Combined genotypic and phenotypic AST through gene expression profiling

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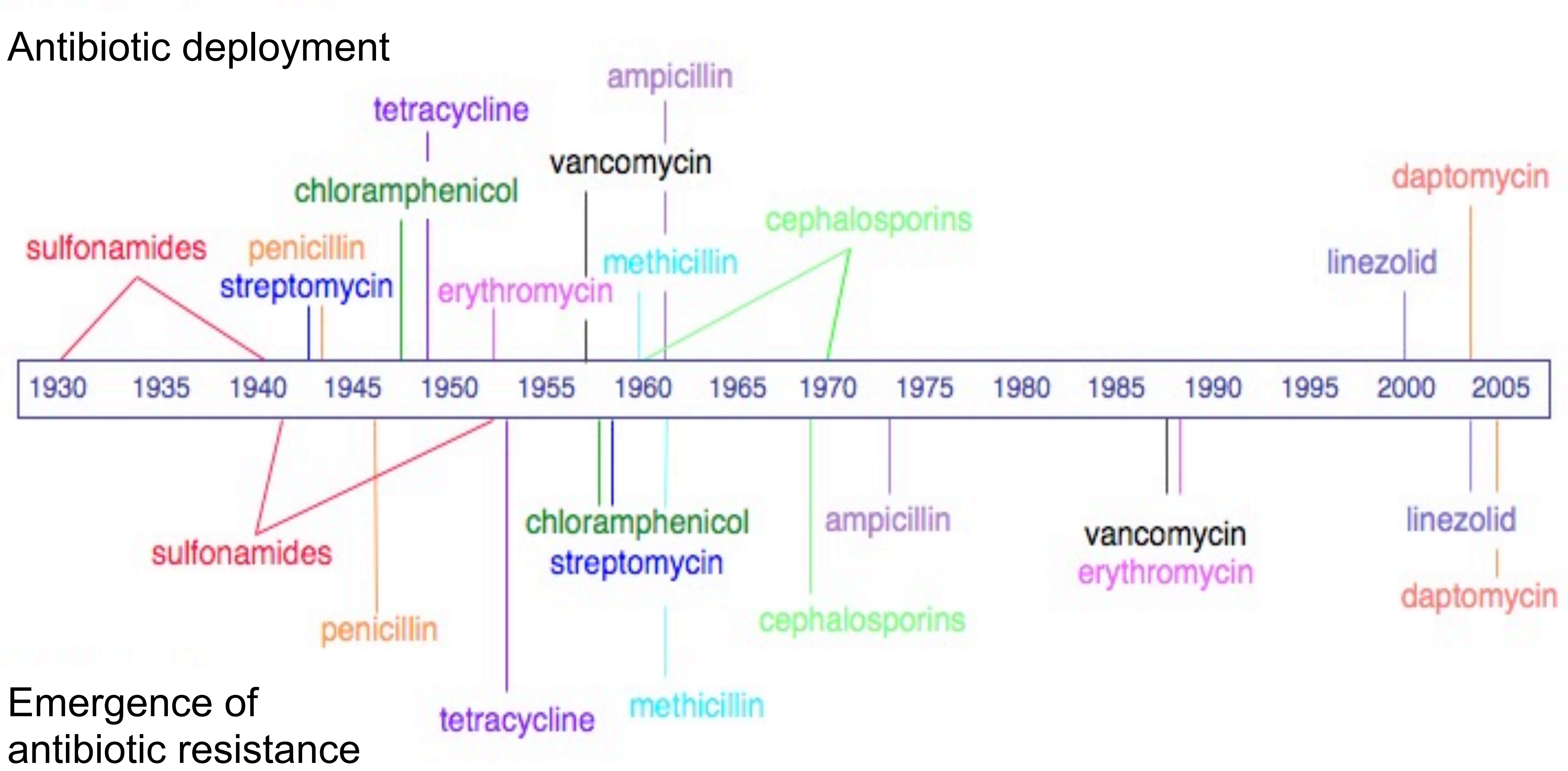
Disclosures

- None

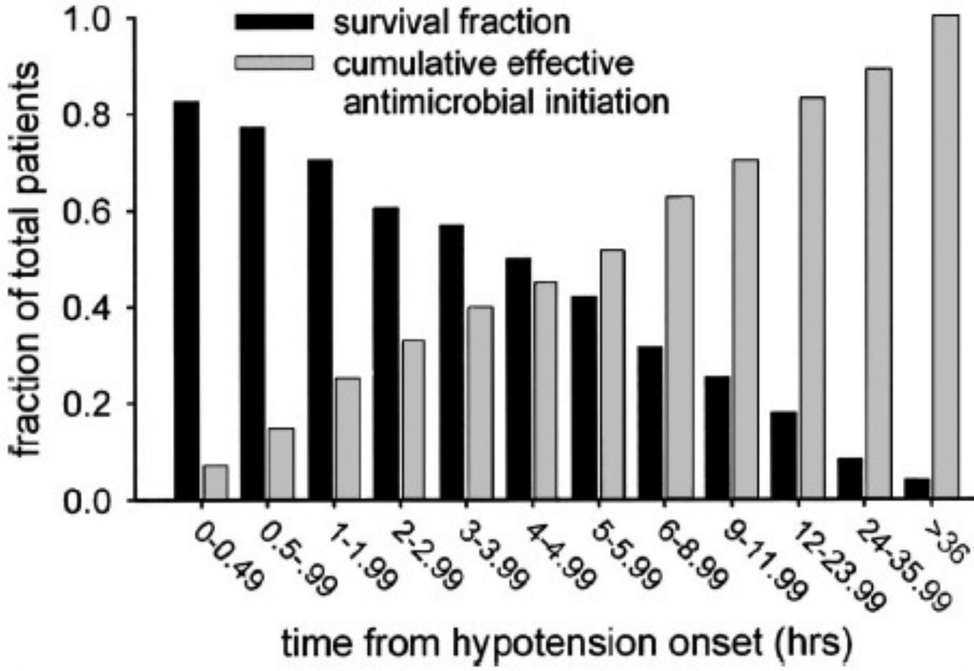
- Some of this work was supported by an R01 from NIH/NIAID, awarded as an academia-industry partnership with NanoString

Antibiotics: a scarce societal good

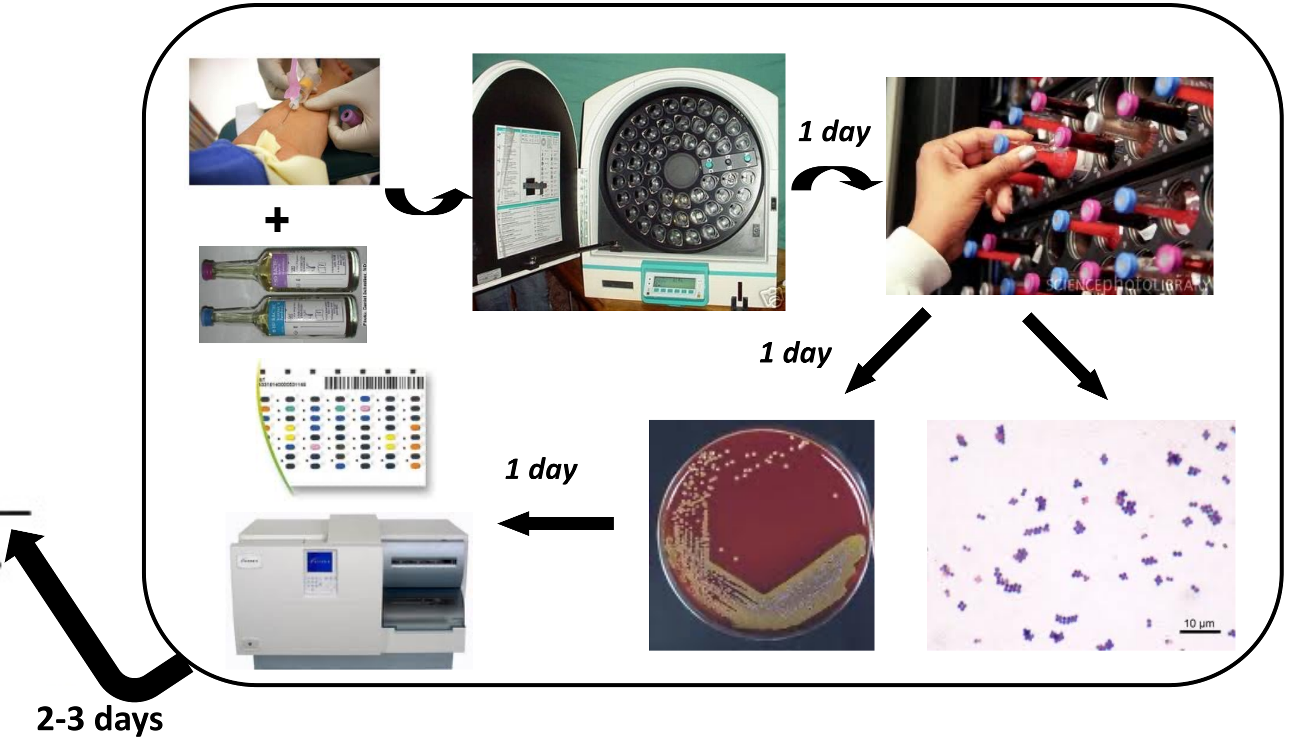


Clatworthy AE et al, *Nat Chem Biol* 2007;3:541

Current bacterial diagnostics are too slow to meet the clinical mandate



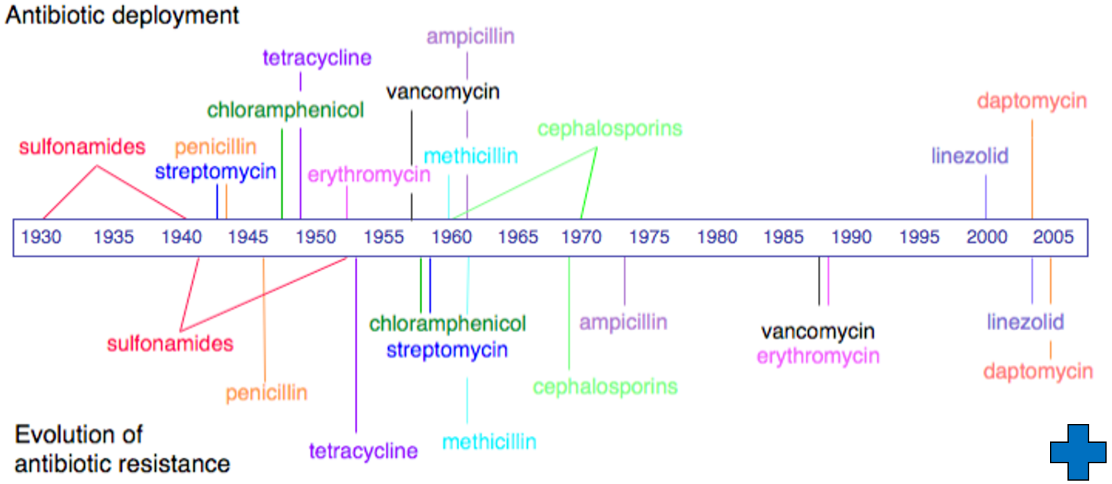
Kumar A et al, *Crit Care Med* 2006;34:1589

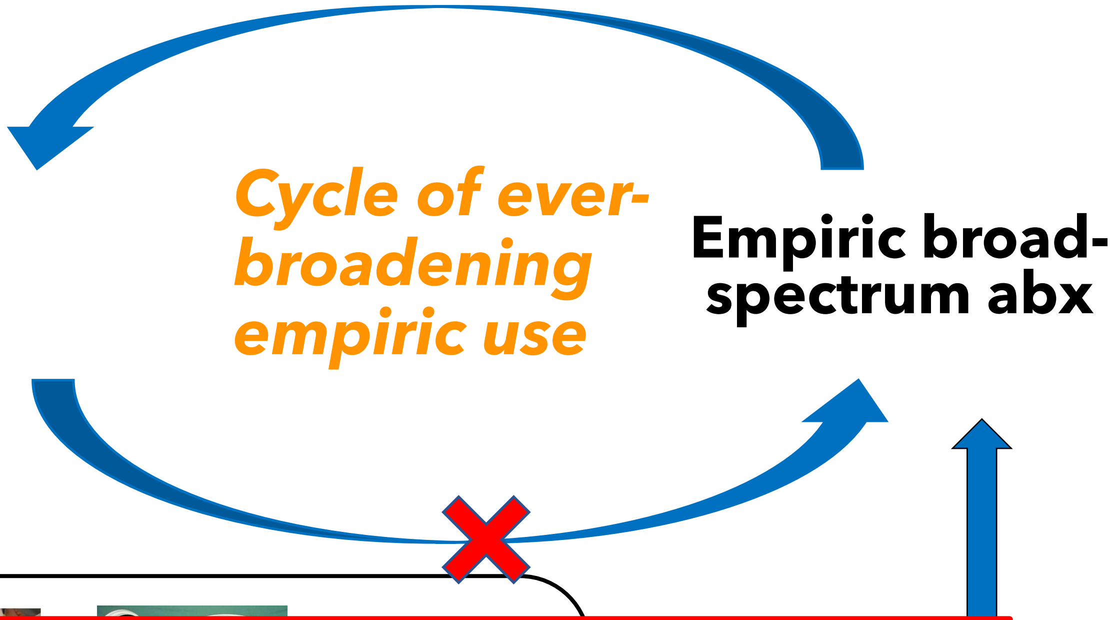


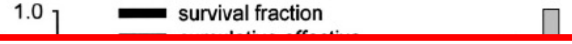
- Every hour's delay to giving appropriate antibiotics increases mortality from severe sepsis by 7%

- Best-case scenario for growth-based AST is 2-3 days from presentation

This combination leads to empiric broad-spectrum antibiotic use, feeding the cycle







- To break the cycle of escalating antibiotic use, diagnostics must be **fast** AND **accurate**
  - Ensure efficient deployment of scarce antibiotics
  - Potential to **resolve tension** between individual and society
  - Consider Oncology: from poisons to targeted therapy with molecular diagnostics

Genomics and AST

- How can we find evidence of **resistance** in genomic data?
  - Hydrolases, acetylases, efflux pumps
    - May even work better than phenotype in some cases, eg carbapenemase producers[1]
  - Target site mutations? Gene inactivations?
  - Changes in expression or copy number??

[1] Humphries RM, *J Clin Micro* 2019

Genomics and AST

- How can we find evidence of **resistance** in genomic data?

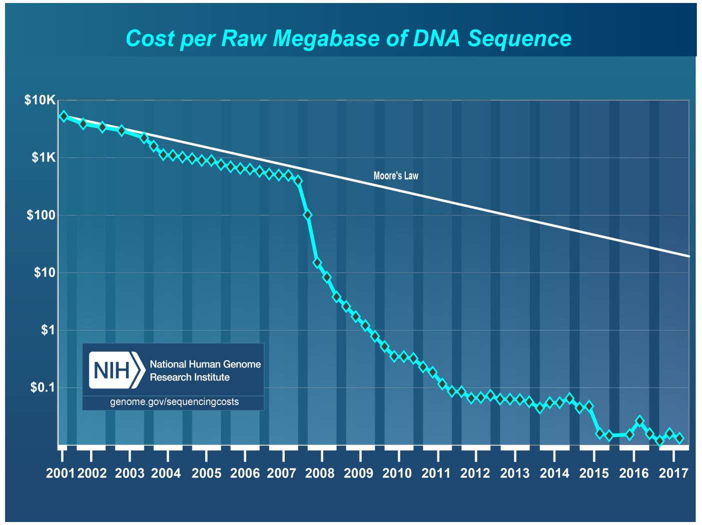
- How can we find evidence of **susceptibility** in genomic data?
  - Is this just the absence of (known) resistance determinants? Is that enough?
    - Works well in certain cases: MRSA, VRE, TB
    - But… these are corner cases
    - Consider: 13-68% of CRE[2,3], and >95% of carba-R *Pseudomonas*[3], do NOT have carbapenemases

[2] Cerqueira GC *et al*, *PNAS* 2017;114:1135
[3] Woodworth KR *et al*, *MMWR* 2018;67:396

Genomics and AST

- How can we find evidence of **resistance** in genomic data?

- How can we find evidence of **susceptibility** in genomic data?
  - Is this just the absence of (known) resistance determinants? Is that enough?
  - Can't we just sequence everything and use machine learning / AI?
    - *Correlates vs surrogates*
    - *Agnostic vs mechanistic*



Genotypic vs phenotypic approaches for antibiotic susceptibility testing (AST)

- **Phenotypic AST**
  - Long the gold standard
  - Detects **susceptibility** – "answers the key question"
    - Also risks of errors – eg carbapenemases: inoculum effect, heteroresistance
  - Speed?
  - Antibiogram as (very) low-res method to infer transmission

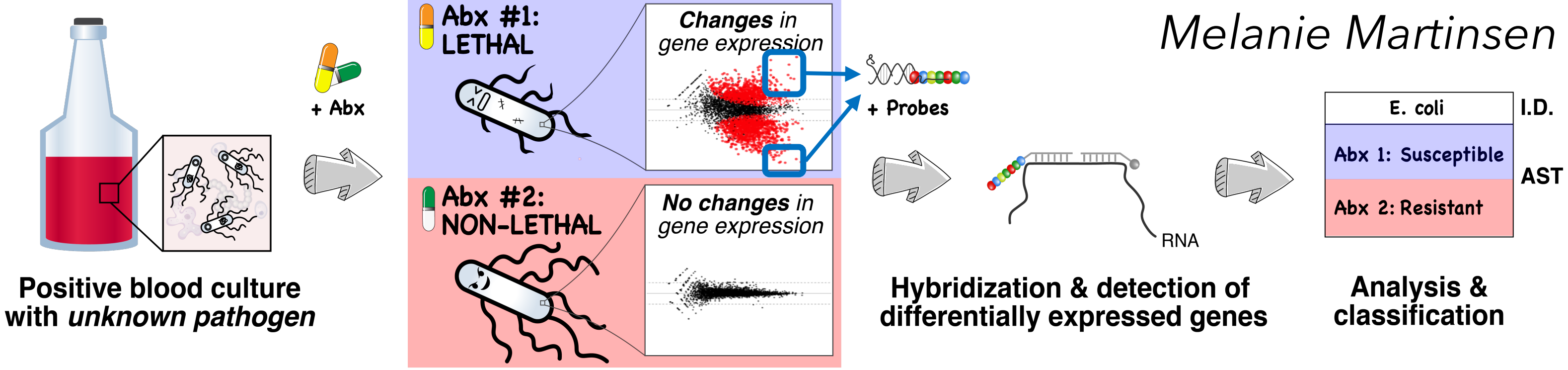
- **Genotypic AST**
  - Capitalizes on WGS revolution
  - Predicts **resistance** – "by proxy" or "by correlate"
    - Risk of errors from undertraining, unexpected diversity, new mechanisms
  - Speed? Cost? Implementation?
  - "Collateral info": potential for built-in molecular epidemiology

What if we could do both in a single assay?

A new approach to AST through RNA detection: *transcription as phenotype*

- RNA **sequence → genotype**
- RNA **abundance → phenotype**
  - Transcriptional changes are among the earliest adaptations to stress

- **Postulate: susceptible** and **resistant** strains will exhibit **differential gene expression upon antibiotic exposure**
  - Rapid (minutes)
  - Agnostic to resistance mechanism



Positive blood culture with *unknown pathogen*

Hybridization & detection of differentially expressed genes

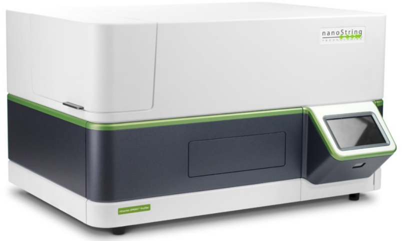
Analysis & classification

Melanie Martinsen

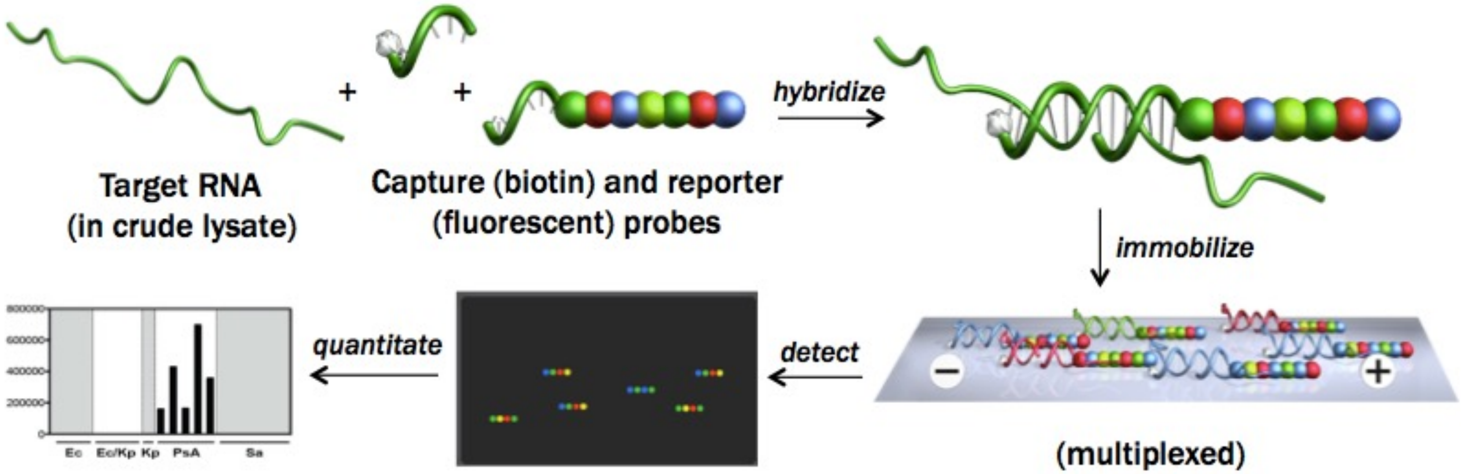
GoPhAST-R: Genotypic + Phenotypic AST through RNA detection

RNA signatures: an early, readily measurable distress signal

- RNA **sequence → genotype**
- RNA **abundance → phenotype**
    - Transcriptional changes are among the earliest adaptations to stress



- Enabling technology: **NanoString**



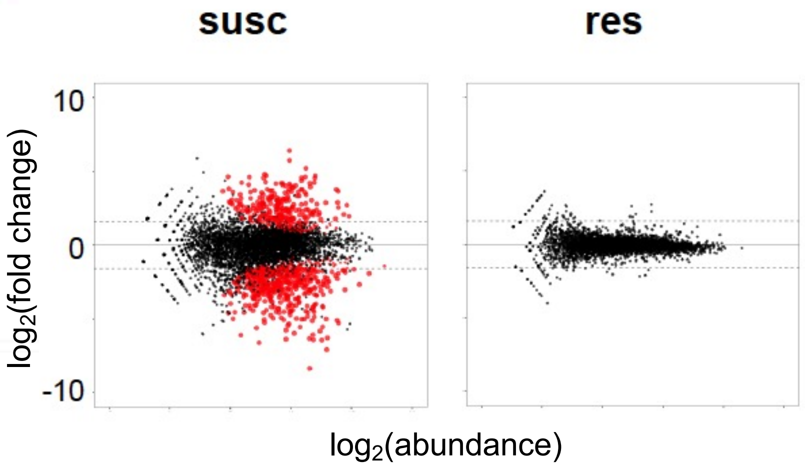
- **<u>Multiplexable</u>** (hundreds of transcripts in "one pot")
- **<u>Quantitative</u>** over 3-4 orders of magnitude
- Total assay time ~hours (hands-on time: ~minutes)
- No enzymology = direct from crude lysates
- Benchtop instrument for detection

Workflow: defining transcriptional signatures

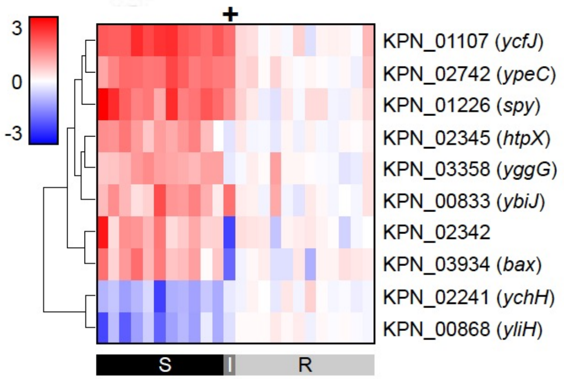
Drug exposure

clinical isolates
(susc, res)
+/- antibiotic
(at clinical breakpoint)

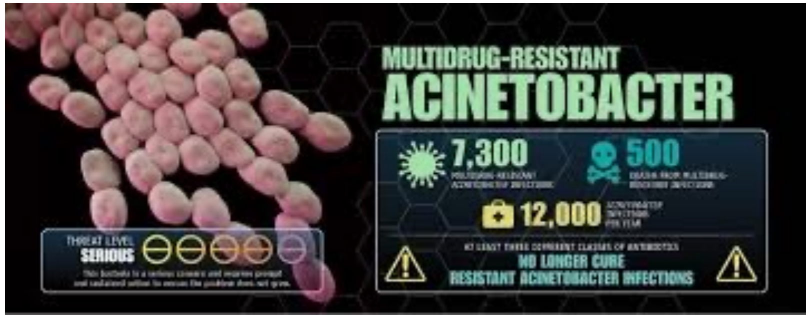
Expression analysis
(RNA-Seq)

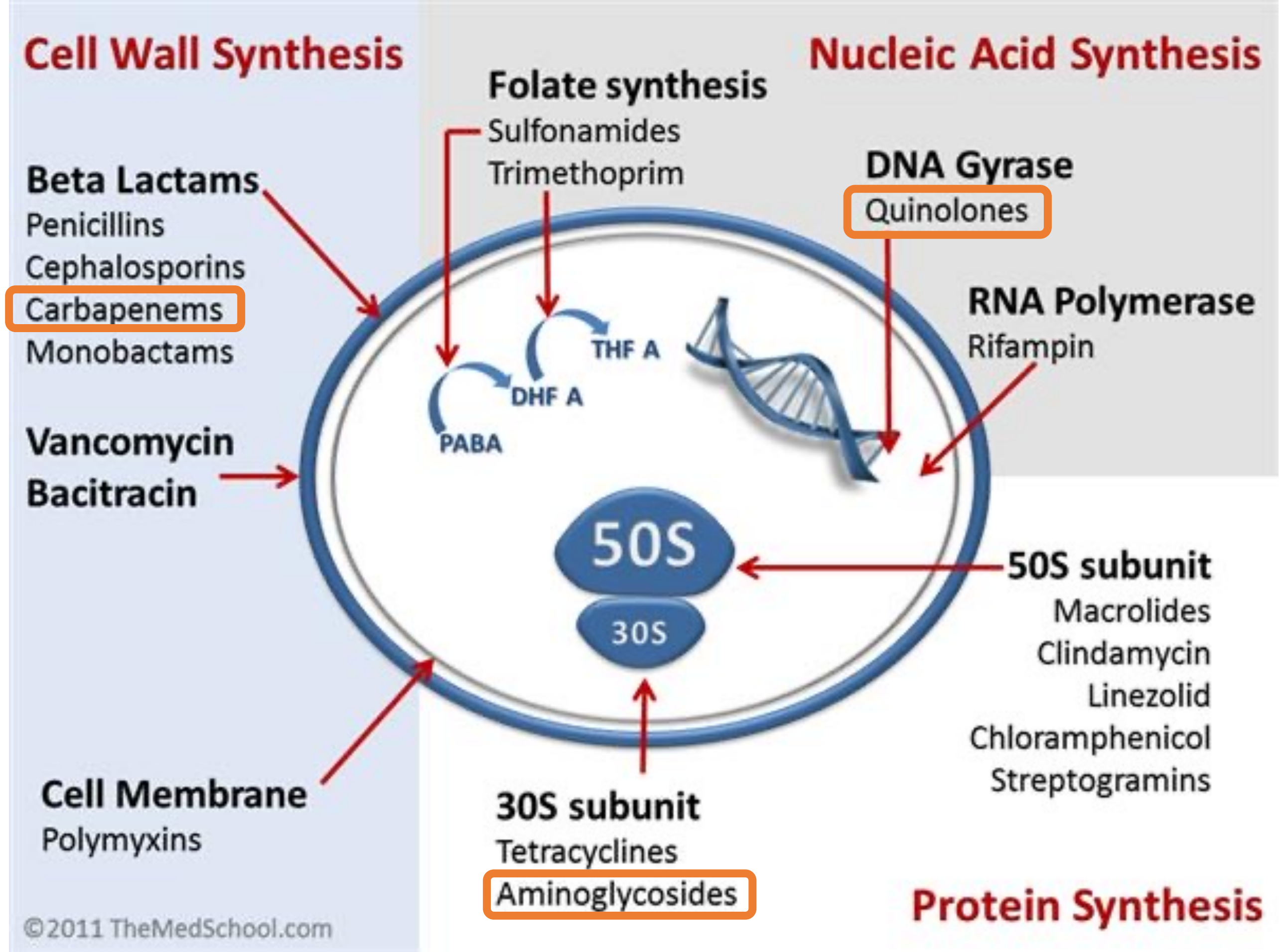


Signature validation
(multiplexed RNA detection)



Application: MDRO GNRs, multiple antibiotic classes





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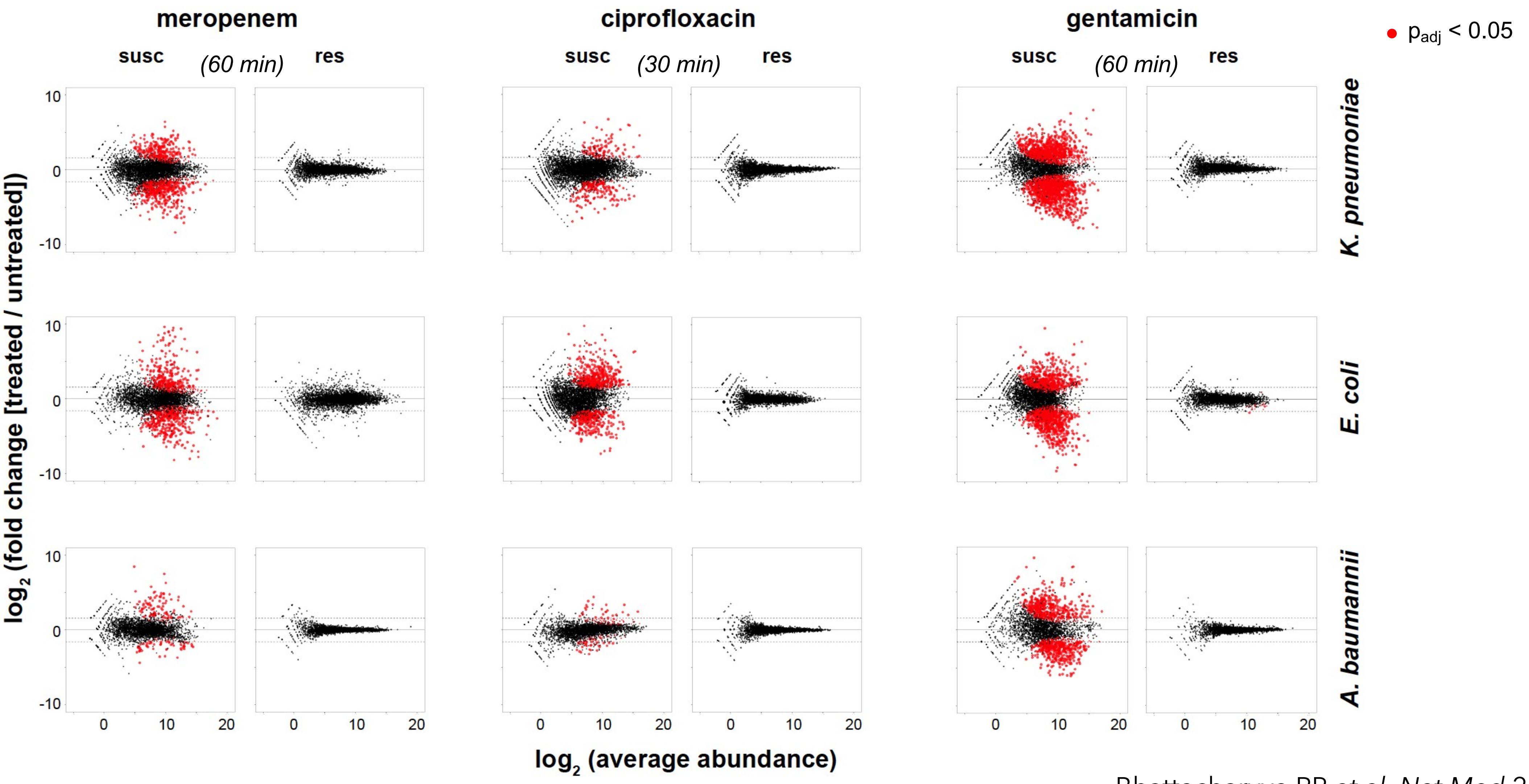
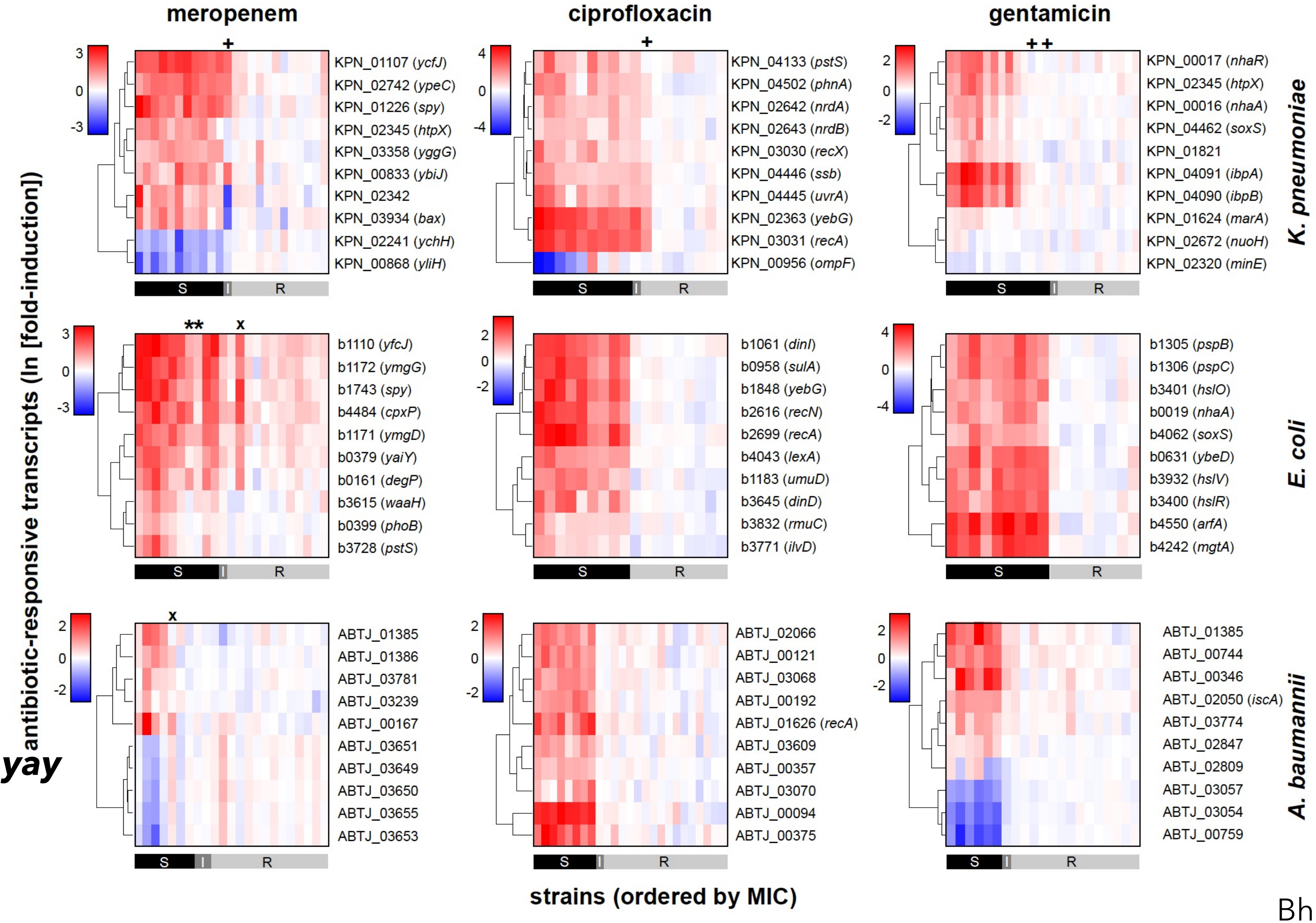
RNA-Seq: S and R strains respond differently to antibiotic exposure



J. Livny
J. Liu
D. Hung

Bhattacharyya RP *et al*, *Nat Med* 2019

GoPhAST-R: a small subset of transcripts predict AST

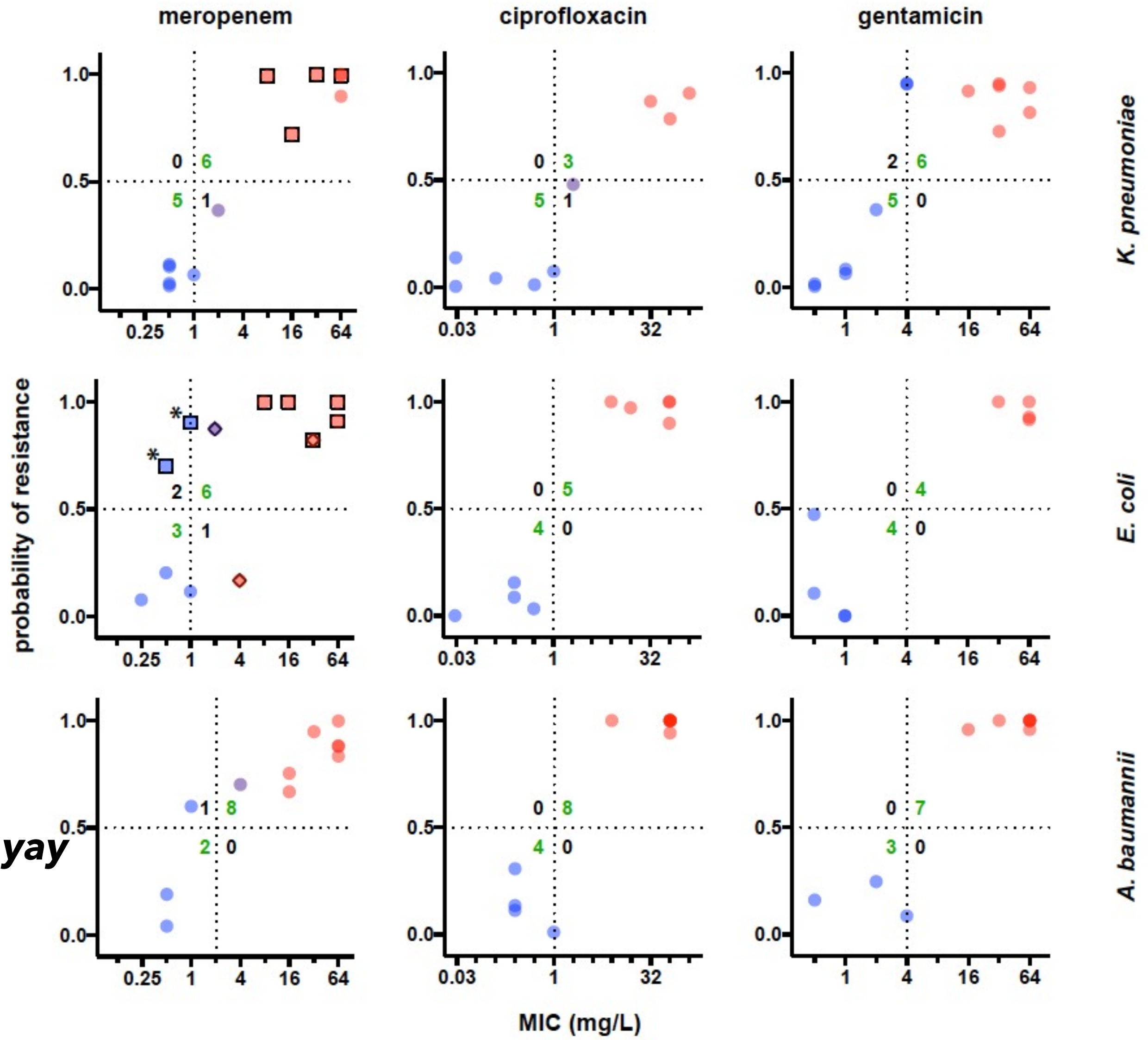


(top 10 antibiotic-responsive transcripts, measured by NanoString hybridization assay)

N. Bandyopadhyay
J. Livny
N. Shoresh
S. Son
D. Hung

Bhattacharyya RP *et al*, *Nat Med* 2019

GoPhAST-R: machine learning predicts susceptibility from NanoString data



Input: NanoString data, top 10 antibiotic-responsive transcripts

Model: random forest

Output: probability of resistance

		Reference (MIC)		
		Susc	Intd	Res
Prediction (GoPhAST-R)	Susc	97	5	1
	Non-susc (I or R)	6	23	107

Categorical agreement: 227/239 (95%)

- *3 discrepancies clearly due to errors w/ gold standard*
- *8 of remaining 9 "missed close" (variability in gold standard too)*

N. Bandyopadhyay
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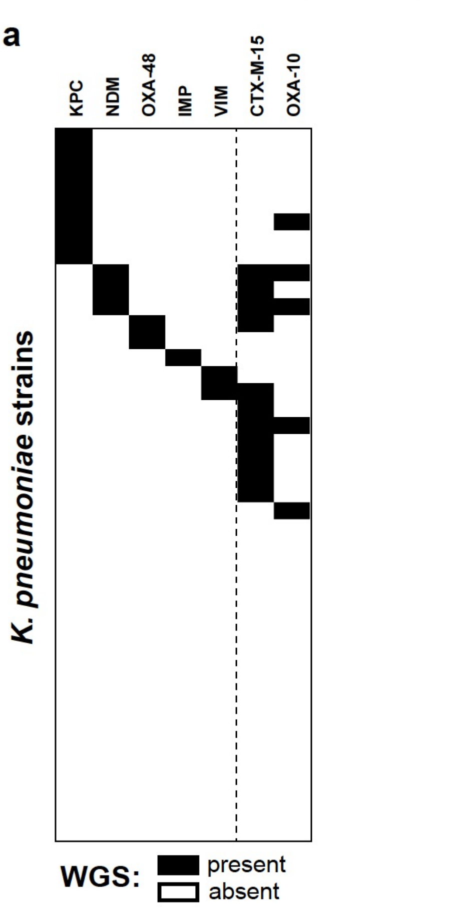
D. Hung

Bhattacharyya RP *et al*, *Nat Med* 2019

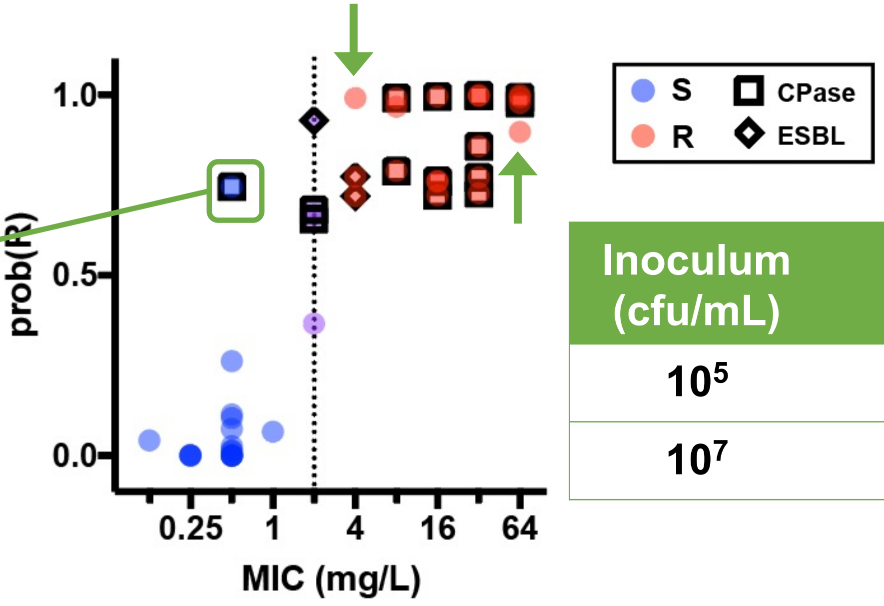
GoPhAST-R: simultaneous resistance gene detection enhances assay

- Simultaneous detection of 5 common carbapenemases:



Detected 67/67 carbapenemases

- Overlay on phenotypic AST



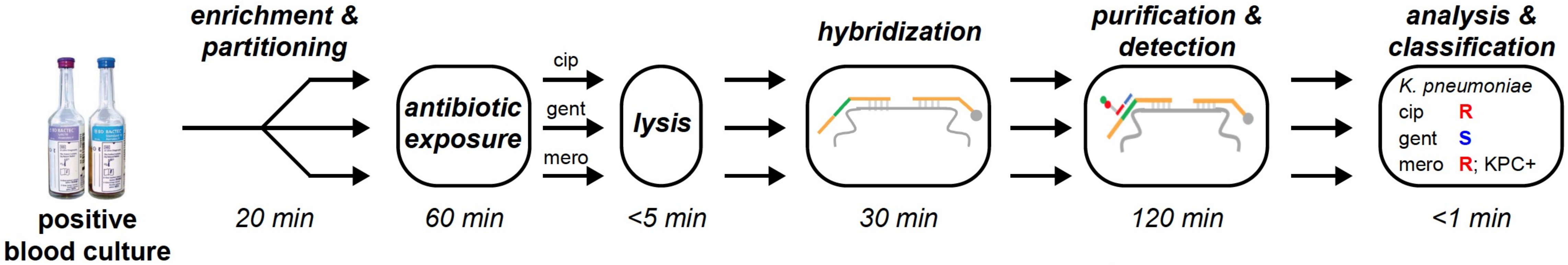
Inoculum (cfu/mL)	MIC (mg/L)
10^5	0.5
10^7	32

- Uncover "hidden" carbapenem-R
- Flag unexplained resistance
- Suggest alternative abx (avibactam; aztreonam)
- Facilitate molecular epidemiology

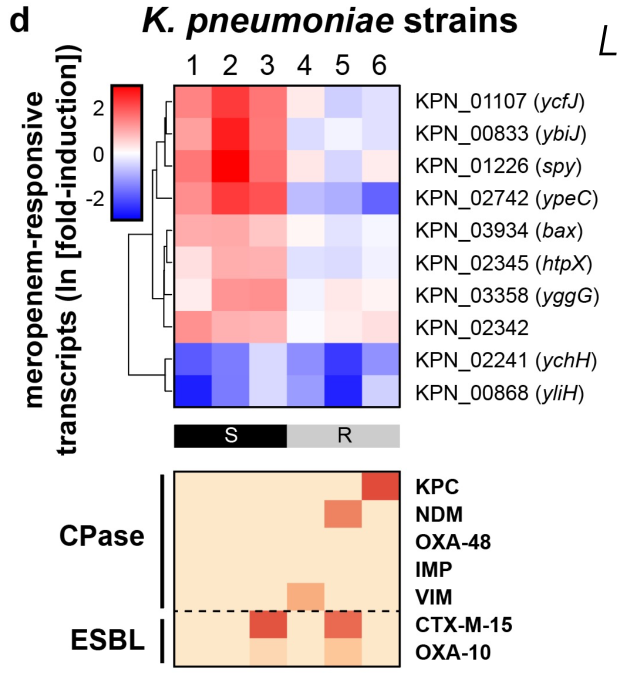
J. Livny
P. Ma
S. Son
G. Cerqueira
A. Earl
D. Hung

Bhattacharyya RP *et al*, *Nat Med* 2019

GoPhAST-R: proposed workflow



- **AST** in <4 hours (<30 min hands-on)
  - vs broth microdilution = 28 hrs from positive BCx
- Success direct from blood cultures
  - 71/72 (99%) correct from spiked BCx
  - 8/8 correct for real BCx
- Genotype: built-in carbapenemase assay
- RNA-Seq data on ~50 bug-drug pairs

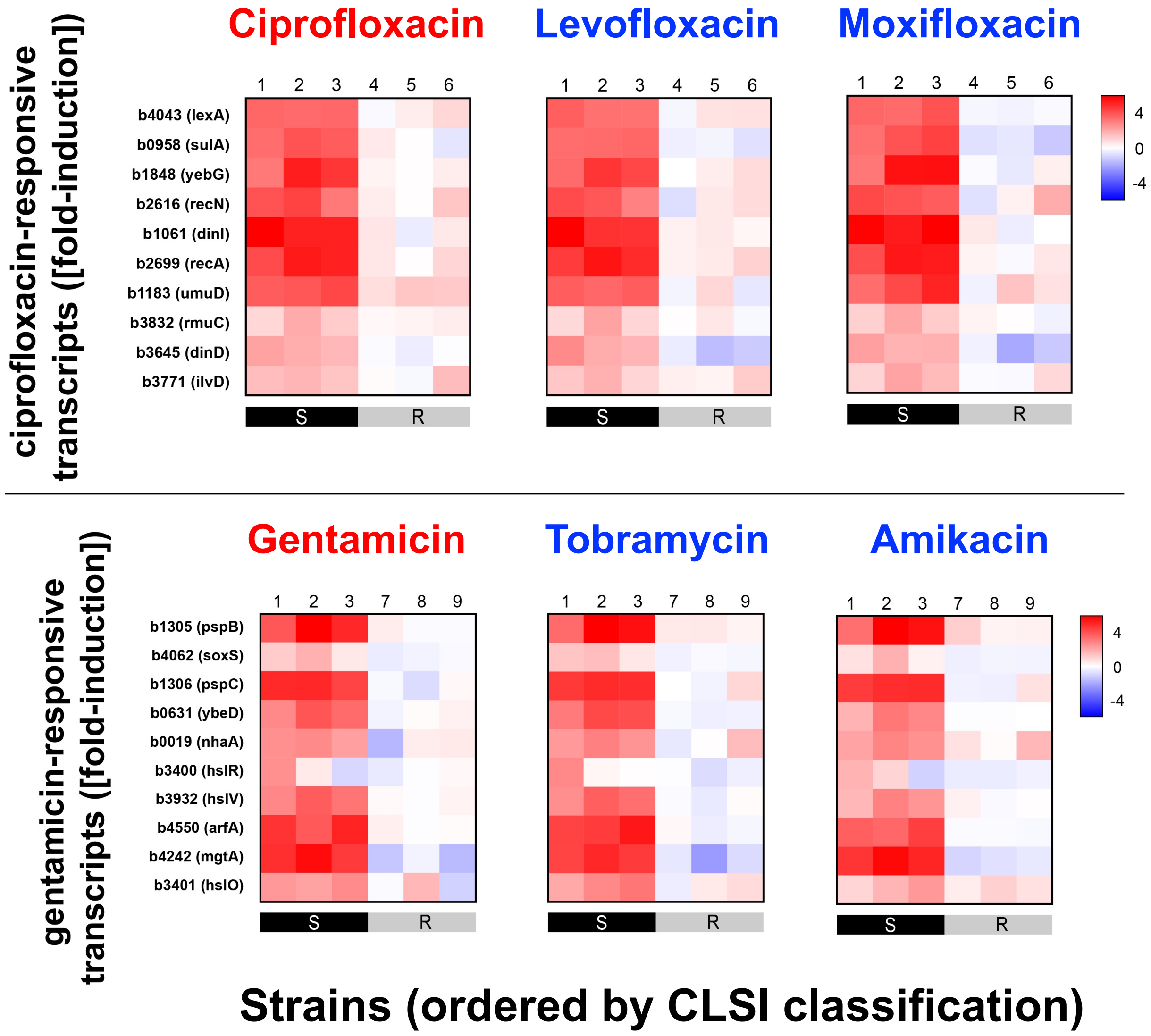


L. Wu

Bhattacharyya RP *et al*, *Nat Med* 2019

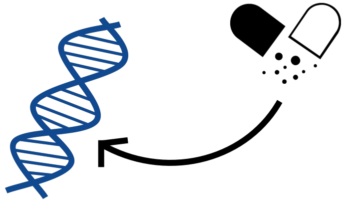
RNA signatures generalize across fluoroquinolones & aminoglycosides

RNA-Seq → NanoString


Ciprofloxacin Levofloxacin Moxifloxacin

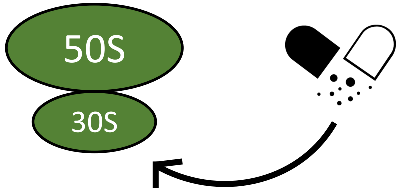
Gentamicin Tobramycin Amikacin

Strains (ordered by CLSI classification)



DNA gyrase inhibitors

NanoString only

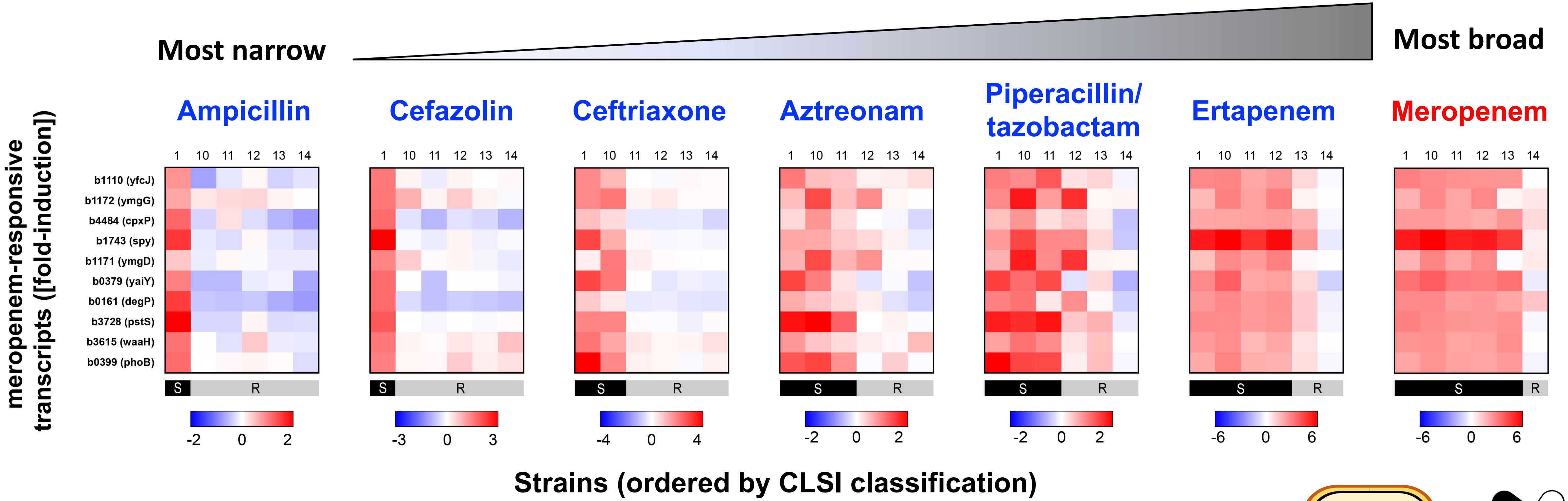


protein synthesis inhibitors

Melanie Martinsen

Martinsen *et al, AAC 2021*

RNA signatures generalize across beta-lactams



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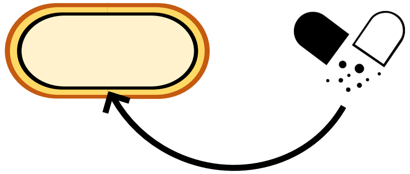
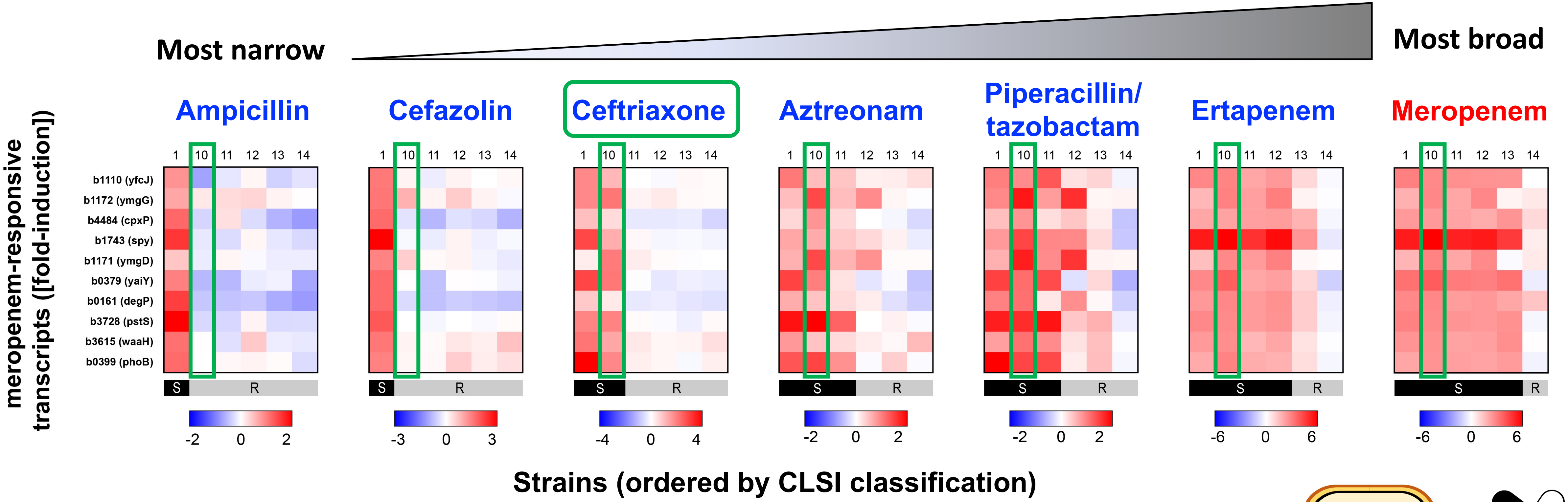
Alex Jaramillo Cartagena



cell wall synthesis inhibitors

Martinsen *et al, AAC 2021*

RNA signatures generalize across beta-lactams



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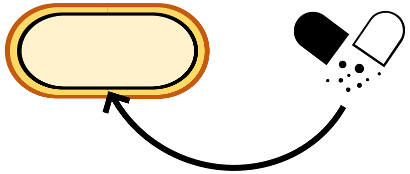


cell wall synthesis inhibitors

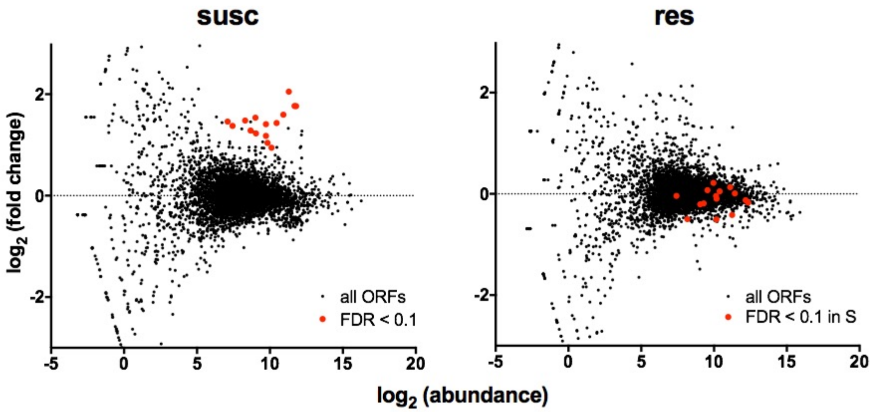
Martinsen *et al*, *AAC 2021*

Beyond typical bacteria: slow-growing species still transcribe fast

- Pilot fungal transcriptional signatures enable rapid AST on the same NanoString platform:

Melanie Martinsen
Eleanor Young
Jose Muñoz
Poppy Sephton-Clark
Michelle Matzko
Christina Cuomo

***Candida albicans* + fluconazole:**



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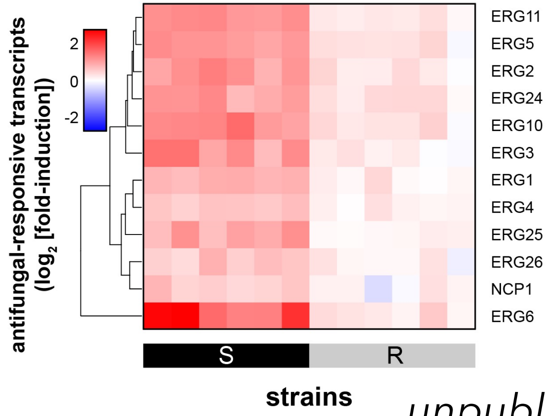
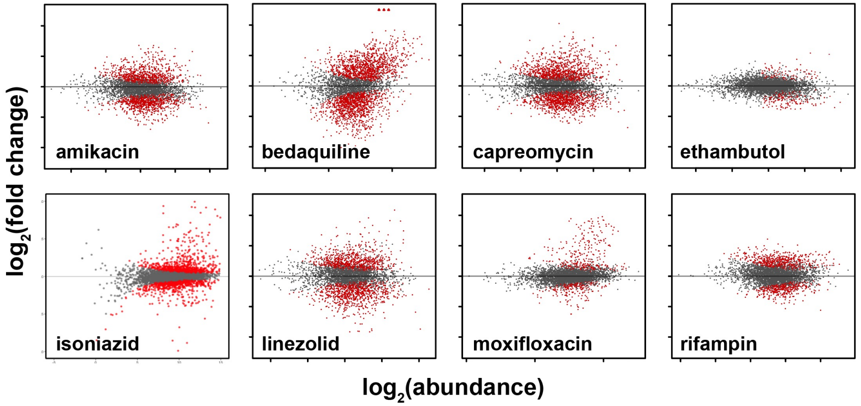


unpublished

- *Mycobacteria* show transcriptional signatures in hours:

***M. tuberculosis* + various abx:**



in prep

with A. van den Bossche, P. Ceyssens (Sciensano, Belgium)

Summary: RNA-based detection for rapid bacterial ID and AST

- **GoPhAST-R:** antibiotic-induced transcriptional signatures are a fast, accurate, **phenotypic** measure of **antibiotic susceptibility**
  - Sensitive to <10^5 bacteria; assay time ~4 hrs
  - Clinical pilot on BCx underway
- Simultaneous detection of key genetic resistance determinants enhances AST accuracy, value
- Accuracy likely to improve with further training/testing
- Success in fungi, mycobacteria = possible **pan-microbial** approach to AST (work in progress)
- Goals: faster, cheaper, more sensitive, & more deployable assay
- What biology underlies these responses?
  - Shared pathways within, among classes? Adaptation? Signs of struggle?

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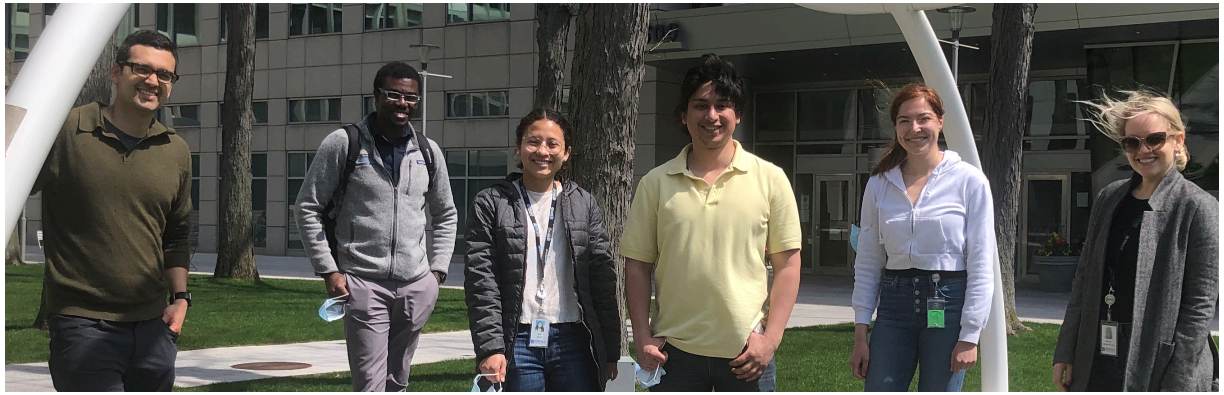
  - Amy Barczak
  - Jim Gomez
  - Jamin Liu
  - Lidan Wu
  - Rob Rudy
  - Sophie Son
- Noam Shoresh



Broad Fungal Genomics
- **Christina Cuomo**
  - **Poppy Sephton-Clark**
  - Jose Muñoz

Broad Bacterial Genomics
- Ashlee Earl
  - Gustavo Cerqueira
  - Alejandro Pironti
  - Abigail Manson

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  - MGH/MIT Innovation Discovery Grant
  - MGH Pilot Translational Grant
  - MGH ID Division

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