

**University of Southampton iGEM 2009 Protocol:**

High Fidelity PCR:

(Invitrogen Platinum® Pfx)<sup>[1]</sup>

High Fidelity PCR:

Component	Amount/ $\mu\text{L}$
<i>5x colourless Gotaq buffer</i>	10
<i>10mM dNTP mix</i>	1
<i>VF2 Primer</i>	1.5
<i>VR Primer</i>	1.5
<i>DNA sample</i>	1
<i>Pfx Polymerase</i>	0.5
<i>H<sub>2</sub>O</i>	34.5

1. Prepare the solutions in the order shown above
2. Vortex the solutions and then centrifuge for 20 seconds
3. Place the vials into the PCR machine and chose the following PCR programme:

1.	95°C	2 minutes
2.	95°C	1 minute
	55°C	1 minute
3.	72°C	1 min (per Kb)
	72°C	5 minutes
4.	4°C	$\infty$

*[Step 2 is repeated for 30 cycles.]*

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

[1] Invitrogen Platinum® Pfx DNA Polymerase Protocol