Improving late blight (*Phytophthora infestans*) resistance in potato using resistance genes from wild potato relatives.

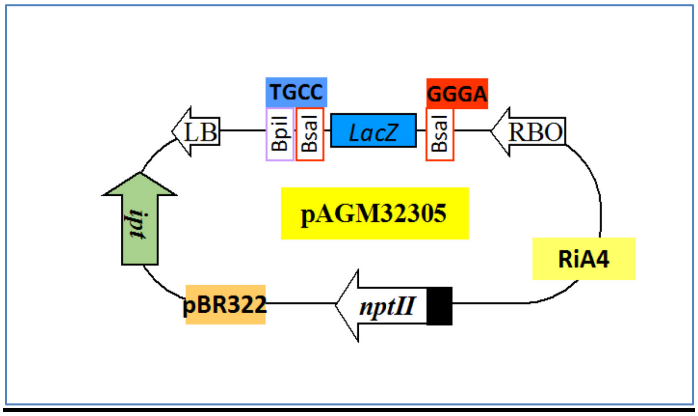
ANNEX 1

This annex contains methods for the experiments that were done to characterise the genetically modified plants referred to in the associated release application by The Sainsbury Laboratory. It also provides vector map and details of the structure of the T-DNAs in each plasmid used for plant transformation.

Section 1: Map of vector pAGM32305.
Section 2: Structure of T-DNAs in plasmids SLJ24904 and SLJ25057.
Section 3: Methods used for the characterisation of transgenic plants.

Table 1: List of primers used in PCR, ddPCR and qPCR tests.

SECTION 1: MAP OF VECTOR pAGM32305

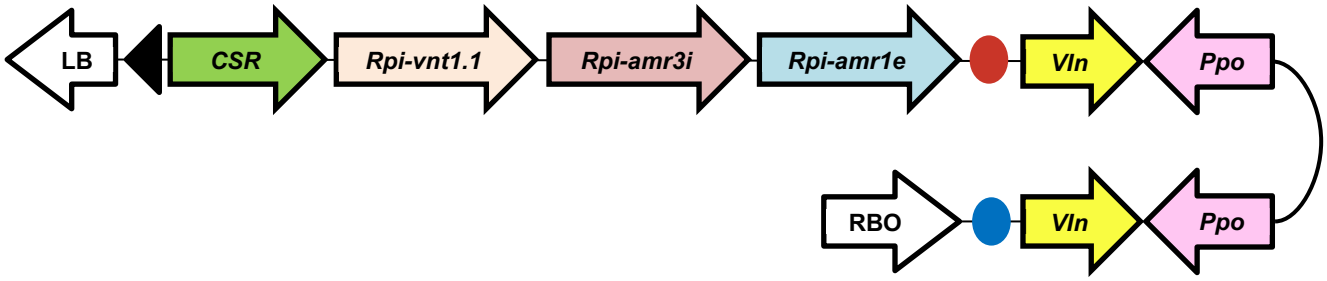


A detailed description of vector elements has been presented in Section 11 of Part A1. Note that the LacZ gene in the vector's Golden Gate cassette is removed upon cloning.

SECTION 2: STRUCTURE OF T-DNAs IN PLASMIDS SLJ24904 AND SLJ25057

SLJ24904)T-DNA

SLJ25057 T-DNA



A detailed description of T-DNA elements has been presented in Section 12 of Part A1, including information on regulatory sequences linked to the cloned genes. Black triangle represents extra left border sequences. Red circles indicate dummy-linkers and blue circles represent end-linkers. Curved line indicates spacer element that creates a loop between sense and antisense sequences.

SECTION 3: METHODS USED FOR CHARACTERISATION OF TRANSGENIC PLANTS

Genomic DNA isolation

Plant genomic DNA extraction is performed according to the Doyle and Doyle CTAB-based method (Doyle and Doyle, 1987) or using Qiagen DNeasy Plant Mini Kit (Cat No./ID: 69106).

PCR amplification from genomic DNA

All PCR reactions are performed in a final volume of 25 µl with 2.5 µl of 10x Standard *Taq* PCR buffer (New England BioLabs), 0.5 µl of 10 mM dNTPs, 1 µl of 10 µM forward and reverse primers, 0.125 µl of *Taq* polymerase (New England BioLabs) and 100-150 ng of genomic DNA per PCR reaction. The following PCR program is used: 95°C 3 min, 30x [95°C 30'' > $T°_{annealing}$ 30'' > 68°C for corresponding elongation time], 68°C 5 min. $T°_{annealing}$ is within 55°C to 60°C depending on the melting temperature of the primer pairs. Elongation time is within 30'' to 50'' depending on the length of the amplicons. After the amplification, 10 µl of the PCR reactions (including DNA loading buffer) are loaded and run on a 1.5 % TAE agarose gel next to a DNA molecular-weight ladder (100 bp, New England BioLabs).

Droplet Digital PCR (ddPCR) amplification for copy number determination

All ddPCR reactions are performed with 15 to 30 ng of genomic DNA as template. The reaction mix is set up using the BIO-RAD QX200 ddPCR 2X EvaGreen Supermix following the manufacturer's recommended protocol. The final concentration of primers is 100nM and 2-5 U of EcoRI enzyme are added per reaction to separate tandem gene copies and improve template accessibility. Droplets are generated with the BIO-RAD QX200 Droplet Generator and PCR amplification is performed in a BIO-RAD C1000 Touch Thermal Cycler. The following PCR program is used: 95°C 5 min, 40x [95°C 30'' – 60.5°C 1 min - 72°C 1 min] - 4°C 5 min - 90°C 5 min. All the steps are performed with a temperature ramp of 2°C/sec and a lid temperature of 105°C. After amplification, droplets are analysed in the BIO-RAD QX200 Droplet Reader device and the number of positive droplets obtained for the gene of interest is compared to the number of positive droplets obtained for the control gene (*Vacuolar Invertase*), which corresponds to 4 copies / potato tetraploid genome.

RNA extraction and qPCR amplification

RNA is isolated using the RNeasy Mini Kit (Qiagen) and treated with DNAse (Qiagen). All qPCR reactions are performed in a final volume of 20 µl with 10 µl of KAPA SYBR® FAST qPCR Mix and 1 µl of 10 µM forward and reverse primers. cDNA synthesis is performed with 2-2.5 µg total RNA using the SuperScript II First-Strand Synthesis SuperMix (Invitrogen) and oligo-dT primer in a final volume of 20 µl. The cDNA is diluted 1/5 and 1 µL of the dilution is used as template for qPCR amplification. The following qPCR program is used: 95°C 2min, [95°C 20'' - 58°C 20'' - 72°C 30''] 40x - 72°C 5min. The result is expressed as [Number of mRNA molecules of *Rpi-amr3i* per 1 Million mRNA molecules of *EF1* control].

Table 1. List of primers used in PCR, ddPCR and qPCR tests

Construct	Gene/Region	Test	Primer ID	Sequence 5'-3'
All gDNAs	*EF1α*	PCR	MP075	GGAAGCTGCTGAGATGAACAAGA
All gDNAs	*EF1α*	PCR	MP076	CCTTCACAATTTCATCATACCTAGCC
pAGM32305 Backbone	*ipt* (outside LB)	PCR	MP115	AAAACTTATGGATCTGCGTC
pAGM32305 Backbone	*ipt* (outside LB)	PCR	MP116	GGAGCTGGTGCAAACTAATAC
pAGM32305 Backbone	*nptII*	PCR	MP112	GAAGAGTATGATTGAACAAGATGG
pAGM32305 Backbone	*nptII*	PCR	MP113	ATATATGAGTAAACTTGGTCTGAC
pAGM32305 Backbone	RiA4 (outside RBO)	PCR	MP110	CAAATAACAGTTGGGTGGAG
pAGM32305 Backbone	RiA4 (outside RBO)	PCR	MP111	CATGCTAACATTCAACTCTGGC
SLJ25057	*CSR terminator*	PCR	MP037	TACAGATGGACAAGATCATTTACC
SLJ25057	*Rpi-vnt1.1 promoter*	PCR	MP081	TGGCTGTGAGTTTGGGCTATTATG
SLJ25057	*Rpi-vnt1.1 terminator*	PCR	LT179	ATGCTTGACTAAGAAGC
SLJ25057	*Rpi-amr3i promoter*	PCR	KW_amr3_prom_seq_R	TAATCTTGTAGCCTTGAACATGCC
SLJ25057	*Rpi-amr3i terminator*	PCR	MP041	CATCTAATGCCATCTTCCAAATGC
SLJ25057	*Rpi-amr1e promoter*	PCR	MP056	CCTCAAAAGTTGCAACTTACATTCCTC
SLJ25057	*Agp promoter*	PCR	MP157	CAAGCTTGTTAACGGATC
SLJ25057	Spacer	PCR	MP148	TTCAGATTCTGGAGCGTCAG
SLJ25057	Spacer	PCR	MP154	TCCATAAGACCTTGACTG
SLJ25057	*GBSS promoter*	PCR	MP160	TTGTAGACCACACATCAC
SLJ24904	*CSR terminator*	PCR	MP037	TACAGATGGACAAGATCATTTACC
SLJ24904	*p-ARSK1*	PCR	MP085	TCTATATTCCACTATCTCGATTTCTA
SLJ24904	*Oc-IΔD86*	PCR	MP088	GAATTGCAAGAATTTAAACCAGTTG
SLJ24904	*pMDK4-20*	PCR	MP125	GGTCCTCATAAATATGACTATGCC
All relevant cDNAs	*EF1α*	qPCR	EF1_F	GGAAGCTGCTGAGATGAACAAGA
All relevant cDNAs	*EF1α*	qPCR	EF1_R	CTCACGTTCAGCCTTAAGTTTGT
SLJ25057	*Rpi-vnt1.1*	qPCR	HD453(F)_SIMPLOT_qPCR_vnt1	ATGTTACTGTGTCTCTTTTGC
SLJ25057	*Rpi-vnt1.1*	qPCR	HD457(R)_SIMPLOT_qPCR_vnt1	ATCAATCGGTGCAACAATCTT
SLJ25057	*Rpi-amr3i*	qPCR	MP015	CTGAGGATTCTGCACGAGAGATTG
SLJ25057	*Rpi-amr3i*	qPCR	MP016	TCATCATAACTTCAAGGAGGTAAG
SLJ25057	*Rpi-amr1e*	qPCR	KW_amr1e_qPCR_F	GAGATTCCGGAGAGTATTGGAGAAAT
SLJ25057	*Rpi-amr1e*	qPCR	MP053	CGGGGGCAACATGCTTATTTCGTC
All relevant gDNAs	*Vacuolar Invertase*	ddPCR	MP013	CTGGGTCAAGTACAAAGGCAAC
All relevant gDNAs	*Vacuolar Invertase*	ddPCR	MP014	CATTTTGGGGTCCGGTCCAA
SLJ25057	*Rpi-amr1e*	ddPCR	MP059	GCTCTGGTGTAGAGACTAGTGC
SLJ25057	*Rpi-amr1e*	ddPCR	MP060	GAATTGGCTAATAATCAAAGATGGAG
SLJ25057	*CSR*	ddPCR	MP035	GAACTGTTATCAAAATCGCTAAAGC
SLJ25057	*CSR*	ddPCR	MP036	AGGAAAAGACTTATTTACCCTACATC

REFERENCES

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.