

Pathogen Genomics in the Era of COVID-19



Laboratory Perspective

Martin Adams
GSSHealth



Sequencing Technologies

First Generation



Sanger Sequencing
Maxam and Gilbert
Sanger Chain-termination

- Infer nucleotide identity using dNTPs then visualize with electrophoresis
- 500-1000 bp fragments

Second Generation (Next Generation Sequencing)



454, Solexa,
Ion Torrent
Illumina

- High throughput from the parallelization of sequencing reactions
- ~50-500 bp fragments

Third Generation



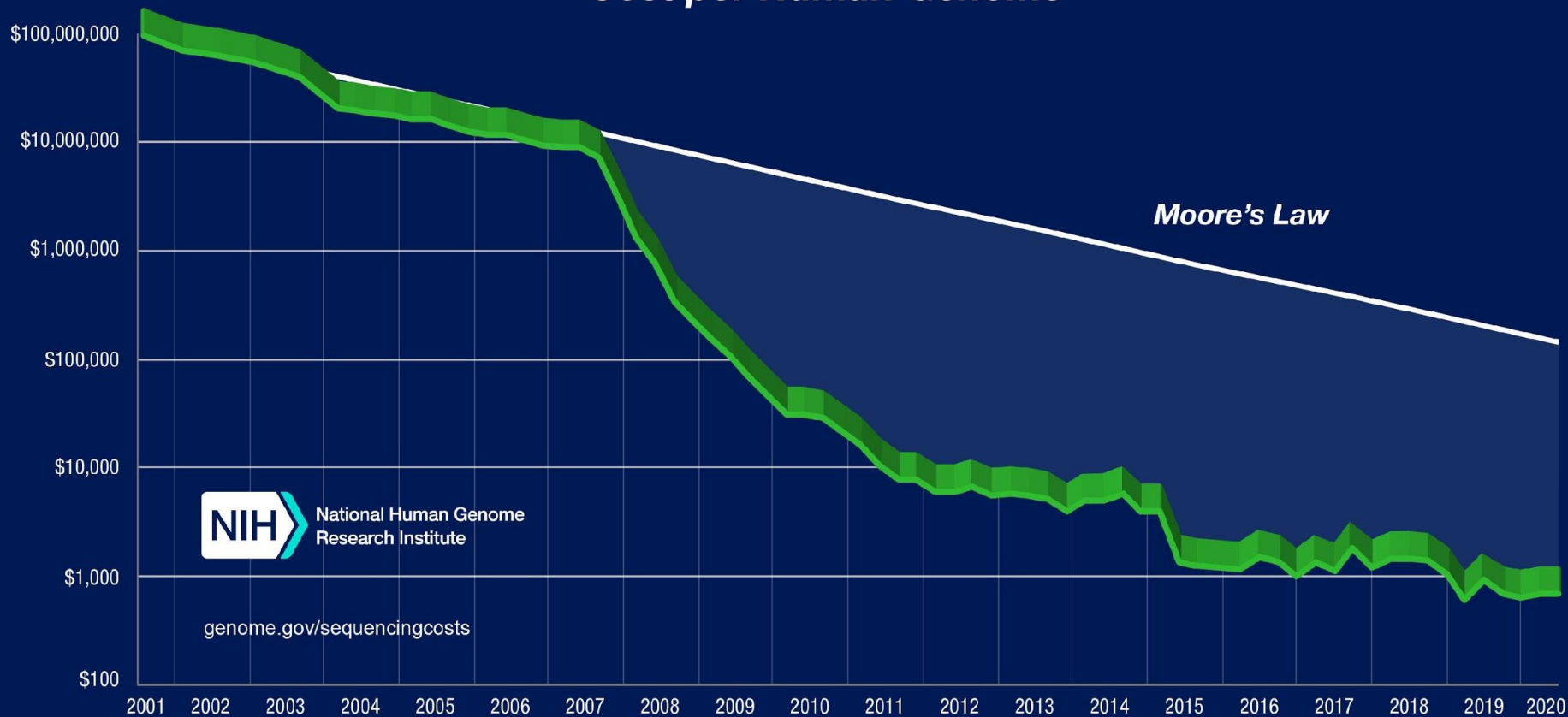
PacBio
Oxford Nanopore

- Sequence native DNA in real time with single-molecule resolution
- Tens of kb fragments, on average

Short-read sequencing

Long-read sequencing

Cost per Human Genome

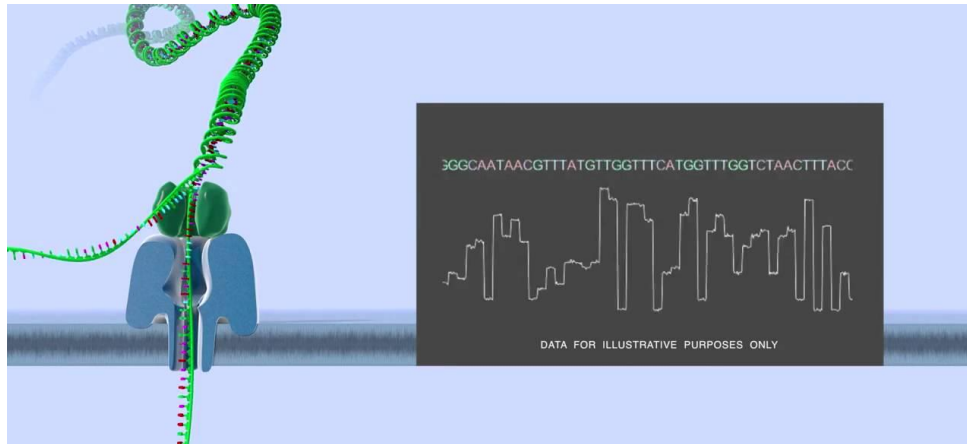


Evolution of Sequencing Laboratories



<https://www.nature.com/news/human-genome-project-twenty-five-years-of-big-biology-1.18436>
And field photographs, Martin Adams, GSSHealth

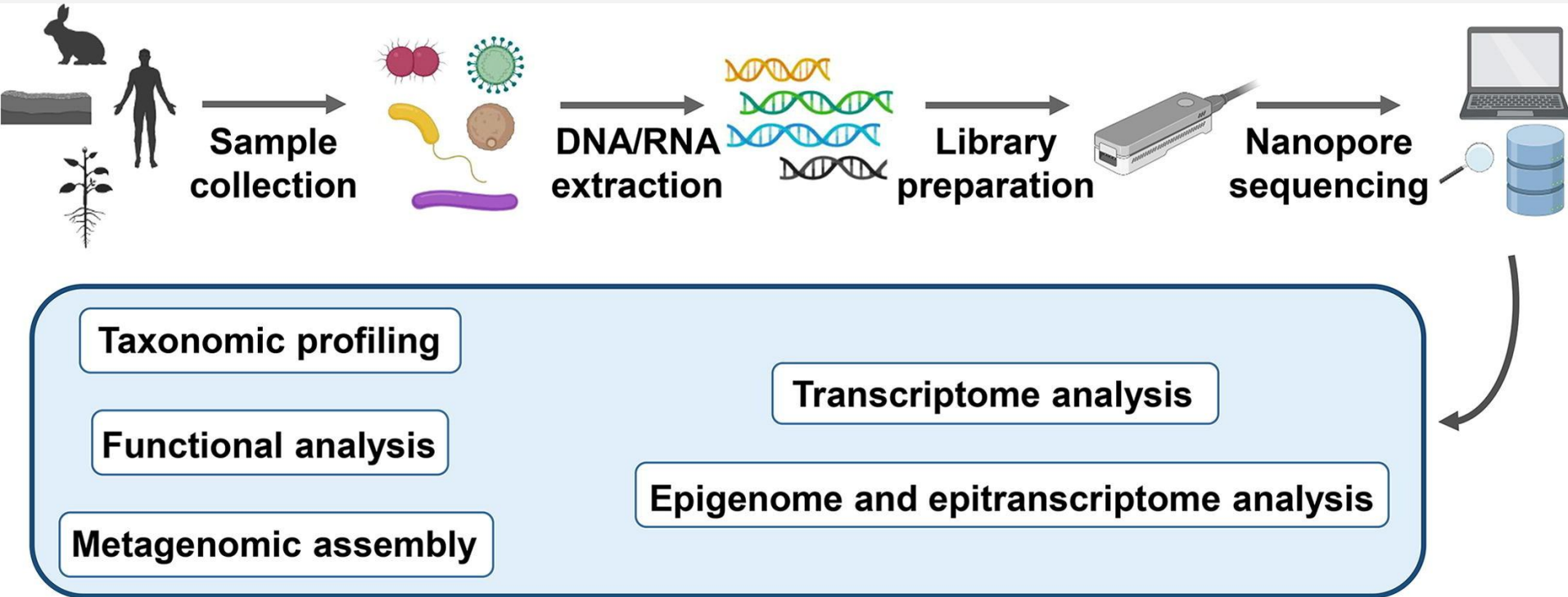
Oxford Nanopore MinION



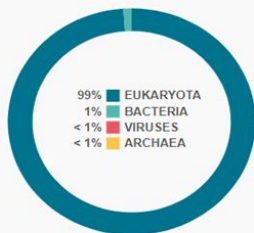
Pictures from Oxford Nanopore



Process of Obtaining Sequencing Data



Project “diarrhea”



READS ANALYSED
200,000

READS CLASSIFIED
197,219

READS UNCLASSIFIED
2,781

Taxa at Rank: Genus

Filter...

Taxon	Cumulative Reads
Homo	194,784
Shigella	1,043
Escherichia	558
Streptococcus	259
Malassezia	187
Bacteroides	10
Salmonella	10
Plesiomonas	10
Phycomyces	7
Lobosporangium	5
Klebsiella	4
Collinsella	4

« 1 2 3 4 5 6 »

Key Figures

READS ANALYSED
200,000

AVG QUALITY SCORE
10.53

TOTAL YIELD
686.8 Mbases

AVG SEQUENCE LENGTH
3,434

Quality Score

EXPORT PNG



Fogarty International Center

Building International Capacity for Genomic Surveillance of SARS-CoV-2 and Other Emerging Pathogens

David J. Spiro, PhD

Director, Division of International Epidemiology and Population Studies

Fogarty International Center



Rapid Advances in Sequencing Technology have made Real-Time Global Genomic Surveillance a Reality

- Increasing portability of sequencing
- Decreasing cost of sequencing
- Increased genomic capacity of sequencing platforms
- Improved bioinformatics capability
- Global platforms for data analysis and data-sharing



illumina®



Oxford
NANOPORE
Technologies



Nextstrain



FIC/JHU APL Genomic Epidemiology Training Program

- **Real-time Genomic Epidemiology Training Workshop**

- Bangkok, Thailand

- **MinION Workshop – Colombia**

- Santa Marta, Colombia



Obtain sample



Prepare DNA/RNA
for sequencing

Days 1-2

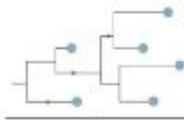


Sequence DNA



Initial sample
processing

Days 2-3



In-depth
analysis

Days 3-5



SARS-COV2 Virtual Trainings-Pre-recorded Material



Property International Center
Advancing Science for Global Health



JOHNS HOPKINS
UNIVERSITY

Deep Dive: SARS-CoV-2 Amplification and Oxford Nanopore Library Construction

Peter Thielen JHU APL



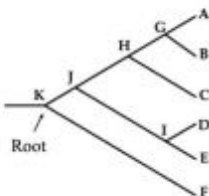
Rooting a phylogenetic Tree

To root an unrooted tree:

- root by outgroup, e.g. use F as an outgroup
- midpoint rooting – the midpoint of the path joining the two most dissimilar taxa

Outgroup should:

- Be the most distantly related of the taxa
- Not belong to ingroup, thus branched off before ingroup (e.g. judged using a priori biological/paleontological information)
- Be homologous to ingroup



James Otieno FIC

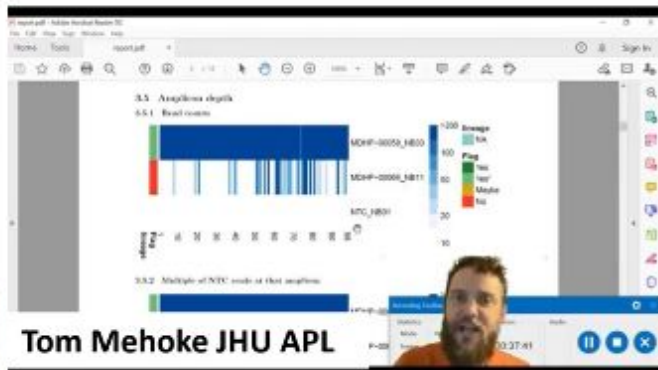


Phylogenetic Assignment of Named Global Outbreak LINEages PANGOLIN



- Local
<https://github.com/cox-lineages/pangolin>
- Webserver
<https://pangolin.cog-uk.io/>

Nidia Trovão FIC



Tom Mehoke JHU APL

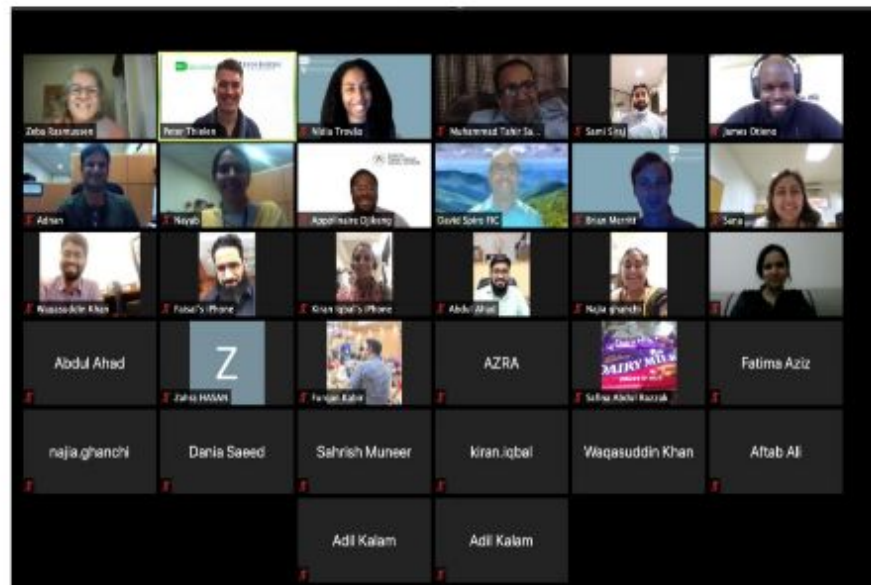


- Ten hours of pre-recorded lectures with tutorials
- Software package consolidates MinION, ARTIC network software into unified user-friendly interface

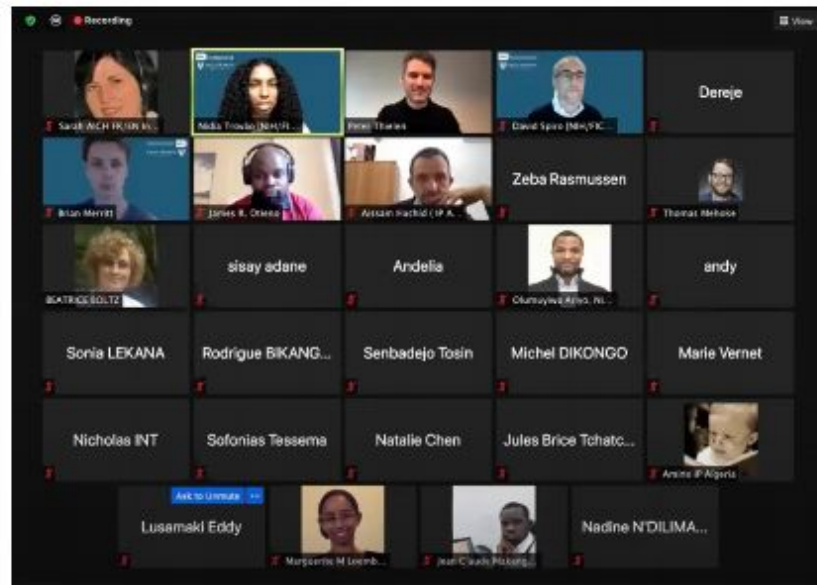
SARS-CoV2 Genomic Epidemiology Virtual Trainings October and December 2020 and January 2021



SARS-COV2 Virtual Trainings-Live Sessions



Pakistan October 2020



Africa CDC/Pasteur Institute January 2021

SARS-COV2 Virtual Trainings-Follow Up

The screenshot shows a Slack interface for the channel #laboratory-and-sequencing. The left sidebar lists various channels and direct messages. The main area shows a conversation from Saturday, February 6th. Three messages are visible: Emmanuel Lokilo (INRB/DRC) at 1:59 AM asking about using Docker for consensus generation; Peter Thielen (Johns Hopkins, USA) at 9:30 AM discussing software for ARTIC protocol on Oxford Nanopore platforms; and James R Otieno at 9:45 AM suggesting a check for viral RNA proportion in reads. A message input box is at the bottom.

Search Genomic_Epidemiology

Genomic_Epidemiology

All DMs
Mentions & reactions
More

Channels

- # general
- # laboratory-and-sequencing
- # phylogenetics
- # random
- # wg_africa_2020
- # wg_pakistan_2020
- + Add channels

Direct messages

- David Spiro you
- akbar
- Asghar Nasir

#laboratory-and-sequencing

Add a topic

124

Saturday, February 6th

New

Emmanuel Lokilo (INRB/DRC) 1:59 AM
Hi team,
WE prepared the library using truseq stranded total RNA protocol with illumina Miseq, using indexes instead of barcodes ...is it possible to use docker to generate consensus with the outputs WE have?

Peter Thielen (Johns Hopkins, USA) 9:30 AM
The software presented in the workshop is specific to the ARTIC protocol on Oxford Nanopore platforms
The analysis approach will be different for data generated using your approach. The first step should be alignment to the reference genome with bwa or similar.
you may find that a very small proportion of data generated originated from viral RNA

James R Otieno 9:45 AM
If this might help, as Peter has said, you might first want to check what proportion of your reads are the virus of interest against a database. I prefer Kraken, and you can just download the smaller version 4/8Gb.

Send a message to #laboratory-and-sequencing

- Slack Channels
- Zoom “Office Hours”
- Individual Zoom calls and emails.
- Access to pre-recorded lectures
- Access to Zoom recordings of live sessions

Future Directions

- Software development and courses targeted toward public health labs-Pakistan
- Advanced Training in Genomic Epidemiology - Pakistan.
- Responsible Conduct in Genomic Sciences Training-Pakistan
- Targeted Bioinformatic Trainings in Genomic Epidemiology for Healthcare Professionals
- Future training programs in Bangladesh, India, Tunisia, Cameroon, Philippines

