

## I Introduction of technology

qEASY qPCR Primer Pairs are designed by using SBI's proprietary primer design algorithm. It sets for use in SYBR Green-based real-time RT-PCR on any cyclor. The primers are designed according to the conserved region of a specific gene with all variants. At least one primer crosses the junction of adjacent exons to avoid genomic DNA amplification directly and effectively. Our primers set cover all genes from human, mouse, rat and can be widely applied to the quantitative analysis of gene expression. Using in conjunction with the SYBR Green mix reagent in the qPCR experiment with single-strand cDNA as the template, each pair of primers can solely acquire a single, correct size and efficient amplified product. Therefore, the characteristic of our primers is high specificity, high amplification efficiency, wide linear range and uniform reaction conditions. Each package for a specific gene is supplied as a lyophilized form of forward and reverse primers that can be easily reconstituted to obtain a primer solution, which can be used directly in SYBR Green-based real-time RT-PCR.

## II Product description

Target genes and primers information

Species	Gene Bank Ref.ID	Gene Symbol	PCR size (bp)	Other Name
Human	NM_019887.5, NM_138930.3, NM_001278342.1, NM_001278302.1, NM_001278303.1, NM_001278304.1	DIABLO	123	SMAC; DFNA64

Shipping and storage information

Catalog No.	Volume	Number of 25 $\mu$ l reactions	Shipping and Storage	Package tube
HP100033	2 nmol	200	lyophilized power	1

Shipping and Storage conditions: Each primer package contains a mix of forward and reverse primers for a specific target gene in a centrifugal tube shipped lyophilized at room temperature.

Upon receipt, store them at  $-20^{\circ}\text{C}$  and avoid repeated freeze-thaw cycles. When stored under these conditions and handled correctly, the product can be kept stably for 12 months in lyophilized power and 6 months in solution.

## III The product using process

The mix of forward and reverse primers of a specific gene is lyophilized power, which is attached to the wall of centrifugal tube. Before use, centrifuge the tube for a few seconds, and then re-suspend the primer mix in  $200\mu\text{l}$   $\text{dH}_2\text{O}$  to make a final concentration of  $10\mu\text{M}$ . We recommend preserve them with small package and store at  $-20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

The customer only needs to take into account the volume of PCR reaction systems when using it. Draw a certain amount of dissolved primer mix to make a final concentration of  $0.2\mu\text{M}$  and use in conjunction with SYBR Green qPCR Mix of other companies. The methods in detail refer to the **part IV "Experimental Procedures"**.

The product is suitable for detection by SYBR Green and not necessarily right for Taqman.



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## IV Experimental Procedures

1. The preparation of related reagents and materials for qPCR experiments: template (first-strand cDNA or plasmid); qPCR Primer mix (10 nM); RNase-free H<sub>2</sub>O; 2×SYBR Green PCR Master Mix. Keep the qPCR mix containing SYBR Green away from light, thaw it at room temperature (if stored at -20°C) and turn it upside down gently to mix, then lay it on the ice.

2. Prepare a master mix of PCR reagents with the components in the table below (e.g. SYBR Green I Master Mix of Roche).

Component	Volume/reaction (μl)	
2×SYBR Green mix (Roche)	10	25
qPCR Primer mix (10 nM)	0.8	2
Template (DNA or cDNA, ≤100ng)	5	5
ddH <sub>2</sub> O	4.2	16
Total	20	50

3. Set up and run the program to carry out the PCR reaction according to the operating manual of machine. Generally, three-step way is employed (annealing temperature is 60 °C). (e.g. Light Cycler 480 II)

Step 1: pre-degeneration

95°C 30s

Step 2: PCR reaction

95°C 10s  
60°C 20s  
72°C 10s } 45 cycles

(Annealing temperature is according to primer. The annealing temperature is 60°C in our experiment)

Step 3: melting curve

95°C 5s

65°C 1min

65°C-97°C (continuous, ramp rate 0.11°C/s, acquisitions 5s/°C)

Step 4: 40°C 30s

4. Analyze the data according to the machine's manual after the reaction.

## V Verification process of qPCR primers and verification report

1. Validation Experiment Instrument: Roche Applied-science LightCycler®480 II

2. Verification experiment materials and reagents

Reagents: Roche SYBR Green I Master mix, etc; qEASY qPCR primer mix

Materials: First-strand cDNA of different tissues or cells of human



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### 3. Validation Process

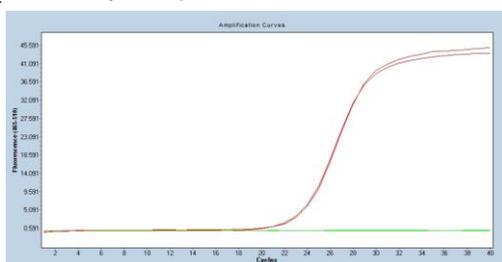
(1) The screening process of primers: design three pairs of primers in conserved regions for each specific gene. Using in conjunction with the SYBR Green mix reagent in the qPCR experiment with single-strand cDNA and ddH<sub>2</sub>O. The protocols in detail refer to the **part IV “Experimental Procedures”**.

(2) Generation of the verification report: through analyzing the amplification curves and dissolution curves of three pairs of primers, selected the pair of primers with high specificity and sensitivity.

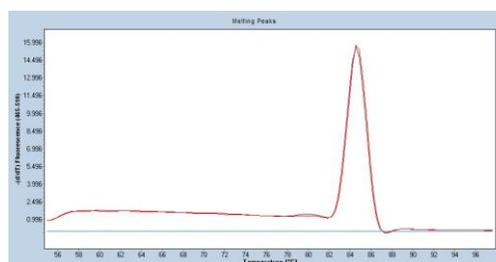
### 4. Validation Report

Positive tissue or cell: liver

The amplification curve and dissolution curve of human gene OTC in the qPCR experiment (cDNA and ddH<sub>2</sub>O as templates).



DIABLO amplification curves



DIABLO dissolution curves

## VI Notes

1. In order to avoid genomic DNA amplification, at least one primer for specific gene crosses the junction of adjacent exons. However, it is necessary to treat with DNase I to remove genomic DNA for genes without introns.

2. The high quality of RNA and optimized reaction conditions are the prerequisites to ensure the accuracy of experiments. It is essential for extraction of RNA to operate carefully and avoid the degradation. Adjusting the template concentration within the detectable linear scope and adding the positive control can avoid the experiment failure.

3. Calculate the amplification efficiency of primers needs to draw standard curve generally. Prepare a dilution series (e.g, 5-fold or 10-fold dilutions) of a RNA or purified plasmid as templates to construct standard curves for primer by SYBR Green-based real-time RT-PCR. Construct a standard curve for the specific gene by plotting CT values (Y-axis) against the log of template amount or dilution (X-axis). Based on the formula  $E = 10^{-1/\text{slope}} - 1$ , the amplification efficiency E was calculated by the slope of standard curve (E lies between 90% to 105%).

4. Carrying out the quantitative analysis based on the data report provided by this product, please refer to the following methods:

(1) absolute quantitative: The amounts of specific gene is calculated using the CT values obtained from the sample of interest and the corresponding standard curve.

(2) relative quantitative: if the amplification efficiency of target gene and reference gene is close to 100%, less than 5%, the Livak method ( $\text{Ratio} = 2^{-\Delta\Delta\text{CT}}$ ) is suitable; if the amplification efficiency of target gene and reference gene is not close, more than 5%,

the Pfaffl method ( $\text{Ratio} = (E_{\text{target}})^{\Delta\text{CT, target (calibrator-test)}} / (E_{\text{ref}})^{\Delta\text{CT, ref (calibrator-test)}}$ ) is suitable.