Purification of Plasmid DNA for Microinjection

1) Prepare plasmid from bacteria using your preferred method (alkaline lysis, lysozyme-triton or other methods), but be sure to perform 2 CsCl gradients. Also note that phenol kills injected embryos so an ethanol precipitation and ethanol wash MUST follow all phenol extractions.
2) Set up an overnight digest using 20 ug of plasmid DNA with the appropriate restriction enzyme to cut the insert from the vector (use 100 ul total volume for digest mix).
3) Prepare a 0.8% agarose minigel (1X TAE buffer with ethidium bromide) using a comb with ~6 mm wide teeth.
4) Add 20 ul of bromophenol blue/sucrose loading dye to the digest mix and load evenly over 4 wells. Don't use a size or concentration standard on this gel in order to prevent possible contamination of the insert DNA.
5) Run in 1X TAE + ethidium bromide at a low voltage (~35) for several hours or until the insert and vector bands are clearly separated.
6) Photograph the gel in its tray in order to prevent contamination from the transilluminator surface. Limit the time of UV light exposure so the DNA does not become damaged.
7) With a new, sterile scalpel blade, excise the insert band from the gel. Trim away excess agarose and avoid dragging the blade or the excised bands across the area of the gel where the vector bands are.
8) For transgenes less than 10 kbp, purify the insert DNA using a Q1Aquick Gel Extraction Kit (Qiagen, 800-426-8157, www.qiagen.com; Cat. No. 28704). Use the vendor's protocol (http://www.qiagen.com/literature/handbooks/qqspin/qqspin_gelextract.pdf or get the protocol from us) using the following modifications:
    a) carry out step 3 at room temperature, or 37^{O}C if necessary, for dissolution of the agarose and mix gently by hand instead of vortexing.
    b) apply equal volumes of the DNA solution into two columns at step 6.
    c) include the optional step 9 and repeat for a total of two Buffer QX1 rinses.
    d) repeat step 10 twice for a total of 3 Buffer PE washes, letting the column stand for 5 min before centrifugation after the first addition of Buffer PE.
    e) at step 13, add 30 ul of injection buffer to one of the columns and let stand 1 min before centrifugation. Use the 30 ul of collected sample for elution from the remaining column. Do a second rinse on this column with 10 ul of fresh injection buffer for a total of 40 ul.

1) On a 0.8% agarose gel, run 2 and 5 ul aliquots of the purified, eluted DNA next to size and concentration standards. Desired injection concentration for most constructs in 2.5 ng/ul. Do not dilute all of the stock. After dilution of a portion of the stock with injection buffer, filter using a Millipore HV 0.45 m unit (attaches to a 1 cc syringe). Particle free ultra clean DNA is a must!

*Injection Buffer is 10mM Tris C1 pH 7.5 and 0.1mM EDTA made from sterile millipore system purified water.