

Sep 09, 2020

Colony PCR -- CHEM 584

DOI

<https://dx.doi.org/10.17504/protocols.io.bk5xky7n>

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Protocol Citation: Ken Christensen 2020. Colony PCR -- CHEM 584. protocols.io

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Protocol status: In development

We are still developing and optimizing this protocol

Created: September 09, 2020



Last Modified: September 09, 2020

Protocol Integer ID: 41879

Keywords: transformants for an insert, transformant, pcr, chem, restriction digest, insert

Abstract

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



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 μ L 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 μ L 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.