Pathogen ID & Surveillance: Sequencing Roadmap Beyond COVID-19 at illumina

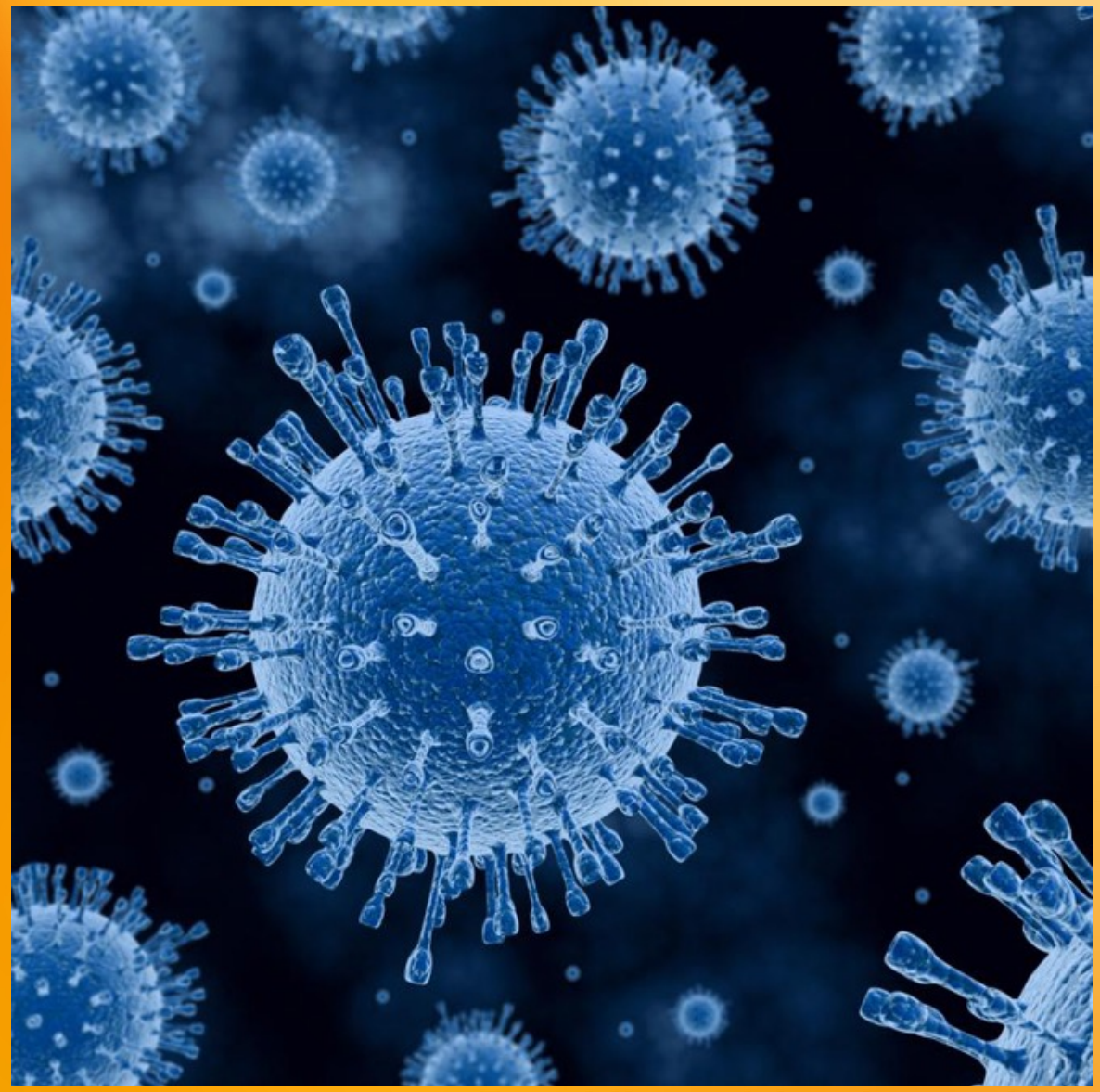
Let's Discuss!!!

Chris Detter – cdetter@illumina.com (505-699-1996)
AMR East & LATAM; Microbial and Infectious Disease Sales Specialist

Genome Trakr Meeting – Technology Talk

College Park, MD

10.20.2022



illumina Infectious Disease & Microbiology Specialist Team

- **Chris Detter** (505-699-1996; cdetter@illumina.com)

ID&M Specialist – US East and LATAM

- DoE Joint Genome Institute & Livermore National Lab
  - Part of Human Genome Program… R&D
- Los Alamos National Lab – Genome Center Director
  - Focus on Infectious Disease Genomics….and SFAF
- Defense Threat Reduction Agency (DTRA)
  - Built Genome centers abroad
- MRIGlobal
  - Chief Scientist R&D for Biosurveillance and Infectious Disease
  - Applied development of Biosurveillance using Genomics

BAYLOR

- **Jason Smith** (858-531-8182; jsmith6@illumina.com)

ID&M Specialist – US West and Canada

- Illumina: Sequencing Specialist – US-West
- Past Companies: PacBio, Ion Torrent, Applied Bio, LLNL
- Location: Reno, NV
- Expertise: Microbiology (UCSB) , Computer Science (CSU Chico), Next-Generation-Sequencing (Field Sales/Support)

UCSB
UC SANTA BARBARA

Emerging and Reemerging Infectious Diseases

List of priority infectious diseases that threaten global health security

Viral infections

Pandemic influenza
COVID-19[1]
Middle East respiratory syndrome[1]
Severe acute respiratory syndrome[1]
Marburg[1]
Ebola[1]
Lassa fever[1]
Viral hemorrhagic fevers
Rift Valley fever[1]
West Nile fever
Dengue
Polio
Zika[1]
Nipah and henipavirus diseases[1]
Chikungunya[1]
Measles
Yellow fever
Viral hepatitis

Bacterial infections

Tuberculosis[1]
Invasive meningococcal disease
Invasive pneumococcal disease
Cholera
Typhoid
Diphtheria
Pertussis (whooping cough)

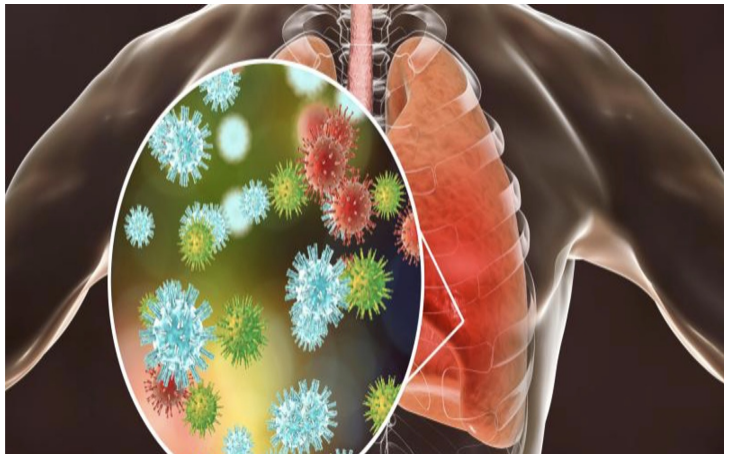
Drug-resistance

Drug-resistant bacterial, viral, and protozoal infections
Drug-resistant malaria
Antiretroviral-resistant human immunodeficiency virus/AIDS

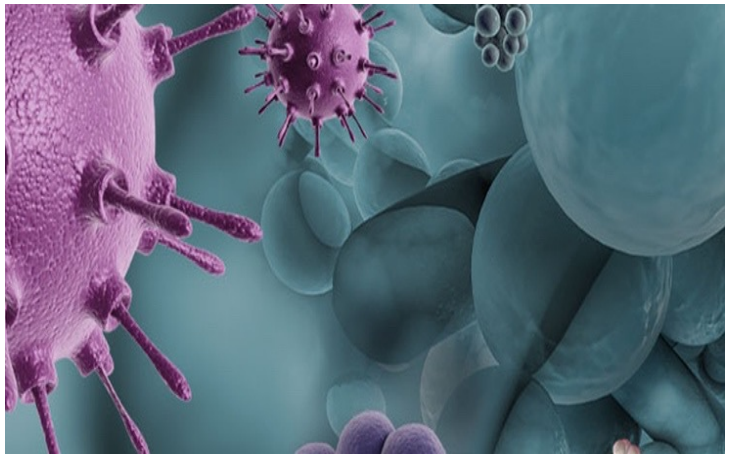
1 - WHO Blueprint priority disease.

Zumla A, Hui DSC. Emerging and Reemerging Infectious Diseases: Global Overview. *Infect Dis Clin North Am*. 2019;33(4):xiii-xix. doi:10.1016/j.idc.2019.09.001

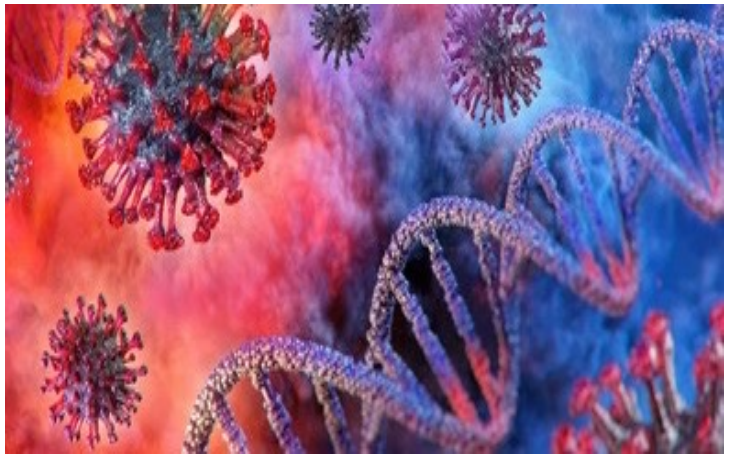
2022 Infectious Disease & Microbial Focus at Illumina



Infectious Disease Testing



Public Health



Microbiology Research

Illumina Developed

Partnerships

Commercial Enablement

Next-Generation Sequencing Unlocking the Power of the Microbial Genome
> APPLICATIONS

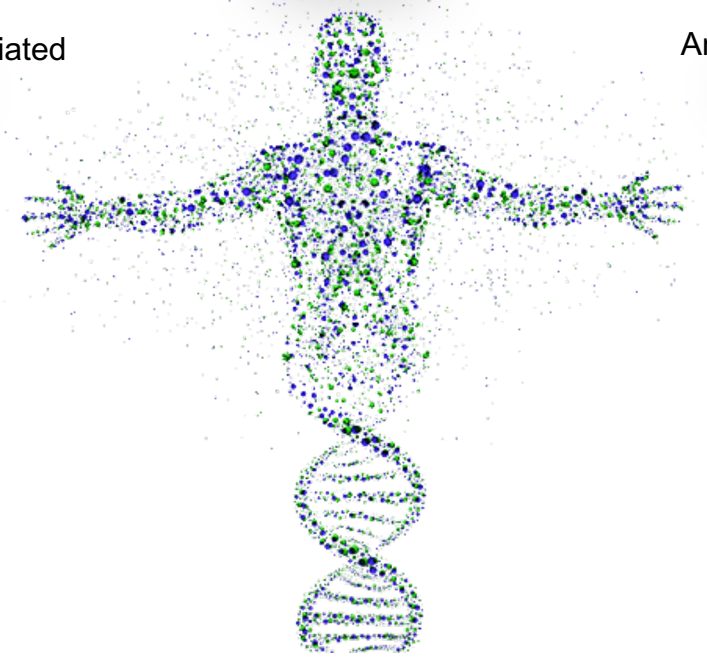
Disease of unknown Etiology

Healthcare Associated Infections

Antibiotic Resistance

Clinical

Respiratory Pathogens



Foodborne Pathogens

Wastewater Surveillance

Illumina technology has been used in the fight against COVID-19 at all stages of the pandemic

JANUARY 2020

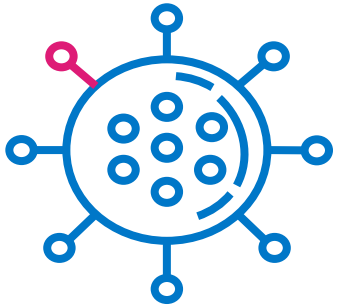
IDENTIFICATION OF NEW VIRUS CAUSING COVID-19 WITH ILLUMINA PLATFORM

Discovery

JUNE 2020

ILLUMIAN DEVELOPS FIRST SEQUENCING BASED COVID-19 DIAGNOSTIC TEST **COVIDSeq™**

Detection



SINCE LATE 2020

PUBLIC HEALTH AND RESEARCH INSTITUTIONS USE ILLUMINA TECHNOLOGY TO CHARACTYERIZE NOVEL SARS-COV-2 VARIANTS **COVIDSeq, RVOP, RPIP, etc.**

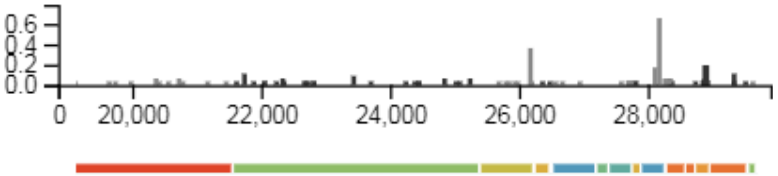
Surveillance

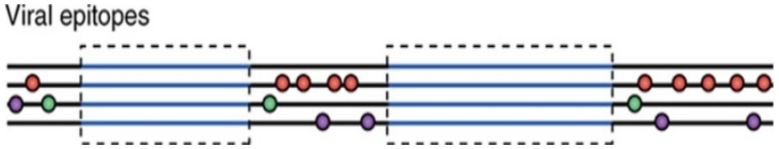
[redacted]

Beyond Positive Detection of Sars-CoV-2 by RT-qPCR or NGS

Are there mutations in the viral sequence?

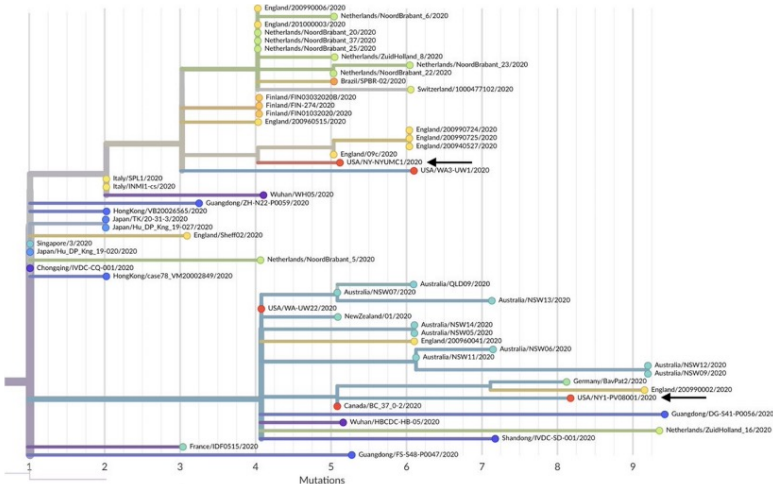
- Guidance for PCR-based testing revision
- Guidance for vaccine targets

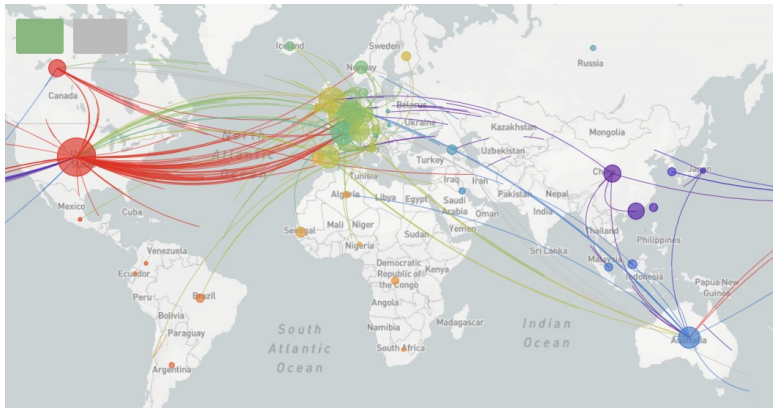




What was the route of transmission?

- Guidance for public health policy decisions





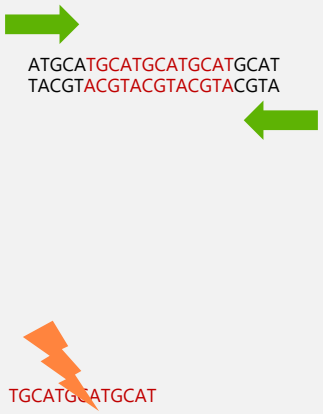
Source: GISAID

How fast is the virus mutating?

Are there any co-infections?

The Spectrum of Molecular Identification & Diagnostics

PCR



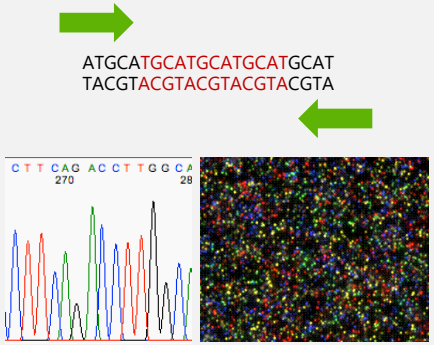
- Sensitive, Specific
- Fast and Cheap
- Very limited scope
- Low/No Mismatch Tolerance

Multiplex PCR

- Sensitive, Specific
- Fast and Cheap
- Limited scope
- Low/No Mismatch Tolerance

Amplicon Sequencing

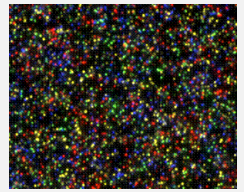
Low Mismatch Tolerance



- Amplicon panels
- Marker genes
- Limited breadth
- Primer interactions
- Amplicon dropout

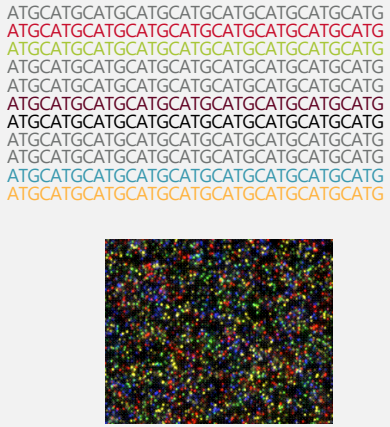
Precision Metagenomics

High Mismatch Tolerance



- Very broad
- All pathogen types detected
- Modest depth
- Very sensitive/specific
- Reliable AMR

Shotgun Metagenomics



- Broadest
- All pathogen types detected
- Deep sequencing
- Variable sensitivity
- Variable AMR detection

Breadth of Test

https://brcf.medicine.umich.edu/cores/advanced-genomics/faqs/sanger-sequencing-faqs/interpretation-of-sequencing-chromatograms/. https://arep.med.harvard.edu/Polonator/

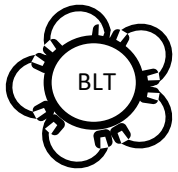
The Illumina RNA (DNA) Prep with Enrichment Workflow

Leveraging BLT technology to achieve rapid turnaround time without compromising quality

cDNA Synthesis

cDNA synthesis kit included (RNA)

Tagmentation



BLT rapid library prep generation

Capture

3-Plex, rapid single-hybridization

Quant & Norm

Completed libraries ready to load on any Illumina sequencer

Library Preparation*

9.5 (6.5) hours

Comparable to Illumina DNA Prep w/ Enrichment + cDNA Synthesis

MiniSeq Rapid 100-cycles kit

<5h

1x100

18 samples

MiSeq V3 150-cycles kit

~12h

1x75

24 samples

21h

2x75

NextSeq 550 Mid-Output 150-cycles kit

~9h

1x75

96 samples

15h

2x75

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*Approximate time for manual processing of 24 samples with 3-plex enrichment is ~9.5 from Total RNA to library with ~2 hours of hands-on time.

RVOP

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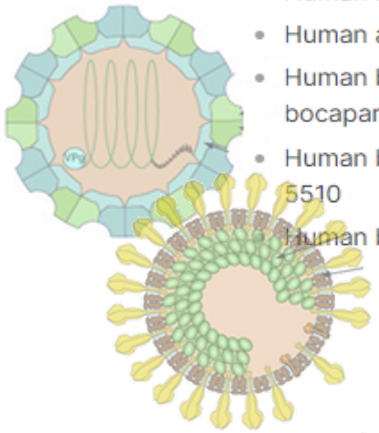
Respiratory Viral Oligo Panel (RVOP)

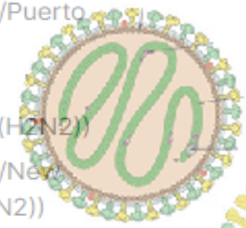
Comprehensive Detection of 40+ Respiratory Viral Whole Genomes

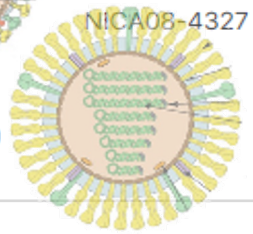
- 7,800 probes (80bp) tiling the full genome of common respiratory viruses, recent flu strains, and SARS-CoV-2
- Includes probes that target human mRNAs to act as positive controls

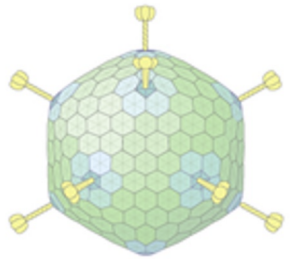
Respiratory Viruses Detected by Respiratory Virus Oligo Panel

- Human coronavirus 229E
- Human coronavirus NL63
- Human coronavirus OC43
- Human coronavirus HKU1
- SARS-CoV-2
- Human adenovirus B1
- Human adenovirus C2
- Human adenovirus E4
- Human bocavirus 1 (Primate bocaparvovirus 1 isolate st2)
- Human bocavirus 2c PK isolate PK-5510
- Human bocavirus 3
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a
- Human metapneumovirus (CAN97-83)
- Respiratory syncytial virus (type A)
- Human Respiratory syncytial virus 9320 (type B)
- Influenza A virus (A/Puerto Rico/8/1934(H1N1))
- Influenza A virus (A/Korea/426/1968(H2N2))
- Influenza A virus (A/New York/392/2004(H3N2))
- Influenza A virus (A/goose/Guangdong/1/1996(H5N1))
- Human bocavirus 4 NI strain HBoV4-NI-385
- KI polyomavirus Stockholm 60
- WU Polyomavirus
- Human parechovirus type 1 PicoBank/HPeV1/a
- Human parechovirus 6
- Human rhinovirus A89
- Human rhinovirus C (strain 024)
- Human rhinovirus B14
- Human enterovirus C104 strain: AK11
- Human enterovirus C109 isolate NICA08-4327
- Influenza A virus (A/Zhejiang/DTID-ZJU01/2013(H7N9))
- Influenza A virus (A/Hong Kong/1073/99(H9N2))
- Influenza A virus (A/Texas/50/2012(H3N2))
- Influenza A virus (A/Michigan/45/2015(H1N1))
- Influenza B virus (B/Lee/1940)
- Influenza B virus (B/Wisconsin/01/2010)
- Influenza B virus (B/Brisbane/60/2008)
- Influenza B virus (B/Colorado/06/2017)
- Influenza B virus (B/Washington/02/2019)
- Human control genes









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RPIP

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2

Respiratory Pathogen ID/AMR Panel (RPIP)

Comprehensive Detection of Respiratory Pathogens and Antimicrobial Resistance Genes

Explore Complete List of Bacteria, Fungi, and Viruses on our webpage

Powered by Explify® Platform IDbyDNA

>280 Pathogens

180+ Bacteria

40+ Viruses*

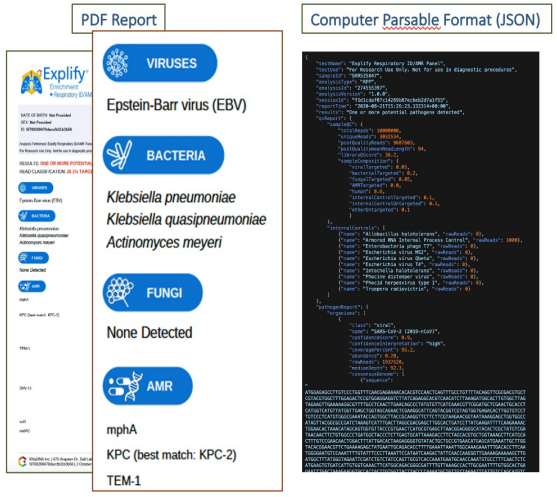
50+ Fungi

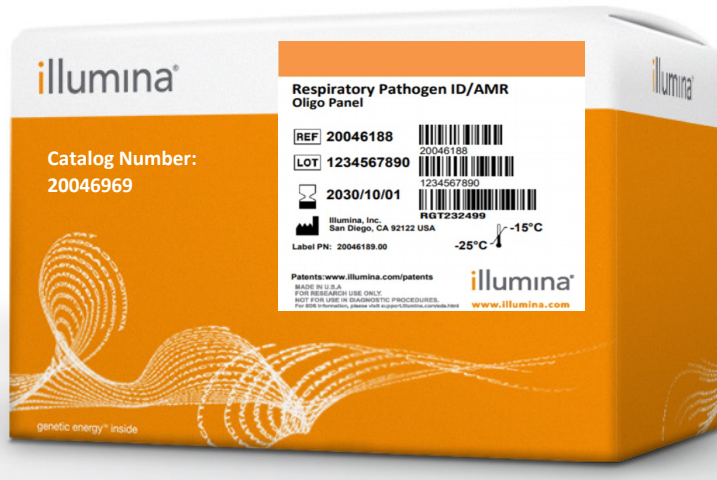
>16 Antibiotic Classes

50+ Antimicrobial Resistant Genes

1200+ Antimicrobial Resistance Alleles

*full genome coverage available for SARS-CoV-2 and flu viruses





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VSP

3

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Introducing Viral Surveillance Panel (VSP)

- 66 Viral species with 216 strains total (numbers in parenthesis)
- Oligo Hybrid Capture-80 bp oligos tiles across each genome for WGS Sequencing in full-length
- Flexible to mutations/strain variation of 20% due to hybrid capture technology
- Free to use DRAGEN Microbial Enrichment BaseSpace® app for analysis- including, consensus sequences, contig assembly, genome coverage and read counts
- Designed for broad viral genomic surveillance for a variety of sample types* including wastewater, environmental and post-clinical for public health

Arthropod transmitted / febrile tropical

Chikungunya(1)
Dengue Virus 1(1)
Dengue Virus 2(1)
Dengue Virus 3(1)
Dengue Virus 4(1)
West Nile Virus(2)
Yellow fever virus(1)
Zika(2)

Bloodborne

Hepatitis A(1)
Hepatitis B (1)
Hepatitis C(7)
Hepatitis E(1)
HIV 1(1)
HIV 2(1)
Torque Teno virus(1)

Encephalitis

Eastern equine encephalitis virus(1)
Hendra henipavirus(1)
Japanese encephalitis Virus(1)
Nipah Virus(1)
Tick-borne encephalitis virus(1)
Venezuelan equine encephalitis virus(1)
Western equine encephalitis virus(1)

Respiratory /Cardiopulmonary

Adenovirus(3)
Coronavirus -229E(1)
Coronavirus -HKU1(1)
Coronavirus- OC43(1)
Coronavirus-NL63(1)
Influenza A(7)
Influenza B(1)
Hantavirus(33)
MERS-CoV(1)
Metapneumovirus(1)
Parainfluenza(4)
Parechovirus(1)
Respiratory syncytial virus(1)
Rhinovirus(3)
SARS-COV(1)
SARS-COV-2(1)

Hemorrhagic fever

Chapare virus(1)
Crimean-congo haemorrhagic fever virus(1)
Ebola(1)
Guanarito Virus(1)
Rift Valley Fever Virus(3)
Junin Virus(1)
Kyasanur Forest disease virus(1)
Lassa fever virus(2)
Lujo Hemorrphagic Fever Virus(1)
Machupo Virus(1)
Marburg Virus(1)
Omsk hemorrhagic fever virus(1)
Sabia virus(1)

Enteric

Aichivirus (3)
Astrovirus(1)
Coxsackieviruses(28)
Enterovirus(28)
Norovirus(14)
Poliovirus(1)
Rotavirus(4)
Salivirus(1)
Sapovirus(1)

Oncolytic

Oncolytic HPV(14)
Polyomavirus(9)

Rash/Lesion

Monkeypox(2 - both clades)
Parvovirus(1)
Rubella(1)
Variola virus(1)

*** Note: Red Box indicates recent public health outbreaks world-wide**

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Viral Surveillance Panel - Applications

* Sample Type: Wastewater, Surfaces, Soil, Air, Clinical, Zoonotic, etc.

* We recommend ~1M reads for simple samples & ~5-10M reads for complex samples

Environmental Surveillance

- Emerging
- Outbreaks

- **Public Health**
- **USDA**
- **FDA**
- **BioDefense**
- **Customs Boarder Patrol**
- **Academic**
- **etc**

Clinical Surveillance

- Outbreaks
- Emerging
- Reflex

- **Public Health Labs**
- **Service Labs**
- **Government Labs**
- **Clinical Hospital Labs**
- **Academic Medical Centers**
- **etc**

Viral Metagenomics

- Research
- WGS

- **Academic**
- **Government**
- **etc**

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Pan-Corona

4

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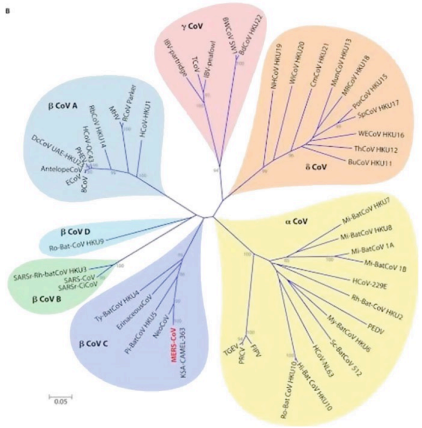
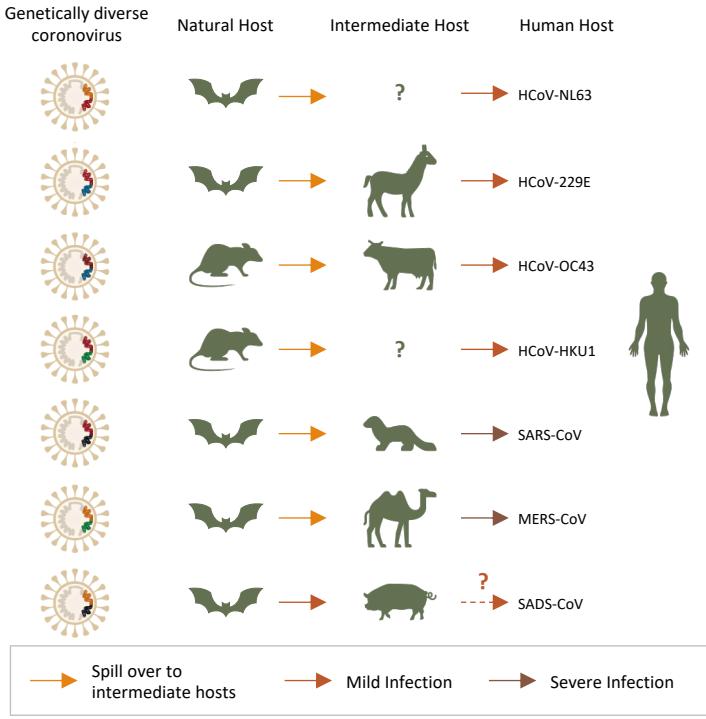
Introducing Pan-Coronavirus (Pan-CoV) Panel

- The Pan-CoV Panel allows for detection and **WGS of 203 known coronaviruses and over 370 strains of animal and closely related novel coronaviruses**, for surveillance of animal reservoir and sequencing of known human coronaviruses
- Hybrid capture panels allow for up to 20% variation in viral genome, allowing for mutations and novel strains to be detected
- At least one reference genome for each "branch" in the coronavirus phylogenetic tree is captured on the panel



Genera tiled end to end for genome enrichment and sequencing	Number of genus
Alphacoronavirus	69
Bafinivirus	1
Betacoronavirus	59
Deltacoronavirus	13
Gammacoronavirus	61
Grand Total	203

Illumina Pan-Coronavirus Panel (Pan-Cov Panel)



Species	Number of genomes In Pan-Coronavirus panel from known hosts
Bat	64
Rodent	32
Mink, Pangolin, Murine, Porcine, snake	28
Bird	25
Bovine/Camel/Goat/Horse	11
Dolphin/whale/fish	9
Human	7
Dog	7
Feline/Ferret	6
Hedgehog	2

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Pan-Coronavirus Panel - Applications

* Sample Type: Zoonotic, Wastewater, Surfaces, Air, etc.

* We recommend ~5-10M reads for complex samples

Environmental Surveillance

- Emerging
- Outbreaks

- **Zoonotic Researchers**
- **Public Health**
- **BioDefense**
- **Academic**
- **etc**

Viral Metagenomics

- Research
- WGS
- Discovery

- **Zoonotic Researchers**
- **Academic**
- **Government**
- **etc**

UPIP

5

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Urinary Pathogen Identification Panel (UPIP)

Powered by Explify™ UPIP

174

Pathogens

- **14** fungi
- **35** viruses
- **4** parasites
- **121** bacteria

46

Antibiotics

3,728
Antimicrobial Resistance Markers

explify

Bonus: Simultaneous detection of STI pathogens

27 sexually transmitted pathogens detectable within the same urine specimen & workflow

Exact numbers of pathogens and AMR markers may change with product updates/improvements.

Urinary Pathogen Identification Panel - Applications

* Sample Type: Urine, Swabs, Blood, Wastewater, etc.

* We recommend ~1M reads for simple samples & ~5-10M reads for complex samples

- UPIP provides **highly sensitive**, **culture-free identification and quantification** of both common and underrecognized uropathogens that can cause urinary tract infections.
- Also provides **Extensive antimicrobial resistance** information.

Clinical ID

- Chronic
- Undiagnosed
- Outbreaks

- **Public Health Labs**
- **Long Term Care Facilities**
- **Academic Medical Centers**
- **Commercial Service Labs**
- **Government Labs**
- **etc**

Clinical Surveillance

- Outbreaks
- Emerging
- Research
- Antimicrobial Resistance Monitoring (AMR)

- **Public Health Labs**
- **Government Labs**
- **Academic Research Centers**
- **etc**

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Illumina Infectious Disease Panel Roadmap – summary comparison

	Illumina Respiratory Oligo Virus Panel **RVOP**	Illumina Respiratory Pathogen ID/AMR Panel **RPIP**	Illumina Urinary Pathogen ID/AMR Panel **UPIP**	Illumina Pan-Coronavirus Panel **Pan-CoV**	Illumina Viral Surveillance Panel **VSP**
Target(s)	40 common respiratory viruses	282 Respiratory pathogens (viral, bacteria, fungi) and 2108 antimicrobial resistance markers	174 Urinary pathogens (virus, bacteria, fungi, parasite) and 3728 antimicrobial resistance markers	225 Coronaviruses, over 370 strains	66 viral pathogens, 216 strains
Whole Genome vs. Detection	Whole Genome	Detection of pathogens and AMR markers Whole genome: SARS-CoV-2, Influenza A/B	Detection of pathogens and AMR markers	Whole Genome	Whole Genome
Library Prep	RNA Prep with Enrichment	RNA Prep with Enrichment	DNA Prep with Enrichment	RNA Prep with Enrichment	RNA Prep with Enrichment
Software	RNA Pathogen App	Explify RPIP	Explify UPIP	DRAGEN Microbial Enrichment App	DRAGEN Microbial Enrichment App
Configuration	Standalone panel	Panel, Library Prep, Indexes Set A, B , C, or D	Panel, Library Prep, Indexes Set A, B , C, or D	Standalone panel (96 reactions)	Standalone panel (96 reactions) Panel, Library Prep and Indexes Set A or B

Apps Galore!

1) Plug & Play, 2) Easy to Share & Collaborate, 3) Simple Push-Button Analysis, 4) Automation & Integration, 5) Secure

Illumina Core Apps

- 16S Metagenomics
- GISAID Submission
- DRAGEN RNA Pathogen Detection
- DRAGEN COVID Lineage

BaseSpace Labs Apps

- Kraken Metagenomics
- Prokka Genome Annotation
- SRST2
- Velvet *de novo* Assembly
- String Graph Assembler
- ITS Metagenomics

BaseSpace 3rd Party Apps

- DRAGEN Metagenomics Pipeline
- MetaPhlAn
- SPAdes Genome Assembler
- CosmosID Metagenomics
- EzBioCloud 16S-based MTP
- DNA Star
- DeepChek-HBV, HCV, HIV
- Explify RPIP Analysis
- SEAR
- GENIUS Metagenomics: Know Now
- QIIME

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https://illumina.box.com/Metagenomics

Breadth of ID and Surveillance @ illumina…..Future Panels?!

A world of use cases for Next-Generation Sequencing (NGS) for infectious disease

Antimicrobial resistance: Microbial sequencing, by NGS, is well established in research and public health settings and is emerging as a component that can be used to predict antimicrobial resistance. (Ranson E., 2020)

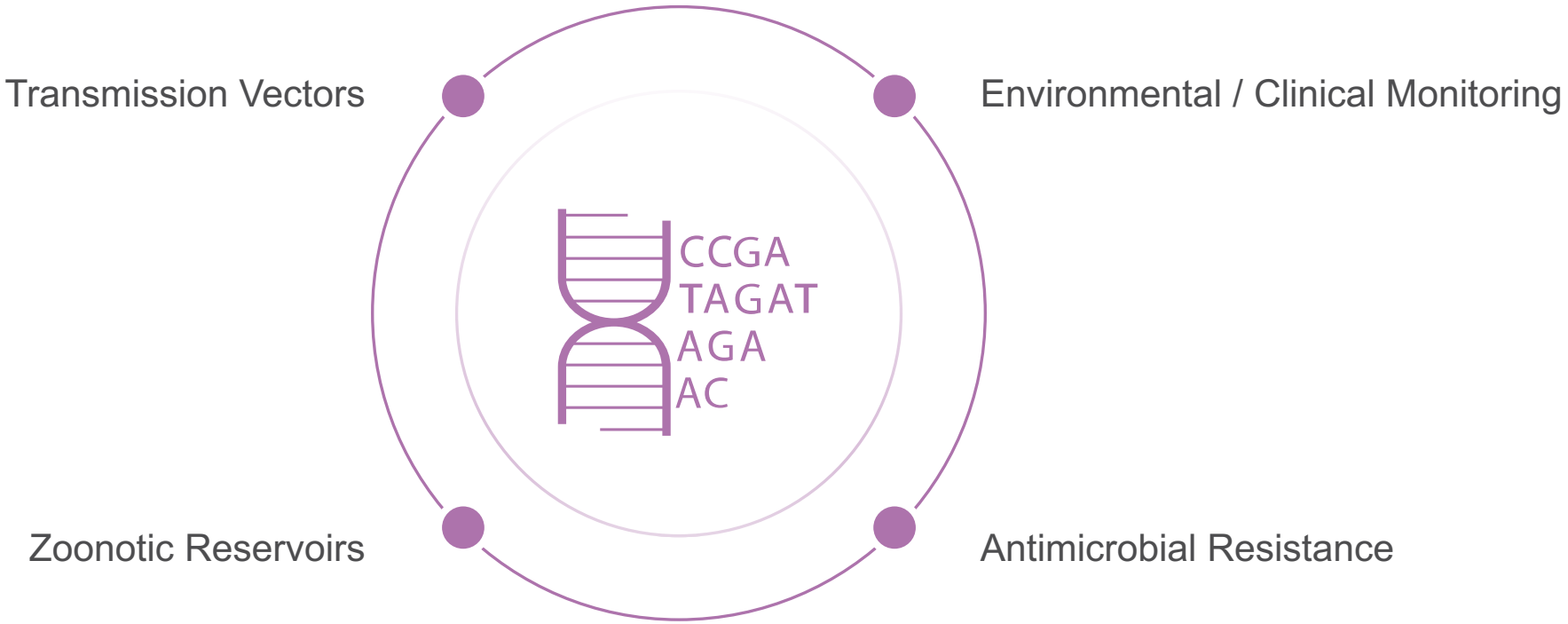
Early detection: NGS has been used in a vast array of analyses for infectious disease research of public health relevance to identify outbreak origins, track transmissions, investigate epidemic dynamics, determine etiological agents of a disease, and discover novel human pathogens (Berry, I. M., 2020)

Endemic Disease Surveillance: NGS is a great tool for vector control programs and public health environmental surveillance (Junqueira, A.C.M., 2017). That technology and bioinformatics tools offer new opportunities for Emerging Infectious Diseases surveillance in quality and in scale. (Kwok, K.T.T, 2020)

Wastewater: NGS can provide a comprehensive insight into microbial communities and their functional capacities in water and wastewater systems, thus eliminating the need to develop a new assay for each target organism or gene. (The Water Research Foundation, 2020)

Foodborne: Whole-Genome Sequencing provides information beyond the identity and relationship of strains; it can also help public health by improving the safety, quality, and shelf life of foods. (Brown, E., 2019)

Applying Genomics in Surveillance of Pathogens



Importance of Wastewater Surveillance for Public Health

Community-level monitoring of infectious diseases and detection of emerging pathogens

- Asymptomatic population is included despite not seeking medical care/getting clinically tested
- Track which variants are dominant in a community
- Whole community-level view of infectious diseases regardless of access to healthcare
- Discover emerging microbes in a population
- Detect pathogens in community before hospitals can detect surges (pandemic preparedness)
- Find antimicrobial resistance (AMR) genes

Wastewater Surveillance with Illumina NGS - Workflow

https://www.illumina.com/destination/wastewater-surveillance-webinar-series.html

Sample Collection

24 hour wastewater influent collected from various treatment plants across the USA

RNA Extraction + Quantification

RNA was extracted using a Qiagen RNAEasy protocol. qPCR according to the N1 CDC protocol

Library Prep-in Duplicates

COVIDSeq™ Assay (included v3 Primers)

OR

COVIDSeq™ Assay with v4 Primers

OR

Illumina RNA Prep with Enrichment Kit + Respiratory Virus Oligo Panel v2

Sequencing

2 × 150 bp read length on the MiSeq, 1M reads/sample

2 × 150 bp read length on the NextSeq, 10 M reads/sample

Data Analysis

BaseSpace DRAGEN™ COVID Lineage app

Kallisto pipeline: Not available on BaseSpace Nature Biotechnology **34**, 525–527 (2016), doi:10.1038/nbt.3519

BaseSpace DRAGEN™ RNA Pathogen App

3rd Party Solutions

- Health Care Acquired Infections
- TB - GenoScreen

Illumina Developed

Partnerships

Commercial Enablement

illumina®

Enablement through 3rd party solutions

Healthcare Acquired Infections (HAI)

BioMerieux

- Web based software solution to process bacterial whole genome sequence data (WGS).
- Detailed genetic characterization to accurately determine and track 13 of the most common bacteria found in healthcare settings.
- Uses illumina's WGS pipeline!

Mycobacterium tuberculosis (mtb)

GenoScreen

- An all-in-one assay based on targeted sequencing
- **DIRECT from Clinical Samples….no culture required!**
- IDs more than 140 mycobacterial species
- Prediction of resistance to 15 anti-TB drugs
- Uses illumina's Library Prep and Sequencers!

Enablement of our Solutions

- MiSeq
- NextSeq 1000/2000
- Infinity Long Reads
- New SBS Chemistry

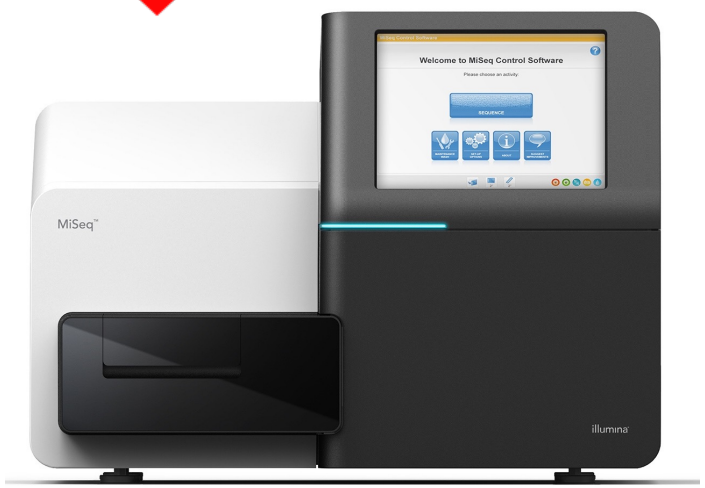
Illumina Developed

Partnerships

Commercial Enablement

illumina®

MiSeq



Key Applications

- Bacterial & viral (small) genomes
- 16S profiling
- Targeted regions, amplicon panels

	# paired reads **Millions**	kits available **Cycles**
Nano	1	300, 500
Micro	4	300
v2	15	50, 300, 500
v3	25	150, 600

Foodborne Pathogen Outbreaks

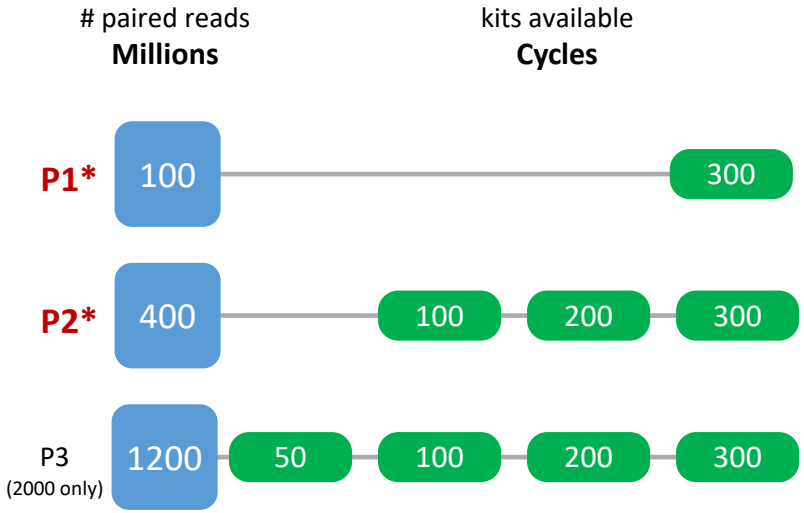
Public Health Genomic Epidemiology

Genomic Epidemiology

NextSeq 1000/2000 - will enable a broader range of applications and cost savings

Key Applications

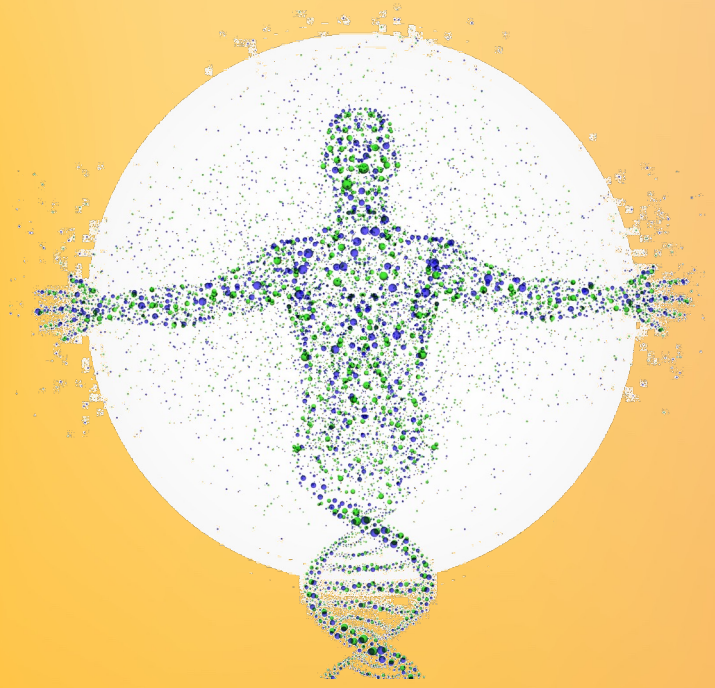
- Larger genomes (i.e fungal)
- Metagenomics
- Whole Genome Sequencing at Scale
- Multiplexing many genomes
- Cost reduction via multiplexing



NEW

- **P1 and P2 with 600 cycle this year**
  - ~35hrs vs 65hrs (MiSeq)
  - ~same price, but 4x more data
- **P4 coming 2023/4 with new SBS Chemistry.**
  - Patterned flow cell like NovaSeq….future proof
- **NO Washes. Single, disposable cassette**
- **DRAGEN on-board for 6x faster secondary analysis**

Thank You!



Questions?