SOP: SP028

PCR of Bacteria for Detection of Recombinant Clones

Materials and Reagents:

1. Bacteria colonies on agar or overnight broth cultures to be tested
2. Sterile H_2O
3. puRe Taq Ready-to-Go PCR Beads in 0.2 ml vials Amersham Biosciences product 27-9559-01
4. Primers, 10 mM (note 1)
5. Thermal cycler – Tetrad PTC225, program COLPCR
6. Sterile 0.5 or 1.5 ml microcentrifuge vials
7. Pipetman p10 and/or p20 pipettors or equivalent
8. Sterile micropipettor tips
9. Sterile toothpicks (optional)
10. puRe Taq Ready-to-Go PCR Bead Product instructions supplied by manufacturer

Protocol:

1. _____ Aliquot 5.0 µl sterile H_2O to sterile 0.5 ml or 1.5 ml microcentrifuge vials for each colony or culture to be assayed. (note 2)

2. _____ Using sterile pipette tips or toothpicks, pick well-isolated single colonies and transfer each to vial with pre-aliquoted sterile H_2O.

3. _____ Overnight bacterial cultures may be used as an alternative. (note 3)

4. _____ **Critical**: retain the remaining bacteria for each clone until gel analysis is complete. (note 4)

5. _____ Add 22.0 µl of water/vial to the required number of puRe Taq Ready-to-go PCR beads.

6. _____ Add 1.0 µl each 10 mM primer or 2.0 µl of a 1:1 mixture of both 10 mM primers.

7. _____ Add 1.0 µl of resuspended bacteria to the PCR mixture. Final volume/vial = 25.0 µl.

8. _____ Process vials in Tetrad thermal cycler via program COLPCR in the Main menu. (note 5)

9. _____ Analyze 5.0 µl/PCR reaction by agarose gel electrophoresis to view insert. (note 6)

10. _____ If a positive clone is detected, spot 2-5 µl of the bacterial stock onto a new agar plate and subsequently grow an overnight culture for preparation of a frozen glycerol stock. (SOP RP001 Recombinant clone glycerol stock)

Notes:

1. Primers used to generate the cloned amplicon are recommended to assure gene specificity. As an alternative, T7 promoter and T7 terminator primers work well with pET 15b and pET 23b. They report insert size only, not presence of correct gene.
2. Reactions required: one for each colony to be tested, one as a positive control (if available), and one as a negative control. Best negative control is a colony taken from a vector alone ligation/transformation.
3. Overnight cultures:
    a. Spin 100 µl culture in 0.5 or 1.5 ml sterile microcentrifuge vial 10 min at 10-12,000 rpm.
    b. Remove and autoclave supernatant.
    c. Resuspend each pellet in 25 ul sterile water.
    d. Add water and primers to PCR vials as described in steps 2 and 3 above.
    e. Add 1.0 µl resuspended bacteria/25 ul PCR reaction.
4. Population viability drops over time in water. If PCR and gel analysis requires > 4-6 hr, adding 100 µl LB broth to bacteria-water stock after sampling for PCR will retain viable bacteria.

5. COLPCR profile on Tetrad, Main menu:

	Temp °C	Time	Cycles
Step 1:	94	10 min	1
Step 2:	94	30 sec	
Step 3:	55	30 sec	1
Step 4:	72	1min 30 sec*	
Step 5:	Goto step 2		29
Step 6	72	7 min	1
Step 7	10	for ever	

*Elongation time (Step 4) can be decreased or increased depending on predicted insert length (general rule: 1 min per 1000 bp).

Programs with the above properties can be entered on alternative cyclers. Step one, a 5-10 minute denaturation at 94°C is required to lyse bacteria, making DNA available for amplification.

6. Refer to SOP SP018 Agarose gel electrophoresis.