clony PCR

Introduction

Colony PCR is a convenient high-throughput method for determining the presence or absence of insert DNA in plasmid constructs. Primers designed to specifically target the insert DNA can be used to determine if the construct contains the DNA fragment of interest. Alternatively, primers targeting vector DNA flanking the insert can be used to determine whether or not the insert is the correct molecular size. Insert specific primers can provide information on both the specificity and size of the insert DNA while the use of vector specific primers allows screening of multiple constructs simultaneously. Colony PCR can also be used to determine insert orientation. PCR amplification of the plasmid using an insert specific primer paired with a vector specific primer can be designed to produce an amplicon of a specific size only if the insert is in the correct orientation. In all experimental designs, presence or absence of a PCR amplicon and size of the product are determined by electrophoresis alongside a DNA size marker on an agarose gel.

Reference:
Bergkessel, M., & Guthrie, C. (2013). Colony PCR. *Methods in enzymology*, *529*, 299–309. https://doi.org/10.1016/B978-0-12-418687-3.00025-2

Materials

- Agar plate with bacterial stock
- MQ Water 1.1ul
- 10X Taq DNA Polymerase PCR Buffer 1ul
- Taq polymerase 0.1ul
- primers_F 1ul
- primers_R 1ul
- 2.5 mM dNTP 0.8ul

Procedure

Preparation

1. Thaw the **PCR buffer, dNTPs, and primers** before starting.

   Keep all thawed reagents on ice during preparation.
   DNA polymerase should be removed from the −20°C freezer only immediately before use and kept in an ice box at all times.

2. Prepare an ice bucket with sufficient ice and place the required reagents on ice.

3. Prepare an ice block/rack for the PCR tubes.

Preparation of Colony Suspension

4. Prepare the required number of **1.5-mL microcentrifuge tubes**. Add **100 µL of MQ water** to each tube. Using a sterile pipette tip, gently pick a single bacterial colony from the agar plate.Resuspend the colony thoroughly in the 100 µL MQ water by stirring the tip in the solution.

5. Vortex briefly to obtain a homogeneous **cell suspension**.

   The resulting suspension will be used directly as the **PCR template**.

PCR Master Mix Preparation

6. **Prepare the PCR master mix**

   The final PCR reaction volume is **10 µL**.
   Do not add the DNA template to the master mix. The template should be added separately after the master mix has been aliquoted.
   To compensate for pipetting loss and improve accuracy, prepare slightly more master mix than theoretically required.

7. Aliquot the appropriate volume of master mix into each labeled PCR tube.

8. Add the corresponding **cell suspension/template** to each PCR tube.

9. Mix gently and ensure that the final reaction volume is **10 µL per tube**.

run PCR

10. Briefly **spin down the PCR tubes** to collect all liquid at the bottom of the tubes and remove droplets from the tube walls.

11. Place the tubes into the PCR machine.

12. Select the appropriate PCR program according to the training protocol.

13. Set the reaction volume to **10 µL**.

14. For the relevant program parameter, select the **3-minute setting** as specified in the laboratory training protocol. Start the PCR run.