Tutorial- Golden Gate Assembly on Benchling

1

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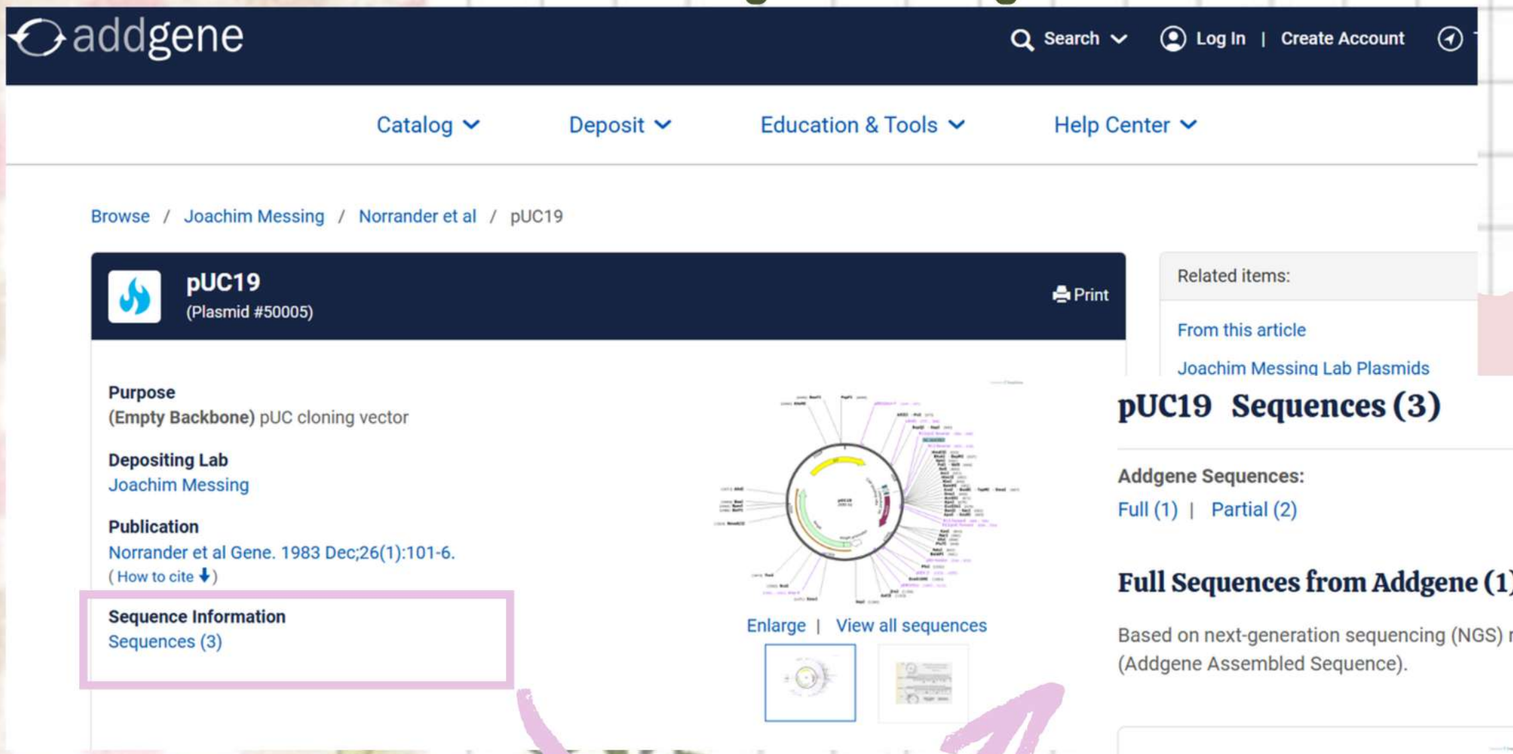
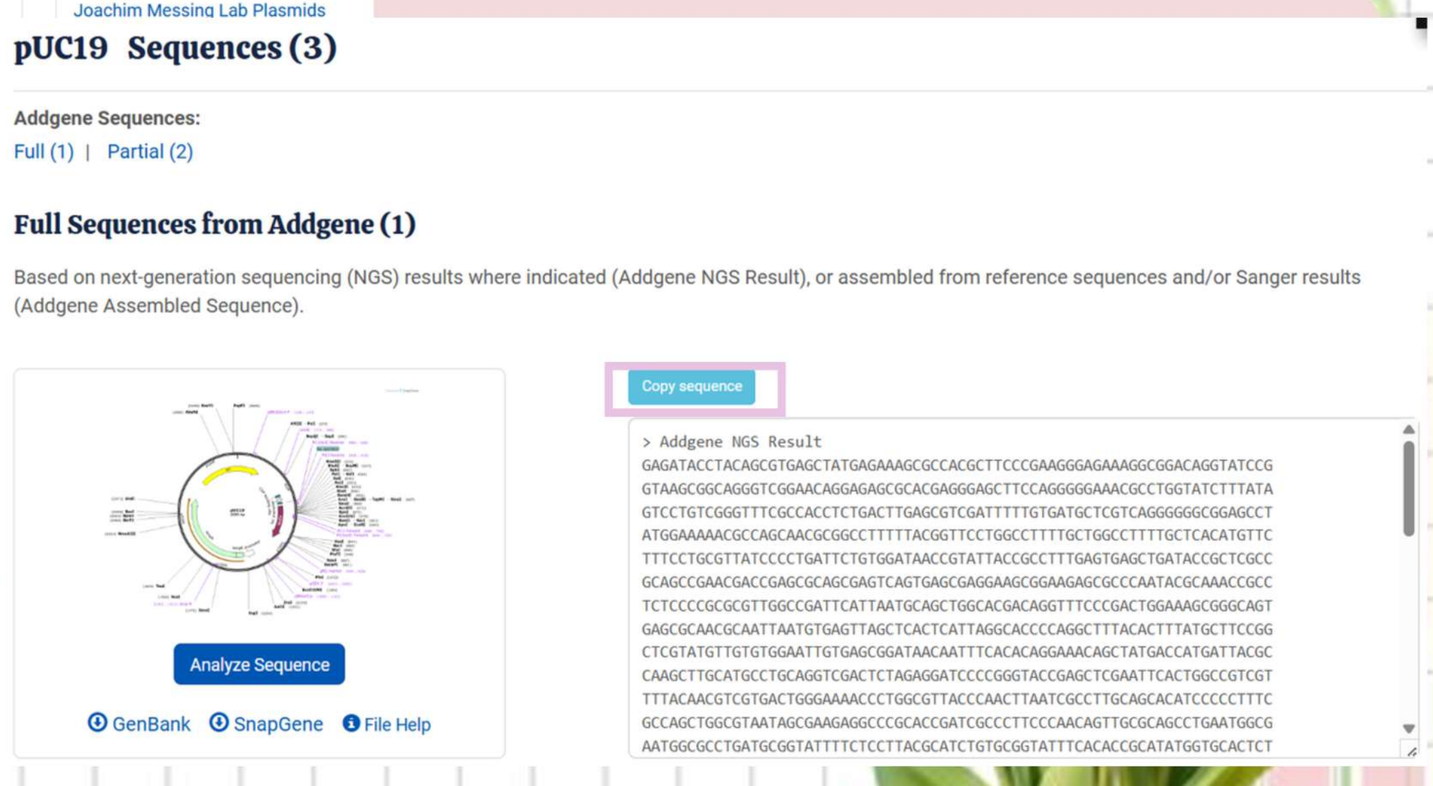
1. Find a Plasmid Sequence: pUC19



Select the full sequence



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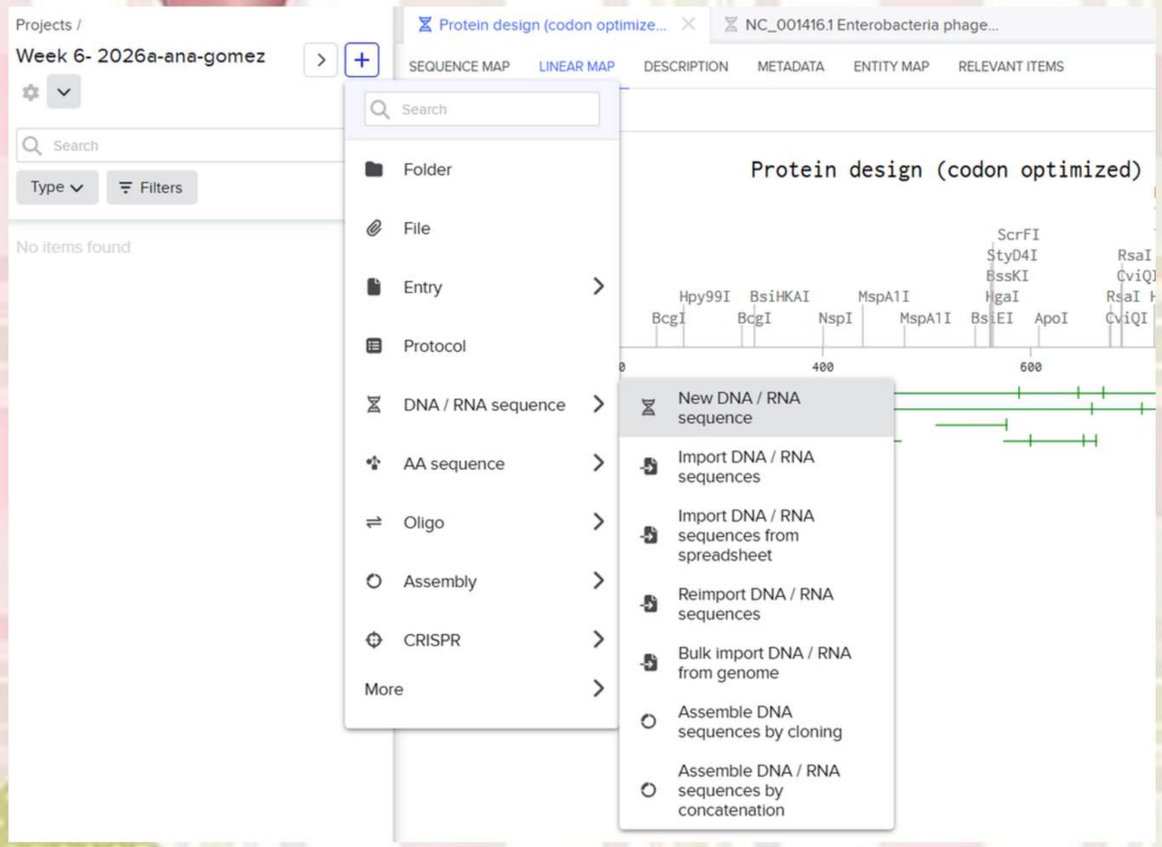
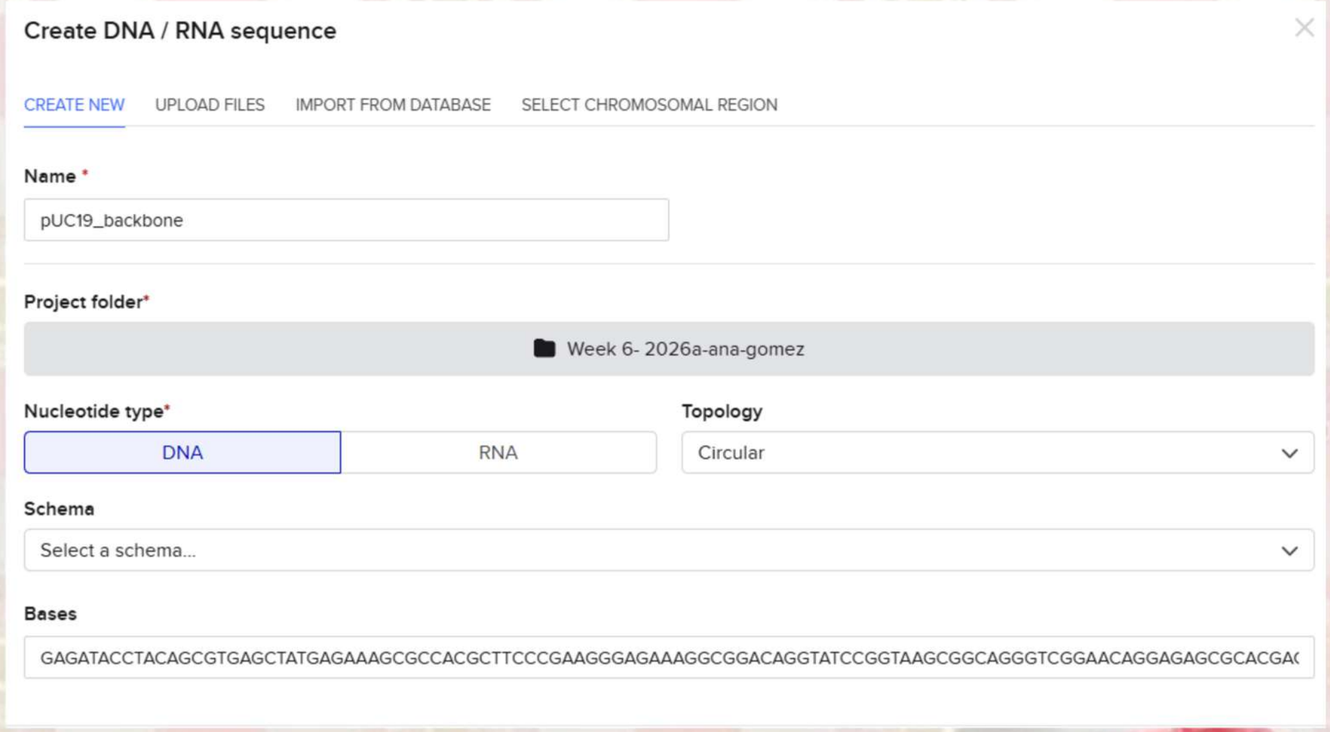
2.Login in Benchling and create a new project. Then select Add New DNA



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



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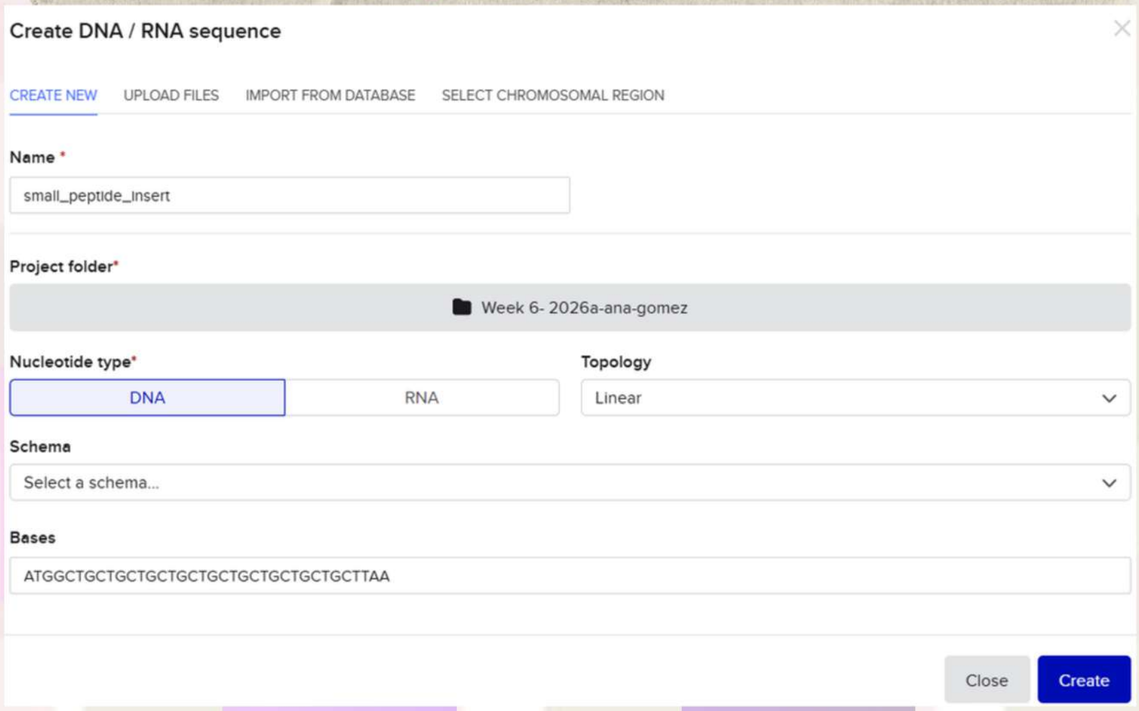
4. Add a New sequence in your project with the small DNA you want to insert

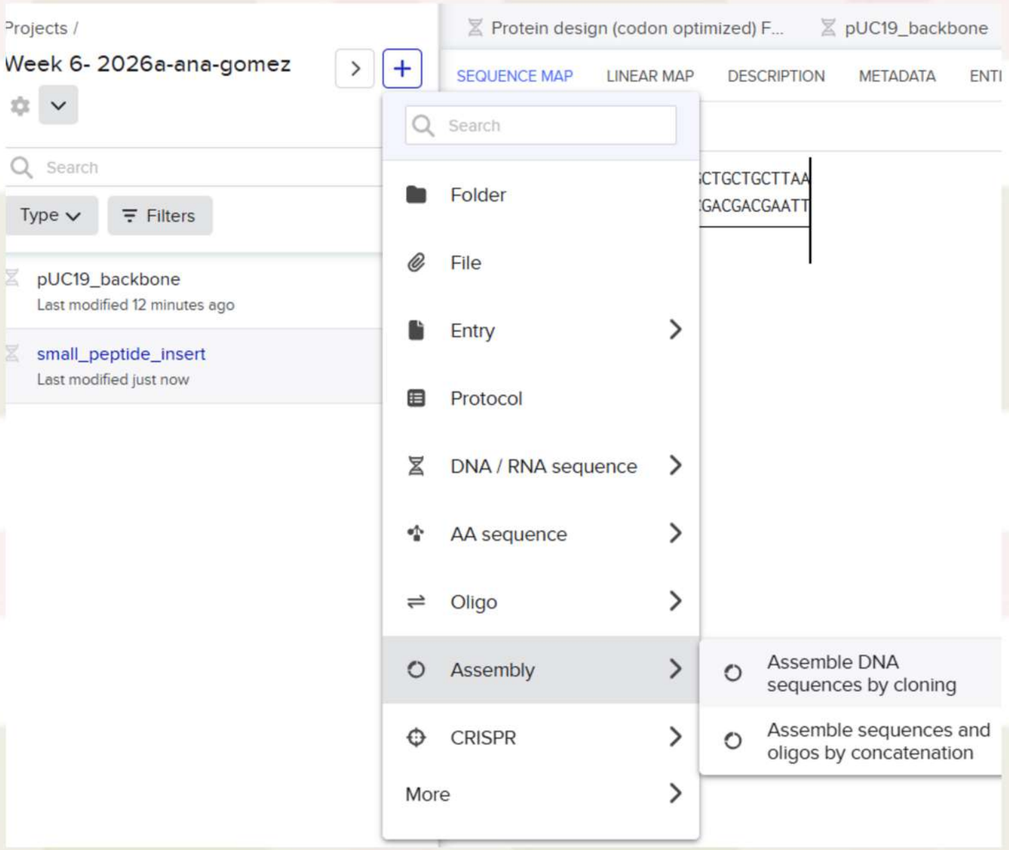


Small peptide structure output

GGTCTCAATGATGGCTGCTGCTGCTGCT
GCTGCTGCTGCTGCTTAAGCTTGAGACC

56 BP

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



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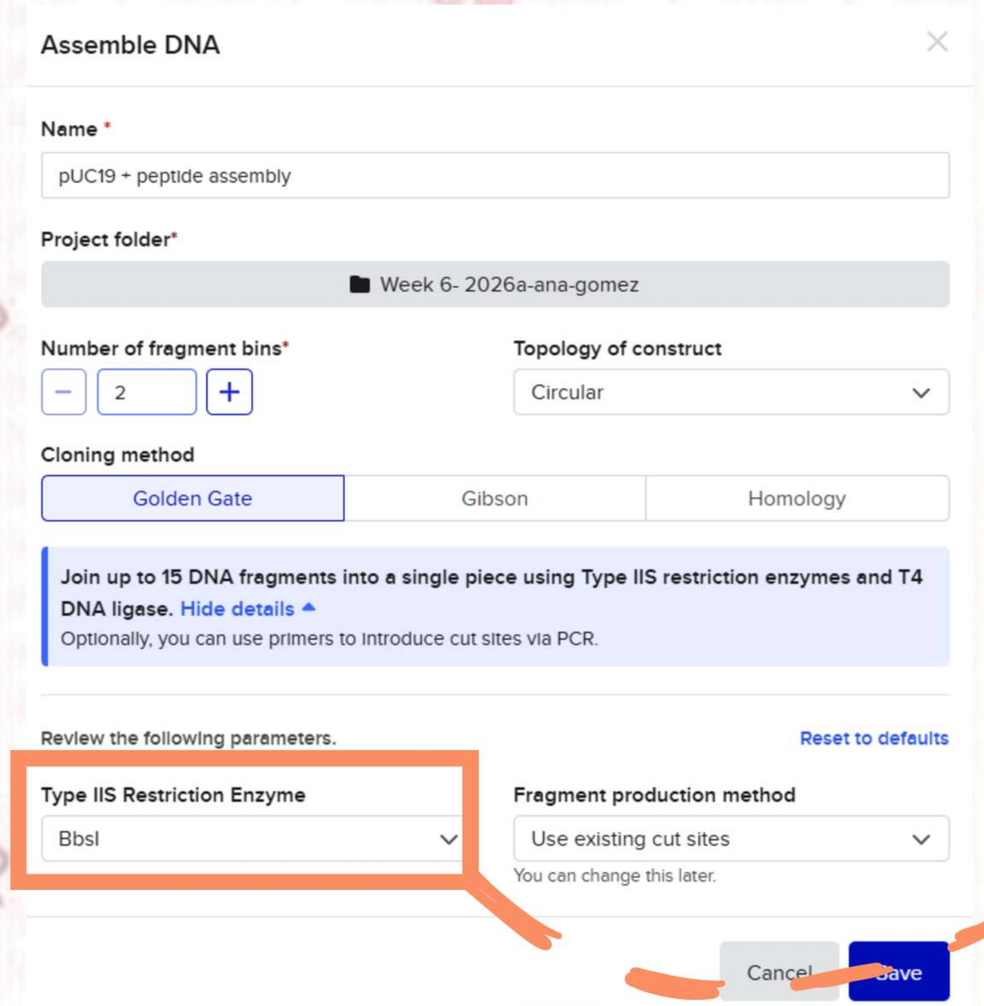
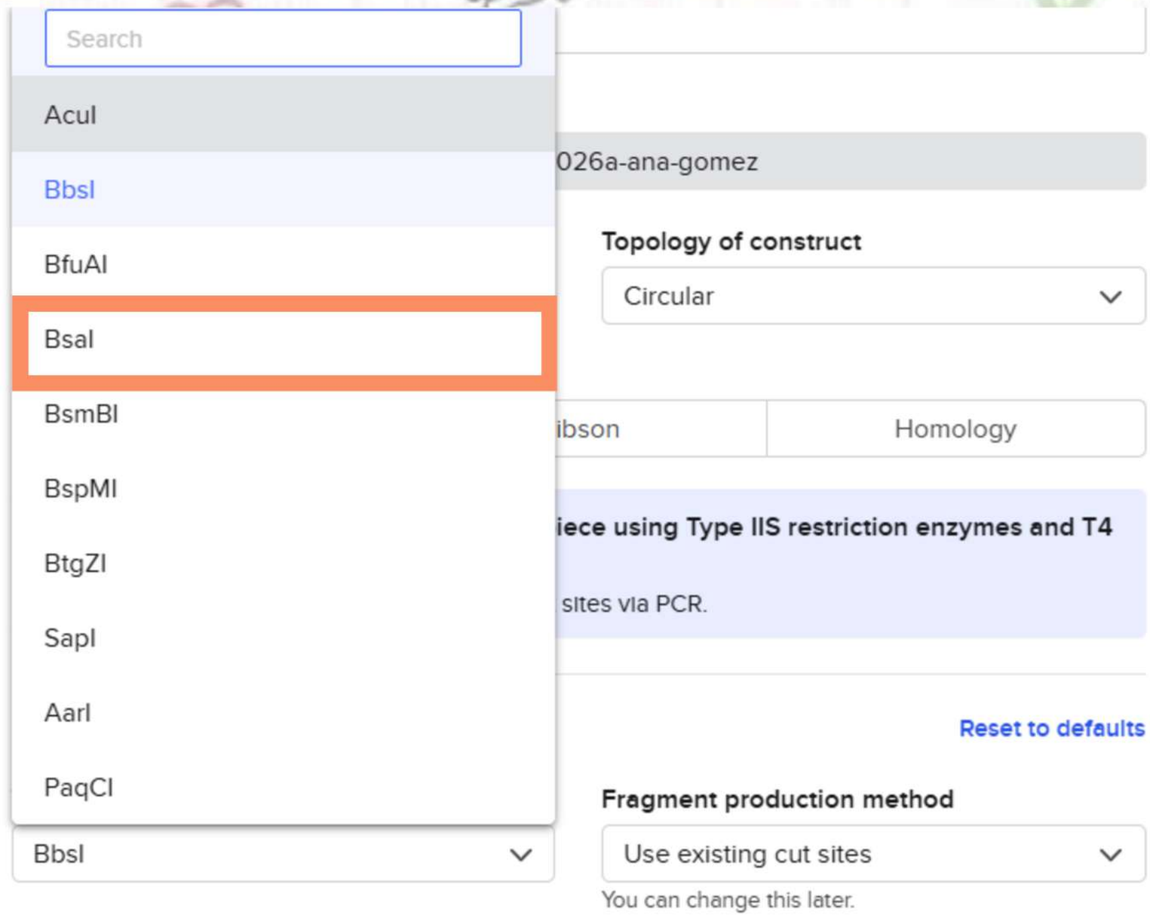
6. Change the name of the assemble DNA project, select the Golden Gate option



7. Change the restriction enzyme from BdsI to BsaI



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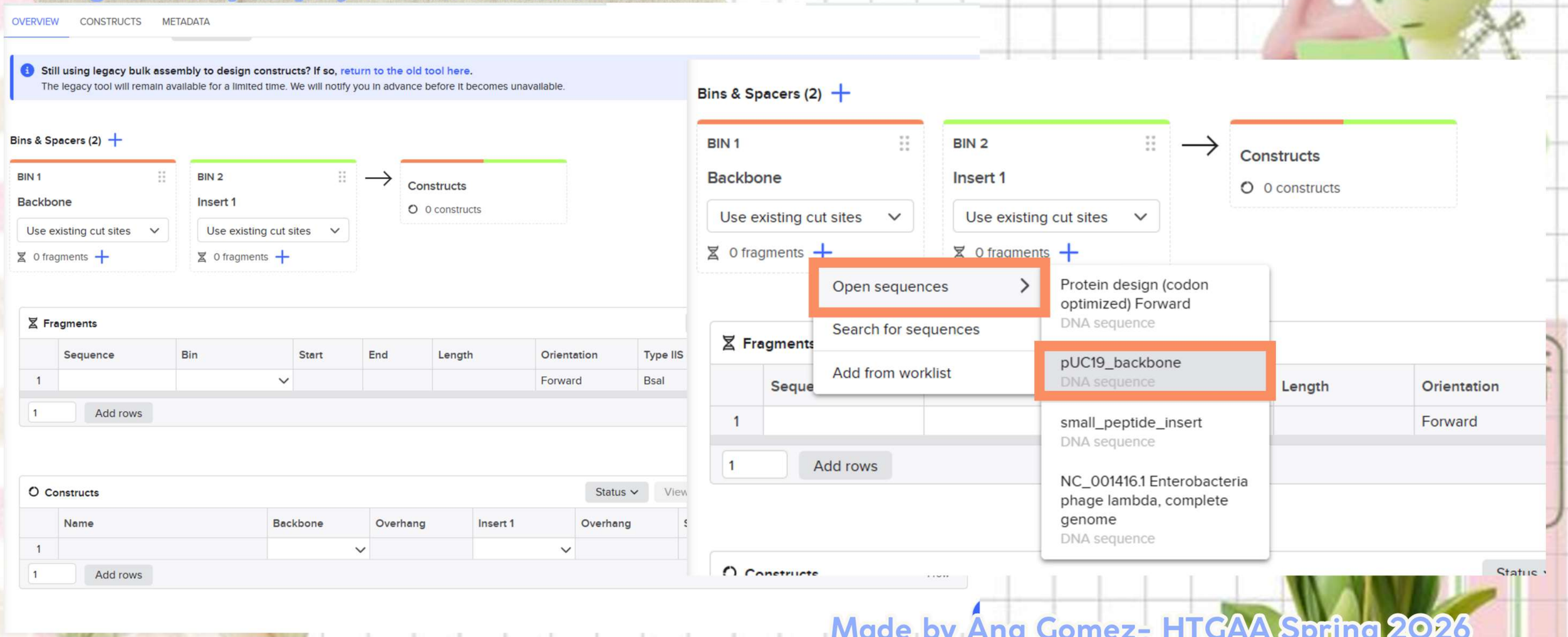
8. The program opens this view, select "+" to add fragments to your project in BIN 1 and BIN 2



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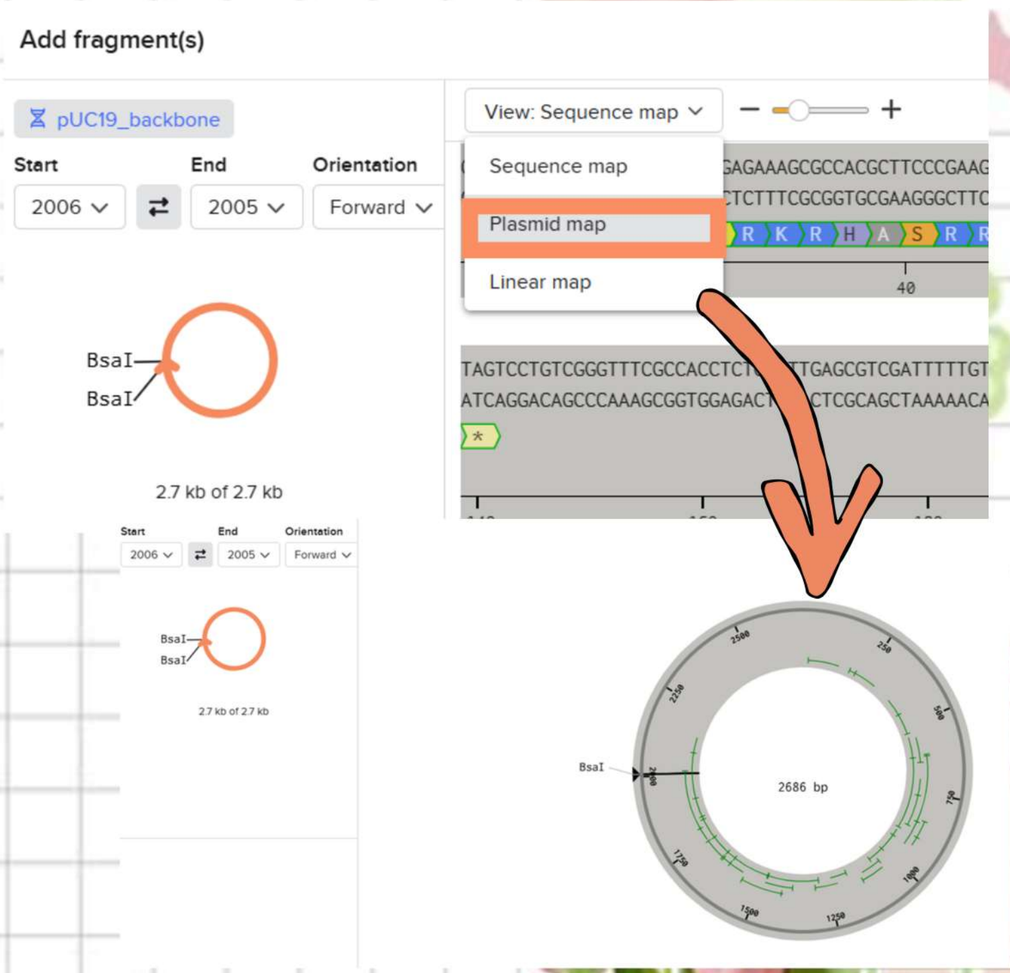
9. Once the sequence is selected, you select the start and end of the restriction enzyme cut sizes



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

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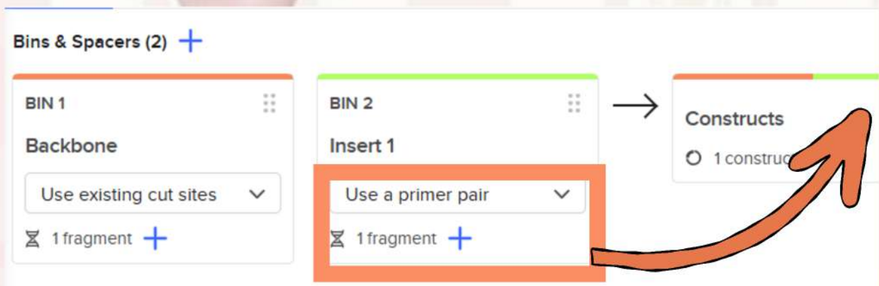
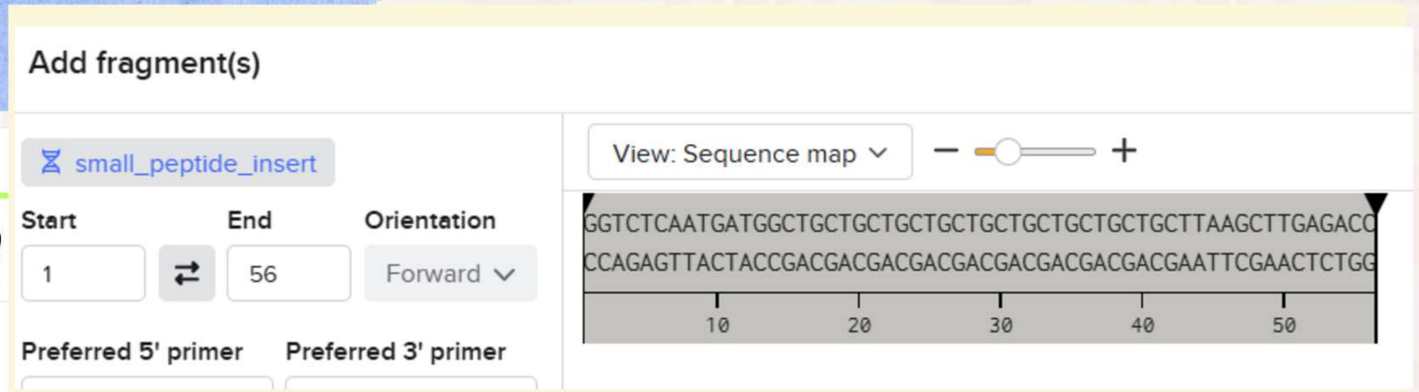
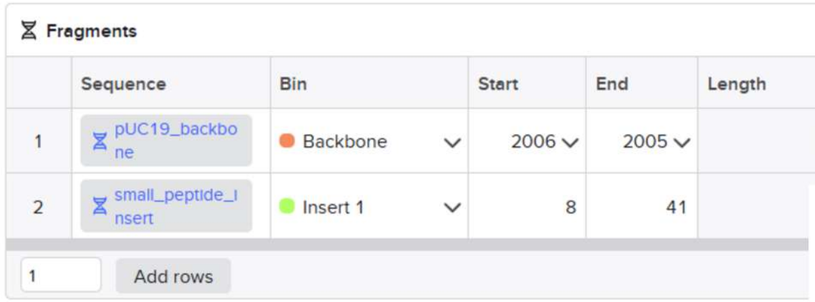
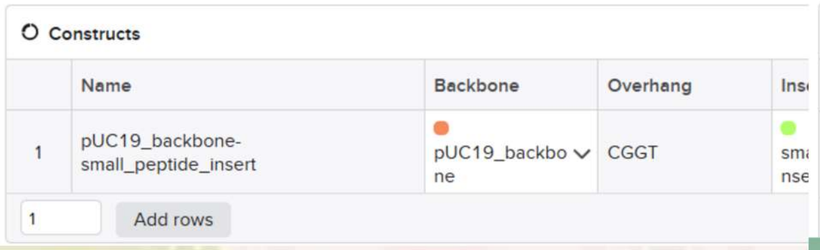
10. For the small peptide insert, the features change to use a primer pairs





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





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

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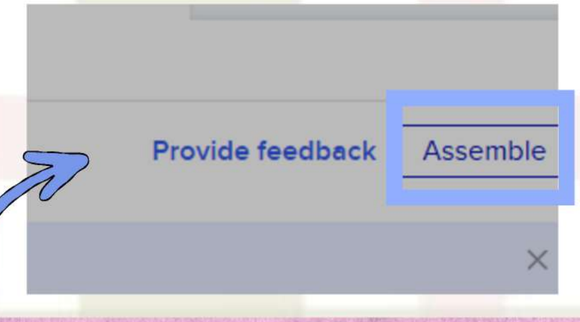
11. After selecting the primers. At the construct Table, you select the option: Autopopulate → select below the status: view constructs

Status | View constructs | Autopopulate

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

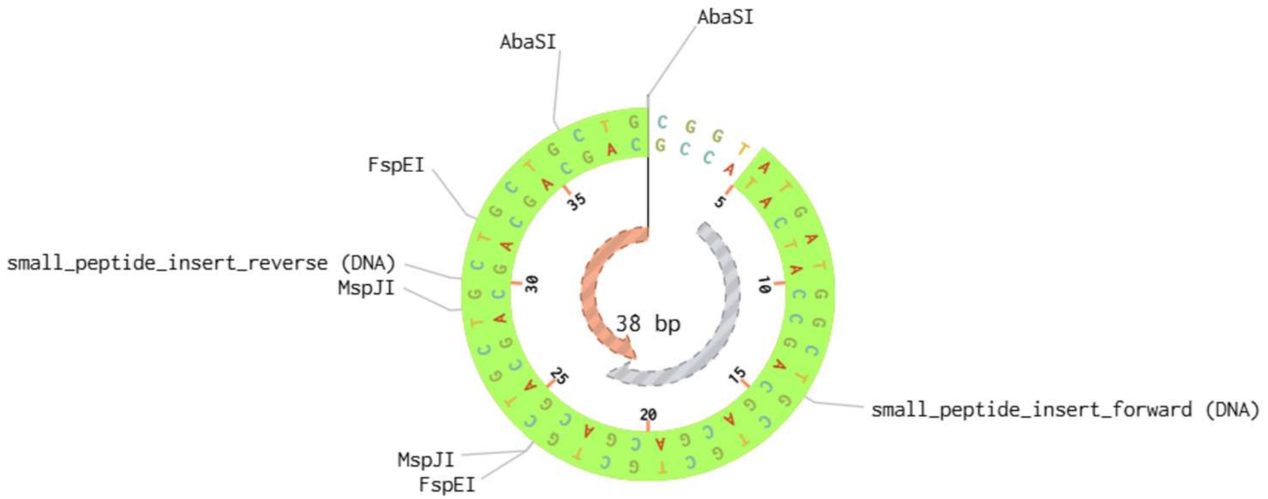
1 Add rows

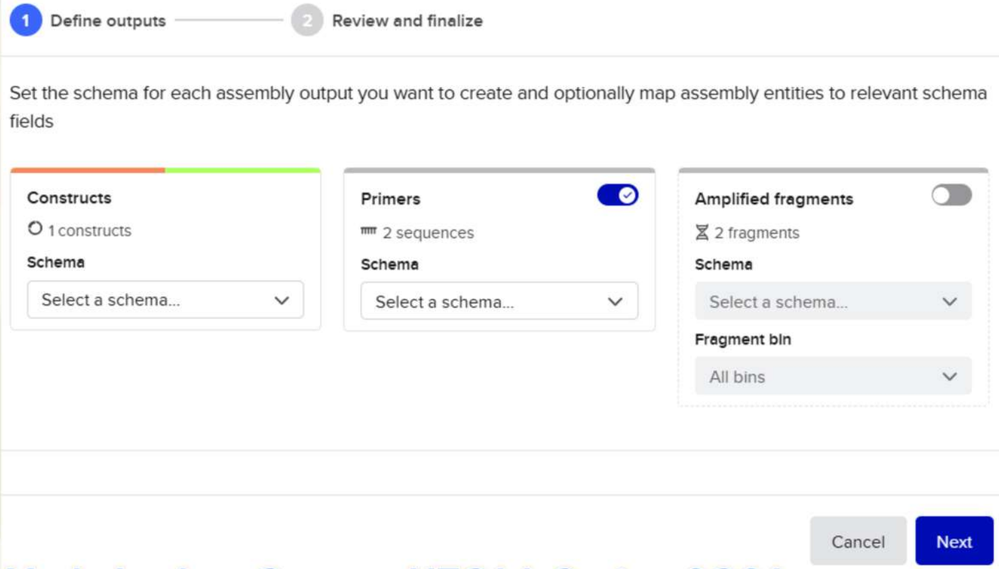


12. To finish the project, select the button at the top "assemble," then finalize the assembly by selecting the features, and then press "next."

View: Plasmid map

Previous transformation plasmid view





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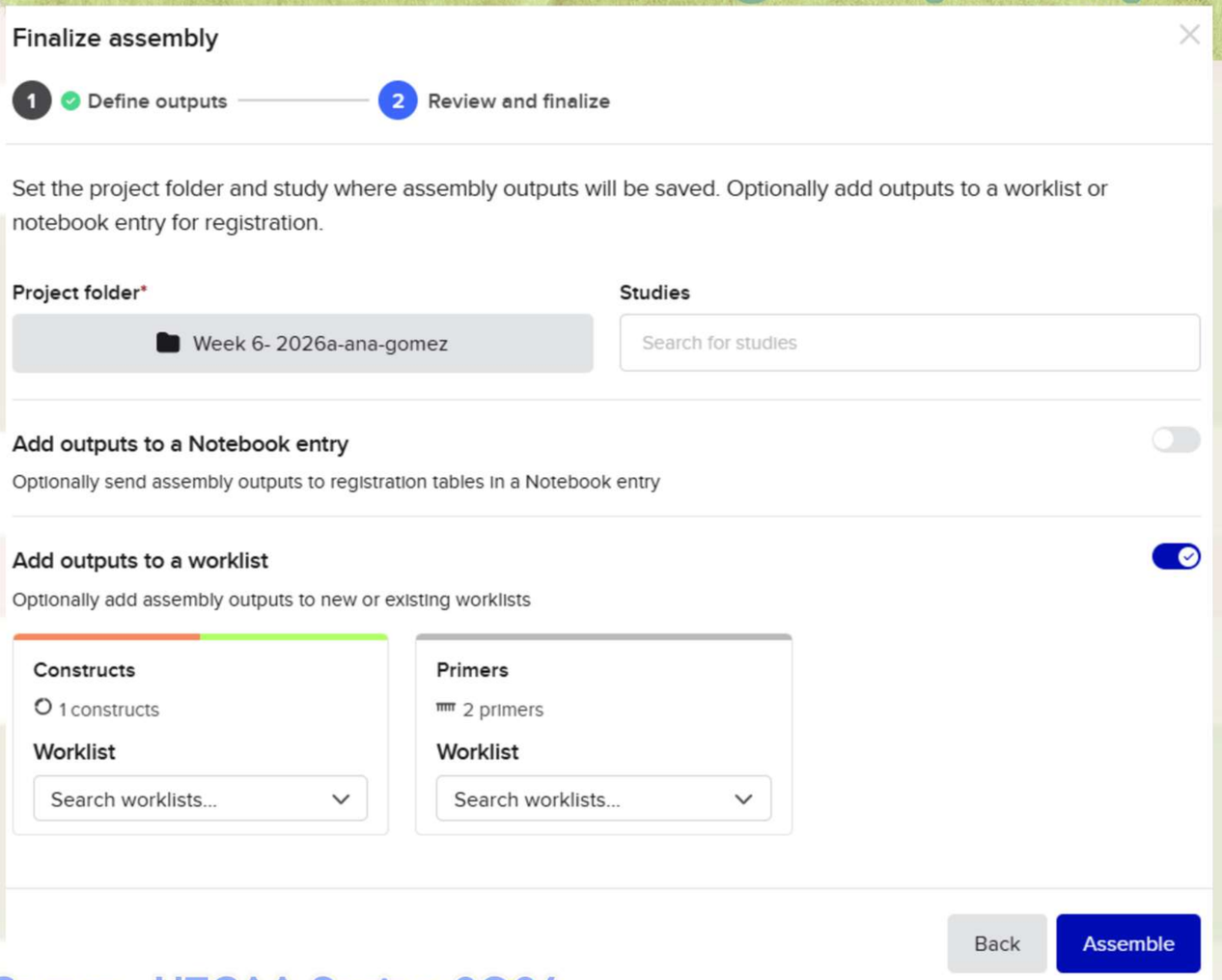
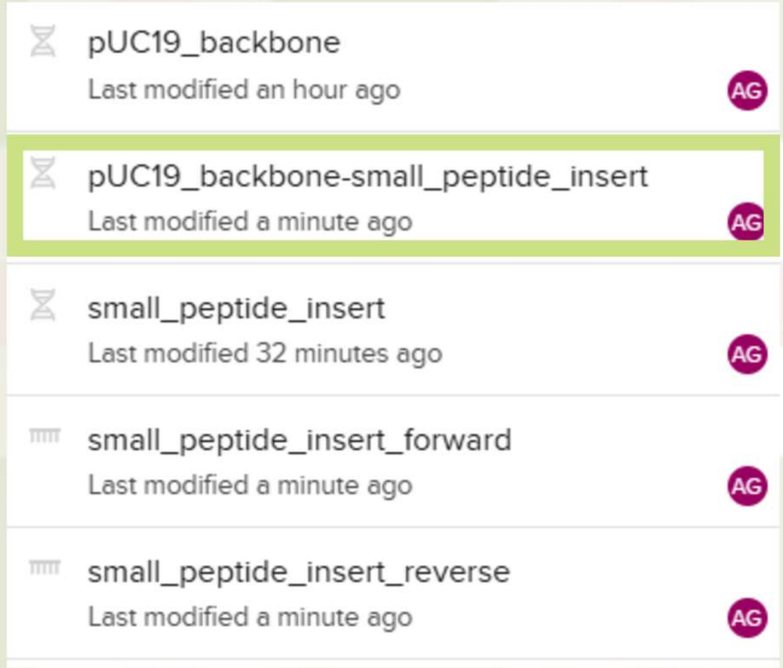
13. Then review and finalize the assembly, make sure the project location. Then after filling the spaces, press Assemble



The files after the assembly:



Final output

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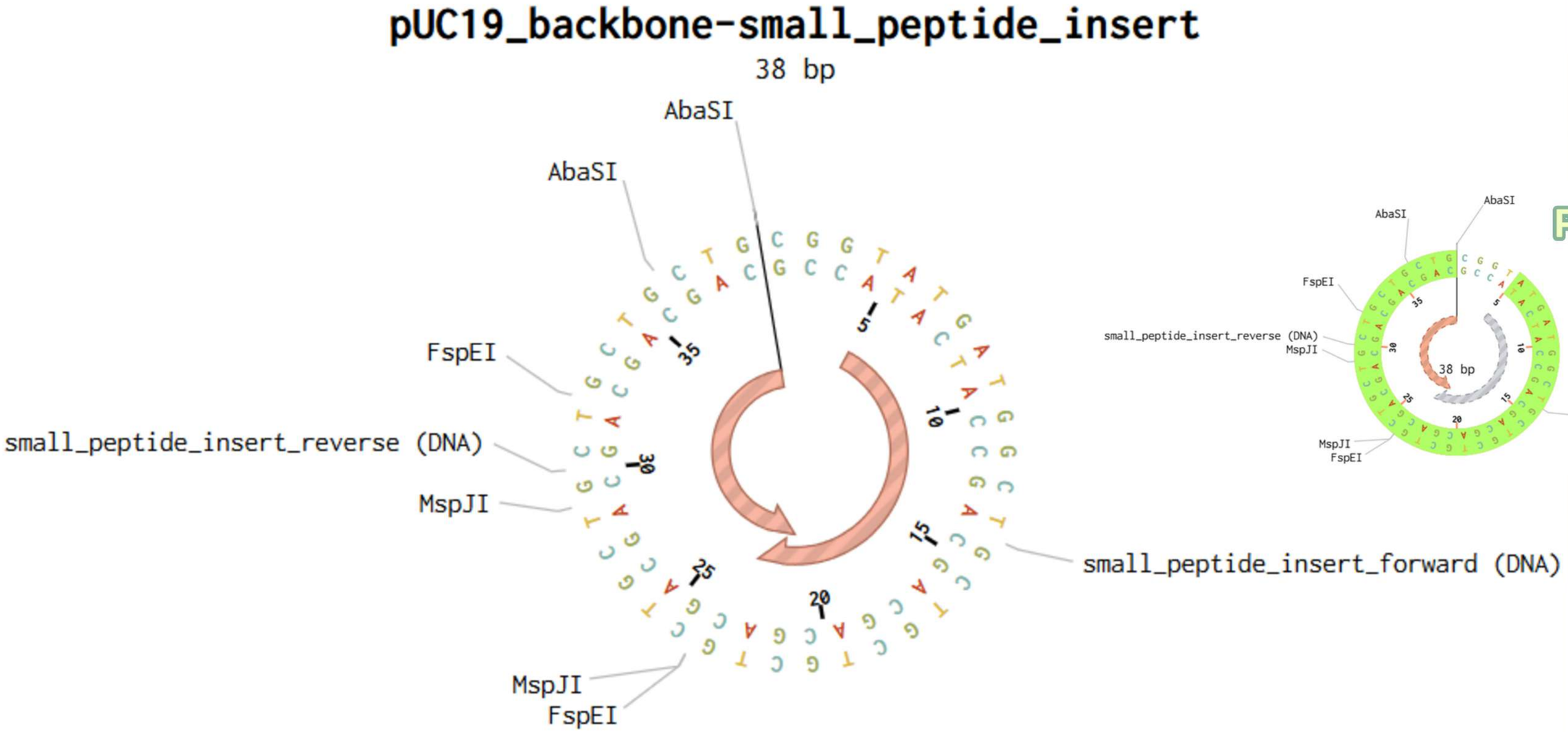
Final View



Previous view

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