Effective genomic DNA removal for accurate gene expression analysis

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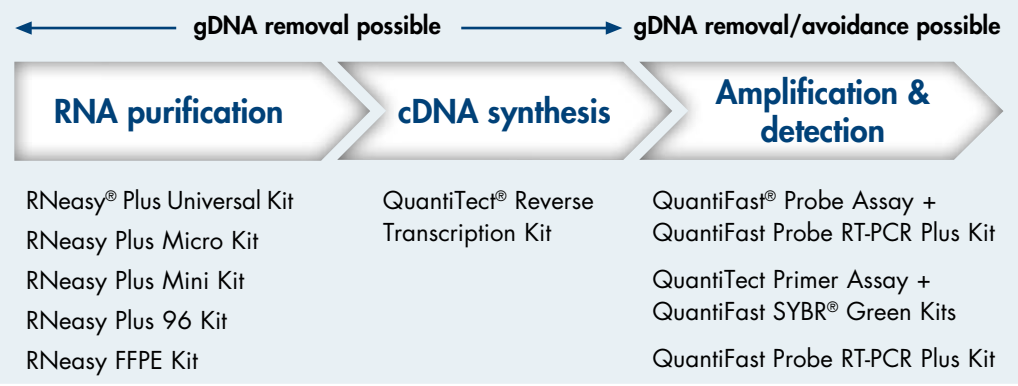
The elimination of genomic DNA (gDNA) contamination in an RNA sample is essential for achieving accurate gene expression results. gDNA contamination may decrease the accuracy of real-time RT-PCR gene expression analysis if the primers used amplify both cDNA and gDNA sequences, resulting in false-positive real-time PCR signals with lower C_T values than true positives. Importantly, RNA from samples containing highly fragmented nucleic acids (e.g., from formalin-fixed, paraffin embedded [FFPE] tissue) often has very high levels of residual gDNA because the small size of fragmented gDNA results in its co-purification with RNA. Here, we examine several solutions for effective gDNA removal at different timelines in the gene expression analysis workflow: during RNA purification, reverse transcription, and data acquisition.



Elimination of gDNA during purification

gDNA removal can be integrated with RNA purification, eliminating the requirement to use DNase after the isolation procedure. Depending on the RNeasy kit, gDNA may be eliminated with gDNA Eliminator spin columns, DNAse treatment to remove DNA from FFPE samples, or improved phase separation. The RNeasy Plus Kit, which uses gDNA Eliminator spin columns to remove gDNA, and an RNA purification kit with integrated genomic DNA removal from Supplier A_v, were tested for gDNA removal efficiency (Figure 1). Duplicate real-time RT-PCR assays for β-actin were performed with (+RT) or without (–RT) reverse transcriptase. The –RT curves demonstrate that RNA purified using the RNeasy Plus Mini Kit was virtually free of gDNA, whereas the RNA purified using the kit from Supplier A_v resulted in a lower C_T value indicating possible gDNA contamination. ▶

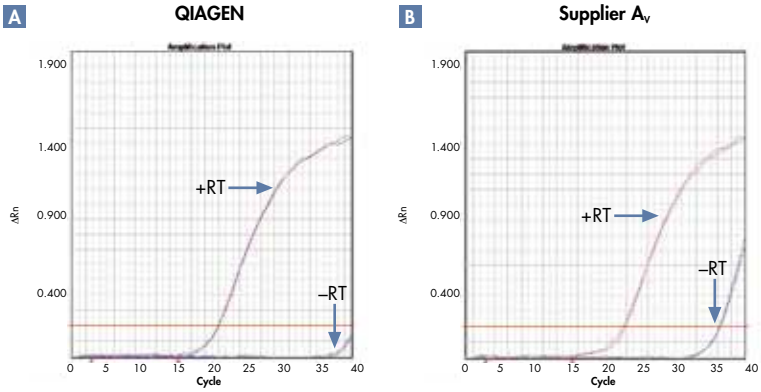


Figure 1. Efficient genomic DNA removal using the RNeasy Plus Mini Kit. Total RNA was purified from Jurkat cell samples (1 x 10^6 cells per sample) using A the RNeasy Plus Mini Kit or B an RNA purification kit with integrated genomic DNA removal from Supplier A_V. Duplicate real-time RT-PCR assays for β-actin were performed with (**+RT**) or without (**–RT**) reverse transcriptase.

Integration of cDNA synthesis with gDNA removal

Following RNA purification, cDNA is synthesized from RNA for use as a template in real-time PCR. Inclusion of gDNA removal during cDNA synthesis can ensure accurate gene expression results and eliminates the need to design cDNA-specific PCR primers, an important consideration since it is not always possible to design exon-spanning primers. gDNA Wipeout Buffer in the QuantiTect Reverse Transcription Kit effectively eliminates gDNA contamination. When two methods for cDNA synthesis were compared using either the QuantiTect Reverse Transcription Kit (with supplied RT primer mix) or a kit from Supplier T (with mix of oligo-dT and random primers), the reactions with and without reverse transcriptase (+RT and –RT) from Supplier T showed signal overlap due to genomic DNA contamination. The –RT signal from cDNA purified by the QuantiTect Reverse Transcription Kit generated no signal, indicating complete elimination of gDNA (Figure 2).

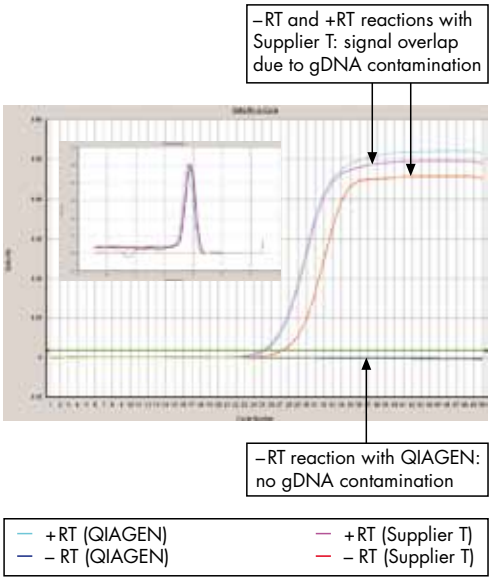


Figure 2. Effective removal of genomic DNA using gDNA Wipeout Buffer. Total RNA was purified from HeLa cells using the RNeasy Mini Kit. cDNA was synthesized from 1 µg RNA using either the QuantiTect Reverse Transcription Kit (with supplied RT primer mix) or a kit from Supplier T (with mix of oligo-dT and random primers). Reactions were carried out either with (**+RT**) or without (**–RT**) reverse transcriptase. Samples equivalent to 16 ng cDNA were analyzed on the Applied Biosystems® 7500 Real-Time PCR System using the QuantiFast SYBR Green PCR Kit and a primer pair specific for the cDNA and genomic DNA sequences of E2F3 (E2F transcription factor 3). Melting curve analysis was also carried out (see inset): all reactions provided a single peak, demonstrating specific amplification of the intended amplicon.

gDNA can impact data from highly degraded RNA samples

Samples containing highly degraded RNA (e.g., RNA from FFPE tissue) often contain a high ratio of gDNA to RNA and may result in false-positive real-time PCR signals if the gDNA is not eliminated. Real-time RT-PCR was performed on RNA isolated from FFPE tissue using the QuantiFast Probe RT-PCR Plus Kit, which contains gDNA Wipeout Buffer, or an alternative kit that does not

remove gDNA. The alternative kit gave inaccurate quantification values due to the detection of both cDNA and gDNA, whereas the QuantiFast Probe RT-PCR Plus Kit provided accurate quantification due to detection of cDNA only (Figure 3).

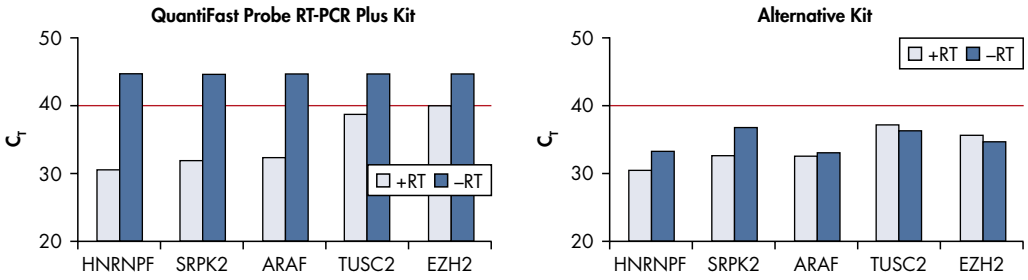


Figure 3. Effective elimination of genomic DNA from FFPE samples. Real-time RT-PCR was performed on RNA isolated from FFPE tissue using the QuantiFast Probe RT-PCR Plus Kit or an alternative kit without gDNA Wipeout Buffer. +RT indicates the signal that is due to mRNA, while –RT corresponds to the signal coming from contaminating gDNA. The alternative kit showed similar C_T values with, or without, the addition of RT leading to highly sensitive, but false positive, results. The QuantiFast Probe RT-PCR Plus Kit provided accurate quantification due to detection of cDNA only. The red line at C_T=40 indicates the limit of detection. Signals that are above the line are not true positives.

Optimized SYBR Green primer design enables specific detection of cDNA

Careful primer design can exclude results from contaminating gDNA during data acquisition. QuantiTect Primer Assays, which are designed to only detect cDNA using primers that span exon/exon boundaries, enable analysis of cDNA targets in the presence of gDNA using SYBR Green (Figure 4A and 4B). High PCR specificity was achieved when the QuantiTect Primer Assay for IL8 (a chemokine), in combination with the QuantiFast SYBR Green PCR Kit, was used to analyze different amounts of leukocyte cDNA (Figure 4C and 4D).

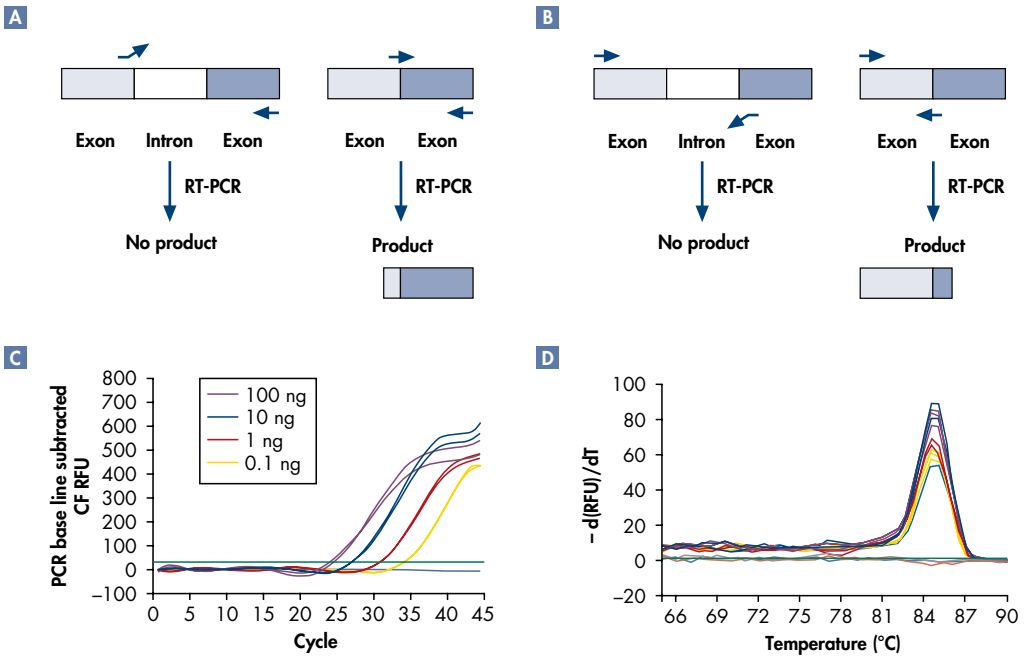


Figure 4. Specific detection of cDNA with QuantiTect Primer Assays. A Primer design to eliminate signals from contaminating gDNA. Forward primer crosses an intron/exon boundary. B Reverse primer crosses an intron/exon boundary. C Different amounts of leukocyte cDNA were analyzed in duplicate using the QuantiTect Primer Assay for IL8 and the QuantiFast SYBR Green PCR Kit. No template control (NTC) reactions were also performed (shown by the flat plot). D Melting curve analysis, demonstrating high PCR specificity on the iCycler® from Bio-Rad.

Ordering Information

Product	Contents	Cat. no.
Products for RNA purification		
RNeasy Plus Mini Kit (50)*	RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water, and Buffers	74134
RNeasy Plus Universal Mini Kit (50)*	RNeasy Mini Spin Columns, gDNA Eliminator Solution, Collection Tubes, RNase-Free Water, and Buffers	73404
RNeasy FFPE Kit (50)	RNeasy MinElute® Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	73504
RNeasy Plus Micro Kit (50)	RNeasy MinElute Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Water, and Buffers	74034
RNeasy Plus 96 Kit (12)	12 RNeasy 96 Plates, 12 gDNA Eliminator 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers	74192
Products for separate cDNA synthesis		
QuantiTect Reverse Transcription Kit (50)*	100 µl 7x gDNA Wipeout Buffer, 50 µl Quantiscript® Reverse Transcriptase, 200 µl 5x Quantiscript RT Buffer, 50 µl RT Primer Mix, 1.9 ml RNase-Free Water	205311
Products for amplification and detection		
QuantiFast Probe RT-PCR Plus Kit (80)*	0.5 ml 2x QuantiFast Mix 1, 0.5 ml 2x QuantiFast Mix 2, 20 µl RT Mix, 45 µl ROX Dye Solution, 45 µl High-ROX Dye Solution, 1.9 ml RNase-Free Water	204482
QuantiFast Probe Assay (80)*	Primer/probe set (FAM™ and/or MAX™ label) for 80 x 25 µl reactions + QuantiFast Probe RT-PCR Plus Kit (80)	Varies
QuantiTect Primer Assay*	10x QuantiTect Primer Assays (lyophilized) supplied in a single tube	Varies
QuantiFast SYBR Green PCR Kit (400)*	3 x 1.7 ml 2x QuantiFast SYBR Green PCR Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water	204054
QuantiFast SYBR Green RT-PCR Kit (400)*	3 x 1.7 ml 2x QuantiFast SYBR Green RT-PCR Master Mix (contains ROX dye), 100 µl QuantiFast RT Mix, 2 x 2 ml RNase-Free Water	204154

* Other sizes available, please inquire.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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