COLONY PCR

This procedure can be used to amplify DNA from bacterial or yeast cells.

Materials:

10X taq buffer
10X dNTP mix
forward and reverse primers (1:10 dilution of 1μg/μl stock)
taq polymerase

Procedure: 2 PCR reactions

1st PCR:

Master Mix (per colony)

10X taq buffer	1.5μl
10X dNTP	1.5μl
forward primer	0.15μl
reverse primer	0.15μl
taq poymerase	0.15μl
ddH_2O	11.55μl
	15μl total

1. Prepare master mix for the number of colonies to be assayed (be sure to include positive and negative controls) in one tube. Keep on ice.
2. Dispense 15μl mix per well of a 96-well PCR plate. Keep on ice.
3. Twirl a sterile toothpick or pipet tip in an individual colony, make an "X" streak onto an agar plate on a grid location, and twirl the tip into one of the wells containing reaction mix. Repeat for as many colonies as required.
4. Overlay each well with 5-10μl of mineral oil to prevent evaporation.
5. Place plate in thermocycler and run:

94 C	5min	
94 C	30s	25 cycles
55 C	30s	25 cycles
72 C	1 min/kb	25 cycles
4 C	∝	

2nd PCR:

<u>Master Mix</u>

Same as first PCR except: 6.55µl water instead of 11.55µl (10µl per reaction).

1. Prepare master mix and dispense into a 96-well PCR plate corresponding to the plate from the first PCR.
2. Add 5µl from each well of the first plate into the corresponding well of the new plate, overlay with mineral oil, and repeat PCR reaction.
3. Run 5µl on agarose gel to check for amplification.

Comments:

This procedure is simple and very useful but takes a little practice so don't get discouraged!