Supplementary Information to:
"Phage biocontrol reduces the burden on plant immunity through suppression of bacterial virulence"

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Summary:

The Supplementary information includes 5 figures and 7 Tables

Figures

Tables

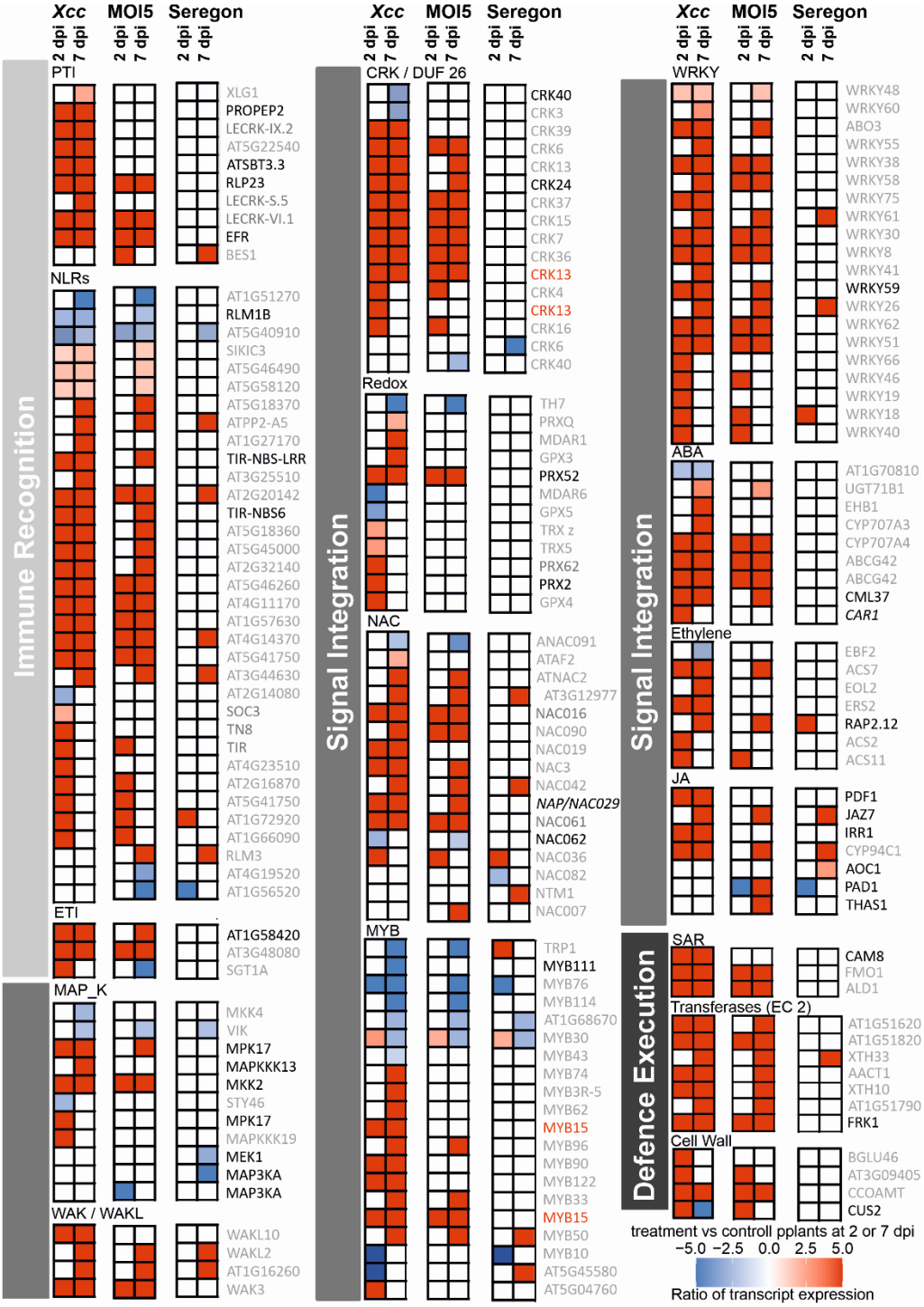

Figure S1: *Arabidopsis* defence regulation in different microbial treatments compared to control plants

Ratio of gene expression of the 3 treatments (Xcc, MOI5 and Seregon) compared to control plants (expressed as log2 (FC)). Shown are selected transcripts thought to be involved in pathogen recognition (light grey), immune signal integration (medium grey) and defense execution (dark grey). The level of gene expression is presented as a heat map, shown bottom right, with shades of blue indicating lower expression, and shares of red indicating higher expression. The heat map is cut off at +/-5 for visual simplification, numerical data can be found in supplemental table S2. Transcripts are grouped in functional groups based on MapMan mapping file X4.1 [1], with adaptations as listed in supplemental table 2. Transcript names mentioned in the text are in black, and additional names are in grey, names of splice isoforms of the same transcript are in red.

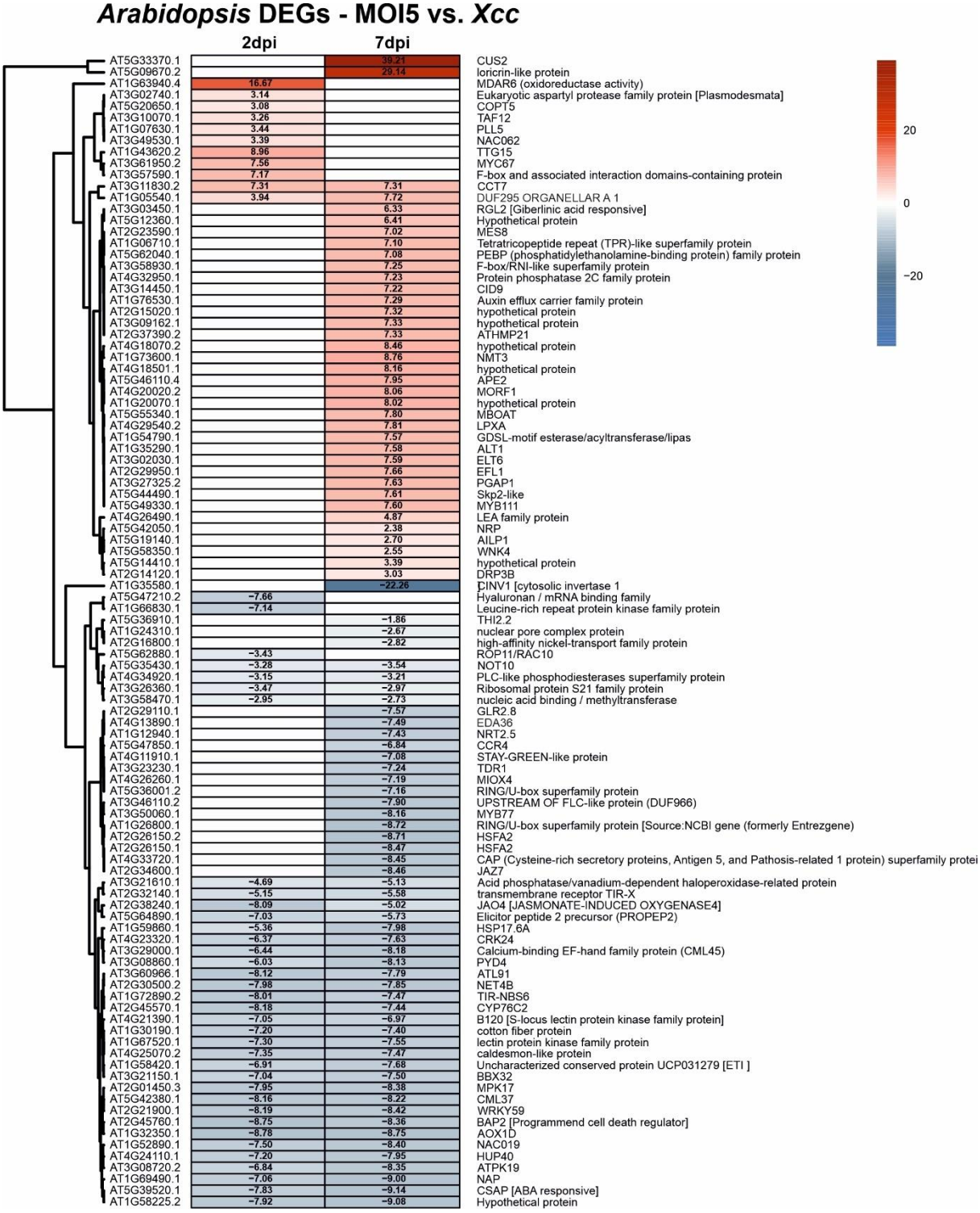

Figure S2. Phage biocontrol (MOI5) leads to altered defence regulation in *Arabidopsis* compared to *Xcc* infected plants. Global *A. thaliana* transcriptome analysis during the tripartite interaction. Heat map of the 100 most significant differentially expressed plant genes. Gene names based on TAIR entries were added. Shown are log2fold changes for three independent biological replicates MOI5 vs *Xcc* at 2 or 7 dpi. Empty cells are not differentially expressed between the two conditions (MOI5 versus *Xcc*) at the given day.

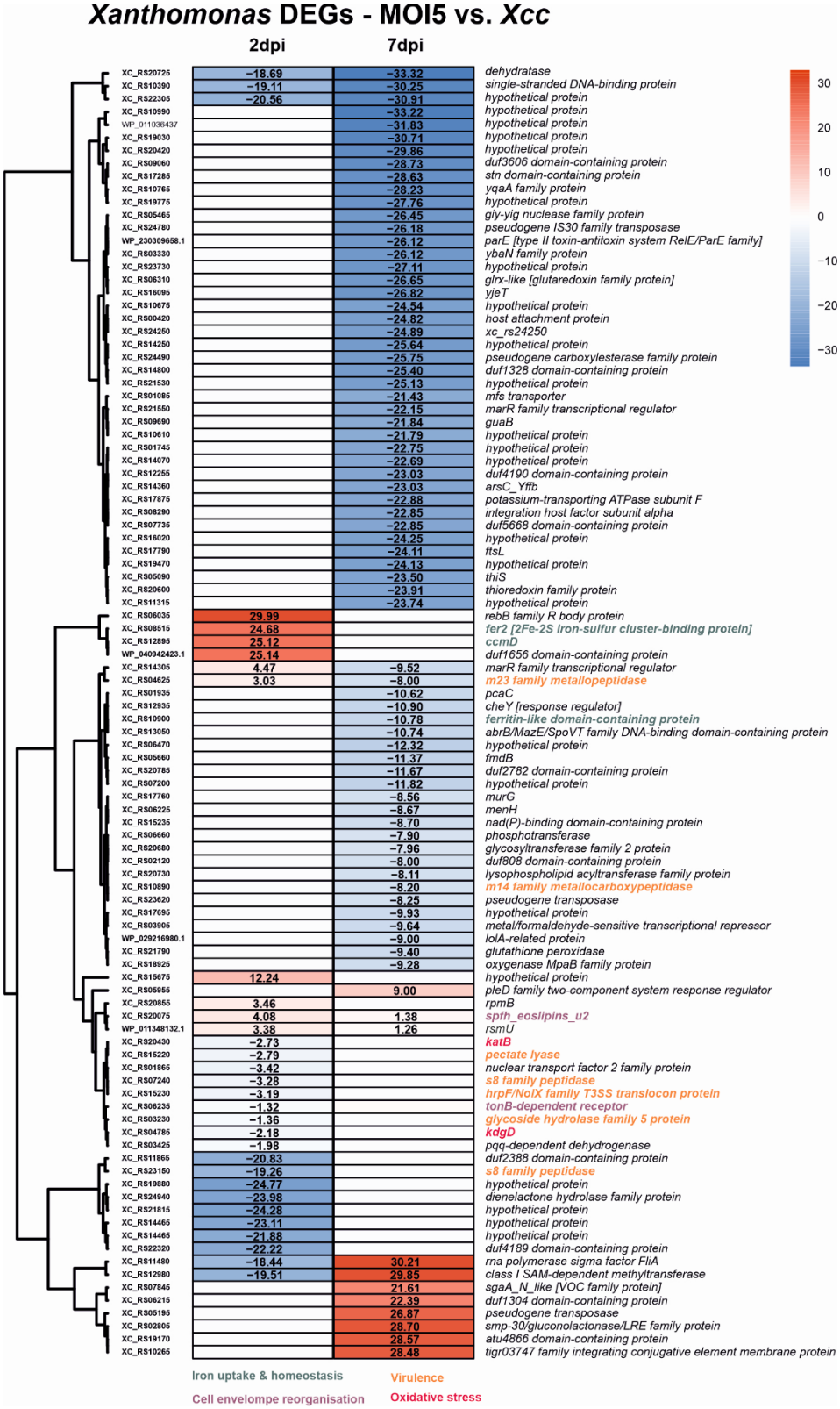

Figure S3: Phage biocontrol leads to reduced expression of virulence-associated genes in *Xanthomonas campestris (Xcc).*

Global bacterial transcriptome analysis during the tripartite interaction. Heat map of the 100 most significant differential expressed bacterial genes in presence or absence of the phage. Gene identifiers were replaced with gene names based on NCBI entries. Shown are log2fold changes of data for three independent biological replicates (MOI5 versus *Xcc* at 2 or 7 dpi).

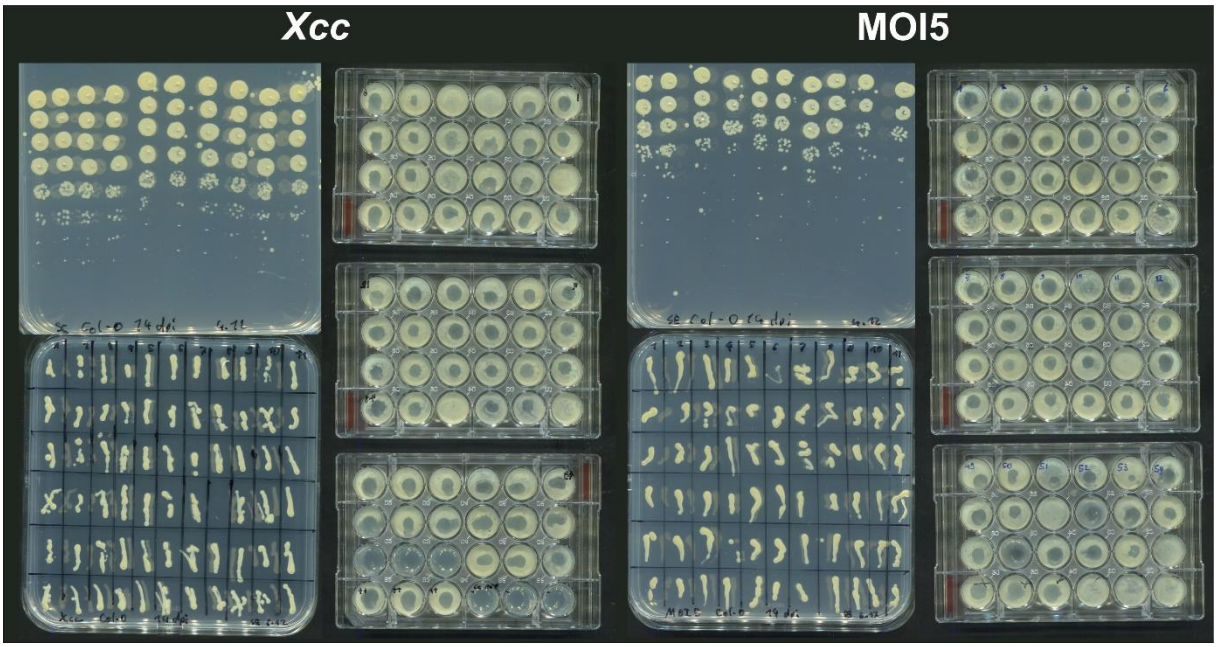

Figure S4: Bacterial survivors after at the end of the in-planta experiment and phage susceptibility testing
After 14 days of growth in the plant environment (on ½ MS plates) individual colonies extracted from plant material. Those "survivors" were re-streaked on nutrient agar and subjected to a 48 well double agar overlay spot assay. Susceptible clones show lysis zones whereas resistant ones don't.

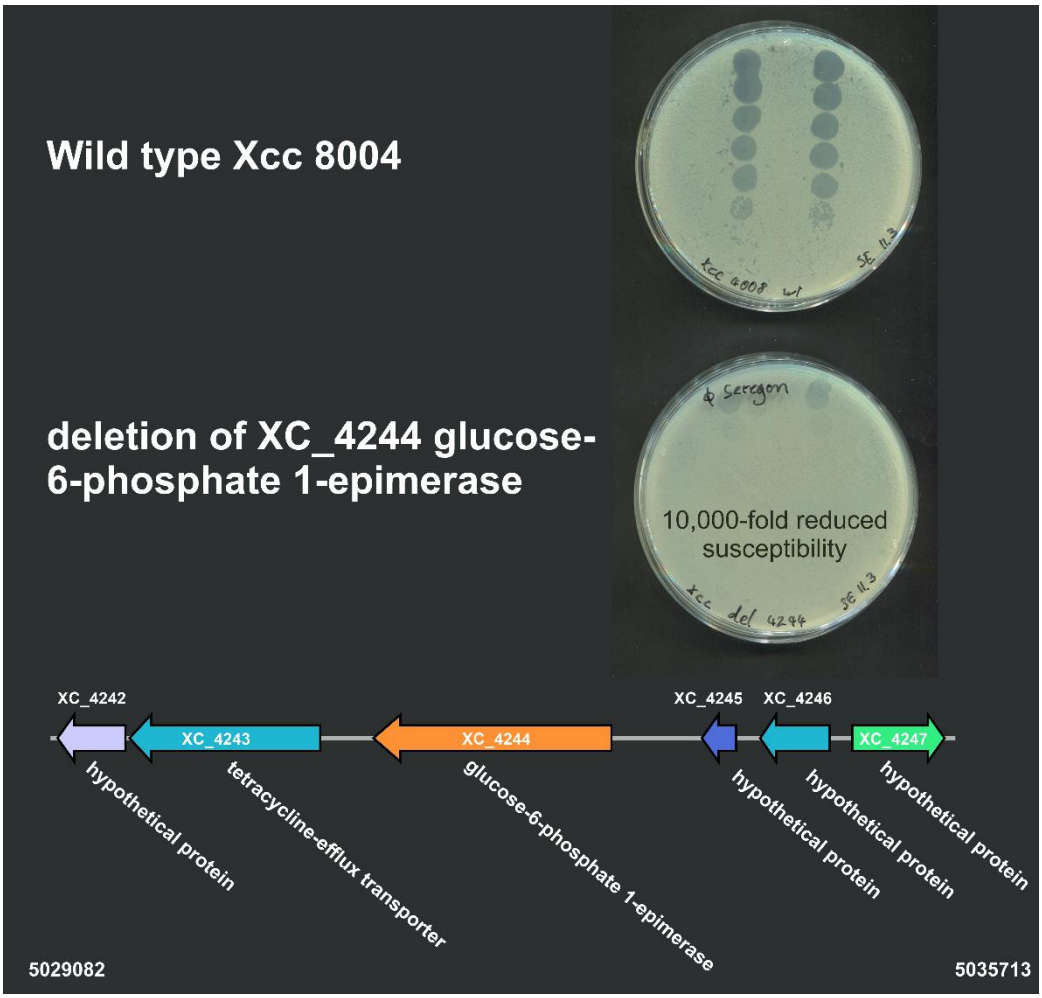

Figure S5. Mutation of XC_4244 encoding a glucose-6-phosphate 1-epimerase significantly reduces susceptibility of *Xcc* to infection with phage Seregon.
To test if the SNPs identified in "bacterial survivors" of the phage biocontrol treatment were relefant for phage infection, we constructed a knockout mutant of XC_4244 by homologous recombination. *Xcc* 8004 wt and *Xcc*ΔXC_4244 were infused into the 0.4%-soft agar of a double agar overlay. Dilution series of *Xanthomonas* phage Seregon was spotted on top and incubated for 24 h at 28°. Displayed below is the genomic context of gene XC_4422.

Supplemental Table 7 Strains used in this study

Organism	Source	Reference
Xanthomonas campestris pv. *Campestris* 8004	AG Narberhaus (RUB, Bochum)	[2]
Xanthomonas campestris pv. *Campestris* Δ XC_4244	This study	-
Xanthomonas phage Seregon	Erdrich et al. 2022	[3]

Supplemental Table 8 Primers and plasmids used in this study

Primer Name	Sequence	Reference
InFusion_Xcc_4244_del_LF_F	cgccaagcttgcatgcctgcagatcaggagagatggacat	This study
InFusion_Xcc_4244_del_LF_R	ccttaattctctagttgagaccccaagacagtaaagaggca	This study
InFusion_Xcc_4244_del_RF_F	ataaagtattgagaccaatcaggcaggcgtct	This study
InFusion_Xcc_4244_del_RF_R	gtaaaacgacggccagttaacacaccgacgtca	This study
InFusion_Xcc_4244_Kan_F	ggtctcaactagagaattaagggag	This study
InFusion_Xcc_4244_Kan_R	ggtctcaatactttatcctagtttg	This study
Seregon_Capsid-F	atggatttgttcagcactgcgg	This study
Seregon_Capsid-R	agcttcagctcgtcgaacatg	This study
pK19mobsacB		[4]

Literature:

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3. Erdrich SH et al. Isolation of Novel Xanthomonas Phages Infecting the Plant Pathogens X. translucens and X. campestris. *Viruses* 2022;**14**:1449. https://doi.org/10.3390/v14071449
4. Schäfer A et al. Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum. *Gene* 1994;**145**:69–73. https://doi.org/10.1016/0378-1119(94)90324-7