LEISH BIO

VENI Hotstart high-fidelity Taq Polymerase 2X

50 mM TAPS-HCl
(pH 9.3 @ 25°C),
100 mM KCl,
4 mM MgCl2,
2mM DTT
400 µM dNTPs
10% glycerol
2mg/ml BSA
Hotstart high-fidelity Taq Polymerase 100U/ml

Reaction Setup:
VENI Hot Start High-Fidelity 2X Master Mix is inhibited at room temperature, allowing flexible reaction setup. It is not necessary to set up the reaction on ice.
All components should be mixed prior to use.

Component	25 µl Reaction	50 µl Reaction	Final Concentration
VENI HOTSTART High-Fidelity 2X Master Mix	12.5 µl	25 µl	1X
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	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.Transfer PCR tubes to a PCR machine and begin thermocycling.

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VENI HOTSTART 2X Master Mix does not require a separate activation step. Standard VENI HOTSTART HIGHFIDELITY cycling conditions are recommended.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	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	

*Gradient PCR should be performed to identify the optimal annealing temperature.

General Guidelines:

1. Template:
   Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	AMOUNT
DNA Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–10 ng

2. Mg^{++} and additives:
   The VENI HOTSTART High-Fidelity Master Mix contains 2.0 mM Mg^{++} when used at a 1X concentration. This is optimal for most PCR products generated with this master mix.

3. Deoxynucleotides:
   The final concentration of dNTPs is 200 µ M of each deoxynucleotide in the 1X VENI HOTSTART HIGHFIDELITY Hot Start High-Fidelity Master Mix. VENI HOTSTART HIGHFIDELITY Hot Start High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil-containing primers or templates.

4. VENI HOTSTART HIGHFIDELITY Hot Start High-Fidelity DNA Polymerase concentration:
   The concentration of VENI HOTSTART HIGHFIDELITY Hot Start High- Fidelity DNA Polymerase in the VENI HOTSTART HIGHFIDELITY Hot Start High-Fidelity 2X Master Mix has been optimized for best results under a wide range of conditions.

5. If genomic DNA are used at template, Veni hotstart high-fidelity Taq Polymerase kit are preferred even the GC content doesn't exceed 60%.

6. Amplification of long products:
   When amplifying products > 6 kb, it is often helpful to increase the extension time to 1min/kb.

7. PCR product:
   The PCR products generated using VENI Hot Start High-Fidelity 2X Master Mix have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, the DNA should be purified prior to A-addition, as VENI HOTSTART HIGHFIDELITY Hot Start High-Fidelity DNA Polymerase will degrade any overhangs generated.