Weekly Homework 6

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About Next Generation Sequencing

(with emphasis on Illumina Technology)

April 10, 2019

As stated by Sherri Millis in a recent article from the American Lung Association, next-generation sequencing has the main advantage that we are now able to analyze multiple genes from body cell at the same time. [1] One of the most well-known technologies at the current time is Illumina. They not only offer a vast range of products and services based solely on genome sequencing but also run continuous efforts for further on researching topics such as Cancer, Microbiology, Complex Diseases and Agrigenomics[15] (a trending topic which tries to apply advanced genomics techniques into agriculture).[2]

The work conducted by Illumina can be summarized into four main steps: sample preparation, cluster generation, sequencing, and data analysis. [3] The first phase could be done in multiple ways, but the one used by Illumina consists of adding adapters to the end of each DNA sequence. Adapters are sticky parts, genetically engineered in order to be attached to either DNA or RNA fragments. [4] Afterward, on the newly attached parts of each DNA segment is run the process called reduced cycle amplification. During this process, on these adapters are placed primers for sequence binding, indices and some regions complementary to the flow cell oligos. These oligos are the abbreviation used in some publications for oligonucletides which are nucleic acid polymers[14] of reduced dimensions used in multiple fields such as research, forensics and so on.[5]

Cluster generation represents the second phase of the process done for genes sequencing. During this part, each fragment of a molecule is isothermally amplified so that it will be

able to go through the flow cell. In this case, the flow cell can be compared to a glass slide with routes covered entirely with two types of oligos. These oligos are the same ones that can be found at the extremities of each sequence after the sample preparation step so that when they initiate the hybridization[9] process they will be matched two by two: one oligo with one fragment which has the same oligo at its extremity. After they are attached, with the help of polymerase[13], the DNA fragment is duplicated over its paired oligo. The newly created double-stranded molecule is separated in two fragments so that in the end we will end up only with copies for each fragment. The clustering generation process continues even further, so that now each fragment bends over the other type of oligo, creating a bridge and then duplicating again. The two fragments of the bridge are denatured again, in order to further create bridges by attaching to other oligos. Thus, one fragment gets to have a multitude of copies.

On another note, sequencing, the third part of the work flow actually happens simultaneously with the second part during each fragment's copy for building bridges with the other oligos and these processes are all wrapped up during the fourth part which is data analysis.

An exhaustive article about Illumina technology was published at the beginning of 2010, in the Bioinformatics journal, where it covers advantages in comparison with the previously used technology by presenting already ran experiments and their results. Their arguments are strongly supported by graphs, plots and detailed analysis. [6]

References

[1] American Lung Association,
`https://www.lung.org/lung-health-and-diseases/lung-disease-lookup/lung-cancer/patient-resources-and-videos/videos/next-generation-sequencing.html`

[2] Illumina website,
`https://emea.illumina.com`

[3] Illumina Sequencing Synthesis Video
`https://www.youtube.com/watch?v=fCd6B5HRaZ8t=82s`

[4] Adapter (Genetics)

https://en.wikipedia.org/wiki/Adapter

[5] What is an oligonucleotide?

https://www.news-medical.net/life-sciences/What-is-an-Oligonucleotide.aspx

[6] SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data

https://doi.org/10.1186/1471-2105-11-485

[7] Illumina dye sequencing

https://en.wikipedia.org/wiki/IlluminadyesequencingReducedcycleamplification

[8] Genome

https://en.wikipedia.org/wiki/Genome

[9] Nucleic acid hybridization

https://en.wikipedia.org/wiki/Nucleic-acid-hybridization

[10] Introduction to Next Generation Sequencing

https://www.youtube.com/watchv=ToKUGzYhC4

[11] Nature: Next generation sequencing

https://www.nature.com/subjects/next-generation-sequencing

[12] Bibliography management with bibtex

https://www.overleaf.com/learn/latex/Bibliographymanagementwithbibtex

[13] Polymerase Wikipedia

https://en.wikipedia.org/wiki/Polymerase

[14] Nucleic acid monomer and polymer

https://socratic.org/questions/what-is-a-nucleic-acid-monomer-and-polymer

[15] Agrigenomics

https://www.genalice.com/applications/agrigenomics/