

Qiagen Prep for BAC DNA

Using Qiagen-tip 100 Plasmid Midi kit # 12143 or 12145. Just columns #10043.

Day 0:

- Streak out onto LB-chloramphenicol plates BAC clone of interest using sterile technique, for single colony isolation. Can prep up to 8 BAC clones in one day (# tubes will fit in SS34 or JA-20 rotor).
- Incubate at 37C overnight, agar side up.

Day 1:

- Inoculate 5 ml LB broth + 3 ul chloramphenicol (34mg/ml) with fresh single colony, using sterile toothpick. (don't use plates older than 10-14 days)
- Incubate in 37C shaking incubator overnight (225-250 rpm)

Day 2:

- Morning, take out 5 ml cultures and store in fridge until evening
- At ~6pm inoculate 100ml of LB + 60ul chloramphenicol with 0.5 ml of the 5 ml o/n culture.
- Incubate in 37C shaking incubator for 14 hours (until ~8am next morning)

Day 3:

- Bring cultures back to Lab.
- Make glycerol freezer stock
 - 0.5 ml of culture + 0.5 ml glycerol (I use 80%) in cryotube to be stored at -80C
- Pour each 100 ml culture into 2, 50ml conical tubes, balancing them.
- Centrifuge at 4000 rpm, 20 min, 4C to pellet cells. (can run 4 samples [8 tubes] at a time)
 - Discard supernatant
 - While tubes are spinning, cool down second cfg.
- Resuspend cells in 10 ml Buffer P1 (5ml per 50ml tube and combine). Transfer to 50ml Oakridge tube.
- Add 10ml Buffer P2 (Lysis), invert 6X, incubate at RT 5 minutes
- Add 10ml Buffer P3 (Neutralization), invert 6X, incubate 15 min on ice.
- Centrifuge at 20,000xg (~13350 rpm in SS34 rotor) for 30 min, 4 C.
- Remove supernatant using pipette and transfer to clean Oakridge tube. Cfg additional 15 min, 4C at 20,000 x g.
- While tubes spinning, prepare column
 - Attach column to 50ml conical collection tube using blue ring stands
 - Equilibrate by applying 4 ml Buffer QBT. Discard flow through
 - Pre-warm Buffer QF to 65C in water bath
- Apply supernatant to column in 50ml conical. Discard flow through. (Can only add 10-12ml at time, watch collection tube so that liquid does not touch bottom of column) Multiple samples usually do not flow through at the same rate.
- Wash column with 2X 10ml Buffer QC
- In new 15 ml falcon snap-cap tube (#35-2059), elute from column with 5 ml pre-warmed Buffer QF

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- Precipitate with 3.5ml isopropanol (RT) and spin 30-60 min, 11.5Krpm, 4C. (Make sure tubes are balanced, outside edge is marked, and have tube adaptors for this rotor—find in SER near microchem)
- Remove supernatant quickly, avoiding pellet, and keep. Allow to air dry for 5 minutes.
- Resuspend pellet in 400ul water and transfer to 1.5ml cfg tube
- Reprecipitate DNA --Add:
 - 55 ul 3M Sodium Acetate
 - 1 ml 95% EtOH
- Incubate at -20C for at least 10 min. (Longer is ok)
- Cfg 13.2K, 4C, 15 minutes in benchtop microcentrifuge
- Remove supernatant and keep (until sure have product). Air dry briefly
- Resuspend in 50ul H₂O, heat at 55C for few minutes. Will be very viscous.
- Check yield using Nanodrop. Be sure to bring water blank, and that samples are resuspended as well as possible. Write concentration, prep date, and initials on each tube.
- Store DNA at -20C in BACs box. Make sure to assign # to each BAC and to log tubes into appropriate sheets for -80C and -20C storage boxes.

Yields over 1ug/ul and under 40ng/ul are usually problematic for use as FISH probes.