

Preparation of DNA for Microinjection

Materials:

- Use gel-purified DNA fragments (or Qiagen-cleaned midiprep fragments with heat-deactivated restriction enzyme)
- Centriprep-30 columns from Millipore (#4306)
- Centrex MF-1.5 (0.2 micron) filter. Schleicher and Schuell (#10467B04)
- rotor with 50 ml Falcon tube insert
- Nanodrop
- Agarose gel

Solutions:

- Microinjection Buffer (MB):

10 mM Tris, pH 7.4 (or 7.5) @ 4°C

0.15 mM EDTA, pH 8.0 @ rm. temp.

For 500 ml, add 5 ml of 1M Tris and 150 ul of 0.5M EDTA. Filter sterilize into 500 ml container.

*Note: Microinjection Buffer does not need to be made fresh.

- Do all spins @ 1500 x g (2800 rpm), @4°C

Procedure:

- 1.) Wash columns by adding 10 ml of MB to outside chamber of columns. Spin for 10 minutes. Pour out both inside and outside chambers. Repeat.
 - This step is to remove glycerol in column membrane.
- 2.) Mix DNA with 15 ml MB and add into outside chamber of column. Spin for 30 minutes. Pour out

inner chamber solution only (*make sure to always turn the groove of the inner chamber up, otherwise the DNA will be poured out, too*).

- 3.) Spin again for 25 minutes. Pour out **inner** chamber solution again, and spin for another 20 minutes. Pour out **inner** chamber solution again.
- 4.) Add 10 ml MB to outside chamber. Spin for 20 minutes. Pour out **inner** chamber solution.
- 5.) Spin again for 15 minutes. Pour out **inner** chamber solution again, and spin for another 20 minutes. Pour out **inner** chamber solution.
- 6.) Add 5 ml MB to outside chamber. Spin for 10 minutes. Pour out **inner** chamber solution again, and spin for another 5 minutes. Pour out **inner** chamber solution.
- 7.) Repeat 5 minute spins and disposal until outer chamber solution volume is <500 ul.
 - Use pipet to measure volume.
- 8.) Transfer solution to new, Centrex column.
- 9.) Spin @3200 rpm for 10 minutes @ rm. temp.
- 10.) Measure sample concentration on Nanodrop.
 - Use MB to blank
- 11.) Run a 50 ng aliquot and a 100 ng of each DNA sample through a 1% agarose gel to verify purity.