

Protocol for Preparation of DNA for Transgenic Mice

For quality control of DNA intended for use in generation of transgenic mice, the following requirements should be met:

1. We need 5-10 µg of pure inserts (no plasmid sequence). The inserts can be purified on low melting agarose or purified using any methods of your choice (when the construct is large, low-melting agarose method is probably the best one). The best quality agarose should be used (i.d. SeaKem agarose). Here is one protocol that works well:

- a. Cut DNA to completion, test it on an analytical gel.
- b. Run on low melt preparative agarose gel.
- c. Cut out the DNA from the agarose and melt at 70° to 75° C for 5 minutes (check to make sure that there is no chunky solid agarose left).
- d. Add equal volume of equilibrated phenol (phenol should be less than one month old). Spin for at least 5 minutes in a microfuge. The upper phase should be clear; if not, spin again.
- e. Take the top phase and avoid any white interface.
- f. Phenol extract again 2 X.
- g. Add 1/10 volume of 4M LiCl solution, upon which a white precipitate should form. Ice for 2 minutes and spin for 2 minutes.
- h. Take the top phase and CHCl₃ extract once.
- i. Take the top phase and add 2 volumes of Ethanol (there is no need for more salt) and spin for 10 minutes.
- j. Take off all the supernatant, spin briefly and take off again residual supernatant. Air dry for 10 minutes.
- k. Resuspend the pellet in tissue culture water.
- l. Measure OD_{260/280}. Repeat the phenol extraction and ethanol precipitation if the ratio is less than 1.8.

2. We need the following record:

- a. Spectrophotometer readings, with the O.D._{260/280} ration greater than 1.8.
- b. DNA concentration of the insert.

- c. EtBr picture of a gel with:
 - insert alone
 - insert + one restriction enzyme
 - insert + another restriction enzyme
- d. A rough map of the plasmid and insert, which show different fragments of the corresponding enzymes used above. The EtBr picture should allow us to judge whether there are any residual fragments containing plasmid sequences.