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# VirAmp Documentation

*Release 1.0*

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VirAmp is a galaxy-based system for fast virus genome assembly and variation discovery.

Quick Start Guide:

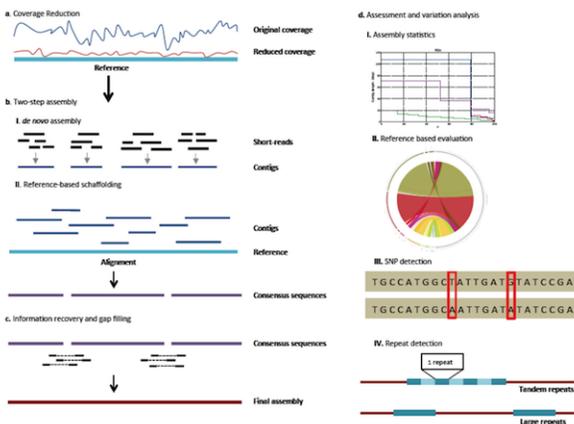
1. Launch the latest version of the “Szpara\_Viramp” AMI from Amazon Web Services
- 2) SSH into the server and start the run.sh script using `screen ./run.sh`

Contents:



## Introduction

The following graphic is an overview of how the VirAmp platform works:



Advances in next generation sequencing make it possible to obtain high-coverage sequence data for large numbers of viral strains in a short time. However, since most bioinformatics tools are developed for command line use, the selection and accessibility of computational tools for genome assembly and variation analysis limits the ability of individual labs to perform further bioinformatics analysis. We have developed a multi-step viral genome assembly pipeline named VirAmp, that combines existing tools and techniques and presents them to end users via a web-enabled Galaxy interface. Our pipeline allows users to assemble, analyze and interpret high coverage viral sequencing data with an ease and efficiency that was not possible previously. Our software makes a large number of genome assembly and related tools available to life scientists and automates the currently recommended best practices into a single, easy to use interface. We tested our pipeline with three different datasets from human herpes simplex virus (HSV). VirAmp provides a user-friendly interface and a complete pipeline for viral genome analysis. We make our software available via an Amazon Elastic Cloud disk image that can be easily launched by anyone with an Amazon web service account. A demonstration version of our system can be found at <http://www.viramp.com>. We also maintain detailed documentation on each tool and methodology at <http://docs.viramp.com>.



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**Usage**

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This is a general description of the usage and function of each tool found in the [VirAmp pipeline](#). A more detailed description can be found at the webpage of each tool.

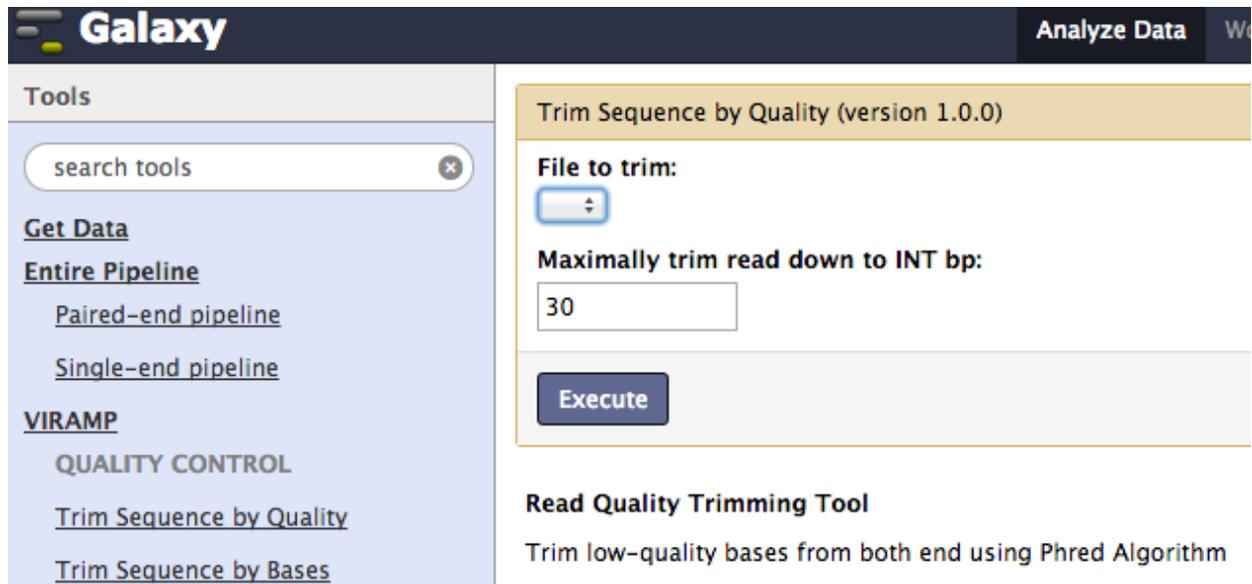
## **2.1 One-click pipeline**

Two general pipelines are provided with a one-click option, one for paired-end data and the other for single-end data. Users are only required to submit read files and a reference file corresponding to their data. Alongside the default settings, users may use the “advanced setting” option to custom configure the pipeline with alternative parameters.

The screenshot shows the Galaxy web interface. On the left, the 'Tools' sidebar is visible with a search bar containing 'search tools'. Under the 'Entire Pipeline' section, the 'Paired-end pipeline' tool is selected. The main panel displays the configuration for 'Paired-end pipeline (version 1.0.0)'. It includes several dropdown menus: 'File of Read 1' (set to 'Read\_1 in paired-end sequencing'), 'File of Read 2' (set to 'Read\_2 in paired-end sequencing'), 'Reference genome' (set to an empty dropdown), 'Create one linear genome sequence:' (set to 'no'), and 'Assembly settings:' (set to 'Default Setting'). An 'Execute' button is located at the bottom of the configuration panel.

## 2.2 Quality Control

First, trim out the low quality bases of the input fastq files. This can be achieved by either removing low quality bases or trimming a certain length from each end.



The screenshot shows the Galaxy web interface. The top navigation bar includes the Galaxy logo and a 'Analyze Data' button. On the left, a 'Tools' sidebar contains a search box and a list of tool categories: 'Get Data', 'Entire Pipeline' (with sub-items 'Paired-end pipeline' and 'Single-end pipeline'), and 'VIRAMP' (with sub-items 'QUALITY CONTROL', 'Trim Sequence by Quality', and 'Trim Sequence by Bases'). The main panel displays the 'Trim Sequence by Quality (version 1.0.0)' tool configuration. It features a 'File to trim:' dropdown menu, a 'Maximally trim read down to INT bp:' input field with the value '30', and an 'Execute' button. Below the tool configuration, the title 'Read Quality Trimming Tool' is followed by the description: 'Trim low-quality bases from both end using Phred Algorithm'.

## 2.3 Diginorm

Next, reduce coverage and bias using Digital normalization. This step reduces the sample variation as well as sample bias.

The screenshot shows the Galaxy web interface. On the left is a 'Tools' sidebar with a search bar and categories: 'Get Data', 'Entire Pipeline' (with links for 'Paired-end pipeline' and 'Single-end pipeline'), 'VIRAMP', 'QUALITY CONTROL' (with links for 'Trim Sequence by Quality' and 'Trim Sequence by Bases'), 'DIGINORM' (with link for 'Reduce the coverage'), 'DE NOVO CONTIG ASSEMBLING', 'Velvet', and 'SPAdes'. The main panel displays the configuration for the 'Reduce the coverage (version 1.0.0)' tool. The configuration includes: 'File to dignorm:' (a dropdown menu), 'shotgun sequencing data of fasta or fastq format', 'sequence type:' (a dropdown menu set to 'paired end'), 'Coverage cutoff:' (a text input field with '10'), 'Number of hash table to use:' (a text input field with '4'), 'Caution: Only change when experiencing error', 'Lower bound on hashsize to use:' (a text input field with '1e8'), 'Caution: Only change when experiencing error', and an 'Execute' button at the bottom.

## 2.4 *de novo* Contig assembly

Now, the pipeline assembles the short reads into longer contigs. By default the **One-click pipeline** uses *velvet*. Two alternatives, *SPAdes* and *VICUNA*, are provided and can be selected as either individual tools or through the advanced options in the one-click pipeline.

The screenshot shows the Galaxy web interface for the 'Velvet (version 1.0.0)' tool. The left sidebar is identical to the previous screenshot. The main panel displays the configuration for 'Velvet (version 1.0.0)'. The configuration includes: 'Single-end sequence to be assembled:' (a dropdown menu with '146: HSV-McKr\_1w\_read1.fq'), 'Paired-end sequence to be assembled:' (a dropdown menu with '148: HSV-McKr\_1w\_read3.fq'), 'k-mer(s) used in velvet assembling:' (a text input field with '35,45,55,65'), 'put one or more k-mer size, like: 21,25,29; k-mer needs to be odd and does not exceed 91', 'Data format:' (a dropdown menu set to 'FASTQ'), and an 'Execute' button at the bottom.

## 2.5 Reference-based scaffolding

The contigs are then assembled into even longer *super-contigs*. This step is a modification of AMOScmp

Tools

search tools

[Get Data](#)

[Entire Pipeline](#)

**VIRAMP**

QUALITY CONTROL

[Trim Sequence by Quality](#)

[Trim Sequence by Bases](#)

DIGINORM

Reference-guided Scaffolding (version 1.0.0)

Contig file:  
171: assembled genome on data 149, data 1, and data 150.fasta

Paired-end sequence:  
Selection is Optional

Reference sequence:  
1: JN55585\_truncated.fasta

Execute

## 2.6 Reference-independent scaffolding

The next step extends the super-contigs and connects them using *SSPACE*. The pipeline will produce a draft genome as a multi-fasta file usually containing 5~15 contigs which are listed in the same order as the reference.

Galaxy

Analyze Data Workflow Shared Data Visualization Admin He

Tools

search tools

[Get Data](#)

[Entire Pipeline](#)

**VIRAMP**

QUALITY CONTROL

[Trim Sequence by Quality](#)

[Trim Sequence by Bases](#)

DIGINORM

[Reduce the coverage](#)

DE NOVO CONTIG ASSEMBLING

[Velvet](#)

[SPAdes](#)

Scaffolding pre-assembled contigs using paired-read data (version 1.0.0)

Contigs to be scaffolded:  
171: assembled genome on data 149, data 1, and data 150.fasta

paired-end dataset READ-1:  
150: HSV-McKr\_read\_3.fastq

paired-end dataset READ-2:  
146: HSV-McKr\_1w\_read1.fq

Insertion size:  
350  
Insertion size of the paired-end reads, please consult the sequencing facility if you are not sure about this value

paired-end dataset format:  
FASTA

Execute

## 2.7 Gap closing

This step connects all the contigs in the multi-fasta from the previous step into one linear genome for the convenience of downstream functional analysis. However, this is **optional** and highly recommended to be done only after assessing the draft genome, as the gaps between the contigs could be from misassembly, sequencing, genome feature, etc.

Generate linear genome from contigs (version 1.0.0)

**Contigs:**

171: assembled genome on data 149, data 1, and data 150.fasta ⇅

**Reference genome:**

1: JN55585\_truncated.fasta ⇅

Execute

---

## Post-Assembly Analysis

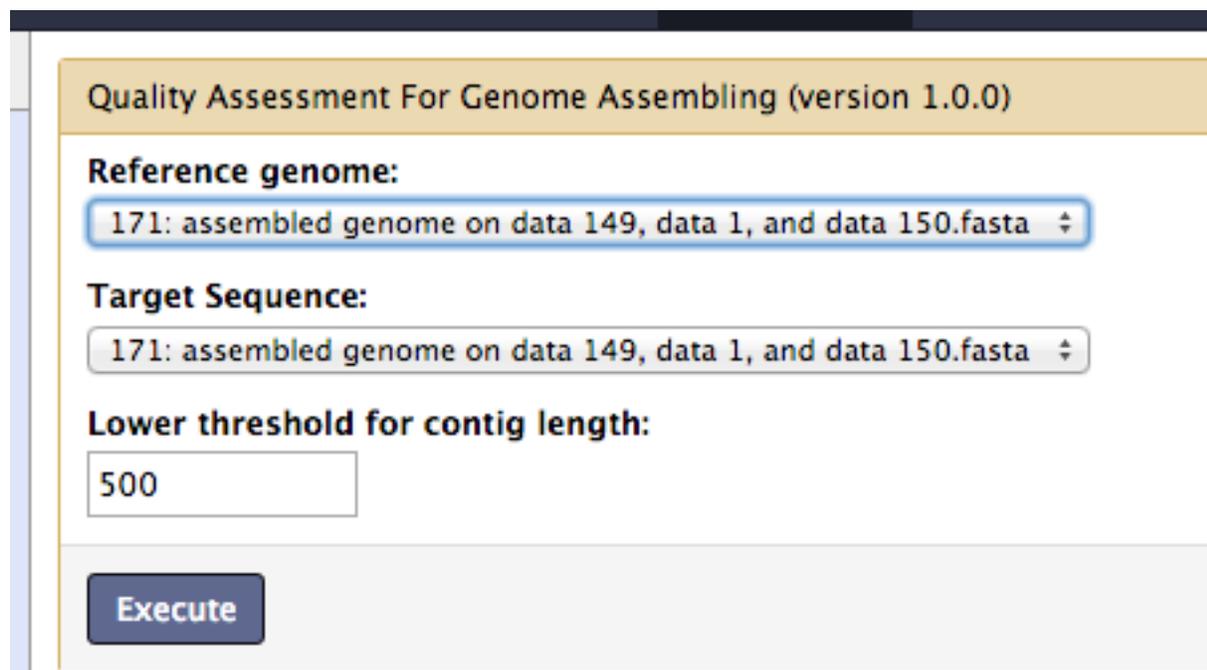
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VirAmp not only provides all the processes related to assembly, but also integrates multiple tools for post-assembly processing including quality assessment and variation analysis.

### 3.1 QUASt REPORT

It is important to evaluate how robust the new assembly is before it is fed into the downstream functional analysis. VirAmp constructs a report of common assembly evaluation metrics based on comparisons with the reference. A detailed [QUAST report](#) can be downloaded for further evaluation.

The inputs required are the reference genome and the newly created assembly.



Quality Assessment For Genome Assembling (version 1.0.0)

**Reference genome:**  
171: assembled genome on data 149, data 1, and data 150.fasta ↕

**Target Sequence:**  
171: assembled genome on data 149, data 1, and data 150.fasta ↕

**Lower threshold for contig length:**  
500

Execute

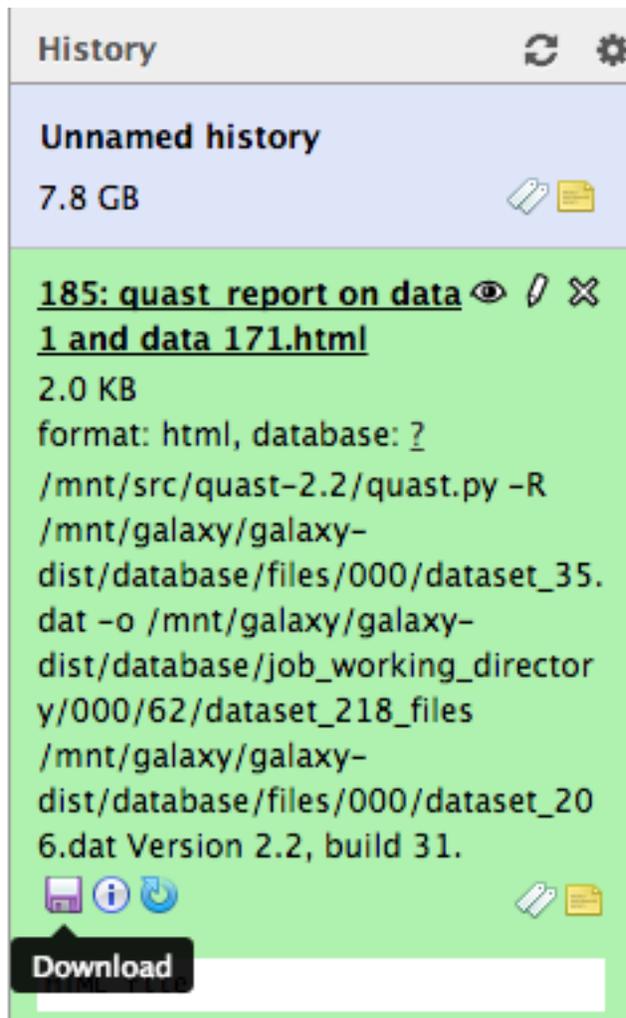
The primary output of QUASt is a summary of common assembly evaluation metrics.

## Assembly Statistics

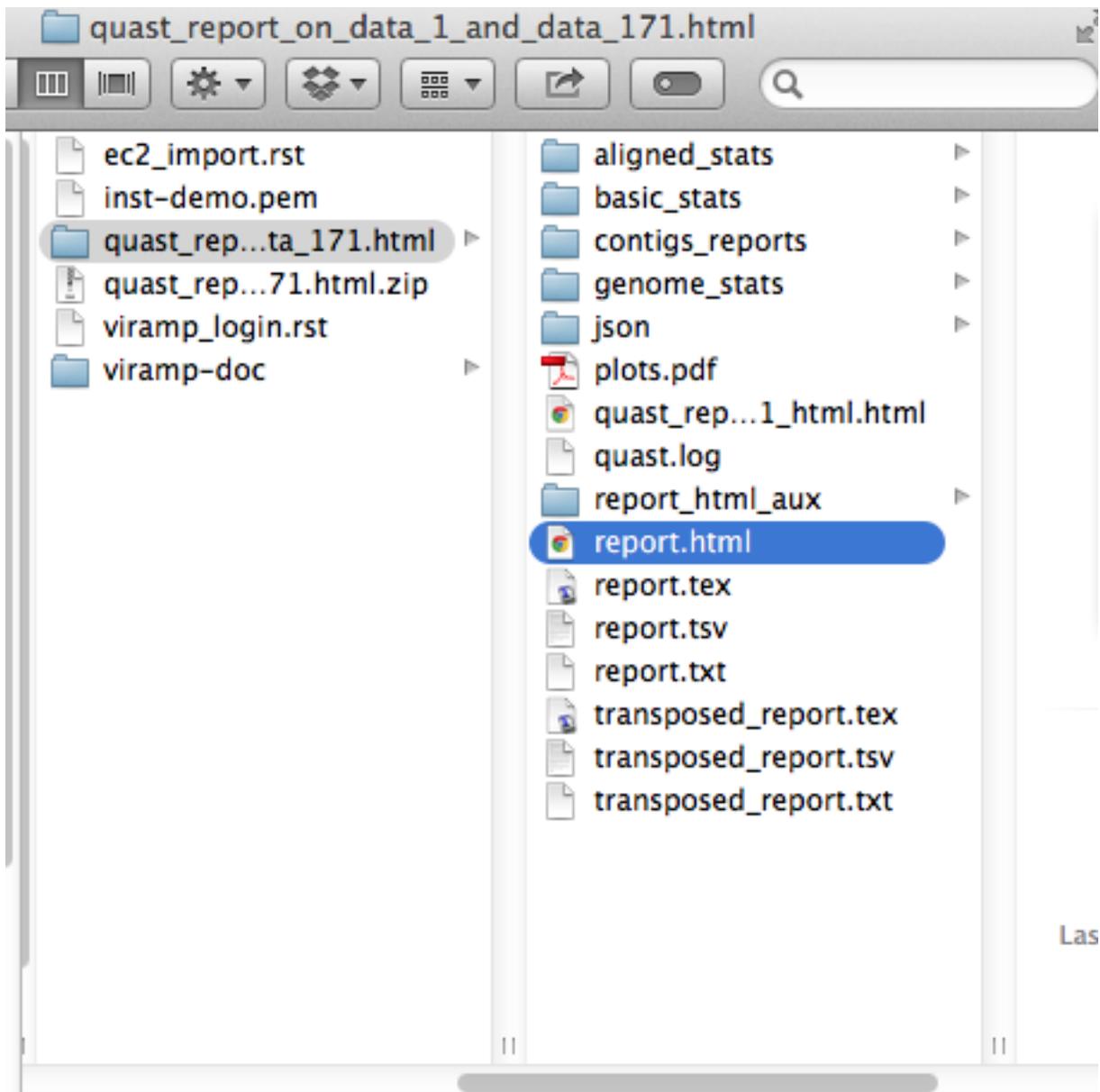
All statistics are based on contigs of size  $\geq 500$  bp, unless otherwise noted (e.g., "# contigs ( $\geq 1000$  bp)" and "Total length ( $\geq 1000$  bp)" include all contigs).

Assembly	dataset_206.dat
# contigs ( $\geq 0$ bp)	7
# contigs ( $\geq 1000$ bp)	5
Total length ( $\geq 0$ bp)	133874
Total length ( $\geq 1000$ bp)	133166
# contigs	6
Total length	133678
Largest contig	71396
Reference length	136376
GC (%)	67.20
Reference GC (%)	67.53
N50	71396
NG50	71396
N75	36562
NG75	36562
L50	1
LG50	1
L75	2
LG75	2
# misassemblies	1
Misassembled contigs length	16097
# local misassemblies	3
# unaligned contigs	1 + 0 part
Unaligned length	512
Genome fraction (%)	97.485
Duplication ratio	1.002
# N's per 100 kbp	120.44
# mismatches per 100 kbp	483.66
# indels per 100 kbp	64.69
Largest alignment	71309
NA50	71309
NGA50	71309
NA75	36560
NGA75	36560
LA50	1
12.LGA50	1
LA75	2
LGA75	2

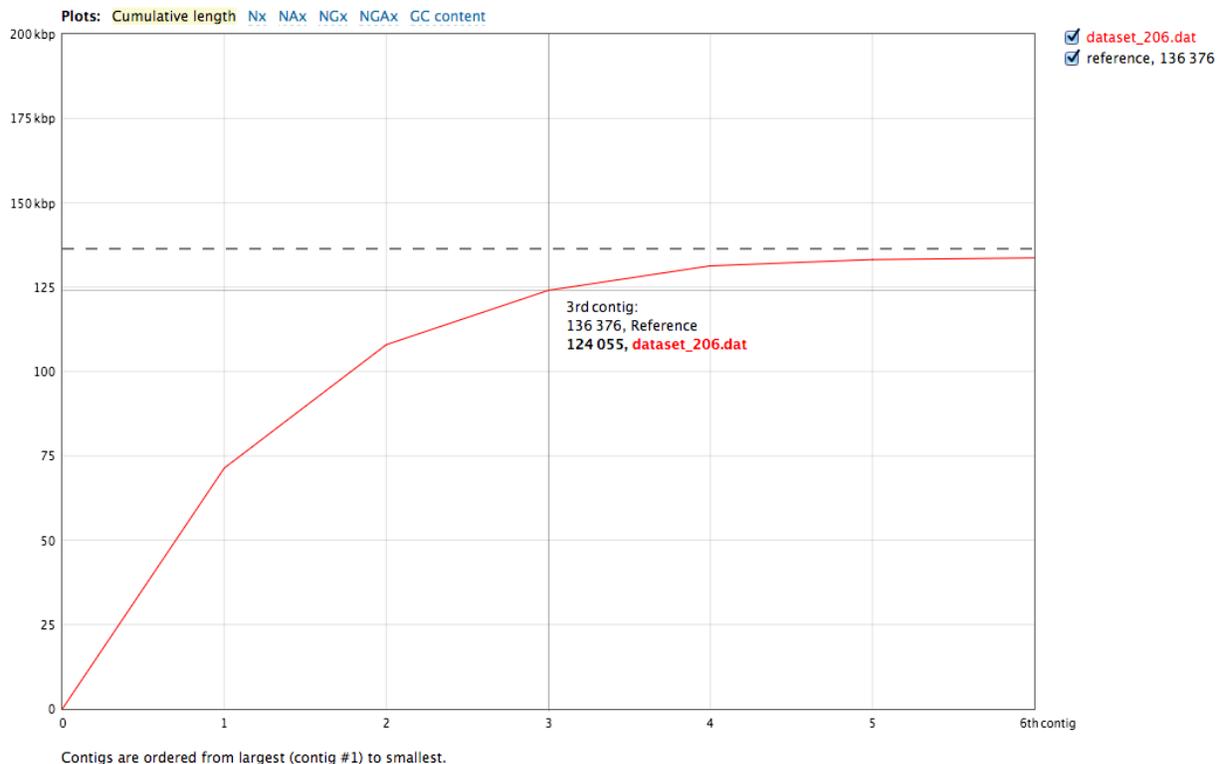
Alternatively, a more detailed QUASt report can also be downloaded.



Unzip and open the report.



A demonstration of a QAST plot:



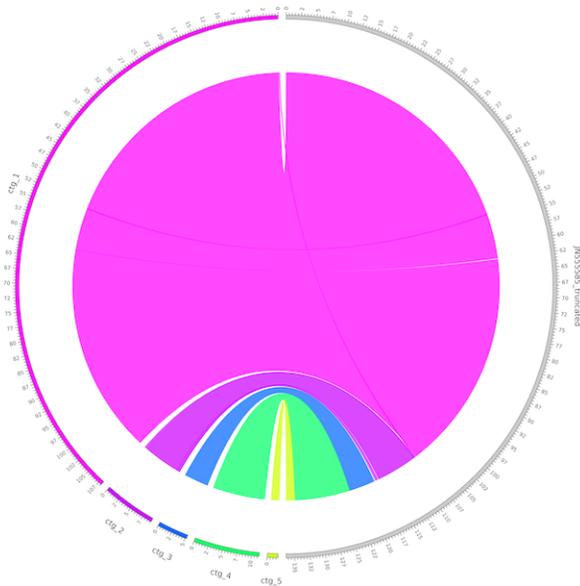
### 3.2 Assembly-Reference Alignment

VirAmp provides information about the difference between the reference and the new assembly based on a MUMmer alignment. Coordinates and percentage identities are displayed for each aligned region between these two sequences. This is useful in identifying large INDELS as well as other complex structural variations. Table 1 demonstrates an example of the comparison report generated by this tool.

[R_St]	[R_Ed]	[T_St]	[T_Ed]	[% IDY]	[LEN_R]	[LEN_T]	[COV_R]	[COV_T]	[REF_ID]	[CGT_ID]
1	62450	47	62569	99.37	136376	71396	45.79	87.57	JN555585_truncated	scaffold2   size71396
53191	53334	53367	53224	100.00	136376	71396	0.11	0.20	JN555585_truncated	scaffold2   size71396
53191	53273	53451	53369	100.00	136376	71396	0.06	0.12	JN555585_truncated	scaffold2   size71396
62633	71434	62611	71396	99.20	136376	71396	6.45	12.31	JN555585_truncated	scaffold2   size71396
71435	108009	3	36562	99.53	136376	36562	26.82	99.99	JN555585_truncated	scaffold1   size36562
108299	108496	5	193	92.96	136376	196	0.15	96.43	JN555585_truncated	scaffold7   size196
108879	116167	1	7268	96.69	136376	7268	5.34	100.00	JN555585_truncated	scaffold4   size7268
116305	116864	1	483	85.71	136376	512	0.41	94.34	JN555585_truncated	scaffold6   size512
117906	119748	1	1843	99.57	136376	1843	1.35	100.00	JN555585_truncated	scaffold5   size1843
120363	123237	16097	13249	97.30	136376	16097	2.11	17.70	JN555585_truncated	scaffold3   size16097
123245	123394	13307	13158	100.00	136376	16097	0.11	0.93	JN555585_truncated	scaffold3   size16097
123383	134543	1	11179	98.86	136376	16097	8.18	69.45	JN555585_truncated	scaffold3   size16097
134332	134565	11181	11413	99.57	136376	16097	0.17	1.45	JN555585_truncated	scaffold3   size16097
134593	136376	11351	13104	97.42	136376	16097	1.31	10.90	JN555585_truncated	scaffold3   size16097

### 3.3 Circos graph visualization

Circos projects the assembled draft genome to the aligned part of the reference genome, creating a straightforward visualization for the above alignment and providing insight into large structural variations.



### 3.4 SNP analysis

Using the alignment between the assembly and the reference, SNP information is displayed in VCF format.

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
Reference	1700	nucmer	T	C	0	PASS	.
Reference	1704	nucmer	G	T	0	PASS	.
Reference	1867	nucmer	G	A	0	PASS	.
Reference	1909	nucmer	T	G	0	PASS	.
Reference	2239	nucmer	C	T	0	PASS	.
Reference	2463	nucmer	C	CC	0	PASS	.
Reference	2466	nucmer	A	G	0	PASS	.
Reference	2467	nucmer	T	C	0	PASS	.
Reference	2558	nucmer	AAG	A	0	PASS	.
Reference	2568	nucmer	G	T	0	PASS	.
Reference	2731	nucmer	T	C	0	PASS	.

### 3.5 Repeat and Tandem repeat analysis

By aligning the assembly against itself, VirAmp additionally provides repeat and tandem repeat information. The starting coordinates and lengths of the repeats are derived from this alignment.

Start	Extent	UnitLen	Copies	Ctg#
18534	20	3	6.7	scaffold1 size36562
36515	48	7	6.9	scaffold1 size36562
1	46	7	6.6	scaffold2 size71396
20931	19	4	4.8	scaffold2 size71396
62510	60	12	5.0	scaffold2 size71396
62570	41	1	41.0	scaffold2 size71396
7819	63	21	3.0	scaffold3 size16097
8474	19	1	19.0	scaffold3 size16097
11139	41	15	2.7	scaffold3 size16097



## Custom installation of the VirAmp AMI

Access <http://aws.amazon.com/>, in a Web browser.

Select ‘My Account/Console’ on the top right if you already have an account; otherwise sign up with a new account.

Go to the ‘AWS Management Console’ option, click the ‘EC2’ at upper left.

Before importing the AMI, make sure you are in the correct Availability zone. Amazon EC2 is hosted in multiple locations world-wide with multiple Availability zones, and resources cannot be replicated across regions until specified. Our AMI is stored in region “US East(N. Virginia)”. Check the upper right corner next to your account name, and make sure it’s set at the correct region. If not, just click and select the correct one from the dropdown menu.

Next, click the blue ‘Launch Instance’ button.

### 4.1 Step-1: Choosing the instance

Click the Community AMIs tab at mid-left and simply search “Szpara\_Viramp”

The screenshot shows the AWS Management Console interface for launching an instance. At the top, there are navigation tabs: 'Services', 'Edit', 'Yinan Wan', 'N. Virginia', and 'Help'. Below this is a progress bar with seven steps: '1. Choose AMI', '2. Choose Instance Type', '3. Configure Instance', '4. Add Storage', '5. Tag Instance', '6. Configure Security Group', and '7. Review'. The main heading is 'Step 1: Choose an Amazon Machine Image (AMI)' with a 'Cancel and Exit' link. A description states: 'An AMI is a template that contains the software configuration (operating system, application server, and applications) required to launch your instance. You can select an AMI provided by AWS, our user community, or the AWS Marketplace; or you can select one of your own AMIs.' On the left, there are tabs for 'Quick Start', 'My AMIs', 'AWS Marketplace', and 'Community AMIs'. Under 'Community AMIs', there is a search bar with 'viramp' entered and a search icon. Below the search bar, a single AMI is displayed: 'VirAmp - ami-3afd1e52' with a Linux logo icon. The description for this AMI is 'Virus Assembly Pipeline'. Below the description, it says 'Root device type: ebs' and 'Virtualization type: paravirtual'. To the right of the AMI details is a blue 'Select' button. At the bottom left, there is a section for 'Operating system' with radio buttons for 'Amazon Linux' and 'Cent OS'.

### 4.2 Step-2: Review Instance type

Due to storage and computational requirements, free tier instances are not usable with our AMI. For trial runs it is possible to choose smaller instance types, but for serious usage it is advised to select at least the m3.large (third option)

## Step 2: Choose an Instance Type

combinations of CPU, memory, storage, and networking capacity, and give you the flexibility to choose the appropriate mix of resources for your applications. [Learn more](#) about instance types and how they can meet your computing needs.

Filter by: All instances Current generation [Show/Hide Columns](#)

Currently selected: t1.micro (up to 2 ECUs, 1 vCPUs, 0.613 GiB memory, EBS only)

	Family	Type	ECUs	vCPUs	Memory (GiB)	Instance Storage (GB)	EBS-Optimized Available	Network Performance
<input checked="" type="checkbox"/>	Micro instances <b>Free tier eligible</b>	t1.micro	up to 2	1	0.613	EBS only	-	Very Low
<input type="checkbox"/>	General purpose	m3.medium	3	1	3.75	1 x 4 (SSD)	-	Moderate
<input type="checkbox"/>	General purpose	m3.large	6.5	2	7.5	1 x 32 (SSD)	-	Moderate
<input type="checkbox"/>	General purpose	m3.xlarge	13	4	15	2 x 40 (SSD)	Yes	Moderate
<input type="checkbox"/>	General purpose	m3.2xlarge	26	8	30	2 x 80 (SSD)	Yes	High
<input type="checkbox"/>	General purpose	m1.small	1	1	1.7	1 x 160	-	Low
<input type="checkbox"/>	Compute optimized	c3.large	7	2	3.75	2 x 16 (SSD)	-	Moderate
<input type="checkbox"/>	Compute optimized	c3.xlarge	14	4	7.5	2 x 40 (SSD)	Yes	Moderate
<input type="checkbox"/>	Compute optimized	c3.2xlarge	28	8	15	2 x 80 (SSD)	Yes	High
<input type="checkbox"/>	Compute optimized	c3.4xlarge	55	16	30	2 x 160 (SSD)	Yes	High
<input type="checkbox"/>	Compute optimized	c3.8xlarge	108	32	60	2 x 320 (SSD)	-	10 Gigabit

## 4.3 Step-3: Launch the Instance

▼ AMI Details [Edit AMI](#)

**VirAmp - ami-06a4426e**  
 Virus Assembly Pipeline  
 Root Device Type: ebs    Virtualization type: paravirtual

▼ Instance Type [Edit instance type](#)

Instance Type	ECUs	vCPUs	Memory (GiB)	Instance Storage (GB)	EBS-Optimized Available	Network Performance
t1.micro	up to 2	1	0.613	EBS only	-	Very Low

▼ Security Groups [Edit security groups](#)

**Security group name**    launch-wizard-1  
**Description**    launch-wizard-1 created on Thursday, May 1, 2014 12:00:19 PM UTC-4

Type ⓘ	Protocol ⓘ	Port Range ⓘ	Source ⓘ
SSH	TCP	22	0.0.0.0/0

▶ Instance Details [Edit instance details](#)

▶ Storage [Edit storage](#)

[Cancel](#)   [Previous](#)   [Launch](#)

## 4.4 Step-4: Create Key-pairs

1. Choose AMI    2. Choose Instance Type    3. Configure Instance    4. Add Storage    5. Tag Instance    6. Configure Security Group    7. Review

**Step 7: Review Instance Launch**

Please review your instance launch details. You can go back to edit changes for each section. Click **Launch** to assign a key pair to your instance and complete the launch process.

**Improve your instance's security.** Your instance may be accessible from any IP address. You can also open additional ports in your security groups.

▼ AMI Details

**Ubuntu Server 12.04 LTS (PV) - an**  
 Ubuntu Server 12.04.3 LTS (PV), EBS-backup  
 Free tier eligible    Root Device Type: ebs    Virtualization type: paravirtual

▼ Instance Type

Instance Type	ECUs	vCPU
t1.micro	up to 2	1

▼ Security Groups

**Select an existing key pair or create a new key pair** ✕

A key pair consists of a **public key** that AWS stores, and a **private key file** that you store. Together, they allow you to connect to your instance securely. For Windows AMIs, the private key file is required to obtain the password used to log into your instance. For Linux AMIs, the private key file allows you to securely SSH into your instance.

Choose an existing key pair

- Create a new key pair
- Proceed without a key pair

**Key pair name**

[Download Key Pair](#)

**You have to download the private key file (\*.pem file) before you can continue. Store it in a secure and accessible location. You will not be able to download the file again after it's created.**

[Cancel](#)   [Launch Instances](#)

▼ AMI Details [Edit AMI](#)

▼ Instance Type [Edit instance type](#)

▼ Security Groups [Edit security groups](#)

[Cancel](#)   [Previous](#)   [Launch](#)

You have now successfully launched your own version of the instance. For information on logging in and starting your instance, please go to VirAmp instance login

## 4.5 Log in to the new instance

Instructions and an overview of the basic steps and parameters you need to login to the instance are provided at the console.

The screenshot shows the AWS Management Console interface for an EC2 instance. At the top, there are buttons for 'Launch Instance', 'Connect', and 'Actions'. Below these is a filter section with 'All instances' and 'All instance types' dropdowns, and a search bar. A table lists instances, with the selected instance 'i-84dbad8d' highlighted. Below the table, the instance details for 'i-84dbad8d' are shown, including its Public DNS, Public IP, and Availability Zone.

Name	Instance ID	Instance Type	Availability Zone	Instance State	Status Checks	Alarm S
	i-3d8be135	t1.micro	us-west-2c	terminated		None
	i-84dbad8d	t1.micro	us-west-2b	running	2/2 checks...	None

Instance: **i-84dbad8d**    Public DNS: **ec2-54-186-170-47.us-west-2.compute.amazonaws.com**

<b>Instance ID</b>	i-84dbad8d	<b>Public DNS</b>	ec2-54-186-170-47.us-west-2.compute.amazonaws.com
<b>Instance state</b>	running	<b>Public IP</b>	54.186.170.47
<b>Instance type</b>	t1.micro	<b>Elastic IP</b>	-
<b>Private DNS</b>	ip-172-31-32-	<b>Availability zone</b>	us-west-2b

Hit the “Connect” button to view information you need to login to the backend of the system.

The screenshot shows a modal dialog titled 'Connect To Your Instance'. It offers two options for connecting: 'A standalone SSH client' (selected) and 'A Java SSH Client directly from my browser (Java required)'. Below, it provides a numbered list of steps to access the instance, including the command to set permissions on the private key file and the example SSH command.

**Connect To Your Instance**

I would like to connect with

- A standalone SSH client
- A Java SSH Client directly from my browser (Java required)

To access your instance:

1. Open an SSH client. (find out how to [connect using PuTTY](#))
2. Locate your private key file (inst-demo.pem). The wizard automatically detects the key you used to launch the instance.
3. Your key must not be publicly viewable for SSH to work. Use this command if needed:
 

```
chmod 400 inst-demo.pem
```
4. Connect to your instance using its Public IP:
 

```
54.186.170.47
```

**Example:**

```
ssh -i inst-demo.pem ubuntu@54.186.170.47
```

Please note that in most cases the username above will be correct, however please ensure that you read your AMI usage instructions to ensure that the AMI owner has not changed the default AMI username.

If you need any assistance connecting to your instance, please see our [connection documentation](#).

**Close**

Start your terminal and type the following command:

```
chmod 400 myPemName.pem
```

Connect to your instance using your public IP:

```
ssh -i myPemName.pem ubuntu@public_IP
```

Change to the galaxy directory:

```
cd /mnt/galaxy/galaxy-dist/
```

Change viramp settings:

```
vi universe_wsgi.ini
```

Line 596: `admin_users = dwr19@psu.edu` should be changed to reflect the current administrators email address  
Line 662: `ftp_upload_site = viramp.com` should be changed from `viramp.com` to your public ip address

Start the viramp server:

```
screen ./run.sh CTRL-a-d
```

- For further information on the individual tools VirAmp utilizes please see the following websites:
  - [seqtk](#)
  - [diginorm](#)
  - [velvet](#)
  - [AMOS](#)
  - [Quast](#)
  - [MUMmer](#)
  - [Circos](#)