

PROTOCOL- Purification of DNA for Pronuclear Injection

Please provide **10 ug plasmid DNA digest** for each pronuclear injection request. Gel documentation confirming complete digestion must accompany the DNA submission.

1. Purify plasmid DNA using ion exchange (i.e. Qiagen or NucleoSpin), silica gel (Sigma, NucleoSpin), other resin-based (Promega) system or CsCl gradient.
2. Quantify the isolated DNA by absorbance. The $A_{260}:A_{280}$ ratio should be ≥ 1.7
3. Digest DNA in an appropriate volume of compatible buffer to digest the construct. Complete digestion is required; please provide gel documentation with the plasmid DNA prep to confirm complete digestion. Indicate the injection fragment on gel documentation of the plasmid DNA digest.
4. The Transgenic/Knockout Core Facility will gel purify the fragment indicated for microinjection using 0.6% agarose gel (Invitrogen UltraPure agarose #15510-019) containing ~200 ng/mL ethidium bromide (Invitrogen #15585-011) or Visual Violet DNA stain (Amresco #N733). Typically the bulk of DNA is loaded into a single wide lane and a small amount is loaded into an adjacent smaller lane. Appropriate markers are loaded into flanking lanes. After electrophoresis the gel is sliced vertically in such a way that the small lane with DNA and marker lane(s) are separated from the lane(s) containing the bulk DNA. After UV exposure in a transilluminator the microinjection fragment is removed from the small lane using a clean razor blade. After re-assembly of the gel a corresponding slice of gel is removed from the bulk DNA lane containing the injection fragment, this avoids exposure of the bulk DNA injection fragment to UV. After excision of the injection fragment the gel slice is placed in a weighed eppendorf tube and the gel weight is determined. Exposure of the remaining gel to UV verifies complete removal of the injection fragment from the gel.
5. The isolated gel slice containing the DNA fragment for injection is removed from gel using NucleoSpin Extract II Kit reagents. Briefly, 200 μ L of buffer NT is added per 100 mg of gel and incubated at 50°C until the gel is completely dissolved (5-10 minutes). The solution is placed into a NucleoSpin column (silica) and DNA is bound, solution is removed by spinning at 11,000xg for 1 minute. Each column has a 400 μ g capacity. The column/DNA is washed with 700 μ L of buffer NT3 by spinning at 11,000xg for 1 minute then dried by spinning for 2 minutes at 11,000xg after removal of the wash solution. DNA is eluted in 30 μ L of microinjection buffer (MIB).
6. Recovered DNA is evaluated for concentration and purity by comparison to λ HindIII marker DNA in a 0.6% agarose gel. Marker lanes contain 50 and 100 ng total DNA respectively.
7. DNA stock solution is then filtered ((Durapore PDVF 0.22 μ m, Ultrafree-MC, Amicon #UFC30GV25) prior to dilution in MIB. Three concentrations are generally made: HI (10 ng/ μ L), MED (5 ng/ μ L) and LO (2.5 ng/ μ L) in filtered MIB. On rare occasions when poor oocyte survival is observed after injection water may be substituted for MIB.
8. DNA dilutions are aliquoted in 10 μ L volumes and stored at -20°C until used. Once thawed DNA aliquots are discarded to avoid freeze-thaw damage.
9. Fertilized oocytes are injected with one or more concentrations of DNA to determine the optimal DNA concentration. Oocyte survival rates after injection of $\geq 70\%$ are considered acceptable.

