Preparation of BAC DNA for Pronuclear Injection

Drop Dialysis

1. Fill the bottom of a Petri dish with injection buffer (MIB)[a].
2. Float the dialysis membrane (VSWP02500, Millipore) on the surface of MIB with shinny face up.
3. Deposit 5–100 μL of DNA on the center of the membrane. Marker dyes may help in determining the dialysis time required. Most samples are dialyzed in less than 30 minutes.
4. Place a tight-fitting lid on the Petri dish to prevent evaporation.
5. Recover the desalted sample, and determine DAN concentration.
6. Before injection, dilute DNA to 3 ng/μl with MIB. Mix 40 μl of DNA with 20 μl of MIB containing 3x polyamines solution[b] (final concentration is 2 ng/μl of DNA, 30 μM spermine and 70 μM spermidine).

[a] Microinjection buffer (MIB): 10 mM Tris, pH7.5, 0.1 mM EDTA, pH8.0 and 100 mM NaCl.

[b] 1000x polyamines solution: 30 mM spermine (Sigma, Tetrahydrochloride, #S-1141) and 70 mM spermidine (Sigma, Trihydrochloride, #S-2501). Stored at -20˚C.

Buffer exchange with Centriprep-30 (alternative to drop dialysis method)

To prepare BAC DNA for injection, buffer exchange is performed with Centriprep-30 concentrators (Amicon-Beverly, MA-product #4306). The following procedures are modified according to instructions come with the concentrators.

1. Add 10 ml microinjection buffer (MIB) to the outside chamber. Spin at 1,500g for 10 min. Discard buffer from both outside and inside chambers. Repeat once.
2. Mix 10 μg or more of BAC DNA with 15 ml of MIB and add into outside chamber of concentrator. Spin at 1,500g for 20 min. Dispose of inner chamber solution and spin again for 10 min. Dispose of inner chamber solution and spin for 10 min. Discard solution in the inner chamber.
3. Add 10 ml of MIB to outside chamber. Spin at 1,500g for 15 min. Dispose inner chamber liquid and spin 10 min. Repeat disposal and spin once more.
4. Add 5 ml of MIB to outside chamber. Spin at 1,500 for 10 min. Repeat disposal and spin as above twice. Collect DNA solution in the outside chamber. The volume should be about 0.5 ml or less.
5. Filter DNA through 0.45 micron Millipore spin filters (eg. #SJHV004NS)
6. Determine integrity and concentration of DNA. Run about 100 ng of DNA in a pulsed field gel to determine the integrity.

The DNA can purified again by by spot-dialysis on a VSWP02500 filter (Millipore) floating on 1/2 or 1/3 TE for 30-60 minutes (during the dialysis the DNA volume increases, and you can concentrate the DNA by spotting the solution on to a Saran wrap laid over a heat-block (40-50˚C) until the volume reduces to 1/2-1/3.