

BioType: A crash course in bioinformatics and custom pipelines

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May 2018

Todo list

Talk about the prior biology knowledge people should have	4
Still need to add information about the inflammatory bowel disease dataset	5
Make URLs footnotes instead of appendix entries	5
Add further software that you end up using (e.g., USEARCH). ALSO, make sure to update the section that mentions installing packages if you do so.	12
write about installing qiime2 https://docs.qiime2.org/2018.4/install/ native/	12
write about installing parallel-fastq-dump	12
write about installing multifastqc	12
add 3rd column to table with link to documentation/download source	12
Write a blurb explaining the benefits of using a virtual environment. .	14
Link to resource for further reading on virtual environments	14
Write blurb about what packages are.	14
Link to resource for further reading about packages.	14
Solve the issue with failing to center figures when they're on their own page	15
Talk about setting up the sra-tools workspace (https://trace.ncbi. nlm.nih.gov/Traces/sra/sra.cgi?view=toolkit_doc&f=std)	15
PYPIPER IS ONLY COMPATIBLE WITH MAC AND LINUX. Start by coding with just subprocess module commands. If the scripts work on Windows computers, then forget about using pypiper. But if the subprocess scripts don't work on windows, then we'll be developing exclusively for Mac anyways, so you could use pypiper without any worries. In that case, talk about installing pypiper here. OTHERWISE, delete this section.	15
show image of what the prompt looks like when it's done initializing. .	15
Reference the figure pypiper-already-installed	17
Figure: pypiper-already-installed	17
reference figure pypiper-wrong-location	17
Figure: pypiper-wrong-location	17
Talk about choosing between PyCharm and other options	18
(Talk about putting all the tools in the same path/directory)	18

****Show the user what the result of the commands are. Right now we show the commands, but not the results. e.g., when we run a command to get a list of accession numbers, follow it up by typing the command that just prints the list of numbers in the terminal. Then the user can see what their results should look like if they did everything correctly.	19
Figure: highlight where to click to download the runinfo table	20
Either shorten this margin-label, or put it in a footnote.	22
Give a specific name to the RunInfo Table file. People might get confused if they needs to fill in the name of the file themselves. .	22
Find resource for explaining the .sra file format that we download from the SRA Database	33

Contents

1	Foreword	3
1.1	Goal	3
1.2	How to use this book	3
1.3	Source Code	4
1.4	Pre-requisites	4
2	Sample Information	5
3	Setting the scene	6
4	Microbiome Analysis	7
4.1	The Gut Microbiome	7
4.2	Relative Abundance Analysis	7
4.3	Metagenomics	8
4.4	Python	9
5	How to Find Tools	10
5.1	Finding Data	10
5.2	Finding Software	10
6	The Plan	11
7	Software and Set-up	12
7.1	Software	12
7.2	Set-up and Install Dependencies	12
7.2.1	Install Anaconda	12
7.2.2	Create a New Virtual Environment	14
7.2.3	Install packages	14
7.2.4	Integrated Development Environment (IDE)	18
7.2.5	PATH	18
7.3	Analysis Pipeline	18

8 The Dataset	19
8.1 Download Dataset	19
8.1.1 Creating the code	24
8.2 Perform Quality Control on Dataset	24
9 Relative Abundance Analysis	25
10 Predict ORFs	26
11 Create Non-redundant Gene Sets	27
12 Align Genes	28
13 Get GenBank Accession Numbers	29
14 Find COG Functional Classes	30
15 New Analysis	31
A Web Resources and Explanations	32

1 Foreword

1.1 Goal

This tutorial aims to improve your general understanding of bioinformatics through several methods:

- Define technical terms commonly used in bioinformatics methods and found in the literature.
- Provide a collection of various useful resources, including...
 1. Resources for finding tools, data, and background information that can help answer your research questions.
 2. Resources that explain details about bioinformatics concepts and techniques in beginner-friendly language.
- Demonstrate how Python can be used to answer your research questions by combining existing bioinformatics tools and automating repetitive or time-consuming tasks.

1.2 How to use this book

Most reference materials are consolidated in the appendices at the end of the book. The main text of this book is written in the right-hand margin. The left-hand margin contains special markers and important notes to the reader. The book can be used as a self-paced tutorial with the help of the markers described below.

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! → The arrow that points to this line is an "Attention" marker. It will indicate key pieces of information that you should pay special attention to. *Curabitur egestas aliquam nisl, pharetra finibus mauris placerat nec. Nam in diam risus. Nulla mollis purus quis feugiat tristique. Vestibulum et sollicitudin ante, at sagittis ipsum. Nunc hendrerit ante sed massa semper eleifend.*

This is a margin label. I will write things here to further explain the main text, define jargon, etc.

→ This kind of annotation will reference appendix entries that you can consult for more-detailed information about the main text.

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1.3 Source Code

The source code for this manual, the accompanying tutorial, and the BioPype package can all be found at github.com/EthanGniot/BioPype.

1.4 Pre-requisites

Things to know

Talk about the prior biology knowledge people should have

Computer requirements

- At least 4GB RAM bare minimum, 16GB RAM if possible (the more RAM, the better)
- The BioPype package was designed to be as operating-system-agnostic as possible. That being said, all tests and analyses from this tutorial were performed on a MacBook Pro (Retina, 13-inch, Late 2013) running macOS 10.13.4 High Sierra. Compatibility with other operating systems have not yet been tested.
- Storage requirements will vary depending on the amount of data you intend to analyze. The analysis performed in this tutorial required at least 500GB and were satisfied by using a 2TB external hard drive.

2 Sample Information

Still need to add information about the inflammatory bowel disease dataset

While we are building the BioType pipeline, we will need a dataset that we can use to test our pipeline throughout the process and make sure it is working as intended. In order to do this, we must use a dataset that's already been analyzed so that we know what the results should look like. When we test our pipeline, if our results match the results of the original analysis, then we will know that our tool is working correctly.

There are several test datasets available to us for testing the feature that analyzes the relative abundance of bacteria in the microbiota.

Make URLs footnotes instead of appendix entries

The first is the dataset used in both the QIIME "Illumina Overview Tutorial" (A.1) and the QIIME 2 "Moving Pictures" tutorial (A.2) derived from the *Moving Pictures of the Human Microbiome* study, where two human subjects collected daily samples from four body sites: the tongue, the palm of the left hand, the palm of the right hand, and the gut (via fecal samples obtained by swapping used toilet paper) Caporaso et al. (2011a). These data were sequenced using the barcoded amplicon sequencing protocol described in *Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample* Caporaso et al. (2011b). A more recent version of this protocol that can be used with the Illumina HiSeq 2000 and MiSeq can be found here.

(Here is information about the untested inflammatory bowel disease dataset that we will analyze using the completed pipeline)

3 Setting the scene

("Here" is a hypothetical situation/research question that a student may have. This is the research question that will be answered by the pipeline we are creating.)

4 Microbiome Analysis

The analysis used in this tutorial will focus on analysis of the human gut microbiome, but BioPype can be used to analyze the microbiomes of other communities as well.

4.1 The Gut Microbiome

→ For more explanation about the **gut microbiota** and **gut microbiome**, see A.3

The human gut is home to trillions of microbes. The collection of physical micro-organisms that live in the gut is referred to as the **gut microbiota**. The **gut microbiome** refers to all of the genes contained within these micro-organisms, as well as their functions and interactions. .

Sometimes the biodiversity of the gut is thrown out of balance, with bacteria that normally have a high relative abundance being underrepresented, while bacteria that normally have a low relative abundance are overrepresented. This phenomenon is referred to as **dysbiosis**. Gut dysbiosis is important to study because it has been found to be associated with a wide range of disorders that manifest in various areas of the body, including diabetes, rheumatoid arthritis, Alzheimer's, inflammatory bowel disease, colon cancer (Buford, 2017; Kennedy et al., 2014; Castellarin et al., 2012), chronic fatigue syndrome (Lakhan and Kirchgessner, 2010), obesity (Turnbaugh et al., 2006, 2009), bacterial vaginosis (Africa et al., 2014), ulcerative colitis (Mandal et al., 2015a; Matsuoka and Kanai, 2015), anxiety, and depression (Evrensel and Ceylan, 2015; Lach et al., 2018)

4.2 Relative Abundance Analysis

Relative abundance is a measure of how common or rare a taxon is, relative to other taxa in its community, and is usually expressed as a percentage. For more information on **relative abundance** analyses, see Appendix A.4.

→ For information on **amplicon** sequencing vs. shotgun sequencing, see Appendix A.5

Operational Taxonomic Unit (OTU): A group of sequences that share significant similarity based on some marker gene. Groups can be treated as a single taxon (e.g., genus, species, etc. depending on the clustering threshold) for analysis purposes. For more info, see Appendix A.6

BioPype lets users examine the **relative abundance** of bacterial taxa in each experimental sample. Relative abundance analyses help visualize the structure of the micro-organism community. Users can use BioPype to analyze bacterial **amplicon** sequencing data to determine which micro-organisms are present and in what proportion. . The idea is to take the sequences and assign them to a taxon. To do that, we group (or cluster) sequences based on their similarity to define **Operational Taxonomic Units (OTUs)**. Using the number of sequences assigned to each OTU/taxon, BioPype can determine the relative abundance of each micro-organism in a sample and plot the results on an interactive bar chart.

For example, studies have found that the genera *Bacteroides* and *Lactobacillus* are both prevalent in the healthy human gut microbiome, but *Bacteroides* has a higher relative abundance than *Lactobacillus* Lloyd-Price et al. (2016). In other words, *Bacteroides* makes up a greater percentage of all the bacteria in the gut than *Lactobacillus* does.

Relative abundance is an important measure of biodiversity in the gut. BioPype can be used to compare the relative abundance of bacteria at different taxonomic levels, which helps users identify instances of gut dysbiosis in sample microbiomes.

4.3 Metagenomics

! → *THIS FUNCTIONALITY HAS NOT BEEN COMPLETED YET, BUT WILL BE ADDED IN FUTURE UPDATES*

BioPype can also be used to predict which genes are present in microbiome samples. From these predicted genes, users can do two things.

First, they can generalize the functions encoded across all bacteria that are present in an experimental condition's microbiome (i.e., at the metagenomic scale) and visualize the relative distribution of these functions with a bar chart. Having this functional snapshot of a metagenome is useful because a functional overview of the experimental condition can be compared to a functional overview of the control condition to see if there are differences in the two groups' functional capabilities. When comparing the microbiomes of disordered vs. non-disordered samples, having a visual depiction of the differences in functional capabilities can make it easier to find the cause of the disorder by narrowing down the potential sources of the condition.

For example, certain G protein-coupled receptors (GPCRs) in the gut are associated with some of the diseases associated with gut dysbiosis, such as inflammatory bowel disease (IBD) (Cohen et al., 2017). Research has also shown that bacteria interact with their environment via the release of small molecules (Li and Tian, 2012). From these premises, one could hypothesize that IBD may be caused by the over-production of certain effector molecules from the gut microbiome. This hypothesis may be tested by using BioPype to compare the functional capabilities of the IBD microbiome with that of a healthy control microbiome. If the results show, for example, that the IBD gut metagenome has a greater proportion of genes associated with lipid production than the control metagenome, it indicates potential support for the user's hypothesis. They can now refine their investigation by narrowing the scope of their search to only analyze genes associated with lipid synthesis.

Second, users can use BioPype to predict the functions of *individual* genes. This is useful because it allows users to identify a specific gene of interest for their research question, which they can then design a validation experiment for.

Continuing with our above example hypothesis about the gut microbiome's role in IBD, the user can now turn their focus to the functions of specific lipid-synthesis genes that are being overrepresented in IBD patients' microbiomes. Upon examining the functions of individual genes, they find that N-acyl amide synthase genes are enriched in the IBD microbiome. Wet-lab validation experiments will confirm that the lipids encoded by these genes are able to interact with GPCRs that regulate GI tract physiology (Cohen et al., 2017). This suggests that these lipids, and their associated genes in the microbiome, have the potential to cause IBD during times of dysbiosis by activating GPCRs in the gut (Cohen et al., 2017), thus supporting the user's original hypothesis. Additionally, one can use these findings to focus future research questions about the role of the human microbiome in health disorders by searching for correlations between N-acyl amide synthase genes and other conditions. For instance, since the products of these genes interact with GPCRs to potentially regulate IBD, and IBD is associated with anxiety and depression, investigation may reveal that the interaction be-

tween these genes and GPCRs potentially regulate anxiety and depression as well.

4.4 Python

5 How to Find Tools

5.1 Finding Data

(Here is where we talk about various databases that users can use to find general information, data files, study results, public datasets, etc.)

5.2 Finding Software

(Here is where we talk about ways/places that people can look for software programs that can help answer their research question.)

6 The Plan

(Break down the sub-tasks required to accomplish the two main tasks: Relative abundance analysis and metagenomic analysis)

1. What is my research question?
2. Is there an existing tool that I can use to directly answer my research question? If not, proceed to Step 3.
3. What is the step-by-step process required to answer my research question?
4. What existing tools are available that can help me accomplish each of these steps?
5. How do I write code that uses these tools to accomplish the steps?

7 Software and Set-up

7.1 Software

Add further software that you end up using (e.g., USE-ARCH). ALSO, make sure to update the section that mentions installing packages if you do so.

(Table of software name, name in PATH, version number, function of the software for each one we're gonna use)

write about installing qiime2 <https://docs.qiime2.org/2018.4/install/native/>

write about installing parallel-fastq-dump

write about installing multifastqc

add 3rd column to table with link to documentation/download source

<i>Software name</i>	<i>Version number</i>
Anaconda	5.1
Biopython	1.70
BLAT	35
matplotlib	2.2.2
pandas	0.22.0
sra-tools	2.8.2
trim-galore	0.4.5

Table 7.1: **Software used to create the tutorial pipeline.**

7.2 Set-up and Install Dependencies

Before we write any code, there are several steps that must be completed to prep your machine for the tasks we will be performing in this tutorial. Without these prerequisites, the code you write during this tutorial will not work correctly:

1. Install and open Anaconda
2. Create a new virtual environment
3. Install packages

7.2.1 Install Anaconda

→ RESOURCE FOR LEARNING ABOUT PYTHON PACKAGES

The Anaconda program will play a key role in this tutorial. Anaconda is essentially Python and a lot of scientific computing tools bundled together, along with many popular add-ons to Python called packages. Downloading all of these tools individually can be difficult, as the quirks of one package may conflict with another when they're installed manually; using Anaconda to install packages greatly simplifies the process because Anaconda can smoothly handle all of the minute details that cause manual installations to fail.

Install and Open:

1. Go to the download page for the Anaconda distribution at <https://www.anaconda.com/download>.
2. Select your preferred operating system from the Windows, macOS, or Linux tabs, then select the Download option for the **Python 3.6 version** (Figure 7.1) and follow the installation instructions.

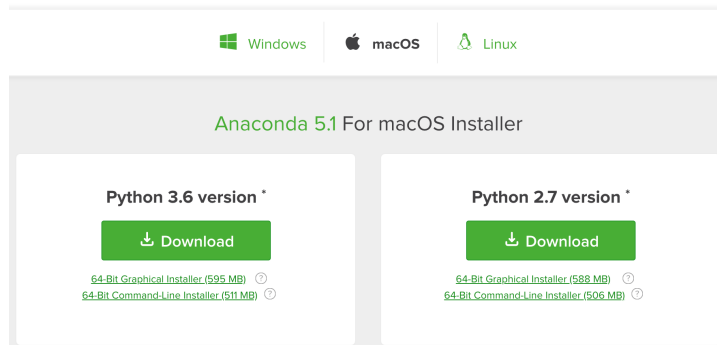



Figure 7.1: The Anaconda download options provided on the Anaconda distribution website at <https://www.anaconda.com/download>

3. After installation is complete, open the application named "Anaconda-Navigator" (the icon looks like ) . After a brief start-up period, you should see the following window (Figure 7.2):

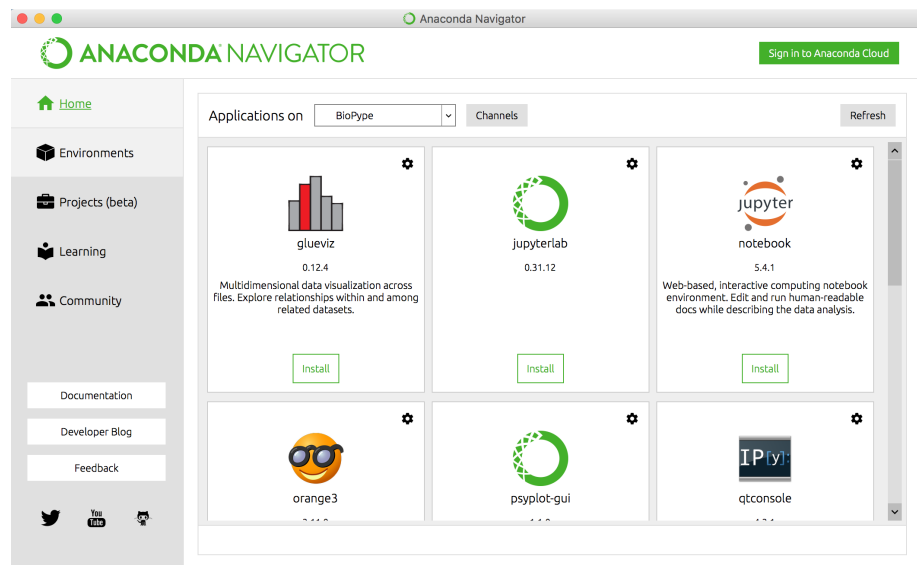


Figure 7.2: The window displayed to the user upon opening Anaconda-Navigator.

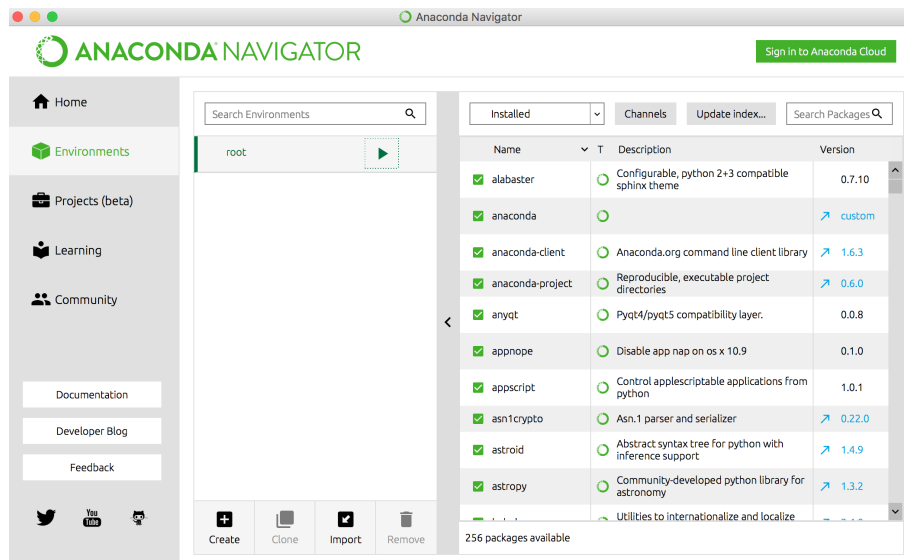


Figure 7.3: The Environments window of the Anaconda-Navigator.

7.2.2 Create a New Virtual Environment

Write a blurb explaining the benefits of using a virtual environment.

Link to resource for further reading on virtual environments

Make sure the computer has an internet connection while completing this section, otherwise Anaconda will not let you create a virtual environment.

1. On the left side of the Anaconda-Navigator window, click on the tab labeled **Environments**. (Figure 7.3)
2. Click the **Create** button on the bottom of the center panel. A new window titled "Create new environment" will appear. (Figure 7.4)
3. Enter a **Name** for the environment. You may choose any name you want, but for the sake of this tutorial we will name the new environment "BioPype".
4. Select the box labeled **Python** next to the **Packages** heading.
5. Choose a version of Python from the adjacent drop-down menu (Python 3.6 is the most current version at the time of this writing, but the packages we use require Python 3.5 so we chose **3.5**. If you are following the tutorial analysis in this manual, choose version 3.5).
6. Click the **Create** button within the "Create new environment window".

7.2.3 Install packages

Write blurb about what packages are.

Link to resource for further reading about packages.

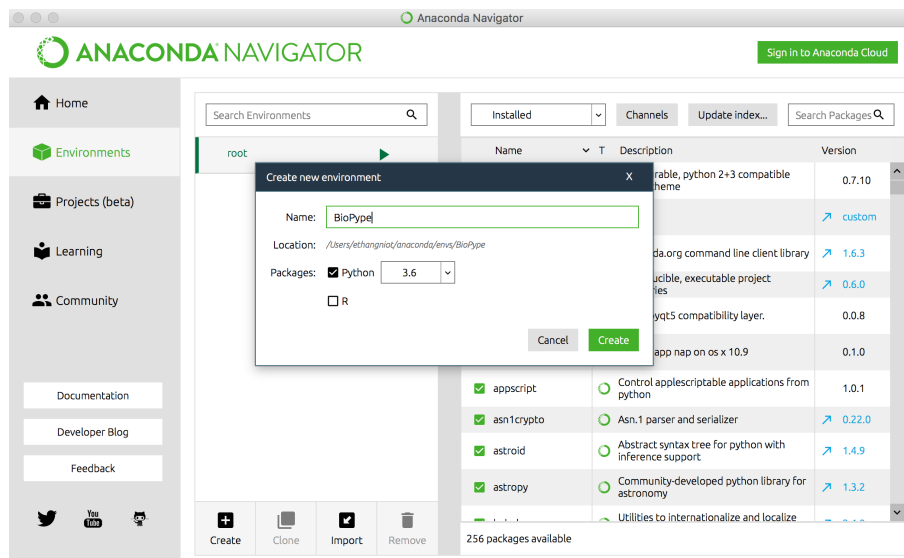


Figure 7.4: The "Create new environment" window.

1. Change Anaconda's current environment from the **root** environment by selecting the **BioPype** tab in the middle panel of the Environments window.
2. Click on the drop-down menu in the right-hand panel that says "Installed" and change it to "All".
3. In the "Search Packages" box, enter "biopython". The search should return a package named "biopython". Select the checkbox to the left of the name. (Figure 7.5)

- A pair of green and red boxes (reading "Apply" and "Clear", respectively) will appear in the bottom-right of the window once the package is selected. Do not click these just yet.

Solve the issue with failing to center figures when they're on their own page

4. Use the search bar to find and select the other packages listed in Table 7.1. Once all packages have been selected, click the green "Apply" button in the bottom right corner of the window, then select "Apply" again within the "Install Packages" window that appears. (Figure 7.6) Anaconda will now install the selected packages.

5. [Talk about setting up the sra-tools workspace \(https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=toolkit_dock&f=std\)](https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=toolkit_dock&f=std)

6. **PYPIPER IS ONLY COMPATIBLE WITH MAC AND LINUX.** Start by coding with just subprocess module commands. If the scripts work on Windows computers, then forget about using pypiper. But if the subprocess scripts don't work on windows, then we'll be developing exclusively for Mac anyways, so you could use pypiper without any worries. In that case, talk about installing pypiper here. OTHERWISE, delete this section.

- (a) Open a terminal window in the BioPype environment by clicking the "play" button on the BioPype environment tab and then selecting "Open Terminal".
- (b) Wait for the terminal window to finish opening. You'll know it's finished when you see

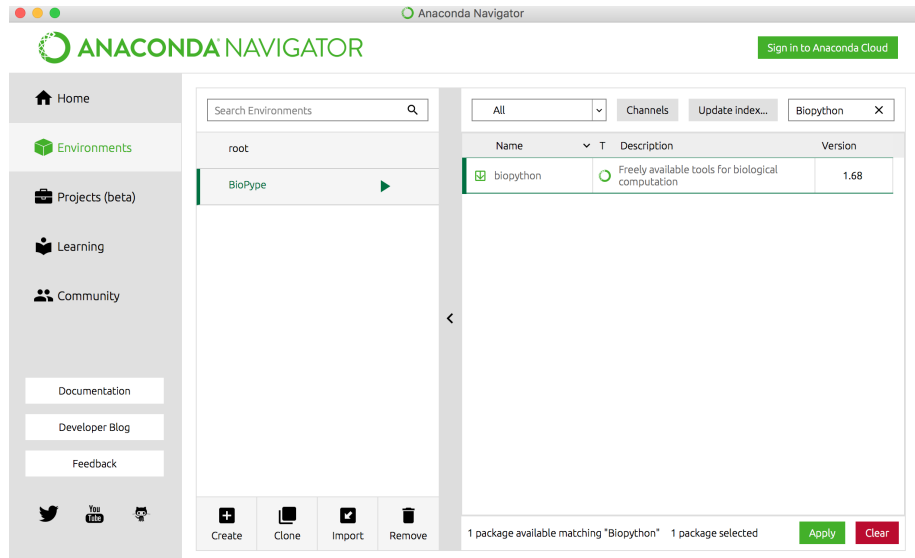


Figure 7.5: Searching for a package. When a package is selected, the check-box next to the package’s name will be green.

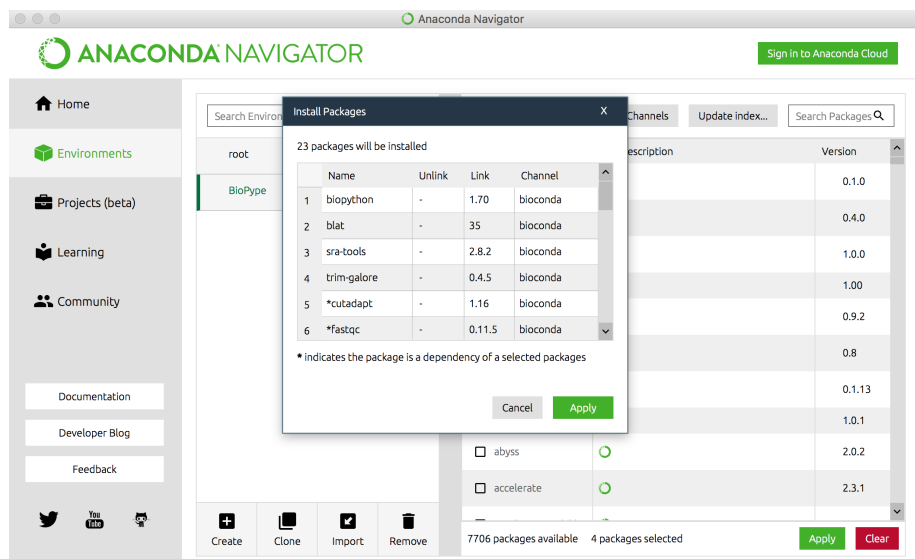


Figure 7.6: The window displaying the packages and dependencies that will be installed.

show image of what the prompt looks like when it's done initializing.

- (c) Install **pypiper** by typing the following at the command prompt, followed by pressing return/enter:

```
pip install --user pypiper
```

- (d) Check that the package was installed correctly by executing the following in the command prompt:

```
conda list
```

This will generate a list of all the packages installed in the current environment. If you see the **pypiper** package listed, the installation was successful and you may skip the rest of this section. If not, proceed with the following steps.

- (e) Execute the install command from step (c) again. This time, the Terminal should return a message similar to the one displayed in

Reference the figure pypiper-already-installed

. The line that reads "Requirement already satisfied: pypiper in" tells us the location where the package was (incorrectly) installed.

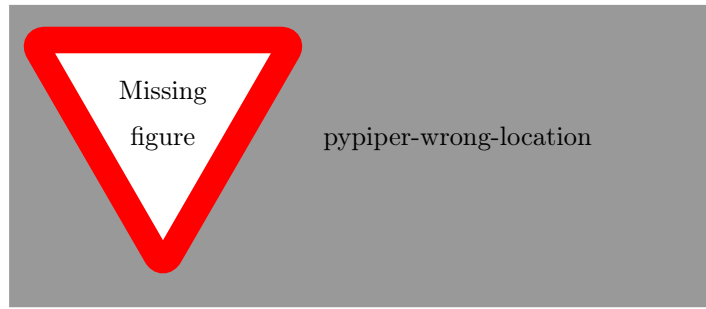


- (f) Open a Terminal window, and navigate to the location indicated by the message from the previous step. For my example, I need to start at my home directory and walk through the following folders: .local | lib | python3.6 | site-packages.

- The folders along the path to the pypiper installation may be hidden. On a Mac, these hidden folders are preceded by a ".". If the path to the pypiper installation includes hidden locations, reveal them by pressing "Cmd + Shift + ." in the Finder window.
- Once you find the site-packages folder containing two pypiper folders

reference figure pypiper-wrong-location

, copy those folders and their contents and paste them into the /anaconda/envs/BioPype/lib/python3.6/site-packages directory. The package should now be installed correctly.



7.2.4 Integrated Development Environment (IDE)

Talk about choosing between PyCharm and other options

7.2.5 PATH

(Talk about putting all the tools in the same path/directory)

7.3 Analysis Pipeline

(Use figures to illustrate the stages of the pipeline)

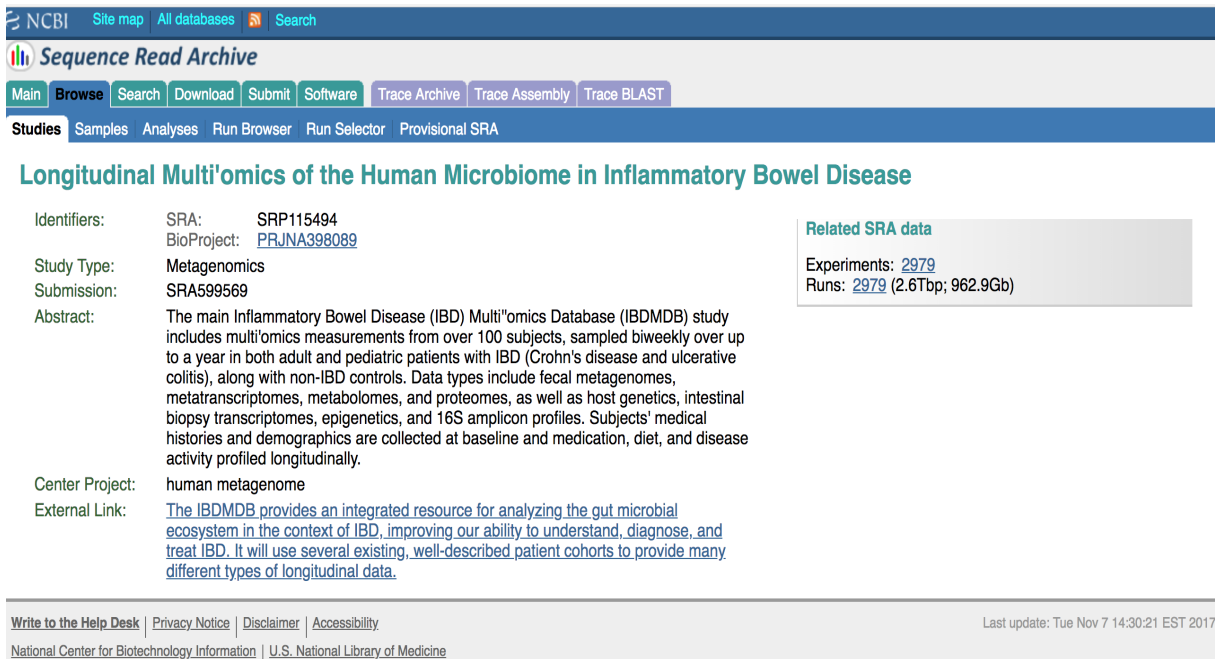
8 The Dataset

Recap

In the previous chapter, we set up our machine so that it has all of the software BioPyPe needs in order to function.

In this chapter, we will use BioPyPe to **download** experimental data, and then prepare them for analysis via a process called "**quality control**". We will also discuss how the BioPyPe functions used in this workflow were created.

As described in Chapter 3, we want to investigate if there are any differences in the gut microbiomes of younger patients with IBD compared to older patients with IBD. Using the methods described in Chapter 5, we found a study in the SRA database with sequencing data that are useful to us. The webpage for the SRA Study is reproduced in Figure 8.1 and can be accessed at <https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP115494>.



NCBI Site map All databases Search

Sequence Read Archive

Main Browse Search Download Submit Software Trace Archive Trace Assembly Trace BLAST

Studies Samples Analyses Run Browser Run Selector Provisional SRA

Longitudinal Multi'omics of the Human Microbiome in Inflammatory Bowel Disease

Identifiers: SRA: SRP115494
BioProject: [PRJNA398089](#)

Study Type: Metagenomics

Submission: SRA599569

Abstract: The main Inflammatory Bowel Disease (IBD) Multi'omics Database (IBDMDB) study includes multi'omics measurements from over 100 subjects, sampled biweekly over up to a year in both adult and pediatric patients with IBD (Crohn's disease and ulcerative colitis), along with non-IBD controls. Data types include fecal metagenomes, metatranscriptomes, metabolomes, and proteomes, as well as host genetics, intestinal biopsy transcriptomes, epigenetics, and 16S amplicon profiles. Subjects' medical histories and demographics are collected at baseline and medication, diet, and disease activity profiled longitudinally.

Center Project: human metagenome

External Link: [The IBDMDB provides an integrated resource for analyzing the gut microbial ecosystem in the context of IBD, improving our ability to understand, diagnose, and treat IBD. It will use several existing, well-described patient cohorts to provide many different types of longitudinal data.](#)

Related SRA data

Experiments: [2979](#)
Runs: [2979](#) (2.6Tbp; 962.9Gb)

[Write to the Help Desk](#) | [Privacy Notice](#) | [Disclaimer](#) | [Accessibility](#)

National Center for Biotechnology Information | U.S. National Library of Medicine

Last update: Tue Nov 7 14:30:21 EST 2017

Figure 8.1: The "SRA Study" webpage.

The dataset is reproduced in Figure 8.2 and can be found by clicking on "Runs" under "Related SRA data" on the right side of the SRA Study web-page, or by going to <https://trace.ncbi.nlm.nih.gov/Traces/study/?acc=SRP115494#>.

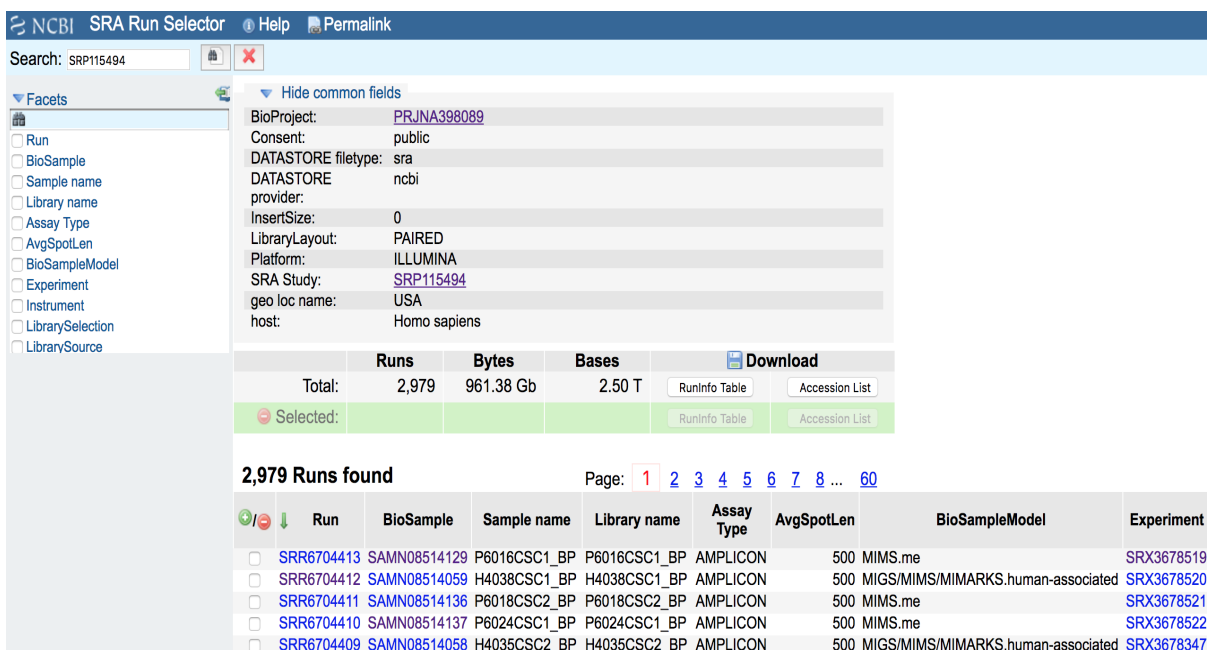


Figure 8.2: The sequencing data collected by the *Longitudinal Multi'omics of the Human Microbiome in Inflammatory Bowel Disease* study. (Note: the table's columns extend beyond the right side of the page because there are 36 metadata categories.)

To learn more about how to use these pages to find information about the study and its methods, please refer to Chapter 5.

8.1 Download Dataset

*****Show the user what the result of the commands are. Right now we show the commands, but not the results. e.g., when we run a command to get a list of accession numbers, follow it up by typing the command that just prints the list of numbers in the terminal. Then the user can see what their results should look like if they did everything correctly.

1) Choose sample criteria

Note: The dataset has a metadata category called "BioSample." This is not to be confused with the generic term "samples", which in this case are equivalent to one run (i.e., one row) of the data table.

- First, we must decide what criteria we will use to choose our experimental samples.
Remember: our research question investigates the effects of age on the gut microbiomes of inflammatory bowel disease patients. Looking at the study abstract from Figure 8.1, we can see that the researchers investigated two types of IBD: Crohn's disease and ulcerative colitis. So one of the metadata categories from the dataset (i.e., the columns from the dataset in Figure 8.2) that we will use to select our samples

is the "host_disease" category. We will also select our samples based on the "host_age" metadata.

2. Now we want to select our samples based on these categories.

Download the RunInfo Table file found on the study's sample page and put it in the project folder you set up in Chapter 7.1. The RunInfo table is the browser's sample table in a .txt file format.



3. Open the command line interface for your machine's operating system. On a mac, this is the Terminal application.
4. Navigate to your project's folder and then confirm that you are in the right location by using the the following commands at the command prompt:

```
$ cd <path\_to\_your\_project>
$ pwd
```

5. Activate the Python interpreter by typing the following into the command prompt:

```
$ python3
```

6. Import BioPype to the current Python environment:

```
>>> import BioPype
```

! →

- If not done already, stop and follow the instructions in Chapter 7 to configure the BioPype working directory and the SRA Toolkit Workspace Location.

7. Import `BioPype.cmds.runtable` to gain access to the `RunTable` class:

Either shorten this margin-label, or put it in a footnote.

Using "as" in an import statement lets you reference the full name of the imported package by some shorter name. It is generally considered better practice to **not** import packages "as" some other name, because it makes the code less explicit (which goes against the purpose of Python), but we do it in this manual for the sake of space.

```
>>> import BioPype.cmds.runtable as runtable
```

8. Create a `RunTable` object called "my_table" out of the RunInfo Table .txt file:

Give a specific name to the RunInfo Table file. People might get confused if they needs to fill in the name of the file themselves.

```
>>> my_table = runtable.RunTable('runinfotable_filename.txt')
```


- With the `my_table` object created, you can remind yourself what the metadata categories are by using the following command:

```
>>> my_table.column_names
['Assay_Type', 'AvgSpotLen', 'BioSample', 'BioSampleModel',
 'Experiment', 'Instrument', 'LibrarySelection', 'LibrarySource',
 'Library_Name', 'LoadDate', 'MBases', 'MBytes', 'Organism',
 'ReleaseDate', 'Run', 'SRA_Sample', 'Sample_Name',
 'collection_date', 'env_biome', 'env_feature', 'env_material',
 'host_age', 'host_disease', 'host_sex', 'host_subject_id',
 'lat_lon', 'BioProject', 'Consent', 'DATASTORE_filetype',
 'DATASTORE_provider', 'InsertSize', 'LibraryLayout', 'Platform',
 'SRA_Study', 'geo_loc_name', 'host']
```

9. We want to first group the samples based on the patients' disease...

```
>>>uc_samples=my_table.filter_data("host_disease", '==', 'ulcerative colitis')
>>>cd_samples=my_table.filter_data("host_disease", '==', "Crohn's disease")
```

! →

- Note that the "Crohn's disease" string within the parentheses in the second line of code has two single-quotes/apostrophes between the "n" and the "s" of "Crohn's". This is because the value we input must exactly match the value that the original study's researchers input for the `host_disease` category, and (for whatever reason) they entered the value for Crohn's disease as: "Crohn's disease"

This is an example of why it's important to check the metadata carefully before you begin the analysis. If the argument does not exactly match the value of the metadata category, it won't select the sample.

10. ... then we want to group them based on age:

```
>>>uc_young = uc_samples.filter_data("host_age", '<=', 21)
>>>uc_old = uc_samples.filter_data("host_age", '>=', 60)
>>>cd_young = cd_samples.filter_data("host_age", '<=', 21)
>>>cd_old = cd_samples.filter_data("host_age", '>=', 60)
```

! →

- Note that the third argument passed to the `filter_data()` method is **not** a string like the previous two arguments. It is simply an integer (no quotation marks around it).

11. Now that we have groups of samples selected, we want to get a list of SRA accession numbers for each group. The accession numbers are what we will use to specify the files we want to download from the SRA database.

```
>>>uc_young_nums = uc_young.get_accession_numbers()
>>>uc_old_nums = uc_old.get_accession_numbers()
>>>cd_young_nums = cd_young.get_accession_numbers()
>>>cd_old_nums = cd_old.get_accession_numbers()
```

→ To learn more about .sra file format, see Appendix A.11.

12. We can now use the lists of accession numbers to download .sra files from the SRA database. However, these are large files that can *easily* take up hundreds of gigabytes of memory. They also take time to download (times will vary based on network speed). To account for hardware limitations that would severely bottleneck the analysis at

→ The "random_sample_subset()" method is preceded by "RunTable." in this case because it is a "@staticmethod". To learn more, see Appendix A.7.

file-handling steps like these, we will randomly select a subset of only 3 accession numbers from each list we created in the previous step. The analysis will go much faster if we analyze fewer samples, so these subsets are what we will analyze going forward.

```
# Shorten the name for the runtable module to save space in the manual:
>>>import BioPyPe.cmds.runtable as rt

>>>rs_uc_young = rt.RunTable.random_sample_subset(uc_young_nums, n=3)
>>>rs_uc_old = rt.RunTable.random_sample_subset(uc_old_nums, n=3)
>>>rs_cd_young = rt.RunTable.random_sample_subset(cd_young_nums, n=3)
>>>rs_cd_old = rt.RunTable.random_sample_subset(cd_old_nums, n=3)
```

13. Now we need to download the .sra files linked to these accession numbers and get them into .fastq format. First, we'll need to import the necessary functions:

```
>>>from BioPyPe.cmds.downloadsra import download_sra,
>>>from BioPyPe.cmds.downloadsra import convert_sra_to_fastq
```

14. Use the `download_sra()` function to download the .sra files into named folders:

```
>>>download_sra(rs_uc_young, 'uc_young')
>>>download_sra(rs_uc_old, 'uc_old')
>>>download_sra(rs_cd_young, 'cd_young')
>>>download_sra(rs_cd_old, 'cd_old')
```

! →

- The .sra files will not be downloaded to the correct location if the SRA Toolkit Workspace Location has not been properly configured (see Chapter 7 for details).

→ To learn more about .fastq format, see Appendix A.8.

15. Use the `convert_sra_to_fastq()` function to convert the .sra files to .fastq format.

```
>>>convert_sra_to_fastq(threads=4)
```

! →

- BioPyPe will not know where to look for the downloaded .sra files if the SRA Toolkit Workspace Location has not been properly configured (see Chapter 7 for details).
- The more threads your computer can divide this process between, the better (it's more complicated than that, but for now let's go with it). The question is: how many threads is your computer capable of using? For that, you'll have to do some Googling. You can find the resources I used to figure out how many threads my MacBook Pro can run (4) in Appendix A.10. To simplify: my computer has 1 CPU, the CPU has 2 cores, and each core can run 2 threads. $1 \times 2 \times 2 = 4$ threads.

8.1.1 Creating the code

8.2 Perform Quality Control on Dataset

9 Relative Abundance Analysis

(How to compare relative abundance of bacterial taxa between experimental conditions using QWRAP.)

10 Predict ORFs

(how to predict the ORFs of the sequencing reads)

11 Create Non-redundant Gene Sets

(How to create non-redundant gene sets using the predicted ORFs and what kind of information they provide) (Align reads using BLAT)

12 Align Genes

13 Get GenBank Accession Numbers

14 Find COG Functional Classes

15 New Analysis

(Walk user through analysis of new, untested dataset looking for age-related differences in patients with Inflammatory Bowel Disease.)

Appendix A Web Resources and Explanations

A.1 QIIME Illumina Overview Tutorial

http://nbviewer.jupyter.org/github/biocore/qiime/blob/1.9.1/examples/ipynb/illumina_overview_tutorial.ipynb

A.2 QIIME 2 Moving Pictures Tutorial

<https://docs.qiime2.org/2018.2/tutorials/moving-pictures/>

A.3 Microbiota vs Microbiome

"What is the gut microbiota? What is the human microbiome?" from medicalnewstoday.com. <https://www.medicalnewstoday.com/articles/307998.php>

A.4 Relative- vs Differential-Abundance Analyses

(For more in-depth discussion of differential abundance analyses, see (Mandal et al., 2015b).)

Differential abundance analysis: seeing which samples are most similar/dissimilar based on the raw sequence counts for each sample (E.g., 5 sequences associated with the *Lactobacillus* OTU were found in Sample A, and 10 sequences were found in Sample B). Differential abundance analysis answers the following questions:

- Is Sample A more similar to Sample B, or to Sample C in terms of its microbiome composition? (has an associated p-value)
- Does sequence variant/OTU/taxon/bacterium "X" appear more/less often in Subject A than in Subject B? (raw count)

Relative abundance analysis: seeing which samples are most similar/dissimilar based on the proportion of sequence counts for each sample (E.g., out of all the sequences obtained from Sample A, %50 were associated with the *Lactobacillus* OTU. Out of all the sequences obtained from Sample B, %100 were associated with the *Lactobacillus* OTU. Relative abundance analysis answers the following question:

- Does Sample A have higher/lower percentage of Bacteria X in its microbiome than Sample B?

A.5 Amplicon vs WGS data

<http://galaxyproject.github.io/training-material/topics/metagenomics/tutorials/general-tutorial/tutorial.html#amplicon-data>

A.6 Operational Taxonomic Units (OTUs)

https://www.drive5.com/usearch/manual/otu_definition.html

A.7 Explanation of @staticmethod decorator vs @classmethod decorator in Python

The Basics: Static methods make code easier to read and let you use a class' methods without needing to have an object of that class first. This is useful when it makes logical sense to place a function within a class (because the function is related to the other tasks that the class handles), but the function doesn't *need* to operate on an object/the data of that class. Normal methods are called by typing: `my_object.method`. Static methods are called by typing: `MyClass().method` or `MyClass.method`.

Basic explanation with slight background on class methods:

<https://julien.danjou.info/guide-python-static-class-abstract-methods/>

More technical explanations:

<https://stackoverflow.com/questions/12179271/meaning-of-classmethod-and-staticmethod-for-beginner>

This Stack Overflow question has some basic, easy to understand explanations mixed in with more in-depth explanations. The top-voted answer isn't the only one that is useful; each of the answers presents their explanation with a different degree of simplicity. Make sure to check several answers if the top ones don't seem helpful.

A.8 FASTQ Format

<https://galaxyproject.org/tutorials/ngs/>

This link contains:

1. An explanation of the FASTQ file format
2. An explanation of PHRED quality scores with an accompanying figure (Fig 4)
3. The following quote: "Fastq format is not strictly defined and its variations will always cause headache for you. See <https://www.ncbi.nlm.nih.gov/books/NBK242622/> for more information."
 - From the NCBI link: "Text formats, such as FASTQ, are supported, but are not the preferred submission medium. Poorly defined specifications and high variability within these formats tend to lead to a higher frequency of failed or problematic submissions."

A.9 How many threads to use?

<https://www.jstorimer.com/blogs/workingwithcode/7970125-how-many-threads-is-too-many>

A.10 How many threads can my computer run?

<https://superuser.com/questions/1101311/how-many-cores-does-my-mac-have>

A.11 SRA/.sra file format

Find resource for explaining the .sra file format that we download from the SRA Database

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