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COVID-19

Scientists say EcoHealth Alliance's DEFUSE proposal was a blueprint for SARS-CoV-2

BY ANNETTE GARTLAND ON JANUARY 19, 2024

The investigative research group 'U.S. Right to Know' (USRTK) has made public the full batch of documents it obtained about the EcoHealth Alliance's 'Defusing the Threat of Bat-borne Coronaviruses' (DEFUSE (https://drasticresearch.files.wordpress.com/2021/09/main-document-preempt-volume-1-no-ess-hr00118s0017-ecohealth-alliance.pdf)) grant proposal that the EHA submitted to the Defense Advanced Research Projects Agency (DARPA) in the US in March 2018.

The release of the 1,417-page file (https://usrtk.org/wp-content/uploads/2024/01/USGS-DEFUSE-2021-006245-Combined-Records_Redacted.pdf) of documents obtained via a Freedom of Information request follows USRTK's earlier release of 235 pages on December 18 last year.

The file contains early drafts of the DEFUSE proposal, which was made to DARPA under the umbrella of the PREventing EMerging Pathogenic Threats (PREEMPT) programme. The documents also include emails about the proposal.

The release of the full batch of documents yesterday (January 18) by USRTK followed the sharing on X (Twitter) earlier in the day, by a person who goes by the name of 'Gumby', of the same batch of documents, which 'Gumby' obtained via a separate Freedom of Information (FOI) request.

Both FOI requests were made to the U.S. Geological Survey (USGS), which is one of the organisations involved in submitting the DEFUSE proposal.

'Gumby' said he made his FOI request because USRTK had only released a portion of the documents it had obtained.

After 'Gumby' scooped USRTK, it rushed to publish its full batch of documents and an article about those documents by its reporter Emily Kopp.

According to leaked documents (https://changingtimes.media/2021/09/22/drastic-investigating-team-releases-leaked-documents-about-gain-of-function-coronavirus-research/) made public by the investigative group DRASTIC in September 2021, the EHA requested in its proposal a total $14,209,245 over 3.5 years ($8,411,546 for phase 1 and $5,797,699 for phase 2).

The EHA proposed injecting chimeric bat coronaviruses collected by researchers at the Wuhan Institute of Virology (WIV) into "batified" mice and humanised mice genetically altered to express the human ACE-2 receptor.

"Batified" mice are mice that have been irradiated and injected with bat bone marrow.

The DEFUSE proposal includes discussion about the planned introduction of human-specific cleavage sites into bat coronaviruses.

Richard H. Ebright, a microbiologist working at Rutgers University, who is a member of the leadership team at the NGO Biosafety Now (https://biosafetynow.org/), tweeted on January 19: "The 2018 EcoHealth proposal provided step-by-step plans for construction of a virus having the sequence and properties of the virus that emerged a year later in Wuhan: SARS-CoV-2.

"The four years of lies by EcoHealth and its associates need to end now."

Ebright tweeted on January 18: "An order line for BsmBI in a draft of EcoHealth's 2018 DARPA proposal is the equivalent of a smoking gun.

> "There is no–zero–remaining room for reasonable doubt that EcoHealth and its associates caused the pandemic."

Ebright was referring to the BsmBI restriction enzyme that can be used in cloning.

"As a Type-IIS restriction endonuclease, BsmBI can be used in both seamless and non-seamless directional-cloning strategies," he tweet

Item	Manufacturer	Number/Descr	Unit Price
Restriction Enzymes small tubes	NE BIO LABS	R0580S	$72.00
Restriction Enzymes large tubes	NE BIO LABS	R0580L	$292.00

Lines that appear in the projected budget of Tonie Rocke, a USGS collaborator on the DEFUSE project. While Rocke was set to collaborate with Baric, it's not clear that she was central to this genetic engineering work, Emily Kopp says.

Writer and lecturer Jan Hommel, who, pre-Covid-19, used to work as a neurologist, tweeted his view of the relevance of the proposed BsmBI purchase.

The finding of the purchasing proposal in the DEFUSE documents was like finding a gun with the fingerprints of the perpetrator in a murder, Hommel tweeted.

Bioengineer and immunologist Valentin Bruttel says the DEFUSE draft documents (*excerpt below*) show that the EcoHealth Alliance planned to use six segments to assemble synthetic viruses, "to use unique endonuclease sites that do not disturb the coding sequence, and to buy BsmBI".

PMC2583659, PMC3791741). We will identify the best consensus candidate and synthesize the genome using commercial vendors (e.g., BioBasic, etc.), as six contiguous cDNA pieces linked by unique restriction endonuclease sites that do not disturb the coding sequence, but allow for full length genome assembly. Full length genomes will be transcribed into RNA and electoration is used to recovery full length recombinant viruses (PMC3977350, PMC240733). Using the full length genomes, we will re-evaluate virus growth in primary human airway epithelial cells at low and high multiplicity of infections and in vivo in hACE2 transgenic mice, testing whether backbone genome sequence alters full length SARSr-CoV pre-epidemic or pathogenic potential in models of human infection. All experiments are performed in triplicate and the data provided to the Modeling Team for the development of risk assessment models, warfighter apps, and models to evaluate potential intervention outcomes. We anticipate recoverying 2-5 full length genomes/yr, reflecting strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis.

Emily Kopp said in her report (https://usrtk.org/covid-19-origins/scientists-proposed-making-viruses-with-unique-features-of-sars-cov-2-in-wuhan/) for USRTK that SARS-CoV-2 emerged highly infectious without evolving much in humans.

"The virus 'came out of the box ready to infect'," she wrote. "The receptor binding domain appeared 'finely tuned' for the human ACE2 receptor yet had little genetic variation when first spilling over into humans, presenting a difficult 'paradox' (https://usrtk.org/covid-19-origins/visual-timeline-proximal-origin/) to virologists who sought to prove it emerged naturally."

The documents obtained by USRTK confirm that the scientists working with the Wuhan lab sought to select for receptor binding domains (RBDs) that bind well to human ACE-2 in their research, she said.

"The genome of SARS-CoV-2 falls within the range of a 25 percent genetic difference (https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(21)00174-9/fulltext) from SARS," Kopp added.

Kopp said that the documents USRTK obtained revealed for the first time that a virologist working with the Wuhan lab planned to engineer new spike proteins – in contrast with the collaboration's public work to insert whole spike proteins into viral backbones.

"Language in the proposal indicates this work may have involved unpublished viruses, generating unpublished engineered spike proteins," she wrote.

Bruttel says that, in his opinion, the DEFUSE proposal has always been "a precise blueprint for how SARS2 came into existence".

The draft documents obtained by USRTK contain "further damning details", he tweeted on January 18.

> "IMO the lab origin of SARS-CoV-2 is proven beyond reasonable doubt," he added.

Bruttel, Alex Washburne, and Tony VanDongen published a preprint (https://www.biorxiv.org/content/10.1101/2022.10.18.512756v1) in October 2022 (updated (https://www.biorxiv.org/content/10.1101/2022.10.18.512756v2) in April 2023) in which they stated that the SARS-CoV-2 genome contained a pattern of restriction sites typically found in synthetic viruses, but not in related natural viruses.

They explained that, to construct synthetic variants of natural coronaviruses in the lab, researchers often used a method called in vitro genome assembly.

> "This method utilizes special enzymes called restriction enzymes to generate DNA building blocks that then can be 'stitched' together in the correct order of the viral genome," they said.
>
> "To make a virus in the lab, researchers usually engineer the viral genome to add and remove stitching sites, called restriction sites. The ways researchers modify these sites can serve as fingerprints of in vitro genome assembly."
>
> Washburne et al. added: "The genome of SARS-CoV-2 contains a peculiar pattern of unique restriction endonuclease recognition sites allowing efficient dis- and re-assembly of the viral genome characteristic of synthetic viruses."

The three scientists postulated that SARS-CoV-2 was assembled from six fragments using BsmBI for the backbone and the Bsal restriction endonuclease for the RBD or furin cleavage site (FCS) variants and that these restriction sites were introduced with, abnormally, many synonymous mutations.

VanDongen tweeted on January 18: "One of the main (obviously unfounded) critiques of our preprint was: 'Why only focus on BsmBI and Bsal?'.

"We explained how these enzymes are the go-to guys for CoV reverse genetics systems and were favored by labs like WIV. Now we have DEFUSE buying BsmBI. Sweet …"

An FCS is a segment of four amino acids that enables a virus to use furin in the human body as an enzyme to dissolve its coating so that it can release its genetic material to infect cells. Furin cleavage sites tend to be more infectious than cleavage sites that use other enzymes.

Bruttel explains that a furin cleavage site enables coronaviruses to infect new hosts and to cause severe diseases as it enables infections of neurons.

"Virologists including [Ralph] Baric and Shi [Zhengli] introduced FCSs into all sorts of dangerous coronavirus spike proteins," he tweeted.

Shi Zhengli is a senior scientist at the Wuhan Institute of Virology. Ralph Baric is in charge of coronavirus research at the University of North Carolina (UNC) at Chapel Hill in the US.

DEFUSE did not receive DARPA funding, Bruttel says, but researchers sometimes start projects before the funding is approved, and very similar research projects at the WIV were funded by US and Chinese institutions.

Making synthetic viruses is also very cheap, he adds. "About $6000 are enough according to R. Baric," he tweeted.

The spike protein of SARS-CoV-2 comprises two sub-units, S1 and S2. S1 recognises and binds to ACE-2 and S2 mediates viral cell membrane fusion. The FCS of the virus is positioned at the S1/S2 junction.

The question that has been posed is 'Did the site evolve naturally or was it inserted at that junction by researchers?'. There has been repeated gain-of-function research by scientists who have experimented in the past with engineering an FSC to the S1/S2 junction.

Some virologists have argued that only FCSs in the S2 region of the spike protein are mentioned in the DEFUSE proposal, Bruttel says, but the draft proposal in fact mentions an FCS exactly at the S1/S2 boundary.

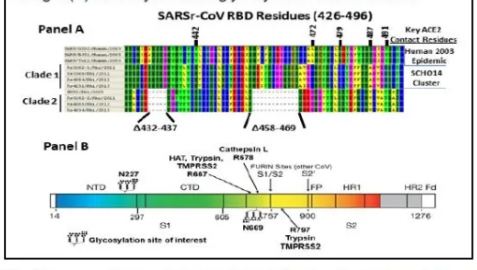
It is stated in an early draft: "In some instances, tissue culture adaptations introduce a furin cleavage site, which can direct entry processes as well, usually by cleaving S at positions 757 and 900 in S2 of other coronaviruses, but not SARS."

Position 757 is the S1/S2 site and position 900 is the S2 site.

The following excerpt from an early draft of the DEFUSE proposal refers to an FCS at the S1/S2 boundary.

TA1.g. Evaluating 2^{ary} S gene Markers for SARSr-CoV Pre-epidemic Potential. 1) ***Identification of high risk/low abundant variants***. RNAseq will identify low abundant quasispecies variants that encode mutations in the RBD and/or residues that bind ACE2 receptor (Fig A). Low abundant mutations, especially in RBD residues that interface with ACE2 receptors, would alter risk assessment calculations as strains identified as low risk, might actually encode high risk, but low abundant variants. To test this hypothesis, we will closely with the modeling core and Dr. Shi's laboratory to identify highly variable residue changes in the SARSr-CoV S RBD, and use commercial gene blocks to introduce these changes singly and then in combination into the S glycoprotein gene of the parental low risk, high abundant strain parent. We will evaluate the ability of these low abundant chimeric viruses to use human, bat, civet and mouse ACE2 receptors, and more importantly, replicate efficiently in human primary cells. 2) ***Impact of RBD deletions on Pre-epidemic Risk Assessment***. SARSr-CoV RBD sequences fall into two larger clades, heavily defined by the presence of small deletions between residues 432-437 and 458-472, which leave the key RBD-ACE2 interface residues intact. (**Fig C**). To improve risk assessment, we will molecularly analyze the functional consequences of these deletions on SARSr-CoV human ACE2 receptor usage, growth in primary cells and in vivo pathogenesis. First, we will delete these regions, sequentially and then in combination, in SCH014, anticipating that the introduction of both deletions will prevent SCH014 growth in Vero and human cells. We also

Figure C. Additional Markers for High/Low Risk Strain Identification. (**A**). Clade 2, but not Clade 1 SARSr-CoV encode programmed deletions which likely impact ACE2 receptor usage. (B) Proteolytic and N-glycosylation sites of interest.



The EHA's final proposal includes the introduction of "human-specific cleavage sites" into bat coronaviruses.

"Human protease-specific site insertion was proposed," DRASTIC said when it released the leaked documents in 2021.

"The proposal does not specify exactly which protease, but does discuss furin in the preceding text."

> *"We will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential Furin cleavage sites[74,75]".*
>
> *SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous Trypsin or Cathepsin L.*
>
> *Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures."*
> (D1, p.13)

HAE (human airway epithelial) cultures effectively mimic the human bronchial environment.

USRTK says Ralph Baric was set to engineer twenty or more "chimeric" SARS-related viral spike proteins per year of the proposal, and two to five full-length engineered SARS-related viruses.

Jan Hommel referred in his comments on X (Twitter) to the preprint published by Bruttel, Washburne and VanDongen on bioRxiv.

There were a number of cutting sites in the genome of SARS-CoV-2 that ensured that the virus could be cut into pieces making it possible to easily exchange them with another gene, as if it were a module, he said.

"Moreover, these cutting sites were much more regularly distributed across the genome than in comparable viruses such as BANAL52 and RaTG13."

This, Hommel said, would fit well with a 'reverse genetics' system, which allowed one to quickly and easily create an artificial virus "because you could easily exchange each of those six pieces for another piece from a different virus", including the part containing the RBD and the FCS.

> "The latter is unique to SARS-CoV-2 and does not occur in any other sarbecovirus," Hommel added. "The entire makeup of SARS-CoV-2 could very well fit a 'reverse genetics system'."

Washburne et al. said in their preprint: "The type of mutations (synonymous or silent mutations) that differentiate the restriction sites in SARS-CoV-2 are characteristic of engineering, and the concentration of these silent mutations in the restriction sites is extremely unlikely to have arisen by random evolution. Both the restriction site fingerprint and the pattern of mutations generating them are extremely unlikely in wild coronaviruses and nearly universal in synthetic viruses. Our findings strongly suggest a synthetic origin of SARS-CoV-2."

They say the restriction map of SARS-CoV-2 is consistent with many previously reported synthetic coronavirus genomes, meets all the criteria required for an efficient reverse genetic system, "differs from closest relatives by a significantly higher rate of synonymous mutations in these synthetic-looking recognitions sites", and has a synthetic fingerprint unlikely to have evolved from its close relatives.

> "We report a high likelihood that SARS-CoV-2 may have originated as an infectious clone assembled in vitro," the researchers wrote.

The head of research and development at the Human Genome Project at the Whitehead Institute/Massachusetts Institute of Technology, Kevin McKernan, tweeted on January 19: "Many people don't want to talk about lab leaks as they see it as part of the narrative and the psyop. But the final evidence conclusively nailing this as a lab origin dropped yesterday. The DEFUSE proposal has listed part numbers for the very enzymes @VBruttel @tony_vandongen @WashburneAlex predicted."

EHA president Peter Daszak and Ralph Baric concealed from the Pentagon their intention to conduct high-risk coronavirus research in Wuhan under lax safety standards, USRTK says.

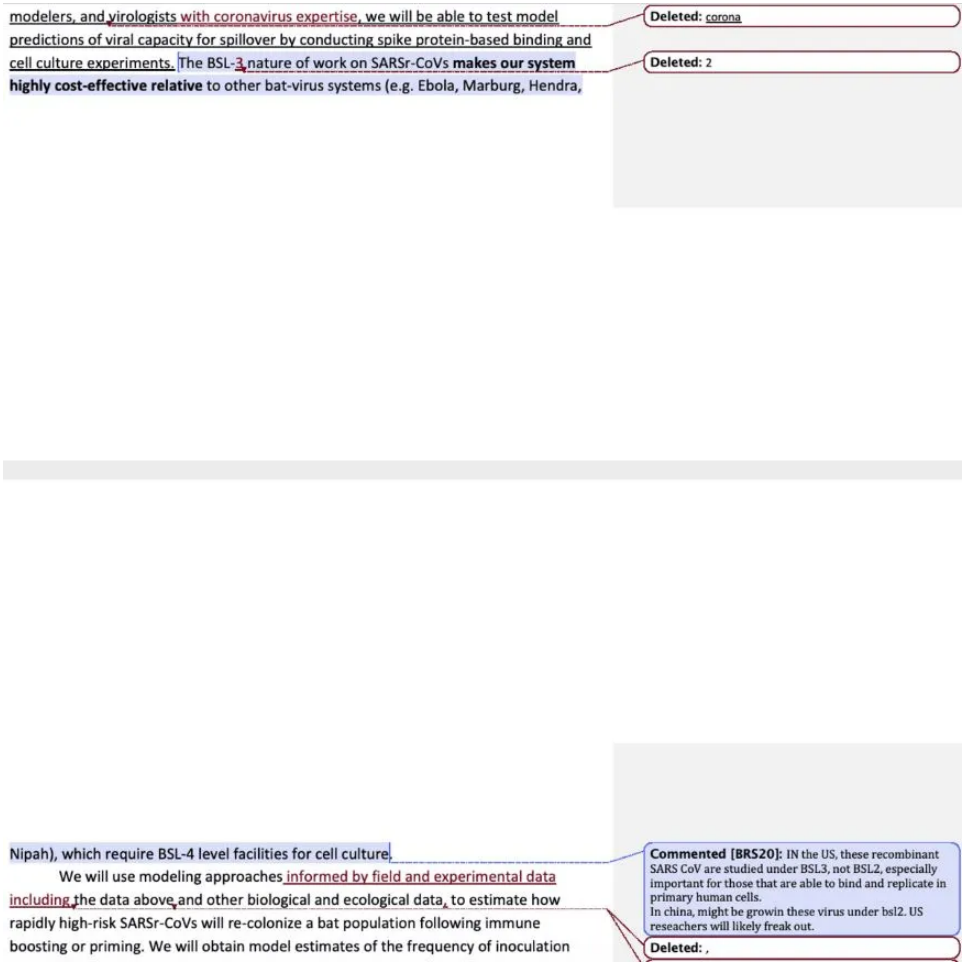
The early draft of the DEFUSE proposal contains comments from "PD" and "BRS" and emails show that PD is Daszak and BRS is Baric.

USRTK says the draft shows that the EHA proposed carrying out DEFUSE experiments in Wuhan with fewer safety precautions than are required in the US – apparently to save on costs.

It is stated in an early draft of the DEFUSE proposal that the engineering and testing of novel coronaviruses would occur in biosafety level two (BSL-2) conditions.

BSL-2 was, however, later edited to BSL-3.

In a comment on the document, Baric said US researchers would likely 'freak out' if they knew the novel coronavirus engineering and testing work would be conducted in a BSL-2 lab.


modelers, and virologists with coronavirus expertise, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-3 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra,

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Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches informed by field and experimental data including the data above and other biological and ecological data, to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation

Commented [BRS20]: IN the US, these recombinant SARS CoV are studied under BSL3, not BSL2, especially important for those that are able to bind and replicate in primary human cells.
In china, might be growin these virus under bsl2. US reseachers will likely freak out.

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In the final DEFUSE proposal it is stated: "Experimental work using bats and or transgenic mice will be conducted at the BSL-3 lab in WIV, Duke-NUS, UNC, or NWHC [the USGS's National Wildlife Health Center]."

It is also stated that live bats would be used at the WIV and labs in Singapore and the US for infection experiments, often using captive bat colonies.

Notes in the newly released documents show that Ralph Baric engineered spike proteins that do not appear in the public scientific literature, "and that this work may have already been underway as the proposal was submitted to DARPA", Emily Kopp writes.

The following is stated in the documents:

- Another idea is...if you build chimera that broadly reduces heterogeneous pop. of SARSr-CoVs in bat cage, this might be something you'd want to develop for humans.
  - RB has already generated SARS-like chimeras w/ RBD from group of bat viruses called 293 (for S1), which is 20% different than epidemic strains, and S2 region from HK3 which is 20% diff.
    - Can drive broad-based rsponese for large no. of family members (but we can test those)
    - **Detailed sequence analysis could be used to engineer broad -based vaccinations for humans**

"While HKU3 bat viruses are known, the reference to 'bat viruses called 293' is ambiguous, and does not appear to refer to any public group of viruses," Kopp writes.

Kopp says the newly released documents challenge an argument made by the National Institutes of Health and some virologists against the relevance of the research proposal to the origins of the pandemic.

"They have argued that this U.S.-China scientific collaboration only planned to engineer viruses starting with viral backbones already in the public literature, and that these viral backbones are too dissimilar to have played a role in the pandemic," she wrote.

"The new documents however reveal that the scientists planned to use new reverse genetics systems and test viruses *in vivo* — in other words, to engineer live novel viruses.

"The documents describe the SARS-related viruses to be studied in the grant as posing 'a clear-and-present danger of a new SARS-like pandemic'."