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<td>June 16, 2017 4:35:11 AM EDT</td>
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(Article begins on next page)
RTeQTL: Real-Time Online Engine for Expression Quantitative Trait Loci Analyses

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Abstract

Our database tool, called Real-Time Engine for Expression Quantitative Trait Loci Analyses (RTeQTL), can efficiently provide eQTL association results that are not available in existing eQTL databases browsers. These functions include (i) single SNP (single-nucleotide polymorphism) and (ii) two-SNP conditional eQTL effects on gene expression regardless of the magnitude of \( P \)-values. The database is based on lymphoblastoid cell lines from >900 samples with global gene expression and genome-wide genotyped and imputed SNP data. The detailed result for any pairs of gene and SNPs can be efficiently computed and browsed online, as well as downloaded in batch mode. This is the only tool that can assess the independent effect of a disease- or trait-associated SNP on gene expression conditioning on other SNPs of interest, such as the top eQTL of the same gene. It is also useful to identify eQTLs for candidate genes, which are often missed in existing eQTL browsers, which only store results with genome-wide significant \( P \)-value. Additional analyses stratifying by gender can also be easily achieved by this tool.

Database URL: http://eqtl.rc.fas.harvard.edu/

Introduction

The ability to interrogate and study the genetics of functional phenotypes that are intermediate between a DNA variant and a disease phenotype of interest can point to the true biological mechanism, critical to disease etiology. Gene expression is one of these key intermediate functional phenotypes (1–3). Numerous studies illuminate significant genetic variation, within and between human populations that affects gene expression levels, and by doing so may underlie phenotypic variation (e.g., 4, 5–8).

depict sex-specific genetic architecture (25), a sex-specific
results in any eQTL browser. As human diseases and traits
morphisms (SNPs) and genes on the genome and store the

analyses are not feasible for existing eQTL browsers
for 2 million SNPs in the HapMap Project filled in using
imputation. These two data sets together identified gen-
ome-wide significant cis and trans eQTLs for 14 177 genes
(27). We will impute the latest version of 1000 Genome
Project variants whenever available and update the Web
site.

Microarray hybridization and normalization
The peripheral blood lymphocytes were transformed by
Epstein-Barr virus, and then cultured in 500-ml roller. The
cell lines were collected when the cell lines reached the log
phase followed by storing at −80°C until use. RNA was ex-
tracted from the samples stored at −80°C in batches using
the RNeasy Maxi Kit, after which the quality and the
quantity of RNA were evaluated. In all, 10 mg of RNA
was used to synthesize cDNA, which was used as a tem-
plate in vitro transcription according to the manufac-
turer’s instruction. Then 15 μg of labeled, fragmented
cRNA was hybridized to Affymetrix U133 Plus 2.0 GeneChips and Illumina Human6 V1 array for MRCA
and MRCE data sets, respectively (27). The MRCA expres-
sion data were normalized using the robust multi-array
average package to remove any technical or spurious back-
ground variation. The MRCE expression data were nor-
malized using quantile normalization based on expression
values from GenomeStudio.

Whole-genome genotyping and imputation
All DNA samples were subjected to stringent quality con-
trol to check for fragmentation and amplification. We
adopted 20 μl of DNA at a concentration of 50 ng/ml for
each array. Whole-genome genotyping was performed ac-
cording to manufacturers’ protocol using the Illumina
HumanHap300 Genotyping BeadChip in a BeadLab with
full automation, and the process was traced in real time.
We excluded SNPs with call rate <95%, Hardy–Weinberg
equilibrium \(P < 10^{-6}\) and MAF <2%. We imputed gen-
otypes from all HapMap2 SNPs using Markov chain haplo-
typing (MaCH) package (28). All imputed SNPs with low
imputation quality score (R-square < 0.3) were excluded from the database.

Statistical analysis model
Linear mixed model is used to account for the family relatedness in the data set. For the sibling data, this model is identical to the model implemented in the multipoint engine for rapid likelihood inference (MERLIN) package (29) that was used in previous publication on the same data sets (4, 27). Specifically, the expression level of an expression probe is modeled as:

\[
\text{probe} = a + \text{SNP1} \cdot \beta_1 + \text{SNP2} \cdot \beta_2 + Z + \epsilon \quad (1)
\]

where \(\beta_1\) is the fixed effect for SNP1 and \(\beta_2\) is the fixed effect for SNP2, \(Z\) is random effect for family and \(\epsilon\) is residual error. R package nlme is used to fit this model and test the SNP effect. The same model excluding the term for SNP2 is used to do single SNP analysis. For analyses stratified by sex, this model is applied to male or female separately. Before fit model (1), inverse normal transformation was applied to expression level to remove outlier’s effect, and batch effects were removed by adjusting principal components calculated based on all genes expression (13, 27).

User input
The user chooses the gene and SNPs in analysis. The probe name for either the Affymetrix or Illumina platform can be chosen by specifying gene names and then adding them to the input box for probes. For analyses involving multiple pairs of gene and SNPs (batch mode), the list of SNP rs names and probe names can be copied and pasted into the corresponding input boxes. There are two cases for SNP columns: (i) when single SNP analysis is desirable, the user inputs the SNP rs name into ‘SNP1’ column and ‘-’ (short dash) in the ‘SNP2’ column. (ii) When two SNPs analyses are desirable, the user inputs the SNP1 rs name into ‘SNP1’ column and SNP2 rs name in the ‘SNP2’ column. When analyses stratified by sex are needed, the user can choose appropriate data sets in the drop-down menu named ‘Stratify by gender’, where ‘Male & Female’ means analysis using full samples without considering SNP-gender interaction effect, ‘Male” or “Female” will only output results for male or female data set, respectively, and ‘Gender Specific’ will perform analysis in male and female separately but output both results. Sanity check for input names will also be performed. We provide a manual on our website and readers will find detailed description on how to use our database http://eqtl.rc.fas.harvard.edu/mrce/static/RTeQTL_manual_20130623.pdf. For example, the input setting shown in Figure 1 will return eQTL results for the following three models:

- Expression of \(211698\_at = rs6809559 + rs1538187\) (Two-SNP analysis)
- Expression of \(121\_at = rs1538187 + rs6809559\) (Two-SNP analysis)
- Expression of \(1007\_s\_at = rs6809559\) (Single-SNP analysis)

\[\text{Results output}\]
Results table will be output on the web page and can be downloaded to desktop computer by clicking the link ‘Download the table as csv file’ on top of the result table. Each row corresponds to a result for each pair of gene and SNPs. Full details for regression results are available, including effect size, standard error, test statistics, \(P\)-value as well as gene annotation of the expression probe and SNPs (chromosomal position, allele label, allele frequency, MaCH imputation quality score, Rsq- see Table 1) and the column to indicate the samples used for analysis. If we compute single SNP, the corresponding outputs of the SNP2 are ‘NA’. If the input SNP or probe name could not be found in our data files, there will be some notes in the last row of the output table. See Figure 2 for an output example.

\[\text{Implementation}\]
Python and HTML languages are used to control workflow and provide efficient access to the data. R function is used to compute the linear mixed model. Original SNP data and expression data were deidentified and stored as binary format. We built efficient index so that specific SNP and expression data can be retrieved in real time. Specifically, one design feature of this engine is that we transform the huge text files of SNPs and gene expression data into multiple smaller binary files to accelerate I/O reading speed. The other feature of this engine is that we used hierarchical index so that the SNPs data corresponding to the SNP1 name input from web page can be quickly located and acquired in the binary files. The analysis for 100 gene-SNPs pairs takes only 20s.

\[\text{Examples}\]
Our eQTL database has been applied to real biological data. The first example is for analysis stratified by gender (26). A genome-wide search for sexually dimorphic associations with height, weight, body mass index, waist circumference, hip circumference and waist-hip ratio was conducted and results demonstrate the value of sex-stratified GWAS to unravel sexually dimorphic genetic underpinning complex traits. The other example is for eQTL
conditional analysis (21–24). The conditional analyses were performed for all expression data, except for cortical tissue, by conditioning the trait-associated SNP on the most significant cis-associated SNP for that particular gene transcript and vice versa.

Commitment to future updates
We will impute genetic variants from the 1000 Genomes panel (phase 1) and update the database. Each following release of 1000G variants will be imputed and incorporated to the database.

Conclusion and Discussion
We developed an efficient web-based database tool for eQTL analysis of any gene and SNPs available. Both single-SNP and two-SNP analyses can be performed, as well as analyses stratified by males and females. The computational result for any pairs of gene and SNPs can be shown online and downloaded in comma separate values (CSV) format. Controlling for multiple testing is important even for candidate gene study. The number of tests to control is determined by the actual number of SNP-gene pairs

Table 1. Headers and description of the online output table

<table>
<thead>
<tr>
<th>Column order</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect</td>
<td>Effect size $\hat{\beta}$ from linear mixed model. The amount of increase/decrease expression by one copy of the Allele 1 in the unit of one standard deviation</td>
</tr>
<tr>
<td>2</td>
<td>SE</td>
<td>Standard error of $\hat{\beta}$</td>
</tr>
<tr>
<td>3</td>
<td>DF</td>
<td>Degrees of freedom of the test</td>
</tr>
<tr>
<td>4</td>
<td>$t$-value</td>
<td>$\beta/SD(\hat{\beta})$</td>
</tr>
<tr>
<td>5</td>
<td>$P$-value</td>
<td>The probability of $P(t &gt;</td>
</tr>
<tr>
<td>6</td>
<td>AL1/2</td>
<td>The allele1/2 label</td>
</tr>
<tr>
<td>7</td>
<td>FREQ1</td>
<td>Frequency for Allele1</td>
</tr>
<tr>
<td>8</td>
<td>Chr</td>
<td>Chromosome</td>
</tr>
<tr>
<td>9</td>
<td>Position</td>
<td>Position on chromosome (NCBI 36)</td>
</tr>
<tr>
<td>10</td>
<td>Rsq</td>
<td>MaCH imputation quality score, which estimates the squared correlation between imputed and true allele counts</td>
</tr>
<tr>
<td>11</td>
<td>Gender</td>
<td>Sample’s gender when analysis stratified by gender</td>
</tr>
</tbody>
</table>

This table provides the header names and description of the columns of the result table for online association analyses output by the RTeqQTL website.
queried from the Web site, instead of the number of available SNPs around the locus of interest. As a general guideline to provide a sense of significance level at genome-wide average, we note that we previously estimated that 5% false discovery rate (FDR) accounting for all cis and trans pairs corresponded to $P < 1.02 \times 10^{-7}$ (1% FDR corresponding to $P < 1.62 \times 10^{-8}$) (27). For cis eQTL defined as SNP and probe within 1 Mb of each other, the 1% FDR corresponded to $P < 6.83 \times 10^{-5}$.

To the best of our knowledge, it is the only online tool that can evaluate the independent effect of a disease- or trait-associated SNP on gene expression conditioning on other SNPs of interest, such as the top eQTL of the same gene. We commit to update the web tool regularly by incorporating more gene expression data sets and imputing the latest panel of variants from the 1000 Genomes Project when available.

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Conflict of interest.
None declared.

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