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Q5 Polymerase PCR Cloning

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MATERIALS

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⊗ Q5 High-Fidelity PCR Kit - 200 rxns **New England Biolabs Catalog #E0555L**

STEP MATERIALS

⊗ Q5 Reaction Buffer Pack - 6.0 ml **New England Biolabs Catalog #B9027S**

⊗ dNTP Mix, PCR Grade (200 µl) **Qiagen Catalog #201900**

⊗ Q5 High-Fidelity DNA Polymerase - 100 units **New England Biolabs Catalog #M0491S**

⊗ Water refers to sterilized deionized water **Contributed by users**

OPEN ACCESS

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Protocol status: Working
 We use this protocol and it's working

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- 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 **Contributed by users**

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

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

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

2 Add 1 µL 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

Note

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.