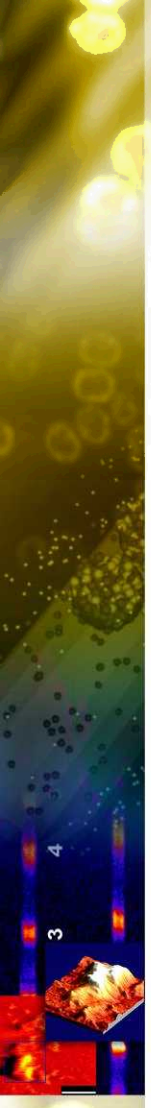
SAND2014-1481C

A Capture-Based Technique for Enhancing RNA-Seq Analyses of Bacterial Transcriptomes During *In Vitro* and *In Vivo* Infections

Zachary W. Bent
Systems Biology
Sandia National Labs

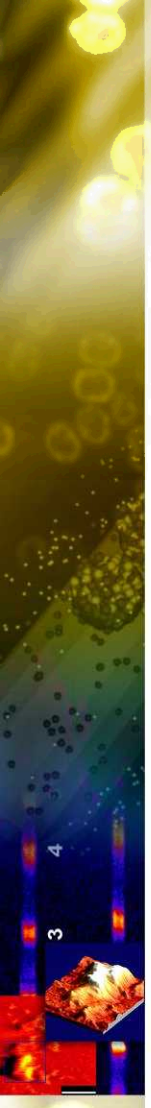


BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories

RNA-Seq

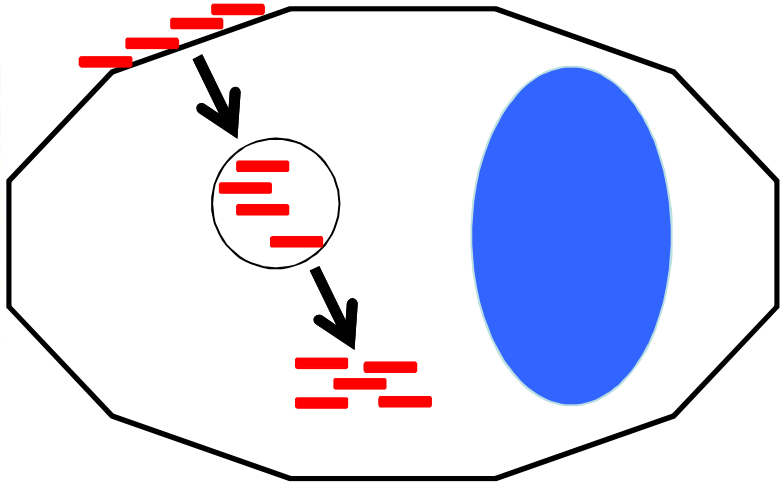
- Sequencing of all RNA transcripts in a sample
  - Coding and non-coding
- Create snap shots of gene expression
  - transcriptomics
- Find regulatory RNAs
  - lncRNA
  - miRNA
- Analyze splice variants
- Large scale SNP analysis



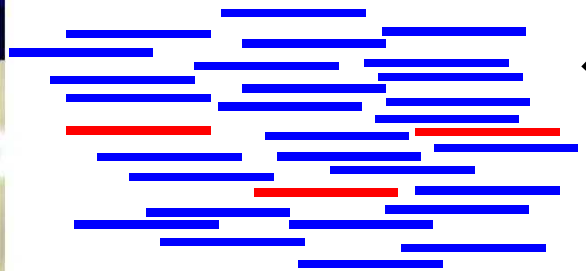
BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories

Host-Pathogen Interactions using RNA-Seq



Total RNA Extraction



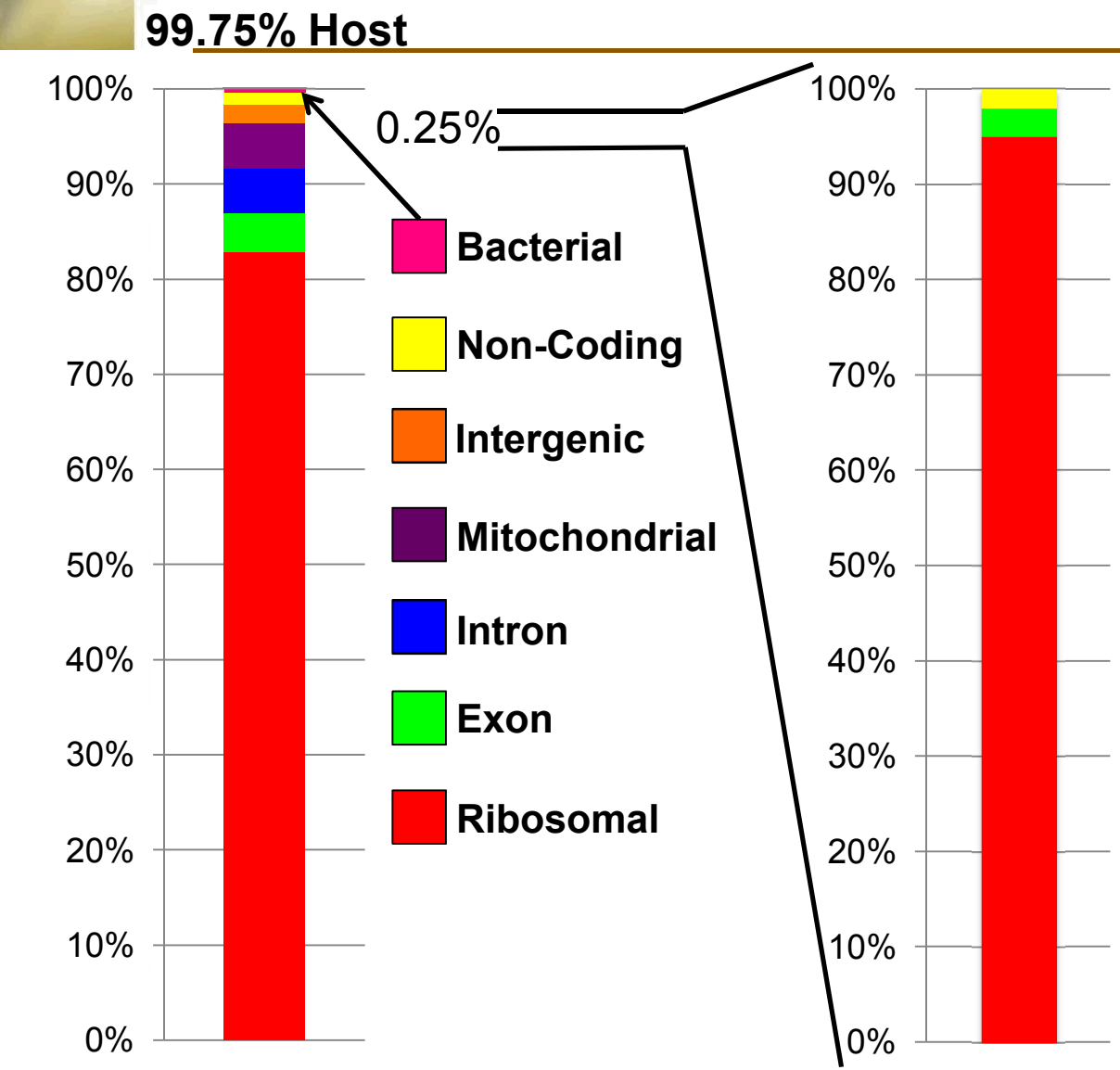
Mix of Host and Pathogen RNA

Prepare RNA-Seq Libraries

HiSeq 2500

Host-Pathogen Transcriptomics

RNA transcripts of MΦ infected at an MOI of 10

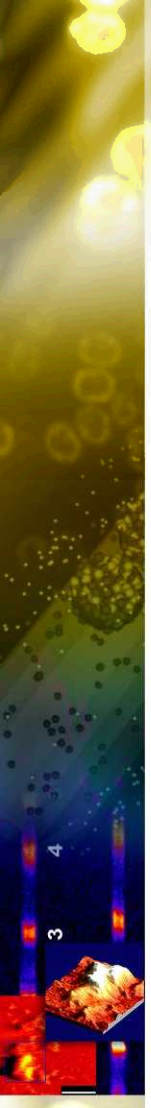


Modified from Langevin et al RNA Biol 2013 10(4)

- 0.25% of transcripts are bacterial
- 3% of bacterial reads are CDS
- 0.0075% of total reads are bacterial CDS
- 12000 reads per HiSeq lane
- ~83 HiSeq lanes = 1,000,000 reads
- ~$125,000 per sample

Non-specific methods to enrich bacterial transcripts

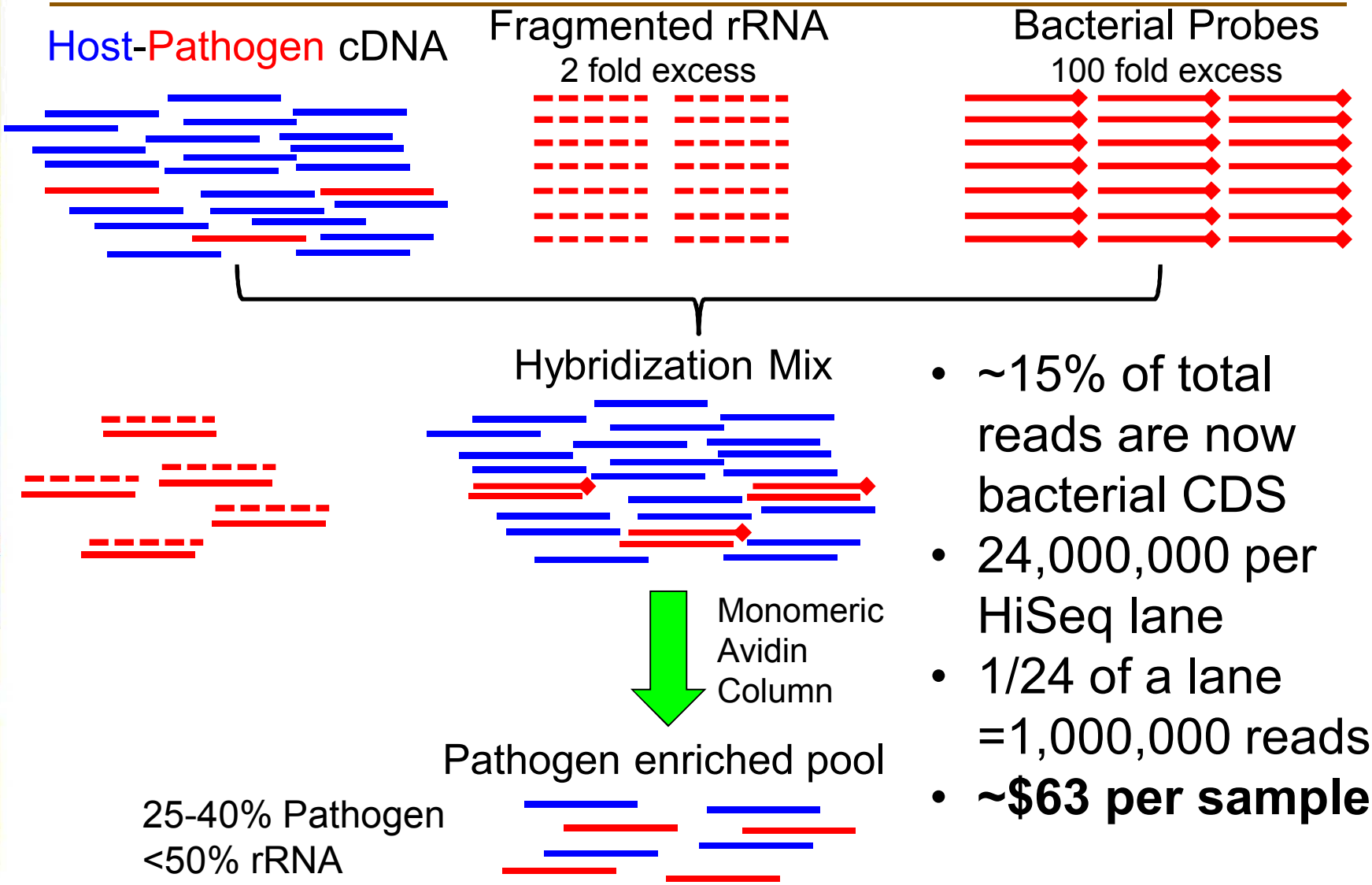
- Pre-filtering of bacteria
  - 5μM filter
  - Density centrifugation
- Ribo-Zero Epidemiology (Epicentre)
  - Removes host and bacterial rRNA
- DSN or HAC treatment
  - Removes most abundant transcripts
    - Typically host rRNA



BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories

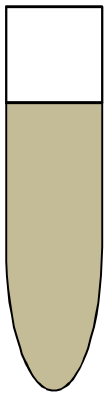
Capture-based bacterial transcript enrichment and rRNA depletion

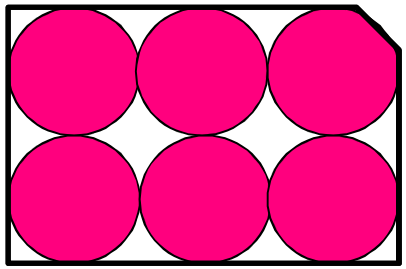


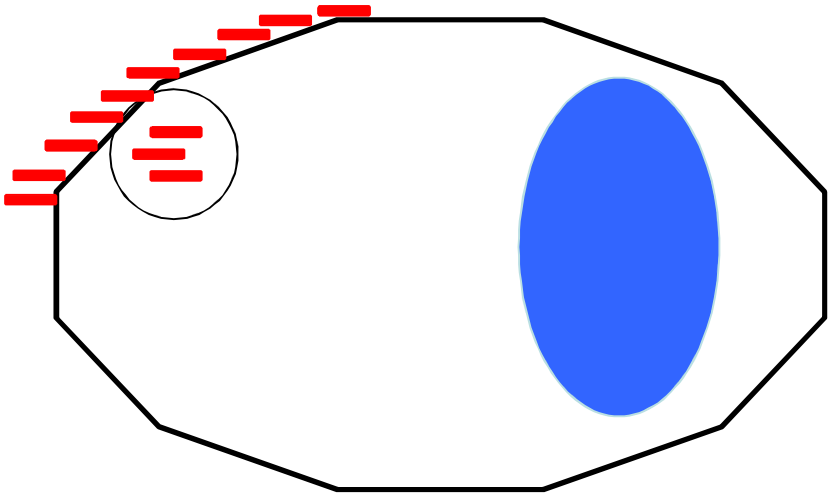
- ~15% of total reads are now bacterial CDS
- 24,000,000 per HiSeq lane
- 1/24 of a lane =1,000,000 reads
- **~$63 per sample**

BIOSCIENCE RESEARCH FOUNDATION

In vitro Y. enterocolitica infections







Growth Media
(LB w/o NaCl)
26° C

Conditioned RPMI
MΦ grown for 2 days
Filter sterilized
5% CO_2
37° C
30min, 1hr, 2hr, 4hr

Infection of MΦ
MOI = 10
37° C
30min, 1hr, 2hr, 4hr

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

Transcriptome of internalized bacteria

37° C and media induction

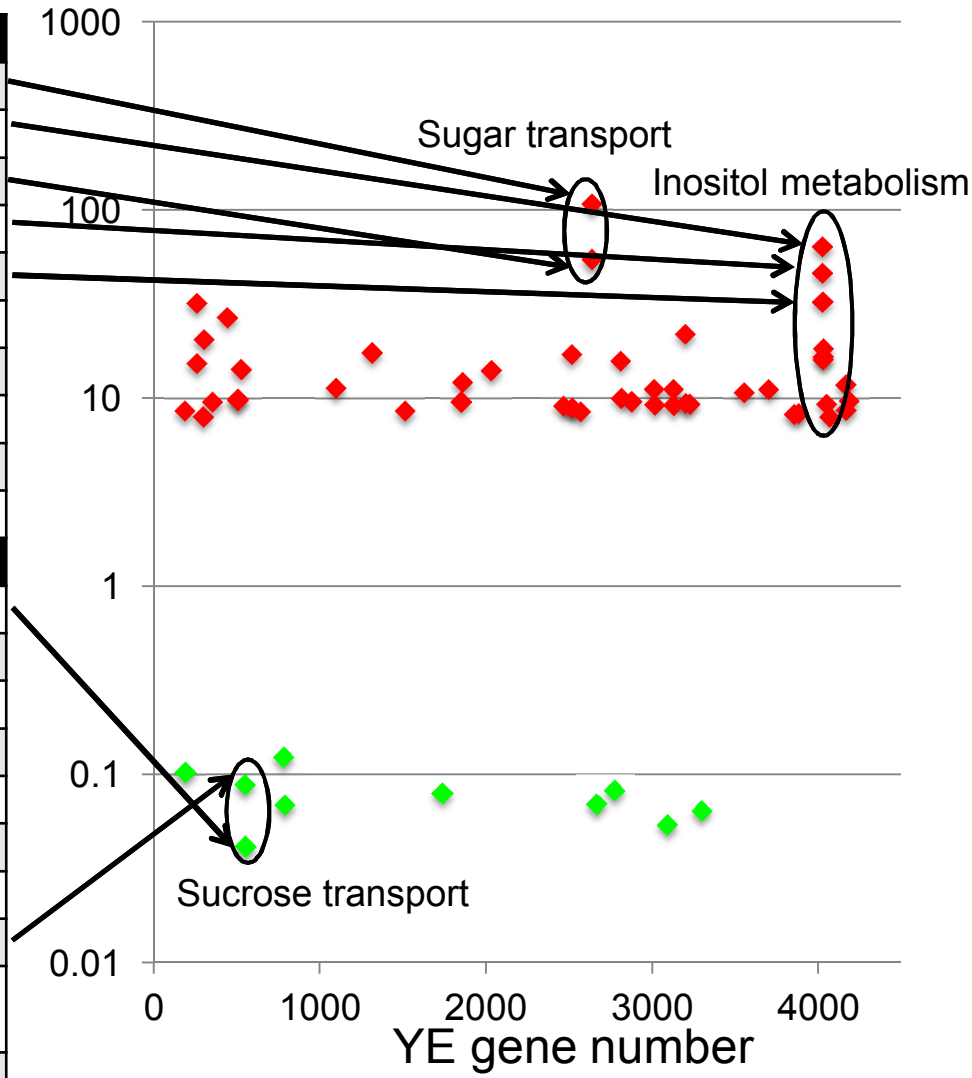
Infection-dependent genes

BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories

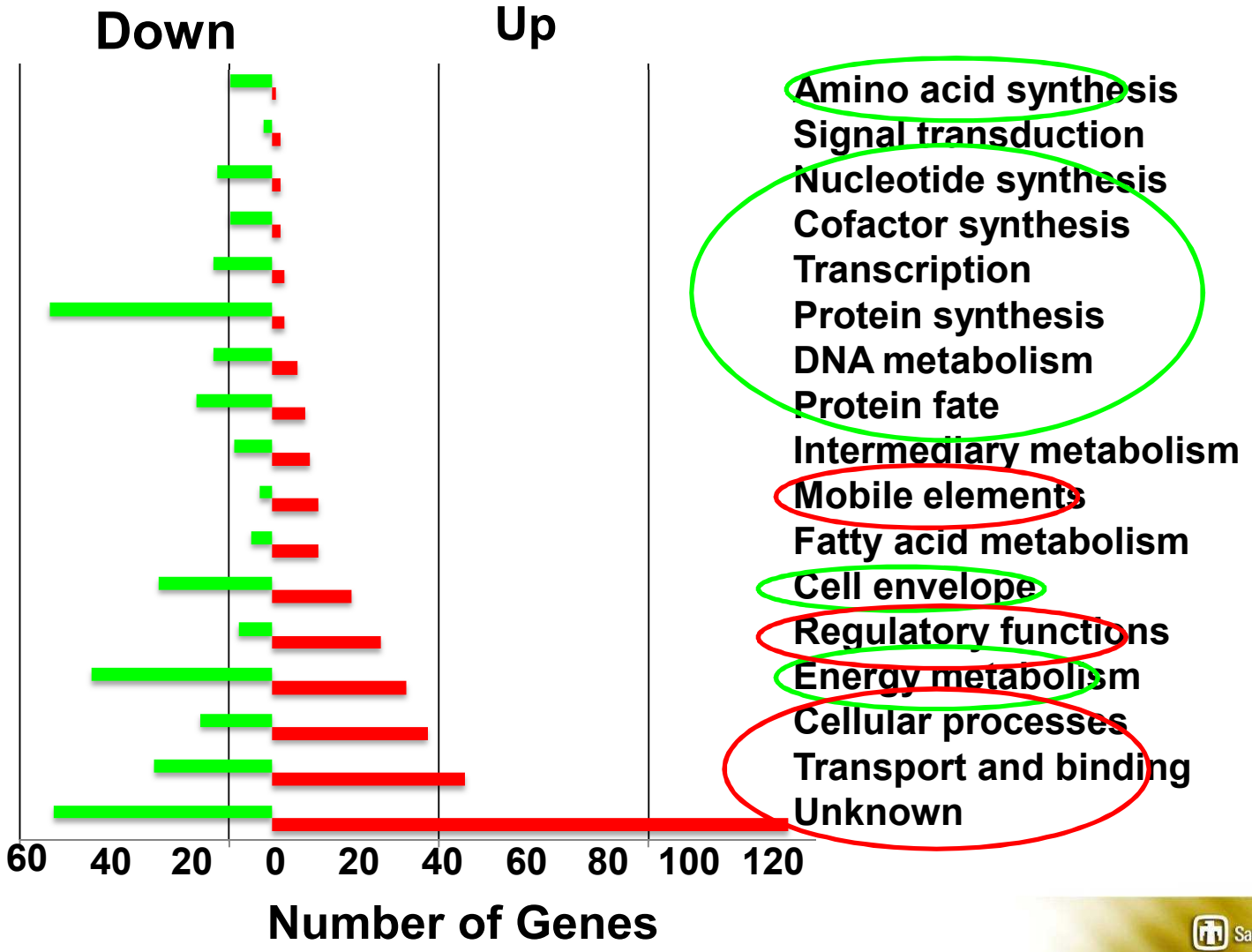
Infection-dependent differential gene expression

Gene ID	Name	Function	Fold Change
YE2639	-	Sugar Transporter	107.65
YE4027	-	Epi-inositol hydrolase	63.44
YE2638	-	Sugar Transport	54.79
YE4025	-	Inosose dehydratase	46.21
YE4026	*idh*	Myo-inositol 2-dehydrogenase	32.73
YE0267	*fadA*	Fatty acid oxidation	31.52
YE0447	-	Unknown membrane transporter	26.63
YE3198	*aglB*	6-phospho-α-glucosidase	21.75
YE0309	*acs*	acetyl-CoA synthetase	20.38
YE4031	-	aldehyde dehydrogenase	18.21
Gene ID	**Name**	**Function**	**Fold Change**
YE0553	*scrY*	sucrose porin	-24.15
YE3092	-	Unknown	-18.47
YE3297	*xni*	exonuclease	-15.63
YE0789	-	thiol:disulfide interchange	-14.42
YE2667	-	Unknown	-14.32
YE1740	-	Unknown	-12.59
YE2772	*hisI*	ATP pyrophosphatase	-12.19
YE0552	*scrA*	Sucrose transporter	-11.30
YE0195	*yigB*	Flavin mononucleotide phosphatase	-9.69

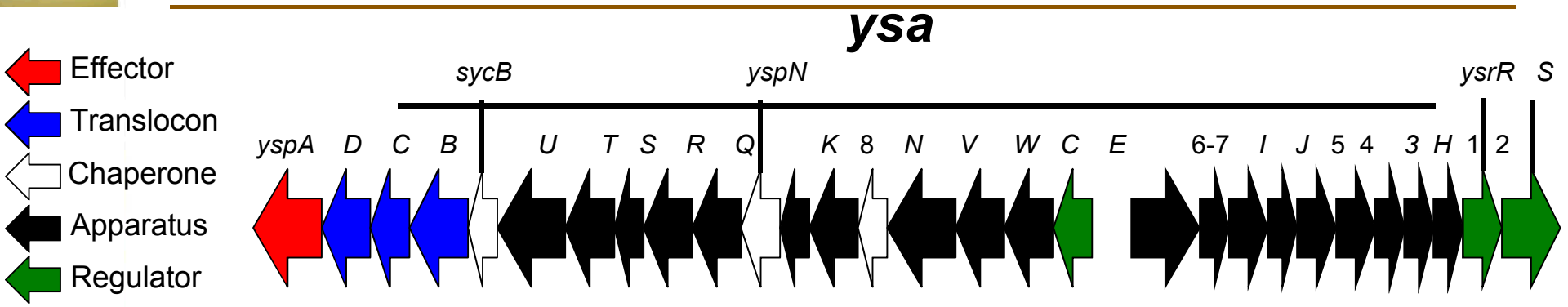


Infection-dependent differential expression by functional category

(Genes differentially expressed between RPMI and infection)



Expression of the Ysa T3SS

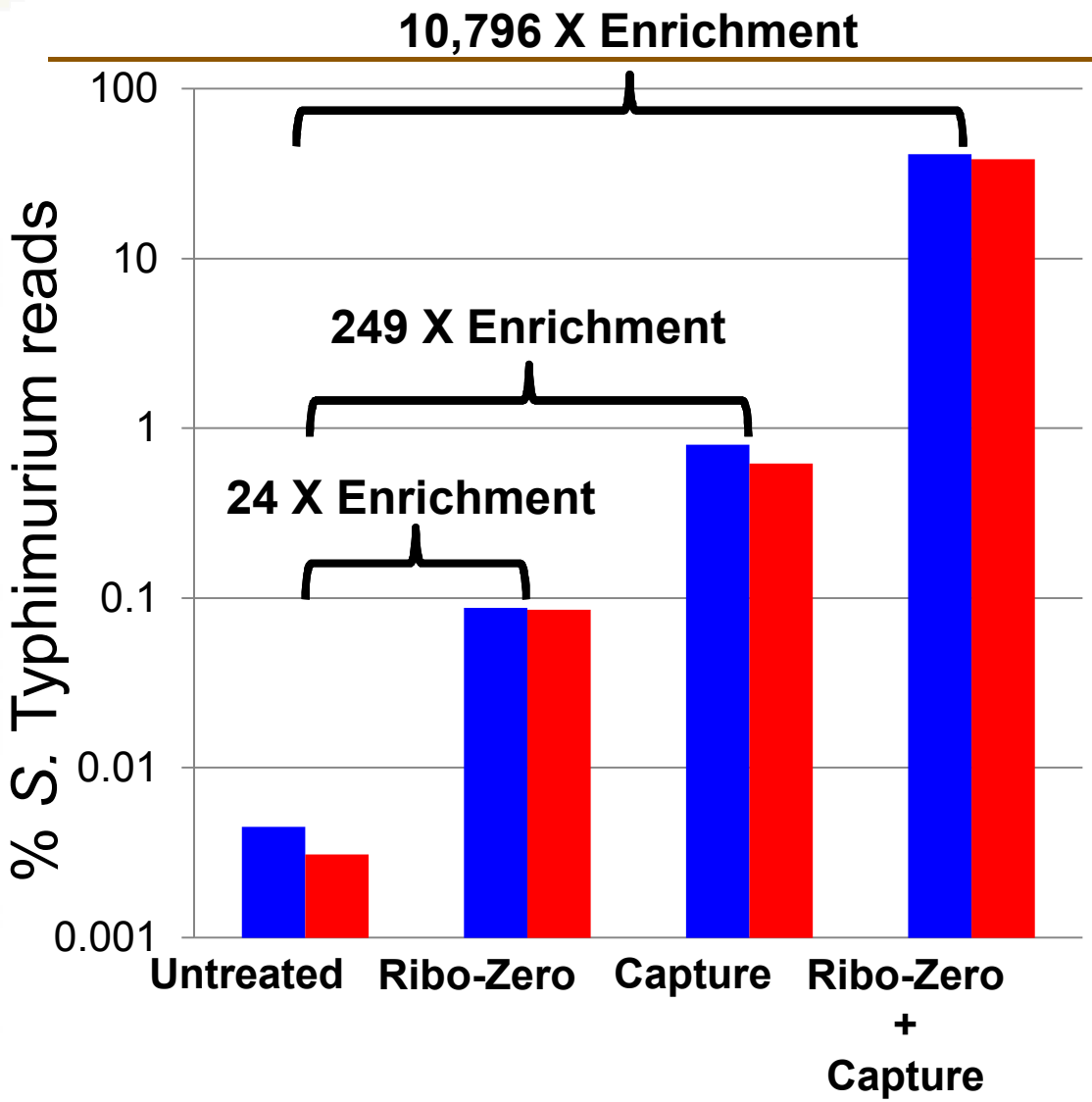


	yspA	D	C	B	scyB	U	T	V	E	6-7	H	1	2	ysrR	S
Control FPKM	5.2	13.6	3.1	1.5	0.8	1.3	1.6	0.5	1.0	2.5	1.7	14.9	15.1	19.4	6.7
RPMI FPKM	41.1	78.4	62.3	54.9	147.2	0.9	0.5	1.0	0	1.3	1.2	16.4	12.4	10.8	2.9
Infection FPKM	97.1	162.2	125.1	119.0	335.6	21.7	7.2	4.3	2.5	16.3	21.7	220.2	144.5	52.1	15.2

In vivo S. Typhimurium infections

Livers	$2.5 \times 10^2 – 5.5 \times 10^3$ CFU/sample
Spleens	$1.3 \times 10^4 – 5.3 \times 10^5$ CFU/sample

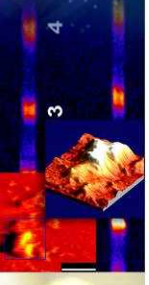
In vivo enrichment



- Synergistic effect of combining Ribo-Zero with capture ~2X
- Despite use of Ribo-Zero, significant amount of bacterial rRNA is present

Conclusions

- RNA from infected samples is dominated by host transcripts
- Capture is a highly effective and cost efficient tool to enrich bacterial transcripts from infected samples
- In combination with Ribo-Zero, capture can achieve over 10,000X enrichment
- Capture technique is highly versatile with potential applications in variety of fields



- Inositol metabolism may play an unrecognized role in Yersinia pathogenesis
- The Ysa T3SS system is expressed during the infection of macrophage cells

BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories

Acknowledgements

Sandia National Labs - CA

Robert Meagher
Stan Langevin
Kunal Poorey
Anupama Sinha
Deanna Curtis
Steve Branda
Victoria VanderNoot
Mary Tran-Gyamfi

University of Colorado

Corrie Detweiler
Eugenia Silva-Herzog
Dave Brazel
Angelika Krivenko

Sequencing

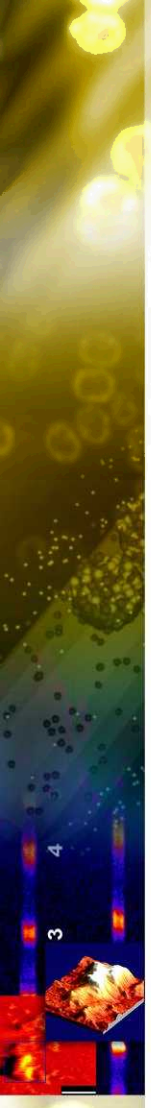
Vincent J. Coates Genomic Sequencing Laboratory (UC Berkeley)
Minyong Chung

UC Davis

Glenn Young
Karen LeGrand
Rachelle Hamblin

Funding

Sandia National Labs LDRD Office



Contact:
zbent@sandia.gov

BIOSCIENCE RESEARCH FOUNDATION

Sandia National Laboratories