

Protocols:

DNA fragment preparation for PN microinjection

The purification of a DNA fragment for microinjection is extremely important. The following protocol provides a guideline for getting DNA that is (1) free of salts, organic solvents, or traces of agarose; (2) in the correct sterile buffer; (3) not sheared or nicked.

- A large prep of the plasmid DNA can be made and purified by cesium chloride (CsCl) gradient or a Qiagen kit. The fragment of interest is then isolated from vector sequences by restriction enzyme digestion, removing as much of the vector sequences as possible.
- Fractionate the digested DNA in an agarose gel by electrophoresis using 1X TAE buffer containing ethidium bromide (0.5 ug/ml).
- View the DNA fragments using a long-wave UV light and carefully excise the fragment of interest using a fresh, sterile scalpel. Dice the agarose block and transfer to one or more 1.5 ml eppendorf tubes depending on the size of the gel slices.
- Further clean the DNA fragment by using a Qiagen QiaexII gel extraction kit (Qiagen Cat #20051). Elute DNA with MiTE (10mM Tris-HCl, 0.1mM EDTA, pH7.4—TKTC provide). Give a total of 1 ug DNA at 50 ng/ul or more in an eppendorf tube to the facility for microinjection. Attach a picture of an agarose gel showing an aliquot of the purified DNA fragment and its concentration.

BAC DNA preparation for PN microinjection

Injection buffer:

- **1000x Polyamine Stock** (please contact TKTC to check if we have stock)
30 mM Spermine (Sigma, tetrahydrochloride, #S-1141)
70 mM Spermidine (Sigma, trihydrochloride, #S-2501)
Dissolve the spermine and spermidine together in autoclaved distilled water, filter sterilize (0.2 micron filters), and store at -20 C. Since the polyamines are very hygroscopic, it is suggested that small quantities (1 gram) should be ordered and then all of it should be prepared at once.
- **Polyamine Microinjection TE Buffer (PMiTE)**
10 mM Tris-HCl, pH 7.5: 0.5 ml of 1 M Tris-HCl, pH 7.5 (autoclaved)
0.1 mM EDTA, pH 8.0: 10 microliters of 0.5 M EDTA, pH 8.0 (autoclaved)
100 mM NaCl: 1 ml of 5 M NaCl (autoclaved) For 50 ml:
1x Polyamines: 50 microliters 1000x Polyamines mix
Autoclaved H₂O up to 50 ml
NOTE: Prepare fresh and discard unused polyamine microinjection TE buffer.

BAC DNA preparation

1. Make a large prep of the BAC DNA.
2. Estimate the DNA concentration by digesting an aliquot of the prep with either Sall or NotI and run the digest on a regular gel. The concentration can be determined by comparing the 7 kb vector fragment with a range of lambda standards.
3. Buffer exchange with Centriprep-30. To prepare the DNA for microinjection, multiple steps of buffer exchange are performed with Centriprep-30 concentrator columns (Amicon, Beverly, MA, CA#4306), all steps must be done at 4 degrees C.
To remove trace glycerol present in the membrane of centriprep-30, rinse the column by adding 10 ml PMiTE to the outside chamber of the column. Spin at 1500g for 10 minutes, decant both inside and outside chambers. Repeat spin and disposal inner chamber solution for two more times.
Mix DNA with 15 ml of PMiTE and add it into the outside chamber of the column. Spin at 1500g for 25 minutes. Dispose the inner chamber solution.

Add 10 ml of PMiTE to the outside chamber and spin at 1500g for 20 minutes. Dispose inner chamber solution and spin again for 15 minutes. Dispose inner chamber solution. Add 5 ml of PMiTE to the outside chamber. Spin at 1500g for 15 minutes. Dispose inner chamber solution. Repeat spins of 5-10 minutes followed by removal of inner chamber solution until outer chamber solution is less than 0.5 ml.

4. Filter the DNA through a small 0.45um Millipore spin filter (Millipore Cat #SLHV004NL).
5. To determine the integrity of DNA, run approximately 200 to 500 ng of DNA on a pulsed-field gel (estimate a 50% yield after buffer exchange columns).
6. To quantitate the concentration of DNA, digest DNA with either Sall or NotI and run digest on a standard 0.8% agarose gel. The concentration can be determined by comparing the 7 kb vector band with lambda standards.
7. Give a total of 1 ug BAC DNA to the facility. Attach pictures of both pulsed-field gel and regular agarose gel showing the integrity and concentration of the prep.