Tips for Growing Single Stranded DNA for Sequencing.

1) Maintain bacterial strains (JM101, JM103...) on minimal agarose plates.
2) Grow bacteria for as brief a time as possible.
3) Grow phage preps for 6-7 hours.
4) Do not freeze phage supernatents.
5) Unlike lambda, do not add chloroform to phage to store.

Minimal Media & Agarose

for 1 liter

15 gm. agarose [omit for media]
10.5 gm. $K_2PO_4$
4.5 gm. $KH_2PO_4$
1.0 gm $[NH_4]SO_4$
0.5 gm Na Citrate
0.2 gm $MgSO_4$ [sterilize separately/add when solution has cooled some/ add 1 ml 1M $MgSO_4$]
5 mg thiamine HCl [filter sterilize separately]
2.0 mg glucose [sterilize separately/add 20 ml 20% glucose]

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.

10. Resuspend in 15 -20ul water.

Check 5ul on minigel.

## Buffers for M13 purification.

20% Polyethanylene Glycol
2.5m NaCl [20gm PEG/100ml 2.5M NaCl]

TES

| | | |
|---|---|---|
| 500mM NaCl | 5M NaCl | 50ml |
| 10mM Tris pH7 | 2M Tris | 2.5ml |
| 1mM EDTA | .5M EDTA | 1ml |
| | H2O | 424ml |
| | ================= | |
| | Total | 500ml |

These solutions are used for preparing M13 single strand DNA for sequencing.