

(b)(3):10 USC 424; (b)(6)

From:

To:

CC:

Subject: Sec. 1.4(c)

Date: 2020/07/08 12:20:00

Priority: Normal

Type: Note

~~Classification: SECRET//NOFORN~~

Thank you, [redacted] Quite a Comment!

From: [redacted]
Sent: Wednesday, July 08, 2020 10:12 AM
To: [redacted]
Subject: [redacted]

~~Classification: SECRET//NOFORN~~

(b)(1); (b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c); (b)(6)

FYSA.

From: [redacted]
Sent: Tuesday, July 07, 2020 2:36 PM
To: [redacted]
Subject: [redacted]

~~Classification: SECRET//NOFORN~~

The next two pages are withheld in full citing (b)(1), (b)(3) 10 USC 424 + 50 USC 3024i, and (b)(6), and are not provided.

Subject: [REDACTED]

~~Classification: SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); (b)(6)

Hi

Here are [REDACTED] questions. I just copied and pasted from what our analysts sent me so I wouldn't lose anything in translation/editing. If you have any questions on the questions, address them to [REDACTED] and [REDACTED] as they were the originators.

(b)(3):10 USC 424; (b)(6)

Thanks,

(b)(3):10 USC 424; Sec. 1.4(c); Sec. 1.4(e); (b)(6)

The next three pages are withheld in full citing (b)(1), (b)(3) 10 USC 424 + 50 USC 3024i, and (b)(6), and are not provided.

That is wonderful! Thank you for reaching out.

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); (b)(6)

From:

Sent: Thursday, June 18, 2020 1:27 PM

To:

Subject:

~~Classification: SECRET//NOFORN~~

(b)(1); (b)(3):10 USC 424;
(b)(3):50 USC 3024(i); Sec. 1.4(c)
(b)(6)

Hi

Just as a heads up, I have been tasked with consolidating questions from on this topic. I will send you our list Friday evening. I am sure there will be interest in all the presentations.

Thanks,

(b)(3):10 USC 424; (b)(6)

From: (b)(3):10 USC 424; (b)(6)

Sent: Thursday, June 18, 2020 6:54 AM

To:

(b)(3):10 USC 424; (b)(6)

Cc:

Subject:

~~Classification: SECRET//NOFORN~~

Please submit to [redacted] by COB Friday.

(b)(3):10 USC 424;
(b)(1); (b)(3):50 USC
3024(i); Sec. 1.4(c);
(b)(6)

From:

Sent: Thursday, June 18, 2020 6:48 AM

To:

Cc:

Subject:

(b)(1); (b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c)

~~Classification: SECRET//NOFORN~~

Sec. 1.4(c); Sec. 1.4(e)

=====

All, please stand down from submitting any

Please draft some questions and send them to

who will consolidate

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); (b)(6)

Sent: Wednesday, June 17, 2020 12:01 PM

To

Cc:

Subject:

~~Classification: SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c); (b)(6)

The next four pages are withheld in full citing (b)(1), (b)(3) 10 USC 424 + 50 USC 3024i, and (b)(6), and are not provided.

From: (b)(3):10 USC 424; (b)(6)
To:

Subject: RE: Question

Date: 2020/04/27 11:54:46

Priority: Normal

Type: Note

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

Cheers,

(b)(3):10 USC 424; (b)(6)

From:

Sent: Monday, April 27, 2020 11:52 AM

To:

Subject: FW: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Thank you for any thoughts!

(b)(3):10 USC 424; (b)(6)

From:

Sent: Monday, April 27, 2020 10:37 AM

To:

Cc:

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

This came in today. While the DR is asking

[redacted] that may be out there. Maybe we can help inform through our channels to the DR?

(b)(3):10 USC 424;
(b)(3):50 USC 3024(i)

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]
Sent: Monday, April 27, 2020 10:21 AM
To: [REDACTED]

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC
424; (b)(6)

From: Ashley Robert P LTG DIA DR [REDACTED]
Sent: Monday, April 27, 2020 10:18 AM
To: [REDACTED]
Cc: [REDACTED]

Johnny O DIA CS

White Suzanne L DIA DD

Sawyer

Subject: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

(b)(1); (b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

The final page is withheld citing (b)(3) 10 USC 424 and (b)(6) and is not provided.

From: (b)(3):10 USC 424; (b)(6)
To:

Subject: RE: Question

Date: 2020/04/27 12:06:00

Priority: Normal

Type: Note

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

Thanks very much!

From: [Redacted]
Sent: Monday, April 27, 2020 11:57 AM
To: (b)(3):10 USC 424; (b)(6)
[Redacted]

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

All,

(b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)
[Redacted]

Thanks,

[redacted]
From: [redacted]
Sent: Monday, April 27, 2020 11:52 AM (b)(3):10 USC 424; (b)(6)
To: [redacted]

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Thank you for any thoughts!

(b)(3):10 USC 424; (b)(6)

From: [redacted] (b)(3):10 USC 424; (b)(6)
Sent: Monday, April 27, 2020 10:37 AM
To: [redacted]
Cc: [redacted]

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(3):10 USC 424; (b)(6)

This came in today. While the DR is asking

that may be out there. Maybe we can help inform through our channels to the DR?

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424;
(b)(3):50 USC 3024(i)

From

Sent: Monday, April 27, 2020 10:21 AM

To:

(b)(3):10 USC 424; (b)(6)

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(3):10 USC 424; (b)(6)

From: Ashley Robert P LTG DIA DR

Sent: Monday, April 27, 2020 10:18 AM

To:

Cc:

White Suzanne L DIA DD

Sawyer

(b)(3):10 USC 424; (b)(6)

Johnny O DIA CS

Subject: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(1); (b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

(b)(3):10 USC 424; (b)(6)

From:

To:

Subject: RE: Question
Date: 2020/04/28 14:22:00
Priority: Normal
Type: Note

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: NS~~

~~Declassify On: 25X1, EO 12951~~

=====

Ah, I see. Thank you for explaining. Actually I happened to be talking with (b)(3):50 USC 3024(i) today; they are following the various variants, especially a few higher-frequency mutations that might possibly influence functional characteristics. Amazing that this can be tracked in close to real-time.

Thank you for the collaboration. Please let us know if we can do anything to support you all.

V/r,

(b)(3):10 USC 424; (b)(6)

From:

Sent: Tuesday, April 28, 2020 11:49 AM

To:

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: NS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Respectfully,

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

The society that separates its scholars from its warriors will have its thinking done by cowards and its fighting by fools.

Thucydides, c. 460 – c. 400 BC

From: [REDACTED]
Sent: Tuesday, April 28, 2020 11:36 AM
To: [REDACTED]
Subject: RE: Question

(b)(3):10 USC 424;
(b)(6)

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

Thank you! Looks great, just one quick follow-up—wondering about the

[REDACTED]

that follows?

(b)(1); (b)(3);50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

From: [REDACTED]
Sent: Tuesday, April 28, 2020 11:30 AM
To: [REDACTED]
Subject: RE: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

Looks good. Edits in red. Thanks for letting me collaborate with you!

Respectfully,

(b)(3):10 USC 424; (b)(6)

The society that separates its scholars from its warriors will have its thinking done by cowards and its fighting by fools.

Thucydides, c. 460 – c. 400 BC

From: [REDACTED]

Sent: Tuesday, April 28, 2020 9:30 AM

To: [REDACTED]

Subject: FW: Question

(b)(3):10 USC 424;
(b)(6)

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: NS~~

~~Declassify On: 25X1, EO 12951~~

=====

Hello [REDACTED]

(b)(3):10 USC 424; (b)(6)

Could we please coordinate with you on a response to the Director on [REDACTED] as my leadership requests below? If you're able to help with this today, I would be grateful. I drafted a few bullets; please edit as needed. Thank you!

(b)(1); (b)(3):50
USC 3024(i);
Sec. 1.4(c);
Sec. 1.4(e)

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(U) Different species of coronavirus also are sometimes referred to as "strains". There are four other endemic human coronaviruses, which typically cause nothing worse than the common cold, and it is possible for a patient to be infected with the COVID-19 virus and one of these other coronaviruses. In a recent US study, 4% of 116 COVID-19 patients also were infected with another coronavirus at the same time.

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(b)(3):10 USC 424; (b)(6)

From:

Sent: Tuesday, April 28, 2020 7:45 AM

To:

Cc:

(b)(3):10 USC 424; (b)(6)

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MC~~

~~Declassify On: 25X1, EO 12958~~

=====

(b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

I'll leave it to you for the coordination. I felt [redacted] had a good and succinct answer to begin with. Can you work with her to come up with an answer that directly covers the DR's questions. I believe he is asking of a [redacted] Please have [redacted] review what you and [redacted] land on. We should have the answer done today.

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

From:

Sent: Monday, April 27, 2020 5:22 PM

To:

Cc:

(b)(3):10 USC 424; (b)(6)

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424;
(b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12958~~

=====

Good afternoon,

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

(b)(1); (b)(3);10 USC 424; (b)(3);50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

Respectfully,

(b)(3);10 USC 424; (b)(6)

The society that separates its scholars from its warriors will have its thinking done by cowards and its fighting by fools.

Thucydides, c. 460 – c. 400 BC

From: [REDACTED]

Sent: Monday, April 27, 2020 2:20 PM

To: [REDACTED]

Cc: [REDACTED]

Subject: RE: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12958~~

=====

(b)(3):10 USC 424; (b)(3):50 USC 3024(i); (b)(6)

[REDACTED] is out today, but our very capable [REDACTED]
can coordinate a response [REDACTED]

has a short and sweet answer below. Perhaps we

v/r

(b)(3):10 USC 424; (b)(6)

Good morning,

(b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Respectfully,

(b)(3):10 USC 424; (b)(6)

The society that separates its scholars from its warriors will have its thinking done by cowards and its fighting by fools.

Thucydides, c. 460 – c. 400 BC

From:

Sent: Monday, April 27, 2020 1:20 PM

To:

Cc:

Subject: FW: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

Were you tracking on the questions from the DR to (b)(3):10 USC 424; (b)(6) ? I do not want to send as is: Our folks provided their views (I wanted them in on the conversation)

Is there a plan for how to answer the DR? If so, we need to clean this up before including...

(b)(3):10 USC 424;
(b)(3):50 USC
3024(i)

[REDACTED]

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]

Sent: Monday, April 27, 2020 1:10 PM

To [REDACTED]

Cc [REDACTED]

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424;
(b)(6)

~~Derived From: MG~~

~~Declassify On: 25X1, EO 12958~~

=====

[REDACTED]

(b)(3):10 USC 424; (b)(6)

Adding [REDACTED] teammates. We're not aware of this particular report, but can review it. We are monitoring these topics and will continue to do so.

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

[REDACTED]

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

V/r,

(b)(3):10 USC 424; (b)(6)

From:

Sent: Monday, April 27, 2020 10:37 AM

To:

Cc:

(b)(3):10 USC 424; (b)(6)

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: NS~~

~~Declassify On: 25X1, EO 12958~~

(b)(3):10 USC 424; (b)(6)

This came in today. While the DR is asking

that may be out there. Maybe we can help inform through our channels to the DR?

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(3):50 USC 3024(i)

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]

Sent: Monday, April 27, 2020 10:21 AM

To: [REDACTED]

Subject: FW: Question

(b)(3):10 USC 424; (b)(6)

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

(b)(3):10 USC 424; (b)(6)

From: Ashley Robert P LTG DIA DR [REDACTED]

Sent: Monday, April 27, 2020 10:18 AM

To: [REDACTED]

Cc: [REDACTED]

(b)(3):10 USC 424; (b)(6)

White Suzanne L DIA DD

Sawyer

Johnny O DIA CS [REDACTED]

Subject: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

The final 2 pages are withheld in full citing (b)(1), (b)(3) 10 USC 424 + 50 USC 3024(i), and (b)(6) and are not provided.

(b)(3):10 USC 424; (b)(6)

From:

To:

CC:

Subject: RE: Question

Date: 2020/04/28 14:36:02

Priority: Normal

Type: Note

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

Great, Much appreciated!

(b)(3):10 USC 424; (b)(6)

From: (b)(3):10 USC 424; (b)(6)

Sent: Tuesday, April 28, 2020 2:27 PM

To:

Cc:

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(3):10 USC 424; (b)(6)

Below please find the response I coordinated with

Standing by to re-work as needed.

V/r,

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(U) Different species of coronavirus also are sometimes referred to as "strains". There are four other endemic human coronaviruses, which typically cause nothing worse than the common cold, and it is possible for a patient to be infected with the COVID-19 virus and one of these other coronaviruses. In a recent US study, 4% of 116 COVID-19 patients also were infected with another coronavirus at the same time.

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

From: [REDACTED]

Sent: Tuesday, April 28, 2020 7:45 AM

To: [REDACTED]

Cc: [REDACTED]

(b)(3):10 USC 424;
(b)(6)

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

I'll leave it to you for the coordination. I felt [REDACTED] had a good and succinct answer to begin with. Can you work with her to come up with an answer that directly covers the DR's questions. I believe he is asking of a [REDACTED] Please have [REDACTED] review what you and [REDACTED]

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(b)(3):50 USC 3024(l);
Sec. 1.4(c); Sec. 1.4(e)

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]

Sent: Monday, April 27, 2020 5:22 PM

To: [REDACTED]

Cc: [REDACTED]

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

(b)(3):10 USC 424; (b)(6)

~~Classified By:~~

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

=====

Good afternoon,

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

(b)(1); (b)(3):10 USC 424; (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

Respectfully,

(b)(3):10 USC 424; (b)(6)

*The society that separates its scholars from its warriors will have its thinking done by cowards
and its fighting by fools.*

Thucydides, c. 460 – c. 400 BC

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]
Sent: Monday, April 27, 2020 2:20 PM
To: [REDACTED]
Cc: [REDACTED]

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12958~~

=====
(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC
424; (b)(6)

is out today, but our very capable [REDACTED] has a short and sweet answer below. Perhaps we
can coordinate a response [REDACTED]

v/r

(b)(3):10 USC 424; (b)(3):50 USC 3024(i); (b)(6)

(b)(3):10 USC 424; (b)(6)

Good morning,

(b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Respectfully,

(b)(3):10 USC 424; (b)(6)

*The society that separates its scholars from its warriors will have its thinking done by cowards
and its fighting by fools.*

Thucydides, c. 460 – c. 400 BC

From:

Sent: Monday, April 27, 2020 1:20 PM

To:

Cc:

(b)(3):10 USC 424; (b)(6)

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MC~~

~~Declassify On: 25X1, EO 12951~~

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(6)

Were you tracking on the questions from the DR to [REDACTED]? I do not want to send as is: Our

(b)(3):10 USC 424

folks provided their views (I wanted them in on the conversation)

Is there a plan for how to answer the DR? If so, we need to clean this up before including...

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(3):50 USC 3024(i)

From:

Sent: Monday, April 27, 2020 1:10 PM

To:

Cc:

(b)(3):10 USC 424;
(b)(6)

Subject: RE: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~ (b)(3):10 USC 424; (b)(6)

~~Derived From: MC~~

~~Declassify On: 25X1, EO 12951~~

=====

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424

Adding [redacted] teammates. We're not aware of this particular report, but can review it. We are monitoring these topics and will continue to do so.

(b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

V/r,

(b)(3):10 USC 424; (b)(6)

From: [REDACTED]

Sent: Monday, April 27, 2020 10:37 AM

(b)(3):10 USC 424; (b)(6)

To: [REDACTED]

Cc: [REDACTED]

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

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(b)(3):10 USC 424; (b)(6)

This came in today. While the DR is asking [REDACTED]

[REDACTED] that may be out there. Maybe we can help inform through our channels to the DR?

(b)(3):10 USC 424; (b)(6)

(b)(3):10 USC 424; (b)(3):5
USC 3024(i)

From: [REDACTED]

Sent: Monday, April 27, 2020 10:21 AM

(b)(3):10 USC 424; (b)(6)

To: [REDACTED]

Subject: FW: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

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(b)(3):10 USC 424; (b)(6)

From: Ashley Robert P LTG DIA DR

Sent: Monday, April 27, 2020 10:18 AM

To:

Cc:

(b)(3):10 USC 424; (b)(6)

White Suzanne L DIA DD

Sawyer

Johnny O DIA CS

Subject: Question

~~Classification: TOP SECRET//NOFORN~~

~~Classified By:~~

(b)(3):10 USC 424; (b)(6)

~~Derived From: MS~~

~~Declassify On: 25X1, EO 12951~~

(b)(3):10 USC 424; (b)(1); (b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e); (b)(6)

~~Classification: TOP SECRET//NOFORN~~

~~Classification: TOP SECRET//NOFORN~~

~~Classification: TOP SECRET//NOFORN~~

~~Classification: TOP SECRET//NOFORN~~

~~SECRET//NOFORN~~

SARS-COV-2 GENOME ANALYSIS

(b)(3)10 USC 424, (b)(6)

June 8, 2020

The following page is withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and is not provided.

~~SECRET//NOFORN~~

~~SECRET//NOFORN~~

Building a Full-Length Clone

Using Type IIS Restriction Enzymes and Golden Gate Assembly System

~~SECRET//NOFORN~~

WIV SARS-CoV Reverse Genetics System

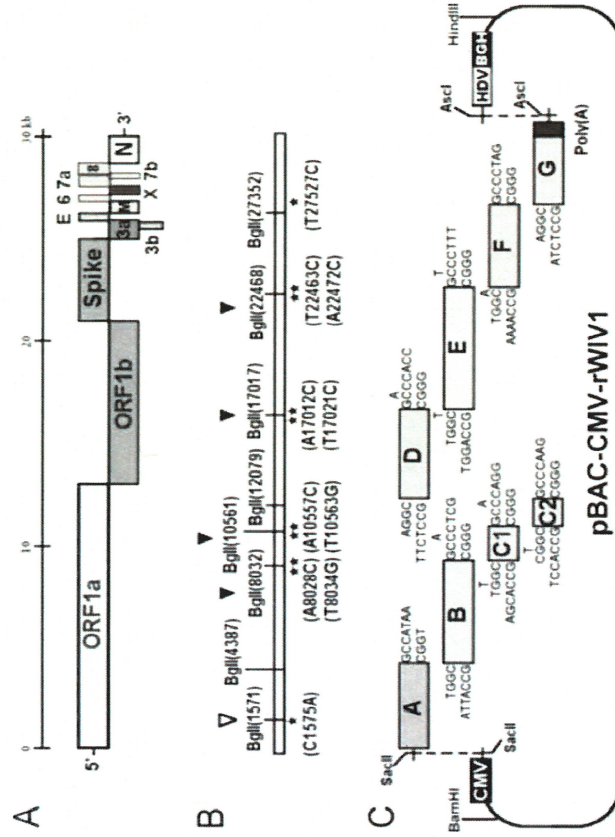


FIG 1 Strategy for construction of an infectious WIV1 BAC clone. (A) Genomic structure of WIV1. (B) The mutations are indicated under the stars. C1575A was used to ablate a natural BglII site at nucleotide 1571 (V), and T27527C was used to disrupt a potential T7 stop site. The others were for introducing BglII sites (▼). (C) The WIV1 genome was split into eight contiguous cDNAs (A to G): A, nt 1 to 4387; B, nt 4388 to 8032; C1, nt 8033 to 10561; C2, nt 10562 to 12079; D, nt 12080 to 17017; E, nt 17018 to 22468; F, nt 22469 to 27352; G, nt 27353 to 30309. Unique BglII sites were introduced into the fragments by synonymous mutations to make these fragments capable of unidirectional ligation along with native BglII sites in the genome. The original nucleotides are shown above the flanking sequences of corresponding fragments. A poly(A) sequence was added to the 3' terminus of fragment G. A CMV promoter, HDV ribozyme, and BGH transcriptional terminal signal were inserted into pBAC1C11 between BamHI and HindIII sites. SacII and Ascl sites were introduced between the CMV promoter and ribozyme. Fragments A to G were inserted into the pBAC-CMV plasmid in a single step.

Eleven Tips For Optimizing Your Golden Gate Assembly Reactions

Looking for a simple, intuitive DNA engineering in a single reaction? Have you ever wish you had a tool that helped you optimize your Golden Gate Assembly reactions?

- 1 Check your sequence**
Check your sequence for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your sequence for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 2 Check your primers**
Check your primers for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 3 Check the right plasmid**
Check the right plasmid for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 4 Choose the right buffer**
Choose the right buffer for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 5 Increase your template assembly**
Increase your template assembly for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 6 Make sure your plasmid prep is good**
Make sure your plasmid prep is good for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 7 Avoid primer dimers**
Avoid primer dimers for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 8 Avoid PCR-induced errors**
Avoid PCR-induced errors for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 9 Increase insert amount for complex assemblies**
Increase insert amount for complex assemblies for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 10 Carefully design every insert's overhang**
Carefully design every insert's overhang for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.
- 11 Check for a sequence error if your assembly becomes non-functional**
Check for a sequence error if your assembly becomes non-functional for any potential issues. Golden Gate Assembly is a highly efficient and accurate method for cloning DNA fragments into plasmids. However, there are some common pitfalls that can lead to failed reactions. Check your primers for any potential issues, such as the presence of restriction sites, and make sure your fragments are the correct size and orientation.

Golden Gate Assembly

20+ FRAGMENT ASSEMBLY NOW ACHIEVABLE
WITH HIGH EFFICIENCY AND ACCURACY

NEW ENGLAND
Biolabs

INSPIRED BY NATURE
RESEARCH BY SCIENCE
DESIGNED BY NATURE

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Unit 10, The Quadrant
Basingstoke, Hampshire RG24 0BA
Tel: 44-1256-333117
uk@neb.com

Featured Tools

Download the NEB AR App for iPhone® or iPad®. Scan the augmented reality barcode to find videos, tutorials and immersive experiences.

For help designing primers, try the new NEB Golden Gate Assembly Tool at GoldenGate.neb.com

Try our ligase fidelity tools for the design of high-fidelity Golden Gate assemblies at neb.com/research/subjects/tools

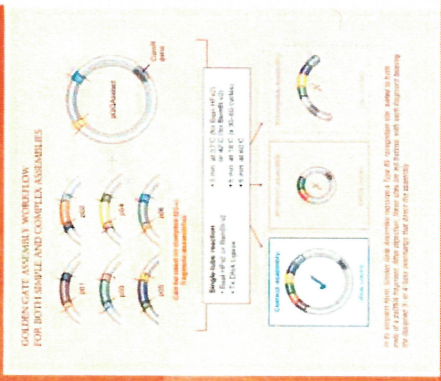
- **Ligate Fidelity Viewer™ (v2)** – Visualize overhang ligation preferences
- **Cricket™** – Predict high-fidelity junction sites
- **SynGene™** – Split DNA sequence for seamless high-fidelity assembly

Push the limits of Golden Gate Assembly

Golden Gate Assembly (1,2) allows for the efficient and seamless assembly of DNA fragments using libraries of Type IIS restriction enzymes and T4 DNA Ligase. With constant advances in both the development of new enzymes and research on automating enzyme functionality (e.g., ligase fidelity), NEB is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

Advantages:

- Clone seamlessly with no scars remaining after assembly
- Perform single insertions, in pairs, 3 fragments using our kit protocols
- Generate libraries with high efficiency
- Assemble multiple fragments (2-20+) in order, in a single reaction
- Experience high efficiency, even with regions of high GC content and areas of repeats
- Use with a broad range of fragment sizes (100 bp to >10 kb)



FEATURED PRODUCTS Type IIS Restriction Enzymes used in Golden Gate Assembly

Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence. Type IIS enzymes commonly used in Golden Gate Assembly are listed below. NEB currently offers over 45 Type IIS restriction enzymes. Please visit www.neb.com for comprehensive table.

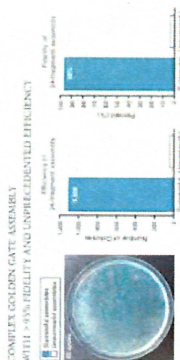
RESTRICTION ENZYME	SEQUENCE	SIZE
BsaI	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000
BsaI-HF	GAATAC (2/6)	302,500,000

What users are saying:

"I've been using Golden Gate Assembly for a while now and it's been a great experience. The efficiency is high and the results are consistent. I've been able to assemble complex constructs with ease. The support from NEB is excellent. I highly recommend this system to anyone looking for a reliable and efficient cloning method." - Dr. John Doe, University of California, Berkeley

NEB® Golden Gate Assembly Kits (BsmBI-v2 or BsaI-HF-v2)

The absence of a central site in a sequence determines the choice of which Type IIS restriction enzyme to use for assembly. For your assembly, choose BsmBI-v2 or BsaI-HF-v2. Both kits are designed to be used for assembly of 2-20+ fragments in a single step.

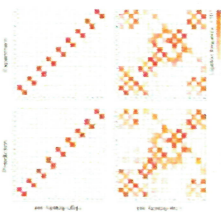


Using NEB Golden Gate Assembly Kits (BsmBI-v2 or BsaI-HF-v2) allows for the efficient and seamless assembly of DNA fragments using libraries of Type IIS restriction enzymes and T4 DNA Ligase. With constant advances in both the development of new enzymes and research on automating enzyme functionality (e.g., ligase fidelity), NEB is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

Advances in Ligase Fidelity

Research at NEB has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling and joining ligase fidelity in order to predict overlap, as well as improved fidelity (1). This research has enabled complex fragment assemblies with high efficiency and >90% accuracy. More information can be found in the NEB website, www.neb.com, under the heading "Ligase Fidelity".

All of these are available at www.neb.com/GoldenGate



Previous research demonstrated ligase fidelity in Golden Gate Assembly (1). This research has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling and joining ligase fidelity in order to predict overlap, as well as improved fidelity (1). This research has enabled complex fragment assemblies with high efficiency and >90% accuracy. More information can be found in the NEB website, www.neb.com, under the heading "Ligase Fidelity".

Visit www.neb.com/GoldenGate to learn more and view related videos



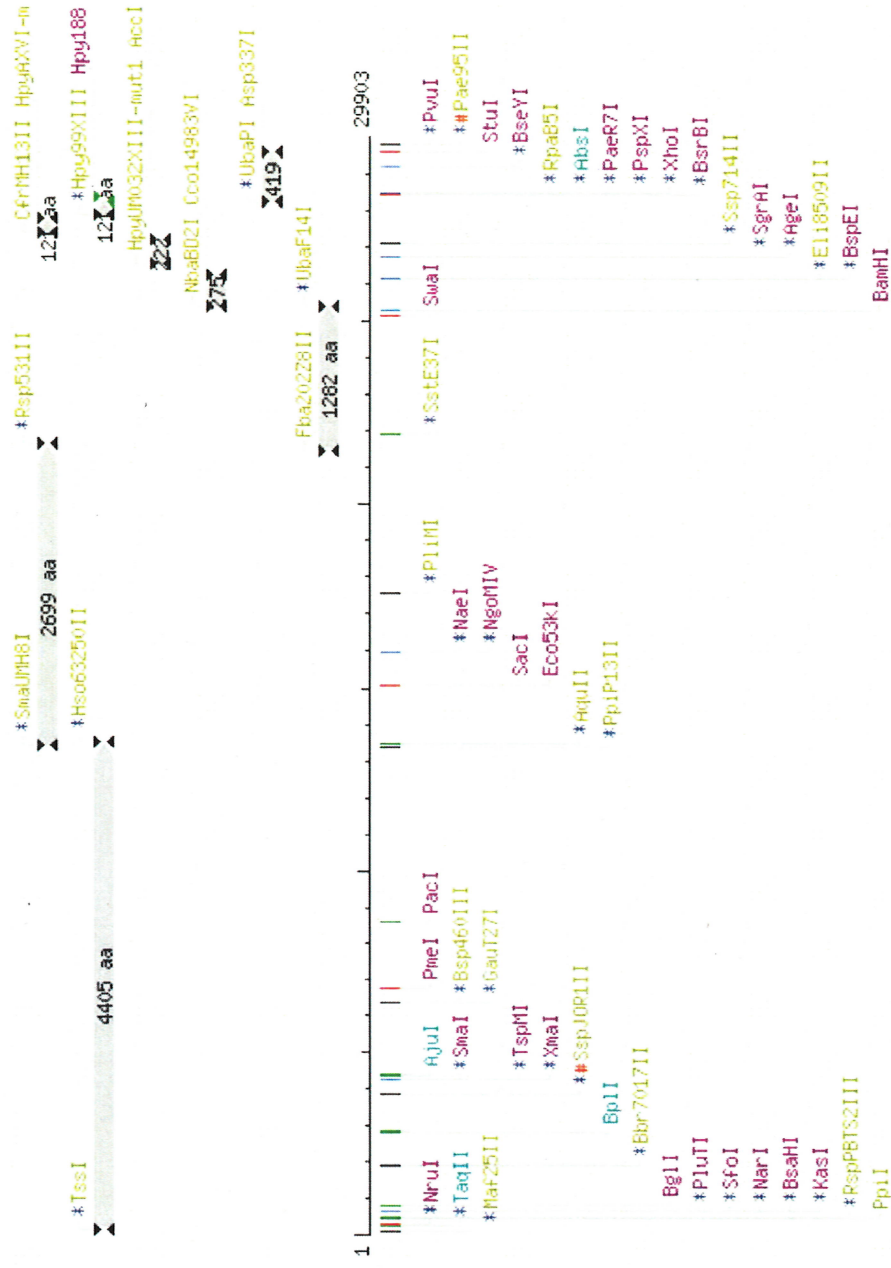
Expanded "assembly standards" for MoClo, GoldenGate 2.0 and Other Modular Golden Gate Assembly Methods

MoClo and GoldenGate 2.0 uses 3 levels of successive assembly. The common standard overhang for each level. Utilizing gathered ligase fidelity information, NEB has expanded each level of assembly, overlap without overhang fidelity. The expanded sets are:

- Level 1 (100% efficiency)
 - BsmBI: 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, 1O, 1P, 1Q, 1R, 1S, 1T, 1U, 1V, 1W, 1X, 1Y, 1Z, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 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1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 1QP, 1QQ, 1QR, 1QS, 1QT, 1QU, 1QV, 1QW, 1QX, 1QY, 1QZ, 1RA, 1RB, 1RC, 1RD, 1RE, 1RF, 1RG, 1RH, 1RI, 1RJ, 1RK, 1RL, 1RM, 1RN, 1RO, 1RP, 1RQ, 1RR, 1RS, 1RT, 1RU, 1RV, 1RW, 1RX, 1RY, 1RZ, 1SA, 1SB, 1SC, 1SD, 1SE, 1SF, 1SG, 1SH, 1SI, 1SJ, 1SK, 1SL, 1SM, 1SN, 1SO, 1SP, 1SQ, 1SR, 1SS, 1ST, 1SU, 1SV, 1SW, 1SX, 1SY, 1SZ, 1TA, 1TB, 1TC, 1TD, 1TE, 1TF, 1TG, 1TH, 1TI, 1TJ, 1TK, 1TL, 1TM, 1TN, 1TO, 1TP, 1TQ, 1TR, 1TS, 1TT, 1TU, 1TV, 1TW, 1TX, 1TY, 1TZ, 1UA, 1UB, 1UC, 1UD, 1UE, 1UF, 1UG, 1UH, 1UI, 1UJ, 1UK, 1UL, 1UM, 1UN, 1UO, 1UP, 1UQ, 1UR, 1US, 1UT, 1UU, 1UV, 1UW, 1UX, 1UY, 1UZ, 1VA, 1VB, 1VC, 1VD, 1VE, 1VF, 1VG, 1VH, 1VI, 1VJ, 1VK, 1VL, 1VM, 1VN, 1VO, 1VP, 1VQ, 1VR, 1VS, 1VT, 1VU, 1VV, 1VW, 1VX, 1VY, 1VZ, 1WA, 1WB, 1WC, 1WD, 1WE, 1WF, 1WG, 1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 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SARS-COV-2 GENOME RESTRICTION MAP



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Type IIS Restriction Enzymes

- BsmBI

5' - CGTCTCNNNNN-3' 

3' - GCAGAGNNNNN - 5' BsaI 

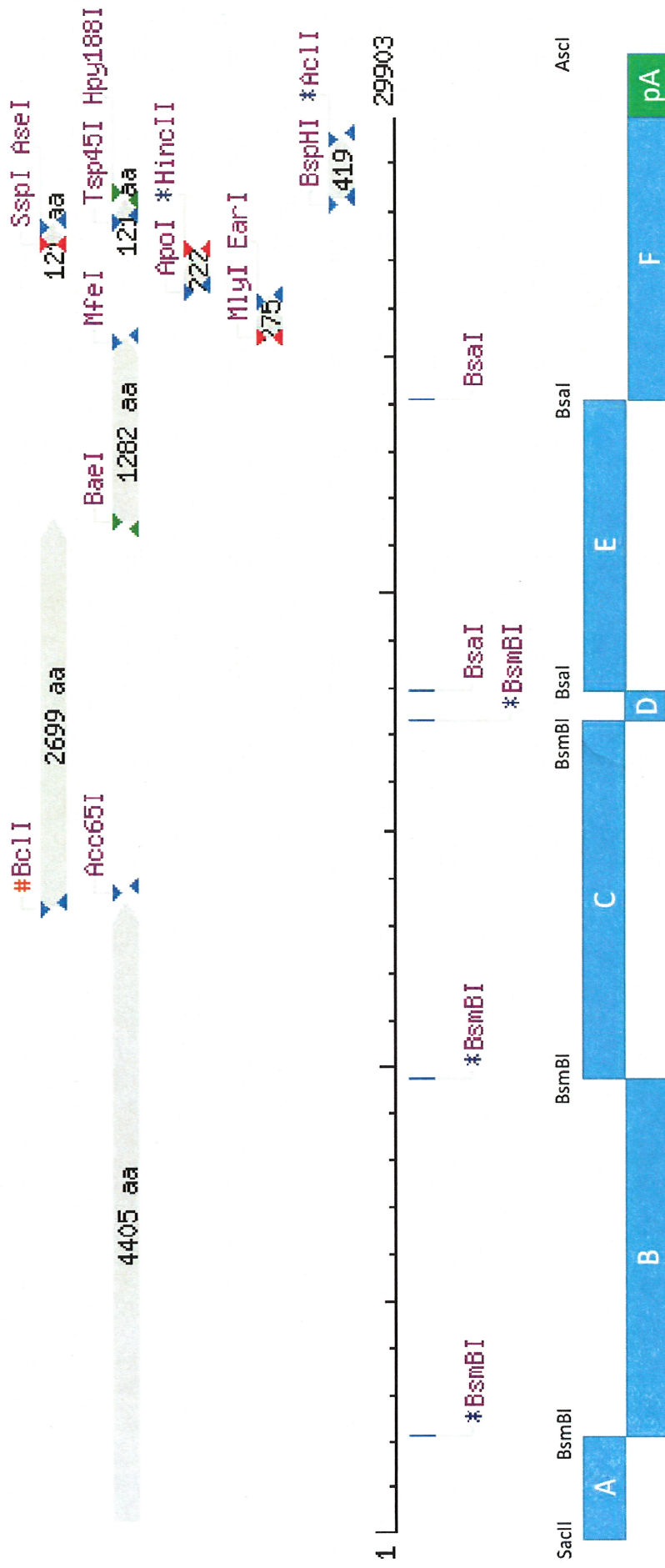
5' - GGTCTCNNNNN-3' 

3' - CCAGAGNNNNN - 5' SARS-CoV-2 genome does not
have any SacII or AscI restriction sites 

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RE-based Fragment Build Option – *BsmBI*/*BsaI* (4 nt overhangs)



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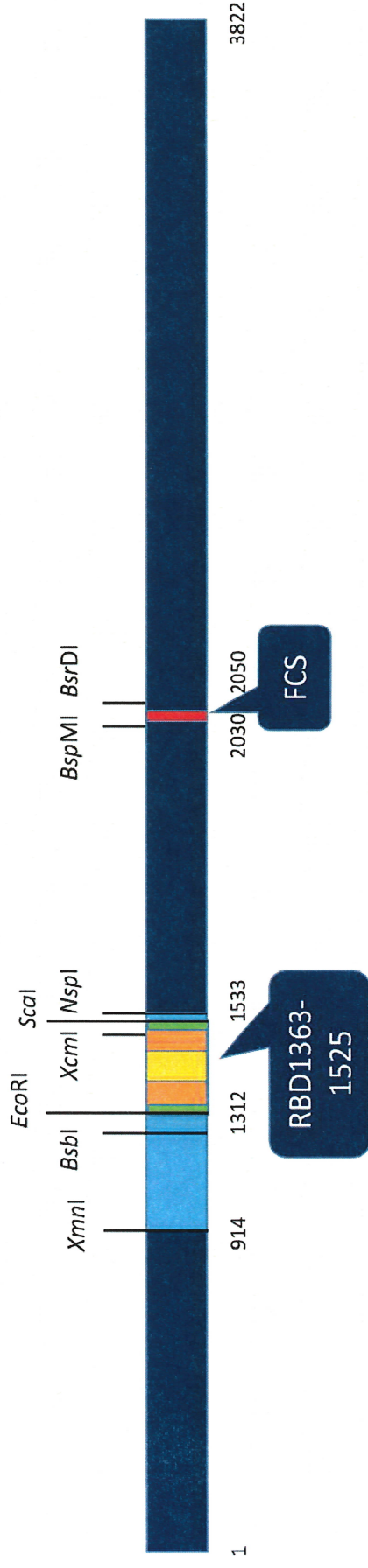
(b)(3);50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

Spike Gene

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SARS CoV-2 SPIKE GENE

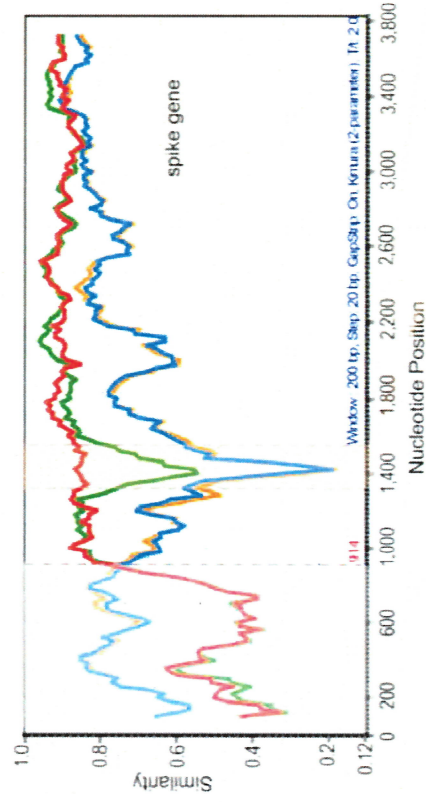


Highest homology to RaTG13Pangolin CoV

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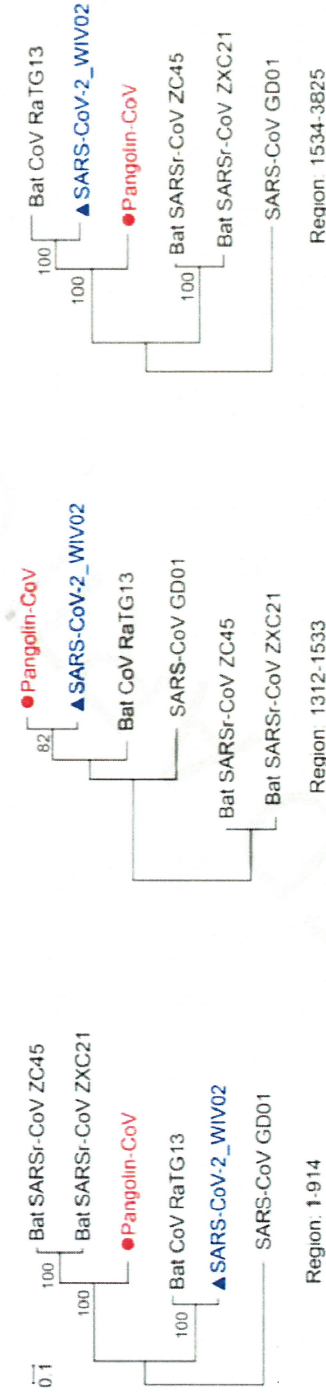
SARS-COV-2 SPIKE GENE SEGMENTS

QUERY: PANGOLIN-COV



— SARS-CoV-2_WIV02
— Bat CoV RaTG13
— Bat SARSr-CoV_ZC45
— Bat SARSr-CoV_ZXC21

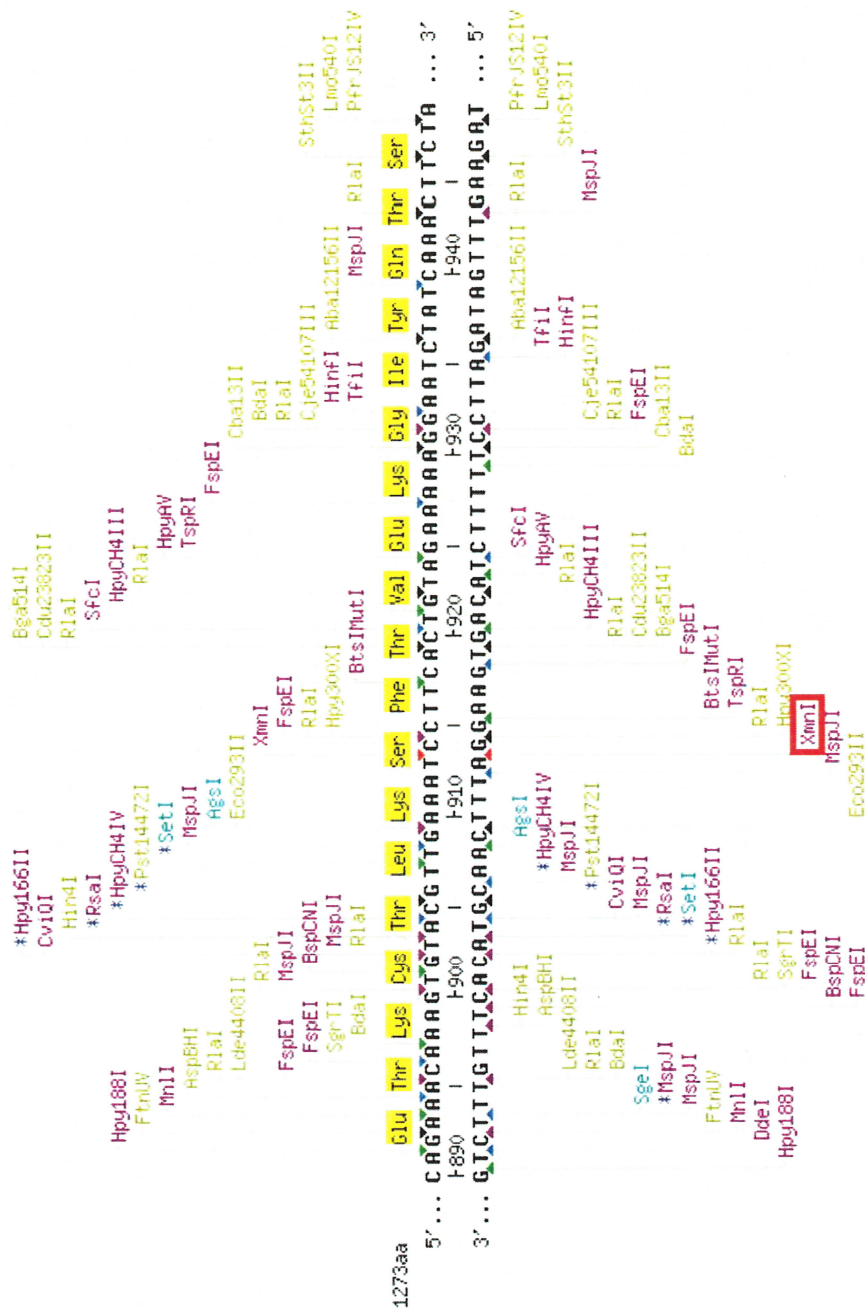
b



Extracted from Xiao *et al.*, 2020

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Nucleotide 914 Region



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Nucleotide 1312 Region

133

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SARS-CoV 1980 CATGTCGACACTTCTTATGAGTGGGACATTCCCTATTGGAGCTGGCATTGTGCTAGTTAC
H V D T S Y E C D I P I G A G I C A S YSARS-CoV-2
CATGTCAAACTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S YBCoV RaTG13
CATGTCAATAACTCGTATGAGTGTGACATACCTATTGGTGCAGGAATATGCGCCAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S Y SARS-CoV CATAAGTTTCTTTATT-----
ACGTAGTACTAGCCAAAATCTATTGTGGCT 2028 H T V S L L R S T S Q K S I
V ASARS-CoV-2 2082 Q T Q T N S P R A R S V A S Q S I I ABCoV RaTG13
CAGACTCAAACTAATTC-----ACGTAGTGTGGCCAGTCAATCTATTATTGCC 2070 Q T Q
T N S R S V A S Q S I I AFu in Cleavage SiteNovel BspMI Restriction SiteNovel NmeAIII
Restriction SiteNovel BsrDI Restriction Site

The following 3 pages are withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i), and are not provided.

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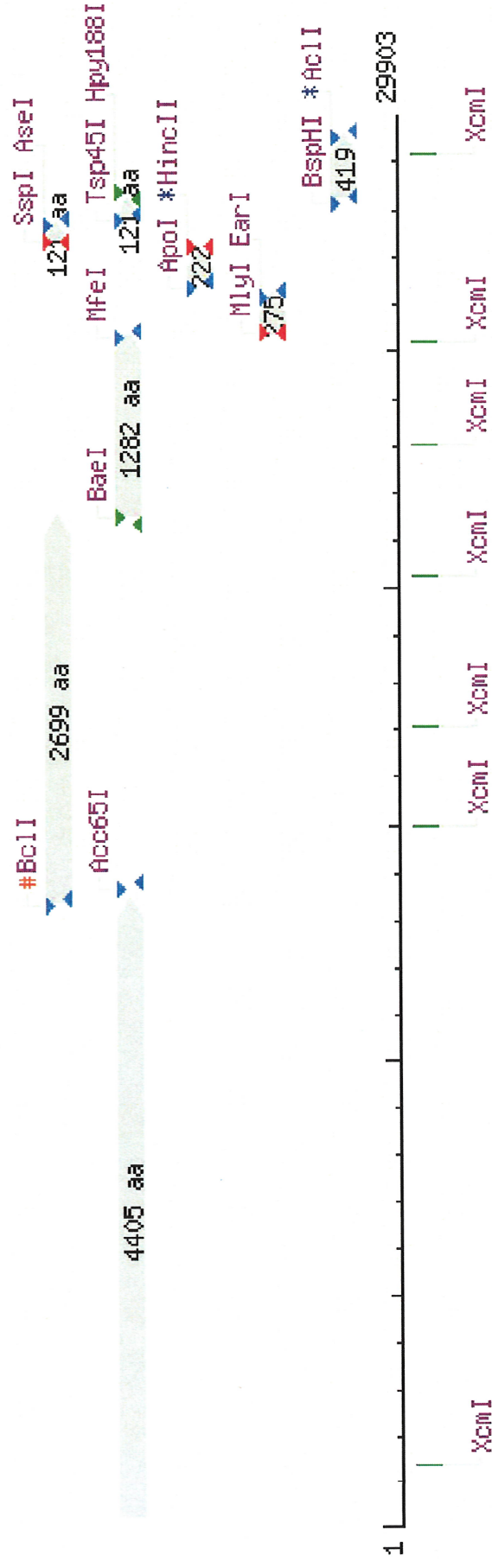
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Back-Up Slides

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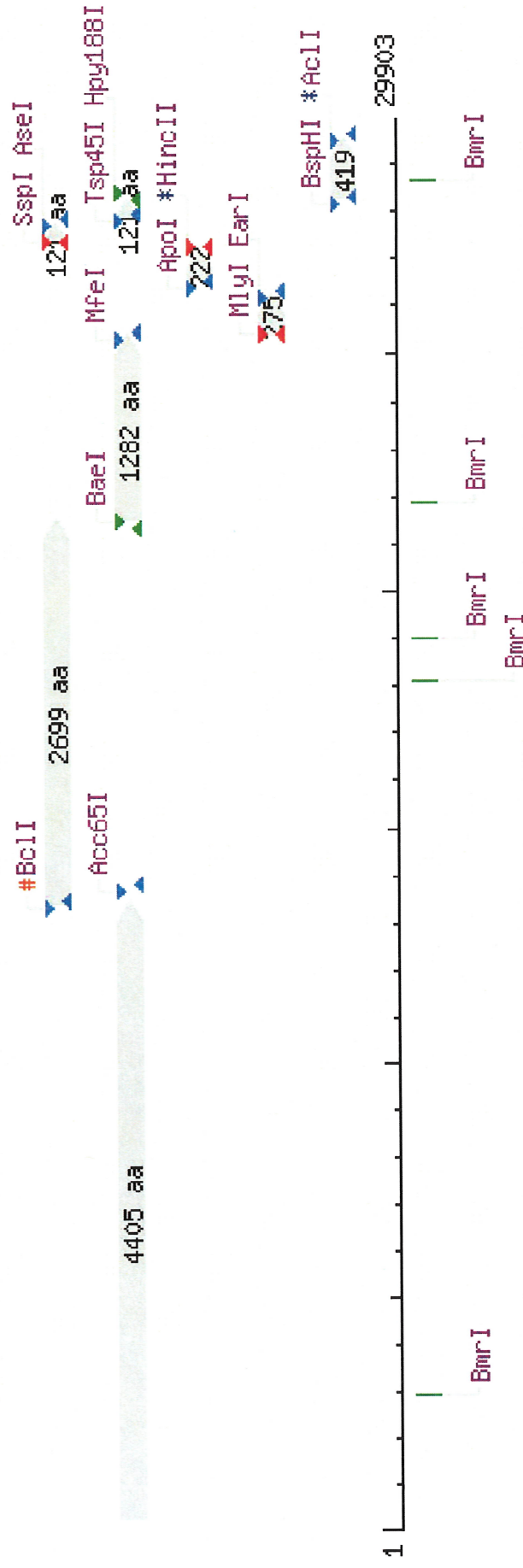
RE-based Fragment Build Option – XcmI (1 nt overhang)



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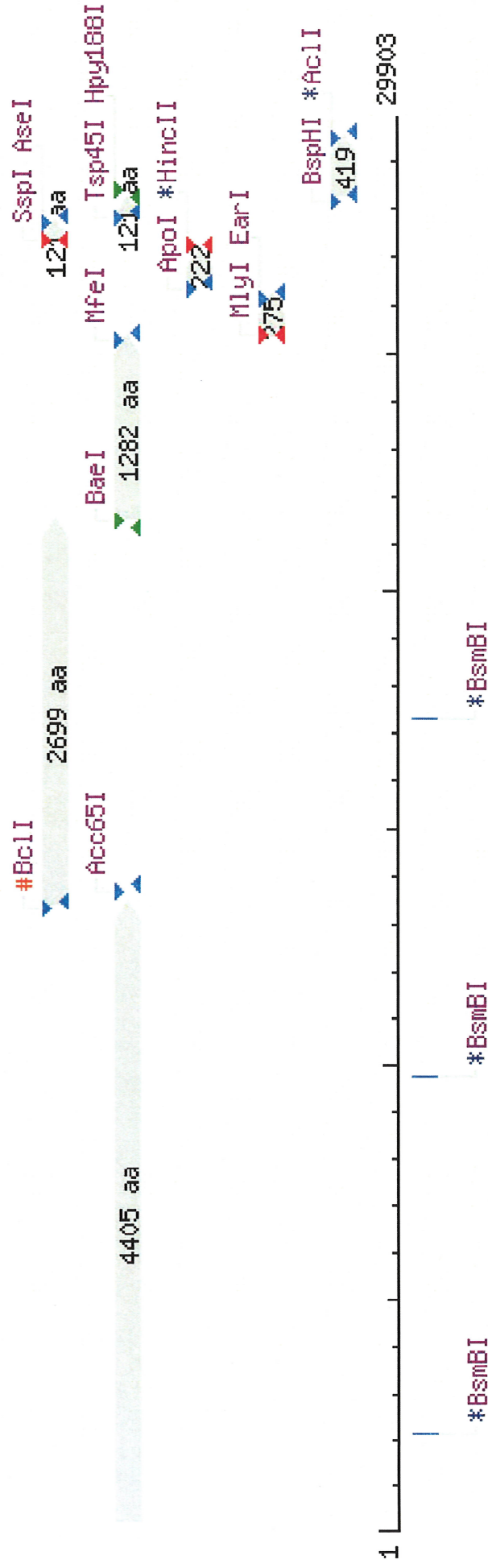
RE-based Fragment Build Option – *BmrI* (1 nt overhang)



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RE-based Fragment Build Option – *BsmBI* (4 nt overhang)



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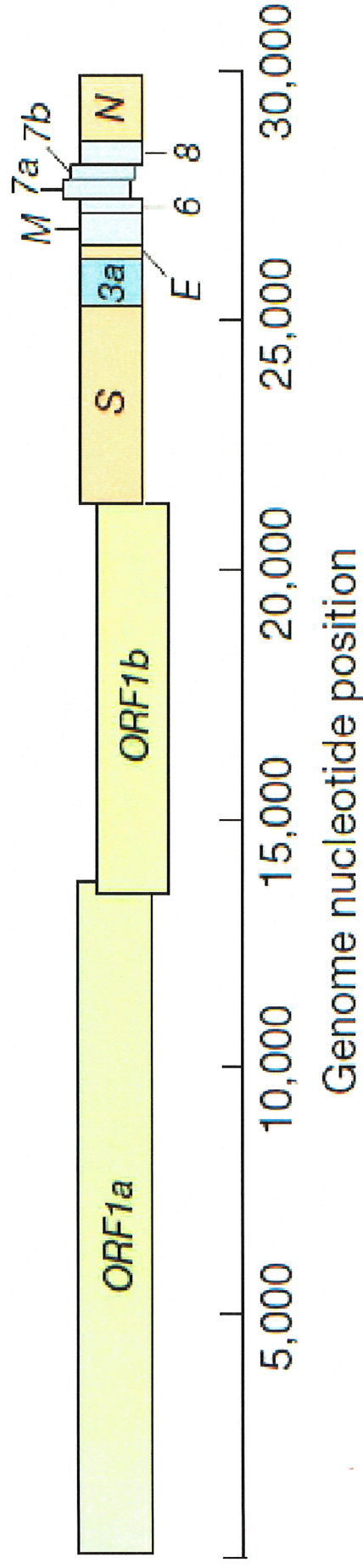
SARS-COV-2 GENOME ANALYSIS

(b)(3)10 USC 424, (b)(6)

June 17, 2020

~~SECRET//NOFORN~~

SARS-CoV-2 Genome



The next page is withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i) and is not provided.

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Methods to construct a Coronavirus Full-Length Clone

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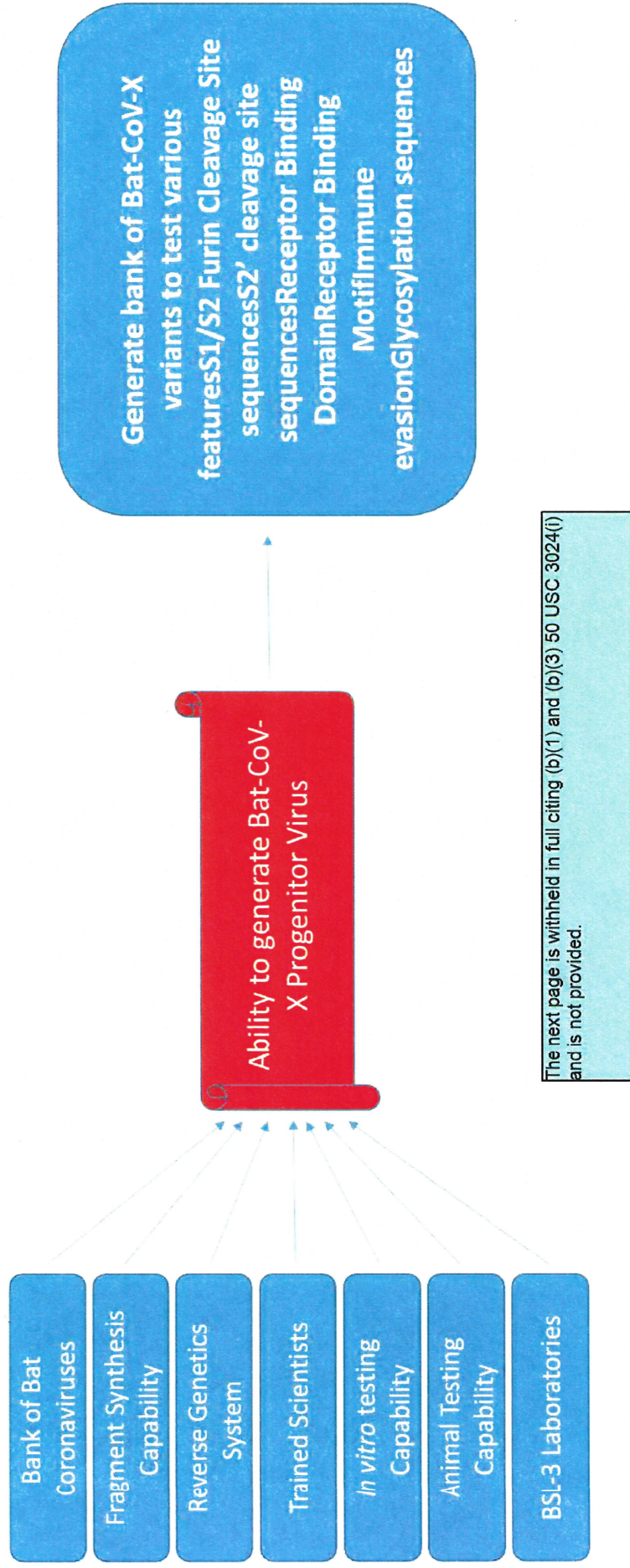
CORONAVIRUS INFECTIOUS CLONE CONSTRUCTION

- 1) Synthesize or PCR amplify 6-10 segments of a Bat Coronavirus Build a 5' transcription initiation fragment "stitch" the fragments together using an infectious clone technology 3' to the transcription initiation fragment Restriction-enzyme-based fragment cloning system Overlapping Fragment system Guided RNA Recombination Clone in a suitable host (E. coli, yeast, etc.) Sequence verify cloned insert

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CAPABILITIES NEEDED TO CONSTRUCT A BAT-LIKE CORONAVIRUS INFECTIOUS CLONE



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Type IIS Restriction Enzymes and Golden Gate Assembly System

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Type II Restriction Enzymes

- Non palindromic recognition site
Cuts at sites outside of recognition site
Each digested location has unique nucleotide overhangs
Example BsaI

5' – GGTCTC NNNNN – 3'

3' – CCAGAG NNNNN – 5'



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Push the limits of Golden Gate Assembly

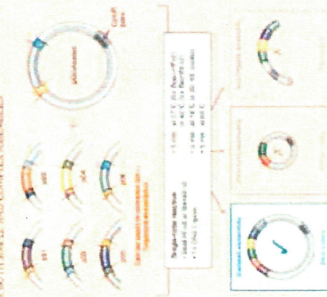
Golden Gate Assembly (1-2) allows for the efficient and seamless assembly of DNA fragments using restriction enzymes and T4 DNA Ligase. With constant efficiency in both the development of new enzymes and research on maintaining enzyme functionality, Golden Gate Assembly is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

Advantages:

- Clones seamlessly with no gaps remaining after assembly
- Perform single insertions in just 5 minutes using our fast protocols
- Generate libraries with high efficiency
- Assemble multiple fragments (2-20) in order to a single reaction
- Experience high efficiency even with regions of high GC content and areas of repeats
- Use with a broad range of fragments sizes (1-100 bp to 3.5 kb)



GOLDEN GATE ASSEMBLY: A POWERFUL TOOL FOR GENETIC ENGINEERING



Golden Gate Assembly is a powerful tool for genetic engineering. It allows for the efficient and seamless assembly of DNA fragments using restriction enzymes and T4 DNA Ligase. With constant efficiency in both the development of new enzymes and research on maintaining enzyme functionality, Golden Gate Assembly is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

FEATURED PRODUCTS

Type IIS Restriction Enzymes used in Golden Gate Assembly

Type IIS restriction enzymes recognize asymmetric DNA sequences and create outside of their recognition sequence. Type IIS enzymes commonly used for Golden Gate Assembly are listed below. NEB currently offers over 40 Type IIS restriction enzymes.

Please visit www.neb.com for comprehensive table.

RESTRICTION ENZYME	NEB #	SEQUENCE	SIZE
BamHI	80301	GAATTC	307,307 bp
BclI	80302	TGATCA	307,307 bp
BglII	80303	AAGCTT	307,307 bp
BspI	80304	GAATTC	307,307 bp
BspII	80305	GAATTC	307,307 bp
BspIII	80306	GAATTC	307,307 bp
BspI	80307	GAATTC	307,307 bp
BspII	80308	GAATTC	307,307 bp
BspIII	80309	GAATTC	307,307 bp
BspIV	80310	GAATTC	307,307 bp
BspV	80311	GAATTC	307,307 bp
BspVI	80312	GAATTC	307,307 bp
BspVII	80313	GAATTC	307,307 bp
BspVIII	80314	GAATTC	307,307 bp
BspIX	80315	GAATTC	307,307 bp
BspXI	80316	GAATTC	307,307 bp
BspXII	80317	GAATTC	307,307 bp
BspXIII	80318	GAATTC	307,307 bp
BspXIV	80319	GAATTC	307,307 bp
BspXV	80320	GAATTC	307,307 bp
BspXVI	80321	GAATTC	307,307 bp
BspXVII	80322	GAATTC	307,307 bp
BspXVIII	80323	GAATTC	307,307 bp
BspXIX	80324	GAATTC	307,307 bp
BspXX	80325	GAATTC	307,307 bp
BspXXI	80326	GAATTC	307,307 bp
BspXXII	80327	GAATTC	307,307 bp
BspXXIII	80328	GAATTC	307,307 bp
BspXXIV	80329	GAATTC	307,307 bp
BspXXV	80330	GAATTC	307,307 bp
BspXXVI	80331	GAATTC	307,307 bp
BspXXVII	80332	GAATTC	307,307 bp
BspXXVIII	80333	GAATTC	307,307 bp
BspXXIX	80334	GAATTC	307,307 bp
BspXXX	80335	GAATTC	307,307 bp
BspXXXI	80336	GAATTC	307,307 bp
BspXXXII	80337	GAATTC	307,307 bp
BspXXXIII	80338	GAATTC	307,307 bp
BspXXXIV	80339	GAATTC	307,307 bp
BspXXXV	80340	GAATTC	307,307 bp
BspXXXVI	80341	GAATTC	307,307 bp
BspXXXVII	80342	GAATTC	307,307 bp
BspXXXVIII	80343	GAATTC	307,307 bp
BspXXXIX	80344	GAATTC	307,307 bp
BspXXXX	80345	GAATTC	307,307 bp
BspXXXXI	80346	GAATTC	307,307 bp
BspXXXXII	80347	GAATTC	307,307 bp
BspXXXXIII	80348	GAATTC	307,307 bp
BspXXXXIV	80349	GAATTC	307,307 bp
BspXXXXV	80350	GAATTC	307,307 bp
BspXXXXVI	80351	GAATTC	307,307 bp
BspXXXXVII	80352	GAATTC	307,307 bp
BspXXXXVIII	80353	GAATTC	307,307 bp
BspXXXXIX	80354	GAATTC	307,307 bp
BspXXXXX	80355	GAATTC	307,307 bp
BspXXXXXI	80356	GAATTC	307,307 bp
BspXXXXXII	80357	GAATTC	307,307 bp
BspXXXXXIII	80358	GAATTC	307,307 bp
BspXXXXXIV	80359	GAATTC	307,307 bp
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BspXXXXXIX	80364	GAATTC	307,307 bp
BspXXXXXX	80365	GAATTC	307,307 bp
BspXXXXXXI	80366	GAATTC	307,307 bp
BspXXXXXXII	80367	GAATTC	307,307 bp
BspXXXXXXIII	80368	GAATTC	307,307 bp
BspXXXXXXIV	80369	GAATTC	307,307 bp
BspXXXXXXV	80370	GAATTC	307,307 bp
BspXXXXXXVI	80371	GAATTC	307,307 bp
BspXXXXXXVII	80372	GAATTC	307,307 bp
BspXXXXXXVIII	80373	GAATTC	307,307 bp
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BspXXXXXXXII	80377	GAATTC	307,307 bp
BspXXXXXXXIII	80378	GAATTC	307,307 bp
BspXXXXXXXIV	80379	GAATTC	307,307 bp
BspXXXXXXXV	80380	GAATTC	307,307 bp
BspXXXXXXXVI	80381	GAATTC	307,307 bp
BspXXXXXXXVII	80382	GAATTC	307,307 bp
BspXXXXXXXVIII	80383	GAATTC	307,307 bp
BspXXXXXXXIX	80384	GAATTC	307,307 bp
BspXXXXXXXX	80385	GAATTC	307,307 bp
BspXXXXXXXXI	80386	GAATTC	307,307 bp
BspXXXXXXXII	80387	GAATTC	307,307 bp
BspXXXXXXXIII	80388	GAATTC	307,307 bp
BspXXXXXXXIV	80389	GAATTC	307,307 bp
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BspXXXXXXXIII	80398	GAATTC	307,307 bp
BspXXXXXXXIV	80399	GAATTC	307,307 bp
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BspXXXXXXXVII	80402	GAATTC	307,307 bp
BspXXXXXXXVIII	80403	GAATTC	307,307 bp
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BspXXXXXXXIII	80408	GAATTC	307,307 bp
BspXXXXXXXIV	80409	GAATTC	307,307 bp
BspXXXXXXXV	80410	GAATTC	307,307 bp
BspXXXXXXXVI	80411	GAATTC	307,307 bp
BspXXXXXXXVII	80412	GAATTC	307,307 bp
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BspXXXXXXXIII	80418	GAATTC	307,307 bp
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BspXXXXXXXIII	80438	GAATTC	307,307 bp
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BspXXXXXXXVII	80442	GAATTC	307,307 bp
BspXXXXXXXVIII	80443	GAATTC	307,307 bp
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BspXXXXXXXXI	80446	GAATTC	307,307 bp
BspXXXXXXXII	80447	GAATTC	307,307 bp
BspXXXXXXXIII	80448	GAATTC	307,307 bp
BspXXXXXXXIV	80449	GAATTC	307,307 bp
BspXXXXXXXV	80450	GAATTC	307,307 bp
BspXXXXXXXVI	80451	GAATTC	307,307 bp
BspXXXXXXXVII	80452	GAATTC	307,307 bp
BspXXXXXXXVIII	80453	GAATTC	307,307 bp
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BspXXXXXXXVIII	80463	GAATTC	307,307 bp
BspXXXXXXXIX	80464	GAATTC	307,307 bp
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BspXXXXXXXII	80467	GAATTC	307,307 bp
BspXXXXXXXIII	80468	GAATTC	307,307 bp
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BspXXXXXXXVIII	80473	GAATTC	307,307 bp
BspXXXXXXXIX	80474	GAATTC	307,307 bp
BspXXXXXXXX	80475	GAATTC	307,307 bp
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BspXXXXXXXIII	80478	GAATTC	307,307 bp
BspXXXXXXXIV	80479	GAATTC	307,307 bp
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BspXXXXXXXVI	80481	GAATTC	307,307 bp
BspXXXXXXXVII	80482	GAATTC	307,307 bp
BspXXXXXXXVIII	80483	GAATTC	307,307 bp
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BspXXXXXXXV	80490	GAATTC	307,307 bp
BspXXXXXXXVI	80491	GAATTC	307,307 bp
BspXXXXXXXVII	80492	GAATTC	307,307 bp
BspXXXXXXXVIII	80493	GAATTC	307,307 bp
BspXXXXXXXIX	80494	GAATTC	307,307 bp
BspXXXXXXXX	80495	GAATTC	307,307 bp
BspXXXXXXXXI	80496	GAATTC	307,307 bp
BspXXXXXXXII	80497	GAATTC	307,307 bp
BspXXXXXXXIII	80498	GAATTC	307,307 bp
BspXXXXXXXIV	80499	GAATTC	307,307 bp
BspXXXXXXXV	80500	GAATTC	307,307 bp

WIV Bat Coronavirus Collection Efforts

• WIV possesses a large bank of Bat Coronaviruses isolated from various bat species in Yunnan Province ChinaGe et al., 2013Yang et al., 2016Hu et al., 2017Five-year longitudinal study to isolate Bat Coronaviruses (April 2011 – October 2015)Only a few sequences have been published

Table 1. Summary of SARS-CoV detection in bats from a single habitat in Kunming, Yunnan.

Sampling time	Sample type	Sample Numbers		SARS-CoV +	SARS-CoV + bat species (No.)
		Total	CoV +		
April, 2011	anal swab	14	1	1	<i>R. sinicus</i> (1)
October, 2011	anal swab	8	3	3	<i>R. sinicus</i> (3)
May, 2012	anal swab & feces	54	9	4	<i>R. sinicus</i> (4)
September, 2012	- feces	39	20	10	<i>R. sinicus</i> (16) <i>R. ferrugineus</i> (3)
April, 2013	feces	52	21	18	<i>R. sinicus</i> (16)
July, 2013	anal swab & feces	115	9	8	<i>R. sinicus</i> (8)
May, 2014	feces	131	8	4	<i>A. stoliczkanus</i> (3) <i>R. affinis</i> (1)
October, 2014	anal swab	19	4	4	<i>R. sinicus</i> (4)
May, 2015	feces	145	3	0	
October, 2015	anal swab	25	6	5	<i>R. sinicus</i> (5)
Total		602	84	64	<i>R. (51) A (3)</i>



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Postulated WIV Bat-CoV-X Full-length Clone Construction Process

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Quote from Zeng *et al.*, 2016

- From Materials and Methods, Virus and cells section
“All experiments using live virus was conducted under biosafety level 2 (BSL2) conditions.”

From the first paragraph of the Discussion

“In this study, we have developed a fast and cost-effective method for reverse genetics of coronaviruses by combining two approaches developed by others (29, 30). Our method allows the genomes of coronaviruses to be split into multiple fragments and inserted into a BAC plasmid with a single step. Recombinant viruses can then be efficiently rescued by direct transfection of the BAC construct. As the genomes can be divided into multiple short fragments, mutations can be introduced into individual fragments easily (31). Using this method, we successfully rescued three recombinant viruses derived from SL-CoV WIV1 (rWIV1, rWIV1-DX, and rWIV1-GFP-DX).”

The next page is withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i) and is not provided.

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WIV SARS-CoV Reverse Genetics System

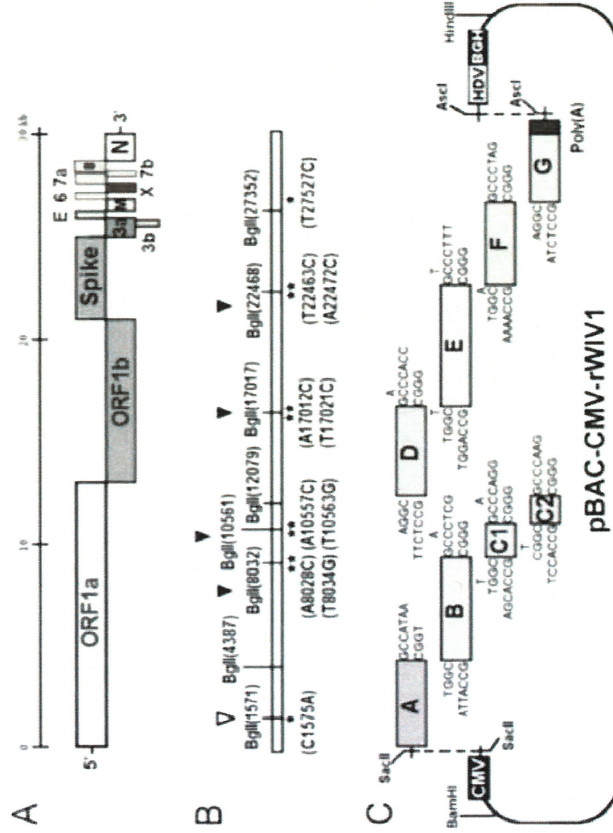
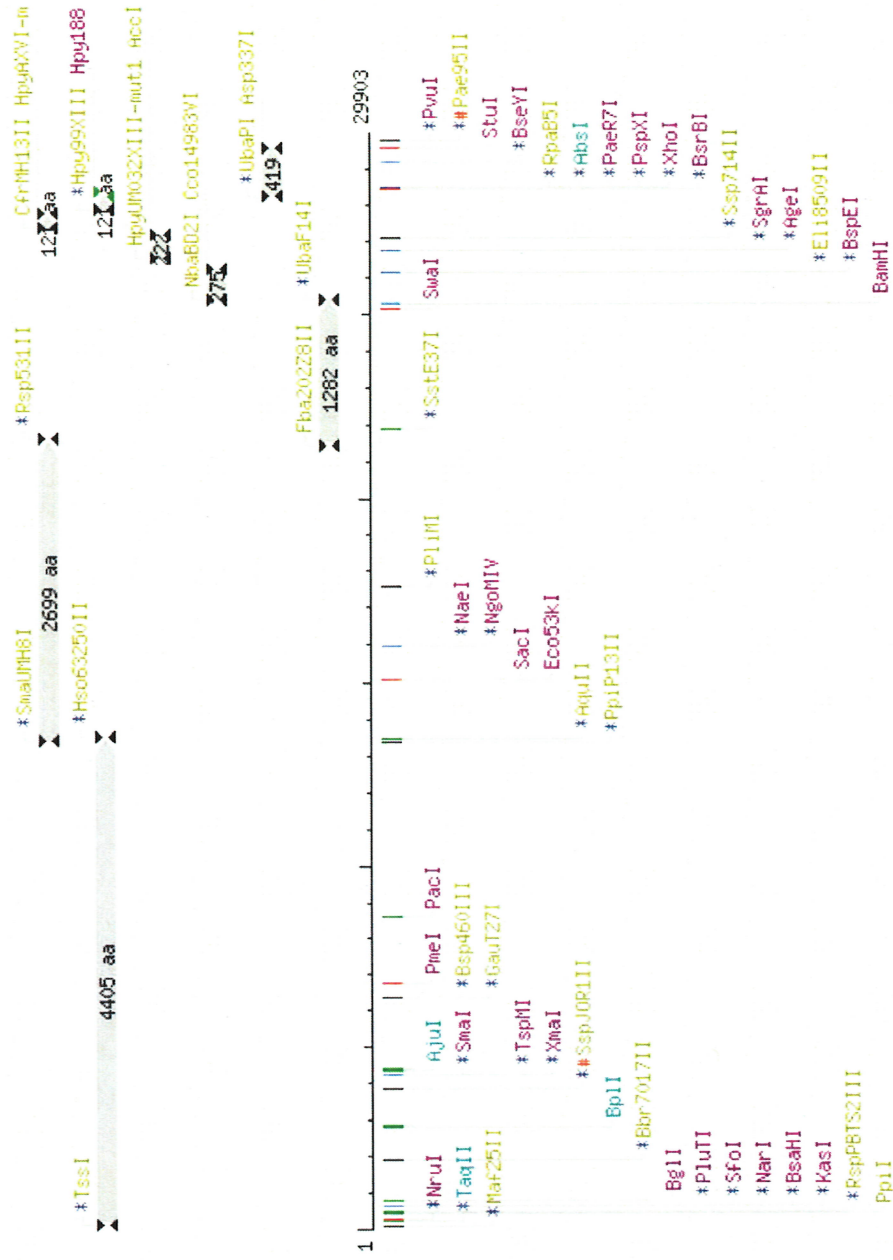


FIG 1 Strategy for construction of an infectious WIV1 BAC clone. (A) Genomic structure of WIV1. (B) The mutations are indicated under the stars. C1575A was used to ablate a natural BglII site at nucleotide 1571 (V), and T27527C was used to disrupt a potential T7 stop site. The others were for introducing BglII sites (V). (C) The WIV1 genome was split into eight contiguous cDNAs (A to G): A, nt 1 to 4387; B, nt 4388 to 8032; C1, nt 8033 to 10561; C2, nt 10562 to 12079; D, nt 12080 to 17017; E, nt 17018 to 22468; F, nt 22469 to 27352; G, nt 27353 to 30309. Unique BglII sites were introduced into the fragments by synonymous mutations to make these fragments capable of unidirectional ligation along with native BglII sites in the genome. The original nucleotides are shown above the flanking sequences of corresponding fragments. A poly(A) sequence was added to the 3' terminus of fragment G. A CMV promoter, HDV ribozyme, and BGLI transcriptional terminal signal were inserted into pBeloBAC1.1 between BamHI and HindIII sites. SacII and Ascl sites were introduced between the CMV promoter and ribozyme. Fragments A to G were inserted into the pBAC-CMV plasmid in a single step.

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SARS-COV-2 GENOME RESTRICTION MAP



Type IIS Restriction Enzymes

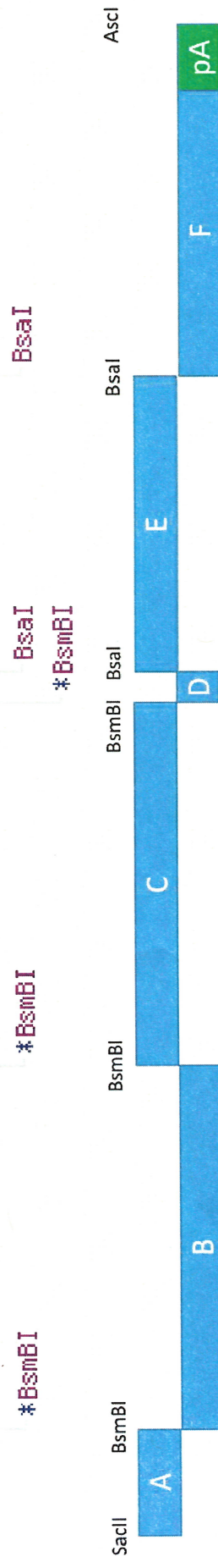
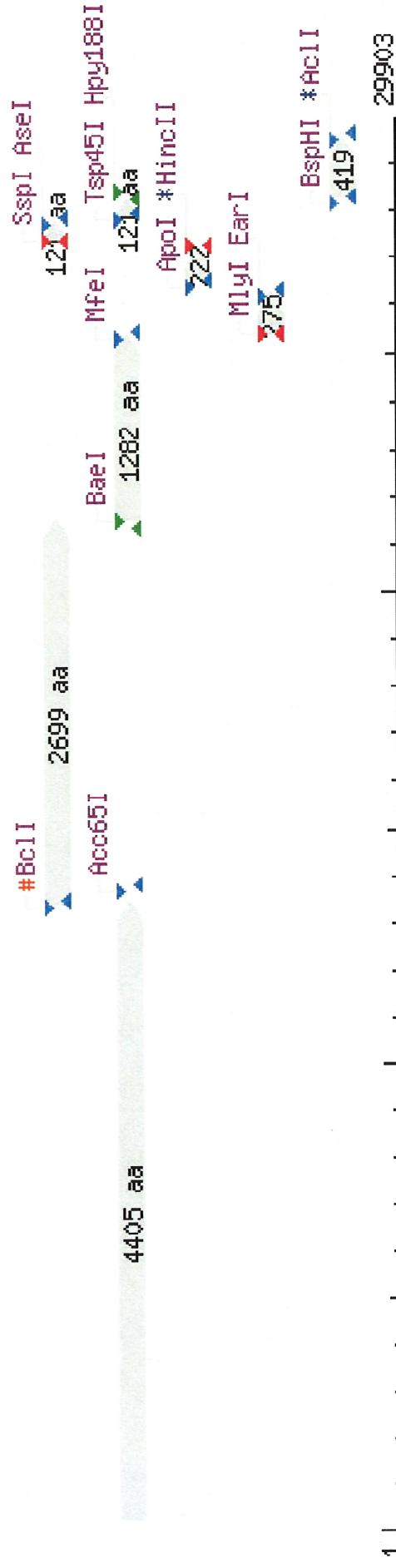
- BsmBI (Plus strand)
 - 5' - CGTCTC>NNNN-3' 5' - NNNNNGAGACG-3'
 - 3' - GCAGAG>NNNN- 5' BsaI^{3'} (Plus strand) GC-
 - 5' - GGTC>NNNN-3' 5' BsaI (Minus strand)
 - 3' - CCAGAG>NNNN- 5' SARS-COV-2 NNNNGAGACG-3' not
- have any SacII or AscI restriction sites

(b)(1), (b)(3), (b)(3)(C) USC 3024(i), Sec 1.4(c), Sec 1.4(e)

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RE-based Fragment Build Option – BsmBI/BsaI

(4 nt overhangs)

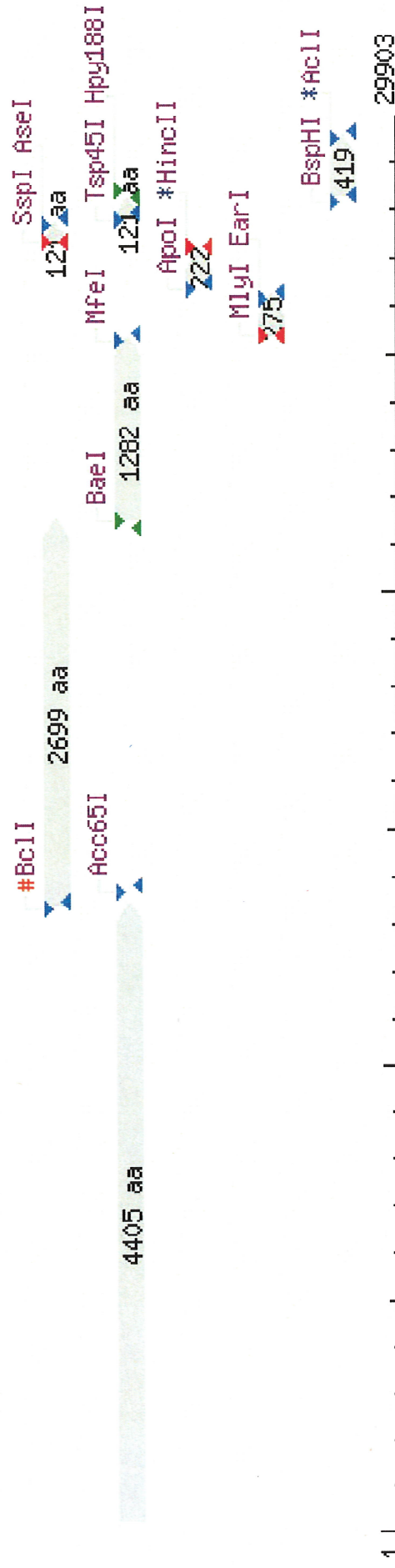


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RE-based Fragment Build Option – BsaI/Esp3I

(Invisible restriction sites)



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Xie et al., 2020 SARS-CoV-2 FLC Assembly

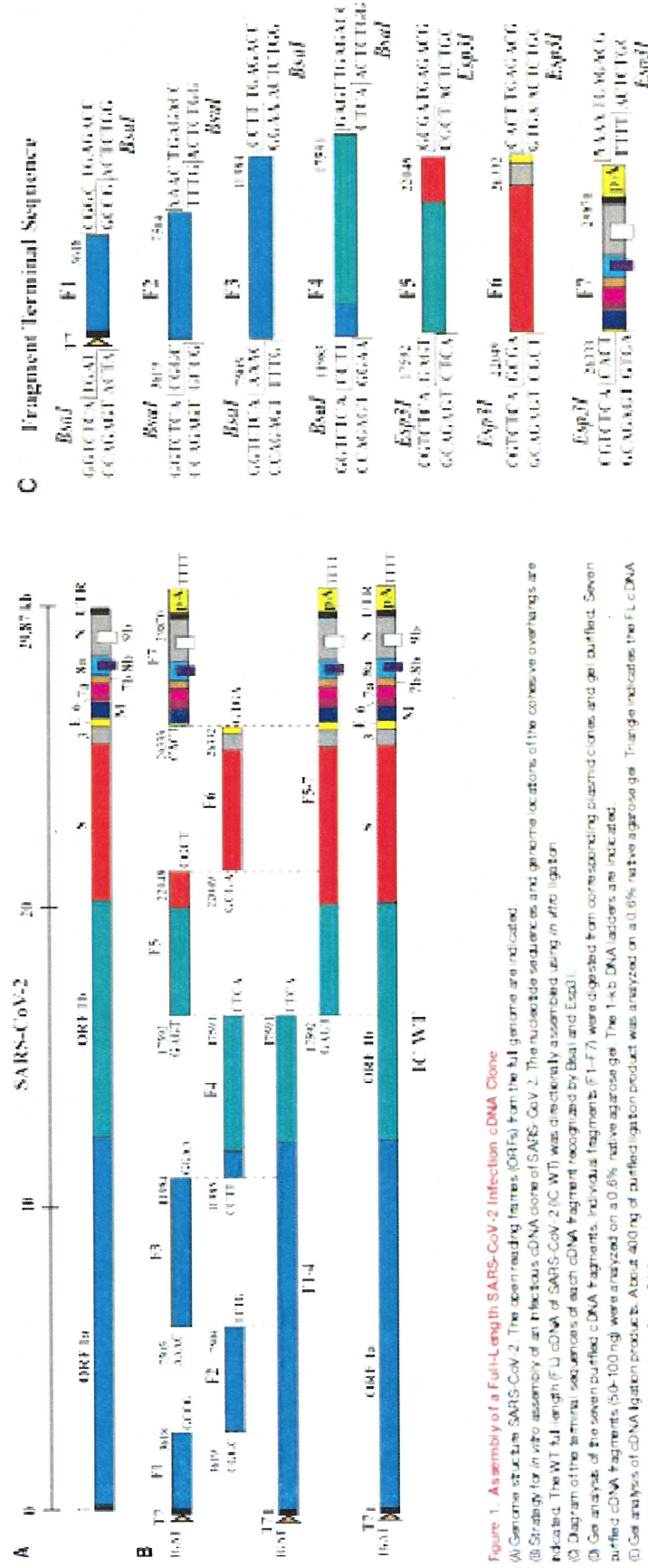


Figure 1. Assembly of a Full-Length SARS-CoV-2 Infection cDNA Clone
(A) Genome structure SARS-CoV-2. The open reading frames (ORFs) from the full genome are indicated.
(B) Strategy for *in vitro* assembly of an infectious cDNA clone of SARS-CoV-2. The nucleotide sequences and genome locations of the cohesive overhangs are indicated. The WT full length (F) cDNA of SARS-CoV-2 (C) WT was directionally assembled using *in vitro* ligation.
(D) Diagram of the terminal sequences of each cDNA fragment recognized by BsaI and EcoRI.
(E) Gel analysis of the seven purified cDNA fragments. Individual fragments (F1-F7) were digested from corresponding plasmid clones and gel purified. Seven purified cDNA fragments (50–100 ng) were analyzed on a 0.6% native agarose gel. The 1 kb DNA ladders are indicated.
(F) Gel analysis of cDNA ligation products. About 400 ng of purified ligation product was analyzed on a 0.6% native agarose gel. Triangle indicates the F1 cDNA product. Circles indicate the intermediate cDNA products.
(G) Gel analysis of RNA transcripts. About 1 µg of *in vitro* transcribed (IT) RNAs were analyzed on a 0.6% native agarose gel. DNA ladders are indicated. Because this is a native agarose gel, the DNA size is not directly correlated to the RNA size. Triangle indicates the genome-length RNA transcript. Circles show the shorter RNA transcripts.

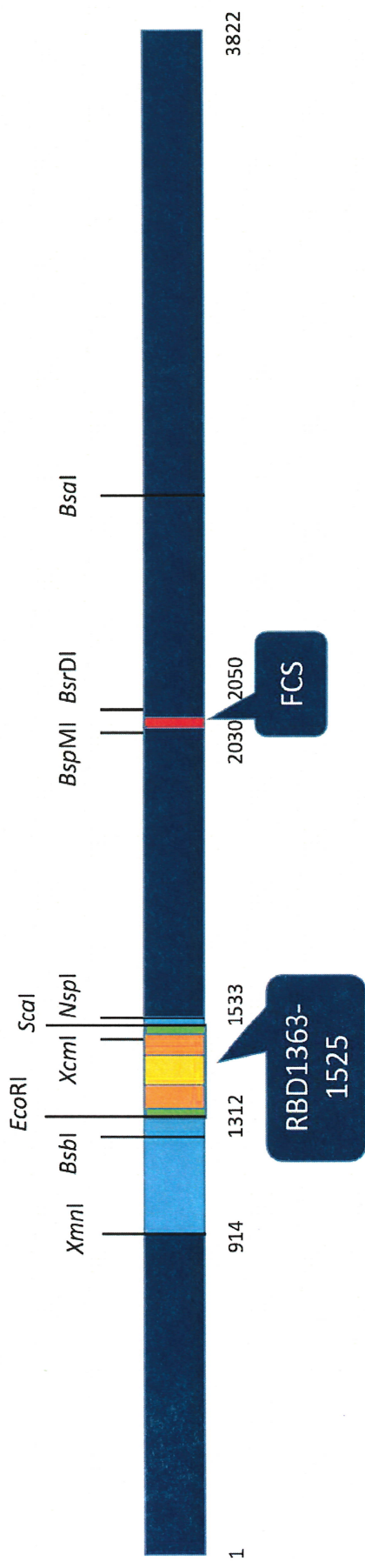
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Spike Gene

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SARS CoV-2 SPIKE GENE



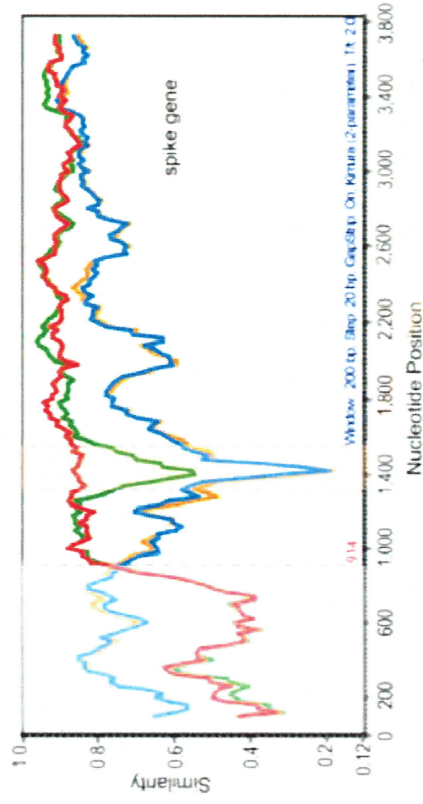
Highest homology to RaTG13Pangolin CoV

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SARS-COV-2 SPIKE GENE SEGMENTS

QUERY: PANGOLIN-COV

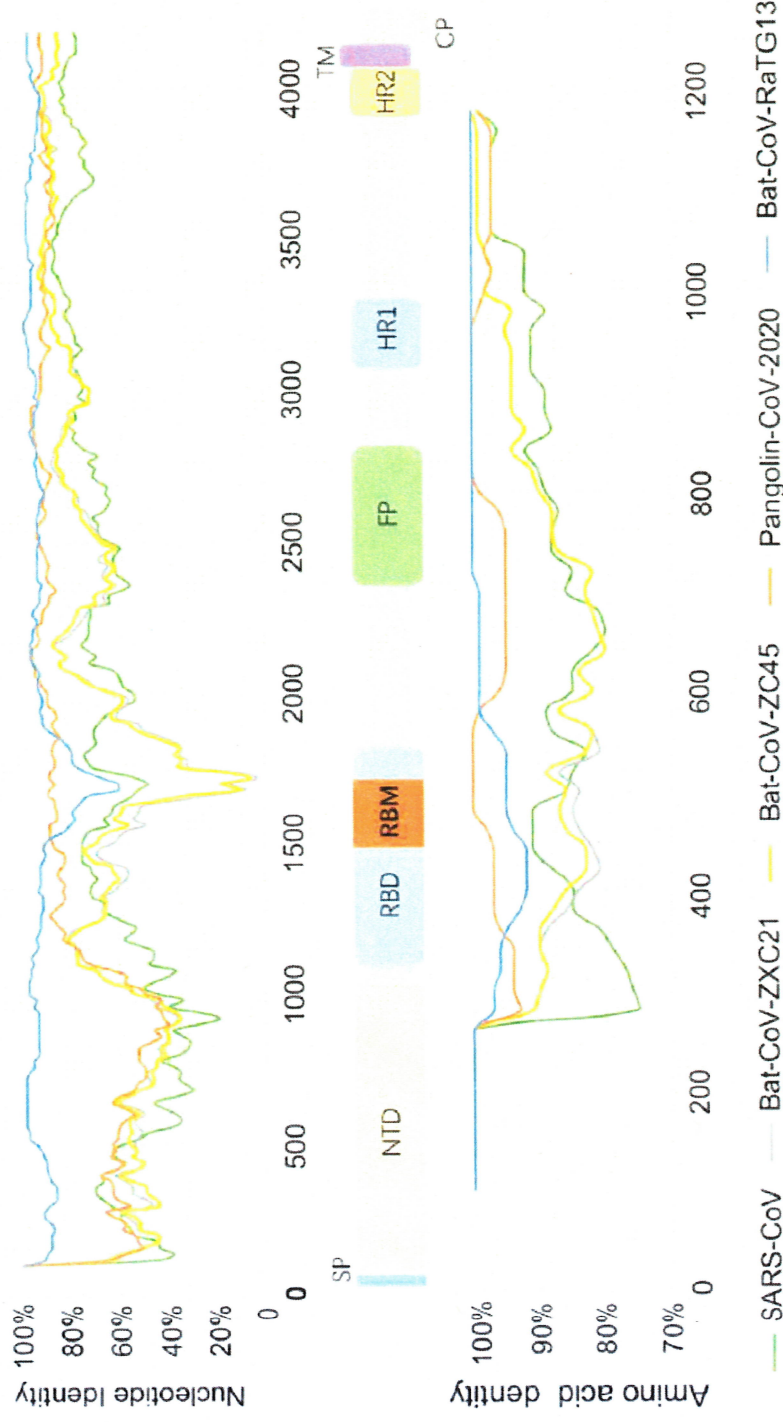
- SARS-CoV-2_WIV02
- Bat CoV RaTG13
- Bat SARSr-CoV_ZC45
- Bat SARSr-CoV_ZXC21



b



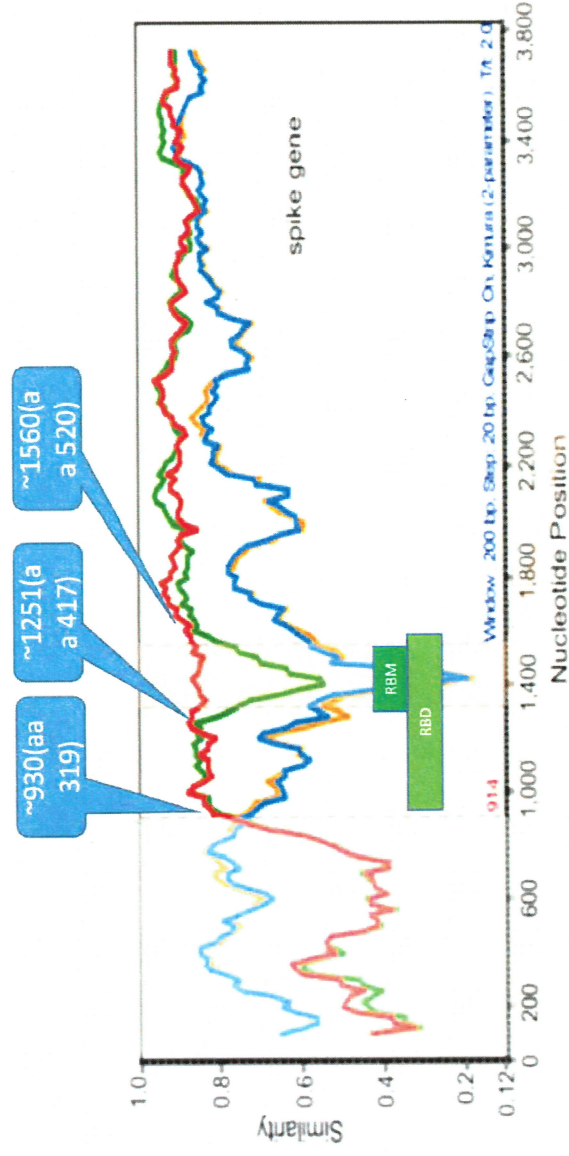
SARS-CoV-2 SimPLOT



- "A recent study found that a human ACE2-binding ridge in SARS-CoV-2 RBD takes a more compact conformation compared with the SARS-CoV RBD; moreover, several residue changes in SARS-CoV-2 RBD may also enhance its human ACE2-binding affinity [13]. The core residues in RBM which may related to higher human ACE2-binding affinity than SARS-CoV are 100% identical between SARS-CoV-2 and CoV-Pangolin-2020. Therefore, pangolin-CoV-2020 (CoV-pangolin/GD) potentially recognizes human ACE2 better than the SARS-CoV."

Minimal Receptor Binding Domain Cassette

- WIV scientists previously defined the minimal Receptor Binding Domain cassette that could functionally transfer ACE2 binding capability from one Spike protein to another SARS Nucleotide: 930-1554 SARS Amino Acid: 310-518 Receptor Binding Motif SARS Nucleotide: 1251-1482 SARS Amino Acid: 417-494 Homology cut points of SARS-CoV-2 coincide with WIV-identified borders of RBD and RBM



Furin Cleavage Site

SARS-CoV CATGTCGACACTTCTTATGAGTGGGACATTCCCTATTGGAGCTGGCAATTTGTGCTAGTTAC
1980 H V D T S Y E C D I P I G A G I C A S YSARS-CoV-2
CATGTCAACAACATCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S YBCoV RaTG13
CATGTCAATAAAGTGTGAGTGTGACATACCTATTGGTGCAGGAATATGCCAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S Y SARS-CoV CATAAGTTTCTTTATT-----
ACGTAGTACTAGCCAAAATCTATTGTGGCT 2028 H T V S L L R S T S Q K S I
V ASARS-CoV-2 CAGACTCAGACTAATTCTCCTCGGCGGCACGTAGTGTAGCTAGTCAATCCATCATTGCC
2082 Q T Q T N S P R R A R S V A S Q S I I ABCoV RaTG13
CAGACTCAAACTAATTC-----ACGTAGTGTGGCCAGTCAATCTATTATTGCC 2070 Q T Q
T N S R S V A S Q S I I AFurin Cleavage SiteNmeAIII Restriction Site

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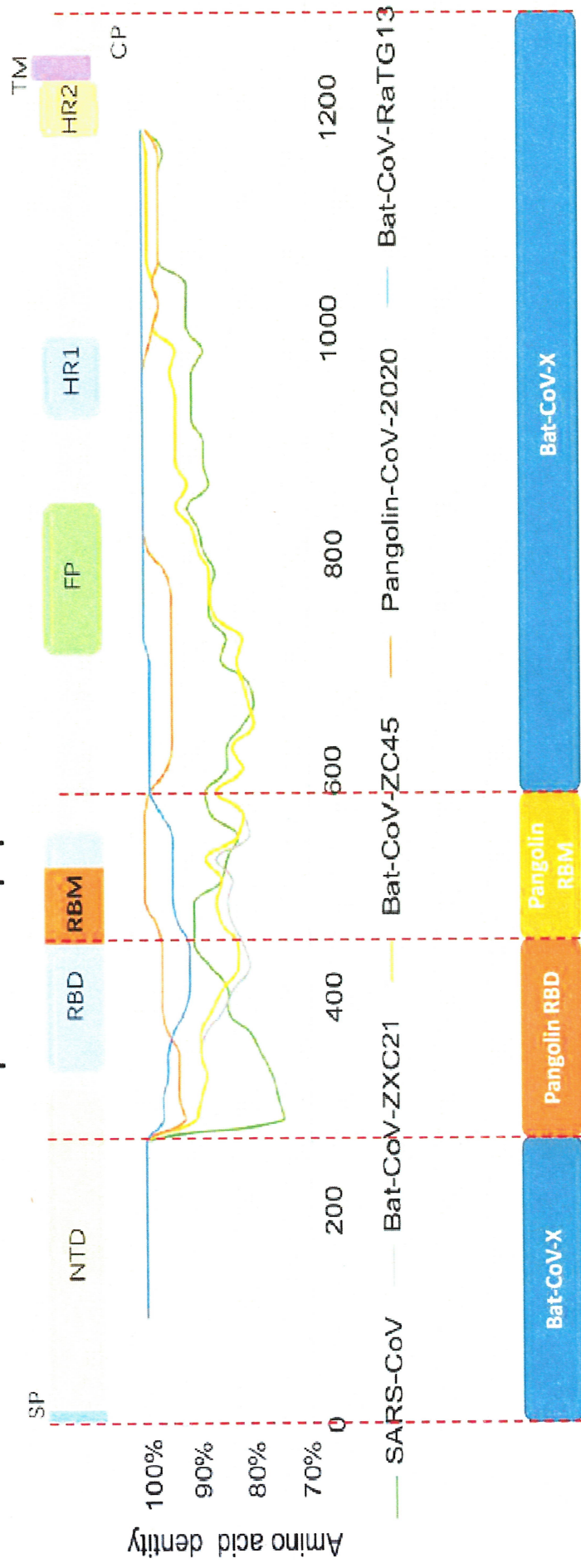
SARS-CoV-2 Furin Cleavage Site GC Content

- The percent GC of the furin cleavage site insert is 77% compared to ~40% of the surrounding DNA. Contains an NmeAIII restriction site. The other CoV's with FCS have a %GC of <55%

Virus	Nucleotide and Amino Acid Sequences
SARS-CoV-2 (47%)	G G T A T A T G C G C T A G T T A T C A G A C T C A G A C T A A T T C F E R K L G G G S A C G T A G T G T A G C T A G T C A A T C C A T C A T T G C C T A C A C T A T G G I C A S Y Q T T Q T N S P F R A R S V A S Q S I I A Y T M %GC: 17/39 = 44%
MERS-CoV (5%)	C T C T G T G C T C T T C C T G A C A C C C T A G T A C T C T C A C A C C C T C G C A G T G T G C G C T C T G T T C C A G G T G A A A T G C G C T T G G C A T C C A T T G C T T L C A L P D T P S T L T P R S S V R S S V P G E M R L A S I A %GC: 21/39 = 54%
BatCoV-HKU5 (47%)	G G T C A A T C A C T T T G T G C T A T T C C A C C A A C T A C T T C T T C A C G C G T T C G A C G T G C T A C T T C T G G T G C A T C G A T G T G T T T C A A A T C G C C C G Q S L C A I P P T T S T S T S C A C C C V R R A T S G A S D V F Q I A %GC: 19/39 = 49%
IBV-Besudette (34%)	G A G A C C A G T T T T A C A T C A A A T C A C T A A T G G A A C A C G C T C G T T T T A G A C G T T C T A T T A C T G A A A A T G T T G C A A A T T G C C C T T A T G T T E N Q F Y I K I T N G T R R F R R S S I T E N V A N C P Y V %GC: 13/39 = 33%

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SARS-CoV-2 Spike Appears to be a Chimera



Break points align with those identified by WIV Scientists in 2008 (Ren *et al.*, 2008)

RmYN02 a Red Herring

- Zhou et al., 2020 publish paper describing Bat CoV rmYN02Next generation sequencing was done on pooled bat samples to develop two genome sequences – RmYN01 and RmYN02. Claim that RmYN02 contains inserted nucleotides at the S1/S2 cleavage site. Assert that the SARS-CoV-2 FCS is therefore of natural origin. No virus is available for peer confirmation.

H

SARS-CoV-2 Numbering	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693
Consensus SARS-CoV-2	G	A	G	I	C	A	S	Y	Q	T	Q	T	N	S	P	R	R	A	R	S	V	A	S	Q	S	I	I
RmYN02	G	A	G	V	C	A	S	Y	-	-	-	-	N	S	P	-	A	A	R	-	V	G	T	N	S	I	I
RaTG13	G	A	G	I	C	A	S	Y	Q	T	Q	T	N	S	-	-	-	R	S	V	A	S	Q	S	I	I	I
ZC45	G	A	G	I	C	A	S	Y	H	T	A	S	I	L	-	-	-	R	S	T	S	Q	K	A	I	V	V
ZXC21	G	A	G	I	C	A	S	Y	H	T	A	S	I	L	-	-	-	R	S	T	G	Q	K	A	I	V	V
pangolin/MP789/2019	G	A	G	I	C	A	S	Y	Q	T	Q	T	N	S	-	-	-	R	S	V	S	Q	X	A	I	I	I
pangolin/GX/PSL/2017	G	A	G	I	C	A	S	Y	H	S	M	S	S	F	-	-	-	R	S	V	N	Q	R	S	I	I	I
SARS-CoV GZ02	G	A	G	I	C	A	S	Y	H	T	V	S	L	L	-	-	-	R	S	T	S	Q	K	S	I	V	V
RmYN01	G	A	G	I	C	A	S	Y	H	T	A	S	L	L	-	-	-	R	N	T	G	Q	K	S	I	V	V

Polybasic cleavage site

O-linked glycan residues

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RmYN02 a Red Herring

Virus	Nucleotide and Amino Acid Sequences
SARS-CoV-2	G G T A T A T G C G C T A G T T A T C A G A C T C A G A C T A A T T C T C C T C G G C G G G C A C G T A G T G T A G C T A G T C A A T C C A T C A T T G C C T A C A C T A T G G I C A S Y Q T T Q T N S P R R A R S V A S Q S I I A Y T M
Delta763	G G A A T A T G C G C C A G T T A T C A G A C T C A A A C T A A T T C A C G T A G T G T G G C C A G T C A A T C T A T T A T T G C C T A C A C T A T G G I C A S Y Q T T Q T N S R S V A S Q S I I A Y T M
BetaCoV2	G G T G T G T G T G C C A G T T A C A A C T C A C C T G C A G C G C G T G T A G G T A C T A A T T C C A T T A T T G C C T A C G C G A T G G V C A S Y N S P A A A R V G T T N S I I A Y A M

The next 3 pages are withheld in full citing (b)(1) and (b)(3) 50 USC 3024(i) and are not provided.

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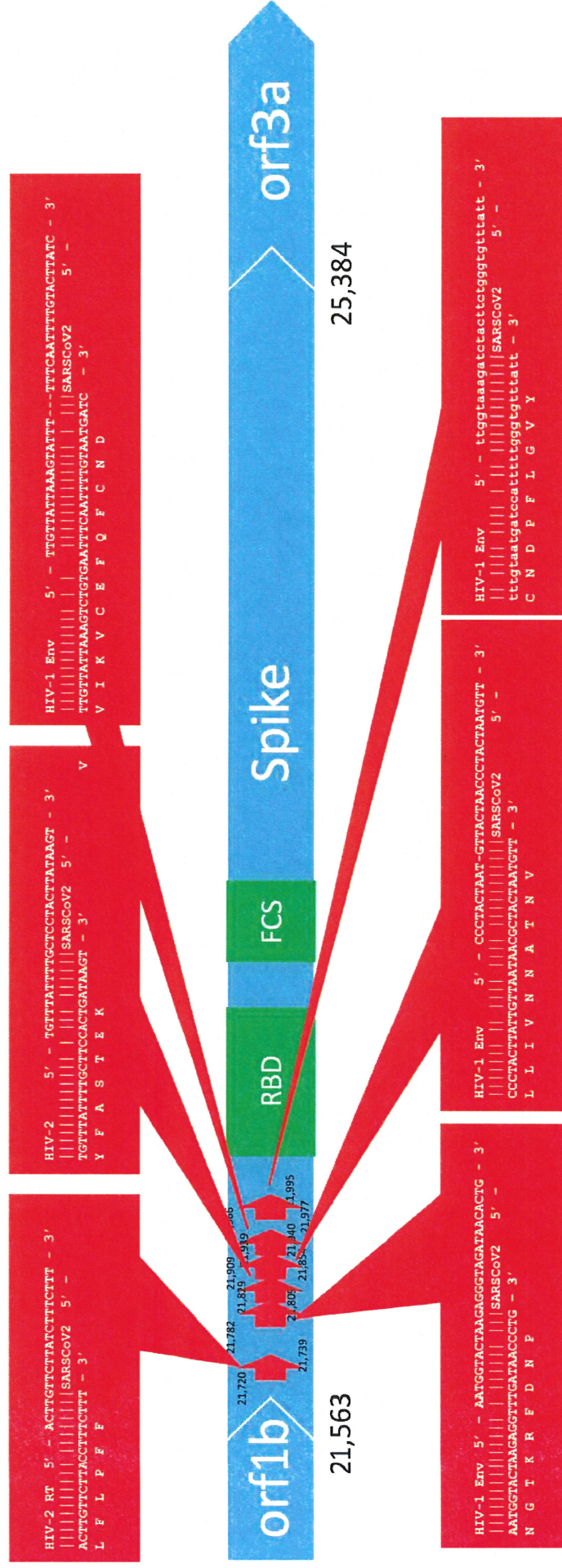
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HIV Epitopes

Perez, 2020

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HIV Sequences in the SARS-CoV-2 Spike Gene



Adapted from Perez, 2020

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Perez, 2020 Scientific Challenges

- None of the six proposed regions are identical at either the nucleotide or amino acid level with the corresponding HIV/SIV segments. None of the six peptides are related to identified immunosuppressive regions of HIV and SIV (Retroviral ISU Domains). The HIV gp41 Immunosuppressive (ISU) Domains sequence is KQLQARILAVERYLKDQQLGG - this sequence does not match any of the six. Four of the six regions either perfectly or almost perfectly match corresponding peptides in multiple Pangolin CoVs - Perez did not account for Pangolin genomes in the paper.

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Alternative Scenario

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Hypothetical Laboratory Origin of SARS-CoV-2

- WIV conducted a longitudinal studies to isolate a large number of bat Coronaviruses from multiple locations in China (2011-2015)WIV Developed Reverse Genetic System, assembled WIV1 full-length infectious clone, and created chimeric viruses exchanging the WIV1 spike gene with the spike gene from other bat Coronaviruses (2015-2017)WIV and other Chinese scientists conduct gain of function studies on SARS, MERS, IBV, and PEDV to insert furin cleavage sites demonstrating increased virulence of the chimeric virusesWIV conducted in vivo and in vitro studies to characterize the bank of bat CoronavirusesWIV conducted the live bat Coronavirus studies under BSL2 conditionsHypothesis: Between 2017 and 2019, WIV created a full-length infectious clone in pBAC-CMV using an unpublished bat Coronavirus genome as template (BatCoVX)Hypothesis: Between 2017 and 2019, WIV created chimeric Bat-CoV-X viruses using the pBAC-CMV-BCoVX backbone and swapping out key cassettes with other bat Coronaviruses (RBD, RBM, etc.) and adding additional features such as a furin cleavage siteHypothesis: In 2018-2019, WIV conducted in vitro and in vivo studies to characterize the BatCoVX chimeric viruses under BSL2 conditionsHypothesis: In mid-2019, one of the not fully characterized Bat-CoV-X chimeric viruses escaped from the WIV facilities and begins infecting civilians in the city of WuhanHypothesis: Starting in mid-2019 through present, WIV and other Chinese laboratories conduct studies to characterize the Chimeric BCoVX virus that escaped (now called SARS-CoV-2)WIV (Zhou et al., 2020) publishes the 2019-nCoV genome sequence showing relatedness to RaTG13 (a previously unpublished genome)BatCoVX likely highly related to RaTG13Hypothesis: Beginning in early 2020, WIV and other government controlled agencies begin to publish obfuscation information to drive the narrative that SARS-CoV-2 is of natural origin and resulted from natural recombinationRaTG13RMYN02Pangolin CoV's

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CONCLUSION

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Concluding Points

- WIV possesses a bank of Bat Coronavirus isolates. WIV has scientists experienced in Coronavirology and Coronavirus Infectious Clone generation. WIV Scientists generated chimeric SARS-CoV and Bat-CoV Spike genes to identify minimal Spike Receptor Binding Domain cassette that could transfer receptor binding specificity (Ren et al., 2008). WIV possesses an existing and published Coronavirus Reverse Genetics System (Zeng et al., 2016) utilizing their pBAC-CMV-WIV1 Full-length clone to generate chimeras with Bat-CoV spike genes (Hu et al., 2017). WIV has BSL2/BSL3/BSL4 animal facilities. WIV has multiple in vitro assays (apoptosis, IFN- β induction, etc.) to characterize their Bat Coronaviruses and chimeric Bat Coronaviruses. WIV and other Chinese researchers have conducted Gain of Function studies in SARS, MERS, IBV, and PEDV to add Furin Cleavage Sites to CoV Spike protein. The absence of a published progenitor virus for SARS-CoV-2 only indicates that it has not been published, not that it does not exist. The genomic sequence of SARS-CoV-2 has Type IIS restriction sites that are consistent with being generated by the Golden Gate Cloning system utilizing the published pBAC-CMV plasmid. The SARS-CoV-2 genome has several break points where homology jumps from Bat Coronaviruses to Pangolin Coronaviruses, which is consistent with a synthesized chimeric virus. The SARS-CoV-2 Spike protein similarity with RaTG13 and Pangolin CoV Spike proteins may also be explained by use of cassettes swapped into the base virus – these break points align with those identified by WIV scientists (Ren et al., 2008). There are no other published Betacoronaviruses that possess a Furin Cleavage Site in their Spike protein (RmYN02 does not have an insertion). Zeng et al., 2016 stated that "All experiments using live virus was conducted under biosafety level 2 (BSL2) conditions" which would make an accidental release of a pathogenic Bat-CoV capable of binding human ACE2 more likely. A chimeric virus comprised of segments from natural Bat-CoV genomes would appear like a recombined virus.

The molecular biology capabilities of WIV and the genome assessment are consistent with the hypothesis that SARS-CoV-2 was a lab-engineered virus that was part of a bank of chimeric viruses in Zhen-Li Shi's laboratory at WIV that escaped from containment

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BACK-UP SLIDES

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SARS-CoV CATGTCGACACTTCTTATGAGTGCACATTCTTATTGGAGCTGGCATTGTGCTAGTTAC
1980 H V D T S Y E C D I P I G A G I C A S YSARS-CoV-2
CATGTCAACAACTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S YBCoV RaTG13
CATGTCAATAACTCGTATGAGTGTGACATACCTATTGGTGCAGGAATATGCGCCAGTTAT 2022
H V N N S Y E C D I P I G A G I C A S Y SARS-CoV CATAAGTTTCTTTATT-----
ACGTAGTACTAGCCAAAATCTATTGTGGCT 2028 H T V S L L R S T S Q K S I
V ASARS-CoV-2 CAGACTCAGACTAATTCCTCGCGGGCACGTAAGTAGCTAGTCAATCCATCATTGCC
2082 Q T Q T N S P R A R S V A S Q S I I ABCoV RaTG13
CAGACTCAAACTAATTC-----ACGTAGTGTGGCCAGTCAATCTATTATTGCC 2070 Q T Q
T N S R S V A S Q S I I AFurin Cleavage SiteBspMI Restriction SiteNmeAIII Restriction SiteBsrDI
Restriction Site

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