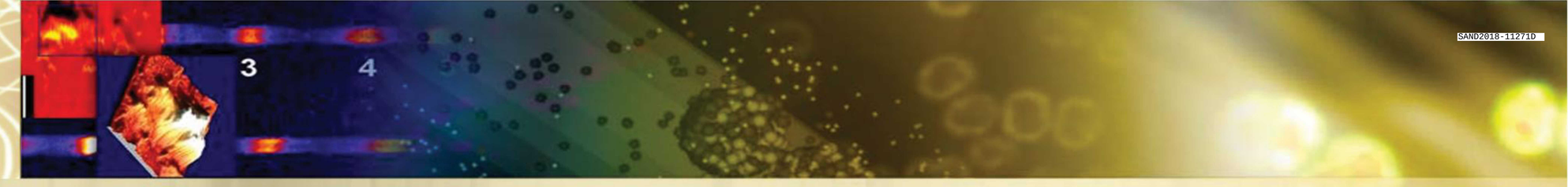
SANDIA BIOSCIENCE




SAND2018-11271D

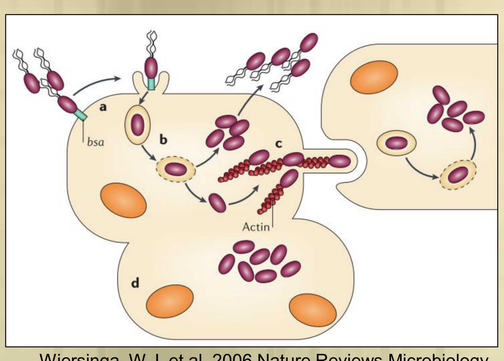
# Elucidating Genetic Mechanisms of Bacterial Pathogenesis for Host-Directed Protection

**Colleen Courtney, Anupama Sinha, Debjit Ray, Kunal Poorey, Nicole Collette (LLNL), Ashlee Fergusen (LLNL), Sahar El-Etr (LLNL), and Steven Branda**

## Motivation and Approach

### Why *Burkholderia*?

- Naturally resistant to most commonly used antibiotics
- Highly virulent, especially via airway
- Readily isolated from environment
- High-risk biothreat agent




Wiersinga, W.J. et al. 2006 Nature Reviews Microbiology

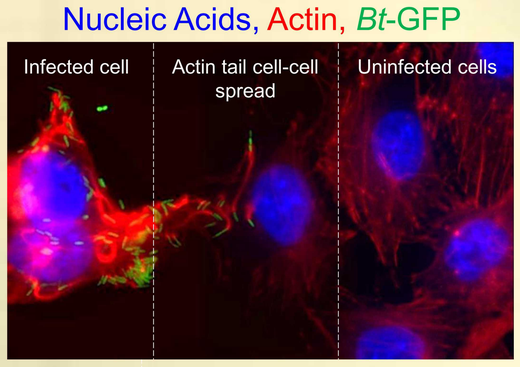
### Our approach:

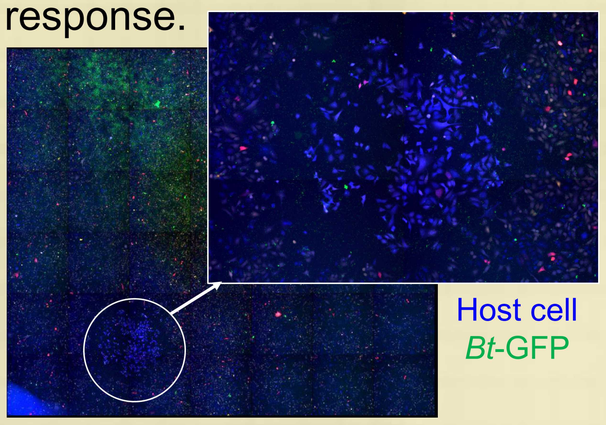
- Use a Genome-Scale CRISPR-Cas9 Knockout (GeCKO) Screen to find genes that aid in host protection
- Develop assays to confirm their protective action and elucidate genetic mechanisms in pathogenesis
- Transition validated, protective genes to murine primary cell, *ex vivo* lung/airway explant, and *in vivo* validation.

## Actin Tail motility

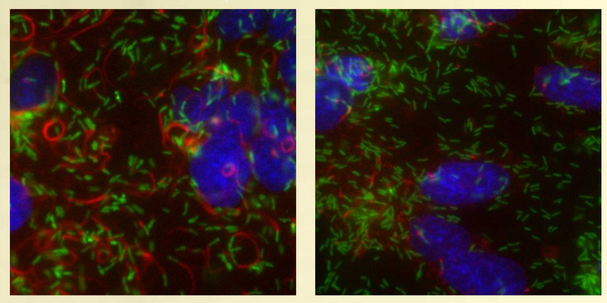
Actin tails are a signature of *Bt* pathogenesis and allow the bacteria to propel within the host and cause cell-cell spread. Presence and length of actin tails (right) and cell-cell spreading (bottom) elucidates host response.

Nucleic Acids, Actin, *Bt*-GFP



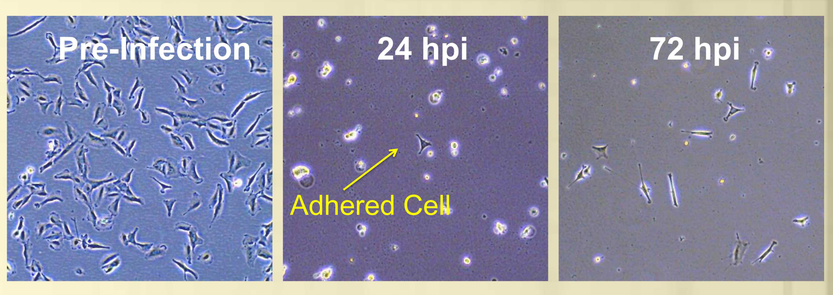



Host cell
*Bt*-GFP



**Control** **ΔFERMT3**

## Host GeCKO Screen




Identify gene knockouts that protect human airway epithelial cells from *B. thailandensis* (*Bt*)

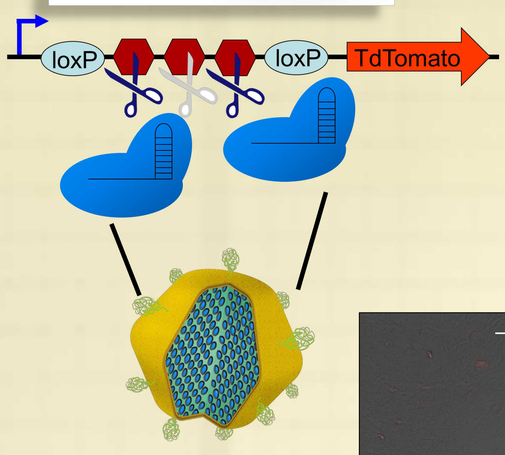
- 8 independent infections in 3 screens
- 24 hpi allow cells to recover
- 72 hpi collect for DNA

| sgRNAs Hitting Protein-Coding Gene | Protein-Coding Genes | Average Number of Infections from which sgRNA was Recovered | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 5325 | 3580 | 325 | 184 | 169 | 184 | 246 | 225 | 412 |
| 2 | 817 | 370 | 101 | 91 | 122 | 93 | 21 | 11 | 8 |
| 3 | 79 | 32 | 11 | 27 | 5 | 2 | 0 | 2 | 0 |
| 4 | 5 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Genes carried on for assay  Second priority gene targets

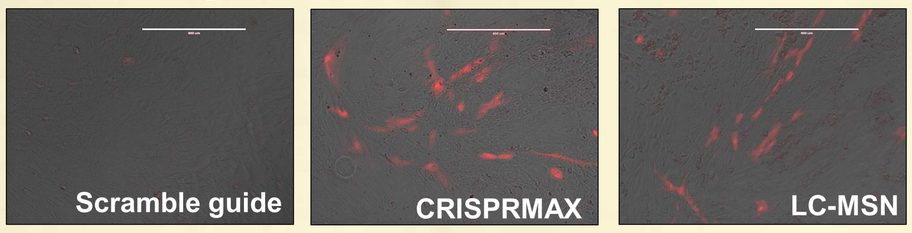
## Model Mouse for Gene Editing

Ai9(RCL-tdT) mouse

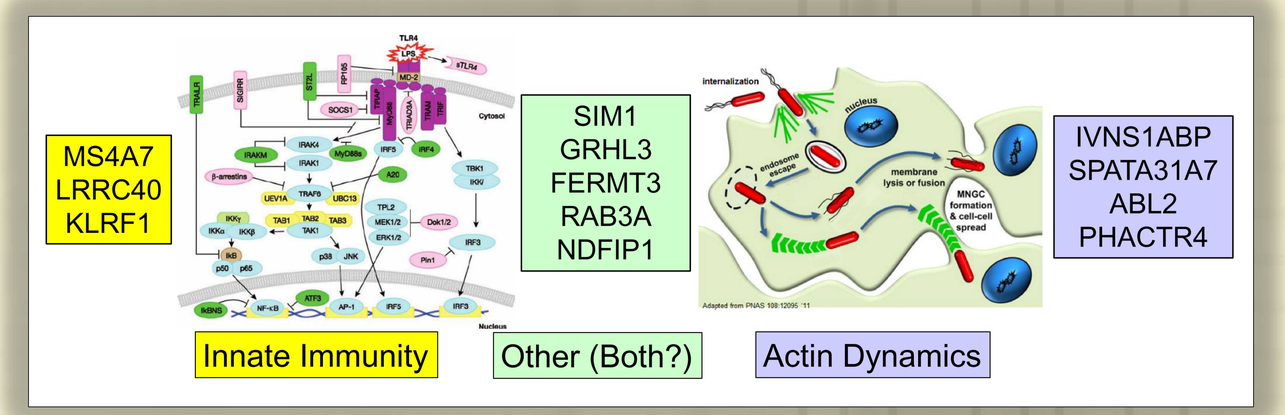



Cas9-sgRNA loaded LC-MSN

The construct in Ai9 mice can be targeted with one sgRNA that cuts twice and removes a stop cassette allowing for constitutive expression of TdTomato (red) fluorescent protein. We are using the Ai9 mouse to evaluate our protein delivery and gene editing efficiency for an easy gain of signal fluorescent readout.




## Host Defense Targets




MS4A7
LRRC40
KLRF1

Innate Immunity

SIM1
GRHL3
FERMT3
RAB3A
NDFIP1

Other (Both?)

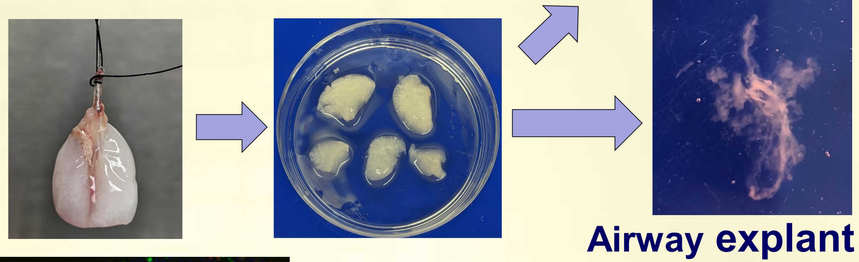



IVNS1ABP
SPATA31A7
ABL2
PHACTR4

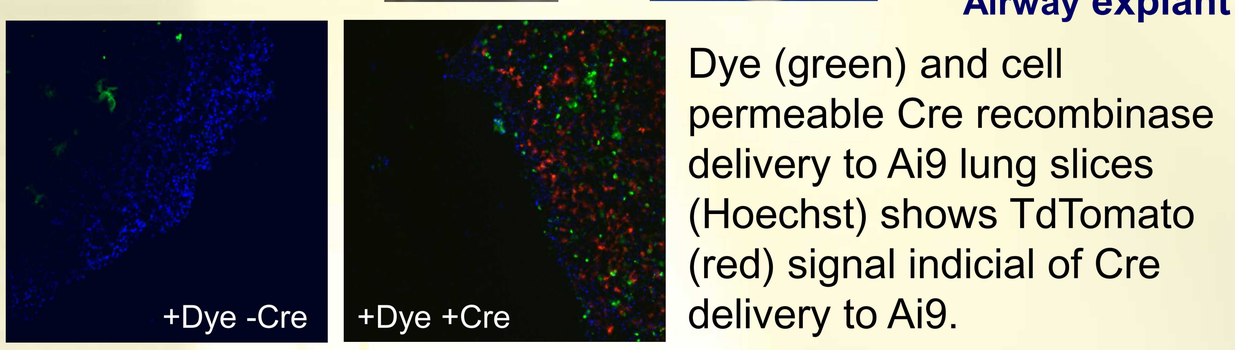
Actin Dynamics

## Lung Explants

Need to achieve 4-7 day viability *ex vivo* to allow for CRISPR/Cas editing of target and infection with *Bt.* Ai9 mice used for protocol development for gain of signal editing and quick protein delivery readout with Cre.



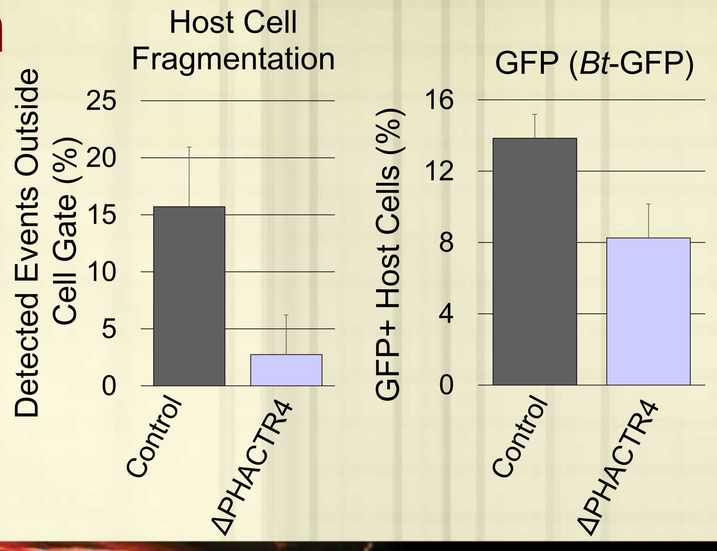

**Airway explant**




Dye (green) and cell permeable Cre recombinase delivery to Ai9 lung slices (Hoechst) shows TdTomato (red) signal indicial of Cre delivery to Ai9.

## Cell Fragmentation and Bacterial Internalization

Cell health and bacterial internalization (GFP signal) are being assayed using flow cytometry. Less fragmentation and GFP, like the PHACTR4 example, indicates greater protection by gene knockout.




## Progress to Date and Future Plan

- Confirmed genome screen identified protective genes
- Continuing multiple assays to elucidate genetic mechanisms of *Bt* pathogenesis
- Working to down select host defense targets to best *in vivo* option for mouse studies
- Transition to CRISPRi and move towards a therapeutic

Sandia National Laboratories is a multi-mission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525. **SAND Number:**

U.S. DEPARTMENT OF ENERGY

Sandia National Laboratories