Introduction to NGS Data Analysis

High Performance
Research Computing
DIVISION OF RESEARCH

Spring 2024

Your Login Password

- Both state of Texas law and TAMU regulations prohibit the sharing and/or illegal use of computer passwords and accounts
- Do not write down passwords
- Do not choose easy to guess passwords
- Change passwords frequently

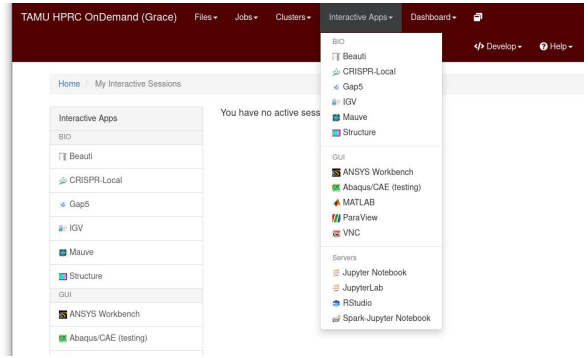
There will be a 10 minute break halfway through today's short course

Introduction to NGS

- NGS Technology
- NGS Tools on Grace
- Quality Control (QC)
- Template Job Scripts
- Visualize BAM Alignment Files
- Sequence Variant Calling
- RNA-seq
- ChIP-seq
- Biocontainers

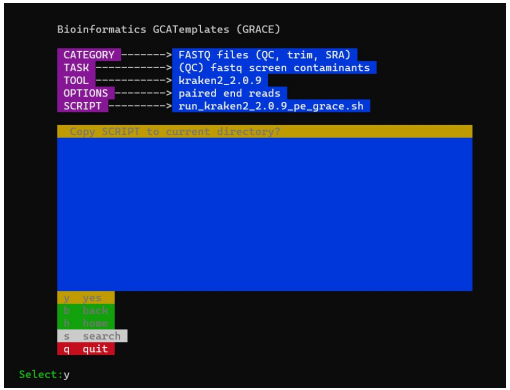
Options for Running Bioinformatics Tools

HPRC Portal



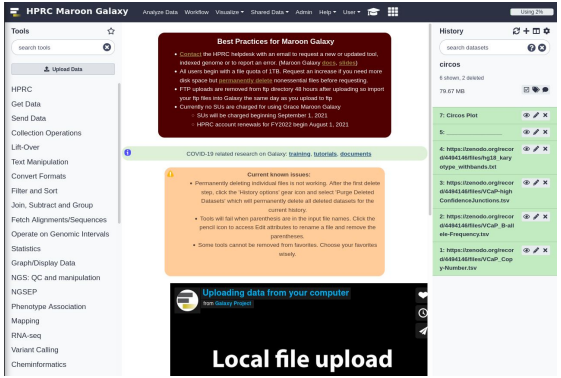
- portal-grace.hprc.tamu.edu
- Access is web-browser-based
- All HPRC software tools are available either as a GUI or via Unix command line
- Can access Unix command line
  - Grace or Terra
- Best for GUI apps
  - RStudio
  - IGV

Unix command line



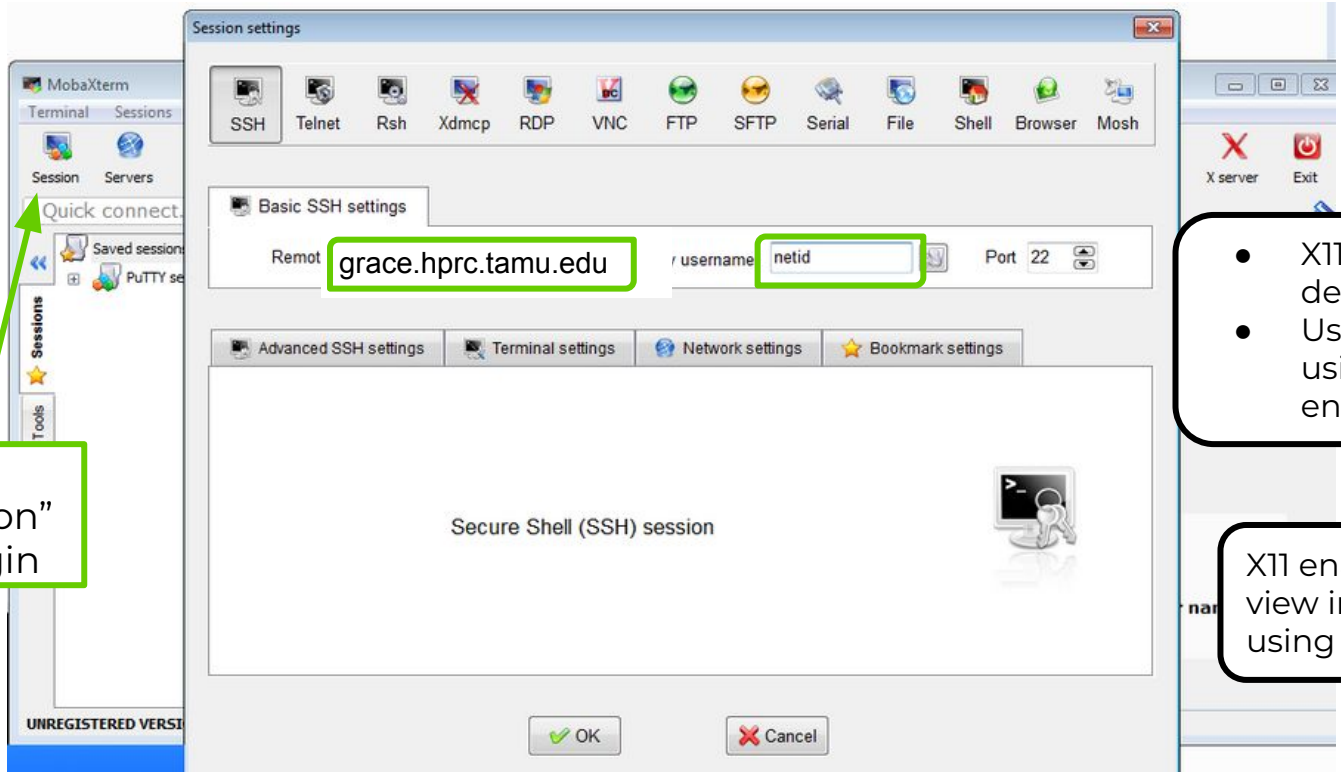
- Need to learn Unix and Slurm
- Bioinformatics template scripts available
- GUI software is not very responsive interactively
- Need SSH client on your Windows computer such as MobaXterm or use HPRC portal

HPRC Maroon Galaxy



- Access is web-browser-based
- First apply for an HPRC account then request a Galaxy account
- Can request HPRC to add tools from the usegalaxy.org toolshed or create a custom tool

Using SSH - MobaXterm (on Windows) to Connect to Grace



click "Session" to begin

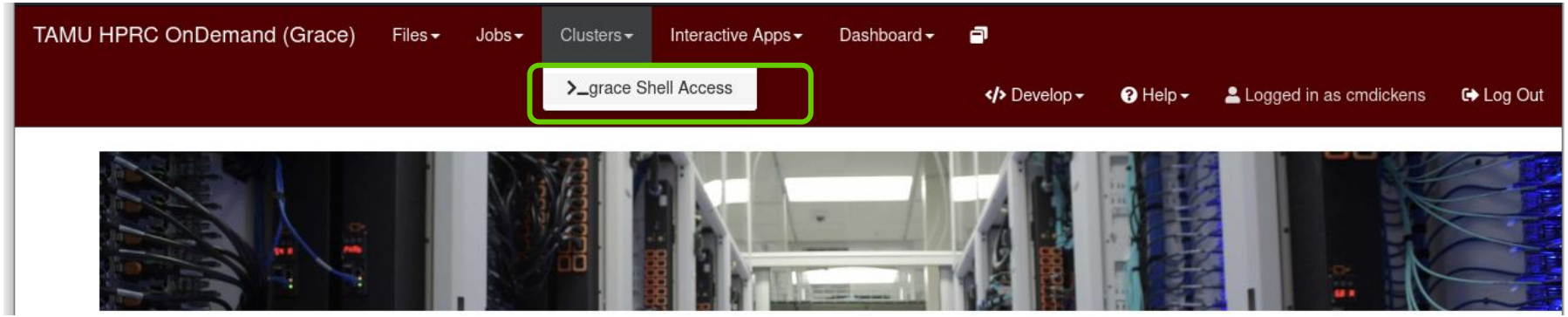
- X11 is enabled by default in MobaXterm
- Use "ssh -X" when using the terminal to enable X11

X11 enables you to view images when using the terminal

https://hprc.tamu.edu/kb/Helpful-Pages/#mobaxterm

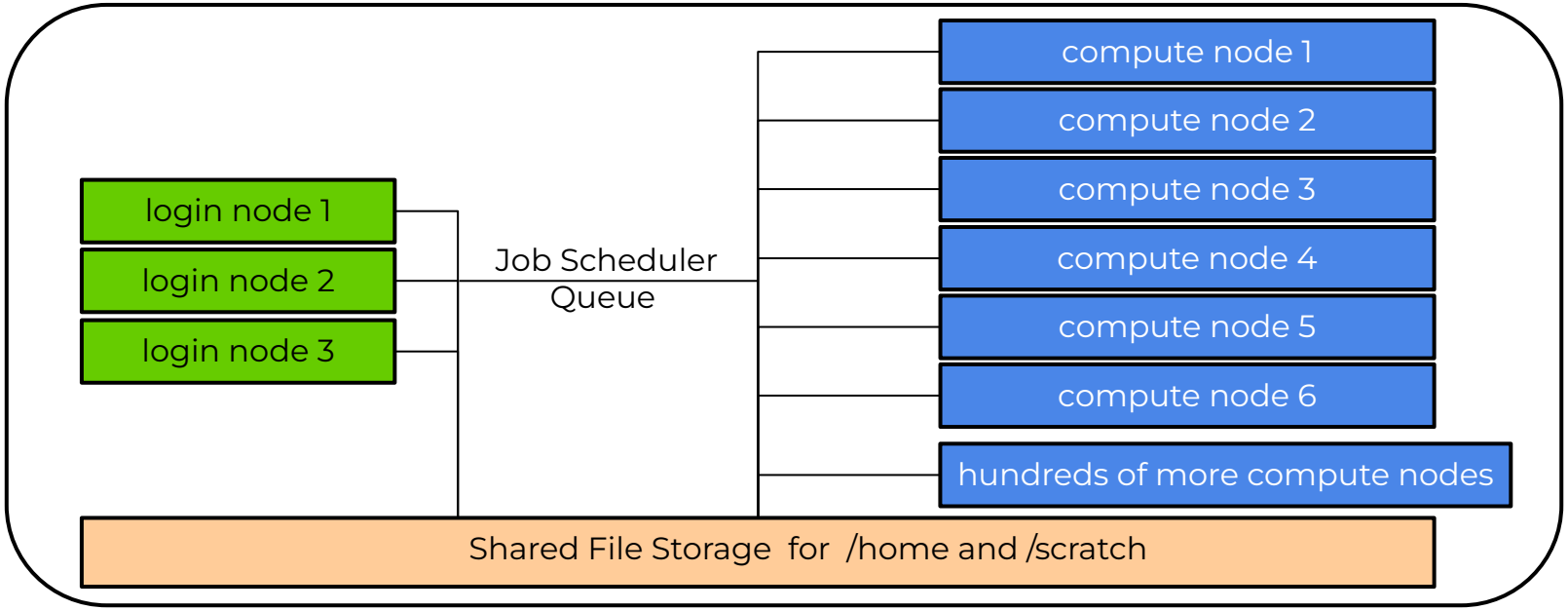
Connect using the HPRC portal

portal-grace.hprc.tamu.edu



There are no SUs charged for using the Shell Access

HPC Diagram



login nodes are for:

- file manipulation and job script preparation
- software installation and testing
- short tasks (< 60 minutes and max 8 cores)
  - also be aware of amount of memory utilized

compute nodes are for:

- computational jobs which can use up to 48 cores and/or up to 360GB memory per Grace compute node.
- all jobs running > 60 minutes

Next Generation Sequencing Technologies

Illumina Sequencing Technology

iSeq 100

MiniSeq

MiSeq Series

NextSeq 550 Series

NextSeq 2000

NovaSeq 6000

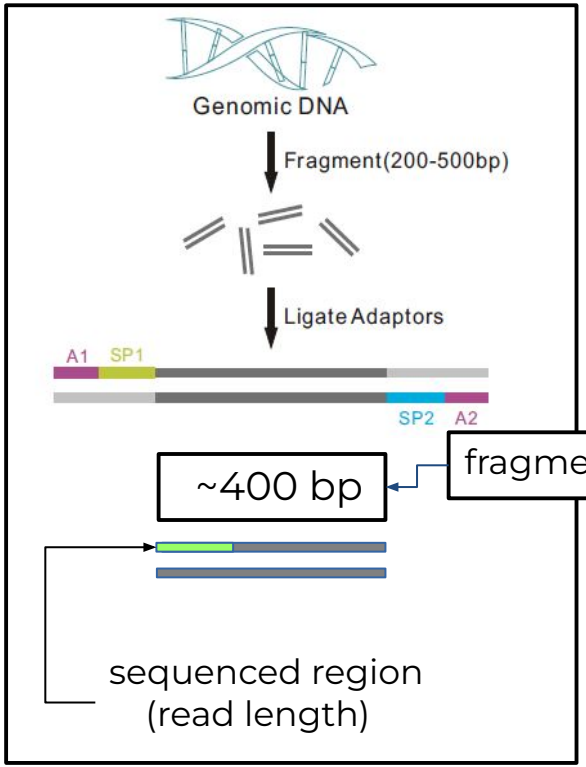
NovaSeq X Series

http://www.illumina.com/systems/sequencing-platforms.html

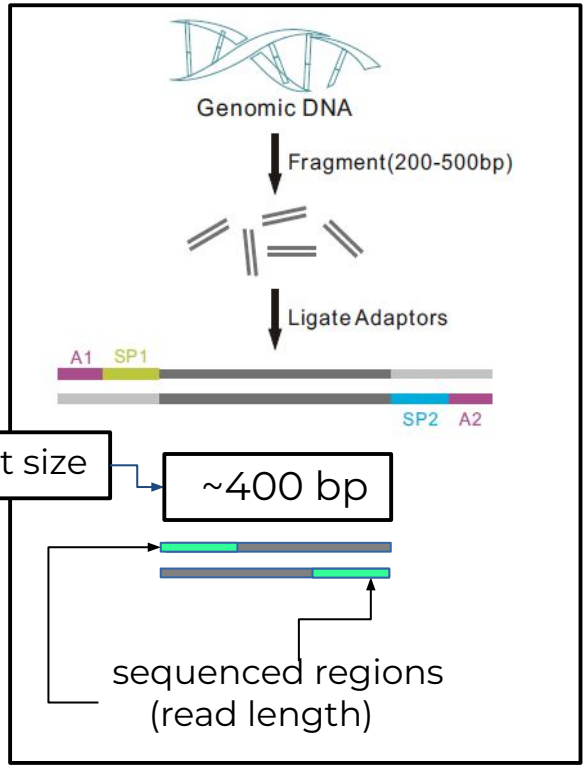
Illumina Sequencing Libraries

illumina.com

single end

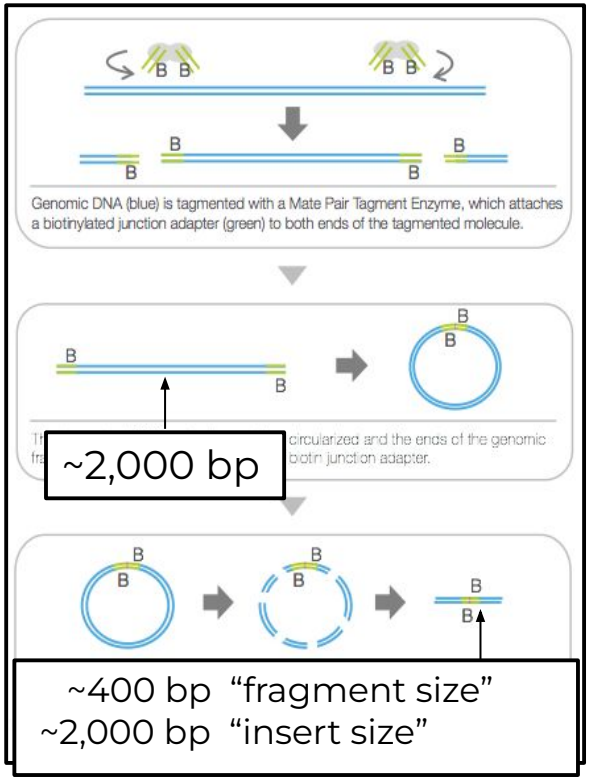


paired ends

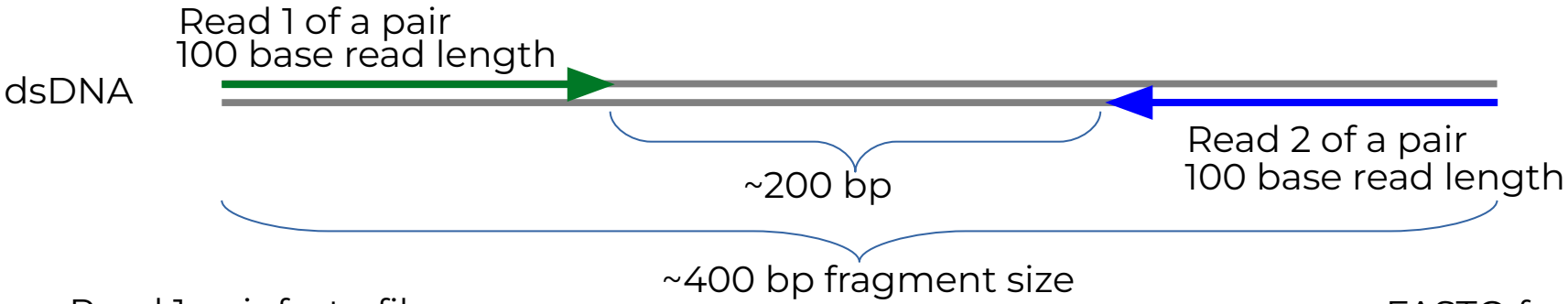


fragment size

mate pairs



Paired End Reads



Read 1 pair fastq file

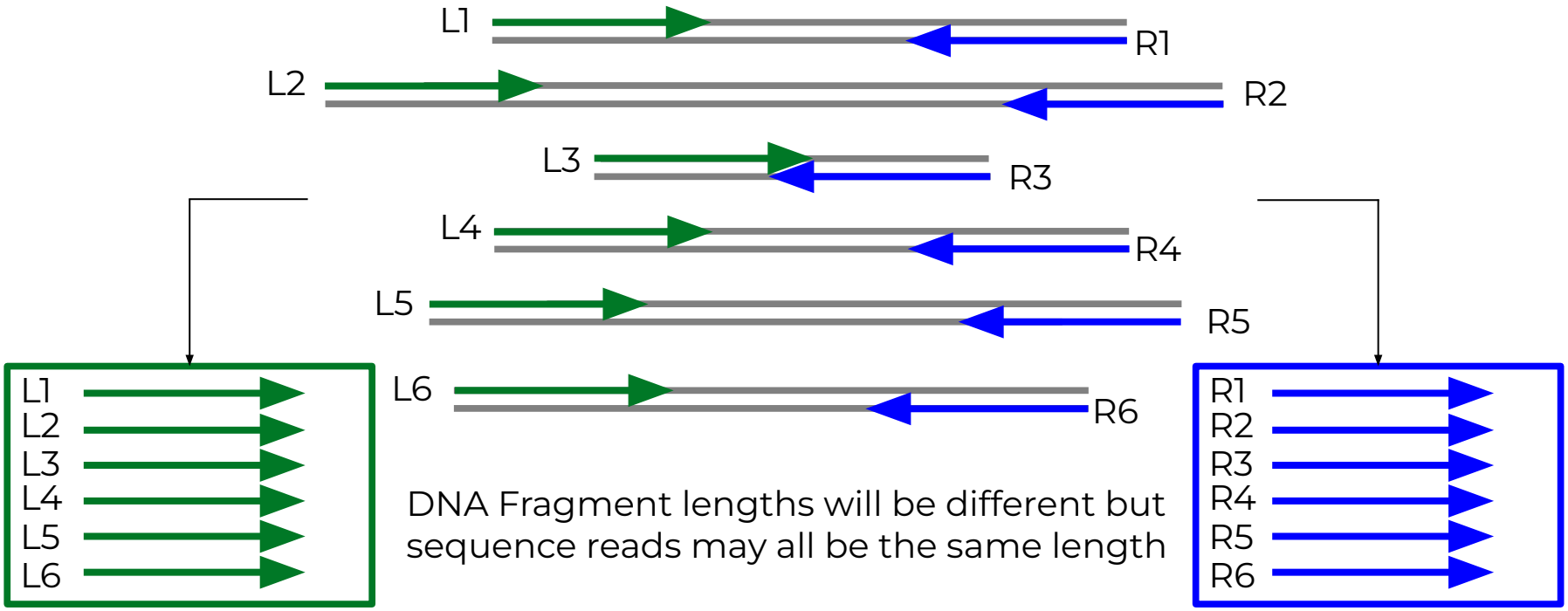
FASTQ format

```
@M00861:1:000000000-A36BE:1:1101:14650:1529 1:N:0:8
TTCTTAAAAATACCATAAAAGGCTTAAACTTGCCATTTACGACGGATTAATTCCAACTCTTTTCGGCTATCTTCATCTTTTAAGGTTAAATGACTCATAACGG
+
FFFHBFFHHIIIIIIHFHHCGEFGHHIHHHIHD/?DGGHHH@DEB,5EGHGHHIIHIF?FGGHHCCBFDGHFHDGHGFFFGDFHH?DFHDFFHHFHFHFFHHH
```

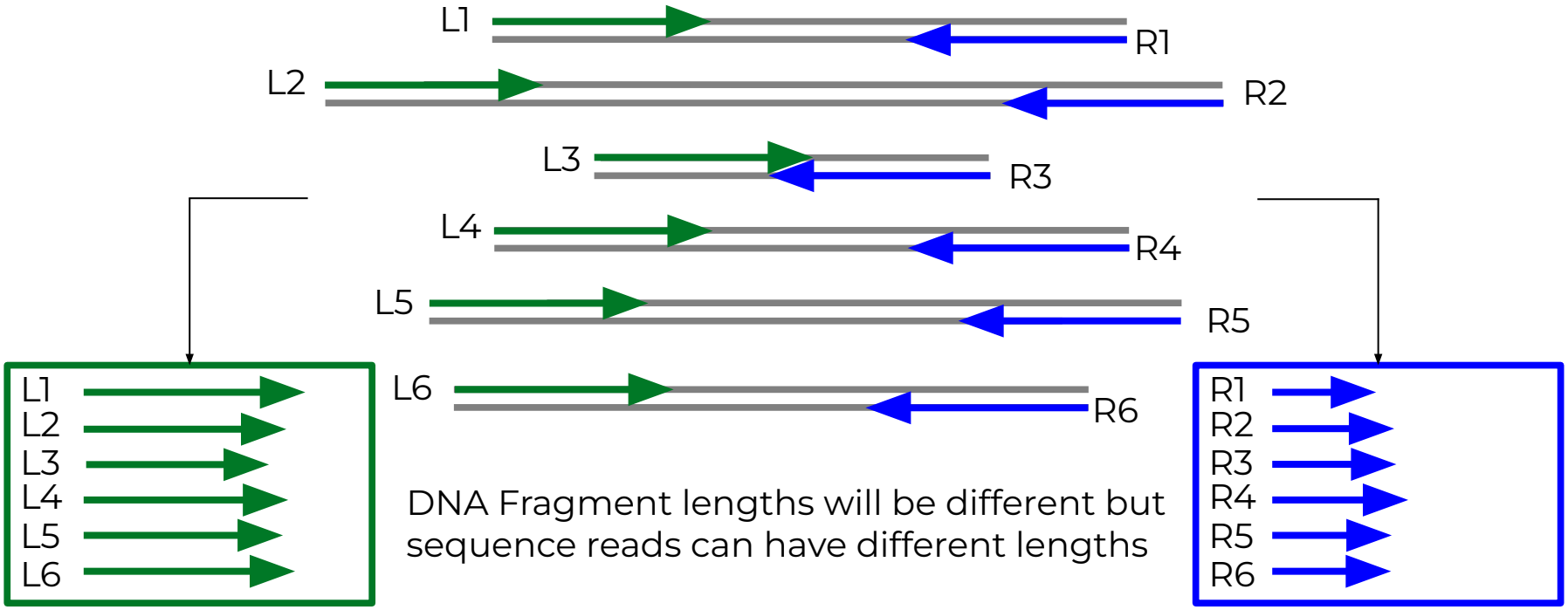
Read 2 pair fastq file

```
@M00861:1:000000000-A36BE:1:1101:14650:1529 2:N:0:8
ACTAAAAATCAATTTTATCAATTTCAAGCTCTACCTTATTTACTCATTATTTTAGTGATGGCCACTTTAATAAAAATATTGGTAGCATATTTTGCAATAGCGG
+
BFFHIHHHFHHDGHIHHIHHHGHHHHHFHHDFFHIHIIIHIHDFHHHIHIIIH=AAFHIIIHFGFHHHHHGGHHIHHFGFFFEGGHHHDGHHH/CGHIFFHHH
```

Maintain Read Pair Order

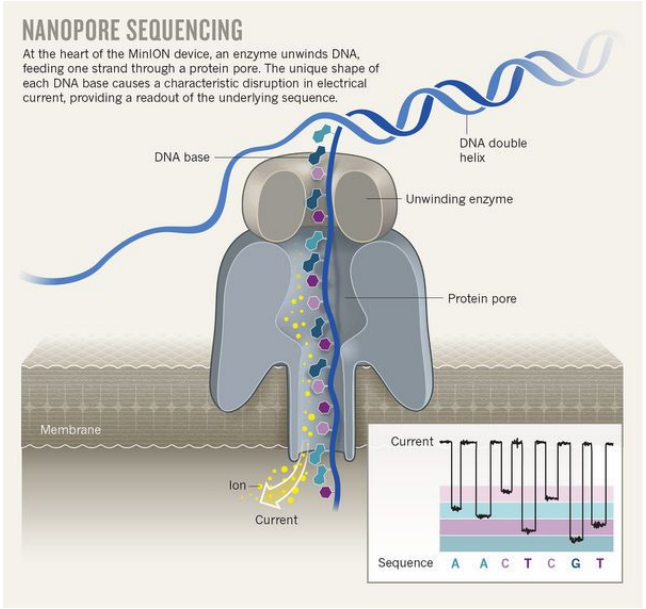


MiSeq Can Perform Initial QC Trimming



Oxford Nanopore Long Read Sequencing

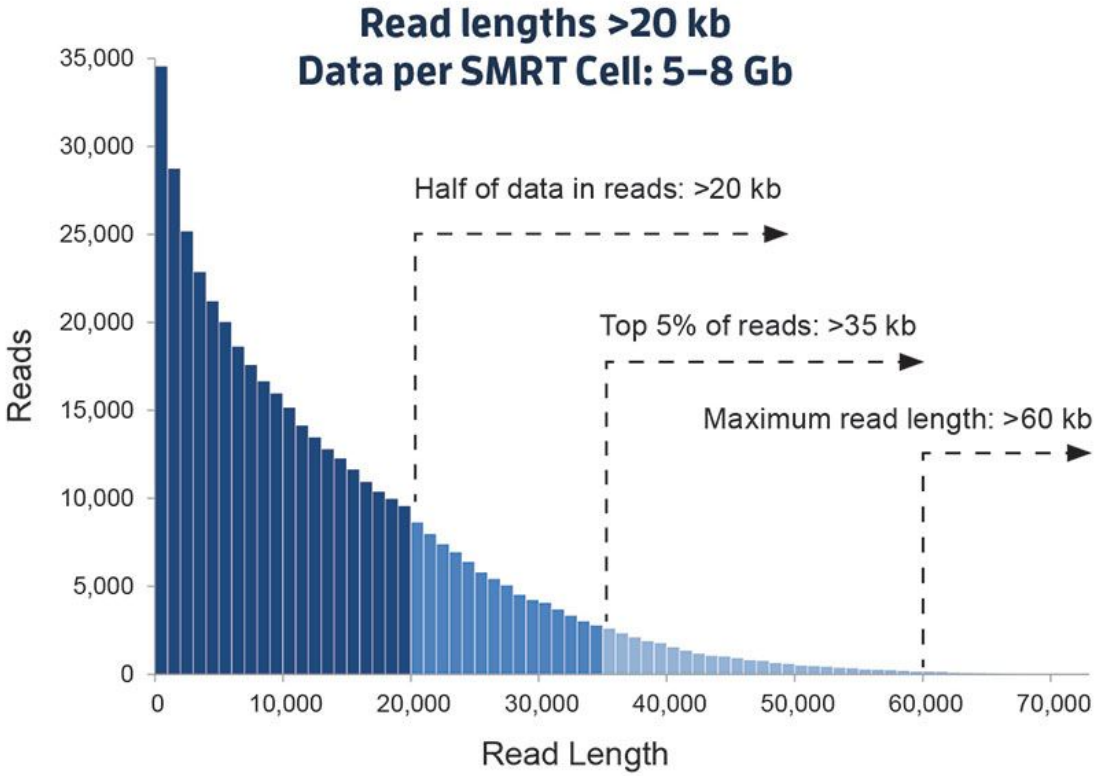
Oxford Nanopore Technologies (ONT)



http://blogs.nature.com
TechBlog: The nanopore toolbox
16 Oct 2017 | 12:00 GMT | Posted by Jeffrey Perkel

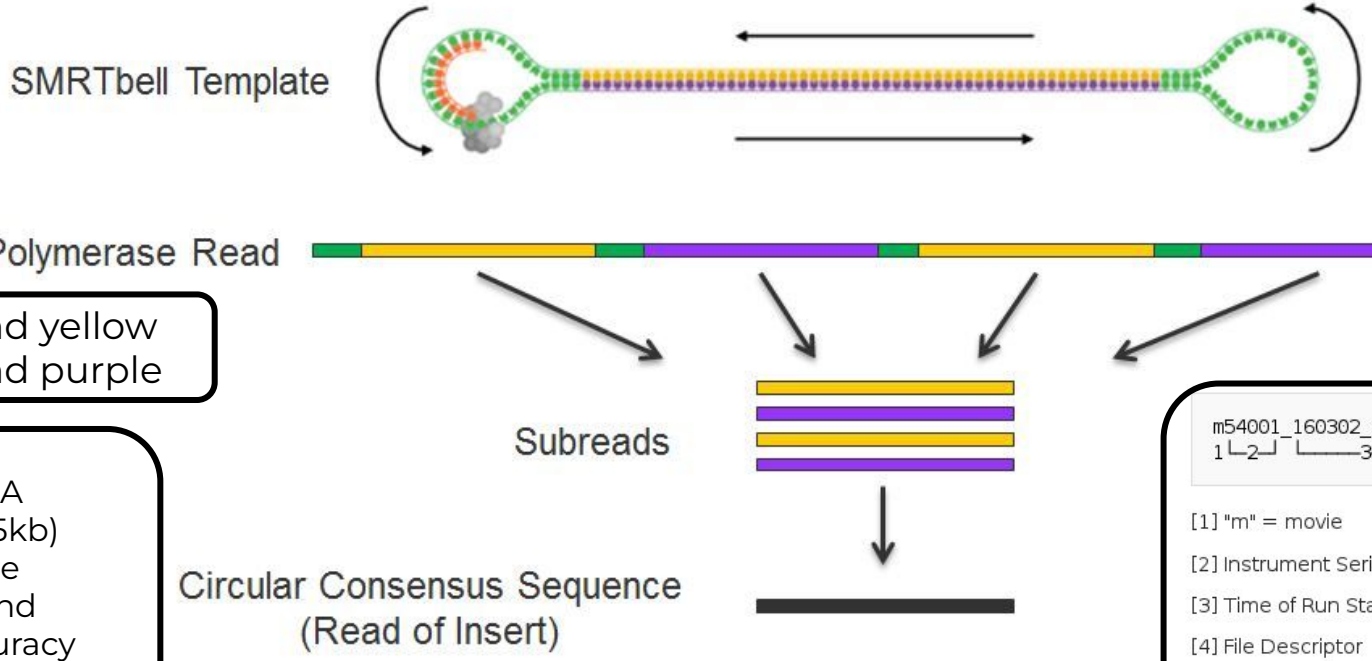
PacBio Long Read Sequencing

Sequel Sequence



pacb.com

PacBio Long Read Sequencing



\+ Strand yellow
\- Strand purple

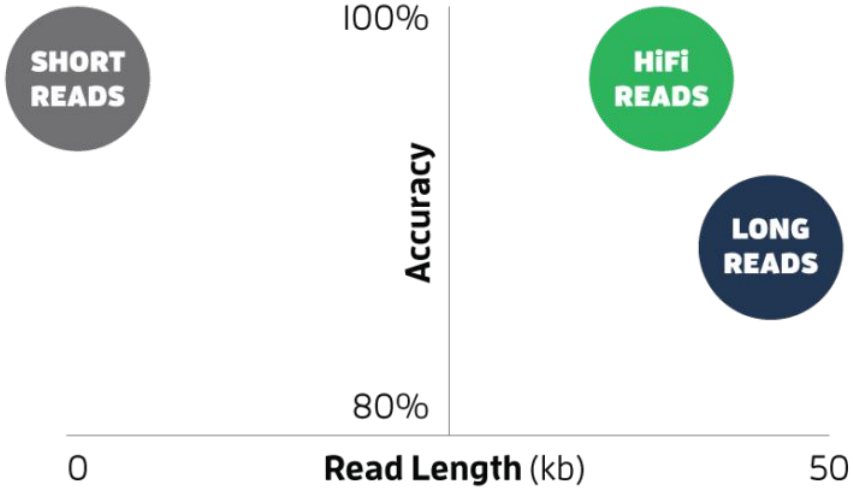
Shorter DNA fragment (5kb) equals more subreads and higher accuracy than longer (60kb)

```
m54001_160302_121501.subreads.bam
1└2┘ └─────3──────┘└──4──┘ └5┘
```

[1] "m" = movie
[2] Instrument Serial Number
[3] Time of Run Start (`yymmdd_hhmmss`)
[4] File Descriptor
[5] File Extension

pacb.com

PacBio HiFi Reads



https://www.pacb.com/blog/hifi-webinar

- HiFi reads are not subreads since they are already error corrected
- HiFi utilize single-molecule consensus rather than multiple-molecule consensus

PacBio Sequencing Tools

- Sequence Alignments
  - Minimap2
- Genome Assembly
  - Canu: PacBio long read assembler
    - grid mode not supported on HPRC clusters
  - Unicycler: bacterial genomes
  - wtdbg2: 10x faster than Canu
    - assembly is very close but not as complete as Canu
  - Flye: PacBio and ONT reads; metagenome also available
- Improve draft assemblies
  - ArrowGrid_HPRC (Terra: retiring May 31, 2024)
  - Purge_Haplotigs (Terra: retiring May 31, 2024)
  - Circlator

https://hprc.tamu.edu/kb/Software/Bioinformatics/PacBio_tools

https://hprc.tamu.edu/software/grace

Tool Suites

- SMRT-Link (PacBio)
  - contains command line versions of additional SMRT Analysis tools
  - PacBio's open-source SMRT Analysis software suite is designed for use with Single Molecule, Real-Time (SMRT) Sequencing data.

bam2fasta	bam2fastq	bamsieve	bax2bam	blasr	ccs	cleric	cromwell
daligne	r	daligner_p	datander	dataset	dazcon	DB2Falcon	DB2fasta
DBdump	DBdust	DBrm	DBshow	DBsplit	DBstats	dexta	falconc
falconcpp	fasta2DB	fuse	gcpp	HPC.daligner	HPC.REPmask		HPC.TANmask
ipdSummary	ipython	ipython2	isoseq3	juliet	julietflow	LA4Falcon	LA4Ice
laa	laagc	LAmerge	LAsort	lima	minimap2	motifMaker	pbalign
pbcromwell	pbdagcon	pbindex	pbmm2	pbservice	pbsv	pbvalidate	ra
REPmask	samtools	sawriter	summarizeModifications		TANmask	undexta	womtool

```
module spider SMRT-Link
```

Genome Hybrid Assemblers (Long-reads + Illumina reads)

- SPAdes
  - subreads as input files
  - no need to correct subreads with short reads prior to assembly
  - uses long reads for gap closure and repeat resolution
- MaSuRCA
  - All long-reads must be in a single fasta file
- Unicycler
  - assembly pipeline for bacterial genomes
  - circularises replicons without the need for a separate tool like Circulator

NGS Tools on Grace

Where to Find NGS Tools

- Search TAMU HPRC Documentation
  - https://hprc.tamu.edu/kb/Software/Bioinformatics
- Type any the following Unix commands to see which tools are already installed on Grace
  - `module spider toolname` (not case sensitive, but read the entire output)
  - `module key assembly` (some modules may be missed because this searches tool descriptions)
- Some tools such as qiime2 are available with Anaconda and do not show up with the module command
- If you are unable to find a tool that you want installed on Grace, send an email with the URL link to: help@hprc.tamu.edu
- Useful websites: long-read-tools.org

Grace Software Toolchains

- search for module names using module spider

```
module spider bowtie2
```

- use module spider with module name for details on how to load module

```
module spider Bowtie2/2.4.2
```

- read output to see which other module(s) to load first

```
module load GCC/10.2.0 Bowtie2/2.4.2
```

show loaded modules

```
module list
```

- see what other modules are compatible with the loaded module(s)

```
module avail samtools
```

- load additional compatible modules

```
module load SAMtools/1.11
```

- unload all loaded modules

```
module purge
```

Compatible Toolchains

- See a short table of compatible toolchains and python versions

toolchains

		GCC version	mpi version	Available Python version
foss/2023b	=	GCC/13.2.0	OpenMPI/4.1.6	Python/3.11.5
foss/2023a	=	GCC/12.3.0	OpenMPI/4.1.5	Python/3.11.3
intel/2023a	=	GCCcore/12.3.0	impi/2021.9.0	Python/3.11.3
foss/2022b	=	GCC/12.2.0	OpenMPI/4.1.4	Python/3.10.8
intel/2022.12	=	GCCcore/12.2.0	impi/2021.8.0	Python/3.10.8
foss/2022	=	GCC/11.3.0	OpenMPI/4.1.4	Python/3.10.4
intel/2022a	=	GCCcore/11.3.0	impi/2021.6.0	Python/3.10.4
foss/2021b	=	GCC/11.2.0	OpenMPI/4.1.1	Python/3.9.6
intel/2022.00	=	GCCcore/11.2.0	impi/2021.5.0	Python/3.9.6
foss/2021a	=	GCC/10.3.0	OpenMPI/4.1.1	Python/3.9.5
intel/2021a	=	GCC/10.3.0	impi/2020.2.0	Python/3.9.5
foss/2020b	=	GCC/10.2.0	OpenMPI/4.0.5	Python/3.8.6
intel/2020b	=	GCC/10.2.0	impi/2019.9.304	Python/3.8.6

- loading foss/2021b will also load GCC/11.2.0, GCCcore/11.2.0 and OpenMPI/4.1.1
- loading GCC/11.2.0 will also load GCCcore/11.2.0 but not foss/2021b

Use $TMPDIR Whenever Possible

- Use the $TMPDIR if the application you are running can utilize a temporary directory for writing temporary files which are deleted when the job ends
- A temp directory (**$TMPDIR**) is automatically assigned for each job which uses the local disk on the compute node–not the /scratch shared file system
  - Especially useful when a computational tool writes tens of thousands of temporary files which are deleted when the job is finished and are not needed for the final results
  - This is useful since files on **$TMPDIR** will not count against your file quota
  - Don't use **$TMPDIR** if your software uses temporary files for restarting where it left off if it should stop before completion
  - Will significantly speed up an mpiBLAST job

```
java -Xmx350g -jar $EBROOTPICARD/FastqToSam.jar TMP_DIR=$TMPDIR \
FASTQ=$pe1_1 FASTQ2=$pe1_2 OUTPUT=$outfile SAMPLE_NAME=$sample_name \
SORT_ORDER=$sort_order MAX_RECORDS_IN_RAM='null'
```

Quality Control (QC)

QC Evaluation

- Use FastQC to visualize quality scores
  - Displays quality score distribution of a subset of ~200,000 reads
    - Input is a fastq file or files
    - Can disable grouping (binning) of sequence regions
  - Will alert you of poor read characteristics
  - Can be run as a GUI or a command line interface

```
module load FastQC/0.11.9-Java-11
```

- FastQC will process using one CPU core per file
  - If there are 10 fastq files to analyze and 4 cores are used, 4 files will start processing and 6 will wait in a fastqc queue
  - If there is only one fastq file to process then using 10 cores does not speed up the process

Digital Normalization for Assembly

- Reduce memory requirements by reducing the number of redundant sequence reads if you have a very high sequencing coverage (> 200x)
- Used for genome and transcriptome assembly, not variant calling or quantitative analysis (ChIP-seq, RNA-seq for expression profiling)
- Trinity 2.4.0+ automatically normalizes reads to a depth of 50x using a modified version of seqtk
- The **`bbnorm.sh`** script in BBMap can normalize reads
  - use reformat.sh to subsample

```
module spider BBMap
```

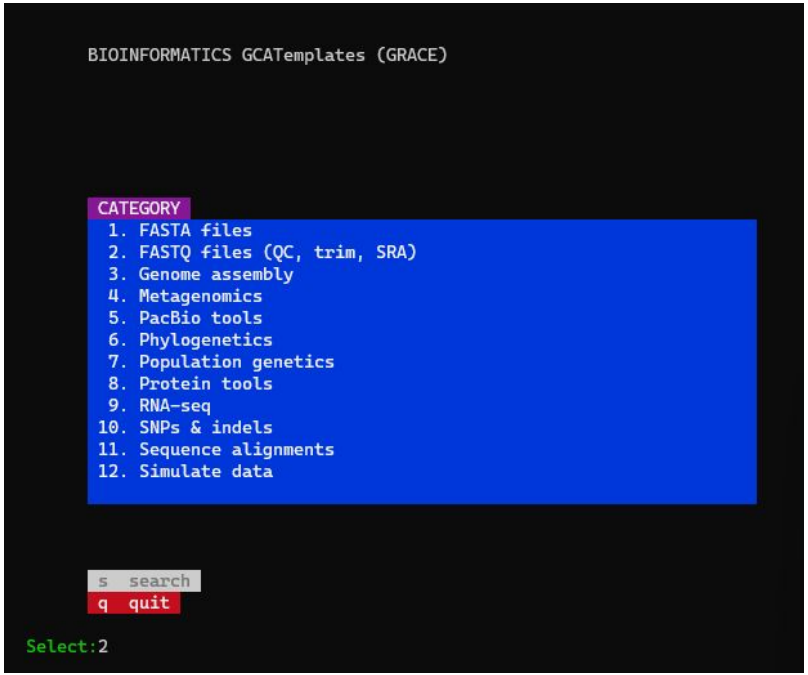
Template Job Scripts

GCATemplates

Genomic Computational Analysis Templates

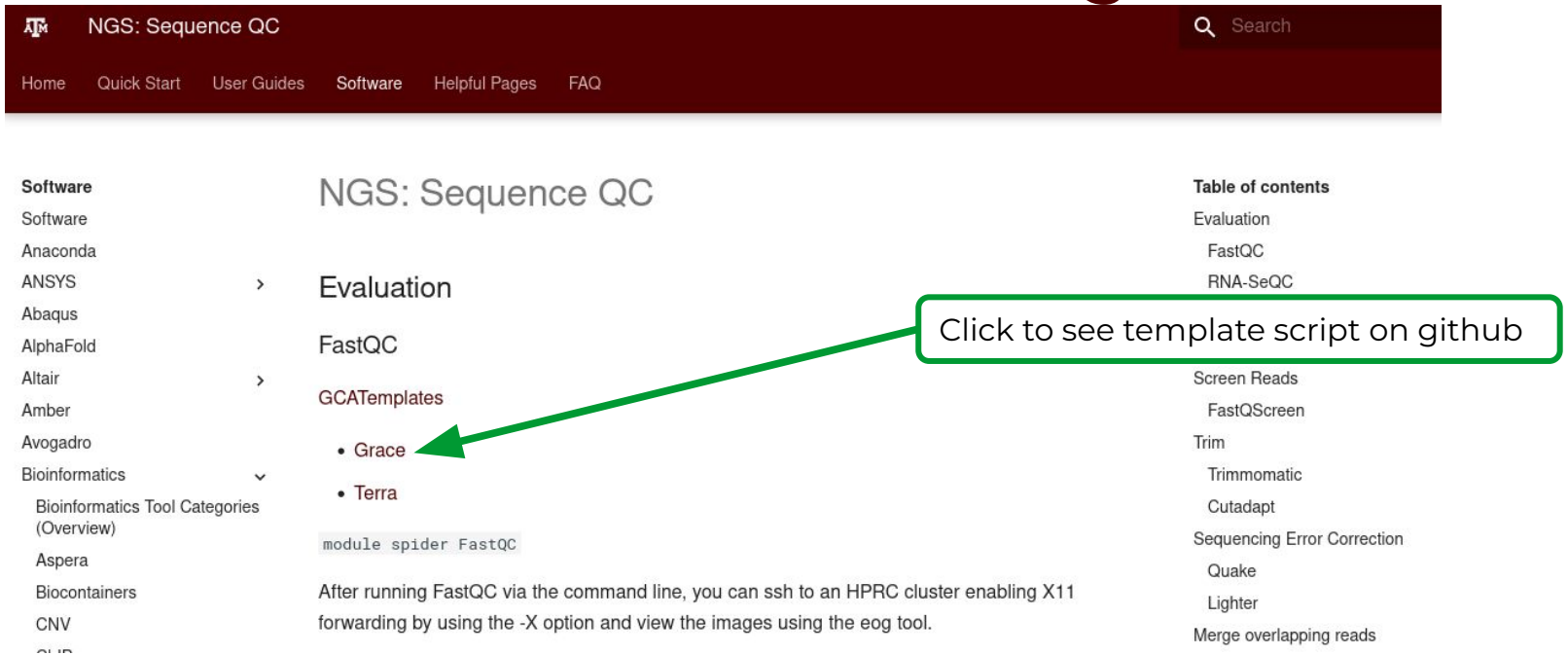
```
gcatemplates
```

- GCATemplates is a collection of template job scripts for each of the HPRC clusters.
- The template scripts are configured with example input files and can be run without changes to get an idea of how the tool and job scheduling work
- Update the template script as needed for larger datasets, different option values or additional options



https://hprc.tamu.edu/kb/Software/useful-tools/GCATemplates

Access GCATemplates Scripts from the HPRC KnowledgeBase



https://hprc.tamu.edu/kb/Software/Bioinformatics/Sequence_QC

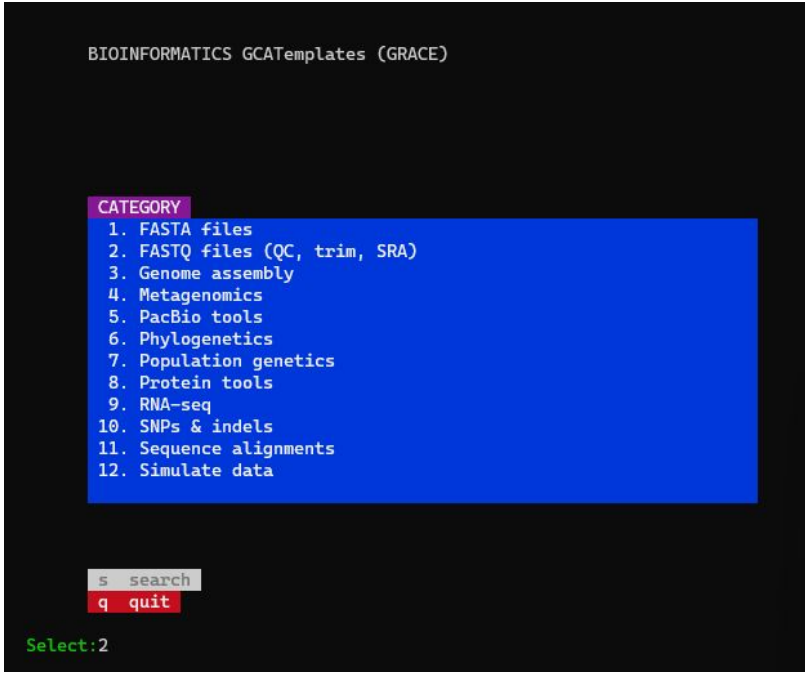
Finding NGS Job Template Scripts Using GCATemplates on HPRC Clusters

```
mkdir $SCRATCH/ngs_class
cd $SCRATCH/ngs_class
gcatemplates
```

For practice, we will copy a template file

- Select #2 then find the template that contains fastqc
  - or use the search function to find fastqc
- Final step will save a template job script file to your current working directory

Genomic Computational Analysis Templates



Sample GCATemplate Job Script (Grace)

```
#!/bin/bash
#SBATCH --job-name=fastqc             # job name
#SBATCH --time=01:00:00               # max job run time dd-hh:mm:ss
#SBATCH --ntasks-per-node=1           # tasks (commands) per compute node
#SBATCH --cpus-per-task=2             # CPUs (threads) per command
#SBATCH --mem=4G                      # total memory per node
#SBATCH --output=stdout.%j            # save stdout to file (%j is jobid)
#SBATCH --error=stderr.%j             # save stderr to file (%j is jobid)

module purge
module load FastQC/0.11.9-Java-11

<<README
        - FASTQC manual: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help
README
################################### VARIABLES ###################################
# TODO Edit these variables as needed:
########## INPUTS ##########
pe1_1='/scratch/data/bio/GCATemplates/data/miseq/c_dubliniensis/DR34_R1.fastq.gz'
pe1_2='/scratch/data/bio/GCATemplates/data/miseq/c_dubliniensis/DR34_R2.fastq.gz'

######## PARAMETERS ########
threads=$SLURM_CPUS_PER_TASK

########## OUTPUTS #########
output_dir='./'

################################### COMMANDS ####################################
fastqc -t $threads -o $output_dir $pe1_1 $pe1_2

<<CITATION
        - Acknowledge TAMU HPRC: https://hprc.tamu.edu/research/citations.html
        - FastQC: http://www.bioinformatics.babraham.ac.uk/projects/fastqc
CITATION
```

Sample GCATemplate Job Script (Grace)

```
#!/bin/bash
#SBATCH --job-name=fastqc              # job name
#SBATCH --time=01:00:00                # max job run time dd-hh:mm:ss
#SBATCH --ntasks-per-node=1            # tasks (commands) per compute node
#SBATCH --cpus-per-task=2              # CPUs (threads) per command
#SBATCH --mem=4G                       # total memory per node
#SBATCH --output=stdout.%j             # save stdout to file (%j is jobid)
#SBATCH --error=stderr.%j              # save stderr to file (%j is jobid)

module purge
module load FastQC/0.11.9-Java-11

<<README
        - FASTQC manual: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help
README
################################### VARIABLES ###################################
# TODO Edit these variables as needed:
########## INPUTS ##########
pe1_1='/scratch/data/bio/GCATemplates/data/miseq/c_dubliniensis/DR34_R1.fastq.gz'
pe1_2='/scratch/data/bio/GCATemplates/data/miseq/c_dubliniensis/DR34_R2.fastq.gz'

######## PARAMETERS ########
threads=$SLURM_CPUS_PER_TASK

########## OUTPUTS #########
output_dir='./'

################################### COMMANDS ####################################
fastqc -t $threads -o $output_dir $pe1_1 $pe1_2

<<CITATION
        - Acknowledge TAMU HPRC: https://hprc.tamu.edu/research/citations.html
        - FastQC: http://www.bioinformatics.babraham.ac.uk/projects/fastqc
CITATION
```

These parameters are read by the job scheduler

Load the required module(s) first

This is a section of comments

This is a single line comment and not run as part of the script

This is the command to run the application

Viewing Maximum Available Resources

The **maxconfig** command will show the recommended Slurm parameters for the maximum available resources (cores, memory, time) per node for a specified accelerator or partition (default Grace partition: long)

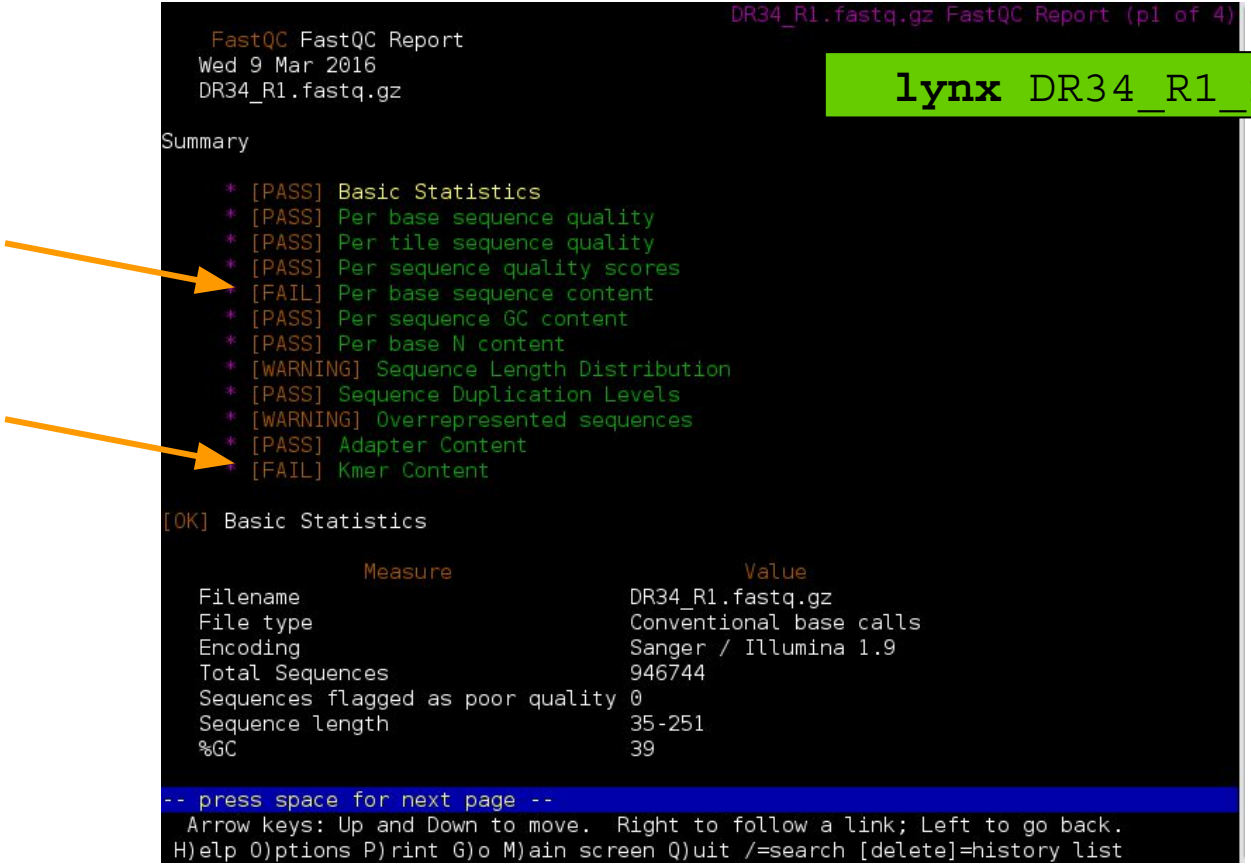
```
[username@grace ~]$ maxconfig

  Grace partitions:   short  medium  long  xlong  vnc  gpu  bigmem  special  gpu-a40
  Grace GPUs in gpu partition:  a100:2  a40:3  rtx:2  t4:4

  Showing max parameters (cores, mem, time) for partition long

  CPU-billing * hours * nodes =   SUs
            48 *   168 *      1 = 8,064

#!/bin/bash
#SBATCH --job-name=my_job
#SBATCH --time=7-00:00:00
#SBATCH --nodes=1              # max 64 nodes for partition long
#SBATCH --ntasks-per-node=1
#SBATCH --cpus-per-task=48
#SBATCH --mem=360G
#SBATCH --output=stdout.%x.%j
#SBATCH --error=stderr.%x.%j
```

FastQC Exercise

- Use the GCATemplate for FastQC to submit a job evaluating the two sequence files
  - `gedit run_fastqc_0.11.9_grace.sh &`
  - `sbatch run_fastqc_0.11.9_grace.sh`
- After your fastqc job is complete, unzip the results file and you can view the results files with the **`lynx`** and **`eog`** Unix commands
  - eog requires X11 login; if using the portal, use the Files app to view images
  - `unzip DR34_R1_fastqc.zip`

FastQC Report Using lynx

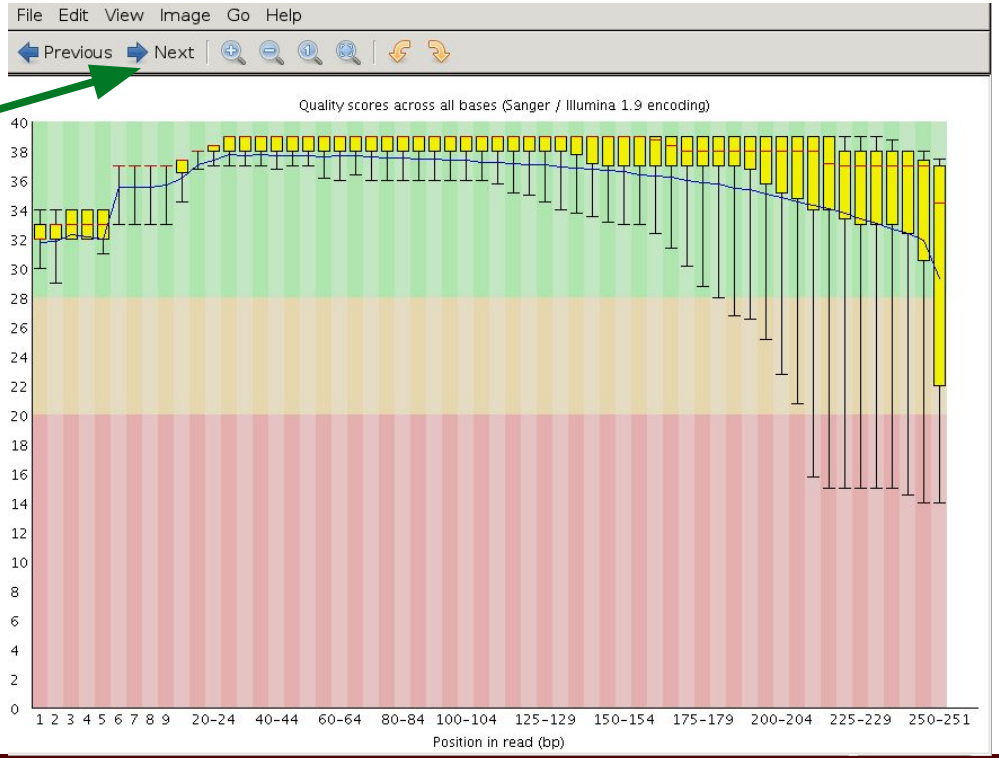
```
lynx DR34_R1_fastqc.html
```



FastQC Output Image Quality Distribution

```
eog DR34_R1_fastqc/Images/per_base_quality.png
```

click for the next image in the same directory, or use the left/right arrow keys

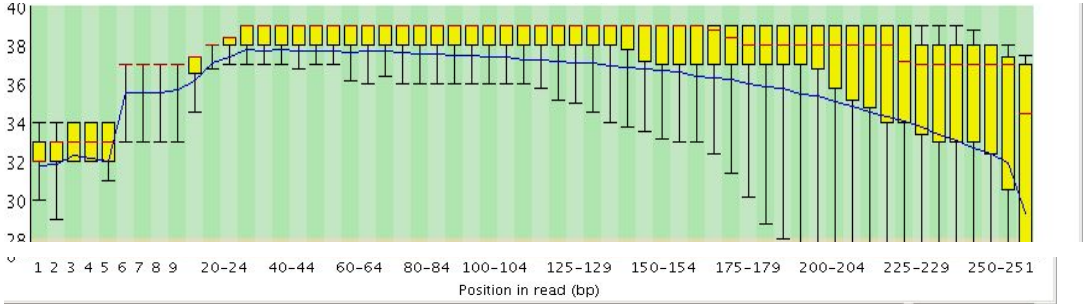


Prior to QC trimming

FastQC Output Image Quality Distribution

FASTQ format

```
@ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAAAAAAACAAAAAACATAATGCCGTAAA
+ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
=:=4AD=B8A:+<A::1<:AE<C3*?F<B???<?:8:6?B*9BD;/638.=-'-.@7=).=A:6?DDDCBBBB9555&&)+((+2&&+(((((((()&&&+
@ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
GATGTTTTGTTACTGATTGGAACCATGATTGGTGCTTTACTTGGTTTCTTCCTATTTAACCACAAGCCTGCCAAAGTATTTATGGGAGATGTAGGTAGTT
+ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
BCCFDEFFHHHHHJJJJIJJJJJJJJJJIJJJFHIJJJIJJJJJIJJJJJJJIJJJIJIIJJJJJFHJJJJIJJHH=CHHFFFFFFEDDEDEEEDCCDCE
@ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
TATTTTAAGTGACCAAGGAATGACTCCCCAATCATGGCTGTATCAACTCCAAAATTTTCTGCAACAGTCGCTGAAATATCTGCAAAATGCCCTTGTGGAA
+ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
CCCFFFFFHHHHHJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJIJJJHIJHHGFFFFFFFEEEEEEDDDDDDDDDCDDDD
```

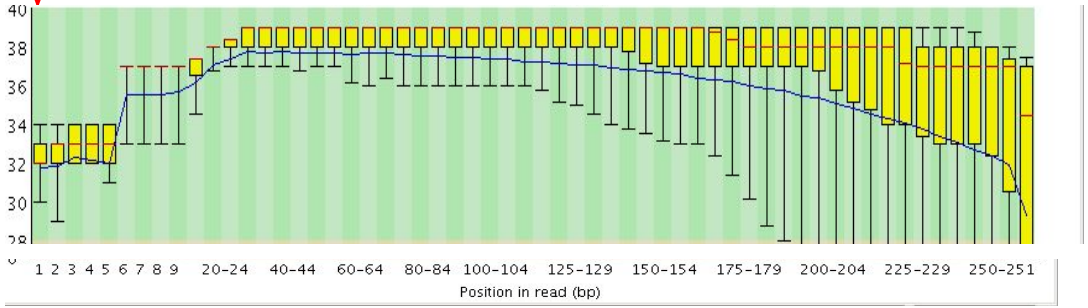


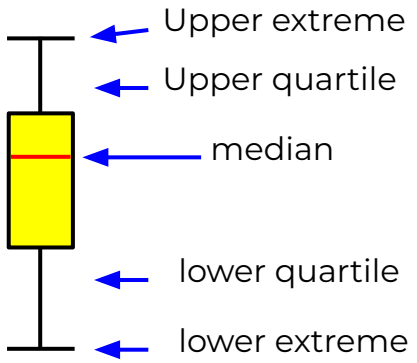
FastQC Output Image Quality Distribution

FASTQ format

```
@ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAAAAAAACAAAAAACATAATGCCGTAAA
+ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
=:=4AD=B8A:+<A::1<:AE<C3*?F<B???<?:8:6?B*9BD;/638.=-'-.@7=).=A:6?DDDCBBBB9555&&)+((+2&&+(((((((()&&&+
@ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
GATGTTTTGTTACTGATTGGAACCATGATTGGTGCTTTACTTGGTTTCTTCCTATTTAACCACAAGCCTGCCAAAGTATTTATGGGAGATGTAGGTAGTT
+ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
BCCFDEFFHHHHHJJJJIJJJJJJJJJJIJJJFHIJJJIJJJJJIJJJJJJJIJJJIJIIJJJJJFHJJJJIJJHH=CHHFFFFFFEDDEDEEEDCCDCE
@ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
TATTTTAAGTGACCAAGGAATGACTCCCCAATCATGGCTGTATCAACTCCAAAATTTTCTGCAACAGTCGCTGAAATATCTGCAAAATGCCCTTGTGGAA
+ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
CCCFFFFFHHHHHJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJIJJJHIJHHGFFFFFFFEEEEEEDDDDDDDDDCDDDD
```

Average quality score distribution at position one



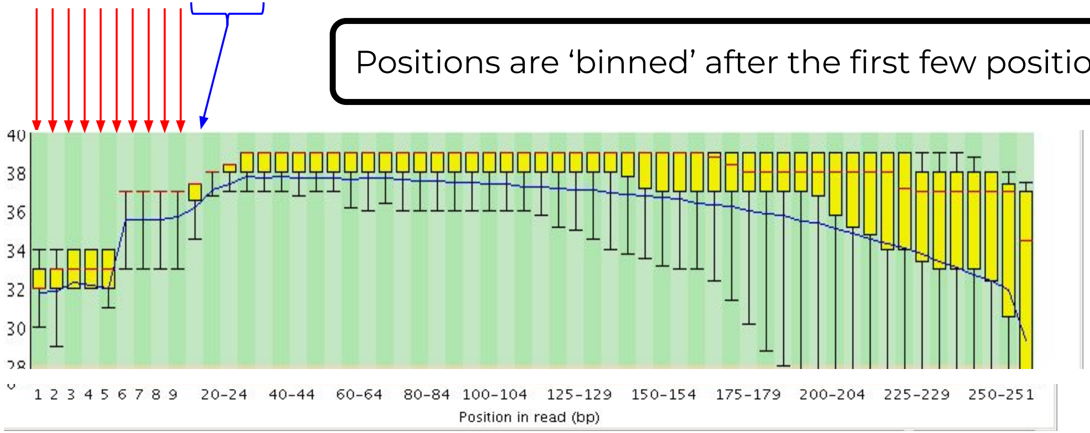


FastQC Output Image Quality Distribution

FASTQ format

```
@ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAAAAAACAAAAAACATAATGCCGTAAA
+ERR504787.2.1 M00368:15:000000000-A0HKH:1:5:21261:10968-1 length=100
=:=4AD=B8A:+<A::1<:AE<C3*?F<B???<?:8:6?B*9BD;/638.=-'-.@7=).=A:6?DDDCBBBB9555&&)+((+2&&+((((((()&&&+
@ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
GATGTTTTGTTACTGATTGGAACCATGATTGGTGCTTTACTTGGTTTCTTCCTATTTAACCACAAGCCTGCCAAAGTATTTATGGGAGATGTAGGTAGTT
+ERR504787.3.1 M00368:15:000000000-A0HKH:1:3:12724:25677-1 length=100
BCCFDEFFHHHHHJJJJIJJJJJJJJJJIJJJFHIJJJIJJJJJIJJJJJJJIJJJIJIIJJJJJFHJJJJIJJHH=CHHFFFFFFEDDEDEEEDCCDCE
@ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
TATTTTAAGTGACCAAGGAATGACTCCCCAATCATGGCTGTATCAACTCCAAAATTTTCTGCAACAGTCGCTGAAATATCTGCAAAATGCCCTTGTGGAA
+ERR504787.5.1 M00368:15:000000000-A0HKH:1:2:16161:12630-1 length=100
CCCFFFFFHHHHHJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJIJJJHIJHHGFFFFFFFEEEEEEDDDDDDDDDCDDDD
```

Positions are ‘binned’ after the first few positions

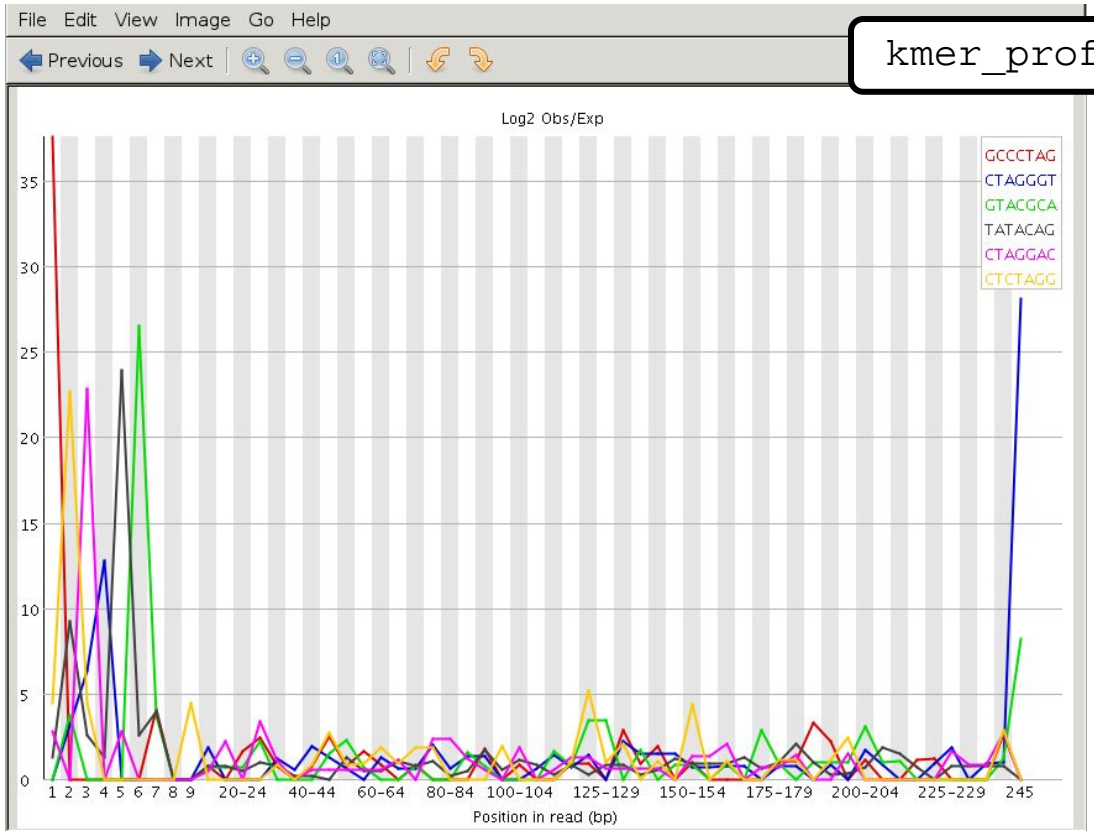


Illumina Transposon Insertion Site

per_base_sequence_content.png

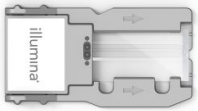
Illumina Transposon Insertion Site

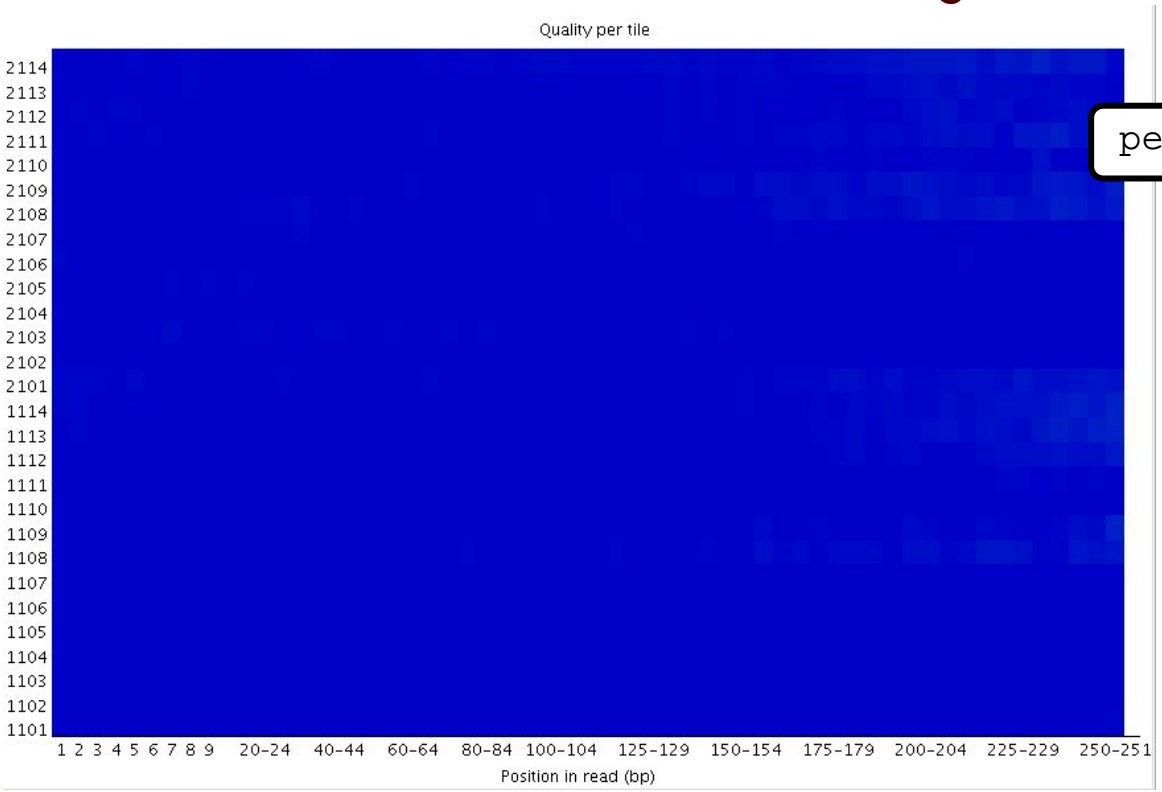
kmer_profiles.png



FastQC Flowcell Quality Image

MiSeq flowcell





good quality — poor quality

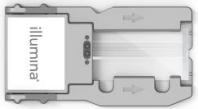
`per_tile_quality.png`

Flowcell quality mapping
Good per_tile quality

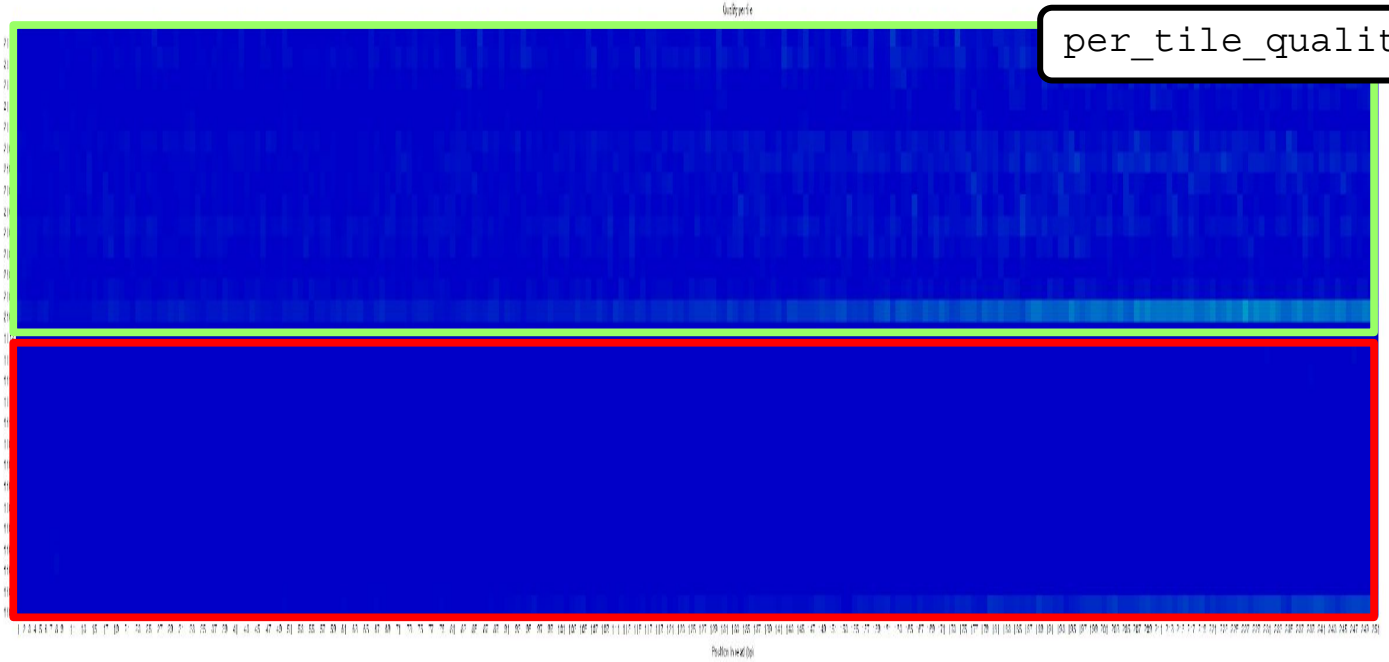
FastQC Flowcell Quality Image

bottom of flowcell

MiSeq flowcell



top of flowcell



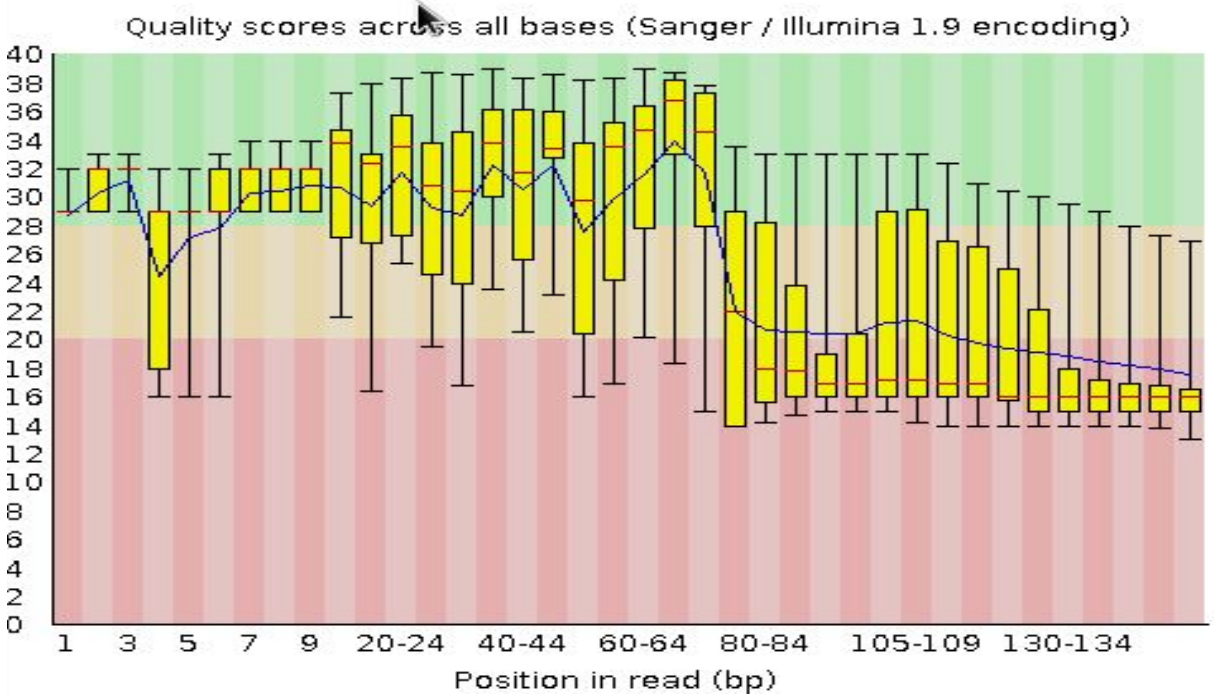
per_tile_quality.png

good quality — poor quality

Failed QC Examples

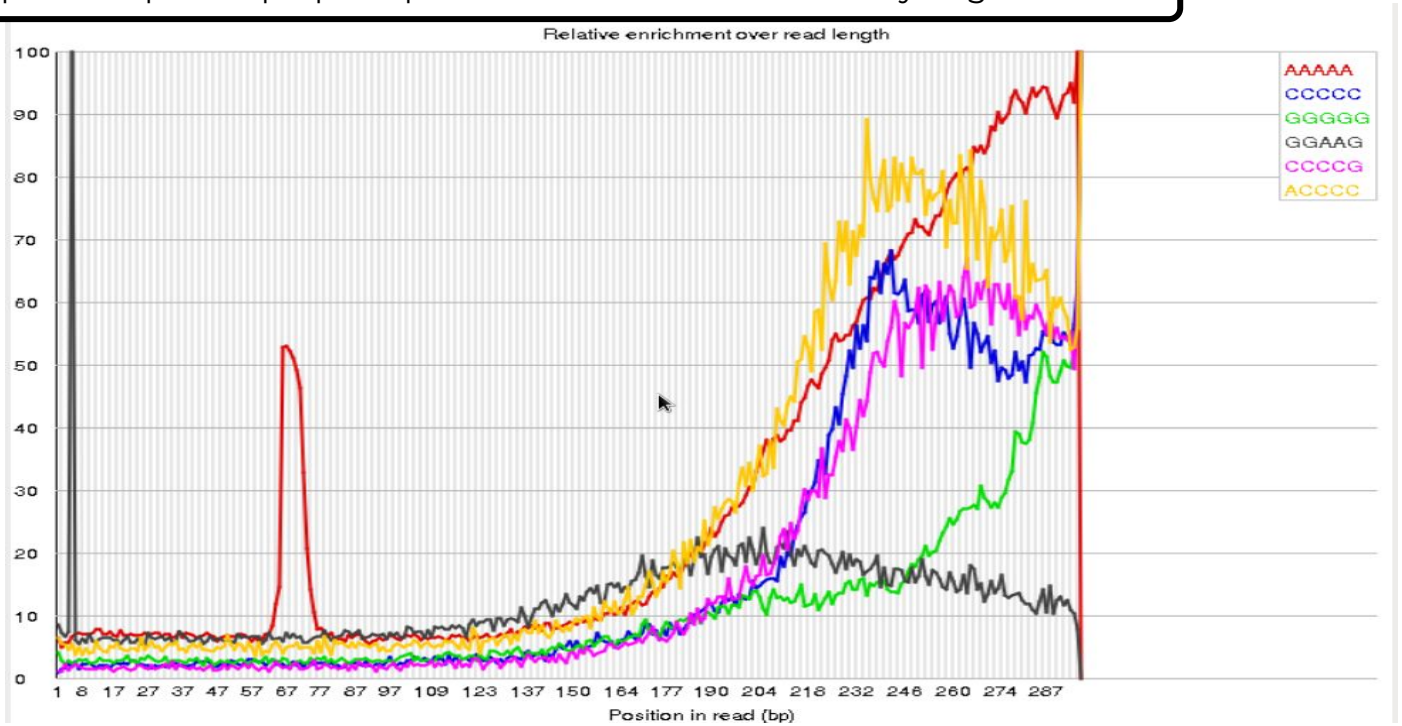
FastQC Output Image
Failed Per Base Sequence Quality

Example 1. Expired MiSeq mate-pair kit (9 months expired)



FastQC Output Image
Failed Kmer Content

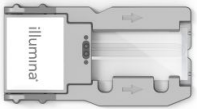
Example 2. Sequence prep adapters still on ends of DNA library fragments

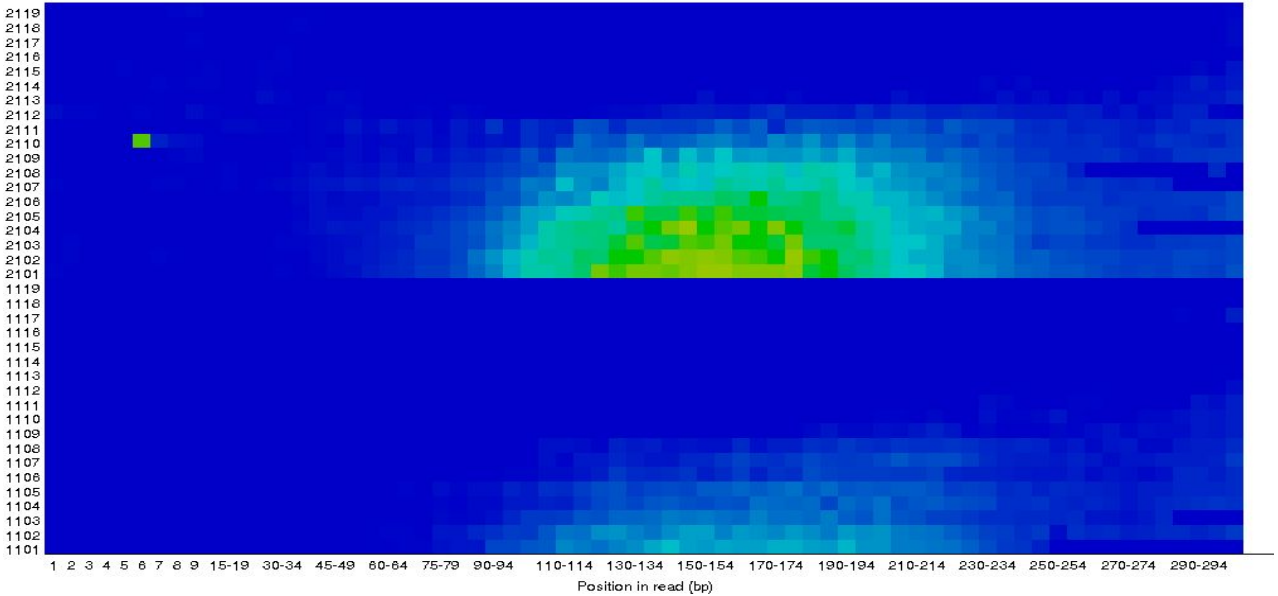


FastQC Output Image
Flowcell: Not Good per_tile Quality

Example 3. Faulty flowcell

MiSeq flowcell

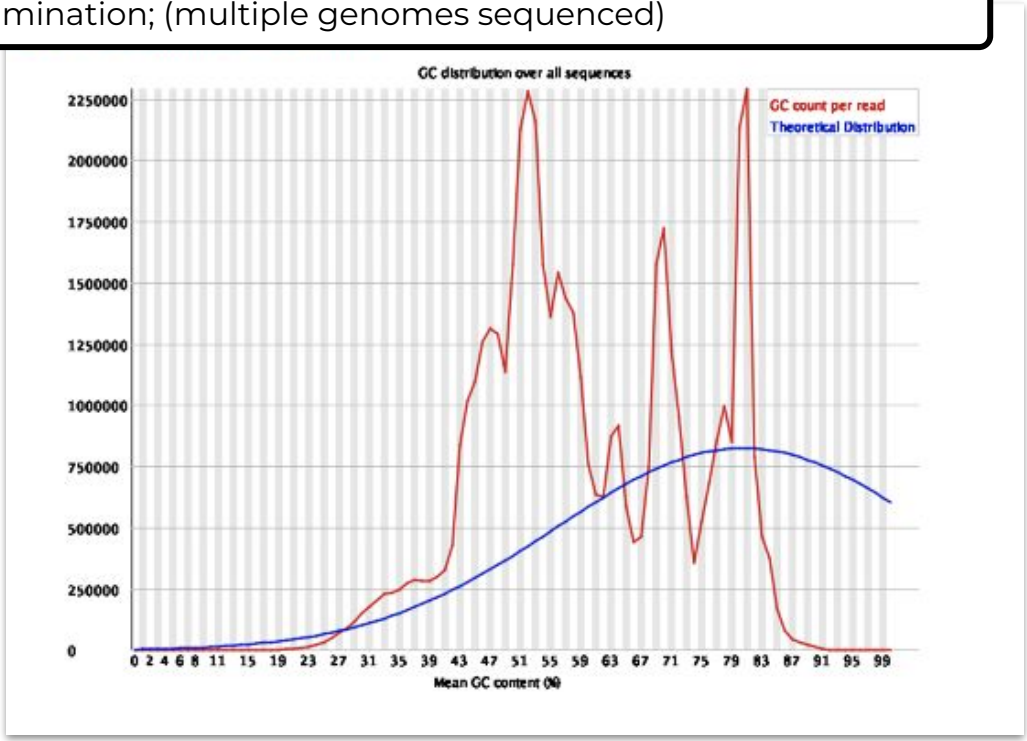




good quality — poor quality

FastQC Output Image
Failed Per Sequence GC Content

Example 4. Contamination; (multiple genomes sequenced)



QC Quality Trimming

- Sequence quality trimming tools

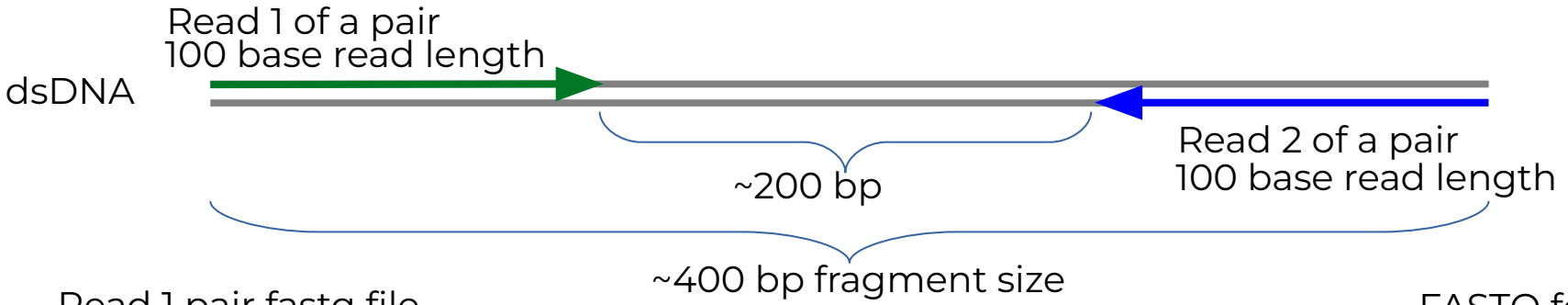
```
module spider Trimmomatic
```

- Trimmomatic will maintain paired end read pairing after trimming
- Trim reads based on quality scores
  - Trim the same number of bases from each read or
  - Use a sliding window to calculate average quality at ends of sequences
- Decide if you want to discard reads with Ns
  - some assemblers replace Ns with As or a random base G, C, A or T
- Trim adapter sequences
  - Trimmomatic has a file of Illumina adapter sequences

```
module load Trimmomatic/0.39-Java-11
```

```
ls $EBROOTTRIMMOMATIC/adapters/
```

Paired End Short Reads



Read 1 pair fastq file

FASTQ format

```
@M00861:1:000000000-A36BE:1:1101:14650:1529 1:N:0:8
TTCTTAAAAATACCATAAAAGGCTTAAACTTGCCATTTACGACGGATTAATTCCAACTCTTTTCGGCTATCTTCATCTTTTAAGGTTAAATGACTCATAACGG
+
FFFHBFFHHIIIIIIHFHHCGEFGHHIHHHIHD/?DGGHHH@DEB,5EGHGHHIIHIF?FGGHHCCBFDGHFHDGHGFFFGDFHH?DFHDFFHHFHFHFFHHH
```

Read 2 pair fastq file

```
@M00861:1:000000000-A36BE:1:1101:14650:1529 2:N:0:8
ACTAAAAATCAATTTTATCAATTTCAAGCTCTACCTTATTTACTCATTATTTTAGTGATGGCCACTTTAATAAAAATATTGGTAGCATATTTTGCAATAGCGG
+
BFFHIHHHFHHDGHIHHIHHHGHHHHHFHHDFFHIHIIIHIHDFHHHIHIIIH=AAFHIIIHFGFHHHHHGGHHIHHFGFFFEGGHHHDGHHH/CGHIFFHHH
```

Trimming PE Short Sequence Reads

File 1 from sequencer

100 bases

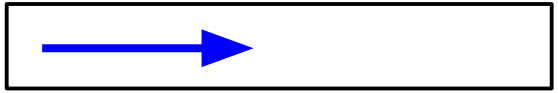
QC trim

100 bases

File 2 from sequencer

100 bases

QC trim



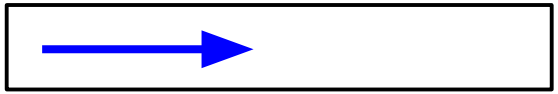
50 bases

minimun read length = 40

Resulting FASTQ Files with trimmed reads

Paired end 1 trimmed file

Paired end 2 trimmed file



Trimming PE Short Sequence Reads

File 1 from sequencer

100 bases

QC trim

100 bases

File 2 from sequencer

100 bases

QC trim

20 bases

minimun read length = 40

Resulting FASTQ Files with trimmed reads

Paired end 1 trimmed file

Paired end 2 trimmed file

Single end reads

Merge Overlapping Paired End Short Reads

fragment 1

dsDNA

---

fragment 2

dsDNA

Merge Overlapping Paired End Short Reads

fragment 1

dsDNA

---

fragment 2

dsDNA

Paired end read 1 (left)

Paired end read 2 (right)

Merge Overlapping Paired End Short Reads

fragment 1

dsDNA

fragment 2

dsDNA

Paired end read 1 (left)

Paired end read 2 (right)

Unpaired 'merged' read

Tools for merging overlapping reads:

```
module spider FLASH
```

```
module spider BBMap
```

```
module spider PEAR
```

Mapping Reads to a Reference Assembly

Mapping Short Reads to a Reference Assembly

- Align reads using bwa

```
module spider BWA
```

- Align reads using bowtie or bowtie2

```
module spider Bowtie
```

```
module spider Bowtie2
```

- genome index files for found here:

```
/scratch/data/bio/genome_indexes/
```

Send an email to help@hprc.tamu.edu if you need and index that is not found in the genome_indexes directory

Visualize bam Alignment Files

Sample bam and Reference Files

```
cd $SCRATCH/ngs_class
```

For this samtools demo, add symbolic links* to the example files in your working directory

```
ln -s /scratch/data/bio/training/alignments/dr34.sam
```

Add a symbolic link to the example reference genome fasta file

```
ln -s /scratch/data/bio/training/genomes/c_dubliniensis.fa
```

Use the tab key when typing these long paths

* The symbolic links are used to make the commands shorter for demonstration purposes only. You do not need to make symbolic links in order to use **`samtools tview`**

Sorting Alignment sam/bam Files

- Sequence Alignment/Map format (sam)
  - view sam files using the UNIX command:

```
more dr34.sam
```

- Binary Alignment/Map format (bam)
  - Compressed (binary) sam files need samtools to view
    -

```
module load GCC/10.2.0 SAMtools/1.11
```

  - Recommended: sort sam/bam file based on coordinate into bam format
  -

```
samtools sort -@ 1 -m 2G -o dr34.bam dr34.sam
```

  - Create an index of the bam file using samtools
    - A samtools index is needed prior to viewing bam files in browsers

```
samtools index dr34.bam
```

```
dr34.bam.bai
```

Viewing sam/bam Files

Viewing bam files using samtools

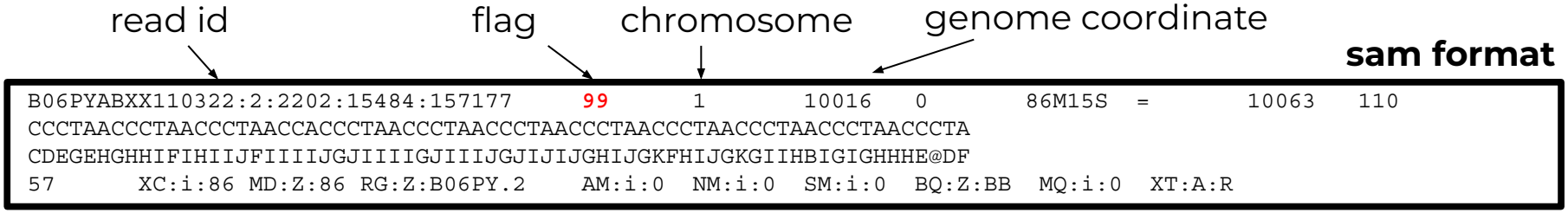
```
samtools view dr34.bam | more
```
view only alignments

```
samtools view -H dr34.bam
```
view only header

```
samtools view -h dr34.bam | more
```
view header + alignments

Sam Flags and Bits

- Flags describe alignments (the flag value is the sum of bits)



bits: **1** **2** 4 8 16 **32** **64** 128 256 512 1024 2048

1 + 2 + 32 + 64 = 99

- Filter bam alignments based on bit in flag (-f and/or -F)
  - Keep only reads that are 'mapped in proper pair'

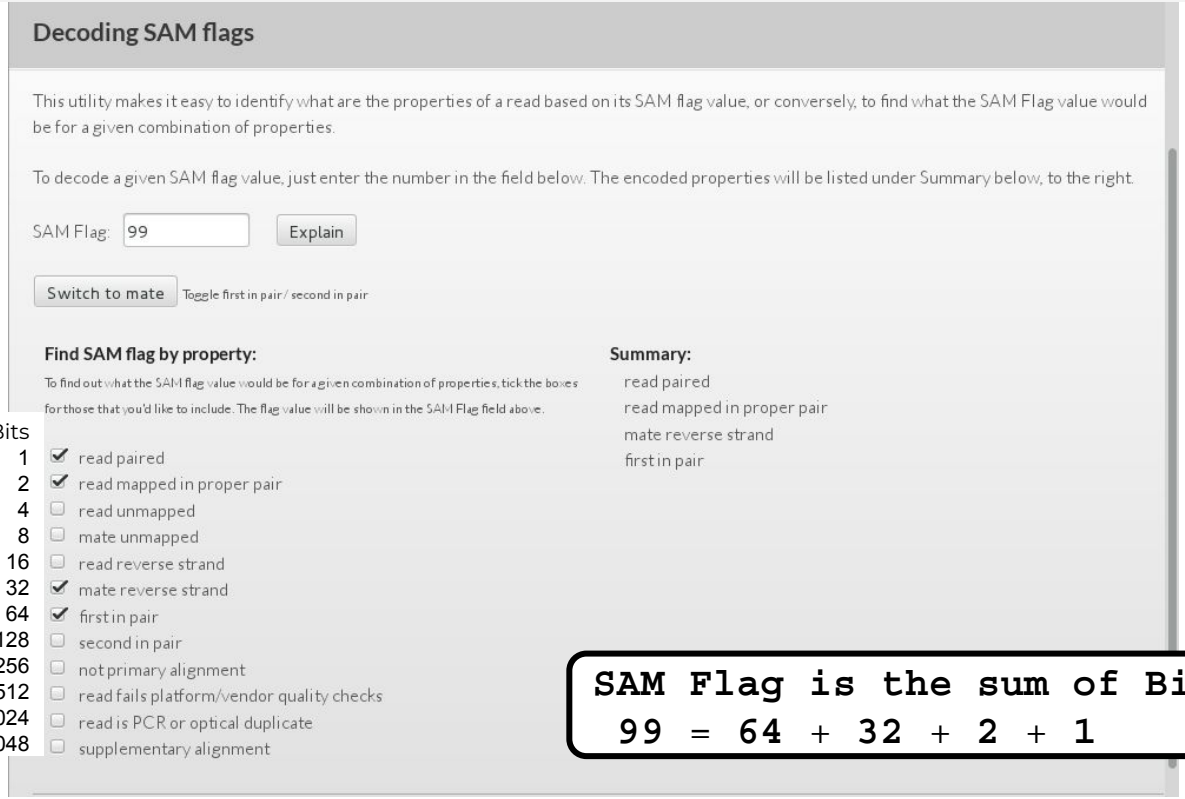
```
samtools view -h -b -f 2 dr34.bam > dr34_paired_reads.bam
```

  - Keep all except reads that are 'PCR or optical duplicate'

```
samtools view -h -b -F 1024 dr34.bam > dr34_dedup_reads.bam
```

Sam Flags and Bits

https://broadinstitute.github.io/picard/explain-flags.html



SAM Flag is the sum of Bits

99 = 64 + 32 + 2 + 1

Alignment Statistics

samtools flagstat dr34.bam

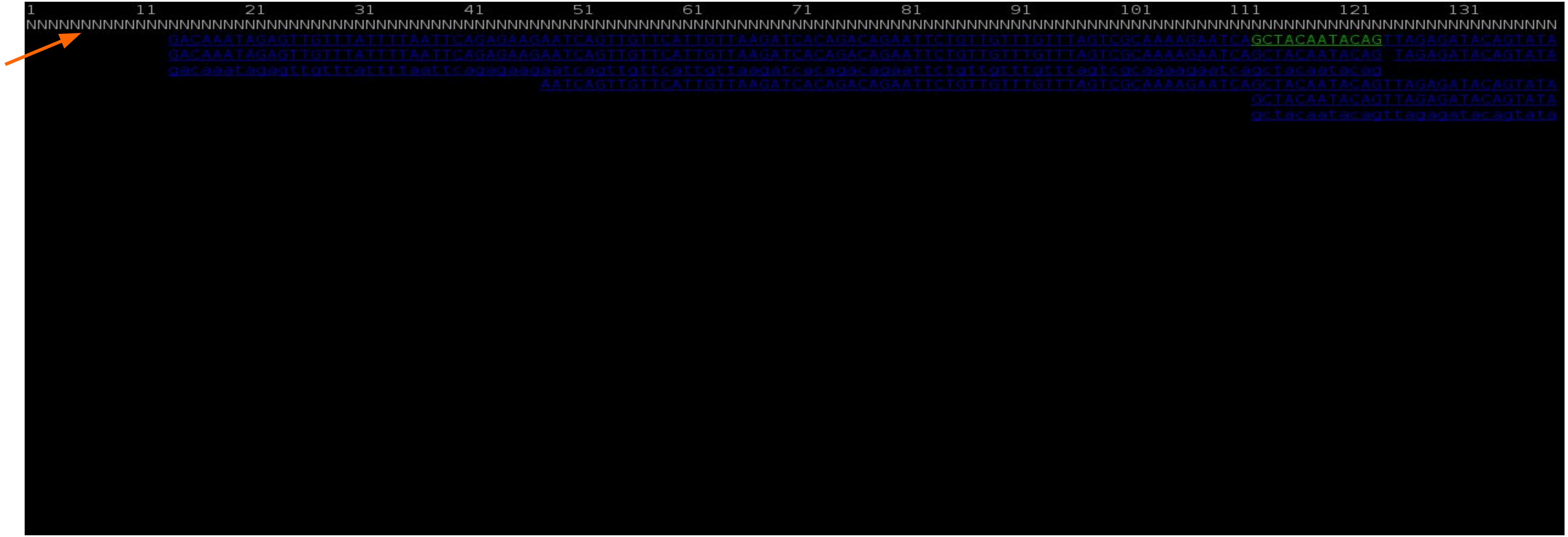
```
150000 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
140150 + 0 mapped (93.43% : N/A)
150000 + 0 paired in sequencing
75002 + 0 read1
74998 + 0 read2
85639 + 0 properly paired (57.09% : N/A)
136854 + 0 with itself and mate mapped
3296 + 0 singletons (2.20% : N/A)
909 + 0 with mate mapped to a different chr
56 + 0 with mate mapped to a different chr (mapQ>=5)
```

Both reads in the pair are mapped on the same chromosome and in FR or RF orientation

SAMtools Without a Reference Genome

Reference genome represented on top as NNNNNNNN since no reference genome was p

```
samtools tview dr34.bam
```



SAMtools with a Reference Genome

Reference genome sequence displayed on top when reference file is provided

```
samtools tview dr34.bam c_dubliniensis.fa
```

SAMtools with a Reference Genome

Type ? for help menu

```
samtools tview dr34.bam c_dubliniensis.fa
```

View at a Specific Coordinate

```
samtools tview dr34.bam c_dubliniensis.fa -p 1:315398
```

homozygous SNP at coordinate 1:315398

. = matches the forward strand
, = matches the reverse strand
A = does not match reference; read on forward strand
a = does not match reference; read on reverse strand
_ = secondary alignment

Sequence Variant Calling

Sequence Variant Calling

- Start with aligning reads to a reference
  - GATK does not require QC trimming
  - Mark PCR duplicates with Picard
- Differentiate between sequencing errors and SNPs
  - Calling SNPs may require a min read depth of 10x (higher for indels)
  - Calling variants may require 1/3 of reads to contain SNP
  - Strand bias may result as a consequence of the sequencing chemistry's response to certain DNA sequence motifs but it can be detected computationally
- BLAST reads with SNPs to identify variant calls due to misalignments especially with duplicated genes
- Variant Call Format (vcf) – standard format of variant calls
- Identify multiple-nucleotide polymorphism (MNP)
  - Two SNPs within a single codon

	codon	translation
Reference:	**TTT**	Phe
SNP 1:	**TTA**	Leu
SNP 2:	**TAT**	Tyr
SNP 1 + 2:	**TAA**	**STOP**

Marking PCR Duplicates

- PCR duplicates are artifacts resulting from a PCR amplification step during NGS library preparations.
- PCR duplicates should be removed/marked as to not bias the frequency of variants or gene expression levels
  - Use picard tools to mark duplicates
  - freebayes will ignore marked duplicates during variant calling

```
module spider picard
```

Variant Calling Tools

Use bam file of sequence reads aligned to a reference as input for the following four work flows

1. GATK

```
module spider GATK picard SAMtools
```

   - No need to QC trim reads–the GATK best practices pipeline will perform the necessary steps, including marking PCR duplicates
   - You need a set of known variants for your species (dbSNP), or you can bootstrap your population to get variant frequency
   - Used in conjunction with other tools
     - samtools
     - picard

2. SAMtools and BCFtools

```
module spider SAMtools BCFtools
```

3. freebayes

```
module spider freebayes
```

4. VarScan

```
module spider VarScan
```

Sample vcf File Format

```
##fileformat=VCFv4.0
##fileDate=20110705
##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF  ALT     QUAL FILTER INFO                              FORMAT      Sample1         Sample2
2      4370    rs6057    G    A       29   .      NS=2;DP=13;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:52,51  1|0:48:8:51,51
2      7330    .         T    A       3    q10    NS=5;DP=12;AF=0.017               GT:GQ:DP:HQ 0|0:46:3:58,50  0|1:3:5:65,3
2      110696  rs6055    A    G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27  2|1:2:0:18,2
2      130237  .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60  0|0:48:4:56,51
2      134567  microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                   GT:GQ:DP    0/1:35:4        0/2:17:2
```

3 more columns not shown due to width of rows

vcf File Column Descriptions

```
##fileformat=VCFv4.0
##fileDate=20110705
##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF  ALT     QUAL FILTER INFO                              FORMAT      Sample1        Sample2
2      4370    rs6057    G    A       29   .      NS=2;DP=13;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:52,51 1|0:48:8:51,51
2      7330    .         T    A       3    q10    NS=5;DP=12;AF=0.017               GT:GQ:DP:HQ 0|0:46:3:58,50 0|1:3:5:65,3
2      110696  rs6055    A    G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2
2      130237  .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:56,51
2      134567  microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                   GT:GQ:DP    0/1:35:4       0/2:17:2
```

variants that are phased are inherited together (paternal)

| indicates phased variants

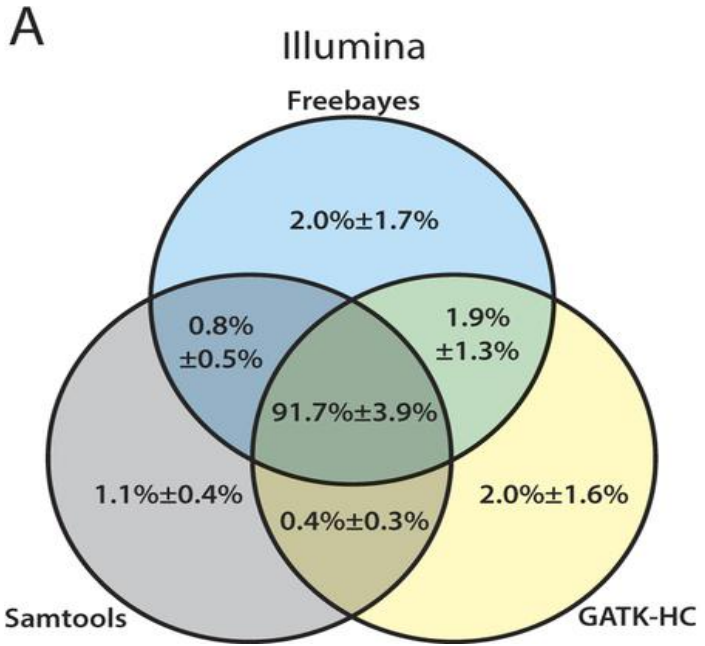
/ indicates non-phased variants

INFO section is for all samples combined

Sample1 haplotypes: **GTGT** and **GTTT**

Sample2 haplotypes: **ATTT** and **GAGT**

Summarizing Variant Calls from Different Tools



The mean percentage with standard deviation of confidence variant calls with equal to or higher than the quality score threshold of 20 are represented for (A) Illumina data sets

Huang et al 2015 doi:10.1038/srep17875

Consequence of Amino Acid Change

- Assess consequence of amino acid change based on sequence conservation across multiple species using the PROVEAN tool
- Variants with a score equal to or below -2.5 are considered “deleterious”

```
module spider PROVEAN
```

```
## PROVEAN v1.1 output ##
# Query sequence file:  CTRG_00013.fa
# Variation file:   CTRG_00013.var
# Protein database: /scratch/datasets/blast/nr
[16:01:13] searching related sequences...
[16:16:36] clustering subject sequences...
# Number of clusters:   30
# Number of supporting sequences used: 245
[16:18:39] computing delta alignment scores...
## PROVEAN scores ##
# VARIATION SCORE
A431S   -0.455
E411K   -3.051
E226Q   -1.564
```

Verify that enough supporting sequences were found

“deleterious”

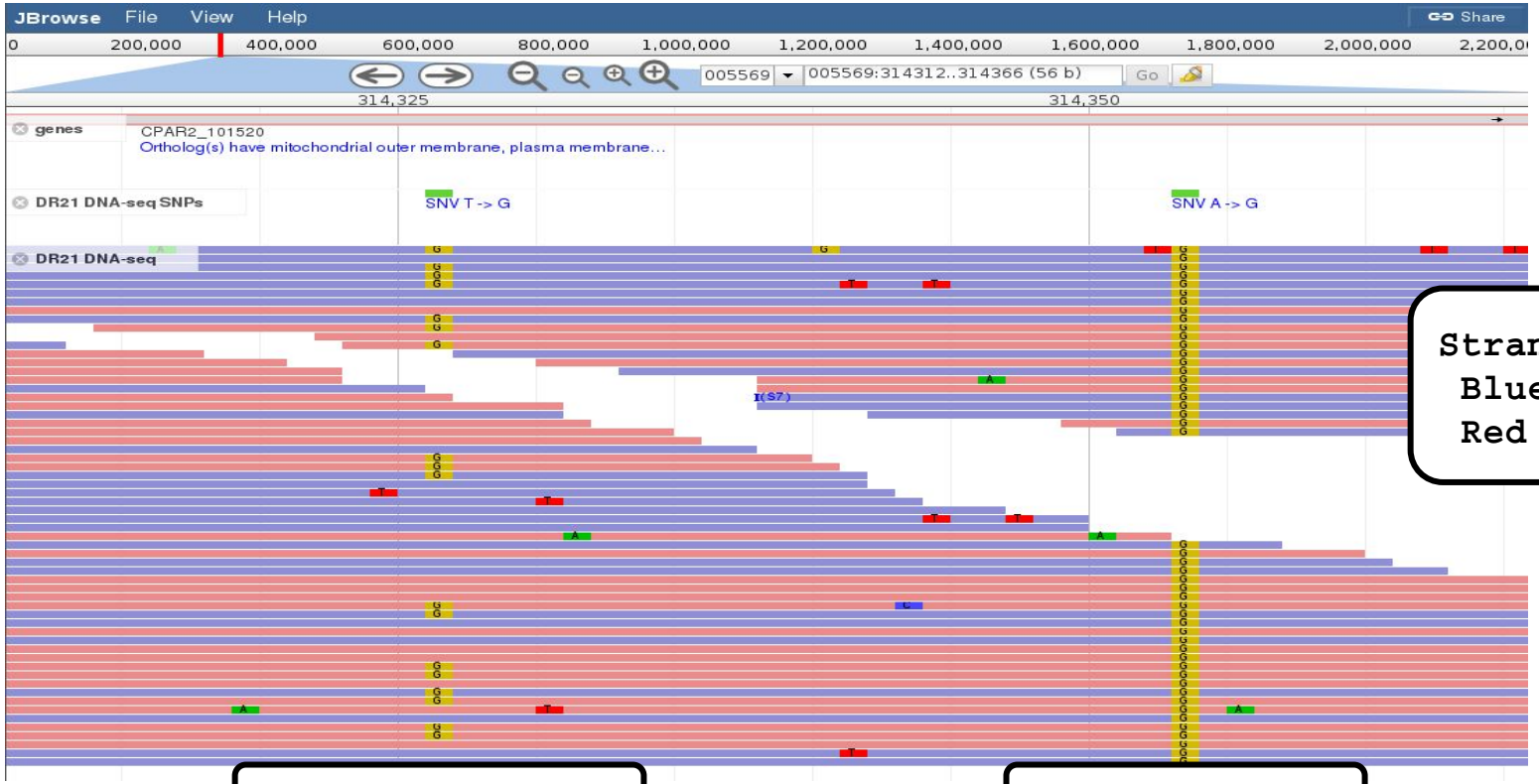
Annotate Variants

```
module spider snpEff
```

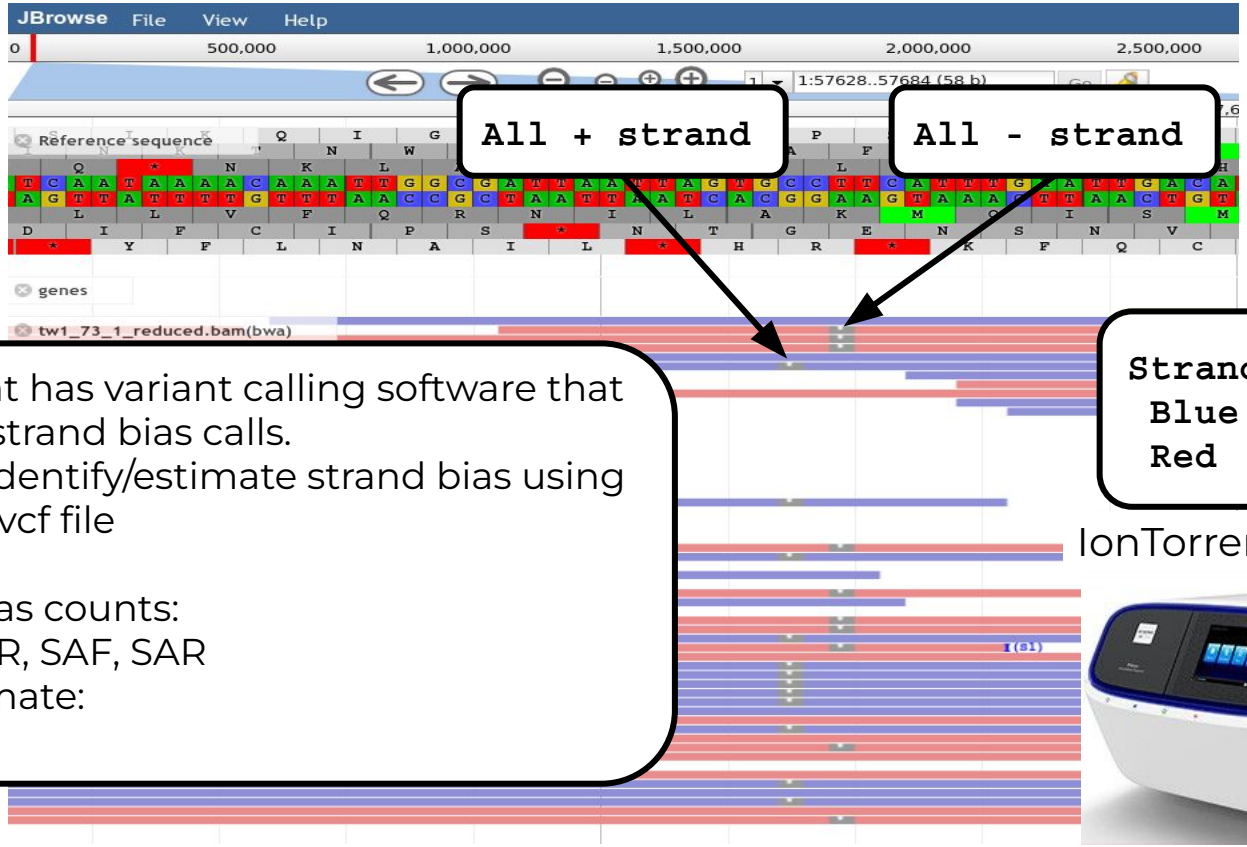
- A file of variant calls in vcf format is needed
- A reference sequence with gene annotations is needed
- snpEff annotates a vcf file
  - There are > 2,500 pre-built databases available and you can build your own if needed
  - Annotates MNP (multiple nucleotide polymorphism)
    - Codon change due to two SNPs: ACA → GGA

```
5       325795  .       AC      GG      23.8901 .
AB=0.428571;ABP=3.32051;AC=1;AF=0.5;AN=2;AO=3;CIGAR=2X;DP=7;DPB=7;DPRA=0;EPP=3.73412;
EPPR=3.0103;GTI=0;LEN=2;MEANALT=1;MQM=33;MQMR=48.5;NS=1;NUMALT=1;ODDS=5.49681;PAIRED=0;
PAIREDR=0.5;PAO=0;PQA=0;PQR=0;PRO=0;QA=114;QR=150;RO=4;RPL=3;RPP=9.52472;RPPR=3.0103;
RPR=0;RUN=1;SAF=2;SAP=3.73412;SAR=1;SRF=2;SRP=3.0103;SRR=2;TYPE=mnp;technology.ILLUMINA=1;
ANN=GG|missense_variant|MODERATE|CD36_51230|CD36_51230|transcript|CAX41505.1|
protein_coding|1/1|c.1657_1658delACinsGG|p.Thr553Gly|1657/1851|1657/1851|553/616||
GT:DP:RO:QR:AO:QA:GL    0/1:7:4:150:3:114:-6.7054,0,-11.1847
```

Viewing SNPs in a Diploid Organism



Example of Sequencing Strand Bias



IonTorrent has variant calling software that can skip strand bias calls.
You can identify/estimate strand bias using values in vcf file

Strand bias counts:
  SRF, SRR, SAF, SAR
Bias estimate:
  SAP

Strand:
Blue = +
Red = -

IonTorrent Proton

RNA-seq Overview

RNA-seq Applications

- Differential Expression (DE) and transcript abundance
  - HISAT2, STAR, TopHat, Cufflinks, Cuffmerge, Cuffdiff
  - DESeq and DESeq2 (R package)
  - EdgeR (R package)
- Transcriptome assembly (find isoforms and rare transcripts)
  - *de novo* (Trinity, SOAPdenovo-Trans)
  - reference based (Trinity, StringTie)
- Genome Annotation
  - Align to assembly for validation of gene models
- Variant Calling
  - STAR/Picard/GATK (Haplotype Caller (HC) in RNA-seq mode)
- *de novo* genome assembly scaffolding
  - L_RNA_scaffolder
- Identify fusion transcripts
  - tophat-fusion

Sequence Depth for RNA-seq Differential Expression

RNA-seq differential expression studies: more sequence or more replication?
Liu, Yuwen, Zhou, Jie and White, Kevin P. Bioinformatics. 2014 Feb 1; 30(3): 301–304.
doi: 10.1093/bioinformatics/btt688 PMCID: PMC3904521

- Using more biological replicates instead of increasing sequencing depth resulted in improved accuracy of expression estimation
- Use more biological replicates at lower sequencing depth is more beneficial than fewer samples at a higher sequencing depth
- Increasing sequence depth is beneficial for exon or transcript-specific expression studies

RNA-seq Transcriptome Assembly

- Assembly with a reference genome

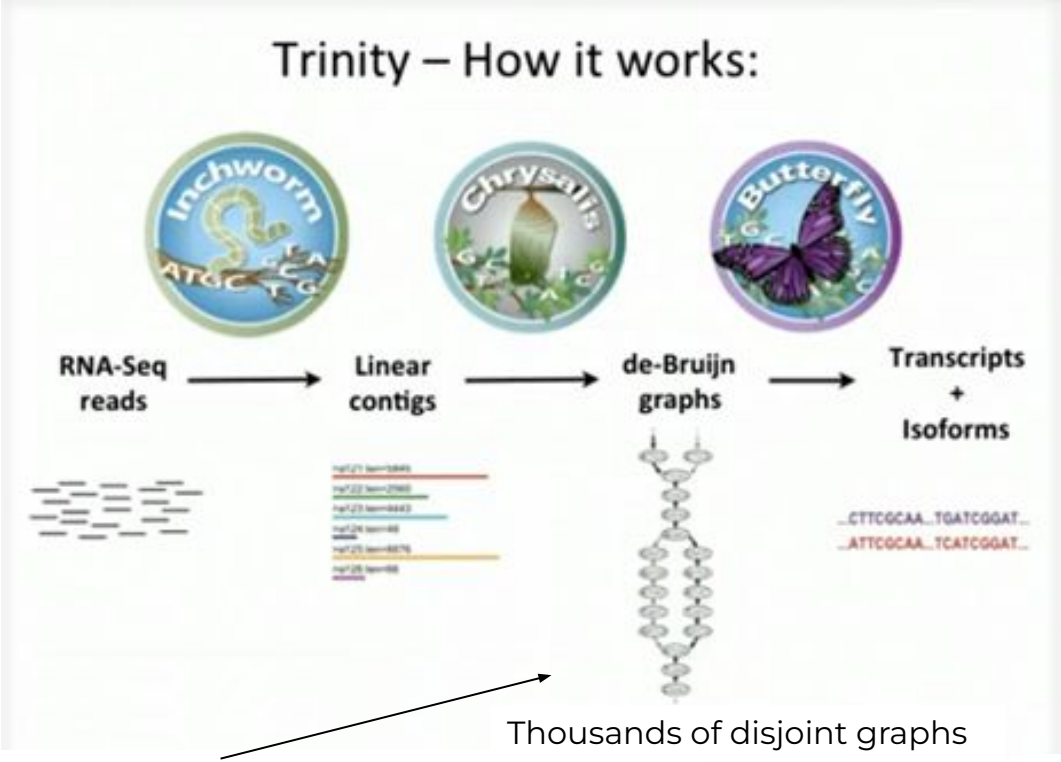
```
module spider Trinity
```

```
module spider HISAT2 Cufflinks
```

```
module spider StringTie
```

- *de novo a*ssembly without a reference genome

```
module spider Trinity
```



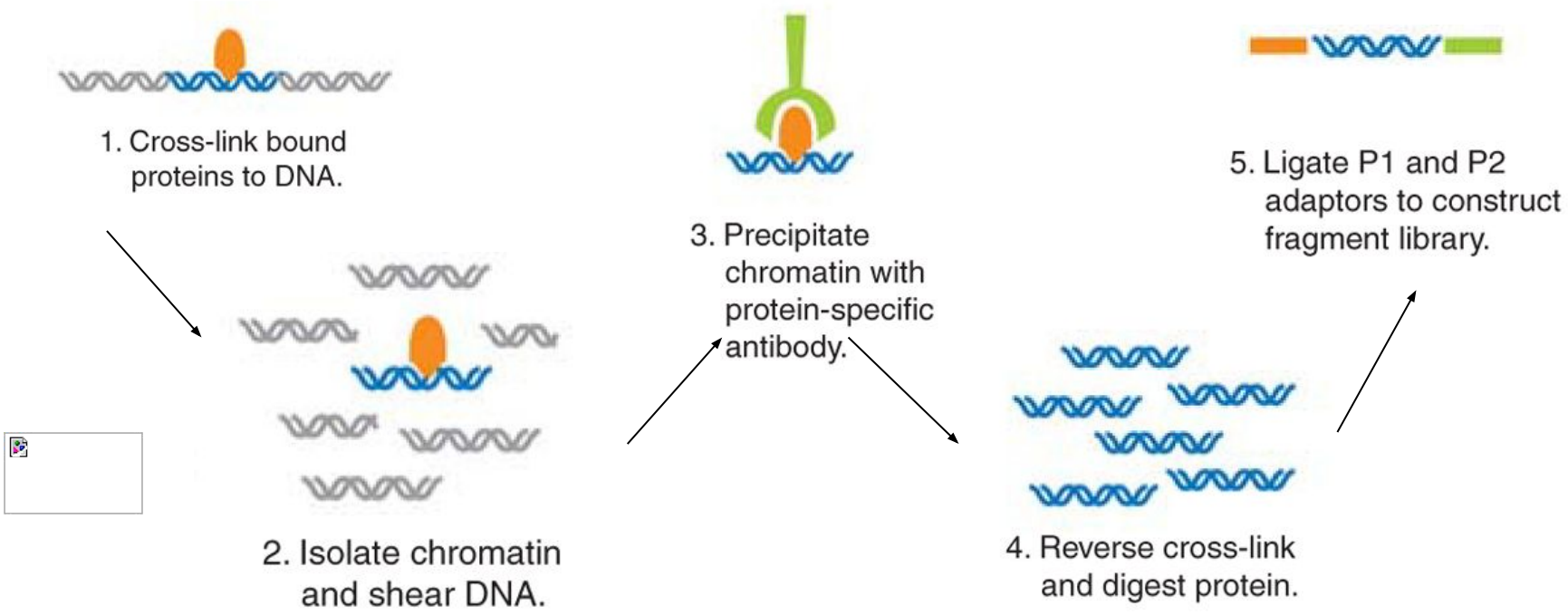
https://www.broadinstitute.org/videos/introduction-de-novo-rna-seq-assembly-using-trinity

Running Trinity on Grace

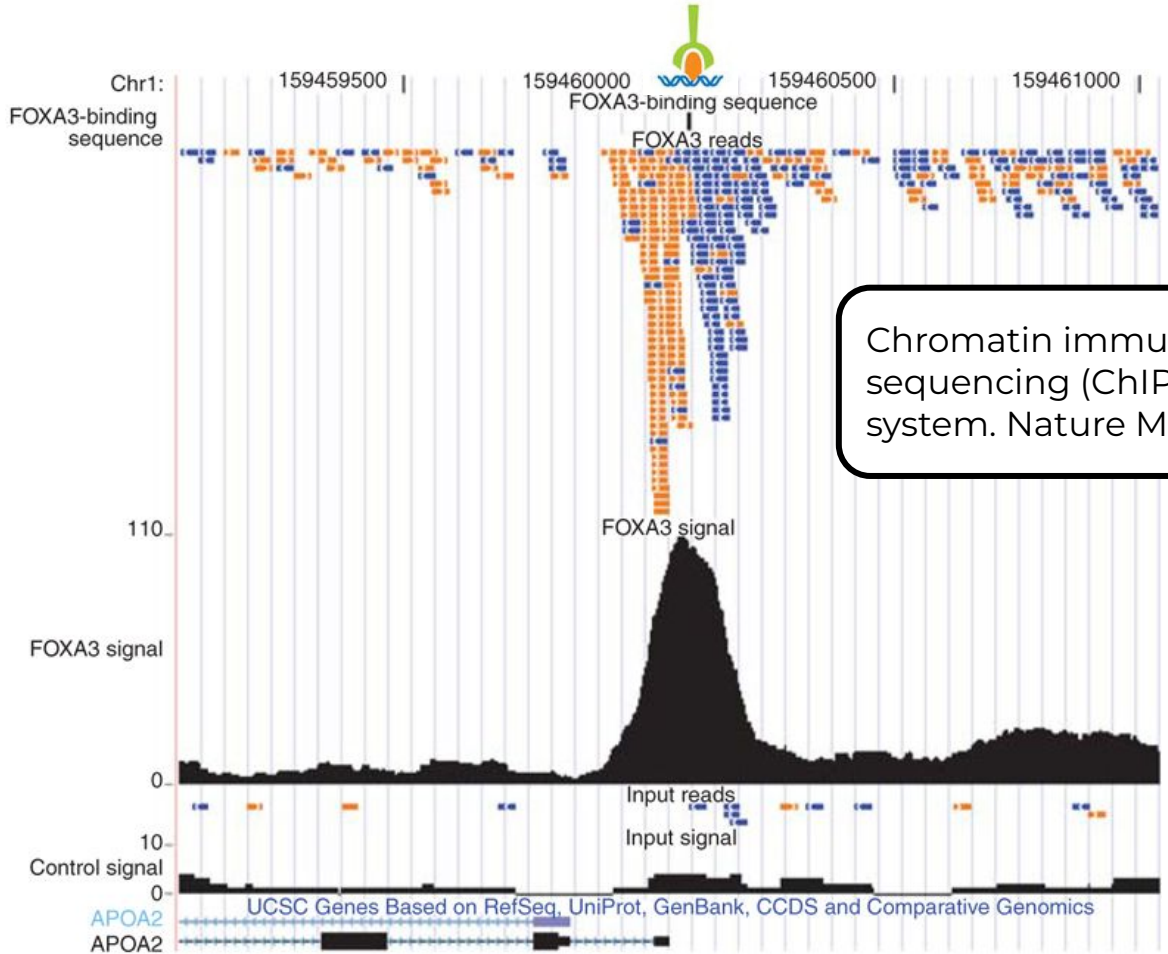
- Trinity creates 100,000s of intermediate files
  - Contact *help@hprc.tamu.edu* and request a file quota increase before running Trinity or use the **`$TMPDIR`** in your job script
  - Run one Trinity job at a time and check resource usage
    - **`showquota`**
    - It is recommended not to run multiple Trinity jobs unless you are using **`$TMPDIR`**
  - Trinity creates checkpoints and can be restarted if it stops due to file/disk quota met, out of memory or runtime
    - Checkpoints are not available when running Trinity in Galaxy
    - Checkpoints are not available if you use **`$TMPDIR`** with Trinity
      - need to rsync results from **`$TMPDIR`** at end of job script
      - checkpoints are stored in **`$TMPDIR`** which is deleted after the job ends
- See GCATemplates for sample Trinity scripts

ChIP-seq

Chromatin immunoprecipitation (ChIP) is a technique for identifying and characterizing elements in protein-DNA interactions involved in gene regulation or chromatin organization.

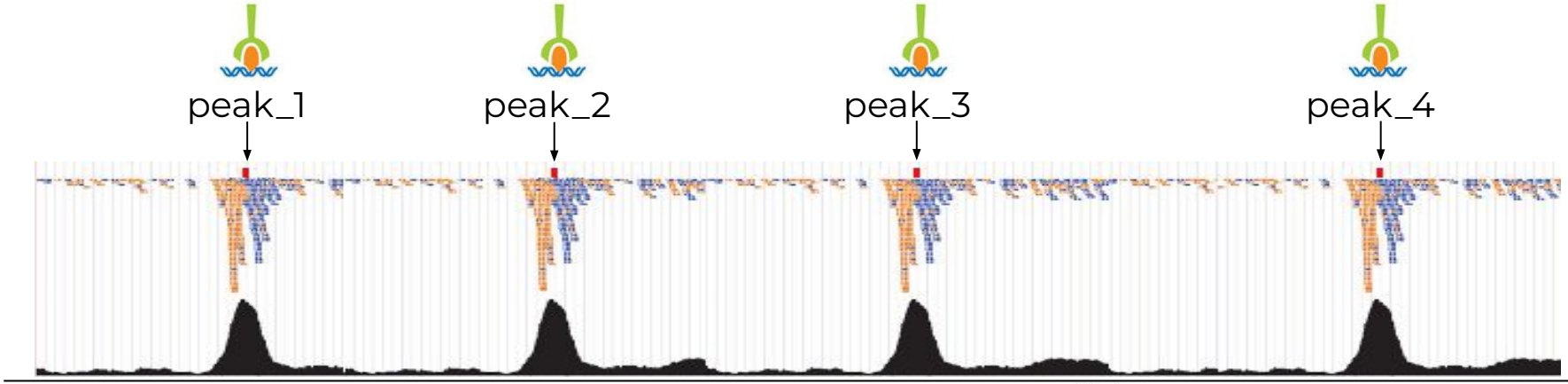


Chromatin immunoprecipitation sequencing (ChIP-Seq) on the SOLiD™ system. Nature Methods 6, (2009)



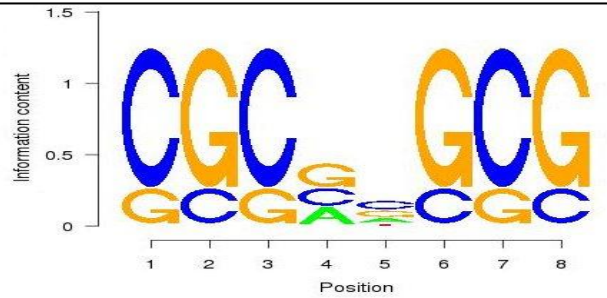
Chromatin immunoprecipitation sequencing (ChIP-Seq) on the SOLiD™ system. Nature Methods 6, (2009)

The goal is to find a consensus DNA sequence among the sequences at each peak which will give us the DNA sequence motif that a protein recognizes and binds



A sequence logo can be used to represent the DNA sequence motif where the protein binds

Generate a sequence logo with the R package seqLogo

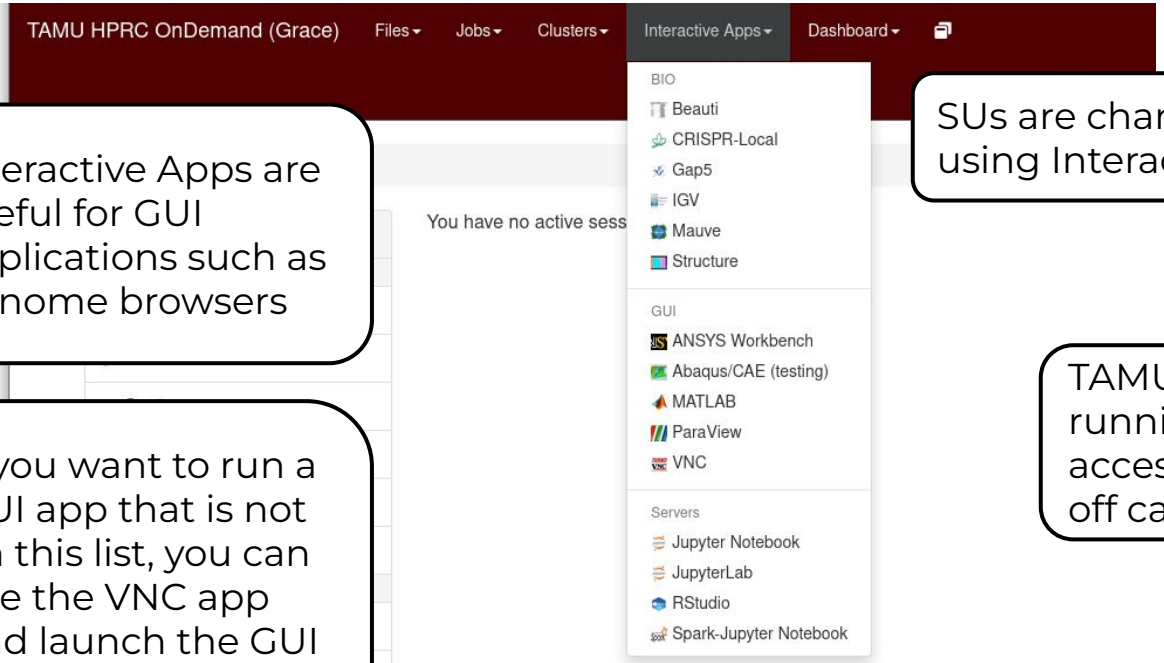



```
module load iccifort/2020.4.304  impi/2019.9.304  R_tamu/4.1.0
```

ChIP-seq Tools

- Protein-DNA interactions
  - `module spider MACS2`
- Background signal differentiation
  - `module spider deepTools`
- Identify enriched domains from histone modification ChIP-seq data
  - `module spider SICER2`

HPRC Portal Interactive Apps



Interactive Apps are useful for GUI applications such as genome browsers

SUs are charged for using Interactive Apps

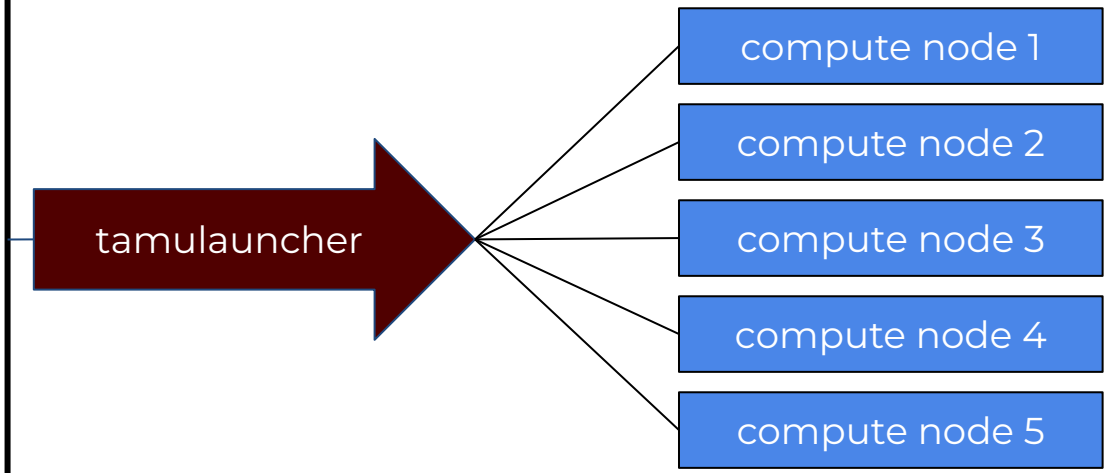
If you want to run a GUI app that is not on this list, you can use the VNC app and launch the GUI from the VNC terminal

TAMU VPN must be running in order to access the portal from off campus

portal-grace.hprc.tamu.edu

TAMULauncher

```
blastn -query chunk0000.fa -db 'nt.bacteria'  -task megablast -out chunk0000.fa.out -outfmt 6
blastn -query chunk0001.fa -db 'nt.bacteria'  -task megablast -out chunk0001.fa.out -outfmt 6
blastn -query chunk0002.fa -db 'nt.bacteria'  -task megablast -out chunk0002.fa.out -outfmt 6
blastn -query chunk0003.fa -db 'nt.bacteria'  -task megablast -out chunk0003.fa.out -outfmt 6
blastn -query chunk0004.fa -db 'nt.bacteria'  -task megablast -out chunk0004.fa.out -outfmt 6
blastn -query chunk0005.fa -db 'nt.bacteria'  -task megablast -out chunk0005.fa.out -outfmt 6
blastn -query chunk0006.fa -db 'nt.bacteria'  -task megablast -out chunk0006.fa.out -outfmt 6
blastn -query chunk0007.fa -db 'nt.bacteria'  -task megablast -out chunk0007.fa.out -outfmt 6
blastn -query chunk0008.fa -db 'nt.bacteria'  -task megablast -out chunk0008.fa.out -outfmt 6
blastn -query chunk0009.fa -db 'nt.bacteria'  -task megablast -out chunk0009.fa.out -outfmt 6
blastn -query chunk0010.fa -db 'nt.bacteria'  -task megablast -out chunk0010.fa.out -outfmt 6
blastn -query chunk0011.fa -db 'nt.bacteria'  -task megablast -out chunk0011.fa.out -outfmt 6
blastn -query chunk0012.fa -db 'nt.bacteria'  -task megablast -out chunk0012.fa.out -outfmt 6
blastn -query chunk0013.fa -db 'nt.bacteria'  -task megablast -out chunk0013.fa.out -outfmt 6
blastn -query chunk0014.fa -db 'nt.bacteria'  -task megablast -out chunk0014.fa.out -outfmt 6
blastn -query chunk0015.fa -db 'nt.bacteria'  -task megablast -out chunk0015.fa.out -outfmt 6
blastn -query chunk0016.fa -db 'nt.bacteria'  -task megablast -out chunk0016.fa.out -outfmt 6
blastn -query chunk0017.fa -db 'nt.bacteria'  -task megablast -out chunk0017.fa.out -outfmt 6
blastn -query chunk0018.fa -db 'nt.bacteria'  -task megablast -out chunk0018.fa.out -outfmt 6
blastn -query chunk0019.fa -db 'nt.bacteria'  -task megablast -out chunk0019.fa.out -outfmt 6
blastn -query chunk0020.fa -db 'nt.bacteria'  -task megablast -out chunk0020.fa.out -outfmt 6
blastn -query chunk0021.fa -db 'nt.bacteria'  -task megablast -out chunk0021.fa.out -outfmt 6
blastn -query chunk0022.fa -db 'nt.bacteria'  -task megablast -out chunk0022.fa.out -outfmt 6
blastn -query chunk0023.fa -db 'nt.bacteria'  -task megablast -out chunk0023.fa.out -outfmt 6
```



- A convenient way to run a large number of single-core jobs
- Available for use from the Unix command line and Galaxy (BLAST+)
- Only need to submit one job instead of many small jobs
- Useful for submitting thousands of BLAST jobs

Biocontainers

What is a Biocontainer?

- A biocontainer is a container that has one or more bioinformatics software packages installed
- A container is a single image file that contains one or more installed software components
- Docker or Singularity is used to build and access a biocontainer
- A container does not contain the entire OS, just components to complement the host OS for running software installed in the container

Pros

- Software is already installed with a specific version together with all software dependencies
- The biocontainer is just one file compared to a software module or conda environment which can have thousands of files

Cons

- The latest software version of a bioinformatics tool may not have a biocontainer available
- Software dependencies within a container may not be the version you want to use

https://biocontainers-edu.readthedocs.io/en/latest/index.html

Using Biocontainers on HPRC Clusters

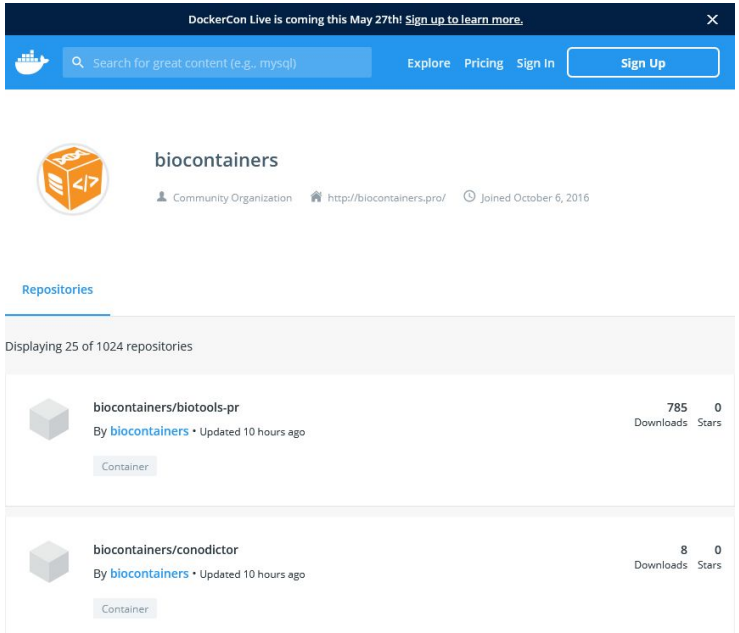
1. Docker is not available on HPRC clusters but Singularity is available
2. Docker images must be converted to a Singularity image
3. Singularity is only available on compute nodes–not login nodes
4. Compute nodes do not have internet access but you can enable a proxy configuration in order to download biocontainers
5. Use VNC portal app or srun to access command line on a compute node in order to convert Docker recipe to a Singularity image
6. Once you have your biocontainer converted to Singularity, you can run it from within a job script

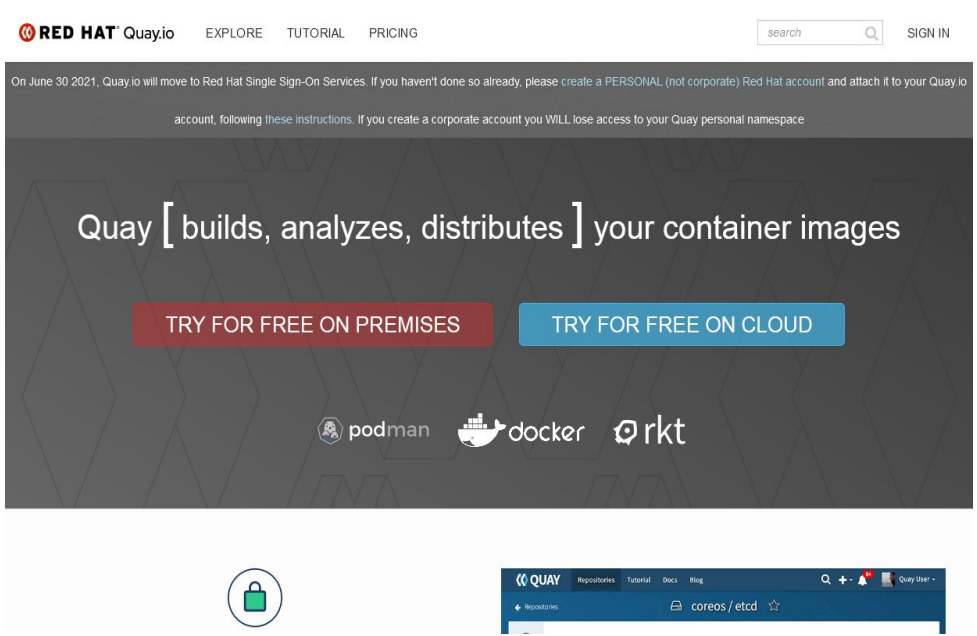
https://hprc.tamu.edu/kb/Software/Bioinformatics/Biocontainers

Finding Biocontainers

https://hub.docker.com/u/biocontainers

https://quay.io





Build the Singularity Biocontainer

1. Connect to a compute node using the HPRC portal VNC app, **`srun`** interactive job, or run as a job script

```
srun --time=01:00:00 --mem=14G --ntasks=1 --cpus-per-task=2 --pty bash
```

2. Run the following on the compute node command line to enable proxy for internet connection

```
module load WebProxy
```

3. Images can be large during the conversion from Docker to Singularity. Run the following:

```
export SREGISTRY_DATABASE=$TMPDIR
export SINGULARITY_CACHEDIR=$TMPDIR
```

4. The following will create a Singularity image from an available Docker container and name it blast_2.2.31.sif (takes about 4 minutes to download with 2 CPUs and convert the blast container)

```
singularity pull docker://biocontainers/blast:2.2.31
```

https://hprc.tamu.edu/kb/Software/Singularity/#singularity-pull-examples

Singularity Build Time

blast_2.2.31.sif	#SBATCH cpus	#SBATCH memory	time to build .sif file
build 1	2 cores	4 GB	3 min 45 sec
build 2	28 cores	54 GB	2 min

```
#!/bin/bash
#SBATCH --job-name=singularity   # job name
#SBATCH --time=01:00:00          # max job run time dd-hh:mm:ss
#SBATCH --ntasks-per-node=1      # tasks (commands) per compute node
#SBATCH --cpus-per-task=2        # CPUs (threads) per command
#SBATCH --mem=4G                 # total memory per node
#SBATCH --output=stdout.%j       # save stdout to file (%j is jobid)
#SBATCH --error=stderr.%j        # save stderr to file (%j is jobid)

module purge
module load WebProxy
export SREGISTRY_DATABASE=$TMPDIR
export SINGULARITY_CACHEDIR=$TMPDIR
singularity pull docker://biocontainers/blast:2.2.31
```

Test the Singularity Biocontainer

Enter the following in the same directory as the .sif file to see the help info for the blastp command

```
singularity exec blast_2.2.31.sif  blastp -h
```

Or run the following to launch a prompt which you can use to explore the commands available in the container. This is not used to update the image with new or additional software.

```
singularity run blast_2.2.31.sif
```

type **exit** to exit the **Singularity>** prompt

Example using blastp in a singularity command

These are just examples not available to run.

Example command to run on the compute node command line after either using **`srun`** or the VNC portal app to launch a job or by putting the command into a job script

singularity exec blast_2.2.31.sif blastp -query seqs.fa -db hg19 -out blastout.csv -num_threads 28

Or if your .sif file is in a different directory than the working directory

singularity exec /sw/hprc/sw/bio/containers/blast_2.2.31.sif blastp -query seqs.fa -db hg19 -out blastout.csv -num_threads 28

https://hprc.tamu.edu/kb/Software/Singularity/#interact-with-container

Useful alias Commands for your ~/.bashrc

```
# list files newest to oldest
alias lhl='ls -lsth'
alias lh='ls -lsth | head'
alias lhh='ls -lsth | head -20'
alias lhhh='ls -lsth | head -30'


# show your running jobs
alias q='squeue -u $USER'
alias p='pestat -u $USER'
```

HPRC Resources

- Free Help
  - Send an email to help@hprc.tamu.edu if you have any questions regarding Bioinformatics tools usage on HPRC clusters or to schedule a Zoom or in-person visit
    - First spend some time investigating the error
      - read log files, stdout file, stderr file, tool manual
      - Google search
      - Google user groups: many are software specific
    - Include details about your issue
      - Which **cluster** or which Galaxy you are using
      - The **JobID**
      - Which software you are using
      - Which modules you have loaded
      - Commands you used in your job script
      - Error messages you are seeing
- HPRC NGS data analysis tools Documentation
  - https://hprc.tamu.edu/kb/Software/Bioinformatics

Let us know when the issue has been resolved so we can close the helpdesk ticket