

Training module

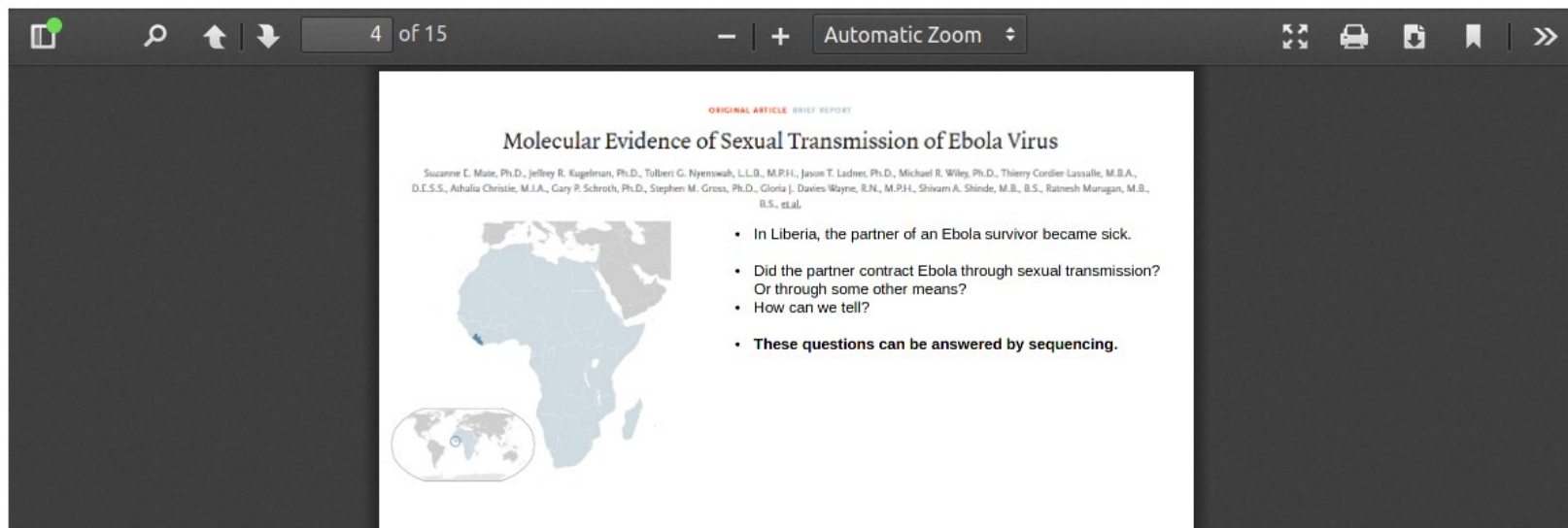
```
#####
# Genomic molecular characterization for viral strains using informatics tools  #
# CGS, USAMRIID                                                                #
# Authors: Raina Kumar (code and training module pipeline),                    #
#           Joushua Richardson (documentation and presentations)                #
# Contact: raina.kumar.ctr@mail.mil                                           #
#####
```

Objective

The training module will provide the complete bioinformatics workflow for analyzing genomics data using open source tools. The training module uses sequence reads generated using genomics tools such as genomic DNA or RNA sequencing using next generation sequencing technology with objective of characterization of viral strains in outbreak setting.

```
from IPython.display import IFrame
IFrame('documentation/final_pdfs/1_training_mod_013120_intro.pdf', width=900, height=300)
```

Out[4]:

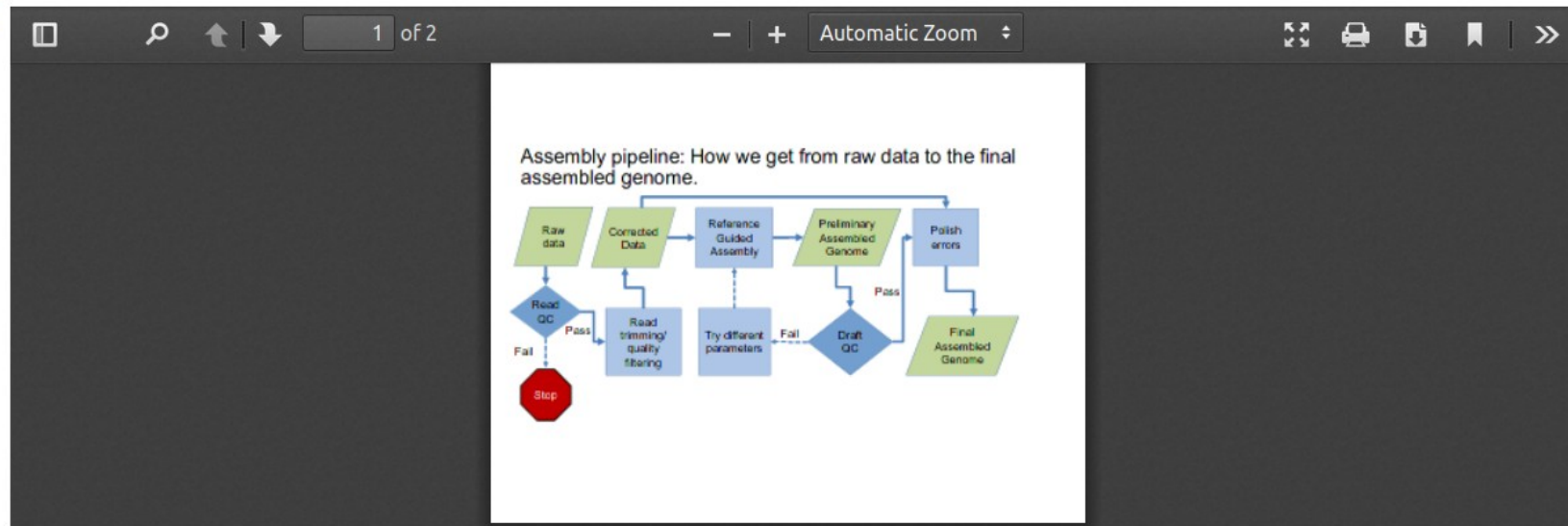


In [3]:

```
## Introduction to genomics assembly workflow
from IPython.display import IFrame
IFrame('documentation/final_pdfs/2_training_mod_013120_AssemblyPipe.pdf', width=900, height=300)
```

Edit Metadata

Out[3]:



In [2]:

Edit Metadata

```
# Step 1
# Define paths for input base directory, work directory and result directory in config.yaml for any new datasets
#

base_dir = "/home/guest/projects/"
work_dir = "/home/guest/projects/makono"
result_dir = "/home/guest/projects/results/"
reference_dir = "/home/guest/projects/makona/references/"
srefindex = "/home/guest/projects/makona/seqindex/"
sreference = "/home/guest/projects/makona/references/GCF_000848505.1_ViralProj14703_genomic.fna"
pri_adaptors = "/home/guest/projects/makona/references/pri_adaptors.fa"
```

```
## Step 2
```

```
##Run following:
```

```
##
```

```
## shell command
```

```
## For paired end data
```

```
## test fastqc read.R1_001.fastq.gz read.R2_001.fastq.gz -f fastq -o results/fastqc > log.txt
```

```
from IPython.display import IFrame
```

```
IFrame('documentation/final_pdfs/3_training_mod_013120_Fastqc.pdf', width=900, height=300)
```

Out[4]:

The screenshot displays a Jupyter Notebook interface. The top toolbar includes icons for file operations, a search icon, a zoom slider set to 'Automatic Zoom', and other standard controls. The main content area shows a slide titled 'Fastqc'. On the left, a flowchart illustrates the sequencing process: 'Raw Data' leads to 'Control Files', 'Read 1', and 'Read 2', which then merge into 'Paired End Reads'. Below this, a diagram shows 'Read1.fastq.gz' and 'Read2.fastq.gz' files being processed by 'Fastqc' to produce 'HTML Reports'. On the right, a bulleted list provides instructions: starting with raw sequencing data in fastq format, remembering two reads per DNA fragment, and running Fastqc to summarize read quality and output metrics. A final note instructs the user to click on HTML files to view reports.

Fastqc

- Start with raw sequencing data, in fastq format and zipped.
- Remember, there are two reads for each DNA fragment. The first read of each fragment is stored in one file, and the second read of each fragment is stored in another.
- Run Fastqc, a program that summarizes the quality of reads. Also outputs a number of useful metrics.

Read1.fastq.gz Read2.fastq.gz → Fastqc → HTML Reports

- Click on one of the HTML files to view the reports

```
!snakemake -s "popgen_fastqc.smk"
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Using shell: /bin/bash
```

```
Provided cores: 3
```

```
Rules claiming more threads will be scaled down.
```

```
Job counts:
```

count	jobs
1	all
1	raw_fastqc
2	

```
[Wed Jan 29 09:54:16 2020]
```

```
rule raw_fastqc:
```

```
    input: samples/raw/Brett424_1_S4_L001_R1_001.fastq.gz, samples/raw/Brett424_1_S4_L001_R2_001.fastq.gz
```

```
    output: results/fastqc/Brett424_1_S4_L001_R1_001_fastqc.html, results/fastqc/Brett424_1_S4_L001_R1_001_fastqc.zip, results/
fastqc/Brett424_1_S4_L001_R2_001_fastqc.html, results/fastqc/Brett424_1_S4_L001_R2_001_fastqc.zip, results/fastqc/Brett424_1_S4
_L001_fastqc.logfc.txt
```

```
    jobid: 1
```

```
input: samples/raw/Brett424_1_S4_L001_R1_001.fastq.gz, samples/raw/Brett424_1_S4_L001_R2_001.fastq.gz, results/fastqc/Brett424_1_S4_L001_R1_001_fastqc.html, results/fastqc/Brett424_1_S4_L001_R1_001_fastqc.zip, results/fastqc/Brett424_1_S4_L001_R2_001_fastqc.html, results/fastqc/Brett424_1_S4_L001_R2_001_fastqc.zip, results/fastqc/Brett424_1_S4_L001_fastqc.logfc.txt
jobid: 0
```

[Wed Jan 29 09:54:30 2020]

Finished job 0.

2 of 2 steps (100%) done

Complete log: /home/guest/projects/.snakemake/log/2020-01-29T095416.164303.snakemake.log

Workflow finished, no error

In [14]:

```
# Step 2 Fastqc results
from IPython.display import FileLink, FileLinks
FileLinks('makona/results/fastqc/.')
```

Edit Metadata

Out[14]: makona/results/fastqc/.

[Brett424_1_S4_L001_fastqc.logfc.txt](#)

[Brett424_1_S4_L001_R2_001_fastqc.html](#)

[Brett424_1_S4_L001_R1_001_fastqc.zip](#)

[Brett424_1_S4_L001_R2_001_fastqc.zip](#)

[Brett424_1_S4_L001_R1_001_fastqc.html](#)

In [15]:

Edit Metadata

```
# Step 3
## Trimming the bait illumina adaptors and primers from Illumina sequencing protocol using tool trimmomatic

##
## shell command
## For Paired end data
# "time java -jar trimmomatic-0.33.jar PE -threads 3 -trimlog logprefix input.read.R1_001.fastq.gz input.read.R2_001.fa
##

from IPython.display import IFrame
IFrame('documentation/final_pdfs/4_training_mod_013120__Trimv2.pdf', width=900, height=300)
```

Out[15]:

1 of 3 Automatic Zoom

Library Preparation

Illumina adapter and barcode (p5) Original viral sequence Illumina adapter and barcode (p7)

primer primer

Add Illumina adapters, which are DNA sequences necessary for sequencing on the MiSeq.

- Remember that sequences were added during sample and library preparations that are not part of the original viral sequence.
- We need to remove those sequences now.


```
# Step 3 Run Trimmomatic on sequence reads using snakemake rule trimmomatics
```

```
!snakemake -s "popgen_trimmomatics.smk"
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [16]:

```
# Sequence read summary after trimming adaptors and primers
```

```
# Reports
```

```
from IPython.display import FileLink, FileLinks  
FileLinks('makona/results/primer_adapt_removed/.')
```

Edit Metadata

Out[16]: makona/results/primer_adapt_removed/.
[Brett424_1_S4_L001_R1_unpaired.fastq](#)
[Brett424_1_S4_L001_trimmolog.txt](#)
[Brett424_1_S4_L001_R2_unpaired.fastq](#)
[Brett424_1_S4_L001_R2_paired.fastq](#)
[Brett424_1_S4_L001_R1_paired.fastq](#)

```
# Step 4

## Reference mapping for Read correction
## Align reads to makona viral genome assembly fasta file

## Shell command
## time bwa mem -t 30 makona/references/GCF_000848505.1_ViralProj14703_genomic.fna input.read.1.fastq input.read.2.fastq

from IPython.display import IFrame
IFrame('documentation/final_pdfs/5_training_mod_013120__Alignmentv2.pdf', width=900, height=300)
```

Out[6]:

We can now align the viral reads to a known reference sequence.

Sequence of the reference strain (already known).

Quality Viral Reads, from the previous step.

- Use the "bwa" program to map the viral reads to the known reference assembly.

In [17]:

Edit Metadata

```
# Run step 4 for reference mapping for read correction using snakemake rule refmapsam
```

```
!snakemake -s "popgen_refmapsam.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [1]:

Edit Metadata

```
# Output from reference mapping
```

```
from IPython.display import FileLink, FileLinks
```

```
FileLinks('makona/results/ref_aligned/')
```

Out[1]: makona/results/ref_aligned/

[Brett424_1_S4_L001_assembly_align_mem_ref_sorted.bam](#)

[Brett424_1_S4_L001_assembly_align_mem_ref.sam](#)

In [7]:

Edit Metadata

```
## Step 5
```

```
## Sort sam file and convert to bam format file using samtools software
```

```
## Shell command:
```

```
## "time samtools sort -O BAM makona.aligned.mem.sam > sample1.assembly_align_mem_ref_sorted.bam"
```

In [18]:

Edit Metadata

```
!snakemake -s "popgen_samsort2bam.smk" -n

/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
['Brett424_1_S4_L001']
|--- Number of samples to analyze: 1
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
Building DAG of jobs...
Nothing to be done.
```

In [17]:

Edit Metadata

```
# Output from reference mapping

from IPython.display import FileLink, FileLinks
FileLinks('makona/results/ref_aligned/.')
```

Out[17]: makona/results/ref_aligned/.

[Brett424_1_S4_L001_assembly_align_mem_ref_sorted.bam](#)
[Brett424_1_S4_L001_assembly_align_mem_ref.sam](#)

In [17]:

Edit Metadata

```
# Step 6

## Reference Guided Assembly graph using velvet assembler

## Shell Command:
## "time velveth out.assembly.dir input.kmernumber -bam -longPaired {output.assembly.dir}"

from IPython.display import IFrame
IFrame('documentation/final_pdfs/', width=900, height=300)
```

```
## Reference Guided Assembly graph using velvet assembler
```

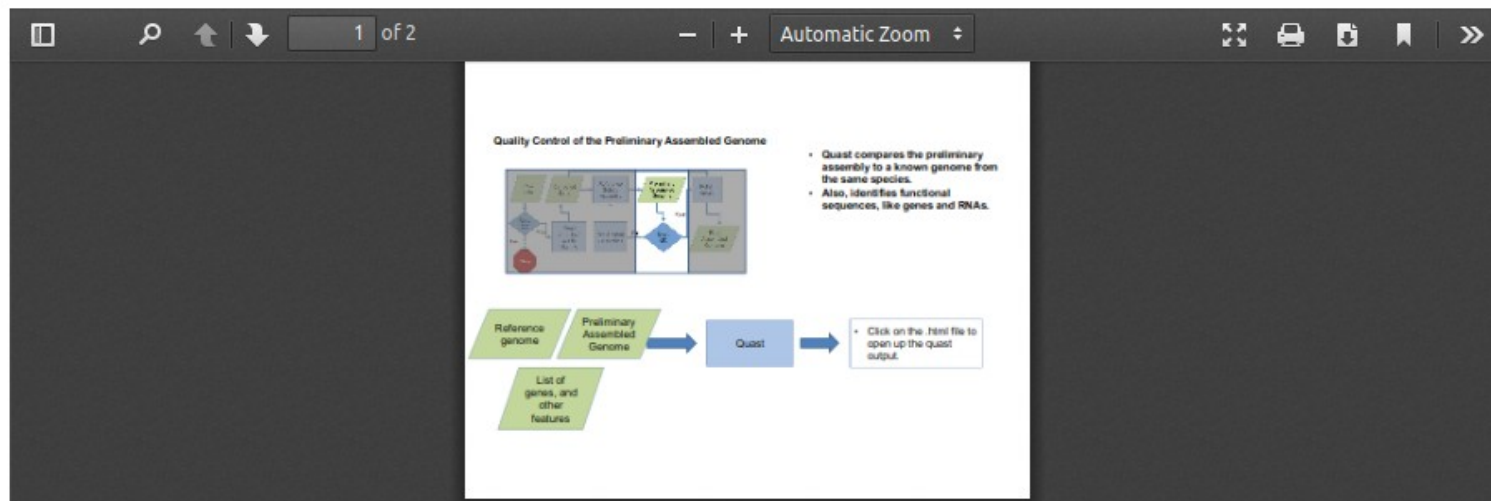
```
## Shell Command:
```

```
## "time velveth out.assembly.dir input.kmernumber -bam -longPaired {output.assembly.dir}"
```

```
from IPython.display import IFrame
```

```
IFrame('documentation/final_pdfs/', width=900, height=300)
```

Out[17]:



In [19]:

```
!snakemake -s "popgen_assembly.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done
```

```
# Output from reference mapping
```

```
from IPython.display import FileLink, FileLinks  
FileLinks('makona/results/velvet_assembly/')
```

```
Out[5]: makona/results/velvet_assembly/  
         Brett424\_1\_S4\_L001\_assembly\_log.txt  
         Brett424\_1\_S4\_L001\_logfile\_assemref\_27.txt  
         Brett424\_1\_S4\_L001\_logfile\_cindex.txt  
         Brett424\_1\_S4\_L001\_reindex.log.txt  
makona/results/velvet_assembly/Brett424_1_S4_L001_AssemRef/  
         contigs.fa.fai  
         contigs.fa  
         Log  
         Roadmaps  
         contigs.fa.bwt  
         contigs.fa.ann  
         PreGraph  
         contigs.fa.pac  
         contigs.fa.sa  
         velvet\_asm.afg  
         LastGraph  
         Sequences  
         stats.txt  
         Graph  
         contigs.fa.amb
```

In [4]:

Edit Metadata

```
# Step 7

## Reference Guided Assembly map using velvet assembler
## Shell Command:

## "time velvetg input.out.assembly.dir -amos_file yes > output.logfile"

from IPython.display import IFrame
IFrame('documentation/final_pdfs/5_training_mod_013120__Alignmentv2.pdf', width=900, height=300)
```

Out[4]:

1 of 3


Automatic Zoom

We can now align the viral reads to a known reference sequence.

Sequence of the reference strain (already known):

Quality Viral Reads from the previous step:

- Use the "bwa" program to map the viral reads to the known reference assembly.



In [20]:

Edit Metadata

```
!snakemake -s "popgen_assembly_sgraph.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

click to expand output; double click to hide output

```
[ Brett424_1_S4_L001 ]
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [6]:

Edit Metadata

```
## Step 7 output
```

```
## # Output from velvet assembly
```

```
from IPython.display import FileLink, FileLinks  
FileLinks('makona/results/velvet_assembly/.')
```

Out[6]: makona/results/velvet_assembly/

[Brett424_1_S4_L001_assembly_log.txt](#)

[Brett424_1_S4_L001_logfile_assemref_27.txt](#)

[Brett424_1_S4_L001_logfile_cindex.txt](#)

[Brett424_1_S4_L001_reindex.log.txt](#)

makona/results/velvet_assembly/Brett424_1_S4_L001_AssemRef/

[contigs.fa fai](#)

[contigs.fa](#)

[Log](#)

[Roadmaps](#)

[contigs.fa.bwt](#)

[contigs.fa.ann](#)

[PreGraph](#)

[contigs.fa.pac](#)

[contigs.fa.sa](#)

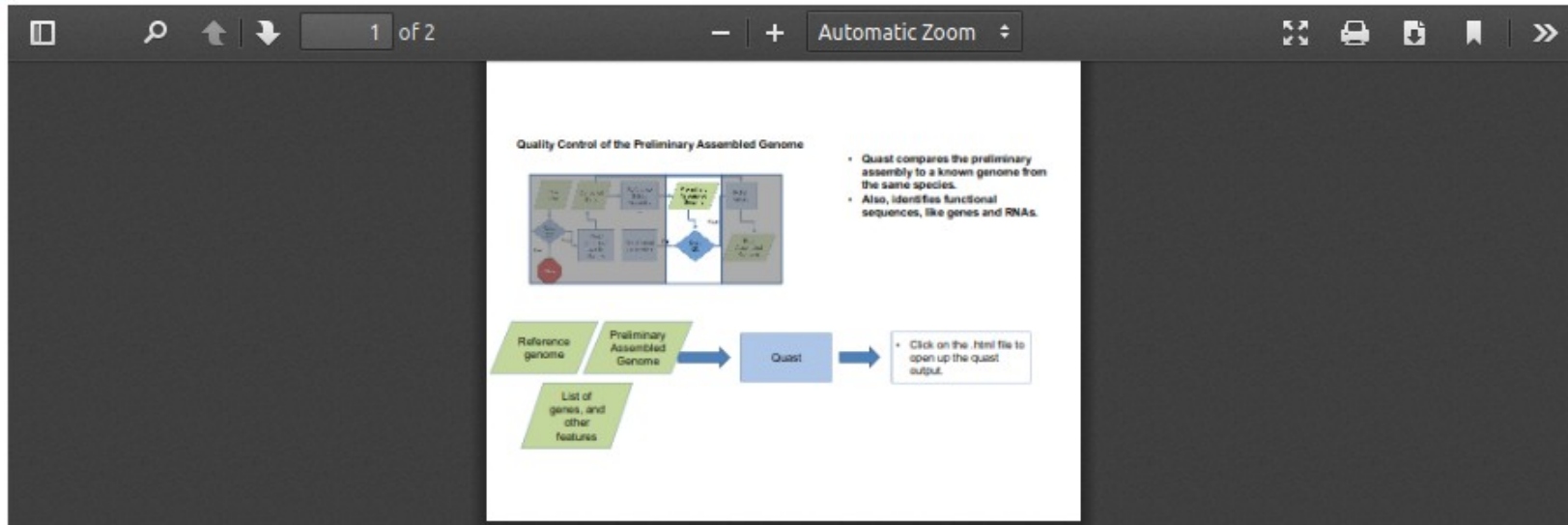

```
# Step 8

## Assembly quality assesment stastics and gene prediction
## Shell Command:

## "time quast.py step7.input.contig.fa -R chk.genome.fa -G chk.genome.gff -o out.assembly.stat.reports --glimmer > out

from IPython.display import IFrame
IFrame('documentation/final_pdfs/6_training_mod_013120__DraftQC.pdf', width=900, height=300)
```

Out[8]:



In [21]:

```
!snakemake -s "popgen_assembly_predictgene.smk" -n
```

Edit Metadata

In [21]:

Edit Metadata

```
!snakemake -s "popgen_assembly_predictgene.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [10]:

Edit Metadata

```
## Step 8 Assembly reports
```

```
from IPython.display import HTML
```

```
HTML(filename="./makona/results/assembl_stats/Brett424_1_S4_L001_reference_stats/report.html")
```

Out[10]:

QUAST

Quality Assessment Tool for Genome Assemblies by [QAE](#)

24 January 2020, Friday, 02:19:26

[View in Icarus contig browser](#)

All statistics are based on contigs of size ≥ 300 bp, unless otherwise noted (e.g., "# contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs).

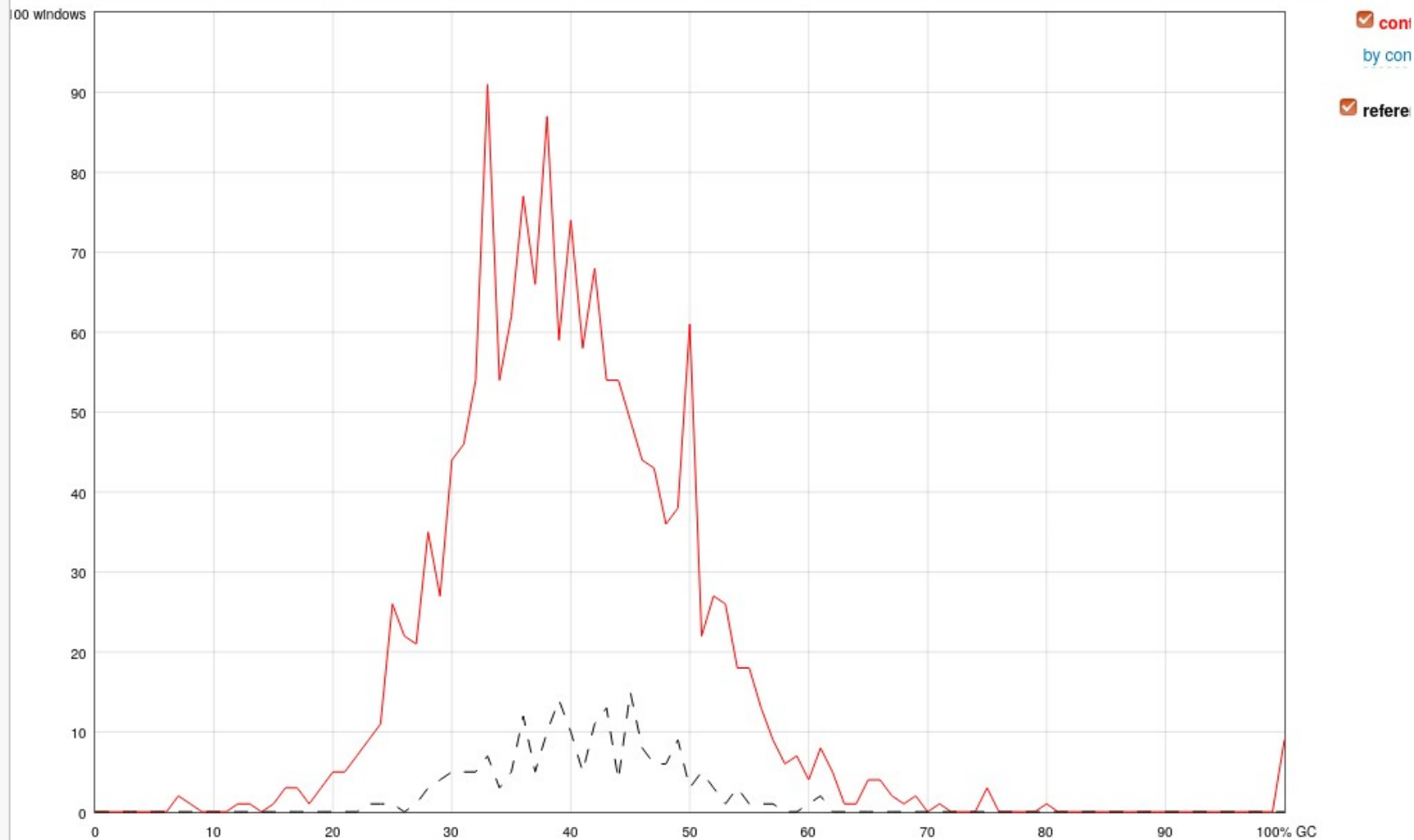
Aligned to "GCF_000848505.1_ViralProj14703_genomic" | 18 959 bp | 1 fragment | 41.07% G+C

55 genomic features

click to expand output; double click to hide output

Cumulative length Nx NAx NGx NGAx Misassemblies Genomic features GC content

Normal / logarithmic scale



In [9]:

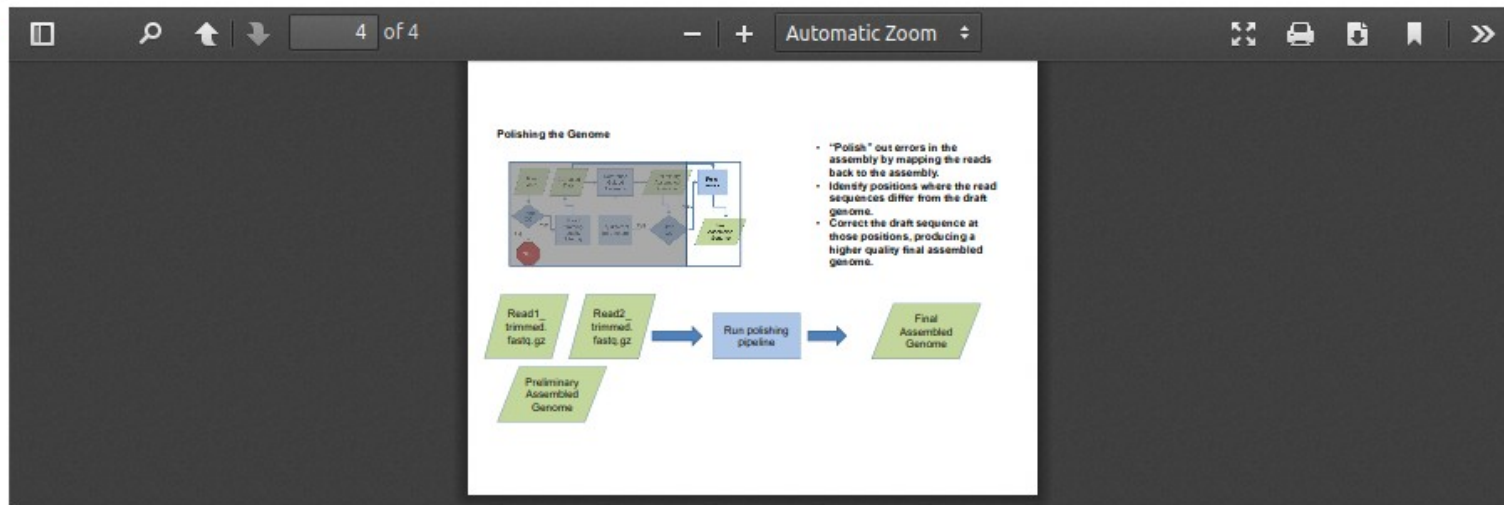
Edit Metadata

```
# Step 9
## Create index of contigs and map reads back to contig
## Shell command:

## "time bwa index -a bwtsv step7.input.contig.fa > output.logfile"

from IPython.display import IFrame
IFrame('documentation/final_pdfs/7_training_mod_013120__Polishv2.pdf', width=900, height=300)
```

Out[9]:



In [22]:

Edit Metadata

```
!snakemake -s "popgen_bwaindex_contig.smk" -n
```

```
/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
['Brett424_1_S4_L001']
|--- Number of samples to analyze: 1
```

```
!snakemake -s "popgen_bwaindex_contig.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [10]:

[Edit Metadata](#)

```
# Step 10
```

```
## "time bwa mem -t 30 step8.input.contig.fa {input.read1p} {input.read2p} > {output.contigalign}"
```

In [28]:

[Edit Metadata](#)

```
!snakemake -s "popgen_alignreads2contig.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

In [28]:

[Edit Metadata](#)

```
# Step 11
```

```
## Coordinate sort sam files and convert to bam file using samtools
```

In [30]:

```
# Step 13

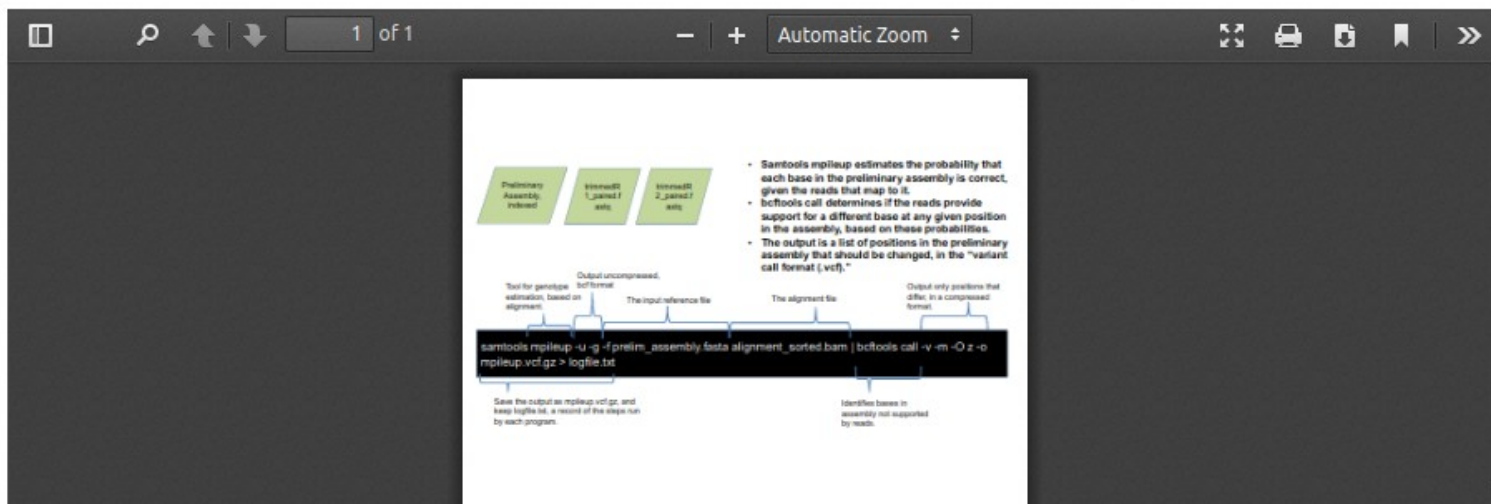
## Variant Calling using samtools mpileup

## Shell Command:

## "time samtools mpileup -u -g -f step8.input.contig.fa step11.contig.read.sorted.aligned.bam | bcftools call -v -m -O z -o

from IPython.display import IFrame
IFrame('documentation/command_pdfs/training_mod_Draft_Sl39.pdf', width=900, height=300)
```

Out[30]:



In [31]:

```
!snakemake -s "popgen_variantsCall.smk" -n
```

```
/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
['Brett424_1_S4_L001']
|--- Number of samples to analyze: 1
```

```
from IPython.display import FileLink, FileLinks
FileLinks('makona/results/variants_calling/')
```

Out[38]: makona/results/variants_calling/

- [Brett424_1_S4_L001_mpileup.vcf.gz](#)
- [Brett424_1_S4_L001_mpileup.vcf.gz.csi](#)
- [Brett424_1_S4_L001_vcfindex.txt](#)
- [Brett424_1_S4_L001_snpcall.txt](#)

In [32]:

Edit Metadata

```
!snakemake -s "popgen_vcfindex.smk" -n
```

/home/guest/projects//makona/results directory exists

|--- Results directory is: /home/guest/projects//makona/results

|--- The current working directory is /home/guest/projects//makona

['Brett424_1_S4_L001']

|--- Number of samples to analyze: 1

|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed

Building DAG of jobs...

Nothing to be done.

In [12]:

Edit Metadata

```
# Step 15
```

```
## Build sequences consensus
```

```
## Shell Command:
```

```
## "time cat step8.input.contig.fa | bcftools consensus output.mpileup.vcf.gz > output.consensus.fa"
```

In [33]:

Edit Metadata

```
!snakemake -s "popgen_buildConsensus.smk" -n
```



```
!snakemake -s "popgen_buildConsensus.smk" -n
```

```
/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
['Brett424_1_S4_L001']
|--- Number of samples to analyze: 1
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
Building DAG of jobs...
Nothing to be done.
```

In [39]:

Edit Metadata

```
## Reports

from IPython.display import FileLink, FileLinks
FileLinks('makona/results/consensus_seq/.')
```

Out[39]: makona/results/consensus_seq/.
[Brett424_1_S4_L001_consensus.fa](#)

In [2]:

Edit Metadata

```
# Step 16

## Consensus multiple alignment

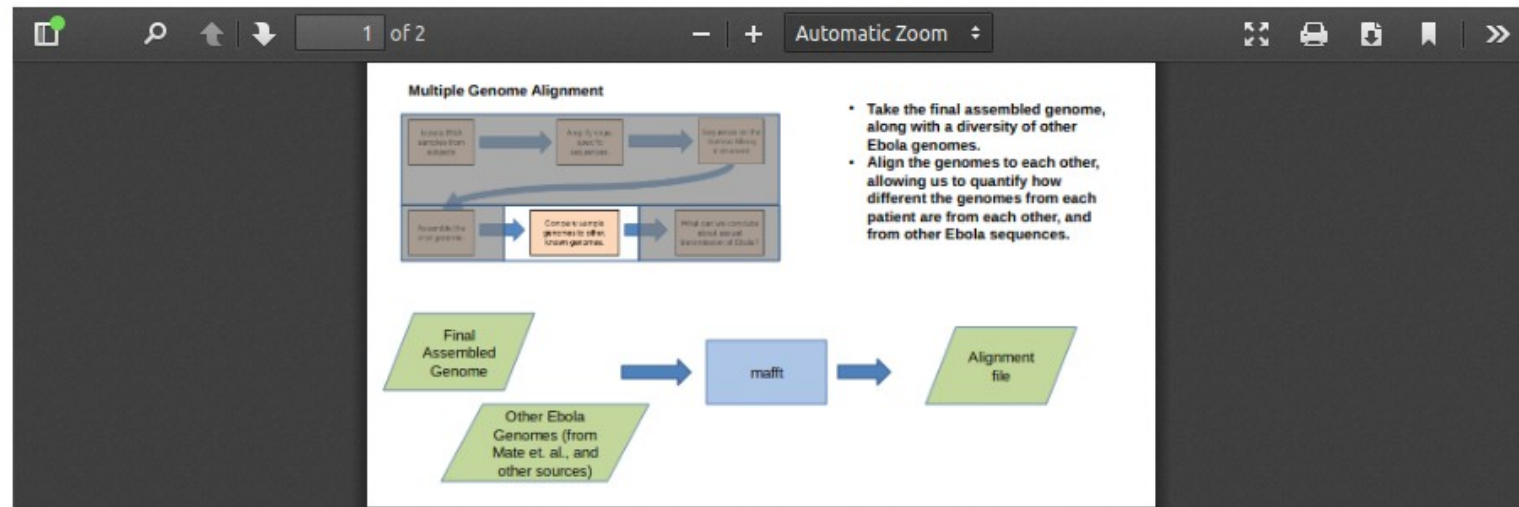
## Shell Command:

## cat final_assembly.fasta | mafft ebola_ref.fasta > Final_alignment.out

from IPython.display import IFrame
IFrame('documentation/final_pdfs/8_training_mod_013120_GenAlignv3.pdf', width=900, height=300)
```

Out[2]:

Out[2]:



In [5]:

Edit Metadata

```
!snakemake -s "popgen_mafft_alignment_view.smk"
```

```
/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
['Brett424_1_S4_L001']
|--- Number of samples to analyze: 1
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
Building DAG of jobs...
Using shell: /bin/bash
Provided cores: 3
Rules claiming more threads will be scaled down.
Job counts:
```

[illegible]

In [*]:

Edit Metadata

```
## View MSA alignment
```

```
library(shiny)
runApp()
```

```
Listening on http://127.0.0.1:6922
```

In [2]:

Edit Metadata

```
## Reports
```

```
from IPython.display import FileLink, FileLinks
FileLinks('makona/results/maff_haplo/.')
```

Out[2]: makona/results/maff_haplo/
[makona_multiple_alignment.out](#)
[final_contactenated_mafft.fa](#)

In [3]:

Edit Metadata

```
## Shell Command:
```

```
# "time bcftools stats -F step8.input.contig.fa -s step11.output.mpileup.vcf.gz > output.variants.stat"
```

```
!snakemake -s "popgen_variants_stat.smk" -n
```

```
/home/guest/projects//makona/results directory exists
```

```
|--- Results directory is: /home/guest/projects//makona/results
```

```
|--- The current working directory is /home/guest/projects//makona
```

```
['Brett424_1_S4_L001']
```

```
|--- Number of samples to analyze: 1
```

```
|--- Sample Brett424_1_S4_L001 (reads: Brett424_1_S4_L001_R1_001.fastq.gz & Brett424_1_S4_L001_R2_001.fastq.gz) will be processed
```

```
Building DAG of jobs...
```

```
Nothing to be done.
```

Out[4]: makona/results/variants_stats/.

click to expand output; double click to hide output

In [5]:

Edit Metadata

```
!head -100 makona/results/variants_stats/Brett424_1_S4_L001_vcf.stats
```

```
# This file was produced by bcftools stats (1.9+htslib-1.9) and can be plotted using plot-vcfstats.
# The command line was: bcftools stats -F results/velvet_assembly/Brett424_1_S4_L001_AssemRef/contigs.fa -s - results/variants_calling/Brett424_1_S4_L001_mpileup.vcf.gz
#
# Definition of sets:
# ID      [2]id      [3]tab-separated file names
ID        0          results/variants_calling/Brett424_1_S4_L001_mpileup.vcf.gz
# SN, Summary numbers:
#   number of records    .. number of data rows in the VCF
#   number of no-ALTs    .. reference-only sites, ALT is either "." or identical to REF
#   number of SNPs       .. number of rows with a SNP
#   number of MNPs       .. number of rows with a MNP, such as CC>TT
#   number of indels     .. number of rows with an indel
#   number of others     .. number of rows with other type, for example a symbolic allele or
#                           a complex substitution, such as ACT>TCGA
#   number of multiallelic sites .. number of rows with multiple alternate alleles
#   number of multiallelic SNP sites .. number of rows with multiple alternate alleles, all SNPs
#
```

In [19]:

Edit Metadata

```
## Haplotype network and SNP analysis
```

```
## Shell
```

```
!snakemake -s "popgen_haplonetwork.smk"
```

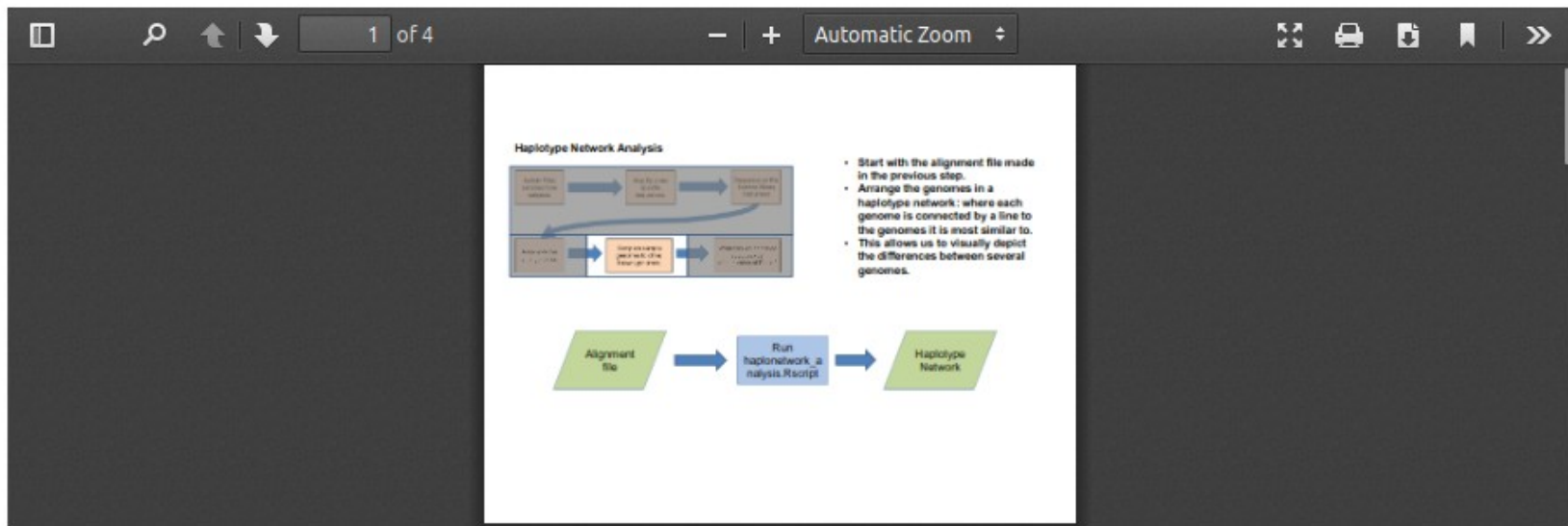
```
/home/guest/projects//makona/results directory exists
|--- Results directory is: /home/guest/projects//makona/results
|--- The current working directory is /home/guest/projects//makona
```

In [20]:

Edit Metadata

```
from IPython.display import IFrame
IFrame('documentation/final_pdfs/9_training_mod_013120_HapNetv2.pdf', width=900, height=300)
```

Out[20]:



In [21]:

Edit Metadata

```
## Reports
```

```
from IPython.display import FileLink, FileLinks
FileLinks('makona/results/haplotype_network/')
```

Out[21]: makona/results/haplotype_network/

[Brett424_1_S4_L001_logfileR.txt](#)

[study_haplonetwork.png](#)

Reports

```
from IPython.display import FileLink, FileLinks
FileLinks('makona/results/haplotype_network/')
```

Out[21]: makona/results/haplotype_network/
[Brett424_1_S4_L001_logfileR.txt](#)
[study_haplonetwork.png](#)

In [11]:

```
from IPython.display import HTML
HTML(filename="./rscript_haplo.nb.html")
```

Out[11]:

R Notebook

Setting up the environment and reading the data and metadata

Haplotype network with:

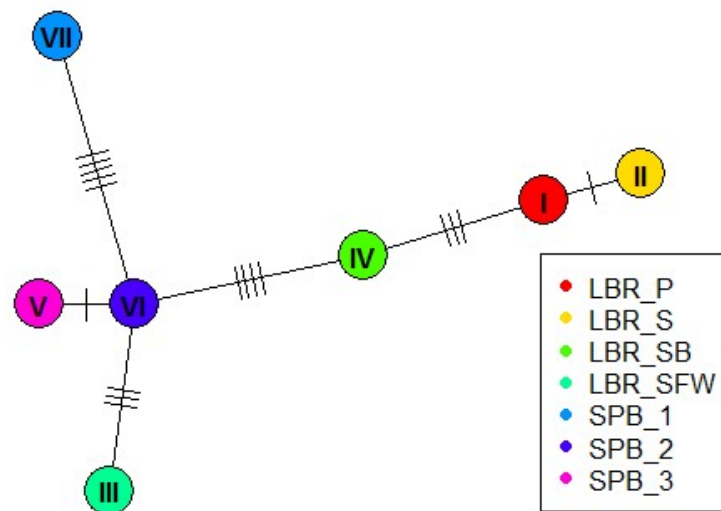
- 7 haplotypes

- 9 links

- link lengths between 1 and 5 steps

Use `print.default()` to display all elements.

Plot the haplonetwork



In [10]:

```
## References
```

```
## Shell
```

```
from IPython.display import IFrame
```

```
IFrame('documentation/final_pdfs/10_training_mod_013120_CommandLine.pdf', width=900, height=300)
```

Out[10]:



In [10]:

Edit Metadata

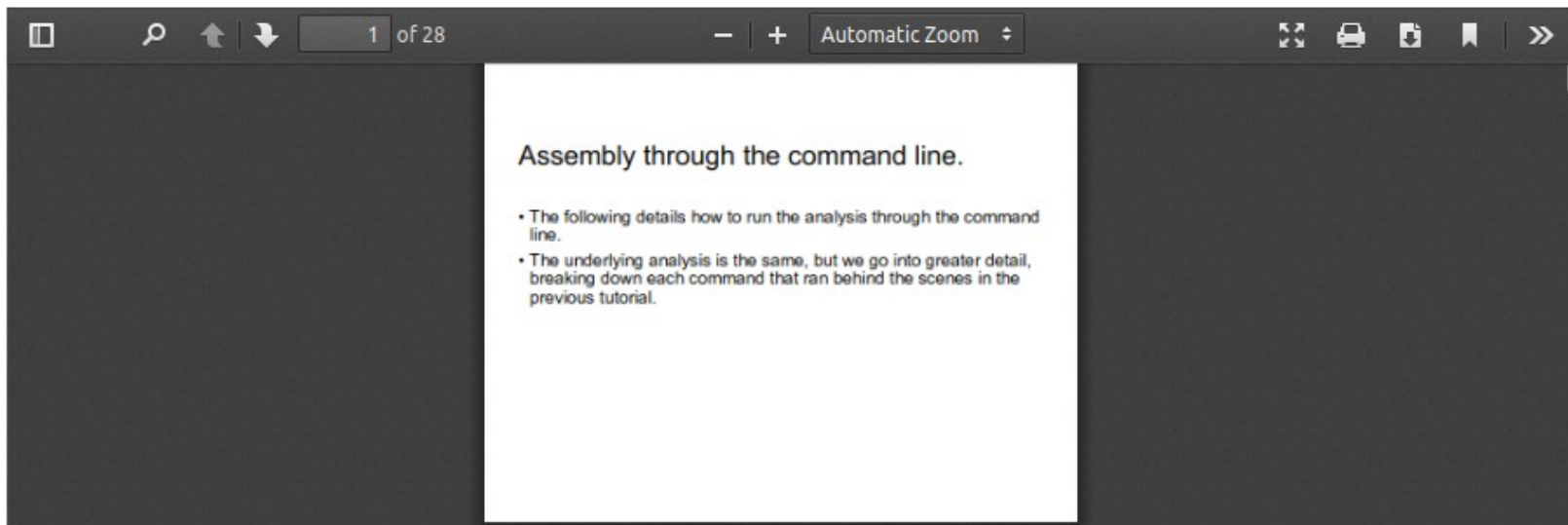
```
## References
```

```
## Shell
```

```
from IPython.display import IFrame
```

```
IFrame('documentation/final_pdfs/10_training_mod_013120__CommandLine.pdf', width=900, height=300)
```

Out[10]:



1 of 28 Automatic Zoom

Assembly through the command line.

- The following details how to run the analysis through the command line.
- The underlying analysis is the same, but we go into greater detail, breaking down each command that ran behind the scenes in the previous tutorial.

In []:

Edit Metadata