Twitter Thread by valentin bruttel

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How SARS2 was stitched together
Nowadays,SARS2 like viruses can be generated in lab within weeks
https://t.co/uWAI0oMrIU
If this was possible in Wuhan 2018/19 is debatable.
However, Shi Zhengli had 2 grants to investigate which manipulations enable bat viruses to go pandemic.
1/x

Grants (recent five years):

01/01/2011-12/31/2015	Mechanism of interspecies transmission of zoonotic viruses, National Basic Research program of China, project no: 2011CB504700. Co-Principal Investigator. 1,300,000 RMB.
01/01/2013-12/31/2017	Identification, genetic evolution and pathogenesis of bat viruses in China. National Natural Science Foundation of China, project no: 81290341. Co-Principal Investigator. 2,900,000 RMB.
01/06/2014-31/05/2019	The ecology of bat coronaviruses and the risk of future coronavirus emergence. National Institutes of Health NIAID R01AI110964. 665,000 US dollars.
01/10/2014-30/09/2019	Emerging Pandemic Threats PREDICT 2_China, United States Agency of International Development, project no: AID-OAA-A-14-00102. Country Coordinator. 559,500 US dollars.
01/01/2016-31/12/2020	Geographical distribution and genetic varation of pathogens in Africa, Sino-Africa Joint Research Center, Chinese Academy of Science, project no: SAJC20165. Principal Investigator. 2,400,000 RMB.
01/01/2018-31/12/2021	Evolution mechanism of the adation of bat SARS-related coronaviruses to host receptor molecules and the risk of interspecies infection, National Natural Science Foundation of China, project no: 31770175. Principal Investigator. 660,000 RMB.
01/07/2018-30/06/2023	Genetic evolution and transmission mechanism of important bat-borne viruses. The strategic Priority research Program of Chinese Academy of Sciences. Principal Investigator. 8,750,000 RMB.
01/01/2019-31/12/2023	Pathogen biology studies on novel swine coronavirus, National Natural Science Foundation of China, project no: 31830096. Principal Investigator. 3,480,000 RMB.

This was pointed before by members of DRASTIC https://t.co/By9hljwjuY, @TheSeeker268 @ydeigin
CV: https://t.co/fXlqRIJoWO
Let's say the WIV did not have yeast artificial cromosomes yet, they would have had to rely on bacteria as described here:
https://t.co/F4C9HfVcXv
2/x

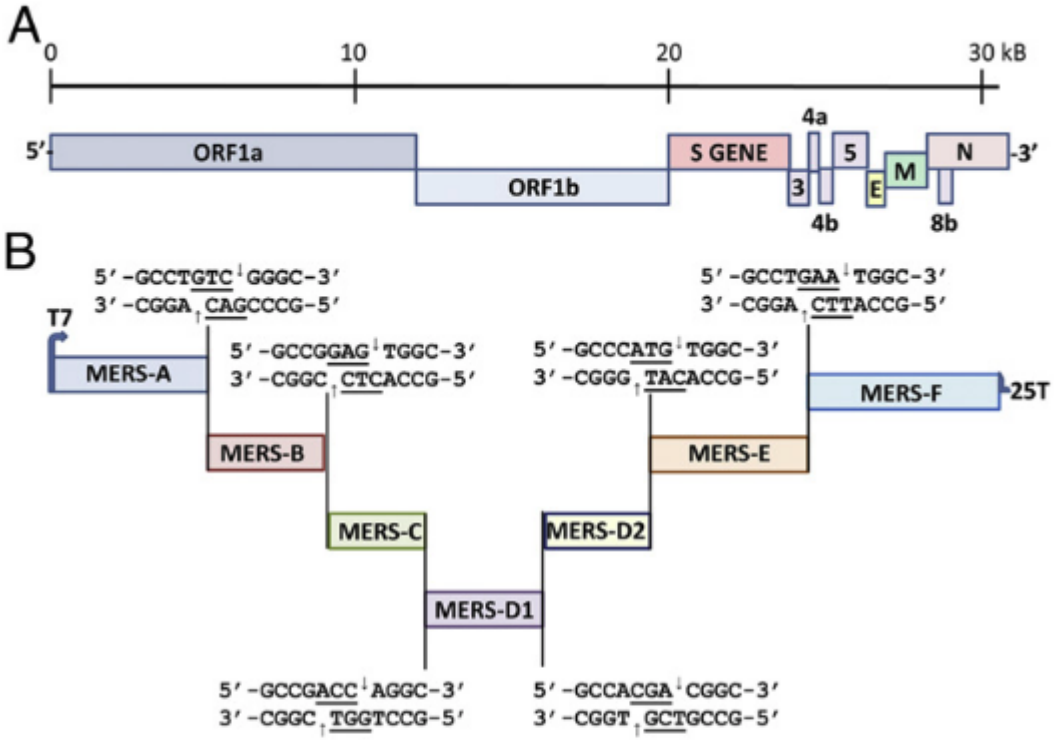
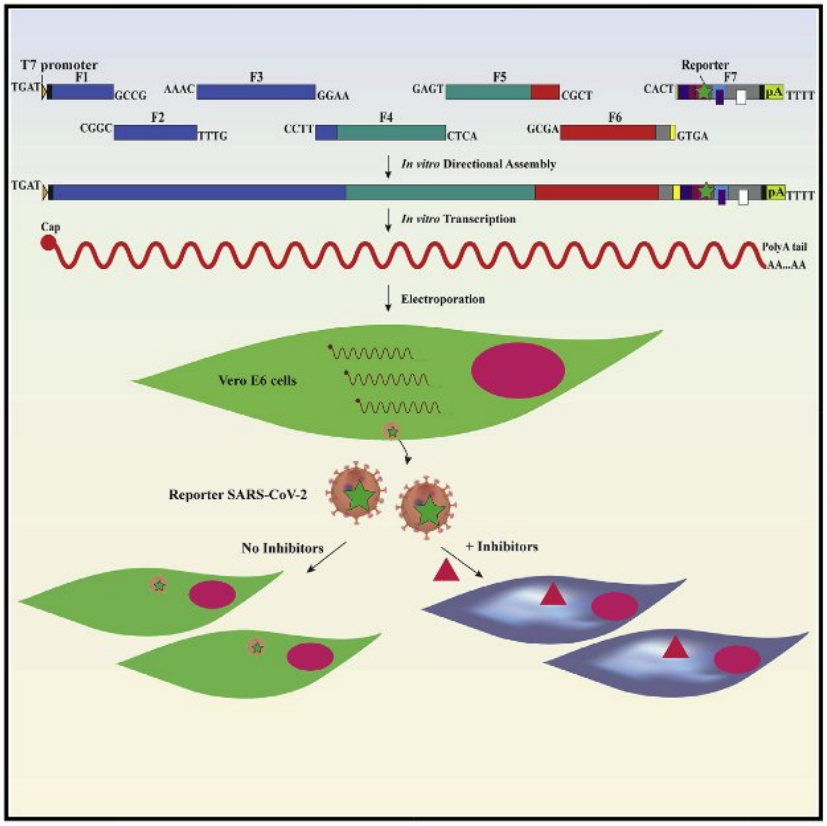


Fig. 1. Organization of the MERS-CoV molecular clone. (*A*) The organization of the MERS-CoV genome. (*B*) The full-length MERS-CoV genome was ultimately divided into seven contiguous cDNAs designated MERS A–F and flanked by unique BglI sites that allow for directed assembly of a full-length cDNA: MERS A (nucleotides 1–4692), MERS-B (4693–8811), MERS-C (8812–12258), MERS-D1 (12259–15470), MERS-D2 (15471–18806), MERS-E (18807–24397), and MERS-F (24398–30119).

This method has now evolved using in vitro assembly and transcription, which significantly speeds up the process and reduces costs, as described here:
https://t.co/Qi180L3c9u
In either case, one would need a special kind of Type IIS endonucleases (DNA cleaving enzymes):
3/x

An Infectious cDNA Clone of SARS-CoV-2

Graphical Abstract



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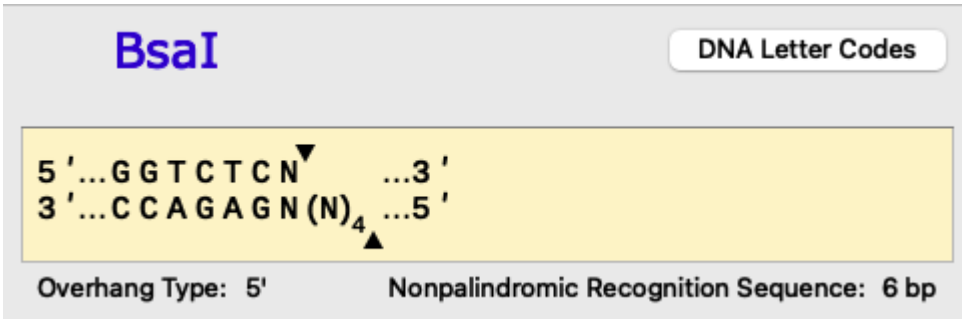
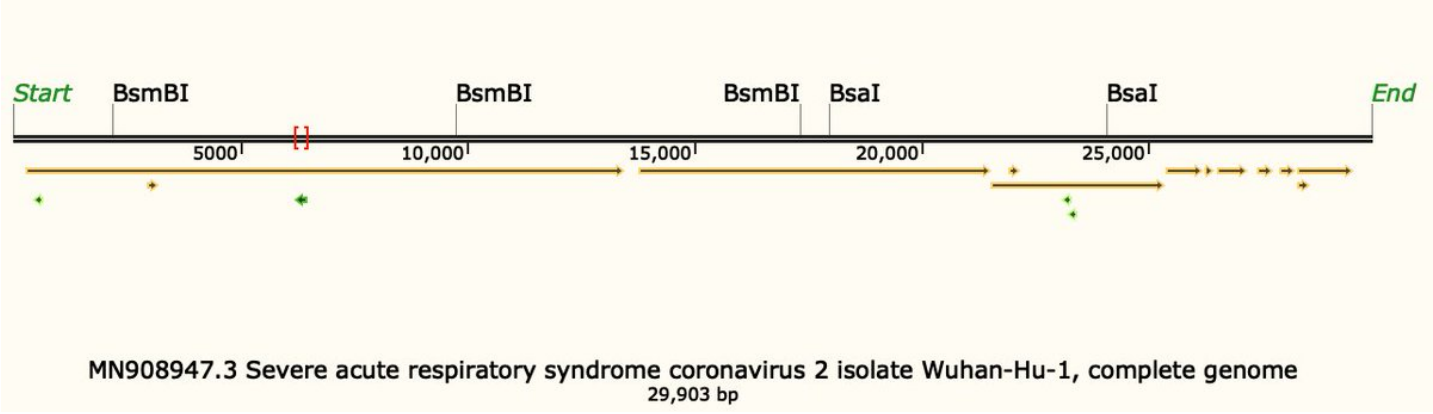
In Brief

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a devastating global pandemic. Xie et al. generated an infectious cDNA clone of SARS-CoV-2 and a mNeonGreen reporter virus. Recombinant SARS-CoV-2 and reporter virus replicate as efficiently as the original clinical isolate.

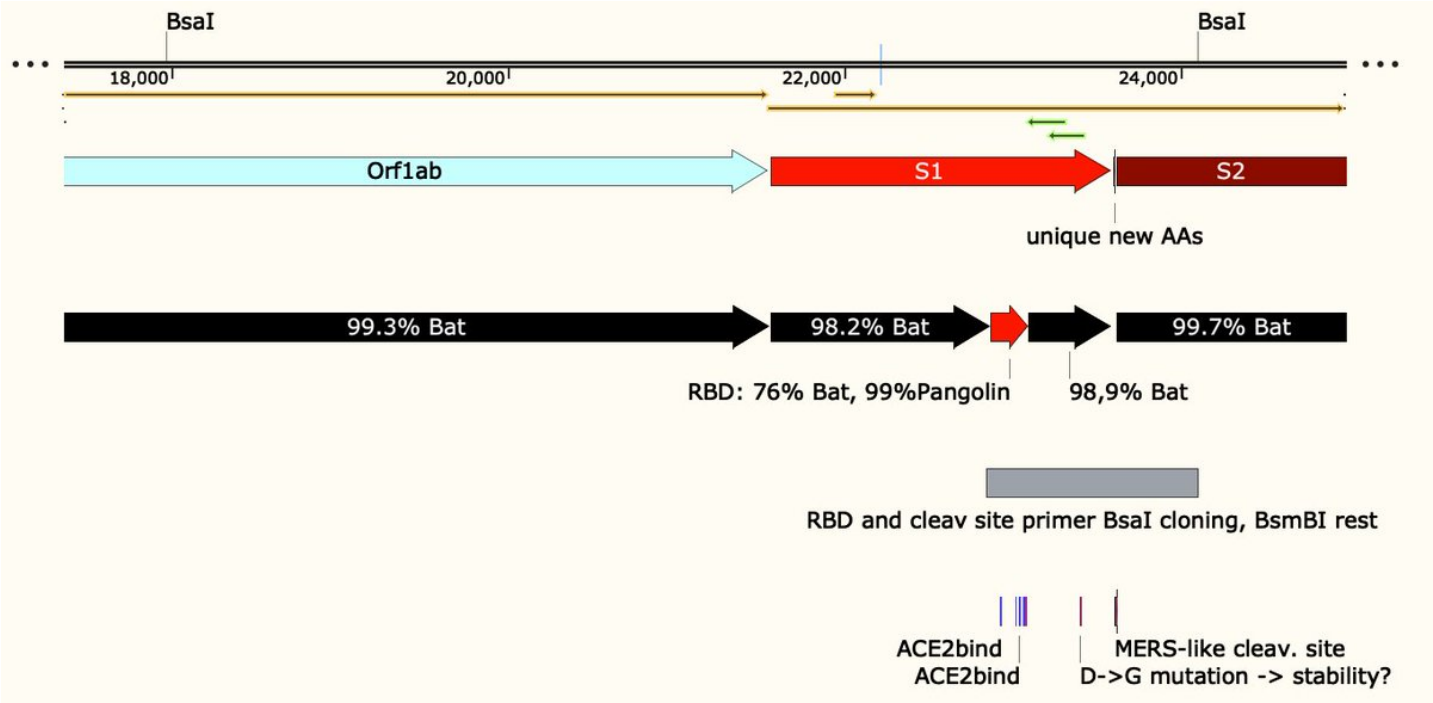
These relatively rare enzymes cut outside of their recognition site and can produce variable 4 nucleotide long DNA sticky ends. Different sticky ends enable an ordered assembly of the full viral genome by ensuring that always the right ends are stitched together by ligases.
4/x



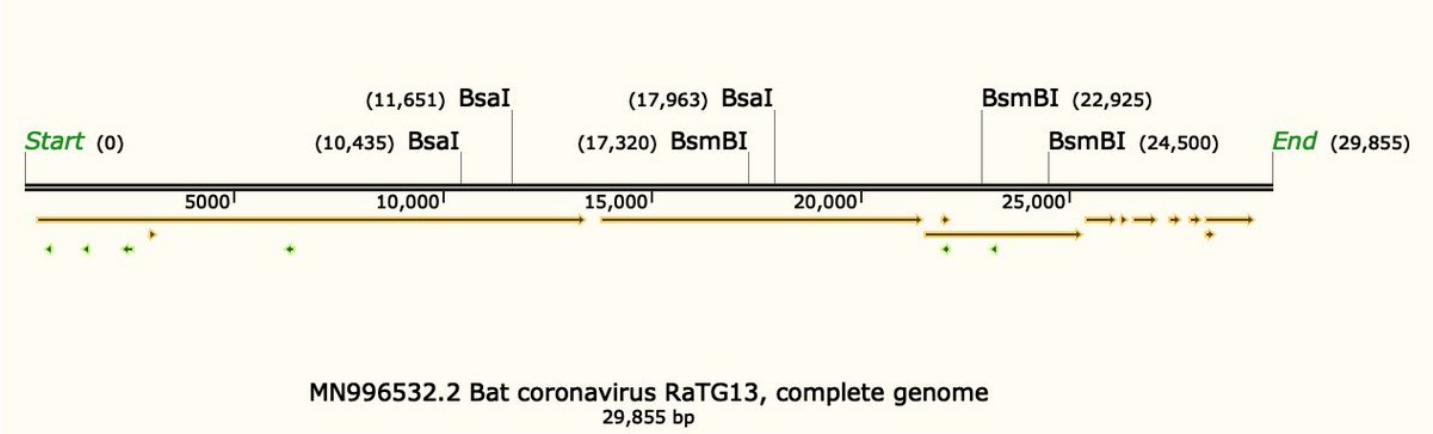
Also, there should be not too many fragments, and fragments should not be longer than ~8 thousand bases.
All these parameters, that do not have any advantage in natural evolution, are miraculously also met by SARS2.
This backbone also enables super easy RBM/FCS manipulations.
5/x



Miraculously, the S1 spike, pangolin-derived RBM, and MERS-like FCS can all be conveniently replaced with simple BsaI digests and new inserts.
Such variants of RaTG13 could be produced for a few $100.
6/x



Lets take a look at RaTG13, the most likely template virus.
The restriction sites are much less irregular, the first fragment would have probably been to big (10.4kb)
Some sites were re-used (doesn't alter the protein sequence), others newly removed/introduced.
smoking gun#4?
7/7



Side note: all sticky ends derived from such a SARS2 digestion are different, which is required for proper reassembly. This in itself is not very unusual given there are 4^4 possible sticky ends.

Side note2: we see 1-2% mutations between RaTG13 and SARS2 on protein level, suggesting that the recombinant RaTG13-PangolinRBM-FCS CoV was subsequently cultured (=tested for infectivity) on human cells/in ACE2 transgenic animals. We could have lost a cleavage site there.

Rescue of bat SARSr-CoVs and virus infectivity experiments

In the current study, we successfully cultured an additional novel SARSr-CoV Rs4874 from a single fecal sample using an optimized protocol and Vero E6 cells [17]. Its S protein shared 99.9% aa sequence identity with that of previously isolated WIV16 and it was identical to WIV16 in RBD. Using the reverse genetics technique we previously developed for WIV1 [23], we constructed a group of infectious bacterial artificial chromosome (BAC) clones with the backbone of WIV1 and variants of S genes from 8 different bat SARSr-CoVs. Only the infectious clones for Rs4231 and Rs7327 led to cytopathic effects in Vero E6 cells after transfection (S7 Fig). The other six strains with deletions in the RBD region, Rf4075, Rs4081, Rs4085, Rs4235, As6526 and Rp3 (S1 Fig) failed to be rescued, as no cytopathic effects was observed and viral replication cannot be detected by immunofluorescence assay in Vero E6 cells (S7 Fig). In contrast, when Vero E6 cells were respectively infected with the two successfully rescued chimeric SARSr-CoVs, WIV1-Rs4231S and WIV1-Rs7327S, and the newly isolated Rs4874, efficient virus replication was detected in all infections (Fig 7). To assess whether the three novel SARSr-CoVs can use human ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (Fig 8).

Side note3:
yes, this is no ultimate proof that SARS2 was build in a lab.
It's yet another BIG smoking gun inconsistant with natural evolution.
I picked a provokative title as virologists seem to be ok with condemning origin theories in @Nature with zero evidence these days.

Side note4:
wow, this has gone viral■.
I do not want to tell anybody how to interpret this.

Still, few personal notes:
-none of this indicates SARS2 was build as a weapon
-the aim of the project may have been understanding pandemics
-warnings were ignored & safety abysmal (BSL2)

-similarly risky research has been done in the US and Europe as well
-it has seen a boom since the pandemic
-it is done mostly unregistered, unregulated and under inappropriate safety conditions
-this mess could be much worse if the technology got into the hands of terrorists
■

Side note5: for specialists:yes, some evidence suggests RaTG13 was manipulted/not the direct template of SARS2. RaTG13 could also be a cousin derived from different passaging experiments with the same, 98-99% RaTG13 identical ancestor (Ra4991?).
https://t.co/efJe7adVFX

> So, in silico, WIV would never make:
> - non-synonymous mutations
> - splicing within genes
> But they could make:
> - synonymous mutations
> - swap genes or the complete genome from other real viruses
>
> — Francisco de Asis (@franciscodeasis) April 16, 2021

1% difference on protein level is not that much.
Delta variant is ~1.5% different in S1 from first SARS2 samples, mutations can accumulate faster during lab passaging.
The reason that we do not know for sure is not the lack of evidence, it's the lack of access to the evidence!