96 Well High Copy Plasmid Preps

(Modified from Wendel lab 2006)

1. Pipette 300ul of Terrific Broth and Kanamycin (10mg/ml) into each well of 96-deep well blocks.

2. Using a 96-long pin replicator, inoculate blocks from a 384-well culture plate. Cover with two layers of AeraSeal tape.

3. Incubate for 18 hours, shaking at 280 rpm, 37°C.

4. Pellet the cells by centrifuging at 3000rpm for 10 minutes. Pour off supernatant. Pellets may be covered and placed in –20°C until ready to use.

5. Add 150ul Solution I with RNaseA to each well. Cover with tape from the tape pad and re-suspend pellets by slowly vortexing.

6. Add 150ul Solution II to each well, with the pipette at an angle to the sample is not directly sprayed. Cover with tape and invert 3-4 times to mix.

7. Quickly add 150ul Solution III to each well. Cover with tape and invert 3-4 times to mix.

8. Let incubate on ice for 10 minutes.

9. Label Uniplates and add 270ul (0.6 volume) Isopropanol to each well while incubating samples. Stack a labeled Unifilter onto Uniplate.

10. Add 450ul of each sample into the Unifilter.
MAKE SURE TO CHECK DIRECTION OF THE PLATES AND FILTER!!

11. Centrifuge the plates at 3200rpm for 30 minutes, 4°C,

12. Remove and throw away Unifilters. Pour off supernatant and let drain upside down for 10-15 seconds.

13. Add 300ul of 70% Ethanol to each well.

14. Centrifuge the plates at 3200rpm for 10 minutes, 4°C.

15. Pour off supernatant and let drain upside down for 10-15 seconds.
**Optional: May invert plate on paper-towel and quick spin at 300rpm for 1 minute.

16. Dry the plates in hood for about 10 minutes, until pellets become clear and no milky color remains at the bottom of the wells.

17. Add 100ul of sterile water to each well, shake plate slightly to spread the water over the pellet.

18. Cover the plate with Thermoseal tape and store at 4°C if going to use that day or store at -20°C for future use.

Solutions for Preps

Terrific Broth:

90% YGT Base
10% Potassium Phosphate
Mix and add 500ul Kanamycin (10mg/ml) per 100ml TB just before use.

YGT Base:

Tryptone	6g	12g	24g
Yeast Extract	12g	24g	48g
Glycerol	2ml	4ml	8ml
Sterile water (start with)	450ml	900ml	1800ml
Final Volume	500ml	1L	2L

Autoclave for 20 minutes, liquid cycle

Potassium Phosphate:

Potassium Phosphate (KH_2PO_4) monobasic	1.155g	2.31g	11.55g
Potassium Phosphate (K_2HPO_4) dibasic	6.27g	12.54g	62.7g
Sterile water (start with)	25ml	50ml	250ml
Final Volume	50ml	100ml	500ml

Autoclave for 20 minutes, liquid cycle

Solution I: 50mM Tris HCl (pH 8.0), 10mM EDTA

1M Tris HCl (pH 8.0)	1.25ml	2.5ml	5ml	7.5ml
0.5M EDTA	0.5ml	1ml	2ml	3ml
RNaseA (10mg/ml)	0.5ml	1ml	2ml	3ml
Sterile water	22.75ml	45.5ml	91ml	136.5ml
Final Volume	25ml	50ml	100ml	150ml

Mix and place in 4°C until use, keep on ice during use.

Solution II: 0.2M NaOH, 1.0% SDS

10M NaOH	0.5ml	1ml	2ml	3ml
20% SDS	1.25ml	2.5ml	5ml	7.5ml
Sterile water	23.25ml	46.5ml	93.0ml	139.5ml
Final volume	25ml	50ml	100ml	150ml

Keep at Room Temp or solution will precipitate out.

Solution III: 3.0M Potassium Acetate (pH 5.5)

Potassium Acetate	7.36g	14.72g	29.44g	44.16g
Sterile water (start with)	10ml	20ml	40ml	60ml
Final Volume	25ml	50ml	100ml	150ml

**Adjust pH to 5.5 with Glacial Acetic Acid. Need approximately 25ml acid per 100ml solution.

Store at 4°C until use. Keep on ice during use.