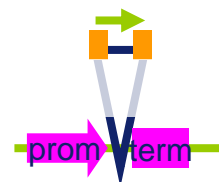


Brassica transformation



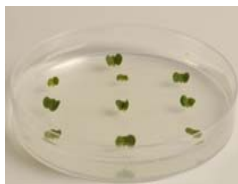
Biotechnology Resources for Arable Crop Transformation

- Brassica resources and facilities
- Introduction to AdVaB



Brassica transformation: resources

- Protocols available for *B. oleracea* and *B. napus* (*B. juncea* and *B. carinata*)
 - Training and support
- Model genotypes – seed available
 - *B. napus* Q6 and Q31
 - *B. oleracea* 1012
- Screening system



Brassica transformation: facilities

- Hotel facilities
- JIC transformation 'service'
 - Production of primary transgenics



- » Basic package starts at £3625
- » Aim for 10 independent transgenic lines
- » Rooting on kanamycin
- » PCR tested for presence of nptII

- Production of T_1 progeny
- Phenotype testing
- Advice on timescales and costings for grant applications



Brassica transformation: requirements

- Kanamycin selection – essential!
 - 35S:KAN (NOS:KAN)
- *A. tumefaciens* strains LBA 4404 and AGL1
- pBRACT₁₀₃, pCAMBIA₂₂₀₀, SLJ₁₇₁₄ or 1711

Collaborative facilities



Quantitative PCR
Estimate copy number (alternative to Southern)
Predicting zygosity (null, hemi or homozygous lines)



DNA extractions

Brassica transformation

AdVaB

Adding Value to Brassica



ROTHAMSTED
RESEARCH

Access to reference populations and linkage map integration



Trait data collation

Collation of QTL unpublished and ongoing datasets



John Innes Centre

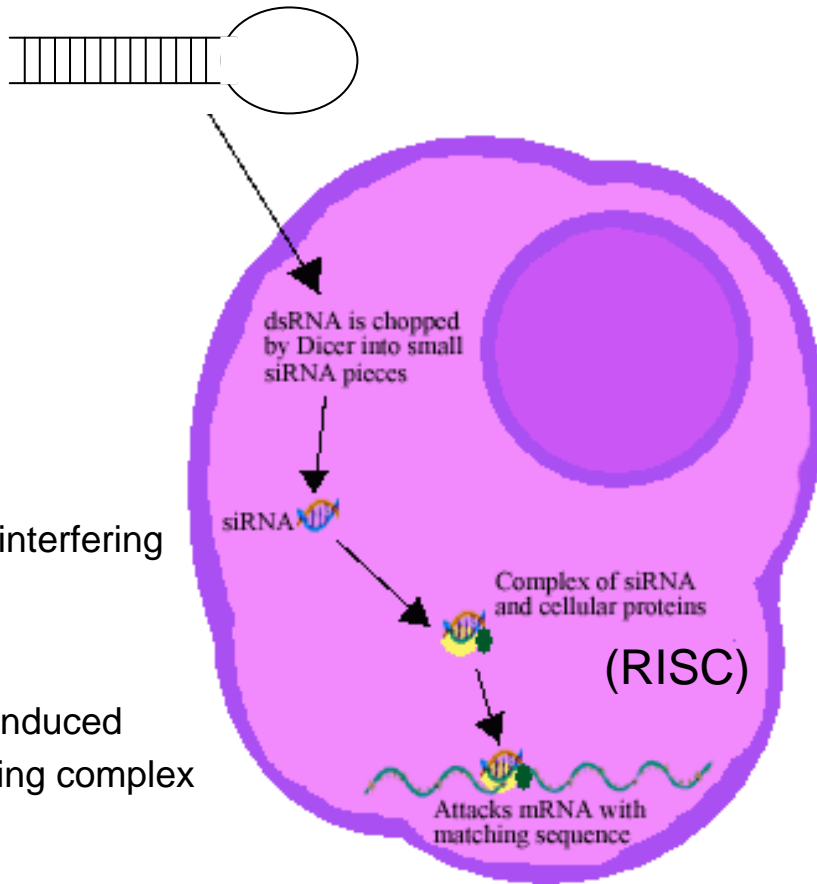
TILLING, RNAi, *B. rapa* transformation



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RNA interference (RNAi) transformation

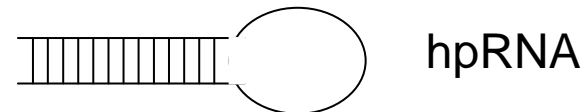
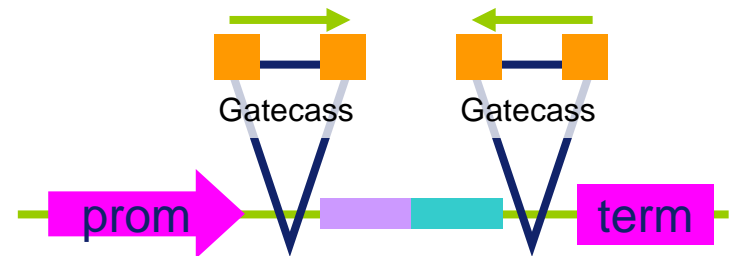
- reverse genetics approach
- construct contain sense/ anti-sense



small interfering RNA

RNA-induced silencing complex

Degrades mRNA = gene silencing



hpRNA

RNAi transformation

- Using candidate genes from UKBRC groups
 - *IND*; *FAE1*; *FCA*
 - RNAi phenotype is dominant – expect to see it in the T1
 - has potential to knock out multiple orthologues
- Initially test in *B. oleracea* (genotype 1012)
- Subsequently in *B. napus* (genotype Q6)

- RNAi transformation added to BRACCT

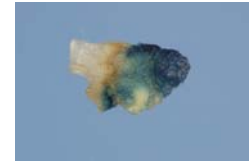
Developing *B. rapa* transformation

- Screened range of *B. rapa* germplasm
 - Most recalcitrant to *in vitro* shoot regeneration

- Best line R018



- Good susceptibility to *Agrobacterium*



- Shoots often small, vitrified and non viable (unable to isolate and root)

- Need to develop robust regeneration system
- Currently exploring alternative methods
 - Collaborations with Canada and S. Korea



Brassica contact

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