CODEX DNA

Harnessing the power of the BioXp™ system to combat COVID-19 caused by SARS-CoV-2

Given the COVID-19 global healthcare emergency at hand, accelerating the pace of innovation and developing tools to combat the spread of COVID-19 is paramount. The scientific community is operating at its creative best to identify and advance solutions for prevention, detection, and treatment. The BioXp™ system is the ideal platform to enable researchers around the world to rapidly synthesize SARS-CoV-2 genome parts, making them readily available for developing vaccines, diagnostics, and therapeutics.

Introduction

SARS-CoV-2 is the causal agent of infectious disease, COVID-2019, that is now considered a global pandemic. SARS-CoV-2 has infected hundreds of thousands of people, claiming thousands of lives around the world[1]. SARS-CoV-2 is also closely related to the virus that caused the SARS pandemic in 2002–2003, sharing about 80% genomic identity[2]. The main route of transmission for the virus is through respiratory droplets produced by infected people. Close contact (< ~6 feet) increases the chances of spreading between people (leading to a community spread at vastly abbreviated timelines), which renders this virus highly contagious. Expectedly, the World Health Organization (WHO) has declared a worldwide public health emergency over the COVID-2019 outbreak, recognizing it as a global pandemic[3].

SARS-CoV-2, like the causal agent of the SARS and MERS outbreaks, has a single-stranded positive-sense RNA genome of about 30 kb in length, making it one of the largest among RNA viruses[2]. So far, no vaccines or therapeutics are available to combat SARS-CoV-2. Synthetic biology, specifically DNA writing technologies allows for the rapid development of prophylactics (vaccines) and therapeutics (monoclonal antibodies). In addition, DNA writing permits the generation of quality-assured material for diagnostic kits and assay development.

Rationally redesigned, full-length attenuated genomes help develop vaccines against the virus. Additionally, viral genome parts facilitate raising antibodies against viral antigens, enabling the development of therapeutic options that could effectively treat infected patients. The development of vaccines, diagnostics, and therapeutics are indispensable to halting the spread of this infection.

In this application note, we describe the on-demand production of the entire genome of SARS-CoV-2. Genome parts were designed and synthesized using the BioXp™ system. We also describe the joining of these parts to assemble the full-length genome of SARS-CoV-2 as a bacterial artificial chromosome (BAC) in *E. coli*. Given the number of genome variants of SARS-CoV-2 that have been identified thus far (https://nextstrain.org/ncov), it may become imperative to incorporate them into the vaccine, therapeutic, and diagnostic development pipelines quickly. The BioXp™ system accelerates this pipeline by enabling rapid synthesis of up to 32 individual, variant genome parts in a single, overnight run of the instrument.

Figure 1: BioXp™ 3250 system

Methods

Design and synthesis of SARS-CoV-2 genome parts on the BioXp™ system

Codex DNA's proprietary design software computed sequence complexity using parameters like repeats, GC flux, GC content, etc., and rationally designed a hierarchical stage-by-stage build of the SARS-CoV-2 genome (~30 kb). The tool enabled the addition of unique Gibson Assembly® handle sequences (30 bp) followed by a restriction endonuclease site. These features allowed the amplification and cloning of the SARS-CoV-2 genome fragments at all three stages of assembly (figure 2). Once designed, we placed the order, receiving the oligonucleotide plates and proprietary reagents in three business days.

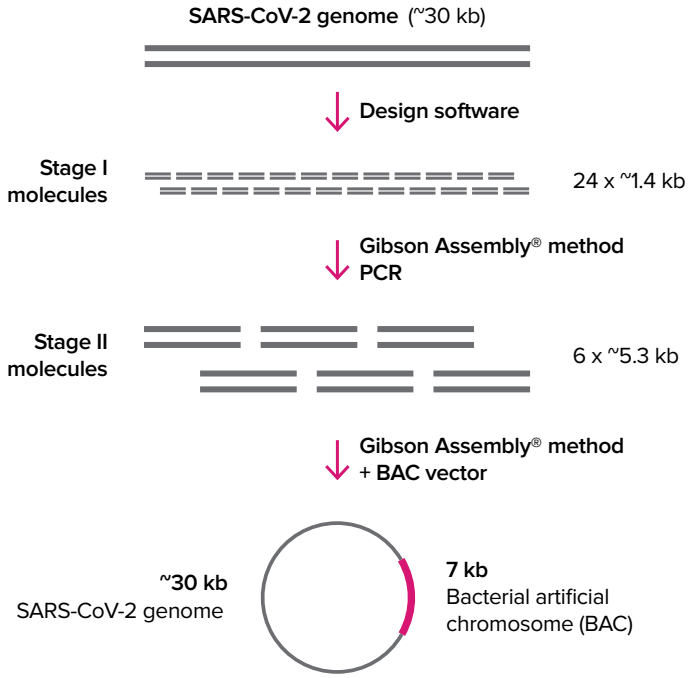

Figure 2: Hierarchical assembly scheme of the SARS-CoV-2 genome. The genome was synthesized using three stages of assembly: Stage I assembly of 24 X ~1.4 kb was completed on the BioXp™ system followed by 6 X ~5.3 kb assembly into stage II molecules and ~30 kb stage III genome.

We loaded the oligonucleotide plates and reagents onto the BioXp™ system and started the auto-generated protocol. The BioXp™ run begins with the initial build of each fragment, followed by error-correction, amplification of the stage I molecules, and purification of the DNA fragments. BioXp™ system generated all 24 fragments comprising the entire SARS-CoV-2 genome successfully (figure 3).

Hierarchical assembly of full-length SARS-CoV-2 genome

Using the Gibson Assembly® HiFi kit (Codex DNA catalog no. GA1100), we cloned stage I molecules (~1.4 kb) and sequenced them using Sanger sequencing (GENEWIZ). We assembled four consecutive, sequence-accurate stage I molecules into stage II molecules using the Gibson Assembly® HiFi kit, amplified the products using PCR, and then selected the products by size. These stage II molecules were further assembled into the full-length genome of SARS-CoV-2 using the Gibson Assembly® Ultra kit (Codex DNA catalog no. GA1200) and cloned using a BAC. We verified the presence of the entire genome cloned in the BAC by colony PCR and field inversion gel electrophoresis (FIGE). The entire cloned genome was visualized further by resolving supercoiled DNA by electrophoresis on a 1% agarose gel without ethidium bromide at 4.5 V/cm for 180 minutes using appropriate molecular weight standards.

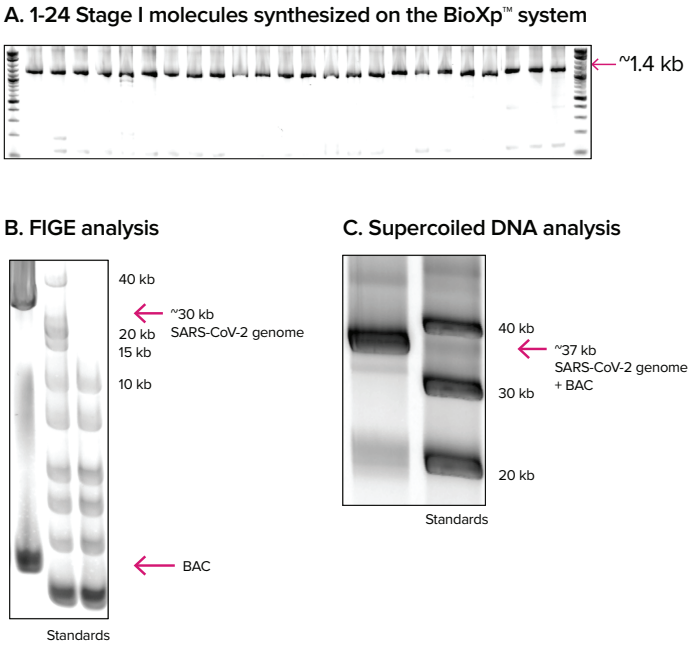

Figure 3: Assembly of SARS-CoV-2 genome using BioXp™ system. (A) 24X stage I molecules synthesized on the BioXp™ system then assembled into stage II and stage III molecules. (B) Analysis of clones carrying stage III molecules using FIGE analysis after releasing the ~30 kb SARS-CoV-2 genome from BAC using restriction endonuclease treatment. (C) Supercoiled DNA analysis of the intact genome cloned into BAC alongside molecular weight standards (DNA analyses from a single, representative clone are shown).

Results

The BioXp™ system and tools enabled us to build the entire genome of SARS-CoV-2 from design to final assembly.

Codex DNA's proprietary design tools were employed to create an *in silico* mapping of the hierarchical three-stage assembly that was necessary to build the SARS-CoV-2 genome. Specifically, we divided the entire ~30 kb genome of SARS-CoV-2, into twenty-four parts of stage I molecules (24 X ~1.4 kb). We ordered these parts with unique ends, via the BioXp™ kit ordering portal. Once delivered, we loaded the reagents onto the BioXp™ system and executed the run. The 16-hour run resulted in the successful generation of all 24 dsDNA fragments comprising the SARS-CoV-2 genome. We cloned the fragments using the Gibson Assembly® method and isolated error-free clones using Sanger sequencing.

Four consecutive stage I clones were combined into stage II molecules (~5.3 kb) through the Gibson Assembly® method, followed by PCR amplification and size selection. We assembled the six stage II molecules thus obtained into a BAC using the Gibson Assembly® method. Next, we screened 96 colonies using colony PCR and verified the presence of stage II molecules in the stage III assembled genome. Then, we selected 24-positively identified clones by colony PCR. We extracted the SARS-CoV-2 BAC from these and released the genome from the BAC using restriction enzyme digest. The released fragment was analyzed using FIGE. All 24 colonies that we tested carried the ~30 kb insert, indicating the presence of the SARS-CoV-2 genome. We subjected a few colonies among these to supercoiled-DNA analysis, which enabled the visualization of the SARS-CoV-2 genome cloned into the BAC as circular molecules. All the colonies tested were verified to contain the full-length genome (figure 3).

Summary

Previously, scientists at Codex DNA developed and deployed synthetic DNA technology to address the annual threat of influenza by generating strain-specific antigen parts[4]. The emerging threat of SARS-CoV-2 genomic variants will trigger similar use of synthetic biology to synthesize multiple, strain-specific variants of antigens, such as the spike protein. The BioXp™ system and tools are a disruptive technology that accelerates the discovery of vaccines, diagnostics, and therapeutics. With the ability to synthesize highly accurate dsDNA molecules and enable rapid iteration cycles of design and synthesis, the BioXp™ system will extend the discovery capabilities of researchers against other infectious agents of humans, livestock, and plants.

References

1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/
2. Taiaroa et. al. Direct RNA sequencing and early evolution of SARS-CoV-2. bioRXiv 2020 doi: https://doi.org/10.1101/2020.03.05.976167
3. https://www.who.int/emergencies/diseases/novel-coronavirus-2019
4. Dormitzer et. al. Synthetic generation of influenza vaccine viruses for rapid response to pandemics. *Sci Transl Med.* 2013 May 15;5(185):185ra68. doi: 10.1126/scitranslmed.3006368

Ordering information

Product	Catalog number
Full-length genomes	
SARS-CoV-2 genome	SC2-FLSG-0000
SARS-CoV-2 genome with T7 promoter and poly(A) tail	SC2-FLSG-1111
SARS-CoV-2 genome-encoding spike protein variant D614G, including a T7 promoter and poly(A) tail	SC2-FLSG-3333
SARS-CoV-2 genome-encoding spike protein with mouse-adapted mutations (Q498Y, P499T); *in vitro* transcription-compatible	SC2-FLSG-4444
SARS-CoV-2 genome-encoding spike protein with mouse-adapted mutations (Q498Y, P499T) and D614G variant; *in vitro* transcription-compatible	SC2-FLSG-4455
SARS-CoV-2 genome-encoding mouse-adapted mutations in NSP4, NSP7, NSP8, ORF6 and spike (D614G variant); *in vitro* transcription-compatible	SC2-FLSG-4466
Our team of experienced scientists can work with you to design and engineer your very own custom SARS-CoV-2 genome	Custom genome
Reporters	
SARS-CoV-2 genome-encoding nanoluciferase reporter and spike D614G variant with deletion of ORF7a gene; *in vitro* transcription-compatible	SC2-FLSG-5555
SARS-CoV-2 genome-encoding nanoluciferase reporter and spike D614G variant with deletions of ORF7a and 7b genes; direct transfection-compatible	SC2-FLSG-6666
Replicons	
SARS-CoV-2 replicon-encoding *Renilla* luciferase reporter and neomycin genes, with deletions of S, E, M and ORFs 6, 7a, 7b, 10 genes; *in vitro* transcription-compatible	SC2-FLSG-5566
SARS-CoV-2 replicon-encoding nanoluciferase and neomycin genes, with deletions of S, E, M and ORFs 3a, 6, 7a, 7b, 8 genes; *in vitro* transcription-compatible	SC2-FLSG-5577

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