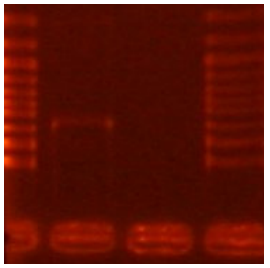
Feb 23, 2019 Version 3

PCR Reaction Optimization V.3



Version 1 is forked from WarmStart LAMP®

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Kenneth Schackart[1]

[1]University of Arizona

Yoon Lab

Kenneth Schackart
University of Arizona

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

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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.

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

MATERIALS

DNAse/RNAse free distilled water **Thermo Fisher Scientific Catalog #**10977023

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

Troubleshooting

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.

> Note
>
> 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 PCR 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 EPCR.
Begin using the following program:

	Step	Temperature	Time	Number Cycles
	Denaturation	95ºC	3 min	1

	Denaturation	95ºC	30 s	30
	Annealing	58ºC	30 s	
	Extension	72ºC	60 s	
	Final Extension	72 º	10 min	1

17 Press PROCEED to begin