Applications of Bioinformatics in the Real-Time Molecular Surveillance of Viral Pathogens

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Computer Science & Engineering
University of California, San Diego

Outline

- Introduction to Viral Molecular Epidemiology
- Sequencing the First Viral Genome
- Annotating a Viral Genome
- Sequencing in the Midst of an Epidemic
- Aligning Viral Genome Sequences
- Phylogenetic Inference and Transmission Clustering

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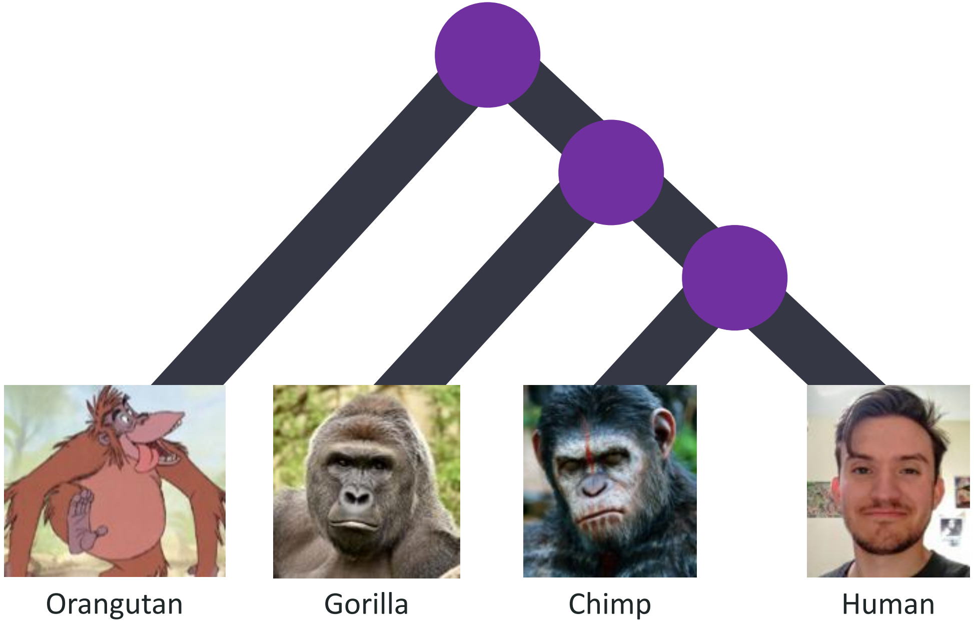
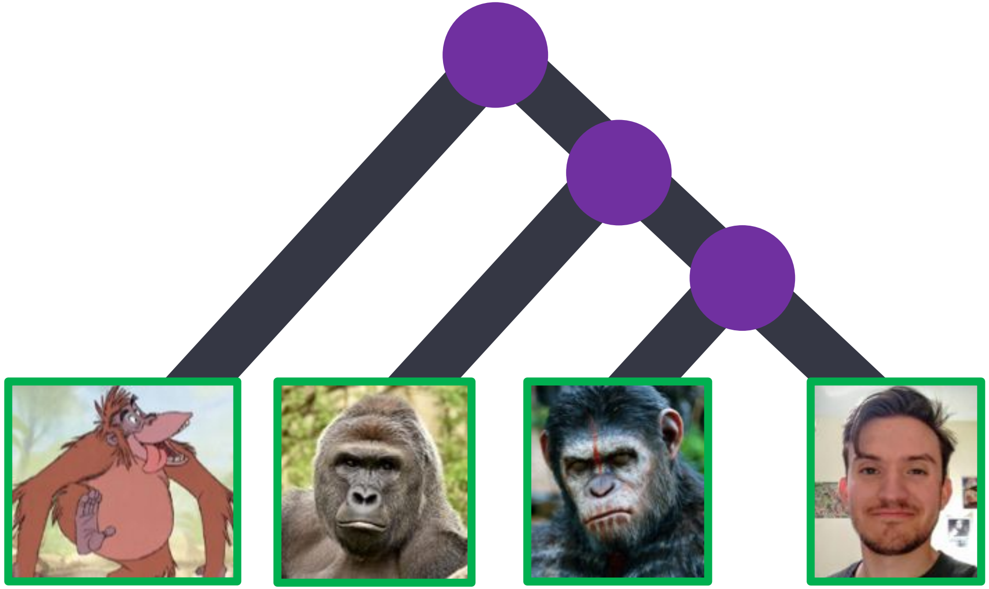
Phylogeny



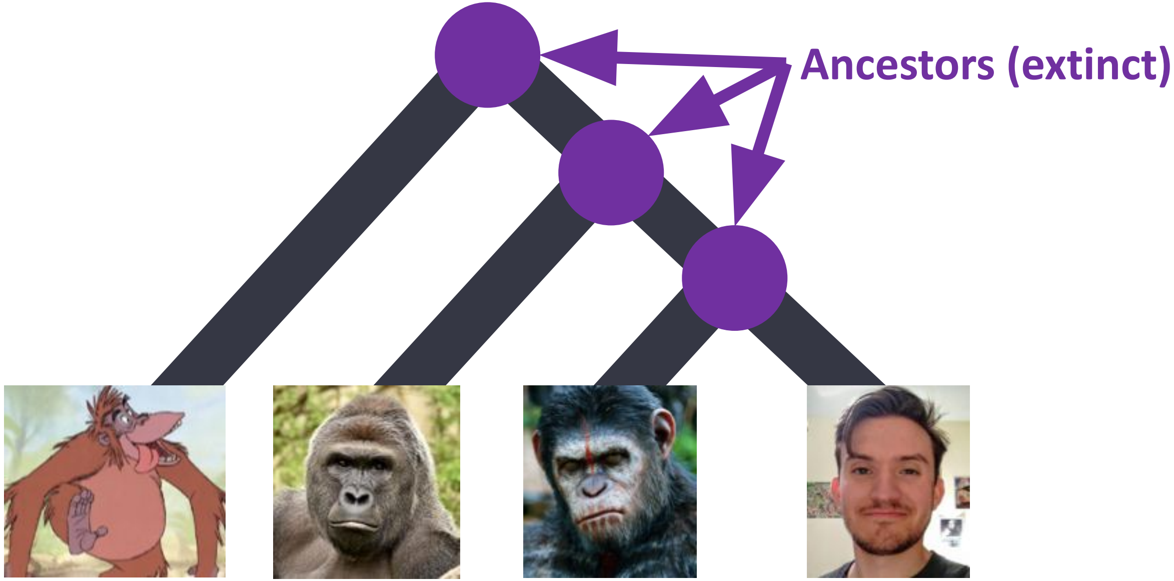
Phylogeny



Orangutan Gorilla Chimp Human

Present-Day Species

Phylogeny



Ancestors (extinct)

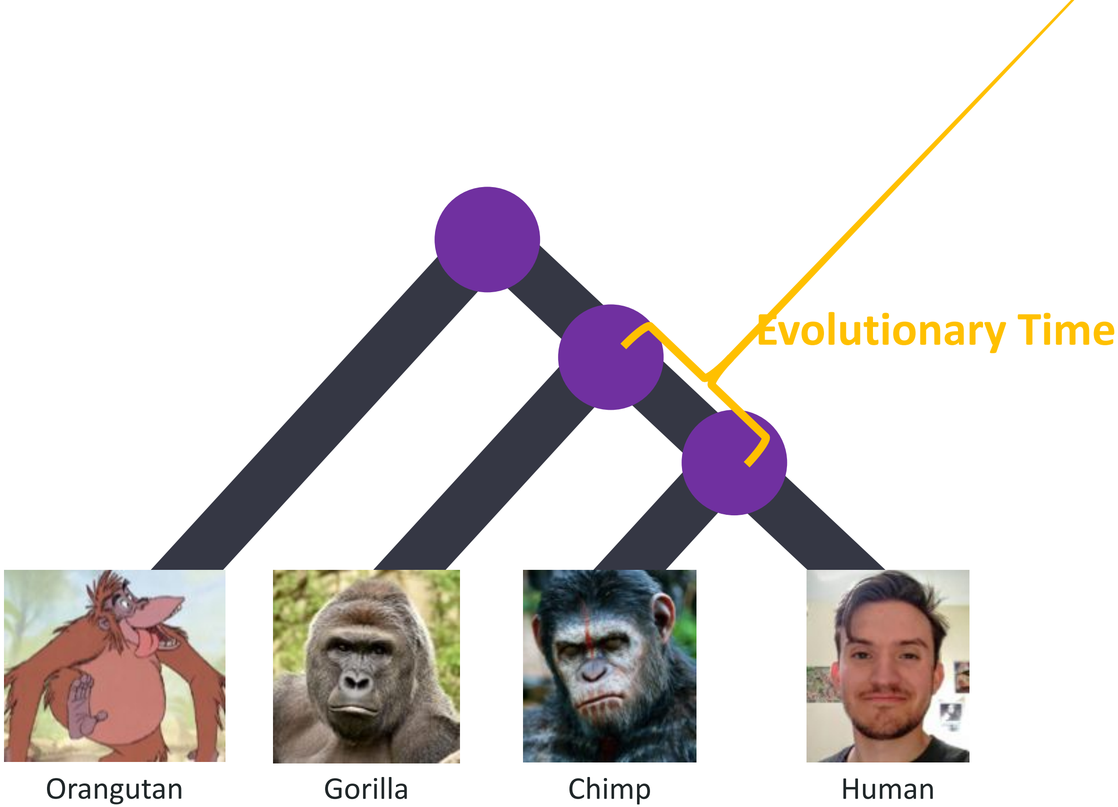
Orangutan

Gorilla

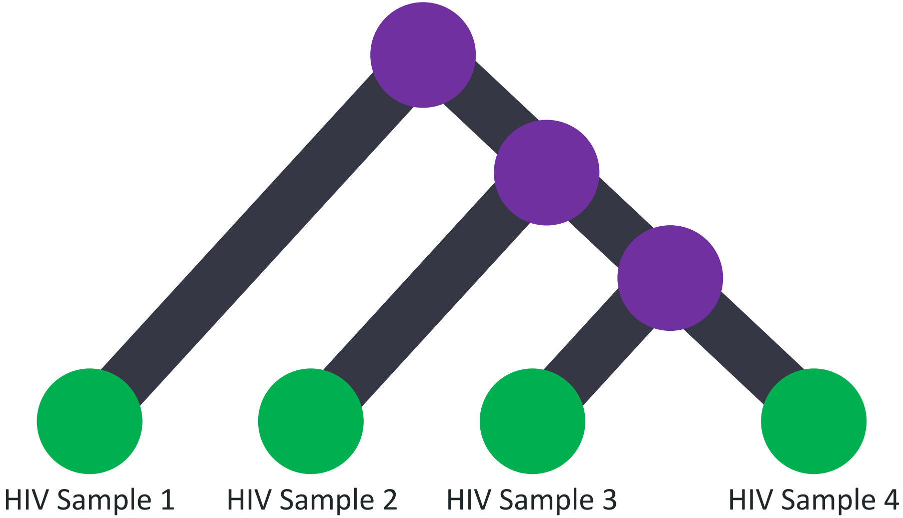
Chimp

Human

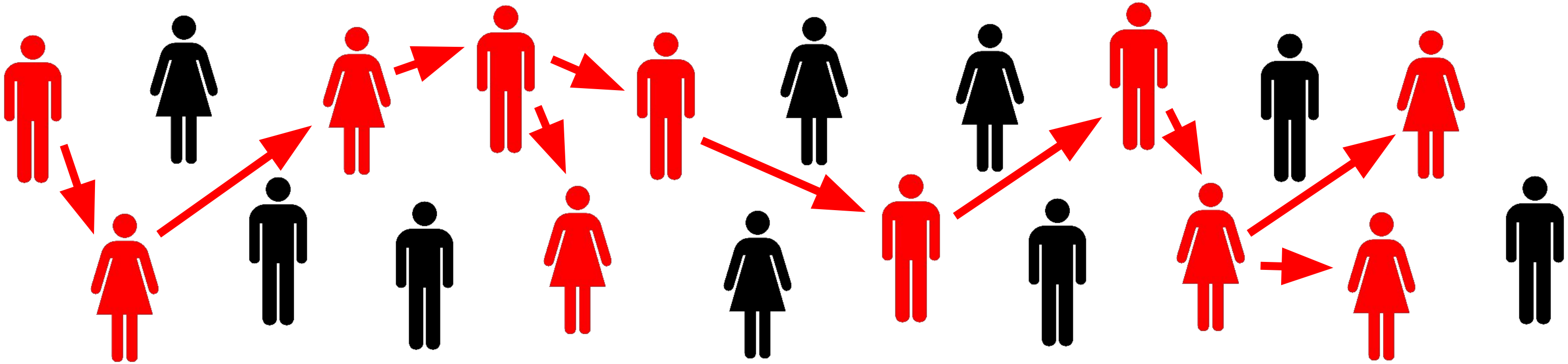
Phylogeny



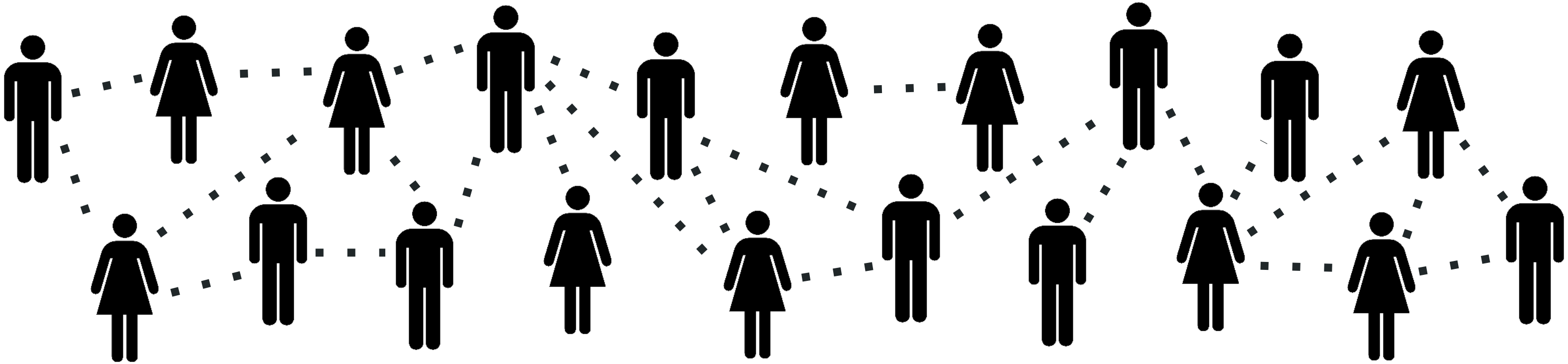
Phylogeny



Epidemic



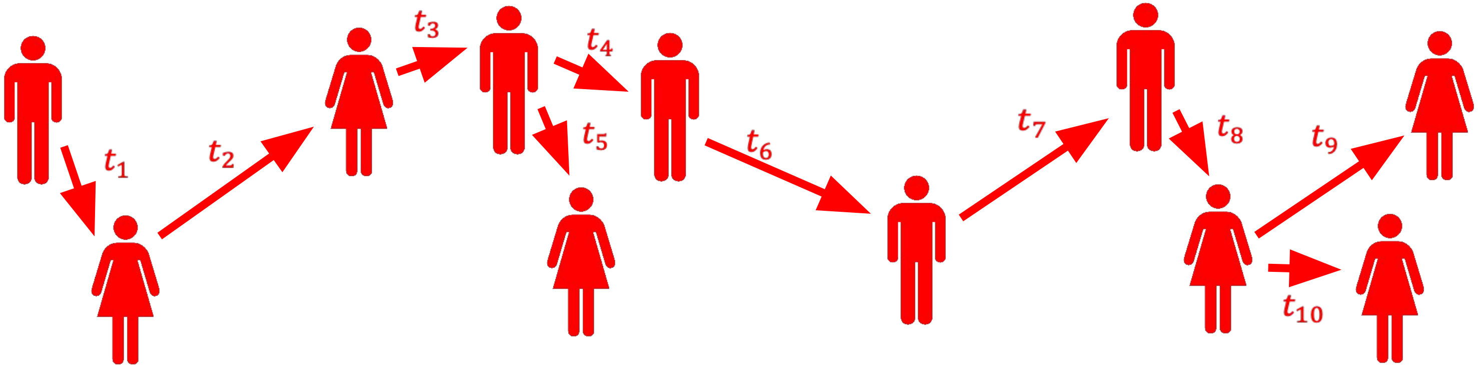
Contact Network



Nodes: Individuals

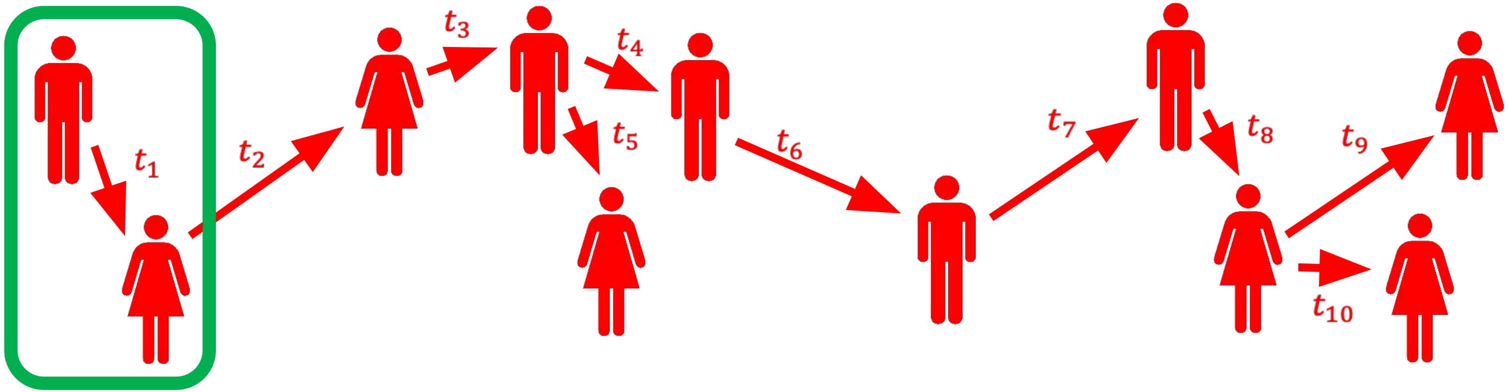
Edges: Risky contacts

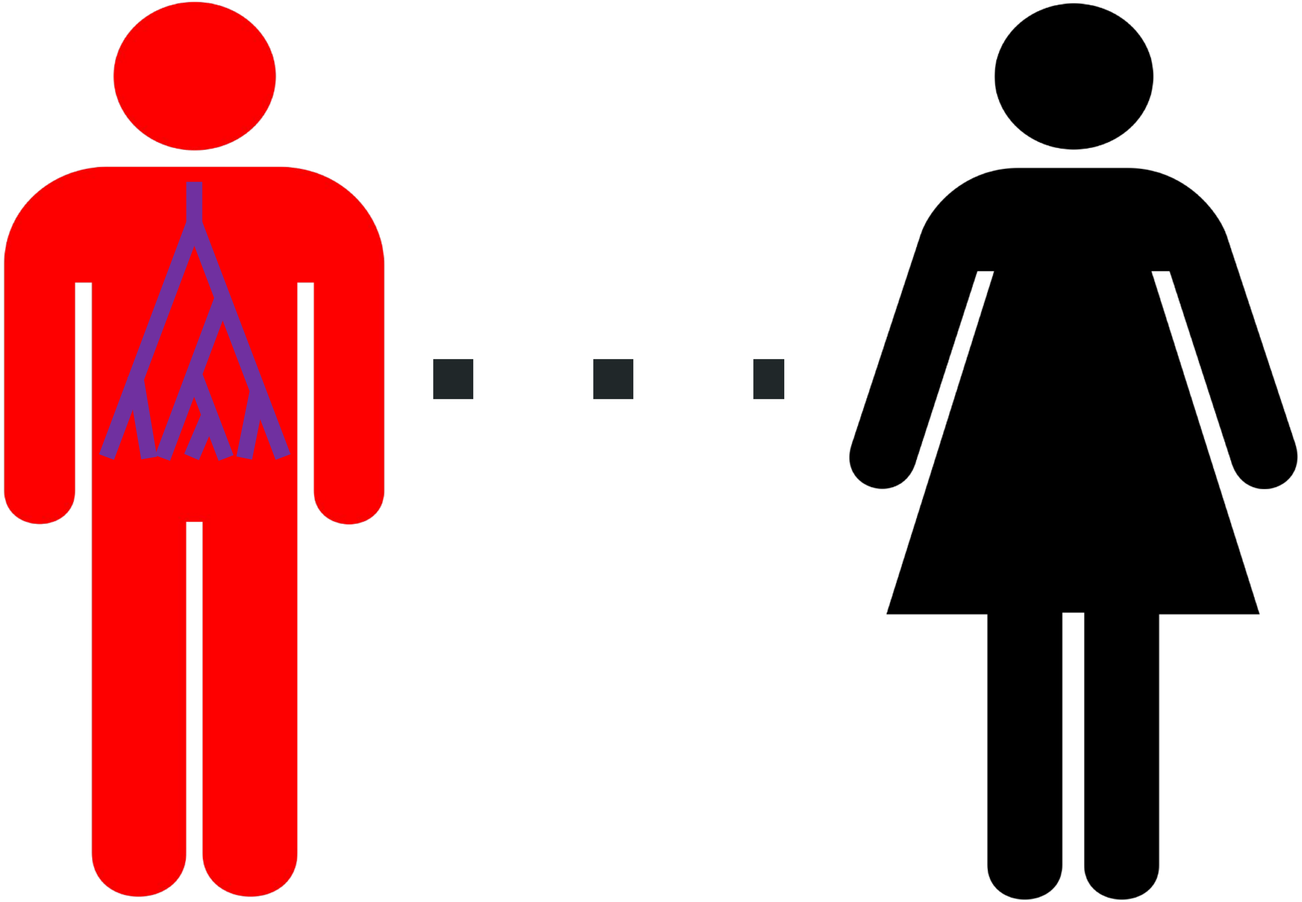
Transmission Network

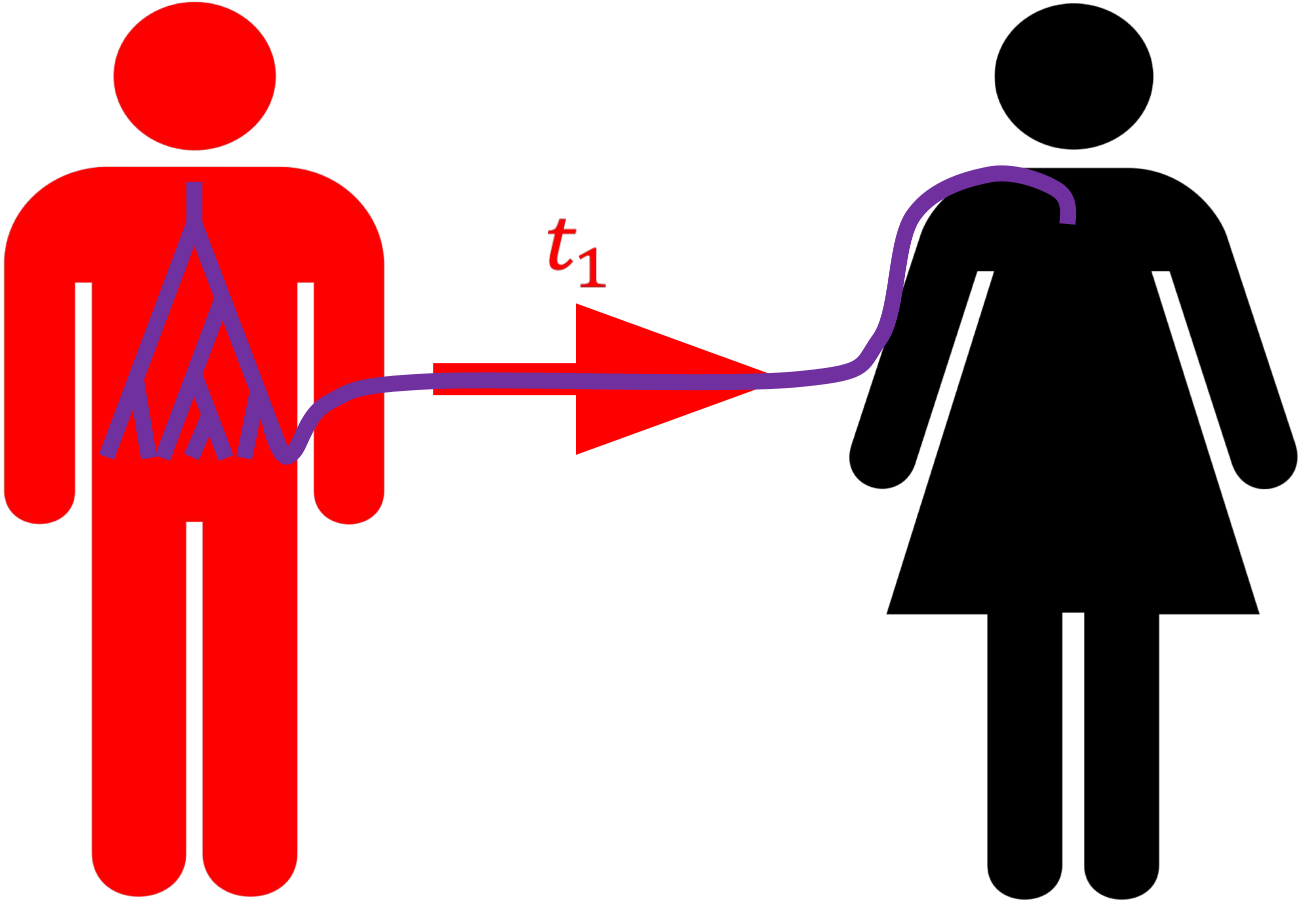


Nodes: Individuals
Edges: Transmissions

Individual Transmission Event



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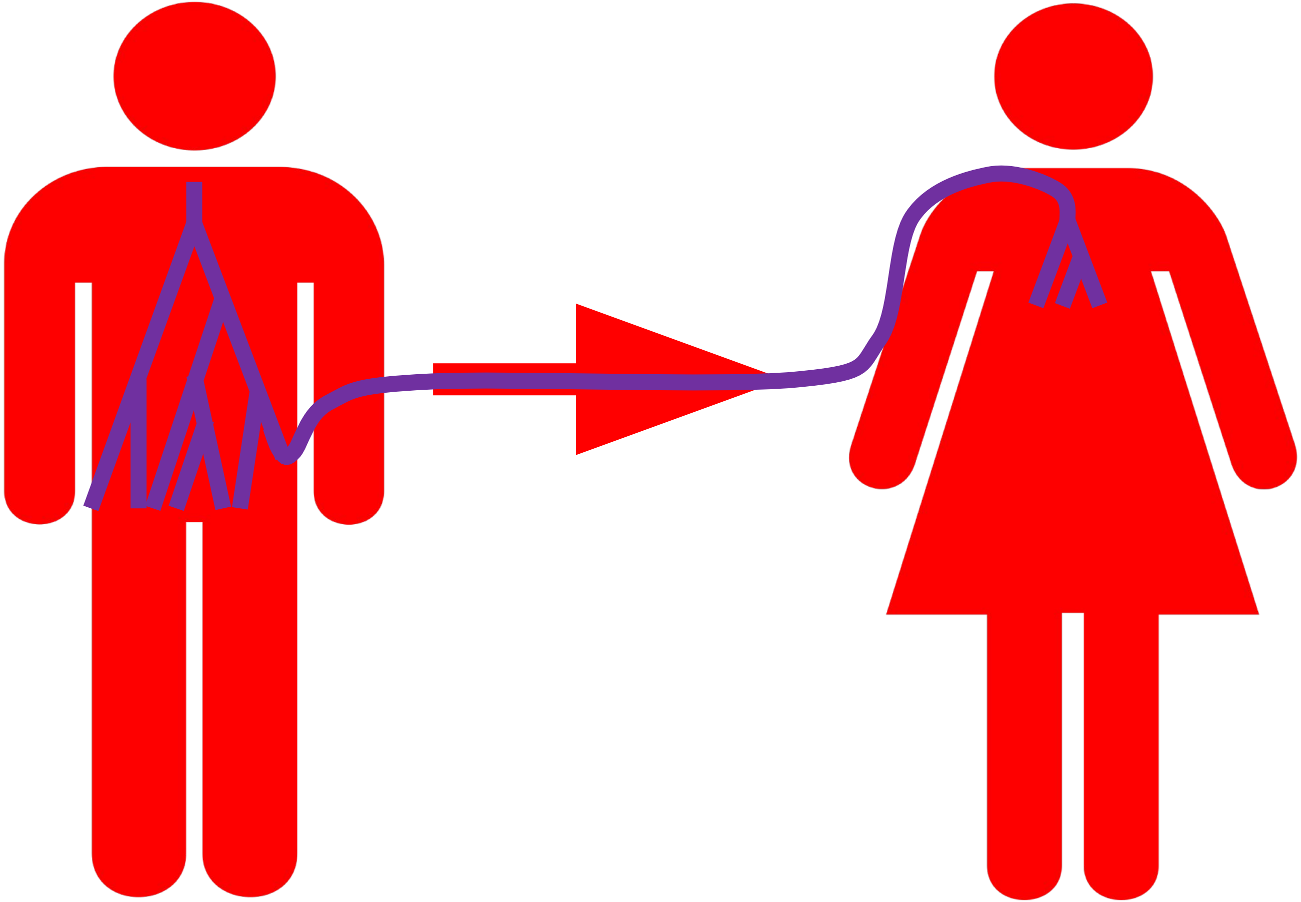
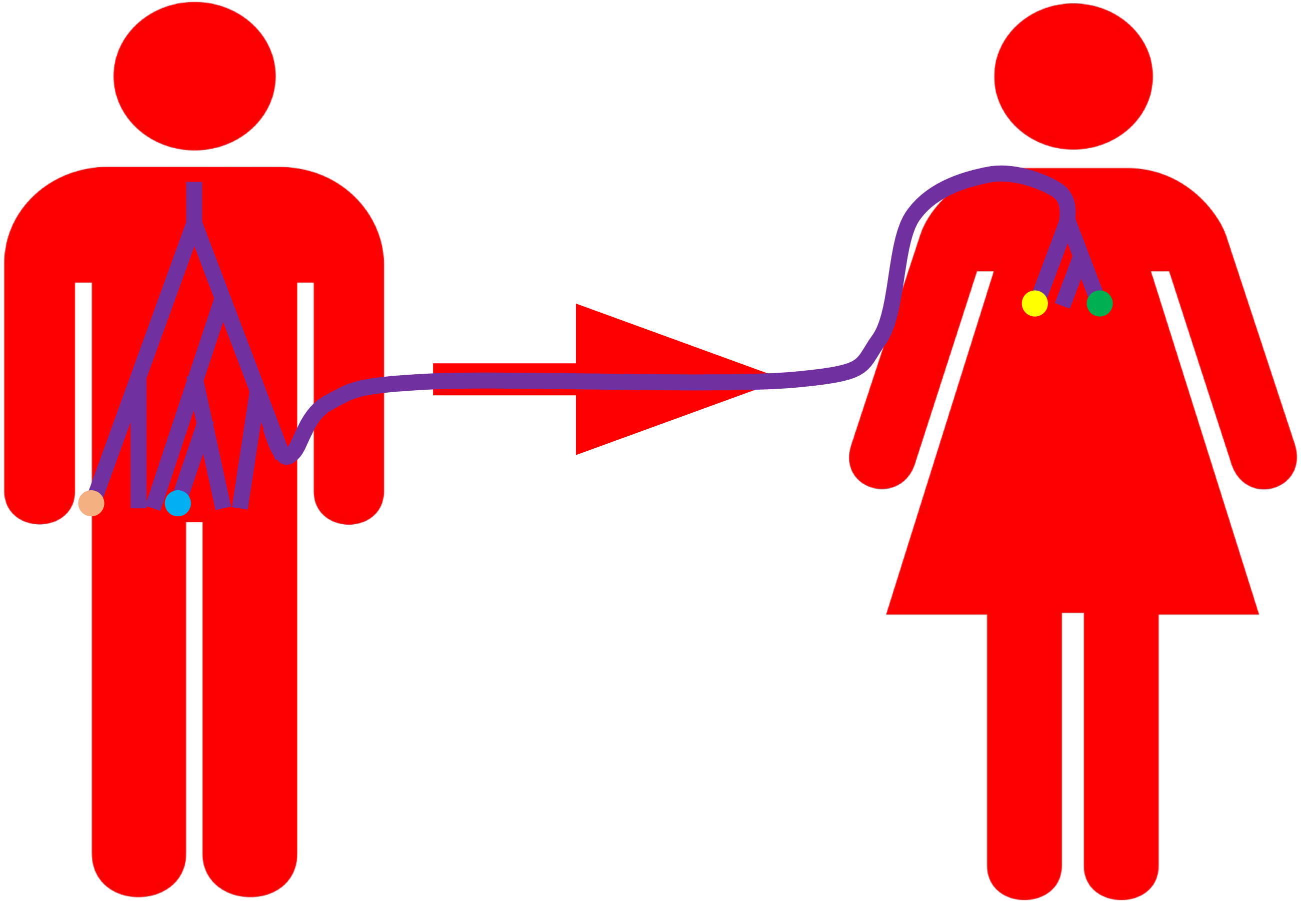
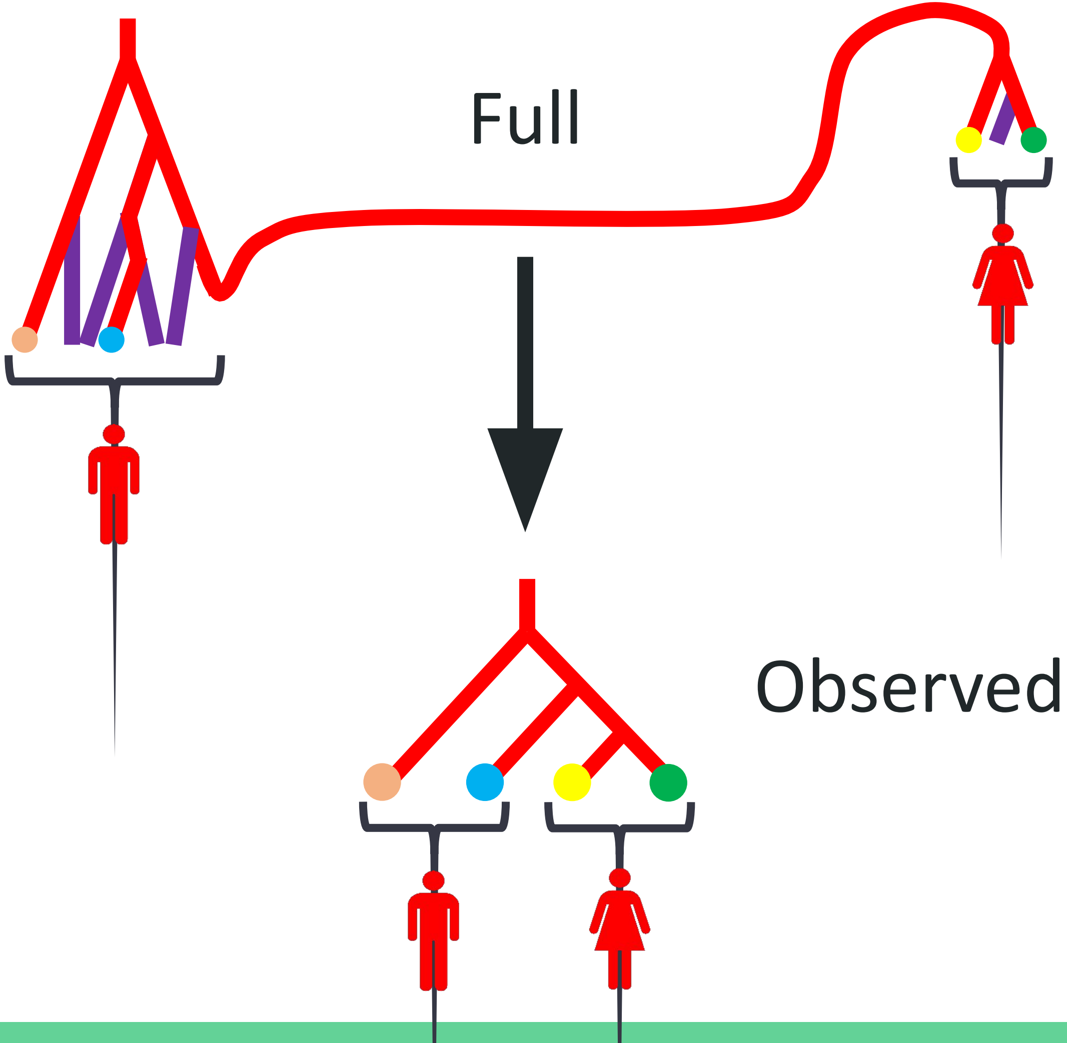
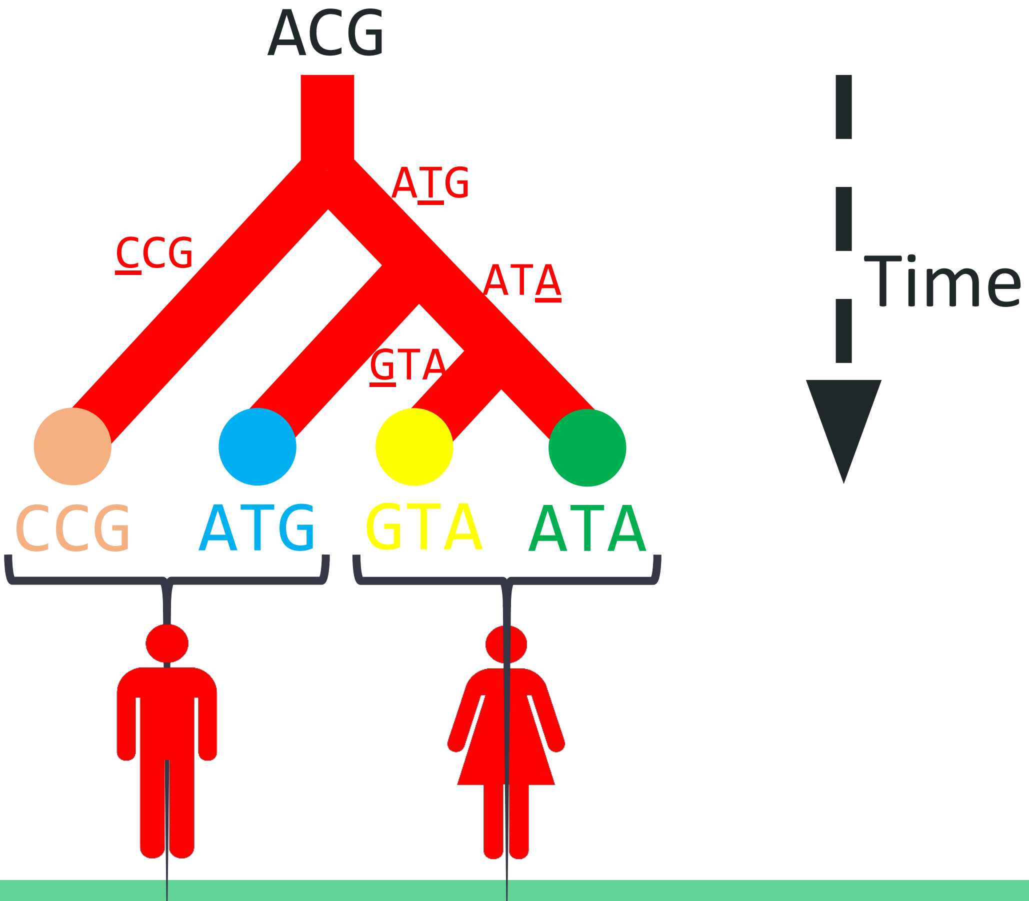






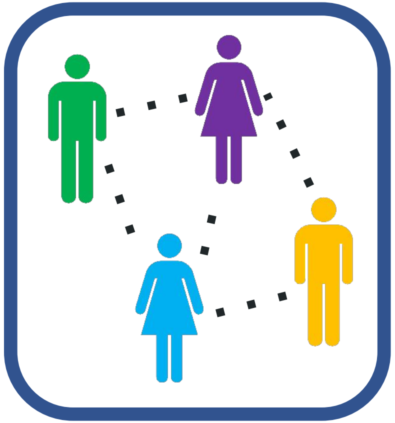
Full

Observed


ACG
ATG
CCG
ATA
GTA
Time
CCG
ATG
GTA
ATA

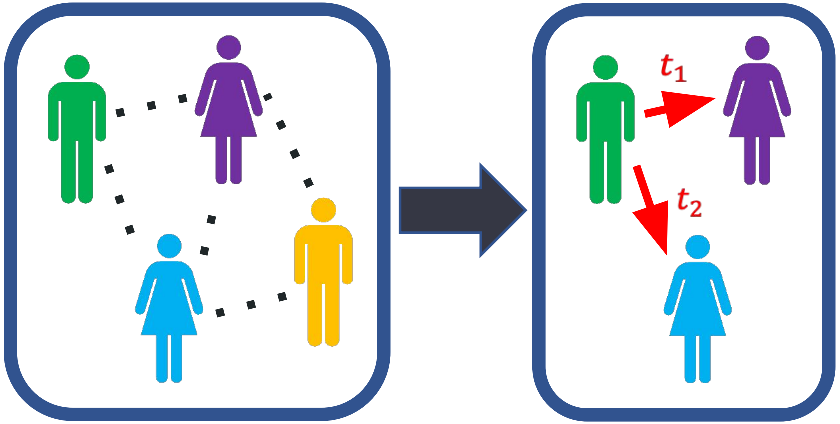
Generative Process

Generative Process



Contact Network

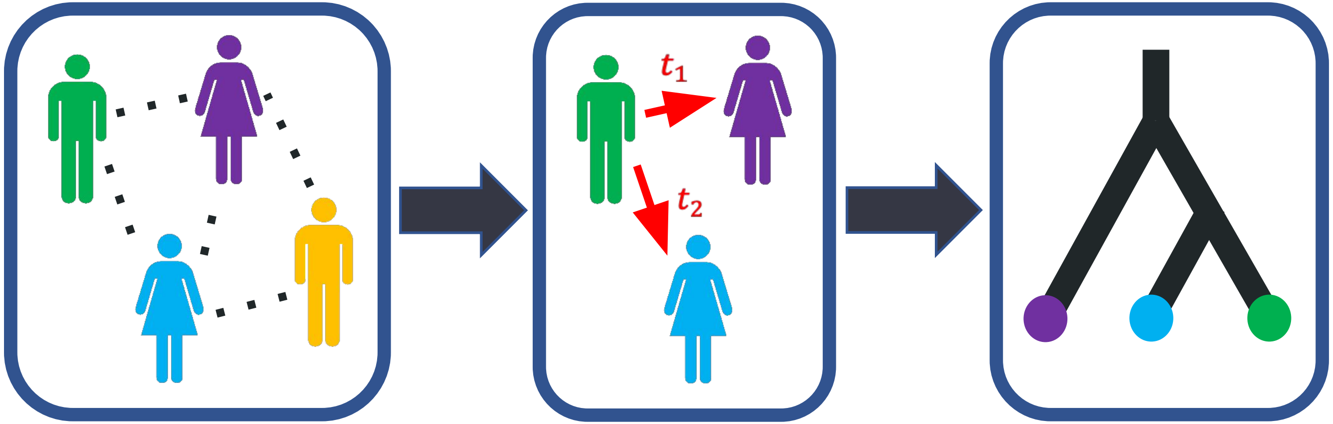
Generative Process



Contact Network

Transmission Network

Generative Process

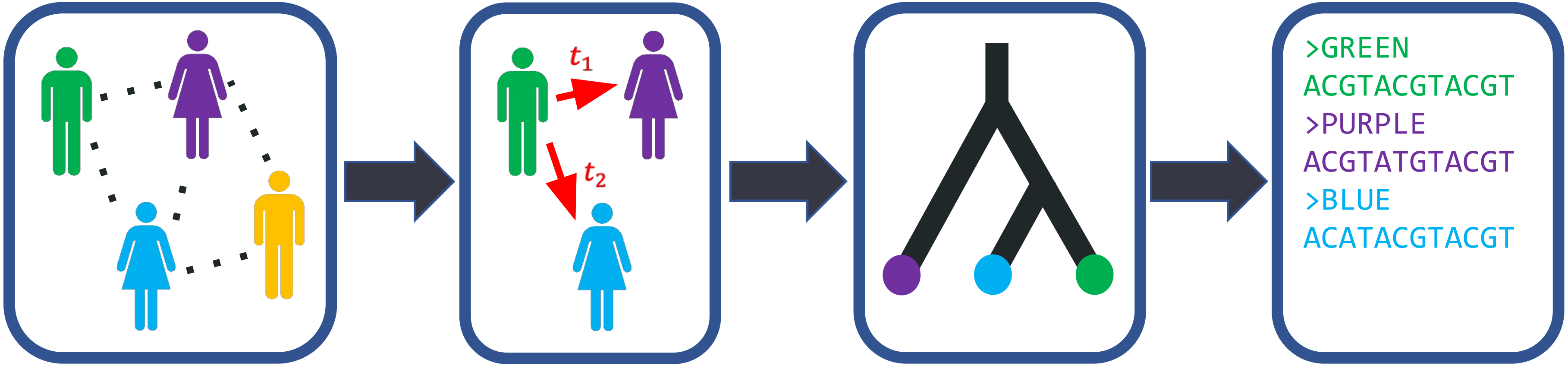


Contact Network

Transmission Network

Viral Phylogeny

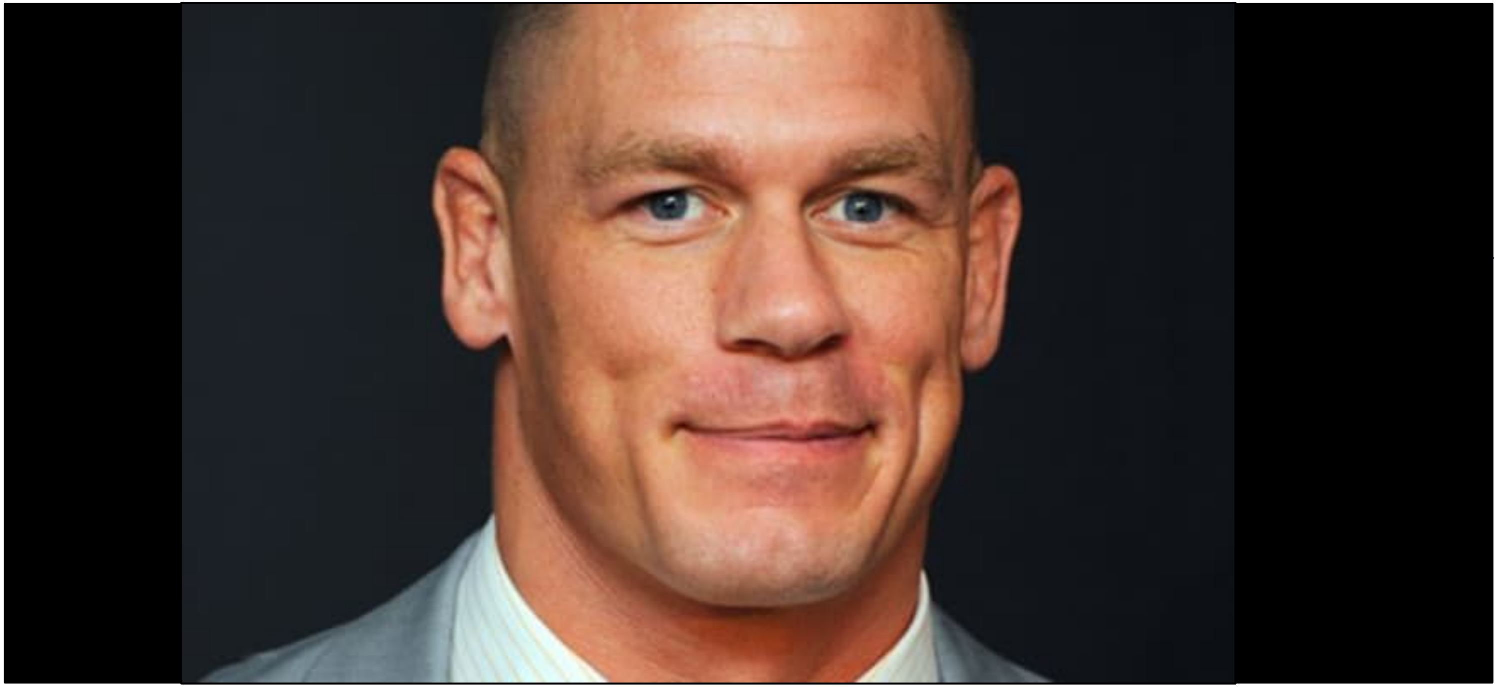
Generative Process



```
>GREEN
ACGTACGTACGT
>PURPLE
ACGTATGTACGT
>BLUE
ACATACGTACGT
```

Contact Network → Transmission Network → Viral Phylogeny → Viral Sequences

Generative Process



```
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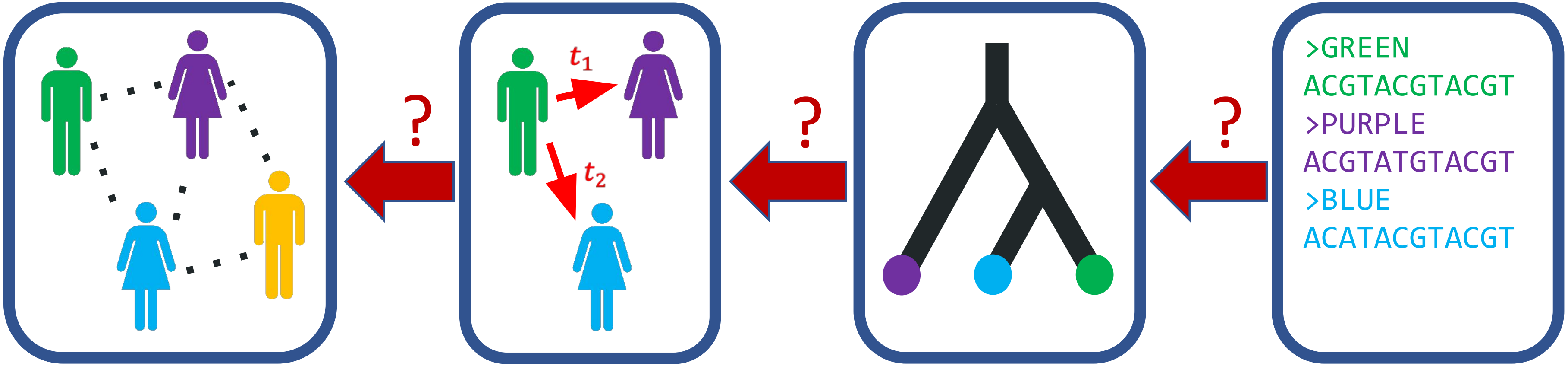
>BLUE
ACATACGTACGT
```

Viral Sequences

Inference



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ACGTATGTACGT
>BLUE
ACATACGTACGT
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Contact Network

Transmission Network

Viral Phylogeny

Viral Sequences

Viral Molecular Epidemiology

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Viral Molecular Epidemiology

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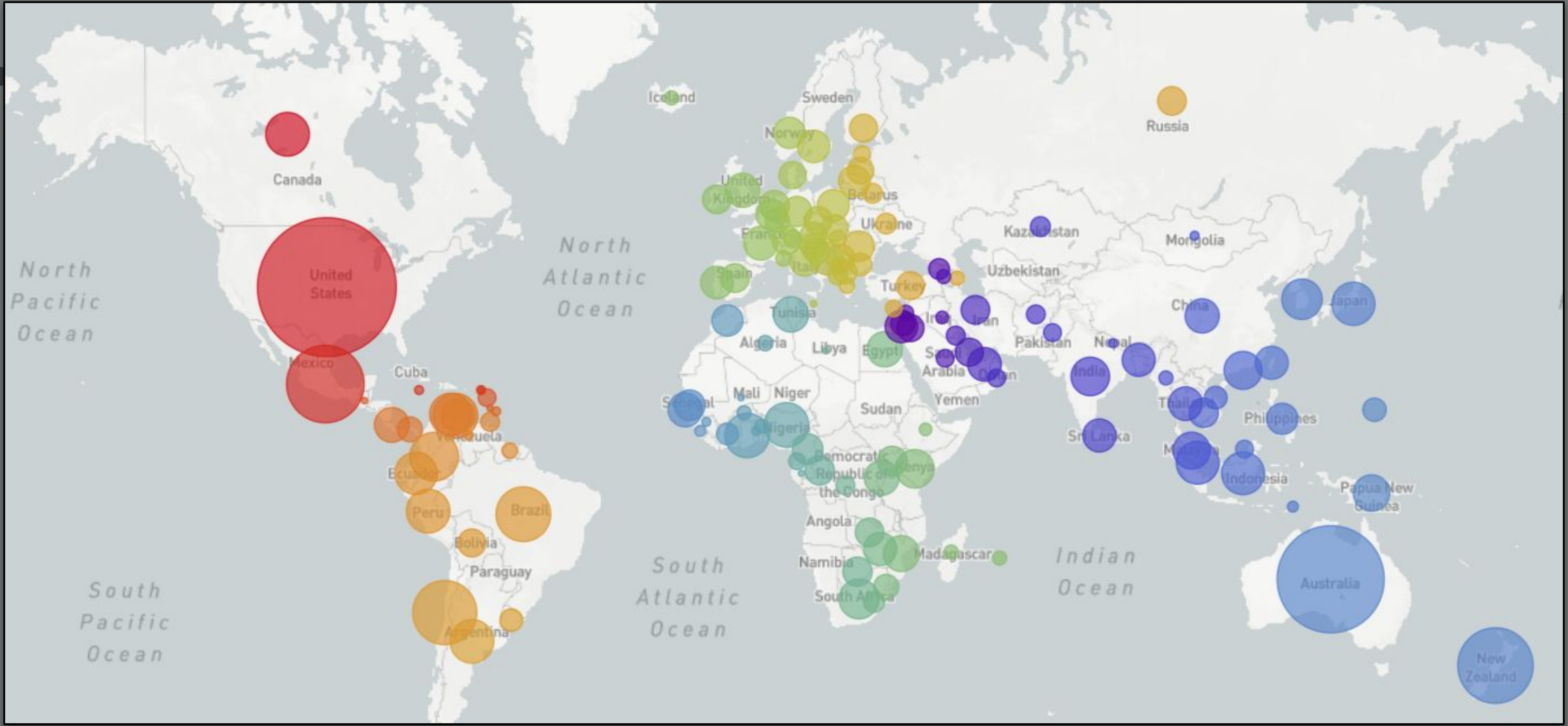
  - How did the virus spread across our communities or across the world?

Viral Molecular Epidemiology



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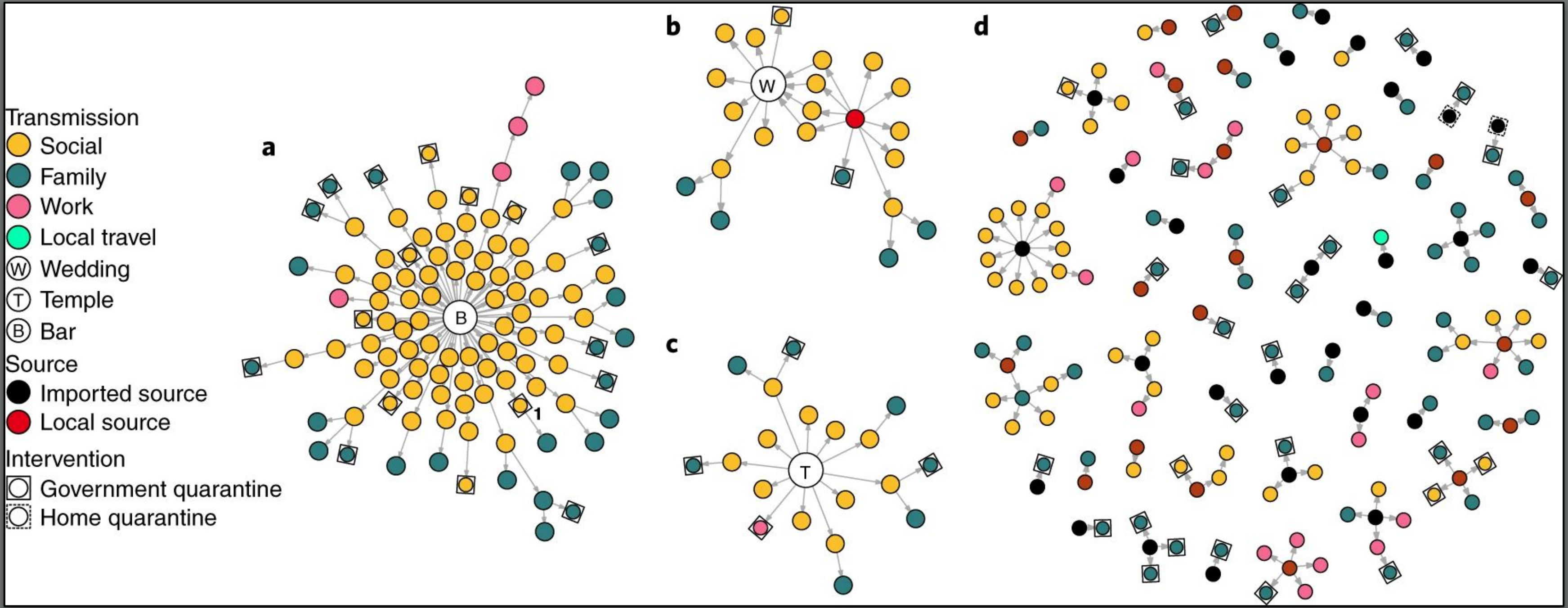
Viral Molecular Epidemiology



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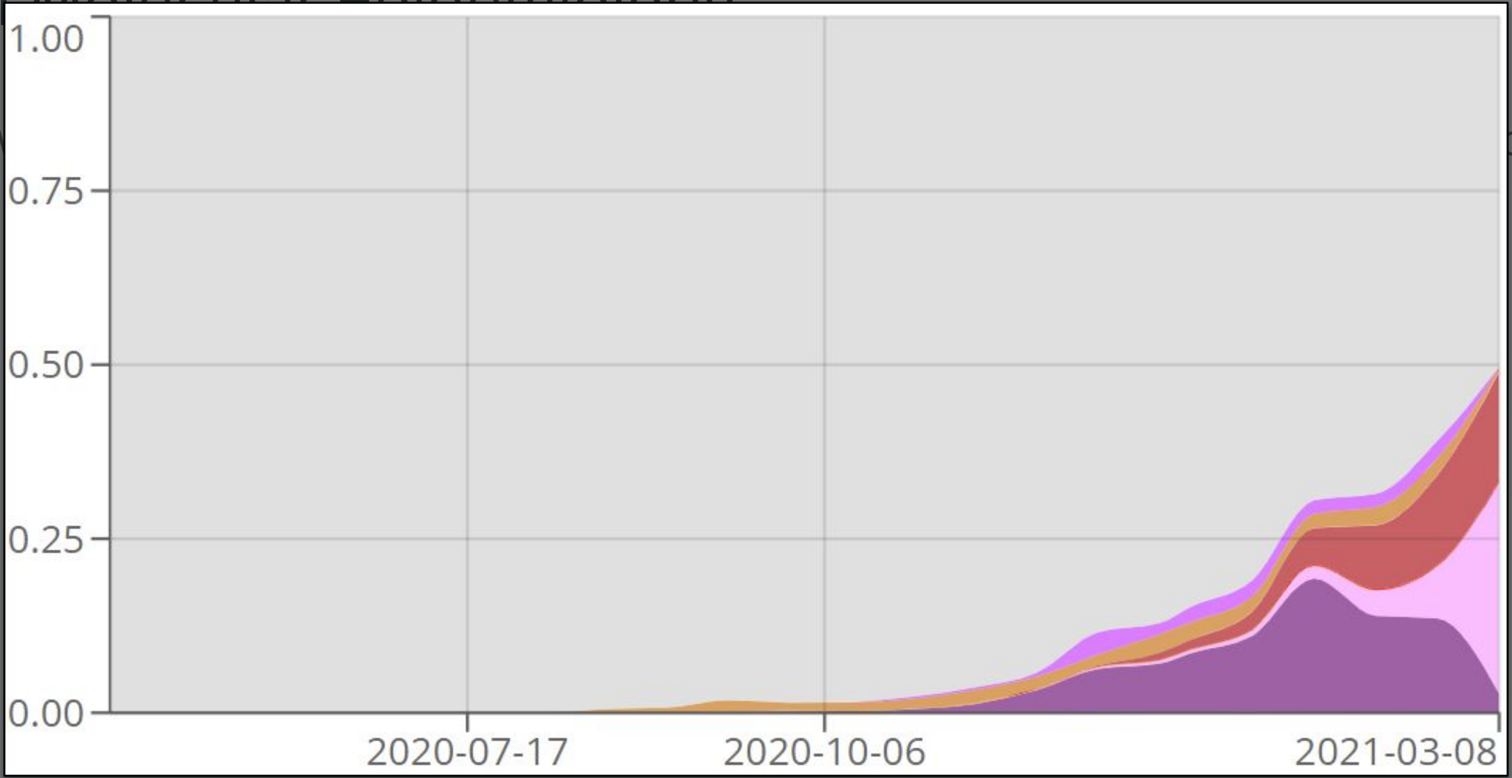
Viral Molecular Epidemiology

- W c



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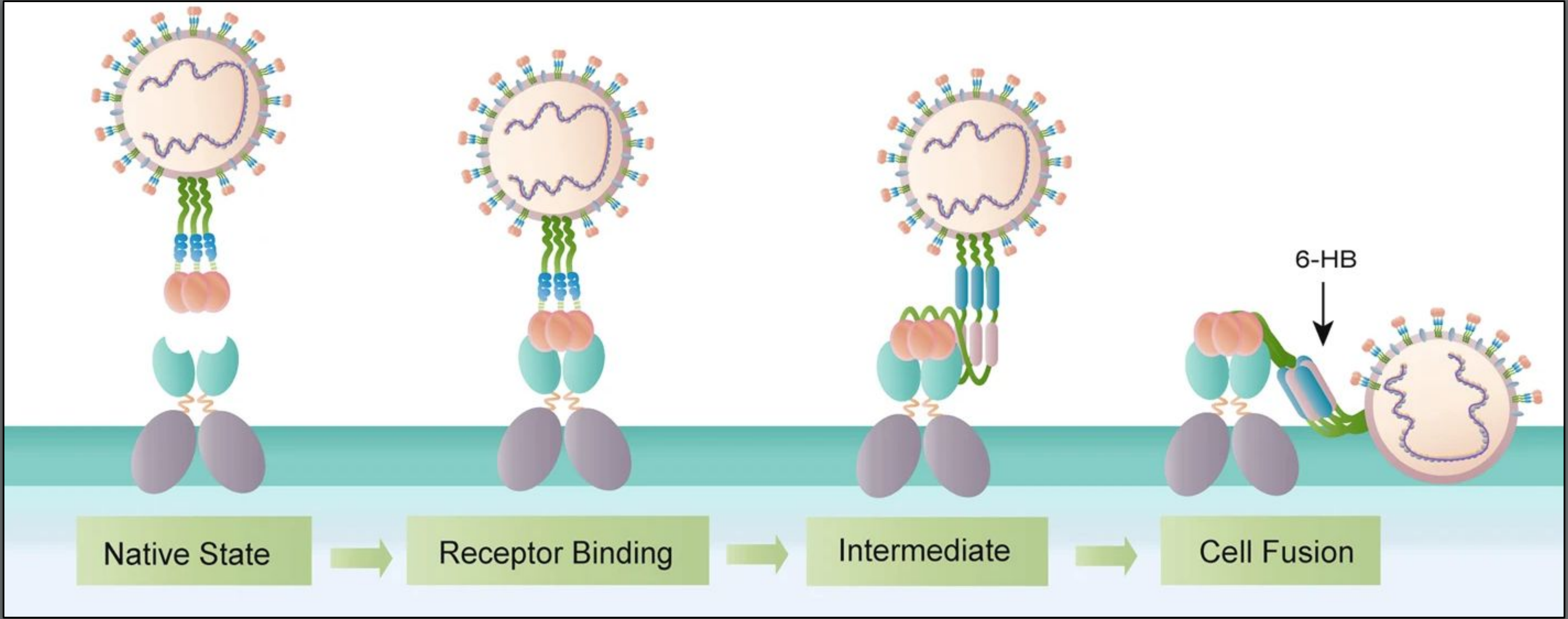
Viral Molecular Epidemiology



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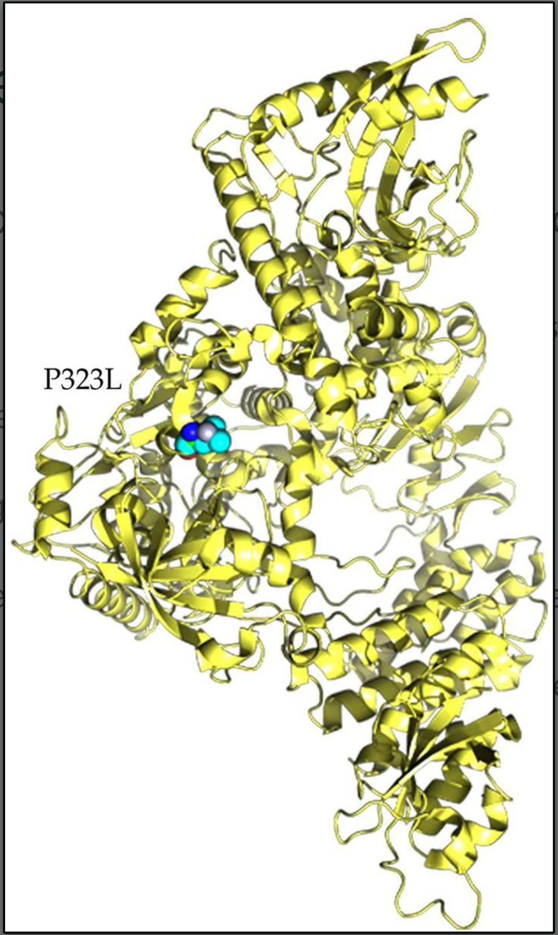

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- How can we translate such questions into formal computational problems?

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stack of NY Times, June 27, 2000

stack of NY Times, June 27, 2000

stack of NY Times, June 27, 2000
on a pile of dynamite

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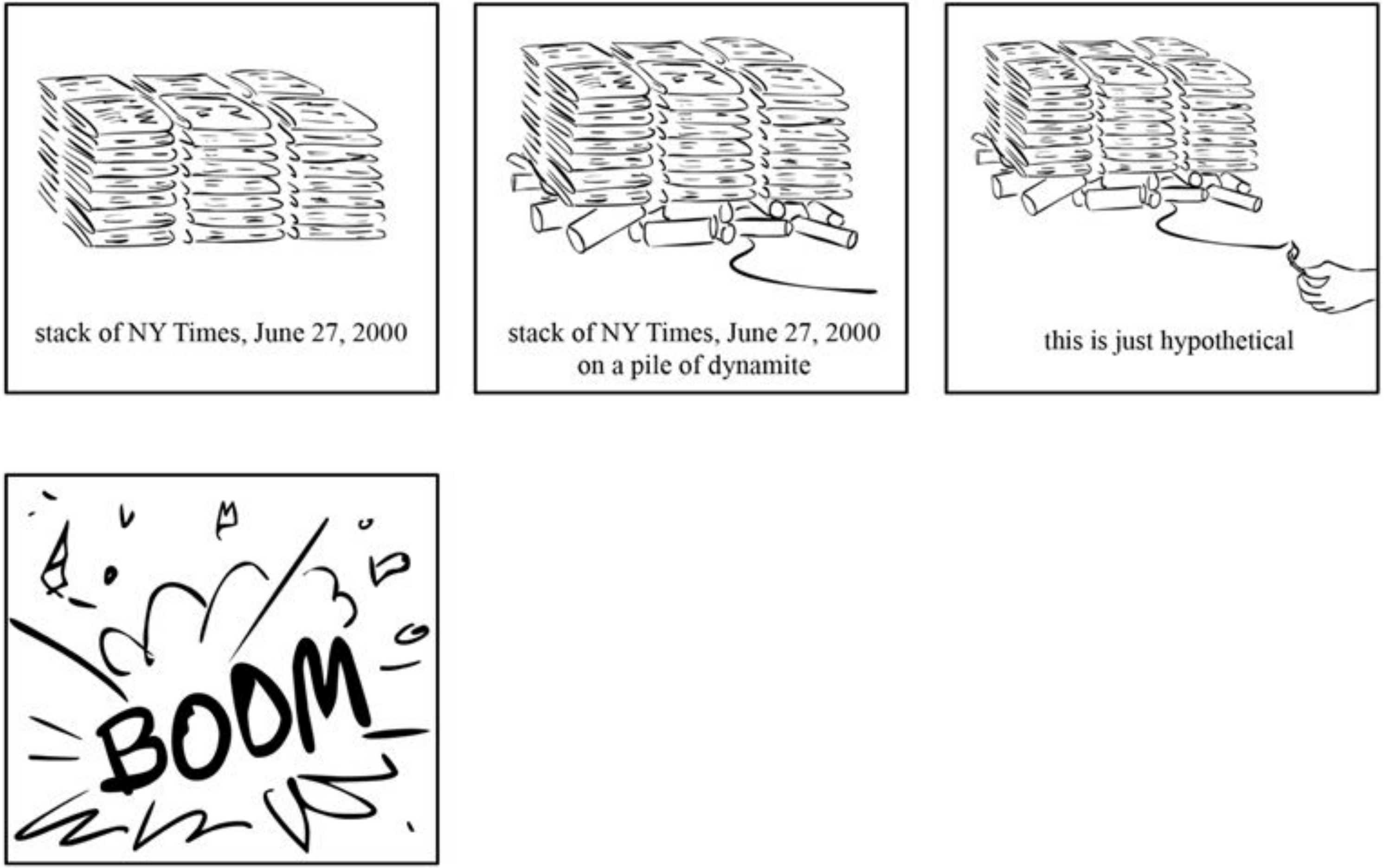
this is just hypothetical


stack of NY Times, June 27, 2000
stack of NY Times, June 27, 2000 on a pile of dynamite
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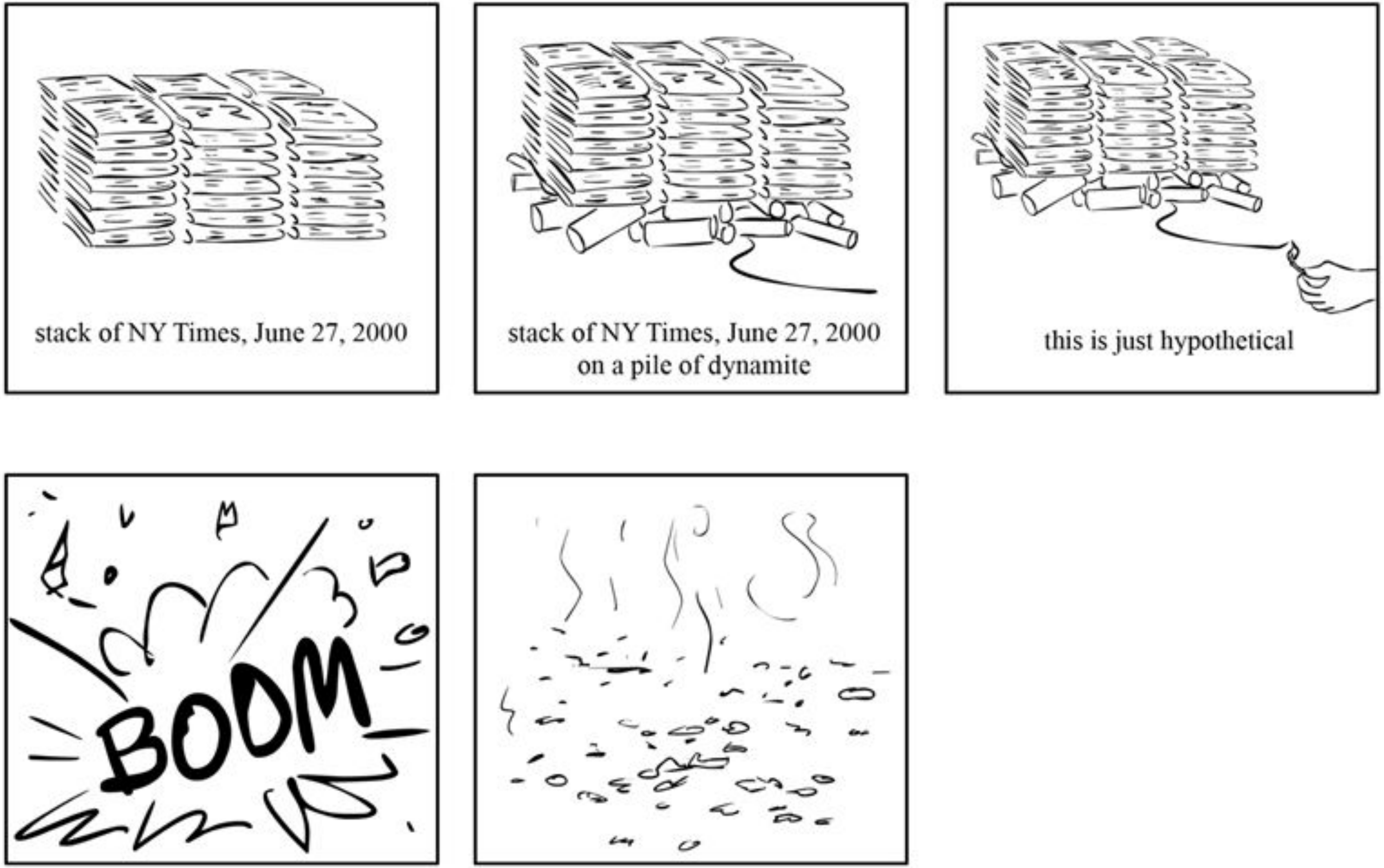
BOOM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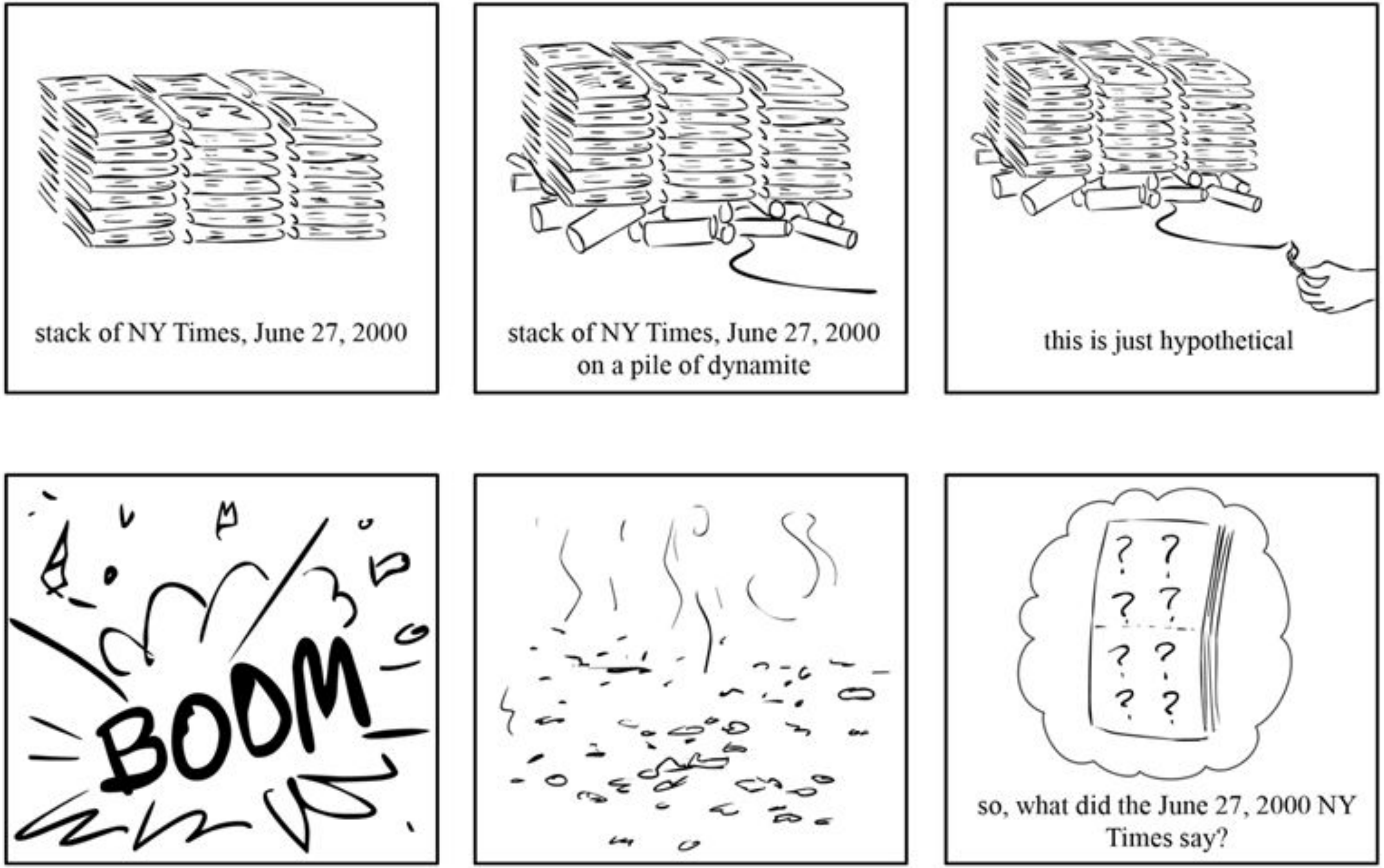
stack of NY Times, June 27, 2000
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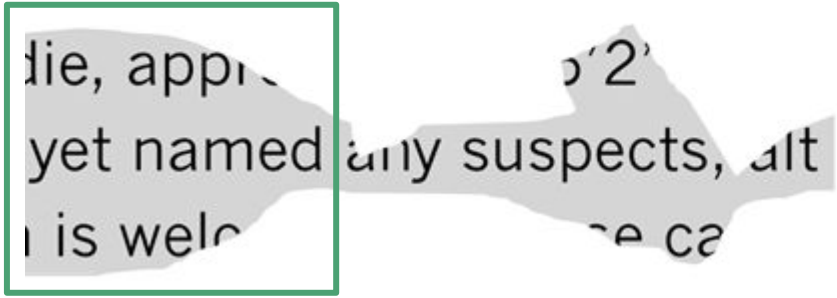
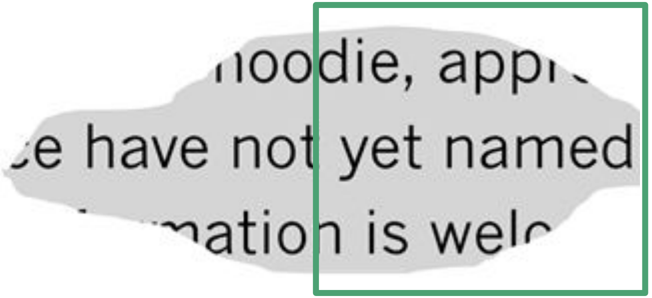
this is just hypothetical
BOOM
so, what did the June 27, 2000 NY Times say?



die, appr ’2’

yet named any suspects, alt

is welc ce ca



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ce have not yet named

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stack of NY Times, June 27, 2000

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CTGATGATGGACTACGCTACTACTGCTAGCTGTATTACGATCAGCTACCACATCGTAGCTACGATGCATTAGCAAGCTATCGGATCAGCTACCACATCGTAGC

CTGATGATGGACTACGCTACTACTGCTAGCTGTATTACGATCAGCTACCACATCGTAGCTACGATGCATTAGCAAGCTATCGGATCAGCTACCACATCGTAGC

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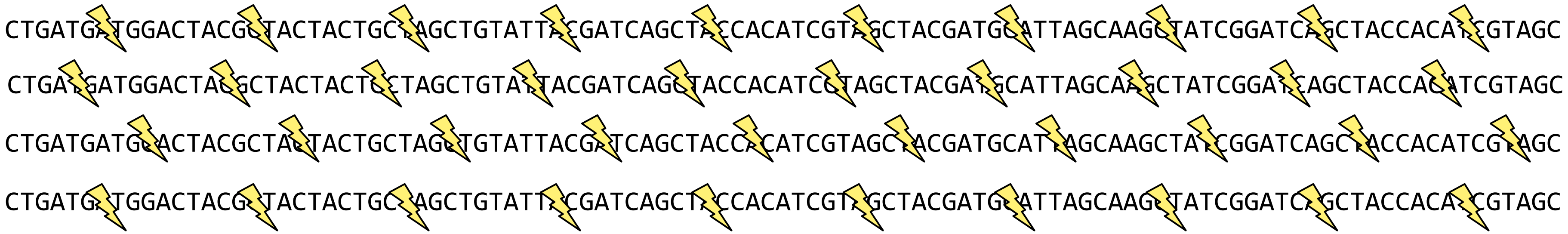
stack of NY Times, June 27, 2000



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stack of NY Times, June 27, 2000

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CTGATGATG ACTACGCT CTACTGCTAG TGTATTACG TCAGCTACC CATCGTAGC ACGATGCAT AGCAAGCTA CGGATCAGC ACCACATCG AGC

CTGAT ATGGACTAC CTACTACTG TAGCTGTATT CGATCAGC ACCACATCGT GCTACGATG ATTAGCAA CTATCGGATCA CTACCAC TCGTAGC

stack of NY Times, June 27, 2000

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CTAGCTGTA TACGATCAG GCATTAGC CAGCTACCA

CTGATGATG ACTACGCT TGTATTACG TCAGCTACC CATCGTAGC AGCAAGCTA ACCACATCG

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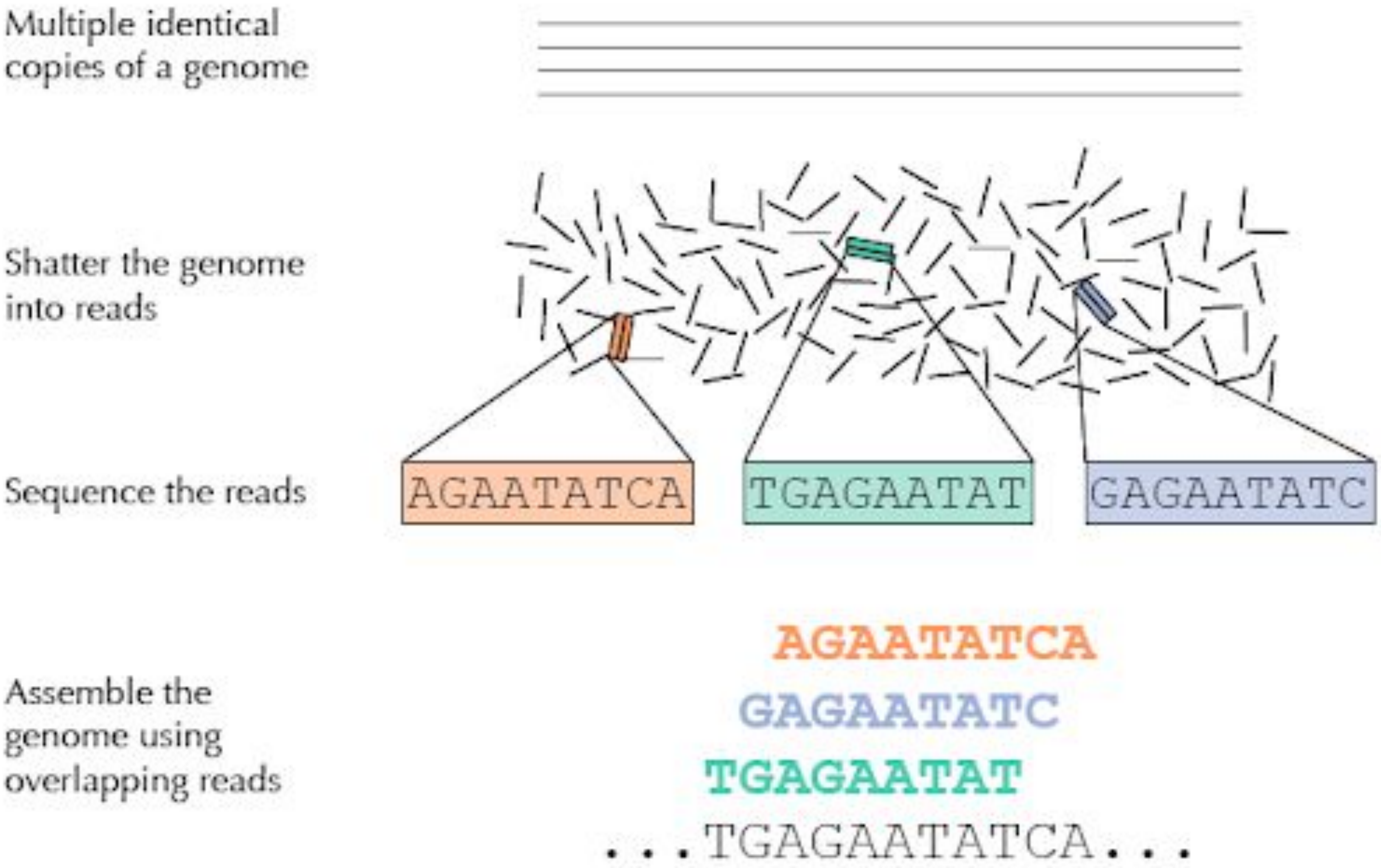


Multiple identical copies of a genome

Shatter the genome into reads

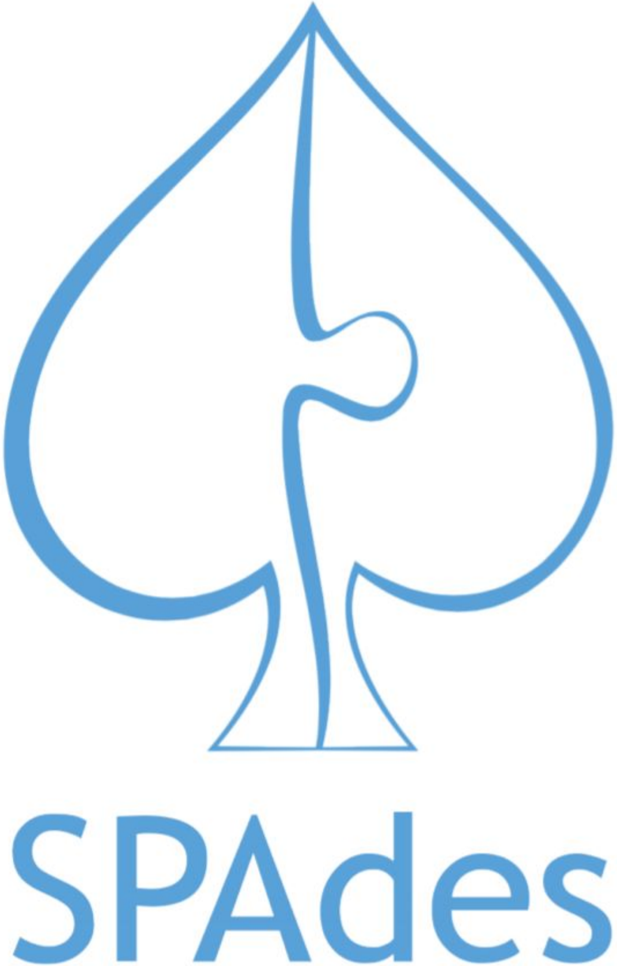
Sequence the reads

AGAATATCA TGAGAATAT GAGAATATC

Assemble the genome using overlapping reads

```
   AGAATATCA
  GAGAATATC
 TGAGAATAT
...TGAGAATATCA...
```

SPAdes Assembler



Center for Algorithmic Biotechnology, St Petersburg University | https://cab.spbu.ru/software/spades/

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Assembled Genome

```
ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGG
CTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTT
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TCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAAC
TTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGG
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TTAAAGCCACTTGCGAATTTTGTGGCACTGAGAATTTGACTAAAGAAGGTGCCACTACTTGTGGTTACTTACCCCAAAATGCTGTTGTTAA
AATTTATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAAAACCATTCTT
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GCGCTAACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGA...
```

Assembled Genome

```
ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGG
CTGTCACTCGGCTGCAT                                                          GTAACTCGTCTATCTT
CTGCAGGCTGCTTACGG                                                          AAGGTAAGATGGAGAG
CCTTGTCCCTGGTTTCA                                                          ACGTGGCTTTGGAGAC
TCCGTGGAGGAGGTCTT                                                          GGCGTTTTGCCTCAAC
TTGAACAGCCCTATGTG                                                          TAGCAGAACTCGAAGG
CATTCAGTACGGTCGTA                                                          CAAGGTTCTTCTTCGT
AAGAACGGTAATAAAGG                                                          GGCACTGATCCTTATG
AAGATTTTCAAGAAAAC                                                          GGGCATACACTCGCTA
TGTCGATAACAACTTCT                                                          AGCTTCATGCACTTTG
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AAAAGAGCTATGAATTG                                                          CAAATTTTGTATTTCC
CTTAAATTCCATAATCA                                                          ATCTGTCTATCCAGTT
GCGTCACCAAATGAATG                                                          CAGACGGGCGATTTTG
TTAAAGCCACTTGCGAA                                                          AAAATGCTGTTGTTAA
AATTTATTGTCCAGCAT                                                          CTTGAAAACCATTCTT
CGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTGTTCTCTTATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCACGTGCTA
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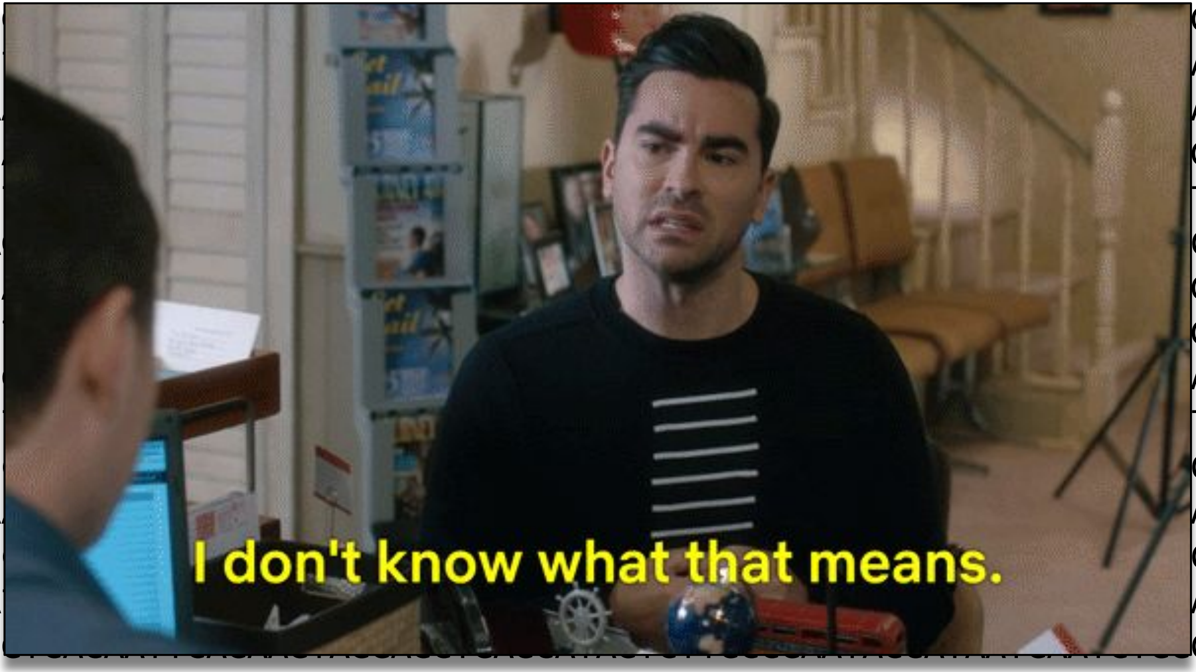
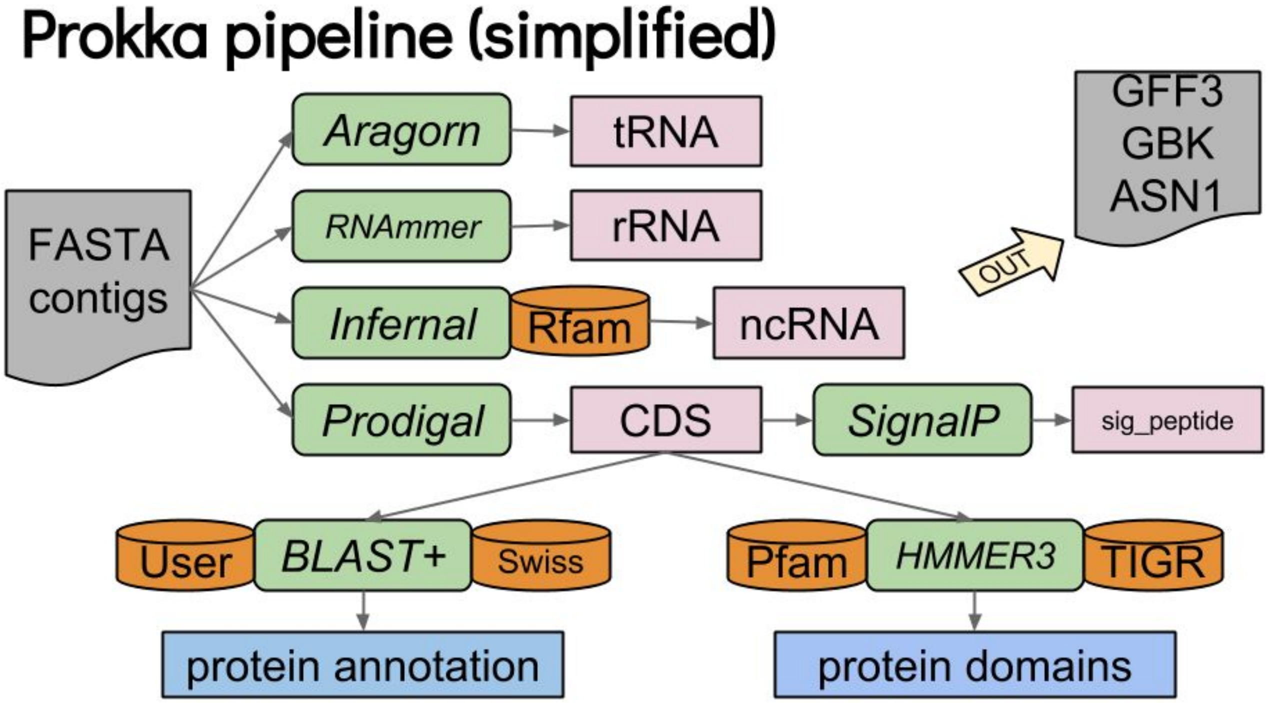


Prokka: Gene Prediction and Functional Annotation



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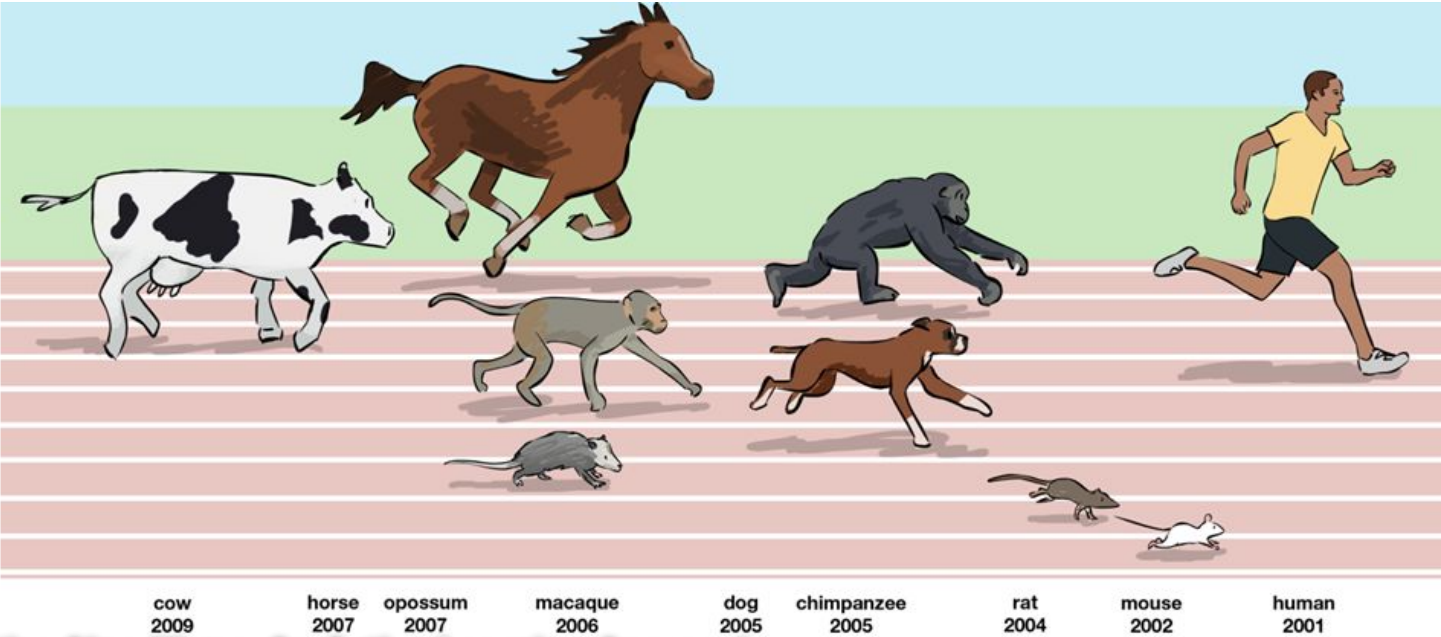
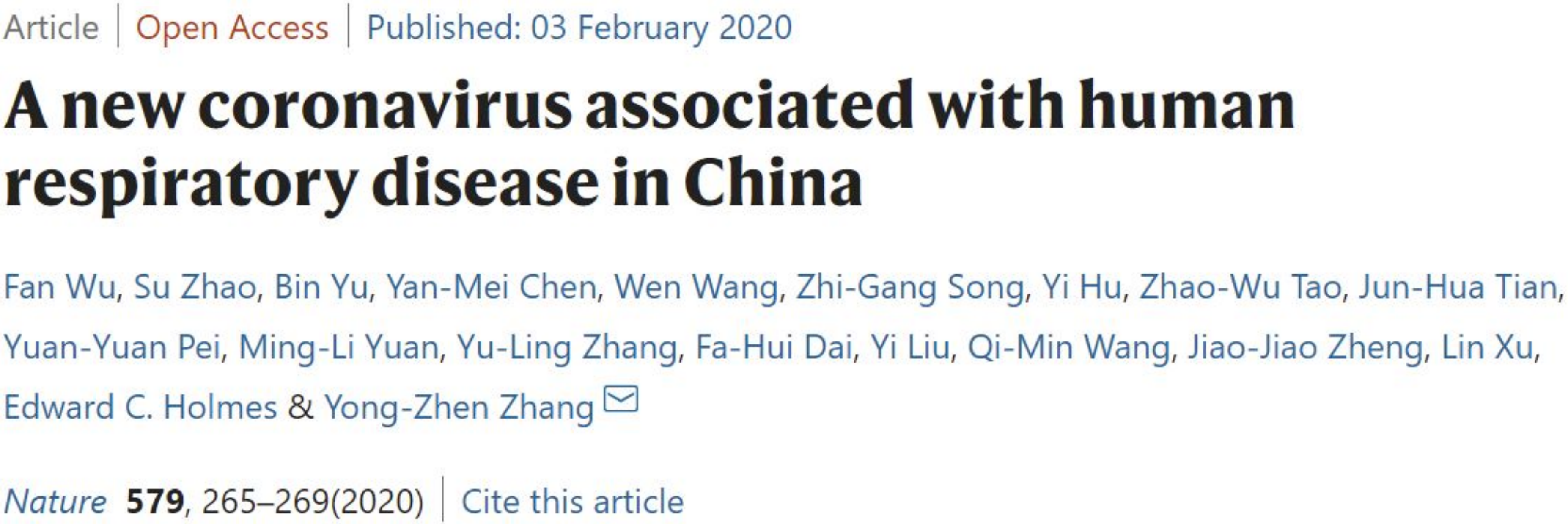


The SARS-CoV-2 Reference Genome



Article | Open Access | Published: 03 February 2020

A new coronavirus associated with human respiratory disease in China

Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang Song, Yi Hu, Zhao-Wu Tao, Jun-Hua Tian, Yuan-Yuan Pei, Ming-Li Yuan, Yu-Ling Zhang, Fa-Hui Dai, Yi Liu, Qi-Min Wang, Jiao-Jiao Zheng, Lin Xu, Edward C. Holmes & Yong-Zhen Zhang ✉

Nature **579**, 265–269(2020) | Cite this article

Mapping Reads to the Reference

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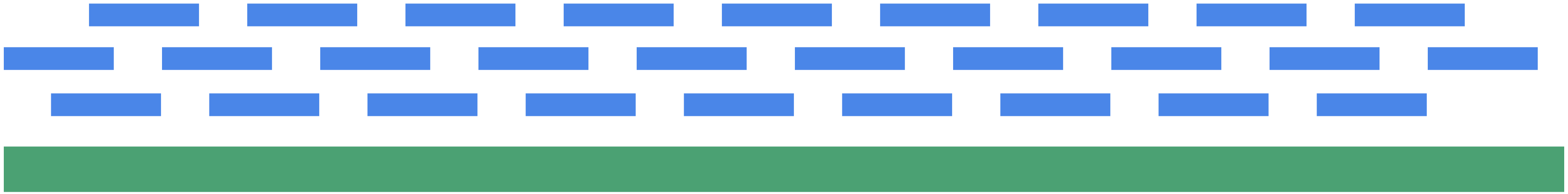

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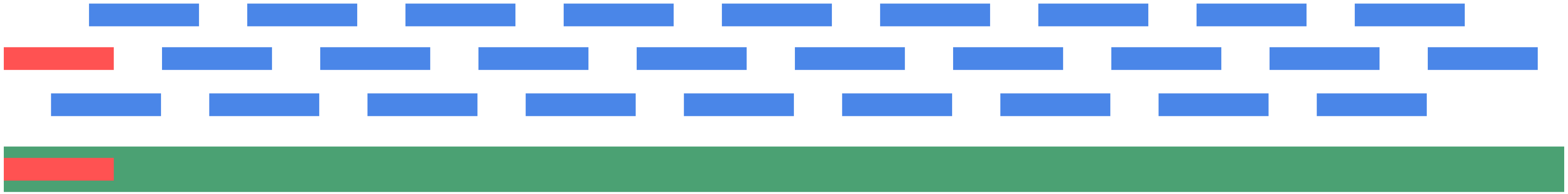

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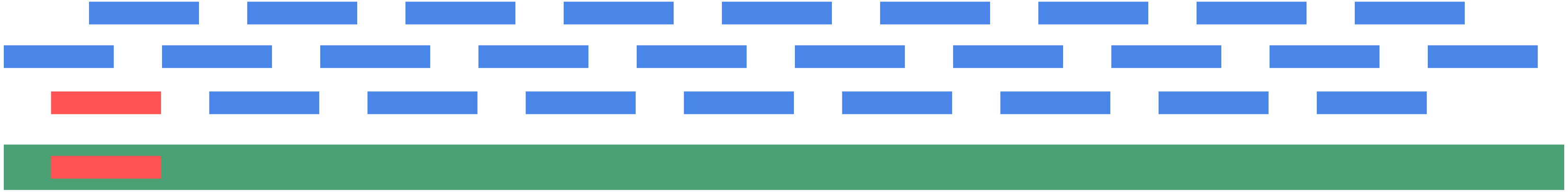

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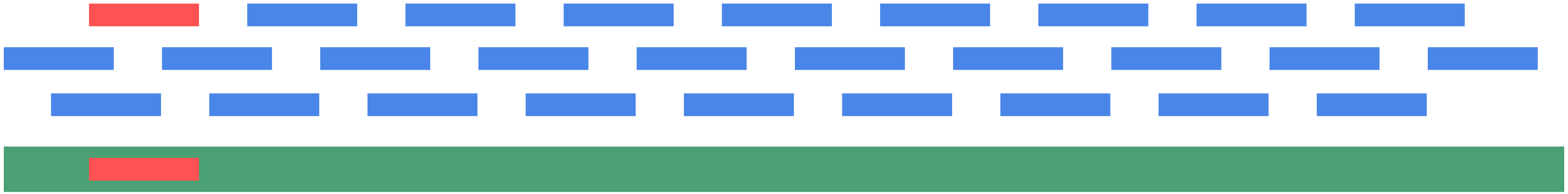

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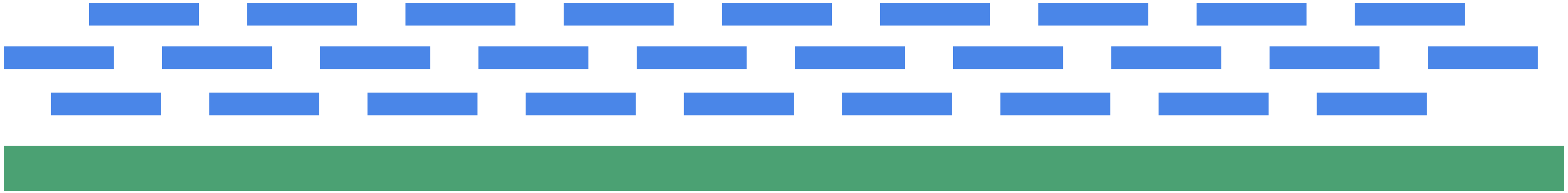

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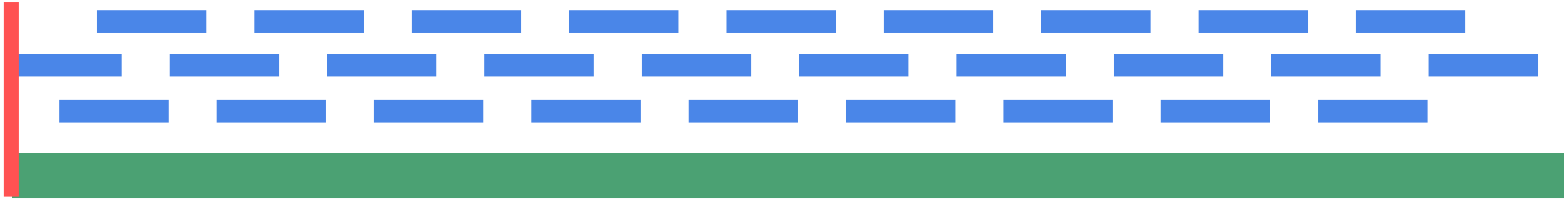

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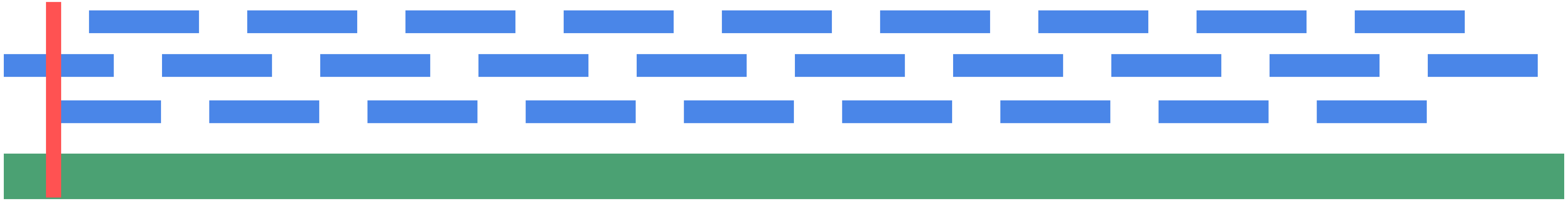

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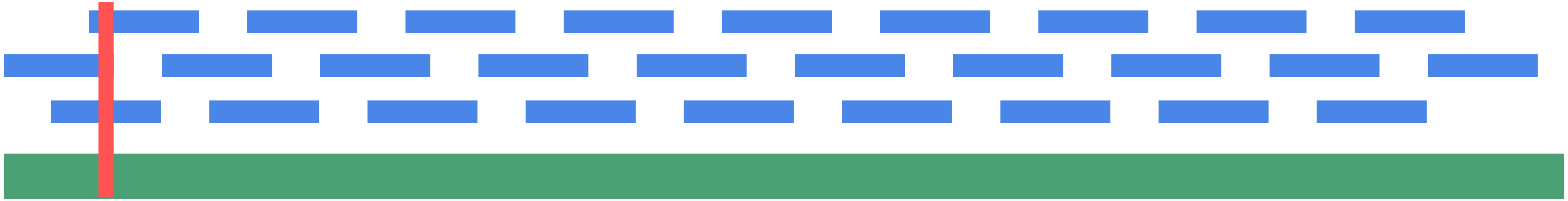

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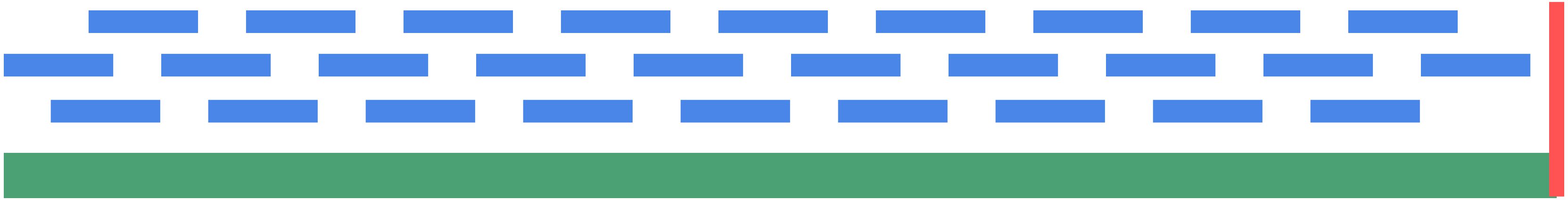

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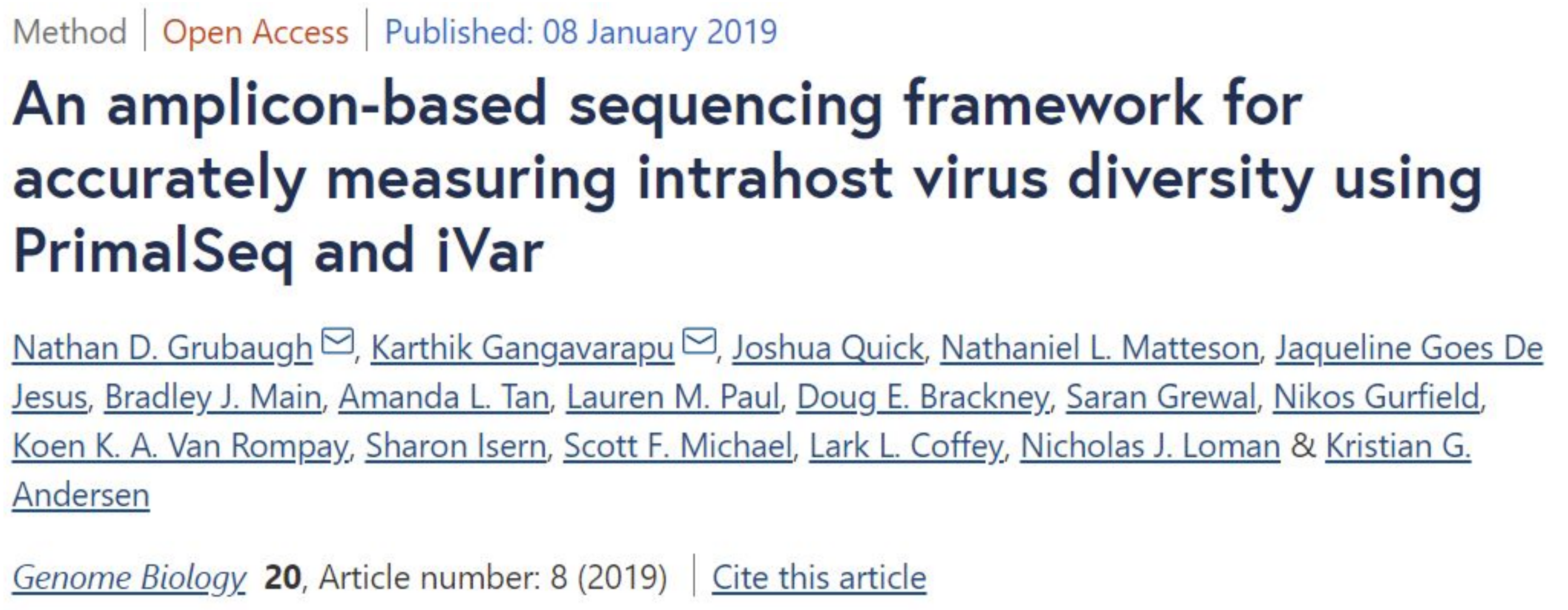

Method | Open Access | Published: 08 January 2019

An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar

Nathan D. Grubaugh, Karthik Gangavarapu, Joshua Quick, Nathaniel L. Matteson, Jaqueline Goes De Jesus, Bradley J. Main, Amanda L. Tan, Lauren M. Paul, Doug E. Brackney, Saran Grewal, Nikos Gurfield, Koen K. A. Van Rompay, Sharon Isern, Scott F. Michael, Lark L. Coffey, Nicholas J. Loman & Kristian G. Andersen

Genome Biology **20**, Article number: 8 (2019) | Cite this article

Outline

- Introduction to Viral Molecular Epidemiology
- Sequencing the First Viral Genome
- Annotating a Viral Genome
- Sequencing in the Midst of an Epidemic
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- Phylogenetic Inference and Transmission Clustering

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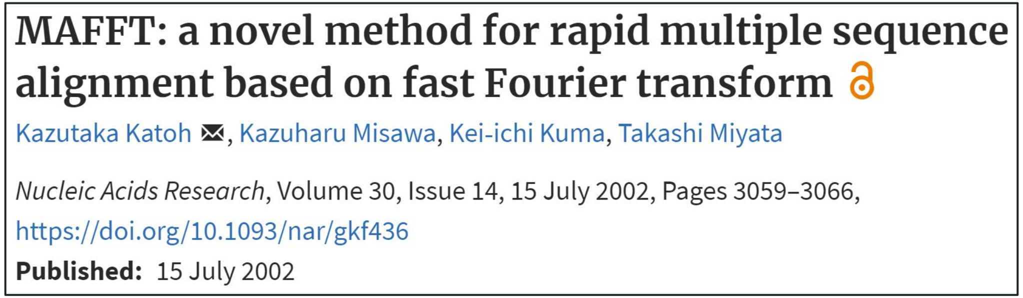
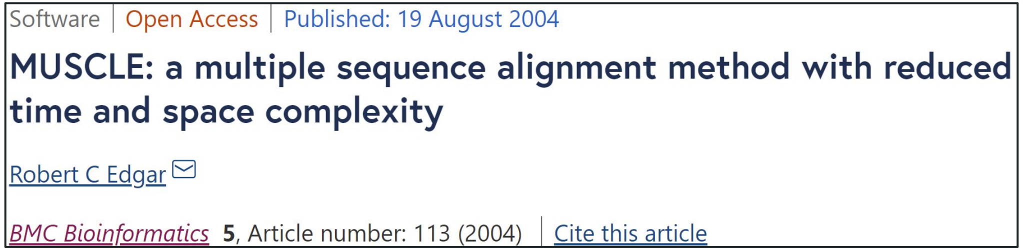


MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform

Kazutaka Katoh ✉, Kazuharu Misawa, Kei-ichi Kuma, Takashi Miyata

Nucleic Acids Research, Volume 30, Issue 14, 15 July 2002, Pages 3059–3066, https://doi.org/10.1093/nar/gkf436

Published: 15 July 2002

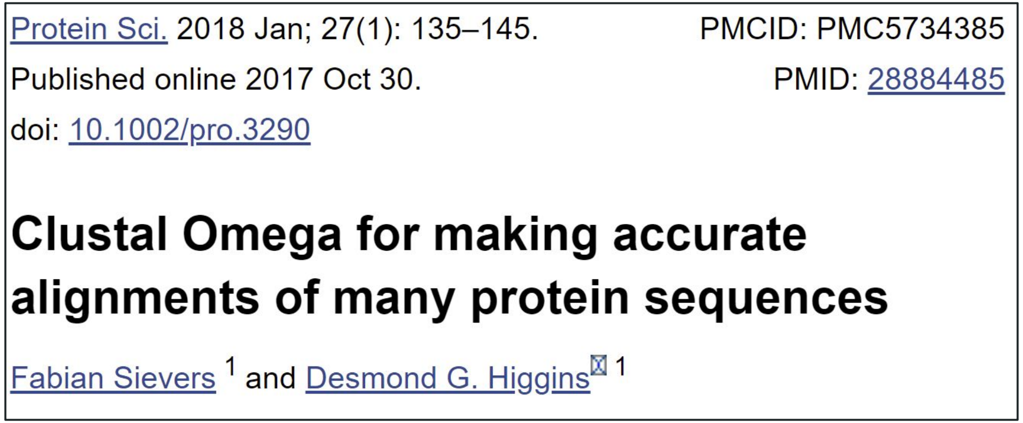


Software | Open Access | Published: 19 August 2004

MUSCLE: a multiple sequence alignment method with reduced time and space complexity

Robert C Edgar ✉

BMC Bioinformatics **5**, Article number: 113 (2004) | Cite this article



Protein Sci. 2018 Jan; 27(1): 135–145. PMCID: PMC5734385

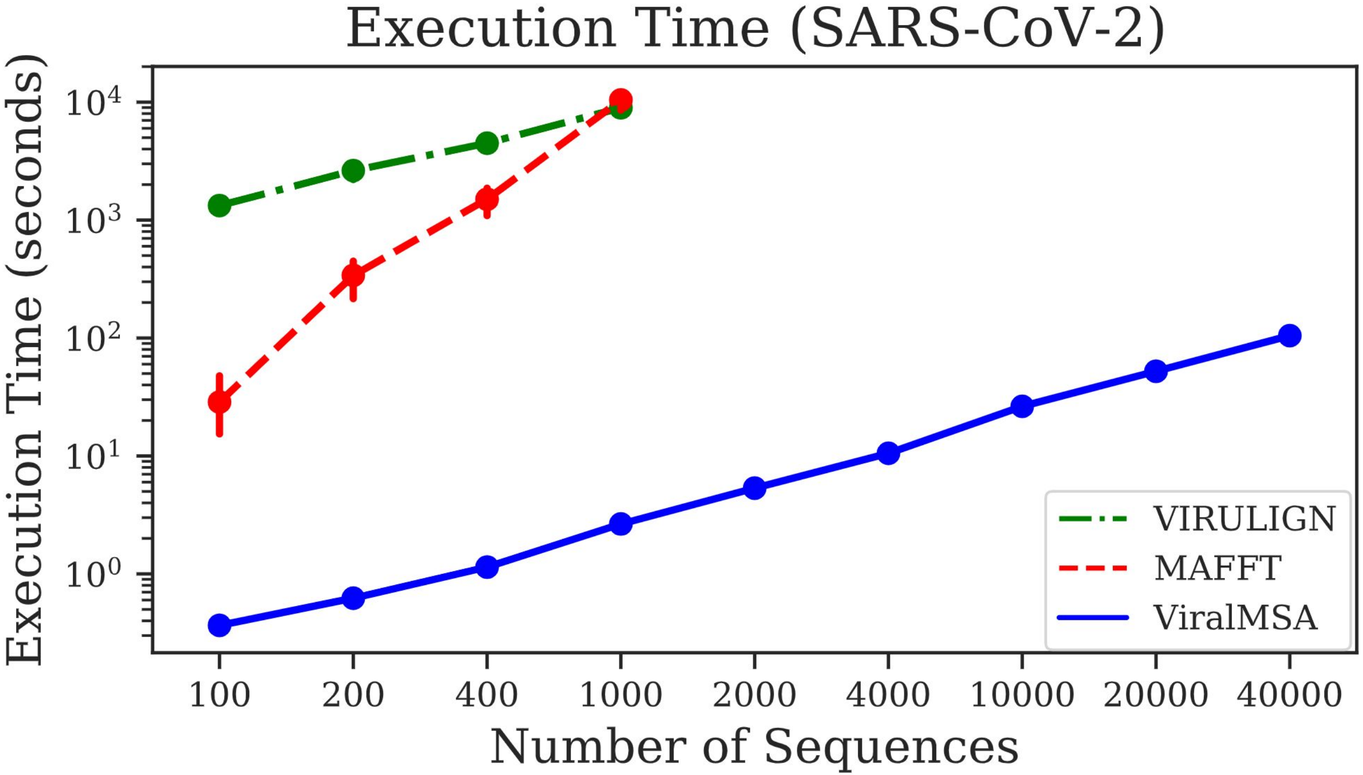
Published online 2017 Oct 30. PMID: 28884485

doi: 10.1002/pro.3290

Clustal Omega for making accurate alignments of many protein sequences

Fabian Sievers [1] and Desmond G. Higgins✉ [1]

Align-to-Reference Approach



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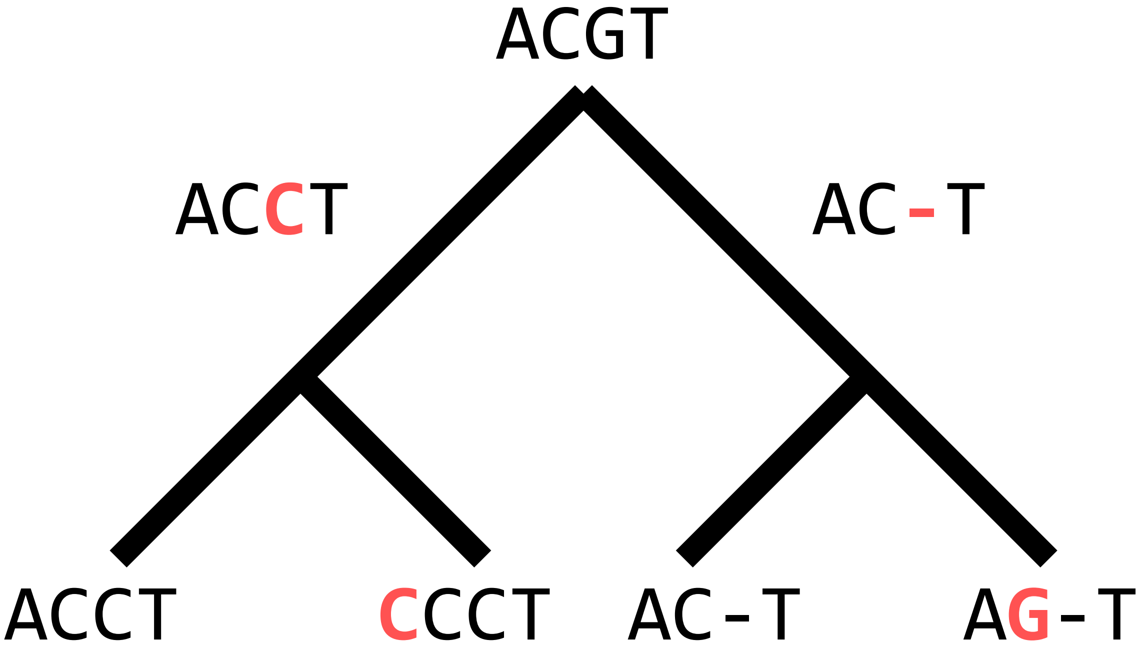
ACGT
ACCT
AC-T
ACCT
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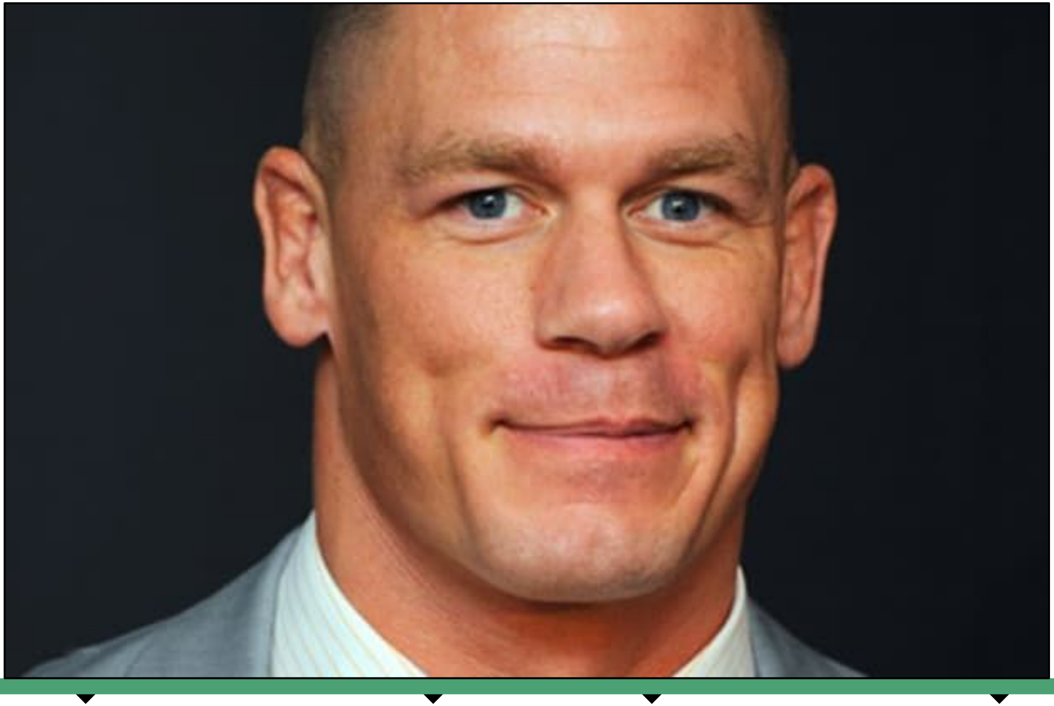
AG-T



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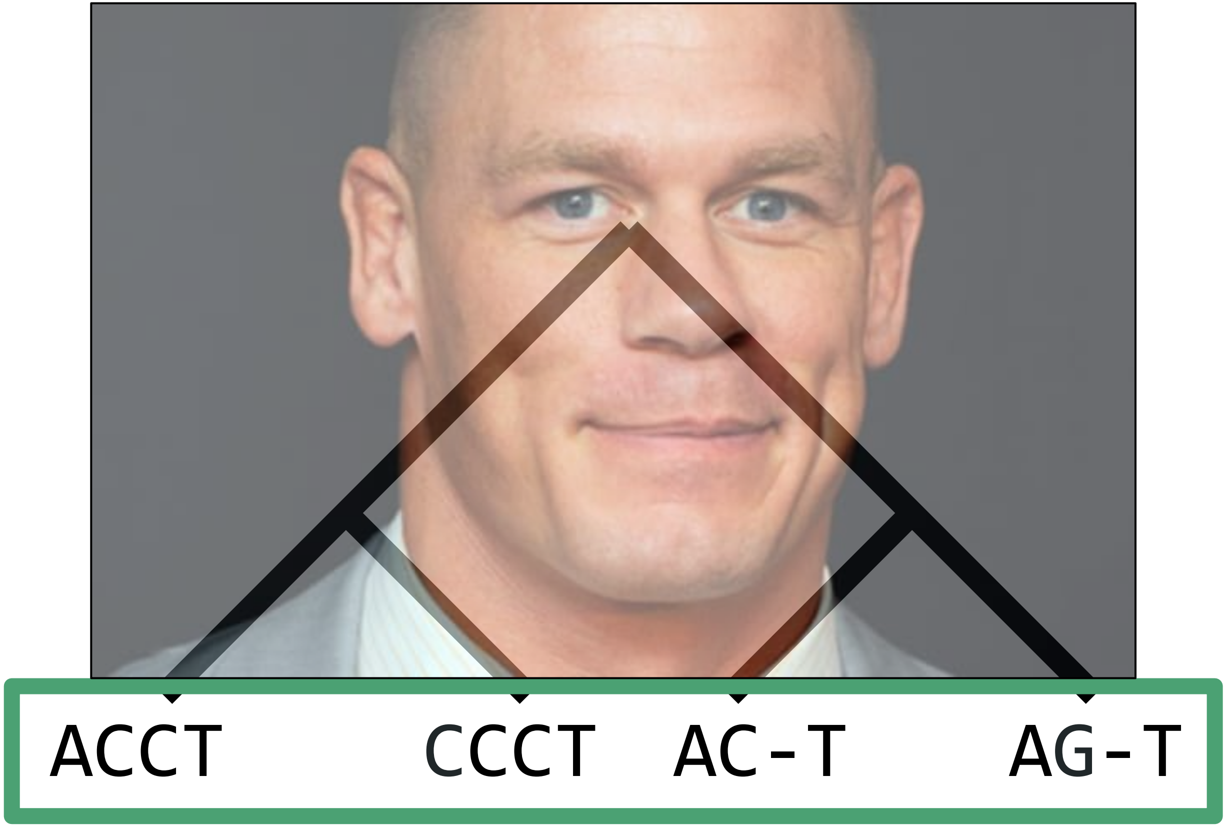

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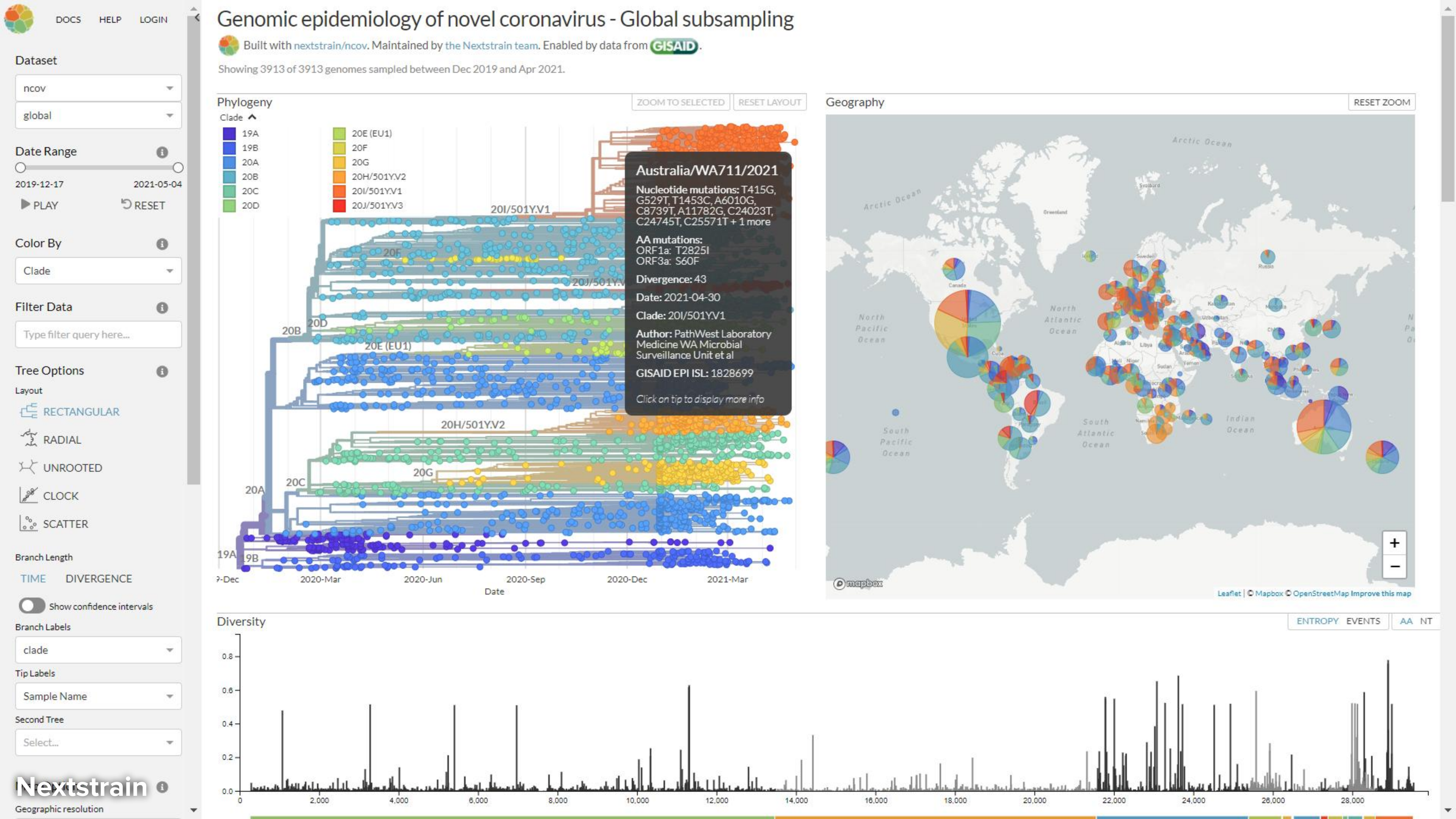

Genomic epidemiology of novel coronavirus - Global subsampling

Built with nextstrain/ncov. Maintained by the Nextstrain team. Enabled by data from GISAID.

Showing 3913 of 3913 genomes sampled between Dec 2019 and Apr 2021.

Dataset: ncov / global

Date Range: 2019-12-17 – 2021-05-04

Color By: Clade

Filter Data: Type filter query here...

Layout: RECTANGULAR

Branch Length: TIME

Branch Labels: clade

Tip Labels: Sample Name

Second Tree: Select...

Australia/WA711/2021

Nucleotide mutations: T415G, G529T, T1453C, A6010G, C8739T, A11782G, C24023T, C24745T, C25571T + 1 more

AA mutations:
ORF1a: T2825I
ORF3a: S60F

Divergence: 43

Date: 2021-04-30

Clade: 20I/501Y.V1

Author: PathWest Laboratory Medicine WA Microbial Surveillance Unit et al

GISAID EPI ISL: 1828699

Click on tip to display more info

Nextstrain

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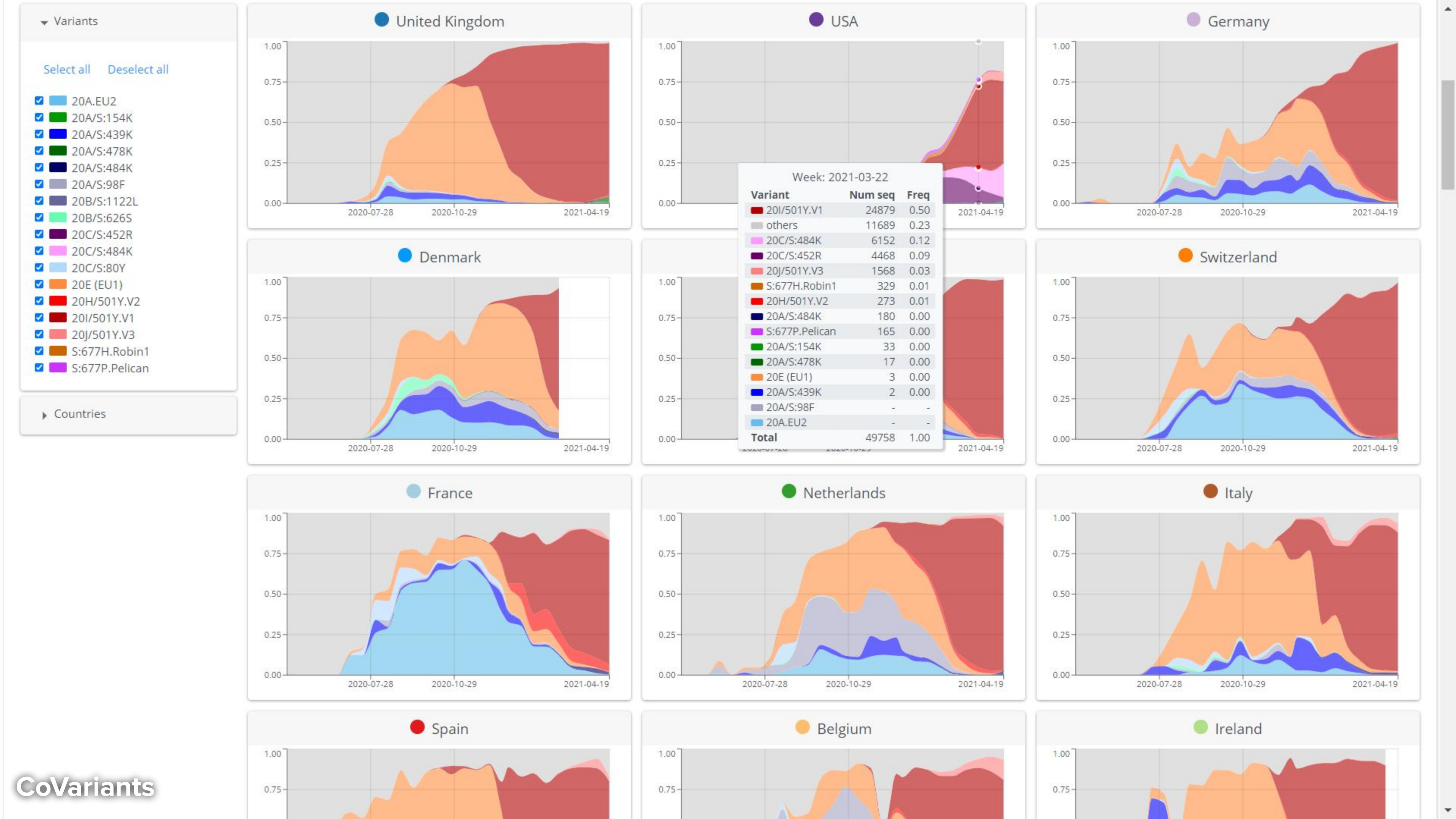

Variants

Select all Deselect all

- ☑ 20A.EU2
- ☑ 20A/S:154K
- ☑ 20A/S:439K
- ☑ 20A/S:478K
- ☑ 20A/S:484K
- ☑ 20A/S:98F
- ☑ 20B/S:1122L
- ☑ 20B/S:626S
- ☑ 20C/S:452R
- ☑ 20C/S:484K
- ☑ 20C/S:80Y
- ☑ 20E (EU1)
- ☑ 20H/501Y.V2
- ☑ 20I/501Y.V1
- ☑ 20J/501Y.V3
- ☑ S:677H.Robin1
- ☑ S:677P.Pelican

Countries

Week: 2021-03-22

Variant	Num seq	Freq
20I/501Y.V1	24879	0.50
others	11689	0.23
20C/S:484K	6152	0.12
20C/S:452R	4468	0.09
20J/501Y.V3	1568	0.03
S:677H.Robin1	329	0.01
20H/501Y.V2	273	0.01
20A/S:484K	180	0.00
S:677P.Pelican	165	0.00
20A/S:154K	33	0.00
20A/S:478K	17	0.00
20E (EU1)	3	0.00
20A/S:439K	2	0.00
20A/S:98F	-	-
20A.EU2	-	-
Total	49758	1.00

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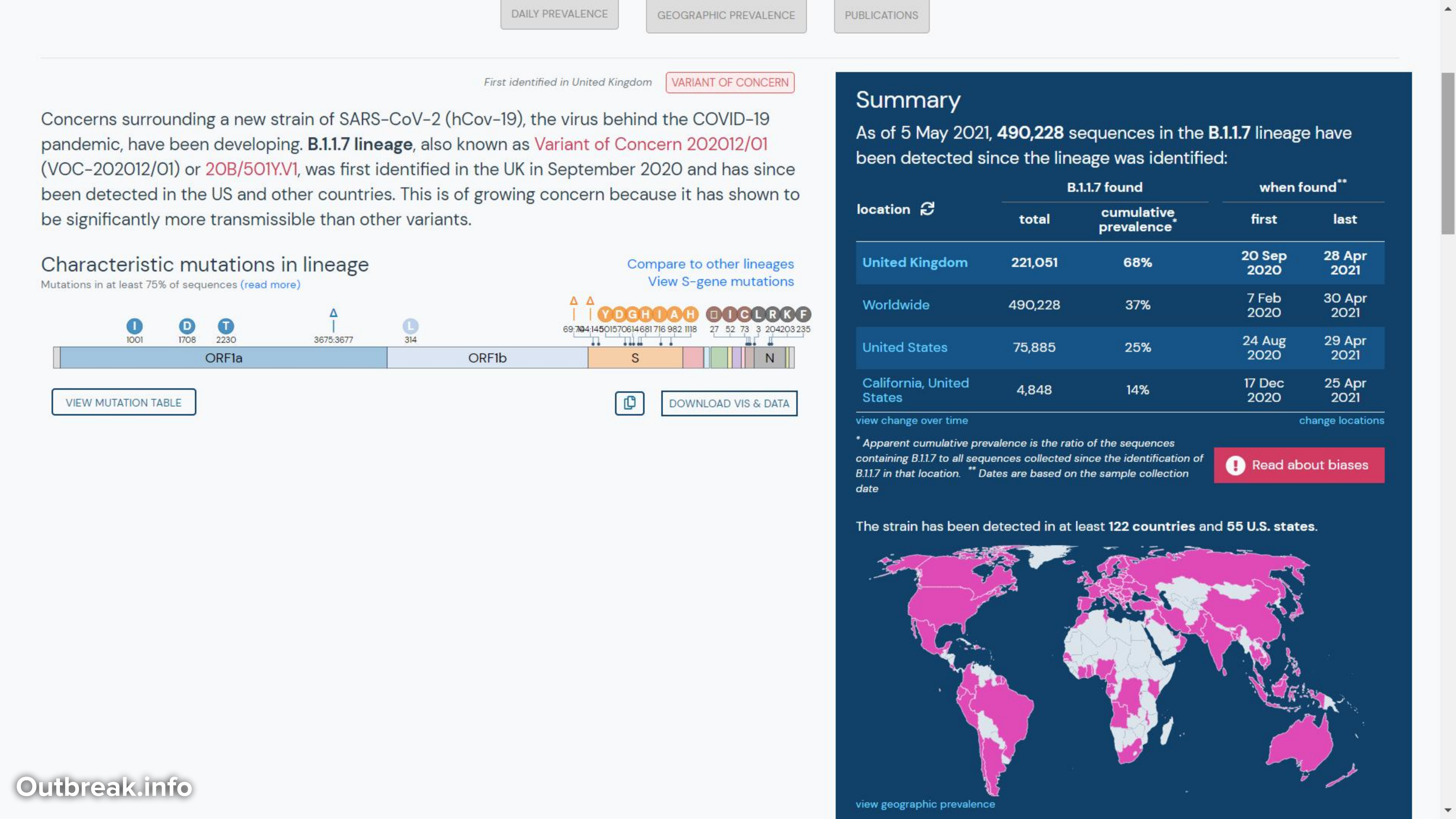

First identified in United Kingdom VARIANT OF CONCERN

Concerns surrounding a new strain of SARS-CoV-2 (hCov-19), the virus behind the COVID-19 pandemic, have been developing. **B.1.1.7 lineage**, also known as Variant of Concern 202012/01 (VOC-202012/01) or 20B/501Y.V1, was first identified in the UK in September 2020 and has since been detected in the US and other countries. This is of growing concern because it has shown to be significantly more transmissible than other variants.

Characteristic mutations in lineage

Mutations in at least 75% of sequences (read more)

Compare to other lineages
View S-gene mutations

Summary

As of 5 May 2021, **490,228** sequences in the **B.1.1.7** lineage have been detected since the lineage was identified:

location	B.1.1.7 found		when found**	
	total	cumulative prevalence*	first	last
United Kingdom	221,051	68%	20 Sep 2020	28 Apr 2021
Worldwide	490,228	37%	7 Feb 2020	30 Apr 2021
United States	75,885	25%	24 Aug 2020	29 Apr 2021
California, United States	4,848	14%	17 Dec 2020	25 Apr 2021

view change over time
change locations

** Apparent cumulative prevalence is the ratio of the sequences containing B.1.1.7 to all sequences collected since the identification of B.1.1.7 in that location. ** Dates are based on the sample collection date*

Read about biases

The strain has been detected in at least **122 countries** and **55 U.S. states**.

view geographic prevalence

Outbreak.info

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Molecular Cluster vs. Transmission Cluster

- **Molecular Cluster:** Group of persons linked by molecular (e.g. sequence) data
- A molecular cluster is a **subset** of a transmission cluster
    - Not every infected person gets their molecular data collected (e.g. sequencing)
    - A molecular cluster only consists of persons in a transmission cluster who got sequenced
- We need to be conscious of people missing from a molecular cluster
    - Diagnosed but not sequenced (in *transmission* cluster, but not *molecular* cluster)
    - Not diagnosed (in *risk network*, but not *transmission cluster*)

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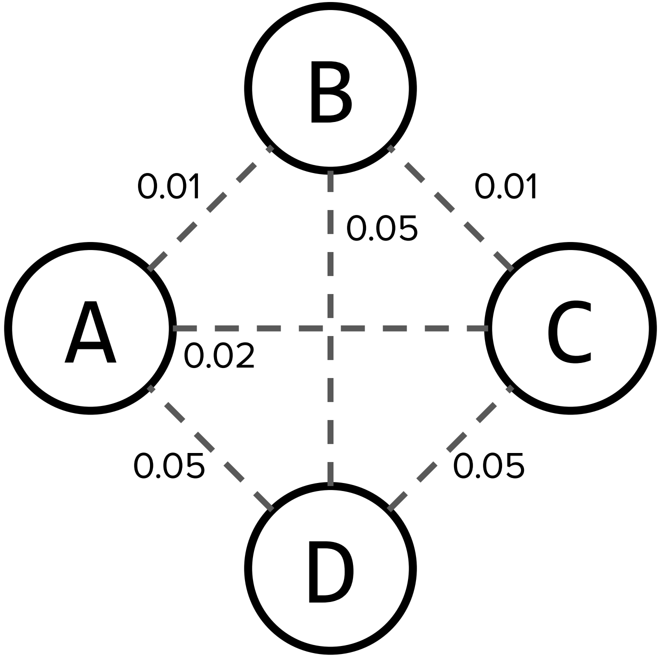
  - Each resulting chain of links defines a molecular cluster

Molecular Clustering from Sequences



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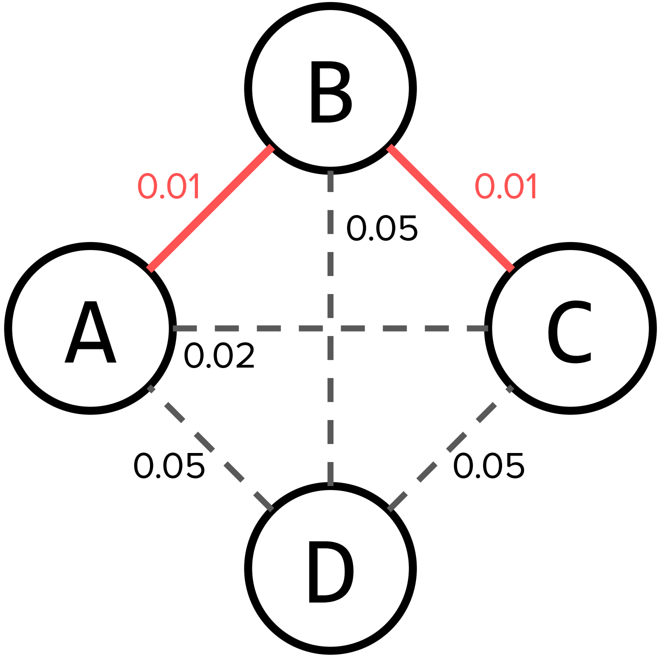

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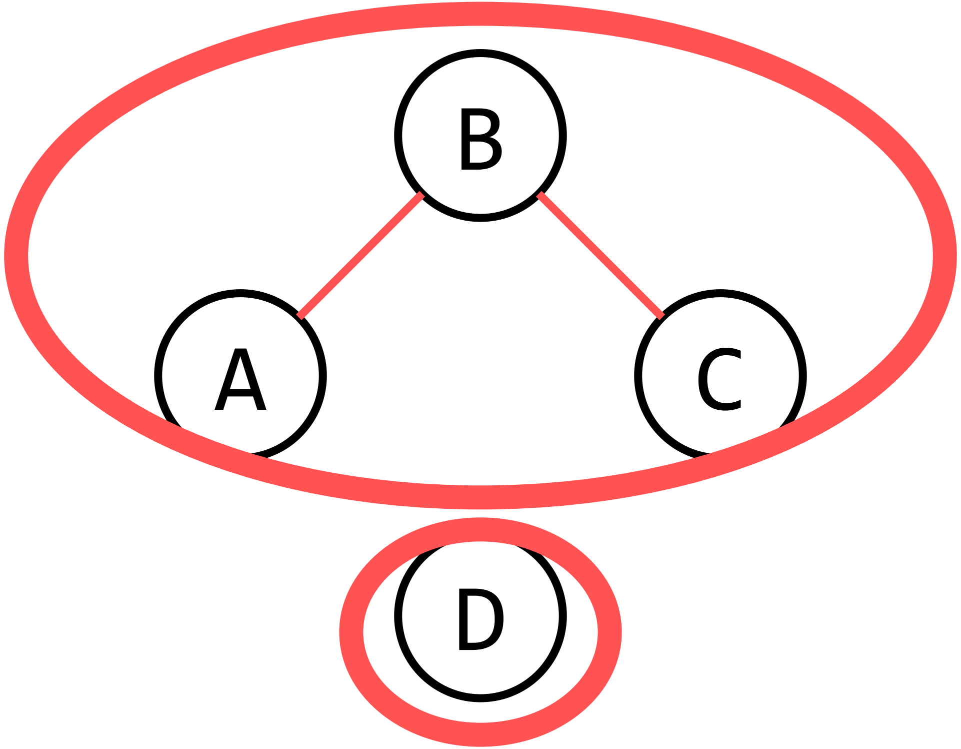

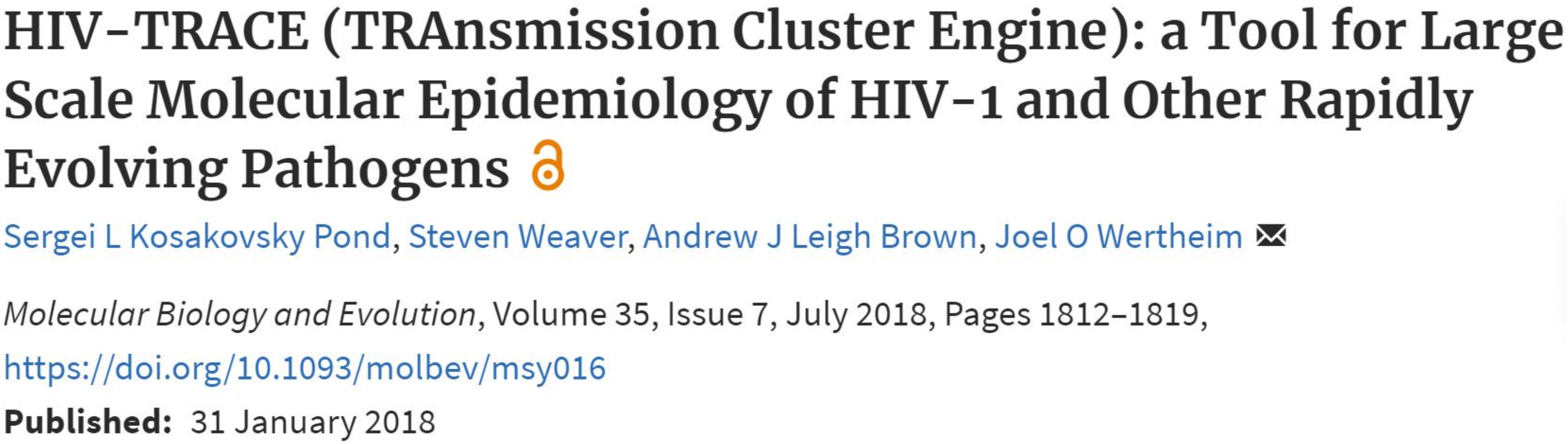

HIV-TRACE (TRAnsmission Cluster Engine): a Tool for Large Scale Molecular Epidemiology of HIV-1 and Other Rapidly Evolving Pathogens

Sergei L Kosakovsky Pond, Steven Weaver, Andrew J Leigh Brown, Joel O Wertheim ✉

Molecular Biology and Evolution, Volume 35, Issue 7, July 2018, Pages 1812–1819, https://doi.org/10.1093/molbev/msy016

Published: 31 January 2018

https://github.com/veg/hivtrace

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  - There are quite a few ways to define “clusters” given a phylogeny
  - Subtree with maximum pairwise distance below some threshold? Cutting the tree in some way?
  - Single-linkage like in HIV-TRACE (but using pairwise distances from the tree)?

TreeCluster: Clustering biological sequences using phylogenetic trees

Metin Balaban, Niema Moshiri, Uyen Mai, Xingfan Jia, Siavash Mirarab ✉

Published: August 22, 2019 • https://doi.org/10.1371/journal.pone.0221068

https://github.com/niemasd/TreeCluster

Questions?