Mimulus lewisii stable transformation (by Janelle Sagawa)

Agro Transformation

1. Thaw agro competent cells on ice (stored at -80 ºC) – 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)
   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 28-30ºC for 1-2 hours
9. Plate 100 µL on LB+Kan+Gent+Rif plate and incubate for 2 days at 28-30ºC

Antibiotics: keep at 4ºC; store stock solutions at -20ºC in aliquots

Antibiotic	Powder storage	Stock solution	Final conc.	µl stock/ ml LB media	Resistance
Kanamycin	RT	50 mg/ml in water	50 µg/ml	1	
Gentamicin	4ºC	50 mg/ml in water	50 µg/ml	1	Ti plasmid marker
Rifampin	-20ºC	25 mg/ml in **methanol**	25 µg/ml	1	Agro chr. marker

Agro Preparation

1. Inoculate 4 individual agro colonies into separate 5 ml tubes of LB+Kan+Gent+Rif
   Shake overnight at 28-30ºC
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 ºC (if left longer, the bacteria may begin to die)
4. Make glycerol stocks by adding 1 ml of agro to glycerol stock tube. Freeze in liquid nitrogen then store in -80 ºC freezer
5. Transfer contents of flask to 500 ml centrifuge bottles and label them
6. Balance the bottles
7. Centrifuge to pellet the Agro
   JA10 rotor and use appropriate lid
   ROTOR = 10
   SPEED = 6000
   TIME = 15 minutes
   TEMP = 4 C
8. While centrifuging, make the re-suspension solution:
   300 ml total for each construct
   5% sucrose = 15 grams sucrose

0.1 M acetosyringone = 2 mL/L = 600 µL
make acetosyringone fresh by dissolving 0.0196 grams/mL in methanol
dissolve sucrose, add acetosyringone, add dH_2O to bring total to 300 mL, and mix

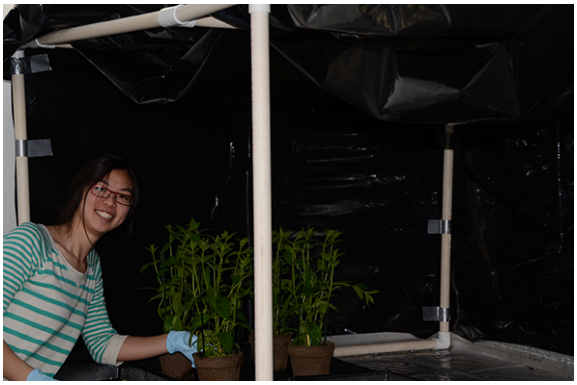
9. After centrifuging, pour off liquid into the original flasks the Agro was grown in and bleach the flasks
10. Resuspend the Agro pellet in 300 mL resuspension solution by adding the solution to the centrifuge bottle and shaking
11. Pour into spray bottle for infiltration
12. Add 300 µL Silwett to the Agro and shake to mix (**note: we found that the amount of Silwett is critically important for transformation success**)

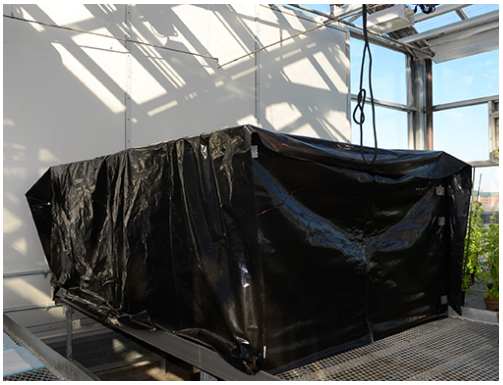
<u>Plant infiltration</u>

Ideally want 12 young healthy plants that have branched and have lots of young flower buds (5mm or smaller) for infiltration (When plants are young, cut/pinch the tip so that they will be short and branched)

1. Trim off large buds (>5mm) 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 **quickly** release the vacuum

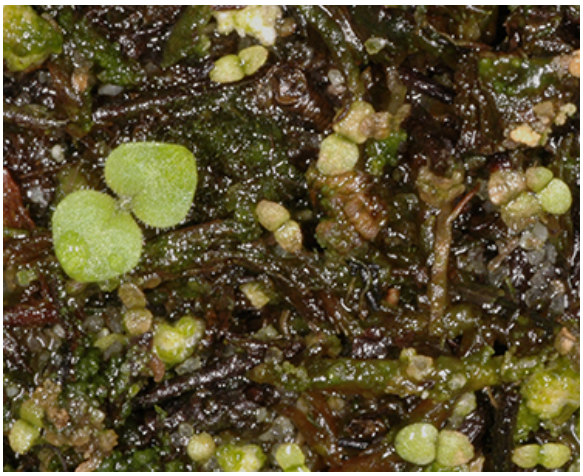
5. Cover the plants with plastic tent overnight





6. Take off plastic and put the plants in tomato cages
7. Pollinate for 2 weeks

8. Wait for 3-4 weeks and collect the seeds
9. Plant the seeds in soil flats using a salt shaker, about 15-20 seed capsules (500-1000 seeds per capsule) per flat
10. When the seeds start to germinate, begin spraying with 1:1000 Basta



11. Continue spraying with Basta every other day
12. Transplant the resistant plants