

BAC Glycerol stock cultures

Purpose: As with any bacterial clone resource, it is imperative to create replica sets for archival purposes. Improper storage, cross contamination between wells and damage to mishandled plates often requires that the plate be replaced. Also, any library that is constructed using a non-phage resistant host (such as DH10B) is always vulnerable to T1 phage contamination, therefore storage of archival plates in a separate -80C freezer is strongly recommended. It is advisable to create a master and replica glycerol stock for each RPCI BAC clone before using the clones for the first time.

Materials:

LB + Glycerol broth: (2000ml)
20g Tryptone (BD 211705)
10g Yeast Extract (BD 212750)
20g NaCl (SIGMA S3014)
150ml glycerol (EM Science GX0185-5)

qs to 2 L with ddH₂O

pH 7.0 with ~400 μ L 5N NaOH

Aliquot into 5 x 500ml bottles and autoclave 25 min on liquid cycle.

Before using, add 400 μ L Chloramphenicol (20mg/mL). LB will expire one week after this addition. Label bottle with date of Chloramphenicol addition.

96-well, deep sterile plates (Costar 3961)
Airpore strips (Qiagen 19571)
96-pin replication tool (V&P Scientific)

Procedure:

- 1 Label 96-well deep block plates using laser printed cryolabels. This protocol describes the procedure for creating a 750 μ l master archive and a 250 μ l distribution copy.
- 2 Add 1000 μ L of LB + glycerol to each well of 96-W block.
- 3 Using long 250 μ L pipet tips, pick clones from the single-colony, individually archived RPCI clone glycerol stock resource, in the same order as the BAC DNA plates were created (see clone order worksheet).
- 4 Cover plates with airpore strips and incubate overnight at 37°C, at slight angle, in environmental shaker.

- 5 Remove airpore strips, and transfer 250ul to another 96-well deep block plate. Discard pipet tip in biohazard waste.
- 6 T1-Phage test the distribution plate using a 96-pin replicating tool (see Support Protocol).
- 7 Freeze and store both sets of plates at -80°C in appropriately labeled cryoracks. Clones must be frozen completely before shipping.