Heat–Labile dsDNase treatment for rapid and complete removal of gDNA from RNA preparations without affecting quantity or quality of the RNA transcriptome

INTRODUCTION

Traditionally, highly expressed mrnas have been the main target for transcriptional research and in the last decade increased attention for the entire transcriptome has emerged. Column based kits are expensive and have a preference for particular sizes of RNA, a fact that has led to a renaissance for Trizol-based RNA isolation. When performing RT-qPCR on targets with low expression, contaminating genomic DNA (gDNA) from RNA isolation can cause erroneous results. DNase treatment of RNA preps has so far been time consuming and/or resulted in loss of RNA, especially as a result of DNase inactivation. These inactivation steps have typically included excessive heat (degrades RNA), use of resin (time consuming and difficult to perform on small volumes) or addition of EDTA (might inhibit downstream applications).

ArcticZymes has developed a heat-labile dsDNase (HL-dsDNase) that efficiently degrades dsDNA in a 10-minute incubation step at 37°C. Irreversible inactivation of HL-dsDNase is achieved by heating the sample to 55°C for 5 minutes. The HL-dsDNase treatment can easily be performed in volumes ranging from 8–50 µl, and is gentle enough to avoid degradation of RNA. Here we show that this rapid and straightforward HL-dsDNase protocol degrades contaminating gDNA in RNA preps to levels below the detection limit of qPCR. The HL-dsDNase treatment does not affect the Cq values of cDNA quantified in downstream reverse transcription and qPCR, and introduces no bias for particular species or sizes of RNA.

RESULTS

HL-dsDNase efficiently removes gDNA

The capacity of HL-dsDNase to degrade sufficient gDNA in the described protocol was investigated. It was shown that HL-dsDNase treatment degraded at least 50 ng of gDNA in a 10µl reaction volume to levels below the detection limit of qPCR (Figure 1).

The HL-dsDNase protocol leaves mRNA unharmed

To get an outline of the impact of HL-dsDNase treatment on the RNA transcriptome, intermediate and low copy mRNA (SDHA and JUN respectively), long non-coding RNA (HOTAIR) and miRNA (miR-17) were quantified after HL-dsDNase treatment and compared to untreated control stored on ice. As shown in Figure 2 and 3, no significant change in any RNA was observed as a result of the HL-dsDNase treatment, while all contaminating gDNA was eliminated.

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 8µl was decontaminated by addition to 1µl HL-dsDNase (2U) and 0.8µl 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 1.8µl H2O) was stored on ice and served as a control.

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 the Agilent Brilliant III probe MasterMix (JUN - Taq-Man assay, Applied Biosystems). 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 triplicate.

CONCLUSION

The described HL-dsDNase protocol enables fast and efficient degradation of gDNA without affecting the quantity or quality of the RNA transcriptome.

The protocol can be performed on volumes ranging from 8-50 µl, and is compatible with both one-step and two-step RT kits.