q•Hot 5X PCR Master Mix with 1.5, 2.0, 2.5, or 3.0 mM $MgCl_2$

for in vitro use only

Protocol

1. Thaw and briefly centrifuge all components
2. Prepare your reactions using the Recommended Reaction Mix
3. Briefly centrifuge your samples
4. Run PCR using Recommended Cycling Settings

Recommended Reaction Mix

Component	Conc.	Amount	Final Conc.
q•Hot MM	5X	4 µL	1X
Primer, F[1]	10 pmol/µL	0.2 - 0.6 µL	100 - 300 nM
Primer, R[1]	10 pmol/µL	0.2 - 0.6 µL	100 - 300 nM
Template DNA[2]		2 - 400 ng	0.1 - 20 ng/µL
Nuclease-free water		variable	
Total		20 µL	

1 To maximize assay sensitivity use lowest concentration possible without compromising reaction efficiency. Doubling the reverse primer concentration may improve performance. Further optimization can be tested using final concentrations of 100 - 400 nM.

2 Genomic DNA, plasmid DNA, or cDNA can be used as template. For cDNA up to 10% of the final reaction may be cDNA (i.e. for a 20 µl PCR reaction, use up to 2.0 µl of undiluted cDNA).

Recommended Cycling Settings

Cycle Step	Temp (°C)	Time	Cycles
Initial denaturation	95	15 min	1
Denaturation	95	10 - 20 sec	25 - 30
Annealing	T_M - 4[1]	30 - 60 sec	
Extension	72	20 sec - 4 min	
Final extension	72	5 -10 min	1

[1] *Set annealing temperature to be 4°C less than T_M of primers or set it to 54-66°C initially.*

Estimating primer melting temperature:
For primers containing less than 25 nucleotides, T_M= 4 (G + C) + 2 (A + T), where G, C, A, T represent the number of respective nucleotides in the primer. If primers contain more than 25 nucleotides use specialized software to calculate T_M.

q•Hot 5X Master Mix Order Information

Cat. No.	Vol. (mL)	No. Rxns 20 µL each	Mix Composition: q•Hot Taq	q•Master Mix Buffer	5X $MgCl_2$ Conc.	Final $MgCl_2$ Conc.	dNTPs	Blue Dye	Yellow Dye	Nuclease free H_2O
QHMM15-S	200 µL	50	✓	5X Buffer-with Proof-Reading Enzyme	7.5 mM	1.5 mM	2 mM each of dATP, dCTP, dGTP, & dTTP	Migration equivalent to 3.5 - 4.5kb DNA fragment	Migration rate in excess of primers in 1% agarose gel: <35-45 bp	1 mL
QHMM15-01	1 mL	250								4 mL
QHMM20-S	200 µL	50			10 mM	2.0 mM				1 mL
QHMM20-01	1 mL	250								4 mL
QHMM25-S	200 µL	50			12.5 mM	2.5 mM				1 mL
QHMM25-01	1 mL	250								4 mL
QHMM30-S	200 µL	50			15 mM	3.0 mM				1 mL
QHMM30-01	1 mL	250								4 mL

Description

q•Hot 5X Master Mix is a ready-to-load cocktail for PCR. It contains all the components necessary for DNA amplification in PCR. Simply add nuclease-free water (supplied with the kit), template and primers.

- **Ready-to-load.** The mix also contains two tracking dyes so that your PCR product can subsequently be directly loaded during gel electrophoresis.
- **High Fidelity.** q•Hot Taq and an additional enzyme create both the 5'→3' exonuclease activity as well as the 3'→5' proofreading activity.

Eco-friendly shipping & storage

- Shipped at ambient temperature without ice to reduce packaging waste
- Use within 2 weeks of arrival or store at -20°C

Some applications of this product are covered by patents issued to parties other than qARTA Bio, and may require a license which is not provided by the purchase of this product. User should obtain a patent license if appropriate.

User Protocol

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