Addressing the Yan report

In September 2020, the above claimed to **be** scientific evidence for SARS-CoV-2 being an engineered bioweapon 😰 Zenodo granting it a Digital Object Identifier (DOI) made the report appear credible, despite a lack of peer review.

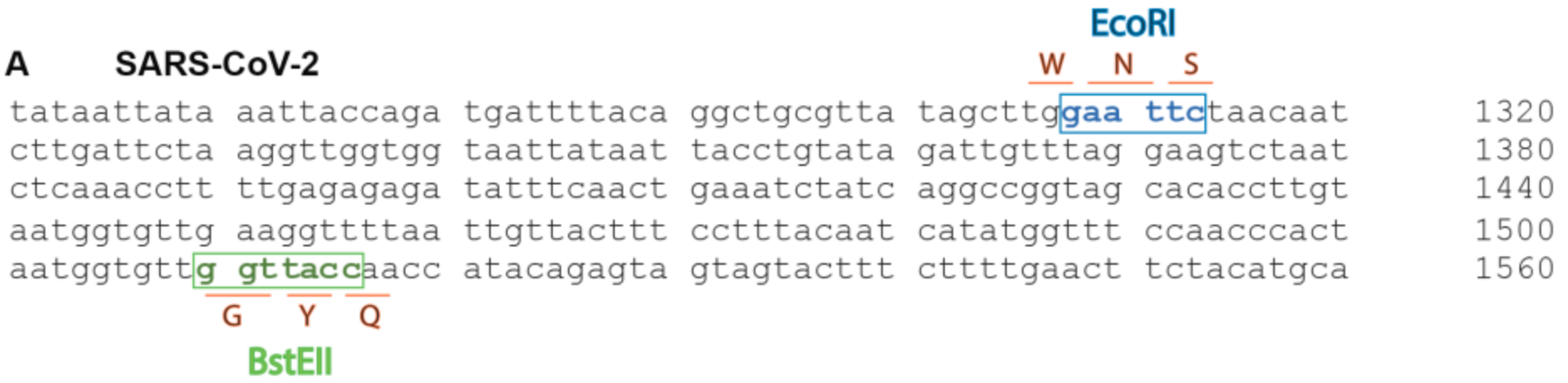
The Johns Hopkins University made up for that lack the same month, explaining why the report was unconvincing. But conspiratorial audiences 🤨🤨 value neither authority, nor being pointed to tonnes of reading material (can't blame them for the latter 😄).

They do value critical thinking, which only requires that **the core claim** be verified. Should it prove false, everything else can be dropped 🤦 What was the report's postulate, then, that sufficed for its authors to be able to seek asylum in 🇺🇸?

Restriction enzymes around the spike's receptor binding motif

Mikolaj Raszek, PhD, was kind enough to elucidate, in *SARS-CoV-2 coronavirus origins alternative theories – do they hold up against science?*, the core claim of the Yan report.

Two **restriction enzymes** (sequences bacteria use to slash viruses to bits, repurposed by humans to glue parts of different genomes together): EcoRI and BstEII. According to Yan et al, the sequence between them allowed to target mammals larger than 🦇🦇.



Download the earliest known SARS-CoV-2 genome ⬇️ (1 of 2)

Yan et al's image caption cites the isolate **Wuhan-Hu-1** (isolate: a population of organisms having little genetic mixing with other organisms of the same species).

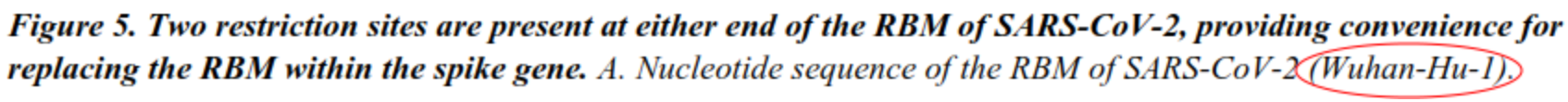


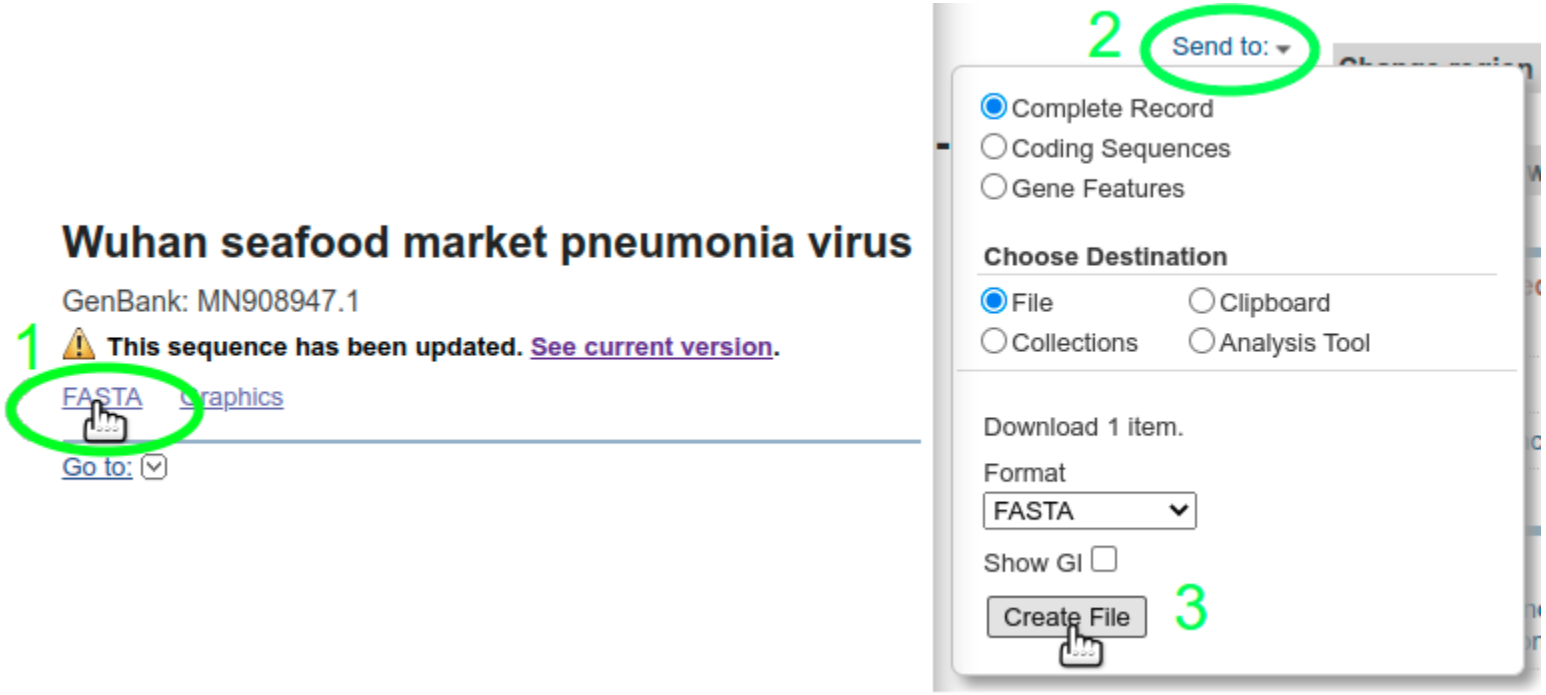
Figure 5. Two restriction sites are present at either end of the RBM of SARS-CoV-2, providing convenience for replacing the RBM within the spike gene. *A. Nucleotide sequence of the RBM of SARS-CoV-2 (Wuhan-Hu-1).*

Viewing the isolate at *NCBI Virus*, the absolutely earliest accession (unique sequence identifier) is MN908947.1, collected in Dec 2019 ➡️ submitted 2020-01-05 ➡️ released 2020-01-12.

That's 2 months until the World Health Organization would declare COVID-19 a pandemic ☣️ (2020-03-11).

Download the earliest known SARS-CoV-2 genome ⬇️ (2 of 2)

In the accession page, switching to the FASTA format (a text format often used for storing reference genomes) allows us to download the troublemaker's genome:



~30k bases (a base is one of `A, C, G, T`) long? What a tiny genome. A human one is 3.1 billion bases, with a single cell taking up between 3.3 GB (reference genome, a measurement standard) and 70 GB (non-reference genome) of your hard drive 🙈

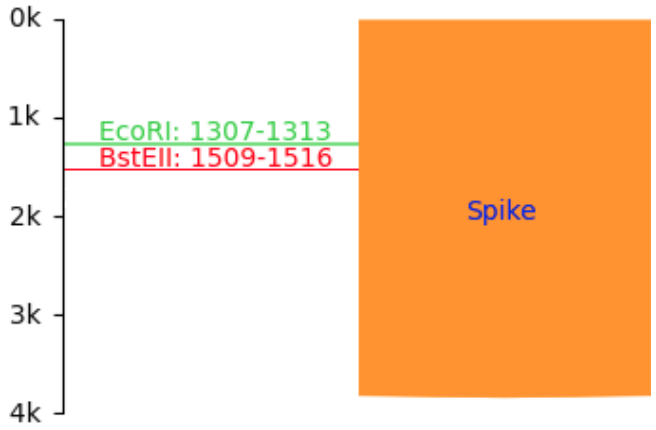
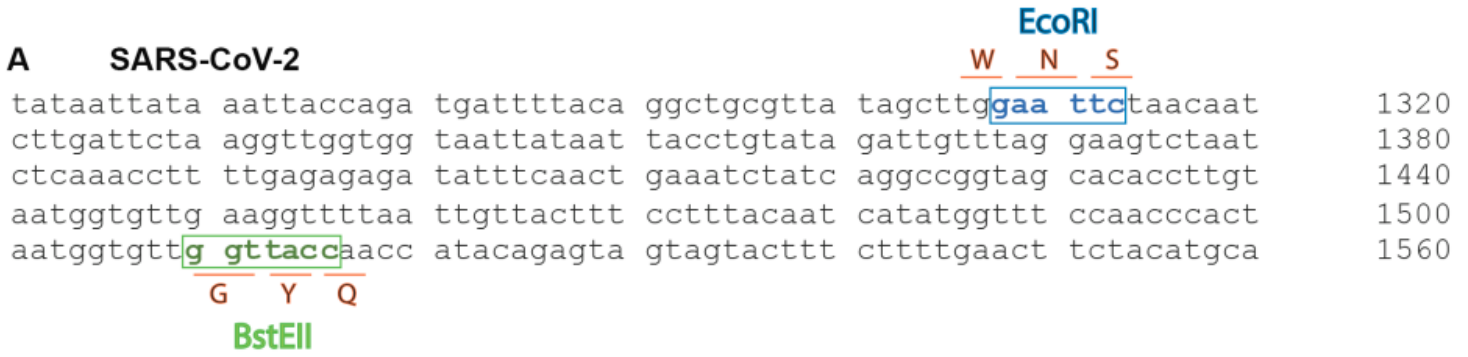
Are EcoRI and BstEII *actually there*? 🔍👀

- **Note:** Sequences identical to those listed **needn't** necessarily come from restriction enzymes - but let's simplify and humour that notion 👌👩

You can open the downloaded SARS-CoV-2 genome in a text editor 📋, and search (`Ctrl+f` / `Cmd+f`) for the occurrences of the EcoRI sequence **GAATTC** yourself. If you fancy a dopamine rush, **stop reading and go ahead now** 😁

The **N** (= whichever base) in BstEII's **GGTNACC** is a tad more problematic, though. If you can locate *regular expression mode* (look for a button marked `.*`) 🤞, this hurdle can be cleared by inputting **GGT[ACGT]ACC**.

Plotting EcoRI & BstEII sequence matches in the spike gene

MN908947.1 spike coordinates	Yan et al's spike coordinates
	

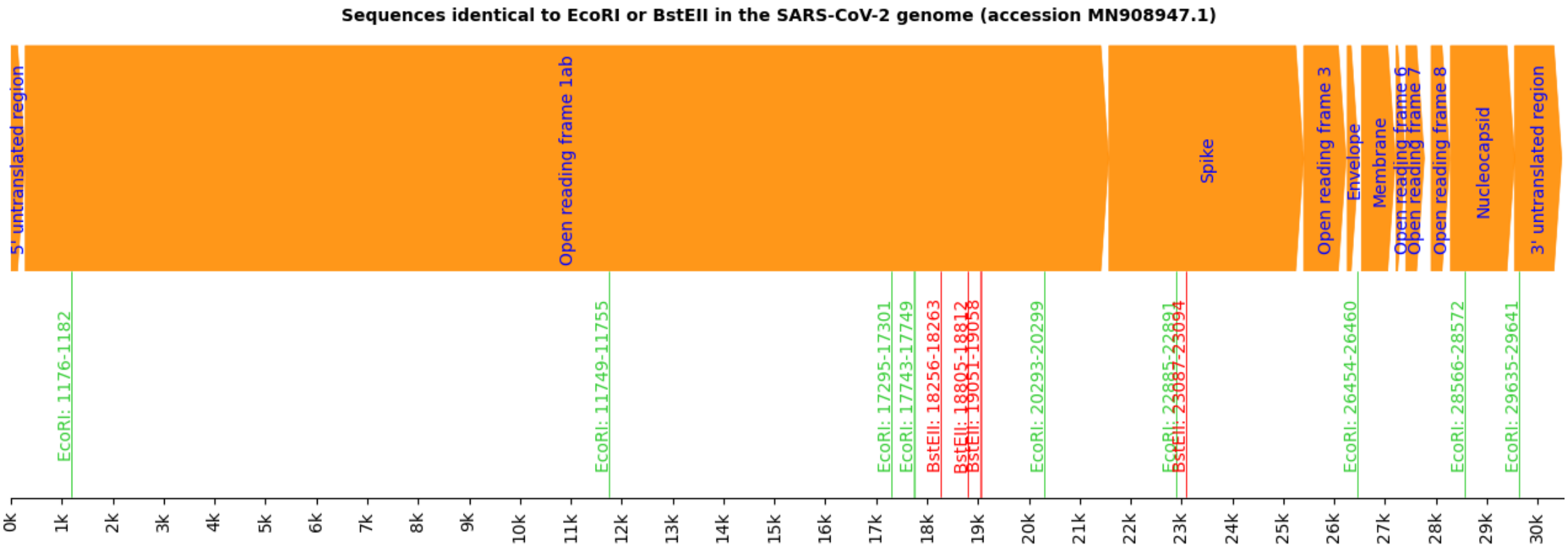
The accession MN908947.1 spike gene **does** contain sequence occurences with 100% identity to EcoRI & BstEII, and that's at the **exact** coordinates specified by Yan et al 🎯

So far so good - let's look at the rest of the genome 🔍

Plotting EcoRI & BstEII matches across the whole genome

But looking at **all the genes** (instead of just the spike), one seems to find more 'genetic modifications' than Yan et al bargained for 🤔

There's even an EcoRI match in the 3' untranslated region (nothing there ever becomes live proteins, hence there's no point in engineering the region).



A restriction enzyme cornucopia? 🦄 Let's find out 🤦

Bioinformatics Algorithms: An Active Learning Approach gives the formula (search for `approximation`) for approximating the likelihood that a **k-mer** (word of size k) occurs in a text *by random chance alone* 🎲

The **lower** ⬇️ that likelihood, the **more probable** ⬆️ any bioengineering 🧬✂️🧬 Customarily, values with `< 5%` chance of being randomly generated, are worthy of investigation.

Click here for the Python version of the approximation formula 🐍. Its code's been tested, so should be reliable. Let's take it for a spin 🧶🐱

⚔️ Theory vs practice: probabilities along the full genome 🧬

1️⃣ A nice property of our approximation formula: if we seek the probability of **just a single occurrence**, any returned number `> 1.0` is the **expected occurrence count**.

2️⃣ BstEII's middle character (GGT**N**ACC) can be anything, so BstEII is considered to have length 6 (the same length as EcoRI), instead of 7.

Restriction enzyme	Expected occurrences	Actual occurrences
EcoRI (**GAATTC**)	7.44	9 (*...are Yan et al onto something?*)
BstEII (**GGT_ACC**)	7.44	4 (*...no they aren't*)

No conclusive evidence either way yet 👱‍♀️ Let's concentrate on the spike 👀

Occurrence probabilities within the spike gene 📌

The accession page informs us that the range of the `"S"` gene is `21579..25400`, which makes for a length of `3821`. Plugging this text length into our formula 🔌, we get:

```
In [3]: ProbabilityOfKmerOccurringNTimesInText(alphabet_size=4)(
   ...:     text_length=3821, kmer_length=6, kmer_occurrence_count=1
   ...: )
Out[3]: 0.931640625
```

There's a 93% probability of at least one sequence of length 6 (doesn't matter if it's EcoRI or BstEII) occurring, in a coronavirus spike gene of that length, just by random chance alone. How about the **joint probability of both of them occurring at once**? 👇👀

Conclusion 🔓🔑

Since BstEII and EcoRI are considered the same length (after disregarding BstEII's arbitrary middle character, they're each 6 bases long), the joint probability of them occurring together in the spike is approximately `93% * 93%`:

```
In [4]: 0.931640625 * 0.931640625
Out[4]: 0.867954241503906
```

➡️ about **87% of all coronaviruses** are going to have - in their spike protein gene - an EcoRI sequence occurring together with a BstEII sequence. Without the need for **any** genetic engineering 🤷‍♀️

Putting it differently: if SARS-CoV-2 was bioengineered ✂️🧬 the way Yan et al suggested, then 17 in 20 coronaviruses occurring in nature **also were**. Why go through the trouble of bioengineering, when nature has already done the work ❓