Colony PCR using Backbone Specific Primers

Introduction

T7 primers will be used to amplify the region between the T7 promoter and terminator to determine whether our Insert has been correctly added using Golden Gate Cloning. Derived from NEB Protocol for OneTaq® 2X Master Mix with Standard Buffer.

Reagents

- T7 forward primer
- T7 reverse primer
- OneTaq 2X Master Mix
- Selected colony on a LB/Kan plate
- Milli-Q H_20

Equipment

- PCR tubes
- Thermocycler
- Pipette and tips

Procedure

1. Add 1.5 µL of Milli-Q H_20 to a sterile PCR tube.
2. Using a sterile pipette tip, remove a colony from the selected LB/Kanamycin plate.
3. Add the following amounts of reagents to a sterile PCR tube:

Reagent	Amount
2.5 µM T7 forward primer	5 µL
2.5 µM T7 reverse primer	5 µL
OneTaq 2X Master Mix	12.5 µL
Milli-Q H_20	2.5 µL
Total	**25 µL**

4. Use the following PCR thermocycler program:

Step	Temperature	Time
Initial Denaturation	94°C	30 seconds
Repeat the following 3 steps for 27 cycles		
Denaturation	94°C	30 seconds
Annealing	41°C	40 seconds
Elongation	68°C	1 minute/kb
Final Elongation	68°C	5 minutes
Hold	4°C	∞