

Transgenic Service

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Preparation of plasmid DNA constructs for microinjection (not for BAC constructs!)

Protocol:

- 1) Digestion of the maxiprep-DNA to liberate the construct from the vector-DNA
- 2) Digestion control of a small amount with agarose gel electrophoresis
- 3) Separation of the fragments with a quantitative agarose gel
- 4) DNA extraktion of the excised gel slice with Quiaex II. Dissolve in 10 mM Tris pH 7.5-8.5.
- 5) Filter-sterilize the Quiaex Extract through a Milipore Millex-GV 0.22 μ filter. (by using a disposable 1 ml syringe with an airbubble as rest-volume, to avoid DNA loss). **Transgenic Service will provide you with Millex filter and syringe.**
- 6) Dialyse the sample with 50-100ml microinjection buffer (i.e. by use of a floating Millipore Membran VMW PO2500 / VM 0.05 μ m, shining/smooth side on top). Take > 50 ml buffer, 50-100 μ l DNA sample, dialyse > 3 hrs, beaker with parafilm covered, at 4°C to avoid evaporation of small DNA volume.
Please use buffer for dialysis which is prepared by Transgenic Service only. Transgenic Service will provide you with dialysis buffer and Millipore Membrane.
- 7) Quantitate the DNA concentration by using agarose gel electrophoresis including DNA markers of known size and concentration or by photometric measurement.
- 8) Store the DNA solution at -20°C (only for plasmid DNA)

Concentration analysis

After dialysis the concentration of DNA should be measured by agarose gel electrophoresis with molecular weight markers. We would like to obtain a gel picture together with the DNA. **We expect dialysed DNA solution with a concentration of 70-100ng/ μ l and a volume of 50-100 μ l.** We will dissolve the concentrated DNA at the Transgenic Service to obtain a final working concentration of 1-3ng/ μ l for injection.