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Fire blight: identification, cloning and functional characterisation of related genes on *Malus ×robusta*

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Fire blight: identification, cloning and functional characterisation of related genes on *Malus* $\times$*robusta*

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List of Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
APS	Ammonium persulfate
bp	Base pair
bZIP	Basic leucine zipper
cDNA	Complementary DNA
CDS	Coding sequence
cfu	Colony-forming unit
Chr	Chromosome
CNRQ	Calibrated normalised relative quantities
C_t	Cycle threshold
cv	Cultivar
ddH_2O	Double-distilled water
DEG	Differential expressed gene
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphate
dpi	Days post inoculation
DTT	Dithiothreitol
ERF	Ethylene responsive factor
ETI	Effector-triggered immunity
FDR	False discovery rate
GDR	Genome database for Rosaceae
GO	Gene Ontology
hpi	Hours post inoculation
HR	Hypersensitive response
HSD	Honest significant difference
ID	Identification

JA	Jasmonic acid
kb	Kilo base
LG	Linkage group
MAB	Marker-assisted breeding
MAPK	Mitogen-activated protein kinase
mRNA	Messenger RNA
Mr5	*Malus* ×*robusta* 5
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PTI	Pattern-triggered immunity
PVDF	Polyvinylidene difluoride
PVE	Phenotypic variation explained
qRT-PCR	Quantitative reverse transcription-PCR
QTL	Quantitative trait locus
RACE	Rapid amplification of cDNA ends
RCS	RIN4 cleavage sites
RNA-seq	RNA sequencing
rpm	Revolutions per minute
RT	Room temperature
SA	Salicylic acid
SAS	Statistical analysis system
SDS	Sodium dodecyl sulfate
SNP	Single nucleotide polymorphism
T3SS	Type III secretion system
T_A	Annealing temperature
TEMED	Tetramethylethylenediamine
Tris	Tris(hydroxymethyl)aminomethane
U	Unit
UTR	Untranslated region
Y2H	Yeast two-hybrid

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1. Introduction

1.1. Description and history of the plant disease fire blight

Fire blight is a bacterial disease, caused by the gram-negative enterobacterium *Erwinia amylovora*, that mainly infects plants of the Rosaceae family. It can spread very quickly and cause enormous damage, which is particularly serious for the economically important fruit crops apple and pear. But also quince and ornamental plants like cotoneaster, hawthorn, pyracantha and mountain ash are affected (Zwet and Beer, 1991).

E. amylovora can infect all parts of the plant including flowers, leaves, branches, stems, fruits and roots (Vanneste, 2000). It overwinters within infected tissue of annual cankers, formed on branches diseased in the previous season. In spring, bacterial ooze, which is composed of millions of bacteria, polysaccharides and plant sap, exudes from these areas and serves as primarily inoculum (Zwet and Beer, 1991). Insects attracted by the ooze, but also wind or rain disseminate the bacteria to blossoms (Schroth et al., 1974). There it multiplies initially at the stigma and later it migrates down to the floral nectaries by moistness, resulting in infection and the production of new bacterial ooze, which serves as secondary inoculum (Thomson et al., 1986). In the secondary phase the pathogen infects also shoots, fruits and rootstocks by entering through the stomata or wounds caused by a number of biotic or abiotic factors. From the infected tissue the pathogen moves through the xylem into the parenchyma and down to the root system (Bogs et al., 1998). Typical symptoms of a shoot blight infection are shown in the right picture of figure 1.1 on page 20. Leaves wilt and turn dark brown, but usually remain attached to the tree. The end of the shoot is bended to a so-called “shepherd’s crook” and droplets of the amber coloured bacterial ooze exude from the infected tissue.

Symptoms of fire blight were first reported by Denning (1794) in North America in the Hudson Valley of New York. However, only a hundred years later initiating research

by T.J. Burrill, J.C. Arthur, and M.B. Waite suggested that a bacterium could be the reason for these symptoms (Griffith et al., 2003). Finally, the pathogen was isolated at the beginning of the 20th century as the first proven bacterial plant pathogen and termed *E. amylovora* (Burrill) Winslow et al. (Baker, 1971; Winslow et al., 1920).

Since the discovery of fire blight the pathogen had moved in a period of approximately 135 years into every region of the USA and from there it finally spread over to Japan and New Zealand and reached Europe in 1957, where it was observed for the first time in pear orchards in Kent, England (Bonn and van der Zwet, 2000; Peil et al., 2009). Around ten years later *E. amylovora* was introduced to the mainland of the European continent by infected plant material from Great Britain and reached four years later West Germany (Van der Zwet et al., 1970; Schroth et al., 1974). Today the disease is present in more than 47 countries around the world and with the exception of Portugal it can be found all over Europe (Van der Zwet et al., 2012; EPPO, 2016). To prevent further introductions and the spread of fire blight, the bacterium is classified in many of these countries, also in Germany as a quarantine disease.

1.2. Conventional control methods of fire blight

Once disease symptoms are visible, it is often too late for control and the only possibility that remains is the removing of infected branches or the entire tree in case of a serious infection. Therefore, precautionary measures as the reduction of the pathogen, the prevention of the successful establishment of it and the improvement of host resistance are the most important tools of an effective disease control (Norelli et al., 2003).

The reduction of the pathogen can be achieved by several chemical and biological compounds, which are mainly applied during bloom time. The most effective chemical agent with an efficacy of around 90 percent is streptomycin. The efficacy of the antibiotic against *E. amylovora* was discovered in the 1950s and changed the fire blight management massively (Schroth et al., 1974). But already 30 years later first streptomycin-resistant *E. amylovora* strains were observed in California, USA and since this time further ones occurred in several regions of the United States as well as in Canada, Israel, Egypt, Syria, Mexico, Lebanon and also New Zealand (McManus et al., 2002; Rezzonico et al., 2009; Miller et al., 1972; Gusberti et al., 2015). These developments, but also the risk to health and environment caused by the application of antibiotics in agriculture, restricted or

even prohibited the use of streptomycin within the EU in 2004 (Phillips, 2007). Another chemical approach and one of the first agents used against fire blight is copper. Varieties of compounds and formulations are available, but their efficacy is far below streptomycin (Zwet and Beer, 1991). Furthermore, most copper compounds are phytotoxic and cause fruit lesions, whereby the application is limited before flowering and after harvesting (Schroth et al., 1974). A relatively new non-antibiotic pesticide with a high efficacy of around 73 percent against fire blight is LMA (potassium-aluminium-sulphate). However, as the effects of aluminium are not entirely known yet, the application is controversial discussed in conventional agriculture and even prohibited in organic production. Also effective against fire blight, especially against shoot blight is the growth regulator prohexadione-calcium, a compound that suppresses tree growth (Yoder et al., 1999). But due to the fact that the application is harmful for developing trees, the use is only recommended for established orchards.

An alternative way to the chemical control of fire blight are biologically based methods like the application of antagonists. The idea is that the antagonistic microorganism colonises the stigma first and hence suppresses the establishment and the growth of *E. amylovora*, which reduces the probability of floral infection and the dissemination of the pathogen (Johnson et al., 1993). However, as multiplication and distribution of *E. amylovora* is strongly influenced by the weather, the same applies for the antagonists, which results in high year-to-year and location-to-location variability. Commercially available products are Blight Ban A506 (*Pseudomonas fluorescens*), Bloomtime Biological (*Pantoea agglomerans*), Serenade Max (*Bacillus subtilis*) and Blossom Protect (*Aureobasidium pullulans*). With an average efficacy between 73 and 78 percent, the yeast-like fungus *Aureobasidium pullulans* is the most promising agent against fire blight by far (Gusberti et al., 2015). But as the EPA registration was made only in 2012, the true potential and durability has to be further tested and confirmed. So far, Kunz et al. (2010) could show that treatments with Blossom Protect in conjunction with fungicides used for apple scab control, can cause fruit russetting, whereby these observations depend on the apple variety treated and on the number of applications.

Another part of the multi-faceted disease control, and perhaps the most important one, is the planting of fire blight resistant cultivars. However, varieties that dominate the market currently are highly susceptible to fire blight, even though resistance breeding has been performed since decades. This contradiction is due to several problems, which rise

during the breeding process of apple. First of all, resistance can be mainly found in wild apple species like *M. robusta*, *M. sublobata*, *M. atrosanguinea*, *M. prunifolia* and *M. fusca* (Aldwinckle and Beer, 1979), which are all characterised by bad fruit quality and small fruit sizes (Aldwinckle and Beer, 1979). Therefore, breeding with wild apples species requires several back crosses to get rid of the genetic drag. Moreover, the process is further aggravated by a long juvenile phase of up to twelve years, a high degree of heterozygosity and the self-incompatibility of apple. To receive an apple with excellent fruit quality combined with resistance to fire blight by classical breeding can thus last between 20 to 50 years and even then it has to be introduced to the market and accepted by the consumers. And finally, replanting an orchard with a new variety is a very expensive process with a high risk for the growers. Since apple plants are in the majority of cases a combination of a certain scion and a size controlling rootstock, resistance is also an important goal of rootstock breeding. However, similar to the grafted varieties the most widely used rootstocks M.9 and M.26, are highly susceptible to *E. amylovora* (Norelli et al., 2003). And though scion breeding is complex, rootstock breeding is even more complex, which is caused by a wider range of selection criteria that require extensive and time-consuming tests (Cousins, 2005). Overall, traditional breeding is not a short-term solution, which, however, can be supported or replaced by genetic engineering.

1.3. Genetic engineering to improve host resistance against fire blight

A faster approach to improve host resistance is the use of genetic engineering, an approach of increasing importance and new possibilities. The breeding process can thereby completely be replaced by the transfer of certain genes into already developed varieties or supported and accelerated by marker-assisted breeding (MAB).

The genetic transformation of apple is mediated by the soil plant pathogen *Agrobacterium tumefaciens*, a molecular method initially reported by James et al. (1989). The first gene introduced to apple plants with the goal to improve resistance to fire blight was *attacin E*, an antibacterial gene from *Hyalophora cecropia* (Aldwinckle and Malnoy, 2009). Since then various strategies were used to improve host resistance of apple: production of antimicrobial proteins, inhibition of bacterial pathogenicity factors and silencing or overexpression of related genes of the plant hosts (Malnoy et al., 2012). However, the

introduction of foreign genes into plant genomes is highly regulated in Europe and commercial use in the near future unlikely. A potential alternative with probably more public acceptance is the cisgenic technology (Schouten et al., 2006). Thereby only genes within the own gene pool of *Malus* controlled by their native regulatory sequences are used for transformation. This implies also the selection of the genetic modified plants without marker sequences or the elimination of them by means of a recombinase system after selection. The first cisgenic apple plant was already developed in 2011 by Vanblaere et al. and gave scab resistance to the susceptible apple cultivar Gala by the *HcrVf2* gene from the wild apple species *Malus ×floribunda* 821. The first step to the development of a cisgenic fire blight resistant apple plant was done with the identification of a quantitative trait loci (QTL) on linkage group 3 of *Malus ×robusta* 5 (Mr5), which explained up to eighty percent of the phenotypic variation (PVE) and indicated, therefore, the presence of a major resistance gene (Peil et al., 2007). Later on the gene was isolated as the first resistance gene against fire blight and termed *FB_MR5* (Fahrentrapp et al., 2013). Virulence analysis of transgenic Gala plants containing the resistance gene could finally confirm the functionality of it and paved, therefore, the way for the first fire blight resistant cisgenic apple plant (Broggini et al., 2014).

Such QTLs like the one from Mr5 can not only help to find resistance genes, they are also useful for MAB. The selection is thereby based on a genetic marker linked to a trait of interest, not by the trait itself. The indirect evaluation enables the analysis already of young seedlings and thus accelerates the breeding process immensely. However, since QTLs often span a region of several centimorgans, a precise localisation as well as a better understanding of the underlying genetics is important for their usability. Until now, 27 QTLs have been reported in apple with significant linkage to fire blight resistance (Khan et al., 2012). Four of them are major QTLs validated in multiple studies with different genetic backgrounds and, therefore, useful for MAB (Malnoy et al., 2012). One is the QTL on linkage group 3 of Mr5 (Peil et al., 2007). The other three were found in 'Fiesta' on linkage group 7 (≈ 46 % PVE), in 'Evereste' on linkage group 12 (≈ 39–57 % PVE) and in *M. fusca* on linkage group 10 (≈ 66 % PVE) (Calenge et al., 2005; Khan et al., 2006; Civetta et al., 2009; Parravicini et al., 2011; Emeriewen et al., 2014).

1.4. General aspects of plant-pathogen interactions

In contrast to humans or animals, plants have no adaptive immune system, but nevertheless most plants are resistant to most pathogens (Nürnberger et al., 2004). To protect themselves against invading organisms, plants developed a broad spectrum of structural, chemical and protein-based defence mechanisms, which can be assigned either to the constitutive or the inducible defence. Preformed structural barriers like cell wall, waxy epidermal cuticles and bark belong thereby to the constitutive defence. But also anti-microbial enzymes and secondary metabolites are part of the continuous defence. One example are Saponins, glycosides that disrupt cellular membranes of invading fungal pathogens (Osbourn, 1996). However, as the production of defence-related chemicals or proteins requires a lot of energy and nutrients, plants often wait until the pathogen is detected.

The recognition of a pathogen attack occurs in two different ways, pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006). The first uses transmembrane pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs), like eubacterial flagellin, fungal cell wall components or specific patterns of gram-negative or -positive bacteria (Nürnberger et al., 2004). The ETI on the other hand is activated mostly inside the cell by the interaction of plant resistance (R) proteins with pathogen-secreted effectors, also called avirulence effectors (Avr effectors). The direct interaction between an effector and a R protein is described in the gene-for-gene hypothesis (Keen, 1990; Flor, 1971). The first direct interaction was proven by a yeast two-hybrid assay between the Pi-ta resistance protein of rice and the effector AVR-Pita of *Magnaporthe grisea* causing rice blast disease. But until today only a few examples of direct interaction have been identified, which led to the supplementation of the gene-for-gene hypothesis by the guard model, explaining an indirect interaction where the avirulence protein is targeted/recognised by a guard (Dangl and Jones, 2001; Van Der Biezen and Jones, 1998). One example of this is the cleavage of the protein kinase PBS1 from *Arabidopsis thaliana* by the *Pseudomonas syringae* effector AvrPphB which activates resistance by the R protein RPS5 (Caplan et al., 2008). Beside PBS1 also RIN4 from *A. thaliana* and Pto from *S. lycopersicum* were identified as guard proteins in the process of pathogen detection (Mackey et al., 2002; Mucyn et al., 2006).

1.5. Genetics of fire blight

First genes related to the pathogenicity of *E. amylovora* were identified in the mid 1980s by transposon mutagenesis and subsequent virulence analysis by Steinberger and Beer (1988). Genes inducing hypersensitive response (HR) and pathogenicity were designated as *hrp* genes, whereby genes which abolished only pathogenicity in host plants, but gave no hypersensitive response in tobacco, were termed *dsp* (disease-specific) genes. The *hrp* and *dsp* genes are clustered within the pathogenicity island (PAI) which was sequenced and characterised in 2005 by Oh et al.. The entire Hrp PAI spans a genomic region of 62 kb and contains around 60 genes (Oh and Beer, 2005). Parts of the PAI code for the Hrp type III secretion system (T3SS), a protein complex found in many gram-negative bacteria. The T3SS enables the pathogen to deliver effector proteins directly into the host cell to modulate plant susceptibility and support growth and spread of the pathogen.

Until today five effector proteins are described for *E. amylovora*: Eop1 (OrfB or EopB), $HopX1_{EA}$ (Eop3), $AvrRpt2_{EA}$ (Eop4), DspA/E and $HopPtoC_{EA}$ (HopC1) (Malnoy et al., 2012; McNally et al., 2012; Khan et al., 2012). Initially the designation followed first the term Erwinia outer protein (Eop) which was later partly changed to Hrp outer protein (Hop) according to the homology to other effectors. A major role in virulence could only be observed for DspA/E, $AvrRpt2_{EA}$, and $HopPtoC_{EA}$ (Zhao et al., 2005, 2006). All effectors identified so far in *E. amylovora* are homologous to known effectors or protein families of *P. syringae* or *Y. pseudotuberculosis*. Except for Eop1 and DspA/E all proteins are located outside the PAI gene cluster.

Another group of proteins secreted by the T3SS with a role in the pathogenicity of *E. amylovora* are harpins, which can be found in all phytopathogenic bacteria possessing a T3SS. However, in contrast to the effectors, harpins are secreted to the intercellular spaces of plant tissues (Oh and Beer, 2005). *E. amylovora* produces two harpins, HrpN and HrpW, both located within the PAI gene cluster. Unlike HrpW, HrpN is necessary for the virulence in plants and furthermore involved in the translocation of DspA/E (Vrancken et al., 2013).

The pathogenesis of *E. amylovora* is also dependent on the production of the exopolysaccharide amylovoran. Amylovoran is the essential part of the bacterial ooze and causes wilting of apple shoots by plugging the vascular tissues (Goodman et al., 1974). Steinberger and Beer could show a correlation between the concentration of amylovoran and the virulence of the pathogen. Furthermore it was demonstrated that amylovoran is

essential for the biofilm formation, which is important for the pathogenesis of *E. amylovora* (Koczan et al., 2009).

1.6. The *E. amylovora* effector AvrRpt2$_{EA}$

AvrRpt2$_{EA}$ is one of the effectors of *E. amylovora* and homologue to AvrRpt2 from *P. syringae* pv. *tomato*. Zhao et al. (2006) showed that the deletion of the effector results in the loss of pathogenicity on immature pear fruits, indicating that *avrRpt2*$_{EA}$ acts as virulence factor. This deletion mutant (ZYRKD3-1) was also used to inoculate the fire blight resistant wild apple accession Mr5 (see figure 1.1).

Figure 1.1.: Shoots of Mr5 infected with the wild type strain Ea1189 (left) or the *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 (right). Pictures were taken 28 days after inoculation (from Vogt et al. 2013).

Interestingly the results showed an avirulent effect of AvrRpt2$_{EA}$ in case of Mr5, as the deletion resulted in a complete breakdown of the resistance (Vogt et al., 2013). This was a strong indication for a gene-for-gene relationship in the host pathogen system Mr5 and *E. amylovora*. Furthermore, the homology between the two effectors AvrRpt2 and AvrRpt2$_{EA}$ suggested a similar resistance mechanism between Mr5 and *A. thaliana*.

In *A. thaliana* the mechanism of the effector can be described as follows. Once the AvrRpt2 effector is delivered into the plant cell it interacts with the host protein ROC1, which activates the self-cleavage via prolyl isomerisation (Coaker et al., 2006). The activated AvrRpt2 cleaves RIN4, thus activating the RPS2 protein and thereby the pathogen defence of the plant (Mackey et al., 2002; Axtell and Staskawicz, 2003; Kim et al., 2005). RIN4 is a membrane bound protein of 211 amino acids and interacts also with three further effectors AvrRpm1, AvrB and HopF2pto (Mackey et al., 2002; Kim et al., 2005; Wilton et al., 2010). Recently it could be shown that $AvrRpt2_{EA}$ from the wild type strain Ea3049 is able to cleave RIN4 of *A. thaliana* in *N. benthamiana* (Vogt et al., 2013).

1.7. Aim of the study

The bacterial plant disease fire blight is one of the main threats of apple production worldwide. Under optimal conditions the pathogen can destroy an entire orchard in a single growing season which causes enormous economic losses. Effective control can be only achieved by the use of the antibiotic streptomycin, but the application is restricted or even prohibited in Europe. Since most of the commercially successful apple cultivars are susceptible, fire blight resistance is one of the main goals of many apple breeding programs. Most described sources for fire blight resistance can be found among wild apple species, which, however, are characterised by bad fruit quality and small fruit size. The introgression of these resistances by classical breeding requires, therefore, several backcrosses to get rid of the undesired traits. This is already very time-consuming by itself, but in case of apple it is additionally extended by the high heterozygosity, the long juvenile phase and the self-incompatibility of apple. Molecular methods like marker assisted breeding can help to speed up this process. The linkage between a genetic marker and a resistance QTL or even a resistance gene accelerates the evaluation of a crossing population enormously. Another advantage is the possibility to pyramid functionally different resistances in one genotype. However, it is essential to determine related genes and to understand the mechanism of resistance. This had let to the aim of this study: the identification, cloning and functional characterisation of fire blight related genes of *Malus* ×*robusta* 5 (Mr5).

The first aim was a more detailed description of the role of AvrRpt2$_{\mathrm{EA}}$, an important effector of *E. amylovora* (see section 1.6). Therefore, the *avrRpt2$_{EA}$* gene of 22 different *E. amylovora* strains differing in origin and isolated from different hosts was sequenced and characterised. Differential alleles of the effector were used for the complementation of the *avrRpt2$_{EA}$* deletion mutant strain ZYRKD3-1. The established strains were evaluated by virulence analysis and Real-Time qRT-PCR.

AvrRpt2$_{\mathrm{EA}}$ from *E. amylovora* is homologue to the effector AvrRpt2 from *P. syringae* pv. *tomato*. In *A. thaliana* AvrRpt2 interacts with the guard protein RIN4, which mediates resistance by activation of the R protein RPS2. The similarity between the two effectors raised the question, if there is a similar resistance mechanism between Mr5 and *A. thaliana*. Therefore, it should be possible to identify *RIN4* in *Malus*. Considering the NCBI and UniProt databases revealed two entries for *Malus* ×*domestica*. Hence, the aim of this study was the identification and characterisation of *RIN4* on the genomic and proteomic level of Mr5. To prove protein-protein interactions between AvrRpt2$_{\mathrm{EA}}$ and the guard protein RIN4, a Y2H assay was performed. Additionally, the recently identified fire blight resistance gene *FB_MR5*, as a potential homologue to the RPS2 gene of *A. thaliana*, was included to the analysis as well.

Another major objective of this study was a RNA-seq analysis of the transcriptome of Mr5, to identify new genes related to the resistance mechanism. Therefore, Mr5 was inoculated with the virulent *avrRpt2$_{EA}$* deletion mutant strain ZYRKD3-1 or with the non-virulent wild type strain Ea1189. The analysis was performed at two time points and the comparison of the transcriptomes enabled the identification of differentially expressed genes (DEGs), which should be related to the disease response. The significant DEGs were assigned to pathways, whereby the focus was on genes belonging to the biotic stress. To confirm the results obtained by the RNA-seq analysis and to verify additional time points, selected gene were further analysed with the BioMark™ HD system.

2. Materials and methods

2.1. Materials

The following section contains all *Escherichia coli* and *Saccharomyces cerevisiae* strains, as well as all used kits, enzymes, vectors and antibodies. Strains of *E. amylovora*, chemicals, lab equipment and the composition of buffers and media are mentioned in appendix A and B.

2.1.1. Plant material

The wild apple accession *Malus* ×*robusta* 5 (Mr5, MAL0991) and the cultivar *Malus* ×*domestica* Borkh. cvs 'Idared' were used for artificial shoot inoculation. Both genotypes were obtained from the apple breeding collection of the Institute for Breeding Research on Fruit Crops in Dresden, DE.

2.1.2. Bacteria and yeast strains

E. amylovora strains used in the study are listed in table 5.1 of appendix B.

E. coli strains:

Library Efficiency® DH5α™	F$^-$ Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-,m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-
One Shot® TOP10	F$^-$ *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara*–*leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

Both chemically competent *E. coli* strains were used for cloning and subcloning of various transformations. The strains were ordered from Life technologies/Thermo Fisher Scientific, Schwerte, DE.

S. cerevisiae strains:

Y2HGold	*MATa, trp1−901, leu2−3, 112, ura3−52, his3−200, gal4*Δ*, gal80*Δ*, LYS2::GAL1*$_{UAS}$*−GAL1*$_{TATA}$*−His3, GAL2*$_{UAS}$*−GAL2*$_{TATA}$*−Ade2, URA3::MEL1*$_{UAS}$*−Mel1*$_{TATA}$*, AUR1-C MEL1*
Y187	*MAT*α*, ura3−52, his3−200, ade2−101, trp1−901, leu2−3, 112, gal4*Δ*, gal80*Δ*, met−, URA3::GAL1*$_{UAS}$*−Gal1*$_{TATA}$*−LacZ, MEL1*

Yeast strains were delivered with the Matchmaker® Gold Yeast Two-Hybrid System (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, FR).

2.1.3. Vectors and plasmids

All vectors and plasmids used for transformation of *E. coli*, *E. amylovora* and *S. cerevisiae* are listed in table 2.1. The selection of recombinant cells was performed by antibiotic in case of bacteria and by single dropout (SD) media in case of yeast.

Table 2.1.: Vectors used for transformation of *E. coli*, *E. amylovora* and *S. cerevisiae*. Recombinant cells were selected by antibiotic (bacteria) or by SD media (yeast).

Vector	Antibiotic [µg/ml]	SD Medium	Organism
pGBKT7[1]	Kanamycin 50	-Trp	*E. coli*, *S. cerevisiae*
pGADT7[1]	Ampicillin 100	-Leu	*E. coli*, *S. cerevisiae*
pRK415[2]	Tetracycline 15	–	*E. coli*, *E. amylovora*
pJET1.2[3]	Ampicillin 100	–	*E. coli*
pCR2.1®-TOPO® TA[3]	Kanamycin 50	–	*E. coli*

[1] Expression vector for Y2H, [2] Expression vector for *E. amylovora*, [3] Sequencing vector

Antibiotic stock solutions were prepared in the following concentration (sterilisation via filtration):

Antibiotics	Ampicillin 50 mg/ml
	Kanamycin 30 mg/ml
	Chloramphenicol 34 mg/ml
	Tetracyclin 12.5 mg/ml

Yeast expression vectors pGBKT7 and pGADT7 were delivered with the Matchmaker® Gold Yeast Two-Hybrid System (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, FR). The two sequencing vectors pJET1.2 and pCR2.1®-TOPO® TA were ordered from Thermo Fisher Scientific (Fermentas/Life Technologies), Schwerte, DE.

2.2. Methods

2.2.1. Inoculation of apple shoots

Shoots of Mr5 and 'Idared' were grafted onto M9 rootstocks. The plants were grown in the greenhouse in Quedlinburg at temperatures between 10 to 15 °C under a natural photo period with extension of day time in spring. After four to six weeks the shoots were inoculated by cutting around one third to a half of the tips of the upper two leaves with a scissor dipped in a bacterial suspension (10^9 cfu/ml). Bacteria for the inoculum were cultivated on bouillon glycerine agar at 26 °C for 48 hours and suspended in sterile water. Inoculation was performed on shoots with a minimum length of 25 cm. The inoculated plants were incubated in the greenhouse at 25 to 27 °C (day) and 22 °C (night). Necrosis rate of each inoculated shoot was measured 28 days post inoculation (dpi). The length of necrotic shoot tissue relative to the total shoot length averaged over all replicates was stated as percent lesion length.

2.2.2. Cultivation of bacteria

E. amylovora and *E. coli* were cultivated on LB medium (composition mentioned in appendix A.2) at 28 and 37 °C, respectively. The *E. amylovora* $avrRpt2_{EA}$ deletion mutant ZYRKD3-1 (Zhao et al., 2006) was cultivated on LB medium supplemented with 20 µg/ml chloramphenicol. Recombinant bacteria were cultivated with the appropriate antibiotics dependent on the used vector (see table 2.1).

2.2.3. Cultivation of yeast

Yeast media were purchased directly from the manufacturer as ready-mixed pouches (media pouches, Takara Bio Europe/Clontech, Saint-Germain-en-Laye, FR). The *S. cerevisiae* strains Y2HGold and Y187, used for the yeast two hybrid (Y2H) assay, were cultivated

on YPDA medium at 30 °C. Cultivation of recombinant *S. cerevisiae* strains is described in section 2.5.1.

2.2.4. DNA extraction

Extraction of genomic DNA from *E. amylovora*

The innuPREP Bacteria DNA Kit (Analytik Jena, Jena, DE) was used for the isolation of genomic DNA from *E. amylovora*. Steps were performed as described in the manual. In order to obtain a high concentration of DNA only 50 µl elution buffer were used.

Extraction of plasmid DNA from *E. coli*

The low copy plasmid pRK415 was isolated with the Qiagen Plasmid Midi Kit (Qiagen GmbH, Hilden, DE), whereas the Miniprep Kit (Fermentas/Thermo Fisher Scientific, Schwerte, DE) was used for the high copy plasmids (pJET1.2, pCR2.1®-TOPO® TA, pGBKT7, pGADT7). Cells were isolated from 25 ml (low copy plasmid) and 2.5 ml (high copy plasmids) LB culture medium respectively. Plasmid DNA was quantified by agarose gel electrophoresis by comparing with several DNA mass standards (λ-DNA, Fermentas/Thermo Fisher Scientific, Schwerte, DE) of known concentration or photometric with the NanoDrop.

2.2.5. RNA extraction

Extraction of RNA from plant material

Approximately 100 mg of plant material was used for the isolation of RNA with the InviTrap® Spin Plant RNA Mini Kit (STRATEC Biomedical, Birkenfeld, DE). Frozen cell tissue was disrupted with 5 mm steel beads in a mixer mill for 2 min and 20 Hz. After complete disruption and homogenisation of the plant tissue, the cells were supplemented with the β-mercaptoethanol containing Lysis Solution RP and thoroughly mixed. Further steps were performed as described in the manual. RNA was eluted with 50 µl elution buffer R and purified with the DNA-free™ Kit according to the manual (Life Technologies, Darmstadt, DE).

Extraction of RNA from *E. amylovora*

0.5 ml of an overnight cell culture was transferred into 5 ml fresh LB medium and incubated (shaking, 28 °C) until the OD_{600} reached 0.5–1. Up to 1 x 10^9 cells were transferred in a micro centrifuge tube and harvested for 2 min at $\geq$ 12 000 g. Further isolation steps were performed with the GeneJET™ RNA Purification Kit as described in the purification protocol for total RNA from bacteria (Fermentas/Thermo Fisher Scientific, Schwerte, DE). RNA was eluted with 50 µl nuclease-free water and purified with the DNA-free™ Kit according to the manual (Life Technologies, Darmstadt, DE).

Extraction of RNA from *S. cerevisiae*

Cells of one yeast colony were transferred into 5 ml YPDA medium and incubated (shaking, 30 °C) until OD_{600} reached 0.5–1. Up to 4 x 10^8 cells were then harvested for 2 min at $\geq$ 12 000 g. Isolation was performed with the GeneJET™ RNA Purification Kit as described in the purification protocol for total RNA from yeast (Fermentas/Thermo Fisher Scientific, Schwerte, DE). The lysis buffer was supplemented with β-mercaptoethanol and 50 units of lyticase. The RNA was eluted with 50 µl nuclease-free water and purified with the DNA-free™ Kit according to the manual (Life Technologies, Darmstadt, DE).

2.2.6. cDNA synthesis

cDNA synthesis was performed with the RevertAid First Strand cDNA Synthesis Kit (Fermentas/Thermo Fisher Scientific, Schwerte, DE). Oligo$(dT)_{18}$ primers were used for the synthesis of plant and yeast cDNA. cDNA from bacteria was reverse transcribed with random hexamer primers. Not more than one microgram was used for transcription. The steps were carried out as described in the manual.

2.2.7. PCR based methods

Standard PCR

Amplification was performed in 20 µl reaction volume with the DreamTaq™ DNA Polymerase (Fermentas/Thermo Fisher Scientific, Schwerte, DE). The reaction mixture contained 10X DreamTaq Buffer, 0.2 mM dNTPs, 0.5 µM forward and reverse primer, 0.4 U DreamTaq™ DNA Polymerase and 1 µl template DNA, RNA or cDNA. In case of a colony PCR the template DNA was replaced with cell material picked from colonies

using a sterile toothpick and dipped into the reaction tube. Initial denaturation at 94 °C was hold for 3 minutes. After the first step the cycles started with denaturation at 94 °C for 30 seconds, followed by annealing for one minute at a primer specific temperature (tabulated in appendix C) and elongation for one minute per up to 1 kb at 72 °C. Final elongation was performed for 5 minutes at 72 °C. The specific number of cycles of the appropriate primers are tabulated in appendix C.

Amplification for cloning was performed in 50 µl reaction volume and unless otherwise specified with the Phusion High-Fidelity DNA Polymerase (Fermentas/Thermo Fisher Scientific, Schwerte, DE). Therefore, one unit of the polymerase was supplemented with 5X HF Buffer as well as dNTPs, primer and template DNA as mentioned above. PCR conditions were not changed.

RACE-PCR

RACE (Rapid amplification of cDNA ends) PCR was performed with the FirstChoice RLM-RACE Kit (Life Technologies, Darmstadt, DE). Steps were performed as described in the manual. The specific inner and outer primer are tabulated in appendix C.4. RNA was isolated from plant material collected for the gene expression analysis described in section 2.8. Samples used for RNA isolation, the amount of RNA, the used polymerase for PCR and nested PCR as well as the purification method of the PCR fragments are mentioned in table 2.2.

Table 2.2.: RACE-PCR of *RIN4* and *FB_MR5* from Mr5: source and amount of RNA, polymerase used for PCR and nested PCR as well as the purification method of the fragments.

Gene	End	Sample and quantity of RNA	Polymerase	Purification method
RIN4	5’	ZYRKD3-1 48 hpi, 10 µg	Phusion	PCR sample
	3’	ZYRKD3-1 48 hpi, 1 µg	–	–
FB_MR5	5’	1189 2 hpi, 23 µg	DreamTaq	Gel extraction
	3’	Mr5_ni[1], 1 µg	Phusion	Gel extraction

[1] Mr5_ni: plant material was collected from shoots of Mr5, which were not inoculated.

DreamTaq™ DNA Polymerase was used if the PCR with the Phusion High-Fidelity DNA Polymerase yielded no fragments. PCR fragments were cloned with the CloneJET PCR Cloning Kit (Fermentas/Thermo Fisher Scientific, Schwerte, DE) as described in section

2.2.8. The cloning protocol was adapted in accordance with the used polymerase for blunt-end PCR products or products with 3'-A overhangs.

Real-Time qRT-PCR

Real-Time qRT-PCR (quantitative reverse transcription-PCR) was performed with the iCycler iQ™ Real-Time PCR Detection System of BioRad. Reaction volume was set to 20 µl, with 2 µl of cDNA (synthesis described in section 2.2.6, diluted 1:50 with ddH_2O), 6 µl ddH_2O, 0.5 µM forward and reverse primer and 10 µl of 2X Maxima SYBR Green qPCR Master Mix (Fermentas/Thermo Fisher Scientific, Schwerte, DE). Initial denaturation at 94 °C was held for 3 minutes. After the first step the cycles started with denaturation at 94 °C for one minute, followed by annealing for one minute at a primer specific temperature (tabulated in appendix C) and elongation for one minute at 72 °C. The final step was performed after 40 cycles for one minute at 94 °C. Melting curve analysis started at 55 °C, with a heating rate of 0.5 °C per ten seconds up to 95 °C. The standard curve was generated by four dilution steps with a factor of 2 (1, 2, 4 and 8 µl cDNA).

Primer pre-test for the gene expression analysis with the BioMark™ HD system

All primers were tested with the same sample of Mr5, isolated one hour after inoculation with the *E. amylovora* wild type strain Ea1189. Since the parameters of the primer design were identical, the establishment occurred only once exemplary for the gene MDP0000327191 in a temperature range between 55 and 65 °C. The best amplification efficiency was achieved with an annealing temperature of 62 °C, whereby further primers as well as different housekeeping genes were tested at this temperature. Each primer test included three technical replicates as well as a water control. Evaluation was performed with the iQycler software (version 3.1.7050). Sample preparation is mentioned in section 2.7.1, primer development and optimization is described in section 2.8.1 and the primer sequences are tabulated in appendix C.5.

Real-Time qRT-PCR with complemented *E. amylovora* strains Four hundred nanograms RNA isolated from *E. amylovora* were used for cDNA synthesis. Primer establishment was performed for the two housekeeping genes EaRproS and EaATP and the specific SNP primers 681/682 and 681/683 with cDNA of *E. amylovora* wild type strain Ea222 (primers are tabulated in appendix C.2). Seven different strains: Ea222, Ea3049, ZYRKD3-1+AvrS_G, Ea222+Avr-S_LacP, Ea222+Avr-S_eP, Ea3049+Avr-C_LacP and

Ea3049+Avr-C_eP were analysed in two technical and three biological replicates. qBase+ was used for the evaluation of the data obtained from the iCycler iQ. The geNorm expression stability value of the reference gene and the coefficient of variation of the normalised reference gene relative quantities was set to 1 and 0.5 respectively (Vandesompele et al., 2002; Hellemans et al., 2007).

2.2.8. Cloning of *E. coli*

Cloning for sequencing

PCR-reaction was performed with the DreamTaq DNA Polymerase from DNA or cDNA with the appropriate primers as described in section 2.2.7. The cloning reaction was carried out with the TOPO® TA Cloning Kit (Life Technologies, Darmstadt, DE) or the CloneJET PCR Cloning Kit (Fermentas/Thermo Fisher Scientific, Schwerte, DE) according to the instructions of the manufacturer. The Transformation was performed with the One Shot® TOP10 and Library Efficiency® DH5α™ competent cells as described in the manuals. Ten microliter of the transformed cells supplemented with 90 µl LB medium were spread on selective LB medium (antibiotics in accordance with the vector, see table 2.1) and incubated over night at 37 °C. For blue/white colony screening with the pCR2.1®-TOPO® TA vector LB medium was further supplemented with 50 µg/ml X-gal. If no colonies obtained, the rest of the transformation mixture was spread on one plate. Recombinant colonies were verified by PCR with M13 or the pJET1.2 primers and send to Eurofins MWG Operon for sequencing (primer sequences are tabulated in appendix C.1).

Cloning in expression vectors

Expression vectors are mentioned in table 2.1. pRK415 was used for the establishment of complemented *E. amylovora* strains with the $avrRpt2_{EA}$ gene (specified in section 2.4). pGBKT7 and pGADT7 were used for the expression of *RIN4*, *FB_MR5* and $avrRpt2_{EA}$ in the Y2H assay (specified in section 2.5). The appropriate genes were amplified from DNA or cDNA with primers containing restriction sites for BamHI and EcoRI as described in section 2.2.7. The PCR products as well as the vectors were digested with the restriction enzymes EcoRI (10 U; Fermentas/Thermo Fisher Scientific, Schwerte, DE) and BamHI (20 U; Fermentas/Thermo Fisher Scientific, Schwerte, DE) for one hour at 37 °C in a water bath (20 µl reaction volume). After digestion, the PCR products were purified with

the QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, DE) and quantified by agarose gel electrophoresis by comparing with several DNA standards of known concentration. Plasmids were purified by ethanol precipitation and quantified with the NanoDrop. Ligation (vector to insert ratio 1:20) was carried out with one unit of T4 DNA Ligase (5 U/µl; Fermentas/Thermo Fisher Scientific, Schwerte, DE) and 2 µl 10X T4 DNA Ligase Buffer at room temperature for 1 hour (20 µl reaction volume). One microliter of the ligation reaction mixture (diluted 1:5 with TE buffer) was used for the transformation of 100 µl Library Efficiency® DH5α™ Competent Cells. The transformation procedure was performed as described in the manual of the competent cells. Ten microliter of the transformed cells supplemented with 90 µl LB medium were spread on selective LB medium (antibiotics in accordance with the vector, see table 2.1) and incubated over night at 37 °C. If no colonies obtained, the rest of the transformation mixture was spread on one plate. Recombinant colonies were verified by PCR and send to Eurofins MWG Operon for sequencing (primers are mentioned in section 2.4 and 2.5 respectively).

2.3. Sequencing of the *avrRpt2*$_{EA}$ gene of different *E. amylovora* strains

The *avrRpt2*$_{EA}$ gene was amplified from single colonies of 22 different *E. amylovora* strains using the avrRpt2-1 and avrRpt2-2 primers (colony PCR is described in section 2.2.7, primers are tabulated in appendix C.2). The PCR product was purified with the MSB® Spin PCRapace Kit (STRATEC Biomedical, Birkenfeld, DE) as described in the manual. Sequencing was performed by Eurofins MWG Operon with the amplification primes and additionally with the avrRpt2-5 and avrRpt2-6 primers. The purified PCR products were sequenced directly, without cloning in a sequencing vector. Nucleotide and derived amino acid sequences were aligned with BioEdit version 7.0.9.0 (Hall, 1999).

2.4. Establishment of complemented *E. amylovora* strains

The *avrRpt2*$_{EA}$ gene was amplified from DNA of *E. amylovora* strains Ea222 and Ea3049 with the avrRpt2-4-Bam5’ and avrRpt2-3-Eco5’ or with the avrRpt2-3-Bam5’

and avrRpt2-4-Eco5' primer pairs. Cloning into the expression vector pRK415 is described in section 2.2.8. To include the own promoter and terminator region, the cloned gene contained 177 bases before start and 98 behind stop codon. The two primer combinations enable the ligation of the *avrRpt2*$_{EA}$ gene in frame with the *lac* promoter of the vector or in the reverse way. This allowed the expression under the *lac* promoter of the vector and under the own promoter of the *avrRpt2*$_{EA}$ gene respectively. Colony PCR and sequencing was performed with the M13 primers (listed in appendix C.1).

The *E. amylovora* wild type strains Ea222, Ea3049 and the *avrRpt2*$_{EA}$ deletion mutant strain ZYRKD3-1 were transformed by electroporation. For that, fresh grown cells of *E. amylovora* were transferred in LB medium (5 ml per transformation) and grown at 28 °C with shaking for around five hours until the OD_{600} reached 0.6–0.8. The cells were harvested by centrifugation (one min, max speed, 4 °C), washed once to twice with cold water and twice with a cold 15 % glycerol solution. After the last washing step the pellet was resuspended in 100 µl cold 15 % glycerol solution and transferred with 1 µl of the vector into an electroporation cuvette (1 mm electrode gap). Electroporation was performed with 800 Ω, 25 µF and 2.5 kV (Gene Pulser® II Electroporation System, Bio-Rad Laboratories). Immediately after the pulse, 1 ml of LB medium (room temperature) was added and the suspension was transferred in a 2 ml Eppendorf tube. After incubation for one additional hour (shaking, 28 °C) the cells were harvested by centrifugation (one min, max speed) and resuspended in 100 µl LB. Ten microliter of the transformed cells supplemented with 90 µl LB medium were spread on plates of selective LB medium (15 µg/ml tetracycline) and incubated at 28 °C. If no colonies obtained, the rest of the transformation mixture was spread on one plate. Colonies were tested for recombination with M13 or gene specific primers.

2.5. Yeast two-hybrid assay

2.5.1. Cloning

The Matchmaker® Gold Yeast Two-Hybrid System (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, FR) was used to detect protein interaction. The Y2H assay was performed between RIN4, the fire blight resistance protein FB_MR5 from Mr5 and the effector protein AvrRpt2$_{EA}$ from *E. amylovora*. Since *RIN4* can be found on chromosome 5 and 10 in the genome of Mr5, both genes were used for the assay (*RIN4*-Chr5 and

RIN4-Chr10). The effector was tested in 3 different versions, the C-allele from Ea222 (*avrRpt2*$_{EA}$-C), the S-allele from Ea3049 (*avrRpt2*$_{EA}$-S) and in a third version, where the reading frame was optimised for eucaryotic translation (*avrRpt2*$_{EA}$-eu, sequence in appendix F).

The development of the fusion constructs with the Gal4 DNA binding domain (bait plasmid pGBKT7) and the Gal4 activation domain (prey plasmid pGADT7) were performed as follows. *FB_MR5* was cloned in frame with the Gal4 DNA binding domain of the bait plasmid pGBKT7, whereas *avrRpt2*$_{EA}$ were used for the generation of the prey construct. *RIN4* was cloned with bait and prey vector to verify an interaction with both, FB_MR5 and AvrRpt2. The genes were cloned full length in frame to the expression vectors as described in section 2.2.8. The synthesis of the bait construct with *FB_MR5* was performed by DNA Cloning Service (DNA Cloning Service, Hamburg, DE). The two alleles of *avrRpt2*$_{EA}$ (*avrRpt2*$_{EA}$-C and *avrRpt2*$_{EA}$-S) and *RIN4* (*RIN4*-Chr5 and *RIN4*-Chr10) were each amplified with the same primers and selected after the transformation in *E. coli* by colony PCR with the allele specific primers. Table 2.3 summarises the designation of the primers used for the amplification of the genes and for the subsequently colony PCR as well as the appropriate vectors (primer sequences are tabulated in appendix C.3). Recombinant vectors were isolated from *E. coli* as described in section 2.2.4. A minimum of three positive colonies were sequenced with the T7 forward primer (T7 Prom) and the appropriate reverse primers pGB-R and pGAD-R (tabulated in appendix C.1).

For the transformation of *S. cerevisiae*, the strains Y2HGold (compatible with the vector pGBKT7) and Y187 (compatible with the vector pGADT7) were grown over night in YPDA medium at 30 °C with shaking. 500 µl of the overnight culture were transferred in fresh YPDA medium (5 ml per transformation) and further incubated until the OD_{600} reached 2 (around 3−5 h). The cells were harvested by centrifugation (5 min, 3500 g), washed once with 1 ml H_2O and once with 1 ml 100 mM lithium acetate solution. The pellet was supplemented with 240 µl polyethylene glycol, 36 µl LiAc, 10 µl carrier DNA (denatured, 10 mg/ml), 1 µg recombinant vector and set with water to a total volume of 360 µl. The reaction mix was vortexed and incubated with shaking in a 42 °C water bath for 40 minutes. The cells were centrifuged (15 s, max speed) and the pellet resolved in 100 µl ddH_2O and spread on single dropout media SD/-Trp (Y2HGold, pGBKT7) and SD/-Leu (Y187, pGADT7). Beside the transformation of the vectors with the candidate

genes, the control vectors pGBKT7-53, pGBKT7-Lam and pGADT7-T delivered with the system were transformed as well.

Table 2.3.: Primers and vectors used in the Y2H assay for the appropriate genes, the two alleles of *avrRpt2*$_{EA}$ (*avrRpt2*$_{EA}$-C and *avrRpt2*$_{EA}$-S) and *RIN4* (*RIN4*-Chr5 and *RIN4*-Chr10) were each amplified with the same primers and selected after transformation in *E. coli* by colony PCR with the allele specific primers.

Gene	**Primer for amplifying**	**Vector**	**Primer for colony PCR**
avrRpt2$_{EA}$-C	Y2H-Avr-F/R	pGBKT7	680, 683
avrRpt2$_{EA}$-S			680, 682
avrRpt2$_{EA}$-eu	Y2H-Avr/eu-F/R		Y2H-Avr/eu-F/R
RIN4-Chr5	Y2H-Rin4-F/R	pGBKT7, pGADT7	3RACE-Rin4-C5, Rin4d-R
RIN4-Chr10			3RACE-Rin4-C10, Rin4d-R
FB_MR5	Y2H-FB_MR5-F/R	pGADT7	cand1-1f, cand1-1r

2.5.2. Mating

For the mating process only fresh grown colonies were used. For the formation of the diploid yeast strain two colonies, each containing the bait or prey vector, were picked with a sterile toothpick and placed both in the same 2 ml Eppendorf tube containing 500 µl of 2X YPDA. The mixture was gently vortexed and incubated with shaking at 200 rpm at 30 °C overnight (20−24 h). After 20 hours one droplet of the culture was examined under the microscope. If zygotes were present, 100 µl of a 1/10 dilution with YPDA were spread on each of the following agar plates.

- SD/-Trp/-Leu (DDO)
- SD/-Trp/-Leu +X-α-Gal (DDO/X)
- SD/-Ade/-His/-Trp/-Leu+X-α-Gal+Aureobasidin A (QDO/X/A)

Plates were incubated at 30 °C for 3–5 days. The control experiments pGBKT7-53 + pGADT7-T (positive) and pGBKT7-Lam + pGADT7-T (negative) were treated in the same way.

To verify the presence of the two corresponding genes on mRNA level RT-PCR was performed. RNA was isolated from colonies grown on DDO medium. For that, cell

material of one inoculation loop was resolved in 1 ml YPDA medium and grown until the OD_{600} reached 0.5–1. RNA isolation and cDNA synthesis were performed as described in section 2.2.5 and 2.2.6. RT-PCR was performed with the same primers used for colony PCR (see table 2.3).

2.6. Western blot

All buffers and solutions used for western blot analysis are mention in appendix A.2.

2.6.1. Protein extraction

Between 50 and 100 mg of plant tissue frozen in liquid nitrogen were disrupted with 5 mm steel beads in a mixer mill for 2 min and 20 Hz. Ten microliter of 2x Lämmli sample buffer (composition mentioned in appendix A.2) were added per 1 mg of plant tissue and incubated at 95 °C for 10 min. The mixture was centrifuged (RT, 13 000 rpm, 5 min) and the supernatant was transferred in a new Eppendorf tube.

2.6.2. Electrophoresis of proteins

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the Mini-PROTEAN® II Electrophoresis Cell system by BioRad.

Table 2.4.: Composition of stacking and resolving gel used in the SDS-PAGE.

	Stacking gel 5 % [ml]	**Resolving gel 15 %** [ml]
H_2O	1.4	2.3
30 % Acryl-/Bisacrylamid (29:1)	0.33	5
Tris 1.5 M (pH 8.8)	-	2.5
Tris 0.5 M (pH 6.8)	0.25	-
10 % SDS	0.02	0.1
10 % APS	0.02	0.1
TEMED	0.002	0.004
Total	4	10

Between 10 and 15 µl of the protein extract as well as a protein weight standard (PageRuler unstained protein ladder, Fermentas/Thermo Fisher Scientific, Schwerte, DE) were loaded

into the wells of the stacking gel and separated initially with 100 V and after the entry of the proteins in the resolving gel with 160 V. Composition of the stacking and resolving gel see table 2.4. 10x SDS-PAGE running buffer was prepared and diluted 1:10 before use.

2.6.3. Membrane transfer and detection

Proteins were transferred onto a pretreated polyvinylidene difluoride (PVDF) membrane using the Trans-Blot® SD Semi-Dry Transfer Cell by BioRad. For that, the PVDF membrane was incubated in methanol for two seconds and then washed in H_2O for two minutes and in anode II buffer for five minutes. The Blot was assembled as described in figure 2.1.

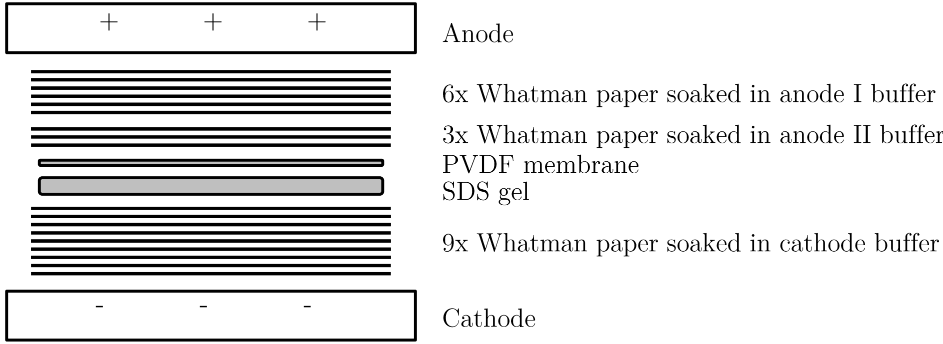

Figure 2.1.: Assembly of the western blot apparatus.

Proteins were transferred from the SDS gel to the PVDF membrane for 30 and up to 120 min at 20 V. For checking purposes the SDS gel was stained with coomassie blue R and destained with acidic acid. The membrane was stained with ready-to-use Ponceau-S solution for two to ten minutes. After destaining with water the membrane was rinsed two times for ten minutes in TBS-T buffer and then blocked with 2 % non-fat dry milk in TBS-T for one hour at room temperature or over night at 4 °C. After washing one time for ten minutes with TBS-T and two times for 10 minutes with TBS the membrane was incubated with the first antibody (RIN4 antibody (aN-13) 200 µg/ml, diluted 1:200 with 1 % non-fat dry milk in TBS, Santa Cruz Biotechnology, Heidelberg, DE) for one hour at room temperature in a hybridisation oven. Washing steps were repeated with TBS-T and TBS as described above. The hybridisation with the second antibody (donkey anti-goat 200 µg/ml, diluted 1:5000 with 1 % non-fat dry milk in TBS, Santa Cruz Biotechnology,

Heidelberg, DE) took place in the same way as described for the first antibody. After further washing steps with TBS-T and TBS as described previously the membrane was incubated with Luminol Reagent for one minute. Protein detection was performed with the Gel Doc™ XRS+ System by BioRad after 10 seconds, 3 and 30 minutes.

2.7. RNA-seq

2.7.1. Sample preparation and RNA extraction

Four samples were analysed by RNA-seq in a differential approach. Therefore, shoots of Mr5 were inoculated with *E. amylovora* wild type strain Ea1189 or the $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1 (described in subsection 2.2.1). The upper two leaves were collected from a minimum of 10 shoots 2 and 48 hours post inoculation (hpi) and immediately frozen in liquid nitrogen. RNA was isolated as described in section 2.2.5. Six microgram (200 ng/µl) were send to GATC Biotech for RNA-seq analysis. The quality of the RNA was verified by GATC with the Bioanalyzer 2100.

2.7.2. cDNA library construction, sequencing and mapping

The cDNA libraries were constructed with the TruSeq RNA Sample Preparation Kit following the manufacturer's instruction (Illumina, San Diego, USA). The four tagged libraries were pooled and sequenced on one paired-end lane with a read length of 50 bp using the Illumina HiSeq2000 system. Reads passing standard filtering of the sequencer were trimmed to cut off the adapter sequences. Mapping to the reference transcriptome of *Malus ×domestica* cv. 'Golden Delicious' (Malus_×_domestica.v1.0.consensus_CDS.fa.gz) was performed by Burrows-Wheeler Alignment tool (BWA) with the default parameters (Li and Durbin, 2009). Reads mapped to more than one site of the genome were excluded. Library construction, sequencing and mapping were done by GATC Biotech AG.

2.7.3. Data analysis and bioinformatics

Quality of the reads was controlled by FastQC (Andrews, 2012). Mapped reads were utilised for differential gene expression analysis using DESeq R package [vers. 3.0.2] (Anders and Huber, 2010). For that, raw counts were imported, normalised and the variance estimated by the assumption of no replicates. Samples infected with the wild

type strain or the mutant strain were compared at 2 and 48 hpi. P-values for the statistical significance of the fold change were adjusted for multiple testing with the Benjamini-Hochberg correction for controlling the false discovery rate of $< 10\,\%$ (Benjamini and Hochberg, 1995). Differentially expressed genes were assigned to pathways by MapMan (Thimm et al., 2004), which is based on the annotation to homologue genes of *A. thaliana*. The mapping file of *Malus* ×*domestica* was downloaded from the MapMan website (http://mapman.gabipd.org/web/guest/mapmanstore, 08.08.2014).

2.8. Gene expression analysis with the BioMark™ HD system

To get more information about gene expression in Mr5 after inoculation with the two different *E. amylovora* strains (Ea1189 and ZYRKD3-1), further time points were included in the analysis. Beside the samples taken at 2 and 48 hpi used for RNA-seq, four more time points per strain were included: 1, 4, 12 and 24 hpi. Additionally the gene expression was analysed in one non-infected sample of Mr5.

2.8.1. Primer development and optimisation

Primer design was performed with the Primer3 software and the following parameters: primer length 18–25 bp (optimum 20); GC content 40–60 %, product size 100–200 bp (optimum 150 bp), melting temperature 60±1 °C, temperature difference < 0.5 °C (Rozen and Skaletsky, 1999). The self-complementarity score was set to three with an increased value if no acceptable primers were found. Primer pairs were also tested by NetPrimer software (PREMIER Biosoft International, Palo Alto, CA) to avoid hairpins, primer dimers and primer cross dimers. The primers were designed on the transcriptome of 'Golden Delicious' and corrected in the case of SNPs to the transcriptome of Mr5. In case of a large number of SNPs, which led to the adoption that some reads could also belong to another gene or allele, up to three wobble bases were used for primer design. The first step in the primer verification was a Reverse Transcriptase-PCR (RT-PCR) with all samples. Annealing was performed for one minute at 57 °C. Positively tested Primers were further analysed by Real-Time qRT-PCR (see paragraph 2.2.7). To use primers with the BioMark™ HD system the C_t value had to be between 25 and 35. Positively

tested primers are listed in appendix C.5.

2.8.2. Differential gene expression analysis

With the BioMark™ 96.96 Dynamic Array it is possible to analyse 96 genes in 96 samples at the same time. 87 out of 96 genes were DEGs identified by RNA-seq, whereby the selection was mainly limited to DEGs with a higher expression level in Mr5 after inoculation with the wild type strain Ea1189. In addition two further candidate genes of Mr5 related to fire blight resistance were selected. The recently identified *FB_MR5* fire blight resistance gene from Mr5 (Fahrentrapp et al., 2013), and a further candidate gene encoding for a class three secretory peroxidase (GenBank: EH034548; (Gardiner et al., 2012)). In case of *FB_MR5* two different primer primers were used. Furthermore the analysis included the five reference genes Ubiquitin, RNA-Polymerase (two different primer pairs), GAPDH, EF1α and Rubisco.

The 96 utilized samples were based on the experimental design of the RNA-seq analysis, but the analysed time points were extended to 1, 2, 4, 12, 24 and 48 hpi and a non-infected sample of Mr5 was added. Three biological and two technical replicates of each sample as well as negative controls (cDNA synthesis without reverse transcriptase) and water were randomly spread on the chip.

The Steps were performed as follows. Forward and reverse primers for all targets were mixed for the pre-amplification step with low TE buffer to a final concentration of 20 µM. 10 µl of each primer pair were combined and filled up with low TE to 1 ml (200 nM pooled primer mix). 4.5 µl Pre-Mix (2.5 µl TaqMan PreAmp Master Mix (Applied Biosystems/Thermo Fisher Scientific, Schwerte, DE) and 1.25 µl pooled primer mix) and 1.5 µl cDNA were pre-amplified with the GeneAmp® PCR 9700 System. Cycling conditions were set to 95 °C for 10 min, followed by 14 cycles: 95 °C for 15 s and 60 °C for 4 min. Reactions were diluted 1:20 with low TE buffer. The 96.96 Dynamic Array Integrated Fluidic Chip (IFC) was prepared according to the manufacturer's instructions and loaded with 6 µl of the primer and the sample assay. For the primer assay 3.85 µl master mix (3.5 µl 2x Assay loading reagent, 0.35 µl low TE) and 3.15 µl 20 µM primer mix were combined. The sample assay consisted of 5.2 µl sample pre-mix solution (3.5 µl 2X TaqMan Gene Expression Master Mix (Applied Biosystems/Thermo Fisher Scientific, Schwerte, DE), 20X DNA Binding Dye Sample Loading Reagent (Fluidigm, South San Francisco, USA), 20X EvaGreen DNA binding dye (Biotium, Fremont, USA) and 1X

low TE) and 1.8 µl pre-amplified cDNA (diluted 1:20). The reaction conditions were set as follows: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. Melt curve analysis was performed after amplification by heating the samples by 1 °C per second from 60−95 °C. Data were analysed by the Fluidigm Real-Time PCR Analysis 3.1.3 software (linear baseline correction, auto Ct threshold determination and quality threshold of 0.65) and by qbase+ 2.5.1. (Hellemans et al. 2007). The fold change was calculated by the $2^{-\Delta\Delta \mathrm{Ct}}$ method and presented relative to the uninfected sample (95 % confidence interval). PCR efficiencies were assumed at 100 % in all samples.

2.8.3. Statistical analysis

The calibrated normalised relative quantities (CNRQ) were exported from qbase+ and statistically evaluated with SAS Enterprise Guide (version 6.100). Normal distribution was verified by Shapiro-Wilk test on log transformed data. If no normal distribution could be observed data was analysed untransformed, square root or multiplicative inverse transformed. Normal distributed data were further analysed by one way analysis of variance (ANOVA) and Tukey's HSD (honest significant difference) test. Without normal distribution Kruskal-Wallis and Mann-Whitney U test was used.

3. Results

3.1. Analysis of the AvrRpt2$_{EA}$ effector

The first step was the sequencing of the *avrRpt2$_{EA}$* gene of different *E. amylovora* strains from different origins and hosts to identify variations in the nucleic and amino acid sequence, respectively. Different alleles of the effector were later used for the complementation of the *E. amylovora avrRpt2$_{EA}$* deletion mutant ZYRKD3-1. Subsequently virulence analysis should provide a more detailed view of the role of the effector within the host pathogen system Mr5 and *E. amylovora*. Furthermore, the expression of the *avrRpt2$_{EA}$* gene of the complemented strains was analysed by Real-Time qRT-PCR.

3.1.1. Sequencing of the *avrRpt2$_{EA}$* gene

The *avrRpt2$_{EA}$* gene of 22 different *E. amylovora* strains (tabulated in appendix B) differing in origin and isolated from different hosts was amplified from single colonies and sequenced by Eurofins MWG Operon. As described in Zhao et al. (2006) the gene started with GTG and stopped after 669 base pairs with TAG. The DNA sequence of all *avrRpt2$_{EA}$* genes was completely identical except for one single nucleotide polymorphism (SNP) at position 467. Three strains Ea110, Ea3049 and Ea3050 possessed a cytosine instead of a guanine at this position. This polymorphism resulted in an exchange of the amino acid sequence at position 156 from cysteine to serine. The two alleles of the *avrRpt2$_{EA}$* gene were further designated as C-allele (cysteine at position 156) and S-allele (serine at position 156). The amino acid sequence from position 150 to 160 from all sequenced *E. amylovora* strains is shown in figure 3.1a. The SNP was also proved by PCR with the specific primer combinations 680/682 for the S-allele and 680/683 for the C-allele (see figure 3.1b). Since the SNP was located at the 3' position of the primers 682 and 683, it was possible to identify clearly the appropriate allele.

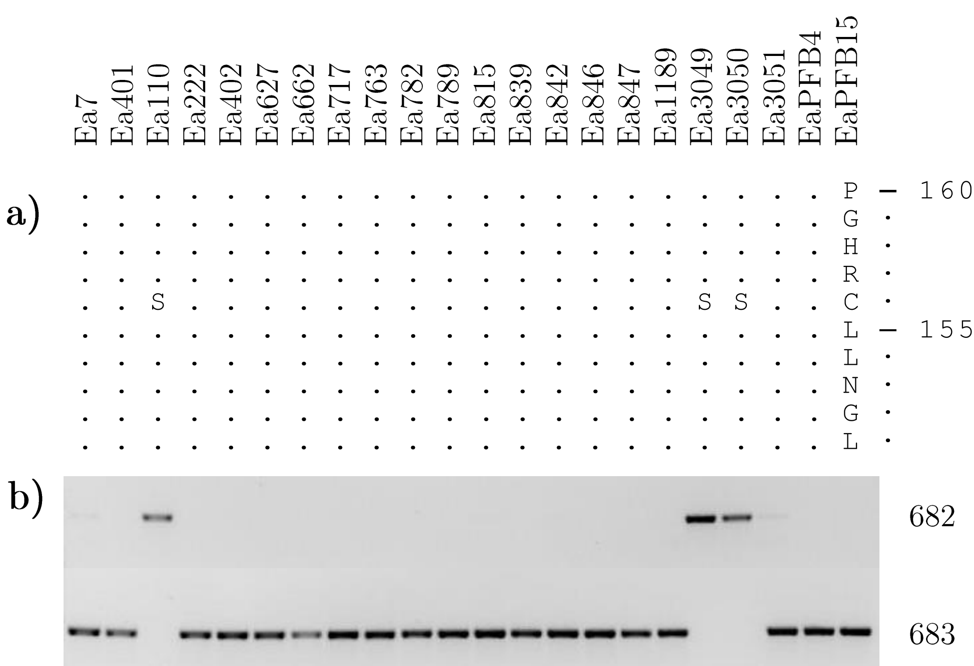

Figure 3.1.: (a) Deduced amino acid sequence of the *avrRpt2$_{EA}$* gene from position 150 to 160 of 22 different *E. amylovora* strains, (b) Verification of the SNP with the allele specific primers 680/682 (S-allele) and 680/683 (C-allele) by PCR.

Virulence analysis with some of these *E. amylovora* strains (Ea7, Ea110, Ea222, Ea3049, Ea3050, Ea3051) and further ones showed a relationship between the allele of the *avrRpt2$_{EA}$* gene and the ability to break the resistance of Mr5 (Vogt et al., 2013). Strains carrying the S-allele of the effector were virulent to Mr5, whereas strains with the S-allele were not able to break the resistance of Mr5.

3.1.2. Development and virulence analysis of the complemented *E. amylovora* strains

In order to verify whether the SNP of the *avrRpt2$_{EA}$* gene indeed determines the virulence of *E. amylovora* against Mr5, the deletion mutant ZYRKD3-1 was complemented with the functional C- or S-allele of the *avrRpt2$_{EA}$* gene. As strains carrying the C-allele are non-virulent to Mr5, the complementation of the virulent mutant strain ZYRKD3-1 with it should lead to the recovery of the resistance in Mr5. ZYRKD3-1 complemented with the S-allele should still break the resistance of Mr5 as the mutant strain by itself and the

S-allele of the effector are virulent. A further point of focus was the analysis of the effect when both alleles are present in one genotype. Therefore, the wild type strains Ea222 (C-allele) and Ea3049 (S-allele) were used for complementation with the appropriate opposite allele as well. The two alleles were ligated to the expression vector pRK415 with the own promoter and terminator region. To enable the expression of the $avrRpt2_{EA}$ gene under the *lac* promoter of the vector or under the own promoter of the gene, the two alleles were cloned in both directions into the expression vector (labelled with LacP and eP). These two versions were designed to verify if the expression level, which should be higher under the lac promoter, influence the virulence.

Beside Mr5, also the susceptible cultivar 'Idared' were used for the virulence analysis. When the apple shoots were ready for inoculation, only the complemented mutant strain ZYRKD3-1+Avr-C_eP was usable. To nonetheless ensure a comparison with the mutant strain complemented with the S-allele, the strain ZYRKD3-1 (pZYR2) published in Zhao et al. (2006) (in this study designated as ZYRKD3-1+Avr-S_G) was used for inoculation as well. The complementation of this strain was performed with the same plasmid pRK415, but without a specific direction of the gene.

Virulence analysis was performed by artificial shoot inoculation of a minimum of ten shoots of Mr5 and 'Idared'. Beside the two complemented mutant strains ZYRKD3-1+Avr-C_eP and ZYRKD3-1+Avr-S_G, the mutant strain ZYRKD3-1 itself and the wild type strains Ea1189 (carrying the $avrRpt2_{EA}$ C-allele), Ea110 and Ea3050 (both carrying the $avrRpt2_{EA}$ S-allele) were used. Prior to the phenotypic evaluation the strains were verified by RT-PCR to prove the transcription of the $avrRpt2_{EA}$ gene (results are shown in appendix D). As shown in the left chart of figure 3.2, 'Idared' was highly susceptible to all *E. amylovora* strains with percent lesion lengths between 40 and 80 %. The inoculation of Mr5 (right chart of figure 3.2) in contrast confirms the strain specific resistance, as already indicated in the introduction. *E. amylovora* strains carrying the S-allele of the effector like Ea110 and Ea3050 as well as the $avrRpt2_{EA}$ deletion mutant ZYRKD3-1 are virulent to Mr5, whereas the wild type strain Ea1189 (carrying the $avrRpt2_{EA}$ C-allele) are non-virulent to Mr5. That this behaviour depends indeed on the allele of the effector could be confirmed by the results obtained from the inoculation of Mr5 with the complemented mutant strains, ZYRKD3-1+Avr-C_eP and ZYRKD3-1+Avr-S_G. The complementation of the virulent $avrRpt2_{EA}$ deletion mutant ZYRKD3-1 with the functional C-allele of the effector results in the complete recovery of resistance of Mr5,

whereas ZYRKD3-1 complemented with the S-allele is still virulent to Mr5. These results confirmed the important role of the *avrRpt2*$_{EA}$ effector in the resistance mechanism of Mr5 and the hypothesis of a gene-for-gene relationship in the host pathogen system Mr5 and *E. amylovora*.

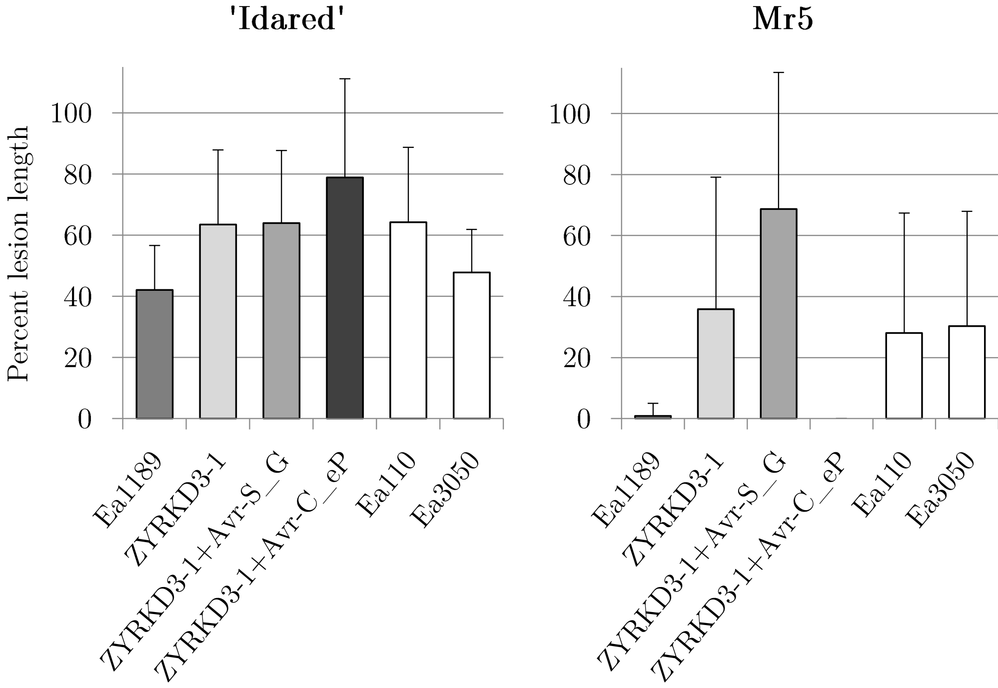

Figure 3.2.: Virulence analysis of the complemented *E. amylovora* strains ZYRKD3-1+Avr-S_G and ZYRKD3-1+Avr-C_eP as well as the wild type strains Ea1189 (carrying the *avrRpt2*$_{EA}$ C-allele) and Ea110 and Ea3050 (both carrying the *avrRpt2*$_{EA}$ S-allele) and the *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1; Percent lesion length: length of necrotic shoot/shoot length*100 % of a minimum of 10 inoculated shoots per strain; error bars represent positive standard deviation.

3.1.3. Real-Time qRT-PCR with the complemented *E. amylovora* strains

The *avrRpt2*$_{EA}$ gene was further analysed by Real-Time qRT-PCR to verify the influence of the promoter used for the complementation (see section 3.1.2) and to compare the expression of the effector to the wild type strains. Therefore, the two wild type strains Ea1189 and Ea3049, as well as the four complemented wild type strains (Ea222+Avr-S_eP, Ea222+Avr-S_LacP, Ea3049+Avr-C_eP, Ea3049+Avr-C_LacP) and the complemented mutant strain (ZYRKD3-1+Avr-S_G) were analysed. To distinguish between the two

alleles of the *avrRpt2*$_{EA}$ gene the specific SNP primers 681/682 (S-allele specific) and 681/683 (C-allele specific) were used for the expression analysis (primers are listed in appendix C.2).

The results obtained by Real-Time qRT-PCR (see figure 3.3) were evaluated with the qbase+ software. The upper chart shows the expression of the S-allele of the *avrRpt2*$_{EA}$ gene, whereas the lower chart shows the expression of the C-allele. The expression is presented relative to the wild type strains Ea3049 in the upper chart and Ea222 in the lower chart. Data normalisation was performed with both reference genes rpoS and pstB. The geNorm expression stability value of the reference genes was 0.61 for both and the coefficient of variation of the reference gene was 0.22 for pstB and 0.2 for rpoS. Error bars denote the 95 % confidence interval of the relative quantities. The significance of the fold changes, analysed with qbase+ statistics by mean comparison (One-way Anova) is tabulated in appendix E.

The results indicate that the orientation of the gene in the vector has no effect of the expression of the *avrRpt2*$_{EA}$ gene, there is no significant difference between the two promoters neither for the C-allele nor for the S-allele. Further, the comparison of the expression of the *avrRpt2*$_{EA}$ gene of transformed strains and untransformed wild type strains shows that the expression is higher in the complemented strains, not only for the gene expressed from the vector, even the genomic ones have a higher expression level. The complemented mutant strain ZYRKD3-1+Avr-S_G showed only expression for the complemented S-allele, as the deletion in the genomic *avrRpt2*$_{EA}$ gene prevent the annealing of the allele specific SNP primers. The expression level is quite similar to the other complemented strains.

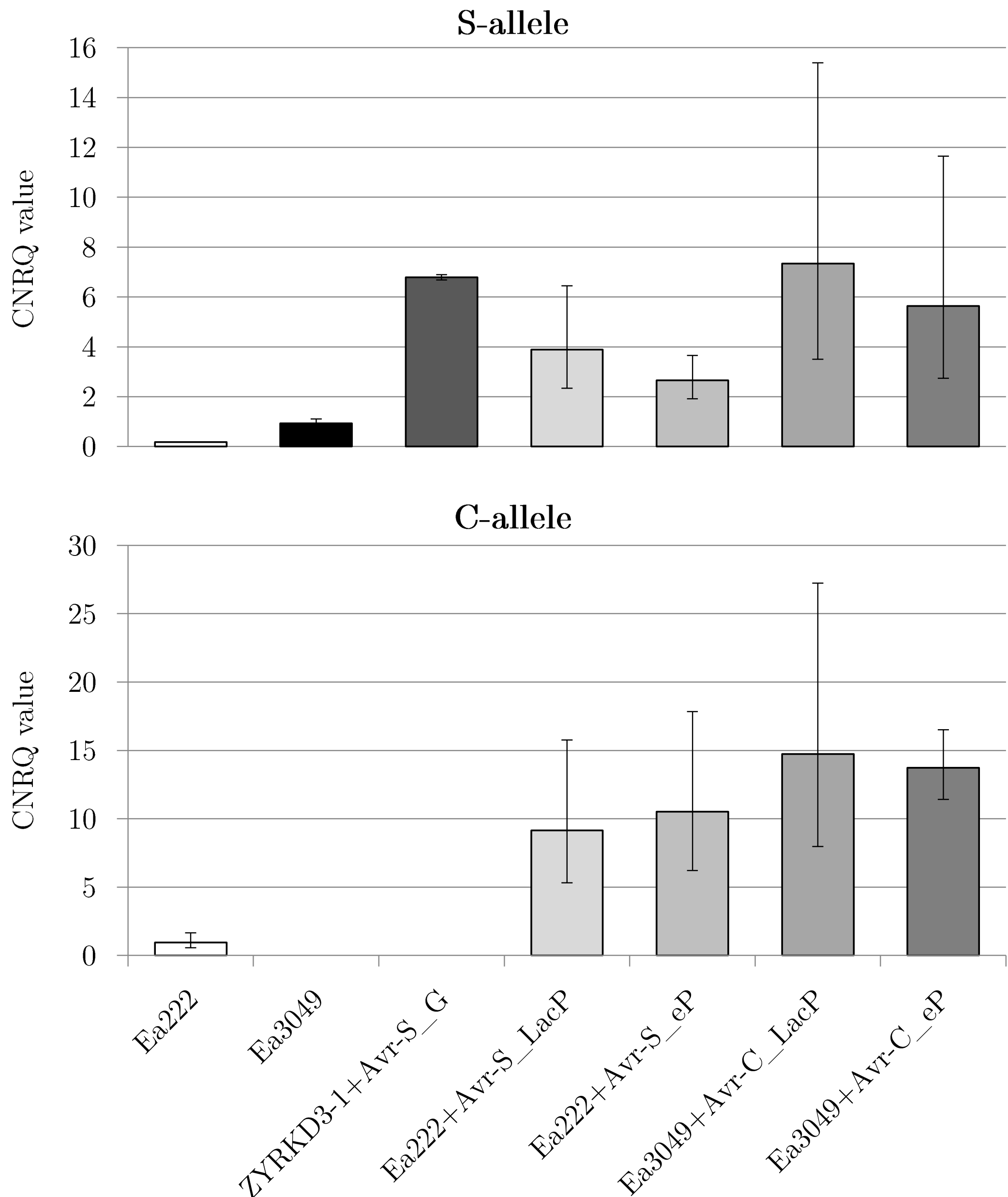

Figure 3.3.: Real-Time qRT-PCR with the complemented *E. amylovora* strains and the corresponding wild type strains. The expression of the appropriate allele of the $avrRpt2_{EA}$ gene was regulated by the *lac* promoter of the vector or by the own promoter (labelled with LacP and eP). ZYRKD3-1+Avr-S_G is the mutant strain ZYRKD3-1 complemented with the S-allele of the effector (designated as ZYRKD3-1 (pZYR2) by Zhao et al. (2006)). Gene expression was analysed with the allele specific SNP primers 681/682 (S-allele, upper chart) or 681/683 (C-allele, lower chart) and evaluated with the qbase+ software. The expression is presented relative to the wild type strains Ea3049 (upper chart) or Ea222 (lower chart). Error bars denote the 95 % confidence interval.

3.2. RIN4 of *Malus ×robusta* 5

RIN4 is a target of the effector AvrRpt2 of *P. syringae* in *A. thaliana* and related to the resistance mechanism by RPS2 or RPM1 (Mackey et al., 2003). After detection of the homologue effector AvrRpt2$_{\mathrm{EA}}$ in *E. amylovora* and the essential role of the *avrRpt2*$_{EA}$ gene in the resistance mechanism of Mr5 the question was raised whether the resistance mechanism of Mr5 is similar to the one of *A. thaliana*. Subsequent literature research revealed several homologous of *RIN4* in different plant genomes, so also in the genome of 'Golden Delicious' on chromosomes 5 and 10. Consequently, the next steps were to identify and to characterise RIN4 in the genome of Mr5. These steps included the sequencing of the gene, the analysis of the UTR via RACE PCR and the comparison of the nucleic and amino acid sequences to other homologues. In addition to the genetic characterisation, the analysis was also focused on the proteomic level, which was done by a western blot and a yeast two-hybrid assay.

3.2.1. Sequence analysis of *RIN4* from Mr5

Two protein sequences of RIN4 from *Malus ×domestica* were already published in the universal protein database (http://www.uniprot.org), *RIN4-1* on chromosome 10 (D2D0J1) and *RIN4-2* on chromosome 5 (D2D0J2). A BLASTn search with the coding sequences of *RIN4-1* and *RIN4-2* in the predicted CDS of the 'Golden Delicious' genome in the database for Rosaceae (http://www.rosaceae.org) identified two similar protein predictions, MDP0000252761 and MDP0000290323, on chromosomes 5 and 10 as well. However, a comparison revealed major differences in the length of the coding sequences, which resulted mainly from the varying definition of the start and stop codon. Table 3.1 shows a comparison of the exon/intron length of the predicted and published sequences of *RIN4* from *Malus* as well as *RIN4* from *A. thaliana*.

The published sequences of *RIN4-1* and *RIN4-2* have the same numbers of exons and introns like *RIN4* from *A. thaliana* and also a similar length of the coding sequence. However, the predicted sequences of MDP0000252761 and MDP0000290323 are considerably longer and MDP0000252761 even comprises of one additional exon. Therefore, it can be assumed that the prediction of MDP0000252761 and MDP0000290323 in the database for Rosaceae is incorrect. In order to confirm this presumption and to determine the right start and stop codon of *RIN4* from Mr5, the ends of the coding sequences were

Table 3.1.: Comparison of the exon/intron length in base pairs of *RIN4* from *Malus ×domestica* published in the universal protein database (http://www.uniprot.org), *RIN4-1* on chromosome 10 (D2D0J1) and *RIN4-2* on chromosome 5 (D2D0J2) with two similar protein predictions MDP0000252761 (chromosome 5) and MDP0000290323 (chromosome 10) identified by BLASTn search in the predicted CDS of the 'Golden Delicious' genome in the database for Rosaceae (http://www.rosaceae.org) as well as *RIN4* from *A. thaliana* (Q8GYN5).

Gene	E1	I1	E2	I2	E3	I3	E4	I4	E5
RIN4-2 (chr 5)	6	2102	507	285	183	93	30		
MDP0000252761 (chr 5)	69	400	507	285	183	1139	78	207	1812
RIN4-1 (chr 10)	6	1605	501	255	183	93	30		
MDP0000290323 (chr 10)	6	1605	501	255	183	1554	1863		
RIN4 A. thaliana	6	472	417	78	183	104	30		

E... exon, I... intron

verified by 3' and 5' RACE-PCR initially. The obtained fragments were cloned and sequenced and aligned to the appropriate contigs of 'Golden Delicious' (see appendix G). An alignment of the sequences obtained from the 3' RACE-PCR was not implemented since the sequences did not match *RIN4*. The results of the 5' RACE-PCR show for both *RIN4* on chromosome 5 and 10 from Mr5 the same first exon of six base pairs in length followed by a large intron. This confirms the sequences of *RIN4-1* and *RIN4-2* and a correct prediction of the start codon of MDP0000290323.

Besides the analysis of the untranslated region (UTR), the entire sequence of both alleles of *RIN4* from Mr5 were defined. Figure 3.4 shows the alignment of the amino acid sequence of RIN4 from *A. thaliana* , the deduced amino acid sequences of RIN4 from Mr5 from chromosome 5 (RIN4-Chr5-Mr5) and 10 (RIN4-Chr10-Mr5) as well as the amino acid sequences of RIN4-1 and RIN4-2 from *Malus ×domestica* . The red arrows in figure 3.4 indicate the RIN4 cleavage sites RCS1 and RCS2 from *A. thaliana* by the effector AvRpt2 from *P. syringae* pv. *tomato*. Both motifs are located in very conserved domains at position 10−11 and 152−153. RCS1 is completely identical in all RIN4 proteins, whereas in RCS2 of Mr5 and *Malus ×domestica* one amino acid has been exchanged from aspartic to glutamic acid in comparison to RIN4 from *A. thaliana*. Another sequence motif, marked with a green box in figure 3.4, is the cysteine-rich membrane-anchoring or S-palmitoylation site in the C-terminal region of RIN4. The three cysteine residues C203, C204 and C205 of RIN4 from *A. thaliana* can also be found on chromosome 10, but not on chromosome 5 of Mr5 and *Malus ×domestica*.

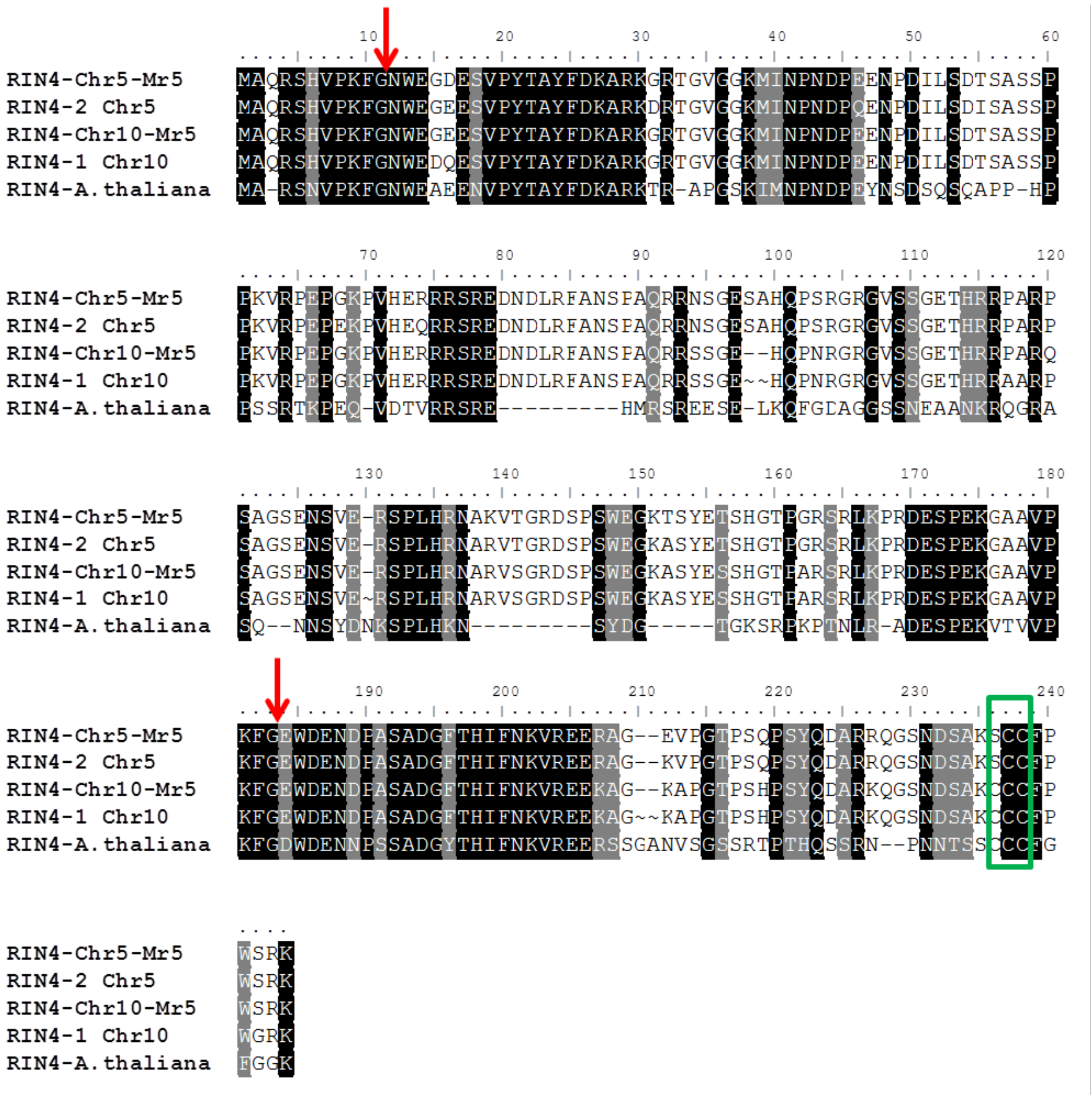

Figure 3.4.: Protein alignment of RIN4: from *A. thaliana* (Q8GYN5), both alleles from Mr5 located on chromosome 5 (RIN4-Chr5-Mr5) and 10 (RIN4-Chr10-Mr5) and both alleles from *Malus* × *domestica*, also located on chromosome 5 (RIN4-2, D2D0J2) and 10 (RIN4-1, D2D0J1). Black background indicates identical amino-acid residues; grey background indicates similar amino acid residues; red arrows indicate cleavage sites of RIN4 from *A. thaliana* by AvRpt2 from *P. syringae* pv. *tomato*; the green box marks the S-palmitoylation side.

A comparison of the protein sequences of RIN4 of Mr5, *Malus ×domestica* (D2D0J2 and D2D0J1) and *A. thaliana* (Q8GYN5) is shown in the form of a identity matrix in percent in table 3.2.

Table 3.2.: Protein sequence identity matrix of RIN4 from Mr5 on chromosomes 5 and 10, from *Malus ×domestica* (M×D) on chromosomes 5 and 10 and from *A. thaliana* (A.t.) in percent.

RIN4		Mr5		M×D		A.t.
		Chr5	**Chr10**	**Chr5**	**Chr10**	
Mr5	**Chr5**	ID	92.9	96.2	91.7	44.2
	Chr10	92.9	ID	92.5	97.4	44
M×D	**Chr5**	96.2	92.5	ID	90.8	44.6
	Chr10	91.7	97.4	90.8	ID	44
A.t.		44.2	44.0	44.6	44	ID

The similarity of the protein sequences between RIN4 of Mr5 and *Malus ×domestica* is very high and ranges between 92 and 97 percent. There are 18 SNPs between *RIN4* on chromosome 5 of Mr5 and *Malus ×domestica*, which result in nine different amino acids. *RIN4* on chromosome 10 is more similar with only 8 SNPs and 5 different amino acids. The comparison of RIN4 from Mr5 and *Malus ×domestica* with RIN4 from *A. thaliana* shows an identity of around 44 %.

3.2.2. Western blot with the RIN4 antibody

Because of the high sequence similarity between RIN4 from *A. thaliana* and Mr5, it was tested to use the N-terminal RIN4 (aN-13) antibody from *A. thaliana* to detect the protein in Mr5 via western blot as well.

For that, proteins were isolated from frozen plant tissue of Mr5, Pinova and *A. thaliana* as control and used for SDS-PAGE. The plant tissue of Mr5 was the same as it was used for the RNA-seq analysis in section 3.4.1. Beside the four samples collected two and 48 hours after inoculation with the non-virulent *E. amylovora* strain Ea1189 or the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 one further non-infected sample of Mr5 was analysed. The plant material of Pinova was non-infected and originated from an *in vitro* culture. Figure 3.5 shows the SDS gel stained with colloidal Coomassie, the membrane after protein transfer stained with Ponceau-S and the membrane developed

with the RIN4 antibody (from a to c). According to the molecular weight, protein bands of RIN4 of *A. thaliana* and Mr5 should appear at 23 and 26 kDa, respectively.

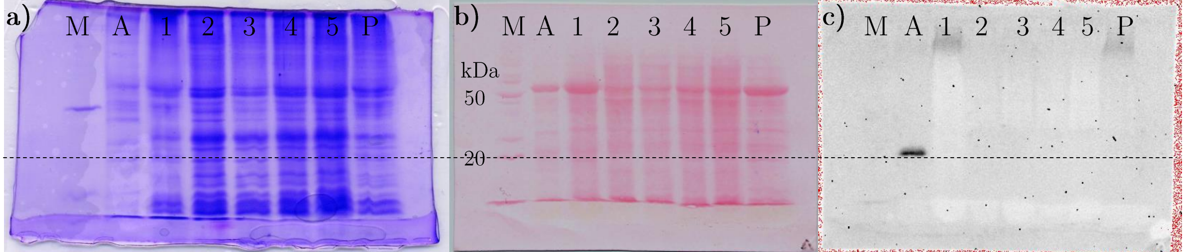

Figure 3.5.: Western blot of Mr5, Pinova and *A. thaliana* with the RIN4 antibody of *A. thaliana*; M molecular weight marker, A *A. thaliana*, 1 Mr5 not inoculated, 2–3 Mr5 at 2 and 48 hpi with Ea1189, 4–5 Mr5 inoculated with ZYRKD3-1 at 2 and 48 hpi, P Pinova; a) SDS gel stained with colloidal Coomassie, b) membrane stained with Ponceau-S and c) membrane with RIN4 antibody after detection with luminol reagent; the dashed line marks 20 kDa.

The SDS gel in figure 3.5a shows an incomplete transfer of the proteins from the gel to the membrane, although the transfer time was increased from 30 to 120 minutes. After the incubation with the antibodies and the detection with luminol reagent it appears that the RIN4 antibody only binds to the control sample of *A. thaliana*. And even though the amount of transferred proteins is higher in the samples of Mr5 and Pinova, no signal can be observed (see figure 3.5b and c). A direct comparison of the antibody binding site and the sequence of Mr5 was not possible, since the manufacturer didn't supply the data.

3.3. Yeast two-hybrid assay to prove protein-protein interactions

Initially, RIN4 was identified in a Y2H screen via its interaction with the *A. thaliana* effector AvrB (Mackey et al., 2003). Today it is known, that RIN4 is targeted by many virulence effectors and associated by several resistance proteins, not only in *A. thaliana* but also in other plant systems. Since a couple of these interactions were identified via a Y2H screen, the method was selected as well to discover potential protein-protein interaction of RIN4 from Mr5 in this study. Two proteins were eligible as interacting partners, the fire blight resistance protein FB_MR5, as Fahrentrapp et al. (2013) postulated a similar mode of action to RPS2, and the effector protein AvrRpt2$_{EA}$ from *E. amylovora*. Therefore, the appropriate genes were fused to the two fragments of the Gal4 transcription factor,

the DNA-binding domain (DNA-BD) or the activation domain (AD). If the so called bait and prey proteins interact with each other, the two domains of the transcription factor are indirectly connected and activate, therefore, the transcription of four independent reporter genes (AUR1-C, ADE2, HIS3, and MEL1).

FB_MR5 was cloned in frame with the Gal4 DNA binding domain of the bait plasmid pGBKT7. Contrary, the $avrRpt2_{EA}$ gene was used for the generation of the prey construct. $avrRpt2_{EA}$ was used in 3 different versions, the C-allele isolated from Ea222, the S-allele isolated from Ea3049 and in a third version, where the reading frame was optimised for eucaryotic translation ($\mathrm{AvrRpt2_{EA}}$-eu, sequence see appendix F). *RIN4* was cloned from chromosomes 5 and 10 with the bait and the prey vector to verify an interaction with both proteins, FB_MR5 and $\mathrm{AvrRpt2_{EA}}$. The constructs were transformed into the appropriate yeast strains and combined for mating. Additionally, a positive (Y2HGold with pGBKT7-53 and Y187 with pGADT7-T) and a negative (Y2HGold with pGBKT7-Lam and Y187 with pGADT7-T) control mating were performed.

The potential protein-protein interaction was verified by the appropriate selective media. The double dropout media (DDO) is the first selection step, only yeast cells containing bait and prey plasmids are able to grow on this media. However, in the last selection step, the Quadruple dropout media supplemented with X-α-Gal and Aureobasidin A (QDO/X/A), only cells containing bait and prey plasmids with interacting proteins are able to grow. Furthermore, due to the α-galactosidase expression the colonies turn blue.

The results are shown in table 3.3. In case of the positive control, colonies grew on all selection steps and turned blue in the presence of α-galactosidase, whereas the colonies of the negative control grew only on the DDO medium. All tested interactions grew on the DDO medium, which means that the transformation with the bait and prey constructs were successful. However, the last selection step was negative in all cases. The tests were repeated several times, always with the same result. To confirm the transcription, RNA was isolated from the individual yeast strains containing one construct, reverse transcribed and analysed by RT-PCR (Data not shown). The transcription of the genes were proven in all cases.

Table 3.3.: Results of the Y2H assay to evaluate potential protein-protein interaction between RIN4 from Mr5 (isolated from chromosome 5 and 10), the fire blight resistance protein FB_MR5 from Mr5 and the effector protein AvrRpt2$_{EA}$ from *E. amylovora* (AvrRpt2$_{EA}$-S: S-allele isolated from Ea3049, AvrRpt2$_{EA}$-C: C-allele isolated from Ea222, AvrRpt2$_{EA}$-eu: optimised reading frame for eucaryotic translation, C-allele), as well as a positive and a negative control, which were delivered with the Matchmaker® Gold Yeast Two-Hybrid System.

Y2HGold (pGBKT7 + X)	**Y187 (pGADT7 + X)**	**DDO**	**QDO/X/A**
Positive Control		+	+ (blue)
Negative Control		+	-
AvrRpt2$_{EA}$-S	FB_MR5	+	-
AvrRpt2$_{EA}$-C	FB_MR5	+	-
AvrRpt2$_{EA}$-eu	FB_MR5	+	-
RIN4 (chr 5/10)	FB_MR5	+	-
AvrRpt2$_{EA}$-S	RIN4 (chr 5/10)	+	-
AvrRpt2$_{EA}$-C	RIN4 (chr 5/10)	+	-
AvrRpt2$_{EA}$-eu	RIN4 (chr 5/10)	+	-

Whether or not colonies were grown on DDO or QDO/X/A medium was marked with + and - respectively. DDO double drop out media, QDO/X/A quadruple dropout media supplemented with X-α-Gal and Aureobasidin A.

3.4. RNA-seq and gene expression analysis with the BioMark™ HD system

Beside the characterisation of already known genes, the study was also focused on the identification of new genes. One approach is a RNA-seq analysis, a method which allows the characterisation of transcriptomes based on next-generation sequencing (NGS) technologies. Since the genome of 'Golden Delicious' is available (Velasco et al., 2010), RNA-seq can be also used for transcriptomic studies of apple. To identify genes related to the disease response the transcriptome of Mr5 was analysed after inoculation with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1 or the non-virulent wild type strain Ea1189 at 2 and 48 hours post inoculation (hpi). Differential expressed genes (DEGs) were identified by statistical evaluation and assigned to functional categories by MapMan. Selected genes were further analysed by gene expression analysis with the BioMark™ HD system to confirm the results received by RNA-seq analysis on the one hand and to verify additional time points on the other. Finally, the log2 fold change of the DEGs obtained by RNA-seq and gene expression analysis was compared. Furthermore, the data was used for a comparison between sequencing results and prediction in case of *RIN4*.

3.4.1. Differential transcriptome analysis by RNA-seq

RNA-seq analysis was primarily performed to identify new proteins related to the resistance mechanism of Mr5 against fire blight. Furthermore, based on the experimental design of the analysis it was possible to study the effect of the existence or the absence of the $\mathrm{AvrRpt2_{EA}}$ effector to the transcriptome of Mr5. Therefore, shoots of Mr5 were inoculated with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1 or with the non-virulent wild type strain Ea1189. To have a comparison between the early and the late disease response, plant material was collected for sequencing 2 and 48 hpi. The RNA was isolated and send to the GATC Biotech for the further steps. The RNA integrity number, verified with the Bioanalyzer 2100, was equal to or above eight in all samples (see appendix J). Since the RIN value ranges between 1 (degraded) and 10 (intact), the values obtained were excellent for plant RNA and, therefore, suitable for the next steps. Sequencing was performed by Illumina HiSeq2000 paired end with a read length of 50 bp. In total, 364.572.150 reads were obtained with a nearly similar distribution within the

four samples (see table 3.4). Quality analysis of the RNA-seq results were implemented by FastQC 0.10.1, which provides a set of modules for the evaluation of high throughput sequencing data (results see appendix K). Since all samples showed a very high sequence quality score with mean values above 35, which means less then one base call in 5000 is predicted to be incorrect, the data has a universally good quality. Modules like basic statistics, sequence GC content and sequence length distribution passed analysis as well, whereas others failed or raised warnings for all or only for individual samples. However, FastQC was mainly designed for DNA-seq, fails or warnings do not necessarily imply poor data in case of RNA-seq.

The alignment of the reads, also called mapping, to the reference transcriptome of *Malus ×domestica* cv. 'Golden Delicious' was done with BWA (Li and Durbin, 2009) and the last step performed by GATC Biotech. Around 50 % of the 364.572.150 reads could be aligned to the reference transcriptome, which included contig crossing reads and singletons but excluded reads that mapped to more than one site. Table 3.4 lists the number of reads per sample received from RNA-seq, but also the mapped reads.

Table 3.4.: Number of reads per sample received from RNA-seq of Mr5 after sequencing and mapping with BWA; transcriptome of Mr5 was sequenced 2 and 48 hpi with the virulent *avrRpt2*$_{EA}$ deletion mutant strain ZYRKD3-1 or with the non-virulent wild type strain Ea1189.

Read Category	Number of Reads			
	ZYRKD3-1		Ea1189	
	2 hpi	48 hpi	2 hpi	48 hpi
All	73 513 950	93 631 410	81 606 040	115 820 750
Mapping to whole genome	54 303 092	66 824 347	56 686 940	80 589 354
Non uniquely mapped[1]	16 687 078	19 616 676	17 699 203	24 009 219
Cross contig[2]	765 520	1 023 886	817 800	1 203 510
Singletons[3]	4 052 216	5 138 139	5 030 528	6 392 451
Resulting Reads	37 616 014	47 207 671	38 987 737	56 580 135

[1]Number of reads mapped to more than one site of the genome, reads have been excluded from analysis. [2]Number of reads with the other end mapped to a different contig. [3]Number of reads with itself or its mate unmapped.

To identify DEGs and thus also genes related to the resistance mechanism, RNA-seq data was statistically evaluated with the DESeq software package (Anders and Huber, 2010). Therefore, the transcriptome was compared between Mr5 inoculated with the wild type strain Ea1189 and Mr5 inoculated with the mutant strain ZYRKD3-1 at 2 and 48 hpi.

Figure 3.6 shows the two scatter plots at 2 (left) and 48 hpi (right) obtained by DESeq, whereby one dot is one gene with the appropriate log2 fold change and the normalised mean read frequency. The red dots represent the significant DEGs, identified with p-values less than 0.1 after they are adjusted for multiple testing with Benjamini-Hochberg correction for controlling the false discovery rate. Overall, it was possible to identify 211 significant DEGs. The plots show the same symmetry in up- and down-regulated genes with a maximum log2 fold change of around 6. As the noise around smaller counts require large log2 fold changes to be significant, it is difficult to identify DEGs with a low level of transcription.

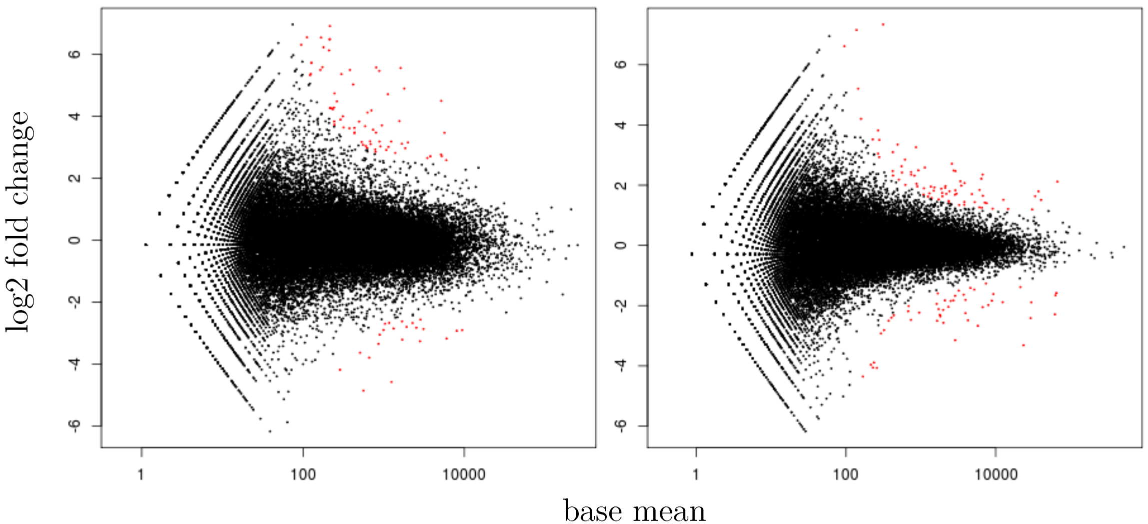

Figure 3.6.: Log2 fold change scatter plot of the DEGs obtained by the transcriptome analysis of Mr5; comparison of the transcriptome was done by the DESeq software package between Mr5 inoculated with the wild type strain Ea1189 and Mr5 inoculated with the mutant strain ZYRKD3-1 at 2 hpi (left) and 48 hpi (right); significant DEGs are coloured in red (10 % FDR).

The distribution of the 211 significant DEGs within the samples is mentioned in table 3.5. 85 out of the 211 significant DEGs were identified at 2 hpi, 106 at 48 hpi and 20 at both time points. Most of these genes had a higher expression level after inoculation of Mr5 with the wild type strain Ea1189: 63 at 2 hpi, 57 at 48 hpi and all of the 20 genes at both time points. The number of genes is thereby nearly identical at 2 and 48 hpi in the incompatible reaction (Mr5 and Ea1189), whereas it increases from 2 to 48 hpi by almost the factor 2 in the compatible reaction (Mr5 and ZYRKD3-1).

Table 3.5.: DEGs with a significant higher expression level in Mr5 at 2 and/or 48 hpi with the non-virulent wild type strain Ea1189 or with the virulent *avrRpt2*$_{EA}$ deletion mutant strain ZYRKD3-1; statistical analysis was implemented with DESeq.

***E. amylovora* strain used for inoculation of Mr5**	**2 hpi**	**DEGs at 2 and 48 hpi**	**48 hpi**
Ea1189	63	20	57
ZYRKD3-1	22	-	49

The 211 DEGs were further analysed by MapMan, which is a tool that enables the assignment of the DEGs to functional categories, so called BINs (Thimm et al., 2004). The left chart in figure 3.7 shows the genes with a significantly higher expression level in Mr5 after inoculation with the *E. amylovora* wild type strain Ea1189. In contrary, the right chart shows the genes significantly higher expressed in Mr5 after inoculation with the mutant strain ZYRKD3-1. Two genes of the left chart were assigned to two different categories simultaneously, which results in 142 instead of 140 genes. The assignment of the DEGs to the BINs is mentioned in more detail in appendix M. In addition to the 211 significant DEGs, the categorisation was also performed with all transcripts obtained from the RNA-seq analysis and with transcripts with a log2 fold change ratio of more than 3 (mentioned only in appendix M).

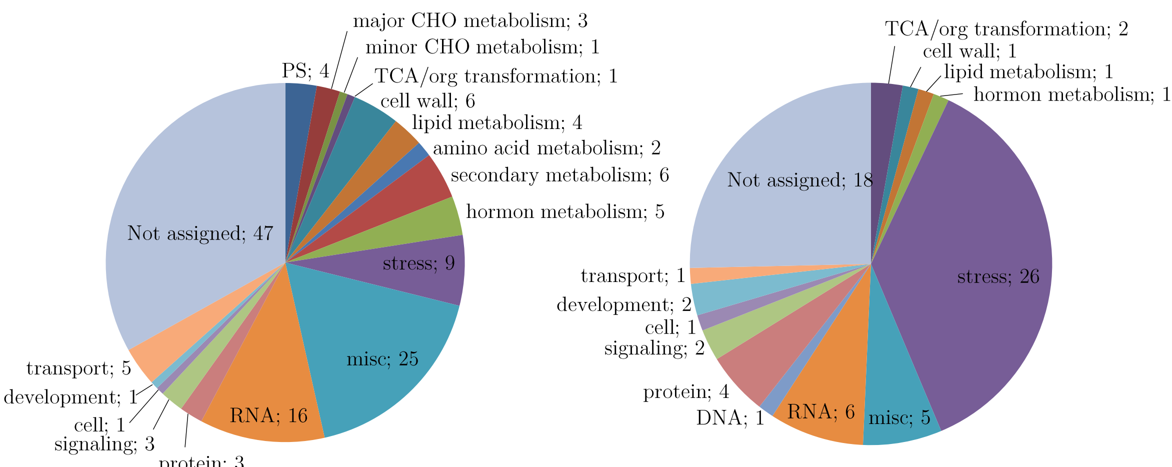

Figure 3.7.: Functional distribution according to the MapMan categories of significant DEGs obtained by the transcriptome analysis of Mr5. DEGs with a higher expression level in Mr5 after inoculation with the wild type strain Ea1189 are shown in the left chart. DEGs with a higher expression level in Mr5 after inoculation with the mutant strain ZYRKD3-1 are shown in the right chart.

Genes of the incompatible reaction in the left chart mainly belong to the miscellaneous enzyme family (misc), to the processing, transcription and regulation of transcription (RNA) or to the stress response (stress). Taking together the major and minor CHO metabolism and the lipid, amino acid, secondary and the hormone metabolism, the incompatible reaction contains another big group of the metabolism related genes with a portion of around 15 percent. Interestingly, the distribution is quite different in the compatible reaction, where the genes are mainly involved in the stress response. 26 out of 71 genes belong to this group, which is a proportion of 37 percent. Most of these genes code for heat shock proteins and except for one, all are active at 48 hpi. The metabolism group in contrast is only of minor significance. Apart from genes involved in the stress response only the protein, RNA or misc category are relevant, however with less importance compared to the incompatible reaction. Between 25 and 33 percent of the significant DEGs could not be assigned to any category.

Beside the assignment via MapMan to the different BINs, the data can be also presented within thematic schemes, where different functional categories are combined. One of these schemes is the biotic stress map of figure 3.8, which combines genes assigned to the biotic stress BIN (coloured in dark grey) and genes which are putative related to the biotic stress (coloured in light grey) (Rotter et al., 2007). Putative related genes belong to cell wall processes, secondary and hormone metabolism, abiotic stress, regulation of transcription, proteolysis, signalling or have miscellaneous functions.

32 percent of the significant DEGs match the biotic stress map at 2 (upper) and 48 hpi (lower). The log2 fold change ratios are described as a gradient from blue (higher expression level in Mr5 inoculated with the non-virulent wild type strain Ea1189) to red (higher expression level in Mr5 inoculated with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1). The map at 2 hpi shows 28 genes, whereby 21 had a higher expression level in Mr5 inoculated with the non-virulent wild type strain Ea1189. In contrast to the distribution at 48 hpi, where 27 out of 39 presented genes have a higher expression level in Mr5 inoculated with the virulent mutant strain ZYRKD3-1. Genes directly related to the biotic stress (coloured in dark grey) were not identified, neither at 2 nor at 48 hpi, only genes putative related to the biotic stress (coloured in light grey). But even there are more genes displayed at 48 hpi, less pathways are involved. Nearly all pathways are active within the light grey area of the upper map, whereas the genes of the lower map are mainly related to the group of the heat shock proteins.

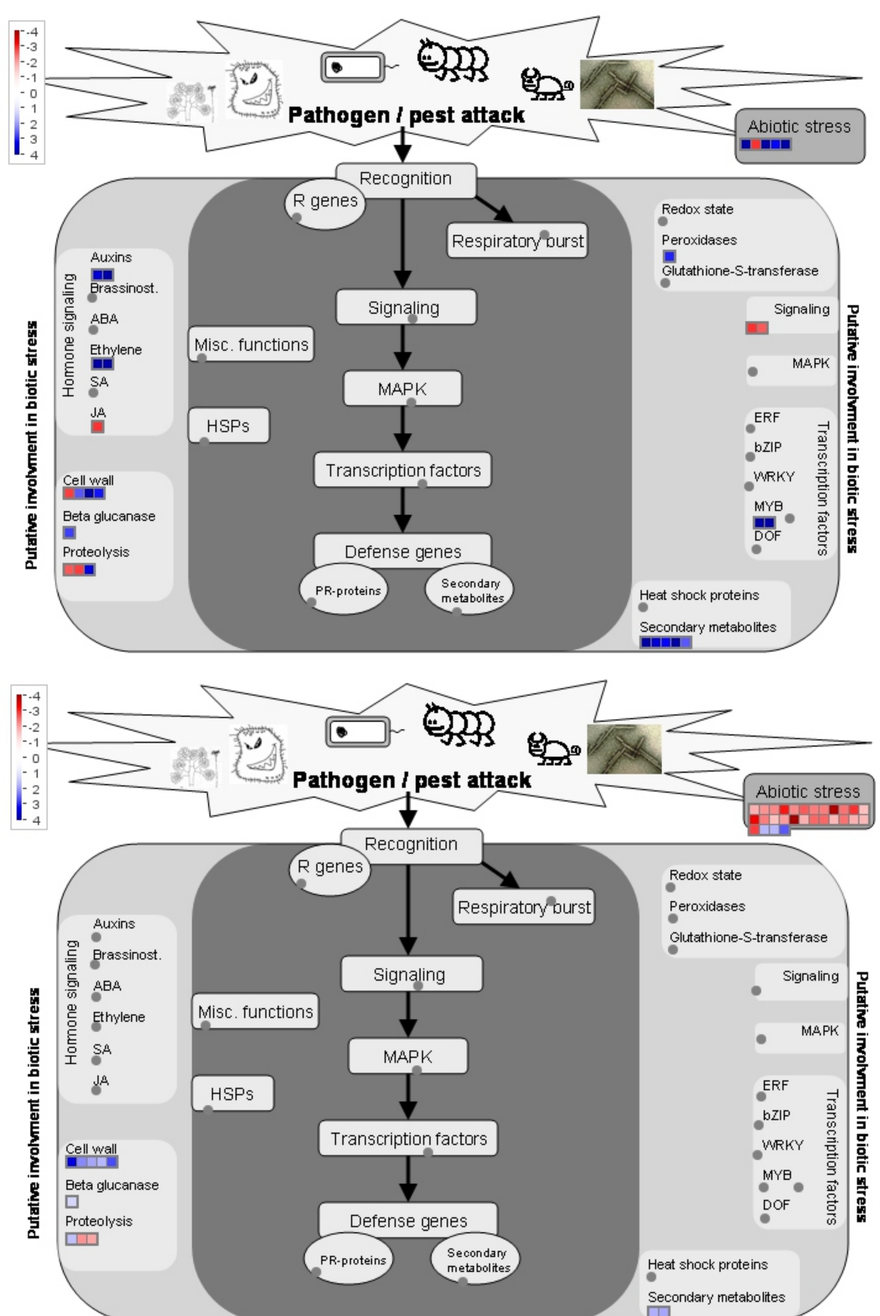

Figure 3.8.: Significant DEGs of Mr5 related to biotic stress map obtained by MapMan at 2 (upper) and 48 hpi (lower). The scheme combines genes assigned to the biotic stress BIN (coloured in dark grey) and genes which are putative related to the biotic stress (coloured in light grey) (Rotter et al., 2007). The log2 fold change ratios are indicated as a gradient from blue (higher expression level in Mr5 inoculated with the non-virulent wild type strain Ea1189) to red (higher expression level in Mr5 inoculated with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1).

3.4.2. Gene expression analysis with the BioMark™ HD system

To confirm the results received by the RNA-seq analysis the expression of selected DEGs was further verified by the Fluidigm BioMark™ HD system. With the 96.96 Dynamic Array Integrated Fluidic Chip it is possible to analyse 96 genes in 96 samples at the same time.

The selection of the 96 samples were based on the experimental design of the RNA-seq analysis, but the analysed time points were extended to 1, 2, 4, 12, 24 and 48 hpi. Additionally, one non-infected sample of Mr5 was added. Three biological and two technical replicates of each sample as well as negative controls and water were analysed.

Primers were mainly designed for DEGs with a higher expression level after inoculation with the wild type strain Ea1189, since these DEGs are highly related to the resistance mechanism. However, the alignment quality of some DEGs with their appropriate reads indicated a wrong assignment, whereas these DEGs were excluded. For the remaining 106 DEGs it was tried to find functional primers. Prior to the analysis with the BioMark™ HD system, the primers were verified by RT-PCR and by Real-Time qRT-PCR. If no functional primers were obtained after a maximum of three attempts the gene was excluded from the expression analysis as well. Finally, it was possible to design 82 usable primers for DEGs with a higher expression level in the incompatible reaction and five more for DEGs with a higher expression level in the compatible reaction. Due to the allopolyploid nature of the apple genome and its high level of heterozygosity some primer pairs matched more than one gene. Furthermore, the expression of the recently identified *FB_MR5* fire blight resistance gene from Mr5 (Fahrentrapp et al., 2013), and a further candidate gene for fire blight resistance of Mr5 encoding for a class three secretory peroxidase (GenBank: EH034548; (Gardiner et al., 2012)) were studied. Two primer pairs were used for *FB_MR5*. For data normalisation, the analysis included five reference genes (Ubiquitin, RNA-Polymerase (two different primer pairs), GAPDH, EF1α and Rubisco). All primers used in the gene expression analysis are listed in appendix C.5.

Data were analysed by the Fluidigm Real-Time PCR Analysis software and subsequently loaded to qbase+. Three of the five reference genes were used for normalisation (GAPDH, EH1α, RNA-Polymerase). The geNorm expression stability value of the reference genes was between 0.26 and 0.31 and the coefficient of variation was between 0.09 and 0.14. The calibrated normalised relative quantities (CNRQ) were exported from qbase+ and statistically evaluated with SAS Enterprise Guide (version 6.100). CNRQ values and the

related confidence intervals are presented in appendix N. The first step in the statistical evaluation of the data was the Shapiro-Wilk test for normal distribution, which applied to all genes, except the housekeeping genes. 75 of the 90 genes showed a normal distribution (partially after transformation) and could, therefore, be analysed by Levene's and Tukey's HSD test (tabulated in appendix P.1 and P.2). The remaining 15 not normal distributed genes were analysed by Kruskal-Wallis and in case of a p-value less than 0.05 further by Mann Whitney U test (tabulated in appendix P.1 and P.3). Finally 24 out of 90 genes, without the housekeeping genes, showed significant differences in the statistical analysis. Table 3.6 shows these 24 genes with the appropriate annotations by GO (Gene ontology), InterPro and MapMan. The following section describes them in more detail.

Table 3.6.: List of the 24 genes, analysed with the BioMark™ HD system, which showed significant differences in the statistical analysis. The annotations by GO, InterPro and MapMan are listed as well.

Gene ID	LG	E	GO	InterPro	MapMan
MDP0000262141	7	↑	CC	Transcription factor, SBP-box	development: squamosa promoter binding like (SPL)
MDP0000940742	3	↑	MF	Glycoside hydrolase, family 1, active site	misc: gluco-, galacto- and mannosidases
MDP0000317974	17	↑	BP	Sulphate anion transporter, conserved site	transport: sulphate
MDP0000163314	17	↓	MF	Cupredoxin	misc: plastocyanin-like
MDP0000225509	3	↓	MF	Tyrosine-protein kinase, catalytic domain	signalling: receptor kinases, leucine rich repeat XI
MDP0000236723	11	↓		FAS1 domain	cell wall: cell wall proteins, AGPs.AGP
MDP0000265729	10	↓		Domain of unknown function DUF641, plant	not assigned: unknown
MDP0000320910	12	↓		Domain of unknown function DUF640	not assigned: unknown
MDP0000750217	5	↓	BP	Cytochrome P450, conserved site	misc: cytochrome P450
MDP0000874252	2	↓	BP	Cytochrome P450, conserved site	misc: cytochrome P450
MDP0000120176	7	↓↑	MF	Terpenoid synthase	secondary metabolism: isoprenoids, terpenoids
MDP0000131100	16	↓↑		BURP domain	cell wall: degradation, pectate lyases and polygalacturonases
MDP0000937986	7	↓↑		CAP160	stress: abiotic, cold
MDP0000151003	14	↓↑	BP	Cytochrome P450, conserved site	misc: cytochrome P450
MDP0000180902	11	↓↑	BP	Oxoglutarate/iron-dependent oxygenase	hormone metabolism: gibberelin, synthesis-degradation

Table 3.6.: (continued)

Gene ID	LG	E	GO	InterPro	MapMan
MDP0000316497	1	↓↑	MF	Homeobox, conserved site	RNA: regulation of transcription, Homeobox transcription factor family
MDP0000628976	11	↓↑	CC	Protein phosphatase 2C	protein: postranslational modification
MDP0000136037	9	↓↑		Lateral organ boundaries, LOB	RNA: regulation of transcription, AS2, Lateral Organ Boundaries Gene Family
MDP0000737128	17	↓↑	MF	Homeobox, conserved site	RNA: regulation of transcription, Homeobox transcription factor family
MDP0000668657	-	↓↑	BP	Inositol oxygenase	minor CHO metabolism: myo-inositol, myo inositol oxygenases
MDP0000296339	15	↓↑	MF	Thiolase-like	lipid metabolism: FA synthesis and FA elongation, beta ketoacyl CoA synthase
MDP0000711911	8	↓↑	MF	Ribosome-inactivating protein subgroup	stress: biotic
MDP0000921319	15	↓↑	BP	Bifunctional inhibitor/plant lipid transfer protein/seed storage helical domain	misc: protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
MDP0000644109	2	↓↑		SPX, N-terminal	stress: abiotic

LG linkage group; E expression: ↑ expression compared to the non-infected sample is increased, ↓ expression compared to the non-infected sample is decreased, ↓↑ expression compared to the non-infected sample is both increased and decreased; GO (gene ontology) annotations: CC cellular component, BP biological process, MF molecular function

Basically, three different expression patterns were observed: a) the expression compared to the non-infected sample is increased, b) the expression compared to the non-infected sample is decreased and c) the expression compared to the non-infected sample is both increased and decreased. The individual patterns and genes will be closer described in the following sections.

The first group with the expression pattern a consists of three genes, which are shown in figure 3.9.

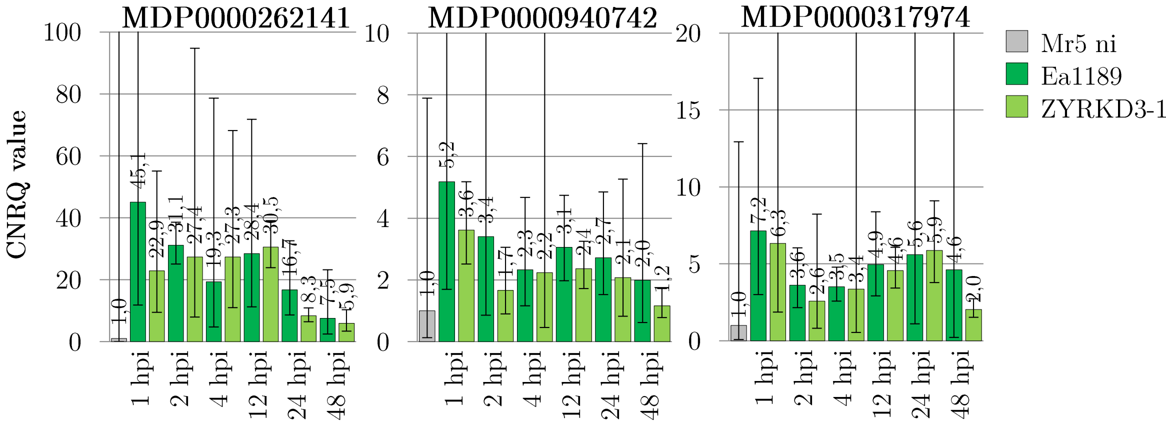

Figure 3.9.: CNRQ values of genes with an increased expression compared to the non-infected sample analysed with the BioMark™ HD system, 95 % confidence interval.

The first gene MDP0000262141 codes for a transcription factor and shows an overall high expression level compared to the non-infected sample. Of particular interest is the difference between the compatible and the incompatible reaction immediately after inoculation, which indicates an involvement in the resistance mechanism. However, this behaviour can be only observed at 1 hpi, whereas later time points show a more similar and decreasing expression level. The same pattern, only less high and with smaller differences, is observable for the glycoside hydrolase MDP0000940742. However, the third gene MDP0000317974 which codes for a sulphate anion transporter shows only rare differences between Mr5 after inoculation with Ea1189 or ZYRKD3-1 suggesting a more common answer.

Seven of the 24 genes show an decreased expression compared to the non-infected sample (see table 3.6, charts in appendix O). Three of them belong to the miscellaneous enzyme family and two are of unknown function. One of the remaining two genes, the gene MDP0000225509 codes for a tyrosine-protein kinase with a leucine rich repeat,

which suggests a specific relation to the defence response. The last gene, MDP0000236723 contains a FAS1 domain, which is a cell adhesion domain. The majority of the genes within this group shows a similar expression between the two strains used for inoculation of Mr5, which indicates a more general answer to the inoculation process itself than a specific one. The expression course of these genes is either low over the entire time or it increases nearly to the same level of the non-infected sample.

The last group of genes shown in figure 3.10 has a lower as well as a higher expression compared to the non-infected sample (expression pattern c). According to the course of expression over the time these genes can be further divided into three groups, recognisable by the colour of the charts. The first seven genes show the highest expression at 2 hpi (green coloured charts) and belong, therefore, to the early disease response. All these genes show a higher expression in Mr5 after inoculation with the *E. amylovora* wild type strain than in Mr5 inoculated with the mutant strain at 1 hpi and interestingly, vice versa at 2 hpi. This behaviour suggests that these genes impact the resistance mechanism in any way. According to the MapMan annotation, each of these genes is assigned to a different category. The second group of genes in figure 3.10 (blue coloured charts) has the highest expression at 4 or 12 hpi and belongs to the late response. In comparison to the rest of this group is the gene MDP0000668657 highly up-regulated and the only one with remarkable differences of the expression between the compatible and the incompatible reaction. It belongs to the minor CHO metabolism, whereas the other three genes are involved in the regulation of transcription and in the lipid metabolism. The last group contains the three remaining genes (yellow coloured charts), whereas the expression over the time is different and does not fit the already mentioned ones. The most interesting gene is MDP0000711911, which is highly up-regulated only in the incompatible reaction and only at one hpi. According to the MapMan annotations, this gene belongs to the biotic stress response. This expression pattern could only be observed once. The other two genes show no strong distinctions in the expression between the compatible and the incompatible reaction and even the course of expression over the time suggests no important role in the pathogen defence.

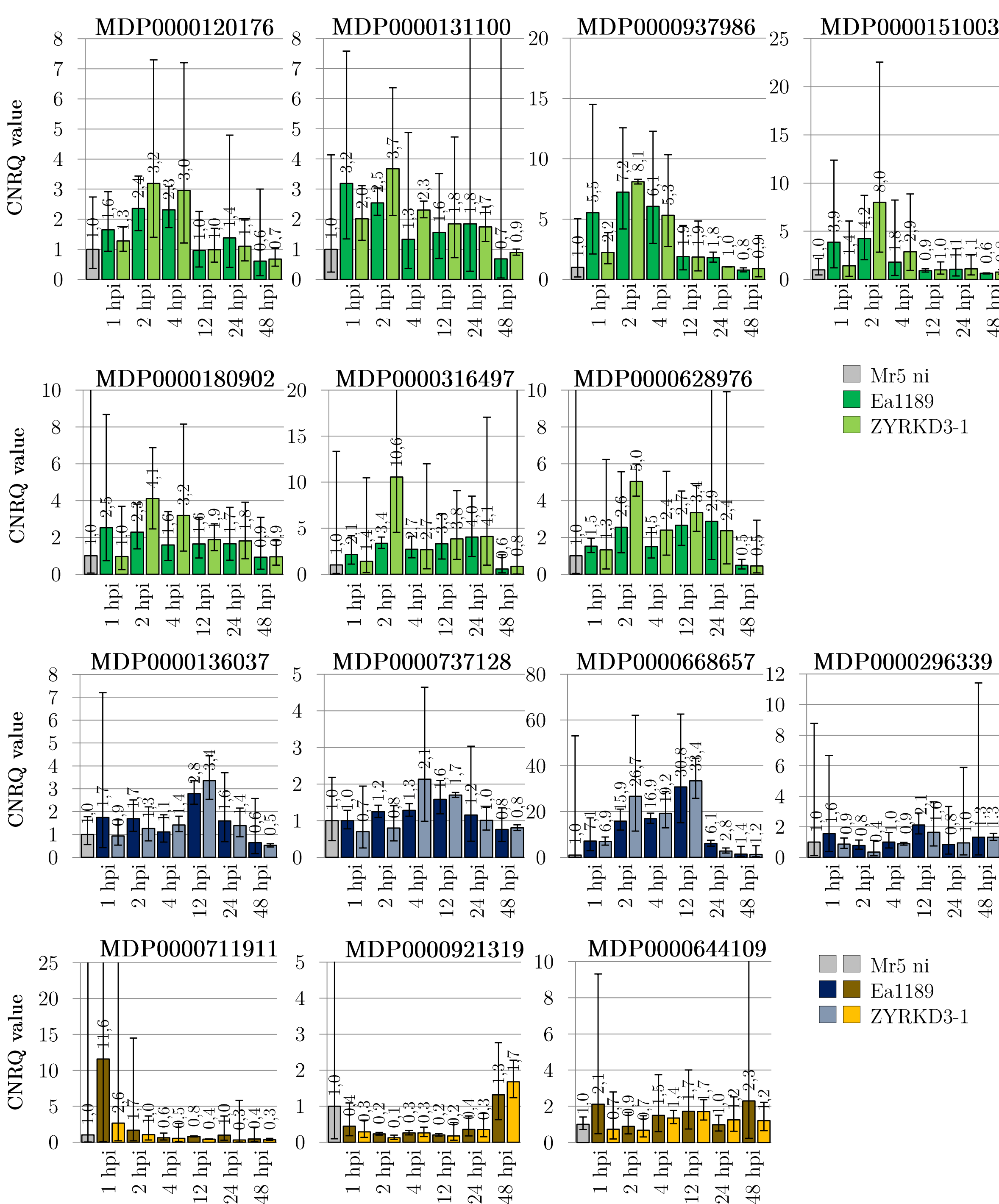

Figure 3.10.: CNRQ values of genes with a lower and higher expression compared to the non-infected sample analysed with the BioMark™ HD system, charts are coloured according to the group defined by the course of expression over the time, 95 % confidence interval.

3.4.3. Comparison of the results obtained by RNA-seq and the BioMark™ HD system

Most genes verified by gene expression analysis with the BioMark™ HD system were chosen from RNA-seq, because of the significant higher expression in Mr5 after inoculation with the *E. amylovora* wild type strain Ea1189 compared to the mutant strain ZYRKD3-1. However, considering the charts from figure 3.9 and 3.10 the expression pattern obtained by the BioMark™ HD system show at least partially a different course. Table 3.7 compares the results of 14 genes obtained by RNA-seq at 2 and 48 hpi and by the BioMark™ HD system at 1, 2, 24 and 48 hpi. The selected genes are the same like the ones from figure 3.10. The designation "Ea1189" and "ZYRKD3-1" in the table means that the expression is higher in Mr5 after inoculation with the non-virulent *E. amylovora* wild type strain Ea1189 or in Mr5 after inoculation with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1, respectively.

Table 3.7.: Comparison of the results obtained by RNA-seq and the BioMark™ HD system of the 14 genes from figure 3.10.

Gene ID	BioMark™ HD		RNA-seq	BioMark™ HD		RNA-seq
	1 hpi	2 hpi		24 hpi	48 hpi	
MDP0000120176	Ea1189	Mut	Ea1189	Ea1189	≈	Ea1189
MDP0000131100	Ea1189	Mut	Ea1189	≈	Mut	Ea1189
MDP0000937986	Ea1189	Ea1189	Ea1189	Ea1189	Mut	Ea1189
MDP0000151003	Ea1189	Mut	Ea1189	=	Mut	Ea1189
MDP0000180902	Ea1189	Mut	Ea1189	Mut	≈	Ea1189
MDP0000316497	Ea1189	Mut	Ea1189	≈	Mut	Ea1189
MDP0000628976	Ea1189	Mut	Ea1189	Ea1189	≈	Ea1189
MDP0000136037	Ea1189	Ea1189	Ea1189	Ea1189	Ea1189	Ea1189
MDP0000737128	Ea1189	Ea1189	Ea1189	Ea1189	Mut	Ea1189
MDP0000668657	=	Mut	Ea1189	Ea1189	Ea1189	Ea1189
MDP0000296339	Ea1189	Ea1189	Ea1189	Mut	=	Ea1189
MDP0000711911	Ea1189	Ea1189	Ea1189	Ea1189	Ea1189	Ea1189
MDP0000921319	Ea1189	Ea1189	Ea1189	=	Mut	Ea1189
MDP0000644109	Ea1189	Ea1189	Mut	Mut	Ea1189	Mut

Ea1189: expression is higher in Mr5 after inoculation with the non-virulent *E. amylovora* wild type strain Ea1189; ZYRKD3-1: expression is higher in Mr5 after inoculation with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1; =/≈ expression is/nearly is the same in Mr5 after inoculation with Ea1189 or with ZYRKD3-1

Comparing the same time points shows especially at 2 hpi wide variations between the two methods. However, if the comparison is performed between 2 hpi from RNA-seq analysis and 1 hpi from the BioMark™ HD system, there are only two mismatches. Considering the situation at 48 hpi it is more difficult since the expression in the compatible and in the incompatible reaction is often the same or at least similar. However, the comparison of the same time points provides four matches, whereby the comparison of 48 hpi from RNA-seq analysis and 24 hpi from the BioMark™ HD system provides 7 matches.

3.4.4. Comparison between prediction, sequencing and RNA-seq data in case of *RIN4* from Mr5

A comparison between the sequencing results of *RIN4* and the prediction from 'Golden Delicious' (http://www.rosaceae.org, (Jung et al., 2014)) was already done in section 3.2.1 and showed big differences, especially in the correct definition of the right start and stop codon. A further comparison with the RNA-seq data should give an indication of the usage possibilities of the RNA-seq data.

Figure 3.11 shows the alignment of the reads obtained from RNA-seq (see chapter 3.4.1) to the nucleic acid sequence of the two predictions of *RIN4* on chromosome 5 (MDP0000252761) and 10 (MDP0000290323) of the 'Golden Delicious' genome. The red lines roughly mark the real start and stop codon obtained by sequencing of *RIN4* from Mr5. The alignment of MDP0000252761 shows the majority of the reads within the red lines, in particular between 74 and 81 percent. Behind the red line of the stop codon decreases the amount of reads considerably. Therefore, the position of the stop codon can be clearly identified. In case of MDP0000290323 from chromosome 10 is the distribution of the reads less obvious, but still 33 to 43 percent of the reads are located within the first 730 base pairs. The consultation of the RNA-seq data is, therefore, very valuable for the gene prediction, but it is restricted to the length of the reference sequence. Hence, it was possible to confirm the right stop codon of RIN4, but in case of the start codon the reference sequence was too short. However, this problem could be solved by a separate mapping, including areas before or behind the reference.

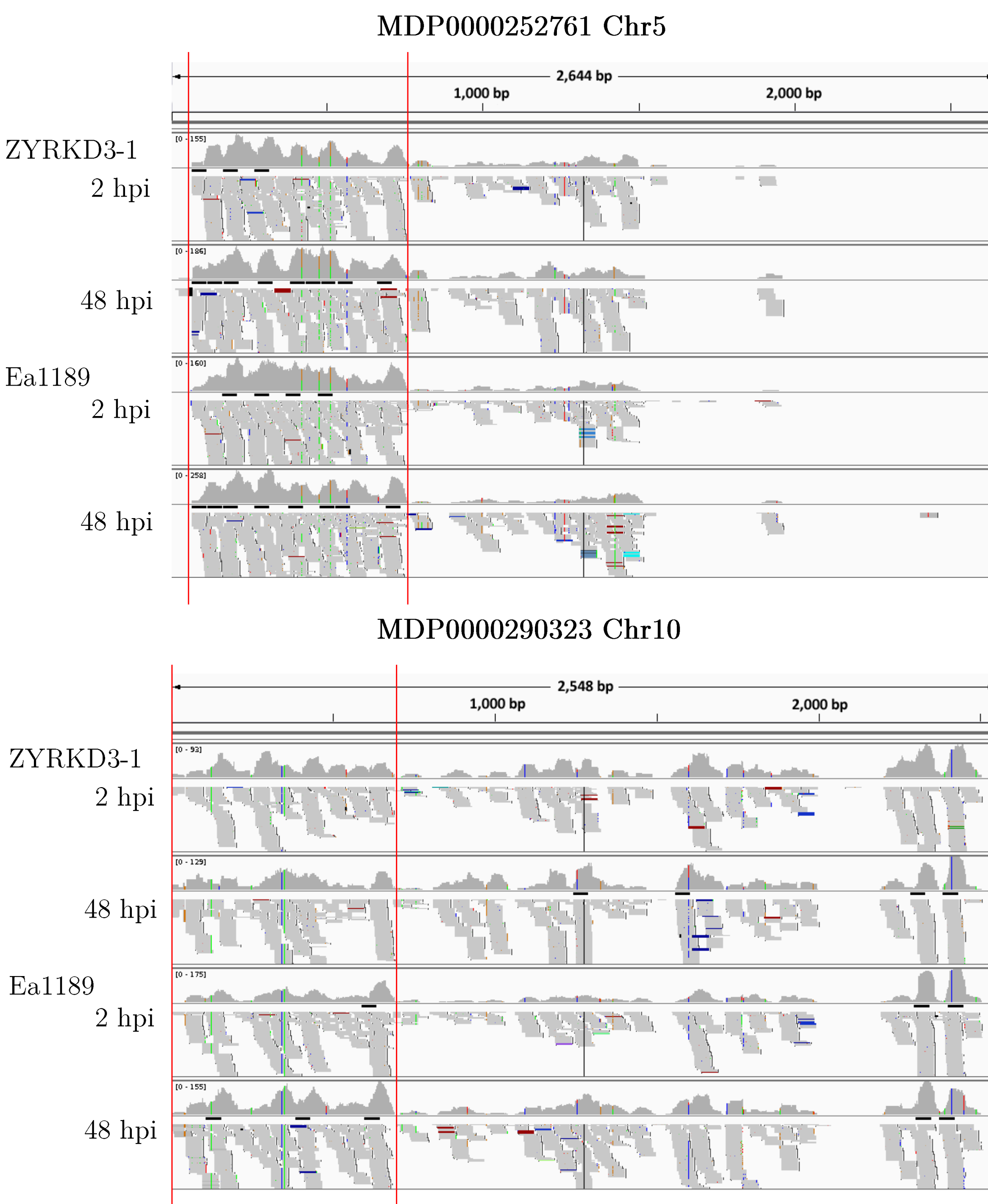

Figure 3.11.: Alignment of the predicted CDS of *RIN4* on chromosome 5 and 10 from 'Golden Delicious' (MDP0000252761 and MDP0000290323; http://www.rosaceae.org, (Jung et al., 2014)) with the matching reads obtained from RNA-seq of Mr5 (see chapter 3.4.1); visualised by IGV. The red lines roughly mark the start and stop codon obtained by sequencing of *RIN4* from Mr5.

3.5. Fire blight resistance gene *FB_MR5* of Mr5

The same problem as described for RIN4 raised for the fire blight resistance gene *FB_MR5*, the prediction of the start and stop codon was uncertain. The first step to verify this assumption was a look at the distribution of the transcripts received by RNA-seq. Since *FB_MR5* is not present in the genome of 'Golden Delicious' it was necessary to do a separate mapping with the fire blight resistance gene including 2 kb before the suspected start codon and 1 kb behind the suspected stop codon. Sequences for the *FB_MR5* gene (accession number: CCH50986.1) and the flanking regions (BAC 16k15, contig2/4, HE805491) were obtained from the European Nucleotide Archive (ENA). The mapping was done with BWA using the default parameters. Table 3.8 shows the amount of all mapped reads of the appropriate sample, including the singletons and the properly paired reads (mapped minus singletons), respectively.

Table 3.8.: Results achieved by the mapping of the transcripts from the RNA-seq analysis of Mr5 after inoculation with the *E. amylovora* wild type strain Ea1189 or the mutant strain ZYRKD3-1 to the fire blight resistance gene *FB_MR5* as reference.

Sample	Mapped	Singletons	Mismatches			
			0	1	2	3
ZYRKD3-1 2 hpi	3354	2762	77	452	1204	1611
ZYRKD3-1 48 hpi	4601	3573	215	615	1610	2153
Ea1189 2 hpi	2189	1677	138	366	725	958
Ea1189 48 hpi	5192	3976	225	757	1712	2485

There is an increase of the transcripts from 2 to 48 hpi in the compatible and in the incompatible reaction, but the amount of mapped reads between the two inoculation approaches is nearly the same. Columns four to seven contain the distribution of these reads in accordance with the number of mismatches. The number of mapped reads increases with the number of mismatches and only 2 to 6 percent contain no mismatches. The visualisation of the mapping by the tool IGV is shown in figure 3.12. All four samples of the RNA-seq analysis were used for the mapping. The red lines mark the assumed start and stop codon published by Fahrentrapp et al. (2013).

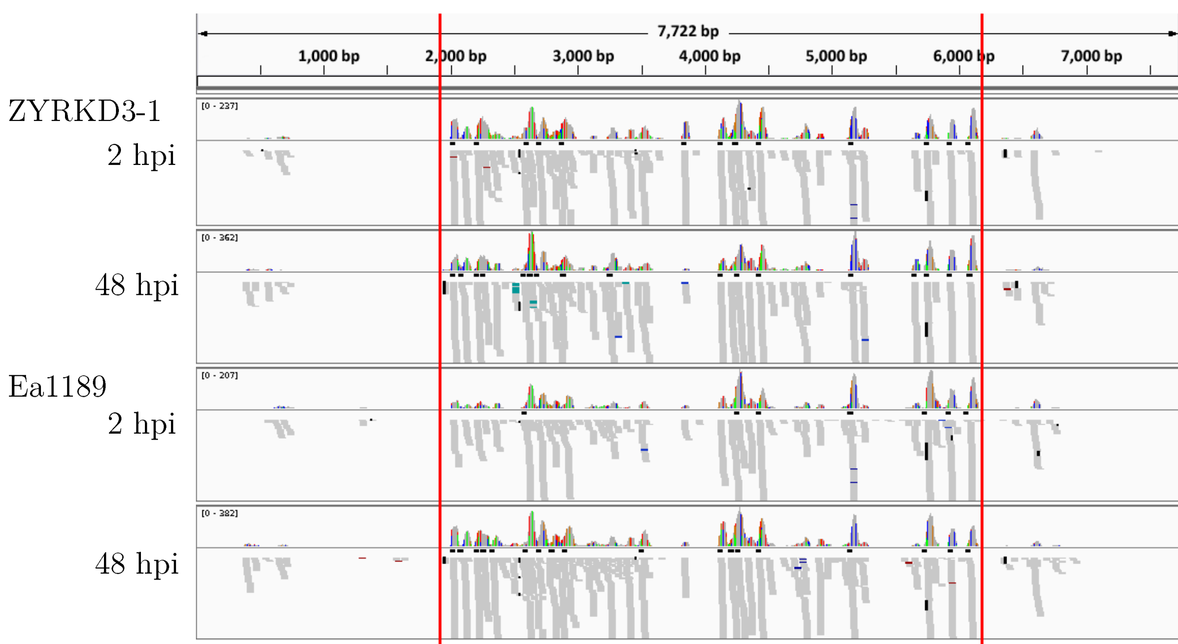

Figure 3.12.: Alignment of the fire blight resistance gene *FB_MR5*, including 2 kb before the suspected start codon and 1 kb behind the suspected stop codon, with the matching reads obtained from the four samples of Mr5 analysed by RNA-seq; visualised by IGV tool.

The number of reads before and behind the red lines gave cause to examine the *FB_MR5* gene additionally by a 3' and 5' RACE-PCR. The analysis of the untranslated region enables the exact determination of the start and stop codon. Details like the samples used for RNA isolation or the amount of RNA are listed in table 2.2. Obtained fragments were cloned and send to Eurofins MWG Operon for sequencing.

The results for the 5' RACE-PCR are shown in appendix Q.1. The sequences of the five analysed colonies suggest a minimum of two alleles. It is moreover unclear whether the sequences match the published *FB_MR5* gene, as even the area behind the start codon possesses mismatches. The sequences before the start codon match the published BAC only within the first 180 base pairs and only with an additional intron. This disagreement could result from a wrong prediction of the BAC sequence or the obtained sequences belong to another gene. However, a comparison of the sequences of the reads before the start codon obtained by the mapping (see figure 3.12) with the sequence of the BAC or the sequences obtained by the RACE-PCR show no accordance. This indicates the right start codon, even the 5' UTR could not entirely determined.

Fragments of the 3' UTR could only be received with a ten times higher amount

of RNA as described in the manual. The results are shown in appendix Q.2. The alignment shows that the suspected stop codon (marked in red) is obviously wrong. The sequences obtained by RACE-PCR extend the *FB_MR5* resistance gene by 52 base pairs, additionally interrupted by an intron of 96 bp in length. This new stop codon (marked in green), however, requires a frame shift of one base pair. In case of the fragment of colony seven the sequence ends after 791 additional nucleotides behind the new stop codon with the poly A tail. The sequences of all colonies are highly identical and match the BAC sequence perfectly.

4. Discussion

4.1. Role of the *E. amylovora* effector AvrRpt2$_{EA}$

The wild apple accession Mr5 had been described as resistant to fire blight by Watkins in 1971. Fifteen years later an inoculation experiment with two different *E. amylovora* isolates showed a strain specific resistance, Mr5 was susceptible to Ea266 but resistant to Ea273 (Norelli et al., 1986). This behaviour depends on the virulence of the strains used for inoculation but also on the oligogenic resistance of Mr5, which is probably less stable than a polygenic (Gardner et al., 1980). Until now three more strains are known to break the resistance of Mr5: Ea3049, Ea400 and Ea110 (Peil et al., 2010; Vogt et al., 2013). Apart from these wild type strains, the resistance of Mr5 was also broken by the *E. amylovora* mutant ZYRKD3-1, where the *avrRpt2$_{EA}$* gene is disrupted by an insertion of an antibiotic resistance cassette (Zhao et al., 2006; Vogt et al., 2013). AvrRpt2$_{EA}$ is one of the effectors of *E. amylovora* and a homologue to AvrRpt2 of *P. syringae* pv. *tomato*. Zhao et al. (2006) identified the effector in the genome of *E. amylovora* and showed that the deletion results in the loss of pathogenicity on immature pear fruits (*Pyrus communis* L. *cv.* Bartlett). Therefore, AvrRpt2$_{EA}$ acts as virulence factor in the system pear and *E. amylovora* and as avirulence factor in the system Mr5 and *E. amylovora*. Accordingly, the AvrRpt2$_{EA}$ effector seems to have an important role in the defence mechanism of apple and pear. Furthermore, it is a strong indication for a gene-for-gene relationship. Based on these results the effector was examined in closer detail.

Hence, the nucleic acid sequence of the *avrRpt2$_{EA}$* gene of twenty-two different *E. amylovora* strains differing in origin and isolated from different hosts was examined. Except for one single nucleotide polymorphism (SNP) obtained in the strains of Ea110, Ea3049 and Ea3050 (identical to Ea266) all sequences were completely identical. The deduced amino acid sequence could show that the SNP results in an exchange from cysteine to serine at position 156. The two alleles of the *avrRpt2$_{EA}$* gene were designated as C-allele

and S-allele in the following. Interestingly, it could be shown, that there is a correlation between the ability to infect Mr5 and the allele of the *avrRpt2*$_{EA}$ gene (Vogt et al., 2013). *E. amylovora* strains carrying the S-allele were able to overcome resistance of Mr5, whereas strains carrying the C-allele were not. As cysteine is able to form disulphide bridges, this exchange can considerably alter the tertiary structure of the effector, thus modifying the downstream acting process in the resistance mechanism of Mr5.

The cystein or serine at position 156 from AvrRpt2$_{\mathrm{EA}}$ of *E. amylovora* is homologue to tyrosine at position 191 in the AvrRpt2 effector protein of *P. syringae* pv. *tomato*. In the system *P. syringae* pv. *tomato* and *A. thaliana* AvrRpt2 cleaves the guard protein RIN4, which mediates resistance by activation of the R protein RPS2. An exchange from tyrosine to cysteine in AvrRpt2 at position 191 leads to the loss of RIN4 cleavage by AvrRpt2, which prevents the RPS2-dependent defence response (Lim and Kunkel, 2004). Therefore, position 156 and 191 in the protein structure of AvrRpt2$_{\mathrm{EA}}$ and AvrRpt2 seems to be an important position in the recognition process.

To verify if the exchange from cysteine to serine at position 156 determines indeed the avirulence activity, the deletion mutant ZYRKD3-1 was complemented with the functional C- or S-allele of the *avrRpt2*$_{EA}$ gene. If the hypothesis of a gene-for-gene relationship is correct, it can be expected that the mutant strain ZYRKD3-1 complemented with the C-allele is not able to break the resistance of Mr5. The complementation with the S-allele is not expected to have an impact on the virulence, as the mutant strain ZYRKD3-1 by itself breaks the resistance of Mr5. Another interesting question was, what happens if both alleles are present in one genotype. Therefore, the wild type strains Ea222 (C-allele) and Ea3049 (S-allele) were used for complementation with the appropriate opposite allele as well.

The two alleles, including the own promoter and terminator region, were cloned in both directions into the expression vector. This should enable the expression of the *avrRpt2*$_{EA}$ gene under the *lac* promoter of the vector or under the own promoter of the gene. These two versions were designed to verify if the expression level, which should be higher under the lac promoter, influence the virulence. To verify the strains not only on a resistant apple cultivar, but also on a susceptible one, 'Idared' was inoculated as well. When the apple shoots were ready for inoculation, only the mutant strain complemented with the C-allele of the *avrRpt2*$_{EA}$ gene under control of its own promoter could be used. To nonetheless ensure a comparison, the missing mutant strain complemented

with the S-allele was obtain from Zhao, who carried out an similar experiment with pears (Zhao et al., 2006). The *avrRpt2*$_{EA}$ gene was thereby cloned in the same plasmid pRK415, but without a specific direction of the gene. For the compatibility, the strain was designated as ZYRKD3-1+Avr-S_G in the following (original designation in Zhao et al. (2006): ZYRKD3-1 (pZYR2)). Beside the complemented strains, the wild type strains Ea1189 (C-allele), Ea110 and Ea3050 (both S-allele) and the mutant strain ZYRKD3-1 itself were used for inoculation.

In case of the susceptible cultivar 'Idared' the virulence analysis (see figure 3.2) showed that all strains, even the deletion mutant ZYRKD3-1, caused infection. This means, that the results of Zhao et al. (2006), where *avrRpt2*$_{EA}$ acts as a virulence gene on susceptible pear fruits, are not transferable to the susceptible apple cultivar 'Idared'. Similar results were obtained with the susceptible apple cultivars 'Royal Gala' and 'Pinova ' (Vogt et al., 2013). In contrast for Mr5, the results show the strain specific resistance as mentioned above and in the introduction. The *E. amylovora* wild type strain Ea1189, carrying the *avrRpt2*$_{EA}$ C-allele, is non-virulent to Mr5, whereas strains carrying the S-allele of the effector like Ea110 and Ea3050 as well as the *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 are virulent to Mr5. The correlation between the allele of the effector and the virulence could be also confirmed by the results obtained from the inoculation of Mr5 with the complemented mutant strains, ZYRKD3-1+Avr-C_eP and ZYRKD3-1+Avr-S_G . The complementation of the deletion mutant ZYRKD3-1 with the C-allele of the *avrRpt2*$_{EA}$ gene resulted in the recovery of the resistance of Mr5, whereas ZYRKD3-1 complemented with the S-allele was still virulent to Mr5. These results confirmed the important role of the *avrRpt2*$_{EA}$ effector in the resistance mechanism of Mr5 and the hypothesis of a a gene-for-gene relationship in the host pathogen system Mr5 and *E. amylovora*. However, these mode of resistance seems to be unique for Mr5, since two other wild apple accessions, *M. baccata* and *M. fusca*, were resistant to all these tested *E. amylovora* strains, regardless of the allele (Vogt et al., 2013).

The complemented strains were further analysed by Real-Time qRT-PCR to verify the effect of the promoter in front of the gene and to compare the expression of the effector to the wild type strains. Therefore, the two wild type strains Ea222 and Ea3049, as well as the four complemented wild type strains (Ea222+Avr-S_eP, Ea222+Avr-S_LacP, Ea3049+Avr-C_eP, Ea3049+Avr-C_LacP) and the complemented mutant strain (ZYRKD3-1+Avr-S_G) were analysed. The distinction between the two alleles of the *avrRpt2*$_{EA}$ gene was achieved

with the two allele specific SNP primers (see figure 3.3).

The expression level of all four complemented wild type strains for both alleles was very similar, only between Ea222+Avr-S_eP and Ea3049+Avr-C_LacP was a significant difference observed. Hence, the direction of the gene within the vector and, therefore, the appropriate active promoter does not clearly affect the expression of the *avrRpt2*$_{EA}$ gene. But since the *avrRpt2*$_{EA}$ gene was ligated in both directions with its own promoter sequences to the expression vector pRK415, this could due to the inactivation of the *lac* promoter and, therefore, to a similar expression level. To investigate this issue, further analysis with complemented strains containing the gene in frame to the *lac* promoter without the own promoter sequences would be necessary. Furthermore, it would be possible to increase the expression by a high copy plasmid. The charts in figure 3.3 also show that the expression of both alleles is always higher in the complemented strains compared to the untransformed wild type strains, not only for the gene expressed from the vector, but also for the genomic one. This means that the expression of the genomic *avrRpt2*$_{EA}$ gene of the complemented strains is enhanced in any way, perhaps by the presence of tetracycline in the media (Migliore et al., 2013). The complemented mutant strain ZYRKD3-1+Avr-S_G behaved like the complemented wild type strains, the only difference was observed to Ea222+Avr-S_eP.

4.2. Identification and characterisation of *RIN4* of Mr5

The strong sequence homology between the effectors AvrRpt2$_{\mathrm{EA}}$ from *E. amylovora* and AvrRpt2 from *P. syringae* pv. *tomato* and the proven gene-for-gene relationship suggests a similar resistance mechanism between Mr5 and *A. thaliana*.

As the system *P. syringae* pv. *tomato* and *A. thaliana* serves as model system to study host-pathogen interactions, the resistance mechanism is well understood and can be described in the following manner. AvrRpt2 interacts within the plant cell with cyclophilin ROC1, which activates the self-cleavage of the effector via prolyl isomerisation (Coaker et al., 2006). Activated AvrRpt2 cleaves the guard RIN4, thus activating the resistance protein RPS2 and thereby the pathogen defence of the plant (Mackey et al., 2002; Axtell and Staskawicz, 2003; Kim et al., 2005). Beside AvrRpt2, RIN4 interacts also with the two *P. syringae* effector proteins AvrRpm1 and AvrB, which cause phosphorylation of RIN4, instead of cleavage, and the associated activation of a further R protein RPM1

(Mackey et al., 2002; Axtell and Staskawicz, 2003).

In 2013, Vogt et al. showed that the S-allele of AvrRpt2$_{EA}$ of *E. amylovora* is able to cleave RIN4 of *A. thaliana*. Therefore, the question was, if *RIN4* is also present in *Malus*, specially in the genome of Mr5. Considering the NCBI and UniProt databases revealed two entries for *Malus* ×*domestica*, RIN4-1 and RIN4-2 on chromosome 10 and 5, respectively. These findings support the hypothesis of a similar resistance mechanism between the two plant species *Arabidopsis* and *Malus* and gave cause for a closer look into the genome of Mr5. And indeed *RIN4* was identified in the genome of Mr5. Similar to *Malus* ×*domestica*, it is localised on two chromosomes, one on chromosome 5 and the other one on chromosome 10. The two loci of *RIN4* in the genome of Mr5 and *Malus* ×*domestica* are probably caused by the genome-wide duplications whereby the two homologue chromosomes 5 and 10 derived from one common ancestor (Velasco et al., 2010). *RIN4* of both loci were sequenced and the protein sequences translated.

To verify the theoretical functionality of RIN4 from Mr5, the amino acid sequence was aligned and compared to the one from *A. thaliana* but also to other RIN4 proteins from different plant species. During the study the alignment was limited to the sequences of Mr5, *Malus* ×*domestica* and *A. thaliana* (see figure 3.4). However, in 2014, Sun et al. published an extended alignment with until then all known RIN4 homologues from 14 different plant species, including *Malus* ×*domestica* and *A. thaliana*. This alignment, extended with the two homologues of RIN4 from Mr5, is shown in appendix H. The subsequently considerations referring to it. The most important sequence motifs of RIN4 proteins are the two cleavage sites (red boxes) and the cysteine-rich membrane-anchoring site (green box) in the C-terminal region (Afzal et al., 2011). Both motifs are elaborated in more detail in the following two paragraphs.

In *A. thaliana* the effector AvrRpt2 from *P. syringae* pv. *tomato* cleaves RIN4 at two positions, RCS1 and RCS2 (red boxes). In all sequences aligned in appendix H both cleavage sites are nearly identical. In case of Mr5 and *Malus* ×*domestica* RCS1 is completely identical in comparison to *A. thaliana*, whereas RCS2 has one amino acid exchange at position 153 from aspartic acid to glutamic acid. Even these amino acids are highly similar, the exchange is located at the exact cleavage site (red arrow). As Kim et al. (2005) demonstrated, that RCS2 is functionally critical for the activation of the RPS2 resistance protein, this exchange could prevent the cleavage by AvrRpt2$_{EA}$. An experiment by Coaker et al. (2005) with RIN4 from *A. thaliana* showed that already an exchange

at position 151 (exact cleavage position between 152 and 153) from phenylalanine to alanine resulted in prevention of cleavage.

The other sequence motif of RIN4 the cysteine-rich membrane-anchoring site (green box) consists of the three cysteine residues C203, C204 and C205, which are required for RIN4 membrane localisation (Kim et al., 2005). They can be found only in the allele of chromosome 10 in the case of Mr5 and *Malus* ×*domestica*. The allele of chromosome 5 shows an exchange of C203 to serine. This could mean that only the *RIN4* allele of chromosome 10 is proper anchored to the membrane.

Beside the cleavage sites and to the membrane-anchoring sites, all RIN4 homologues contain two so-called nitrate-induced (NOI) domains, one in the C-terminal and another one in the N-terminal region. Proteins of the NOI family can be found in mosses as well as in monocots or dicots, but in plants exclusively. The function of these proteins is not entirely clear, but beside RIN4 from *A. thaliana* homologues from other species are involved in defence mechanisms as well (Selote and Kachroo, 2010; Sun et al., 2014; Luo et al., 2009). The domains span around 30 amino acids and include two conserved motifs PXFGXW and Y/FTXXF (underlined in orange) (Afzal et al., 2013). Moreover, they include the two cleavage sites RCS1 and RCS2, as well as the AvrB and AvrRpm1 binding site (blue box) with the phosphorylation site at position 166 (green arrow) (Kim et al., 2005). So far no AvrB or AvrRpm1 homologues are mentioned in *E. amylovora* and a protein-BLAST shows no results as well.

The last part of the functional characterisation was the generation of a identity matrix in order to determine similarities between the RIN4 homologues. The identity matrix of the 26 RIN4 proteins is shown in appendix I. The highest identity of RIN4 proteins are found between *A. thaliana*, *Arabidopsis lyrata* and *Brassica juncea* with 93 and 79 percent identity respectively. All three belong to the family Brassicaceae. The relation between protein identity and taxonomy is also confirmed by the high similarity between *N. benthamiana* and *Solanum lycopersicum*, both members of the Solanaceae family, and between *Zea mays* and *Oryza sativa*, both members of the Poaceae family and monocots. RIN4 from *Malus* shows the highest similarity to RIN4 from *Fragaria vesca*, *G. max* and *V. vinifera*. All belong to the rosids, whereby apple and strawberry even share the same family of the Rosaceae.

Since an antibody for RIN4 of *A. thaliana* was available, the question was raised, whether the high level of similarity between these two plant systems was sufficient enough

to use it for Mr5 as well. This would allow to identify RIN4 of Mr5 not only on the genetic level, but also on the proteomic level. Unfortunately, it was not possible to obtain precise information of the exact binding site of the antibody, but in consultation with the manufacture the antibody binds within amino acids 1–50 and should work for Mr5. The first 50 amino acids of RIN4 from *Malus* and *A. thaliana* contain 35 identical amino acids, 5 similar and 10 different ones, with areas of identical amino acids ranging from five to twelve (see protein alignment in figure 3.4). Normally, the epitope of an antibody is about 5–11 amino acids in length. Hence, depending on the specificity of the RIN4 antibody from *A. thaliana*, it could also be used for Mr5. The results of the western blot are shown in figure 3.5. The SDS gel as well as the membrane indicate an incomplete transfer of the proteins from the gel to the membrane. However, the positive control from *A. thaliana* shows a clear band in the size range of RIN4, even though the amount of the transferred protein is apparently less compared to the remaining samples. Therefore, despite high sequence similarity it was not possible to detect RIN4 from *Malus* with the antibody from *A. thaliana*. Perhaps, depending on the taxonomic relationship it would work for RIN4 from *Arabidopsis lyrata* and *Brassica juncea*.

4.3. Protein-protein interaction in the system Mr5 and *E. amylovora*

Due to the easy handling and the relatively low costs, the yeast two-hybrid system is the method of choice to detect protein-protein interactions (Brückner et al., 2009). It allows to detect interaction in a cellular environment and it can be easily automated for high throughput studies. It is based on the fusion of potential interacting proteins to the DNA binding domain (DBD) and to the activation domain (AD) of a transcriptional activator. These so called prey and bait proteins are co-expressed in yeast. The transcription factor is rebuild in case of an interaction, which leads to the activation of reporter genes and allows the growth under selective conditions. It is possible to test for specific interactions, but also to screen a cDNA library with an specific bait protein for interacting partners.

The identification for example of RIN4 from *A. thaliana* was a result of a yeast two-hybrid screen with the AvrB effector from *P. syringae* as bait protein (Mackey et al., 2002). However, further important interacting partners of RIN4 from *A. thaliana* were identified by Y2H screen, for example the resistance proteins RPM1 and RPS2,

the putative resistance regulator NDR1 and the two proteins AHA1 and AHA2, both regulating the stomatal apertures (Mackey et al., 2002; Day et al., 2006; Liu et al., 2009). And even in other plant species like tomato, soy bean and barley was the Y2H screen used to detect RIN4 interacting partners (Luo et al., 2009; Selote and Kachroo, 2010; Gill et al., 2012).

Hence, the Y2H system was selected, to discover potential protein-protein interaction of RIN4 from Mr5. Two proteins were eligible as interacting partners, the fire blight resistance protein FB_MR5 from Mr5 and the effector protein $\text{AvrRpt2}_{\text{EA}}$ from *E. amylovora*. FB_MR5 is the first isolated resistance gene against fire blight (detailed information in the introduction see section 1.3). Since Fahrentrapp et al. (2013) postulated a similar mode of action to RPS2 from *A. thaliana*, FB_MR5 should interact with RIN4. This interaction, but also the activation of RIN4 from Mr5 by $\text{AvrRpt2}_{\text{EA}}$, would confirm the hypothesis of a similar resistance mechanism between Mr5 and *A. thaliana*. In addition, also the interaction between $\text{AvrRpt2}_{\text{EA}}$ and FB_MR5 was tested. To cover all available options, both homologues of RIN4 and both alleles of the $avrRpt2_{EA}$ gene (C- and S-allele) were used for the assay. As the $avrRpt2_{EA}$ gene was already used for apple transformation at the JKI, it was also available with an optimised reading frame for eucaryotic translation and therefore included in the Y2H analysis as well.

Unfortunately, it was not possible to detect any interaction between all tested combinations (see table 3.3). The simplest reason for this could be that there is just no interaction. However, a common problem of the yeast two-hybrid system is the generation of false negatives, which leads to undetectable interactions. One reason, which could prevent at least the interaction with the effector $\text{AvrRpt2}_{\text{EA}}$, is the potential self cleavage of it within the host cell. At least, in the system *P. syringae* pv. *tomato* and *A. thaliana* results the interaction of AvrRpt2 with cyclophilin ROC1 in the self-cleavage of the amino terminal 71 amino acids. (Coaker et al., 2006). This activation process of AvrRpt2 was observed in yeast as well, because it contains its own cyclopilin (Coaker et al., 2006; Jin et al., 2003). Since, the self cleavage would eliminate the fusion protein, which is required for the Y2H system, no protein-protein interaction could be observed. To prevent this, the DNA-binding or the activation domain has to be fused to the C-terminus of the protein. However, the common yeast two-hybrid vector systems, so also the Matchmaker® Gold Yeast Two-Hybrid System, carry the multiple cloning site downstream of the fusion domains. Another reason of false negatives could be the required localisation of the inter-

acting proteins in the nucleus, which is not suitable for membrane associated proteins. AvrRpt2 and RIN4 in the system *P. syringae* pv. *tomato* and *A. thaliana* are both proven to be associated to the membrane (Axtell and Staskawicz, 2003). If AvrRpt2$_{EA}$ from *E. amylovora* and RIN4 from Mr5 act in the same way it would be possible that potential membrane binding domains prevent the protein-protein interaction. However, in case of RIN4 from *A. thaliana* it was proven, that a full length version of the protein was able to interact with NDR1 (Day et al., 2006), which in this case suggests no negative effect of the membrane binding residues. The localisation of FB_MR5 within the cell is unclear. Fahrentrapp et al. (2013) postulated no indications for membrane-spanning elements for the fire blight resistance gene *FB_MR5* by a hydropathy blot. However, the same blot with the same conditions carried out for the proven membrane-associated proteins RPS2, RPM1 and RIN4 from *A. thaliana* identified transmembrane regions only for RPS2. Thus the actual localisation of FB_MR5 remains to be clarified. At least if FB_MR5 interacts with RIN4 in a manner similar to RPM1 or RPS2 in *A. thaliana* it would be expected to bound to the membrane.

4.4. Differential transcriptome analysis of Mr5 by RNA-seq

RNA-seq allows the characterisation of transcriptomes based on next generation sequencing methods like Illumina. Based on the sequencing of the genome of 'Golden Delicious' by Velasco et al. (2010) a couple of transcriptomic studies of apple were carried out. Until now investigations were focused on columnar tree architecture (Krost et al., 2012, 2013; Zhang et al., 2012; Petersen et al., 2015), CO_2 injury during postharvest storage (Gapper et al., 2013; Mellidou et al., 2014) and apple scab infection (Gusberti et al., 2013).

The aim of this study was the identification of proteins related to the resistance mechanism of Mr5 against fire blight and to study the effect of the AvrRpt2$_{EA}$ effector protein on the transcriptome of Mr5. Hence, Mr5 was inoculated with the virulent *avrRpt2$_{EA}$* deletion mutant strain ZYRKD3-1 or with the non-virulent wild type strain Ea1189. The comparison of the two transcriptomes enables the identification of differentially expressed genes (DEGs), which should be related to the disease response. The comparison of a compatible with an incompatible reaction is not new. But normally it contains two different genotypes of the plant, one susceptible and one resistant, or two different strains

of the pathogen, one virulent and one non-virulent. However, in this study the same genotype of apple and the same strain of *E. amylovora* were used. The only difference between Ea1189 and ZYRKD3-1 is the mutation in the effector gene $avrRpt2_{EA}$.

After inoculation of Mr5, the RNA was isolated from the upper two leaves from a minimum of 10 shoots and send to GATC Biotech for further steps. The transcriptome was sequenced paired end with a read length of 50 bp at 2 and 48 hpi. Received reads were mapped to the reference transcriptome of 'Golden Delicious', which consisted at that time of 63 541 predicted genes characterised by a MDP (Malus × domestica protein) number. Over 360 million reads were generated and around 50 percent were mapped with the software package BWA to the reference (see table 3.4). This mapping rate excludes non uniquely mapped reads and is comparable to other studies that work with *Malus* and the reference transcriptome of 'Golden Delicious'. Gusberti et al. (2013) reported a mapping rate of 35.8 ± 3.7 % with paired end 101 bp sequencing, whereas Gapper et al. (2013) and Mellidou et al. (2014) reached mapping rates of around 65 % with single end 50 bp sequencing. These differences can be attributed to the sequencing mode, the read length and also the parameters used in the mapping. The rather low mapping efficiencies in case of *Malus* result from the low coverage of the reference transcriptome, which is one of the major limitations in RNA-seq studies. In 2014, Bai et al. published an improved version of the apple reference transcriptome with 17 524 novel transcripts which would increase the RNA-seq mapping rates considerably. Another limitation is the different genotype, even though the genome of Mr5 is highly similar to the one of 'Golden Delicious' it is not the same. Instead of the 'Golden Delicious' genome as reference, a de novo assembled reference transcriptome of Mr5 could be used, however, generating this is highly challenging and time-consuming.

The quality control of the reads obtained from Illumina HiSeq2000 was performed with FastQC (results see appendix K). Because of the paired end sequencing mode two fastq files were analysed per sample. All samples passed the sequence quality scores, sequence GC content and the sequence length distribution. The error for the per base sequence quality of the second paired read is the consequence from the decreased quality within the last three bases of the C-terminal end, which is the result of the sequencing process and also causes errors for the per base N content. The duplication level is very high in all samples, which is a consequence of the high degree of overrepresented sequences and Kmers, caused by Illumina PCR primer and adapter sequences. Additionally, this

also causes the errors for the per base sequence content and the per base GC content. However, the low-quality ends are removed by trimming during the mapping process by BWA.

In order to identify differentially expressed genes (DEGs), the huge amount of data was statistical evaluated with the DESeq software package (Anders and Huber, 2010). In total, it was possible to identify 211 significant differentially expressed genes in Mr5 inoculated with the non-virulent wild type strain Ea1189 in comparison with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1 at both time points. The scatter plots in figure 3.6 show the appropriate log2 fold change of each gene with the normalised mean read frequency, whereby the red dots represent the significant DEGs. The shape of the plots show high variability of the fold-change at lower count levels and, therefore, no detectable significant DEGs. Deeper sequencing and the use of biological replicates would help to detect even more lower expressed genes. 85 of the 211 DEGs were identified at 2 hpi, 106 at 48 hpi and 20 at both time points. 66 percent of the 211 genes showed a higher expression level in the incompatible reaction with the non-virulent wild type strain Ea1189. In general, it must be borne in mind that a higher expression level can also stand for the suppression of this gene in the other sample. The DEGs were analysed with MapMan and assigned to functional categories, so called BINs (see figure 3.7 and appendix M). The assignment via MapMan was not only performed with the 211 significant DEGs, but also with all transcripts obtained from the RNA-seq analysis and with transcripts with a log2 fold change ratio of more than 3 (appendix M). The look on genes in dependence of the log fold change without statistical evaluation, is just an approach to include some more DEGs. It allows to compare the distribution of the significant DEGs to the global distribution of all transcripts and to other DEGs.

Since the main objective of this study was the identification of genes related to the resistance mechanism of Mr5 against fire blight, only the transcripts matching the biotic stress map generated by MapMan (see figure 3.8) will be discussed in detail below. At least 32 percent of all the DEGs match this map. The log2 fold change ratio is indicated as a gradient from blue (higher expression level in Mr5 inoculated with the non-virulent wild type strain Ea1189) to red (higher expression level in Mr5 inoculated with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1). The middle part of the map (coloured in dark grey) presents genes assigned directly to the biotic stress BIN, whereas the areas coloured in lighter grey show the genes putative related to the biotic stress

(Rotter et al., 2007). The genes identified in this study are all putative related and belong to cell wall processes, secondary and hormone metabolism, abiotic stress, regulation of transcription, proteolysis. The following sections describe the individual genes in more detail.

DEGs within the first BIN, the hormone signalling pathway, can only be observed at 2 hpi. All of them, except of one, have a higher expression level in the incompatible reaction. Beside the classical plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) there are also auxins, abscisic acid (ABA) and brassinosteroids involved in the defence mechanism of plants. Their molecular mechanisms are partially still unknown and, furthermore, differ depending on the system. The first group of involved hormones are auxins. Both genes of the auxin metabolism, are highly similar to the auxin-responsive GH3-like protein family, a group of genes induced by a high auxin level. The overproduction of the GH3 enzyme causes negative feedback regulation of auxin, which results in growth reduction and enhanced resistance to biotic and abiotic stress (Park et al., 2007). The second group of hormones are ethylene. The high expression level of the two ethylene related genes in the incompatible system suggests a positive effect on plant resistance in case of the system *E. amylovora* and *Malus*. In literature the role of ethylene in plant defence is not always described as positive, it can promote resistance as well as disease (Kunkel and Brooks, 2002). For apple it has been shown, that the infection with *E. amylovora* leads to an ethylene burst, which is also related to the hypersensitive response (HR) in leaves (Iakimova et al., 2013). However, the increase of ethylene was observed in resistant as well as susceptible apple hosts inoculated with *E. amylovora*. Similar results are published by the group of Spinelli et al. (2011) who analysed the ethylene production in apple plants after inoculation with *E. amylovora* and mock. The last group of plant hormones, and the only group with a higher expression level in the compatible reaction, is the jasmonate class. Within this class, only one gene was identified. It is highly similar to lipoxygenase 2 (LOX2). The *LOX2* gene was detected to be necessary for wound-induced JA accumulation in *Arabidopsis* leaves by Bell et al. (1995). But since the experimental design of the transcriptome analysis of this study excluded DEGs by wounding, LOX2 seems to be involved in the defence response. An analysis by De Bernonville et al. (2012) showed, in contrast to the results of this study, a strong down-regulation of LOX2 for the susceptible apple genotype MM106 and no effect for the resistant apple genotype Evereste after inoculation with an *E. amylovora* wilde

type strain. This contrary results can be explained by the fact that within this study the compatible as well as the incompatible reaction were triggered in the same system, which prevents not only the occurrence of DEGs caused by wounding, but also the occurrence of DEGs as a result of different genotypes. Another explanation is the absence of the $AvrRpt2_{EA}$ effector in the mutant strain ZYRKD3-1, as LOX2 modulation depends on T3SS effectors (De Bernonville et al., 2012). If the consideration is not only restricted to the 211 DEGs, but also extended to transcripts with a log2 fold change ratio of more than 3, all identified *LOX* genes are up-regulated in the incompatible reaction (data not shown). This confirms at least the involvement of *LOX* genes in the plant defence mechanism in general and suggest a specific role for the *LOX2* gene.

Another interesting group of genes putative involved in biotic stress are genes related to cell wall processes. The ones identified in this study are similar to genes coding for cell wall proteins or to genes involved in the cell wall degradation and modification. Except for one, all of these genes show a higher expression level in Mr5 after inoculation with the non-virulent wild type strain Ea1189. All genes related to the cell wall degradation contain a BURP domain with similarity to the gene RD22 of *A. thaliana*. A differential RNA-seq experiment with the apple cultivar Florina infected with the fungus *Venturia inaequalis* showed a high expression level of RD22 as well (Beck, 2003). This suggests that RD22 is important for the pathogen defence in apple in general, not only in case of infection with *E. amylovora*. Interestingly, the genes involved in the cell wall degradation are present at both time points, whereby other genes related to cell wall processes are present either at 2 or 48 hpi. Also, Sarowar et al. (2011) showed in a microarray experiment that up-regulation of cell wall biosynthesis- and modification-related genes was observed in blossoms of the fire blight-susceptible apple cultivar Gala during infection with *E. amylovora*. The only up-regulated gene in Mr5 inoculated with the virulent mutant strain ZYRKD3-1 belongs to the cellulose synthesis.

Further defence proteins assigned to the biotic stress map have miscellaneous functions. The two DEGs within this group belong to the pathogenesis-related (PR) proteins, or more precisely to the β-1,3-glucanases and peroxidases. Both DEGs have a higher expression level in the incompatible reaction, whereby β-1,3-glucanase is even still active at 48 hpi. PR proteins are induced during a pathogen attack, which is not surprising, but they are not active in the compatible reaction. Therefore, a closer examination and characterisation of these genes is highly interesting.

The fourth group of DEGs belongs to the proteolysis active proteins. It is the first group with a higher expression level in the compatible reaction. This could result from the successful colonisation of Mr5 with the mutant strain and the consequently increasing demand to eliminate pathogenic proteins. Proteins involved are subtilases, E3 ubiquitin-ligases and AAA proteins, three large protein families all effecting the plant defence in some way.

Within the fifth group, the signalling pathway, two genes were identified. Both were identified only at 2 hpi and only in Mr5 after inoculation with the virulent mutant strain ZYRKD3-1. One of them is nearly identical to a leucine-rich repeat (LRR) protein kinase, whereby the other one belongs to phototropic-responsive NPH3 family proteins. LRR protein kinases regulate a wide variety of defence-related processes, which makes the found DEG an interesting candidate for further analysis.

A further important part of the biotic stress response is the regulation of transcription. The two MYB transcription factors, shown in the biotic stress map in figure 3.8, are only detectable in the incompatible reaction and only at 2 hpi. Defence related transcription factors in apple have not been published until now, however, Sarowar et al. (2011) showed a high up-regulation of WRKY transcription factors and zinc finger family proteins in apple blossoms following *E. amylovora* treatment. In this study, within the significant DEGs no WRKY transcription factors were identified. Apart from the genes matching the biotic stress map, 18 further genes belonging to the regulation of transcription were identified within the 211 DEGs. 11 of them are identified at 2 hpi and all have a higher expression in Mr5 after inoculation with the non-virulent wild type strain. 7 out of 11 belong to the homeobox transcription factor family. The 7 transcription factors identified at 48 hpi belong all, except of two, to the heat-shock transcription factor family and all of them have a higher expression level in Mr5 after inoculation with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1.

The abiotic stress response is the seventh group and consists mainly 24 heat shock proteins (HSPs). Similar to the heat-shock transcription factors mentioned in the preceding paragraph, the HSPs have a higher expression at 48 hpi in the compatible reaction. Beside high temperature and other stress stimuli, HPSs are involved in the response to pathogenic attack as well (Vierling, 1991). These proteins function as chaperones, assisting the correct folding of stress-accumulated misfolded proteins and preventing their aggregation (Gupta et al., 2010). However, until now the exact role of HSPs within

the pathogen defence mechanism is not clearly understood. As the expression level is highly up-regulated in the compatible reaction, HSPs seems to be a result of the successful colonisation of Mr5 with the mutant strain. Interestingly, the up-regulation of the HSPs occur relatively late, whereas literature describes it as a part of the early defence mechanism.

Secondary metabolites are the eighth and last group of DEGs within the biotic stress map and can be found only in Mr5 after inoculation with the non-virulent wild type strain. These genes belonging to the isoprenoids, phenylpropanoids and flavonoids are mainly up-regulated at 2 hpi, which suggests a positive effect on the resistance of Mr5. According to the literature the phenylpropanoid-flavonoid pathway in the system *Malus* and *E. amylovora* depends highly on the cultivar, the bacterial strain, the inoculation method and the time after inoculation (Vrancken et al., 2013).

4.5. Gene expression analysis of Mr5 with the BioMark™ HD system

To confirm the results obtained by the RNA-seq analysis and to verify additional time points, gene expression was further analysed with the BioMark™ HD system. The advantage compared to the classical Real-Time qRT-PCR is the high throughput of samples within a short time. With the BioMark™ 96.96 Dynamic Array it is possible to analyse 96 genes in 96 samples at the same time.

The 96 tested genes consisted of DEGs identified by RNA-seq, two further candidate genes of Mr5 for fire blight resistance and five reference genes for data normalization. The selected DEGs were mainly DEGs with a higher expression level in Mr5 after inoculation with the wild type strain Ea1189. The two additional genes were the recently identified *FB_MR5* fire blight resistance gene from Mr5 (Fahrentrapp et al., 2013), and a further candidate gene encoding for a class three secretory peroxidase (GenBank: EH034548; (Gardiner et al., 2012)).

The 96 utilized samples were based on the experimental design of the RNA-seq analysis, but the analysed time points were extended to 1, 2, 4, 12, 24 and 48 hpi and a non-infected sample of Mr5 was added. Three biological and two technical replicates of each sample as well as negative controls and water were analysed.

The results were statistically verified by the SAS software. From the 96 analysed genes,

only 24 showed significant differences in the expression pattern. These genes and their annotations obtained by GO, InterPro and MapMan are listed in table 3.6. Three different expression patterns were observed: a) the expression compared to the non-infected sample is increased, b) the expression compared to the non-infected sample is decreased and c) the expression compared to the non-infected sample is both increased and decreased.

The first group (expression pattern a), which includes genes with an higher expression level compared to the non-infected sample, consists of three genes (see figure 3.9). Two of them, MDP0000262141 and MDP0000940742, showed differences in gene expression between the incompatible and the compatible reaction, which indicates an involvement in the resistance mechanism. MDP0000262141 contains a SBP (*SQUAMOSA* promoter binding protein) domain and functions as a transcription factor. Padmanabhan et al. (2013) identified an interaction between the (SBP)-domain transcription factor NbSPL6 from *N. benthamiana* and the immune receptor N, which mediates the resistance against *Tobacco mosaic virus* (TMV). Furthermore, they showed a positive effect of AtSPL6 from *A. thaliana* to the RPS4 mediated resistance against *P. syringae*. If the transcription factor plays a similar role in *Malus* it could have an essential part within the defence response. MDP0000940742 is highly similar to BGLU13 (beta glucosidase 13) and, therefore, most likely related to the cell wall reinforcement process during pathogen infection.

The second group (expression pattern b) consisted of seven of the 24 genes (charts in appendix O). However, the expression level in Mr5 is very similar between the two strains used for inoculation, which indicates a more common answer to the inoculation process itself rather than a specific one. Hence, this group will not be further discussed.

The remaining 14 genes of the third group (expression pattern c) have a lower as well as a higher expression compared to the non-infected sample and can be further divided into three groups by the course of expression over the time (see figure 3.10). The first seven genes show the highest expression at two hpi already (green coloured charts) and belong therefore to the early response. At one hpi all these genes have a higher expression in the incompatible than in the compatible reaction and interestingly, vice versa at two hpi. This delayed reaction in the compatible reaction could result from the missing virulence effector *avrRpt2*$_{EA}$ and lead to the successful colonisation of the bacteria. The difference at one hpi is particularly high for the genes MDP0000937986, MDP0000151003 and MDP0000180902. These three genes are described in more detail in the following section. MDP0000937986 belongs to the low-temperature-induced proteins. It has been

shown that cold signals lead to the synthesis of PR proteins and are, therefore, linked to pathogen resistance (Seo et al., 2010). The second gene MDP0000151003 codes for a cytochrome P450 protein, which belongs to the largest superfamilies of proteins with enzymatic activity. P450 genes can be found in all organisms, but with a greater diversity in plants. Their function includes many processes, but the main reason of this variety seems to be the chemical defence (Werck-Reichhart and Feyereisen, 2000). Two more genes coding for cytochrome P450 proteins can be found within the 24 genes, but in contrary in the group of genes with expression pattern b (see table 3.6). The third gene MDP0000180902 is related to the gibberelin synthesis within the hormone metabolism. Several experiments showed that gibberellic acid and its signalling components have an important role in regulating the defence response, but the exact mechanism is largely unknown (Bari and Jones, 2009). Inhibiting the gibberellin biosynthesis by the plant growth regulator prohexadione-calcium is a common method in the control of fire blight (Norelli et al., 2003). Therefore, the up-regulation of MDP0000180902 causes possibly a negative feedback regulation of the gibberellin biosynthesis and thereby enhanced resistance against *E. amylovora*.

The next group of expression pattern c, which contains four of the 14 genes (blue coloured charts in figure 3.10), has the highest expression at four or 12 hpi and belongs to the late response. The gene MDP0000668657 is highly up-regulated between one and 12 hpi and is part of the minor CHO metabolism. The other three genes are involved in the regulation of transcription or in the lipid metabolism. Strong distinctions in the expression between the compatible and the incompatible reaction are not observable for the hole group, which suggests a more common defence mechanism.

The last group of expression pattern c contains the three remaining genes (yellow charts in figure 3.10). The most interesting gene is MDP0000711911, which is highly up-regulated only in the incompatible reaction at one hpi. This gene belongs to the Ribosome-inactivating proteins (RIPs), an enzyme class found mainly in plants. In plants RIPs have antiviral and antitumor activity and play, therefore, an essential role in the defence mechanism (Puri et al., 2012). More RIPs with an similar expression pattern were also found within the significant DEGs from RNA-seq analysis. In view of the expression pattern and the classification, MDP0000711911 is an interesting candidate for further analysis. The second gene of this last group MDP0000921319 is highly down-regulated until 48 hpi, where the expression level reaches more or less the level of the non-infected

sample. The expression of the last gene of this group MDP0000644109 shows no clear course and is similar to the non-infected sample. The last two genes show no strong distinctions in the expression between the compatible and the incompatible reaction and can be assigned to the common defence mechanism.

The following section compares the results obtained by the BioMark™ HD system and the RNA-seq analysis. Mostly all of the genes analysed by the BioMark™ HD system were selected because of the significant higher expression level in the incompatible reaction in the RNA-seq analysis. However, considering the charts from figure 3.9 and figure 3.10 the expression pattern obtained by the BioMark™ HD system show at least partially a different course. This is also shown in table 3.7, which directly compares the results obtained from RNA-seq and from the gene expression analysis with the BioMark™ HD system. The selected genes are the same like the ones from figure 3.10. Comparing the two methods at 2 and 48 hpi, it appears especially for the early disease response a wide variation between the two methods. The reason could be that the RNA used in the two methods came not from the same samples. This unfavourable circumstance was attributed to the failure of the -80 ℃ freezer, whereupon the RNA was thawed and, therefore, no longer usable for the second experiment. Another possible reason could be the missing biological replicates in the RNA-seq analysis. As the results of the gene expression analysis with the BioMark™ HD system show, is the variance very high between the replicates, even a bulk sample is used.

But apart from these possible reasons, the different results could be also caused by the short interval of time for the sampling after inoculation. If the comparison of the results is performed between 2 hpi from RNA-seq with 1 hpi from the BioMark™ HD system, the results are more consistent. A similar behaviour shows the comparison between 48 hpi from RNA-seq with 24 hpi from the BioMark™ HD system, even though it is less clearly. This might mean that the results are not as different as assumed, only time-shifted.

4.6. Fire blight resistance gene *FB_MR5* of Mr5

The identification and isolation of the fire blight resistance gene *FB_MR5* of the wild apple species Mr5 was already described in the section 1.3 of the introduction (Peil et al., 2007; Fahrentrapp et al., 2013). In order to verify the functionality of the resistance gene, Broggini et al. (2014) transferred the gene into the fire blight susceptible apple

cultivar *Malus* ×*domestica* Borkh. cv. 'Gala '. Two gene constructs were used for the *A. tumefaciens*-mediated transformation. One was designed with the *FB_MR5* gene driven by the constitutive 35S promoter and the ocs terminator and the other construct was designed with the *FB_MR5* gene driven by its native promoter and terminator sequences. The obtained transgenic lines were subsequently analysed by artificial shoot inoculation with different strains of *E. amylovora*. Broggini et al. (2014) described susceptibility in case of *FB_MR5* under the 35S promoter whereas the transgenic lines expressing the gene by the native promoter and terminator sequences were mainly resistant. *FB_MR5* driven by the 35S promoter was cloned with the start and stop borders as predicted by the gene finder software FGENESH with algorithms of tomato. The reason why the transgenic lines with *FB_MR5* under the 35S promoter showed disease symptoms could be a wrong prediction of the gene.

One possibility to verify the prediction was a mapping of the transcripts obtained by the RNA-seq analysis of this study to the DNA of the fire blight resistance gene *FB_MR5*. Since *FB_MR5* is not present in the genome of 'Golden Delicious' it was necessary to do a separate mapping. Therefore, the gene including 2 kb before the suspected start codon and 1 kb behind the suspected stop codon, were used as reference. The alignment of the mapping in figure 3.12 shows reads before the start and behind the stop codon. These results gave cause for a 3' and 5' RACE-PCR to obtain a full length sequence of the gene and to predict the right start and stop codon. The results of the 5' RACE-PCR were not entirely clear regarding to the UTR, but the sequences of the reads obtained by the mapping could not be confirmed, which suggest the right prediction of the start codon so far. On the contrary, the results of the 3' RACE-PCR. Therefore, it was possible to identify another stop codon after an additional intron, which results in a shift of the reading frame by one base pair. Since the transgenic Gala lines driven by the 35S promoter contain, therefore, an incomplete version of the *FB_MR5* resistance gene, the pathogen can successfully infect the host. A virulence analysis of new transgenic lines could verify these results, however it is a very time-consuming process.

5. Conclusion

The aim of the study was the identification, cloning and functional characterisation of genes related to the defence mechanism of *Malus* ×*robusta* 5 against the bacterial disease fire blight. Beside the identification of new genes, the study also focused on a closer description of already known genes. One gene with an essential role in the pathogenesis of fire blight is the effector AvrRpt2$_{\mathrm{EA}}$. It was identified in the genome of *E. amylovora* in 2006 by Zhao et al. as a homologue to the already known effector AvrRpt2 of *P. syringae* pv. *tomato*. Inoculation experiments on immature pear fruits with an *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 resulted in the reduction of pathogenicity, indicating that the effector acts as a virulence factor. The infection of the highly resistant wild apple species Mr5 with the same mutant strain, however, showed the opposite effect with a complete breakdown of the resistance and, therefore, an avirulent interaction of *avrRpt2*$_{EA}$ with Mr5. Accordingly, the AvrRpt2$_{\mathrm{EA}}$ effector seems to play an important role in the defence mechanism of apple and pear. Furthermore, it was a strong indication for a gene-for-gene relationship in the host pathogen system Mr5 and E. amylovora. Based on these results the effector was examined in greater detail.

First, the *avrRpt2*$_{EA}$ gene of twenty-two different *E. amylovora* strains differing in origin and isolated from different hosts was sequenced. An alignment of all sequences showed one single nucleotide polymorphism (SNP), which occurred in three strains. Apart of this SNP, the sequences were completely identical. The deduced amino acid sequences showed that the SNP resulted in an exchange from cysteine to serine at position 156. The two alleles of the *avrRpt2*$_{EA}$ gene were designated as C-allele and S-allele. Considering the virulence data of these strains, Vogt et al. (2013) discovered a dependence between the appropriate allele of the effector and the strain specific resistance of Mr5 . Strains with a serine broke the resistance of Mr5, whereby strains with cystein were non-virulent to Mr5. In view of the ability of cysteine to form disulphide bridges this exchange can alter the tertiary structure of the effector considerably. This could modify the downstream

acting process in the resistance mechanism of Mr5 enormously and might be, therefore, the reason for the strain specific virulence in case of Mr5. In order to verify if the SNP of the *avrRpt2*$_{EA}$ gene determines indeed the virulence of *E. amylovora* against Mr5, the virulent deletion mutant ZYRKD3-1 was complemented with the functional C- or S-allele of the effector. The hypothesis was, that the complementation of the virulent mutant strain with the C-allele of the *avrRpt2*$_{EA}$ gene results in avirulence. And in fact, subsequent inoculation experiments confirmed this assumption and suggested, therefore, the first gene-for-gene relationship in the system *Malus* and *E. amylovora*.

The strong sequence homology between the effectors AvrRpt2$_{\mathrm{EA}}$ and AvrRpt2 suggested a similar resistance mechanism between Mr5 and *A. thaliana*. Within the system *A. thaliana* and *P. syringae* pv. *tomato* AvrRpt2 cleaves the guard protein RIN4, thus, activating the resistance protein RPS2 and thereby the pathogen defence (Mackey et al., 2002; Axtell and Staskawicz, 2003; Kim et al., 2005). Since RIN4 has a central role within the plant cell, the question raised if it is also present in the genome of apple and especially in Mr5. A NCBI and UniProt database search of RIN4 homologues yielded two entries for *Malus* ×*domestica*, RIN4-1 and RIN4-2 on chromosome 10 and 5. Therefore, it could be assumed that it is present in the genome of Mr5 as well. And indeed *RIN4* was identified in the genome of Mr5 and similar to *Malus* ×*domestica* on chromosome 5 and 10. A comparison of the protein sequences of RIN4 from *A. thaliana*, *Malus* ×*domestica* and Mr5 showed a high degree of similarity, particularly in the important motif sequences of the cleavage sites RCS1 and RCS2 and the cysteine-rich membrane-anchoring site in the C-terminal region. But although there is high similarity between the two homologues, the RIN4 antibody of *A. thaliana* was not usable for the detection of RIN4 within Mr5.

The next step was the verification of potential protein-protein interactions of RIN4 of Mr5 similar to RIN4 of *A. thaliana*. Initially, RIN4 was identified in a yeast two-hybrid screen via its interaction with the *A. thaliana* effector AvrB (Mackey et al., 2003). Hence, the yeast two-hybrid system was selected as well to verify, if there is an interaction between RIN4 from Mr5, AvrRpt2$_{\mathrm{EA}}$ from *E. amylovora*, but also the recently discovered fire blight resistance protein FB_MR5 from Mr5 (Fahrentrapp et al., 2013). However, within this study it was not possible to detect any interaction between all tested combinations. Beside the reason of no interaction in fact, it can be prevented by a couple of parameters and lead to false negative results, which is a common problem of the Y2H system.

Another important aim of the study was the identification of new genes related to the

defence mechanism of Mr5 against the bacterial disease fire blight. This was achieved by a differential transcriptome analysis of Mr5 after inoculation with the virulent $avrRpt2_{EA}$ deletion mutant strain ZYRKD3-1 or with the corresponding non-virulent wild type strain Ea1189. The possibility to compare a transcriptome of a compatible and an incompatible reaction within the same genotype caused by just one single gene deletion within the bacteria is unique so far. Differential expressed genes (DEGs) are, therefore, clearly related to the resistance mechanism. The comparison of the transcriptomes of Mr5 at two time points post inoculation yielded more than 360 million reads, whereby 50 percent could be mapped to the reference transcriptome of 'Golden Delicious'. After statistical evaluation of this data, 211 significant DEGs remained. With the tool MapMan it was possible to assign these DEGs to functional categories and metabolic pathways such as the biotic and abiotic stress response. To confirm the results obtained by the RNA-seq analysis and to verify additional time points, gene expression was further analysed with the BioMark™ HD system. Since the DEGs with a higher expression level in the incompatible reaction might be particularly important within the defence mechanism of Mr5 against *E. amylovora*, they were selected for the further steps. Among the 90 analysed genes, 24 showed significant differences in the statistical analysis. Summarising the results of both methods, results in several candidate genes with quiet promising expression pattern. However, the results with the BioMark™ HD system did not clearly confirm the results obtained by the RNA-seq analysis.

An additional part of this study was a more detailed investigation of the recently published fire blight resistance gene *FB_MR5*. Functionality tests by Broggini et al. (2014) suggested a wrong prediction of the gene. Therefore, a mapping with the transcripts of the RNA-Seq analysis to the resistance gene including 2 kb before the suspected start codon and 1 kb behind the suspected stop codon as reference was carried out. Since the alignment showed transcripts before the start and behind the stop codon, it was necessary to do a 3’ as well as a 5’ RACE-PCR. The results of the 5’ RACE-PCR were not entirely clear, but the sequences of the reads obtained by the mapping could not be confirmed, which suggest the right prediction of the start codon so far. The sequences obtained by the 3’ RACE-PCR identified another stop codon after an additional intron, which resulted in a shift of the reading frame by one base pair.

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Appendix

A. Materials

E. coli and *S. cerevisiae* strains are listed in the material and methods section 2.1, as well as all kits, enzymes, vectors and antibodies. Strains of *E. amylovora*, chemicals, lab equipment and the composition of buffers and media are mentioned below.

A.1. Used chemicals

Acetic acid, glacial	VWR, Radnor, PA, USA
Agarose	Biodeal, Markkleeberg, DE
Ampicillin	Duchefa Biochemie, Haarlem, NL
Bacto Agar	Difco Laboratories, Heidelberg, DE
Bacto yeast extract	Otto Nordwald,Hamburg, DE
Bromophenol blue	Carl Roth, Karlsruhe, DE
Chloramphenicol	Duchefa Biochemie, Haarlem, NL
Coomassie blue R	Sigma-Aldrich, München, DE
dNTPs	Fermentas/Thermo Fisher Scientific, Schwerte, DE
Dry milk	Santa Cruz Biotechnology, Heidelberg, DE
DTT	SERVA Electrophoresis, Heidelberg, DE
$EDTA\text{-}Na_2$	Sigma-Aldrich, München, DE
EDTA	Merck, Darmstadt, DE
Ethidium bromide	Carl Roth, Karlsruhe, DE
Glycerin	Carl Roth, Karlsruhe, DE
Hydrochloric acid	Carl Roth, Karlsruhe, DE

Isopropanol	Carl Roth, Karlsruhe, DE
IPTG	Thermo Fisher Scientific, Schwerte, DE
Kanamycin	Duchefa Biochemie, Haarlem, NL
Luminol Reagent	Santa Cruz Biotechnology, Heidelberg, DE
β-Mercaptoethanol	SERVA Electrophoresis, Heidelberg, DE
Methanol	Carl Roth, Karlsruhe, DE
Nitrogen, liquid	AIR LIQUIDE, Düsseldorf, DE
Phenol	Carl Roth, Karlsruhe, DE
Ponceau-S solution	Sigma-Aldrich, München, DE
Sodium chloride	Merck, Darmstadt, DE
Sodium dodecyl sulfate	Sigma-Aldrich, München, DE
Sodium hydroxide	Carl Roth, Karlsruhe, DE
TEMED	AMRESCO, Solon, USA
Tetracycline	Duchefa Biochemie, Haarlem, NL
Tris	Carl Roth, Karlsruhe, DE
Tris-HCl	Carl Roth, Karlsruhe, DE
Triton X-100	Sigma-Aldrich, München, DE
Tryptone/Peptone ex casein	Carl Roth, Karlsruhe, DE
Tween-20	Sigma-Aldrich, München, DE

A.2. Composition of buffers and media

Culture media

LB medium	Bacto-tryptone 10 g, Bacto-yeast extract 5 g, NaCl 10 g adjusted to one litre and a pH of 7.2; autoclaved
LB agar	LB medium, Bacto agar 15 g/l

Buffers for western blot

Anode I buffer	0.3 M Tris, 20 % Methanol
Anode II buffer	25 mM Tris, 20 % Methanol
Cathode buffer	0.40 mM 6-Aminohexan acid, 0.01 % SDS, 20 % Methanol
2X Lämmli sample buffer	0.1 M Tris, 4 % SDS, 20 % Glycerin, 20 mM DTT, 0.015 % Bromphenol blue
10X SDS running buffer	250 mM Tris-HCl, 1.92 M Glycine, 1 % SDS pH adjusted to 8.3
10X TBS buffer	20 mM Tris, 150 mM NaCl pH adjusted to 7.4
TBS-T buffer	TBS buffer, 0.1 % Tween
Ponceau-S staining solution	0.2 % Ponceau, 3 % Trichloracetic acid

Other buffers

50X TAE buffer	Tris 242 g, Acetic acid (glacial) 57.1 ml, 0.5 M EDTA-Na_2 100 ml adjusted to one litre and a pH of 8.0
1X TAE buffer	50X TAE-Puffer : ddH_2O (v/v) (1:50)
TE buffer	10 mM Tris-HCl, 1 mM EDTA pH adjusted to 8.0
low TE buffer	10 mM Tris, 0.1 mM EDTA pH adjusted to 8.0

A.3. Laboratory equipment

Autoclave	Systec VX-150, Systec, Linden, DE Systec 3870 ELV, Tuttnauer Europe, Breda, NL
Balance	Sartorius Basic, Sartorius, Göttingen, DE BA 110 S, Sartorius, Göttingen, DE LC 620 P, Sartorius, Göttingen, DE

Centrifuge	Beckman Coulter Microfuge 16, Beckman Coulter, Krefeld, DE 5415R, Eppendorf, Hamburg, DE Universal 32R, Andreas Hettich, Tuttlingen, DE SIGMA 3-18K, SIGMA Laborzentrifugen, Osterode am Harz, DE GeneVac SF50, Biometra®, Göttingen, DE
Clean bench	HERAGUARD HPH 15, Kendro Laboratory Products, Hanau, DE
Electrophoresis	Chamber: Mini/Midi/Maxi, neoLab Migge, Heidelberg, DE Mini-PROTEAN II, BioRad, München, DE Power Supply: Owl™ EC-105 Compact, Thermo Fisher Scientific, Schwerte, DE Pharmacia EPS 300, GE Healthcare Europe, Freiburg, DE Power Pac 200, BioRad, München, DE Consort E865, CONSORT, Turnhout, BE
Electroporator	Gene Pulser® II Electroporation System, BioRad, München, DE
Freezer, Fridge	Glass line, Liebherr, Liebherr-International, Bulle, CH Comfort, Liebherr, Liebherr-International, Bulle, CH SC-50, Bartscher, Salzkotten, DE Active Soft, SNAIGĖ, Alytus, LT HERAfreeze HFU 686 Top, Thermo Fisher Scientific, Schwerte, DE HERAfreeze HFU Basic, Kendro Laboratory Products, Hanau, DE
Gel/Blot visualisation	ChemiDoc™ XRS+ System, BioRad, München, DE
Hybridisation oven	Mini10, MWG Biotech, Ebersberg, DE
Incubator	Heraeus® HERAcool, Kendro Laboratory Products, Hanau, DE Heraeus® B15, Thermo Fisher Scientific, Schwerte, DE Heraeus UT 6420, Kendro Laboratory Products, Hanau, DE INCUBAT® 85, MELAG Medizintechnik, Berlin, DE Kelvitron® t, Kendro Laboratory Products, Hanau, DE

Magnetic stirrer	RH3, VEB MLW, Leipzig, DE
Microscope	JENAVAL, Carl Zeiss Microscopy, Göttingen, DE
Microwave	HF 12M240, Siemens, München, DE
Mixer mill	Retsch® MM300, Retsch, Haan, DE
PCR-Cycler	Unocycler, VWR International, Darmstadt, DE MyCycler™ BioRad, München, DE FlexCycler, Analytik Jena, Jena, DE Arktik, Thermo Fisher Scientific, Schwerte, DE iCycler iQ™ BioRad, München, DE GeneAmp® PCR 9700 System, Applied Biosystems/Thermo Fisher Scientific, Schwerte, DE)
Pipettes	Research/Research plus, Eppendorf, Hamburg, DE
pH meter	ph 211, Hanna Instruments Deutschland, Vöhringen, DE
Photometer	GeneQuant II, Pharmacia Biotech, Piscataway, NJ, USA NanoDrop 2000c, Thermo Fisher Scientific, Schwerte, DE
Pure water system	LaboStar, SG Wasseraufbereitung und Regenerierstation, Barsbüttel, DE
Thermomixer	ThermoStat plus, Eppendorf, Hamburg, DE Thermomixer comfort, Eppendorf, Hamburg, DE TS-100, BioSan, Riga, LV
Vortex	Heidolph REAX 2000, Heidolph Instruments, Schwabach, DE neoLab 7-2020, neoLab Migge, Heidelberg, DE
Washer-Disinfector	26-03, Riebesam, Genthin, DE
Water bath	GFL® 1083, Gesellschaft für Labortechnik, Burgwedel, DE Grant GLS400, Grant Instruments (Cambridge) Ltd, Shepreth, Cambridgeshire, GB
Western blot	Trans-Blot® SD Semi-Dry Transfer Cell, BioRad, München, DE

A.4. Consumables

Blotting paper	Whatman, Göttingen, DE
Electroporation cuvettes	PEQLAB/VWR, Erlangen, DE
Fermacidal D2	DROL, Reichertshausen, DE
Disposable cuvettes (2.5 ml)	Carl Roth, Karlsruhe, DE
Disposable gloves	VWR, Radnor, PA, USA
PCR reaction tubes	Biozym, Hessisch Oldendorf, DE Kisker Biotech, Steinfurt, DE
Petri dishes	Greiner Bio One, Kremsmünster, AT
Pipette tips	Carl Roth, Karlsruhe, DE
PVDF membrane	Santa Cruz Biotechnology, Heidelberg, DE
Reaction tubes (1.5/2 ml)	Carl Roth, Karlsruhe, DE
Reaction tubes (15/50 ml)	VWR, Radnor, PA, USA Corning, Corning NY US
Reaction tubes safe lock (1.5/2 ml)	Eppendorf, Hamburg, DE
Skinman® clear	Ecolab, Monheim am Rhein, DE
Sterile filters	Carl Roth, Karlsruhe, DE

B. *Erwinia amylovora* strains

Table 5.1.: Description of the used *E. amylovora* strains.

Strains (alternative name)	**Host and Origin**	**Reference**	$\mathbf{AA}_{156}$
Ea7	*Pyrus*, Brandenburg, DE; 1972	H.-J. Schaefer	C
Ea110	*Malus*, Michigan, US	A.L. Jones	S
Ea222 (50/92)	*Cotoneaster*, Havlickuv Brod, CZ; 1992	V. Kudela	C
Ea401 (Ea1/79)	*Cotoneaster*, DE; 1979	W. Zeller	C
Ea402 (Ea7/74)	*Cotoneaster*, DE; 1974	W. Zeller	C
Ea627	*Pyrus*, Dresden, DE; 2003	SMUL, Dresden	C
Ea662	*Pyrus*, Tundersleben (Magdeburg), DE; 2003	D. Beyme	C
Ea717	*Crataegus*, Slany, CZ; 1997	J. Korba	C
Ea763	*Pyrus*, Baden-Württemberg,DE; 2006	E. Moltmann	C
Ea782	*Crataegus*, Quedlinburg, DE; 2007	K. Richter	C
Ea789	*Malus*, NZ; 2007	M. Horner	C
Ea815	*Malus*, Freising, DE; 2008	G. Poschenrieder	C
Ea839	*Pyrus*, Baden-Württemberg, DE; 2008	E. Moltmann	C
Ea842	*Malus*, CH; 2008	ACW Wädenswil	C
Ea846	*Malus*, PL; 1986	P. Sobiczewski	C
Ea847 (CFBP 1430)	*Crataegus*, FR; 1973	J.-P. Paulin	C
Ea898 (Ea1189)	*Malus*, DE	Burse et al. (2004)	C
Ea3049 (CFBP 3049, CUCPB 265, E2002A, Ea395)	*Malus*, Ontario, CA (over France)	W.G. Bonn	S
Ea3050 (CFBP 3050, CUCPB 266, E4001A, Ea77, Ea396)	*Malus*, Ontario, CA (over France)	W.G. Bonn	S
Ea3051 (CFBP 3051, CUCPB 273, Ea78)	*Malus*, FR		C
PFB4 (INRA 2653-1)	*Prunus*, Idaho, US; 1995	McManus & Jones, 1995	C
PFB15 (INRA 2655-1)	*Prunus*, Idaho, US	K. Mohan via J. P. Paulin	C
ZYRKD3-1		Zhao et al. (2006)	

Table 5.1.: (continued)

Strains (alternative name)	**Host and Origin**	**Reference**	**AA_{156}**
ZYRKD3-1+Avr-S_G (ZYRKD3-1 (pZYR2))		Zhao et al. (2006)	
ZYRKD3-1+Avr-S_LacP		this study	
ZYRKD3-1+Avr-C_eP		this study	
Ea3049+Avr-C_eP		this study	
Ea3049+Avr-C_LacP		this study	
Ea222+Avr-S_eP		this study	
Ea222+Avr-S_LacP		this study	

AA_{156} is the amino acid at position 156

C. Tables of primers

C.1. General primers

Table 5.2.: Sequences of primers used for colony PCR and for RT-PCR with the reference gene EF1-α.

Primer	Target	Sequence 5' → 3' direction	T_A [°C]	Cycles
M13_F	pCR2.1®-TOPO® TA	AGGGTTTTCCCAGTCACGACGTT	55	30
M13_R		GAGCGGATAACAATTTCACACAGG		
pJET1.2_F	pJET1.2	CGACTCACTATAGGGAGAGCGGC	55	30
pJET1.2_R		AAGAACATCGATTTTCCATGGCAG		
T7 Prom	pGBKT7	TACGACTCACTATAGGGCGA	60	30
pGB_R	pGADT7	AGAAATTCGCCCGGAATTAG		
pGAD_R		AGATGGTGCACGATGCACAG		
EF1α_F	Elongation factor-1	ATTGTGGTCATTGGYCAYGT	56	30
EF1α_R		CCAATCTTGTAVACATCCTG		

C.2. Primers for the synthesis of the complemented strains

Table 5.3.: Sequences of primers used for the synthesis of the complemented strains.

Primer	Target gene	Sequence 5' → 3' direction*	T_A [°C]	Cycles
avrRpt2-1	*avrRpt2$_{EA}$*	GATCCTGGCCTGAAAGGTGATAC	55	35
avrRpt2-2		AGCGGATAGCCATTCTGGATCAG		
avrRpt2-5[1]		GTATGCCTGCACCAGAATGC	-	-
avrRpt2-6[1]		GGGCCTGAAGAGTCATAGAG		
avrRpt2-3-Eco5'		ccgGAATTCCGTGCAGATTGGCGAAGTGATTA	55	30
avrRpt2-4-Bam5'		cgcGGATCCGGTGCTTATCCATGCGGTCGTTC		
avrRpt2-3-Bam5'		cgcGGATCCCGTGCAGATTGGCGAAGTGATTA		
avrRpt2-4-Eco5'		ccgGAATTCGGTGCTTATCCATGCGGTCGTTC		
680		GAGCACCAGCCTCGTCAATC	67	30
682		CATAATGGGTCCATGGCGAG		
683		CATAATGGGTCCATGGCGAC		
681[2]		GAGCACCAGCCTCGTCAATC	62 (682) 64 (683)	40
682[2]		CATAATGGGTCCATGGCGAG		
683[2]		CATAATGGGTCCATGGCGAG		
EaRproS-F[2]	*proS*	TACAACAAGTGAGGGCAAAATG	62	40
EaRproS-R[2]		CGATACCTTTATTGCCACCTTC		
EaATP-F[2]	*pstB*	ATGGTTGATAATACTGCCGCTCAGAC	60	40
EaATP-R[2]		CGTATCGGTATCGCTGTACTCTATC		

[1]Primers used for Sequencing; [2]primers used for Real-Time qRT-PCR; *lower case characters are nonbinding overhangs, underlined characters present restriction sites for EcoRI (GAATTC) and BamHI (GGATCC)

C.3. Primers used in Y2H assay

Table 5.4.: Sequences of primers used in Y2H assay.

Primer	Target gene	Sequence 5' → 3' direction*	T_A [°C]	Cycles
Y2H-Avr-F	*avrRpt2$_{EA}$*	ccgGAATTCGTGAAAGTCAGTCATC	55	35
Y2H-Avr-R		cgcGGATCCGCTAATTTTCACTGTA		
Y2H-Avr/eu-F	*avrRpt2$_{EA}$-eu*	ccgGAATTCATGAAGGTTAGCCAC	52	35
Y2H-Avr/eu-R		cgcGGATCCGTTAGTTCTCGCTGTACTT		
Y2H-FB_MR5-F	*FB_MR5*	cccGAATTCATGGGGGGAGAGGCTTT	-	35
Y2H-FB_MR5-R		cccGGATCCATCAGCTTCAAATCATCTTCC		
Y2H-Rin4-F	*RIN4-1*	ccgGAATTCATGGCACAACGTTCACATGTACC	58	35
Y2H-Rin4-R		cgcGGATCCCGATTCAATCTCATTTTCTGCTCC		
680	*avrRpt2$_{EA}$*	GAGCACCAGCCTCGTCAATC	67	30
682		CATAATGGGTCCATGGCGAG		
683		CATAATGGGTCCATGGCGAC		
Rin4d-R	*RIN4-1*	CAGCGCAGAAGTTCTGGTGAACAT	65 (chr5) 67 (chr10)	30
3RACE_Rin4_C5		GCGCAGAAATTCTGGTGAATCAGC		
3RACE_Rin4_C10		CGACGAAAGGTACCTCGTCTTTTACT		
cand1-1r	*FB_MR5*	TCAGGGCACTTAGAGAACTTGAC	60	30
cand1-1f		CATATCCGGGTCTTCACCAC		

*Lower case characters are nonbinding overhangs, underlined characters present restriction sites for EcoRI (GAATTC) and BamHI (GGATCC)

C.4. Primers for RACE-PCR of *FB_MR5* and *RIN4*

Table 5.5.: Sequences of primers used for RACE-PCR of *FB_MR5* and *RIN4*.

Primer	Gene	Sequence 5' → 3' direction	UTR
5'RACE_FBMR5-188	*FB_MR5*	GTTCTTAGCCGTCAGTTGCC	5'
5'RACE_FBMR5-438		CTTGTAGTCAACGCCCCAGT	
cand1-3f		CTTTCCGACTCCAGCCATAC	
3'RACE_FB_MR5-1		TCATTGTGGGAGTTGGAGTG	3'
3'RACE_FB_MR5-2		CAGGAAAAGGACGCTACTGG	
cand1-1r		TCAGGGCACTTAGAGAACTTGAC	
cand1-2r		GTTTGTGGTGAGTGGTGGTG	
5'RACE_R outer Chr5 (Rin4_Chr5_R)	*RIN4*	TAGTCGTGTAGTTGGATCGTCTCC	5'
5'RACE_R outer Chr10		CACTTGTAGTTGGATCGTCTCC	
RACE_R inner		TTTTCCTGGTTCCGGTCTAA	
3'RACEo_Rin4_C10		CAGCGCAGAAGTTCTGGTGAACAT	3'
3'RACEo_Rin4_C5		GCGCAGAAATTCTGGTGAATCAGC	
3RACEi_Rin4		GGACTCCTTCTCATCCGTCTT	

C.5. Primers used in gene expression analysis with the BioMark™ HD system

Table 5.6.: Sequences of primers used in the gene expression analysis with the BioMark™ HD system.

Gene/Primer		Sequence 5' → 3' direction[1]	Size[2]
Ubiquitin	F	ATCACCCTAGAGGTCGAGTCTTCC	76
	R	GCGGAATGCCTTCCTTGTCTTG	
GAPDH	F	GGTTGCTAGGGTCGCTCTTCAG	73
	R	GTCCGTGGTGATGAAGGGATCG	
EF1-α	F	TCAAGCGTGGGTACGTTGCTTC	77
	R	GATGACCTGAGCGATAAAGTTGGC	
Rubisco	F	TCCCTGTCACCAGAAAGAGCAAC	80
	R	AGGCCACACCTGCATACATTGC	
RNA-Polymerase	F	AGTTTGCTGAAGCTCCGTGTGC	76
	R	CGGAAGCGATTTGGAGGGATCAAG	
RNA-Polymerase	F	ATATGCCACCCCGTTCTCTACT	286
	R	CACGTTCCATTTGTCCAAACTT	
MDP0000047589	F	TCGGTGGAGATTCRGCTA	151
	R	GCAGGCTCAWACCARTGYGAC	
MDP0000095637	F	TGGAGACGCAGATARAACARC	147
	R	GAATGAGRCCGCAGGGTTC	
MDP0000119630	F	TTCGTTTGTGCTGCTGGATA	130
	R	CAACCCTTTCAGTTCCGATG	
MDP0000120176	F	AGAAGGCAAGTGAACAAAGCA	159
	R	TAGCCCACCCTyAGCAATAG	
MDP0000126761	F	CAACRCCAAGCTAGTCTGC	123
	R	TCTGGGTCKGTCATCACCAA	
MDP0000131100	F	RGGTGGTGAGGATGGAGATMGA	142
	R	ATCAGCRGGAAARAAATGG	
MDP0000136037	F	GAYAGCAAGGAATCGGAGAG	140
	R	AACCCAAGCGTCAGCTCYAA	
MDP0000139165	F	CCCACCAGATGGAGCCTAT	141
	R	AAGTTGCCACATACCCCTAGC	
MDP0000151003	F	ATCCCTGCGAAAACACARGT	132
	R	CAAACTCCTGACCYACGAAACC	
MDP0000159251	F	YGAGAAGGTGAAGGGGTTTG	155
	R	GGCGTGAAAGMACYGGCTTA	
MDP0000159572	F	AGCAACCTAACCGACGCTAA	147
	R	GGTATTGAAGGGGACGCATA	
MDP0000163314	F	SACCACCATCCYCCTCAAG	178
	R	CCTGACSCTACAGWCACAGAAA	
MDP0000165381	F	AGGAGCTTTGTAGCGGTTCA	153
	R	TGTGGTTTCCTTCACAACCA	
MDP0000166138	F	CRTCCTGGTCWCCGAAAAAC	195
	R	ACCACCTTCAACCTCCCTTT	
MDP0000174537	F	CCaAAACAYCACAAGCAGTC	169
	R	TATCAACAGGACCCCCATTT	
MDP0000180043	F	TGGGACWACATTTGGGAAGC	113
	R	TGGGATCAAACCACAAGTGA	
MDP0000180902	F	TGGAGCACAAAGTGGAACAA	155
	R	TGTATGGCTCGCAAATCTCA	

Table 5.6.: (continued)

Gene/Primer		Sequence 5' → 3' direction[1]	Size[2]
MDP0000181339	F	CCTGTTGGACATCAAGTTCG	146
	R	ATTGGAGACGGTTTTCTTGG	
MDP0000196394	F	CGCCGATCAAATGGTTCT	134
	R	TCTCTTCCCCTTCCTTCTCC	
MDP0000197472	F	AGGCTCAAGCATTTKGGTGT	134
	R	AGATTTTGGGGGTGAAGTGA	
MDP0000204381	F	TGTTGTCACGGGGTCYAT	163
	R	CGGCAAAAGGGTAAACGAC	
MDP0000204699	F	TGGYTRTACGGATGCTAGTGC	146
	R	CCTCTGAGTYGTAAACCAAAACATC	
MDP0000204794	F	AAAGCTGTGGCTGACGTTCT	144
	R	CTCCCTTCCCTTGATTGGTT	
MDP0000205617	F	CATGCCCGTAAGAAACAAGAA	147
	R	TTCACTTGCGAAATGGTACG	
MDP0000206461	F	GGTTGGCWGCGTCATAGAG	148
	R	TGTGGCTAGAAAGCGAGTCA	
MDP0000211981	F	GCTTGGGAGGAKYACTTTAAC	160
	R	CAGTCCCGAAYGTGAGAGTT	
MDP0000212178	F	GGAATCATCGRCTTTTCG	171
	R	TTTCTTTCTCCTCCTCCCATC	
MDP0000219522	F	CTTCCAATATCCCTTGCTGCT	132
	R	TCATGGTGCTGAACCTGCT	
MDP0000219684	F	GSKCRGATTTCGCATTTG	160
	R	CCGCTTGTCCAGGTTACTGT	
MDP0000225509	F	YAATYAGGCACAAGAACATCA	139
	R	TCCCAATCTGCTCCTCCYT	
MDP0000232616	F	TTTTGGGAATCCAACAGGAC	154
	R	GGCAGATGCAGCATAATTGA	
MDP0000233546	F	GCGTTGCCCACTTATCAAAC	102
	R	CGTCCAAGACCGATTCCAT	
MDP0000236390	F	GCCTCAATCCTCCTCACACT	157
	R	GAGACCGAYTGGGAAYACA	
MDP0000236723	F	ACCCGCTAAAYATCACCACATC	153
	R	KCTTGGGAGCAAAAAYACCR	
MDP0000250070	F	ATCAGCTTCTTGCCGAGTTC	115
	R	CATGGGGGCAGAACAATTA	
MDP0000262141	F	ACTGGTGGTCACGCTCTCTT	152
	R	TCCTCAAAGTAGTGCCTCTTCC	
MDP0000264060	F	ATCTGCCTCCGCACTCTATC	150
	R	CAGCTTCGTCCCCTTGAA	
MDP0000264668	F	TGGTGATGGTCGTGGAGTT	116
	R	TCTCATCCCTCCTTGCTCTC	
MDP0000265729	F	CAGCAGGCSMTGATGAAA	175
	R	CGAAAAYGGAGAGAGACGRAG	
MDP0000265874	F	GATTCCAGGAACTGGTGGTG	145
	R	GTCCATCATCCCCCTTTCTT	
MDP0000268523	F	GCACCCCAGCTGAAGTAGAG	150
	R	CGTAGGGCCAGTTCATTGTT	
MDP0000272542	F	ATGGACGGAGGATTRATGG	172
	R	TGCTGCTGGGCACTTTCYA	
MDP0000277718	F	AAAGGGTCRTCGGCTCTTG	143
	R	GCCATCCTCATCGTCTCAAT	

Table 5.6.: (continued)

Gene/Primer		Sequence 5' → 3' direction[1]	Size[2]
MDP0000286136	F	GCCAAATACGCAAGATAGCC	162
	R	TCTCCACCAACACCTTAGTCG	
MDP0000289300	F	GGAAAGCCYAGAGATGC	160
	R	GGGAAATCAAGCAGCACAAT	
MDP0000294096	F	GGCAGTCATCTCRGMACCT	102
	R	MRYACTCCACCAATCAAGTAACG	
MDP0000296339	F	CACCGAAGAAAGCGAAGAAT	170
	R	TGGCAAGGAGTGAGTTGATG	
MDP0000307705	F	AAGTTCCACCAGTCCCACAG	174
	R	GCTCCGCAAAGAAAAAGTTG	
MDP0000309976	F	TGTTAGTGGTGTCGCTTGGA	143
	R	ATTGGTTGCTCGGGATTGT	
MDP0000311359	F	TATGCAAGRATTGGGAAAGC	157
	R	TCCTCAGCTCCGTATTGTCC	
MDP0000316497	F	GAYGACAGCMGCATAAAAG	112
	R	ATTTCCYCCAATCCTCAGYT	
MDP0000317974	F	GCTGTGCTTRTCACTCTGCT	185
	R	ATGAAGAGAACGCCMARGAA	
MDP0000320910	F	CGGGACTGGAACACCTTCT	147
	R	GAAYGGGCAGTTSTGGWTGT	
MDP0000324831	F	TCCAAAACTAAGCCCCTCCT	150
	R	TCCAGACTCCTCCTTCATCG	
MDP0000327191	F	ATGGCGAAACCAAGTTCATC	151
	R	CTTGGGGTGGTAAGTCTCCA	
MDP0000343634	F	CAGGAGATCCAGGAAAAGGAC	112
	R	CAGTTCTGAAACAAGCCACAAC	
MDP0000353793	F	ATTATTACCCCACGCcCATT	100
	R	ATTACGCTGACCGaGAGATGTT	
MDP0000364885	F	GGGATTAGTGGTGGAGTTTA	147
	R	GGCACGTTACATGTCTTCATT	
MDP0000404331	F	TTGGTGATGGAAGTGGAGATT	121
	R	GCYTTGATGAGTAAYGGGTTTT	
MDP0000440654	F	TGAAGGCATGCCAGAAAGTT	111
	R	CTCCAGTTGGATTCGTCGTAA	
MDP0000551952	F	CCCTCTTTGGATTTGCCTAA	163
	R	ACGCTGGATTTACGGATTGT	
MDP0000597996	F	GGGGGTTCCAATCGTAATG	158
	R	CACGAAAGTGCATACCATGATT	
MDP0000609876	F	CARCAGATGACACCgCAATC	170
	R	CTSCCTCCAGCATTATTTCC	
MDP0000609966	F	MTTCTTCCCGTTCCATCGT	171
	R	TTTCTRTWCTGGTCGTAGAGGGATG	
MDP0000612660	F	CTCAACCTCAAGCCAATGTG	154
	R	CAACTTCGASATCAGCAAGG	
MDP0000617684	F	CCACCGAGGGCTAATCAKC	133
	R	ATGGAARTGGTCTCYGGGCTR	
MDP0000628976	F	TTCSGACGAAGAGAAAATCC	152
	R	ACACAGCGTCCTCCATATCTC	
MDP0000644109	F	TCCTTCAACTTCTGCCCCKA	137
	R	CGCTCCKRATTTCCTTCAAC	
MDP0000668657	F	CATTTGGGAATGCTGTGAACT	136
	R	GTGCAGCCAATCTTCATTAGG	

Table 5.6.: (continued)

Gene/Primer		Sequence 5' → 3' direction[1]	Size[2]
MDP0000680997	F	GTGCCAAACAAAGATGATGC	178
	R	CGAATGRGACCGARTGAGTG	
MDP0000696168	F	TTCTYAATGACACTGGGRTTCA	144
	R	GGAGGACGAGTGCTTGATTT	
MDP0000711911	F	CCTGTCCTGAACCCATCTGT	160
	R	GGGCGTCAGCAAGAAAGTAA	
MDP0000737128	F	AGRAGGGCRAGGTGGAAGAC	100
	R	GAGCCTGAAGRGCATCATT	
MDP0000750217	F	GGGCAGGATGAGTTTATTGG	131
	R	CGAGCCTTAGCGAGTCTATTG	
MDP0000750789	F	TTAAATTGCCTCCACACAAGC	163
	R	AATGGCTTTTCTTCCACAACC	
MDP0000782642	F	GAGCAGTTCCACCAGCAAG	108
	R	CAACAGACACGGGCAAGTT	
MDP0000784187	F	CAAAGTCCACACCCCAATCT	144
	R	AGGCTTSCCTCCGTTTTCT	
MDP0000858763	F	TTCCACTTTAYGAYGACAGCA	196
	R	TAYTTTCCGTTCCCCATTTG	
MDP0000868044	F	GGCACACTTGGTCACGATTA	162
	R	GCCTGRGAGCTTCTCATTTA	
MDP0000874252	F	GTCGTTTCGGTCCTCAAAAA	162
	R	AATCGGCAAATAATCGTCSA	
MDP0000891117	F	TCTRGGATAYCTYGGTGAGC	154
	R	GCCGAGGAGCTTCAWCTGT	
MDP0000919962	F	GGAGCAAGAGACGATCAAGG	117
	R	TTGAGAACACGCATGAGGAC	
MDP0000921319	F	AAGGGCTTGYTGAACTTGAA	130
	R	CCAGGAGGAGGAYTCTTGC	
MDP0000929055	F	CAGCTCCTCTGCAATTTATGG	151
	R	GGCGGTCGTCGTAACAATA	
MDP0000937986	F	CACTCCTCTGCCTTCTTTGG	153
	R	TCAGCGACATTGGTTTTCTG	
MDP0000940742	F	CACCACCAACTTCTTGCTCA	109
	R	GCTCAAACCAGTGCGACA	
MDP0000944210	F	TGAAWATTGGGACTACTGYTCA	114
	R	TGTCCTGRTGGGCAAGAT	
FB_Mr5q1	F	TTTATGGAGAGTGCTCCTTGC	186
	R	AGCGAATCAAGGTTCTCTGG	
FB_Mr5q2	F	TCAATCACATCTCRRCAATR	158
	R	RTCACGAAAAGGGGACWGC	
EH034548	F	TTGCTCCAAAGCGATCAAG	164
	R	TCTAATCTCCCCCTCGGTTC	

[1]Wobbles: M: A or C, R: A or G, W: A or T, S: G or C, Y: C or T, K: G or T; [2]Size of the PCR fragment

D. RT-PCR of the complemented *E. amylovora* strains

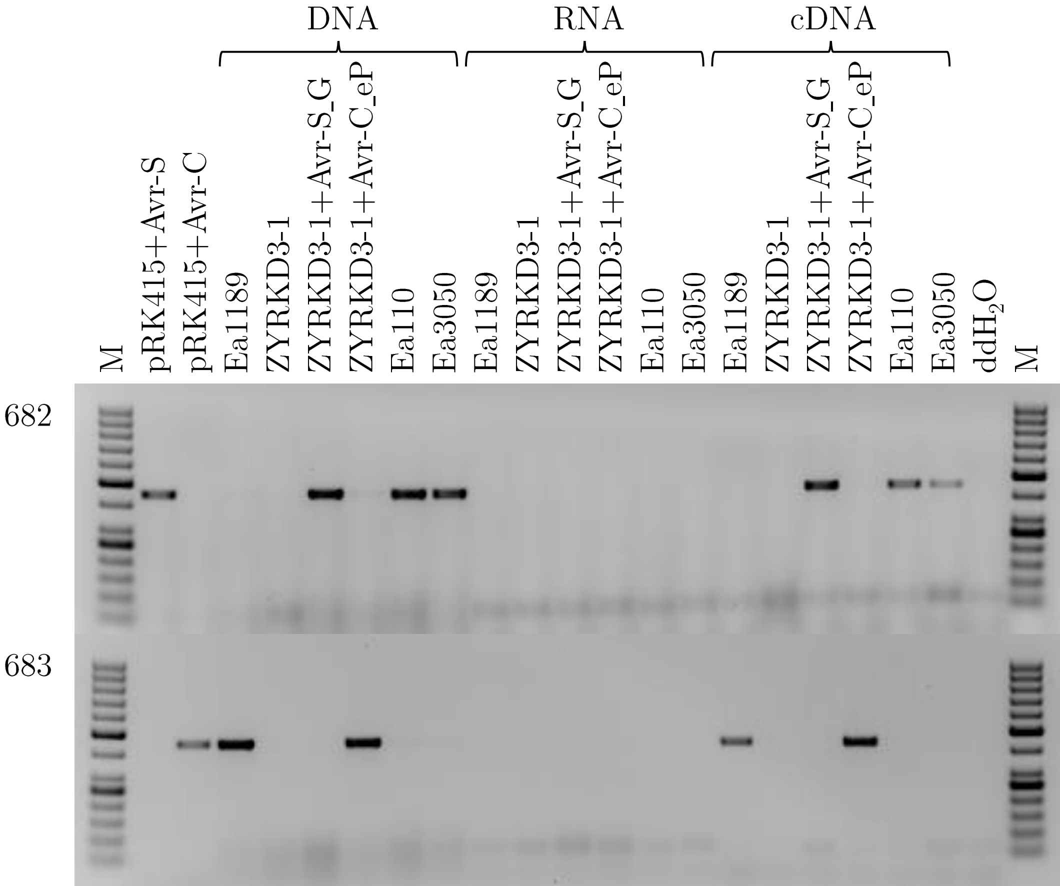

Figure 5.2.: PCR with the specific SNP primers 680/682 (S-allele) and 680/683 (C-allele) to control transcription of the *avrRpt2*$_{EA}$ gene: plasmid pRK415 with the C- and S-allele of the *avrRpt2*$_{EA}$ gene as positive control; ddH$_2$O as negative control; DNA, RNA and cDNA of the wild type strains Ea1189 (carrying the *avrRpt2*$_{EA}$ C-allele), Ea110 and Ea3050 (both carrying the *avrRpt2*$_{EA}$ S-allele), the *avrRpt2*$_{EA}$ deletion mutant strain ZYRKD3-1 and the complemented mutant strains ZYRKD3-1+Avr-S_G and ZYRKD3-1+Avr-C_eP; M molecular weight marker (GeneRuler™50 bp, Fermentas/Thermo Fisher Scientific, Schwerte, DE).

E. Analysis of Real-Time q-RT data of the complemented *E. amylovora* strains

Table 5.7.: One-way ANOVA of the Real-Time qRT-PCR data of the complemented *E. amylovora* strains performed with qbase+.

Comparison		682				683			
		Ratio[1]	95% ci low[2]	95% ci high[2]	Sig[3]	Ratio	95% ci low	95% ci high	Sig
Ea222+Avr-S_LacP	Ea222+Avr-S_eP	1.52	0.61	3.75	No	0.84	0.40	1.76	No
Ea222+Avr-S_LacP	Ea3049	4.88	1.97	12.09	Yes	NaN	NaN	NaN	
Ea222+Avr-S_LacP	Ea3049+Avr-C_LacP	0.50	0.20	1.23	No	0.61	0.29	1.28	No
Ea222+Avr-S_LacP	Ea3049+Avr-C_eP	0.67	0.27	1.67	No	0.66	0.32	1.40	No
Ea222+Avr-S_LacP	ZYRKD3-1+Avr-S_G	0.50	0.18	1.38	No	NaN	NaN	NaN	
Ea222+Avr-S_eP	Ea3049	3.22	1.30	7.97	Yes	NaN	NaN	NaN	
Ea222+Avr-S_eP	Ea3049+Avr-C_LacP	0.33	0.13	0.81	Yes	0.73	0.35	1.54	No
Ea222+Avr-S_eP	Ea3049+Avr-C_eP	0.45	0.18	1.10	No	0.80	0.38	1.68	No
Ea222+Avr-S_eP	ZYRKD3-1+Avr-S_G	0.33	0.12	0.91	Yes	NaN	NaN	NaN	
Ea222	Ea222+Avr-S_LacP	0.03	0.01	0.10	Yes	0.08	0.03	0.18	Yes
Ea222	Ea222+Avr-S_eP	0.04	0.01	0.16	Yes	0.07	0.03	0.15	Yes
Ea222	Ea3049	0.14	0.04	0.51	Yes	NaN	NaN	NaN	
Ea222	Ea3049+Avr-C_LacP	0.01	0.00	0.05	Yes	0.05	0.02	0.11	Yes
Ea222	Ea3049+Avr-C_eP	0.02	0.01	0.07	Yes	0.05	0.02	0.12	Yes
Ea222	ZYRKD3-1+Avr-S_G	0.01	0.00	0.06	Yes	NaN	NaN	NaN	
Ea3049+Avr-C_LacP	Ea3049+222_eP	1.36	0.55	3.37	No	1.09	0.52	2.30	No
Ea3049+Avr-C_LacP	ZYRKD3-1+Avr-S_G	1.01	0.37	2.79	No	NaN	NaN	NaN	
Ea3049+Avr-C_eP	ZYRKD3-1+Avr-S_G	0.74	0.27	2.05	No	NaN	NaN	NaN	
Ea3049	Ea3049+222_LacP	0.10	0.04	0.25	Yes	NaN	NaN	NaN	
Ea3049	Ea3049+222_eP	0.14	0.06	0.34	Yes	NaN	NaN	NaN	
Ea3049	ZYRKD3-1+Avr-S_G	0.10	0.04	0.28	Yes	NaN	NaN	NaN	

[1]Fold change between groups, [2]lower and upper value of the 95 % confidence interval of the ratio, [3]Significance, indication if two groups are statistically different ($p < 0.05$); NaN–Not a Number.

F. Sequence of the *avrRpt2*$_{EA}$-eu gene

Nucleotide sequence of the *avrRpt2*$_{EA}$ gene with an optimised reading frame for eucaryotic translation:

```
1    ATGAAGGTTA GCCACTTGAC CAGCCCAGCT CCAGTTGTTA TTGAGCACCA
51   GCCAAGACAG AGCGAGAAGG TTAGCAGAGA TGGAGATGTT ATTAAGCCTT
101  GGAGCCAGTT GCCAGCAGCT GCTCCTAGCT TCGGAGGATG CTTCGGAAAG
151  AGCAAGAAGA GCAGAGGATA CGATAGCGGA AGCAGCAGCG GAAGCAGAAG
201  CAACGCTGGA TTCAGATTGA ACCACGTTCC ATACGTTAGC CAGCAGAACG
251  AGAGAATGGG ATGCTGGTAC GCTTGCACCA GAATGTTGGG ACACAGCATT
301  AGCAGCGGAC CAAGATTGGG ATTGCCAGAG TTGTACGATA GCAGCGGACC
351  ACAGGGATTG CAGCAGAGAG AGGATGTTTT GAGATTGATG AGAAACGAGA
401  ACTTGGCTGA GGTTAGCTTG CCAGAGAGCA GACAGTTCAG CGCTAACGAG
451  TTGGGAAACT TGTTGTGCAG ACACGGACCA ATTATGTTCG GATGGCAGAC
501  CCCTGCTGGA AGCTGGCACA TGAGCGTTTT GACCGGAATT GATAAGCCAA
551  ACGATGCTAT TATTTTCCAC GATCCACAGA GAGGACCAGA TTTGACCATG
601  CCATTGGATA GCTTCAACCA GAGATTGGCT TGGAGAGTTC CACACGCTAT
651  GTTGTACAGC GAG
```

G. Alignment of the sequences received by 5' RACE-PCR of *RIN4* from Mr5

The alignment contains partly the two contigs including the *RIN4* gene from *Malus ×domestica* cv. 'Golden Delicious' from chromosome 5 and 10 (MDC017238.86 and MDC010910.201), the CDS of two protein predictions similar to *RIN4* from the database for Rosaceae (http://www.rosaceae.org; MDP0000252761 and MDP0000290323), the two gene sequences of *RIN4* from 'Golden Delicious' published in the universal protein database (http://www.uniprot.org) *RIN4-1* on chromosome 10 (D2D0J1) and *RIN4-2* on chromosome 5 (D2D0J2) and the sequences received from 5' RACE-PCR of the *RIN4* gene of Mr5 from chromosome 5 (Sequence-1 chr5, Sequence-2 chr5) and chromosome 10 (Sequence-3 chr10, Sequence-4 chr10, Sequence-5 chr10, Sequence-6 chr10). For reasons of clarity the sequences are not shown in their full length, the break is marked by a zig-zag line.

```
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         5         15         25         35         45         55
MDC017238.86         AATTAGAAAA CAAGTAATGA AAAAATTGAA TTATACAGGG GATAAATTAA TTAATAAAAA
MDP0000252761        ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2               ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5      ---------- ---------- ---------- ---------- ---------- ----------
Sequence-2 chr5      ---------- ---------- ---------- ---------- ---------- ----------
MDC010910.201        ------AAAT GTAGCAATAT ATAATTTGAA TTATACAAGG AATAA----- -TA--AAAAA
MDP0000290323        ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1               ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10     ---------- ---------- -------TCG GATGGCTCGA GTTTTTCAGC AAGATCGCGG
Sequence-4 chr10     ---------- ---------- ---------- ---------- ---------- ----------
Sequence-5 chr10     ---------- ---------- ---------- ---------- ---------- ----------
Sequence-6 chr10     ---------- ---------- ---------- ---------- ---------- ----------

                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        65         75         85         95        105        115
MDC017238.86         AAGAGCATAT TATCAACCAA TAGCATT--- ---------- -----ATCGC AAATAGACGG
MDP0000252761        ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2               ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5      ---------- ---------- ---------- ---------- ---------- ----------
Sequence-2 chr5      ---------- ---------- ---------- ---------- ---------- ----------
MDC010910.201        GAGCATATAT TATCAACCAA CAGCAATTCA CGTAAAATTG AAGAAATGGC AAATAGGGAC
MDP0000290323        ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1               ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10     ATCCGAACAC TGCGTTTGCT GGCTTTGATG AAAAAAATTG AAGAAATGGC AACTAGAG--
Sequence-4 chr10     ---------- ---------- ---------- ---------- ---------- ----------
Sequence-5 chr10     ---------- ---------- ---------- ---------- ---------- ----------
Sequence-6 chr10     ---------- ---------- ---------- ---------- ---------- ----------
```

```
                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        125        135        145        155        165        175
MDC017238.86      AGAGTCTTAA CTCGCTGAGG GAGATGTTTG AAC------- ---AGAGAAG CAG-GACTAT
MDP0000252761     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5   ---------- ---------- ---------- ---------- ---------- ----------
Sequence-2 chr5   ---------- ---------- -----GTTTG AACTTTGAAC AGAGAAGAAG CAGAGACTTT
MDC010910.201     GGAGTCTTAA CTCGCTGAGA GAGATGTTTG AACTTTGAAC AGAGAAGAAG CAGAGACTTT
MDP0000290323     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10  --------AA CTCGCTGAGA GAGATGTTTG AACTTTGAAC AGAGAAGAAG CAGAGACTTT
Sequence-4 chr10  --------AA CTCGCTGAGA GAGATGTTTG AACTTTGAAC AGAGAAGAAG CAGAGACTTT
Sequence-5 chr10  ---------- ---------- ---------- ---------- ---------- ----------
Sequence-6 chr10  ---------- ---------- ---------- ---------- ---------- ----------

                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        185        195        205        215        225        235
MDC017238.86      TACATAGAGA GAAAGGTCAA ACATGACGGG TAGGGTCCGT CAATGGTTGA TAAACCCCTC
MDP0000252761     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5   ---------- ---------- ---------- ---------- ---------- ----------
Sequence-2 chr5   CACAGAGAGA GAATGGTCAA ACATGACGGG TAGCGTCCGT CAATGG---- ----------
MDC010910.201     CACAGAGAGA GAATGGTCAA ACATGACGGG TAGCGTCCGT CAATGGTTGA TAAAACCCTC
MDP0000290323     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10  CACAGAGAGA GAATGGTCAA ACATGACGGG TAGCGTCCGT CAATGGTTGA TAAAACCCTC
Sequence-4 chr10  CACAGAGAGA GAATGGTCAA ACATGACGGG TAGCGTCCGT CAATGGTTGA TAAAACCCTC
Sequence-5 chr10  ---------- ---------- ---------- ---------- ---------- ----------
Sequence-6 chr10  ---------- ---------- ---------- ---------- ---------- ----------

                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        245        255        265        275        285        295
MDC017238.86      TATAACTCTC TCTCCTTTCT CTCTCTACAC CCTCTTCCTC TCTCTCCCTC CCTCTCTCTC
MDP0000252761     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5   ---------- ---------- ---------- -CTCTTCCTC TCTCTCCCTC CCTCTCTCTC
Sequence-2 chr5   ---------- ---------- ---------- ---------- ---------- ----------
MDC010910.201     TAGAAATATT T--CCTTTCT CTTTCTACAC CCTCATCCTC TCTCTCTCTC ----------
MDP0000290323     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10  TAGAAATAGT T--CCTTTCT CTTTCTACAC CCACATCCTC TCTCTCTC-- ----------
Sequence-4 chr10  TAGAAATAGT T--CCTTTCT CTTTCTACAC CCACATCCTC TCTCTCTC-- ----------
Sequence-5 chr10  ---------- ---------- ----CTACAC CCTCATCCTC TCTCTCTC-- ----------
Sequence-6 chr10  ---------- ---------- ---------- ---------- ---------C ----------

                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        305        315        325        335        345        355
MDC017238.86      TCTAAAATAA TTGCTCTCTC TCTTGGTGGT TCGTAGAAGG GTTTCGTGGA GGAGCTACTG
MDP0000252761     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5   TCTAAAATAA TTGCTCTCTC TCTTGGTGGT TCGTAGAAGG GTTTCGTGGA GGAGCTACTG
Sequence-2 chr5   ---------- ---------- ---------- ---------- GTTTCGTGGA GGAGCTTTTG
MDC010910.201     ----AAATAA TTCCTCTCTC TCTTGGTGCT TCGTTGAAGG GTTTCGTGGA GGAGCTTTTG
MDP0000290323     ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1            ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10  ----TAATAA TTCCTCTCTC TCTTGGTGCT TCGTTGAAGG GTTTCGTGGA GGAGCTTTTG
Sequence-4 chr10  ----TAATAA TTCCTCTCTC TCTTGGTGCT TCGTTGAAGG GTTTCGTGGA GGAGCTTTTG
Sequence-5 chr10  ----TAATAA TTCCTCTCTC TCTTGGTGCT TCGTTGAAGG GTTTCGTGGA GGAGCTTTTG
Sequence-6 chr10  ----TAATAA TTCCTCTCTC TCTTGGTGCT TCGTTGAAGG GTTTCGTGGA GGAGCTTTTG
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                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           365        375        385        395        405        415
MDC017238.86          GGGTTGC-GT GTTTGTGGTG GGT---GGTC CAGGAGATTC TACTCTGACA TTACTTTCCT
MDP0000252761         ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2                ---------- ---------- ---------- ---------- ---------- ----------
Sequence-1 chr5       GGGTTGC-GT GTTTGTGGTG GGT---GGTC CAGGAGATTC TACTCTGACA TTACTTTCCT
Sequence-2 chr5       GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG~TTC TACTCTGACA TTAGTTTCCT
MDC010910.201         GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG-TTA TACTCTGACA TTAATTTCCT
MDP0000290323         ---------- ---------- ---------- ---------- ---------- ----------
RIN4-1                ---------- ---------- ---------- ---------- ---------- ----------
Sequence-3 chr10      GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG~TTC TACTCTGACA TTAGTTTCCT
Sequence-4 chr10      GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG~TTC TACTCTGACA TTAATTTCCT
Sequence-5 chr10      GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG~TTC TACTCTGACA TTAATTTCCT
Sequence-6 chr10      GGGTTGCCAT ATTCGTGGAG GGTTTTGGTC CAGGAG~TTC TACTCTGACA TTAATTTCCT

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           425        435        445        455        465        475
MDC017238.86          GGGTTTTTTC TAAATCTGGT GGACAGCAAA ATGGCAGTAA GTTCACAACT TTCTCTGGGT
MDP0000252761         ---------- ---------- ---------- ---------- ---------- ----------
RIN4-2                ---------- ---------- ---------- ATGGCA---- ---------- ----------
Sequence-1 chr5       GGGTTTTTTC TAAATCTGGT GGACAGCAAA ATGGCA---- ---------- ----------
Sequence-2 chr5       GGGTTTTTTC TAAATCTGGT GGACAGCAAA ATGGCA---- ---------- ----------
MDC010910.201         GGGGTTTTTC TAAATCTGGT GGACAGAAAA ATGGCAGTGA GTTCACAA~T CTTTWTGGG~
MDP0000290323         ---------- ---------- ---------- ATGGCA---- ---------- ----------
RIN4-1                ---------- ---------- ---------- ATGGCA---- ---------- ----------
Sequence-3 chr10      GGGGTTTTTC TAAATCTGGT GGACAGAAAA ATGGCA---- ---------- ----------
Sequence-4 chr10      TGGGTTTTTC TAAATCTGGT GGACAGAAAA ATGGCA---- ---------- ----------
Sequence-5 chr10      TGGGTTTTTC TAAATCTGGT GGACAGAAAA ATGGCA---- ---------- ----------
Sequence-6 chr10      TGGGTTTTTC TAAATCTGGT GGACAGAAAA ATGGCA---- ---------- ----------

                      ....|....| /\/\/\/\/\ ....|....| ....|....| ....|....| ....|....|
                            485                  2605       2615       2625       2635
MDC017238.86          TTTTTTGTGC /\/\/\/\/\ TTTCTTAATG TGGCAGCAAC GTTCACATGT ACCAAAGTTT
MDP0000252761         ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
RIN4-2                ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-1 chr5       ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-2 chr5       ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
MDC010910.201         TTTTTTGTGC /\/\/\/\/\ TTTCTTATTG TGGCAGCAAC GTTCACATGT ACCAAAGTTT
MDP0000290323         ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
RIN4-1                ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-3 chr10      ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-4 chr10      ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-5 chr10      ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT
Sequence-6 chr10      ---------- /\/\/\/\/\ ---------- ------CAAC GTTCACATGT ACCAAAGTTT

           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            2645       2655       2665       2675       2685       2695
MDC017238.86          GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
MDP0000252761         GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
RIN4-2                GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
Sequence-1 chr5       GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
Sequence-2 chr5       GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCC-GTAAG
MDC010910.201         GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
MDP0000290323         GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
RIN4-1                GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-3 chr10      GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-4 chr10      GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-5 chr10      GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-6 chr10      GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
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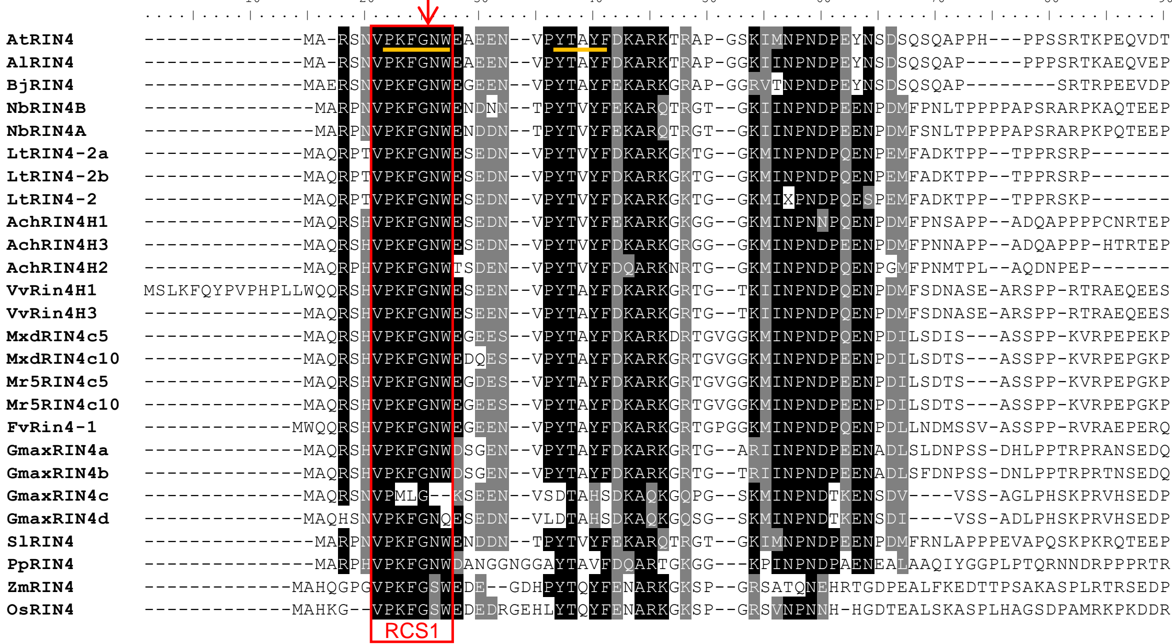
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         2645       2655       2665       2675       2685       2695
MDC017238.86        GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
MDP0000252761       GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
RIN4-2              GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
Sequence-1 chr5     GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCCCGTAAG
Sequence-2 chr5     GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACAGCCT ATTTTGATAA GGCC-GTAAG
MDC010910.201       GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
MDP0000290323       GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
RIN4-1              GGCAATTGGG AAGACCAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-3 chr10    GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-4 chr10    GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-5 chr10    GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG
Sequence-6 chr10    GGCAATTGGG AAGGCGAAGA AAGTGTTCCT TACACTGCCT ATTTTGATAA GGCCCGTAAG

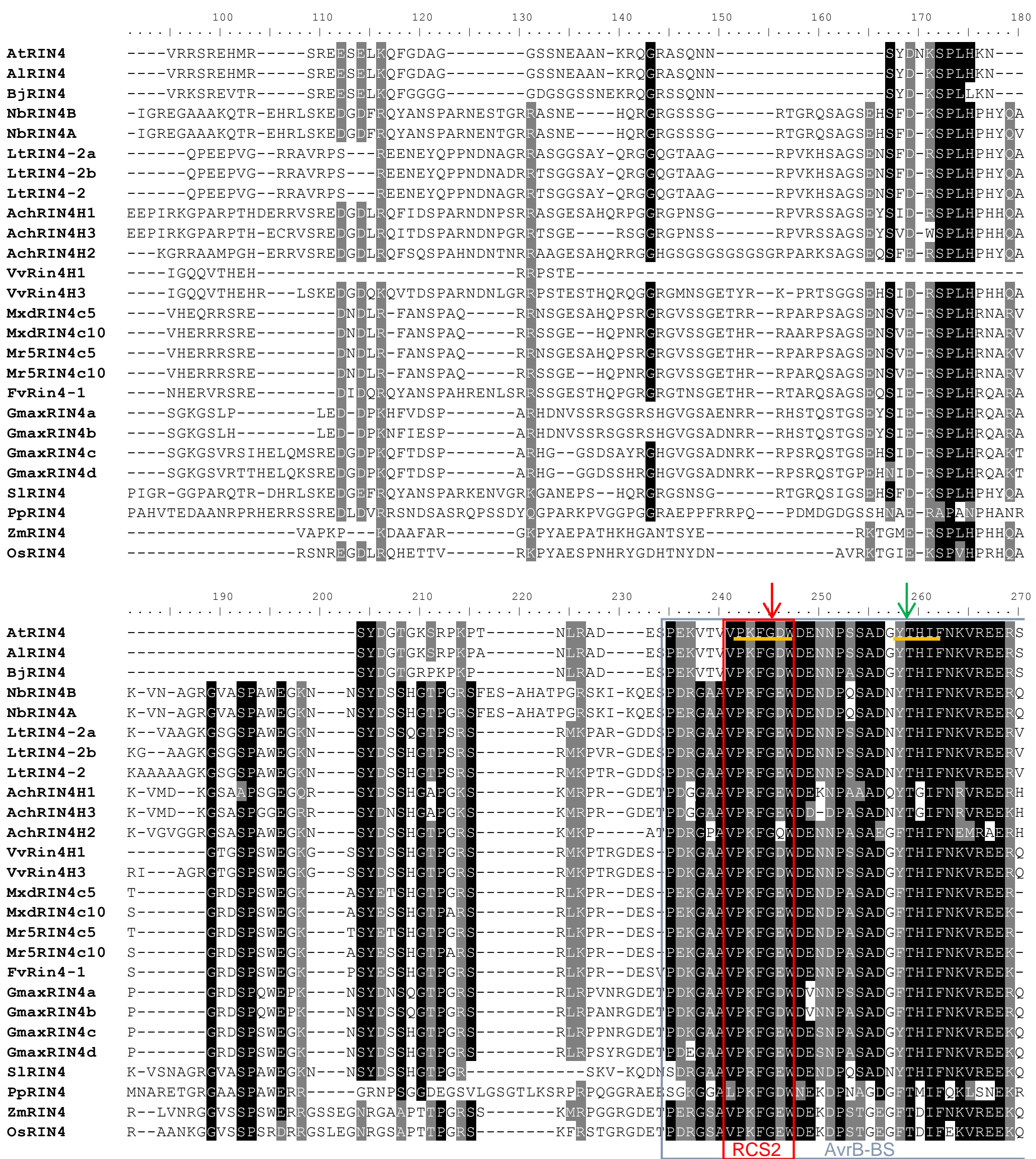
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         2705       2715       2725       2735       2745       2755
MDC017238.86        GATCGAACTG GTGTCGGGGG AAAGATGATT AATCCAAATG ACCCCCAAGA GAACCCGGAC
MDP0000252761       GATCGAACTG GTGTCGGGGG AAAGATGATT AATCCAAATG ACCCCCAAGA GAACCCGGAC
RIN4-2              GATCGAACTG GTGTCGGGGG AAAGATGATT AATCCAAATG ACCCCCAAGA GAACCCGGAC
Sequence-1 chr5     GATCGAACTG GTGTCGGGGG AAAGATGATT AATCCAAATG ACCCCCAAGA GAACCCGGAC
Sequence-2 chr5     GATCGAACTG GTGTCGGGGG AAAGATGATT AATCCAAATG ACCCCCAAGA GAACCCGGAC
MDC010910.201       GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCTAATG ACCCCGAAGA GAATCCAGAC
MDP0000290323       GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCTAATG ACCCCGAAGA GAATCCAGAC
RIN4-1              GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCTAATG ACCCCGAAGA GAATCCAGAC
Sequence-3 chr10    GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCAAATG ACCCCGAAAA GAATCCAGAC
Sequence-4 chr10    GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCAAATG ACCCCGAAGA GAATCCAGAC
Sequence-5 chr10    GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCAAATG ACCCCGAAGA GAATCCAGAC
Sequence-6 chr10    GGTCGAACTG GTGTTGGGGG AAAGATGATT AATCCAAATG ACCCCGAAGA GAATCCAGAC

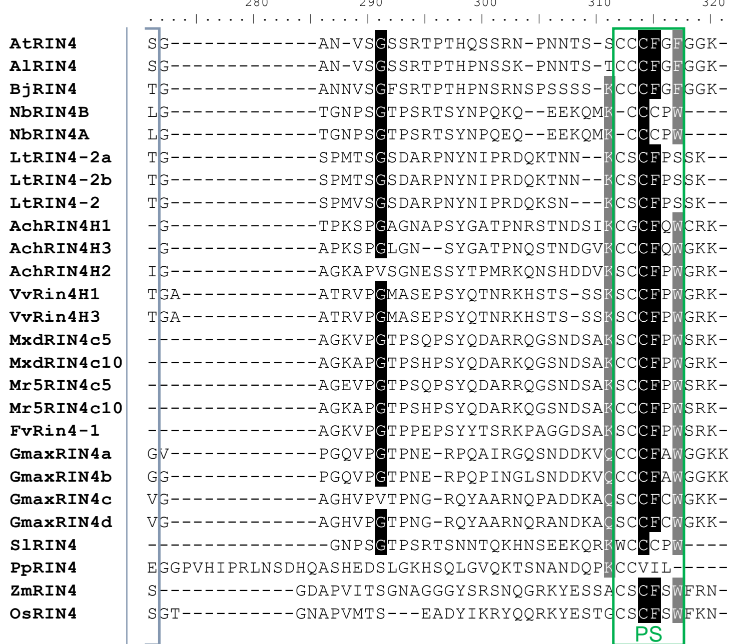
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         2765       2775       2785       2795       2805       2815
MDC017238.86        ATCCTTTCTG ACATATCTGC ATCTTCTCCT CCAAAAGTTA GACCAGAACC AGAAAAACCA
MDP0000252761       ATCCTTTCTG ACATATCTGC ATCTTCTCCT CCAAAAGTTA GACCAGAACC AGAAAAACCA
RIN4-2              ATCCTTTCTG ACATATCTGC ATCTTCTCCT CCAAAAGTTA GACCAGAACC AGAAAAACCA
Sequence-1 chr5     ATCCTTTCTG ACATATCTGC ATCTTCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
Sequence-2 chr5     ATCCTTTCTG ACATATCTGC ATCTTCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
MDC010910.201       ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAACCA
MDP0000290323       ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAACCA
RIN4-1              ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAACCA
Sequence-3 chr10    ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
Sequence-4 chr10    ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
Sequence-5 chr10    ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
Sequence-6 chr10    ATCCTCTCTG ACACATCTGC ATCATCTCCT CCAAAAGTTA GACCGGAACC AGGAAAA...
```

H. Protein sequence alignment of RIN4

Protein sequence alignment of 26 RIN4 homologues; Black background indicates identical amino-acid residues; grey background indicates similar amino acid residues; red boxes indicate cleavage sites RCS1 and RCS2 of AvRpt2 from *Pseudomonas syringae* pv. *tomato*, red arrows mark the exact cleavage site; the green box marks the S-palmitoylation side, the two conserved motifs of the C- and N-terminal NOI domains are underlined in orange, the C-terminal NOI domain contains also the AvrB and AvrRpm1 binding site marked with a blue box with the phosphorylation site T166 (green arrow); plant species: *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brassica juncea*, *Nicotiana benthamiana*, *Lactuca tatarica*, *Actinidia chinensis*, *Vitis vinifera*, *Malus* ×*domestica* cv. 'Golden Delicious' , *Malus* ×*robusta* 5, *Fragaria vesca*, *Glycine max*, *Solanum lycopersicum*, *Physcomitrella patens*, *Zea mays* and *Oryza sativa*; Alignment based on Sun et al. (2014)




AtRIN4
AlRIN4
BjRIN4
NbRIN4B
NbRIN4A
LtRIN4-2a
LtRIN4-2b
LtRIN4-2
AchRIN4H1
AchRIN4H3
AchRIN4H2
VvRin4H1
VvRin4H3
MxdRIN4c5
MxdRIN4c10
Mr5RIN4c5
Mr5RIN4c10
FvRin4-1
GmaxRIN4a
GmaxRIN4b
GmaxRIN4c
GmaxRIN4d
SlRIN4
PpRIN4
ZmRIN4
OsRIN4
RCS2
AvrB-BS



AtRIN4
AlRIN4
BjRIN4
NbRIN4B
NbRIN4A
LtRIN4-2a
LtRIN4-2b
LtRIN4-2
AchRIN4H1
AchRIN4H3
AchRIN4H2
VvRin4H1
VvRin4H3
MxdRIN4c5
MxdRIN4c10
Mr5RIN4c5
Mr5RIN4c10
FvRin4-1
GmaxRIN4a
GmaxRIN4b
GmaxRIN4c
GmaxRIN4d
SlRIN4
PpRIN4
ZmRIN4
OsRIN4
PS

I. Protein sequence identity matrix of RIN4

Table 5.8.: Protein sequence identity matrix of 26 RIN4 homologues; plant species: see caption of appendix H.

	AtRIN4	AlRIN4	BjRIN4	NbRIN4B	NbRIN4A	LtRIN4-2a	LtRIN4-2b	LtRIN4-2	AchRIN4H1	AchRIN4H3	AchRIN4H2	VvRin4H1	VvRin4H3	MxdRIN4c5	MxdRIN4c10	Mr5RIN4c5	Mr5RIN4c10	FvRin4-1	GmaxRIN4a	GmaxRIN4b	GmaxRIN4c	GmaxRIN4d	SlRIN4	PpRIN4	ZmRIN4	OsRIN4
AtRIN4	ID	93	79	32	34	34	35	33	33	34	31	37	40	39	39	39	39	35	38	37	34	34	31	20	24	23
AlRIN4	93	ID	80	34	35	34	35	33	34	35	32	38	41	39	40	39	40	36	38	38	33	34	31	20	24	23
BjRIN4	79	80	ID	31	32	32	33	32	33	33	30	35	38	39	38	39	39	35	37	37	35	35	30	19	24	24
NbRIN4B	32	34	31	ID	97	49	49	48	50	52	51	35	50	43	43	44	44	45	39	40	39	40	79	29	26	27
NbRIN4A	34	35	32	97	ID	49	49	48	50	51	50	35	50	44	44	45	45	44	39	40	38	39	78	29	25	27
LtRIN4-2a	34	34	32	49	49	ID	97	95	54	53	54	41	52	46	44	45	45	46	41	41	38	40	47	24	29	29
LtRIN4-2b	35	35	33	49	49	97	ID	95	54	53	54	41	52	46	45	46	46	46	42	41	39	41	46	25	29	29
LtRIN4-2	33	33	32	48	48	95	95	ID	53	52	54	41	52	46	44	46	45	45	40	40	39	40	47	24	29	29
AchRIN4H1	33	34	33	50	50	54	54	53	ID	84	59	37	55	49	47	48	48	49	42	43	44	45	48	28	30	32
AchRIN4H3	34	35	33	52	51	53	53	52	84	ID	58	38	53	48	49	48	49	47	45	44	45	47	51	29	28	31
AchRIN4H2	31	32	30	51	50	54	54	54	59	58	ID	38	54	51	50	51	50	52	44	45	43	43	46	28	27	28
VvRin4H1	37	38	35	35	35	41	41	41	37	38	38	ID	69	49	48	48	48	51	44	44	37	38	34	20	26	26
VvRin4H3	40	41	38	50	50	52	52	52	55	53	54	69	ID	60	58	58	58	64	53	53	49	49	49	27	30	31
MxdRIN4c5	39	39	39	43	44	46	46	46	49	48	51	49	60	ID	91	96	92	77	54	54	51	51	42	26	29	26
MxdRIN4c10	39	40	38	43	44	44	45	44	47	49	50	48	58	91	ID	92	98	77	53	53	49	50	42	26	29	27
Mr5RIN4c5	39	39	39	44	45	45	46	46	48	48	51	48	58	96	92	ID	93	76	54	53	50	51	42	26	28	26
Mr5RIN4c10	39	40	39	44	45	45	46	45	48	49	50	48	58	92	98	93	ID	78	53	53	50	51	43	26	29	27
FvRin4-1	35	36	35	45	44	46	46	45	49	47	52	51	64	77	77	76	78	ID	55	55	50	50	45	25	28	28
GmaxRIN4a	38	38	37	39	39	41	42	40	42	45	44	44	53	54	53	54	53	55	ID	93	64	62	38	25	29	28
GmaxRIN4b	37	38	37	40	40	41	41	40	43	44	45	44	53	54	53	53	53	55	93	ID	64	62	38	25	29	28
GmaxRIN4c	34	33	35	39	38	38	39	39	44	45	43	37	49	51	49	50	50	50	64	64	ID	90	38	25	26	27
GmaxRIN4d	34	34	35	40	39	40	41	40	45	47	43	38	49	51	50	51	51	50	62	62	90	ID	39	25	28	29
SlRIN4	31	31	30	79	78	47	46	47	48	51	46	34	49	42	42	42	43	45	38	38	38	39	ID	27	26	26
PpRIN4	20	20	19	29	29	24	25	24	28	29	28	20	27	26	26	26	26	25	25	25	25	25	27	ID	20	21
ZmRIN4	24	24	24	26	25	29	29	29	30	28	27	26	30	29	29	28	29	28	29	29	26	28	26	20	ID	62
OsRIN4	23	23	24	27	27	29	29	29	32	31	28	26	31	26	27	26	27	28	28	28	27	29	26	21	62	ID

ID...identical; Values of greater than or equal to 60 % are marked with a grey background

J. RNA Integrity Number of the samples used for RNA-seq

The RNA integrity number was determined by GATC with the Bioanalyzer 2100 for all four RNA samples in order to ensure reliable data of the further expression analysis. RNA was isolated from leaf tissue collected 2 and 48 hpi from Mr5 inoculated with the virulent $avrRpt2_{EA}$ deletion mutant ZYRKD3-1 or with the none-virulent wild type strain Ea1189.

J.1. RIN value of the sample Ea1189 2 hpi

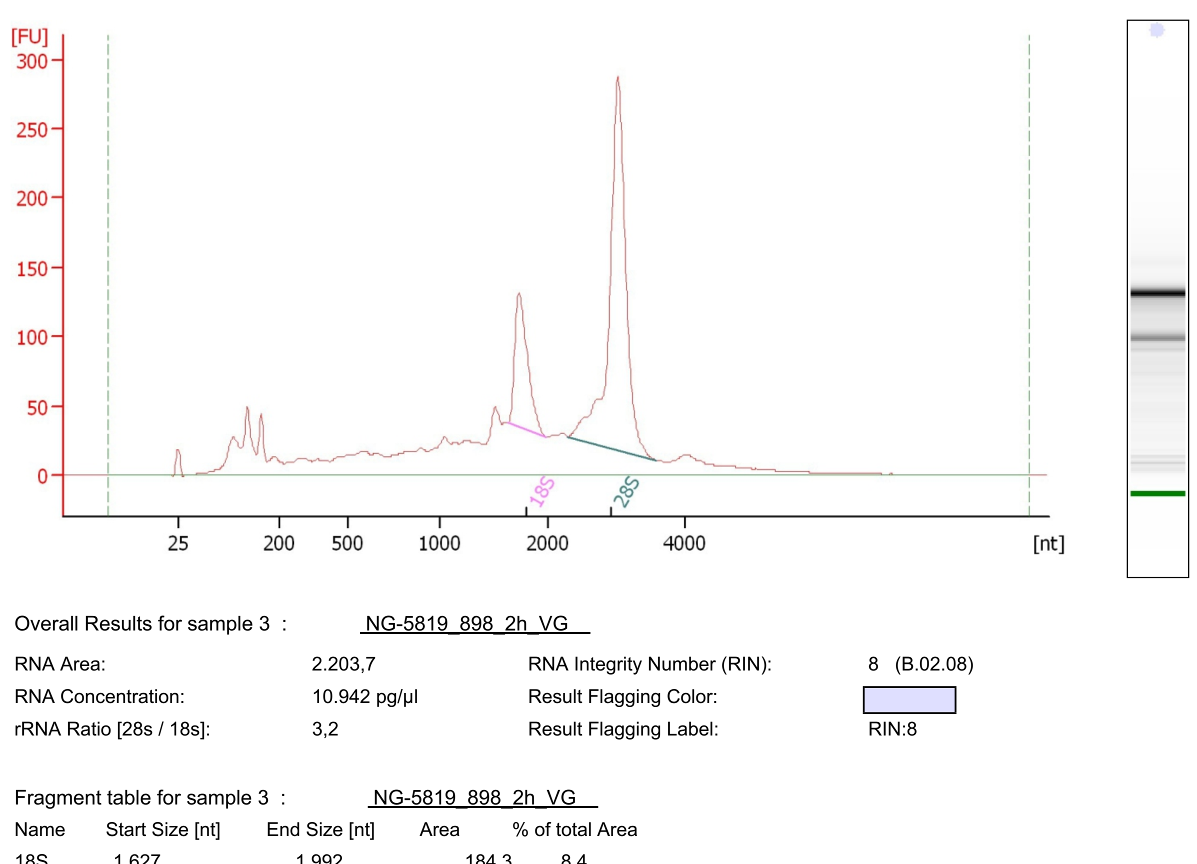
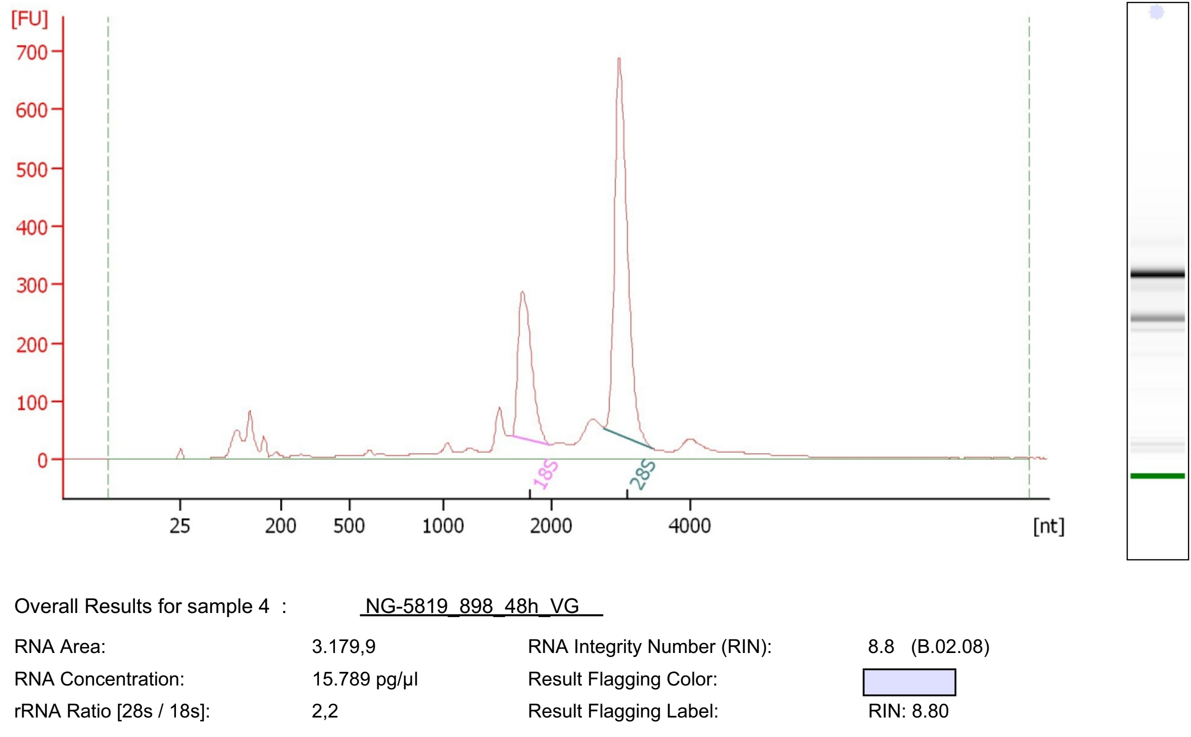


Figure 5.2.: RIN value of the RNA isolated from Mr5 after inoculation with the *E. amylovora* strain Ea1189 at 2 hpi.

J.2. RIN value of the sample Ea1189 48 hpi



Overall Results for sample 4 : NG-5819_898_48h_VG

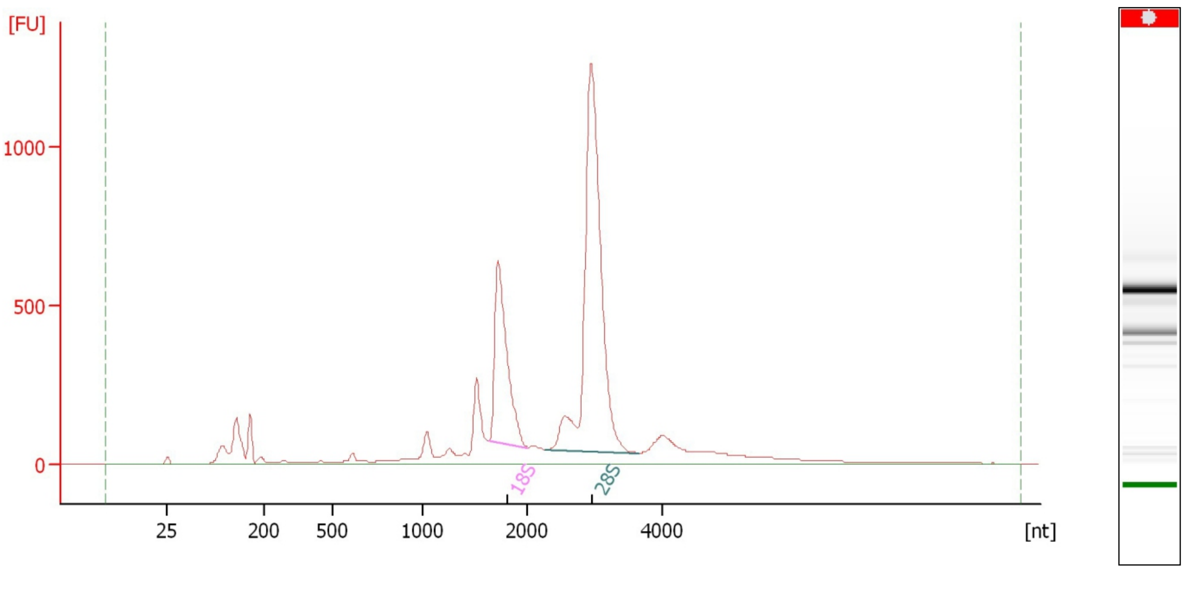
RNA Area:	3.179,9	RNA Integrity Number (RIN):	8.8 (B.02.08)
RNA Concentration:	15.789 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2,2	Result Flagging Label:	RIN: 8.80

Fragment table for sample 4 : NG-5819_898_48h_VG

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1.631	1.995	501,6	15,8
28S	2.770	3.467	1.094,9	34,4

Figure 5.3.: RIN value of the RNA isolated from Mr5 after inoculation with the *E. amylovora* strain Ea1189 at 48 hpi.

J.3. RIN value of the sample ZYRKD3-1 2 hpi



Overall Results for sample 1 : NG-5819_817_2h_VG

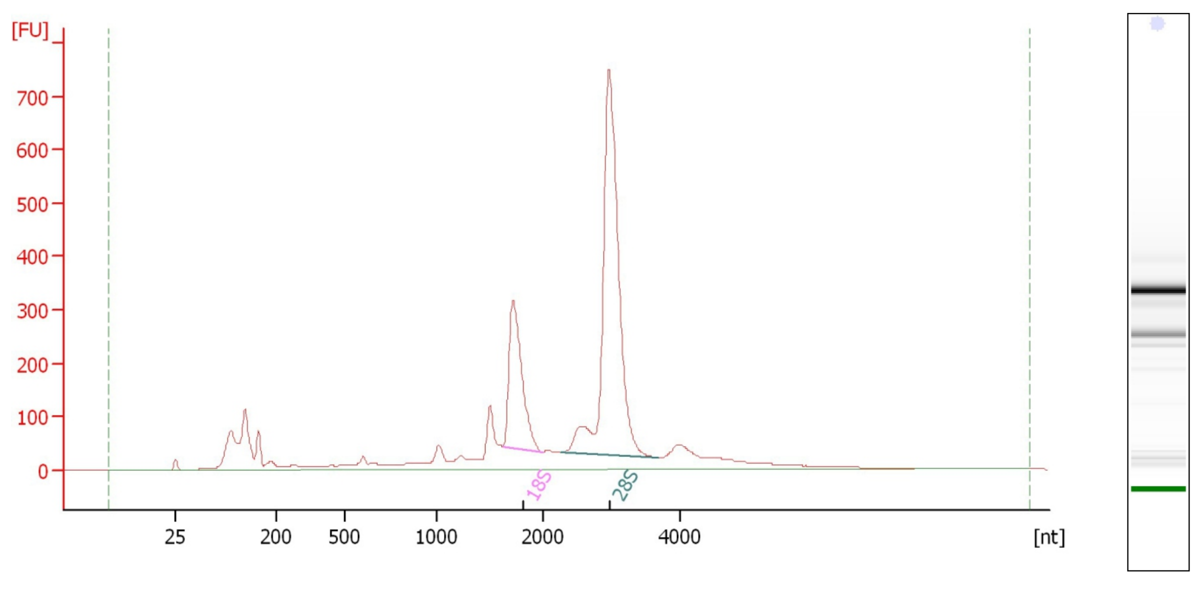
RNA Area:	6.845,4	RNA Integrity Number (RIN):	N/A (B.02.08)
RNA Concentration:	33.990 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2,5	Result Flagging Label:	RIN N/A

Fragment table for sample 1 : NG-5819_817_2h_VG

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1.635	2.011	1.080,2	15,8
28S	2.286	3.659	2.720,1	39,7

Figure 5.4.: RIN value of the RNA isolated from Mr5 after inoculation with the *E. amylovora* *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 at 2 hpi.

J.4. RIN value of the sample ZYRKD3-1 48 hpi



Overall Results for sample 2 : NG-5819_817_48h_VG

RNA Area:	4.095,7	RNA Integrity Number (RIN):	8.7 (B.02.08)
RNA Concentration:	20.337 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2,8	Result Flagging Label:	RIN: 8.70

Fragment table for sample 2 : NG-5819_817_48h_VG

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1.621	1.997	536,2	13,1
28S	2.267	3.690	1.511,4	36,9

Figure 5.5.: RIN value of the RNA isolated from Mr5 after inoculation with the *E. amylovora* $avrRpt2_{EA}$ deletion mutant ZYRKD3-1 at 48 hpi.

K. FastQC results of the transcriptome analysis

Quality analysis of the RNA-seq results by FastQC 0.10.1.

Table 5.9.: Quality analysis of the RNA-seq results by FastQC 0.10.1. software. Because of the paired end mode two fastq files had to be analysed per sample.

	ZYRKD3-1				**Ea1189**			
Quality parameters	**2 hpi**		**48 hpi**		**2 hpi**		**48 hpi**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Basic Statistics	pass	pass	pass	pass	pass	pass	pass	pass
Per base sequence quality[1]	pass	fail	pass	fail	pass	fail	pass	fail
Per sequence quality scores	pass	pass	pass	pass	pass	pass	pass	pass
Per base sequence content[2]	fail	fail	fail	fail	fail	fail	fail	fail
Per base GC content[3]	fail	fail	fail	fail	fail	fail	fail	fail
Per sequence GC content	pass	pass	pass	pass	pass	pass	pass	pass
Per base N content[4]	pass	warn	pass	warn	pass	warn	pass	warn
Sequence Length Distribution	pass	pass	pass	pass	pass	pass	pass	pass
Sequence Duplication Levels[5]	fail	fail	fail	fail	fail	fail	fail	fail
Overrepresented sequences[6]	warn	warn	warn	warn	fail	warn	warn	pass
Kmer Content[7]	warn	warn	fail	fail	fail	warn	fail	warn

[1]This module will raise a failure if the lower quartile for any base is less than 5 or if the median for any base is less than 20. [2]This module will fail if the difference between A and T, or G and C is greater than 20 % in any position. [3]This module will fail if the GC content of any base strays more than 10 % from the mean GC content. [4]This module raises a warning if any position shows an N content of >5 %. [5]This module will issue an error if non-unique sequences make up more than 50 % of the total. [6] This module will issue a warning if any sequence is found to represent more than 0.1 % of the total; This module will issue an error if any sequence is found to represent more than 1 % of the total. [7]This module will issue a warning if any k-mer is enriched more than 3 fold overall, or more than 5 fold at any individual position; This module will issue an error if any k-mer is enriched more than 10 fold at any individual base position.

L. Significant DEGs obtained from RNA-seq

L.1. DEGs at 2 or 48 hpi

Table 5.10.: List of the 191 significant DEGs obtained from RNA-seq analysis of Mr5 at 2 or 48 hpi with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 or with the none-virulent wild type strain Ea1189. The columns contain the number of reads per gene and sample after mapping with the BWA software and the p/padj value obtained by statistical analysis with the DESeq package.

	Gene ID	Number of reads per gene					DESeq	
		ZYRKD3-1		Ea1189				
		2 hpi	48 hpi	2 hpi	48 hpi	log2 FC	p	padj
	MDP0000119630	55	15	864	228	3,8	9,1E-06	0,011
	MDP0000126761	0	0	328	39	Inf	2,8E-08	0,000
	MDP0000128578	2	16	175	24	6,3	2,0E-04	0,091
	MDP0000136037	148	235	1488	890	3,2	3,1E-05	0,026
	MDP0000136226	0	0	162	0	Inf	4,3E-05	0,032
	MDP0000138651	61	208	751	605	3,5	6,1E-05	0,039
	MDP0000144864	459	636	28	604	-4,2	2,4E-05	0,021
	MDP0000151003	794	295	9712	442	3,5	4,9E-07	0,001
	MDP0000156226	1517	3055	259	2609	-2,7	2,3E-04	0,099
	MDP0000158507	0	0	163	123	Inf	4,1E-05	0,031
	MDP0000159070	3313	620	575	936	-2,7	1,0E-04	0,053
	MDP0000159251	4	34	397	270	6,5	2,9E-07	0,001
	MDP0000161200	1959	1498	301	1904	-2,9	7,5E-05	0,043
	MDP0000163314	0	40	163	235	Inf	4,1E-05	0,031
	MDP0000166138	213	862	1897	2432	3,0	4,4E-05	0,032
	MDP0000175481	4114	2988	771	3088	-2,6	1,5E-04	0,074
	MDP0000180043	99	264	924	935	3,1	1,6E-04	0,075
	MDP0000180902	213	429	1774	1280	2,9	7,9E-05	0,045
	MDP0000181339	294	402	2815	1319	3,1	1,4E-05	0,014
	MDP0000184034	5	4	222	20	5,3	1,7E-04	0,080
2 hpi	MDP0000194060	2025	6918	94	5808	-4,6	6,0E-09	0,000
	MDP0000197472	0	26	145	100	Inf	1,1E-04	0,055
	MDP0000203352	9130	8453	1120	7530	-3,2	2,9E-06	0,004
	MDP0000204381	19	37	469	186	4,5	1,6E-05	0,015
	MDP0000204699	0	0	238	36	Inf	1,1E-06	0,002
	MDP0000204794	4	37	331	427	6,2	2,2E-06	0,003
	MDP0000205617	208	1	2092	151	3,2	1,5E-05	0,015
	MDP0000206461	12	79	545	311	5,4	4,2E-07	0,001
	MDP0000208152	168	594	1515	1712	3,0	6,2E-05	0,039
	MDP0000211981	1324	1462	144	1507	-3,3	1,4E-05	0,014
	MDP0000212178	154	505	1295	1299	2,9	1,4E-04	0,067
	MDP0000217497	1702	2098	296	2347	-2,7	2,2E-04	0,097
	MDP0000217948	1476	2181	219	2426	-2,9	9,4E-05	0,050
	MDP0000219522	15	111	443	257	4,7	1,0E-05	0,011
	MDP0000225472	0	45	136	49	Inf	1,7E-04	0,081
	MDP0000225509	4615	1984	701	1586	-2,9	2,7E-05	0,023
	MDP0000229093	18	17	383	74	4,3	8,2E-05	0,046
	MDP0000236390	0	6	328	247	Inf	2,8E-08	0,000
	MDP0000237619	915	1100	35	1269	-4,9	4,8E-08	0,000
	MDP0000241440	795	955	71	1139	-3,6	1,8E-05	0,017
	MDP0000246085	1338	1444	179	1607	-3,1	5,6E-05	0,038
	MDP0000254847	0	4	461	2	Inf	3,2E-10	0,000

Table 5.10.: (continued)

	Gene ID	Number of reads per gene ZYRKD3-1 2 hpi	ZYRKD3-1 48 hpi	Ea1189 2 hpi	Ea1189 48 hpi	log2 FC	DESeq p	DESeq padj
2 hpi	MDP0000257349	4292	5076	496	3780	-3,3	3,0E-06	0,004
	MDP0000262141	112	63	1375	255	3,5	9,5E-06	0,011
	MDP0000264668	99	69	3258	374	4,9	3,0E-10	0,000
	MDP0000265187	19	99	402	244	4,3	6,6E-05	0,040
	MDP0000265729	6	51	318	157	5,6	1,1E-05	0,012
	MDP0000266004	293	1898	2282	4231	2,8	8,6E-05	0,047
	MDP0000272542	31	200	548	435	4,0	3,3E-05	0,027
	MDP0000275716	2834	484	451	432	-2,8	6,1E-05	0,039
	MDP0000277666	11982	5831	1749	6526	-2,9	1,2E-05	0,013
	MDP0000277718	113	143	1142	392	3,2	5,6E-05	0,038
	MDP0000280307	0	0	430	0	Inf	8,3E-10	0,000
	MDP0000282209	2	16	207	65	6,5	5,1E-05	0,035
	MDP0000286136	4	1	395	20	6,5	3,1E-07	0,001
	MDP0000289300	1167	3154	8470	9072	2,7	4,9E-05	0,035
	MDP0000293835	2582	3199	309	3413	-3,2	7,6E-06	0,010
	MDP0000294068	3	20	309	129	6,5	2,3E-06	0,003
	MDP0000294096	0	0	304	1	Inf	7,1E-08	0,000
	MDP0000294180	0	2	182	14	Inf	1,6E-05	0,015
	MDP0000294279	4	13	233	175	5,7	6,9E-05	0,040
	MDP0000296410	341	1095	3357	3461	3,2	8,6E-06	0,011
	MDP0000304236	2446	2650	440	2607	-2,6	1,8E-04	0,081
	MDP0000311359	61	140	741	414	3,5	6,9E-05	0,040
	MDP0000311705	4	6	234	131	5,7	6,6E-05	0,040
	MDP0000317974	121	879	1149	1591	3,1	8,2E-05	0,046
	MDP0000320910	31	103	510	449	3,9	6,4E-05	0,039
	MDP0000322439	1038	1252	83	1381	-3,8	3,5E-06	0,005
	MDP0000324831	20	14	414	169	4,2	6,3E-05	0,039
	MDP0000440622	28	16	1476	241	5,6	2,2E-10	0,000
	MDP0000521048	13966	15451	2067	11480	-2,9	1,3E-05	0,013
	MDP0000609876	119	168	1034	547	3,0	1,8E-04	0,082
	MDP0000609966	114	542	1645	1794	3,7	1,8E-06	0,003
	MDP0000612660	40	269	551	425	3,6	1,1E-04	0,055
	MDP0000628976	29	46	426	381	3,7	2,3E-04	0,098
	MDP0000696168	72	206	1020	846	3,7	9,3E-06	0,011
	MDP0000716457	0	0	224	17	Inf	2,1E-06	0,003
	MDP0000733653	5	87	228	255	5,4	1,4E-04	0,068
	MDP0000737128	55	132	749	474	3,6	3,5E-05	0,029
	MDP0000784187	5	67	386	301	6,1	7,9E-07	0,001
	MDP0000827400	6	62	299	309	5,5	2,0E-05	0,018
	MDP0000858763	3746	4466	585	3860	-2,8	4,0E-05	0,031
	MDP0000891117	110	582	1001	991	3,0	1,5E-04	0,073
	MDP0000919962	97	0	1271	18	3,6	7,7E-06	0,010
	MDP0000944210	58	323	1115	916	4,1	9,3E-07	0,002
48 hpi	MDP0000047589	1882	4322	8681	13998	1,4	3,5E-05	0,019
	MDP0000053760	25316	17140	19669	65065	1,6	4,2E-07	0,001
	MDP0000095637	3583	5702	12626	16245	1,2	2,6E-04	0,096
	MDP0000119199	347	4924	356	2129	-1,5	4,8E-05	0,024
	MDP0000120176	4820	2772	10520	12171	1,8	1,3E-07	0,000
	MDP0000122210	170	257	790	1267	2,0	2,6E-04	0,096
	MDP0000122734	713	44686	994	5499	-3,3	1,0E-21	0,000
	MDP0000122783	32	657	8	142	-2,5	2,1E-04	0,083
	MDP0000125300	27	586	52	102	-2,8	1,0E-04	0,046
	MDP0000139058	778	1307	1641	7002	2,1	1,3E-08	0,000

Table 5.10.: (continued)

	Gene ID	Number of reads per gene					DESeq	
		ZYRKD3-1		Ea1189				
		2 hpi	48 hpi	2 hpi	48 hpi	log2 FC	p	padj
48 hpi	MDP0000142997	152	707	66	164	-2,4	2,2E-04	0,087
	MDP0000144125	429	986	842	3733	1,6	5,3E-05	0,026
	MDP0000149486	5	420	3	33	-4,0	1,4E-05	0,008
	MDP0000150261	286	6422	145	2242	-1,8	6,2E-07	0,001
	MDP0000151161	87	2	730	349	7,2	4,6E-07	0,001
	MDP0000153978	387	7529	166	1643	-2,5	1,6E-11	0,000
	MDP0000158520	54	1632	169	425	-2,2	4,2E-06	0,003
	MDP0000159572	453	733	1075	2667	1,6	2,6E-04	0,096
	MDP0000160256	16	8	296	361	5,2	1,2E-05	0,007
	MDP0000166796	89	3184	168	761	-2,4	1,6E-08	0,000
	MDP0000173059	1129	905	3297	4344	2,0	8,2E-07	0,001
	MDP0000174537	1416	923	2217	3786	1,7	1,6E-05	0,010
	MDP0000175388	2220	49179	2819	16234	-1,9	6,4E-09	0,000
	MDP0000197501	0	449	10	33	-4,1	5,2E-06	0,004
	MDP0000206705	1688	2380	1408	13492	2,2	3,2E-10	0,000
	MDP0000207407	244	6799	261	1751	-2,2	1,1E-09	0,000
	MDP0000207799	1412	864	4313	5929	2,5	2,1E-10	0,000
	MDP0000208496	173	3904	281	1163	-2,0	2,3E-07	0,000
	MDP0000214382	305	5388	94	744	-3,1	3,0E-15	0,000
	MDP0000215062	3173	32240	4222	15119	-1,4	1,9E-05	0,011
	MDP0000216647	1046	1414	1061	5556	1,7	8,5E-06	0,005
	MDP0000217508	652	106899	1250	26804	-2,3	2,6E-12	0,000
	MDP0000219684	118715	21877	261914	76152	1,5	2,6E-06	0,002
	MDP0000220190	80722	19459	76033	54576	1,2	1,8E-04	0,076
	MDP0000232080	1319	6947	1126	3200	-1,4	6,5E-05	0,031
	MDP0000233546	64	191	533	1062	2,2	2,3E-04	0,090
	MDP0000236723	22	47	249	653	3,5	1,1E-05	0,007
	MDP0000240189	3301	1322	3390	5751	1,8	1,4E-06	0,001
	MDP0000243895	775	10388	390	2001	-2,7	1,4E-13	0,000
	MDP0000243954	37969	9197	46470	26031	1,2	2,1E-04	0,084
	MDP0000245635	699	2639	138	1071	-1,6	1,1E-04	0,051
	MDP0000254260	172	99821	551	38749	-1,7	2,3E-07	0,000
	MDP0000254930	3534	10928	4574	5570	-1,3	1,8E-04	0,076
	MDP0000264060	243	1123	1148	4779	1,8	3,9E-06	0,003
	MDP0000265157	84	3563	99	734	-2,6	5,1E-10	0,000
	MDP0000265759	31	3097	24	767	-2,3	3,5E-08	0,000
	MDP0000267897	110	156	301	1377	2,8	6,1E-07	0,001
	MDP0000278885	2483	1537	1134	7905	2,1	1,8E-08	0,000
	MDP0000278972	131	4425	178	1526	-1,8	1,7E-06	0,002
	MDP0000290546	523	11213	623	3313	-2,1	3,9E-09	0,000
	MDP0000291831	25	460	13	38	-3,9	7,9E-06	0,005
	MDP0000292132	464	2952	1189	9788	1,4	3,9E-05	0,020
	MDP0000292492	2845	3389	2911	13003	1,6	1,7E-06	0,002
	MDP0000296339	98	38	403	533	3,5	5,4E-05	0,026
	MDP0000303430	663	101203	2014	41498	-1,6	7,7E-07	0,001
	MDP0000307705	0	0	72	168	Inf	1,9E-04	0,076
	MDP0000309976	1139	925	2025	3268	1,5	2,0E-04	0,081
	MDP0000316497	272	241	694	1595	2,4	4,0E-06	0,003
	MDP0000323296	200	5876	222	2312	-1,6	6,7E-06	0,005
	MDP0000324604	1547	658	1409	3061	1,9	6,7E-06	0,005
	MDP0000334368	288	1153	180	310	-2,2	4,7E-05	0,024
	MDP0000353793	1675	1359	1108	4417	1,4	2,6E-04	0,096
	MDP0000361180	32943	17389	22094	73799	1,8	3,0E-08	0,000
	MDP0000361944	434	254	1220	1584	2,3	7,9E-06	0,005

Table 5.10.: (continued)

	Gene ID	Number of reads per gene ZYRKD3-1 2 hpi	ZYRKD3-1 48 hpi	Ea1189 2 hpi	Ea1189 48 hpi	log2 FC	DESeq p	padj
48 hpi	MDP0000362505	45	2916	53	630	-2,5	5,2E-09	0,000
	MDP0000365549	301	1148	404	348	-2,0	1,5E-04	0,063
	MDP0000370964	160	2912	190	823	-2,1	4,3E-07	0,001
	MDP0000374881	2483	20341	2027	6735	-1,9	1,7E-08	0,000
	MDP0000404331	793	2696	449	859	-1,9	3,9E-06	0,003
	MDP0000465593	434	3375	302	935	-2,1	1,4E-07	0,000
	MDP0000489886	31	334	1	20	-4,4	2,9E-05	0,016
	MDP0000499035	33592	26019	35033	138235	2,1	7,1E-11	0,000
	MDP0000510213	2038	7925	843	3508	-1,5	2,7E-05	0,015
	MDP0000549793	192	4548	261	1744	-1,7	8,9E-06	0,006
	MDP0000551952	488	536	318	2116	1,7	2,4E-04	0,092
	MDP0000563385	35	17	288	383	4,2	8,4E-05	0,039
	MDP0000565027	3346	2283	3281	14257	2,3	2,6E-11	0,000
	MDP0000597996	1708	1453	1311	7047	2,0	9,1E-08	0,000
	MDP0000604702	2	539	11	87	-2,9	1,1E-04	0,049
	MDP0000617684	636	685	1401	2954	1,8	2,2E-05	0,013
	MDP0000644109	477	3400	263	1176	-1,8	4,8E-06	0,004
	MDP0000661381	3731	4055	5714	13283	1,4	3,0E-05	0,017
	MDP0000668657	4008	290	6870	1998	2,5	4,9E-07	0,001
	MDP0000683913	90	285	237	1738	2,3	5,5E-06	0,004
	MDP0000700383	328	13261	406	4112	-2,0	7,9E-09	0,000
	MDP0000714257	0	0	13	201	Inf	3,5E-05	0,019
	MDP0000750217	21	37	332	639	3,8	3,7E-06	0,003
	MDP0000750789	360	581	218	2756	2,0	8,5E-06	0,005
	MDP0000757585	1110	637	2965	2400	1,6	2,5E-04	0,096
	MDP0000768256	1851	14634	2733	5846	-1,6	1,5E-06	0,002
	MDP0000782642	2988	6859	16001	23962	1,5	4,7E-06	0,004
	MDP0000791550	20	1303	69	287	-2,5	2,8E-06	0,002
	MDP0000794528	1620	1066	1513	4274	1,7	1,5E-05	0,009
	MDP0000795157	857	14472	835	4964	-1,8	6,3E-08	0,000
	MDP0000810697	13	755	23	185	-2,3	2,3E-04	0,089
	MDP0000836170	952	833	5175	3759	1,9	4,1E-06	0,003
	MDP0000868044	114	486	691	2215	1,9	4,0E-05	0,020
	MDP0000874252	1375	4029	3981	11735	1,2	2,5E-04	0,094
	MDP0000878878	656	927	2135	6276	2,5	1,9E-10	0,000
	MDP0000915991	438	51402	1222	11804	-2,4	3,5E-13	0,000
	MDP0000921319	2538	2239	8573	6974	1,3	1,9E-04	0,076
	MDP0000925901	149	4087	193	1394	-1,8	1,9E-06	0,002
	MDP0000929055	116	67	178	625	2,9	1,6E-04	0,068
	MDP0000936525	1879	1920	1701	5953	1,3	2,6E-04	0,096
	MDP0000937142	12	507	2	37	-4,1	1,5E-06	0,002
	MDP0000937986	23193	2352	22751	9386	1,7	1,6E-06	0,002

FC Fold change

L.2. DEGs at 2 and 48 hpi

Table 5.11.: List of the 20 significant DEGs obtained from RNA-seq analysis of Mr5 at 2 and 48 hpi with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 or with the none-virulent wild type strain Ea1189. The columns contain the number of reads per gene and sample after mapping with the BWA software and the p/padj value obtained by statistical analysis with the DESeq package.

Gene ID	Number of reads per gene				DESeq					
	ZYRKD3-1		Ea1189		2 hpi			48 hpi		
	2 hpi	48 hpi	2 hpi	48 hpi	log2 FC	p	padj	log2 FC	p	padj
MDP0000131100	1442	1458	9560	5872	2,6	9,8E-05	5,2E-02	1,7	4,8E-06	3,5E-03
MDP0000139165	21	91	757	1136	5,0	1,5E-07	3,9E-04	3,3	1,3E-07	1,9E-04
MDP0000165381	90	160	896	1212	3,2	1,2E-04	5,9E-02	2,6	7,5E-06	5,0E-03
MDP0000166991	14	134	698	908	5,5	4,6E-08	1,6E-04	2,5	1,3E-04	5,5E-02
MDP0000196394	27	60	425	608	3,8	1,7E-04	8,0E-02	3,0	1,2E-04	5,3E-02
MDP0000232616	33	169	1609	1983	5,5	2,4E-10	2,4E-06	3,3	5,0E-10	1,5E-06
MDP0000242456	354	147	3065	899	3,0	2,7E-05	2,3E-02	2,3	2,8E-04	9,9E-02
MDP0000250070	62	336	1249	2469	4,2	4,3E-07	8,9E-04	2,6	4,0E-08	6,7E-05
MDP0000265874	3	2	399	240	6,9	1,2E-07	3,5E-04	6,6	5,3E-05	2,6E-02
MDP0000266393	163	628	1299	2548	2,8	1,9E-04	8,5E-02	1,7	8,6E-05	4,0E-02
MDP0000268523	370	717	9239	5757	4,5	5,7E-10	3,7E-06	2,7	9,4E-12	6,0E-08
MDP0000288425	70	356	2029	1798	4,7	4,4E-09	2,2E-05	2,0	3,5E-05	1,9E-02
MDP0000327191	1113	3707	8371	10903	2,8	3,7E-05	2,9E-02	1,3	2,3E-04	9,0E-02
MDP0000343634	172	1292	2735	4400	3,8	2,4E-07	6,2E-04	1,5	1,4E-04	6,1E-02
MDP0000351523	20	92	429	962	4,3	4,5E-05	3,3E-02	3,1	2,9E-06	2,5E-03
MDP0000364885	57	382	2969	1817	5,6	7,6E-12	1,7E-07	2,0	6,4E-05	3,0E-02
MDP0000440654	805	922	5595	4254	2,6	8,7E-05	4,7E-02	1,9	1,7E-06	1,6E-03
MDP0000680997	48	183	649	1199	3,6	6,3E-05	3,9E-02	2,4	3,0E-05	1,6E-02
MDP0000711911	0	4	681	792	Inf	1,0E-12	4,6E-08	7,3	1,2E-13	1,6E-09
MDP0000940742	847	2141	6136	7132	2,7	5,9E-05	3,9E-02	1,4	6,4E-05	3,0E-02

FC Fold change

M. Assignment of RNA-seq data via MapMan

Table 5.12.: Distribution of all genes/genes with log2 fold change > 3/significant differentially expressed genes achieved by the RNA-seq analysis within the 35 bins of MapMan. RNA-seq was performed with RNA isolated from leaf tissue collected 2 and 48 hpi from Mr5 inoculated with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1 or with the none-virulent wild type strain Ea1189.

		All genes						Genes with log fold change > 3						Sig genes					
	Bins of MapMan		2 hpi			48 hpi			2 hpi			48 hpi			2 hpi			48 hpi	
		∑	↑	↓	∑	↑	↓	∑	↑	↓	∑	↑	↓	∑	↑	↓	∑	↑	↓
1	PS	355	75	280	357	139	218	9	4	5	8	5	3				4	4	0
2	major CHO metabolism	247	120	127	249	128	121	10	5	5	10	6	4	1	1	0	3	3	0
3	minor CHO metabolism	259	135	124	262	155	107	9	5	4	14	11	3				1	1	0
4	glycolysis	121	71	50	125	70	55	2	1	1	8	7	1						
5	fermentation	59	33	26	60	43	17	4	3	1	1	0	1						
6	gluconeogenesis	30	18	12	27	18	9	1	1	0	2	2	0						
7	OPP	39	24	15	41	30	11	1	0	1	2	2	0						
8	TCA	182	107	75	185	113	72	6	4	2	5	5	0	1			1	0	1
9	mitochondrial electron transport	185	141	44	191	113	78	2	1	1	4	2	2				1	1	0
10	cell wall	659	334	325	659	463	196	58	31	27	54	45	9	4	3	1	5	5	0
11	lipid metabolism	722	372	350	708	386	322	40	23	17	46	35	11	3	2	1	3	3	0
12	N-metabolism	48	29	19	47	29	18	3	2	1	3	2	1						
13	amino acid metabolism	570	303	267	563	280	283	22	14	8	17	10	7	2	2	1			
14	S-assimilation	24	15	9	24	13	11	1	0	1	0								
15	metal handling	91	56	35	96	62	34	6	5	1	7	6	1						
16	secondary metabolism	680	358	322	656	425	231	61	41	20	51	42	9	5	5	0	2	2	0
17	hormone metabolism	880	506	374	855	538	317	71	51	20	69	51	18	6	5	1			
18	Co-factor and vitamine metabolism	134	68	66	132	55	77	4	4	0	5	1	4						
19	tetrapyrrole synthesis	129	43	86	127	32	95	7	2	5	6	2	4						
20	stress	2115	915	1200	2153	1122	1031	114	59	55	150	94	56	7	6	1	30	5	25
21	redox	340	209	131	346	189	157	20	16	4	11	6	5						
22	polyamine metabolism	42	24	18	43	28	15	2	2	0	3	2	1						
23	nucleotide metabolism	311	193	118	311	147	164	6	5	1	11	6	5						
24	Biodegradation of Xenobiotics	32	21	11	29	16	13	1	1	0	1	1	0						
25	C1-metabolism	53	27	26	53	27	26	1	0	1	2	1	1						
26	misc	2450	1389	1061	2412	1499	913	192	137	55	216	150	66	16	13	3	19	17	2
27	RNA	4515	2447	2068	4442	2539	1903	227	176	51	225	174	51	14	13	1	8	3	5
28	DNA	712	363	349	704	313	391	24	12	12	26	13	13				1	0	1
29	protein	6173	3439	2734	6162	3302	2860	223	116	107	272	168	104	4	2	2	3	1	2

Table 5.12.: (continued)

Bins of MapMan	all genes						genes with log fold change > 3						sig genes					
	2 hpi			48 hpi			2 hpi			48 hpi			2 hpi			48 hpi		
	$\sum$	↑	↓	$\sum$	↑	↓	$\sum$	↑	↓	$\sum$	↑	↓	$\sum$	↑	↓	$\sum$	↑	↓
30 signalling	2996	1430	1566	3043	1748	1295	188	85	103	204	140	64	2	0	2			
31 cell	1387	754	633	1374	708	666	41	25	16	52	34	18				2	1	1
33 development	947	506	441	920	535	385	58	34	24	66	39	13	3	3	0	3	1	2
34 transport	1894	984	910	1877	1074	803	133	86	47	106	75	31	6	5	1	1	1	0
35 not assigned	16722	9354	7368	16706	9010	7696	990	586	404	1266	809	457	32	24	8	40	30	10
sum of transcripts in all bins	46103	24863	21240	45939	25210	20372	2537	1537	1000	2923	1946	963	106	84	22	127	74	49
multiple annotation	1539						70						2					
transcripts at both time points	42505						453						20					
total amount of transcripts	47998						4937						211					

↑ higher expression level in Mr5 inoculated with the none-virulent wild type strain Ea1189, ↓ higher expression level in Mr5 inoculated with the virulent *avrRpt2*$_{EA}$ deletion mutant ZYRKD3-1

N. CNRQ values of the genes analysed by BioMark™ HD system

Table 5.13.: CNRQ values and the related confidence intervals of the 96 genes analysed with the BioMark™ HD system.

Samples	Hpi	Housek1	Housek2	Housek3	Housek4	Housek5	Housek6
Mr5_ni	0	1 (0.4-2.52)	1 (0.8-1.25)	1 (0.56-1.8)	1 (0.15-6.58)	1 (0.68-1.47)	1 (0.01-88.49)
Ea1189	1	1.55 (0.88-2.72)	1.19 (0.84-1.69)	0.83 (0.7-0.98)	0.32 (0.09-1.15)	1.02 (0.77-1.34)	1.88 (0.71-5.01)
ZYRKD3-1		2.01 (1.12-3.59)	1.23 (0.95-1.58)	0.85 (0.76-0.95)	0.3 (0.09-1.01)	0.96 (0.73-1.28)	1.29 (0.04-38.68)
Ea1189	2	1.19 (0.65-2.2)	1.08 (0.87-1.34)	0.96 (0.89-1.02)	0.31 (0.16-0.57)	0.97 (0.84-1.12)	0.75 (0.08-7.12)
ZYRKD3-1		0.92 (0.64-1.31)	0.89 (0.84-0.96)	1.17 (1.15-1.18)	0.22 (0.08-0.58)	0.96 (0.89-1.03)	1.23 (0.17-8.92)
Ea1189	4	0.95 (0.71-1.27)	1.01 (0.93-1.1)	0.97 (0.87-1.08)	0.28 (0.17-0.45)	1.02 (0.98-1.06)	1.3 (0.76-2.23)
ZYRKD3-1		1.03 (0.39-2.73)	1 (0.7-1.42)	0.97 (0.87-1.07)	0.25 (0.1-0.6)	1.04 (0.79-1.35)	1.12 (0.01-101.31)
Ea1189	12	0.84 (0.55-1.29)	0.88 (0.76-1.03)	1.04 (0.99-1.1)	0.24 (0.12-0.52)	1.09 (0.9-1.31)	1.46 (0.08-27.05)
ZYRKD3-1		0.84 (0.78-0.9)	0.84 (0.79-0.89)	1.04 (0.85-1.27)	0.23 (0.08-0.68)	1.15 (0.92-1.42)	1.49 (0.04-51.69)
Ea1189	24	1.06 (0.49-2.31)	1.04 (0.84-1.3)	0.87 (0.78-0.97)	0.34 (0.15-0.78)	1.1 (0.97-1.26)	1.53 (0.06-39.32)
ZYRKD3-1		1.28 (0.51-3.24)	1.04 (0.69-1.58)	0.92 (0.63-1.34)	0.38 (0.27-0.52)	1.04 (0.91-1.19)	1.61 (0.17-15.28)
Ea1189	48	1.52 (0.98-2.36)	1.13 (0.97-1.32)	0.88 (0.67-1.17)	0.34 (0.14-0.83)	1 (0.82-1.22)	0.55 (0-451.37)
ZYRKD3-1		1.25 (1.06-1.47)	1.01 (0.95-1.07)	0.92 (0.81-1.03)	0.63 (0.45-0.89)	1.08 (0.92-1.28)	1.35 (0.79-2.3)

Samples	Hpi	EH034548	FB_Mr5q1	FB_Mr5q2	MDP0000047589	MDP0000095637	MDP0000119630
Mr5_ni	0	1 (0.36-2.77)	1 (0.35-2.83)	1 (0.12-8.48)	1 (0.06-17.71)	1 (0.64-1.57)	1 (0.06-15.65)
Ea1189	1	2.32 (0.9-5.98)	1.8 (1.11-2.92)	1.28 (0.2-8.14)	5.85 (2.1-16.35)	0.98 (0.7-1.39)	1.07 (0.72-1.58)
ZYRKD3-1		1.01 (0.77-1.32)	1.14 (0.5-2.58)	1.03 (0.18-5.98)	5.33 (1.87-15.21)	0.83 (0.47-1.47)	1.07 (0.13-8.8)
Ea1189	2	1.45 (0.55-3.83)	1.24 (0.2-7.88)	0.83 (0.1-7.01)	4.08 (1.8-9.21)	1.32 (0.6-2.93)	2.84 (1.46-5.53)
ZYRKD3-1		1.52 (1.07-2.18)	1.24 (0.64-2.43)	1.5 (0.38-5.94)	3.19 (1.86-5.46)	2.36 (1.38-4.05)	2.63 (0.37-18.67)
Ea1189	4	1.96 (1.4-2.76)	1.53 (0.46-5.12)	1.1 (0.4-3)	4.33 (1.97-9.49)	1.89 (1.03-3.49)	1.04 (0.24-4.59)
ZYRKD3-1		2.54 (1.01-6.4)	1.63 (0.67-4)	0.77 (0.14-4.21)	4.47 (1.41-14.23)	2.64 (0.93-7.51)	1.94 (0.35-10.78)
Ea1189	12	2.15 (0.65-7.09)	1.78 (0.39-8.09)	1.37 (0.38-4.94)	4.17 (1.57-11.07)	1.62 (0.93-2.83)	4.15 (2.34-7.36)
ZYRKD3-1		2.44 (1.19-4.99)	1.63 (0.43-6.14)	0.89 (0.23-3.45)	3.6 (2.66-4.87)	1.78 (1.13-2.8)	7.73 (0.51-116.71)
Ea1189	24	2.04 (1.17-3.56)	1.53 (1.1-2.13)	1.12 (0.85-1.47)	4.75 (4.23-5.34)	0.96 (0.53-1.74)	3.16 (0.58-17.08)
ZYRKD3-1		1.65 (0.95-2.88)	1.21 (1.04-1.41)	1.16 (0.47-2.87)	2.9 (2.21-3.8)	0.94 (0.56-1.59)	2.65 (0.4-17.35)
Ea1189	48	1.33 (0.11-16.15)	1.11 (0.29-4.26)	0.69 (0.04-10.83)	4.01 (1.15-13.91)	0.94 (0.46-1.93)	1.7 (0.52-5.55)
ZYRKD3-1		0.94 (0.22-3.94)	1.06 (0.52-2.15)	1.32 (0.44-3.98)	2.54 (1.52-4.23)	0.97 (0.78-1.21)	1.23 (0.27-5.67)

Table 5.13.: (continued)

Samples	Hpi	MDP0000120176	MDP0000126761	MDP0000131100	MDP0000136037	MDP0000139165	MDP0000151003
Mr5_ni	0	1 (0.37-2.74)	1 (0.25-3.97)	1 (0.24-4.13)	1 (0.56-1.78)	1 (0.01-179)	1 (0.46-2.18)
Ea1189	1	1.65 (0.93-2.91)	11.96 (0.14-1012.47)	3.19 (1.34-7.58)	1.75 (0.42-7.2)	40.19 (4.67-346.05)	3.87 (1.21-12.39)
ZYRKD3-1		1.28 (0.93-1.74)	1.21 (0.4-3.66)	2.01 (1.3-3.11)	0.93 (0.53-1.64)	24.09 (7.23-80.33)	1.41 (0.33-6.07)
Ea1189	2	2.36 (1.62-3.43)	1.72 (1.52-1.93)	2.54 (2.13-3.04)	1.69 (1.13-2.51)	12.96 (1.73-96.99)	4.24 (2.06-8.72)
ZYRKD3-1		3.19 (1.4-7.3)	1.67 (0.62-4.45)	3.67 (2.12-6.37)	1.26 (0.72-2.21)	20.22 (7.98-51.24)	8 (2.84-22.56)
Ea1189	4	2.31 (1.72-3.1)	1.86 (0.9-3.84)	1.33 (0.36-4.88)	1.12 (0.67-1.86)	15.01 (4.76-47.32)	1.81 (0.4-8.24)
ZYRKD3-1		2.95 (1.21-7.2)	2.73 (0.35-21.36)	2.31 (2.05-2.6)	1.42 (1.12-1.8)	17.62 (7.39-42)	2.87 (0.93-8.87)
Ea1189	12	0.96 (0.41-2.26)	4.39 (2.46-7.85)	1.56 (0.69-3.51)	2.79 (2.32-3.35)	23.06 (3.83-138.99)	0.91 (0.76-1.09)
ZYRKD3-1		0.99 (0.58-1.7)	3.19 (1.92-5.29)	1.85 (0.72-4.73)	3.35 (2.54-4.44)	16.82 (5.07-55.81)	1 (0.54-1.82)
Ea1189	24	1.38 (0.4-4.79)	4.2 (0.51-34.68)	1.84 (0.27-12.68)	1.59 (0.68-3.71)	19.67 (2.55-151.84)	1.07 (0.36-3.2)
ZYRKD3-1		1.1 (0.62-1.97)	3.07 (1.26-7.51)	1.75 (1.27-2.41)	1.39 (0.9-2.15)	8.37 (0.65-107.4)	1.09 (0.47-2.54)
Ea1189	48	0.61 (0.12-3)	2.04 (0.31-13.23)	0.68 (0.05-10.23)	0.64 (0.16-2.57)	9.8 (0.03-3324.15)	0.63 (0.57-0.68)
ZYRKD3-1		0.67 (0.44-1.03)	1.25 (0.89-1.75)	0.9 (0.8-1)	0.52 (0.46-0.6)	2.73 (0.16-46.68)	0.75 (0.55-1.03)

Samples	Hpi	MDP0000159251	MDP0000159572	MDP0000163314	MDP0000165381	MDP0000166138	MDP0000174537
Mr5_ni	0	1 (0.43-2.35)	1 (0.05-18.32)	1 (0.13-7.48)	1 (0.05-22.22)	1 (0.26-3.82)	1 (0.39-2.56)
Ea1189	1	1.67 (0.47-5.88)	0.94 (0.24-3.7)	0.4 (0.21-0.74)	5.38 (0.57-50.76)	2.52 (1.15-5.53)	1.83 (1.33-2.53)
ZYRKD3-1		1.54 (1.1-2.16)	1.48 (0.64-3.44)	0.64 (0.02-20.2)	3.88 (1.18-12.72)	2.26 (1.12-4.56)	0.99 (0.46-2.14)
Ea1189	2	1.11 (0.75-1.66)	1.58 (0.49-5.13)	0.26 (0.14-0.47)	3.64 (1.22-10.82)	2.66 (1.71-4.14)	1.94 (0.64-5.87)
ZYRKD3-1		0.98 (0.48-2.01)	0.9 (0.37-2.18)	0.18 (0.08-0.42)	1.89 (0.88-4.1)	1.74 (0.7-4.33)	2.89 (1.95-4.28)
Ea1189	4	1.55 (1.02-2.35)	1.03 (0.74-1.46)	0.28 (0.13-0.61)	1.82 (0.8-4.16)	1.96 (1.37-2.81)	2.25 (1.01-4.98)
ZYRKD3-1		1.43 (0.38-5.43)	1.18 (0.24-5.82)	0.22 (0.15-0.34)	2.35 (0.44-12.62)	2.52 (1.29-4.94)	2.5 (1.26-4.96)
Ea1189	12	1.55 (1.15-2.08)	0.66 (0.05-8.83)	0.36 (0.2-0.65)	3.81 (2.72-5.33)	2.48 (1.87-3.3)	1.83 (0.65-5.17)
ZYRKD3-1		1.16 (0.92-1.47)	1.17 (0.52-2.64)	0.36 (0.27-0.48)	3.13 (1.69-5.8)	2.24 (1.84-2.72)	2.07 (0.77-5.6)
Ea1189	24	1.36 (0.64-2.87)	0.76 (0.04-13.7)	0.43 (0.34-0.54)	3.23 (1.11-9.36)	2.18 (1.18-4.04)	1.84 (1.29-2.64)
ZYRKD3-1		1.1 (0.82-1.46)	0.63 (0.11-3.76)	0.33 (0.24-0.45)	2.19 (0.99-4.86)	2.04 (0.84-4.95)	1.5 (0.62-3.65)
Ea1189	48	1.25 (0.26-6.12)	0.75 (0.22-2.52)	0.29 (0.18-0.48)	1.46 (0.18-12.11)	0.89 (0.37-2.15)	0.93 (0.43-2)
ZYRKD3-1		0.75 (0.3-1.91)	1.91 (1.44-2.54)	0.35 (0.15-0.81)	1 (0.42-2.37)	0.9 (0.66-1.23)	1.27 (1.14-1.41)

Table 5.13.: (continued)

Samples	Hpi	MDP0000180043	MDP0000180902	MDP0000181339	MDP0000196394	MDP0000197472	MDP0000204381
Mr5_ni	0	1 (0.25-4.03)	1 (0.06-16.8)	1 (0.4-2.48)	1 (0.13-7.94)	1 (0.37-2.68)	1 (0.23-4.35)
Ea1189	1	1.11 (0.39-3.21)	2.52 (0.73-8.68)	1.86 (0.7-4.96)	1.4 (0.33-5.99)	4.09 (0.01-1596.98)	3.33 (1.04-10.65)
ZYRKD3-1		0.95 (0.31-2.93)	0.96 (0.25-3.69)	1.18 (0.43-3.23)	1.19 (0.56-2.55)	3.42 (0.02-608.35)	4.33 (1.81-10.34)
Ea1189	2	1.27 (0.7-2.32)	2.29 (1.37-3.81)	1.71 (0.99-2.95)	0.92 (0.31-2.68)	5.02 (0.01-1951.86)	2.77 (0.77-9.96)
ZYRKD3-1		0.25 (0.07-0.87)	4.11 (2.46-6.88)	1.82 (0.54-6.13)	0.44 (0.12-1.64)	2.19 (0.09-51.6)	1.1 (0.29-4.21)
Ea1189	4	0.92 (0.56-1.53)	1.59 (0.74-3.41)	1.09 (0.61-1.96)	0.56 (0.25-1.24)	19.93 (7.95-49.95)	1.19 (0.56-2.5)
ZYRKD3-1		1 (0.14-7.34)	3.19 (1.25-8.15)	1.62 (1.09-2.4)	0.86 (0.33-2.23)	1.03 (0-1479.44)	1.38 (0.18-10.58)
Ea1189	12	0.78 (0.36-1.65)	1.65 (0.88-3.1)	1.17 (1.14-1.21)	0.62 (0.3-1.27)	5.05 (0.15-171.48)	2.92 (1.3-6.53)
ZYRKD3-1		0.71 (0.37-1.34)	1.87 (1.28-2.74)	1.25 (1.08-1.44)	0.54 (0.35-0.83)	3.48 (0.15-83.21)	2.14 (0.88-5.19)
Ea1189	24	0.66 (0.13-3.43)	1.66 (0.76-3.63)	1.6 (0.85-3.02)	0.69 (0.14-3.4)	2.76 (0.07-107.4)	2.02 (0.63-6.49)
ZYRKD3-1		0.76 (0.19-3.01)	1.81 (0.84-3.91)	1.43 (0.81-2.5)	0.71 (0.16-3.14)	0.77 (0.04-16.27)	1.76 (0.73-4.23)
Ea1189	48	0.82 (0.44-1.54)	0.92 (0.28-3.09)	1.07 (0.34-3.38)	0.59 (0.06-5.42)	11.34 (0.72-177.95)	1.21 (0.22-6.78)
ZYRKD3-1		0.7 (0.32-1.52)	0.95 (0.5-1.81)	0.89 (0.51-1.56)	0.84 (0.72-0.99)	8.94 (0.66-120.55)	0.84 (0.26-2.76)

Samples	Hpi	MDP0000204699	MDP0000204794	MDP0000205617	MDP0000206461	MDP0000211981	MDP0000212178
Mr5_ni	0	no value	1 (0.15-6.49)	1 (0.11-9.41)	no value	1 (0.18-5.64)	1 (0.05-18.38)
Ea1189	1	0.67 (0-1151.79)	3.94 (0.93-16.64)	7.91 (2.28-27.48)	10.1 (0.02-4252.33)	0.37 (0.03-4.2)	1.58 (0.44-5.62)
ZYRKD3-1		no value	1.76 (0.74-4.14)	8.2 (2.64-25.5)	7.55 (0.57-99.61)	0.28 (0.09-0.89)	0.99 (0.48-2.05)
Ea1189	2	0.1 (0.01-1.68)	2.98 (1.17-7.58)	14.34 (5.58-36.82)	1.78 (0.05-63.42)	0.39 (0.12-1.23)	0.9 (0.45-1.81)
ZYRKD3-1		0.06 (0-0.92)	3.61 (1.56-8.37)	10.79 (2.94-39.51)	no value	0.38 (0.09-1.66)	0.59 (0.29-1.23)
Ea1189	4	no value	4.08 (2.64-6.3)	6.38 (1.7-24)	0.31 (0.04-2.15)	0.3 (0.14-0.65)	0.74 (0.38-1.45)
ZYRKD3-1		no value	4.98 (3.3-7.51)	13.31 (6.64-26.66)	0.55 (0-77.39)	0.34 (0.12-0.95)	1.22 (0.52-2.89)
Ea1189	12	0.03 (0.01-0.08)	7.29 (4.26-12.48)	4.54 (1.43-14.36)	0.68 (0.02-19.52)	0.24 (0.07-0.84)	1.23 (1.19-1.28)
ZYRKD3-1		no value	8.11 (4.54-14.47)	4.67 (0.26-83)	no value	0.21 (0.11-0.43)	1.23 (0.95-1.59)
Ea1189	24	0.04 (0.01-0.1)	6.44 (4.66-8.91)	4.48 (0.74-27.26)	5.19 (0.22-122.48)	0.44 (0.21-0.93)	1.11 (0.17-7.43)
ZYRKD3-1		no value	3.33 (1.59-6.95)	4.83 (1.07-21.71)	1.51 (0.01-226.24)	0.49 (0.18-1.3)	1.07 (0.66-1.75)
Ea1189	48	no value	2.17 (0.51-9.17)	0.66 (0.05-8.7)	7.36 (1.22-44.52)	0.3 (0.16-0.55)	0.67 (0.02-20.49)
ZYRKD3-1		no value	1.33 (0.39-4.58)	2.04 (0.17-24.16)	6.47 (1.52-27.59)	0.53 (0.37-0.78)	0.78 (0.52-1.15)

Table 5.13.: (continued)

Samples	Hpi	MDP0000219522	MDP0000219684	MDP0000225509	MDP0000232616	MDP0000233546	MDP0000236390
Mr5_ni	0	1 (0.08-12.47)	1 (0.07-15.09)	1 (0.14-7.39)	1 (0-977.58)	1 (0.16-6.16)	no value
Ea1189	1	3.37 (0.62-18.46)	6.55 (2.91-14.72)	0.38 (0.05-2.84)	12.79 (2.22-73.6)	1.25 (0.18-8.86)	6.15 (0.01-4531.9)
ZYRKD3-1		1.54 (0.55-4.27)	5.85 (3.14-10.91)	0.28 (0.17-0.46)	15.99 (3.67-69.66)	0.7 (0.42-1.18)	1.41 (0.01-144.55)
Ea1189	2	1.79 (0.33-9.65)	6 (2.18-16.57)	0.38 (0.2-0.71)	9.52 (2.05-44.22)	1.48 (0.58-3.74)	1.4 (0.19-10.46)
ZYRKD3-1		1.26 (0.63-2.54)	8.55 (5.59-13.07)	0.26 (0.08-0.85)	6.1 (1.68-22.19)	1.48 (0.6-3.64)	1.51 (0.31-7.5)
Ea1189	4	1.55 (1.28-1.88)	4.88 (2.23-10.65)	0.36 (0.26-0.48)	1.94 (0.51-7.41)	1.06 (0.37-3.09)	0.23 (0.1-0.53)
ZYRKD3-1		1.68 (0.59-4.78)	4.22 (1.88-9.48)	0.42 (0.19-0.92)	4.12 (1.86-9.12)	0.76 (0.51-1.14)	0.21 (0.01-9.05)
Ea1189	12	1.3 (0.44-3.81)	3.43 (1.46-8.09)	0.33 (0.11-0.96)	2.39 (0.19-30.42)	0.47 (0.29-0.75)	0.22 (0.01-4.12)
ZYRKD3-1		1.4 (0.56-3.5)	2.35 (0.61-9.14)	0.29 (0.08-1.03)	3.21 (1.61-6.4)	0.61 (0.26-1.42)	0.19 (0.08-0.45)
Ea1189	24	2.29 (1.17-4.49)	2.01 (0.39-10.24)	0.39 (0.13-1.14)	3.77 (0.4-35.83)	0.76 (0.19-3.02)	0.73 (0.38-1.4)
ZYRKD3-1		1.59 (1.25-2.02)	1.58 (0.32-7.78)	0.5 (0.3-0.82)	2.46 (0.05-110.4)	0.56 (0.39-0.79)	0.11 (0.02-0.75)
Ea1189	48	2.58 (0.3-22.17)	0.94 (0.03-28.1)	0.58 (0.21-1.57)	1.92 (0.09-39.64)	0.91 (0.23-3.64)	0.21 (0.01-4.43)
ZYRKD3-1		1.01 (0.3-3.38)	1.34 (0.78-2.31)	0.86 (0.66-1.14)	4.21 (0.91-19.46)	0.66 (0.08-5.61)	0.15 (0.01-3.48)

Samples	Hpi	MDP0000236723	MDP0000250070	MDP0000262141	MDP0000264060	MDP0000264668	MDP0000265729
Mr5_ni	0	1 (0.13-7.59)	1 (0.02-51.18)	1 (0-466.75)	1 (0.07-14.26)	1 (0.17-5.93)	1 (0.1-10.43)
Ea1189	1	0.31 (0.2-0.46)	0.99 (0.06-16.53)	45.08 (11.84-171.6)	2.16 (1-4.68)	7.86 (3.49-17.72)	0.48 (0.09-2.67)
ZYRKD3-1		0.26 (0.09-0.77)	0.32 (0.08-1.34)	22.85 (9.48-55.06)	1.97 (1.33-2.91)	3.02 (1.45-6.27)	0.28 (0.18-0.42)
Ea1189	2	0.25 (0.11-0.55)	0.65 (0.1-4.21)	31.09 (25.09-38.53)	1.08 (0.35-3.36)	2.79 (0.89-8.77)	0.32 (0.12-0.84)
ZYRKD3-1		0.18 (0.06-0.52)	0.45 (0.09-2.22)	27.37 (7.91-94.75)	1 (0.38-2.63)	4.34 (2.08-9.03)	0.19 (0.06-0.6)
Ea1189	4	0.36 (0.19-0.71)	0.5 (0.15-1.64)	19.35 (4.76-78.63)	1.54 (1.46-1.63)	1.84 (1.01-3.36)	0.62 (0.39-1)
ZYRKD3-1		0.38 (0.32-0.44)	0.68 (0.53-0.87)	27.33 (10.96-68.15)	1.58 (0.78-3.2)	2.45 (1.13-5.27)	0.55 (0.45-0.67)
Ea1189	12	0.35 (0.21-0.6)	0.68 (0.17-2.68)	28.4 (11.23-71.79)	2.75 (0.62-12.15)	1.29 (0.62-2.69)	0.55 (0.29-1.04)
ZYRKD3-1		0.37 (0.32-0.43)	0.81 (0.66-0.99)	30.51 (23.86-39.02)	2.02 (1.46-2.8)	1.07 (0.5-2.33)	0.46 (0.21-1.04)
Ea1189	24	0.4 (0.23-0.7)	0.65 (0.1-4.26)	16.74 (8.6-32.58)	2.35 (0.73-7.58)	0.94 (0.35-2.5)	0.5 (0.33-0.76)
ZYRKD3-1		0.27 (0.2-0.35)	0.76 (0.5-1.17)	8.35 (6.43-10.84)	2.03 (0.92-4.49)	0.65 (0.39-1.09)	0.49 (0.31-0.78)
Ea1189	48	0.24 (0.17-0.33)	0.53 (0.07-3.86)	7.48 (2.41-23.16)	1.49 (0.02-116.8)	0.78 (0.24-2.51)	0.58 (0.08-3.95)
ZYRKD3-1		0.29 (0.1-0.84)	0.37 (0.25-0.55)	5.9 (3.39-10.26)	1.59 (1.02-2.47)	0.49 (0.12-1.97)	0.82 (0.4-1.68)

Table 5.13.: (continued)

Samples	Hpi	MDP0000265874	MDP0000268523	MDP0000272542	MDP0000277718	MDP0000286136	MDP0000289300
Mr5_ni	0	1 (0.06-15.64)	1 (0.31-3.19)	1 (0.11-9.32)	1 (0.23-4.36)	1 (0.17-6.02)	1 (0.12-8.6)
Ea1189	1	0.38 (0.03-4.32)	5.22 (1.04-26.23)	0.82 (0.39-1.74)	1.96 (1.41-2.72)	1.49 (0.37-6)	1.23 (0.67-2.28)
ZYRKD3-1		0.34 (0-256.03)	1.73 (0.46-6.47)	0.36 (0.21-0.63)	1.74 (1.35-2.24)	1.18 (0.81-1.7)	1.13 (0.96-1.33)
Ea1189	2	6.74 (0.05-978.62)	2.23 (1.39-3.6)	0.61 (0.3-1.24)	1.71 (0.93-3.14)	1.31 (0.29-5.82)	0.64 (0.58-0.72)
ZYRKD3-1		3.49 (0.06-188.14)	1.27 (0.47-3.43)	0.64 (0.33-1.27)	1.23 (0.72-2.09)	3.12 (0.87-11.15)	0.42 (0.14-1.27)
Ea1189	4	0.68 (0.09-5)	1.72 (1.13-2.63)	0.74 (0.44-1.24)	1.51 (1.15-1.99)	0.19 (0.01-3.26)	0.49 (0.26-0.95)
ZYRKD3-1		0.84 (0.13-5.55)	1.66 (0.79-3.48)	1.13 (0.71-1.79)	2.09 (1.03-4.22)	0.65 (0.11-3.84)	0.73 (0.21-2.54)
Ea1189	12	26.09 (7.62-89.34)	3.32 (1.3-8.51)	0.75 (0.36-1.59)	1.83 (0.79-4.25)	0.29 (0.08-0.98)	0.94 (0.79-1.12)
ZYRKD3-1		3.89 (0-113793.81)	3.12 (1.71-5.72)	0.71 (0.51-1)	2.04 (1.12-3.7)	0.32 (0.02-6.52)	0.92 (0.53-1.59)
Ea1189	24	3.5 (0.9-13.58)	2.65 (1.11-6.35)	0.79 (0.38-1.65)	2.14 (0.86-5.32)	0.6 (0.28-1.25)	1.15 (0.21-6.39)
ZYRKD3-1		4.3 (0.23-81.3)	1.83 (0.92-3.64)	0.82 (0.58-1.17)	1.82 (1.02-3.24)	0.77 (0.25-2.33)	1.1 (0.47-2.6)
Ea1189	48	3.25 (0.07-150.41)	1.85 (0.28-12.37)	0.58 (0.08-4.25)	1.56 (0.66-3.71)	0.13 (0.03-0.47)	0.78 (0.06-10.27)
ZYRKD3-1		1.84 (0.02-141.11)	0.88 (0.2-3.82)	0.58 (0.35-0.99)	1.58 (1.05-2.37)	0.37 (0.07-2.05)	0.83 (0.56-1.25)

Samples	Hpi	MDP0000294096	MDP0000296339	MDP0000307705	MDP0000309976	MDP0000311359	MDP0000316497
Mr5_ni	0	1 (0.64-1.57)	1 (0.11-8.77)	1 (0.17-5.89)	1 (0.16-6.34)	1 (0.19-5.25)	1 (0.07-13.35)
Ea1189	1	3.53 (0.44-28.55)	1.56 (0.37-6.67)	1.31 (0.39-4.45)	1.35 (0.78-2.33)	2.65 (0.86-8.14)	2.15 (1.1-4.18)
ZYRKD3-1		1.6 (1.22-2.08)	0.86 (0.59-1.27)	0.76 (0.27-2.12)	0.9 (0.84-0.97)	1.97 (0.48-7.98)	1.42 (0.19-10.46)
Ea1189	2	1.35 (0.43-4.16)	0.77 (0.53-1.13)	0.85 (0.52-1.39)	1.11 (0.65-1.89)	1.89 (1.29-2.77)	3.36 (2.8-4.05)
ZYRKD3-1		1.63 (0.65-4.08)	0.35 (0.11-1.11)	1.29 (0.81-2.03)	0.5 (0.33-0.74)	2.64 (1.65-4.24)	10.57 (4.56-24.51)
Ea1189	4	1.53 (0.62-3.75)	1 (0.61-1.63)	0.56 (0.33-0.97)	1.09 (0.57-2.07)	1.64 (1-2.68)	2.71 (1.78-4.13)
ZYRKD3-1		2.44 (1.85-3.22)	0.89 (0.81-0.99)	0.74 (0.14-3.91)	1.13 (0.45-2.86)	2.23 (1.49-3.34)	2.66 (0.59-12.01)
Ea1189	12	1.71 (1-2.92)	2.11 (1.54-2.91)	0.58 (0.29-1.18)	0.99 (0.53-1.85)	2 (0.97-4.12)	3.31 (1.66-6.61)
ZYRKD3-1		1.68 (0.76-3.74)	1.64 (0.74-3.64)	0.68 (0.3-1.52)	0.97 (0.4-2.3)	1.86 (1.1-3.15)	3.83 (1.61-9.09)
Ea1189	24	1.71 (0.94-3.1)	0.84 (0.21-3.33)	0.61 (0.28-1.36)	0.69 (0.17-2.88)	1.73 (0.42-7.1)	4.04 (1.92-8.49)
ZYRKD3-1		1.38 (0.74-2.56)	0.95 (0.15-5.89)	0.49 (0.35-0.69)	0.74 (0.41-1.33)	1.52 (0.68-3.4)	4.11 (0.99-17.06)
Ea1189	48	1.34 (0.41-4.39)	1.32 (0.15-11.42)	0.42 (0.06-3.17)	1.19 (0.35-4.05)	1.65 (0.26-10.42)	0.57 (0.16-2.11)
ZYRKD3-1		0.94 (0.71-1.24)	1.32 (1.11-1.58)	0.71 (0.31-1.6)	0.91 (0.4-2.04)	1.09 (0.75-1.59)	0.85 (0.02-33.47)

Table 5.13.: (continued)

Samples	**Hpi**	**MDP0000317974**	**MDP0000320910**	**MDP0000324831**	**MDP0000327191**	**MDP0000343634**	**MDP0000353793**
Mr5_ni	0	1 (0.08-12.94)	1 (0.06-17.75)	1 (0.1-10.36)	1 (0.19-5.25)	1 (0.33-3.06)	1 (0.11-8.84)
Ea1189	1	7.15 (3-17.06)	0.41 (0.25-0.68)	1.59 (0.27-9.49)	0.81 (0.6-1.11)	0.77 (0.41-1.45)	0.73 (0.19-2.85)
ZYRKD3-1		6.33 (1.86-21.51)	0.2 (0.05-0.76)	2.32 (1.54-3.5)	1.17 (0.53-2.56)	0.4 (0.19-0.82)	0.43 (0.24-0.77)
Ea1189	2	3.6 (2.15-6.05)	0.37 (0.17-0.8)	1.5 (0.65-3.44)	1.38 (0.79-2.44)	0.46 (0.15-1.37)	0.61 (0.5-0.74)
ZYRKD3-1		2.57 (0.8-8.22)	0.45 (0.22-0.94)	1.36 (0.36-5.11)	2.03 (0.86-4.82)	0.67 (0.49-0.91)	0.47 (0.17-1.26)
Ea1189	4	3.51 (2.58-4.78)	0.41 (0.26-0.64)	0.81 (0.58-1.14)	0.67 (0.18-2.51)	0.31 (0.14-0.67)	0.55 (0.35-0.84)
ZYRKD3-1		3.36 (0.54-20.96)	0.68 (0.34-1.36)	1.09 (0.28-4.27)	1.45 (0.87-2.4)	0.69 (0.48-1)	0.46 (0.14-1.5)
Ea1189	12	4.95 (2.92-8.38)	0.42 (0.34-0.52)	1.29 (1.18-1.42)	0.84 (0.42-1.71)	0.33 (0.13-0.86)	0.63 (0.35-1.13)
ZYRKD3-1		4.56 (3.42-6.08)	0.56 (0.5-0.64)	0.91 (0.66-1.27)	1.19 (0.5-2.81)	0.56 (0.39-0.8)	0.53 (0.17-1.62)
Ea1189	24	5.61 (1.11-28.42)	0.63 (0.46-0.86)	1 (0.33-3.02)	1.03 (0.55-1.95)	1.26 (0.29-5.44)	0.55 (0.29-1.04)
ZYRKD3-1		5.87 (3.79-9.1)	0.56 (0.2-1.57)	1.04 (0.28-3.81)	1.12 (0.72-1.77)	0.86 (0.23-3.26)	0.56 (0.12-2.58)
Ea1189	48	4.62 (0.21-101.16)	0.59 (0.2-1.74)	0.87 (0.14-5.25)	0.77 (0.23-2.57)	1.16 (0.73-1.83)	0.66 (0.25-1.76)
ZYRKD3-1		2.03 (1.52-2.73)	0.51 (0.15-1.72)	0.78 (0.36-1.69)	1.28 (0.57-2.9)	1.1 (0.41-2.97)	0.91 (0.63-1.29)

Samples	**Hpi**	**MDP0000364885**	**MDP0000404331**	**MDP0000440654**	**MDP0000551952**	**MDP0000597996**	**MDP0000609876**
Mr5_ni	0	1 (0.02-54.89)	1 (0.16-6.38)	1 (0.27-3.72)	1 (0.05-19.52)	1 (0.15-6.56)	1 (0.03-34.7)
Ea1189	1	19.84 (5.47-71.97)	2.7 (0.56-13.1)	4.28 (1.23-14.91)	1.09 (0.16-7.44)	1.61 (1.09-2.39)	4.24 (3.06-5.89)
ZYRKD3-1		12.56 (3.69-42.7)	0.73 (0.2-2.75)	3.37 (1.26-9.02)	0.53 (0.16-1.73)	0.77 (0.48-1.22)	4.91 (3.68-6.56)
Ea1189	2	16 (6.49-39.42)	0.99 (0.78-1.24)	4.03 (2.12-7.65)	0.77 (0.13-4.47)	0.97 (0.41-2.29)	3.27 (0.98-10.94)
ZYRKD3-1		9.07 (2.05-40.06)	0.68 (0.44-1.04)	2.77 (1.38-5.57)	0.71 (0.13-3.82)	0.97 (0.25-3.7)	3.03 (1.29-7.11)
Ea1189	4	7.17 (1.88-27.33)	1.19 (0.56-2.53)	2.48 (0.73-8.45)	0.78 (0.22-2.76)	1.12 (0.89-1.41)	2.18 (1.09-4.38)
ZYRKD3-1		8.21 (1.05-64.36)	1.2 (0.69-2.09)	2.52 (0.72-8.87)	0.62 (0.1-3.93)	0.75 (0.22-2.6)	2.53 (0.7-9.13)
Ea1189	12	18.15 (14.79-22.28)	1.63 (0.59-4.5)	2.68 (0.97-7.37)	0.94 (0.2-4.39)	1.08 (0.83-1.4)	3.23 (1.14-9.12)
ZYRKD3-1		9.5 (4.96-18.17)	1.5 (1.19-1.9)	2.28 (0.93-5.56)	0.73 (0.14-3.85)	0.79 (0.35-1.81)	3.05 (2.23-4.19)
Ea1189	24	11.42 (2.78-46.92)	1.01 (0.55-1.86)	4.38 (1.33-14.46)	0.74 (0.34-1.58)	0.9 (0.41-1.98)	4.25 (2.09-8.66)
ZYRKD3-1		8.77 (7.3-10.54)	1.03 (0.52-2.03)	2.04 (0.54-7.65)	0.78 (0.17-3.65)	0.78 (0.34-1.77)	3.75 (2.44-5.78)
Ea1189	48	7.42 (0.56-98.35)	2.11 (0.09-51.84)	2.05 (0.49-8.61)	0.64 (0.08-5.33)	0.79 (0.18-3.52)	4.27 (1.36-13.36)
ZYRKD3-1		4.78 (1.58-14.51)	0.99 (0.48-2.04)	1.01 (0.29-3.46)	1.06 (0.49-2.27)	1.11 (0.7-1.74)	3.85 (2.84-5.21)

Table 5.13.: (continued)

Samples	Hpi	MDP0000609966	MDP0000612660	MDP0000617684	MDP0000628976	MDP0000644109	MDP0000668657
Mr5_ni	0	1 (0.18-5.54)	1 (0.01-85.54)	1 (0.38-2.63)	1 (0.04-23.3)	1 (0.71-1.41)	1 (0.02-53.04)
Ea1189	1	3.47 (1.22-9.86)	5.7 (2.03-16.04)	0.84 (0.43-1.63)	1.52 (1.18-1.95)	2.12 (0.48-9.31)	7.12 (2.95-17.22)
ZYRKD3-1		2.1 (0.58-7.69)	5 (1.42-17.65)	0.8 (0.39-1.66)	1.32 (0.28-6.24)	0.73 (0.19-2.79)	6.91 (5.37-8.88)
Ea1189	2	3.08 (1.09-8.7)	2.56 (0.61-10.69)	1 (0.79-1.27)	2.56 (1.17-5.57)	0.89 (0.48-1.67)	15.86 (11.94-21.06)
ZYRKD3-1		3.45 (2.25-5.28)	2.38 (1.13-5.04)	0.77 (0.64-0.93)	5.04 (4.24-5.99)	0.67 (0.31-1.47)	26.69 (11.48-62.07)
Ea1189	4	2.53 (1.15-5.53)	1.88 (0.37-9.57)	0.95 (0.48-1.9)	1.5 (0.88-2.54)	1.5 (0.6-3.74)	16.9 (14.81-19.29)
ZYRKD3-1		3.37 (1.98-5.73)	2.61 (0.77-8.85)	0.92 (0.64-1.32)	2.4 (1.03-5.59)	1.35 (1.04-1.76)	19.24 (12.88-28.74)
Ea1189	12	1.92 (0.82-4.46)	1.46 (0.73-2.92)	1.22 (0.85-1.75)	2.66 (1.57-4.52)	1.72 (0.74-4)	30.79 (15.15-62.58)
ZYRKD3-1		1.66 (0.7-3.91)	1.18 (0.41-3.42)	1.11 (0.82-1.52)	3.35 (2.33-4.81)	1.71 (1.23-2.36)	33.38 (25.75-43.28)
Ea1189	24	2.13 (0.64-7.16)	4.32 (1.32-14.12)	0.97 (0.58-1.6)	2.87 (0.79-10.43)	0.98 (0.64-1.51)	6.05 (4.91-7.47)
ZYRKD3-1		1.72 (0.92-3.23)	2.47 (0.71-8.67)	0.83 (0.4-1.73)	2.37 (0.56-9.92)	1.25 (0.62-2.52)	2.77 (1.88-4.1)
Ea1189	48	1.17 (0.18-7.69)	3.67 (0.32-41.95)	0.73 (0.38-1.37)	0.48 (0.29-0.81)	2.3 (0.23-22.88)	1.4 (0.41-4.8)
ZYRKD3-1		1.05 (0.67-1.66)	2.48 (0.47-13.04)	0.76 (0.52-1.12)	0.45 (0.07-2.94)	1.2 (0.65-2.21)	1.23 (0.3-5.1)

Samples	Hpi	MDP0000680997	MDP0000696168	MDP0000711911	MDP0000737128	MDP0000750217	MDP0000750789
Mr5_ni	0	1 (0.21-4.74)	1 (0.14-6.91)	1 (0.01-199.71)	1 (0.46-2.18)	1 (0.39-2.58)	1 (0.03-31.41)
Ea1189	1	1.4 (0.45-4.34)	1.13 (0.49-2.63)	11.58 (0.04-3358.81)	1 (0.78-1.28)	0.47 (0.12-1.84)	0.15 (0.02-1.34)
ZYRKD3-1		0.78 (0.44-1.39)	0.67 (0.23-1.98)	2.65 (0.19-37.08)	0.7 (0.25-1.94)	0.33 (0.04-2.68)	0.03 (0.01-0.12)
Ea1189	2	1.07 (0.66-1.74)	0.72 (0.49-1.05)	1.66 (0.19-14.51)	1.24 (1.09-1.42)	0.36 (0.13-0.94)	0.09 (0.03-0.23)
ZYRKD3-1		1.04 (0.65-1.69)	0.58 (0.46-0.74)	1.05 (0.3-3.64)	0.8 (0.45-1.41)	0.25 (0.1-0.61)	0.2 (0.02-2.13)
Ea1189	4	0.88 (0.49-1.56)	0.65 (0.49-0.87)	0.6 (0.29-1.25)	1.28 (1.12-1.47)	0.39 (0.17-0.86)	0.12 (0.07-0.19)
ZYRKD3-1		1.38 (0.86-2.22)	0.85 (0.38-1.88)	0.52 (0.11-2.58)	2.14 (0.98-4.64)	0.47 (0.14-1.55)	0.19 (0.01-2.72)
Ea1189	12	1.01 (0.71-1.44)	0.92 (0.85-0.99)	0.79 (0.72-0.87)	1.58 (1.19-2.1)	0.43 (0.34-0.54)	0.21 (0.05-0.9)
ZYRKD3-1		1.13 (0.89-1.44)	0.9 (0.64-1.26)	0.41 (0.38-0.44)	1.7 (1.64-1.77)	0.35 (0.21-0.56)	0.29 (0.04-2.44)
Ea1189	24	1.18 (0.39-3.59)	0.78 (0.7-0.87)	0.97 (0.26-3.65)	1.15 (0.44-3.03)	0.44 (0.28-0.71)	0.18 (0.05-0.67)
ZYRKD3-1		1.09 (0.95-1.26)	0.64 (0.57-0.73)	0.3 (0.02-5.84)	1.01 (0.75-1.37)	0.22 (0.15-0.33)	0.2 (0.12-0.34)
Ea1189	48	0.77 (0.06-9.83)	0.79 (0.4-1.54)	0.45 (0.1-2.09)	0.76 (0.43-1.35)	0.46 (0.24-0.9)	0.16 (0.04-0.63)
ZYRKD3-1		0.66 (0.5-0.87)	0.74 (0.32-1.7)	0.33 (0.21-0.53)	0.81 (0.73-0.89)	0.49 (0.11-2.27)	0.26 (0.02-3.6)

Table 5.13.: (continued)

Samples	Hpi	MDP0000782642	MDP0000784187	MDP0000858763	MDP0000868044	MDP0000874252	MDP0000891117
Mr5_ni	0	1 (0.05-19.47)	1 (0.12-8.2)	1 (0.08-12.24)	1 (0.12-8.24)	1 (0.17-5.98)	1 (0.03-36.78)
Ea1189	1	1.07 (0.29-3.91)	3.09 (0.62-15.39)	1.62 (0.03-91.35)	1.85 (0.97-3.51)	0.44 (0.12-1.56)	4.58 (1.57-13.3)
ZYRKD3-1		1.84 (0.67-5.08)	1.14 (0.46-2.8)	1.15 (0.35-3.82)	1.63 (0.86-3.11)	0.24 (0.15-0.37)	2.81 (0.69-11.47)
Ea1189	2	1.82 (0.53-6.25)	1.54 (0.37-6.39)	0.82 (0.24-2.82)	0.97 (0.4-2.36)	0.35 (0.16-0.76)	3.09 (1.71-5.6)
ZYRKD3-1		1.44 (0.55-3.79)	1.18 (0.25-5.44)	0.84 (0.07-10.87)	0.62 (0.34-1.13)	0.43 (0.2-0.89)	2.43 (0.86-6.86)
Ea1189	4	1.22 (0.8-1.86)	2.18 (1.44-3.31)	1.04 (0.33-3.31)	1.43 (1.09-1.87)	0.48 (0.34-0.68)	2.08 (0.76-5.69)
ZYRKD3-1		1.49 (0.79-2.82)	3.86 (2.23-6.69)	0.68 (0.21-2.21)	1.74 (1.55-1.95)	0.48 (0.24-0.97)	2.56 (1.15-5.71)
Ea1189	12	0.93 (0.17-5.12)	2.24 (0.46-10.87)	0.86 (0.48-1.52)	2.14 (0.7-6.49)	0.45 (0.24-0.84)	3.05 (1.87-4.99)
ZYRKD3-1		1.24 (0.81-1.9)	2.34 (1.04-5.25)	0.43 (0.33-0.58)	2.07 (0.94-4.58)	0.46 (0.27-0.79)	2.81 (1.99-3.96)
Ea1189	24	1.16 (0.2-6.72)	2.6 (1.03-6.53)	0.68 (0.1-4.52)	1.77 (0.64-4.93)	0.5 (0.24-1.02)	2.68 (0.51-14.09)
ZYRKD3-1		0.75 (0.42-1.36)	2.44 (1.49-3.99)	1.41 (0.58-3.41)	1.55 (1.03-2.33)	0.45 (0.34-0.61)	2.19 (1.62-2.96)
Ea1189	48	1.22 (0.69-2.14)	2.61 (0.24-28.63)	0.26 (0-24.92)	1.38 (0.18-10.32)	0.67 (0.22-1.98)	1.92 (0.14-26)
ZYRKD3-1		2.51 (1.7-3.71)	1.24 (0.3-5.04)	0.93 (0.08-11.17)	0.94 (0.38-2.35)	0.84 (0.29-2.44)	1.41 (0.81-2.46)

Samples	Hpi	MDP0000919962	MDP0000921319	MDP0000929055	MDP0000937986	MDP0000940742	MDP0000944210
Mr5_ni	0	1 (0.28-3.52)	1 (0.1-10.31)	1 (0.14-6.94)	1 (0.2-5.03)	1 (0.13-7.89)	1 (0.04-22.92)
Ea1189	1	5.61 (1.6-19.64)	0.44 (0.18-1.09)	1.25 (0.12-12.8)	5.54 (2.12-14.51)	5.19 (1.7-15.86)	2.07 (0.38-11.19)
ZYRKD3-1		5.99 (2.47-14.56)	0.29 (0.13-0.61)	0.78 (0.07-9)	2.24 (1.29-3.88)	3.61 (2.52-5.18)	2.13 (0.99-4.59)
Ea1189	2	12.06 (6.48-22.45)	0.23 (0.19-0.27)	0.99 (0.09-10.27)	7.25 (4.18-12.56)	3.4 (0.86-13.54)	1.79 (0.73-4.39)
ZYRKD3-1		8.48 (1.99-36.18)	0.12 (0.08-0.2)	0.99 (0.16-6.23)	8.1 (7.91-8.3)	1.66 (0.9-3.06)	0.93 (0.42-2.07)
Ea1189	4	5.16 (1.14-23.41)	0.26 (0.2-0.32)	0.97 (0.12-7.84)	6.07 (3-12.28)	2.32 (1.16-4.68)	1.36 (0.62-2.97)
ZYRKD3-1		11.8 (6.54-21.28)	0.26 (0.16-0.42)	0.85 (0.08-8.66)	5.31 (2.73-10.34)	2.24 (0.45-11.05)	1.87 (0.3-11.63)
Ea1189	12	3.59 (1.21-10.67)	0.2 (0.17-0.24)	1.03 (0.09-12.11)	1.88 (0.79-4.47)	3.06 (1.97-4.74)	2.44 (1.1-5.42)
ZYRKD3-1		3.89 (0.23-65.3)	0.17 (0.06-0.54)	0.93 (0.08-10.73)	1.86 (0.71-4.85)	2.36 (1.72-3.25)	1.38 (1.03-1.85)
Ea1189	24	3.74 (0.48-29.01)	0.35 (0.17-0.72)	0.9 (0.12-6.64)	1.8 (1.43-2.27)	2.72 (1.53-4.85)	1.97 (0.34-11.48)
ZYRKD3-1		4.11 (1.04-16.21)	0.35 (0.15-0.8)	0.88 (0.08-10.09)	1.05 (1.04-1.06)	2.07 (0.82-5.27)	1.86 (0.63-5.48)
Ea1189	48	0.75 (0.08-7.01)	1.31 (0.63-2.76)	0.88 (0.07-10.81)	0.78 (0.62-0.97)	1.99 (0.62-6.42)	1.69 (0.08-35.27)
ZYRKD3-1		1.85 (0.16-21.6)	1.68 (1.24-2.27)	0.94 (0.12-7.32)	0.9 (0.22-3.64)	1.16 (0.78-1.72)	1 (0.59-1.71)

O. Genes analysed by BioMark™ HD system

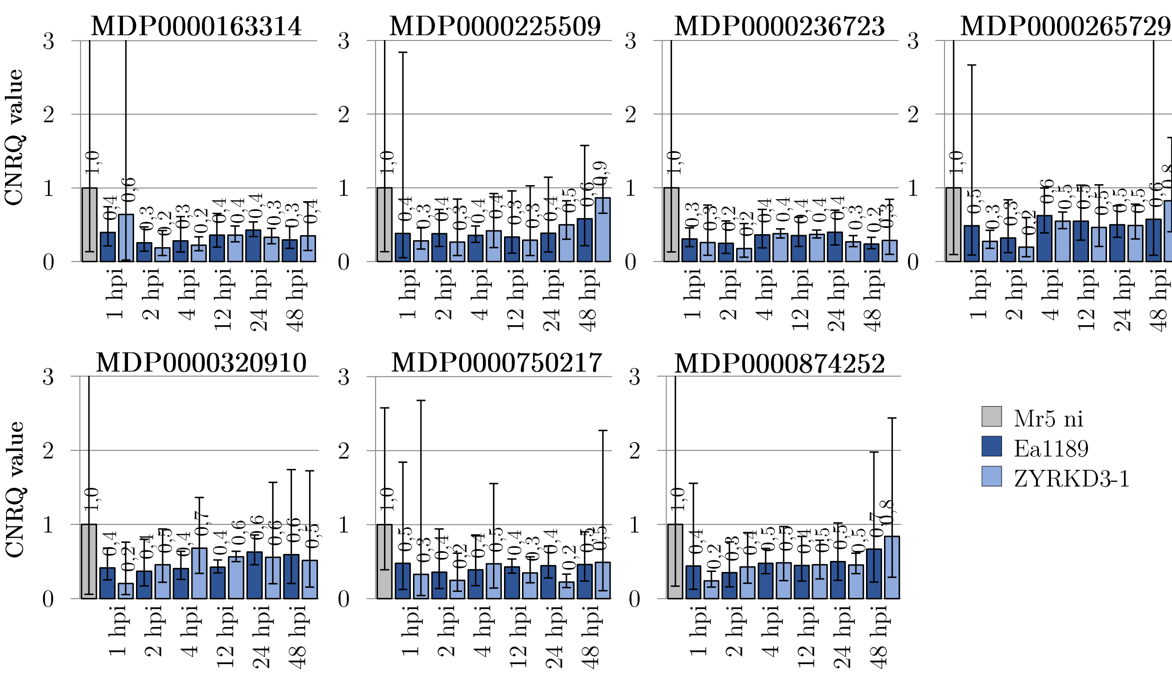


Figure 5.6.: CNRQ values of genes with a lower expression compared to the non-infected sample analysed with the BioMark™ HD system, 95 % confidence interval.

P. Statistical analysis by SAS of the CNRQ values of the genes analysed by BioMark™ HD system

The CNRQ values of the genes analysed by BioMark™ HD system were statistically evaluated by SAS.

P.1. Shapiro-Wilk, Kruskal-Wallis and Levene's test

Table 5.14.: Shapiro-Wilk, Kruskal-Wallis and Levene's test with the CNRQ values of the genes analysed by BioMark™ HD system. Shapiro-Wilk normality test was applied to all genes, except the housekeeping genes. Normal distributed data were further analysed by Levene's test, whereby not normal distributed data were further analysed by Kruskal-Wallis test.

Gene	**Shapiro-Wilk test**[1]	**Kruskal-Wallis test**[2]	**Levene's test**[3]	Gene	**Shapiro-Wilk test**	**Kruskal-Wallis test**	**Levene's test**	Gene	**Shapiro-Wilk test**	**Kruskal-Wallis test**	**Levene's test**	Gene	**Shapiro-Wilk test**	**Kruskal-Wallis test**	**Levene's test**
MDP0000047589	+		-	MDP0000205617	+		-	MDP0000296339	+		+	MDP0000680997	+		+
MDP0000095637	+		-	MDP0000206461	+		-	MDP0000307705	+		+	MDP0000696168	+		+
MDP0000119630	+		-	MDP0000211981	+		-	MDP0000309976	+		+	MDP0000711911	-	+	
MDP0000120176	+		+	MDP0000212178	+		+	MDP0000311359	+		+	MDP0000737128	+		+
MDP0000126761	-	-		MDP0000219522	+		+	MDP0000316497	+		+	MDP0000750217	+		+
MDP0000131100	+		+	MDP0000219684	+		-	MDP0000317974	+		+	MDP0000750789	+		-
MDP0000136037	+		+	MDP0000225509	+		+	MDP0000320910	+		+	MDP0000782642	+		+
MDP0000139165	-	-		MDP0000232616	+		+	MDP0000324831	+		+	MDP0000784187	+		-
MDP0000151003	-	+		MDP0000233546	+		-	MDP0000327191	+		-	MDP0000858763	-	-	
MDP0000159251	+		+	MDP0000236390	-	-		MDP0000343634	+		-	MDP0000868044	+		+
MDP0000159572	+		+	MDP0000236723	-	+		MDP0000353793	-	-		MDP0000874252	+		+
MDP0000163314	+		+	MDP0000250070	-	-		MDP0000364885	+		-	MDP0000891117	+		+
MDP0000165381	+		+	MDP0000262141	+		+	MDP0000404331	+		+	MDP0000919962	+		-
MDP0000166138	+		-	MDP0000264060	-	-		MDP0000440654	+		-	MDP0000921319	-	+	
MDP0000174537	+		-	MDP0000264668	+		-	MDP0000551952	-	-		MDP0000929055	-	-	
MDP0000180043	+		-	MDP0000265729	+		+	MDP0000597996	+		+	MDP0000937986	-	+	
MDP0000180902	+		+	MDP0000265874	+		+	MDP0000609876	+		-	MDP0000940742	+		+
MDP0000181339	+		-	MDP0000268523	+		-	MDP0000609966	+		-	MDP0000944210	+		+
MDP0000196394	+		-	MDP0000272542	+		+	MDP0000612660	+		+	EH034548	+		+
MDP0000197472	+		-	MDP0000277718	+		+	MDP0000617684	+		-	FB_Mr5q1	+		-
MDP0000204381	+		-	MDP0000286136	+		-	MDP0000628976	+		+	FB_Mr5q2	+		-
MDP0000204699	-	-		MDP0000289300	+		+	MDP0000644109	+		+				
MDP0000204794	+		-	MDP0000294096	+		-	MDP0000668657	+		+				

[1]The test was performed on log transformed data. If no normal distribution could be observed data were analysed untransformed, square root or multiplicative inverse transformed. Normal distributed data were marked with +, not normal distributed data were marked with -; [2]only not normal distributed data were analysed, data with a p value less then 0.05 were marked with +, data with a p value more then 0.05 were marked with -; [3]only normal distributed data were analysed, data with a p value less then 0.05 were marked with +, data with a p value more then 0.05 were marked with -

P.2. ANOVA and Tukey's HSD test

Table 5.15.: Tukey's HSD test in conjunction with an ANOVA of the normal distributed CNRQ values of the genes analysed by BioMark™ HD system.

Gene	ANOVA	Mr5_not inoculated	Ea1189 1 hpi	ZYRKD3-1 1 hpi	Ea1189 2 hpi	ZYRKD3-1 2 hpi	Ea1189 4 hpi	ZYRKD3-1 4 hpi	Ea1189 12 hpi	ZYRKD3-1 12 hpi	Ea1189 24 hpi	ZYRKD3-1 24 hpi	Ea1189 48 hpi	ZYRKD3-1 48 hpi
MDP0000120176	<0.0001		A	A	A	A	A	A			A			
		B	B	B	B		B		B	B	B	B		
		C	C	C					C	C	C	C		C
		D		D					D	D	D	D	D	D
MDP0000131100	0.0056	A	A	A	A	A	A	A	A	A	A	A		
		B	B	B	B		B	B	B	B	B	B		B
		C		C	C		C	C	C	C	C	C	C	C
MDP0000136037	<0.0001		A		A				A	A	A			
			B		B	B		B	B		B	B		
		C	C	C	C	C	C	C			C	C		
		D		D		D	D	D				D	D	
		E		E			E						E	E
MDP0000159251	0.2265	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000159572	0.6763	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000163314	0.0325	A	A	A	A		A		A	A	A	A	A	A
			B	B	B	B	B	B	B	B	B	B	B	B
MDP0000165381	0.0348	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000180902	0.0055		A		A	A	A	A	A	A	A	A		
		B	B	B	B		B	B	B	B	B	B	B	B
MDP0000212178	0.7755	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000219522	0.2935	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000225509	0.0219	A	A	A	A		A	A	A	A	A	A	A	A
			B	B	B	B	B	B	B	B	B	B	B	B
MDP0000232616	0.1534	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000262141	0.0002		A	A	A	A	A	A	A	A	A	A	A	A
		B										B	B	B
MDP0000265729	0.0163	A	A	A	A		A	A	A	A	A	A	A	A
			B	B	B		B	B	B	B	B	B	B	
MDP0000265874	0.217	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000272542	0.1909	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000277718	0.1239	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000289300	0.2336	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000296339	0.0263	A	A	A	A		A	A	A	A	A	A	A	A
		B		B	B	B	B	B			B	B	B	B
MDP0000307705	0.1082	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000309976	0.1811	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000311359	0.1977	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000316497	0.0007		A		A	A	A	A	A	A	A	A		
		B	B	B	B		B	B	B	B	B	B		B
		C	C	C	C		C	C	C	C			C	C
MDP0000317974	0.0191		A	A	A	A	A	A	A	A	A	A	A	A
		B			B	B	B	B	B	B			B	B
MDP0000320910	0.05	A		A	A	A	A	A	A	A	A	A	A	A
			B	B	B	B	B	B	B	B	B	B	B	B
MDP0000324831	0.3563	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000404331	0.118	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000597996	0.5565	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000612660	0.1538	A	A	A	A	A	A	A	A	A	A	A	A	A

Table 5.15.: (continued)

Gene	ANOVA	Mr5_not inoculated	Ea1189 1 hpi	ZYRKD3-1 1 hpi	Ea1189 2 hpi	ZYRKD3-1 2 hpi	Ea1189 4 hpi	ZYRKD3-1 4 hpi	Ea1189 12 hpi	ZYRKD3-1 12 hpi	Ea1189 24 hpi	ZYRKD3-1 24 hpi	Ea1189 48 hpi	ZYRKD3-1 48 hpi
MDP0000628976	0.0001		A	A	A	A	A	A	A	A	A	A		
		B	B	B	B		B	B	B	B	B	B		
		C	C	C			C						C	C
MDP0000644109	0.0159	A	A	A	A		A	A	A	A	A	A	A	A
		B	B	B	B	B	B	B	B	B	B	B		B
MDP0000668657	<0.0001		A		A	A	A	A	A	A				
			B	B	B	B	B	B	B					
			C	C	C	C	C	C			C			
			D	D							D	D		
											E	E	E	
		F										F	F	F
MDP0000680997	0.5449	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000696168	0.3716	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000737128	<0.0001				A		A	A	A	A	A			
		B	B		B		B		B	B	B	B		
		C	C	C	C	C	C				C	C	C	C
MDP0000750217	0.0398	A	A	A	A		A	A	A	A	A		A	A
			B	B	B	B	B	B	B	B	B	B	B	B
MDP0000782642	0.3638	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000868044	0.042	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000874252	0.0085	A	A		A	A	A	A	A	A	A	A	A	A
			B	B	B	B	B	B	B	B	B	B	B	
MDP0000891117	0.3506	A	A	A	A	A	A	A	A	A	A	A	A	A
MDP0000940742	0.0037		A	A	A	A	A	A	A	A	A	A	A	
				B	B	B	B	B	B	B	B	B	B	B
		C			C	C	C	C	C	C	C	C	C	C
MDP0000944210	0.7068	A	A	A	A	A	A	A	A	A	A	A	A	A
EH034548	0.0694	A	A	A	A	A	A	A	A	A	A	A	A	A

P.3. Mann-Whitney U test

Table 5.16.: Mann-Whitney U test of not normal distributed CNRQ values of the genes analysed by BioMark™ HD system to find differences.

Gene	Ea1189 1 hpi	ZYRKD3-1 1 hpi	Ea1189 2 hpi	ZYRKD3-1 2 hpi	Ea1189 4 hpi	ZYRKD3-1 4 hpi	Ea1189 12 hpi	ZYRKD3-1 12 hpi	Ea1189 24 hpi	ZYRKD3-1 24 hpi	Ea1189 48 hpi	ZYRKD3-1 48 hpi
MDP0000151003	0.08	0.38	0.08	0.08	0.19	0.08	0.66	1.00	1.00	0.66	1.00	0.08
MDP0000236723	0.08	0.19	0.08	0.08	0.19	0.19	0.19	0.19	0.38	0.08	0.08	0.66
MDP0000711911	0.19	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	1.00	0.19	0.38
MDP0000921319	0.19	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.19	0.19	1.00	0.38
MDP0000937986	0.08	0.19	0.08	0.08	0.08	0.08	0.38	0.38	0.38	0.66	0.08	1.00

Q. Sequences of the *FB_MR5* gene obtained by 3' and 5' RACE-PCR

Q.1. 5' RACE-PCR of the *FB_MR5* gene

Alignment of the nucleic acid sequence of the *FB_MR5* gene (accession number: CCH50986.1) including 600 bp before the start codon with the sequences received from 5' RACE-PCR of the *FB_MR5* gene; sequences for the *FB_MR5* gene (accession number: CCH50986.1) and the flanking regions (Bac 16k15, contig2/4, HE805491) were obtained from the ENA; yellow marks the vector sequence, green marks the primer sequence, red marks the start codon.

```
                         -590       -580       -570       -560       -550       -540       -530
5RACE_K17    AAGATCGCGG ATCCGAACAC TGCGTTTGCT -GGCTTTGAT GA-----AA- ---------- ----GATTTT
5RACE_K12e   -AGATCGCGG ATCCGAACAC TGCGTTTGCT GGGCTTTGAT GA-----AA- ---------- ----------
5RACE_K2     AAGAT-GCGG ATCCGAACAC TGCGTTTGCT -GGCTTTGAT GAAAAG-AA- ---------T TGTTGATTTT
5RACE_K6     AAGATCGCGG ATCCGAACAC TGCGTTTGCT -GGCTTTGAT GA-----CA- ---------- -GTTGACTTT
5RACE_K14    AAGATCGCGG ATCCGAACAC TGCGTTTGCT -GGCTTTGAT GA-----AA- ---------C TGTTGACTTT
FB_Mr5       CTGAAAGCTT CAATTAACGC AGTAAGTTCT TCCTGCAAAT ATATGGGAAA TCCATTATTT ATGGATTAAT

                         -520       -510       -500       -490       -480       -470       -460
5RACE_K17    CATAAGCGAT CAGATCGATG GAAACGCGTC CCTTCATCTT CTTGATCATC CGAAAACTTC AATTAATTCA
5RACE_K12e   -ATAAGCGAT CAGATCGATG GAAACGCGTC CCTTCATCTT CTTGATCATC CGAAAACTTC AATTAATTCA
5RACE_K2     CATAAGCGAT CAGATCGATG GAAACGCGTC CCTTCATCTT CTTGATCATC TGAAAACTTC AATGAATGCA
5RACE_K6     CATAAACGAT CAGATAGATG GAAACGCGTC CCTTCATCTT CTTGATCATC TGAAAACTTC AATGAATGCA
5RACE_K14    CATAAACGAT CAGATAGATG GAAACGCGTC CCTTCATCTT CTTGATCATC TGAAAACTTC AATGAATGCA
FB_Mr5       TATTTCATTT TCTTCATCTT AATTGGAGCT ATCTATATAT ATGGGGTACC GTTTCAGGGT CAATTTGTGC

                         -450       -440       -430       -420       -410       -400       -390
5RACE_K17    TCTTAA--GC TCGAGAGCAC AAGCTTCCAG GGAACTACTT AAA--TCTTA ATTGCGCTCA AGTTTCCAAC
5RACE_K12e   TCTTAA--GC TCGAGAGCAC AAGCTTCCAG GGAACTACTT AAA--TCTTA ATTGCGCTCG AGTTTCCAAC
5RACE_K2     TCTTAA--GC TCGAGAGCTC AAGCTTCCAG GGAACTACTT AAA--TCGTA ATTGCACTAA AGTTTTCCAC
5RACE_K6     TCTTAA--GC TCGAGAGCTC AAGCTTCCAG GGAACTACTT AAA--TCTTA ATTGCACTAA GGTTTTCCAC
5RACE_K14    TCTTAA--GC TCGAGAGCTC AAGCTTCCAG GGAACTACTT AAA--TCTTA ATTGCACTAA AGTTTTCCAC
FB_Mr5       AGTTTTACGG TCGAGAGCTC AAGCTTCCAG GGAACAACTC CTTTATCTTA ATTGCA---A ACTTTTCAAC

                         -380       -370       -360       -350       -340       -330       -320
5RACE_K17    TTCAAGTCTT CAACTGATCC TCGGAAGC-- --GTCCTCGT GATCCGACGA GTCCTAT--- ----------
5RACE_K12e   TTCAAGTCTT CAACTGATCC TCGGAAGC-- --GTCCTCGT GATCCGACGA GTCCTAT--- ----------
5RACE_K2     TTCAGGTCTT CAAATGATCC TCGGAAGCAA GTGTCCTCGT GATCCGACGA CTTCCAT--- ----------
5RACE_K6     TTCAGGTCTT CAAATGATCC TCGGAGGCAA GTGTCCTCGT GATCCGACGA CTTCCAT--- ----------
5RACE_K14    TTCAGGTCTT CAAATGATCC TCGGAAGCAA GTGTCCTCGT GATCCGACGA CTTCCAT--- ----------
FB_Mr5       TTCAACTGAA -ACCTGATCC TTTGAAGCAA GTGTCCTCTT GATCCGACGA CTTCCATGTA AGTTCTTACT

                         -310       -300       -290       -280       -270       -260       -250
5RACE_K17    ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K12e   ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K2     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K6     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K14    ---------- ---------- ---------- ---------- ---------- ---------- ----------
FB_Mr5       ACATATCCGG TCCTATACCG ACGTGAGAGT TTCGAGTTGT GTAACTTGTT GCTTCCTAAA TTAATACTCT
```

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                -240      -230      -220      -210      -200      -190      -180
5RACE_K17    ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K12e   ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K2     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K6     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K14    ---------- ---------- ---------- ---------- ---------- ---------- ----------
FB_Mr5       TACTTTTAAA AGTAAAACAT ATATAATTTT TATGTACCCG ATTAAGCTCA TGTGCAGCCA CGATTATAGA

                -170      -160      -150      -140      -130      -120      -110
5RACE_K17    ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K12e   ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K2     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K6     ---------- ---------- ---------- ---------- ---------- ---------- ----------
5RACE_K14    ---------- ---------- ---------- ---------- ---------- ---------- ----------
FB_Mr5       ATTGTATAAA TTTGTATTAC AATAACTAGT TTCTTCCAAC CCTCGCAAAG TAGAAAGGGT TGTTGGCTTG

                -100       -90       -80       -70       -60       -50       -40
5RACE_K17    ---------- ---------- ---------- ---------- ------CTGT GACTCCTTGT GAGTCTTGAT
5RACE_K12e   ---------- ---------- ---------- ---------- ------CTGT GACTCCTTGT GAGTCTTGAT
5RACE_K2     ---------- ---------- ---------- ---------- ------CTGT GACTACTTGT GAATCTTAAT
5RACE_K6     ---------- ---------- ---------- ---------- ------CTGT GACTACTTGT GAATCTTAAT
5RACE_K14    ---------- ---------- ---------- ---------- ------CTGT GACTACTTGT GAATCTTAAT
FB_Mr5       GGGTCGTCCT TTTTTTAAAT TGGTTTCATC TGTTTTAATA GTGCAGCTGT GATTAGTTGT GAGT~TTGAT

                 -30       -20        -10        0         10        20        30
5RACE_K17    TGGTATTGGT CGGCGGATCA AACTTCC-AT TCATTAACCT ATGGGGGGAG AGGCTTTTCT TGTGGCACTC
5RACE_K12e   TGGTATTGGT CAGCGGATCA AACTTCC-AT TCATTAACCT ATGGGGGGAG AGGCTTTTCT TGTGGCATTC
5RACE_K2     TGGTATTGCT CCACGGATCA GACTTCCAAT TCATAAAACT ATGTCAGGAG AGGCCTTTCT TGTGGCATTC
5RACE_K6     TGGTATTGCT CCACGGATCA GACTTCCAAT TCATAAAACT ATGTCAGGAG AGGCCTTTCT TGTGACATTC
5RACE_K14    TGGTATTGCT CCACGGATCA GACTTCCAAT TCATAAAACT ATGTCAGGAG AGGCCTTTCT TGTGGCATTC
FB_Mr5       TGGTATTGCT CAACGGATCA AACTTCCAAT TCAATAAACT ATGGGGGGAG AGGCTTTTCT TGTGGCATTC

                  40        50        60        70        80        90       100
5RACE_K17    CTCCAAGTGC TGGTTGACAA GTTGGCGTAT CGCGAGGCCT TCAACTACTT CGGACTCGTA GAAGGCGTCG
5RACE_K12e   CTCCAAGTGC TGGTTGACAA GTTGGCGTAT CGCGGGGTCT TCAACTACTT TGGACTCGTA AAAGGCGTCG
5RACE_K2     CTCAAGATGC TGGTTGACAA GTTGGCGCAA CGCGAGGTCT TGAAGTACTT TGGACTCGTA AAGGGCGTTG
5RACE_K6     CTCAAGATGC TGGTTGACAA GTTGGCGCAA CGCGAGGTCT TGAAGTACTT TGGACTCGTA AAGGGCGTTG
5RACE_K14    CTCAAGATGC TGGTTGACAA GTTGGCGCAA CGCGAGGTCT TGAAGTACTT TGGACTCGTA AAGGGCGTTG
FB_Mr5       CTCCAAGTGC TGGTTGACAA GTTGGCGCAT CGCGAGGTCT TCAAGTACTT TGGACTTGTA AAGGGCGTAG

                 110       120       130       140       150       160       170
5RACE_K17    ATAAGAAACT GAAGAAATGG AATGCCACCT TGTCTGCAAT TGGAGCGGTT CTGAATGACG CGGAGGAAAG
5RACE_K12e   ATAAGAAACT GAAGAAATGG AATGCCACCT TGTCTGCAAT TGGAGCGGTT CTGAATGACG CGGAGGAAAG
5RACE_K2     ATCAAAACCT GAAGAAATGG AGTGCCACCT TGTCTGCAAT TGGAGCGGTT CTGAATGACG CGGAGGAAAG
5RACE_K6     ATCAAAACCT GAAGAAATGG AGTGCCACCT TGTCTGCAAT TGGAGCGGTT CTGAATGACG CGGAGGAAAG
5RACE_K14    ATCAAAACCT GAAGAAGTGG AGTGCCACCT TGTCTGCAAT TGGAGCGGTT CTGAATGACG CGGAGGAAAG
FB_Mr5       ATCAAAAACT GAAGAAATGG AGTGCCACCT TGTCTGCGAT TGGAGCGGTG CTGAATGACG CAGAGGAGAG

                 180       190
5RACE_K17    GCAACTGACG GC~TAAGAAC ATCTTGCT
5RACE_K12e   GCAACTGACG GC~TAAGAAC ATCTTTCT
5RACE_K2     GCAACTGACG GC~TAAGAAC ATCTGCT.
5RACE_K6     GCAACTGACG GC~TAAGAAC ATCTTTCT
5RACE_K14    GCAACTGACG GCCTAAGAAC ATCTTGCT
FB_Mr5       GCAACTGACG GC~TAAGAAC AACACACT
```

Q.2. 3' RACE-PCR of the *FB_MR5* gene

Alignment of the nucleic acid sequence of the *FB_MR5* gene (accession number: CCH50986.1) from position 4100 (start codon is position 1) up to 1 kb behind the suspected stop codon with the sequence received from 3' RACE-PCR of the *FB_MR5* gene; sequences for the *FB_MR5* gene (accession number: CCH50986.1) and the flanking regions (Bac 16k15, contig2/4, HE805491) were obtained from the ENA; red marks the old and green the new stop codon, blue marks the poly A tail, yellow marks the vector and primer sequences.

```
                   4110       4120       4130       4140       4150       4160       4170
FB_Mr5      AAAAGGACGC TACTGGCACA AAATATCCCA CATCCCTTAC ATAGATATAG ATTGGAAGAT GATTTGAAGC
3RACE_K7    AAAAGGACGC TACTGGCACA AAATATCCCA CATCCCTTAC ATAGATATAG ATTGGAAGAT GATTTGAAGC
3RACE_K5    AAAAGGACGC TACTGGCACA AAATATCCCA CATCCCTTAC ATAGATATAG ATTGGAAGAT GATTTGAAGC
3RACE_K16   AAAAGGACGC TACTGGCACA AAATATCCCA CATCCCTTAC ATAGATATAG ATTGGAAGAT GATTTGAAGC
3RACE_K8    AAAAGGACGC TACTGGCACA AAATATCCCA CATCCCTTAC ATAGATATAG ATTGGAAGAT GATTTGAAGC

                   4180       4190       4200       4210       4220       4230       4240
FB_Mr5      TGATGGTCGA TTTGATCCCA TTCGCCATGT CCAGGTATGA TCTTCTCAAG GAACGTTTTA TTATTATTAT
3RACE_K7    TGATGGTCGA TTTGATCCCA TTCGCCATGT CCAG------ ---------- ---------- ----------
3RACE_K5    TGATGGTCGA TTTGATCCCA TTCGCCATGT CCAG------ ---------- ---------- ----------
3RACE_K16   TGATGGTCGA TTTGATCCCA TTCGCCATGT CCAG------ ---------- ---------- ----------
3RACE_K8    TGATGGTCGA TTTGATCCCA TTCGCCATGT CCAG------ ---------- ---------- ----------

                   4250       4260       4270       4280       4290       4300       4310
FB_Mr5      TATTATTATT ATTATTATTG TTATATTGTT AATCATCTTT ATTCTTGCTG CATTGGGCAG GTTTTTGGCG
3RACE_K7    ---------- ---------- ---------- ---------- ---------- ---------- GTTTTTGGCG
3RACE_K5    ---------- ---------- ---------- ---------- ---------- ---------- GTTTTTGGCG
3RACE_K16   ---------- ---------- ---------- ---------- ---------- ---------- GTTTTTGGCG
3RACE_K8    ---------- ---------- ---------- ---------- ---------- ---------- GTTTTTGGCG

                   4320       4330       4340       4350       4360       4370       4380
FB_Mr5      GGAGAAGGAT TGTGCCGAAA TGACTGCTTG TCTGAGGAAC TGTTTGGAAG GATGGATCTG TGGTCGGTGA
3RACE_K7    GGAGAAGGAT TGTGCCGAAA TGACTGCTTG TCTGAGGAAC TGTTTGGAAG GATGGATCTG TGGTCGGTGA
3RACE_K5    GGAGAAGGAT TGTGCCGAAA TGACTGCTTG TCTGAGGAAC TGTTTGGAAG GATGGATCTG TGGTCGGTGA
3RACE_K16   GGAGAAGGAT TGTGCCGAAA TGACTGCTTG TCTGAGGAAC TGTTTGGAAG GATGGATCTG TGGTCGGTGA
3RACE_K8    GGAGAAGGAT TGTGCCGAAA TGACTGCTTG TCTGAGGAAC TGTTTGGAAG GATGGATCTG TGGTCGGTGA

                   4390       4400       4410       4420       4430       4440       4450
FB_Mr5      GGCTGAAAAT TTAAATCGCA ATGGCTTGGA GCCGTCATCA TTGGTGGTTT TAGGGGAGCG AATTTTGGAA
3RACE_K7    GGCTGAAAAT TTAAATCGCA ATGGCTTGGA GCCGTCATCA TTGGTGGTTT TAGGGGAGCG AATTTTGGAA
3RACE_K5    GGCTGAAAAT TTAAATCGCA ATGGCTTGGA GCCGTCATCA TTGGTGGTTT TAGGGGAGCG AATTTTGGAA
3RACE_K16   GGCTGAAAAT TTAAATCGCA ATGGCTTGGA GCCGTCATCA TTGGTGGTTT TAGGGGAGCG AATTTTGGAA
3RACE_K8    GGCTGAAAAT TTAAATCGCA ATGGCTTGGA GCCGTCATCA TTGGTGGTTT TAGGGGAGCG AATTTTGGAA

                   4460       4470       4480       4490       4500       4510       4520
FB_Mr5      CAGTTCCCAG AAATGTTGCT TGCAGAGATG TTGATAGAGA CGTAGAAGGA AGTCCTGCTT GAGGCCGTGG
3RACE_K7    CAGTTCCCAG AAATGTTGCT TGCAGAGATG TTGATAGAGA CGTAGAAGGA AGTCCTGCTT GAGGCCGTGG
3RACE_K5    CAGTTCCCAG AAATGTTGCT TGCAGAGATG TTGATAGAGA CGTAGAAGGA AGTCCTGCTT GAGGCCGTGG
3RACE_K16   CAGTTCCCAG AAATGTTGCT TGCAGAGATG TTGATAGAGA CGTAGAAGGA AGTCCTGCTT GAGGCCGTGG
3RACE_K8    CAGTTCCCAG AAATGTTGCT TGCAGAGATG TTGATAGAGA CGTAGAAGGA AGTCCTGCTT GAGGCCGTGG

             4530       4540       4550       4560       4570       4580       4590
FB_Mr5      AATAGTATAT GCAATAGGAT GGTTTACAGA AAATACCATA TTCACGGTTG ATATATTTGA AGGTGGTGGC
3RACE_K7    AATAGTATAT GCAATAGGAT GGTTTACAGA AAATACCATA TTCACGGTTG ATATATTTGA AGGTGGTGGC
3RACE_K5    AATAGTATAT GCAATAGGAT GGTTTACAGA AAATACCATA TTCACGGTTG ATATATTTGA AGGTGGTGGC
3RACE_K16   AATAGTATAT GCAATAGGAT GGTTTACAGA AAATACCATA TTCACGGTTG ATATATTTGA AGGTGGTGGC
3RACE_K8    AATAGTATAT GCAATAGGAT GGTTTACAGA AAATACCATA TTCACGGTTG ATATATTTGA AGGTGGTGGC
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                   4600       4610       4620       4630       4640       4650       4660
FB_Mr5      ATGGACGCAA CACCTGGTTG TGGAGCTGGT GGACCGCTTG ACCATCTAGT AGAGCTCCCA GCAGGCAGTG
3RACE_K7    ATGGACGCAA CACCTGGTTG TGGAGCTGGT GGACCGCTTG ACCATCTAGT AGAGCTCCCA GCAGGCAGTG
3RACE_K5    ATGGACGCAA CACCTGGTTG TGGAGCTGGT GGACCGCTTG ACCATCTAGT AGAGCTCCCA GCAGGCAGTG
3RACE_K16   ATGGACGCAA CACCTGGTTG TGGAGCTGGT GGACCGCTTG ACCATCTAGT AGAGCTCCCA GCAGGCAGTG
3RACE_K8    ATGGACGCAA CACCTGGTTG TGGAGCTGGT GGACCGCTTG ACCATCTAGT AGAGCTCCCA GCAGGCAGTG

                   4670       4680       4690       4700       4710       4720       4730
FB_Mr5      ATCCAGGAGA ATTACTAGAC AAATGTTGCG GAAATGTAGG TGTAAACGGG CCAGGTGTGG TGGTCGATTT
3RACE_K7    ATCCAGGAGA ATTACTAGAC AAATGTTGCG GAAATGTAGG TGTAAACGGG CCAGGTGTGG TGGTCGATTT
3RACE_K5    ATCCAGGAGA ATTACTAGAC AAATGTTGCG GAAATGTAGG TGTAAACGGG CCAGGTGTGG TGGTCGATTT
3RACE_K16   ATCCAGGAGA ATTACTAGAC AAATGTTGCG GAAATGTAGG TGTAAACGGG CCAGGTGTGG TGGTCGATTT
3RACE_K8    ATCCAGGAGA ATTACTAGAC AAATGTTGCG GAAATGTAGG TGTAAACGGG CCAGGTGTGG TGGTCGATTT

                   4740       4750       4760       4770       4780       4790       4800
FB_Mr5      CCCGTGAATG TGAGTGGAGC TGAAACATTA GTTTGAACTG ATGCAGGCTT GGGAGCCATA CATGCATGCG
3RACE_K7    CCCGTGAATG TGAGTGGAGC TGAAACATTA GTTTGAACTG ATGCAGGCTT GGGAGCCATA CATGCATGCG
3RACE_K5    CCCGTGAATG TGAGTGGAGC TGAAACATTA GTTTGAACTG ATGCAGGCTT GGGAGCCATA CATGCATGCG
3RACE_K16   CCCGTGAATG TGAGTGGAGC TGAAACATTA GTTTGAACTG ATGCAGGCTT GGGAGCCATA CATGCATGCG
3RACE_K8    CCCGTGAATG TGAGTGGAGC TGAAACATTA GTTTGAACTG ATGCAGGCTT GGGAGCCATA CATGCATGCG

                   4810       4820       4830       4840       4850       4860       4870
FB_Mr5      CCAAAATTTG TGTTGTTGGT GGGTTTTGTC TAGGGGAAAT TCTCAAATTT GATTACTCGG TACTGCTGGC
3RACE_K7    CCAAAATTTG TGTTGTTGGT GGGTTTTGTC TAGGGGAAAT TCTCAAATTT GATTACTCGG TACTGCTGGC
3RACE_K5    CCAAAATTTG TGTTGTTGGT GGGTTTTGTC TAGGGGAAAT TCTCAAATTT GATTACTCGG TACTGCTGGC
3RACE_K16   CCAAAATTTG TGTTGTTGGT GGGTTTTGTC TAGGGGAAAT TCTCAAATTT GATTACTCGG TACTGCTGGC
3RACE_K8    CCAAAATTTG TGTTGTTGGT GGGTTTTGTC TAGGGGAAAT TCTCAAATTT GATTACTCGG TACTGCTGGC

                   4880       4890       4900       4910       4920       4930       4940
FB_Mr5      TTATGTTTAC AAAAAAATTA TCTTCAATTT TCTTGAGAAA GCATAAGACC AACGATCGCA CCATTTATCG
3RACE_K7    TTATGTTTAC AAAAAAATTA TCTTCAATTT TCTTGAGAAA GCATAAGACC AACGATCGCA CCATTTATCG
3RACE_K5    TTATGTTTAC AAAAAAATTA TCTTCAATTT TCTTGAGAAA GCATAAGACC AACGATCGCA CCATTTATCG
3RACE_K16   TTATGTTTAC AAAAAAATTA TCTTCAATTT TCTTGAGAAA GCATAAGACC AACGATCGCA CCATTTATCG
3RACE_K8    TTATGTTTAC AAAAAAATTA TCTTCAATTT TCTTGAGAAA GCATAAGACC AACAATCCTA CCAT~~~~~~

                   4950       4960       4970       4980       4990       5000       5010
FB_Mr5      GAAGTGGCTG AAGGCACGAC ATGCTCATTA AATTACAGTT AAGCTAACTT GCTCATTAGA TTCTTTGCTT
3RACE_K7    GAAGTGGCTG AAGGCACGAC ATGCTCATTA AATTACAGTT AAGCTAACTT GCTCATTAGA TTCTTTGCTT
3RACE_K5    G--------- ---------- ---------- ---------- ---------- ---------- ----------
3RACE_K16   ---------- ---------- ---------- ---------- ---------- ---------- ----------
3RACE_K8    ~~-------- ---------- ---------- ---------- ---------- ---------- ----------

                   5020       5030       5040       5050       5060       5070       5080
FB_Mr5      GATGATTACA TTCACCACAC ATTACTCCAA ATTAGTGCCT ATATGTTACC TAATGTAACC TCATTTGTAC
3RACE_K7    GATGATTACA TTCACCACAC ATTACTCCAA ATTAGTGCCT ATATGTTACC TAATGTAACC TCATTTGTAC
3RACE_K5    ---------- ---------- ---------- ---------- ---------- ---------- ----------
3RACE_K16   ---------- ---------- ---------- ---------- ---------- ---------- ----------
3RACE_K8    ---------- ---------- ---------- ---------- ---------- ---------- ----------

                   5090       5100       5110       5120       5130       5140       5150
FB_Mr5      TAGCTTCTGA CAGTAATGCG GTTGCACCAT GTCGATGTTT GTTCATGATG TGCCTTTGCA CCCTGTCCTA
3RACE_K7    TAGCTTCTGA CAGTAATGCG GTTGCACCAT GTCGATGTTT GTTCAAAAAA AAAAAAA--- CCTATAGTGA
3RACE_K5    ---------- ---------- ---------- ---------- ----AAAAAA AAAAAAAGAA CCTATAGTGA
3RACE_K16   ---------- ---------- ---------- ---------- ----AAAAAA AAAAAAA--- CCTATAGTGA
3RACE_K8    ---------- ---------- ---------- ---------- ---------- ---------- --------GA

                   5160       5170
FB_Mr5      CTGCATTTGC AACTACACTC TCTTTCT
3RACE_K7    GTCGTATTAA TTC-GGATCC GCGATCT
3RACE_K5    GTCGTATTAA TTC-GGATCC GCGATCT
3RACE_K16   GTCGT----- ---------- ---ATCT
3RACE_K8    GTCGTATTAA TTCTGAATCC GCGATCT
```

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Declaration of Authorship

Versicherung gemäß §5A der Promotionsordnung der Fakultät Mathematik und Naturwissenschaften an der Technischen Universität Dresden

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Datum, Unterschrift

List of publications

Articles

Vogt, I., Wöhner, T., Richter, K., Flachowsky, H., Sundin, G. W., Wensing, A., Savory, E. A., Geider, K., Day, B., Hanke, M.-V., Peil, A. Gene-for-gene relationship in the host–pathogen system *Malus* ×*robusta* 5 – *Erwinia amylovora*. *New Phytologist*, 197 (4): 1262-1275, 2013

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Curriculum vitae

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