Q5 DNA Polymerase PCR

Aim:

- Amplifying sequences using NEB Q5 DNA polymerase

Timeframe:

- Preparation: 15 minutes
- Wait-time: 90 min
- Overall: 1hr 45 min

Materials:

- Q5 DNA polymerase
- Q5 Reaction Buffer (5x)
- dNTP mix (10 mM each)
- Primers (10 µM each)
- DNA template (1 ng/µL)
- Milli Q water

Procedure:

1. Add the following to a 0.2 mL tube (1x 50 µL reaction):

Component (concentration)	Volume (µl)
Q5 reaction buffer (5x)	10
Forward primer (10 µM)	1
Reverse primer (10 µM)	1
dNTP (10 mM each)	1
Milli Q water	Adjust so that final reaction volume is 50 µl.

2. Ensure components are thoroughly mixed then add:

Component (concentration)	Volume (µl)
Template (1 ng/µL)	1
Hot start Q5	0.5

3. Ensure all components are well mixed

Reaction Conditions

4. Typical reaction conditions are shown below

Step	Temperature (˚C)	Time (s)	
1	98	30	
2	98	10	
3	Tm*	10	Tm will be dependent on the specific primer set used in the reaction. Online calculators are available such as http://tmcalculator.neb.com/#!/
4	72	Et*	Extension times depend on length of desired amplicon. NEB typically recommends 20-30s per kb of amplified DNA.
5	Repeat 2 to 4	32x*	Number of PCR cycles are a key determinant in yield and biases. Higher number of cycles tend to give higher yield but also correlate with higher number of errors. 32 cycles generally give good yields for reactions from plasmid DNA.
6	72	5’00"*	Polishing step. This is still widely used and is thought to give the polymerase the opportunity to finish any incomplete extensions.
7	20*	Hold	Q5 Hot-start is not active at temperatures below 4°C and therefore does not require the reaction to be stored at 4°C until processing. This is also true for a number of other modern (post-2012) DNA polymerases.