**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**

1. **1st PCR:**
   - Master Mix (per colony)
   - 10X taq buffer 1.5μl
   - 10X dNTP 1.5μl
   - forward primer 0.15μl
   - reverse primer 0.15μl
   - taq polymerase 0.15μl
   - ddH₂O 11.55μl
   - Total 15μl

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:

   \[
   \begin{align*}
   94^\circ C & \quad 5\text{min} \\
   94^\circ C & \quad 30\text{s} \\
   55^\circ C & \quad 30\text{s} \\
   72^\circ C & \quad 1\text{ min/kb} \\
   4^\circ C & \quad \mu \\
   \end{align*}
   \]

   \(25 \text{ cycles}\)
2nd PCR:

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

1. Prepare master mix and dispense into a 96-well PCR plate corresponding to the plate from the first PCR.
2. Add 5 μl 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 5 μl 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!