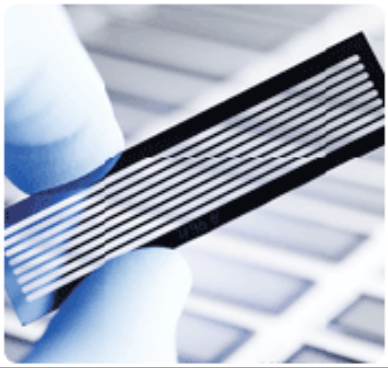
Illumina SOLEXA sequencing in the GenePool



The GenePool offers sequencing on Illumina GAII and IIx instruments.

The technology

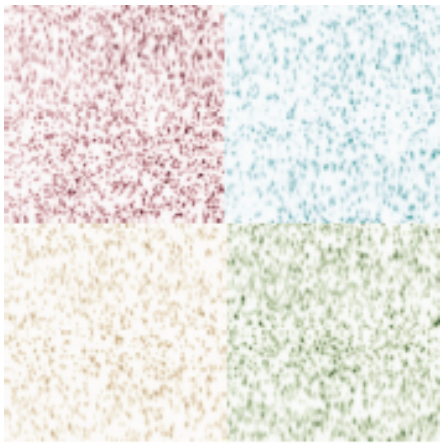
The Illumina GAII and IIx use SOLEXA short read sequencing technology. This sequencing method relies on two innovations. The first is 'in vitro' cloning, the amplification of single target DNA molecules by solid phase PCR on a microfluidics slide or flow cell (see left). The DNA to be sequenced is first sheared to a uniform length (200-500 bases) before being modified by the addition of adapters and processed in the 'flow cell'. This results in clusters of ~1000 DNA molecules about 1 nm across. The GAII uses a density of up to 80 million clusters per run while the IIx permits higher densities.

The second innovation is the use of a set of fluorescently labeled *reversible* terminating nucleotides. In each sequencing cycle, the DNA template is elongated by one nucleotide. This is read after illumination by a laser by a fixed camera, and the base-addition cycle is then repeated after deblocking and removal of the fluorophore from the terminal base. The sequence for any one cluster is determined by first aligning the 18-50 individual sets of four images (one for each base see below) from each cycle and then reading off the sequence.

The GenePool standardly runs up to 50 base sequencing. We can also perform 'paired end' sequencing, where up to 50 base reads are derived from both ends of each template. Thus each lane of the a GAII flow cell can currently yield up to 375 Mb in a single-end 50 base run, or 600 Mb in paired end 50 base reads. Longer reads and higher read numbers are achieved on the IIx system: up to 75 base reads.

Analysis

The task of turning the images of the clusters into DNA sequence is achieved by on-instrument software, and yields a simple text file of each sequence and the estimated quality of each base called. This quality is derived from the intensity of the fluorescent signal measured, and the level of interfering' signal from the other, competing bases. The GenePool bioinformaticians will perform this task for you. Further analysis depends on the kind of data being generated (genome sequencing, RNA sequencing, etc) and can also be performed for you by GenePool staff. The GenePool securely archives all analysed sequence data for at least 1 year after generation.



Contact us for more information and a quotation **genepool@ed.ac.uk**

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