Contamination control with the heat-labile cod UNG ensures accurate and reliable results in your post-PCR analysis

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INTRODUCTION

The sensitivity of PCR renders the method prone to false positives, in particular from previous PCRs; so called “carry-over contamination” (1). By employing the uracil-DNA glycosylase (UNG) de-contamination technology, carry-over contamination can be eliminated. However, post-PCR reactivation of UNG hampers this method, resulting in rapid degradation of the newly synthesized PCR products, and thus impairing any downstream analysis (2,3,4,5).

In our recent studies, residual UNG activity from various commercially available UNGs have been estimated and compared by means of analyzing the quality of the PCR products following UNG treatment. While all the commercially available UNGs tested reactivated post-PCR and gave poor and unreadable sequences, cod UNG did not reactivate. Hence, cod UNG was the only UNG that was completely and irreversibly inactivated upon moderate heat treatment, enabling downstream analysis, such as cloning and sequencing

METHODS

Residual UNG activity was determined by performing a PCR with uracil (10mM ACG, 20mM U) and 1U of five commericallyavaiable UNGs. Post-PCR, samples were incubated at room temperature for 0h, 1h, 3h, 6h and 24h, succeeded by heating the PCR products to 95^{o}C for 10 minutes before cooling them down . The extent of DNA degradation were evaluated on an agarose-gel.

Post-PCR sequence quality and integrity were further evaluated by performing a PCR and including 1U of four commericallyavaiable UNGs in the mastermix. Ensuing PCR, samples were incubated at either room temperature or 4^{o}C at various time intervals, including 0h, 3h, 6h and 24h, before the PCR products were purified and sequenced. Sequence data was thoroughly analysed with emphatis on reduced sequence quality as a result of UNG reactivation.

REFERENCES

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RESULTS

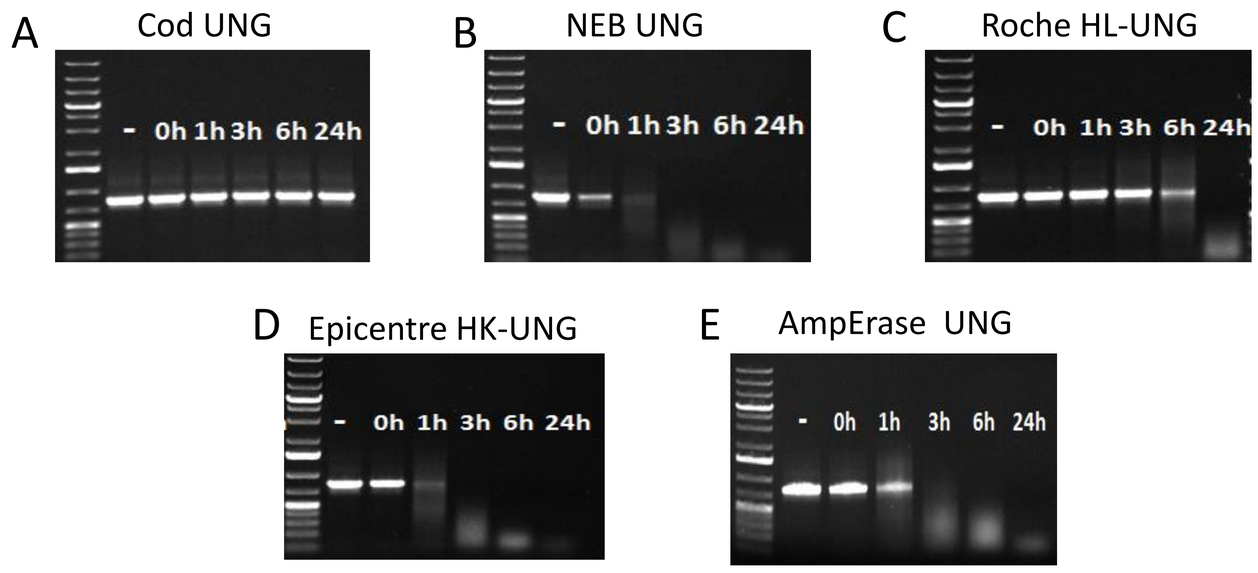

Figure 1. The only UNG that became completely and irreversibly heat-inactivated was cod UNG.
PCR pre-treated with 1U of (A) cod UNG (ArcticZymes) (B) *E.coli* UNG (New England BioLabs) (C) HL-UNG (Roche) (D) HK-UNG (Epicentre, Illumina) (E) AmpErase UNG (Applied Biosystems). Following PCR, products were incubated at room temperature at various time intervals before being analysed on an agarose gel.

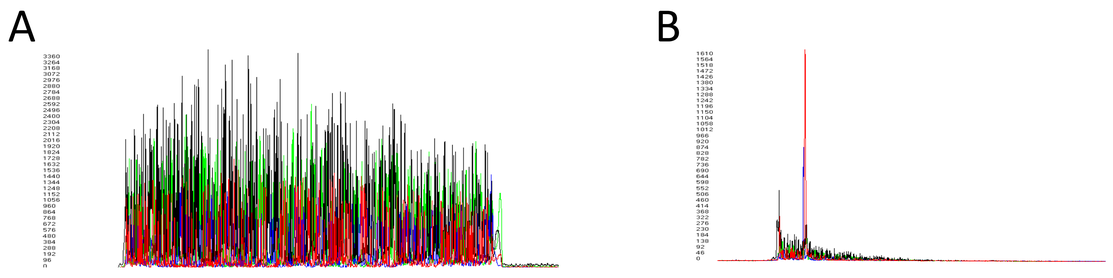

Figure 2. Only cod UNG leaves sequence quality intact
Chromatograms of sequenced PCR products pretreated with 1U of (A) cod UNG (ArcticZymes) or (B) *E.coli* UNG (New England Biolabs) and incubated at room temperature for 3h

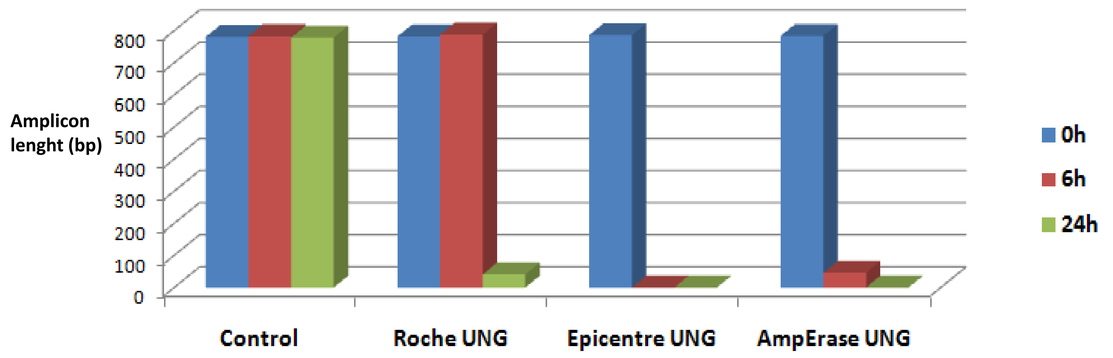

Figure 3. UNG reactivation resulted in degraded sequences
Sequence data of PCR products that had been pretreated with 1U Roche UNG, 1U Epicentre UNG or 1U AmpErase UNG and incubated at room temperature all demonstrated reactivation of UNG and severe degradation of PCR products.

CONCLUSION

UNG can eliminate carry-over contamination, but UNG-reactivation can hamper your post-PCR analysis

Cod UNG is the only commercially avaiable UNG that is completely and irreversibly inactivated by moderate heat treatment, enabeling post-PCR analysis such as cloning and sequencing

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