Part BBa_K314110 http://parts.igem.org/Part:BBa_K314110
Kit Plate 1 Well 1A

Thawed 100µL of DH5alpha from -80C on ice.

To get DNA:

1. Suck up 10µL Milli-Q
2. Pipette into plate well
3. Leave on shaker
4. Ensure the well you want is red
5. Pipette out DNA into DH5alpha

Followed transformation protocol, and spread plates. The two plates were then left in the 37˚C incubator overnight. Plates moved into the cold room in the morning. Set up 3 overnights from each plate, all in 5mL LB+Chl.

The next day the overnights were miniprepped using the QIAGEN kit.

When the primers arrived from the Warwick team 100ng/mL stock solutions were made up.

Reaction mixture of 20µL in total were made up according to the following set up:

Solution	Volume (µL)
Milli-Q	12.4
5xHF Buffer	4
10µM dNTPs	0.4
10µM Forward Primer	1
10µM Reverse Primer	1
Template	1
Phusion	0.2

The tubes were then placed into the PCR machine and run according to the following protocol:

Stage	No. of Cycles	Temperature (°C)	Time (min)
Initial denaturation	1	98	2
Denaturation		98	0.5
Annealing	30	62 or 72	0.5
Extension		72	0.5
Final extension	1	72	5

The annealing temperatures were calculated using http://tmcalculator.neb.com/#!/

12 different primers were sent to us from Warwick in order to PCR the part in its entirety and then to cut the part into sections. These sections also were PCRed to obtain slight modifications to the ends.

No.	Purpose	Sequence
1	Forward primer for BBa_K314110	ACG CGC CCT GTA GCG G
2	Reverse primer for BBa_K314110	AAT TGT AAA CGT TAA TAT TTT GTT AAA ATT CG
3	Forward primer for sequence 1 with EcoRI cut site	GAA TTC GCG TGG GCG ACG CGC CCT GTA GCG G
4	Forward primer for sequence 1 with EcoRI cut site	CAT ATG CTT TGA TTG TGC GAG A TTA GAG CTT GAC GGG GAA
5	Reverse primer for sequence 1 with NdeI cut site and Linker 2	CAT ATG CAA TGC TAC CTT ACC G TTA GAG CTT GAC GGG GAA
6	Forward primer for sequence 2 with EcoRI cut site	GAA TTC GCG TGG GCG ATC GGG GGC TCC CTT T
7	Forward primer for sequence 2 with NdeI cut site and Linker 2	CAT ATG CAA TGC TAC CTT ACC G ATC GGG GGC TCC CTT T
8	Reverse primer for sequence 2 with PstI cut site	CTG CAG GCG TGG GCG CAC TAT TAA AGA ACG TGG ACT CC

9	Reverse primer for sequence 2 with NdeI cut site and Linker 3	CAT ATG GTC GGA ACT CGA TCG G CAC TAT TAA AGA ACG TGG ACT CC
10	Forward primer for sequence 3 with NdeI cut site and Linker 1	CAT ATG CTT TGA TTG TGC GAG A GAC TCT TGT TCC AAA CTG GAA C
11	Forward primer for sequence 3 with NdeI cut site and Linker 3	CAT ATG CTT TGA TTG TGC GAG A GAC TCT TGT TCC AAA CTG GAA C
12	Reverse primer for sequence 3 with PstI cut site	CTG CAG GCG TGG GCG AAT TGT AAA CGT TAA TAT TTT GTT AAA ATT CG

In order to simplify the process, we assigned labels to each tube so therefore the part could be easily identified:

Forward Primer	Reverse Primer	Label	Annealing Temperature (°C)
1	2	A	62
3	4	B	72
3	5	C	72
6	9	D	72
7	8	E	72
10	12	F	72
11	12	G	72

2% agarose gel was then made up using 1g of agarose and 50mL of TBE buffer. The gel was left to set and covered with TBE buffer. The samples were mixed with 5µL of loading dye and loaded into the gel according to the layout below. The ladder used was a 50bp ladder as one part was 455bp long and the others were 150bp.

Ladder	A	B	C	D	E	F	G

From the photo of the gel it can be seen that G failed therefore the PCR was repeated. The second PCR was successful so the parts were then sent off to Warwick to complete their oligo assembly.