COG-train

Primers used for amplicon sequencing of Covid

Naomi Park (nh4@sanger.ac.uk)

SARs-CoV-2 Artic PCR

- Primer design (Artic and others)
- What's a few degrees between primers?
- How to improve genome coverage
- Keeping up with pandemic evolution

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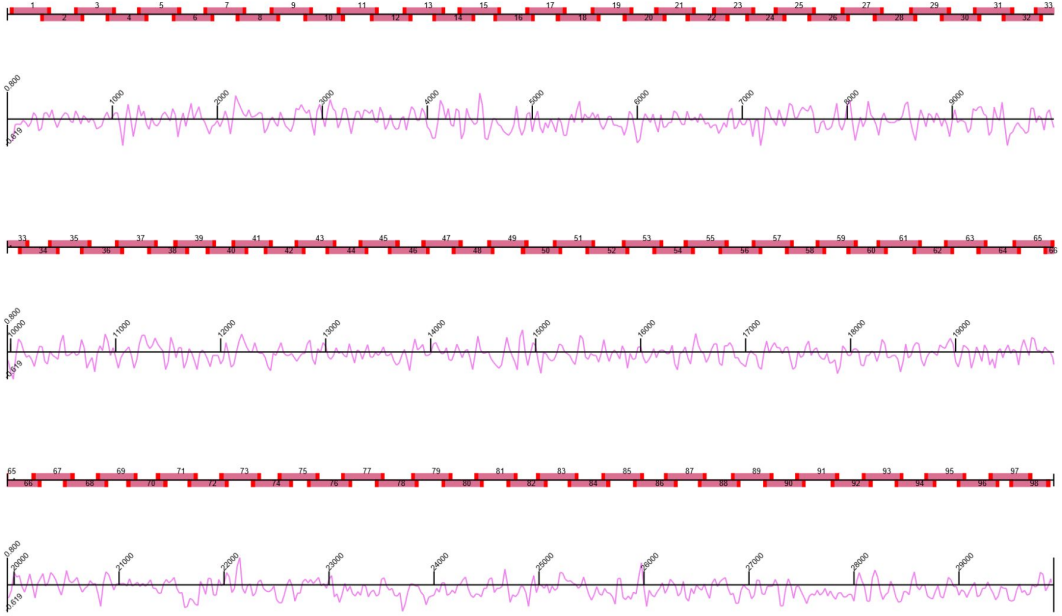
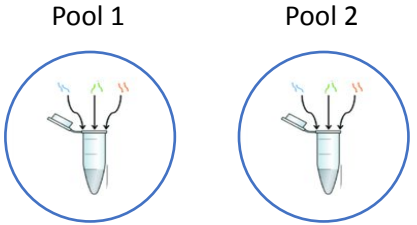
Artic Network PCR design





- SARS-CoV-2 genome is ~30,000 bases
- https://primalscheme.com/ used for design
- 99 Amplicons of ~400bp
- Split into 2 pools - Odd and even primer pairs are physically separated
- 35 cycles of PCR

ARTIC NETWORK

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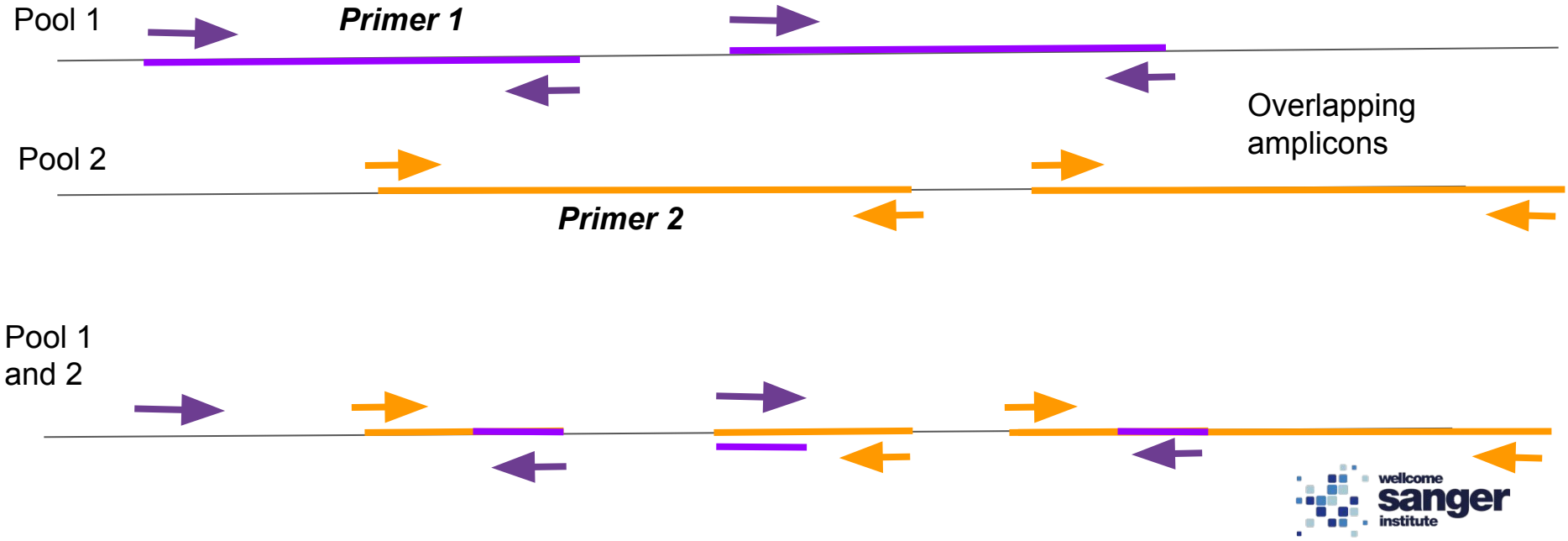
Artic PCR - Why two overlapping pools?



Other Amplicon Schemes

NEBNext® ARTIC products for SARS-CoV-2 sequencing

Product Listing | **Application Overview**

Especially with the ongoing emergence of SARS-CoV-2 variants that affect virus transmission and other metrics significant to public health, there is an increasing need for reliable, accurate and fast methods for sequencing SARS-CoV-2. The NEBNext ARTIC kits are based on the original work of the ARTIC Network, who quickly adapted their protocols to SARS-CoV-2 (Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt)).

The ARTIC method is a multiplexed amplicon-based whole-viral-genome sequencing approach (Figure 1), and the NEBNext ARTIC kit options are compatible with Illumina and Oxford Nanopore Technologies sequencing platforms. The two kits compatible with Illumina sequencing generate library inserts of ~150 bp or ~400 bp, for 2 x 75 or 2 x 250 sequencing, respectively (Figure 2). The NEBNext ARTIC SARS-CoV-2 RT-PCR Module contains only the reagents required for cDNA synthesis and targeted cDNA amplification from SARS-CoV-2 genomic RNA.

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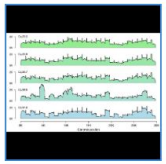
Other Amplicon Schemes



Version 6

Jul 29, 2021

Run

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SARS-CoV2 genome sequencing protocol (1200bp amplicon "midnight" primer set, using Nanopore Rapid kit) V.6

Version 1 is forked from nCoV-2019 sequencing protocol v2 (GunIt)

Nikki Freed[1], Olin Silander[2]

[1]University of Auckland; [2]Massey University

5 *Works for me*

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dx.doi.org/10.17504/protocols.io.bwyppfvn

Coronavirus Method Development Community

Nikki Freed

Steps | Guidelines | Warnings | Materials | Forks | Metadata | Metrics

ABSTRACT

To enable faster, easier sequencing of SARS-COV2 genomes with fewer steps than current methods, we use multiplexed 1200 base pair PCR amplicons with the Oxford Nanopore RAPID barcoding kit.

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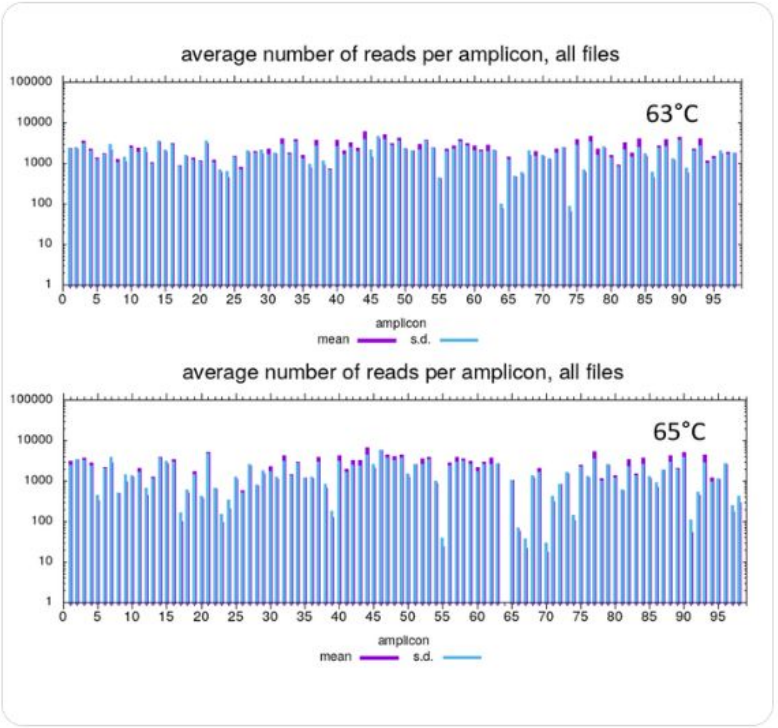
Importance of PCR annealing temp

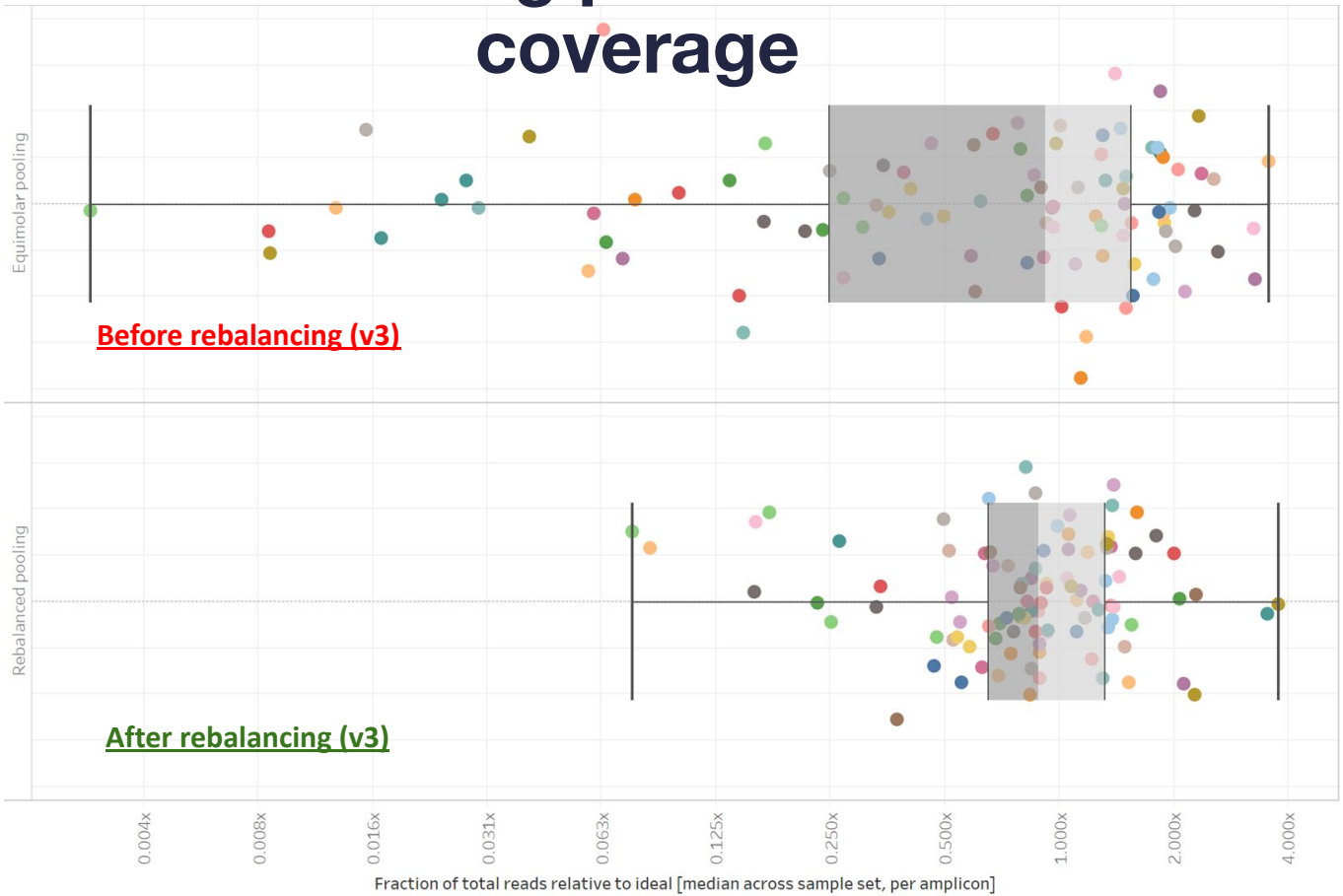
Dr Naomi Park
@SequencingPark

Update to COVID-19 ARTIC v3 Illumina library construction and sequencing protocol @sangerinstitute. A change in annealing temperature from 65°C to 63°C recovers amplicon 64 dropout and reduces variation in coverage. @CovidGenomicsUK dx.doi.org/10.17504/proto...

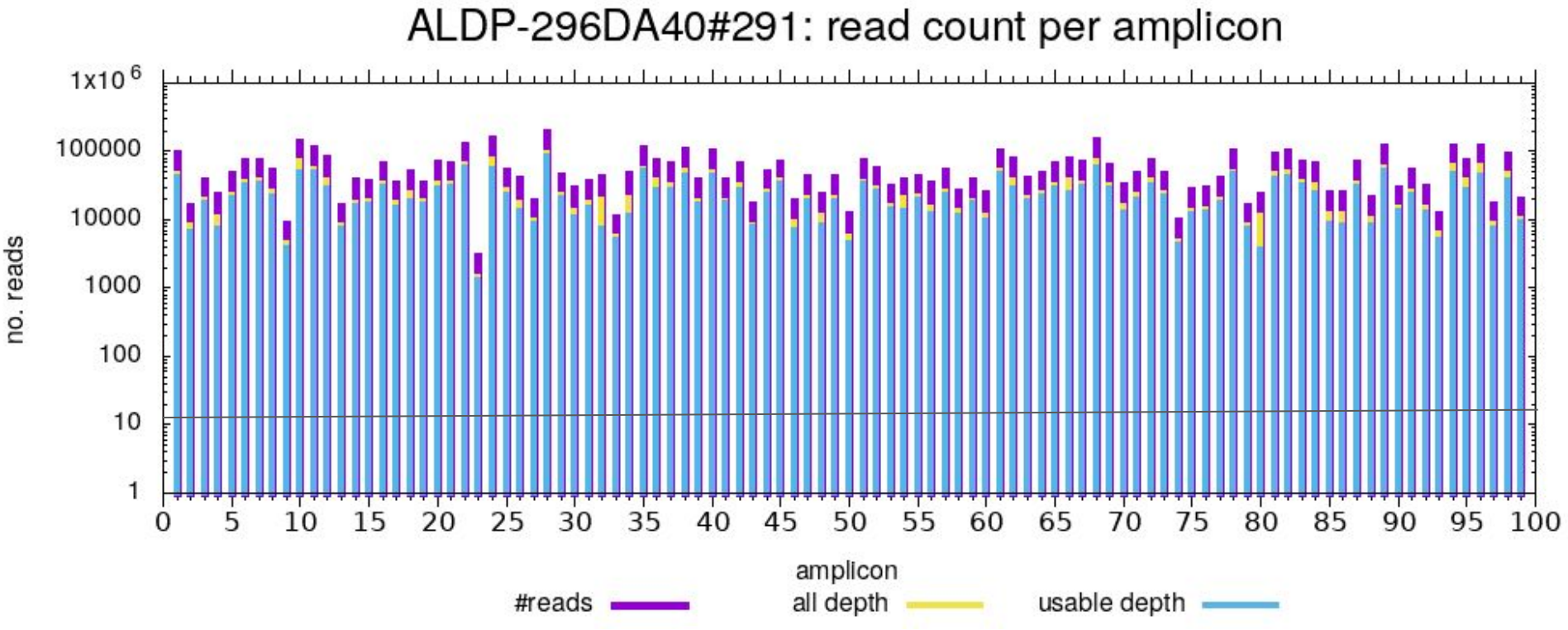


1:01 PM · May 22, 2020 · Twitter Web App

Rebalancing primers for even coverage



Keeping up with Pandemic evolution



https://primer-monitor.neb.com/lineages

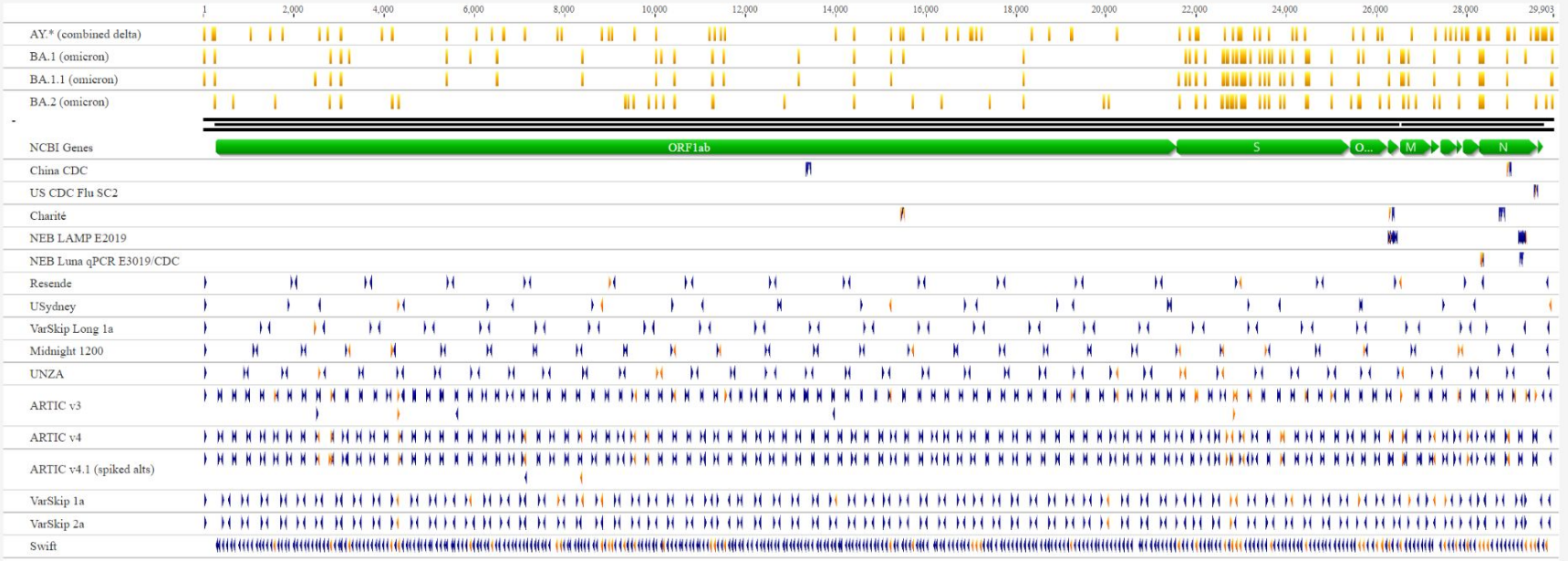
Primer Monitor Tool

Enabled by data from GISAID

Primer Variant Summary | Primer Sets | Lineage Variants | About

Create Account

SARS-CoV-2 Lineage Variant Summary



Nucleotide variants related to lineages of concern are indicated in yellow above a schematic SARS-CoV-2 genome. Primers that do not overlap with variants associated with these lineages are shown in blue. Overlapping primers are indicated in orange.