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Poster Abstract – 6.06

TRANSCRIP PROFILING USING NEXT-GEN SEQUENCING TECHNOLOGIES

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mRNA-seq, transcriptome analysis

We are developing transcriptome analyses using mRNA-Seq. cDNAs libraries obtained by retrotrascription of mRNA random fragments, were sequenced with Illumina Genome Analyzer II who provides million of short sequences 36 or 75 nt long in paired-ends or single reads. By using two different softwares for alignment of sequences on the reference genomes (ELAND and BOWTIE) we mapped the GAII reads to exons, introns or intergenic regions, hence identifying new exons or new genes not predicted. We then adapted modules of the ERANGE program for digital gene expression analysis, detection of alternative splicing (annotated or new) and SNPs discovery. Assigned expression values showed a high reproducibility among technical replicates. Specific features of this values are that they are not subjected to background noises or saturation as this simply derives from a the number of reads obtained from GAII for each gene, and this number is directly proportional to the copies number of that transcript. Starting from mRNA isolated from total RNA is therefore possible, with this analysis, to estimate gene expression level of all gene transcripts in a cell in a given biological moment or in a particular physiological or pathological state. A strong bioinformatics engagement was necessary to develop a modified module in ERANGE software dedicated to the alternative splicing detection.