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CBrowse: a SAM/BAM-based contig browser for transcriptome assembly visualization and analysis

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ABSTRACT

Summary: To address the impending need for exploring rapidly increased transcriptomics data generated for non-model organisms, we developed CBrowse, an AJAX-based web browser for visualizing and analyzing transcriptome assemblies and contigs. Designed in a standard three-tier architecture with a data pre-processing pipeline, CBrowse is essentially a Rich Internet Application that offers many seamlessly integrated web interfaces and allows users to navigate, sort, filter, search and visualize data smoothly. The pre-processing pipeline takes the contig sequence file in FASTA format and its relevant SAM/BAM file as the input; detects putative polymorphisms, simple sequence repeats and sequencing errors in contigs and generates image, JSON and database-compatible CSV text files that are directly utilized by different web interfaces. CBowse is a generic visualization and analysis tool that facilitates close examination of assembly quality, genetic polymorphisms, sequence repeats and/or sequencing errors in transcriptome sequencing projects.

Availability: CBrowse is distributed under the GNU General Public License, available at http://bioinfolab.muohio.edu/CBrowse/

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1 INTRODUCTION

Web-based genome browsers, such as GBrowse (Stein *et al.*, 2002) and UCSC Genome Browser (Kent *et al.*, 2002), are widely utilized for visualizing genomes and their sequence features to facilitate various data analyses that address interesting biological questions. For non-model organisms without sequenced genomes, transcriptome sequencing is definitely the most efficient way to explore the transcribed portions of genomes and determine their dynamics (Bräutigam *et al.*, 2011; Feldmeyer *et al.*, 2011; Parchman *et al.*, 2010). Many bioinformatics programs have been developed/improved to address the challenges in transcriptome assembly, especially *de novo* assembly without a reference genome, using complementary DNA (cDNA)/messenger RNA (mRNA) data from next-generation sequencing and Sanger sequencing (Bräutigam *et al.*, 2011; Feldmeyer *et al.*, 2011; Martin and Wang, 2011; Zheng *et al.*, 2011). So far, there is no open-source, web-based contig browser yet that allows users to navigate transcript assembly, visualize contigs and examine genetic polymorphisms, simple sequence repeats and sequencing errors embedded in the assembly. To address the impending need for exploring rapidly increased transcriptomics data for non-model organisms, we developed CBrowse (contig browser), an AJAX-based web browser to visualize and analyze transcriptome assemblies and their individual contigs.

2 IMPLEMENTATION

As shown in Supplementary Figure S1, CBrowse is designed to follow a standard three-tier software architecture composed of Data Layer, Business Logic Layer and Presentation layer, with a data pre-processing pipeline. The data pre-processing pipeline detects simple sequence repeats for contigs, makes inferences from read alignments about putative polymorphisms and sequencing errors and stores resultant data in a hard-drive file system (HDFS), which can be optionally imported into a SQL-based database (e.g. MySQL or PostgreSQL). Data layer enables data accessing through HDFS or a database, Business Logic Layer processes users' requests submitted from Presentation Layer and Presentation Layer displays the desired data in different web interfaces.

Since Sequence Alignment/Map (SAM) format and its sister format Binary Sequence Alignment/Map (BAM) are widely adopted in presenting sequence alignment information for both genome and transcriptome assembly (Barnett *et al.*, 2011; Li *et al.*, 2009), the input files for the pre-processing pipeline are as follows: (i) a SAM/BAM file that contains alignment information for all individual cDNA/mRNA reads mapped to the contigs, (ii) a sequence file in FASTA format that contains all contigs within a transcriptome assembly and (iii) a Extensible Markup Language (XML) configure file that provides necessary information (e.g. species name, assembly name and data location) for data processing (Supplementary Fig. S1). Implemented in C++ with Perl wraps, the pipeline can process input data; detect polymorphisms, simple sequence repeats and sequencing

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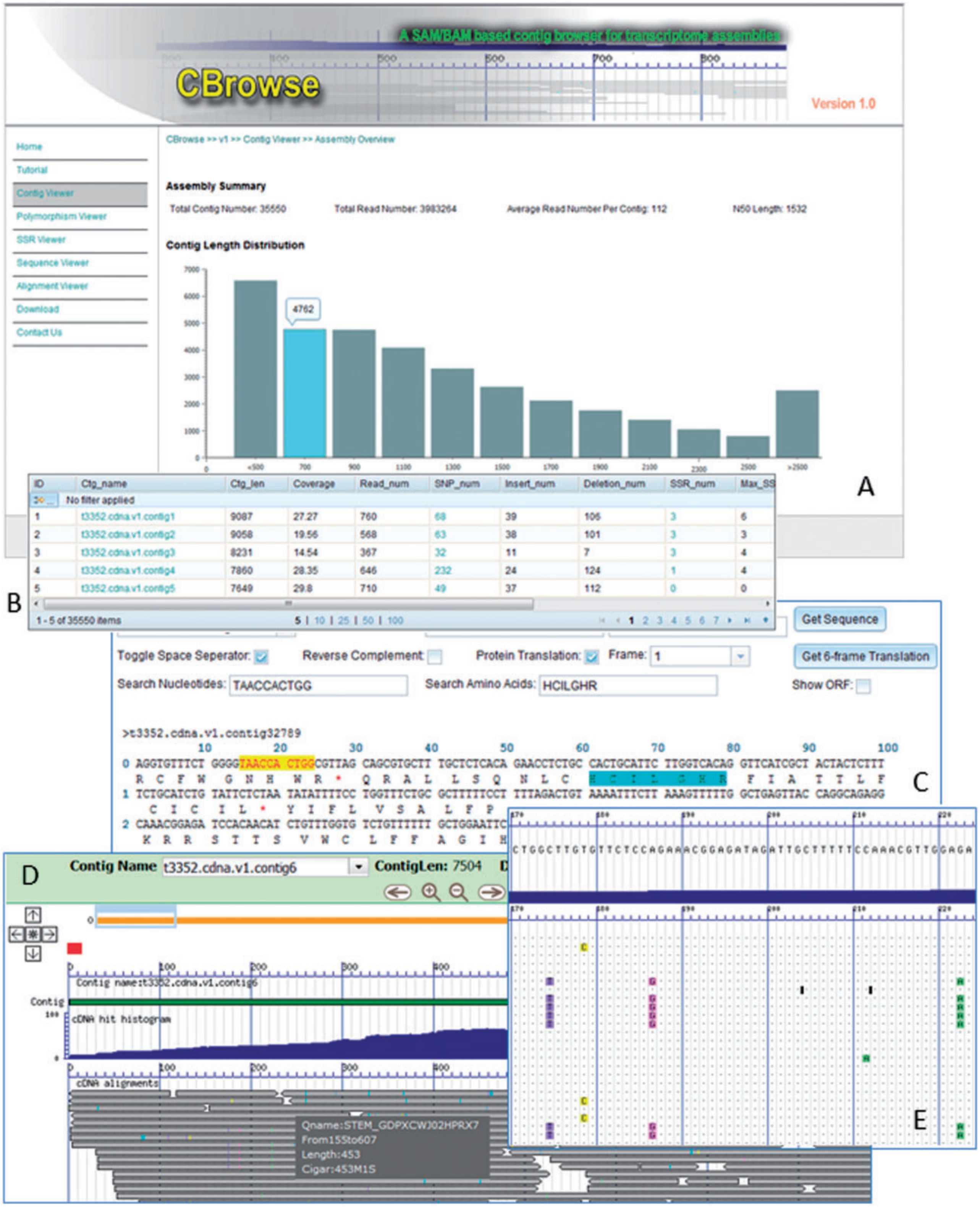

Assembly Summary

Total Contig Number: 35550 Total Read Number: 3983264 Average Read Number Per Contig: 112 N50 Length: 1532

ID	Ctg_name	Ctg_len	Coverage	Read_num	SNP_num	Insert_num	Deletion_num	SSR_num	Max_SS
	No filter applied								
1	t3352.cdna.v1.contig1	9087	27.27	760	68	39	106	3	6
2	t3352.cdna.v1.contig2	9058	19.56	568	63	38	101	3	3
3	t3352.cdna.v1.contig3	8231	14.54	367	32	11	7	3	4
4	t3352.cdna.v1.contig4	7860	28.35	646	232	24	124	1	4
5	t3352.cdna.v1.contig5	7649	29.8	710	49	37	112	0	0

1 - 5 of 35550 items

Toggle Space Seperator: ☑ Reverse Complement: □ Protein Translation: ☑ Frame: 1

Search Nucleotides: TAACCACTGG Search Amino Acids: HCILGHR Show ORF: □

```
>t3352.cdna.v1.contig32789
         10        20        30        40        50        60        70        80        90        100
0 AGGTGTTTCT GGGGTAACCA CTGGCGTTAG CAGCGTGCTT TGCTCTCACA GAACCTCTGC CACTGCATTC TTGGTCACAG GTTCATCGCT ACTACTCTTT
   R  C  F  W   G  N  H   W  R  *   Q  R  A  L   L  S  Q   N  L  C   H  C  I  L   G  H  R   F  I  A   T  T  L  F
1 TCTGCATCTG TATTCTCTAA TATATTTTCC TGGTTTCTGC GCTTTTTCCT TTTAGACTGT AAAATTTCTT AAAGTTTTTG GCTGAGTTAC CAGGCAGAGG
    C  I  C   I  L  *   Y  I  F  L   V  S  A   L  F  P
2 CAAACGGAGA TCCACAACAT CTGTTTGGTG TCTGTTTTTT GCTGGAATTC
   K  R  R   S  T  T  S   V  W  C   L  F  F   A  G  I  H
```

Contig Name t3352.cdna.v1.contig6 ContigLen: 7504

Qname:STEM_GDPXCWJ02HPRX7
From155to607
Length:453
Cigar:453M1S

Fig. 1. The snapshots of CBrowse web interfaces

Panel A: the major web portal shows contig length distribution for an assembly. **Panel B**: The data grid view shows contig summary information. **Panel C**: the sequence viewer shows its functionality with one contig. **Panel D**: the alignment view in bird's-eye resolution. **Panel E**: the alignment view in nucleotide resolution that displays and highlights the differences between contig sequence and individual sequence reads errors and generate image, JSON and database-compatible CSV text files that are utilized by different web viewers of CBrowse (Fig 1). Our C++ program relies on the application programming interface (API) of BamTools (Barnett *et al.*, 2011) to access BAM files, uses tinyXML library (http://www.grinninglizard.com/tinyxml/) to generate and parse configuration files and map index files in XML format and utilizes GD library (http://www.libgd.org) to draw alignment graphics in PNG format. The pipeline not only extracts overall information for a transcriptome assembly (e.g. total number of contigs and associated reads, average reads per contig and contig length distribution) and calculates its N50 length but also retrieves summary information for each contig and computes its sequence coverage. For simple sequence repeats, our pipeline invokes Phobos (Mayer *et al.*, 2010) to identify perfect/imperfect repeats and generates results in GFF format. The repeat unit size and the minimum repeat number are customizable using the configuration XML file. By default, the repeat unit size is between 1 and 12 nt, while the minimum repeat number is set to be 8 for mono-nucleotides, 5 for dimers, 4 for triplets and 3 for repeats with a unit size of 4–12 nt. For putative polymorphisms and sequencing errors, our C++ program examines base by base for any discrepancy between each contig and its component sequence reads. Along a given contig, the C++ program identifies all putative polymorphic positions, which must be covered by $\geq$10 individual sequence reads and the accumulated occurrence of any polymorphic type is $\geq$5. The valid polymorphism types include single-nucleotide polymorphisms (SNPs, single-base mismatch), single base indel and multiple-base mismatch and indels. The frequency of any valid polymorphism type needs to be at least 2 for any putative polymorphic position along a contig. Our pipeline also invokes SAMTools and BCFTools to call SNPs and short indels and generate results in VCF format, which can be explored through our Polymorphism Viewer (see below).

Implemented in PHP and JavaScript (i.e. Dojo and MapEasy libraries), CBrowse web interfaces are AJAX-based and compatible with Mozilla Firefox (8.0 or above), Google Chrome and Internet Explorer (9.0 or above). As shown in Figure 1, there are five major viewers in CBrowse: (i) Contig Viewer presents summary information about the assembly as well as individual contigs; (ii) Sequence Viewer allows users to manipulate, examine and scan individual nucleotides of each contig; (iii) Polymorphism Viewer provides the polymorphism types (SNPs, single indels and other multiple-base mismatches/indels), positions and frequencies for each contig; (iv) SSR Viewer displays the detected simple sequence repeats in each contig and (v) Alignment Viewer offers both bird's eye and nucleotide resolution of sequence alignments in Google-map style, with color-coded nucleotide differences between contigs and their component sequence reads. In particular, all these interfaces allow users to search and navigate data easily.

3 CONCLUSION

Similar to GBrowse 2.0 (http://gmod.org/wiki/GBrowse), X-MAP (Yates *et al.*, 2008), Genome Projector (Arakawa *et al.*, 2009) and JBrowse (Skinner *et al.*, 2009), CBrowse essentially is an AJAX-based Rich Internet Application that decouples interactions with users from interactions with the server. Such decoupling empowers web applications with rich graphic user interface (GUI) characteristics such as desktop application, enables a asynchronous client–server communication and offers faster and smoother user experience by partial updates in web pages. Different from these genome browsers, CBrowse is designed for analyzing and visualizing transcriptome assembly and contigs, with unique functionality such as seamlessly integrated data grid viewer, alignment viewer and sequence viewer that allow users to navigate, sort, filter, search and visualize transcriptome data efficiently. As an open source project, our web-based CBrowse can be utilized by the research community to disseminate and release transcriptome data over the Internet.

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Conflict of Interest: none declared.

REFERENCES

Arakawa,K. *et al.* (2009) Genome Projector: zoomable genome map with multiple views. *BMC Bioinformatics*, **10**, 31.

Barnett,D.W. *et al.* (2011) BamTools: a C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics*, **27**, 1691–1692.

Bräutigam,A. *et al.* (2011) Critical assessment of assembly strategies for non-model species mRNA-Seq data and application of next-generation sequencing to the comparison of C(3) and C(4) species. *J. Exp. Bot.*, **62**, 3093–3102.

Feldmeyer,B. *et al.* (2011) Short read Illumina data for the de novo assembly of a non-model snail species transcriptome (*Radix balthica*, *Basommatophora*, *Pulmonata*), and a comparison of assembler performance. *BMC Genomics*, **12**, 317.

Kent,W.J. *et al.* (2002) The human genome browser at UCSC. *Genome Res.*, **12**, 996–1006.

Li,H. *et al.* (2009) The Sequence alignment/map (SAM) format and SAMtools. *Bioinformatics*, **25**, 2078–2079.

Martin,J.A. and Wang,Z. (2011) Next-generation transcriptome assembly. *Nat. Rev. Genet.*, **12**, 671–682.

Mayer,C. *et al.* (2010) Genome-wide analysis of tandem repeats in *Daphnia pulex*–a comparative approach. *BMC Genom.*, **11**, 277.

Parchman,T.L. *et al.* (2010) Transcriptome sequencing in an ecologically important tree species: assembly, annotation, and marker discovery. *BMC Genom.*, **11**, 180.

Skinner,M.E. *et al.* (2009) JBrowse: a next-generation genome browser. *Genome Res.*, **19**, 1630–1638.

Stein,L.D. *et al.* (2002) The generic genome browser: a building block for a model organism system database. *Genome Res.*, **12**, 1599–1610.

Yates,T. *et al.* (2008) X-Map: annotation and visualization of genome structure for Affymetrix exon array analysis. *Nucleic Acids Res.*, **36**, D780–D786.

Zheng,Y. *et al.* (2011) iAssembler: a package for de novo assembly of Roche-454/Sanger transcriptome sequences. *BMC Bioinformatics*, **12**, 453.