Repair of FFPE DNA damage

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BACKGROUND:

Formalin-fixed, paraffin-embedded (FFPE) samples represent the most comprehensive collections of patient material in hospital pathology archives. With the advent of high throughput sequencing, FFPE material represents a valuable trove of source material for genomic analysis. However, the effects of the FFPE process on DNA must be taken into account for reliable analysis.

DNA damages is known to be in the form of: (i) Cross-links (DNA-DNA, Protein-DNA), (ii) depurination, leading to (iii) DNA fragmentation and (iv) sequence alterations (chimeras, SNPs).

These negatively affect sequencing outputs, by reducing: a) the sequencing depth, b) sequencing uniformity, c) read length, d) ratio of reads passing quality filtering; and increasing a) the number of chimeric reads. b) FFPE derived single nucleotide polymorphisms (SNPs), translocations, and insertions and deletions (indels) .

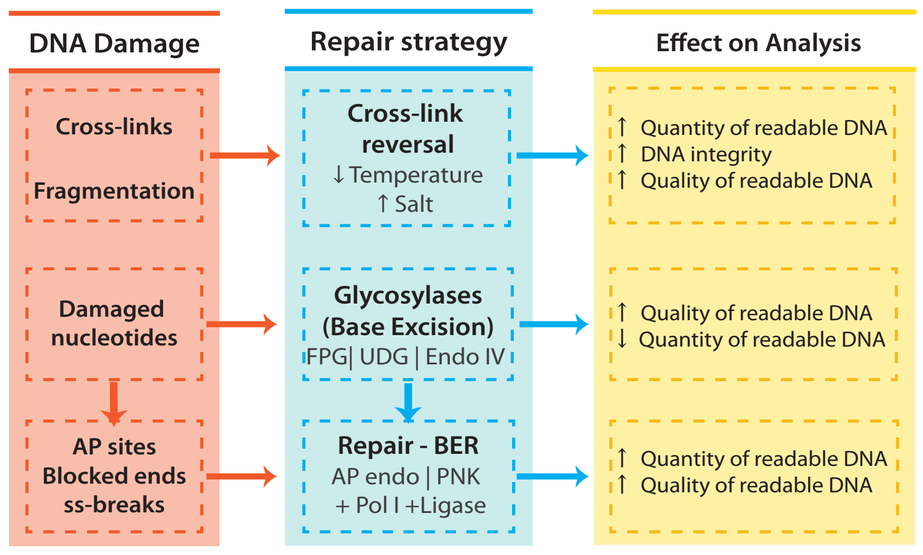
To address FFPE induced DNA damage, the Base Excision Repair pathway, represents a unique opportunity. This pathway is the main pathway for repair of lesions, such as damaged bases, AP sites and ss-breaks.

AIMS:

i. Characterise the damage to FFPE-induced damage and its impact on downstream analyses.

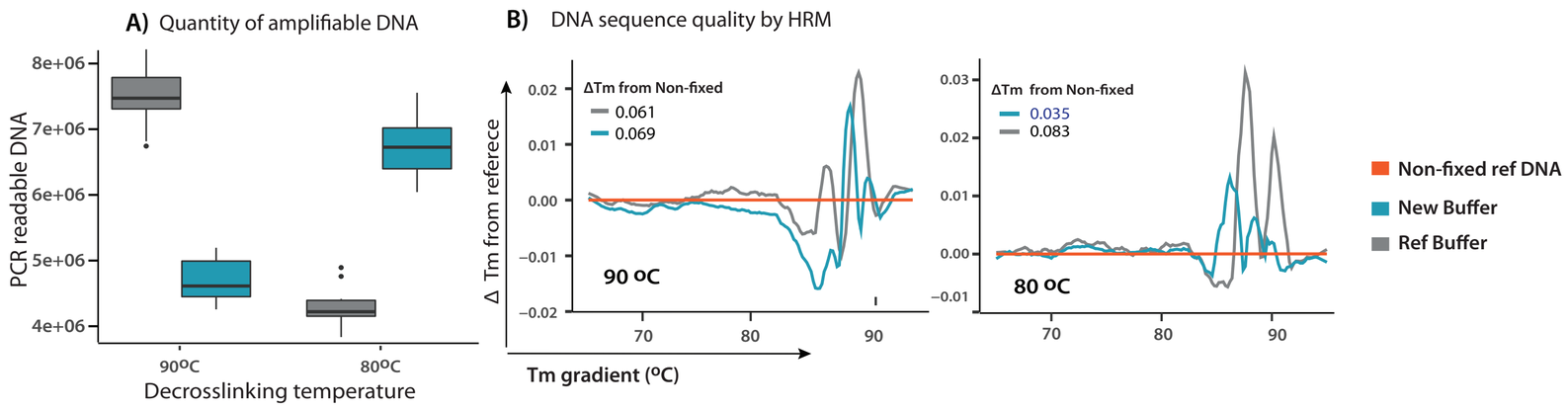
ii. Develop a method to repair damage in both bacterial and mammalian DNA to improve the fidelity of analyses.

STRATEGY:



RESULTS:

1. Optimising formalin crosslinks removal

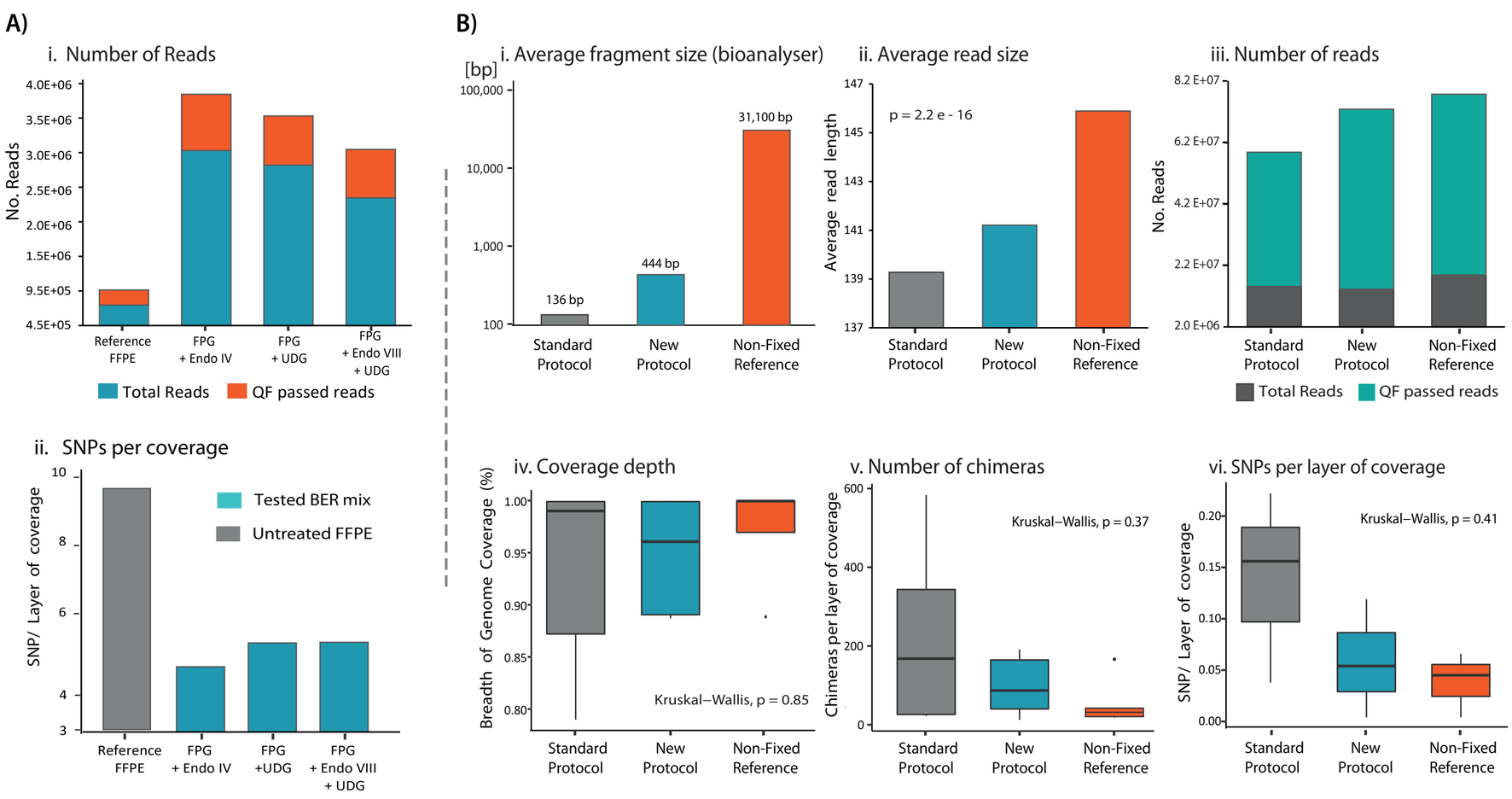


A new decrosslinking strategy [Chaotropic Salt Buffer at 80ºC for 1h] ***(cyan)*** **was compared to a reference protocol [90 ºC x 1 h with QIAGEN ATL buffer]** ***(grey)*****.**

(A) Yield: absolute quantity of amplifiable DNA or a 500 bp DNA fragment from 10^6 genomes (n=6).
(B) Sequence quality: HRM plot – ΔTm of tests plotted against the Tm of NF sample (orange). Average ΔTm from NF shown

The new strategy improves FFPE DNA seqeunce quality. The Tm of DNA fragments amplified were closer to that of paired-NF DNA, with [ΔTm (%)] = 2.82 (not significant) versus 3.02 ($p < 0.05$) for the reference protocol.

2. Repair of FFPE DNA



Reconstitution of the BER system for repair of FFPE DNA

A) Trial of glycosylases mixes

WGS. analysis. 6 replicates treated with each mix were pooled (n = ∑6) and analysed by WGS. Data validated that all mixes improved the sequence (i) coverage and number of reads and QP reads and reduced the amount of SNPs (iii).

The best performance in all cases was observed in the BER mix with FPG and Endo VIII.

B) Validating the combined protocol

Outputs of Bioanalyser and WGS for bacterial FFPE DNA exposed to the combined treatment *(cyan)*, labelled as New Protocol, (∑n = 6), compared to paired-samples decrosslinked with reference (QIAGEN) protocol and unrepaired ***(grey),*** (∑n = 6), and paired Non-Fixed samples (***orange***), (n = ∑3).

Altogether, the results shown here consistently indicate an improvement in the sequence integrity, readability and quality of readable bacterial

CONCLUSION:

This study provides value for the metagenomics field by providing an understanding of the nature of damage to bacterial DNA in FFPE samples and on the related impact on analyses.

Furthermore, given the paucity of published information on mammalian FFPE DNA repair, and none on bacterial repair, the strategy devised here utilising bacterial DNA as an optimisation model, provides all users of FFPE samples (both human and bacterial) with a thoroughly-characterised methodology for DNA repair.

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