

EST Plasmid Preps

Terrific Broth (TB)

12 g Bacto-tryptone

24 g yeast extract

4 ml glycerol

Add Milli-Q H₂O to total 900 ml, and autoclave.

10X TB salts:

2.31 g KH₂PO₄

12.54 g K₂HPO₄ (potassium phosphate, dibasic)

Add Milli-Q H₂O to total 100ml

Autoclave, cool and add to Terrific Broth along with selective antibiotic.

1. Pick colonies into 96-well block containing 0.5ml of TB + salts with the appropriate antibiotics and incubate for 16 hours at 37°C, shaking at 550 rpm according the HiGro protocol.
2. Centrifuge 3000 rpm for 10 minutes and decant supernatant into disposal bucket in the hood. Cover with an acetate seal and freeze pellets at -20°C or continue to the next step.
3. The Biomek NX program “Plasmid prep 4–blocks” will add 250µl TE, 250ul Lysis buffer (1% SDS, 0.2 M NaOH), and 250ul 3M NaOAc (pH4.8).
4. Shake for 15 minutes at a setting of 6, then freeze at least 4 hours or overnight at -20°C.
5. Allow about 30 minutes at room temperature for plates to thaw, then centrifuge at 3000 rpm for 45 minutes.
6. Transfer 70µl of supernatant into new Corning 3790 plate, and add 95% Ethanol by using the Biomek NX program “Transfer 70µl from 4 blocks into 4 plates & EtOH.”
7. Place at -20°C for at least 3 hours.
8. Centrifuge at 3000 rpm for 30 minutes.
9. Decant by inverting into sink and blot dry on paper towels.
10. Wash with 70% EtOH by using the Biomek NX program “70% EtOH wash 4-plates” and centrifuge at 3000 rpm for 15 minutes, and repeat from step 9.
11. Decant and air dry inverted on the bench top.

12. Resuspend DNA in 150µl of H₂O (Add 100µl of 40µg/ml RNaseA to each 100ml AccuGene water.) using the Biomek NX program “Dissolve in H2O-4_plates” then shake for 10 minutes at a setting of 5, and allow to sit for at least 30 minutes before quantifying.

1) TE (50mM Tris-HCl, pH 7.6, 10mM EDTA, pH 8.0)

1M Tris pH 7.6

Dissolve 12.1 g in 80ml ddH₂O

Add about 5.5ml HCl until pH7.6 then bring to 100ml with ddH₂O.

Use 25ml for 500ml TE

0.5M EDTA pH8.0

Add 18.6 g of EDTA-2H₂O to 80ml H₂O.

Adjust pH to 8.0 with NaOH (about 2.0g of NaOH pellets)

Use 10ml for 500ml TE

2) Lysis Buffer (0.2M NaOH/ 1% SDS solution) *Make fresh*

100ml:

40ml (0.5M NaOH stock)

10ml (10% SDS stock)

50ml H₂O

0.5 M NaOH

2 g NaOH pellets/100ml H₂O

10% SDS

10g/100ml H₂O

3) 3M NaOAc, pH 4.8

408.24g NaOAc-3H₂O

Dissolve in 300ml Milli-Q H₂O, adjust with glacial acetic acid and bring final volume to 1L with Milli-Q H₂O. (Does not fully go into solution until addition of 500ml of glacial acetic acid)