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Medi Quest BRS Hospital

A monthly News letter from BRS Hospital

NEXT GENERATION SEQUENCING (NGS)

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Next generation sequencing is a technology for determining the sequence of DNA or RNA variation associated with diseases or other biological phenomena.

Introduced for commercial use in 2005, this method was initially called "massively parallel sequencing" Because it enabled the sequencing of many DNA strands at the same time, in contrast to one strand at a time with the conventional Sanger sequencing. NGS helps the sequencing of hundreds of thousands of genes at the time in multiple samples and can identify single nucleotide variants copy number variants, structural variants.

Next generation sequencing technology

Several platforms for next generation were developed, which included Illumina sequencing, Pyro sequencing ION torrent sequencing. Illumina sequencing technology emerged as the dominant player and will be discussed in this issue.

Illumina sequencing.

It is an imaging based method and can read the human genome in 48hrs.

In NGS, Vast numbers of short reads are sequenced in a single run.

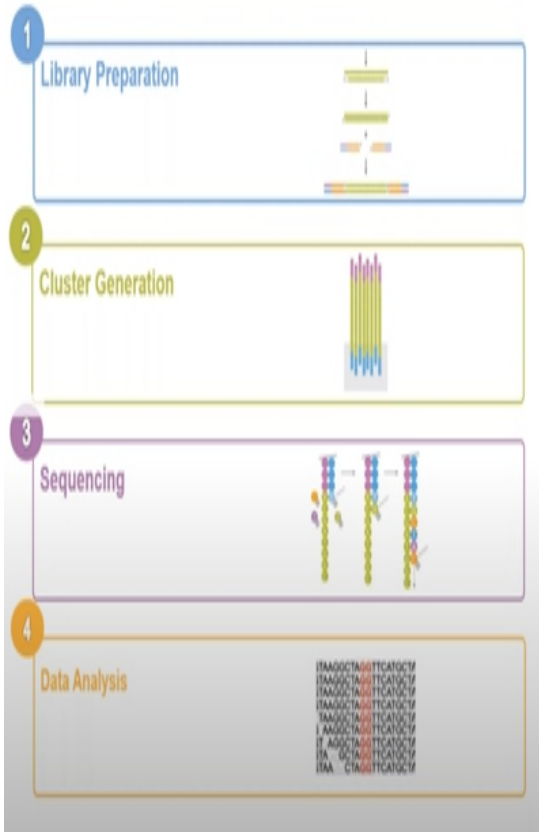


Fig.1 The Four steps in Illumina Sequencing Workflow

1. Library Preparation

The DNA to be sequenced is broken into smaller fragments by enzymes or high frequency sound waves.

To the DNA fragments the following are added to either end,

i. Primer binding regions.

ii . Unique index or bar code to identify the fragment.

iii. Adapter sequences at either end of the DNA fragment which bind to homologous adapter capture sequences in the flow cell.

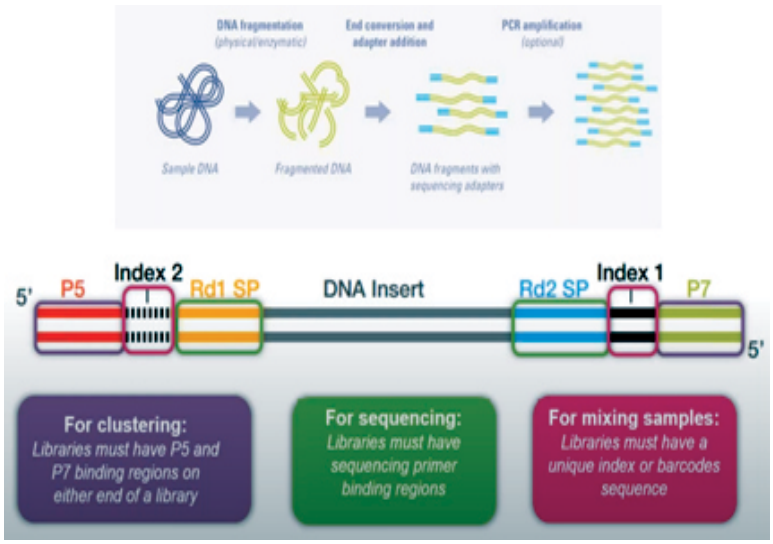


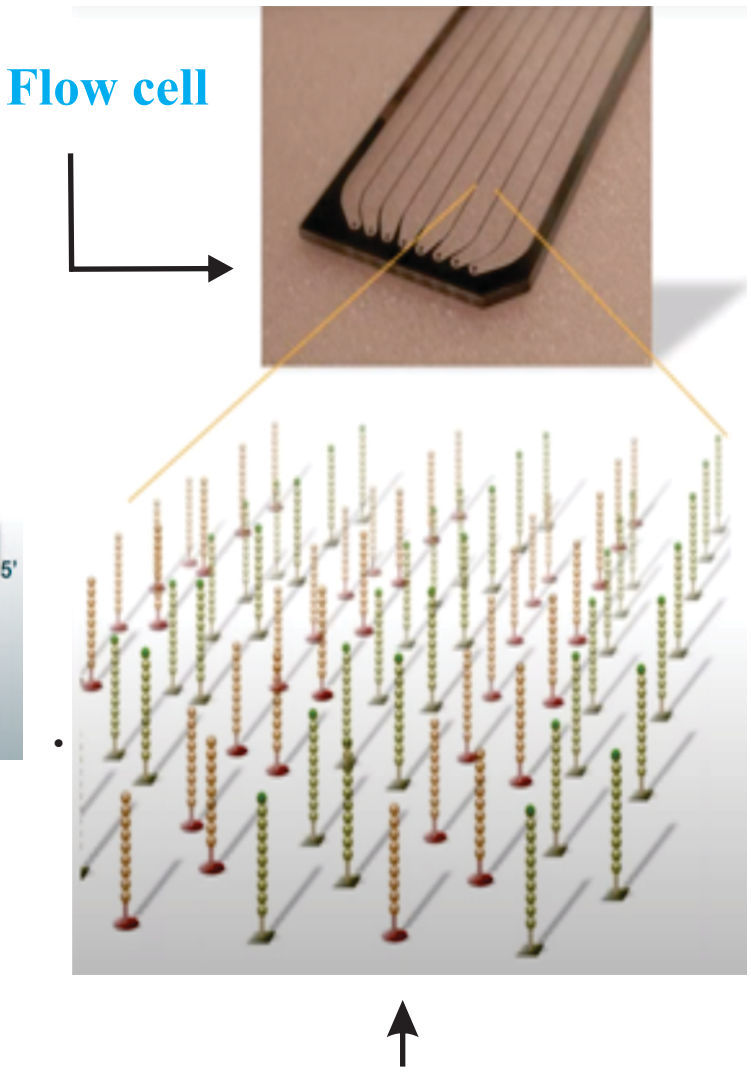
Fig.2 - Library Prepration

What is a flow cell?

Cluster generation occurs on a flow cell

A flow cell is a thick glass slide with channels or lanes

Each lane is coated with a lawn of oligos complementary to library adapters



Capture Oligos on the flow cell

Fig.3 Flow cell

Cluster Generation

The figure below (Fig.4) gives the steps numbered 1-10, involved in cluster generation.

1. and 2. involves library preparation referenced earlier.

3. The DNA library is denatured and the single stranded DNA fragments bind or hybridize to oligos on the flow cell (Capture)

4. This is the forward strand, DNA polymerase attaches to the primer binding site in the forward strand and synthesises the complementary DNA. strand which can also be referred as the reverse strand

5.The forward strand is washed away, leaving behind the reverse strand.

6. The reverse strand folds over and bridges with adjacent capture oligo (bridge hybridisation).

7. DNA polymerase attaches to reverse strand and the forward strand is created.

8. The forward and reverse strand are denatured and linearised.

9. Now the forward and reverse strands under go bridge hybridisation followed by synthesis of complementary DNA strands by DNA polymerases. The process is repeated continuously to create clusters , of forward and reverse strands.

10. The reverse strands are washed away , leaving behind amplified copies of the orignal DNA strand (the forward strand)

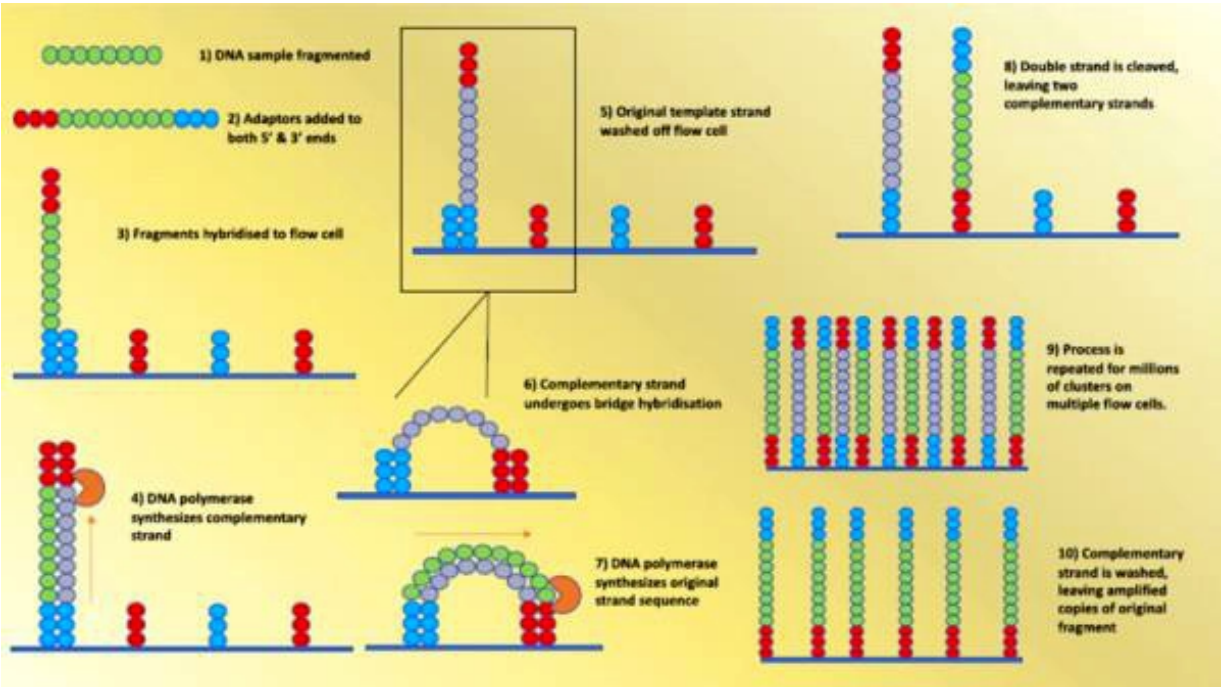


Fig. 4 Cluster Generation

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Sequencing by Synthesis

This is a proprietary method devised by Illumina, and uses Fluorescent Reversible Terminator Chemistry.

The fluorescent tagged nucleotides with terminators are incorporated into clusters. Only one nucleotide is attached , and when it attaches it emits a light signal and a picture taken and the base is read . This is one cycle. The terminators and fluorescent tags are removed which allows the next fluorescent tagged complementary nucleotide to be added and the base is read and the process continues.

The number of cycles determine the length of the read , this is the read one sequence.

The read one sequences are then washed away. Next the Index at either end of Forward strand are read, Index 1 read and Index 2 read and are washed away.

Forward strand undergoes bridge hybridisation, and reverse strand is synthesised, denaturing takes place and strands are linearised, the process repeats, clusters are generated, forward strands are washed away, and reads of reverse strand done as earlier. Completing the process of sequencing of all DNA strands in the library.

Alignment and Data Analysis

The sequencing reads are the raw data , which are now aligned and analysed by professionals in Bioinformatics.

Bioinformatics, as related to genetics and genomics, is a scientific subdiscipline that involves using computer technology to collect, store, analyze and disseminate biological data and information, such as DNA and amino acid sequences or annotations about those sequences. The reads are stored in the FAST Q format.

The sequencing reads are mapped to the reference genome and differences if any identified . These genetic variants are copy number variants and sequence variants.

Next issue

How to read a NGS report ?

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