Agrobacterium Ti Plasmid Prep + PEG 6000 Precipitation

Stephen Farrand Lab (modification of Sambrook et al's mini-prep method) Modified by David Neece April 2016 to include RNase step, Chloroform cleanup, and PEG 6000 precipitation

1) Inoculate single colony into 50 mls of MGL + antibiotic. Grow in MGL at 28°C , 250 rpm, overnight.

- 2) Set on ice 10 min.
- 3) Centrifuge 10 ml of the culture at 6000 RPM for 5 min.

4) Resuspend pellet in 1 ml Agro-wash and transfer to a 1.5ml microfuge tube. Add 5 μ l of 10% Sodium Sarkosyl. Incubate on ice for 5 min. Spin as in step 3. Aspirate off supernatant.

5) Add Solution 1: 225 μl

Resuspend by pipetting or vortexing Add 20 μ l of lysozyme solution and mix by inversion. Let sit 5 minutes on ice.

6) Add **Solution 2: 450µl** (freshly made). Mix gently by invertion several times. Incubate at RT for 5 min.

7) Add **Solution 3: 350** μ I (ice cold). Mix by inverting tube quickly back and forth. Incubate on ice for 10-15 min, mixing every few min.

- 8) Centrifuge at 12,000 RPM for 10 mins at 4°C.
- 9) Transfer supernatant into new microfuge tube. Add 5μl of 10mg/ml RNase and incubate at 37°C for 30 min. Transfer to a pre-spun 2.0ml Phase Lock Gel (Heavy) tube (volume will be about 900 μl).

10) Add 500 μ l of phenol:chloroform:isoamyl-alcohol (25:24:1). Mix by gently shaking tube 40 times.

11) Centrifuge at 10,000 RPM for 5 min at RT, then transfer the top, aqueous phase to a new pre-spun 2.0ml Phase Lock Gel (Heavy) tube ml tube.

12) Add 200 μ l of Chloroform:Isoamyl-OH (24:1) and shake gently 40 times.

13) Centrifuge at 10,000 RPM for 5 min at RT, then transfer the top, aqueous phase to a new 1.5ml tube. Volume should be about 700-900 μ l.

14) Add 0.313 volumes of 42% PEG 6000 and mix gently by inversion. Solution should become cloudy.

15) Incubate 6 hours to overnight on ice in fridge, or at -20° C (may freeze at -20° C).

16) Spin at 10,000 RPM for 10 min at 4° C, then pour off supernatant.

17) Wash pellet with 70% ethanol and spin again as in previous step. Pour off ethanol and dry pellet at room temp.

18. Resuspend in 50-100 μ l of TE.

Agro-wash: 0.50 M NaCl 0.05 M Tris-HCl (pH 8.0) 0.02 M EDTA(pH 8.0) (or 2.9% NaCl in TE pH 8.0)

Solution #1:

2.25 ml 20%	Glucose
1.25 ml	1M TRIS pH8
1.0 ml	0.5M EDTA pH8
45.0 ml	Nanopure water

Solution #2 (prepare fresh): 2.5 ml 10% SDS (1% final) 12.5 ml 0.4M NaOH (0.2M final) 10.0 ml Nanopure water

Solution #3:

11.8 g CH₃COOK (potassium acetate; 3M final)
4.6 ml CH₃COOH (glacial acetic acid 17.4M; 2M final)
Bring volume up to 40 ml with Nanopure water.

Lysozyme Solution

Add 500 μ l sterile H₂O to a 1.5ml tube Add a few flakes of lysozyme to 500 μ l H₂O -- can be made by sticking sterile blue 1ml pipette tip into powder about 0.5-1cm deep, then add this to water dissolving whatever stuck to the outside of the tip

42% PEG 6000

Dissolve 16.8 g PEG6000 in a total volume of 40 ml Nanopure water Filter sterilize using 0.2uM syringe filter

MGL medium	(1 liter)
Tryptone	5.00 g
Yeast Extract	2.50 g
NaCl	2.27 g
Mannitol	10.00 g
Glutamic acid	2.32 g
KH ₂ PO ₄	0.50 g

pH may be adjusted to 7 with NaOH, but not necessary.