NEXT GENERATION SEQUENCING: METHODS FOR SHOTGUN SEQUENCING AND AMPLICON SEQUENCING

Kristoffersen J.B. [1], BobrovaO.E[2]
[1]*Institute of Marine Biology, Biotechnology and Aquaculture,Hellenic Centre for Marine Research, 71500 Gournes, Heraklion, Greece*
[2] *Department of Microbiology, Virology and Biotechnology, Odesa National I.I.Mechnikov University, Dvoryanska str.2, 65082 Odesa, Ukraine*
E-mail: jonbent@hcmr.gr

Next-generation sequencing technologies have evolved rapidly during the last decade. Here's an overview of the current leading sequencing technologies and examples of their applications. Illumina, Inc., currently has a leading position among sequencing technology companies. Illumina has a range of instruments, from the low-throughput MiniSeq for small labs to HiSeq *x10* for very high-throughput. Pacific Biosciences Inc. is another sequence technology company, whose instruments delivered greater read lengths than Illumina. Oxford Nanopore also delivers long read lengths, but with sequence instruments that are far smaller than Pacific Biosciences.

Whole-genome shotgun sequencing attempts to sequence random pieces of DNA from the whole genome. With sufficient amount of sequencing, or coverage, it may be possible to reconstruct large pieces of a genome from numerous small sequence pieces. The current optimum method for whole-genome shotgun library construction is a PCR-free approach, since this avoids biases associated with PCR-amplification. A drawback with the PCR-free approach is the need for a higher starting amount of high-quality DNA. In the Illumina TruSeq library protocol, shotgun library construction consists of DNA fragmentation to desired length; end-repair; A-tailing; adapter ligation; then optional PCR enrichment. Magnetic bead cleanups are performed between most of these steps. Quality control and accurate quantification of the finished library follows. Regardless of library protocol, every Illumina library consists of a piece of DNA, the "insert", flanked by two different adapters, with indexes either on one or both sides. The adapters contain sequences complementary to oligos on the flowcell surface, while the indexes allow multiplexing of several samples in the same flowcell lane.

In amplicon sequencing, a tiny proportion of the genome is targeted by PCR amplification with primers. Since this target will be very small compared to the whole genome, one can typically sequence numerous samples simultaneously and still get a high coverage of the target region. Numerous strategies exist for the construction of amplicon libraries. Signifcant cost-savings can be made on primer costs by using clever indexing strategies. A two-step PCR library protocol is an effective and economic method. In the first PCR round, specific primers with a 5'-end tail are used to amplify the targeted region. In the second step PCR, indexed adapters with a 3'-end complemetary to the tail from the 1st PCR are added. The stock of indexed adapters can be used for other loci and experiments; only a single pair of tailed primers need to be obtained for each new locus. For even greater

multiplexing capabilities, a second level of indexing can be added by inserting a secondary index between the specific primer and the tail. To optimize the quality and scientific value of an amplicon experiment, care must be taken regarding factors such as primer target region, fragment lengths, optimization of amplification conditions to minimize PCR artefacts, and library diversity. It is recommended to include in every experiment a Mock community with known composition in order to assess biases and error rates. In addition, extraction blanks and PCR-blanks should be included to assess possible contaminations.

Shotgun metagenome sequencing avoids most of the potential problems of amplicon library sequencing, but require a much greater number of reads per sample, hence are far costlier. With an expectation of continuously falling sequencing costs, shotgun metagenome sequencing may largely replace amplicon sequencing in the near future.