

# Transformation of Potato Stems with *Agrobacterium*

## I. *Agrobacterium* Transformation

Prepare frozen competent *Agrobacterium* cells.

- Inoculate 2 ml of LB with a single colony of the *Agrobacterium* strain (e.g. CP2760) to be transformed with the binary plasmid. Grow overnight at 30°C.
- Transfer the culture to 50 mL of L-Broth. Grow for 4 hours.
- Centrifuge at 3000 xg for 20 minutes.
- Wash the pellet with chilled TE pH 7.2.
- Suspend in 5 ml of sterile L-Broth.
- Aliquot 500 µl portion into sterile tubes, freeze in liquid nitrogen, and store at -80°C. STORE FOR A MAXIMUM OF THREE MONTHS.

To transform plasmids into competent *Agrobacterium* cells.

- Thaw cells on ice. Mix with 1 µg of plasmid DNA. Incubate on ice for 5 min.
- Place the tube in liquid nitrogen for 5 minutes.
- Transfer the tube to 37°C water bath for 5 minutes.
- Add 1 ml of L-broth and incubate at 28-30°C with shaking for 2-4 hours. Plate 200 µl per plate on L-Agar with appropriate antibiotics and grow at 28°C for 2 to 4 days (until visible colonies form).
- Select colonies and grow overnight in L-broth containing antibiotics. Verify the presence of the transformed plasmid by doing minipreps and digests.
- After confirming the successful transformation, prepare cultures for accession into the culture collection. (They will be stored at -80°C as 15% glycerol stocks)

## II. POTATO TRANSFORMATION

- Grow up and induce the *Agrobacterium*
  - Inoculate the transformed *Agro* strain in 10 ml L-Broth containing the appropriate selection (Kanamycin, etc.).
  - Incubate for 48 hours at 28-30°C & 200 rpm.
  - Dilute 1:50 in fresh medium and grow for 16 hours at 28-30°C & 200 rpm.
  - Transfer overnight culture to fresh medium (10% v/v inoculum). Add 100 mM acetosyringone in DMSO to a final concentration of 100 µM and grow for 4-6 hours. [e.g. 1 µl stock per ml culture]
- Prepare the *Agro* for transformation
  - Pellet the *Agro* by centrifugation at 5,000 x g for 10 min. Suspend in an equal volume of sterile MS with 3% sucrose, pH 7.
  - Pellet again, as above. Suspend in sterile MS+Suc (half the original volume)
  - Measure the OD<sub>595</sub> and prepare a dilution in MS + Suc that has an OD<sub>595</sub> = 0.4
- Prepare the tissue and perform the transformation
  - Prepare internodal stem segments by cutting in vitro grown plants into ~5 mm sections. Be careful to avoid the apical meristem where the petiole joins the stem.

- Stem segments may be pooled into sterile MS + Suc in a Petri dish to avoid desiccation.
- Drain and transfer the stem segments into the *Agro* solution. Incubate at room temperature for 10 minutes.
- Blot the stems gently on sterile 3MM filters
- Initiate the Agrobacterium infection the plant.
  - Using sterile instruments, transfer each stem segment to the surface of Co-Cultivation Medium.
  - Incubate for 2-3 days at 23°C with 16 hour photoperiod (8 hour dark).

### III. REGENERATION

Day 1: Transfer the stem segments to Stage 1 medium in tall Petri dishes.

Day 14: Transfer to fresh Stage I medium.

Day 28 and every 2-3 weeks thereafter: Remove shoots and place on Stage 3 medium. There may not be many shoots.

- Transfer the stem segments to Stage 2 medium.
- Keep track of which stem segments the various shoots come from by assigning a number to each disk that forms shoots.
- As shoots appear, cut them off and transfer to Stage 3 medium.
- Leave the stems on Stage 2 to form more shoots.

When shoots grow and form roots on Stage 3, slice the shoot portion into segments with a node in each segment and transfer to Stage 3 medium again.

- (This increases the number of shoots and insures that the shoots were real transformants. It is also the time to do a Northern analysis to make absolutely sure that the plants you have are really true transformants.
- BE EVER VIGILANT! WATCH FOR SIGNS OF BACTERIAL CONTAMINATION.

After rooting (when 2-3 inches tall), plants may be transferred to Vermiculite wetted with MS and sterilized.

- Allow plants to form roots (usually 10 days at 28°C with 16 hour photoperiod. Roots should become apparent at the bottom of the pot.
- Gradually open the top of the Magenta vessel to "harden" the plantlet.  
(Day 1, 1 hour; Day 2, 2 hours; Day 3, all day; Day 4 all day & overnight; Day 5 plant in the greenhouse. )
- Beware of desiccation! Vermiculite must be kept moist.

When the plant has had a couple of days of hardening off in this fashion, transfer it to soil.

## IV. MEDIA

|  | <b>[Final]</b> | <b><u>1 Liter</u></b> | <b><u>500 ml</u></b> |
|--|----------------|-----------------------|----------------------|
| <b>A. Co-cultivation Medium</b>  |                |                       |                      |
| MS with Vitamins (Phytotech M404)  |                | 4.4 grams             | 2.2 g                |
| Sucrose  | 2%             | 20.0 grams            | 10 g                 |
| Adjust pH to 5.4 pH is important for <i>vir</i> induction. Bring to final volume. Then add:  |                |                       |                      |
| Gelzan CM (or agar, or Phytigel)   | 0.2%           | 2.0 grams             | 1.0 g                |
| Autoclave 20 minutes. Cool to 55°C. Move to hood and add.                                    |                |                       |                      |
| Zeatin Riboside (freezer) ( <b>1 mg/ml stock [4.56mM]</b> )                                  | 9.12 µM        | 2.0 ml                | 1.0 ml               |
| NAA (1mg/ml Phytotech)   | 0.54µM         | 0.1 ml                | 0.05 ml              |
| <b>B. Shoot Multiplication Medium (stock plants)</b>   |                |                       |                      |
| MS with Vitamins (Phytotech M404)  |                | 4.4 grams             | 2.2 g                |
| Sucrose  | 3%             | 30.0 grams            | 15 g                 |
| Adjust pH to 5.6 & Bring to final volume. Then add:  |                |                       |                      |
| Gelzan CM  | 0.2%           | 2.0 grams             | 1.0 g                |
| <b>C. Stage I Medium (Shoot Induction)</b>   |                |                       |                      |
| MS with Vitamins (Phytotech M404)  |                | 4.3 grams             | 2.15 g               |
| Sucrose  | 2%             | 20.0 grams            | 10 g                 |
| Adjust pH to 5.6 & Bring to final volume. Then add:  |                |                       |                      |
| Gelzan CM  | 0.2 %          | 2.0 grams             | 1.0 g                |
| Autoclave 20 minutes. Cool to 55°C. Move to hood and add.                                    |                |                       |                      |
| Carbenicillin (50 mg/ml stock)   | 500µg/ml       | 10 ml                 | 5.0 ml               |
| Kanamycin (50 mg/ml stock)   | 100µg/ml       | 2.0 ml                | 1.0 ml               |
| Zeatin Riboside (freezer) ( <b>1 mg/ml stock [4.56mM]</b> )                                  | 4 mg/L         | 4.0 ml                | 2.0 ml               |
| NAA (1mg/ml stock from Phyto)  | 0.1 mg/L       | 0.1 ml                | 0.05 ml              |
| ± Vancomycin (100 mg/ml)   | 100 µg/ml      | 1.0 ml                | 0.5 ml               |
| Dispense into Falcon 1005 petri dishes.  |                |                       |                      |
| <b>D. Stage II Medium (Root Induction)</b>   |                |                       |                      |
| MS with Vitamins (Phytotech M404)  |                | 4.4 grams             | 2.2 g                |
| Sucrose  | 2%             | 20.0 grams            | 10 g                 |
| Adjust pH to 5.6 & Bring to final volume. Then add:  |                |                       |                      |
| Add Gelzan CM  | 0.2%           | 2.0 grams             | 1.0 g                |
| Autoclave 20 minutes. Cool to 55°C. Move to hood.  |                |                       |                      |
| Aseptically, add:  |                |                       |                      |
| Gibberellic Acid (10 mg/ml in EtOH)  |                | 1.0 ml                | 0.5 ml               |
| Zeatin Riboside (freezer) ( <b>1 mg/ml stock [4.56mM]</b> )                                  | 4 mg/L         | 4.0 ml                | 2.0 ml               |
| Kanamycin (freezer) (50 mg/ml Stk)   |                | 2 ml                  | 1.0 ml               |
| Carbenicillin (freezer) (50 mg/ml Stk)   |                | 10 ml                 | 5.0 ml               |
| <i>(OPTIONAL, add Cefotaxime or Vancomycin). Dispense to sterile plates or Magenta boxes</i> |                |                       |                      |
| <b>E. Stage III Medium (Kanamycin Selection)</b>   |                |                       |                      |
| MS with Vitamins (Phytotech M404)  |                | 4.4 grams             | 2.2 g                |
| Sucrose  | 3%             | 30.0 grams            | 15 g                 |
| Adjust pH to 5.6 & Bring to final volume. Then add:  |                |                       |                      |
| Add Gelzan CM  | 0.2%           | 2.0 grams             | 1.0 g                |
| Autoclave 20 minutes. Cool to 55°C. Move to hood.  |                |                       |                      |
| Add Kanamycin (50 mg/ml Stk)   | 200µg/ml       | 4 ml                  | 2 ml                 |
| Add Carbenicillin (50 mg/ml Stk)   | 500µg/ml       | 10 ml                 | 5 ml                 |
| Dispense to sterile Magenta boxes.   |                |                       |                      |