Feb 21, 2019

In devel.

PCR Reaction Optimization

Version 2

Forked from WarmStart LAMP®

Kenneth Schackart[1]

[1]University of Arizona

dx.doi.org/10.17504/protocols.io.yezftf6

Kenneth Schackart

ABSTRACT

How to run nucleic acid amplification using the Thermo scientific PCR Master Mix kit.
Each reaction produces 50 µL.
For the original protocol, look at: PCR Master Mix Manual.pdf .

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

GUIDELINES

Gloves must be worn at all times.
Use all precautions to avoid contamination when making reaction mixture.
Always pipette mix each reagent in aliquot before pipetting.

MATERIALS

NAME	CATALOG #	VENDOR
DNAse/RNAse free distilled water	10977023	Thermo Fisher Scientific

MATERIALS TEXT

- 70% ethanol solution in DI water
- RNAway
- Thermo Scientific Master Mix (2x)
- PCR primer mix (25 µM)
- Target DNA or RNA
- RNAse free water

Prepare Work Area

1 Spray entire work area with 70% EtOH including pipettes, tip holder used for holding PCR tubes, and work surface. Wipe with a paper towel.

2 Spray entire work area with RNAway.

Gather Materials

3 Take styrofoam container to Marley 527 (directly across from Marley 509) and fill halfway with ice.

4 Set PCR tube holder on ice, and allow to cool for **00:03:00** .

5 Transfer Master Mix, primers, RNAse free water, and target tubes from freezer to PCR tube holder on ice.

6 Allow reagents to thaw on ice

7 Carefully obtain (2) 0.2 mL PCR tubes. Label one with "NTC" and the other "TARG". These will be your reaction vessels.

To avoid contamination when grabbing PCR tubes, only touch the outside of tubes. Avoid touching the inside of the caps of other tubes in this process. This is critical.

8 Vortex mix all reagents for approximately **00:00:05** .

9 Spin down all reagents for approximately **00:00:05** .

Prepare Reaction

10 Add the following to your two tubes:

	Target	NTC
PCR Master Mix	25 µL	25 µL
Primer Mixture (25 µM)	0.2 - 2.0 µL	0.2 - 2.0 µL
Target	1 µL	-
Water	to 50 µL	to 50 µL
Total	50 µL	50 µL

Various primer concentrations are to be optimized. Start with 1.0 µL (0.5 µM).

11 Vortex mix the reaction mixture.

12 Spin down reaction mixture.

Run LAMP Reaction

13 Place reaction vessels into thermocycler.

14 Turn on thermocycler

15 Hit PROCEED to select a reaction cycle.

16 Scroll using the '<' and '>' keys to get to PCR.
Begin using the following program:

Step	Temperature	Time	Number Cycles
Denaturation	95°C	3 min	1
Denaturation	95°C	30 s	30
Annealing	58°C	30 s	
Extension	72°C	60 s	
Final Extension	72°C	10 min	1

17 Press PROCEED to begin