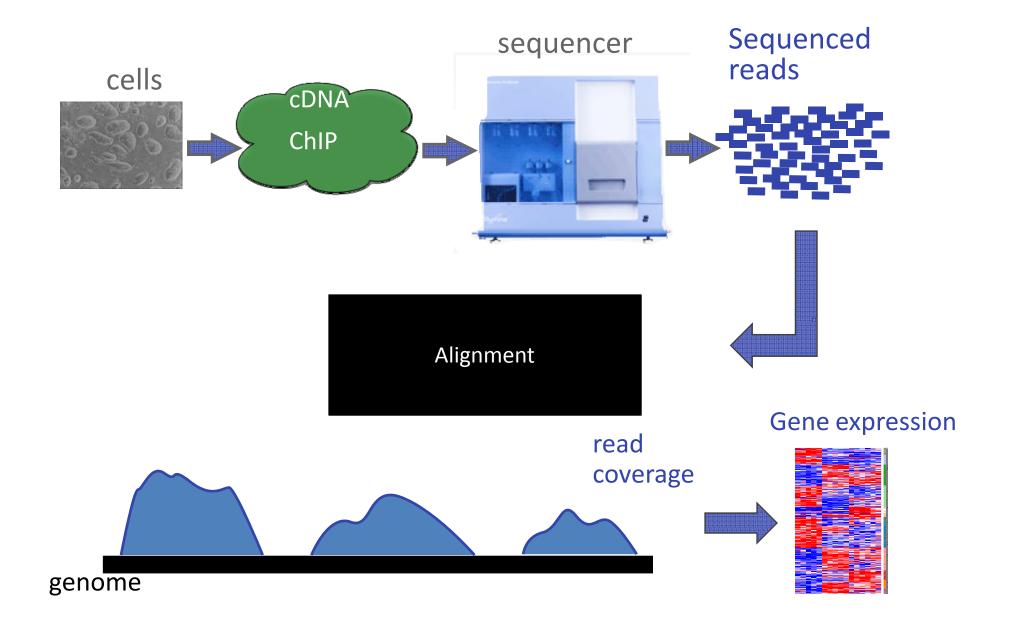
Data: databases, annotations, genomic resources

Week 2

Presenter: Hennady Shulha



Setting up

Log in into ghpcc06.umassrc.org

```
$ ssh <username>@ghpcc06.umassrc.org
```

Check that you are in your home directory:

```
$ pwd
```

\$ cd

• Create "biocourse" directory

```
$ mkdir biocourse
```

\$ cd biocourse

Setting up

Put into current folder file from web

```
$ wget http://biocore.umassmed.edu/biocourse/week2.tar.gz
```

Unpack compacted files

```
$ tar -xvzf week2.tar.gz
$ cd week2
```

Unzip this given file

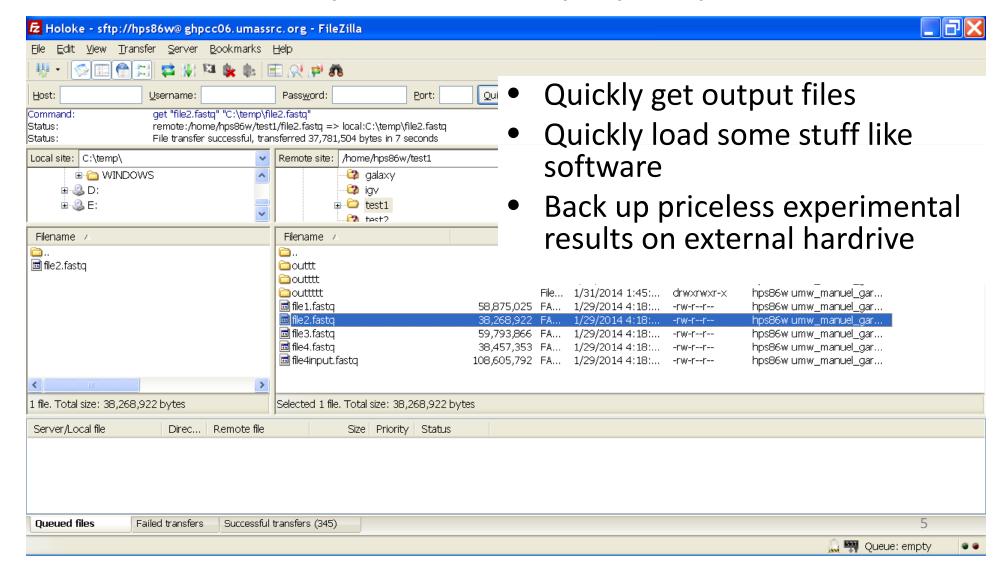
```
$ qunzip reads.fastq.qz
```

alternative and more common commands for download: "sftp", "ftp"

sftp/ftp software: Filezilla. https://filezilla-project.org/

File transfer software

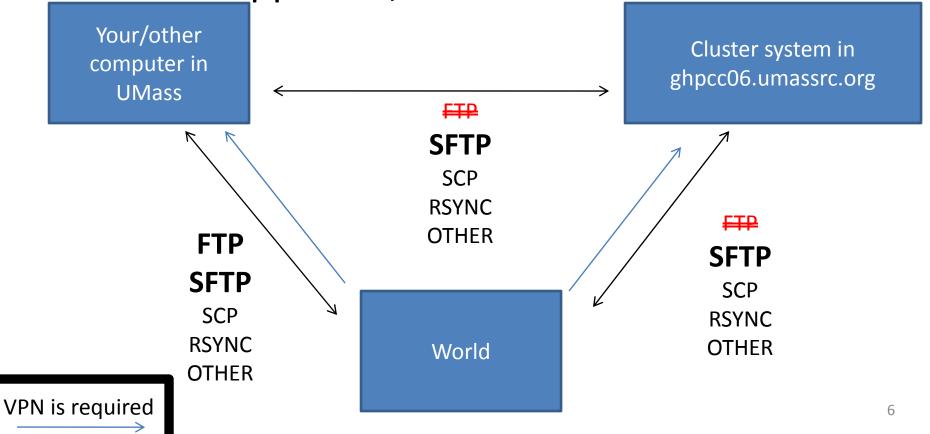
Used to connect your PC to any ftp/sftp location



Protocols availability

SFTP-supported by Filezilla

scp - not supported;



VPN

https://ssl.umassmed.edu

(contact UMass support if you do not have VPN account)

Used to connect your PC through UMass firewall if you are outside of UMass campus.



How we represent genomic data?

- Genomic sequence
 - Genomes
 - PCR products
- Genomic annotations
 - Genes
 - miRNAs
- Experimental results
 - Sequencing experiment
 - Array hybridization
- Process data for visualization
 - How many reads per base?
 - Probes are on

File formats

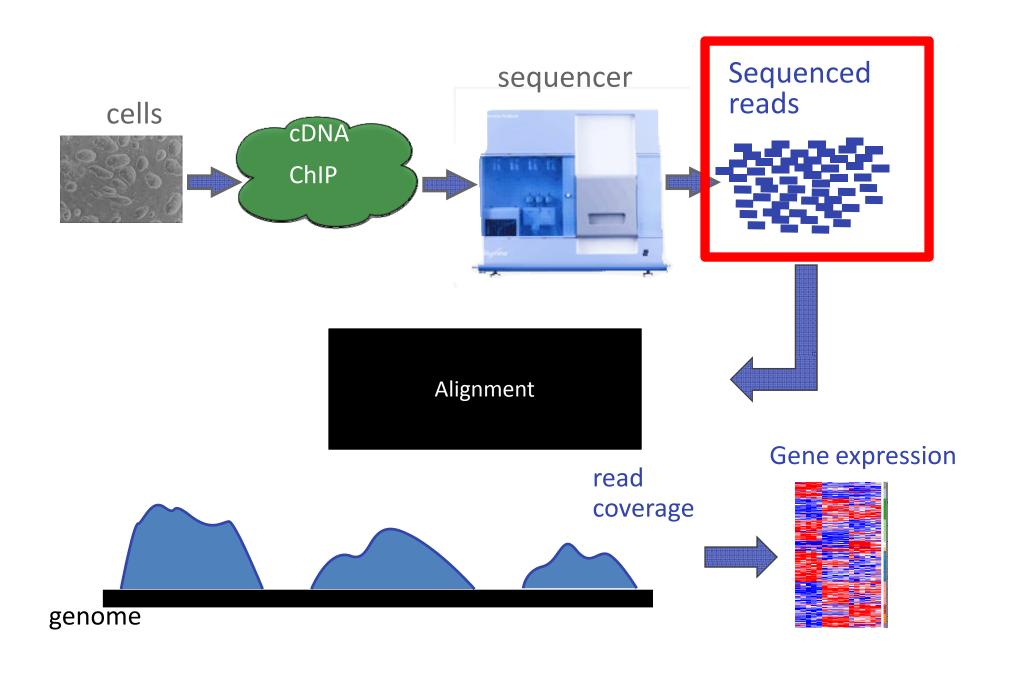
Binary

 Compressed to save space; not directly readable by human; advantage is that only part of file is needed to do visual display

Text readable

Can be opened in any text editor; occupy large space (2-3x comparing with binary)

Good command to test what is inside is "head"



File formats: Fasta

Used to keep sequences of genomes, proteins etc.

Example:

File formats: Fastq

Used mainly to store reads and information about quality

Example:

File formats: Fastq quality

Quality values

FASTQ (Phred)

 $Q = NUMERICS_ID_of_SYMBOL - 33$

 $Q = -10 \log_{10} P$ where P is probability of incorrect base calling

Solexa

 $Q = 10 * log(1 + 10 ** (NUMERICS_ID_of_SYMBOL - 64) / 10.0)) / log(10)$

 $Q_{
m solexa-prior\ to\ v.1.3} = -10\ \log_{10}rac{p}{1-p}$ where P is probability of incorrect base calling

@EAS54_6_R1_2_1_413_324
CCCTTCTTGTCTTCAGCGTTTCTCC
+
?;3;;;;;;;;;;;7;;;;88

@EAS54_6_R1_2_1_443_348
GTTGCTTCTGGCGTGGGTGGGGGGGG+EAS54_6_R1_2_1_443_348
:::::::::::::::9:7::.7:393333

File formats: Fastq versions

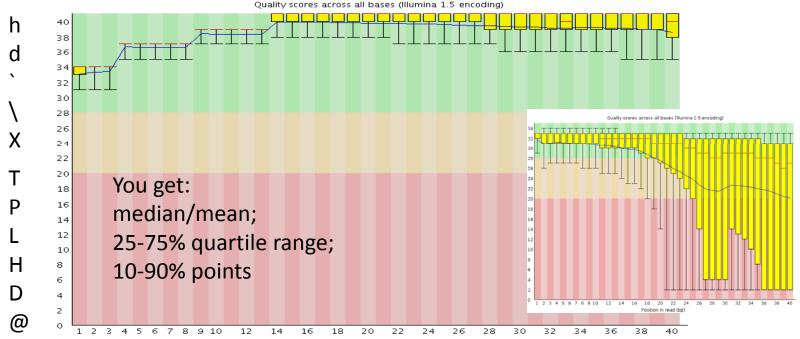
File formats: Fastq versions

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
33
                  59
                                                 104
                                                                126
0.....9.....40
                        S - Sanger Phred+33, raw reads typically (0, 40)
       Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64, raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64, raw reads typically (3, 40)
  with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
   (Note: See discussion above).
L - Illumina 1.8+ Phred+33, raw reads typically (0, 41)
```

I.e. if you are going to use quality scores (by aligners) – would be better to know a source that generated it!!!

Fastq quality

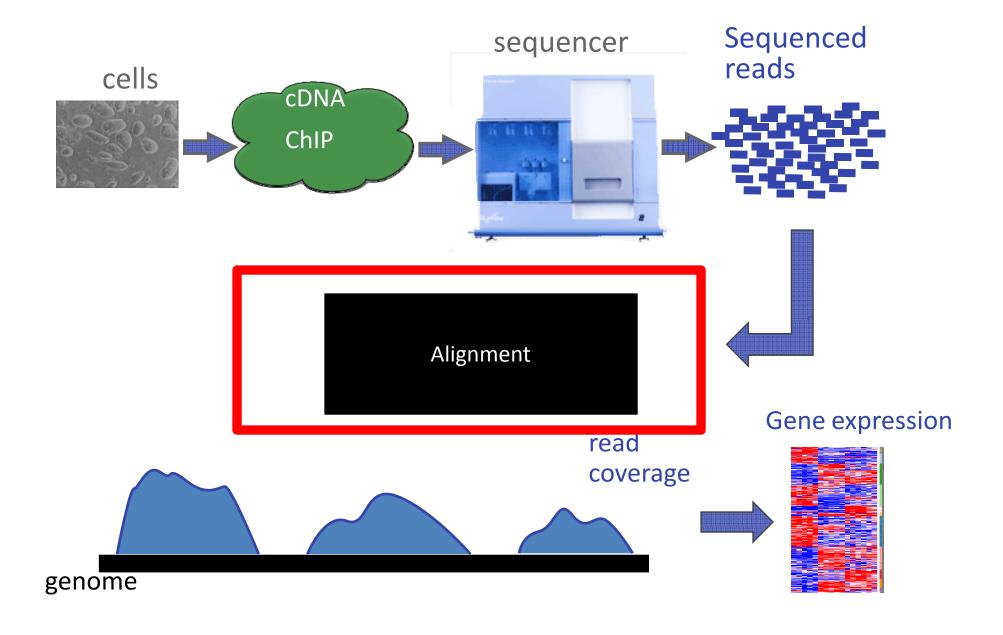
 Alternative: check if general picture is fine and not use it.



Very useful software: "FastQC". It would do basic analysis for your FASTQ files.

```
$ module load fastqc/0.10.1
$ fastqc -help
$ fastqc reads.fastq
```

module avail



BAM/SAM: Read alignment format

- Source of a bam file
 - Fastq → aligner → BAM: Alignment results
- Aligners:
 - Bowtie: Aligns contiguous reads
 - Tophat: Aligns spliced reads

To load bowtie:

```
$ module load bowtie/1.0.0
```

Information about genomic locations, plus other useful things like base qualities, number of mismatches etc.

BAM contains the same info but in compressed, binary format.

@HD VN:1.0

@SQ SN:1 LN:249250621

Header: basic description; sizes of chromosomes; other

@HD VN:1.0

@SQ SN:1 LN:249250621

Sequence1

Sequence name: whatever the read was named in fastq file

@HD VN:1.0

@SQ SN:1 LN:249250621

Sequencel 113 chr1 497

Genomic position: position how it was mapped by a mapper

```
@HD VN:1.0
```

@SQ SN:1 LN:249250621

Sequence1 113 chr1 497 37 37M

CIGAR string: contains information about mapping like gaps.

@HD VN:1.0

@SQ SN:1 LN:249250621

Sequencel 113 chr1 497 37 37M 15 100338662 0

CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG

Sequence by itself: mapped sequence

```
@HD VN:1.0
```

@SQ SN:1 LN:249250621

Sequencel 113 chrl 497 37 37M 15 100338662 0

CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG

Quality: quality from FASTQ file

Other info: for example, NM:i:0 means 0 mismatches

File conversion

SAMTOOLS

\$ module load samtools/0.0.19

```
Program: samtools (Tools for alignments in the SAM format)
Version: 0.1.18 (r982:295)
         samtools <command> [options]
Usaqe:
Command: view
                     SAM<->BAM conversion
                     sort alignment file
         sort
         mpileup
                     multi-way pileup
         depth
                     compute the depth
         faidx
                     index/extract FASTA
         tview
                     text alignment viewer
         index
                     index aliqnment
         idxstats
                     BAM index stats (r595 or later)
         fixmate
                     fix mate information
         flagstat
                     simple stats
                     recalculate MD/NM tags and '=' bases
         calmd
                     merge sorted alignments
         merge
                     remove PCR duplicates
         rmdup
         reheader
                     replace BAM header
         cat
                     concatenate BAMs
                     cut fosmid regions (for fosmid pool only)
         tarqetcut
                     phase heterozygotes
         phase
```

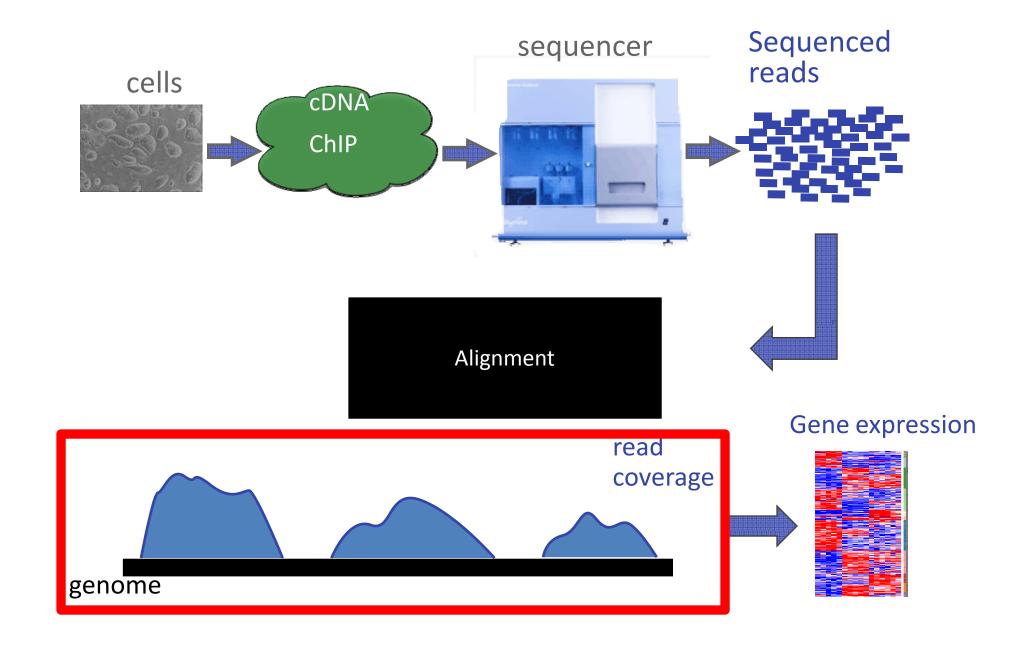
File conversion

SAM<->BAM conversion

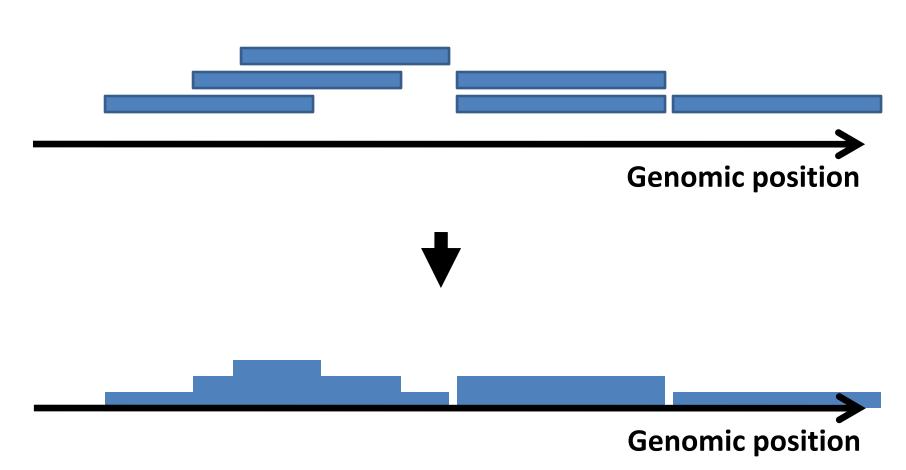
```
$ samtools view -b -S reads.sam > reads.bam
[samopen] SAM header is present: 25 sequences.

$ samtools view -h reads.bam | less

$ samtools sort
Usage: samtools sort [-on] [-m <maxMem>] <in.bam> <out.prefix>
$ samtools sort reads.bam sorted
```



Aggregation



File formats: WIG/bigWIG



Aggregated information about genomic locations.

VARIABLE Step

variableStep chrom=chr2

300701 12.5

300702 12.5

300703 12.5

300704 12.5

300705 12.5

is equivalent to:

variableStep chrom=chr2 span=5 300701 12.5

File formats: WIG/bigWIG



Aggregated information about genomic locations.

FIXED Step

fixedStep chrom=chr3 start=400601 step=100

11

22

33

displays the values 11, 22, and 33 as single-base regions on chromosome 3 at positions 400601, 400701, and 400801, respectively. Adding span=5 to the declaration line:

fixedStep chrom=chr3 start=400601 step=100 span=5

11

22

33

Tools

Bedtools (BED manipulation but BAM support is available)
 https://bedtools.googlecode.com/files/BEDTools-User-Manual.v4.pdf

```
$ module load bedtools/2.17.0

$ head a.bed
chr1 100 200
chr1 1000 2000

$ head b.bed
chr1 150 250

$ intersectBed -a a.bed -b b.bed
chr1 150 200
```

Tools

Bedtools

GFF/VCF files.
GFF/VCF files within a "window".
ED/GFF/VCF file.
of features in one BED/GFF file (e.g.,
ned windows).
,
erage.
egular BED/GFF/VCF file.
_ , ,
formats.
d and unblocked) to BAM format.
each interval defined in a BED/GFF/
res.
apped by another feature.
).
vals.
rdinates.
me.
pairs.
DED (CIEE 6)
BED/GFF file.
s) or distance (negative values) between d of the same line.
non column groupings. Akin to the SQL
file, allowing coverage/other 34
34
os from many others.
E C I S E C I

File formats: bedGraph



CHR	Start	End	Value
chr1	1	100	100
chr1	200	300	20

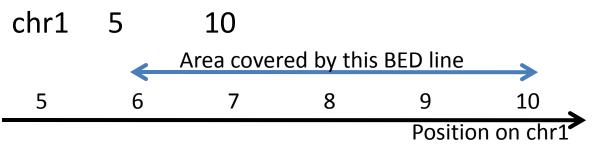
\$ genomeCoverageBed -bg -ibam sorted.bam -g mm10.chrom.sizes >
out.bg

track type=bedGraph name=test_track

File formats: BED

Information about genomic locations. TAB delimited.

• Simple case:



Advanced (12 columns, details in the link on slide9):

chr22 100 500 cloneA 96 + 100 500 0 2 4,5, 0,35 chr22 200 600 cloneB 90 - 200 600 0 2 4,5, 0,36

File formats: GTF

Gene information (usually from some external databases)

TAB separated information

<seqname> <source> <feature> <start> <end> <score> <strand> <frame> [attributes] [comments]

```
AB000381 Twinscan CDS 700 707 . + 2 gene_id "001"; transcript_id "001.1"; AB000381 Twinscan start_codon 380 382 . + 0 gene_id "001"; transcript_id "001.1"
```

UCSC browser

http://genome.ucsc.edu

http://biocore.umassmed.edu/ucsc.html

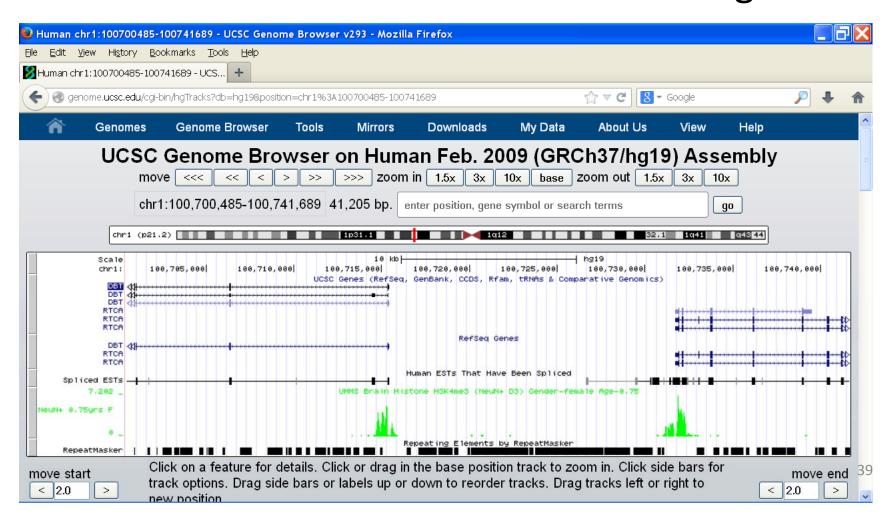
ENSEMBL

http://www.ensembl.org/index.html

IGV browser

http://www.broadinstitute.org/igv

UCSC – handles bunch of different things

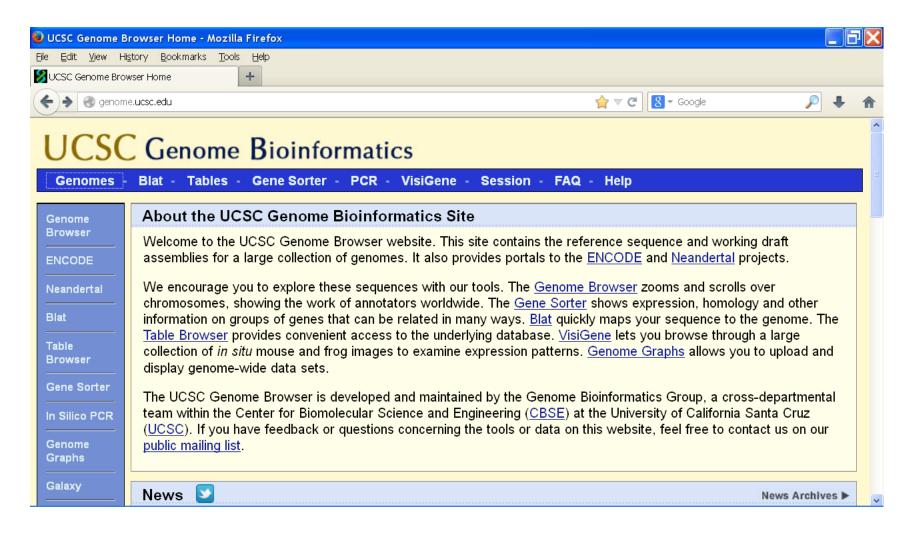


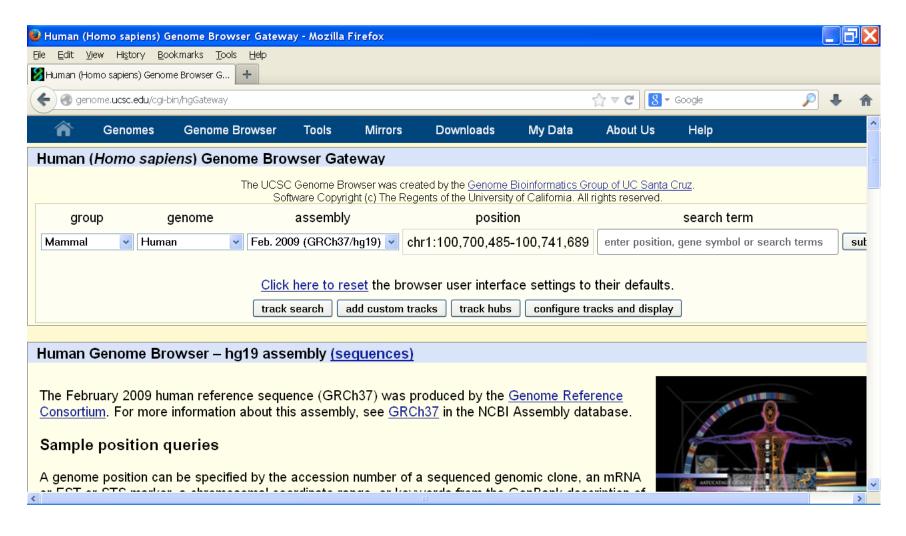
http://biocore.umassmed.edu/ucsc.html

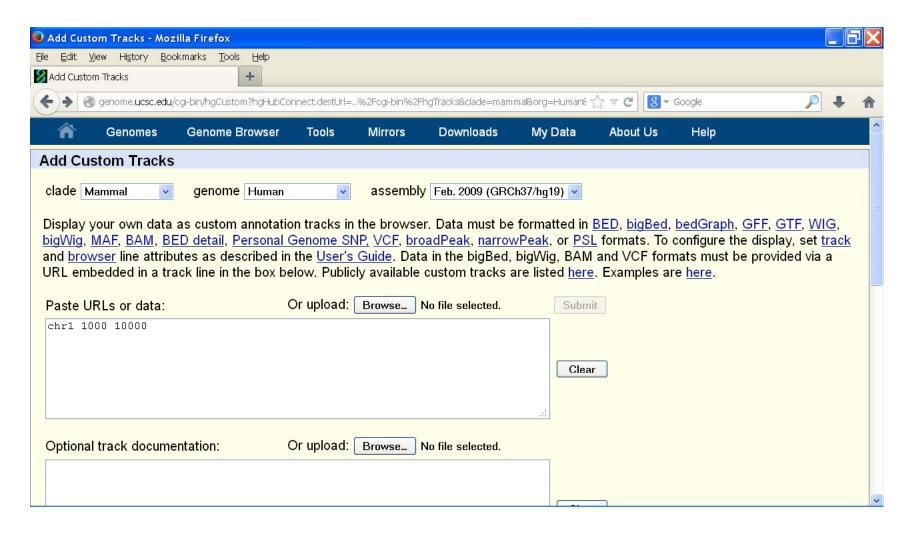
to use for visualization, any other activities where you would expect heavy competition on ucsc.edu.

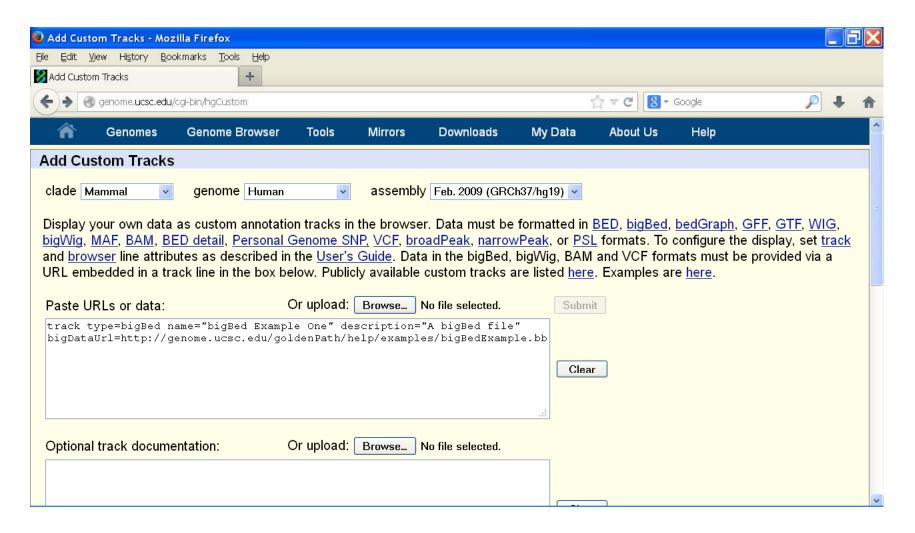
http://genome.ucsc.edu/index.html

to use for tools that are not under heavy competition like BLAT, datatables download.

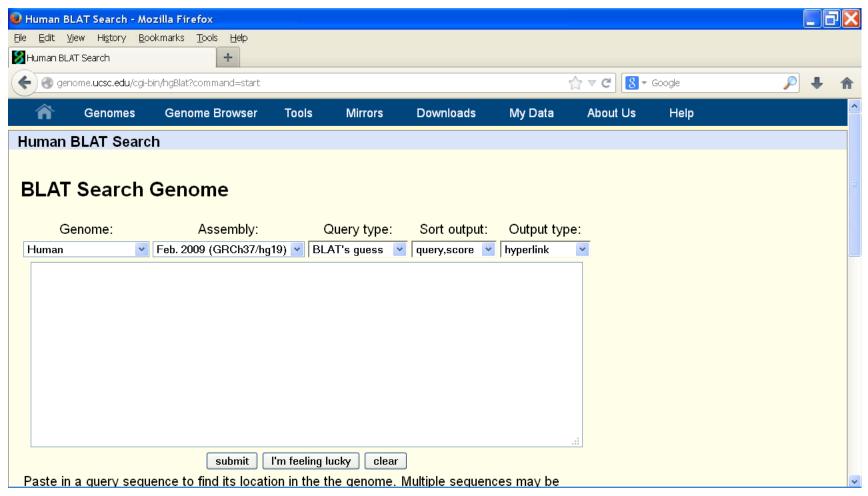




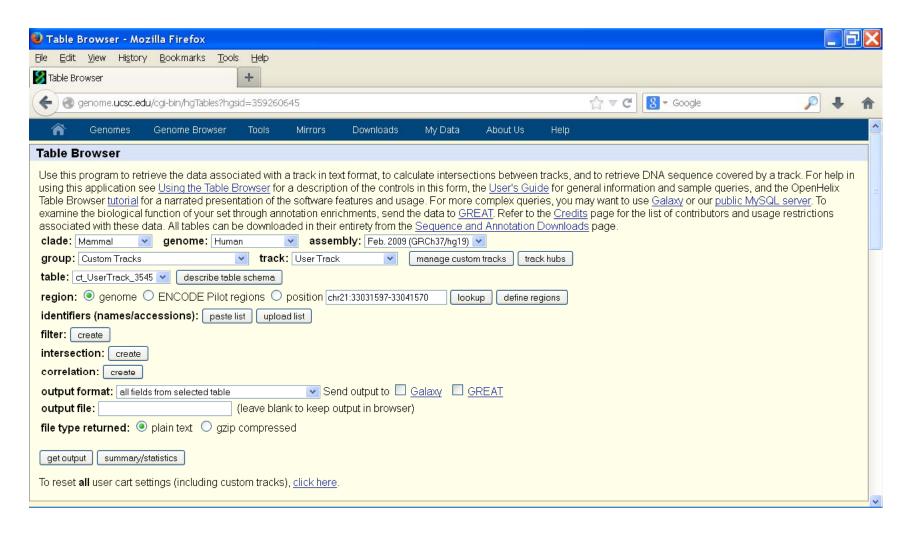




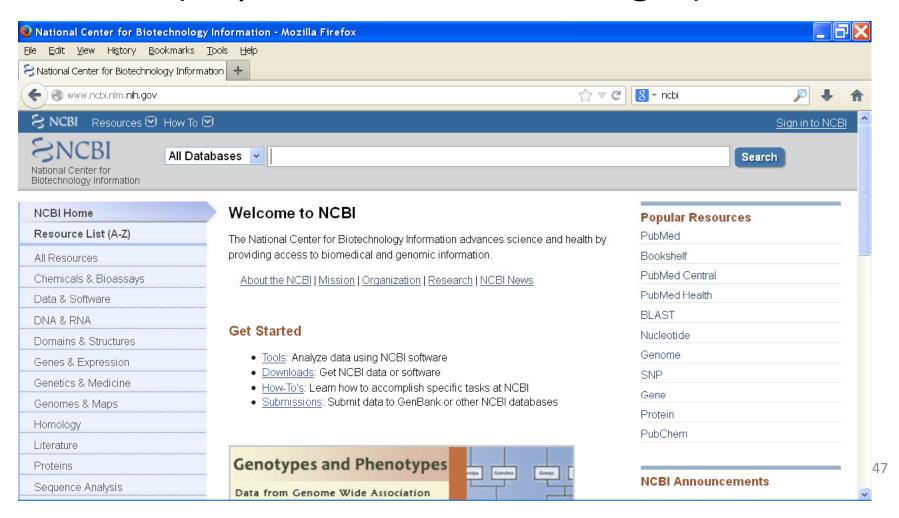
http://genome.ucsc.edu/index.html

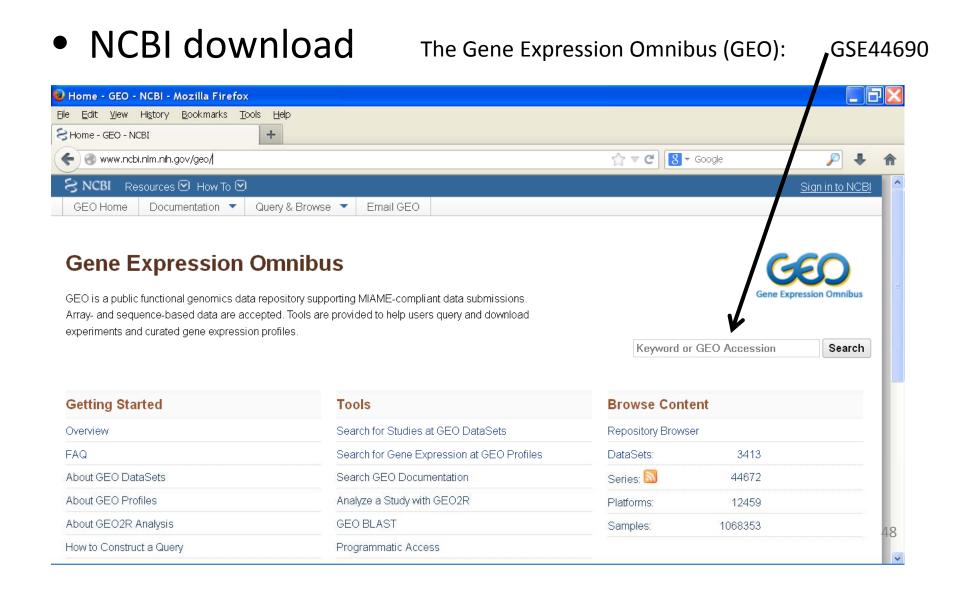


Visualization/Databases



NCBI (http://www.ncbi.nlm.nih.gov)

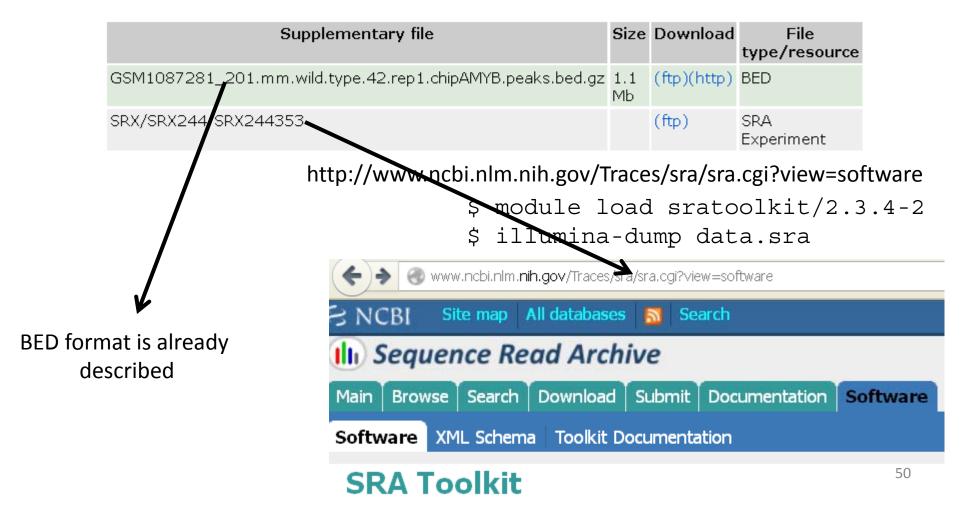




NCBI download



NCBI download



Questions?

Homework

Use information from today's seminar and get instructions from this location:

Server: ghpcc06.umassrc.org

Folder/file: /project/umw_biocore/seminar/Step2.docx