

# M3D I: Growth of Phage Materials

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4/15/15

1. Discussion presentation
2. Purify M13 phage — ~2 hours
3. Pre-lab during 60 min incubation
4. Measure concentration of M13 phage
5. Complex M13 phage with AuNPs

 Welcome Cherry (and David)!! 

# Comments on Mod2 flow cytometry figure draft:

- (1) Relabel your figure axes so that they are easier to read – better to use “Green fluorescence (MFI)” where MFI = mean fluorescence intensity and is the actual data that is shown in each plot.
  
- (2) Try to cite the figure parenthetically throughout your Results section versus wasting text on text such as “Figure 2 shows...” For example, “CHO-K1 transfected with the intact pMAX-BFP-MCS plasmid was utilized to determine positive GFP fluorescence (Figure 2A).”
  
- (3) Strive to write in a professional manner. Be concise and use technical language. “To-Pro3 was used to eliminate non-viable cells through exclusion from the ‘live cell’ gate (Figure 1X).”

# Important upcoming dates:

Browser tabs: ndar, SD Actin Flows M..., Evernote Web, Search results ..., Login :: Identif..., e-QIP\_Reques..., e-QIP: Investig..., 20.109(S15... x

Address bar: i/20.109(S15):\_Spring\_2015\_schedule

Browser tabs: Maps, equipment, 20.109(S14) - Ope..., 20.109(S15): Sprin..., 20.109(F14) - Ope..., Atlas - Navigating ..., COLD-PCR - Wiki...

3	1	Tue/Wed Apr 14/15	AB	CG	Growth of phage nanowires		
3	2	Thur/Fri Apr 16/17	AB	CG	Biotemplating on phage nanowires	Yes	lab quiz
<b>Patriot's Day April 20-21</b>						<b>No lab Apr 21 or Apr 22</b>	
		Thu/Fri Apr 23/24					<b>No lecture or lab today.</b>
3	3	Tue/Wed Apr 28/29	AB	CG	TEM	Yes	lab quiz
3	4	Thur/Fri Apr/May 30/1	AB	CG	Solar cell assembly	Yes	
3	5	Tue/Wed May 5/6	AB	CG	Solar cell testing	Yes	lab quiz
3	6	Thur/Fri May 7/8	AB	CG	Wrap-up and data summary		<b>Mod 3 Mini-report Due THU or FRI at 5:00 pm</b>
3	7	Tue/Wed May 12/13	AB	CG	Student presentations		
		Thur May 14				No lab session. Meet in <b>16-220</b> from 11am-1pm: feedback/discussion about class, then celebratory lunch etc.	

# Main ideas behind Module 3:

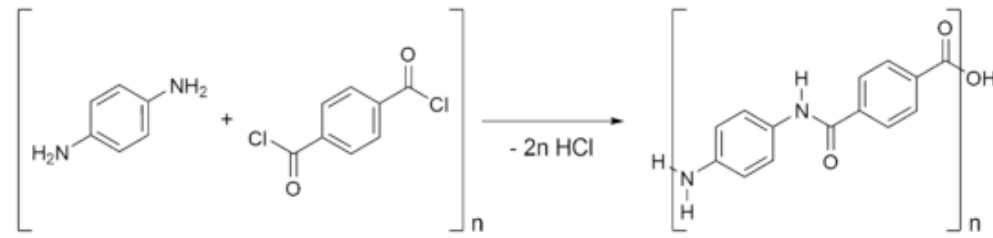
- Use biology to create functional nanomaterials
- New properties can emerge at different scales
- Our biological nanomaterial is the M13 phage

## Giant's Causeway — Ireland



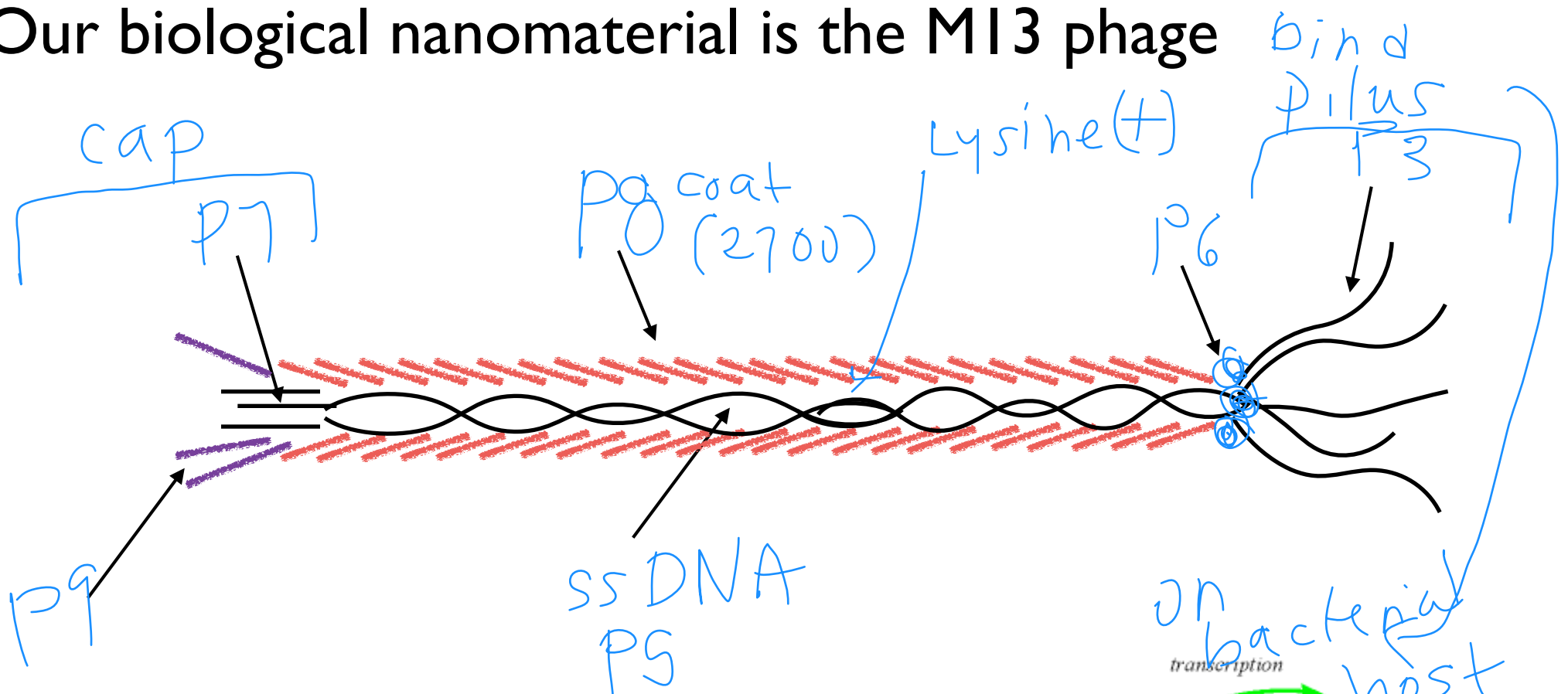
macvb

poly paraphenylene terephthalamide



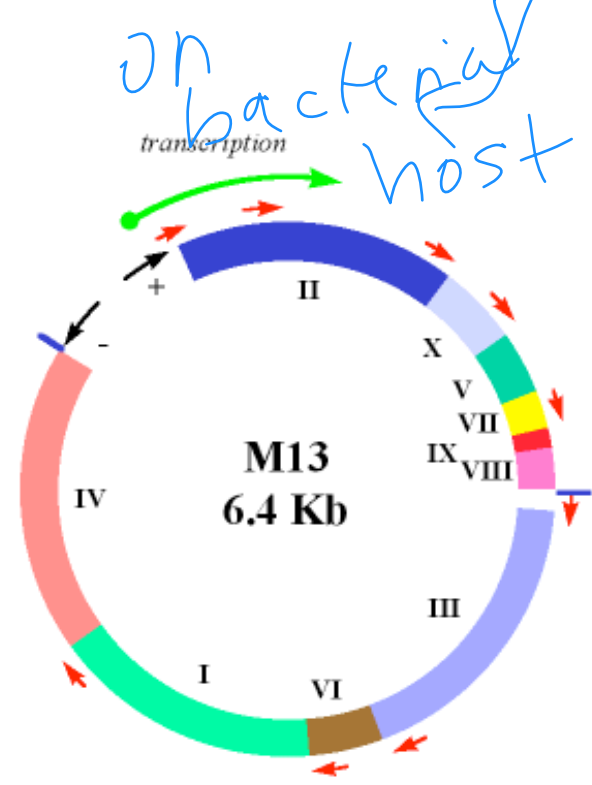
hano

# Our biological nanomaterial is the M13 phage



p3, 6, 7, 8, 9 packaging for DNA

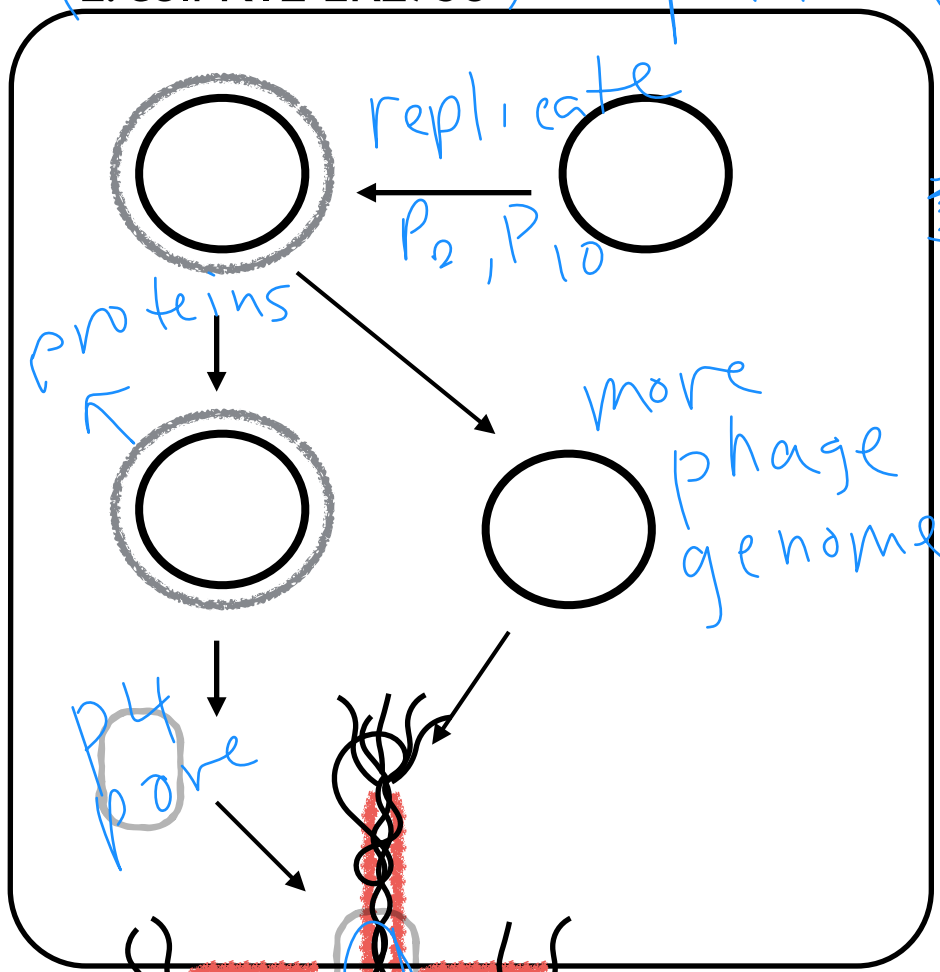
- promoters
- terminators
- ori



# Bacteriophage are 'high copy' obligate organisms. =

(E. coli K12 ER2738) ← spun out

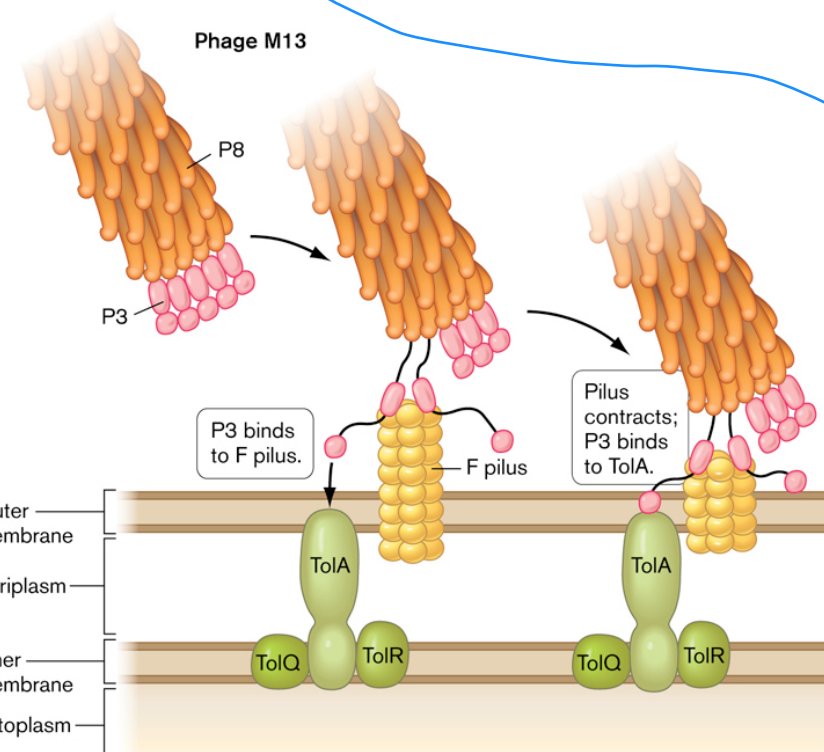
need host to replicate



F pilus

$10^{12} - 10^{13}$  particles

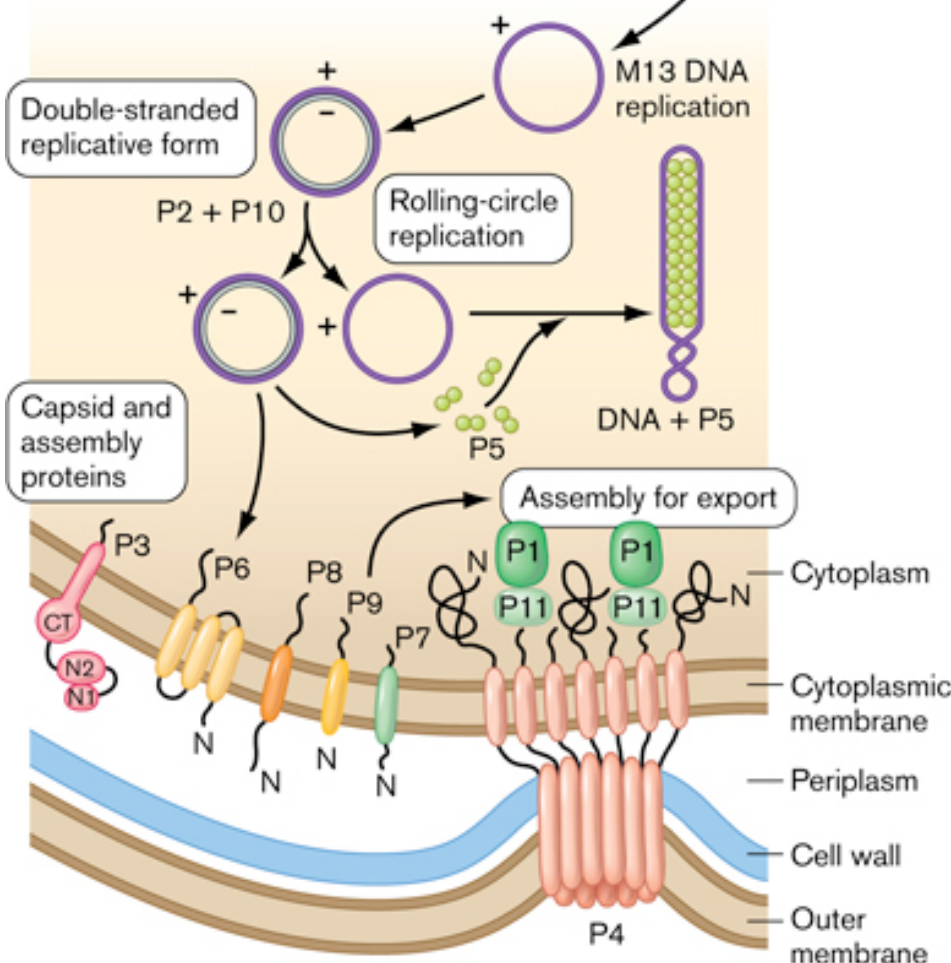
phage stays in tail pore complete



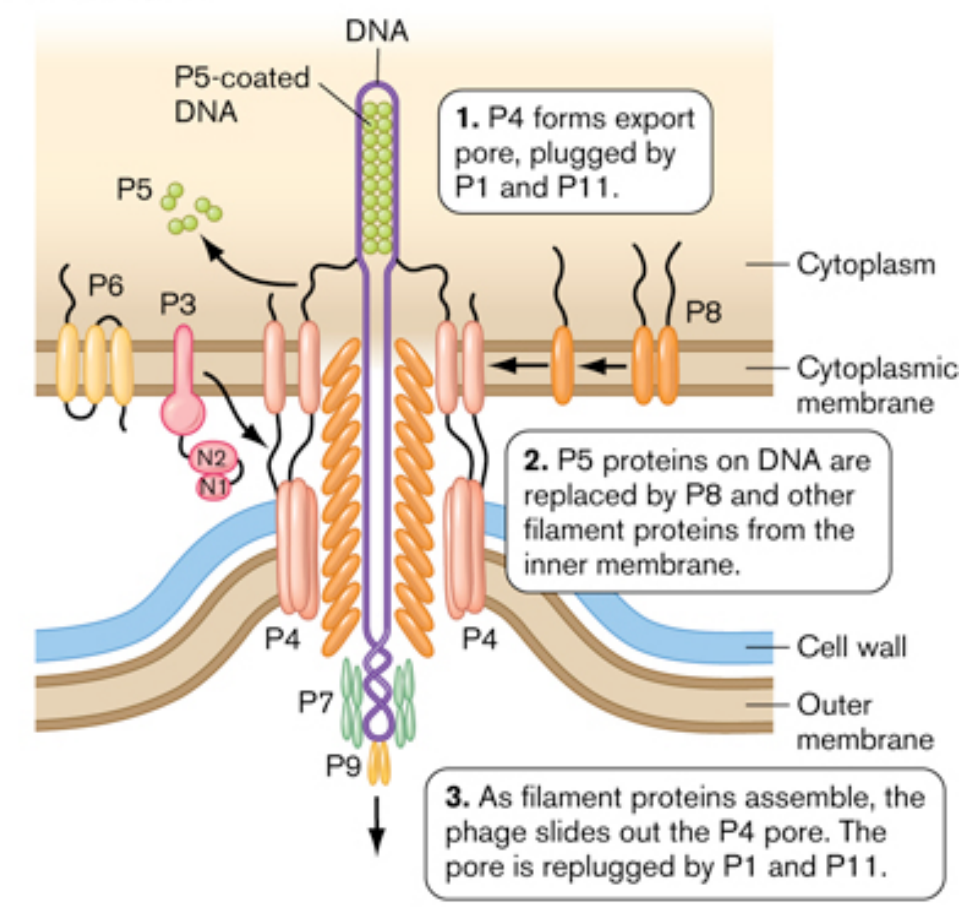
<http://www.norton.com/college/biology/microbiology2/ch/11/etopics.aspx>

# Bacteriophage are 'high copy' obligate organisms.

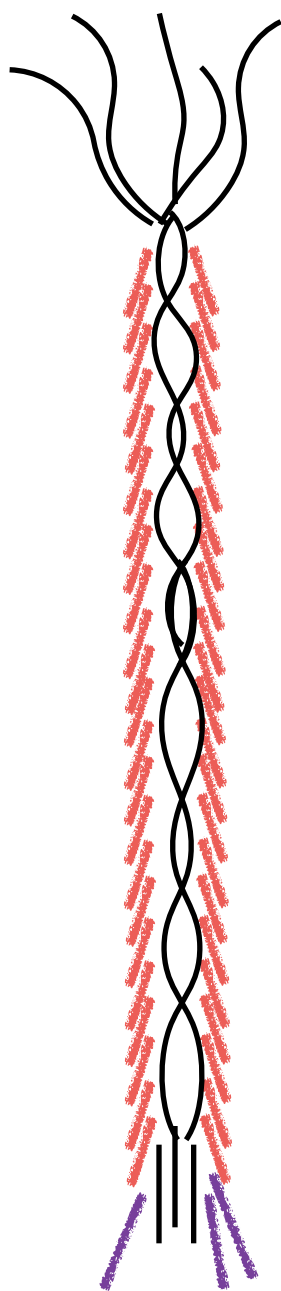
A. M13 assembly



B. M13 export



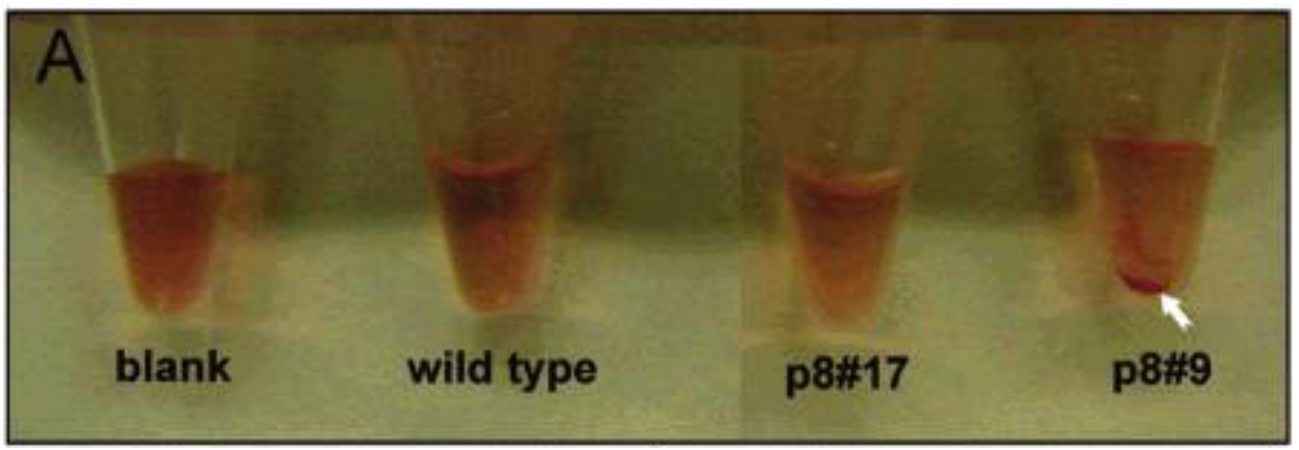
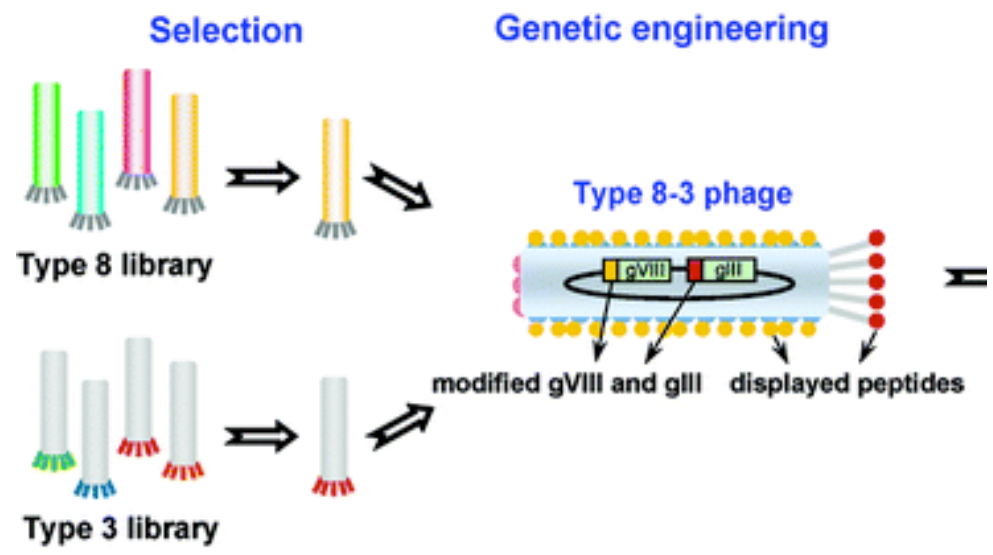
# Phage are engineer-able biomaterials



SWDPYSHLLQHPQ  
 Streptavidin  
 L7 purification

#9  
VSGSSPDS

binds  
 gold  
 (Au)



Programmable Assembly of Nanoarchitectures Using Genetically Engineering Viruses, *Nano Letters*, Yu et al. 2005



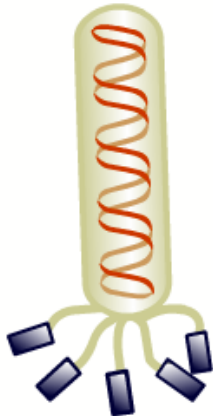
M13 phage were engineered to bind gold — best candidates were selected using phage display.

www.dyax.com/our-technology.html

close or Esc Key

### Phage Display

What is a Phage? | **Phage Display** | Phage Display Leader



#### What is a Phage Display?

In phage display, new genetic material is inserted into a phage gene. The bacteria process the new gene so that a new protein or peptide is made. This protein or peptide is exposed on the phage surface.

← Back | Next →

# Phage titer: plaque assay or spec.

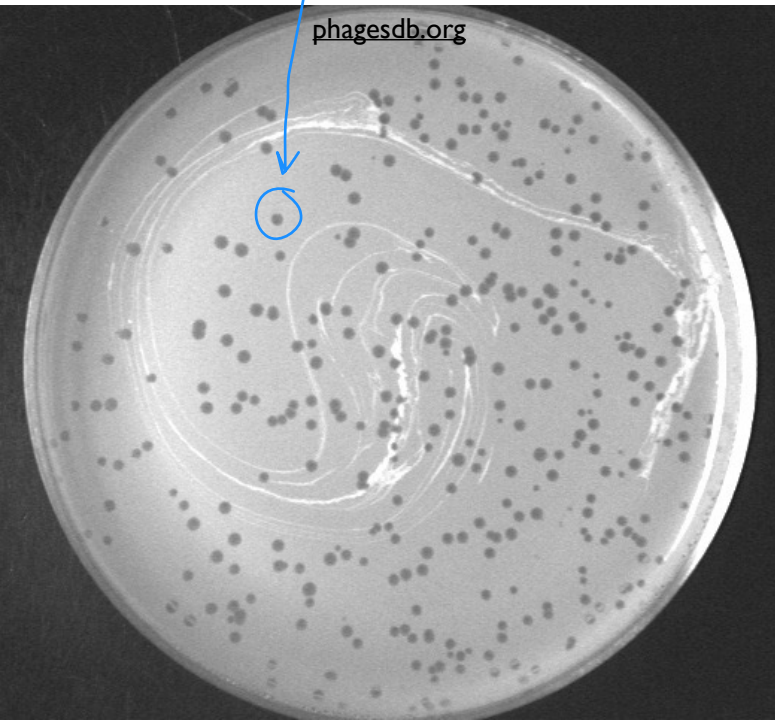
plaque = PFU = plaque forming unit =

1 virus

By plating:

Phage slow *E. coli* growth upon infection

bacteriophage = bacterial virus



phagesdb.org

20.109

By spectroscopy:

# phage particles =

quartz  
\$\$

protein + DNA

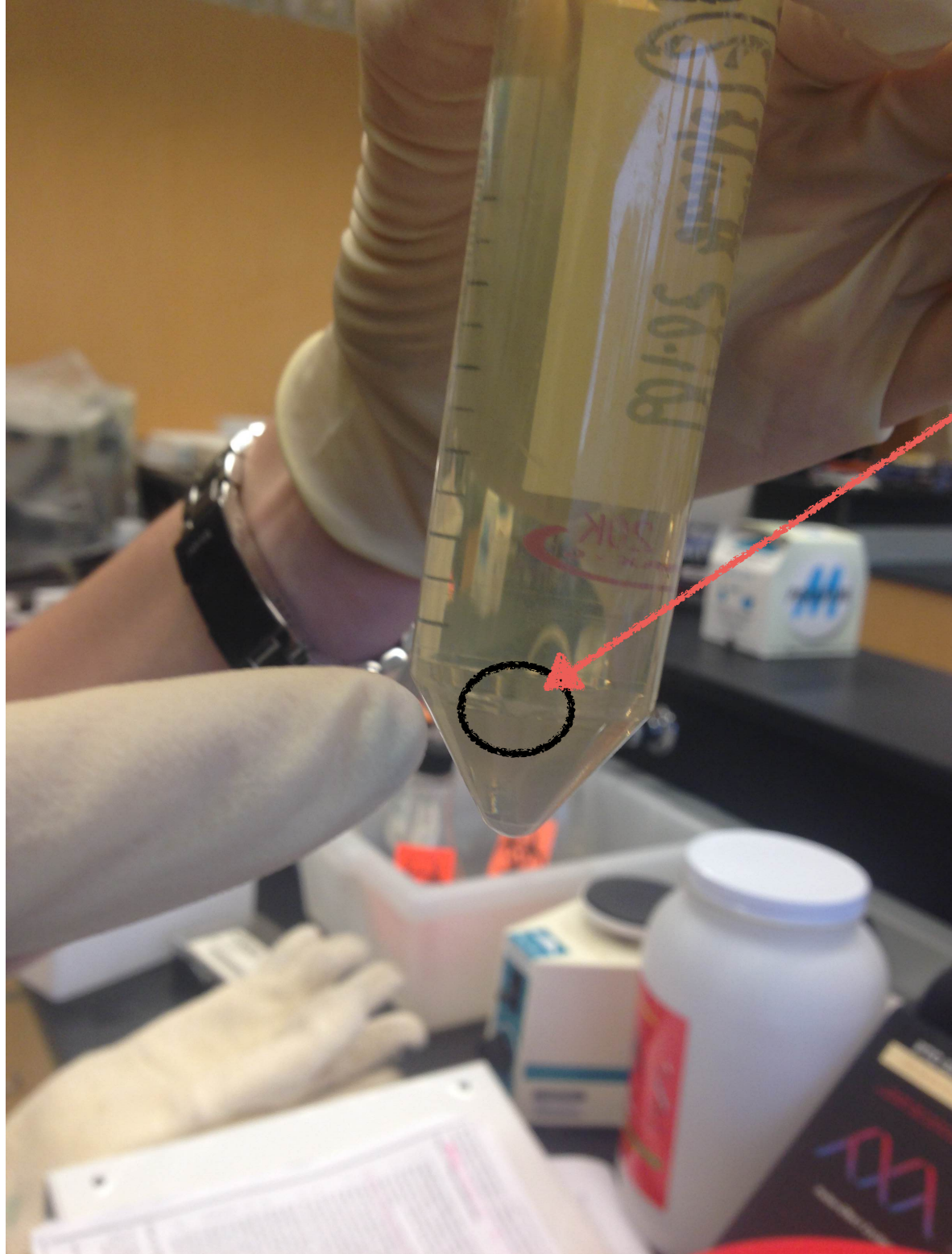
background

$$6 \times 10^{16} (A_{269} - A_{320})$$

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$$\# \text{ DNA bases in phage genome}$$

7220 bases



That is a phage pellet.

Maybe make a circle to mark where you think this should be....

## Today in the lab:

- Purify phage — **PAY ATTENTION** — the phage is in the supernatant!
- Measure concentration — **BE CAREFUL** — quartz cuvettes are fragile.
- Work on Mod2 Paper, write a blog post, or start to think about your Research Proposal during down time

- Office Hours: Extra Mod2  
Noreen 9-11 AM Friday } 16-429b  
Leslie 11-1 PM Friday }

Shannon+Noreen 10 AM-12 PM Sunday } 56-382  
Next time in the lab:  
Leslie 2-4 Sunday }

- Complex AuNP:phage with titania
- Set up TEM grids
- Later: TEM analysis and build/test DSSC

# Major assignment in Module 3: Research Proposal

openwetware.org/wiki/20.109(S15):Research\_proposal\_presentation

Search

st Visited Google Maps equipment 20.109(S14) - Ope... 20.109(S15): Sprin... 20.109(F14) - Ope... Atlas - Navigating ... COLD-PCR - Wiki...

with a person from another lab group. This person will offer you a fresh ear to consider your proposal. You can rework your proposal based on the conversations you've had.

## Logistics

[edit]

Prepare a 12 minute powerpoint talk that describes the research question you have identified, how you propose to study the question, and what you hope to learn. A general outline for your research proposal presentation is:

- a brief project overview (scientific and social context)
- sufficient background information for everyone to understand your proposal
- a statement of the research problem and goals (specific research aims)
- project details and methods
- predicted outcomes if everything goes according to plan and if nothing does
- needed resources to complete the work
- societal impact if all goes well

On the day you present (see announcements on front page for when and where) your team should print out and bring **two copies** of your powerpoint slides. Black and white is fine and you can print more than one slide per page if you like (4-6 slides per page tends to be ideal for my note-taking). You should also write (and print out) your "talking points" in the comments box of each slide or in a separate document. These are speaking notes for your presentation, and should include short phrases to remind you of the key points to cover on each slide, as well as the transitions you've planned between them. For example, in a previous year's presentation one slide's talking points were:

*As you can see from this image, taken from a review on hydrocarbon metabolism in marine bacteria, the *alcanivorax* species is the first to grow in population after an oil spill, and its growth correlates with a decrease in aliphatic hydrocarbons.*

- *After most alkanes have been degraded, the *Cycloclasticus* species blooms while aromatic hydrocarbon levels decrease*
- *One thing to note is that as soon as they have done their job, both species return to their normal population levels.*
- *One problem with using *Alcanivorax* and *Cycloclasticus* to clean oil spills, however is that they can only be found in specific locations*

The next slide (transition statement) began: *To remedy this, we decided to look into other bacteria into which we could move the hydrocarbon metabolic pathways*

You don't have to use complete sentences in your own talking points, but the above example should give you a sense of what content is expected.

***You will be graded on the integrated success of your presentation: concepts, slides, talking points, and presentation.***

## Rubric

[edit]

[Here you go](#)