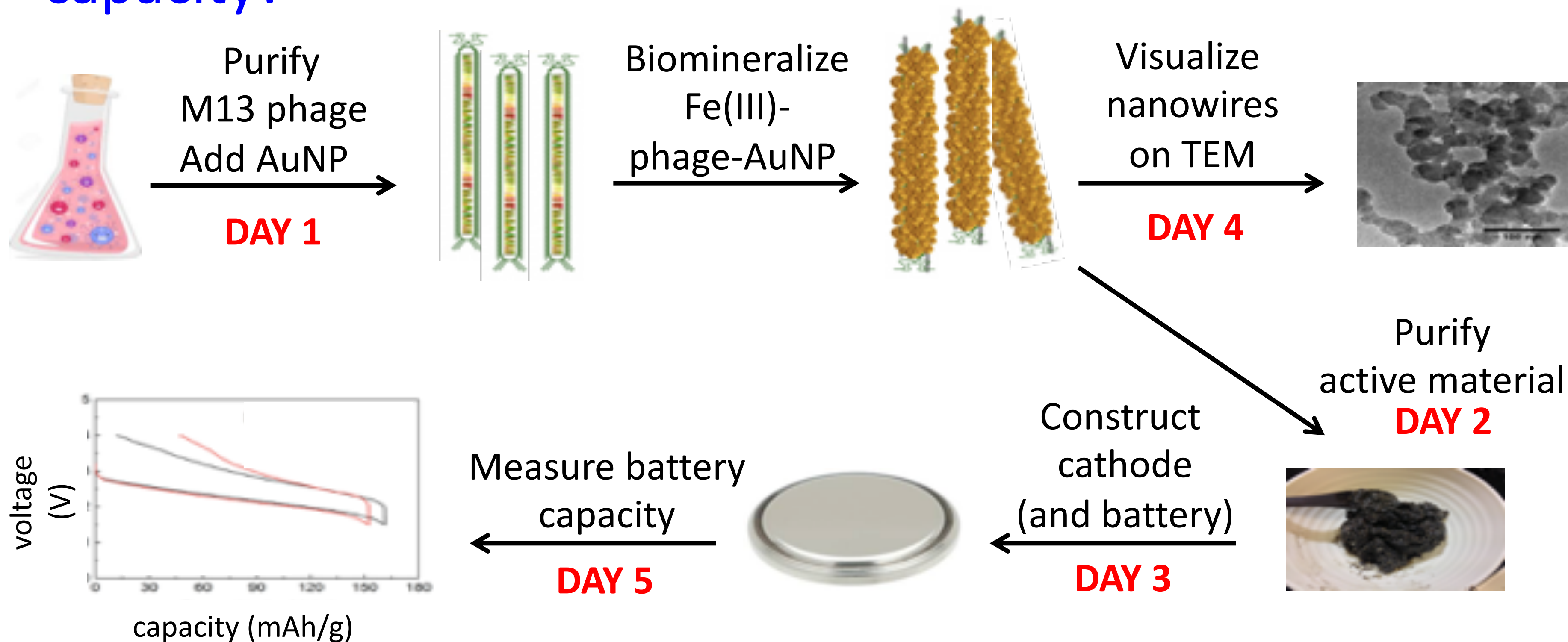
M3D1:Growth of phage materials

1. Purify M13 bacteriophage (phage)
2. Prelab during 60min incubation
3. Finish M13 purification and measure concentration
4. Incubate phage with nanoparticles (AuNP)

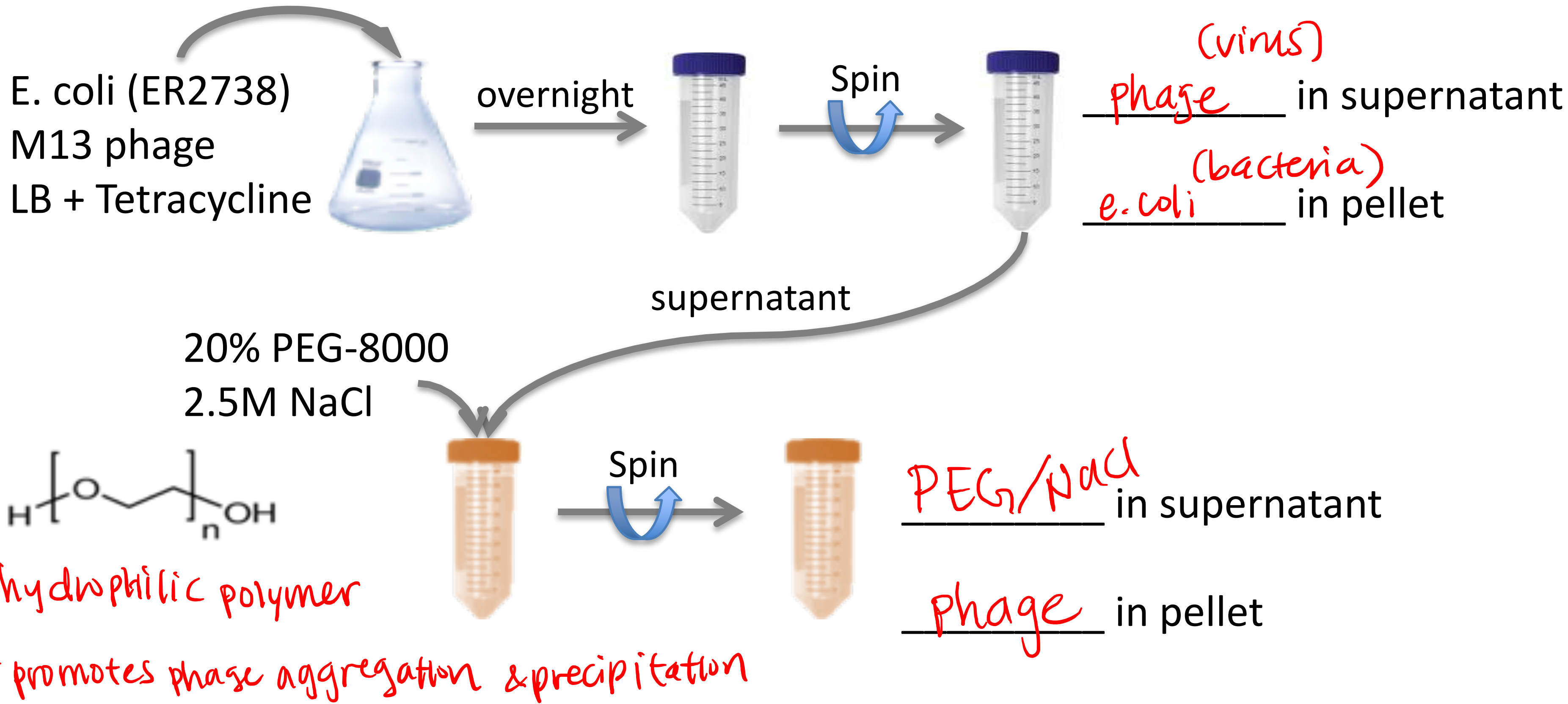
Thank you, Jifa Q. (Belcher Laboratory)!

Module 3: biomaterials engineering

How do nanoparticle size and quantity affect battery capacity?

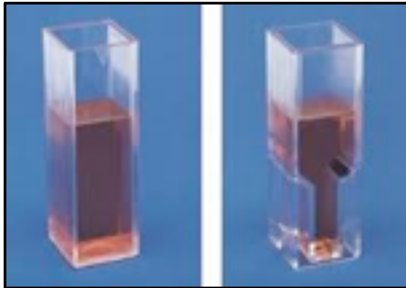


Phage purification using polyethylene glycol (PEG) in 2.5M NaCl



Determining phage titer (number of virus):

- By plating: plaque assay
  - Phage slows *E. coli* growth = plaque (cleared zone)
  - Plaque-forming units: PFU/mL



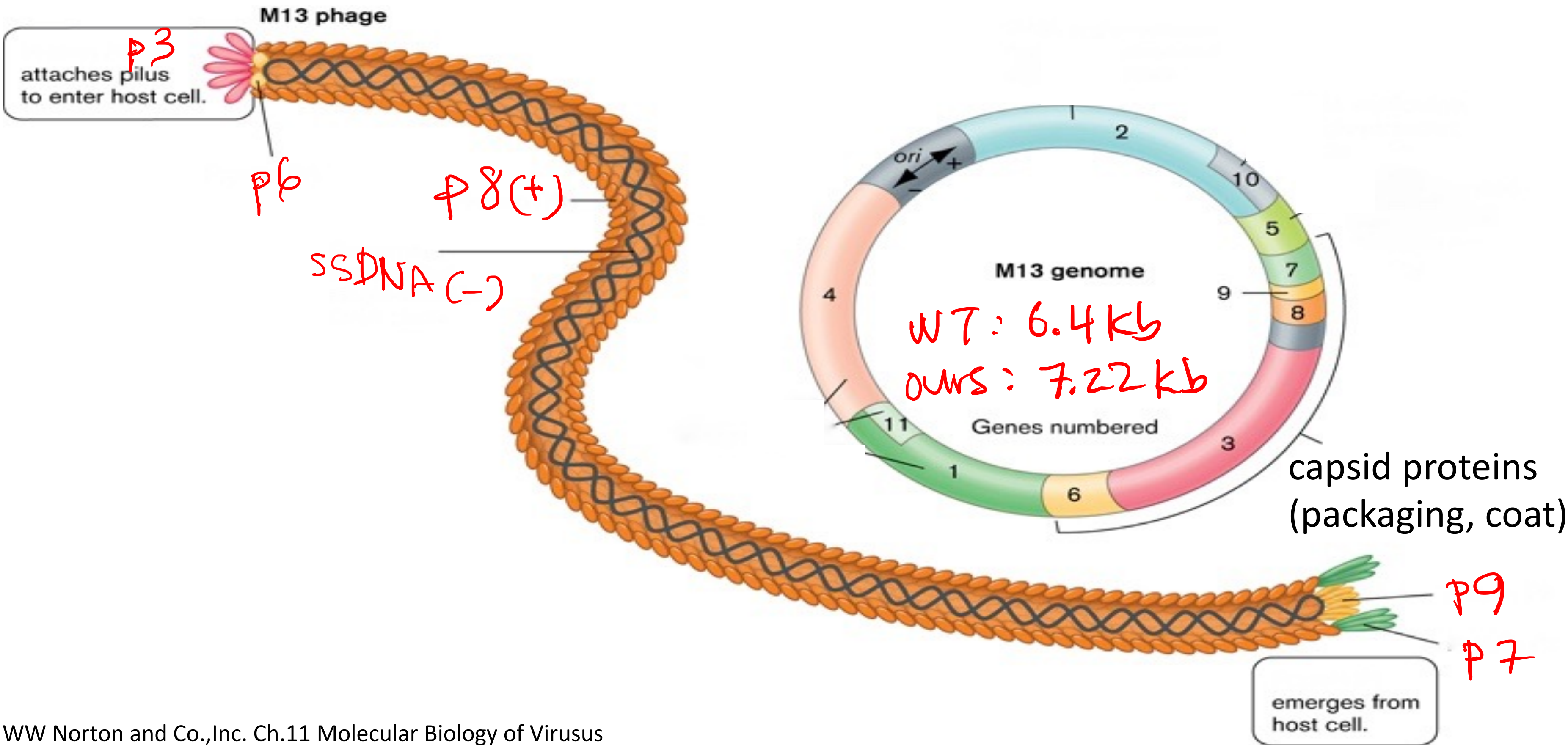
- By spectrophotometry

$$\text{\# phage / mL} = \frac{(6 \times 10^{16})\ (A269 - A320)}{\text{\# bases in phage genome}}$$

* dilution factor

❖ Quartz cuvettes are expensive!

M13 is a high aspect ratio phage coated in proteins encoded by ssDNA loop



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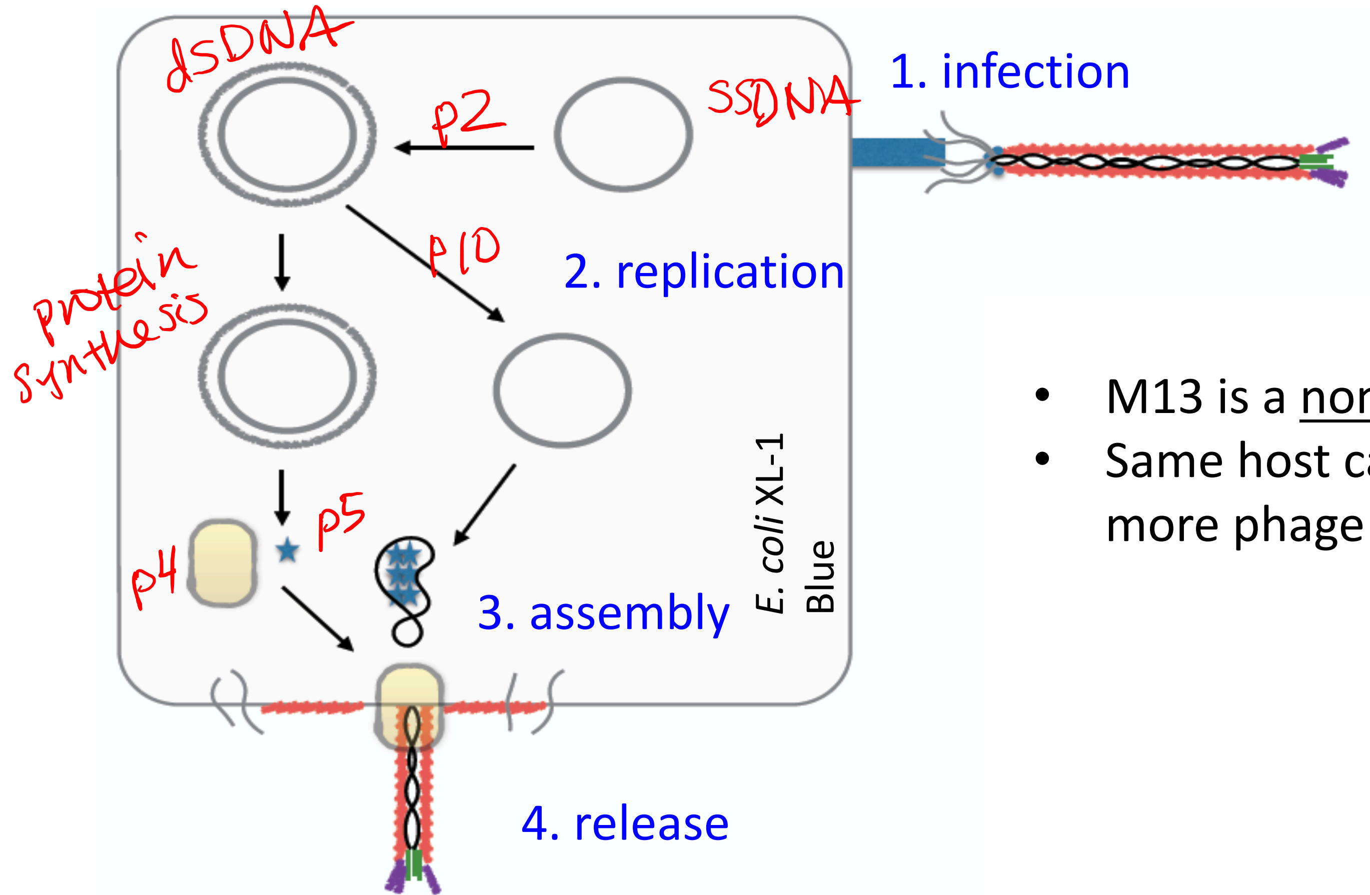
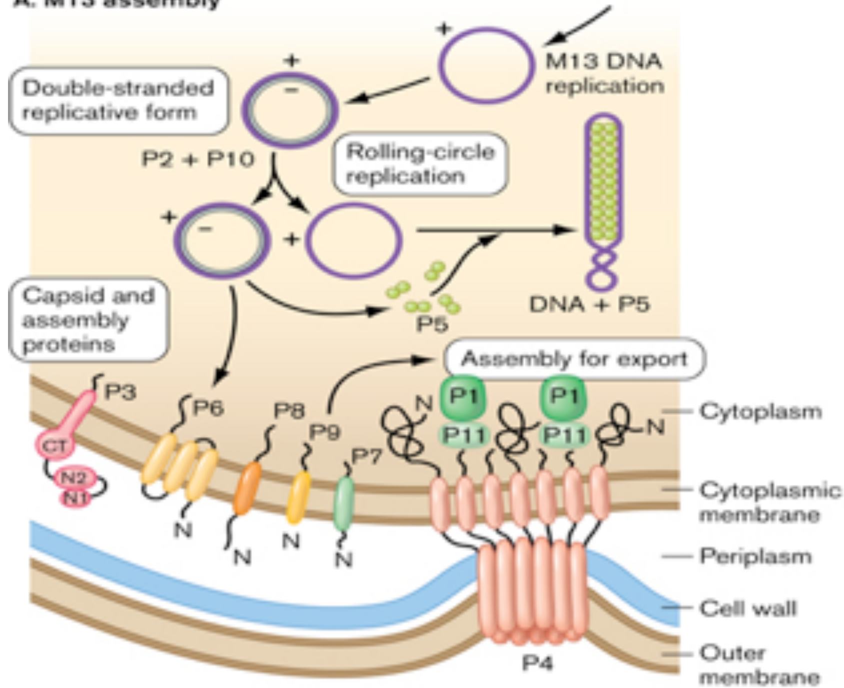
M13 virus life-cycle has four essential steps

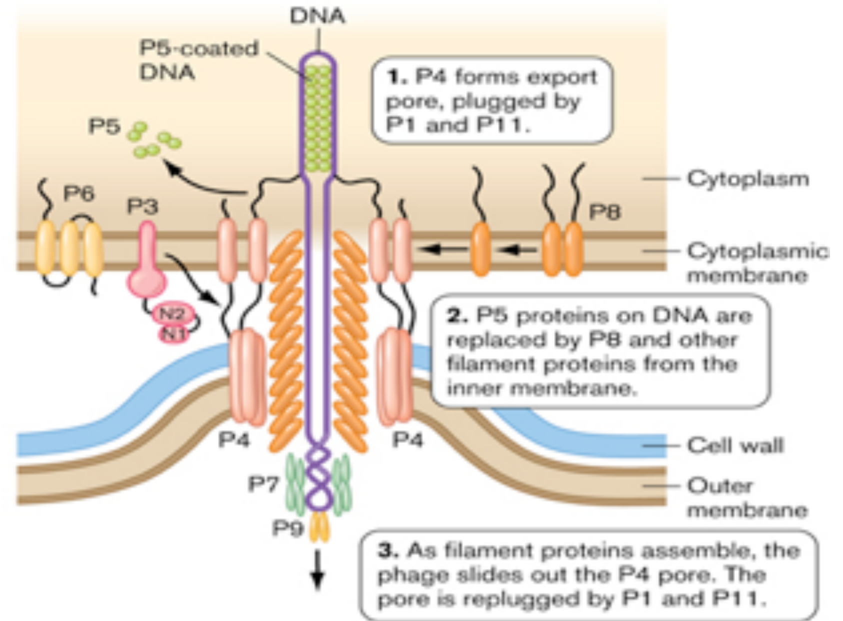


- M13 is a nonlytic phage
- Same host can keep producing more phage

M13 is a nonlytic bacteriophage

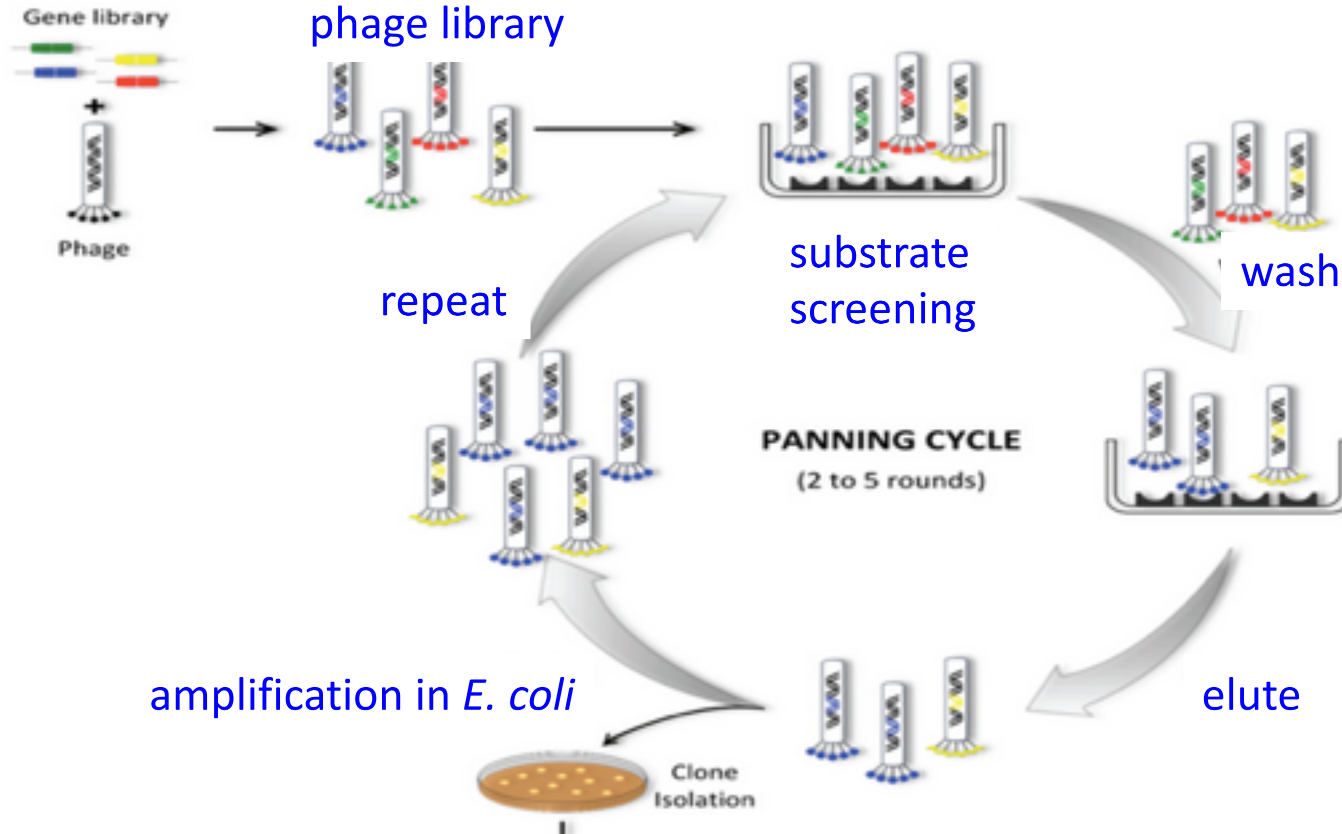
(so we can easily get lots of it)





http://www.wwnorton.com/college/biology/microbiology2/ch/11/etopics.aspx

Phage display allows agnostic selection of useful peptide sequences (typically binding)

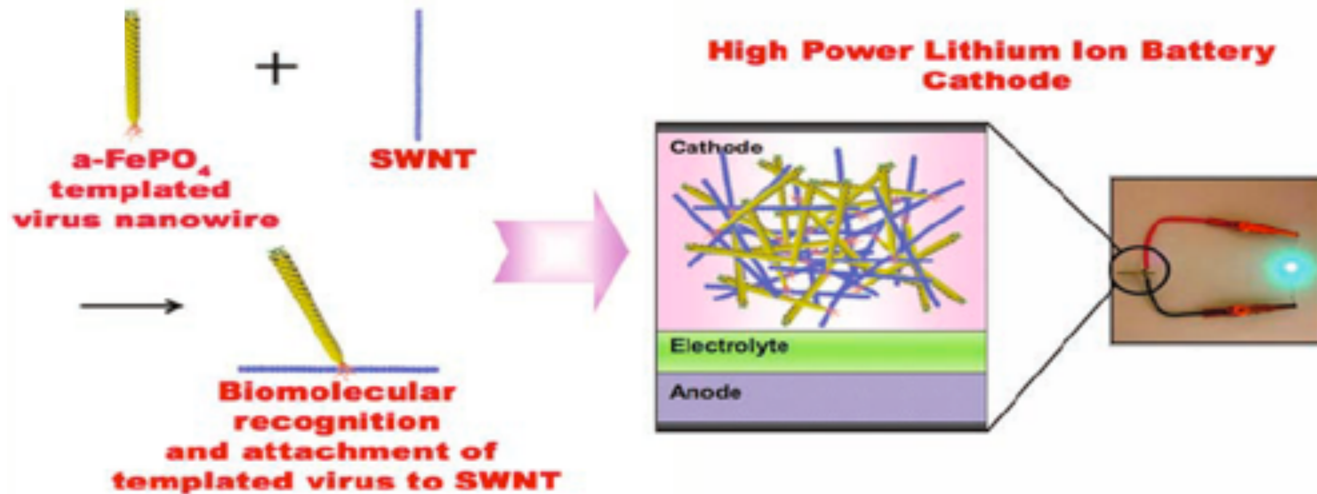


Biomolecules 2015, 5(3), 1783-1809;
doi:10.3390/biom5031783

M13 are engineer-able biomaterials

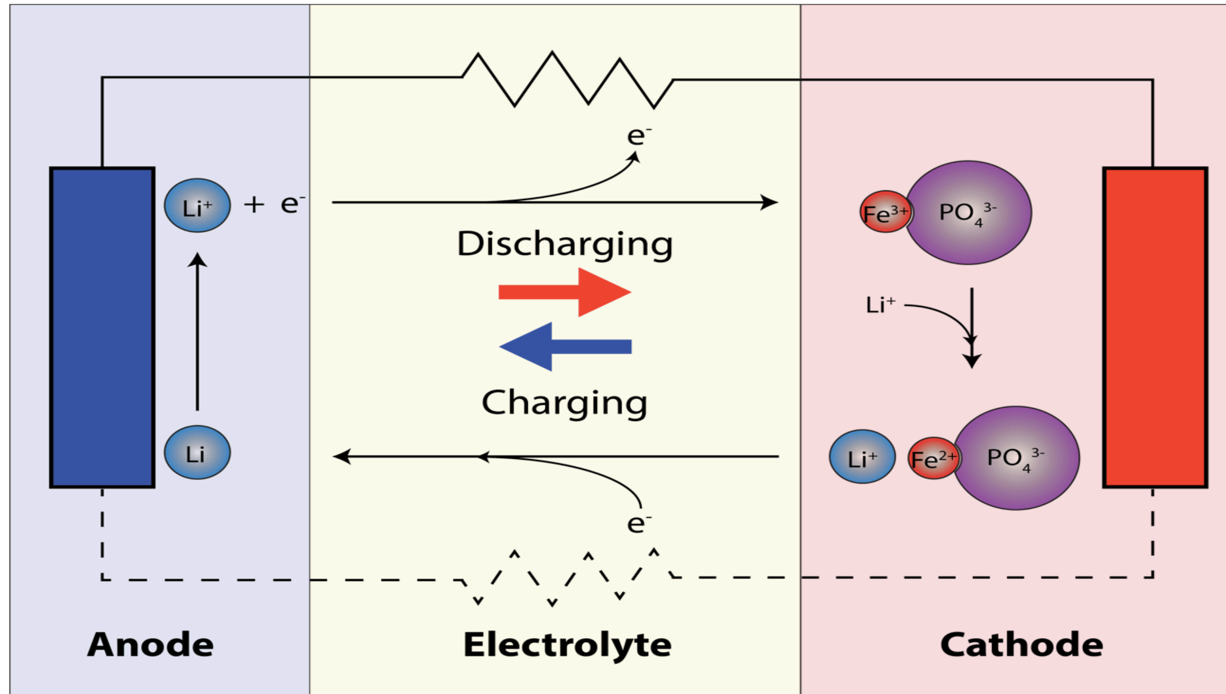
negatively charged

- Our p8 coat protein was mutated to contain sequence DSPHTELP
- Modified p8 proteins bind single wall carbon nanotubes (SWCNT), iron, gold, and other cationic metals
- Example of this virus in literature (Science, 2009):



Lee et al. Fabricating Genetically Engineered High-Power Lithium-Ion Batteries Using Multiple Virus Genes. Science. 2009

M13 nanowires as battery cathode



Cathode needs to be a good conductor of:

- ions
- electrons

Image: George Sun

You will make a "gold standard" battery and an experimental battery

- Gold standard: 3.8 nm AuNPs, 40 AuNP/phage
- Experimental: choice of sizes & quantities
  - Size: 3.8 nm, 5 nm, 9 nm diameter AuNP (can mix them)
  - Quantity of AuNPs
  - Constraint: up to 50 mL total volume (phage + NPs)

Considerations for experimental battery: nanoparticle material and size

- Surface area to volume ratio of AuNPs
  - Conductivity (consider volume of Au in cathode)
  - More surface area may be beneficial if Au has a catalytic function (Au may facilitate intercalation of Li^+ in $FePO_4$ cathode)
- Phage surface area available for Au and Fe binding
  - Too many AuNPs may reduce # binding sites for $FePO_4$

Design with your lab partner. What is your **hypothesis**?

You will make two flasks—one for each battery

Gold standard

1) 4e13 Phage
2) 3.8 nm Au NPs
   (40 NPs/phage)
\+ 3) Water

Final volume 50 mL

Experimental

1) 4e13 Phage
2) ____________nm Au NPs
   (_______NPs/phage)
\+ 3) Water

Final volume 50 mL

Today in lab

1. Finish phage purification
2. Calculate phage number
3. Begin construction of phage-NP-$FePO_4$ nanowires (2 flasks, one per battery)
   - **Choose Au NP size, quantity**

M3D2 HW: Describe **FIVE** recent findings that could potentially define an interesting research question.

- Formally cite the finding
- Write 3-5 sentences summarizing the finding

RNA-seq vs. qPCR

RNA-seq:

- Measures every expressed gene
- You *enrich* for mRNA
- Requires sequencing

qPCR:

- Measures single gene
- No mRNA enrichment
- Does not require sequencing

Q1:

Why are you more likely to observe sequence from the 3’ end of a gene in RNA-seq data (relative to sequence from the 5’ end)?

Why are you more likely to observe sequence from the 3’ end of a gene in RNA-seq data?

- What is special about the 3’-end? of mRNA only

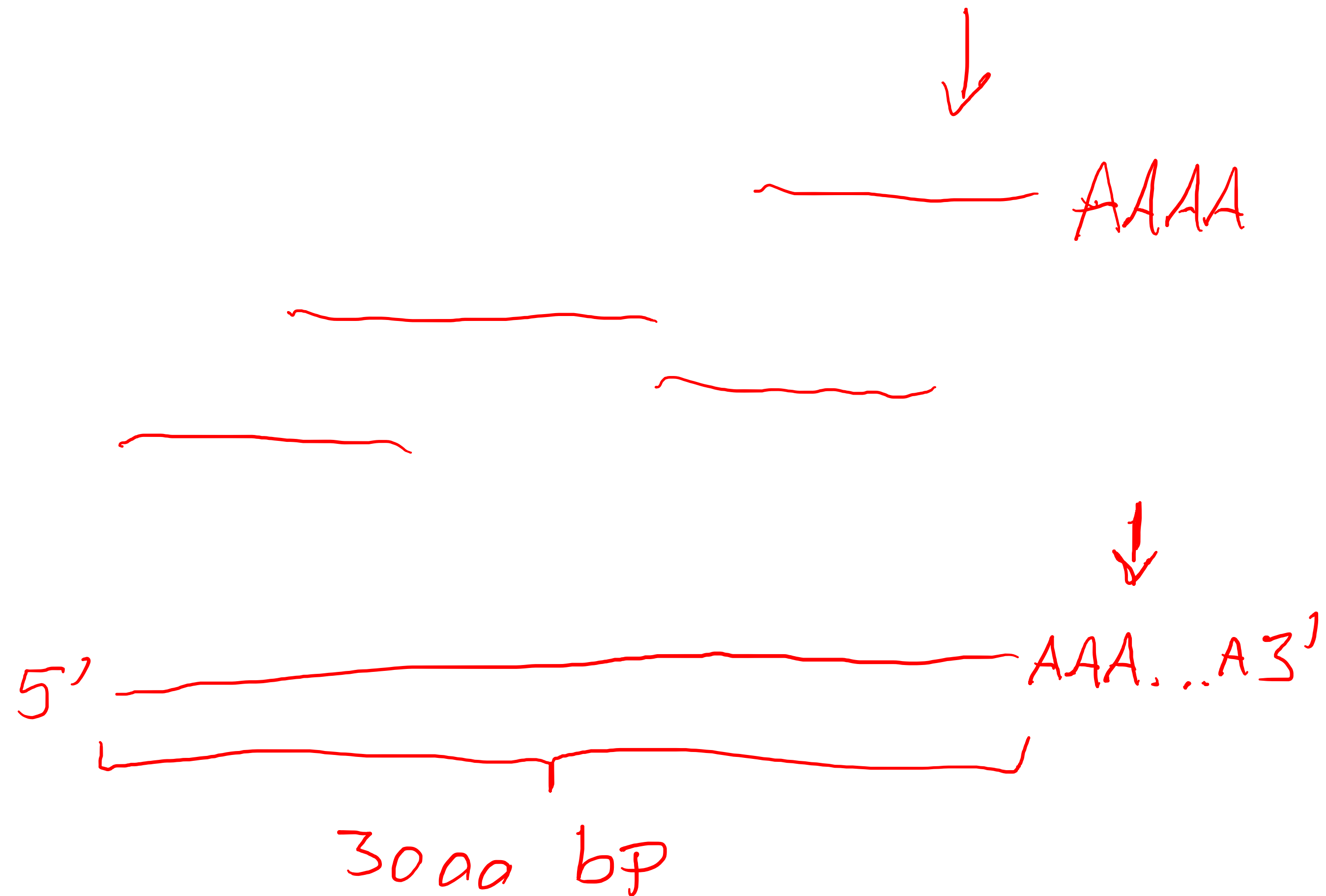
Has a polyA-tail

- What is special about mRNA?

Has the p(A) tail

- How do you enrich for mRNA?

Use oligo(dT)



Q2:

To compare two sets of RNA-seq data, you first normalize the results by calculating the RPKM value for each gene. What are the two factors to which you normalize (hint: how do you normalize between experiments AND how do you normalize between genes)?

Calculating the RPKM

- RPKM = Reads Per Kilobase Million

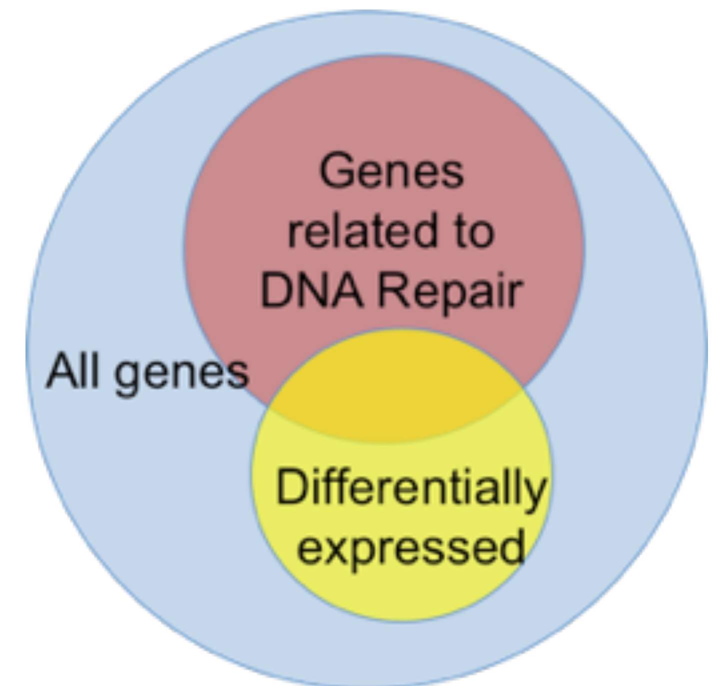
Total reads / 1,000,000 = **per million (PM)** scaling factor

Reads / **PM** = RPM

RPM / gene length in Kb = RPKM

Q3:

When analyzing RNA-seq data you identify a group of differentially expressed genes (yellow circle). You already know which genes are involved in DNA repair (red circle).w



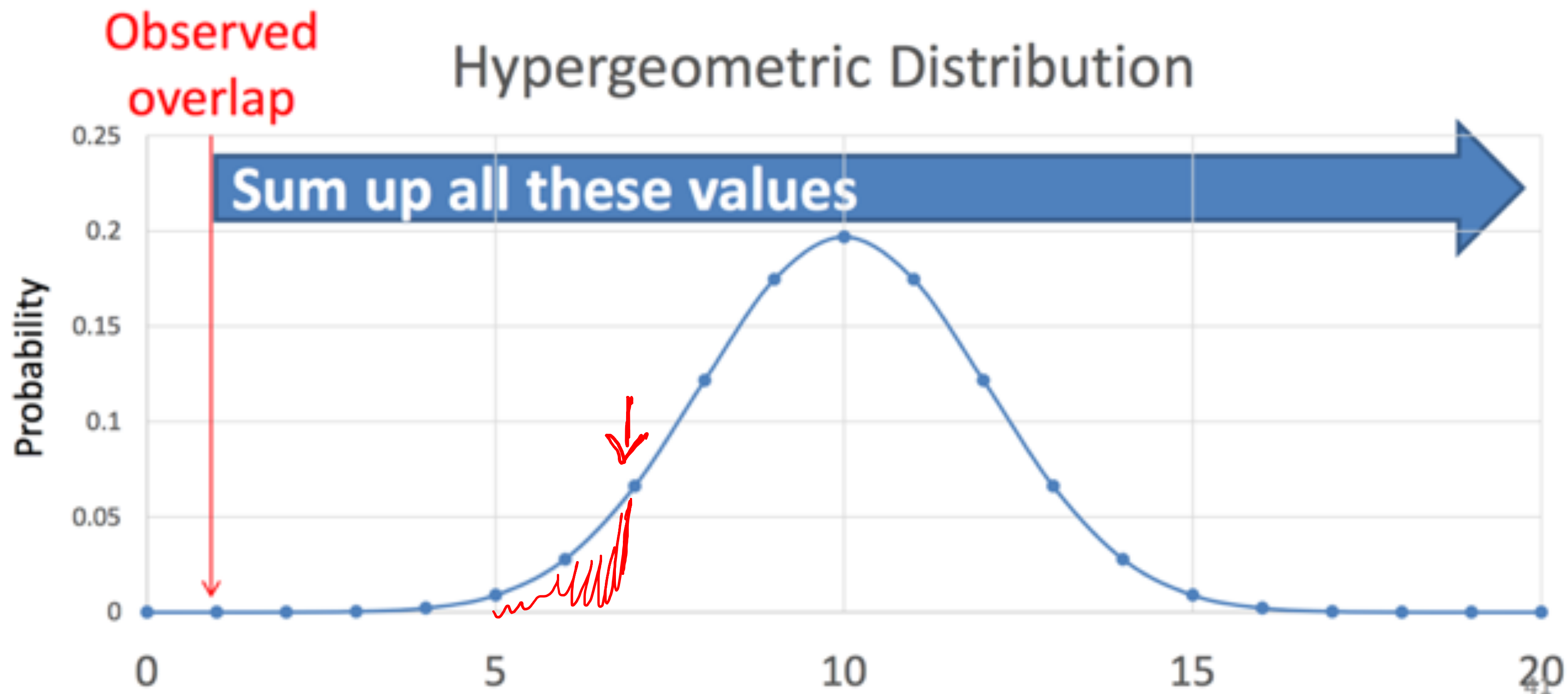
Which probability distribution will tell you the probability of overlap?

- Hypergeometric distribution

$$P(overlap) = \frac{\binom{DNA\ repair}{Overlap}\binom{Genome - DNA\ repair}{Diff.\ expr. - overlap}}{\binom{Genome}{Diff.\ expr.}}$$

What statistical function can you use to test if the overlap is significant?

- Cumulative density function (CDF)
- Fisher's Exact Test



Q4:
qPCR is used to measure expression levels of specific genes.

qPCR is used to measure expression levels of specific genes

Why measure p21?

It lets us track the cell cycle in response to DNA damage

Why measure GAPDH?

- Normalize p21 expression
- "Housekeeping" gene

Q5:

Briefly describe “synthetic lethality” and how it applies to your cell viability experiment

Briefly describe "synthetic lethality" and how it applies to your cell viability experiment

What is synthetic lethality?

The combination of 2 or more gene defects → cell death.

Individual mutations do not kill the cell

How does it apply to our experiment?

If we knock out NHEJ and HR we expect cells to die when we induce DNA damage. We do not expect cells to die from DNA damage if only a single pathway has been knocked out