Jan 11, 2022 Version 3

General Taq PCR Master Mix -- CHEM 384/584 V.3

DOI
https://dx.doi.org/10.17504/protocols.io.b3npqmdn

Ken Christensen[1]

[1]Brigham Young University

Ken Christensen
Brigham Young University

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Protocol Citation: Ken Christensen 2022. General Taq PCR Master Mix -- CHEM 384/584. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b3npqmdnVersion created by **Ken Christensen**

Protocol status: In development
We are still developing and optimizing this protocol

Created: January 11, 2022

Last Modified: January 11, 2022

Protocol Integer ID: 56751

Keywords: general taq pcr master mix, most pcr master mix, necessary pcr components except the template, necessary pcr component, pcr product, pcr, direct loading of pcr product, master mix format simplifies workflow, agarose gel for electrophoresi, sample handling, primer, electrophoresi, agarose gel

Abstract

2X PCR Master Mixes are convenient to use as they include all the necessary PCR components except the template and primers. Most PCR Master Mixes also include agarose gel running dyes and a density reagent that allows direct loading of PCR products on an agarose gel for electrophoresis. The master mix format simplifies workflows and sample handling; simply add primers, template, and water and then begin PCR.

Guidelines

Storage: -20°C for long-term storage. 4°C for short-term storage (up to 3 months). (Note) If used frequently, store at 4°C ; the activity of the Master Mix may decrease with repeated freezing and thawing. Gently mix well before use and centrifuge briefly.

Application:
- DNA amplification by PCR
- Colony PCR

PCR Products: Since most PCR products amplified Taq PCR Master Mix have an A overhang added at 3′-termini, the obtained PCR product can be used directly for cloning into a TA cloning vector. Additionally, it is possible to clone the product in a blunt-end vector after blunting and phosphorylation.

Dye Migration during Electrophoresis: Check individual manufacturers information on the migration of the included running dyes.

Setup Reaction

1 To a 25 µL aliquot of a 2X Taq PCR Master Mix (e.g. TaqDog, or Sapphire Amp), add template (10-20 µl cleared lysate for colony PCR or 20-50 ng of purified DNA for typical PCR), forward and reverse primers to a final concentration of [M] 200 nanomolar (nM). Adjust final volume to 50 µL with nuclease free water or autoclaved water.

	A	B
	2X Master Mix	25 ul (pre-aliquoted and stored in the freezer)
	Template	10-20 ul of bacterial lysate or 20-50 ng purified DNA
	Forward Primer	1 ul of 10 uM primer dilution
	Reverse Primer	1 ul of 10 uM primer dilution
	ddH2O	to a final volume of 50 ul

Run Reaction

2 followed by 30 cycles of 98°C, 5 sec; 55°C, 5 sec; and 72°C, 40 sec.

	A	B	C
	Initial denature	94C	1 minute
	Denature	94C	30 seconds
	Anneal	55C	30 seconds
	Extension	72C	1 min/kb
	Repeat steps 2-4		25-40x
	Final extension	72	minutes

	A	B	C
	Cool	4C	Until cancelled

A typical thermocycling program for a PCR for amplicons less than 1 kb is 1 minute. For longer amplicons, adjust the program to 1 minute/kb seconds for the extension and final extension times.