A. tumefaciens mediated transformation of *B. oleracea*

- The full *B. oleracea* protocol is available to download as a .pdf from this website
- The doubled haploid genotype DH AG1012 is shown here as the model genotype*
- As an aid, the following photos highlight the various stages of the transformation process
- The note page attached to each slide of this presentation provide additional information

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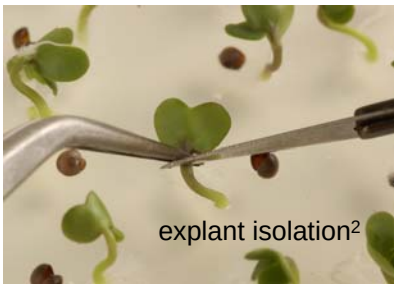
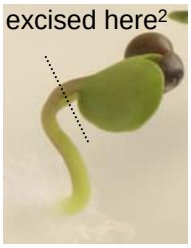
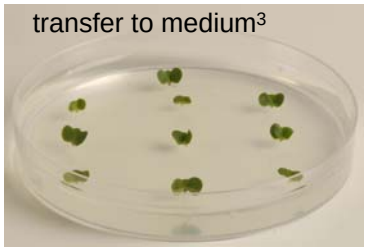
This presentation should be viewed after first reading the Brassica protocol

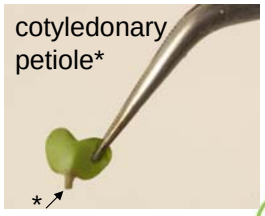
* PAC Sparrow, Dale PJ and Irwin JA (2004). The use of phenotypic markers to identify *Brassica oleracea* genotypes for routine high-throughput *Agrobacterium*-mediated transformation. Plant Cell Reports 23:64-70

Isolation of cotyledonary petioles from 4 day-old-seedlings









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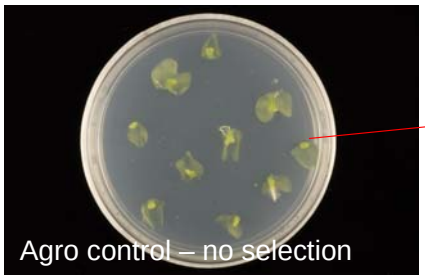
1: As culture room conditions may vary, the size of the seedlings is more important than the age. The explants shown here are of an ideal size.

2: Cotyledonary petioles are isolated just above the meristem. It is important that the scalpel blade is sharp, as petioles isolated with a good 'clean' cut surface (i.e. when the tissue is not torn) respond best. Aim for a petiole length of 1-2mm. If after excision the two cotyledonary petioles are still attached together, it is likely that you have included some meristematic tissue, this is not desirable.

3: All explants are isolated and transferred to co-cultivation medium. Cotyledonary explants are picked up (as shown) and the petiole dipped into the *Agrobacterium* suspension. The petiole base should not be immersed in the suspension for more than a second, before being transferred back to the co-cultivation medium (5cm deep petri-dishes are ideal for holding the bacterial suspension).

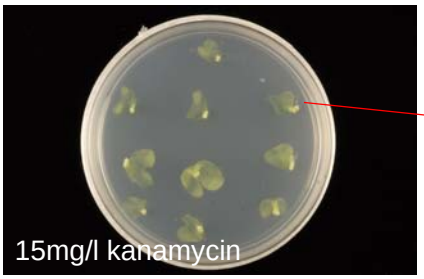
By the time explants are moved onto selection medium (72 hours after inoculation), petioles will have lengthened. It should then be possible to embed the petiole into the selection medium, with the cotyledonary lamella clear of the medium.

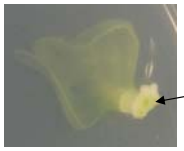
DH AG1012 @ 1 week



Petiole base
fleshy and swollen

On kanamycin free medium





Green region visible

Petiole base bleaching
on selection

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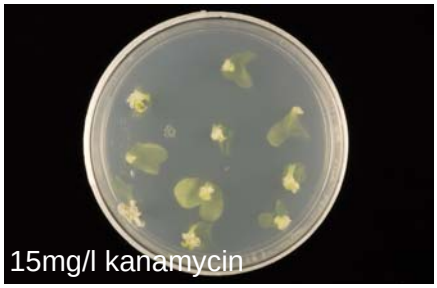
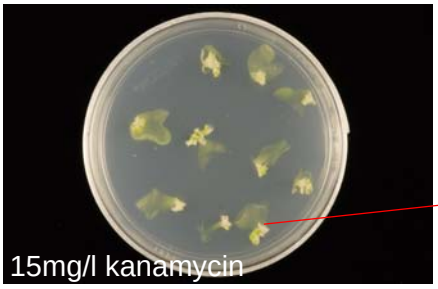
A week after transfer to selection (or selection free medium for the controls) petiole bases will begin to swell.

In the absence of selection (control plates) the petiole base will look fleshy and green, while the experimental explants in the presence of kanamycin will be showing signs of chlorosis. Hopefully a few green spots (putative transformed cells) should be visible.

DH AG1012 @ 2 weeks

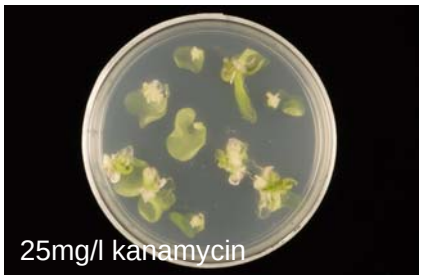


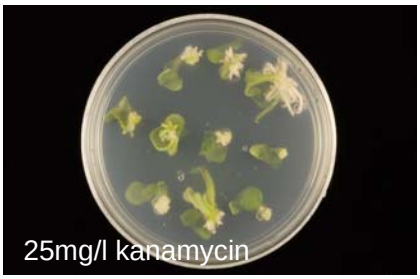


After 2 weeks on selection shoots are beginning to form. DH AG1012 is sensitive to kanamycin and 15mg/l* is high enough to cause chlorosis (or bleaching out) of non transformed shoots, but not high enough to result in necrosis of the explants.

* 25mg/l can be used at this stage for more robust genotypes

DH AG1012 @ 3 weeks





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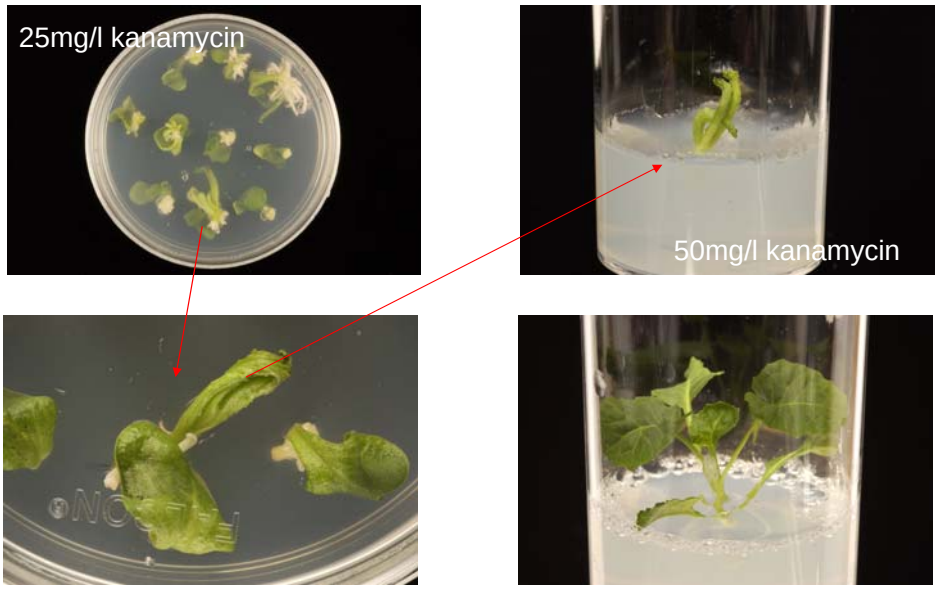
Explants are transferred to fresh selection medium after 3 weeks (selection is increased from 15 to 25mg/l kanamycin at this stage).

While transferring explants to new media, white regenerating shoots (escapes) are removed and discarded where ever possible at this stage.

Green shoots have been isolated at this stage, but in general shoots start to develop after this subculture onto fresh medium.

Shoot isolation



Shoots are isolated and transferred to Gamborgs B5 medium – for shoot elongation and rooting. Isolated shoots can often be vitrified at this stage – but the reduced sucrose levels generally overcome this problem. Vitrification is absent by the time plantlets are moved into sterile peat pots.

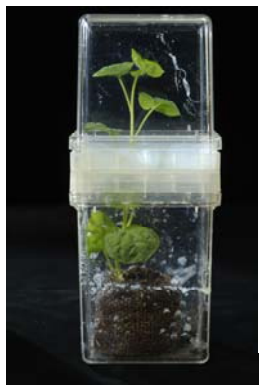
Selection is maintained (and can be increased to 50mg/l kanamycin) while shoots are on B5 medium as it is still possible for escapes to survive to this stage. Shoots that are escapes should start to bleach out on this medium, and do not tend to root.

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)

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John Innes Centre