UNIX Data Tools

Buffalo Chapter 7

Overview

In Chapter 3 we learned the basic operations within the Unix shell:

- standard out and standard error streams of data
- how to redirect our data streams
- how to efficiently run a series of commands using pipes
- how to manage command processes

Here, we'll learn a number of UNIX tools that will allow us to inspect and process data

Inspecting a data file for the first time: `head`

- Use the `cd` command to navigate into the `chapter-07-unix-data-tools` folder in the Buffalo online resources
- We can inspect a file by using the `cat` command to print its contents to the screen:

```
$ cat Mus_musculus.GRCm38.75_chr1.bed
```

- That's a little unwieldly...perhaps we just want to see the first few lines of a file to see how it's formatted. Let's try:

```
$ head Mus_musculus.GRCm38.75_chr1.bed
```

- If we want to see less or more of a given file, we can specify the number of lines using the `-n` option:

```
$ head -n 3 Mus_musculus.GRCm38.75_chr1.bed
```

Inspecting a data file for the first time: `tail`

- Similar to `head`, you can use the `tail` command to inspect the end of a file:

```
$ tail -n 3 Mus_musculus.GRCm38.75_chr1.bed
```

- `tail` can also be useful for removing the header of a file; this is particularly useful when concatenating files for an analysis:

```
$ tail -n +2 genotypes.txt
```

- And here's a handy trick for inspecting both the head and tail of a file simultaneously:

```
$ (head -n 2; tail -n 2) < Mus_musculus.GRCm38.75_chr1.bed
1     3054233    3054733
1     3054233    3054733
1     195240910    195241007
1     195240910    195241007
```

Additional uses of `head`

- We can also use `head` to inspect the first bit of output of a UNIX pipeline:

```
$ grep 'gene_id "ENSMUSG00000025907"' Mus_musculus.GRCm38.75_chr1.gtf | head -n 1
```

- When including head at the end of a complex UNIX pipeline, the pipeline will only run until it produces the number of lines dictated by `head`
- Why is this important or useful? This dummy pipeline may help:

```
$ grep "some_string" huge_file.txt | program1 | program2 | head -n 5
```

Inspecting files and pipes using `less`

- `less` is what is known as a "terminal pager"; it allows us to view large amounts of text in our terminal
- Whereas with `cat` the contents of our file flash before our eyes, with `less` we can view and scroll through the file's contents
- Let's observe the difference between `cat` and `less` using a file from the Buffalo Chapter 7 materials:

Try:

```
$ cat contaminated.fastq
```

Then try:

```
$ less contaminated.fastq
```

- While viewing the file in `less` try navigating with the space bar and the `b`, `j`, `k`, `g`, and `G` keys. To exit the file, press `q`

Using `less` to highlight text matches and check pipes

- Highlighting text matches can allow us to search for potential problems in data
- For example, imagine we download useful Illumina data from another study and it's not clear from the documentation whether adapter sequence has been trimmed
- We can search for a known 3' adapter sequence using `less`:

```
$ less contaminated.fastq

# then press / and enter AGATCGG
```

- `less` can also be used to check the individual components of a pipe under construction:

```
$ step1 input.txt | less
$ step1 input.txt | step2 | less
$ step1 input.txt | step2 | step3 | less
```

- The commands will only run until a page of your terminal is full, limiting computation time

Inspecting files using the `wc` command

- The default of `wc` is to provide the number of lines, words, and bytes (characters) in a file:

```
$ wc Mus_musculus.GRCm38.75_chr1.bed Mus_musculus.GRCm38.75_chr1.gtf
```

- Each line of data entry in the .bed file should correspond to a single line of data entry in the .gtf file. Notice any problems?
- Using `head`, see if you can inspect the two files and resolve this issue
- The discrepancy in the line numbers, may have been more clear had we only inspected the number of lines:

```
$ wc -l Mus_musculus.GRCm38.75_chr1.bed Mus_musculus.GRCm38.75_chr1.gtf
```

Inspecting file size using the `ls` and `du` commands

- Before downloading or moving or running an analysis on a file, it is useful to know the file size
- There are a few ways we can extract this information
- First, we can use our old friend, the `ls` command with the `-l` and `-h` options:

```
$ ls -lh Mus_musculus.GRCm38.75_chr1.bed
```

- Or we can use the `du` command, also with the `-h`, or "human readable" option:

```
$ du -h Mus_musculus.GRCm38.75_chr1.bed
```

- Personally, I prefer the less verbose format of `du`, particularly when inspecting a large number of files

Inspecting the number of columns in a file with `awk`

- Another useful piece of information we may want to know about a file is its number of columns
- We could find this by visually inspecting the first line of the file, but this opens us up to human error:

```
$ head -n 1 Mus_musculus.GRCm38.75_chr1.bed
```

- A better solution is to have our computers count the columns for us using an `awk` one-liner:

```
$ awk -F "\t" '{print NF; exit}' Mus_musculus.GRCm38.75_chr1.bed
```

- `awk` is a bit different than some of the basic UNIX commands we've been learning...it is actually a simple programming language in itself...we'll come back to it in more depth later

Number of columns in files with headers

- Our handy `awk` script works well for the `Mus_musculus.GRCm38.75_chr1.bed` file, but what about for the `Mus_musculus.GRCm38.75_chr1.gtf file`?
- We can get around this issue by employing the `tail` command we learned earlier:

```
$ tail -n +6 Mus_musculus.GRCm38.75_chr1.gtf | awk -F "\t" '{print NF; exit}'
```

- In the Buffalo book, this one-liner outputs that there are 16 columns...is this what you get?
- Thinking back to the first few chapters in Buffalo and our discussion regarding "robust" and "reproducible" code, why might this be considered a "brittle" solution?
- Can you think of a more robust solution?

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | awk -F "\t" '{print NF; exit}'
```

- How might this be a brittle solution?

Using the `cut` command to extract specific columns

- On occasion, we will want to extract a subset of specific information from a file
- The `cut` command assumes tab delimitation and allows us to extract specific columns of a tab-delimited file
- For example, say we wanted just the start positions of the windows in our .bed file:

```
$ cut -f 2 Mus_musculus.GRCm38.75_chr1.bed | head -n 3
```

Using the `cut` command to extract specific columns

- The `-f` option allows us to specify columns in ranges (e.g., `-f 3-8`) and sets (e.g., `-f 1,3,5`) but ***DOES NOT*** allow us to order columns (e.g., `-f 7,3,1`)
- For example, we can extract chromosome, start site, and end site from our .gtf file by first removing the header and then cutting out the first, fourth, and fifth columns:

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f 1,4,5 | head -n 5
```

- We can also specify the delimiter in differently formatted files like .csv:

```
$ cut -d "," -f 2,3 Mus_musculus.GRCm38.75_chr1_bed.csv | head -n 3
```

Tidying things up with `column`

- Often times, when we inspect a tab-delimited file with `head`, the results are fairly messy:

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f1-8 | head -n3
```

- This can make it difficult to understand file contents
- Fortunately, there's a UNIX program/option combination to tidy things up: `column -t`

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f 1-8 | column -t \
 | head -n 3
```

- `column` should only be used for file inspection in the terminal, redirecting its standard out to a file will introduce variable numbers of spaces which could cause problems in downstream analysis
- `column` can also be used with files with other delimiting characters:

```
$ column -s "," -t Mus_musculus.GRCm38.75_chr1_bed.csv | head -n 3
```

`grep`: one of the most powerful UNIX tools

- Thus far we've only scratched the surface of the utility of `grep`
- In addition to being useful, `grep` is *fast*

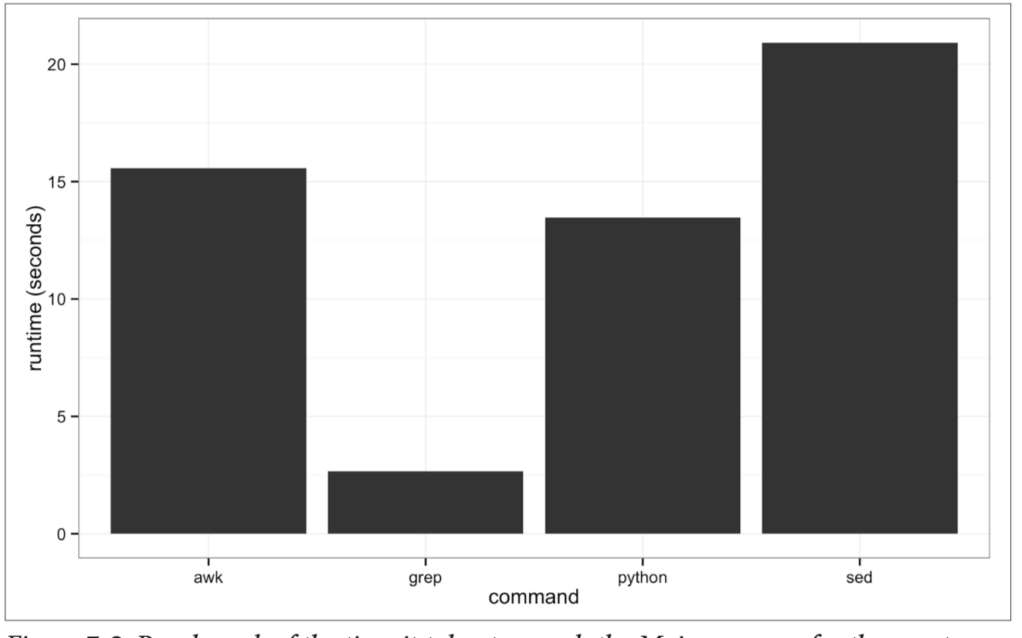


Figure 7-2. Benchmark of the time it takes to search the Maize genome for the exact string "AGATGCATG"

`grep`: one of the most powerful UNIX tools

- The program `grep` requires a *pattern* to search for and a *file* to search through:

```
$ grep "Olfr418-ps1" Mus_musculus.GRCm38.75_chr1_genes.txt
```

- Quotes around the pattern prevent our shell from trying to interpret symbols in the pattern
- `grep` will also return partial matches:

```
$ grep Olfr Mus_musculus.GRCm38.75_chr1_genes.txt | head -n 5
```

- If a partial match is not desired, we can prevent this using the `-w` option which matches entire words
- For example, in the `example.txt` file we want to match everything but "bioinfo":

```
$ cat example.txt
$ grep -v "bioinfo" example.txt
$ grep -v -w "bioinfo" example.txt
```

`grep`: one of the most powerful UNIX tools

- General `grep` rule: always be as restrictive as possible to avoid unintentional matches
- If the matching line itself does not provide enough context, the `-B` and `-A` options can be helpful:

```
$ grep -B1 "AGATCGG" contam.fastq | head -n 6
$ grep -A2 "AGATCGG" contam.fastq | head -n 6
```

- `grep` search patterns can also be made more flexible and powerful with *Basic Regular Expressions* (BRE) and *Extended Regular Expressions* (ERE)
- An example of a BRE:

```
$ grep "Olfr141[13]" Mus_musculus.GRCm38.75_chr1_genes.txt
```

- An example of an ERE:

```
$ grep -E "(Olfr218|Olfr1416)" Mus_musculus.GRCm38.75_chr1_genes.txt
```

Additional `grep` uses with various options

- Say we're interested in the number of small nuclear RNAs in our set of genes:

```
$ grep -c 'gene_biotype "snRNA"' Mus_musculus.GRCm38.75_chr1.gtf
```

- Or perhaps we only want `grep` to extract the word matches to our search pattern, not the entire line:

```
$ grep -o "Olfr.*" Mus_musculus.GRCm38.75_chr1_genes.txt | head -n 3
```

Identification of non-ASCII files and characters

- In bioinformatics, many programs will assume that our input text files are encoded in ASCII
- Occasionally, often due to human manipulation of data files, our data can include include an invisible non-ASCII character that throws our program for a loop
- To easily determine whether a given file is encoded in something other than ASCII, the `file` command can be quite useful:

```
$ file Mus_musculus.GRCm38.75_chr1.bed Mus_musculus.GRCm38.75_chr1.gtf
$ file improper.fa
```

Illustrating the trouble a non-ASCII character can cause

- To show how non-ASCII characters can cause problems, we'll install the program `bioawk` from github
- If you're working on hpc-class:

```
module load bioawk
```

- If you're working on a directory on your own machine:

```
$ git clone git://github.com/lh3/bioawk.git
$ cd bioawk
$ make
$ sudo cp bioawk /usr/local/bin/
```

- Or if you've installed `Homebrew`:

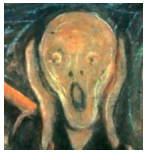
```
$ brew tap homebrew/science
$ brew install bioawk
```

Illustrating the trouble a non-ASCII character can cause

- Now let's apply the following `bioawk` one-liner which should produce the reverse complement of our sequences:

```
$ bioawk -cfastx '{print revcomp($seq)}' improper.fa
```

- Shoot...`bioawk` choked on our second sequence...non-ASCII character!!



Sleuthing out our non-ASCII character with `hexdump`

- `hexdump` will identify the problematic character and the `-c` option will print the character as well:

```
$ hexdump -c improper.fa
0000000 > g o o d - s e q u e n c e \n A
0000010   G   C   T   A   G   C   T   A   C   T   A   G   C   A   G   C
0000020   T   A   C   T   A   C   G   A   G   C   A   T   C   T   A   C
0000030   G   G   C   G   C   G   A   T   C   T   A   C   G  \n   >   b
0000040   a   d   -   s   e   q   u   e   n   c   e  \n   G   A   T   C
0000050   A   G   G   C   G   A   C   A   T   C   G   A   G   C   T   A
0000060   T   C   A   C   T   A   C   G   A   G   C   G   A   G   221
0000070   G   A   T   C   A   G   C   T   A   T   T  \n
000007c
```

Sorting plain-text data with `sort`

- Sorting plain text data can be necessary because:

```
1. Some operations are more efficient when working on sorted data

2. In order to find unique lines, we must first have sorted data
```

- First, let's sort the `example.bed` file without options to see if we can figure out how the default program works:

```
$ sort example.bed
```

- Options allow us to sort by specific columns in various orders and to tell `sort` that our data are numeric rather than alpha-numeric:

```
$ sort -k1,1 -k2,2n example.bed
```

- Now see if you can figure out how to sort the `Mus_musculus.GRCm38.75_chr1_random.gtf` file, first by chromosome, then by window start site

Additional features of `sort`

- Since sorting very large files can be computationally intensive, we may want to check whether a file is already sorted first using the `-c` option:

```
$ sort -k1,1 -k2,2n -c example.bed
$ echo $?
$ sort -k1,1 -k2,2n example.bed > example_sorted.bed
$ sort -k1,1 -k2,2n -c example_sorted.bed
$ echo $?
```

- We can also sort files in reverse order using the `-r` option:

```
$ sort -k1,1 -k2,2n -r example.bed
```

- But how is this sorting?
- Can you think of a way to sort in reverse order based on both columns 1 and 2?
- What if we want to sort in forward order by column 1 and reverse order by column 2?

Advanced sorting options in GNU

- The `-V` option can recognize numbers inside of strings...how might this be useful?
- Inspect the entire `example2.bed` file:

```
$ cat example2.bed
```

- Why might we want to recognize numbers within a string here?

```
$ sort -k1,1 -k2,2n example2.bed

$ sort -k1,1V -k2,2n example2.bed
```

- In the event that you want to sort a truly enormous file, there are modifications to `sort` that can be applied to allocate more memory to the program:

```
$ sort -k1,1 -k4,4n -S2G Mus_musculus.GRCm38.75_chr1_random.gtf

$ sort -k1,1 -k4,4n --parallel 4 Mus_musculus.GRCm38.75_chr1_random.gtf
```

Finding unique values using the program `uniq`

- After first inspecting the entire `letters.txt` file, run the `uniq` program on this file and see if you can understand how this program works

```
$ cat letters.txt

$ uniq letters.txt
```

- What do we need to do to get a truly unique list of letters?

```
$ sort letters.txt | uniq
```

- And what if we want unique values but still want want a count of each letter?

```
$ sort letters.txt | uniq -c
```

- And if you're still not convinced that this could be useful, try this:

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f3 | sort | uniq -c
```

Finding unique values using the program `uniq`

- The `uniq` output can also be sorted based on entry counts by piping to `sort` and using the `-n` option:

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f3 | sort | uniq -c | \
    sort -n
```

- What if you wanted these listed from most to least common in the file?
- We can also use the combination of `sort` and `uniq` to gather information from multiple columns in a file:

```
$ grep -v "^#" Mus_musculus.GRCm38.75_chr1.gtf | cut -f3,7 | sort | uniq -c
```

- Or we can use these programs to process and inspect a subset of data from a file…for example, all the features associated with a particular gene:

```
$ grep "ENSMUSG00000033793" Mus_musculus.GRCm38.75_chr1.gtf | cut -f3 | sort \
    | uniq -c
```

Merging the contents of two files with the program `join`

- The contents of two files can be merged by joining the files based on a common column
- In the following two files, what would be the common column to use for a join?

```
$ cat example.bed

$ cat example_lengths.txt
```

- In order to complete the join, we must first sort *both* files on the common column

```
$ sort -k1,1 example.bed > example_sorted.bed

$ sort -c -k1,1 example_lengths.txt
```

- Let's talk through the following syntax to make sure it's clear:

```
$ join -1 1 -2 1 example_sorted.bed example_lengths.txt > example_with_lengths.txt
```

Merging the contents of two files with the program `join`

- Let's also look at the number of lines in our files to see if the join was complete:

```
$ wc -l example_sorted.bed example_lengths.txt example_with_lengths.txt
```

- Now let's see what happens when there is not complete overlap in our common columns:

```
$ head -n2 example_lengths.txt > example_lengths_alt.txt

$ join -1 1 -2 1 example_sorted.bed example_lengths_alt.txt

$ join -1 1 -2 1 example_sorted.bed example_lengths_alt.txt | wc -l
```

- Because chr3 is absent from the `example_lengths_alt.txt` file, it is omitted entirely from the join
- The GNU `join` option `-a` allows us to include these "unpairable" lines in our output file:

```
$ join -1 1 -2 1 -a 1 example_sorted.bed example_lengths_alt.txt
```

Processing data with the `awk` programming language

- Unlike the UNIX programs we've been learning, `awk` is a full-fledged programming language
- `awk` is simpler then `python` and not built for complicated tasks, but it's great for quick data-processing tasks
- To learn `awk` we must understand how it:

    1. Processes records
    2. Uses pattern-action pairs

- `Awk` processes data a record at a time and records are composed of fields
- `Awk` assigns the entire record to variable `$0`, field 1 to `$1`, field 2 to `$2`, etc...
- In pattern-action pairs, `awk` first tries to match a specified pattern in a record or field and, if this is successful, the specified action is carried out

Processing data with the `awk` programming language

- We can mimic the `cat` program with `awk` by omitting the pattern component of a pattern-action pair:

```
$ awk '{ print $0 }' example.bed
```

- Similarly, we can also mimic `cut`:

```
$ awk '{ print $2 "\t" $3 }' example.bed
```

- Standard arithmetic operators (+, -, *, /, etc...) can be used in the pattern component of pattern-action pairs
- For example, here our pattern is matching .bed file features that are at least 18bp long and the implicit action is to print matches to standard out:

```
$ awk '$3 - $2 > 18' example.bed
```

Processing data with the `awk` programming language

- We can also link patterns in a chain to apply multiple conditions in our pattern using the `&&` (AND), `||` (OR), and `!` (NOT) operators
- For example, if we want the .bed features that are on chromosome 1 AND at least 10bp long:

```
$ awk '$1 ~ /chr1/ && $3 - $2 > 10' example.bed
```

- We can also include more explicit actions than just printing an entire record to standard out:

```
$ awk '$1 ~ /chr2|chr3/ { print $0 "\t" $3 - $2 }' example.bed
```

Additional functionality of the `awk` programming language

- The pattern-process `awk` tools we have learned thus far are very useful for processing files, but `awk` has many more useful tools
- The `BEGIN` and `END` commands can allow us to initialize variables before implementing our pattern-process across records (`BEGIN`) and use this variable afterwards (`END`):

```
$ awk 'BEGIN{ s = 0 }; { s += ($3-$2) }; END{ print "mean: " s/NR };' example.bed
```

- Here we initialize the variable `s` and increment (`+=`) this variable by the length of each feature across all records and then divide this by `NR`...what is `NR`?
- `NR` can also be used to extract intermediate records (i.e., lines) in a file (the same process we discussed using `head` and `tail` in a pipe):

```
$ awk 'NR >= 3 && NR <= 5' example.bed
```

Additional functionality of the `awk` programming language

- `awk` can also be used to convert a .gtf file into a .bed file:

```
$ awk '!/^#/ { print $1 "\t" $4-1 "\t" $5 }' Mus_musculus.GRCm38.75_chr1.gtf |
    \ head -n 3
```

- Note that the start site of features in the .bed file is 1 less than the start site of features in the .gtf file: .bed uses 0-indexing and .gtf uses 1-indexing
- Associative arrays (similar to `Python` dictionaries) can also be very useful in `awk`:

```
$ awk '/Lypla1/ { feature[$3] += 1}; \
    END { for (k in feature) \
    print k "\t" feature[k] }' Mus_musculus.GRCm38.75_chr1.gtf
```

- Could this also be done with basic UNIX commands?

`bioawk`: `awk` functionality more tailored to bioinformatics

- `bioawk` is similar to `awk` but it can recognize common bioinformatics file formats (e.g., .bed, .sam, .vcf, .gff, .fastx) and includes useful programs for bioinformatics

```
$ bioawk -c gff '$feature ~ /gene/ && $source ~ /protein_coding/ \
    {print $seqname,$end-$start}' Mus_musculus.GRCm38.75_chr1.gtf | head -n 4
```

- You could also use `bioawk` to convert a `fastq` into a `fasta` file:

```
$ bioawk -c fastx '{print ">"$name"\n"$seq}' contam.fastq | head -n 4
```

- Or to print the number of sequences in a `fastq/fasta` file, something you couldn't do with `wc`:

```
$ bioawk -c fastx 'END{print NR}' contam.fastq
```

`bioawk`: `awk` functionality more tailored to bioinformatics

- Finally, the option `-c hdr` can be very useful as it sets the field variables to the names given in a file header
- For example, take another look at the `genotypes.txt` file:

```
$ head -n 4 genotypes.txt
```

- Let's use the `-c hdr` option to find the markers where ind_A and ind_B have the same genotype:

```
$ bioawk -c hdr '$ind_A == $ind_B {print $id}' genotypes.txt
```

Using the `sed` program to edit text in a stream

- In addition to many other functions, we can use `sed` to make simple "find and replace" edits to our files:

```
$ head -n 3 chroms.txt

$ sed 's/chrom/chr/' chroms.txt | head -n 3
```

- If this file were many Gb in size, this stream editing would be much, much faster than opening the file and doing a find and replace in a text editor
- The above syntax only substitutes the first occurence of "chrom" on a line, to do this across all "chrom" values we'd need to use the global option of `sed`:

```
$ sed 's/chrom/chr/g' chroms.txt
```