# The Wonderful World of Mycobacteriophages: Discovery, dynamics, collusion, and therapy

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# Two very sick patients

## 9th October 2017

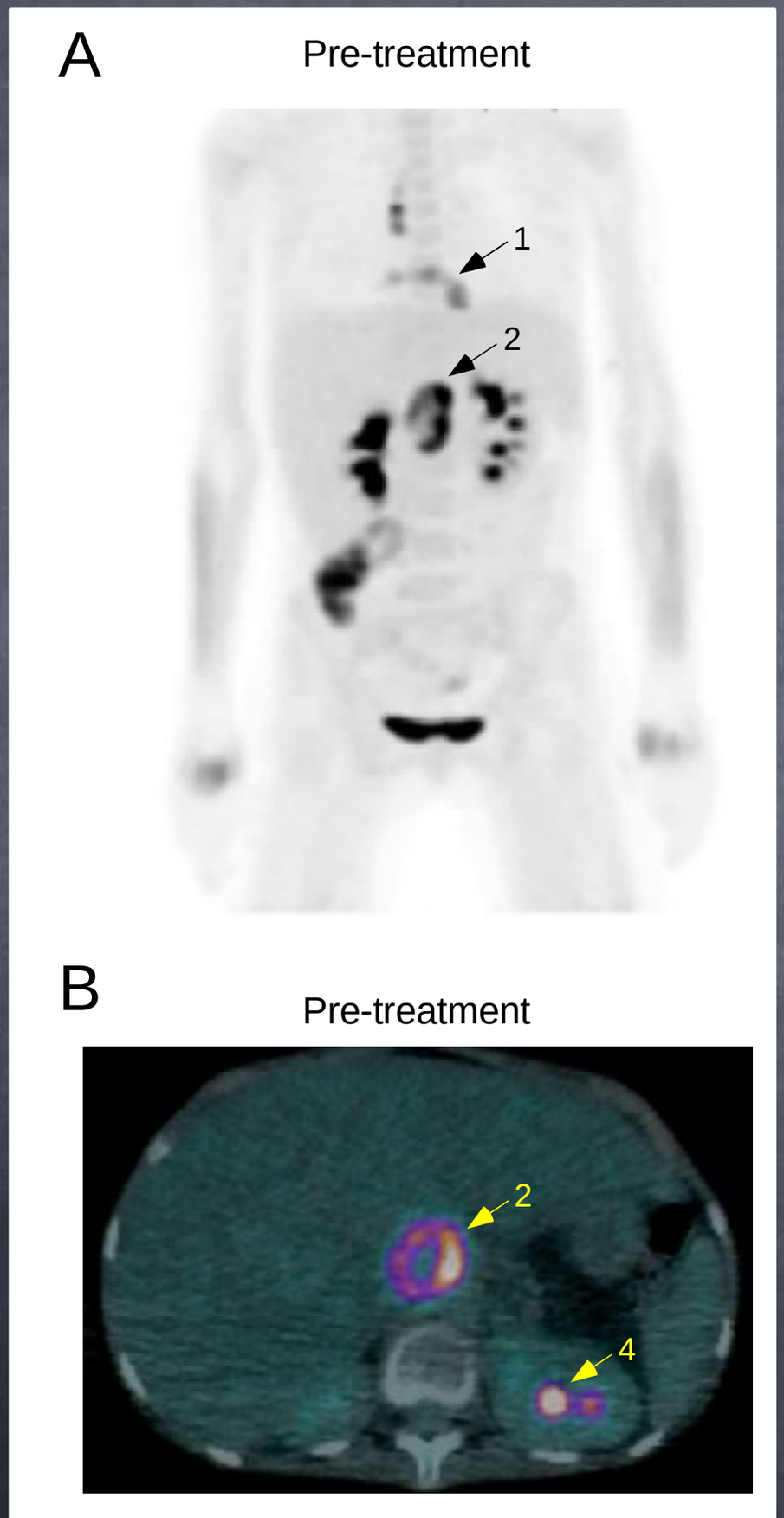
- Email message from Dr. James Soothill in London:
- Two patients with difficult to treat disseminated *Mycobacterium abscessus* infections:
- Cystic Fibrosis; Double lung transplant

## 2nd November 2017

- Strains received in Pittsburgh; subcultured

Both strains highly antibiotic resistant
Patients are at high risk
Could phages be used therapeutically?

# CT-PET: Pre-treatment



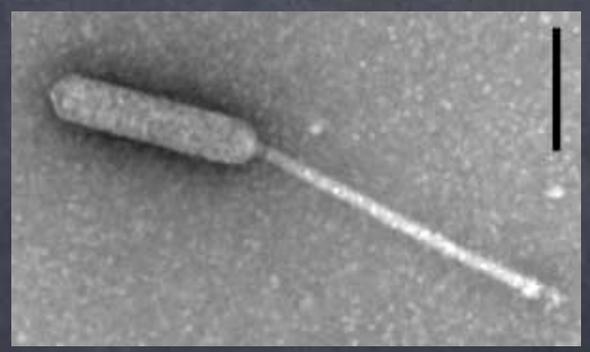
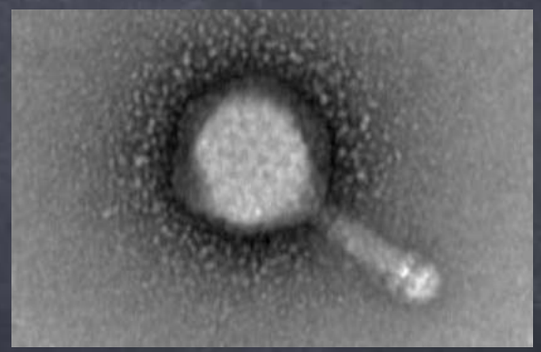

tigecycline
imipenem
amikacin
moxifloxacin
clarithromycin
linezolid
amphotericin
clofazimine
Bedaquiline
Rituximab
basiliximab
tacrolimus
mycophenolate mofetil
methylprednisolone

# Sternal wound and skin nodules

Pre-treatment

# What are bacteriophages?

- Bacteriophages (phages) are viruses that infect bacteria
- Replication is host-dependent
- They contain their own genetic material
- Phages are host-specific; sometimes at strain level
- Phages are small (need an electron microscope to see)
- Many different types; mostly dsDNA tailed phages
- Maybe lytic or temperate
- Population is vast, dynamic, old, and highly diverse

# Bacteriophages: The numerical majority

## The phage population is vast

$10^6$ - $10^7$ phage particles/ml

$10^{31}$ phage particles in biosphere

## The phage population is dynamic

5:1 - 10:1 phage:bacteria

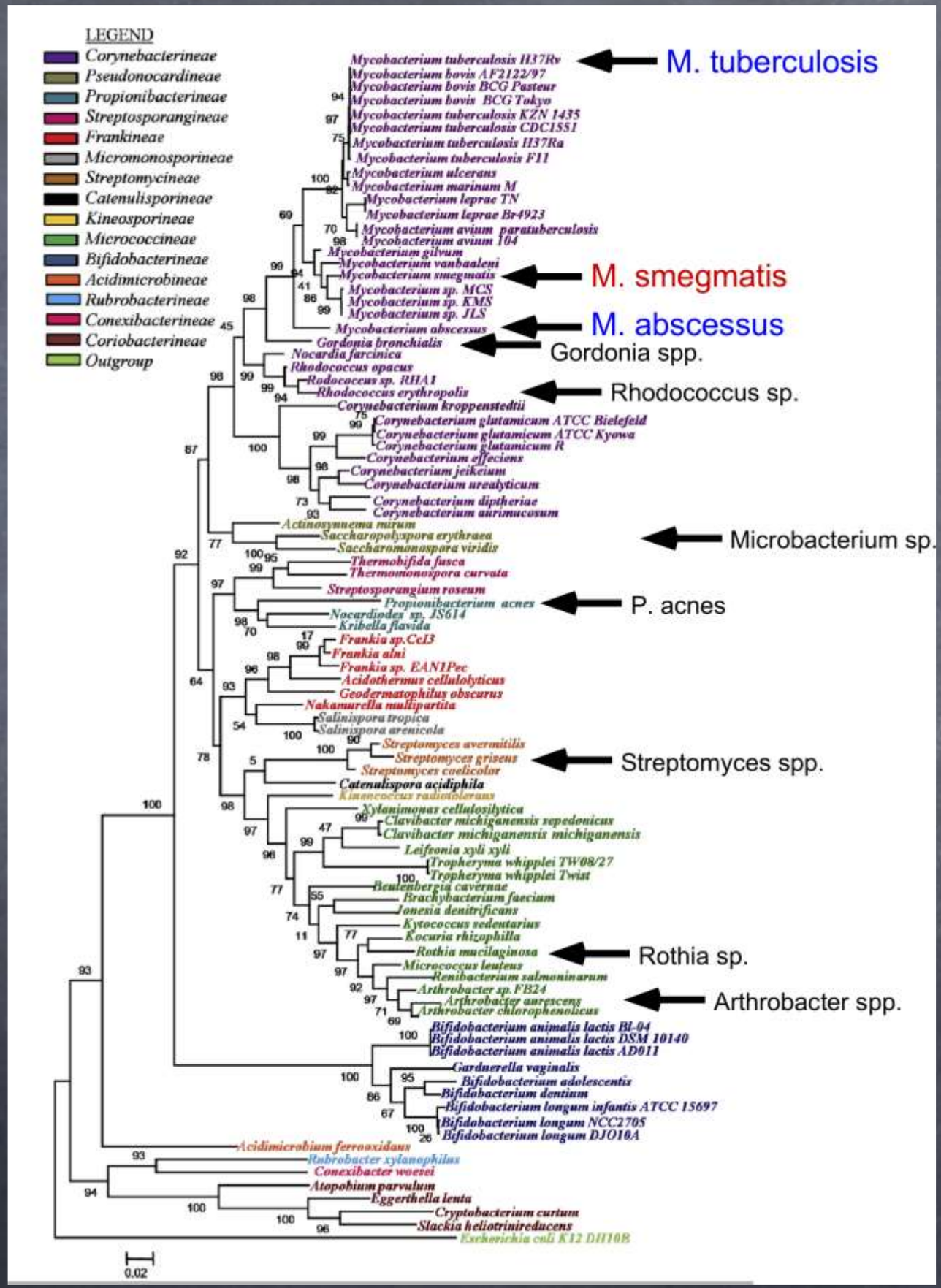
$10^{23}$ phage infections per second

## The phage population is old

Capsid structural conservation suggests ancient origins

# The Freezer!

# Bacterial hosts for phage isolation: Targeting Actinobacteria



# SEA-PHAGES: Phage discovery and genomics
# A platform for integrating research and education

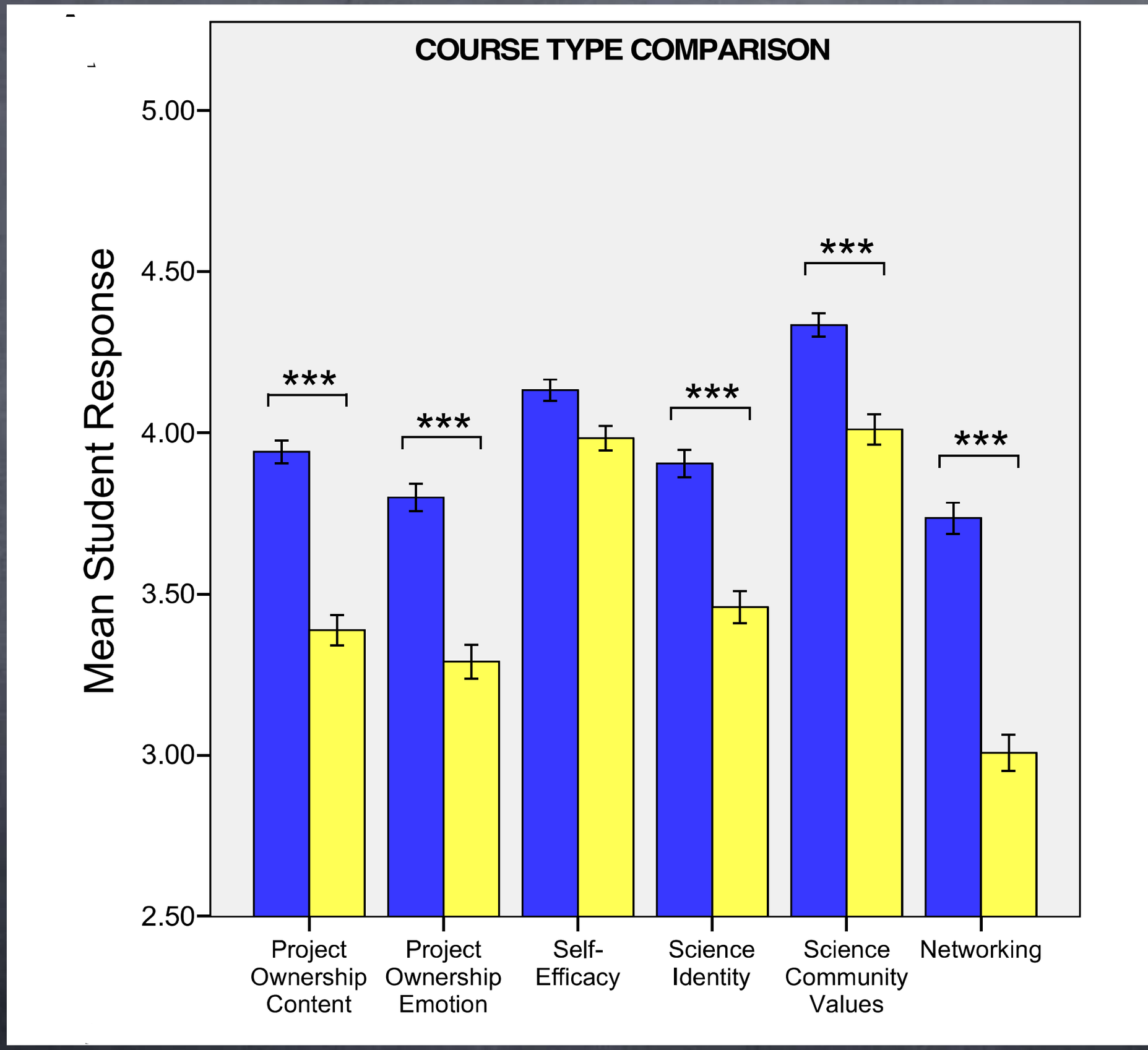
## Fall (microbiology) — Spring (bioinformatics)

Concrete → Representational

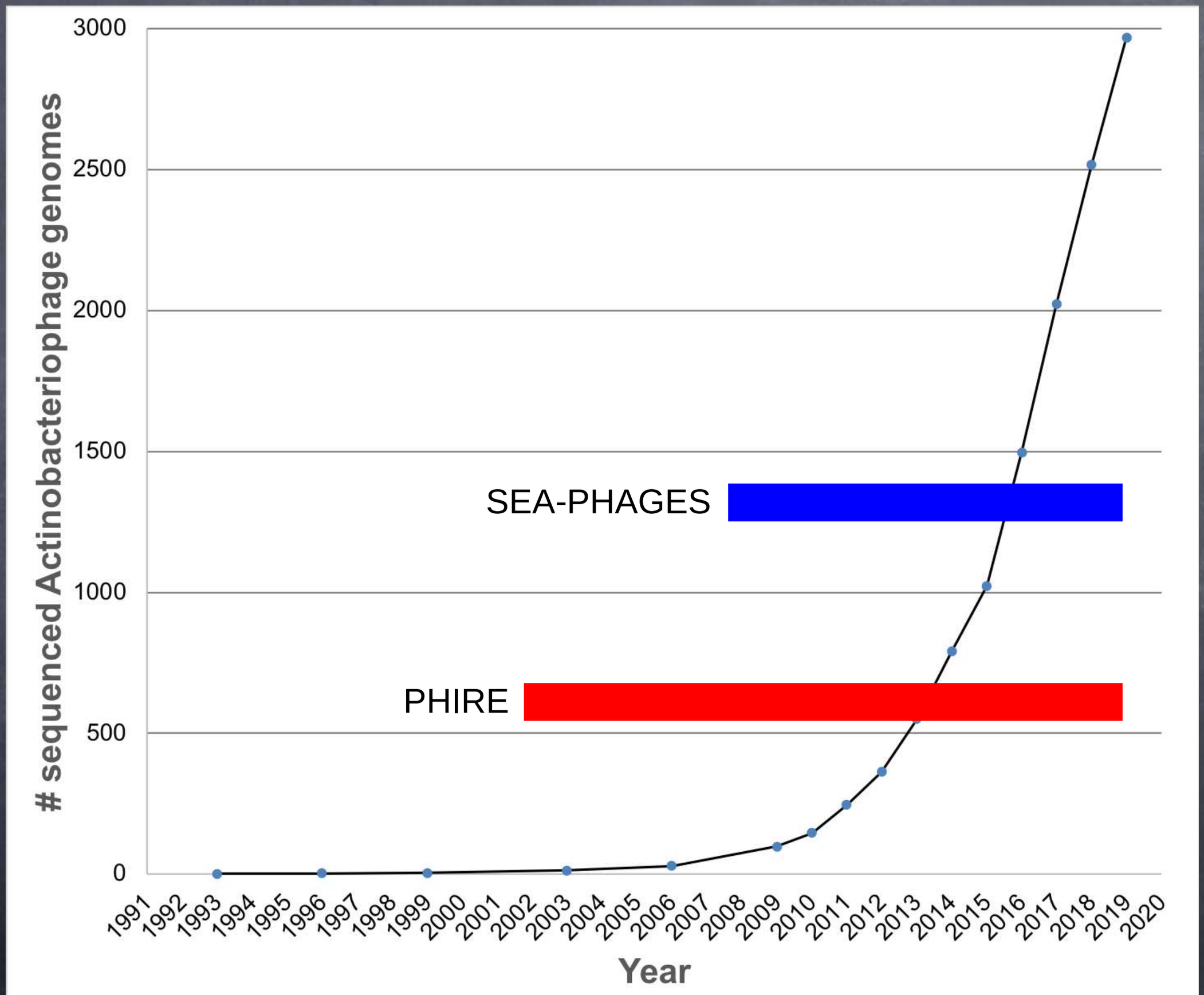
- Concrete beginnings offer equal opportunity of engagement
- Complex abstract processes contextualized for constructed learning
- Parallel projects facilitate peer-mentoring and development of flourishing scientist/educators

HHMI PHIRE program 2002-2019 (Pittsburgh); K-RITH/UKZN
HHMI SEA-PHAGES, 2008-2019 150 schools, >5,000 students (2019)

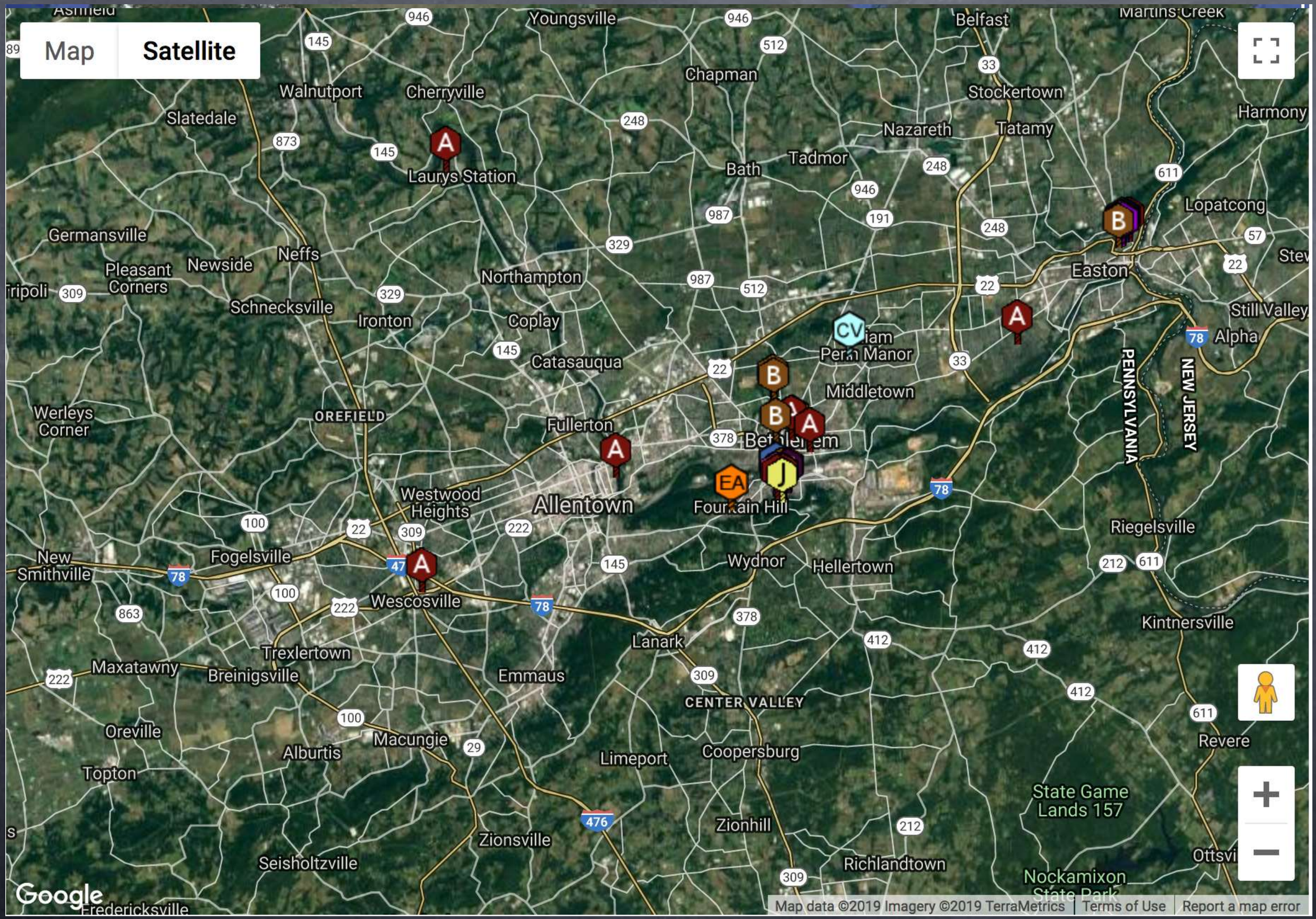
# SEA-PHAGES vs Traditional Lab



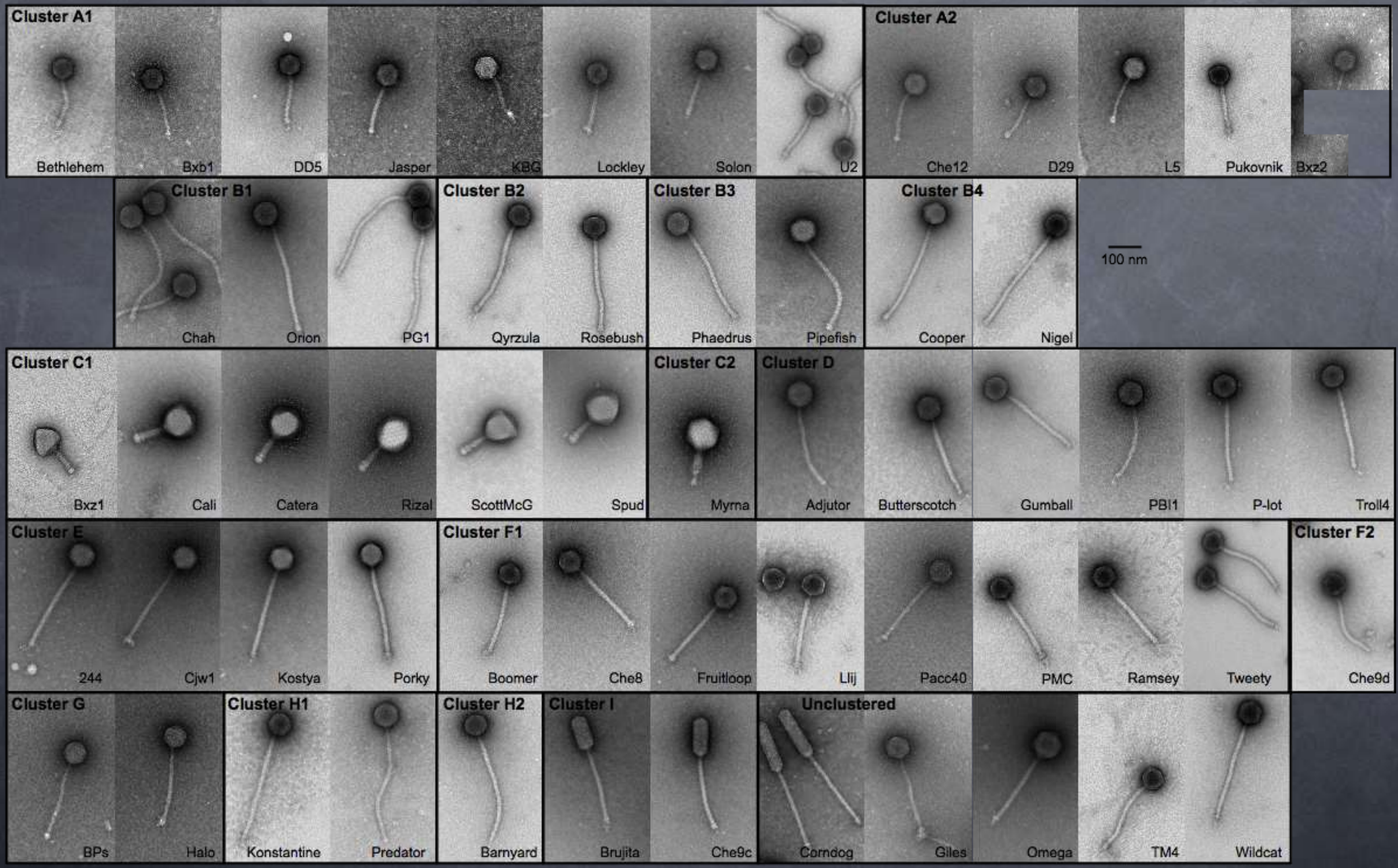
# Growth in the number of sequenced Actinobacteriophages



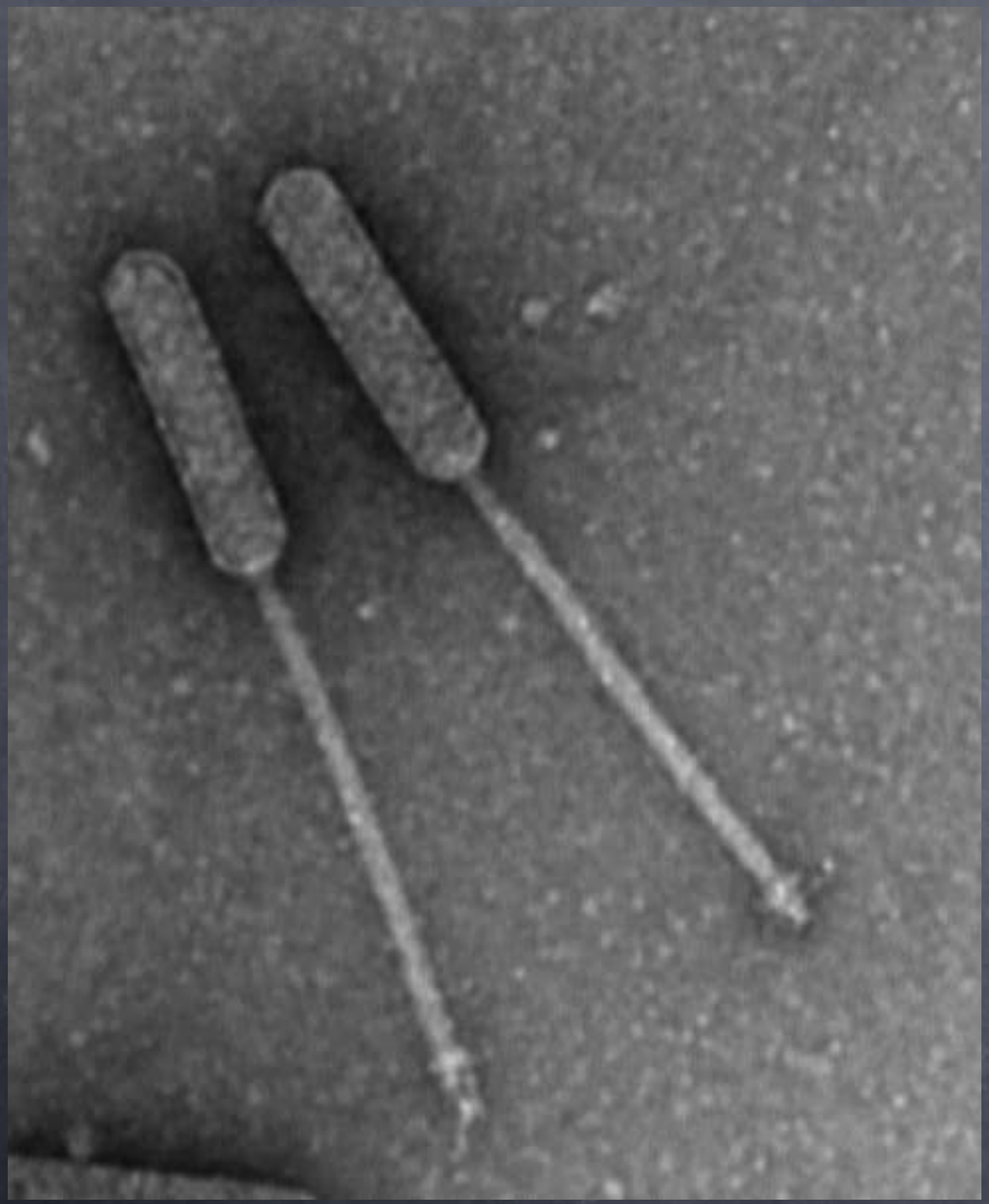
# Geographical distribution of sequenced actinobacteriophages

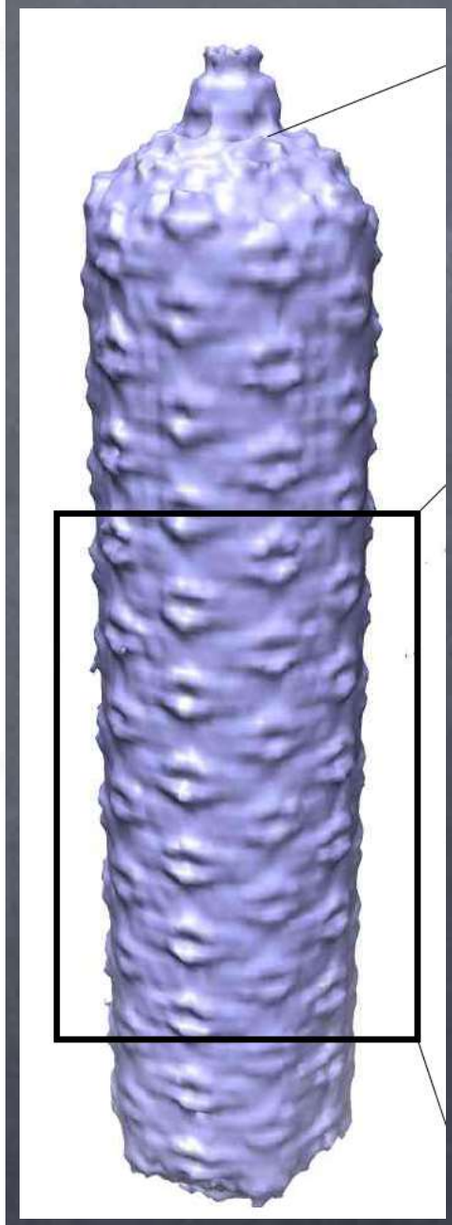


# Limited diversity of mycobacteriophage morphologies



# Mycobacteriophage Corndog





# The Actinobacteriophage Database

at PhagesDB.org

Home | Phages | Hosts | Data | BLAST | Publications | Resources | Software | Social | About

Type phage name

Search

**Recently Added Phages**

- CRB2
- Tentickles
- KodiBodie
- RocPanther
- RosaDiaz

**Recently Modified Phages**

- BirdsNest
- LilMcDreamy
- Indlovu
- Quesadilla
- Imvubu

**Recently Finished Phages**

- Bexan (A1)
- Indigenous (BI1)
- ShakeNBake (BN)
- Danforth (A8)
- Jerm2 (A1)

Signed in as **Graham Hatfull**

**Phagehunting in South Africa**

Teaching Assistants preparing for the 2013 K-RITH Mycobacterial Genetics Course at University of KwaZulu Natal, Durban, South Africa.

Welcome to the Actinobacteriophage Database at PhagesDB.org, an interactive site that collects and shares information related to the discovery, characterization, and genomics of phages that infect bacterial hosts within the phylum Actinobacteria.

## Gene Content Comparisons Now Available on PhagesDB

**Dan Russell** | Jan 23, 2018

**Live Phage Stats**

| Sequenced Phages | |
|---|---|
| *Actinoplanes* | 1 |
| *Arthrobacter* | 270 |
| *Brevibacterium* | 2 |
| *Corynebacterium* | 21 |
| *Gordonia* | 382 |
| *Microbacterium* | 229 |
| *Mycobacterium* | 1800 |
| *Propionibacterium* | 55 |
| *Rhodococcus* | 55 |
| *Rothia* | 1 |
| *Streptomyces* | 222 |
| *Tetrasphaera* | 1 |
| *Tsukamurella* | 2 |
| Total Phages | 15575 |
| Found in 2019 | 520 |
| Found in 2018 | 2472 |
| Found in 2017 | 2817 |
| Finished Genomes | 3041 |
| Available Fasta Files | 3041 |
| Phamerated | 3031 |
| # Phams | 30382 |
| In GenBank | 2716 |

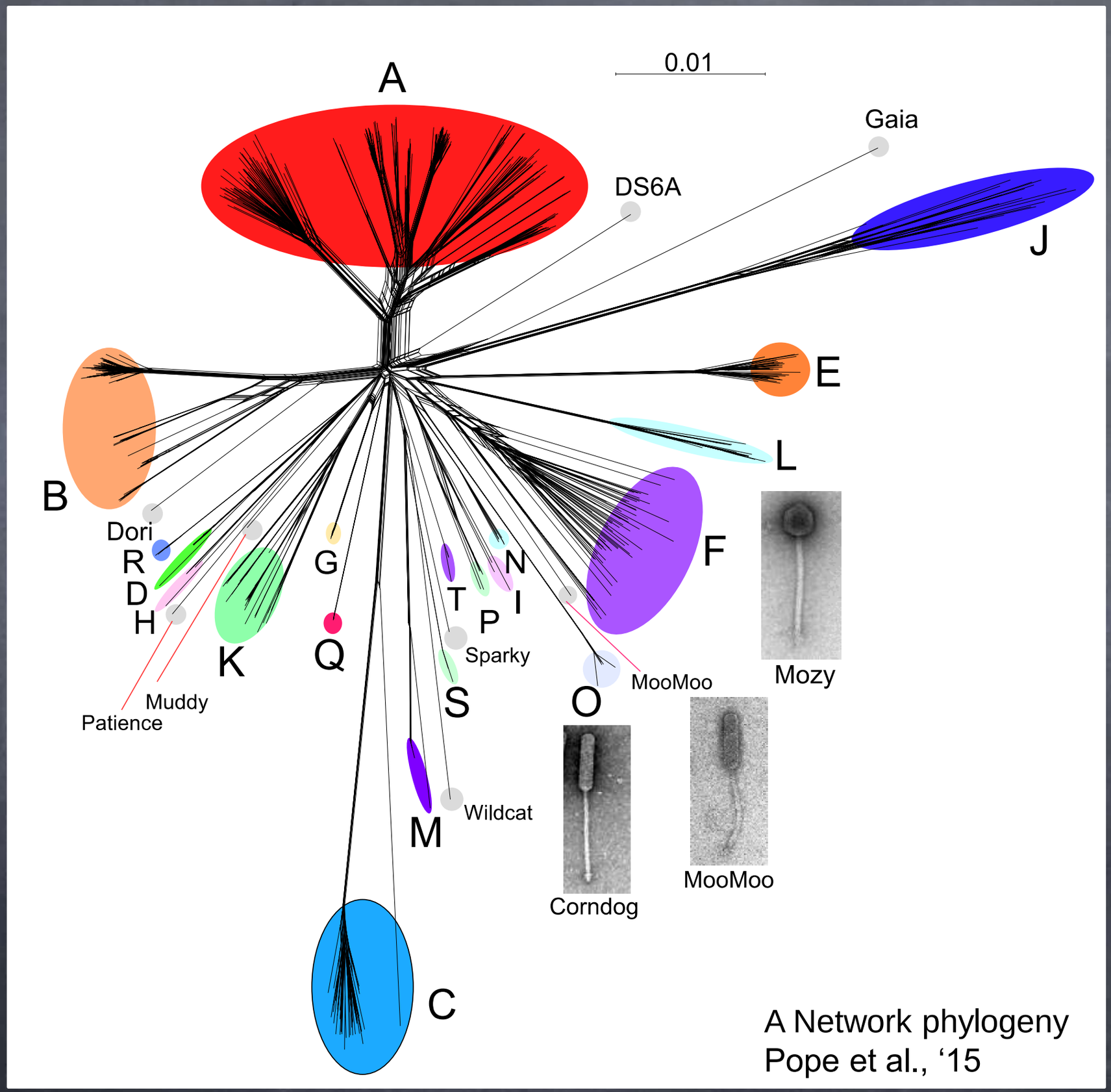
# Mapping the diversity of Actinobacteriophages

Definitions:

- Cluster: A group of phages with substantial nucleotide sequence similarity and shared gene content; e.g. Cluster A
- Subcluster: Subdivision within a cluster (by distinct ANI and other parmeters) e.g. Subcluster A2
- Singleton: One of a kind phage with no close relatives

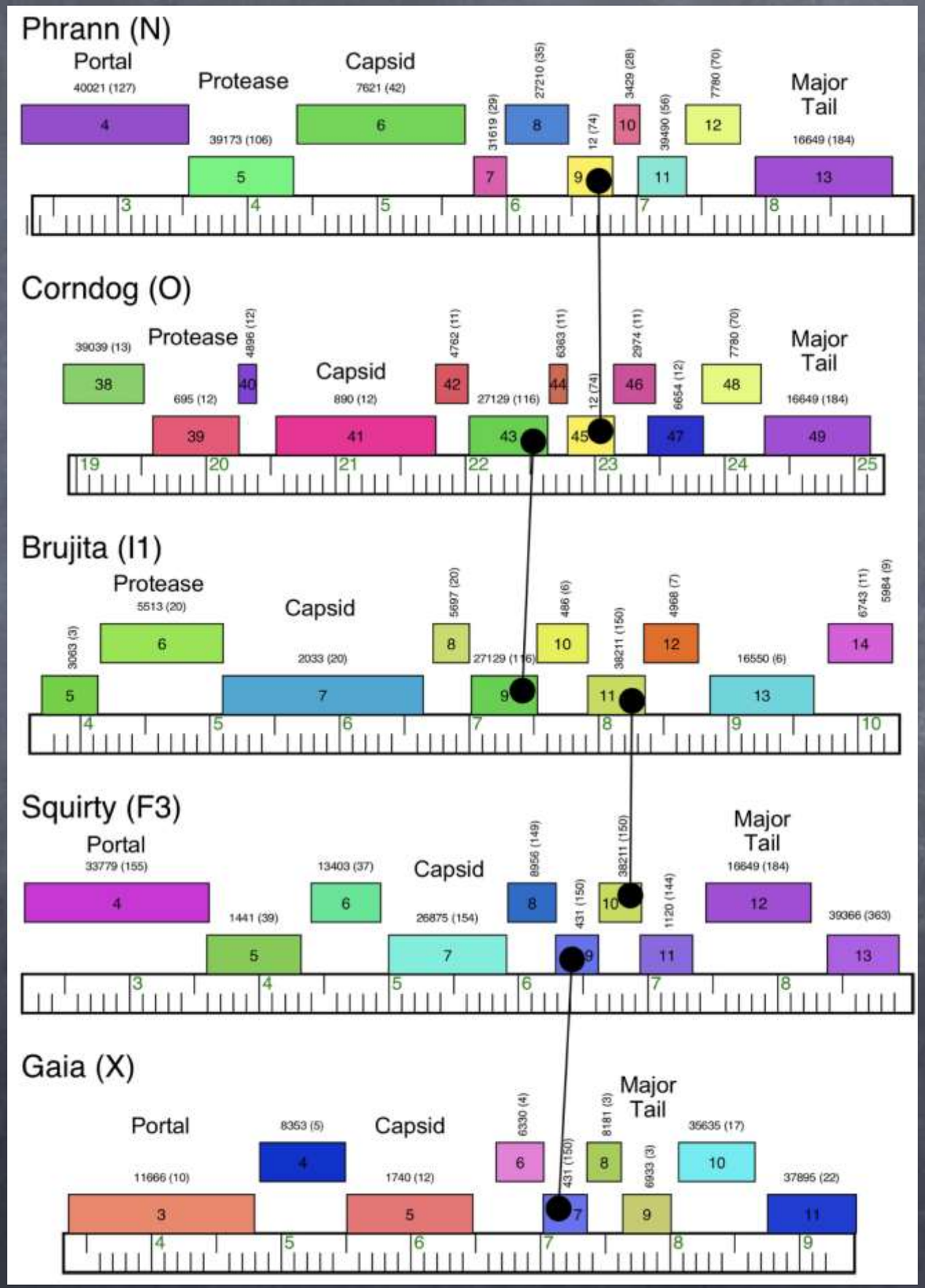
| Number of Clusters, Subclusters, and Singletons | | | | | | |
|---|---|---|---|---|---|---|
| | Clusters | Singletons | Clusters + Singletons | Subclusters | Clusters + Subclusters | Clusters + Subclusters + Singletons |
| Overall | 125 | 68 | 193 | 143 | 235 | 303 |
| Mycobacterium | 29 | 10 | 39 | 62 | 79 | 89 |
| Gordonia | 33 | 9 | 42 | 39 | 62 | 71 |
| Streptomyces | 15 | 12 | 27 | 20 | 29 | 41 |
| Arthrobacter | 23 | 7 | 30 | 8 | 28 | 35 |
| Microbacterium | 16 | 8 | 24 | 14 | 27 | 35 |
| Rhodococcus | 3 | 13 | 16 | 0 | 3 | 16 |
| Corynebacterium | 3 | 2 | 5 | 0 | 3 | 5 |

# Fantastic diversity of bacteriophages

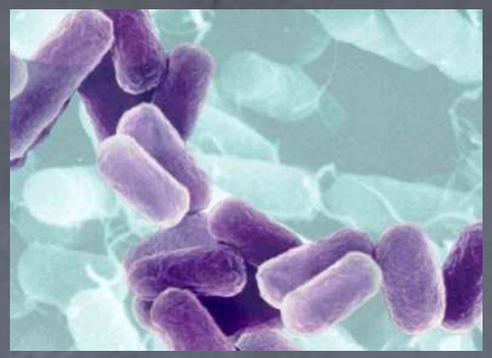


# Mosaicism, Mosaicism, Mosaicism

# Mosaic construction of phage genomes



# More than 50% of Actinobacteriophages are temperate



## Lysogeny
(parasitism: no viral reproduction, no cell death)

(spontaneous/UV)
Induction

## Lytic growth
(viral production/cell lysis)

Lytic (virulent) phages only do lytic growth
Temperate phages choose between lytic growth and lysogeny

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Prophage (integrated)

Temperate

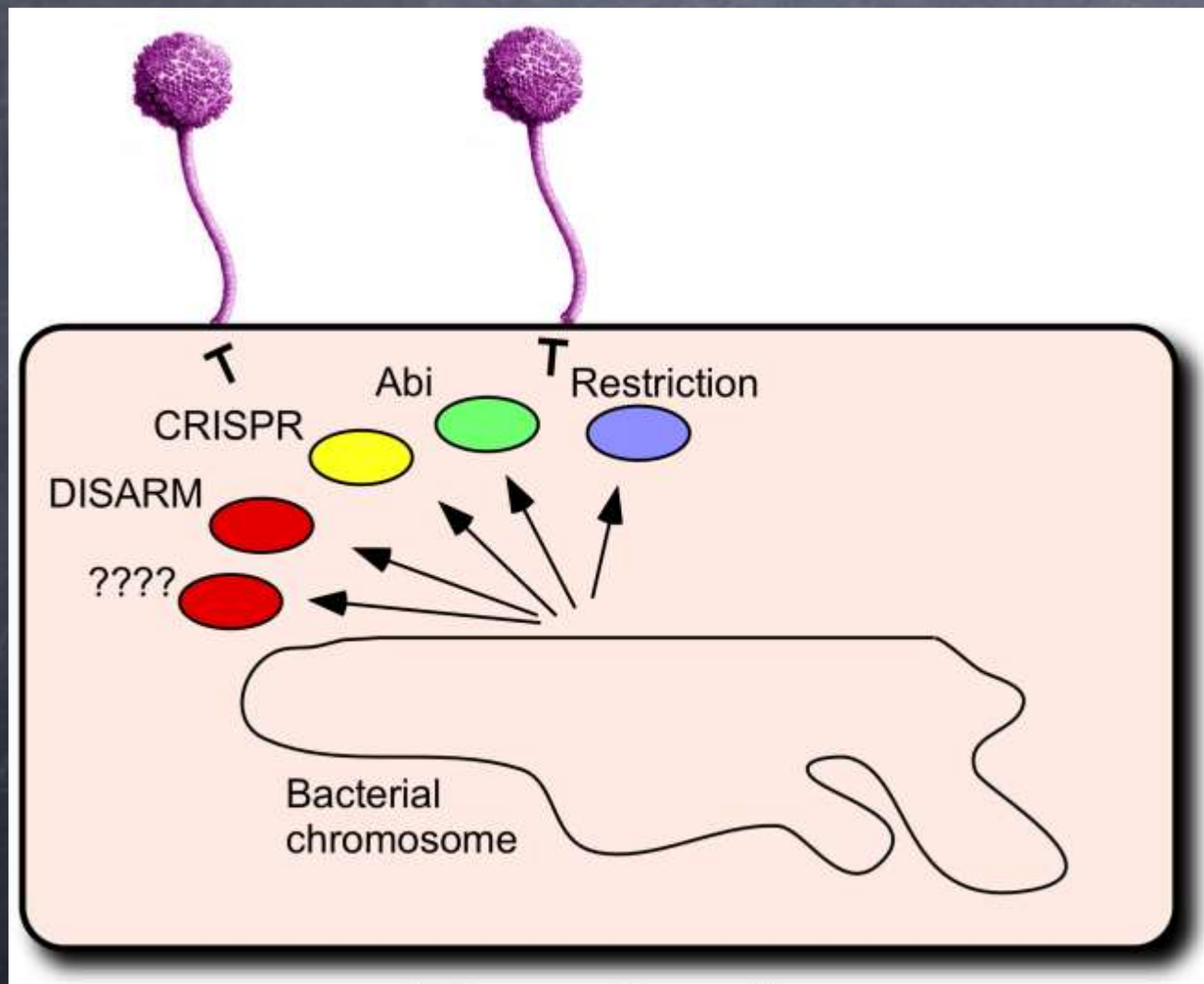
Turbid plaque

Lytic

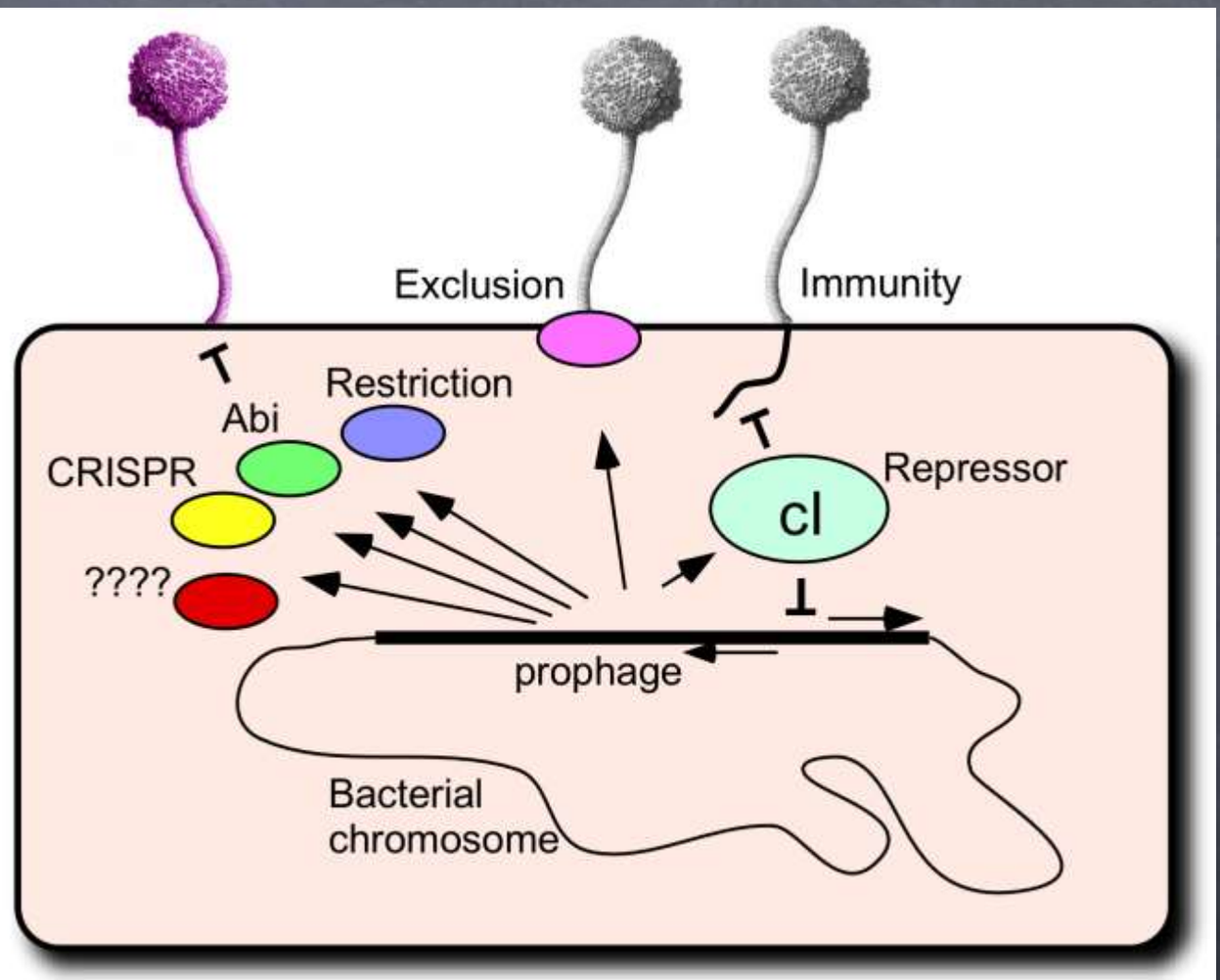
Clear plaque

# Evidence of collusion: Collaboration between phages and bacteria to defend against phage attack

- Bacteria have numerous systems to protect against viral attack
- Temperate phages contribute many additional defense systems
- Defense is typically heterotypic (against a phage different to prophage




Bacteria

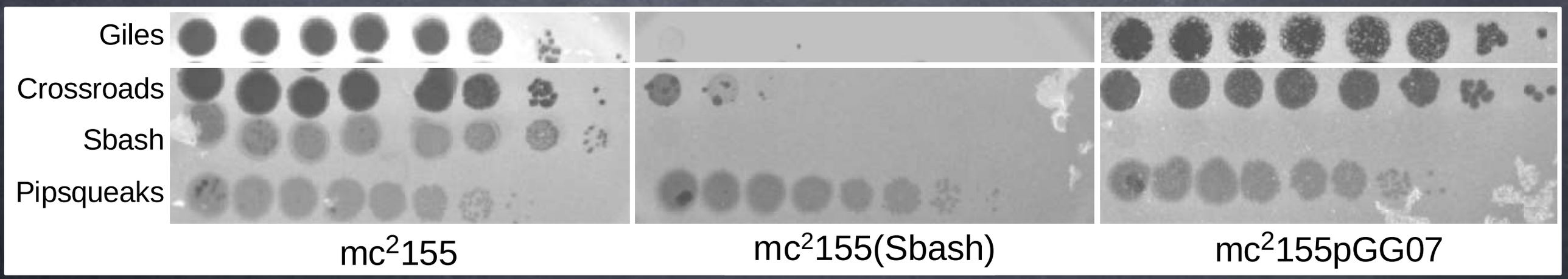



Lysogen

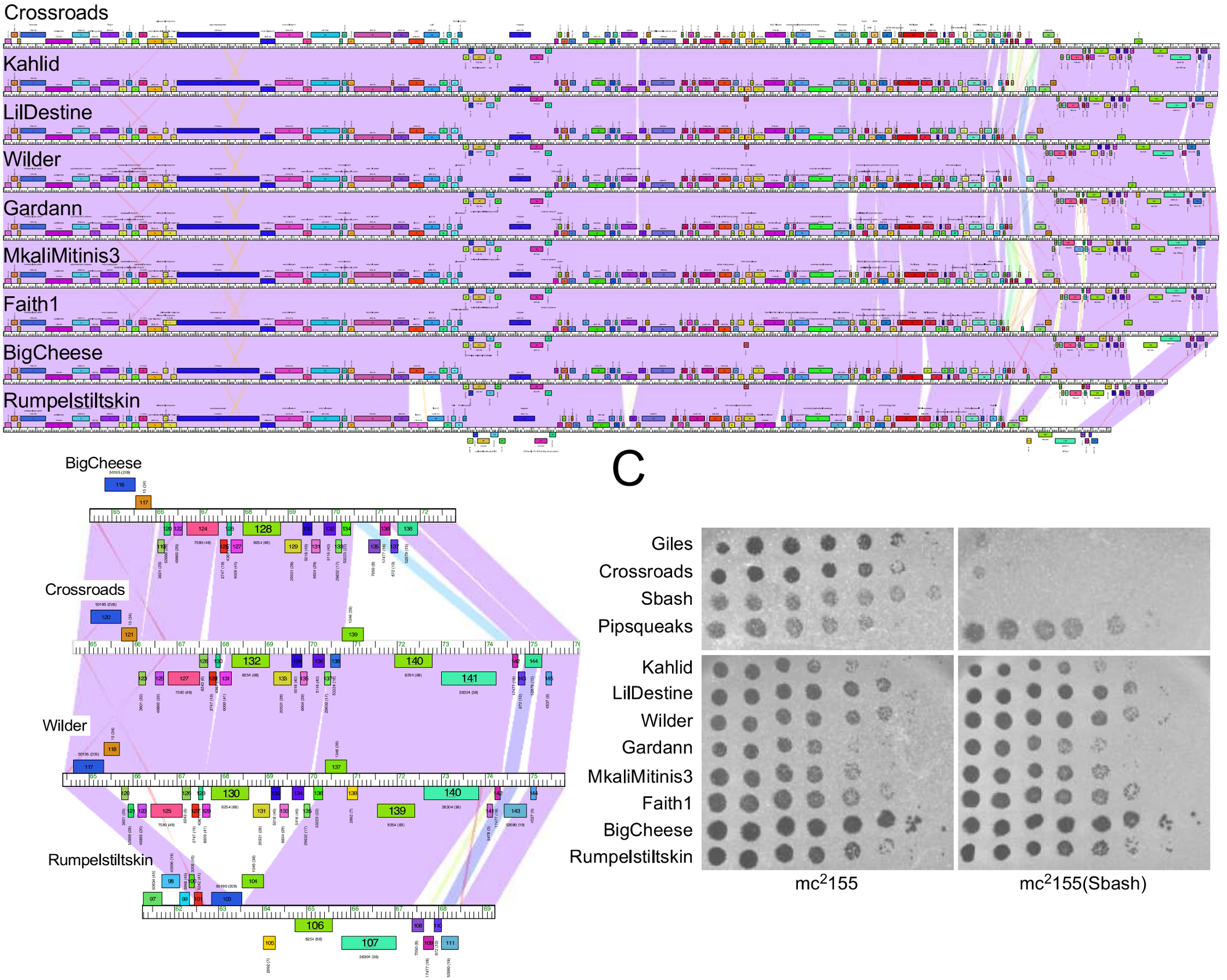
# Discovering prophage-mediated defense systems

Because these can be highly specific, need:

- A large collection of phages known to infect a common bacterial strain
- A subset of temperate phages forming stable lysogens in that strain
- Genomic definition of the phages
- Expansive diversity of the phages that spans completely distinct genome sequences, and variants.



# Prophage-mediated defenses are often highly specific



# The search for bacteriophages for GD01 & GD02

## Goals:

- Identify three or more phages that infect *M. abscessus* GD01 and GD02
- Phages should be genomically distinct to minimize cross-resistance to the phages
- Phages should be lytic, and not temperate
- Phages should be grown to high-titer and stable

# Strategies for identifying of *M. abscessus* phages

## Three main approaches:

1. Screen individual *M. smegmatis* phages in the archive for that infect *M. abscessus* GD01/GD02
2. Enrichment of large pools of extant phages
3. De novo phage isolation

Bad news: One patient died ~6 weeks after starting phage search

Good news: Within ~2 months identified one good phage and engineered/genetics two other phages for other patient

# Narrowing the search:
# Genome type correlates with host range

- A subset of phages isolated on *M. smegmatis* also infect other mycobacterial species
- ~10% of all *M. smegmatis* phages also infect *M. tuberculosis* H37Rv, but constrained to genomic types:
- Clusters A2, A3, G, K, AB are best candidates for broader host ranges (spans total ~200 isolates)
- Correlation is imperfect, and not all phages of a cluster/subcluster have identical host ranges

De novo isolation identified few new phages for M. ab GD01, and no new genomic types

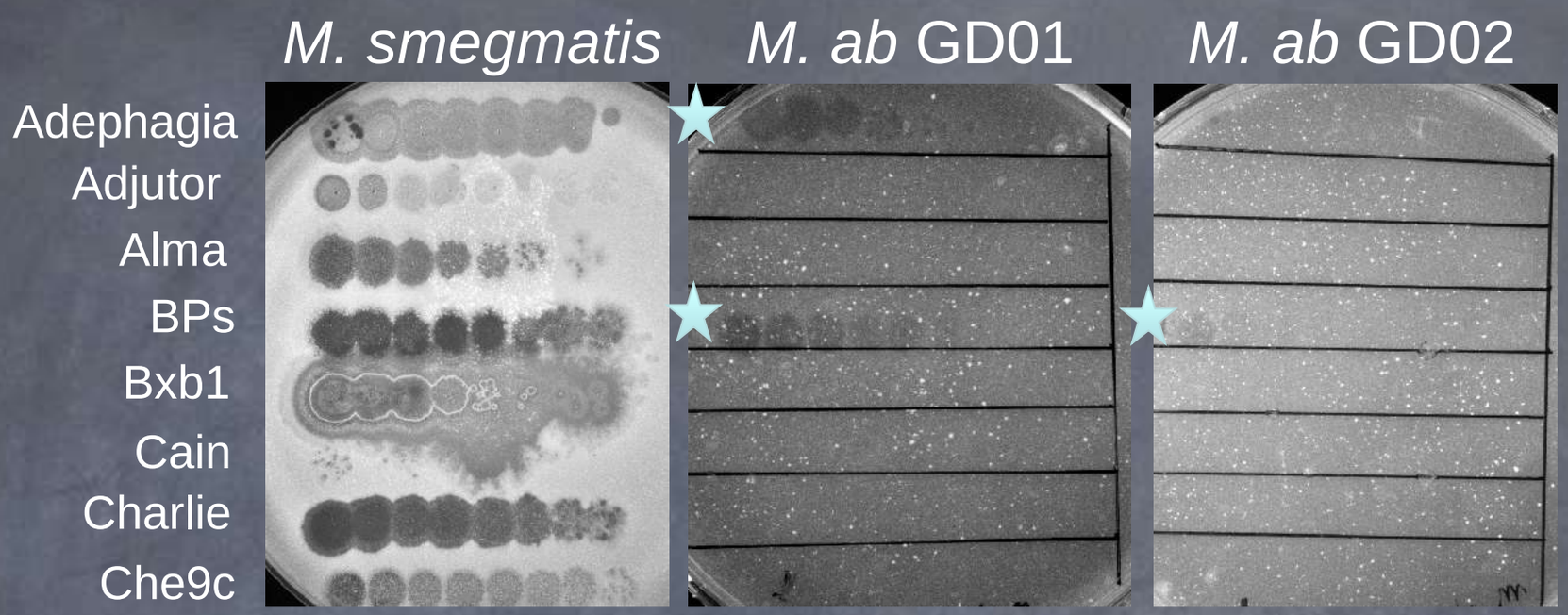
# Few *M. smegmatis* phages infect *M. abscessus* GD01

Table S1. Plating efficiencies of phages on *M. smegmatis* and *M. abscessus* GD01

| Phage[1] | Cluster | *M. smegmatis* mc²155[2] | *M. abscessus* GD01[2] | EOP |
|---|---|---|---|---|
| [3]Bxb1 | A1 | $1 \times 10^{9}$ | $1 \times 10^{9}$ | $<1 \times 10^{-9}$ |
| [3]D29 | A2 | $1 \times 10^{6}$ | 0 | $<1 \times 10^{-6}$ |
| [3]Elmo HRM$^{mc2\ 155}$ | A3 | $1 \times 10^{10}$ | $1 \times 10^{5}$ | $1 \times 10^{-5}$ |
| [3]Fernando | A3 | $1 \times 10^{9}$ | $1 \times 10^{3}$ | $1 \times 10^{-6}$ |
| [3]Isca | A3 | $1 \times 10^{8}$ | $1 \times 10^{8}$ | 1 |
| [3]Peaches | A4 | $3 \times 10^{8}$ | 0 | $<3 \times 10^{-8}$ |
| [3]DaVinci | A6 | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Et2Brutus | A11 | $1 \times 10^{8}$ | 0 | $<1 \times 10^{-8}$ |
| [3]EagleEye | A16 | $2 \times 10^{8}$ | 0 | $<2 \times 10^{-8}$ |
| [3]Muddy | AB | $1 \times 10^{10}$ | $1 \times 10^{10}$ | 1 |
| [3]Hedgerow | B2 | $2 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Dandelion | C1 | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Adjutor | D1 | $3 \times 10^{8}$ | 0 | $<3 \times 10^{-8}$ |
| [3]Courthouse | E6 | $1 \times 10^{8}$ | 0 | $<1 \times 10^{-8}$ |
| [3]Tweety | F1 | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-8}$ |
| [3]BPs | G1 | $1 \times 10^{10}$ | $1 \times 10^{7}$ | $1 \times 10^{-3}$ |
| [3]BPs HRM10 | G1 | $1 \times 10^{10}$ | $1 \times 10^{10}$ | 1 |
| [3]Halo HRM20 | G1 | $1 \times 10^{10}$ | $1 \times 10^{10}$ | 1 |
| [3]Halo | G1 | $2 \times 10^{10}$ | $2 \times 10^{7}$ | $1 \times 10^{-3}$ |
| [3]Island3 | I1 | $1 \times 10^{9}$ | 0 | $<1 \times 10^{-9}$ |
| [3]Che9c | I2 | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Adephagia | K1 | $2 \times 10^{9}$ | $1 \times 10^{7}$ | $5 \times 10^{-3}$ |
| [3]Adephagia*Δ42Δ43* | K1 | $1 \times 10^{10}$ | $2 \times 10^{5}$ | $2 \times 10^{-5}$ |
| [3]TM4 | K2 | $2 \times 10^{9}$ | $1 \times 10^{6}$ | $5 \times 10^{-4}$ |
| [3]TM4Δ | K2 | $1.3 \times 10^{10}$ | $2 \times 10^{5}$ | $1.5 \times 10^{-5}$ |
| [3]ZoeJ | K2 | $1 \times 10^{9}$ | $1 \times 10^{5}$ | $1 \times 10^{-4}$ |
| [3]ZoeJ*Δ45* | K2 | $2 \times 10^{10}$ | $2 \times 10^{10}$ | 1 |
| [3]ShedlockHolmes | K3 | $3 \times 10^{10}$ | $1 \times 10^{8}$ | $3 \times 10^{-3}$ |
| [3]Fionnbharth | K4 | $1 \times 10^{11}$ | $1 \times 10^{8}$ | $1 \times 10^{-3}$ |
| [3]Fionnbharth *Δ47* | K4 | $1 \times 10^{11}$ | $1 \times 10^{8}$ | $1 \times 10^{-3}$ |
| [3]Wintermute | K4 | $2 \times 10^{10}$ | $1 \times 10^{6}$ | $5 \times 10^{-5}$ |
| [3]Larva | K5 | $1 \times 10^{9}$ | 0 | $<1 \times 10^{-9}$ |
| [3]PegLeg | M1 | $2 \times 10^{10}$ | 0 | $<2 \times 10^{-10}$ |
| [3]Nanosmite | M3 | $1 \times 10^{9}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Charlie | N | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Giles | Q | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [3]Jeon | W | $1 \times 10^{9}$ | 0 | $<1 \times 10^{-9}$ |
| [3]Gaia | X | $2 \times 10^{9}$ | 0 | $<2 \times 10^{-9}$ |
| [3]Rem711 | Z | $1 \times 10^{10}$ | 0 | $<2 \times 10^{-10}$ |
| [3]Cuke | Singleton | $1 \times 10^{5}$ | 0 | $<1 \times 10^{-5}$ |
| [3]DS6A | Singleton | $1 \times 10^{9}$ | 0 | $<1 \times 10^{-9}$ |
| [3]Kumao | Singleton | $3 \times 10^{9}$ | 0 | $<3 \times 10^{-9}$ |
| [3]Moomoo | Singleton | $3 \times 10^{6}$ | 0 | $<3 \times 10^{-6}$ |
| [3]Sparky | Singleton | $2 \times 10^{6}$ | 0 | $<2 \times 10^{-6}$ |
| [4]BabyRay | A3 | $1 \times 10^{9}$ | $1 \times 10^{9}$ | 1 |
| [4]Fred313 | A3 | $1 \times 10^{7}$ | $1 \times 10^{7}$ | 1 |
| [4]Fred313cpm1 | A3 | $1 \times 10^{10}$ | $1 \times 10^{10}$ | 1 |
| [4]HelDan | A3 | $2 \times 10^{7}$ | $1 \times 10^{5}$ | $5 \times 10^{-3}$ |
| [4]Puppy | A3 | $1 \times 10^{8}$ | $1 \times 10^{8}$ | 1 |
| [4]TNguyen7 | A3 | $1 \times 10^{9}$ | $1 \times 10^{8}$ | $10^{-1}$ |
| [4]Cambiare | G2 | $1 \times 10^{10}$ | 0 | $<1 \times 10^{-10}$ |
| [4]Flagstaff | G2 | $2 \times 10^{10}$ | 0 | $<2 \times 10^{-10}$ |
| [4]Pinnie | G3 | $2 \times 10^{8}$ | 0 | $<2 \times 10^{-10}$ |
| [4]Validus | K1 | $3 \times 10^{10}$ | $1 \times 10^{5}$ | $3 \times 10^{-6}$ |
| [4]Keshu | K3 | $2 \times 10^{10}$ | $2 \times 10^{10}$ | 1 |
| [4]Keshu*Δ47* | K3 | $1 \times 10^{10}$ | $1 \times 10^{10}$ | 1 |
| [4]SamScheppers | K4 | $2 \times 10^{10}$ | 0 | $<2 \times 10^{-10}$ |
| [4]Amgine | K6 | $3 \times 10^{10}$ | 0 | $<3 \times 10^{-10}$ |
| [4]Krueger | K6 | $2 \times 10^{10}$ | 0 | $<2 \times 10^{-10}$ |
| [4]Gabriela | L2 | $1 \times 10^{10}$ | $1 \times 10^{9}$ | $1 \times 10^{-1}$ |
| [4]Itos | L2 | $3 \times 10^{10}$ | $1 \times 10^{10}$ | $3 \times 10^{-1}$ |

[1]Phage lysates were grown on *M. smegmatis* mc²155, except Isca and DS6A, grown on GD01 and M. tb respectively.
[2]Plaque forming units $ml^{-1}$
[3]Phages tested in an initial screen from which phages for a therapeutic cocktail were selected
[4]Additional phages tested after the initial therapeutic phages were selected

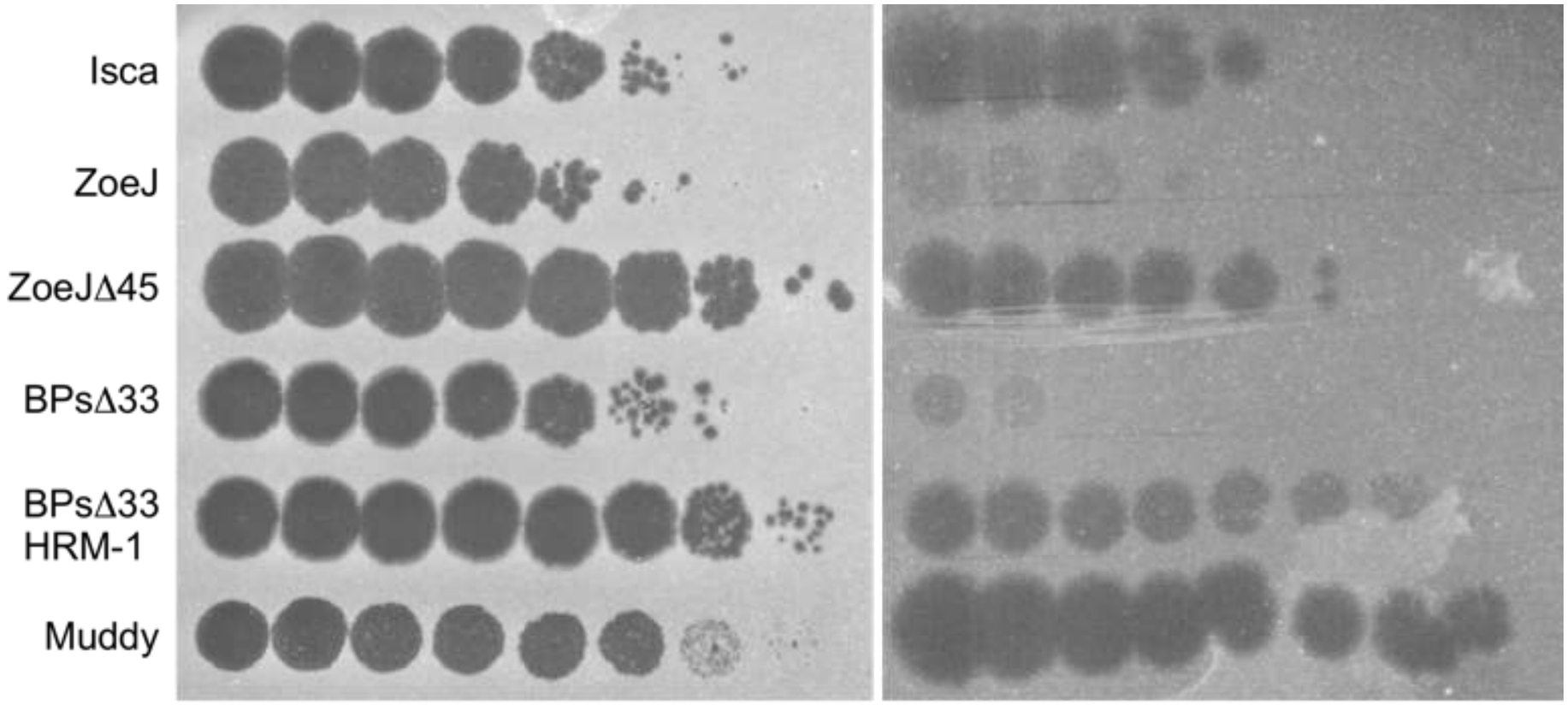
# Screening the extant collection of *M. smegmatis* phages: Phage Muddy infects and kills *M. abscessus* GD01




#1

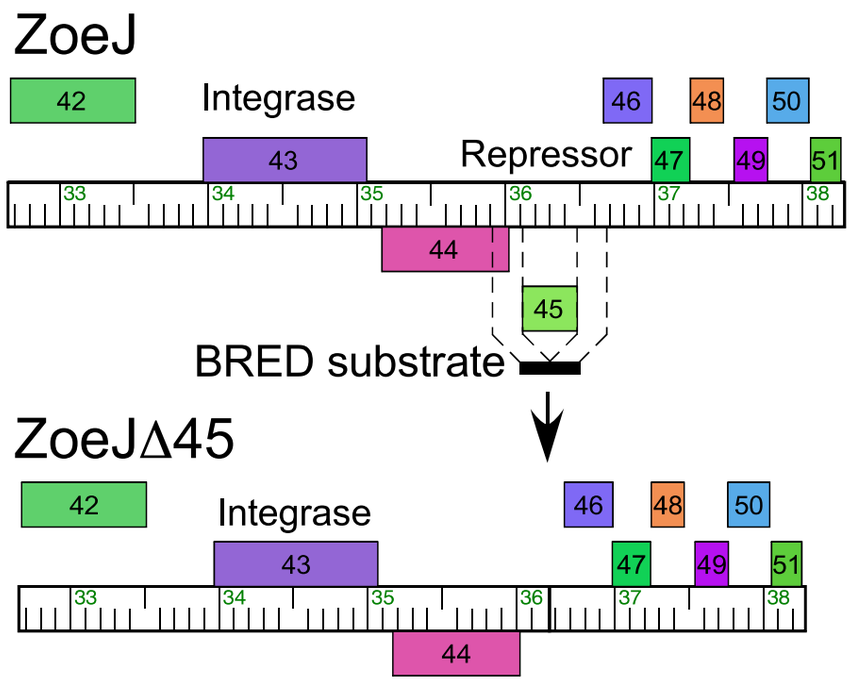
Few *M. smegmatis* phages efficiently infect *M. abscessus*
Muddy efficiently infects *M. abscessus* GD01
M. ab GD01 and M. ab GD02 differ in their susceptibilities

# Phage #2: Engineering a lytic derivative, ZoeJΔ*45*

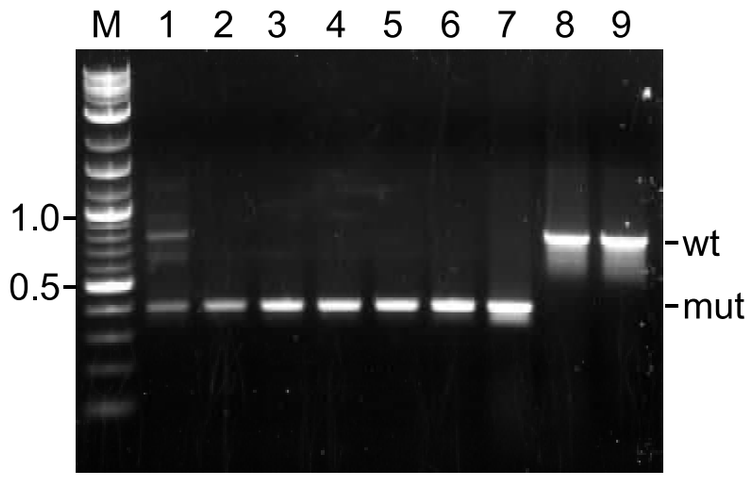



B M. smegmatis mc$^2$155

C M. abscessus GD01



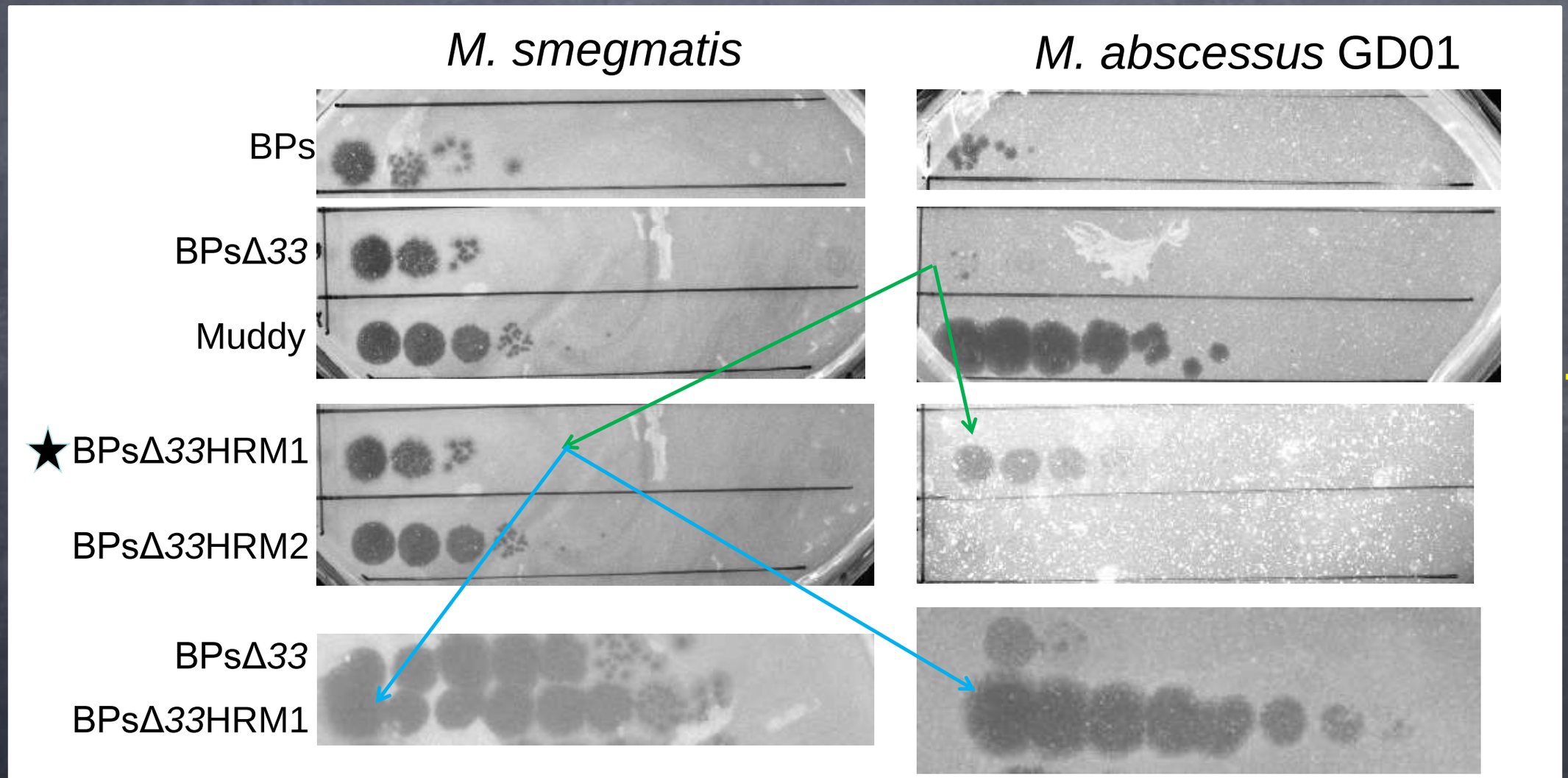

#2




Kathleen Cornerly
Rebekah Dedrick

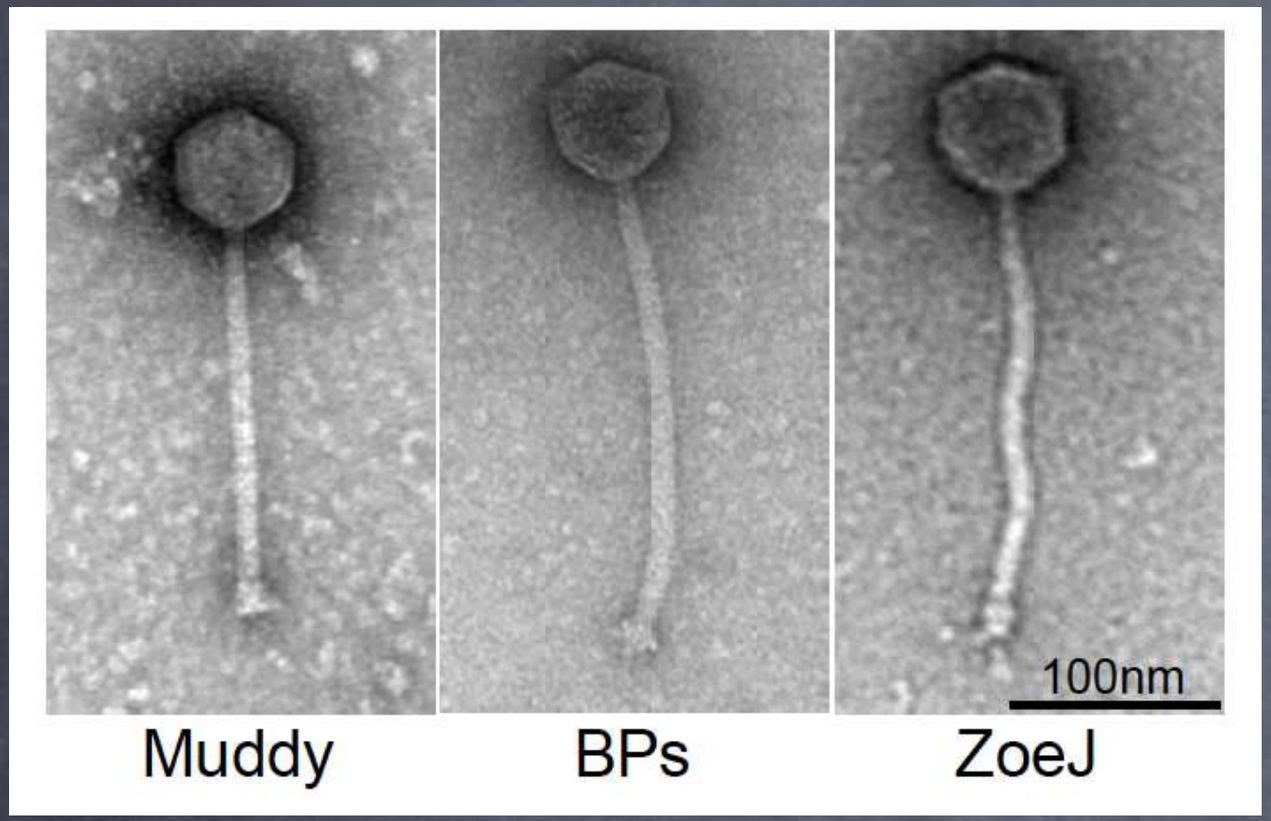
# Phage #3: Mutational Expansion of host range of a lytic derivative of phage BPs

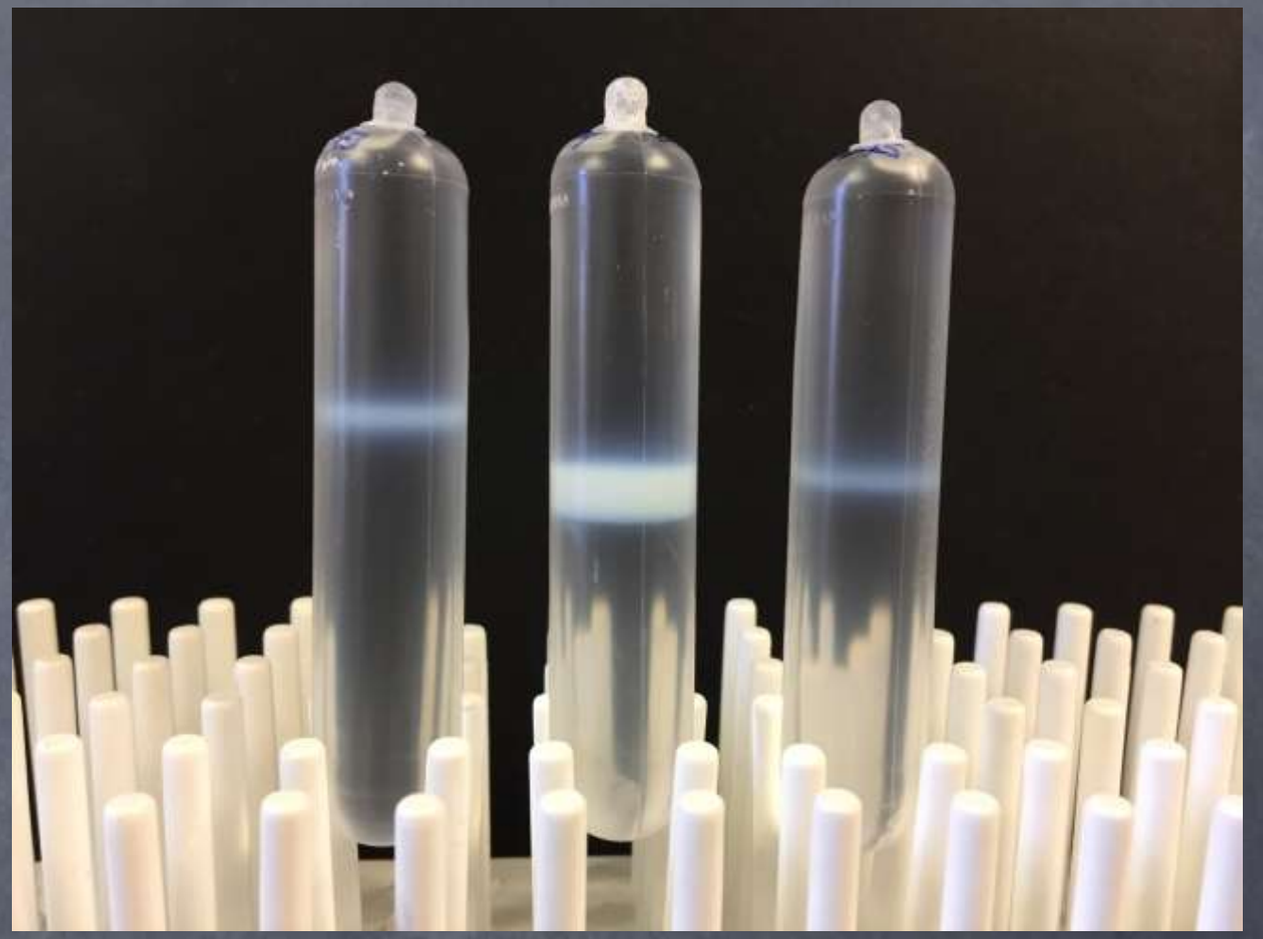
- BPs & BPs∆*33* plate inefficiently on *M. abscessus* GD01
- Plaques picked from *M. abscessus* have expanded host range
- Mutants grown on *M. smegmatis* infect *M. abscessus* GD01




BPs HRM's have mutations in the phage portal gene (gene *3*)

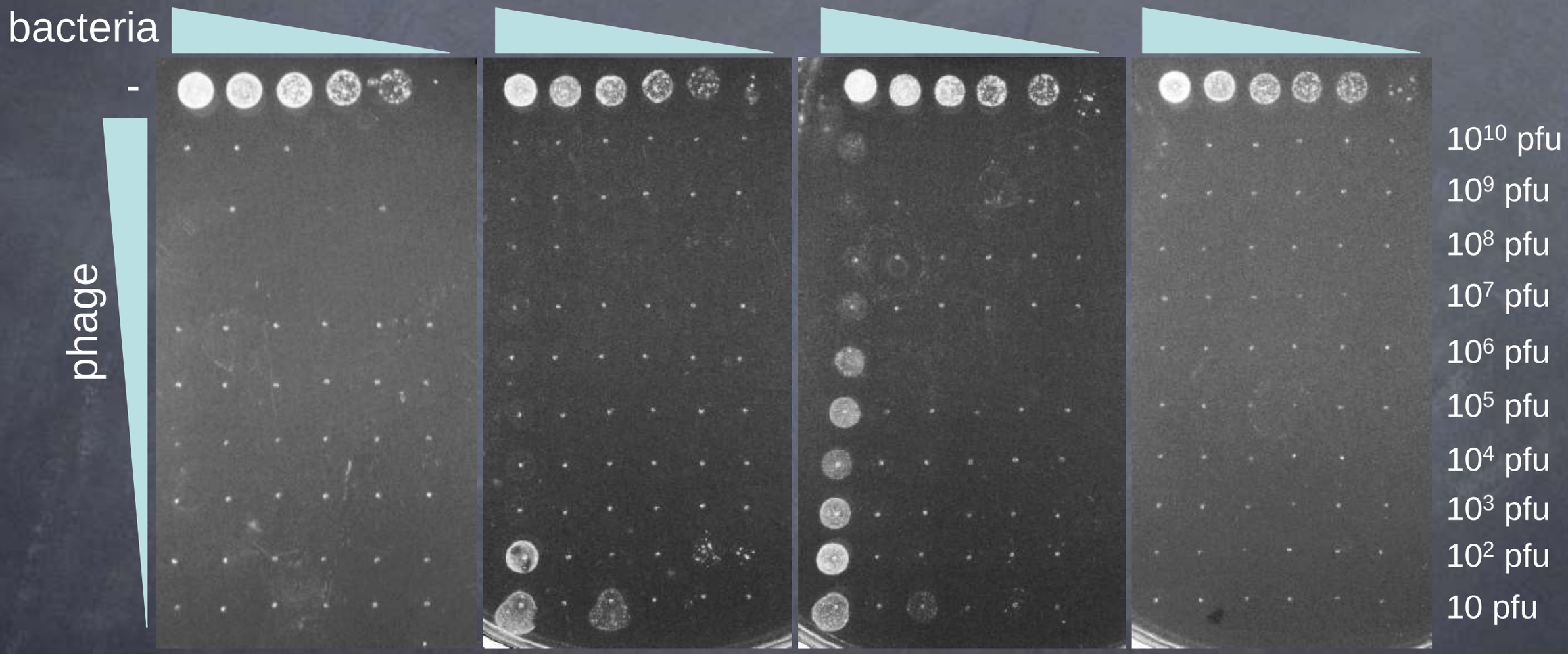
# The three musketeers



# Phage killing of *M. abscessus* GD01

Bacteria & phages were incubated in liquid culture for 96 hours
Then plated for survivors on solid medium




Good killing by each individual phage with some variation
Complete killing and no survivors with cocktail

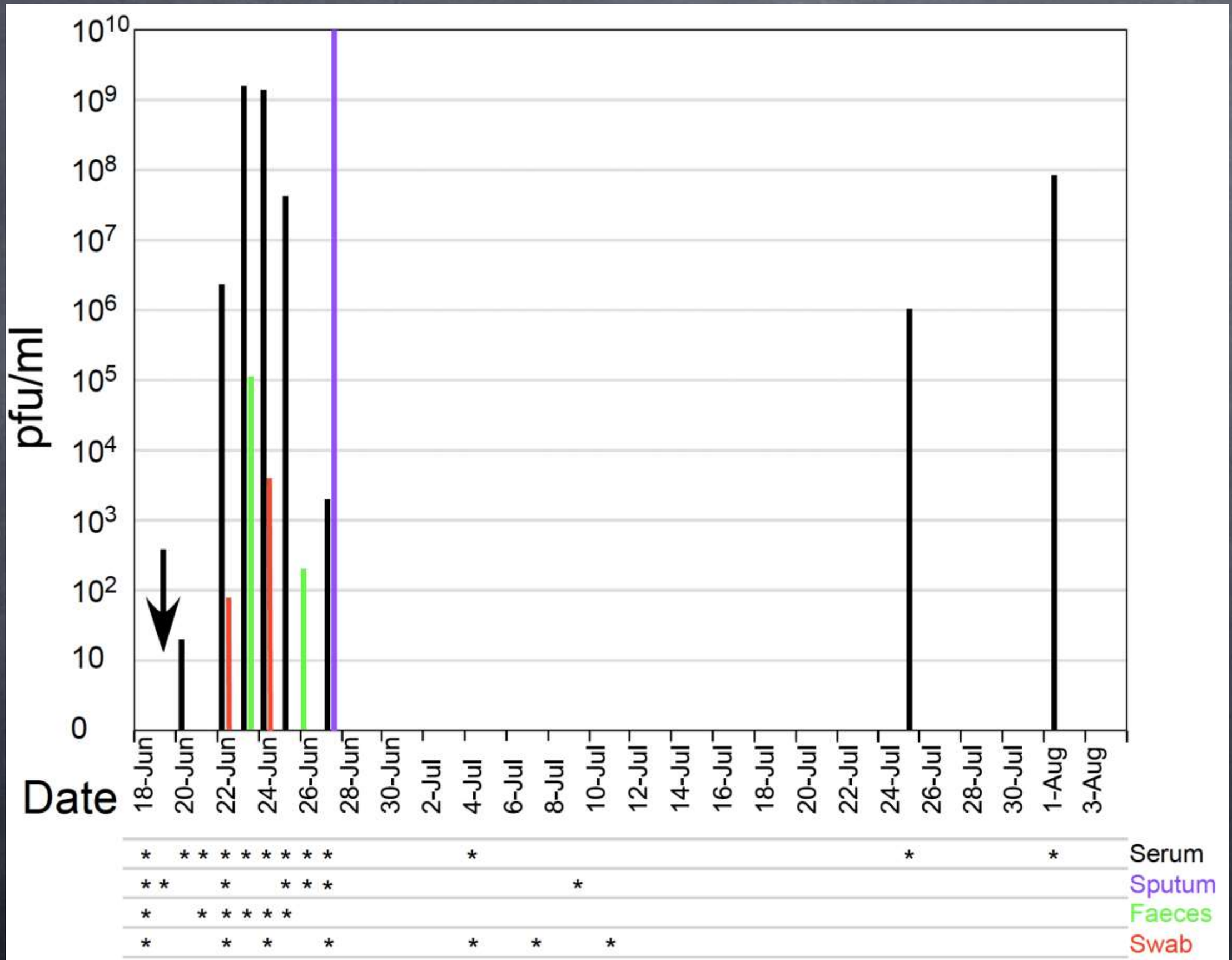
# The first phage therapy of a mycobacterial infection

- A three-phage cocktail against *M. abscessus* GD01: Muddy, ZoeJΔ45, BPsΔ33HRM
- Phages were grown to high titer; purified through two rounds of CsCl equilibrium density centrifugation
- Stocks at $10^{12}$ – $10^{14}$ pfu/ml. Cocktail at $10^{11}$ pfu/ml; stable.
- Dialyzed extensively into PBS; demonstrated to have negligible CsCl remaining; negative for LPS toxicity.
- Multi-stage approval process for importing and emergency compassionate use of unlicensed drugs in the UK
- Approved as non-GMO
- Administered IV 2x-daily; $10^{9}$ pfu/dose; Topical application to sternal wound and skin nodules
- Monitor clinical response; phage in sputum/serum; immune response; phage resistance

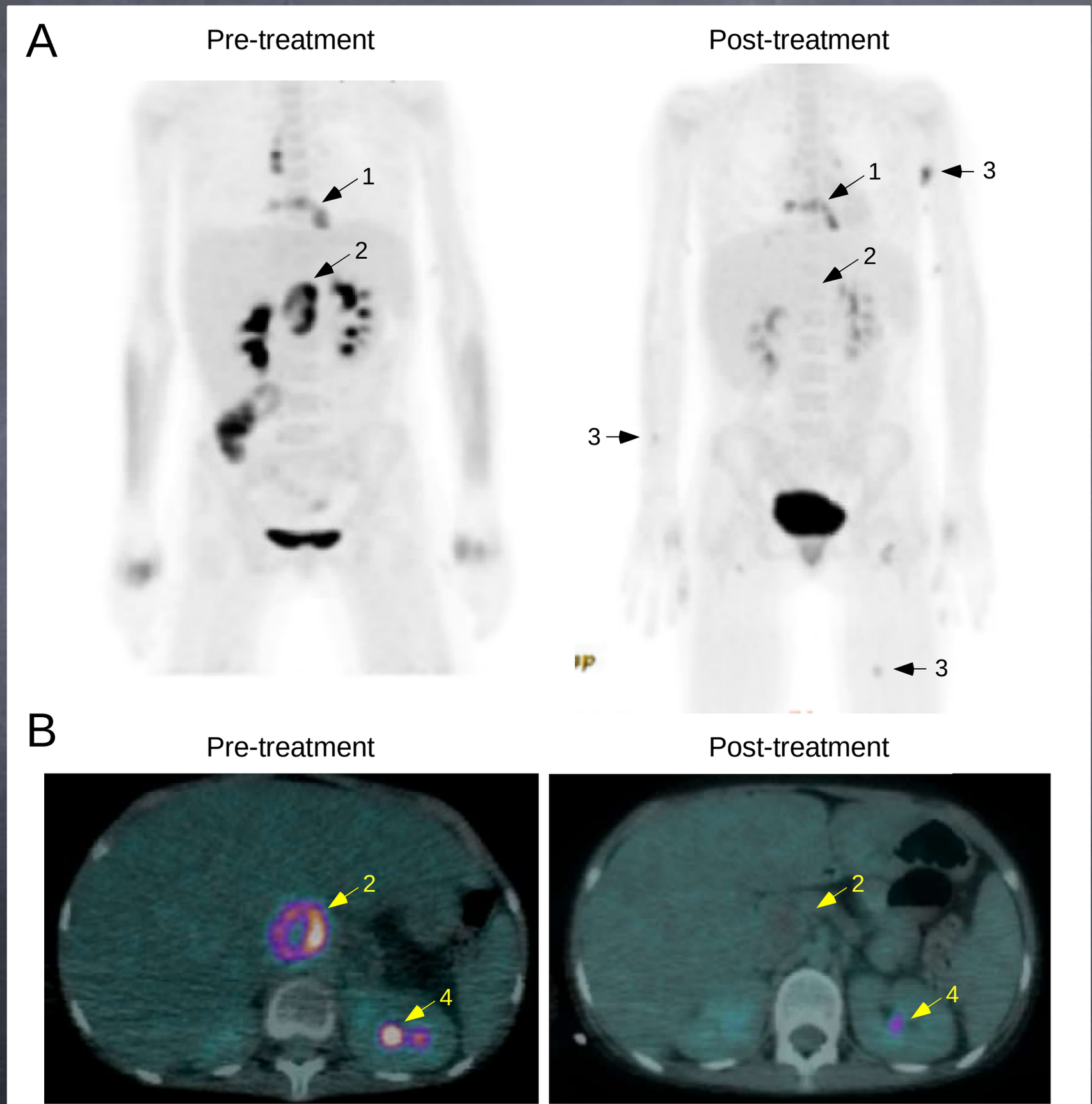
# Summary of outcomes

- No adverse reactions
- Phage recovered from some patient samples, consistent with *in vivo* replication
- Improved lung function
- Weight gain
- Infected liver node resolved by 6-weeks
- Closure of the sternal wound
- Skin nodule resolution (incomplete)
- No neutralizing antibodies detected
- Phage resistance not observed in any subsequent isolate
- Patient able to return to normal routine

# Recovery of phage from patient samples



# CT-PET: Pre- and post-treatment



# Resolution of wounds and skin nodules

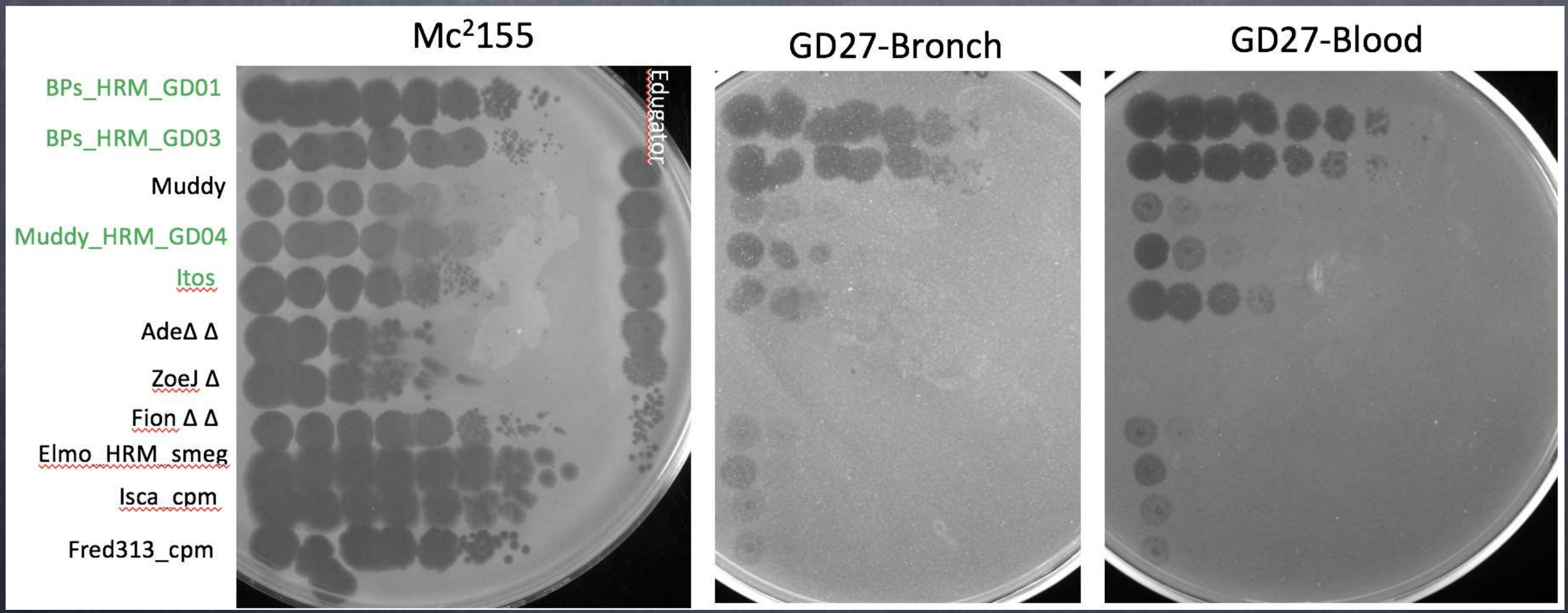
Pre-treatment

Post-treatment

# Helen, GH, IC, Mum

# Not one cure for them all

- Received about 40 NTM clinical isolates (mostly M. abscessus)
- Screened with ~20 ‘best’ phages
- ~40% of strains not susceptible to any of the phages
- Of other ~60%, many (~40%) only infected by one phage



# Opportunities and challenges for phage treatment of NTM infections

## Opportunities

- Personalized cocktails of matched phages may be effective for treating M. ab and other NTM infections
- IV administration is safe
- Resistance not observed (yet)
- Possibility of synergies with antibiotics
- Have started therapy on three addition patients.

## Challenges

- Only ~60% of M. ab clinical isolates are sensitive to at least one phage; similar expected to MAC
- Screening is relatively time-consuming, long, tedious
- Engineering/genetics may be required
- Resistance profiles and mechanisms are unknown
- Monotherapy possibilities are unclear
- Response in immune-competent patient is unclear
- Aerosol administration may be possible

# Summary

- SEA-PHAGES promotes student engagement and phage discovery
- Considerable potential for phage therapy of NTM infections
- Personalized treatments in progress
- Cocktail for general treatment of *M. abscessus* infections not currently possible
- Large collection of phages fuels therapeutic advancement
- Potential for therapy of TB considerable
- Need to understand phage dynamics *in vivo*

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Monty Montgomery

Connie Benson
Steffanie Strathdee

David Hanauer

SEA-PHAGES Faculty
SEA-PHAGES Students

Phage Hunters everywhere!

HHMI, NIH

# SEA-PHAGES Annual Symposium 2018

# Summary

SEA-PHAGES is an inclusive Research Education Community

Integration of research and educational missions promotes sustainable success in both

Bacteriophage discovery and genomics is a well-suited platform for student engagement

The number and diversity of phages promotes project ownership and an endless supply of novelty

SEA-PHAGES addresses basic questions in phage population but fuels translations including therapeutic interventions

# TB phage therapy: challenges and opportunities

- Potential for TB combination treatment with antibiotics to a) reduce treatment length, b) reduce antibiotic resistance
- Potential for controlling MDR-TB and XDR-TB infections
- Need phages that infect broad spectrum of clinical isolates
- Phages must be lytic; engineering is relatively simple
- Orthogonal phage resistance profiles
- LPS contamination is not a safety issue

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- M. tb is genetically more homogenous than other pathogens (core vs pan-genome: ~93%)
- M. tb strains do not carry prophages
- M. tb drug resistance derives from single resistance combinations; not MDR transporters
- Phage-resistance may be associated with reduced fitness
- IV versus inhaled delivery; little known about access in vivo
- Clinical trials (I/II) should be low risk, phages have been identified, GMP needed, immune-compromised vs -competent