



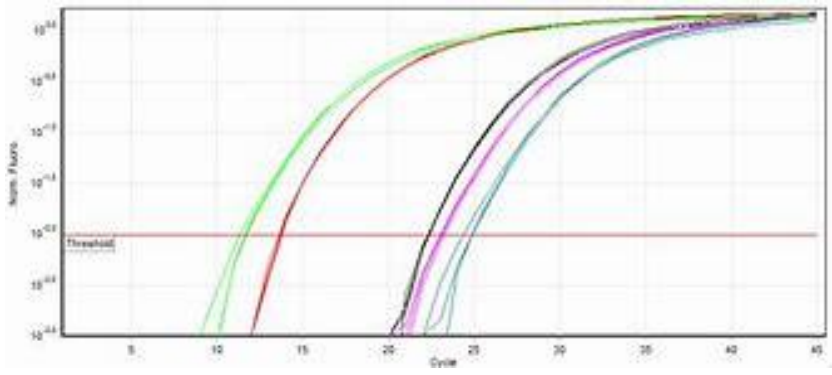
SARS-CoV-2

NGS Sequencing and Bioinformatics
Approaches for Genomic Epidemiology

Kevin G. Libuit, MS
DCLS Bioinformatics Lead Scientist

DIAGNOSTIC TESTINGS ARE PRIORITY

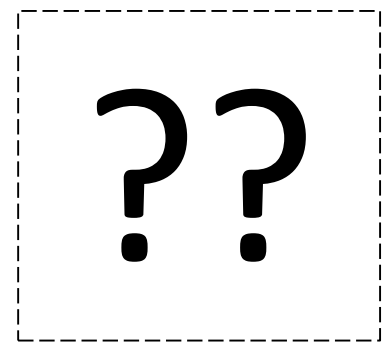
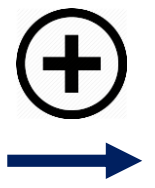




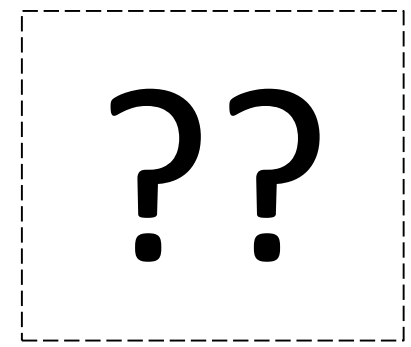
RT PCR Diagnostics



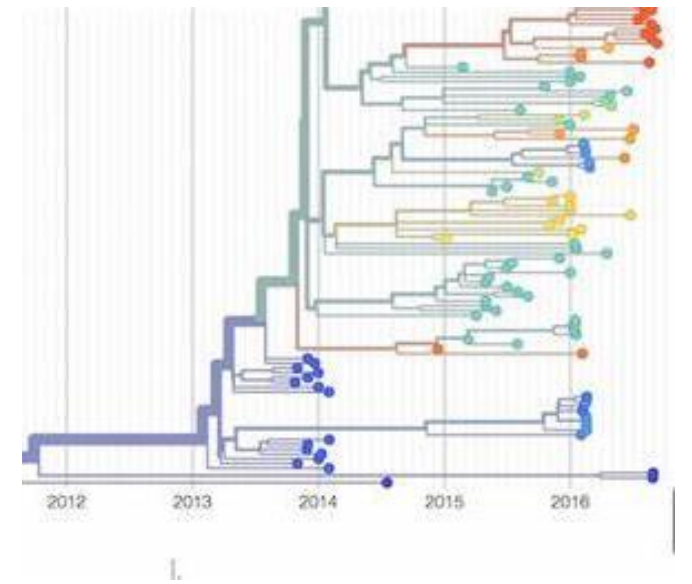
RNA Extract



SARS-CoV-2 (SC2)
Sequencing

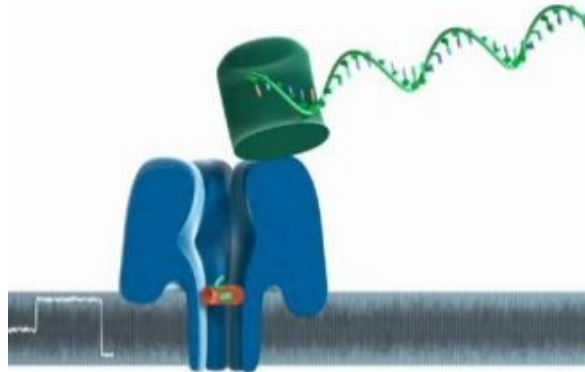


SC2 Sequence
Assembly

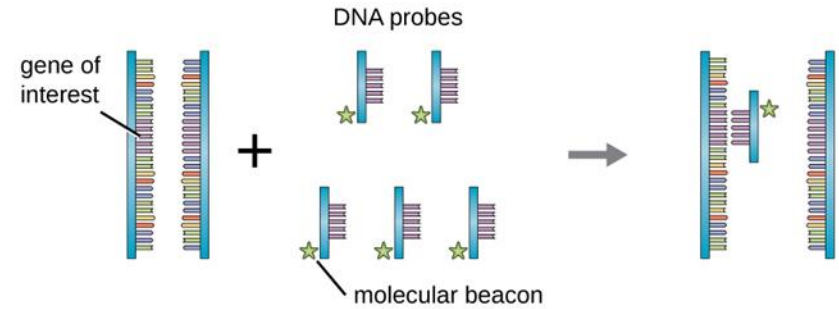


Genomic
Epidemiology

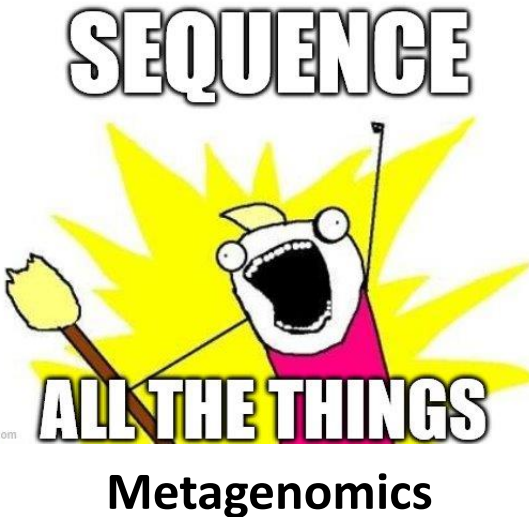
SC2 Sequencing Approaches



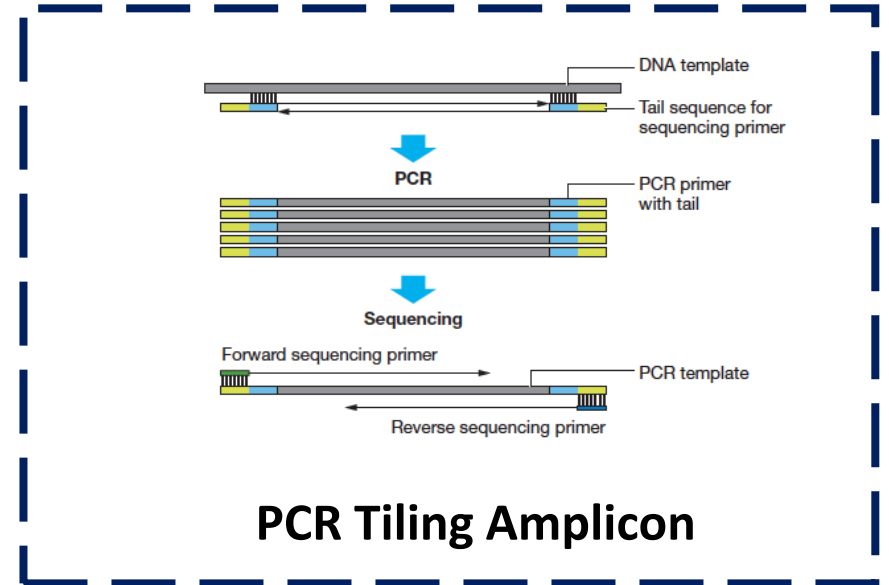
Direct RNAseq



SC2 Enrichment



Metagenomics



PCR Tiling Amplicon



RNA Extract

ARTIC Network Tile Amplicon Sequencing



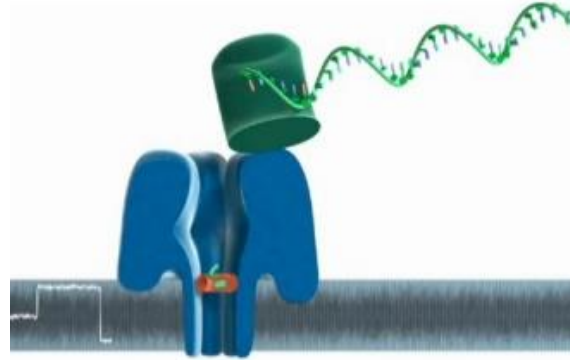
Developed complete primer set for SC2 genome sequencing

- V1 made available in Jan 2020
 - 2 US States utilized the ARTIC PCR protocol to sequence SC2
- V2 released this month
- Twitter talks of V3 coming soon

ARTIC Amplicon Sequencing



**ARTIC PCR
Amplicons**



ONT MinIon



Illumina *Seq

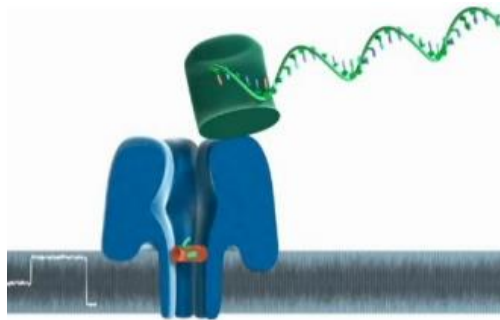
```
AAAGAAACTATAGCTGAGAGCGGCG  
ATCGTACGATGCATGCTAGCTAGCG  
AGAGCGGCGATCGTACGATGCATGC  
TAGCTAGCGAGAGCGGCGATCGTAC  
GATGCATGCTAGCTAGCGAGAGCGG  
CGATCGTACGATGCATGCTAGCTAG  
CGAGAGCGGCGATCGTACGATGCAT
```

SC2 Genome Assembly

ARTIC Amplicon Sequence Analysis, ONT MinION



**ARTIC PCR
Amplicons**



ONT MinION



**ARTIC Bioinformatics
Pipeline**



```
AAAGAAACTATAGCTGAGAGCGGCG  
ATCGTACGATGCATGCTAGCTAGCG  
AGAGCGGCGATCGTACGATGCATGC  
TAGCTAGCGAGAGCGGCGATCGTAC  
GATGCATGCTAGCTAGCGAGAGCGG  
CGATCGTACGATGCATGCTAGCTAG  
CGAGAGCGGCGATCGTACGATGCAT
```

SC2 Genome Assembly

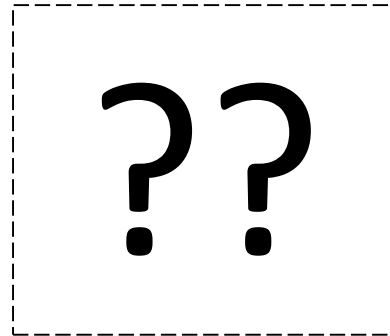
ARTIC Amplicon Sequence Analysis, Illumina Instruments



**ARTIC PCR
Amplicons**



Illumina *Seq



**Amplicon
Sequence Analysis**



```
AAAGAAACTATAGCTGAGAGCGGCG  
ATCGTACGATGCATGCTAGCTAGCG  
AGAGCGGCGATCGTACGATGCATGC  
TAGCTAGCGAGAGCGGCGATCGTAC  
GATGCATGCTAGCTAGCGAGAGCGG  
CGATCGTACGATGCATGCTAGCTAG  
CGAGAGCGGCGATCGTACGATGCAT
```

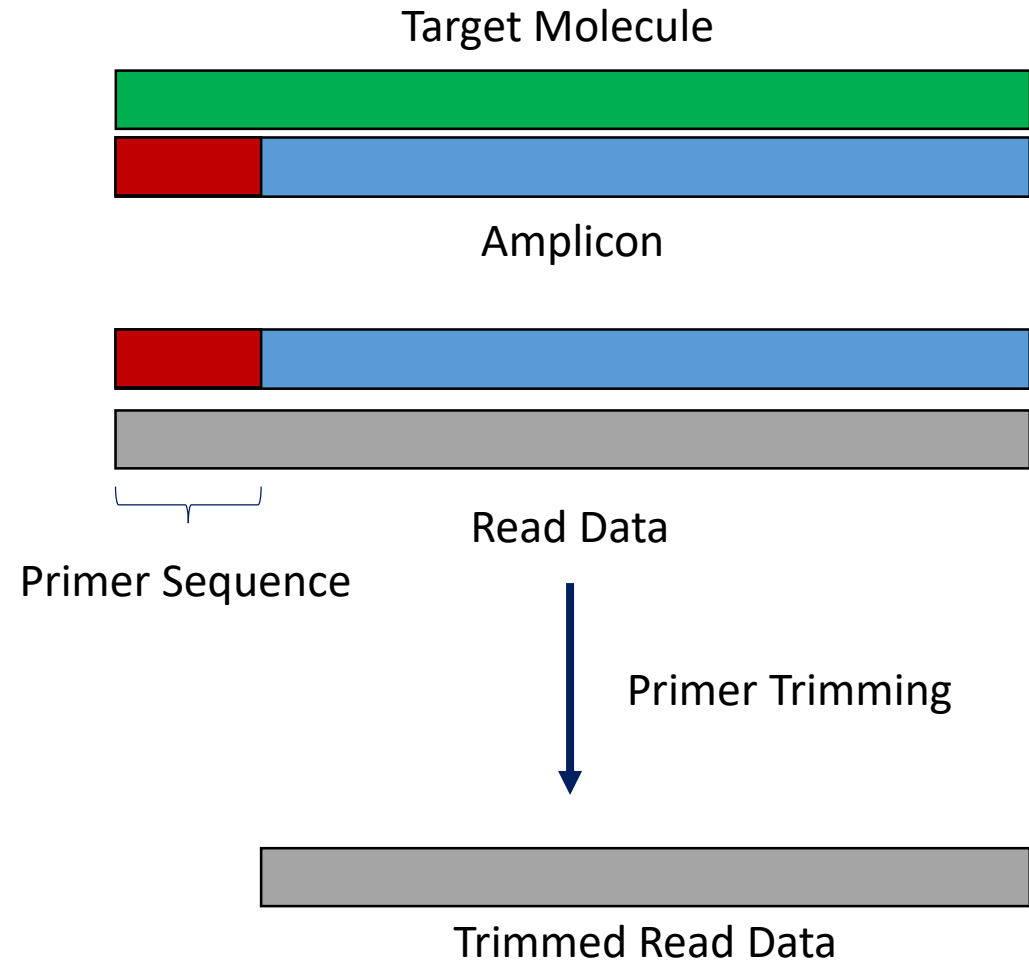
SC2 Genome Assembly

ARTIC Amplicon Sequence Analysis

Map to a reference genome to generate consensus sequence

Read mapping will not be complete

- V1 PCR Failure may occur
- Sequence data of primers should be excluded



ARTIC Amplicon Sequence Analysis, Illumina Instruments



**ARTIC PCR
Amplicons**



Illumina *Seq



1. Quality trimming and adapter removal (SeqClean)
2. Map to reference genome (MN908947.3; minmap2)
3. Trim adapter regions from read data & generate consensus sequence (iVar)

ARTIC Amplicon Sequence Analysis, Illumina Instruments

Quality of Read Alignment

sample	aligned_bases	percent_cvg	mean_depth	mean_base_q	mean_map_q
SAMPLE_001	29892	99.9632	2245.75	37.7	58.9
SAMPLE_002	29449	98.4818	2244.14	37.9	58.8
SAMPLE_003	29781	99.592	1902.06	38	59
SAMPLE_004	24838	83.0619	1828.47	37.8	58.5
SAMPLE_005	29544	98.7995	1895.36	37.6	58.8

SARS-CoV-2 Genomic Data Sharing

```
AAAGAACTATAGCTGAGAGCGGCG  
ATCGTACGATGCATGCTAGCTAGCG  
AGAGCGGCGATCGTACGATGCATGC  
TAGCTAGCGAGAGCGGCGATCGTAC  
GATGCATGCTAGCTAGCGAGAGCGG  
CGATCGTACGATGCATGCTAGCTAG  
CGAGAGCGGCGATCGTACGATGCAT
```

SC2 Genome Assembly

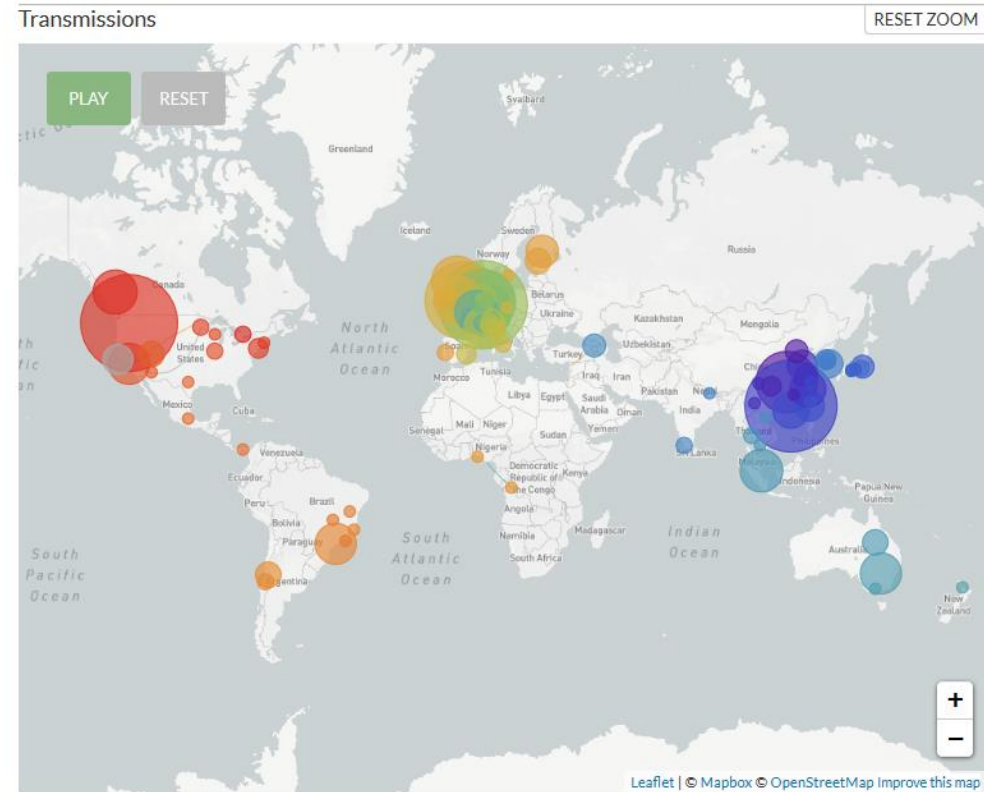
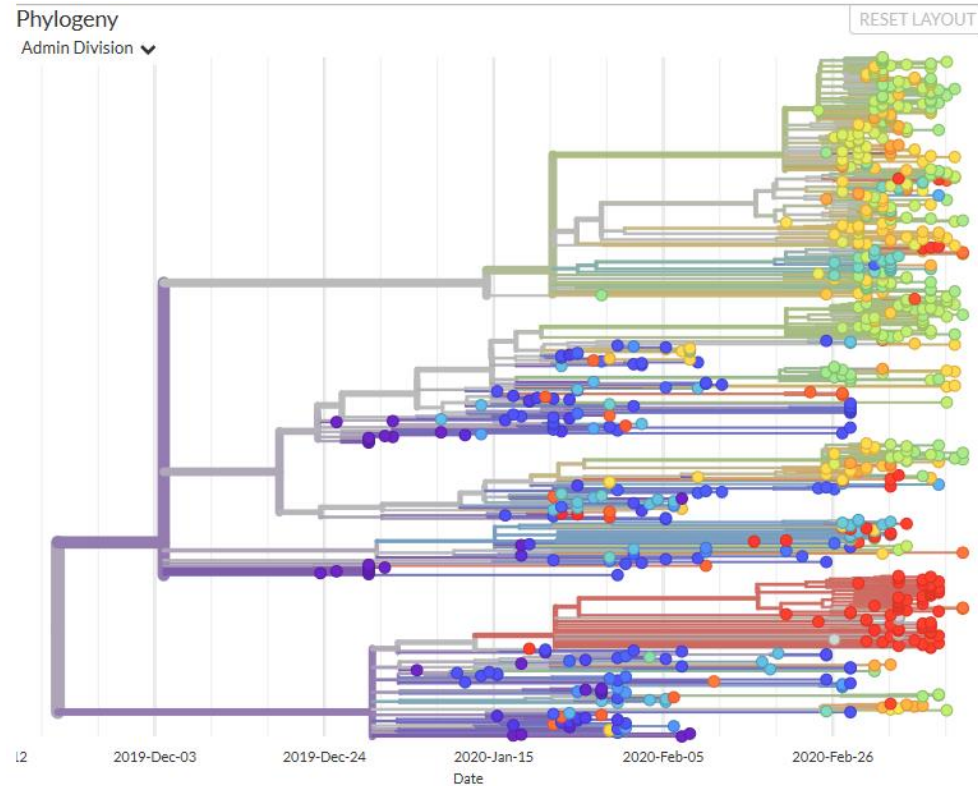


National
Center for
Biotechnology
Information

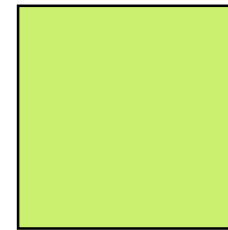
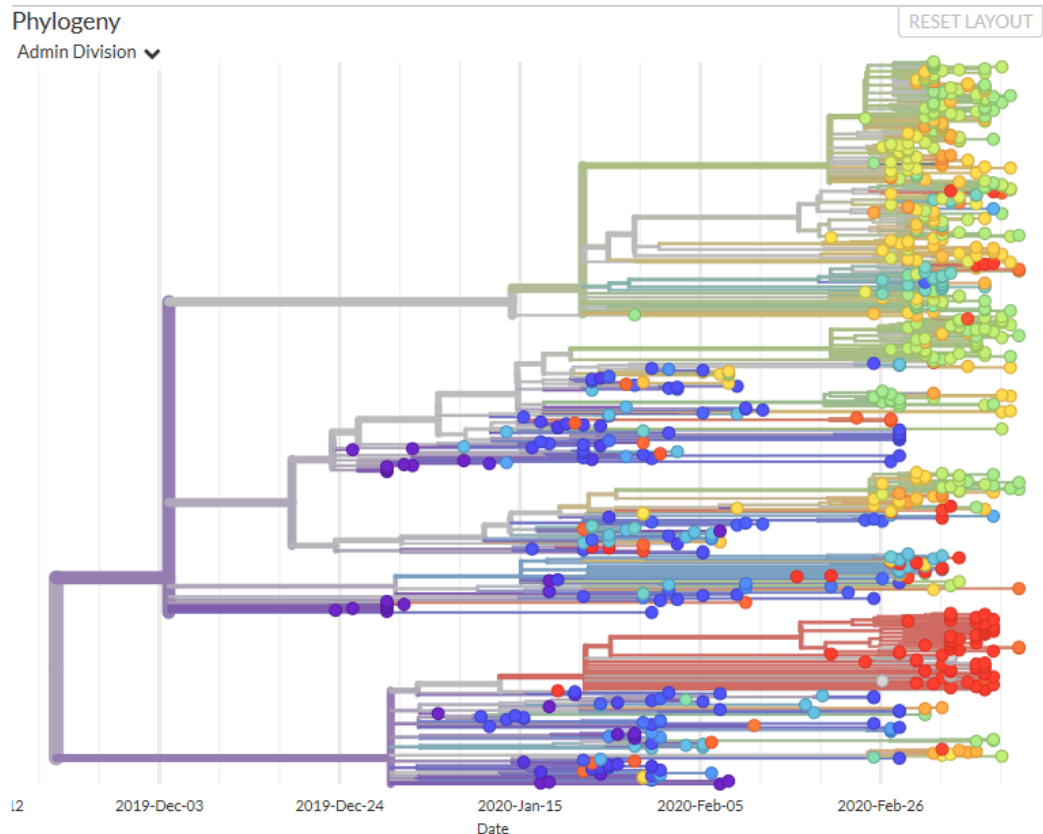


Nextstrain

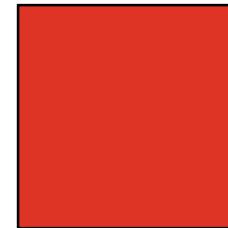
SARS-CoV-2 Genomic Epidemiology, International Surveillance



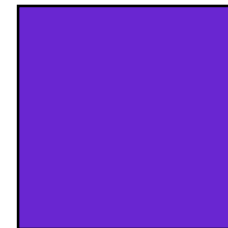
SARS-CoV-2 Genomic Epidemiology, Local Surveillance



Facility A

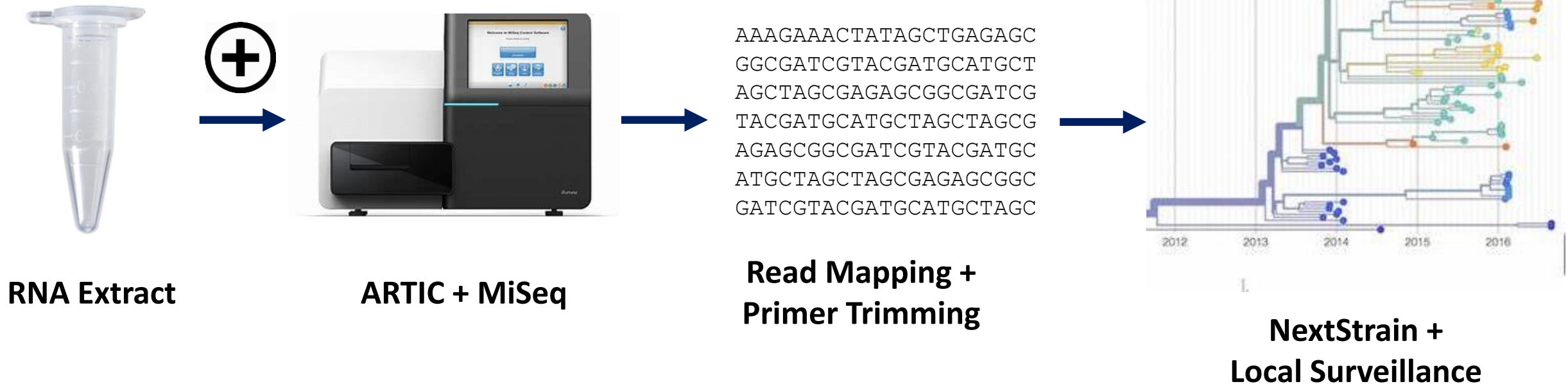


Facility B



Facility C

SARS-CoV-2 Sequencing and Analysis Workflow



Things to Consider:

- TAT: 2-4 Days
- Sequencing Priority
- VTM from Industry Laboratories
- Sequencing/Bioinformatics Capabilities

SC2 Sequencing/Analysis Resources

1. CDC's Open Repository
- https://github.com/CDCgov/SARS-CoV-2_Sequencing
2. [NextStrain.org](https://nextstrain.org) & [GISAID.org](https://gisaid.org)
3. ARTIC Network Protocols and Tutorials:
- <https://artic.network/ncov-2019>
4. StaPH-B ToolKit (Dev Branch):
- https://github.com/StaPH-B/staphb_toolkit/tree/dev

