Title:	Reconstruction of an Attenuated SARS-CoV2 Genome Using Synthetic Genomics
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Abstract:	There is a need to establish a platform that would enable one to reconstruct attenuated RNA viruses just from their genomic sequence information. This is crucial to respond to the new emerging variants of RNA viruses. To this end, we are attempting to create a synthetic attenuated SARS-CoV2 (aSARS-CoV2) virus, building-up on the previous work with a native virus created synthetically. Towards this goal, ~29 kb of the complete genome, except for the nucleocapsid-encoding gene (N gene) of a pre-delta variant of SARS-CoV2 (B.1.5), was custom synthesized in 15 blocks by previous researchers in the lab. The custom synthesis has been done so that each block overlaps with its adjacent blocks, except the 5' end of the first and the 3' end of the last block, which overlap with the pRS313 yeast vector. To increase the transformation success rate, pRS313 was split into two fragments, one containing the ARS/CEN6 sequence and the other containing the HIS3 selection marker. Out of the 15 blocks, a specific set of four and three blocks were first transformed into the Saccharomyces cerevisiae BY4741 along with the pRS313 vector fragments. The highly efficient recombination machinery of Saccharomyces cerevisiae then recombines all these overlapping blocks together to form a stable circular vector. The two recombined vectors were then isolated and digested to get the two recombined blocks of interest. Hence, a total of 10 blocks were then transformed with the linearized pRS313 vector fragments into the BY4741 strain to fully recombine the attenuated SARS-CoV2 genome in DNA form. We have now successfully recombined the genomic blocks of aSARS-CoV2 in yeast. The proper assembly and orientation of the aSARS-CoV2 genome with the yeast vector have been established by junction-confirming PCR (JnPCR) over all junctions. We have also succeeded in purifying the DNA of recombined aSARS-CoV2 genome in yeast plasmid with minimal genomic DNA contamination. This has also been confirmed with JnPCR. We are currently working on increasing the yield and integrity of the isolated plasmid containing the aSARS- CoV2 genome by various means, such as using the long and accurate PCR (LA-PCR) or multicopy vector pRS423, and finally getting the in vitro transcription of ~28.5 kb DNA to work. This is being attempted using Promega's T7 RiboMAX TM Express Large-Scale RNA Production System. Once this is achieved, this ~28.5 kb RNA will be introduced into a mammalian cell line already expressing the N gene, yielding the full attenuated SARS-CoV2.
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