Twitter Thread by Billy Bostickson ■■&■ ■

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Let Uncle Ralph educate you
Synthetic Viral Genomics:
Risks and Benefits for Science and Society

"Alternatively, “No See’m” sites can be used to insert foreign genes into viral, eukaryotic, or microbial genome or vector, simultaneously removing all evidence of the restriction sites that were used in the recombinant DNA manipulation"

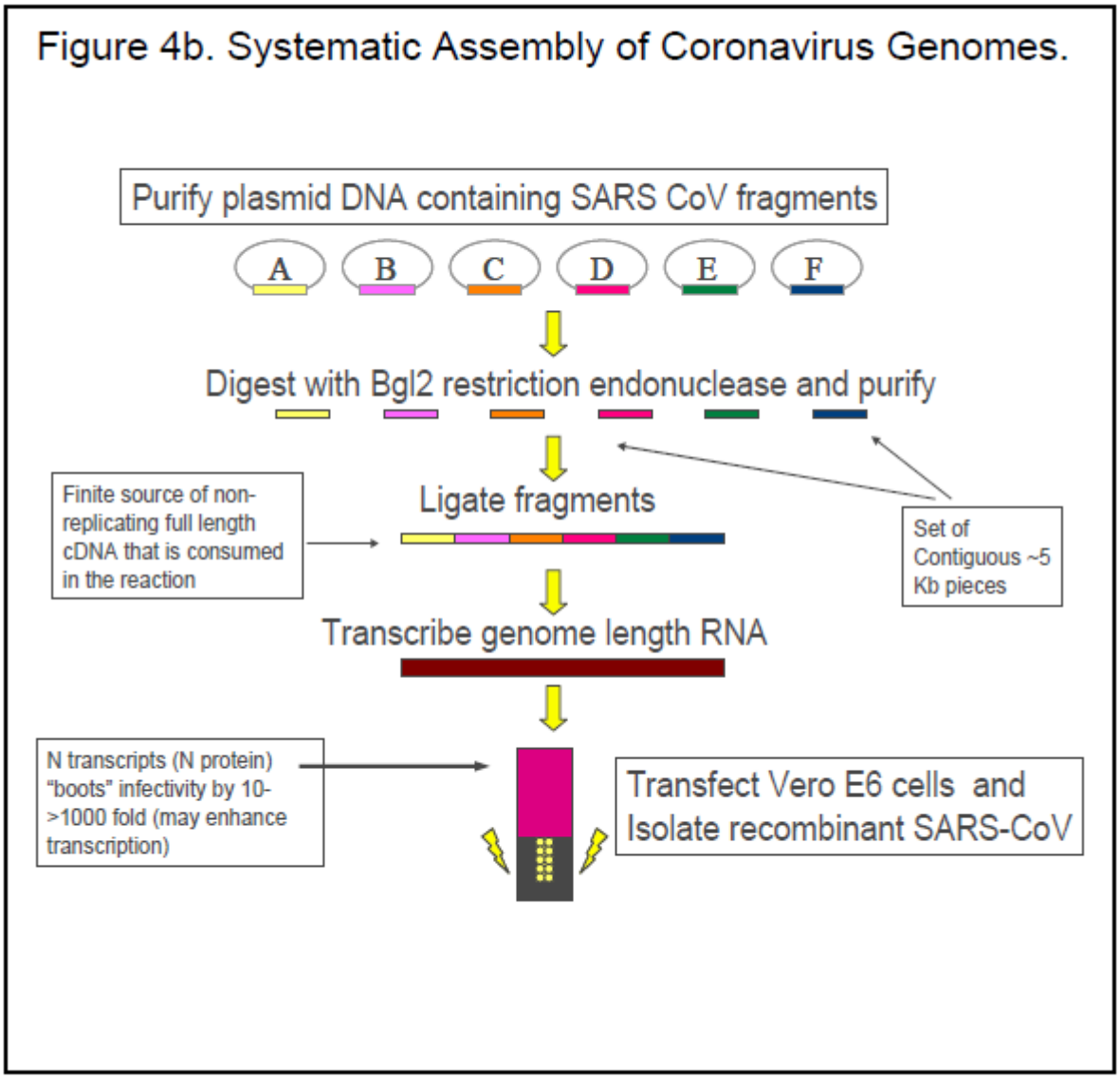
By orientating the restriction sites as “No See’m”, the sites are removed during reassembly, leaving only the desired mutation in the final DNA product.

The dual properties of strand specificity & a variable end overhang that can be tailored to match any sequence allow for Esp3I sites to be engineered as “universal connectors” that can be joined with any other four nucleotide restriction site overhangs (e.g. EcoRI, PstX1, BamH1)

Seamless assembly (also called No See’m Sites (85)) cascades have been used to assemble full length cDNAs of the coronaviruses mouse hepatitis virus, transmissible gastroenteritis virus, infectious bronchitis virus and SARS-CoV (Refs: 85,86,87)

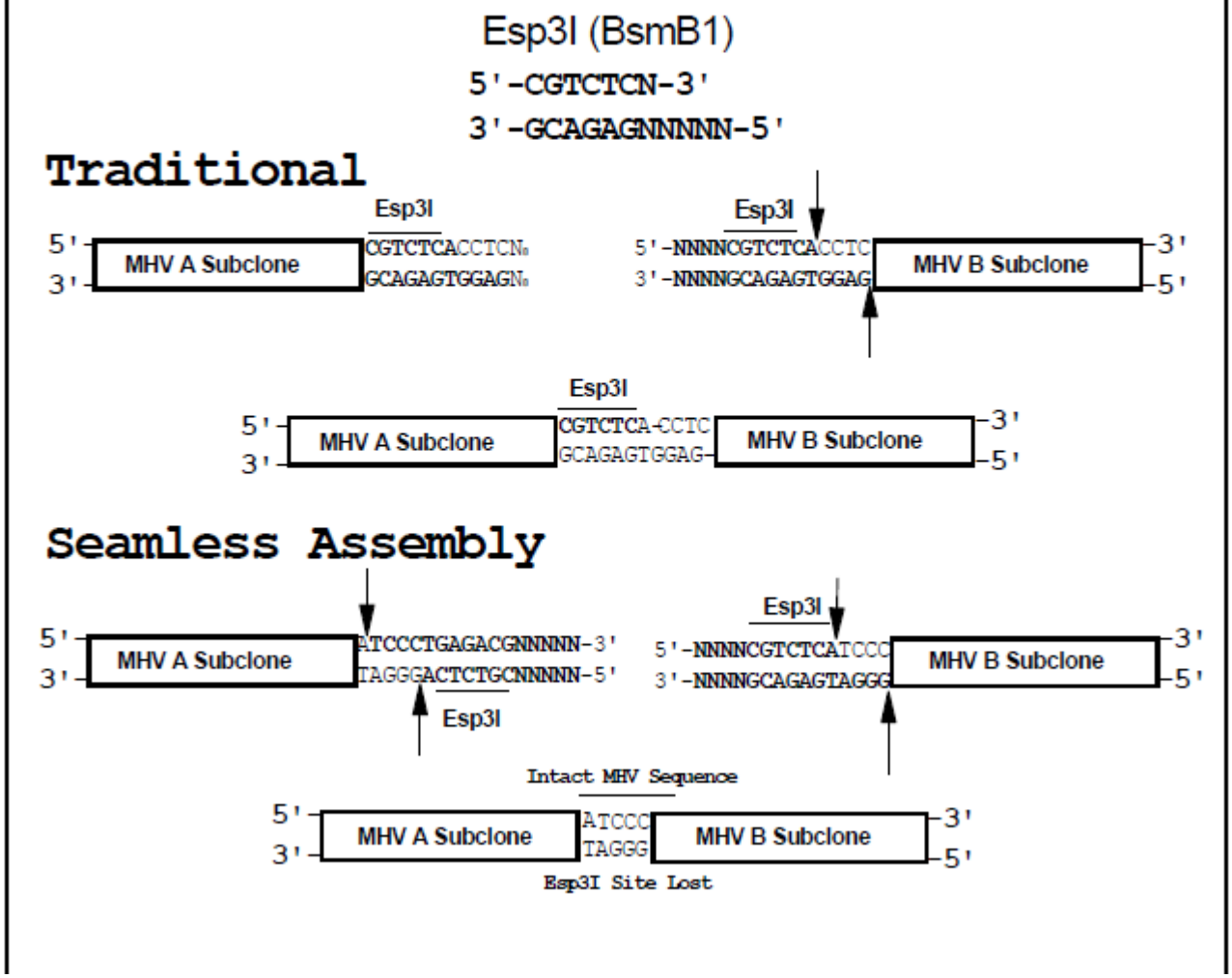
recovery using DNA microarrays. PLoS Biology **1:**257-260.
85. **Yount, B., C. Curtis, and R. S. Baric.** 2000. A strategy for the assembly of large RNA and DNA genomes: the transmissible gastroenteritis virus model. J. Virology **74**.
86. **Yount, B., Denison MR, Weiss, SR and Baric RS.** 2002. Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59. J. Virol. **76:**11065-11078.
87. **Yount, B., K Curtis, E Fritz, L Hensley, PJahrling, E Prentice, M Denison, T Geisbert and R Baric.** 2003. Reverse genetics with a full length infectious cDNA of the severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A **100:**12995-13000.

Type IIS restriction endonucleases recognize asymmetric binding sites & leave asymmetric ends
These enzymes can be used to create unique interconnecting junctions, which can be subsequently removed from final assembly product allowing seamless reconstruction of an exact sequence



With enzymes like Esp3I, interconnecting restriction site junctions can be located at ends of each cDNA & systematically removed during assembly of complete full-length cDNA product

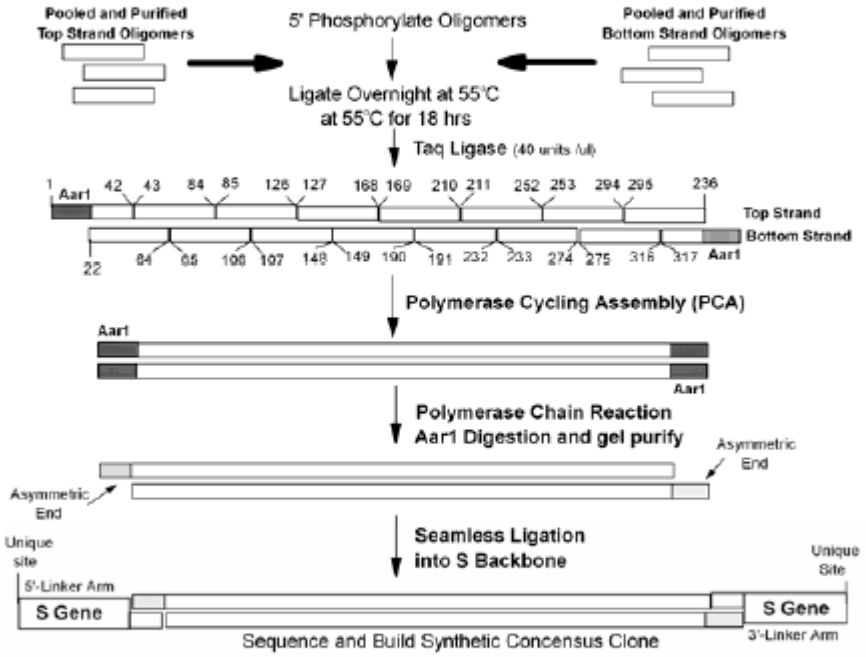
Figure 4a. Systematic Whole Genome Assembly Techniques.



Consequently, knowledgeable experts can theoretically reconstruct full length synthetic genomes for any of the high priority virus pathogens, although technical concerns may limit the robustness of these approaches.

Figure 9. PCA Technique. Synthetic Reconstruction of Exotic SARS-CoV Spike Glycoproteins.

Synthetic S glycoproteins are synthesized and inserted into the SARS-CoV molecular clone; allowing for recovery of recombinant viruses encoding zoonotic S glycoproteins.



Another approach might be to “humanize” zoonotic viruses by inserting mutations into virus attachment proteins or constructing chimeric proteins that regulate virus species specificity (viral attachment proteins bind receptors, mediating virus docking and entry into cells).

Another approach might be to "humanize" zoonotic viruses by inserting mutations into virus attachment proteins or constructing chimeric proteins that regulate virus species specificity (viral attachment proteins bind receptors, mediating virus docking and entry into cells). For example, the mouse hepatitis virus (MHV) attachment protein, the S glycoprotein, typically targets murine cells and is highly species specific. Recombinant viruses contain chimeric S glycoproteins that are composed of the ecto-domain of a feline coronaviruses fused with the c-terminal domain of MHV S glycoproteins targets feline, not murine cells for infection. The pathogenicity of these chimeric coronaviruses is unknown (39). As information regarding the structure and interactions between virus attachment proteins and their receptors accumulate, data will provide detailed predictions regarding easy approaches to humanize zoonotic strains by retargeting the attachment proteins to recognize human, not the animal receptors (43-45). Conversely, it is not clear whether species retargeting mutations will result in viruses that produce clinical disease in the human host.

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"Scapegoat option"
This powerful technique provides bioterrorists with a "scapegoat" option; leaving a sequence signature that misdirects efforts at tracking the true originators of the crime. Even better, the approach could be used to build mistrust &/or precipitate open warfare

Synthetic DNAs and systematic assembly approaches also provide unparalleled power for building genomes of any given sequence, simultaneously providing novel capabilities for nefarious use. For example, genome sequences represent fingerprints that allow geographic mapping of the likely origin of a given virus. Recombinant viruses generated from classic recombinant DNA techniques will carry the signature of the parental virus used in the process as well as novel restriction sites that were engineered into the genome during the cloning process. In contrast, synthetic viral genomes can be designed to be

Dual Use

result after the release of designer pathogens in US cities. Given the reported findings and the large repertoire of host, viral and microbial virulence genes identified in the literature, the most robust defense against the development of designer viral pathogens for malicious use is basic research into the mechanisms by which viral pathogenesis might be manipulated and applied counter measures that ameliorate these pathogenic mechanisms. This justification, however, blurs the distinction between fundamental academic research and bio-weapon development.

Uncle Ralph summarises his findings (1)

4. Summary

Chemical synthesis of viral genomes will become less tedious over the coming years. Costs will likely decrease as synthesis capabilities increase. Moreover, the technology to synthesize DNA and reconstruct whole viral genomes is spreading across the globe with dozens of commercial outfits providing synthetic DNAs for research purposes. DNA synthesizers can be purchased through on-line sites such as eBay. It is likely that engineering design improvements will allow for simple construction of larger genomes. The technology to synthetically reconstruct genomes is fairly straightforward and will be used, if not by the United States, then by other Nations throughout the world. It is also likely that synthetic genes and synthetic life forms will be constructed for improving the human condition and they will be released into the environment. As with most

Uncle Ralph Concludes (2)

Hence Ecohealth DARPA/DTRA spooks & virus thieves collaboration with Baric (UNC) Lipkin (Mailman) NIchols (Atlanta CDC) and USAMRIID (Bavari, Totura et al) & Jonathan Epstein's palpable concern about dual use references in the @USRightToKnow FOIA emails

human condition and they will be released into the environment. As with most technology, synthetic biology contains risks and benefits ranging from a network to protect the public health from new emerging diseases to the development of designer pathogens. Synthetic genome technology will certainly allow for greater access to rare viral pathogens and allow for the opportunity to attempt rationale design of super pathogens. It is likely that the threat grows over time, as technology and information provide for more rational genome design. The most robust defense against the development of designer viral pathogens for malicious use may be basic research into the

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mechanisms by which viral pathogenesis might be manipulated so that applied counter-measures can be developed.

A scary read for an experimental monkey!
Delving into the cold and calculating mind of a twisted genius?
https://t.co/MrwxYK93cx
Bring Uncle Ralph and his transgenic mice in for questioning!
unroll @threadreaderapp

You just read the Baric Paper, one of 6 in a series, the rest are available here in a 191 page document https://t.co/rjNZ8aDNDl

Baric RS. 2006. Synthetic Viral Genomics. In: *Working Papers for Synthetic Genomics: Risks and Benefits for Science and Society*, pp. 35-81. Garfinkel MS, Endy D, Epstein GL, Friedman RM, editors. 2007.

Collett MS. 2006. Impact of Synthetic Genomics on the Threat of Bioterrorism with Viral Agents. In: *Working Papers for Synthetic Genomics: Risks and Benefits for Science and Society*, pp. 83-103. Garfinkel MS, Endy D, Epstein GL, Friedman RM, editors. 2007.

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Jones R. 2005. Sequence Screening. In: *Working Papers for Synthetic Genomics: Risks and Benefits for Science and Society*, pp. 1-16. Garfinkel MS, Endy D, Epstein GL, Friedman RM, editors. 2007.

Sanghvi Y. 2005. A Roadmap to the Assembly of Synthetic DNA from Raw Materials. In: *Working Papers for Synthetic Genomics: Risks and Benefits for Science and Society*, pp. 17-33. Garfinkel MS, Endy D, Epstein GL, Friedman RM, editors. 2007.

Related Papers

1. Synthetic Genomics: Options for Governance (2008) https://t.co/A32jEfL1TQ
2. Sequence Screening - Robert Jones (2005)
https://t.co/zOqMroYbVU
3. Synthetic Biology as a Field of Dual-Use Bioethical Concern - Alexander Kelle
https://t.co/Va1movPqvs

Related papers (2)

4. Sanghvi Y. A Roadmap to the Assembly of Synthetic DNA from Raw Materials.
https://t.co/4kWu4vzcXC
5. Collett MS. Impact of Synthetic Genomics on the Threat of Bioterrorism with Viral Agents.
https://t.co/OcaTmuCUBy

Related Papers (3)

6. Fleming DO. Risk Assessment of Synthetic Genomics: A Biosafety & Biosecurity Perspective.
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7. Risk Governance of Synthetic Biology
https://t.co/9BE9X5s0Fb
8. US Competitiveness in Synthetic Biology

https://t.co/OCZJdsXSjl

Related papers (4)
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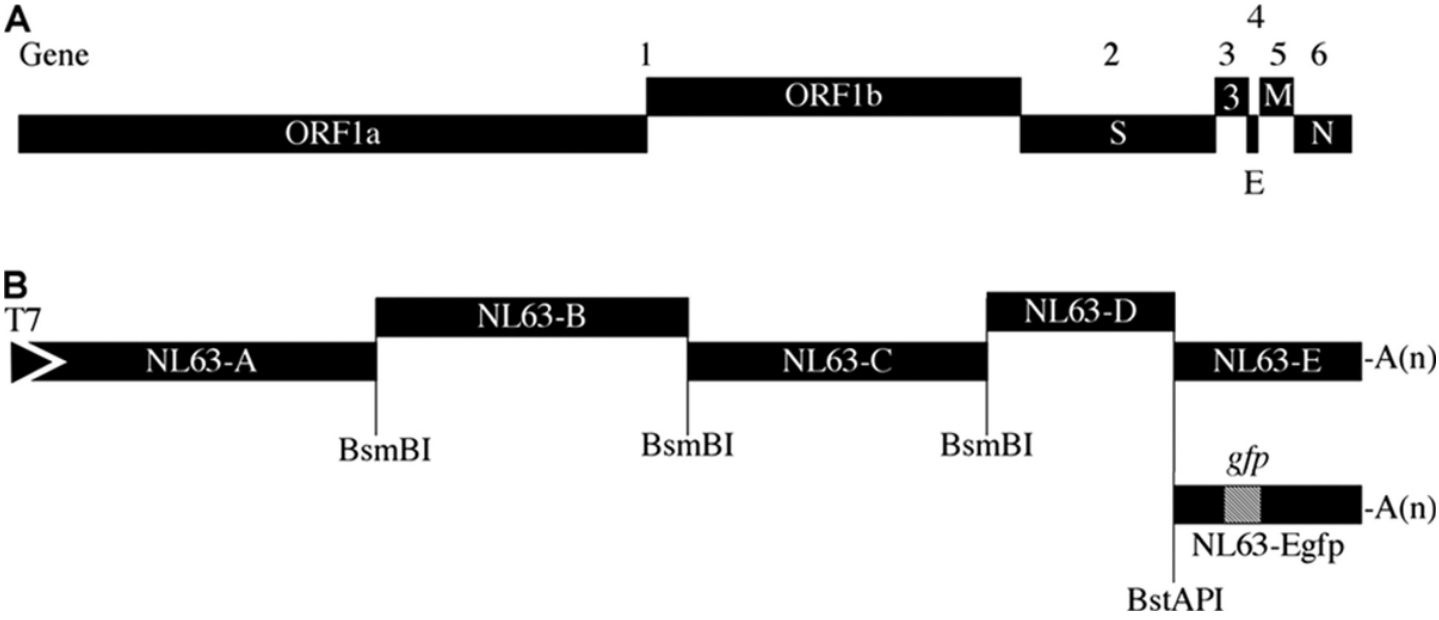
https://t.co/7kPE1OpbrS
10. Synthetic biology: emerging research field in China
https://t.co/T3BcaDvUsa
11.
What rough beast? Synthetic biology, uncertainty,& the future of biosecurity (2016)
https://t.co/XTlBXILEWh

Abstract

Synthetic biology seeks to create modular biological parts that can be assembled into useful devices, allowing the modification of biological systems with greater reliability, at lower cost, with greater speed, and by a larger pool of people than has been the case with traditional genetic engineering. We assess the offensive and defensive security implications of synthetic biology based on the insights of leading synthetic biologists into how the technology may develop, the projections of practicing biosecurity authorities on changes in the security context and potential security applications of synthetic biology, and joint appraisals of policy relevant sources of uncertainty. Synthetic biology appears to have minimal security implications in the near term, create modest offensive advantages in the medium term, and strengthen defensive capabilities against natural and engineered biological threats and enable novel potential offensive uses in the long term. To maximize defensive and minimize offensive effects of synthetic biology despite uncertainty, this essay suggests a combination of policy approaches, including community-based efforts, regulation and surveillance, further research, and the deliberate design of security and safety features into the technology.

Back to Baric et Al
Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus (2003)
https://t.co/k8PI1rbcm8
Systematic Assembly of a Full-Length Infectious Clone of Human Coronavirus NL63 (2008)
https://t.co/bWhAv1wsC0



For the Record (WIV)
In 2016, Shi and her team at the WIV, in conjunction with the New York-based EcoHealth Alliance, constructed a full-length clone of a bat coronavirus called SL-CoV WIV1. They assembled it in discrete segments.

They genetically engineered the virus using the pGEM®-T Easy Vector Systems to join the segments. This system, also available on the internet, gives researchers several options for how to remove GM inserts that can be seen as signatures of a lab-made virus.18

pGEM®-T Easy Vector Systems:
“Thus, several options exist to remove the desired insert DNA with a single restriction digestion.”
This shows that researchers at the WIV have the ability to genetically engineer viruses and remove the signatures of the genetic engineering.

WIV & EcoHealth Alliance published a paper in 2017 on how they genetically modified spike proteins of 8 bat coronaviruses, by cutting & pasting genetic material from other coronaviruses, so that the viruses infected the human ACE2 receptor

They used pGEM®-T Easy Vector Systems to join the segments to genetically engineer these viruses.
They showed how they can insert new spikes into viruses. The researchers state:
“Then any spike could be substituted into the genome of SARSr-CoV WIV1 through this strategy.”

This shows that researchers at WIV have the ability to genetically modify multiple coronaviruses to insert new spikes, and these new viruses cannot be detected as genetically engineered.

The research clearly shows that GOF researchers at WIV could assemble SARS-CoV-2 from bat coronaviruses, such as RaTG13 or similar & spike protein from Malayan Pangolins & insert multi-basic cleavage sites into precise regions of spike & leave no evidence of genetic engineering.

All the above about WIV research is taken verbatim from an excellent article by Andre Leu on the website of @OrganicConsumer
COVID 19: The Spike and the Furin Cleavage

https://t.co/bDOMmhpJ1t