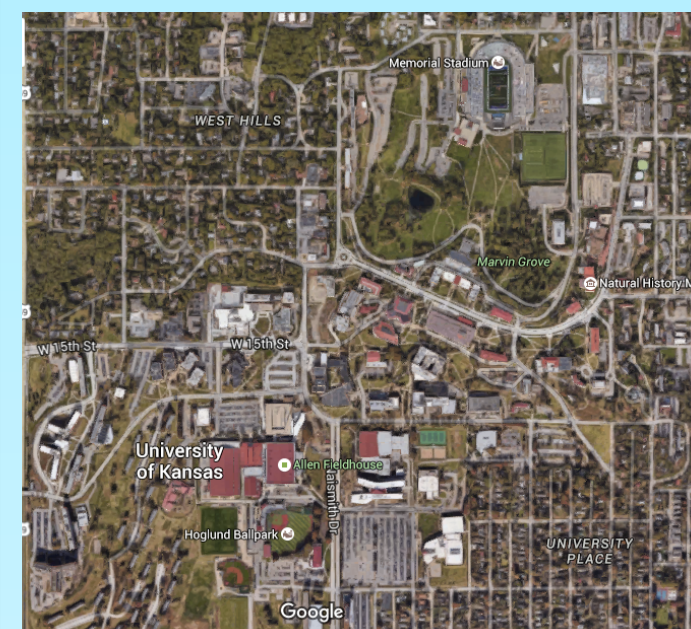


# Isolation and characterization, including host range, immunity, excision and potential splicing in the temperate J-cluster mycobacteriophage HokkenD

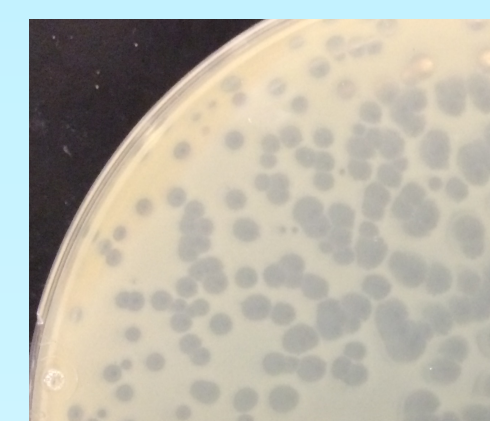
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## HokkenD is a Cluster J mycobacteriophage

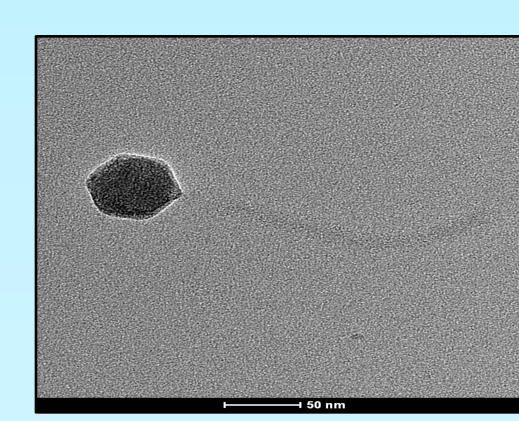


University of Kansas

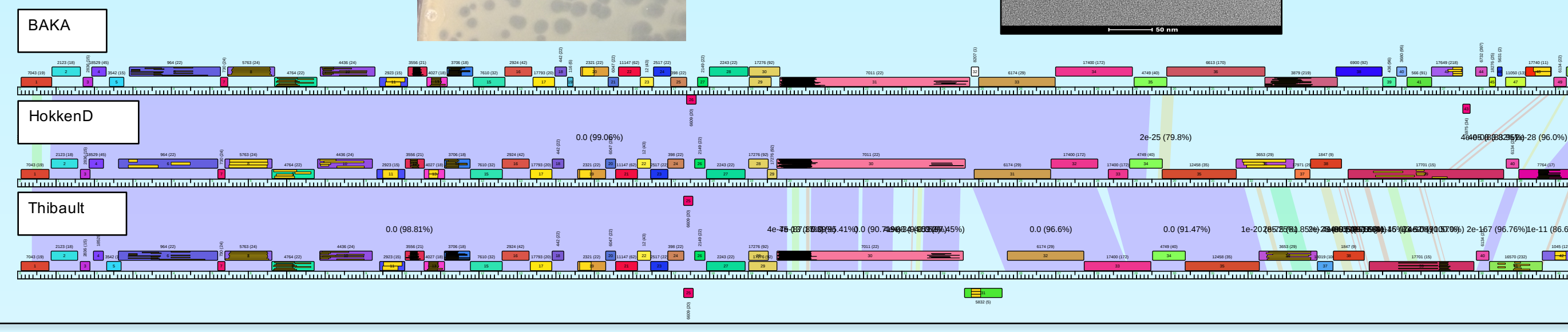
- HokkenD was isolated in 2014 from soil on the campus of the University of Kansas in Lawrence KS.
- HokkenD is a temperate phage with ~1mm turbid plaques.
- EM revealed a phage with a 65 nm head and a 200 nm tail.
- Genome sequencing revealed it to be a Cluster J phage, closely related to BAKA and Thibault.



HokkenD plaques



HokkenD EM



## What is the natural host of HokkenD?

**Observation:** The GC content of HokkenD is 60.8%, whereas *M. smeg* is 67.4%.

**Question:** If *M. smeg* is not HokkenD's natural host, can we find a better candidate?

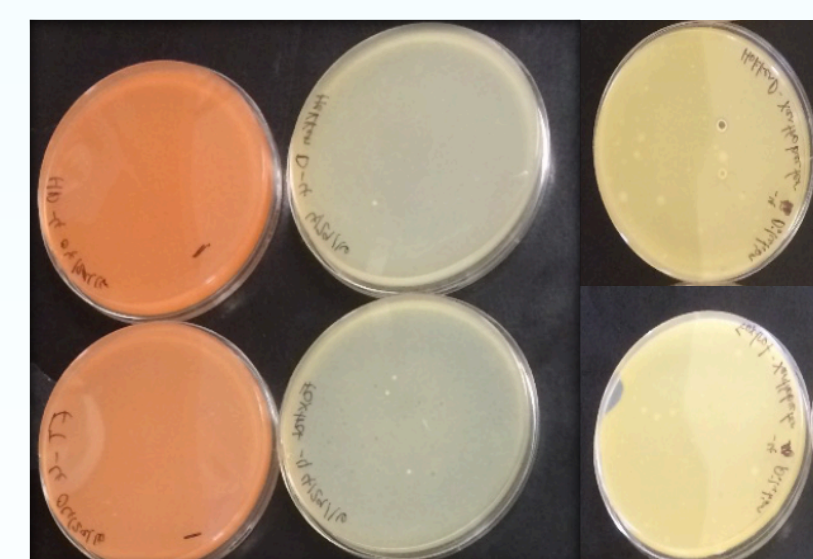
**Approach:** BLAST the genomes of Cluster J phages against the NCBI Microbe database

**Expectation:** Since Cluster J phages are temperate, there may be residual genomic signatures of past hosts in the phage genomes.

### Top 5 BLAST hits for Cluster J phages

HokkenD	Omega	Pound	Redno2	Optimus
<i>Mycobacterium Pheli</i>	<i>Nocardia Thailandica</i>	<i>Nocardia Thailandica</i>	<i>Nocardia Brasiliensis</i>	<i>Corynebacterium Aur</i>
<i>Mycobacterium Gilyum</i>	<i>Leucobacter</i>	<i>Corynebacterium Aur</i>	<i>Nocardia Asteroides</i>	<i>Bifidobacterium Pseudo</i>
<i>Nocardia Brasiliensis</i>	<i>Herbaspirillum Chloropholic</i>	<i>Bifidobacterium Pseud</i>	<i>Gordonia Nambiensis</i>	<i>Aquamicrobium Defluvil</i>
<i>Nocardia Asteroides</i>	<i>Nocardia Ottidiscavarium</i>	<i>Aquamicrobium Defluvil</i>	<i>Nocardia Thailandica</i>	<i>Xanthobacter</i>
<i>Mycobacterium sp.</i>	<i>Nocardia Asteroides</i>	<i>Methylobacterium</i>	<i>Nocardia Seriolae</i>	<i>Paracoccus Aminophilus</i>

<i>Nocardia corynebacteroides</i>	<i>Xanthobacter sp</i>
Gram positive	Gram negative
Growth temperature: 28°C	Growth temperature: 30°C
Medium: Yeast malt extract agar	Medium: nutrient agar or nutrient broth
Colonies: orange/red pigmented colonies	Colonies: circular, raised, moist, and yellow



Since *Nocardia* and *Xanthobacter* were overrepresented in the BLAST results, we obtained strains from NRRL and ATCC and tested them to see if they could be infected by a range of different phage strains.

Phage	HokkenD	BAKA	Thibault	D29	Potter	Foxtrot P1
Cluster	J	J	J	A2	B1	C1
Infect <i>M. smeg</i>	YES	YES	YES	YES	YES	YES
Infect <i>Nocardia</i>	NO	NO	NO	NO	NO	NO
Infect <i>Xanthobacter</i>	NO	NO	NO	NO	NO	NO

**Conclusion:** These stains of *Xanthobacter* and *Nocardia* are unlikely to be the natural host of Cluster J phages.

## Testing immunity of HokkenD

**Observation:** HokkenD is a temperate phage of Cluster J.

**Question:** Does HokkenD provide superinfection immunity when it forms a lysogen?

**Approach:** Generate a HokkenD lysogen, and challenge it with a variety of phages.

Creating a HokkenD lysogen

L-plate      L-plate with *M. smeg*

Infection test

*M. smeg*      HokkenD lysogen

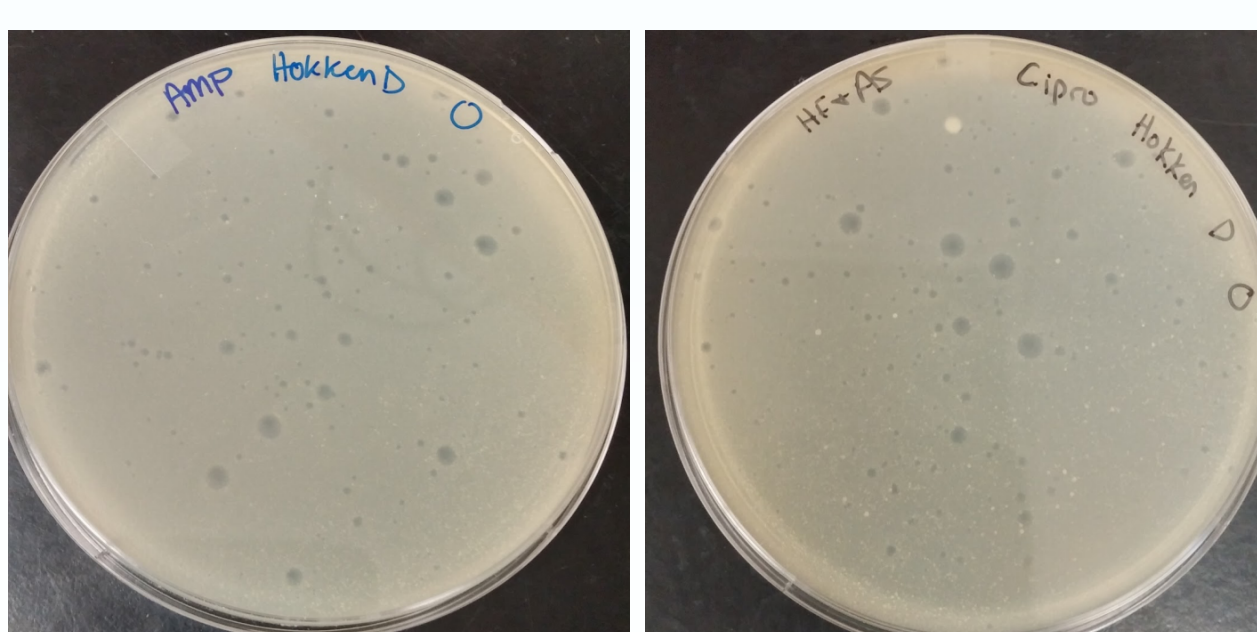
Phage	Cluster	Infect <i>M. smeg</i>	Infect HokkenD lysogen
HokkenD	J	+	-
Courthouse	J	+	-
BAKA	J	+	-
Thibault	J	+	+
D29	A2	+	+
Turbido	A2	+	+
Apparition	A2	+	+
Rockstar	A3	+	+
Potter	B1	+	+
FoxtrotP1	C1	+	+
Polka14	F1	+	+

**Conclusion:** HokkenD is immune to some, but not all Cluster J phages, and not immune to representative phages from other clusters.

## Testing induction of HokkenD

**Question:** What conditions give the best induction of HokkenD from the lysogen

**Approach:** Grow HokkenD lysogen to log phase in a liquid culture, challenge it with environmental factors that may promote induction for 2 hours, filter the lysate, and plate on *M. smeg* to quantify the titer of released phage.

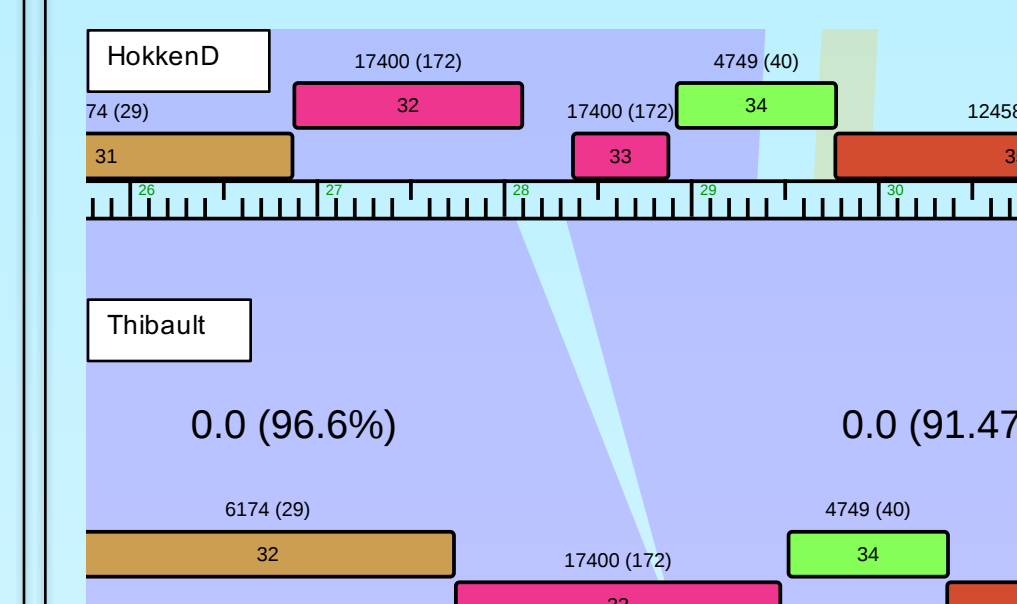


Plaque assay

Experimental treatment	Titer (pfu/ml)
37°C (control)	0
42°C (high temperature)	0
UV (120,000 μJ)	0
UV (6,000,000 μJ)	0
Ampicillin (50 μg/ml)	8 x 10 <sup>4</sup>
Ciprofloxacin (0.5 μg/ml)	2 x 10 <sup>4</sup>

**Conclusion:** Antibiotics produced the greatest induction of prophage from the HokkenD lysogen, but the lack of any phage shedding from control conditions was troubling.

## Splicing of the minor tail protein gene in HokkenD

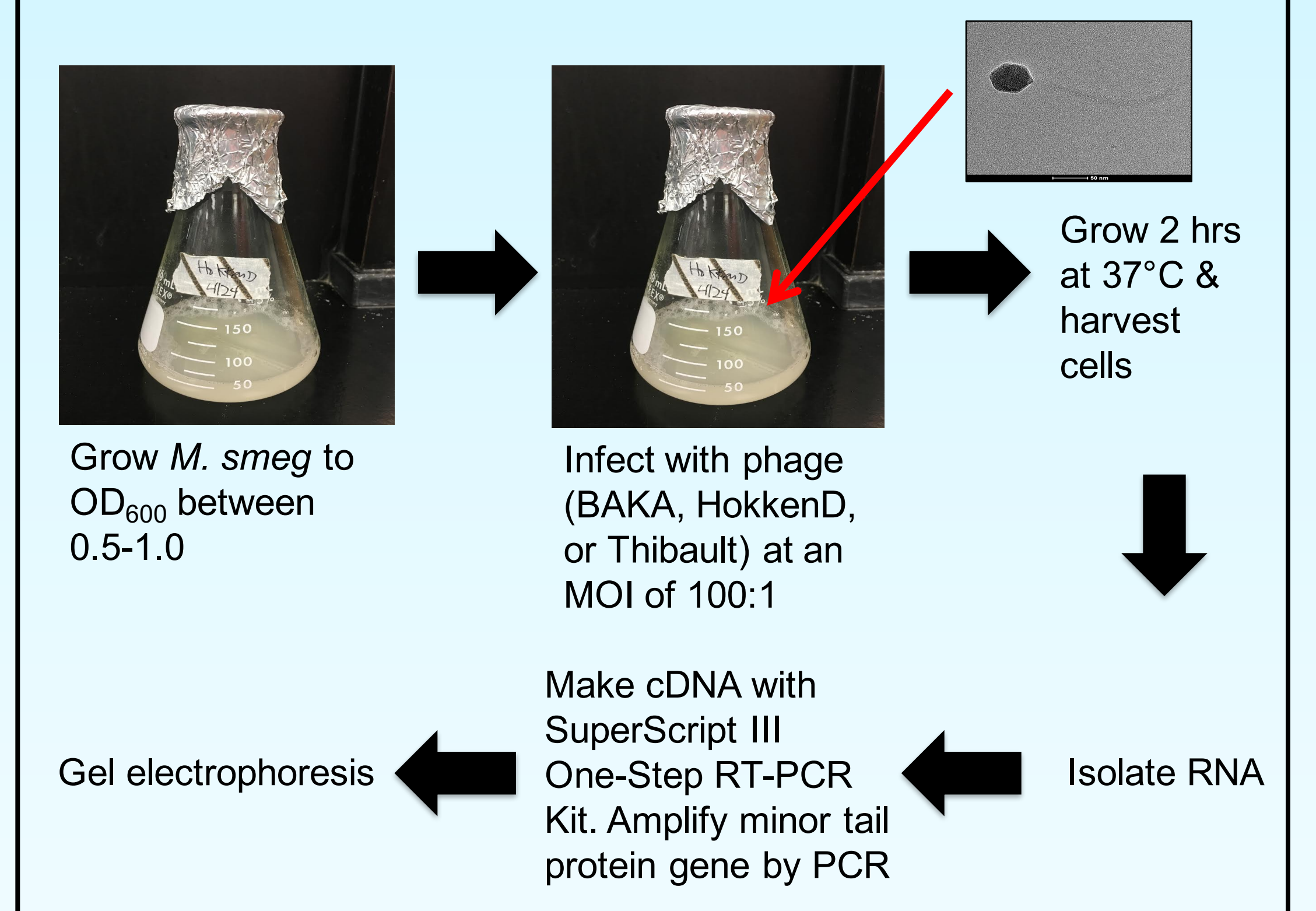


**Observation:** HokkenD gp32 and 33 encode the N-terminal half and C-terminal half a minor tail protein (pham 17400). Pope et al., 2013 demonstrated that the same gene is spliced in phage BAKA.

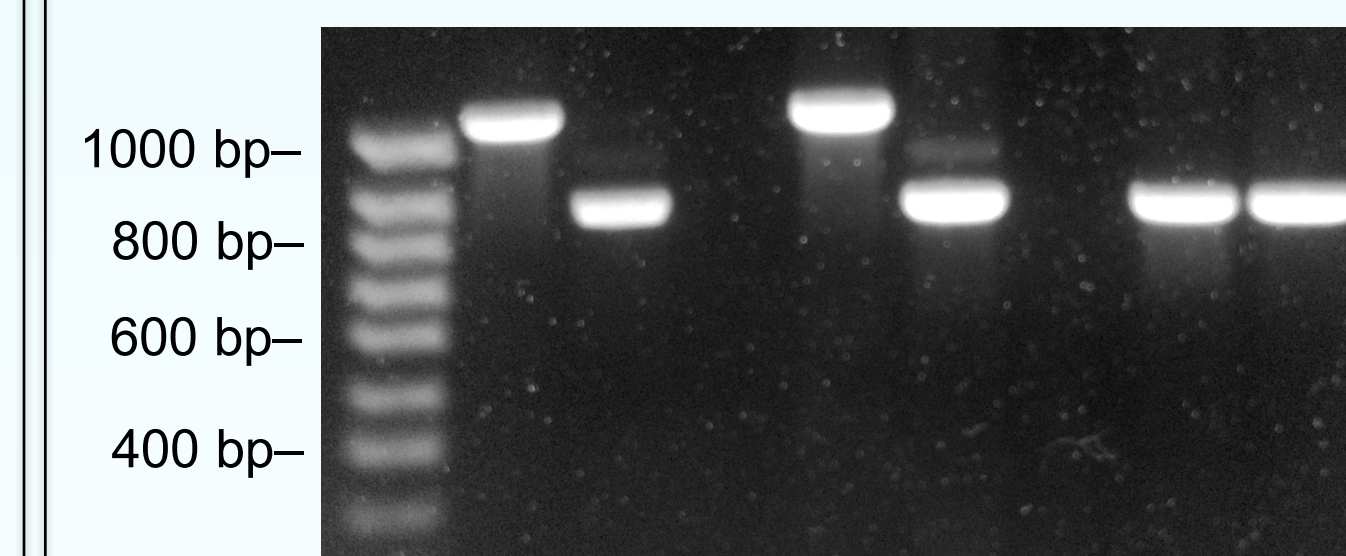
**Question:** Is the minor tail protein in HokkenD spliced?

**Approach:** Isolate genomic and cDNA from HokkenD and amplify a region spanning the space between gp32 and 33.

### Experimental procedure



BAKA      HokkenD      Thibault  
MWGen cDNA      Gen cDNA      Gen cDNA



**Conclusion:** Similar to what is observed in BAKA, the HokkenD minor tail protein gene is spliced.

## Conclusions

- HokkenD is a temperate bacteriophage of Cluster J.
- HokkenD shares superinfection immunity with some, but not all Cluster J phages.
- The GC content of HokkenD suggests that *M. smeg* may not be its natural host, although the identity of its natural host remains unknown.
- The minor tail protein gene of HokkenD is interrupted by an intron that is spliced out during gene expression.

## Acknowledgements

Thanks to funding and support from HHMI and KU's Undergraduate Biology Program, 20 undergraduate students enrolled in the SEA Phage Lab were able to participate in authentic research, gain valuable research and communication skills, and experience teamwork and camaraderie in a laboratory setting.