

Mimulus in planta transformation (Yuan Lab)

Agro Transformation

Using Electroporator:

1. Thaw agro competent cells on ice (stored at -80 °C) – Agrobacterium GV3101
2. Chill 2-mm electroporation cuvette on ice
3. Aliquot 1 mL LB into eppie tube
4. Add 1.5 µL plasmid DNA to agro cells
5. Transfer the agro + plasmid mixture to the cuvette
6. Electroporate: (Bio-Rad Electroporator program Agr)
Capacitance: 25 µF
Voltage: 2.4 kV
Resistance: 200 Ohm
Pulse length: 5 msec
7. Immediately add 1 mL LB to the cuvette
8. Transfer to an eppie tube and shake at 28-30°C for 1-2 hours
9. Plate 100 µL on LB+Kan+Gent+Rif plate and incubate for 2 days at 28-30°C

Using Freeze-Thaw Method:

1. Thaw competent Agrobacterium on ice (use 250 ul per transformation reaction) and add 10 ul of standard E. coli miniprep plasmid.
2. Incubate the mixture on ice for 5 minutes
3. Transfer mixture to liquid nitrogen and incubate for 5 minutes.
4. Incubate the mixture for a further 8 minutes in a 37 °C dry bath.
5. Add 1 ml of LB to each tube, shake at 28 °C for at least 2 hours.
6. Collect the cells by spinning 5000g for 1 min in a microcentrifuge and take 750 ul LB out, resuspend the pellet with the remaining LB, spread them on LB agar plates containing the appropriate antibiotic.
7. Incubate cells for 2 days at 28 °C.

Agro Preparation

1. Inoculate 4 agro colonies into separate 5 mL tubes of LB+Kan+Gent+Rif
Shake overnight at 28-30°C
2. Colony PCR from the 5 mL culture to check that it has your insert
3. Inoculate correct agro colony into a flask of 300 mL LB+Kan+Gent+Rif, cover with foil
Shake for 12-16 hours at 28-30 °C (if left longer, the bacteria will begin to die)
4. Make glycerol stocks by adding 1 mL of agro to glycerol stock tube. Put in liquid nitrogen then store in -80 °C freezer
5. Transfer contents of flask to 500 mL centrifuge bottles and label them

6. Balance the bottles (use the scale and spray bottle of water to balance)
7. Centrifuge to pellet the Agro (on the first floor)
 - SPEED = 6000g
 - TIME = 15 minutes
 - TEMP = 4 C
8. While centrifuging, make the resuspension solution:
 - 300 mL total for each construct
 - 5% sucrose = 15 grams sucrose
 - 0.1 M acetosyringone = 2 mL/L = 600 μ L
 - make acetosyringone fresh by dissolving 0.0196 grams/mL in methanol
 - dissolve sucrose, add acetosyringone, add dH₂O to bring total to 300 mL, and mix
9. After centrifuging, pour off liquid into the original flasks the Agro was grown in and bleach the flasks
10. Resuspend the Agro pellet in 300 mL resuspension solution by adding the solution to the centrifuge bottle and shaking
11. Pour into spray bottle for infiltration
12. Add 300 μ L Silwett to the Agro and shake to mix

Plant infiltration

Ideally want 8-12 young healthy plants in square pots that have branched and have lots of buds for infiltration (When plants are young, cut/pinch the plants so that they will be short and branched)

1. Trim off mature buds from the plants
2. Gently peel back the leaves around the young buds
3. Spray the young buds 2-3 times with the Agro (spray right before they will be put in the vacuum)
4. Vacuum infiltrate 1-2 plants at a time: pull a vacuum until 29 inches Hg, seal the vacuum and turn off the pump, wait 2 minutes, then release the vacuum
5. Mark the plant with a colored sword
6. Cover the plants with plastic tent overnight
7. Take off plastic and put in tomato cage
8. Pollinate for 2 weeks
9. Collect the seeds
10. Plant in flats using a salt shaker, about 15-20 seed capsules per flat
11. When the seeds start to germinate, begin spraying with 1:1000 Basta
12. Continue spraying with Basta every other day

Transplant the healthy looking, resistant plants