

FISH Protocol

PAC/BAC culture for DNA isolation

PAC culture

Day 1

1. Seed 3 ml of LB containing 25 µg / ml kanamycin with bacteria harboring P1 plasmid.
2. Grow overnight to stationary phase. For highest yield use a P1 transductant.

Day 2

1. Seed 50 ml of media containing 25 µg / ml kanamycin in a 125 ml Erlenmeyer flask with 1.7 ml of overnight culture (1/30 dilution).
2. Shake for 1.5 hrs.
3. For maximal yields, add IPTG to a final concentration of 0.5 mM.
4. After induction with IPTG continue growth for 5 hrs then harvest bacteria.
5. Centrifuge culture at 10,000 x g for 10 minutes (CELL PELLETT MAY BE FROZEN WITHOUT DAMAGE TO THE P1 PLASMID)
6. Isolate DNA using BD Clontech NucleoBond Plasmid Purification kit (PT3178-2) according to manufacturer's instructions

BAC culture

Details can be found at <http://bacpac.chori.org/protocols.htm>

IPTG stock; 0.05M = 50 mM

Add 1 / 100 vol. of IPTG stock to 50 ml of culture = Add 0.5ml of 0.05 M IPTG

IPTG stock; 0.2 g/ ml = 840 mM

Add 1 / 100 vol. of IPTG stock to 50 ml of culture = Add 30 µl of 0.84 M IPTG