# 16S NGS DATA ANALYSIS PIPELINE USER GUIDE
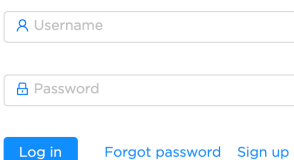
## VERSION 1.0

For Research Use Only

# INTENDED USE

The YouSeq NGS data analysis pipeline is designed to generate easy and simple reports from the fastq.gz or bam file data produced by Illumina and Ion torrent sequencers.

The analysis pipeline cleans the data and attempts to assign the reads (or paired reads) to a specific bacterial species. The output of the pipeline is summarized in a sunburst plot along with pdf reports and .csv files. The raw and summarized data can be downloaded and further interrogated in a spreadsheet.
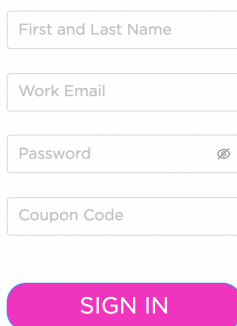
# SIGNING UP

1.  Go to https://youseq.basepairtech.com/

2.  Click on 'Sign up':



3.  Enter your Name, email address and a memorable password, then click 'sign in':
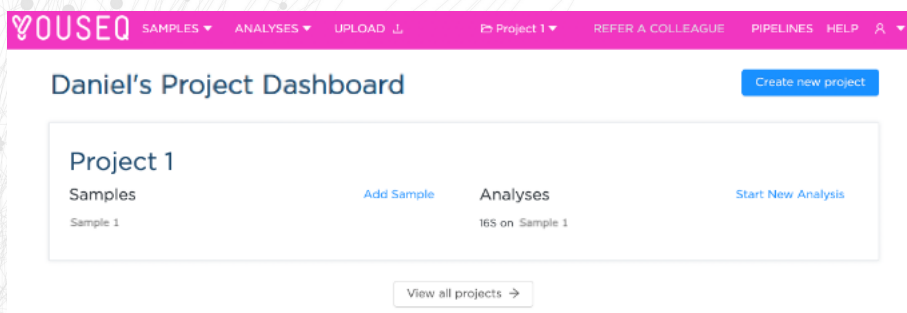


4.  The project dashboard will then open:

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# APPLYING COUPON CODES

Before analysing the data, a coupon must be activated on your account. Contact us on support@youseq.com, along with the kit serial number, to request a coupon code. The kit serial number can be found on the pouch label.

1. Go to your account menu in the top right corner

2. Click on 'Billing & Coupons'

3. Click on the 'Coupons' tab

4. Click on 'Activate coupon'

5. Enter the provided coupon code and click 'activate'
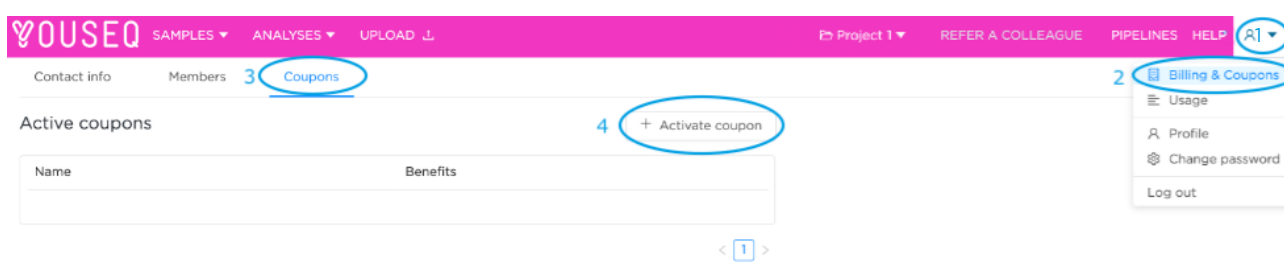


# CREATING A NEW PROJECT

1. Click on the blue "Create New Project" button

2. Enter your Project Name

3. This will create the project overview on the project dashboard:
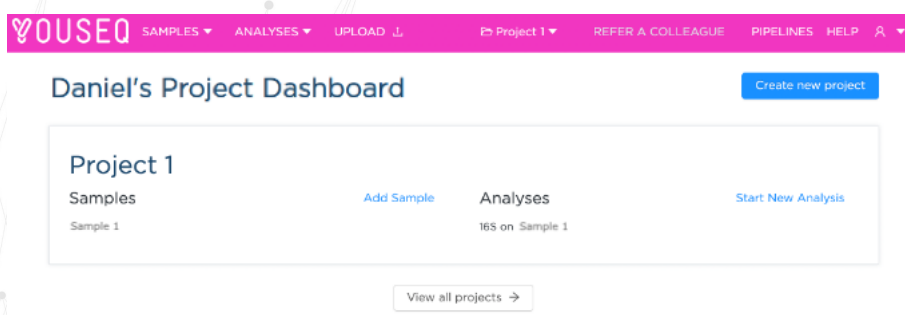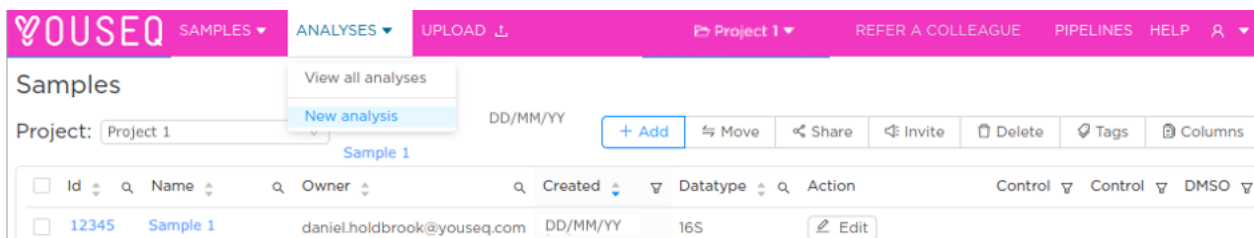


# UPLOADING SAMPLE SEQUENCE DATA

From the project dashboard, sample sequence data can be uploaded using the 'Add Sample' button or 'Upload' tab on the top menu bar. This will bring up a pop-up window that allows files to be drag and dropped for uploading. Sequence data can be uploaded from multiple samples at once. This will upload the files to the current project.

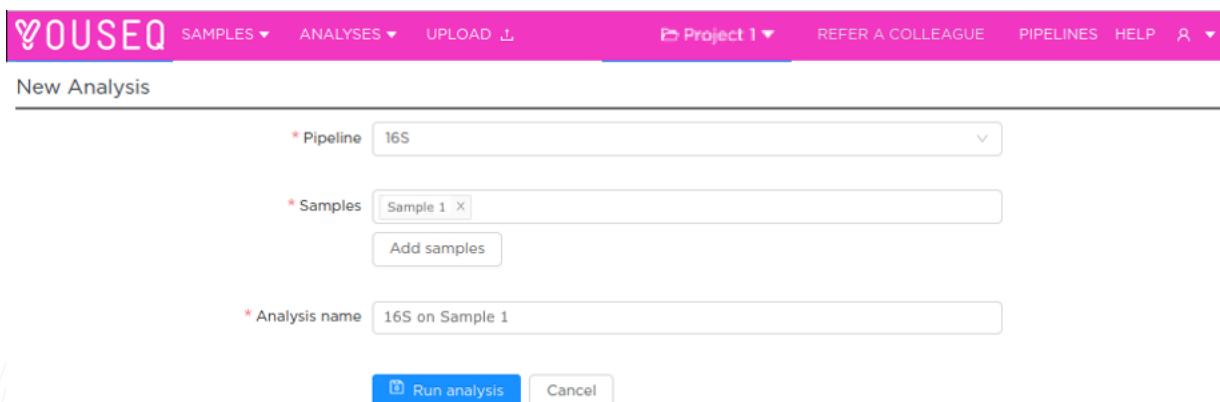The following file types will be accepted: .bam, .fastq, fastq.gz

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# ANALYSING SAMPLE SEQUENCE DATA

1.  Hover over the 'Analyses' tab in the top menu bar

2.  Click on 'New Analysis':



3.  Click on the dropdown menu in the 'Pipeline' field

4.  Select the '16S' pipeline

5.  Select a sample, or several samples (each sample will be analysed individually) by clicking on the 'Add samples' button

6.  Choose a name for the analysis run (a suggested name will be automatically provided)

7.  Click on the blue 'Run analysis' button:

# DOWNLOADING RESULTS

After the samples have been analysed the results can be viewed in a number of formats. The main results page will display the read assignments as a sunburst plot, and as a series of summary tables that contain the top 10 highest occurring assignments at each taxonomic level: Kingdom, Phylum, Class, Order, Family, Genus and Species.

The downloadable PDF report contains a summary of how each read was assigned at each taxonomic level. The raw assignments and summary tables can also be downloaded in csv format.

Option 1: Results can be reviewed online with the interactive sunburst plot: This will be automatically displayed when the analysis window is opened.
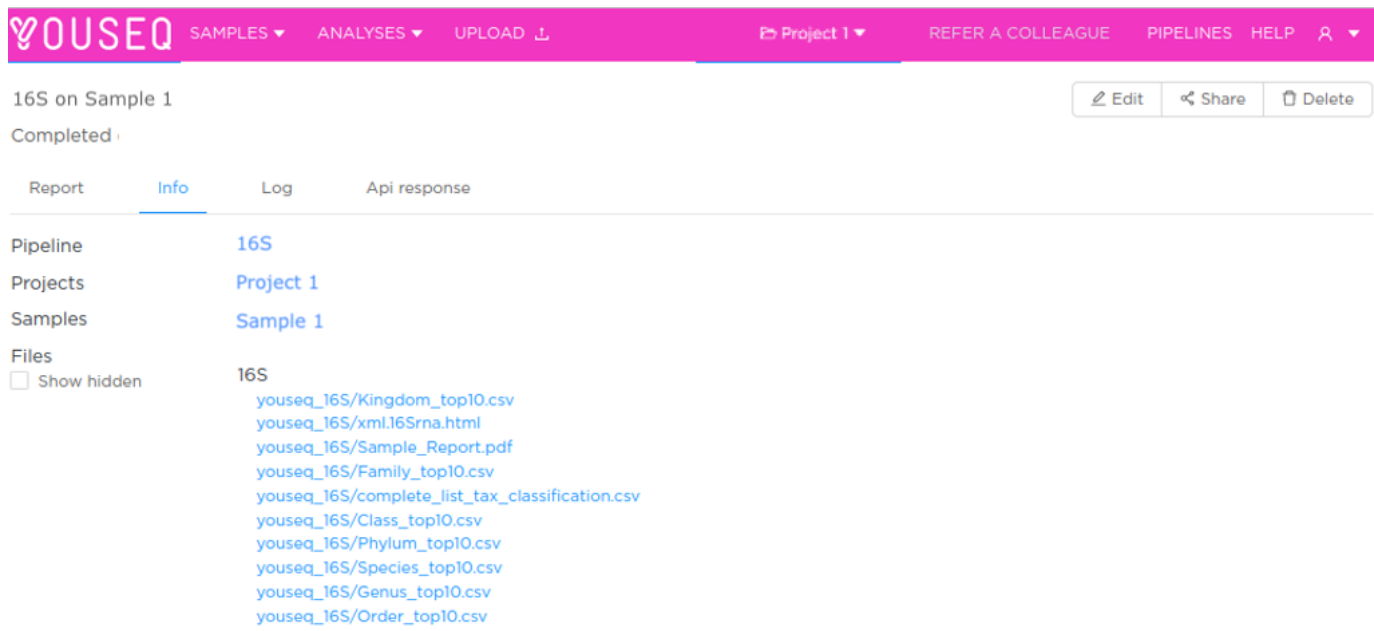
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Option 2: A PDF report of the data can be downloaded by clicking on the 'Download PDF' button, located above the sunburst plot.

Option 3: A number of reports and file types are produced by the analysis pipeline and can be downloaded by clicking on the 'Info' tab and then the name of file from the 'Files' list. The download will start automatically.

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