

Sugden Lab.

Screening Colonies by PCR

Aliquot 30-50 μl of H_2O in wells of a microtiter dish corresponding to the number of colonies to be screened

Or

Make a master plate. Pick colony w/pipette tip and make a small patch on new plate. Dip the tip into PCR tube.

Prepare PCR mixture (20 μl RXNs): for 10 RXNs

20 μl 10x Taq Buffer

20 μl 2 mM dNTPs

50-100 pm of each primer

2 μl of Taq (10U)

158 μl H_2O

Aliquot 20 μl of PCR mix into PCR tubes

Pick colony with pipette tip or toothpick.

Number the colony on the plate or make a master plate with all the isolated colonies.

Dip the pipette tip or tooth pick in microtiter dish well and transfer 1 μl to PCR tube.

Run Touchdown PCR program: 30 seconds elongation time for every 500 bp.

Run 8-10 μl out on a gel.