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

ANNEX 1

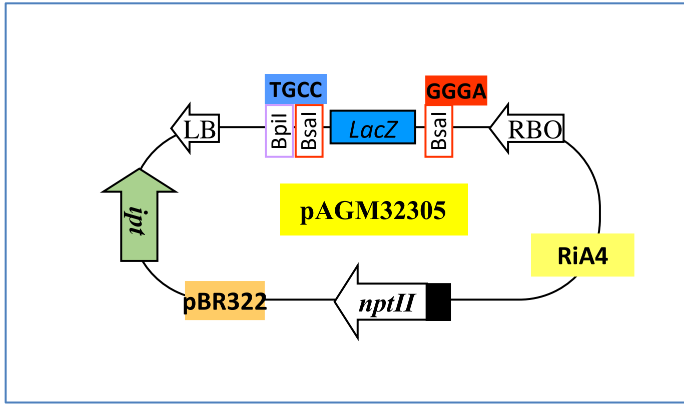
This annex contains methods for the experiments that were done to characterise the genetically modified plants referred to in the associated release application by The Sainsbury Laboratory. It also provides vector map and details of the structure of the T-DNAs in each plasmid used for plant transformation.

Section 1: Map of vectors pAGM32305 and pICSL32281_LacZ.
Section 2: Structure of T-DNAs in plasmids SLJ25057, SLJ25606, SLJ25586 and SLJ25587.
Section 3: Methods used for the characterisation of transgenic plants.

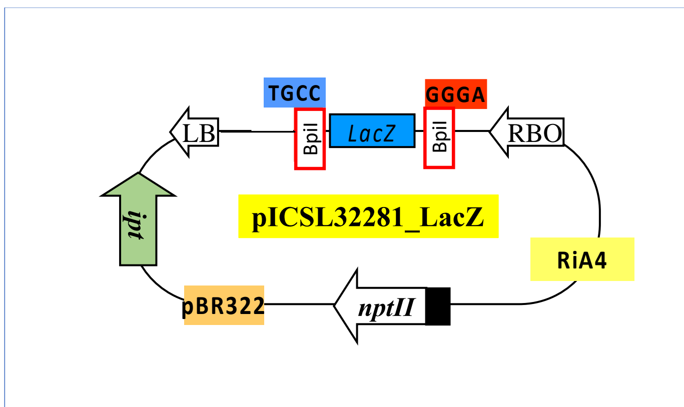
Table 1: List of primers used in PCR.

SECTION 1:

A) MAP OF VECTOR pAGM32305



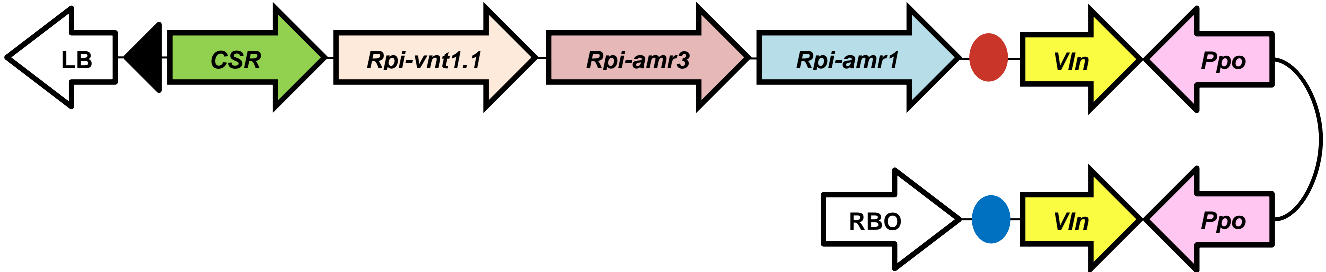
B) MAP OF VECTOR pICSL32281_LacZ



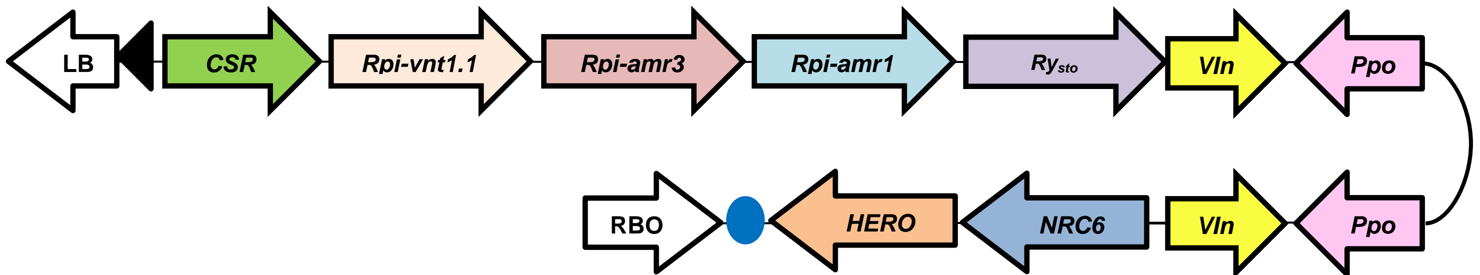
A detailed description of vector elements has been presented in Section 11 of Part A1. Note that the LacZ gene in the vectors' Golden Gate cassette is removed upon cloning.

<u>SECTION 2</u>: STRUCTURE OF T-DNAs IN PLASMIDS SLJ25057, SLJ25606, SLJ25586 AND SLJ25587.

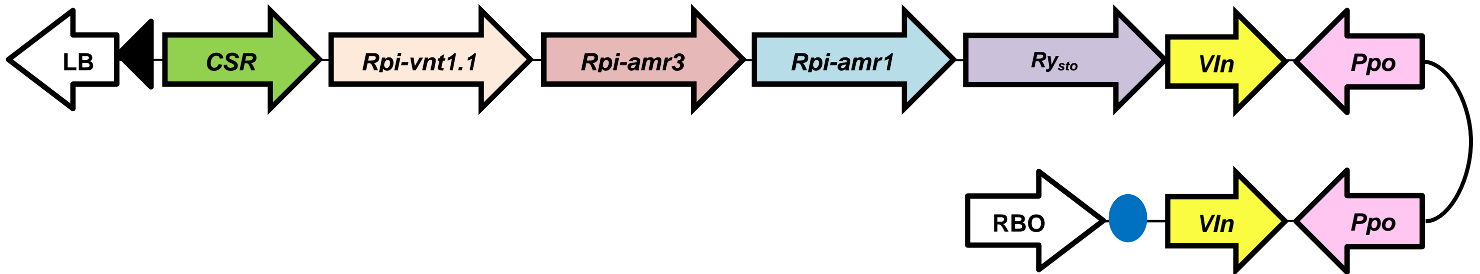
SLJ25057 T-DNA



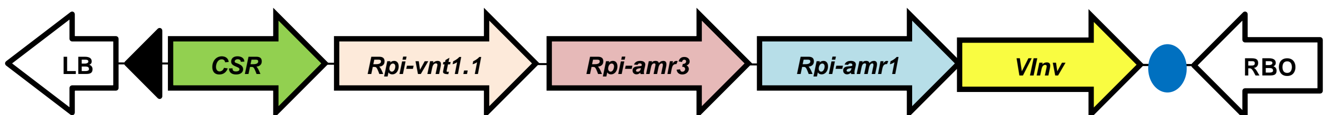
SLJ25606 T-DNA



SLJ25586 T-DNA



SLJ25587 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 triangles represent extra left border sequences. Red circles indicate dummy-linkers and blue circles represent end-linkers. Curved lines indicate spacer elements that create 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'' > $T^{o}_{annealing}$ 30'' > 68°C for corresponding elongation time], 68°C 5 min. $T^{o}_{annealing}$ 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).

Table 1. List of primers used in PCR tests

Construct	Gene/Region	Test	Primer ID	Sequence 5'-3'
All gDNAs	*EF1α*	PCR	MP075	GGAAGCTGCTGAGATGAACAAGA
All gDNAs	*EF1α*	PCR	MP076	CCTTCACAATTTCATCATACCTAGCC
Vector Backbones	*ipt* (outside LB)	PCR	MP115	AAAACTTATGGATCTGCGTC
Vector Backbones	*ipt* (outside LB)	PCR	MP116	GGAGCTGGTGCAAACTAATAC
Vector Backbones	*nptII*	PCR	MP112	GAAGAGTATGATTGAACAAGATGG
Vector Backbones	*nptII*	PCR	MP113	ATATATGAGTAAACTTGGTCTGAC
Vector Backbones	RiA4 (outside RBO)	PCR	MP110	CAAATAACAGTTGGGTGGAG
Vector Backbones	RiA4 (outside RBO)	PCR	MP111	CATGCTAACATTCAACTCTGGC
All SLJs	*CSR terminator*	PCR	MP037	TACAGATGGACAAGATCATTTACC
All SLJs	*Rpi-vnt1.1* promoter	PCR	MP081	TGGCTGTGAGTTTGGGCTATTATG
All SLJs	*Rpi-vnt1.1* terminator	PCR	LT179	ATGCTTGACTAAGAAGC
All SLJs	*Rpi-amr3* promoter	PCR	KW_amr3_prom_seq_R	TAATCTTGTAGCCTTGAACATGCC
All SLJs	*Rpi-amr3* terminator	PCR	MP041	CATCTAATGCCATCTTCCAAATGC
All SLJs	*Rpi-amr1* promoter	PCR	MP056	CCTCAAAAGTTGCAACTTACATTCCTC
All SLJs	*Rpi-amr1* terminator	PCR	AS206	GCAATGATGCGACGATATGGTTG
All SLJs	Ry_{sto} promoter	PCR	AW_Ry_F1	CTATGTGATTGTTATAACTTACGCATG
All SLJs	Ry_{sto} terminator	PCR	AW_Ry_R1	ACCTTATGCTAATGTACGCGTAAG
SLJ25606	*NRC6* promoter	PCR	AW_NRCH_R4	TGATTAAACTACTAAATACATGACTC
SLJ25606	*NRC6* terminator	PCR	AW_NRCHF4	GATGTTTGGATATCTATGTATTGTTCGA
SLJ25606	*Hero* promoter	PCR	AW_pHERO_R	TTCCATAAATTTAGCATATAATCCTTC
SLJ25606	*Hero* terminator	PCR	AW_HeroTer_F2	GTTGATGAAGCCATGAAAGAT
All SLJs except SLJ25687	*Agp* promoter	PCR	MP157	CAAGCTTGTTAACGGATC
All SLJs except SLJ25687	Spacer	PCR	MP148	TTCAGATTCTGGAGCGTCAG
All SLJs except SLJ25687	Spacer	PCR	MP154	TCCATAAGACCTTGACTG
All SLJs except SLJ25687	*GBSS* promoter	PCR	MP160	TTGTAGACCACACATCAC
SLJ24904	*CSR* terminator	PCR	MP037	TACAGATGGACAAGATCATTTACC

REFERENCES

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