Week 4: Next Generation Sequencing Questions

BIFX 504, Advanced Molecular Biology for Bioinformatics

Name: James Jedediah Smith

1. Describe the fluorescent terminators that are used in Sanger sequencing.

They are nucleotides that have been fluorescently color-coded and include a terminator.

1. How does Sanger sequencing work?

DNA is denatured so DNA polymerase can bind. The fluorescent terminators are incorporated by the polymerase. Results in different lengths of DNA transcripts that have a fluorescently color-coded terminator on the end. Sequencer machine detects theses bits from longest to shortest to build up a coherent sequence.

1. Why don't we use Sanger sequencing for whole genome sequencing anymore?

It takes a long time and is not as efficient as more modern techniques.

1. How much does it cost to sequence a human genome using Illumina Next Generation Sequencing?

Only $1,000 and just 48 hours.

1. What do the adapters that are added to the end of a DNA sequences contain?

They contain primer binding sites that allow sequencing reactions to occur and capture sequences that allow the sample to be capture on flow cells for sequencing.

1. What are the steps in Illumina Next Generation Sequencing?

Steps that happen to create the clusters:

1. Load denatured single strands into flow cells where their capture sites bind to short DNA sequences located on inside of flow cell.
2. Polymerase is added to synthesize a copy attached directly to the flow cell.
3. Original strand is washed out, free end of the copy binds to flow cell. Polymerase is added again to synthesize more strands.
4. After repeating the process several times, should have around 1000 molecules of the same sequence. Add sequencing primer and it is complete.

Steps that happen in the HiSeq or NovaSeq:

1. Clustered flow cell mounted on the stage.
2. Pumps pull reagents from fridge to flow cell.
3. Powerful microscope used to take picture after each fluorescent reversible terminator is added.
4. Terminator is removed, next one is added, picture is taken, repeat until done.
5. How are the Fluorescent Reversible Terminators that are used in Illumina sequencing different than Fluorescent Terminators that are used in Sanger sequencing?

They can be removed after the fact, which allows polymerase to continue sequencing once the result has been recorded.

1. Why can't Illumina NGS sequence more than 300 bases of DNA per cluster?

The enzymes and chemistry are not perfect. Some strands may lag behind or jump ahead. These errors add up over time. Limited to 300 bases to ensure accuracy.

1. How does Nanopore sequencing work?

Single strand of DNA or RNA is threaded through a pore embedded in lipid membrane. Different bases going through results in different currents that can be detected via machine to determine which base it was.

1. What are the benefits and drawbacks of nanopore sequencing

It can read really long sequences, no need to convert RNA to DNA, and super portable! Only needs a laptop and sequencer the size of a remote control. Problem is that it does have high error rates of 10 – 15%. Often biased errors too.

1. How are the building blocks that are used for PacBio sequencing different than those that are used for Illumina sequencing?

The fluorescent bit is attached to the phosphate group. There also isn’t any blocking or terminating group.

1. How does PacBio Sequencing work?

Tiny array of wells on a plate. DNA polymerase is at the bottom and starts sequencing a template strand using the modified bases that have fluorescent bits attached on the phosphate. The phosphate gets removed and there is no terminator, so sequencing occurs continuously without extra chemistry steps. Camera located beneath the well records the reaction. When a nucleotide binds, its color gets a big jump in intensity.

1. What makes PacBio Sequencing better for genotyping than Nanopore sequencing?

PacBio Sequencing has random errors. This means you can re-read the same sequence a bunch of times eliminate unmatched errors and form a consensus sequence.

1. What are the benefits and drawbacks of using long reads to sequence a whole genome?

Draw backs are that it costs more and is harder to prep. But benefits are that it is easier to assemble genomes, detect structural variations, and identify the chromosomes of phase variations.