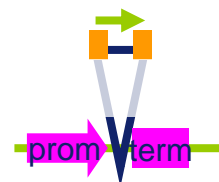


# Brassica transformation



## 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<sub>1</sub> progeny
- Phenotype testing
- Advice on timescales and costings for grant applications



# Brassica transformation: requirements

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- Kanamycin selection – essential!
  - 35S:KAN (NOS:KAN)
- *A. tumefaciens* strains LBA 4404 and AGL1
- pBRACT<sub>103</sub>, pCAMBIA<sub>2200</sub>, SLJ<sub>1714</sub> or 1711

# Collaborative facilities

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Quantitative PCR

Estimate copy number (alternative to Southernns)

Predicting zygosity (null, hemi or homozygous lines)



DNA extractions

# Brassica transformation

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## 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Collation of QTL unpublished and ongoing datasets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

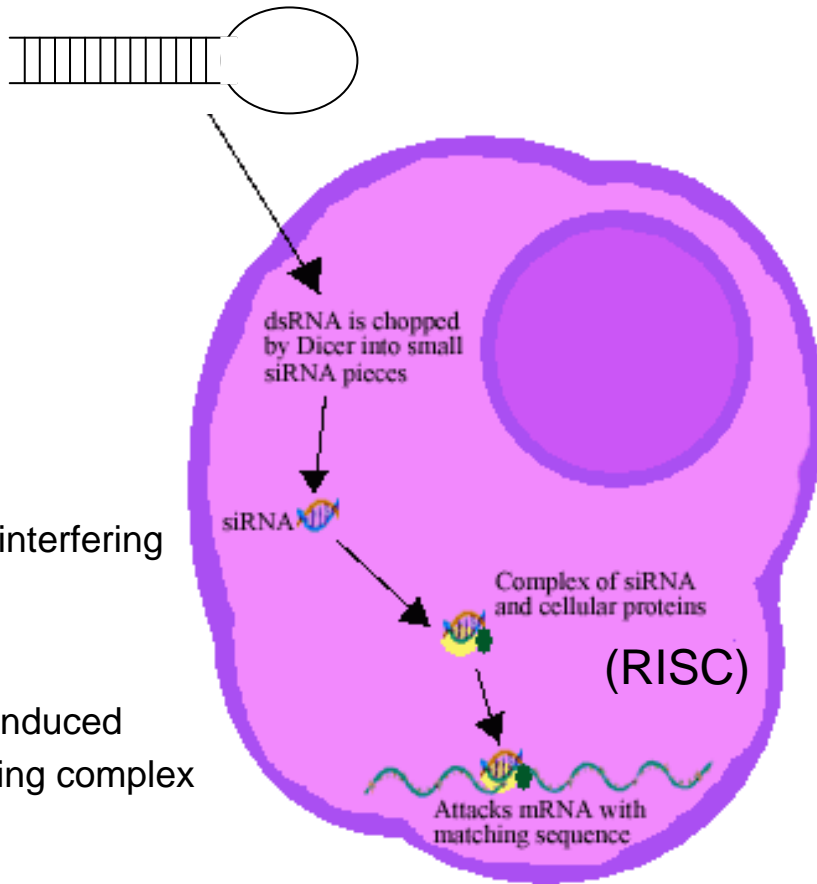
TILLING, RNAi, *B. rapa* transformation



John Innes Centre

# RNA interference (RNAi) transformation

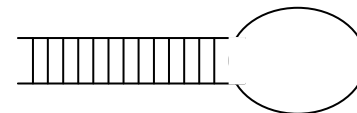
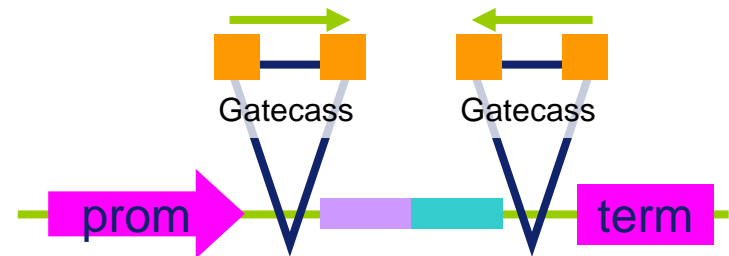
- reverse genetics approach
- construct contain sense/ anti-sense



small interfering RNA

RNA-induced silencing complex

Degrades mRNA = gene silencing



hpRNA

# RNAi transformation

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- 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

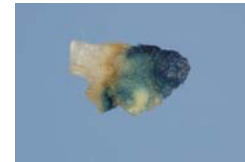
# Developing *B. rapa* transformation

- 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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## Acknowledgements

JIC - Judith Irwin; Lars Ostergaard; Ian Bancroft  
- Wendy Harwood and Mark Smedley

CPB Twyford - Peter Werner  
iDNA - Peter Isaac