Three generations of sequencing: An overview of sequencing chemistry

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Abstract

The DNA sequencing methods were developed by Sanger & Coulson and Maxam & Gilbert in 1977. Sanger's sequencing has been evolved into current automated DNA sequencing that is also referred to as the 'First- Generation Sequencing' (FGS), where the terminator ddNTP is tagged with specific fluorescent dyes. Second and third generation sequencing technologies referred commonly to as next generation sequencing (NGS) technology, has evolved significantly with increase in sequencing speed , decrease in sequencing cost, since its inception in 2004. Second generation sequencing (SGS), involves massively parallel sequencing of number of templates of same sample in a single run and produces an enormous volume of data economically. Genome Analyzer, HiSeq, MiSeq and NextSeq by Illumina, Inc., Ion Torrent by Life Technologies are the sequencing platforms currently available for second generation sequencing. In contrast to the short read length of SGS technologies, third generation sequencing (TGS) results in longer read length at low cost. TGS is the ultimate dream of biologists, as it will bring freedom from amplification artifacts and bias. The platforms available for the third generation sequencing are Helicos™ Genetic Analysis System by SeqLL, LLC, SMRT Sequencing by Pacific Biosciences, Nanopore sequencing by Oxford Nanopore's, to name few. NGS has catalyzed the number of breakthroughs, such as advancing scientific knowledge in human disease research to agriculture, microbial ecology to evolutionary science. Present talk will be focussed on the different sequencing chemistry of FGS, SGS and TGS platforms with brief description of the workflows used by second generation Illumina sequencers.