Genome Sequencing

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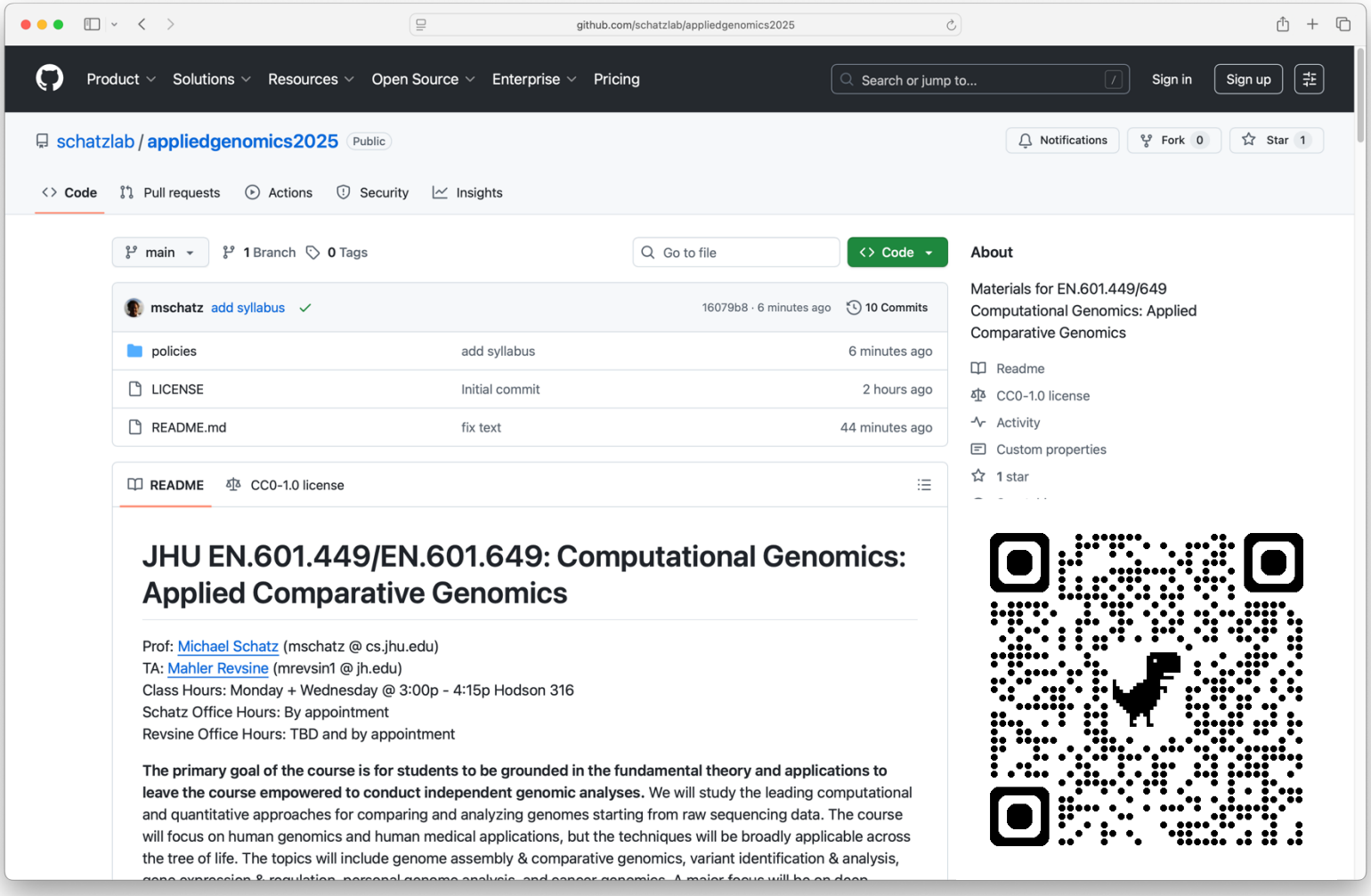
August 27 2025

Lecture 2: Applied Comparative Genomics

Course Webpage

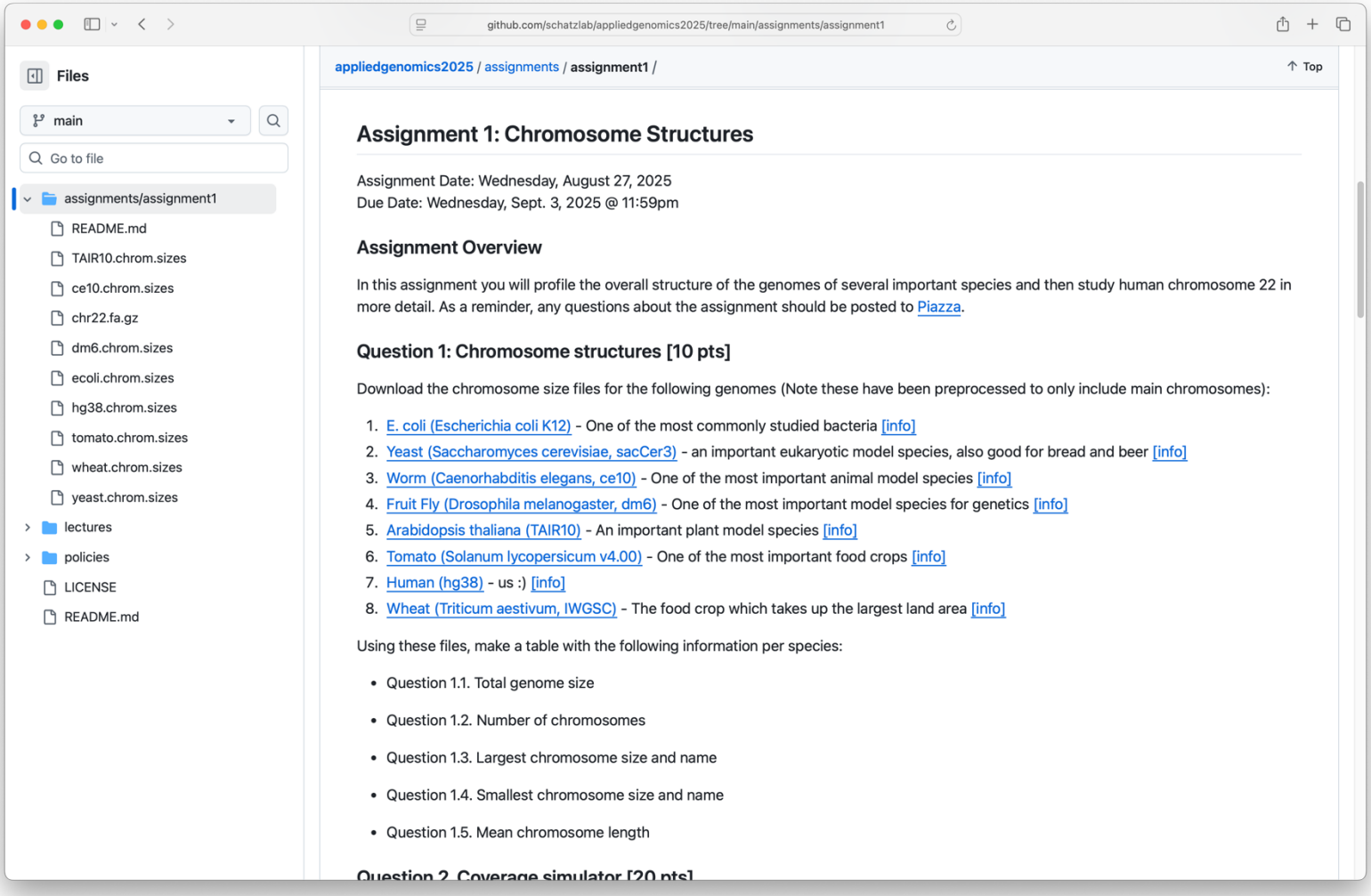


https://github.com/schatzlab/appliedgenomics2025

TA: Mahler Revsine

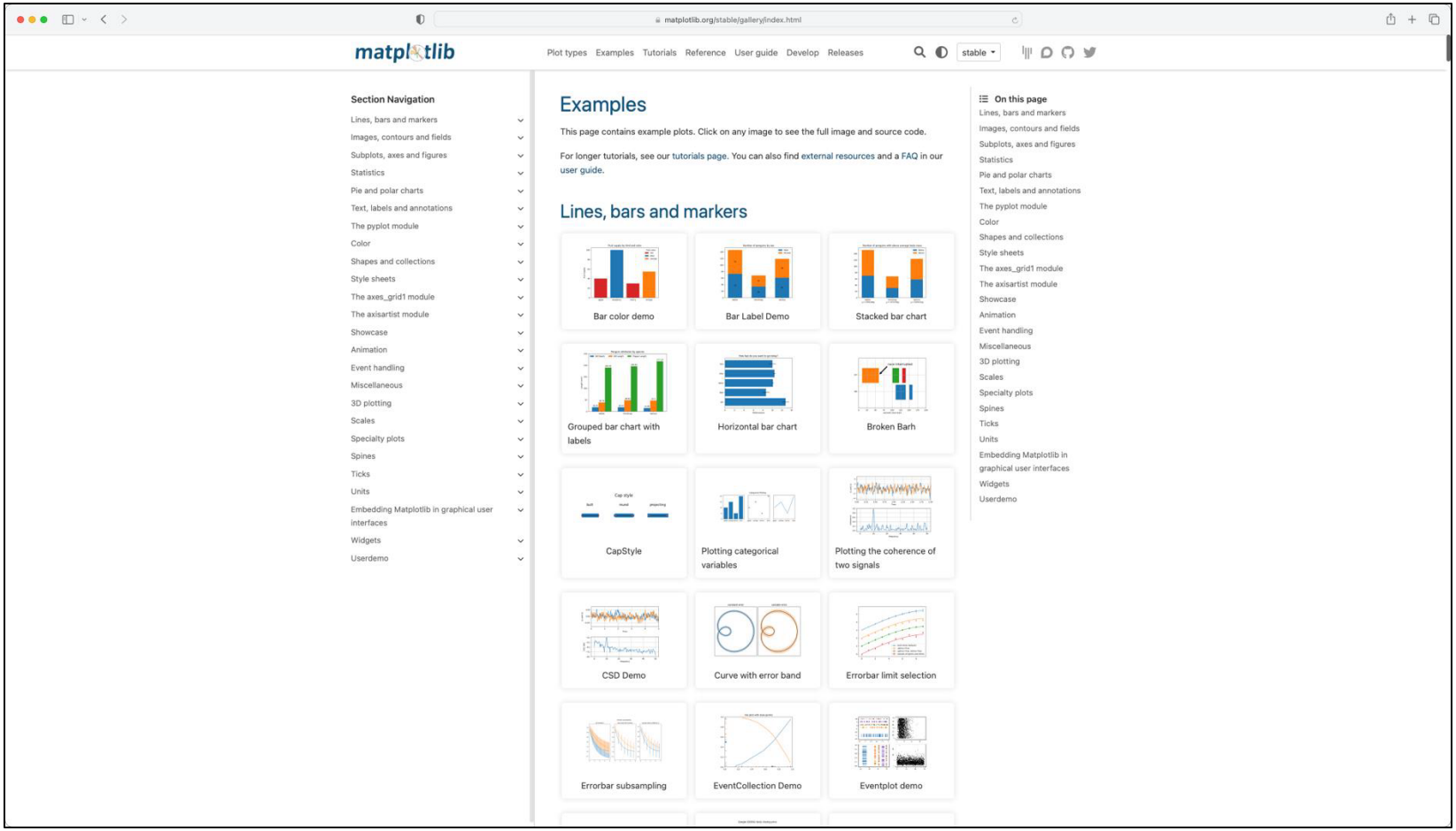
Office Hours: Mondays at 4:30pm
@ Malone 216 and Zoom

Assignment 1



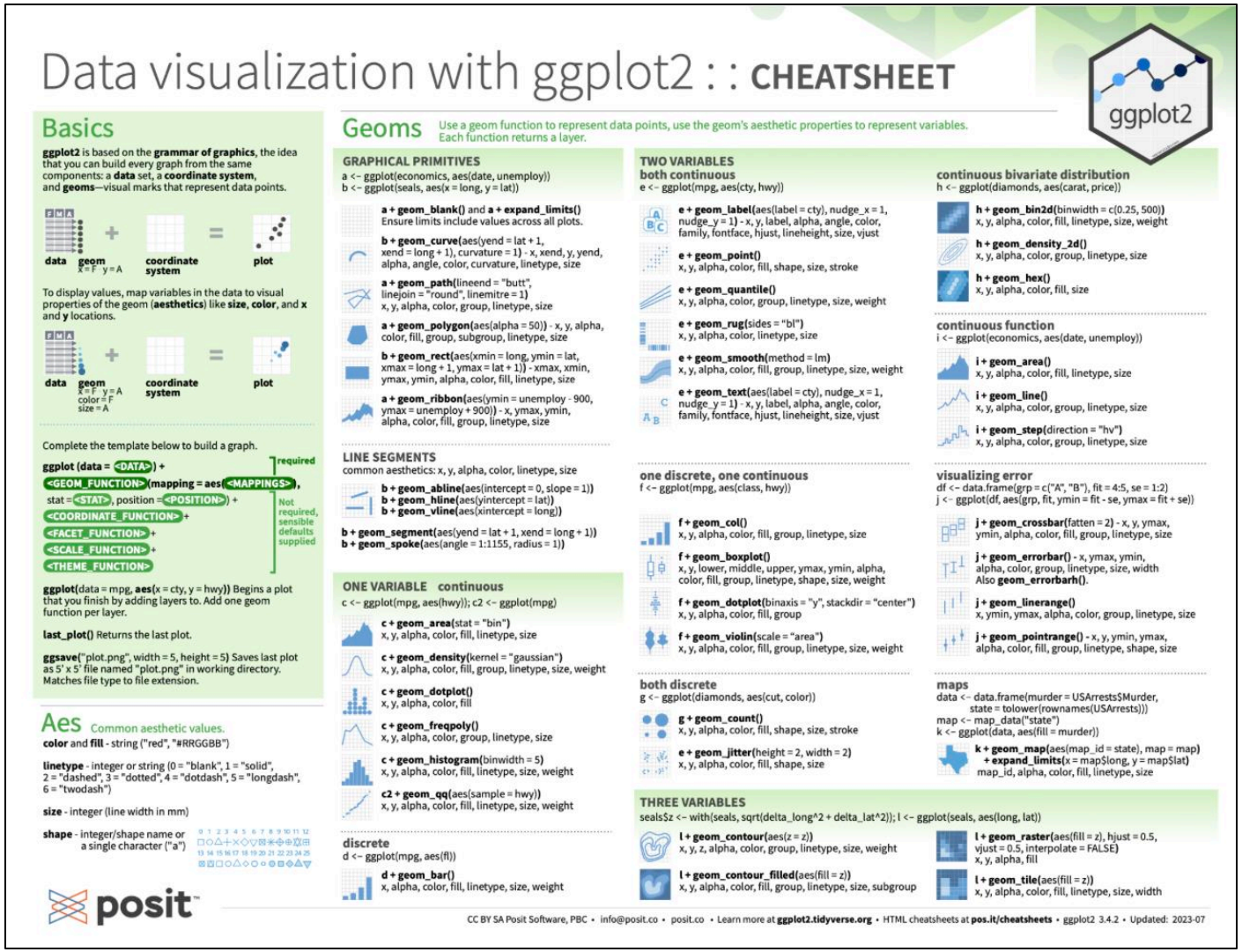
https://github.com/schatzlab/appliedgenomics2025/tree/main/assignments/assignment1
Due end of day on Wednesday Sept 3 (right before midnight)

Plotting in Python



https://matplotlib.org/

Plotting in R / ggplot2



Data visualization with ggplot2 : : CHEATSHEET

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>) +
  <GEOM_FUNCTION>(mapping = aes(<MAPPINGS>),
  stat = <STAT>, position = <POSITION>) +
  <COORDINATE_FUNCTION> +
  <FACET_FUNCTION> +
  <SCALE_FUNCTION> +
  <THEME_FUNCTION>
```

required

Not required, sensible defaults supplied

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.

Aes

Common aesthetic values.

color and **fill** - string ("red", "#RRGGBB")

linetype - integer or string (0 = "blank", 1 = "solid", 2 = "dashed", 3 = "dotted", 4 = "dotdash", 5 = "longdash", 6 = "twodash")

size - integer (line width in mm)

shape - integer/shape name or a single character ("a")

0 1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24 25

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

c + geom_freqpoly() x, y, alpha, color, group, linetype, size

c + geom_histogram(binwidth = 5**)** x, y, alpha, color, fill, linetype, size, weight

c2 + geom_qq(aes(sample = hwy)**)** x, y, alpha, color, fill, linetype, size, weight

discrete

```
d <- ggplot(mpg, aes(fl))
```

d + geom_bar() x, alpha, color, fill, linetype, size, weight

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))
```

g + geom_count() x, y, alpha, color, fill, shape, size, stroke

e + geom_jitter(height = 2, width = 2**)** x, y, alpha, color, fill, shape, size

THREE VARIABLES

```
seals$z <- with(seals, sqrt(delta_long^2 + delta_lat^2)); l <- ggplot(seals, aes(long, lat))
```

l + geom_contour(aes(z = z)**)** x, y, z, alpha, color, group, linetype, size, weight

l + geom_contour_filled(aes(fill = z)**)** x, y, alpha, color, fill, group, linetype, size, subgroup

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

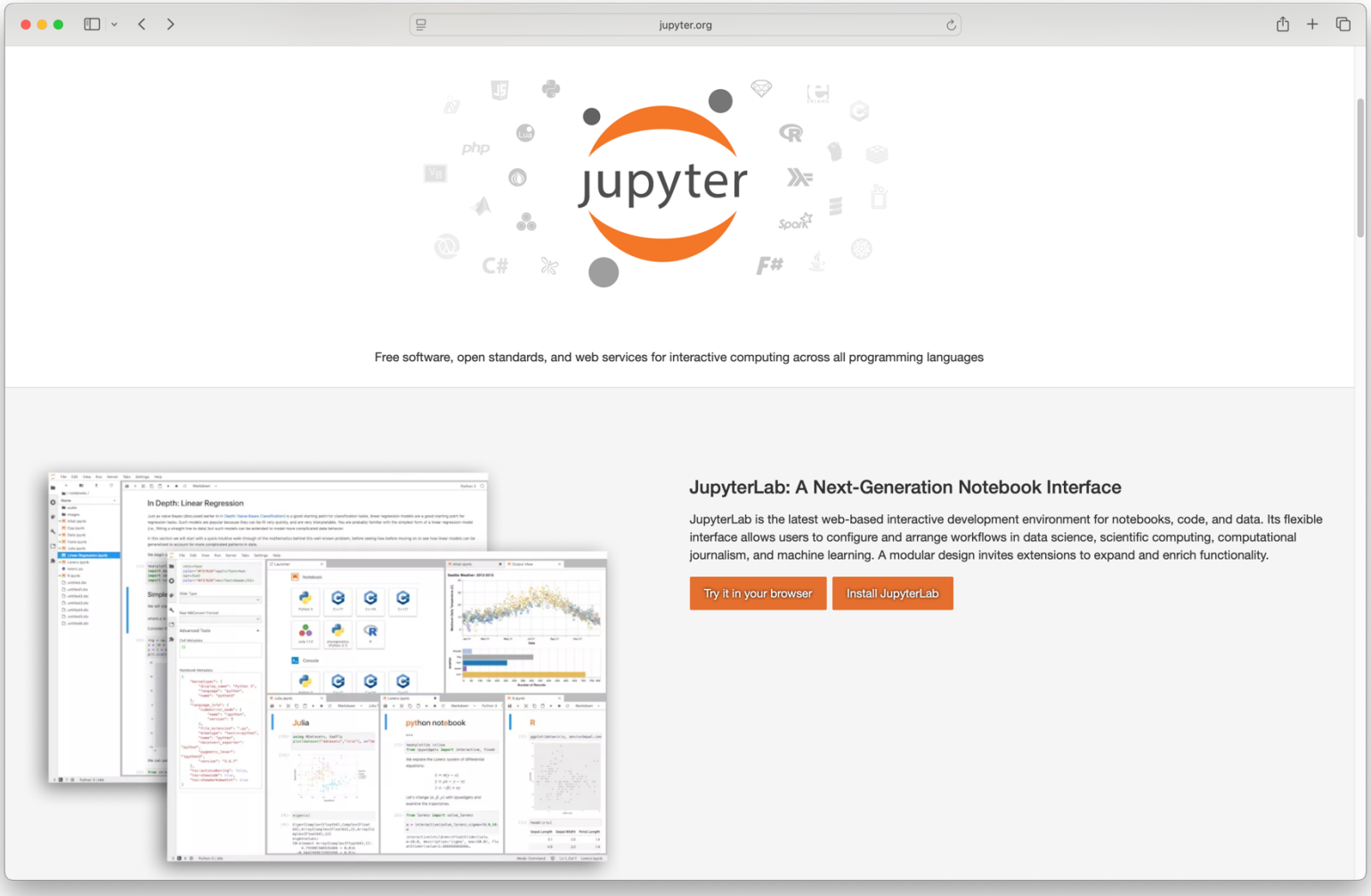
```
data <- data.frame(murder = USArrests$Murder,
  state = tolower(rownames(USArrests)))
map <- map_data("state")
k <- ggplot(data, aes(fill = murder))
```

k + geom_map(aes(map_id = state), map = map**)** **+ expand_limits(**x = map$long, y = map$lat**)** map_id, alpha, color, fill, linetype, size

posit

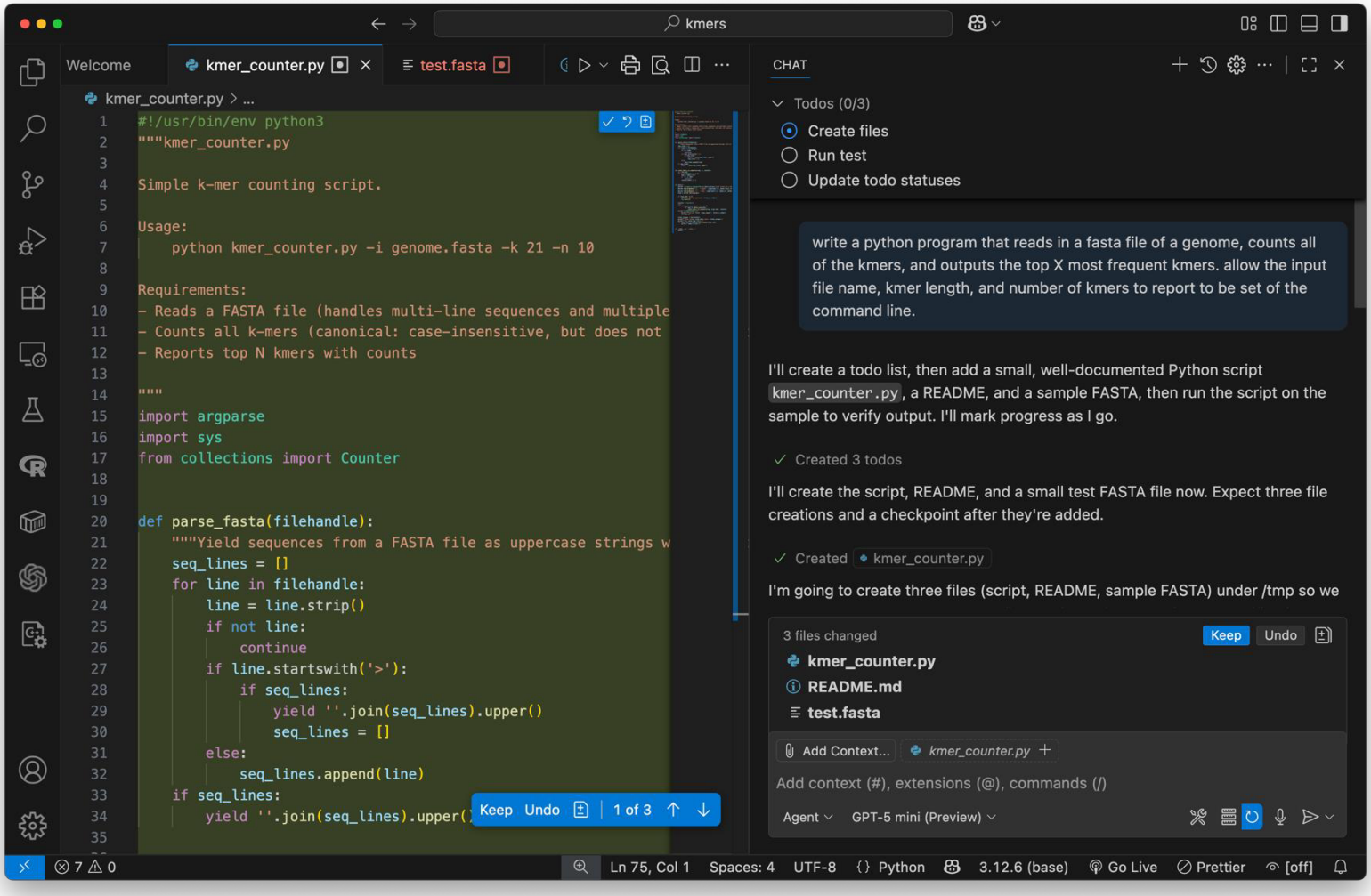
https://ggplot2.tidyverse.org/

Jupyter Notebooks



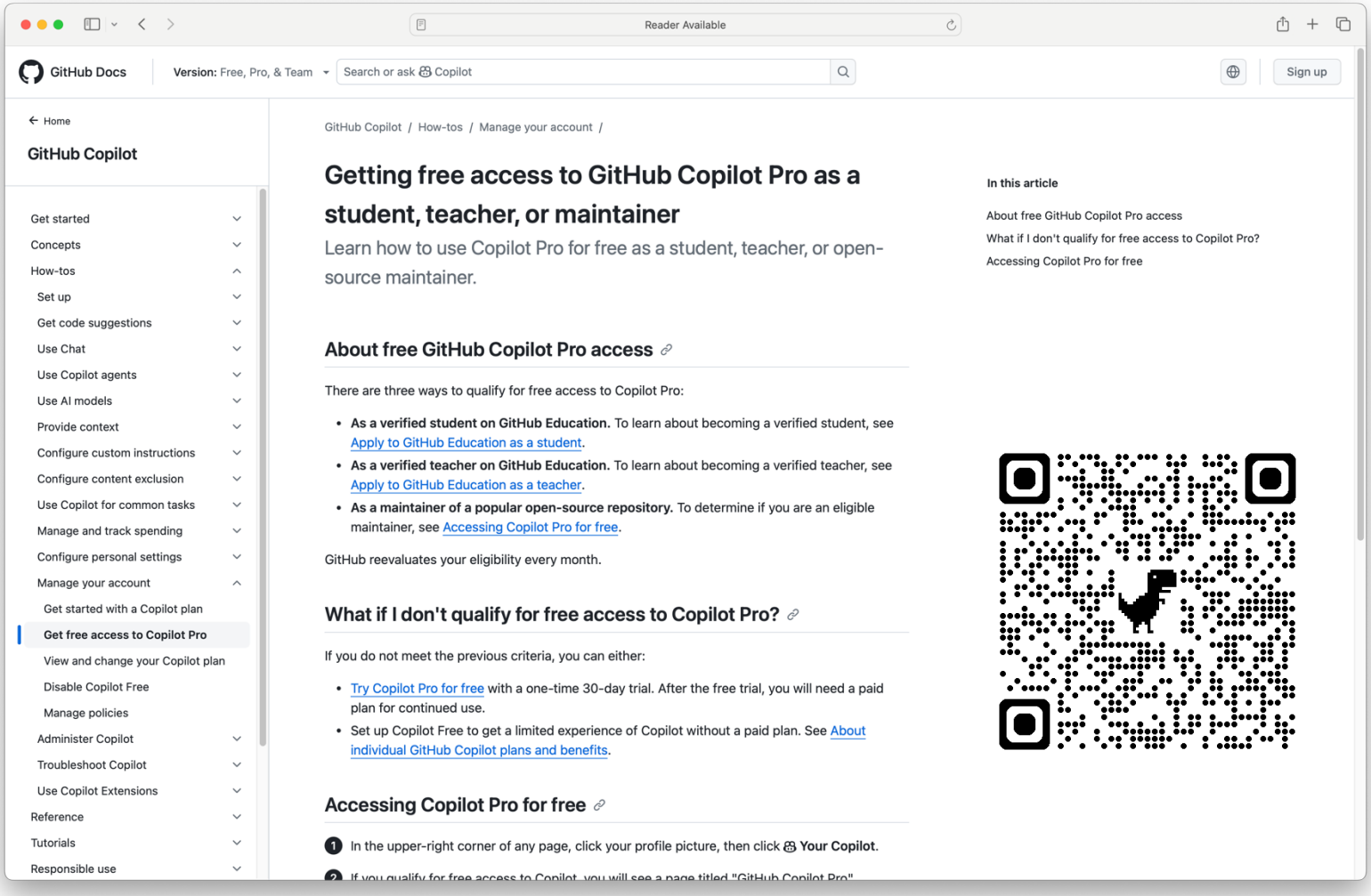
https://jupyter.org

VS Code



“write a python program that reads in a fasta file of a genome, counts all of the kmers, and outputs the top X most frequent kmers. allow the input file name, kmer length, and number of kmers to report to be set of the command line.”

Github Copilot Pro



https://docs.github.com/en/copilot/how-tos/manage-your-account/get-free-access-to-copilot-pro

Unsolved Questions in Biology

- What is your genome sequence?
- How does your genome compare to my genome?

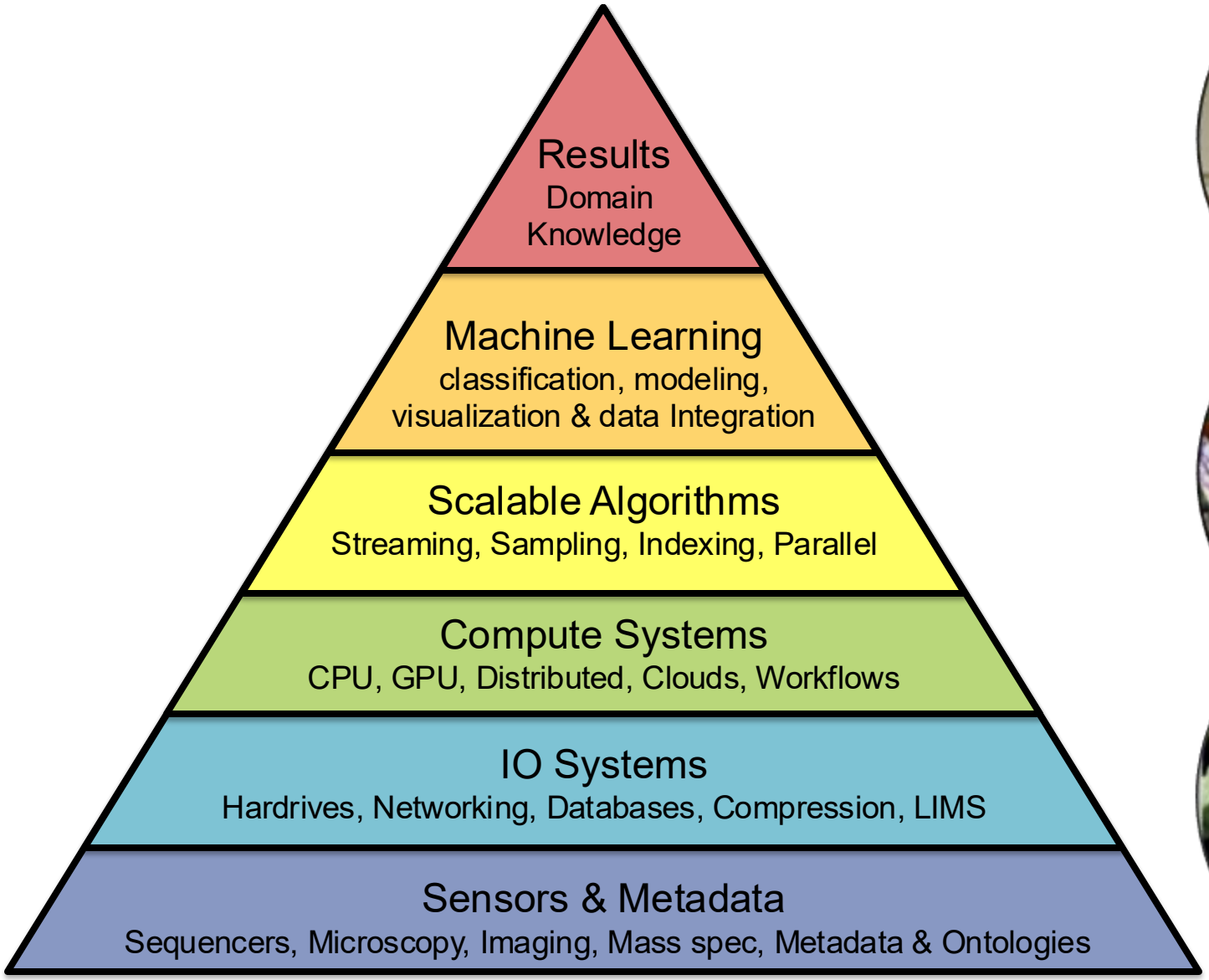
- Where are the genes and how active are they?
- How does gene activity change during development?
- How does splicing change during development?

- How does methylation change during development?
- How does chromatin change during development?
- How does is your genome folded in the cell?
- Where do proteins bind and regulate genes?

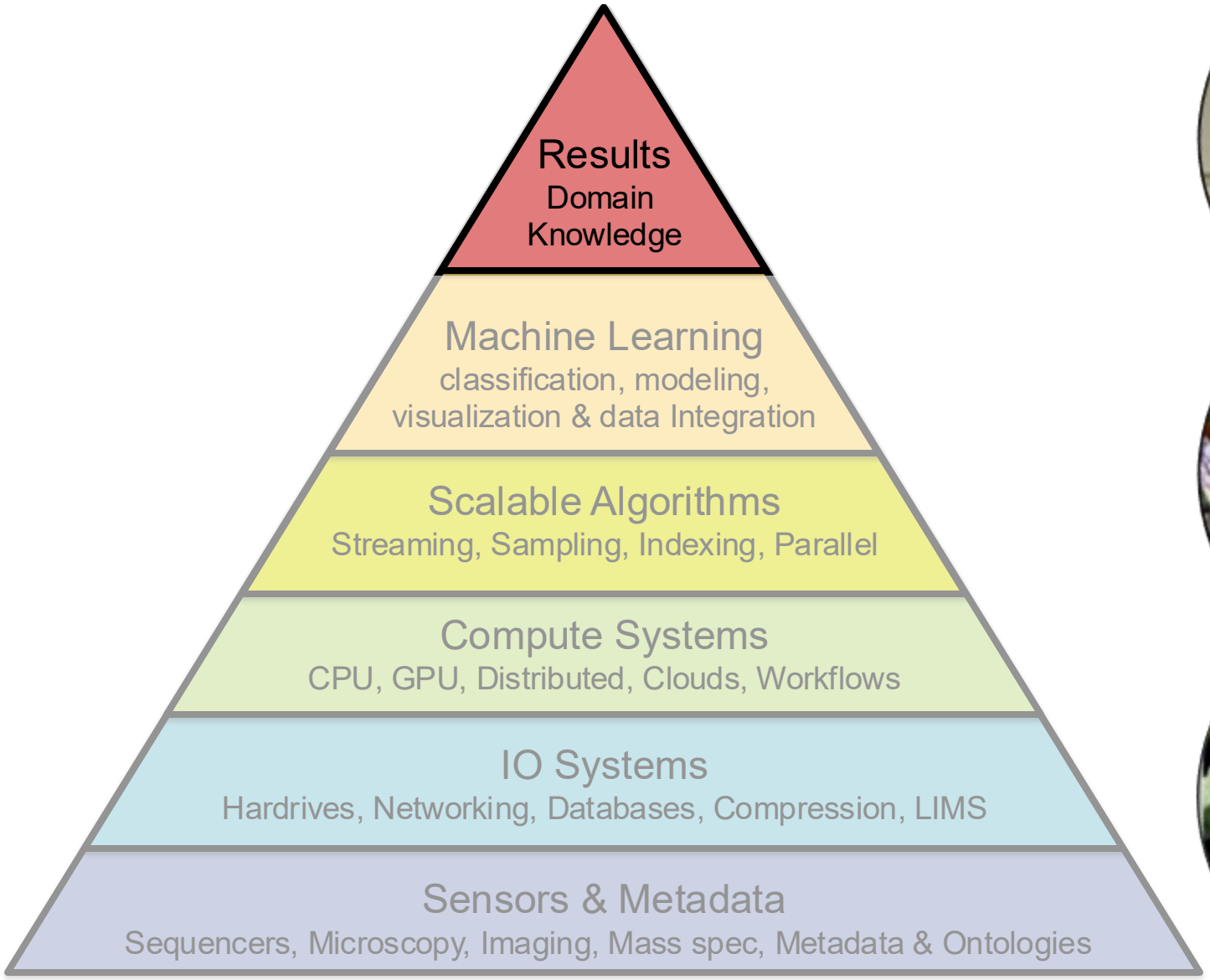
- What virus and microbes are living inside you?
- How do your mutations relate to disease?
- What drugs and treatments should we give you?

- ***Plus thousands and thousands more***

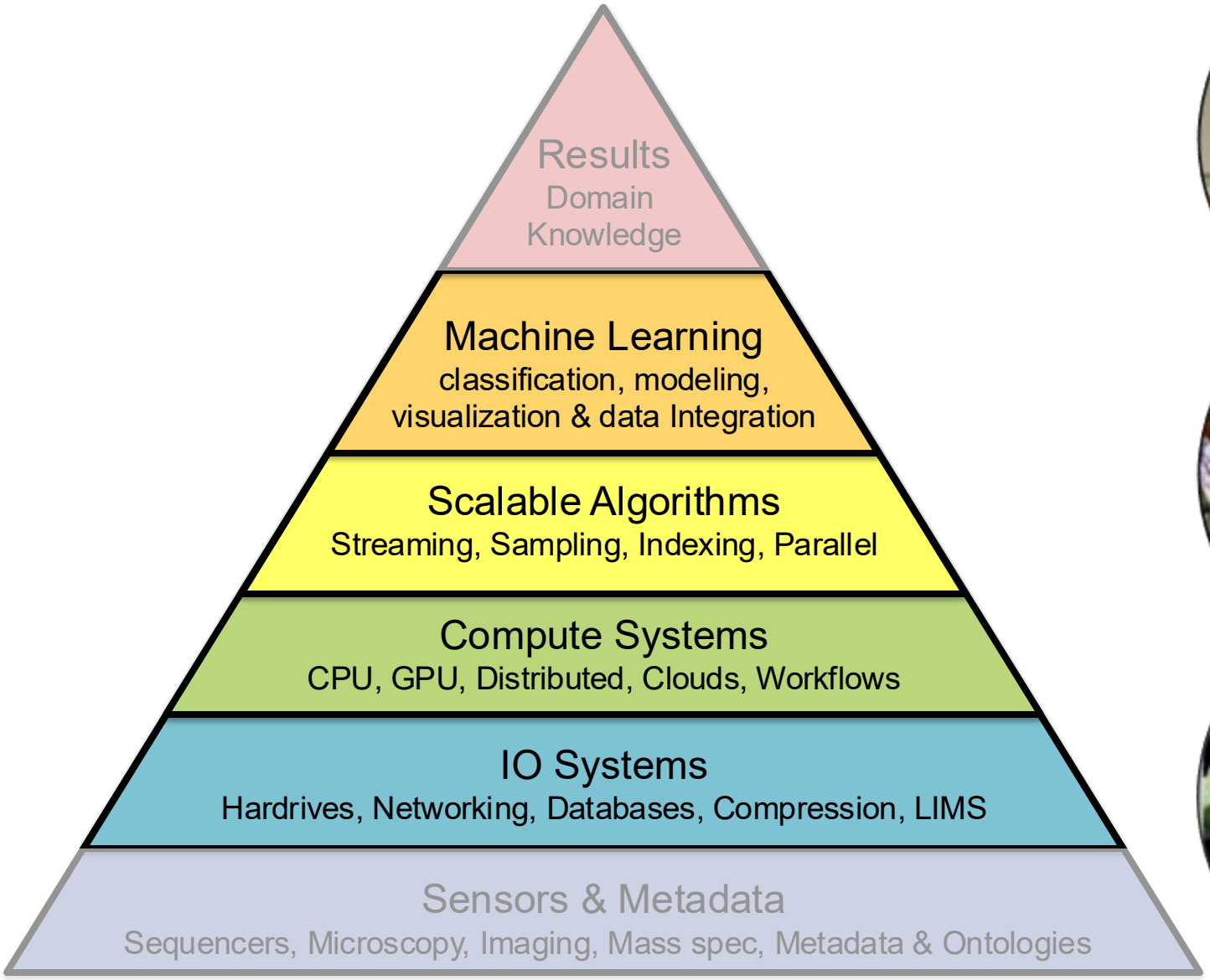
Comparative Genomics Technologies



Comparative Genomics Technologies



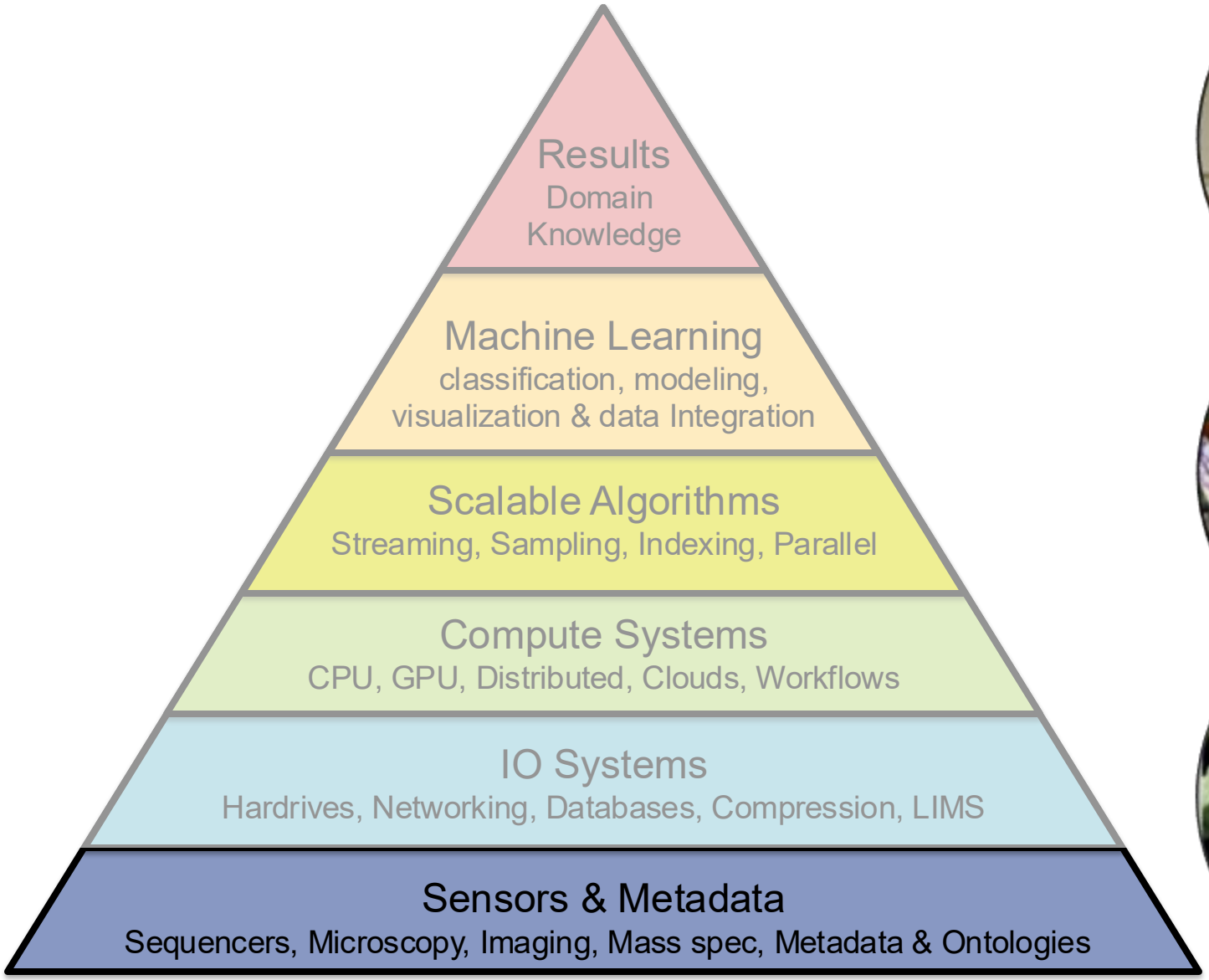
Comparative Genomics Technologies



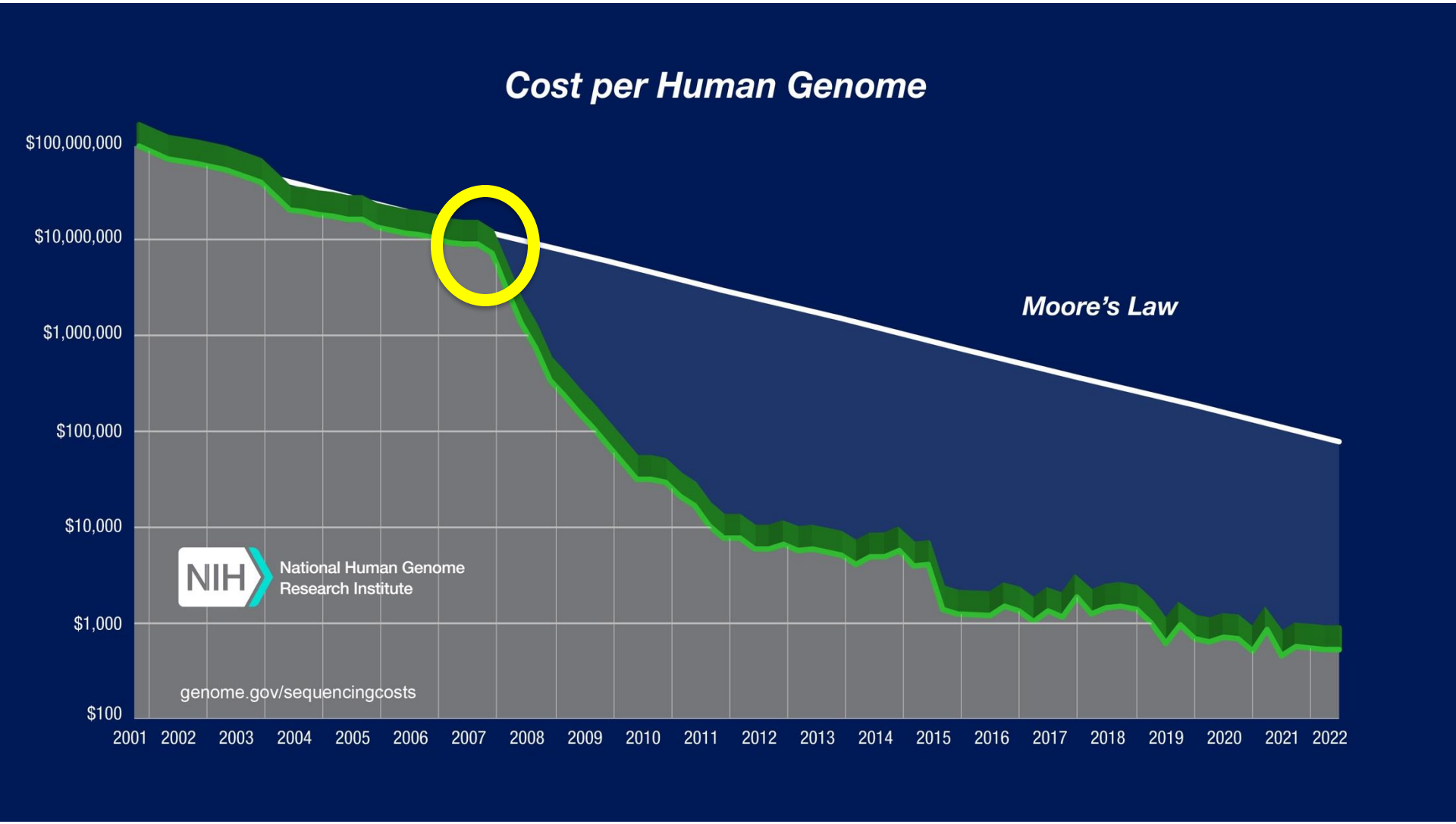
Selected Topics

- Genome assembly, whole genome alignment
- Full text indexing: Suffix Trees, Suffix Arrays, FM-index
- Dynamic Programming: Edit Distance, sequence similarity
- Read mapping & Variant identification
- Gene Finding: HMMs, Plane-sweep algorithms
- RNA-seq: mapping, assembly, quantification
- ChIP-seq: Peak finding, motif finding
- Methylation-seq: Mapping, CpG island detection
- HiC: Domain identification, scaffolding
- Chromatin state analysis: ChromHMM, Enformer, etc
- Scalable genomics: Cloud computing, scalable data structures
- Population & single cell analysis: clustering, pseudotime
- Disease analysis, cancer genomics, Metagenomics
- Deep learning in genomics

Comparative Genomics Technologies



Cost per Genome

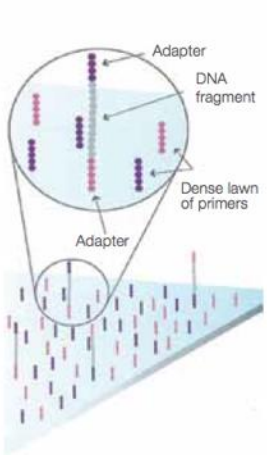


https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost

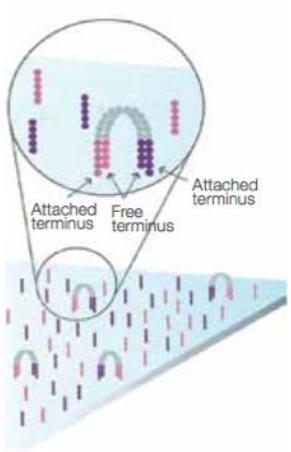
Second Generation Sequencing

Illumina NovaSeq 6000
Sequencing by Synthesis

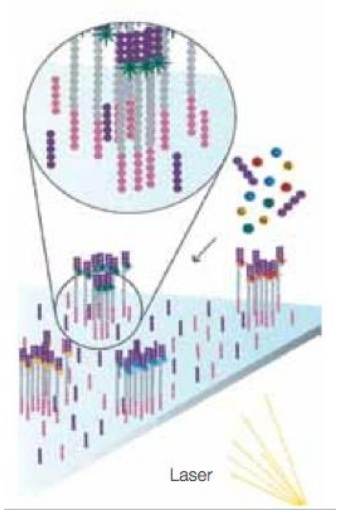
>3Tbp / day
(JHU has 4 of these!)



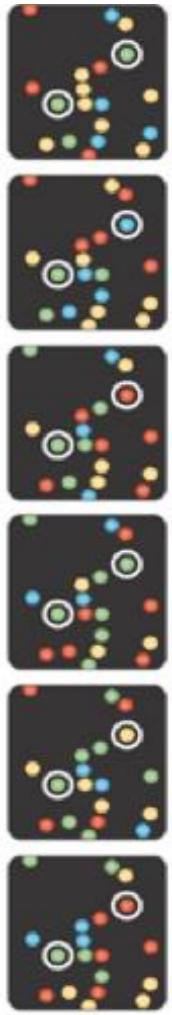
1. Attach



2. Amplify

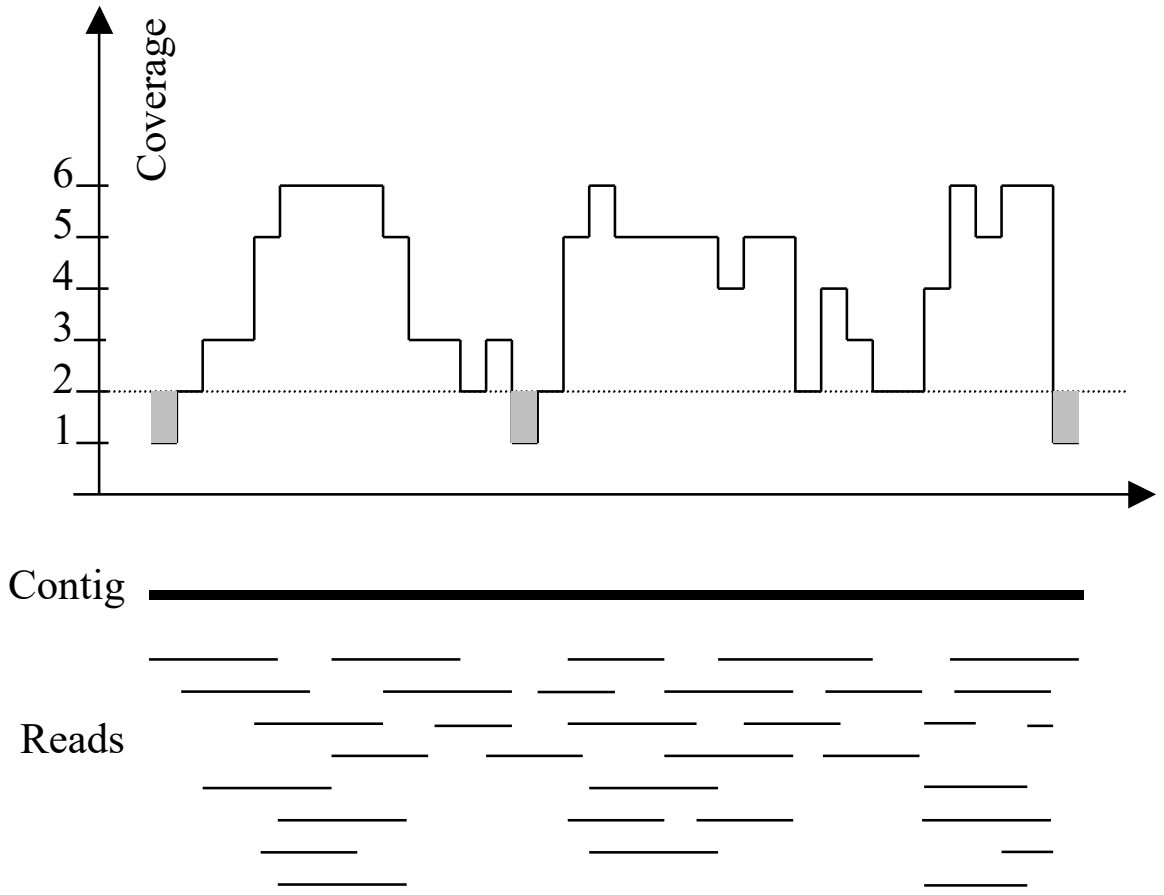


3. Image



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

Typical sequencing coverage

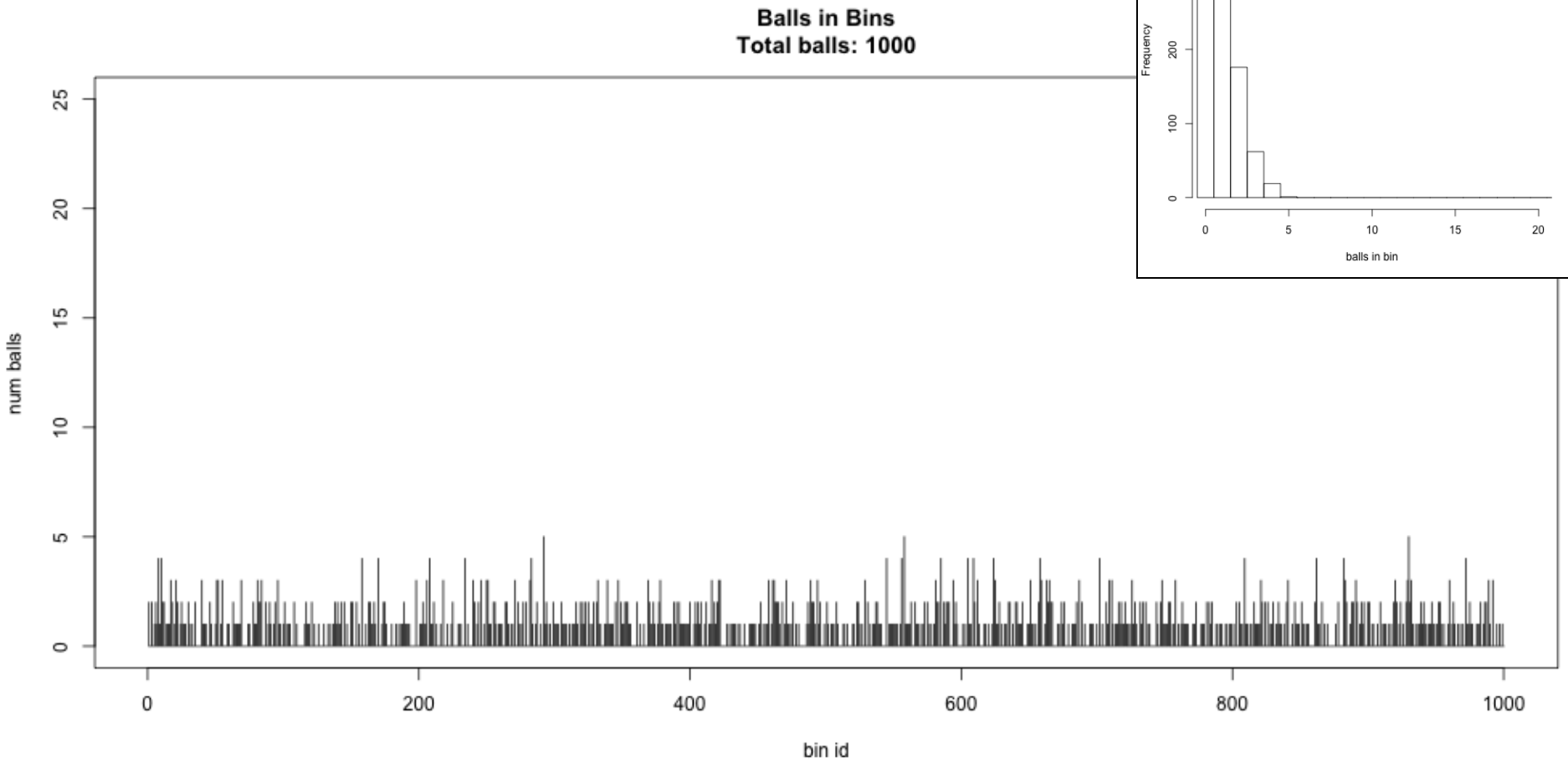


Imagine raindrops on a sidewalk

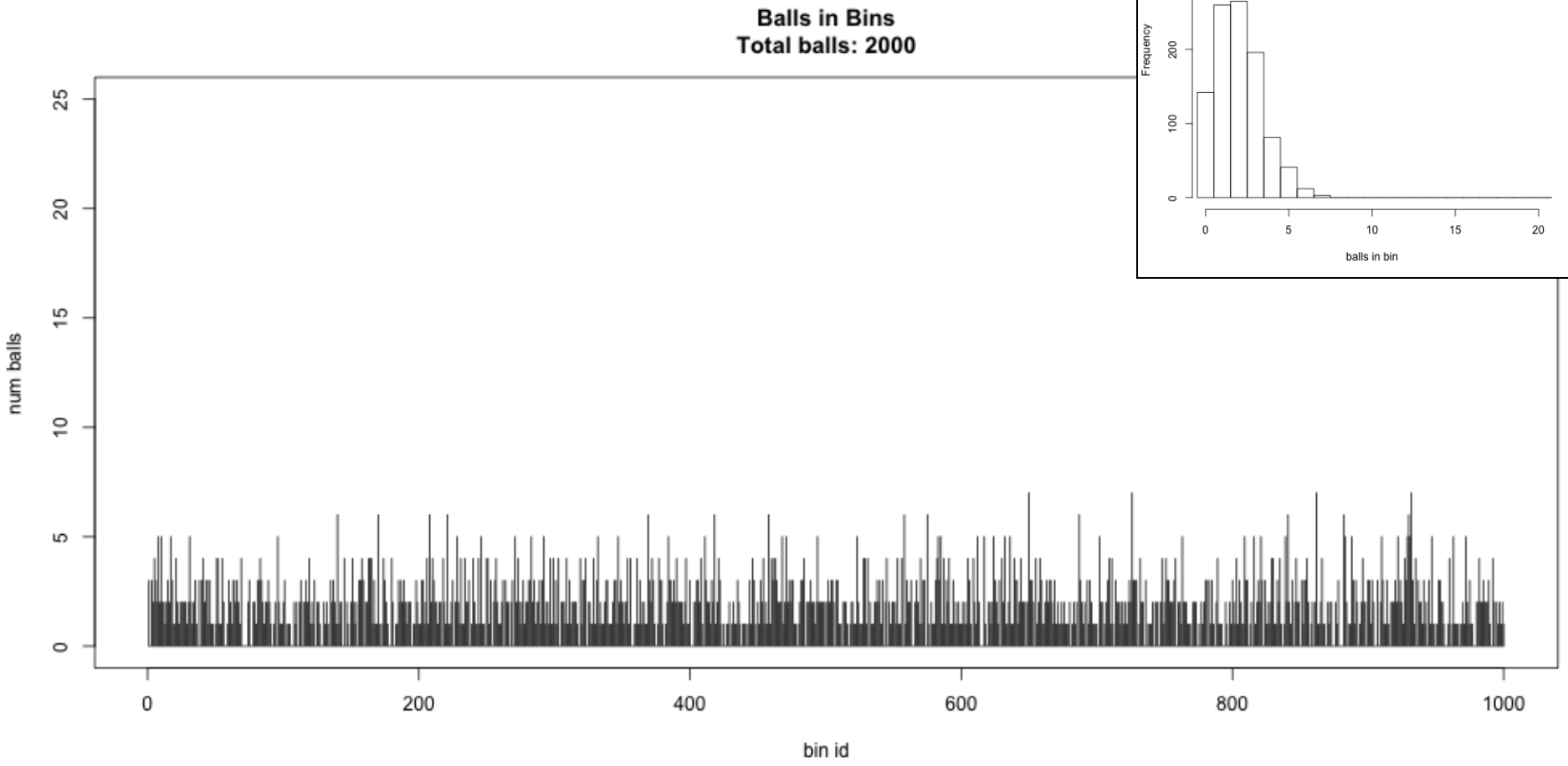
We want to cover the entire sidewalk but each drop costs $1

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

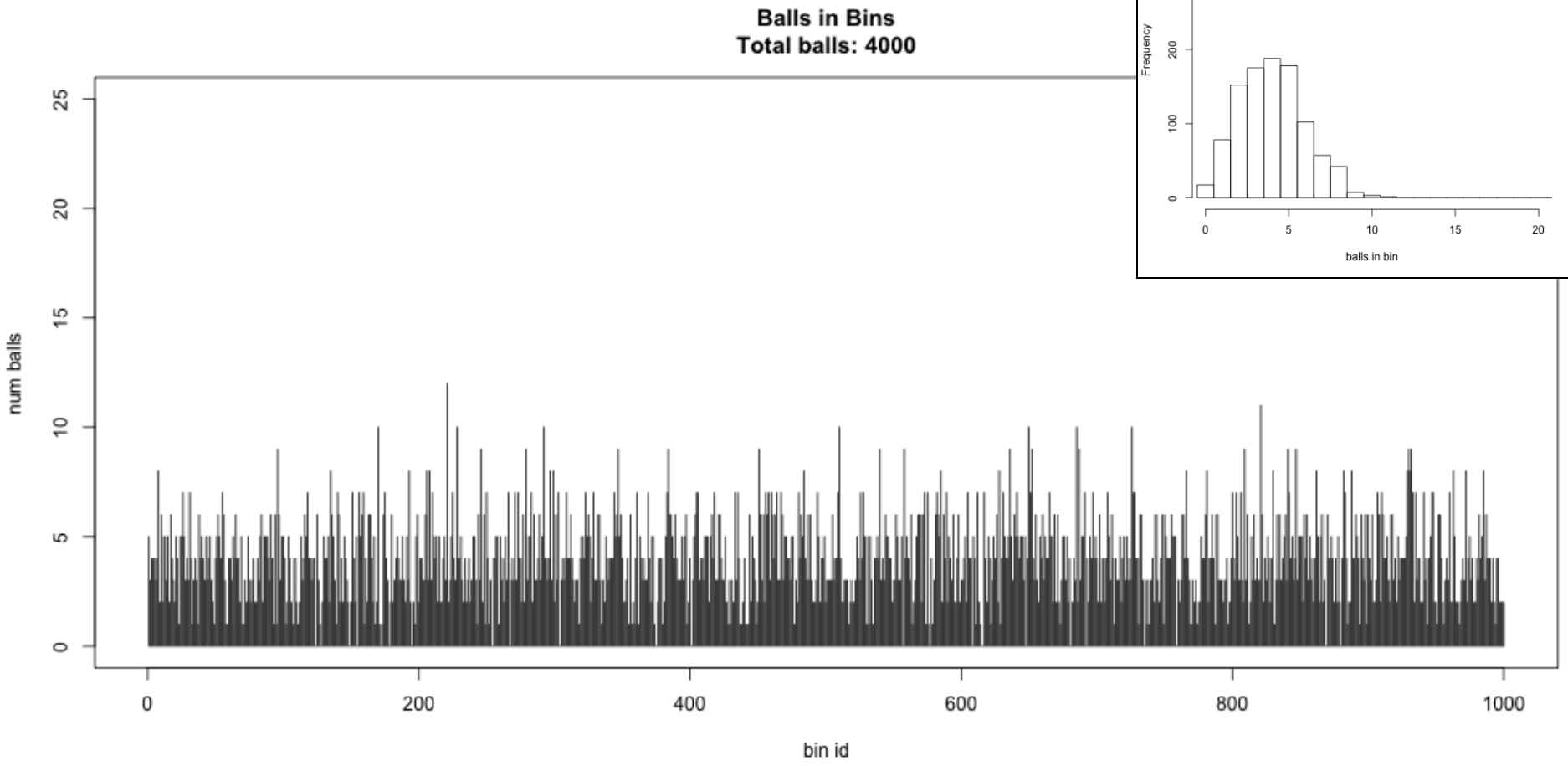
1x sequencing



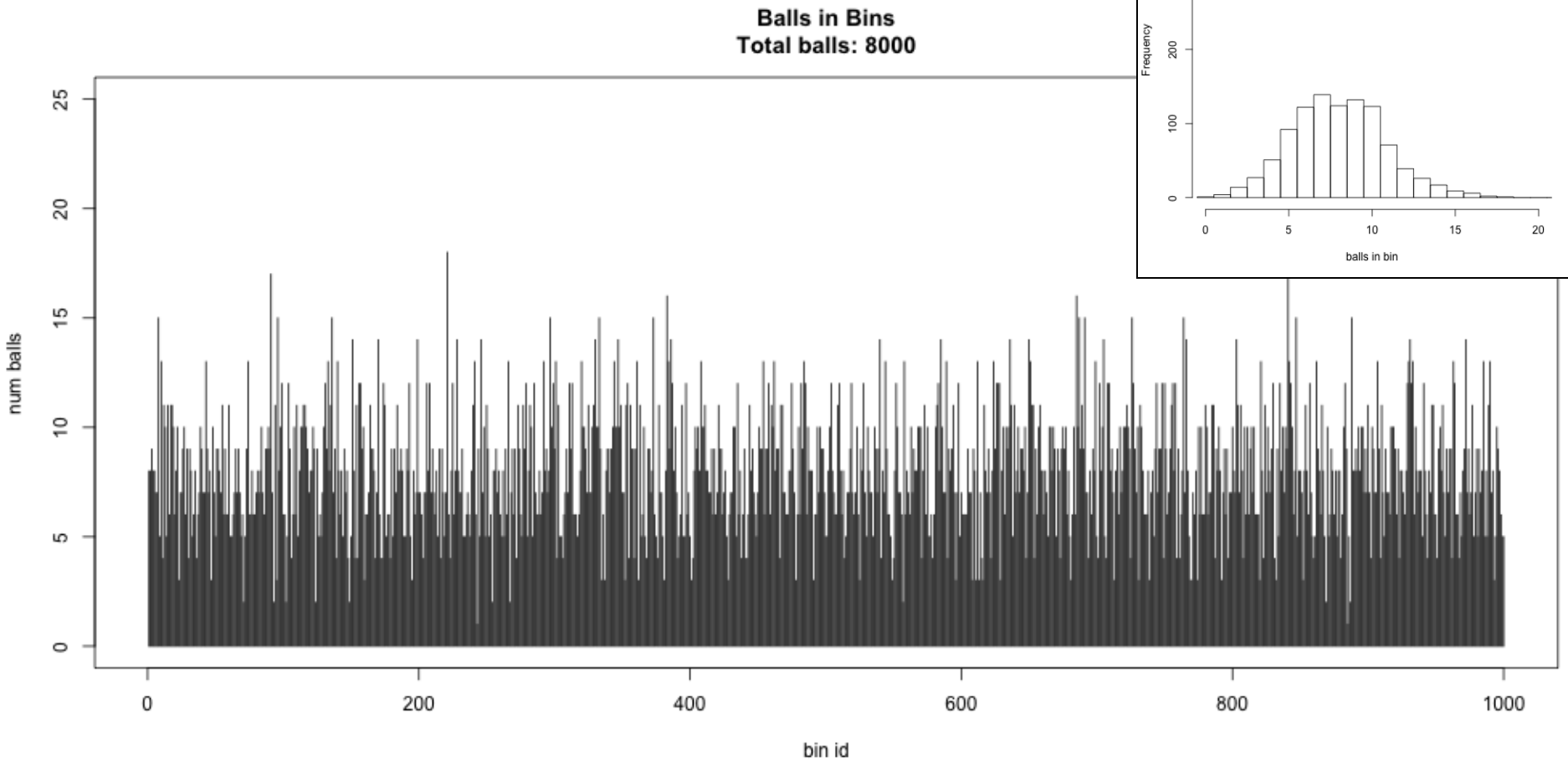
2x sequencing



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.

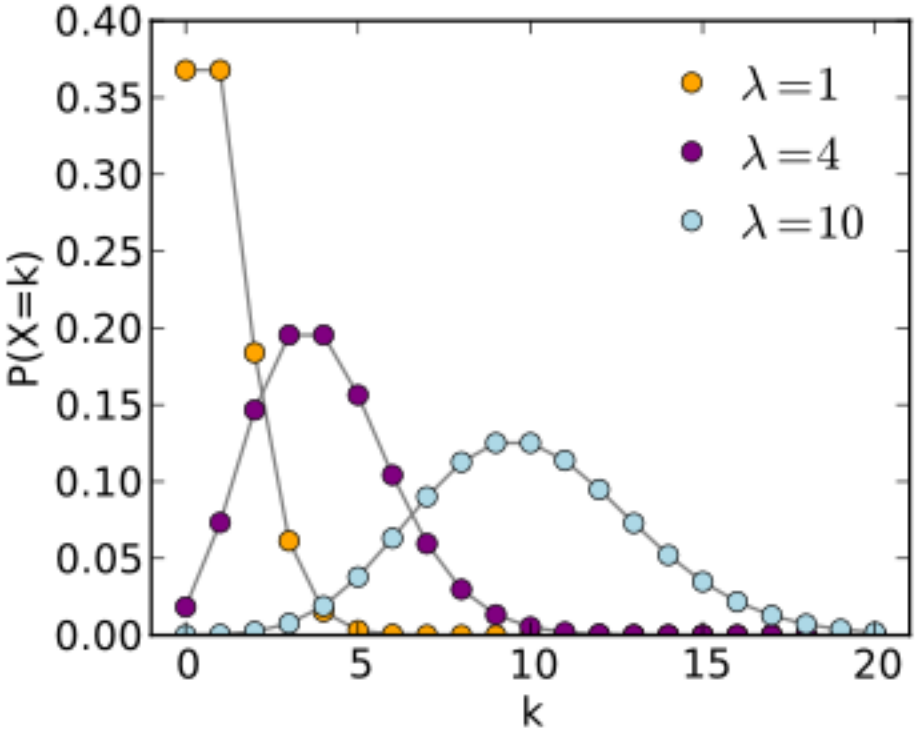
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

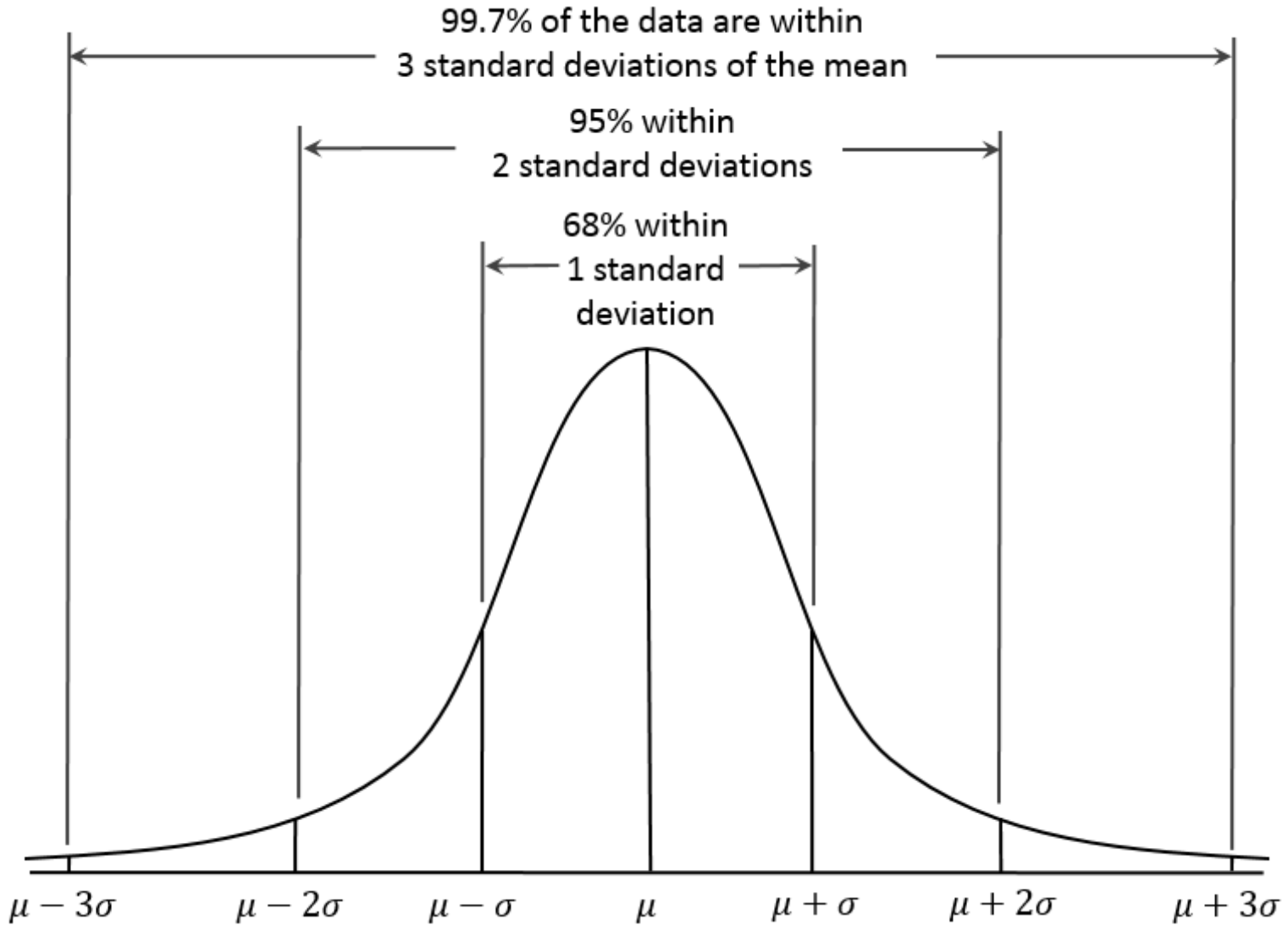
Resembles a normal distribution, but over the positive values, and with only a single parameter.

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 $\lambda > 10$