

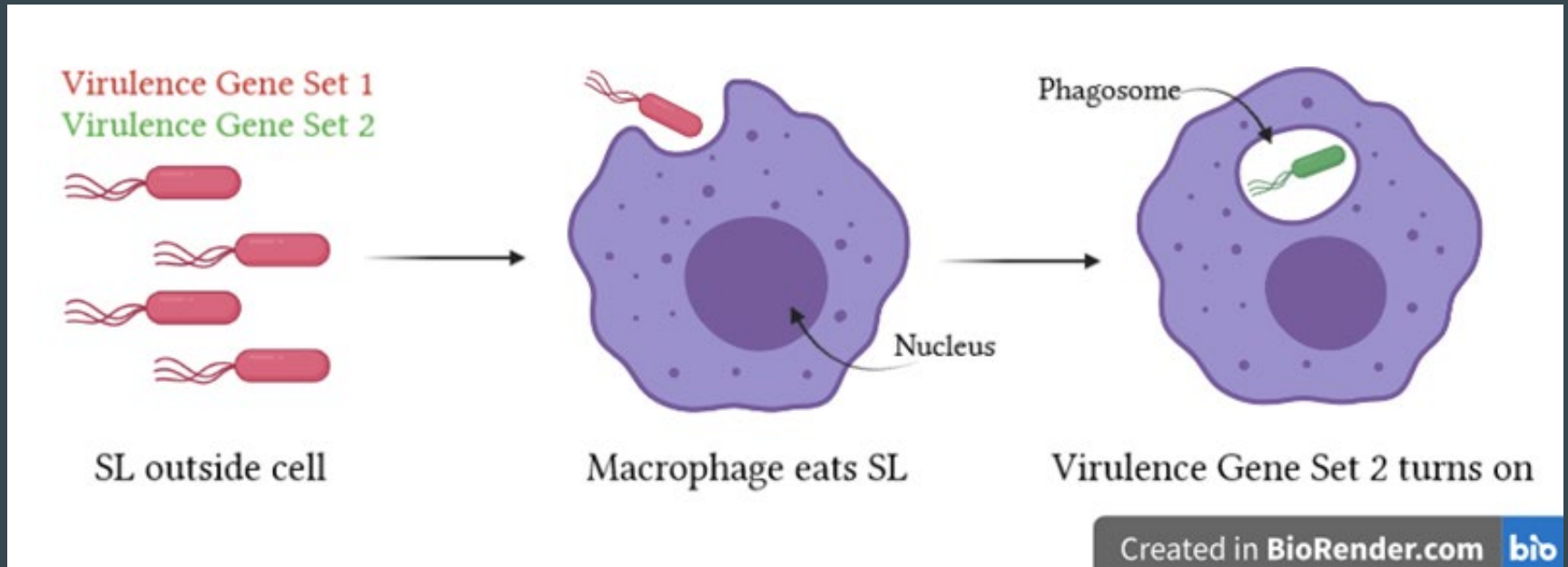
Heterogeneity of Virulence Gene Expression in *Salmonella typhimurium*



July Chen
Lane Lab (Molecular Biosciences)
05.27.2021 Research Expo

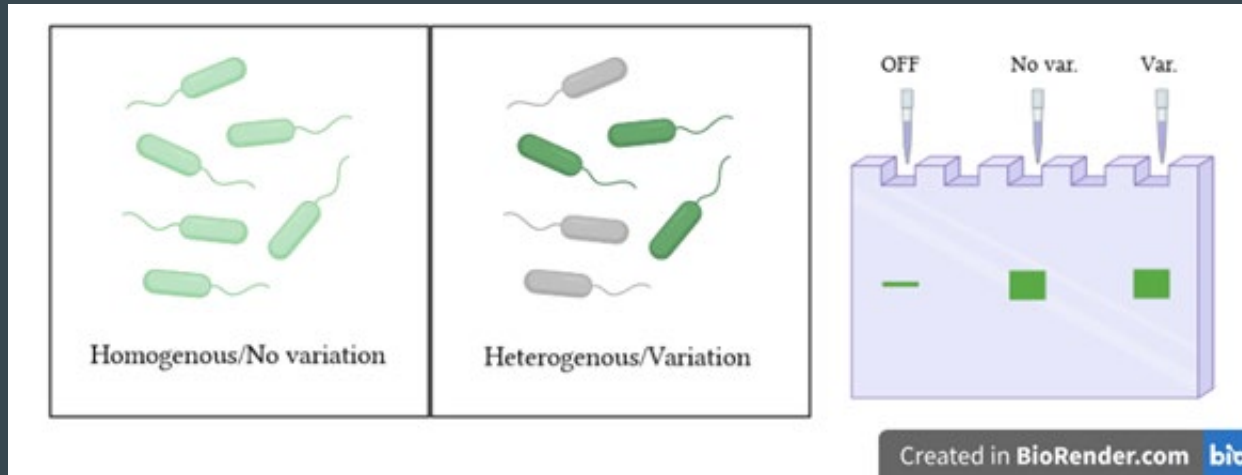
Virulence in *Salmonella Typhimurium*

- Virulence genes help bacteria like Salmonella (SL) invade and damage hosts



Cell-to-cell variation is found in virulence gene expression

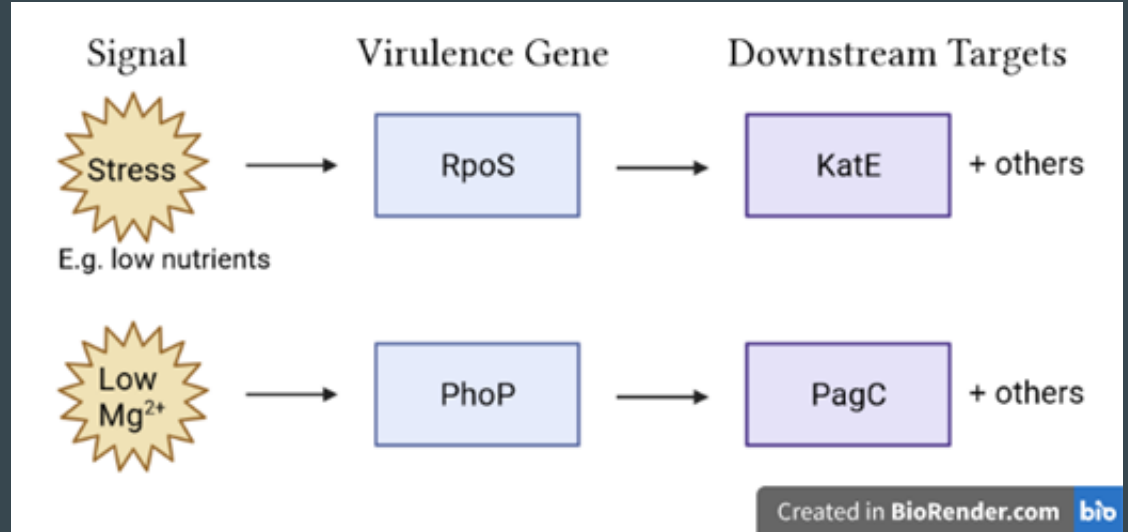
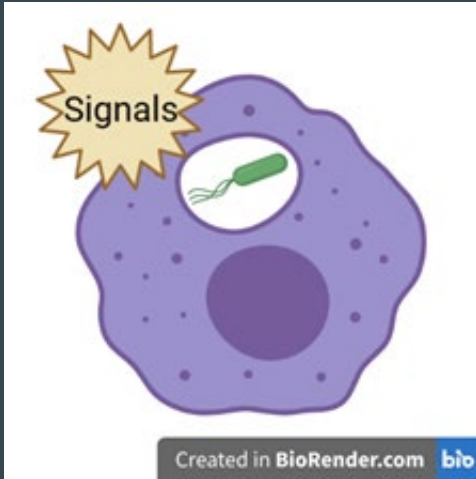
- Gene expression levels may vary among genetically identical bacteria



- This variation cannot be distinguished at the population level
- Single-cell variation found in one set of Salmonella virulence genes (SPI)

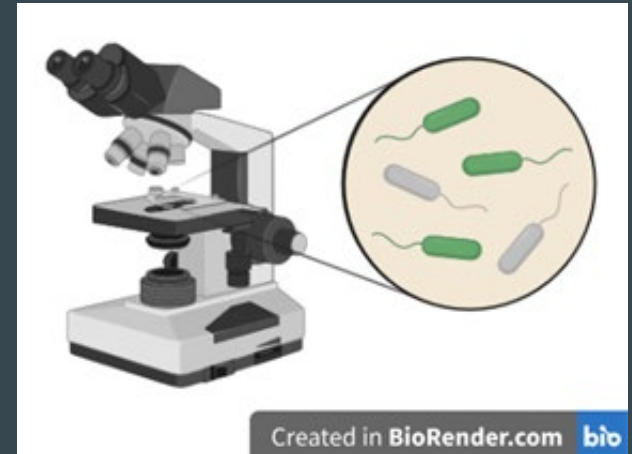
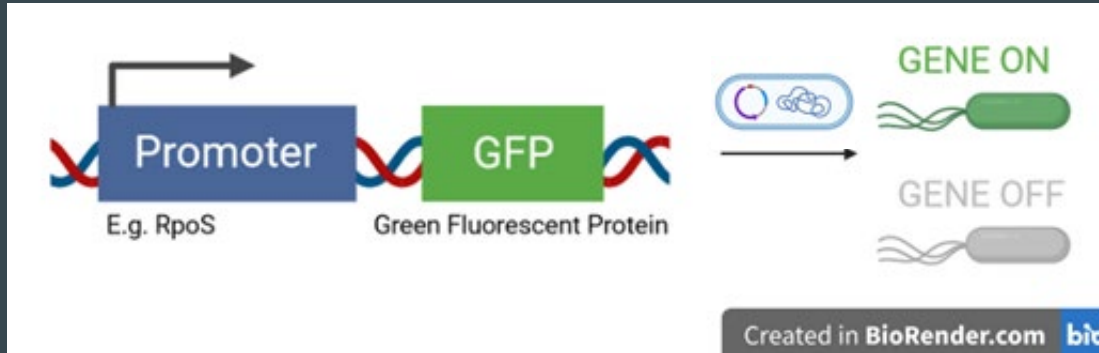
Question/Hypothesis

- To what extent do other virulence genes (beyond ~~Slp~~ **Slp**) have variation in SL?
 - RpoS, PhoP



Fluorescent reporters to detect variation

- Promoters: Gene region for initiating transcription (gene expression)
- Fluorescent reporters using promoters
 - Attaching promoter to fluorescent protein lets us track gene expression
- Gene expression → fluorescence visible under microscope



Automating Reporter Design

- Created Python code to help extract DNA sequence of promoters
- Prints 2000 DNA bases upstream of gene of interest where promoter region is
- Facilitates the reporter design process

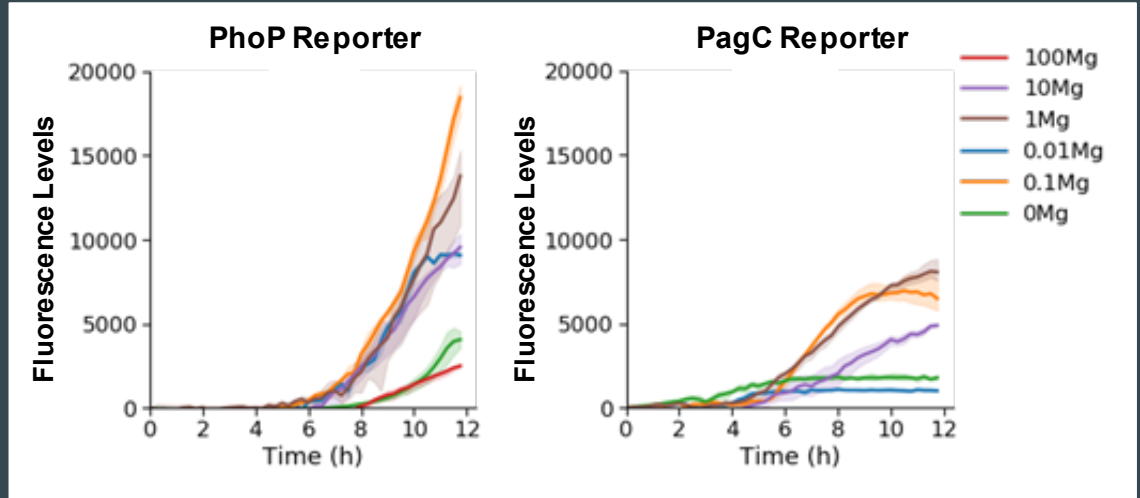
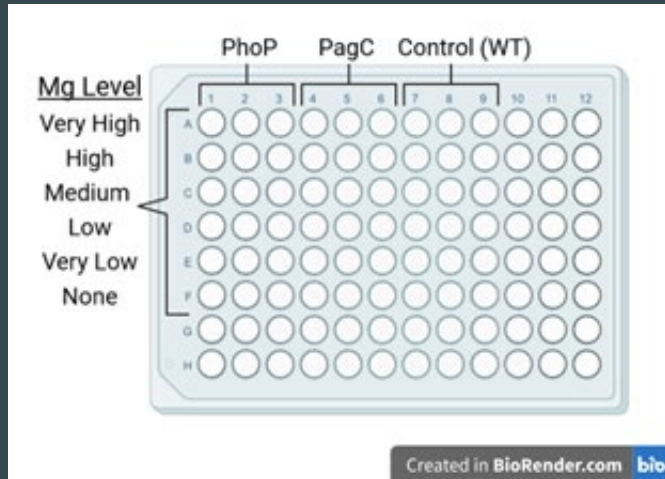


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This is the color key:  
Upstream sequence without the CDS of the nearest gene  
CDS of the nearest upstream gene  
Where the CDS of the interest with nearest overlapping gene and the gene of interest overlap  
CDS of the gene of interest with no overlap with the nearest genes  
How to interpret the results:  
Upstream region: GREEN and BLACK  
CDS region: RED and BLUE  
ATCCGAGCCGATCACTGACGACGCTGTTGATATCTAGTTCCTCAACCGTTGACCTTGTAGCTTTCACCGCTACGACGCTTTCCTGACGGTACCCAGGTTGATGTTACGGGACG  
AGGTTGCGGGACAGGATGTCATTTGCGCTGCTTAAACGGTTTGGTGAATGATCCGCGCGATTTGAGAGCCGAGAAATTTTGTGACTGTTGTATGTCGGCGCCCGTCAAGG  
ACATCAGCGCAAGCTTAGCCCTGTCACGCACTTCCGCGCTAACAGGAGACCGTTTTTCCCTGCGCAGATGGATCCATGATCACCAAGTTGATGTCATATTCAGAGAGAGATCT  
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TGCACTTTTGTCACTTAATTTGCACTAAGCTCAAAACAGGATACAGAAAGTCCCTGACCTGCGTGTGATGATGCGCAATTAACAGATGCGCAATTAACAGATGCGCAATTAACAG  
AATTTGCGCTCTTGTATGCTGCTTGCATCAAGCTCAACCAACATCATTAGCTTGGTGGTACTTTCCTCTGCGCCGACAGTGTCAAAAAGCGCTGTCACTCACTTACCATTTAA  
CAGCAACATTAACAGGCTAAGAGCTAGCGGACCTAAATAAATCACGCTTGGTGGATATATCAAGTTCAATTTGTACAGCTTTAAGCACTTTGATGAATTTGATGCAATTTAA  
CTAGCTTTCATCACAAATTTGCAATATTTCCACACTAGTTAGCTAAGCCAATTAATAATGATGATCCAAAGAACAGGATCAATTTAAATTAATAATCTCAATTAACAGAT  
GGATAGGATGTTCTGTTACATAAAGCAGCAATAGTACAGATACGCAATAGTGTAGCGCTCTTTACGAAATCAAAAATGCTTTTTCACTGATATGATGATTAATAATTTGTA  
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GCGTCCGATAGCGCGGATACCGTTCCGCTCAATCTGGCTGAGCGGCTCATGGTGTATTTGCTATCAATTAGCGGTTAAATGCAACAGCAGCCAGAGATCCGTTGATATGCTG  
TGAATCAGGATTAACAGCGGTTACGCGCGCGCTTTTACGCGCTGCTAACGACTCTGGAGCGCGCGGATGACCAAAATTAACGACTCTACCAACAGCGAAATCGCGCTCGTGGG  
CAGCGAGATACGGAATGTTGACCGTTTGGCTCATGATATGAAAAAAATTAACAAAAATAAGAGATTACTGCTGTTGCGAGAGATCAACAGCGGGAATTTGGTGGAAAA  
TAAATATCTGCGCAGCAGCAGATGAACAGGTTTTGGGATGTGATCAATTTAAAAATTTTGGACTAGCGCGGAGATACCTTTAACCAATATAGGAATAGAGACAGCAAAATA  
AAATGAGAGATACAGCAATCTGATGAGCGCATCAGCACCGCCACTTTACAGCATCACTTACCAAGATAGCGGATAGCGGCTGCGCGCTG
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Acknowledgements: Elizabeth Hora, Baker Undergraduate Faculty Grant

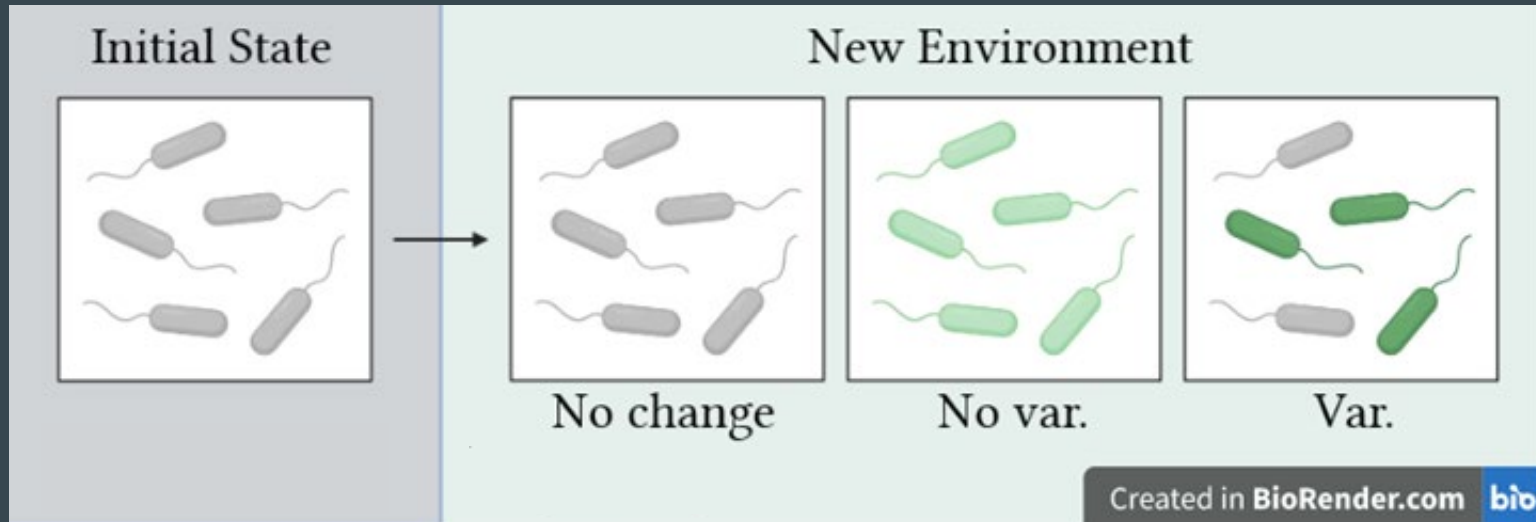
Experiment Plate Reader

- Plate reader: Allows experiments using many media conditions
- Tested PhoP and PagC at different Mg levels
- Highest activation for 0.1mM and 1.0mM Mg



Experiment-Validation

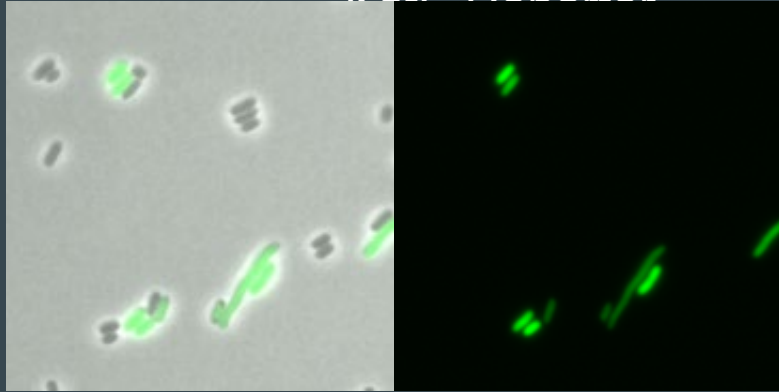
- Validate reporters with known signals
 - Nutrient deprivation activates RpoS, low Mg activates PhoP



Experiment-Validation

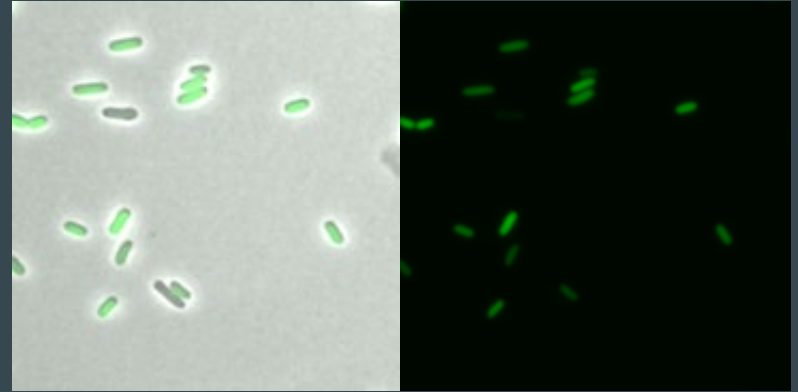
RpoS Reporter

KatE Reporter



Phase

Phase



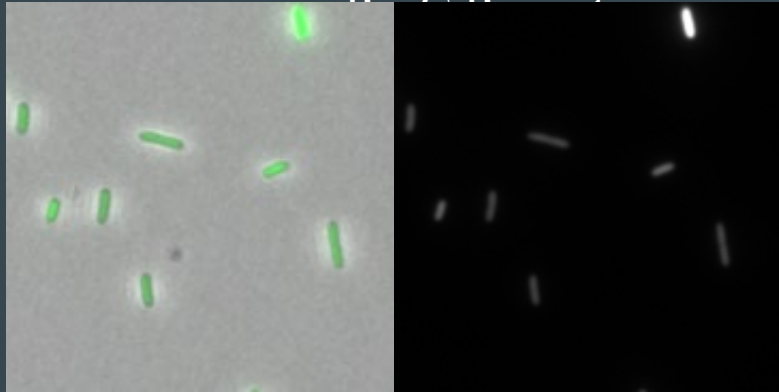
GFP

GFP

- SL with RpoS and KatE reporters after 12 hours in low nutrient environment
- Gene expression levels vary noticeably among cells

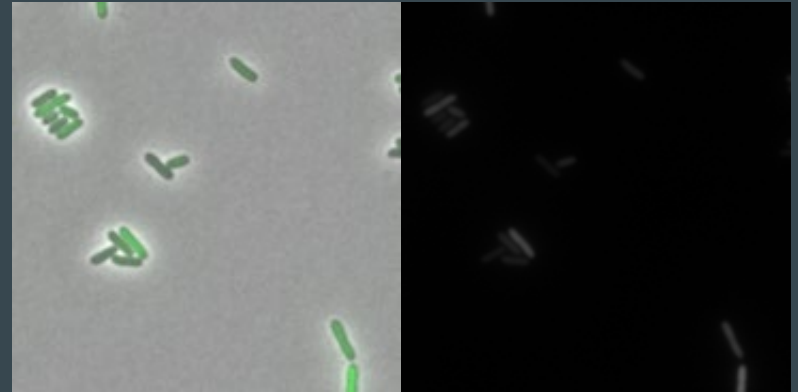
Data and Results Validation

PhoP Reporter



Phase

Phase



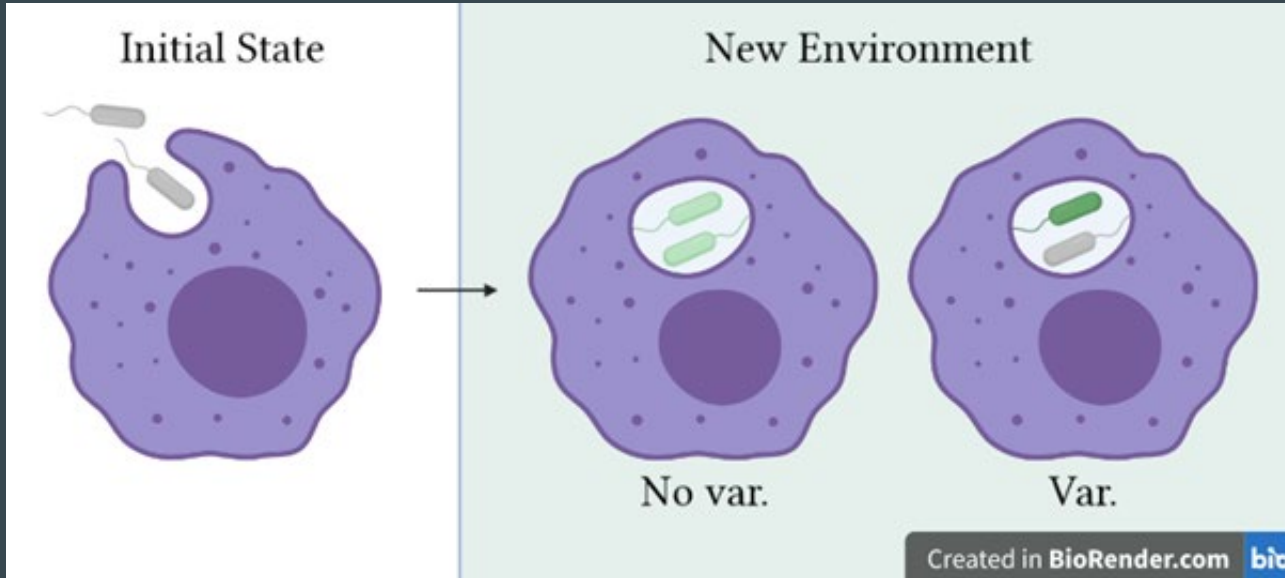
GFP

GFP

- SL with PhoP and PagC reporters after 8 hours in low Mg^{2+} environment
- Less pronounced gene expression variation - variability is more continuous

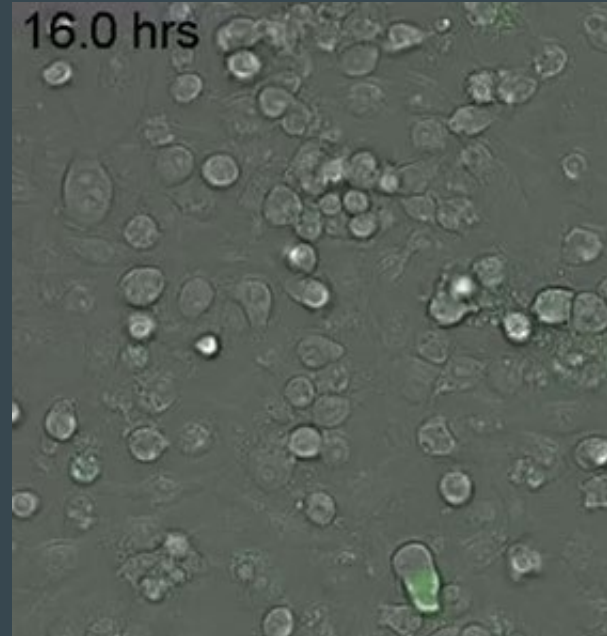
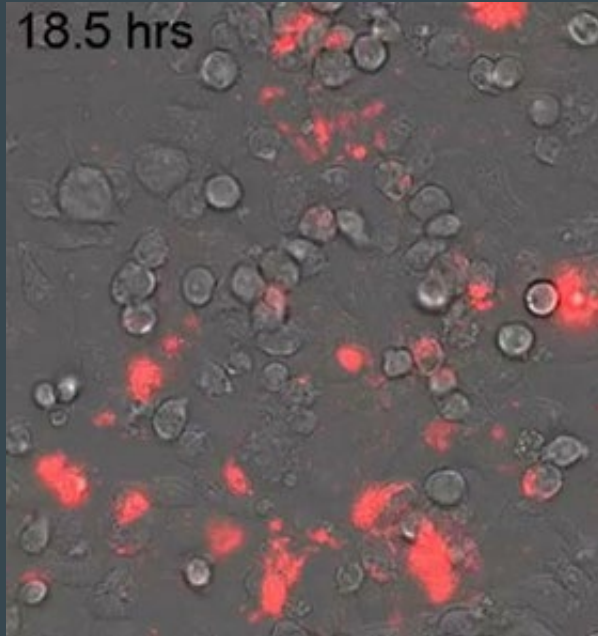
Experiment 3 Infection

- Infect macrophages with RpoS and KatE reporters



Experiment 3 Infection

- Timelapse of KatE reporters inside macrophages

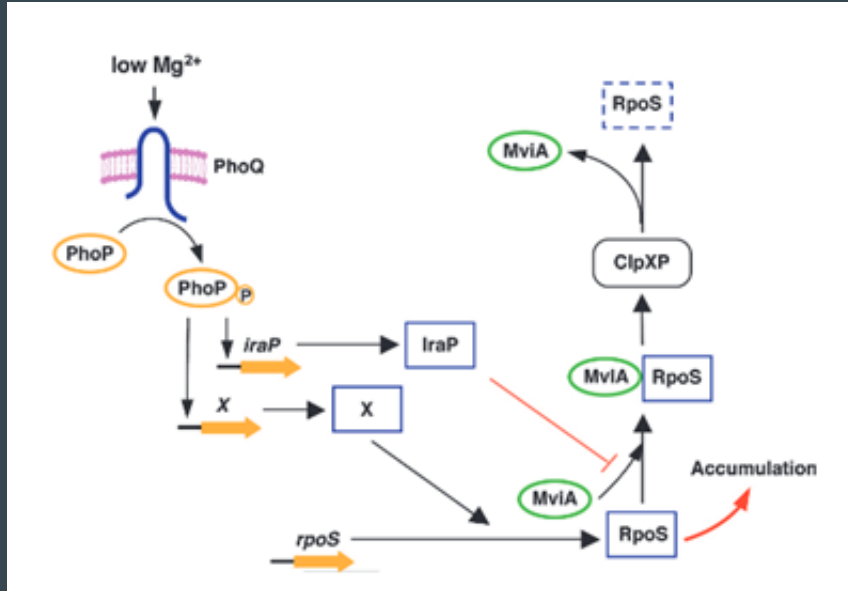


Phase + TRITC

Phase + GFP

Conclusions/Current Work

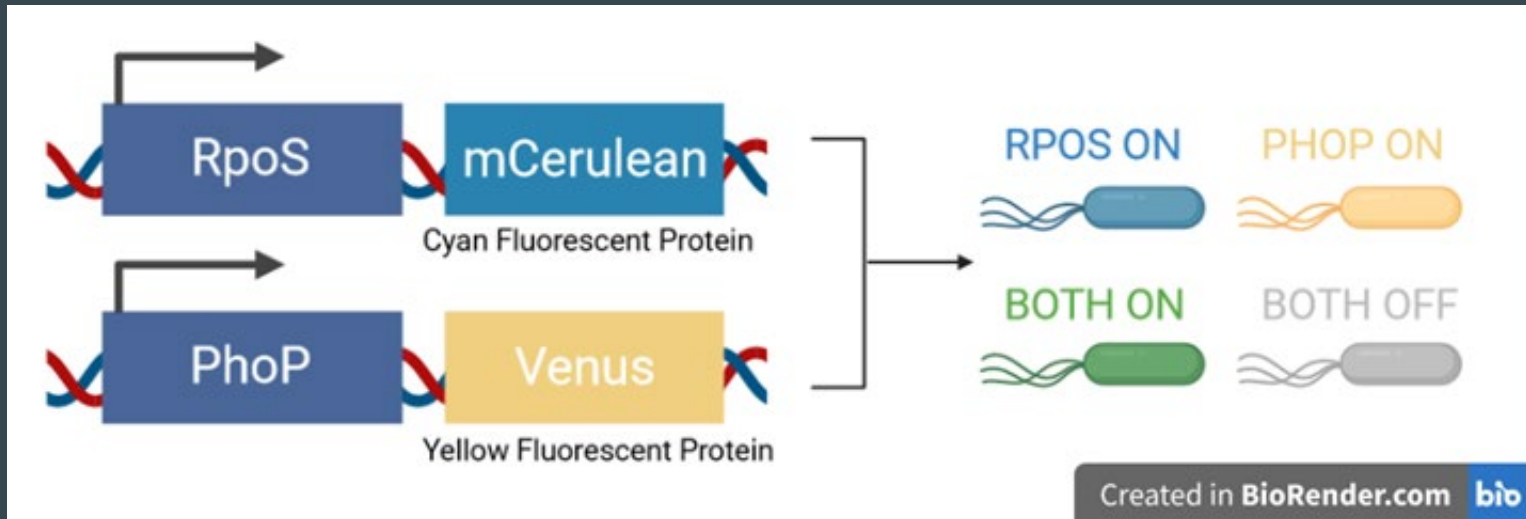
- Data thus far suggests single cell variation in RpoS and PhoP
- Next steps: investigate relationship between RpoS and PhoP activation



Tu, X., Latifi, T., et al. (2006). The PhoP/PhoQ Two-Component System Stabilizes the Alternative Sigma Factor RpoS in *Salmonella Enterica*. *PNAS*, 103(36), 13503-13508. <https://doi.org/10.1073/pnas.0606026103>

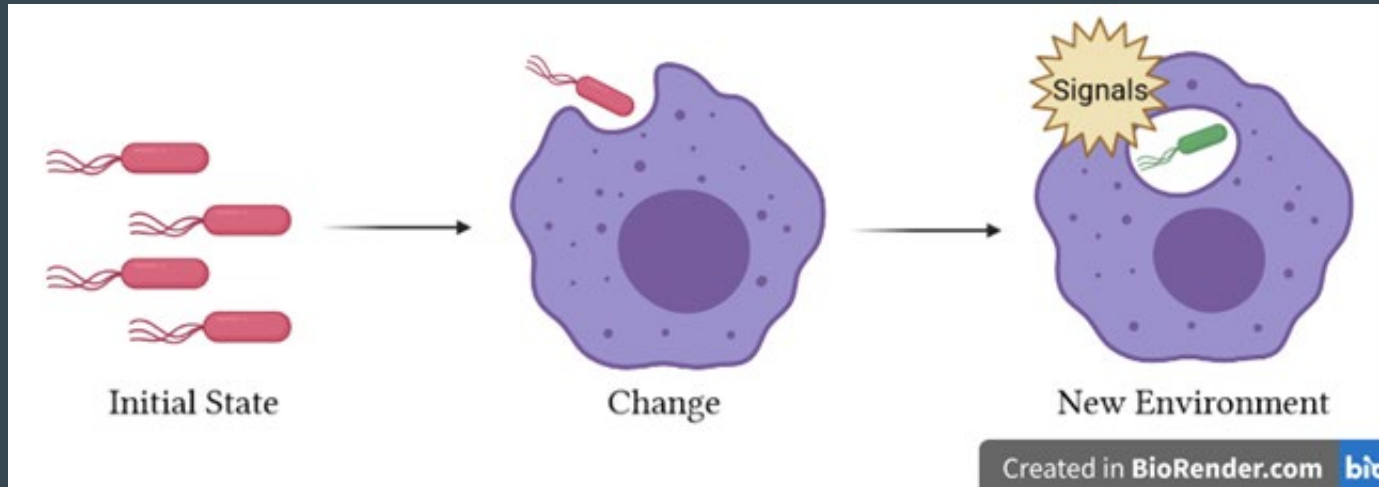
Conclusions/Current Work

- Data thus far suggests single cell variation in RpoS and PhoP
- Next steps: investigate relationship between RpoS and PhoP activation
- Studies suggest interregulation between RpoS and PhoP, do they vary together?



Overall Impact

- Leads to broader questions:
 - Why do genetically identical cells express different phenotypes?
 - How do bacteria survive and adapt in changing environments?
- Insight into infection strategies used by SL and other bacteria



Acknowledgements

- Lane Lab (Molecular Biosciences)
 - Dr. Keara Lane, Madison Smith, Elizabeth Hora, and others
- Baker Undergraduate Faculty Grant



<https://sitn.hms.harvard.edu/seminars/2016/food-fights-back-exploring-salmonellas-journey-body/>