DNA Purification

General Considerations

The quality of DNA for microinjection is essential to the success of transgenic experiments. DNA that is not purified properly will make the injections difficult and/or reduce the survival. Any traces of phenol, ethanol, salts, or enzymes are toxic for embryos. Glassware with possible detergent residue should be avoided and plastic ware should be rinsed with filtered microinjection buffer prior to use.

1. The injected fragment should be free of flanking plasmid sequences (which may be toxic). Therefore the construct should be designed so that the insert for microinjection can be excised without vector sequences.
2. The DNA must be intact to prevent integration of partial constructs.
3. The DNA must be free of any contaminants that may harm the embryo.
4. All solutions used in DNA purification should utilize fresh, high quality sterile reagents. Embryo tested water (Sigma W1503) must be used.
5. It is essential that DNA solutions do not contain any particulate matter that may clog injection needles.
6. DNA concentration must be measured exactly. A higher than optimum concentration will cause embryo death. A lower than optimum concentration will result in fewer or no transgenic pups.
7. Determine the DNA concentration. The pure DNA A260/A280 should be in the range of 1.80-1.88. Leave the DNA in concentrated form in the TE microinjection buffer.

Purification of DNA for microinjection (Random integration)

1. Perform a restriction digest of at least 10 microgram of the construct to remove the vector sequences.

2. Separate the DNA fragment for microinjection (gene of interest) from the vector on an agarose gel run in 1 x TAE (not TBE).
3. Excise the gel slice containing the DNA fragment of interest.
4. Purify the DNA either by using a DNA purification column or by phenol-chloroform extraction and ethanol precipitation.
5. Final DNA should be dissolved in the TE microinjection buffer (10mM Tris-0.1mM EDTA, pH 7.5). We will need 1-2ug of DNA at a concentration of at least 100 ng/ul.
6. The core facility will dilute your sample for microinjection.

Preparation of targeting construct for electroporation (Gene-Targeting)

1. The goal is to have 50-100 µg of purified, linear plasmid DNA at a final concentration of approximately 1ug/ul.
2. Grow and purify plasmid DNA.
3. Digest plasmid DNA with appropriate enzymes to linearize and to remove vector backbone DNA
4. Extract DNA of interest with pheno/chloroform, precipitate with ethanol and re-suspend in TE (or PBS).
5. OR: Purify using DNA purification column.
6. Re-suspend final purified, linearized DNA in TE (allow overnight at 4°C, followed by vigorous 'flicking').
7. Measure concentration and A260/280 ratio.