New developments for streamlined gene expression analysis

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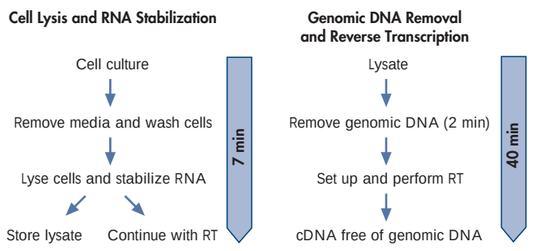
Introduction

QIAGEN has developed new tools that enable faster and more reliable results in gene expression analysis. The tools are suited for many tasks, such as monitoring of gene silencing efficiency following siRNA transfection.

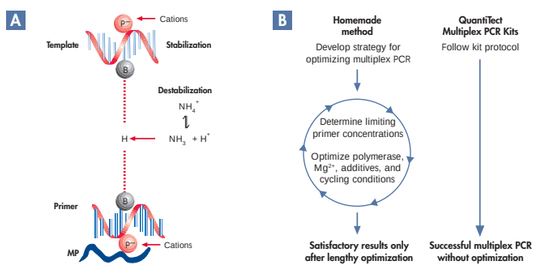
A new direct cDNA kit provides a simple, rapid procedure for preparing cDNA from cultured cells without the need for RNA purification. Cell lysis, RNA stabilization, genomic DNA removal, and first-strand cDNA synthesis are all integrated in one kit. The synthesized cDNA accurately represents the in vivo gene expression profile, and is free of genomic DNA that can distort real-time PCR results if primers for detection of cDNA only are not available.

From Cell Lysis to First-Strand cDNA



QuantiTect® Multiplex PCR Kits are preoptimized master mixes (available with or without ROX dye) that enable success in real-time, multiplex PCR at the first attempt. Up to 4 cDNA or genomic DNA targets can be accurately quantified in the same tube. The kits are compatible with different probe systems (e.g., TaqMan® probes) and with QuantiTect Assays, which are ready-to-use primer–probe sets.

Real-Time, Multiplex PCR without Optimization

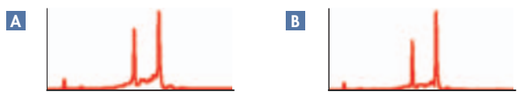


A QuantiTect Multiplex PCR Buffer contains NH_4^+ ions and Factor MP to ensure specific primer annealing and efficient extension. NH_4^+ destabilizes the weak hydrogen bonds at mismatched bases (**B**) of nonspecifically bound primers. Factor MP (**MP**) increases the concentration of primers at the template and stabilizes specifically bound primers. B QuantiTect Multiplex PCR Kits eliminate the need for multiplex PCR assay optimization.

High-Quality RNA for Reliable Real-Time PCR Analysis

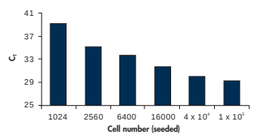
In real-time RT-PCR assays where it is not possible to design RNA-specific primers (e.g., the target is a single-exon gene), it is critical to remove contaminating genomic DNA in the RNA sample. The direct cDNA kit incorporates a short genomic DNA removal step to ensure only RNA is detected.

Stabilization of RNA in Cell Lysates



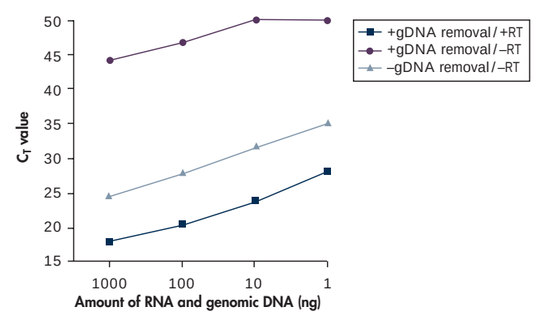
Intact RNA obtained from A Huh7 and B 293 cells after cell lysis and RNA stabilization using the direct cDNA kit. Profiles were generated on the Agilent 2100 bioanalyzer.

Wide Linear Range in Real-Time PCR Analysis



Linearity between cell number seeded and C_T value in real-time RT-PCR. Different amounts of HepG2 cells were seeded and cultured. Using the direct cDNA kit, the cells were lysed and first-strand cDNA was synthesized. Real-time PCR was performed using the QuantiTect Probe PCR Kit and the QuantiTect Gene Expression Assay for human tubulin.

Efficient Removal of Genomic DNA from RNA Samples



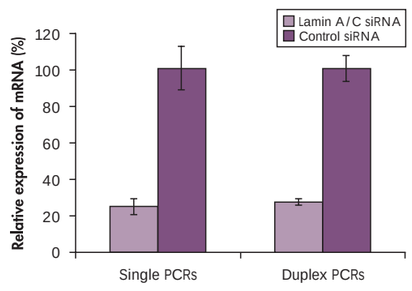
Effective removal of contaminating genomic DNA from small to large RNA samples, ensuring quantification of RNA only in real-time RT-PCR analysis. Using the QuantiTect Reverse Transcription Kit (which is a component of the direct cDNA kit), a 1:1 mixture of RNA and genomic DNA was processed in 3 different ways: genomic DNA removal followed by reverse transcription (**+gDNA removal/+RT**); no genomic DNA removal and no reverse transcription (**–gDNA removal/–RT**); genomic DNA removal with no reverse transcription (**+gDNA removal/–RT**). Real-time PCR was performed using the QuantiTect Probe PCR Kit and the QuantiTect Gene Expression Assay for GAPDH.

Effective Monitoring of Gene Silencing Using Real-Time, Multiplex PCR

In RNA interference experiments, gene silencing efficiency can be determined using quantitative, real-time PCR. The expression of the gene being knocked down is quantified and normalized to the expression of a control gene in the same sample. To enable reliable quantification and to save time and reduce costs, targets can be amplified together in real-time, multiplex PCR.

QuantiTect Multiplex PCR Kits provide a simple solution for real-time, duplex PCR of target and control genes which is compatible with various sequence-specific probe technologies (e.g., TaqMan probes and QuantiProbes). There is no need for optimization by the user, and reliable results are obtained at the first attempt, with threshold cycle (C_T) values equivalent to those obtained when performing amplifications in separate reactions. For further convenience, QuantiTect Multiplex PCR Kits can be used in combination with QuantiTect Endogenous Control Assays and QuantiTect Gene Expression Assays, which are validated, ready-to-use primer–probe sets for reference and target genes.

Reliable Monitoring of siRNA-Mediated Gene Silencing Using Real-Time, Duplex PCR



Analysis of relative expression of lamin A/C after siRNA-mediated knockdown, showing that the QuantiTect Multiplex PCR Kit produces similar results in real-time, single PCR and real-time, duplex PCR. Single PCRs were performed using the QuantiTect Gene Expression Assays for lamin A/C and GAPDH (both with FAM labeled QuantiProbe™). Duplex PCR was performed using the QuantiTect Gene Expression Assay for lamin A/C (with FAM labeled QuantiProbe) and the QuantiTect Endogenous Control Assay for GAPDH (with Yakima Yellow™ labeled QuantiProbe). Expression of lamin A/C was normalized to the expression of GAPDH and quantified using the $\Delta\Delta C_T$ method.

Comparable Results Between Multiplex PCR and Corresponding Single PCRs

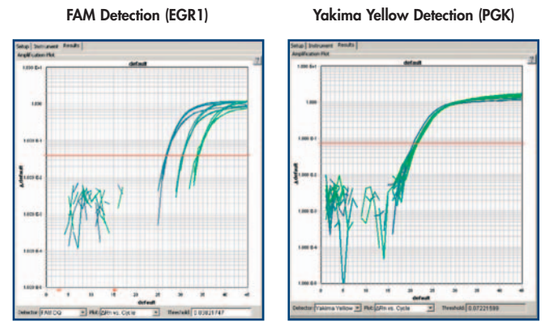
For a real-time, multiplex PCR assay to be successful, the C_T values generated must be equivalent to those obtained in real-time, single PCR. This can be achieved at the first attempt using QuantiTect Multiplex PCR Kits, even in multiplex analysis of targets which vary greatly in abundance.

Comparable C_T Values Between Triplex PCR and Single PCRs

	Detection of		
	t(8;14) (20 copies)	GAPDH (10^6 copies)	NFκB (copies in parentheses)
Triplex PCR	34.31	20.37	21.92 (10^5)
Single PCRs	34.07	20.54	21.83 (10^5)
Triplex PCR	34.61	20.62	25.03 (10^4)
Single PCRs	34.00	20.46	25.19 (10^4)
Triplex PCR	35.17	19.94	28.38 (10^3)
Single PCRs	34.43	20.50	28.65 (10^3)

Real-time, triplex PCR of high, low, and variable amounts of target genes using the QuantiTect Multiplex PCR Kit and TaqMan probes, showing C_T values similar to those obtained in real-time, single PCR.

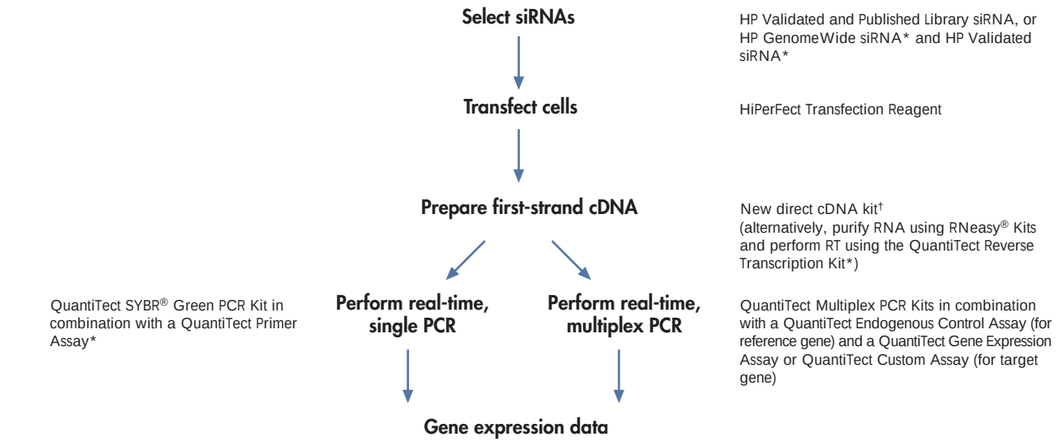
Comparable C_T Values Between Duplex PCR and Single PCRs



Real-time, duplex PCR of different ratios of targets using the QuantiTect Multiplex PCR Kit, producing C_T values similar to those obtained in real-time, single PCR (in the figure, the amplification plots for duplex and single PCR are overlaid). The targets were variable amounts of human EGR1 sequence mixed with constant amounts of human PGK sequence (10^6 copies). Duplex PCR and single PCRs of each template mixture were performed in duplicate using the QuantiTect Gene Expression Assay for EGR1 and the QuantiTect Endogenous Control Assay for PGK.

Complete Solution for Validation of RNA Interference

The new direct cDNA kit and the QuantiTect Multiplex PCR Kits form part of a complete system that streamlines the procedure for validation of RNA interference, saving time and reducing costs. The latest developments are shown below. For more information, contact your local QIAGEN office.



* Available from QIAGEN from April 2005.

† Available from QIAGEN later in 2005.

Summary

- Using the new direct cDNA kit, first-strand cDNA can be prepared directly from cultured cells in less than one hour
- Genomic DNA contamination of RNA samples, which may affect the accuracy of real-time RT-PCR analysis, is eliminated by the direct cDNA kit or the QuantiTect Reverse Transcription Kit
- Using QuantiTect Multiplex PCR Kits, quantitative gene expression data obtained in multiplex PCR are equivalent to those obtained in single PCRs
- QuantiTect Multiplex PCR Kits are compatible with all types of primer–probe systems (e.g., TaqMan probes) and can be used immediately with primer–probe sets that have already been established
- The combination of QuantiTect Multiplex PCR Kits with QuantiTect Assays provides a ready-to-use solution for real-time, duplex PCR
- QuantiTect Multiplex PCR Kits enable success in real-time, multiplex PCR at the first attempt with no need for lengthy optimization steps, such as determining limiting primer concentrations

1030455 02/2005