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The Three Main Approaches to High-Throughput Sequencing

Sequencing by synthesis is influenced by the traditional methods involving radio-labeling. Specifically the Maxam-Gilbert sequencing technique, which requires radioactively labeling one end of a DNA sequence. This is done in order to visualize the chemically separated fragments within the electrophoresis gel. The sanger sequencing method also used radioactivity because it doesn't use regular nucleotides but instead a radioactive nucleotide called ddNTP. Similar to these previous methods, sequencing by synthesis involves four fluorescently labeled nucleotides that can be used to sequence millions of clusters on a flow cell surface.

Sequencing by ligation is similar to traditional sequencing approaches mainly in its use of enzymes. Sequencing by ligation involves using DNA ligase to identify nucleotides on a specific DNA sequence position. Both the Maxam-gilbert method and the Sanger sequencing method can use enzymes like DNA polymerase or kinases to bind certain molecules and nucleotides to the sequence that is being studies

I would say that Single-molecule/Real-time sequencing is most influenced by clone-by-clone sequencing. This is because both methods involve sequencing on a much smaller scale where nucleotides and bases are observed on a more tedious singular/individual basis.

The Pros and Cons of High-Throughput Sequencing

One of the pros I noticed with high-throughput sequencing techniques is the homogenization of fragment sizes during sequencing. Fragments that are too large or too small are often rejected, which definitely affects the accuracy of results. The biggest reason to use high-throughput sequencing over low throughput methods is the ability to sequence many strands of DNA simultaneously in parallel. This enable millions of DNA strands to be sequenced at once.

There are negative aspects as well associated with high throughput. Due to its nature of being able to sequence large numbers of strands, it is inefficient and not cost-effective when sequencing a smaller number of targets. If 1-20 DNA targets are being sequenced, a lower throughput method like Sanger Sequencing is faster and more efficient.

Experiment

HTS is used very commonly in DNA-SIP technology which studies how microorganisms convert carbons-based molecules into cellular biomass (similar to how we use DNA/RNA and create proteins.) One experiment studying and using this method is the “flourescent probes” experiment (Crombie et al., 2018; Pan, Gao, Fan, Li, & Dai, 2018), where cells are broken down in labeled and unlabelled fractions (or fragments.) Then HTS is used after the cellular components are separated by density in an aqueous medium through centrifuge.