A novel NGS library preparation method that preserves and reveals the native ends

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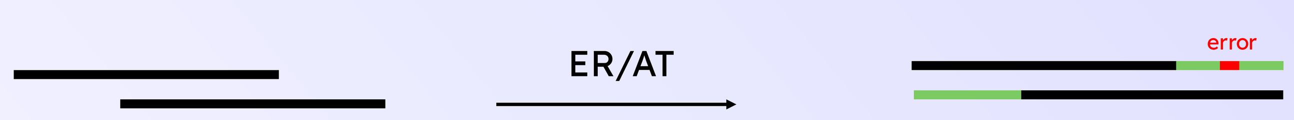
Abstract

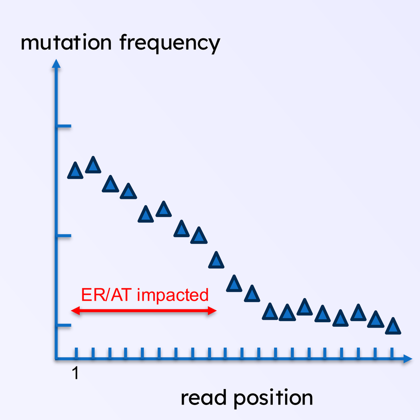
Most NGS library preparation workflows rely on end repair/A-tailing (ER/AT) to prepare the ends of the DNA templates for adapter ligation. However, these enzymatic steps not only result in the loss of valuable end sequence information by modifying the native DNA termini, but also bear the risk of introducing mutational artifacts. For biologically derived DNA, such as cell-free DNA, the restoration of native ends can serve as another orthogonal and informative candidate biomarker, in addition to those such as somatic mutations and epigenetic modifications. By using a unique library preparation method that does not rely on ER/AT, we show here that it is feasible to preserve and restore the original template end information. Validation of our method was performed on a variety of DNA samples with contrived ends. We then applied our method to a panel of circulating cell-free plasma DNA and report the profiles of the native end population in this poster. Our method provides a unique and high-fidelity tool to uncover the potential use of native ends for liquid biopsy applications.

Introduction

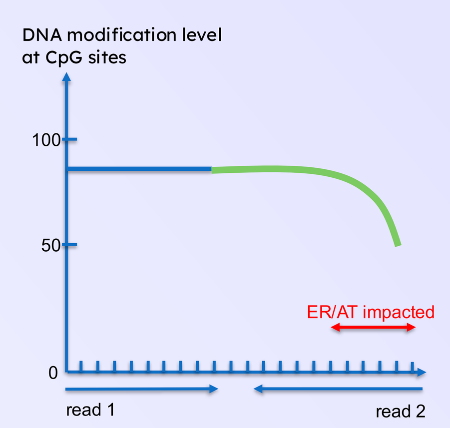
- Conventional NGS library preparation often includes ER/AT step. Early errors introduced during ER/AT are difficult to correct after sequencing, resulting in uncertainty.



- Errors introduced by ER/AT impact both genetics and epigenetics sequencing results



Recreated from Xinyue, Y. *et al*, Archives in Toxicology, 94(2) (2020).

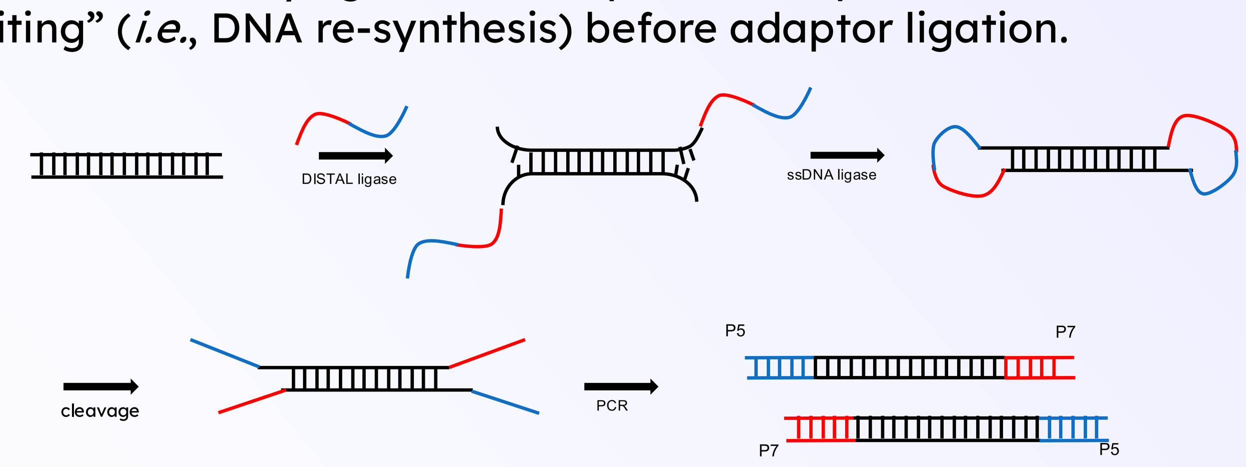


Recreated from Jiang P. *et al*, Genome Research, 30(8) (2020)

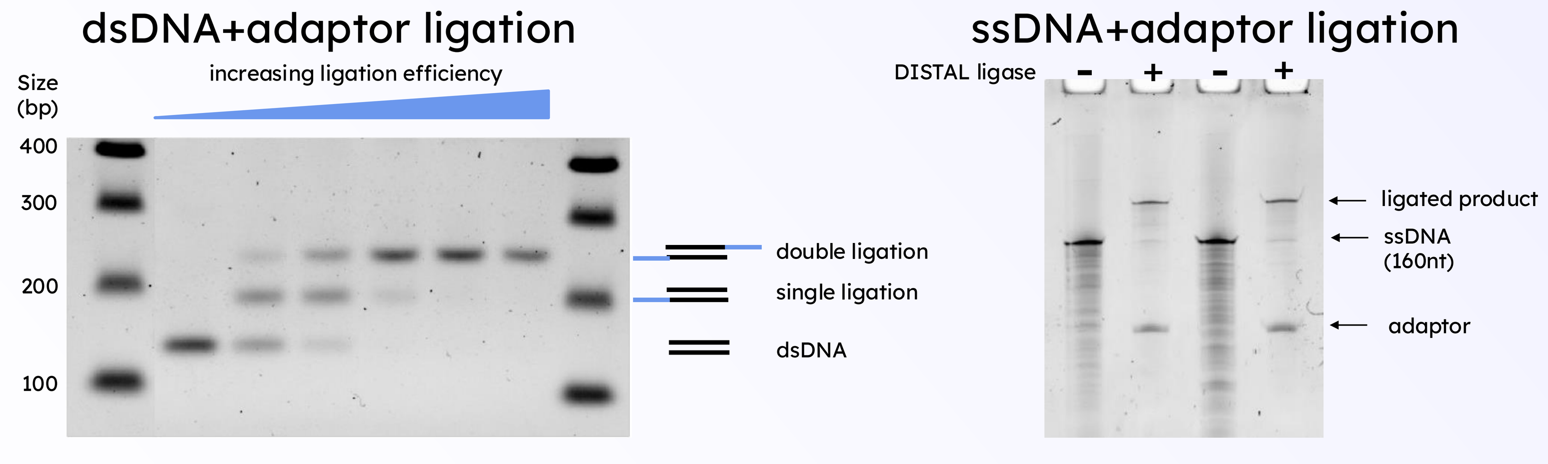
- ER/AT re-synthesizes a substantial portion of the input DNA, compromising sample authenticity. According to a study by Broad Institute, 7–17% and 32–57% of interior 'duplex base pairs' from cfDNA and FFPE DNA, respectively, could be re-synthesized. (Kan Xiong, *et al.*, NAR, 50(1), 2022)
- ER/AT erases fragment end configuration, which could be used as an informative fragmentomic biomarker for liquid biopsy (Spencer D. and Dennis L., Diagnostics (Basel), 2022, 12(4))

TrueLink™ technology

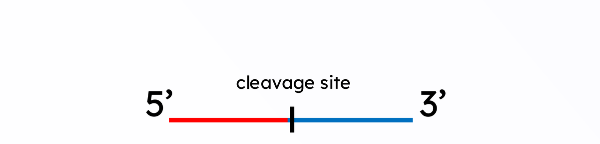
- TrueLink™ directly ligates an adaptor to template DNA, with no ER/AT "writing" (*i.e.*, DNA re-synthesis) before adaptor ligation.

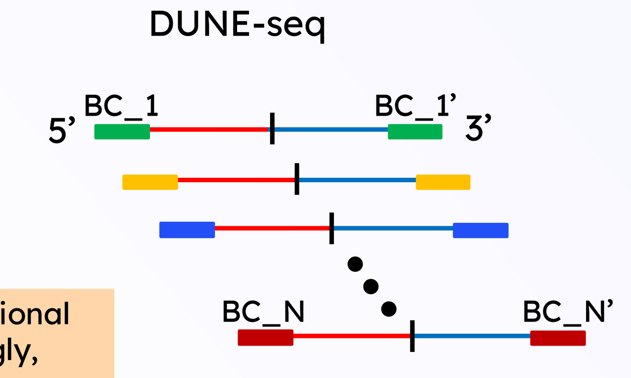


- TrueLink™ 3' ligation: novel direct ligation to 3' end of dsDNA and ssDNA, catalyzed by a proprietary ligase – DISTAL (duplex-retaining single-strand tail ligation) ligase (Canal Biosciences)



- Design of TrueLink™ single strand DNA adaptors and placement of barcodes

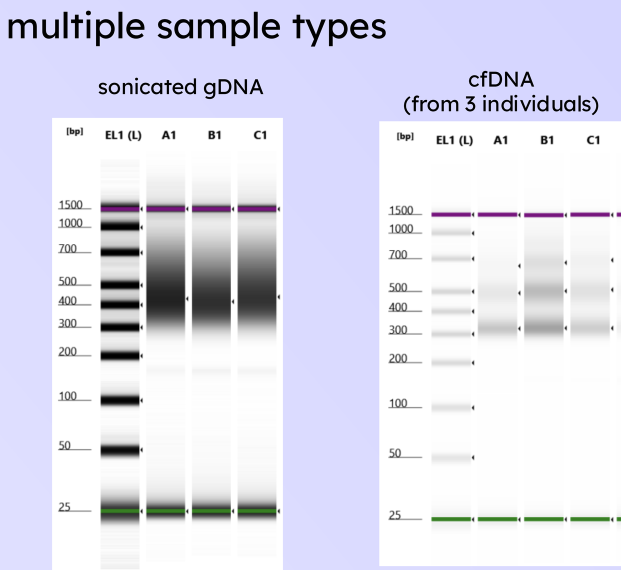




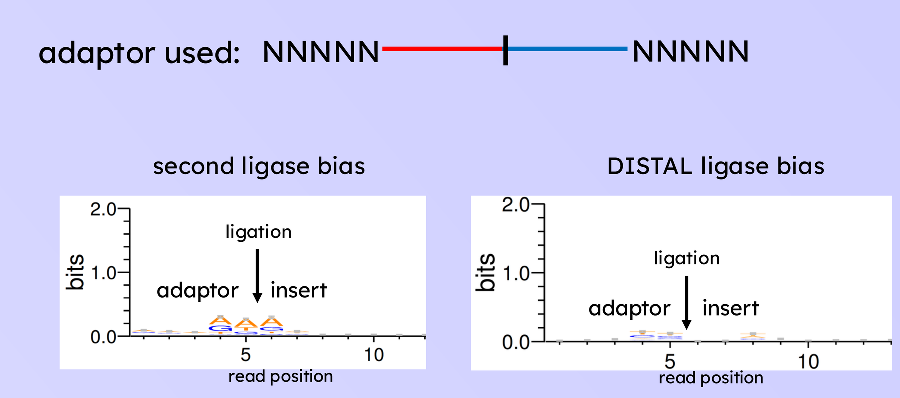
Note: double-stranded region used in conventional Illumina Y-adaptors is not required. Accordingly, BC_1 and BC_1' can be non-complementary.

TrueLink™ NGS libraries

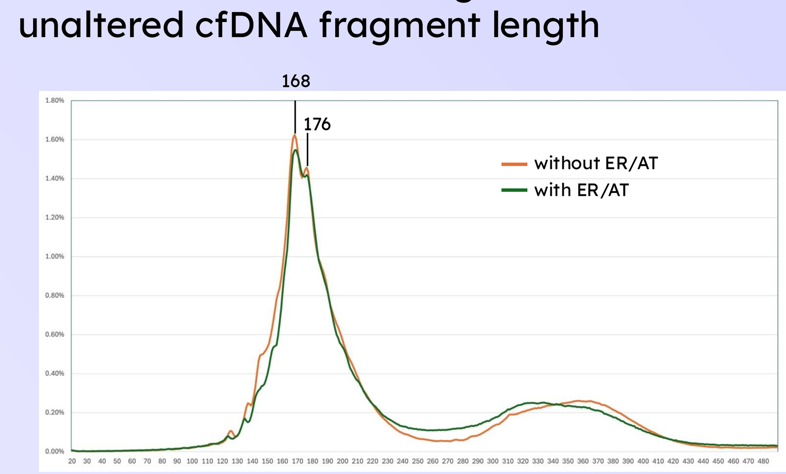
A. TrueLink™ NGS libraries from multiple sample types



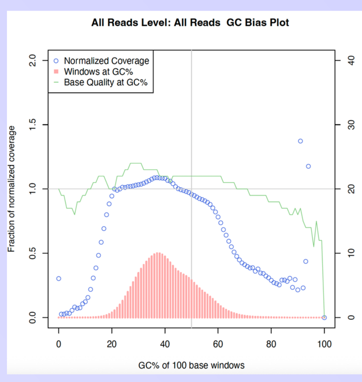
B. TrueLink™ ligations exhibit minimal sequence bias



C. TrueLink™ uncovers high-resolution and unaltered cfDNA fragment length

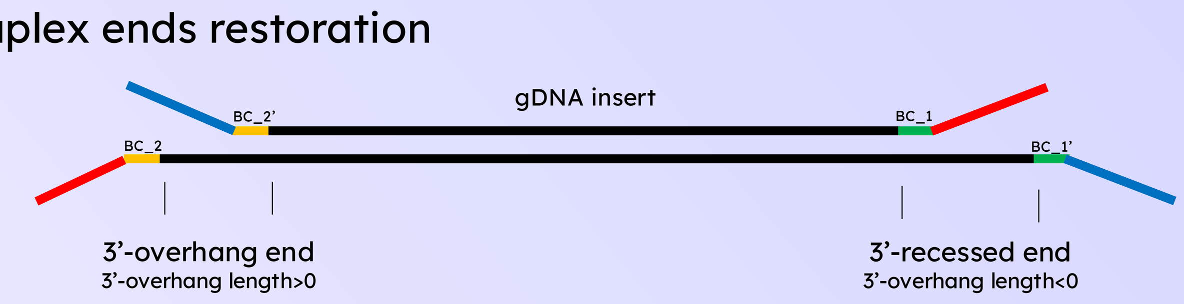


D. GC bias plot on sonicated gDNA library (NA12878)

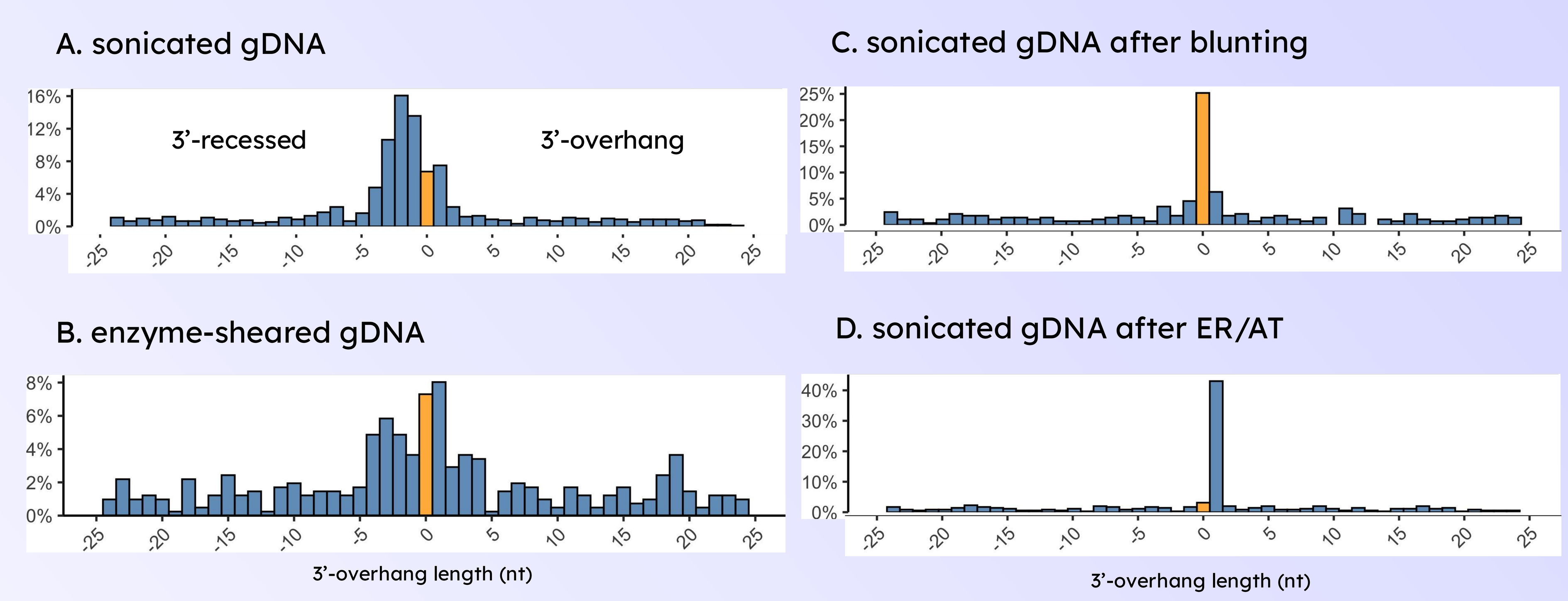


DUNE-seq: Profiling of native duplex ends

- Native duplex ends restoration

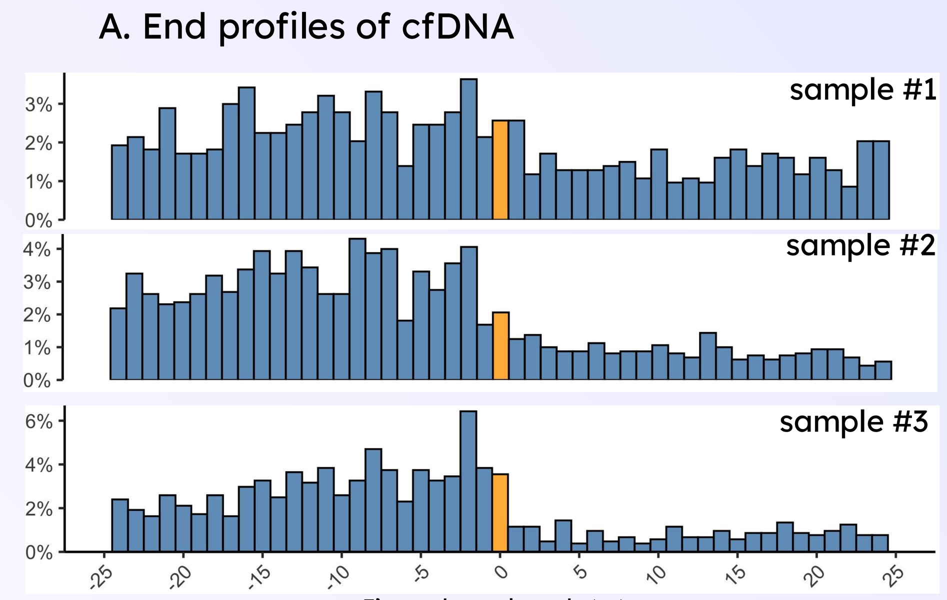


- Duplex end profiles on gDNA (NA12878) with contrived ends

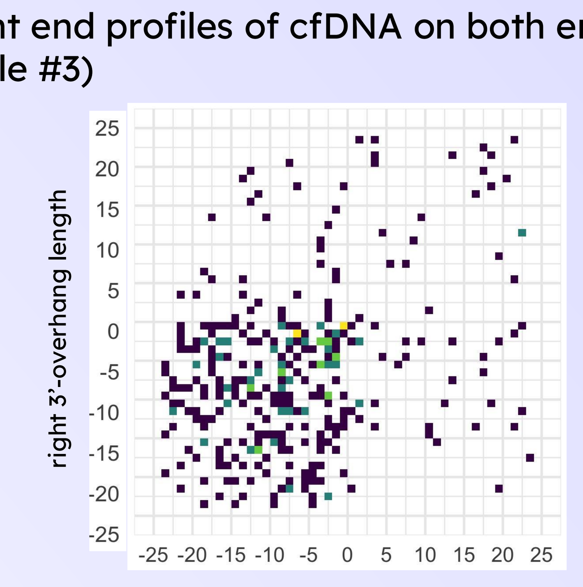


- Native duplex end profiles on circulating cfDNA (3 individuals)

A. End profiles of cfDNA



B. Joint end profiles of cfDNA on both ends (sample #3)



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