PCR AMPLIFICATION USING PHUSION DNA POLYMERASE

Aim: To reproduce (amplify) selected sections of DNA (inserts A1, A2, B1, B2, C1, C2, E1, E2).

Material:

- Template DNA
- Phusion HF buffer (5X)
- Phusion DNA Polymerase
- dideoxy nucleotide triphosphate mix 10 mM (dNTPs)
- 0.2 ml thin Wall Tubes PCR tubes
- EDTA disodium salt dehydrate
- Forward primer
- Reverse primer
- Nuclease free water

Protocol:

Primers:

Forward 5' GCT CGT GGA TCC GAA TTC G 3'

Reverse 5' GGC CGC TAC TAG TAC AAA AAA C 3'

	Tube	Control	Control	Control	Control
Phusion HF Buffer (5X)	10 µl	10 µl	10 µl	10 µl	10 µl
dNTPs (10 mM)	1 µl	1 µl	1 µl	1 µl	1 µl
Template DNA	<250 ng	<250 ng	<250 ng	<250 ng	<250 ng
10 µM forward Primer	2.5 µl		2.5 µl		2.5 µl
Nuclease free water	To 50 µl	To 50 µl	To 50 µl	To 50 µl	To 50 µl
Phusion DNA Polymerase	0.5 µl	0.5 µl	0.5 µl	0.5 µl	0.5 µl
Total	50 µl	50 µl	50 µl	50 µl	50 µl

1. In a 0.2 ml tube, set up the following reaction:

2. Set up the following cycles in a PCR machine
   - Initial denaturation: 98°C for 2 min
   - 30 cycles:
     1. 94°C for 30 sec
     2. 45°C - 72°C for 30 sec depending on your annealing temperature
     3. 72°C for 30-60 sec per kb
     4. Final extension: 72°C for 5 min.