

Identification of regulatory genetic variation that affects drug response in acute leukemia by new generation ultra high-throughput sequencing

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The novel ultra high-throughput sequencing technology that has recently become available allows comprehensive identification and genotyping of rare genetic variants in large genomic regions in hundreds of samples per experiment at reasonable costs. The Illumina 1G genome analysis system uses the Solexa technology for sequencing by synthesis using cleavable fluorescent terminating nucleotides, and allows the sequencing of up to one millions of bases in a single run. The Solexa technology can also be used for gene expression analysis and for sequencing of nucleic acids tags in various applications. In this project, we will establish this new technology at the Department of Medical Sciences in Uppsala. The first application of the technology will be to identify regulatory genetic variants that affect drug response patterns in children with acute leukemia. The *in vitro* drug response of the ALL patients against ten anti-cancer drugs has been determined in cell cultures of the patient samples (Frost et al. 2003), and the clinical outcome of the patients is well documented.

The strategy of the project is to use allelic imbalance in gene expression as a guide to genes with *cis*-acting regulatory variation (Pastinen et al. 2005, Milani et al. 2007). We will sequence a set of genes that display allelic imbalance in gene expression, based on a genome-wide survey of allele specific expression in samples from 200 patients with acute lymphoblastic leukemia (ALL) performed in Dr Syvänen's group using Illumina technology. In the regulatory regions of these genes, we will screen for genetic variation that would explain the differences in expression of the two alleles, such as SNPs in transcription factor or micro-RNA binding sites or DNA methylation using the newly established Solexa high-throughput sequencing technology. Regulatory regions, in which we find variation that might explain the allele specific expression using full sequencing, will be further analyzed by genotyping and methylation analysis in a larger set of patients with acute leukemia available for this study. Regulatory variation will be correlated with the already known drug response patterns of the patients. The regulatory nature of the identified variants will be further verified by functional assays. In cases where mono-allelic expression is observed, methylation of the promoter regions will also be analyzed using the Solexa technology to sequence bisulphate treated DNA. In addition to the analysis of regulatory regions, we will perform digital expression profiling with the Solexa technology on the subset of the ALL samples from which RNA is available.

References

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