Quick Taq™ HS DyeMix

DTM-101 100 reactions

Store at -20°C

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

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FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

Quick Taq™ HS DyeMix is a Taq-based 2× master mix PCR reagent that contains an electrophoresis dye (BPB; bromophenol blue) and anti-Taq antibodies for hot start PCR. This reagent contains all components for PCR except primers and template DNA. This reagent shows specific and efficient amplification. The amplified products can be directly loaded in the wells of agarose or acrylamide gels.

Features

-As this reagent contains bromophenol blue (BPB) as an electrophoresis dye; the PCR products can be analyzed directly with an agarose or acrylamide gel.

-This reagent exhibits greater PCR performance than conventional rTaq DNA polymerase.

-This reagent contains anti-Taq antibodies for hot start PCR. Hot start technology realizes highly specific and sensitive PCR.

-This reagent is stable for at least three months at 4°C. No decrease in reaction efficiency is observed following 30 freeze-thaw cycles.

-This reagent is suitable for a colony-direct PCR (see [6], Example 2).

[2] Components

This reagent includes the following components for 100 reactions, 50 µL total reaction volume:

2× Quick Taq™ HS DyeMix	1.25mL× 2

*In the case of the long-term storage (>3 months), this reagent should be stored at -20°C.

[3] Primer Design

Primers should be 22–35 bases long, with a melting temperature (Tm) > 60°C.

[4] Analysis and Cloning of PCR products

-As this reagent contains bromophenol blue (BPB) as an electrophoresis dye and by adjusting its relative density, the PCR products can be applied directly to an agarose or acrylamide gel.

-The PCR products can be cloned using general TA cloning technology.

-The PCR products can be used as templates for sequencing after an appropriate treatment.

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[5] Protocol

1. Standard reaction

Before preparing the reaction mixture, the master mix solution should be completely thawed.

	Reaction volume		Final concentration
	50 µL	20 µL	
Autoclaved, distilled water	X µL	X µL	
2x Quick Taq™ HS DyeMix	25 µL	10 µL	1 ×
10 pmol /µL Primer #1	1.0 µL	0.4 µL	0.2 µM
10 pmol /µL Primer #2	1.0 µL	0.4 µL	0.2 µM
Template DNA	Y µL	Y µL	Genomic DNA -200 ng /50 µL Plasmid DNA -50 ng /50 µL *E. coli* colony
Total	50 µL	20 µL	

*For the PCR reaction, thin-wall tubes are recommended. A total reaction volume of 50 µL is also recommended.

2. PCR cycle conditions

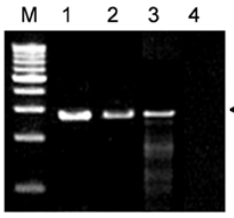
3-step cycle		
Predenaturation:	94°C, 2min.	
Denaturation:	94°C, 30sec.	25-40 cycles
Annealing:	(Tm-5) °C, 30sec.	
Extension:	68°C, 1min. /kb	

2-step cycle		
Predenaturation:	94°C, 2min.	
Denaturation:	94°C, 30sec.	25-40 cycles
Extension:	68°C, 1min. /kb	

[6] Examples

Example 1. Amplification of the human p53 gene (2.9 kb)

The human p53 gene (2.9 kb) was amplified using 50 ng of human genomic DNA.Quick Taq™ HS DyeMix successfully amplified the targets.



◀ 2.9 kb

M : 1kb Ladder
1: Quick Taq HS DyeMix
2: rTaq DNA polymerase (hot start)
3: rTaq DNA polymerase
4: Taq Master Mix (A company)

<Cycling condition>
94 °C 2 min.
94 °C 30 sec.
60°C 30 sec.
68°C 3 min.
40 cycles

Template: Human genomic DNA 50 ng/ 50 µl reaction
Forward Primer : AATGGATGATTTGATGCTGTCCC
Reverse Primer : ATAAGAGCTCCCAAGACTTAG
*Final concentration 0.2 µM

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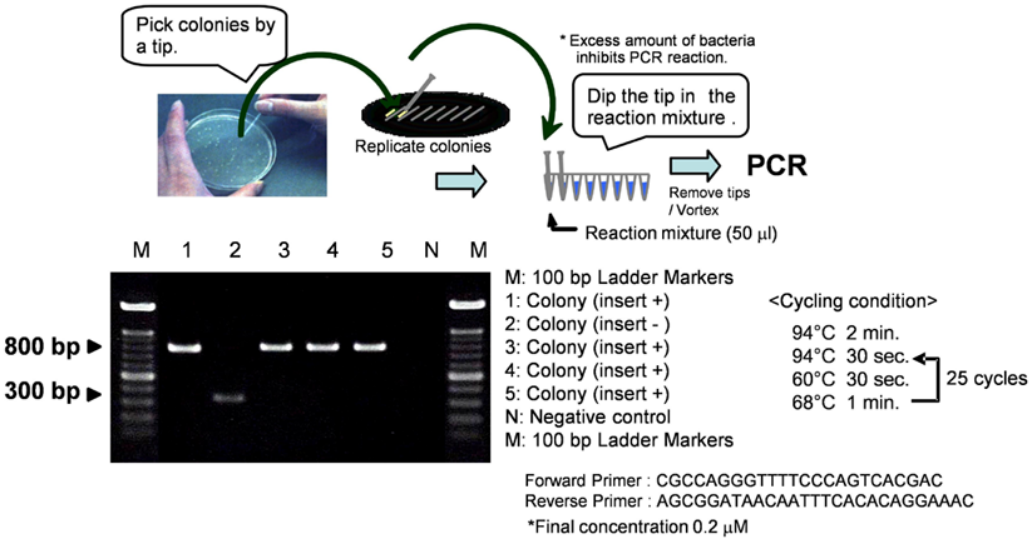
Example 2. Insert amplification by a colony-direct PCR

The inserts were amplified using Quick Taq™ HS DyeMix with universal primers from *E. coli* DH5α colonies bearing pTA2 plasmid (insert size: 500 bp). Quick Taq™ HS DyeMix successfully and efficiently amplified all targets.



[7] Trouble shooting

Symptom	Cause	Solution
No PCR product / low yield	Cycling conditions are not suitable.	Increase the number of cycles by 2-5 cycles.
	Primer is not good.	Check the quality of the primers.
		Redesign the primers.
	Template DNA is of insufficient quality and/or quantity.	Check the quality of the template DNA.
		Increase the amount of the template DNA
	Too much sample	Excessive amounts of bacterial cells may inhibit amplification. Decrease the sample volume.
Smearing / Extra band	Cycling conditions are not suitable.	Decrease the number of cycles by 2-5 cycles.
	Primer concentration is not appropriate	Optimize the primer concentration to around 0.1-0.2 µM.
	Annealing temperature is too low.	Optimize the annealing temperature to around 55°C -65°C.
	Primer is not good.	Check the quality of the primers.
		Redesign the primers.
	Too much template DNA	Reduce the amount of template DNA

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