Judelson, 9-1-97

PLAQUE LIFTS

1. Plate the phage using standard procedures. Aim for a maximum of 40,000 pfu/plate for 150 mm plate, or 12,000 per 100 mm plate. The object is to obtain plaques that are barely touching each other. Use >1-day oíd plates (to avoid condensation smearing plaques and to obtain better adherence between top and bottom agar).

2. Incubate at **37C** for ca. 8 hrs.

3. ChiII the plates for 2 hr at 4C to firm up the top agar.

4. Transfer the phage to filters: Gently lay a nitrocellulose membrane on the plate for 2 minutes. Orient with ink.

   If making duplicate lifts, let the second filter transfer for 5 mm. Wrap the plates and store at 4^0C.

5. Denature the membrane by placing (plaque side up) on 1.5 M NaCí, 0.5 M NaOH, 2 min.

6. Neutralize by placing on 1.5 M NaCl, 0.5 M Tris pH 8.0, 5 min.

7. Rinse by submerging in 0.2 M Tris-Cl PH 7.5, 2 X SSC (or 3X SSPE) for 30 sec maximum.

8. Biot briefly on Whatman paper.

9. Oven bake 80C, 1.5 hr.

10. Hybridize as usual.

11. Picking positives:

    Using a steriie pasteur pipette, carefuíiy remove the plaque(s) from the original piate. GENTLY expel the plaque into a 1.5 mi tube containing 300 µl SM. Add 20 µl chlorofornm and gently hit the tube with your fingers. Let sit 6-8 hr at 4C (or 2 hr at room temp). Asstuning 10^7 pfu/piaque, plate dilutions and do secondary (and tertiary) screens. It wiil usually take 3 rounds to purify phage due to the ease by which they diffuse. Just in case, store the primary piaque suspension at 4C (NEVER freeze lambda phage suspensions).