Introduction to the Scientific Method with a Vignette in Phage Research

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The Rise in Antibiotic Resistance is Inciting a Global Public Health Crisis

In 2013, the US Centers for Disease Control declared antibiotic resistance as one of the nation's most serious health threats:

➢ 2 million people per year in the US acquire serious antibiotic resistant infections.

➢ At least 23,000 people each year die as a direct result of these infections.

➢ Many more die from complications caused by the antibiotic resistant infection.

➢ Antibiotic resistant infections prolong hospital stays and add considerable cost to the healthcare system:

   ✧ $20 billion a year in excess direct healthcare costs

   ✧ $35 billion a year in indirect cost (eg. loss of productivity at work)

CDC, *"Antibiotic Resistance Threats in the US"*, 2013
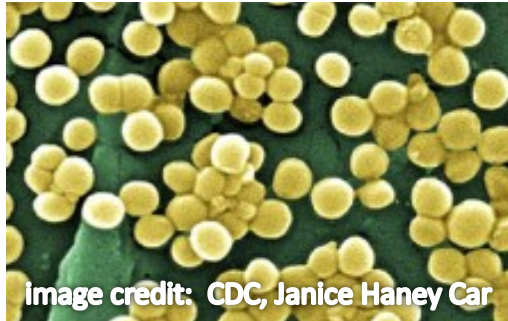
Drug Resistant Staphylococci

Methicillin Resistant
Staphylococcus aureus
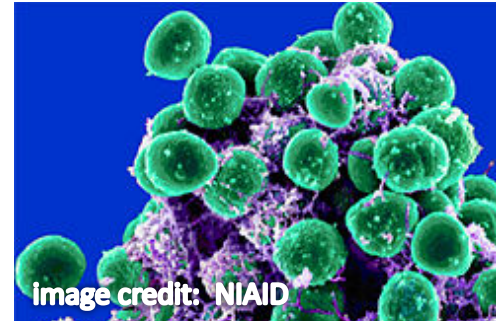

Biofilm forming
Staphylococcus epidermidis

- Drug-resistant *Staphylococcus aureus* (MRSA) causes both hospital- and community- acquired infections. In 2011:
  - ✧ caused 80,461 severe hospital-acquired infections
  - ✧ 11,285 of these resulted in death

- *Staphylococcus epidermidis* is the most common cause of drug-resistant infections associated with medical implants (ie. cardiac devices, prosthetic joints; CNS shunts, catheters etc.)

- Today there exist Staphylococcal strains that are resistant to all known antibiotics

CDC, "*Antibiotic Resistance Threats in the US*", 2013

Bacterial viruses (aka Phages): Alternatives to antibiotics
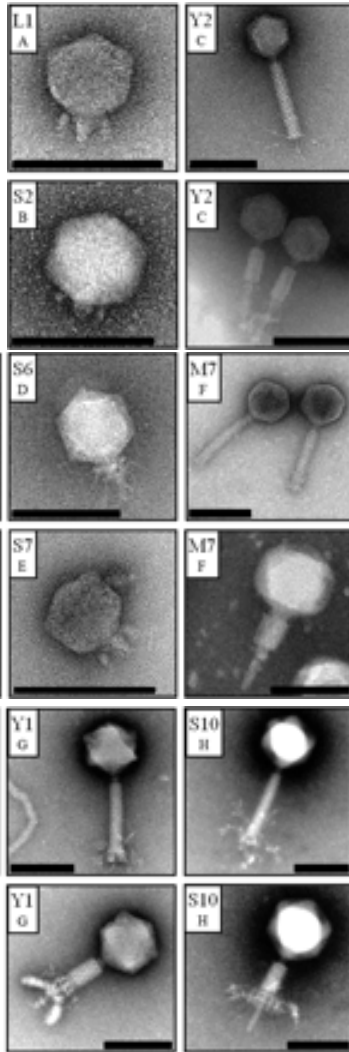
**Erwinia phages**. Born *et al*, *App and Env Eng*, 2011

- Bacterial viruses (bacteriophages) are the most abundant entities in the biosphere.

- In terrestrial and aquatic environments, phages outnumber their bacterial prey by a factor of 10.

- Many are lethal to their bacterial hosts.

- "Phage Therapy" was commonly used to treat infections prior to the advent of antibiotics, and still practiced today in parts of Eastern Europe.

- Currently a renewed interest in using phages or their products as alternatives to antibiotics.

Enlisting Bacterial Viruses to Combat Drug Resistant Staphylococci

- Fishing for Phages at the Tuscaloosa Wastewater Treatment Plant:

- Novel *Staphylococcus epidermidis viruses* isolated from Tuscaloosa wastewater:
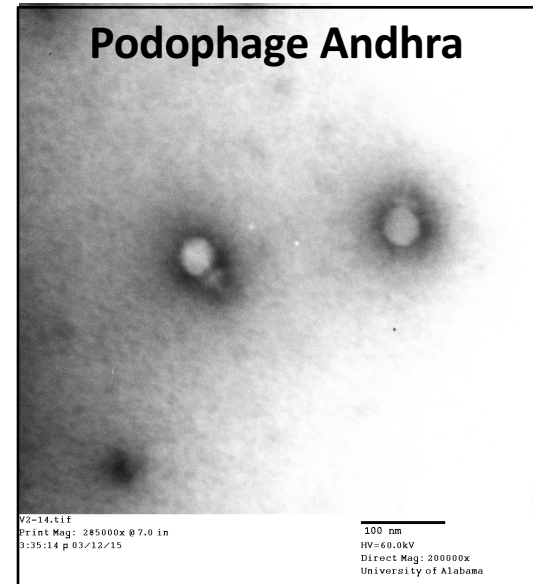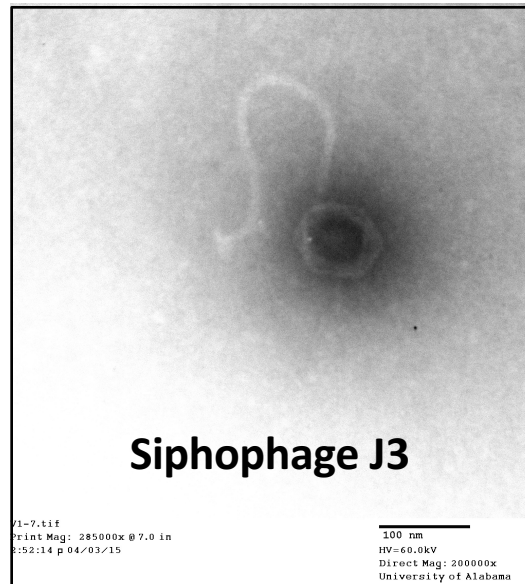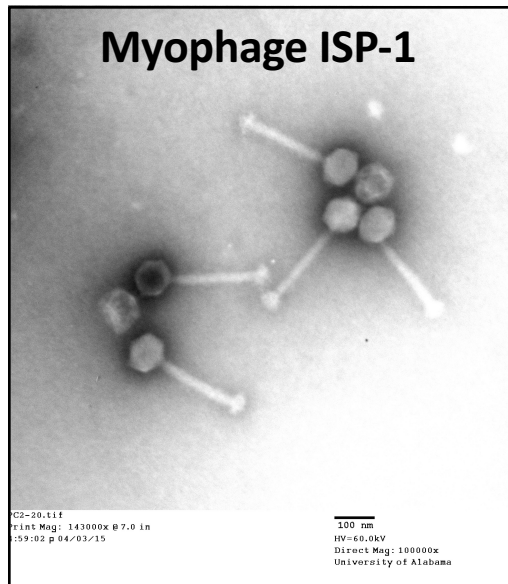
Phage characterization using the scientific method, a series of steps that researchers use to answer questions

Ask a question

Do background research

Form a hypothesis
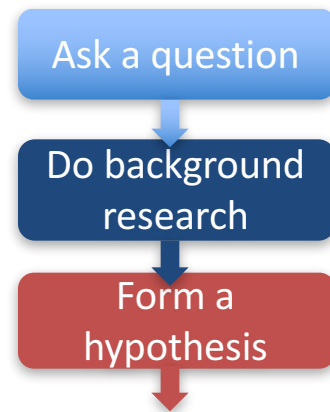
-> Which phage is more effective in bacterial killing (Myophage ISP or Podophage Andhra?)

-> No examples of *S. epidermidis* podophages have been reported, but we observed larger "plaques" on bacterial lawn:

ISP-1
head = 85.7 nm

Andhra
head = 42.7 nm

100 nm

100 nm

-> Hypothesis: Podophage Andhra is more effective in bacterial killing than Myophage ISP.

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Test with an experiment

Experiment failed ☹

Experiment worked!

-> Designed a "phage challenge" experiment – bacteria grown in liquid culture and 1×10^7 phage particles are added after 2h.
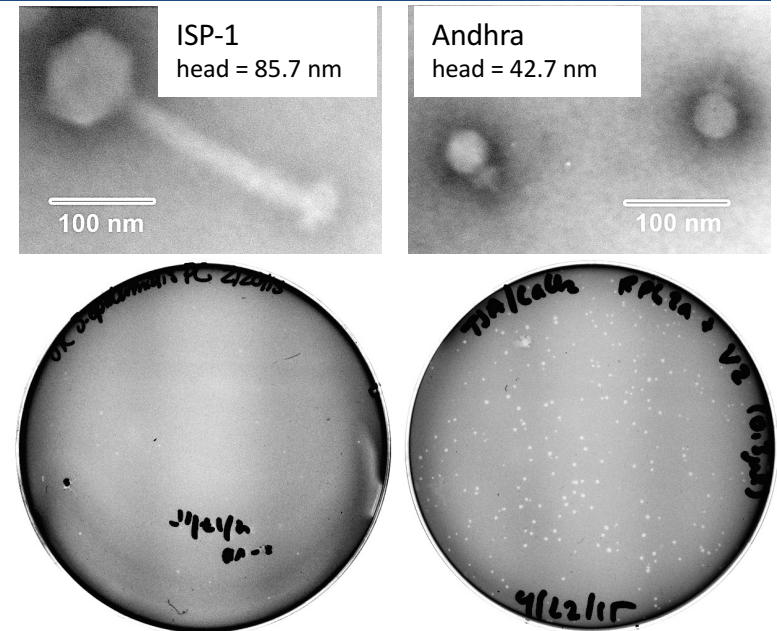

Andhra

Phage ISP-1

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Repeat!

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Andhra

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Analyze data & draw conclusions

Results align well with hypothesis

Results DO NOT align with hypothesis

Communicate results

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Staph growth (OD600)

Andhra

hours

Staph growth (OD600)

Phage ISP-1

hours

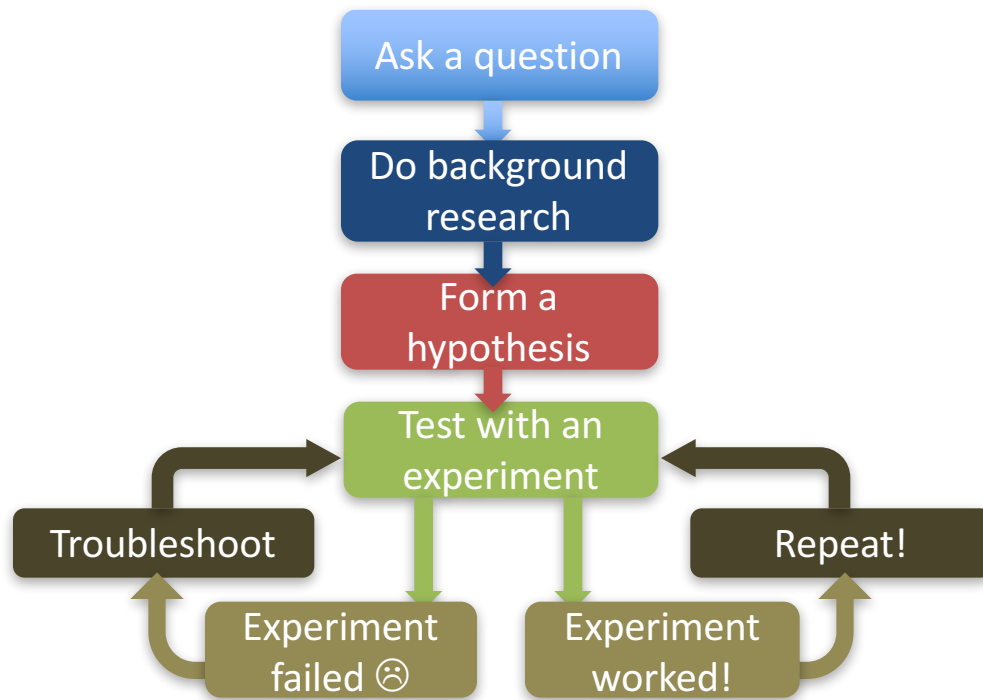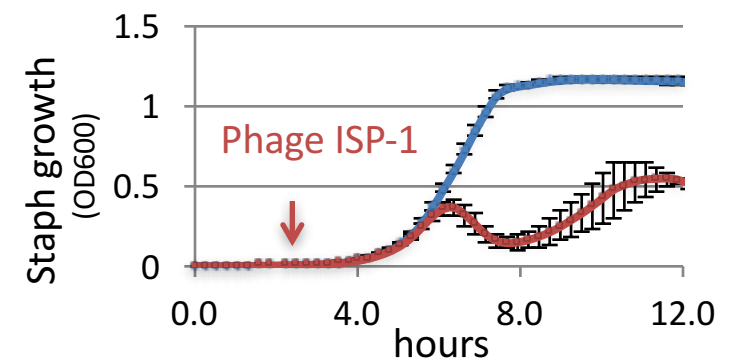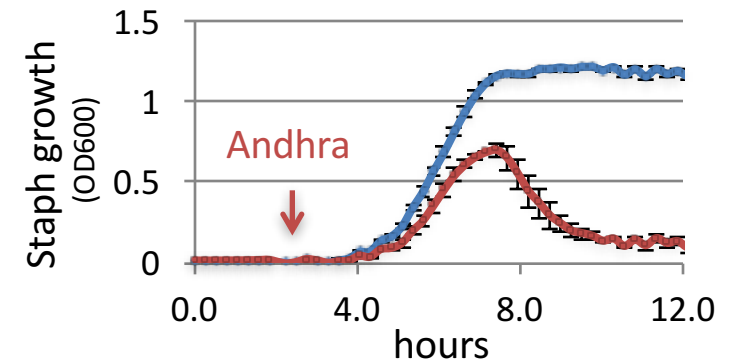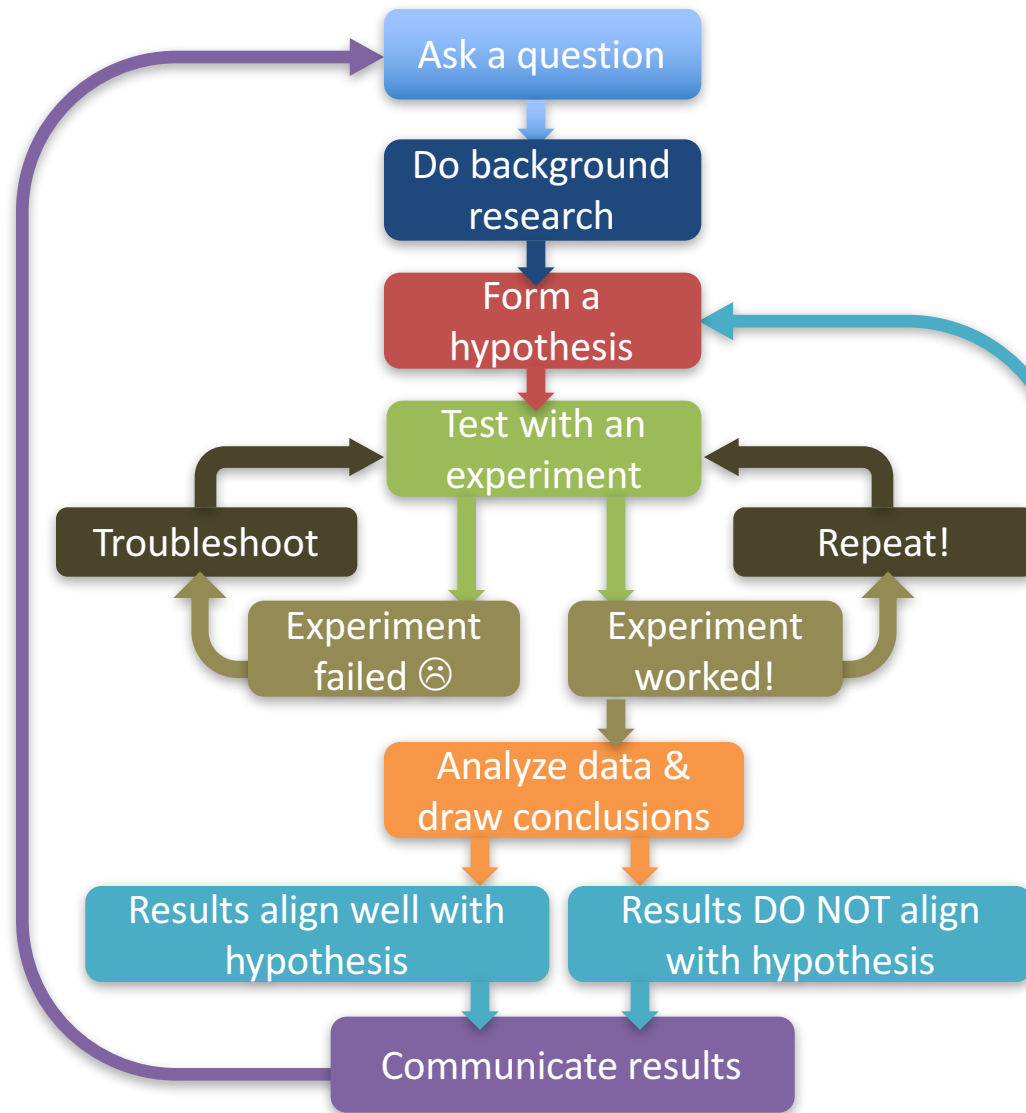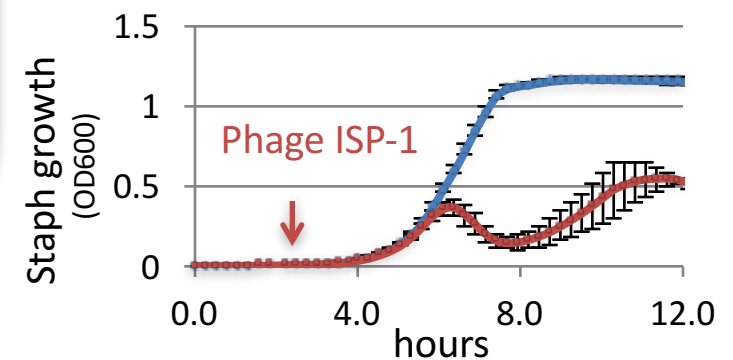-> Andhra appears to kill *S. epidermidis* more effectively than ISP.

Phage characterization using the scientific method, a series of steps that researchers use to answer questions

Ask a question

-> Which protein in Andhra is responsible for bacterial killing?

Do background research

-> Other phages are known to kill bacteria with proteins called "holins" and "lysins", which rupture membranes and degrade bacterial cell walls.

Form a hypothesis

-> Hypothesis: Andhra encodes lysins that can be used as potent anti-bacterial agents.

Test with an experiment

-> We sequenced Andhra's genome and searched for proteins that share homology with known lysins.

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Phage Andhra Genome

Andhra genome characteristics:

- linear, double-stranded DNA
- 18,546 base pairs
- 20 genes
- 45% of genes have unknown function

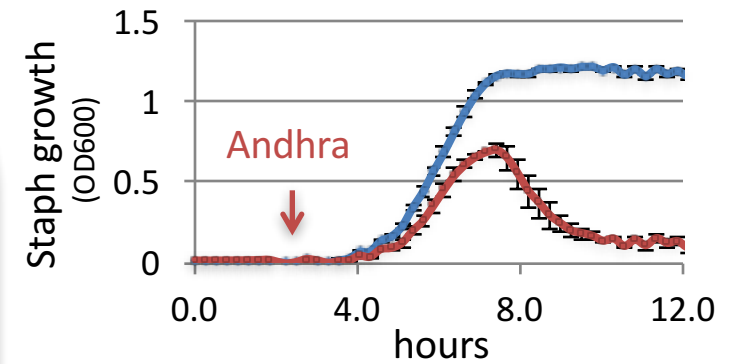Andhra genome schematic:

* gp = gene product; gp10 and gp14 are predicted lysins

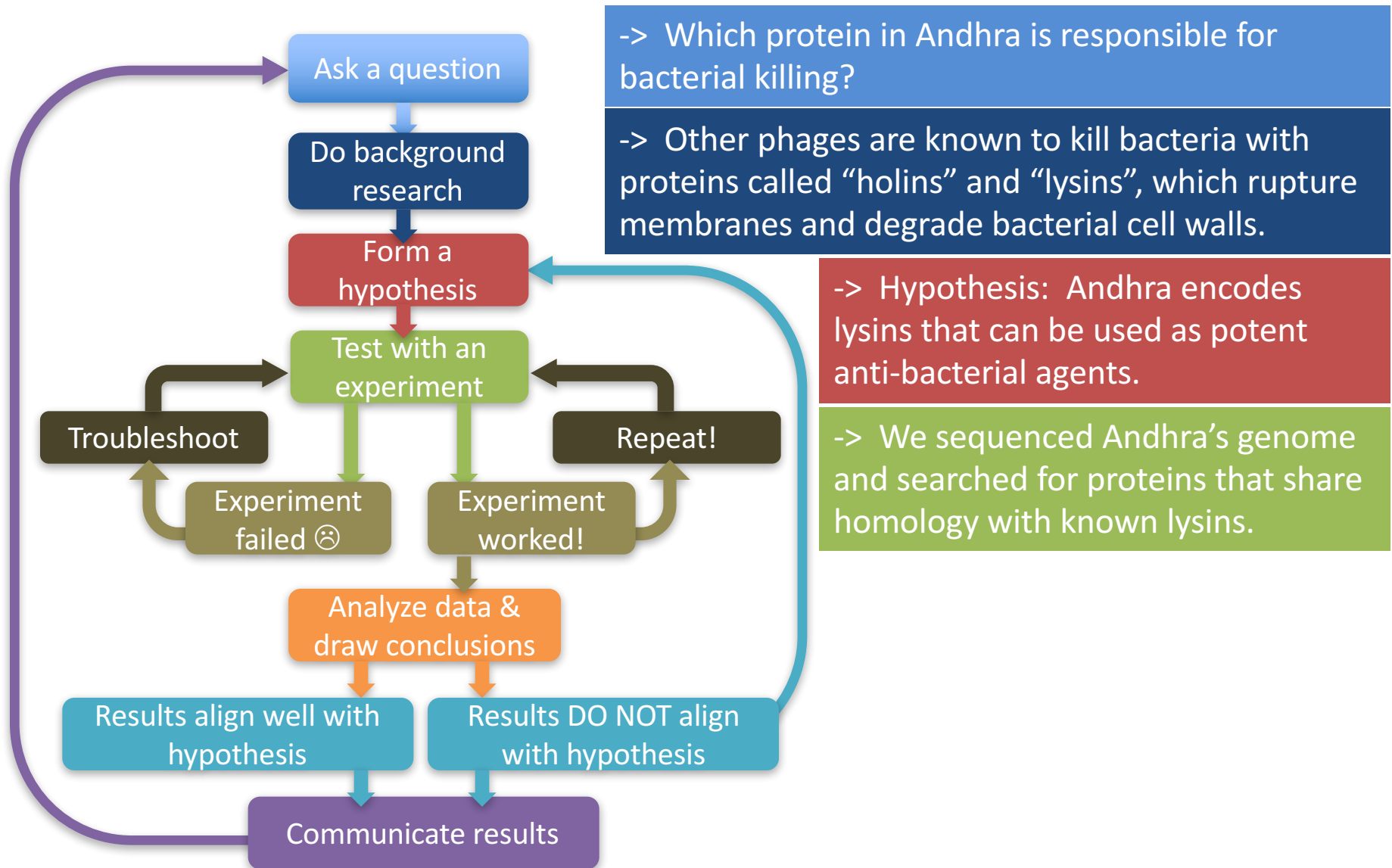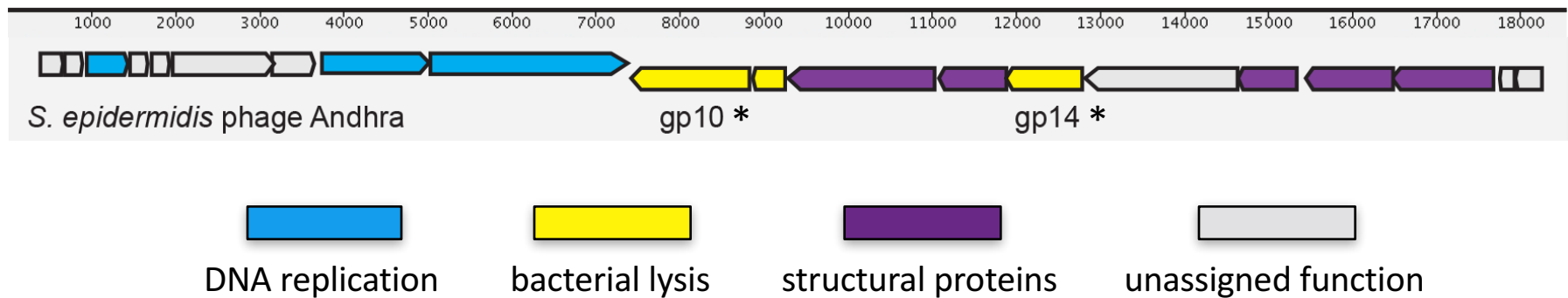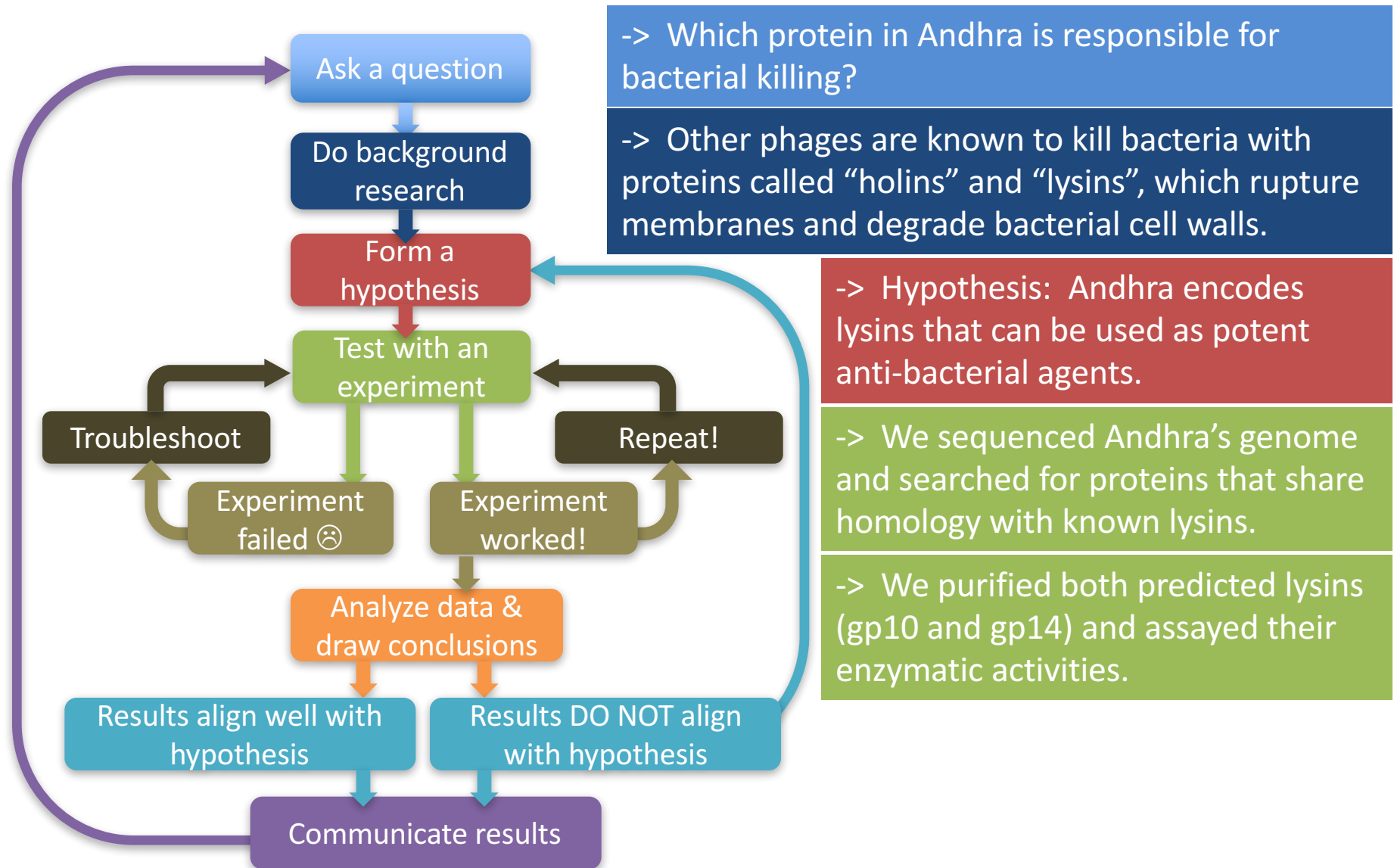# Phage characterization using the scientific method, a series of steps that researchers use to answer questions

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-> Hypothesis: Andhra encodes lysins that can be used as potent anti-bacterial agents.

-> We sequenced Andhra's genome and searched for proteins that share homology with known lysins.

-> We purified both predicted lysins (gp10 and gp14) and assayed their enzymatic activities.

Purification and characterization of Andhra's gene products 10 and 14

1. Purified proteins:
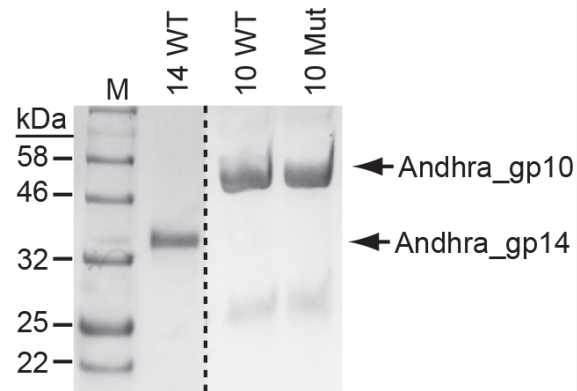
3. Effects of purified proteins on cell walls:

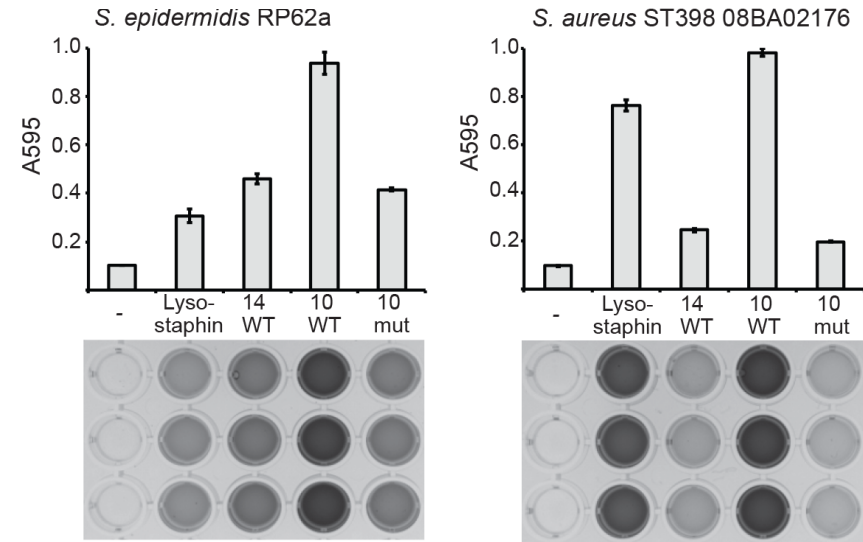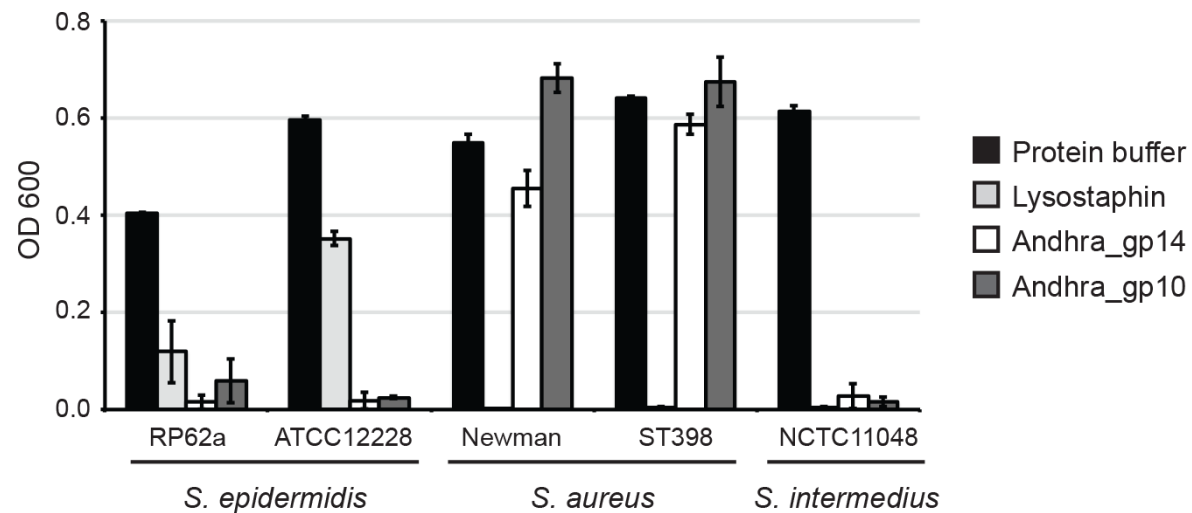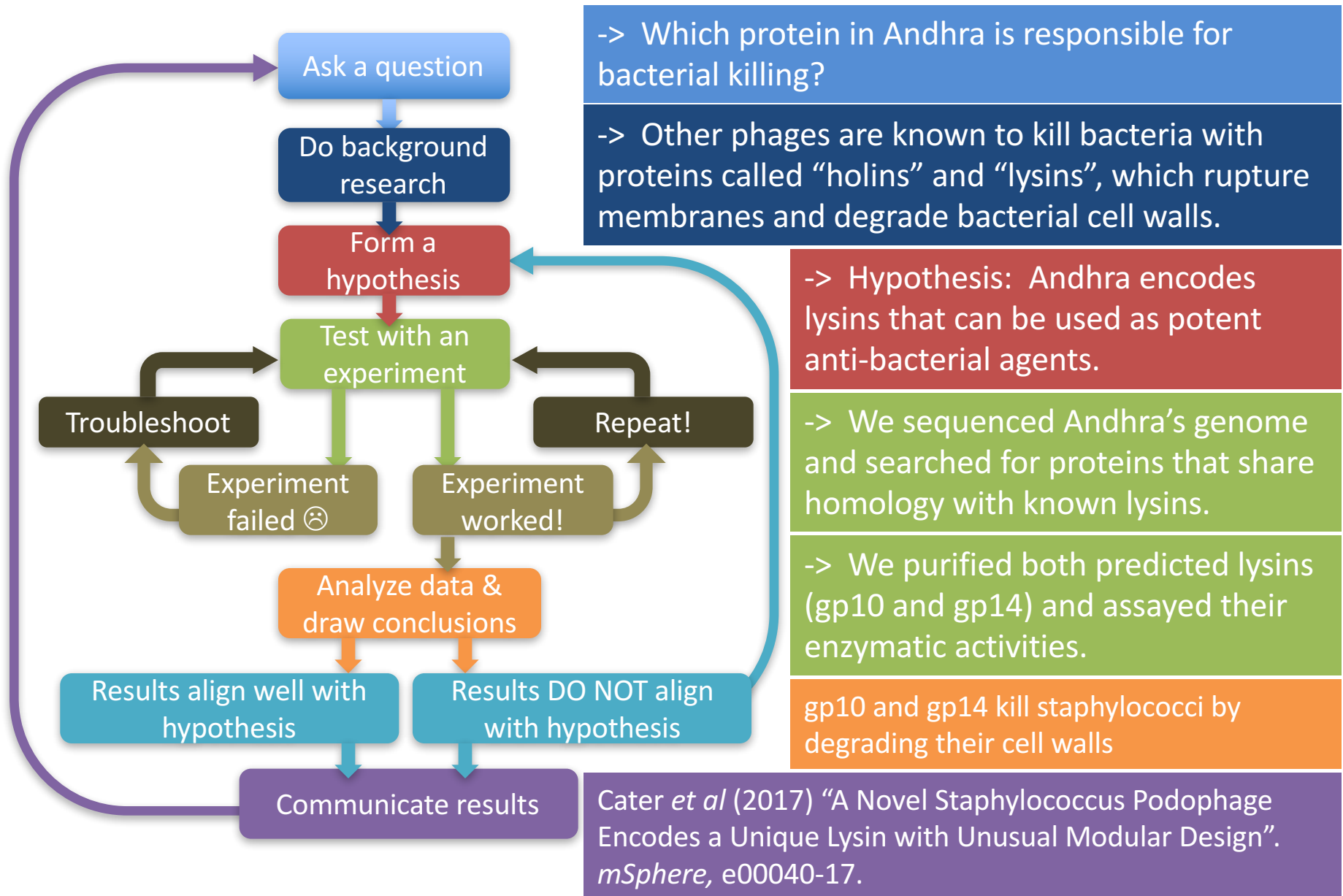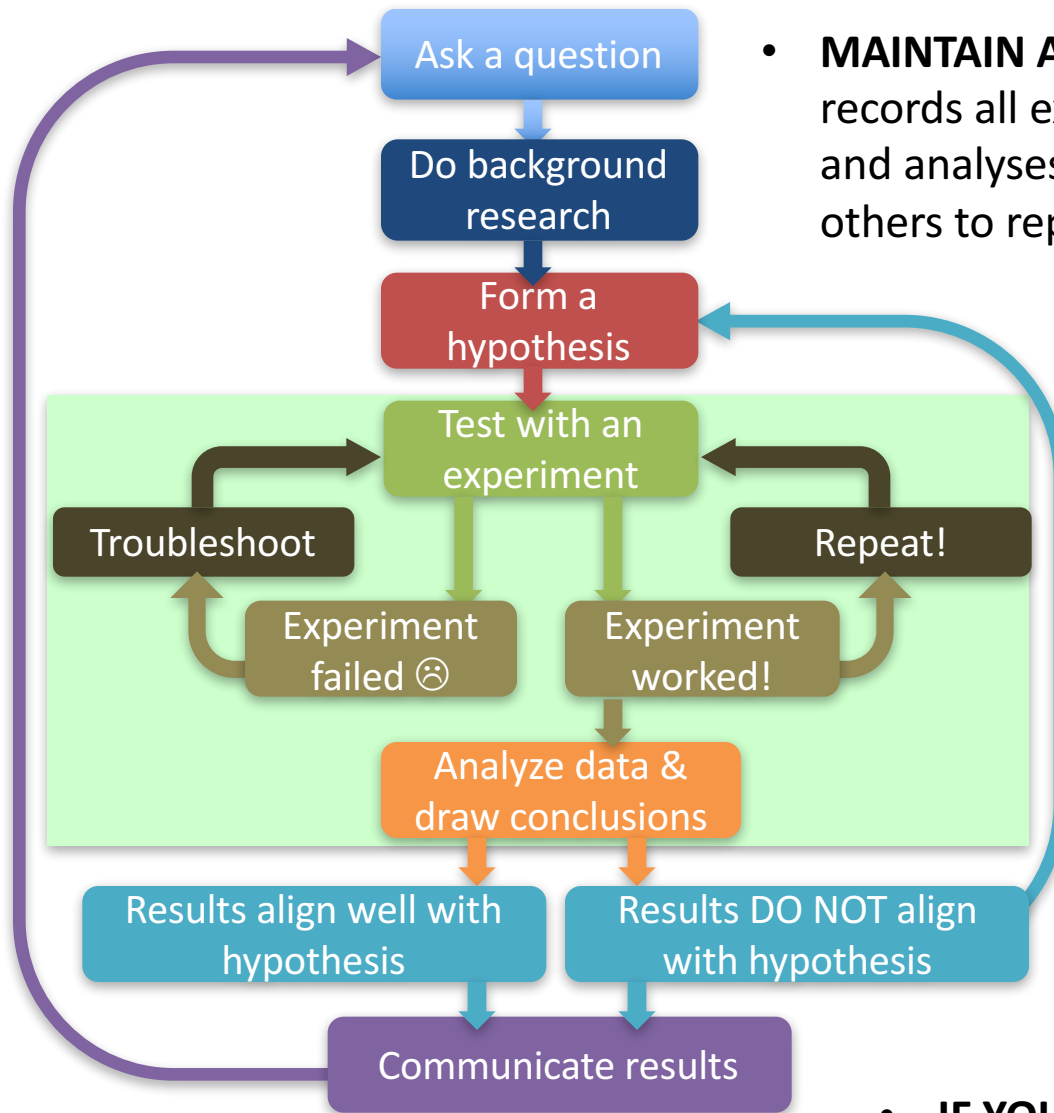2. Effect of purified proteins on cell growth:

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gp10 and gp14 kill staphylococci by degrading their cell walls

Cater *et al* (2017) "A Novel Staphylococcus Podophage Encodes a Unique Lysin with Unusual Modular Design". *mSphere,* e00040-17.

General Lab Responsibilities and Tips

- **SAFETY FIRST!**

- **MAINTAIN A DETAILED LAB NOTEBOOK** that records all experiments conducted, data collected, and analyses in real time! This will allow you and others to reproduce your experiments.

  - **STICK TO THE PROTOCOL**. If changes to the protocol are made, record details in your lab notebook.

  - **RECORD OBSERVATIONS ON A REGULAR BASIS**. Anything that appears out of the ordinary should be faithfully recorded.

  - **RECORD DATA WITHOUT BIAS.** Even if a data point does not appear to agree with the trend, it must be recorded.

  - **WHEN TROUBLESHOOTING, CHANGE ONLY ONE VARIABLE AT A TIME.**

- **IF YOU HAVE ANY QUESTIONS, JUST ASK!**