Isolation of linearized plasmid DNA constructs for microinjection to produce transgenic animals

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Equipment and reagents

- DNA preparation kit (e.g. from Qiagen)
- Agarose, e.g. Seakem (FMC Bioproducts)
- 1× TAE buffer, prepared from a 50× stock (50× TAE buffer is 2 M Tris-acetate, 0.05 M EDTA pH 8.0)
- Ethidium bromide solution (10 mg/ml)
- Absolute and 70% ethanol
- 3 M sodium acetate, pH 5.2
- 10 mM Tris-HCl pH 7.4, 0.1 mM EDTA
- DNA electroelution system, e.g. Biotrap BT 1000 (Schleicher and Schuell)
- 0.22 μm sterile filter, e.g. Millex-GV_4 (Millipore)

Method

1. Prepare high-quality plasmid DNA according to standard protocols. DNA preparation kits can be used, e.g. from Qiagen. Digest 10–20 μg DNA with appropriate restriction enzyme(s) to release the insert.
2. Load the digested sample onto a standard agarose gel in 1× TAE buffer.
3. Stain the gel in ethidium bromide solution after electrophoresis.
4. Cut out the desired fragment from the gel under long-wave UV light.
5. Put the gel piece into the Biotrap electroelution chamber, which is set up according to the manufacturer's instructions, and cover it with 1× TAE buffer.
6. Place the Biotrap system in a horizontal electrophoresis tank filled with pre-cooled 1× TAE buffer.
7. Run the electrophoresis for 90 min at 10 V/cm. Within that time the DNA should be collected in the trap limited by the two membranes. The gel piece can be

stained again in ethidium bromide solution and checked under UV light to ensure no DNA remains.

8 Reverse the polarity of the electrodes for 30–60 sec to detach any DNA that has stuck to the DNA-impermeable membrane on the exit side of the trap.

9 Transfer the DNA solution from the trap into a 2 ml microcentrifuge tube and precipitate the DNA by adding 2.5 vols of absolute ethanol and 0.1 vol. of 3 M sodium acetate (pH 5.2) and placing at –20 °C for at least 30 min.

10 Pellet DNA by centrifugation, wash in 70% ethanol, and air dry.

11 Resuspend in the desired amount of injection buffer containing 10 mM Tris-HCl (pH 7.4) and 0.1 mM EDTA.

12 Check the DNA concentration as described below (Testing the exact concentration of DNA constructs used for microinjection to produce transgenic animals) and dilute the DNA solution with injection buffer to a final concentration of 2 ng/μl for injection.

13 To remove any particles, spin the DNA through a 0.22 μm sterile filter. Store at 4 °C until use.