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GermSAGE: a comprehensive SAGE database for transcript discovery on male germ cell development

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ABSTRACT

GermSAGE is a comprehensive web-based database generated by Serial Analysis of Gene Expression (SAGE) representing major stages in mouse male germ cell development, with 150 000 sequence tags in each SAGE library. A total of 452 095 tags derived from type A spermatogonia (Spga), pachytene spermatocytes (Spcy) and round spermatids (Sptd) were included. GermSAGE provides web-based tools for browsing, comparing and searching male germ cell transcriptome data at different stages with customizable searching parameters. The data can be visualized in a tabulated format or further analyzed by aligning with various annotations available in the UCSC genome browser. This flexible platform will be useful for gaining better understanding of the genetic networks that regulate spermatogonial cell renewal and differentiation, and will allow novel gene discovery. GermSAGE is freely available at http://germsage.nichd.nih.gov/

INTRODUCTION

Spermatogenesis is a complex and tightly regulated developmental process that involves mitotic expansion and proliferation of spermatogonia (Spga), meiosis in spermatocytes (Spcy) and postmeiotic differentiation in spermatids (Sptd). It provides an informative model system for studying the underlying molecular mechanisms of the physiological changes occurring during self-renewal and differentiation of germ cell development. Despite its biological importance, little is known about stage-specific gene regulation in spermatogenesis. The information on male germ cell specific transcripts is very limited, with only 1962 transcripts in type A Spga, 4385 in pachytene Spcy and 4014 in round Sptd in the Unigene database (1). To provide a comprehensive understanding of male germ cell development, we profiled and analyzed the transcriptome of male germ cells at Spga, Spcy and Sptd stages by Serial Analysis of Gene Expression (SAGE) (2–5). SAGE provides several advantages over other gene expression profiling methods including microarray analysis. It is a high-throughput method, which simultaneously detects and measures the expression level of genes including rare genes, in a cell at a given time. It does not depend on the prior availability of transcript information (6) and provides an unbiased method to examine both known and unknown genes. We identified a wide variety of genes specifically expressed during male germ cell development (Table 1). Importantly, we identified novel regulatory and expression patterns such as an extensive presence of antisense transcripts and spliced variants. These antisense transcripts had multiple origins, including processed sense transcripts, intronic and exonic sequences of a single gene or multiple genes, intergenic sequences and pseudogenes. One-third of the identified genes demonstrated evidence of alternative splicing (3,4).

The GermSAGE database is a comprehensive web-based database containing male germ cell transcript data. The tag coverage for each germ cell SAGE library is over 150 000 and contains a total of 452 095 SAGE tags derived from type A Spga, pachytene Spcy and round Sptd. It is a flexible platform which is integrated with the UCSC genome browser allowing browsing, search and comparative analysis of male germ cell data with customizable search parameters. GermSAGE leads to a better understanding of male germ cell development and may provide insight on male infertility, and allows development of genetic tests and therapies.

DESIGN AND IMPLEMENTATION

Displaying features

The GermSAGE application provides an organized approach for sharing genomic data in the form of a Browser Extensible Display (BED) format. It utilizes the

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UCSC Genome Browser (7) to visualize experimental datasets with respect to the mouse genome (NCBI Build 35 assembly). Genome coordinate information in the BED file was obtained by BLASTN analysis of the SAGE tag sequences (8) against the mouse genome (NCBI Build 35 assembly); only perfectly matched tags were retained. In case a tag match to more than one Unigene clusters, the complete Unigene list can be retrieved by using 'Full text or specific field search' in the main page. Users can compare the GermSAGE data to other genome annotations or upload their own data using UCSC Genome Browser's custom annotation track feature. The custom annotation track is viewable on top of the GermSAGE dataset, thus making it an ideal mechanism for displaying and analyzing personalized data.

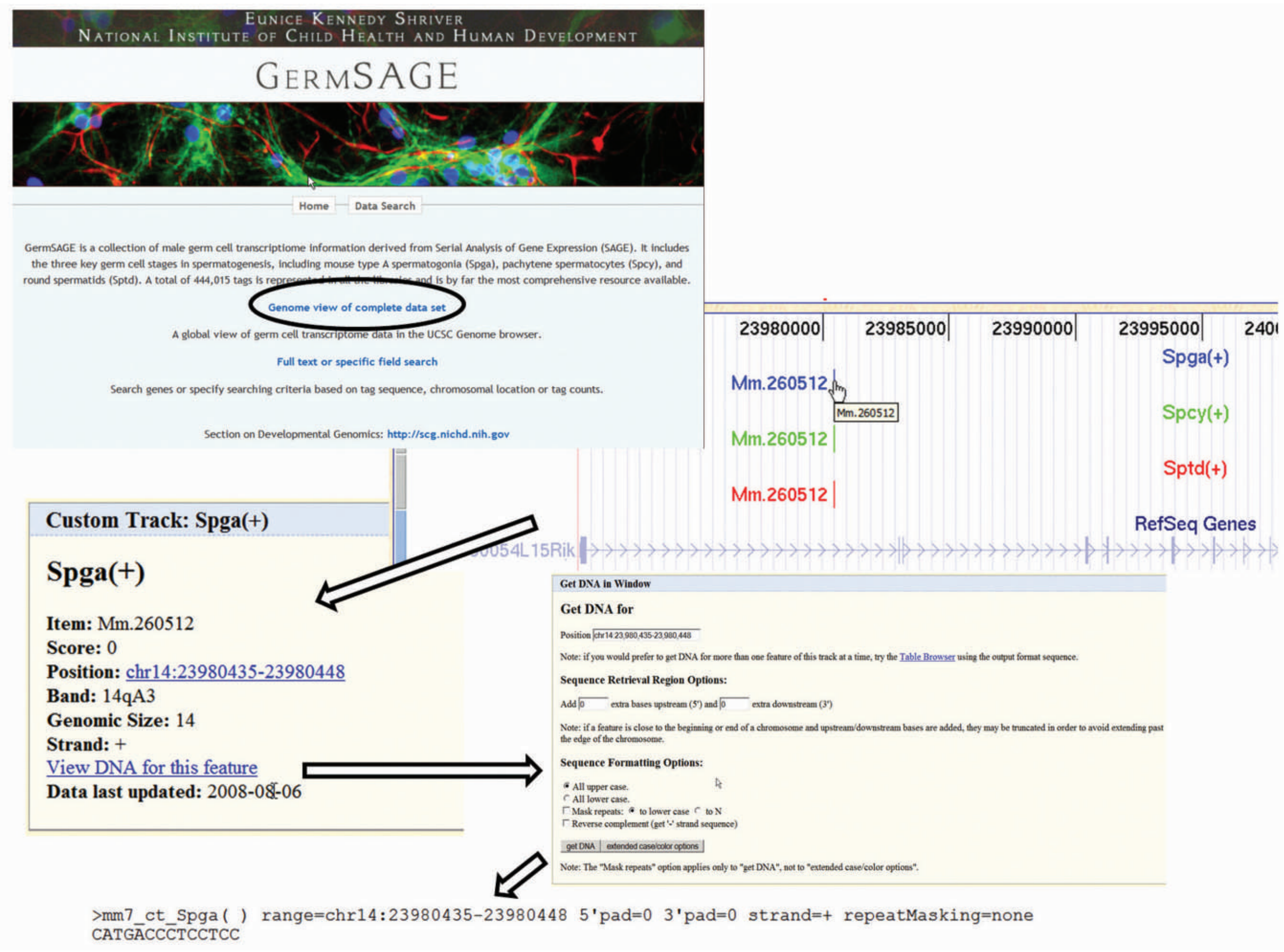
Table 1. Statistics of germ cell SAGE libraries

SAGE tag	Spga	Spcy	Sptd
Reliable tags	110 872	11 384	11 214
Total unique tags	31 514	36 147	34 000
Known genes	12 508	13 691	12 368
Uncharacterized cDNA	3020	4406	4234
Novel	563	1058	1155
Transcripts identified	16 091	19 155	17 757

Reliable tags refers to SAGE tags without ambiguous nucleotide base. Known genes are defined as those that match Unigene clusters with at least one known mRNA; uncharacterized cDNA refers to the full cDNAs from RIKEN, IMAGE, expressed clones or any cDNAs without annotation. Novel tag refers to no Unigene cluster match. Each transcript is covered with an average of 3.6 SAGE tags.

Architecture and technologies

GermSAGE is a web based application constructed using components and services that work together to form a multi-layered architecture. The core of the system is based on a domain model that is comprised of Java objects that describe the business, operations and object relationships (9). The domain model is established using Hibernate (10). This framework organizes mapping of the domain model to the underlying relational database of the application. The result is a transparent back-end layer that facilitates data query and retrieval features. The view layer of the application is rendered primarily using Java Server Pages (JSP, v2.0). This layer is XHTML 1.0 strict compatible. Client-side functionality is coded using JavaScript. The application layers are joined using Spring (11),



GermSAGE is a collection of male germ cell transcriptiome information derived from Serial Analysis of Gene Expression (SAGE). It includes the three key germ cell stages in spermatogenesis, including mouse type A spermatogonia (Spga), pachytene spermatocytes (Spcy), and round spermatids (Sptd). A total of 444,015 tags is represented in all the libraries and is by far the most comprehensive resource available.

Genome view of complete data set

A global view of germ cell transcriptome data in the UCSC Genome browser.

Full text or specific field search

Search genes or specify searching criteria based on tag sequence, chromosomal location or tag counts.

Section on Developmental Genomics: http://scg.nichd.nih.gov

Custom Track: Spga(+)

Spga(+)

Item: Mm.260512
Score: 0
Position: chr14:23980435-23980448
Band: 14qA3
Genomic Size: 14
Strand: +
View DNA for this feature
Data last updated: 2008-08-06

Get DNA in Window

Get DNA for

Position chr14:23,980,435-23,980,448

Note: if you would prefer to get DNA for more than one feature of this track at a time, try the Table Browser using the output format sequence.

Sequence Retrieval Region Options:

Add 0 extra bases upstream (5') and 0 extra downstream (3')

Note: if a feature is close to the beginning or end of a chromosome and upstream/downstream bases are added, they may be truncated in order to avoid extending past the edge of the chromosome.

Sequence Formatting Options:

● All upper case.
○ All lower case.
□ Mask repeats: ● to lower case ○ to N
□ Reverse complement (get '-' strand sequence)

get DNA | extended case/color options

Note: The "Mask repeats" option applies only to "get DNA", not to "extended case/color options".

```
>mm7_ct_Spga( ) range=chr14:23980435-23980448 5'pad=0 3'pad=0 strand=+ repeatMasking=none
CATGACCCTCCTCC
```

Figure 1. Genome view in GermSAGE. The genome view allows free navigation on male germ cell transcriptome data in UCSC genome browser. SAGE tags for Spga, Spcy and Sptd are marked in blue, green and red, respectively. SAGE tag tracks can be turn on and off through the custom track option. The sequences on how to retrieve the tag sequence are shown. Users can modify and process the sequence before exporting in FASTA format.

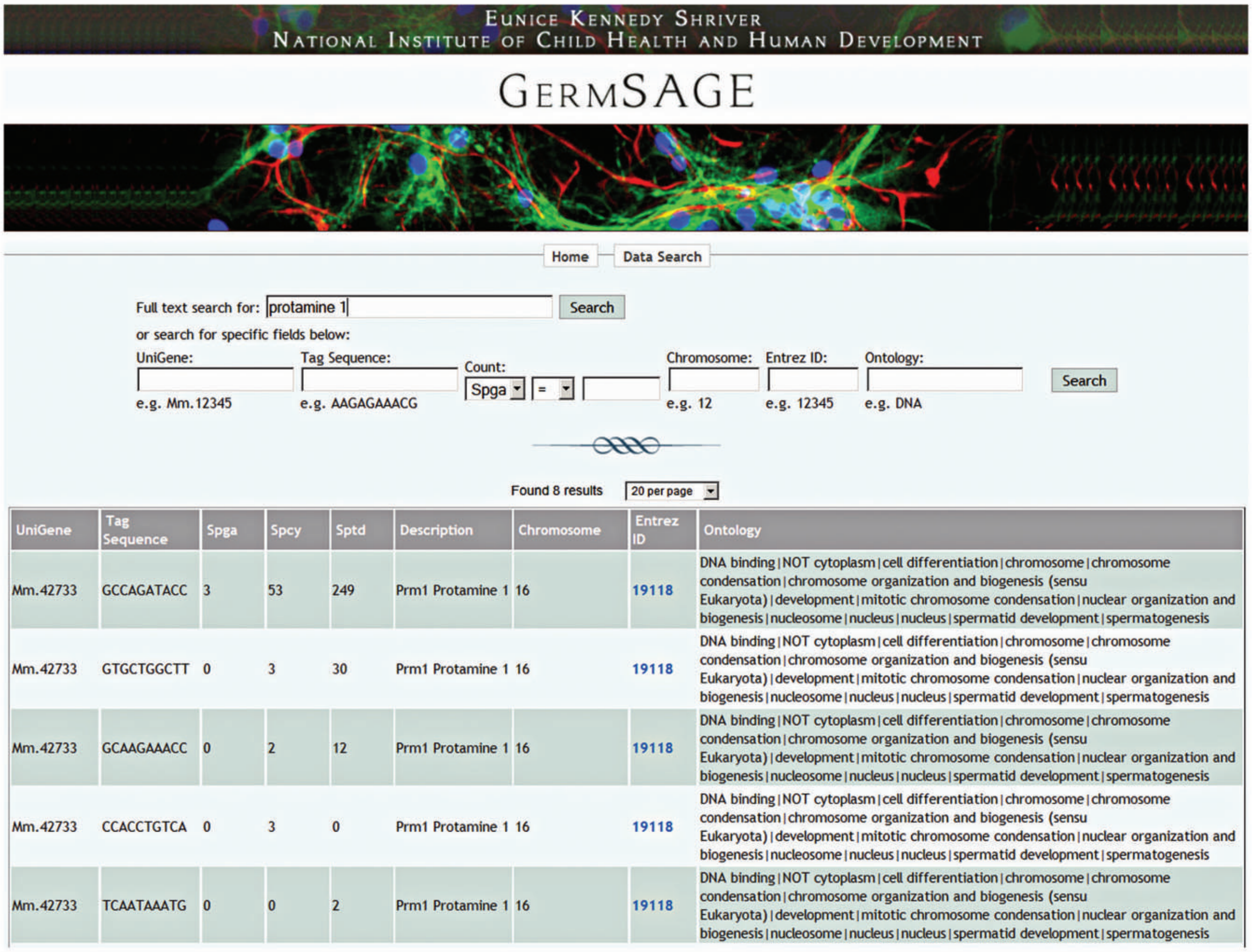
a highly customizable and extensible framework which provides common infrastructures. The system will check the availability of the host site (http://genome.ucsc.edu) before rendering the BED files.

USING GERMSAGE

The entry page of the GermSAGE database provides two analysis strategies: (i) a global view of germ cell transcriptome data by selecting 'Genome view of complete data set'; and (ii) search genes or specify searching criteria based on tag sequence, chromosomal location or tag counts via the tab 'Full text or specific field search'. All results generated from both approaches will be displayed in UCSC, a tutorial on how to use the UCSC genome browser is available (12,13).

Genome view of complete data set

This is the 'free view' mode of GermSAGE that will open a new browser window that directs the users to the UCSC genome browser; this provides both graphical and text-based views of the SAGE tag data and annotations (Figure 1). Users can freely navigate in the genome browser by using the display navigation toolbar on the top to examine the overall expression patterns of transcripts. The position/search box allows users to locate the expression of germ cell transcripts at a particular location or in association with the keyword found in the genome browser database. SAGE tag sequences at different germ cell stages are aligned in the genome browser and presented with different colors (Spga: blue; Spcy: green; Sptd: red). The SAGE tags are also marked with + or – on the track to indicate the strand orientation, and can be turned on or off by changing the custom tracks setting. The Unigene assignment can be retrieved by clicking on the color tag or select 'pack' option in the custom tracks item below the annotation window. For more details about the tag and its sequence, click on the tag again to bring up a window that shows the tag information, including Unigene assignment, chromosomal position/band and strand orientation. Click on 'view DNA for this feature' will bring up 'Get DNA in Window', which retrieves the tag sequence. A number of manipulations on the tag sequence are available before exporting to the web browser in FASTA format (Figure 1).



Full text search for: protamine 1 Search

or search for specific fields below:

UniGene: e.g. Mm.12345

Tag Sequence: e.g. AAGAGAAACG

Count: Spga =

Chromosome: e.g. 12

Entrez ID: e.g. 12345

Ontology: e.g. DNA

Search

Found 8 results 20 per page

UniGene	Tag Sequence	Spga	Spcy	Sptd	Description	Chromosome	Entrez ID	Ontology
Mm.42733	GCCAGATACC	3	53	249	Prm1 Protamine 1	16	19118	DNA binding | NOT cytoplasm | cell differentiation | chromosome | chromosome condensation | chromosome organization and biogenesis (sensu Eukaryota) | development | mitotic chromosome condensation | nuclear organization and biogenesis | nucleosome | nucleus | nucleus | spermatid development | spermatogenesis
Mm.42733	GTGCTGGCTT	0	3	30	Prm1 Protamine 1	16	19118	DNA binding | NOT cytoplasm | cell differentiation | chromosome | chromosome condensation | chromosome organization and biogenesis (sensu Eukaryota) | development | mitotic chromosome condensation | nuclear organization and biogenesis | nucleosome | nucleus | nucleus | spermatid development | spermatogenesis
Mm.42733	GCAAGAAACC	0	2	12	Prm1 Protamine 1	16	19118	DNA binding | NOT cytoplasm | cell differentiation | chromosome | chromosome condensation | chromosome organization and biogenesis (sensu Eukaryota) | development | mitotic chromosome condensation | nuclear organization and biogenesis | nucleosome | nucleus | nucleus | spermatid development | spermatogenesis
Mm.42733	CCACCTGTCA	0	3	0	Prm1 Protamine 1	16	19118	DNA binding | NOT cytoplasm | cell differentiation | chromosome | chromosome condensation | chromosome organization and biogenesis (sensu Eukaryota) | development | mitotic chromosome condensation | nuclear organization and biogenesis | nucleosome | nucleus | nucleus | spermatid development | spermatogenesis
Mm.42733	TCAATAAATG	0	0	2	Prm1 Protamine 1	16	19118	DNA binding | NOT cytoplasm | cell differentiation | chromosome | chromosome condensation | chromosome organization and biogenesis (sensu Eukaryota) | development | mitotic chromosome condensation | nuclear organization and biogenesis | nucleosome | nucleus | nucleus | spermatid development | spermatogenesis

Figure 2. Full text or specific field search in GermSAGE. GermSAGE output for protamine 1 was illustrated. The search fields allow qualitative lookup based on keyword, ID, position and gene ontology. Quantitative analysis based on tag count is also available. A combination of different search criteria is allowed.

One of the powerful features of GermSAGE is that it provides users with an interaction interface in genome browser format. The user can overlay the male germ cell transcriptome data with a variety of annotated tracks below the genome view window, and create a custom map by adding tracks to view various types of data and specific genomic landmarks. The browser offers a broad list of options. It includes: (1) mapping and sequencing tracks that contains information about the position, marker and GC percentage of the annotated gene region; (2) genes and gene prediction tracks on gene annotation and predictions from various sources; (3) mRNA and EST tracks that contains information on transcripts, CAGE (Cap Analysis of Gene Expression) tags to identify potential transcription start sites and alternatively spliced RNA species; (4) expression and its regulation that includes expression data from different microarray platforms and regulatory information such as CpG islands and microRNA; (5) comparative genomics shows the sequence conservation among species; and (6) variation and repeats.

This information will lead to better insights about gene regulation, and facilitate the generation of hypotheses.

Full text or specific field search

This option provides a more focused and quantitative analysis of male germ cell transcripts based on the inputs defined by the users. Inputs include gene name/symbol, tag sequence, Unigene ID, chromosomal region or gene function in terms of gene ontology. Here, we use *Protamine 1* (*Prm1*) as an example to show the power of GermSAGE in dissecting the complexity of antisense transcription in the male germ trancriptome. Recent study of the human genome suggested 61% of loci expressed

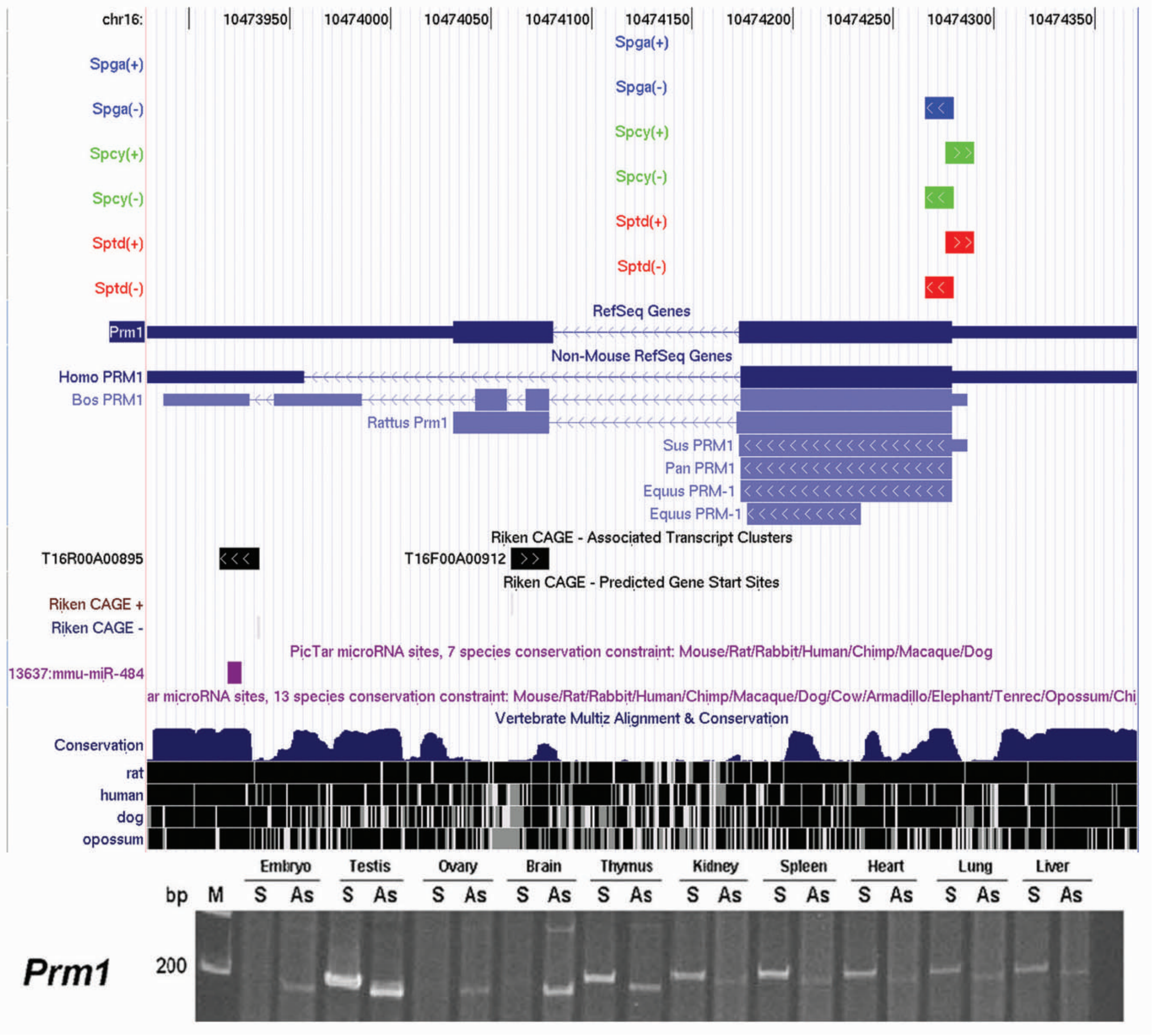
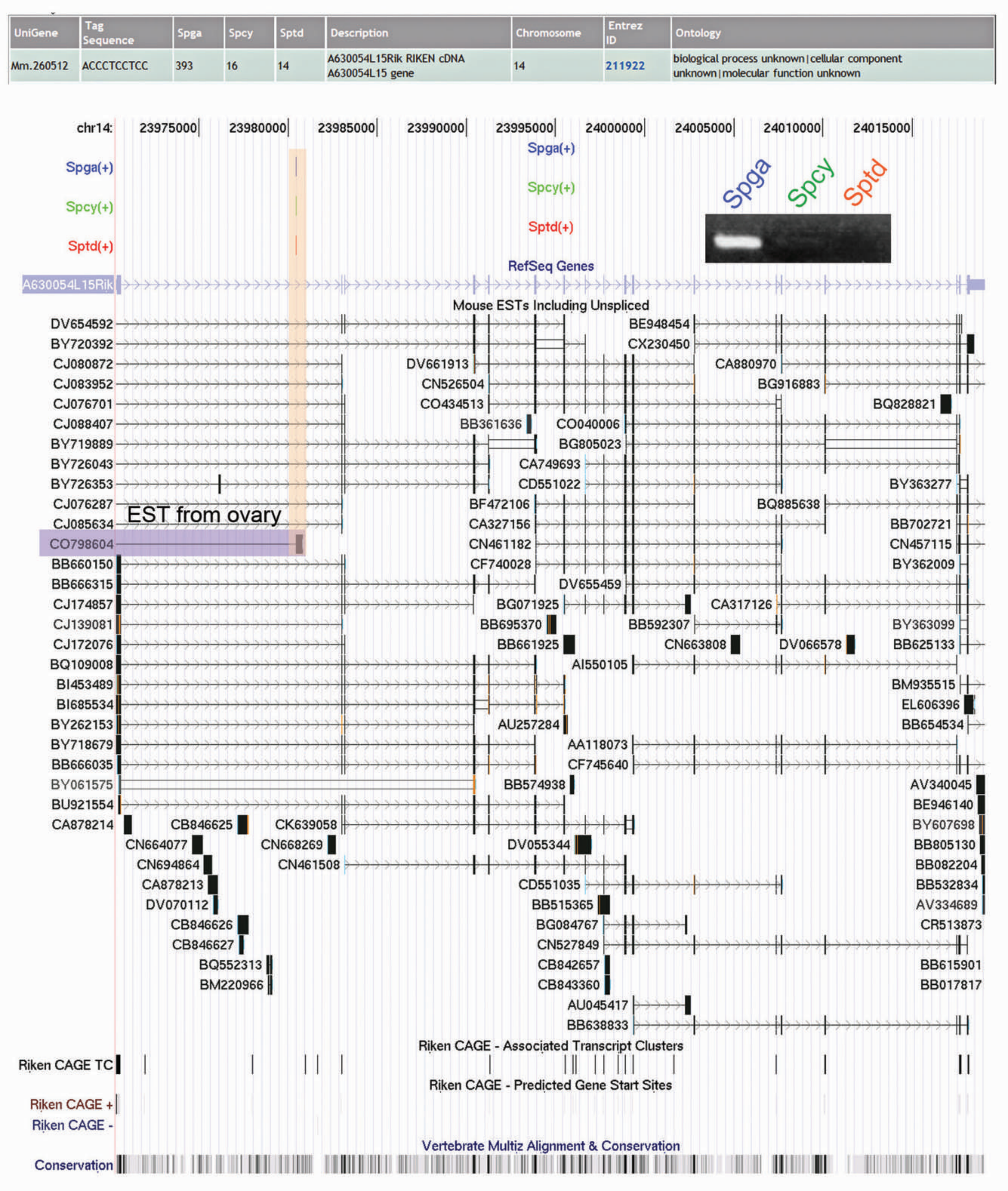


Figure 3. SAGE tags mapped to protamine 1. SAGE tags mapped to *Prm1* are overlaid with additional annotation tracks, such as conservation among species, Riken CAGE and microRNA to get better insights on potential gene regulation. The bottom panel shows validation of *prm1* sense (S) and antisense transcripts (AS) in different tissues. M indicates the marker.

antisense transcripts (14). We have demonstrated frequent antisense transcription events in the male germ cell transcriptome could be classified into three groups (4). Protamines are arginine-rich nuclear proteins that replace histones late in the haploid phase of spermatogenesis, and are important for sperm head condensation and DNA stabilization. They are abundantly expressed in spermatid stage (15). There are a total of five SAGE tags mapped to *Prm1* and Sptd showed the highest expression (Figure 2), as previously reported (15). A click on the



UniGene	Tag Sequence	Spga	Spcy	Sptd	Description	Chromosome	Entrez ID	Ontology
Mm.260512	ACCCTCCTCC	393	16	14	A630054L15Rik RIKEN cDNA A630054L15 gene	14	211922	biological process unknown | cellular component unknown | molecular function unknown

Figure 4. Novel gene search in GermSAGE. A tag preferentially expressed in Spga was identified. The tag indicated the presence of a potential short isoform. This observation was supported by EST and CAGE tag evidence. RT–PCR confirmed the tag was exclusively expressed in Spga.

Entrez ID will show the SAGE tags associated with *Prm1* in the genome browser. *Prm1* is highly conserved among species as seen on the conservation track and might be regulated by microRNA miR-484 (16) at the 3′-end. Three tags are demonstrated to be derived from the sense transcript, while two tags (one in Spcy and one in Sptd) are derived from the antisense transcript. This is also suggested by the presence of the putative transcription start site in the antisense CAGE tag cluster (T16F00A00912) (Figure 3). The existence of both sense and antisense transcripts was confirmed by orientation specific RT–PCR (Figure 3).

GermSAGE can also be applied for novel gene discovery in male germ cell development. The total number of uncharacterized cDNA and novel transcripts in the GermSAGE database is 20 707 and 2776, respectively. To highlight this capability, we used the search tool to identify a tag preferentially expressed in spermatogonial stage. The expression level is over 24- and 28-fold compared to Spcy and Sptd, respectively (Figure 4). The genome browser showed the tag oriented in the sense direction to the uncharacterized cDNA A630054L15Rik. Importantly, the tag alignment suggested the transcript might be an alternative splice variant of the cDNA. To increase the predictive evidence, the EST track and CAGE tag annotations were turned on to support the potential existence of the transcript. It turned out that the tag mapped perfectly to an EST sequence derived from ovary (CO798604, Figure 4) at the 3′-end and a transcription start site supported by CAGE tag at the 5′-end. RT–PCR confirmed the prediction and it is highly expressed in Spga (Figure 4). The same approach can be applied to other novel gene discovery as well.

GermSAGE allows a combination of search parameters. Transcripts may also be retrieved by tag count number. This is useful for refining output based on expression level. For example, one can retrieve Spga expressed transcripts with tag counts more than 10 involved in DNA binding on chromosome 12 by selecting Spga >10, keying in 12 in the chromosome field and DNA binding in the ontology field. The data can be sorted in ascending or descending order by clicking on the items in the first row. This function is also useful in revealing transcripts that demonstrate stage-specific gene expression.

FUTURE DEVELOPMENTS

GermSAGE is created as a flexible platform for male germ cell transciptome analysis. In addition to static data analysis, we previously leveraged the power of SAGE by developing algorithms to extract cellular dynamics contained in the SAGE data to reveal transcriptional regulation and gene interaction networks at particular male germ cell stages and during cellular transition (5). We are working on the pipeline for such dynamic data analysis and this will be included in the next version of GermSAGE. In the current version, the rendering of transcriptome data takes up to 2 min. To address this performance issue, we are currently planning to modify and deploy the genome browser source code on servers at the National Institute of Child Health and Human Development, National Institutes of Health. The long-term scientific vision of GermSAGE is to serve as a centralized platform to scan the entirety of dynamic genomic changes in male germ cell development, as well as the manifestations of those changes through gene expression patterns.

DATABASE AVAILABILITY

The database is freely available and can be accessed from http://germsage.nichd.nih.gov. GermSAGE is compatible with common web browsers in the market, including Microsoft internet explorer, Mozilla firefox and Apple Safari, and is platform independent. All feedbacks are welcome and should be forwarded to Dr Tin-Lap Lee at leetl@mail.nih.gov.

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