

## DNA for microinjection

### DNA Requirements for microinjection

The most critical factors affecting integration efficiency is purity of the DNA.

It is essential that microinjection DNA be absolutely free of contaminants that are toxic to embryos as trace of phenol, ethanol and enzymes.

The efficiency of integration is also influenced by endotoxins plasmids contamination.

### Plasmid DNA preparation

Cesium Chloride gradient centrifuge preparation yields the purest plasmid DNA.

We are routinely recommended to use Qiagen Endo Free Plasmid kit (sit: cat.12362),

NucleoSpin TM Extract kit (sit: K3051-1) or NucleoBond AX 500Tip (sit: cat 4003-1).

Preparation of microinjection samples DNA.

DNA concentration must be measured exactly in order that final concentration for injection 4,5 mg/ml can be determined.

Generally we provide the helper plasmid.

We recommend also to use high quality sterile Water for Embryo Transfer, (Sigma W1503), to dilute DNA.

It is also very important that DNA solution do not contain any small particles, which can clog the microinjection needles.

### DNA shipping

Send 45µg of plasmids under 2.5 vol of 100% EtOH and 1/20 vol of 3M NaAcet or as precipitated pellet or dissolved in ultra pure water.

In any case indicate very clear your choice.