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APPLYING SINGLE MOLECULE REAL TIME DNA SEQUENCING

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SMRT (single molecule real time) DNA sequencing is a novel, high throughput method for sequencing DNA. It harnesses the intrinsic power of DNA polymerase enzymes as sequencing engines by eavesdropping on template-directed synthesis in real-time. Two critical technology components enable this process: The first is phospholinked nucleotides where, in contrast to other sequencing approaches, the fluorescent label is attached to the terminal phosphate rather than the base. The enzyme cleaves away the fluorophore as part of the incorporation process, leaving behind completely natural double-stranded DNA. The second critical component is zero-mode waveguide (ZMW) observation confinement technology that allows single-molecule detection at concentrations of labeled analogs relevant to the enzyme. Through the combination of these innovations, our technology allows the speed, processivity, efficiency and fidelity of the enzyme to be exploited. We show application of this technology to shotgun sequencing of human and bacterial DNA resulting in high consensus accuracy and unprecedented readlength. Because with Phospholink nucleotides the polymerase reverts completely to the initial state after each base sequenced the accuracy profile as a function of position within a read is flat. We will present a novel sample prep concept based on DNA hairpin ligation to double-stranded DNA that facilitates whole genome shotgun sequencing directly from genomic DNA with near-Poisson limited coverage uniformity and practically no GC bias. This sample prep will be demonstrated to enable consensus sequencing based on data extracted from just one molecule, allowing high accuracy sequencing at the molecular limit and without amplification.

Objectives

- The attendee should be able to distinguish 2 technologies enabling SMRT DNA sequencing and characteristics of each.
- They should be able to define what SMRT DNA sequencing is and how it differs from other sequencing technologies.
- Attendees should be able to explain how high accuracy without amplification is achieved through sample prep – ie, what methods
- Attendees will have learned about a unique sample prep process involving DNA hairpin ligation and double-stranded DNA.