Colony PCR

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Abstract

Use PCR to screen positive colonies after transformation of assembled DNA.

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Summary

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Materials

Reagents

- OneTaq® Hot Start Quick-Load® 2X Master Mix with Standard Buffer (NEB; M0488L)
- LB plate with antibiotic *OR* LB broth with antibiotics
- Bacterial culturing tubes
- Agarose

Procedures

At least two days prior to the day of colony PCR

1. Design and order primers. The goal is to distinguish positive clones (i.e., with insert(s)) from negative clones that might result from the plasmid used for backbone preparation, or from the carried-over template DNA used in insert preparation. There are two types of designs suggested:
    1. Have both forward and reverse primers anneal to the backbone, but will produce amplicons of different M.W., so one can distinguish positive clones from negative ones.
    2. Have one primer anneal to the backbone, while the other anneal only to the insert. Negative clones should not give a clear band, but a control condition is still suggested because primers could still bind to other parts of the bacteria genome.

Day 1

1. Use PCR table to determine the master mix recipe and the thermo-cycling program.
    - Reaction size: 8 or 10 μL per colony.
    - The following parameters are used for OneTaq HS-QL 2X MM Standard Buffer:

polymerase	denature temp. (degC)	initial denature time (s)	denature time (s)	annealing time (s)	extension temp. (degC)	extension rate (s/kb)	final extension time (m)	primer conc. (nM)
OneTaq HS-QL 2X MM Standard Buffer	94	240	20	30	68	60	300	200

2. Label PCR tubes and distribute master mix into each tube.

3. Pick colony, inoculate, and put some cells into the PCR reaction mix.

    - Can use marker to select & label the colonies.
    - Use pipette tip (P2 or P20) to pick colony. Gentle touch is good, and don't dip too deep to avoid picking up the gel.
    - Dip the tip into the reaction mixture, and shake/stir a little bit to deposit some cells into the solution. Keep the tip.
    - Inoculate the colony onto an LB plate (can incubate at 37 degC for later inoculation into liquid broth) or directly into liquid broth in a bacterial culture tube.

4. Prepare 1% agarose gel while waiting for the reaction to complete.

5. Run the product directly on the gel. No extra loading dye is needed if using the "Quick-Load" products.

6. If do colony PCR in the morning with a starter culture on an LB plate, then inoculate a liquid culture (for mini-prep) with positive clones at a later time of the day.

Reference and related files