Purification of Restriction Fragments for Microinjection

DNA restriction endonuclease fragments to be used for microinjection may be purified by many different protocols, although it is necessary to include a final purification step to remove particulate material which may clog the injection needles. Transgenes are usually designed so that the gene to be microinjected can be isolated as cleanly as possible from plasmid sequences (early publications indicated that there are some plasmid sequences which can inhibit the expression of various transgenes).

The following protocol yields DNA which is clean enough to microinject from plasmids which are 20 kb or less**. If larger plasmids, BAC or PAC DNAs are to be microinjected, please contact the Mouse Genetics SRF director for more specific protocols.**

1. Digest plasmid DNA to completion with the appropriate restriction endonuclease(s). Digest enough plasmid DNA to generate 1-2 ug of fragment containing the transgene.

2. Separate the restriction fragments by electrophoresis through an agarose gel.

3. Under ultraviolet illumination, identify and isolate the band containing the gene to be microinjected.

4. Extract and purify the DNA according to the manufacturer's protocols for the Qiagen gel-purification kit.

5. Elute the DNA from the Qiagen filter using injection buffer (**5 mM Tris, pH 7.4, 0.1 mM EDTA**). Alternatively, the DNA can be eluted using the manufacturer's instructions, and then the transgene fragment can be ethanol precipitated, washed with 70% ethanol, and resuspended in the injection buffer listed above. (The mouse genetics and gene targeting facility can provide this buffer). Check the concentration of serial dilutions on a gel, and then calculate the stock concentration of the eluted fragment.

   Submit the fragment to the Mouse Genetics SRF at 50-100 ng/microliter (1-2 micrograms total).

6. The Mouse Genetics SRF staff will prepare and filter the final dilution that will be used for microinjection.