SOP 010 - Colony PCR（cPCR）

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1. Scope

This SOP applies to all laboratory personnel performing colony PCR for screening transformed bacterial colonies.

2. Summary of Methods

Colony PCR (cPCR) is a rapid screening technique used to verify the presence of target DNA fragments directly from bacterial colonies following transformation. In this procedure, a small portion of a colony is transferred into a PCR reaction mix and used as the DNA template, eliminating the need for plasmid extraction. The PCR products are then analyzed by agarose gel electrophoresis to confirm the presence and size of the desired insert. This SOP describes step-by-step methods for sample collection, PCR setup, thermocycling, and result interpretation to ensure reliable and efficient colony screening.

3. Definitions

Colony PCR (cPCR): A PCR method using cells from a single bacterial colony as the DNA template for amplification.

PCR (Polymerase Chain Reaction): A technique to amplify specific DNA sequences.

Agarose Gel Electrophoresis: A method to separate DNA fragments by size.

Template: The source of DNA for PCR, here provided by a bacterial colony.

Insert: The DNA fragment intended to be screened for presence/absence.

4. Health and Safety

Laboratory personnel must wear appropriate PPE (lab coat, gloves, eye protection) at all times.

Handle all bacterial cultures and PCR reagents according to institutional biosafety guidelines.

Dispose of all waste, especially gels and used tips, in accordance with the laboratory's biological and chemical waste disposal protocols.

In case of spillage or exposure, follow the laboratory's incident response procedures.

5. Personnel Qualifications

Personnel performing cPCR must have basic molecular biology training and must have completed training on PCR techniques, pipetting, and biosafety.

New personnel must be supervised until competence is demonstrated.

6. Equipment

Thermal cycler (PCR machine)

Benchtop microcentrifuge

Gel electrophoresis apparatus

Gel imaging system or UV transilluminator

Vortex mixer

Micropipettes and filter tips

7. Materials

Fresh bacterial colonies on LB agar plates

Sterile toothpicks or pipette tips

PCR tubes or strips

PCR master mix (in this protocol, we refer to Ampliqon DNA Taq polymerases)

Ammonium Buffer / 5X PCR Buffer RED (Ampliqon)

Forward and reverse primers (10 µM)

Nuclease-free water

Agarose

DNA gel stain (e.g., SYBR Safe, GelRed)

DNA ladder (e.g., 1kb DNA ladder)

$MgCl_2$ (25mM)

dNTP mix

Loading dye

Gloves, lab coat, and eye protection

8. Procedure

8.1 Setup

8.1.1 Calculate the number of PCR reactions required based on colonies to be screened. (colonies + parental)

8.1.2 Prepare the PCR master mix as follows (total volume of the master mix is 24 µl per PCR reaction):

8.1.2.1 Thaw all the frozen materials as recommended

8.1.2.2 Dilute the primers with nuclease-free water until the concentration is 10 µM

8.1.2.3 Per PCR reaction, pipette into the master mix (prepare for 1-2 more reactions than you need):

8.1.2.3.1 15.8 µl nuclease-free water

8.1.2.3.2 0.5 µl forward primer (10 µM)

8.1.2.3.3 0.5 µl reverse primer (10 µM)

8.1.2.3.4 1.5 µl $MgCl_2$ (25mM)

8.1.2.3.5 0.5 µl dNTP mix (10mM)

8.1.2.3.6 5 µl Ammonium Buffer / 5X PCR Buffer RED

8.1.2.3.7 0.2 µl 2x Taq DNA polymerase (always add enzyme in the last step)

8.2 Sample Collection

8.2.1 Choose at least half of the colonies available (i.e there are 12 colonies on the plate, then choose 6 colonies out of 12).

8.2.2 Label the selected colonies.

8.2.3 Label all the PCR tubes.

8.2.4 Using a sterile toothpick or pipette tip, pick a single colony (picked half of that colony, not all the colony, so that we still have the biomass left to preserve). Also, prevent taking a very large colony. Pick a nicely separated 1 colony only.

8.2.5 Dip the tip directly into the PCR tube containing the master mix or first suspend the colony in 10 µl nuclease-free water, then add 1 µl as template to the PCR mix.

8.3 PCR Thermocycling Program (Recommended, optimize as needed)

8.3.1 Initial denaturation: 95°C for 10 min (to lyse cells and denature DNA)
8.3.2 Denaturation: 95°C for 30 sec
8.3.3 Annealing: 55–60°C for 30 sec (depends on the primer itself, so change accordingly)
8.3.4 Extension: 72°C, 30 sec per kb (for short complex targets, <3 kb, use 10 sec/kb. For long amplicons, >3kb, use 30-60 sec/kb).
8.3.5 Number of cycles: 30-35
8.3.6 Final extension: 72°C for 5 min
8.3.7 Hold: 4°C
8.3.8 Store the PCR products in fridge (4ºC)

Note:

Annealing temperature and extension time should be optimized based on primer design and expected insert length. Use the Tm values provided by the primer manufacturer and adjust as necessary for optimal specificity and yield.
If this above method fails (i.e lots of unspecific band, no band, smears), consider using the following method:

8.4 Cell lysis and plasmid extraction before PCR (alternative method)
8.4.1 Using a sterile toothpick or pipette tip, pick a single colony (picked half of that colony, not all the colony, so that we still have the biomass left to preserve). Also, prevent taking a very large colony. Pick a nicely separated 1 colony only
8.4.2 Dip the toothpick into 50 µl of sterile milli-q water in an eppendorf tube.
8.4.3 Heat the solution in 90-100ºC for 10 minutes
8.4.4 Collect the supernatant which contain the DNA
8.4.5 Take 1 µl and mix it with the PCR Master Mix from step 8.1 and proceed to step 8.3.

8.5 Agarose Gel Electrophoresis
8.5.1 Prepare 1% agarose gel with suitable DNA stain. Prepare the agarose gel as follows:
8.5.1.1 Prepare the gel tray, adjust the balance if needed.
8.5.1.2 Prepare 1% agarose gel (i.e dissolve 1 gram of agarose powder in 100 ml TAE buffer. Adjust this according to your needs).
8.5.1.3 Microwave it shortly at a high temperature, until it's boiled.
8.5.1.4 Let it cool down until it's ok for you to touch it (around 60ºC)
8.5.1.5 Add suitable DNA stain (EtBr/SYBR Safe/SafeRed. Commonly, 5 µL for SYBR Safe in 50 ml of agar solution).
8.5.1.6 Pour the gel to the gel tray (around 5-7mm in thickness).
8.5.1.7 Add the suitable comb.

8.5.1.8 Let it sit for 20 minutes until it's solidified.

8.5.1.9 After the gel is solidified, put it in the gel electrophoresis machine and take out the comb.

8.5.1.10 Pour the TAE buffer until it covers all the wells (maximum volume should be as indicated in the machine).

8.5.1.11 Mix in 1:5 ratio of loading dye:PCR products (but it depends on what dye is used, so read the label as well before using it. I.e 25 µl PCR product + 5 µl loading dye for 6x loading dye. This step is not necessary if you used PCR master mix that already contained the dye like OneTaq 2x Master Mix).

8.5.2 Load 15 µl PCR product mixed with loading dye into each well.

8.5.3 Run gel alongside an appropriate DNA ladder (e.g., 1kb ladder) with the following gel electrophoresis machine setting:

8.5.3.1 V: 100-110

8.5.3.2 A: 400

8.5.3.3 Time: 20 minutes

8.5.3.4 Make sure you put the gel in the direction from - (black) to +(red)

8.5.4 Visualize DNA bands using gel imaging system/Gel dock.

8.6 Clean-Up

8.6.1 Properly dispose of gel waste and used tips according to biosafety and chemical waste protocols.

8.6.2 Clean the workspace and return equipment to designated storage.

8.6.3 Store any backup plates at 4°C.

9 Data and Records Management

9.1 colony numbers, PCR conditions, primer information, and gel results in the designated laboratory notebook or electronic record system.

9.2 Save gel images and link corresponding samples and date in digital storage.

9.3 Retain physical or digital records for a minimum of 2 years or according to lab policy.

10 References

10.1 List of related SOPs

10.1.1 SOP for PCR Reagent Preparation

10.1.2 SOP for Agarose Gel Electrophoresis

10.2 List of equipment manuals

10.2.1 Manufacturer's manual for PCR thermal cycler

10.2.2 Gel documentation system user guide

10.3 List of literature for references

10.3.1 Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition

10.4 List of related record templates

10.4.1 Colony PCR Result Sheet

10.4.2 Gel Documentation Record Form

10.5 Ampliqon Taq DNA Polymerase (protocol from supplier)

11 Appendix

If there needs to be an appendix or several appendices, add as many as needed.