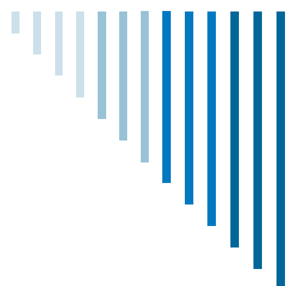
TaKaRa



PRODUCT NOTE

TAKARA BIO EUROPE

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TaKaRa Ex Taq R-PCR

Improved conditions using Smart Cycler (Cepheid) and LightCycler (Roche) Systems

TaKaRa Ex Taq R-PCR allows more accurate and higher sensitivity real time PCR. An innovative buffer system improves denaturation, thus minimizing non-specific amplification derived from primer dimer formation.

We have compared the Takara enzyme for Real Time PCR on several human genes, which could not be successfully amplified through the conventional method, using two different instruments dedicated to Real-Time PCR: Cepheid's Smart Cycler System and Roche's LightCycler.

Reaction condition

Basic Reaction Mixture

TaKaRa Ex Taq HS (5 units/µl)	0.25 µl
10× Basic R-PCR Buffer	2.5 µl
Mg^{2+} Solution (250 mM)	0.3 µl
dNTP Mixture (each 10 mM)	0.75 µl
SYBR Green I (3000-fold dilution)	2.5 µl
Forward Primer (15 µM)	0.5 µl
Reverse Primer (15 µM)	0.5 µl
Template (< 30 ng)*	2 µl
dH_2O	15.7 µl
Total**	25 µl

New Reaction Mixture

TaKaRa Ex Taq HS (5 units/µl)	0.25 µl
5×Real Time PCR Buffer (2.1)	5 µl
Mg^{2+} solution (250 mM)	0.5 µl
dNTP Mixture (each 10 mM)	0.75 µl
SYBR Green I (3,000-fold dilution)	2.5 µl
Forward Primer (10 µM)	0.5 µl
Reverse Primer (10 µM)	0.5 µl
Template (< 30 ng)*	2 µl
dH_2O	13 µl
Total**	25 µl

* Templates are the cDNAs synthesized with M-MLV Reverse Transcriptase using total RNA extracted from human culture cells and oligo dT primer. The cDNA used as templates result from reverse transcription of 0 pg (Negative Control), 1 pg, 10 pg, 100 pg, 1 ng, and 10 ng total RNA.

** When using LightCycler, the reaction mixture was prepared to the total volume of 20 µl.

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Condition of thermal cycling

95°C	10 seconds		Hot Start
95°C	5 seconds	45 cycles	PCR
55°C	20 seconds		
72°C	15 seconds		
60°C ↓ 95°C	0.2°C/sec.		Melting curve

Results

Graphs presented below were obtained with each instruments. Primers used are :

(1) Primer 147 (for RAB32, member RAS oncogene family)

(2) Primer 150 (for protein kinase, DNA-activated, catalytic polypeptide).

Each figure exhibits same template amplification with TaKaRa Ex Taq HS in Basic or Improved R-PCR conditions. Amplification curve and Melting curve are shown for each pair of primers.

Figures 1 and 2 were obtained with Smart Cycler System whereas figures 3 and 4 are from the LightCycler. Upper panels show amplification curve and lower panels melting curve.

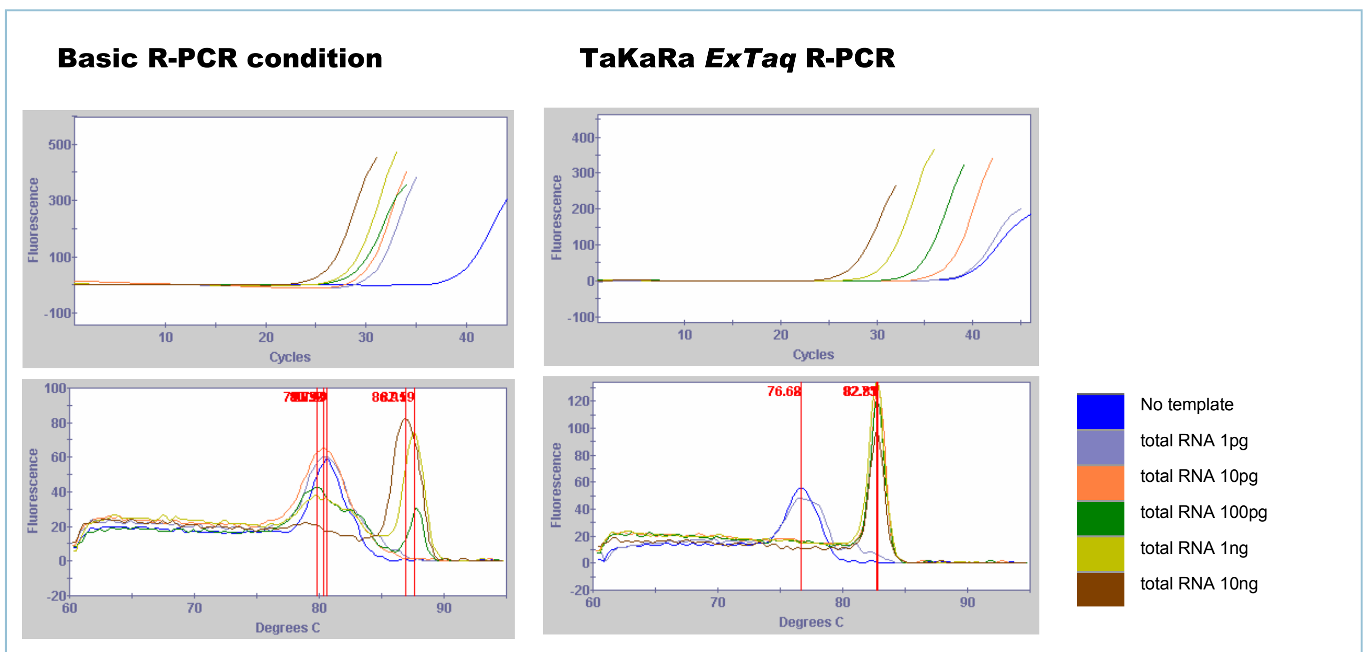

Figure 1 : RAB32, member of RAS oncogene family, amplification with Smart Cycler. (Primer ID : 147)

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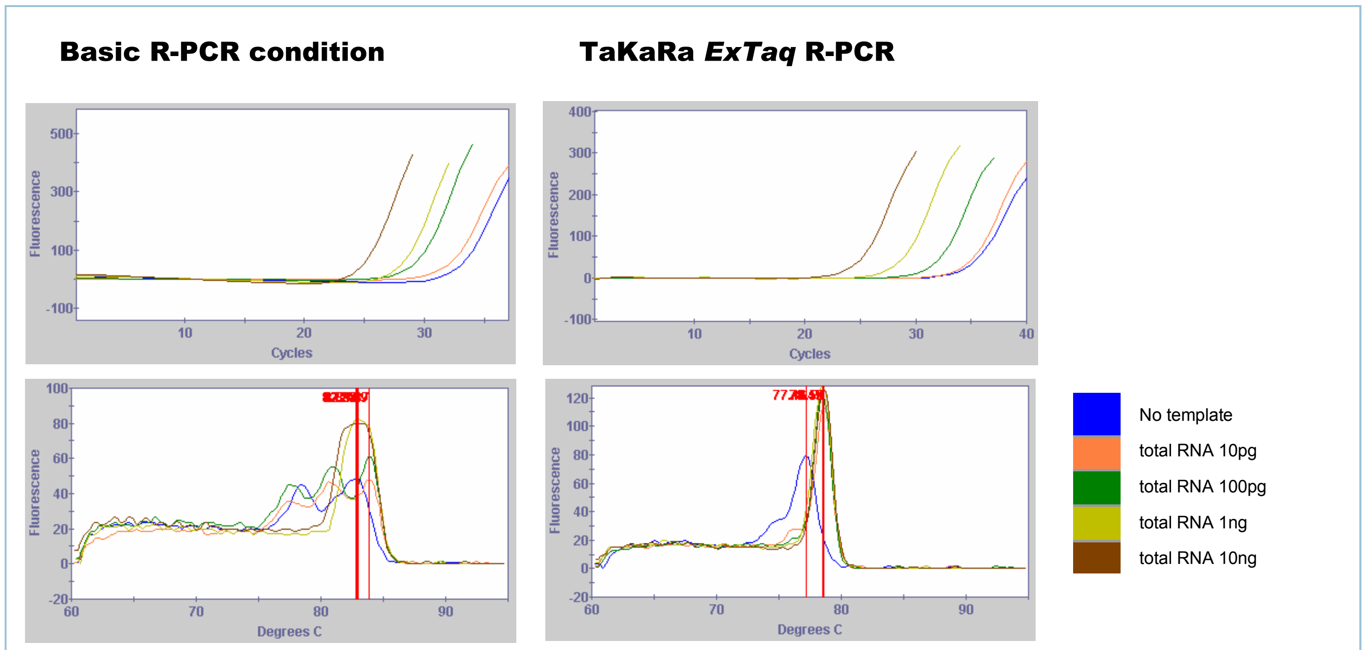

Figure 2 : Protein kinase, DNA-activated catalytic polypeptide, amplification with Smart Cycler. (Primer ID : 150)

Basic R-PCR condition

TaKaRa *ExTaq* R-PCR

Basic R-PCR condition	TaKaRa *ExTaq* R-PCR
No template	No template
total RNA 1pg	total RNA 1pg
total RNA 10pg	total RNA 10pg
total RNA 100pg	total RNA 100pg
total RNA 1ng	total RNA 1ng
total RNA 10ng	total RNA 10ng

Figure 3 : RAB32, member of RAS oncogene family, amplification with LightCycler. (Primer ID : 147)

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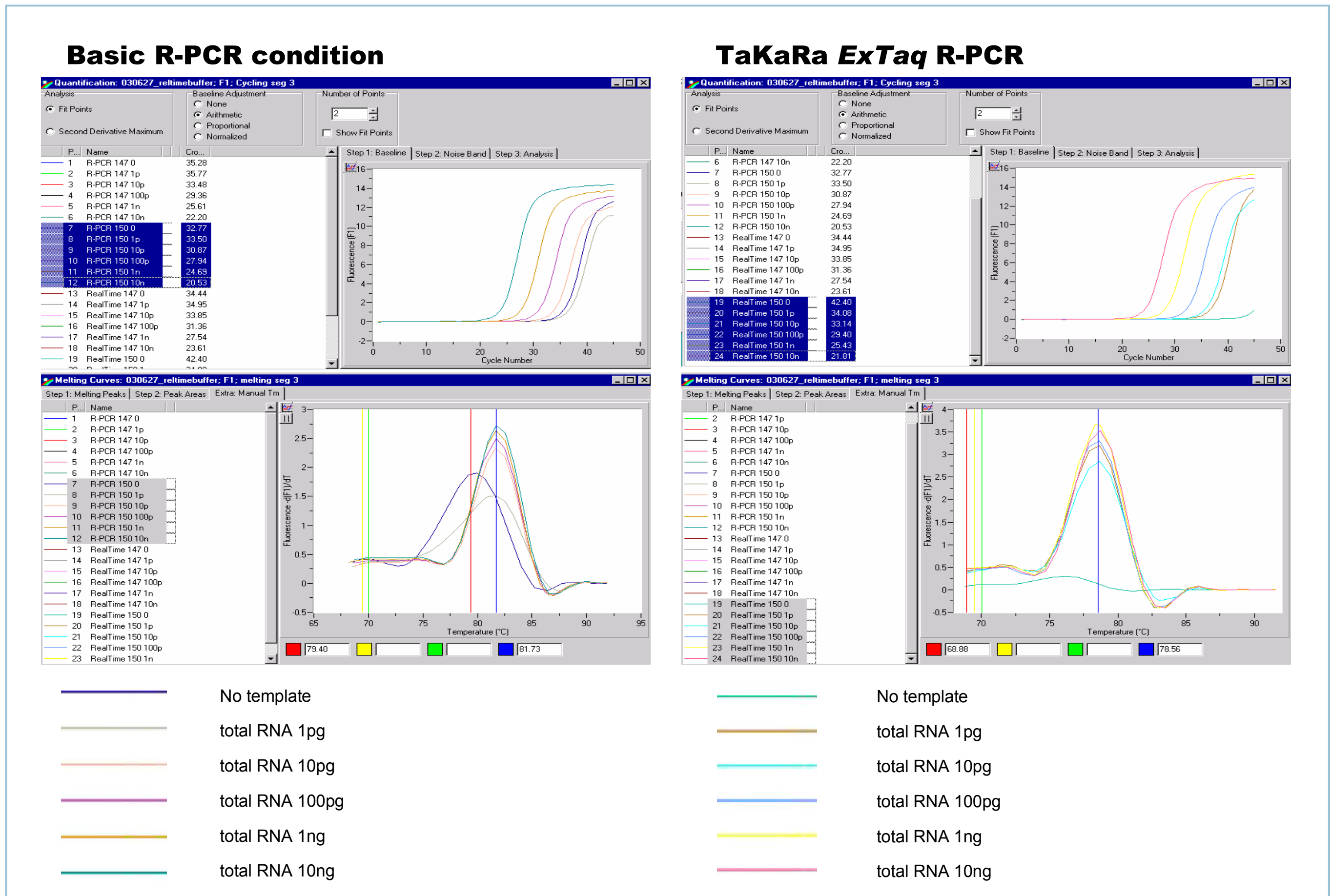

Figure 4 : Protein kinase, DNA-activated catalytic polypeptide, amplification with LightCycler. (Primer ID : 150)

Conclusion

TaKaRa Ex Taq R-PCR reduces non-specific amplification and improves efficiency. Melting curves show that less or no non-specific amplification occurs with TaKaRa Ex Taq R-PCR. This improvement is already observed for low copy number of starting material.

In addition, new conditions allow better performance with LightCycler.

Ex Taq R-PCR achieved effective real time PCR of several human genes which basic conditions did not allow successful amplification.

Highly engineered buffer allows more effective denaturation that minimizes primer dimer formation. Accordingly, specificity is greater and reaction speed is increased.

TaKaRa Ex Taq R-PCR optimizes the use of SYBR Green I, thanks to higher specificity. It is furthermore well suited for use with Fluorescent Probes.

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