Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Oral Communication Abstract – 1.02

NEW DNA SEQUENCING TECHNOLOGIES: PRINCIPLES, APPLICATIONS AND PERSPECTIVES

VALLE G.

CRIBI Biotechnology Centre, University of Padua, Via Ugo Bassi 58/b, 35121 Padova

DNA sequencing, structural genomics, gene prediction, transcriptome analysis

For more than 30 years the Sanger method has been essentially the only approach to DNA sequencing. Even now, it is still unsurpassed both for quality and for read length, reaching up to one thousand bases in a single electrophoretic run. However, two main bottlenecks make it difficult to apply the Sanger method to large scale parallel sequencing: firstly, the need of specific DNA preparation, required for each sample, typically obtained by bacterial cloning; secondly, the electrophoretic step that is difficult to miniaturize.

This presentation covers some recent technological advances in DNA sequencing. Three "second generation" instruments are now available: the Roche-454, the Illumina-Solexa and the ABI-SOLiD. In general, second generation DNA sequencers have solved the two problems indicated above: 1) they do not need an individual DNA preparation for each sample since the amplification of DNA is performed by solid-phase PCR; and 2) the electrophoretic step is not required as the sequencing reaction is done on a solid-phase and monitored by high resolution image processing.

Two different strategies have been implemented to overcome the step of bacterial cloning. SOLiD and 454 use a method called emulsion PCR that allows the amplification of single DNA fragments onto micro-beads that are virtually kept separated from each other in the droplets of an emulsion. Solexa uses a different approach called Bridge-PCR, based on the amplification of single DNA fragments on a slide coated with primers, thus producing a tiny spot of identical double stranded sequences from each DNA fragment.

For the solid-phase sequencing the three instruments adopted different solutions: the 454 uses pyrosequencing, Solexa uses a modification of the Sanger method, while Solid uses a more complex procedure based on ligation. The 454 is able to produce about 400,000 reads per run, 200-300 bases long (that is about 100 million bases), while Solexa and SOLiD produce 30-100 million reads per run, about 35-50 bases long, totalling several gigabases.

The possible applications of these new technologies go far beyond basic sequence analysis. In particular it is now possible to analyze the transcriptome at a very high coverage, making much easier the identification of genes, alternative splicing and non-coding RNAs. A preliminary analysis of the transcriptome of grape was performed with 454, Solexa and SOLiD, producing interesting results. A critical comparison of these three technologies will be presented.