Genomic Med. (2008) 2:149–150
DOI 10.1007/s11568-009-9110-9

ABSTRACTS

HGM2008 new technologies: genome sequencing to molecular imaging symposium abstracts

018: Single-cell genotyping and targeted genome sequencing techniques

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Most genetic studies of multi-factorial disease generate a large set of loci associated with disease phenotypes. To identify the exact molecular alteration behind the association, full sequence information from these loci is often required from a large number of affected and control subjects. Even with current development in sequencing capacity, it will remain too expensive to sequence whole genomes. We have developed a technique to efficiently extract relevant parts of genomes for sequencing. We have further developed a rapid, simple, and cost-effective assay to detect copy-number variation, which is a common cause of multi-factorial disease. Mutations often cause aberrant expression in specific cells. Ultimately, expression levels should be measured on a single-cell level, and preferably in the preserved context of tissues, not to overlook alterations in minority cell populations. We are developing an in situ genotyping technique that allows detection of individual transcript molecules with allelic resolution in tissue samples.

019: Design and implementation of a high-throughput, high content RNAi screen for endocytosis in metazoan cells

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It is increasingly evident that endocytosis in metazoan cells occurs through multiple specialized yet interconnected pathways. For many of these though, in particular clathrin independent (CI) modes of endocytosis, little is known about their regulatory circuits and molecular machinery. We have used the adherent Drosophila SR + cell line and a custom designed high-throughput, high content fluorescence microscopy to screen endocytic phenotypes using RNAi to 7,216 genes, chosen for their conservation between human and flies. We have simultaneously monitored two endocytic pathways, the canonical clathrin mode marked by endocytosis of transferrin and the CI-GEEC pathway marked by fluorescent dextran. Cells were grown and assayed on a customized glass slide followed by semi-automated image acquisition. Custom-written MATLAB routines were used to identify single cells and extract quantitative measures of endocytic uptake. The design of this screen provides quantitative data at both the single cell and population level. Heterogeneous responses to RNAi within a single population of cells allowed us to develop novel statistical indicators of perturbation. These indicators are robust to the systematic noise characteristic of such large-scale experiments. Candidate genes from the primary screen are being channelled through independent confirmatory experiments which have served to validate both the set of 'hits' and the statistical methodology, and provide a comprehensive genetic landscape for the understanding of this fundamental cellular process. The analysis of quantitative phenotypic data from cells and their populations in large-scale screens may be generally useful for exploring biological networks.

020: Technologies for personal genomics: exomes, allele-specific-RNAs, microbiomes, VDJomes

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Relative to the 2004 reference human genome, a personal genome shows about 10,000 DNA variations which affect protein structure and three million which do not. While association studies of common alleles with diseases mostly yield, so far, weak predictive power and few causative alleles. Most researchers expect that this will be soon remedied by genome-wide sequencing and aggregating alleles by system functions. Second-generation sequencing (e.g. http://www.Polonator.org/–uniquely open-source for hardware, software, wetware) has brought costs down by 10,000-fold (from $300 M to $30 K), but to get to a true mass market requires a cost less than $1,000—which for now requires targeted sequencing including coding variants ($\sim$1% of the genome), regulatory variants (RNA quantitation by sequencing), microbiome and VDJ-ome). We use solution hybridization capture for >10 kbp contiguous regions, but for more precision (e.g. 100 bp exons) we can cut cost by 10-fold by using circle-capture methods. http://www.PersonalGenomes.org/is a unique effort to integrate these with comprehensive sets of medical and non-medical traits and environmental measures and provide these in an open-access format. To

gain access to personal variation in RNAs in a broad set of cell-types, we establish pluripotent stem cells from skin. We have IRB approval to expand our current cohort to 100,000 volunteers.

021: Clinical molecular diagnosis in the era of massively parallel sequencing: PAP/MAP for monitoring of therapy or recurrence in breast cancer

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Mortality from breast cancer may be reduced substantially if personalized therapy is instituted. Whole exome capture followed by massively parallel sequencing will soon be available to enumerate all the cancer-specific protein and microRNA structural changes. With this information, therapy can be instituted to leverage the enumerated "weaknesses" of the cancer. Once therapy is instituted, monitoring of therapy or recurrence (MOTOR) can be performed by utilizing the cancer signature in circulating cells. As a model system to demonstrate the efficacy of MOTOR, we analyzed plasma to detect breast cancer mutation signatures of DNA fragments released from apoptotic or necrotic cancer cells and the DNA of circulating cancer epithelial cells. Initially, candidate cancer genes were sequenced in breast cancer tissue samples to identify in each patient a personalized cancer signature of somatic mutations. Subsequently, pyrophosphorolysis activated polymerization (PAP) (http://www.cityofhope.org/PAP), a method for detecting ultra-rare mutations, was used to detect the cancer-specific signature in DNA isolated from the plasma and circulating epithelial cells of patients with non-metastatic breast cancer. Our data demonstrate the rapid development of PAP assays that routinely detect even a single copy of the cancer-specific somatic mutations in circulation. The selectivity of PAP is extremely high, often ranging from at least one part per million to as high as one part per billion. Circulating levels of cancer signature mutations and their rates of increase are being measured at multiple intervals in a multi-year follow up. Our ultimate goal is to achieve effective monitoring of chemotherapy and to predict recurrence months to years earlier than currently possible.