Hot Start *Taq* DNA polymerase Colony PCR

. **Aim:**

- Identify the presence of inserts in colonies which passed the screening

Timeframe:

- Preparation: 15 minutes
- Wait-time: 90 min
- Overall: 1 hr 45 min

Materials:

- 10X Standard Taq Reaction Buffer
- 10 mM dNTPs
- 10 µM Forward Primer
- 10 µM Reverse Primer
- Hot Start Taq DNA Polymerase
- Template DNA
- Milli Q water

Procedure:

1. Combine the following components in a PCR tube and mix gently:
    - Use primers suitable for the sequence you are testing for

Component (concentration)	**Volume (µl)**
Standard Taq Reaction Buffer (10X)	5
dNTPs (mM)	1
Forward Primer (µM)	1
Reverse Primer (µM)	1
Hot Start *Taq* DNA Polymerase	0.25
Template DNA	From the colony
Milli Q water	Adjust volume to a total volume of 50 µl

2. Pick a colony with a pipette tip and dip the tip into the PCR tube. Use the same pipette tip to streak an agar plate and inoculate an overnight culture.

Reaction Conditions

Step	Temperature (˚C)	Time (s)
1	95	300
2	95	30
3	Tm*	60
4	68	1 min/kb
5	Repeat 2 to 4	32x*
6	68	300
7	4	Hold

3. Visualise the results through agarose gel electrophoresis.