**A Short Tutorial for M3T Web Server**

Last updated by JH Zhang and C.Liu on December 30th, 2012

A Twenty Minutes Crash Course for M3T:

M3T stands for Mitochondria Genome Annotator, also called MiToTaTor. It allows automated annotation and analysis of newly sequenced mitochondria genome. The web sever can be accessed at *http://www.herbalgenomics.org/m3t*.

**Step I. Upload a sequence for annotation.**

1. Go to **"AnnoGenome"** page, download the sample fasta file.
2. Fill in the information for Project Name, Kingdom and Species Name.
3. Upload the sample fasta sequences for your genome.
4. Only PNG format for the output image is supported at this time, so you can just use the default selection.
5. Click the "Submit" button and you will see a message like the following.
6. *"You job xxx has been submitted and it is under processing. It usually takes up to 60 minutes to finish the annotation of a 150kb long mitochondria genome sequence. Please keep a note of your project id, as this is the only information by which you can access your data through http://www.herbalgenomics.org/m3t under "ViewAnno".*
7. The best practice is to keep a note of the job ID or the URL and comes back, say, 60 minutes later to check the results.

**Step II. Visualize and editing the annotations.**

1. Once you come back, go to the **"ViewAnno"** page, enter the ID for your job. A report page will show up. Browse through this page to see the results for gene models in GFF3 format, circular map image in png format, sequences for predicted DNA and proteins in FASTA format and analysis reports organized in Tables.
2. Through our own research work, we become big fans of Apollo genome editor and believe that there is no need or it is not possible to reinvent a genome annotation editor just as powerful. As a result, we make M3T output compatible with Apollo for editing. Download the gene models in GFF format. Open it up in Apollo, please select GFF3 format for "**Choose Data Source**". And Check the "**Embed FASTA in GFF**".
3. Edit the annotations using Apollo. Apollo is a very powerful editor that supports all sorts of editing of the predicted gene models. Of course, other editors can be used as well.
4. Export the annotation to a file in GFF3 format for updating of the analysis (see below).

**Step III. Re-analyze the annotations again and again.**

1. Go to the **"UpdateAnno"** page,
2. Fill in the Project Name, Kingdom and Species Name.
3. Upload the GFF3 files you have just edited using Apollo or any other editors.
4. Again, we only support the PNG format for the circular map at this time, so leave this selection as the way it is.
5. Click "Submit", you should see a message similar to what you see after you click the "Submit" button on the "Update**Anno**" page. Keep a note of the ID and come back later.
6. Repeat the steps described above: browsing the gene prediction models, results, view the circular map and then edit them again in compatible editors like Apollo. Please note that a new job ID will be assigned to each update. Essentially, all versions of your annotations are stored on the server and can be accessed using their corresponding job ID.

**Step IV. Re-draw the circular map again and again.**

1. Go to the **"QuickDraw"** page.
2. Enter the Species Name.
3. Select the file to upload.
4. Select the type of the input file. Two different formats are supported. One is the GFF3 format, which is the format of the annotation file you have been working on. The other one is a tab-delimited file you can prepare using Excel or Notepad.
5. Specify the output image format. We only support the output image in PNG format for now.
6. Click "Submit".

**Step V. Generate GenBank submission file.**

1. Go to **"PrepDBSub"** page,
2. Upload the gene bank submission template you created earlier. Otherwise, follow the link to create a new submission template.
3. Upload the GFF3 file you created earlier.
4. Fill in the Sample Information.
5. Click "Submit" to create the files.
6. A page will pop up containing links to two files. Download these files for submission to GenBank.

**Step VI. Retrieve sequences from other mitochondria genome for comparative study.**

1. Go to **"ExtractSeq"** page,
2. Select the species and the gene ids for which you would like to retrieve the sequences.
3. Select the type of sequence you would like to retrieve.
4. Select if you want the output sequences to be concatenated or not.
5. Click "submit". The corresponding sequences will be retrieved and can be used for comparative studies.

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