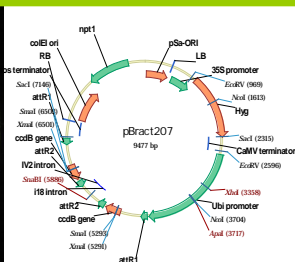




# BRACT Transformation Resources & An update on *B. rapa* transformation

Tom Lawrenson



# Summary of BRACT resources

- Efficient and reliable transformation protocols ([www.bract.org](http://www.bract.org))
  - Brassica (*B. oleracea* and *B. napus*), barley and wheat
- Training (via our website or as hosted training programmes)
- Germplasm (seed of readily transformable genotypes)
- Constructs (pBRACT series vectors, tried and tested in the crops)
- Full range of transformation services (to produce and test transgenics)
- BRACT operates on a non-profit cost recovery basis
- Help with grant applications/ project planning
  - Text for grant proposals/ letters of support
  - General advice on project approach/ scale/ timescale feasibility and cost breakdowns

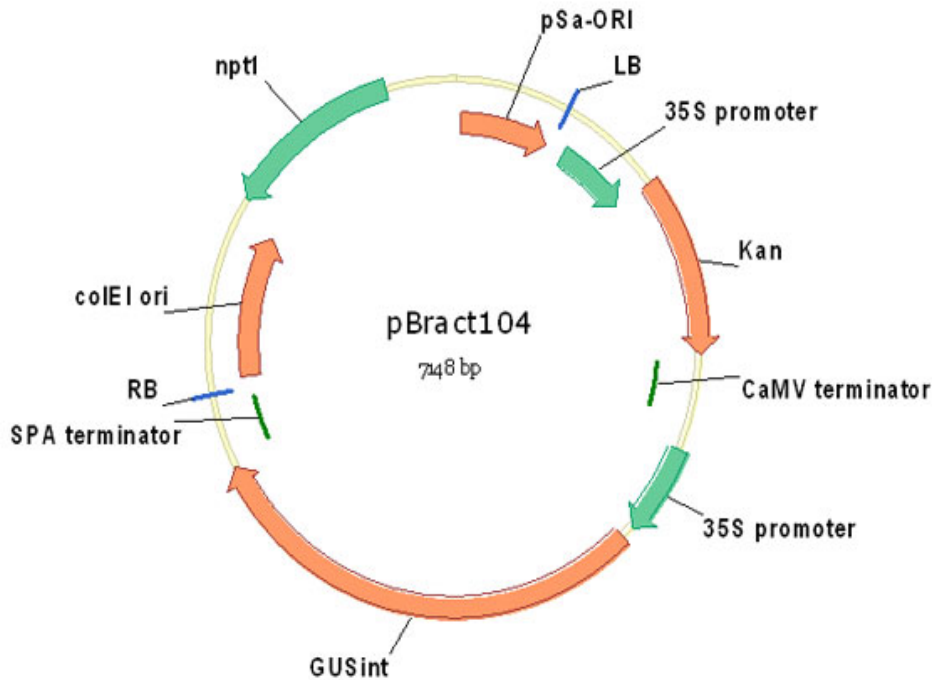


# Project objectives

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1. Produce a GUS reporter gene plasmid
2. Increase frequency of transformed cells
3. Increase the robustness of the shoot regeneration regime
4. Optimise selection criteria to combine the above and enable successful transformation
5. Evaluate molecular approach to increase transformation success
6. Demonstrate success with 'useful' genes!

# Produce a GUS reporter gene plasmid



GUS expression in a transgenic leaf of R-0-18

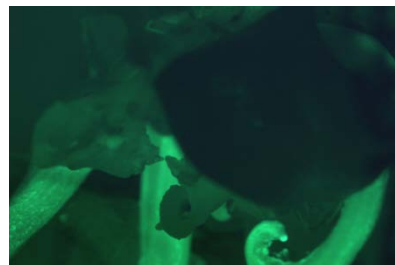


- 35S::KAN 35S::GUS
- Suitable for establishing and developing transformation protocols for use in Brassica
- Available via [www.bract.org](http://www.bract.org)

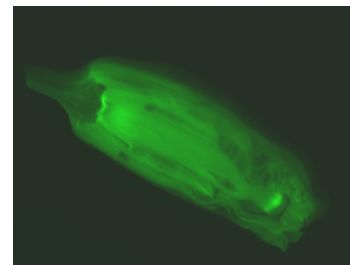
- In addition we have also tested GFP as a suitable reporter gene plasmid



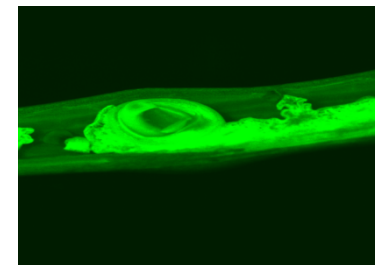
*B. rapa* R-o-18 callus - 14 days



*B. oleracea* DH 1012 shoot - 1 month



*B. oleracea* DH 1012 flower



*B. oleracea* DH 1012 fruit

# Starting point:

based on the protocol for *B.oleracea*  
further developed through the AdVAB project

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## Main lines of approach

- Growth regulators (cytokinins/auxins)
- Ethylene inhibitors
- Inhibitors of browning/vitrification/necrosis
- Promoters of *Agrobacterium* mediated transformation

# Increasing frequency of transformed cells, and shoot quality

Old *B. rapa*  
media  
BAP 3mg/L



New media  
BAP 4mg/L  
NAA 0.1mg/L

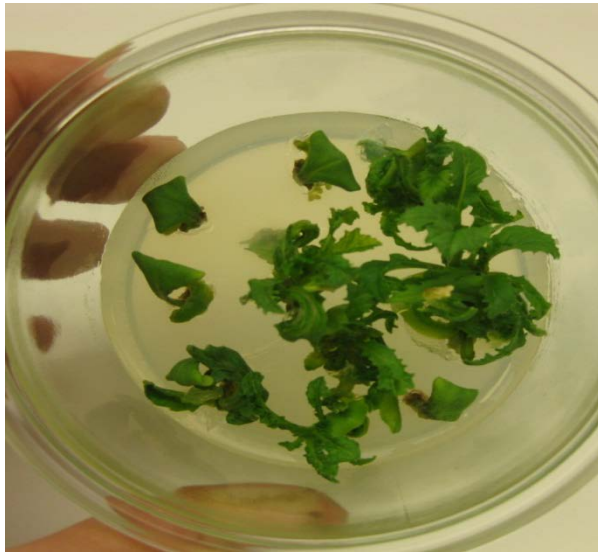


# Timing of transition to elongation media

Old media  
20-30% good shoots



New media  
90-100% good shoots



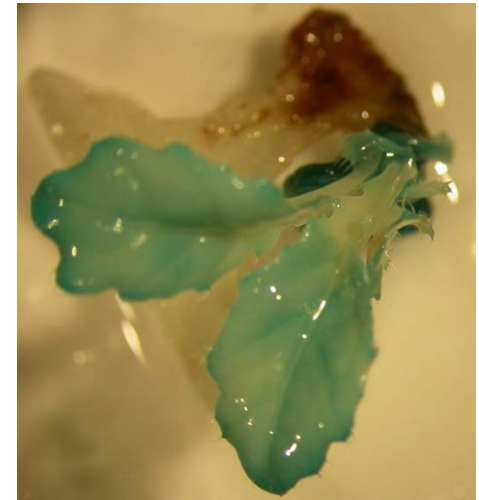
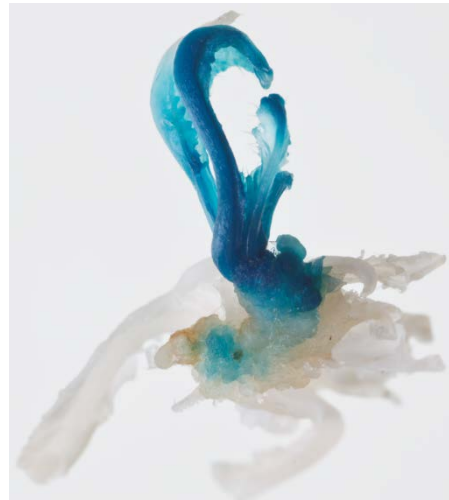
Elongation at 5 days



Elongation at 10 days

# Optimising selection

- Kanamycin <1%



- Gentamicin 1-5% using pPZP222
  - Also offers ‘Gene stacking’ potential in *B. oleracea* and *B. napus*



# A molecular approach to increase transformation success

- *In vitro* ethylene presence can reduce transformation success
- ACC deaminase breaks down ACC the direct precursor to ethylene
- We have introduced the ACC deaminase gene into our helper plasmid pSoup (naming it pSoupPlus)
- Evaluated in *B. oleracea*

ACC present/absent	% independent transgenic shoots	Improvement with ACC
Present	21.5	26%
Absent	17	

# Summary

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- Protocol for R-o-18 transformation will soon be available on [www.bract.org](http://www.bract.org) based on gentamicin selection
- Utility of GFP as a reporter gene demonstrated in *Brassica*
- Demonstrated a successful alternative selectable marker for Brassica (Gentamicin) – gene stacking potential
- Increased transformation efficiency in *B. oleracea* using pSoupPlus
- Additional BRACT constructs available: pSoupPlus; pBRACT35s::mGFP5ER; pBRACT35s::GUS

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- Alison Hinchliffe (Lab support)
- Tom Lawrenson (BRACT Maternity cover for Penny) [tom.lawrenson@jic.ac.uk](mailto:tom.lawrenson@jic.ac.uk) (and for *B. rapa* enquiries)