Biological Databases Final Project Proposal

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1 Project Overview

Trypansoma brucei (*T. brucei*) is a single cell protist parasite that gives rise to the African trypanosomiasis, or sleeping sickness. The mitochondrial genome of *T. brucei* consists of a network of circular structures called minicircles (1kb) and maxicircles (10kb). Maxicircles encode for protein-coding genes. However, such transcripts can be translated only after posttranscriptional deletion and insertion of uridines through a process called RNA-editing. This editing process is directed by short stretches of guideRNAs, which are encoded in minicircles. Understanding the organizational structures of minicircles and its encoded guideRNAs is an important step for deciphering the RNA-editing mechanism in *T. brucei*.

Here, we create a database for visualising next-generation sequencing data from mitochondrial minicircle DNA and small RNAs (putative guideRNAs) isolated from *T. brucei*. The database will facilitate the storage and analysis of minicircle sequences. Analyses to be performed include aligning reads and categorizing minicircles based on the minicircle conserved regions they contain.

Using MSAD, users would be able to retrieve and download information on minicircle sequences based on specific filtering criteria, including presence of conserved regions (CSBs), alignment coverage by smallRNA, etc. In addition, we wish to provide visualization for the minicircle sequence alignment information such as displaying the pileup of mapped sRNA reads along a specified minicircle query. If time permits, we will also visualize alignment for a given minicircle cluster.

With this database, users will be able to answer questions such as:

1. Which putative minicircle sequences contain conserved regions (CSB1, CSB2, CSB3)?
2. What is the multiple sequence alignment (MSA) for the specific cluster of sequences?
3. For a given minicircle sequence, which regions have high coverage of mapped sRNA reads? Where are the CSB regions (if any)?

Upon completion of the project, the database will be made accessible to the Afasizhev lab and BU affiliated students working on this dataset. If successful in this initial deployment, there is a possibility that the database could be made publicly available for other researchers to use.

Our database will contain data from the following:

1. PacBio Minicircle Sequencing Dataset - this dataset contains 39,939 filtered minicircle DNA sequences isolated from the mitochondria of *Trypanosoma brucei*. These reads are on average around 1kb long. For each sequence, we will have a unique sequence identifier, the actual DNA sequence, and a cluster assignment. The cluster assignment has already been precomputed using Connected Component Clustering, based on sequence similarity. We are currently in the process of applying other clustering methods, so we are interested in adding additional cluster assignments later on.
2. Illumina smallRNA-seq dataset - this contains 3,937,040 unique processed smallRNA reads which are putative guideRNAs. Each read has a duplication number (how many duplicate sequences of

itself is found in the original sequence file), an RNA sequence, and alignment information: where each sRNA read aligned to each of the minicircle sequences (some align to more than 1 or no minicircle sequences, and can align at more than two different positions within the same minicircle sequence). This is a very big file, so we may just store alignment information (processed from BED format) into the database and not each individual smallRNA sequence.

3. The sequences for the known minicircle conserved regions CSB1, CSB2, and CSB3. These are short sequences that are commonly found in curated minicircles (CSB3 is associated with the origin of replication). We'd like to be able to query which putative minicircle sequences contain each or combinations of these regions.

Multiple Sequence Alignments: given a cluster id, we can retrieve minicircles associated with such cluster and generate a Multiple Sequence Alignment of these sequences on the fly (or precomputed).

2 Tasks to accomplish

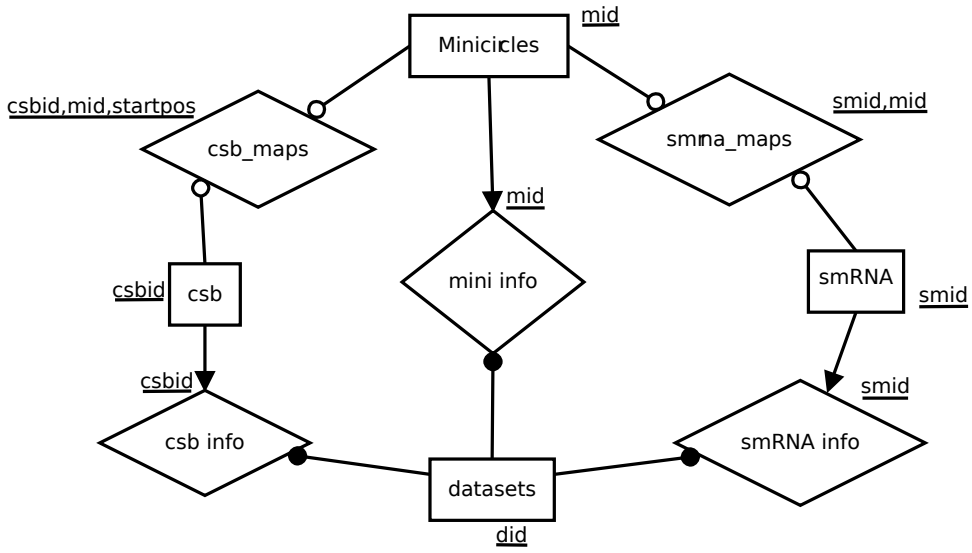
We plan to implement the following:

1. Create database tables (on bioed)
2. Ensure database conforms to ER diagram
3. Write cgi interface to database
   (a) About page (introduction)
   (b) A separate page for data retrieval/search, including:
      i. CSB based filtering
      ii. Coverage based filtering
      iii. Auto-complete features for searches
      iv. Dataset download ability
   (c) Multiple sequence alignment page:
      i. Display multiple sequence alignments
      ii. Download
4. Data download
5. Testing

In order to get our project on bioed we'd like to have ClustalW2 installed for performing multiple sequence alignments. Other than that what is currently available on bioed should be sufficient.

3 ER diagram

Here we present an entity-relationship diagram that represents how we plan to build our database:



4 Tables

These are the tables we plan to implement in our project, with all fields listed. Primary keys are underlined, and foreign keys are italicized.

- **CSB: csbid, sequence**
- **smallRNA: smid, sequence, copynumber**
- **dataset: did**
- **minicircles: mid, *did*, sequence, clusternumber**
- **csb_maps: *csbid*,*mid*,startpos, endpos, strandinfo, quality**
- **smrna_maps: *smid*, *mid*, startpos, endpos, strandinfo, quality**

As indicated by the ER diagram above we will have tables for smRNAs, minicircles, CSBs, datasets, and the maps of both CSBs and smRNAs onto minicircles. These last two tables represent many-to-many relationships, and so they need their own table. The relationships between our three sequence tables (minicircles, smRNAs, and CSBs) and the dataset table are all one to many, as each sequence may only belong to one dataset, a dataset being here defined as describing the origin of the sequences, the platform they were sequenced on, and other similar metadata. This relationship will be contained in the table structure by having a dataset field holding a did in each of these sequence tables.

For all of the sequence data tables (CSB, smallRNA, minicircles) the ID being used is a pre-supplied ID, which we are going to pull from the FASTA files containing our dataset on data import.

4.1 Indexes

We plan to use the following indexes on our tables:

- **CSB: primary key index on csbid**
- **smallRNA: primary key index on smid**
- **dataset: primary key index on did**
- **minicircles: primary key index on mid**
- **csb_maps: Unique index on (csbid,mid,startpos)**
- **smrna_maps: Unique index on (smid,mid)**

As we refine our queries and develop the web interface to our database it is likely that we will add to this and adopt other indexes.

5 Three SQL Queries

Here we present three sample SQL queries which could be used to answer a particular question about the data contained in our database.

5.1 Minicircle in largest cluster

Here we'd like to find the minicircle that belongs in the largest cluster, and return the minicircle id, the sequence, and the cluster number.

```
SELECT mid, sequence, clusternum
FROM minicircles
GROUP BY clusternum
ORDER BY count(*) DESC
LIMIT 1;
```

5.2 All minicircles containing 'csb3'

Now we'd like to find all of the minicircles which contain the CSB 'csb3', and we'd like to return the minicircle id, the minicircle sequence, and the cluster number:

```
SELECT m.mid, m.sequence, m.clusternum
FROM csb_maps c NATURAL JOIN minicircle m
WHERE c.csbid = 'csb3';
```

5.3 All smRNAs mapping to cluster 4 minicircles

We want to find all the smRNAs which map to minicircles that are in cluster 4, and return the minicircle id, the smRNAid, and the smRNA sequence:

```
SELECT m.mid, s.smid, s.sequence
FROM smrna_maps s NATURAL JOIN minicircles m
WHERE m.clusternum = 4;
```

6 External data processing

- Clustalw2 will be used to perform multiple sequence alignment on a specified cluster number. Multiple sequence alignments are computed on the fly when the user specifies an input cluster number.
- Alignment of smallRNA to minicircles was done using Bowtie2. This alignment is precomputed and stored in the database.
- Cluster assignments to each minicircle was done using BLAST for each pairwise minicircle sequences, then clustered as connected components. The clustering is precomputed and cluster assignments are stored in the database.

7 Links to Other databases

The data for our database comes from the following datasets:

- Minicircles published from Hong/Simpson Paper: http://dna.kdna.ucla.edu/tbrucei/Default.htm

- Curated list of *T.brucei* minicircles from Genbank. We have the fasta file containing all the sequences from Genbank available to download.
- KISS database: reference link to another database that contains minicircles and guideRNA mappings: http://gmod.mbl.edu/kiss/

We will link back to the Genbank sequences we use.

8 Graphical Output

An overview of our plans for graphical output:

- Display multiple sequence alignments for minicircle reads belonging to a specified cluster (color-coded by bases).
- Display alignment of smallRNA that maps to a specified minicircle. We will show the minicircle sequence as a single track, then below the minicircle track, we will show the smallRNA sequences, aligned by the start and end positions of where the smallRNA maps to the given minicircle.

9 Data Download

We plan to offer the ability for users of our database to download data from it:

- After the user runnings a specific query to narrow down the list of minicircles of interest (ie. by CSB mapping or clusternumber), there will be an option to download the minicircles from the query in fasta format. We will also provide the option to download the smallRNA alignments that map to the minicircles of interest (alignment file in BED format, smallRNA sequences in fasta format).
- The user will be able to download the multiple sequence alignment file for a specified cluster (file in aln format, output from clustalw2)