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high resolution transcriptome of diverse cell types in mammals

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Update of the FANTOM web resource: high resolution transcriptome of diverse cell types in mammals

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ABSTRACT

Upon the first publication of the fifth iteration of the Functional Annotation of Mammalian Genomes collaborative project, FANTOM5, we gathered a series of primary data and database systems into the FANTOM web resource (http://fantom.gsc.riken.jp) to facilitate researchers to explore transcriptional regulation and cellular states. In the course of the collaboration, primary data and analysis results have been expanded, and functionalities of the database systems enhanced. We believe that our data and web systems are invaluable resources, and we think the scientific community will benefit for this recent update to deepen their understanding of mammalian cellular organization. We introduce the contents of FANTOM5 here, report recent updates in the web resource and provide future perspectives.

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INTRODUCTION

Recent advances in transcriptomics have improved the coverage as well as the detection accuracy of profiled RNA molecules. This means that several new opportunities are available for studying molecular function, gene regulation, embryogenesis, response to environmental stimuli and more.

The FANTOM project, one of the longest-lived collaborative projects in genomics, aims at the functional characterization of mammalian genomes. It started in the early 2000 with the generation of more than 100 000 mouse full-length cDNAs that revealed that the portion of the genome encoding for proteins is small, whereas the majority of it is involved in producing non-coding RNAs (1,2). Those full-encoding for proteins is small, whereas the majority of it is represented by microarray data and microarray data. The FANTOM4 web resource also incorporates genome browsers and bioinformatics analysis results.

Within the FANTOM5 project, the consortium profiled nearly 2000 human and 1000 mouse samples, representative of the majority of cell types and tissues, using CAGE followed by next generation single molecule sequencing (Helicos) (9). The project uncovered the first comprehensive promoter landscape of a mammalian genome (3) and the existence of anti-sense transcription (4). FANTOM4 adopted CAGE and 454 Life Science sequencing combined to Illumina microarrays to study a model of differentiation in human THP-1 (myeloid leukemia) cells, and to define the transcriptional regulatory network based on TSS activity that explained such timely process (5).

Several databases were developed to collect the results from those four FANTOM iterations: the FANTOM-db (6) to store the mouse cDNA clones; the RIKEN Expression Array Database (READ) (7) containing the expression profile data for the clones; and the FANTOM4 web resources (8) to integrates CAGE expression profiles with short RNA sequencing data and microarray data. The FANTOM4 web resource also incorporates genome browsers and bioinformatics analysis results.

The second phase of FANTOM5 aimed at studying dynamic states of cells

Dynamic states of cells

The second phase of FANTOM5 aimed at studying dy-
namic changes in the transcriptional landscape over time, complementing the collection of steady cellular states of the first phase. We collected 19 human and 14 mouse time series, covering development (mouse visual cortex and cerebellum), in vitro differentiation (iPS to neurons, ES cells to cardiomyocytes, calcification), response to drugs (MCF7 cells response to HRG and EGF, macrophage response to LPS) and infection (rinderpest, influenza), which resulted in additional nearly 1000 human and 600 mouse samples. The complete sets of FANTOM5 human and mouse samples are listed in Supplementary Tables S1 and S2, respectively.

Identification of additional promoters and enhancers

Given the increase in CAGE profiles number, we extended the list of promoters and transcribed enhancers. As a result, the total number of identified peaks (that correspond to a promoter) has increased by 10% in human and 30% in mouse to a total of ~200 000 and 158 000, respectively. Although the samples profiled in the phase 2 make up roughly 50% of the entire FANTOM5 data collection, the number of distinct cell types that was added is small and as a result does not expand the set of identified human promoters to the same extent as the previous phase. Transcribed enhancers were also identified by using the added CAGE profiles based on bi-directionality of transcription initiation (10), resulting in additional 20 000 human regions, while mouse enhancers were identified all at once in the second phase (Table 1).

Table 1. Summary of the number of samples, promoters and enhancers for human and mouse

<table>
<thead>
<tr>
<th>Archived data</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 1+2</td>
</tr>
<tr>
<td>Samples</td>
<td>975</td>
<td>1816</td>
</tr>
<tr>
<td>CAGE peaks</td>
<td>184 827</td>
<td>201 802</td>
</tr>
<tr>
<td>Enhancers</td>
<td>43 011</td>
<td>65 423</td>
</tr>
</tbody>
</table>

Upgrade to the latest genome assemblies

All data were originally processed based on the reference genomes GRCh37 (hg19) for human and GRCm38 (mm9) for mouse. Thanks to the continued efforts to improve the reference genome sequences, GRCh38 (hg38) and GRCm38 (mm10) have recently become available as the new target reference genome sequences, GRCh37 (hg19) for human and GRCm37 (mm9) for mouse. Thanks to the continued efforts to improve the reference genome sequences, GRCh38 (hg38) and GRCm38 (mm10) have recently become available as the new target reference genome sequences. We therefore compiled a data set that consists of (i) TSS activities at a single base-pair resolution from re-alignment of the CAGE reads with the latest genome assemblies, representing the most accurate TSS profiles (ii) CAGE peaks consistent with those defined on the old genome assemblies by using liftOver, a tool for conversion of genomic coordinates across different assemblies (https://genome.ucsc.edu/cgi-bin/hgLiftOver), and (iii) expression tables based on (i) and (ii) with dedicated normalization, representing the most accurate expression profiles of consistent promoters between two genome builds. Gene-promoter associations were also redone in order to account for changes in the coordinates of (ii) after migration to the latest genome assemblies.

NEW FEATURES, DATABASES AND TOOLS IN THE SECOND PHASE OF FANTOM5

Incorporation of the latest data and time series navigation

Contents of all databases and interfaces (ZENBU, SSTAR, TET, Track Hub and BioMart) were expanded to cover the new data without the need to change their data structure. Besides the increase in data content, navigation interfaces to the 33 time series data sets were implemented in SSTAR, where a clickable chart representing the set of time courses (22) redirects users to individual time points or to a dedicated page for one set of time series (Figure 1A).

Partner databases and tools

As a result of extensive use of the FANTOM5 data, multiple databases and tools were also developed by collaborating researchers to share their own results (Table 2). Most of them were published independently, but were hyperlinked with SSTAR at the content level (deep linking), as in Figure 1B. Here, we give a brief introduction of each, so to help users to explore contents and derived results, and developers to design their future studies based on the FANTOM data set.

CAGEdbPOSSUM (23) implements TF binding site (TFBS) enrichment predictions within cis-regulatory regions derived from TSSs identified by CAGE to infer key transcriptional regulators. The user selects TSSs associated with their cell or tissue type of interest and the tool predicts TFBSs within putative cis-regulatory regions surrounding the TSSs to assess their over-representations as compared to what would be expected by chance. CAGEdbPOSSUM has been applied to all phase 1 FANTOM5 samples using TF binding motifs from the JASPAR database (24). By combining motif enrichment analysis with CAGE-derived cis-regulatory regions, CAGEdbPOSSUM helps power insight into the TFs that act as key regulators in specific mammalian cells and tissues.

EpiFactors (25) is a manually curated database providing information about epigenetic regulators, their complexes, targets and products in human. It contains information on 815 proteins, including 95 histones and protamines and 69 complexes. For 789 of the related genes, expressions values across all FANTOM5 samples are presented. The protein (gene) page contains a summary of the available data with external links, including mouse orthologous from MGI (26) if available, and the complex page provides information on proteins involved in complex formation, their molecular function and specific targets and products.

Ligand Receptor Connectome (27) is a web-based visual and interactive guide to cell-cell communication networks between 144 major human primary cells (profiled in FAN-
TOM5) using 2442 ligand–receptor pairs. Users can select their cells, ligands, receptors or interacting pairs of interest and visualize them as a network with cells being nodes and interactions being edges. This helps to uncover which cells are communicating the most via selected ligand-receptor pair(s), shows the top paths used to communicate between any given two cells or yields information on most specific ligands and receptors expressed by a given cell. Visualized networks can be downloaded as an .svg image or in a format compatible with Cytoscape (28) for further exploration.

Mogrify (29) is a tool that can predict TFs that may be used for the reprogramming of cells by taking advantage of regulatory network information and gene expression data. The pre-calculated results, key regulators influencing the change of cellular states are based on the FANTOM5 data and are available in the database.

SlideBase is a web-based tool that enables users to select enhancers, promoters and more from the FANTOM project upon user-defined expression thresholds for each sample, through the usage of interactive sliders. This allows for on-the-fly selection of tissue-specific enhancers or promoters, with definitions set by the user. The tool also reports overlaps with SNPs, enhancer-TSS associations by co-expression and allows for genome-browser visualizations of selected sets.

RefEx (Reference Expression Data set) is a curated reference data set of mammalian gene expression measured by four different but complementary technologies (EST, GeneChip, CAGE and RNA-seq) from publicly available
<table>
<thead>
<tr>
<th>Database or tool name</th>
<th>Description</th>
<th>update from the initial release (Lizio et al.)</th>
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<td>web-accessible directories of genomic data that can be viewed on the UCSC Genome Browser tool for the visualization and analysis of network graphs</td>
<td>updated to include phase 2 data</td>
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<tr>
<td>CAGEEd-oPOSSUM</td>
<td>motif enrichment analysis from CAGE-derived TSSs</td>
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<td>Epifactors</td>
<td>database for epigenetic factors, corresponding genes and products</td>
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<td>Ligand Receptor Connectome</td>
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FUTURE PLANS

Additional data

The published data so far have described samples derived from human and mouse. In the course of the FANTOM5 project, we attempted to achieve cross-species comparisons in a few selected cell types. Studies on rat, dog, chicken and macaque samples are under preparation for publication and will be incorporated to the FANTOM web resource.

A current limitation of the published data in FANTOM5, besides the coverage of species, lies in the approaches we take to explore RNAs. Since CAGE protocol is designed to capture only the 5′-end of capped long RNA molecules, the internal structure of long RNAs and small regulatory RNAs remains unexplored. To complement the CAGE profiles, CAGEscan (31), RNA-seq and small RNA sequencing data are being analyzed and will also be added to the FANTOM web resource.

Additional databases and tools

As introduced above, the data set provided by FANTOM5 forms a foundation for unique analysis and tool development. We foresee efforts in the development of additional databases and interfaces, within and outside of the FANTOM consortium, and won’t exclude the possibility to interconnect external tools with our databases; this would increase both their and the FANTOM web resource overall utility.

Upgrade of the existing databases and interfaces

We are also actively working on upgrading the existing databases and interfaces. In particular, functionalities of ZENBU are being enhanced to empower users with more data manipulation and visualization tools. The backend engine of SSTAR, Semantic MediaWiki, is going to be upgraded to the latest version to improve responsiveness. These changes, as well as expansion of the contents to cover additional data, will further facilitate exploration and characterization of mammalian genomes in the context of cellular states.

Lastly, the consortium is already focused on the next FANTOM project. For its sixth iteration, we aim to uncover the function of long non-coding RNAs by high-throughput screening coupled with CAGE.
SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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REFERENCES


