University of California
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Pathogen Detection Using Cell-free DNA in Body Fluids

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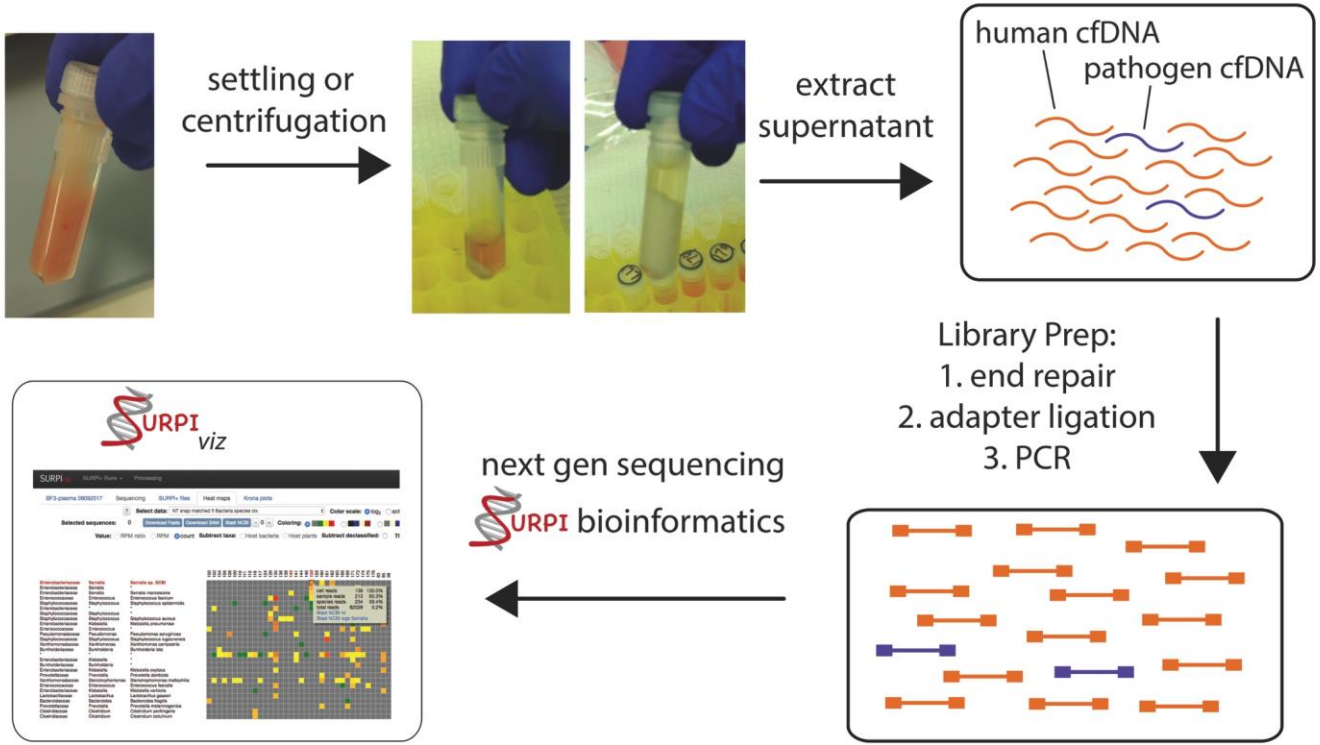
Disclosures

- No financial conflict of interest
- Testing discussed may be pursued as a laboratory-developed test (LDT) validated in the CLIA-certified UCSF Clinical Microbiology Laboratory and is not FDA-approved. Testing may be marketed and sold by UCSF.

Intro Case:

- 33 year old woman transferred from outside hospital
- 2 weeks productive cough -> respiratory failure.
- All blood culture and respiratory cultures were negative at UCSF.

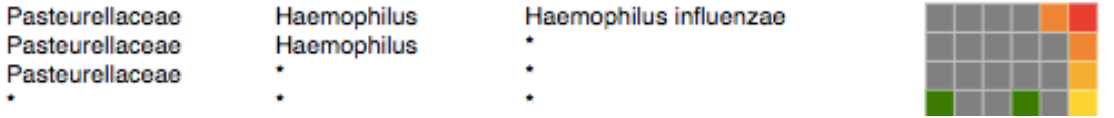
Cell-free (cf)DNA Workflow



Low Microbial Background: eg.170-4700X less Propionibacterium *acnes*

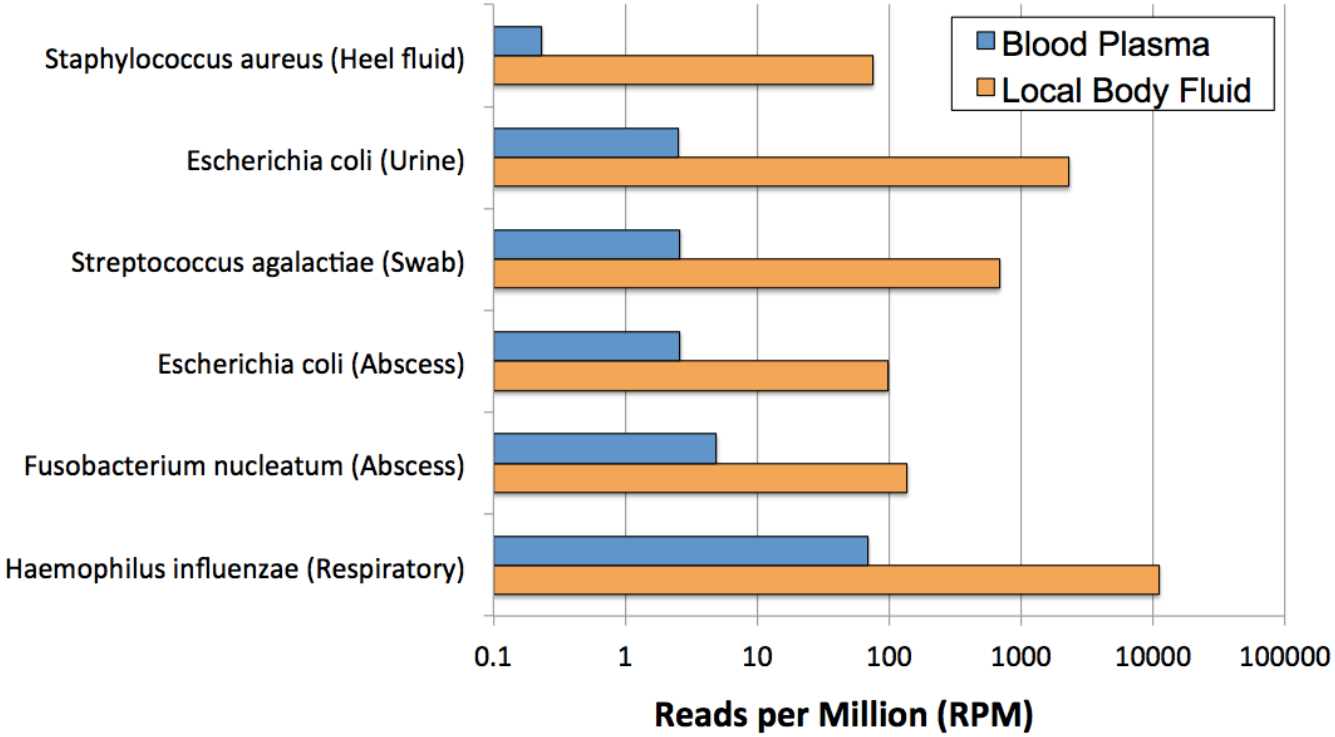
NGS Results

- NGS Miseq:



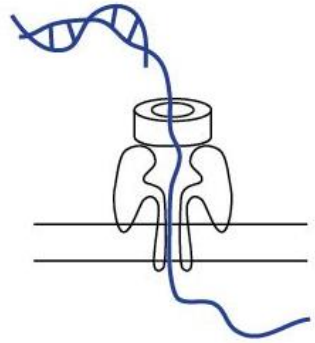
  - BAL cell-free DNA: 11096 reads / million (RPM) to *Haemophilus influenza*
  - Plasma cell-free DNA : 69 reads / million to *Haemophilus influenza*
- Subsequently, 1 of 4 blood culture taken before antibiotics at OSH was reportedly positive for H. *influenza*. Also confirmed by 16S sequencing on BAL.

Local Body Fluid vs. Plasma



Nanopore NGS Results

- BAL: RPM 4159 (Hiseq: 11096)
- Plasma: RPM 37 (Hiseq: 69)



SURPIviz SURPI+ Runs Processing

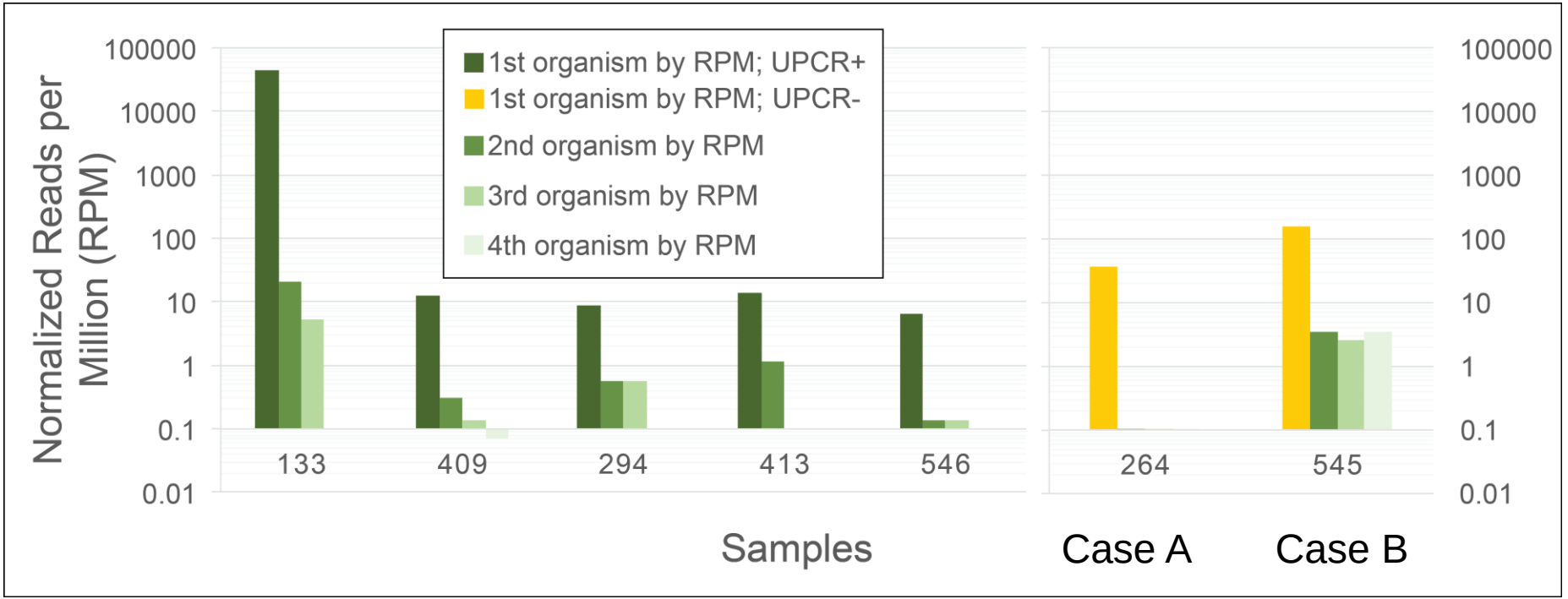
BF13 BF15 BF69 BF69 BF78 BF81 BF87 BF105 BF121 ***BF133-BAL*** BF133-plasma BF137 BF139 BF149 BF152 BF176 BF185 BF198 BF202 BF206 BF208 BF239 BF256 BF261 BF262 BF264

Haemophilus influenzae
Haemophilus parainfluenzae
Escherichia coli
Klebsiella pneumoniae
Shigella sonnei
Neisseria lactamica
Neisseria meningitidis
Neisseria gonorrhoeae
Citrobacter freundii
Pasteurella multocida

RPM	37.293	
cell reads	9	100.0%
sample reads	9	100.0%
species reads	1553	0.6%
total reads	18137	0.0%

Blast NCBI nt
Blast NCBI wgs Haemophilus

mNGS Vs. 16S (UPCR)

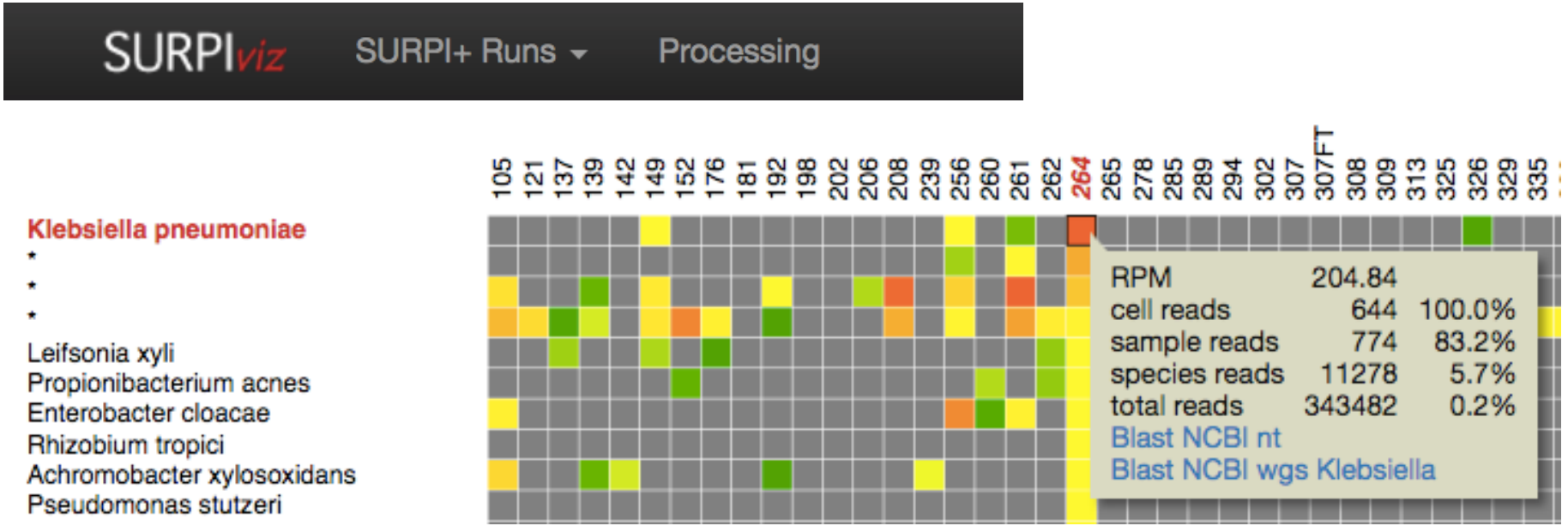


Vs. 16S - Case A

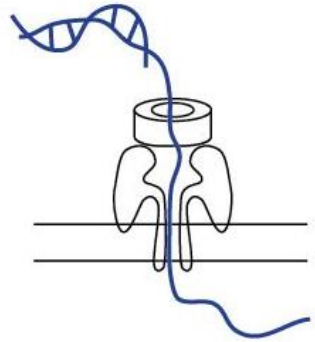
- 3 year old boy with necrotizing pneumonia
- 16S - Pleural Fluid: Streptococcus *mitis* group
- Improved on vancomycin/meropenem

Vs. 16S - Case A
Illumina Hiseq

- Pleural Fluid cfDNA WGS -> SURPI:



Vs. 16S - Case A
Oxford Nanopore



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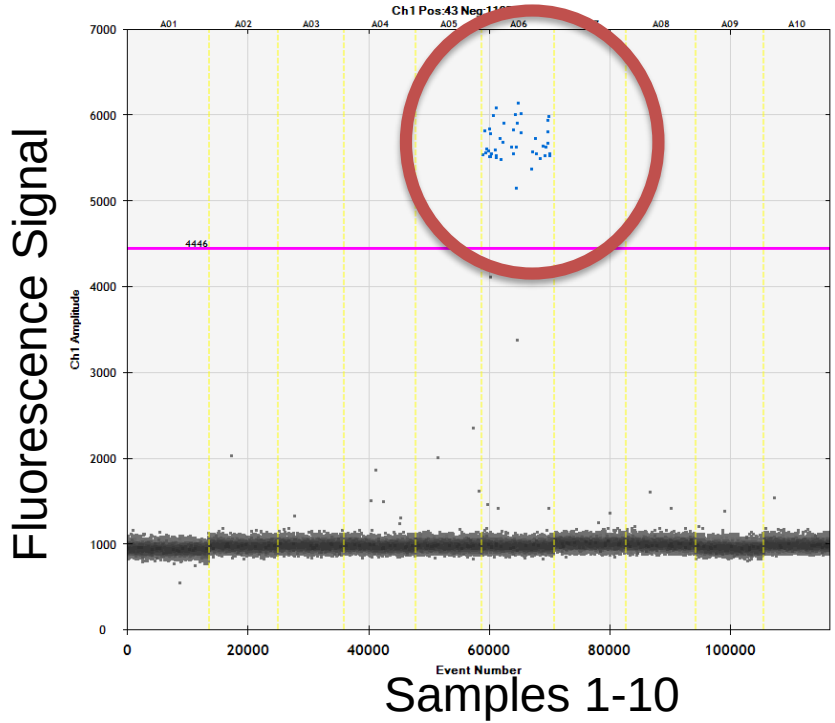
BF13 BF15 BF69 BF69 BF78 BF81 BF87 BF105 BF121 BF133 BF133 BF137 BF139 BF149 BF152 BF176 BF185 BF198 BF202 BF206 BF208 BF239 BF256 BF261 BF262 BF264 BF265 BF278 BF285 BF289 BF294 BF302 BF307 BF307-F BF309 BF313 BF325 BF326 BF329 BF335 BF339

Klebsiella pneumoniae
Klebsiella quasipneumoniae
Klebsiella variicola
Escherichia coli
Shigella sp. PAMC 28760
Human alphaherpesvirus 1
[Enterobacter] lignolyticus
Klebsiella aerogenes
Citrobacter rodentium
Acinetobacter johnsonii

RPM	124.36	
cell reads	122	100.0%
sample reads	157	77.7%
species reads	1020	12.0%
total reads	18137	0.7%

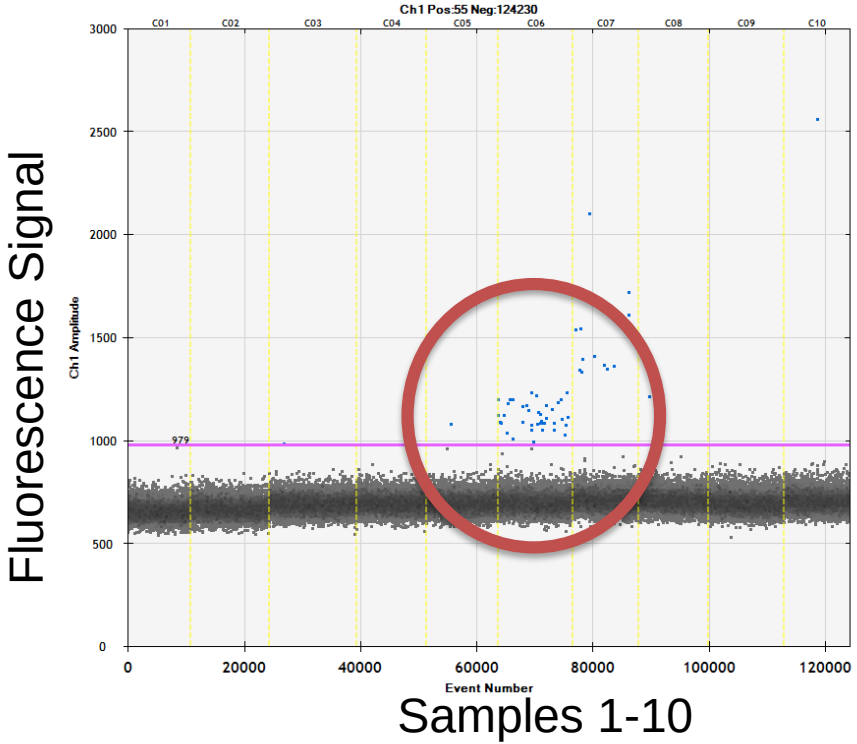
Blast NCBI nt
Blast NCBI wgs Klebsiella

Digital PCR Verification



Samples 1-10

Klebsiella *pneumoniae*



Samples 1-10

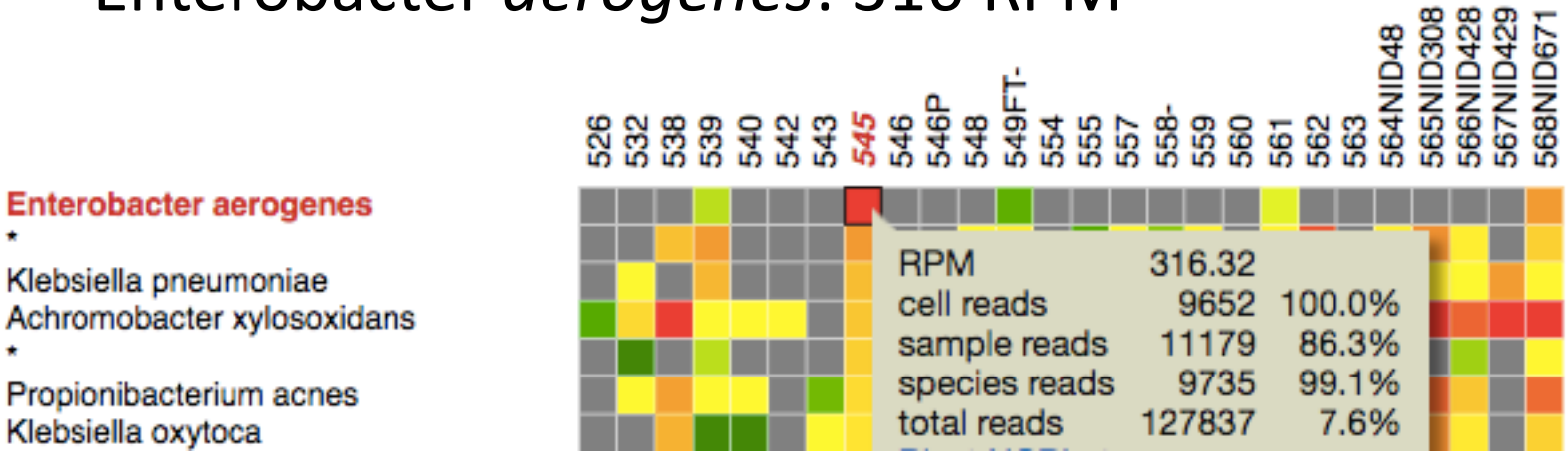
Streptococcus *mitis*

Vs. 16S - Case B

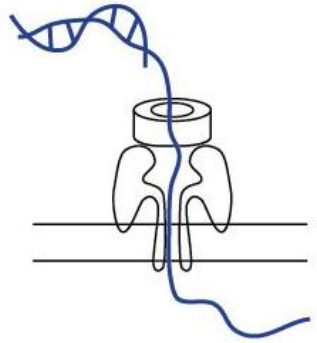
- ~70 year old man with fever and encephalopathy, placed on empiric antibiotics. Has Deep Brain Stimulator implant for Parkinson's.
- CSF: WBC 28->760 in 1 month.
- 16S, ITS sequencing on CSF:
  - negative 2X

Vs. 16S - Case B

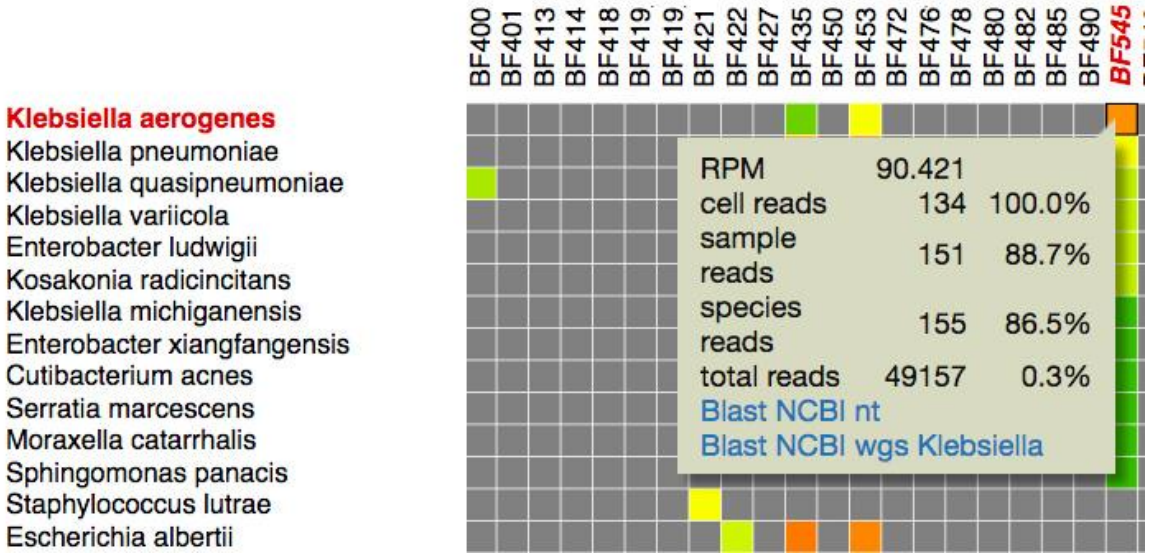
- CSF 2 days prior to implant removal:
  - 16S sequencing: negative
  - Enterobacter *aerogenes*: 316 RPM



Vs. 16S – Case B
Oxford Nanopore

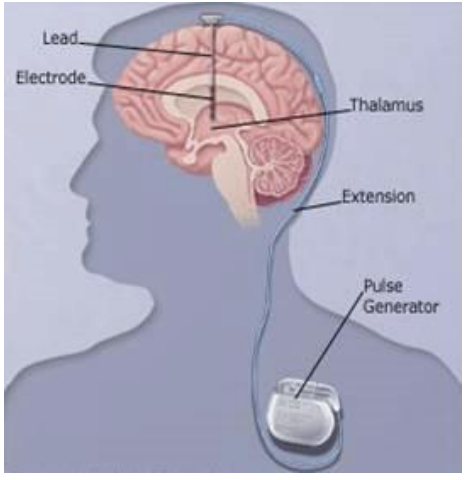


SURPIviz SURPI+ Runs Processing

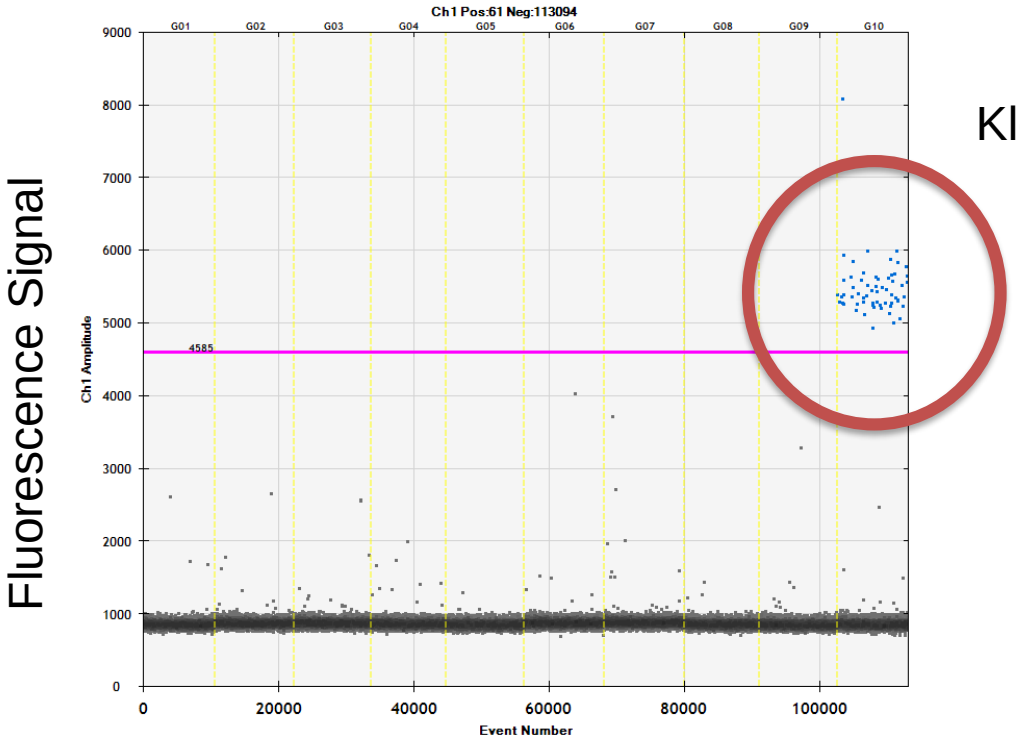


Vs. 16S - Case B

- Brain surgery to remove infected implant
  - Culture: Klebsiella (Enterobacter) *aerogenes*



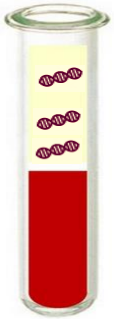
Digital PCR Verification



Klebsiella *aerogenes*

Bonus Cancer Diagnostics

Copy Number Variation (CNV) Detection



Patient Sample

Reference Sample

99% of data is human!

CELL-FREE DNA IN PATIENT PLASMA

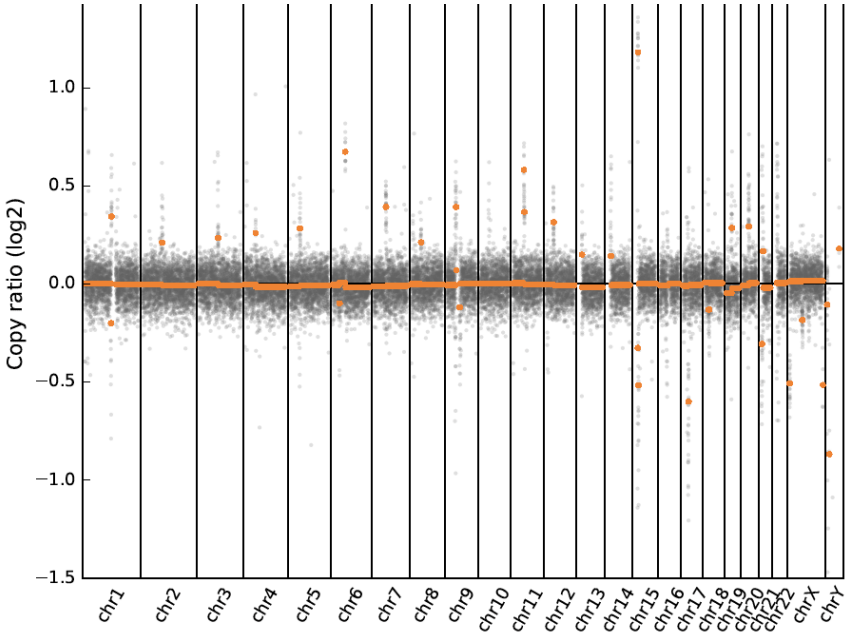
CCCTTAGCGCTTTAACGTACGTAAAACCCTT
AACGTACGTAAAAACGGGGTCAAAGGTTCCC
GACTTAAAATCGGAATCGATGCCCAAACTT
GAATCGATGCCCAAACGGGGTCAAAGTTCCC

MASSIVELY PARALLEL SEQUENCING

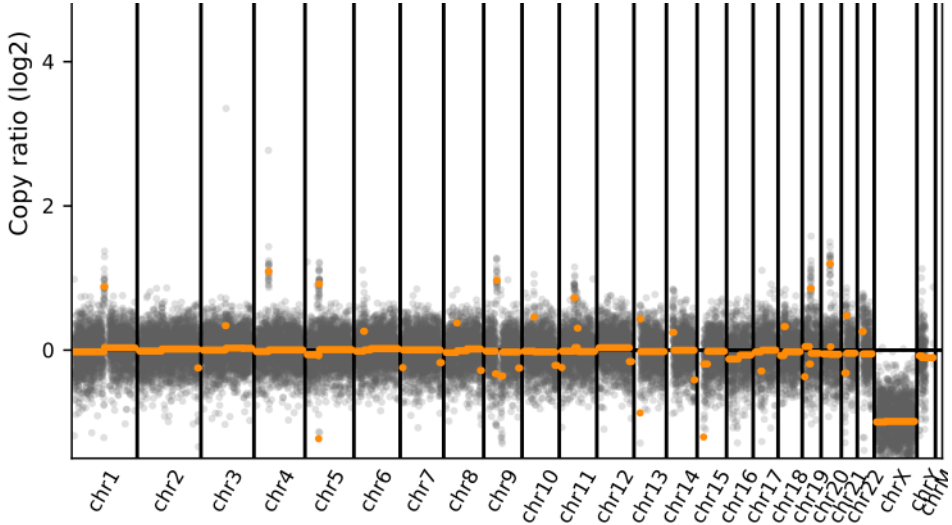
Whole Genome Sequencing & Alignment

Normalize Patient Sample with Reference Sample

CNV of Normal Control

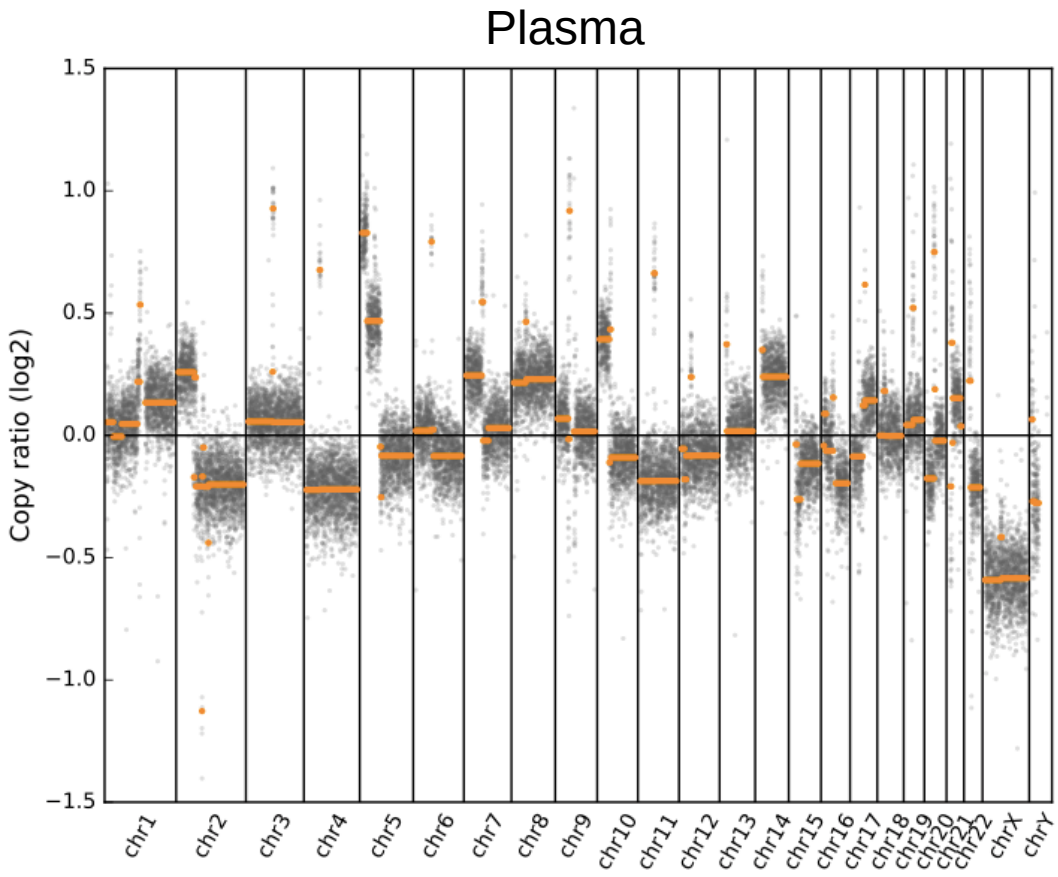


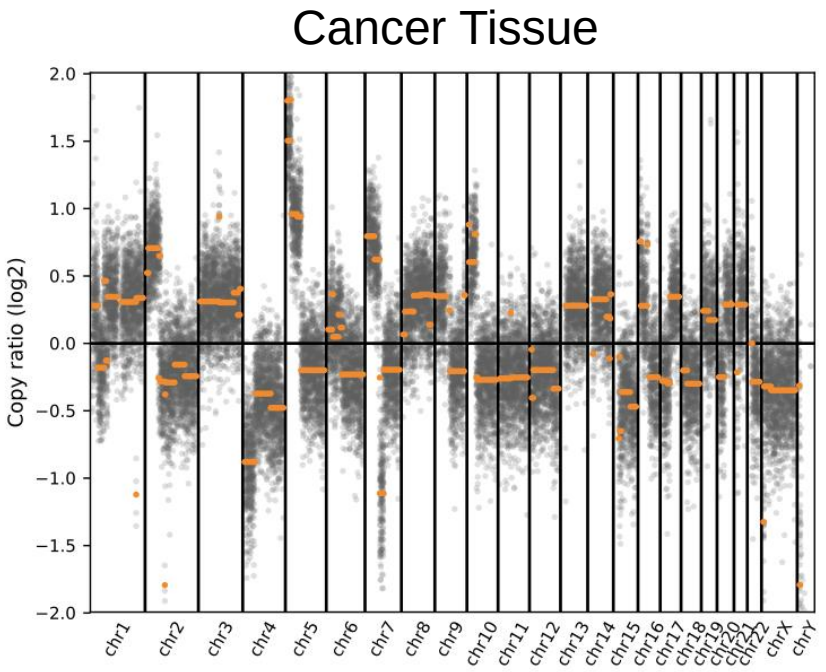
Plasma cfDNA



Tissue FFPE

Plasma: Metastatic Neuroendocrine





Conclusions

- ✓ Same Sample
- ✓ Same Data

- One test, any body fluid
  - Pathogens:

    High Signal, Low Microbial Contamination
  - Cancers:

    Suggested by Abundant Copy Number Variations

UCSF Next-Gen Precision Diagnostics

Acknowledgements

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- Eric Talevich, Iwei Yeh
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- Yasemin Sucu, Shaun Arevalo, Allan Gopez
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UCSF Next-Gen Precision Diagnostics

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Questions?