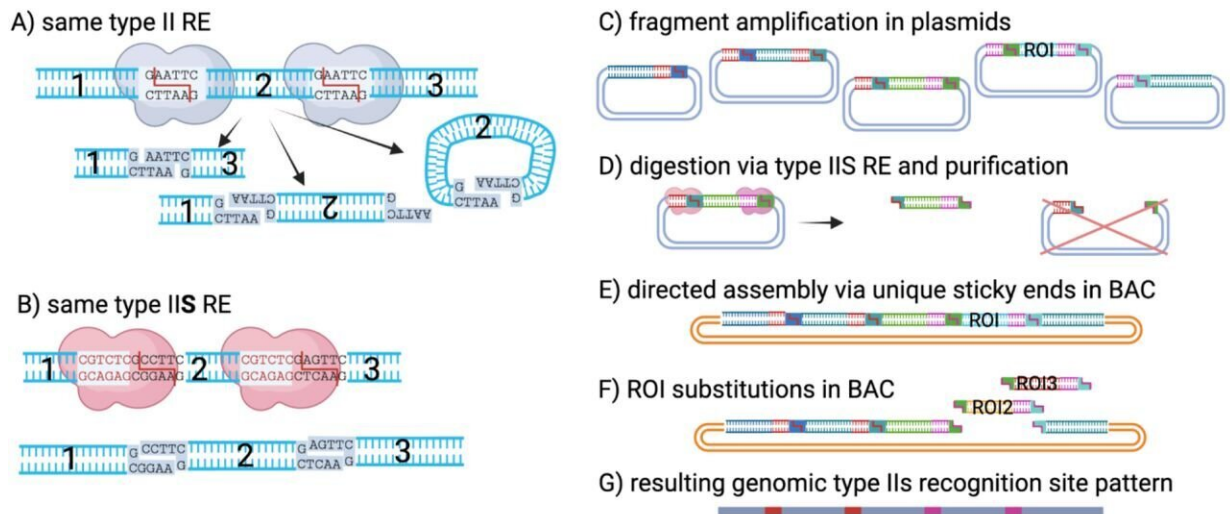


A scientific response to the recent reporting on the origin of SARS-CoV-2

October 26 2022, by Stefan Dreising



Synthetic RNA virus assembly. Directed assembly of ~30kb CoV genomes requires several design considerations A) Several identical type II enzymes cannot be used for directed genome assembly as this leads to random fragment sequences, inverted fragments, and loops. Use of different type II enzymes that cut in their recognition sequence for every junction requires working with numerous buffers, running numerous reactions at different temperatures, and may require modifying numerous recognition sites in the genome. The use of fewer distinct enzymes is preferred. B) Endonucleases that cleave outside of their recognition sequence (type II shifted or type IIS) can produce distinct sticky ends allowing for directed assembly of complex viral genomes. C) For IVGA, individual fragments derived from PCR or DNA synthesis are first amplified in bacterial plasmids. D) Fragments are then cut out of the plasmids using type IIS endonucleases. E) Unique sticky ends at each section enable directed assembly in a full-length cDNA or bacterial artificial chromosome. F)

Use of a different type IIS endonuclease with sites flanking a region of interest (ROI) allows for efficient substitutions of that region. G) This method does not alter viral proteins. However, it does leave a distinct pattern (fingerprint) of regularly spaced type IIS recognition sites of the endonucleases that were used for synthetic assembly. Credit: *bioRxiv* (2022). DOI: 10.1101/2022.10.18.512756

The origin of SARS-CoV-2 remains unresolved. In a non-peer-reviewed preprint published on *bioRxiv* on Oct. 20, 2022 three authors present analyses that, according to their interpretation, suggest a "synthetic emergence" of SARS-CoV-2 and its release in the context of a "laboratory accident".

Experts from the University of Wuerzburg and the University Hospital have reviewed the preprint on the origin of SARS-CoV-2. In summary, the analyses presented in the study do not provide sufficient evidence for the authors' conclusion that SARS-CoV-2 is of synthetic origin.

Valentin Bruttel (affiliation: Department for Obstetrics and Gynecology, University Clinics of Würzburg, Germany), Alex Washburne, and Antonius Van Dongen present analyses that, according to their interpretation, suggest a "synthetic emergence" of SARS-CoV-2 and its release in the context of a "laboratory accident".

Summary of the preprint

The core message of the preprint is that the [genome](#) of SARS-CoV-2 has an "abnormal pattern" of recognition sites for certain restriction enzymes (BsaI and BsmBI) and therefore is highly unlikely to have arisen by [natural evolution](#).

Based on statistical analyses, the authors conclude that this pattern most likely arose as a "fingerprint" in the SARS-CoV-2 genome during the establishment of a reverse genetics system for the (most likely bat-derived SARS-CoV-2 predecessor) in a research laboratory.

Background

Restriction enzymes such as BsaI and BsmBI are used to clone genomes of coronaviruses isolated from animals such as bats and render them accessible for systematic research.

Such so-called reverse genetics systems are of great importance for virological research. They allow the genetic conservation of otherwise mutation-prone viruses as well as the investigation of individual viral gene functions. Using these tools, coronavirus genomes, which are relatively large at just under 30,000 nucleotides, can be cloned and modified in bacteria in 5-8 small subfragments.

For the cloning of the various fragments, individual bases of the viral genome occasionally have to be exchanged in order to insert the restriction sites necessary for cloning, or to remove unwanted restriction sites. Subsequently, the individual DNA fragments can be re-assembled to give rise to a complete viral genome.

Scientific evaluation

1. Contrary to the authors' claim, the restriction site pattern of SARS-CoV-2 may well have arisen naturally—similar patterns are also found in coronaviruses closely related to SARS-CoV-2

All 5 restriction sites—BsmBI (n=3) and BsaI (n=2)—central to the analyses in the preprint are also commonly found in closely related

coronaviruses. Thus, the existence of these 5 sites in the SARS-CoV-2 genome can be explained without human manipulation. Some known coronaviruses closely related to SARS-CoV-2 were obviously not included in the analyses performed in the preprint.

The authors of the preprint further argue that restriction sites in the SARS-CoV-2 genome that are unfavorable for genetic work are absent and presumably have been artificially altered ("deleted"). However, while many other coronaviruses actually show significantly more restriction sites for the two restriction enzymes analyzed, some closely related bat coronaviruses such as BANAL-20-103 and BANAL-116 also show only 5 and 7 restriction sites, respectively, with similarly sized genome fragments.

2. The position of the two BsaI restriction sites in the region of the S gene does not indicate genetic manipulation of the SARS-CoV-2 genome.

The Spike protein of coronaviruses is of particular interest because it determines whether human cells can be infected or not. For reverse genetics models, it was therefore of particular interest for researchers to be able to exchange or modify the Spike protein coding region of coronavirus genomes.

As shown by the authors, the two restriction sites for BsaI could be used to easily manipulate the most important part of the Spike protein of SARS-CoV-2, namely the receptor-binding domain (RBD) and the furin cleavage sites (FCS). For no other coronavirus isolate does this appear to be so easily possible with BsaI, since either the appropriate restriction sites are missing or additional sites would lead to additional unwanted fragments upon digestion with BsaI.

The authors argue that this suggests that the SARS-CoV-2 genome has

been optimized for easy replacement and manipulation of the most important parts of the Spike protein. In fact, the combination of BsaI and BsmBI has been used in the past by research groups in Wuhan to clone coronavirus genomes from bats and perform so-called gain-of-function experiments. However, in this case, the two BsaI restriction sites were each positioned to allow the exchange of the entire Spike protein.

This is, however, not possible for SARS-CoV-2. If the two BsaI restriction sites had been in exactly the same positions as in previously published reverse genetics models, this would indeed have provided strong evidence for human manipulation. The two observed BsaI restriction sites are, however, also frequently found in closely related coronaviruses of SARS-CoV-2.

It is noteworthy, however, that these coronaviruses usually possess at least one further BsaI site, which would have to be eliminated (i.e., mutated). Given the high mutation rate of circulating SARS-CoV-2 variants, one would also expect that artificially inserted synonymous (wobble) mutations, inserted to create or eliminate defined restriction sites, would disappear and artificially eliminated ones would reappear over the course of the more than two-year pandemic.

In fact, however, the Omicron variants still have the same interface distribution pattern as the original Wuhan virus.

3. The statistical analyses of the paper on the distribution of restriction sites are flawed or incomplete in important respects.

The combination of BsaI and BsmBI analyzed in the preprint is indeed not suitable for the vast majority of coronaviruses to dissect their genomes into an appropriate number of fragments (5 to 7) of suitable size (

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