

**A multi-track RNA-seq browser for visualization of Arabidopsis thaliana transcription patterns from different growth states and conditions.**

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**Date:** October 16, 2015

**Agenda:**

1. Check if having local BAM files can speed up data retrieval using samtools' mpileup () call.

**Protocol:**

1. Check if local BAM files can speed up data retrieval using samtools' mpileup () call.
  - a. Downloaded the BAM file for experiment SRR547531 using wget ().
  - b. Executed mpileup () through SSH.
    - i. Used local BAM file and compare to iPlant BAM file
  - c. Ran the output.cgi script with the two BAM files (local vs. iPlant).
    - i. Used Chrome Dev Tools to analyze the TTFB (time to first byte).

**Results:**

1. Check if local BAM files can speed up data retrieval using samtools' mpileup () call.
  - a. Done, file size = 662MB.
  - b. Local BAM file mpileup call returns data very quickly (< 1 second), iPlant BAM file takes 10-20 seconds.
  - c. Local BAM file returns data in ~600ms! The iPlant BAM file takes ~60 seconds!
    - i. Only calling the generate\_rnaseq\_graph() function once to produce a single image.

**Notes/Questions:**

- Figure out why a URL to the BAM file doesn't work. Currently it works with a relative path..!
- See if BAM files hosted on Drop box result in data retrieval that is just as fast as local BAM files. This might implicate the iPlant server as the bottleneck and prove the HTTP request's innocence.

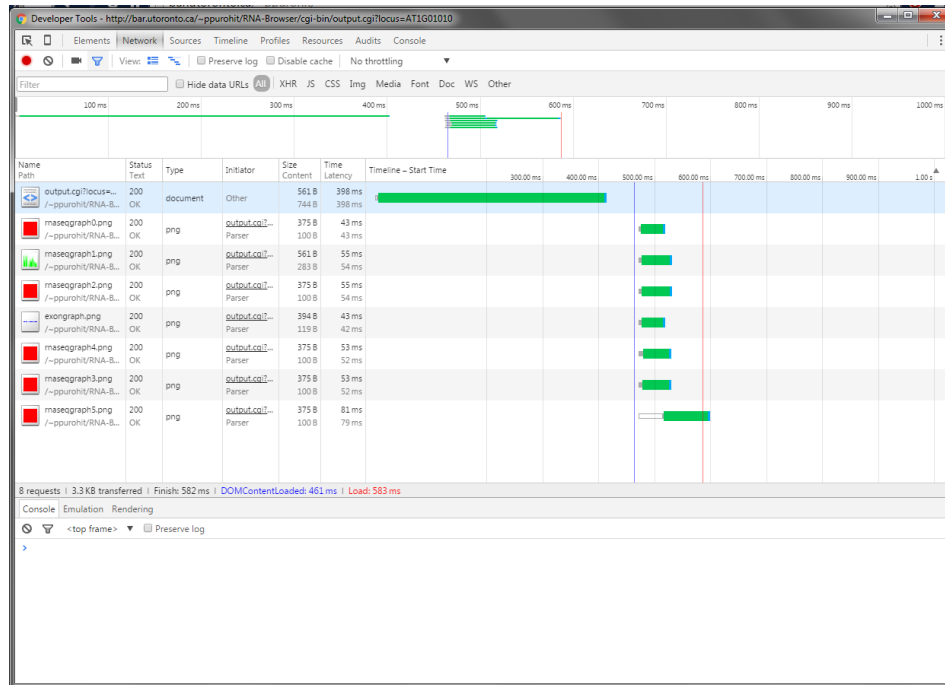


Figure 1: ~600ms for local BAM file.

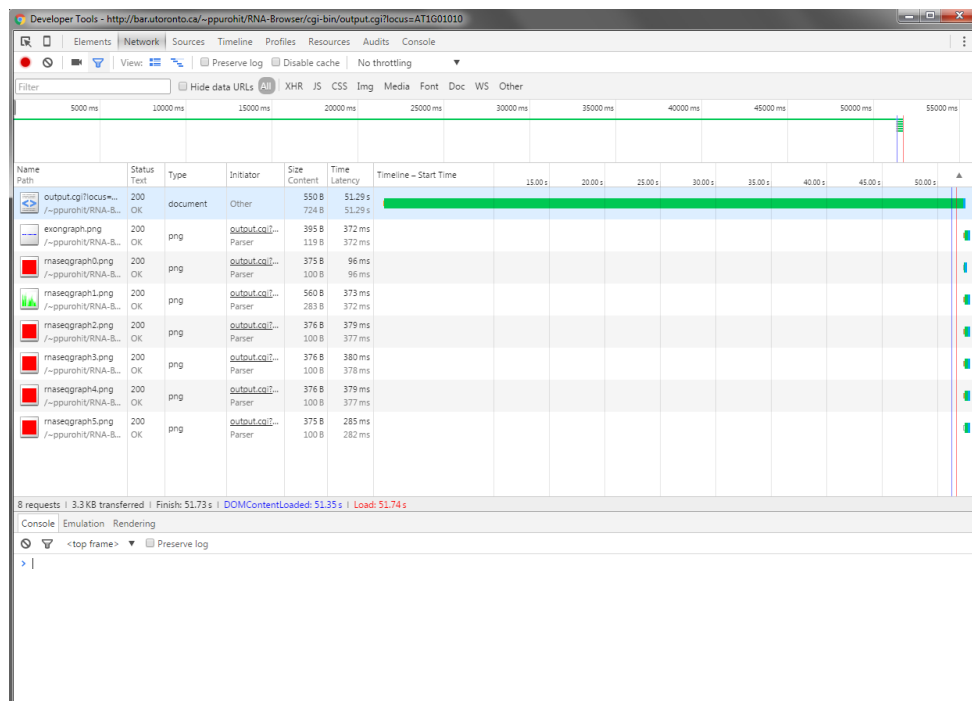


Figure 2: ~60 seconds for iPlant BAM file.

**Date:** October 21, 2015

**Agenda:**

1. Check if BAM files stored on other servers are just as fast as local BAM for the mpileup() call.

**Protocol:**

1. Executed 3 mpileup () commands through SSH.

- a. `time samtools mpileup -r chr2:8032000-10329941`  
`http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeSydhRnaSeq/wgEncodeSydhRnaSeqK562Ifna6hPolyaAln.bam > ucsc.txt`
- b. `time samtools mpileup -r Chr2:10327050-10329941`  
`http://vision.iplantcollaborative.org/iplant/home/araport/rnaseq_bam/aerial/SRR547531/accepted_hits.bam > iplant.txt`
- c. `time samtools mpileup -r Chr2:10327050-10329941`  
`http://bar.utoronto.ca/~ppurohit/RNA-Browser/cgi-bin/data/iplant/home/araport/rnaseq_bam/aerial/SRR547531/accepted_hits.bam > bar.txt`

**Results:**

1. Done.
2. Executed mpileup () through SSH.
  - a. UCSC.edu BAM file:
    - i. 1.322 s for 6 260 175 bytes
  - b. iPlant BAM file:
    - i. 15.294 s for 1 248 931 bytes
  - c. bar.utoronto.ca BAM file:
    - i. 0.060 s for 1 248 931 bytes

**Notes/Questions:**

- The UCSC BAM file returned only ~1500 bytes for the chr2:10327050-10329941 region. This smaller size could be the reason why the command is fast. The query region was therefore increased to get 6x more data.
  - o ... and it's still fast!

**Date:** October 23, 2015

**To-do List:**

1. Research proposal (Oct 29)
2. XML File Updates
  - a. Change the Newland links to their new Vision links
  - b. Add colours as discussed in Oct 22 meeting w/ NP
    - i. Try to make them web safe colours without changing the shade too much
  - c. Add the missing information in the XML file (some pictures missing)
3. Download the mpileup data for the default locus from all of the BAM files on iPlant
  - a. Spread this out over 3-4 days
4. RNA Browser:
  - a. Start working with locally stored mpileup data
  - b. Change the image dimensions for RNA-Seq graph to ~250 x 50.
  - c. Make the exon graph slim and add a horizontal line through the middle
  - d. Start making dynamic requests for RNA-Seq graphs
    - i. Sample flow:
      1. User comes on our app
      2. The RNA-Seq graphs of the default gene are pre-made and loaded on page load
      3. When the user enters a particular locus, the app will load 3 graphs, the rest are dynamically generated after page load ...
  - e. Image read map heights:
    - i. Start with default height of 1000 reads
    - ii. Have a button that allows the user to re-generate all images such that the max height is the maximum read for any base pair
  - f. FPKM calculations:
    - i. Genie is doing this, make sure to have the information she needs for these calculations

**Date:** October 25, 2015

**Agenda:**

1. Get Richard's new BAM Locator XML file and update the newland links to vision links.
2. Change the displayxml.cgi to account for changes made in the attribute names.

**Protocol:**

1. Got Richard's new BAM Locator XML file and updated the newland links to vision links.
  - a. Wrote a [java program](#) to go through an XML file, find the correct new link and replace it.
2. The attribute name changed to svgname from subunitname (correct name is there now).
  - a. Changed the correct IF statement in displayxml.cgi to look for svgname as opposed to subunitname.

**Results:**

1. Success. The code replaced all files correctly.
2. Success. The displayxml.cgi script works correctly with the new BAM locator XML file.

**Date:** October 29, 2015

**Agenda:**

1. Add colours picked out by Dr. Provert to the BAM locator XML file's foreground column.
2. Update the BAM file links to the new Amazon S3 links.

**Protocol:**

1. Add colours picked out ...
  - a. Manually copy-pasted the new HEX colour codes.
  - b. Fixed cases where the colour attribute was missing.
2. Update BAM file links to Amazon ...
  - a. Ran the Java code from Oct 25, 2015 w/ Amazon S3 prefix instead of the iPlant prefix

**Results:**

1. Add colours picked out...
  - a. Successful.
  - b. Live on BAR @ <http://bar.utoronto.ca/~ppurohit/RNA-Browser/cgi-bin/displayxml.cgi>
2. Update BAM file links to Amazon
  - a. Success. It is live on BAR at the link shown above.

**Notes:**

1. The images still look incorrect for some of the experiments (i.e. the image should be root but is not)
  - a. Look into this and fix it.

**Date:** November 1, 2015

**Agenda:**

1. Get the mpileup for a default gene of interest from all BAM files.
  - a. Investigate the issue of remote BAMs not returning data but same local files would (reported by Vivek)

**Protocol:**

1. Get the mpileup for default gene of interest from all BAM files.
  - a. Wrote a shell script to iterate over the BAM files in the `iplant_path_to_rnaseq_bam_files.txt` file.
  - b. Each time, it executes the `samtools mpileup` call on the BAM file and outputs it to a smaller BAM file.
    - i. Getting the mpileup for the first locus only (Chr1:3631-5899).
2. Based on the result for #1, the questions arise: are these files not returning data because there is no data? Or is it because there is some issue with the remote vs. local file?
  - a. Executed mpileup through command line on a single BAM file that did not return data.
    - i. `samtools mpileup -r Chr1:3631-5899`  
[http://s3.amazonaws.com/iplant-cdn/iplant/home/araport/rnaseq\\_bam/leaf/SRR446034/accepted\\_hits.bam](http://s3.amazonaws.com/iplant-cdn/iplant/home/araport/rnaseq_bam/leaf/SRR446034/accepted_hits.bam)
  - b. To see if the issue was resolved in the latest version of samtools, downloaded and installed the latest v1.2.1 of SAM Tools.
    - i. Downloaded the source code
    - ii. Executed the `makefile`
    - iii. Installed by: `make -prefix=/path_to/install_folder/ install`.
    - iv. Executed the call from 2(a)(i) with the new samtools exe (be sure to specify with a relative path to the new executable).
      1. `./samtools-1.2/exe/bin/samtools mpileup`  
`http://s3.amazonaws.com/iplant-`



```
cdn/iplant/home/araport/rnaseq_bam/leaf/SRR446034/accepted_hits.bam -r Chr1:3631-5899 -d 8000
```

- c. To see if BamView can show anything, the SRR446034 BAM file's Amazon link was used to see if there is any data in the file.
  - d. Tried to redo 2(b)(iv) but when I'm in the directory of the newly installed BAM file
    - i. `./samtools mpileup http://s3.amazonaws.com/iplant-cdn/iplant/home/araport/rnaseq_bam/leaf/SRR446034/accepted_hits.bam -r Chr1:3631-5899 -d 8000`
3. Rerun the shell script from the same directory as the latest SAM Tools ... (repeat #1)

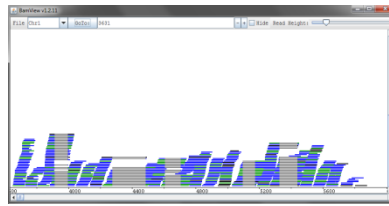
## Result:

1. Get the mpileup for default gene of interest from all BAMs.
  - a. Got back data from the following BAM files (increase text size/zoom in to read):

```
i. dark_SRR1019456_accepted_hits.bam
ii. dark_SRR1019457_accepted_hits.bam
iii. dark_SRR495258_accepted_hits.bam
iv. dark_SRR495257_accepted_hits.bam
v. root_SRR314814_accepted_hits.bam
vi. reeptacle_SRR401418_accepted_hits.bam
vii. reeptacle_SRR401416_accepted_hits.bam
viii. aerial_SRR547531_accepted_hits.bam
ix. reeptacle_SRR401415_accepted_hits.bam
x. reeptacle_SRR401421_accepted_hits.bam
xi. reeptacle_SRR401419_accepted_hits.bam
xii. reeptacle_SRR401415_accepted_hits.bam
xiii. aerial_ERR274510_accepted_hits.bam
xiv. aerial_SRR847505_accepted_hits.bam
xv. aerial_SRR847506_accepted_hits.bam
xvi. aerial_SRR847504_accepted_hits.bam
xvii. aerial_SRR548277_accepted_hits.bam
xviii. reeptacle_SRR401420_accepted_hits.bam
xix. flower_SRR800753_accepted_hits.bam
xx. reeptacle_SRR401414_accepted_hits.bam
xxi. flower_SRR800754_accepted_hits.bam
xxii. leaf_SRR1159837_accepted_hits.bam
```

2. Try to get mpileup from a BAM that did not return data in 1(a)
  - a. Command line mpileup call with SAM Tools v0.1.18
    - i. open: No such file or directory
    - ii. Segmentation fault
  - b. Command line mpileup call with SAM Tools v1.2.1
    - i. [knet\_seek] SEEK\_END is not supported for HTTP. Offset is unchanged.
    - ii. [mpileup] 1 samples in 1 input files
  - c. Checking the file with BamView program

- i. Figure 3: SRR446034 BAM file's first locus in BamView program.



- d. GOT BACK DATA! For some reason this works and returns data from that same BAM file.
  - i. But doesn't work for another experiment, SRR949989's BAM file...
- 3. Rerun the shell script from the same directory as the latest SAM Tools ...
  - a. Got even less number of BAM files returning data

**Date:** November 5, 2015

**Agenda:**

1. Put together a rough version of the multi-track viewer.

**Protocol:**

1. Combined the relevant code from output.cgi and displayxml.cgi to read the mini-BAM files from the mpileups directory and output to files in the /img/ directory where all the image files are...
  - a. The img files were generated using a shell script that generates blank images and chmods them to 766.

**Results:**

1. Works well, but the mpileups are local. Doesn't tell is anything about what the end product will be like.

**Date:** November 12, 2015

**Agenda:**

1. Fix up the displayxml.cgi file and email a link to NP.
2. Add a horizontal line to the exon graph.

**Protocol:**

1. Cleaned up code, minor programming changes.
  - a. Added missing parts as outlined by NP.
  - b. Added colours as outlined by NP.
2. Added the horizontal line w/ another filledRectangle() call.

**Results:**

1. <http://bar.utoronto.ca/~ppurohit/RNA-Browser/cgi-bin/displayxml.cgi>

2. 

**Date:** November 12, 2015

**Agenda:**

1. Produce the total number of mapped reads from a BAM file.

**Protocol/Thought Process:**

1. Information on total number of mapped reads should be available in the index file.
  - a. Search the samtools manual for a convenient command to find this number
    - i. Found and executed two potential commands to get this information on SRR547531 (the BAM file on BAR server):
      1. `samtools idxstats <bam file>`
        - a. OUTPUT (tab delimited): seq name, seq length, # of mapped reads, # of unmapped reads
      2. `samtools view -c -F 4 <bam file>`
        - a.  $\Sigma$  of all mapped reads in the BAM file from all sequence names...
        - b. This produces a single number that is the  $\Sigma$  of all mapped reads from 1(a)(i)(1).

**Result:**

```
samtools idxstats accepted_hits.bam
```

Chr1	30427671	1892640	0
Chr2	19698289	2015247	0
Chr3	23459830	2539031	0
Chr4	18585056	1181593	0
Chr5	26975502	1580658	0
ChrC	154478	1792538	0
ChrM	366924	68400	0
*	0	0	0

```
samtools view -c -F 4 accepted_hits.bam
```

```
11070107
```

- Note that the  $\Sigma$  of mapped reads = 11070107.

**Date:** November 18, 2015

**Agenda:**

1. Get the number of mapped reads for a given region rather than the whole file.

**Protocol:**

1. The number of lines returned by the `samtools view` command is correlated with the number of reads. Therefore, `samtools view Chr1:3631-5899 | wc -l` produces the number of reads mapped for that region.
  - a. **NEED TO VERIFY THIS ASSUMPTION.**
2. Ran the following:
  - a. `Samtools view accepted_hits.bam Chr1:3631-5899 | wc -l`
  - b. `Samtools view accepted_hits.bam | wc -l`
  - c. `Samtools view -c -F 4 accepted_hits.bam`

**Results:**

1. VERIFY, TO DO.
2.
  - a. 120
  - b. 11070107
  - c. 11070107

**Date:** November 25, 2015

**Agenda:**

1. Create the front end of multitrack-rnaseq.html.
2. Display exon image by having the cgi script return a base64 image string. This image should be changing based on locus.
3. Display the RNA-Seq coverage images (not base64 for now, rather just the images generated by the CGI script). These images will have to be for the first locus since mpileups exist for that locus only.

**Protocol:**

1. Done, see November 25 and 26's commits to GitHub.
2. Done, see November 25 and 26's commits to GitHub.
3. Done, see November 25 and 26's commits to GitHub.

**Results:**

