Agrobacterium-mediated *Arabidopsis* cell suspension culture transformation

Protocol obtained from VIB

Material

- *Arabidopsis* cell suspension culture PSB-D (grows in MSMO medium and always in dark) or PSB-L (grows in MSMO medium and needs a 16h light/8h dark-cycle)
- *Agrobacterium* strain C58C1Rif^r[pMP90M])
- MSMO (1l) medium
  - 4.43 g MS (minimal organics Sigma #M6899)
  - 30g sucrose
  - 500 µl NAA (1mg/ml stock in 100mM NaOH)
  - 50 µl Kinetin (1mg/ml stock in DMSO)
  - pH 5.7 using 1M KOH and autoclave
- MSMO (1l) medium for plates
  - Idem above
  - Add 0,8% plant agar
  - Autoclave
  - Add antibiotics
- Antibiotics per liter MSMO medium:
  - 4ml Km (stock 12.5mg/ml) or 400µl Hg (stock 50mg/ml) → plant selection
  - 10 ml Carbenicillin (stock 50mg/ml) → for killing *Agrobacteria*
  - 10 ml Vancomycin (stock 50mg/ml) → for killing *Agrobacteria*
- YEB (1l)
  - 0.5% beef extract (=lab lemco) 5g
  - 0.1% yeast extract 1g
  - 0.5% peptone 5g/l
  - 0.5% sucrose 5g/l
  - 2mM MgSO4 (2ml/l <1M stock)
- acetosyringone (Fluka cat nr. 38766-1G)
- YEB + Agar
  - Idem above
  - Add 1.5% agar LabM and autoclave
  - Add antibiotics:
    - Rifampicin (100µg/ml)
    - Gentamycin (40 µg/ml)
    - Spectinomycin (100µg/ml)

Day 1(Wednesday)

- Grow 2ml of *Agrobacterium* culture in YEB (with Rif, Genta and Spec) in a falcon tube (28^{o}C o/n with shaking)
- Dilute 20ml of a 7d old *Arabidopsis* cell suspension culture in 80ml fresh MSMO (1in5 dilution)

Day 2 (Thursday)

- Transfer the *Agrobacterium* culture to 20ml medium (YEB with adequate antibiotics)

Day 3 (Friday)

- *Agrobacterium* wash
    - Spin the bacterial culture @4000 rpm 15min (Eppendorf 5810R centrifuge)
    - Discard the supernatant
    - Add 40ml of MSMO (washing), vortex until pellet dissolves
    - Spin @4000rpm 15min
    - Discard the supernatant
    - Add 40ml of MSMO (washing), vortex again until pellet dissolves
    - Check the O.D.^{600nm} (make first 1:1 dilution with MSMO in cuvette)
    - Spin @4000rpm 15min
    - Discard supernatant and dilute with MSMO until you reach an O.D of 1.0
- Cocultivation/ transformation (every construct in duplo)
    - Take a 6well multiwell plate
    - Add per well:
        - 3ml of the 2-day old *Arabidopsis* cell suspension culture
        - 200µl of *Agrobacterium* culture (OD 1.0)
        - 6µl of acetoseringone (100mM stock)
    - Close the muliwell plate, tape it with Micropore^{TM} surgical tape, and leave it over weekend at 130 rpm in an orbital shaker at 25 ^{o}C

Day 6 (Monday)

DIRECT TRANSFORMATION:

- Bring one well (so 3ml of transformed plant cells) into 8 ml of MSMO with the 3 antibiotics (Vc, Cb and Km or Hg) – all in a 25ml shake flask
- Tape the flask with Micropore^{TM} surgical tape
- Put flask on orbital shaker at 130 rpm , 25 ^{o}C for 9 days

TRANSFORMATION VIA PLATE:

- Bring one well (3ml) into a 50 ml falcon tube
- Add 40 ml MSMO (with Vc, Cb and Km or Hg) and wash by inverting the tubes several times.
- Centrifuge 5 min at 800 rpm.
- Take as much cells as you can (max 2 ml) and put it on a plate with KVC.

Day 15 (Wednesday) Only for the direct transformation

- Transfer the 10ml into a 100ml shake flask containing 25 ml of fresh MSMO with Vc,Cb,Km or Hg

Day 22 (Wednesday) Only for the direct transformation

- Transfer as much cells as possible (let them sink to the bottom) into 35ml of MSMO containing Vc, Cb, Km or Hg. in a 100 ml shake flask;

Day 29 (Wednesday) Only for the direct transformation

- Transfer as much cells as possible into +/- 35 ml of MSMO containing Km or Hg. (So only containing plant-selection antibiotic! No Vc and Cb anymore).
- **Critical step**: By now the *Agrobacteria* should be dead. You can check this by taking one ml of your culture and put it on a YEB plate. When you don't see growth of bacteria after three days at 28 ^{o}C you culture is bacteria-free. When there is growth you should keep the culture in MSMO (with Vc,Cb,Km or Hg) for one week extra
- After 7 days transfer +/- 7 ml cells into +/- 43 ml of fresh medium (at this stage expressionanalysis can be done)
  Keep at 25^{o}C with shaking 130rpm, transfer into new medium every week.

REMARK WHEN WORKING WITH CELLSUSPENSIONCULTURES

!!!!! When you see the cells are not grown dense, you can always reduce the amount of medium.
Or: let all the cells sink to the bottom and try to take 6 or 7 ml cells only to the next week.

Week 2 or 3 after co-cultivation Only for the transformation via plate

- Scrape off the plant cell tissue and chop it with a scalpel. Transfer the plant cells in 30 ml MSMO + (Vc,Cb,Km or Hg) in a 100 ml shake flask. After one week transfer ±7 ml into 45ml of MSMO (but only containing plant-selection antibiotic!) in a 100 ml shake flask; keep in 25^{o}C with shaking, transfer into the new medium every week.