



Functional Genomics

Program/Abstract # 359

GXD: A gene expression resource for developmental biologists

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The Gene Expression Database (GXD) is an extensive and easily searchable database of gene expression information about the mouse that is freely available online. GXD collects different types of expression data from wild-type and mutant mice, including RNA in situ hybridization and immunohistochemistry results. A strong emphasis is on gene expression during development. The GXD staff reads the scientific literature and enters the expression data from those papers into the database on a daily basis. GXD also acquires expression data directly from researchers, including laboratories doing large-scale expression studies. GXD currently contains over 420,000 expression results for more than 9200 genes. In addition, it has over 70,000 images of expression data, allowing users to search for these data and to interpret the experiments themselves. By being an integral part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with other genetic and disease-oriented data. Thus, users can evaluate expression data in a larger context and search by a wide variety of biologically and biomedically relevant parameters. GXD is unique because it collects complex data from disparate sources, integrates it, and then provides tools that make it possible for researchers to search these data in ways unavailable elsewhere. GXD is available through the MGI web site at <http://www.informatics.jax.org>. GXD is funded by NIH grant HD033745.

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Quantitative analysis of cis-regulatory genes and networks

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cis-Regulatory components of the genome are crucial players of gene regulatory networks. Efficient discovery and functional characterization of cis-regulatory modules (CRMs), and their integration into gene regulatory networks have remained a major challenge in genomic regulatory biology. To accelerate these processes, we recently developed a set of “barcoded” reporters that enable us to track in vivo activities of ≥ 100 CRMs simultaneously. These reporters are powerful tools for high-throughput and quantitative discovery and characterization of CRMs. We will present methods for efficiently studying interactions between cis and trans-regulatory networks using this system.

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Gene regulatory network model of sea urchin ectoderm formation

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Gene regulatory networks (GRNs) consist of interacting regulatory genes and provide a mechanistic understanding of developmental processes. Here we conducted a systematic approach to construct the sea urchin ectodermal GRN during early embryogenesis. We first identified genes involved in ectodermal formation by whole mount in situ hybridization. To see the interaction among those regulatory genes, we carried out large-scale perturbation assays in which translation of each gene was interrupted by introduction of morpholino antisense oligonucleotides (MASO). Resultant expression changes were quantified by QPCR and nCounter Analysis System. Perturbation data and spatiotemporal expression patterns were combined to infer network linkages, and construct a provisional GRN model. We also carried out cis regulatory analysis to characterize modules that define specific expression patterns of regulatory genes. Our current ectodermal GRN includes 22 transcription factor genes, 4 genes encoding known signaling ligands, and 3 signal genes with unknown function. Key GRN features relevant to the ectoderm formation include: 1) a nodal pathway that initiates the oral ectoderm formation; 2) a double negative gate of *gsc* and *sip1* that specifies the lineage of the oral ectodermal cells; and 3) a number of aboral genes of the homeobox family that lock the fate of aboral ectoderm through mutual feedback activation.

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Program/Abstract # 362

Transcriptomics during maternal-to-zygotic transition in the zebrafish: An mRNA-seq approach

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Maternally deposited mRNAs direct early development before zygotic transcription initiation during the mid-blastula transition (MBT). In order to gain insights into the mechanisms involved in maternal-to-zygotic transition in the zebrafish (*Danio rerio*), we performed high throughput mRNA sequencing of oocytes, cleavage-, blastula-, and early

gastrula-stage embryos using the SOLiD3 sequencing platform (ABI). About 23–55 million reads were generated from poly(A)-selected RNA libraries. While ~58% of the unique reads map to annotated sequences, the remaining accounts for putative novel transcribed regions (NTRs) in the zebrafish genome — an average of 1800 NTRs in each library, across all chromosomes. By mapping splice-junction reads, our data support known splicing patterns of characterized genes such as *tp53*, and reveal novel splicing patterns in others. Furthermore, a cohort of ~1000 maternal transcripts with delayed polyadenylation pattern was identified, which is a novel observation in zebrafish. In summary, our work represents the first application of next generation sequencing technology to study early development in zebrafish, and reveals novel elements which should contribute to the annotation of its genome and the understanding of early developmental mechanisms.

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Transposon-mediated insertional mutagenesis in the rat

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We have established a piggyBac (PB)/Sleeping Beauty (SB) transposon (Tn)-mediated insertional mutagenesis system in the

rat. Single copy PB Tn insertions were introduced into the rat genome by coinjection into albino zygotes of circular plasmids carrying the Tn and mRNA encoding PB transposase (Tase). The Tn designed to act as a gene trap carries a tyrosinase pigmentation gene that can rescue the albinism. We also generated albino transgenic rats expressing PB or SB Tase ubiquitously by standard methods. “Seed” rats are generated by crosses that carry both the Tn and Tase transgenes. These seed rats have random and mosaic coat color pigmentation, presumably due to somatic Tn mobilization. These seed rats were then bred with wild-type albinos to segregate germline transposition events. These are visualized by a change in coat color because the expression levels of the tyrosinase reporter gene are sensitive to chromosomal location. Progeny with novel coat colors were analyzed by inverse PCR (iPCR) to molecularly identify the novel insertion sites. DNA sequence analysis of the iPCR products has identified Tn insertions into the 12 defined genes, indicating that we have generated 12 gene-trap mutant rats. The frequency of germline transposition ranges around 8%, approximately one new insertion per litter. This simple insertional mutagenesis system should lead to the generation of a library of new mutations in the rat. The generation of new mutations in the rat should open up this fundamental model system for developmental studies.

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