

ASA³P

User Manual

Version 1.3.0

Oliver Schwengers

30.04.2020

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[This page provides an overview on the number of virulence factors and categories detected in each genome.](#)

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[Content - Widgets](#)

[Downloads](#)

[Links](#)

[Single Nucleotide Polymorphism \(SNP\) genome details](#)

[Content - Widgets](#)

[Downloads](#)

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[Core - pan genome](#)

[Content - Widgets](#)

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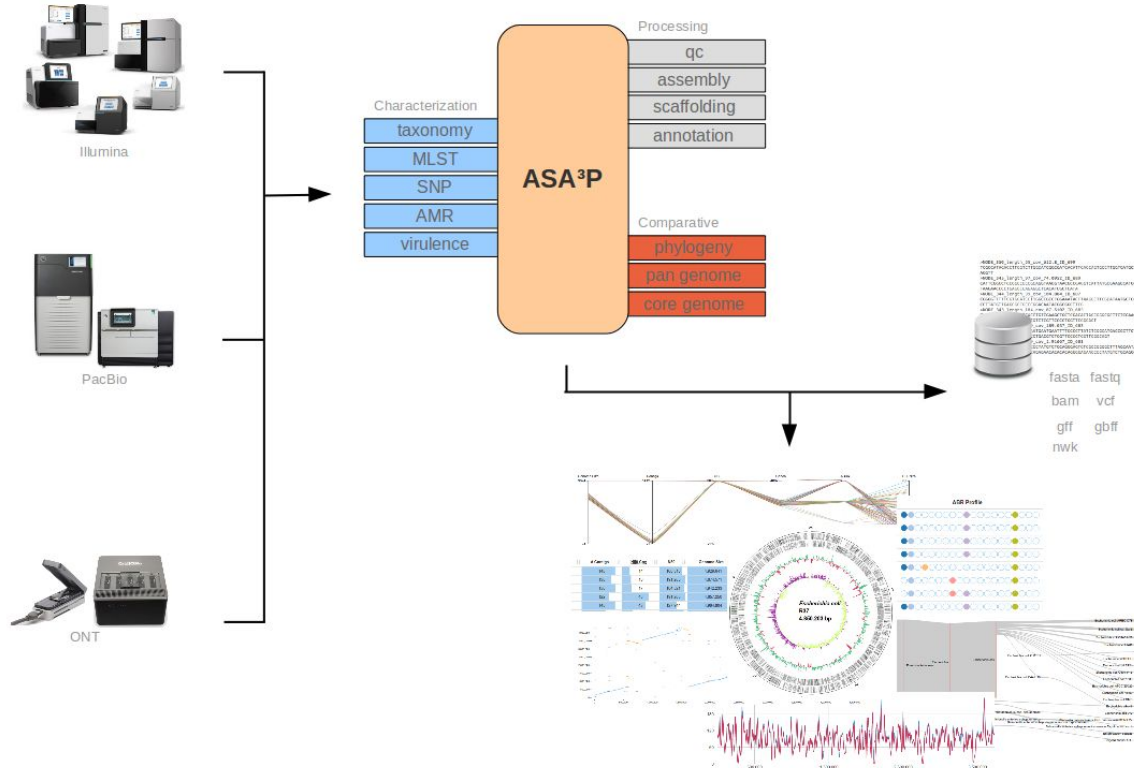
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Introduction

ASA³P is an automatic and highly scalable assembly, annotation and higher-level analysis pipeline for closely related bacterial isolates. It is developed as a command line tool creating standard bioinformatics file formats as well as sophisticated HTML5 documents.

Its main purpose is the automatic processing of large scale NGS data from all major sequencing platforms and thus transforming raw sequencing reads into assembled and annotated genomes and finally gathering as much information on each single bacterial genome as possible. Per-isolate analyses are finally complemented by first comparative insights. Hereby, the software incorporates many best-in-class open source bioinformatics tools and thus takes away the burden of ever repeating tasks. Envisaged as an upfront tool, it provides comprehensive insights as well as a general overview and comparisons of analysed genomes along with all necessary result files for subsequent deeper analyses, presenting all this by interactive modern HTML5 documents to the user.



Processing big data created by modern NGS technologies easily outscales traditional compute resources. Targeting this bottleneck, ASA³P is able to take advantage of already installed SGE compute clusters or even to automatically create such on cloud computing infrastructures. Using modern bioinformatic frameworks ([BiBiGrid](#)) combined with state of the

art cloud computing, ASA³P easily scales up underlying compute nodes and thus adopts to project sizes at any scale. Hence, processing and analyzing even thousands of bacterial genomes becomes a routine task.

This manual is intended for both normal users without deeper computer skills and advanced bioinformaticians who like to set up their own installations.

Normal users analyzing rather small projects (< 200 genomes) are highly encouraged to use our Docker based version as it is by far the simplest, easiest and most straightforward way to analyze a given set of genomes.

Facing bigger projects or higher demands in terms of runtime and/or throughput one should take advantage of the ASA³P cloud version.

Workflow

For the user

Although conducting a rather complex set of analyses ASA³P was developed to hide as much complexity from the user as possible. Therefore, for each run (subsequently referred to as **project** as we call it) it expects a distinct directory (**<project-dir>**) containing a

- config.xls
- data/
 - reference genome files
 - isolate raw reads, contigs, genome files

Internals

In order to speed up overall runtime, ASA³P executes as many analyses as possible in parallel. Hereby, its internal workflow is divided into five stages:

- 1) directory setup and creation of reference genome indices
- 2) processing per-isolate input data
- 3) per-isolate analyses and genome characterizations
- 4) comparative analyses
- 5) creating HTML5 reports

As a first step the pipeline sets up the internal directory structure and builds necessary reference genome indices. In a second step it incorporates raw sequencing reads, pre-assembled contigs as well as annotated genomes and subsequently conducts diverse quality control, assembly, scaffolding and annotation steps in order to process input data into assembled and annotated genomes as a starting point for step 3.

Based on these annotated genomes ASA³P performs several genome characterization steps, e.g. taxonomic classifications, MLST typing and detection of antibiotic resistances. Hereby, ASA³P tries to gather as much information as it is possible for an automatic pipeline. In a fourth stage, ASA³P performs comparative analyses such as a calculation of the core and pan genome as well as the creation of a phylogenetic tree.

Finally, all information and results get collected and presented in HTML5 documents taking advantage of modern interactive visualizations and widgets.

Input

ASA³P is able to incorporate sequencing reads from all major sequencing platform manufacturers, i.e. Illumina (Illumina), Pacific Bioscience (PacBio) and Oxford Nanopore Technologies (ONT). Illumina reads can either be provided as single or paired-end reads and are accepted as zipped fastq files. Both PacBio RSII and Sequel reads are supported and can be provided in the new Sequel unmapped BAM format as well as the legacy bax.h5 format. In order to process ONT reads, the raw data has to be transformed, i.e. base-called and demultiplexed and can then be provided as regular zipped fastq files, one per isolate. Due to the currently available plethora of available combinations of sequencing kits, barcodes and sequencing devices we decided to leave this pre-analysis to the users in order to skip an otherwise overwhelming amount of information necessary to appropriately analyze ONT raw reads.

In order to integrate published or third party genomes, ASA³P is also able to incorporate genome data at any level, e.g. assembled contigs, ordered and rearranged contigs and annotated genomes. Externally assembled genomes (contigs, scaffolds, ordered contigs)

are accepted as fasta files. Pre-annotated genomes are accepted in the following formats: GFF, GFF + Fasta, GenBank flat file, EMBL. For GFF only, Genbank flat file and EMBL format files must contain the genome sequences.

Output

tl; dr

Just open your browser and open the `index.html` file located within the **<project-dir>/reports** folder.

Following a convention-over-configuration approach ASA³P organizes all input and intermediate data as well as result files in a standardized directory structure. Thus, all analyzed projects have the same directory and file structure allowing users to easily find and extract all created data files.

As described before ASA³P expects a distinct directory for each project containing an ASA³P configuration file named `config.xls` and a directory called `data` comprising all input data:

- `config.xls`
- `data/`
 - reference genome files
 - isolate raw reads

After ASA³P has successfully finished, the project directory contains the listed additional files and directories:

- `asap.log`: a log file for debugging purposes
- `config.json`: a technical internal configuration file
- `references/`: all provided reference genomes, necessary file format conversions and mapping indices
- `reads_raw/`: raw reads and quality information files
- `reads_qc/`: quality clipped read files and quality information files
- `assemblies/`: one subfolder for each isolate containing assembled contigs and discarded contigs
- `scaffolds/`: one subfolder for each isolate containing scaffolded contigs and a pseudo genome containing linked scaffolds and contigs
- `annotation/`: one subfolder for each isolate containing genome annotation files (`.gbk`, `.gff`, `.ffa`, `.ffn`)
- `taxonomy/`: special information on each genome in distinct JSON files
- `mlst/`: special information on each genome in distinct JSON files
- `abr/`: special information on each genome in distinct JSON files
- `vf/`: special information on each genome in distinct JSON files

- mappings/: special information on each genome in distinct JSON files
- snps/: special information on each genome in distinct JSON files
- corepan/: core and pan genome as fasta files, a pan-genome-matrix file, a single JSON file for each isolate containing information on accessory and singleton genes
- phylogeny/: a newick file and the consensus sequences of all isolates
- reports/: HTML5 documents along with necessary CSS, JavaScripts and linked data files

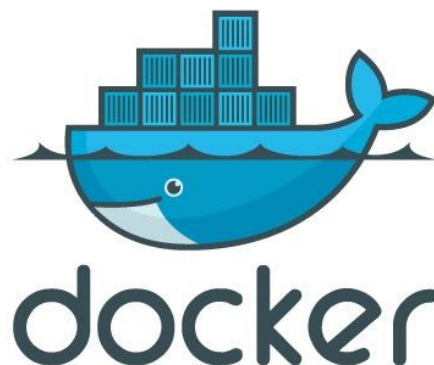
Versions

ASA³P is a complex software with many external dependencies which makes it hard to distribute and install. To overcome this bottleneck we provide two portable versions, i.e. a Docker based container image and a highly scalable OpenStack cloud version with fully-automatic internal cluster lifecycle management (full orchestration: create, configure, run, terminate).

Docker

For users without access to cloud infrastructures or without the need thereof, ASA³P provides an easy to use version based on the famous Docker[®] containerization software. The necessary container image is publicly hosted at Docker Hub[®]:

<https://hub.docker.com/r/oschwengers/asap>. Hereby, users can take advantage of utmost simplicity. In principle, using Docker, ASA³P can run on a powerful laptop albeit in most cases a high-class desktop machine or fairly large virtual machine in terms of hardware capacity will be needed.



Docker itself is an open-source and free software which creates and provides software containers which contain applications as well as necessary dependencies, e.g. software libraries, system tools, etc... Therefore, containers isolate the software from its surrounding environment and ensure the same conditions apply for every execution of the software. For further information please have a look at the official Docker manual: <https://www.docker.com/what-docker>.

Setup

To setup the ASA³P Docker version, users need to perform two steps:

- 1) pull the Docker image from Docker Hub
- 2) download and extract the ASA³P directory

Pull the ASA³P Docker image:

```
sudo docker pull oschwengers/asap
```

Due to its huge size the container itself **does not** include the actual ASA³P software and necessary database which are ~142 Gb in size. These components must be downloaded (once) and mounted to the container at execution.

Download and extract the ASA³P directory:

```
wget https://zenodo.org/record/3606300/files/asap.tar.gz?download=1
tar -xzf asap.tar.gz
rm asap.tar.gz
```

Execution

Execute ASA³P via Docker:

```
sudo asap/asap-docker.sh -p <project-dir> [-s <scratch-dir>] [-a  
<asap-dir>] [-c] [-z] [-d]
```

This is the preferred and recommended way to start an ASA³P analysis. By this all internal paths, environment variables and mount points will be set in an automatic manner.

Parameters:

- -p <project-dir>: mandatory: path to the actual project directory (containing `config.xls` and `data` directory)
- -a <asap-dir>: optional: path to the ASA³P dir in case the script was moved/copied somewhere else
- -s <scratch-dir>: optional: path to a distinct scratch/tmp dir
- -z: optional: path to a distinct scratch/tmp dir
- -c: optional: skip comparative analysis steps

- -d: optional: enable verbose debugging logs

If you have Docker installed on a rather slow or tiny device it is recommended to provide a different scratch directory for temporary files, e.g. assembly tmp files.

Manually start Docker container:

```
USER=$(id -u)
GROUP=$(id -g)
sudo docker run \
--rm \
--privileged \
--user $USER:$GROUP \
-v <asap-dir>:/asap/:ro \
-v <project-dir>:/data/ \
[-v <scratch-dir>:/var/scratch/] \
--volume="/etc/group:/etc/group:ro" \
--volume="/etc/passwd:/etc/passwd:ro" \
oschwengers/asap
```

Docker parameters:

1. --rm: removes the container after the run
2. --privileged: give Docker additional permissions in order to internally trigger Singularity containers
3. --user \$(id -u):\$(id -g): passes user and group ids to the Docker container
4. -v: mounts a folder from the host system into the container

Mounted volumes:

- <asap-dir>: absolute path to downloaded and extracted ASA³P directory (asap.tar.gz)
- <project-dir>: absolute path to local ASA³P project directory (containing config.xls and data subdirectory)
- <scratch-dir>: optionally path to a distinct scratch/tmp dir
- /etc/group: necessary to execute ASA³P as the current user instead of root within the Docker container
- /etc/passwd: necessary to execute ASA³P as the current user instead of root within the Docker container

Now, ASA³P runs in the background within a new container and saves result and log files into the mounted project directory on the local system. When the container has finished, the docker client will shut it down and remove all obsolete temporary files. Of course, mounted volumes will not be removed!

Example

Setup (only execute once):

```
cd ~
```

```
wget https://zenodo.org/record/3606300/files/asap.tar.gz?download=1
tar -xzf asap.tar.gz
rm asap.tar.gz
sudo docker pull oschwengers/asap
```

Start ASA³P analysis of an exemplary project:

```
wget \
https://zenodo.org/record/3606761/files/example-lmonocytogenes-4.tar.gz?download=1
tar -xzf example-lmonocytogenes.tar.gz
sudo asap/asap-docker.sh -p example-lmonocytogenes/
```

After ASA³P has successfully finished you can use your browser to open the HTML5 report index page located at:

```
~/example-lmonocytogenes/reports/index.html
```

Cloud - OpenStack

Introduction

As ASA³P Docker containers lack horizontal scalability via a compute cluster a scalable OpenStack based cloud solution has been developed. Especially, analyses of larger projects often need massive compute resources in order to finish within a reasonable amount of time. Therefore, the ASA³P cloud version has a built in support for the Sun Grid Engine (SGE) which automatically sets up all necessary infrastructure. Hereby, ASA³P also assesses and configures the optimal amount of slave nodes taking into account configurable limits and thus meeting specific cloud project quotas.

Technical background

In order to horizontally scale out and to distribute underlying analyses ASA³P needs a quite complex technical setup. The figure below shows the hardware/software setup during a pipeline execution:

Setup and configuration of the SGE based compute cluster is conducted via the [BiBiGrid](#) framework, developed at the university of Bielefeld. The BiBiGrid framework is implemented in Java and only requires user cloud credentials as well as some cloud/project specific information. For the [de.NBI](#) Openstack cloud the login credentials (except your password) can be downloaded in form of the Openstack RC v3 file. This file can be found under 'Access & Security' -> 'API Access' in your Openstack web interface. As the BiBiGrid framework is currently compatible with Openstack and [Amazon AWS](#) support for Amazon AWS is planned for the future.

Note:

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experience to read detailed external documents or to ask your administrators and colleagues for further help.

Note:

Please, bear in mind ASA³P was developed and tested on Ubuntu 16.04. Due to uncountable combinations of Linux distributions and versions we cannot give support for other setups than the tested one.

OpenStack configurations

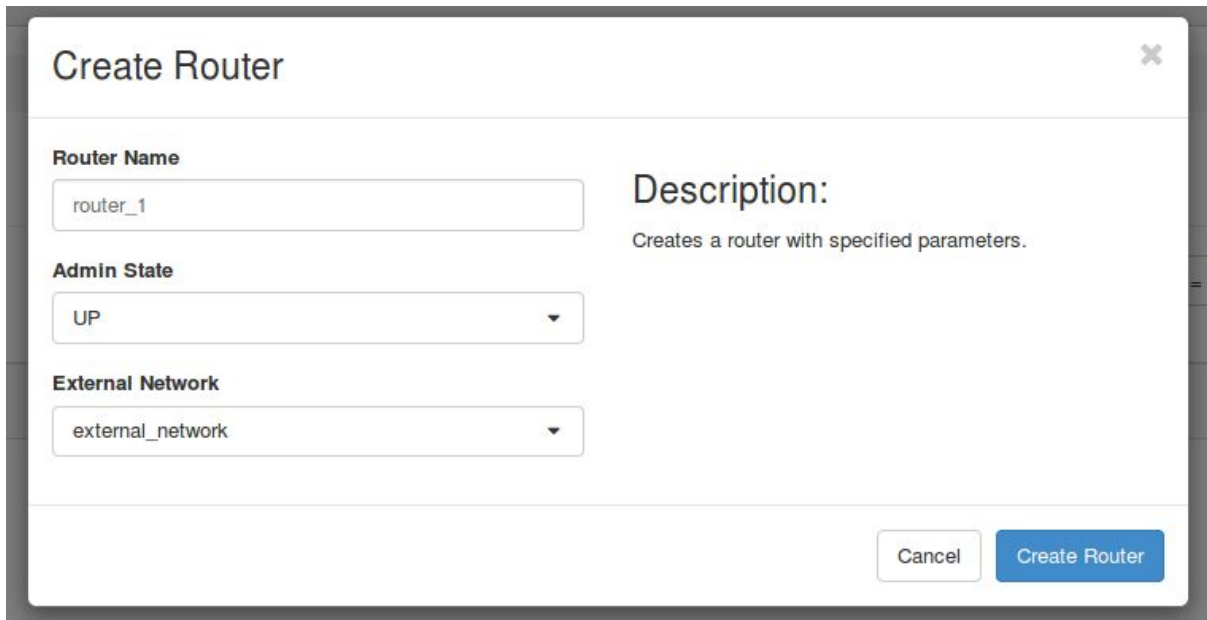
Before ASA³P can be executed in the cloud, users need to set up their OpenStack project. This step has to be performed only once for each OpenStack project

- 1) setup a network within your project
- 2) configure the default security group
- 3) create a SSH key pair
- 4) setup and start a **gateway** instance

Setup a network

Just like a physical network is needed to connect your local computer to the internet, virtual machines inside a cloud project need a virtual network. Such networks consist of a **router**, an **internal network** and a **subnet** to connect to each other and allow user access via SSH. All the following exemplary steps are shown inside the OpenStack web interface.

- a. set up a **router** by choosing '**Network**' -> '**Routers**' and click on '**+ Create Router**'. Set a name for the router, choose '**Admin State** **UP**' and **select** an **external network**.
(**Note:** the external network should already have been set up by your cloud administrator.)



Create Router [X]

Router Name

Admin State

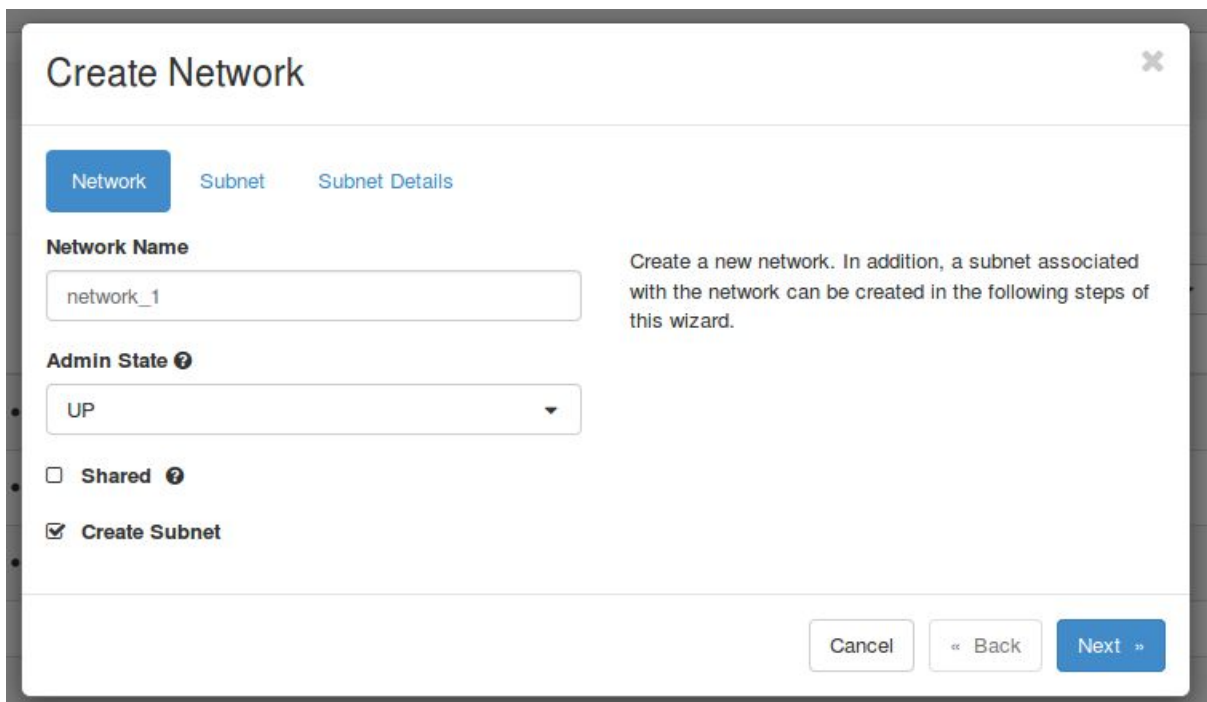
External Network

Description:
 Creates a router with specified parameters.

Cancel Create Router

- b. Set up a **network** and a **subnet** by selecting '**Network**' -> '**Networks**' and click on '+ **Create Network**'. Choose a name for the network, select '**UP**' as '**Admin State**', **uncheck** '**Shared**' and **check** '**Create Subnet**'.

Next select '**Subnet**' and choose a name for the subnet. Fill in a '**Network Address**', e.g. 192.168.0.0/24, select **IPv4** and leave the **Gateway IP empty**. Also leave the '**Subnet Details**' unchanged and click on '**Create**'.



Create Network [X]

Network Subnet Subnet Details

Network Name

Admin State ⓘ

☐ **Shared** ⓘ

☒ **Create Subnet**

Create a new network. In addition, a subnet associated with the network can be created in the following steps of this wizard.

Cancel « Back Next »

Create Network

Network

Subnet

Subnet Details

Subnet Name

Network Address ?

IP Version

Gateway IP ?

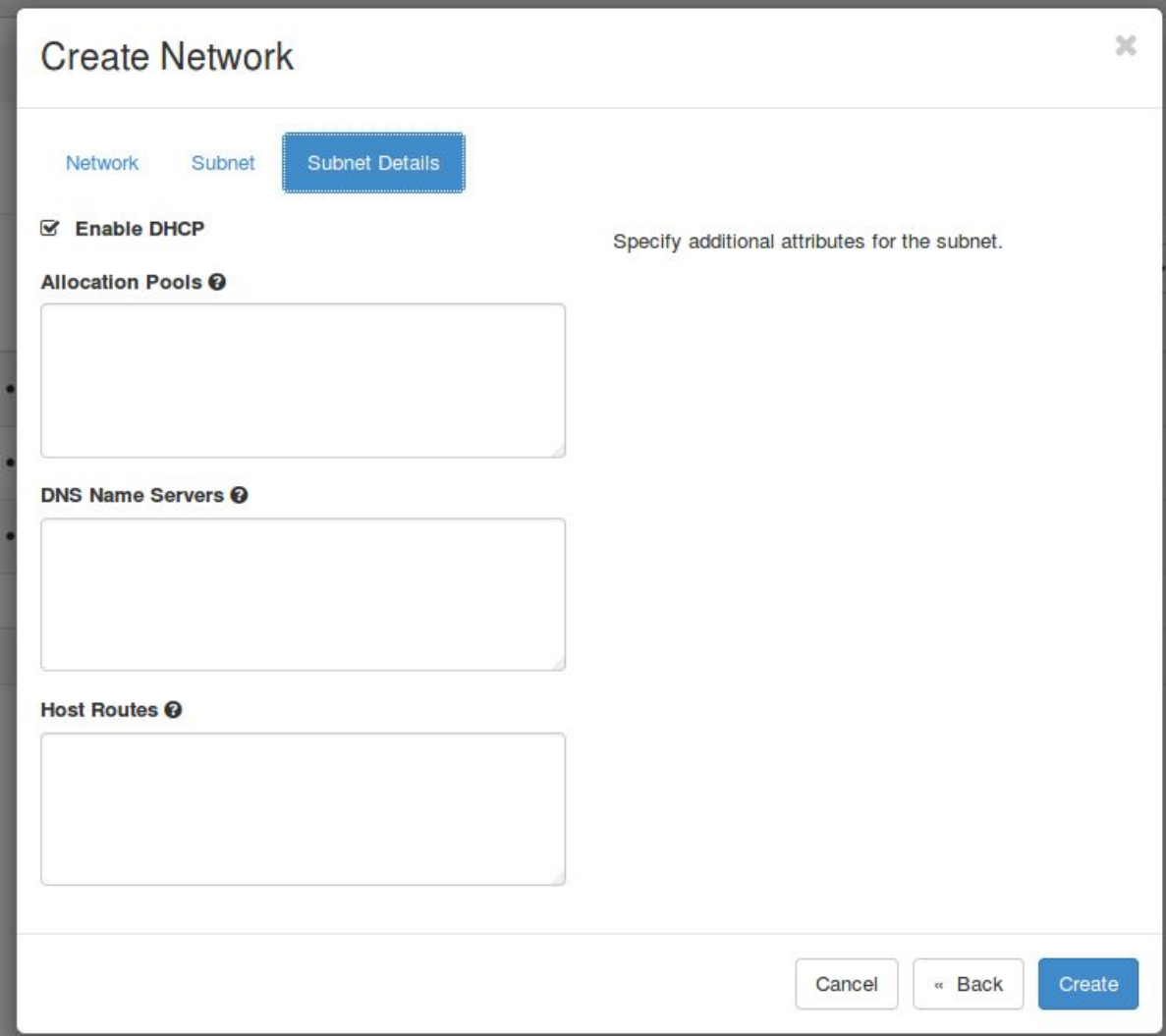
☐ **Disable Gateway**

Creates a subnet associated with the network. You need to enter a valid "Network Address" and "Gateway IP". If you did not enter the "Gateway IP", the first value of a network will be assigned by default. If you do not want gateway please check the "Disable Gateway" checkbox. Advanced configuration is available by clicking on the "Subnet Details" tab.

Cancel

« Back

Next »



The image shows a 'Create Network' dialog box with a close button (X) in the top right corner. It features three tabs: 'Network', 'Subnet', and 'Subnet Details', with 'Subnet Details' being the active tab. A checkbox labeled 'Enable DHCP' is checked. To the right of this checkbox is the text 'Specify additional attributes for the subnet.' Below the checkbox are three text input fields, each preceded by a label and a help icon: 'Allocation Pools', 'DNS Name Servers', and 'Host Routes'. At the bottom right of the dialog are three buttons: 'Cancel', '<< Back', and 'Create'.

Create Network

Network Subnet Subnet Details

☒ Enable DHCP Specify additional attributes for the subnet.

Allocation Pools ?

DNS Name Servers ?

Host Routes ?

Cancel << Back Create

- c. Finally, the router needs to be connected to the newly created network. Choose **'Network' -> 'Routers'** and click on the created router. Select **'Interfaces' -> '+ Add Interface'**, choose your subnet and click on **'Submit'**.

Configure the default security group

In OpenStack security groups define the allowed in- or outgoing traffic for your virtual machines. Each OpenStack project has its own default security group which usually does not allow ssh access to the virtual machines (Port 22). As access to the **gateway** instance via SSH is mandatory to run ASA³P, an extra rule has to be added to the default security group.

- login to the Openstack web interface and choose '**Access & Security**' -> '**Security Groups**'
- Select '**Manage Rules**' for the default security group
- Select '**+ Add Rule**', choose **SSH** as '**Rule**' and click on '**Add**'

Add Rule

Rule *

SSH

Remote * ?

CIDR

CIDR ?

0.0.0.0/0

Description:

Rules define which traffic is allowed to instances assigned to the security group. A security group rule consists of three main parts:

Rule: You can specify the desired rule template or use custom rules, the options are Custom TCP Rule, Custom UDP Rule, or Custom ICMP Rule.

Open Port/Port Range: For TCP and UDP rules you may choose to open either a single port or a range of ports. Selecting the "Port Range" option will provide you with space to provide both the starting and ending ports for the range. For ICMP rules you instead specify an ICMP type and code in the spaces provided.

Remote: You must specify the source of the traffic to be allowed via this rule. You may do so either in the form of an IP address block (CIDR) or via a source group (Security Group). Selecting a security group as the source will allow any other instance in that security group access to any other instance via this rule.

Cancel

Add

Create an SSH key pair

To access the **gateway** instance later via a secure shell (SSH), a SSH key pair has to be generated. This can easily be done via the OpenStack web interface. Therefore, login and choose '**Access & Security**' -> '**Key Pairs**' and click '**+ Create Key Pair**'. Then fill in a name and select '**Create Key Pair**'. Afterwards, the newly created public key will be automatically stored in OpenStack and a dialogue will open to download the private key file.

It is also possible to **import** an already created SSH key to OpenStack. Therefore, choose '**Access & Security**' -> '**Key Pairs**' and click '**Import Key Pair**'. Fill in a name for the key pair and paste the public key in the text field. Afterwards, choose '**Import Key Pair**' to upload the SSH key.

Setup and start a gateway instance

Start a new virtual machine instance. This VM merely acts as a starting point to transfer all data and finally execute ASA³P within the cloud project. Therefore, this VM is subsequently referred to as a gateway **instance**. All analyses will be carried out on additional VMs orchestrated by the ASA³P cloud script. Thus, this VM only needs rather small hardware capacities:

- min. 1 vCPU
- min. 1 GB RAM

After starting up the **gateway instance**, assign a **Floating IP** to it via the OpenStack web interface. Therefore, select '**Compute**' -> '**Instances**' and click the 'arrow-down button' in the '**Action**' column of the **gateway instance**. In the drop down menu choose '**Associate Floating IP**' and select an IP address. If no IP address is available, a new address can be assigned with the '+' button. The **gateway instance** is now accessible via ssh and the assigned **Floating IP address**.

ASA³P installation and configuration

- 1) create two volumes storing the ASA³P directory and project data
- 2) download the Openstack RC v3 file
- 3) install and configure the ASA³P cloud version

Create ASA³P and data volumes

- a) create two new volumes:
 - **volume 1**: 250 GB size for the ASA³P directory
 - **volume 2**: depends on the size of your project
- b) attach both volumes to the **gateway instance** via the Openstack web interface
- c) login into the **gateway instance**
- d) lookup identifier of attached volumes:


```
sudo fdisk -l
```

- e) create an **ext4** file system on both volumes:

```
sudo mkfs -t ext4 /dev/<asap-device>
sudo mkfs -t ext4 /dev/<data-device>
```
- f) mount **volume 1** to /asap/

```
sudo mkdir /asap/
sudo mount /dev/<asap-device> /asap/
sudo chmod 777 /asap/
```
- g) download **ASA³P** and extract it into the volume:

```
cd /asap/
wget https://zenodo.org/record/3606300/files/asap.tar.gz?download=1
tar -xzf asap.tar.gz
rm asap.tar.gz
mv ./asap/* .
rmdir ./asap/
```
- h) unmount **volume 1**

```
sudo umount /asap/
```
- i) detach **volume 1** from the **gateway instance** via the OpenStack web interface
- j) mount **volume 2** to /data/

```
sudo mkdir /data/
sudo mount /dev/<data-device> /data/
sudo chmod 777 /data/
```
- k) upload your project data directory (containing config.xls and data subdirectory) via scp

Install and configure the ASA³P cloud version

- a) download the Openstack RC v3 file (via the OpenStack web interface)
 - login to the Openstack web interface
 - choose '**Access & Security**' -> '**API Access**' and select '**Download OpenStack RC File v3**'
 - the OpenStack RC File contains required environment variables for the subsequent cluster creation
 - copy the file to the **gateway** instance (e.g. via scp)
- b) login to the **gateway instance**
- c) install Java OpenJDK 8 and OpenStack client

```
sudo apt-get update
sudo apt-get install openjdk-8-jdk python-openstackclient
```
- d) download and extract necessary **ASA³P** cloud files to the home directory

```
wget \
https://zenodo.org/record/3606300/files/asap-cloud.tar.gz?download=1
tar -xzf asap-cloud.tar.gz
```
- e) fill out the **asap.properties** file (~/.asap-cloud/asap.properties)
 Edit the file with a command line editor (e.g. nano) replacing the bold values to your OpenStack project.
 - cloud.region=<**openstack-name-region**>
region of the used cloud
 - cloud.zone=<**openstack-name-zone**>

zone of the used cloud

- project.subnet.id=<openstack-id-subnet>
* subnet in your cloud to host the BiBiGrid cluster
- project.cpu=<openstack-quota-vcpus>
* quota of max. number of available vCPUs (cores)
- project.mem=<openstack-quota-vmem>
* quota of max. number of available Gb memory (RAM)
- vm.flavour=<openstack-name-flavour>
name of the instance flavor for the cluster VMs
- vm.image.id=<openstack-id-base-image>
* ID of the OpenStack Ubuntu 16.04 base image
- vm.cpu=<openstack-vm-cpu>
number of vCPUs (cores) for the VM flavour
- vm.mem=<openstack-vm-mem>
number of Gb memory (RAM) for the VM flavour
- volume.asap.id=<openstack-id-asap-volume>
* ID of the volume containing ASA³P (**volume 1**)
- volume.data.id=<openstack-id-data-volume>
* ID of the volume containing the project directory (**volume 2**)

If you run ASA³P within the de.NBI cloud at Justus-Liebig-University Gießen the `asap-denbi-giessen.properties` template can be used and only marked (*) property entries have to be changed. Rename the template to `asap.properties` before you proceed.

Example:

```
cloud.region=RegionOne
cloud.zone=nova
project.cpu=100
project.mem=60
project.subnet.id=01ddceed-59d7-491a-a857-6f78b3893a0b
vm.instance=de.NBI.small
vm.image.id=a200e6fb-caf7-41b2-97ae-954d8294f0c9
vm.cpu=8
vm.mem=16
volume.asap.id=e1dd6746-65c5-4172-b1bb-5d3137ce9295
volume.data.id=b046fc0e-5f1f-4e6a-9fce-f84fec4d4dbb
```

Start ASA³P in the cloud

Once everything is setup and configured correctly, you merely have to log into the **gateway instance** (if not already done) and execute the ASA³P cloud script:

```
~/asap-cloud/asap-cloud.sh -i <instance-id> -o <openstack-rc> -p
<project-dir>
```

The script accepts three parameters:

<instance-id>: OpenStack ID of the **gateway** instance

<openstack-rc> : path to Openstack RC v3 file

<project-dir> : path to local ASA³P project directory (containing config.xls and data subdirectory, e.g. /data/my-first-project)

At runtime the script will ask for the OpenStack user password. This is a necessity in order to perform all internal steps such as attaching/detaching volumes, starting/stopping VMs, etc...

Note:

As the script waits until ASA³P has finished execution, we strongly encourage users to execute the script inside a detachable session with tmux or screen. For further information please read the official documentations.

After ASA³P has finished, the script will automatically stop all started SGE and NFS servers and terminate all cluster VMs. Now, all data, results as well as HTML5 reports are stored within the project directory and accessible again from:

/data/<your-project-dir>/

Custom installation on private cluster systems

As ASA³P comes with a very high number of external dependencies, we highly discourage from custom installations. Nevertheless, if you need to do so, please note that we cannot offer any support for this! We apologize for any inconvenience but this would simply be way out of our capabilities.

Before setting everything up step by step please, take notice of the following pre requirements:

- 1) a shared directory (e.g. NFS) for
 - a) ASA³P home directory
 - b) your project data
- 2) a working compute cluster based on either Sun Grid Engine or Open Grid Scheduler
- 3) access to all shared directories mentioned in 1) from the executing machine as well as all cluster slave nodes

We developed and tested ASA³P on Ubuntu LTS 16.04. All installation instructions are related to this specific Linux distribution. If you use an alternative one, please bear in mind that certain packages might be missing. In this case you would need to figure out all lacking dependencies by your own.

If you meet all listed pre requirements follow the subsequent steps:

- 1) Install all necessary Ubuntu packages via apt-get:
 - a) gnuplot-nox
 - b) less
 - c) libdatettime-perl
 - d) libxml-simple-perl
 - e) libdigest-md5-perl
 - f) bioperl
 - g) libtbb2

- h) openjdk-8-jdk
 - i) python3
 - j) python3-setuptools
 - k) python3-pip
 - l) roary
 - m) singularity-container
- 2) Install python dependencies via pip3:
 - a) biopython
 - b) networkx
 - c) numpy
 - d) filetype
 - e) pytest
 - f) mock
 - g) pandas
 - h) matplotlib
 - i) seaborn
 - j) pyfaidx
 - k) pyahocorasick
 - 3) Download and extract the ASA³P directory containing all scripts, configs and shared executables to a shared directory:
<https://zenodo.org/record/3606300/files/asap.tar.gz?download=1>
 - 4) Set and export an environment variable called "ASAP_HOME" pointing to the aforementioned directory.

Download Files

All necessary files can be downloaded from our public Zenodo repository at:

<https://doi.org/10.5281/zenodo.3606299>

Common files

- ASA³P directory (software & databases):
<https://zenodo.org/record/3606300/files/asap.tar.gz?download=1>
- Excel[®] configuration template
<https://zenodo.org/record/3606300/files/config.xls?download=1>
- This manual
<https://zenodo.org/record/3606300/files/manual.pdf?download=1>

For local containerized execution you need to pull the container image from Docker Hub:

<https://hub.docker.com/r/oschwengers/asap>

For the OpenStack based cloud version you need a set of cloud orchestration files bundled in the following tarball:

<https://zenodo.org/record/3606300/files/asap-cloud.tar.gz?download=1>

In either case you will need to download the ASA³P directory tarball and extract it.

Exemplary Data

To get started we provide an exemplary configuration spreadsheet:

<https://zenodo.org/record/3606300/files/config-example.xls?download=1>

and 2 exemplary projects:

<https://zenodo.org/record/3606761/files/example-lmonocytogenes-4.tar.gz?download=1>

<https://zenodo.org/record/3606761/files/example-ecoli-input.tar.gz?download=1>

The first project is a subset of 4 *Listeria monocytogenes* strains collected, isolated and uploaded to SRA by the FDA. The related bioproject is PRJNA215355.

The latter one is a collection of different public *Escherichia coli* strains sequenced on different sequencing platforms to merely show the broad range of support input data types.

Analyses

Quality Control / Clipping

This step provides a quality overview of all sequenced reads before and after the actual quality clipping. In the quality clipping process reads unsuitable for subsequent analysis steps are filtered out. Quality of sequenced reads is measured via [FastQC](#). A check for potential contaminations is conducted via [FastQ Screen](#). Reads which were sequenced on an Illumina platform are quality clipped with [Trimmomatic](#) using the following settings: "ILLUMINACLIP: and :2:30:10", 'LEADING:15', 'TRAILING:15', 'SLIDINGWINDOW:4:20', 'MINLEN:20', 'TOPHRED33'. Reads which were sequenced on a Pacific Bioscience platform are not quality clipped as this is internally performed by the HGAP 4 assembler. Reads which were sequenced on an Illumina platform are quality clipped with [Filtlong](#) using the following settings: "--min_length 500 --min_mean_q 85 --min_window_q 65".

Assembly

Reads that passed quality control get assembled into contigs. PacBio, Illumina and Oxford Nanopore reads are assembled with [HGap4](#), [SPAdes](#) and [Unicycler](#), respectively.

Scaffolding

Orders and orientations of assembled contigs are somewhat arbitrary. During a scaffolding step ASA³P tries to map those contigs onto a set of closely related (user provided) reference genomes in order to rearrange them. With this additional information scaffolders can fix the order and orientation and merge multiple contigs into scaffolds. As a modern multi-reference scaffolder ASA³P internally takes advantage of the tool [MeDuSa](#). As joined contigs pose an

artificial bridge an artificial six frame stop codon sequence is used to mark such positions 'NNNNNNNNNNCTAGCTAGCTAGCNNNNNNNNNN'. By using this sequence to link all scaffolds and contigs ASA³P also provides pseudo genomes. Finally, raw contigs as well as oriented and linked scaffolds are mapped onto all provided reference genomes in order to compare the results of this step.

Annotation

To annotate contigs and scaffolds ASA³P internally uses [Prokka](#) and [Barrnap](#). For high quality annotation genus specific information is used. Therefore, ASA³P uses genus specific Blast databases comprising all [RefSeq](#) genome annotations related to a certain genus. In order to further increase annotation quality ASA³P uses a combination of small specialised high quality databases such as [CARD](#) for antimicrobial resistance genes and [VFDB](#) for virulence factors.

Taxonomic Classification

For the taxonomic classification of bacterial isolates ASA³P uses three distinct methods:

- Kmer profile & ANI values
- 16S sequence homology
- Comparison of average nucleotide identities ([ANI](#))

The first two are user provided reference-free solutions where the last one is a reference based approach.

Kmer profiles are compared against representative and reference genomes from [RefSeq](#) and for promising candidates [ANI](#) and conserved DNA values are computed. based database. In order to search for 16S homology the pipeline uses [Infernal](#) to extract the best scoring 16S sequence and subsequently queries it against the [RDP](#) 16S database.

Finally, the pipeline uses a proprietary [ANI](#) implementation based on [Nucmer](#) to calculate whole genome sequence identity as a reference based solution.

Multilocus Sequence Typing (MLST)

[MLST](#) is a typing method for closely related bacterial strains within a species. Therefore, genomes are blasted against public databases containing 5 to 7 thoroughly selected loci for each typed organism. Each combination of alleles determines a unique sequence type.

ASA³P uses a proprietary implementation based on BLASTn and the public database [PubMLST](#). If a genome contains exactly one reference loci set the classification was successful. Otherwise, the most similar reference is shown in case there were sufficient matches.

Antibiotic Resistance Detection (ABR)

There are many different molecular mechanisms for ABR posing a major bioinformatic challenge. Addressing this issue ASA³P takes advantage of the Comprehensive Antibiotic Resistance Database ([CARD](#)) and its corresponding search tool. The database is manually curated and updated on a monthly basis. Additionally, CARD provides its own sophisticated ontology in order to classify detected ABRs. To our best knowledge it's the only database/tool which can detect, classify and describe several different types of ABR, e.g. gene homology and mutations driven mechanisms.

Virulence Factor (VF) Detection

As VF have a major impact on whether a bacterial strain is harmless or a severe pathogen ASA³P provides a detection of potential VFs. Therefore, the pipeline identifies VFs via a BLASTn search against the virulence factor database ([VFDB](#)). Hits with a coverage of at least 80 % and a percent identity of 90 % or higher are taken into account. The corresponding loci are only assigned with their highest scoring hit.

Reference Mapping

In order to assess an isolate genome size compared to a reference genome and subsequently enable the calling of single nucleotide variants sequenced and quality clipped reads are mapped to the reference genome at the first position in a project. For Illumina, Pacific Bioscience and Oxford nanopore reads ASA³P uses [Bowtie 2](#), [blasr](#) and [Minimap2](#), respectively. Finally, generated Sequence Alignment/Map (SAM) files are converted to ordered Binary Alignment/Map (BAM) files via [SAMtools](#).

Single Nucleotide Polymorphism (SNP)

This analysis provides information on SNPs compared to the reference genome. Via the mpileup function of [SAMtools](#) mapped BAM files together with the reference genome are used to compute the likelihood of each possible genotype. The resulting likelihoods containing genomic positions are stored as Binary Variant Call Format (BCF) files. [BCFtools](#) is then used to call variants in the sequence compared to the reference. Genomic variants in the resulting Variant Call Format (VCF) file are then filtered via [SnpSift](#). Finally, filtered variants get annotated via [SnpEff](#) to predict resulting effects.

Core - pan genome

Coding sequences (CDS) of the analysed genomes get clustered and assigned to gene abundance groups via [Roary](#). These groups consist of genes present in all analysed genomes ('core'), genes present at least in one other analysed genome ('accessory') and

genes unique to one a single genome ('singletons'). Internally, Roary uses [CD-HIT](#) and [BLAST+](#) and is provided with .gff files resulting from prior annotation.

Phylogeny

Based on a consensus sequence created via [BCFtools](#) ASA³P uses [FastTreeMP](#) to calculate a phylogenetic tree of all analyzed genomes. FastTreeMP follows an approximately-maximum-likelihood approach on nucleotide level. The resulting newick file (.nwk) contains the tree representation including edge lengths. ASA³P provides a comprehensive visualization of such trees via the web based JavaScript library [PhyloCanvas](#).

Results

The ASA³P workflow comprises several steps which each of output their own data files and information. All results are stored in a standardized directory structure explained before for follow-up analyses. Additionally, ASA³P creates interactive and responsive HTML5 reports providing aggregated information in a dense and user friendly manner. Therefore, ASA³P presents certain information via specialized HTML5/Javascript widgets from both open source and private sources. Result pages also inform users on failed or skipped analyses. The following sections provide comprehensive information on which reports contain which results and how to interact with related widgets.

Quality clipping overview

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in '# Reads' column. Their data field filling ratio corresponds to the ratio of field value to column maximum. Mouse over on underlined table headers to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- **Details** on the quality control of a particular genome can be accessed via click on the magnifying glass in the overview table.
- [FastQC](#); Simon Andrews (2010). FastQC: A quality control tool for high throughput sequence data.
- [FastQ Screen](#); Steven Wingett (2011). FastQ Screen allows you to screen a library of sequences in FastQ format against a set of sequence databases so you can see if the composition of the library matches with what you expect.
- [Trimmomatic](#); Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. Bioinformatics, btu170. [PubMed](#).
- [Filtlong](#); Wick, R. (2017)

Glossary

- **GC**: GC content in percent.
- **Genome**: Name of the processed genome.
- **Length**: Minimal/ mean/ maximal read length for this particular genome.
- **PC**: Read percentage of potential contaminations. Based on a 10% random subset mapping against a contamination references data base (e.g. containing phiX sequences).
- **Quality**: Minimal/ mean/ maximal PHRED score of sequenced reads for this particular genome (error probability; PHRED 20: 1 in 100; PHRED 30: 1 in 1000).
- **# Reads**: Number of sequenced reads for this particular genome.

Quality clipping genome details

Content - Widgets

Table raw

Displays the properties of the raw data, including File names, the # Reads, read Lengths, Quality and GC percentage.

Table QC

Displays the properties of data after quality control, including File names, the # Reads, read Lengths, Quality and GC percentage.

Boxplot potential contaminations

The percentage of reads that could not be mapped to the reference but to different contamination targets is shown per target. The different targets include human, mouse PhiX and vectors.

Interactive diagram groups

The first diagram of each quartet refers to the forward reads of raw data, the second to forward quality controlled data, the third to reverse reads of raw data and the fourth to

reverse reads of quality controlled data. Via mouseover on the diagram the according filename is displayed.

Per base qualities

Diagrams with the quality scores across all bases. On the x-axis the base position in the reads is displayed. On the y-axis the Quality as PHRED score is shown.

Per sequence qualities

Diagrams with the quality score distribution over all sequences. On the x-axis the mean sequence Quality as PHRED score of a read is shown. On the y-axis the number of reads is display.

Per base sequence contents

Diagrams with the sequence content across all bases. On the x-axis the base position in the reads is displayed. On the y-axis the percentage of each base (A, C, G, T) across all reads is displayed.

Per sequence GC contents

Diagrams with the GC distribution over all sequences. The red graph shows the GC count per read, the blue graph shows the theoretical distribution. On the x-axis the mean GC content of the reads is display. On the y-axis the number of reads is display.

Per base N contents

Diagrams with the N content across all bases. On the x-axis the base position in the reads is displayed. On the y-axis the percentage of bases characterised as 'N' (not assignable) is displayed.

Sequence length distributions

Diagrams with the distribution of sequence lengths over all sequences. On the x-axis the sequence lengths of the reads are displayed. On the y-axis the number of reads is displayed.

Kmer profiles

Diagrams with the log₂ ratio from observations to expected kmers. The six kmers with the highest log₂ obs/exp are displayed. On the x-axis the base position in the reads is display. On the y-axis the log₂ ratio from observations to expected kmers is displayed.

Glossary

- **GC:** GC content in percent.
- **Length:** Minimal/ mean/ maximal read length for this particular file.
- **Potential Contaminations:** Read percentage of potential contaminations. Based on a 10% random subset mapping against a contamination references data base (e.g. containing phiX sequences).

- **Quality:** Minimal/ mean/ maximal PHRED score of sequenced reads for this particular genome (error probability; PHRED 20: 1 in 100; PHRED 30: 1 in 1000).
- **# Reads:** Number of sequenced reads for this particular file.

Assembly overview

Content - Widgets

Interactive dotplot

Via the radio buttons on the right key data for X and Y axis can be selected. Mouse over a dot of interest to display the according genome name as well as horizontal and vertical value extensions. Zooming can be applied via marking the area of interest with left mouse button down. To reset the view right click.

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in most columns containing numeric values. Their data field filling ratio corresponds to the ratio of field value to column maximum. Mouse over on underlined table headers to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file a via click on the csv button (search and sorting are contained in the downloaded file). To download the fasta file of a particular genome assembly click on fasta in the data table.

Links

- **Details** on the assembly of a particular genome can be accessed via click on the magnifying glass in the overview table.
- **HGap:** Chin, Chen-Shan, et al. "Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data." *Nature methods* 10.6 (2013): 563-569. [PubMed](#).
- **SPAdes:** Bankevich A., Nurk S., Antipov D., Gurevich A., Dvorkin M., Kulikov A. S., Lesin V., Nikolenko S., Pham S., Prjibelski A., Pyshkin A., Sirotkin A., Vyahhi N., Tesler G., Alekseyev M. A., Pevzner P. A. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology*, 2012. [PubMed](#).
- **Unicycler:** Wick R. R., Judd L. M., Gorrie C. L., and Holt K. E. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol*. 2017 Jun; 13(6): e1005595.

Glossary

- **# Contigs**: Number of contigs (set of overlapping DNA segments).
- **GC**: GC content in percent.
- **Genome**: Name of the processed genome.
- **Genome size**: Genome size in 1000 bases [kb].
- **Mean contig lengths**: Mean contig lengths of this particular genome.
- **Median contig lengths**: Median contig lengths of this particular genome.
- **N50**: Given ordered contigs from longest to smallest, length of the contig at 50% of the genome length.
- **N50 coverage**: Length weighted mean coverage of sequences with N50 length or longer.
- **N90**: Given ordered contigs from longest to smallest, length of the contig at 90% of the genome length.
- **N90 coverage**: Length weighted mean coverage with sequenced reads of N90 contigs.

Assembly genome details

Content - Widgets

Histograms of contig specifications

Contig lengths

Histogram of contig length in kb. Via mouse over the number of contigs in each bin is displayed.

Contig coverage

Histogram of the average read coverage per contig. Via mouse over the average coverage of each bin is displayed.

Contig GC contents

Stacked histogram of GC contents per contig. Via mouse over the GC content of each individual contig is displayed.

Basic assembly statistics

Provides information on the assembly in general and on the contig length.

Interactive data table contigs

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Mouseover on underlined table headers to display further information on it.

Downloads

The contigs and scaffolds used in this assembly as well as the ones discarded (not used for assembly) can be downloaded as fasta on the right below the histograms. The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Glossary

- **Contigs:** Set of overlapping DNA segments (reads).
- **Coverage:** Mean read coverage of this contig.
- **# Gaps:** Amount of space (bp) between assembled nucleotides in this contig.
- **GC:** GC content in percent.
- **Length:** Length of the contig in base pairs.
- **N50 length:** Given ordered contigs from longest to smallest, length of the contig at 50% of the genome length.
- **N90 length:** Given ordered contigs from longest to smallest, length of the contig at 90% of the genome length.
- **Name:** Name of this contig.
- **Scaffolds:** Consists of aligned contigs with the sequence 'NNNNNNNNNNCTAGCTAGCTAGCNNNNNNNNNN' in between them.

Scaffolding overview

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in columns containing numeric values. Their data field filling ratio corresponds to the ratio of field value to column maximum. Mouse over on underlined table headers to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) a file via click on the csv button (search and sorting are contained in the downloaded file). To download a fasta file containing the Scaffolds or the generated Pseudo genome click on the according name in the data table.

Links

- **Details** on the contig layout of a particular genome can be accessed via click on the magnifying glass in the overview table.
- **MeDuSa**: E Bosi, B Donati, M Galardini, S Brunetti, MF Sagot, P Lió, P Crescenzi, R Fani, and M Fondi. MeDuSa: a multi-draft based scaffolder. Bioinformatics (2015): btv171. [PubMed](#).
- **MUMmer/Nucmer**: Open source MUMmer 3.0 is described in "Versatile and open software for comparing large genomes." S. Kurtz, A. Phillippy, A.L. Delcher, M. Smoot, M. Shumway, C. Antonescu, and S.L. Salzberg, Genome Biology (2004), 5:R12. [PubMed](#).

Glossary

- **# Contigs**: Number of contigs (set of overlapping DNA segments).
- **Genome**: Name of the processed genome.
- **N50**: Given ordered contigs from longest to smallest, length of the contig at 50% of the genome length.
- **Pseudo genome**: Genome generated via joining all sequence elements after scaffolding with the sequence 'NNNNNNNNNNCTAGCTAGCTAGCNNNNNNNNNN'.
- **# Scaffolds**: Number of scaffolds (joined, aligned and assigned contigs) after polishing. Joined with the sequence 'NNNNNNNNNNCTAGCTAGCTAGCNNNNNNNNNN'.

Scaffolding genome details

Provides information on contig alignment and assignment to reference genome(s). The contigs of the particular Whole Genome Assembly (WGA) are compared to each of the reference genomes via Synteny plots. In order to visualize the scaffolding quality the comparison is done before and after the scaffolding process.

Content - Widgets

Basic scaffolding statistics

Provides information on scaffolding in general and on the scaffold length.

DNA synteny plots

The upper synteny plot of each genome comparison displays the position of all contigs in both genomes before the scaffolding process (Pre Scaffolding). The lower synteny plot after scaffolding (Post Scaffolding). On the x-axis the contig position in the reference genome is displayed. On the y-axis the contig position in the WGA is displayed. Contigs referenced to the minus strand are displayed in orange the ones referenced to the plus strand are displayed in blue. Mouse over a contig to receive information on its name, length assigned strand as well as start and end position in the reference.

Downloads

The scaffolds and the generated pseudo genome can be downloaded as fasta on the top right.

Glossary

- **# Contigs**: Number of contigs (set of overlapping DNA segments).
- **Genome Size [Mb]**: Size of the WGA in million/mega bases.
- **N50**: Given ordered contigs from longest to smallest, length of the contig at 50% of the genome length.
- **N90**: Given ordered contigs from longest to smallest, length of the contig at 90% of the genome length.
- **# Scaffolds**: Number of scaffolds (joined, aligned and assigned contigs) after polishing.
- **WGA**: Whole Genome Assembly generated via joining all sequence elements after scaffolding with the sequence 'NNNNNNNNNNCTAGCTAGCTAGCNNNNNNNNNN'.

Annotation overview

Content - Widgets

Interactive dotplot

Via the radio buttons on the right key data for X and Y axis can be selected. Mouse over a dot of interest to display the according genome name as well as horizontal and vertical value extensions. Zooming can be applied via marking the area of interest with left mouse button down. To reset the view right click.

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in columns containing numeric values. They visualize the relative relation of this value compared to the according values of the other genomes.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file). To download the GenBank (gbk) or General Feature Format (gff) file of a particular genome assembly click on gbk or gff in the data table.

Links

- [Barrnap](#); Barrnap predicts the location of ribosomal RNA genes in genomes. It supports bacteria (5S,23S,16S), archaea (5S,5.8S,23S,16S), mitochondria (12S,16S) and eukaryotes (5S,5.8S,28S,18S). [GitHub](#).
- **Details** on the annotation of a particular genome can be accessed via click on the magnifying glass in the overview table.
- [Prokka](#): Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014 Jul 15;30(14):2068-9. PMID:24642063. [PubMed](#).
- [RefSeq](#): O'Leary, Nuala A., et al. "Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation." Nucleic acids research (2015): gkv1189. [PubMed](#).
- [CARD](#); Jia et al. 2017. CARD 2017: expansion and model-centric curation of the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 45, D566-573. [PubMed](#).
- [VFDB](#): Chen LH, Zheng DD, Liu B, Yang J and Jin Q, 2016. VFDB 2016: hierarchical and refined dataset for big data analysis-10 years on. Nucleic Acids Res. 44(Database issue):D694-D697. [PubMed](#).

Glossary

- **# CDS**: Number of coding DNA sequences found.
- **# CRISPR/CAS**: Number of CRISPR cassettes found.
- **# Genes**: Number of genes found.
- **Genome**: Name of the processed genome.
- **# Hyp. Proteins**: Number of hypothetical protein coding genes found.
- **# ncRNA**: Number of non coding RNA genes found.
- **# rRNA**: Number of ribosomal RNA genes found.
- **# tRNA**: Number of transfer RNA genes found.

Annotation genome details

Content - Widgets

Interactive genome plot

The circular genome plot is generated utilising the BioCircos.js library. The most outer circle displays the position reference in million base pairs. The most outer gene feature circles display all annotated gene features from forward and reverse strand. Mouse over the gene features to show feature start, end, type, gene name and product. The CDSs are displayed in grayscale, RNAs in green and misc features in orange. The outer circular box plot visualizes the GC content of 1 kb bins. GC contents above the genome mean are colored in green and the ones below are colored in red. The inner circular box plot visualizes the GC Skew of 1 kb bins. GC Skews above the genome mean are colored in purple and the ones below are colored in neon green. Positioning of the whole genome plot can be applied via drag and drop and Zooming can be applied via mouse wheel.

Basic annotation statistics

Abundance of the annotated feature types found in this genome. Visualization of the annotation prediction rate.

Interactive data table features

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table.

Downloads

Several annotation based files can be downloaded, including the genome as gbk, annotations as gff, gene sequences as ffn, coding sequences as faa and the circular genome plot as svg file. The features table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- [BioCircos.js](#); BioCircos.js: an Interactive Circos JavaScript Library for Biological Data Visualization on Web Applications. Cui, Y., et al. Bioinformatics. (2016). [PubMed](#).

Glossary

- **End**: End position of the feature in base pairs.
- **Gene**: Gene name in case it is provided by the feature reference.
- **Inference**: Source the feature prediction is based on.
- **Locus**: Designation of the annotated genomic region.
- **Misc features**: Miscellaneous feature an annotated genomic area that is neither CDS nor RNA.
- **Product**: Short description of the product associated with the feature.
- **Start**: Start position of the feature in base pairs.
- **Strand**: The forward/plus strand is marked via '+' and the reverse/minus strand is marked with '-'.
- **Type**: Designated group of this gene feature.

Taxonomic classification overview

Here an overview on the taxonomy of the analysed genomes with key data from reference free classification and highest reference average nucleotide identity is provided.

Content - Widgets

Interactive data tables

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Mouse over on underlined table headers to display further information on it.

Reference free classifications

The results from Mash / ANI and Infernal / Blastn are displayed.

Highest reference ANIs

The results from Nucmer based ANI classification are displayed.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- [ANI](#): Goris, Johan, et al. "DNA–DNA hybridization values and their relationship to whole-genome sequence similarities." International journal of systematic and evolutionary microbiology 57.1 (2007): 81-91. [PubMed](#).
- **Details** on the taxonomy of a particular genome can be accessed via click on the magnifying glass in the overview table.
- **Kmer** column value redirects to kmer taxonomic classification in the ncbi Taxonomy Browser.
- **16S rRNA** column value redirects to 16S rRNA taxonomic classification in the ncbi Taxonomy Browser.
- [Mash](#): Brian D. Ondov, Todd J. Treangen, Páll Melsted, Adam B. Mallonee, Nicholas H. Bergman, Sergey Koren & Adam M. Phillippy: Mash: fast genome and metagenome distance estimation using MinHash. Genome Biology 2016
- [Infernal](#): E. P. Nawrocki and S. R. Eddy, Infernal 1.1: 100-fold faster RNA homology searches, Bioinformatics 29:2933-2935 (2013). [PubMed](#).
- [MUMmer/Nucmer](#): Open source MUMmer 3.0 is described in "Versatile and open software for comparing large genomes." S. Kurtz, A. Phillippy, A.L. Delcher, M. Smoot, M. Shumway, C. Antonescu, and S.L. Salzberg, Genome Biology (2004), 5:R12. [PubMed](#).
- [RDP](#): Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis Nucl. Acids Res. 42(Database issue):D633-D642; doi: 10.1093/nar/gkt1244. [PubMed](#).

Glossary

- **16S Classification:** Rfam 16S based taxonomic classification via Infernal.
- **ANI [%]:** Percent average nucleotide identity. Based on the ANI publication the sequenced genome is split into 1020 bp fragments which are compared against the reference (in our approach Nucmer was used instead of blastN). For the calculation the length of the fragments with less than 30% non identities and an alignment length higher than 70% are summed and divided by the total length of the sequenced genome.
- **Conserved DNA [%]:** Percent conserved DNA. Based on the ANI publication the sequenced genome is split into 1020 bp fragments which are compared against the reference (in our approach Nucmer was used instead of blastN). For the calculation the length of the fragments that matched with 90% sequence identity or higher are summed and divided by the total length of the sequenced genome.
- **Genome:** Name of the processed genome.
- **Kmer / ANI Classification:** Kmer based taxonomic classification via Mash with subsequent ANI computation against candidates.
- **Reference:** ID of the reference genome used for taxonomic classification.

Taxonomic classification genome details

Content - Widgets

Interactive phylogeny visualization

The height of the phylogenetic levels symbolizes the number of contigs classified as such. The number of classified contigs may decrease with classification depth. On mouse over the current and the next lower phylogenetic level together with the number of contigs classified (weight) is displayed.

Kmer contig classifications

Here the phylogeny was calculated based on kmers and ANI values.

16S rRNA classifications

Here the phylogeny was calculated based on 16S rRNAs.

Interactive data table features

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Mouse over on underlined table headers to display further information on it.

Kmer contig classifications

Contains the set of kmer classification results of all contigs.

16S rRNA classifications

Contains the set of 16S rRNA classification results of all contigs based on highest scoring 16S RNA.

Reference ANIs

Table of reference genomes and their percent average nucleotide identity and percentage of conserved DNA.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Glossary

- **ANI [%]:** Percent average nucleotide identity. Based on the ANI publication the sequenced genome is split into 1020 bp fragments which are compared against the reference (in our approach Nucmer was used instead of blastN). For the calculation the length of the fragments with less than 30% non identities and an alignment length higher than 70% are summed and divided by the total length of the sequenced genome.
- **Classification:** Deepest phylogenetic classification level for a single or group of contigs/16S RNAs.
- **Contigs [#]:** Number of contigs that have been identified to this phylogenetic level depth.
- **Contigs [%]:** Percentage out all contigs that have been identified to this phylogenetic level depth.
- **Hits [#]:** Number of 16S RNAs in the analysed genome that match this 16S RNA database entry.
- **Hits [%]:** Percentage of all 16S RNAs in the analysed genome that match this 16S RNA database entry.
- **Linage:** List of phylogenetic levels this particular level and the according contigs are included.
- **Reference:** Accession of the reference genome.
- **Conserved DNA [%]:** Percent conserved DNA. Based on the ANI publication the sequenced genome is split into 1020 bp fragments which are compared against the reference (in our approach Nucmer was used instead of blastN). For the calculation the length of the fragments that matched with 90% sequence identity or higher are summed and divided by the total length of the sequenced genome.

Multilocus Sequence Typing (MLST) overview

Content - Widgets

Interactive donut chart

The distribution of the different Sequence Types, Clonal Clusters and Lineages are displayed.

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. In green the found classification elements are displayed.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- [MLST](#); R. Urwin & M.C. Maiden, 2003, Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol., 11, 479-487. [PubMed](#).
- [PubMLST](#); Database.

Glossary

- **Alleles**: Contiguous nucleotide sequence 350 to 600 base pairs in length of a housekeeping gene fragment used in MLST analysis.
- **Clonal Cluster**: Group of related sequence types.
- **Genome**: Name of the processed genome.
- **Lineage**: Members of particular clonal complexes.
- **Scheme**: Group of bacterial variants.
- **Sequence Type**: Unique combination of MLST allele designations used in an MLST scheme.

Antibiotic Resistance Detection (ABR) overview

The antibiotic resistance profile of each genome is visualized on this page.

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in columns containing numeric values. They visualize the relation of this value compared to the according values of the other genomes. In the ABR Profile column found antibiotic drug resistances are visualized as colored circles. A popup appears on mouse-overs on the circles to display the individual resistances. Mouse-overs on underlined terms display further information on it.

Downloads

The table can be saved in comma separated value (csv) file format via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- **Details** on the resistance of a particular genome can be accessed via click on the magnifying glass in the overview table.
- [CARD](#); Jia et al. 2017. CARD 2017: expansion and model-centric curation of the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 45, D566-573. [PubMed](#).

Glossary

- **# ABR Genes**: Number of antibiotic resistance genes found.
- **ABR Profile**: Found antibiotic agent resistances.
- **# ABR Target Drugs**: Number of antibiotic agent resistances.
- **Genome**: Name of the processed genome.
- **# Potential ABR Genes**: Number of potential antibiotic resistance genes found.

Antibiotic Resistance Detection (ABR) genome details

Content - Widgets

Interactive data tables

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. To display additional model information mouse over a model. The 'Seq Identity' is categorised into four groups based on value. Entries below 80% sequence identity

are highlighted in red, blow 95% in yellow, blow 98% in light green and above in green. To display the aligned sequence mouse over the bit score value. Mouse over on underlined term to display further information on it.

ABR Genes

Provides information on the genes with a perfect reference match (100%) in the ABR database.

Potential ABR genes - best hits

Provides information on genes and their best non perfect reference ABR database match (40% < match <=100%).

Potential ABR genes - all hits

Provides information on genes with all their non perfect reference ABR database matches (40% < match <=100%).

Links

Click on a model redirects to this model reference in the CARD database.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Glossary

- **Model:** Name of the resistance mechanism.
- **ABR Target Drugs:** The drug or drug family the resistance is associated with.
- **Start:** Start position of this resistance gene in this genome.
- **End:** End position of this resistance gene in this genome.
- **Length:** Length of this resistance gene in this genome.
- **Strand:** The forward/plus strand is marked via '+' and the reverse/minus strand is marked with '-'.
- **Bit Score:** Normalized chance to find the score or a higher one of this match by chance given in bit (bit score of 3 equals a chance of $2^3 = 8 \rightarrow 1 : 8$).
- **eValue:** Expected number of alignments in the database used with a score equivalent or higher than this match.
- **Seq Identity:** Percentage of identical positioned nucleotides in the alignment.

Virulence factor detection overview

This page provides an overview on the number of virulence factors and categories detected in each genome.

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in columns containing numeric values. They visualize the relative relation of this value compared to the according values of the other genomes. Mouse over on underlined term to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Links

- **Details** on the virulence factors of a particular genome can be accessed via click on the magnifying glass in the overview table.
- **VFDB**: Chen LH, Zheng DD, Liu B, Yang J and Jin Q, 2016. VFDB 2016: hierarchical and refined dataset for big data analysis-10 years on. Nucleic Acids Res. 44(Database issue):D694-D697. PubMed.

Glossary

- **Genome**: Name of the processed genome.
- **Locus**: Designation of the annotated genomic region.
- **# VFs**: Number of assigned virulence factors hits per genome.
- **# VF categories**: Number of virulence factor categories per genome.

Virulence factor detection genome details

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term

in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Glossary

- **Category:** Virulence factor category designation according to its function.
- **Coverage:** Sequence coverage of this data base hit in percent.
- **eValue:** Expected number of virulence factors in the database used with a score equivalent or higher than this match.
- **Gene:** Gene name in case it is provided by the virulence factor database.
- **Locus:** Designation of the annotated genomic region.
- **Product:** Short description of the product associated with the locus.
- **# VFs:** Number of assigned virulence factors hits per genome.
- **# VF categories:** Number of virulence factor categories per genome.

Reference mapping

Content - Widgets

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Mouse over on underlined term to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file). To download the bam file of a particular genome mapping click on bam in the data table.

Links

- [SAMtools](#); Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, 25, 2078-9. [PubMed](#).

- [Bowtie 2](#); Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012, 9:357-359. [PubMed](#).
- [Minimap2](#): Li H., Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics, 2018, 34(18):3094-3100

Glossary

- **Genome**: Name of the processed genome.
- **# Multiple**: Number of reads that mapped multiple times.
- **Ratio**: Ratio of total reads that could be mapped to the reference.
- **# Reads**: Total number of analysed reads.
- **# Unique**: Number of reads that mapped once.
- **# Unmapped**: Number of reads that could not be mapped to the reference.

Single Nucleotide Polymorphism (SNP) overview

This page provides an average SNP distribution mapping and a SNP comparison of the analysed genome.

Content - Widgets

SNP distribution graph

The mean number of SNPs per 10 kb compared to the reference genome are displayed. Mouse over the graph to display the position and mean SNP number of an individual peak.

Interactive data table

Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in most columns containing numeric values. They visualize the relative relation of this value compared to the according values of the other genomes. Mouse over on underlined term to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file). The vcf file of each genome can be downloaded.

Links

- **Details** on the SNPs of a particular genome can be accessed via click on the magnifying glass in the overview table.

- [SAMtools](#); Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, 25, 2078-9. [PubMed](#).
- [BCFtools](#); Included in SAMtools.
- [SnpSift](#); "Using Drosophila melanogaster as a model for genotoxic chemical mutational studies with a new program, SnpSift", Cingolani, P., et. al., Frontiers in Genetics, 3, 2012. [PubMed](#).
- [SnpEff](#); "A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3.", Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. Fly (Austin). 2012 Apr-Jun;6(2):80-92. [PubMed](#).
- [HTSlib](#); Included in SAMtools.
- Glossary
- **Change Range**: Ratio of number single nucleotide polymorphisms to genome size.
- **Genome**: Name of the processed genome.
- **HI SNPs**: Number of high impact single nucleotide polymorphisms. SNPs are considered high impact if they result in the gain or loss of a start or stop codon.
- **SNPs**: Number of single nucleotide polymorphisms.
- **TS/TV**: Ratio of number nucleotide transitions to number nucleotide transversions.

Single Nucleotide Polymorphism (SNP) genome details

Content - Widgets

SNP distribution graph

Displays the number of SNPs per 10 kb of this particular genome in red and of the mean of all analysed genomes in blue. Mouse over the graph to display the position and the number of SNPs of an individual peak.

Histograms

Mouse over the individual bar to display the number of SNP occurrences for this individual category.

Region

Displays the position distribution of SNPs relative to known genes.

Classes

Display the effect type distribution of the SNPs of this genome.

Impacts

Display the severity type distribution of the SNPs of this genome.

Statistics

Statistical summary of the SNPs and their effects of this genome. Mouse over on underlined term to display further information on it.

Interactive data table high impact SNPs

The table contains all SNPs that have been rated as 'high' by SnpEff. This includes the SnpEff categories: chromosome_number_variation, exon_loss_variant, frameshift_variant, rare_amino_acid_variant, splice_acceptor_variant, splice_donor_variant, start_lost, stop_gained, stop_lost, transcript_ablation. Individual sorting can be applied via clicking on the respective column header. Use the Search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Mouse over on underlined term to display further information on it.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file).

Glossary

- **Change Range:** Ratio of number single nucleotide polymorphisms to genome size.
- **Contig:** Reference genome accession of the contig this SNP was found.
- **Downstream:** Number of SNPs that are located 3' toward the transcription direction of the closest gene.
- **Alt:** Base(s) at the SNP position.
- **Coverage:** Number of reads that display this SNP.
- **Effect:** Of High Impact SNPs including stop gain and lost and start lost.
- **Exon:** In this eucaryotic setting referring to the number of SNPs that are located in a translated region of the genome.
- **Gene:** Reference gene name for this SNP.
- **HI SNPs:** Number of high impact single nucleotide polymorphisms.
- **High:** Includes the SnpEff categories: chromosome_number_variation, exon_loss_variant, frameshift_variant, rare_amino_acid_variant, splice_acceptor_variant, splice_donor_variant, start_lost, stop_gained, stop_lost, transcript_ablation.
- **Intergenic:** Number of SNPS that are located in non transcribed regions of this genome.
- **Low:** Includes the SnpEff categories: 5_prime_UTR_premature_start_codon_gain_variant, initiator_codon_variant, splice_region_variant, start_retained, stop_retained_variant, synonymous_variant.
- **Mean Qual:** Mean quality of the detected SNP base as PHRED score (error probability; 20: 1 in 100; 30: 1 in 1000).
- **Missense:** Number of SNPs that lead to a different amino acid in the resulting protein.

- **Moderate:** Includes the SnpEff categories: 3_prime_UTR_truncation +exon_loss, 5_prime_UTR_truncation +exon_loss_variant, coding_sequence_variant, disruptive_inframe_deletion, disruptive_inframe_insertion, inframe_deletion, inframe_insertion, missense_variant, regulatory_region_ablation, splice_region_variant, TFBS_ablation.
- **Modifier:** Includes the SnpEff categories: 3_prime_UTR_variant, 5_prime_UTR_variant, coding_sequence_variant, conserved_intergenic_variant, conserved_intron_variant, downstream_gene_variant, exon_variant, feature_elongation, feature_truncation, gene_variant, intergenic_region, intragenic_variant, intron_variant, mature_miRNA_variant, miRNA, NMD_transcript_variant, non_coding_transcript_exon_variant, non_coding_transcript_variant, regulatory_region_amplification, regulatory_region_variant, TF_binding_site_variant, TFBS_amplification, transcript_amplification, transcript_variant, upstream_gene_variant.
- **Nonsense:** Number of SNPs that lead to a new stop codon in the translated sequence.
- **Position:** Position in the reference genome this SNP occurred in base pairs.
- **Ref:** Base at the reference position.
- **SNPs:** Number of single nucleotide polymorphisms.
- **Silent:** Number of SNPs with no direct effect on the resulting amino acid sequence.
- **Start lost:** This SNP causes start codon loss of the associated gene.
- **Stop Gained:** This SNP causes stop codon gain of the associated gene.
- **Stop lost:** This SNP causes stop codon loss of the associated gene.
- **Synonymous Variant:** Numbers of SNPs that do not lead to a change in the encoded amino acid.
- **TS/TV:** Ratio of number nucleotide transitions to number nucleotide transversions.
- **Upstream:** SNPs that are located 5' toward the transcription direction of the closest gene.

Core - pan genome

Content - Widgets

Interactive donut chart

The percentage distribution of Core, Accessory and Singleton genes is displayed.

Gene Numbers

Provides absolute numbers on Core, Pan, Accessory and Singleton genes.

Interactive PAN / Core / Singleton Development chart

Displays changes in number of CDS (loci) in pan, core and singletons with increasing numbers of genomes included (x-axis). For each comparison the number of genomes is picked randomly ten times and the average values are displayed. Pan and core genome size is referenced by the left y-axis. The number of singletons is referenced by the right y-axis. Highlighting of an individual graph can be done via clicking on the graph or the according

legend. Individual values on the graphs can be accessed via mouseover. Individual data points can be highlighted via clicking on them.

Skipped Genome

In case a sequenced genome could not be analysed this frame is displayed and shows the affected genomes.

Interactive data tables

Individual sorting can be applied via clicking on the respective column header. Use the search function (top right of the table) to display only genomes that contain the search term in any of their table fields. The number of entries displayed per page can be chosen on the top left of the table. Blue horizontal bar plots are displayed in columns containing numeric values. They visualize the relation of this value compared to the according values of the other genomes.

Overview

Provides information on the accessory genome size and number of singletons genes for each genome.

Core Genome

Provides information on the product (function) for each loci of the core genome.

Accessory Genome

Provides information on the product (function) and the abundance for each loci of the accessory genome.

Singletons

Provides information on each locus, its product (function) and the genome it was found.

Downloads

The table can be saved as a comma separated value (csv) file via a click on the csv button (search and sorting are contained in the downloaded file). A fasta file with all core gene sequences and a file with all the pan gene sequences can be downloaded. The matrix maps which gene is present in which sequenced organism (present = 1, absent = 0) can be downloaded as tab separated value 'tsv' file.

Links

- **Details** on the core and pan genome distribution of a particular genome can be accessed via click on the magnifying glass in the overview table.
- [Roary](#): "Roary: Rapid large-scale prokaryote pan genome analysis", Andrew J. Page, Carla A. Cummins, Martin Hunt, Vanessa K. Wong, Sandra Reuter, Matthew T. G. Holden, Maria Fookes, Daniel Falush, Jacqueline A. Keane, Julian Parkhill, Bioinformatics, (2015). [PubMed](#).

Glossary

- **Abundance:** Number of locus occurrence in this analysis.
- **Accessory:** Number of genes that are contained in at least one other analysed organism (also known as dispensable genome).
- **Core:** Number of genes contained in all analysed genomes.
- **Genome:** Name of the processed genome.
- **Locus:** Defined contiguous nucleotide sequence in the genome.
- **Pan:** Total number of individual genes in this analysis.
- **Pan Genome Matrix:** The matrix maps which gene is present in which sequenced organism (present = 1, absent = 0).
- **Product:** Functional information on the associated locus.
- **Singletons:** Number of genes contained only in this genome out of the analysed set.

Phylogeny

Content - Widgets

Phylogenetic tree display

A tree type (rectangular, radial, circular, diagonal and hierarchical) can be chosen via the drop down menu. The tree can be positioned via mouse drag and drop. The zoom function is controlled via mouse wheel. Via right clicks in a blank area of the diagram further display and export options show up (like Export as Image). Via a mouseover on a tree node the number of leaves associated with this subtree is displayed. Via a left click on a tree node the subtree is highlighted in blue. Via a right click on a tree node additional display and export options are available (including Collapse/Expand Subtree and Export Subtree as Newick File)

Downloads

The SNP based phylogenetic distances can be downloaded in newick file format on the top right ('Downloads').

Links

- [FastTreeMP](#); Price, M.N., Dehal, P.S., and Arkin, A.P. (2010) FastTree 2 -- Approximately Maximum-Likelihood Trees for Large Alignments. PLoS ONE, 5(3):e9490. doi:10.1371/journal.pone.0009490. [PubMed](#).
- [Phylocanvas](#); Centre for Genomic Pathogen Surveillance (2016). Interactive tree visualisation for the web.