Robertson Laboratory
Standard Protocol for DNA Mini-prep

IMPORTANT: sterile techniques must be employed for efficient and successful DNA extraction

I - BAC CLONES

1. BACs are supplied in agar (2.5mL screw tops filled with LB agar, stabbed with a toothpick which has been put into a bacterial colony). These are then frozen; at -70C they will keep indefinitely, at -20C for a year or more, at 4C for several months and at room temperature for a week.
2. Make up 10mL of LB and add antibiotic(s) as needed according to the following concentrations:

   NOTE: for wild type selection, only Chloramphenicol is employed

   a. Chloramphenicol – [12.5 ug/mL]
      - supplied as 1000x stock (250mg in 20mL ethanol)
   b. Kanamycin – [25 ug/mL]
      - supplied as 1000x stock (250mg in 10mL mQH_2O)
   c. Ampicillin – 50 ug/mL
      - Supplied as 1000x stock (1g in 20mL MQH_2O)
   d. Tetracycline – 12.5 ug/mL
3. Dip a sterile toothpick into the stab and initiate the culture by dropping the whole toothpick in a flask containing LB media
4. Cap the flask and shake vigorously overnight at 30C

II - SINGLE COLONY PREPS

1. Make LB agar plates containing Chloramphenicol (12.5 ug/mL final concentration)
2. Dip a flame-sterilised inoculating loop into the BAC clone culture
3. Begin plating the bacteria by streaking three parallel lines on LB agar; remember to perform this without redipping the loop
4. Flame sterilise the inoculating loop and make three more adjoining parallel lines on the plate to spread the bacteria (the idea is to serially inoculate cells until they are dilute enough to form single colonies).
5. Grow colonies overnight at 32C

   NOTE: ideal appearance is continuous line of colonies in the first group of three parallel lines, going to individual colonies clearly separated in lines belonging to group four

III – SMALL SCALE ISOLATION OF BAC DNA

NOTES: The succeeding steps are adapted from the protocol by Zhang from Texas A&M University (http://hbz.tamu.edu/manual.pdf); gentle shaking and inversion during the indicated DNA extraction steps prove to be useful in the differential precipitation of unwanted bacterial genomic DNA (4.6 megabases) and the BAC DNA (40-200 kilobases), ensuring that the DNA is kept intact. DO NOT VORTEX or VIGOROUSLY MIX DNA SOLUTIONS!!!

1. Using a sterile tip, stab a single colony from the LB agar plate that have been growing overnight; pipette the colony in and out of the tip into 10 mL of LB media with the appropriate antibiotic(s)
   NOTE: Use a 50mL Falcon tube at this point to ensure sufficient aeration while shaking
2. Grow cells overnight with rapid shaking at 32C (24 hours of shaking/incubating is likewise acceptable)
3. Transfer LB media containing cells into a 15mL Falcon tube
4. Spin at 4100 rpm for 5 minutes; discard supernatant and resuspend pellet in the remaining ~100uL of LB
5. Transfer ~150uL of cells+LB into a sterile 1.7mL Eppendorf tube
6. Spin at 3000RPM to pellet cells; aspirate excess LB
7. Add 100uL of **SOLUTION I** to the cells and mix vigorously (vortex may be used) to completely lyse the cells
8. Incubate resulting lysate on ice for 5 minutes
9. Add 200uL of **SOLUTION II**. At this point, the resulting lysate must be manipulated GENTLY; agitation is carried out by inverting the tubes 12-15 times, DO NOT VORTEX
10. Incubate the resulting lysate on ice for 2 minutes and 30 seconds
11. Add 300uL of **SOLUTION III** and invert tubes 12-15 times, followed by incubation on ice for 12-15 minutes
12. Cold centrifuge (4C) the lysate at 13000 rpm for 10 minutes to separate out the pellet
13. Transfer the CLEAR supernatant to a new, sterile 1.7mL Eppendorf (if the supernatant is not clear, supernatant MUST be centrifuged again at 13000 rpm for 10 minutes and then transferred to a new tube)
14. Add 600uL of isopropanol to the supernatant, mix gently and centrifuge at 13000 rpm for 5 minutes
15. Aspirate the isopropanol (a fairly large pellet must be visible at this point) and wash the DNA pellet with with 300uL of 70% Ethanol (volume of Ethanol added is commensurate with pellet size; usually, 300uL is sufficient)
16. Centrifuge DNA pellet with the 70% Ethanol wash at 13000 rpm for 2 minutes
17. Aspirate excess solvent and dry the pellet by inverting the tube and air-drying the pellet for 10 minutes
18. Resuspend pellet in 50uL of sterile mQH_2O; allow the pellet to dissolve overnight at room temperature or for 10 minutes at 37C
19. Treat with 2uL of RNAse at 10mg/mL concentration and incubate for 1 hour at 37C
20. Use immediately or keep on ice (4C)

III – RESTRICTION DIGEST SET-UP

1. Set up a test digest using
    a. 10.0uL of isolated BAC DNA in mQH_2O
    b. 25.5uL of sterile mQH_2O
    c. 4.0uL of restriction enzyme buffer
    d. 0.5uL of restriction enzyme
2. Digest overnight at 37C (at least 14 hours)
3. Run samples on 0.8% agarose GPL

IV – SOLUTIONS

1. Solution I (Tris-Glucose-EDTA Cell Suspension Buffer)
    a. 9g Glucose (Dextrose)
    b. 25ml 1M Tris, pH 8.0
    c. 20ml 0.5M EDTA, pH 8.0
    d. Bring up volume to 1L
2. Solution II (SDS/NaOH Lysis Solution)
    a. 40ml 5N NaOH
    b. 50ml 20% SDS
    c. Bring up volume to 1L
3. Solution III (Precipitation Buffer)
    a. 294g K acetate
    b. Dissolve acetate well prior to acetic acid addition
    c. 115ml Glacial acetic acid
    d. Bring up volume to 1L