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Reviving classic molecular scissors for DNA-based data storage: restriction endonuclease-based ONT nanopore sequencing library preparation

Abstract

DNA data storage allows sequences to be defined without biological constraints, yet sequencing workflows typically employ universal protocols designed for genomic DNA. Standard Oxford Nanopore Technologies (ONT) library preparation relies on indiscriminate end-repair and dA-tailing, treating all sequences identically. This generic approach ignores the potential to program retrieval functions into the DNA itself. We introduce NinjaSeq, a method that utilizes restriction endonucleases (REases) to enable cost-efficient, simplified, and programmable library preparation.

The utilization of REases in this context represents a conceptual revival of "legacy" molecular tools in the era of programmable gene editing. While the last decade has been defined by the rise of programmable nucleases such as CRISPR-Cas9, which can be targeted to arbitrary sequences, REases have traditionally been viewed as rigid tools limited by fixed recognition motifs. However, the paradigm of DNA data storage fundamentally inverts this constraint. In this domain, the data carrier itself is synthetic and fully programmable. Consequently, rather than requiring an enzyme that adapts to a fixed biological target, the target sequences can be adapted to match the specific requirements of highly characterized, robust, and commercially available enzymes.

NinjaSeq utilizes PCR to embed restriction recognition sites (RRS) into the flanking regions of DNA constructs. Digestion with specific REases produces overhangs directly compatible with sequencing adapters, bypassing the need for end-repair enzymes. To ensure library integrity, graph-based constrained coding algorithms were utilized to exclude RRS motifs from the data payloads.

We demonstrate that REase-mediated cleavage yields valid sequencing libraries that match the decoding performance of standard protocols when RRS constraints are applied. The enzymatic compatibility of digestion and ligation facilitated a functional ”one-pot” reaction. Furthermore, we showed the random access capability of NinjaSeq during the library preparation step. By targeting unique file-specific flanking sites, we selectively retrieved a target file from a mixed pool with a 16-fold enrichment.

NinjaSeq establishes a robust alternative to standard end-repair/dA-tailing by replacing non-specific enzymatic processing with precise, sequence-directed cleavage. This approach reduces reagent costs, allows for simplified processing, and provides a scalable mechanism for physical data retrieval in DNA storage systems.

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Kornelija Kaminskaitė, Ignas Galminas and Vakarė Gruodytė would be participating.