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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.

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Building a Full-Length Clone

Using Type IIS Restriction Enzymes and Golden Gate Assembly System

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WIV SARS-CoV Reverse Genetics System

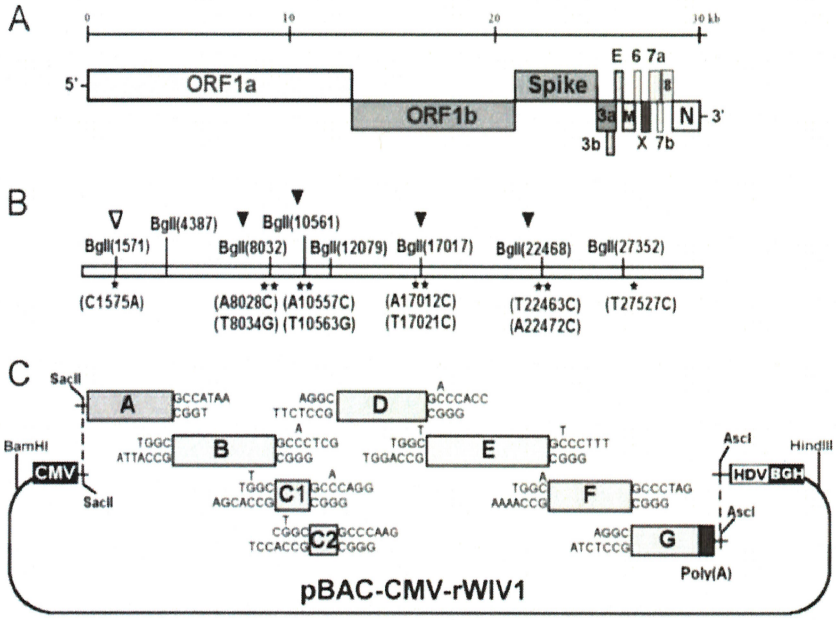

FIG 1 Strategy for construction of an infectious WIV1 BAC clone. (A) Genomic structure of WIV1. (B) The mutations are indicated under the stars. C1575A was used to ablate a natural BglII site at nucleotide 1571 (▽), and T27527C was used to disrupt a potential T7 stop site. The others were for introducing 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 pBeloBAC11 between BamHI and HindIII sites. SacII and AscI sites were introduced between the CMV promoter and ribozyme. Fragments A to G were inserted into the pBAC-CMV plasmid in a single step.

Zeng et al., 2016

Eleven Tips For Optimizing Your Golden Gate Assembly Reactions

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your Golden Gate Assembly experiment.

1 Check your sequences

2 Orient your primers

3 Choose the right plasmid

4 Choose the right buffer

5 Increase your complex assembly efficiency by increasing the Golden Gate cycling levels

6 Make sure your plasmid prep is RNA-free

7 Avoid primer dimers

8 Avoid PCR-induced errors

9 Decrease insert amount for complex assemblies

10 Carefully design EVERY insert's overhang

11 Check for a sequence error if your assembly becomes non-functional

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Mixed Sources

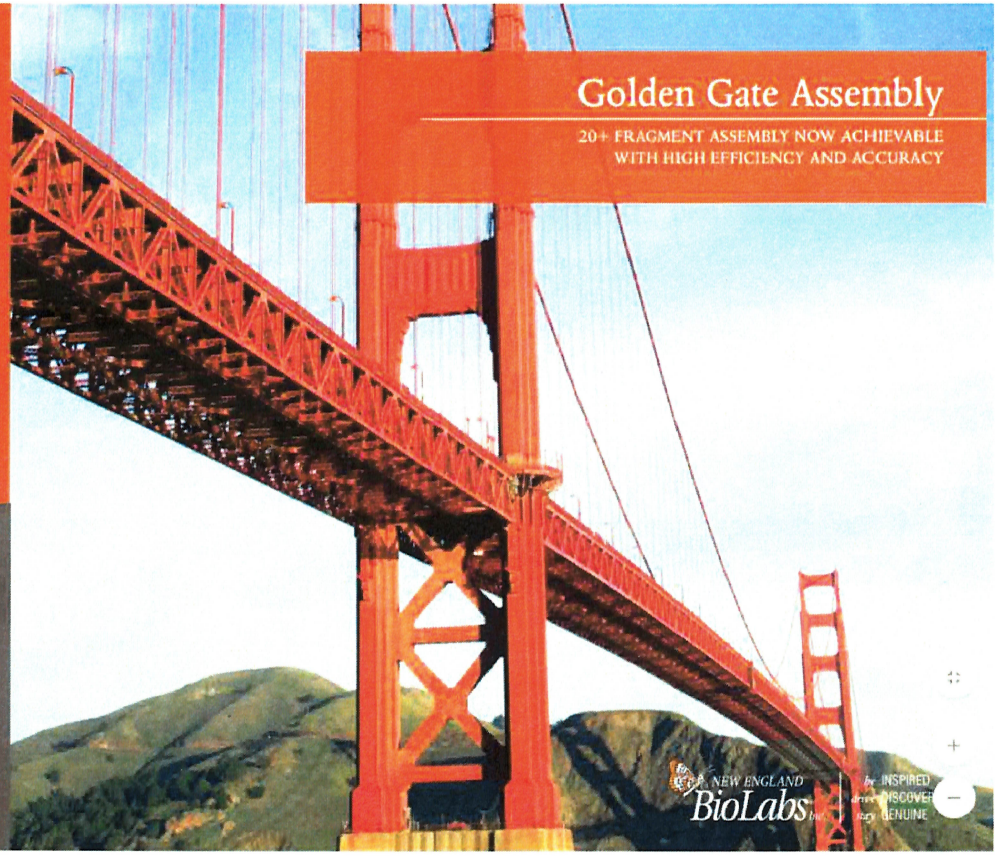
Featured Tools

Download the **NEB AR App** for iPhone® or iPad®. Scan the augmented reality butterfly icon 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/nebeta-tools**

- Ligase Fidelity Viewer™ (v2) – Visualize overhang ligation preferences
- GetSet™ – Predict high-fidelity junction sets
- SplitSet™ – Split DNA sequence for scarless high-fidelity assembly



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Push the limits of Golden Gate Assembly

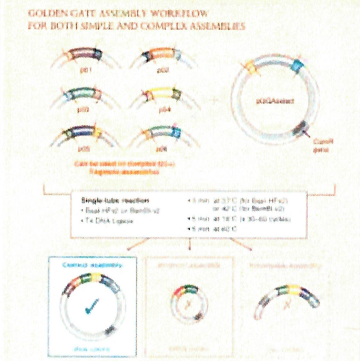
Golden Gate Assembly (1,2) allows for the efficient and seamless assembly of DNA fragments using activities of Type IIS restriction enzymes and T4 DNA Ligase.

With constant advances in both the development of new enzymes and research on maximizing 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 insert cloning in just 5 minutes using our fast protocols
- Generate libraries with high efficiencies
- 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 >15 kb)

GOLDEN GATE ASSEMBLY WORKFLOW
FOR BOTH SIMPLE AND COMPLEX ASSEMBLIES



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.

PRODUCT	NEB #	SEQUENCE	SIZE
BbsI	R0539S/L	GAAGAC(2/6)	300/1,500 units
BbsI-HF	R3539S/L	GAAGAC(2/6)	300/1,500 units
BsaI	R0535S/L	GGTCTC(1/5)	1,000/5,000 units
BsaI-HFv2	R3733S/L	GGTCTC(1/5)	1,000/5,000 units
BsmBI-v2	R0739S/L	CGTCTC(1/5)	200/1,000 units
Esp3I	R0734S/L	CGTCTC(1/5)	300/1,500 units
BtgZI	R0703S/L	GCGATG(10/14)	100/500 units
SapI	R0569S/L	GCTCTTC(1/4)	250/1,250 units
BspQI	R0712S/L	GCTCTTC(1/4)	500/2,500 units

BsaI-HFv2

5´GGTCTCNNNNN 3´
3´CCAGAGNNNNN 5´

What users are saying:

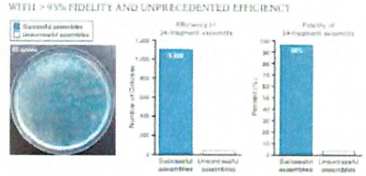
FEATURED KITS

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

The absence of internal sites in a sequence determines the choice of which Type IIS restriction enzyme to drive the assembly. For your convenience, NEB now offers two kits for Golden Gate Assembly featuring BsaI-HFv2 or BsmBI-v2. Both kits incorporate digestion followed by ligation with T4 DNA Ligase into a single reaction, and can be used to assemble 2-20+ fragments in a single step.

PRODUCT	NEB #	SIZE
NEB Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 rxns
NEB Golden Gate Assembly Kit (BsaI-HFv2)	E1601S/L	20/100 rxns

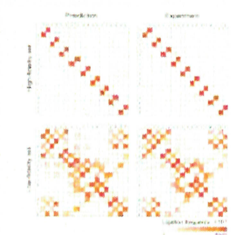
COMPLEX GOLDEN GATE ASSEMBLY
WITH >95% FIDELITY AND UNPRECEDENTED EFFICIENCY



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 end-joining ligation fidelity in order to predict overhang sets with improved fidelity (3). This research has enabled complex fragment assemblies with high efficiency and >90% accuracy. More information can be found in the NEB publication, *Comprehensive Profiling of Four Base Overhang Ligation Fidelity by T4 DNA Ligase and Application to DNA Assembly* (3) or in our webinar, *Fidelity and Bias in End-Joining Ligation: Enabling complex, multi-fragment Golden Gate DNA Assembly*. We also encourage you to try our free Ligase Fidelity Tools.

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



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

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

MoClo (and GoldenBraid 2.0) uses 3 levels of successive assembly. The community has agreed upon a set of common standard overhangs for each level. Utilizing gathered ligase fidelity information, NEB has expanded each level of assembly overhangs without sacrificing fidelity. The expanded sets are:

- Level 0 (Basic parts)
- Level 1 (Transcriptional units)
- Level 2 (Multigene constructs)

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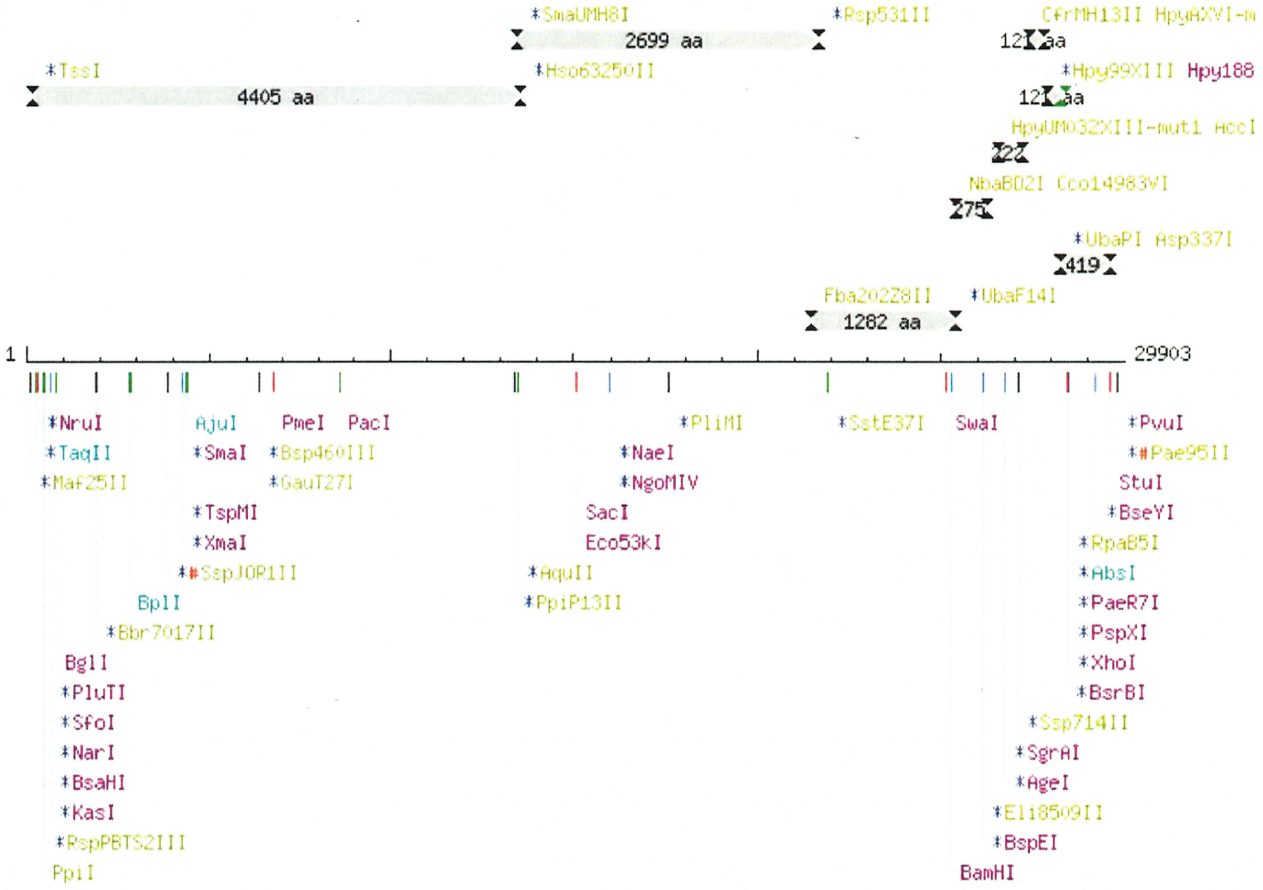
SARS-COV-2 GENOME RESTRICTION MAP



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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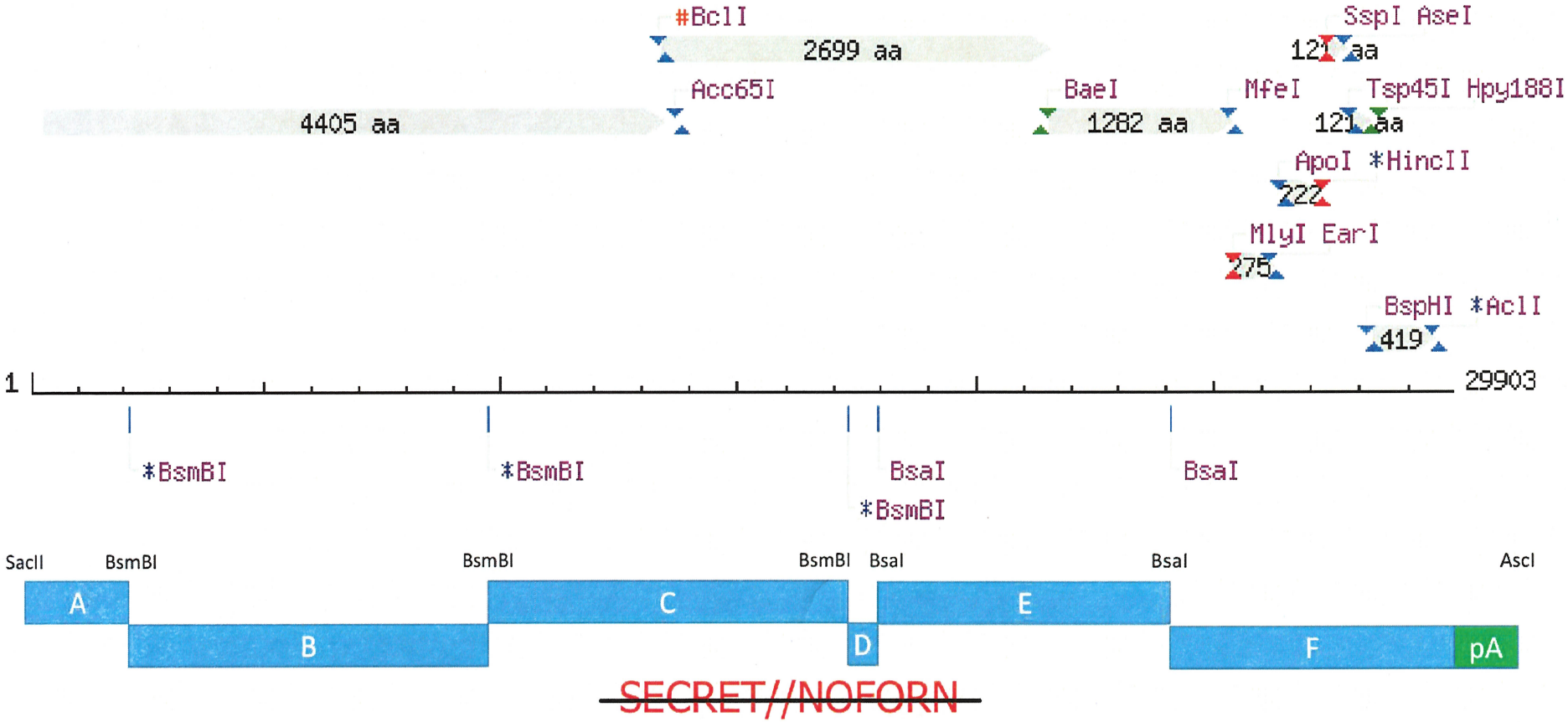
RE-based Fragment Build Option – *Bsm*BI/*Bsa*I (4 nt overhangs)



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

Spike Gene

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