Cod UNG PCR decontamination enables post-PCR analysis

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INTRODUCTION

Contamination of PCR samples with amplicon DNA generated by previous reactions, “carry over contamination”, is considered to be one of the major sources of false positive results (1). Use of the uracil-DNA glycosylase (UNG) decontamination method has since its invention been hampered by post-PCR reactivation of UNG (2, 3, 4). As a result of this reactivation, the newly produced PCR products quickly degrade, making downstream analysis , such as sequencing of the PCR product, challenging.

The heat-labile cod UNG is completely and irreversably inactivated by moderate heat-treatment. In this study we have tested the quality of PCR products treated with various commercially available UNGs and analyzed the quality of the sequencing data.

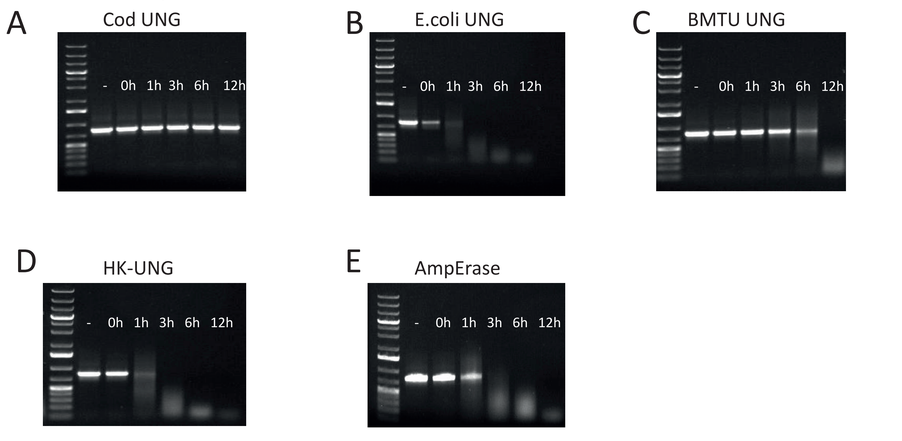
METHODS

To determine residual UNG activity a PCR was performed with uracil (10mM ACG, 20mM U) and 1 unit of 5 different commercially available Uracil DNA glycosylases. The PCR product was then incubated at room temperature for 0h, 1h, 3h, 6h and 24h, post PCR. The PCR product was heated to 95°C for 10 min, cooled down, and then analyzed on a agarose-gel to evaluate the extend of DNA degradation.

To determine the ability to do downstream analysis of UNG treated PCR products the products were set up for sequencing and the quality of the sequence determined.

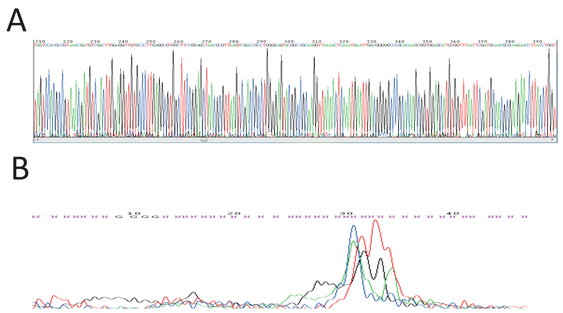
RESULTS

Figure 1. CodUNG is the only UNG which is completely and irreversibly heat-inactivated



PCR reactions pre-treated with (A)1 U codUNG (Marine Biochemicals), (B) E.coli UNG (New England Biolabs), (C) BMTU UNG (Roche), (D) HK-UNG (Epicentre) and (E) AmpErase (Applied Biosystems). After PCR the products were incubated at room temperature at different time-points before they were analyzed on a agarose gel to monitor product degradation.

Figure 2. CodUNG treated PCR reactions can be sequenced post PCR



Chromatogram of sequenced PCR products pretreated with cod UNG (A) and E.coli UNG (B). Only codUNG treated PCR products gave readable sequences.

CONCLUSION

Use of the uracil-DNA-glycosylase decontamination method is hampered by the reactivation of UNG after PCR.

Cod UNG is the only commercially available UNG that is completely and irreversibly inactivated

PCR reactions pre-treated with codUNG remains intact after PCR making downstream analysis, such as cloning and sequencing, possible.

REFERENCES

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