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Q5 PCR DNA Amplificaiton (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 µl Reaction**	**50 µl Reaction**	**Final concentration**
	Q5 High-Fidelity 2X Master Mix	12.5 µl	25 µl	1X
	Forward Primer (10 µM)	1.25 µl	2.5 µl	0.5 µM
	Reverse Primer (10 µM)	1.25 µl	2.5 µl	0.5 µM
	Template DNA	variable	variable	< 1,000 ng
	Nuclease-Free Water	to 25 µl	to 50 µl	

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.

Troubleshooting

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

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.