NEXT GENERATION SEQUENCING *on table*

DNA and RNA sequencing became routine tool in Patients´ diseases follow up and either for diagnosis and prognosis clarification.

Clarification of **theoretical understanding** and the **practical development** through the ways the process should be executed using all the specialized equipment for DNA sequencing, will be here explained using a simple model. ThermoFisher Formers and Theory Basis transmit eager laboratory assurance.

Practical Development for DNA Sequencing

The sequencing process has the following steps: to prepare our sample and libraries and to actually sequence our target DNA and read its code. First we use the Qubit, a small fluorimeter who has the ability to read DNA concentration (dsDNA, ssDNA) thanks to its reagent kits specific for each DNA types, RNA, micro RNA and protein.

In order to achieve the best results with sequencing, 10 nanograms of the DNA are wanted to prepare the DNA libraries. Library preparation of fragmented DNA, for use with the Ion Torrent™ sequencing platform, involves repair of 3' and 5' ends before ligation to an adaptor. After ligation, adaptor-DNA constructs are purified and size selected, and amplified via polymerase chain reaction (PCR).

The Ion OneTouch™ 2 uses three breakthrough technologies that enable automated delivery of templated Ion Sphere™ particles. The first is the reaction filter that creates millions of micro reactors in which clonal amplification occurs. The second is the the fully integrated thermal cycler and disposable path amplification plate system that enables robust thermal cycling of the micro reactors. The third is the integrated centrifuge, which recovers the templated Ion Sphere™ particles. Combined, these technologies deliver massively parallel clonal amplification and recovery automatically.

The Ion Torrent, a Robot that would remove all the interferings from the sample being those unbound DNA and unbound beads (more explanation on the process when described in the theoretical part) has a robotic arm with a pipette on the tip, and he would resuspend our sample until all the bound DNA was tagged with biotin who will bind streptavidin. Then after this, it would turn a magnetic to attract all streptavidin to the vial's walls making it easy to remove the interfering beads and unbound DNA from the sample.

The libraries are finally sequenced with the Personal Genome Machine, the DNA sequencer.

Theoretical Fundamentals

The theory behind this process is very simple: we have a semiconductor chip with 1.100.000 pits where the beads we bind our target ssDNA fit and we pump a solution with a nucleotide that will bind or not with the first nucleotide of the target strand, then

the PGM washes that nucleotide and pumps a solution with a different one (being the nucleotides timine, adenine, cytosine and guanosine) until the nucleotides solution binds with the nucleotide from the substituted DNA sequence, releasing hydrogen atoms, will lower the medium´s pH and such variation will be detected by the semiconductor chip (and since we should have approximately 1.100.000 pits with each a bead with bound DNA we are actually getting approximately 1.100.000 readings from the same sample and getting the average result) and the machine will wash and keep repumping nucleotides until the target DNA is fully sequenced up to a 400 base pairs maximum.

Now, how do we prepare our sample? It's quite simple: we bind our target dsDNA to the microbeads that will fit the pits of the semiconductor chip through a centrifugation.

Then we tag the beads with biotin (a DNA single strand can only be bound to the sphere or to biotin which means that each dsDNA strand has one connection to the bead on one strand and one connection to biotin on the other strand or is unbound from one or all of these reagents) in a reactor and tag that biotin with streptavidin (who has magnetic properties and is attracted by biotin) and submit that reactor to a magnetic field that will immobilise all the DNA bound to the microbeads and to biotin and remove the medium with all the interferent unbound DNA and microbeads. The double bounded DNA will then be denaturised separating it to single strand DNA where we will have 2 types of ssDNA ones bound to spheres and the others bound to biotin and streptavidin.

We will then submit the reactor vase to a magnetic field and at this time we would just transfer it to a different vial (since the biotin will be immobilised we are transferring the bound beads suspension and leaving behind the biotin bound strand) that can now be injected on the Personal Genome Machine and sequenced.

We hope you still keep your breath. From our side, we deeply desire diseases undoing and Patients ever luck.

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