Sep 25, 2019

Q5 Polymerase PCR Cloning

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1 Works for me dx.doi.org/10.17504/protocols.io.7pqhmmw

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MATERIALS

NAME	CATALOG #	VENDOR
Q5 High-Fidelity PCR Kit - 200 rxns	E0555L	New England Biolabs

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Water refers to sterilized deionized water		
Q5 Reaction Buffer Pack - 6.0 ml	B9027S	New England Biolabs
dNTP Mix, PCR Grade (200 µl)	201900	Qiagen
Q5 High-Fidelity DNA Polymerase - 100 units	M0491S	New England Biolabs

1 Prepare 6-reaction PCR mix by adding together **244.2 µl** deionized water, **66 µl** Q5 reaction buffer, **6.6 µl** dNTP mix, and **3.3 µl** Q5 DNA polymerase

Water refers to sterilized deionized water

Q5 Reaction Buffer Pack - 6.0 ml
by New England Biolabs
Catalog #: B9027S

dNTP Mix, PCR Grade (200 µl)
by Qiagen
Catalog #: 201900

Q5 High-Fidelity DNA Polymerase - 100 units
by New England Biolabs
Catalog #: M0491S

2 Add **1 µl** 1 of template DNA and **0.5 µl** of primer pairs into specified PCR tube

3 Aliquot **48 µl** of PCR mix into each PCR tube

4 Load PCR tubes in thermocycler

Protocol is set to run at **98 °C** for **00:00:40** followed by **00:00:30** for the desired annealing temperature before **72 °C** for x amount of time (depending on fragment length). The cycle repeats for 34 rounds starting with **00:00:10** at **98 °C** followed by annealing and elongation, before incubating at **72 °C** for **00:05:00** and then **4 °C** infinitely. In this PCR run, the elongation time and annealing temperature depends on specific fragment length and primer annealing temperature.

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