Rapid and complete removal of gDNA from both small and large volume RNA preparations

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INTRODUCTION

The presence of genomic DNA (gDNA) has the potential to introduce incorrect results when performing highly sensitive applications, such as RT-qPCR. Thus, removal of gDNA from RNA preps is often a necessity for obtaining correct quantification of RNA, a procedure most commonly achieved by DNase I treatment. DNase I must however be inactivated prior to reverse transcription using cumbersome protocols that usually includes excessive heat (degrades RNA), use of resin (time consuming and difficult to perform on small volumes) or addition of EDTA (might inhibit downstream applications). Consequently, there are very few options available for convenient removal of gDNA from small volumes of RNA without affecting the quantity and quality of the RNA transcriptome.

ArcticZymes has developed a heat-labile double-strand specific DNase (HL-dsDNase) that is easily inactivated by heating to 55˚C, a temperature leaving RNA intact. The DNase treatment is fully scalable, thereby enabling the removal of gDNA from minor volumes of low-concentration RNA. The treatment does not introduce any loss or bias to the RNA. In the Heat&Run kit, the unique properties of HL-dsDNase are used to ensure continuous same-tube DNase treatment and reverse transcription, thereby minimizing pipetting steps and making the protocol very adaptable to high-throughput workflows. Here we show that this rapid and straightforward Heat&Run protocol degrades contaminating gDNA to levels below the detection limit of qPCR. It is also shown that the RNA transcriptome, here represented by mRNA, micro RNA and long non-coding RNA, remains completely unharmed by the treatment.

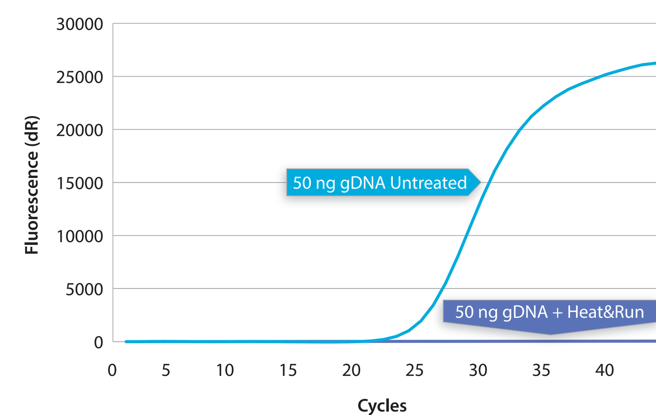
METHODS

Total RNA was isolated from human breast carcinoma cells using a Trizol protocol. Purified RNA (400 ng) spiked with gDNA (1.2 ng) in a volume of 8 µl was decontaminated by addition to 1 µl HL-dsDNase (2U) and 0.8 µl 10x reaction buffer in a PCR tube on ice. The sample was incubated for 10 minutes at 37°C followed by 5 minutes at 55°C. Untreated control (identical amount of template added 1.8 µl H2O) was stored on ice during the HL-dsDNase treatment protocol. Decontamination using the Turbo DNA-free kit (Life) was performed according to the manufacturers protocol. In comparison studies, inactivation resin was substituted with similar amounts of water in untreated controls and Heat&Run samples.

Reverse Transcription was performed in the same tube by adding RT reagents directly to HL-dsDNase treated samples and untreated control. The Roche Transcriptor cDNA synthesis kit was used for synthesizing cDNA from mRNA and noncoding RNA. SYBR Green qPCR was performed using Eurogentec MESA SYBR MasterMix (SDHA, HOTAIR, and ValidPrime (genome specific primers, TATAA Biocenter)). The Qiagen miScript RTII kit was used for synthesizing cDNA from miRNA. miR-17 was quantified using the miR-17 primer assay with miScript SYBR Green PCR Kit (both from Qiagen).

All qPCR were performed on a Stratagene Mx3005P instrument (Agilent). All experiments were repeated in triplicates.

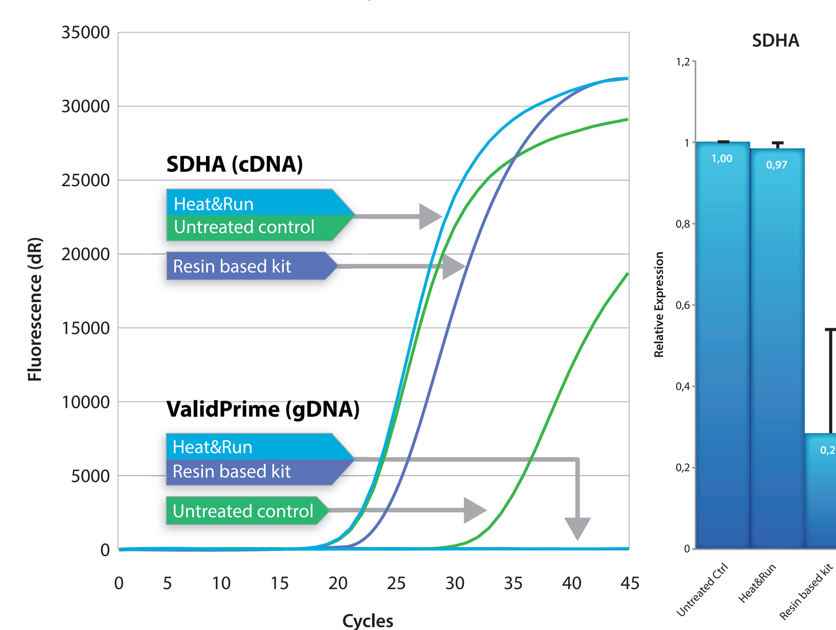
RESULTS



The Heat&Run kit efficiently removes gDNA

The capacity of the Heat&Run kit to degrade sufficient amounts of gDNA was investigated. In Figure 1 it is shown that 50 ng of gDNA in a 10 µl reaction volume is removed to levels below the detection limit of qPCR.

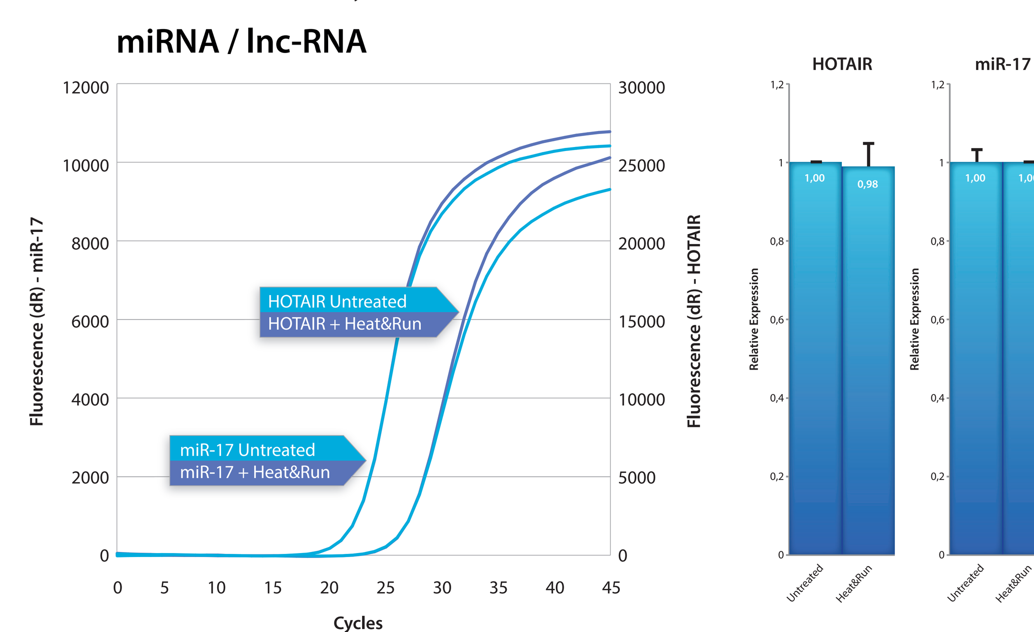
Figure 1: Amplification plots of Heat&Run treated gDNA and untreated control. Heat&Run treatment digested all DNA present.



The quantity and quality of mRNA is not affected by the treatment

The performance of the Heat&Run kit when decontaminating RNA in a total reaction volume of 10 µl was compared to that of a resin based DNase I kit. Both treatments succeeded in removing all gDNA present. However, it was also shown that the amount of cDNA produced was unaffected by the Heat&Run protocol, while two thirds of the mRNA quantified by qPCR was lost when a DNase I kit with resin based inactivation was used (Figure 2).

Figure 2: Amplification plots and histogram of SDHA mRNA. The standard deviation of the histogram is determined from two biological replicates. Both Heat&Run and Turbo DNA-free treatment succeeded in removing all present gDNA. No loss of RNA was observed using the Heat&Run kit, while Turbo DNA-free treatment caused a significant loss from low-volume samples.

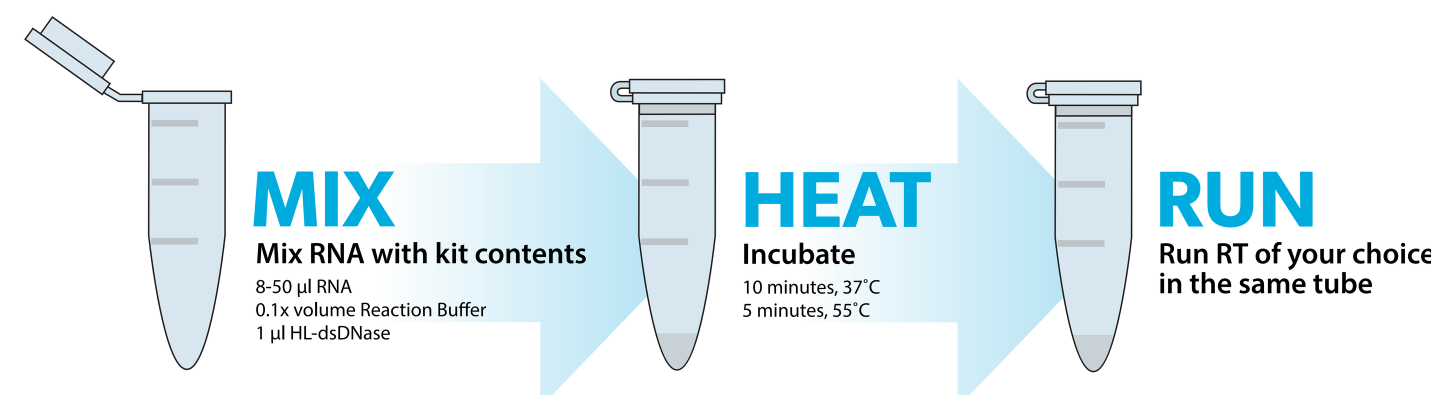


The Heat&Run kit does not affect the RNA transcriptome

To get an outline of the impact of Heat&Run treatment on other classes of the RNA transcriptome, long non-coding RNA (HOTAIR) and miRNA (miR-17) were quantified after Heat&Run treatment and compared to untreated controls stored on ice. As shown in Figure 3, no impact on these classes of RNA was observed as a result of the treatment.

Figure 3: Amplification plots and histogram of miR-17 (miRNA) and HOTAIR (lnc-RNA). The standard deviation of the histogram is determined from two biological replicates. Heat&Run treatment did not cause any loss of miRNA or lnc-RNA.

PROTOCOL



CONCLUSION

- The Heat&Run protocol enables fast and efficient degradation of gDNA without affecting the quantity and quality of RNA.
- Removal of gDNA can be performed on RNA volumes ranging from 8-50 µl.
- Reverse Transcription can be completed in the same tube, ensuring an easy and efficient workflow

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