

Preparation of DNA for microinjection

1. Purify plasmid DNA for microinjection by CsCL centrifugation or Qiagene kit.
2. * Gel purify DNA fragment with TAE buffer.
3. Purify DNA fragment by electroelution, followed by desalting and concentration by GeneClean Kit.
4. Suspend the DNA in microinjection buffer (10mM Tris HCL, 0.1mMEDTA, PH7.4).
5. Extract DNA with phenol/chloroform 1X.
6. Extract DNA with chloroform 2X.
7. Add 1/10 of 2M NaCL (v/v) and 3 volumes of 100% Ethanol and precipitate.
8. Wash twice in 70% Ethanol and dry at RT.
9. Resuspend in 50ng/ul, 50 ul total microinjection buffer.

* Purification of DNA should avoid overdigestion or partial digestion of DNA, excessive heat DNA, vigorous vortexing or pipetting, exposure to short-wavelength UV light, exposure to varying temperatures and DNase activity.