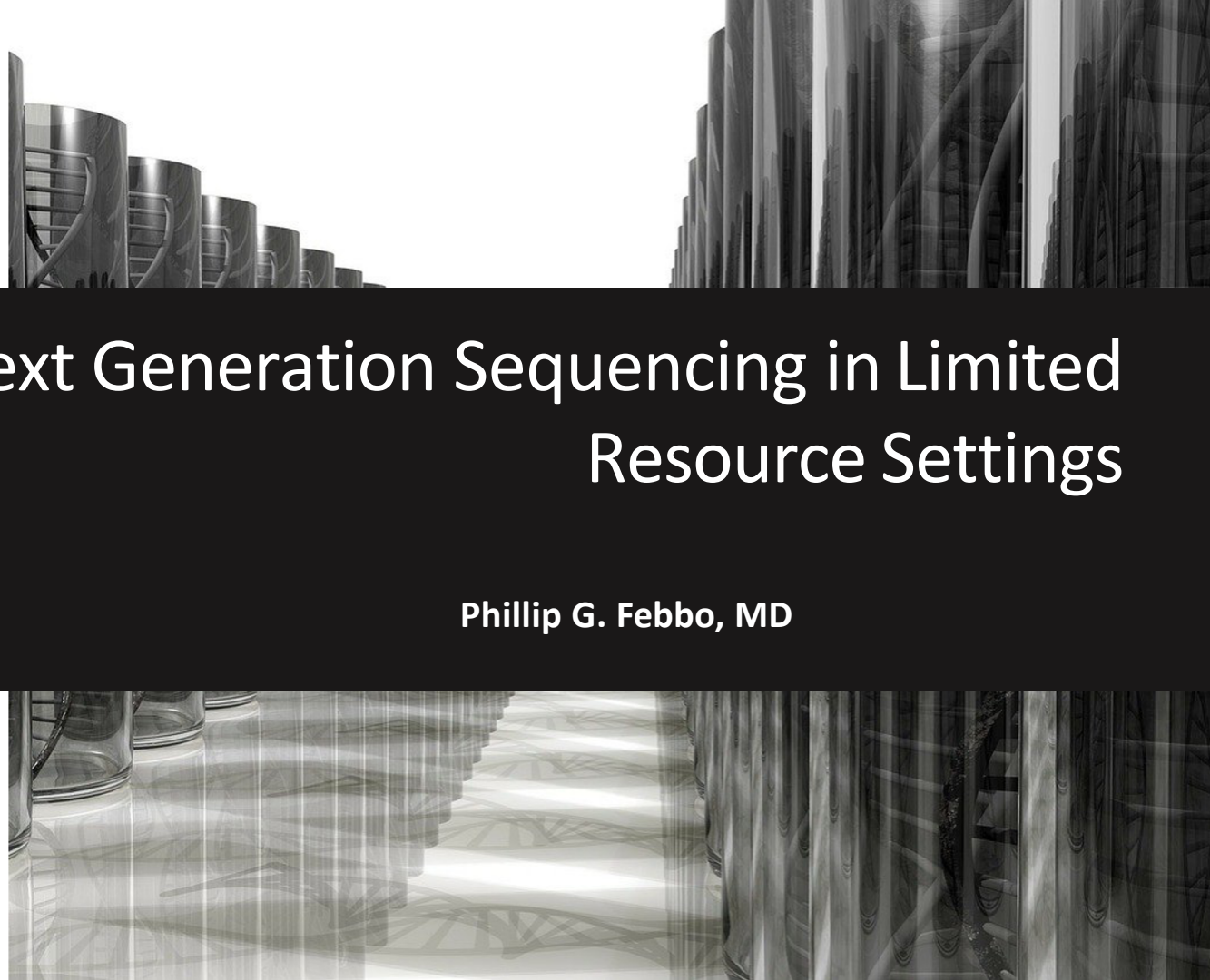
May 8, 2020

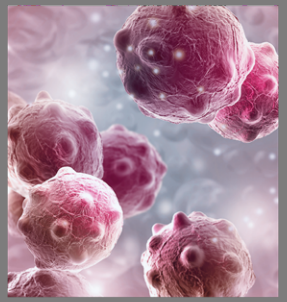


Next Generation Sequencing in Limited Resource Settings

Phillip G. Febbo, MD

Global Genomic Medicine Collaborative

Illumina's Mission

Unlocking the power of the genome to improve human health



illumina®

Genomic Research & Testing is Unlocking Insights

Empowering genetic analysis and facilitating a deeper understanding of genetic variation and function to drive advancements in medicine, agriculture, and many other areas

Genomics Research Today

Takes place in government, university, pharmaceutical, biotechnology, and agrigenomics laboratories around the world, to better our understanding of the relationship between gene sequence and biological processes

Advancing Healthcare

Researchers who investigate human and non-human genetic variation to understand the mechanisms of disease are enabling the development of more effective diagnostics and therapeutics

Advancing Quality of Life

Research also provides greater insight into genetic variation in plants (e.g., food and biofuel crops) and animals (e.g., livestock and domestic), enabling improvements in crop yields and animal breeding programs.

illumina®

Potential Genomics Use Cases in Country

Infectious Disease

Drug resistance testing to influence best course of treatment – improvement over current culture-based testing

Infectious Disease Surveillance

More comprehensive surveillance that monitors pathogens and antimicrobial resistance

Agriculture

Advance breeding programs for local plant & animal species

Oncology

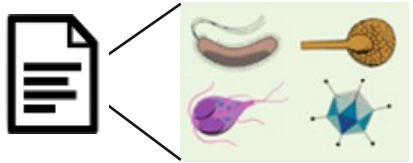
Screening test for hereditary risk of cancer to identify high-risk individuals in population (eg, breast, colon cancer)

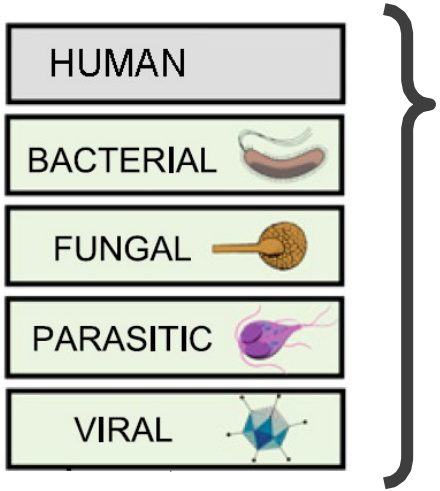
Genetic Disease

Facilitate early detection and intervention

illumina®

Interrogation of Nucleic Acid Sequences Allows for Unbiased Detection of Organisms





Total Nucleic Acid Extraction

DNA

RNA → cDNA

Molecular assay & Sequencing

?

Sequence Data

Sequence Database

illumina®

Clinical Microbiology Workflow and Applications using NGS

Isolate Sequencing

Whole genome sequencing of a single culture

de-novo assembly and gene annotation

Targeted Panels

Enrichment of a specific set of targets (organism, functional genes, antimicrobial resistance)

Shotgun Metagenomics

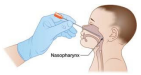
Whole genome sequencing directly from microbiology clinical samples

Comparison to reference microbial genome databases

illumina®

Basic Workflows for Cultured/Direct from Patient Samples

Specimen



NPS, BAL, etc

Viral culture

Extraction

Viral Extraction Kit

Library Prep

Shotgun:
Nextera XT, TruSeq Stranded Total RNA, Nextera DNA Flex

Enrichment:
Nextera DNA Flex for Enrichment, RNA Exome

Library

Sequencing

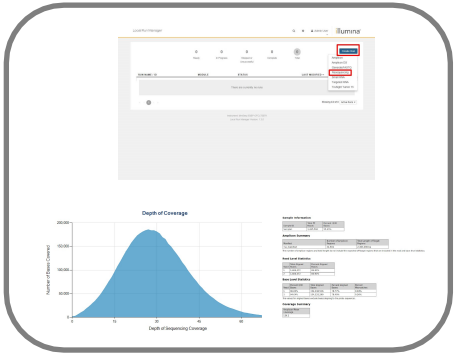
NextSeq

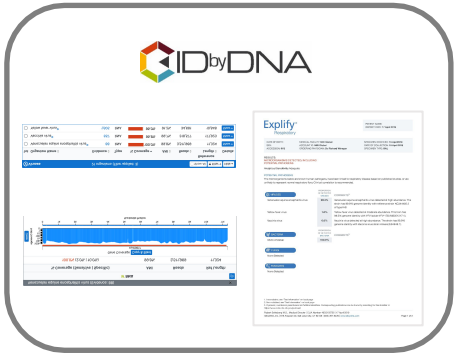
MiSeq

MiniSeq

iSeq

Local Data Analysis





Cloud-Based Data Analysis

illumina





Popular Applications & Methods	iSeq 100	MiniSeq	MiSeq Series ✪	NextSeq 550 Series ✪	NextSeq 2000
	Key Application	Key Application	Key Application	Key Application	Key Application
Large Whole-Genome Sequencing (human, plant, animal)					
Small Whole-Genome Sequencing (microbe, virus)	●	●	●	●	●
Exome & Large Panel Sequencing (enrichment-based)				●	●
Targeted Gene Sequencing (amplicon-based, gene panel)	●	●	●	●	●
miRNA & Small RNA Analysis	●	●	●	●	
DNA-Protein Interaction Analysis (ChIP-Seq)			●	●	●
Methylation Sequencing				●	●
16S Metagenomic Sequencing		●	●	●	●
Metagenomic Profiling (shotgun metagenomics, metatranscriptomics)			●	●	●
Cell-Free Sequencing & Liquid Biopsy Analysis				●	●
Run Time	9.5–19 hrs	4–24 hours	4–55 hours	12–30 hours	24-48 hours
Maximum Output	1.2 Gb	7.5 Gb	15 Gb	120 Gb	300 Gb*
Maximum Reads Per Run	4 million	25 million	25 million †	400 million	1 billion*
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp	2 × 150 bp

- **Shotgun metagenomic workflows are most efficiently performed on the MiSeq and NextSeq550**
- **Enrichment workflows can be performed on lower-throughput instruments, such as MiSeq, MiniSeq, and iSeq**

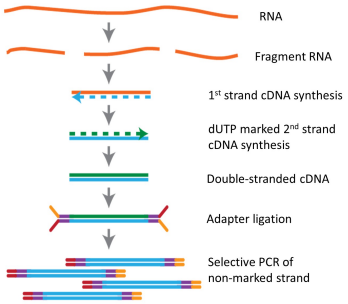
illumina®

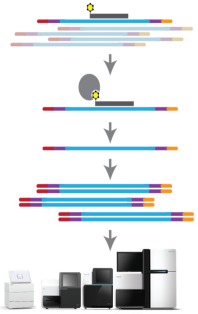
Hybridization Capture - Management of Disease Outbreaks

Pelican box with foam padding used for transport of the iSeq100

iSeq100 running samples in the DRC genomics lab

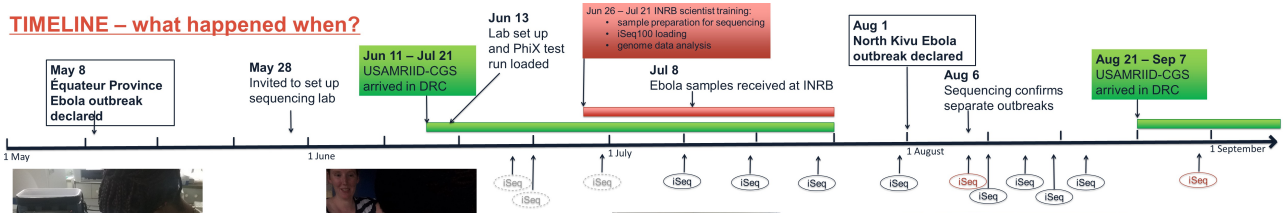
METHOD – stranded RNA-seq with targeted enrichment





iSeq100:
- 17 hour run time
- Dual-indexing
- up to 2 x 151 cycles

TIMELINE – what happened when?

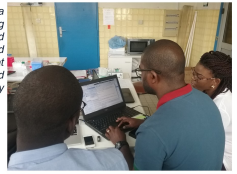


Preparing samples for sequencing

Power failure - loading the iSeq100 reagent cartridge by torchlight

Starting a run on the iSeq100

Performing data analysis using pipelines and scripts pre-loaded on a laptop that was hand-carried into country



iSeq100 loaded with:
- iSeq Non-Ebola samples
- iSeq Samples from Équateur outbreak (May 8 – Jul 24)
- iSeq Samples from North Kivu outbreak (Aug 1 – ongoing)

illumina®

Example: Infectious Disease

TB Drug Resistance Testing to Influence the Best Course of Treatment

- Drug-resistant TB is a public health crisis and focus for the WHO and country TB programs
  - Globally, 3.4% of new TB cases and 18% of previously treated TB cases had drug-resistant TB[1]
- Rapid detection & screening is done with cheaper methods. **However, these methods cannot resolve complex drug resistance, which is essential to guide patient care.**
  - Comprehensive drug susceptibility testing (DST) is performed after patients test positive for TB and then positive for resistance to first-line therapy (rifampicin-resistant TB)
  - Today, comprehensive DST is performed using culture, which takes 4+ weeks
- Opportunity for NGS tests to replace conventional DST: accurate and more rapid results for both first-line and second-line anti-TB drug[1] vs. culture

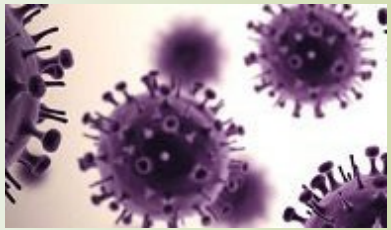
1(1)WHO 2019 report, pg. 2 and pg. 57, respectively

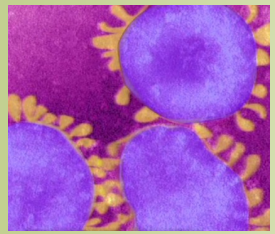
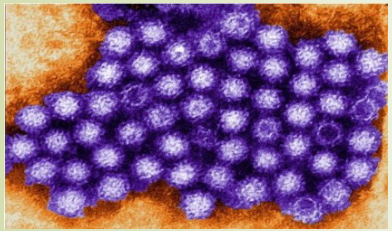
Example: Infectious Disease Surveillance

Tracking Pathogens and Antimicrobial Resistance

- Traditional methods of assessing infectious agents are typically limited to a small & defined set of organisms
- NGS provides a universal, hypothesis-free method for infectious disease surveillance that can be used with viruses, bacteria, or parasites
- NGS is also able to better identify & monitor antimicrobial resistance

- **Future opportunity in HIV drug resistance testing**
  - HIV/TB co-infection rates are high, and HIV is another global health priority (95-95-95 UNAIDS goal)
- **Additional opportunity for Surveillance panel**
  - Public Health (e.g. CDC Africa; Flemming Fund) and professional societies are strengthening surveillance efforts in Africa from One-Health perspective
  - Long-term opportunity for a “pan-infectome” and antimicrobial resistance (AMR) NGS panel approaches for broad detection of pathogen AMR, mainly starting in surveillance

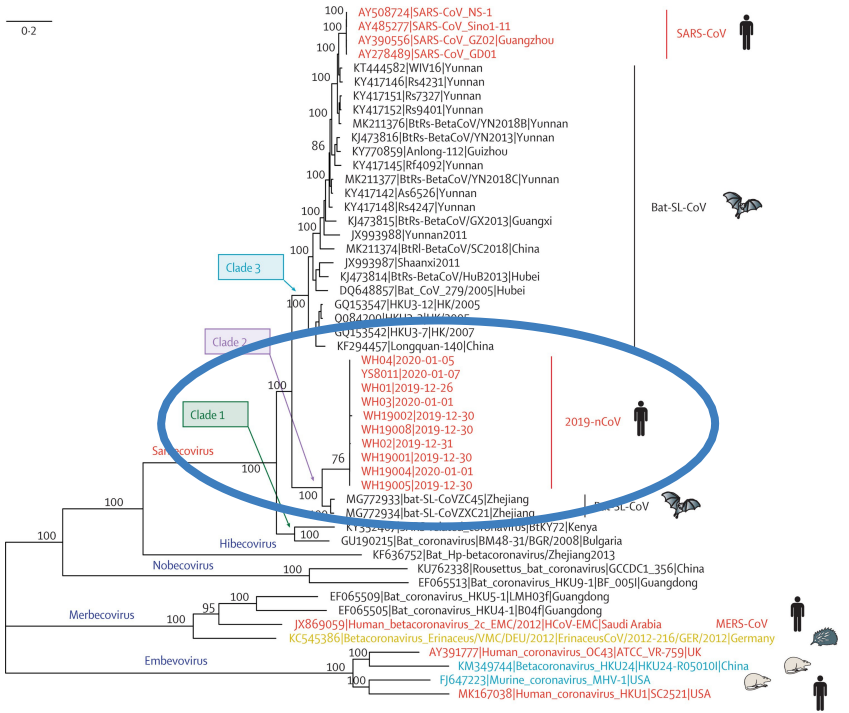


(1) WHO 2019 report, pg. 2 and pg. 57, respectively

Use of Clinical Metagenomics in the 2019-nCoV Outbreak

- **NGS was used to initially identify the unknown virus from Wuhan Province, China**
  - Shotgun metagenomic workflow enabled unbiased characterization of unknown viral pathogen
- **Bioinformatic analysis linked sequence to Betacoronavirus genus**
- **Phylogenetic tree showed a distinct species related to bat CoV and relatively distant from SARS and MERS**
- **Information concerned public health officials, as novel viruses have unpredictable transmissibility, morbidity, and mortality**

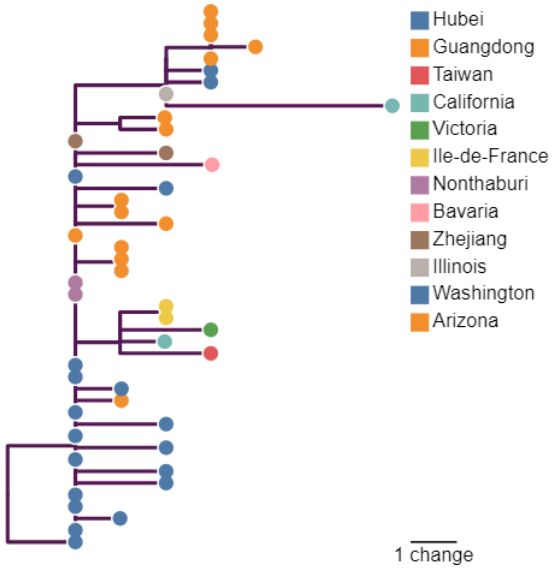


Lu, et al. The Lancet. https://doi.org/10.1016/S0140-6736(20)30251-8

illumina®

NGS in 2019-nCoV Outbreak Management

- **The use of NGS remains vital to ongoing outbreak management, to enable:**
  - Confirmation of all PCR positive samples, per guidance by WHO[1]
  - Viral evolution tracking
  - Monitoring for viral shift or potential to escape PCR diagnostic
  - Development of vaccine candidates
- **Sequencing can be performed from viral culture or directly from clinical specimens, such as BAL**



http://virological.org/t/phylodynamic-analysis-46-genomes-31-jan-2020/356

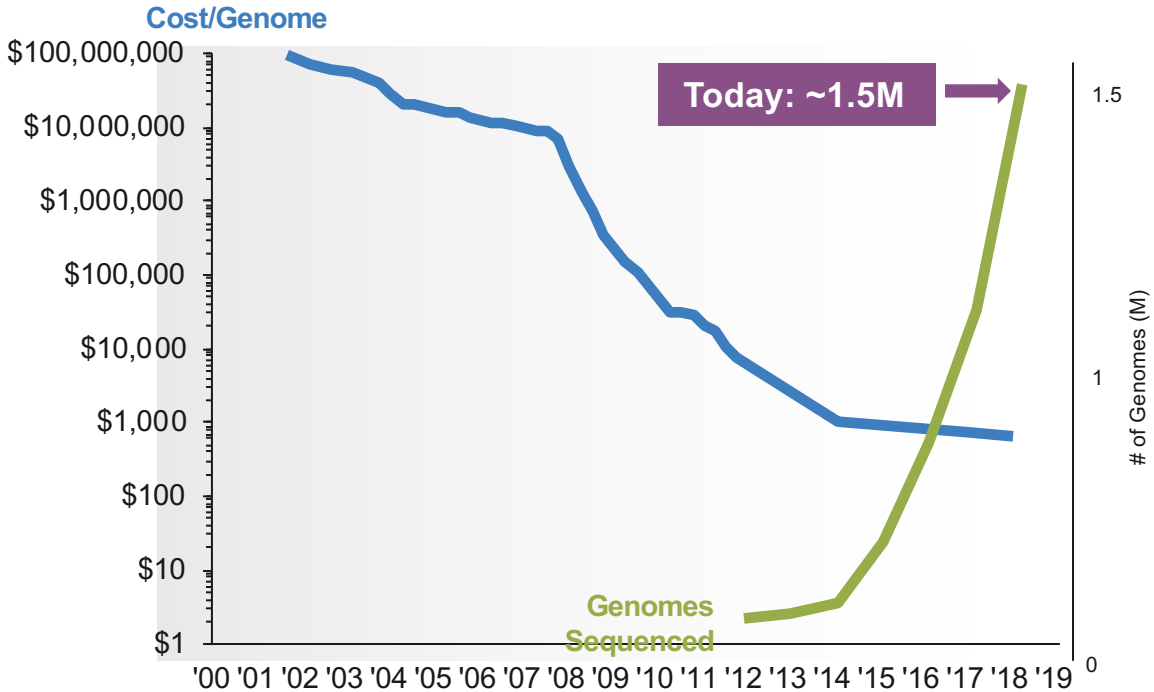
[1] WHO: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases Interim guidance 17 January 2020

illumina®

Illumina NGS Technology Has Helped Reduce the Cost of Sequencing While Providing Ability to Scale

Ability to **cost-effectively** sequence **large sample sizes quickly and accurately**, generating vast amounts of high-quality data.

These break-throughs in **cost** & **throughput** allow for greater adoption of sequencing, which further enables communities to unlock genomic insights that drive advancements

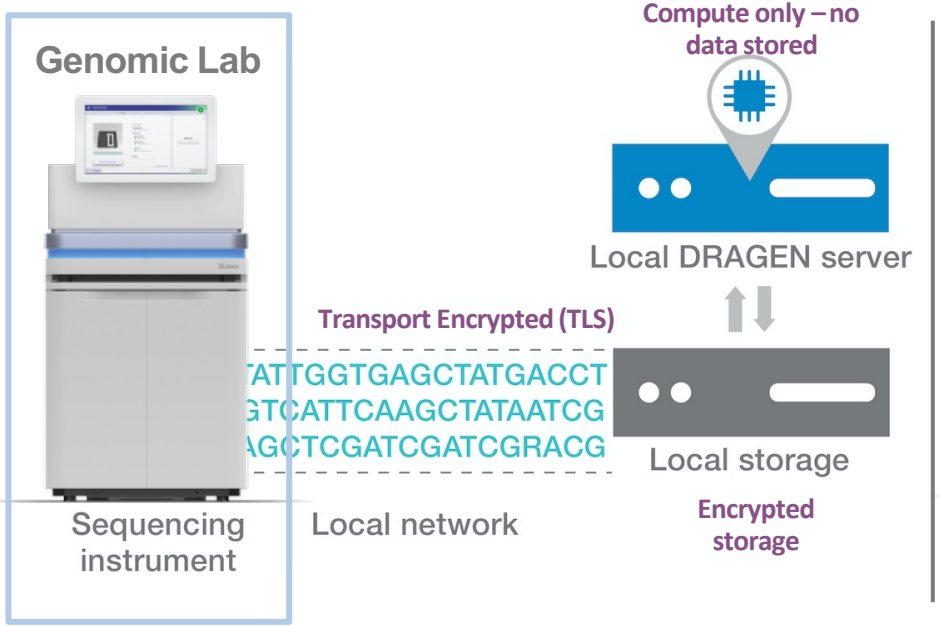


Country Data Infrastructure

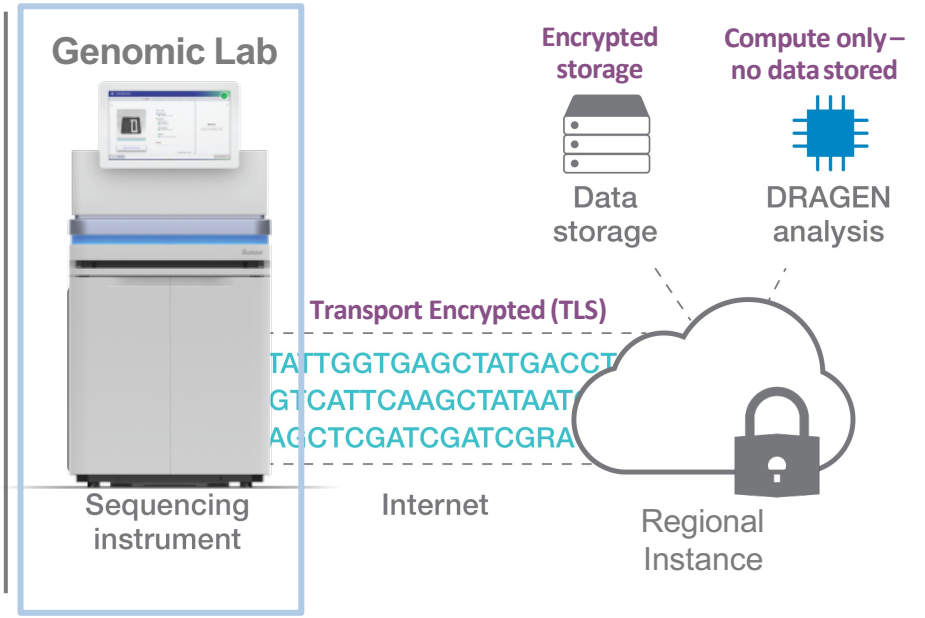
Local data stays local, and is encrypted and protected to international security standards

Two Potential Options for Storage & Security

Local In-Country Storage



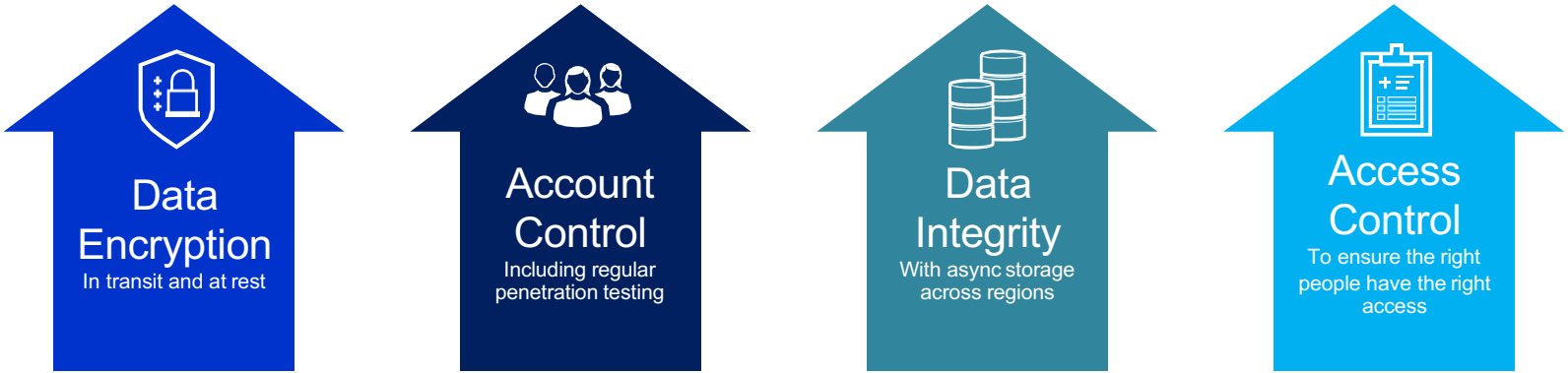
Country Cloud Instance*



15 *A cloud-based data center will be set up in-country, meaning that the data will physically reside within that country's borders

illumina®

Embedded Commitment to Data Security



illumina®

Rare and Undiagnosed Genetic Disease

When considered as a whole, genetic diseases are a global public health crisis

Individuals with HIV: **~40M**[4] | Number of Malaria cases/year: **212M**[4]

[1] https://www.omim.org
[2] Global Genes RARE Facts & Statistics: https://globalgenes.org/rare-diseases-facts-statistics/
[3] Rare Disease Impact Report: Insights from patients and the medical community. Shire. 2013
[4] World Health Organization. http://www.who.int/

illumina®

Diagnostic Yield Across Studies

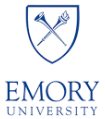
Study*	Diagnostic Yield	Notes
Yang et al 2013 NEJM	**~22%**	Clinic-based WES pilot study (N=250)
Yang et al 2014 JAMA	**~25%**	Follow-up to the above; N=2000, 504 with diagnosis; ~5% ACMG incidental findings return
Lee et al 2014 JAMA	**~25–30%**	Rare disease cohort of 814 patients; proband-only and trio sequencing
Srivastava et al 2014 Ann Neurology	**41%**	WES in a child neurology clinic
Gilissen et al 2014 Nature	**60%**	WGS in children with Intellectual Disability
Taylor et al 2015 Nature Genetics	**34% (57%)**	WGS of 68 Mendelian disorders (14 with trios)
Stavropoulos et al 2016 Genomic Medicine	**34%**	WGS of 100 Neurodevelopmental Delay patients; direct comparison to array
Retterer et al. 2016 Genetics in Medicine	**28–55% per indication**	3,040 consecutive WES cases at GeneDx
Vanderver et al. 2016 Annals of Neurology	**42% (+)**	WES of 71 children with white matter disease; family trio+
Tarailo-Graovac et al 2016 NEJM	**68%**	WES of 41 patients with intellectual developmental disorder and unexplained metabolic phenotypes. 44% had a change in care.
Tan et al 2017 JAMA Pediatrics	**52%**	WES of ambulant children with suspected monogenic conditions
Lionel et al 2017 GIM	**41%**	WGS and panel assessment of pediatric outpatients from diverse subspecialities

*Full citations available upon request

iHope Network

Donating clinical whole genome sequencing tests (cWGS) to help find answers for underserved families with children facing rare and undiagnosed diseases

https://www.illumina.com/company/ihope.html

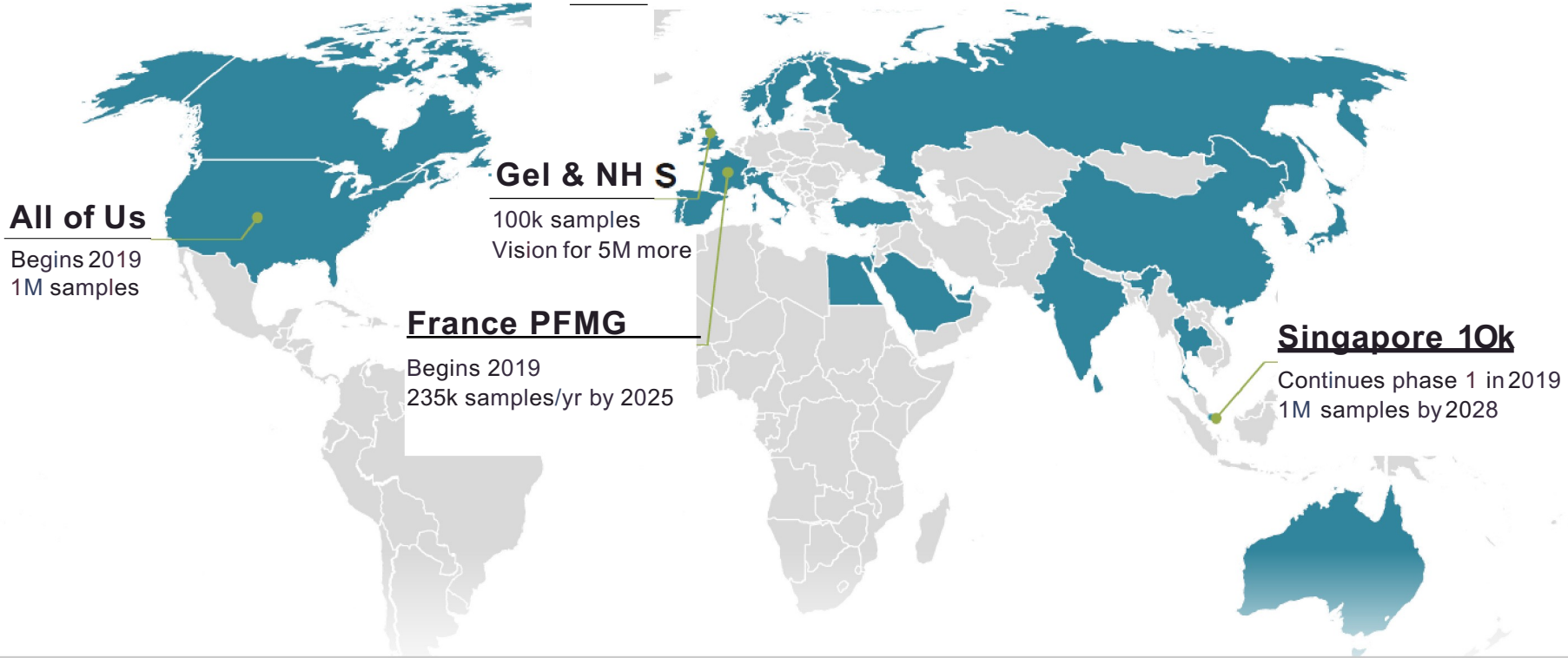


iHope: Hospital Infantil de Las Californias

- Clinical team is led by Dr Marilyn Jones and Diane Masser-Frye MS MSW
- Phenotyping and sample collection take place on “Genome Days”
- Thus far 60 cases have been processed
- Majority had no previous genetic testing
- Mutations span the mutational spectrum, but more than half were structural variations that would have been missed by many other tests
- Returned likely causal variants in 41 cases (68% diagnostic yield)
- Despite resource limitations, 29% had a change in management

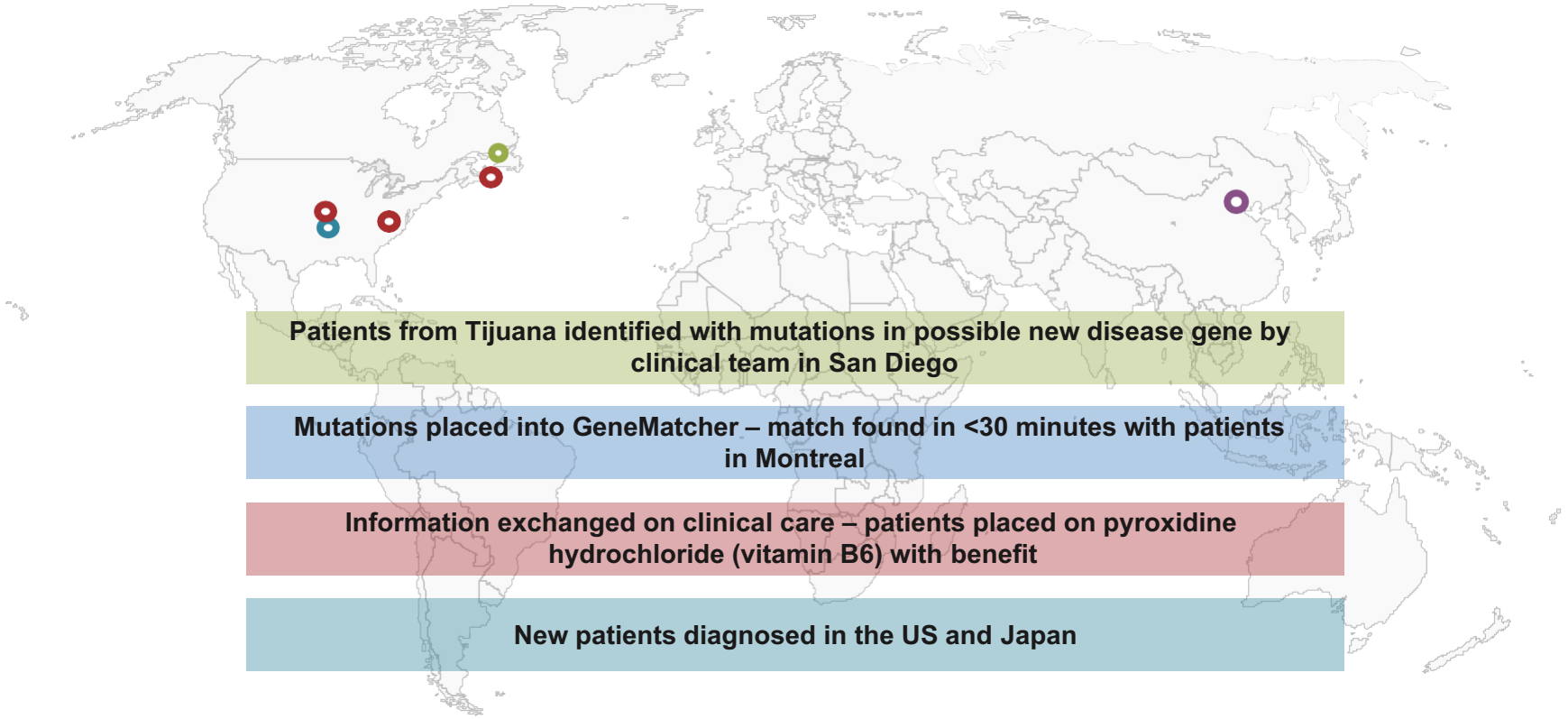
illumina

Global Emeirgence of Population Genomics



—50 Programs

A New Disease…a New Treatment



Patients from Tijuana identified with mutations in possible new disease gene by clinical team in San Diego

Mutations placed into GeneMatcher – match found in <30 minutes with patients in Montreal

Information exchanged on clinical care – patients placed on pyroxidine hydrochloride (vitamin B6) with benefit

New patients diagnosed in the US and Japan

illumina®

Why WGS is Ready for Prime Time

1. Most **comprehensive** methodology
2. **Simpler, faster** workflow than WES
3. The **best way to interrogate the exome** is through WGS
4. Better ability to detect ***de novo*** variants
5. Ability to identify **multiple genetic variants in single patient** contributing to phenotype
6. **Capable** of re-analysis of non-diagnosed patients

illumina®

Illumina Commitment to Quality and Patient Care

iHOPE Network

- Illumina launched consortium of member institutions in 2017
- iHope Network members have committed to a minimum philanthropic donation of 10 whole-genome tests per year (patients from a clinical referral network of >30 US and international sites)
- Agreed to donate the variants to public databases (e.g., Clinvar)

Undiagnosed Diseases Network

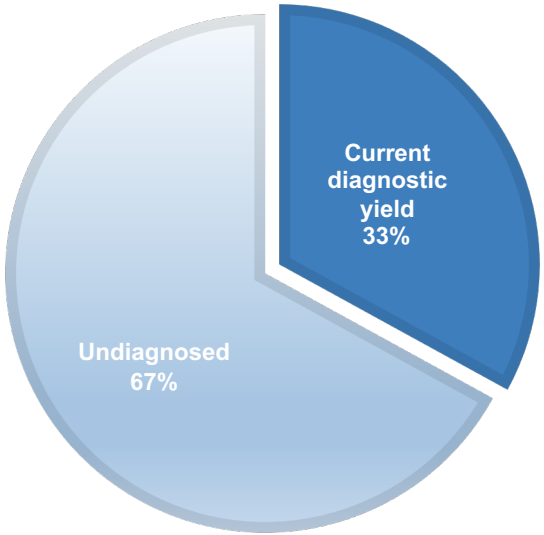
- Illumina is a supporter of the Undiagnosed Diseases Network (UDN)
- The UDN made up of clinical and research centers across the United States working to improve diagnosis and care of patients with undiagnosed diseases
- To date, the UDN has completed exome or genome sequencing for over 1000 patients and has published over 50 manuscripts

Medical Genome Initiative

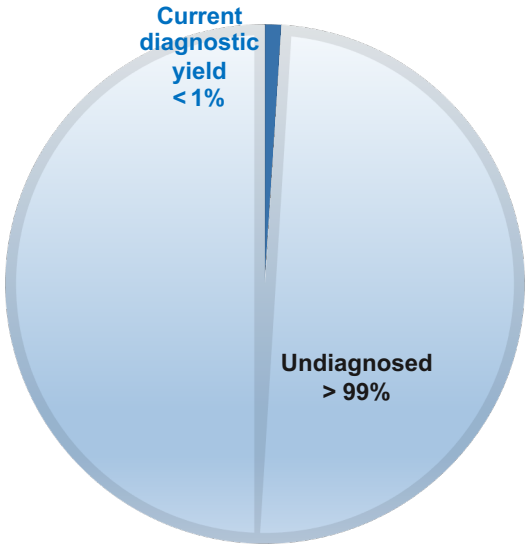
- Consortium from 8 leading health care and research organizations in the U.S. and Canada
- Focus on the publication of common laboratory and clinical best practices for the application of clinical Whole-Genome Sequencing
  - Baylor Genetics
  - Broad Institute of MIT and Harvard
  - HudsonAlpha Institute for Biotechnology
  - Illumina
  - Mayo Clinic
  - Rady Children's Institute for Genomic Medicine
  - The Hospital for Sick Children (SickKids Toronto)
  - Stanford Medicine

For Research Use Only. Not for use in diagnostic procedures.

Diagnostic yields remain very low



Current RUGD diagnostic yield:
33%

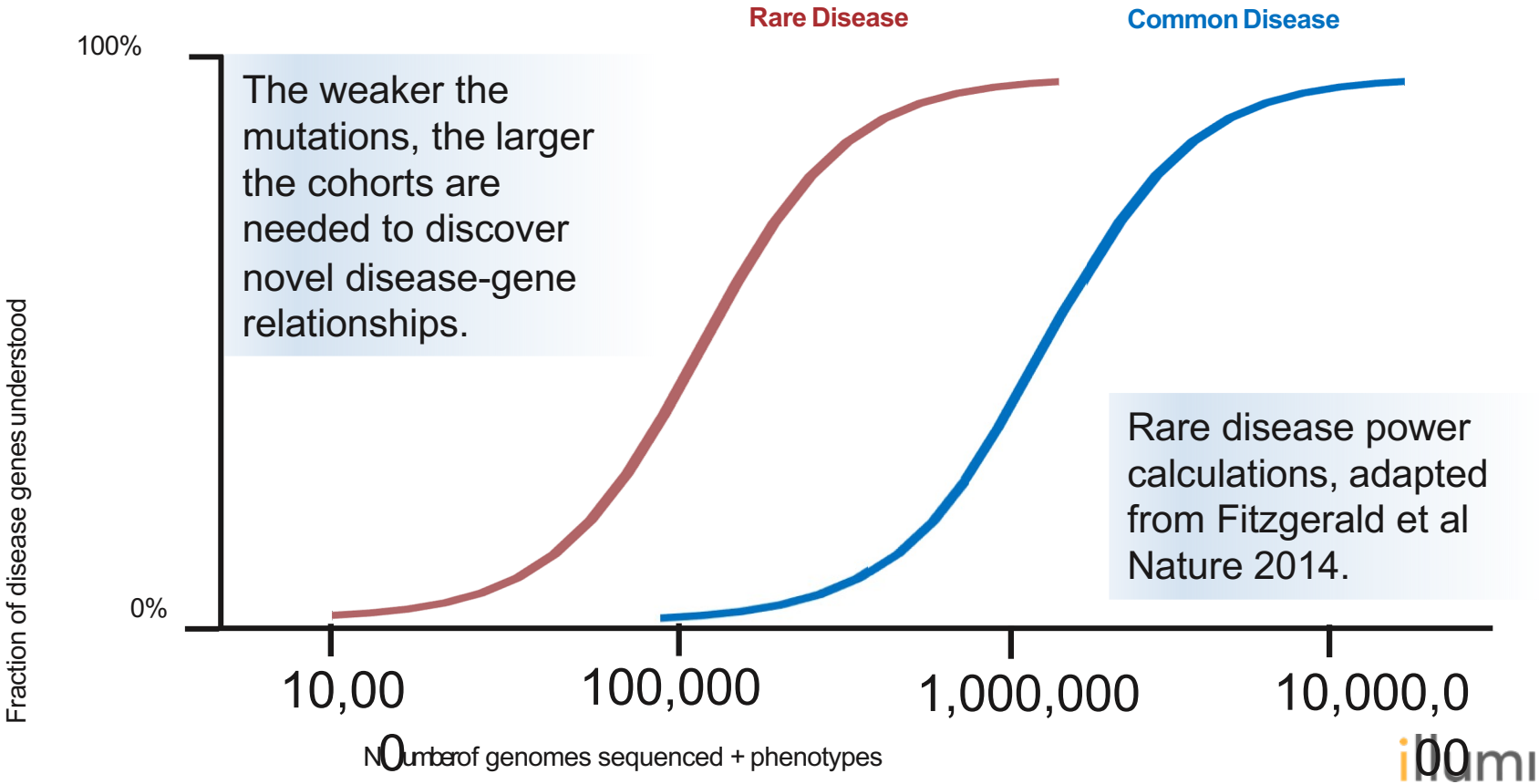


Current common genetic disease diagnostic yield:
< 1%

Yang et al, JAMA 2014, Clark et al, Genomic Medicine 2018, Fitzgerald et al, Nature 2014, Franke et al, Nature Genetics 2010, Sanders et al, Neuron 2015, Khera et al, Nature Genetics 2018

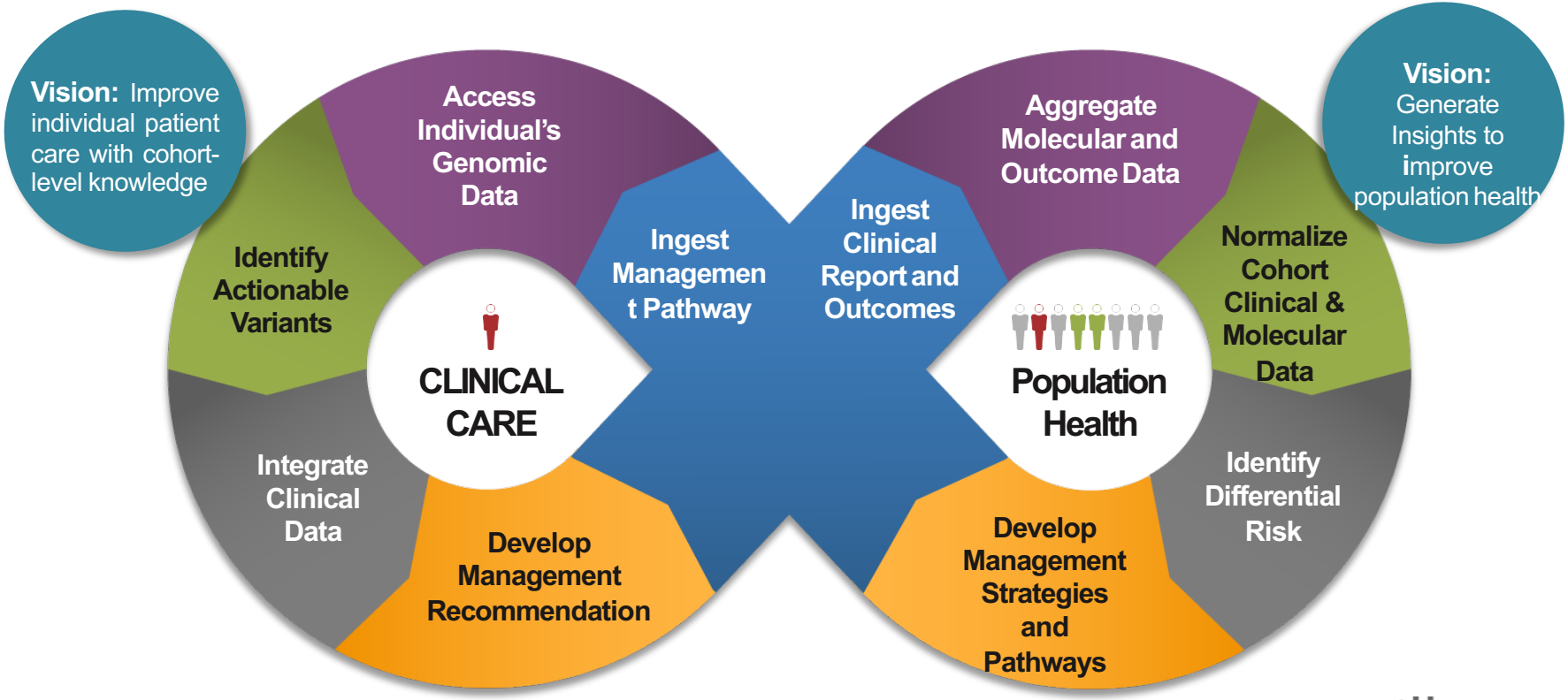
illumina®

> 50% of disease genes are yet to be discovered



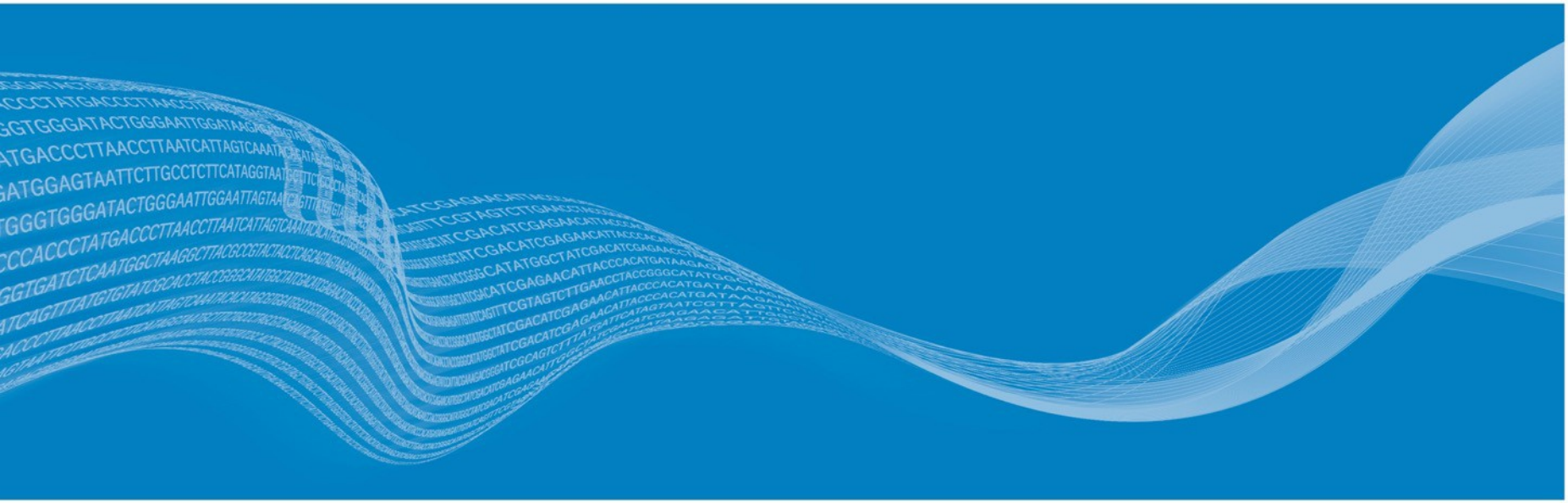
Concept of a Learning Health System

The Infinite Cycle of Genomic Medicine: Fully Reap the Benefits of Big Data in the Context of Clinical Care



illumina®

Thank you



illumina®