ABSTRACTS (in alphabetical order)

Gregor GILFILLAN
Norwegian Sequencing Centre, Oslo NO

"ChIP-seq from low cell numbers: challenges and opportunities"

Chromatin immunoprecipitation coupled with high-throughput DNA sequencing (ChIP-seq) offers high resolution, genome-wide analysis of DNA-protein interactions. However, current standard methods require abundant starting material in the range of 1-20 million cells per immunoprecipitation. In this respect, ChIP-seq has so far failed to match the older, and widely regarded as inferior, ChIP-chip (microarray) method - which could be performed with as few as 1000 cells.

To obtain ChIP-seq results from lower cell numbers, protocol improvements can either be implemented during the immunoprecipitation itself, or the "sample prep" used for sequencing library construction.

Gilfillan lab has recently enhanced a native ChIP (non-cross-linked) protocol, to allow ChIP-seq of histone modifications to reliably be performed from 100,000 cells. However, wasted sequencing capacity caused by PCR-duplicate reads and un-mapped reads become an increasing problem as ever-lower input amounts are used. The lab is now comparing the performance of a number of alternative sample prep methods that have been developed, and promise to lower input requirements considerably.

Michael KERTESZ
Technology Development, Illumina Inc, San Diego (CA) US

"Moleculo Sequencing for Human Genome Phasing and De-Novo Assembly of Complex Genomes"

Moleculo is a novel DNA sequencing approach that delivers synthetic reads up to 10Kb long at an extremely low error rate (Q50) – thus improving the efficiency and accuracy of many existing sequencing applications and enabling a range of new applications, ranging from de-novo assembly of complex animal and plant genomes, to human genome phasing, cancer genomics and direct measurement of gene isoforms.

Moleculo's technology is based on a proprietary method including a molecular biology protocol, library prep kit and analysis algorithm that are used in conjunction with Illumina's high-throughput sequencing instruments.

Rob KLOSE
Department of Biochemistry, Oxford University, Oxford UK

"Epigenetic conservation in vertebrates revealed by non-methylated DNA profiling"

Rob Klose will describe a simplified approach to isolate non-methylated DNA from vertebrate genomic DNA. By coupling this strategy to massively-parallel sequencing,

regions on non-methylated DNA can easily be identified within virtually any sequenced vertebrate genome. Using this approach, his lab has mapped regions of non-methylated DNA, classically called CpG islands, in two independent tissues in seven diverse vertebrate species. This has revealed and unexpected level of epigenetic conservation among species and revealed unifying features shared amongst tissue specific non-methylated loci.

Marco-Antonio MENDOZA-PARRA
Functional genomics & cancer, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch FR

"A quality control system for the certification of ChIP-seq and enrichment-related massive parallel sequencing generated profiles"

The absence of a quality control system is a major weakness for the comparative analysis of genome-wide profiles generated by next generation sequencing (NGS). This concerns particularly genome binding/occupancy profiling assays, like ChIP-seq, but also related enrichment-based studies like MeDIP-seq/MBD-seq, GRO-seq or RNA-seq.

Importantly, quality control assessment may significantly improve multi-dimensional comparisons, which have great promise for extracting information from combinatorial analyses of the global profiles established for chromatin modifications, the bindings of epigenetic and chromatin-modifying enzymes/machineries, RNA polymerases and transcription factors, and total, nascent or ribosome-bound RNAs. Marco-Antonio will present a bioinformatics-based approach that associates global and local QC indicators (QCis) to virtually any type of ChIP-seq datasets as well as to a variety of enrichment-based studies by NGS. This QC system was used to certify more than 2,600 publicly available datasets, which are hosted in a database for data mining and comparative QC analyses.

Shankar PATTABHIRAMAN
Functional genomics & cancer, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch FR

“Linear DNA Amplification: recent developments”

LinDA (Linear DNA Amplification) is a versatile single-tube DNA amplification strategy, which utilizes the processivity and fidelity of the T7 RNA polymerase and has been used for transcription factor and epigenetic profiling with as low as 1,000 cells or 30 pg of starting material. The amplification procedure did not introduce any GC bias as is the case with other PCR based strategies and consecutive steps are performed in the same tube, thus eliminating the need for column purification and minimizing the risk of sample losses and enabling automation. Several developments to the original procedure have been introduced making it more cost effective by integrating the sequencing library step and amenable to different NGS strategies such as exome and methylome sequencing. In addition, the lab is developing LinDA for single cell analysis.