Ultra High-Fidelity Single Molecule Sequencing

High-fidelity DNA sequencing method detecting base substitutions at a single-molecule level, able to explore mutation origins in cancer and aging.

Technology

HiDEF-seq, or Hairpin Duplex Enhanced Fidelity Sequencing, is an advanced single-molecule DNA sequencing technology carried out by employing a multi-faceted approach to enhance fidelity. It is the first single-molecule sequencing technology that achieves single-molecule accuracy. It is also the first DNA sequencing method that can distinguish base changes that are in only one versus both strands of the DNA double helix. HiDEF-seq accomplishes this by increasing the number of independent sequencing passes per DNA molecule during Pacific Biosciences sequencing and by incorporating additional innovations in DNA library preparation. Specifically, HiDEF-seq eliminates *in vitro* artifacts during library preparation by a novel nick ligation procedure and by either using the NanoSeq A-tailing approach or avoiding A-tailing altogether. A novel computational pipeline is developed specifically for HiDEF-seq further eliminates analytical artifacts. HiDEF-seq achieves single-molecule fidelity for single base substitutions, since these have an orthogonal error profile to the prevalent insertion and deletion sequencing errors of single-molecule sequencing. This method enables the accurate detection of both double-stranded and single-stranded DNA changes, even in challenging sample types like post-mortem tissues. HiDEF-seq is also the only long-read sequencing method with single-molecule accuracy, and its unmatched fidelity makes it an invaluable tool for genomic research and clinical diagnostics.

Background

The global NGS market was valued at $13 billion in 2022 and is projected to reach $27 billion by 2027, growing at a compound annual growth rate (CAGR) of 15.7% during this period. The sequencing market has seen continuous advancements in technologies aimed at improving read length, depth, and accuracy. Our latest technology, HiDEF-seq, represents a significant breakthrough as the first of its kind to eliminate all artifacts associated with single-strand nicks, a common source of errors. This is accomplished via a nick ligation step, and in cases of degraded DNA, eliminating A-tailing from the novel library preparation method. The method's fidelity has been established by profiling sperm, which harbor the lowest mutation burden of any accessible tissue, and by profiling tissues from individuals with cancer-predisposition syndromes that show characteristic single- and double-strand patterns of DNA changes. This method holds promise for a wide range of applications including cancer research, aging studies, and mutagenesis research.

Advantages

Technology ID

EVR01-05

Category

Life Sciences/Research tools/Sequencing

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- High-Fidelity: Profiling of ssDNA mismatch and cytosine deamination damage events with a single-molecule fidelity of < 1 error per 1 billion bases (10^{-9}) or greater fidelity, and profiling of dsDNA single-base substitution mutations with computationally estimated fidelity of < 1 error per 100 trillion (10^{-14}) base pairs.
- Origins of mutations: This approach detects initiating single-strand mismatch and damage events at single-molecule resolution to enable an understanding of mutation origins.
- Concomitant analysis of ssDNA and dsDNA: Direct sequencing of single DNA molecules without any prior amplification that achieves, for single-base substitutions, single-molecule fidelity detection of dsDNA mutations simultaneously with ssDNA mismatches and damage.

Applications

- Detecting and characterizing defects in DNA replication and damage repair on a single base level in diseased and healthy tissues.
- In experimental systems to dissect the kinetics of the DNA damage, repair, and replication equilibrium - combined with *in vitro* genetic and other manipulations, with synchronization of the cell cycle, and in reconstituted enzyme systems.

Intellectual Property

NYU has a U.S. patent pending covering this novel sequencing method including its components and processes.

References

1. Liu, M.H., Costa, B.M., Bianchini, E.C. et al. , https://doi.org/10.1038/s41586-024-07532-8