‹ (../01-background/index.html) Data Wrangling and Processing for Genomics (../) › (../03-trimmi

Assessing Read Quality

Overview

Teaching: 30 min
Exercises: 20 min
Questions

- How can I describe the quality of my data?

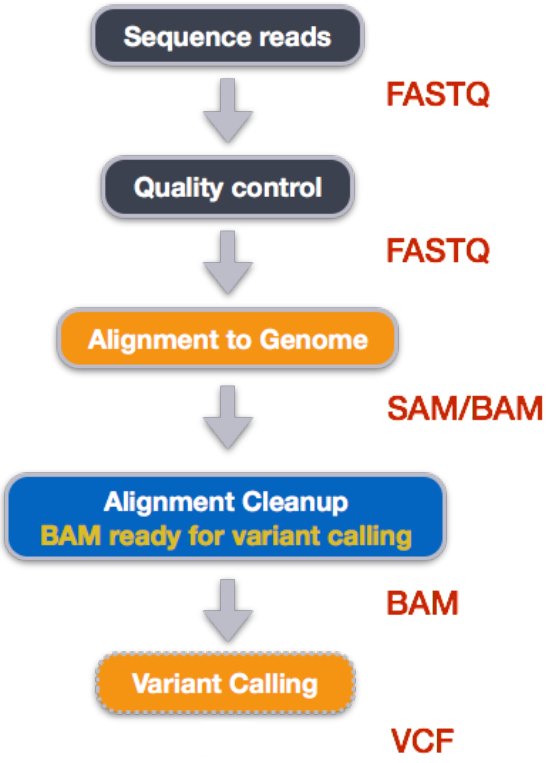
Objectives

- Explain how a FASTQ file encodes per-base quality scores.
- Interpret a FastQC plot summarizing per-base quality across all reads.
- Use `for` loops to automate operations on multiple files.

Bioinformatic workflows

When working with high-throughput sequencing data, the raw reads you get off of the sequencer will need to pass through a number of different tools in order to generate your final desired output. The execution of this set of tools in a specified order is commonly referred to as a *workflow* or a *pipeline*.

An example of the workflow we will be using for our variant calling analysis is provided below with a brief description of each step.



1. Quality control - Assessing quality using FastQC
2. Quality control - Trimming and/or filtering reads (if necessary)
3. Align reads to reference genome
4. Perform post-alignment clean-up
5. Variant calling

These workflows in bioinformatics adopt a plug-and-play approach in that the output of one tool can be easily used as input to another tool without any extensive configuration. Having standards for data formats is what makes this feasible. Standards ensure that data is stored in a way that is generally accepted and agreed upon within the community. The tools that are used to analyze data at different stages of the workflow are therefore built under the assumption that the data will be provided in a specific format.

Starting with Data

Often times, the first step in a bioinformatic workflow is getting the data you want to work with onto a computer where you can work with it. If you have outsourced sequencing of your data, the sequencing center will usually provide you with a link that you can use to download your data. Today we will be working with publicly available sequencing data.

We are studying a population of *Escherichia coli* (designated Ara-3), which were propagated for more than 50,000 generations in a glucose-limited minimal medium. We will be working with three samples from this experiment, one from 5,000 generations, one from 15,000 generations, and one from 50,000 generations. The population changed substantially during the course of the experiment, and we will be exploring how with our variant calling workflow.

The data are paired-end, so we will download two files for each sample. We will use the European Nucleotide Archive (https://www.ebi.ac.uk/ena) to get our data. The ENA "provides a comprehensive record of the world's nucleotide sequencing information, covering raw sequencing data, sequence assembly information and functional annotation." The ENA also provides sequencing data in the fastq format, an important format for sequencing reads that we will be learning about today.

To download the data, run the commands below.

Here we are using the `-p` option for `mkdir`. This option allows `mkdir` to create the new directory, even if one of the parent directories doesn't already exist. It also supresses errors if the directory already exists, without overwriting that directory.

It will take about 15 minutes to download the files.

Bash

```
mkdir -p ~/dc_workshop/data/untrimmed_fastq/
cd ~/dc_workshop/data/untrimmed_fastq

curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/004/SRR2589044/SRR2589044_1.fastq.gz
curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/004/SRR2589044/SRR2589044_2.fastq.gz
curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/003/SRR2584863/SRR2584863_1.fastq.gz
curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/003/SRR2584863/SRR2584863_2.fastq.gz
curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/006/SRR2584866/SRR2584866_1.fastq.gz
curl -O ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR258/006/SRR2584866/SRR2584866_2.fastq.gz
```

📌 Faster option

If your workshop is short on time or the venue's internet connection is weak or unstable, learners can avoid needing to download the data and instead use the data files provided in the `.backup/` directory.

Bash

```
$ cp ~/.backup/untrimmed_fastq/*fastq.gz .
```

This command creates a copy of each of the files in the `.backup/untrimmed_fastq/` directory that end in `fastq.gz` and places the copies in the current working directory (signified by `.`).

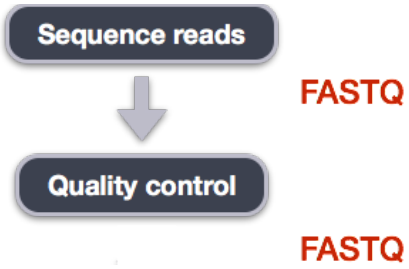
The data comes in a compressed format, which is why there is a `.gz` at the end of the file names. This makes it faster to transfer, and allows it to take up less space on our computer. Let's unzip one of the files so that we can look at the fastq format.

Bash

```
$ gunzip SRR2584863_1.fastq.gz
```

Quality Control

We will now assess the quality of the sequence reads contained in our fastq files.



Details on the FASTQ format

Although it looks complicated (and it is), we can understand the fastq (https://en.wikipedia.org/wiki/FASTQ_format) format with a little decoding. Some rules about the format include…

Line	Description
1	Always begins with '@' and then information about the read
2	The actual DNA sequence
3	Always begins with a '+' and sometimes the same info in line 1
4	Has a string of characters which represent the quality scores; must have same number of characters as line 2

We can view the first complete read in one of the files our dataset by using `head` to look at the first four lines.

Bash

```
$ head -n 4 SRR2584863_1.fastq
```

Output

```
@SRR2584863.1 HWI-ST957:244:H73TDADXX:1:1101:4712:2181/1
TTCACATCCTGACCATTCAGTTGAGCAAAATAGTTCTTCAGTGCCTGTTTAACCGAGTCACGCAGGGGTTTTTGGGTTACCTGATCCTGAGAGTTAACGGTAGAAACGGTCAGTACGTCAGAATTTACGCGTTGTTC
GAACATAGTTCTG
+
CCCFFFFFGHHHHJIJJJJIJJJIIJJJJIIIJJGFIIIJEDDFEGGJIFHHJIJJDECCGGEGIIJFHFFFACD:BBBDDACCCCAA@@CA@C>C3>@5(8&>C:9?8+89<4(:83825C(:A#############
#############
```

Line 4 shows the quality for each nucleotide in the read. Quality is interpreted as the probability of an incorrect base call (e.g. 1 in 10) or, equivalently, the base call accuracy (e.g. 90%). To make it possible to line up each individual nucleotide with its quality score, the numerical score is converted into a code where each individual character represents the numerical quality score for an individual nucleotide. For example, in the line above, the quality score line is:

Output

```
CCCFFFFFGHHHHJIJJJJIJJJIIJJJJIIIJJGFIIIJEDDFEGGJIFHHJIJJDECCGGEGIIJFHFFFACD:BBBDDACCCCAA@@CA@C>C3>@5(8&>C:9?8+89<4(:83825C(:A#############
#############
```

The numerical value assigned to each of these characters depends on the sequencing platform that generated the reads. The sequencing machine used to generate our data uses the standard Sanger quality PHRED score encoding, using Illumina version 1.8 onwards. Each character is assigned a quality score between 0 and 41 as shown in the chart below.

Output

```
Quality encoding: !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJ
                  |         |         |         |         |
Quality score:    01........11........21........31........41
```

Each quality score represents the probability that the corresponding nucleotide call is incorrect. This quality score is logarithmically based, so a quality score of 10 reflects a base call accuracy of 90%, but a quality score of 20 reflects a base call accuracy of 99%. These probability values are the results from the base calling algorithm and depend on how much signal was captured for the base incorporation.

Looking back at our read:

Output

```
@SRR2584863.1 HWI-ST957:244:H73TDADXX:1:1101:4712:2181/1
TTCACATCCTGACCATTCAGTTGAGCAAAATAGTTCTTCAGTGCCTGTTTAACCGAGTCACGCAGGGGTTTTTGGGTTACCTGATCCTGAGAGTTAACGGTAGAAACGGTCAGTACGTCAGAATTTACGCGTTGTTC
GAACATAGTTCTG
+
CCCFFFFFGHHHHJIJJJJIJJJIIJJJJIIIJJGFIIIJEDDFEGGJIFHHJIJJDECCGGEGIIJFHFFFACD:BBBDDACCCCAA@@CA@C>C3>@5(8&>C:9?8+89<4(:83825C(:A############
############
```

we can now see that there is a range of quality scores, but that the end of the sequence is very poor (`#` = a quality score of 2).

Exercise

What is the last read in the `SRR2584863_1.fastq` file? How confident are you in this read?

Solution

Bash

```
$ tail -n 4 SRR2584863_1.fastq
```

Output

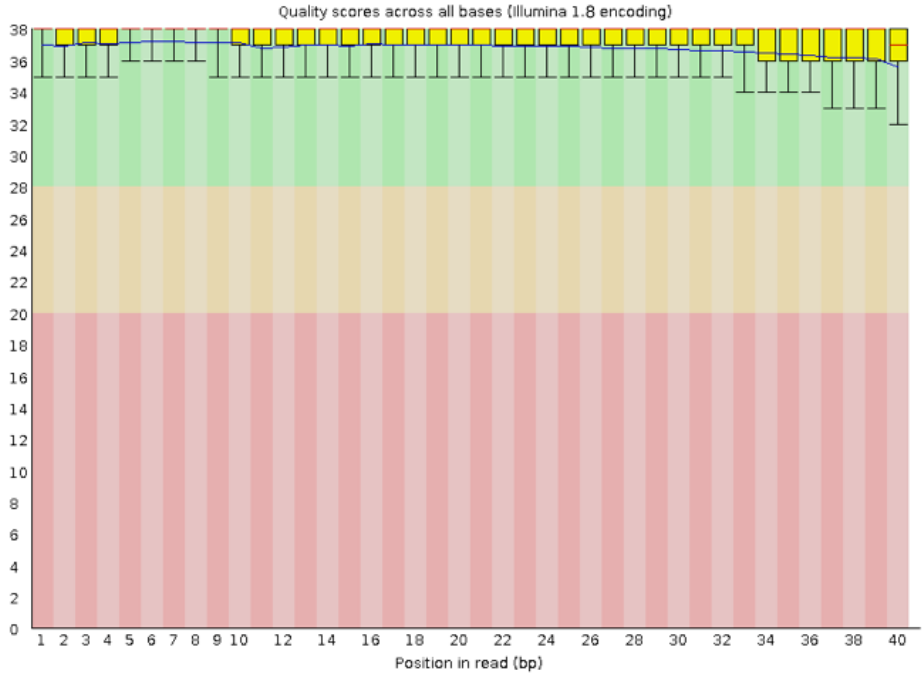
```
@SRR2584863.1553259 HWI-ST957:245:H73R4ADXX:2:2216:21048:100894/1
CTGCAATACCACGCTGATCTTTCACATGATGTAAGAAAAGTGGGATCAGCAAACCGGGTGCTGCTGTGGCTAGTTGCAGCAAACCATGCAGTGAACCCGCCTGTGCTTCGCTATAGCCGTGACTGATGAGGATC
GCCGGAAGCCAGCCAA
+
CCCFFFFFHHHHGJJJJJJJJJHGIJJJIJJJJIJJJJIIIIJJJJJJJJJJJJJJIIJJJHHHHHFFFFFEEEEEDDDDDDDDDDDDDDDDCDEDDBDBDDBDDDDDDDDDBDEEDDDD7@BDDDDDD>AA>?
B?<@BDD@BDC?BDA?
```

This read has more consistent quality at its end than the first read that we looked at, but still has a range of quality scores, most of them high. We will look at variations in position-based quality in just a moment.

Assessing Quality using FastQC

In real life, you won't be assessing the quality of your reads by visually inspecting your FASTQ files. Rather, you'll be using a software program to assess read quality and filter out poor quality reads. We'll first use a program called FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to visualize the quality of our reads. Later in our workflow, we'll use another program to filter out poor quality reads.

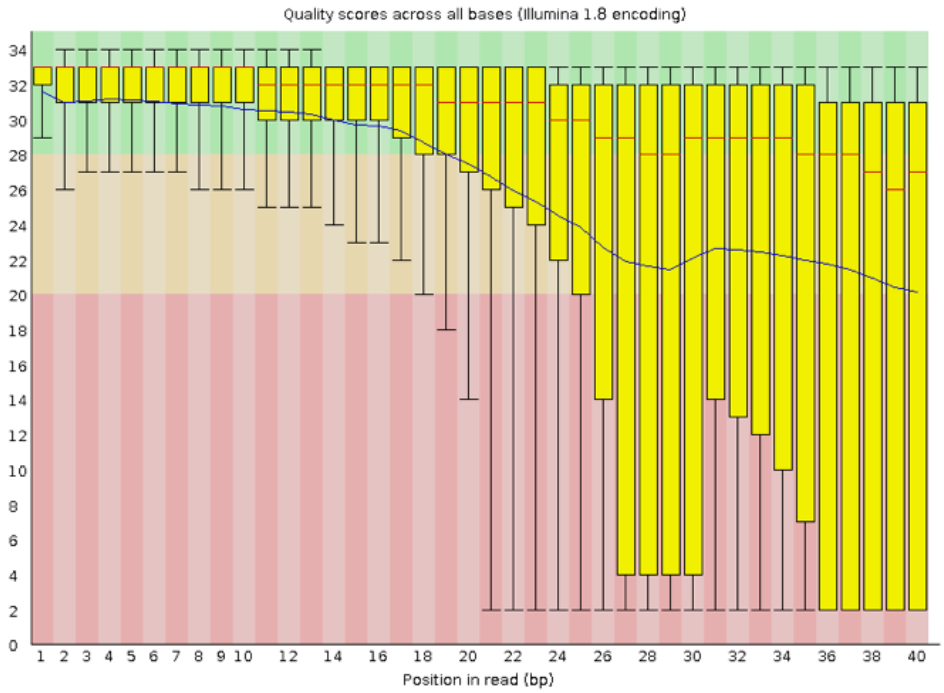
FastQC has a number of features which can give you a quick impression of any problems your data may have, so you can take these issues into consideration before moving forward with your analyses. Rather than looking at quality scores for each individual read, FastQC looks at quality collectively across all reads within a sample. The image below shows one FastQC-generated plot that indicates a very high quality sample:



The x-axis displays the base position in the read, and the y-axis shows quality scores. In this example, the sample contains reads that are 40 bp long. This is much shorter than the reads we are working with in our workflow. For each position, there is a box-and-whisker plot showing the distribution of quality scores for all reads at that position. The horizontal red line indicates the median quality score and the yellow box shows the 1st to 3rd quartile range. This means that 50% of reads have a quality score that falls within the range of the yellow box at that position. The whiskers show the absolute range, which covers the lowest (0th quartile) to highest (4th quartile) values.

For each position in this sample, the quality values do not drop much lower than 32. This is a high quality score. The plot background is also color-coded to identify good (green), acceptable (yellow), and bad (red) quality scores.

Now let's take a look at a quality plot on the other end of the spectrum.



Here, we see positions within the read in which the boxes span a much wider range. Also, quality scores drop quite low into the "bad" range, particularly on the tail end of the reads. The FastQC tool produces several other diagnostic plots to assess sample quality, in addition to the one plotted above.

Running FastQC

We will now assess the quality of the reads that we downloaded. First, make sure you're still in the `untrimmed_fastq` directory

Bash

```
$ cd ~/dc_workshop/data/untrimmed_fastq/
```

Exercise

How big are the files? (Hint: Look at the options for the `ls` command to see how to show file sizes.)

Solution

Bash

```
$ ls -l -h
```

Output

```
-rw-rw-r-- 1 dcuser dcuser 545M Jul  6 20:27 SRR2584863_1.fastq
-rw-rw-r-- 1 dcuser dcuser 183M Jul  6 20:29 SRR2584863_2.fastq.gz
-rw-rw-r-- 1 dcuser dcuser 309M Jul  6 20:34 SRR2584866_1.fastq.gz
-rw-rw-r-- 1 dcuser dcuser 296M Jul  6 20:37 SRR2584866_2.fastq.gz
-rw-rw-r-- 1 dcuser dcuser 124M Jul  6 20:22 SRR2589044_1.fastq.gz
-rw-rw-r-- 1 dcuser dcuser 128M Jul  6 20:24 SRR2589044_2.fastq.gz
```

There are six FASTQ files ranging from 124M (124MB) to 545M.

FastQC can accept multiple file names as input, and on both zipped and unzipped files, so we can use the *.fastq* wildcard to run FastQC on all of the FASTQ files in this directory.

Bash

```
$ fastqc *.fastq*
```

You will see an automatically updating output message telling you the progress of the analysis. It will start like this:

Output

```
Started analysis of SRR2584863_1.fastq
Approx 5% complete for SRR2584863_1.fastq
Approx 10% complete for SRR2584863_1.fastq
Approx 15% complete for SRR2584863_1.fastq
Approx 20% complete for SRR2584863_1.fastq
Approx 25% complete for SRR2584863_1.fastq
Approx 30% complete for SRR2584863_1.fastq
Approx 35% complete for SRR2584863_1.fastq
Approx 40% complete for SRR2584863_1.fastq
Approx 45% complete for SRR2584863_1.fastq
```

In total, it should take about five minutes for FastQC to run on all six of our FASTQ files. When the analysis completes, your prompt will return. So your screen will look something like this:

Output

```
Approx 80% complete for SRR2589044_2.fastq.gz
Approx 85% complete for SRR2589044_2.fastq.gz
Approx 90% complete for SRR2589044_2.fastq.gz
Approx 95% complete for SRR2589044_2.fastq.gz
Analysis complete for SRR2589044_2.fastq.gz
$
```

The FastQC program has created several new files within our `data/untrimmed_fastq/` directory.

Bash

```
$ ls
```

Output

```
SRR2584863_1.fastq        SRR2584866_1_fastqc.html  SRR2589044_1_fastqc.html
SRR2584863_1_fastqc.html  SRR2584866_1_fastqc.zip   SRR2589044_1_fastqc.zip
SRR2584863_1_fastqc.zip   SRR2584866_1.fastq.gz     SRR2589044_1.fastq.gz
SRR2584863_2_fastqc.html  SRR2584866_2_fastqc.html  SRR2589044_2_fastqc.html
SRR2584863_2_fastqc.zip   SRR2584866_2_fastqc.zip   SRR2589044_2_fastqc.zip
SRR2584863_2.fastq.gz     SRR2584866_2.fastq.gz     SRR2589044_2.fastq.gz
```

For each input FASTQ file, FastQC has created a `.zip` file and a `.html` file. The `.zip` file extension indicates that this is actually a compressed set of multiple output files. We'll be working with these output files soon. The `.html` file is a stable webpage displaying the summary report for each of our samples.

We want to keep our data files and our results files separate, so we will move these output files into a new directory within our `results/` directory.

Bash

```
$ mkdir -p ~/dc_workshop/results/fastqc_untrimmed_reads
$ mv *.zip ~/dc_workshop/results/fastqc_untrimmed_reads/
$ mv *.html ~/dc_workshop/results/fastqc_untrimmed_reads/
```

Now we can navigate into this results directory and do some closer inspection of our output files.

Bash

```
$ cd ~/dc_workshop/results/fastqc_untrimmed_reads/
```

Viewing the FastQC results

If we were working on our local computers, we'd be able to display each of these HTML files as a webpage:

Bash

```
$ open SRR2584863_1_fastqc.html
```

However, if you try this on our AWS instance, you'll get an error:

Output

```
Couldn't get a file descriptor referring to the console
```

This is because the AWS instance we're using doesn't have any web browsers installed on it, so the remote computer doesn't know how to open the file. We want to look at the webpage summary reports, so let's transfer them to our local computers (i.e. your laptop).

To transfer a file from a remote server to our own machines, we will use `scp`, which we learned yesterday in the Shell Genomics lesson.

First we will make a new directory on our computer to store the HTML files we're transfering. Let's put it on our desktop for now. Open a new tab in your terminal program (you can use the pull down menu at the top of your screen or the Cmd+t keyboard shortcut) and type:

Bash

```
$ mkdir -p ~/Desktop/fastqc_html
```

Now we can transfer our HTML files to our local computer using `scp`.

Bash

```
$ scp dcuser@ec2-34-238-162-94.compute-1.amazonaws.com:~/dc_workshop/results/fastqc_untrimmed_reads/*.html ~/Desktop/fastqc_html
```

As a reminder, the first part of the command `dcuser@ec2-34-238-162-94.compute-1.amazonaws.com` is the address for your remote computer. Make sure you replace everything after `dcuser@` with your instance number (the one you used to log in).

The second part starts with a `:` and then gives the absolute path of the files you want to transfer from your remote computer. Don't forget the `:`. We used a wildcard (`*.html`) to indicate that we want all of the HTML files.

The third part of the command gives the absolute path of the location you want to put the files. This is on your local computer and is the directory we just created `~/Desktop/fastqc_html` .

You should see a status output like this:

Output

```
SRR2584863_1_fastqc.html                    100%  249KB 152.3KB/s   00:01
SRR2584863_2_fastqc.html                    100%  254KB 219.8KB/s   00:01
SRR2584866_1_fastqc.html                    100%  254KB 271.8KB/s   00:00
SRR2584866_2_fastqc.html                    100%  251KB 252.8KB/s   00:00
SRR2589044_1_fastqc.html                    100%  249KB 370.1KB/s   00:00
SRR2589044_2_fastqc.html                    100%  251KB 592.2KB/s   00:00
```

Now we can go to our new directory and open the HTML files.

Bash

```
$ cd ~/Desktop/fastqc_html/
$ open *.html
```

Your computer will open each of the HTML files in your default web browser. Depending on your settings, this might be as six separate tabs in a single window or six separate browser windows.

Exercise

Discuss your results with a neighbor. Which sample(s) looks the best in terms of per base sequence quality? Which sample(s) look the worst?

Solution

All of the reads contain usable data, but the quality decreases toward the end of the reads.

Decoding the other FastQC outputs

We've now looked at quite a few "Per base sequence quality" FastQC graphs, but there are nine other graphs that we haven't talked about! Below we have provided a brief overview of interpretations for each of these plots. For more information, please see the FastQC documentation here (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/)

- **Per tile sequence quality** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/12%20Per%20Tile%20Sequence%20Quality.html): the machines that perform sequencing are divided into tiles. This plot displays patterns in base quality along these tiles. Consistently low scores are often found around the edges, but hot spots can also occur in the middle if an air bubble was introduced at some point during the run.
- **Per sequence quality scores** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/3%20Per%20Sequence%20Quality%20Scores.html): a density plot of quality for all reads at all positions. This plot shows what quality scores are most common.
- **Per base sequence content** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/4%20Per%20Base%20Sequence%20Content.html): plots the proportion of each base position over all of the reads. Typically, we expect to see each base roughly 25% of the time at each position, but this often fails at the beginning or end of the read due to quality or adapter content.
- **Per sequence GC content** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/5%20Per%20Sequence%20GC%20Content.html): a density plot of average GC content in each of the reads.
- **Per base N content** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/6%20Per%20Base%20N%20Content.html): the percent of times that 'N' occurs at a position in all reads. If there is an increase at a particular position, this might indicate that something went wrong during sequencing.

- **Sequence Length Distribution** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/7%20Sequence%20Length%20Distribution.html): the distribution of sequence lengths of all reads in the file. If the data is raw, there is often on sharp peak, however if the reads have been trimmed, there may be a distribution of shorter lengths.
- **Sequence Duplication Levels** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/8%20Duplicate%20Sequences.html): A distribution of duplicated sequences. In sequencing, we expect most reads to only occur once. If some sequences are occurring more than once, it might indicate enrichment bias (e.g. from PCR). If the samples are high coverage (or RNA-seq or amplicon), this might not be true.
- **Overrepresented sequences** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/9%20Overrepresented%20Sequences.html): A list of sequences that occur more frequently than would be expected by chance.
- **Adapter Content** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/10%20Adapter%20Content.html): a graph indicating where adapater sequences occur in the reads.
- **K-mer Content** (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/3%20Analysis%20Modules/11%20Kmer%20Content.html): a graph showing any sequences which may show a positional bias within the reads.

Working with the FastQC text output

Now that we've looked at our HTML reports to get a feel for the data, let's look more closely at the other output files. Go back to the tab in your terminal program that is connected to your AWS instance (the tab label will start with `dcuser@ip`) and make sure you're in our results subdirectory.

Bash

```
$ cd ~/dc_workshop/results/fastqc_untrimmed_reads/
$ ls
```

Output

```
SRR2584863_1_fastqc.html  SRR2584866_1_fastqc.html  SRR2589044_1_fastqc.html
SRR2584863_1_fastqc.zip   SRR2584866_1_fastqc.zip   SRR2589044_1_fastqc.zip
SRR2584863_2_fastqc.html  SRR2584866_2_fastqc.html  SRR2589044_2_fastqc.html
SRR2584863_2_fastqc.zip   SRR2584866_2_fastqc.zip   SRR2589044_2_fastqc.zip
```

Our `.zip` files are compressed files. They each contain multiple different types of output files for a single input FASTQ file. To view the contents of a `.zip` file, we can use the program `unzip` to decompress these files. Let's try doing them all at once using a wildcard.

Bash

```
$ unzip *.zip
```

Output

```
Archive:  SRR2584863_1_fastqc.zip
caution: filename not matched:  SRR2584863_2_fastqc.zip
caution: filename not matched:  SRR2584866_1_fastqc.zip
caution: filename not matched:  SRR2584866_2_fastqc.zip
caution: filename not matched:  SRR2589044_1_fastqc.zip
caution: filename not matched:  SRR2589044_2_fastqc.zip
```

This didn't work. We unzipped the first file and then got a warning message for each of the other `.zip` files. This is because `unzip` expects to get only one zip file as input. We could go through and unzip each file one at a time, but this is very time consuming and error-prone. Someday you may have 500 files to unzip!

A more efficient way is to use a `for` loop like we learned in the Shell Genomics lesson to iterate through all of our `.zip` files. Let's see what that looks like and then we'll discuss what we're doing with each line of our loop.

Bash

```
$ for filename in *.zip
> do
> unzip $filename
> done
```

In this example, the input is six filenames (one filename for each of our `.zip` files). Each time the loop iterates, it will assign a file name to the variable `filename` and run the `unzip` command. The first time through the loop, `$filename` is `SRR2584863_1_fastqc.zip`. The interpreter runs the command `unzip` on `SRR2584863_1_fastqc.zip`. For the second iteration, `$filename` becomes `SRR2584863_2_fastqc.zip`. This time, the shell runs `unzip` on `SRR2584863_2_fastqc.zip`. It then repeats this process for the four other `.zip` files in our directory.

When we run our `for` loop, you will see output that starts like this:

Output

```
Archive:  SRR2589044_2_fastqc.zip
   creating: SRR2589044_2_fastqc/
   creating: SRR2589044_2_fastqc/Icons/
   creating: SRR2589044_2_fastqc/Images/
  inflating: SRR2589044_2_fastqc/Icons/fastqc_icon.png
  inflating: SRR2589044_2_fastqc/Icons/warning.png
  inflating: SRR2589044_2_fastqc/Icons/error.png
  inflating: SRR2589044_2_fastqc/Icons/tick.png
  inflating: SRR2589044_2_fastqc/summary.txt
  inflating: SRR2589044_2_fastqc/Images/per_base_quality.png
  inflating: SRR2589044_2_fastqc/Images/per_tile_quality.png
  inflating: SRR2589044_2_fastqc/Images/per_sequence_quality.png
  inflating: SRR2589044_2_fastqc/Images/per_base_sequence_content.png
  inflating: SRR2589044_2_fastqc/Images/per_sequence_gc_content.png
  inflating: SRR2589044_2_fastqc/Images/per_base_n_content.png
  inflating: SRR2589044_2_fastqc/Images/sequence_length_distribution.png
  inflating: SRR2589044_2_fastqc/Images/duplication_levels.png
  inflating: SRR2589044_2_fastqc/Images/adapter_content.png
  inflating: SRR2589044_2_fastqc/fastqc_report.html
  inflating: SRR2589044_2_fastqc/fastqc_data.txt
  inflating: SRR2589044_2_fastqc/fastqc.fo
```

The `unzip` program is decompressing the `.zip` files and creating a new directory (with subdirectories) for each of our samples, to store all of the different output that is produced by FastQC. There are a lot of files here. The one we're going to focus on is the `summary.txt` file.

If you list the files in our directory now you will see:

```
SRR2584863_1_fastqc       SRR2584866_1_fastqc       SRR2589044_1_fastqc
SRR2584863_1_fastqc.html  SRR2584866_1_fastqc.html  SRR2589044_1_fastqc.html
SRR2584863_1_fastqc.zip   SRR2584866_1_fastqc.zip   SRR2589044_1_fastqc.zip
SRR2584863_2_fastqc       SRR2584866_2_fastqc       SRR2589044_2_fastqc
SRR2584863_2_fastqc.html  SRR2584866_2_fastqc.html  SRR2589044_2_fastqc.html
SRR2584863_2_fastqc.zip   SRR2584866_2_fastqc.zip   SRR2589044_2_fastqc.zip
```

The `.html` files and the uncompressed `.zip` files are still present, but now we also have a new directory for each of our samples. We can see for sure that it's a directory if we use the `-F` flag for `ls`.

Bash

```
$ ls -F
```

Output

```
SRR2584863_1_fastqc/      SRR2584866_1_fastqc/      SRR2589044_1_fastqc/
SRR2584863_1_fastqc.html  SRR2584866_1_fastqc.html  SRR2589044_1_fastqc.html
SRR2584863_1_fastqc.zip   SRR2584866_1_fastqc.zip   SRR2589044_1_fastqc.zip
SRR2584863_2_fastqc/      SRR2584866_2_fastqc/      SRR2589044_2_fastqc/
SRR2584863_2_fastqc.html  SRR2584866_2_fastqc.html  SRR2589044_2_fastqc.html
SRR2584863_2_fastqc.zip   SRR2584866_2_fastqc.zip   SRR2589044_2_fastqc.zip
```

Let's see what files are present within one of these output directories.

Bash

```
$ ls -F SRR2584863_1_fastqc/
```

Output

```
fastqc_data.txt  fastqc.fo  fastqc_report.html  Icons/  Images/  summary.txt
```

Use `less` to preview the `summary.txt` file for this sample.

Bash

```
$ less SRR2584863_1_fastqc/summary.txt
```

Output

```
PASS    Basic Statistics        SRR2584863_1.fastq
PASS    Per base sequence quality       SRR2584863_1.fastq
PASS    Per tile sequence quality       SRR2584863_1.fastq
PASS    Per sequence quality scores     SRR2584863_1.fastq
WARN    Per base sequence content       SRR2584863_1.fastq
WARN    Per sequence GC content SRR2584863_1.fastq
PASS    Per base N content      SRR2584863_1.fastq
PASS    Sequence Length Distribution    SRR2584863_1.fastq
PASS    Sequence Duplication Levels     SRR2584863_1.fastq
PASS    Overrepresented sequences       SRR2584863_1.fastq
WARN    Adapter Content SRR2584863_1.fastq
```

The summary file gives us a list of tests that FastQC ran, and tells us whether this sample passed, failed, or is borderline (`WARN`). Remember, to quit from `less` you must type `q` .

Documenting Our Work

We can make a record of the results we obtained for all our samples by concatenating all of our `summary.txt` files into a single file using the `cat` command. We'll call this `fastqc_summaries.txt` and move it to `~/dc_workshop/docs` .

Bash

```
$ cat */summary.txt > ~/dc_workshop/docs/fastqc_summaries.txt
```

Exercise

Which samples failed at least one of FastQC's quality tests? What test(s) did those samples fail?

Solution

We can get the list of all failed tests using `grep` .

Bash

```
$ cd ~/dc_workshop/docs
$ grep FAIL fastqc_summaries.txt
```

Output

```
FAIL    Per base sequence quality       SRR2584863_2.fastq.gz
FAIL    Per tile sequence quality       SRR2584863_2.fastq.gz
FAIL    Per base sequence content       SRR2584863_2.fastq.gz
FAIL    Per base sequence quality       SRR2584866_1.fastq.gz
FAIL    Per base sequence content       SRR2584866_1.fastq.gz
FAIL    Adapter Content SRR2584866_1.fastq.gz
FAIL    Adapter Content SRR2584866_2.fastq.gz
FAIL    Adapter Content SRR2589044_1.fastq.gz
FAIL    Per base sequence quality       SRR2589044_2.fastq.gz
FAIL    Per tile sequence quality       SRR2589044_2.fastq.gz
FAIL    Per base sequence content       SRR2589044_2.fastq.gz
FAIL    Adapter Content SRR2589044_2.fastq.gz
```

Other notes – Optional

Quality Encodings Vary

Although we've used a particular quality encoding system to demonstrate interpretation of read quality, different sequencing machines use different encoding systems. This means that, depending on which sequencer you use to generate your data, a `#` may not be an indicator of a poor quality base call.

This mainly relates to older Solexa/Illumina data, but it's essential that you know which sequencing platform was used to generate your data, so that you can tell your quality control program which encoding to use. If you choose the wrong encoding, you run the risk of throwing away good reads or (even worse) not throwing away bad reads!

Same Symbols, Different Meanings

Here we see `>` being used as a shell prompt, whereas `>` is also used to redirect output. Similarly, `$` is used as a shell prompt, but, as we saw earlier, it is also used to ask the shell to get the value of a variable.

If the *shell* prints `>` or `$` then it expects you to type something, and the symbol is a prompt.

If *you* type `>` or `$` yourself, it is an instruction from you that the shell should redirect output or get the value of a variable.

Key Points

- Quality encodings vary across sequencing platforms.
- `for` loops let you perform the same set of operations on multiple files with a single command.

< (../01-background/index.html)

> (../03-trimmi