Suitable for difficult targets such as GC-rich targets.

High efficient Real-time PCR Master Mix

New !!

TOYOBO
Ideas & Chemistry

KOD SYBR® qPCR Mix

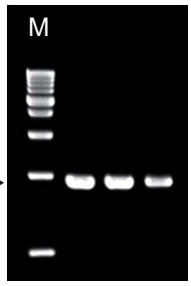
High GC　Long　Crude

New suggestions from TOYOBO

Flexible primer setting

Q: Should we redesign the primers less than 200 bp for real-time PCR?

➡ A: If the target is to be less than 2 kb, you can use conventional primers.

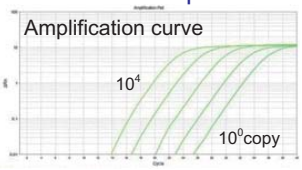


Electrophoresis analysis

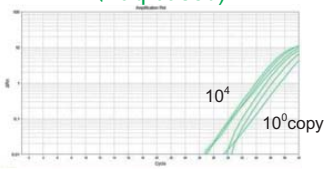
Same primers

Quantitative amplification (~ 2 kb)

KOD SYBR® qPCR Mix



Conventional master mix (Taq based)



Enables PCR fragment length polymorphism analysis using melting curve assay

Amplification of difficult targets, such as promoter regions

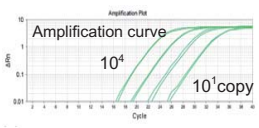
Q: Our target contains a promoter region that forms a secondary structure.

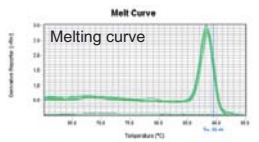
➡ A: This reagent is suitable for a difficult target such as this.

CCCGCCCGAGAGAGTGACTCTCACGAGAGCCGCGAGAGTCAGCTTGGCCAAT
CCGTGCGGTCGGCGGCCGCTCCCTTTATAAGCCGACTCGCCCGGCAGCGCAC
CGGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGCCATTTTTTGTCTAACC
CTAACTGAGAAGGGCGTAGGCGCCGTGCTTTTGCTCCCCGCGCGCTGTTTTTC
TCGCTGACTT

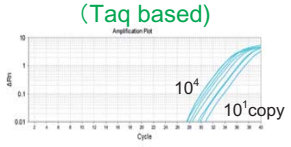
Target: GC content：64%, 219 bp：
Homo sapiens telomerase RNA (TR) gene, promoter and complete squence
Template: human genomic DNA
Primer: (from a paper using the ChIP technique)：
Red: primer sequnce

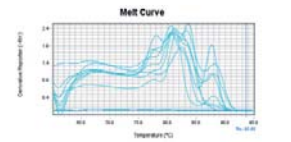
KOD SYBR® qPCR Mix





Conventional master mix (Taq based)





Conventional primer sets can be used. Primers can be set more flexibly.

■ Concept of KOD SYBR® qPCR Mix

KOD SYBR® qPCR Mix was developed based on the unique properties (high efficiency, robustness) of KOD DNA Polymerase to enhance the convenience and versatility of the SYBR® Green I assay.

● Comparison of properties with the conventional master mix

	Conventional (Taq based)	KOD SYBR® qPCR Mix
Enzyme	Taq DNA Polymerase	KOD DNA Polymerase [exo(-) mutant]
Amplification size	70 ~ 150 bp (Maximum: 300 bp)	70 bp ~ 2 kb
High GC Targetgets	Susceptible	Not susceptible
Inhibition by impurities in crude samples	Susceptible	Not susceptible (Suitable for amplification from crude specimens)

■ Applicable real-time cyclers

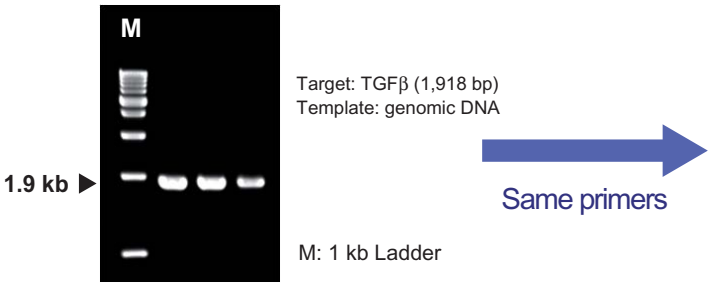
The Rox reagent is supplied separately for use in various cyclers.

Maker	Real-time cycler
Applied Biosystems	ABI PRISM® 7000/7700
	Applied Biosystems 7300
	Applied Biosystems 7500/7500 Fast
	Applied Biosystems 7900HT
	Applied Biosystems StepOne™/StepOnePlus™
Bio-Rad/MJ	MiniOpticon™
	CFX96 Touch™
Roche Diagnostics	LightCycler® 1.x / 2.0
	LightCycler® Nano
	LightCycler® 480
Agilent Technologies	Mx3000P / Mx3005P / Mx4000
TaKaRa	Thermal Cycler Dice® Real Time System
BioFlux	LineGene

KOD SYBR® qPCR Mix is based on KOD exo(-) DNA Polymerase, a 3' -5' exonuclease deficient KOD DNA Polymerase, and a buffer optimized for it. The reagent exhibits the following special features.

Feature1 Long target amplification (~2 kb)

● Quantitative amplification can be achieved using long targets, up to 2kb. Therefore, primers for conventional PCR can be used. This aids primer design.



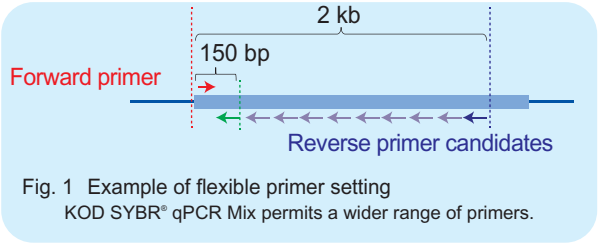


Fig. 1 Example of flexible primer setting
KOD SYBR® qPCR Mix permits a wider range of primers.

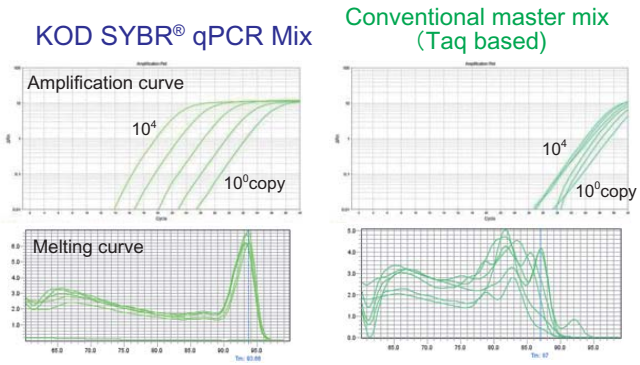
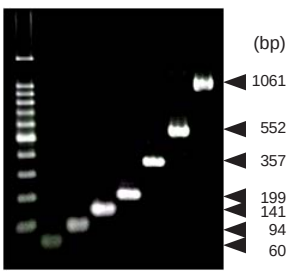
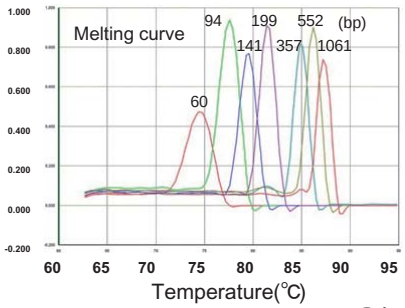


Fig. 2 Long target amplification [ABI StepOnePlus™]
A real-time PCR assay was performed using primers for conventional PCR. KOD SYBR® qPCR Mix exhibited quantitative amplification.

● This reagent enables PCR fragment length polymorphism assay in one tube using melting curve analysis. In this analysis, the differences in Tms between the fragments should be > 3°C (optimally >5°C).



Electrophoresis analysis
M: 100 bp Ladder



● Relationship between target size and Tm

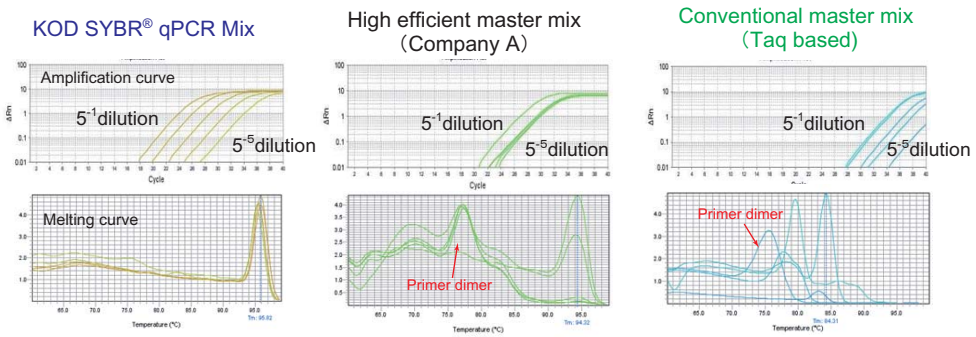
Target size (bp)	Tm [Predicted] (℃)	Tm [Mesured] (℃)
60	72	74
94	76	77.5
141	79	79
199	82	82
357	84	85
552	86	86
1,061	88	87

Fig.3 Comparison of the calculated and measured melting temperatures (Tm) [ABI 7500]

Various targets of human β-actin gene were amplified and analized.

Feature 2 Efficient for GC-rich targets

KOD SYBR® qPCR Mix is able to amplify high GC targets (GC content : >70%) quantitatively.



Target: IGF2R (189 bp / GC content: 83%)
Template: HeLa cDNA
cDNA was synthesized using ReverTra Ace® qPCR RT Kit (Code No.FSQ-101) with total RNA from HeLa cells.

Fig.4 Amplification of GC rich targets [ABI StepOnePlus™]

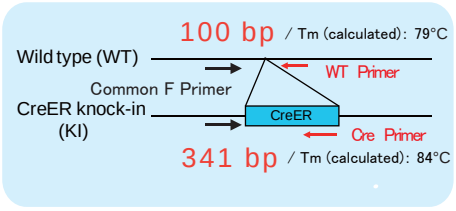
GC rich targets (GC content: >70%) were amplified using various real-time PCR master mixes. The targets were amplified successfully and quantitatively using KOD SYBR® qPCR Mix.

Feature 3 Amplification from crude samples

KOD SYBR® qPCR Mix is able to amplify targets from crude samples (e.g. mouse tail and plant lysates).
The reagent can be used for genotyping and SNP analyses.

Genotyping using mouse tail lysates

Alkaline lysis method



Target: Region contains the targeting site (Cre ER)
(WT: 100 bp, KI: 341 bp)

Template: Mouse tail lysate (alkaline lysis method)

Composition: Primer: F: WT: Cre= 0.2: 0.2: 0.67 µM (final)
Rox (0.1x)
Mouse tail lysate 2 µl / 20 µl

Cycling condition:

98°C, 2 min

98°C, 10 sec
60°C, 10 sec
68°C, 30 sec*] 40 cycles

Melting curve analysis

* Extension time should be set 30 sec/ 500 bp

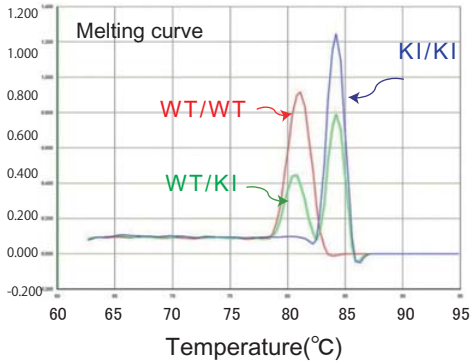
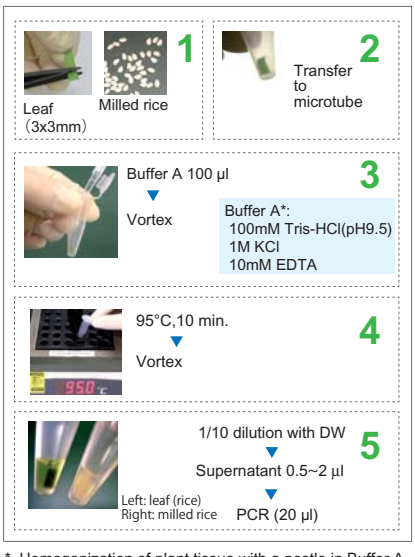


Fig. 5 One-tube mouse genotyping using melting curve analysis [ABI 7500]

Primers were designed so that the amplicons were 100 bp (Tm: 79°C) and 341 bp (Tm: 84°C) for wild-type and knock-in, respectively. All genotypes were successfully detected.

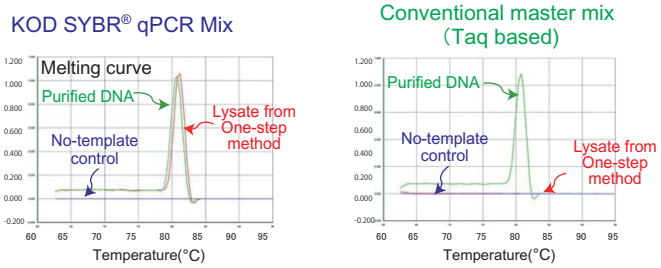
Amplification from plant lysates

One-step method



* Homogenization of plant tissue with a pestle in Buffer A enhances the efficiency. In this case, heating is not necessary.

(A) Short target: Rice rbcL(257bp)



(B) Long target: Rice/tobacco rbcL(1.3kb)

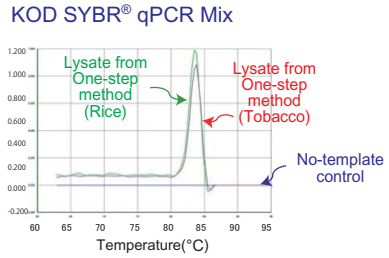


Fig. 6 Amplification from plant lysates [ABI 7500]

(A) Targets were amplified from crude lysates and genomic DNA from rice leaves using KOD SYBR® qPCR Mix.

(B) The long targets (1.3 kb) were amplified successfully from crude lysates from rice and tobacco leaves.

SNP (Single nucleotide polymorphism) analysis by ASP (Allele specific primer)-PCR

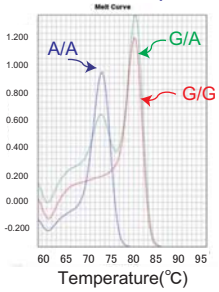
The melting temperature of a PCR product can be increased by 3–5°C by adding a GC tail at the 5' end. This method permits one tube ASP (Allele specific primer)-PCR for SNP analysis. In this case, SNP and mismatch sites were set at the second and third bases from 3' end of the primer, respectively.

Whole blood 2 µl
↓
Suspend in 48 µl DW
↓ 5 µl
20 µl reaction

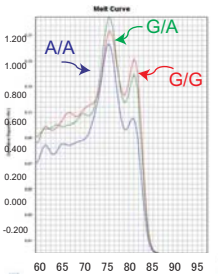
Fig.7 One-tube ASP-PCR analysis using whole blood specimen.
[ABI 7500 Fast real-time PCR system]

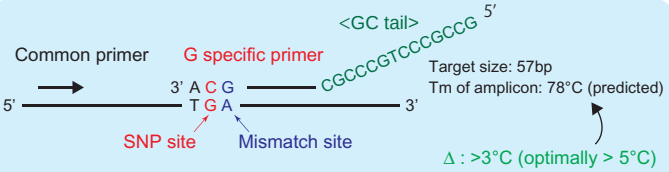
SNP analysis was performed with a GC tailed primer from whole blood samples using KOD SYBR® qPCR Mix, high efficient master mix (Company A) and conventional Taq based master mix. All types of SNP were successfully determined by KOD SYBR® qPCR Mix. No signal was detected using the Taq-based conventional master mix.

KOD SYBR® qPCR Mix



High efficient master mix
(Company A)





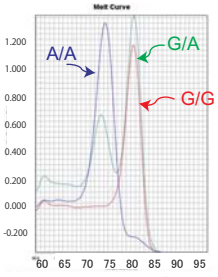
Common primer : 5'-GTACGGGCTGCAGGCATAC-3'
G specific primer : 5'-GCCGCCCTGCCCGCCACACTCACAGTTTTCACTGCA-3'
A specific primer : 5'-CCCACACTCACAGTTTTCACTATA-3'

*In this case, the bases at mismatch sites can be any bases except T which corresponds to A. Actually, G and A were used in this case.
*This method can be applied to various kinds of ASP-PCR. The sequence of the GC tail can be flexible. Various kinds of GC tails have been reported.

Cotton swab
with oral
↓
Suspend in 200 µl DW
↓ 5 µl
20 µl reaction

Fig. 8 One-tube ASP-PCR analysis using cotton swab an oral sample.
[ABI 7500 Fast real-time PCR system]

KOD SYBR® qPCR Mix



Product name	Package	Storage	Code No.	Price
KOD SYBR® qPCR Mix ・KOD SYBR® qPCR Mix ・50× ROX reference dye*	3 X 1.67 ml (200 rxn)**	-20℃	QKD-201	

* 50× ROX reference dye is supplied separately.
** Based on 50 µl reaction.