Colony PCR

1. Pick small amount of colony and dilute in 25µL of ddH20.
2. Use 1µL of this dilution to carry out the PCR amplification below.
   * Note: This is done on ice.

Forward primer- 5'GGCTCAGAATTCGCGGCCGCTTCTAGAG3' (Prefix +6)
Reverse primer- 5'CTGGACCTGCAGCGGCCGCTACTAGTA3' (Suffix +6)

Table 1: Assemble reaction in a thin walled tube in the following order:

Component	25µL rxn	Final conc.
ddH_2O	19.75 µL	-
10x taq buffer	2.5 µL	1x
10mM dNTPs	0.5 µL	200µM
Forward Primer	0.5 µL	200µM
Reverse Primer	0.5 µL	200µM
Template DNA*	1 µL	-
Taq polymerase	0.25 µL	0.25U/µL

3. Run with the described thermocycler program

Table 2: Cycling instructions

Cycle Step	Temperature (°C)	Time (sec)	Cycles
Initial Denaturation	95	180	1
Denaturation	95	10	30
Annealing	56	20	
Extension	72	120	
Final Extension	72	600	1

Stop	4	∞	1