

Protocols

Preparation of DNA for Electroporation into ES Cells

1. Prepare at least 30 μg of the targeting vector plasmid. We recommend the Qiagen EndoFree Plasmid Maxi kit. Alternatively, one can use standard plasmid protocols followed by CsCl banding
2. Linearize the targeting vector by digesting with a unique enzyme
3. After digestion, check for complete linearization by running a small fraction on a mini-gel
4. Precipitate DNA with two volumes of ethanol and 0.3 volume of 7.5 M ammonium acetate
5. Wash pellet 2X with clean 70% ethanol
6. Resuspend DNA in 0.1X TE* at concentration of 1.0 mg/ml. A total of 25 micrograms of linearized DNA is needed for electroporation.

***0.1X TE**

Prepare 1X TE:

10mM Tris-HCl, pH 8.0

1mM EDTA, pH 8.0

Sterilize.

Dilute to 0.1X with sterile water.

Note:

- It is very important to precipitate DNA with ammonium acetate rather than the commonly used sodium acetate
- It is equally important to resuspend the DNA pellet in 0.1X TE according to the protocol above and not water or PBS
- Not adhering to either of the above will greatly affect transformation efficiency

Preparation of DNA for Pronuclear Injection

1. Prepare 30-50 μg of DNA. We suggest that you use a plasmid preparation protocol that results in endotoxin-free, clean plasmid DNA. The EndoFree Qiagen kit is suitable for this purpose.
2. Digest enough transgene construct to release 20 μg of fragment to be injected. (The fragment to be injected has to be free of all vector sequences.)
3. After digestion, check for complete digestion by running a dilute portion of the DNA on a gel. Compare to a lane of uncut plasmid and a lane where the digestion is overloaded (to check for additional unwanted products). Take a clear picture of this gel

Bring the entire digestion reaction to the facility for injection along with the picture of the gel, clearly indicating the fragment to be injected. The facility will further purify the fragment for injection.

Please contact the facility director for special preparation procedures for large DNAs (YACs, BACs, etc.)

Tail-DNA Extraction

1. Place tail sample in 1.5-ml tube with 0.75 ml tail buffer*.
2. Incubate at 55°C overnight with shaking.
3. Extract digestion solution with equal volume of 1:1 phenol:chloroform then equal volume of chloroform.
4. Precipitate DNA with equal volume of isopropanol.
5. Spin down precipitate for 10 minutes.
6. Invert tubes to discard supernatant.
7. Dry pellet by placing tubes inverted over a paper towel at room temperature for an hour.
8. Resuspend DNA pellet by adding 80 µl TE.

***Tail buffer**

100mM NaCl

10mM Tris-HCl, pH 8.0

25mM EDTA, pH 8.0

0.5% SDS

- 0.8 mg/ml Proteinase K