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Commentary

Clinical uses of Single-Molecule Sequencing Tools

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DESCRIPTION

Single-molecule sequencing facilitates DNA or RNA to be sequenced directly from biological samples, making it well-suited for diagnostic and clinical applications. Single-molecule sequencing tools can create multikilobase sequences with the potential to greatly progress genome and transcriptome assembly. However, the error rates of single-molecule reads are high, which has limited their use thus far to resequencing bacteria. To address this constraint, present a correction algorithm and assembly strategy that uses short, high-fidelity sequences to correct the error in single-molecule sequences. Demonstrate the efficacy of this technique using reads obtained by a Pacific Biosciences RS instrument from phage, bacterial, and eukaryotic complete genomes.

Short read technologies have intrinsic limitations such as GC bias, difficulties mapping to repetitive components, trouble discriminating paralogous sequences, and complications in phasing alleles. Long read single molecule sequencers resolve these difficulties. Moreover, they offer higher consensus accuracies and can detect epigenetic alterations from native DNA. The first commercially available long read single molecule platform was the RS system based on Pacific Biosciences single molecule real-time (SMRT) sequencing technology, which has since progressed into their RSII and Sequel systems. Long reads that permit direct sequencing through regions of the genome inaccessible or problematic to analyze by short-read platforms. Long-read correction achieves>99.9% base-call accuracy, leading to considerably better assemblies than current sequencing strategies: in the best example, the median counting size was quintupled relative to high-coverage, second-generation assemblies. Greater gains are predicted if read lengths continue to increase, including the prospect of single-counting bacterial chromosome assembly. Single molecule sequencing is based on the successive enzymatic degradation of fluorescently labelled single DNA molecules, and the detection and identification of the released monomer molecules according to their sequential order in a microstructured channel. The detection technique is evolved from confocal fluorescence microscopy, with two different laser sources to excite the individual mononucleotides that are either labelled with tetramethylrhodamine (TMR) or Cyanine5 (Cy5). The handling of DNA which is immobilized on carrier beads, and the recognition of the cleaved monomers is performed in optically transparent and biochemically inert microstructures (glass or PMMA) with detection channels of 7 μ × 10 μm. The projected rate of sequencing is ≈100 bases min−1, dependent solely on the rate of the enzymatic DNA cleavage.

Applications

- Recent advances in sequencing technologies show a tendency towards single-molecule sequencing, which ultimately will lead to the profitable implementation of such platforms.
- Single-molecule sequencing displays great promise for future genomic medicine. These techniques offer long read lengths, high consensus accuracies, direct identification of base modifications, direct RNA sequencing, portability, and more democratized access to sequencing platforms.
- Long reads enable proximate reference-quality genome assemblies, detection of novel disease-causing structural variation, and the capability to sequence through previously 'unsequenceable' repetitive DNA contents of clinical utility.
- Single-molecule sequencing is developing clinical tests, such as direct sequencing of FMR1 alleles for length determination and identifying AGG interruptions, HLA typing at unparalleled resolution, disentangling chromothripsis genomes, determining the allelic distribution of low-frequency mutations in cancer genes such as TP53 and BCR-ABL1, and enabling in-field genetic testing.

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