SOUTHWESTERN

THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
AT DALLAS

TRANSGENIC TECHNOLOGY CENTER

Preparation of BAC DNA for the Creation of Transgenic Mice: Takara/ Macherey-Nagel NucleoBond BAC 100 Methodology
1-16-26

Reagents

BAC Buffer
Chloramphenicol, 10mg/ml (ThermoFisher #J67273.AC)
70 % ethanol (room temperature)
Ice
Isopropanol (room temperature)
NucleoBond BAC 100 kit (Takara #740579)
SeaKem GTG Agarose, Lonza (Fisher #BMA50071)

***The Core can supply BAC Buffer if needed.

BAC Buffer	for 50mls
10mM Tris-HCl pH 7.5 (autoclaved)	500ul of 1M Tris-HCl
0.1mM EDTA pH 8.0 (autoclaved)	10ul of 0.5M EDTA
0.1 M NaCl (autoclaved)	1ml of NaCl
1x Polyamines	50ul of 1000x Polyamine mix

1000x Polyamine Stock
30 mM Spermine (Sigma tetrahydrochloride, #S-1141)
70mM Spermidine (Sigma, trihydrochloride, #S-2501)

Dissolve the spermine and spermidine together in autoclaved distilled water, filter sterilize (0.2 micron filters, Whatman Anotop 25 syringe filter). 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. Store at -20^{o}C in 100ul aliquots.

Equipment

Centrifugation tubes or vessels with suitable capacity for the volumes specified
Funnels to hold the NucleoBond Folded Filters for lysate filtration
NucleoBond Rack (Takara #740563) or equivalent holder
Refrigerated centrifuge
Standard microbiological equipment for growing and harvesting bacteria

Method

1. Harvest bacteria from a 250ml LB culture + chloramphenicol by centrifugation at 3700 x g (5900 rpm) for 20 min at 4°C.

2. Resuspend the pellet of bacterial cells in 30mls **Buffer S1 + RNase A**. Then split evenly between 3-50ml round bottom centrifuge tubes.

3. Add 10mls **Buffer S2** to each tube. Mix gently by inverting the tube 6-8 times. Incubate the mixture at room temperature for 2-3 min.

4. Add 10mls pre-cooled **Buffer S3 (4°C)** to each tube. Immediately mix the lysate gently by inverting 6-8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min. Then spin 15000 x g (11900 rpm) for 10 min at 4°C. This helps so there is less flocculate material going onto the filter and clogging/slowing the process.

5. Equilibrate the column with 6mls **Buffer N2**, allowing the column to empty by gravity. Discard flowthrough.

6. Place a **NucleoBond Folded Filter** in a funnel of appropriate size. (See section 3.4 of manual on how to set up the funnel and filter.)

7. Pour 1 centrifuge tube at a time into the funnel and filter. Let drain by gravity. Repeat for remaining two tubes through the same filter and column. This is a very slow process.

8. Add 30mls **Buffer N3** to column and let drain by gravity. Discard flowthrough.

9. Using a clean round bottom tube, place column over tube in rack. Elute with 15mls **(pre-warmed 50°C) Buffer N5** to the column. Let drain by gravity.

10. Add 11mls **room temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge 10000 x g (9700 rpm) for 40 min at 4°C. Discard supernatant.

11. Add 3mls of **70% ethanol** to the pellet. Vortex briefly and split between 2 microcentrifuge tubes. Centrifuge 20000 x g (14000 rpm) for 10 min at room temperature. Carefully discard supernatant using a pipette tip. Let dry about 3-5 min at room temperature.

12. Resuspend pellet in 50ul **BAC Buffer** per tube, then combine and place in clean 1.5ml microcentrifuge tube.

13. Read concentration on the fluorometer. Run 200ngs on 0.8% GTG agarose gel.