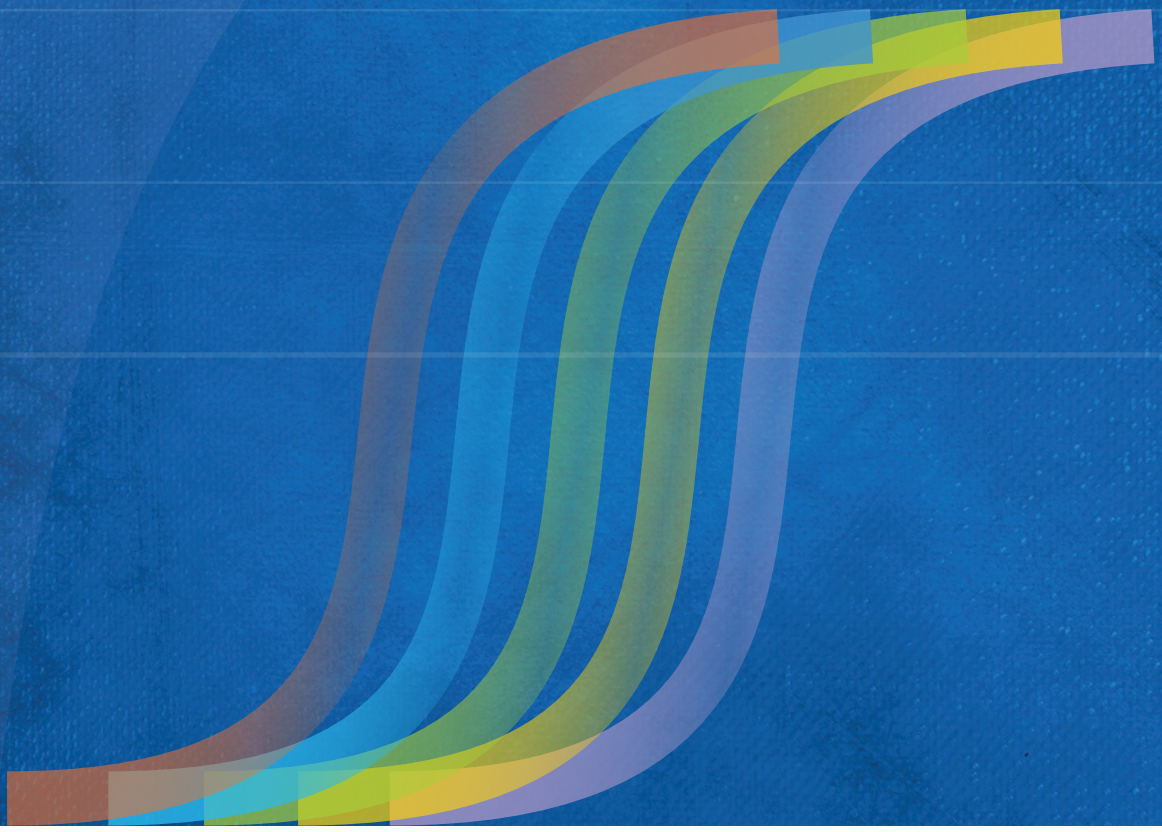
Real-Time PCR and Reverse Transcription

that's GOOD science!®



TaKaRa

Clontech TaKaRa cellartis

1842
Predecessor to Takara Bio begins as a small sake brewery business

1925
Formally incorporates as Takara Shuzo Co., Ltd

1950
Takara Bio founds a biotechnology R&D center

1979
Commences sales of the first domestically produced restriction enzymes in Japan

1984
Clontech Laboratories, Inc. is established in the United States

Real-time PCR reagents for the most demanding qPCR experiments

When you use real-time PCR reagents that are sensitive with high specificity, you can spend less time on qPCR troubleshooting and more time on moving your research forward. We offer reverse transcription and real-time PCR kits for both dye- and probe-based detection. Compatible with all major real-time PCR instrument systems, these products allow you to obtain accurate, consistent results from a wide variety of sample types, even when other enzymes fail. Whether your experiments are routine, complicated, or proving difficult, we have the right qPCR tools to push your experiment to its fullest potential and that's good science!

1993
Higuchi, ***et al.*** detect increases in EtBr fluorescence in real-time PCR with a CCD camera

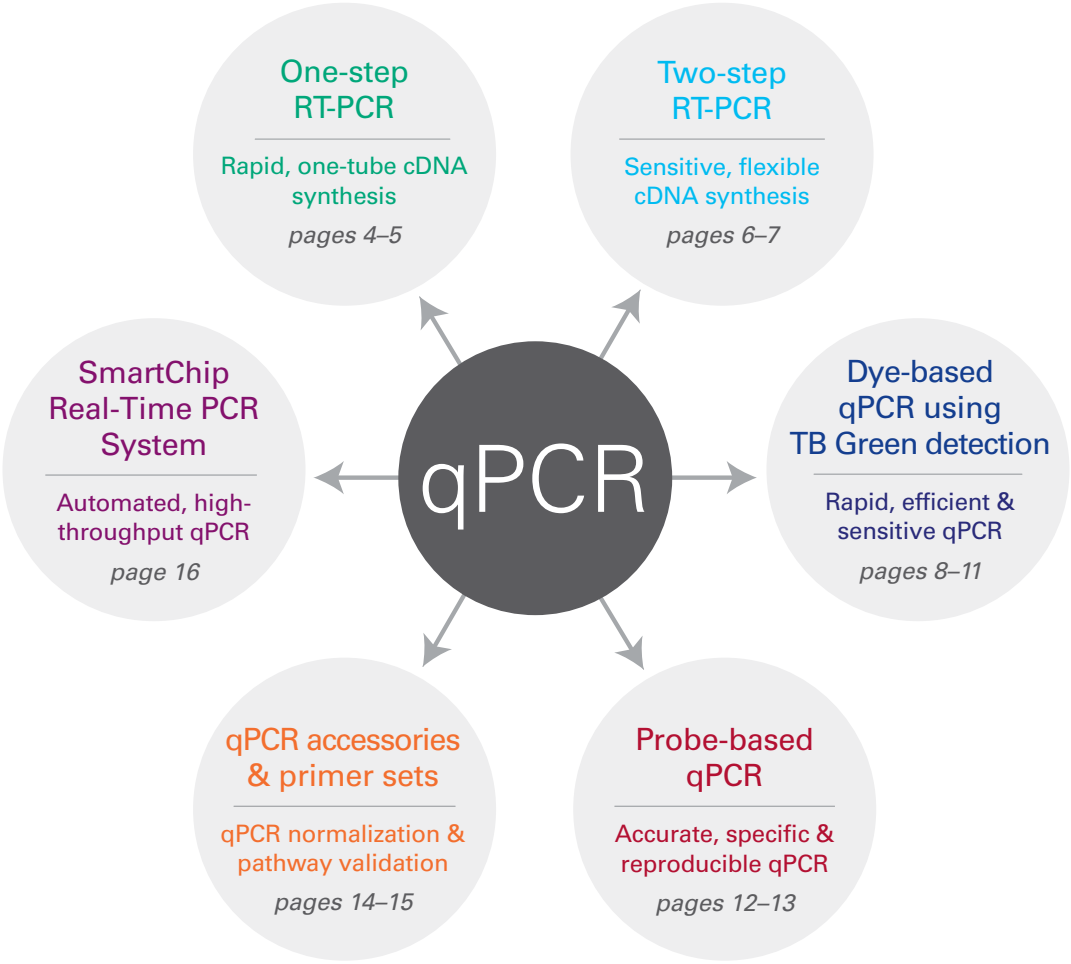
2005
Takara Bio acquires Clontech Laboratories, Inc. of the United States

2013
Over 2,500 articles published to date using qPCR products from Takara Bio Inc.

2017
TBUSA acquires WaferGen Bio-systems, Inc. and the SmartChip™ Real-Time PCR System

2018
Launch of TB Green™ intercalating dye

Find the best tool for your research

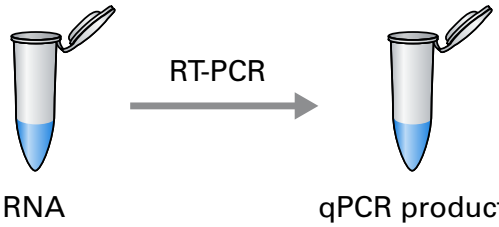


Need commercial or custom products?

We offer a range of OEM and customization services, including custom packaging and product configurations. Please inquire at bd_oem@takarabio.com.

One-step reverse transcription kits

One-step reverse transcription kits perform the reverse transcriptase step in the same tube as the qPCR reaction.



Advantages:

- Simple and rapid
- Works well with small assay numbers
- Amenable to high throughput

Limitations:

- Inability to optimize reverse transcription step
- Does not generate stock of cDNA

Probe-based detection: accurate, specific, and reproducible

Cat. #	Product	Size
RR064A	One-Step PrimeScript™ RT-PCR Kit (Perfect Real Time)	100 rxns
RR064B	One-Step PrimeScript RT-PCR Kit (Perfect Real Time)	500 rxns

Faster and more efficient than other one-step kits

	PrimeScript kit	Company A	Company Q
Reverse transcription	5 min at 42°C	15 min at 48°C	10 min at 50°C
Denaturation/activation (95°C)	10 sec (denaturation)	10 min (activation)	5 min (activation)
PCR denaturation (95°C) PCR annealing/extension (60°C) cycles	3 sec 25 sec x 40	15 sec 60 sec x 40	10 sec 30 sec x 40
Approx. total run time	**41 min**	**114 min**	**60 min**

Highlighted citations:

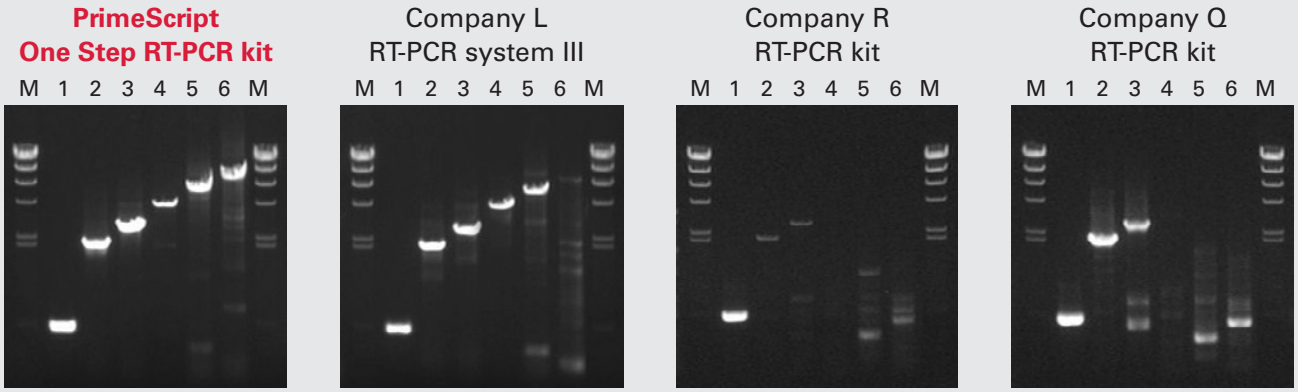
Xie, Y., Wang, M., Xu, D., Li, R. & Zhou, G. Simultaneous detection and identification of four sugarcane viruses by one-step RT-PCR. *J. Virol. Methods* **162**, 64–68 (2009).

In collaboration with the USDA, the authors successfully developed a one-step quadruplex RT-PCR method for detecting viruses in sugarcane using Cat. # RR064A from Takara Bio. This rapid and sensitive technique greatly reduces cost and labor, since multiple infections can be tested in one sample.

Dye-based detection with TB Green kits: rapid and efficient

Cat. #	Product	Size
RR086A	One-Step TB Green PrimeScript RT-PCR Kit II (Perfect Real Time)	100 rxns
RR086B	One-Step TB Green PrimeScript RT-PCR Kit II (Perfect Real Time)	500 rxns

Better amplification and longer products than other one-step kits



Comparison of Takara Bio one-step kit with competitor kits for long amplification. 1 µg of total human RNA was used as a template for cDNA synthesis. Each RT-PCR was performed according to the supplier's recommended conditions. Lane 1 is *TFR*, 0.5 kb. Lane 2 is *CCND2*, 2.1 kb. Lane 3 is *CCND2*, 2.8 kb. Lane 4 is *TFR* 4.4 kb. Lane 5 is Dystrophin, 6 kb. Lane 6 is Dystrophin, 8 kb. M is a control which contained a λ-HindIII digest.

Highlighted citations:

Ma, W. *et al.* Zika Virus Causes Testis Damage and Leads to Male Infertility in Mice. *Cell* **167**, 1511–1524.e10 (2016).

Cat. # RR064A was used to sensitively detect Zika viral RNA levels from multiple tissues in Zika virus-infected mice. These data were able to discern higher viral levels in testis, associated with infertility.

Zou, Q. *et al.* Use of Praziquantel as an Adjuvant Enhances Protection and Tc-17 Responses to Killed H5N1 Virus Vaccine in Mice. *PLoS One* **7**, e34865 (2012).

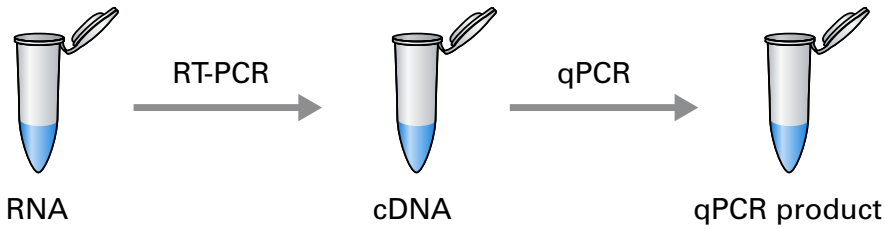
Cat. # RR084A was used to measure H5N1 infection levels in mouse lung tissue. This enabled the testing of a novel adjuvant, PZQ, which was able to reduce virus loads and prolong survival.

Zhang, N. *et al.* Development of one-step SYBR Green real-time RT-PCR for quantifying bovine viral diarrhea virus type-1 and its comparison with conventional RT-PCR. *Virol. J.* **8**, 374 (2011).

Cat. # RR084A was used to develop a specific, sensitive, and reproducible assay for bovine viral diarrhea virus, a surrogate model for hepatitis C virus. The assay was 10-fold more sensitive than conventional assays and showed no primer dimers or nonspecific products.

Two-step reverse transcription kits

Two-step reverse transcription kits perform the reverse transcriptase step to generate cDNA and can be combined with our probe- and dye-based qPCR kits for full analysis.



Advantages:

- High sensitivity facilitates quality results from limited sample input
- Ability to optimize reverse transcription and qPCR steps separately
- Can generate cDNA stocks

Limitations:

- More time-consuming
- Less amenable to high throughput

Convenience: complete master mix with primers and reverse transcriptase

Cat. #	Product	Size
RR036A	PrimeScript RT Master Mix (Perfect Real Time)	200 rxns
RR036B	PrimeScript RT Master Mix (Perfect Real Time)	800 rxns

Flexibility: primers and reverse transcriptase provided as separate components

Cat. #	Product	Size
RR037A	PrimeScript RT Reagent Kit (Perfect Real Time)	200 rxns
RR037B	PrimeScript RT Reagent Kit (Perfect Real Time)	800 rxns

Specificity: includes genomic DNA removal reagent

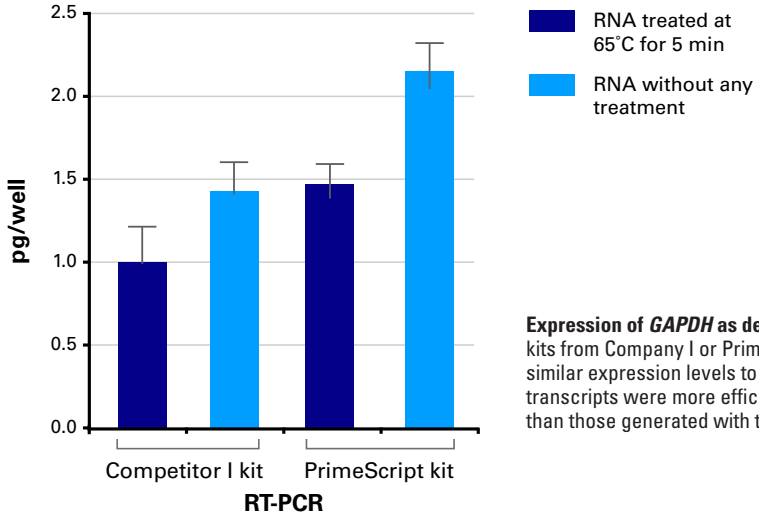
Cat. #	Product	Size
RR047A	PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time)	100 rxns
RR047B	PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time)	400 rxns

Highlighted citations:

Mizutani, R. *et al.* Identification and Characterization of Novel Genotoxic Stress-Inducible Nuclear Long Noncoding RNAs in Mammalian Cells. *PLoS One* **7**, e34949 (2012).

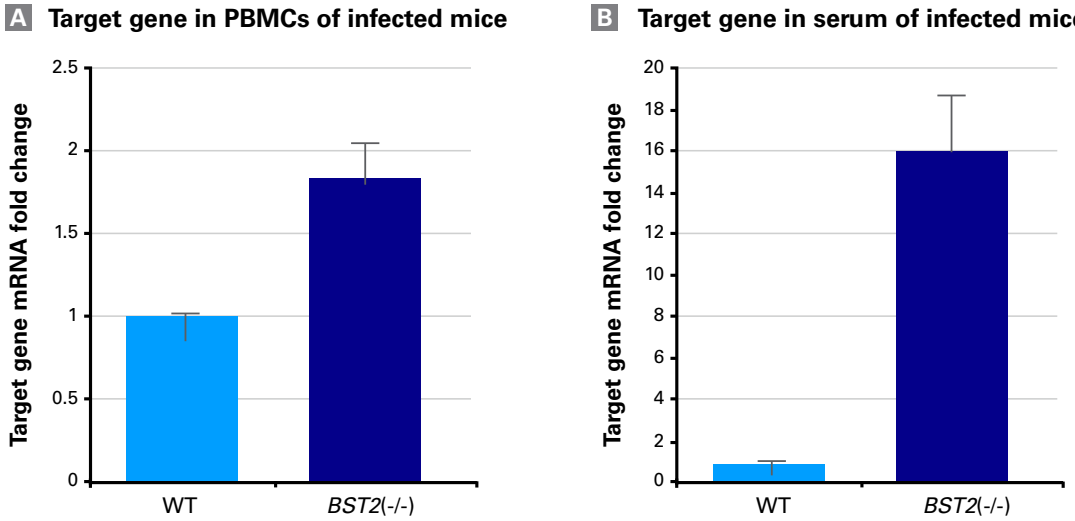
The authors identified a variety of novel lncRNAs using transcriptome sequencing and bioinformatics. Cat. # RR036A was used to confirm these species in a variety of tissues and cells.

Superior reverse transcription efficiency over other kits



Expression of *GAPDH* as determined by qPCR. Expression levels of human *GAPDH* generated with kits from Company I or PrimeScript RT master mix. Heat-denatured samples (dark blue bars) show similar expression levels to samples that were not heat-denatured (light blue bars). Human *GAPDH* transcripts were more efficiently detected from cDNA generated with PrimeScript RT master mix than those generated with the Company I kit.

Fast reverse transcription in just 15 minutes



Difference in target gene expression levels in wild-type and *BST2*(-/-) mice. Relative expression levels of the target gene as determined by qPCR. The fold increase in expression of the target gene in *BST2*(-/-) mice is shown for PBMC (**Panel A**) and serum (**Panel B**) samples. The PrimeScript RT Reagent Kit (Perfect Real Time) was able to generate cDNA from total RNA isolated from mouse PBMCs and serum samples in ~15 minutes.

Highlighted citations:

Makino, K. *et al.* Inhibition of Uterine Sarcoma Cell Growth through Suppression of Endogenous Tyrosine Kinase B Signaling. *PLoS One* **7**, e41049 (2012).

The authors used Cat. # RR037A to analyze gene expression levels in frozen and FFPE uterine leiomyosarcoma samples. The findings implicated TrkB signaling, providing potential drug targets for cancer therapies.

Sato, Y., Inoue, M., Yoshizawa, T. & Yamagata, K. Moderate Hypoxia Induces β-Cell Dysfunction with HIF-1–Independent Gene Expression Changes. *PLoS One* **9**, e114868 (2014).

The authors used Cat. # RR047A to detect alterations of gene expression in primary islet cells under hypoxic conditions. These findings could implicate novel roles in β-cell dysfunction in type 2 diabetes.

Dye-based qPCR kits using TB Green detection

Our dye-based qPCR kits utilize our proprietary TB Green intercalating dye. This versatile dye can be used with standard methods and equipment with no need for protocol modifications.

Advantages:

- Rapid and simple protocol
- More cost-effective than probes
- Sensitive: 1,000X detection

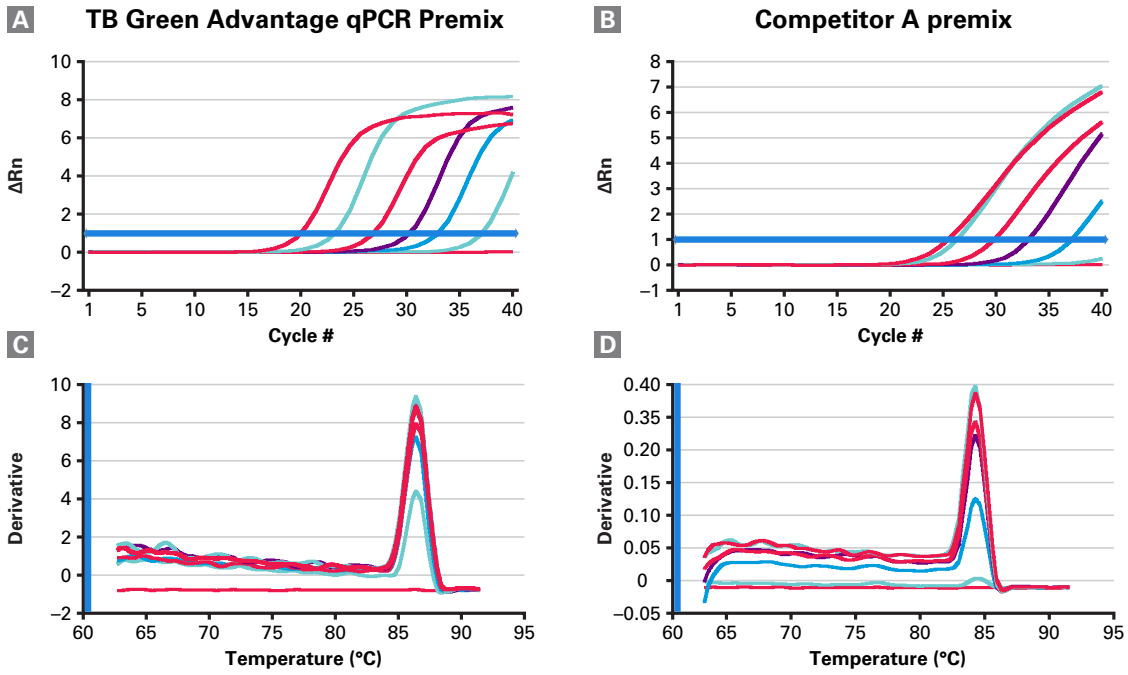
Limitations:

- Can result in background due to nonspecific PCR products
- May require more optimization

Efficient and versatile: 2X master mix works with most templates

Cat. #	Product	Size
639676	TB Green Advantage® qPCR Premix	200 rxns

More efficient than other enzyme mixes



Comparison of amplification efficiency. Takara Bio TB Green Advantage qPCR Premix outperforms Competitor A's SYBR mix on an ABI PRISM 7000 Sequence Detection System. The results for the Takara Bio reagent are shown in **Panels A** and **C**, and those for Competitor A's reagent are shown in **Panels B** and **D**. The TB Green Advantage premix showed higher amplification efficiency, indicated by Ct values shifted to the left.

Highlighted citations:

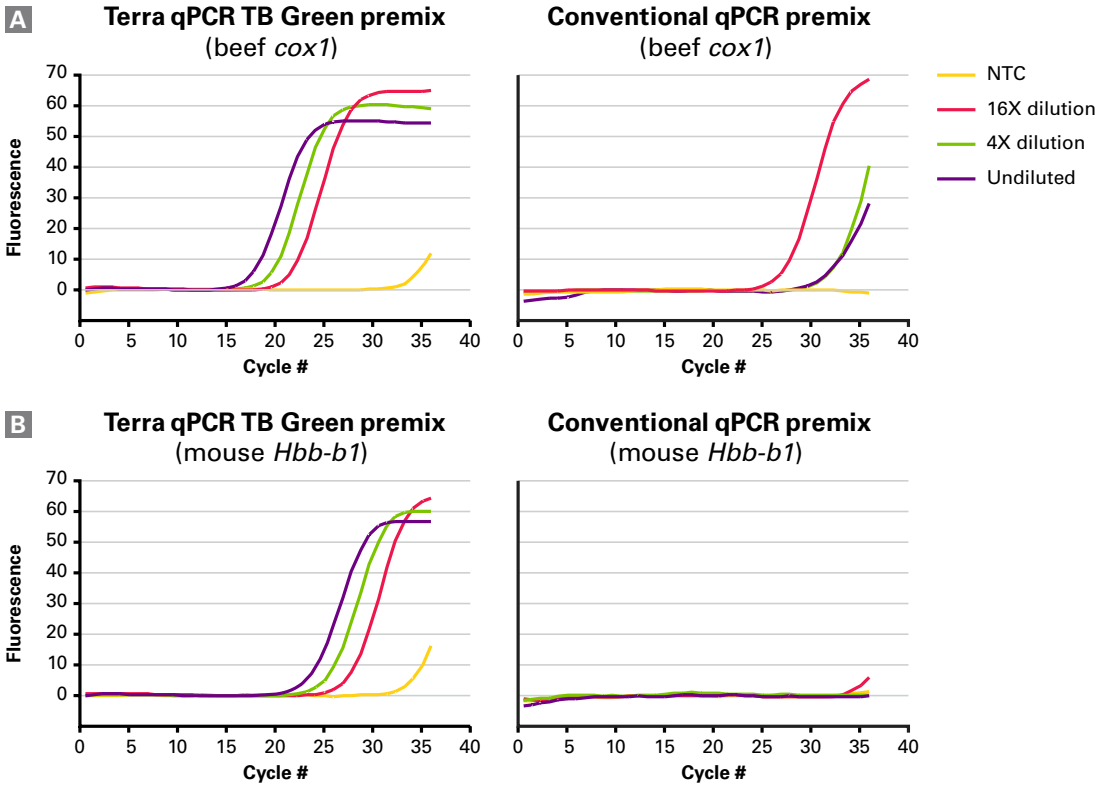
He, C. *et al.* The Differential Expression and Possible Function of Long Noncoding RNAs in Liver Cells Infected by Dengue Virus. *Am. J. Trop. Med. Hyg.* **97**, 1904–1912 (2017).

Researchers performed RNA-seq to identify candidate lncRNAs implicated in liver injury following dengue virus infection. Cat. # 639676 was used by researchers to confirm differentially expressed lncRNAs and identify novel diagnostic markers for disease.

Convenience: direct qPCR on crude extracts without purification

Cat. #	Product	Size
638319	Terra™ qPCR Direct TB Green Premix	200 rxns

Achieve amplification in the presence of inhibitors



Crude extract amplification comparison between Takra Bio and conventional premixes. Real-time PCR was performed using undiluted, 4X diluted, and 16X diluted crude alkaline-heat extracts of mouse spleen or cow muscle using either Terra qPCR Direct TB Green Premix or a conventional qPCR premix. Data generated by Terra qPCR Direct TB Green Premix corresponded to the theoretical quantities of each gene, while the conventional product was clearly affected by inhibitors present in the crude samples.

Highlighted citations:

Chen, X. *et al.* The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues. *Gene* **595**, 221–226 (2016).

Researchers tested the effects of differential miRNA expression and drug resistance in 55 breast cancer FFPE tissues. Cat. # 639676 was used to identify key miRNAs that could serve as biomarkers for breast cancer treatment.

Li, Y. *et al.* MAF1 suppresses AKT-mTOR signaling and liver cancer through activation of PTEN transcription. *Hepatology* **63**, 1928–42 (2016).

Cat. # 638319 was used by researchers to directly assess *MAF1* expression in primary human hepatocellular carcinoma tumors. These data identified novel tumor-suppression activities with potential prognosis prediction value.

Dye-based qPCR kits using TB Green detection

Greater specificity and longer amplicons with Tli RNase H Plus

Flexibility: master mix with two ROX dyes for maximum instrument coverage

Cat. #	Product	Size
RR420A	TB Green Premix *Ex Taq*™ (Tli RNase H Plus)	200 rxns
RR420B	TB Green Premix *Ex Taq* (Tli RNase H Plus)	400 rxns

Bulk: master mix with one ROX dye in up to 25 ml for maximum throughput

Cat. #	Product	Size
RR420L	TB Green Premix *Ex Taq* (Tli RNase H Plus), 1 x 5 mL	200 rxns
RR420W	TB Green Premix *Ex Taq* (Tli RNase H Plus), 5 x 5 mL	1,000 rxns

Convenience: complete master mix containing ROX dye in bulk

Cat. #	Product	Size
RR42LR	TB Green Premix *Ex Taq* (Tli RNase H Plus), 1 x 5 mL, ROX Plus	200 rxns
RR42WR	TB Green Premix *Ex Taq* (Tli RNase H Plus), 5 x 5 mL, ROX Plus	1,000 rxns

Maximum specificity and GC-rich targets with Tli RNase H Plus

Flexibility: master mix with two ROX dyes for maximum instrument coverage

Cat. #	Product	Size
RR820A	TB Green Premix *Ex Taq* II (Tli RNase H Plus)	200 rxns
RR820B	TB Green Premix *Ex Taq* II (Tli RNase H Plus)	400 rxns

Bulk: master mix with one ROX dye in up to 25 ml for maximum throughput

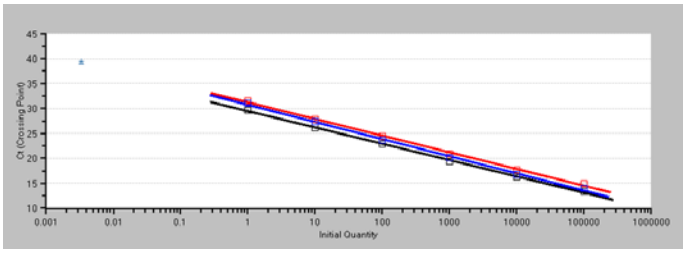
Cat. #	Product	Size
RR820L	TB Green Premix *Ex Taq* II (Tli RNase H Plus), 1 x 5 mL	200 rxns
RR820W	TB Green Premix *Ex Taq* II (Tli RNase H Plus), 5 x 5 mL	1,000 rxns

Convenience: complete master mix containing ROX dye in bulk

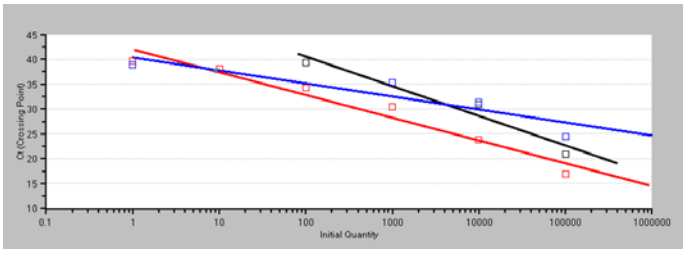
Cat. #	Product	Size
RR047A	PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time)	100 rxns
RR047B	PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time)	400 rxns

Improved specificity and efficiency compared to other mixes

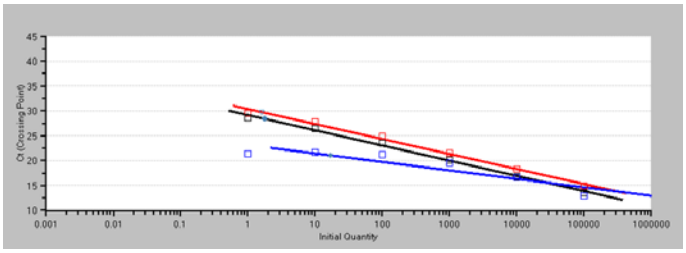
Takara Bio



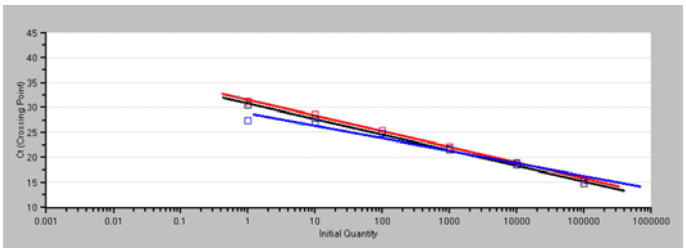
Company A



Company B

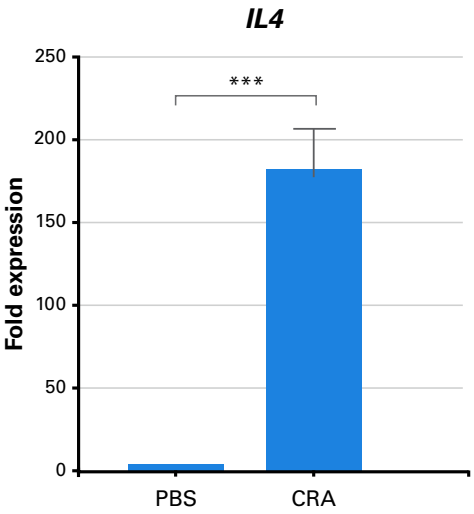


Company C



qPCR efficiency was assessed using Cat. # RR420A and other commercially available SYBR master mixes (Companies A, B, and C). Three portions of the *ACTB* gene were amplified (red: 186 bp; black: 381 bp; blue: 533 bp) from cDNA synthesized using 10 pg to 1 µg of human testes total RNA, and the standard curves were plotted. Takara Bio's TB Green mix outperformed all other mixes.

Accurate detection from *in vivo* samples



Assessment of expression levels of *IL4* from *in vivo* samples. Detection of IL-4 was performed via qPCR using Cat. # RR820A in mouse lung samples from mice treated with either PBS or cockroach allergen (CRA), which initiates lung inflammation. Cat. # RR820A efficiently and specifically amplified *IL4* in the CRA-treated mice.

Highlighted citations:

Sung, H.Y. *et al.* Amyloid Beta-Mediated Hypomethylation of Heme Oxygenase 1 Correlates with Cognitive Impairment in Alzheimer's Disease. *PLoS One* **11**, e0153156 (2016).

> Cat. # RR420A was used by researchers to quantify expression levels of *HMOX1*, as well as methylation levels of particular CpG sites, implicated in Alzheimer's disease.

Sato, Y. *et al.* Anks4b, a novel target of HNF4α protein, interacts with GRP78 protein and regulates endoplasmic reticulum stress-induced apoptosis in pancreatic β-cells. *J. Biol. Chem.* **287**, 23236–45 (2012).

> Cat. # RR820A was used by researchers to identify *Anks4b*, a novel target implicated in β-cell apoptosis.

Chen, Q., Gu, Y. & Liu, B. Expression and mechanism of action of the SARI tumor suppressor in prostate cancer. *Int. J. Clin. Exp. Pathol.* **8**, 7953–60 (2015).

> Cat. # RR820A was used by researchers to identify *SARI*, a key regulator of prostate cancer proliferation, in tissues and cells.

Probe-based qPCR kits

Our probe-based qPCR kits are optimized for accurate target quantification and detection over a broad dynamic range, enabling highly reproducible qPCR. These premixes are compatible with all fluorescent oligonucleotide probes.

Advantages:

- High specificity, sensitivity, and reproducibility
- Compatible with multiplexing
- Ideal for genotyping and CNV analysis

Limitations:

- Each sequence requires design and optimization of different probes
- Predesigned probes can be costly to purchase

Choose the format that suits your experiments

Flexibility: master maximum with two ROX dyes for maximum instrument support

Cat. #	Product	Size
RR390A	Premix *Ex Taq* (Probe qPCR)	200 rxns
RR390B	Premix *Ex Taq* (Probe qPCR)	400 rxns

Bulk: master mix with one ROX dye in up to 25 ml for maximum throughput

Cat. #	Product	Size
RR390L	Premix *Ex Taq* (Probe qPCR)	200 rxns
RR390W	Premix *Ex Taq* (Probe qPCR)	1,000 rxns

Convenience: complete master mix containing ROX dye in bulk

Cat. #	Product	Size
RR39LR	Premix *Ex Taq* (Probe qPCR), ROX Plus	200 rxns
RR39WR	Premix *Ex Taq* (Probe qPCR), ROX Plus	1,000 rxns

Highlighted citations:

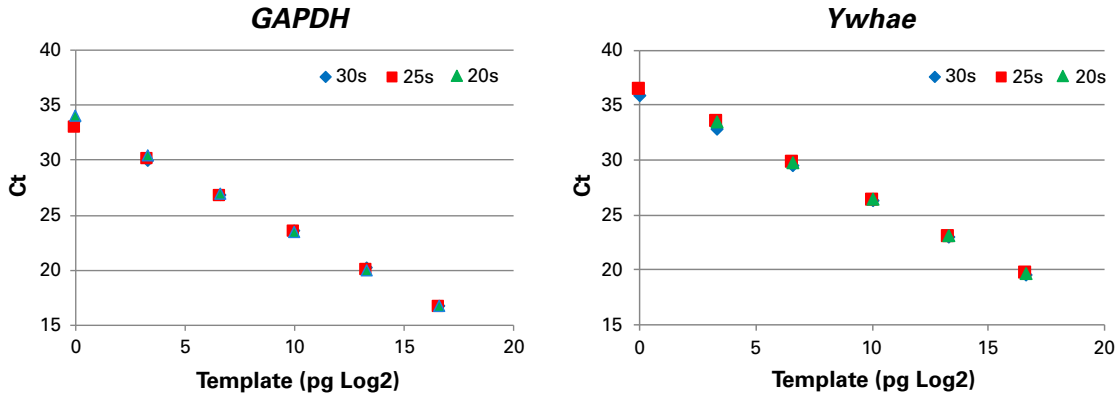
Teng, Q. *et al.* Development of a TaqMan MGB RT-PCR for the rapid detection of H3 subtype avian influenza virus circulating in China. *J. Virol. Methods* **217**, 64–69 (2015).

Cat. # RR390A was used with minor groove binder probes to develop a sensitive and rapid assay for H3 avian influenza virus. This assay was 1,000X more sensitive than conventional qPCR, with a detection limit of 10 copies per reaction.

Kim, H.-R. *et al.* Multiplex real-time polymerase chain reaction for the differential detection of porcine circovirus 2 and 3. *J. Virol. Methods* **250**, 11–16 (2017).

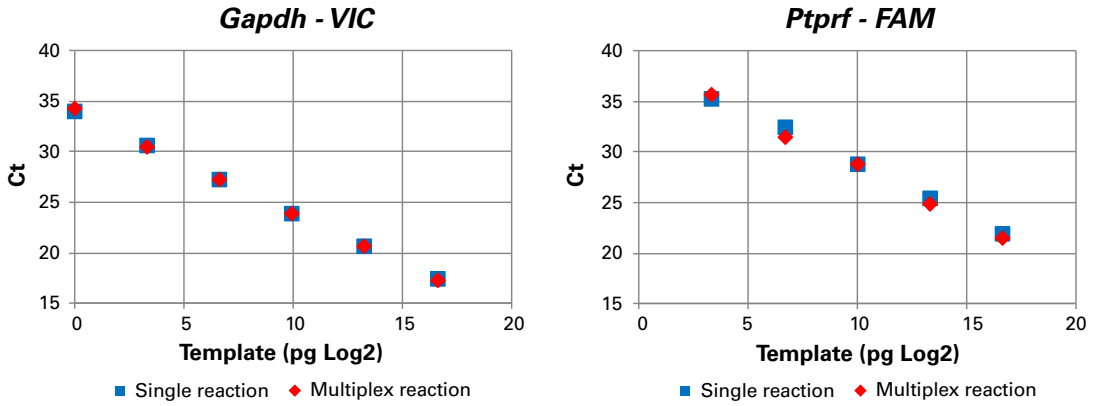
A multiplex qPCR assay was developed for the rapid and differential detection of porcine circovirus 2 and 3 using Cat. # RR390A. This sensitive and reproducible assay had a limit of detection below 50 copies and had coefficients of intra-assay and inter-assay variation less than 4%.

Save time by using shorter extension times without compromising efficiency



Assessment of shorter extension times. A portion of the *GAPDH* and *Ywhae* genes were amplified by real-time PCR using Cat. # RR390A from cDNA synthesized using 1 pg to 100 ng of total RNA. The standard curves were plotted and reaction efficiency was assessed using various extension times. The same efficiency was obtained with extension times of 20, 25, and 30 seconds.

Perform single and multiplex reactions with the same master mix



Cat. # RR039A works well in single and multiplex reactions. Real-time PCR was performed on mouse cDNA for Mouse *Gapdh* or *Ptprf* using Cat. # RR039A in single and multiplex reactions. Ct values were nearly the same regardless of the type of reaction tested, indicating that the mix works for both experimental setups.

Highlighted citations:

Jordan, J. A. & Durso, M. B. Real-time polymerase chain reaction for detecting bacterial DNA directly from blood of neonates being evaluated for sepsis. *J. Mol. Diagn.* **7**, 575–81 (2005).

Cat. # RR039A was used to develop a rapid assay capable of detecting bacterial DNA directly from blood samples.

Jiang, Y. *et al.* Aberrant expression of RSK4 in breast cancer and its role in the regulation of tumorigenicity. *Int. J. Mol. Med.* **40**, 883–890 (2017).

Cat. # RR390A was used to accurately and specifically detect altered expression of *RSK4* in multiple cell and tissue samples.

qPCR accessories and primer sets

qPCR normalization: eliminate housekeeping genes with pooled reference sets

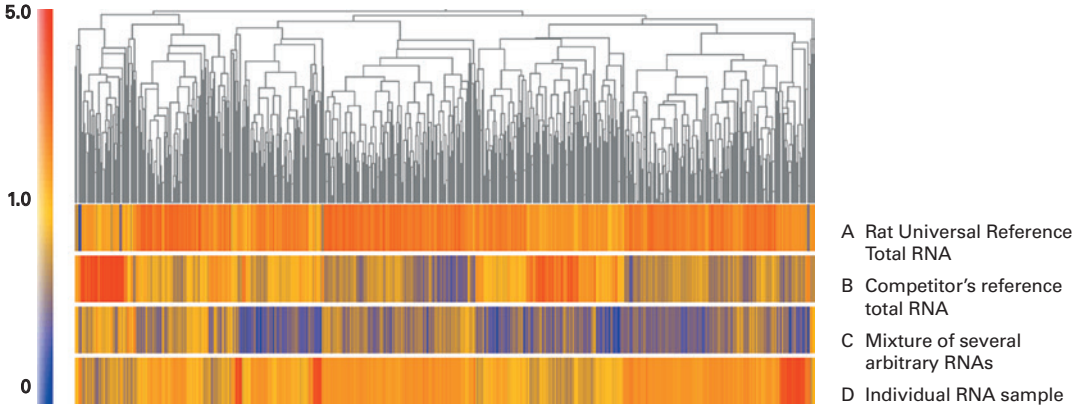
Reference RNA

Cat. #	Product	Size
636690	qPCR Human Reference Total RNA	25 ug
636657	Mouse Universal Reference Total RNA	2 x 200 ug
636658	Rat Universal Reference Total RNA	2 x 200 ug

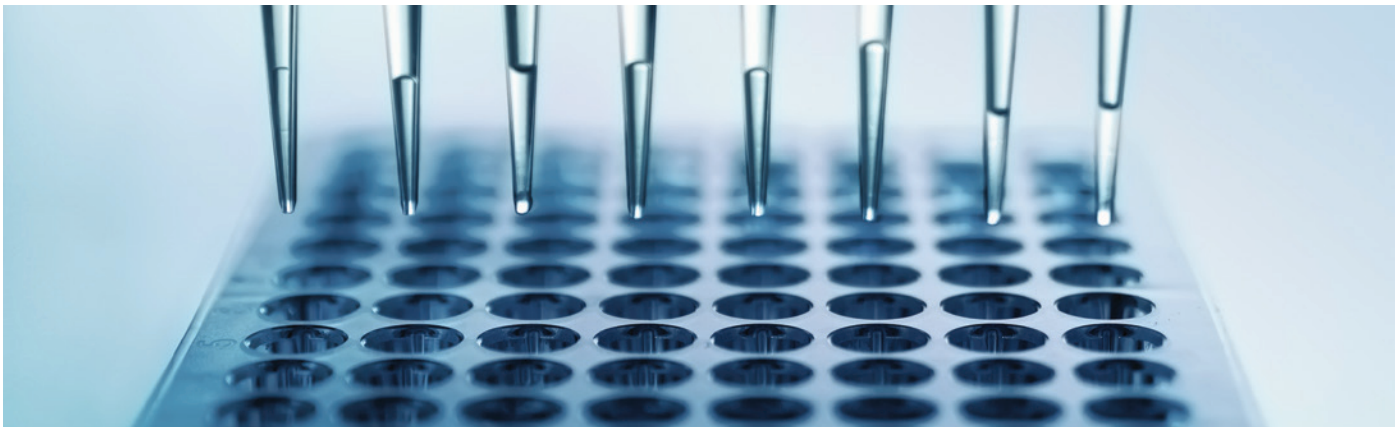
Reference cDNA

Cat. #	Product	Size
636692	qPCR Human Reference cDNA, Oligo(dT)-primed	25 rxns
636693	qPCR Human Reference cDNA, Oligo(dT)-primed	100 rxns
639653	qPCR Human Reference cDNA, Random-primed	25 rxns
639654	qPCR Human Reference cDNA, Random-primed	100 rxns

Better gene coverage (>90%) than other controls



Takara Bio's Rat Universal Reference Total RNA demonstrates more than 90% gene coverage. Cy3 labeled probes were generated using our Rat Universal Reference Total RNA (Row A), another vendor's reference total RNA (Row B), a random RNA mixture (Row C), and an individual RNA sample (Row D). Probes were hybridized to a glass rat array containing 3,766 long oligonucleotides. Expression results were analyzed using GeneSpring software (version 5.0) to cluster genes according to their expression patterns. Varying colors reflect the ratio of the intensity of any gene on each array to its median intensity across all arrays. The red and blue colors reflect high and low ratios, respectively. Our results indicate that the Reference Total RNA has more than 90% gene coverage with even distribution and outperforms another vendor's universal RNA mixture.



Prevalidated qPCR primer sets: convenient 96-well plates containing 88 target primers and 8 controls

Disease pathways

Cat. #	Product
PH011	PrimerArray® Colorectal Cancer & Pancreatic Cancer (Human)
PH012	PrimerArray Prostate Cancer & Melanoma (Human)
PH013	PrimerArray Small Cell Lung Cancer & Non-small Lung Cancer (Human)
PH014	PrimerArray Asthma & Rheumatoid Arthritis (Human)
PH015	PrimerArray Diabetes Mellitus, Type I & Type II (Human)

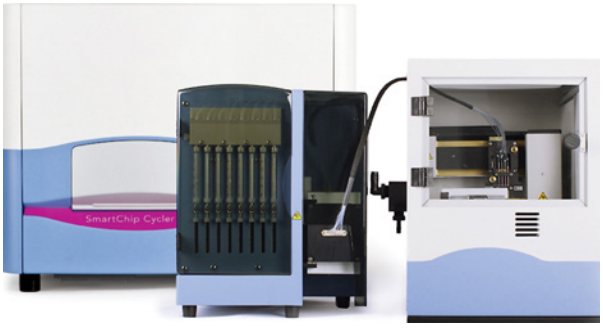
Biological pathways

Cat. #	Product
PH001	PrimerArray Cytokine-Cytokine Receptor Interaction (Human)
PH002	PrimerArray Cell Cycle (Human)
PH003	PrimerArray Cell Adhesion Molecules (Human)
PH004	PrimerArray Jak-STAT Signaling Pathway (Human)
PH005	PrimerArray Natural Killer Cell Mediated Cytotoxicity (Human)
PH006	PrimerArray Axon guidance (Human)
PH007	PrimerArray Focal adhesion (Human)
PH009	PrimerArray TGF-beta Signaling Pathway (Human)
PH010	PrimerArray Wnt Signaling Pathway (Human)

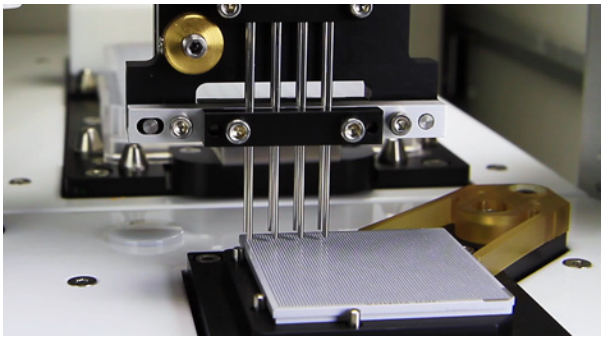
Note: Corresponding mouse versions of primer sets are available as catalog number PN### instead of PH###.

Doing a lot of qPCR?

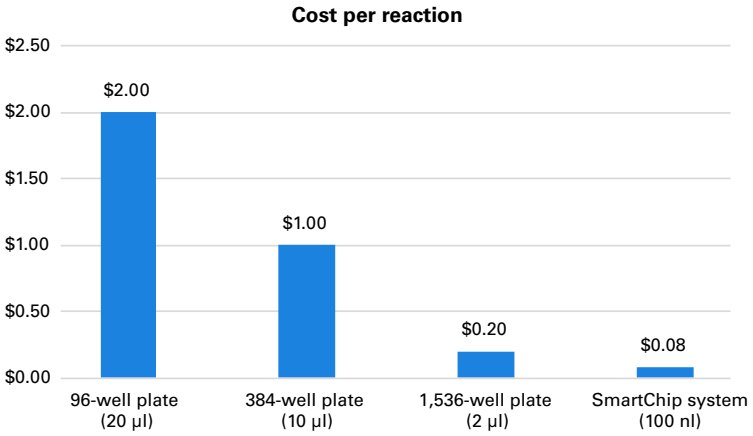
Utilize the SmartChip Real-Time PCR System to automate your qPCR workflow



- High throughput: 5,184 individual qPCR reactions per chip
- Maximum flexibility: one chip accommodates 14 different assay and sample options
- Significant cost savings: <10 cents per reaction
  - Minimize reagent usage with 100-nl reactions
  - Preserve sensitivity while only using 3–10 ng/µl of input



200X reduction in reaction costs with the SmartChip system



The SmartChip system utilizes 100-nl reactions which offer significant reagent and cost savings over conventional plates. A typical experiment performed with the SmartChip system has a cost of $0.08/reaction, compared to up to $2/reaction in a 96-well plate.

Learn more at: https://go.takarabio.com/SmartChip_Brochure

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