README.md

The eDNA-container app

Introduction

A Docker image containing a eDNA pipeline based on QIIME2. The pipeline is controlled via flask GUI that runs in the users browser.

The advantages of the pipeline are:

- simple to setup and run using point and click with a browser based GUI
- should run on any machine where Docker-desktop can be installed
- adaptable to any primer combination or taxonomic database
- snakemake is used to confirm successful completion of each stage of the pipeline
- species summary tables with counts are created, including the ASV sequence for manual confirmation of the taxonomic classification
- rarefaction and taxonomic barplots are generated that can be viewed using the QIIME viewer (drag and drop)
- A PDF report is generated containing QC plots and important information about the ASV generation so that QC parameters can be optimised

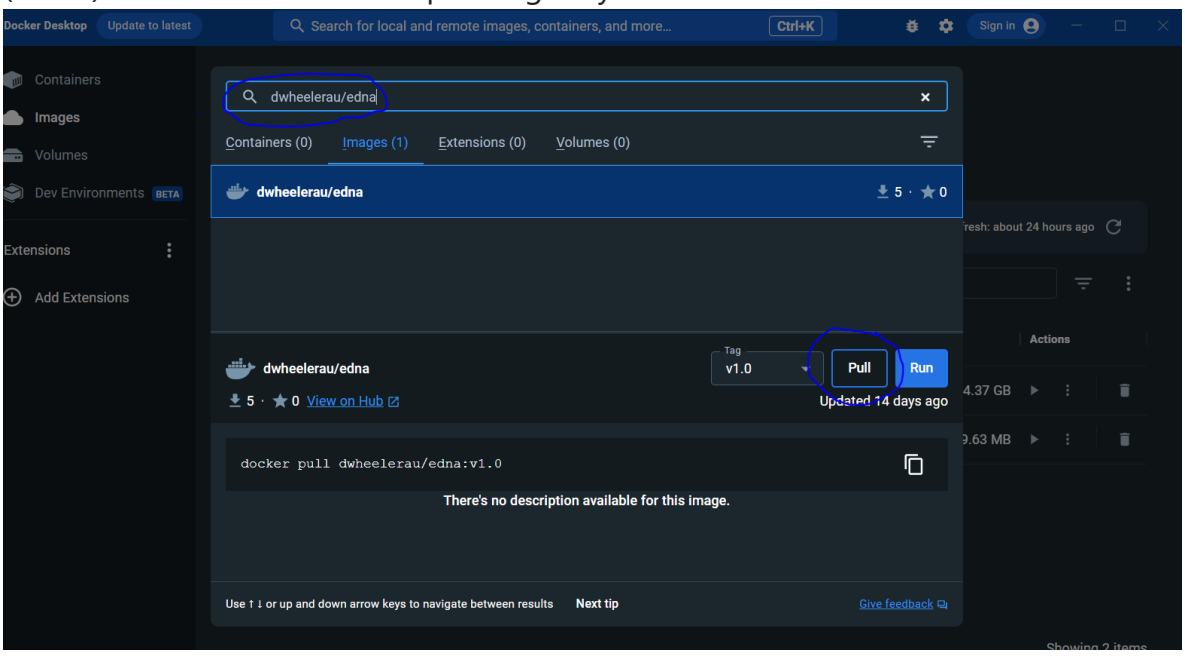
The pipeline can also be used without Docker as described at this repo.

Running the image in a container on Windows

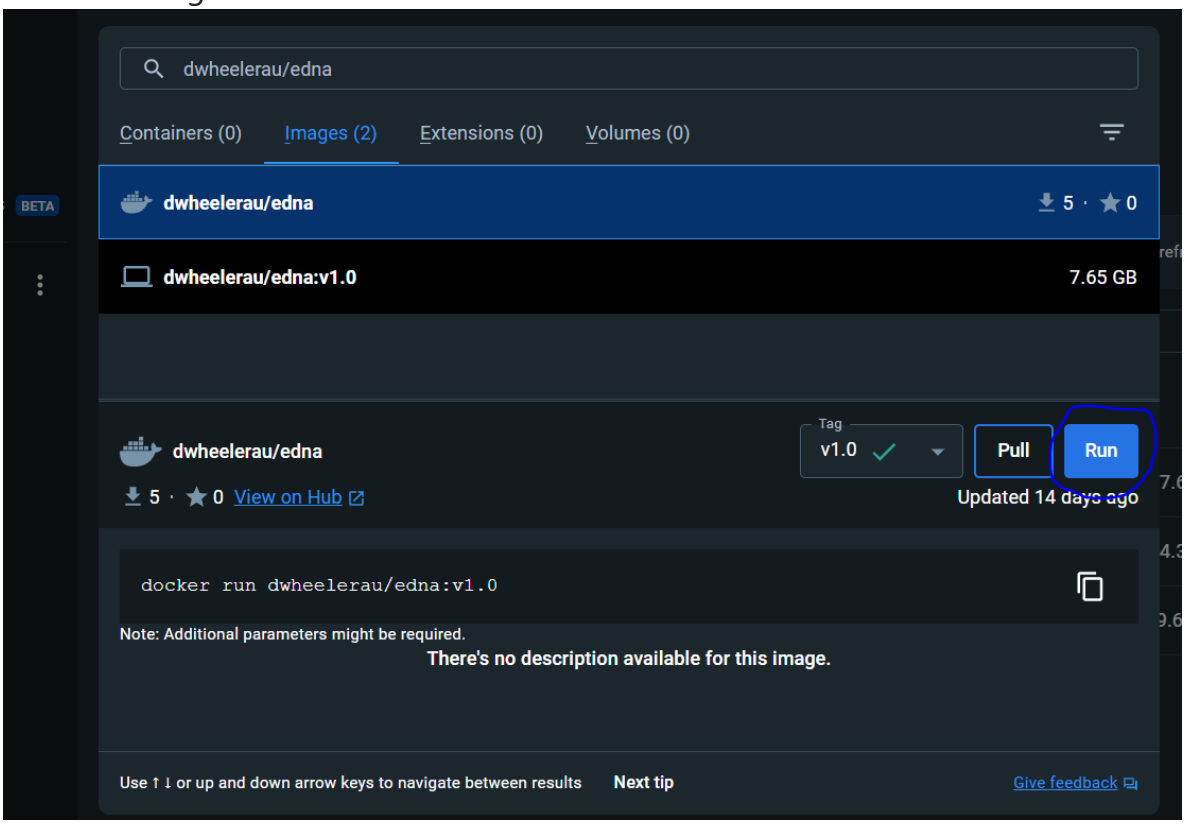
Ensure that the Docker-desktop app is installed on your windows computer. The offical guide for installing Docker-desktop can be found here. If you run into any issues a more complete guide is available here.

1. Start by opening the Docker-desktop app.

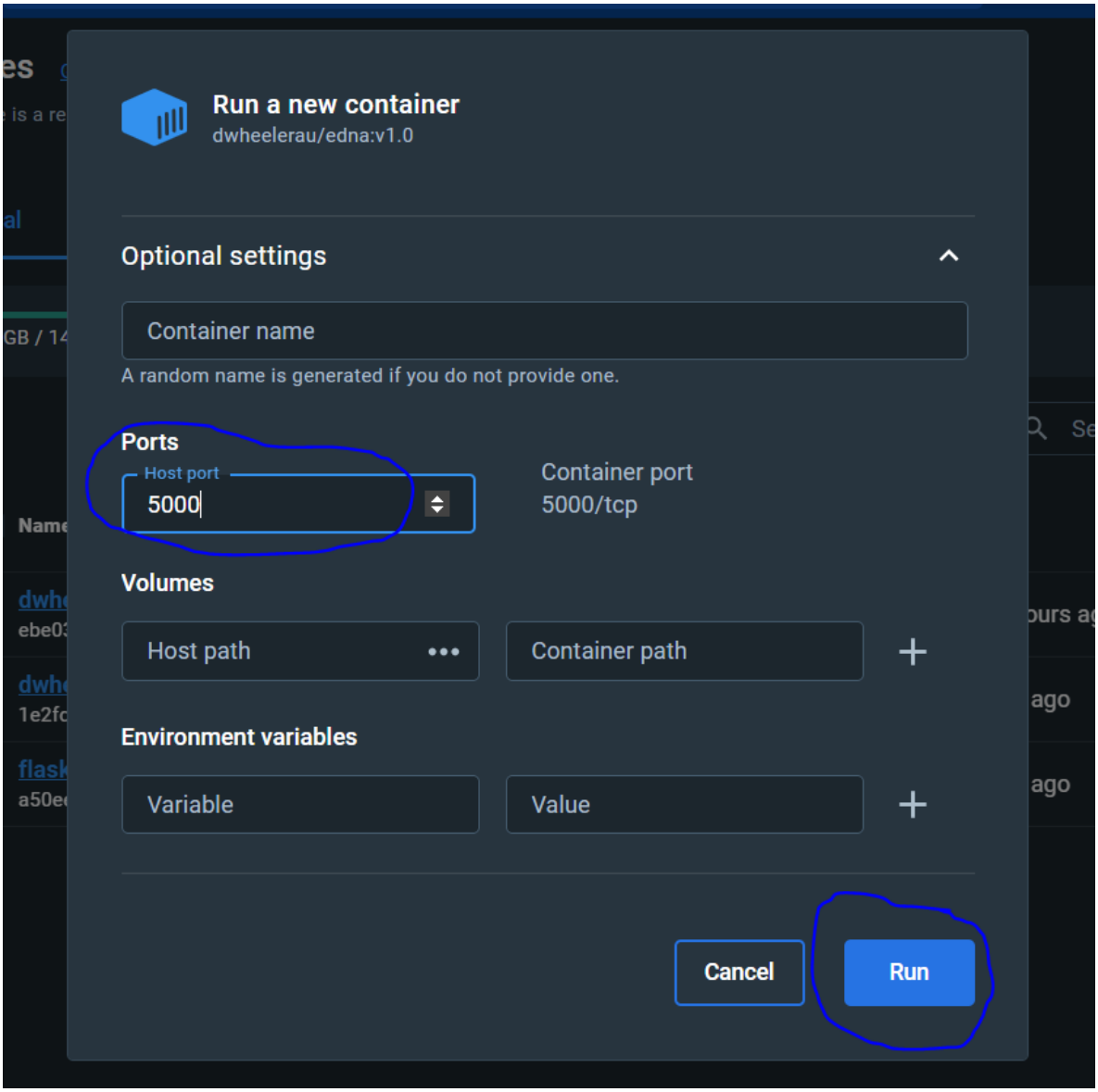
2. In the search bar at the top of the page search for `dwheelerau/edna`, then use the "Pull" button to obtain a copy of the pipeline image. Downloading the image (~7GB) will take some time depending on your internet connnection.



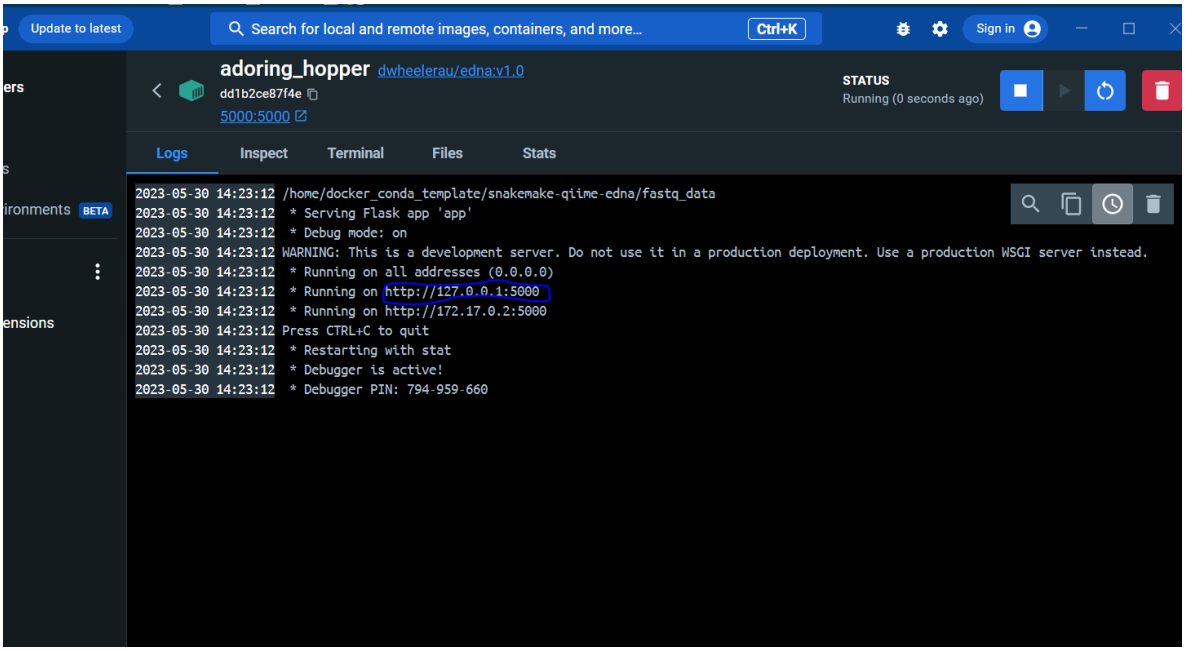
3. After the image has downloaded click the "Run" button.



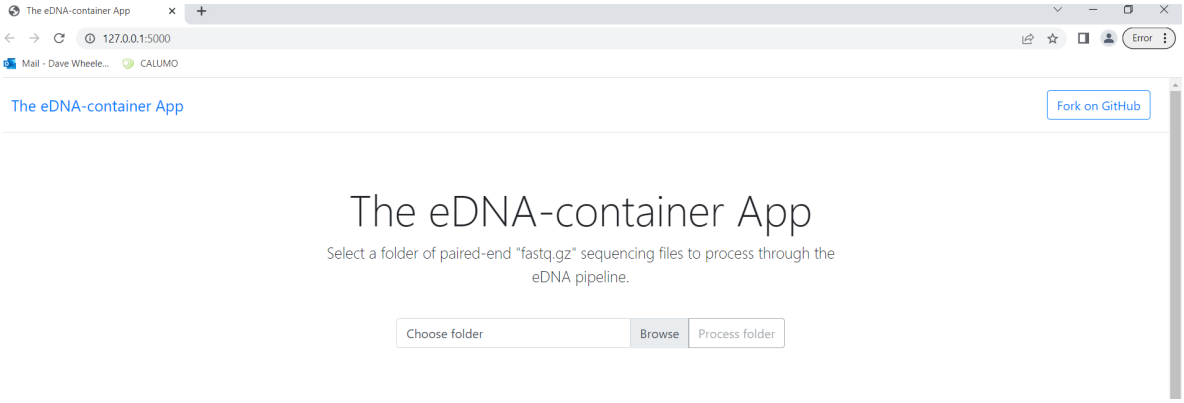
4. Use the "Optional settings" drop down to open host port 5000 as shown in the image below, then click "Run".



5. The log section of the container should look like the image below, this is showning the IP address for The eDNA-container app user interface, click on the top link or type `http://127.0.0.1:5000` in an internet browser (**Note:** No data is transfered over the internet, the pipeline will run using the local computer resources).



6. Please wait a few seconds while the pipeline setup beings, if this has been successful the following screen should display.



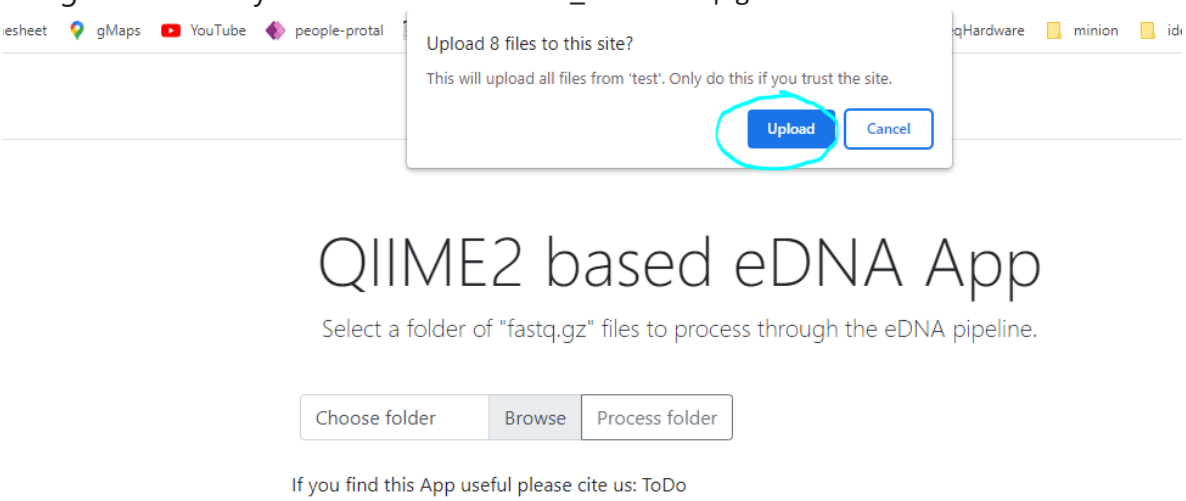
Follow the instructions below to carry out an eDNA analysis using the pipeline. **Note** for future runs on the pipeline all that is required is to click the "Play" Button next to the container ID in the Docker-desktop app. Remember to shutdown the container using the "Stop" button to free up system resources after you have completed the analysis.

Analysing paired-end sequencing data using The eDNA-container app

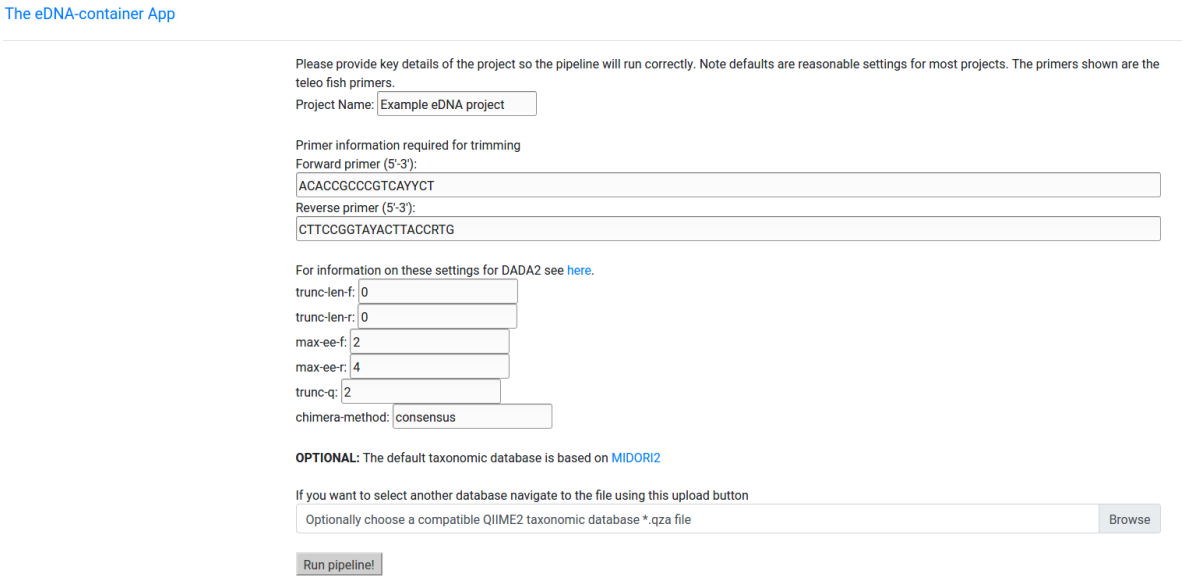
The pipeline is currently only configured to process paired-end fastq.gz sequencing files. Ensure that the target directory only contains sequencing data for the samples you wish to analyse.

If this is your first time using the app, we recommend that you initially try using the test data. To do this download the fastq_data. folder to your computer and select this when prompted by the app. This is only a small dataset so it should run reasonably quickly even on modest systems. An example of the results when the pipeline runs successfully can also be found at the previous link as a zip file. If you have any trouble running the app on this dataset please see the Trouble shooting section below.

1. Using the browser based user interface select the target folder of `fastq.gz` files that you wish to process through the pipeline and click the "Upload" button (note no data is transfered over the internet). **Important note about the infiles**. The app extracts the sample name from the sequence filenames, so you may need to rename your sequencing files so they fit the following pattern: `SAMPLENAME_R1_001.fastq.gz` and `SAMPLENAME_R2_001.fastq.gz` . The SAMPLENAME needs to be the same for both forward (R1) and reverse (R2) reads. It is important to include the underscore separator (_) between the SAMPLENAME and the R1/R2 designation. Also you must include the `_001.fastq.gz` tail in the filename as well.



2. Click the "Process folder" button to open the settings page.



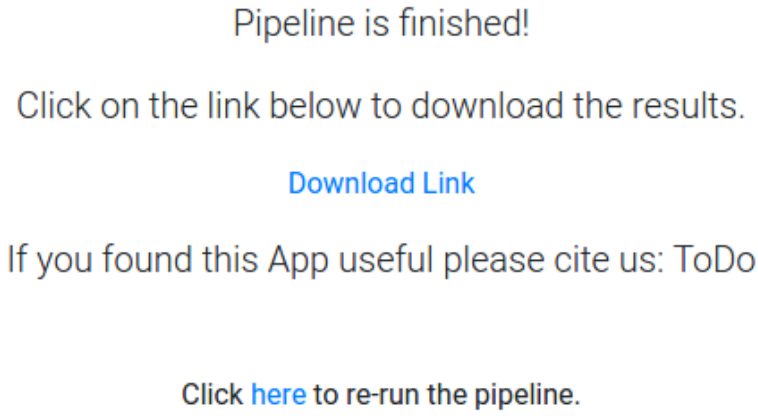
3. The next page is the settings page, the following table details each option. **Note** be sure to set `trunc-len-f` and `trunc-len-r` to 0 when using variable length amplicons in order to avoid introducting trimming biases. We recommend running the pipeline initially with the default quality settings and then adjusting these based on the outputs pressented in the `final-report.pdf` (specifically the raw read QC plots and DADA2 tables).

Setting	Explanation
Project name	A name for your project (will be used as the project title)
Forward primer	Forward PCR primer sequence for cutadapt primer/adapter removal
Reverse primer	Reverse PCR primer sequence for cutadapt primer/adapter removal
trunc-len-f	Remove 3' end of forward read at this position due to low quality (0=no trim)
trunc-len-r	Remove 3' end of reverse read at this position due to low quality (0=no trim)
max-ee-f	Forward reads with > number expected errors will be discarded
max-ee-r	Reverse reads with > number expected errors will be discarded
trunc-q	Truncate reads at first instance of quality score <= value
chimera-method	chimera removal method: consensus, pooled, or none
Taxonomic database	File location for a QIIME2 compatible (.qza) naive_bayes clasifier (optional)

Table describing the key settings; for additional information see the DADA2 and naive_bayes classifier pages. A classifier based on the MIDORI2 database (12S rRNA) and the Telo fish primer amplicon is provided as a default (F:5'ACACCGCCCGTCAYYCT3'/R:5'CTTCCGGTAYACTTACCRTG3').

4. When you are ready click the "Run pipeline!" command to start the analysis. A data submitted screen (below) will be replaced by a Download data link once the pipeline has completed. As noted before this analysis is performed on your own computer inside your local Docker environment with the download link a shortcut

to data stored inside the eDNA-container app.



A summary of the key outputs are below. If your analysis fails you will be notified by a message on the user interface, if this occurs see the FAQ. Any file ending in .qzv can be viewed by drag and drop into the QIIME2 viewer. You can also test the pipeline using the defaults with the example data in the testing_data folder.

Key outputs

file path	Explanation
final_results/final-report.pdf	A PDF report describing some key outputs from your run
final_results/asv_count_tax_seqs_summary.csv	Final spreadsheet of eDNA taxa counts
final_results/barchart.qzv	species barplot
final_results/alpha_rarefaction.qzv	Alpha diversity rarefaction plot viewable using the qiime2 viewer
paired-end-demux.qzv	Read quality plots viewable using the qiime2 viewer
final_results/asvs	Amplified Sequence Variant files
logs	DADA2 and cutadapt plugin log files
Report_data/boxplot-forward.png	Boxplot of forward read used in report

file path	Explanation
Report_data/boxplot-reverse.png	Boxplot of reverse read used in report
manifest/manifest.tsv	Sample metadata used to assign sequence files to samples

The taxonomic database

A classifier based on the MIDORI2 database (12S rRNA) and the Telo fish primers (F:5'ACACCGCCCGTCAYYCT3'/R:5'CTTCCGGTAYACTTACCRTG3') amplicon is provided as a default database. This database was built using only the amplicon generated using this primer combination, so if you have used another primer set you will need to build your own database or obtain a pre-built `QZA` database file from a reliable source (ie Silva 16S sequences and Taxonomy.

Building a custom database requires two files, the first being the reference library of sequence barcodes in FASTA format, and the second being a corresponding taxonomy file with IDs that match the FASTA headers. Please see the [QIIME2 documentation] ((https://library.qiime2.org/plugins/q2-feature-classifier/3/) for additional information on building a custom database `QZA` file that is compatible with the eDNA app. We have also provided an example script that demonstrates the basic workflow.

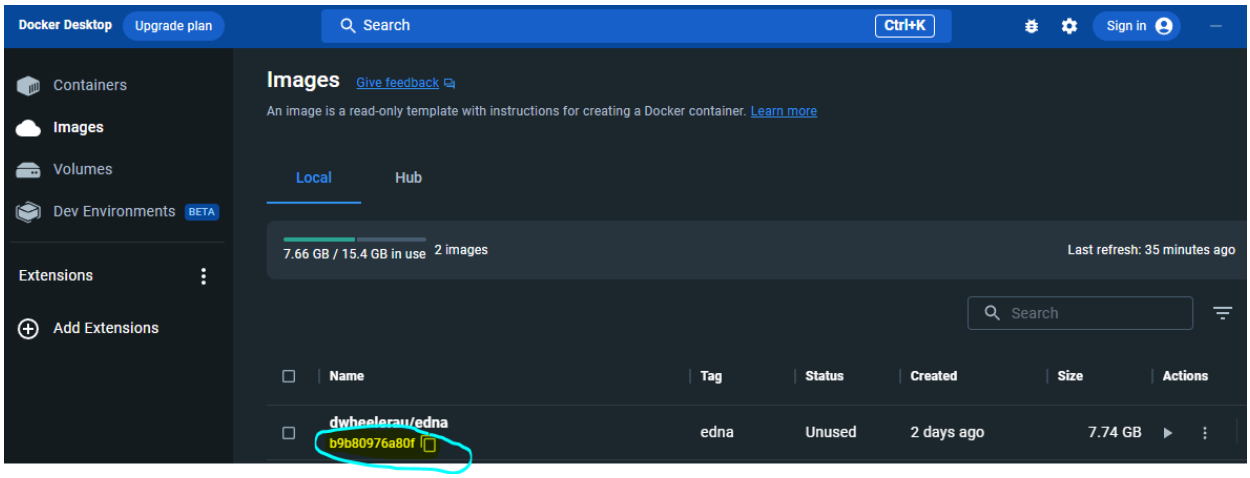
Running the pipeline using terminal commands (Windows/Linux)

If you are confortable using the command line and Docker is installed the following can be used to run the pipeline without the user interface.

```
docker pull dwheelerau/edna
```

Downloading the image (~7GB) will take some time depending on your internet connnection.

4. The image should appear in your Docker desktop app under the 'images' section (see screen shot below). Click the copy icon next to the iamge id code as shown below (this code will be used in the next command).



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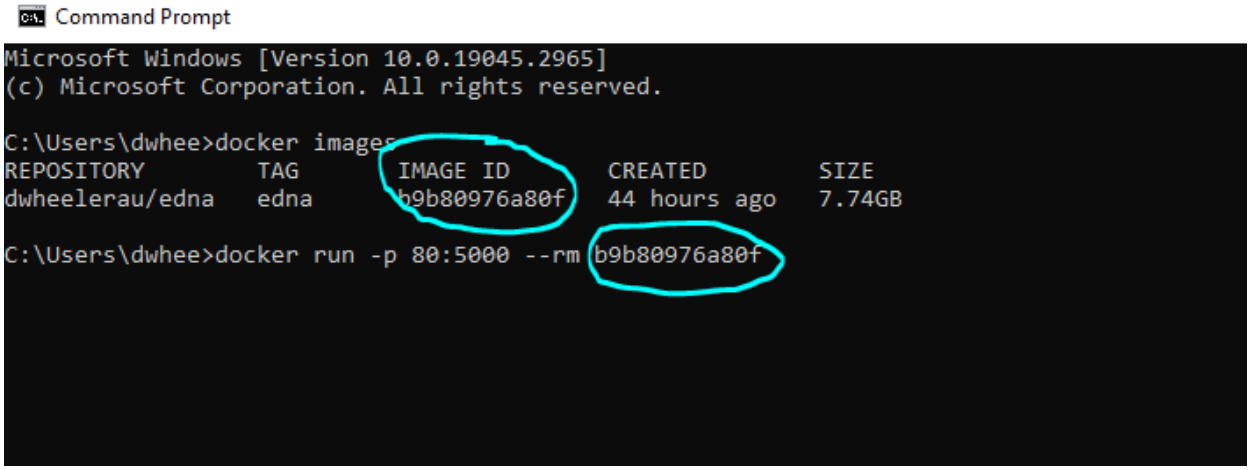
5. Type the following command in the terminal window replacing "IMAGEID" with the code you copied above (you can "paste" by right clicking on the command prompt window boarder and selecting edit->paste).

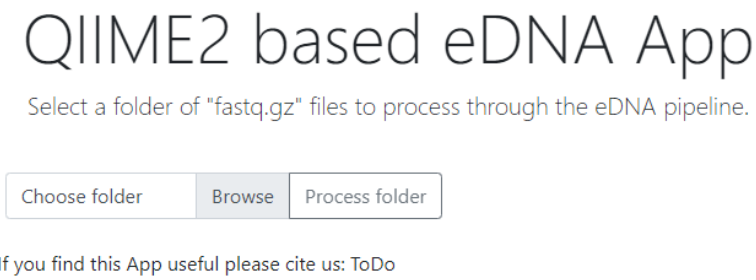
```
docker run -p 80:5000 --rm IMAGEID
```



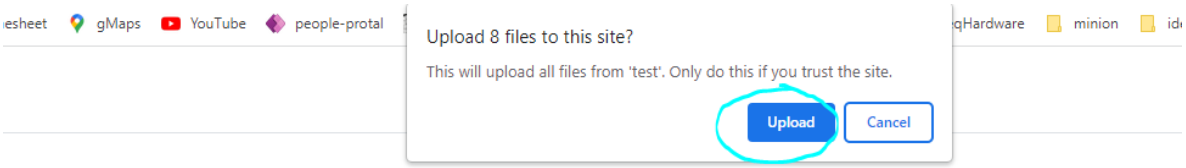
6. Open a internet browser (we recommend chrome or firebox) and enter the following IP address

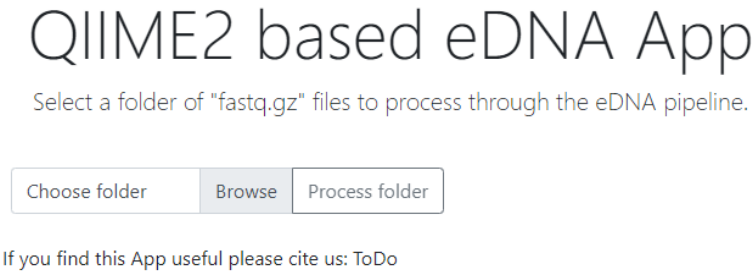
```
http://127.0.0.1:80
```

The following window should open (after a short wait for the app to start). Any errors during the run should appear in the command prompt window.

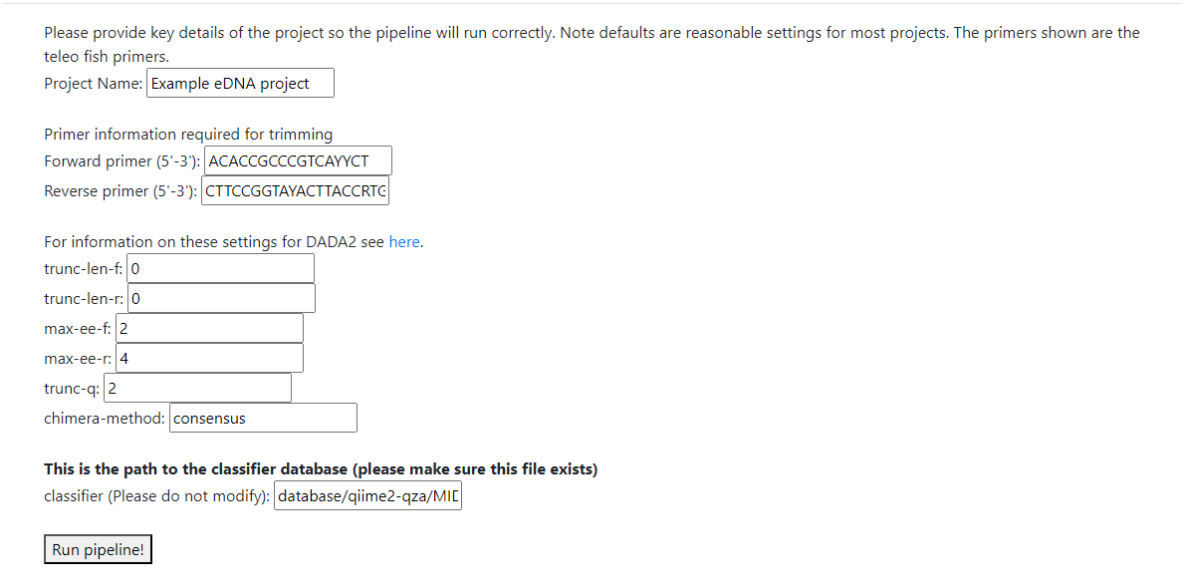


7. Using the 'select' or 'browse' button select the folder where the fastq.gz sequencing files for this project are located and accept the image upload dialogue.

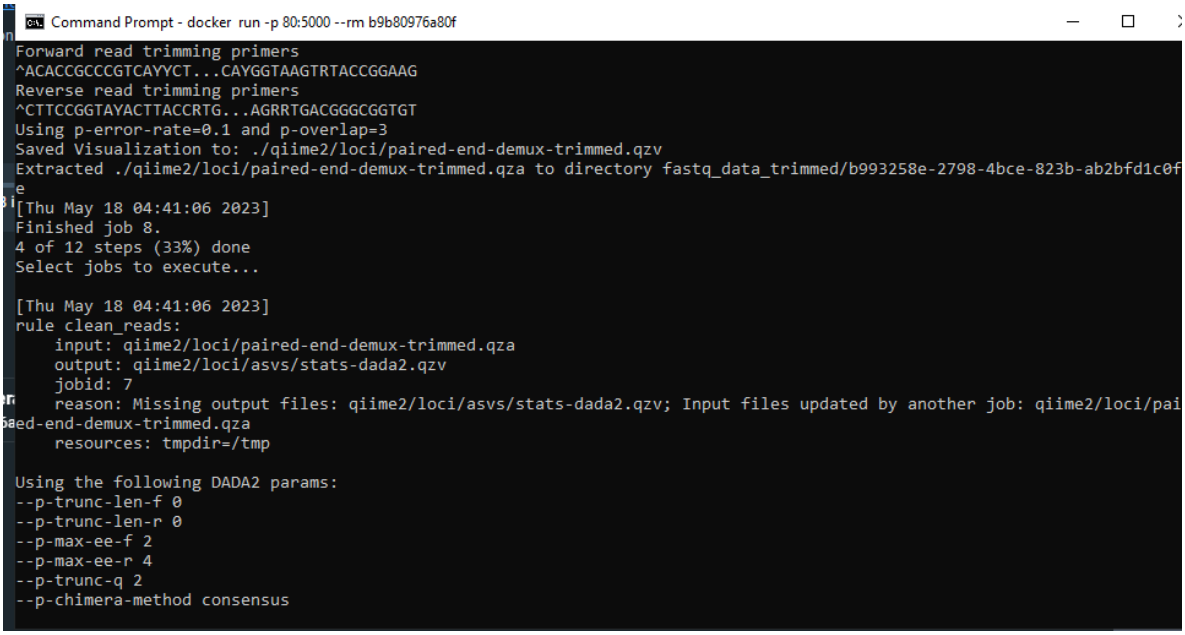




8. Click 'process folder' button and update the settings on the next page as required (the default if the telo fish eDNA primers)



9. When you are ready use the 'Run pipeline!' button to run the application on your data (the progress will be logged to the command prompt terminal window).



10. A running screen will be replaced by a download link to the results (zipped folder). Any errors will be reported in the command prompt window.

If you wish to re-use the pipeline in the future, simply open the Docker-desktop app and follow the instructions from Step 5.

Running the image in a container on Linux

1. Make sure you have Docker installed on your OS
2. Pull the image from a terminal window

```
docker pull dwheelerau/edna:edna
```

3. Find out the image ID using

```
docker images
```

4. Run the image replacing IMAGEID with the number in the first column from the above command.

```
docker run -p 80:5000 --rm IMAGEID
```

5. Open a firefox/chrome browser window and naviate to the following IP address (or localhost on port 80):

```
http://127.0.0.1:80
```

The app should open in the browser after a short delay. Any errors during pipeline running should appear in the terminal window.

6. Follow the instructions from step 7 in the windows section above.

Building the latest version of the pipeline

Quick start after cloning this repository:

```
cd edna-contained
sudo docker build -f Dockerfile . -t dwheelerau/edna:edna
```

Once the image is stored on your computer the `docker run` command can be used to run the app.

Trouble shooting

Key outputs are not included in the ZIP file? The most likely explanation is that the analysis has failed. Error message will be printed to the Docker terminal window (logs tab), check here for any messages. The success or otherwise of each rule in the pipeline should highlight what went wrong. If the PDF report is generated also check the results in the tables, if they report that 0% reads passed filtering then this would indicate an issue with the filtering and QC settings. The most common errors are:

- Overly stringent trimming removing the region of overlap between the forward and reverse read
- Overly stringent quality trimming removing the region of overlap between the forward and reverse read
- providing a taxonomic sequence database that does not overlap your amplicon

- incorrect primer sequences provided (the app will discard all reads which don't contain the expected primers)

The dada2 step keeps failing. This is the most resource intensive step of the pipeline and it can fail if the hsot computer does not have enough memory to process the provided data. One solution here is to make more memory available to Docker by changing the memory settings (under resources in the settings) in docker desktop. If this does not work you may need to access a more powerful computer.

Another common reason for the dada2 step to fail is that there are no ASV's available after QC and trimming. This could be caused by over-stringent QC settings, or due to providing the incorrect primer sequences during the setup process for the app.

ToDo

- Building database help