University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	PCR Protocol		
	Description:	For amplification of DNA. Taken from the Q5 High-Fidelity 2X Master Mix instructions. PCR Video		
	Trigger:			
Last updated	Name:	Elizabeth Brookes	Date:	15.08.14
You will need	Time:	2 hours		
	PPE:	Standard equipment (lab coat, gloves, etc.)		
	Equipment:	○ Thermocycler/PCR machine ○ Ice box		
	Materials:	○ 4 x PCR tubes ○ 1mL of Q5 High-Fidelity 2X Master Mix ○ 10µL of 10µM Forward Primer ○ 10µL of 10µM Reverse Primer ○ 8µL of Template DNA ○ 72µL of Nuclease-Free Water		

Step 1	For a standard 50µL reaction, mix the following into a PCR tube **on ice** and repeat for all 4 tubes: ○ 25µL of Q5 High-Fidelity 2X Master Mix ○ 2.5µL of 10µM Forward Primer ○ 2.5µL of 10µM Reverse Primer ○ 2µL of Template DNA ○ 18µL of Nuclease-Free Water to make up to 50µL
Step 2	Gently mix the reagents. If required, collect all liquid to the bottom of the PCR tubes with a quick spin.
Step 3	Place the PCR tubes in the PCR machine and close the lid.
Step 4	Program the PCR machine: 1. Press <Create>. 2. Press <Start>. 3. Using the arrow keys and number pad, program the following temperatures and times: • Initial denaturation: 98°C, 5 minutes. • 30 cycles of: • Denaturation: 98°C, 30 seconds. • Annealing: 57°C, 30 seconds. • Extension: 72°C, 30 seconds. • Final extension: 72°C, 5 minutes. • Hold: 4°C, infinite time. 4. Press <Start>. 5. Enter reaction volume (50µL) when prompted. 6. Press <Start> to begin the reaction.
Step 5	Wait for the PCR machine to finish (approximately 40 minutes). The screen will show which stage the machine is up to.
Step 6	Collect the tubes from the machine. If they are not to be used immediately, place in the -20°C freezer. Otherwise, place in ice.

Version history	*V1 replaced by the Q5 High-Fidelity 2X Master Mix protocol to create V2.*