

# Computational Analysis Primer

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QB Lecture 2



# Outline

## Part 1: Overview & Fundamentals

- Why Computers?
- Overview of Computation Systems
- Unix and Scripting Primer

## Part 2: Example Analysis



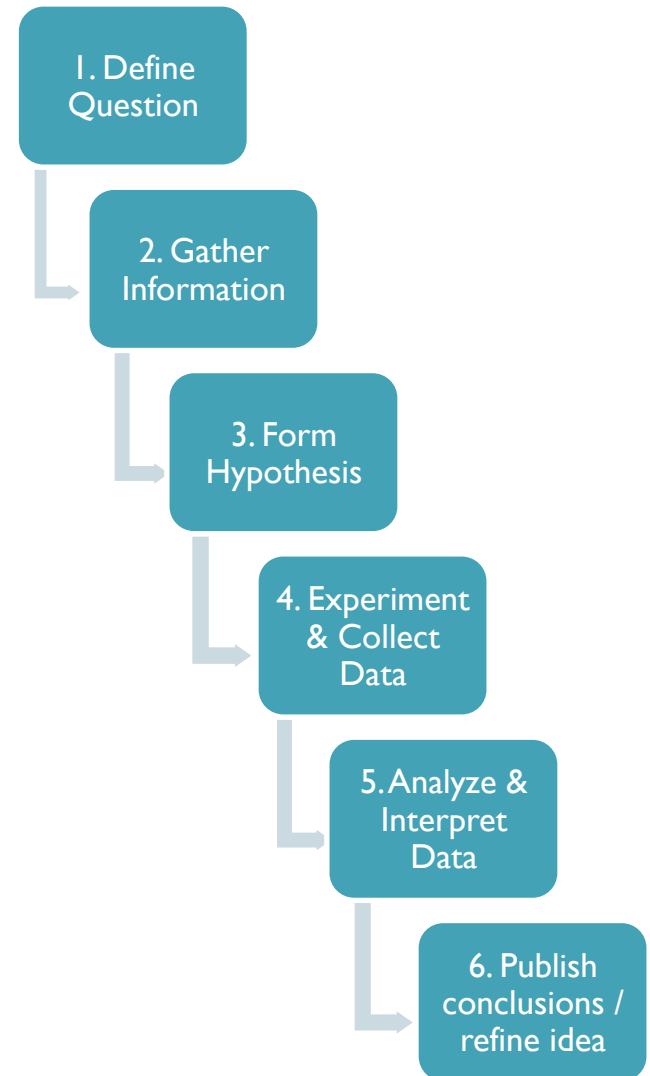
# Scientific Method

## What is analysis?

- Experimental design
  - Frame the question so that it can be quantitatively answered
- Assay design
  - Statistical, mathematical, computational methods to improve the sensors
- Drawing conclusions
  - Identify trends, patterns, correlations, and causal links

## How do we analyze?

- Paradigms of science:
  1. Make observations
  2. Formulate mathematical models
  3. Simulate processes
  4. Data-intensive discovery



# How do we draw conclusions?

- Comparison & Triangulation: How does X compare to Y?

X	Y
Exomes of kids with autism	Exomes of kids that do not
Genomes of Europeans	Genomes of non-Europeans, mammals, ...
Gene expression in mutants	Gene expression in wild type
Firing patterns of mutant fly neurons	Firing patterns of wild type

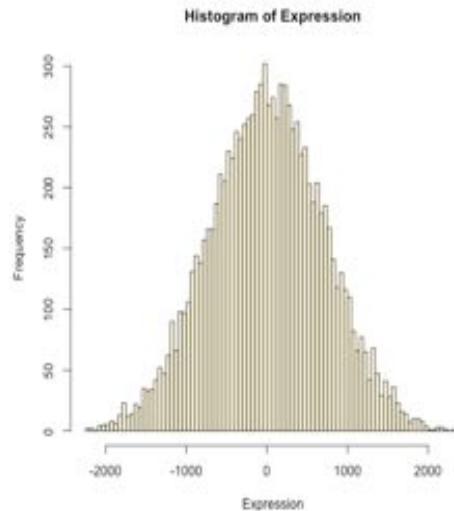
- Modeling & Predictions: How will X respond to Y?

X	Y
Mutant tomatoes	Increased temperatures
Human Microbiome	Probiotic treatments
Gene expression in mice	Knockout of transcription factor
Firing rate in flies	Decreased sodium levels

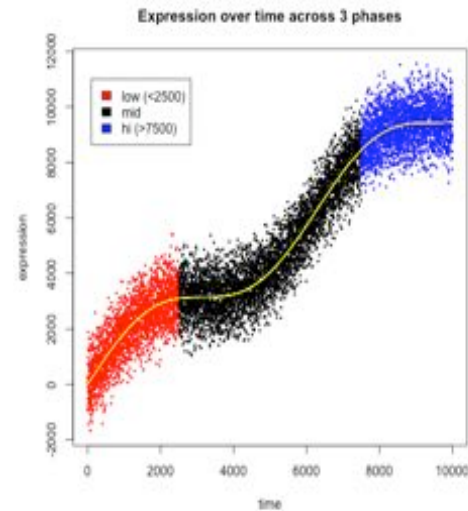
# How do we DRAW conclusions?

-902.473  
242.817  
-872.453  
73.9297  
236.169  
46.7525  
975.014  
716.563  
-533.971  
-120.282  
725.12  
-736.76  
176.156  
189.224  
1847.46  
-159.099  
-56.4754  
-973.626  
1181.9  
-315.455  
-1480.43  
215.293  
-747.505  
682.577  
...

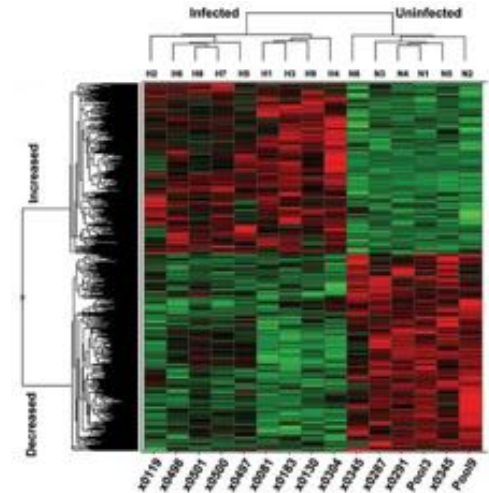
Histogram



Scatterplot



Heatmap



Data and data transformations are ubiquitous in science  
Data are too numerous and transformations are too complex to do by hand  
==> Mendel: 100k observations, 10 years  
==> HiSeq 2000: 600B observations, 10 days  
==> Make friends with your computational tools

# What is a computer?

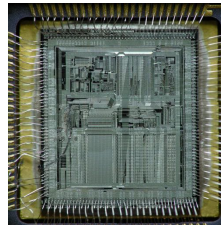
[hardware]



**Hard Drive**  
Permanent Storage – 1TB  
(big, slow, cheap)



**RAM**  
Working Storage – 8 GB  
(small, fast, expensive)



**Processor**  
Arithmetic, logic  
# cores, clock speed



**Display**  
Human Interface



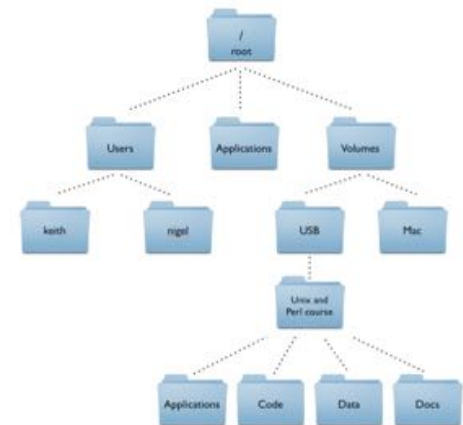
**Network**  
Computer Interface  
Home: 10Mb/s, CSHL: 1Gb/s

# What is a computer?

[software]

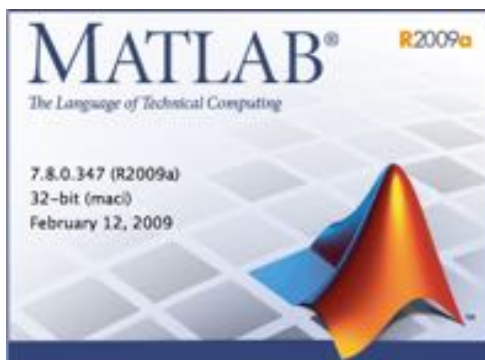


**Office Applications**  
Presentations, Documents  
Simple statistics and plots



**Files / Data**  
Papers, sequences,  
measurements

**Operating System**  
Mission Control  
Windows, Mac, Unix, iOS



**Scientific Applications**  
Specialized Analysis  
Commercial



**Code / Scripts**  
Research Applications  
Academic

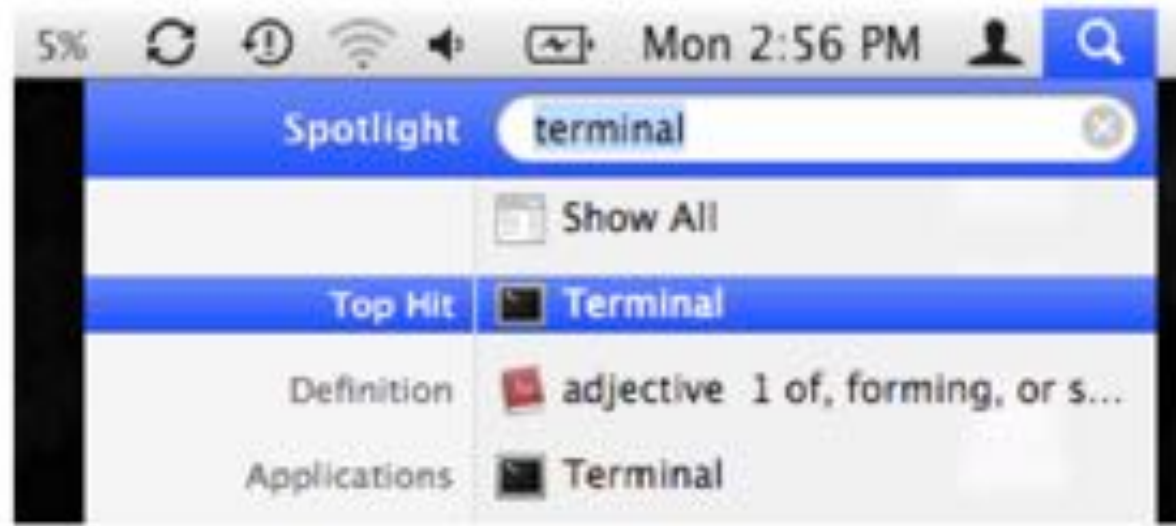
# How does (scientific) software operate?



- The software we need to run is very specialized, there is no 'align genomes' button in Excel
  - Data files are huge, so probably wouldn't want one anyways
- It takes a lot of work (and time/money) to create a graphical interface to software, so most scientific software uses a 'command line' interface
  - Important to become comfortable using command line tools
- Scientific analyses tend to use workflows consisting of several applications where the output of one phase becomes the input to the next
  - Develop a workflow for dataset X, apply again to dataset Y



# Where is the command line?



- Your Mac has a very powerful command line interface hidden just beneath the graphical environment
  - This command line interface is (basically) the same as that used by our scientific cluster BlueHelix
  - Big data files are stored on our central storage system BlueArc
- This environment has a universe of programs you can use to manipulate files and data in novel ways
  - Learning to use this environment is a lot like learning a new language
  - [http://korflab.ucdavis.edu/Unix\\_and\\_Perl/index.html](http://korflab.ucdavis.edu/Unix_and_Perl/index.html)

# Hola, como estas?

Command	Output
man	Look up something in the manual (also try Google)
ls	List the files in the current directory
cd	Change to a different directory
pwd	Print the working directory
mv, cp, rm	Move, copy, remove files
mkdir, rmdir	Make or remove directories
cat, less, head, tail, cat	Display (parts) of a text file
echo	Print a string
sort, uniq	Sort a file, get the unique lines
find, grep	Find files named X, or containing X
chmod	Change permissions on a file
wc	Count lines in a file
jot / seq	Output numbers from 1 to X (on Linux use seq)
(pipe), > (redirect)	Send output to a different program, different file

# File Hierarchy

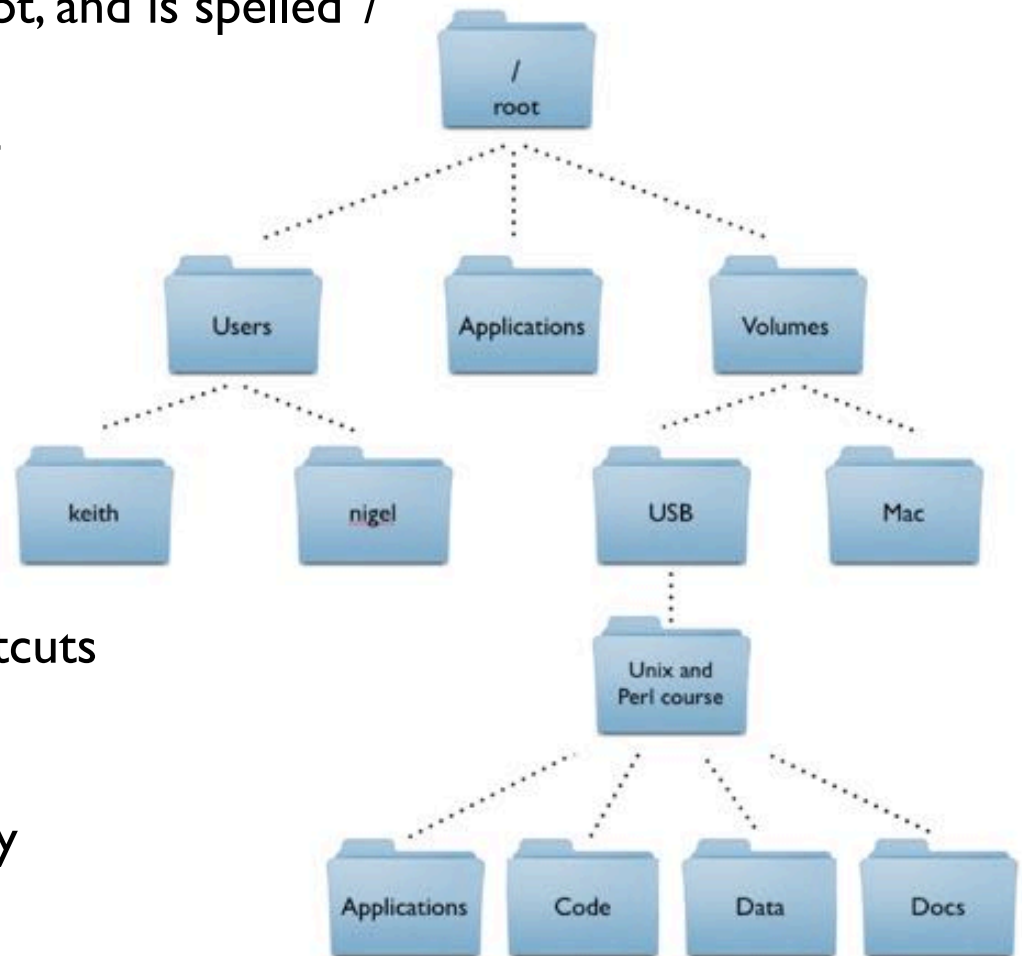
Files are stored in nested directories (folders) that form a tree

- The top of the tree is called the root, and is spelled '/'

- Your home directory (on mac) is at  
/Users/username

- Command line tools are at  
/bin/  
/usr/bin/  
/usr/local/bin/

- A few special directories have shortcuts  
~ = home directory  
~bob= bob's home directory  
. = current working directory  
.. = parent directory  
- = last working directory



# Working with the shell

- The shell is interactive and will attempt to complete your command as soon as you press enter

```
$ pwd  
/Users/mschatz
```

```
$ echo "Hello, World"  
Hello, World
```

- Here are a few tips that will make your life easier

Command	Effect
Left/Right arrow	Edit your current command
Up/Down arrow	Scroll back and forth through your command history
Control-r	Search backwards through your command history
history	What commands did I just run?
Control-c	Cancel the command
Control-u	Clear the current line
Control-a, Control-e	Jump to the beginning and end of the line

# Files and permissions

- Every file has an owner and a group, you can only read/write to a file if you have permission to do so

```
$ pwd
```

```
/Users/mschatz/Desktop/Unix_and_Perl_course/Data/Arabidopsis
```

```
$ ls -l
```

```
total 193976
```

```
-rw-r--r--@ 1 mschatz  staff  39322356 Jul  9  2009 At_genes.gff
-rw-r--r--@ 1 mschatz  staff  17836225 Oct  9  2008 At_proteins.fasta
-rw-r--r--@ 1 mschatz  staff  30817851 May  7  2008 chr1.fasta
-rw-r--r--@ 1 mschatz  staff  11330285 Jul 10  2009 intron_IME_data.fasta
```

- These files can be read by anyone, but only written by me
  - Change permissions with 'chmod'

```
$ chmod g+w At_*
```

```
$ man chmod
```

- Programs and scripts have the execute bit set

```
$ ls -l /bin/ls
```

```
-r-xr-xr-x  1 root  wheel  80688 Feb 11  2010 /bin/ls*
```

# Working with files and directories

- Create directories and copies of the working files

```
$ mkdir myfiles
$ cd myfiles/
$ cp ../At_* .
$ ls -l
total 111648
-rw-r--r--@ 1 mschatz  staff  39322356 Nov  8 01:37 At_genes.gff
-rw-r--r--@ 1 mschatz  staff  17836225 Nov  8 01:37 At_proteins.fasta
```

- Rename files

```
$ mv At_genes.gff Arabidopsis_genes.gff
```

- See how long the files are

```
$ wc -l *
531497 Arabidopsis_genes.gff
214021 At_proteins.fasta
745518 total
```

- Clean up

```
$ cd ..
$ rm -rf myfiles/
```

[WARNING!!! Double check rm]

# Working with text files

- Display the first few lines of a file

```
$ head -5 At_proteins.fasta
>AT1G51370.2 | Symbols: | F-box family protein | chr1:19049283-19050416 FORWARD
MVGKKKTKICDKVSHEEDRISQLPEPLISEILFHLSTKDSVRTSALSTKWRYLWQSVPGLDLDPYASSNTNTIVSFVES
FFDSHRDSWIRKLRLDLGYHHDKYDLMSWIDAATTRRIQHLDVHCFHDNKIPLSIYTCTTLVHLRLRWAVLTNPEFVSLP
CLKIMHFENVSYPNETTLOKLISGSPVLEELILFSTMYPKGNVLQLRSDTLKRLDINEFIDVVIYAPLLQCLRAKMYSTK
NFQIISSGFPAKLDIDFVNTGGRYQKKKVIEDILIDISVRDLVISSNTWKEFFLYSKSRPLLQFRYISHLNARFYISDL
```

- Show the first few proteins names in the file

```
$ grep '>' At_proteins.fasta | head -5
>AT1G51370.2 | Symbols: | F-box family protein | chr1:19049283-19050416 FORWARD
>AT1G50920.1 | Symbols: | GTP-binding protein-related | chr1:18874223-18876238 FORV
>AT1G36960.1 | Symbols: | similar to unknown protein [Arabidopsis thaliana] (TAIR:7
>AT1G44020.1 | Symbols: | DC1 domain-containing protein | chr1:16719132-16721096 RI
>AT1G15970.1 | Symbols: | methyladenine glycosylase family protein | chr1:5486538-!
```

- Count how many proteins are present, excluding hypothetical proteins

```
$ grep '>' At_proteins.fasta | wc -l
32825
$ grep '>' At_proteins.fasta | grep -v 'hypothetical' | wc -l
31267
```

## Working with text files 2

- Create a file of just hypothetical proteins

```
$ grep '>' At_proteins.fasta | grep 'hypothetical' > hypotheticals
$ wc -l hypotheticals
    1558 hypotheticals
```

- Count hypotheticals per chromosome

```
$ cut -f4 -d'|' hypotheticals | head -3
chr1:11437249-11439801 FORWARD
chr1:5167349-5168146 REVERSE
chr1:16717096-16717944 FORWARD
$ cut -f4 -d'|' hypotheticals | cut -f1 -d':' | head -3
chr1
chr1
chr1
$ cut -f4 -d'|' hypotheticals | cut -f1 -d':' | sort | uniq -c
    382 chr1
    234 chr2
    260 chr3
    204 chr4
    384 chr5
     9  chrC
    84 chrM
     1 CAB12631.1 (PTHR11061
```

[What happened here?]



# Scripting basics

- A bash script is just a list of commands

```
$ cat simple_script.sh
#!/bin/sh
```

```
echo "Hello, World"
echo "Shall we play a game?"
```

```
$ chmod +x simple_script.sh
$ ./simple_script.sh
```

[What does this do?]

- Things get interesting when we add variables and loops

```
$ cat loop_script.sh
#!/bin/sh
```

```
for name in "Mike" "Justin" "Mickey"
do
    echo "Hello, $name" >> people.txt
    everyone="$name $everyone"
done
echo "Hello: $everyone" >> people.txt
```

```
$ chmod +x loop_script.sh
$ ./loop_script.sh
$ ./loop_script.sh
$ ./loop_script.sh
```

[What does this do?]

## Scripting basics 2

- Conditionals and loops let us work over any number and type of file

```
$ cat conditional_script.sh
#!/bin/sh

for filename in `ls */bin/*`
do
    type=`echo $filename | cut -f2 -d'.'`
    echo "Processing $filename, type is $type"
    echo "====="

    if [[ $type == "fasta" ]]
    then
        protein_count=`grep -c '>' $filename`
        hypo_count=`grep -c hypothetical $filename`
        echo "$filename has $protein_count total proteins, $hypo_count are hypothetical"
    elif [[ $type == "gff" ]]
    then
        echo "$filename stats"
        cut -f3 $filename | sort | uniq -c
    else
        echo "Unknown file type"
    fi

    echo "====="
    echo
done
```

[What does this do?]

# Scripting Challenges

- Create 1000 files named mutantA.X.txt with X in [1,1000] that each contain 'gene'
  - That each contain the numbers 1 to X
- How do I rename 1000 files named mutantA.X.txt to mutantB.X.txt?
- How can I create a directory with just the files that contain 'special gene'

# Break





# Outline

## Part 1: Overview & Fundamentals

## Part 2: Example Analysis

- Background on tracking DNA replication with next-gen sequencing
- Walk-through of analysis steps
- Visualization of discovered replication sites

# ~300 separate loci direct DNA replication initiation in *Saccharomyces cerevisiae*

ARS: autonomously replicating sequence

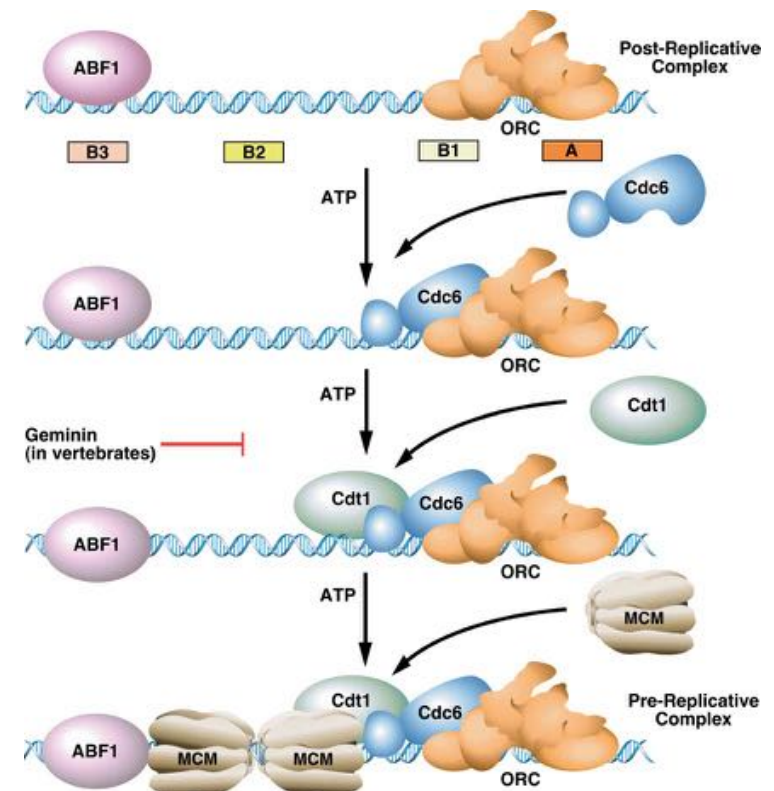
G1



S



The Stillman lab is interested, in part,  
in the signaling mechanisms  
governing pre-RC firing  
-> genome-wide replication tracking



# Tracking replication with EdU pulldown + sequencing

DNA of cells arrested in G1 with  $\alpha$ -factor

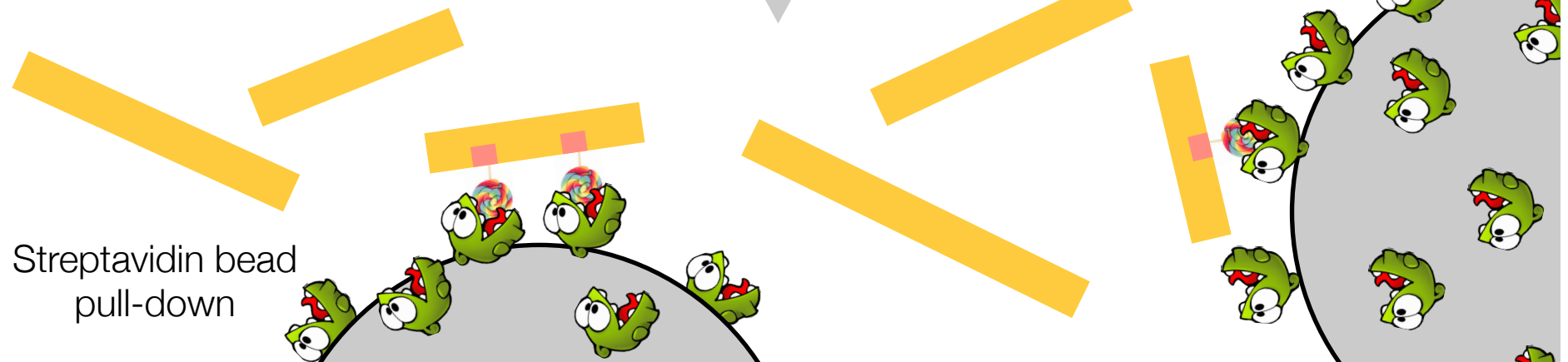


Release cells into S-phase  
EdU incorporation during replication

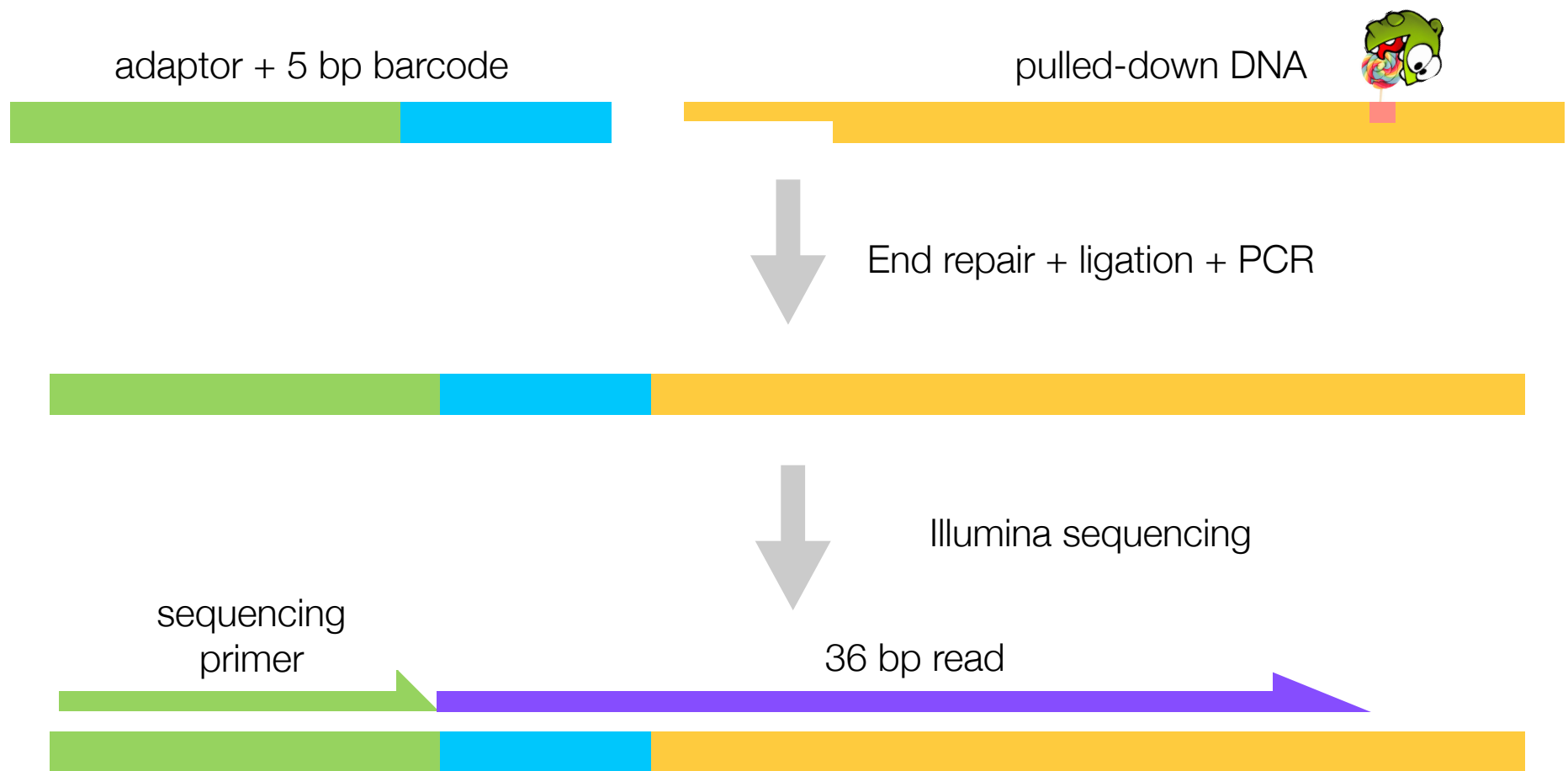


Click-iT linking of EdU to biotin

DNA sonication



# Barcoding samples for sequencing



~15 M reads for 14 barcoded samples

**Thanks Yi-Jun!**



# What we will do

- Today
  - Map reads to the yeast genome
  - Compute “replication profiles”: # of reads covering each genomic position
  - View these data using the UCSC genome browser; compare to known ARSs
- Tomorrow
  - Matlab tutorial
  - Load replication profiles into Matlab
  - Smooth and plot replication profiles
- Homework: compare replication profiles for 3 different strains

# Analysis Pipeline



- No single application available that will let us analyze these data
  - Just 4 steps to go from raw observations to biological discovery
- Each step requires selection, tuning, and debugging
  - Analogous to a wetlab protocol for running an experiment
- The components of the pipeline can be used in many other assays
  - Reads => Comparative Genomics, Transcriptome Analysis, de novo sequencing, Protein binding sites, Chromatin regulation...
  - Alignment => Forms the basis for almost every assay
  - SAMTools => Filtering, selection, interpretation of alignments

# Analysis Pipeline



- Get the files (curl dash Capital-O)

```
$ curl -O http://schatzlab.cshl.edu/data/challenges/replication_exercise.tgz
```

- Unpack the files

```
$ tar xzvf replication_exercise.tgz
```

- Check out the files

```
$ cd replication_exercise/  
$ ls -R  
$ less *.txt  
$ less reads/A1.fastq
```

[What is the secret phrase?]

# Analysis Pipeline



- Check out the analysis script

```
$ cat course_pipeline.sh
```

- We have already done the first steps to partition reads into batches

```
# Quality filter reads
# fastq_quality_filter -q 10 -p 90 -i /data/kinney/data/illumina_sequencing/
11.01.24_sheu_edu/reads.fastq -o reads/reads_qual.fastq

# Split reads by batch
# cat reads/reads_qual.fastq | fastx_barcode_splitter.pl --bcfile /data/
kinney/data/illumina_sequencing/11.01.24_sheu_edu/barcodes.txt --prefix reads/
tmp1_ --suffix .fastq --mismatches 0 -bol
```

- You can embed comments into scripts with '#'

# Analysis Pipeline



- Now that the reads are prepared, next step is to align

```
# Create bwa index for genome  
# bwa index genome/genome.fasta
```

```
# Align reads using bwa  
bwa aln genome/genome.fasta reads/A1.fastq > mappings/A1.sai  
bwa samse genome/genome.fasta mappings/A1.sai reads/A1.fastq > mappings/A1.sam
```

- BWA (Li & Durbin, 2009) is one of the most popular tools for aligning short reads to a reference genome. It is used in almost every sequencing assay that start from short reads. It takes a few steps to run because it uses a special index of the genome for making the alignments fast. We will talk about it in detail at the end of the course

# Analysis Pipeline



- Now that the reads are aligned, need to transform and sort them

```
# Create pileup using samtools
```

```
samtools view -bS mappings/A1.sam > mappings/A1.bam
```

```
samtools sort mappings/A1.bam mappings/A1.sorted
```

```
samtools index mappings/A1.sorted.bam
```

```
samtools pileup -c -f genome/genome.fasta mappings/A1.sorted.bam > pileups/A1.pileup
```

- The pileup file encodes how many reads align to each position in the genome

```
$ less pileups/A1.pileup
```

- Run a quick command to find positions with deep coverage

```
$ awk '{if ($8>50){print}}' A1.pileup | less
```

[AWK is a really powerful, if arcane filter]

# Analysis Pipeline



- Now run a custom script to summarize the depth information

```
$ ./pileup2bedfile.py pileups/A1.pileup 31  
$ less pileups/A1.pileup.bed
```

- This file can then be loaded into the UCSC Genome Brower for inspection, and relate it to known annotations

See <http://genome.ucsc.edu/>

# Homework

- Replication Analysis
  - Modify `course_pipeline.sh` to analyze BI, CI, DI
  - Load the bed files into the UCSC genome browser
  - See if you can spot and interesting variations between the data sets
- Read the Matlab Getting Started Guide. This is available as a pdf here:  
[http://www.mathworks.com/help/pdf\\_doc/matlab/getstart.pdf](http://www.mathworks.com/help/pdf_doc/matlab/getstart.pdf)
- Focus on these sections
  - Introduction
  - Matrices and Arrays
  - Graphics, starting with Basic Plotting Functions
  - Programming
  - Data Analysis
  - Desktop Tools and Development Environment



# Resources

- Much like learning a new spoken language, computer languages have their own syntax and grammar that will be unfamiliar at first, but get easier and easier over time
  - There are many ways to accomplish the same task
  - You can quickly become a data magician
- The way to learn a new computer language is to practice speaking it
  - The ~30 commands you have seen today can be combined together into an infinite number of combinations
  - Lots of good resources available online:
    - [http://www.molvis.indiana.edu/app\\_guide/unix\\_commands.html](http://www.molvis.indiana.edu/app_guide/unix_commands.html)
    - <http://tldp.org/LDP/abs/html/index.html>
    - <http://stackoverflow.com/>
    - <http://google.com>
- WARNING: Computers can be very unforgiving
  - `'rm -rf /'` <= delete every file on your computer
  - `'cp junk.doc thesis.doc'` <= overwrite your thesis with junk.doc
  - `'cat results.partial > results.all'` <= oops, should have appended with >>