

The Role of Genomics in Outbreak Investigations

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Disclosures.

- Member of the Strategic committee for the Ontario COVID-19 Genomics Network (OCGN).

“If I have seen further, it is by standing on the shoulders of giant” - Sir Issac Newton

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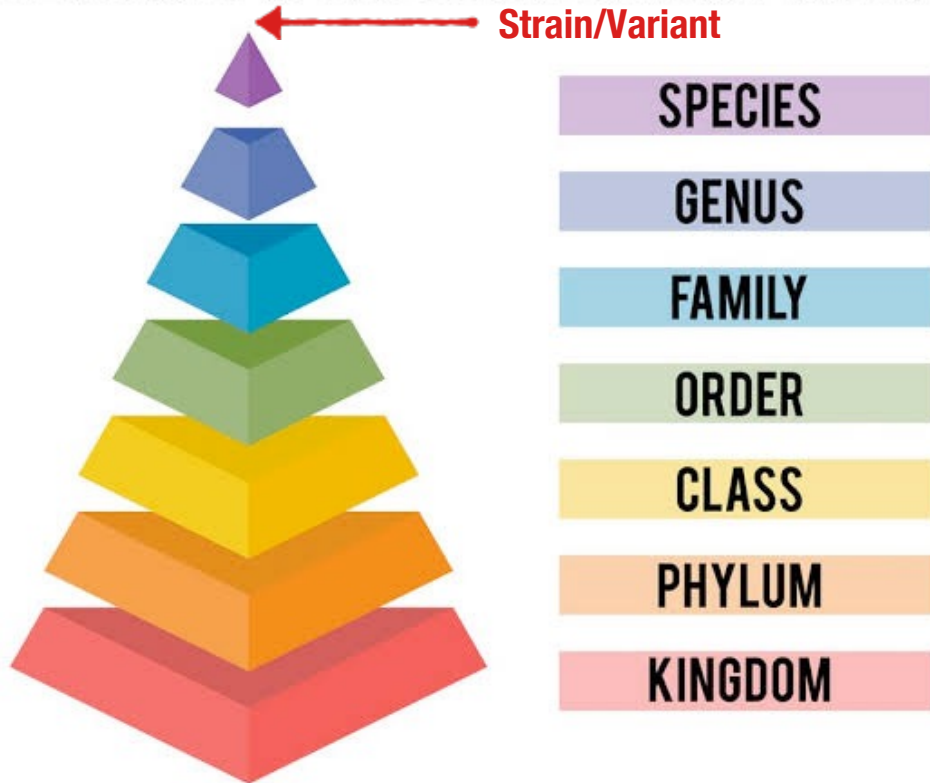
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Learning Objectives.

- Summarize the different genomic assays used in “genomics”.
- Recognize methods used to distinguish organisms at the species/strain level.
- Apply knowledge of the principles of health and disease surveillance.
- Assess different methods to distinguish disease clusters and the limitations of genomic typing.

Taxonomic Differentiation of Organisms.

HIERARCHY OF BIOLOGICAL CLASSIFICATION

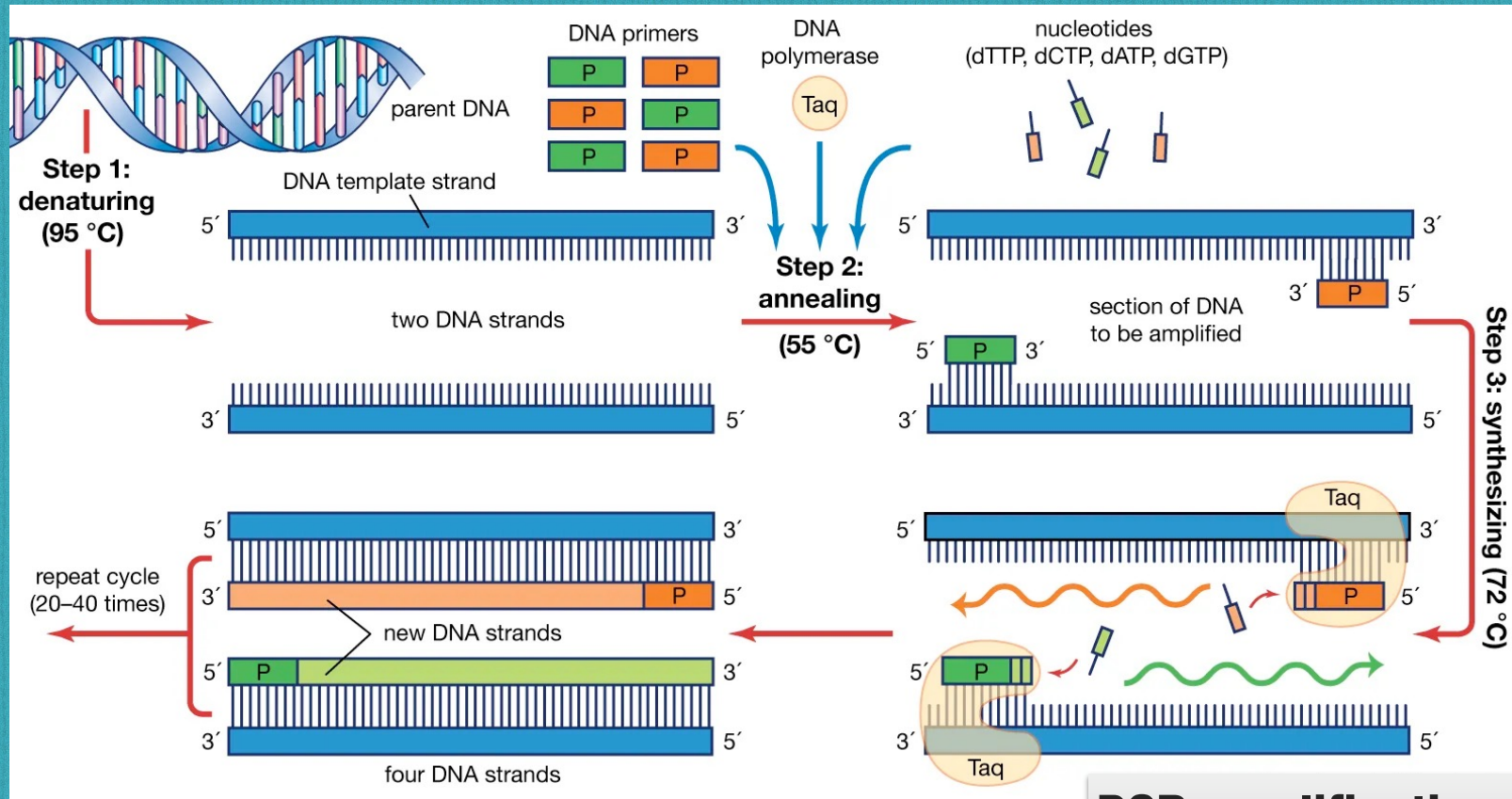


STRAIN VERSUS SPECIES

STRAIN	SPECIES
A genetic variant or subtype of a biological species	A group of organisms that can reproduce with one another in nature and produce fertile offspring
A subtype of species	The basic taxonomic group of classification of organisms
An isolate of a given species with a specific genetic characteristic	Organisms in a species have a genetic similarity sufficient for mating, producing a fertile offspring
Can be characterized by serotyping, enzyme type, functional traits, and protein plasmid characterization, etc.	Can be characterized by genetic, biochemical and phenotypic criteria

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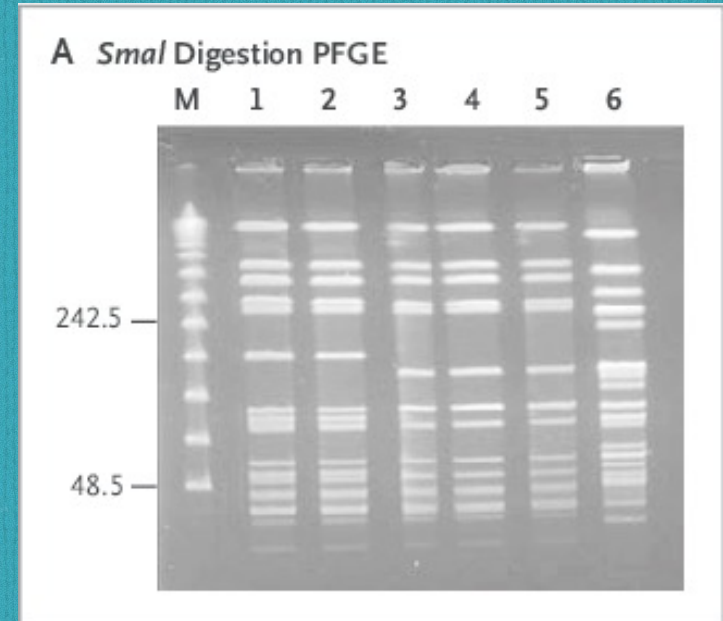
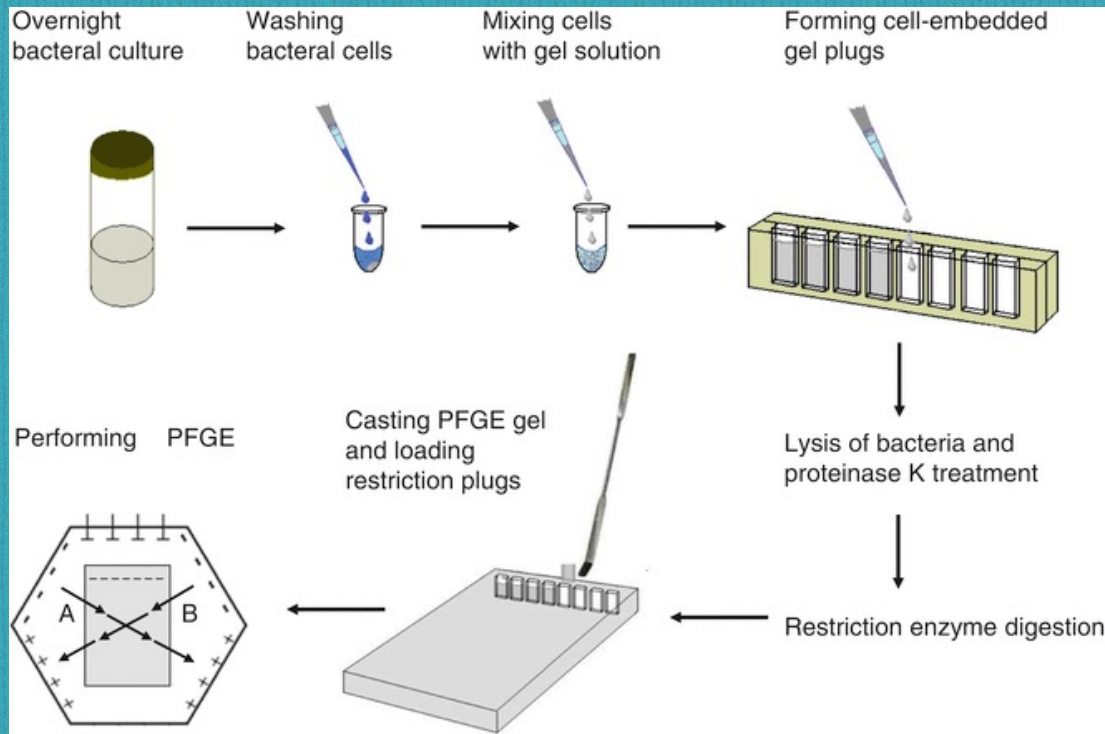
PCR Amplification.



PCR amplification.

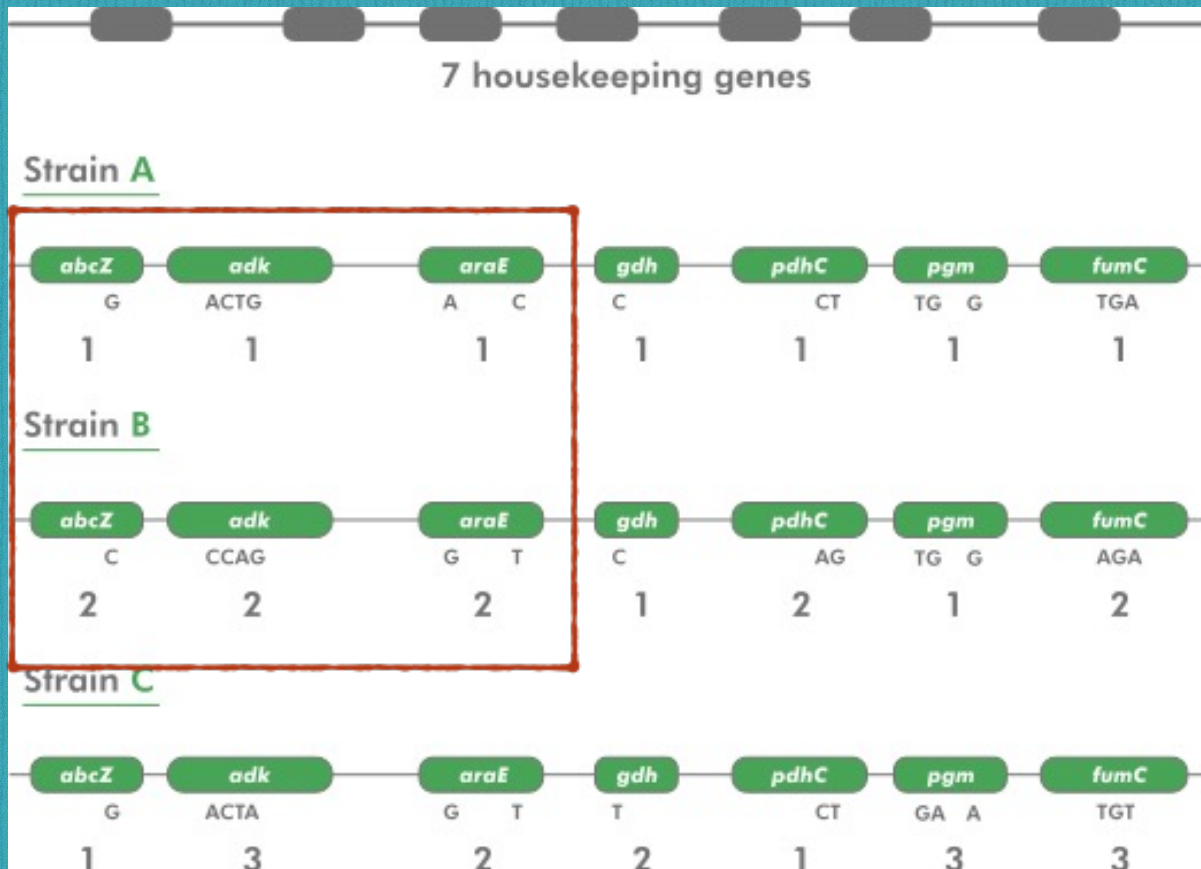
- Inexpensive (\$), limited computing power required (*)
- Require expertise for molecular assays - less of an issue now post COVID-19.
- Need to know your target(s) ahead of time.

Pulse-Field Gel Electrophoresis



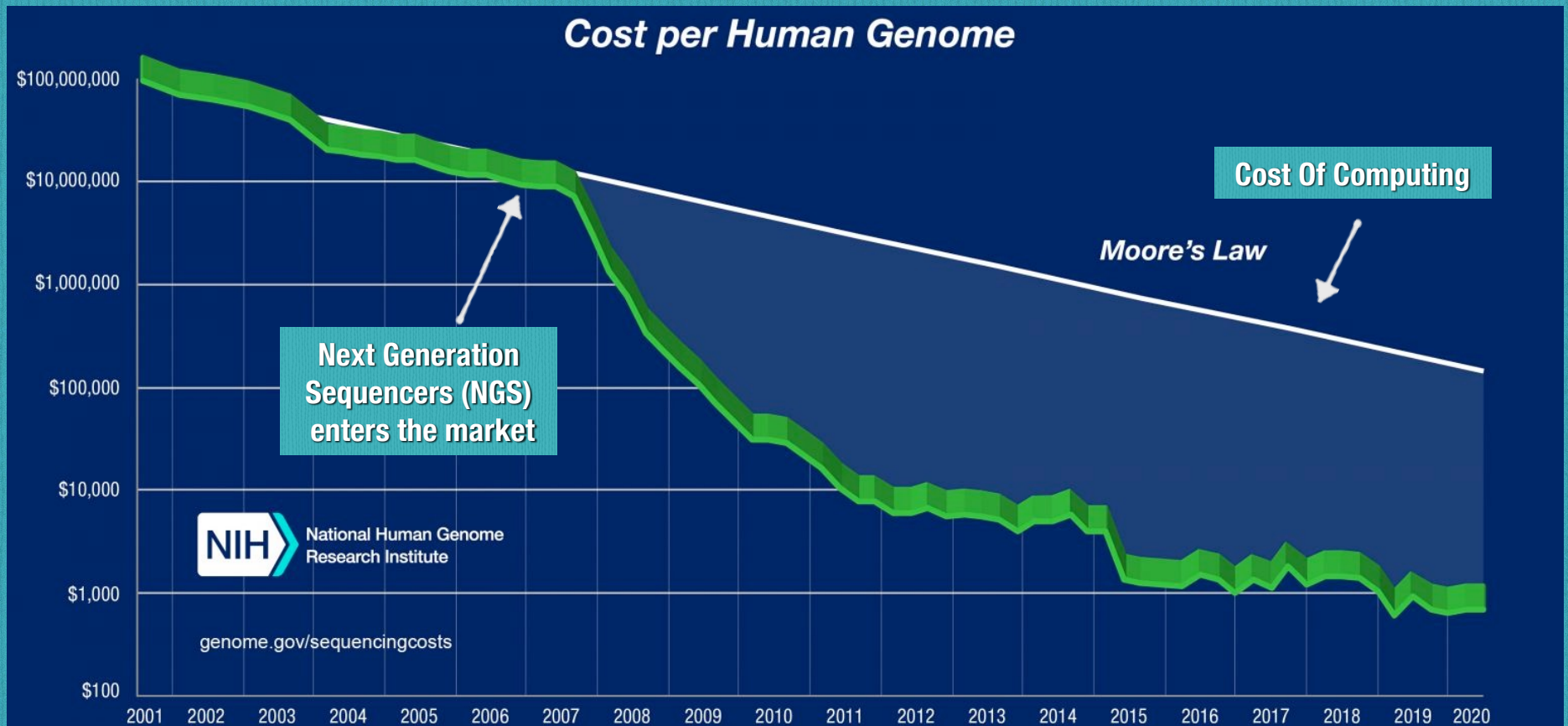
- Only be used for Bacteria and/or Fungi.
- Labor Intensive and require laboratory space.
- Limited number of samples can be interrogated at one time.
- Limited resolution - unclear what the changes were and how to assign them.

Multilocus Sequence Typing (MLST)



- Distinguished organisms base on highly conserved “house-keeping genes”.
- Look for changes in house keeping genes to determine strain level difference.
- Cheap (\$\$) but significant human resources required.
- Isolates need to be cultured and need to know what to look for.
- Different organisms have different levels of ‘basal’ mutations in house keeping genes.

The Changing Landscape of Genomics.



Human Genome Project - 12 donors, Sequenced

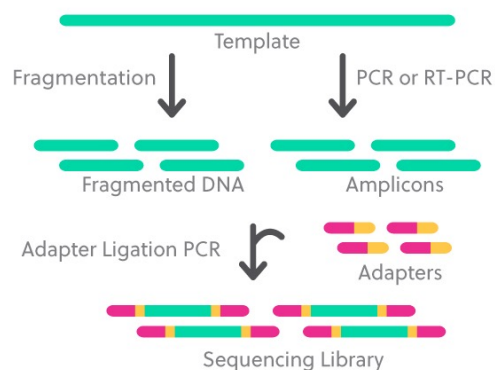
90% of the human genome - \$3 Billion. → Today's cost for 12 donor genomes - \$12,000

Amplicon Based Sequencing (NGS)

STEP 1: Extraction



STEP 2: Library Prep



STEP 3: Sequencing



STEP 4: Analysis

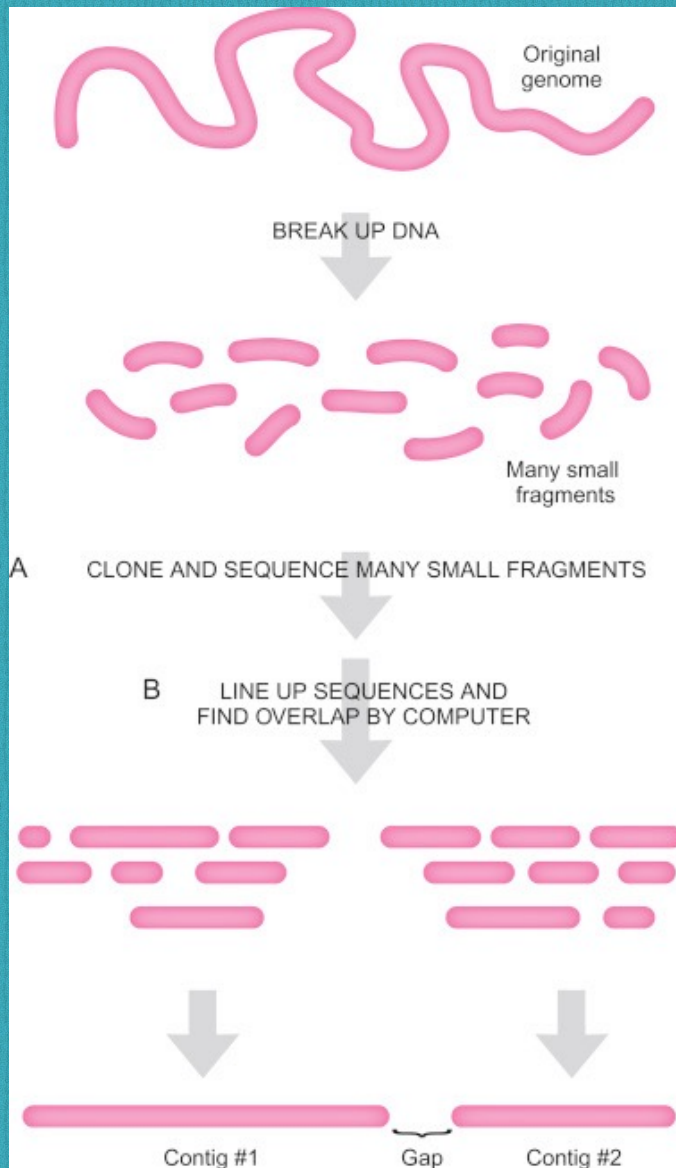


iRepertoire

- Need to know what you are looking for.
- Relatively inexpensive (\$\$) and computing intensive (**).

**Most Common type of
“Genomic assay”.**

“Shot-gun” Sequencing



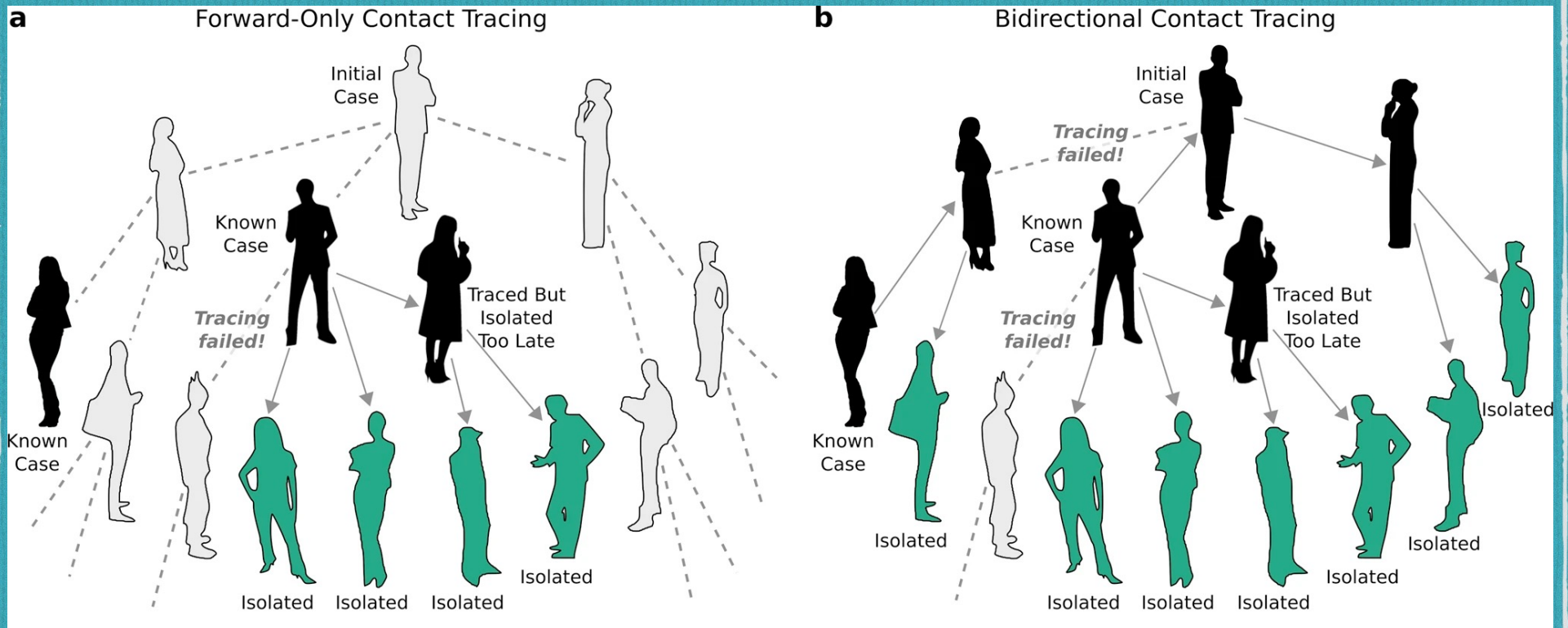
- Does not require prior knowledge of what to look for, can be used for new pathogens. (pathogen discovery)
- Expensive (\$\$\$\$\$).
- Significant computing power required (*****).
- Significant expertise in bioinformatics required (****).

For now...rarely used in a clinical setting.

Communicable Disease Transmission.

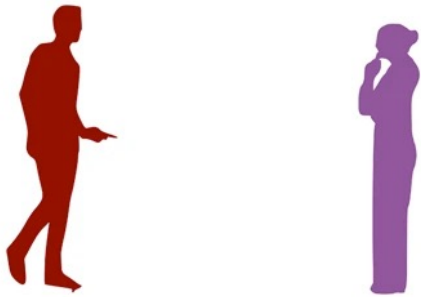


Contact Tracing



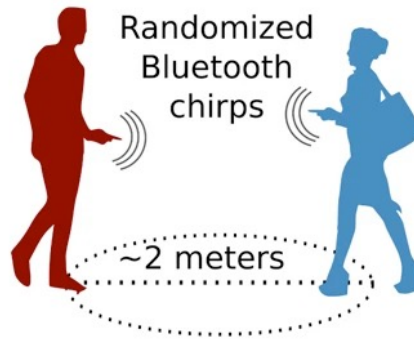
Digital and Analog Contact Tracing

c Manual Contact Tracing

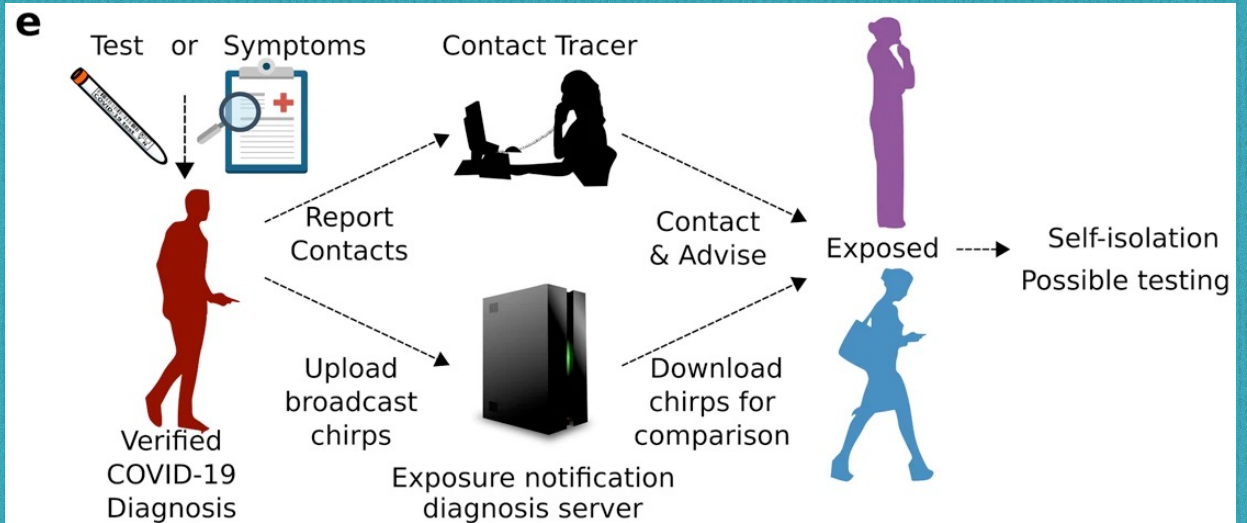


Individuals try to remember the people they encountered

d Private Digital Exposure Notification

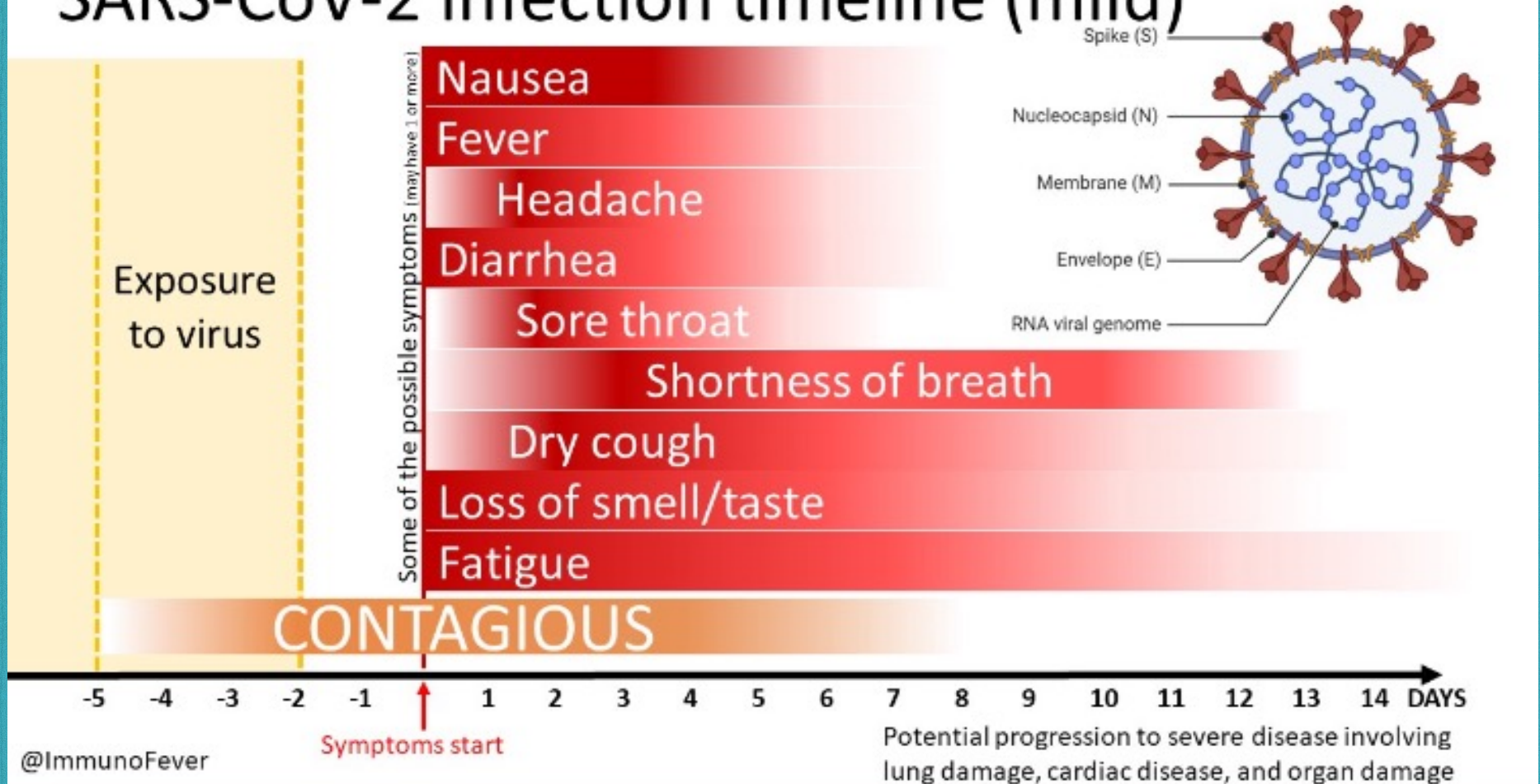


Phones locally store logs of broadcasted and received chirps

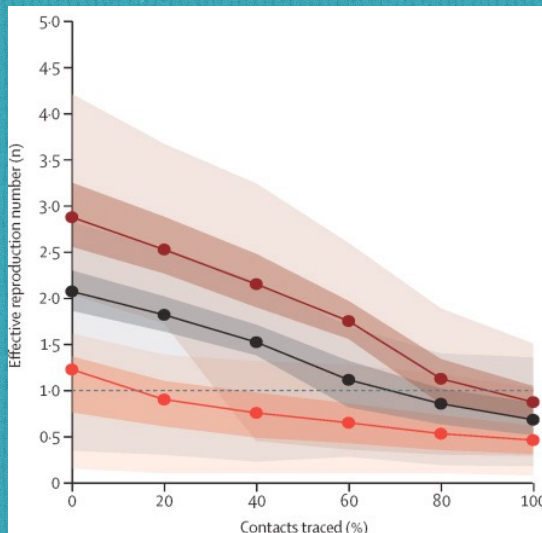
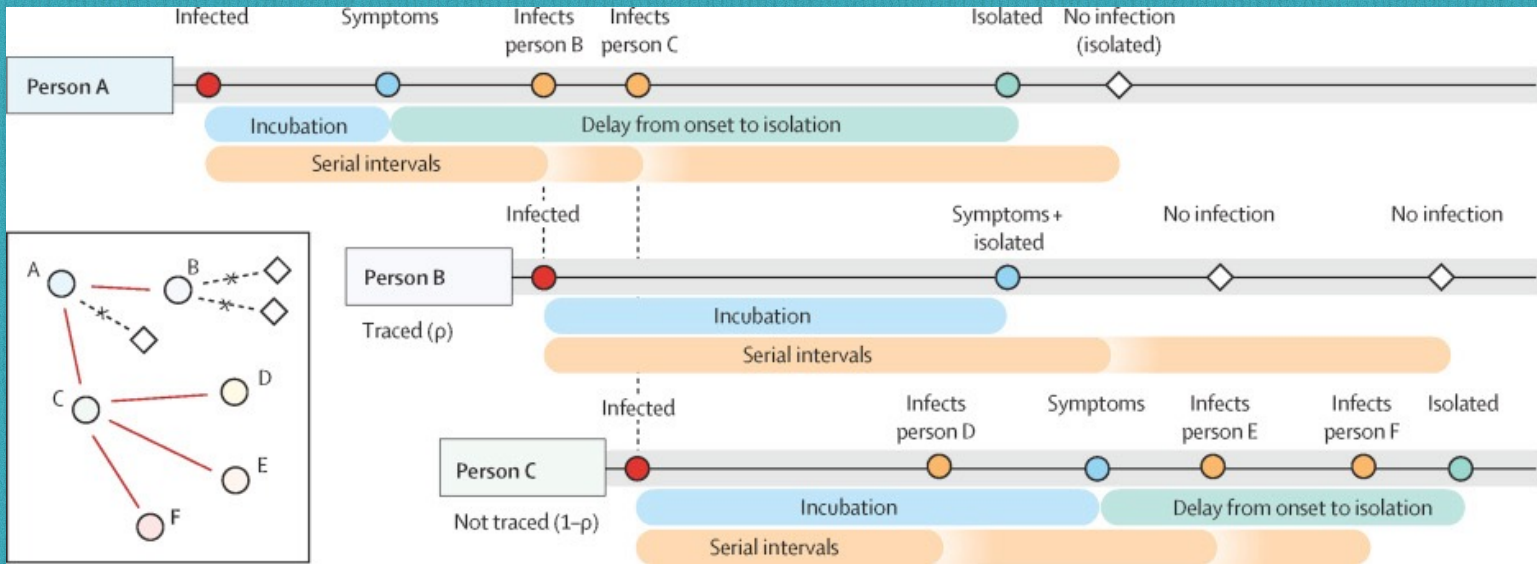


The Incubation Period.

SARS-CoV-2 infection timeline (mild)



Challenges with Contact Tracing



The higher the R_0 , the greater need for better contact tracing.

But once the R_0 is > 3 - the efficacy of contact tracing is close to 0% - at least according to this model in a community setting.

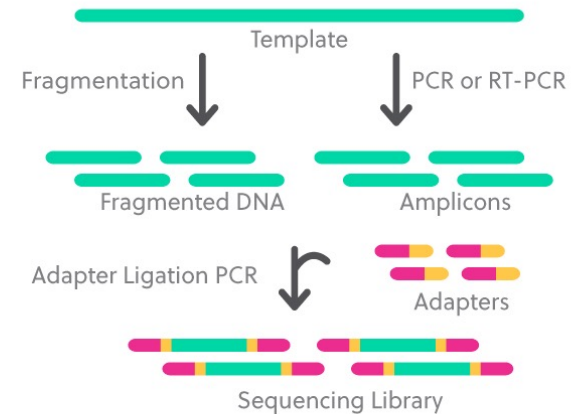
Genomic Community Surveillance

Amplicon-based Sequencing

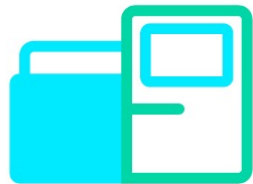
STEP 1: Extraction



STEP 2: Library Prep



STEP 3: Sequencing

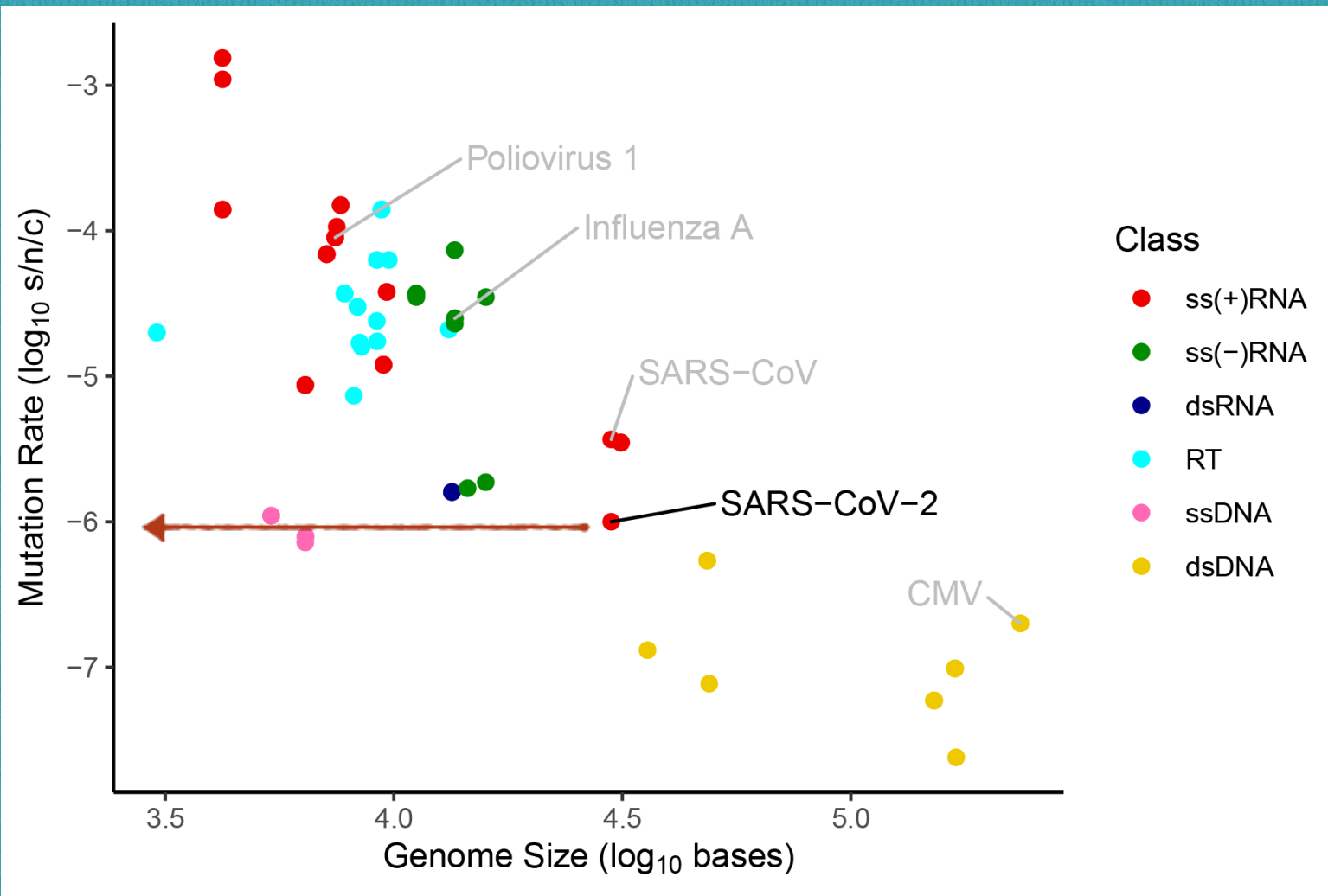


STEP 4: Analysis



1

Base Mutation rate



2

Genomics based epidemiology.

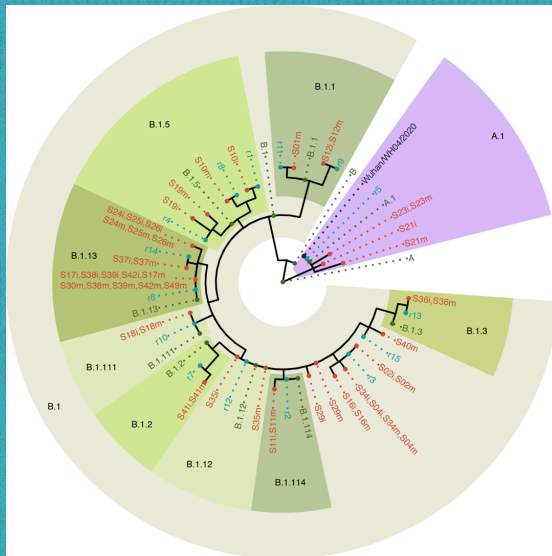
www.nature.com/scientificreports

scientific reports

OPEN Phylogenomics reveals viral sources, transmission, and potential superinfection in early-stage COVID-19 patients in Ontario, Canada

Calvin P. Sjaarda^{1,2,3}, Nazneen Rustom^{3,4}, Gerald A. Evans^{5,6}, David Huang⁷, Santiago Perez-Patrigeon⁸, Melissa L. Hudson^{1,2}, Henry Wong⁹, Zhengxin Sun⁷, T. Hugh Guan⁹, Muhammad Ayub^{10,11}, Claudio N. Soares^{12,13}, Robert I. Colautti^{17,21} & Prameet M. Sheth^{5,6,10,11}

- Introduction of 2 distinct lineages of the SARS-CoV-2 virus into Eastern Ontario.
- Lineage A - Directly from Wuhan
- Lineage B - Multiple Variants from Europe, UK and the US.
- Blinded genomics analysis and contact tracing done by separate team members.



Viral phylogeny and Prevalence of SARS-CoV-2 strains in Eastern Ontario.

Number of Samples

20760

▲ 20760 of prior Max

Last Updated - 9/26/2022

7 Days

30 Days

180 Days

1 Year

Max

Custom



06/18/2020

09/26/2022

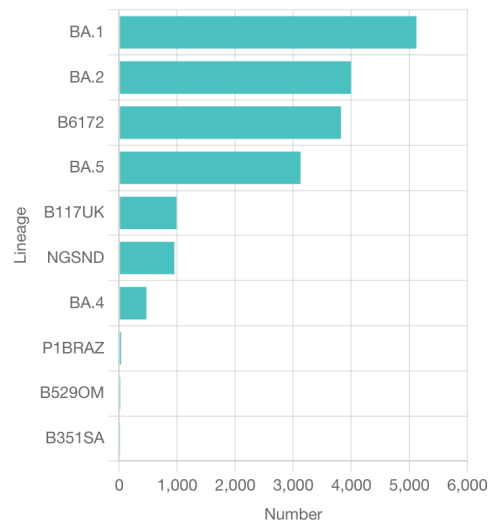
Sample Count

Sample Lineage

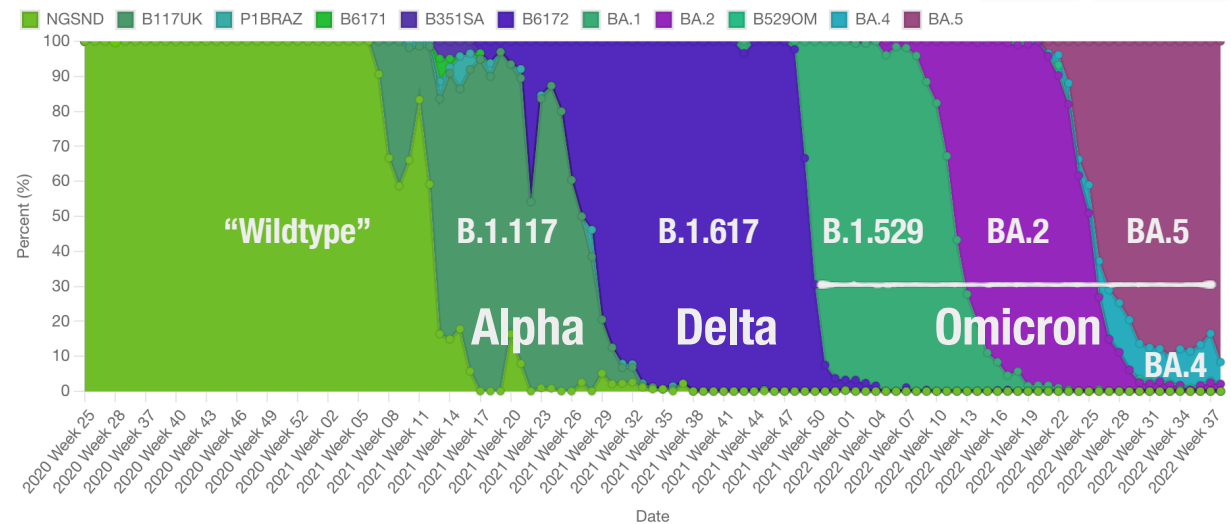
Key Performance Indicators

Genomic Coverage

Top 10 Lineages of Sample Number of Selected Locations



Sample Number/Percent by Lineages Over Time



Monitoring Emerging Variants of Concern.

	RUN_ID	Unique_ID	LINEAGE_old	LINEAGE	SAMPLE_COLLECTION_DATE
1	Run329	ON-KHS-22-16700-v1	XBB.1	XBB.1.5	2022-12-11
2	Run329	ON-KHS-22-16712-v1	XBB.1	XBB.1.5	2022-12-12
3	Run329	ON-KHS-22-16718-v1	XBB.1	XBB.1.5	2022-12-12
4	Run332	ON-KHS-22-16946-v1	XBB.1	XBB.1.5	2022-12-17
5	Run335	ON-KHS-22-17170-v1	XBB.1	XBB.1.5	2022-12-24
6	Run335	ON-KHS-22-17206-v1	XBB.1	XBB.1.5	2022-12-22
7	Run335	ON-KHS-22-17222-v1	XBB.1	XBB.1.5	2022-12-23
8	Run337	ON-KHS-23-00015-v1	XBB.1.5	XBB.1.5	2022-12-28
9	Run337	ON-KHS-23-00029-v1	XBB.1.5	XBB.1.5	2022-12-29
10	Run337	ON-KHS-23-00032-v1	XBB.1.5	XBB.1.5	2022-12-29
11	Run337	ON-KHS-23-00081-v1	XBB.1.5	XBB.1.5	2022-12-29
12	Run338	ON-KHS-23-00145-v1	XBB.1.5	XBB.1.5	2022-12-29
13	Run338	ON-KHS-23-00150-v1	XBB.1.5	XBB.1.5	2022-12-29
14	Run338	ON-KHS-23-00155-v1	XBB.1.5	XBB.1.5	2022-12-29
15	Run338	ON-KHS-23-00166-v1	XBB.1.5	XBB.1.5	2022-12-30

- Allows for continuous surveillance for new and emerging variants of concern.
- Changes in epidemiology.

3

How Different is Different Enough?

Best of a Bad Method: Optimal use of SNP distance thresholds for SARS-CoV-2 transmission clustering

Peter C. Jentsch^{1,2}, Calvin P. Sjaarda^{3,4}, Jennifer L. Guthrie^{5,6}, Robert A. Kozak^{1,7}, Chris Kandel^{7,9}, Prameet M. Sheth^{3,4}, Henry Wong^{3,4}, Allison McGeer^{7,9}, Samira Mubareka^{1,7}, and Finlay Maguire^{1,8}

¹Sunnybrook Research Institute, Toronto, Canada

²Simon Fraser University, Burnaby, Canada

³Queen's University, Kingston, Canada

⁴Kingston Health Sciences Centre, Kingston, Canada

⁵Western University, London, Canada

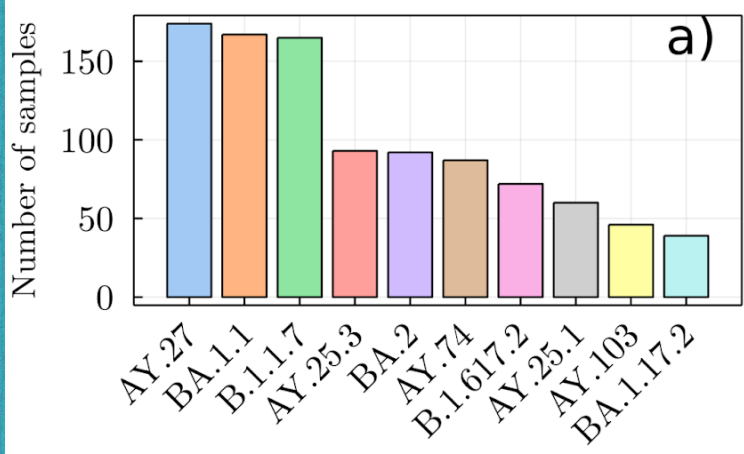
⁶Public Health Ontario, Toronto, Canada

⁷University of Toronto, Toronto, Canada

⁸Dalhousie University, Halifax, Canada

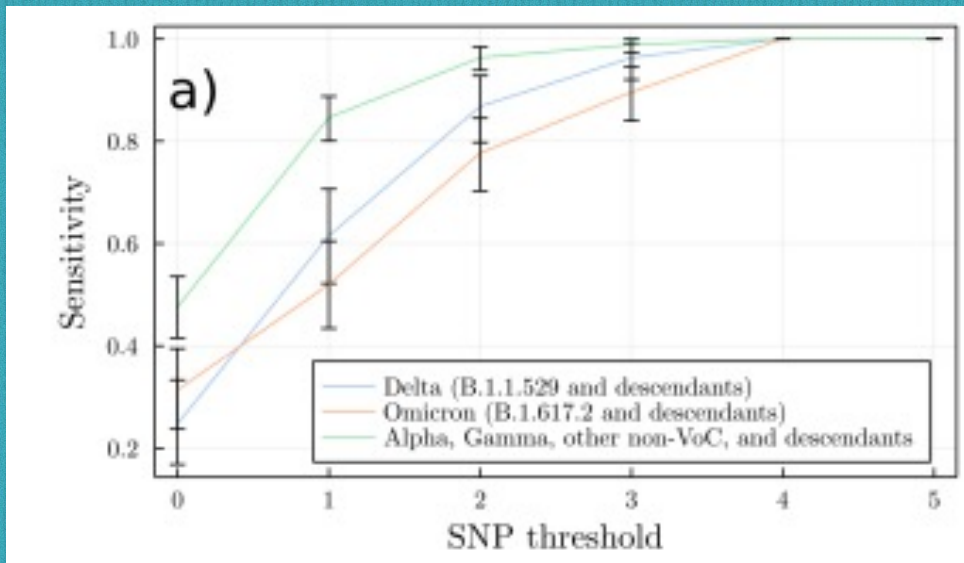
⁹Mount Sinai Hospital, Toronto, Canada

- Used 15,504 sequences from KHSC from the beginning of the pandemic to the Omicron BA.2 lineage (Included Alpha, Delta, Omicron).
- 636 outbreak clusters from 1395 patients.



How Different is Different Enough?

- Using 2 nucleotide change as a threshold had a sensitivity of >80% for Alpha/Gamma but < 60% of Delta and Omicron isolates
- Using 3 nucleotide differences allow us to differentiate between viruses within a set time with an accuracy of >90% for all the VOC variants.
- So for our outbreak reports we chose to use 3 SNP's to call out a virus as being “different”.



Challenges / Limitation of using Genomics in Institutional Outbreaks.

- Require infrastructure and personnel
- Timeliness - takes ~ 4 - 7 days from sample collection.
- Need to establish a threshold for calling a strain 'related' vs. 'unrelated'.
- Cannot determine direction of transmission. - mutations are not acquired in one direction.

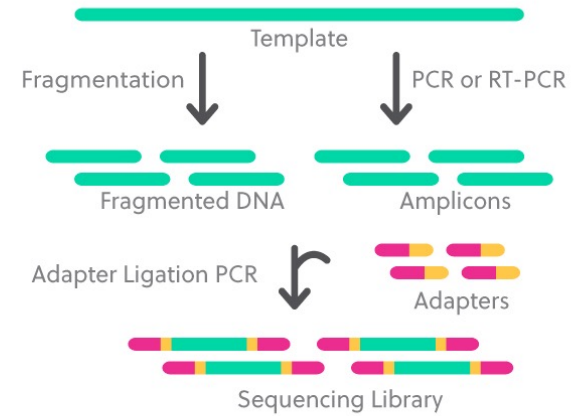
Genomics for Outbreak Management

SARS-CoV-2 Genomics

STEP 1: Extraction



STEP 2: Library Prep



STEP 3: Sequencing



STEP 4: Analysis



Genomic Informed Outbreak Investigations - SARS-CoV-2

- 19 person outbreak at KHSC. Patients and staff involved.
- Time and place analysis suggested all of the patients were part of one outbreak cluster.
- Genomics verified that the viruses were almost identical in all of the individuals.
- Evaluate what changes can be made to mitigate further spread and transmission.

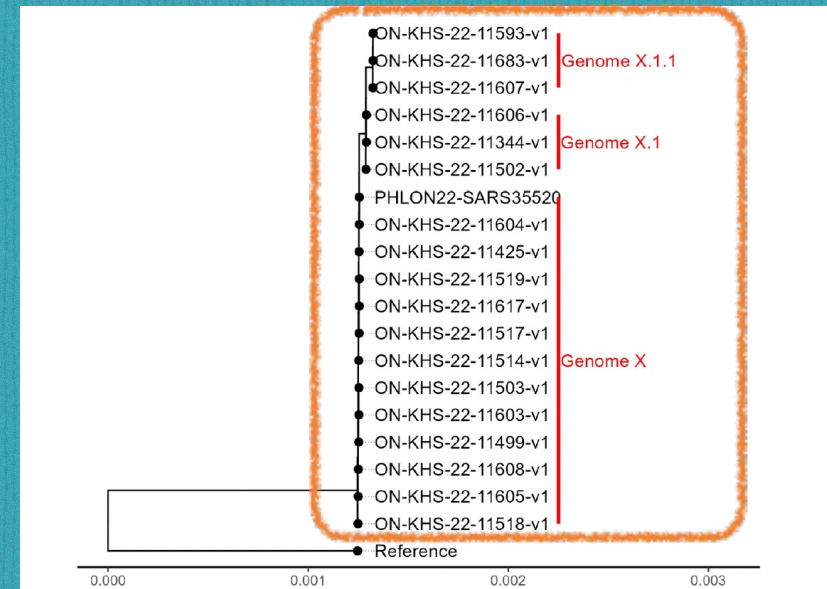


Figure 1. Phylogenetic shows how samples are related to each other based on viral genome sequencing.

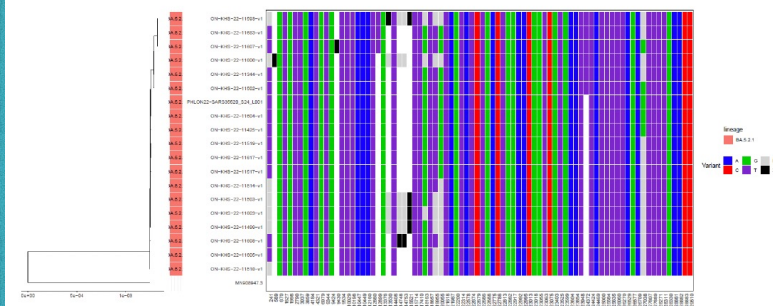


Figure 2. Phylogenetic tree and variant map shows the SNPs detected in each sample.

Genomic Informed Outbreak Investigations - SARS-CoV-2.

- 10 person outbreak at KHSC - time and place analysis suggested that all the cases were “related”.
- The IPAC team called an outbreak and started investigating a break down in process / protocol.
- Genomics revealed that there were 4 - distinct viruses circulating amongst the 10 people involved.
- led to significant anxiety amongst the staff on the floor.

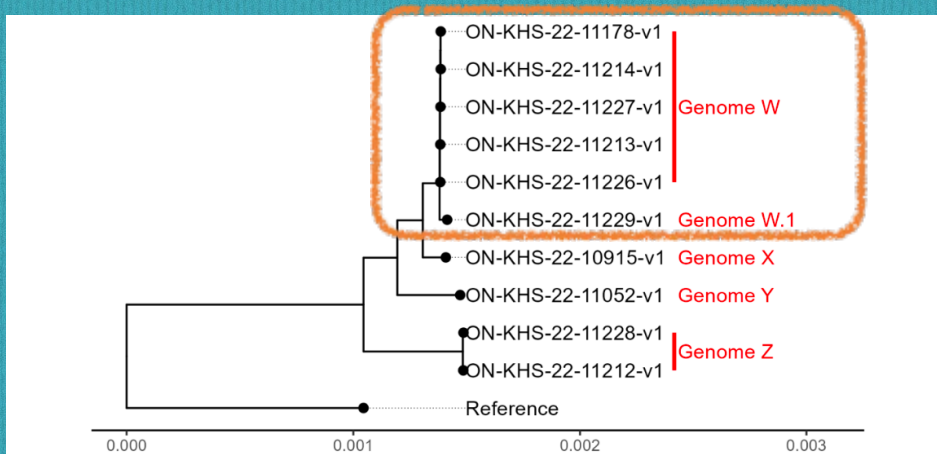


Figure 1. Phylogenetic shows how samples are related to each other based on viral genome sequencing.

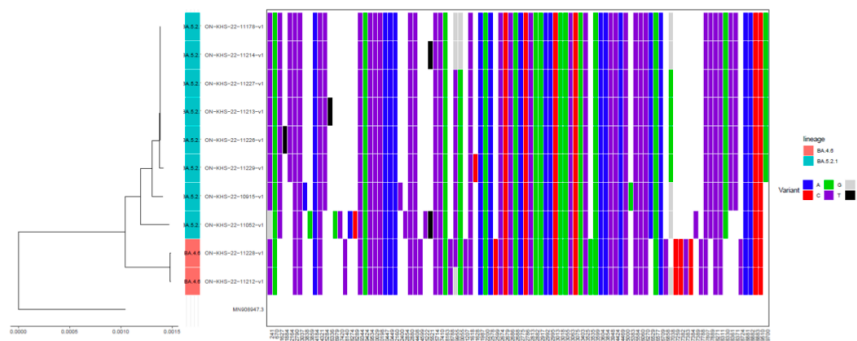


Figure 2. Phylogenetic tree and variant map shows the SNPs detected in each sample.

Outbreak Reports to IPAC

Kingston Health
Sciences Centre

SARS-CoV-2 Outbreak Whole Genome Sequencing Report

Introduction

Outbreaks in health care or institutional settings are particularly challenging as infection prevention and control, occupational health and safety, and public health must quickly investigate, assess, isolate infected cases and trace potential exposures to mitigate further spread of the pathogen within the facility. Time-resolved SARS-CoV-2 whole genome sequencing can facilitate a deeper understanding of the dynamics of localized spread of SARS-CoV-2 to aid organizations understand and control the outbreak dynamics.

Reports Overview

In-depth genomic analysis can be performed in cases where the PHU, hospital, or organization is investigating the outbreak for specimen relatedness to assess the possibility of multiple introductions, nosocomial transmission, etc.

Sample Prerequisites

Specimen Eligibility

1. Specimen Volume: 1ml (0.5 ml as absolute minimum)
2. Ct \leq 30 from the e-gene PCR test

Outbreak Reports.

- 31 person outbreak at KHSC – 23 patients – one cluster.

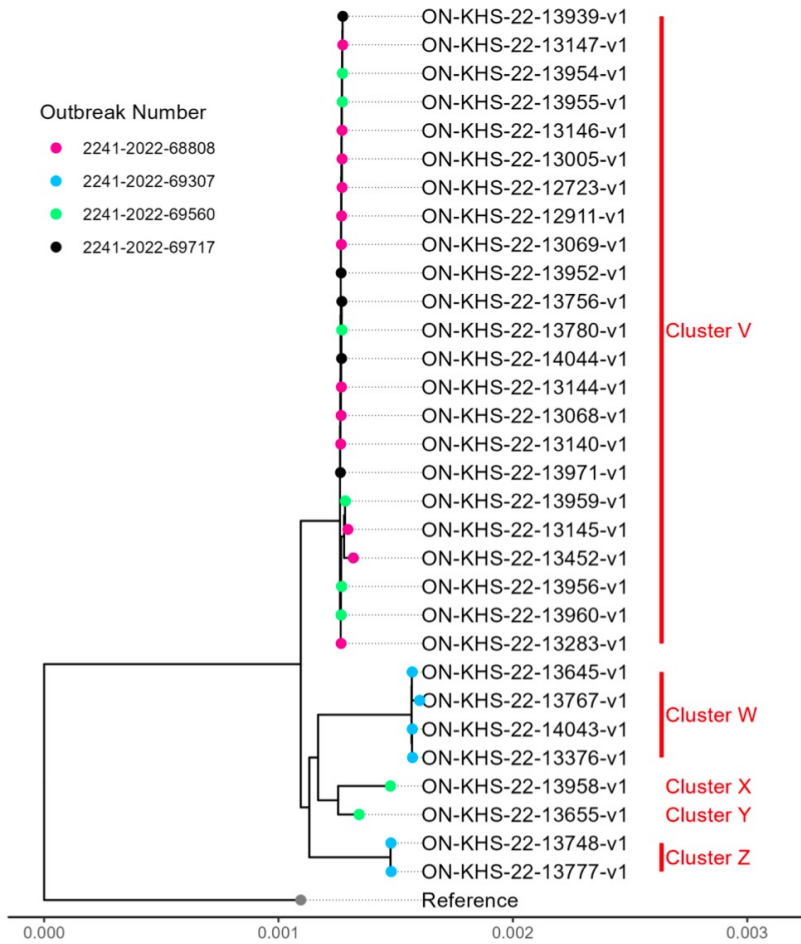


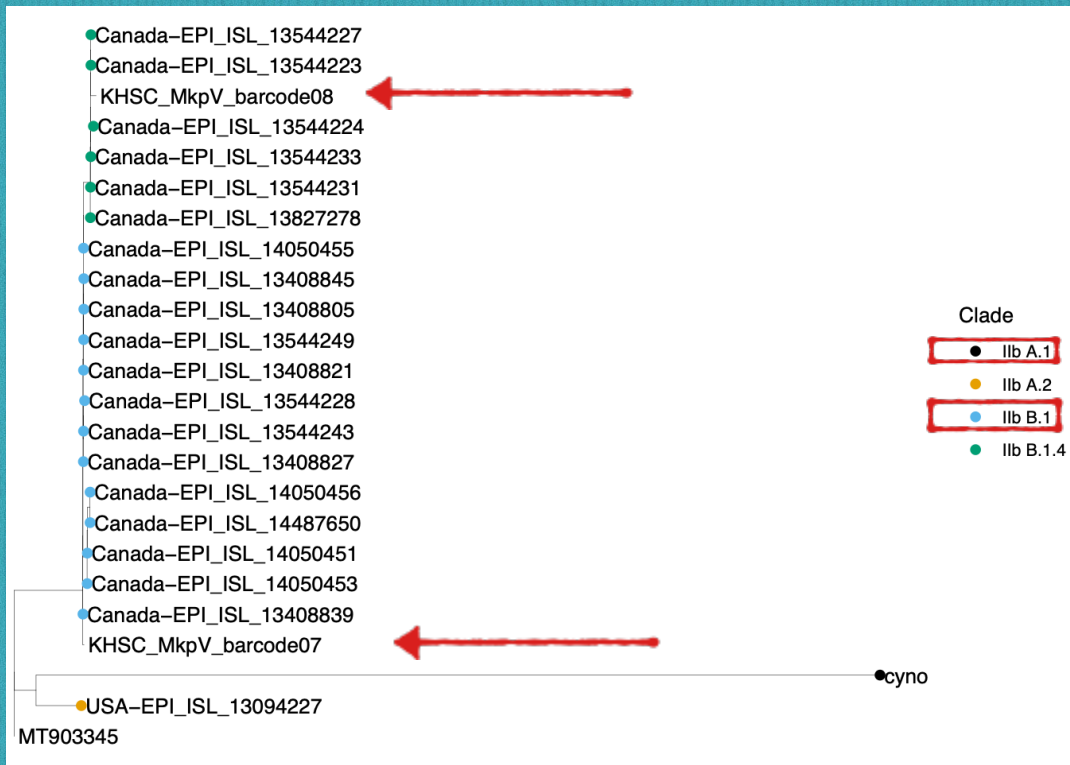
Figure 1. Phylogenetic shows how samples are related to each other based on viral genome sequencing.

Table 2. Lineage and genome assignment for cases associated with outbreak

Case	NGS No	Genome Completeness	Pango Lineage (WHO label)	Cluster	Loc	Outbreak Number
1	ON-KHS-22-12723-v1	0.9865	BA.5.2 (Omicron)	V	S3	2241-2022-68808
2	ON-KHS-22-12911-v1	0.9887	BA.5.2 (Omicron)	V	S3	2241-2022-68808
3	ON-KHS-22-13005-v1	0.9786	BA.5.2 (Omicron)	V	S3	2241-2022-68808
4	ON-KHS-22-13068-v1	0.9955	BA.5.2 (Omicron)	V	S3	2241-2022-68808
5	ON-KHS-22-13069-v1	0.9928	BA.5.2 (Omicron)	V	S3	2241-2022-68808
6	ON-KHS-22-13140-v1	0.9931	BA.5.2 (Omicron)	V	S3	2241-2022-68808
7	ON-KHS-22-13144-v1	0.9958	BA.5.2 (Omicron)	V	S3	2241-2022-68808
8	ON-KHS-22-13145-v1	0.9774	BA.5.2 (Omicron)	V	S3	2241-2022-68808
9	ON-KHS-22-13146-v1	0.9873	BA.5.2 (Omicron)	V	S3	2241-2022-68808
10	ON-KHS-22-13147-v1	0.9286	BA.5.2 (Omicron)	V	S3	2241-2022-68808
11	ON-KHS-22-13283-v1	0.9359	BA.5.2 (Omicron)	V	S3	2241-2022-68808
13	ON-KHS-22-13452-v1	0.8420	BA.5.2 (Omicron)	V	S3	2241-2022-68808
12	ON-KHS-22-13376-v1	0.9446	BF.5 (Omicron)	W	S4	2241-2022-69307
14	ON-KHS-22-13645-v1	0.7581	BF.5 (Omicron)	W	S4	2241-2022-69307
16	ON-KHS-22-13748-v1	0.9475	BE.1.2.1 (Omicron)	Z	S4	2241-2022-69307
18	ON-KHS-22-13767-v1	0.9596	BF.5 (Omicron)	W	S4	2241-2022-69307
19	ON-KHS-22-13777-v1	0.9587	BE.1.2.1 (Omicron)	Z	S4	2241-2022-69307
32	ON-KHS-22-14043-v1	0.9929	BF.5 (Omicron)	W	S4	2241-2022-69307
15	ON-KHS-22-13655-v1	0.9385	BA.5.2.1 (Omicron)	Y	S5	2241-2022-69560
20	ON-KHS-22-13780-v1	0.9289	BA.5.2.6 (Omicron)	V	S5	2241-2022-69560
24	ON-KHS-22-13954-v1	0.9796	BA.5.2 (Omicron)	V	S5	2241-2022-69560
25	ON-KHS-22-13955-v1	0.9851	BA.5.2 (Omicron)	V	S5	2241-2022-69560
26	ON-KHS-22-13956-v1	0.9744	BA.5.2 (Omicron)	V	S5	2241-2022-69560
27	ON-KHS-22-13957-v1	0.2569	FAILED		S5	2241-2022-69560
28	ON-KHS-22-13958-v1	0.9000	BA.5.2.1 (Omicron)	X	S5	2241-2022-69560
29	ON-KHS-22-13959-v1	0.9142	BA.5.2 (Omicron)	V	S5	2241-2022-69560
30	ON-KHS-22-13960-v1	0.9508	BA.5.2 (Omicron)	V	S5	2241-2022-69560
31	ON-KHS-22-13971-v1	0.9874	BA.5.2 (Omicron)	V	S5	2241-2022-69560
17	ON-KHS-22-13756-v1	0.9438	BA.5.2.6 (Omicron)	V	M4	2241-2022-69717
21	ON-KHS-22-13939-v1	0.9676	BA.5.2 (Omicron)	V	M4	2241-2022-69717
22	ON-KHS-22-13952-v1	0.9938	BA.5.2 (Omicron)	V	M4	2241-2022-69717
23	ON-KHS-22-13953-v1	0.0264	FAILED		M4	2241-2022-69717
33	ON-KHS-22-14044-v1	0.9930	BA.5.2 (Omicron)	V	M4	2241-2022-69717

Notes: 'FAILED' includes samples that were not successfully sequenced (genome completeness < 90%). A cluster refers to a group of SARS-CoV-2 sequences that are genetically related (≥ 3 mutations). Genome designations are arbitrary letters assigned to delineate genomes containing related sequences and are specific to this WGS request.

Amplicon-based Sequencing for Mpox

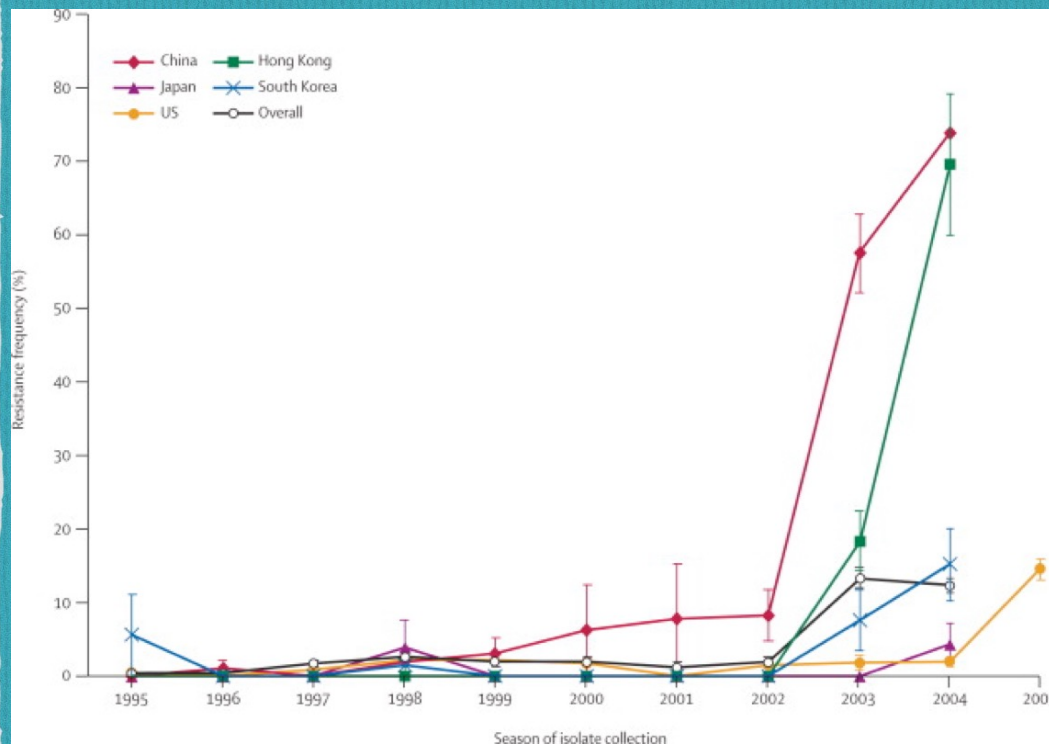


- Two-patients tested positive for Mpox in the community.
- Health unit was contact tracing to determine community transmission.
- Genomics identified that the strains were very different.
- Allowed us to inform our health unit sure that this was not community transmission.

Monitoring Anti-Viral Resistance to SARS-CoV-2

- Nirmatrelvir-ritonavir (Paxlovid) is a newly developed protease inhibitor that is the leading antiviral drug against SARS-CoV-2.
- Paxlovid is an oral drug that is taken twice daily for 5 days in non-hospitalized adults with mild to moderate COVID-19 who are at high risk of disease progression.
- Short dose duration of Paxlovid, is resistance really an issue??

Lessons from the Past : Influenza and Amantadine



- Amantadine was a drug used against Influenza A.
- Amantadine was administered within 24 - 48 hours after symptom onset and continued for 24 to 48 hours after symptom resolution.
- Short dose, resistance developed rapidly...drug is no longer indicated for Influenza A.

Monitoring Anti-Viral Resistance to SARS-CoV-2



Original Investigation | Infectious Diseases

Prevalence of Low-Frequency, Antiviral Resistance Variants in SARS-CoV-2 Isolates in Ontario, Canada, 2020-2023

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Abstract

IMPORTANCE Nirmatrelvir-ritonavir is an oral antiviral medication that improves outcomes in SARS-CoV-2 infections. However, there is concern that antiviral resistance will develop and that these viruses could be selected for after treatment.

OBJECTIVE To determine the prevalence of low-frequency SARS-CoV-2 variants in patient samples that could be selected for by nirmatrelvir-ritonavir.

DESIGN, SETTING, AND PARTICIPANTS This retrospective cohort study was conducted at 4 laboratories that serve community hospitals, academic tertiary care centers, and COVID-19 assessment centers in Ontario, Canada. Participants included symptomatic or asymptomatic patients who tested positive for SARS-CoV-2 virus and submitted virus samples for diagnostic testing between March 2020 and January 2023.

EXPOSURE SARS-CoV-2 infection.

MAIN OUTCOMES AND MEASURES Samples with sufficient viral load underwent next-generation genome sequencing to identify low-frequency antiviral resistance variants that could not be identified through conventional sequencing.

RESULTS This study included 78 866 clinical samples with next-generation whole-genome sequencing data for SARS-CoV-2. Low-frequency variants in the viral *nsp5* gene were identified in 128 isolates (0.16%), and no single variant associated with antiviral resistance was predominate.

CONCLUSIONS AND RELEVANCE This cohort study of low-frequency variants resistant to nirmatrelvir-ritonavir found that these variants were very rare in samples from patients with SARS-CoV-2, suggesting that selection of these variants by nirmatrelvir-ritonavir following the initiation of treatment may also be rare. Surveillance efforts that involve sequencing of viral isolates should continue to monitor for novel resistance variants as nirmatrelvir-ritonavir is used more broadly.

Key Points

Question What is the prevalence of low-frequency variants in SARS-CoV-2 isolates from patient samples that could confer resistance to nirmatrelvir-ritonavir?

Findings In this cohort study, 78 866 SARS-CoV-2 isolates from patients underwent next-generation sequencing, and low-frequency variants were detected in 128 samples (0.16%).

Meaning These findings suggest that SARS-CoV-2 variants that could be selected for by treatment with nirmatrelvir-ritonavir are rare and that surveillance efforts that involve sequencing of viral isolates should continue to monitor for novel resistance variants.

+ Supplemental content

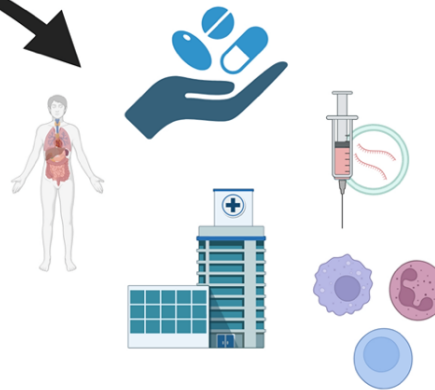
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- We evaluated >78,000 SARS-CoV-2 sequences from patients in Ontario, Canada between March 2020 and Jan 2023.
- Paxlovid resistance was found in 128/78,866 (0.16%) of clinical isolates.
- Levels of resistance were evaluated using the genomic sequences from the Ontario COVID-19 genomics laboratories led by Dr. Kozak (Toronto) and Dr. Sheth (Eastern and Northern Ontario).
- Currently doing a follow up study with over 100,000 patients and using provincial data to monitor Paxlovid Resistance.

Combining Genomics and Patient Outcomes

Combine genomics data
to clinical data

> 95,000 Genomes currently available



COVID - 19 genomic information from
Dr. Sheth and Dr. Kozak's Labs

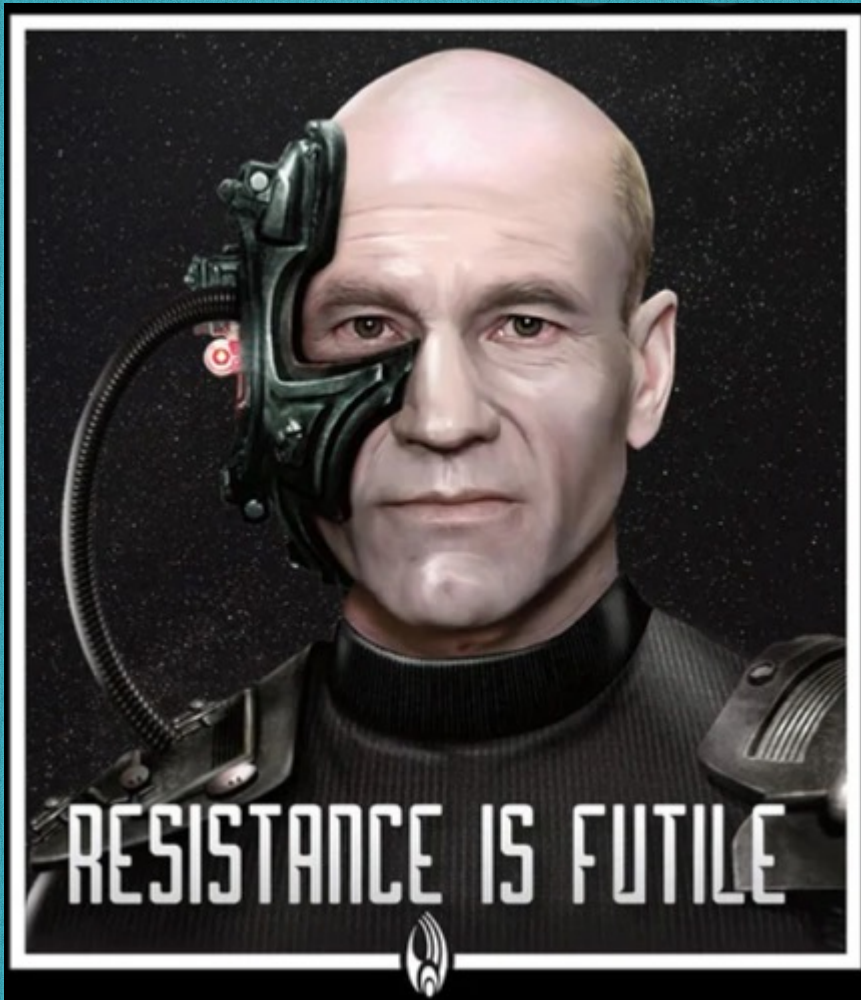
Members of the OCGN (ON COVID-19
Genomics Network Lab)

- Patient immune status
- Vaccination uptake
- Hospitalizations
- Antiviral dispersment

Ongoing Antiviral
Resistance Surveillance

ICES Databases using HCN

1. RPDB (patient demographics and vital status)
2. COVAXON (Immunizations)
3. ODB (paxlovid prescriptions)
4. CIHI-DAD (Hospitalization and co-morbidities data)
5. CCM (COVID-19 outcomes)
6. OHIP (Healthcare utilization)
7. IPDB (prescriber information)



The Borg were wrong !!

RESISTANCE IS NOT FUTILE

....IT IS INEVITABLE

Wong, Sjaarda and Sheth, Unpublished data

Summary

- Genomics is very useful for population level surveillance for emerging and endemic pathogen.
- The wide adoption of Genomics is currently impeded by costs linked to personnel and computing.
- Analysis is challenging as it is organism dependent and need to know the stability of the genome.
- Need to incorporate genomics into outbreak investigations to help with Public Health and Institutional Contact Tracing for a wider array of pathogens (Syphilis, Influenza, C. difficile, Salmonella, Shigella, Multidrug Resistant Organisms (MDRO), TB etc).



**Thank you for your time.
Happy to take questions !**

(FREE Teleclass)

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