SYNTHETIC BIOLOGY

Yeast-based synthetic genomics brewing up a storm against COVID-19

A switch from *Escherichia coli* to *Saccharomyces cerevisiae* as the recombination vessel allows synthetic construction of stable large RNA virus genomes without clinical samples – paving the way for rapid responses to viral threats.
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Over the past decade, large RNA viruses, such as the Zika or Ebola virus, have become an increasing threat to the human population on our planet[1]. The current COVID-19 pandemic, caused by the large, positive sense single-stranded RNA virus SARS-CoV-2, has cost the lives of 4.5 million people to date[2], and severely impacted the economic and political landscape worldwide[3]. If the responses by scientists of sequencing the virus, generating vaccines, and advocating to reduce the spread had not been as prompt as it was, the current state of the world could be much worse[1]. Thao *et al.*[4] contributed to this response by constructing the full 30kb genome of SARS-CoV-2 in just 7 days by using a yeast-based synthetic genomics platform, as well as assessing the feasibility of this platform for COVID-19 and future pathogens.

In synthetic genomics platforms, microbes are used to "reverse-engineer" the genome of a virus by forcing their replication machinery to join chemically synthesized fragments of the viruses' genome into a vessel, such as an artificial chromosome[5]. The genome within the artificial chromosomes is extracted, capped, and enveloped to construct a synthetic version of the virus. This technique has been a vital tool in the development of vaccines and understanding pathogenicity of various viruses[4,5]. Historically, synthetic genomes have been generated using the *E. coli* replication machinery[5]. However, using *E. coli* to generate the SARS-CoV-2 genome is not feasible for several reasons. *E. coli* takes significantly longer than other microbes to produce a full genome, slowing down the ability to take quick action against a viral pathogen[4]. Additionally, synthetic genomes of large RNA viruses akin to SARS-CoV-2 that have been constructed by the *E. coli* replication machinery have been found to be unstable, thus introducing mutations at a frequency dissimilar to the virus *in vivo*[6].

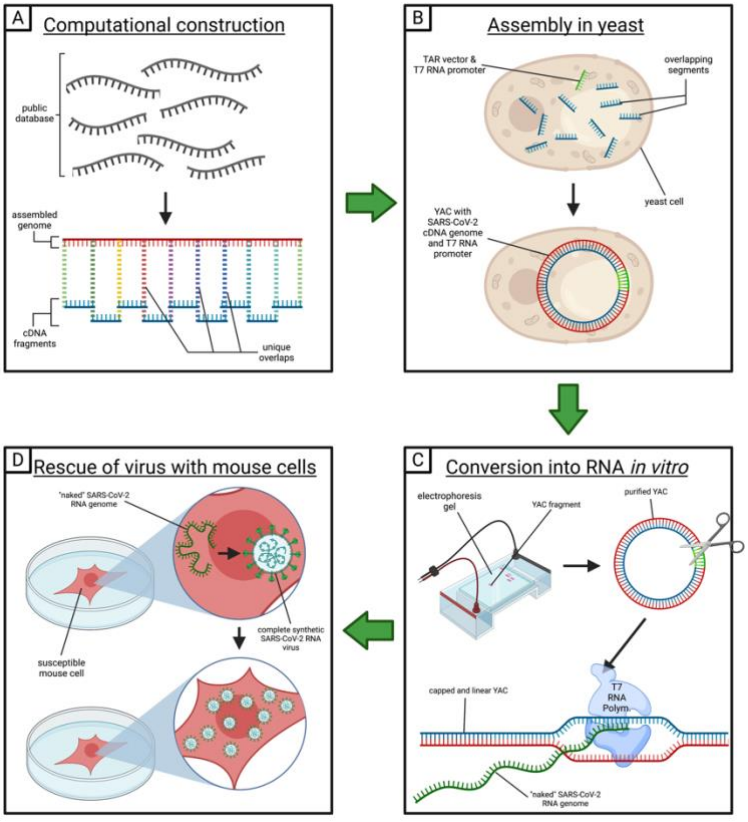

Figure 1. Workflow of the yeast-based synthetic genetics platform used to construct synthetic SARS-CoV-2. **A.** Thao *et al.* computationally constructed a SARS-CoV-2 genome by collating parts from public databases and splitting this genome into overlapping segments of cDNA. The fragments were chemically synthesized from these segments. **B.** Synthesized fragments, including a fragment at the 5' end carrying sequences for the TAR vector and T7 RNA Polymerase promoter, were assembled into a Yeast Artificial Chromosome (YAC) in one step using homologous recombination in yeast. **C.** The YAC was extracted using gel electrophoresis. It was purified and linearised, and a T7 RNA Polymerase translated the cDNA YAC into a SARS-CoV-2 RNA genome. **D.** The genome was cultured in susceptible mouse cells where it synthesized its envelope and replicated. *Image created with Biorender.com*

Due to these caveats, Thao *et al*. used the replication machinery of *S. cerevisiae* (yeast) as a synthetic genomics platform. This technique had recently been found to be faster and more reliable in large RNA viruses[5]. Here, they collated all parts of the SARS-CoV-2 genome from different public databases, translated these into a single cDNA "genome" and split it into 12 overlapping segments (Fig. 1A). Thao *et al*. then obtained chemically synthesised fragments of these computational constructs (Fig. 1A). The fragments were joined in a single step by using yeast's replication machinery (Fig. 1B). During yeast replication, homologous recombination allows for

the overlapping segments to be joined in the correct order of the viruses' genome. Thao *et al*. used transformation-associated recombination (TAR) cloning for this process, meaning that the genome is assembled into a yeast artificial chromosome (YAC) and a T7 RNA polymerase promoter is attached in the 5' untranslated region. This means that the viruses' genome, upon completion of the replication process, is not integrated into the genome of the yeast but rather a stand-alone "unit" with a T7 RNA polymerase promoter that can be extracted for processing *in vitro* (Fig. 1B). The extraction took place using electroporation, which allows the circular YAC to run down a gel and be physically cut out of the gel and purified (Fig. 1C). The YAC was then linearised and exposed to T7 RNA polymerase (Fig. 1C). T7 RNA polymerase translates cDNA into RNA, thus generating the "naked" single-stranded SARS-CoV-2 RNA genome from the YAC. The polymerase also caps the 5' and 3' ends of this naked genome, allowing the synthetic virus to be mistaken for mRNA in the host. Finally, Thao *et al*. cultured the naked genome in SARS-CoV-2-susceptible cells of mice, enabling the virus to produce its envelope components and replicate (Fig. 1D). This whole process was repeated with genomes of various other viruses by extracting either virus isolates, cloned or synthetic DNA or clinical samples, and all generated viruses were assessed for their stability, replicability, and similarity to the original viruses. This showed that all viruses were functionally and phenotypically the same as the original viruses.

Thao *et al*. illustrated that the yeast-based synthetic genomic platform is a feasible and rapid alternative to its *E. coli* counterpart[4]. They dared to use and adapt this relatively new technique to provide a functioning, synthetic SARS-CoV-2 genome as a tool for other scientists without access to clinical samples, enabling the wider scientific community to participate in reducing the impact of COVID-19 on our population[7-9]. Subsequently, the produced synthetic genome allowed researchers to understand SARS-CoV-2 more holistically[7], determine the effect of SARS-CoV-2 on immune responses[8], and develop a treatment that reduces the severity of symptoms[9]. In addition to the advances made with the SARS-CoV-2 genome, Thao *et al*. also leaped forward in the field of synthetic genomics by illustrating that yeast-based synthetic genomic platforms are a feasible way to rapidly react to emerging pathogens, thanks to yeast's remarkable replication machinery. The only downfall, and perhaps the most terrifying thought, is that this technique may fall into the wrong hands.

References

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