# Contents

## 1. EDGE ABCs
- 1.1 About EDGE Bioinformatics ........................................ 1
- 1.2 Bioinformatics overview ........................................... 1
- 1.3 Computational Environment ...................................... 3

## 2. Introduction
- 2.1 What is EDGE? ..................................................... 5
- 2.2 Why create EDGE? ............................................... 5

## 3. System requirements
- 3.1 Hardware Requirements .......................................... 7
- 3.2 Ubuntu 14.04 .................................................... 7
- 3.3 CentOS 7 .......................................................... 8

## 4. Installation
- 4.1 EDGE Installation ............................................... 10
- 4.2 Configure SELinux on CentOS ................................ 24
- 4.3 EDGE Docker image ............................................. 25
- 4.4 EDGE VMware/OVF Image ..................................... 25

## 5. Graphic User Interface (GUI)
- 5.1 User Login ....................................................... 29
- 5.2 Upload Files ...................................................... 30
- 5.3 Initiating an analysis job ........................................ 31
- 5.4 Choosing processes/analyses .................................... 34
- 5.5 Submission of a job ............................................. 42
- 5.6 Checking the status of an analysis job ......................... 42
- 5.7 Monitoring the Resource Usage ................................. 44
- 5.8 Management of Jobs ............................................ 44
- 5.9 Project List Table ............................................... 45
- 5.10 Other Methods of Accessing EDGE ......................... 46

## 6. Command Line Interface (CLI)
- 6.1 Configuration File ............................................... 50
- 6.2 Test Run .......................................................... 52
- 6.3 Descriptions of each module ................................. 54
- 6.4 Other command-line utility scripts ......................... 61
# 7 Output 62

7.1 Example Output .............................................................. 63

# 8 Databases 64

8.1 EDGE provided databases .................................................. 64
8.2 Building bwa index .......................................................... 67
8.3 SNP database genomes ....................................................... 67
8.4 Ebola Reference Genomes .................................................. 73

# 9 Third Party Tools 74

9.1 Assembly ........................................................................... 74
9.2 Annotation ......................................................................... 75
9.3 Alignment ........................................................................... 77
9.4 Taxonomy Classification ...................................................... 78
9.5 Phylogeny ........................................................................... 78
9.6 Specialty Genes ................................................................... 79
9.7 Visualization and Graphic User Interface ............................... 79
9.8 Utility ................................................................................. 81
9.9 Amplicon Analysis .............................................................. 84

# 10 FAQs and Troubleshooting 85

10.1 FAQs ................................................................................. 85
10.2 Troubleshooting ............................................................... 87
10.3 Discussions / Bugs Reporting .............................................. 88

# 11 Copyright 89

# 12 Contact Us and Citation 90

12.1 Citation .............................................................................. 90
A quick About EDGE, overview of the Bioinformatic workflows, and the Computational environment

1.1 About EDGE Bioinformatics

EDGE bioinformatics was developed to help biologists process Next Generation Sequencing data (in the form of raw FASTQ files), even if they have little to no bioinformatics expertise. EDGE is a highly integrated and interactive web-based platform that is capable of running many of the standard analyses that biologists require for viral, bacterial/archaeal, and metagenomic samples. EDGE provides the following analytical workflows: pre-processing, assembly and annotation, reference-based analysis, taxonomy classification, phylogenetic analysis, and PCR analysis. EDGE provides an intuitive web-based interface for user input, allows users to visualize and interact with selected results (e.g. JBrowse genome browser), and generates a final detailed PDF report. Results in the form of tables, text files, graphic files, and PDFs can be downloaded. A user management system allows tracking of an individual’s EDGE runs, along with the ability to share, post publicly, delete, or archive their results.

While EDGE was intentionally designed to be as simple as possible for the user, there is still no single ‘tool’ or algorithm that fits all use-cases in the bioinformatics field. Our intent is to provide a detailed panoramic view of your sample from various analytical standpoints, but users are encouraged to have some knowledge of how each tool/algorithm workflow functions, and some insight into how the results should best be interpreted.

1.2 Bioinformatics overview

1.2.1 Inputs:

The input to the EDGE workflows begins with one or more illumina FASTQ files for a single sample. (There is currently limited capability of incorporating PacBio and Oxford Nanopore data into the Assembly module) The user can also enter SRA/ENA accessions to allow processing of publically available datasets. Comparison among samples is not yet supported but development is underway to accommodate such a function for assembly and taxonomy profile comparisons.
1.2.2 Workflows:

Pre-Processing
Assessment of quality control is performed by FAQCS. The host removal step requires the input of one or more reference genomes as FASTA. Several common references are available for selection. Trimmed and host-screened FASTQ files are used for input to the other workflows.

Assembly and Annotation
We provide the IDBA, Spades, and MegaHit (in the development version) assembly tools to accommodate a range of sample types and data sizes. When the user selects to perform an assembly, all subsequent workflows can execute analysis with either the reads, the contigs, or both (default).

Reference-Based Analysis
For comparative reference-based analysis with reads and/or contigs, users must input one or more references (as FASTA or multi-FASTA if there are more than one replicon) and/or select from a drop-down list of RefSeq complete genomes. Results include lists of missing regions (gaps), inserted regions (with input contigs if assembly was performed), SNPs (and coding sequence changes), as well as genome coverage plots and interactive access via JBrowse.

Taxonomy Classification
For taxonomy classification with reads, multiple tools are used and the results are summarized in heat map and radar plots. Individual tool results are also presented with taxonomy dendograms and Krona plots. Contig classification occurs by assigning taxonomies to all possible portions of contigs. For each contig, the longest and best match (using BWA-MEM) is kept for any region within the contig and the region covered is assigned to the taxonomy of the hit. The next best match to a region of the contig not covered by prior hits is then assigned to that taxonomy. The contig results can be viewed by length of assembly coverage per taxa or by number of contigs per taxa.

Phylogenetic Analysis
For phylogenetic analysis, the user must select datasets from near neighbor isolates for which the user desires a phylogeny. A minimum of two additional datasets are required to draw a tree. At least one dataset must be an assembly or complete genome. RefSeq genomes (Bacteria, Archaea, Viruses) are available from a dropdown menu, SRA and FASTA entries are allowed, and previously built databases for some select groups of bacteria are provided. This workflow (see PhaME) is a whole genome SNP-based analysis that uses one reference assembly to which both reads and contigs are mapped. Because this analysis is based on read alignments and/or contig alignments to the reference genome(s), we strongly recommend only selecting genomes that can be adequately aligned at the nucleotide level (i.e. ~90% identity or better). The number of ‘core’ nucleotides able to be aligned among all genomes, and the number of SNPs within the core, are what determine the resolution of the phylogenetic tree. Output phylogenies are presented along with text files outlining the SNPs discovered.

Gene Family Analysis
For specialty gene analysis, the user selects read-based analysis and/or ORF(contig)-based analysis.

For read-based analysis antibiotic resistance genes and virulence genes are detected using Huttenhower lab’s progam ShortBRED. The antibiotic resistance gene database was generated by the developers of ShortBRED using genes from ARDB and Resfams. The virulence genes database was generated by the developers of EDGE using VFDB.

For ORF-based analysis, antibiotic resistance genes are detected using CARD’s (Comprehensive Antibiotic Resistance Database) program RGI (Resistance Gene Identifier). RGI uses CARD’s custom database of antibiotic resistance genes. The virulence genes are detected using ShortBRED with a database generated by the developers of EDGE using VFDB.

Primer Analysis
For primer analysis, if the user would like to validate known PCR primers in silico, a FASTA file of primer sequences must be input. New primers can be generated from an assembly as well.

QIIME analysis
QIIME is an open-source bioinformatics pipeline for performing microbiome analysis from raw DNA sequencing data. EDGE implementation is based on Qiime v1.9.1 and includes demultiplexing and quality filtering, OTU picking, taxonomic assignment, and phylogenetic reconstruction, and diversity analyses and visualizations. Currently, EDGE supports three amplicon types, 16s using GreenGenes database, 16s/18s using SILVA database, and Fungal ITS.

**TargetedNGS analysis**

This is a pipeline for assay design evaluation.

This tool been designed to be robust enough to handle a range of assay designs. Therefore, no major assumptions of input reads are made except that they represent amplicons from a multiplexed targeted amplification reaction and that the reference is comprised of only target regions in the assay, instead of whole genomes. The idea is to survey the reads and delineate whether each reference sequence, or target, is present or absent.

All commands and tool parameters are recorded in log files to make sure the results are repeatable and traceable. The main output is an integrated interactive web page that includes summaries of all the workflows run and features tables, graphical plots, and links to genome (if assembled, or of a selected reference) browsers and to access unprocessed results and log files. Most of these summaries, including plots and tables are included within a final PDF report.

### 1.2.3 Limitations

**Pre-processing**

For host removal/screening, not all genomes are available from a drop-down list, however

**Assembly and Taxonomy Classification**

EDGE has been primarily designed to analyze microbial (bacterial, archaeal, viral) isolates or (shotgun) metagenome samples. Due to the complexity and computational resources required for eukaryotic genome assembly, and the fact that the current taxonomy classification tools do not support eukaryotic classification, EDGE does not fully support eukaryotic samples. The combination of large NGS data files and complex metagenomes may also run into computational memory constraints.

**Reference-based analysis**

We recommend only aligning against (a limited number of) most closely related genome(s). If this is unknown, the Taxonomy Classification module is recommended as an alternative. If the user selects too many references, this may affect runtimes or require more computational resources than may be available on the user’s system.

**Phylogenetic Analysis**

Because this pipeline provides SNP-based trees derived from whole genome (and contig) alignments or read mapping, we recommend selecting genomes within the same species or at least within the same genus.

### 1.3 Computational Environment

#### 1.3.1 EDGE source code, images, and webservers

EDGE was designed to be installed and implemented from within any institute that provides sequencing services or that produces or hosts NGS data. When installed locally, EDGE can access the raw FASTQ files from within the institute, thereby providing immediate access by the biologist for analysis. EDGE is available in a variety of packages to fit various institute needs. **EDGE source code** can be obtained via our GitHub page. To simplify installation, a VM in OVF or a Docker image can also be obtained. A demonstration version of EDGE is currently available at https://bioedgelanl.gov with example data sets available to the public to view and/or re-run. This webserver has 24 cores, 512GB ram with Ubuntu 14.04.3 LTS, and also allows EDGE runs of SRA/ENA data. This webserver does not...
currently support upload of data (due in part to LANL security regulations), however local installations are meant to be fully functional.
2.1 What is EDGE?

EDGE is a highly adaptable bioinformatics platform that allows laboratories to quickly analyze and interpret genomic sequence data. The bioinformatics platform allows users to address a wide range of use cases including assay validation and the characterization of novel biological threats, clinical samples, and complex environmental samples. EDGE is designed to:

- Align to real world use cases
- Make use of open source (free) software tools
- Run analyses on small, relatively inexpensive hardware
- Provide remote assistance from bioinformatics specialists

2.2 Why create EDGE?

EDGE bioinformatics was developed to help biologists process Next Generation Sequencing data (in the form of raw FASTQ files), even if they have little to no bioinformatics expertise. EDGE is a highly integrated and interactive web-based platform that is capable of running many of the standard analyses that biologists require for viral, bacterial/archaeal, and metagenomic samples. EDGE provides the following analytical workflows: quality trimming and host removal, assembly and annotation, comparisons against known references, taxonomy classification of reads and contigs, whole genome SNP-based phylogenetic analysis, and PCR analysis. EDGE provides an intuitive web-based interface for user input, allows users to visualize and interact with selected results (e.g. JBrowse genome browser), and generates a final detailed PDF report. Results in the form of tables, text files, graphic files, and PDFs can be downloaded. A user management system allows tracking of an individual’s EDGE runs, along with the ability to share, post publicly, delete, or archive their results.

While the design of EDGE was intentionally done to be as simple as possible for the user, there is still no single ‘tool’ or algorithm that fits all use-cases in the bioinformatics field. Our intent is to provide a detailed panoramic view of your sample from various analytical standpoints, but users are encouraged to have some insight into how each tool or workflow functions, and how the results should best be interpreted.
Fig. 2.1: Four common Use Cases guided initial EDGE Bioinformatic Software development.
NOTE: There is a demo version of EDGE, found on https://bioedge.lanl.gov/edge_ui/ is run on our own internal servers and is recommended only for testing and demo purposes only.

The current version of the EDGE pipeline has been extensively tested on a Linux Server with Ubuntu 14.04 and CentOS 6.5/7 operating system and will work on 64-bit Linux environments. Perl v5.8 or above is required.

### 3.1 Hardware Requirements

Due to the involvement of several high memory and high CPU consuming steps Minimum requirement: 16GB memory and at least 8 computing CPUs. A higher computer spec is strongly recommended: 256GB memory and 64 computing CPUs. Please ensure that your system has the essential software packages installed properly before running the installing script. The following should be installed by a system administrator (requires sudo).

**Note:** If your system OS is neither Ubuntu 14.04 or Centos 6.5 or 7.0, it may have different packages/libraries name and the newer compiler (gcc5) on newer OS (ex: Ubuntu 16.04) may fail on compiling some of thirdparty bioinformatics tools. We would suggest to use EDGE VMware image or Docker container.

### 3.2 Ubuntu 14.04

1. Install build essential libraries and dependencies:

```bash
sudo apt-get -y update
sudo apt-get install -y build-essential libreadline-gplv2-dev libx11-dev
  libxt-dev libgs10-dev libfreetype6-dev libnrcurses5-dev gfortran
  inkscape libwwww-perl libxml1-libxml-perl libperlio-gzip-perl
  zlib1g-dev zip unzip libjson-perl libpng12-dev cpanminus default-jre
  firefox wget curl csh liblapack-dev libblas-dev libatlas-dev
  libcairo2-dev libssh2-1-dev libssl-dev libcurl4-openssl-dev bzip2
```
2. Install Apache2 for EDGE UI:

```bash
sudo apt-get install apache2
sudo a2enmod cgi proxy proxy_http headers
```

3. Install packages for user management system:

```bash
sudo apt-get install sendmail mysql-client mysql-server phpMyAdmin tomcat7
```

### 3.3 CentOS 7

1. Install libraries and dependencies by yum:

```bash
# add epel repository
sudo yum -y install epel-release

sudo yum install -y libX11-devel readline-devel libXt-devel ncurses-devel
inkscape \ exat expat-devel freetype freetype-devel zlib zlib-devel perl-App-cpanminus \ perl-Test-Most blas-devel atlas-devel lapack-devel libpng12 libpng12-devel \ perl-XML-Simple perl-JSON csh gcc gcc-c++ make binutils gd qsl-devel git
```

2. Update perl tools:

```bash
sudo cpanm App::cpanoutdated
sudo su -
cpan-outdated -p | cpanm
exit
```

3. Install perl modules by cpanm:

```bash
sudo cpanm -f Bio::Perl Net::Ping
sudo cpanm Graph Time::Piece Hash::Merge PerlIO::gzip Heap::Simple::XS File::Next
sudo cpanm Algorithm::Munkres Archive::Tar Array::Compare Clone Convert::Binary::C
sudo cpanm HTML::Template HTML::TableExtract List::MoreUtils PostScript::TextBlock
sudo cpanm SOAP::Lite SVG SVG::Graph Set::Scalar Sort::Naturally
Spreadsheet::ParseExcel
sudo cpanm CGI::Simple GraphViz XML::Parser::PerlSAX XML::Simple Term::ReadKey
```

4. Install package for httpd for EDGE UI:

```bash
sudo yum -y install httpd
sudo systemctl enable httpd && sudo systemctl start httpd
```
5. Install packages for user management system:

```
sudo yum -y install sendmail mariadb-server mariadb php phpMyAdmin tomcat
sudo systemctl enable tomcat && sudo systemctl start tomcat
```

6. Configure firewall for ssh, http, https, and smtp:

```
sudo firewall-cmd --permanent --add-service=ssh
sudo firewall-cmd --permanent --add-service=http
sudo firewall-cmd --permanent --add-service=https
sudo firewall-cmd --permanent --add-service=smtp
sudo firewall-cmd --reload
```

7. Disable SELinux:

```
As root edit /etc/selinux/config and set SELINUX=disabled

Restart the server to make the change
```

**Warning:** This is for development version of EDGE. Stable version (v1.5) is here.
CHAPTER 4

Installation

Note: These instructions assumes Ubuntu 14 and CentOS 7

4.1 EDGE Installation

Note: A base install is ~8GB for the code base and ~330GB for the databases.

1. Please ensure that your system has the essential software building packages (page 7) installed properly before proceeding following installation.

2. Download the codebase, databases and third party tools:

```
## Codebase is ~107Mb and contains all the scripts and HTML needed to make EDGE run

## Third party tools is ~2.9Gb and contains the underlying programs needed to do the analysis

## Pipeline database is ~11.7Gb and contains the other databases needed for EDGE

## BWA index is ~33Gb and contains the databases for bwa taxonomic identification pipeline

## HOST genomes BWA index is ~41Gb for Host removal, including human, bacteria, phiX, viruses, invertebrate vectors of human pathogens
```

## NCBI Genomes is ~16.4Gb and contain the full genomes for prokaryotes and some viruses

## GOTTCHA database is ~16Gb and contains the custom databases for the GOTTCHA taxonomic identification pipeline

## Amplicon database is ~78Mb and contains the databases for Qiime 16s and 18s ITS pipeline

## NT database is ~25Gb and contains the NCBI nt database for contig identification

## ShortBRED database is ~27Mb and contains the databases used by ShortBRED for virulence factors and read based antibiotic resistance analysis

## PanGIA database is ~15Gb and contains the databases used for the PanGIA taxonomic identification pipeline

## Diamond database is ~15Gb and contains the databases from RefSeq for protein based taxonomic identification

(Optional)
## Other Host bwa index ~17.2Gb for host removal, including pig, sheep, cow, monkey, hamster. and goat.
wget -c ftp://ftp.lanl.gov/public/genome/EDGE/dev/edge_dev_otherHostIndex.tgz

## GOTTCHA2 databases are 23.8Gb, 28.5Gb and 1.7Gb and contains the custom databases for the GOTTCHA2 taxonomic identification pipeline

## For machine with < 32Gb memory, we suggest to use the smaller BWA index (~13Gb) and contains the databases for bwa taxonomic identification pipeline

### Warning: Be patient; the database files are huge.

3. Unpack main archive:

    tar -xvzf edge_dev_main.tgz

### Note: The main directory, edge_dev, will be created.

Create a link from edge to that directory:
4. Unpack the third party software into main directory (edge):

```
ln -sf edge_dev edge
```

**Note:** You should see a thirdParty directory inside the edge directory.

5. Unpack the databases:

```
# unpack databases
bin -xvzf edge_dev_thirdParty_software.tgz -C edge/
```

**Note:** At this point, you should see a database directory and the edge directory.

6. Create the symlink from edge to the database directory:

```
ln -s 'pwd'/database edge/database
```

**Note:** This will keep the database directory outside of the edge install location. Should you need to reinstall the code base you will not need to redownload/install the databases.

7. Installing pipeline:

```
cd edge
./INSTALL.sh
```

**Note:** When installing JBrowse, it may require internet connection.

**Note:** If the machine is shared and used with others, the system installed tools version may not be compatible with EDGE. In this case, we would suggest to use force option ./INSTALL.sh force to install all list tools locally.

It will install the following depended tools (page 74).

- Assembly
  - idba
  - spades
  - megahit
• Annotation
  – prokka
  – RATT
  – tRNAscan
  – barrnap
  – BLAST+
  – blastall
  – phageFinder
  – glimmer
  – aragorn
  – prodigal
  – tbl2asn
  – ShortBRED

• Alignment
  – hmmer
  – infernal
  – bowtie2
  – bwa
  – mummer
  – RAPSearch2

• Taxonomy
  – kraken
  – metaphlan
  – kronatools
  – gottcha

• Phylogeny
  – FastTree
  – RAxML

• Utility
  – bedtools
  – R
  – GNU_parallel
  – tabix
  – JBrowse
  – primer3
  – samtools
- sratoolkit
- ea-utils

- Perl_Modules
  - perl_parallel_forkmanager
  - perl_excel_writer
  - perl_archive_zip
  - perl_string_approx
  - perl_pdf_api2
  - perl_html_template
  - perl_html_parser
  - perl_JSON
  - perl_bio_phylo
  - perl_xml_twig
  - perl_egi_session

- Python_Packages
  - Anaconda2
  - Anaconda3

8. Restart the Terminal Session to allow $EDGE_HOME to be exported.

**Note:** After running INSTALL.sh successfully, the binaries and related scripts will be stored in the ./bin and ./scripts directory. It also writes EDGE_HOME environment variable into .bashrc or .bash_profile.

## 4.1.1 Testing the EDGE Installation

After installing the packages above, it is highly recommended to test the installation:

```
> cd $EDGE_HOME/testData
> ./runAllTest.sh
```
There are 17 module/unit tests which took around 2 hours 14 mins in our testing environments. (64 cores 2.30GHz, 512GB ram with CentOS-7.1.1503). You will see test output on the terminal indicating test successes and failures. The Specialty Genes Profiling test will fail in this stage since it requires virulence database imported and configured. You can test it again after database created and configured. Some tests may fail due to missing external applications/modules/packages or failed installation. These will be noted separately in the $EDGE_HOME/testData/runXXXXTest/TestOutput/error.log or log files in each modules. If these are related to features of EDGE that you are not using, this is acceptable. Otherwise, you’ll want to ensure that you have the EDGE installed correctly. If the output doesn’t indicate any failures, you are now ready to use EDGE through command line. To take advantage of the user friendly GUI, please follow the section below to configure the EDGE Web server.

4.1.2 Apache Web Server Configuration

1. Modify/Check sample apache configuration file:

```text
For Ubuntu

Double check $EDGE_HOME/edge_ui/apache_conf/edge_apache.conf alias directories match the EDGE installation path at line 2,5,6,16,17,29,59.

The default is configured as http://localhost/edge_ui/ or http://www.yourdomain.edge_ui/

For CentOS

Double check $EDGE_HOME/edge_ui/apache_conf/edge_httpd.conf alias directories the match EDGE
```

4.1. EDGE Installation
2. Confirm apache/httpd user and groups are edge:

For Ubuntu

The user and group can be edited at /etc/apache2/envvars and the variables are APACHE_RUN_USER and APACHE_RUN_GROUP.

For CentOS

The User and Group on lines 66 and 67 in $EDGE_HOME/edge_ui/apache_conf/centos_httpd.conf should be edge

```bash
## Make APACHE_RUN_USER have Permission to write
> sudo chown -R xxxxx $EDGE_HOME/edge_ui $EDGE_HOME/edge_ui/JBrowse/data
#(xxxxx is the APACHE_RUN_USER value)

> sudo chgrp -R xxxxx $EDGE_HOME/edge_ui $EDGE_HOME/edge_ui/JBrowse/data
#(xxxxx is the APACHE_RUN_GROUP value)
```

3. (Optional) If users are behind a corporate proxy for internet:

Please add proxy info into $EDGE_HOME/edge_ui/apache_conf/edge_apache.conf or $EDGE_HOME/edge_ui/apache_conf/edge_httpd.conf

```bash
# Add following proxy env
SetEnv http_proxy http://yourproxy:port
SetEnv https_proxy http://yourproxy:port
SetEnv ftp_proxy http://yourproxy:port
```

4. Copy configuration files to the appropriate directories:

For Ubuntu

```bash
> sudo cp $EDGE_HOME/edge_ui/apache_conf/edge_apache.conf /etc/apache2/conf-available/
> sudo ln -s /etc/apache2/conf-available/edge_apache.conf /etc/apache2/conf-enabled/
```

For CentOS

```bash
> sudo cp $EDGE_HOME/edge_ui/apache_conf/edge_httpd.conf /etc/httpd/conf.d/
> sudo cp $EDGE_HOME/edge_ui/apache_conf/centos_httpd.conf /etc/httpd/conf/httpd.conf
```

5. Restart the apache2/httpd to activate the new configuration:

For Ubuntu

```bash
> sudo service apache2 restart
```

For CentOS

```bash
> sudo systemctl restart httpd
```
4.1.3 User Management System Installation: MySQL

**Note:** Setup two temporary environmental variables:

<table>
<thead>
<tr>
<th>UN</th>
<th>username</th>
</tr>
</thead>
<tbody>
<tr>
<td>PW</td>
<td>password</td>
</tr>
</tbody>
</table>

These will be used when setting up the user management system.

**Note:** If you were using the user management system and are updating from EDGE v1.1 to this version. You only need to run the commands below and continue to install tomcat:

```sql
mysql -u $UN -p userManagement
mysql> ALTER TABLE projects ADD full_name VARCHAR(255);
mysql> ALTER TABLE users_projects add display varchar(25) NOT NULL DEFAULT 'yes';
mysql> ALTER TABLE users_projects ADD CONSTRAINT displayChk CHECK (display IN ('yes', 'no'));
```

1. Start mysql (if it is not already running):

   For Ubuntu
   ```bash
   > sudo service mysql start
   ```
   For CentOS
   ```bash
   sudo systemctl start mariadb.service && sudo systemctl enable mariadb.service
   ```

2. Secure mysql:

   **Note:** The root password here is for the mysql root and not the system root.

   ```bash
   > sudo mysql_secure_installation
   ```
   (a) Enter root password (likely none)
   (b) Set root password? Yes
   (c) Enter new root password.
   (d) Re-enter new root password.
   (e) Remove anonymous users? Yes
   (f) Disallow root login remotely? Yes
   (g) Remove test database and access to it? Yes
   (h) Reload privilege table now? Yes

3. Create database: userManagement:

   ```bash
   > cd $EDGE_HOME/userManagement
   > mysql -p -u root
   ```

4.1. EDGE Installation
mysql> create database userManagement;
mysql> use userManagement;

4. Load userManagement_schema.sql:

mysql> source userManagement_schema.sql;

5. Load userManagement_constrains.sql:

mysql> source userManagement_constrains.sql;

6. Create an user account and grant all privileges to user:

Note: This is the database user (not an individual account).
Replace with the appropriate values:

username: yourDBUsername
password: yourDBPassword

mysql> CREATE USER 'yourDBUsername'@'localhost' IDENTIFIED BY 'yourDBPassword';
mysql> GRANT ALL PRIVILEGES ON userManagement.* to 'yourDBUsername'@'localhost';
mysql> exit;

4.1.4 User Management System Installation: Tomcat

1. Configure tomcat basic auth to secure /user/admin/register web service:

Warning: Run this code only once!

Note: The username and password here should be the same as the database user.
Update the values for the username and password accordingly before running the code.
This adds the following to /usr/share/tomcat/conf/tomcat-users.xml:

For Ubuntu

```bash
sudo sed -i 's@</tomcat-users>@<role rolename="admin"/>@'yourAdminName'" password=""${PW}" roles="admin"/@g' /var/lib/tomcat7/conf/tomcat-users.xml
```

For CentOS

```bash
sudo sed -i 's@<!-- <role rolename="admin"/> -->@<!-- <role rolename="admin"/> -->
<role rolename="admin"/>
<user username=""${UN}"" password=""$PW"" roles="admin"/>@g' /usr/share/tomcat/conf/tomcat-users.xml
```
2. Update inactive timeout to a more reasonable number 4320 min (3 days) from default (30mins) in /var/lib/tomcat7/conf/web.xml or /etc/tomcat/web.xml

**Note:** This is modifying the following code:

```
<!-- <session-config>
   <session-timeout>30</session-timeout>
</session-config> -->
```

For Ubuntu

```bash
sudo sed -i 's@<session-timeout>.*</session-timeout>@<session-timeout>4320</session-timeout>@g' /var/lib/tomcat7/conf/web.xml
```

For CentOS

```bash
sudo sed -i 's@<session-timeout>.*</session-timeout>@<session-timeout>4320</session-timeout>@g' /usr/share/tomcat/conf/web.xml
```

3. Add memory constrains to Java:

**Warning:** Run this code only once!

**Note:** This will add the following line to the appropriate file:

```
JAVA_OPTS=" -Xms256M -Xmx1024M -XX:PermSize=256m -XX:MaxPermSize=512m"
```

For Ubuntu

```bash
sudo sed -i 's@#JAVA_OPTS@JAVA_OPTS="-Xms256m -Xmx1024m -XX:PermSize=256m -XX:MaxPermSize=512m"
#JAVA_OPTS@g' /usr/share/tomcat7/bin/catalina.sh
```

For CentOS

```bash
sudo sed -i 's@#JAVA_OPTS@JAVA_OPTS="-Xms256m -Xmx1024m -XX:PermSize=256m -XX:MaxPermSize=512m"
#JAVA_OPTS@g' /usr/share/tomcat/conf/tomcat.conf
```

4. Restart tomcat server:

For Ubuntu

```bash
sudo service tomcat7 restart
```

For CentOS

```bash
sudo systemctl restart tomcat
```

5. Copy database connector clients to appropriate lib directory:

For Ubuntu

```bash
sudo cp mysql-connector-java-5.1.34-bin.jar /usr/share/tomcat7/lib/
```
6. Centos Only: Update the MySQL database driver to be used:

```
sed -i 's@driverClassName=.*$@driverClassName="org.mariadb.jdbc.Driver"@' $EDGE_HOME/userManagement/userManagementWS.xml
```

7. Deploy userManagement to tomcat server:

**Note:** For CentOS the userManagementWS.xml should have:

```
driverClassName="org.mariadb.jdbc.Driver"
```

Please check and confirm this before deploying userManagement.

For Ubuntu

```
sudo cp userManagementWS.war /var/lib/tomcat7/webapps/
sudo cp userManagement.war /var/lib/tomcat7/webapps/
sudo chown 755 /var/lib/tomcat7/webapps/* war
sudo cp userManagementWS.xml /var/lib/tomcat7/conf/Catalina/localhost/
sudo chown 744 /var/lib/tomcat7/conf/Catalina/localhost/userManagementWS.xml
```

For CentOS

```
sudo cp userManagementWS.war /var/lib/tomcat/webapps/
sudo cp userManagement.war /var/lib/tomcat/webapps/
sudo chown 755 /var/lib/tomcat/webapps/* war
sudo cp userManagementWS.xml /etc/tomcat/Catalina/localhost/
sudo chown 744 /etc/tomcat/Catalina/localhost/userManagementWS.xml
```

8. Modify the username/password in userManagementWS.xml:

For Ubuntu

```
sudo sed -i 's@username=.*$@username="'${UN}'"@' /var/lib/tomcat7/conf/Catalina/localhost/userManagementWS.xml
sudo sed -i 's@password=.*$@password="'${PW}'"@' /var/lib/tomcat7/conf/Catalina/localhost/userManagementWS.xml
```

For CentOS

```
sudo sed -i 's@username=.*$@username="'${UN}'"@' /etc/tomcat/Catalina/localhost/userManagementWS.xml
sudo sed -i 's@password=.*$@password="'${PW}'"@' /etc/tomcat/Catalina/localhost/userManagementWS.xml
```

9. Update sys.properties in the userManagement deployment:

**Note:** Tomcat should automatically unarchive the .war files.
The default configuration is to have the user management system on localhost with email notifications turned off.

Modify the user management sys.properties if you want to change the default behavior.

You will need to copy the sys.properties files to the directory of the userManagement deployment.

For Ubuntu

```
sudo cp $EDGE_HOME/userManagement/sys.properties /var/lib/tomcat7/webapps/
    →userManagement/WEB-INF/classes/sys.properties
sudo chmod 744 /var/lib/tomcat7/webapps/userManagement/WEB-INF/classes/
    →sys.properties
```

For CentOS

```
sudo cp $EDGE_HOME/userManagement/sys.properties /usr/share/tomcat/
    →webapps/userManagement/WEB-INF/classes/sys.properties
sudo chmod 744 /usr/share/tomcat/webapps/userManagement/WEB-INF/classes/
    →sys.properties
```

10. Restart tomcat server:

For Ubuntu

```
sudo service tomcat7 restart
```

For CentOS7

```
sudo systemctl restart tomcat
```

11. Setup admin user:

```
Note: The script createAdminAccount.pl creates an admin user account for EDGE userManagement.

Update email (-e), First Name (-fn), and Last Name (-ln) appropriately.

It will ask tomcat service username and password (tomcat-users.xml:) before creating EDGE user account (email).

If “HTTP Status 401” error shows, please make sure the tomcat username and password in the first step match with what entered here.

Should this script fail, the userManagement is not set up correctly.

```
perl createAdminAccount.pl -e <email> -fn <first name> -ln <last name>
```

12. Enable userManagement in EDGE sys.properties:

```
Note: See EDGE Configuration (page 23) below

> sed -i 's@user_management=.*$@user_management=1@g' $EDGE_HOME/edge_ui/
    →sys.properties
> sed -i 's@edge_user_management_url=.*$@edge_user_management_url=http://
    →localhost/userManagement@0g' $EDGE_HOME/edge_ui/sys.properties
```

13. Optional: configure social (facebook, google, windows live, linkedin) login function:
• modify $EDGE_HOME/edge_ui/javascript/social.js, change apps id you created on each social media.

**Note:** You need to register your EDGE’s domain on each social media to get apps id. e.g.: A FACEBOOK app needs to be created and configured for the domain and website set up by EDGE. see [https://developers.facebook.com/](https://developers.facebook.com/) and StackOverflow Q&A

Google+

Windows

LinkedIn

14. Optional: configure sendmail to use SMTP to email out of local domain:

• edit /etc/mail/sendmail.cf and edit this line:
  
  # “Smart” relay host (may be null) DS

• and append the correct server right next to DS (no spaces);
  
  # “Smart” relay host (may be null) DSmail.yourdomain.com

• Then, restart the sendmail service

  > sudo service sendmail restart

4.1.5 MYSQL Databases CREATION

**Note:** This requires that MySQL is installed and running.

**Note:** EDGE provides Virulence Factors, Metadata, and Pathogen sql dump files which will be used for Speciality Gene Profiling module, Sample MetaData module and Pathogen Detection module, respectively. You will need configure the database info in the $EDGE_HOME/edge_ui/sys.properties. See [EDGE Configuration](#) (page 23) below

1. Change directory into database:

   ```sh
cd $EDGE_HOME/SQLdbfile
   ```

2. Run install script for databases and Grant privilege database user to have access to the databases:

   ```bash
   mysql -u root -p
   mysql> source virulence_db.sql ;
   mysql> GRANT ALL PRIVILEGES ON virulenceFactors.* to 'yourDBUsername'@'localhost';
   
   mysql> create database edgeDB;
   mysql> use edgeDB;
   mysql> source edge_db.sql ;
   mysql> GRANT ALL PRIVILEGES ON edgeDB.* to 'yourDBUsername'@'localhost';
   
   mysql> create database pathogens ;
   mysql> use pathogens;
   mysql> source pathogen_db.sql ;
   ```

4.1. EDGE Installation
mysql> GRANT ALL PRIVILEGES ON pathogens.* to 'yourDBUsername'@'localhost';
mysql> exit;

3. Configure Virulence, Metadata and Pathogen Database information:

Edit $EDGE_HOME/edge_ui/sys.properties with the appropriate database username and password.

# Virulence Factor database
VFDB_dbhost = localhost
VFDB_dbport = 3306
VFDB_dbname = virulenceFactors
VFDB_dbuser = edge_user
VFDB_dbpasswd = edge_user_password

## configure edge pathogen detection 1: with 0: without
edge_pathogen_detection=0
pathogen_dbhost=localhost
pathogen_dbname=pathogens
pathogen_dbuser=edge_user
pathogen_dbpasswd=edge_user_password

## configure edge sample metadata option 1: with 0: without
edge_sample_metadata=0
edge_dbhost=localhost
edge_dbname=edgeDB
edge_dbuser=edge_user
edge_dbpasswd=edge_user_password

4.1.6 EDGE configuration

Note: EDGE system configuration file is $EDGE_HOME/edge_ui/sys.properties. You can edit this file to turn on/off EDGE functions/modules here. (on=1, off=0);

1. Add EDGE GUI admin info:

According to User Management system installation step 11:

edgeui_admin=admin@my.com
edgeui_admin_password=admin

2. Turn on user management system:

Note: This assumes localhost is the domain. Update the domain as necessary. If user management system is not in the same domain with EDGE:

edge_user_management_url=http://www.someother.com/userManagement

# If you have User Management system enabled.
user_management=1
edge_user_management_url=http://localhost/userManagement

4.1. EDGE Installation
3. Turn on upload function:

```plaintext
user_upload=1
user_upload_maxFileSize='5gb'
```

4. Turn on project intermediate files clean up:

```plaintext
#Clean up old bam/sam/fastq/gz files (based on file age) in project directories
edgeui_proj_store_days=10
```

5. Set up the archive directory:

```plaintext
#The archive space is for offload the main computational disk space
edgeui_archive=/path/to/archive_SPACE
```

6. Turn on Social Login function:

```plaintext
#If you have User Management system installation step 18 done.
user_social_login=1
```

7. Turn on job submission for SGE/UGE cluster environment:

Note: make sure the user/apache user running EDGE is a cluster user.

qconf -suserl to check cluster user list

```plaintext
#Configure cluster system 1: with 0: without
cluster=1

## sge environment configuration
sge_bin=/cm/shared/apps/sge/2011.11p1/bin/linux-x64
sge_root=/cm/shared/apps/sge/2011.11p1
sge_cell=default

## edge job submission configuration
cluster_job_notify=edge@yourdomain.com
cluster_job_prefix=EDGE_pipeline_
cluster_qsub_options=
cluster_job_resource=h_vmem=6G -pe smp <CPU> -binding linear:<CPU/2>
cluster_job_max_cpu=64
```

### 4.2 Configure SELinux on CentOS

Warning: This is not complete.

1. Install semanage (if not already installed):

```bash
> sudo yum install -y policycoreutils-python setroubleshoot
```

2. Allow httpd to access $EDGE_HOME, the databases, and read/write to the EDGE_input/EDGE_output:

```
```
3. Allow httpd to execute cgi-scripts in $EDGE_HOME/edge_ui/cgi-bin/:

```
> sudo semanage boolean -m --on httpd_enable/cgi
> sudo semanage fcontext -a -t httpd_sys_script_exec_t "$/EDGE_HOME/edge_ui/cgi-bin(/.*)?"
```

4. Allow httpd to connect to mysql database:

```
> sudo semanage boolean -m --on httpd_can_network_connect_db
```

5. Optional: Allow httpd to work with nfs and send mail:

```
> sudo semanage boolean -m --on httpd_use_nfs
> sudo semanage boolean -m --on httpd_can_sendmail
```

6. REQUIRED: Apply the rules:

```
> sudo restorecon -R $EDGE_HOME
> sudo restorecon -R $EDGE_HOME/database/
> sudo restorecon -R $EDGE_HOME/edge_ui/EDGE_input/
> sudo restorecon -R $EDGE_HOME/edge_ui/EDGE_output/
```

### 4.3 EDGE Docker image

**Note:** Not updated for develop.

EDGE has a lot of dependencies and can (but doesn’t have to) be very challenging to install. The EDGE docker gets around the difficulty of installation by providing a functioning EDGE full install on top of official Ubuntu 14.04.3 LTS. You can find the image and usage at docker hub.

### 4.4 EDGE VMware/OVF Image

**Note:** Not updated for develop.

You can start using EDGE by launching a local instance of the EDGE VM. The image is built by VMware Fusion v8.5.0. The pre-built EDGE VM is provided in Open Virtualization Format (OVA/OVF) which is supported by major virtualization players, such as VMware / VirtualBox / Red Hat Enterprise Virtualization, etc. Unfortunately, this may not always work perfectly, as each VM technology seems to use slightly different OVA/OVF implementations that aren’t entirely compatible. For example, the auto-deploy feature and the path of auto-mount shared folders between host and guest which are used in the EDGE VMware image may not be compatible with other VM technologies (or may need advanced tweaks). Therefore, we highly recommended using VMware Workstation Player which is free for non-commercial, personal and home use. The EDGE databases are not included in the image. You will need to
Download and mount the databases, input and output directories after you launch the VM. Below are instructions to run EDGE VM on your local server:

1. Install VMware Workstation player.
2. Download the EDGE VM image (EDGE_vm_1.5_RC1.ova) from LANL FTP site.
3. Download the EDGE databases and follow instruction to unpack them.
4. Import the EDGE VM image. If the first time import fails (due to strict OVF specification), click “Retry”; this will allow import of the image.

5. Configure your VM.
   • Allocate at least 10GB memory to the VM
   • Share/Mount the database, input and output directory to the “database”, “EDGE_input” and “EDGE_output” directory in the VM guest OS.
6. Start EDGE VM.
7. Access EDGE VM using host browser (http://<IP_OF_VM>/edge_ui/).

Note that the IP address will also be provided when the instance starts up.

8. Control EDGE VM with default credentials
   • OS Login: edge/edge
   • EDGE user: admin@my.edge/admin
   • MariaDB root: root/edge
Importing the Virtual Machine

The import failed because /Users/218819/Downloads/EDGE_vm_1.5_RC1.ova did not pass OVF specification conformance or virtual hardware compliance checks.

Click Retry to relax OVF specification and virtual hardware compliance checks and try the import again, or click Cancel to cancel the import. If you retry the import, you might not be able to use the virtual machine in VMware Fusion.

☐ Do not show this message again

Cancel  Retry

Enable Shared Folders

<table>
<thead>
<tr>
<th>On</th>
<th>Name</th>
<th>Folder</th>
<th>Permissions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>database</td>
<td>database</td>
<td>Read &amp; Write</td>
</tr>
<tr>
<td>✔</td>
<td>EDGE_input</td>
<td>EDGE_input</td>
<td>Read &amp; Write</td>
</tr>
<tr>
<td>✔</td>
<td>EDGE_output</td>
<td>EDGE_output</td>
<td>Read &amp; Write</td>
</tr>
</tbody>
</table>

Add Device...
CentOS Linux 7 (Core)
Kernel 3.10.8-229.el7.x86_64 on an x86_64

===================================================================
EDGE Bioinformatics Virtual Machine RC1
===================================================================
Access to EDGE web-interface from host OS:
   http://192.168.185.132/edge_ui/

===================================================================
CPU usage...........: 1.70, 0.41, 0.14 (1, 5, 15 min)
Memory used.........: 225 MB / 11566 MB
Swap in use.........: 0 MB
Processes running...: 467
System uptime.......: 0 days 0 hours 0 minutes 36 seconds
Disk space ROOT.....: 27G remaining
Disk space INPUT....: 1.4T remaining
Disk space OUTPUT...: 116G remaining

===================================================================

localhost login:
CHAPTER 5

Graphic User Interface (GUI)

The User Interface was mainly implemented in JQuery Mobile, CSS, javascript and perl CGI. It is a HTML5-based user interface system designed to make responsive web sites and apps that are accessible on all smartphone, tablet and desktop devices. (see How to make an app icon on the mobile device (page 85))

See GUI page

5.1 User Login

A user management system has been implemented to provide a level of privacy/security for a user’s submitted projects. When this system is activated, any user can view projects that have been made public, but other projects can only be accessed by logging into the system using a registered local EDGE account or via an existing social media account (Facebook, Google+, Windows, or LinkedIn). The users can then run new jobs and view their own previously run projects or those that have been shared with them. Click on the upper-right user icon will pop up an user login window.
5.2 Upload Files

For LANL security policy, the function is not implemented at https://bioedge.lanl.gov/edge_ui/.

EDGE supports input from NCBI Sequence Reads Archive (SRA) and select files from the EDGE server. To analyze users’ own data, EDGE allows users to upload fastq, fasta and genbank (which can be in gzip format) and text (txt). Max file size is ‘5gb’ and files will be kept for 7 days. Choose “Upload files” from the navigation bar on the left side of the screen. Add users files by clicking “Add Files” button or drag files to the upload feature window. Then, click “Start Upload” button to upload files to EDGE server.
5.3 Initiating an analysis job

Choose “Run EDGE” or “Run Qiime” from the navigation bar on the left side of the screen.

5.3.1 Run EDGE

Click “Run EDGE” will cause a section to appear called “Input Raw Reads.” Here, you may browse the EDGE Input Directory and select FASTQ files containing the reads to be analyzed. EDGE supports gzip compressed fastq files. At minimum, EDGE will accept two FASTQ files containing paired reads and/or one FASTQ file containing single reads as initial input. Alternatively, rather than providing files through the EDGE Input Directory, you may decide to use as input reads from the Sequence Read Archive (SRA). In this case, select the “yes” option next to “Input from NCBI Sequence Reads Archive” and a field will appear where you can type in an SRA accession number.

In addition to the input read files, you have to specify a project name. The project name is restricted to only alphanumerical characters and underscores and requires a minimum of three characters. For example, a project name of “E.
coli. Project” is not acceptable, but a project name of “E_coli_project” could be used instead. In the “Description” fields you may enter free text that describes your project. If you would like, you may use as input more reads files than the minimum of 2 paired read files or one file of single reads. To do so, click “additional options” to expose more fields, including two buttons for “Add Paired-end Input” and “Add Single-end Input”.

In the “additional options”, there are several more options, for output path, number of CPUs, and config file. In most cases, you can ignore these options, but they are described briefly below.

5.3.2 Run Qiime

Click “Run Qiime” will cause a section to appear for Qiime input and parameters. Currently, EDGE supports three amplicon types, 16s using GreenGenes database, 16s/18s using SILVA database, and Fungal ITS. Similar to “Run EDGE”, input can be either from the Sequence Read Archive (SRA, internet required) or browse the EDGE Input Directory based on the reads type. The Qiime pipeline support one Reads Type in a run, paired-reads, single end reads, or de-multiplexed reads directory. There is also a mapping file input requirement which is adapted from QIIME Metadata mapping file. This mapping file contains all of the information about the samples necessary to perform the
data analysis. It is in tab-delimited format. In general, the header for this mapping file starts with a pound (#) character, and generally requires a “SampleID”, “BarcodeSequence”, and a “Description”.

Mapping File Example:

<table>
<thead>
<tr>
<th>#SampleID</th>
<th>BarcodeSequence</th>
<th>SampleType</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>ACATAACCGTCTA</td>
<td>Stool</td>
<td>MiSeq,metagenome</td>
</tr>
<tr>
<td>Sample2</td>
<td>ACCATGCGTCTA</td>
<td>Blood</td>
<td>MiSeq,clinical</td>
</tr>
<tr>
<td>Control1</td>
<td>AGCCATCGTCTA</td>
<td>Control</td>
<td>Negative</td>
</tr>
<tr>
<td>Control2</td>
<td>CGTCTAACCATG</td>
<td>Control</td>
<td>Spike-in Control</td>
</tr>
</tbody>
</table>

When the reads type is “De-multiplexed Reads Directory”, the mapping file needs a ‘Files’ column with FASTQ filenames for each sampleID. It can be paired-end or single-end FASTQ file and paired-end FASTQ files are comma-separated.

<table>
<thead>
<tr>
<th>#SampleID</th>
<th>Files</th>
<th>SampleType</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>S1.R1.fastq,S1.R2.fastq</td>
<td>Stool</td>
<td>MiSeq,metagenome</td>
</tr>
<tr>
<td>Sample2</td>
<td>S2.R1.fastq,S2.R2.fastq</td>
<td>Blood</td>
<td>MiSeq,clinical</td>
</tr>
<tr>
<td>Control1</td>
<td>C1.R1.fastq,C1.R2.fastq</td>
<td>Control</td>
<td>Negative</td>
</tr>
<tr>
<td>Control2</td>
<td>C2.R1.fastq,C2.R2.fastq</td>
<td>Control</td>
<td>Spike-in Control</td>
</tr>
</tbody>
</table>

5.3.3 Output path

You may specify the output path if you would like your results to be output to a specific location. In most cases, you can leave this field blank and the results will be automatically written to a standard location, $EDGE_HOME/edge_ui/EDGE_output. In most cases, it is sufficient to leave these options to the default settings.
5.3.4 Number of CPUs

Additionally, you may specify the number of CPUs to be used. The default and minimum value is one-fourth of total number of server CPUs. You may adjust this value if you wish. Assuming your hardware has 64 CPUs, the default is 16 and the maximum you should choose is 62 CPUs. Otherwise, if the jobs currently in progress use the maximum number of CPUs, the new submitted job will be queued (and colored in grey. Color-coding see Checking the status of an analysis job (page 42)). For instance, if you have only one job running, you may choose 62 CPUs. However, if you are planning to run 6 different jobs simultaneously, you should divide the computing resources (in this case, 10 CPUs per each job, totaling 60 CPUs for 6 jobs).

5.3.5 Config file

Below the “Use # of CPUs” field is a field where you may select a configuration file. A configuration file is automatically generated for each job when you click “Submit.” This field could be used if you wanted to restart a job that hadn’t finished for some reason (e.g. due to power interruption, etc.). This option ensures that your submission will be run exactly the same way as previously, with all the same options.

See also:
Example of config file (page 50)

5.3.6 Batch project submission

The “Batch project submission” section is toggled off by default. Clicking on it will open it up and toggle off the “Input Sequence” section at the same time. When you have many samples in “EDGE Input Directory” and would like to run them with the same configuration, instead of submitting several times, you can compile a Excel file with project name, fastq inputs and optional project descriptions (you can download the example excel file and fill it with your own data) and submit through the “Batch project submission” section.

5.4 Choosing processes/analyses

Once you have selected the input files and assigned a project name and description, you may either click “Submit” to submit an analysis job using the default parameters, or you may change various parameters prior to submitting the job. The default settings include quality filter and trimming, assembly, annotation, and community profiling. Therefore, if you choose to use default parameters, the analysis will provide an assessment of what organism(s) your sample is composed of, but will not include host removal, primer design, etc. Below the “Input Your Sample” section is a section called “Choose Processes / Analyses”. It is in this section that you may modify parameters if you would like to use settings other than the default settings for your analysis (discussed in detail below).
5.4.1 Pre-processing

Pre-processing is by default on, but can be turned off via the toggle switch on the right hand side. The default parameters should be sufficient for most cases. However, if your experiment involves specialized adapter sequences that need to be trimmed, you may do so in the Quality Trim and Filter subsection. There are two options for adapter trimming. You may either supply a FASTA file containing the adapter sequences to be trimmed, or you may specify N number of bases to be trimmed from either end of each read.
**Note:** Trim Quality Level can be used to trim reads from both ends with defined quality. “N” base cutoff can be used to filter reads which have more than this number of continuous base “N”. Low complexity is defined by the fraction of mono-/di-nucleotide sequence. Ref: FaQCs.

The host removal subsection allows you to subtract host-derived reads from your dataset, which can be useful for metagenomic (complex) samples such as clinical samples (blood, tissue), or environmental samples like insects. In order to enable host removal, within the “Host Removal” subsection of the “Choose Processes / Analyses” section, switch the toggle box to “On” and select either from the pre-build host list (Human, Invertebrate Vectors of Human Pathogens, PhiX, RefSeq Bacteria and RefSeq Viruses) or the appropriate host FASTA file for your experiment from the navigation field. The Similarity (%) can be varied if desired, but the default is 90 and we would not recommend using a value less than 90.

5.4. Choosing processes/analyses
5.4.2 Assembly And Annotation

The Assembly option by default is turned on. It can be turned off via the toggle button. EDGE performs iterative kmers de novo assembly by IDBA-UD. It performs well on isolates as well as metagenomes but it may not work well on very large genomes. By default, it starts from kmer=31 and iterative step by adding 20 to maximum kmer=121. When the maximum k value is larger than the input average reads length, it will automatically adjust the maximum value to average reads length minus 1. User can set the minimum cutoff value on the final contigs. By default, it will filter out all contigs with size smaller than 200 bp.

The Annotation module will be performed only if the assembly option is turned on and reads were successfully assembled. EDGE has the option of using Prokka or RATT to do genome annotation. For most cases, Prokka is the appropriate tool to use, however, if your input is a viral genome with attached reference annotation (GenBank file), RATT is the preferred method. If for some reason the assembly fails (ex: run out of Memory), EDGE will bypass any modules requiring a contigs file including the annotation analysis.

5.4.3 Reference-based Analysis

The reference-based analysis section allows you to map reads/contigs to the provided references, which can be useful for known isolated species such as cultured samples, to get the coverage information and validate the assembled contigs. In order to enable reference-based analysis, switch the toggle box to “On” and select either from the pre-
build Reference list (Ebola virus genomes (page 73), E.coli 55989, E.coli O104H4, E.coli O127H6 and E.coli K12 MG1655) or the appropriate FASTA/GenBank file for your experiment from the navigation field.

Given a reference genome fasta file, EDGE will turn on the analysis of the reads/contigs mapping to reference and JBrowse reference track generation. If a GenBank file is provided, EDGE will also turn on variant analysis.

### 5.4.4 Taxonomy Classification

Taxonomic profiling is performed via the “Taxonomy Classification” feature. This is a useful feature not only for complex samples, but also for purified microbial samples (to detect contamination). In the “Community profiling” subsection in the “Choose Processes / Analyses section,” community profiling can be turned on or off via the toggle button.
There is an option to “Always use all reads” or not. If “Always use all reads” is not selected, then only those reads that do not map to the user-supplied reference will be shown in downstream analyses (i.e. the results will only include what is different from the reference). Additionally, the user can use different profiling tools with checkbox selection menu. EDGE uses multiple tools for taxonomy classification including GOTTCHA (bacterial & viral databases), MetaPhlAn, Kraken and reads mapping to NCBI RefSeq using BWA.

Turning on the “Contig-Based Taxonomy Classification” section will initiate mapping contigs against NCBI databases for taxonomy and functional annotations.

5.4.5 Phylogenomic Analysis

EDGE supports 5 pre-computed pathogen databases (E.coli, Yersinia, Francisella, Brucella, Bacillus (page 67)) for SNP phylogeny analysis. You can also choose to build your own database by first selecting a build method (either FastTree or RAxML), then selecting a pathogen from the “Search Genomes” search function. You can also add FASTA files or SRA Accessions.

5.4.6 Specialty Genes Profiling

For specialty gene analysis, the user selects read-based analysis and/or ORF(contig)-based analysis.
For read-based analysis antibiotic resistance genes and virulence genes are detected using Huttenhower lab’s program ShortBRED. The antibiotic resistance gene database was generated by the developers of ShortBRED using genes from ARDB and Resfams. The virulence genes database was generated by the developers of EDGE using VFDB.

For ORF-based analysis, antibiotic resistance genes are detected using CARD’s (Comprehensive Antibiotic Resistance Database) program RGI (Resistance Gene Identifier). RGI uses CARD’s custom database of antibiotic resistance genes. The virulence genes are detected using ShortBRED with a database generated by the developers of EDGE using VFDB.

### 5.4.7 PCR Primer Tools

EDGE includes PCR-related tools for use by those who want to use PCR data for their projects.
• **Primer Validation**

The “Primer Validation” tool can be used to verify whether and where given primer sequences would align to the genome of the sequenced organism. Prior to initiating the analysis, primer sequences in FASTA format must be deposited in the folder on the desktop in the directory entitled “EDGE Input Directory.”

In order to initiate primer validation, within the “Primer Validation” subsection switch the “Run Primer Validation” toggle button to “On”. Then, within the “Primer FASTA Sequences” navigation field, select your file containing the primer sequences of interest. Next, in the “Maximum Mismatch” field, choose the maximum number of mismatches you wish to allow per primer sequence. The available options are 0, 1, 2, 3, or 4.

• **Primer Design**

If you would like to design new primers that will differentiate a sequenced microorganism from all other bacteria and viruses in NCBI, you can do so using the “Primer Design” tool. To initiate primer design switch the “Run Primer Design” toggle button to “On”. There are default settings supplied for Melting Temperature, Primer Length, Tm Differential, and Number of Primer Pairs, but you can change these settings if desired.
5.5 Submission of a job

When you have selected the appropriate input files and desired analysis options, and you are ready to submit the analysis job, click on the “Submit” button at the bottom of the page. Immediately you will see indicators of successful job submission and job status below the submit button, in green. If there is something wrong with the input, it will stop the submission and show the message in red, highlighting the sections with issues.

5.6 Checking the status of an analysis job

Once an analysis job has been submitted, it will become visible in the left navigation bar. There is a grey, red, orange, green color-coding system that indicates job status as follow:

<table>
<thead>
<tr>
<th>Status</th>
<th>Color</th>
<th>Not yet begun</th>
<th>Error</th>
<th>In progress (running)</th>
<th>Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status</td>
<td>Color</td>
<td>Not yet begun</td>
<td>Error</td>
<td>In progress (running)</td>
<td>Completed</td>
</tr>
<tr>
<td>Not yet begun</td>
<td>Grey</td>
<td>Red</td>
<td>Orange</td>
<td>Green</td>
<td></td>
</tr>
</tbody>
</table>

While the job is in progress, clicking on the project in the left navigation bar will allow you to see which individual steps have been completed or are in progress, and results that have already been produced. Clicking the job progress widget at top right opens up a more concise view of progress.
5.6. Checking the status of an analysis job
5.7 Monitoring the Resource Usage

In the job project sidebar, you can see there is an “EDGE Server Usage” widget that dynamically monitors the server resource usage for %CPU, %MEMORY and %DISK space. If there is not enough available disk space, you may consider deleting or archiving the submitted job with the Action tool described below.

5.8 Management of Jobs

Below the resource monitor is the “Action” tool, used for managing jobs in progress or existing projects.
The available actions are:

- **View live log** A terminal-like screen showing all the command lines and progress log information. This is useful for troubleshooting or if you want to repeat certain functions through command line at edge server.

- **Force to rerun this project** Rerun a project with the same inputs and configuration. No additional input needs.

- **Interrupt running project** Immediately stop a running project.

- **Delete entire project** Delete the entire output directory of the project.

- **Remove from project list** Keep the output but remove project name from the project list.

- **Empty project outputs** Clean all the results but keep the config file. User can use this function to do a clean rerun.

- **Move to an archive directory** For performance reasons, the output directory will be put in local storage. User can use this function to move projects from local storage to a slower but larger network storage, which are configured when the edge server is installed.

- **Share Project** Allow guests and other users to view the project.

- **Make project Private/Public** Restrict access to viewing the project to only yourself. Or open it everyone.

### 5.9 Project List Table

When you click “My Project List”, all your projects or projects shared to you will show in a table. It lists the projects status, submission time, running time, type and owner. User can select one or more jobs from the checkbox in the project table and perform actions similar to “Action” Widget described in the previous section. The action will apply to all checked projects.
When mouse over the action buttons on the project list page, it will show a pop up info for the action buttons. There is a special action button for multiple projects, “Compare Selected Projects Taxonomy Classification (HeatMap)” which will draw heatmaps of taxonomy profiling results for multiple projects using MetaComp.

5.10 Other Methods of Accessing EDGE

5.10.1 Internal Python Web Server

EDGE includes a simple web server for single-user applications or other testing. It is not robust enough for production usage, but it is simple enough that it can be run on practically any system.

To run gui, type:

```
$EDGE_HOME/start_edge_ui.sh
```

This will start a localhost and the GUI html page will be opened by your default browser.

5.10.2 Apache Web Server

The preferred installation of EDGE uses Apache 2 (See Testing the EDGE Installation (page 14)), and serves the application as a proper system service. A sample httpd.conf (or apache2.conf, depending on your operating system) is provided in the root directory of your installation. If this configuration is used, EDGE will be available on any IP or hostname registered to the machine, on ports 80 and 8080.

You can access EDGE by opening either the desktop link (below), or your browser, and entering http://localhost:80 in the address bar.
Note: If the desktop environment is available, after installation, a “Start EDGE UI” icon should be on the desktop. Click on the green icon and choose “Run in Terminal.” Results should be the same as those obtained by the above method to start the GUI.

The URL address is 127.0.0.1:8080/index.html. It may not be that powerful, as it is hosted by Apache HTTP Server, but it works. With system administrator help, the Apache HTTP Server is the suggested method to host the gui interface.

Note: You may need to configure the edge_wwwroot and input and output in the edge_ui/edge_config.tmpl file while configuring the Apache HTTP Server and link to external drive or network drive if needed.

A Terminal window will display messages and errors as you run EDGE. Under normal operating conditions you can minimize this window. Should an error/problem arise, you may maximize this window to view the error.
Warning: IMPORTANT: Do not close this window!

The Browser window is the window in which you will interact with EDGE.
The command line usage is as followings:

```
Usage: perl runPipeline.pl [options] -c config.txt -p 'reads1.fastq reads2.fastq' -o out_directory
Version 1.1
Input File:
  -u Unpaired reads, Single end reads in fastq
  -p Paired reads in two fastq files and separate by space in quote
  -c Config File
Output:
  -o Output directory.
Options:
  -ref Reference genome file in fasta
  -primer A pair of Primers sequences in strict fasta format
  -cpu number of CPUs (default: 8)
  -version print verison
```

A config file (example in the below section, the Graphic User Interface (GUI) (page 29) will generate config automatically), reads Files in fastq format, and a output directory are required when run by command line. Based on the configuration file, if all modules are turned on, EDGE will run the following steps. Each step contains at least one command line scripts/programs.

1. Data QC
2. Host Removal QC
3. *De novo* Assembling
4. Reads Mapping To Contig
5. Reads Mapping To Reference Genomes
6. Taxonomy Classification on All Reads or unMapped to Reference Reads
7. Map Contigs To Reference Genomes
8. Variant Analysis
9. Contigs Taxonomy Classification
10. Contigs Annotation
11. ProPhage detection
12. PCR Assay Validation
13. PCR Assay Adjudication
14. Phylogenetic Analysis
15. Generate JBrowse Tracks
16. HTML report

6.1 Configuration File

The config file is a text file with the following information. If you are going to do host removal, you need to build host index (page 67) for it and change the fasta file path in the config file.

```
[Count Fastq]
DoCountFastq=auto

[Quality Trim and Filter]
## boolean, 1=yes, 0=no
DoQC=1
## Targets quality level for trimming
q=5
## Trimmed sequence length will have at least minimum length
min_L=50
## Average quality cutoff
avg_q=0
## "N" base cutoff. Trimmed read has more than this number of continuous base "N" will be discarded.
N=1
## Low complexity filter ratio, Maximum fraction of mono-/di-nucleotide sequence
lc=0.85
## Trim reads with adapters or contamination sequences
adapter=/PATH/adapter.fasta
## phiX filter, boolean, 1=yes, 0=no
phiX=0
## Cut # bp from 5 end before quality trimming/filtering
5end=0
## Cut # bp from 3 end before quality trimming/filtering
3end=0

[Host Removal]
## boolean, 1=yes, 0=no
DoHostRemoval=1
## Use more Host= to remove multiple host reads
Host=/PATH/all_chromosome.fasta
similarity=90
```
### Boolean, 1=yes, 0=no

- **DoAssembly=1**
- **assembledContigs**
- **minContigSize=200**
- **assembler=idba_ud**
- **idbaOptions="--pre_correction --mink 31"**

---

### Bypass assembly and use pre-assembled contigs
- **assembledContigs**
- **minContigSize=200**

---

### Spades or idba_ud
- **assembler=idba_ud**
- **idbaOptions="--pre_correction --mink 31"**
- **singleCellMode=**
- **pacbioFile=**
- **nanoporeFile=**

---

### Reads Mapping To Contigs
- **DoReadsMappingContigs=auto**

---

### Reads Mapping To Reference
- **DoReadsMappingReference=0**
- **bowtieOptions=**
- **reference=**
- **MapUnmappedReads=0**

---

### Reads Taxonomy Classification
- **DoReadsTaxonomy=1**
- **AllReads=0**
- **enabledTools=gottcha-genDB-b,gottcha-speDB-b,gottcha-strDB-b,gottcha-genDB-v,gottcha-speDB-v,gottcha-strDB-v,metaphlan,bwa,kraken_mini**

---

### Contigs Mapping To Reference
- **DoContigMapping=auto**
- **identity=85**
- **MapUnmappedContigs=0**

---

### Variant Analysis
- **DoVariantAnalysis=auto**

---

### Contigs Taxonomy Classification
- **DoContigsTaxonomy=1**

---

### Contigs Annotation
- **DoAnnotation=1**
- **kingdom: Archaea Bacteria Mitochondria Viruses**
- **kingdom=Bacteria**
- **contig_size_cut_for_annotation=700**
- **support tools: Prokka or RATT**
- **annotateProgram=Prokka**
- **annotateSourceGBK=**
6.2 Test Run

EDGE provides an example data set which is an E. coli MiSeq dataset and has been subsampled to ~10x fold coverage reads.

In the EDGE home directory,

```
cd testData
sh runTest.sh
```

See Output (page 62)
Project Start: 2015 Oct 15 11:26:30
Version: 1.1
The output directory path exists
If you use different input, it may mess up the result with existing files.
[Quality Trim and Filter]
Quality Trim and Filter Finished
[Assembly]
IDBA Assembly Finished
[Reads Mapping to Contigs]
Reads Mapping to Contigs Finished
[Reads Mapping To Reference]
Reads Mapping to Reference Finished
Unmapped reads retrieved
[Reads Taxonomy Classification]
Reads Taxonomy Classification Finished
[Contigs Mapping To Reference]
Contigs Mapping to Reference Finished
[Variant Analysis]
GF3 file not exists, skip Variant Analysis
[Contigs Taxonomy Classification]
Contigs Taxonomy Classification Finished
[Contigs Annotation]
Contig Annotation Finished
[ProPhage Detection]
ProPhage Detection Finished
[Phylogenetic Analysis]
Phylogenetic Analysis Finished
[Primer Validation]
Primer Validation Finished
[Generate JBrowse Tracks]
Generate JBrowse Tracks Finished
Produce Final PDF Report
Running time: 00:00:02

[HTML Report]
HTML Report Finished
Total Running time: 00:00:02

All Done.

Fig. 6.1: Snapshot from the terminal.
6.3 Descriptions of each module

Each module comes with default parameters and user can see the optional parameters by entering the program name with –h or -help flag without any other arguments.

1. Data QC
   • Required step? No
   • Command example
     ```perl
     perl $EDGE_HOME/scripts/illumina_fastq_QC.pl -p 'Ecoli_10x.1.fastq Ecoli_10x.2.fastq' -q 5 -min_L 50 -avg_q 5 -n 0 -lc 0.85 -d QcReads -t 10
     ```
   • What it does
     – Quality control
     – Read filtering
     – Read trimming
   • Expected input
     – Paired-end/Single-end reads in FASTQ format
   • Expected output
     – QC.1.trimmed.fastq
     – QC.2.trimmed.fastq
     – QC.unpaired.trimmed.fastq
     – QC.stats.txt
     – QC_qc_report.pdf

2. Host Removal QC
   • Required step? No
   • Command example
     ```perl
     perl $EDGE_HOME/scripts/host_reads_removal_by_mapping.pl -p 'QC.1.trimmed.fastq QC.2.trimmed.fastq' -u QC.unpaired.trimmed.fastq -ref human_chromosomes.fasta -o QcReads -cpu 10
     ```
   • What it does
     – Read filtering
   • Expected input
     – Paired-end/Single-end reads in FASTQ format
   • Expected output
     – host_clean.1.fastq
     – host_clean.2.fastq
     – host_clean.mapping.log
     – host_clean.unpaired.fastq
     – host_clean.stats.txt
3. IDBA Assembling
   • Required step? No
   • Command example
     
     ```
     fq2fa --merge host_clean.1.fastq host_clean.2.fastq pairedForAssembly.fasta
     idba_ud --num_threads 10 -o AssemblyBasedAnalysis/idba --pre_correction
     → pairedForAssembly.fasta
     ```
   
   • What it does
     – Iterative k-mers de novo Assembly, it performs well on isolates as well as metagenomes. It may not work well on very large genomes.
   
   • Expected input
     – Paired-end/Single-end reads in FASTA format
   
   • Expected output
     – contig.fa
     – scaffold.fa (input paired end)

4. Reads Mapping To Contig
   • Required step? No
   • Command example
     
     ```
     perl $EDGE_HOME/scripts/runReadsToContig.pl -p 'host_clean.1.fastq host_clean.2.
     →fastq' -d AssemblyBasedAnalysis/readsMappingToContig -pre readsToContigs -ref
     → AssemblyBasedAnalysis/contigs.fa
     ```
   
   • What it does
     – Mapping reads to assembled contigs
   
   • Expected input
     – Paired-end/Single-end reads in FASTQ format
     – Assembled Contigs in Fasta format
     – Output Directory
     – Output prefix
   
   • Expected output
     – readsToContigs.alnstats.txt
     – readsToContigs_coverage.table
     – readsToContigs_plots.pdf
     – readsToContigs.sort.bam
     – readsToContigs.sort.bam.bai

5. Reads Mapping To Reference Genomes
   • Required step? No
   • Command example:
perl $EDGE_HOME/scripts/runReadsToGenome.pl -p 'host_clean.1.fastq host_clean.2.fastq' -d ReadsBasedAnalysis -pre readsToRef -ref Reference.fna

- What it does
  - Mapping reads to reference genomes
  - SNPs/Indels calling

- Expected input
  - Paired-end/Single-end reads in FASTQ format
  - Reference genomes in Fasta format
  - Output Directory
  - Output prefix

- Expected output
  - readsToRef.alnstats.txt
  - readsToRefplots.pdf
  - readsToRef.refID.coverage
  - readsToRef.refID.gap.coords
  - readsToRef.refID.window_size_coverage
  - readsToRef.ref_windows.gc.txt
  - readsToRef.raw.bcf
  - readsToRef.sort.bam
  - readsToRef.sort.bam.bai
  - readsToRef.vcf

6. Taxonomy Classification on All Reads or unmapped to Reference Reads

- Required step? No

- Command example:

```
perl $EDGE_HOME/scripts/microbial_profiling/microbial_profiling_configure.pl →$EDGE_HOME/scripts/microbial_profiling/microbial_profiling.settings.tmpl
→gottcha-speDB-b > microbial_profiling.settings.ini
perl $EDGE_HOME/scripts/microbial_profiling/microbial_profiling.pl -o Taxonomy -→s microbial_profiling.settings.ini -c 10 UnmappedReads.fastq
```

- What it does
  - Taxonomy Classification using multiple tools, including BWA mapping to NCBI Refseq, metaphlan, kraken, GOTTCHA.
  - Unify varies output format and generate reports

- Expected input
  - Reads in FASTQ format
  - Configuration text file (generated by microbial_profiling_configure.pl)

- Expected output
- Summary EXCEL and text files.
- Heatmaps tools comparison
- Radarchart tools comparison
- Krona and tree-style plots for each tool.

7. **Map Contigs To Reference Genomes**

- **Required step? No**
- **Command example:**

```bash
perl $EDGE_HOME/scripts/nucmer_genome_coverage.pl -e 1 -i 85 -p contigsToRef - Reference.fna contigs.fa
```

- **What it does**
  - Mapping assembled contigs to reference genomes
  - SNPs/Indels calling

- **Expected input**
  - Reference genome in Fasta Format
  - Assembled contigs in Fasta Format
  - Output prefix

- **Expected output**
  - contigsToRef_avg_coverage.table
  - contigsToRef.delta
  - contigsToRef_query_unUsed.fasta
  - contigsToRef.snps
  - contigsToRef.coords
  - contigsToRef.log
  - contigsToRef_query_novel_region_coord.txt
  - contigsToRef_ref_zero_cov_coord.txt

8. **Variant Analysis**

- **Required step? No**
- **Command example:**

```bash
perl $EDGE_HOME/scripts/SNP_analysis.pl -genbank Reference.gbk -SNP contigsToRef -snps -format nucmer
perl $EDGE_HOME/scripts/gap_analysis.pl -genbank Reference.gbk -gap contigsToRef -ref_zero_cov_coord.txt
```

- **What it does**
  - Analyze variants and gaps regions using annotation file.

- **Expected input**
  - Reference in GenBank format
  - SNPs/INDELs/Gaps files from “Map Contigs To Reference Genomes”

---

**6.3. Descriptions of each module**
• Expected output
  – contigsToRef.SNPs_report.txt
  – contigsToRef.Indels_report.txt
  – GapVSReference.report.txt

9. Contigs Taxonomy Classification
• Required step? No
• Command example:
  ```
  perl $EDGE_HOME/scripts/contig_classifier_by_bwa/contig_classifier_by_bwa.pl --db
  →$EDGE_HOME/database/bwa_index/NCBI-Bacteria-Virus.fna --threads 10 --prefix
  →OutputCT --input contigs.fa
  ```
• What it does
  – Taxonomy Classification on contigs using BWA mapping to NCBI Refseq
• Expected input
  – Contigs in Fasta format
  – NCBI Refseq genomes bwa index
  – Output prefix
• Expected output
  – prefix.assembly_class.csv
  – prefix.assembly_class.top.csv
  – prefix.ctg_class.csv
  – prefix.ctg_class.LCA.csv
  – prefix.ctg_class.top.csv
  – prefix.unclassified.fasta

10. Contig Annotation
• Required step? No
• Command example:
  ```
  prokka --force --prefix PROKKA --outdir Annotation contigs.fa
  ```
• What it does
  – The rapid annotation of prokaryotic genomes.
• Expected input
  – Assembled Contigs in Fasta format
  – Output Directory
  – Output prefix
• Expected output
  – It produces GFF3, GBK and SQN files that are ready for editing in Sequin and ultimately submitted to Genbank/DDJB/ENA.
11. **ProPhage detection**

- **Required step?** No
- **Command example:**
  ```
  perl $EDGE_HOME/scripts/phageFinder_prepare.pl -o Prophage -p Assembly Annotation/PROKKA.gff Annotation/PROKKA.fna
  $EDGE_HOME/thirdParty/phage_finder_v2.1/bin/phage_finder_v2.1.sh Assembly
  ```
- **What it does**
  - Identify and classify prophages within prokaryotic genomes.
- **Expected input**
  - Annotated Contigs GenBank file
  - Output Directory
  - Output prefix
- **Expected output**
  - phageFinder_summary.txt

12. **PCR Assay Validation**

- **Required step?** No
- **Command example:**
  ```
  perl $EDGE_HOME/scripts/pcrValidation/validate_primers.pl -ref contigs.fa -primer primers.fa -mismatch 1 -output AssayCheck
  ```
- **What it does**
  - In silico PCR primer validation by sequence alignment.
- **Expected input**
  - Assembled Contigs/Reference in Fasta format
  - Output Directory
  - Output prefix
- **Expected output**
  - pcrContigValidation.log
  - pcrContigValidation.bam

13. **PCR Assay Adjudication**

- **Required step?** No
- **Command example:**
  ```
  perl $EDGE_HOME/scripts/pcrAdjudication/pcrUniquePrimer.pl --input contigs.fa --gff3 PCR.Adjudication.primers.gff3
  ```
- **What it does**
  - Design unique primer pairs for input contigs.

6.3. **Descriptions of each module**
14. Phyllogenetic Analysis

- Required step? No

- Command example:

  perl $EDGE_HOME/scripts/prepare_SNP_phylogeny.pl -o output/SNP_Phylogeny/Ecoli -
  -tree FastTree -db Ecoli -n output -cpu 10 -p QC.1.trimmed.fastq QC.2.trimmed.fastq
  -c contigs.fa -s QC.unpaired.trimmed.fastq
  perl $EDGE_HOME/scripts/SNPphy/runSNPphylogeny.pl output/SNP_Phylogeny/Ecoli/
  -SNPphy.ctrl

- What it does

  - Perform SNP identification against selected pre-built SNPdb or selected genomes
  - Build SNP based multiple sequence alignment for all and CDS regions
  - Generate Tree file in newick/PhyloXML format

- Expected input

  - SNPdb path or genomesList
  - Fastq reads files
  - Contig files

- Expected output

  - SNP based phylogenetic multiple sequence alignment
  - SNP based phylogenetic tree in newick/PhyloXML format.
  - SNP information table

15. Generate JBrowse Tracks

- Required step? No

- Command example:

  perl $EDGE_HOME/scripts/edge2jbrowse_converter.pl --in-ref-fa Reference.fna --in-
  ref-gff3 Reference.gff --proj_outdir EDGE_project_dir

- What it does

  - Convert several EDGE outputs into JBrowse tracks for visualization for contigs and reference, respectively.

- Expected input

  - EDGE project output Directory

- Expected output

  - EDGE post-processed files for JBrowse tracks in the JBrowse directory.
  - Tracks configuration files in the JBrowse directory.
16. **HTML Report**

- Required step? No
- Command example:

```bash
perl $EDGE_HOME/scripts/munger/outputMunger_w_temp.pl EDGE_project_dir
```

- What it does
  - Generate statistical numbers and plots in an interactive html report page.
- Expected input
  - EDGE project output Directory
- Expected output
  - report.html

### 6.4 Other command-line utility scripts

1. To extract certain taxa fasta from contig classification result:

```bash
cd /home/edge_install/edge_ui/EDGE_output/41/AssemblyBasedAnalysis/Taxonomy
perl /home/edge_install/scripts/contig_classifier_by_bwa/extract_fasta_by_taxa.pl -fasta ../contigs.fa -csv ProjectName.ctg_class.top.csv -taxa "Enterobacter cloacae" > Ecloacae.contigs.fa
```

2. To extract unmapped/mapped reads fastq from the bam file:

```bash
cd /home/edge_install/edge_ui/EDGE_output/41/AssemblyBasedAnalysis/
# extract unmapped reads
perl /home/edge_install/scripts/bam_to_fastq.pl -unmapped readsToContigs.sort.bam
# extract mapped reads
perl /home/edge_install/scripts/bam_to_fastq.pl -mapped readsToContigs.sort.bam
```

3. To extract mapped reads fastq of a specific contig/reference from the bam file:

```bash
cd /home/edge_install/edge_ui/EDGE_output/41/AssemblyBasedAnalysis/
perl /home/edge_install/scripts/bam_to_fastq.pl -id ProjectName_00001 -mapped
```
The output directory structure contains ten major sub-directories when all modules are turned on. In addition to the main directories, EDGE will generate a final report in portable document file format (pdf), process log and error log file in the project main directory.

- AssayCheck
- AssemblyBasedAnalysis
- HostRemoval
- HTML_Report
- JBrowse
- QcReads
- ReadsBasedAnalysis
- ReferenceBasedAnalysis
- Reference
- SNP_Phylogeny

In the graphic user interface, EDGE generates an interactive output webpage which includes summary statistics and taxonomic information, etc. The easiest way to interact with the results is through the web interface. If a project run finished through the command line, user can open the report html file in the HTML_report subdirectory off-line. When a project run is finished, user can click on the project id from the menu and it will generate the interactive html report on the fly. User can browse the data structure by clicking the project link and visualize the result by JBrowse links, download the pdf files, etc.
7.1 Example Output

See http://lanl-bioinformatics.github.io/EDGE/example_output/report.html

Note: The example link is just an example of graphic output. The JBrowse and links are not accessible in the example links.
8.1 EDGE provided databases

8.1.1 NCBI Refseq

EDGE prebuilt blast db and bwa_index of NCBI RefSeq genomes.

**Warning:** NCBI restructure the ftp site. The link for Bacteria below is an archive.

- **Bacteria:** NCBI all complete bacteria download method
  - Version: NCBI 2017 Oct 3
  - 245 Archaea + 7917 Bacteria genomes
- **Virus:** NCBI Virus
  - Version: NCBI 2017 Oct 3
  - 7458 complete genomes + Neighbor Nucleotoides (118039 sequences)

see SEDGE_HOME/database/bwa_index/id_mapping.txt for all gi/accession to genome name lookup table.

8.1.2 Krona taxonomy

- **website:** [http://sourceforge.net/p/krona/home/krona/](http://sourceforge.net/p/krona/home/krona/)

**Update Krona taxonomy db**

Download these files from ftp://ftp.ncbi.nih.gov/pub/taxonomy:

Transfer the files to the taxonomy folder in the standalone KronaTools installation and run:


8.1.3 Metaphlan database

MetaPhlAn relies on unique clade-specific marker genes identified from 3,000 reference genomes.

  - website: http://huttenhower.sph.harvard.edu/metaphlan

8.1.4 Human Genome

The bwa index is prebuilt in the EDGE. The human hs_ref_GRCh38 sequences from NCBI ftp site.


8.1.5 MiniKraken DB

Kraken is a system for assigning taxonomic labels to short DNA sequences, usually obtained through metagenomic studies. MiniKraken is a pre-built 4 GB database constructed from complete bacterial, archaeal, and viral genomes in RefSeq (as of Mar. 30, 2014).

  - website: http://ccb.jhu.edu/software/kraken/

8.1.6 GOTTCHA DB

A novel, annotation-independent and signature-based metagenomic taxonomic profiling tool.

  - website: http://lanl-bioinformatics.github.io/GOTTCHA/
  - version: v20150825

8.1.7 SNPdb

SNP database based on whole genome comparison. Current available db are Ecoli, Yersinia, Francisella, Brucella, Bacillus (page 67).

8.1.8 Invertebrate Vectors of Human Pathogens

The bwa index is prebuilt in the EDGE.

• website: https://www.vectorbase.org
version: 2014 July 24

8.1.9 NCBI Nucleotide database (NT) database
• version: 2016 April 26

8.1.10 VFDB
A Microbial database of virulence factors
• paper: http://www.ncbi.nlm.nih.gov/pubmed/?term=26578559
• website: http://www.mgc.ac.cn/VFs/main.htm
• version: 20160818

8.1.11 ARDB
Antibiotic Resistance Genes Database
• website: http://ardb.cbcb.umd.edu/index.html
• version: 1.1

8.1.12 CARD
The Comprehensive Antibiotic Resistance Database
• website: https://card.mcmaster.ca/
• Version: 1.0.6

8.1.13 Amplicon: 16s/18s/ITS
For QIIME (Quantitative insights into Microbial Ecology) analysis
• Greengenes OTUs (16s)
  – website: http://greengenes.secondgenome.com/
  – version: 2013 May
• SILVA OTUs (16S/18S)
  – website: http://www.arb-silva.de/download/archive/qiime/
  – version: 119
• UNITE OTUs (ITS)
  – website: https://unite.ut.ee/repository.php
  – version: 12_11
8.2 Building bwa index

Here take human genome as example.

1. Download the human hs_ref_GRCh38 sequences from NCBI ftp site.

   perl $EDGE_HOME/scripts/download_human_refseq_genome.pl output_dir

2. Gunzip the downloaded fasta file and concatenate them into one human genome multifasta file:

   gunzip hs_ref_GRCh38.*.fa.gz
   cat hs_ref_GRCh38.*.fa > human_ref_GRCh38.all.fasta

3. Use the installed bwa to build the index:

   $EDGE_HOME/bin/bwa index human_ref_GRCh38.all.fasta

Now, you can configure the config file with “host=/path/human_ref_GRCh38.all.fasta” for host removal step.

8.3 SNP database genomes

SNP database was pre-built from the below genomes.

8.3.1 Ecoli Genomes

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>URL</th>
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<tbody>
<tr>
<td>Ecoli_042</td>
<td>Escherichia coli 042, complete genome</td>
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<td>Ecoli_536</td>
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<td>Ecoli_55989</td>
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<td>Ecoli_APEC_O1</td>
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<td>Ecoli_ATCC_8739</td>
<td>Escherichia coli ATCC 8739 chromosome, complete genome</td>
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<tr>
<td>Ecoli_BL21_DE3</td>
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<td>Ecoli_CE10</td>
<td>Escherichia coli O7:K1 str. CE10 chromosome, complete genome</td>
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<td>Ecoli_CFT073</td>
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<td>Escherichia coli DH1, complete genome</td>
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Table 8.1 – continued from previous page

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<td>Ecoli_HS</td>
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<td>Ecoli_IAI1</td>
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<td>Ecoli_IHE0334</td>
<td>Escherichia coli IHE0334 chromosome, complete genome</td>
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<tr>
<td>Ecoli_FF82</td>
<td>Escherichia coli FF82, complete genome</td>
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<td>Ecoli_P12b</td>
<td>Escherichia coli P12b chromosome, complete genome</td>
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<tr>
<td>Ecoli_Sakai</td>
<td>Escherichia coli SMS-3-5 chromosome, complete genome</td>
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<tr>
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<td>Sflexneri_5_8401</td>
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<tr>
<td>Ssonnei_S046</td>
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8.3. SNP database genomes
## 8.3.2 Yersinia Genomes

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<td>Ypestis_Angola</td>
<td>Yersinia pestis Angola chromosome, complete genome</td>
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<td>Ypestis_Antiqua</td>
<td>Yersinia pestis Antiqua chromosome, complete genome</td>
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<td>Ypestis_KIM_10</td>
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<td>Ypestis_Microtus_91001</td>
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## 8.3.3 Francisella Genomes

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<td>Ftularen-sis_holarctica_FTNF002</td>
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<td>Ftularen-sis_holarctica_LVS</td>
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<td>Ftularen-sis_tularensis_WY9634</td>
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## 8.3.4 Brucella Genomes

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<td>Brucella melitensis bv. 1 str. 16M</td>
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### 8.3.5 Bacillus Genomes

<table>
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<td>Bthuringiensis_chinensis_CT43</td>
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<td>Bthuringiensis_finitimus_YBT020</td>
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<td>Bthuringiensis_konkukian_9727</td>
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## 8.4. Ebola Reference Genomes

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</thead>
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CHAPTER 9

Third Party Tools

9.1 Assembly

• IDBA-UD
  - Citation: Peng, Y., et al. (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth, Bioinformatics, 28, 1420-1428.
  - Site: http://i.cs.hku.hk/~alse/hkubrg/projects/idba_ud/
  - Version: 1.1.1
  - License: GPLv2

• SPAdes
  - Site: http://bioinf.spbau.ru/spades
  - Version: 3.9.1
  - License: GPLv2

• MEGAHIT
  - Citation: Li D. et al. (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015 May 15;31(10):1674-6
  - Site: https://github.com/voutcn/megahit
  - Version: 1.0.3
  - License: GPLv3
9.2 Annotation

- **RATT**
  - Site: http://ratt.sourceforge.net/
  - Version:
  - License:
  - Note: The original RATT program does not deal with reverse complement strain annotations transfer. We edited the source code to fix it.

- **Prokka**
  - Citation: Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation, Bioinformatics, 30,2068-2069.
  - Site: http://www.vicbioinformatics.com/software.prokka.shtml
  - Version: 1.11
  - License: GPLv2
  - Note: The NCBI tool tbl2asn included within PROKKA can have very slow runtimes (up to several hours) while it is dealing with numerous contigs, such as when we input metagenomic data. We modified the code to allow parallel processing using tbl2asn.

- **tRNAscan**
  - Site: http://lowelab.ucsc.edu/tRNAscan-SE/
  - Version: 1.3.1
  - License: GPLv2

- **Barrnap**
  - Citation:
  - Site: http://www.vicbioinformatics.com/software.barrnap.shtml
  - Version: 0.42
  - License: GPLv3

- **BLAST+**
  - Citation: Camacho, C., et al. (2009) BLAST+: architecture and applications, BMC bioinformatics, 10, 421.
  - Version: 2.5.0
  - License: Public domain

- **blastall**
- Version: 2.2.26
- License: Public domain

**Phage_Finder**
- Site: http://phage-finder.sourceforge.net/
- Version: 2.1
- License: GPLv3

**Glimmer**
- Site: http://ccb.jhu.edu/software/glimmer/index.shtml
- Version: 302b
- License: Artistic License

**ARAGORN**
- Citation: Laslett, D. and Canback, B. (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences, Nucleic acids research, 32, 11-16.
- Site: http://mbio-serv2.mbioekol.lu.se/ARAGORN/
- Version: 1.2.36
- License:

**Prodigal**
- Site: http://prodigal.ornl.gov/
- Version: 2.60
- License: GPLv3

**tbl2asn**
- Citation:
- Version: 25.3 (2016 Dec 2rd)
- License:

**Warning:** tbl2asn must be compiled within the past year to function. We attempt to recompile every 6 months or so. Most recent compilation is 2 Dec 2016
9.3 Alignment

- **HMMER3**
  - Citation: Eddy, S.R. (2011) Accelerated Profile HMM Searches, PLoS computational biology, 7, e1002195
  - Site: http://hmmer.janelia.org/
  - Version: 3.1b1
  - License: GPLv3

- **Infernal**
  - Citation: Nawrocki, E.P. and Eddy, S.R. (2013) Infernal 1.1: 100-fold faster RNA homology searches, Bioinformatics, 29, 2933-2935.
  - Site: http://infernal.janelia.org/
  - Version: 1.1rc4
  - License: GPLv3

- **Bowtie 2**
  - Version: 2.2.6
  - License: GPLv3

- **BWA**
  - Citation: Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform, Bioinformatics, 25, 1754-1760.
  - Site: http://bio-bwa.sourceforge.net/
  - Version: 0.7.12
  - License: GPLv3

- **MUMmer3**
  - Citation: Kurtz, S., et al. (2004) Versatile and open software for comparing large genomes, Genome biology, 5, R12.
  - Site: http://mummer.sourceforge.net/
  - Version: 3.23
  - License: GPLv3

- **RAPSearch2**
  - Site: http://omics.informatics.indiana.edu/mg/RAPSearch2/
  - Version: 2.23
  - License: GPL
9.4 Taxonomy Classification

- **Kraken**
  - Site: http://ccb.jhu.edu/software/kraken/
  - Version: 0.10.4-beta
  - License: GPLv3

- **Metaphlan**
  - Site: http://huttenhower.sph.harvard.edu/metaphlan
  - Version: 1.7.7
  - License: Artistic License

- **GOTTCHA**
  - Citation: Tracey Allen K. Freitas, Po-E Li, Matthew B. Scholz, Patrick S. G. Chain (2015) Accurate Metagenome characterization using a hierarchical suite of unique signatures. Nucleic Acids Research (DOI: 10.1093/nar/gkv180)
  - Site: http://lanl-bioinformatics.github.io/GOTTCHA/
  - Version: 1.0b
  - License: GPLv3

- **GOTTCHA2**
  - Citation:
  - Site: https://gitlab.com/poeli/GOTTCHA2
  - Version: 2.2.0
  - License:

9.5 Phylogeny

- **FastTree**
  - Site: http://www.microbesonline.org/fasttree/
  - Version: 2.1.9
  - License: GPLv2

- **RAxML**
  - Citation: Stamatakis,A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30:1312-1313
9.6 Specialty Genes

- **Bio::Phylo**
  
  
  - Site: http://search.cpan.org/~rvosa/Bio-Phylo/
  
  - Version: 0.58
  
  - License: GPLv3

- **PhaME**
  
  - Citation: Sanaa Afroz Ahmed, Chien-Chi Lo, Po-E Li, Karen W Davenport, Patrick S.G. Chain. From raw reads to trees: Whole genome SNP phylogenetics across the tree of life. bioRxiv doi: http://dx.doi.org/10.1101/032250
  
  - Site: https://github.com/LANL-Bioinformatics/PhaME/
  
  - Version: 1.0
  
  - License: GPLv3

9.7 Visualization and Graphic User Interface

- **jsPhyloSVG**
  
  
  - Site: http://www.jsphylosvg.com
  
  - Version: 1.55
• JBrowse
  – Citation: Skinner, M.E., et al. (2009) JBrowse: a next-generation genome browser, Genome research, 19, 1630-1638.
  – Site: http://jbrowse.org
  – Version: 1.11.6
  – License: Artistic License 2.0/LGPLv.1

• KronaTools
  – Citation: Ondov, B.D., Bergman, N.H. and Phillippy, A.M. (2011) Interactive metagenomic visualization in a Web browser, BMC bioinformatics, 12, 385.
  – Site: http://sourceforge.net/projects/krona/
  – Version: 2.7
  – License: BSD

• JQuery
  – Site: http://jquery.com/
  – Version: 1.10.2
  – License: MIT

• JQuery Mobile
  – Site: http://jquerymobile.com
  – Version: 1.4.3
  – License: CC0

• DataTables
  – Site: https://datatables.net
  – Version: 1.10.11
  – License: MIT

• jQuery File Tree
  – Site: http://www.abeautifulsite.net/jquery-file-tree/
  – Version: 1.01
  – License: GPL and MIT

• Raphael - JavaScript Vector Library
  – Site: http://dmitrybaranovskiy.github.io/raphael/
  – Version: 1.4.3
  – License: MIT

• Tooltipster
  – Site: http://iamcege.github.io/tooltipster/
  – Version: 3.2.6
  – License: MIT
• Lazy Load XT
  – Site: http://ressio.github.io/lazy-load-xt/
  – Version: 1.0.6
  – License: MIT

• Plupload
  – Site: http://www.plupload.com
  – Version: 2.1.7
  – License: GPLv2 and OEM

• hello.js
  – Site: http://adodson.com/hello.js/
  – Version: 1.8.1
  – License: MIT

### 9.8 Utility

• BEDTools
  – Citation: Quinlan, A.R. and Hall, I.M. (2010) BEDTools: a flexible suite of utilities for comparing genomic features, Bioinformatics, 26, 841-842.
  – Site: https://github.com/arq5x/bedtools2
  – Version: 2.19.1
  – License: GPLv2

• R
  – Site: http://www.r-project.org/
  – Version: 3.3.2
  – License: GPLv2

• R_3.3.2_Packages
  – Custom built directoy containing all the packages required to install R packages offline
  – The majority of the packages were downloaded automatically using the following R commands.

```
# Function to get dependencies and imports for a given list of packages.
getPackages <- function(packs){
  packages <- unlist(
    tools::package_dependencies(packs, available.packages(), which=c("Depends", "Imports"), recursive=TRUE))
  packages <- union(packs, packages)
  packages
}
```
# Use the function by providing the names of the desired packages.

```r
packages <- getPackages(c("packageName", "packageName2"))
# For example
packages <- getPackages(c("MetaComp","gtable","gridExtra","devtools",
                         "phylseq","webshot","plotly","shiny","DT"))
```

# Download packages to current/desired directory.

```r
download.packages(packages, destdir="./", type="source")
```

- The packages specific to bioconductor needed to be manually downloade from the site
- stringi defaults to downloading icudt55I.zip from online, the following method, from their documenta-
  tion, was used to build a custom stringi package to avoid connecting to the internet :  

  1. File the `git clone https://github.com/gagolews/stringi.git` command.
  2. Edit the `.Rbuildignore` file and get rid of the `^src/icu55/data` line.
  3. Run `R CMD build stringi_dir_name`.

- **MetaComp: EDGE Taxonomy Assignments Visualization**
  - Citation: 
  - Site: https://cran.r-project.org/ 
  - Version: 1.0.1
  - License: 

- **GNU_parallel**
  - Citation: O. Tange (2011): GNU Parallel - The Command-Line Power Tool, ;login: The USENIX Maga-
    zine, February 2011:42-47
  - Site: http://www.gnu.org/software/parallel/ 
  - Version: 20140622
  - License: GPLv3

- **tabix**
  - Citation: 
  - Site: http://sourceforge.net/projects/samtools/files/tabix/ 
  - Version: 0.2.6 
  - License: 

- **Primer3**
  - Citation: Untergasser, A., et al. (2012) Primer3–new capabilities and interfaces, Nucleic acids research,
    40, e115.
  - Site: http://primer3.sourceforge.net/ 
  - Version: 2.3.5
  - License: GPLv2

- **SAMtools**

---

**9.8. Utility**

---
– Citation: Li, H., et al. (2009) The Sequence Alignment/Map format and SAMtools, Bioinformatics, 25, 2078-2079.
– Site: http://samtools.sourceforge.net/
– Version: 0.1.19
– License: MIT

• FaQCs
  – Citation: Chienchi Lo, Patrick S.G. Chain (2014) Rapid evaluation and Quality Control of Next Generation Sequencing Data with FaQCs. BMC Bioinformatics. 2014 Nov 19;15
  – Site: https://github.com/LANL-Bioinformatics/FaQCs
  – Version: 2.02
  – License: GPLv3

• wigToBigWig
  – Citation: Kent, W.J., et al. (2010) BigWig and BigBed: enabling browsing of large distributed datasets, Bioinformatics, 26, 2204-2207.
  – Site: https://genome.ucsc.edu/goldenPath/help/bigWig.html#Ex3
  – Version: 4
  – License:

• sratoolkit
  – Citation:
  – Site: https://github.com/ncbi/sra-tools
  – Version: 2.8.1
  – License:

• ea-utils
  – Citation: Erik Aronesty (2011) ea-utils : “Command-line tools for processing biological sequencing data”
  – Site: https://code.google.com/archive/p/ea-utils/
  – Version: 1.1.2-537
  – License: MIT License

• Anaconda2 (Python 2)
  – Citation:
  – Site: https://anaconda.org
  – Version: 4.1.1
  – License: 3-clause BSD

• Anaconda2Packages
  – Custom built directory containing all the required python2 packages for offline installation.
  – This was generated primarily using the command:
    
    ```
    pip download packageName
    ```
  – Some packages were manually downloaded into the directory to install via conda

9.8. Utility
- Dependencies were manually downloaded as they were discovered

- Anaconda3 (Python 3)
  - Citation:
  - Site: https://anaconda.org
  - Version: 4.1.1
  - License: 3-clause BSD

- Anaconda3Packages
  - Custom built directory containing all the required python3 packages for offline installation.
  - This was generated primarily using the command:

```
pip download packageName
```

### 9.9 Amplicon Analysis

- QIIME
  - Citation: Caporaso et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010 May;7(5):335-6
  - Site: http://qiime.org/
  - Version: 1.9.1
  - License: GPLv2
10.1 FAQs

• Can I speed up the process?
  You may increase the number of CPUs to be used from the “additional options” of the input section. The default and minimum value is one-eighth of total number of server CPUs.

• There is no enough disk space for storing projects data. How do I do?
  There is an archive project action which will move the whole project directory to the directory path configured in the $EDGE_HOME/sys.properties. We also recommend a symbolic link for the $EDGE_HOME/edge_ui/EDGE_input/public/ directory which points to the location where the users’ (or sequencing centers’) raw data are stored, obviating unnecessary data transfer via web protocol and saving local storage.

• How to decide various QC parameters?
  The default parameters should be sufficient for most cases. However, if you have very depth coverage of the sequencing data, you may increase the trim quality level and average quality cutoff to only use high quality data.

• How to set K-mer size for IDBA_UD assembly?
  By default, it starts from kmer=31 and iterative step by adding 20 to maximum kmer=121. Larger K-mers would have higher rate of uniqueness in the genome and would make the graph simpler, but it requires deep sequencing depth and longer read length to guarantee the overlap at any genomic location and it is much more sensitive to sequencing errors and heterozygosity. Professor Titus Brown has a good blog on general k-mer size discussion.

• How many reference genomes for Reference-Based Analysis and Phylogenetic Analysis can be used from the EDGE GUI?
  The default maximum is 20 and there is a minimum 3 genomes criteria for the Phylogenetic Analysis. But it can be configured when installing EDGE.

• How to make an app icon on the mobile device?
Launch the Safari browser on Apple’s iOS and navigate to the https://bioedge.lanl.gov/edge_ui/ or your EDGE instance website. (Please refresh the page a few times to update the cache) Tap the Share button on the browser’s toolbar — that’s the rectangle with an arrow pointing upward. It’s on the bar at the top of the screen on an iPad, and on the bar at the bottom of the screen on an iPhone or iPod Touch. Tap the Add to Home Screen icon in the Share menu.

Launch Chrome for Android and open the https://bioedge.lanl.gov/edge_ui/ or your EDGE instance website. (Please refresh the page a few times to update the cache) Tap the menu button and tap Add to homescreen. You’ll be able to enter a name for the shortcut and then Chrome will add it to your home screen.

- Why a job is queued and never autostarted?

The queued job had too much CPUs request. The autorun feature will start running queued job when there is available CPU resource. The queued job CPUs usage plus running jobs CPUs usage should be less than (\(<\) \texttt{edgeui\_tol\_cpu}) configured in the SEDGE\_HOME/edge_ui/sys.properties.

- Why some of the taxonomy profiling result are N.A.?

<table>
<thead>
<tr>
<th>Tool</th>
<th>#Reads</th>
<th>%Reads</th>
<th>Rank</th>
<th>Top1</th>
<th>Top2</th>
<th>Top3</th>
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</thead>
<tbody>
<tr>
<td>gottcha-speDB-b</td>
<td>189,392</td>
<td>3.2</td>
<td>species</td>
<td>Enterococcus faecium</td>
<td>Parabacteroides distasonis</td>
<td>Bacteroides vulgatus</td>
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<tr>
<td>gottcha-speDB-v</td>
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<td>0.0</td>
<td>species</td>
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<td>Streptococcus phage 20617</td>
<td>Paprika mild mottle virus</td>
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<tr>
<td>bwa</td>
<td>0</td>
<td>0.0</td>
<td>species</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Please check the log file to give us more information. For above example on BWA result, at web UI, you can open log file by Clicking the link next to “Output Directory” at “General” section -> ReadsBasedAnalysis -> Taxonomy -> log -> allReads-bwa.log.
In this case, it is out of memory. EDGE requires at least 16G memory. see System requirements
For machine with < 32Gb memory, we suggest to use the smaller BWA index (~13Gb) and contains
the databases for bwa taxonomic identification pipeline

```
```

### 10.2 Troubleshooting

- Process.log and error.log files may help on the troubleshooting.

#### 10.2.1 EDGE WEB GUI

- In the GUI, if you are trying to enter information into a specific field and it is grayed out or won’t let you, try
  refreshing the page by clicking the icon in the right top of the browser window.
- After installation, I can login but cannot select any files for input. the selection pop-up is empty.

This could be the permission issue on the EDGE_input/EDGE_output directory for Apache user.
Please see Apache Web Server Configuration

- I can not login to EDGE, it keeps saying Session expired.

The login session will expire in 12 hours. If you keeps get session expired message. It may indicate
the ‘/’ (root) space is full. Please try to clean up log files or others you/admins can delete. For
example, /var/log/message-2016xxxx is the archived log rotations which can be deleted for the space.

#### 10.2.2 Coverage Issues

- Average Fold Coverage reported in the HTML output and by the output tables generated in `{output directory}/AssemblyBasedAnalysis/ReadsMappingToContigs/` are calculated with mpileup using the default options
for metagenomes. These settings discount reads that are unpaired within a contig or with an insert size out of the expected bounds. This will result in an underreporting of the average fold coverage based on the generated BAM file, but one that the team feels is more accurate given the intended use of this environment.

10.2.3 Data Migration

- The preferred method of transferring data to the EDGE appliance is via SFTP. Using an SFTP client such as FileZilla, connect to port 22 using your system’s username and password.
- In the case of very large transfers, you may wish to use a USB hard drive or thumb drive.
- If the data is being transferred from another LINUX machine, the server will recognize partitions that use the FAT, ext2, ext3, or ext4 filesystems.
- If the data is being transferred from a Windows machine, the partition may use the NTFS filesystem. If this is the case, the drive will not be recognized until you follow these instructions:
  - Open the command line interface by clicking the Applications menu in the top left corner (or use SSH to connect to the system).
  - Enter the command: ‘sudo yum install ntfs-3g ntfs-3g-devel -y’
  - Enter your password if required.
- After a reboot, you should be able to connect your Windows hard drive to the system, and it will mount like a normal disk.

10.2.4 Known Issues

- Installations on CentOS 6.4 with Apache 2.2 are known to have difficulty executing jobs that have “.real” anywhere in the name. This is due to a CGI execution issue. The recommended resolution is to use an underscore in place of the period, or simply name your job something else.

10.3 Discussions / Bugs Reporting

- We have created a mailing list for EDGE users. If you would like to receive notifications about the updates and join the discussion, please join the mailing list by becoming the member of edge-users groups.
  EDGE user’s google group
- We appreciate any feedback or concerns you may have about EDGE. If you encounter any bugs, you can report them to our GitHub issue tracker.
  Github issue tracker
- Any other questions? You are welcome to Contact Us and Citation (page 90)
Questions? Concerns? Please feel free to email our google group at edge-users@googlegroups.com or contact a dev team member listed below.

<table>
<thead>
<tr>
<th>Name</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patrick Chain</td>
<td><a href="mailto:pchain@lanl.gov">pchain@lanl.gov</a></td>
</tr>
<tr>
<td>Chien-Chi Lo</td>
<td><a href="mailto:chienchi@lanl.gov">chienchi@lanl.gov</a></td>
</tr>
<tr>
<td>Paul Li</td>
<td><a href="mailto:po-e@lanl.gov">po-e@lanl.gov</a></td>
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<td>Karen Davenport</td>
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<td>Joe Anderson</td>
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<tr>
<td>Kim Bishop-Lilly</td>
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12.1 Citation

Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform

Po-E Li; Chien-Chi Lo; Joseph J. Anderson; Karen W. Davenport; Kimberly A. Bishop-Lilly; Yan Xu; Sanaa Ahmed; Shihai Feng; Vishwesh P. Mokashi; Patrick S.G. Chain

Nucleic Acids Research 2016;
doi: 10.1093/nar/gkw1027