Homework #10

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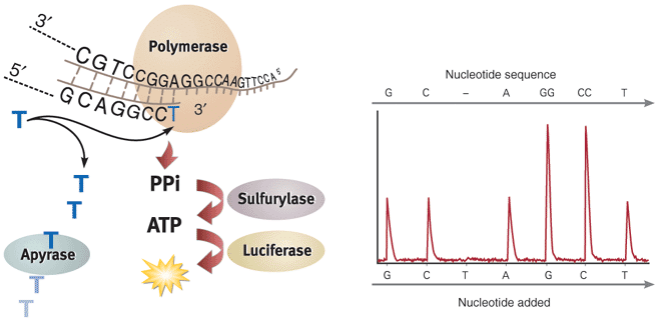
Context

Sequencing is a method of determining the order of nucleotides (A, T, C, G) in a DNA sequence. Sanger sequencing is the first sequencing method to selectively incorporate dideoxy nucleotides (OH is missing at the 3 'end and is replaced by H2) during in vitro DNA replication. So as to stop the process of complementarity of the entire DNA molecule to a certain sequence (the date of the nucleotide at which it is desired to stop A, T, C or G). In this way molecules of different sizes are obtained. Using electrophoresis on the 4 mixtures (ending in A, C, G or T) the order of nucleotides in a DNA segment can be obtained. For this sequencing method it is necessary to know the first nucleotides that will constitute the primer for DNA polymerase (starting point). With this sequencing method, in 2001 the sequencing of the human genome was successful for the first time within the Human Genom Project.[2]

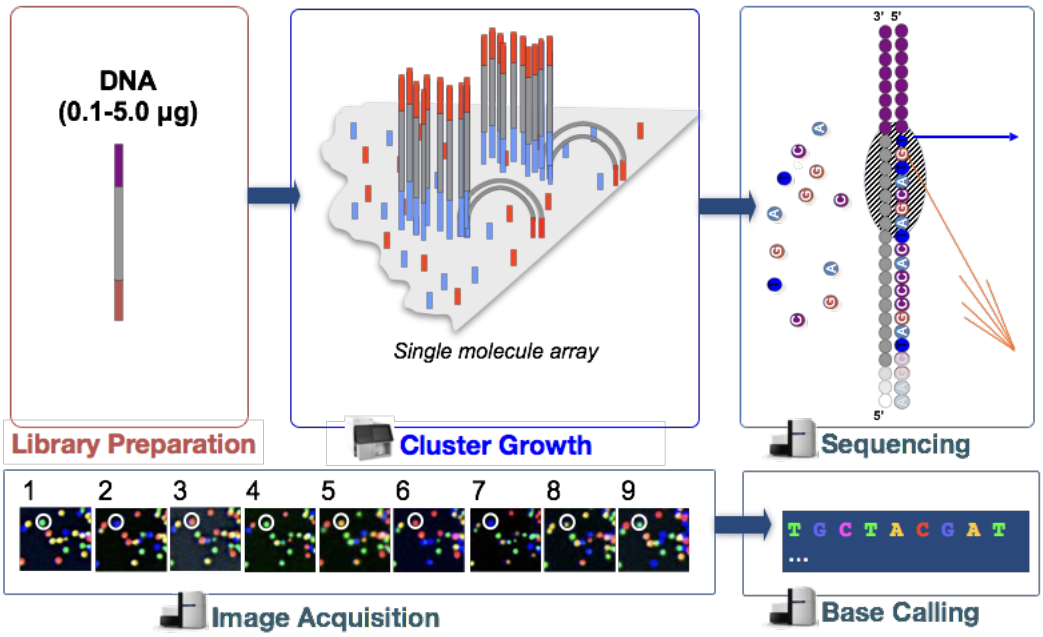
Next Generation sequencing (NGS) consists of new DNA or RNA sequencing technologies that are intended to be more productive in terms of time and cost than Sanger sequencing. A sequencing method can only be called NGS if in a single experiment, for a DNA molecule with more than 1 million bases on single stranded, the sequencing succeeds or for an RNA molecule larger than 1 million bases. Among the new sequencing techniques are: Pyrosequencing, Sequencing by Synthesis, Ion Semiconductor Sequencing, etc.[2]

Pyrosequencing



It is based on Sanger sequencing: it needs a primer that will be the primer for DNA polymerase. Then each time a series of identical nucleotides that are known in advance will be introduced into the mixture of interest (the one containing the semi-replicated DNA molecule). Once the DNA polymerase binds a series of nucleotides to the semi-replicated DNA molecule (say nucleotides) they will be released into pyrophosphate molecules. AT sulfurylase will react with those in pyrophosphate molecules that will generate in ATP molecules. The n ATP molecules will react with luciferase which will generate a light wave of a certain intensity (higher intensity for a larger n and lower intensity for a smaller n). Reading these sound signals reconstructs the nucleotide sequence in the DNA molecule we want to sequence.[1, 3]

Sequencing by Synthesis

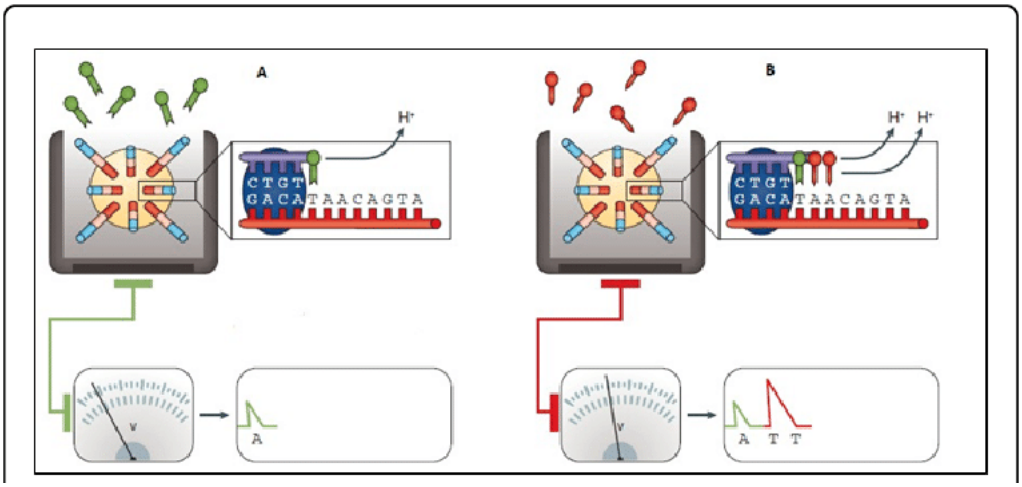


In this sequencing technique, primers must be known from both ends of the DNA molecule to be sequenced (called oligos), as opposed to the first two sequencing methods in which it was necessary to know only one primer. Sequencing begins on a lamella on which these oligos exist and where the DNA molecule to be sequenced is inserted (single stranded). Due to hybridization, the newly introduced DNA molecule will hybridize to one of the oligos, after which the protein DNA polymerase will begin to complete replication. At this point there is a DNA molecule that we want to sequence. By heating to 90-98 degrees the molecule will degenerate into a single stranded DNA molecule that will hybridize to a complementary oligos and another single stranded DNA molecule that will be washed (because it does not bind to any oligo).

DNA polymerase will help complete replication by making a new DNA molecule. This process is repeated simultaneously on the slide several times. Finally, by heating to 90-98

degrees, all DNA molecules will turn into several single stranded DNA molecules. Wash backward strands and keep forward strands. Then sequencing takes place fixedly like the Sanger with one exception, instead of di-deoxynucleotides, here there are fluorescent nucleotides that emit several (4) light signals of different intensities for each nucleotide.[4]

Ion Semiconductor Sequencing



Ion Semiconductor Sequencing is a type of synthetic sequencing that is based on the release of hydrogen ions during DNA replication with DNA polymerase. Unlike the above techniques where modified nucleotides are used, normal nucleotides are used here. And here we need a primer from which the protein DNA polymerase will start. One or more nucleotides (for identical nucleotides that are continuous) of a single type are introduced into the solution containing the DNA molecule. A sensor sensitive to the release of hydrogen ions is used to see the presence or absence of the reaction. Then the solution is washed and the process is resumed.[5]

References

[1] Robert England, Monica Pettersson, Pyro Q-CpG™: quantitative analysis of methylation in multiple CpG sites by Pyrosequencing, Nature Methods, 2005
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[2] Ivo Glynne Gut New sequencing technologies, Springer, 2013
`https://link.springer.com/article/10.1007/s12094-013-1073-6`

[3] 454 Sequencing
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[4] Illumina
http://www.illumina.com/

[5] Ion Torrent
http://www.lifetechnologies.com/ca/en/home/brands/ion-torrent.html