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## Q5 Polymerase PCR Cloning

DOI

<https://dx.doi.org/10.17504/protocols.io.7pqhmmw>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** September 25, 2019

**Last Modified:** September 12, 2023

**Protocol Integer ID:** 28112

**Keywords:** q5 polymerase pcr cloning, pcr

## Materials

### MATERIALS

 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

## Protocol materials

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

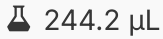
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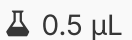
- 1 Prepare 6-reaction PCR mix by adding together  244.2  $\mu\text{L}$  deionized water,  66  $\mu\text{L}$  Q5 reaction buffer,  6.6  $\mu\text{L}$  dNTP mix, and  3.3  $\mu\text{L}$  Q5 DNA polymerase

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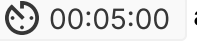
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- 2 Add  1  $\mu\text{L}$  1 of template DNA and  0.5  $\mu\text{L}$  of primer pairs into specified PCR tube
- 3 Aliquot  48  $\mu\text{L}$  of PCR mix into each PCR tube
- 4 Load PCR tubes in thermocycler

#### Note

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