Colony PCR

By Penn iGEM 2013 and Spencer Glantz

Goal: PCR Amplification directly from a colony rather than a DNA sample can be used to verify an insertion of a sizeable chunk of DNA in a vector by using primers that bind on either side of the insertion. This allows users to get an answer back right away (2-3 hours) and avoid time-cost of miniprepping and sequencing.

Comments:

(1) Colony PCR is even more specific if you use a combination of vector-specific and gene-specific primers. Always run a positive control colony PCR (some insert with known size in the same backbone) and a negative control colony PCR (backbone without insert).

(2) When picking colonies, use a sterile toothpick or sterile pipet tip. Angle the tip down towards a colony and just barely touch the colony. DO NOT scoop up LB agar since this inhibits the PCR reaction. Recall that ~10^9 cells are in each colony - just barely touching the colony and transferring a tiny fraction of it is more than sufficient for running a PCR.

(3) After touching the colony, place the tip in 20 uL of MilliQ Water/Sterile (in a clean PCR tube) and pipet up and down several times. Label the tube with a colony number and store the colony in water at 4C. Note that this colony will only last ~12 hours, so make sure ONLY pick colonies if you will be running and analyzed the colony-PCR that day. Put ONLY ONE colony in each PCR tube - do not store multiple colonies in the same water. If colonies are TOO small to pick one at a time reliably, then put the plate back in the incubator and allow the cells to grow some more. If colonies are too crowded, re-plate the cells at a lower concentration.

(4) The only difference between this and regular PCR is (A) your source of DNA template and (B) the first step of the Cycler protocol. Having initial denaturation step at 95C for 6 minutes lyses the bacterial cells that you add to the reaction. This releases plasmid DNA in the cell into the tube (as well as genomic DNA). Now it can be used as a template for PCR as usual.

Protocol:

<u>Reaction Mixture (20 uL):</u>

1 uL Template [Pick colonies and add a stab of each colony to 20 uL of water- see comments above]
10 uL 2x Taq Master Mix
1 uL FWD Primer (@10 uM)
1 uL REV Primer (@10 uM)
7 uL H2O

OR IF 2x Taq Master Mix is not available:

Reaction Mixture (20 uL):

1 uL "Template" [Pick colonies and add a stab of each colony to 20 uL of water- see comments above, take 1 uL of this colony-water suspension]
2 uL 10x Taq Reaction Buffer
1 uL FWD Primer (@10 uM)
1 uL REV Primer (@10 uM)
0.5 uL 10 mM dNTPs
0.5 uL Taq Polymerase
14 uL H2O

Cycler Protocol:

95°C for 6 minutes
30x [95°C for 30 sec, 55°C for 30 sec, 68°C for 1 kb/min]
68°C for 20:00 min
Hold at 4°C