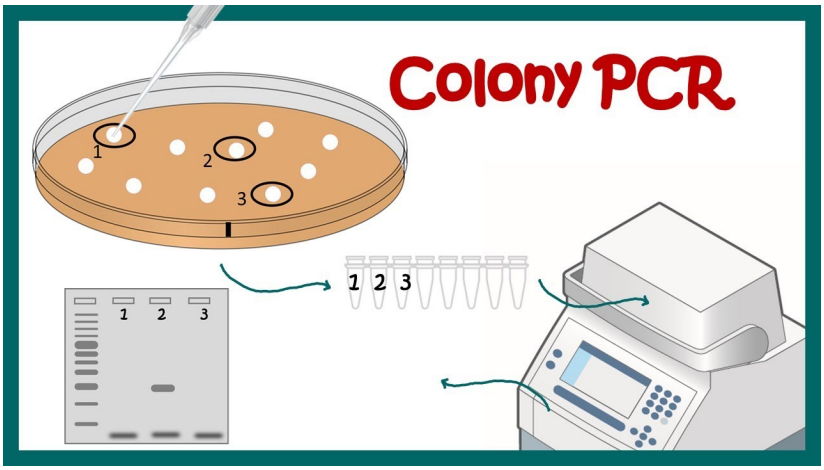
Bacterial colony PCR

Background:
Transformation of bacteria with transgenic DNA often requires verification beyond successful growth on a selection media to ensure that colonies growing on these plates are true transformant. One common approach is to test for the presence of exogenous genes included in the incorporated genetic material via PCR. While this procedure can be performed following extraction of DNA using chemical methods, e.g., alkaline based chemical extraction, kits and/or materials for these procedures can be costly and often require extra time to complete. For situations where simple verification of transformation is needed without any desire for further use of extracted plasmids, colony PCR followed by gel electrophoresis can be used as a quick and reliable alternative. Rather than requiring chemical or enzymatic lysis to yield high quality DNA, this protocol simply uses high temperatures to lyse cells, releasing enough of the cytoplasmic DNA for PCR.

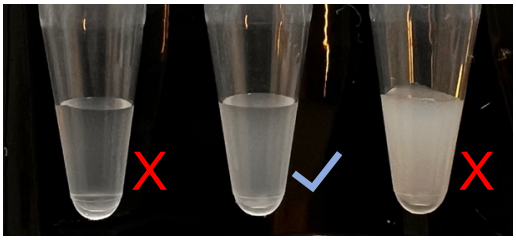


Materials:

- Sterile Milli-Q water
- 200 µL microcentrifuge tubes
- A thermocycler
- Microcentrifuge

Protocol:

- Suspend a small amount of your putative transformant colonies using 50 µL of Milli-Q water in a 200 µL microcentrifuge tube via pipetting.



    - Note: The liquid should appear cloudy but not be completely opaque.
- Incubate the tube in a thermocycler set to 99°C for 5 min.
    - Alternatively, if a thermocycler is not available, a 1.5 mL microcentrifuge tube can be used and placed in boiling water, but make sure to seal the lid closed.
- Immediately quick spin the tube at max speed for 30 sec, and use the resulting supernatant in place of the water and DNA template components of your PCR reaction.
- Proceed to **DreamTaq polymerase chain reaction protocol**.