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Q5 PCR DNA Amplification (Protocol for Q5® High-Fidelity 2X Master Mix)

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

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ABSTRACT

This protocol is for PCR with Q5® High-Fidelity 2X Master Mix

GUIDELINES

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed prior to use.

MATERIALS

MATERIALS

Q5 High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog**
#M0492L

Q5 High-Fidelity 2X Master Mix - 100 rxns **New England Biolabs Catalog**
#M0492S

Reagent	25 μ l Reaction	50 μ l Reaction	Final concentration
Q5 High-Fidelity 2X Master Mix	12.5 μ l	25 μ l	1X
Forward Primer (10 μ M)	1.25 μ l	2.5 μ l	0.5 μ M
Reverse Primer (10 μ M)	1.25 μ l	2.5 μ l	0.5 μ M
Template DNA	variable	variable	< 1,000 ng
Nuclease-Free Water	to 25 μ l	to 50 μ l	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

SAFETY WARNINGS

PCR reagents are classified as non-hazardous. Follow the specified handling and disposal considerations included in the safety data sheets provided by the manufacturer.

BEFORE START INSTRUCTIONS

Please note that protocols with Q5 High-Fidelity DNA Polymerase may differ from protocols with other polymerases. Conditions recommended below should be used for optimal performance.

- 1 Add all components in a 250 µL tube making up to a 25 or 50 µL reaction. If performing various PCR with different templates, a Master Mix is recommended to be done.

When doing a Master Mix always add Q5 enzyme last, then vortex the solution briefly and centrifugate before use.

'Pro-Tip': Use DMSO 3% or 5X GC enhancer as PCR additives when amplifying particularly difficult or high GC amplicons.

- 2 Gently mix the PCR reactions and transfer the tubes to a thermocycler. Thermocycling conditions for a routine PCR:

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
*50–72°C	10–30 seconds	
72°C	20–30 seconds/kb	

Final Extension	72°C	2 minutes
Hold	4– 10°C	

*The use of the NEB_{Tm} Calculator is highly recommended.