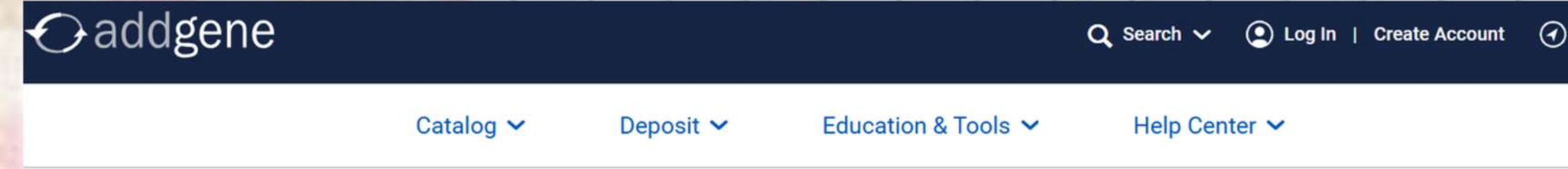


Tutorial- Golden Gate Assembly on Benchling

1

Made by Ana Gomez- HTGAA Spring 2026

1. Find a Plasmid Sequence: pUC19



Browse / Joachim Messing / Norrander et al / pUC19

pUC19
(Plasmid #50005)

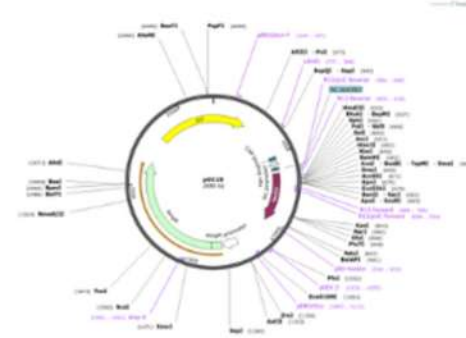
Print

Purpose
(Empty Backbone) pUC cloning vector

Depositing Lab
Joachim Messing

Publication
Norrander et al Gene. 1983 Dec;26(1):101-6.
(How to cite ↓)

Sequence Information
Sequences (3)



Enlarge | View all sequences



Related items:

From this article
Joachim Messing Lab Plasmids

pUC19 Sequences (3)

Addgene Sequences:
Full (1) | Partial (2)

Full Sequences from Addgene (1)

Based on next-generation sequencing (NGS) results where indicated (Addgene NGS Result), or assembled from reference sequences and/or Sanger results (Addgene Assembled Sequence).

Analyze Sequence

GenBank | SnapGene | File Help

Copy sequence

```
> Addgene NGS Result
GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCAGAAAGGAGAAAGCGGCAGGTATCCG
GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATA
GTCCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCT
ATGGAAAAACGCCAGCAACCGGCCTTTTACGGTTCCTGGCCTTTTCTGGCCTTTTCTCACATGTTT
TTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC
GCAGCCGAACGACCGAGCGCAGCGAGTCACTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC
TCTCCCAGCGCTTGGCCGATTATTAATGAGCTGGCACGACAGGTTTCCGACTGGAAAGCGGGCAGT
GAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGG
CTCGTATGTTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGC
CAAGCTGCGTGCAGGTCGACTTAGAGGATCCCCGGGTACCGAGCTCGAATTCAGTGGCCGTCGT
TTTACAACGTCGTGACTGGGAAACCTGGCGTTACCAACTTAATCGCCTTGCAGCACATCCCCCTTT
GCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCAGTCCCTTCCCAACAGTTGCGCAGCCTGAATGGCG
AATGGCGCCTGATCGGGTATTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCCT
```

Select the full sequence

Made by Ana Gomez- HTGAA Spring 2026



Tutorial- Golden Gate Assembly on Benchling

2

Made by Ana Gomez- HTGAA Spring 2026

2. Login in Benchling and create a new project. Then select Add New DNA

3. Fill the backbone info | Select circular DNA | Paste the plasmid sequence

The screenshot shows the Benchling interface. On the left, there's a sidebar with a search bar and a list of project items. The main area displays a 'Protein design (codon optimized)' with a linear map showing various restriction enzyme sites (BcgI, Hpy99I, BsiHKAI, NspI, MspA1I, BsiEI, ApoI, ScrFI, StyD4I, BssKI, HgaI, RsaI, CviQI). A dropdown menu is open over the 'DNA / RNA sequence' option, listing several options for adding new DNA/RNA sequences, with 'New DNA / RNA sequence' highlighted.

The screenshot shows the 'Create DNA / RNA sequence' form. It has tabs for 'CREATE NEW', 'UPLOAD FILES', 'IMPORT FROM DATABASE', and 'SELECT CHROMOSOMAL REGION'. The 'Name' field contains 'pUC19_backbone'. The 'Project folder' is set to 'Week 6- 2026a-ana-gomez'. Under 'Nucleotide type', 'DNA' is selected. Under 'Topology', 'Circular' is selected. The 'Schema' dropdown is set to 'Select a schema...'. The 'Bases' field contains the sequence: GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAC

Made by Ana Gomez- HTGAA Spring 2026



Tutorial- Golden Gate Assembly on Benchling

Made by Ana Gomez- HTGAA Spring 2026

4. Add a New sequence in your project with the small DNA you want to insert

5. After having the two sequences, add the Assembly DNA sequences by cloning option

Create DNA / RNA sequence

CREATE NEW | UPLOAD FILES | IMPORT FROM DATABASE | SELECT CHROMOSOMAL REGION

Name *
small_peptide_insert

Project folder*
Week 6- 2026a-ana-gomez

Nucleotide type*
DNA | RNA | Topology: Linear

Schema
Select a schema...

Bases
ATGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTTAA

Close | Create

Projects / Week 6- 2026a-ana-gomez

SEQUENCE MAP | LINEAR MAP | DESCRIPTION | METADATA | ENTI

Search

Type | Filters

- pUC19_backbone (Last modified 12 minutes ago)
- small_peptide_insert (Last modified just now)

Folder | File | Entry | Protocol | DNA / RNA sequence | AA sequence | Oligo | Assembly | CRISPR | More

Assembly options:
 Assemble DNA sequences by cloning
 Assemble sequences and oligos by concatenation

Small peptide structure output

GGTCTCAATGATGGCTGCTGCTGCTGCT
GCTGCTGCTGCTGCTTAAGCTTGAGACC

56 BP

Made by Ana Gomez- HTGAA Spring 2026

Tutorial- Golden Gate Assembly on Benchling

4

Made by Ana Gomez- HTGAA Spring 2026

6. Change the name of the assemble DNA project, select the Golden Gate option

7. Change the restriction enzyme from BbsI to BsaI

Assemble DNA

Name *
pUC19 + peptide assembly

Project folder*
Week 6- 2026a-ana-gomez

Number of fragment bins*
- 2 +

Topology of construct
Circular

Cloning method
Golden Gate Gibson Homology

Join up to 15 DNA fragments into a single piece using Type IIS restriction enzymes and T4 DNA ligase. [Hide details](#)
Optionally, you can use primers to introduce cut sites via PCR.

Review the following parameters. [Reset to defaults](#)

Type IIS Restriction Enzyme
BbsI

Fragment production method
Use existing cut sites
You can change this later.

Cancel Save

Search

AcuI

BbsI

BfuAI

BsaI

BsmBI

BspMI

BtgZI

SapI

AarI

PaqCI

BbsI

2026a-ana-gomez

Topology of construct
Circular

Gibson Homology

Join up to 15 DNA fragments into a single piece using Type IIS restriction enzymes and T4 DNA ligase. [Hide details](#)
Optionally, you can use primers to introduce cut sites via PCR.

[Reset to defaults](#)

Fragment production method
Use existing cut sites
You can change this later.

Made by Ana Gomez- HTGAA Spring 2026

Tutorial- Golden Gate Assembly on Benchling

5

Made by Ana Gomez- HTGAA Spring 2026

8. The program opens this view, select "+" to add fragments to your project in BIN 1 and BIN 2



OVERVIEW CONSTRUCTS METADATA

Still using legacy bulk assembly to design constructs? If so, return to the old tool here. The legacy tool will remain available for a limited time. We will notify you in advance before it becomes unavailable.

Bins & Spacers (2) +

BIN 1 Backbone Use existing cut sites 0 fragments +

BIN 2 Insert 1 Use existing cut sites 0 fragments +

Constructs 0 constructs

Open sequences > Protein design (codon optimized) Forward DNA sequence

Search for sequences

Add from worklist

pUC19_backbone DNA sequence

small_peptide_insert DNA sequence

NC_001416.1 Enterobacteria phage lambda, complete genome DNA sequence

Sequence	Bin	Start	End	Length	Orientation	Type IIS
1					Forward	Bsal

Name	Backbone	Overhang	Insert 1	Overhang
1				

Made by Ana Gomez- HTGAA Spring 2026

Tutorial- Golden Gate Assembly on Benchling

Made by Ana Gomez- HTGAA Spring 2026

9. Once the sequence is selected, you select the start and end of the restriction enzyme cut sizes

Add fragment(s)

pUC19_backbone

View: Sequence map

2006 2005 Forward

2.7 kb of 2.7 kb

Cancel Add

Add fragment(s)

pUC19_backbone

Start End Orientation

2006 2005 Forward

2.7 kb of 2.7 kb

View: Sequence map

Sequence map

Plasmid map

Linear map

2686 bp

Made by Ana Gomez- HTGAA Spring 2026

Extra! You can change the view of the sequence map → to a circular map

Tutorial- Golden Gate Assembly on Benchling

Made by Ana Gomez- HTGAA Spring 2026

10. For the small peptide insert, the features change to use a primer pairs

Bins & Spacers (2) +

BIN 1

Backbone

Use existing cut sites

1 fragment +

BIN 2

Insert 1

Use a primer pair

1 fragment +

Constructs

1 construct

Add fragment(s)

small_peptide_insert

View: Sequence map

GGTCTCAATGATGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTTAAGCTTGAGACC
CCAGAGTTACTACCGACGACGACGACGACGACGACGACGACGACGAATTCGAACTCTGG

Start: 1, End: 56, Orientation: Forward

Preferred 5' primer, Preferred 3' primer

Note* I select a section for Bsal NOT to interfere: start: 8 / End: 47 → Otherwise, it will be an error in the Construct section

Fragments

	Sequence	Bin	Start	End	Length
1	pUC19_backbone	Backbone	2006	2005	2686
2	small_peptide_insert	Insert 1	8	41	

2	small_peptide_insert	Insert 1	8	45	38	Forward	Bsal	Use existing cut sites
---	----------------------	----------	---	----	----	---------	------	------------------------

Constructs

	Name	Backbone	Overhang	Insert
1	pUC19_backbone-small_peptide_insert	pUC19_backbone	CGGT	small_peptide_insert

Constructs

	Name	Backbone	Overhang	Insert 1	Overhang	Status
1	pUC19_backbone-small_peptide_insert	pUC19_backbone		small_peptide_insert		<p>✖ Sticky ends of pUC19_backbone (CGGT) and small_peptide_insert (ATGA) are incompatible.</p>

For this case, it appeared because I'm using an imaginary/invented sequence of a small peptide. The best way to approach is to select the space by pairing ourselves

Tutorial- Golden Gate Assembly on Benchling

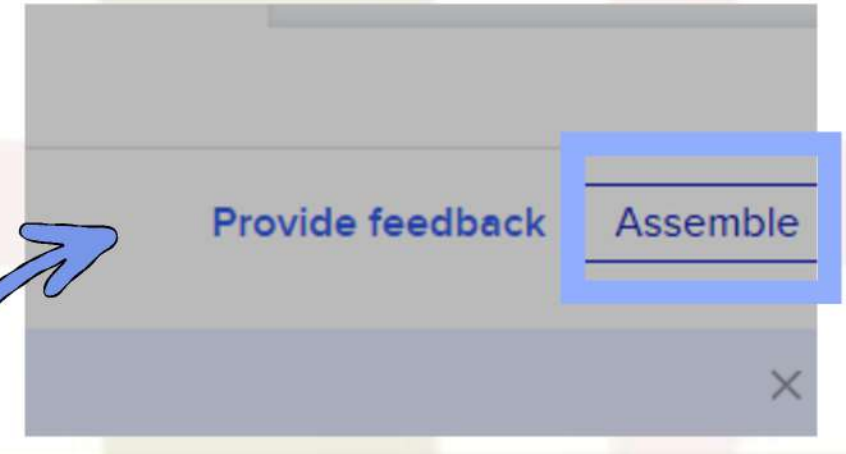
Made by Ana Gomez- HTGAA Spring 2026

11. After selecting the primers. At the construct Table, you select the option: Autopopulate → select below

the status: view constructs

	Name	Backbone	Overhang	Insert 1	Overhang	Status
1	pUC19_backbone-small_peptide_insert	pUC19_backbone	CGGT	small_peptide_insert	CGGT	Ready to assemble

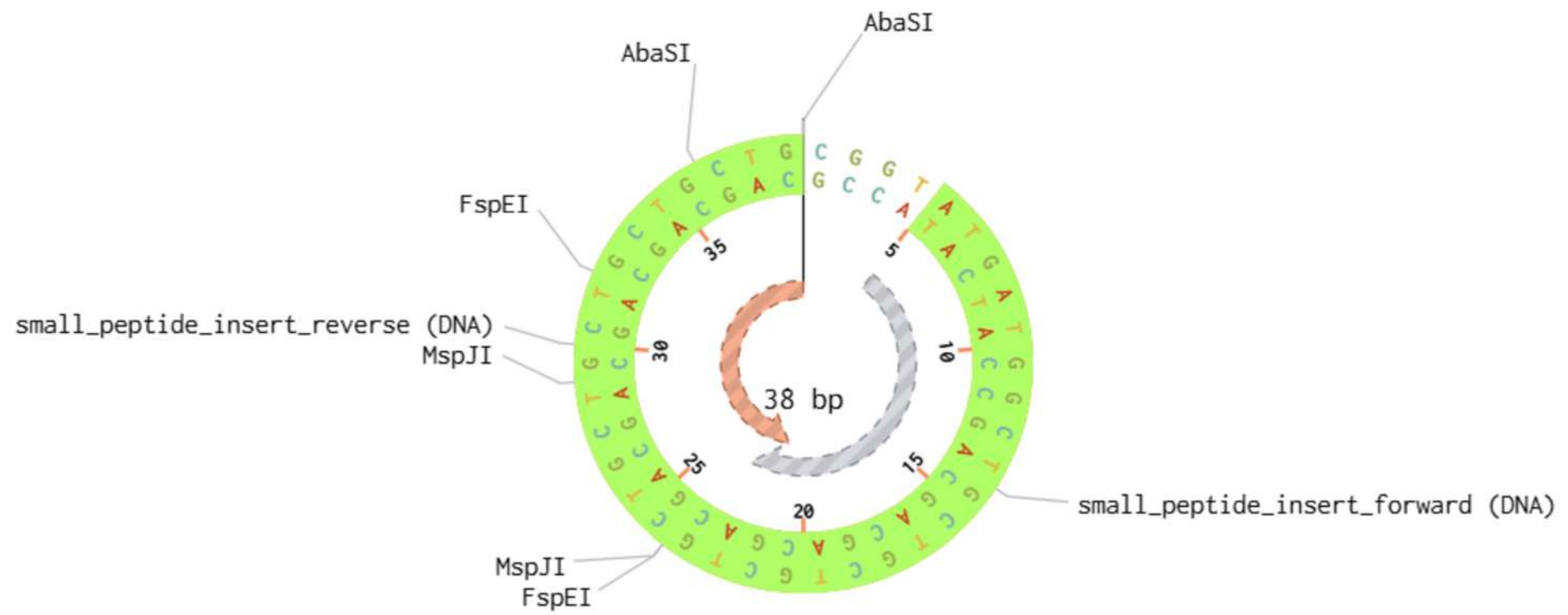
1 Add rows



12. To finish the project, select the button at the top "assemble," then finalize the assembly by selecting the features, and then press "next."

View: Plasmid map

Previous transformation plasmid view



1 Define outputs — 2 Review and finalize

Set the schema for each assembly output you want to create and optionally map assembly entities to relevant schema fields

Constructs <input type="radio"/> 1 constructs Schema Select a schema...	Primers <input checked="" type="checkbox"/> <input checked="" type="checkbox"/> 2 sequences Schema Select a schema...	Amplified fragments <input type="checkbox"/> <input checked="" type="checkbox"/> 2 fragments Schema Select a schema... Fragment bin All bins
--	--	--

Cancel Next

Tutorial- Golden Gate Assembly on Benchling

9

Made by Ana Gomez- HTGAA Spring 2026

13. Then review and finalize the assembly, make sure the project location. Then after filling the spaces, press Assemble

Finalize assembly

1 Define outputs — 2 Review and finalize

Set the project folder and study where assembly outputs will be saved. Optionally add outputs to a worklist or notebook entry for registration.

Project folder* Week 6- 2026a-ana-gomez **Studies**

Add outputs to a Notebook entry
Optionally send assembly outputs to registration tables in a Notebook entry

Add outputs to a worklist
Optionally add assembly outputs to new or existing worklists






Constructs
1 constructs

Worklist

Primers
2 primers

Worklist

The files after the assembly:

 pUC19_backbone Last modified an hour ago	AG
 pUC19_backbone-small_peptide_insert Last modified a minute ago	AG
 small_peptide_insert Last modified 32 minutes ago	AG
 small_peptide_insert_forward Last modified a minute ago	AG
 small_peptide_insert_reverse Last modified a minute ago	AG

Final output

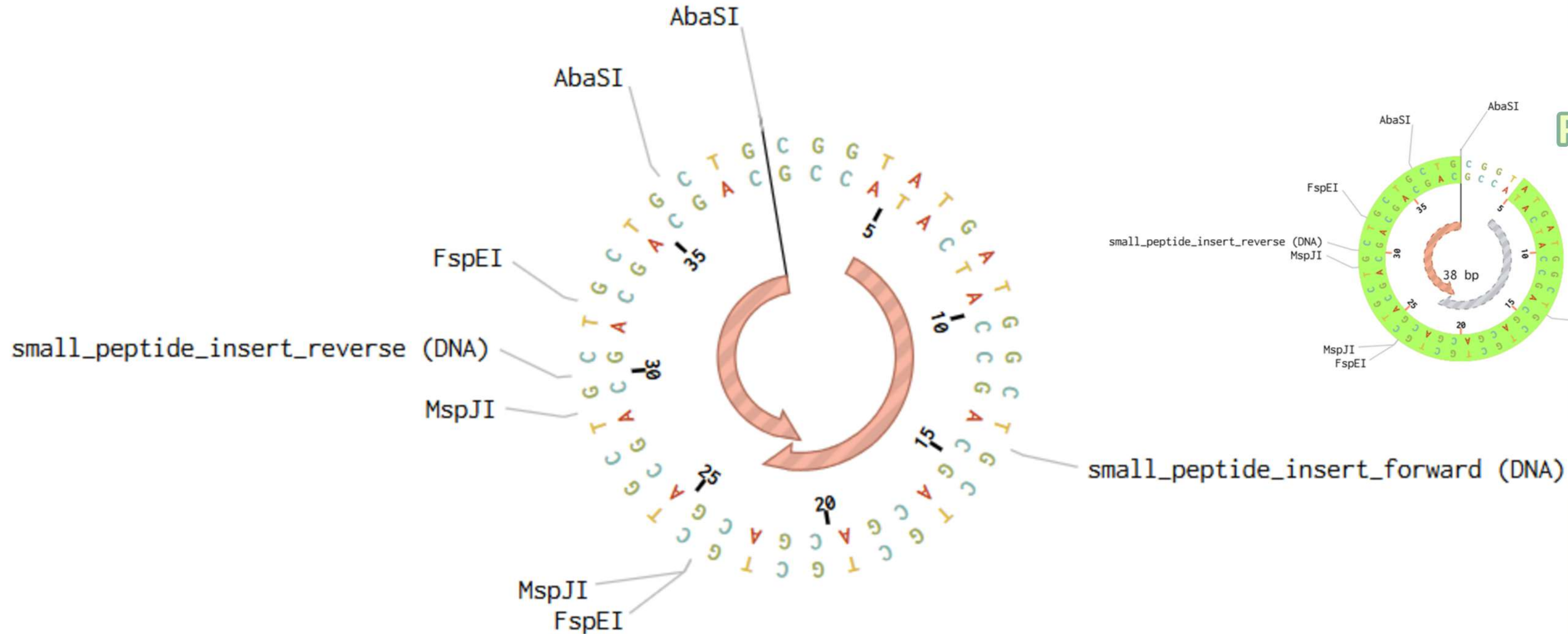
Tutorial- Golden Gate Assembly on Benchling

Made by Ana Gomez- HTGAA Spring 2026

Final View

pUC19_backbone-small_peptide_insert

38 bp



Previous view

