Preparation of DNA for microinjection by CTC

It is essential to provide CTC with the highest quality DNA to increase the success of your project. 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. It is also important to get rid of any particles that could clog the injection needles. Pre-filtration of all DNA suspension solutions and microinjection buffer through 0.02 um filters prior to diluting the DNA for microinjection (the 0.02 µm filters will trap DNA molecules) is highly recommended (i.e. Anotop syringe filter from Whatman). In addition, sterile endotoxin-free ultra-pure water or embryo-tested water (e.g. Sigma W1503) should be used for making up the microinjection buffer (MIB).

There are a number of commercially available kits that provide a simple and fast way to obtain microinjection quality DNA.

- NucleoSpin® Extract II Kit (Clontech)
- Geneclean II kit (Bio 101, Inc.).
- QIAquick gel extraction kit (Qiagen, Inc.)

In each case you will need to:

1. Perform restriction digest to release the transgene from plasmid vector sequences. The final yield should be 10 - 20 micrograms of transgene insert.
2. Separate restriction digest products on a TBE or TAE agarose gel.
3. Place gel on transilluminator and excise desired band from the gel with a sterile scalpel. It is important to removes as much excess agarose as possible and minimize DNA exposure to UV light to prevent photochemical damage (less than 1 minute).
4. Transfer agarose slice(s) to a pre-weighed tube. Reweigh tube to determine weight of agarose in tube.
5. Process the gel fragment through your endotoxin-free commercial gel extraction kit according to the manufacturer's instructions.
6. When eluting the DNA from the commercial column, elute into the following Microinjection Injection Buffer (MIB): 10 mM Tris-HCl, pH 8.5 filtered through a 0.02 micron syringe filter.
7. Microdialyse the DNA fragment on a 0.05µm membrane (Millipore type VM, Cat# VMWP02500) against 10-50ml of filtered Microinjection Buffer for 3 hours.
8. Quantitate the DNA concentration by comparing the ethidium bromide staining of a sample run on an agarose gel next to a standard of known concentration. Also, measure concentration on a Nanodrop, or DNA fluorometer.
9. Store eluted and microdialysed DNA at -20°C until use.
10. At the time of microinjection, the stock DNA (approx. 100ng/µl) is adjusted to 5 ng/µl, using injection pre-filtered MIB.

NOTE: It is strongly recommended that powder-free gloves be used when handling the DNA preparation. Powdered gloves are most often associated with the presence of particulate matter that gets lodged at the tip of the injection needle and renders it useless.