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PCR (Toehold) Fragment Protocol for a 25ul reaction

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Protocol status: Working
We use this protocol in our group and it is a working PCR protocol.

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Keywords: fragment protocol, pcr, reaction, protocol

Materials

Q5 reaction buffer- 5ul
dNTPs- 1ul
Primer mix- 2.5ul

- (1.25ul per primer)

Q5 High Fidelity Polymerase- .25ul
Template DNA- *** varies 1000ng<

- Lambert iGEM: 1.5ul

H20- to 25ul

1 Pipette all reaction agents into pcr tube

2 put pcr tube in thermal cycler and set the annealing temperature to 55 degrees celsius

3 Annealing temperatures vary depending on DNA

4 Set elongation time to 30 seconds

5 1 min/2kb for Q5 High Fidelity → may vary