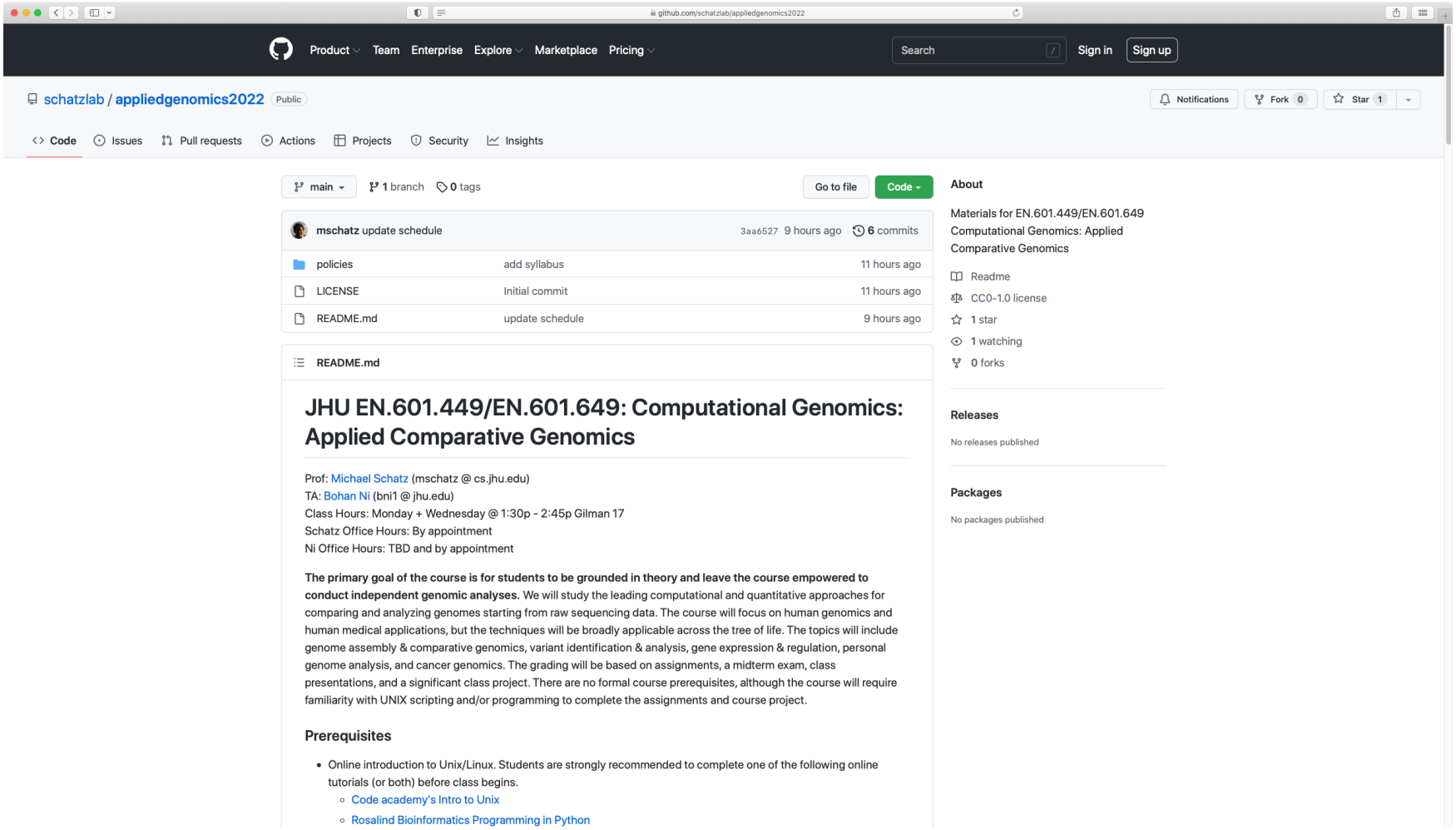
Genomic Technologies

Michael Schatz

August 31, 2022

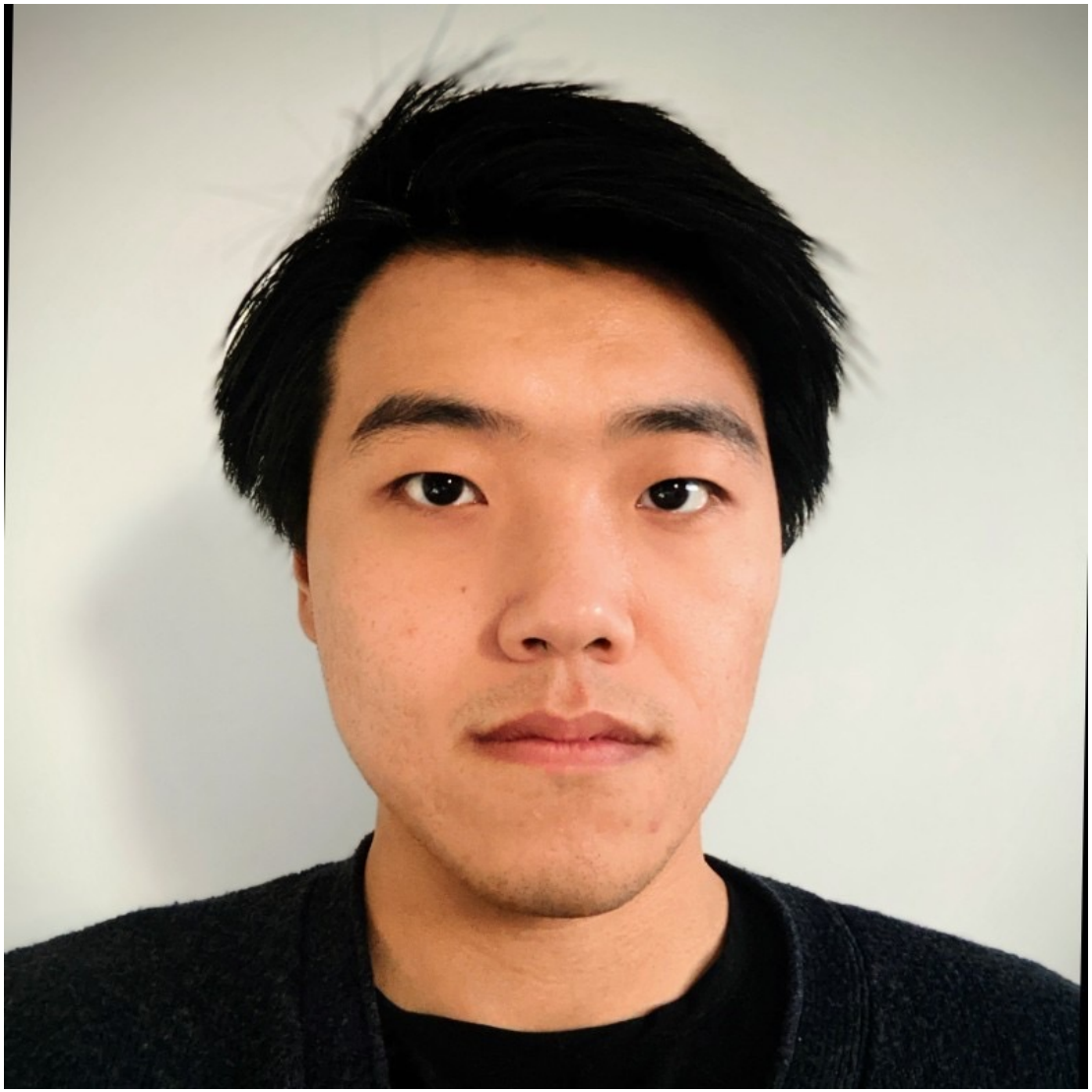
Lecture 2: Applied Comparative Genomics

Course Webpage



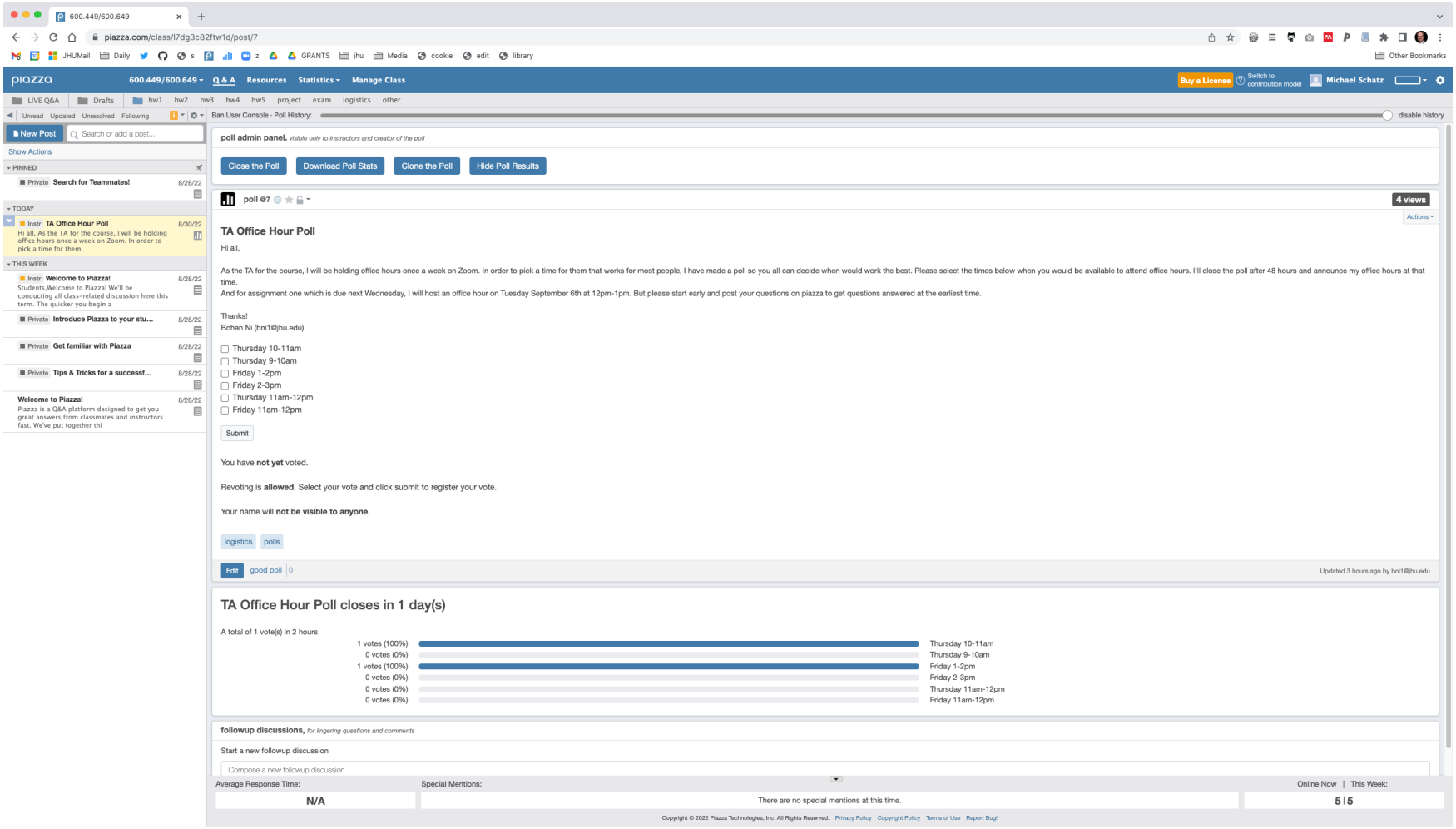
https://github.com/schatzlab/appliedgenomics2022

TA: Bohan Ni



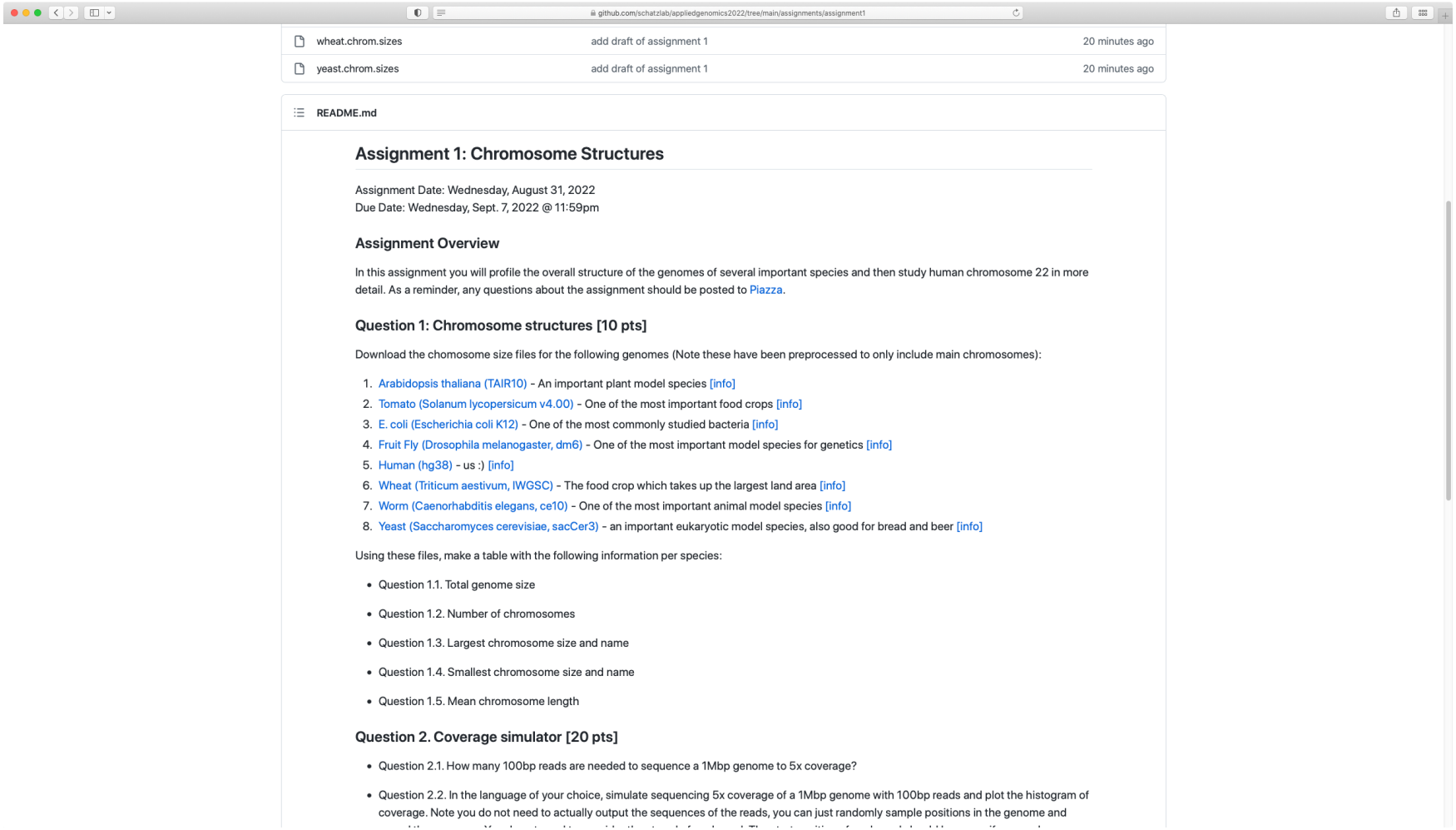
Check Piazza for Office Hours Poll

TA: Bohan Ni



Check Piazza for Office Hours Poll

Assignment 1



github.com/schatzlab/appliedgenomics2022/tree/main/assignments/assignment1

wheat.chrom.sizes	add draft of assignment 1	20 minutes ago
yeast.chrom.sizes	add draft of assignment 1	20 minutes ago

README.md

Assignment 1: Chromosome Structures

Assignment Date: Wednesday, August 31, 2022
Due Date: Wednesday, Sept. 7, 2022 @ 11:59pm

Assignment Overview

In this assignment you will profile the overall structure of the genomes of several important species and then study human chromosome 22 in more detail. As a reminder, any questions about the assignment should be posted to Piazza.

Question 1: Chromosome structures [10 pts]

Download the chomosome size files for the following genomes (Note these have been preprocessed to only include main chromosomes):

1. Arabidopsis thaliana (TAIR10) - An important plant model species [info]
2. Tomato (Solanum lycopersicum v4.00) - One of the most important food crops [info]
3. E. coli (Escherichia coli K12) - One of the most commonly studied bacteria [info]
4. Fruit Fly (Drosophila melanogaster, dm6) - One of the most important model species for genetics [info]
5. Human (hg38) - us :) [info]
6. Wheat (Triticum aestivum, IWGSC) - The food crop which takes up the largest land area [info]
7. Worm (Caenorhabditis elegans, ce10) - One of the most important animal model species [info]
8. Yeast (Saccharomyces cerevisiae, sacCer3) - an important eukaryotic model species, also good for bread and beer [info]

Using these files, make a table with the following information per species:

- Question 1.1. Total genome size
- Question 1.2. Number of chromosomes
- Question 1.3. Largest chromosome size and name
- Question 1.4. Smallest chromosome size and name
- Question 1.5. Mean chromosome length

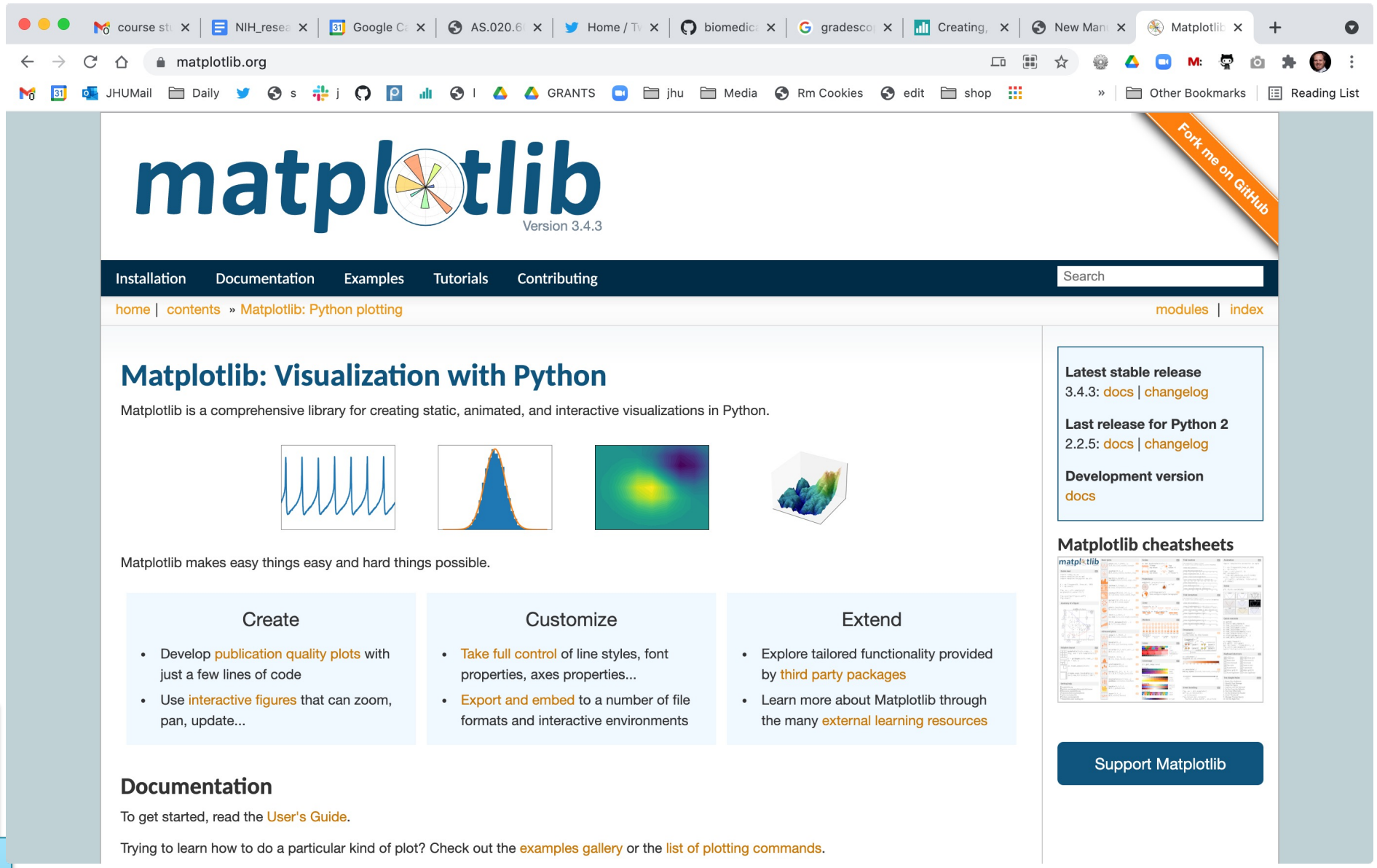
Question 2. Coverage simulator [20 pts]

- Question 2.1. How many 100bp reads are needed to sequence a 1Mbp genome to 5x coverage?
- Question 2.2. In the language of your choice, simulate sequencing 5x coverage of a 1Mbp genome with 100bp reads and plot the histogram of coverage. Note you do not need to actually output the sequences of the reads, you can just randomly sample positions in the genome and

https://github.com/schatzlab/appliedgenomics2022/tree/main/assignments/assignment1

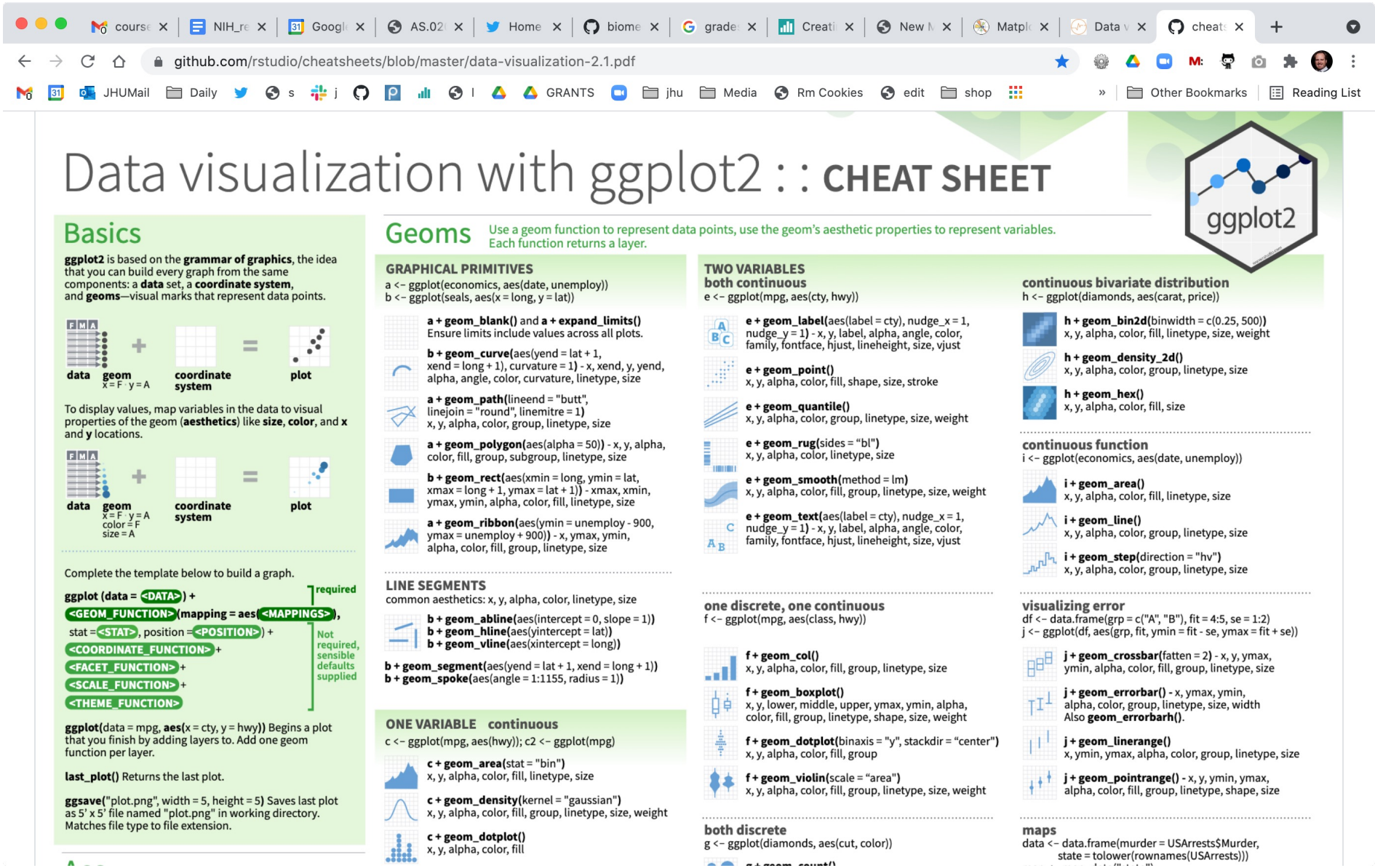
Due end of day on Sept 7 (right before midnight)

Plotting in Python



https://matplotlib.org/

Plotting in R / ggplot2



github.com/rstudio/cheatsheets/blob/master/data-visualization-2.1.pdf

Data visualization with ggplot2 : : CHEAT SHEET

Basics

ggplot2 is based on the **grammar of graphics**, the idea that you can build every graph from the same components: a **data** set, a **coordinate system**, and **geoms**—visual marks that represent data points.

data + geom (x = F, y = A) = coordinate system → plot

To display values, map variables in the data to visual properties of the geom (**aesthetics**) like **size**, **color**, and **x** and **y** locations.

data + geom (x = F, y = A, color = F, size = A) = coordinate system → plot

Complete the template below to build a graph.

```
ggplot (data = <DATA>) +                                   required
<GEOM_FUNCTION>(mapping = aes(<MAPPINGS>),
 stat = <STAT>, position = <POSITION>) +                   Not required,
<COORDINATE_FUNCTION> +                                    sensible
<FACET_FUNCTION> +                                         defaults
<SCALE_FUNCTION> +                                         supplied
<THEME_FUNCTION>
```

ggplot(data = mpg, **aes(**x = cty, y = hwy**))** Begins a plot that you finish by adding layers to. Add one geom function per layer.

last_plot() Returns the last plot.

ggsave("plot.png", width = 5, height = 5**)** Saves last plot as 5' x 5' file named "plot.png" in working directory. Matches file type to file extension.

Geoms

Use a geom function to represent data points, use the geom's aesthetic properties to represent variables. Each function returns a layer.

GRAPHICAL PRIMITIVES

```
a <- ggplot(economics, aes(date, unemploy))
b <- ggplot(seals, aes(x = long, y = lat))
```

a + geom_blank() and **a + expand_limits()** Ensure limits include values across all plots.

b + geom_curve(aes(yend = lat + 1, xend = long + 1), curvature = 1**)** - x, xend, y, yend, alpha, angle, color, curvature, linetype, size

a + geom_path(lineend = "butt", linejoin = "round", linemitre = 1**)** x, y, alpha, color, group, linetype, size

a + geom_polygon(aes(alpha = 50)**)** - x, y, alpha, color, fill, group, subgroup, linetype, size

b + geom_rect(aes(xmin = long, ymin = lat, xmax = long + 1, ymax = lat + 1)**)** - xmax, xmin, ymax, ymin, alpha, color, fill, linetype, size

a + geom_ribbon(aes(ymin = unemploy - 900, ymax = unemploy + 900)**)** - x, ymax, ymin, alpha, color, fill, group, linetype, size

LINE SEGMENTS

common aesthetics: x, y, alpha, color, linetype, size

b + geom_abline(aes(intercept = 0, slope = 1)**)**
b + geom_hline(aes(yintercept = lat)**)**
b + geom_vline(aes(xintercept = long)**)**

b + geom_segment(aes(yend = lat + 1, xend = long + 1)**)**
b + geom_spoke(aes(angle = 1:1155, radius = 1)**)**

ONE VARIABLE continuous

```
c <- ggplot(mpg, aes(hwy)); c2 <- ggplot(mpg)
```

c + geom_area(stat = "bin"**)** x, y, alpha, color, fill, linetype, size

c + geom_density(kernel = "gaussian"**)** x, y, alpha, color, fill, group, linetype, size, weight

c + geom_dotplot() x, y, alpha, color, fill

TWO VARIABLES both continuous

```
e <- ggplot(mpg, aes(cty, hwy))
```

e + geom_label(aes(label = cty), nudge_x = 1, nudge_y = 1**)** - x, y, label, alpha, angle, color, family, fontface, hjust, lineheight, size, vjust

e + geom_point() x, y, alpha, color, fill, shape, size, stroke

e + geom_quantile() x, y, alpha, color, group, linetype, size, weight

e + geom_rug(sides = "bl"**)** x, y, alpha, color, linetype, size

e + geom_smooth(method = lm**)** x, y, alpha, color, fill, group, linetype, size, weight

e + geom_text(aes(label = cty), nudge_x = 1, nudge_y = 1**)** - x, y, label, alpha, angle, color, family, fontface, hjust, lineheight, size, vjust

one discrete, one continuous

```
f <- ggplot(mpg, aes(class, hwy))
```

f + geom_col() x, y, alpha, color, fill, group, linetype, size

f + geom_boxplot() x, y, lower, middle, upper, ymax, ymin, alpha, color, fill, group, linetype, shape, size, weight

f + geom_dotplot(binaxis = "y", stackdir = "center"**)** x, y, alpha, color, fill, group

f + geom_violin(scale = "area"**)** x, y, alpha, color, fill, group, linetype, size, weight

both discrete

```
g <- ggplot(diamonds, aes(cut, color))
```

continuous bivariate distribution

```
h <- ggplot(diamonds, aes(carat, price))
```

h + geom_bin2d(binwidth = c(0.25, 500)**)** x, y, alpha, color, fill, linetype, size, weight

h + geom_density_2d() x, y, alpha, color, group, linetype, size

h + geom_hex() x, y, alpha, color, fill, size

continuous function

```
i <- ggplot(economics, aes(date, unemploy))
```

i + geom_area() x, y, alpha, color, fill, linetype, size

i + geom_line() x, y, alpha, color, group, linetype, size

i + geom_step(direction = "hv"**)** x, y, alpha, color, group, linetype, size

visualizing error

```
df <- data.frame(grp = c("A", "B"), fit = 4:5, se = 1:2)
j <- ggplot(df, aes(grp, fit, ymin = fit - se, ymax = fit + se))
```

j + geom_crossbar(fatten = 2**)** - x, y, ymax, ymin, alpha, color, fill, group, linetype, size

j + geom_errorbar() - x, ymax, ymin, alpha, color, group, linetype, size, width Also **geom_errorbarh()**.

j + geom_linerange() x, ymin, ymax, alpha, color, group, linetype, size

j + geom_pointrange() - x, y, ymin, ymax, alpha, color, fill, group, linetype, shape, size

maps

```
data <- data.frame(murder = USArrests$Murder,
                   state = tolower(rownames(USArrests)))
```

https://ggplot2.tidyverse.org/

Unsolved Questions in Biology

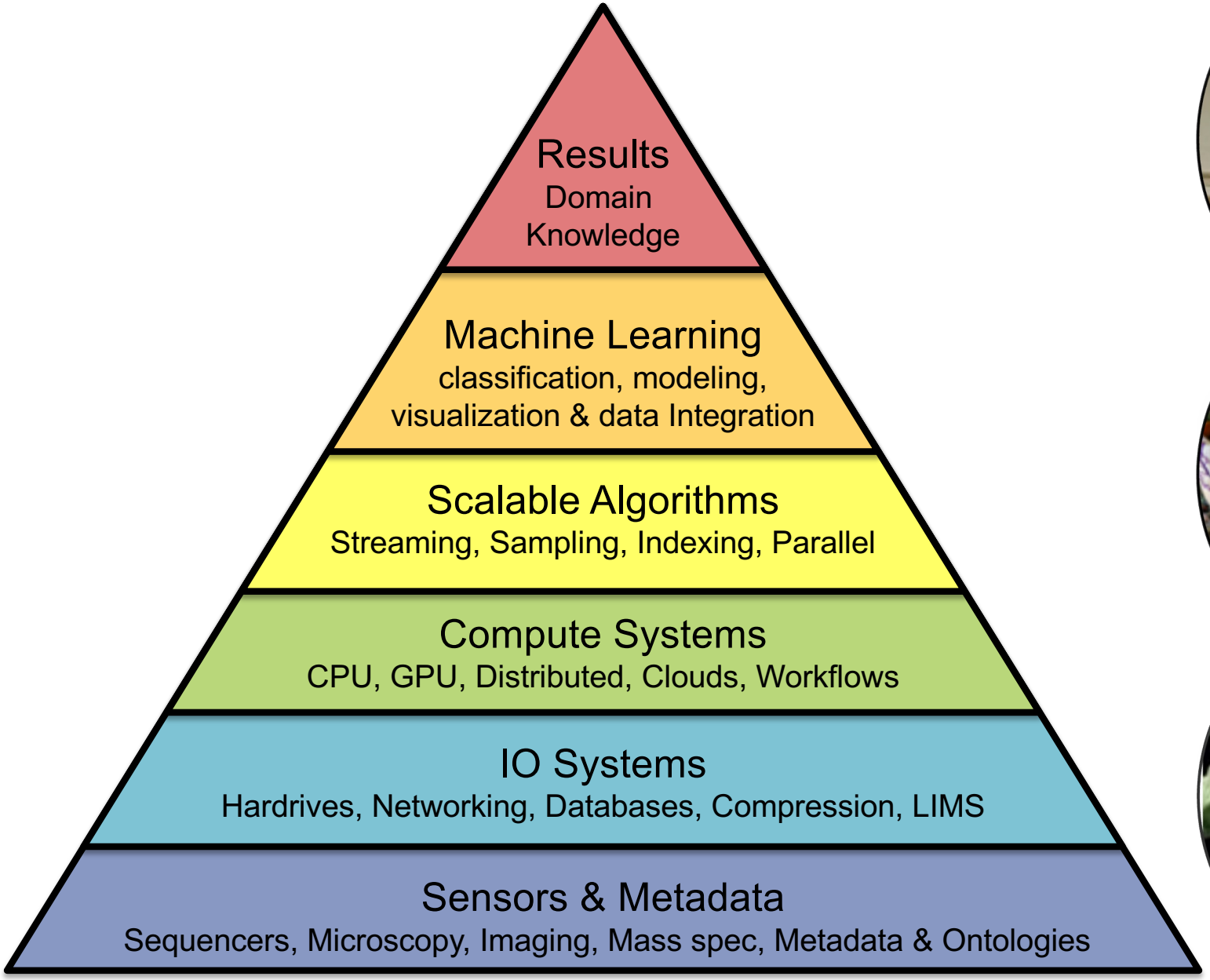
- What is your genome sequence?
-
-
-
-
-
-
-
-
-
-
-
-
-
- ***Plus thousands and thousands more***

The instruments provide the data, but none of the answers to any of these questions.

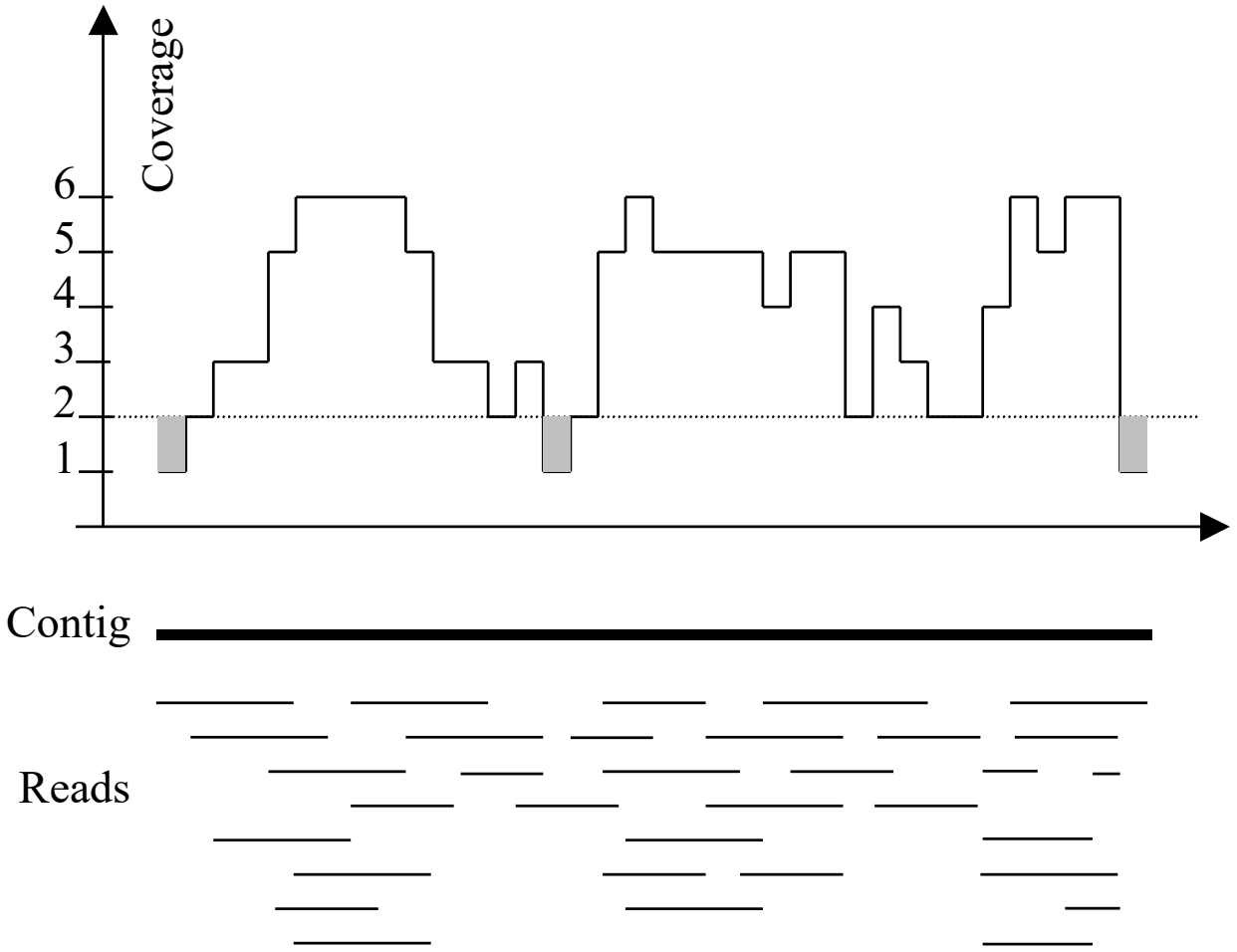
What software and systems will?

And who will create them?

Comparative Genomics Technologies



Typical sequencing coverage

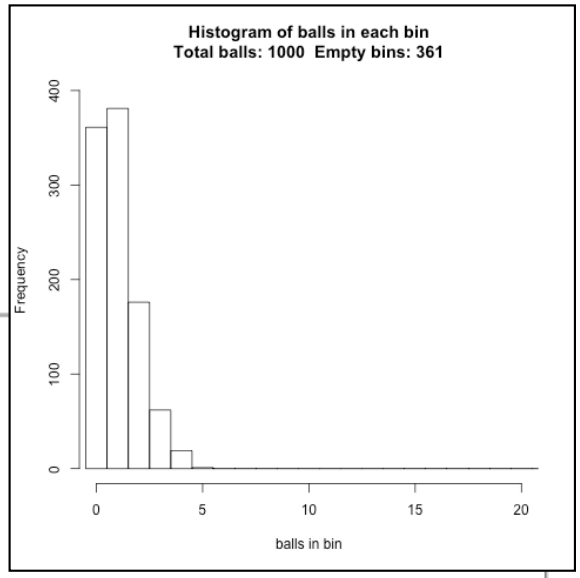


Imagine raindrops on a sidewalk

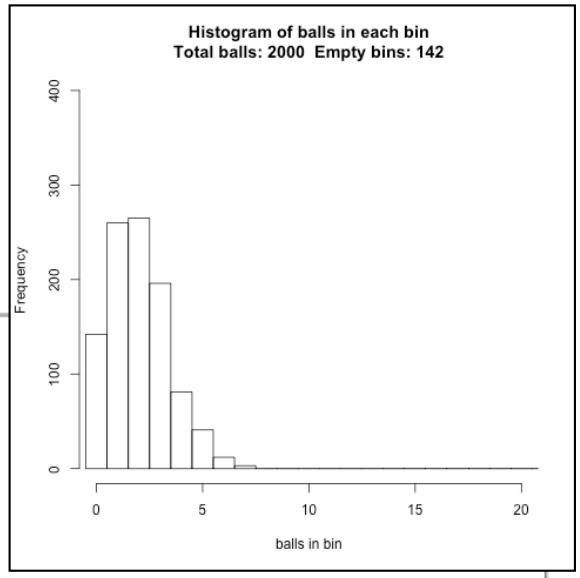
We want to cover the entire sidewalk but each drop costs $1

If the genome is 10 Mbp, should we sequence 100k 100bp reads?

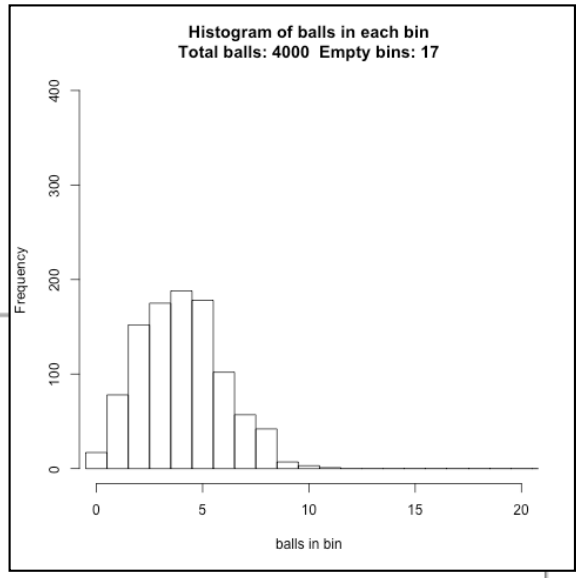
1x sequencing



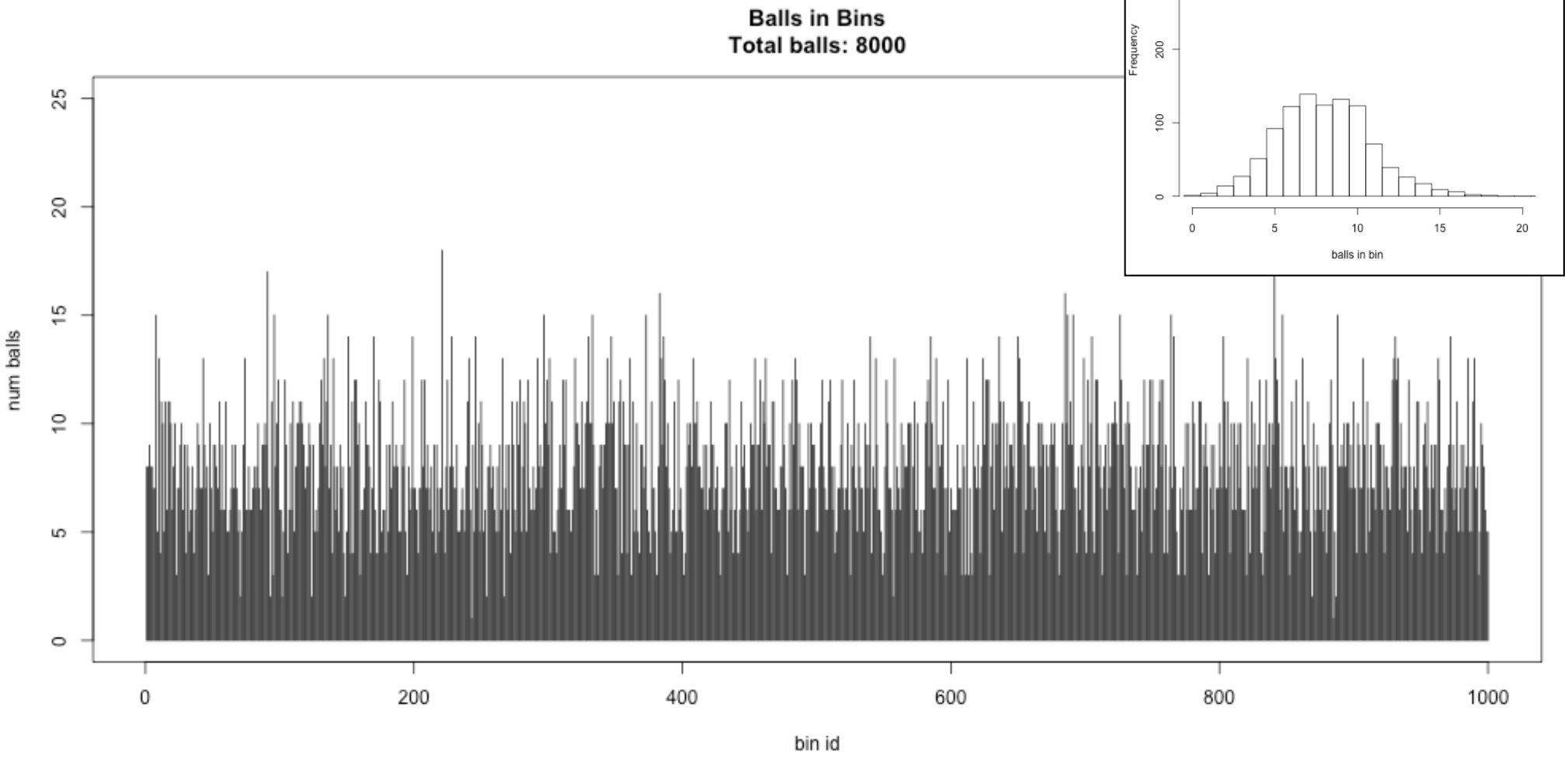
2x sequencing



4x sequencing



8x sequencing



Poisson Distribution

The probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.

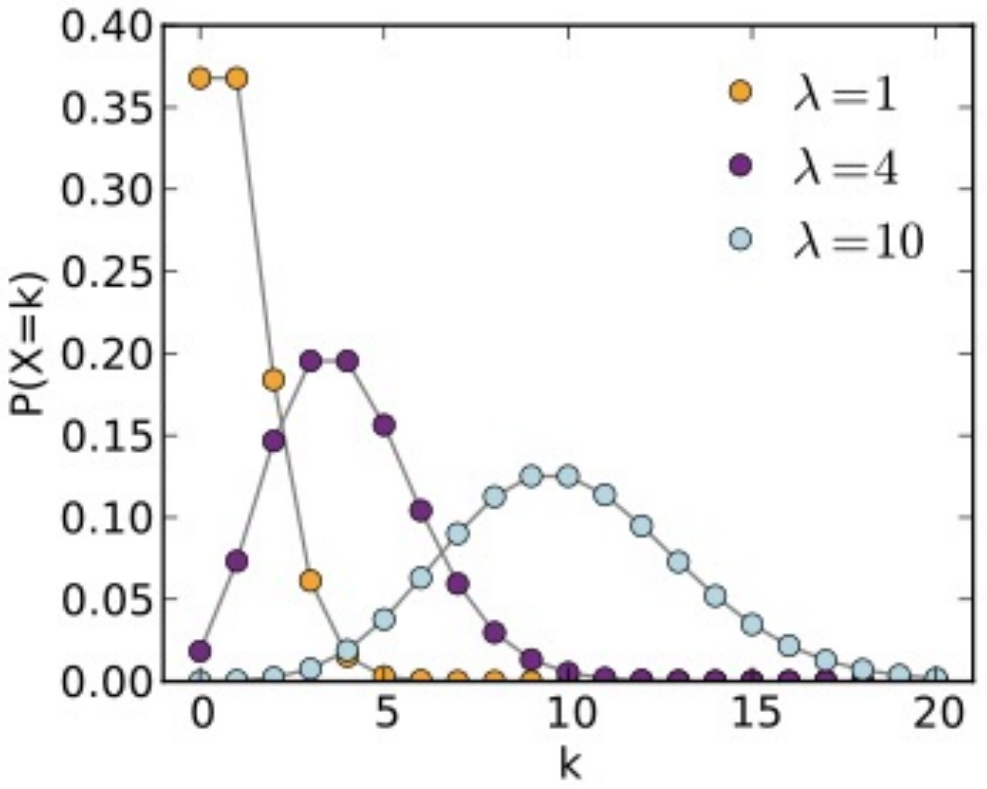
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

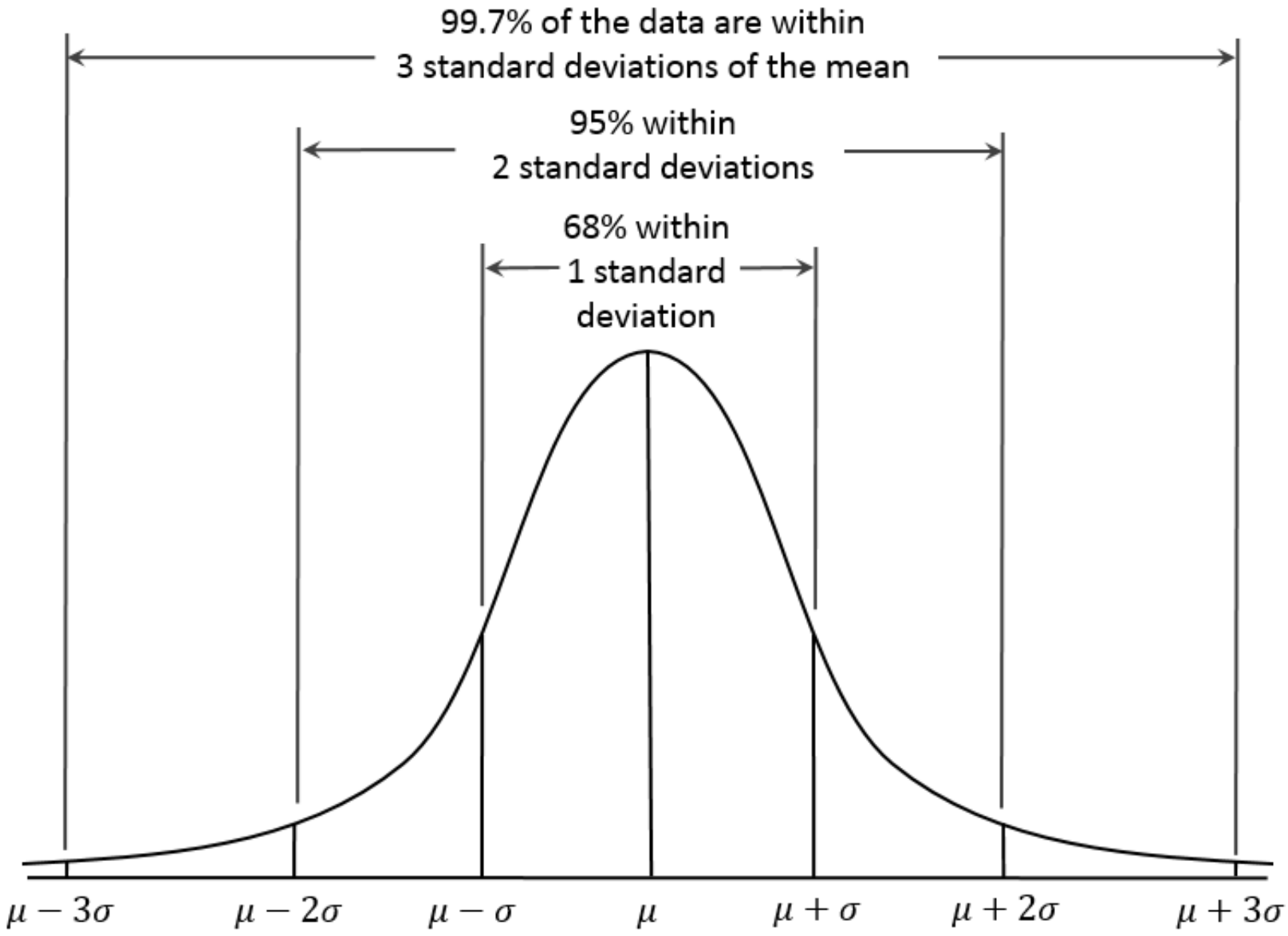
Resembles a normal distribution, but over the positive values, and with only a single parameter.

Key properties:

- ***The standard deviation is the square root of the mean.***
- ***For mean > 5, well approximated by a normal distribution***



Normal Approximation



Can estimate Poisson distribution as a normal distribution when $\lambda > 10$

Pop Quiz!

I want to sequence a 10Mbp genome to 24x coverage.
How many 120bp reads do I need?

I need 10Mbp x 24x = 240Mbp of data
240Mbp / 120bp / read = 2M reads

I want to sequence a 10Mbp genome so that
>97.5% of the genome has at least 24x coverage.
How many 120bp reads do I need?

Find X such that X-2*sqrt(X) = 24

36-2*sqrt(36) = 24

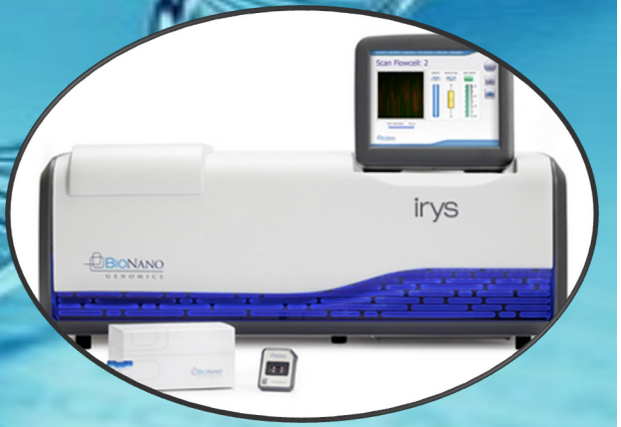
I need 10Mbp x 36x = 360Mbp of data
360Mbp / 120bp / read = 3M reads

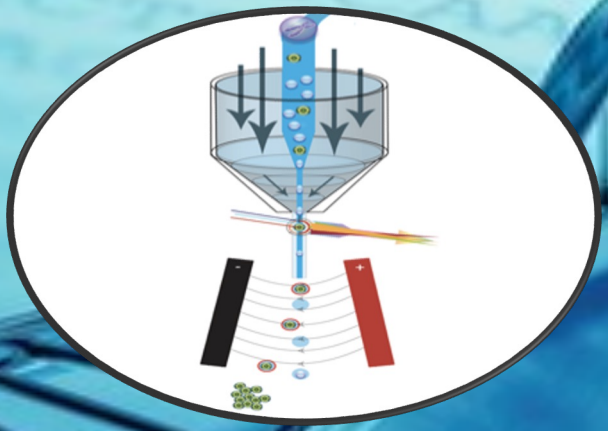
Genomics Arsenal in the year 2022

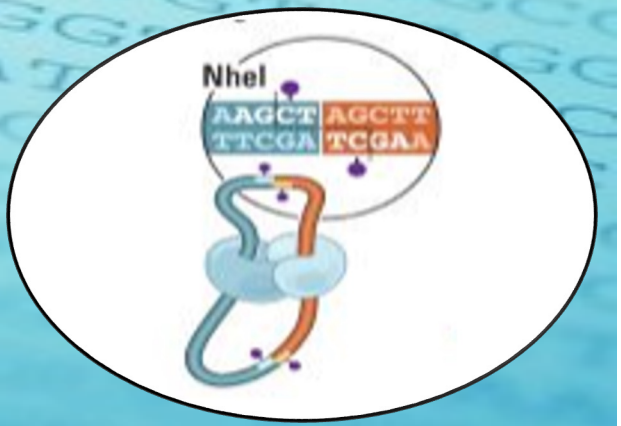
Sample Preparation

Sequencing

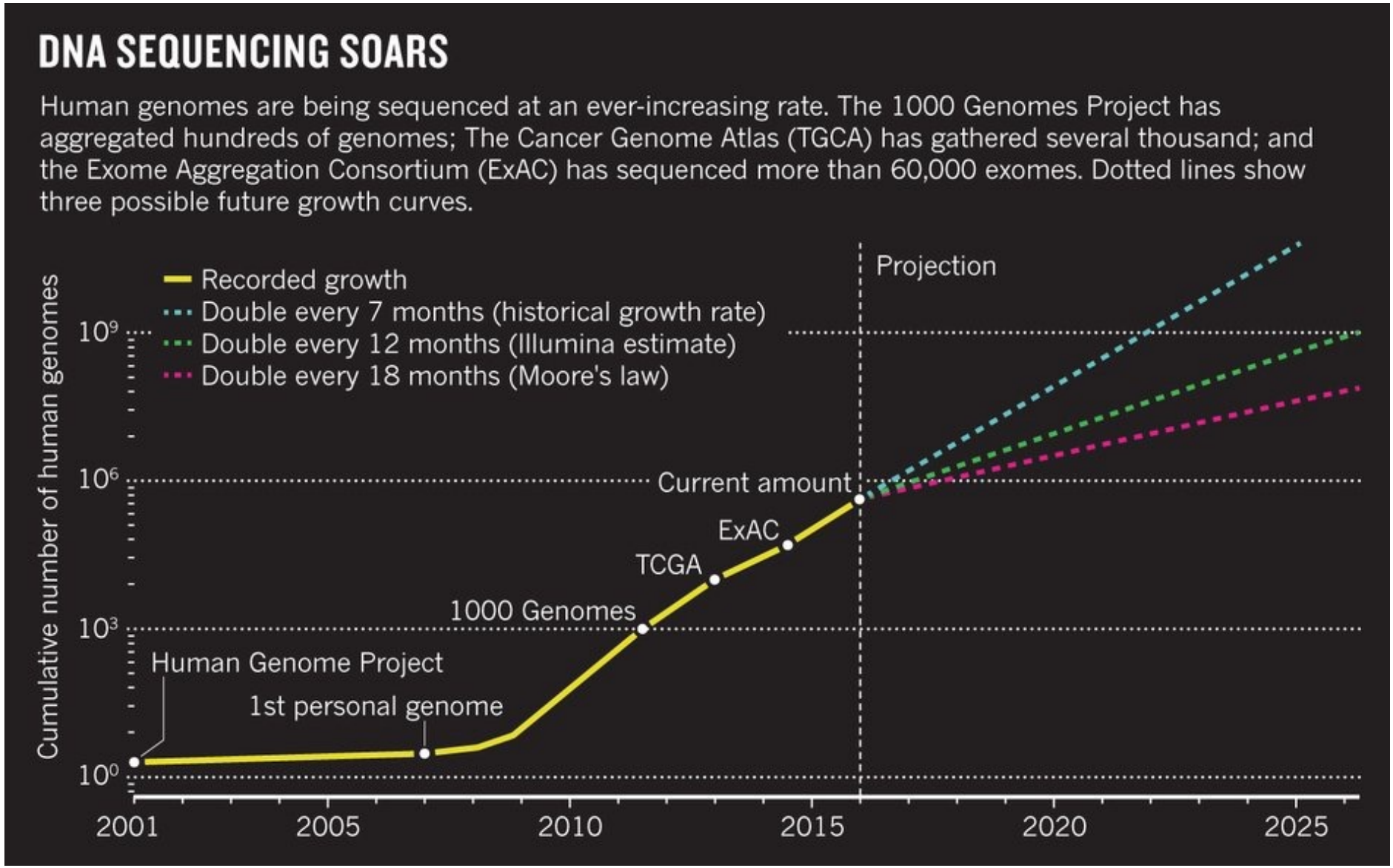
Chromosome Mapping







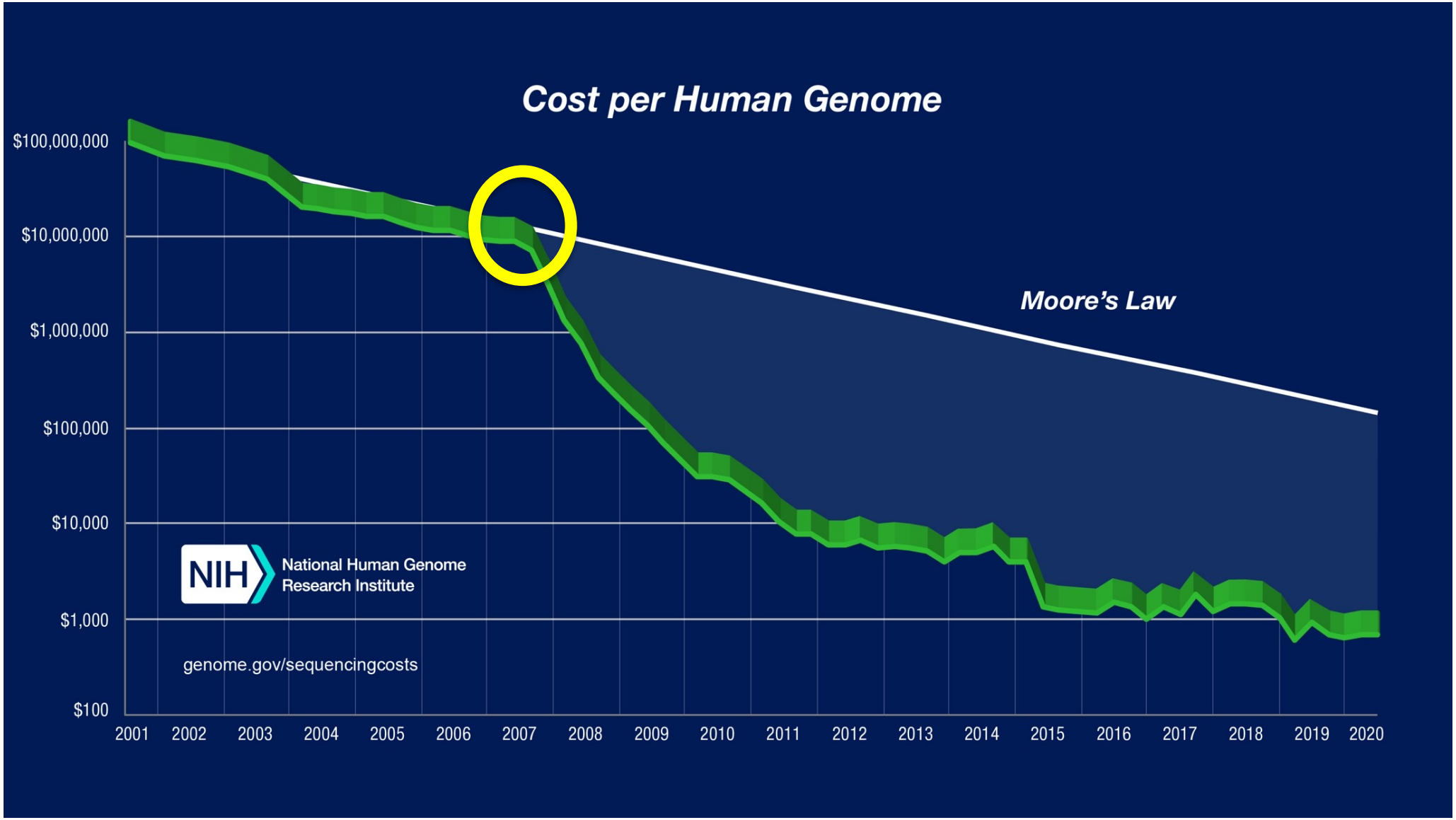
Sequencing Capacity



Big Data: Astronomical or Genomical?

Stephens, Z, et al. (2015) PLOS Biology DOI: 10.1371/journal.pbio.1002195

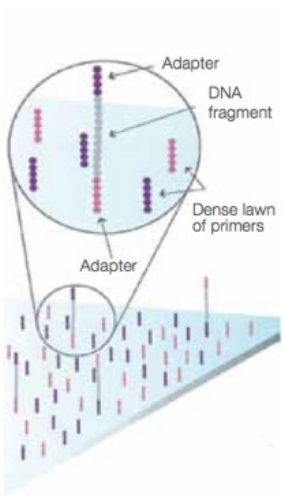
Cost per Genome



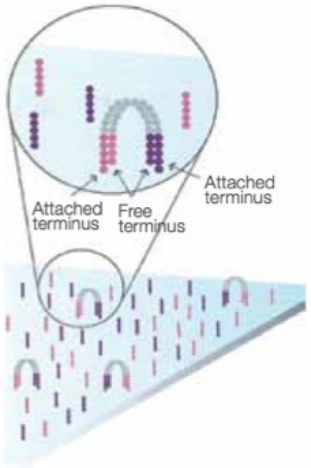
Second Generation Sequencing

Illumina NovaSeq 6000
Sequencing by Synthesis

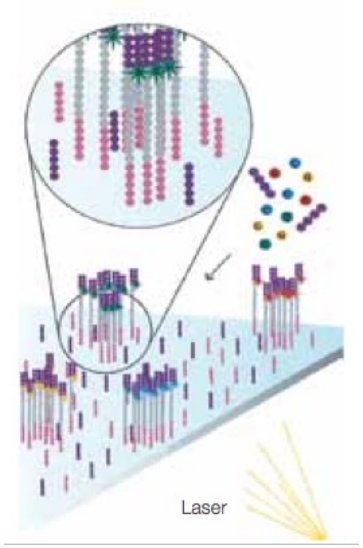
>3Tbp / day



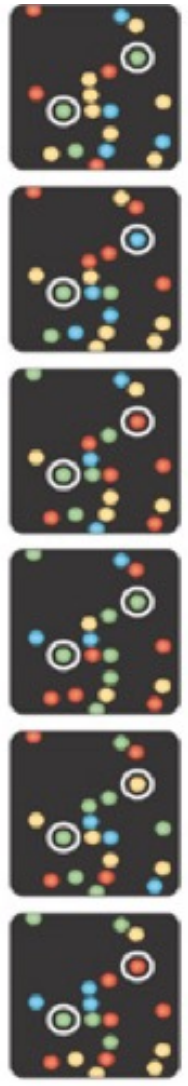
1. Attach



2. Amplify



3. Image



Metzker (2010) Nature Reviews Genetics 11:31-46
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Next Steps

1. Reflect on the magic and power of DNA ☺
2. Check out the course webpage
3. Register on Piazza
4. Work on Assignment 1
    1. Set up Linux, set up Virtual Machine, set up Ubuntu
    2. Set up Dropbox for yourself!
    3. Get comfortable on the command line