AN OVERVIEW OF MAKING AND SCREENING PHAGE λ LIBRARIES

1. Prepare genomic DNA by Sau3A digestion or prepare cDNA.

2. Buy or prepare phage arms. Buying is cost effective particularly if you digest them yourself.

3. Ligate arms and insert as with any DNA ligation.

4. Package ligation mix. It is best to buy extracts to plate the library but to test ligation efficiency, home-made extracts are fine and are significantly cheaper.

5. Titer phage. (This is where you start if you buy a library or if someone has sent you one!).

6. Depending how many recombinants you get from the ligation (or from the person who sent it to you) you can either: amplify the library before screening or: screen one aliquot of the 1° library and amplify the rest. It is best to screen the 1° library if possible as some phage may not amplify well and will thus be under-represented in an amplified library. For a genomic library, you should aim to screen the equivalent of 3 genomes, assuming an average insert size of 15 kb. For a cDNA library you should screen 1 – 4 million recombinants depending on the abundance of the message.

7. Plate out phage on 20 cm^2 plates and perform 1° screen.

8. Pick positive phage pools and replate and screen until you plaque purify the phage of interest.

9. If the phage vector has a plasmid vector within it that can be excised by recombination you are essentially done – just ZAP it out. If not, you need to purify phage DNA so that you can subclone the insert. Phage

DNA can be prepared from liquid lysates or plate lysates and can be made ultrapure by fractionation on CsCl gradients.