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Colony Screening of Agrobacterium

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We use this protocol and it's working

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Abstract

This protocol provides a rapid and reliable method for screening Agrobacterium colonies for the presence of a construct of interest using PCR. Crude lysates generated by alkaline lysis are suitable for direct PCR amplification, allowing high-throughput colony screening without genomic DNA purification.

Guidelines

Notes
1. Buffer A must be prepared **fresh** for optimal lysis efficiency.
2. Avoid transferring excessive bacterial material, which may inhibit PCR.
3. Inclusion of PVP and BSA improves amplification by reducing PCR inhibitors.

Materials

Reagents
1. LB agar plates with appropriate antibiotics
2. Transformed Agrobacterium colonies
3. Buffer A: 100 mM NaOH, 2% (v/v) Tween 20 (prepare **fresh**)
4. Buffer B: 100 mM Tris-HCl, 2 mM EDTA (pH = 2.0; **do not adjust**)
5. Buffer C: 10% (w/v) PVP-40, 1% (w/v) BSA
6. PCR reagents and gene- or construct-specific primers

Equipment
1. PCR tubes (0.2 mL)
2. Micropipettes and sterile tips
3. Thermal cycler with heated lid
4. Vortex mixer (optional)
5. Agarose gel electrophoresis system

Colony Preparation (Timing: ~5 minutes)

1 Identify Agrobacterium colonies of interest on selective LB plates.

2 Replica plate or streak a small amount of each colony onto a labeled grid plate for identification and later recovery.

Cell Lysis (Timing: ~15 minutes)

3 Transfer a small amount of colony into a PCR tube containing 50 µL Buffer A. The bacterial mass should be just visible.

4 Repeat for all colonies to be screened.

5 Mix thoroughly by pipetting, vortexing briefly, or flicking the tubes to ensure bacteria are fully suspended. (Critical Step)

6 Incubate tubes at 95 °C for 10 minutes in a thermal cycler with the heated lid on. (Critical Step)

Neutralization (Timing: ~5 minutes)

7 Add 50 µL Buffer B to each tube to neutralize the lysate.

8 Mix thoroughly by pipetting, vortexing, or flicking the tubes.

PCR Amplification (Timing: 1.5–2.5 hours)

9 Prepare 25 µL PCR reactions according to the Taq polymerase manufacturer's instructions, adding Buffer C at a 1:10 dilution and 1 µL lysate as template.

10 Run PCR using cycling conditions appropriate for the primers and target sequence.

Analysis (Timing: ~45 minutes)

11 Analyze PCR products by agarose gel electrophoresis.

12 Identify positive colonies and recover them from the replica or grid plate for downstream applications.

Protocol references

Xin Z, Velten JP, Oliver MJ, Burke JJ (2003). High-throughput DNA extraction method suitable for PCR. BioTechniques 34(4): 820–826.