

Protocol: BAC DNA Preparation for Transgenic Microinjection

****Note: With BAC DNA: Do not vortex, do not filter, and do not freeze. Handle it gently; it is large, delicate and easily damaged.**

You will need:

Your sequence confirmed BAC

Slide-a-lyzer cassettes from Pierce (catalog # 66415)

18 gauge needles

4L of TE (10mM Tris pH 7.2, 0.1mM EDTA)

Appropriate Restriction enzymes

CHEF gel apparatus

Markers for CHEF gel: (a) low range PFG marker (NEB catalog#N03505) and (b) serial dilutions of lambda DNA from Promega (catalog # D1501)

- 1) Take a glycerol stock of your confirmed BAC to Jeff Conroy and Mike Henry at the Roswell Park Microarray/Genomics Resource Center (<http://microarrays.roswellpark.org/>). Give them pertinent information on culturing your sample (Ask for a minimum of 4 DNA preps).
- 2) Pick up your sample from the Microarray Center.
- 3) You should receive approximately 400uL of DNA in TE. Split the sample in half. Keep half at 4C. Dialyze the other half against 4L TE (10mM Tris pH 7.2, 0.1mM EDTA) + 100mM NaCl. Autoclave the dialysis solution. Dialyze at room temperature against 2L for the afternoon. Change the solution to the remaining fresh 2L and let the dialysis go overnight.

****Dialyze in Slide-a-lyzer cassettes from Pierce (10,000MWCO 0.1-0.5mL capacity, catalog # 66415) ****

****Use a 18 gauge needle to inject the DNA into the cassette-slowly and gently. The use of the 18 gauge needle and inject rate is critical to not shearing the BAC****

- 4) Set up restriction digests with the appropriate enzymes to check your DNA (usually 37C for 3 hours). Digest dialyzed and undialyzed DNA samples and also run lanes of undigested DNA for each sample. Usually 15uL of undialyzed DNA is enough to see on a CHEF gel.

5) CHEF gel: Set up in the late afternoon for an overnight run.

a. 1% agarose in 0.5XTBE, NO Ethidium Bromide

10X TBE:

108 g Tris base

55g Boric acid

40mL 0.5MEDTA

pH to 8.3

Bring to a final volume of 1L

Notes on preparing and running the CHEF gel:

We like to use bromophenol blue. The low range marker is a gel provided in a syringe. Use a clean razor blade to cut the thinnest slice possible. Put it in a well. Cover with warm agarose so it doesn't float out.

Dilute Lambda DNA to run various concentrations to determine your DNA concentration. 5-50ng is usually a good range.

Use 2L of 0.5XTBE for running buffer

The CHEF gel apparatus should be turned on before loading samples so the buffer is cooled to 14C.

Our program parameters:

For a 5-150kb digested fragment:

0.2 initial switch time

12.9 final switch time

Running time: 15 hours, 16 minutes

Run at 6 volts, and a 120 degree included angle

Use a pump to circulate the buffer...cold buffer, 14C.

6) The next morning soak the gel in 0.5ug/mL of Ethidium Bromide for 20-30 minutes

7) Rinse gel in dH2O for 10 minutes and then photograph

- 8) Give the TG facility both lots of DNA (dialyzed and non-dialyzed) as well as the photo.
- 9) Stay in contact with the TG facility for timing the microinjection. It is best to inject circular rather than linearized DNA.
- 10) The BAC DNA once prepared must be used within 2 weeks of delivery to the TG facility.