

A New Approach to DNA analysis from FFPE: NGS library preparation methods for Single-strand and Double-strand DNA



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COI Disclosure Information
All presenters are employees of Takara Bio Inc.

Abstract

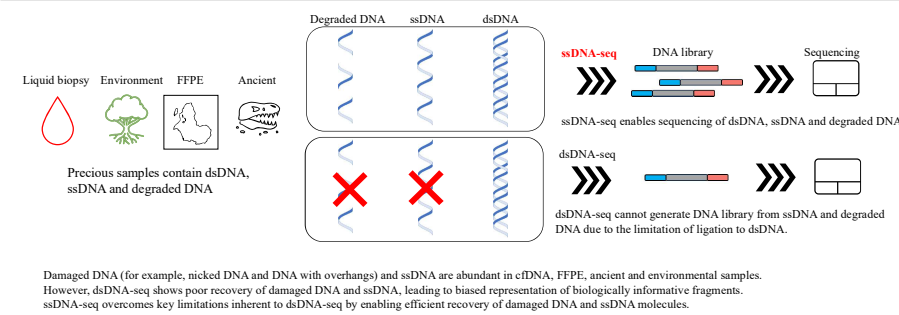
Background: DNA analysis from formalin-fixed paraffin-embedded (FFPE) samples and cell-free DNA (cfDNA) is essential in various fields such as cancer gene panel analysis, clinical sequencing, infectious disease research, genetic disease research, and forensic science. FFPE-derived DNA and cfDNA are often highly fragmented and consist of a mixture of double-strand DNA (dsDNA) with overhangs, nicked dsDNA, and single strand DNA (ssDNA), which conventional dsDNA-oriented library preparation methods may not fully capture. Therefore, a single strand DNA NGS library preparation method using a single strand DNA ligase (SDL) was developed and optimized for FFPE-derived DNA and cfDNA.

Results: The SDL-based approach yielded sufficient libraries for sequencing from high-degradation FFPE DNA and cfDNA. It improved key metrics compared with commercial kits, including higher mapping rates and lower PCR duplicate rates. With 50 base synthetic ssDNA, the mapping rate increased from ~30% using a conventional ssDNA-seq method to >90% with the SDL-based approach, demonstrating effective recovery of short ssDNA.

Conclusion: These findings suggest that the single strand DNA NGS library preparation method is highly effective at recovering a broader diversity of DNA fragments, including ssDNA and damaged molecules from degraded samples such as FFPE and cfDNA, underscoring its utility for sensitive DNA analysis.

Introduction

① ssDNA-seq resolves challenges that are difficult to overcome with dsDNA-seq in precious samples



Damaged DNA (for example, nicked DNA and DNA with overhangs) and ssDNA are abundant in cfDNA, FFPE, ancient and environmental samples. However, dsDNA-seq shows poor recovery of damaged DNA and ssDNA, leading to biased representation of biologically informative fragments. ssDNA-seq overcomes key limitations inherent to dsDNA-seq by enabling efficient recovery of damaged DNA and ssDNA molecules.

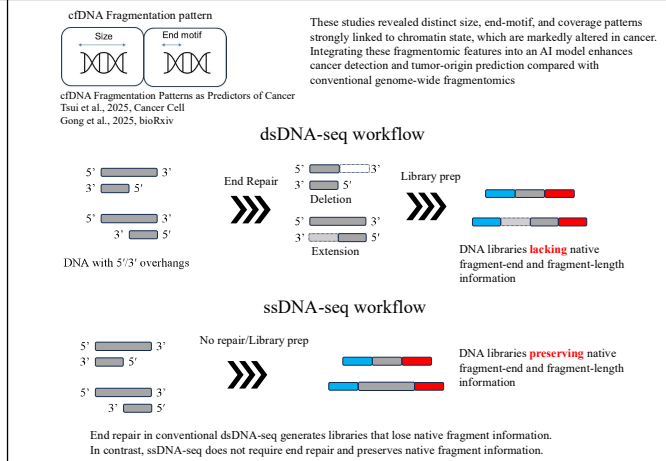
Challenges of Conventional dsDNA-seq

- Underrepresentation of ultra-short, nicked, overhang-containing, and ssDNA fragments
- Causing end-size biases and altering native fragmentation patterns
- Lower conversion efficiency for crosslinked, chemically damaged DNA (e.g., FFPE DNA)
- Poor recovery of degraded and modified molecules in ancient or environmental samples

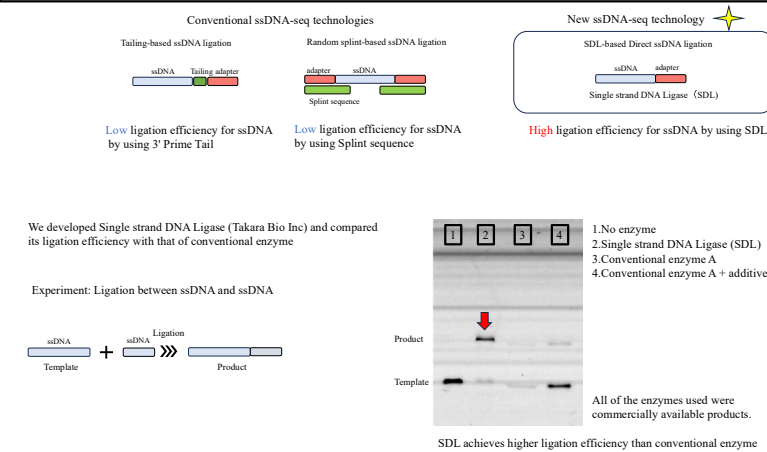
Advantages of ssDNA-seq

- Efficient recovery of nicked DNA, DNA with overhangs, and ssDNA
- High-resolution capture of termini, size profiles, and fragmentation motifs
- Improved library complexity for severely degraded samples
- High compatibility with ultra-short cfDNA (<50 nt), microbial cfDNA, mtDNA
- Suitable for low-input and damage-rich sample types (FFPE, ancient DNA)

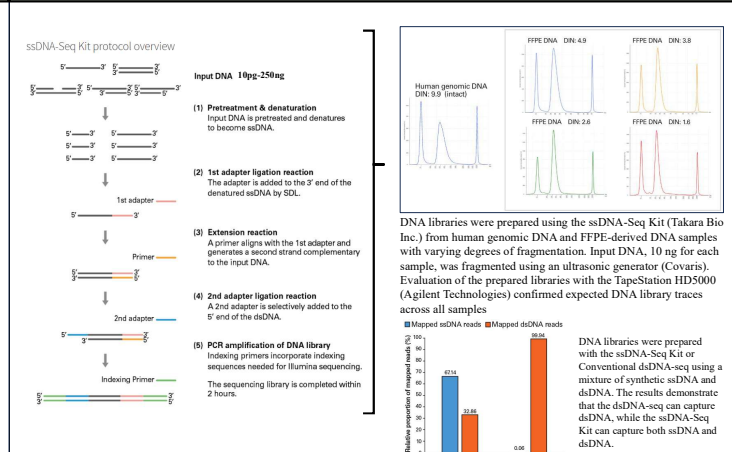
② ssDNA-seq provides high-quality data for fragmentomics analyses.



③ Single strand DNA Ligase is a novel enzyme that shows high ligation efficiency for ssDNA



④ The SDL-based NGS library preparation performance: ssDNA-Seq Kit



Performance Comparison of ssDNA-seq Kit and Competitor Kits

