Lesson in Transfiguration

Rapid Adoption of Sequencing in Public Health Labs

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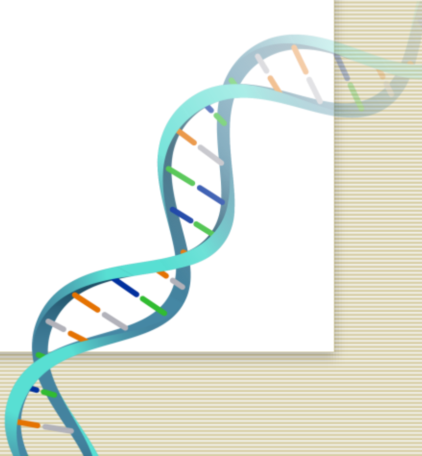
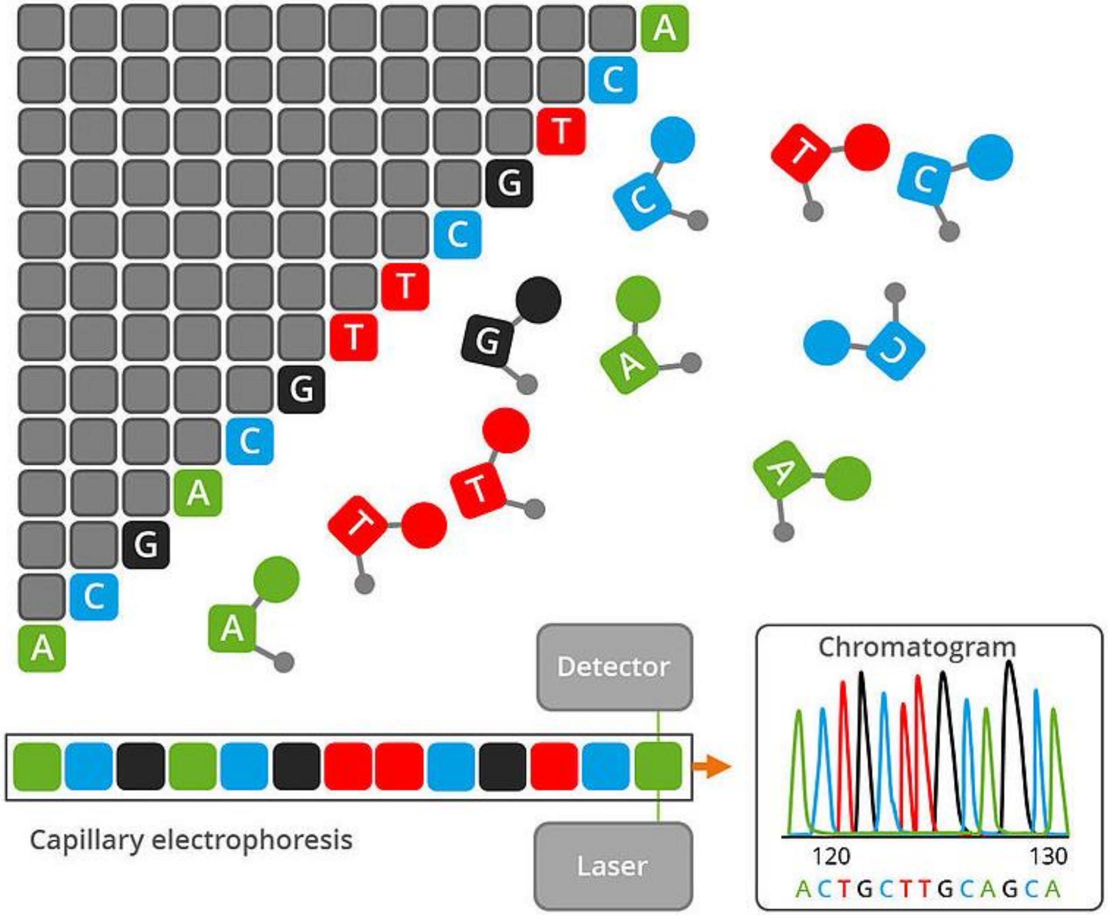


Outline

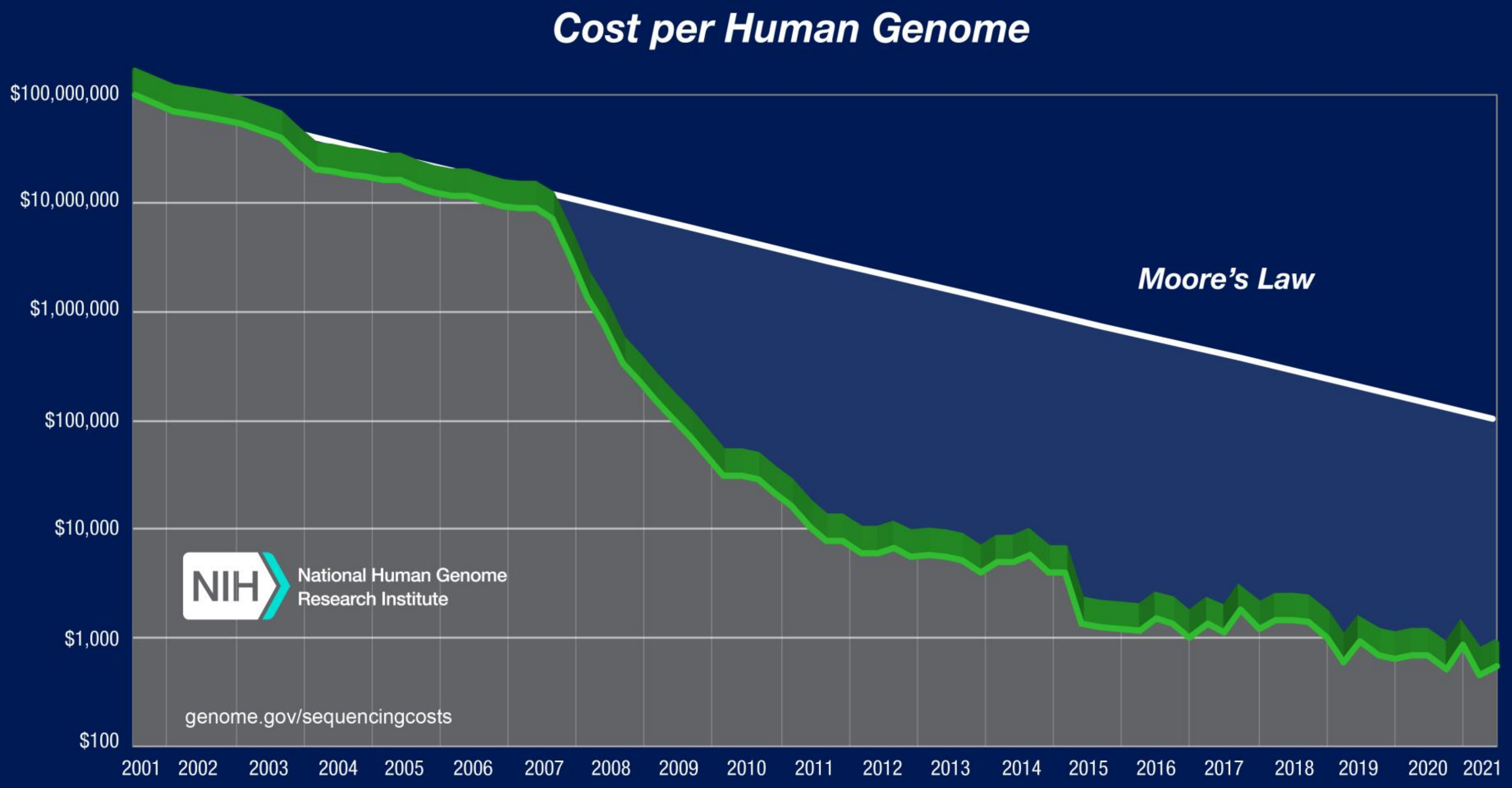
- Next-generation sequencing
  Whole-genome sequencing
- Primary uses of genomic surveillance at WSLH
  - SARS-CoV-2
  - Influenza
  - Enteric bacteria
  - Antibiotic resistant bacteria
- Additional uses of genomic surveillance
  - *Mycobacterium tuberculosis*
  - *Cyclospora/Cryptosporidium*
  - *Enteroviruses* and *Streptococcus pneumoniae*
  - Hepatitis C virus
  - *Candida auris*
- Future of NGS testing for infectious diseases

Sanger method of sequencing (“First generation”)



https://www.gatc-biotech.com/en/expertise/sanger-sequencing.html

https://www.nature.com/news/human-genome-project-twenty-five-years-of-big-biology-1.18436


Cost per Human Genome
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
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2017
2018
2019
2020
2021
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts

https://www.illumina.com

Next-generation sequencing (NGS)

- Massively parallel sequencing
- Millions of short DNA strands (‘reads’) per specimen
  - ATCGTAATGCCGTATCGTACTAAAGTTTT…
- BIG DATA: one run > 800,000 book novels of information
- Whole Genome Sequencing (WGS) is a common use of NGS

4 Main Steps in NGS Testing

- Nucleic acid extraction
- Library preparation
- Sequencing
- Analysis

My experience with next-generation sequencing is:

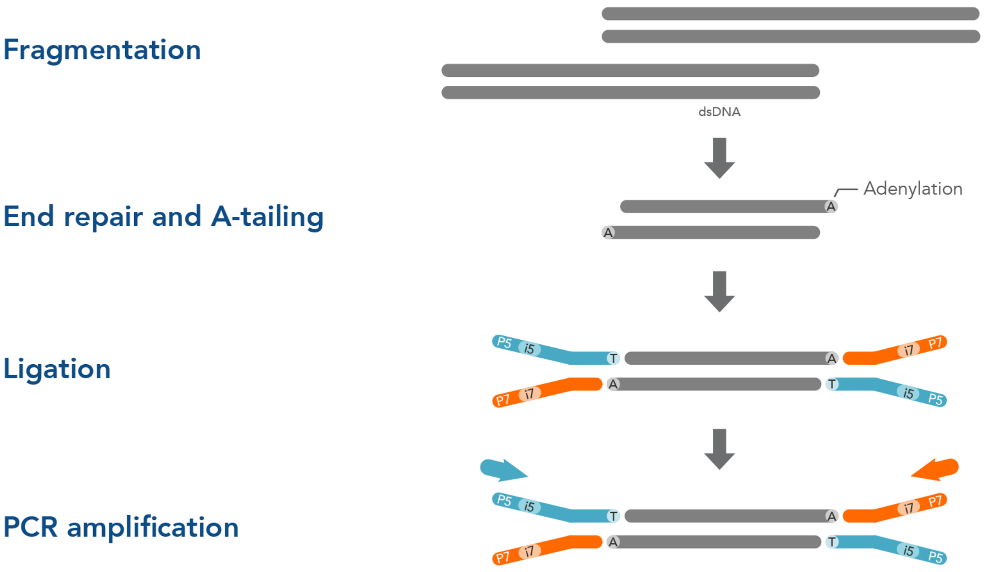
A) I’ve never heard of it
B) I’ve heard of it but don’t know much
C) I’ve learned a decent amount about it
D) I’ve performed it myself
E) I could give this lecture

4 Main Steps in NGS Testing

- Nucleic acid extraction

4 Main Steps in NGS Testing

- Library preparation
  - Break nucleic acid into small fragments (50bp-250bp)
  - Use primers to add “barcodes” to the ends of each fragment



Fragmentation

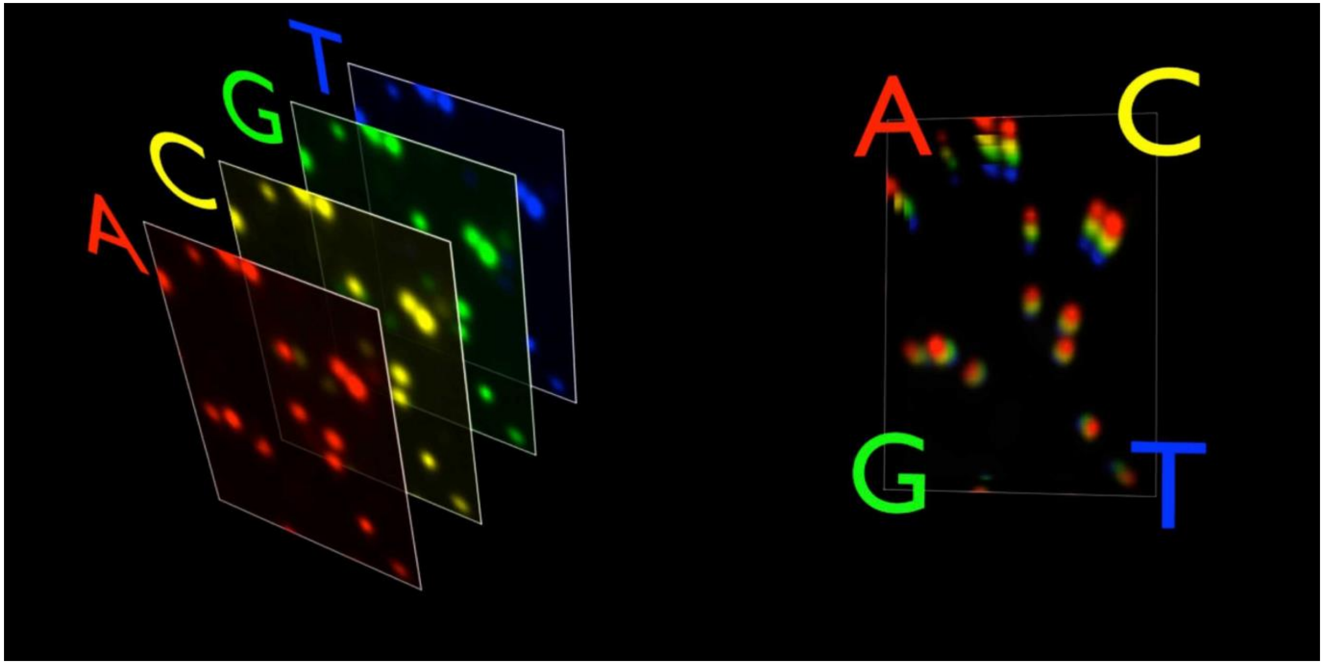
End repair and A-tailing

Ligation

PCR amplification

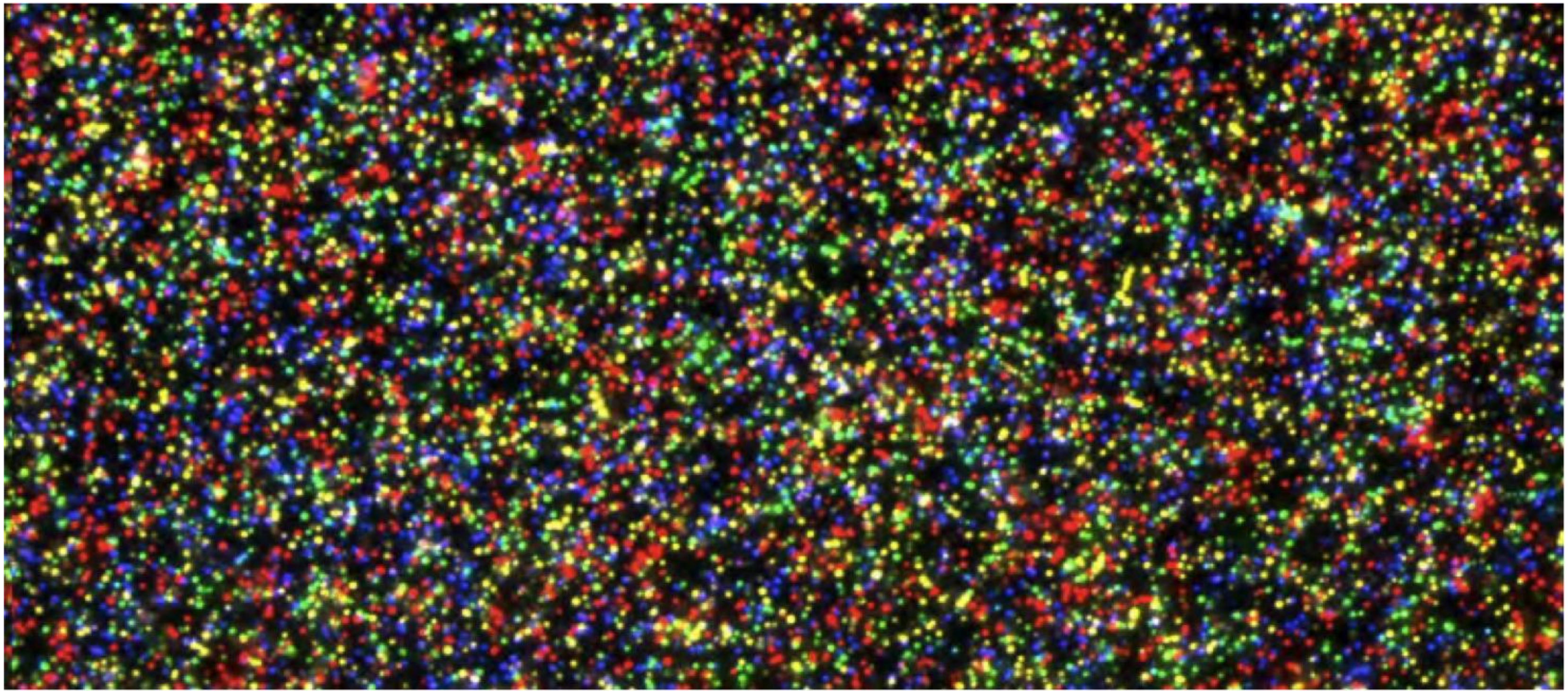
4 Main Steps in NGS Testing

- Sequencing



4 Main Steps in NGS Testing

- Sequencing: massively parallel!

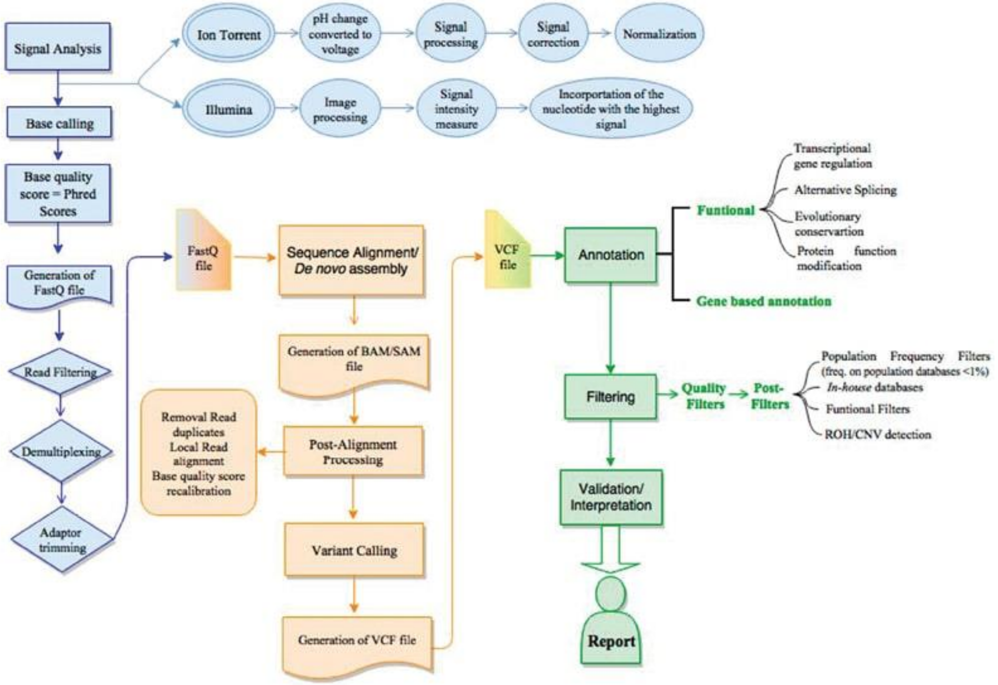


4 Main Steps in NGS Testing

- Analysis
  - Put all the different “reads” into one combined genome (“consensus sequence”)
  - Analyze the consensus sequence for what you’re looking for
    - Relatedness with other bacteria
    - Subtype/genotype
    - Antibiotic resistance genes
    - Primer/probe binding sites
    - Etc.

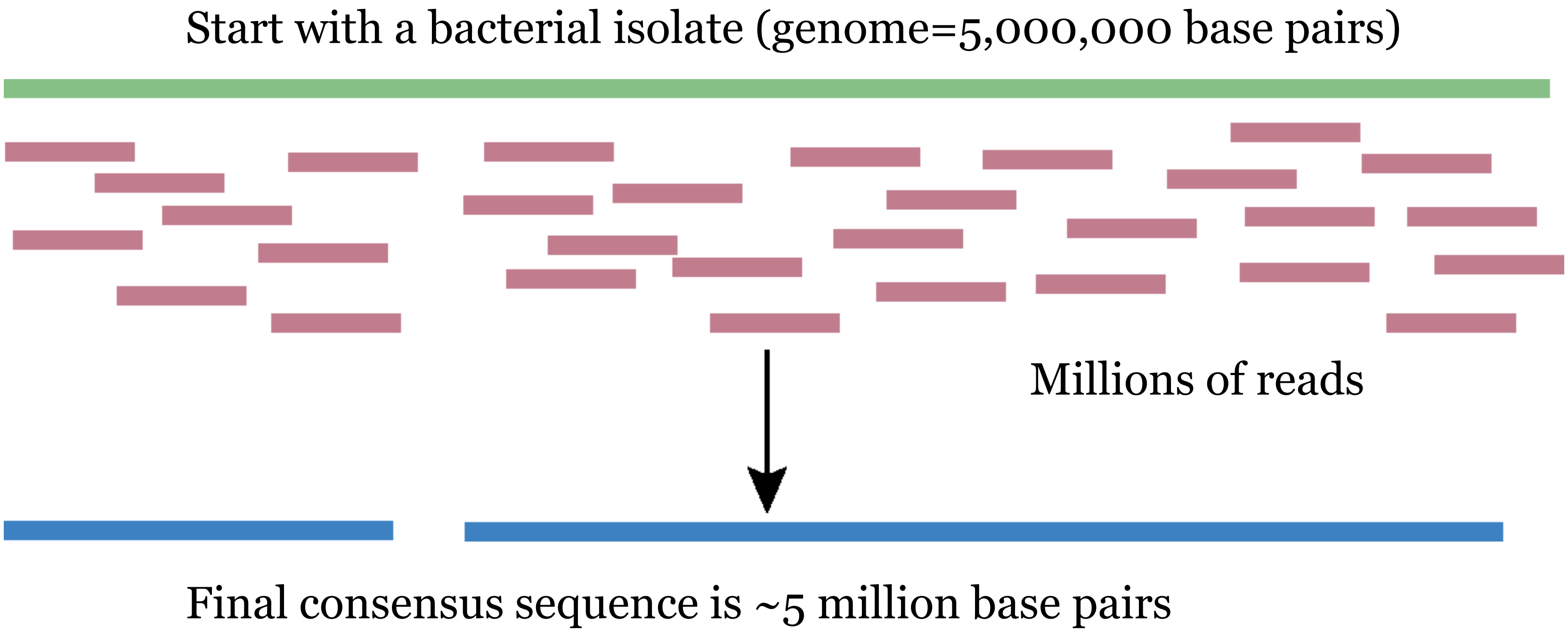
4 Main Steps in NGS Testing

- Analysis
  - Some point-and-click software with easy user interface
  - Much is Linux-based coding
  - Lots of quality steps!



https://diagnostech.co.za/intro-to-ngs-data-analysis-workflow/

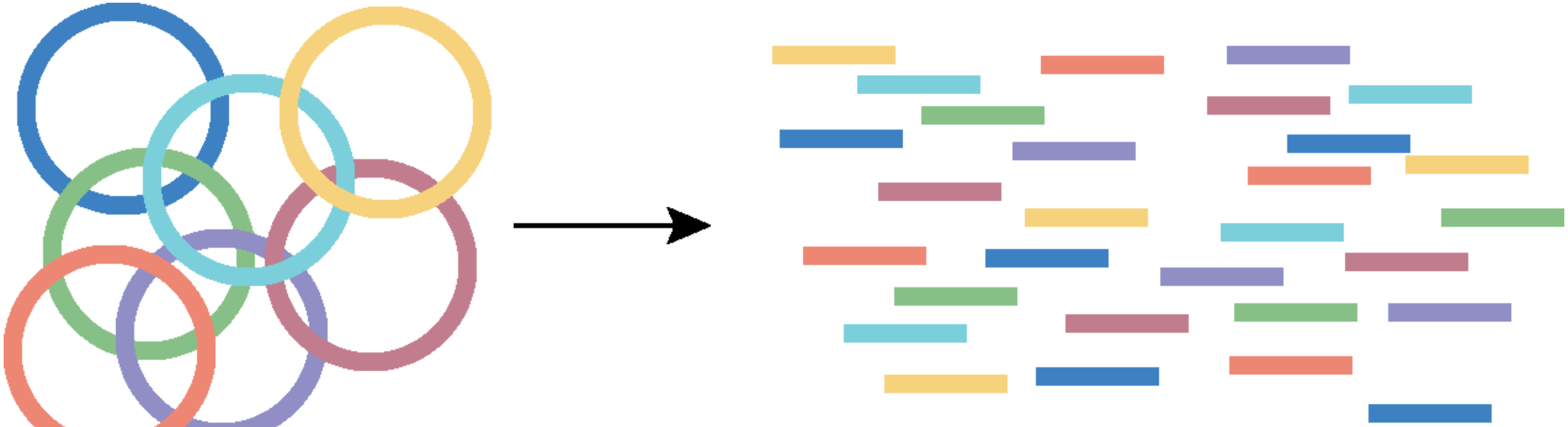
Whole Genome Sequencing of a Bacterial Isolate


Start with a bacterial isolate (genome=5,000,000 base pairs)

Millions of reads

Final consensus sequence is ~5 million base pairs

Metagenomics



reads

Bacterial genomes,
Human DNA,
Human RNA,
Virus DNA/RNA

Comparison of methods

	PCR	**Sanger sequencing**	**Next-generation sequencing**
Percentage of genome targeted	<1%	<1% to 10%	100%
Result	Positive vs. negative	Genotype, Subtype, Relatedness (potentially)	Genotype, Subtype, Relatedness using entire genome

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Main Applications of NGS in Infectious Disease

- **Identify and respond to outbreaks**
  - Comprehensive genomic characterization to determine pathogen relatedness
  - National surveillance
    - PulseNet model for *Salmonella*/STEC/*Listeria*
    - National TB approach
  - Smaller outbreaks: is a cluster of disease due to transmission of a strain within a facility, or multiple introductions?

- **Ensure that vaccines are effective**
  - Track strains of influenza and SARS-CoV-2, as part of monitoring vaccine effectiveness
  - Use WGS for bacterial serotyping (*S. pneumoniae*) to ensure circulating serotypes are covered in the vaccine

- **Ensure PCR test accuracy**
  - WGS data allows test developers, FDA, and CDC to monitor changes in pathogens that may lead to false negative or false positive results

Main Applications of NGS in Infectious Disease

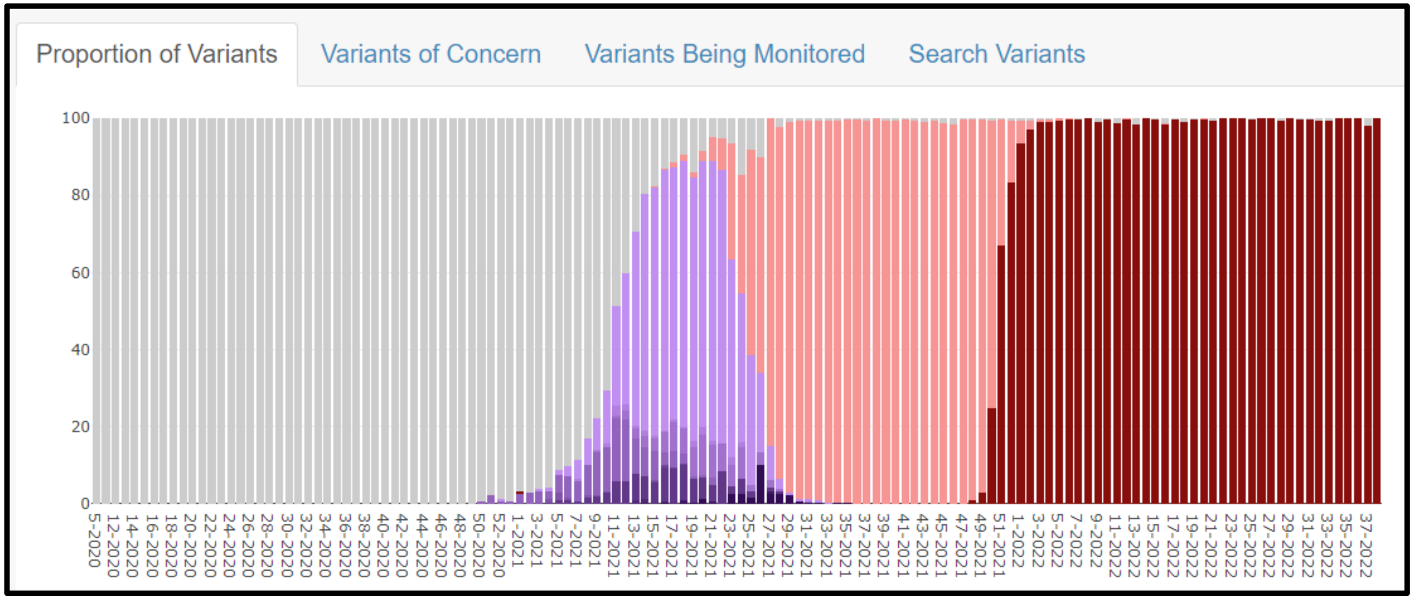
- **Ensure that viral treatments are effective**
  - Predict influenza antiviral resistance
  - Guide monoclonal antibody use for SARS-CoV-2 (before Omicron messed it up!)

- **Guide treatment in other ways**
  - Use NGS to identify pathogens that other methods fail to identify
    - Targeted on a bacterial/yeast isolate
    - Metagenomics approach if all other tests are negative

  - Predict antibiotic susceptibility for faster targeted treatment
    - TB as a great example
    - For non-fastidious organisms, the cost and TAT of NGS don’t currently match conventional method

SARS-CoV-2 Genomic Surveillance

- Many uses of the same sequence data!
  - Track virus lineages/variants
  - Inform monoclonal antibody use
  - Inform vaccine strain selection
  - Ensure test results remain accurate



https://dataportal.slh.wisc.edu/sc2dashboard

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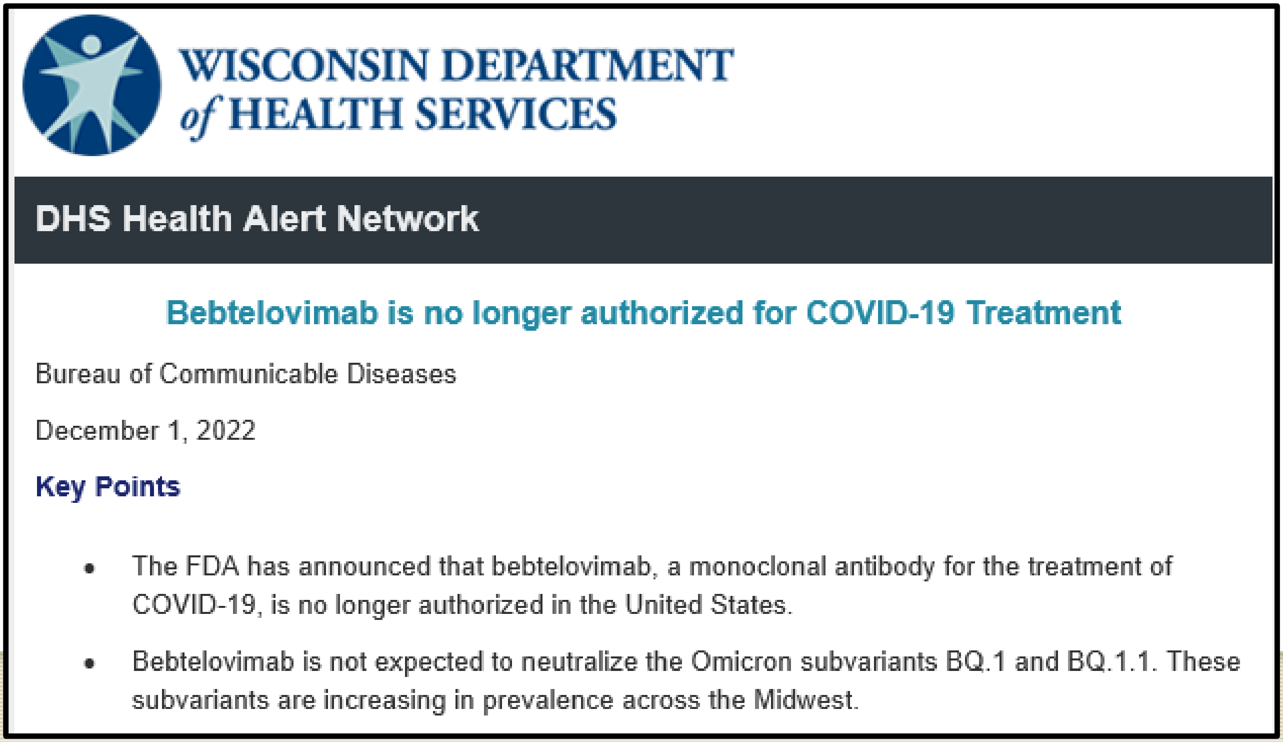

WISCONSIN DEPARTMENT *of* HEALTH SERVICES

DHS Health Alert Network

Bebtelovimab is no longer authorized for COVID-19 Treatment

Bureau of Communicable Diseases

December 1, 2022

Key Points

- The FDA has announced that bebtelovimab, a monoclonal antibody for the treatment of COVID-19, is no longer authorized in the United States.
- Bebtelovimab is not expected to neutralize the Omicron subvariants BQ.1 and BQ.1.1. These subvariants are increasing in prevalence across the Midwest.

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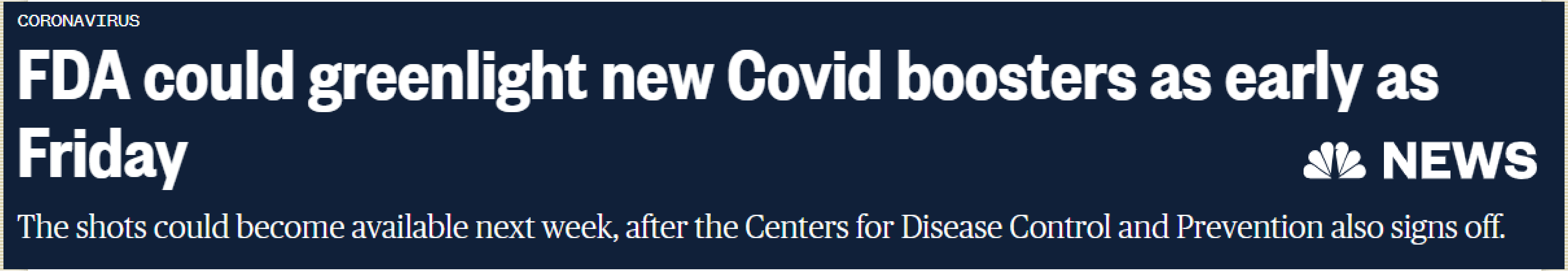


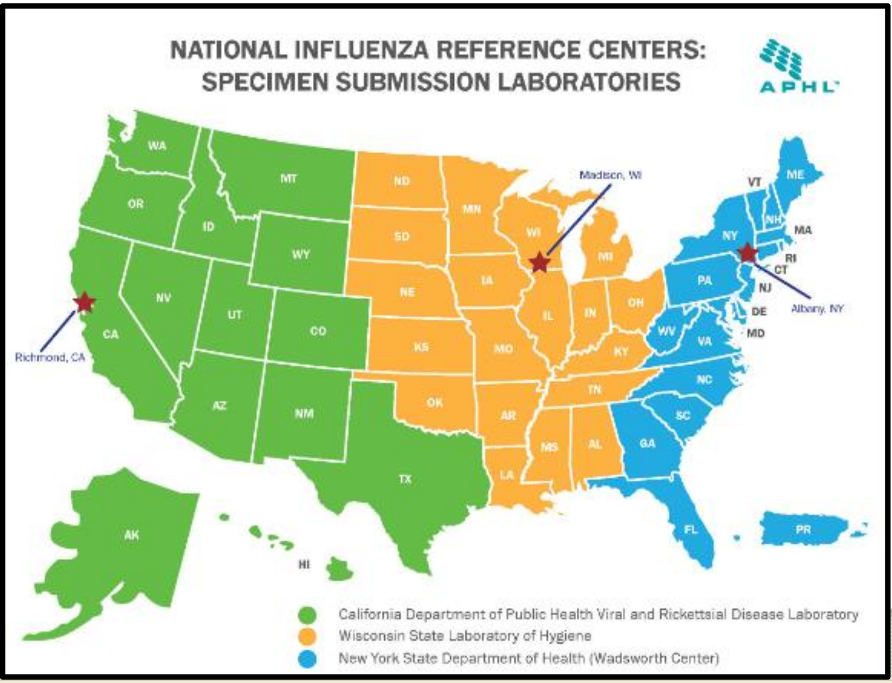
CORONAVIRUS

FDA could greenlight new Covid boosters as early as Friday

The shots could become available next week, after the Centers for Disease Control and Prevention also signs off.

Influenza Genomic Surveillance: National Influenza Reference Centers

- Subset of influenza positive samples from clinical labs to PHL to NIRC to CDC
- NGS at NIRC sites allows for
  - Strain characterization
  - Antiviral resistance tracking
  - Detecting novel strains with pandemic potential
  - Track virus mutations that may affect diagnostic test accuracy

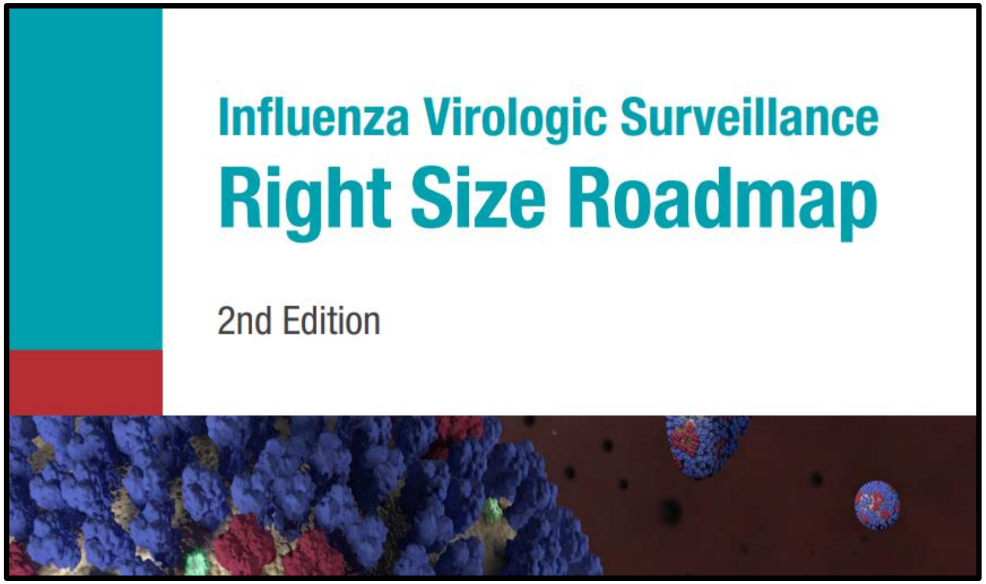


NATIONAL INFLUENZA REFERENCE CENTERS: SPECIMEN SUBMISSION LABORATORIES

🟢 California Department of Public Health Viral and Rickettsial Disease Laboratory
🟠 Wisconsin State Laboratory of Hygiene
🔵 New York State Department of Health (Wadsworth Center)

Sequence Every Positive? No

- Surveillance
  - Influenza and SARS-CoV-2 surveillance
    - Right-sizing
    - You don’t need to count every raindrop to know that it’s raining



Influenza Virologic Surveillance

Right Size Roadmap

2nd Edition

“Cripes Alfrighty! Ya don’t hafta count all the snowflakes to know it’s snowin,’ doncha know!”

Charlie Berens:

https://www.patrickdurkinoutdoors.com/post/cripes-alfrighty-charlie-berens-knows-the-outdoors

Enteric Bacterial Genomic Surveillance: PulseNet

PulseNet USA™

The National Molecular Subtyping Network for Foodborne Disease Surveillance

CDC

Annual PulseNet numbers in WI:

- ~1,000 Salmonella
- ~400 Shiga-toxin producing E. coli (STEC)
- handful of *Shigella*

Antibiotic Resistant Bacteria: AR Lab Network

Outbreak of Extensively Drug-resistant *Pseudomonas aeruginosa* Associated with Artificial Tears

Distributed via the CDC Health Alert Network
February 1, 2023, 7:00 PM ET
CDCHAN-00485

- Use sequencing to determine:
  - Mechanism of resistance (KPC, NDM, etc.)
  - If isolates are related
    - if related, likely transmission at facility
    - if unrelated, likely multiple introductions into facility

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Additional Uses of NGS

- Current projects at WSLH
  - *Mycobacterium tuberculosis*
    - National surveillance to identify clusters/outbreaks
    - Use mutations to predict drug susceptibility/resistance faster than growth-based methods
  - *Cyclospora/Cryptosporidium*
    - National surveillance to identify clusters/outbreaks
  - *Enteroviruses* and *Streptococcus pneumonia*
    - Subtyping/genotyping using NGS
  - Hepatitis C virus
    - Statewide surveillance to identify clusters/outbreaks
  - *Candida auris*
    - Surveillance and outbreak response

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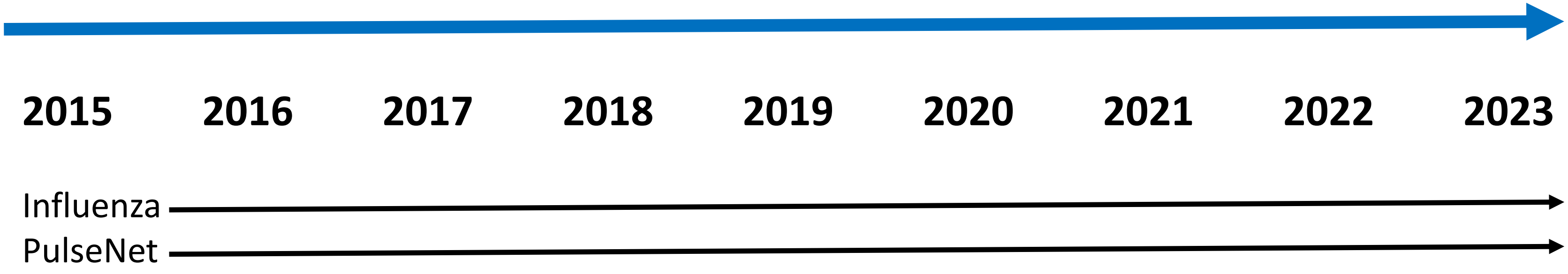
    - Statewide surveillance to identify clusters/outbreaks
  - *Candida auris*
    - Surveillance and outbreak response
- Future projects at WSLH
  - *Legionella* to determine relatedness of clinical and environmental isolates
  - Rabies typing
  - RSV surveillance
  - Norovirus surveillance

Rapid Adoption of Sequencing in Public Health Labs: Timeline of NGS at WSLH



2015 2016 2017 2018 2019 2020 2021 2022 2023

Influenza

PulseNet

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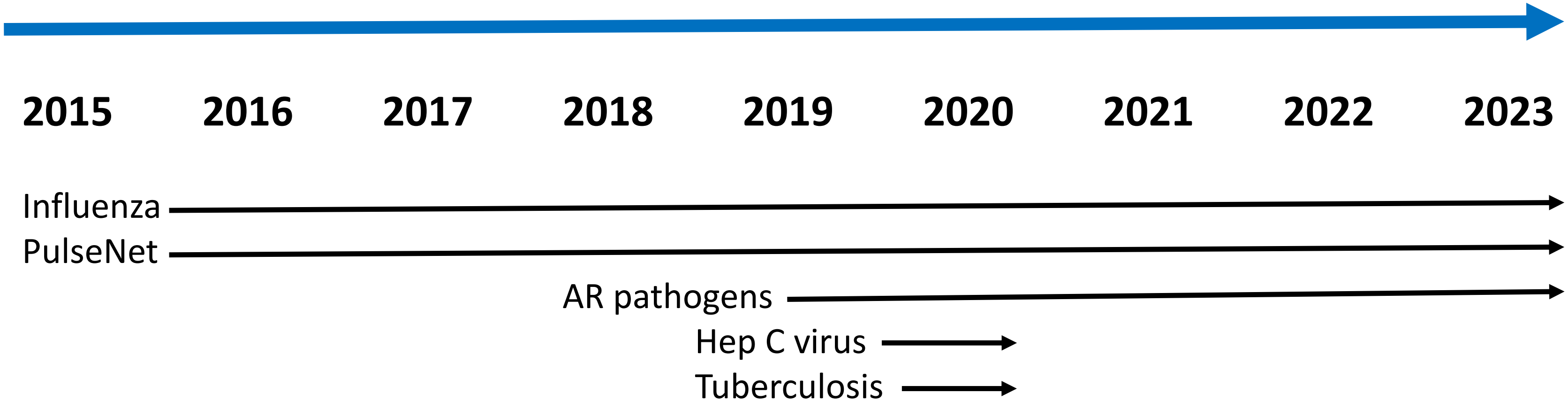


2015 2016 2017 2018 2019 2020 2021 2022 2023

Influenza

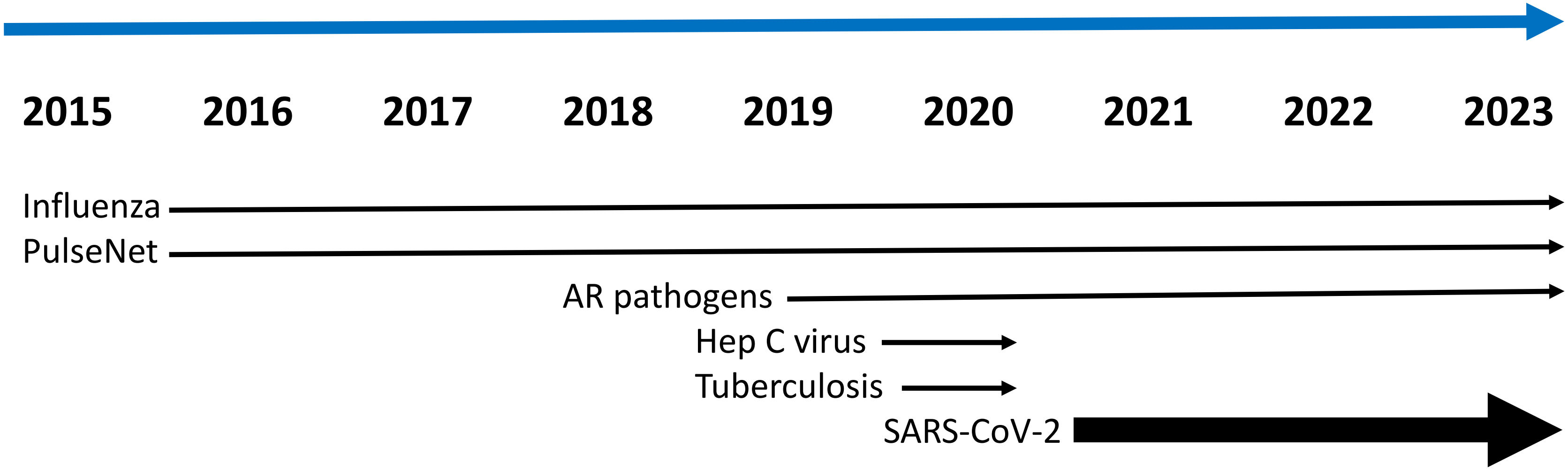
PulseNet

AR pathogens

Hep C virus

Tuberculosis

Rapid Adoption of Sequencing in Public Health Labs: Timeline of NGS at WSLH



2015 2016 2017 2018 2019 2020 2021 2022 2023

Influenza

PulseNet

AR pathogens

Hep C virus

Tuberculosis

SARS-CoV-2

Rapid Adoption of Sequencing in Public Health Labs: Timeline of NGS at WSLH

20 **2023**

Inf

Pul

All surveillance and outbreak response work that we do is only possible because of samples that you submit.

THANK YOU!

Cryptosporidium

S. pneumoniae

C. auris

Enteroviruses

etc…

Future of NGS testing for Infectious Diseases

- Public health labs continue to expand use of NGS
- NGS will become cheaper, more automated, higher-throughput
- Clinical microbiology labs will do more NGS
  - Perhaps: submit data to a public health portal for analysis (and public health then has the data for surveillance)
- NGS will replace some (but not all) traditional test methods in clinical labs
  - Cost and TAT will largely drive implementation in clinical labs

Future talks about next-generation sequencing should be:

A) At a simpler level than this talk
B) At a similar level to this talk
C) At a more advanced level than this talk

https://www.spreadshirt.com/shop/design/select+difficulty+funny+gamer+meme+2020+snapback+cap-D5efdedf6f937643f11d4fc4f?sellable=N0pwMQGq0ruV5nydYvXx-803-34
https://www.redbubble.com/i/poster/Difficulty-Level-Knitting-With-A-Cat-Funny-by-CroyleC/39108905.LVTDI


THANK
YOU

Sequence every organism out there?

- Questionable pathogenicity
    - *M. gordonae*
    - Bocavirus
    - Bacillus non-anthracis
- Cost and TAT of NGS don’t make sense compared to other methods
    - STIs
- No great reason to sequence
    - PIV, hMPV, rhinovirus
- No pathogen available (antibody testing)
    - Arboviruses, syphilis, Lyme

Examples: Four Main Testing Teams in WSLH Communicable Disease Division

- Bacteriology, Mycobacteriology, Serology, Virology

- **Bacteriology**
  - *Salmonella*/STEC/*Listeria*
    - WGS of all isolates
  - *Haemophilus influenzae* ID and serotyping
    - Currently real-time PCR
    - Considering if NGS could replace
  - LRN biothreat rule-out testing
    - Real-time PCR faster and cheaper for rule-outs
    - If positive, ship to CDC for additional testing
      - Likely involves WGS
    - If negative, don't need to sequence the isolate
      - Bacillus non-anthracis almost always environmental contaminants

https://www.cdc.gov/vaccines/vpd/anthrax/photos.html

Examples: Four Main Testing Teams in WSLH Communicable Disease Division

- **Mycobacteriology**
  - TB: WGS of all isolates
    - National surveillance
    - Identify cross-contamination
    - AST prediction
  - *M. gordonae*
    - Do not sequence; not a pathogen
    - Second-most common AFB identified
  - Other NTM
    - Perhaps sequence for ID or outbreak response
    - Routine WGS not needed
      - May or may not be clinically relevant

https://www.cdc.gov/vaccines/vpd/tb/public/photos.html

Examples: Four Main Testing Teams in WSLH Communicable Disease Division

- **Serology**
  - Hepatitis C
    - WGS for outbreak detection

  - Sexually transmitted infections
    - Don’t sequence regularly
      - Cost and TAT can’t compete with high-throughput instruments
      - Perhaps sequence for research projects

https://www.molecular.abbott/int/en/products/instrumentation/alinity-m-instrument
https://diagnostics.roche.com/us/en/products/instruments/cobas-5800-ins-6638.html
https://www.hologic.com/hologic-products/diagnostic-solutions/panther-scalable-solutions

Examples: Four Main Testing Teams in WSLH Communicable Disease Division

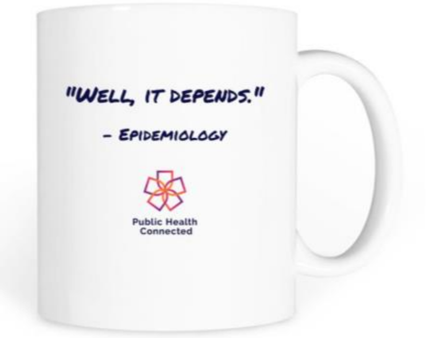
- **Virology**
  - SARS-CoV-2 and influenza
    - WGS for surveillance, but not every positive
  - Other respiratory viruses (PIV, hMPV, rhinovirus bocavirus)
    - Don’t sequence regularly
    - Perhaps sequence to support outbreak investigations
      - If one LTCF outbreak: not needed
      - If want pathogen relatedness across multiple facilities: yes
  - RSV
    - WGS for surveillance becoming more important
      - New vaccines and treatments

FDA NEWS RELEASE

FDA Approves First Respiratory Syncytial Virus (RSV) Vaccine

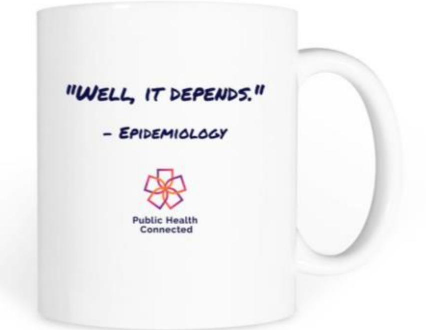
For Immediate Release: May 03, 2023

Sequence Every Positive?



- **Surveillance**
  - PulseNet (yes)
  - Influenza and SARS-CoV-2 surveillance (no)
- **Outbreak response**
  - CRAB in WI (yes)
  - *Cyclospora* outbreaks of 2018 (no)

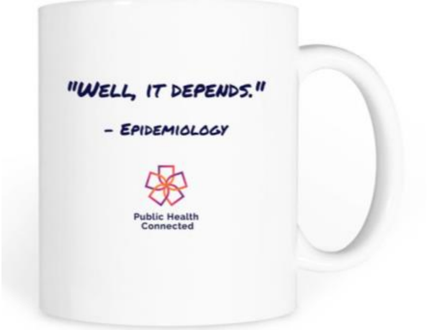
Sequence Every Positive?



- Surveillance
  - PulseNet (yes)
    - National surveillance for *Salmonella*/STEC/*Listeria*
    - Relatedness identifies clusters of cases with common exposures

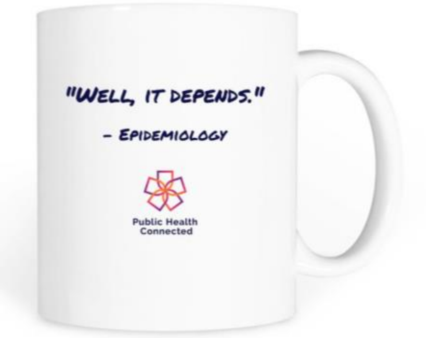
https://www.cdc.gov/pulsenet/index.html

Sequence Every Positive?



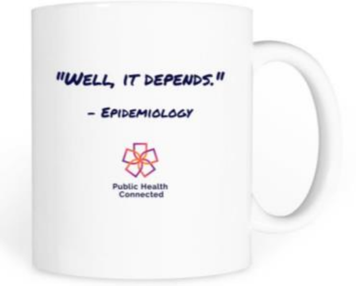
- Surveillance
  - Influenza and SARS-CoV-2 surveillance (no)
    - Be mindful of the burden on clinical labs sending positives to PHLs
      - Avoid heavy lifts on short-staffed clinical labs
      - Need strong relationships between clinical and PHL based on trust
      - Relationships and trust disappear if we ask for unreasonable things

Sequence Every Positive?



- Outbreak response
  - CRAB in WI (yes)
    - Increase in CRAB at a hospital and multiple LTCFs
    - Epidemiology showed patient transfers between facilities
    - WGS confirmed clonal outbreak of CRAB

Sequence Every Positive?



- Outbreak response
  - *Cyclospora* outbreaks of 2018 (no)
    - McDonalds (511 cases) and gas station (250 cases)
    - NGS useful to confirm two separate outbreaks
    - Once sources identified, NGS not needed on the other 500+ cases