

Colony PCR

1. Pick small amount of colony and dilute in 25 μ L of ddH₂O.
2. Use 1 μ L 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	25 μ L rxn	Final conc.
ddH ₂ O	19.75 μ L	-
10x taq buffer	2.5 μ L	1x
10mM dNTPs	0.5 μ L	200 μ M
Forward Primer	0.5 μ L	200 μ M
Reverse Primer	0.5 μ L	200 μ M
Template DNA*	1 μ L	-
Taq polymerase	0.25 μ L	0.25U/ μ L

3. Run with the described thermocycler program

Table 2: Cycling instructions

Cycle Step	Temperature ($^{\circ}$ C)	Time (sec)	Cycles
Initial Denaturation	95	180	1
Denaturation	95	10	30
Annealing	56	20	
Extension	72	120	
Final Extension	72	600	1



Lethbridge iGEM 2017

Stop	4	∞	1
------	---	----------	---