Exercise 8 - String manipulation with regex in Python

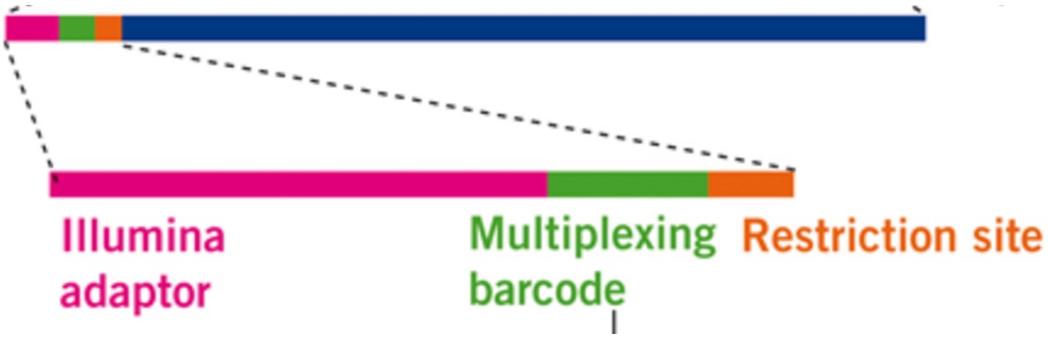
Work with a partner to complete the tasks below and submit your results via a pull request on GitHub by the beginning of tutorial next Friday.

To begin this week, one of the partners should fork the TA's Exercise 8 Github repo and provide collaborative access to the other partner. Clone the forked repo so that you have the required files. Be sure to commit regularly to avoid merge conflicts and demonstrate effort by both partners.

Background

RADseq

The cost of high-throughput sequencing technologies continue to decrease... but sometimes grant funding does, too... Rather than sequencing the whole genome of each sample, many population geneticists strech their sequencing dollars furthur with reduced representation techniques. In this tutorial, you will be working with RADseq (restriction site associated DNA sequencing) data generated by me (Meredith) in the Feder Lab! Briefly, genomic DNA from each sample was digested with two restriction enzymes (EcoRI is the important one today), fragmenting the genome. Adapters with restriction enzyme "sticky ends" were then ligated to the genomic DNA fragments. As part of these adapters, a unique sequence of ~8 bp for each individual sample, sits just 5' of the EcoRI cut site (of which the 'AATTC' remains in the sequence).



After library preparation, all of these individual samples are pooled together (sometimes 100+) in a single sequencing run. It sounds like a mess, but luckily, we have bioinformatic tools and **regex** to "unpool" or demultiplex individuals, based on the ~8 bp barcode found preceding an 'AATTC' in each (well, hopefully most) of the 100s of millions of raw sequences.

Fastq file

Along with the next-generation sequencing and big data revolution, came the `fastq` file format (similar to `fasta`, but with additonal lines of sequence quality scores). The raw data files we now receive from sequencing facilities are nearing 100 Gb a piece, necessitating efficient automated processing. You'll want to be familiar with the file format for this tutorial.

```
@FCC638CACXX:5:1105:14309:52971#ATCNCGATC/1
CCAGTCTAAATTCCAAAGCCTCACCGCATTGACGAAACACAGCCTCGAGGAAGACAAATAGGAATATTTAGGGATTATAAGATTTGTGTGTTTGCAAGAC
+
bbbeeeeeggggggifhiiiiiiiiiiiiiifiiiiiiiiiiiiiiiiiiiiiifgggggeeeeedededddcc`bcddccccccdcbcccccccdcccc
@FCC638CACXX:5:1105:14154:53737#ATCNCGATC/1
GCTATTGGACAATTCATTACATGTGGCGGCATGTTACGCATATTGGGATTCAGCGGGTTAGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGA
+
[\^ccccceeeeeehehh_eaSbKRb^Y^_eeeehhhbedeab\NWbeeehbHV\aaW^^^_ZZ\N^^^XXXY^^[_WWX^_``GJS]YY]Y]`R]`a^B
@FCC638CACXX:5:1105:8119:53833#ATCNCGATC/1
TGCTGCTAAATTCGTTTCGTTCGAATAAAACTAAATGCGTTCAACAAAAAATTGATTTATTCGTTTATGGAACAACAAAACCGTTCGAAAATAAAAATTT
+
bbbeeeeeggggigiiihghhiiiiihiiiiiiiiiigihiiiiiiiiiihfiiiiiiiggggeeeeeebd`ccccccacccaccccccccccc
@FCC638CACXX:5:1105:13834:54314#ATCNCGATC/1
CCTCCAACAATTCCCGTTTTTGAGATATTTAGGTGGCCCGGTTGACATGGAATGCACGATATGTATGTAGTTAGATCGGAAGAGCACACGTCTGAACTCC
+
bbbeeeecg[bbcefgdehghedfffhhfhff]b`eggfhf_ege]egfdegfbcddggag]c]bZ^ZacZb]]_]`Z]^_ac^^bab^]^ab_Y]`Y_b
```

As you can see above, the `fastq` is organized in 4 line units:

`@ Header with sequencing information, but no sample information yet!`

`sequence data`

`+`

`quality scores associated with sequence base pairs`

For the purposes of this tutorial, we are going to focus on the first two lines.

VCF file

Biologists handling genomic data, also frequently encounter the `VCF` file format. `VCFs` are used to store SNP (single nucleotide polymorphism) genotypes and associated statistics for many individuals (columns) at many positions (rows) in the genome. Below is a truncated example of SNP data from a RADseq data set. The majority of the header and position statistics have been removed for clarity.

```
##Deleted a large header, all lines starting with ##
#CHROM  POS     INFO    FORMAT  CF.A.003        CF.A.004        CF.A.005        CF.A.006        CF.A.007        CF.A.008
Contig23        34      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,40      0/0:4,0:4:12:0,12,160   0/0:3,0:3:9:0,9,120
Contig150       37      DeletedStuff    GT:AD:DP:GQ:PL  0/0:5,0:5:15:0,15,123   0/0:5,0:5:15:0,15,121   0/0:4,0:4:12:0,12,99
Contig234       63      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,35      0/0:1,0:1:3:0,3,39      0/0:1,0:1:3:0,3,39
Contig286       18      DeletedStuff    GT:AD:DP:GQ:PL  0/0:5,0:5:15:0,15,199   0/0:1,0:1:3:0,3,32      0/0:1,0:1:3:0,3,40
Contig286       76      DeletedStuff    GT:AD:DP:GQ:PL  0/0:5,0:5:15:0,15,190   0/0:1,0:1:3:0,3,37      0/0:1,0:1:3:0,3,39
Contig286       84      DeletedStuff    GT:AD:DP:GQ:PL  0/0:5,0:5:15:0,15,196   0/0:1,0:1:3:0,3,39      0/0:1,0:1:3:0,3,39
Contig319       49      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,40      0/0:1,0:1:3:0,3,40      ./.:.:.:.:.     0/0:2
Contig319       64      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,40      0/0:1,0:1:3:0,3,40      ./.:.:.:.:.     0/0:2
Contig355       39      DeletedStuff    GT:AD:DP:GQ:PL  0/0:2,0:2:6:0,6,55      0/0:2,0:2:6:0,6,55      0/0:3,0:3:9:0,9,83
Contig355       83      DeletedStuff    GT:AD:DP:GQ:PL  0/0:2,0:2:6:0,6,55      0/0:2,0:2:6:0,6,55      0/0:3,0:3:9:0,9,83
Contig426       46      DeletedStuff    GT:AD:DP:GQ:PL  0/0:2,0:2:6:0,6,55      0/0:2,0:2:6:0,6,55      0/0:1,0:1:3:0,3,28
Contig426       60      DeletedStuff    GT:AD:DP:GQ:PL  0/0:2,0:2:6:0,6,55      0/0:2,0:2:6:0,6,55      0/0:1,0:1:3:0,3,28
Contig449       19      DeletedStuff    GT:AD:DP:GQ:PL  0/0:5,0:5:15:0,15,194   0/0:3,0:3:9:0,9,120     0/0:4,0:4:12:0,12,166
Contig454       2       DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,37      ./.:.:.:.:.     0/0:1,0:1:3:0,3,40      0/0:5
Contig454       58      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,37      ./.:.:.:.:.     0/0:1,0:1:3:0,3,34      0/0:5
Contig500       27      DeletedStuff    GT:AD:DP:GQ:PL  0/0:2,0:2:6:0,6,80      0/0:3,0:3:9:0,9,107     0/0:2,0:2:6:0,6,80
Contig538       36      DeletedStuff    GT:AD:DP:GQ:PL  ./.:.:.:.:.     0/0:2,0:2:6:0,6,80      0/0:5,0:5:15:0,15,200   0/0:2
Contig588       70      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,39      0/0:1,0:1:3:0,3,39      0/0:2,0:2:6:0,6,77
Contig588       78      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,39      0/0:1,0:1:3:0,3,39      0/0:2,0:2:6:0,6,77
Contig588       84      DeletedStuff    GT:AD:DP:GQ:PL  0/0:1,0:1:3:0,3,39      0/0:1,0:1:3:0,3,39      0/0:2,0:2:6:0,6,77
```

This data set included biallelic SNPs only and the data for each individual and position are listed in the following format:

`genotype : read count per allele : total read depth : genotype quality : genotype likelihoods`

For the purposes of this tutorial, don't worry about understanding each field, just the **pattern** in which they are arranged.

Writing files line by line

When dealing with very large data files, you can easily slow down your program by storing too much information in memory. Therefore, whenever possible, write what you can to an output file frequently. For example:

```
file = open("filename"","w")
for each step in your loop:
  get or create string
  file.write(string + "\n")
file.close()
```

Using a dictionary

A dictionary is an unordered set of keys and associated values, which is useful in python because it is easy to search quickly. There are many ways to populate a dictionary, but for this exercise we will consider populating an empty dictionary with a loop.

```
dictionary = {}
for line in file:
  line = line.strip()
  cols = line.split()
  if cols[0] in dictionary:
   print("Duplicate: " + cols[1])
   break
  else:
   dictionary[cols[0]] = cols[1]
```

This code loops over a text file, checking to to see if a key from the first column is in the dictionary, adding the key and associated value (from the second column) if it is not. It's always good to add a check to avoid duplication errors (there are additional ways to deal with key errors that we won't cover here). We can then later retrieve the value associated with a key with:

```
dictionary[key]
```

Challenge

1. For this challenge, you'll be performing two string manipulations on the modified VCF file `Cflorida.vcf`. This file contains the SNP information for two populations of *Rhagoletis* flies collected from flowering dogwood (*Cornus florida*) fruits in Steven F. Austin Forest, TX and Gainesville, FL. Unfortunatley, all collaborators did not follow a standardized naming convention, resulting in individual samples from TX named:

CF.A.XXX

CF.A2.XXX

CF07.A.XXX

cf.a.XXX

and individuals from FL named:

CF.G2.XXX

CF.GAI.XXX

cf.gai.XXX

with XXX representing 3 digit IDs.

Your first objective is to use regular expressions to standardize all TX samples to Cf.Sfa.XXX and all FL samples to Cf.Gai.XXX.

The VCF file also contains many different fields of information for each individual and SNP, as explained above. Today, we are interested only in the allele counts (number of reference and alternate alleles sequenced for each individual), bolded in the example below:

0/0:**7,0**:7:21:0,21,194

Your second objective is to use regular expressions to extract only the allele counts for each SNP for each individual (e.g. 7,0). If the data are missing, indicated by `.`, replace with `NA`.

The goal is to create a new file `CfloridaCounts.txt`, with the same structure as `Cflorida.vcf`, but with corrected sample names and allele counts only.

A couple tips on this one:

Combine these objectives into one for loop over `Cflorida.vcf`.

Work out your regex on paper first.

2. For this challenge, you'll be implementing a simple version of a demultiplexing program for RADseq data (explained above). You will start with two files `indivIDs.txt` and `seqFastq.fq`:

*`indivIDs.txt` is tab-delimited with 8 bp DNA "barcodes" in the left column and the associated sample ID in the right column

*`seqFastq.fq` is a fastq file (described above), containing raw sequence data that lacks associated sample IDs.

Your objective is to write a fasta file, `IDseq.fasta`. The header line should contain the sample ID (e.g. `> ID`) and the sequence line should contain the associated DNA sequence from the fastq file, with the "barcode" and `AATTC` cut site removed. Also, plot a histogram of the start position of the `AATTC` cut site match in each barcoded sequence.

A couple tips on this one:

Break this down into a few chunks for easier management:

a. Store the sample IDs in a dictionary, with the DNA "barcodes" as the keys and the sample IDs as the values.

b. Write a regular expression to match the 8 bp barcode, followed by the `AATTC` cut site, followed by the rest of the sequence. Note that you will need to reference each of these pieces later on.

c. Use a while loop to scan the fastq file line by line, searching each sequence line for the regular expression. Then:

* Check the sample ID dictionary for the 8 bp barcode
  * Store the position of the cutsite match
  * Write the header with sample ID to the fasta file
  * Write the trimmed sequence to the fasta file

Devise a plan for splitting up the work and generating the required code. Do this in parrallel, not sequentially. Don't forget to check and edit each other's code. Remember to frequently `add-commit` locally and `push-pull` to GitHub to avoid conflicts. Also, remember you don't have to be in the same place at the same time to work on this collaboratively thanks to GitHub!!!

Turning in your assignment via GitHub

Once you have committed all changes to your local Git repos and pushed all of those commits to the forked repo on GitHub, you can "turn in" your assignment using a `pull request`. This can be done from the GitHub repo website. When viewing the forked repo, select "Pull requests" in the upper middle of the screen, then click the green "New pull request" button in the upper right. You'll then see a screen with a history of commits for you and your collaborator, select the green "Create pull request button". In the text box next to your user icon near the top of the page, remove whatever text is there and add "owner's last name - collaborator's last name submission", but obviously substitute your last names. If I and Ann Raiho worked on the project together the text would read "jones-raiho submission". Then click the green "Create pull request" button. **Only one of you will need to create a pull request.**