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Abstract

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Introduction

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Results

Experiment 1

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These data show us that we successfully silenced our favourite gene **Figure 1**. All individual measurements are listed in **Table ??**.

Experiment 2

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Figure 1 | CRISPR loci and HDR constructs. (a) *PiAvr1*, **(b)** *PiTubA1*, and **(c)** *PiAP5*. Orange arrowheads indicate expected DSB sites upon Cas9 nuclease activity; black arrows indicate start codons (ATG); grey blocks mark homologous regions between the genes and the HDR constructs (referred to as repair template or ssODN); interpuncts (•) represent cropped sequences; PAM: Protospacer Adjacent Motif.

Experiment 3

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Experiment 4

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Figure 2 | In vivo cleavage assay. a) gRNAs 1, 2, and 3 with Avr1 as target DNA. **b)** gRNA8 with PiTubA2 as target DNA (**right**) and gRNA183 with PiAP5 as target DNA (**left**).

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Acknowledgements

We are grateful to our colleagues who generously shared their constructs and experiences. This work was supported by xxx with financial aid from the xxx in the framework of the xxx programme (project # 1234).

Materials and methods

Culturing conditions

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Experimental procedures

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Constructs

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Cas9 *in vitro* activity assay

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Molecular analysis of transformants

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T7EI assay

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Data availability

All data is public available under xxx.

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Supplementary files

Figure S1 | Detection limit assays. a) PCR amplicons used for the detection limit assays. ΔAvr1 contains a 29 bp deletion. **b)** T7EI assay. Varying amounts of Avr1 and ΔAvr1 PCR amplicons were annealed and digested by T7EI. **c)** Example output from TIDE, confirming the presence of the 29 bp deletion in ΔAvr1 (left red bar). Here, a sequence chromatogram obtained from sequencing a molar ratio of 998:2 Avr1:ΔAvr1 was compared to a sequence chromatogram of Avr1. The predicted frequency of chromatograms with a deletion (1.5%) deviates from the actual molar ratio of Avr1:ΔAvr1 (0.2%).

Figure S2 | Expression analysis of Cas9 in 11 selected transformed lines. cDNA and genomic DNA (gDNA) of *P. infestans* strain T30-2 were used as negative control and gDNA from a *P. infestans* Cas9 transformant as positive control.

Figure S3 | Plasmids used in this study. Plasmids starting with 'pYF' are obtained from Francis Fang (Fang & Tyler 2017), plamids starting with 'pJH' are modified versions as described in this report.

Table S1 | Primers used in this study.

Name	Sequence (5' - 3')	Purpose
Avr1_F	ATGCACCGCGTATTGCTGC	*P. infestans* T30-2 Avr1
Avr1_F	TTAAAATGGTACCACAACATGTCCACC	*P. infestans* T30-2 Avr1
Avr1_Nested F	GACGTTTGCCCTGTTGTGTA	*P. infestans* T30-2 Avr1 - nested
Avr1_Nested R	ACAACATGTCCACCAAGCATG	*P. infestans* T30-2 Avr1 - nested
pRPL41_seq_F	CAAGCCTCACTTTCTGCTGACTG	Sequence analysis of gRNA constructs
sgRNA_Col_R	AAAAGCACCGACTCGGTGC	Sequence analysis of gRNA constructs
Cas9_RT_F	CCGAAGAGGTCGTGAAGAAG	Expression analysis of Cas9
Cas9_RT_R	GCCTTATCCAGTTCGCTCAG	Expression analysis of Cas9
pHAM34_Pseq_F	TCGCCCGACTCGCCCAC	Sequence analysis of Cas9
Cas9_Seq_757_F	CTGTTCGGAAACCTGATTGCCC	Sequence analysis of Cas9
Cas9_Seq_1439_F	GGATGACCAGAAAGAGCGAGG	Sequence analysis of Cas9
Cas9_Seq_2163_F	AGAGGACATCCAGAAAGCCCAGG	Sequence analysis of Cas9
Cas9_Seq_2865_F	GAACACTAAGTACGACGAGAATGAC	Sequence analysis of Cas9
Cas9_Seq_3600_F	CAAGGGCTACAAAGAAGTGAAAAAG	Sequence analysis of Cas9
eGFP_F	ATGGGCAAGGGCGAGGAA	Detection of eGFP
eGFP_R	TCACTTGTAGAGTTCATCCATGCCA	Detection of eGFP
Hyg_ApaI_R	GGGCCCCTATTCCTTTGCCCTC	Cloning Hygromycin into pYF2.3
Hyg-AflII_F	CTTAAGATGAAAAAGCCTGAACTCAC	Cloning Hygromycin into pYF2.3
M13F	CGTTGTAAAACGACGGCCAG	General primers
M13R	TGCCAGGAAACAGCTATGACC	General primers
PiNLS_SacII_F	ACACCCGCGGATGCACAAGCGCAAG	Cloning PiNLS in pYF2.2
PiNLS_SpeI_R	ACACACTAGTCTCGCCCATTGCCGCGTC	Cloning PiNLS in pYF2.2
PiNLS_AgeI_F	ACACACCGGTATGCACAAGCGCAAG	Cloning PiNLS in pGFPN
PiNLS_NheI_R	ACACGCTAGCCTCGCCCATTGCCGCGTC	Cloning PiNLS in pGFPN
PiAP5_EcoRI_F	ACACGAATTCATGCGTCTCGGTCTGCTC	PiAp5 full-length
PiAP5_NotI_R	ACACGCGGCCGCATTTGGTCCCATGAGACGCG	PiAp5 full-length
PcS9_F_EcoRI	cacaGAATTCCGTCAATACGGCTGTAAACCAC	*P. capsici S9* promoter
PcS9_F_NheI	cacaGCTAGCTTTGGCGACTTCTTTTGTTCAGG	*P. capsici S9* promoter
Avr1_gRNA_1_pT7_F	GAAATTAATACGACTCACTATAGGTGGCCAAAGCAATGATATTG	In vitro transcription gRNA
Avr1_gRNA_2_pT7_F	GAAATTAATACGACTCACTATAGGTCGTTTATCGAGTCCTTCGT	In vitro transcription gRNA
Avr1_gRNA_3_pT7_F	GAAATTAATACGACTCACTATAGGGAATCCAAGACTCGATTTTT	In vitro transcription gRNA
PiTubA2_gRNA_8_pT7_F	GAAATTAATACGACTCACTATAGGACGCATGTTGCTTTAAGCTT	In vitro transcription gRNA
PiAP5_gRNA_183_pT7_F	GAAATTAATACGACTCACTATAGGGATAAGACGGTGAACAGCAA	In vitro transcription gRNA
gRNA3_Gibson_F	TCGGCATGGCGAATGGGACAGGATTCCTGATGAGTCCGTGA	gRNA2-3 plasmid assembly
gRNA3_Gibson_R	GCTAAGTATTCTAGTCGACAGTCCCATTCGCCATGCCGAA	gRNA2-3 plasmid assembly