Polymerase Chain Reaction (PCR)

Materials

- 10 mM dNTP mix
- 10 µM Forward primer
- 10 µM Reverse primer
- DNA template (10 pg to 1 µg)
- Water, nuclease free
- Thin walled PCR tubes

For High Fidelity enzyme amplification:

- High Fidelity Enzyme Mix (5U/µL)
- 10X High Fidelity PCR Buffer with 15 mM $MgCl_2$

For Taq DNA Polymerase amplification:

- 10X Taq Buffer with $(NH_4)_2SO_4$
- 25 mM $MgCl_2$
- Taq Polymerase (5U/µL)

Apparatus

- Thermocycler (Applied Biosystems)

Method

1. After thawing and vortexing the solutions (except the enzyme solution), add the components in a sterile PCR tube in this following order and keep it on ice.

10X High Fidelity PCR Buffer with 15 mM $MgCl_2$	5 µL	Taq Polymerase (5U/µL)	5 µL
10 mM dNTP mix	1 µL	10 mM dNTP mix	1 µL
10 µM Forward primer	1 µL	10 µM Forward primer	1 µL
10 µM Reverse primer	1 µL	10 µM Reverse primer	1 µL
DNA template Genomic DNA	10 pg- 1 µg	25 mM $MgCl_2$	4 µL
High Fidelity Enzyme Mix	0,5 µL (2.5 U)	DNA template Plasmid DNA	10 pg- 1 µg
Water, nuclease free	to 50 µL	Taq DNA Polymerase	0.5 (2.5U)
		Water, nuclease free	to 50 µL

2. Spin the PCR tubes to collect all drops and immediately place the PCR tubes in the thermocycler, start the PCR reaction.

3. Thermal cycling protocol:

   Obs: Because the primers used have more than 12 pb (including restriction sites) that don't anneal with the template in the first PCR steps, we decided to add 10 cycles with a lower temperature to favor the best annealing.

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	3 min	1
Denaturation	94-95	30 s	10
Annealing	*Tm_1-5	30 s	
Extension	72	1 min	
Denaturation	94-95	30 s	25
Annealing	**Tm_2-5	30 s	
Extension	72	1 min	
Final Extension	72	10 min	1
Keep	4	∞	

*Tm_1 = Melting temperature considering just the annealing primer region. Theoretically calculated by the IDT tools.
**Tm_2= Melting temperature considering the restrictions sites

4. Load a 5 µL sample and run an agarose gel electrophoresis (**see the section Agarose Gel Electrophoresis and DNA Gel Purification**) [http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf].
5. If you see just one band in the gel of the expected size, you can purify the PCR product using Wizard SV Gel and PCR Clean-Up System kit (Promega). But if you see more than one band, you need to run a new gel with total sample, excise the band of interest and then purify (**see the section Agarose Gel Electrophoresis an DNA Gel Purification**) [http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf].
6. Use Nanodrop Spectrometer (Thermo Scientific) to quantify the DNA fragment.
7. Store at -20°C.