Library Preparation And Normalization Of Copied DNA

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BACKGROUND

Monitoring of viral infections such as with the SARS-CoV-2 virus was vital to detection and characterization of new variants before they became widespread and allowed public health agencies to deploy resources and develop policies in advance of new waves of the virus. I

The ARTIC Network developed a panel of primers and a workflow for whole genome sequencing of SARS-CoV-2 using multiplex PCR. This became a popular strategy for sequencing. The ARTIC protocol generates overlapping PCR amplicons that span the SARS-CoV-2 genome using a defined multiplex PCR primer set. These were sequenced and mapped to the SARS-CoV-2 genome to generate a high quality consensus sequence of the variant in the sample.

While ARTIC was developed for SARS-CoV-2, the protocol is readily adaptable to a wide array of viruses.

Despite its clear utility, challenges arose for ARTIC: new variants would arise that the consensus primers would not recognize and all testing for those new variants would be compromised. Normalization of samples with high variation of starting template proved difficult and sequencing library preparation was not optimized for convenience, speed, or cost.

TECHNOLOGY DESCRIPTION

The invention is a specialized genomic sequencing workflow that couples the **ARTIC Network** primer set with a modified **Santa Cruz Reaction (SCR)** library preparation protocol. The new workflow includes features such as

- **Modified Primer Design**: The protocol uses a modified ARTIC multiplex PCR primer set where primers carry a defined **4-base pair (4bp) 5-prime tail**. This creates amplicons with common, fixed ends that serve as targets for ligation.
- **Simplified Splint Ligation**: By using 4bp tails, the SCR process replaces complex random 7-mer splints (over 16,000 species) with a single, highly efficient splint species complementary to the primer tails. This ensures all library molecules are **full-length amplicons** without losing sequencing "real estate".
- **Enzymatic Library Normalization**: The invention introduces a novel "throttling" method for normalization. By limiting the concentration of the **P7 adapter** relative to the **P5 adapter**, the reaction naturally plateaus, producing consistent library yields regardless of variable DNA input amounts.

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INVENTORS

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OTHER INFORMATION

KEYWORDS

Viral sequencing, PCR amplification, splint ligation, short read sequencing

CATEGORIZED AS

- **Biotechnology**
  - Genomics

RELATED CASES

2022-844-0

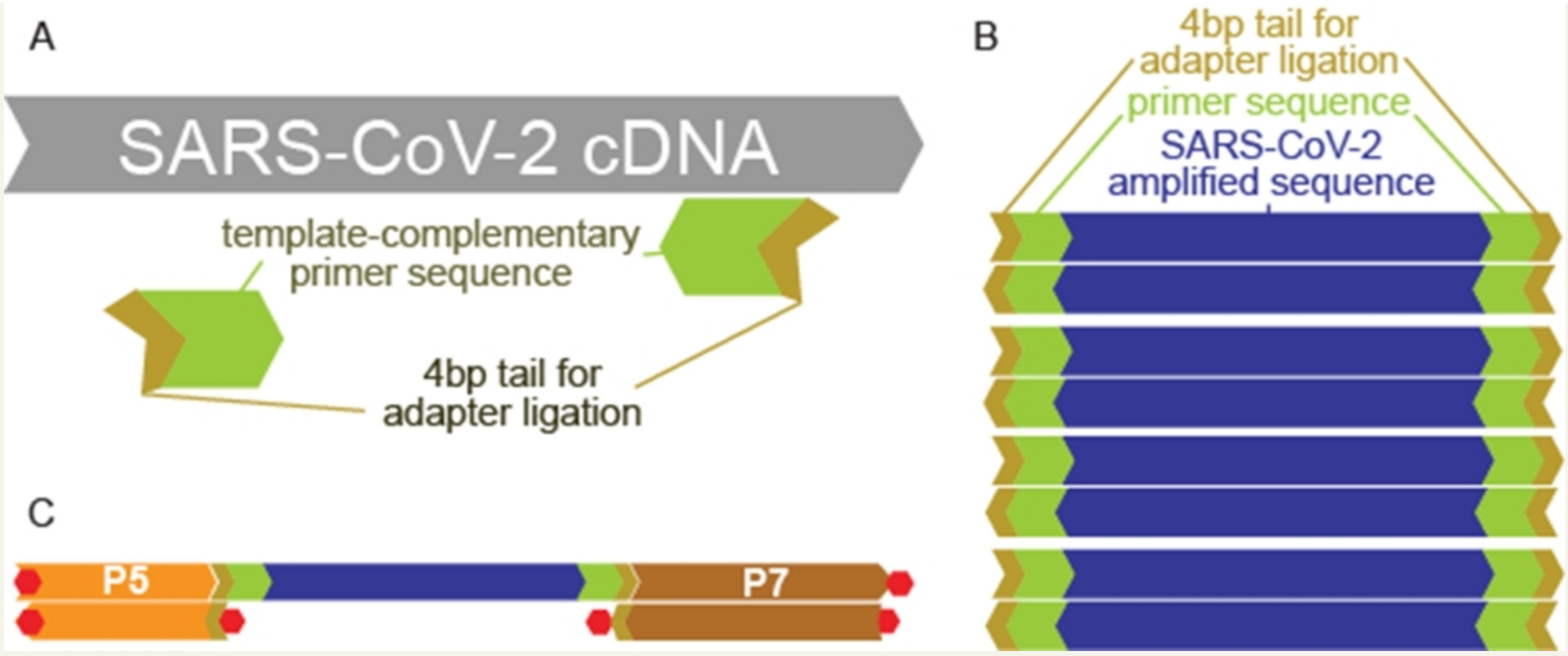

Figure 1 - Schematic of invention – In this example, the SARS-CoV-2 genome is the target to be amplified. The approach works for any target for which PCR primers can be generated. (A) PCR primers are designed, each with a 4 basepair 5-prime tail that is **not** complimentary to the target DNA. These 4 basepairs are the last 4 basepairs of the Illumina sequencing adapter. (B) After PCR, each amplified molecule will contain these 4 basepairs at their ends. (C) Libraries are made by completing the adapter via splint ligation or other ligation. In this way, **only** PCR amplicon products make sequencable, full-length adapters.

APPLICATIONS

- High-throughput genome surveillance of transmissible viruses
- Public health monitoring of evolving viral variants.
- Automated, production-scale sequencing on platforms like the Agilent Bravo liquid handling robot.

ADVANTAGES

Efficiency & Speed:

The one-tube reaction uses a limited number of reagents and completes library preparation in approximately **2.5 hours**

Cost Reduction:

Removal of PNK: By using 5-prime phosphorylated primers, the need for T4 Polynucleotide Kinase (PNK) is eliminated, reducing costs by **~15%**.

Reduced Ligase: The efficiency of fixed-sequence splints allows for lower units of T4 DNA ligase (the most expensive component).

Miniaturization: The protocol is flexible and can be scaled down to sub-10 µL reaction volume

Dimer Suppression: The design uses full-length indexing primers that prevent the amplification of adapter-dimers, improving PCR efficiency by over **4000X**

INTELLECTUAL PROPERTY INFORMATION

Patent Pending

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- Methods and Computational System for Genetic Identification and Relatedness Detection
- Methods For Generating Target Enrichment Probes For Genome Sequencing Applications
- Barcoded Solid Supports and Methods of Making and Using Same

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