Docket #: S22-283

Simplified sequencing library preparation for digested DNA and improvements to cDNA library priming

The cost of DNA and RNA sequencing have decreased in recent years to aid effective research and clinical applications; however, the labor time and throughput of preparing DNA and RNA sequencing libraries remains a challenge. To address these challenges, inventors at Stanford created a simplified protocol for DNA sequencing library preparation as well as a reinforced primer for improved cDNA library priming.

The simplified protocol for DNA sequencing library preparation works by combining the reaction conditions for multiple steps. This protocol produces an amplified sequencing library from fragmented DNA using only a single master mix for a single incubation program in a standard thermal cycler. One application of the invention is called fragmentation at methylated loci and sequencing (FML-seq) and involves only three benchtop steps. Genomic DNA is digested by a methylation-dependent restriction endonuclease. Then, a unique master mix is added that combines reagents for adapter ligation, adapter loop breaking, and indexing PCR. Lastly, a single cleanup without size selection is used to purify the library. Using FML-seq, the inventors have successfully profiled DNA methylation—a crucial epigenetic mark. Compared to existing DNA methylation profiling methods, the invention enables FML-seq to reduce the risks of error, reagent costs, and especially labor time due to its short protocol. The invention is also suitable for other sequencing targets via the substitution of different enzymes and adapters.

The reinforced cDNA library primer* is intended to replace standard oligo(dT) primers. Many RNA sequencing approaches target the recovery of a diverse library of different reverse-transcribed complementary DNA (cDNA) molecules. They often

use a short sequence of deoxythymidines (oligo(dT)) as the primer because it hybridizes to the longer tail of adenines (poly(A)) found on almost every mature non-ribosomal RNA. A downside of this approach is that every base sequence in the resulting cDNA library then includes a homopolymer of continuous dT bases, which creates a variety of problems. This invention's optimized, reinforced primer sequence alleviates those problems, reducing loss of the primer's affinity for its poly(A) target, increasing base diversity, and making it less prone to being lost or misread by the sequencer than pure oligo(dT). In optimization experiments, using the reinforced primer increased the proportion of sequence reads passing the sequencer's quality filter from 50%-60% to 80%-90%. The primer was designed by well-established technology and can be synthesized for additional applications from a different design with the same cost and ease.

The additional technology mentioned in this abstract refers to **Stanford docket no. S22-285*

Applications

- DNA sequencing library preparation kits for research and clinical use
- DNA methylation profiling kits
- Genomic sequencing kits
- RNA sequencing library preparation kits for research and clinical use, such as for single-cell RNA-seq

Advantages

- Standardized storage conditions across salt solutions are safe in a standard freezer, simplifying reagent supply chain
- More cost-effective and less labor-intensive compared to existing protocols
- Reduces errors due to its short single-step program
- Increased proportion of sequence reads that pass the sequencer's quality filter
- oligo(dT) tends to be used when only a small amount of RNA is available, a specialized niche with fewer competitive products on the market

Publications

- Joseph W. Foley, Shirley X. Zhu and Robert B. West. "Cost-effective DNA methylation profiling by FML-seq." bioRxiv. January 13, 2023.

Patents

- Published Application: WO2024073034

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