

The New Zealand Institute for Plant & Food Research Limited

Plant & Food
RESEARCH

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A close-up photograph of several green leaves. The leaves are vibrant green and have serrated edges. The focus is sharp on the central leaf, showing its intricate vein structure. The background is softly blurred, showing more green foliage.

Intragenic transformation for genetic improvement of root and tuber crops

Tony Conner

Introduction



- Considerable concern among general public and politicians about the use of GM crops in agriculture
- A main underlying concern involves transfer of genes across very wide taxonomic boundaries
- Raises perceived risks for environmental and food safety, as well as ethical issues associated with interfering with nature

Gene transfer within species: a solution?



- Public opinion surveys repeatedly find gene transfer within species more acceptable
- Sequences from genomics programmes have immense value for within species gene transfer
- Provides a vast source of genes to effect transfers of ‘cisgenes’ or ‘intragenes’

An underlying issue for intragenic transfers



However – the transfer of genes within species still requires the use of vector systems based on DNA from highly divergent species:

- T-DNA border regions
- Selectable marker genes or recombination sites for their subsequent removal
- “Base” DNA with unique restriction sites to clone the gene-of-interest

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Can we circumvent the use of foreign DNA?

The challenge



- Key requirement is to find appropriate DNA fragments in genomes of each crop with the functional equivalence of important vector components
- Usually achieved by joining two DNA fragments together so that each provides half the desired component
- Non-transgenic “GM” crops?

Potato T-DNA region



1 GTCGACAGTA AAAGTTGCAC CTGGAATAAG GTTTTTCATTC TTCACAGGAG GCATCTCACT
61 CTTTCTAGCA GGTCTTGAAC GCTTAGATTG AACAGATGTA GGACTCACAT CTGATATGGA
121 GGATTCTTGA CTTGTTTCAG CAGCATCAGA TGAAGCTTCT GAGACTTCAC CTGATCCATC
181 ATCTGTAGCA GTTGCTTCTA CTTCTTCCAC TGCTACATCA GTCTCAGTTG CTGATACTAT
241 AAGACCTCTT AATTTAGGTC GTAAAATGCA ACCAACTCTA AAATGGGGAA ACAATTTAAT
301 AGATGTTGAC AGAGGCAGGA TATATTTTGG GGTA

Potato T-DNA region

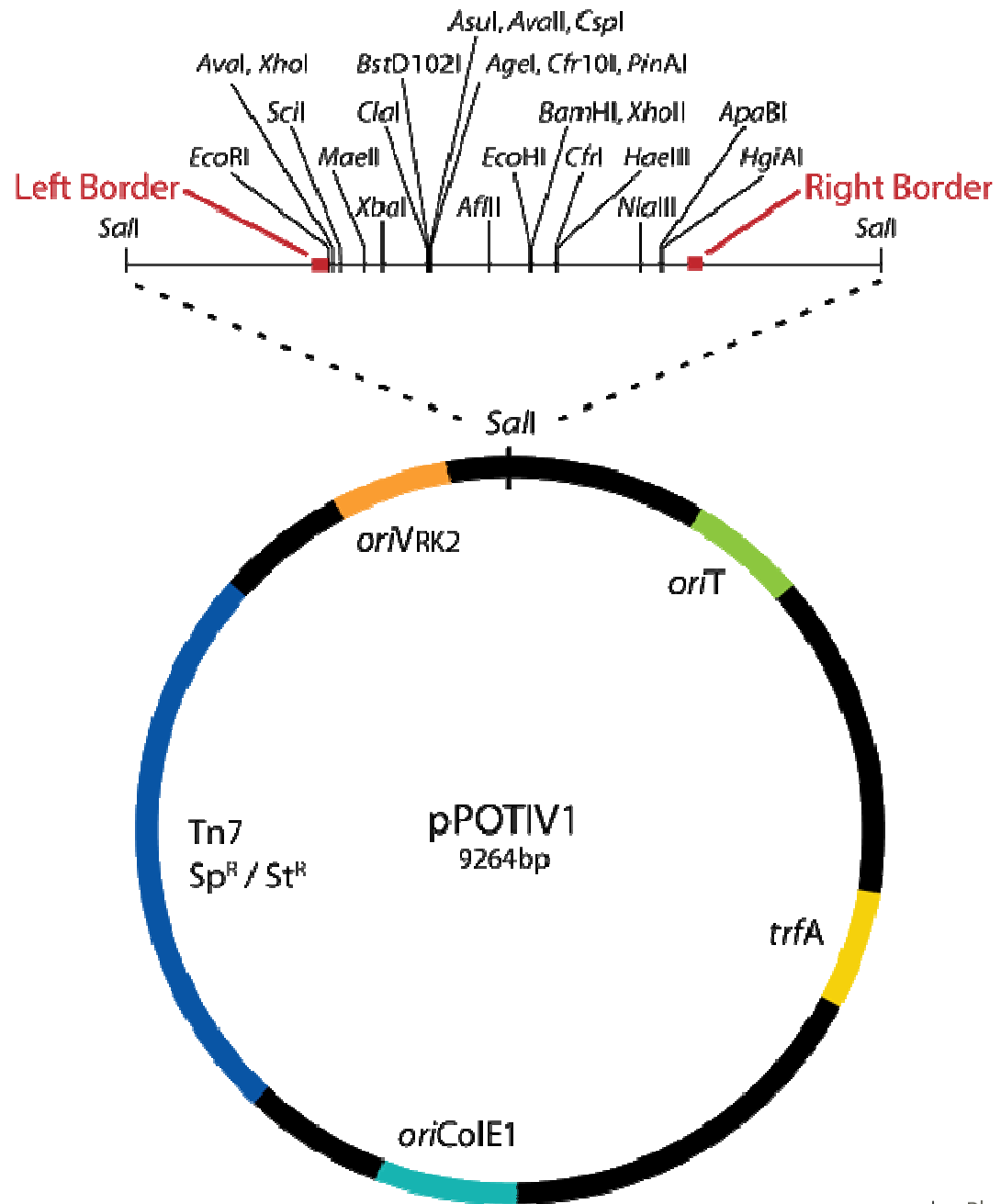


1 GTCGACAGTA AAAGTTGCAC CTGGAATAAG GTTTTTCATTC TTCACAGGAG GCATCTCACT
61 CTTTCTAGCA GGTCTTGAAC GCTTAGATTG AACAGATGTA GGACTCACAT CTGATATGGA
121 GGATTCTTGA CTTGTTTCAG CAGCATCAGA TGAAGCTTCT GAGACTTCAC CTGATCCATC
181 ATCTGTAGCA GTTGCTTCTA CTTCTTCCAC TGCTACATCA GTCTCAGTTG CTGATACTAT
241 AAGACCTCTT AATTTAGGTC GTAAAATGCA ACCAACTCTA AAATGGGGAA ACAATTTAAT
301 AGATGTTGAC AGAGGCAGGA TATATTTTGG GGTAAACGGG AATTCTTCAG CAGTTGCTCG
361 AGGGAGATTG GCGGTGCTTT CAGCTCACCT TGCAGCTTCA CTCAACGTCT CCGATTTAAC
421 AACCTTCAAA CTTCTAGAAA CTTCCGGTGT ATCCGCCGTT TCCGGCGTTG CACCTCCGCC
481 GAATCTAAAA GGTGCGTTGA CGATCATCGA TGAGCGGACC GGTAAGAAGT ATCCGGTTCA
541 GGTTCCTGAG GATGGCACTA TCAAAGCCAC CGACTTAAAG AAGATAACAA CAGGACAGAA
601 TGATAAAGGT CTTAAGCTTT ATGATCCAGG CTATCTCAAC ACAGCACCTG TTAGGTCATC
661 AATATGCTAT ATAGATGGTG ATGCCGGGAT CCTTAGATAT CGAGGCTACC CTATTGAAGA
721 GCTGGCCGAG GGAAGTTCCT TCTTGAAGT GGCATATCTT TTGTTGTATG GTAATTTACC
781 ATCTGAGAAC CAGTTAGCAG ACTGGGAGTT CACAGTTTCA CAGCATTCAG CGGTTCCACA
841 AGGACTCTTG GATATCATAc AGTCAATGCC CCATGATGCT CATCCAATGG GGGTTCTTGT
901 CAGTGCAATG AGTGCTCTTT CCGTTTTTCA TCCTGATGCA AATCCAGCTC TGAGAGGACA
961 GGATATATAC

Potato T-DNA region



1 GTCGACAGTA AAAGTTGCAC CTGGAATAAG GTTTTTCATTC TTCACAGGAG GCATCTCACT
61 CTTTCTAGCA GGTCTTGAAC GCTTAGATTG AACAGATGTA GGACTCACAT CTGATATGGA
121 GGATTCTTGA CTTGTTTCAG CAGCATCAGA TGAAGCTTCT GAGACTTCAC CTGATCCATC
181 ATCTGTAGCA GTTGCTTCTA CTTCTTCCAC TGCTACATCA GTCTCAGTTG CTGATACTAT
241 AAGACCTCTT AATTTAGGTC GTAAAATGCA ACCAACTCTA AAATGGGGAA ACAATTTAAT
301 AGATGTTGAC AGAGGCAGGA TATATTTTGG GGTAAACGGG AATTCTTCAG CAGTTGCTCG
361 AGGGAGATTG GCGGTGCTTT CAGCTCACCT TGCAGCTTCA CTCAACGTCT CCGATTTAAC
421 AACCTTCAAA CTTCTAGAAA CTTCCGGTGT ATCCGCCGTT TCCGGCGTTG CACCTCCGCC
481 GAATCTAAAA GGTGCGTTGA CGATCATCGA TGAGCGGACC GGTAAGAAGT ATCCGGTTCA
541 GGTTTCTGAG GATGGCACTA TCAAAGCCAC CGACTTAAAG AAGATAACAA CAGGACAGAA
601 TGATAAAGGT CTTAAGCTTT ATGATCCAGG CTATCTCAAC ACAGCACCTG TTAGGTCATC
661 AATATGCTAT ATAGATGGTG ATGCCGGGAT CCTTAGATAT CGAGGCTACC CTATTGAAGA
721 GCTGGCCGAG GGAAGTTCCT TCTTGAAGT GGCATATCTT TTGTTGTATG GTAATTTACC
781 ATCTGAGAAC CAGTTAGCAG ACTGGGAGTT CACAGTTTCA CAGCATTGAG CGGTTCCACA
841 AGGACTCTTG GATATCATA AGTCAATGCC CCATGATGCT CATCCAATGG GGGTTCTTGT
901 CAGTGCAATG AGTGCTCTTT CCGTTTTTCA TCCTGATGCA AATCCAGCTC TGAGAGGACA
961 GGATATATAC AAGTGTAAC AATTTAAAAG CATATGGTGG CACTGCTCAA TATATGAGGT
1021 GGGCGCGAGA AGCAGGTACC AATGTGTCCT CATCAAGAGA TGCATTCTTT ACCAATCCAA
1081 CGGTCAAAGC ATACTACAAG TCTTTTGTCA AGGCTATTGT GACAAGAAAA AACTCTATAA
1141 GTGGAGTTAA ATATTCAGAA GAGCCCGCCA TATTTGCGTG GGAATCATA AATGAGCCTC
1201 GTTGTGAATC CAGTTCATCA GCTGCTGCTC TCCAGGCGTG GATAGCAGAG ATGGCTGGAT
1261 TTGTCGAC



Plant-derived bacterial plasmids



- We have established that potato-derived sequences retain expected function of important vector components such as:
 - T-DNA borders
 - bacterial origins of replication
 - selectable markers for use in bacteria
 - regions for site-specific recombination
- Provides tools to build the complete bacterial plasmids out of potato DNA
- Concept extendable to virtually all crops; only requires minimal DNA sequence resources

Options for sweetpotato



Accession & location	First segment	Second segment	Accession & location
T-DNA borders			
EE881471 (575-583)	TGGCAGGAT	ATATCCAATGGTAAAC	DC880504 (108-123)
EE883720 (rc, 355-363)	TGGCAGGAT	ATATCCAATGGTAAAC	DC879607 (104-119)
EE881527 (rc, 162-170)	TGGCAGGAT	ATATCCAATGGTAAAC	EE874747 (554-569)
DC881838 (242-250)	TGACAGGAT	ATATCCAATGGTAAAC	DC880504 (108-123)
FRT recombination sites:			
CO500980 (583-599)	TTT GTT CG TATTCTCTA	GAAAGTATA AGAACA GA	DC880157 (rc, 184-200)
DV038047 (224-240)	CAAGTTT CTATTCTCTA	GAAAGTATA AGAACA GA	DC880157 (rc, 184-200)
LoxP recombination site:			
EE874787 (482-498)	ATAACT TTCTT ATTGCAT	ACATA AATACA AAAGTTAT	DC881678 (30-46)

Options for cassava



Accession & location	First segment	Second segment	Accession & location
T-DNA borders:			
GR421427 (549-557)	TGGCAGGAT	ATATCTTCTGGTCATC	FG806565 (148-163)
FF536695 (395-403)	TGGCAGGAT	ATATTCCTCGGTCAAG	FF536559 (144-159)
DB928281 (rc, 241-249)	TGACAGGAT	ATATAACCATCTAAAG	CK649311 (165-180)
DB952809 (504-512)	TGGCAGGAT	ATATAGCTATCTCATC	DB921435 (rc, 155-170)
FRT recombination sites:			
DB930086 (117-133)	TTT GTTTC G TATTCTCTA	GAAAGTATA AGAACAGA	DV452209 (637-653)
CK648822 (rc, 376-392)	CAAGTTT CTATTCTCTA	GAAAGTATA AGAACAGA	DB928670 (586-602)
LoxP recombination site:			
DB933318 (382-415)	ATAACTTCGTATAGCAT	ACATTATACGAAGTTAT	DB948195 (rc, 601-634)

Options for taro



Accession & location	First segment	Second segment	Accession & location
<i>FRT</i> recombination sites:			
FD509778 (10-26)	TTGAAAAACCTT CTCTA	GAAAGTAG TTGGTGGG TC	FD509778 (183-199)
FD509778 (114-130)	AAAAATATCATCAT CTA	GAAAGTATA AGAACA GA	FD480279 (410-426)
EU368044 (rc, 385-401)	GTTGCTTGCACCATCTA	GAAATTGTT CGCACTTC	EU368044 (rc, 259-275)
<i>LoxP</i> recombination site:			
EU369669 (rc, 18-34)	ATAAC AACGGATATA AAT	ACAT GATTCGAATTGTG	EU369669 (284-300)

Options for yam (*Dioscorea*)



Accession & location	First segment	Second segment	Accession & location
<i>FRT</i> recombination site			
DN792562 (rc, 332-348)	ATTGTTCTTACA TCTA	GAAA A TAA A GAAG A T A	DN792567 (rc, 284-300)
<i>LoxP</i> recombination site			
DN792554 (60-74)	CTACCATCGTCT GGCAA	ACC AGACA AGAAG CCAT	DN792579 (89-107)

Options for oca (*Oxalis tuberosa*)



Accession & location	First segment	Second segment	Accession & location
<i>FRT</i> recombination site			
AF470273 (85-101)	AGTGCCACTAATA TCTA	GAAAG AATTGTAAGAAC	AF470273 (579-595)
<i>LoxP</i> recombination site			
AF470300 (rc, 91-107)	ATAA TTCTTCTAGCATA	ACA ACATGCGAACGTAT	Z66546 (38-54)

Issues to be resolved



- Efficient plant-derived selectable marker genes
- To date, entirely plant-derived plasmids are low copy and difficult to use (require specific mutants)
- Inadvertent transfer of plasmid backbone sequences, especially from binary vectors with plant-derived T-DNAs
- New gene transfer tools based only on regions for site-specific recombination and a single T-DNA border (minicircle transformation)

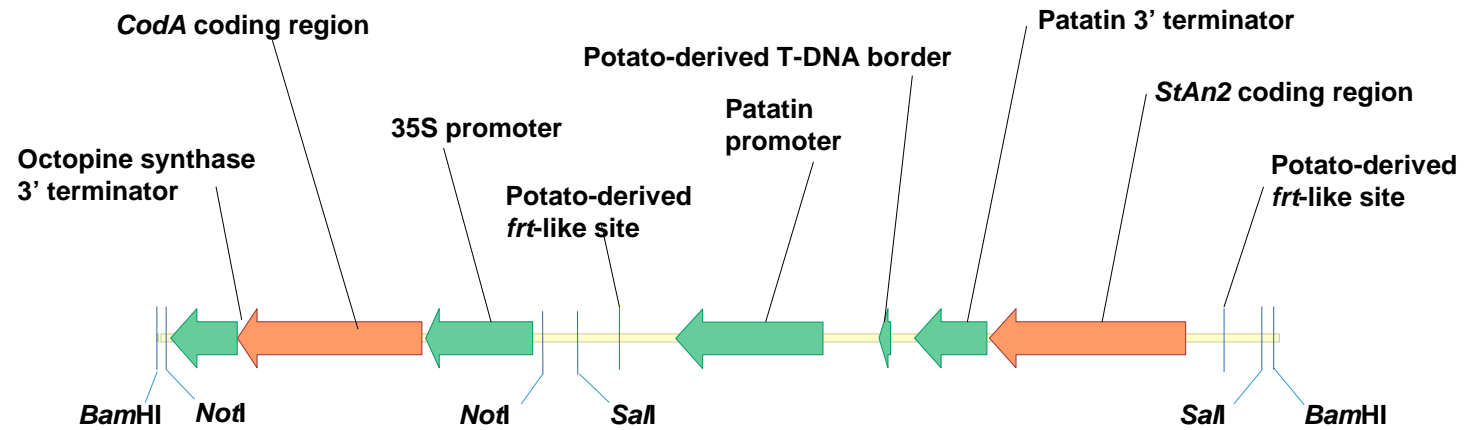
Plant-derived marker genes



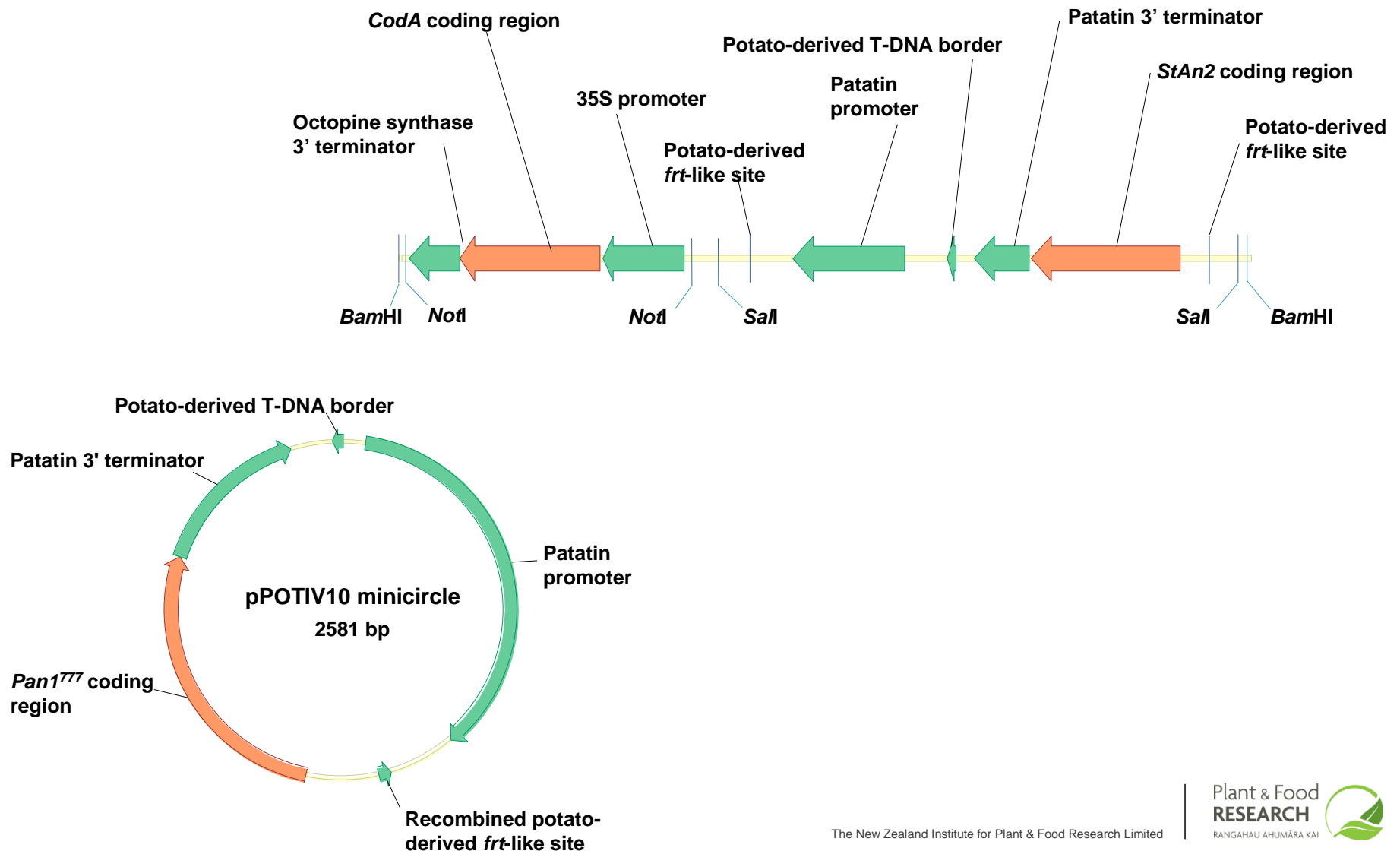
- Pigmentation genes (*StAn2* – *D* locus)
 - R2R3 myb transcription factor that controls the anthocyanin pathway in potato
 - under the expression signals of potato GBSS and patatin genes
- Mutant acetohydroxyacid synthase gene



Potato intragenic minicircle T-DNA



Potato intragenic minicircle T-DNA



Current targets for intragenic gene transfer in potato



- Disease resistance, especially late blight
- Improved abiotic stress tolerance
- Tuber-specific anthocyanin pigmentation
- For proof-of-concept this strategy involves:
 - allele mining from germplasm collections to identify variant versions of genes with novel function
 - confirm gene function using a transgenic approach
 - insert genes into desired expression cassette
 - achieve gene transfer via potato-derived T-DNAs

Breeding outcomes



- Intragenic vectors allow the targeted design of minor rearrangements in crop genomes
- Very similar to micro-translocations that could occur naturally or induced via mutation breeding
- Provides a valuable breeding tool for all crops by targeted genome rearrangement
- Especially applicable for the highly heterozygous clonal crops

Conclusions



- Intragenic vectors may help alleviate public concerns over deployment of “GM crops”
- Especially ethical issues associated with transfer of DNA sequences across wide taxonomic boundaries
- Anticipated to provide a more socially acceptable and responsible way forward for the deployment of GM crops
- Less stringent oversight and reduced cost of compliance to allow GM approaches to be deployed in ‘minor crops’

Acknowledgements



- Julie Pringle – vector design, construction & transformation
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