SAND2015-9668C

Exceptional service in the national interest

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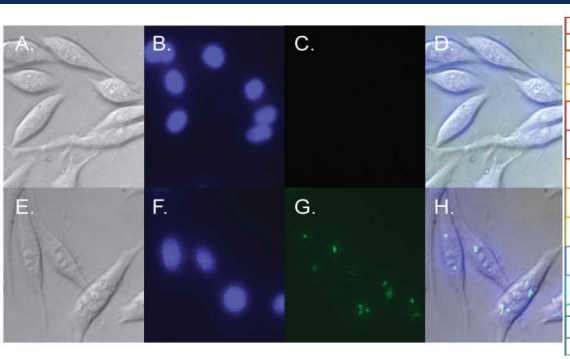

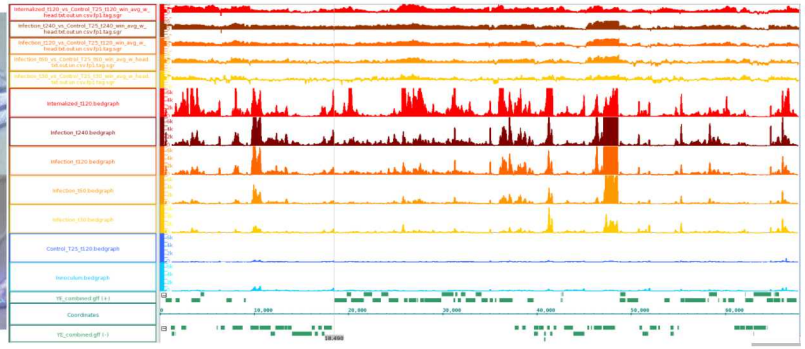

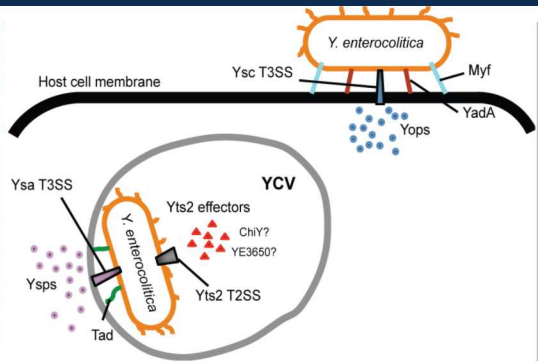

Understanding the Spatial and Temporal Dynamics of Pathogenesis

Kunal Poorey, Sandia National Labs
Systems Biology

U.S. DEPARTMENT OF ENERGY | NNSA National Nuclear Security Administration

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Host-Pathogen Interactions using RNA-Seq

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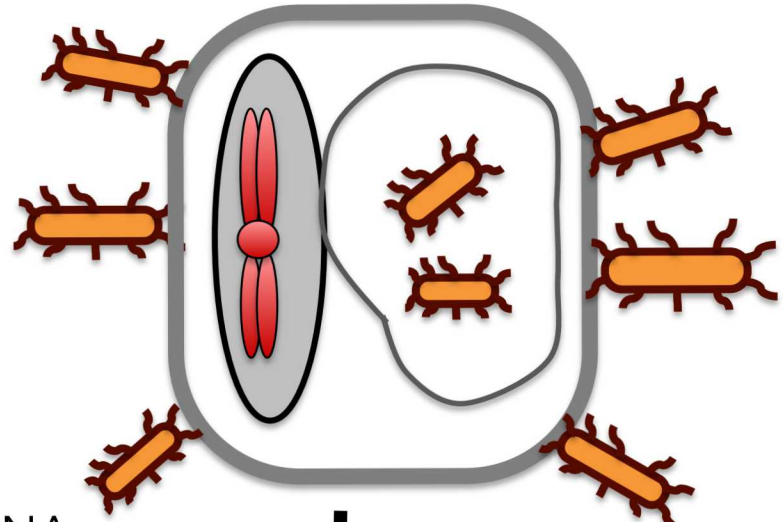
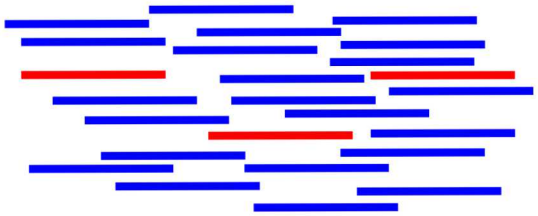


Total RNA Extraction



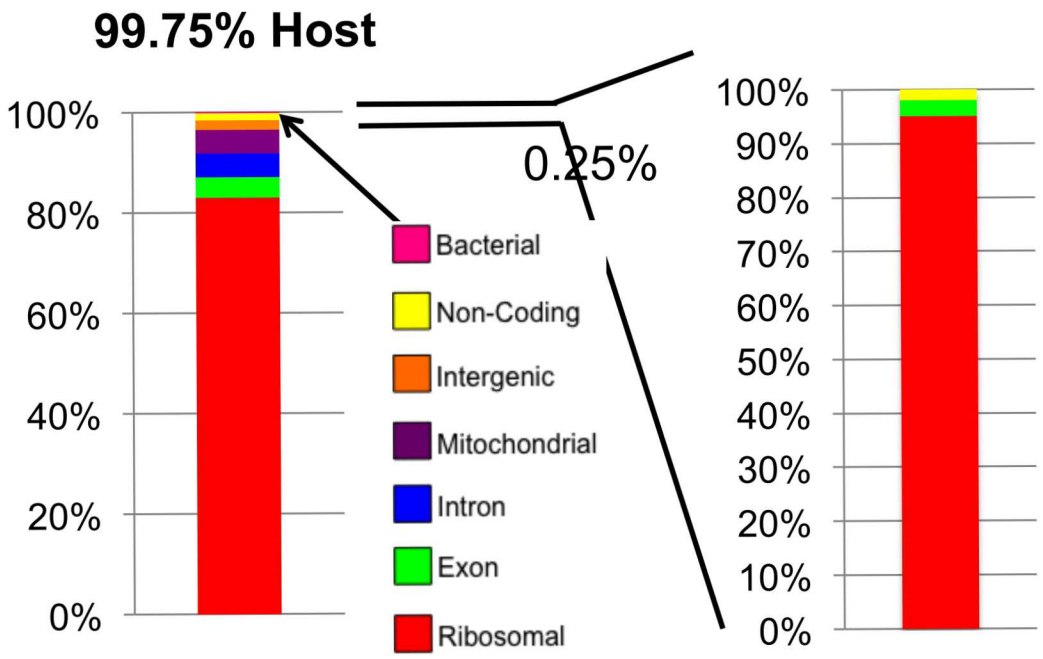
Mix of Host and Pathogen RNA

Prepare RNA-Seq Libraries

Host-Pathogen Transcriptomics

HiSeq 2500

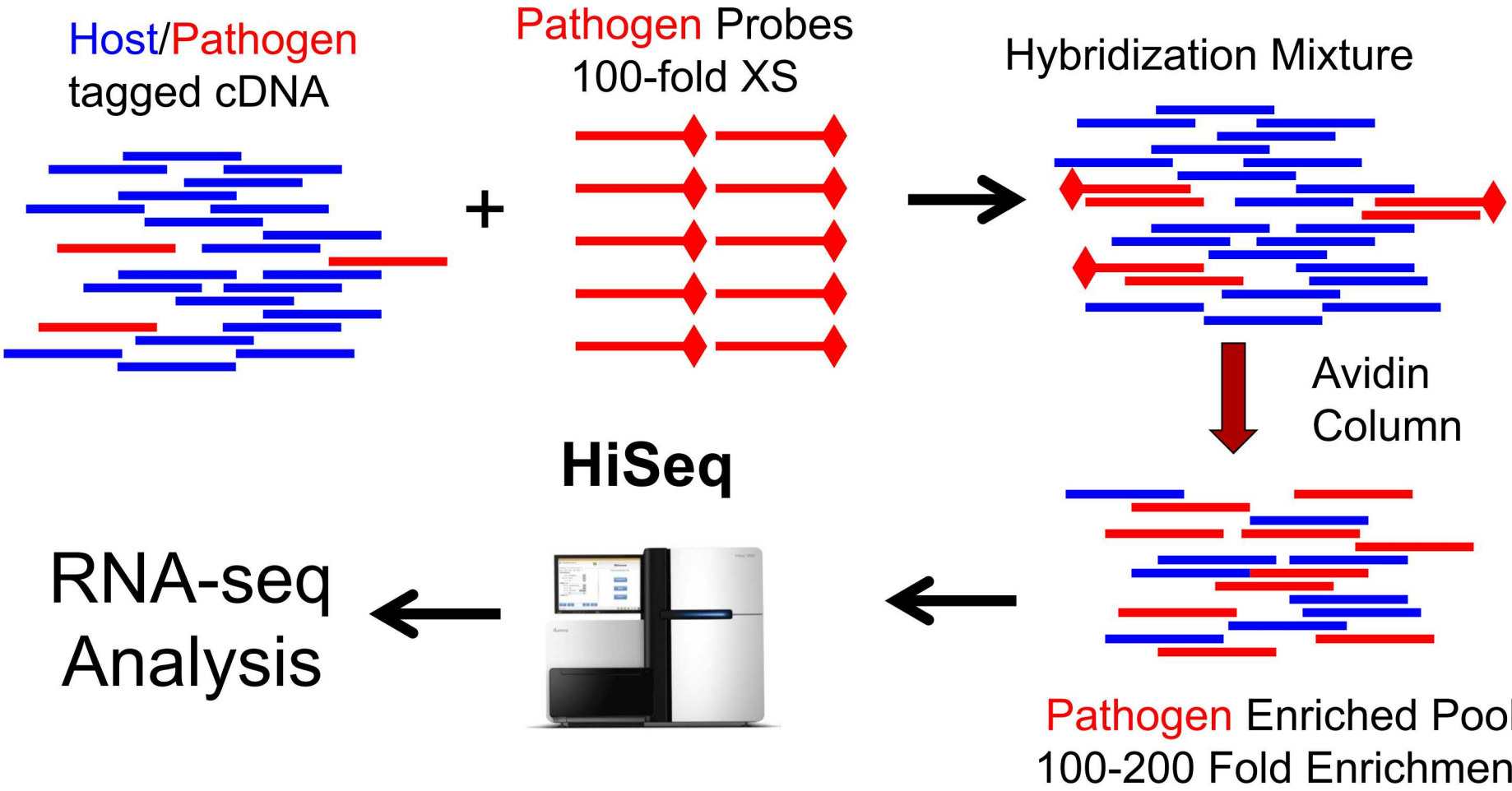
Brute Force Sequencing is NOT Feasible for Bacterial Transcriptomes



- 0.0075% of total reads are bacterial CDS
- 12000 reads per HiSeq lane
- ~83 HiSeq lanes = 1,000,000 reads

Problem : To study pathogen action/response in infection we need a cost effective method to enrich for bacterial cDNA.

Pathogen Capture



Version 1 – Syringe Pumps

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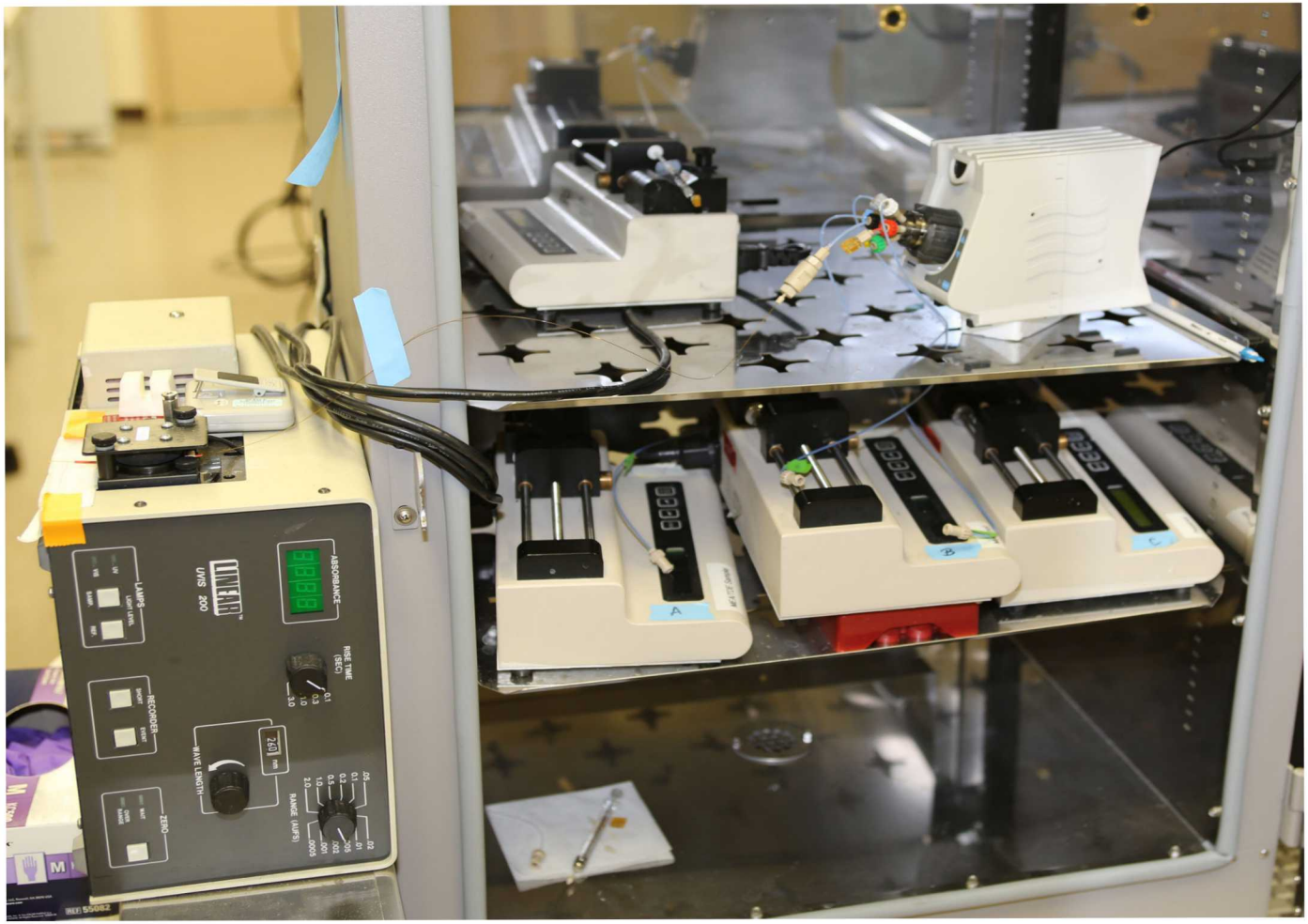

The Diseases of *Yersinia*

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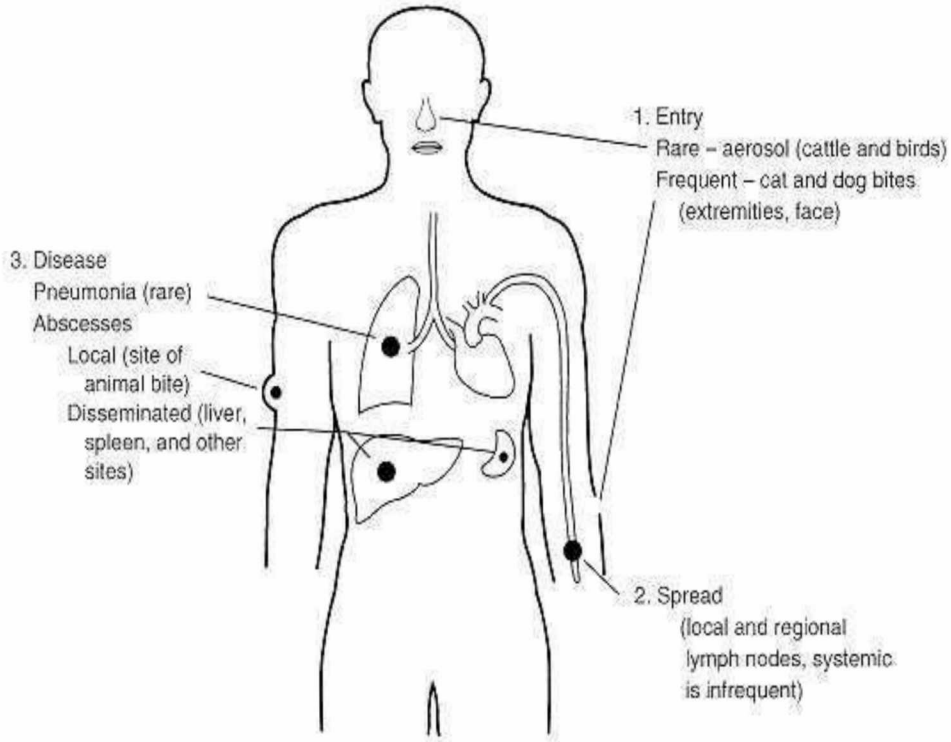
Plague

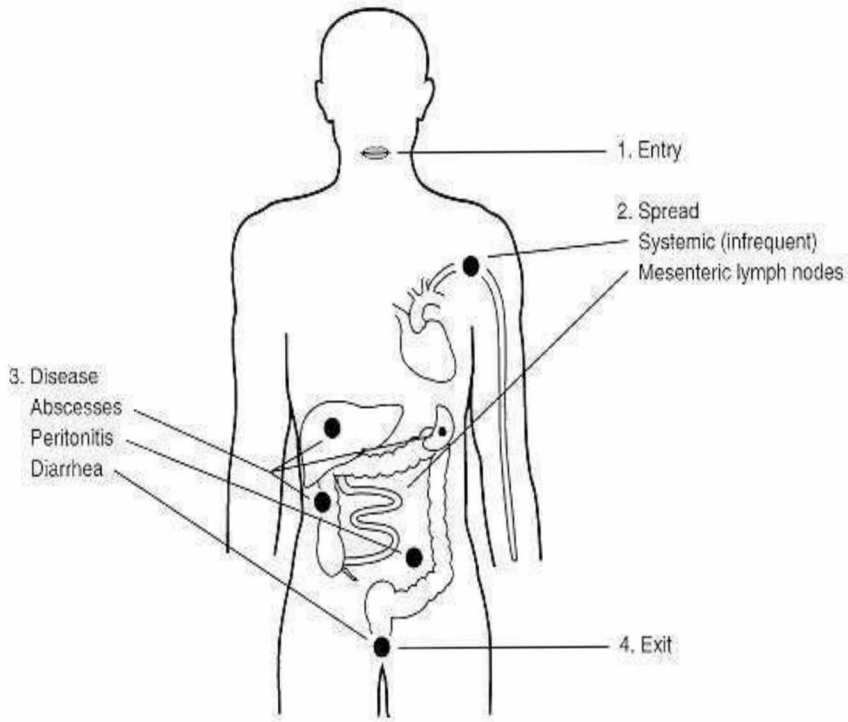
Y. pestis



Gastrointestinal syndromes

Y. pseudotuberculosis

Y. enterocolitica



All *Yersinia* are typically considered extracellular pathogens

Y. enterocolitica Remains Viable within MΦ

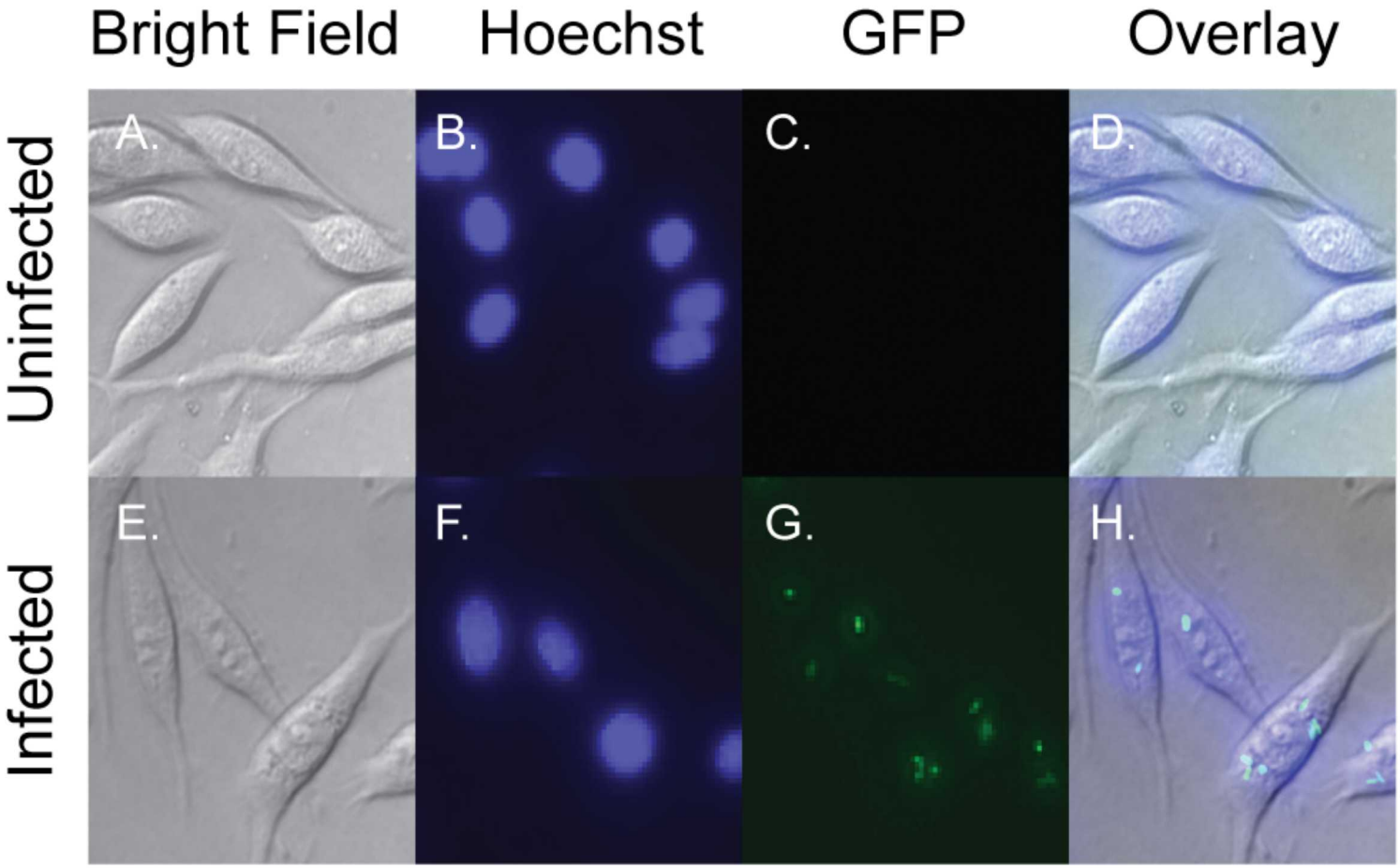
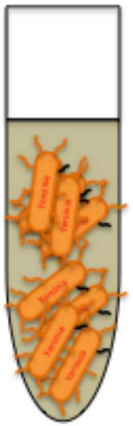
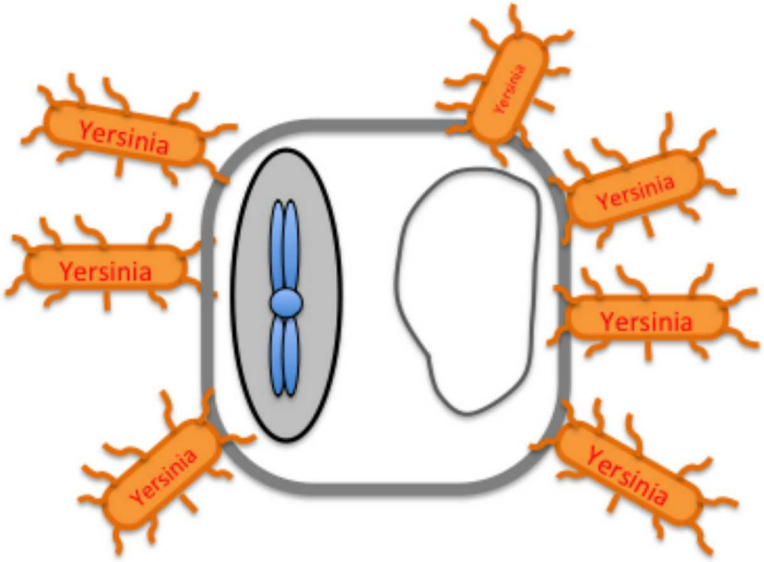


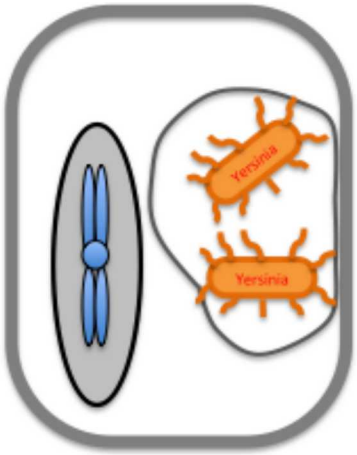
Plate counts show 19% of inoculum is viable after 3 hours within macrophage

Bent et al, I & I, accepted

Y. enterocolitica Infections







Pathogen Transcription in media

Growth Medium 25°C
& Conditioned RPMI 37°C

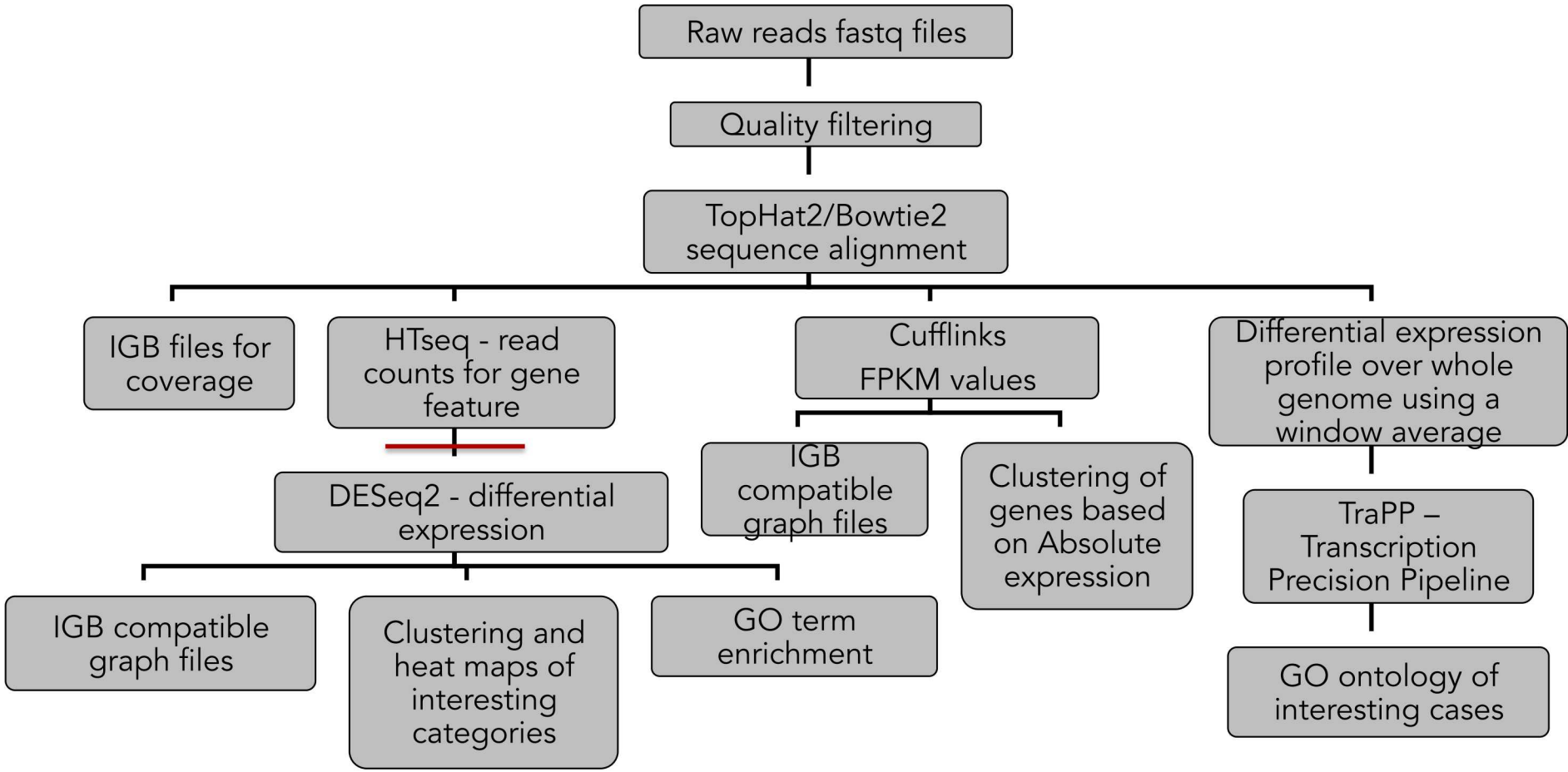
Pathogen Transcription during the time course of infection

Infection of MΦ
MOI = 10 37°C
Time : 30, 60, 120, 240 min

Pathogen Transcription when surviving internalization within host cells

Gentamicin Treated
MOI = 10
1hr of infection
1hr of gentamicin

RNA-seq Analysis by YAnTra (Yet Another Transcriptomics pipeline) ☺



Genome wide map of reads

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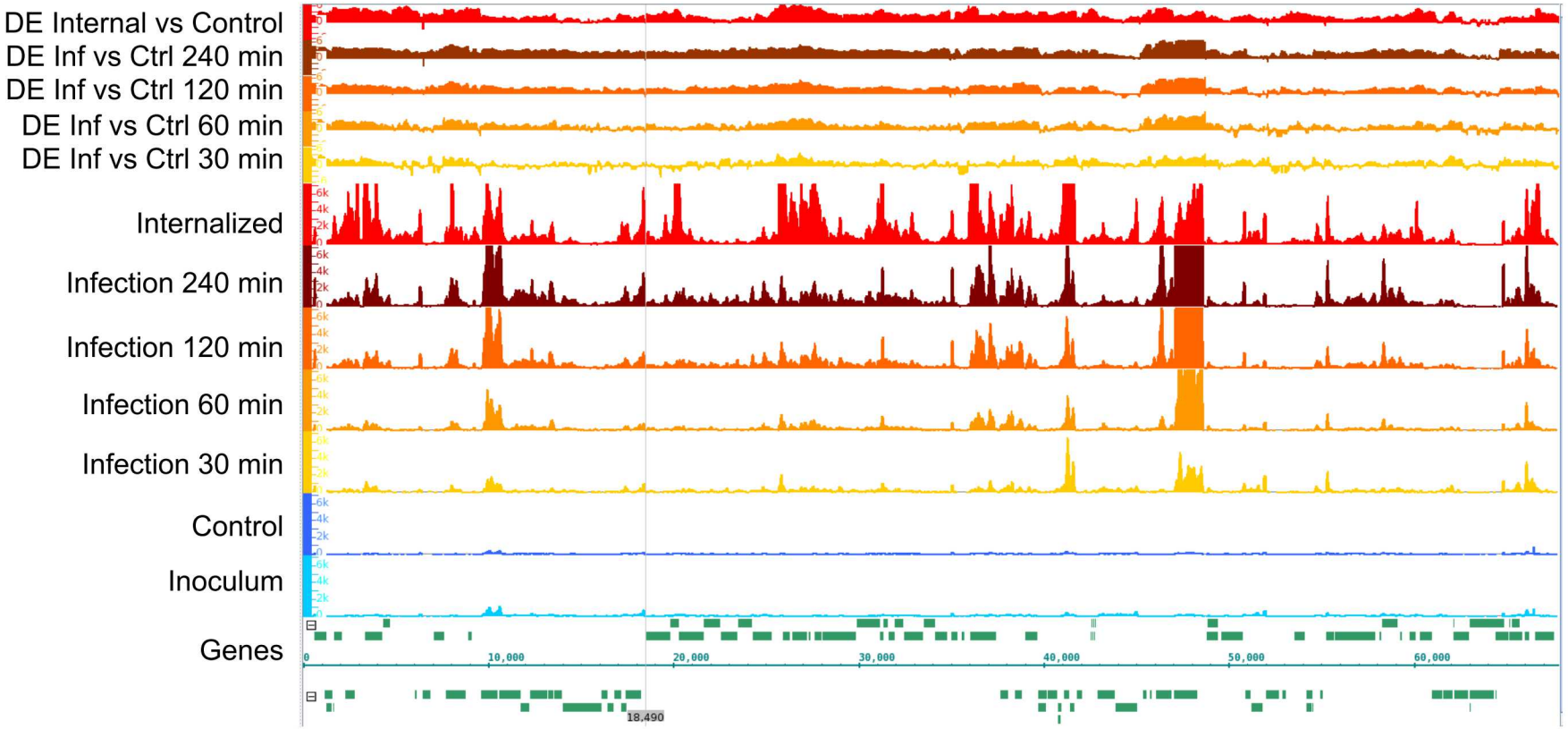
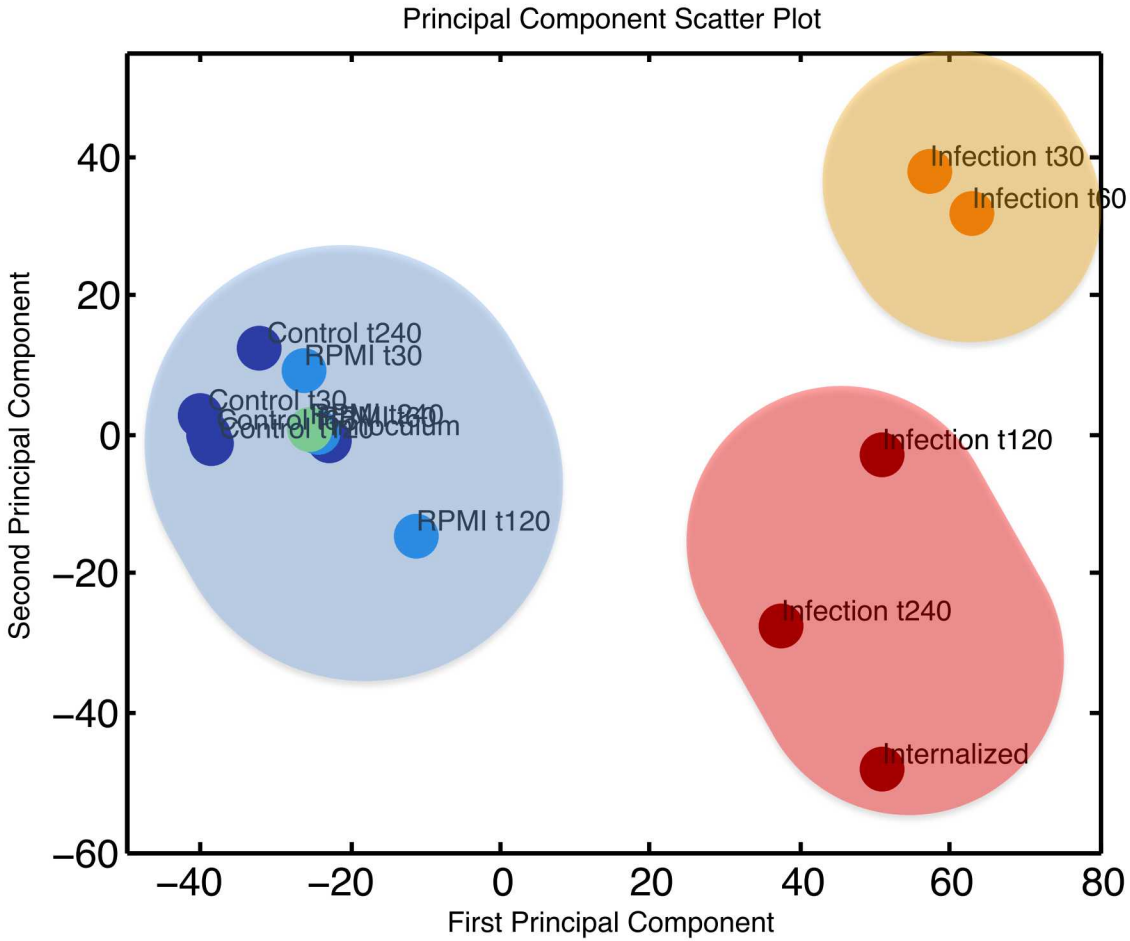
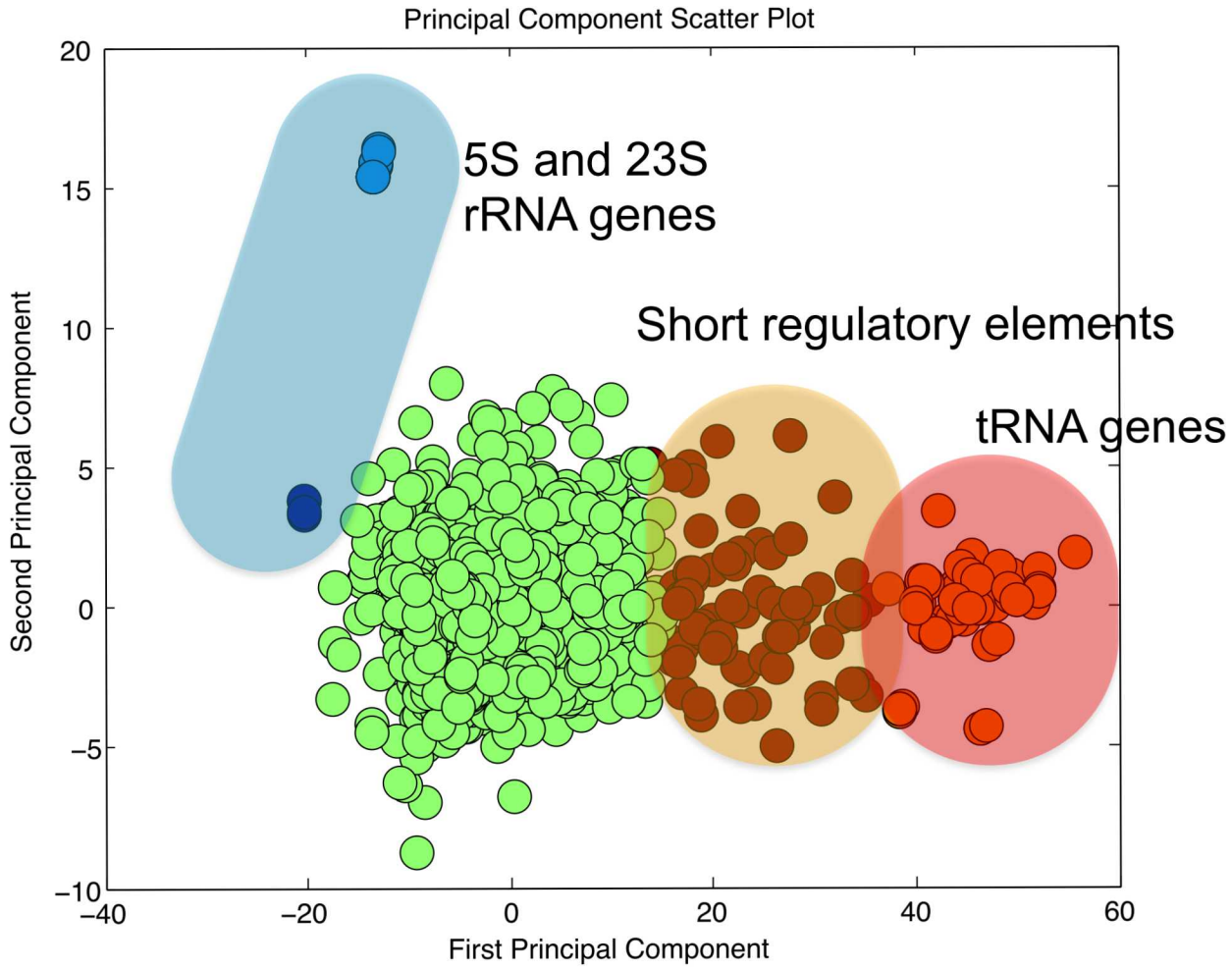
Screen shot of Virulence plasmid



Gene expression profile in infection is widely different than growth media



Gene expression profile of different gene categories are very different as well



Gene Expression during Infection is Temporally Dynamic

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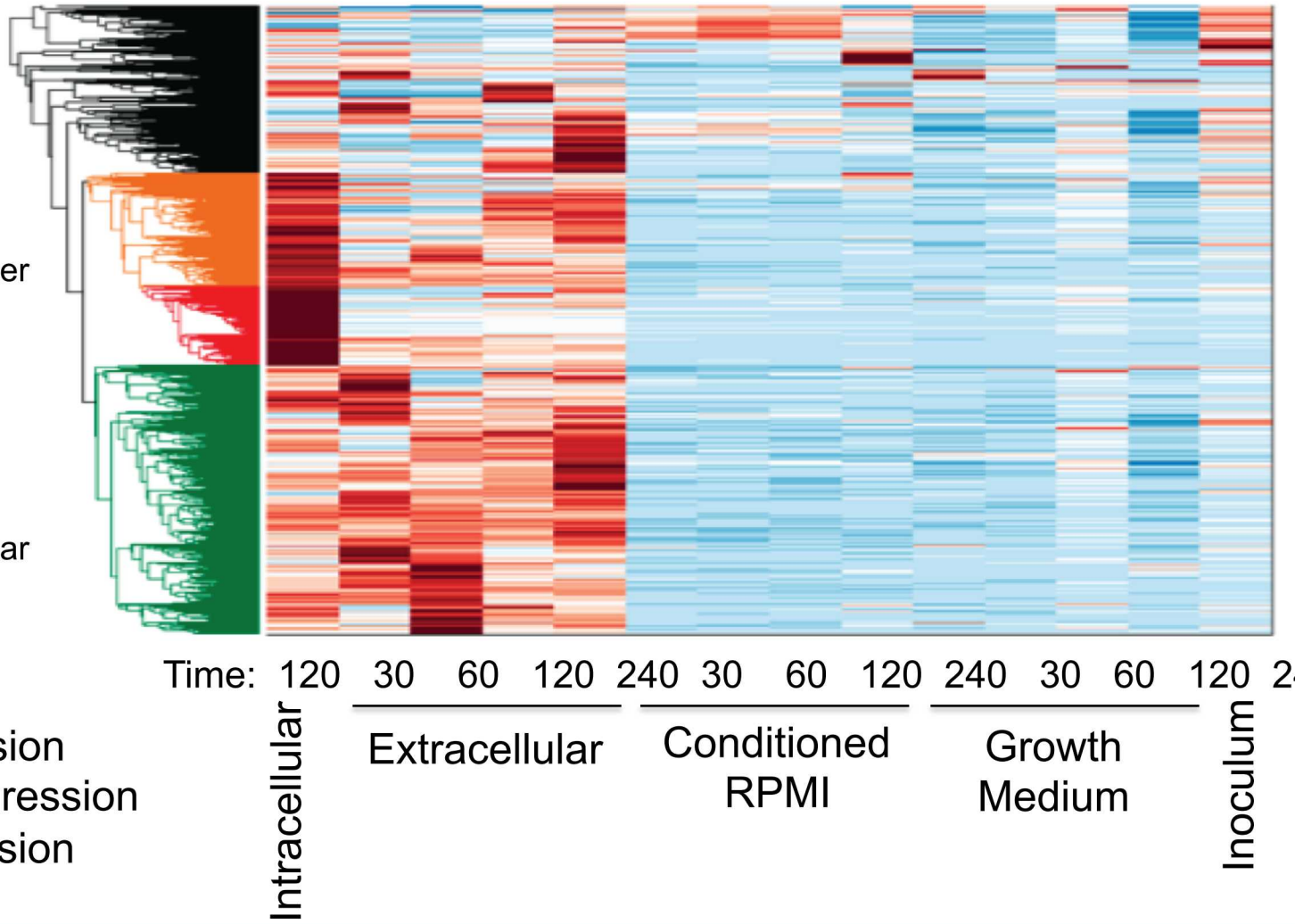
Black: Low expression all conditions

Orange: Highly expressed intracellular, increased extracellular expression over time

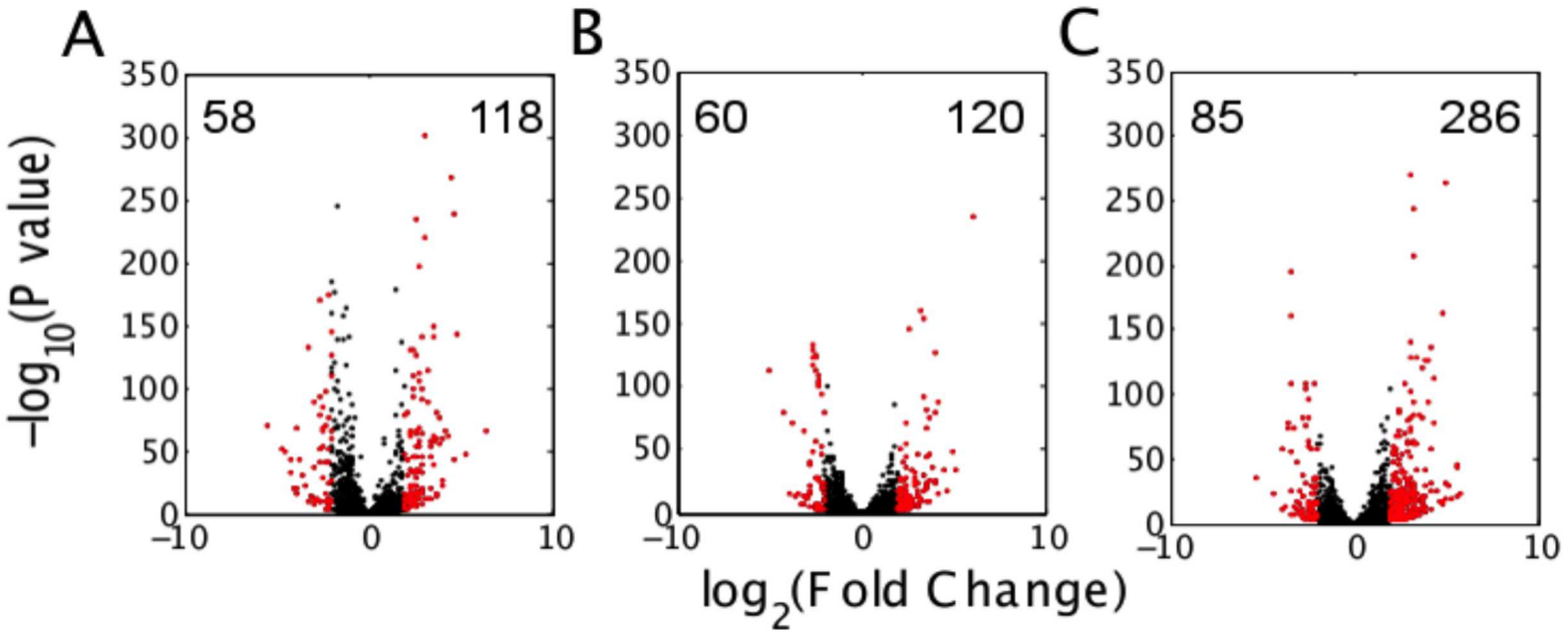
Red: Highly expressed intracellular

Green: Highly expressed extracellular and intracellular

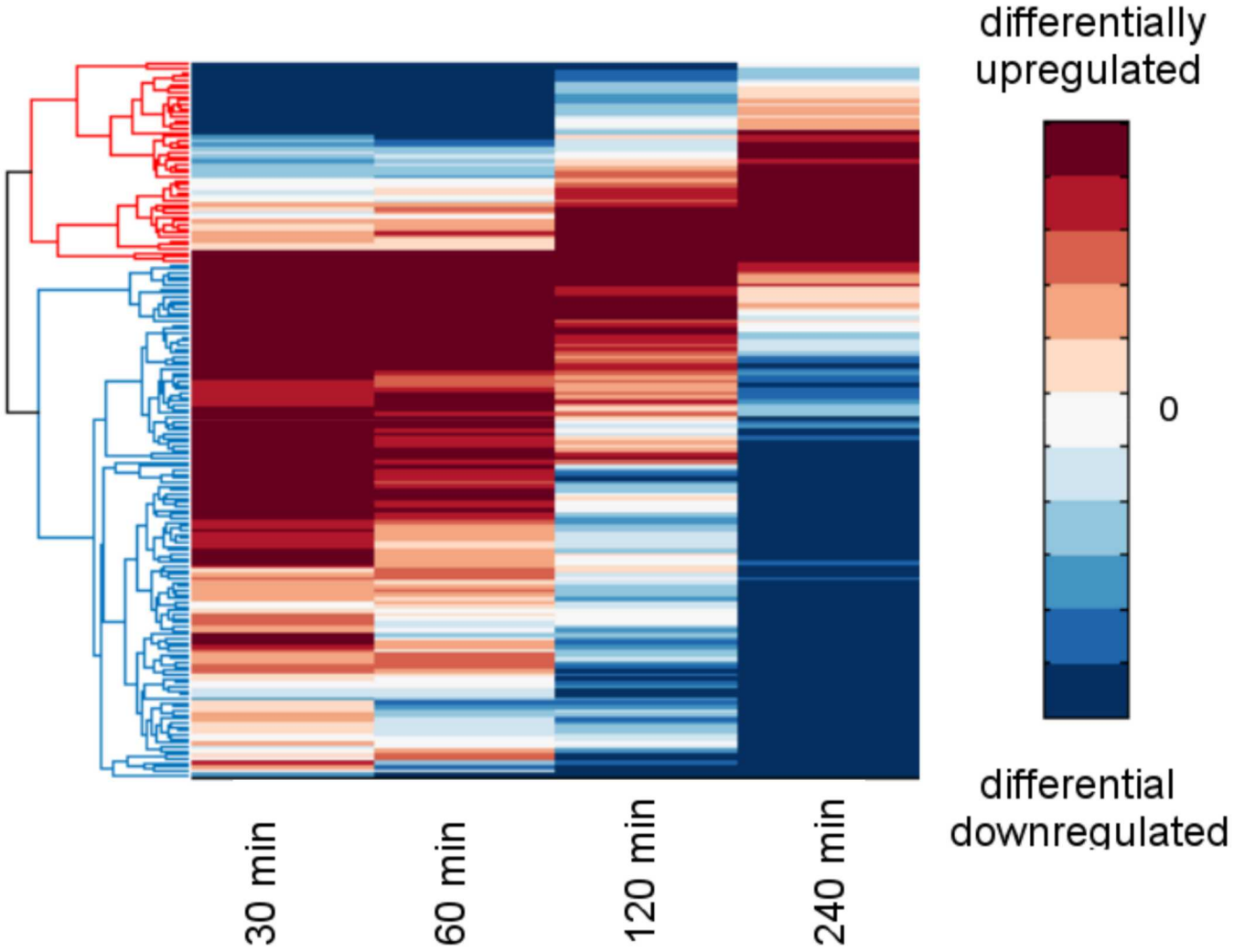


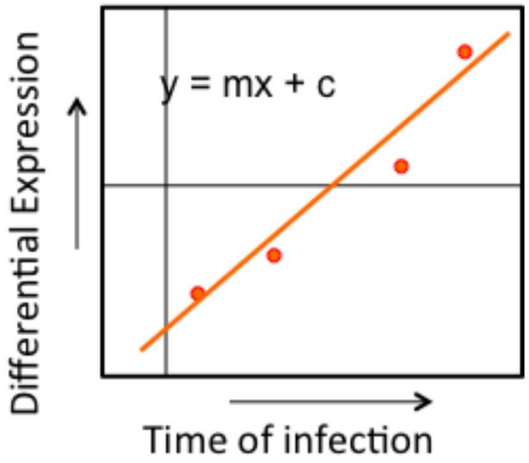
Differential Expression

- Differential expression was calculated by DESeq2 R package which has been wrapped into YANTra Pipeline



Differential expression profile





The Ysa T3SS and Yts2 T2SS are Expressed Intracellularly

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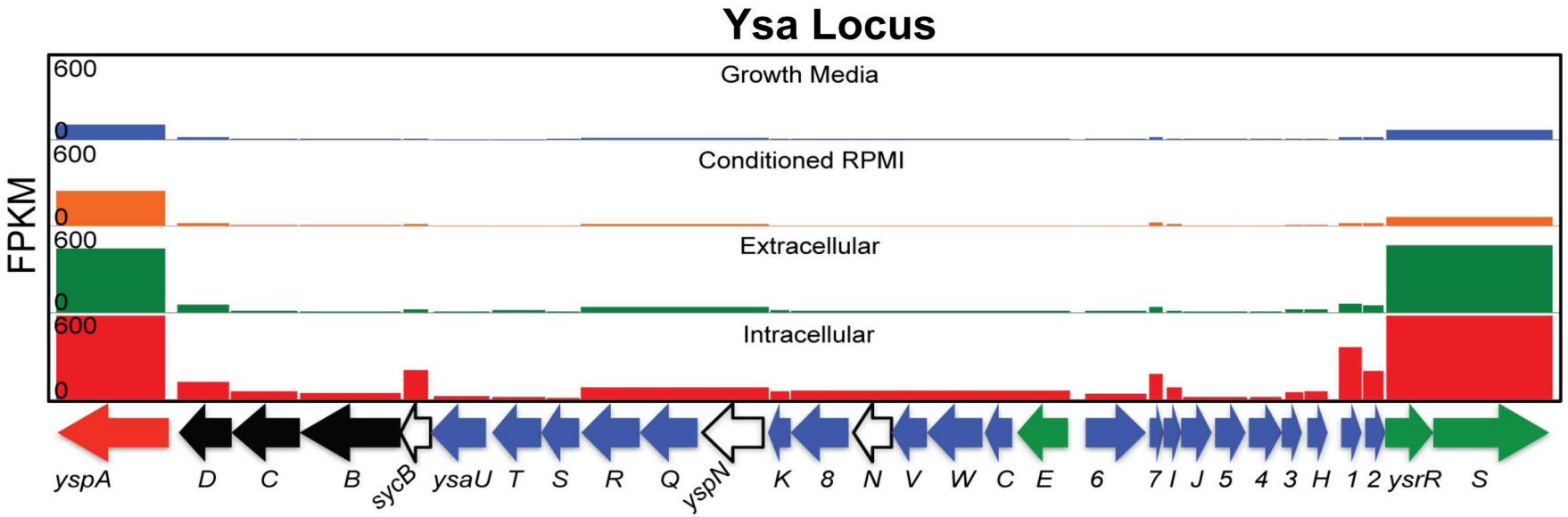

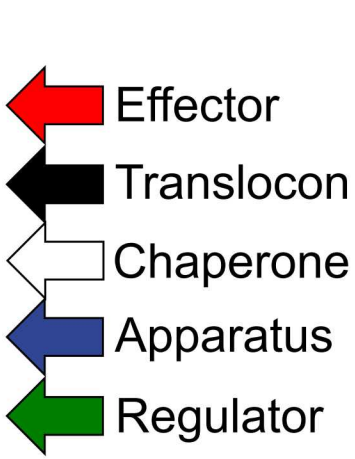

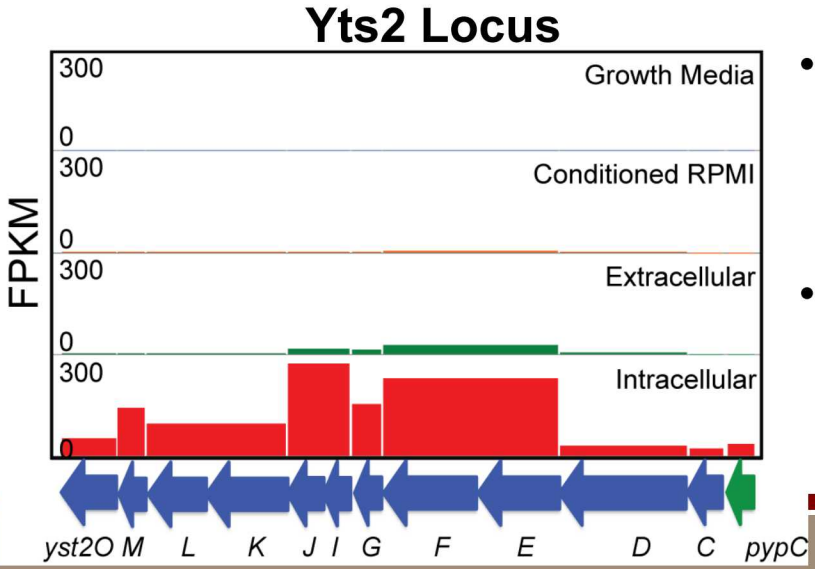

- First evidence Ysa T3SS is expressed intracellularly in mammalian cells
- First example of native expression of the Yts2 T2SS

A New Model of *Y. enterocolitica* Intracellular Infection

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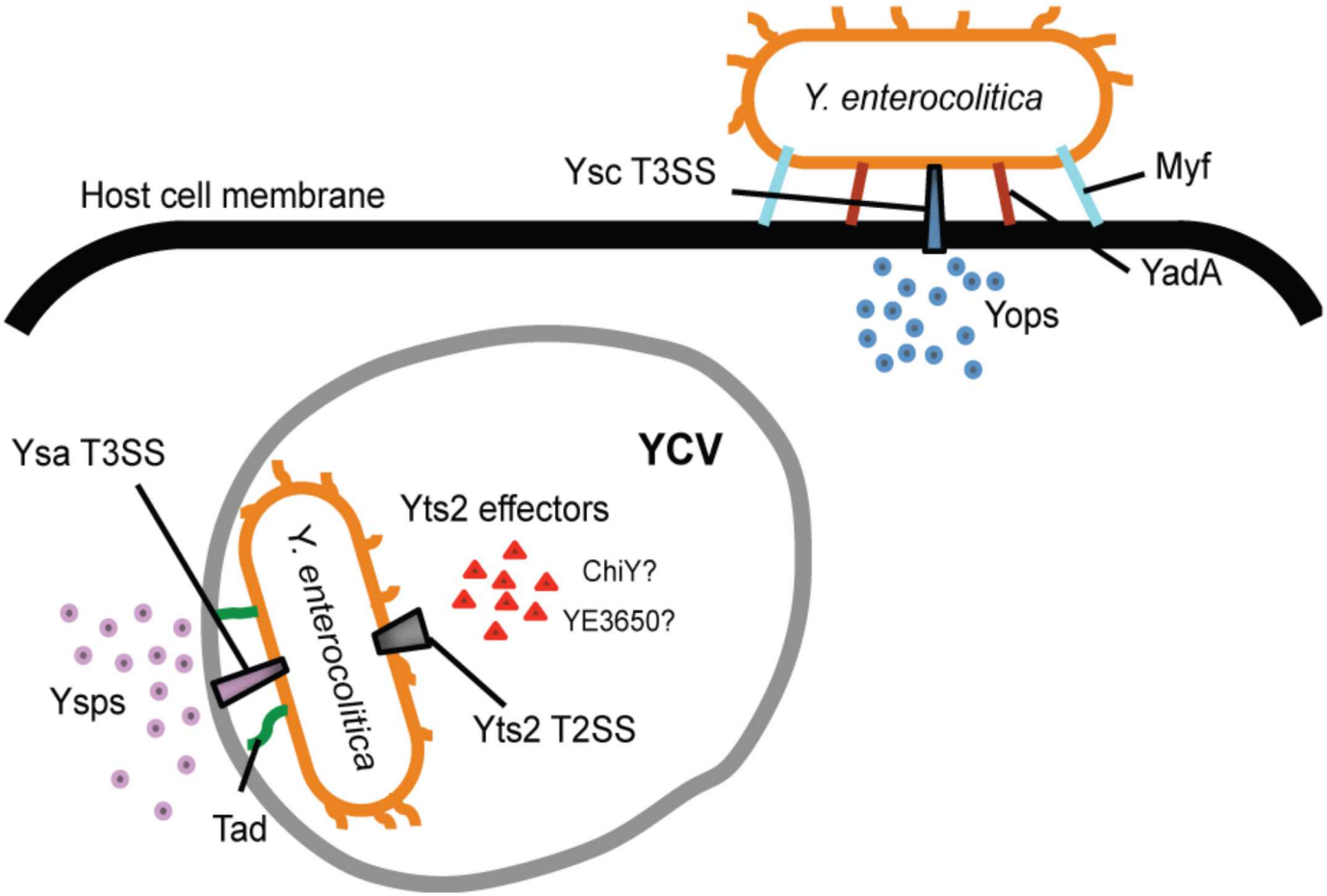


Y. enterocolitica Summary

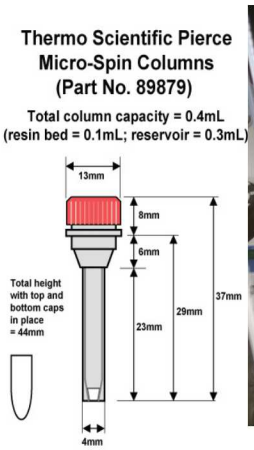
- Despite extracellular classification, *Y. enterocolitica* is viable within macrophages
- Infection is a dynamic process, even *in vitro*
- Differential gene expression analysis reveals systems that are important during different stages of infection
- Ysa T3SS, Yts2 T2SS, and Tad pilus are highly expressed after internalization and mutation in this pathway alter its survival.

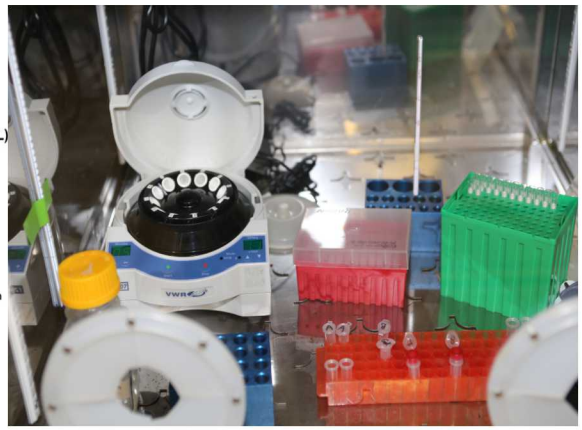
- Deeper analysis of Yersinia transcriptomics is under way with gene network analysis and TraPP
  - TraPP is a Trancription Precision Pipeline which was developed to understand fidelity in transcription process. (Poorey K et.al Genome Res 2010)

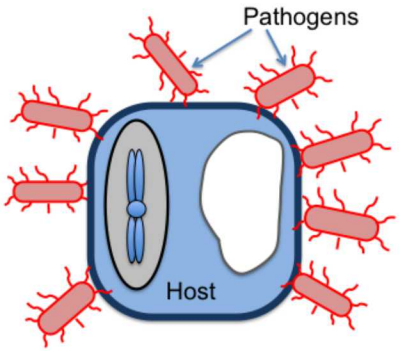
Dynamics of infection in Antibiotic resistant *Klebsiella pneumoniae*

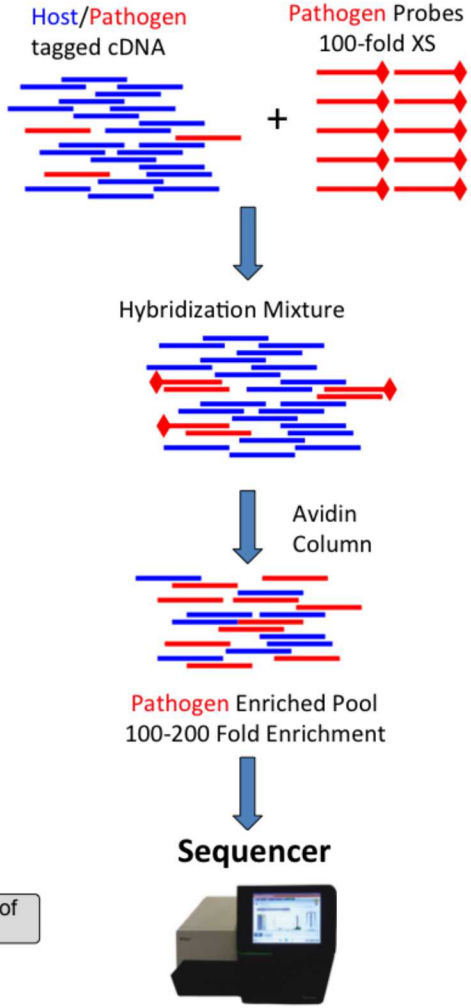
Sandia National Laboratories

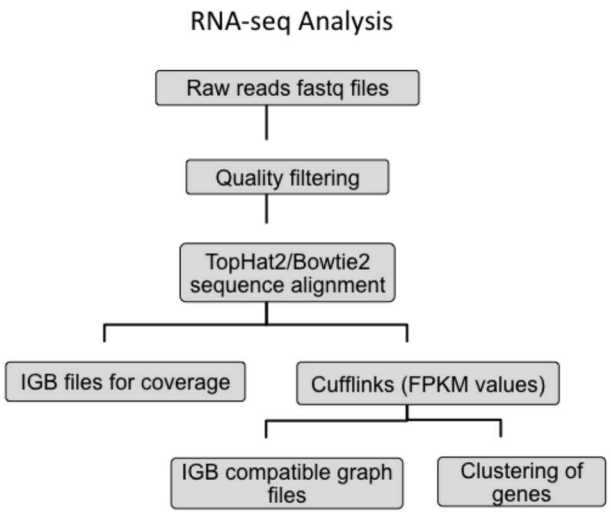
- New version of user friendly Capture method is developed used to study the host pathogen interaction dynamics of antibiotic resistant *Klebsiella pneumoniae* over a time course of infection











Version 2 – Spin Columns

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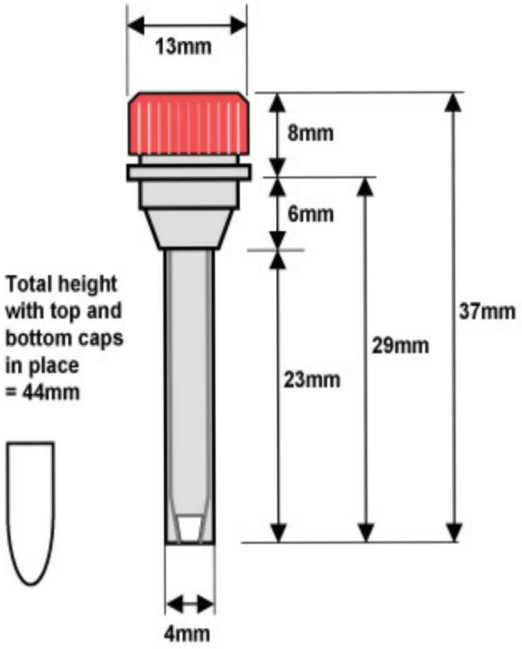
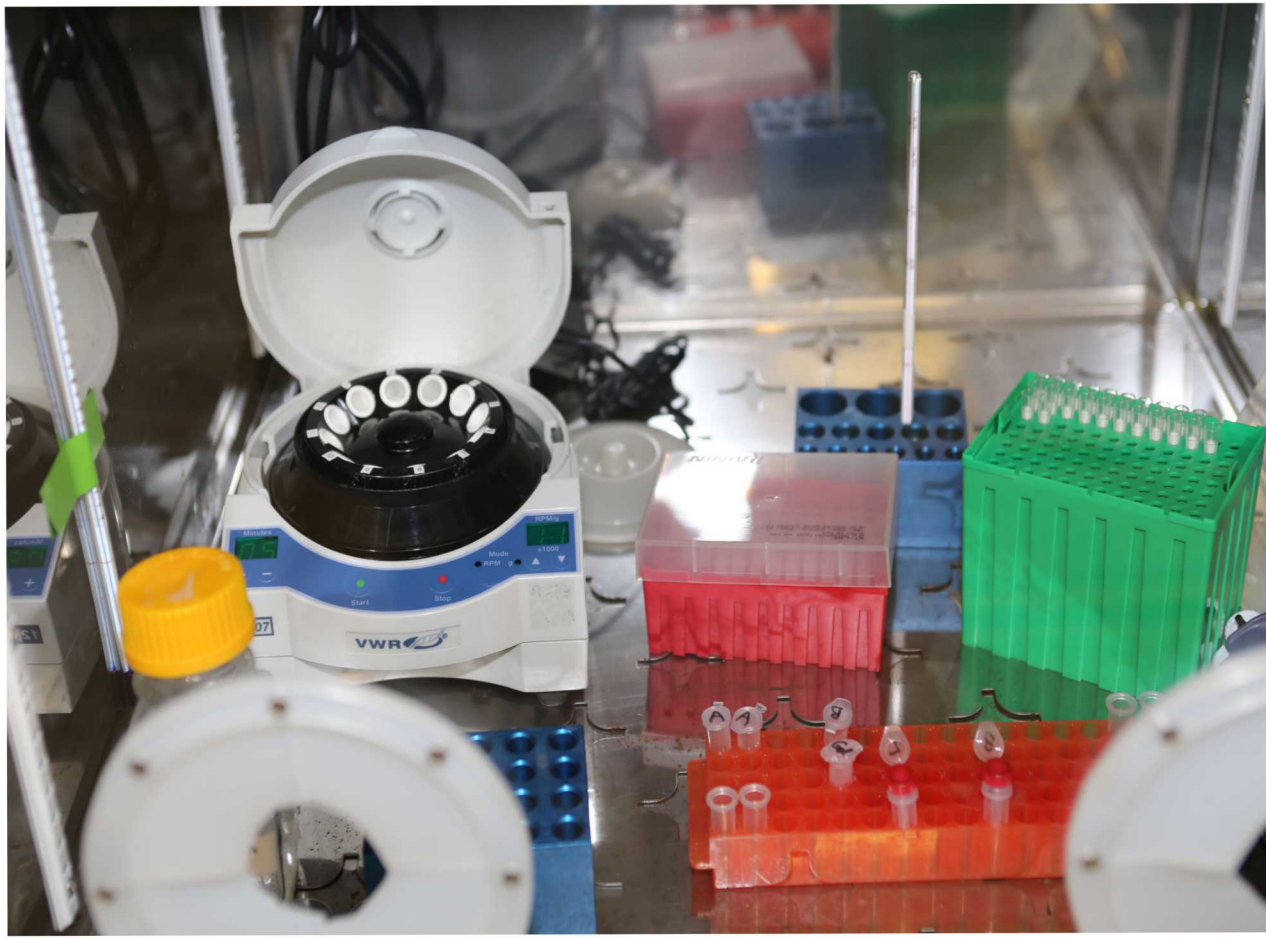
Thermo Scientific Pierce Micro-Spin Columns (Part No. 89879)

Total column capacity = 0.4mL (resin bed = 0.1mL; reservoir = 0.3mL)





Capture increases the coverage of Transcriptomics

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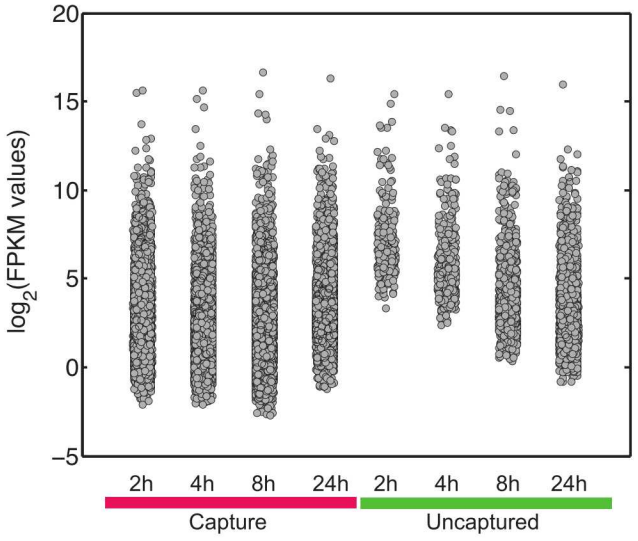
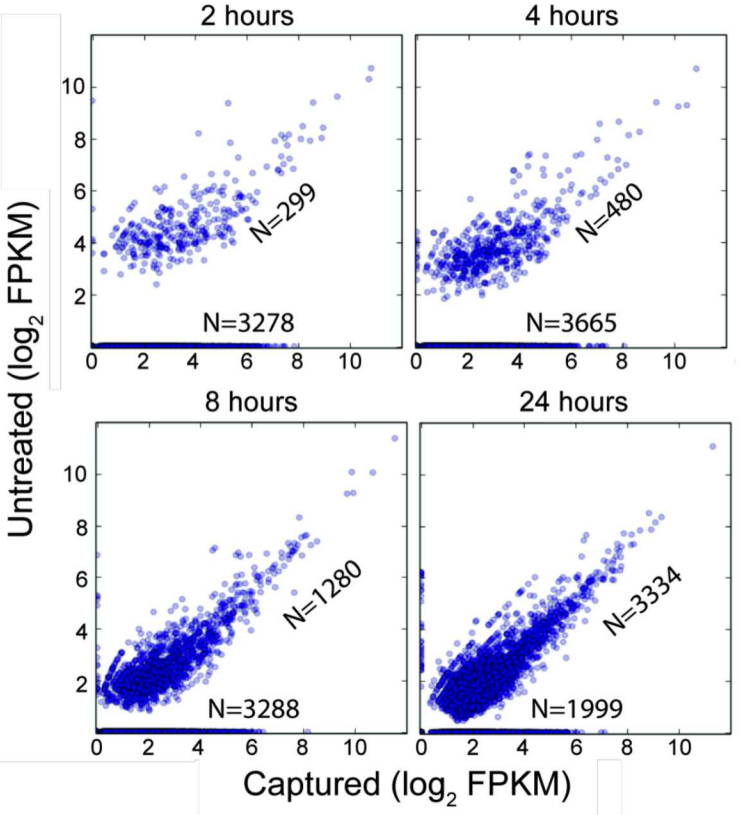
- The overall coverage for RNA-seq is vastly improved with Capture.
  2 - 11X increase of coverage
- Capture helps in studying low expressed genes





Expression in Interesting categories

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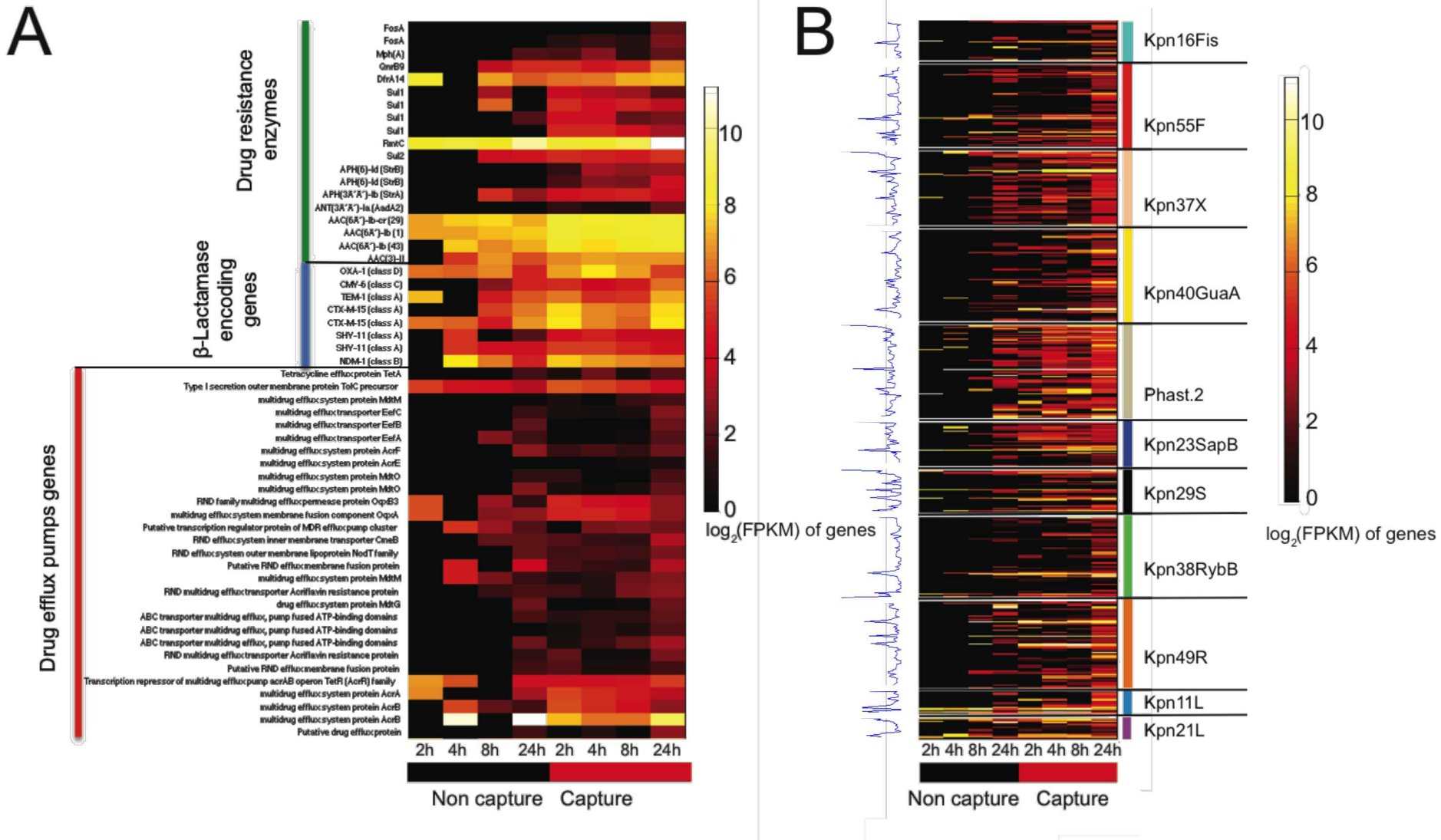
Antibiotic resistance apparatus

Genomic Islands



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Annette LaBauve Steve Branda Anupama Sinha
Deanna Curtis & Cathy Branda

Questions?

Funding

Sandia National Laboratories