

PCR Screening of Transformation Colonies

Overview: This is a useful screening method for transformed colonies. It is nice because it has a built in control for the PCR reaction: even negatives (vector religation) will yield an ~200 bp amplification product (depending on where the primers are located on the plasmid). If you see nothing on the gel, the PCR reaction didn't work. Positives will be the size of the insert + 200 bp. A Master Mix greatly improves the consistency between samples.

Materials:

RedTaq DNA Polymerase	(available in Biochem stockroom)
Sigma Deoxynucleotide Mix	(also in stockroom)
10X RedTaq Buffer	(comes with RedTaq)
5' primer	(sequencing primer of vector)
3' primer	(" " " ")
Master Plate	(see below)

Day One:

After obtaining colonies from a transformation reaction, transfer each colony to a grid on a fresh plate that you have annotated. This avoids satellite colonies and gives more colony material to work with the next day. Incubate overnight.

Day Two:

For each isolate (and cells with vector alone), scoop a small bit of colony from the plate with a P200 pipette tip and add to 50 μ l of ddH₂O in a 1.5 ml eppendorf. Pipette up and down with the P200 to mix, then boil for 5 minutes. Spin for 5 minutes at topspeed in a desktop microfuge, 14,000g

Create a Master Mix for all PCR reactions to be done as follows:

For each reaction (multiply by number of reactions):

5 μ l	10X REDTaq PCR Buffer
1 μ l	Deoxynucleotide Mix
X μ l	5' Primer*
Y μ l	3' Primer*
2.5 μ l	REDTaq Polymerase
36.5-(X+Y) μ l	H ₂ O

*Use ~ 50 pmol of each primer per reaction. This can vary from 20-200 pmol based on prior experience with those primers.

To each PCR tube, add:

45 μ l	Master Mix
5 μ l	Water from centrifuged eppendorf (contains template)

Run standard 55° PCR program (30 cycles) unless you know you need different for your primer pair, and analyze 10-20 μ l of the completed reaction (straight from the tube) on an agarose gel.