M13 Single Strand Template Purification for Sequencing.

1. Pick plaque and grow in 2.5ml 2xYT with 1-2 drops of fresh JM101 [JM103 or JM109 are also acceptable] for 6-7 hrs.

2. Spin out cells in microfuge 5 min. 2x.

3. To 1ml supernatent add 250ul 2.5M NaCl 20% PEG. Incubate 30 min. at R.T.

4. Spin in microfuge 5 min-pour off supernatent. (Better- suck off with drawn out pasture pipette)

5. Quickly spin tubes again and remove residual supernatent.

6. Resuspend pellet in 100-200ml TES.

7. Extract: a) 2+ times phenol/chloroform mixture  
   b) chloroform

8. Add: a) 50ul Na Acetate  
   b) 250-500ul Etoh  
   c) ppt. on dry ice.  
   d) gently wash pellet with 70% Etoh.

9. Spin 5min. pour off supernatent & dry in vacuum.


    Check 5ul on minigel.