

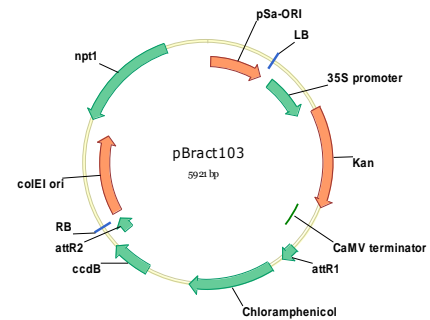


Transformation Resources

(for Brassica, Barley and Wheat)

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BIOTECHNOLOGY RESOURCES FOR ARABLE CROP TRANSFORMATION

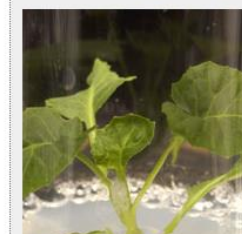


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Welcome to BRACT: Biotechnology Resources for Arable Crop Transformation

Supporting Barley, Wheat and Brassica transformation, the BRACT facility offers the research community access to:

- Easy to follow transformation protocols
- Germplasm (of readily transformable genotypes)
- Hosted training programmes
- pBRACT crop designed constructs (and construct building services)
- A full range of transformation services (from supply of primary transgenics, through to seed of subsequent generations, molecular analysis and basic phenotyping)
- And help and advice with grant applications/ project planning



Genetic modification of plants for research purposes is an invaluable tool used by scientists globally to gain a better understanding of the functions of certain genes in plants. It is now used routinely in model plants where the ability to modify genes or switch them on and off has led to a wealth of

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- The majority of our users cost BRACt into their research grants
 - Can provide text for grant applications/ letters of support/ advice on timescales
- Full range of transformation services: From the design and build of constructs; to production of primary transgenics, next generation transgenics (T1/T2) to basic phenotyping



Primary transgenics
8-12 weeks



GH facilities for generating T1 seed



Basic Phenotyping (case-by-case)



BRACT contacts

wendy.harwood@jic.ac.uk (PL: barley/ wheat)

penny.hundleby@jic.ac.uk (Brassica)

mark.smedley@jic.ac.uk (constructs)

tom.lawrenson@jic.ac.uk (CRISPR advice)



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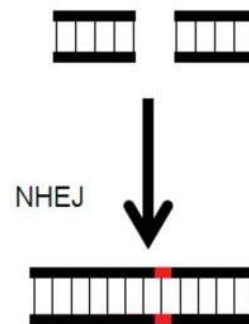
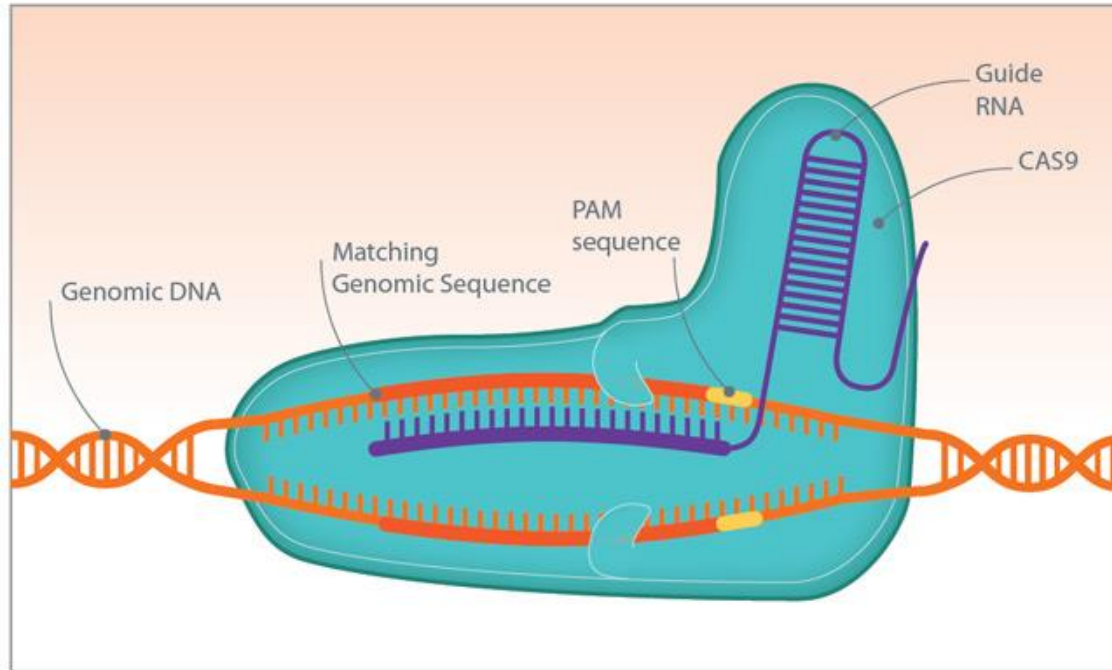


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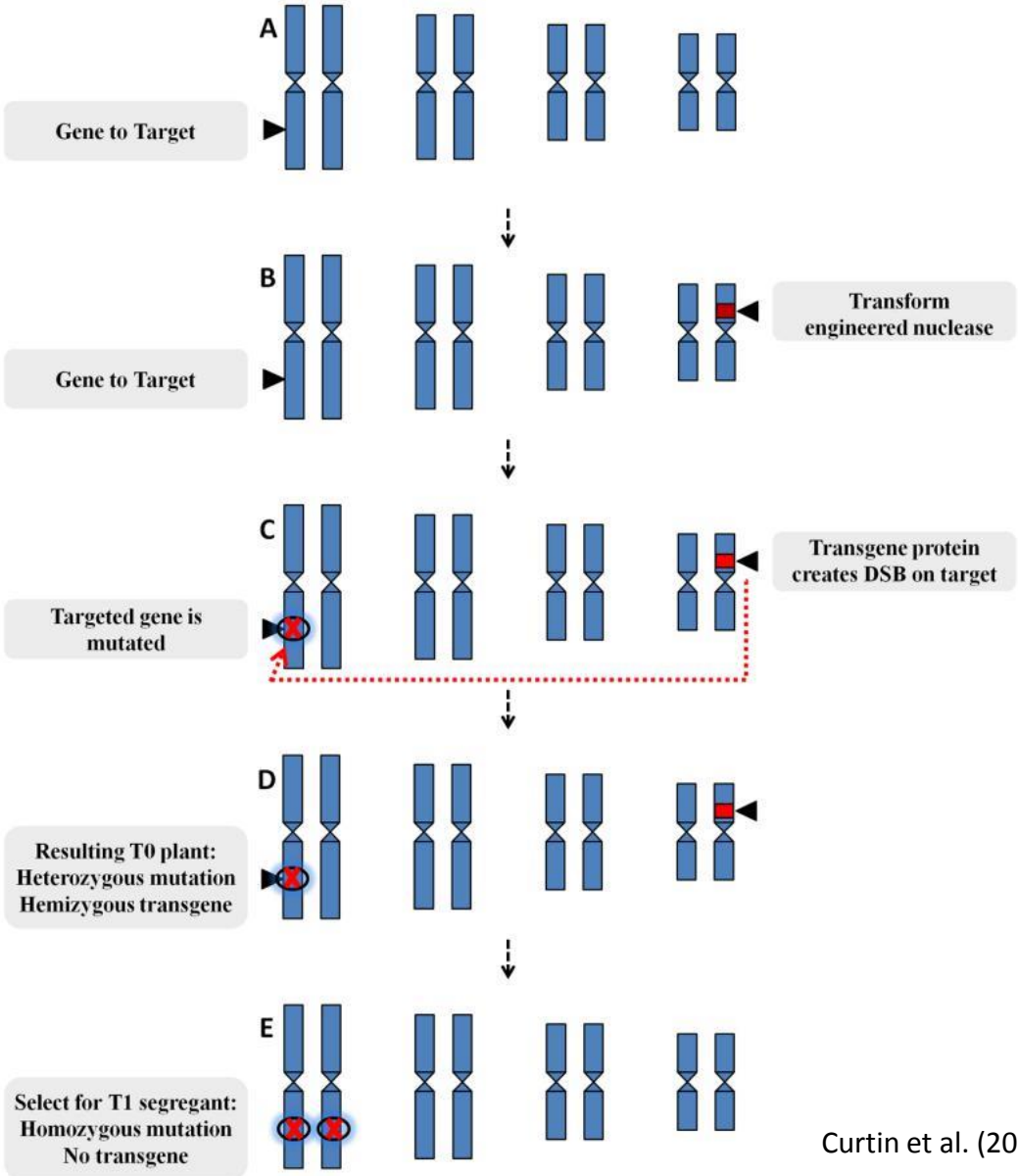


Evaluation of CRISPR/Cas9 as a tool to generate knock out mutants in your favourite gene

Double strand break and error prone repair by non-homologous end joining to create indels (small insertions or deletions)



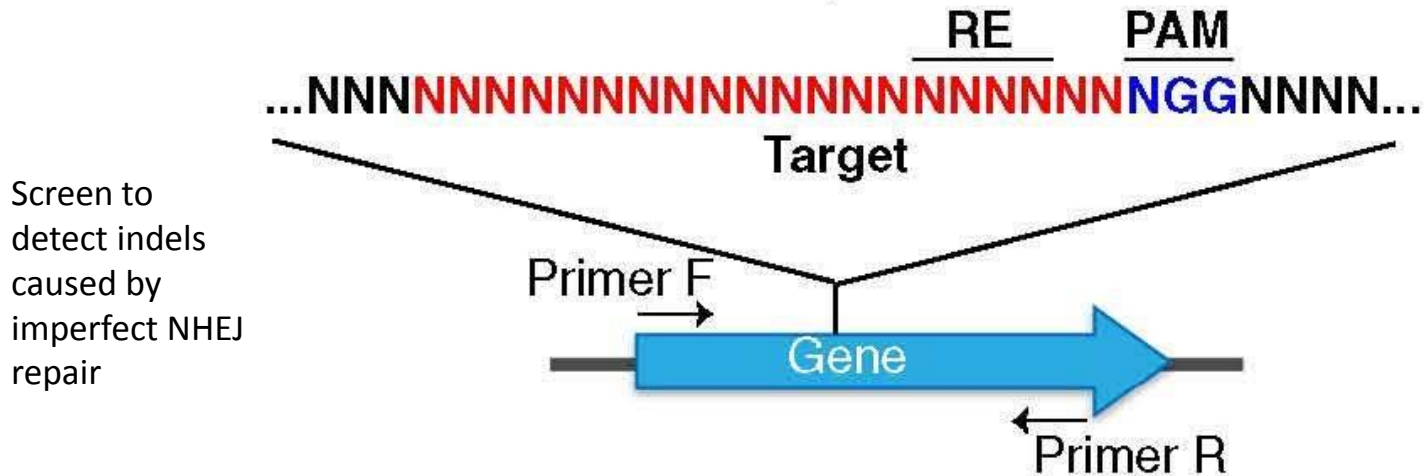
Recovering plants containing only the target mutation



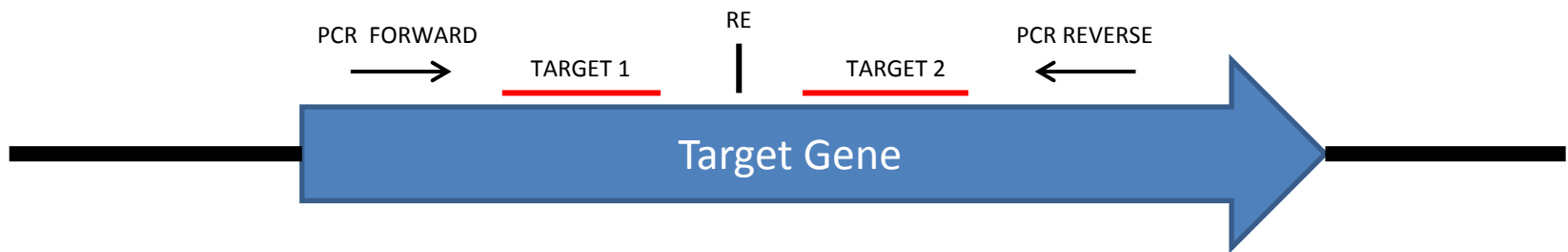
- CRISPR targeting of GA4 on chromosome 5 *Brassica oleracea* DH1012
- Constructs easily assembled using GoldenGate cloning
- Level 1 Cas9 transcriptional unit available
- Level 1 gRNA cassettes synthesised for around £30 each
- 2 constructs: 1 with single GA4 gRNA and 1 with pair of GA4 gRNAs



Single guide RNA strategy



Paired guide RNA strategy

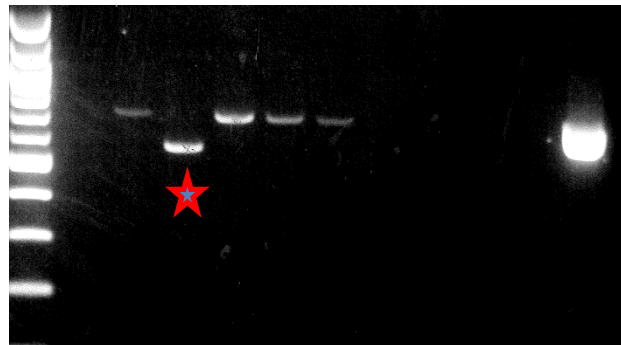


Screen to detect deletions corresponding to distance between two targets

Single guide T0 screen. Enriched for mutations by restriction digestion followed by PCR. Bands in + (restriction) lanes were cloned and sequenced to reveal indels at target locus.



Paired guide T0 screen. Enrich for mutations by restriction digest followed by PCR. Band shift to lower position indicates deletion at target locus confirmed by sequencing



Results

- 80 primary T0 plants generated
- A subset of 20 were screened for indels/deletions
- 2/20 lines screened were active
indicating 10% of transgenics will be active for the knockout

Exon 1 of GA4 oxidase on chromosome 5 *B.oleracea* DH1012

Guide sequence

PAM

Single guide

WT ACGATCCCCTCTTGACGCCGCTCCTTCCCCTCCGCGCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
Mt1 ACGATCCCCTCTTGACGCCGCTCCTTCCCCTT-GCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
Mt3 ACGATCCCCTCTTGACGCCGCTCCTTCCCCTCA-GCCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
Mt4 ACGATCCCCTCTTGACGCCGCTCCTTCCCCT-----CCGCAAGTGAAAACATCCCTCTCATTGACCTGAA
Mt5 ACGATCCCCTCTTGACGCCGCTCCTTCCC-----CAAGTGAAAACATCCCTCTCATTGACCTGAA

% ARE FRAMESHIFT

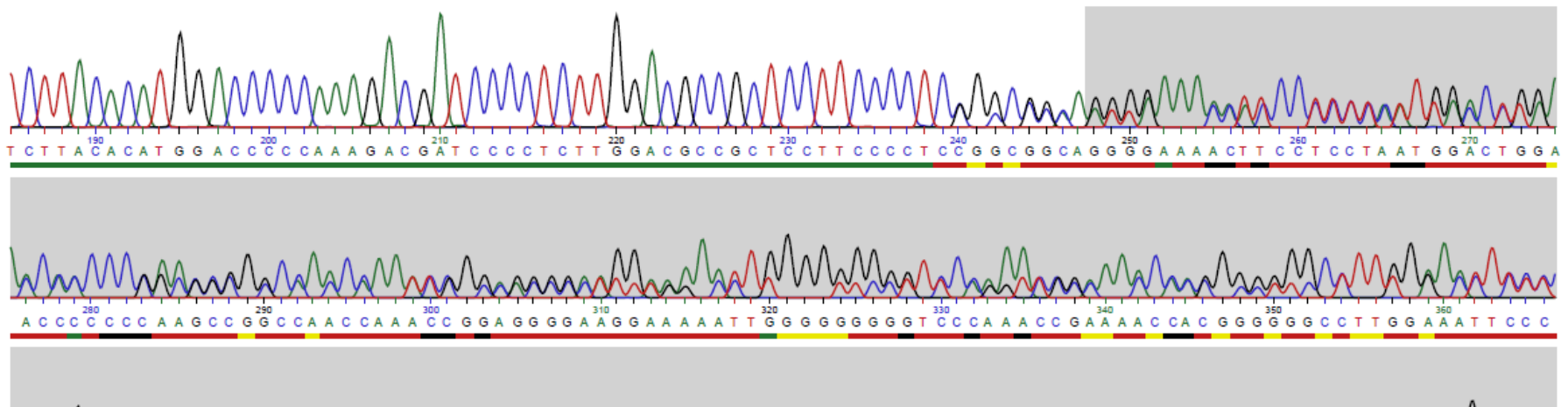
Pair of guides

CCCCTCCGCGCCGCAAGTGAAAAC-----280BP-----CCATCACC GGCTCCCCTCTCAAG

Mutant	1	CCTGATTCTTACACATGGACCCCAAGACGATCCCCTCTTGGACGCCGC	50
WT	1	CCTGATTCTTACACATGGACCCCAAGACGATCCCCTCTTGGACGCCGC	50
Mutant	51	TCCTTCCCCTC-----	61
WT	51	TCCTTCCCCTCCGCGCCGCAAGTGAAAACATCCCTCTCATTGACCTGAAC	100
Mutant	62	-----	61
WT	101	ACCCGACGCGGCCAACCAATCGGCAGCGCATGTAGAACTGGGGTGCG	150
Mutant	62	-----	61
WT	151	TTCCAGATCGCAAACCACGGCGTGCCTTGGAACTTCTCCAAGGCATTGA	200
Mutant	62	-----	61
WT	201	GTTTTCACAGGCAGTCTTTTCAGCTACCTGTCCACCGCAAGCTTAAGG	250
Mutant	62	-----	61
WT	251	CGGCTCGGTCGGAGACAGGTTTCTCTGGCTACGGCGTCGCTCGTAICTCA	300
Mutant	62	-----CCGGCTC	68
WT	301	TCTTCTTCAATAAGCAAATGTGGTCCGAAGGTTTATCCATCACC GGCTC	350
Mutant	69	CCCTCTCAACGACTTCGGTAACTTTGGCCCAA	102
WT	351	CCCTCTCAACGACTTCGGTAACTTTGGCCCAA	384

282 bp deletion = 94 amino acids removed

- T1 screening for homozygotes and heterozygotes via PCR and direct sequencing
- 96 T1 plants per active line
- One T0 active line showed no indel activity in T1
- Second active line showed high frequency of multiallelic events (50/96)



2/80 T0 plants showed dwarf phenotype consistent with GA4 knockout mutant in Arabidopsis



PCR and direct sequencing (no enrichment) showed C5 GA4 homozygous for frameshift mutations. Checking C8 copy showed only WT sequence.

Two gRNAs used to target chromosome 5 GA4 and relation to chromosome 8 copy

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
	C	C	C	C	T	C	C	G	G	C	C	G	C	A	A	G	T	G	A	A	A	A	C	gRNA 1	
c5																									
c8																									
	C	C	A	T	C	A	C	C	G	G	C	T	C	C	C	C	T	C	T	C	A	A	C	gRNA 2	
c5																									
c8																									



PAM



Mismatch



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