BIONEER
Innovation • Value • Discovery

Taq PCR MasterMix

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I. Introduction

Taq PCR MasterMix is a convenient PCR master mix containing *Taq* DNA polymerase, dNTPs, reaction buffer, tracking dye, and stabilizer. The Master mix retains its activity for over six month in -20°C freezer. *Taq* PCR MasterMix is available with or without tracking dye, depending on your application. If purchased with tracking dye, reactions can be loaded on agarose gels without adding loading buffer.

II. Application

- Routine PCR
- Primer extension
- TA cloning
- Gene sequencing

III. Contents

Component	Concentration
Taq DNA polymerase	1 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 μM
Reaction buffer, (with 1.5 mM $MgCl_2$)	1 X
Stabilizer and tracking dye[1)]	Trace

1) *Taq* PCR MasterMix is premixed with Xylene Cyanol. Xylene Cyanol migrates at approximately 4 kb on a 1% agarose gel.

IV. Storage

For long term storage, *Taq* PCR MasterMix should be stored at -20°C upon receipt and is stable until the date stated on the label.

V. Additional Required Materials & Devices

- Thermal cycler for PCR
- Calibrated micropipette
- Sterilized micropipette tips

VI. General Precautions

- Wear gloves throughout experimental set-up to prevent contamination.
- Store positive materials, such as samples and control templates, in separate freezer from the kit.
- Add templates to the reaction mixture in a hood or a spatially separated facility.

VII. Protocol

1. Thaw template DNA and primers before use.
2. Mix the Taq PCR Master Mix by vortexing briefly and dispense 25 μl into each PCR tube.
3. Add template DNA and primers into the PCR tubes containing the Master Mix.

♦ Recommended amount of template and primers

Reaction volume		50 μl reaction
Template DNA	Bacteriophage Plasmid DNA	0.1 pg-500 ng
	Total genomic DNA	1 ng-1 ug
Primer	Forward primer (10 pmol/μl)	1-5 μl
	Reverse primer (10 pmol/μl)	1-5 μl

4. Add distilled water into the *Taq* PCR MasterMix tubes to a total volume of 50 μl.
5. Mix the reaction mixture by vortexing and spin down either by using Bioneer's *ExiSpin* Vortex/Centrifuge (15 second vortex on high followed by 5 second spins at 1,500 rpm x 4 cycles) or by pipetting up and down several times and then briefly spinning down.
6. Perform the reaction under the following conditions.

• In case of Standard PCR

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	1 min**	1 cycle
Denaturation	95°C	30 sec	25-35 cycles
Annealing*	45-65°C	30 sec	
Extension	72°C	30 sec-1 min/kb	
Final extension	72°C	Optional. Normally 3~5 min	1 cycle

* The optimal annealing temperature depends on the melting temperature of the primers.
** 5 minutes when human DNA is used as a template.

• In case primer's Tm value is higher than 65°C or PCR product size is bigger than 5 kb.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	1 min*	1 cycle
Denaturation	95°C	30 sec	25-35 cycles
Annealing/extension	68°C	1 min/kb	
Final extension	68°C	Optional. Normally 3~5min	1 cycle

* 5 minutes when human DNA is used as a template.

7. Maintain the reaction at 4°C after the completion of amplification. It is recommended to store the sample at -20°C until use.
8. Load 5 μl of the reaction mixture directly on agarose gel to analyze the PCR products. If purchased with tracking dye, the reaction can be directly loaded onto the gel.

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VIII. Experimental Data

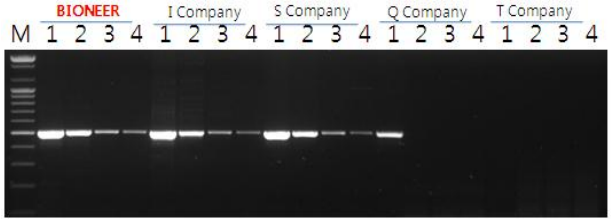

Figure 1. Comparison of PCR amplification efficiency between *Taq* PCR MasterMix from Bioneer and other suppliers' PCR master mix. The cycling conditions for *Taq* PCR MasterMix were 95°C for 5 min, 35 cycles of 95°C for 20 sec, 55°C for 20 sec, and 72°C for 30 sec. PCR reactions using other suppliers' PCR master mix were performed according to each supplier's protocol.
Target Gene: IRGC (Immunity-related GTPase family, cinema)
Lane M : 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1 : 10 ng human DNA Lane 2 : 1 ng human DNA
Lane 3 : 100 pg human DNA Lane 4 : 10 pg human DNA

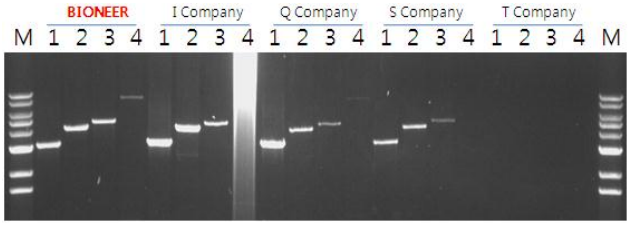

Figure 2. Comparison of PCR amplification of long targets between *Taq* PCR MasterMix from Bioneer and other suppliers' PCR master mix. The cycling conditions for *Taq* PCR MasterMix were 95°C for 5 min, 30 cycles of 95°C for 20 sec, 65°C for 20 sec, and 68°C for 4 min. PCR reactions using other suppliers' PCR master mix were performed according to each supplier's protocol.
Lane M : 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)
Lane 1 : 3 kb fragment (human tumor protein p53 gene)
Lane 2 : 4 kb fragment (human beta globin region)
Lane 3 : 4.5 kb fragment (human DNA cross-link repair 1A gene)
Lane 4 : 8 kb fragment (human hemoglobin epsilon 1 gene)

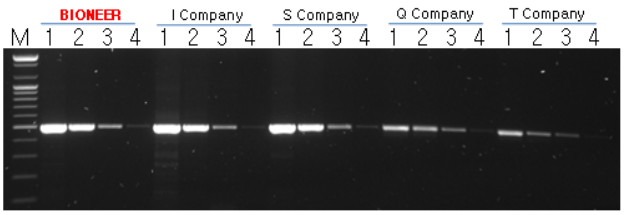

Figure 3. Comparison of PCR amplification efficiency between *Taq* PCR MasterMix from Bioneer and other suppliers' PCR master mix. The cycling conditions for *Taq* PCR MasterMix were 95°C for 5 min, 35 cycles of 95°C for 20 sec, 55°C for 20 sec, and 72°C for 30 sec. PCR reactions using other suppliers' PCR master mix were performed according to each supplier's protocol.
Target Gene : Human myc.
Lane M : 100 bp DNA Ladder (Bioneer, Cat. No. D-1030)
Lane 1 : 10 ng of human total cDNA
Lane 2 : 1 ng of human total cDNA
Lane 3 : 100 pg of human total cDNA
Lane 4 : 10 pg of human total cDNA

IX. Troubleshooting

● No product or low yield

Possible Cause	Recommendation
Insufficient template	Increase the amount of template used in PCR. High quality templates are essential for amplification of long targets. Check the purity of template or repeat purification of template.
$MgCl_2$ concentration is too low	Increase the amount of $MgCl_2$ concentration in steps.
Primer design is not optimal	Design alternative primers.
Cycle conditions are not optimal	Reduce the annealing temperature. Increase the number of cycles.
Amplification of GC-rich genes	Add 0.5-1 M Betaine or 2-8% DMSO.

● Product is multi-banded or smeared

Possible Cause	Recommendation
Annealing temperature is too low	Increase annealing temperature according to primer length.
Incorrect extension time	Adjust the length of time for the extension step according to the size of the expected PCR product.
Primer design is not optimal	Design alternative primers.
Problems with template	Check the concentration, storage conditions, and quality of template.
Too many cycles	Reduce the number of cycles.

● Products in negative control lane(s)

Possible Cause	Recommendation
Carry-over contamination	Set up PCR reactions in an area separate from that used for PCR product analysis.

X. Ordering Information

Cat. No.	Description
K-2609	*Taq* PCR MasterMix, 2.5 ml of 2X Master mix solution
K-2610	*Taq* PCR MasterMix, 25 ml of 2X Master mix solution
K-2601	*Taq* PCR MasterMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube, 96 tubes
K-2602	*Taq* PCR MasterMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 20 µl reaction/tube,480tubes
K-2603	*Taq* PCR MasterMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction/tube, 96 tubes
K-2604	*Taq* PCR MasterMix, 0.2 ml thin-wall 8-strip tubes with attached cap, 50 µl reaction/tube,480tubes