Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics Supplement Series 1 (2008) 19–21
www.elsevier.com/locate/FSIGSS

Research article

Single primer extension (SPEX) amplification to accurately genotype highly damaged DNA templates

Paul Brotherton [a,b,*], Phillip Endicott [b], Mark Beaumont [c], Ross Barnett [d], Jeremy Austin [a], Alan Cooper [a], Juan J. Sanchez [e]

[a] *Australian Centre for Ancient DNA, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia*
[b] *Henry Wellcome Ancient Biomolecules Centre, University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK*
[c] *School of Animal and Microbial Sciences, University of Reading, Reading RG6 6AG, UK*
[d] *Department of Archaeology, University of Durham, South Road, Durham DH1 3L, UK*
[e] *National Institute of Toxicology and Forensic Sciences, Canary Islands delegation, 38320, Tenerife, Spain*

Received 20 August 2007; accepted 9 October 2007

Abstract

High levels of non-authentic sequence data can be generated by traditional PCR-based methodologies when DNA is damaged, template numbers are small and/or the target amplification size too large. We therefore present an alternate methodology based on single primer extension (SPEX) amplification; that places no pre-defined size constraints on amplification and interacts with only one of the DNA strands at the target locus.

Keywords: DNA damage; Genotyping; Low copy number DNA; SNPs

1. Introduction

DNA sequences from forensic and ancient samples can only be inferred indirectly following PCR amplification. As these sequences are the primary input into further enquiries, the generation of accurate sequence data is a critical issue and likely to come under increasing scrutiny. Traditional PCR-based methodologies can be inherently unsuited to the job of deriving quantitative conclusions and accurate consensus sequence data from damaged and degraded templates. The basic unreliability of PCR-derived sequences from damaged and fragmented DNA is highlighted by the inability of a large number of studies to fully resolve the molecular nature of DNA miscoding lesion damage over a 20-year period.

2. Materials and methods

We developed a novel single primer extension (SPEX) amplification methodology, specifically designed to avoid many of the pitfalls of PCR-based approaches (Fig. 1). In direct contrast to traditional PCR, SPEX specifically targets only one of the template strands at a locus-of-interest and imposes no predefined target length. SPEX has successfully amplified human and extant and extinct animal species with a spread of carbon-dated ages from 200-year-old temperate samples to 60,000-year-old frozen permafrost samples [1].

3. Results and discussion

All polymerase-based methodologies introduce a background level of polymerase misincorporation errors, but the use of SPEX, followed by homopolymer tailing, avoids the potential creation of additional PCR-generated artefacts. SPEX needs no specialised equipment and has successfully generated sequence data of unprecedented accuracy from specimens up to

* Corresponding author. Tel.: +441457858605.
E-mail address: paul.brotherton@virgin.net (P. Brotherton).

1875-1768/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigss.2007.10.111

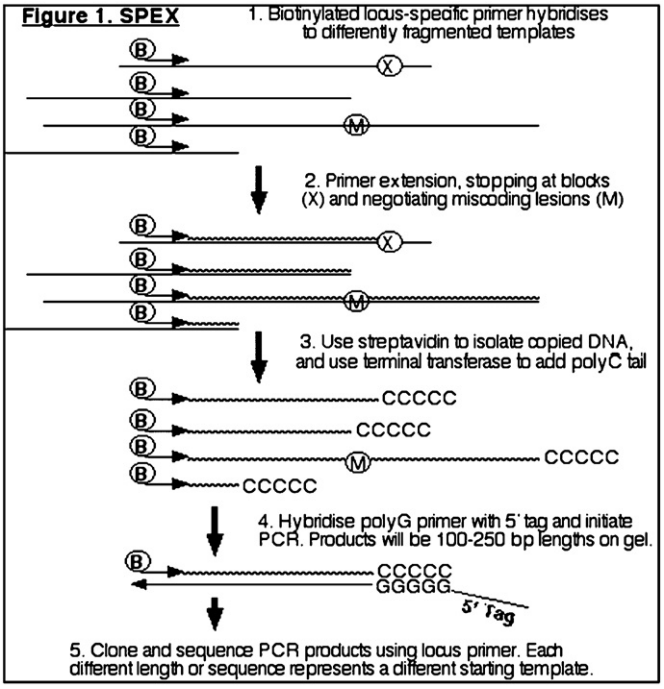

Fig. 1.

60,000-years-old (without the generation of sequence artefacts often produced by PCR). The first-generation copies of DNA templates produced by SPEX provide direct information about the effective amplifiable length of damaged DNA, and can produce a quantifiable (up to 40-fold or more) depth-of-coverage from a single aliquot of extract – a level far greater than with any other current methodology [1].

Various biochemical explanations for several different kinds of miscoding lesion DNA damage were previously proposed based on PCR-derived sequence variation [2,3]. However, SPEX-derived data showed that the majority of previously proposed miscoding lesions do not occur in ancient DNA, with some mistakenly inferred from significant levels of wholly PCR-generated sequence artefacts [1]. SPEX instead demonstrated that C > U-type base modifications (observed as complementary G > A transitions in SPEX sequence traces; Fig. 2) are the sole cause of endogenous miscoding lesions in ancient DNA [1].

Preliminary data also shows that SPEX can preferentially amplify endogenous DNA templates from ancient, archaeological and forensic specimens over modern contamination. DNA was extracted from modern hair and deliberately mixed with DNA extracted from a >5000-year-old archaeological specimen (with known multiple SNP differences) so that a ~50:50 ratio of the two genotypes was observed following traditional PCR and cloning. However, >98% of SPEX-generated sequences corresponded to the endogenous genotype of the ancient specimen. Presumably this effect occurs because most SPEX primer extensions on damaged and degraded templates from ancient specimens (Fig. 2) are shorter than those on modern contaminants; therefore these shorter SPEX amplicons are subsequently preferentially amplified.

SPEX can target a far greater number of highly damaged, up to 60,000-year-old, DNA templates than PCR (as short as 35–50 bases in length) and, unlike PCR, is capable of clearly identifying which sequence traces originated from separate DNA template molecules. This makes SNP genotyping and sequence inferences following SPEX amplification, as well as quantitative analyses of DNA damage, inherently more accurate than those based on traditional PCR amplification.

Funding source

The work was supported by grants from NERC, Wellcome and Leverhulme Trusts. J.J. Sanchez was supported by an interministerial grant (MJU-MEC 2007). The sponsors had no involvement in the development of the paper or decisions related to the paper.

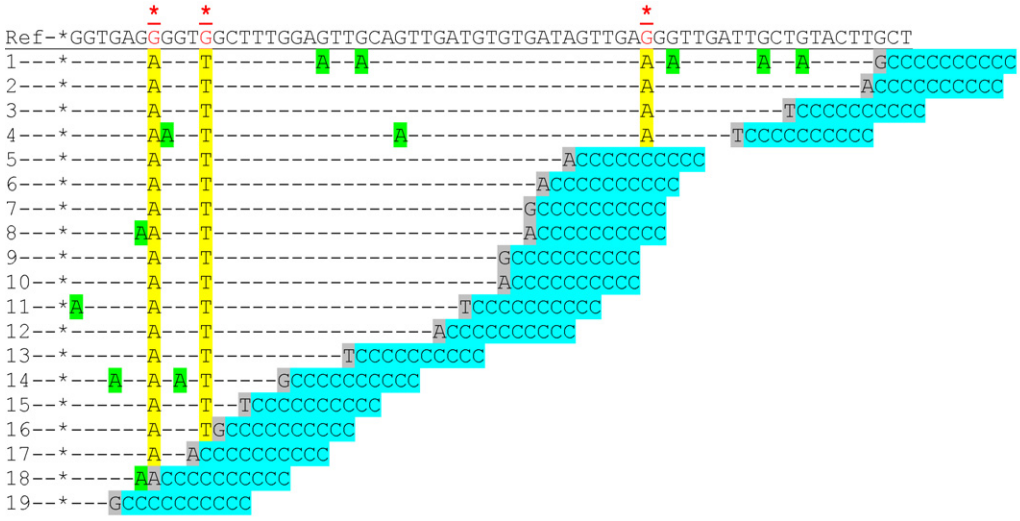

Fig. 2.

Conflict of interest

None.

References

[1] P. Brotherton, P. Endicott, J.J. Sanchez, M. Beaumont, R. Barnett, J. Austin, A. Cooper, Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions, Nucl. Acid. Res. (2007), doi:10.1093/nar/gkm588.

[2] M.T. Gilbert, A.J. Hansen, E. Willerslev, L. Rudbeck, I. Barnes, N. Lynnerup, A. Cooper, Characterization of genetic miscoding lesions caused by postmortem damage, Am. J. Hum. Genet. 72 (2003) 48–61.

[3] M. Stiller, R.E. Green, M. Ronan, J.F. Simons, L. Du, W. He, M. Egholm, J.M. Rothberg, et al., Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA, Proc. Natl. Acad. Sci. USA 103 (2006) 13578–13584.