

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 supernatant add 250ul 2.5M NaCl 20% PEG. Incubate 30 min. at R.T.
4. Spin in microfuge 5 min-pour off supernatant.
(Better- suck off with drawn out pasture pipette)
5. Quickly spin tubes again and remove residual supernatant.
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.
10. Resuspend in 15 -20ul water.

Check 5ul on minigel.