University of Southampton iGEM 2009 Protocol:
Colony PCR

Colony PCR protocol:

Component	Amount/ µL
5x green Gotaq buffer	2
10mM dNTP mix	1
VF2 Primer	0.2
VR Primer	0.2
H_2O	6.55
GOTaq	0.05
DNA sample	1

1. The DNA sample is prepared by diluted a colony in 50 µL of water.
2. Prepare the solutions in the order shown above. Generally a solution mix is created for all the samples, which is then separated out so that there is 9 µL per sample and then the DNA is added.
3. Vortex the solutions and then centrifuge for 20 seconds
4. Place the vials into the PCR machine and chose the following PCR programme:

1.	95°C	2 minutes
2.	95°C	1 minute
	53°C	1 minute
	72°C	1 min (per Kb)
3.	72°C	5 minutes
4.	4°C	∞

[Step 2 is repeated for 30 cycles.]

5. Analyse the samples by Gel electrophoresis.
6. The samples can be stored at -20 °C until they are used.