

## PCR SCREENING OF BACTERIAL COLONIES

by TCH 8/13/01

1. Prepare **PCR master mix (10ul/ reaction)** as the follows (in microliter):

	<u>1 X</u>
10 x PCR Buffer	1.0
dNTPS(10mM each)	1.2
DMSO	0.6
Primer #1 (330ng/ul)	0.2
Primer #2 (330ng/ul)	0.2
ddH <sub>2</sub> O	6.7
Taq polymerase (5U/ul)	0.1
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	10.0

2. Aliquot 10 ul of the master mix to each well of a 96-well PCR plate (note: an 8- or 12-channel pipetter can be used to facilitate the aliquoting process);
3. Set up a 96-well tissue culture plate containing 30-50 ul of LB/antibiotic medium (Note: Label the PCR plate and the LB plate in the same order);
4. Hand-pick up well-isolated bacterial clones with pipette tips (p20-200 tips without filter), and attach the tips to an 8- or 12-channel pipetter;
5. Dip the tips into the PCR plate, and rinse off the plasmid DNA by gently pipetting up and down several times;
6. Inoculate the LB/antibiotic (tissue culture) plate by dipping/rinsing the same tips in LB medium of each well (in the same orientation as the PCR plate so you can track down potential positive clones);
7. Place the LB plate in the 37<sup>0</sup>C bacteria incubator;
8. To the PCR plate, add 15-20 ul of PCR-grade mineral oil to each well and seal the plate with PCR plate seal films;
9. Perform PCR amplification. A typical cycling program (on Hybaid Omn-E thermocycler) is listed as the follows:  
95<sup>0</sup>C x 2 min x1 cycle

92<sup>0</sup>C x 20 sec, 55<sup>0</sup>C (or T<sub>m</sub>) x 30 sec, 70<sup>0</sup>C x 30-60sec (or 1 kb/min) x23-28 cycles

10. Add 2 ul 6x DNA sample buffer and load to 0.8% agarose gel (along with 1Kb plus ladder);

11. Transfer the LB/antibiotic medium of corresponding positive clones to 2 ml miniprep culture for overnight incubation at the 37<sup>0</sup>C bacterial shaker.

**Note:** 1) Only strong/intense amplification can be considered as potential positive clones;

2) PCR cycle numbers usually depend on the size of clones. **25 cycles** are standard although 28 cycles may be needed if the clones are tiny (or the product size is > 1kb).

3) Depending on cloning background, one can screen for 30-80 colonies.