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Colony PCR -- CHEM 584

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ABSTRACT

A quick way to screen transformants for an insert. This is faster and less expensive than doing a restriction digest.

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After you have colonies from a transformation

1 Draw grid on clean agar plate (use the appropriate antibiotic). You will use this to inoculate an overnight culture for a positive clone.

2 Select colonies to pick. Streak portion of colony to numbered sector and place the remainder in a correspondingly numbered PCR tube with 50 µL of autoclaved water.

3 Heat at 95 °C for 10 minutes. *** This can be done in PCR machine, a heat block, or a boiling water bath*

4 Spin solution on high for 10 minutes to pellet cellular debris, and remove 4 µL for PCR.

5 Setup and run the PCR reaction, then assess the results using agarose gel electrophoresis. Be sure to include a positive control (e.g., plasmid) if available. Negative controls are also useful if one is available.

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