

Brassica transformation



Biotechnology Resources for Arable Crop Transformation

- Brassica resources and facilities

Dr Penny Sparrow

Bract objectives & resources

To provide the UK research community with:

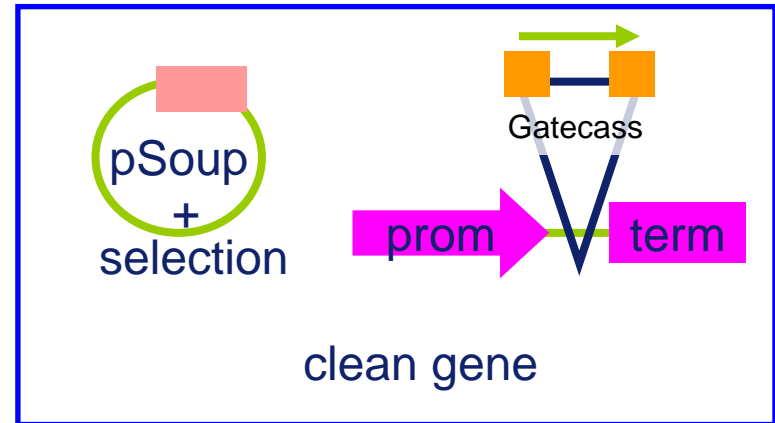
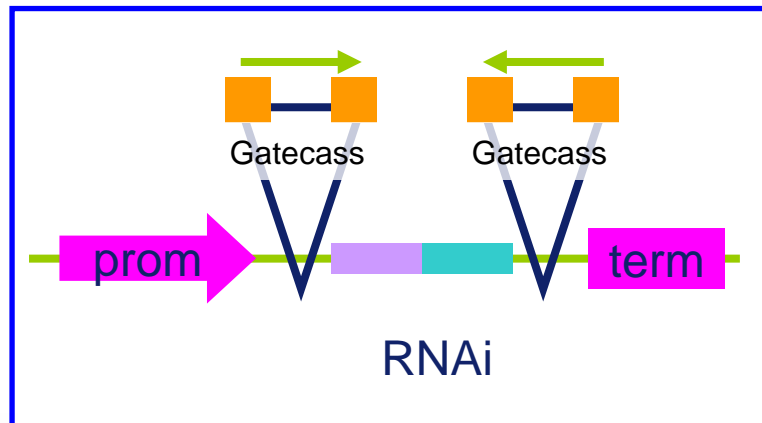
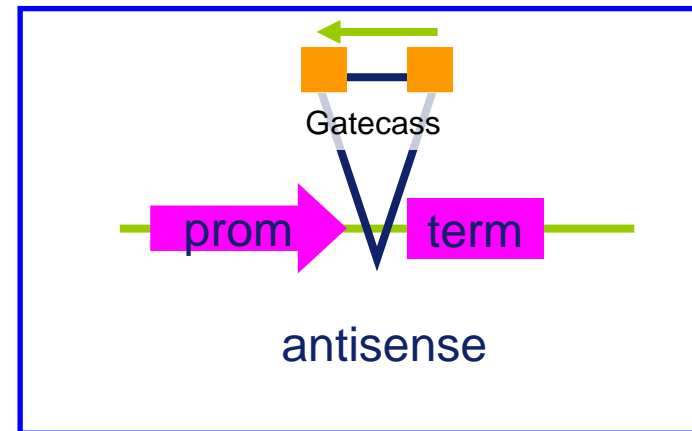
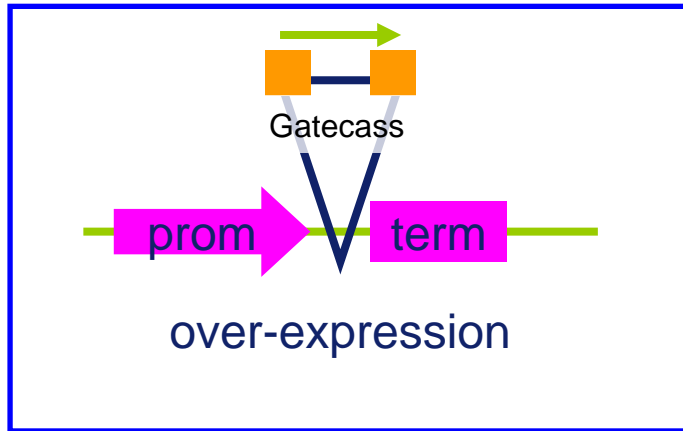


- up to date transformation protocols for the major UK crops. Wheat, Barley, Brassica and Pea www.bract.org



- access to a range of transformation vectors and ready made constructs
- a range of transformation resources and facilities

BRACT constructs



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Brassica objectives & outputs

- identify candidate genotypes for routine transformation work
 - *B. napus* and *B. oleracea* (>10%)
 - make germplasm available to the research community
- develop easy to follow protocols www.bract.org
- develop bract as centre of excellence for brassica transformation in the UK
 - offering a range of transformation facilities and resources

Three *B. napus* genotypes available



Q6_(PAC3) = 12.0%

Q31_(PAC2) = 17.1%

W10 = 7.6%

average
transformation
efficiency



Q6 and W10 = SOSR

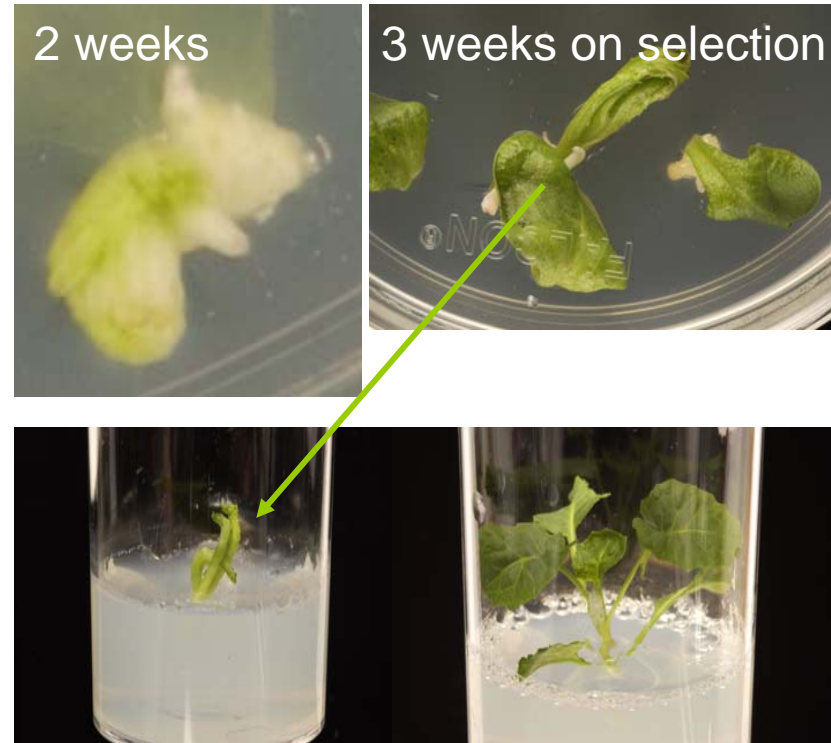
Q31 = WOSR

All doubled haploid, and self compatible

Q lines from a DH mapping population
(Bancroft Group)

B. oleracea: AG DH 1012*

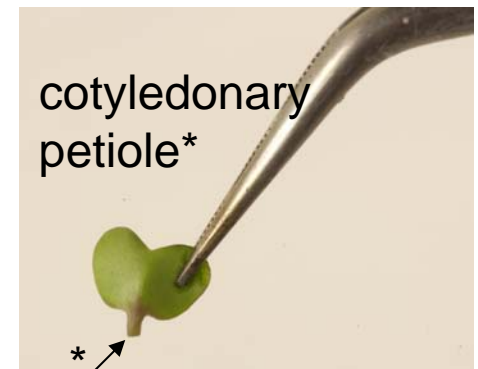
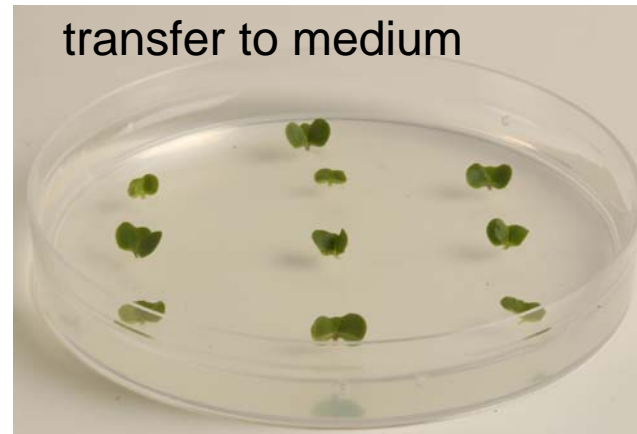
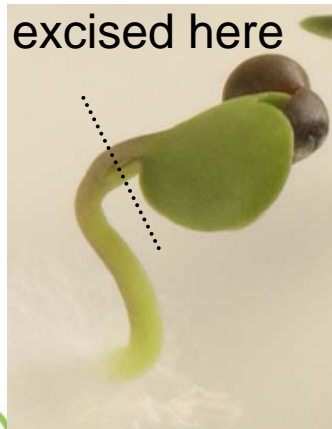
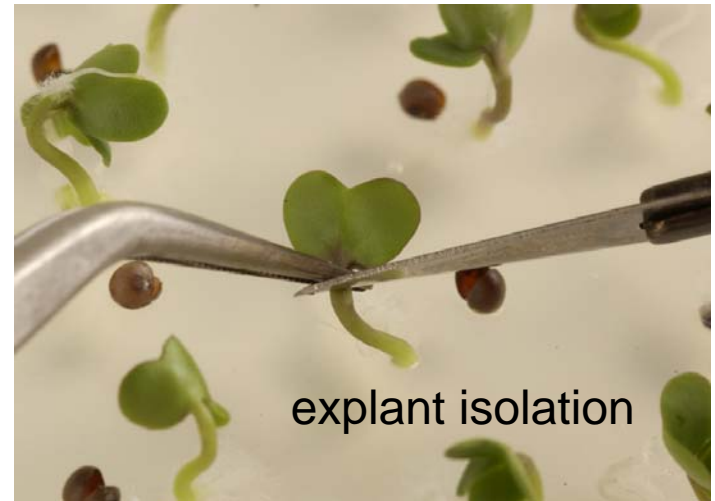
- transgenic shoots recovered after 4-5 weeks, at rates of >20% (previous in-house best 7%)
 - this genotype is rapid-cycling and highly self-compatible
 - associated genetic mapping population**
 - ‘model genotype’ for testing gene function



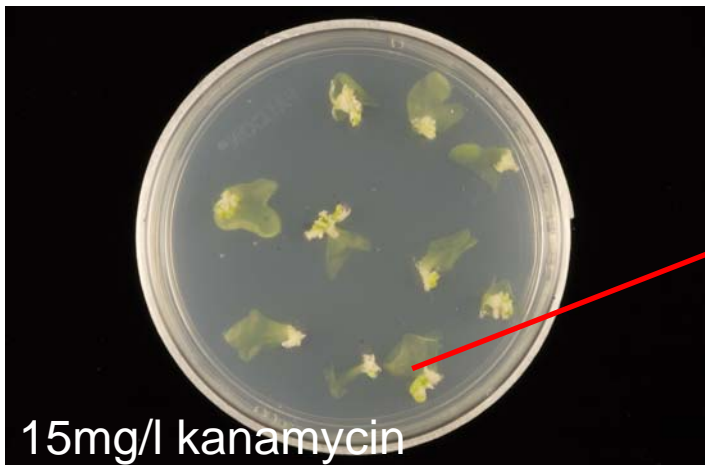
* Sparrow et al. 2004 Plant Cell Reports 23:64-70

** Bohuon et al. 1996 Theor Appl Genet 93: 833-839

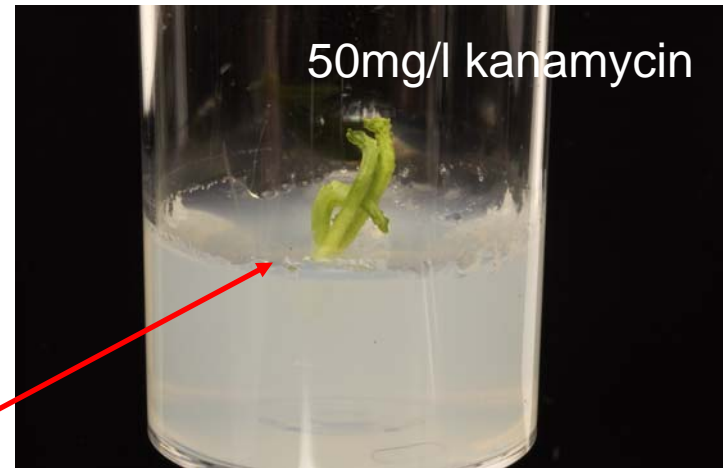
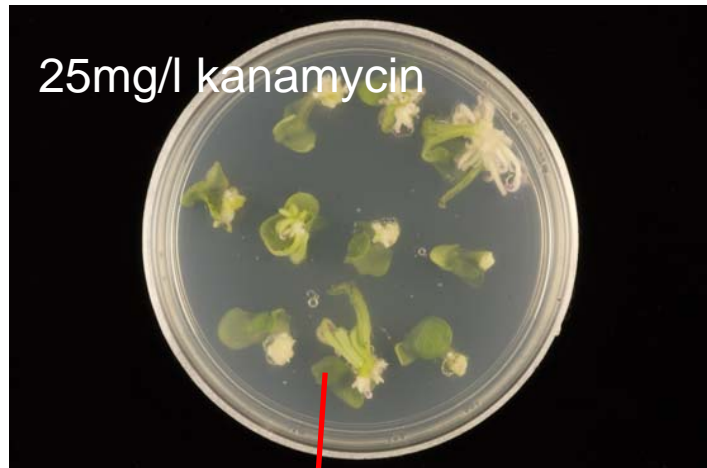
Protocol and photo-board now online



DH AG1012 @ 2 weeks



DH AG1012 @ 3 weeks



Approximate timescales



Rooted shoots transfer to sterile peat pots



~ 7-8 weeks



A fast turn over could see plants transferred to the glass house 10-12 weeks from explant isolation



Seed set ~
+ 6 weeks

harvest @
~32 weeks

Flower in 6-8 weeks
(~20 weeks after explant isolation)

Requirements and costs

- 35S kanamycin selection
- *A. tumefaciens* LBA 4404 and AGL1 favoured
- costs start at £3625 per experiment; aim to deliver 10 independent T₀ lines
- T₁ seed can also be supplied, as well as progeny testing at an additional cost
- advice on timescales and help with costings for grant applications



Summary

- up and running as a full transformation facility
 - provider &/ or collaborative programmes
 - provide users with T₀ plantlets, T₁ or T₂ seed
 - progeny testing
- protocol and trouble shooting guide www.bract.org
- seed of all genotypes freely available
- free training and advice
 - request feedback from users; e.g. use of alternative selectable markers, *Agrobacterium* strains etc.

Still room for improvement

Expand the

- range of *Agrobacterium* strains
- selectable markers; hygromycin, bar etc.
- range of promoters, constructs - RNAi
- range of Brassica species – *B. rapa*

- keep ahead of the field
 - input from science community



Brassica contacts

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