

< > 1-50 of 1159 items

<input type="checkbox"/> Barcode	Name	Location	Modified	Schema
4C012	4C EE&SB fridge transient storage	4°C Fridge	09/08/2018	4°C Fridge
4C002	4C Fridge 00271	4°C Fridge	09/08/2018	4°C Fridge
4C009	4C Fridge 01223	4°C Fridge	09/08/2018	4°C Fridge
4C007	4C Fridge 01233	4°C Fridge	09/08/2018	4°C Fridge
4C014	4C Fridge 01871	4°C Fridge	09/08/2018	4°C Fridge
4C015	4C Fridge Aaron	4°C Fridge	15/04/2021	4°C Fridge
4C016	4C Fridge Adam	BioInnovati...	15/04/2021	4°C Fridge
4C005	4C Fridge ANALYTICS	4°C Fridge	19/11/2018	4°C Fridge
4C011	4C Fridge CFB00266	4°C Fridge	09/08/2018	4°C Fridge
CFB01478	4C Fridge CFB01478	4°C Fridge	19/11/2018	4°C Fridge
CFB01653	4C Fridge CFB01653	4°C Fridge	09/08/2018	4°C Fridge
4C003	4C Fridge DSP1	4°C Fridge	09/08/2018	4°C Fridge

Reach out when struggling with the platform:

Biosustain Benchling support
lims_support@biosustain.dtu.dk



Access Benchling:

biosustain.benchling.com



(login with DTU credentials)

Additional resources:

[LIMS Help guides](#)



[Benchling Help Center: Molecular Biology](#)



Hands-on Benchling support

Mondays 13:00 -14:00 (Room 222)



Research Data Management Team

DROP-IN HOURS

Get hands-on support for **Benchling** and other **data management** tasks.



MONDAYS
13:00 – 14:00
2nd FLOOR

Image by Joomp on Freepik



**Mía López Portillo
Ontiveros**
RDM/LIMS support

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Come back to this page by clicking on the icon!



1. Introduction to the Molecular Biology Suite



Functionalities and tools overview

Sequence Alignment

- ✓ Alignment to template
- ✓ Consensus alignment
- ✓ Benchling BLAST

Sequence Visualization

- ✓ Plasmid map
- ✓ Annotations and feature libraries creation (*Bulk auto-annotation*)
- ✓ Sequence search

Construct Design

- ✓ RE-based cloning
- ✓ Golden Gate and Gibson assembly
- ✓ Bulk assembly
- ✓ Codon optimization
- ✓ Worklists integration
- ✓ *In silico* PCR and digestions
- ✓ Customizable enzyme lists

AA / Protein Analysis

- ✓ AA alignment
- ✓ Auto-fill, back and bulk translations
- ✓ Electrochemical properties overview

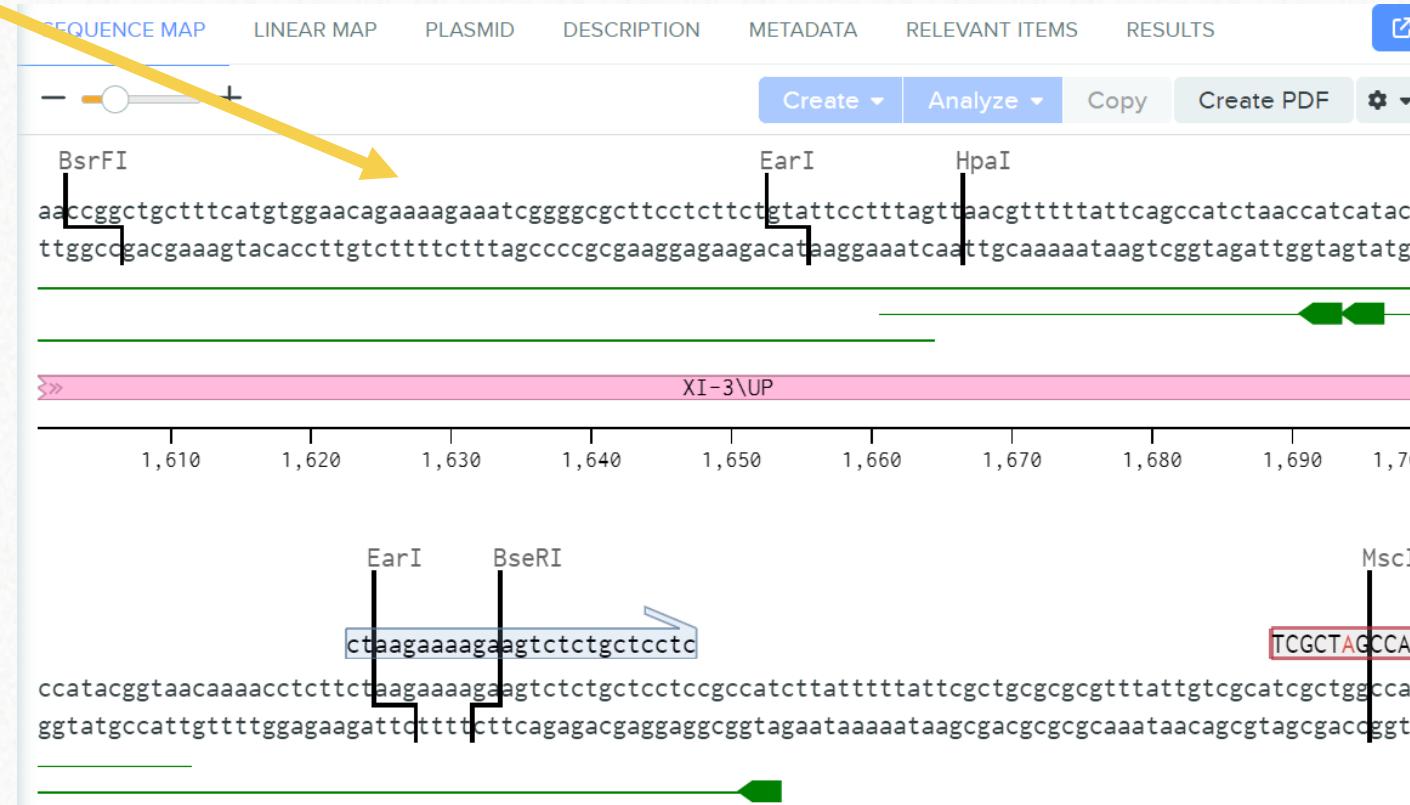
CRISPR

- ✓ Guide RNA design
- ✓ On/Off-target scoring
- ✓ HR template design



Functionalities and tools overview

Your sequence



Functionalities



Functionalities and tools overview



Features (annotations and translations)

Digests

Primers

History

Alignments

CRISPR

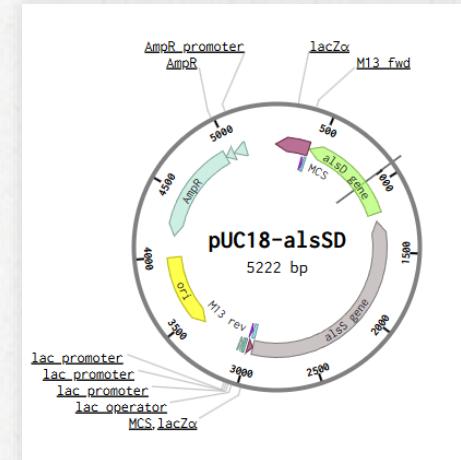
Information (topology, tags)

2. Training overview



Training goals:

The basics of...



A search results page titled "Constructs" showing three items: AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-T7 terminator, AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-T7 terminator, and AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-rmB T1 terminator. Each item has a circular diagram and a link to "View".

- How to navigate the **sequence visualization** window and the workspace
- How to **assemble** simple constructs
- How to perform bulk assemblies with the **Combinatorial Assembly** tool



Training goals:

The basics of...



- How to **create a sequence alignment**
- How to use Benchling's **CRISPR** tools



Today's work example:

Hypothetical scenario: **Production of acetoin in *E. coli***



Scenario inspired by:

Journal of the Taiwan Institute of Chemical Engineers 167 (2025) 105895



Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.journals.elsevier.com/journal-of-the-taiwan-institute-of-chemical-engineers



Metabolic engineering of *Escherichia coli* for improved cofactor regeneration in lactate to acetoin via whole-cell conversion

Chan-Hsiang Hsu, Sefli Sri Wahyu Effendi, Wan-Wen Ting, Yu-Hsiu Li, I-Son Ng *

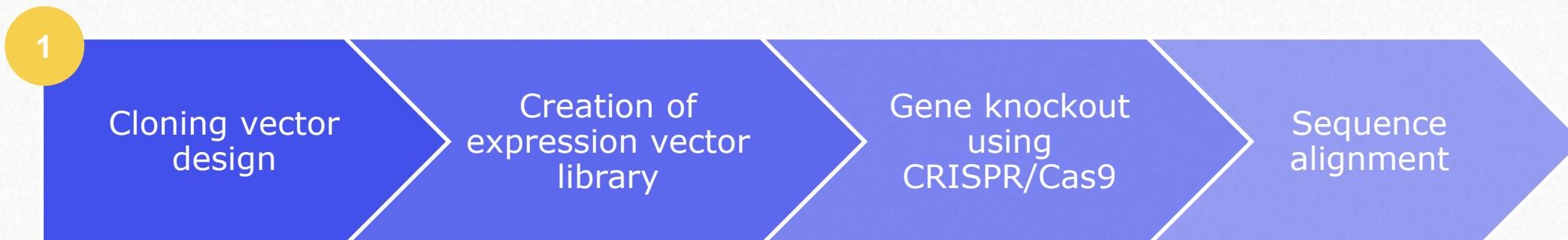
Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan



[Link to article](#)

Today's work example:

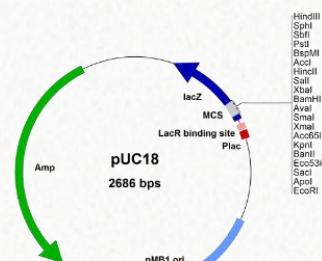
Hypothetical scenario: **Production of acetoin in *E. coli***



alsS and ***alsD*** from *Bacillus subtilis*



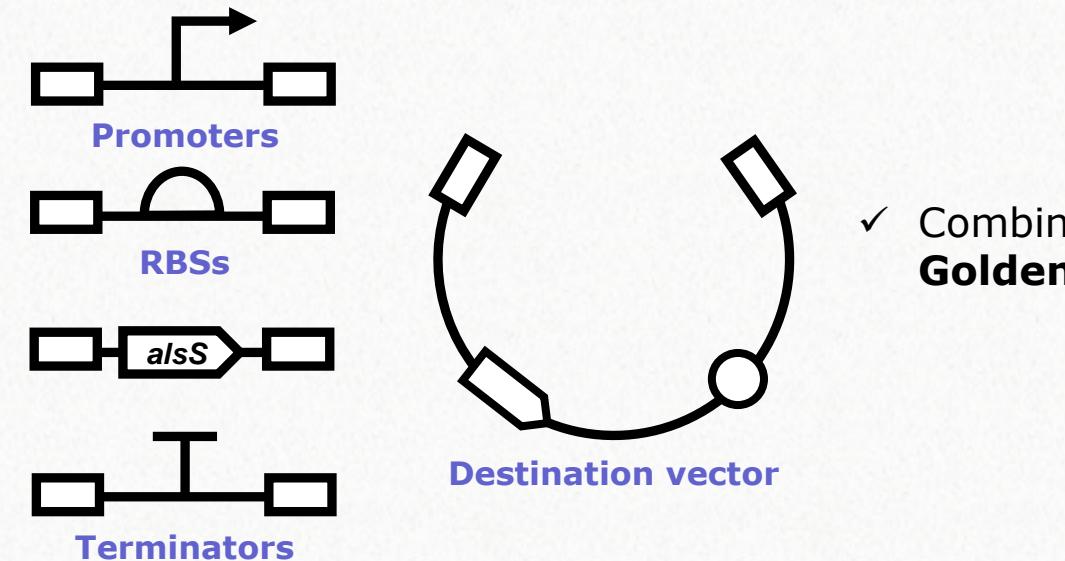
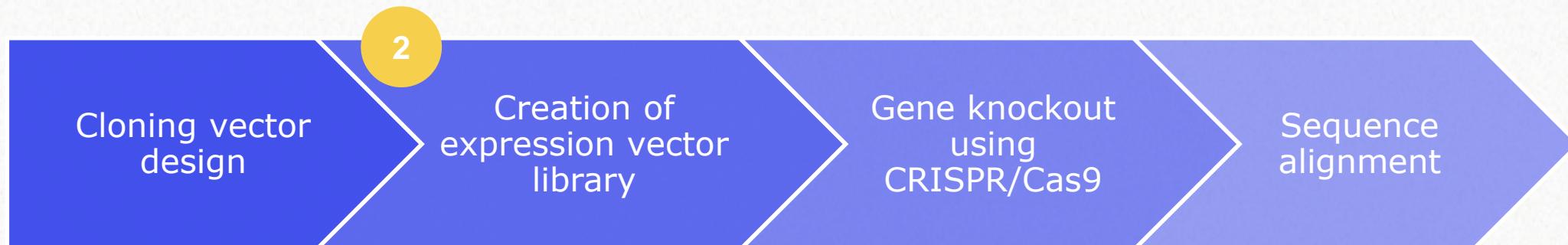
pUC18 cloning vector



- ✓ Primer design
- ✓ *In silico* PCR
- ✓ Virtual digestion
- ✓ RE-based cloning

Today's work example:

Hypothetical scenario: **Production of acetoin in *E. coli***



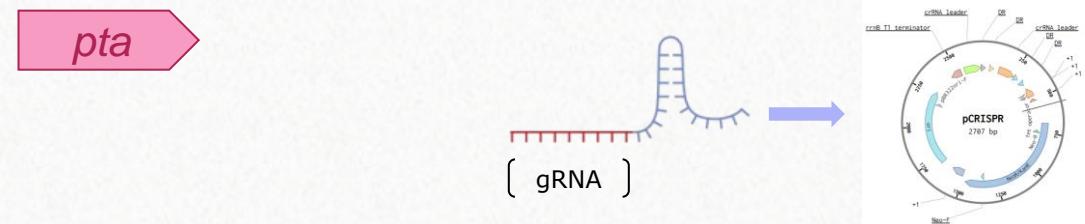
✓ Combinatorial cloning:
Golden Gate

Today's work example:

Hypothetical scenario: **Production of acetoin in *E. coli***

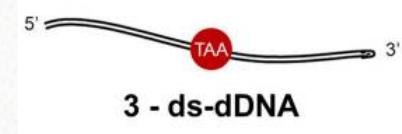


Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR



- ✓ gRNA design
- ✓ HR template design

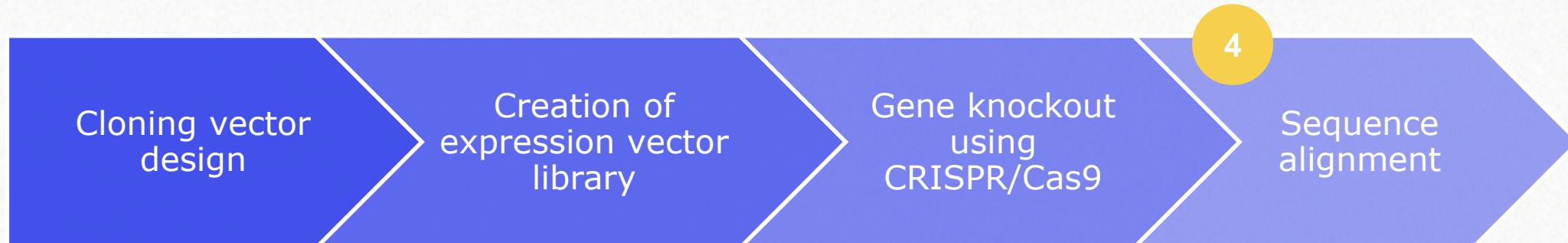
HR template design to KO *pta*



König, E., Zerbini, F., Zanella, I., Fraccascia, D., & Grandi, G. (2018). Multiple Stepwise Gene Knockout Using CRISPR/Cas9 in *Escherichia coli*. *Bio-protocol*, 8(2), e2688. <https://doi.org/10.21769/BioProtoc.2688>

Today's work example:

Hypothetical scenario: **Production of acetoin in *E. coli***



✓ Multisequence alignment

3. The basics of sequences





This section will give you an overview of how to **import**, **visualize**, and **annotate** sequences. It also shows how to **optimize** a coding sequence's codons.



3. The basics of sequences

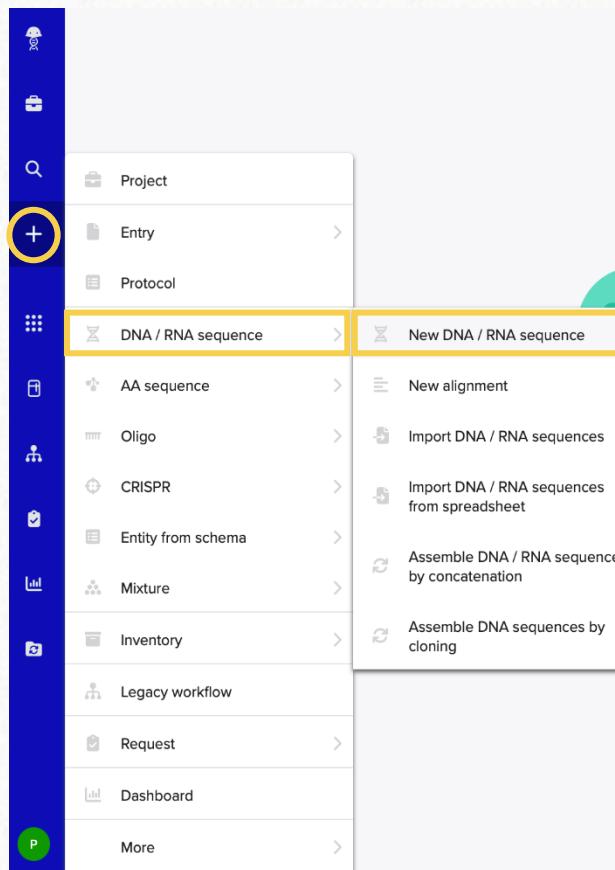
3.1 Sequence creation and import



Create and import a sequence

How to create a new entity from a nucleotide sequence

1 Create a new DNA sequence



2 You can paste or write down any nucleotide sequence of your interest, and you must assign the right topology and schema.

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Name*
pCAT

Set nucleotide type*
DNA RNA

Set folder*
Patricia B.

Set topology
Linear

Set schema
DNA Fragment

Bases
ggcacgtaaagagggttccaactttcaccataatgaaaca

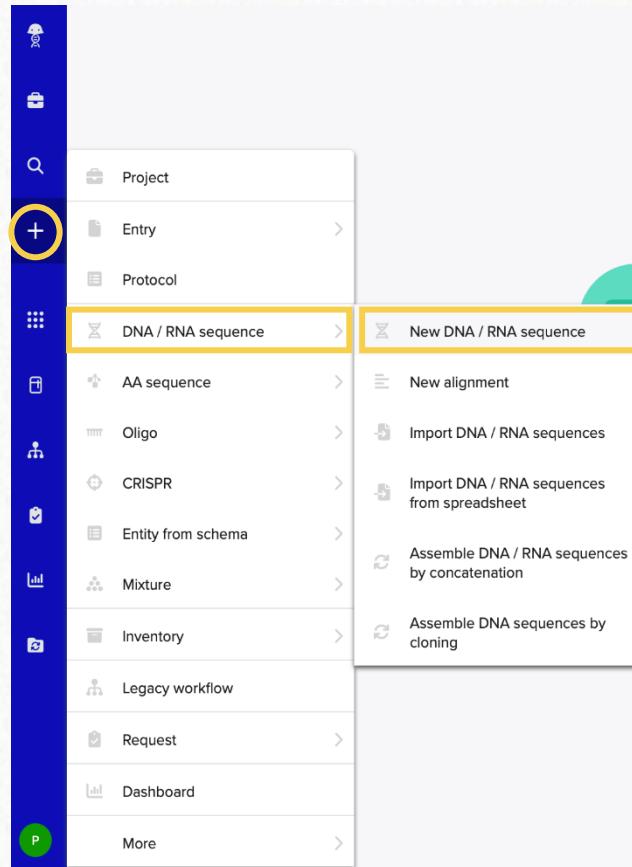
i You can leave the **Bases** field **empty** and add your sequence later. This can be useful if you wish to copy and paste a sequence with its annotations.

Create

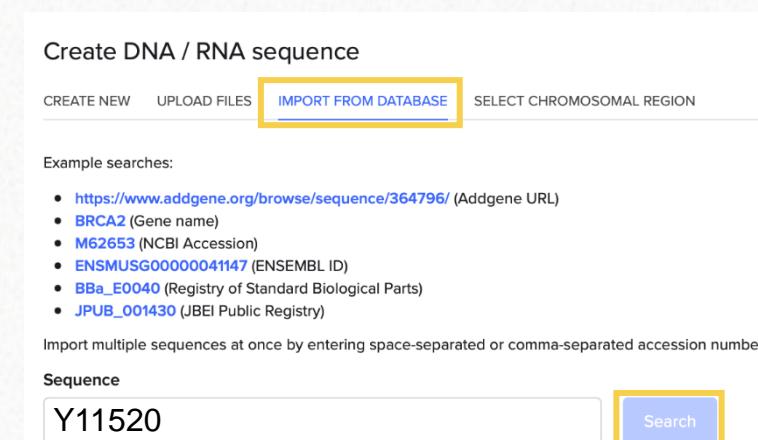
Create and import a sequence

How to import of sequences from a database

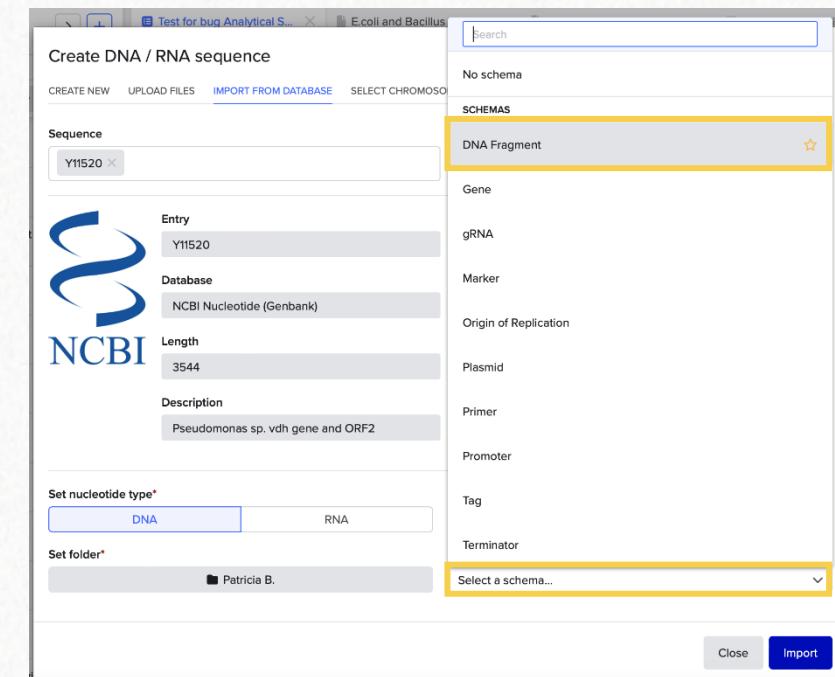
1 Create a new DNA sequence



2 You can write or paste a valid accession number from databases like GenBank, Addgene or the iGEM Registry

A screenshot of the 'Create DNA / RNA sequence' dialog. The 'IMPORT FROM DATABASE' tab is selected. It shows a list of example searches: https://www.addgene.org/browse/sequence/364796/ (Addgene URL), BRCA2 (Gene name), M62653 (NCBI Accession), ENSMUSG00000041147 (ENSEMBL ID), BBa_E0040 (Registry of Standard Biological Parts), and JPB2_001430 (JBEI Public Registry). Below this is a 'Sequence' input field containing 'Y11520' and a 'Search' button.

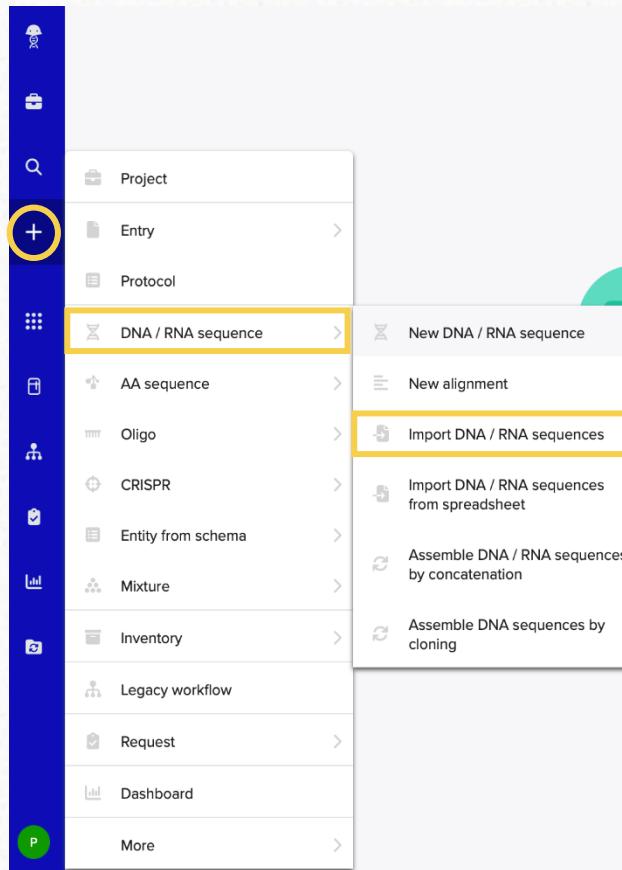
3 If the ID is valid, Benchling will show you the gene's description. You can set its schema and import it.

A screenshot of the 'Create DNA / RNA sequence' dialog. The 'IMPORT FROM DATABASE' tab is selected. The 'Sequence' field contains 'Y11520'. The 'Entry' field shows 'Y11520', 'Database' is 'NCBI Nucleotide (Genbank)', 'Length' is '3544', and 'Description' is 'Pseudomonas sp. vdh gene and ORF2'. Under 'Set nucleotide type*', 'DNA' is selected. In the 'Set folder*' section, 'Patricia B.' is listed. On the right, a sidebar titled 'SCHEMAS' shows 'DNA Fragment' selected (highlighted with a yellow box). Other options include Gene, gRNA, Marker, Origin of Replication, Plasmid, Primer, Promoter, Tag, and Terminator, with 'Select a schema...' also highlighted with a yellow box.

Create and import a sequence

How to import sequences **from a file**

- 1 Choose the **Import** sequences option



- 2 Choose the correct nucleotide type and select the sequence files. The sequences will be uploaded automatically to the folder you set.

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!

Nucleotide type* DNA RNA

Project folder

Drag and drop files to upload or

Open Show errors only

GFP.dna

pUC18.dna

pUC18 - circular DNA

- i Remember to set the folder **before** uploading your files.

If you made a mistake, you can fix it by using the **Move to** option.

Create and import a sequence

How to import sequences **from a file**

Create DNA / RNA sequence

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

Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!

Nucleotide type* Set folder

DNA RNA Patricia

Drag and drop files to upload or choose a file

Note: GenBank sequences use the *LOCUS* for the sequence name. To use the filename instead, [click here](#).

Open Move To **Set Topology** Edit Tags Auto-annotate Show errors only

Vector_pBR322.gb Linear Circular

Exported - circular DNA ACCESSION DEFINITION ORGANISM SOURCE

ORGANISM synthetic DNA ... SOURCE Create New Tag

UPLOAD DONE OPEN SEQUENCE · UPLOADED TO PATRICIA

i Change its **topology** and **edit** the **tags** attached to your entity to make it easier to find.

When uploading a sequence, it is possible to:

- i** Change its **topology** and **edit** the **tags** attached to your entity to make it easier to find.

Create and import a sequence

How to import sequences from a file

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!

Nucleotide type*

DNA RNA

Set folder

Patricia

Drag and drop files to upload or choose a file

Note: GenBank sequences use the LOCUS for the sequence name. To use the filename instead, [click here](#).

Open Move To Set Topology Edit Tags Auto-annotate Show errors only

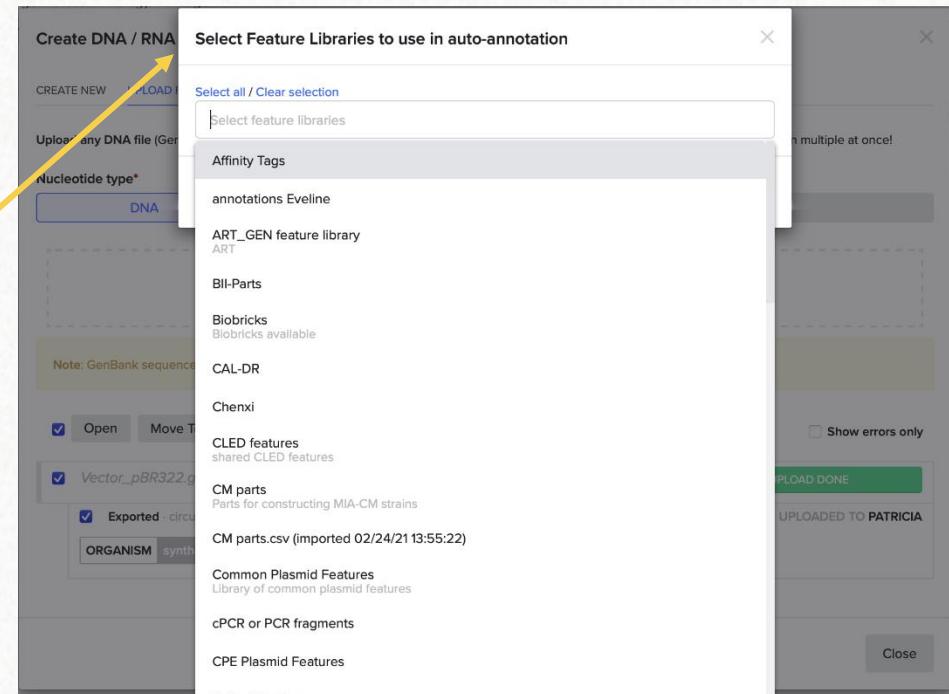
Vector_pBR322.gb

Exported · circular DNA ACCESSION J01749 DEFINITION Cloning vector... OPEN SEQUENCE · UPLOADED TO PATRICIA

ORGANISM synthetic DNA ... SOURCE synthetic DNA ...

UPLOAD DONE

Close



You can **auto – annotate** the sequence from an existing list of features.

- This can also be done **in bulk** when using the expanded view of the selecting multiple entities at once

First steps: Create and import the building blocks to create the DNA construct

Import of sequences from a file

The screenshot shows the 'Create DNA / RNA sequence' interface. At the top, there are tabs for 'CREATE NEW', 'UPLOAD FILES' (which is selected), 'IMPORT FROM DATABASE', and 'SELECT CHROMOSOMAL REGION'. Below this, a text input field says 'Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!'. A 'Nucleotide type*' dropdown is set to 'DNA'. A 'Set folder' button is followed by a dropdown menu showing 'Patricia'. A central area has a dashed box for dragging files, with a placeholder 'Drag and drop files to upload' and a 'or choose a file' button. Below this is a note: 'Note: GenBank sequences use the LOCUS for the sequence name. To use the filename instead, click here.' At the bottom, there are buttons for 'Open', 'Move To', 'Set Topology', 'Edit Tags', 'Auto-annotate', and a highlighted '≡' icon. A red square highlights the '≡' icon. There is also a 'Show errors only' checkbox. The sequence 'Vector_pBR322.gb' is listed with details: 'Exported · circular DNA', 'ACCESSION J01749', 'DEFINITION Cloning vector...', 'ORGANISM synthetic DNA ...', and 'SOURCE synthetic DNA ...'. A green 'UPLOAD DONE' button is visible, along with an 'OPEN SEQUENCE - UPLOADED TO PATRICIA' link. A 'Close' button is at the bottom right.

The screenshot shows a 'Create DNA / RNA' dialog box with a yellow arrow pointing to the 'Add items to entity worklist' button. This button opens a modal window titled 'Add items to entity worklist'. Inside, there are tabs for 'New worklist' (selected) and 'Existing worklist'. A 'Worklist Name*' input field contains 'Project_training'. Below it, 'Selected items' show 'Exported' with a red square highlighting the 'X' icon. A blue 'Add items to worklist' button is at the bottom right. The background shows a list of entities with a checked 'Vector_pBR322.gb' entry. It includes fields like 'ACCESSION J01749', 'DEFINITION Cloning vector...', 'ORGANISM synthetic DNA ...', and 'SOURCE synthetic DNA ...'. Buttons for 'Open', 'Move To', 'Set Topology', 'Edit Tags', 'Auto-annotate', and a trash can icon are visible. A 'Show errors only' checkbox is also present. A green 'UPLOAD DONE' button is at the top right, and a 'CLOSE' button is at the bottom right of the modal.

i You can also **create worklists or add to existing ones** to find your currently used entities faster.

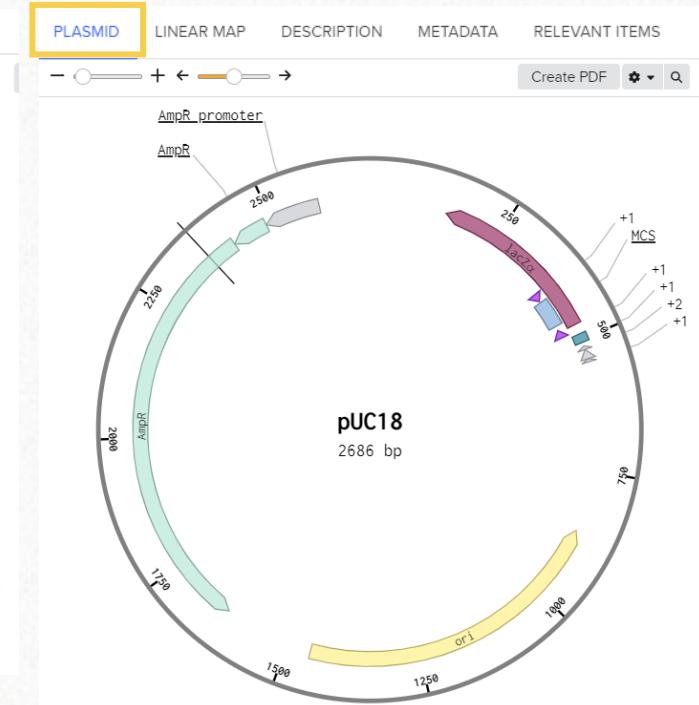
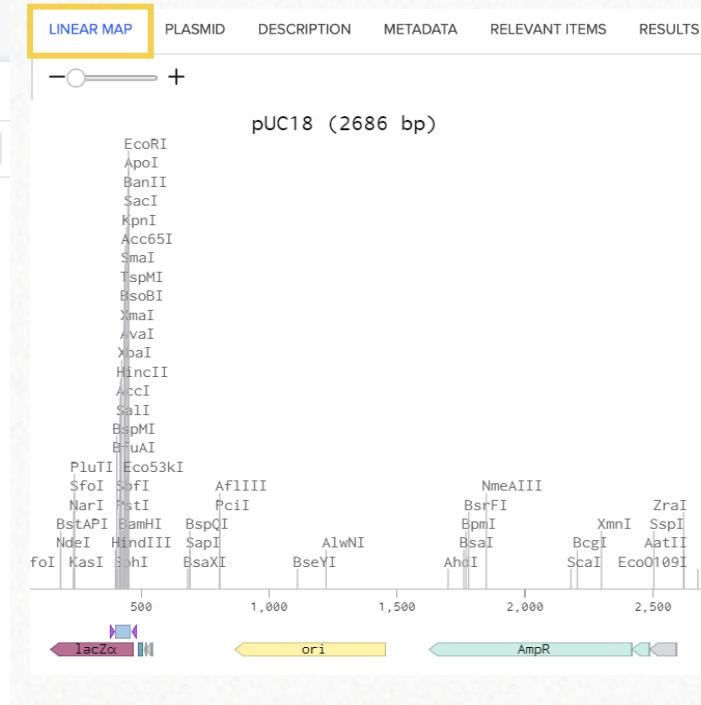
3. The basics of sequences

3.2 Sequence visualization



View, annotate and edit your sequences

Different viewing options:



- ✓ For circular sequences, a plasmid viewing option is available
- ✓ You can click on the different elements or annotations in any of the views to select the corresponding sequence fragment

View, annotate and edit your sequences

Different viewing options:

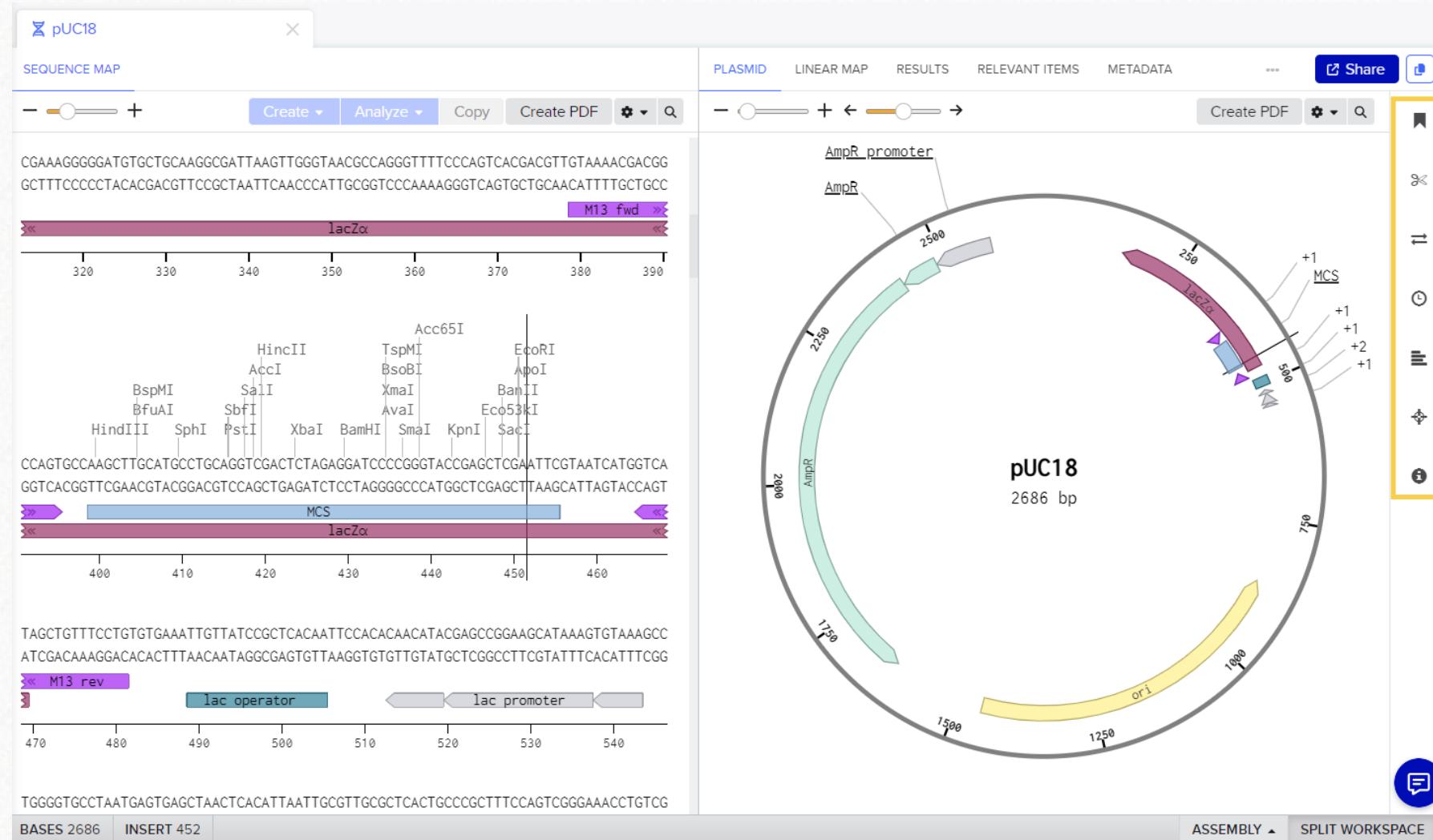


PRO TIP:

Click on “**split workspace**” to change the viewing mode to split screen/full screen

View, annotate and edit your sequences

Sequence navigation:

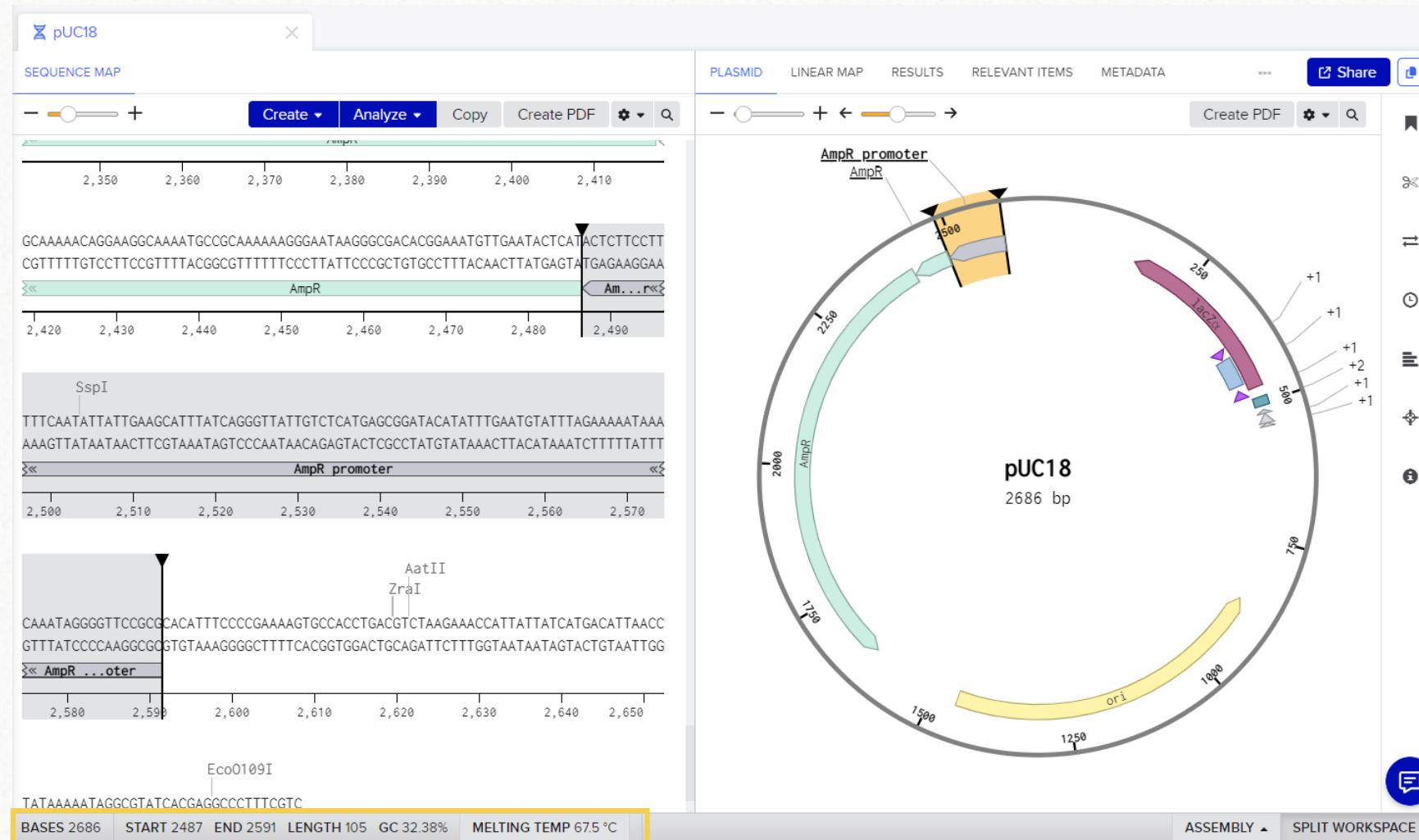


Functionalities

- Features (annotations and translations)
- Digests
- Primers
- History
- Alignments
- CRISPR
- Information (topology, tags)

View, annotate and edit your sequences

Sequence navigation:



- ✓ Click on any element or annotation in any of the views to select the corresponding sequence fragment
- ✓ See the **electrochemical properties** of the fragment on the bottom



PRO TIP:

Click on “*melting temperature*” to access the parameter settings. Different calculation algorithms are available.

View, annotate and edit your sequences

Sequence navigation:

SEQUENCE MAP

Annotations for the sequence: lacZα

Annotations for the sequence: lacZα

Annotations for the sequence: Acc65I

Create **Analyze**

Annotation
Primer
Translation
New AA sequence
New DNA
New RNA
New part

Run Primer3
Run Benchling BLAST
Submit to NCBI BLAST
Analyze as translation
Optimize codons

BstAPI
NdeI

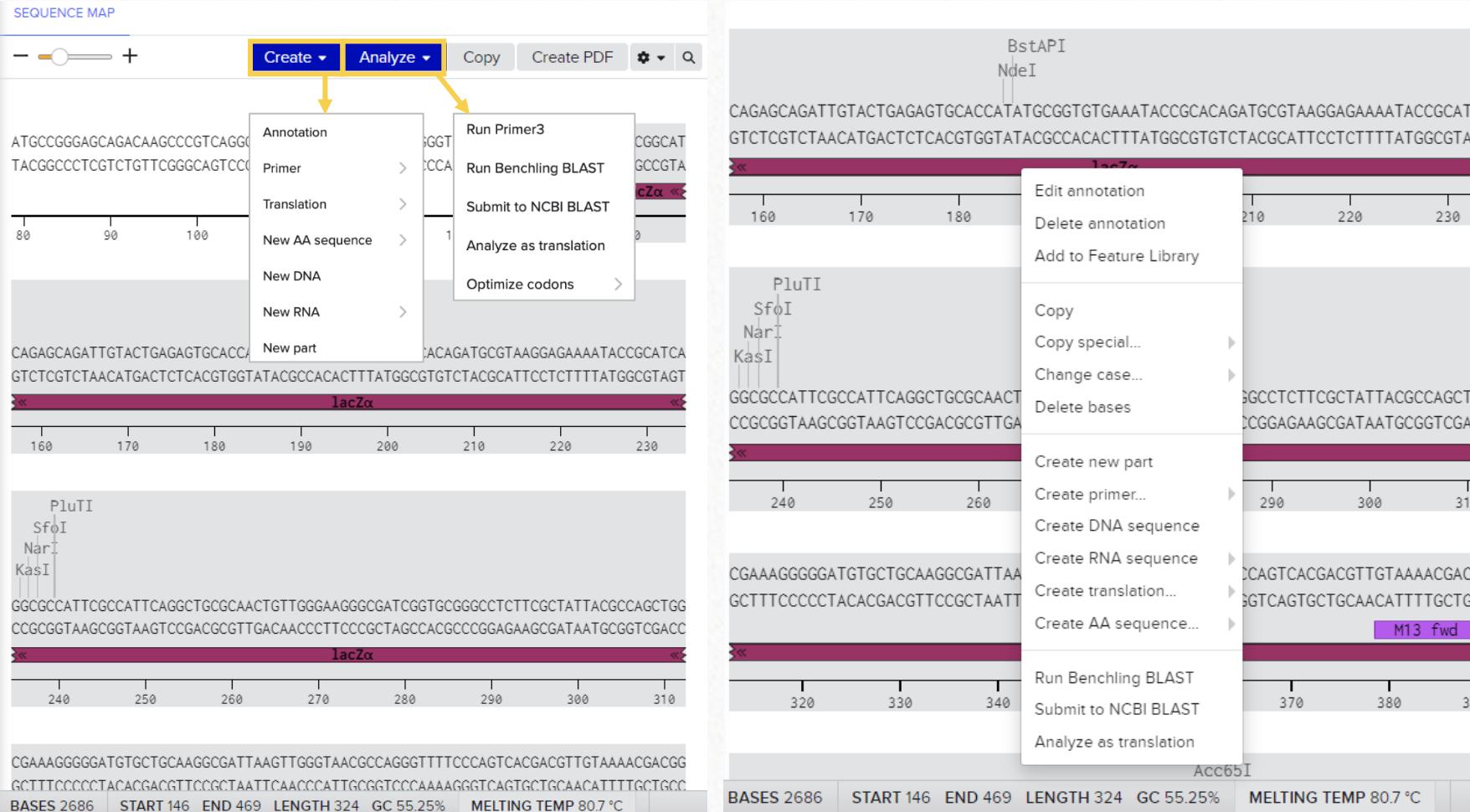
PluTI
SfoI
NarI
KasI

M13 fwd

CGGCCTTTCGCTATTACGCCAGCTGC
CCGGAGAAGCGATAATGCGGTGACCG

Acc65I

BASES 2686 START 146 END 469 LENGTH 324 GC 55.25% MELTING TEMP 80.7 °C



- ✓ With a sequence fragment selected, see the "create" and "analyze" functions
- ✓ Right-click on a selection to unlock a new set of editing options



PRO TIP:
Click directly on any part of the sequence (not a fragment) and paste or write new bases directly.

3. The basics of sequences

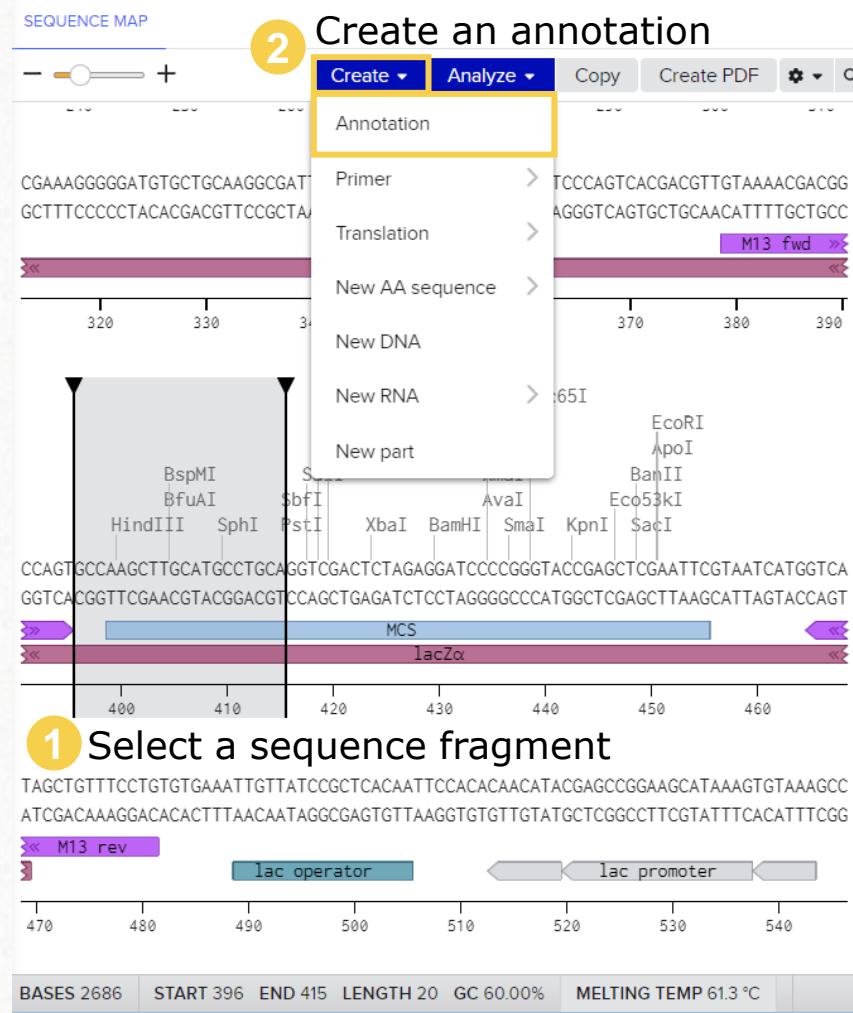
3.3 Sequence annotation



View, annotate and edit your sequences

Sequence annotations

1 Select a sequence fragment



SEQUENCE MAP

Annotation menu (highlighted):

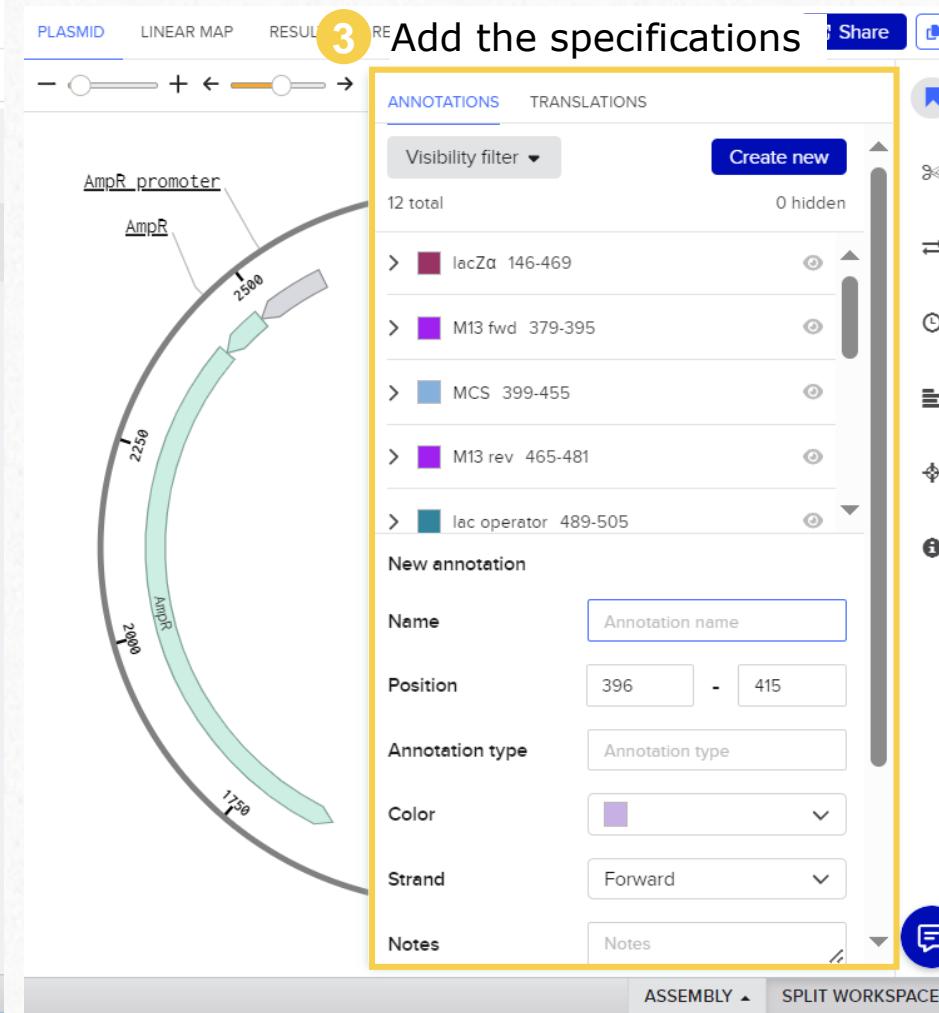
- Create
- Analyze
- Annotation
- Primer
- Translation
- New AA sequence
- New DNA
- New RNA
- New part

Sequence details:

- BASES 2686
- START 396
- END 415
- LENGTH 20
- GC 60.00%
- MELTING TEMP 61.3 °C

2 Create an annotation

3 Add the specifications



PLASMID LINEAR MAP RESULTS Share

Annotations (12 total):

- lacZα 146-469
- M13 fwd 379-395
- MCS 399-455
- M13 rev 465-481
- lac operator 489-505

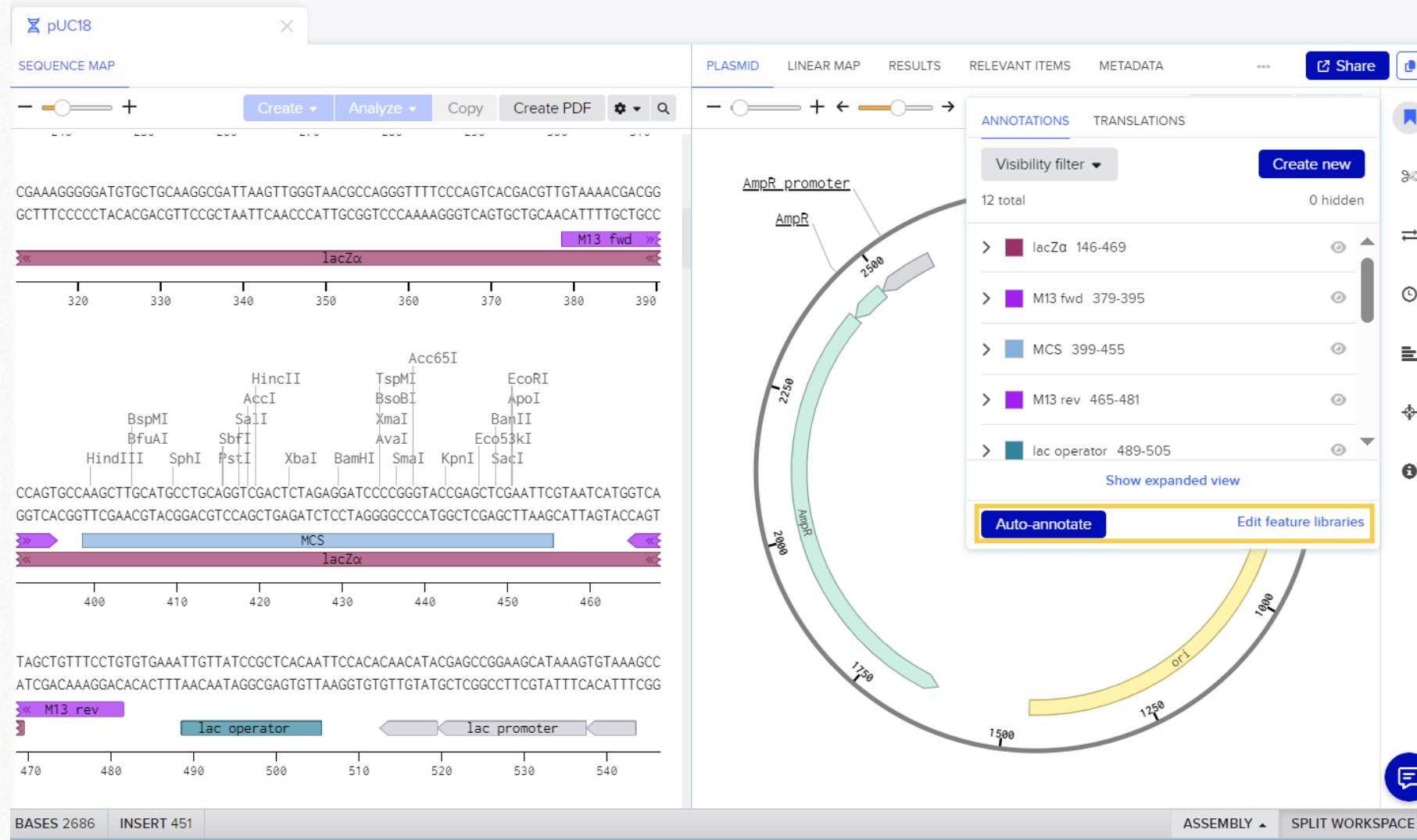
New annotation:

- Name: Annotation name
- Position: 396 - 415
- Annotation type: Annotation type
- Color: Purple
- Strand: Forward
- Notes: Notes

- ✓ Annotations are automatically imported with your sequences when uploading from databases and files

View, annotate and edit your sequences

Sequence annotations



i You can access the “**edit feature libraries**” and “**auto-annotate**” options at any time to create your own annotations list or use an existing one on your sequence

Be aware that the **libraries are shared within the Center** so don’t edit libraries that don’t belong to you

3. The basics of sequences

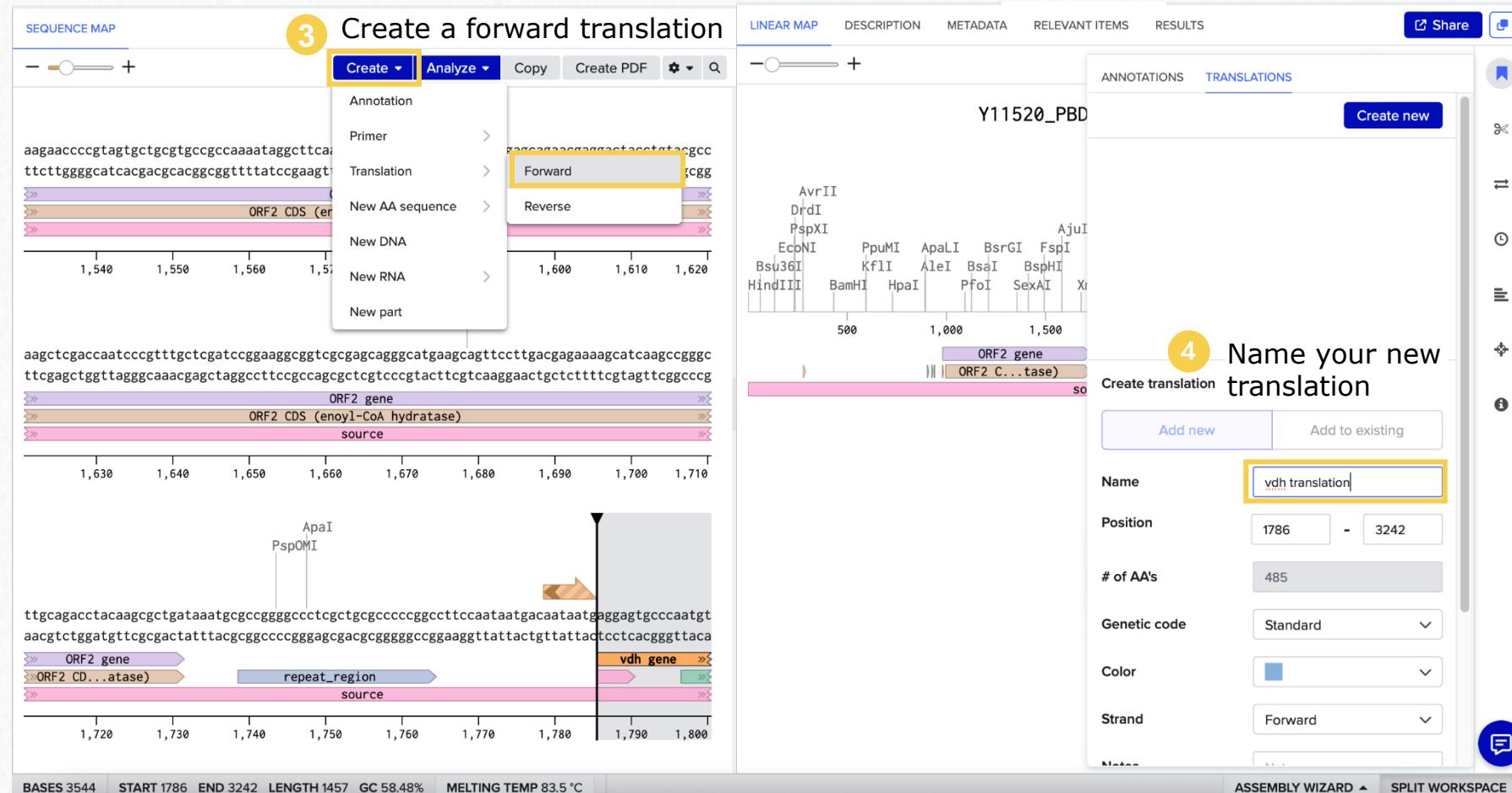
3.4 Codon optimization



View, annotate and edit your sequences

How to **codon optimize** a gene of interest for the host you want to express it in

- 1 Open the file with your gene of interest
- 2 Select the gene (for example, by clicking its annotation)



The screenshot shows the Bioworkshop software interface. On the left, there's a sequence map with two DNA strands. The top strand has a primer and a forward translation highlighted. The bottom strand has an ORF2 gene, a repeat region, and a vdh gene. In the center, a linear map shows restriction sites like AvrII, DrdI, PspXI, EcoNI, PpuMI, ApaI, BsrGI, FspI, Bsu36I, KfII, HpaI, AleI, BsaI, BspHI, HindIII, BamHI, PfoI, SexAI, and XbaI. A zoomed-in view of the vdh gene region is shown on the right, with a 'Create new' button and a 'Name your new translation' input field containing 'vdh translation'. Other fields include Position (1786 - 3242), # of AA's (485), Genetic code (Standard), Color (blue), Strand (Forward), and Notes.

SEQUENCE MAP

LINEAR MAP DESCRIPTION METADATA RELEVANT ITEMS RESULTS Share

Annotations TRANSLATIONS Create new

BASES 3544 START 1786 END 3242 LENGTH 1457 GC 58.48% MELTING TEMP 83.5 °C ASSEMBLY WIZARD ▾ SPLIT WORKSPACE

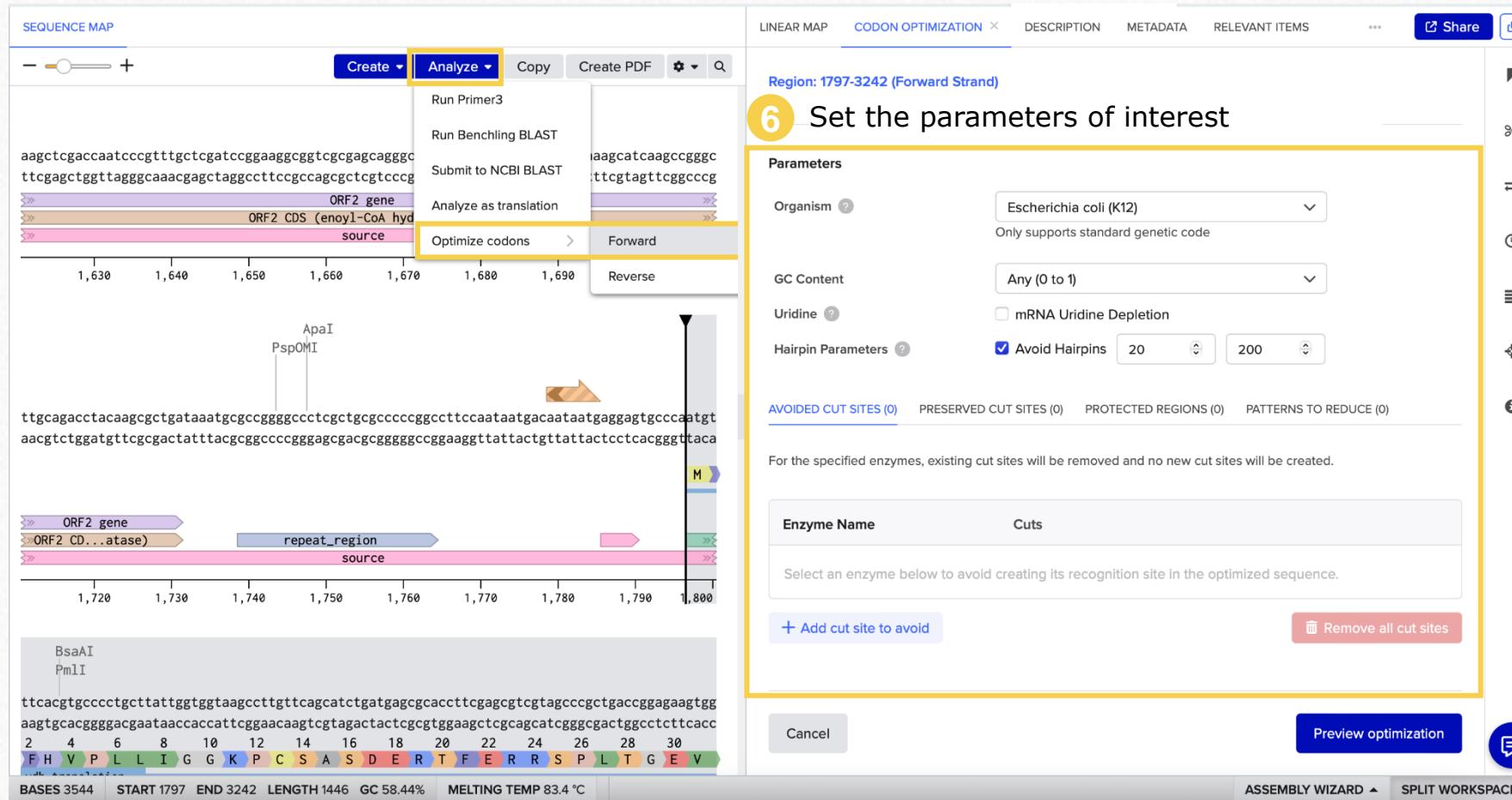
✓ Before codon optimization, the DNA sequence must be translated

i If the sequence fragment selected is not a multiple of 3, the codon optimization will not be possible

View, annotate and edit your sequences

How to **codon optimize** a gene of interest for the host you want to express it in

- Select the newly created translation and codon optimize it



The screenshot shows the Bioworkshop interface with the following components:

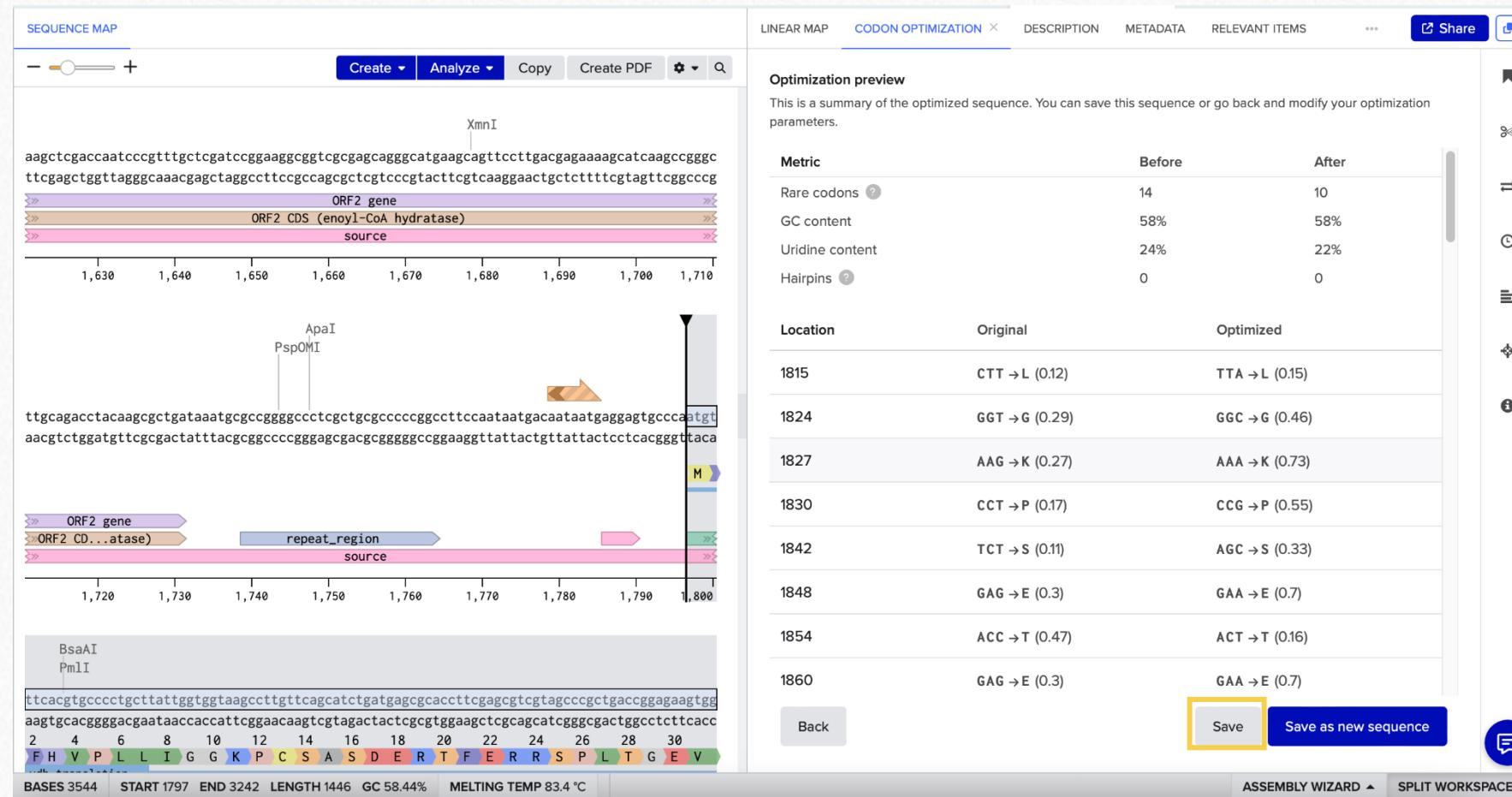
- SEQUENCE MAP:** Displays two sequence regions. The top region spans from 1,630 to 1,690, containing an **ORF2 gene** (purple) and **ORF2 CDS (enoyl-CoA hyd)** (brown). The bottom region spans from 1,720 to 1,800, containing an **ORF2 gene** (purple), **repeat_region** (blue), and **source** (pink). Enzyme restriction sites are marked: ApaI at 1,640, PspOMI at 1,645, BsaAI at 1,720, and PmlI at 1,790.
- CODON OPTIMIZATION:** A context menu is open over the top sequence region, with the "Optimize codons" option highlighted.
- Parameters Panel (highlighted with a yellow box):**
 - Organism:** Escherichia coli (K12)
 - GC Content:** Any (0 to 1)
 - Uridine:** mRNA Uridine Depletion
 - Hairpin Parameters:** Avoid Hairpins (values 20 and 200)
- AVOIDED CUT SITES (0):** A section where users can add or remove cut sites for enzymes like BsaAI and PmlI.
- Buttons:** Cancel, Preview optimization, and a message icon.
- Bottom Status Bar:** BASES 3544, START 1797, END 3242, LENGTH 1446, GC 58.44%, MELTING TEMP 83.4 °C, ASSEMBLY WIZARD ▾, and SPLIT WORKSPACE.

- ✓ When codon optimizing, its possible to select the GC content and other details
- ✓ You can select cut sites to avoid or remove in your optimized sequence

View, annotate and edit your sequences

How to **codon optimize** a gene of interest for the host you want to express it in

- Take a look at the changes made and save the new optimized CDS sequence



- You can keep the changes by saving the new sequence as a new entity or overwriting/editing your original sequence

4. Benchling access and folder setup





LET'S MOVE TO BENCHLING TO START THE HANDS-ON!

Access Benchling:

biosustain.benchling.com

(login with DTU credentials)



Create a training folder to work in

The screenshot shows the Benchling application interface. On the left is a vertical toolbar with various icons. A yellow circle with the number 1 is on the folder icon. The main workspace shows a list of existing projects and a dropdown menu. A yellow circle with the number 2 is on the 'Molecular Biology Training' project in the list, and another on the 'Projects / Biosustain Training / Molecular Biology Training' path in the dropdown. A yellow circle with the number 3 is on the 'Folder' option in the dropdown menu. To the right, a 'Create folder' dialog box is open, containing fields for 'Name*' (with 'Your name' typed in), 'Location*' (set to 'Molecular Biology Training'), and a 'Description' field. A yellow circle with the number 4 is on the 'Create' button.

1

2

3

4

Projects / Biosustain Training / Molecular Biology Training

Mía Last modified 4 days ago

Agata Last modified 21/03/2024

BS Last modified 21/03/2024

Dushica Last modified 18/06/2024

Ester Last modified 20/03/2024

Ingrid Last modified 21/03/2024

JY Last modified 18/06/2024

Kostas test folder Last modified 21/03/2024

Lilos Last modified 21/03/2024

Max Last modified 21/03/2024

Search

Type ▾ Filters

Folder

Entry

Protocol

DNA / RNA sequence

AA sequence

Oligo

Assembly

CRISPR

Entity from schema

Mixture

More

Create folder

Name* Your name

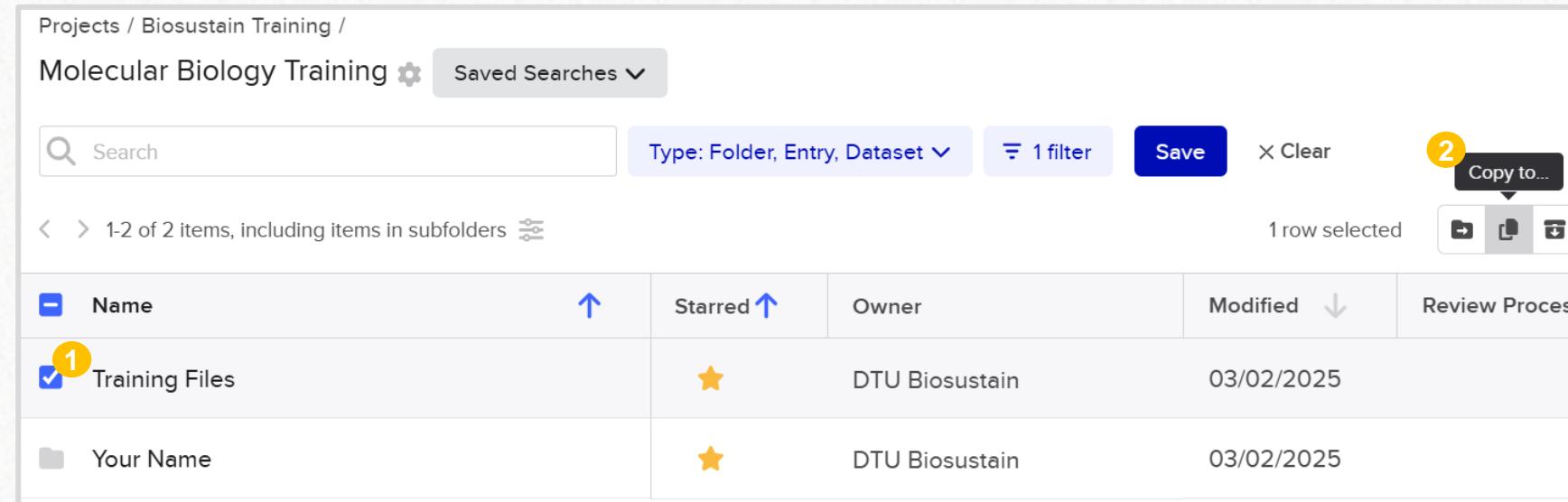
Description

Location* Molecular Biology Training

Create

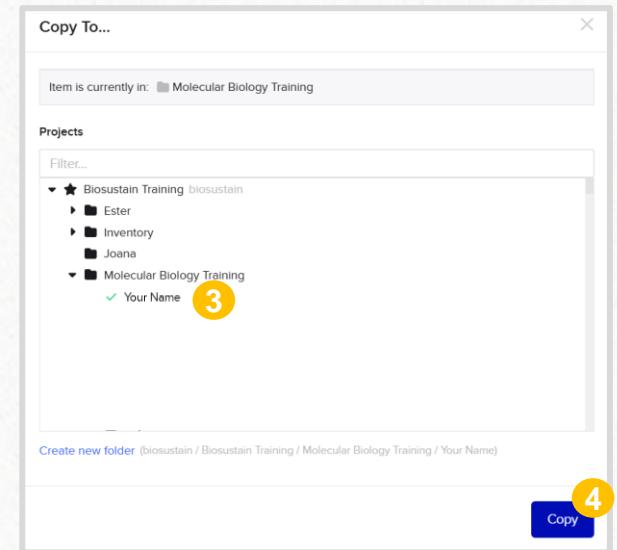
- ✓ Remember to select your own training folder when creating or importing sequences

Copy the *Training Files* folder into your own



The screenshot shows a list of items in a project. The 'Training Files' folder is selected, indicated by a checkmark icon (1). The 'Copy to...' button is highlighted with a yellow circle (2).

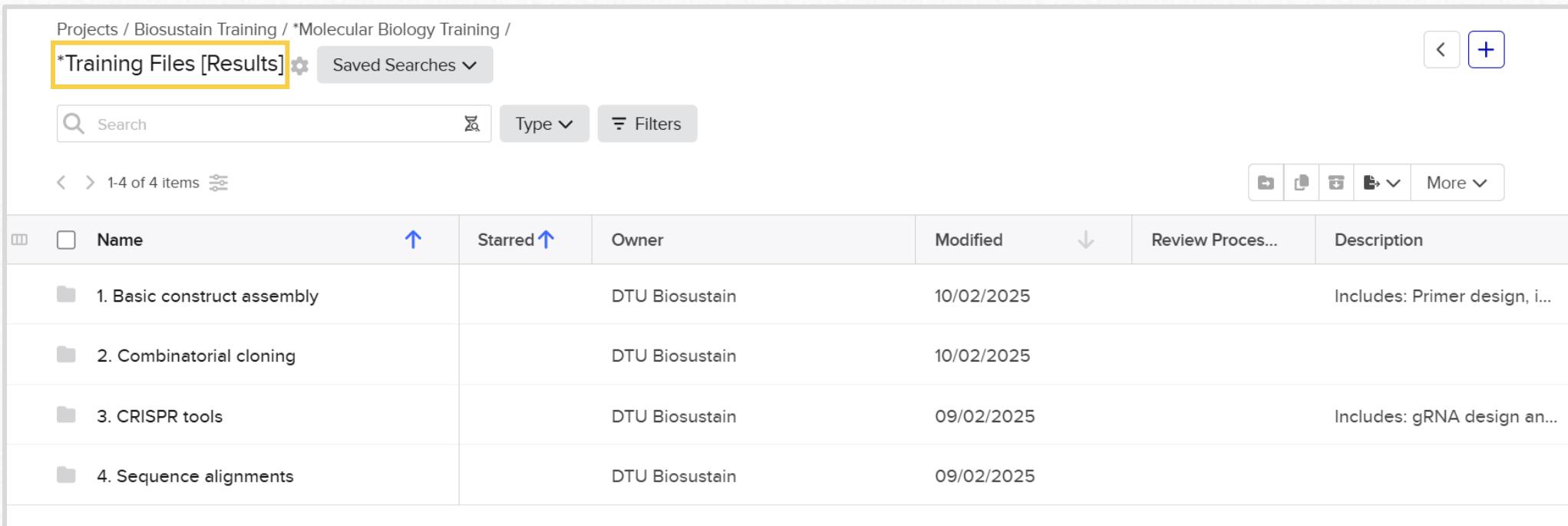
Name	Starred	Owner	Modified	Review Process
Training Files	★	DTU Biosustain	03/02/2025	
Your Name	★	DTU Biosustain	03/02/2025	



Do not modify the *Training Files* folder! Make sure you are **copying it, and **not moving** its contents.**

The *Training Files [Results]* folder

- i** You can find the **expected outputs** for each part of the hands-on in this folder, such as annealed primers, finalized assemblies and resulting constructs.



The screenshot shows a digital interface for managing training files. At the top, there's a breadcrumb navigation: Projects / Biosustain Training / *Molecular Biology Training / *Training Files [Results]. Below this, there are search and filter options. The main area displays a table of four items:

Name	Starred	Owner	Modified	Review Proces...	Description
1. Basic construct assembly		DTU Biosustain	10/02/2025		Includes: Primer design, i...
2. Combinatorial cloning		DTU Biosustain	10/02/2025		
3. CRISPR tools		DTU Biosustain	09/02/2025		Includes: gRNA design an...
4. Sequence alignments		DTU Biosustain	09/02/2025		



Do not modify the contents of this folder!

5. Basic construct assembly



This is the first part of the *hands-on* example.

1

Cloning vector design

Creation of expression vector library

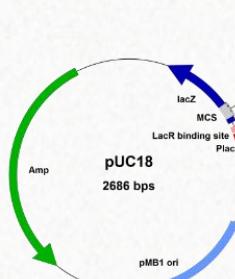
Gene knockout using CRISPR/Cas9

Sequence alignment

alsS and **alsD** from *Bacillus subtilis*



pUC18 cloning vector



- ✓ Primer design
- ✓ *In silico* PCR
- ✓ Virtual digestion
- ✓ RE-based cloning

Expected output:

- alsSD fwd and rev primers
- alsSD PCR product
- Saved BamHI + Sall digestions for the alsSD PCR product and pUC18
- pUC18-alsSD plasmid

You will need the files in the **Basic construct assembly** subfolder.



The screenshot shows the Bioworkshop software interface with two main panels:

Left Panel (Sequence Map):

- Header: ... / Your Name / Training Files / 1. Basic construct assembly
- Search bar: Search (Type: Filters)
- File list:
 - alsSD source (Last modified 9 minutes ago) - highlighted with a yellow box
 - pUC18 (Last modified 9 minutes ago) - highlighted with a yellow box

Right Panel (Linear Map):

- Header: alsSD source (3326 bp)
- Panel tabs: METADATA, DNA FRAGMENT BATCH, LINEAR MAP, RESULTS, Share
- Sequence: tagtgaaacttatcacaagatattaaaattttacgtttaaatgc atcaacttgaatagtgttctataaattttaaaatgcaaattttacg
- Annotations:
 - alsS CDS (blue arrow, positions 145-165, amino acids M T K A T K E Q K)
 - alsS gene (grey arrow, positions 140-180)
 - alsD CDS (blue arrow, positions 165-180, amino acids S L V K N R G A E L V V D C L)
 - alsD gene (green arrow, positions 165-180, amino acids yw...S)
 - yw...e (orange arrow, positions 180-185)
- Restriction sites: Tth11I, Pfl1FI, StuI, MlyI, PfeI, EagI, PciI, XbaI, KpnI, Acc65I, HpaI, NmeAIII, XmnI, BsrGI, BsrDI, BspHI, EcoRI, PsiHAI, AgeI, BsmFI, BaeI, BsaAI, BpuEI, PsII, SmII, BtsOI, BtsI, BtgI, Bsp1286I, BsiHKAI, BlpI, FspI, SphI, BbvCI, Bpu10I, AlwNI, BssSI, BsrBI, BssSXI, BssSI, BsrBI, AlwNI.
- Base statistics: BASES 3326, INSERT 154
- Assembly buttons: ASSEMBLY ▲, SPLIT WORKSPACE

5. Basic construct assembly

5.1 Primer design

5.1.1 Manual primer design

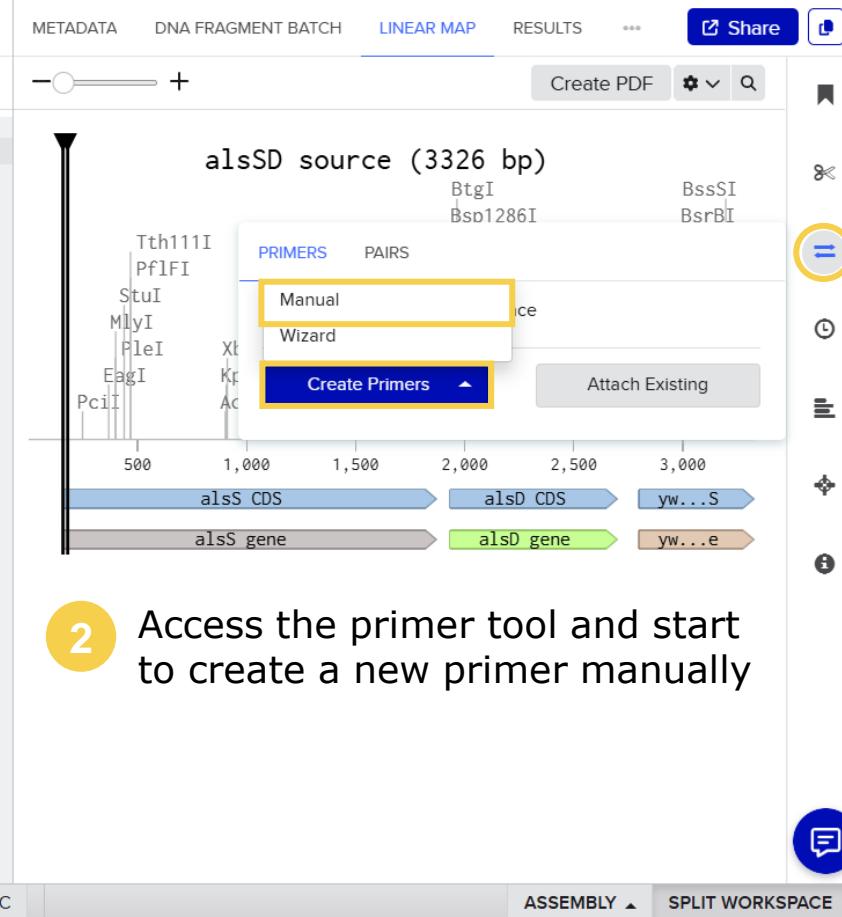
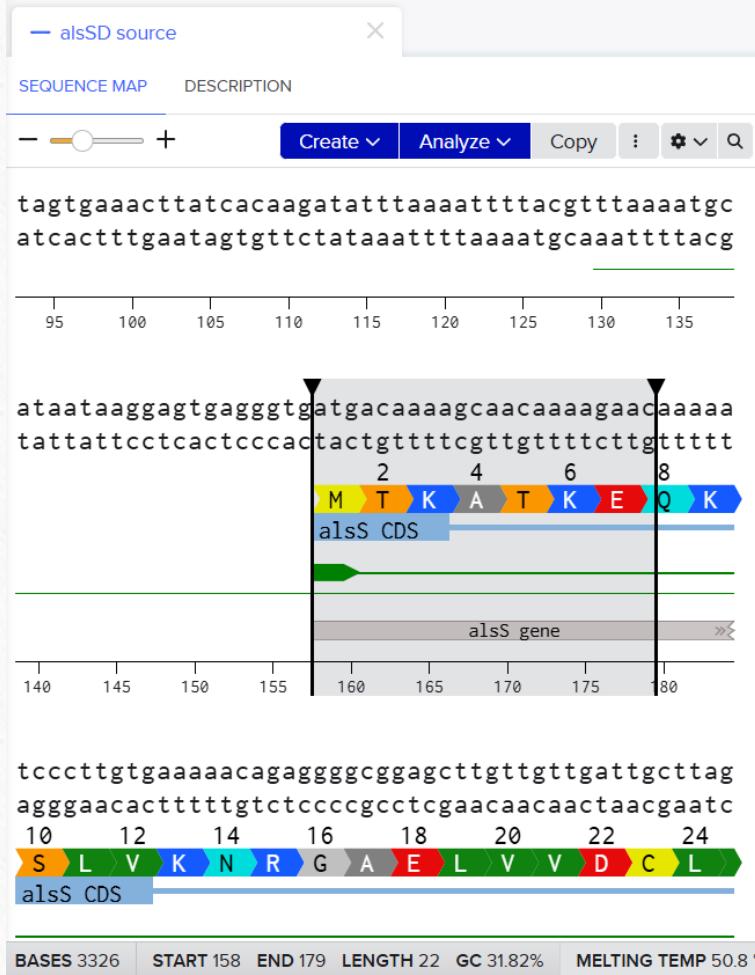


Construct design

Manual primer creation

Scenario: Creating primers to add restriction sites to *a/sSD*

- 1 Select ~ 22 bases at the start of *a/sS*



- 1 You can also attach **already existing** primers to your sequence if the entities are uploaded on Benchling

- 2 Access the primer tool and start to create a new primer manually

Construct design

Manual primer creation

3 Select primer pair creation

alsSD source

SEQUENCE MAP DESCRIPTION

tagtggaaacttatcacaagatattttacgtttaaatgc
atcaactttgaatagtgttctataaattttaaaatgcaatttacg

95 100 105 110 115 120 125 130 135

ataataaggagtgagggtgtatgacaaaaagcaacaaaaagaacaaaaa
tattatttcctcactcccaactactgtttcggtttttttttttttttttttt
2 4 6 8
M T K A T K E Q K
alsS CDS

3' Location 1 1

Overhang 0 bp 0 bp

Cut Site Aanl

Verify Check Secondary Structure at 50 °C

T_m GC Content

Bases 5' 3'

Strand Forward Reverse

Jump to Primer Set from Selection

Share

4 Set the 3' selected bases as forward (**start of alsS**)

5 Set the 5' selected bases as reverse (**end of alsD**)

✓ Make sure to select the **start of alsS** and the **end of alsD**

BASES 3326 START 158 END 179 LENGTH 22 GC 31.82% MELTING TEMP 50.8 °C

ASSEMBLY SPLIT WORKSPACE

Construct design

Manual primer creation

- 6 Look up **BamHI** restriction site in the *Cut site* dropdown menu
- 7 Copy and paste the site at the beginning of the forward primer, and set the **overhang** to 6

Strand Forward Reverse

Bases	5'	3'	5'	3'
GGATCC	atgacaaaagca		ttattcagggttccttc	agt
acaaaagaac				

3' Location 179 2678

Overhang 6 0

Cut Site BamHI GGATCC

Use the dropdown above to look up restriction sites.

Strand Forward Reverse

Bases	5'	3'	5'	3'
GGATCC	atgacaaaagca		GTCGAC	tattcagggttccttc
acaaaagaac			tccttcagtt	

3' Location 179 2678

Overhang 6 6

Cut Site Sall GTCGAC

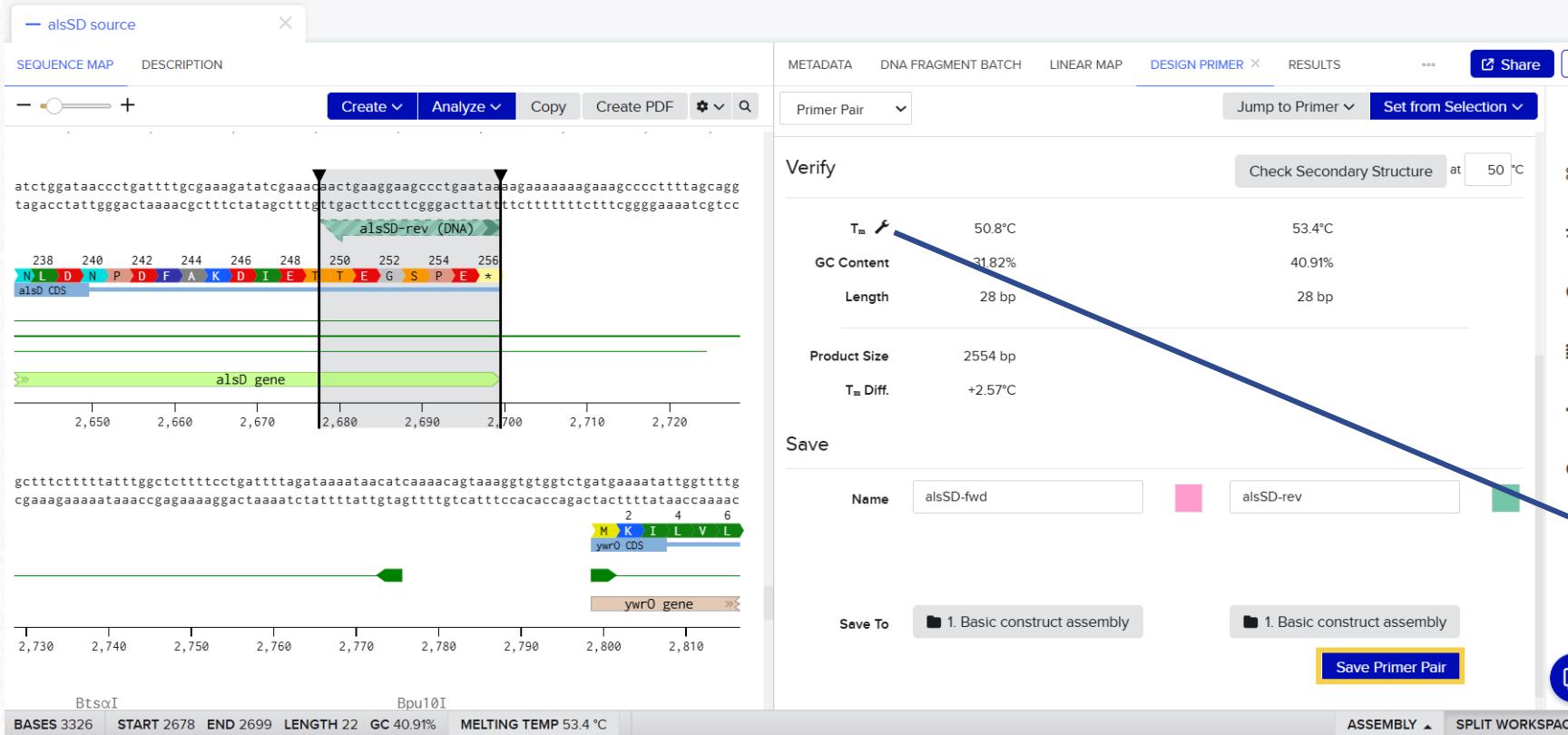
Use the dropdown above to look up restriction sites.

- 8 Repeat the process to add a **Sall** site at the beginning of the reverse primer

Construct design

Manual primer creation

9 Name, select a location for your primers and save them



- ✓ Make sure to check that the melting temperatures of your primer pair are within an acceptable range

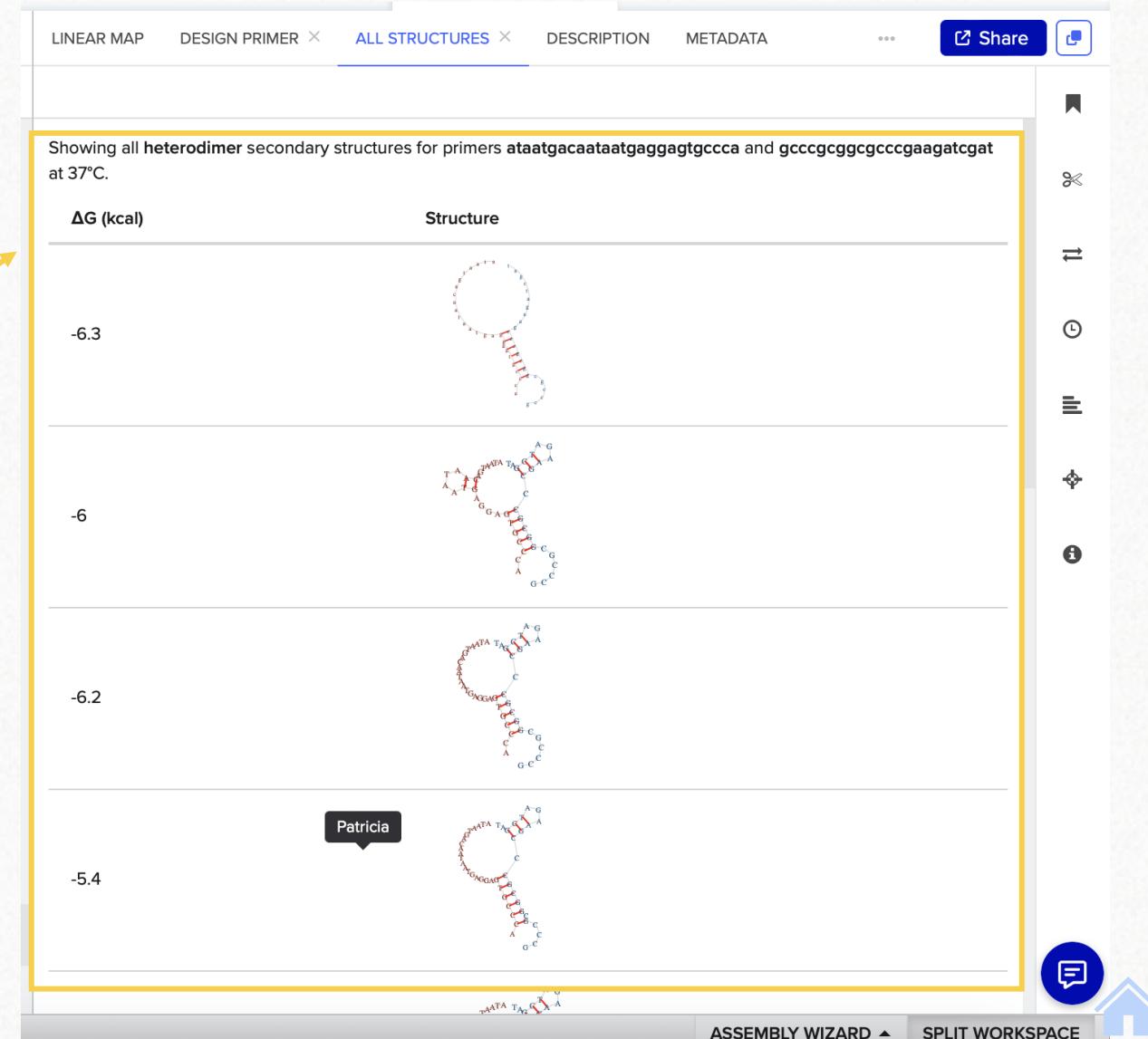
PRO TIP: You can adjust the default parameters for thermodynamic calculations

Construct design

Manual primer creation

i PRO TIP: Benchling offers the possibility to visualize **secondary structures** of your primers

The screenshot shows the Benchling software interface for primer design. The top navigation bar includes tabs for LINEAR MAP, DESIGN PRIMER (selected), ALL STRUCTURES (highlighted with a yellow box), DESCRIPTION, and METADATA. Below the tabs are dropdowns for Primer Pair, Overhang (0 bp), Cut Site (Aanl), and a note to use the dropdown above to look up restriction sites. A 'Verify' section displays various metrics: T_m (56.1°C and 69.8°C), GC Content (38.46% and 73.91%), Length (26 bp and 23 bp), Min ΔG Homodimer (-3.3 kcal and -13.8 kcal), Min ΔG Monomer (-0.1 kcal and -2.5 kcal), Product Size (1495 bp), T_m Diff. (+13.77°C), and Min ΔG Heterodimer (-6.3 kcal). A 'Check Secondary Structure' button is highlighted with a yellow box and has a tooltip indicating it's set to 37 °C. The 'Save' section allows naming the primers as fwd_vdh and rev_vdh. At the bottom are buttons for ASSEMBLY WIZARD and SPLIT WORKSPACE.



5. Basic construct assembly

5.1 Primer design

5.1.2 Primer wizard

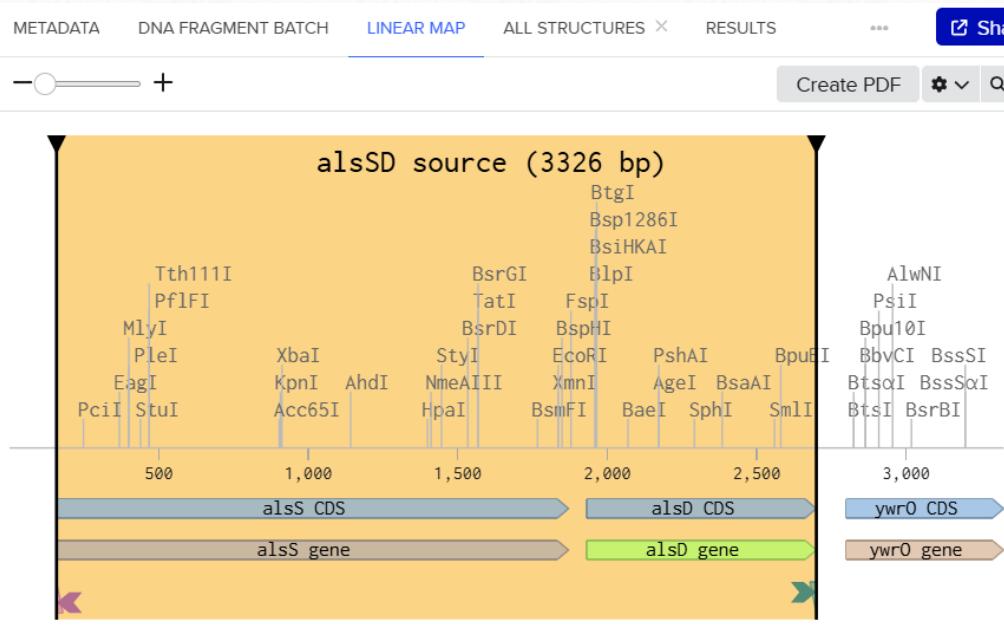


Construct design

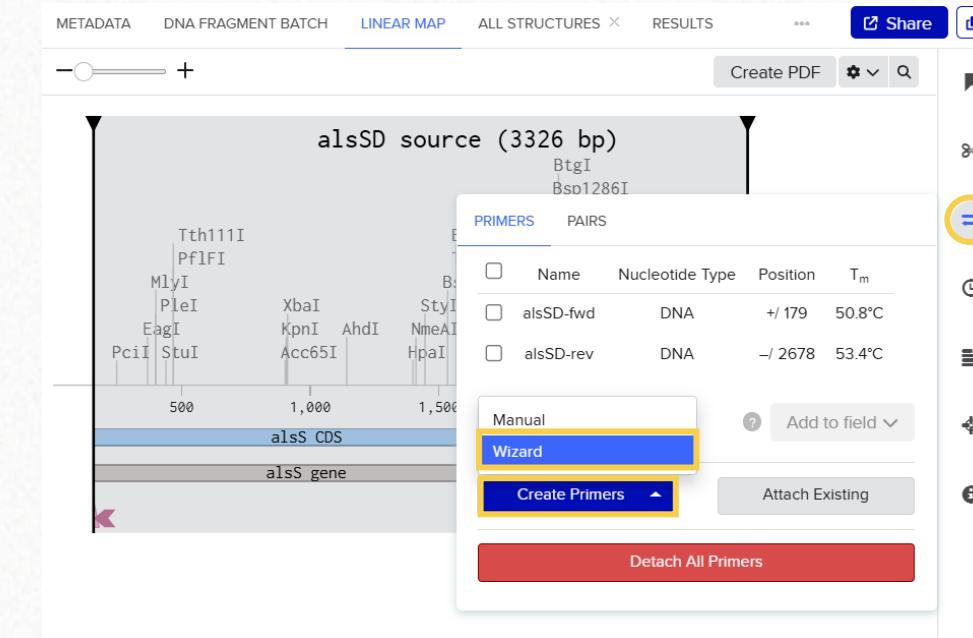
Automatic primer creation – Primer Wizard

- ✓ Benchling has a tool for automatic primer creation called the **Primer Wizard**. To try it out, follow these steps:

1 Select the CDS of *alsS* and *alsD*



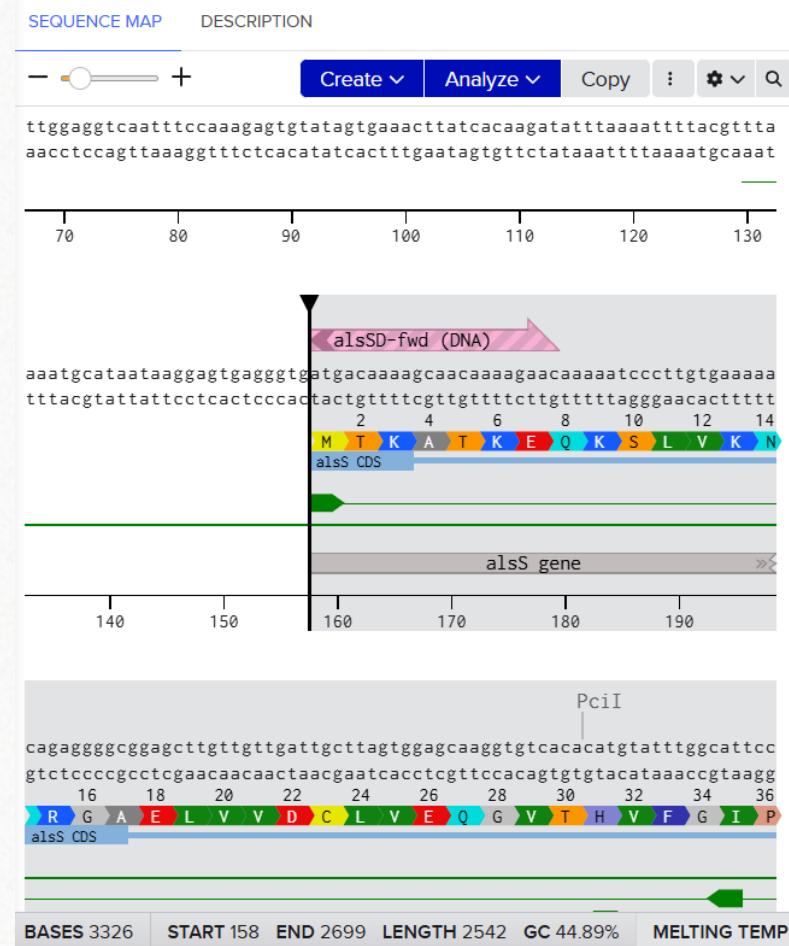
2 Access the primer tool and select *wizard*



PRO TIP: Select both sequences by holding **Shift** while you click on the second one

Construct design

Automatic primer creation – Primer Wizard



3 Select PCR as sequencing task

METADATA DNA FRAGMENT BATCH PRIMER WIZARD X Share

Task PCR T_m Reset

Region

Target 158 2699 Use selection

Primer

	Min	Opt	Max
GC%	30	50	65
T _m	45	62	65
Size	15	22	31
3' GC clamp	0		

5 Generate Primers

4 Use your selected sequence

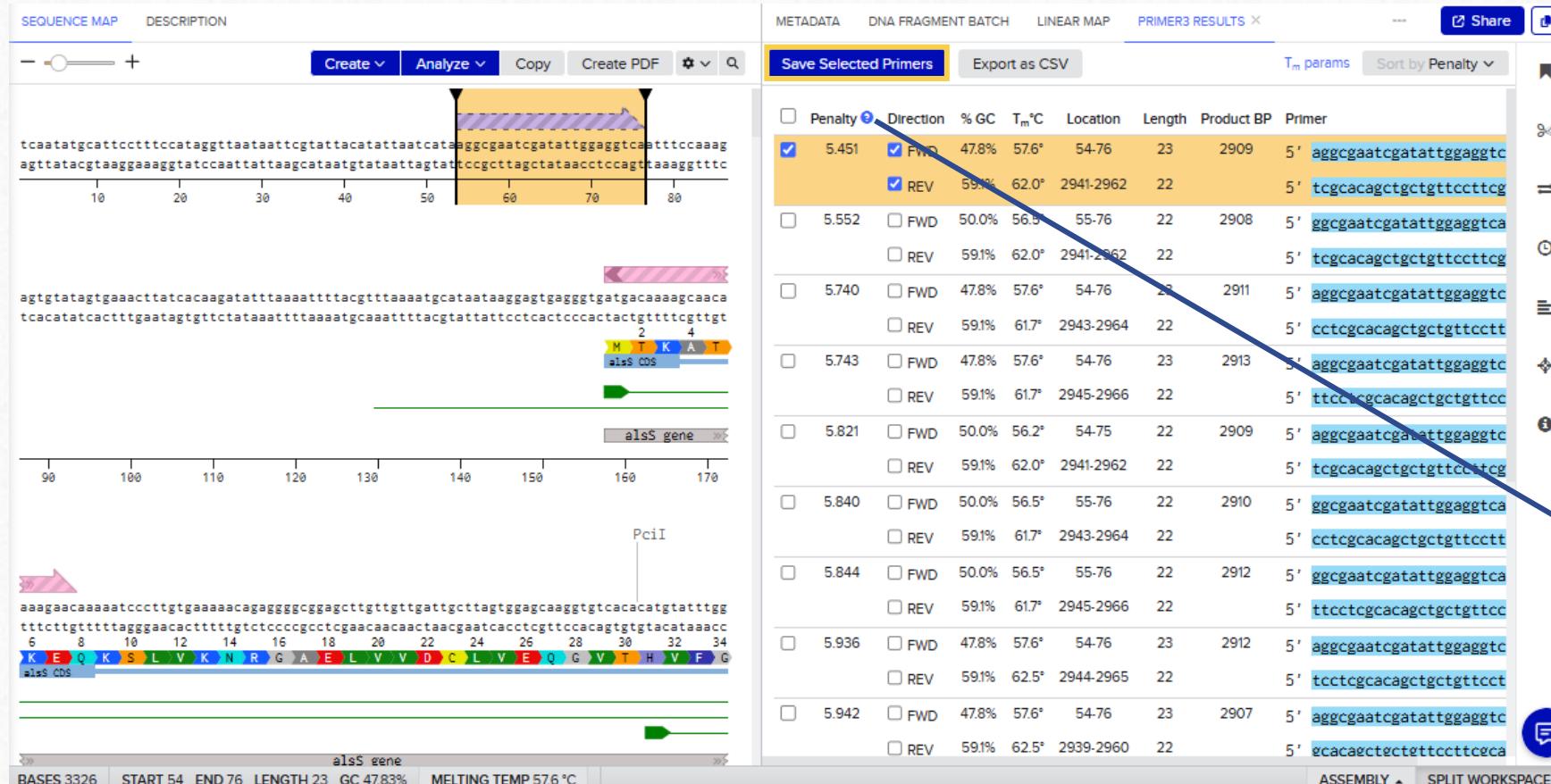
- ✓ Primer Wizard allows for different sequencing tasks
- ✓ Primer Wizard is powered by Primer3

 If you find any problem in the creation of the primers, choose a higher maximum amplicon size

Construct design

Automatic primer creation – Primer Wizard

- 6 Explore the primer options. You do not need to save them to continue with the next part of this tutorial.



The screenshot shows the Primer Wizard software interface. On the left, there are three sequence maps for genes alsS, alsS gene, and PciI. The alsS gene map highlights a region from position 54 to 76. The PciI map highlights a region from position 6 to 34. Below the maps, the following parameters are shown: BASES 3326, START 54, END 76, LENGTH 23, GC 47.83%, and MELTING TEMP 57.6 °C. On the right, the "PRIMER3 RESULTS" tab is active, displaying a table of primer pairs. The table includes columns for Penalty, Direction, % GC, T_m °C, Location, Length, Product BP, and Primer sequence. The first primer pair is selected with checkboxes for FWD and REV directions. A blue arrow points from the "Save Selected Primers" button in the top bar to the "Sort by Penalty" button in the table header.

	Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
<input checked="" type="checkbox"/>	5.451	<input checked="" type="checkbox"/> FWD	47.8%	57.6°	54-76	23	2909	5' aggcgaatcgatattggaggtc
<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/> REV	59%	62.0°	2941-2962	22		5' tcgcacagctgctgtccitcg
<input type="checkbox"/>	5.552	<input type="checkbox"/> FWD	50.0%	56.5	55-76	22	2908	5' ggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgtccitcg
<input type="checkbox"/>	5.740	<input type="checkbox"/> FWD	47.8%	57.6°	54-76	23	2911	5' aggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	61.7°	2943-2964	22		5' cctcgcacagctgctgtccitcg
<input type="checkbox"/>	5.743	<input type="checkbox"/> FWD	47.8%	57.6°	54-76	23	2913	5' aggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	61.7°	2945-2966	22		5' ttccgcacagctgctgtccitcg
<input type="checkbox"/>	5.821	<input type="checkbox"/> FWD	50.0%	56.2°	54-75	22	2909	5' aggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgtccitcg
<input type="checkbox"/>	5.840	<input type="checkbox"/> FWD	50.0%	56.5°	55-76	22	2910	5' ggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	61.7°	2943-2964	22		5' cctcgcacagctgctgtccitcg
<input type="checkbox"/>	5.844	<input type="checkbox"/> FWD	50.0%	56.5°	55-76	22	2912	5' ggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	61.7°	2945-2966	22		5' ttccgcacagctgctgtccitcg
<input type="checkbox"/>	5.936	<input type="checkbox"/> FWD	47.8%	57.6°	54-76	23	2912	5' aggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	62.5°	2944-2965	22		5' ttccgcacagctgctgtccitcg
<input type="checkbox"/>	5.942	<input type="checkbox"/> FWD	47.8%	57.6°	54-76	23	2907	5' aggcgaatcgatattggaggtc
<input type="checkbox"/>		<input type="checkbox"/> REV	59.1%	62.5°	2939-2960	22		5' gcacaactcttcccttccea

✓ It is possible to select primers independently of their pair, so you can mix and match as you need!

i By default, sorting is done based on Primer3 penalty score.
The lower the penalty, the better the primer pair

5. Basic construct assembly

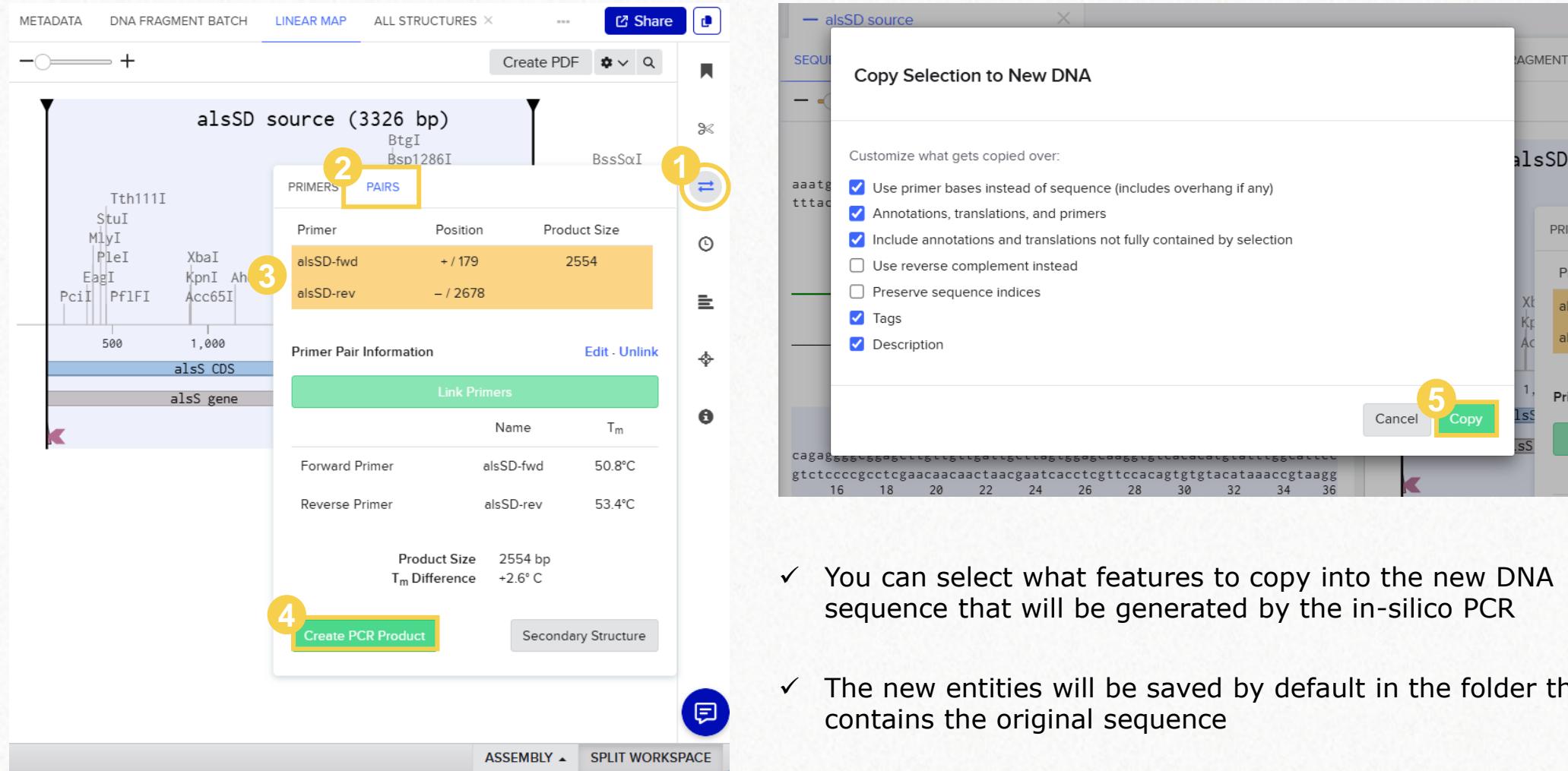
5.2 *In-silico* PCR



Construct design

In-silico PCR: Create a PCR product

- ✓ We will do an *in-silico* PCR using the primers created **manually**, to add the **BamHI** and **SalI** restriction sites.

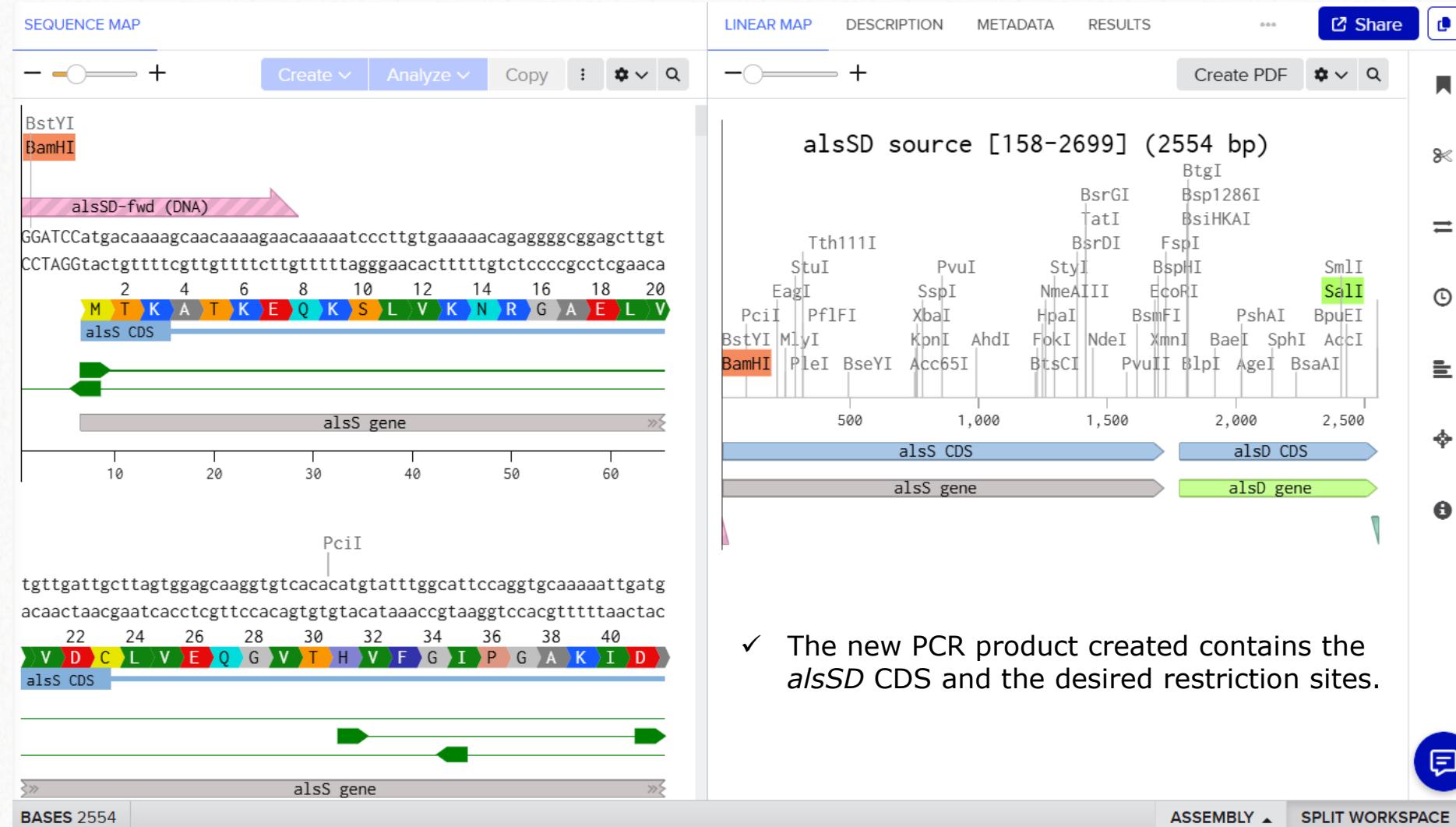


The screenshot shows the BioEdit software interface for creating an in-silico PCR product. On the left, a linear map of the *alsSD source* (3326 bp) is displayed, showing various restriction sites (BtgI, Bsp1286I, BssSsI, Tth111I, StuI, MlyI, PteI, XbaI, EagI, PciI, Pf1FI, KpnI, Ah, Acc65I) and the *alsS CDS* and *alsS gene*. A primer pair is selected: **alsSD-fwd** at position + 179 and **alsSD-rev** at position - 2678. The product size is 2554 bp, with a T_m difference of +2.6°C. On the right, a modal window titled "alsSD source" is open, showing the "PAIRS" tab selected. It contains options for "Use primer bases instead of sequence (includes overhang if any)", "Annotations, translations, and primers", "Include annotations and translations not fully contained by selection", "Use reverse complement instead", "Preserve sequence indices", "Tags", and "Description". At the bottom right of the modal is a green "Copy" button. Numbered circles (1 through 5) highlight specific steps: 1 points to the copy icon in the main toolbar; 2 points to the "PAIRS" tab; 3 points to the forward primer; 4 points to the "Create PCR Product" button; and 5 points to the "Copy" button in the modal.

- ✓ You can select what features to copy into the new DNA sequence that will be generated by the in-silico PCR
- ✓ The new entities will be saved by default in the folder that contains the original sequence

Construct design

In-silico PCR: Create a PCR product



- ✓ The new PCR product created contains the *alsSD* CDS and the desired restriction sites.

5. Basic construct assembly

5.3 Virtual digestion

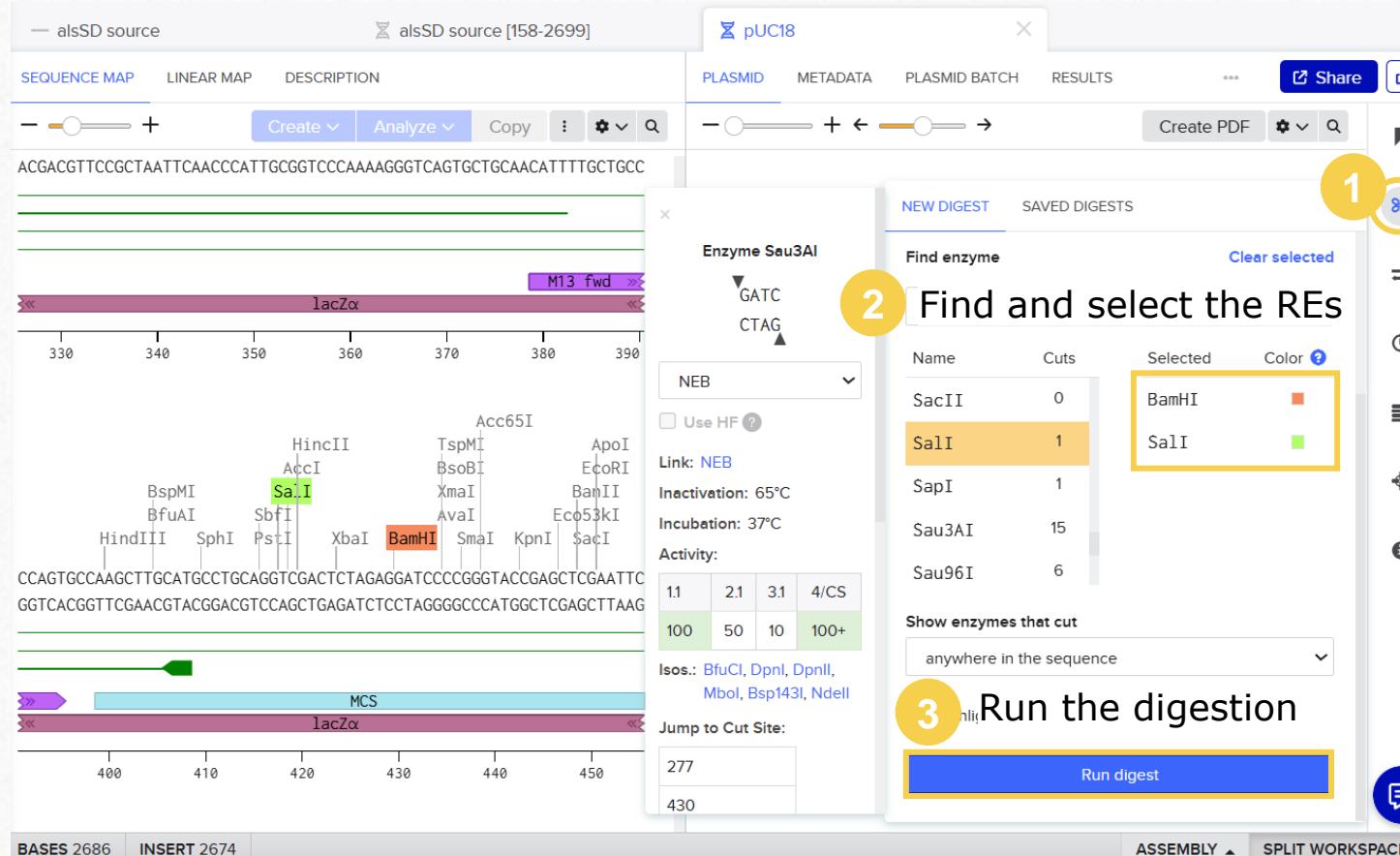


Construct design

Virtual digestion

We will run two virtual digestions to create the **compatible sticky ends** for RE-based cloning in our gene of interest and the backbone (pUC18)

Digestion of the backbone (open the pUC18 sequence)



The screenshot shows the alsSD software interface for designing a construct. On the left, a sequence map of the pUC18 backbone is displayed, showing restriction sites for various enzymes. A specific site for **BamHI** is highlighted in red. In the center, a 'NEW DIGEST' dialog box is open, listing enzymes and their properties. The enzyme **Sau3AI** is selected. The 'Selected' column shows that **BamHI** and **SalI** are selected, indicated by orange boxes around their rows. At the bottom right of the dialog, a blue button labeled 'Run digest' is visible.

1  Find and select the REs

2  Find and select the REs

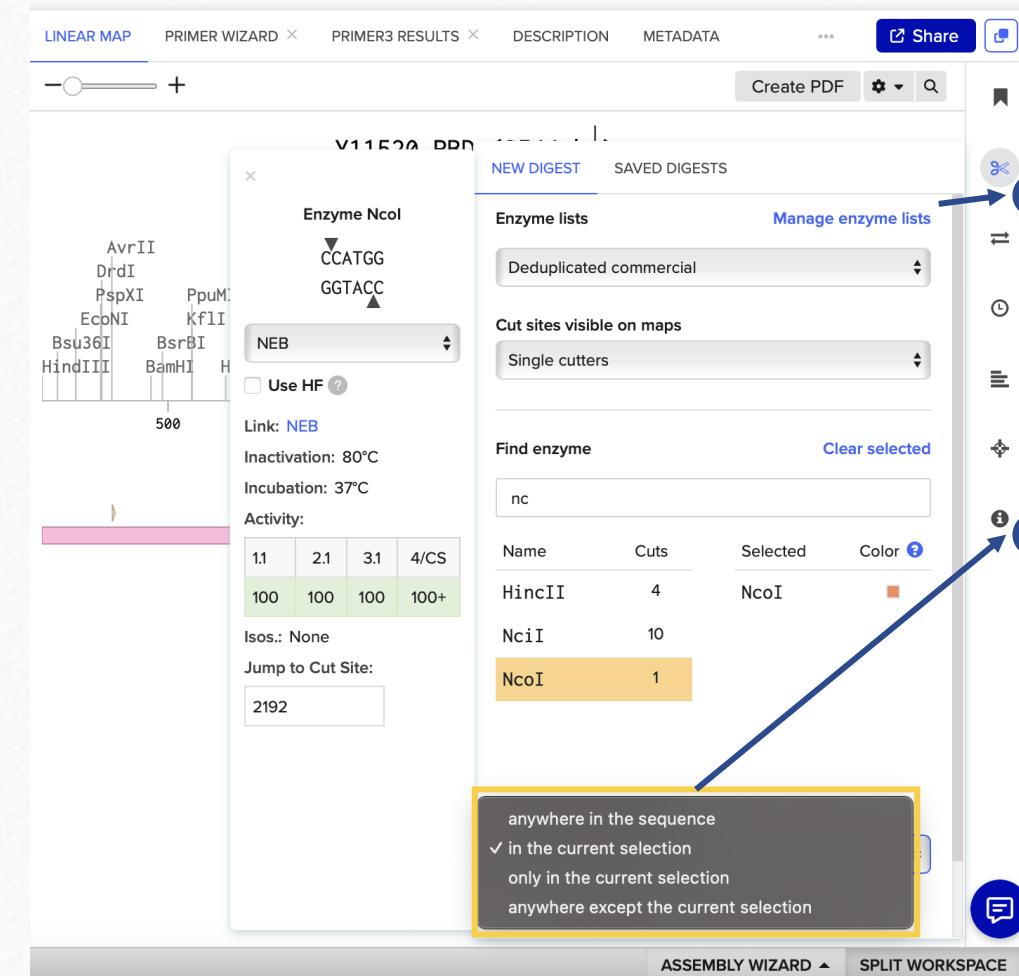
3  Run the digestion

Name	Cuts	Selected	Color
SacII	0		
SalI	1		
SapI	1		
Sau3AI	15		
Sau96I	6		

- ✓ The REs selected for this example are **BamHI** and **SalI**, which are single cutters in the MCS of pUC18.

Construct design

Virtual digestion



The screenshot shows the BioEdit software interface with the 'LINEAR MAP' tab selected. A context menu is open over a sequence fragment, showing enzyme information for NcoI. The sequence CCATGG is shown with a restriction site GGTACC highlighted. The enzyme details include:

- Enzyme NcoI
- Cuts: CCATGG, GGTACC
- NEB
- Link: NEB
- Inactivation: 80°C
- Incubation: 37°C
- Activity:

1.1	2.1	3.1	4/CS
100	100	100	100+
- Iso.: None
- Jump to Cut Site: 2192

The right panel displays enzyme lists and a find enzyme search. A tooltip at the bottom right of the enzyme list panel provides options for selecting enzymes based on their location in the sequence:

- anywhere in the sequence
- ✓ in the current selection
- only in the current selection
- anywhere except the current selection

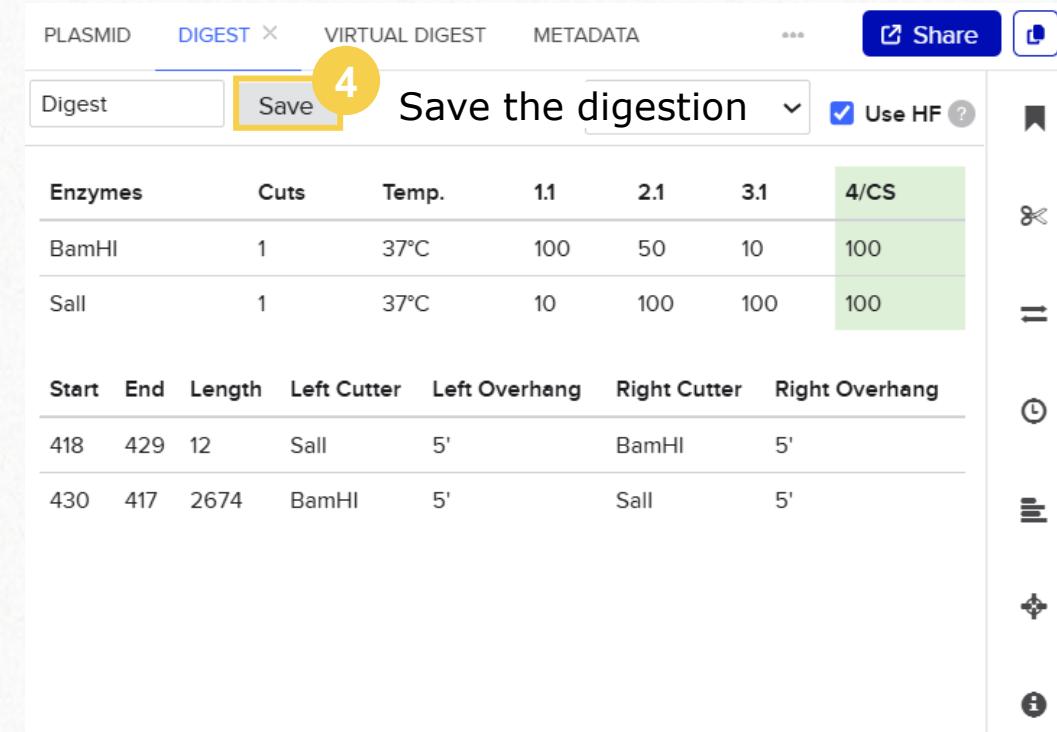
PRO TIP: The enzyme lists available can be managed, similarly to the features libraries and are shared within the Center.

PRO TIP: Click on any fragment of the sequence to select the enzyme list relevant to that fragment

Construct design

Virtual digestion

Digestion of the backbone



The screenshot shows the BioEdit software interface with the 'Virtual Digest' tab selected. At the top, there are tabs for 'PLASMID', 'DIGEST X', 'VIRTUAL DIGEST' (which is highlighted in blue), and 'METADATA'. Below the tabs is a toolbar with a 'Share' button and a 'Save' button, which is highlighted with a yellow circle containing the number '4' to indicate unsaved changes. To the right of the save button is a dropdown menu labeled 'Save the digestion' with a 'Use HF' checkbox. The main workspace displays two rows of restriction enzyme digestions:

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
BamHI	1	37°C	100	50	10	100
Sall	1	37°C	10	100	100	100

Below this, a table provides detailed cut information:

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
418	429	12	Sall	5'	BamHI	5'
430	417	2674	BamHI	5'	Sall	5'

To the right of the workspace are several vertical icons: a bookmark, a share icon, a copy icon, a double arrow icon, a clock icon, a list icon, a diamond icon, and an info icon.

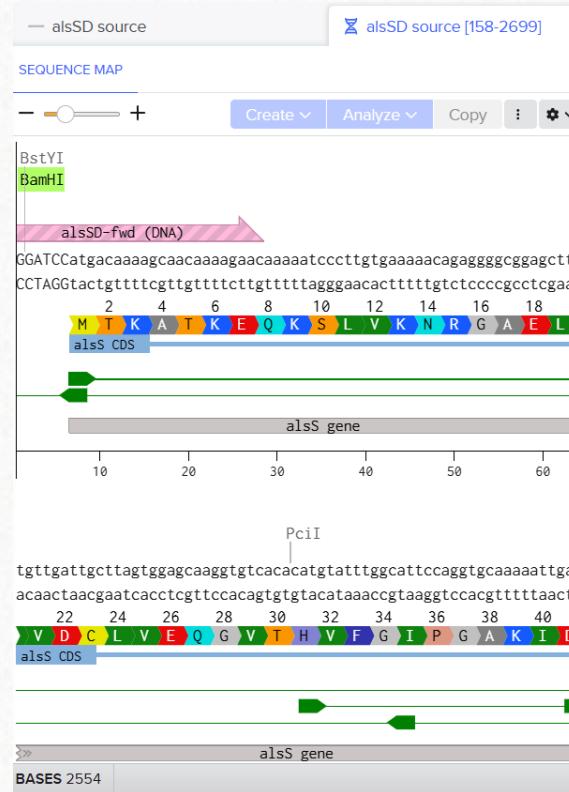
- ✓ A saved digestion will allow you to easily find the fragments you need to work with for the assembly

Construct design

Virtual digestion

Digestion of the insert

1 Open the amplified *alsSD* sequence



Enzyme BamHI

GGATCC
CCTAGG

NEB

Use HF

Find enzyme

Selected

BamHI

SalI

Show enzymes that cut

anywhere in the sequence

Highlight enzymes with compatible sticky ends

Run digest

LINEAR MAP DIGEST VIRTUAL DIGEST DESCRIPTION Share

Digest Save

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
BamHI	1	37°C	100	50	10	100
SalI	1	37°C	10	100	100	100

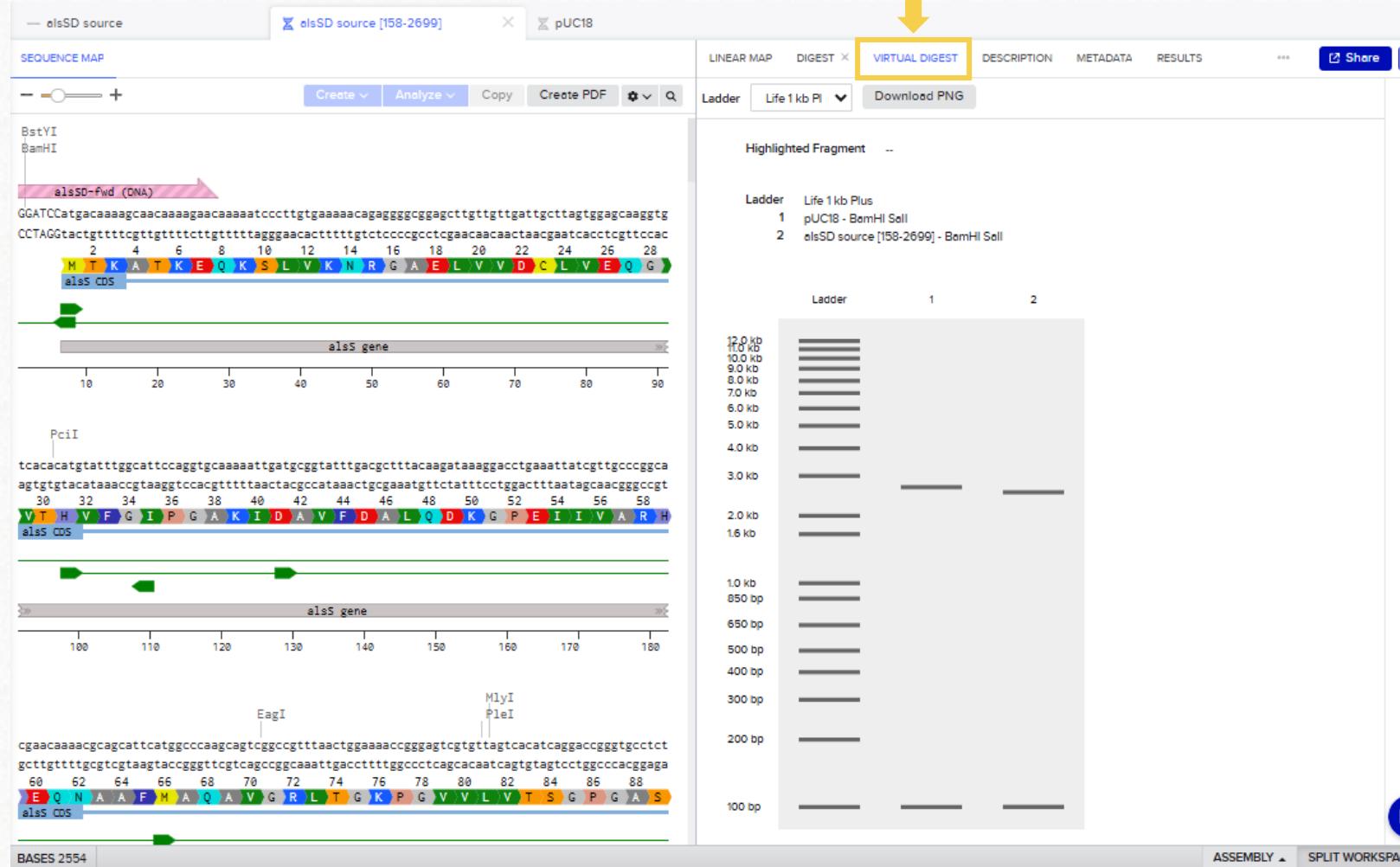
Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	BamHI	5'
2	2549	2548	BamHI	5'	SalI	5'
2550	2554	5	SalI	5'	None	blunt

Run the digestion

Construct design

Virtual digestion

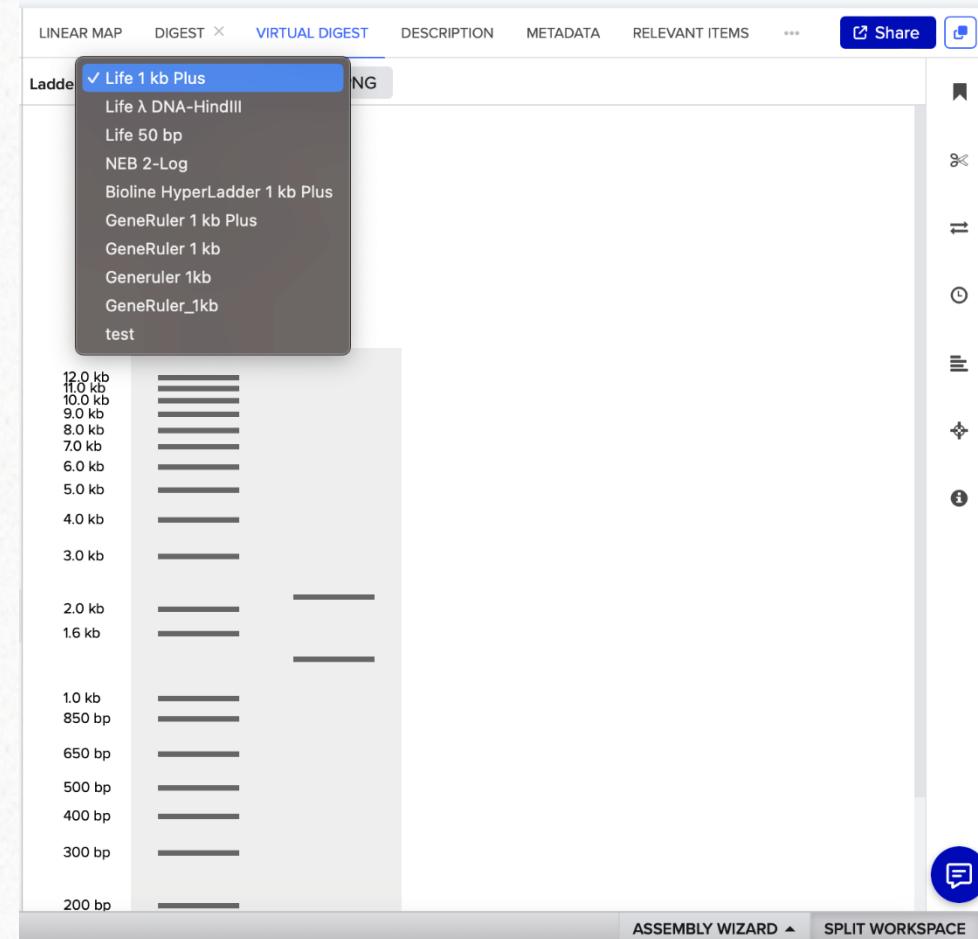
Gel visualization



- ✓ After running both digestions, you can easily visualize the resulting fragments in a simulated electrophoresis gel.
 - 1st lane: **Ladder**
 - 2nd lane: **Backbone**
 - 3rd lane: **Insert**
- ✓ If you click on the bands, you can easily select the DNA sequences that correspond to the digested fragments

Construct design

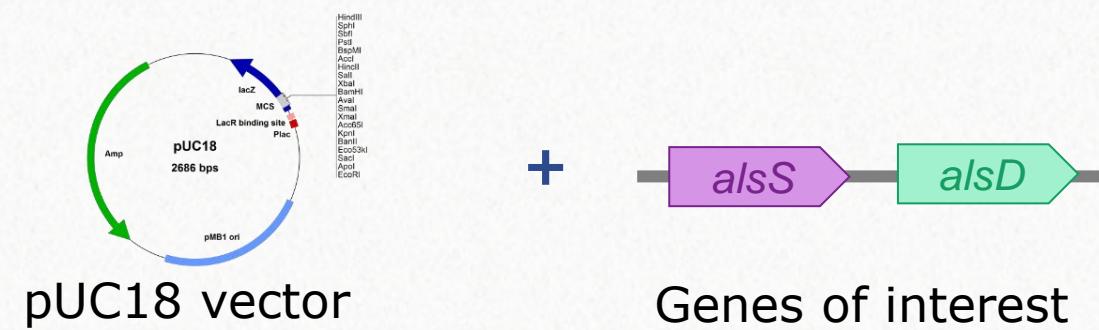
Virtual digestion



 **PRO TIP:** It's possible to choose between different ladders

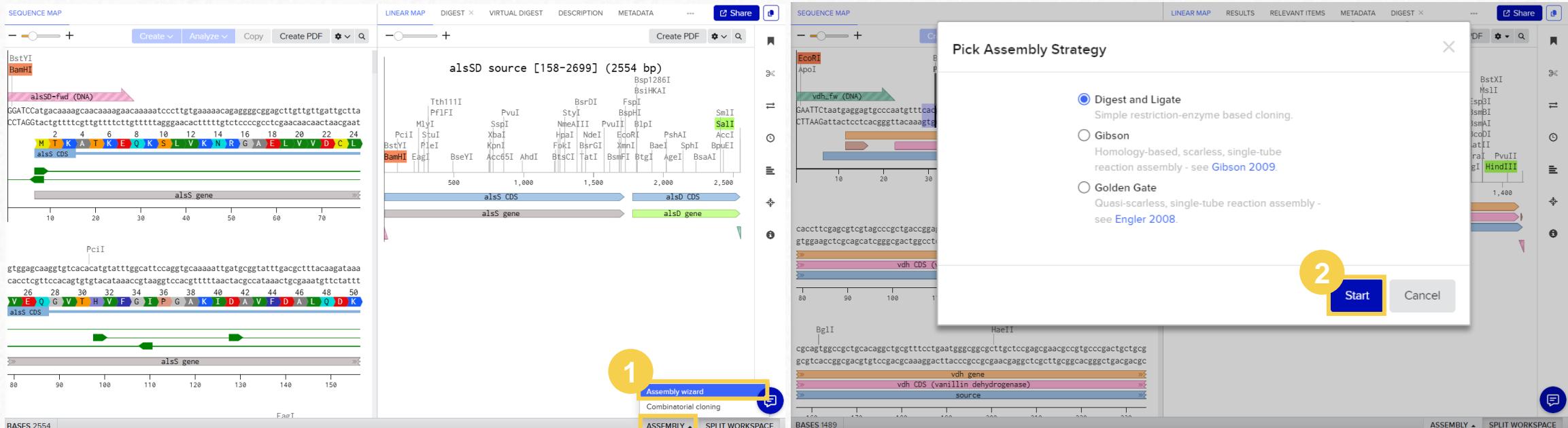
5. Basic construct assembly

5.4 Assembly Wizard



Construct Assembly

Assembly Wizard



The screenshot shows the Assembly Wizard interface with two sequence maps and a central dialog for picking an assembly strategy.

Left Sequence Map: Shows restriction enzyme sites (BstYI, BamHI) and a gene (alsS) with its CDS. A pink arrow labeled "alsSD-fwd (DNA)" points to a sequence of DNA bases. A green arrow labeled "alsS gene" points to the gene's location on the map.

Middle Sequence Map: Shows restriction enzyme sites (Tth11I, PflMI, PvuI, StyI, BsrDI, FspI, SmaII, EcoRI, BpuI, SalI, etc.) and genes (alsSD, alsS, alsD). A pink arrow labeled "alsSD source [158-2699] (2554 bp)" points to a sequence of DNA bases. A green arrow labeled "alsD CDS" points to the gene's location on the map.

Right Sequence Map: Shows restriction enzyme sites (EcoRI, ApoI, PvuII, BpuI, SalI, etc.) and genes (vdh, vdh CDS, vdh gene, vdh CDS (vanillin dehydrogenase), vdh source). A pink arrow labeled "vdh fw (DNA)" points to a sequence of DNA bases. A green arrow labeled "vdh CDS" points to the gene's location on the map.

Pick Assembly Strategy Dialog:

- Digest and Ligate
Simple restriction-enzyme based cloning.
- Gibson
Homology-based, scarless, single-tube reaction assembly - see [Gibson 2009](#).
- Golden Gate
Quasi-scarless, single-tube reaction assembly - see [Engler 2008](#).

Buttons: Start (highlighted with a yellow circle and number 2) and Cancel.

Bottom Buttons: ASSEMBLY ▲, SPLIT WORKSPACE.

The Assembly Wizard allows you to use the following assembly strategies:

- ✓ Digest and Ligate (restriction enzyme-based cloning)
- ✓ Gibson assembly (no need for restriction enzymes)
- ✓ Golden Gate

Construct Assembly

Digest and Ligate: Locate the Assembly Wizard work environment

The screenshot shows the QFB Assembly Wizard interface. On the left, there's a circular plasmid map of pUC18 with various restriction sites and features labeled: AmpR_promoter, AmpR, M13_fwd, MCS, lacZ, +3 lac_promoter, ori, and several insertion points (2500, 2250, 2000, 1750, 1500, 1250, 1000). Above the map are tabs for PLASMID, SEQUENCE MAP, LINEAR MAP, and DESCRIPTION. A 'Create PDF' button is also present. To the right of the map is a 'DIGEST' table:

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
BamHI	1	37°C	100	50	10	100
Sall	1	37°C	10	100	100	100

Below the digest table is a 'SET FRAGMENT' section with a yellow border, containing a Backbone-Insert diagram and assembly statistics: BASES 2686, INSERT 693. To the right is an 'OVERALL ASSEMBLY' section with a yellow border, showing a status message: 'The backbone or an insert is unset.' It includes a text input field 'pUC18-alsSD' with a delete icon, an 'Assemble' button, and a '+' icon. A blue arrow points from the text 'Name your construct' to the 'pUC18-alsSD' field.

✓ This will remain open even if you go from one file to another

Name your construct

Construct Assembly

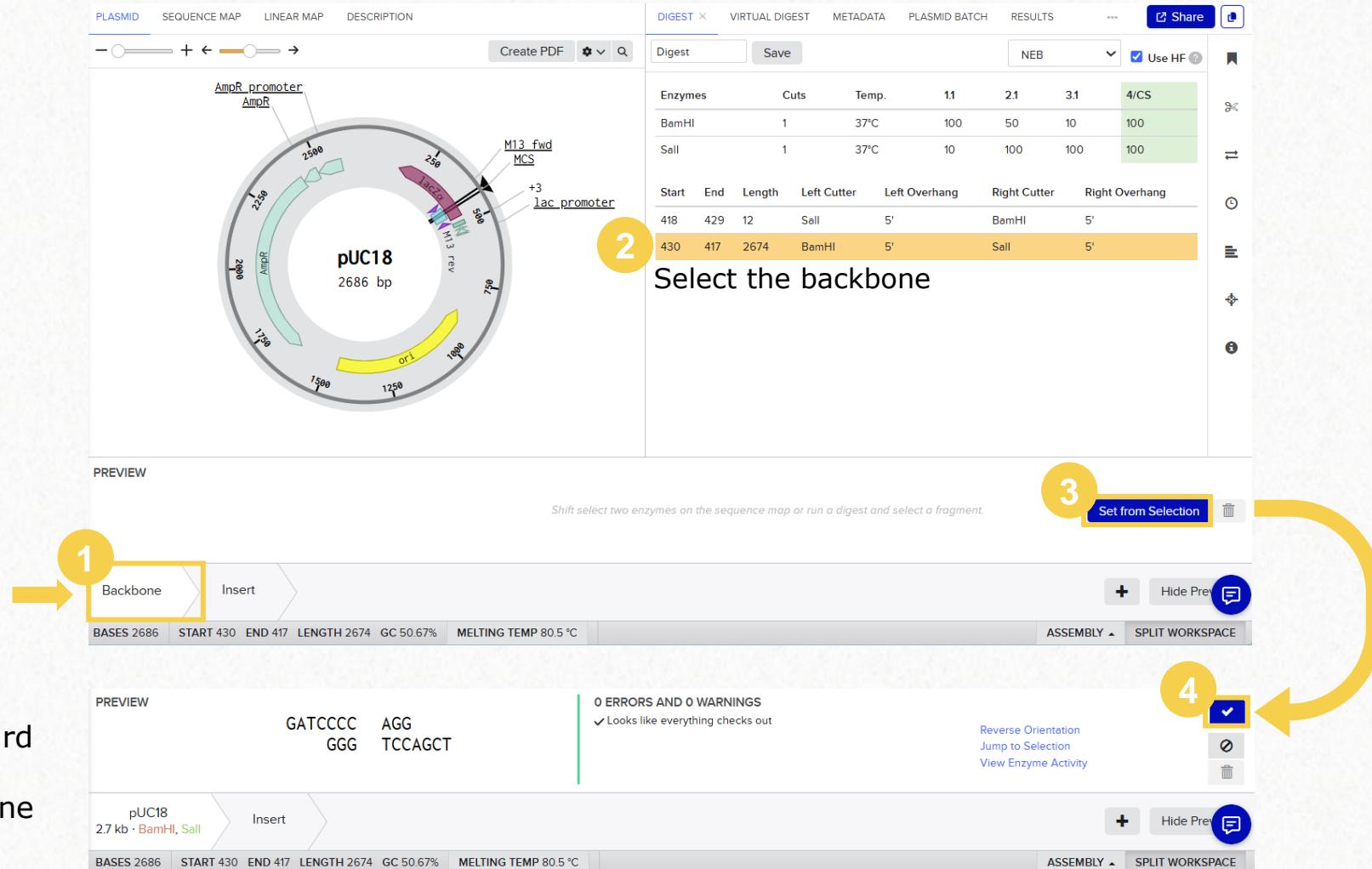
Digest and Ligate: Add the backbone

1 Backbone

2 Select the backbone

3 Set from Selection

4 ✓



The screenshot shows the Assembly Wizard interface with the following details:

- PLASMID:** pUC18 (2686 bp) sequence map.
- SEQUENCE MAP:** Shows restriction sites for AmpR promoter, AmpR, M13 fwd MCS, M13 rev, +3 lac promoter, and ori.
- LINEAR MAP:** Shows the linearized plasmid with the same features.
- DESCRIPTION:** Create PDF, Share, Digest, Save, NEB, Use HF.
- DIGEST:** Enzymes: BamHI, Sall. Cuts: 1. Temp: 37°C. NEB: 1.1, 2.1, 3.1, 4/CS. Start: 418, End: 429, Length: 12, Left Cutter: Sall, Left Overhang: 5', Right Cutter: BamHI, Right Overhang: 5'.
- PREVIEW:** Shift select two enzymes on the sequence map or run a digest and select a fragment. ASSEMBLY, SPLIT WORKSPACE.
- PREVIEW (Bottom):** GATCCCC (GGG) AGG TCCAGCT. 0 ERRORS AND 0 WARNINGS. Looks like everything checks out. Reverse Orientation, Jump to Selection, View Enzyme Activity.
- Bottom Navigation:** BASES 2686, START 430, END 417, LENGTH 2674, GC 50.67%, MELTING TEMP 80.5 °C. ASSEMBLY, SPLIT WORKSPACE.

- ✓ The Assembly Wizard shows the digested ends of the backbone

Construct Assembly

Digest and Ligate: Add the insert

The screenshot illustrates the 'Digest and Ligate' step in the Assembly Wizard. The interface is divided into several sections:

- SEQUENCE MAP**: Shows the **alsSD source [158-2699] (2554 bp)**. It displays restriction sites (e.g., Tth111I, PflFI, SspI, PvuI, StyI, BsrDI, FspI, BspHAI, BsiHKAI, SmlI, SalI) and genes (**alsS CDS**, **alsD CDS**, **alsS gene**, **alsD gene**). A yellow circle labeled **2** highlights the **SalI** site.
- DIGEST**: Shows the digest setup. Enzymes selected are **BamHI** and **SalI**. Conditions: Temp. 37°C, NEB 100. A checkbox for **Use HF** is checked. A yellow circle labeled **2** is positioned over the **SalI** entry in the digest table.
- PREVIEW**: Shows the resulting construct. A pUC18 vector (2.7 kb) is digested with **BamHI** and **SalI**, and an **Insert** sequence (AGG TCCAGCT GATCCCC GGG) is inserted. A yellow circle labeled **1** highlights the **Insert** sequence. A yellow arrow points from the **SalI** site on the sequence map to the **Insert** sequence in the preview.
- Select the insert**: A text instruction located between the digest table and the preview area.
- Set from Selection**: A button located in the preview area, highlighted by a yellow circle labeled **3**.

Construct Assembly

Digest and Ligate: Check for compatibility

- ✓ The assembly wizard will check for compatibility between sticky ends.
- ✓ Depending on the orientation of your backbone and insert, you might need to make adjustments – such as in this case!

The screenshot shows the QFB Assembly Wizard interface with two preview panels and a central status bar.

Top Preview Panel: Shows a backbone (pUC18) with restriction sites BamHI and Sall, and an insert (alsSD source [158-2699]) with restriction sites BamHI and Sall. The sticky ends are shown as AGGGATCC and TCCAGCT. The status bar indicates "2 ERRORS AND 0 WARNINGS" with two error icons: "The left sticky end does not match" and "The right sticky end does not match". A yellow circle labeled "1" points to the "Reverse Orientation" button in the toolbar, which is highlighted with a yellow box.

Bottom Preview Panel: Shows the same backbone and insert, but the insert has been reversed. The sticky ends are now AGGTTCGACT and ATGGATCCC. The status bar indicates "0 ERRORS AND 0 WARNINGS" with a green checkmark icon: "Looks like everything checks out". A yellow arrow points from the top panel to this panel, indicating the result of the orientation change. A yellow circle labeled "2" points to the "Reverse Orientation" button in the toolbar of the bottom panel, which is also highlighted with a yellow box.

- ✓ In this scenario, it is necessary to click on "Reverse Orientation" so the ends match.

Construct Assembly

Digest and Ligate: Assemble

The screenshot shows the Assembly Wizard interface. At the top left is the 'SET FRAGMENT' section, which displays two fragments: 'pUC18' (2.7 kb, BamHI, SalI) and 'alsSD source [158-2699]' (2.5 kb, SalI, BamHI). At the top right is the 'OVERALL ASSEMBLY' section, which says 'Looks like everything checks out' and contains a button labeled 'Assemble' with a yellow circle containing the number '1' above it. Below these are 'Create PDF', 'Share', and 'Hide Preview' buttons. A large yellow arrow points from the overall assembly section down to the 'SEQUENCE MAP' and 'LINEAR MAP' sections.

SEQUENCE MAP: This panel shows two sequence alignments. The top alignment covers positions 320 to 390, and the bottom alignment covers positions 400 to 540. It includes restriction sites for AccI, SalI, SbfI, and MCS (multiple cloning site). Gene regions for 'lacZα' and 'alsD gene' are indicated with arrows and color-coded boxes.

LINEAR MAP: This panel displays a circular map of the construct pUC18-alsSD. The map shows the 5222 bp circular DNA molecule with various genes and features: 'AmpR_promoter' (green), 'AmpR' (light green), 'lacZα' (purple), 'MCS' (blue), 'M13_fwd' (grey), 'M13_rev' (yellow), 'lac_promoter' (red), 'lac_promoter' (red), 'lac_promoter' (red), 'MCS_lacZα_+1' (green), and 'alsD gene' (green).

At the bottom of the interface, there are buttons for 'BASES 5222', 'INSERT 710', 'ASSEMBLY ▾', and 'SPLIT WORKSPACE'.

- ✓ You will be asked to choose a folder to save the construct in

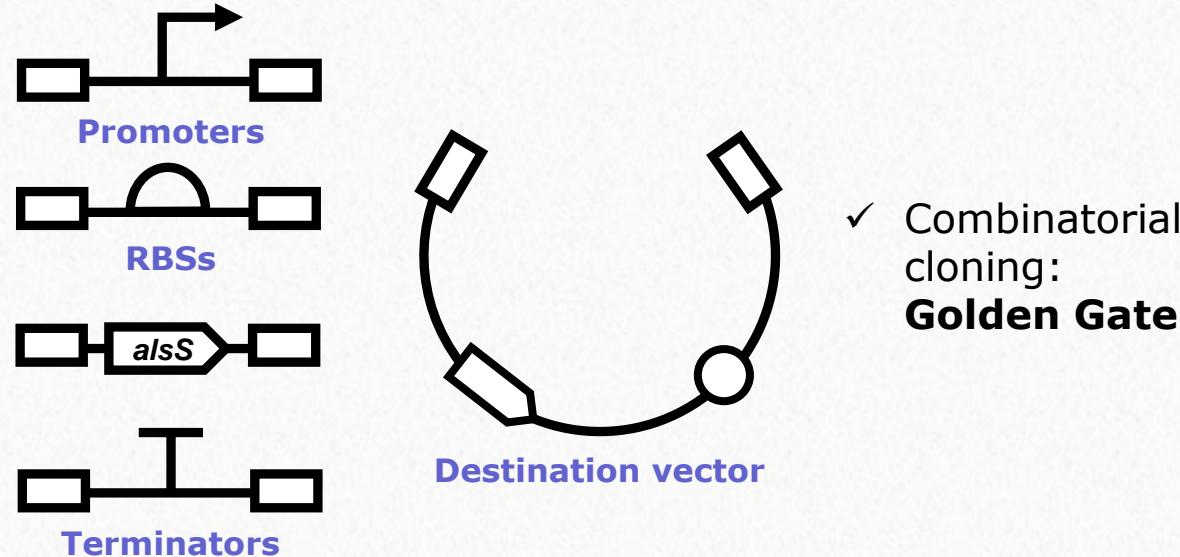
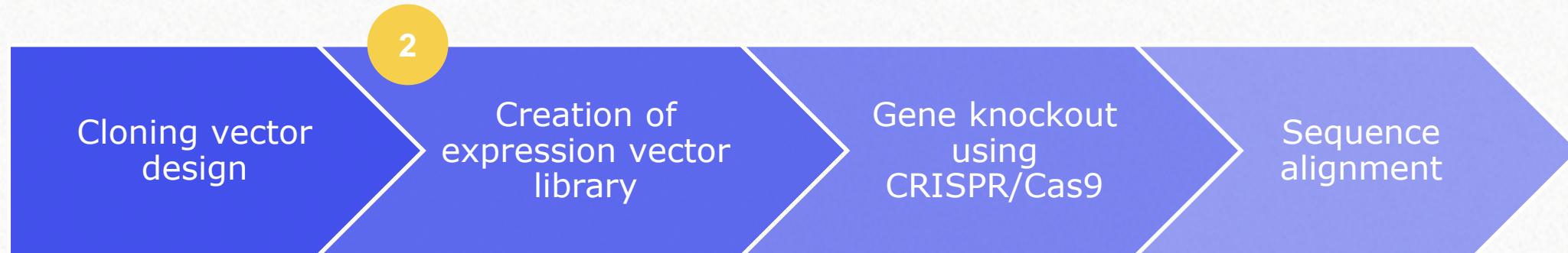
- ✓ The assembly is now done!

6. Combinatorial cloning: Golden Gate





This is the second part of the *hands-on* example.



✓ Combinatorial cloning:
Golden Gate

Expected output:

- alsS expression vector library (combinatorial cloning assembly file)
- 27 resulting vector combinations

You will need the files in the **Combinatorial cloning** subfolder.

... / Your Name / Training Files /

2. Combinatorial cloning

alsS

SEQUENCE MAP

DESCRIPTION METADATA RESULTS **LINEAR MAP** RELEVANT ITEMS

alsS (1713 bp)

BASES 1713 ASSEMBLY ▾ SPLIT WORKSPACE

The screenshot shows a bioinformatics interface for a gene named 'alsS'. On the left, a sidebar lists various files under '2. Combinatorial cloning', including 'alsS', 'pET-Ori-KanR', 'promoter-01-T5', 'promoter-02-tac', 'promoter-03-T7', 'RBS-01-B0030', 'RBS-02-B0032', 'RBS-03-B0034', 'terminator-01-rnnBT1', and 'terminator-02-T0'. The main panel displays two sequence maps for the 'alsS' gene. The top map shows the full 1713 bp sequence with restriction sites for BssSaiI, BssSI, MboII, SacII, and EcoI. The bottom map shows a shorter segment from position 80 to 150 with restriction sites for NspI and PstI. Both maps include a color-coded amino acid sequence below the DNA sequence, showing the 'alsS CDS' (Coding DNA Sequence) with positions 1 through 26 indicated above it. The right side of the interface shows a detailed list of all restriction enzymes available for the 'alsS' gene, ranging from 200 bp to 1,600 bp.

Construct Assembly

Worklist creation

PRO TIP:

Creating a **worklist** can make it easier to find your most used files!

... / Your Name / Training Files /
2. Combinatorial cloning Saved Searches

Search Type Filters

1-12 of 12 items

Name	Inventory	ID	Modified	Authors	Description
alsS			11/02/2025	Mía López Portillo ...	
alsS expression vector library			17/02/2025		
pET-Ori-KanR	No inventory availa...		11/02/2025	Mía López Portillo ...	
promoter-01-T5			11/02/2025	Mía López Portillo ...	
promoter-02-tac			11/02/2025	Mía López Portillo ...	
promoter-03-T7			11/02/2025	Mía López Portillo ...	
RBS-01-B0030	No inventory availa...		11/02/2025	Mía López Portillo ...	
RBS-02-B0032	No inventory availa...		11/02/2025	Mía López Portillo ...	

Add items to worklist

Add items to entity worklist

New worklist Existing worklist

Worklist Name*

alsS EVL

Selected items

alsS pET-Ori-KanR promoter-01-T5 promoter-02-tac
promoter-03-T7 RBS-01-B0030 RBS-02-B0032 RBS-03-B0034
terminator-01-rrnBT1 terminator-02-T0 terminator-03-T7

Add items to worklist

Worklist 'alsS EVL' was created with 11 items

- 1 Select the sequence files in the **Combinatorial cloning** folder
- 2 Select "Add items to worklist"

Construct Assembly

Combinatorial Cloning Tool

- ✓ An alternative to the Assembly Wizard is the Combinatorial Cloning tool

It allows you to work with several cloning methods:

- ✓ **Golden Gate**
- ✓ **Gibson**
- ✓ **Homology**
- ✓ This tool is especially useful for **designing many constructs at once**

The screenshot shows the Combinatorial Cloning Tool interface. On the left, a detailed view of a 'Golden Gate assembly' record is displayed, showing bins for Backbone, Promoter, and Gene, and a table of fragments with their sequence, bin, start, end, length, orientation, and restriction enzyme type. On the right, a grid of circular construct visualizations is shown, each representing a different combination of backbone, promoter, and gene elements.

Bins & Spacers (3)

BIN 1	BIN 2	BIN 3	Constructs
Backbone	Promoter	Gene	24 constructs

Fragments

Sequence	Bin	Start	End	Length	Orientation	Type IIS enzyme	Frag
1 backbone	Backbone	2248	3314	1067	Forward	Bsal	Use
2 promoter001	Promoter	8	328	321	Forward	Bsal	Use
3 promoter002	Promoter	8	366	359	Forward	Bsal	Use
4 promoter003	Promoter	8	315	308	Forward	Bsal	Use
5 gene001	Gene	8	4007	4000	Forward	Bsal	Use
6 gene002	Gene	8	4191	4184	Forward	Bsal	Use
7 gene003	Gene	8	4188	4181	Forward	Bsal	Use
8 gene004	Gene	8	4004	3997	Forward	Bsal	Use
9 gene005	Gene	8	4188	4181	Forward	Bsal	Use
10 gene006	Gene	8	4004	3997	Forward	Bsal	Use
11 gene007	Gene	8	4001	3994	Forward	Bsal	Use
12 gene008	Gene	8	4185	4178	Forward	Bsal	Use

Constructs

Name	Backbone	Overhang	Promoter	Overhang	Gene
1 backbone-promoter001-gene001	backbone	AACA	promoter001	CGAT	gene001
2 backbone-promoter001-gene002	backbone	AACA	promoter001	CGAT	gene002
3 backbone-promoter001-gene003	backbone	AACA	promoter001	CGAT	gene003
4 backbone-promoter001-gene004	backbone	AACA	promoter001	CGAT	gene004

Construct Assembly

Combinatorial Cloning Tool: How to access it

The screenshot shows the Combinatorial Cloning Tool interface. On the left, a sidebar lists various projects and files, with 'alsS' selected. A central panel displays a sequence map for the 'alsS' gene, showing restriction sites like BssS α I, MboII, SacII, and EciI. The sequence is shown as a DNA string with amino acid translations below it. A dropdown menu is open at the bottom of this panel, with three numbered steps indicating the cloning process:

1. **Assembly**: Shows options to "Assemble DNA sequences by cloning" or "Assemble sequences and oligos by concatenation".
2. **CRISPR**: Shows an option to "Entity from schema".
3. **Mixture**: Shows an option to "Bases 1713".

To the right, a detailed assembly map for the 'alsS' gene is shown, spanning from 200 to 1,600 bp. It highlights the 'alsS CDS' and 'alsS gene' regions, along with various restriction sites and their corresponding enzymes.

Construct Assembly

Combinatorial Cloning Tool: Configuration

The screenshot shows the Combinatorial Cloning Tool interface. On the left, there's a sequence map for the *alsS* gene, which is 1713 bp long. The gene has a CDS from position 24 to 28. On the right, a detailed linear map of the *alsS* gene is shown with various restriction enzyme sites labeled along its length.

The central part of the screen displays the "Assemble DNA" configuration dialog:

- Step 1:** Name the project as "alsS expression vector library".
- Step 2:** Set the "Number of fragment bins" to 5.
- Step 3:** Select the "Cloning method" as "Golden Gate".
- Step 4:** Choose "Bsal" as the "Type IIS Restriction Enzyme".
- Step 5:** Click the "Save" button to confirm the settings.

i You can modify these parameters later (before finalizing the assembly)

i The only thing you will not be able to modify later is the **cloning method**

Construct Assembly

Combinatorial Cloning Tool: Full view

The screenshot shows the Combinatorial Cloning Tool interface for assembling an *alsS* expression vector library. The top navigation bar includes a search bar, tabs for METADATA, OVERVIEW (selected), and CONSTRUCTS, and a title *alsS expression vector library*. Below the tabs, there are five bins labeled BIN 1 through BIN 5, each containing a backbone and one or more inserts. The right side shows a summary of constructs and an 'Assemble' button. Below the bins is a 'Fragments' table with columns for Sequence, Bin, Start, End, Length, Orientation, Type IIS enzyme, Fragment production method, and Status. A single row is shown with values: Sequence 1, Bin 1, Start, End, Length, Forward, BsAl, Use existing cut sites, and Status. At the bottom is a 'Constructs' table with columns for Name, Backbone, Overhang, Insert 1, Overhang, Insert 2, Overhang, Insert 3, Overhang, and Insert 4. A single row is shown with dropdown menus for each column.

i You can add multiple fragments to each bin to create several combinations

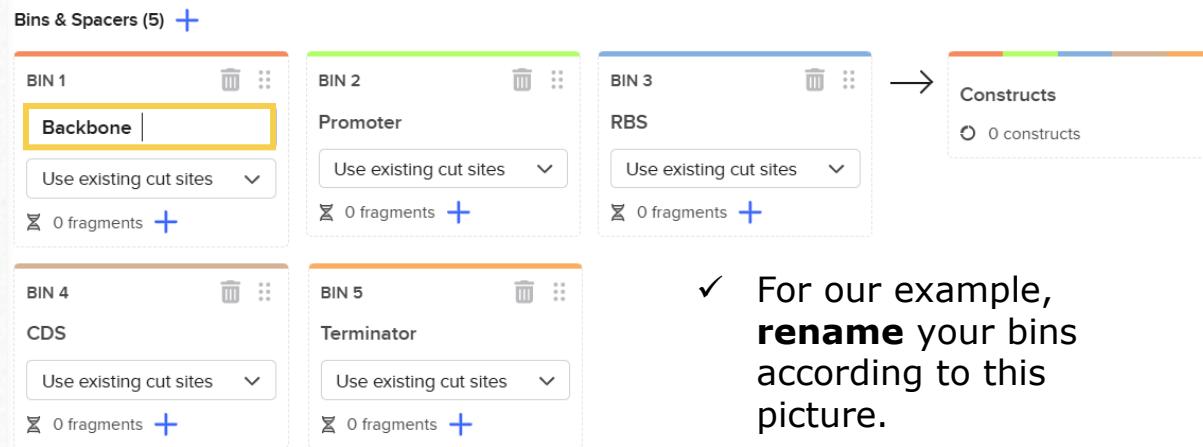
i All added fragments will show up here
(You can change some configurations)

i When you're done adding your fragments, you can autopopulate this table with all possible combinations!

Construct Assembly

Combinatorial Cloning Tool: Bins and spacers

i You can rename the bins for better organization.



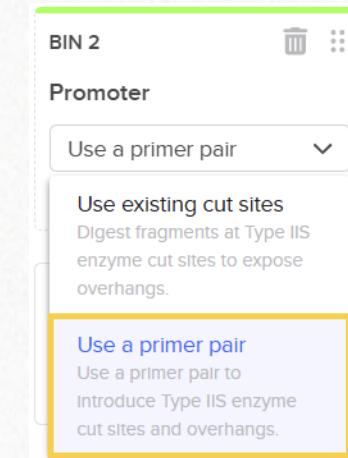
Bins & Spacers (5) +

- BIN 1** Backbone | Use existing cut sites 0 fragments +
- BIN 2** Promoter | Use existing cut sites 0 fragments +
- BIN 3** RBS | Use existing cut sites 0 fragments +
- BIN 4** CDS | Use existing cut sites 0 fragments +
- BIN 5** Terminator | Use existing cut sites 0 fragments +

→ Constructs
0 constructs

✓ For our example, **rename** your bins according to this picture.

i You can choose whether to use **existing cut sites** or a **primer pair** in each bin



BIN 2

Promoter

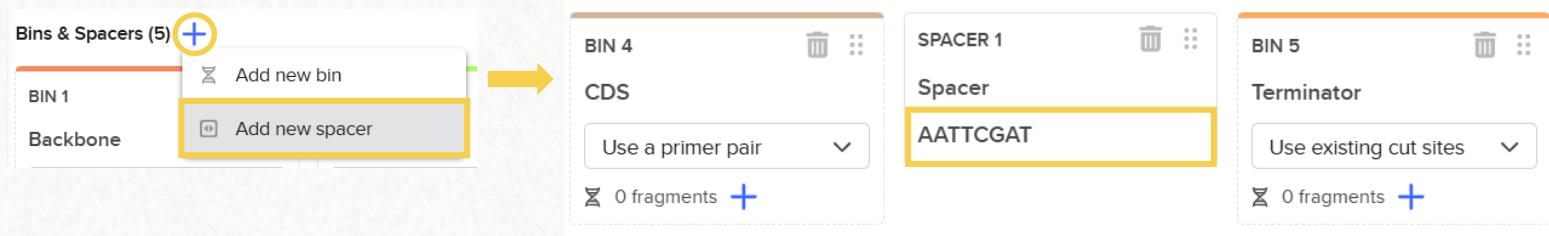
Use a primer pair

Use existing cut sites
Digest fragments at Type IIS enzyme cut sites to expose overhangs.

Use a primer pair
Use a primer pair to introduce Type IIS enzyme cut sites and overhangs.

- ✓ For our example, set all bins except for the **Backbone** to use a **primer pair**.

i It is possible to add **spacers** (max. 20 nt) between bins, which will be incorporated in the primer design. At least one of the bins next to the spacer must be set to use a primer pair.



Bins & Spacers (5) +

Add new bin

BIN 1 Backbone

Add new spacer

SPACER 1 Spacer | AATTCGAT

BIN 4 CDS | Use a primer pair 0 fragments +

BIN 5 Terminator | Use existing cut sites 0 fragments +

- ✓ Spacers will not be used in our example.

Construct Assembly

Golden Gate: Set fragments in corresponding bins

1. Backbone

- 1 Find and select the **backbone** file (pET-Ori-KanR)

The screenshot shows the 'Bins & Spacers' section with 6 items. A yellow circle highlights the '+' button at the top right. Below it, under 'BIN 1', the 'Backbone' file is selected. A yellow box highlights the 'Add from worklist' button.

Bins & Spacers (6) +

BIN 1

Backbone

Add from worklist

0 fragments +

Open sequences >

Search for sequences

Use existing cut sites

0 fragments

Add from worklist

alsS EVL

Item

pET-Ori-KanR

promoter-01-T5

Cancel Next

- 2 Verify the selection is correct and click "Add"

The screenshot shows the 'Add fragment(s)' dialog. It displays a circular backbone sequence with two BsaI restriction sites. The linear map shows the sequence from 1,000 to 5,000 bp, with regions labeled 'medium copy (pET) ori/KanR' and 'pET-Ori-KanR'. The 'ori' and 'bla' genes are also indicated. The 'Add' button is highlighted with a yellow box.

Add fragment(s)

pET-Ori-KanR

Start: 28 End: 3327 Orientation: Forward

BsaI

BsaI

3.3 kb of 5.4 kb

(5420 bp)

View: Linear map

medium copy (pET) ori/KanR

pET-Ori-KanR

ori

bla

Back Add

- ✓ Since this bin was configured to use **existing cut sites**, Benchling has detected the **BsaI** sites in the sequence and automatically selected the region between them.
- ✓ If you choose the option to **create a primer pair** for a sequence, you will be able to freely select the region you'd like to use.

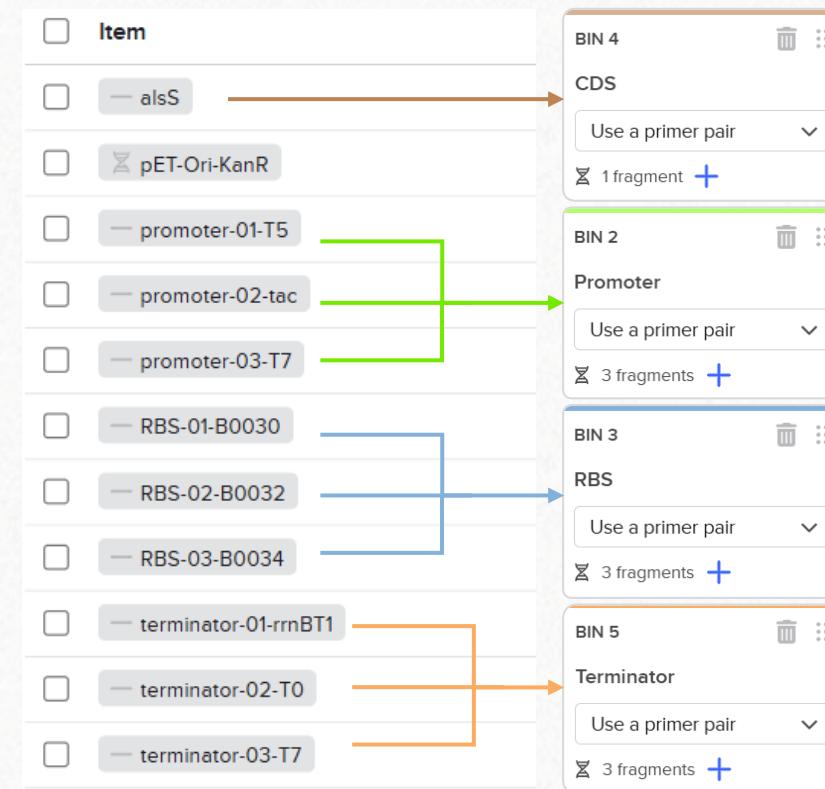
Construct Assembly

Golden Gate: Set fragments in corresponding bins

2. Inserts

3

Repeat the process for each bin following each category.
Keep the entire sequences.



- ✓ Primers with appropriate overhangs will be designed for the assembly of these fragments following the position of the bins.

Construct Assembly

Golden Gate: Verify the fragments

- ✓ You should obtain a table like this one.
- ✓ By clicking on a specific row, you will be able to edit the fragments if you need to do so. You can also change the bin a sequence corresponds to, and even remove sequences.

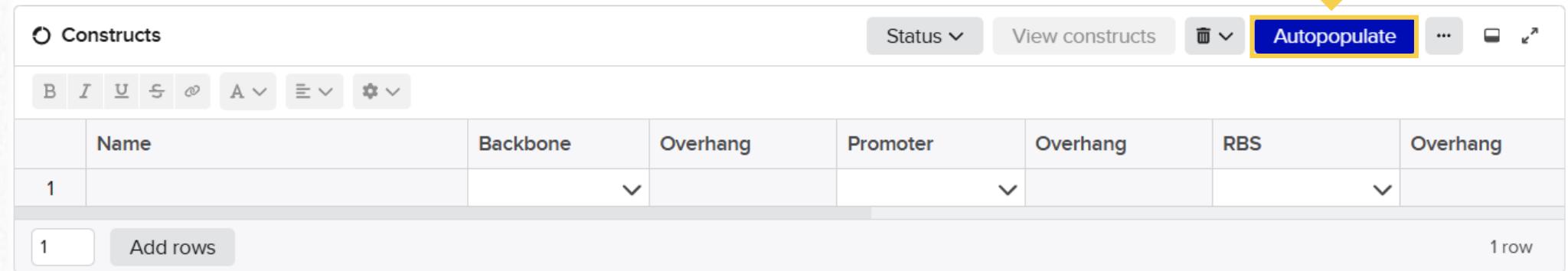
Fragments												Status	Edit fragments	⋮	Close
	Sequence	Bin	Start	End	Length	Orientation	Type IIS enzyme	Fragment production method	Preferred 5' primer	Preferred 3' primer	Status				
1	pET-Ori-KanR	Backbone	28	3327	3300	Forward	Bsal	Use existing cut sites			Looks good				
2	promoter-01-T5	Promoter	1	45	45	Forward	Bsal	Use a primer pair			Looks good				
3	promoter-02-tac	Promoter	1	46	46	Forward	Bsal	Use a primer pair			Looks good				
4	promoter-03-T7	Promoter	1	36	36	Forward	Bsal	Use a primer pair			Looks good				
5	RBS-01-B0030	RBS	1	52	52	Forward	Bsal	Use a primer pair			Looks good				
6	RBS-02-B0032	RBS	1	50	50	Forward	Bsal	Use a primer pair			Looks good				
7	RBS-03-B0034	RBS	1	49	49	Forward	Bsal	Use a primer pair			Looks good				
8	alsS	CDS	1	1713	1713	Forward	Bsal	Use a primer pair			Looks good				
9	terminator-01-rrnBT1	Terminator	1	110	110	Forward	Bsal	Use a primer pair			Looks good				
10	terminator-02-T0	Terminator	1	126	126	Forward	Bsal	Use a primer pair			Looks good				
11	terminator-03-T7	Terminator	1	71	71	Forward	Bsal	Use a primer pair			Looks good				

Construct Assembly

Golden Gate: Populate the “constructs” table

4

Click the “Autopopulate” button to fill the **Constructs** table with all possible combinations of your fragments.



	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang
1		v		v		v	

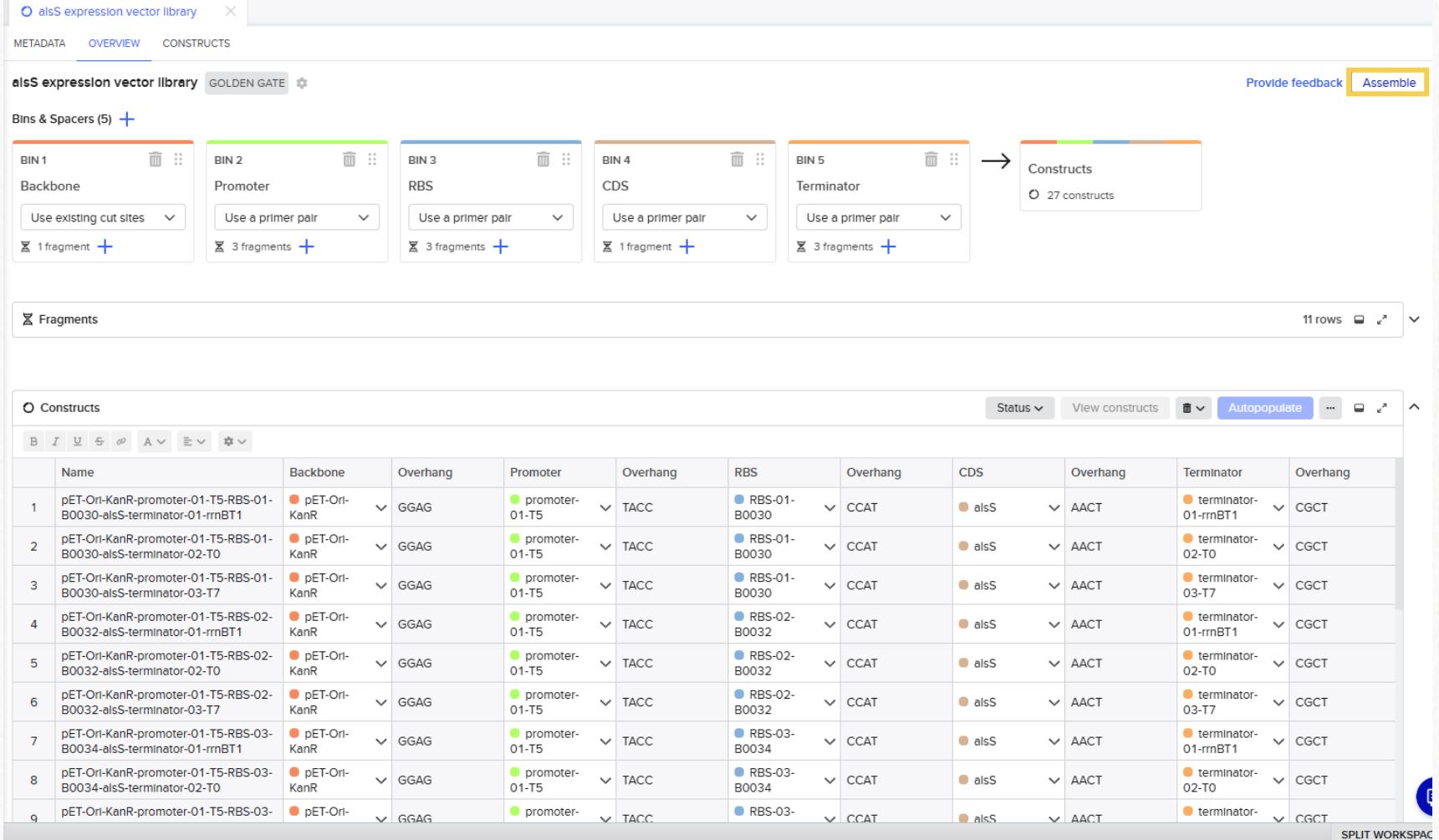
1 Add rows 1 row

- ✓ You can also create combinations **manually**, with the option of **skipping** bins if you wish to do so
- ✓ It's also possible to **remove** rows that you are not interested in.

Construct Assembly

Golden Gate: Finalize the assembly

- 5 Click the “**Assemble**” button to create **primer** (optional), **fragment** (optional) and **plasmid** files for all of your constructs.



The screenshot shows the Combinatorial Cloning software interface. At the top, there's a navigation bar with tabs for METADATA, OVERVIEW, and CONSTRUCTS. The CONSTRUCTS tab is selected, showing a workspace titled "alsS expression vector library GOLDEN GATE". The workspace contains several bins and spacers: BIN 1 (Backbone), BIN 2 (Promoter), BIN 3 (RBS), BIN 4 (CDS), and BIN 5 (Terminator). Each bin has dropdown menus for primer selection and a count of fragments (e.g., 3 fragments for Promoter). An arrow points from the bins to a "Constructs" panel on the right, which displays "27 constructs". Below the workspace is a "Fragments" section with a table of 11 rows. At the bottom of the workspace is a detailed construct table with columns for Name, Backbone, Overhang, Promoter, Overhang, RBS, Overhang, CDS, Overhang, Terminator, and Overhang. The table lists 9 rows of constructs, each with unique identifiers and specific sequence details. A yellow arrow points to the "Assemble" button in the top right corner of the workspace.

	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang	CDS	Overhang	Terminator	Overhang
1	pET-Ori-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-01-rnBT1	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-01-B0030	▼ CCAT	● alsS	▼ AACT	● terminator-01-rnBT1	▼ CGCT
2	pET-Ori-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-02-T0	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-01-B0030	▼ CCAT	● alsS	▼ AACT	● terminator-02-T0	▼ CGCT
3	pET-Ori-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-03-T7	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-01-B0030	▼ CCAT	● alsS	▼ AACT	● terminator-03-T7	▼ CGCT
4	pET-Ori-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-01-rnBT1	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-02-B0032	▼ CCAT	● alsS	▼ AACT	● terminator-01-rnBT1	▼ CGCT
5	pET-Ori-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-02-T0	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-02-B0032	▼ CCAT	● alsS	▼ AACT	● terminator-02-T0	▼ CGCT
6	pET-Ori-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-03-T7	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-02-B0032	▼ CCAT	● alsS	▼ AACT	● terminator-03-T7	▼ CGCT
7	pET-Ori-KanR-promoter-01-T5-RBS-03-B0034-alsS-terminator-01-rnBT1	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-03-B0034	▼ CCAT	● alsS	▼ AACT	● terminator-01-rnBT1	▼ CGCT
8	pET-Ori-KanR-promoter-01-T5-RBS-03-B0034-alsS-terminator-02-T0	● pET-Ori-KanR	▼ GGAG	● promoter-01-T5	▼ TACC	● RBS-03-B0034	▼ CCAT	● alsS	▼ AACT	● terminator-02-T0	▼ CGCT
9	pET-Ori-KanR-promoter-01-T5-RBS-03-	● pET-Ori-	▼ GGAG	● promoter-	▼ TACC	● RBS-03-	▼ CCAT	● alsS	▼ AACT	● terminator-	▼ CGCT

i After assembling the construct(s), this Combinatorial Cloning file cannot be edited anymore.

Construct Assembly

Golden Gate: Save the constructs and related files

The figure consists of three side-by-side screenshots of a software interface titled "Assemble DNA".

- Screenshot 1:** Shows the initial step where the user is prompted to "Save constructs", "Save fragments", and "Save primers". Below this, there is a note: "Add constructs to a folder and optionally set a schema". It includes fields for "Set location*" (set to "Mia") and "Set schema" (set to "Plasmid"). A checkbox "Add constructs to a worklist" is also present. At the bottom are "Cancel" and "Next" buttons, with "Next" highlighted by a yellow arrow.
- Screenshot 2:** Shows the second step where "Save constructs" is completed (indicated by a green checkmark). The "Save fragments" button is now highlighted in blue. A tooltip message "Saving fragments is optional." is displayed above the "Save fragments" button. A checkbox "Create DNA Sequences to represent amplified fragments" is present. At the bottom are "Back" and "Next" buttons, with "Next" highlighted by a yellow arrow.
- Screenshot 3:** Shows the third step where both "Save constructs" and "Save fragments" are completed (both have green checkmarks). The "Save primers" button is now highlighted in blue. A tooltip message "Saving primers is optional." is displayed above the "Save primers" button. A checkbox "Create DNA Oligos to represent newly designed primers" is present. At the bottom are "Back" and "Assemble" buttons, with "Assemble" highlighted by a yellow arrow.

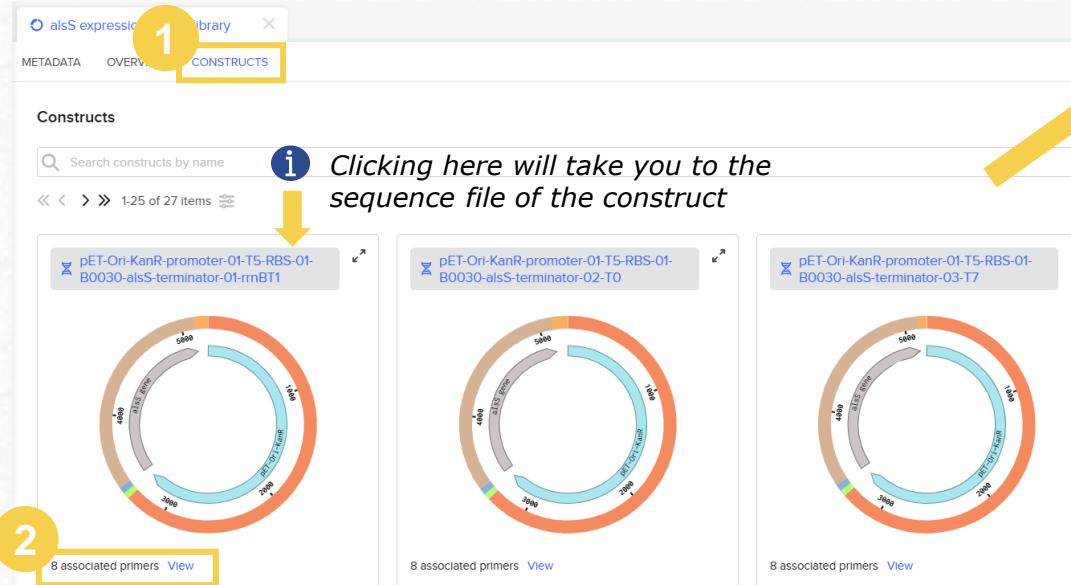
i You can choose whether to create files for every primer and related amplicon.

i If you choose not to create the primer files, you will still be able to find them later.

Construct Assembly

Golden Gate: Results

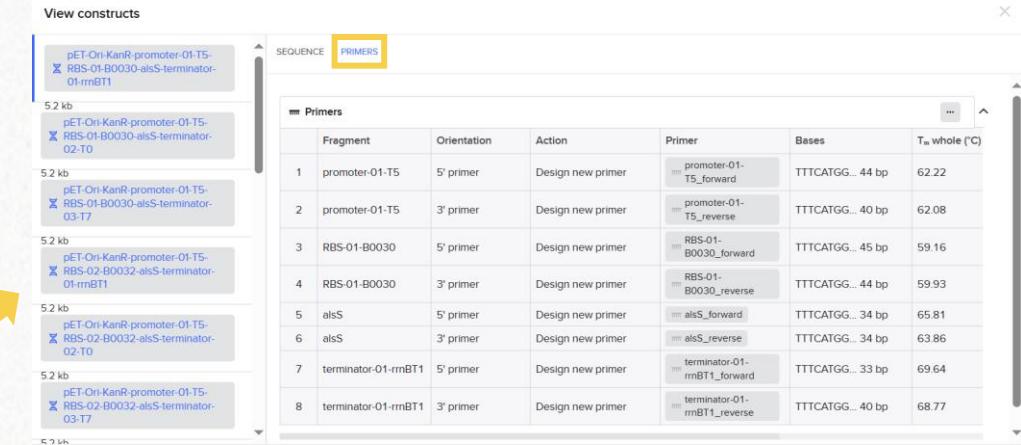
- ✓ After you finalize the assembly, you can move over to the “Constructs” tab to see the resulting constructs.
- ✓ You can view the primer information summarized in a table.



1 Clicking here will take you to the sequence file of the construct

2 8 associated primers View

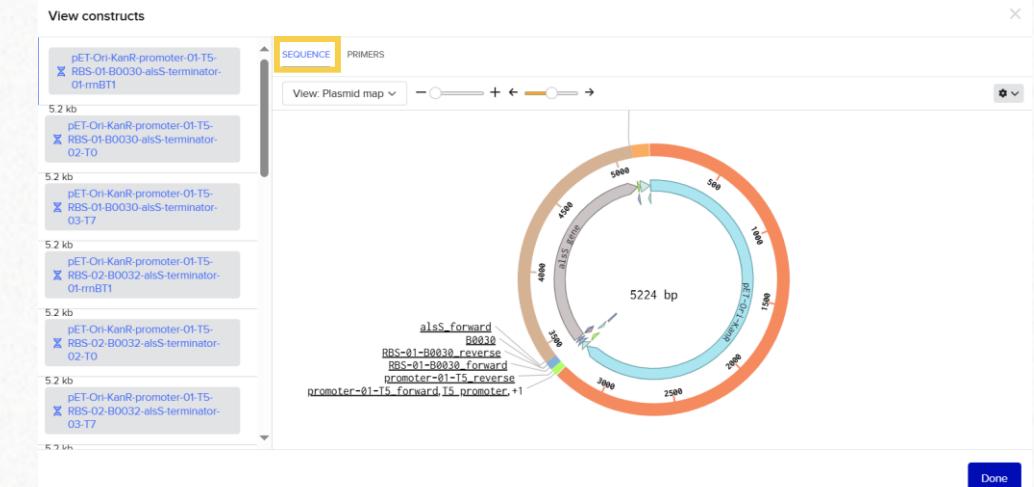
Primer view



Primers					
Fragment	Orientation	Action	Primer	Bases	T _m whole (°C)
1	5' primer	Design new primer	promoter-01-T5_forward	TTTCATGG... 44 bp	62.22
2	3' primer	Design new primer	promoter-01-T5_reverse	TTTCATGG... 40 bp	62.08
3	5' primer	Design new primer	RBS-01-B0030_forward	TTTCATGG... 45 bp	59.16
4	3' primer	Design new primer	RBS-01-B0030_reverse	TTTCATGG... 44 bp	59.93
5	5' primer	Design new primer	alsS_forward	TTTCATGG... 34 bp	65.81
6	3' primer	Design new primer	alsS_reverse	TTTCATGG... 34 bp	63.86
7	5' primer	Design new primer	terminator-01-rmBT1_forward	TTTCATGG... 33 bp	69.64
8	3' primer	Design new primer	terminator-01-rmBT1_reverse	TTTCATGG... 40 bp	68.77

1 You can copy this table or download it as a CSV file.

Sequence view



Construct Assembly

Golden Gate: Results

- ✓ You will also be able to find a file with the resulting construct. By going to the "Assembly History" tab, you will see the fragments that were used to create it, and you can also find a link to the Combinatorial Cloning file.



7. CRISPR tools

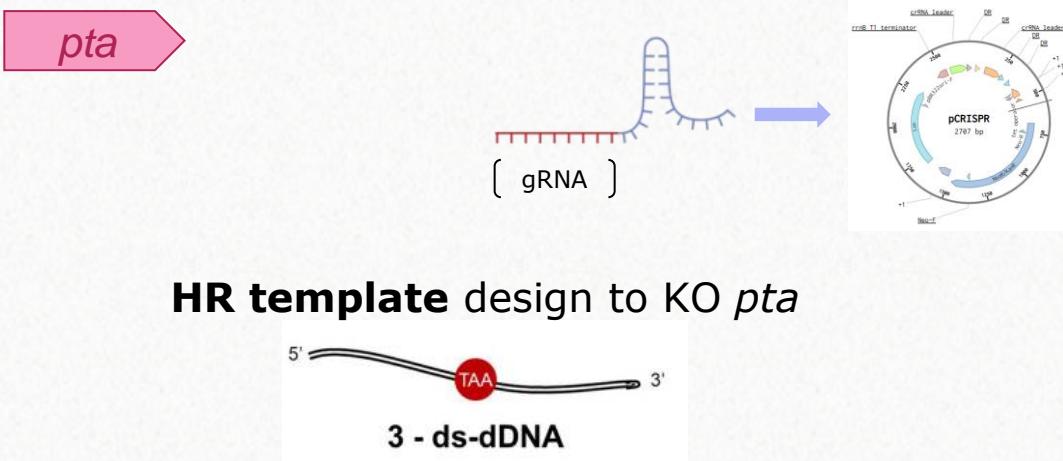




This is the third part of the *hands-on* example.



Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR



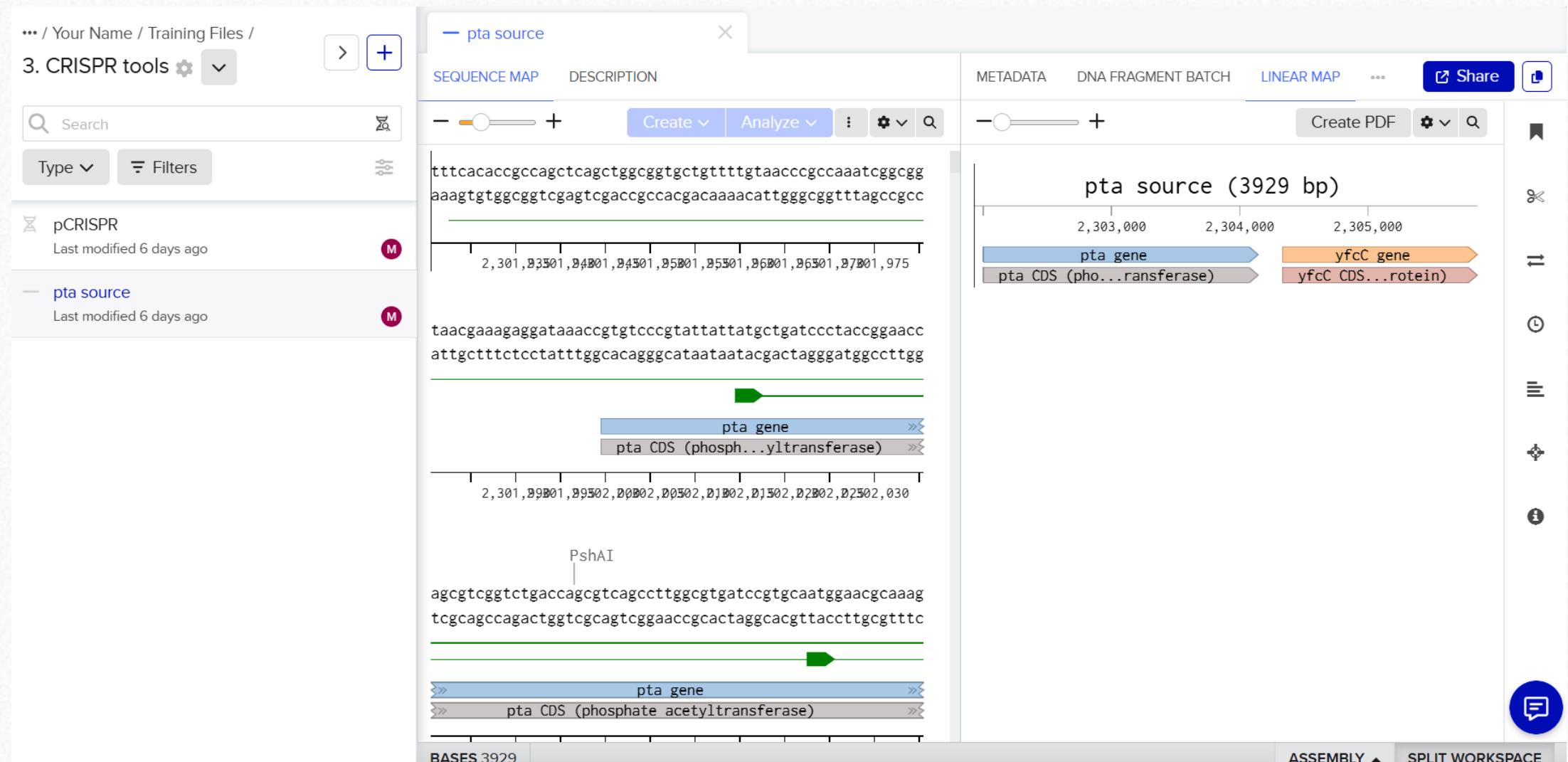
- ✓ gRNA design
- ✓ HR template design

Expected output:

- Selected gRNA for the *pta* gene
- Forward and reverse primers to clone the gRNA into pCRISPR via Bsal
- pCRISPR-*pta*-gRNA construct
- Modified *pta* sequence for KO
- HR template for KO

König, E., Zerbini, F., Zanella, I., Fraccascia, D., & Grandi, G. (2018). Multiple Stepwise Gene Knockout Using CRISPR/Cas9 in *Escherichia coli*. *Bio-protocol*, 8(2), e2688. <https://doi.org/10.21769/BioProtoc.2688>

You will need the files in the **CRISPR tools** subfolder.



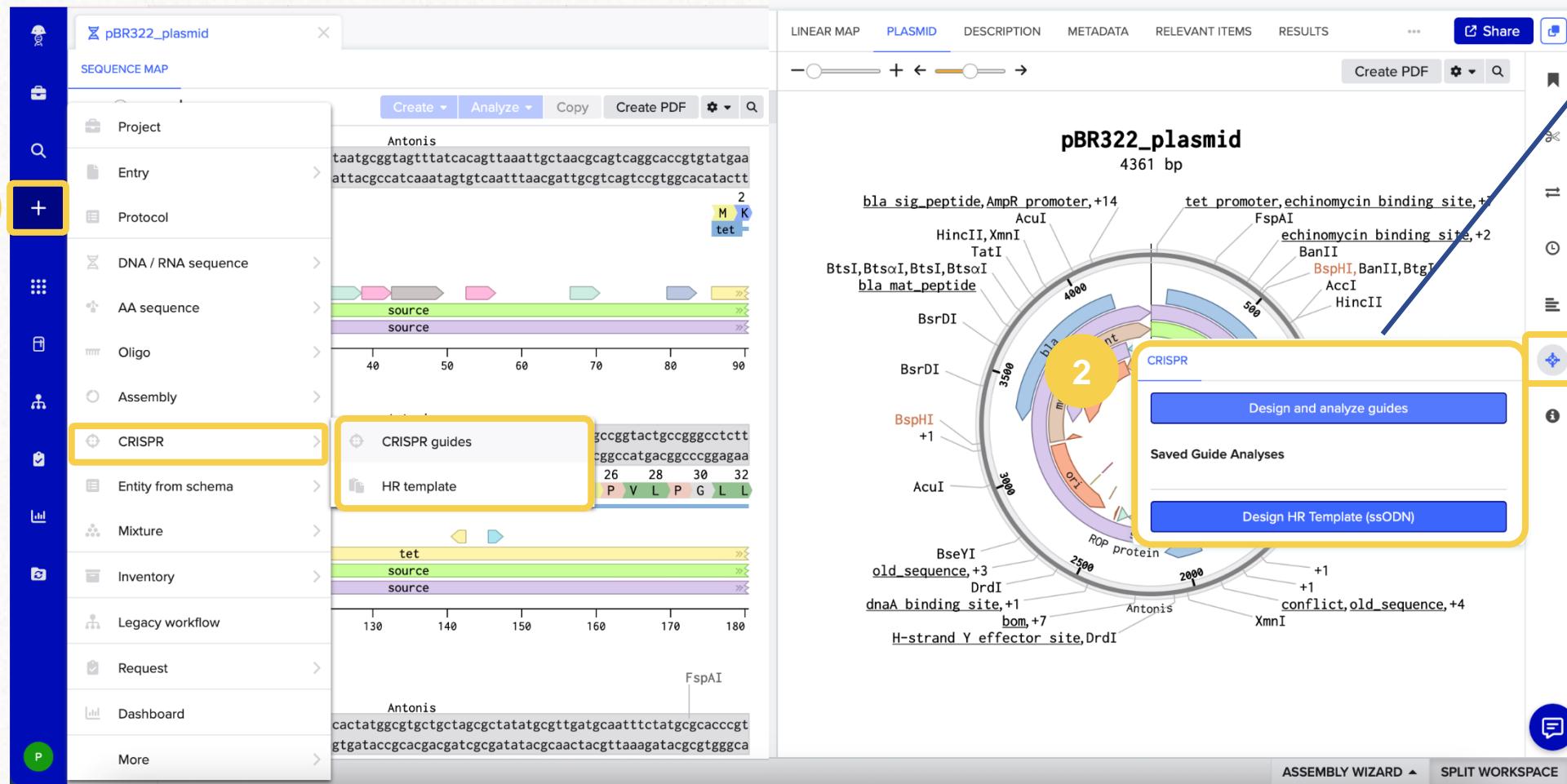
The screenshot shows the CRISPR tools interface with the following details:

- Left Sidebar:** Shows a file tree with "Your Name / Training Files / 3. CRISPR tools". Subfolders include "pCRISPR" (modified 6 days ago) and "pta source" (modified 6 days ago).
- Top Bar:** Includes a search bar, type/filters dropdown, and a "Create" button.
- Sequence Map View:** Displays three sequence maps:
 - pta source:** A 3929 bp sequence with two genes: "pta gene" (blue arrow) and "yfcC gene" (orange arrow). The "pta CDS (phosphate acetyltransferase)" is also labeled.
 - PshAI:** A sequence with one gene: "pta gene" (blue arrow) and "pta CDS (phosphate acetyltransferase)" (grey arrow).
- Right Panel:** Shows detailed gene annotations for "pta source":
 - Linear map view from 2,303,000 to 2,305,000 bp.
 - Genes: "pta gene" (blue), "yfcC gene" (orange).
 - CDS: "pta CDS (phosphate acetyltransferase)" (grey), "yfcC CDS (phosphate acetyltransferase)" (brown).
- Bottom:** Buttons for "ASSEMBLY ▲" and "SPLIT WORKSPACE".

CRISPR tools

Tool overview

- It is possible to create guide RNA sequences and Homologous recombination templates using the CRISPR tool. There are 2 ways to access it:



The screenshot shows the Benchling software interface. On the left, there is a sidebar with various tools and a main workspace. In the main workspace, a plasmid named "pBR322_plasmid" is shown as a circular map with restriction sites and gene regions. A yellow box labeled "1" highlights the "+" button in the sidebar, which is used to add new entries. Another yellow box labeled "2" highlights the "CRISPR" section in the sidebar, which is where the CRISPR tool is located. A callout box with a blue arrow points from the text "By default, Benchling will use the open sequence as to design the gRNA on" to the "CRISPR" section in the sidebar.

CRISPR tools

Tool overview

Design CRISPR Guides: Guide parameters

Design Type

- Single guide
Wild-type Cas9, single gRNA (higher efficiency)
- Paired guides
Double Cas9 nuclease, two gRNAs (lower off-target effects)
- Guides for "base editing" (Komor et al., 2016)
C > T (or G > A) substitution, no dsDNA breaks

Guide Length: 20

Genome: GRCm38 (mm10, Mus musculus)

PAM: NGG (SpCas9, 3' side)

Save these as my default

Custom PAM dropdown menu:

- NGG (SpCas9, 3' side) (selected)
- NAG (SpCas9, 3' side)
- NG (SpCas9 NG, 3' side)
- NNNNGATT (NmeCas9, 3' side)
- NNAGAAW (StCas9, 3' side)
- NAAAAC (TdCas9, 3' side)
- NNGRR (SaCas9, 3' side)
- NNGRRT (SaCas9, 3' side)

SEQUENCE MAP view showing Brca2 genomic regions and assembly details.

RESULTS panel:

It's possible to design single guide RNA, paired guides or guides for base editing.

Benchling supports various Cas enzymes targeting different PAM sites. To specify your custom PAM, select the "Custom PAM" option in the "PAM" dropdown selection.

7. CRISPR tools

7.1 gRNA design



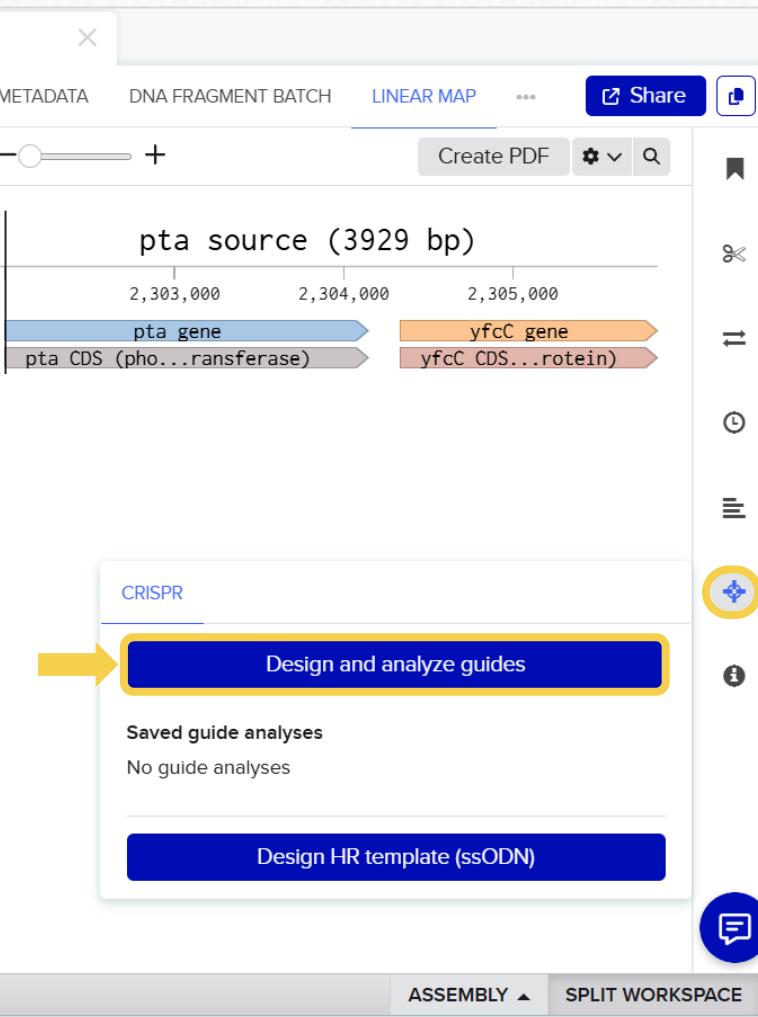
CRISPR tools

gRNA design

1 Open the **pta source** file.



2 Access the **gRNA design** menu.



gRNA design

3

Change the genome to ***E. coli* BL21(DE3)**.

4

Click **Finish** and continue.

Design CRISPR guides: Guide parameters

Design type Single guide
Wild-type Cas9, single gRNA (higher efficiency)
 Paired guides
Double Cas9 nickase, two gRNAs (lower off-target effects)
 Guides for "base editing" (Komor et al., 2016)
C > T (or G > A) substitution, no dsDNA breaks

Guide length

Genome

PAM

Show advanced settings

Save these as my default CRISPR settings

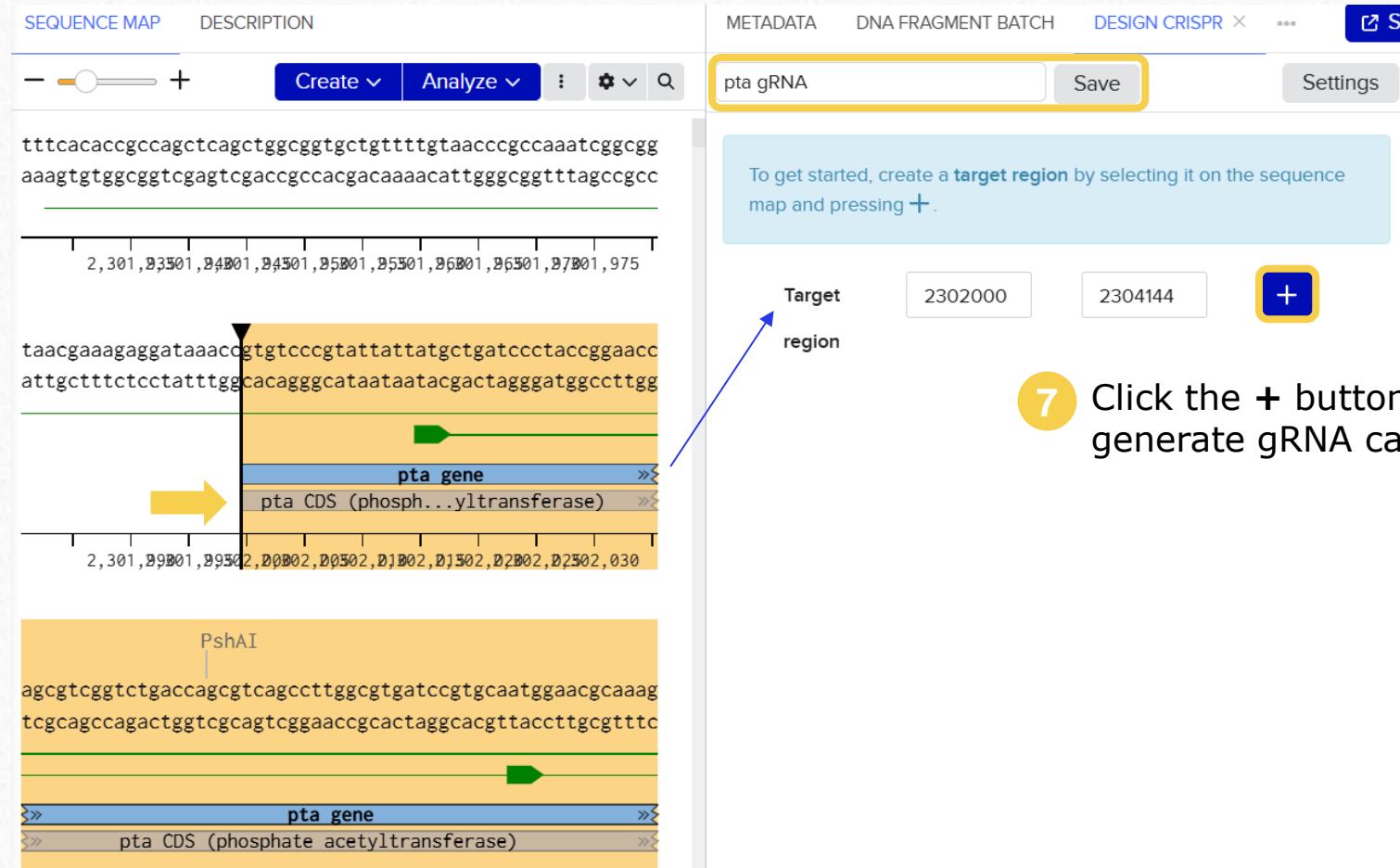
Finish

 Setting the genome is important for **off-target** calculations.

CRISPR tools

gRNA design

- 5 Give a name to your CRISPR design tab and save it so you can come back to it if you need to.
- 6 Select the **pta CDS** annotation. The target region will be set automatically.

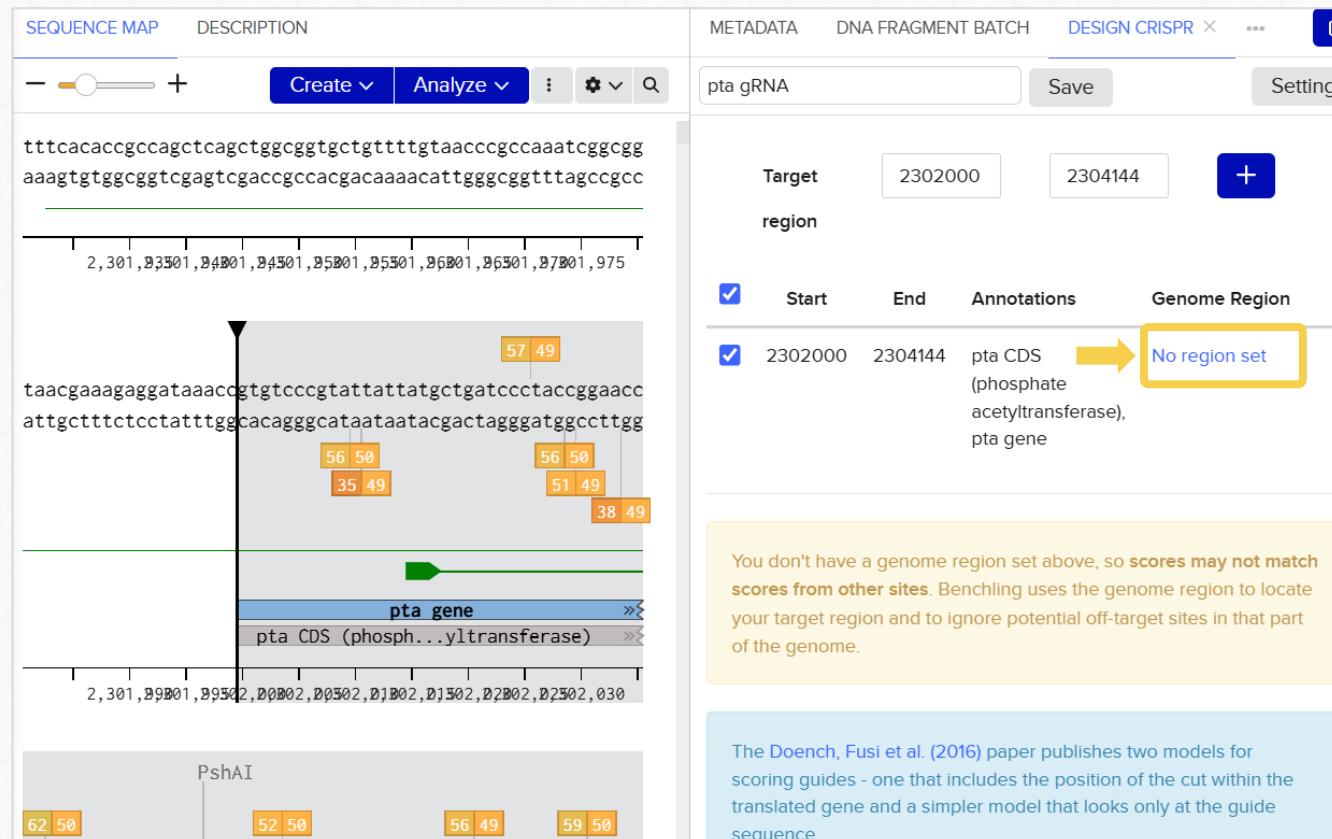


The screenshot shows the CRISPR tools interface with two main panels. On the left, the 'SEQUENCE MAP' panel displays a DNA sequence with a 'pta gene' and 'pta CDS (phosphate acetyltransferase)' annotation. A yellow arrow points from the 'pta CDS' label to the 'Target region' button in the 'DESIGN CRISPR' panel on the right. The 'DESIGN CRISPR' panel has tabs for 'METADATA', 'DNA FRAGMENT BATCH', and 'DESIGN CRISPR'. The 'DESIGN CRISPR' tab is active, showing a search bar with 'pta gRNA' and a 'Save' button. Below the search bar is a message: 'To get started, create a target region by selecting it on the sequence map and pressing +.' To the right of the message are two boxes containing '2302000' and '2304144', with a yellow-bordered '+' button next to them. A blue arrow points from the 'Target region' label to the '+' button.

CRISPR tools

gRNA design

- Set the genome region as shown to obtain accurate **off-target** scores for the gRNA candidates.



The screenshot shows the CRISPR tools interface. On the left, the "SEQUENCE MAP" tab displays a DNA sequence with several potential target sites highlighted by orange boxes with coordinates (e.g., 57 49, 56 50, 35 49, 56 50, 51 49, 38 49). A gene annotation for "pta gene" is shown with its corresponding CDS. On the right, the "DESIGN CRISPR" tab shows the target regions 2302000 and 2304144. In the "Genome Region" section, a message states "No region set". A yellow arrow points from this message to the "Chromosome" dropdown menu in a modal window. The modal window also contains a "Find genome matches" button and "Set genome region" and "Cancel" buttons.

SEQUENCE MAP

DESCRIPTION

tttcacaccgcagctcagctggcggtgtttgttaaccgcataatccggcgaaagtgtggcggtcgagtgcaccgcacaaaaatggcggttagccgc

2,301,93501,94001,94501,95001,95501,96001,96501,97001,975

taacgaaagaggataaacgtgtccgttattatgtctatccctaccggaaaccttgcttctctatttggcacaggcataataatcgtactggatggccttgg

2,301,99001,99502,00002,00502,01002,01502,02002,02502,030

METADATA **DNA FRAGMENT BATCH** **DESIGN CRISPR** **...** **SI**

pta gRNA Save Settings

Target region

2302000 2304144 +

<input checked="" type="checkbox"/> Start	<input checked="" type="checkbox"/> End	Annotations	Genome Region
2302000	2304144	pta CDS (phosphate acetyltransferase), pta gene	No region set

You don't have a genome region set above, so scores may not match scores from other sites. Benchling uses the genome region to locate your target region and to ignore potential off-target sites in that part of the genome.

The Doench, Fusi et al. (2016) paper publishes two models for scoring guides - one that includes the position of the cut within the translated gene and a simpler model that looks only at the guide sequence.

Genome region

Setting a genome region will remove it from off-target analysis.

None

Chromosome 2302000 2304144

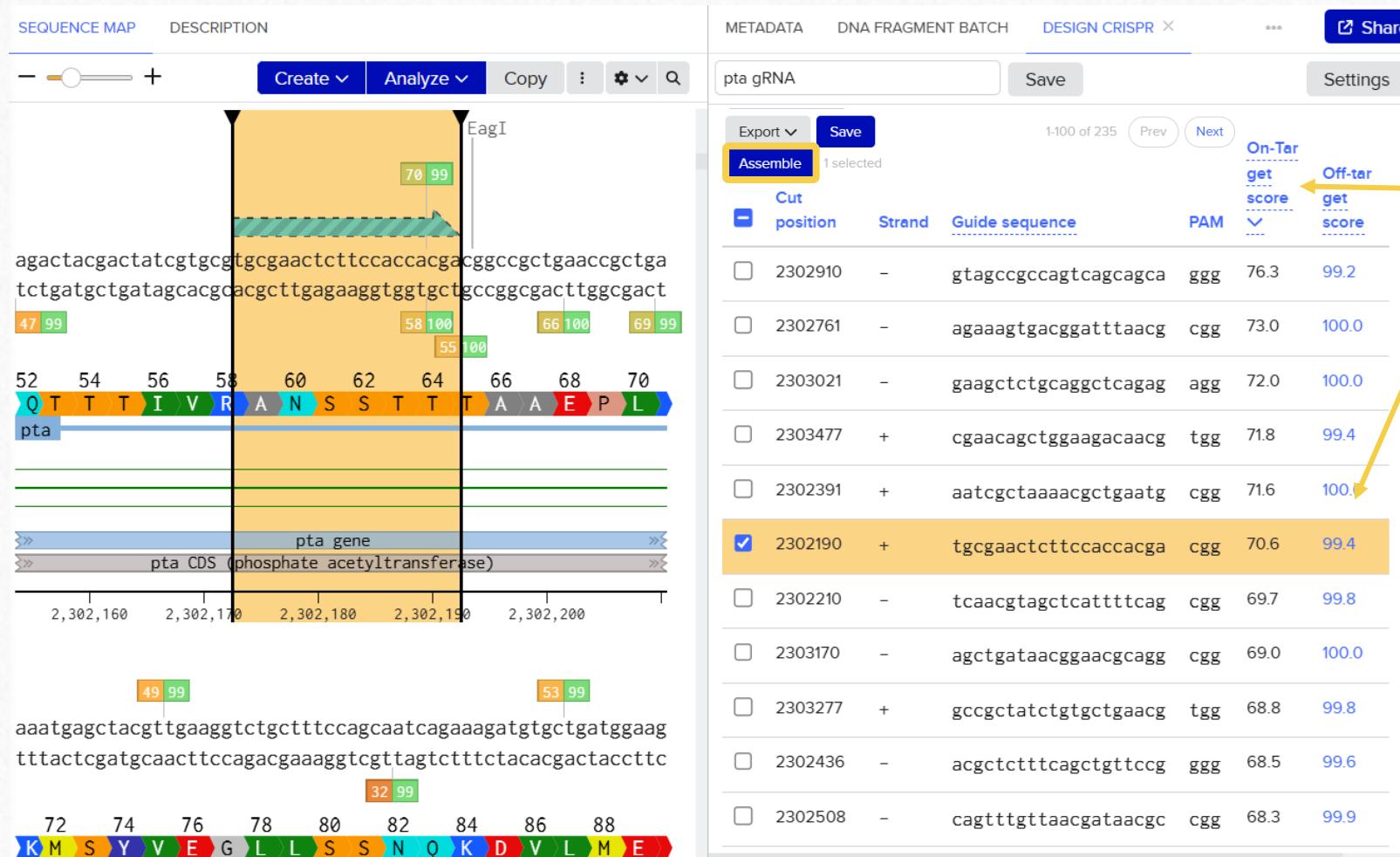
Find genome matches

Set genome region **Cancel**

CRISPR tools

gRNA design

- i** Benchling will show you a list of potential gRNAs to choose from. You can sort them by **on-target** or **off-target** score, or browse your sequence and select the best one for your needs based on its location.



The screenshot shows the Benchling CRISPR tool interface. On the left, there is a "SEQUENCE MAP" view of a DNA sequence (pta gene) with restriction sites (EagI) and amino acid translations. The sequence starts at position 2,302,160 and ends at 2,302,200. A "pta CDS (phosphate acetyltransferase)" is indicated. On the right, the "DESIGN CRISPR" tab is active, showing a list of potential gRNAs. The columns include: Cut position, Strand, Guide sequence, PAM, On-Tar get score, and Off-tar get score. The "On-Tar get score" column is sorted in descending order. One gRNA (2302190) is selected, highlighted in yellow, and has a blue checkmark next to it. The "Save" button is also visible.

Cut position	Strand	Guide sequence	PAM	On-Tar get score	Off-tar get score
2302910	-	gtagccgccagtca...ca	ggg	76.3	99.2
2302761	-	agaaaagtgacggat...a	cgg	73.0	100.0
2303021	-	gaagctctgcaggctc...	agg	72.0	100.0
2303477	+	cgaacagctggaaga...	tgg	71.8	99.4
2302391	+	aatcgctaaaacgctg...	cgg	71.6	100.0
2302190	+	tgcgaaactttccacc...	cgg	70.6	99.4
2302210	-	tcaacgtagctcat...t	cgg	69.7	99.8
2303170	-	agctgataacggaacg...	cgg	69.0	100.0
2303277	+	gccgctatctgtgctg...	tgg	68.8	99.8
2302436	-	acgctttcagctgttcc...	ggg	68.5	99.6
2302508	-	cagtttgtaacgataac...	cgg	68.3	99.9

9 Sort by **on-target** score.

10 Select the gRNA as shown.

i By clicking the blue **Save** button, you can create a file with your selected gRNA(s). You should do it for this example.

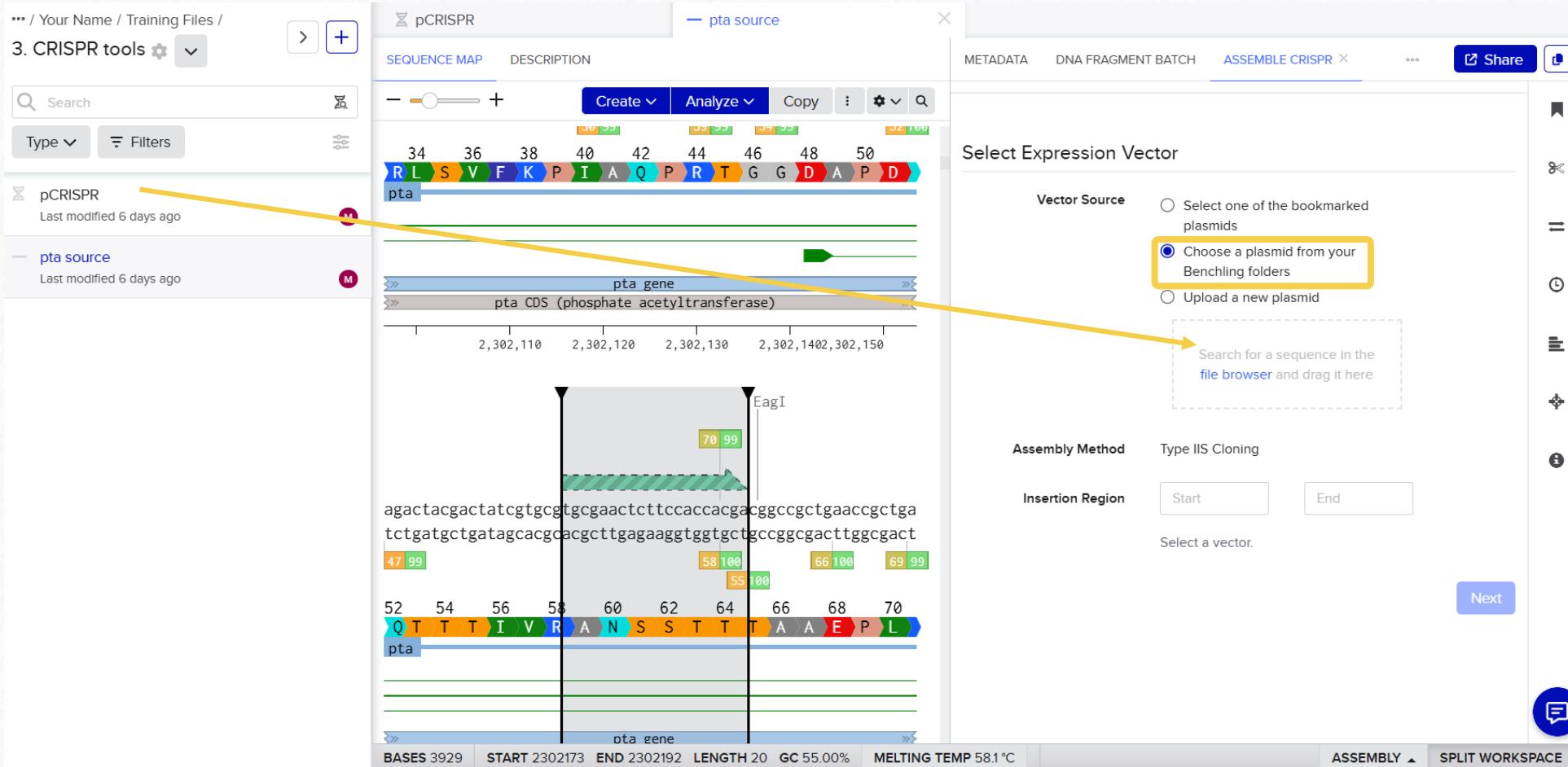
11 Click **Assemble**.

i This option will allow you to place the chosen gRNA into a plasmid with Type IIS restriction sites.

CRISPR tools

gRNA design

12 Select the **Choose a plasmid from your Benchling folders** option and drag the **pCRISPR** file into the box.



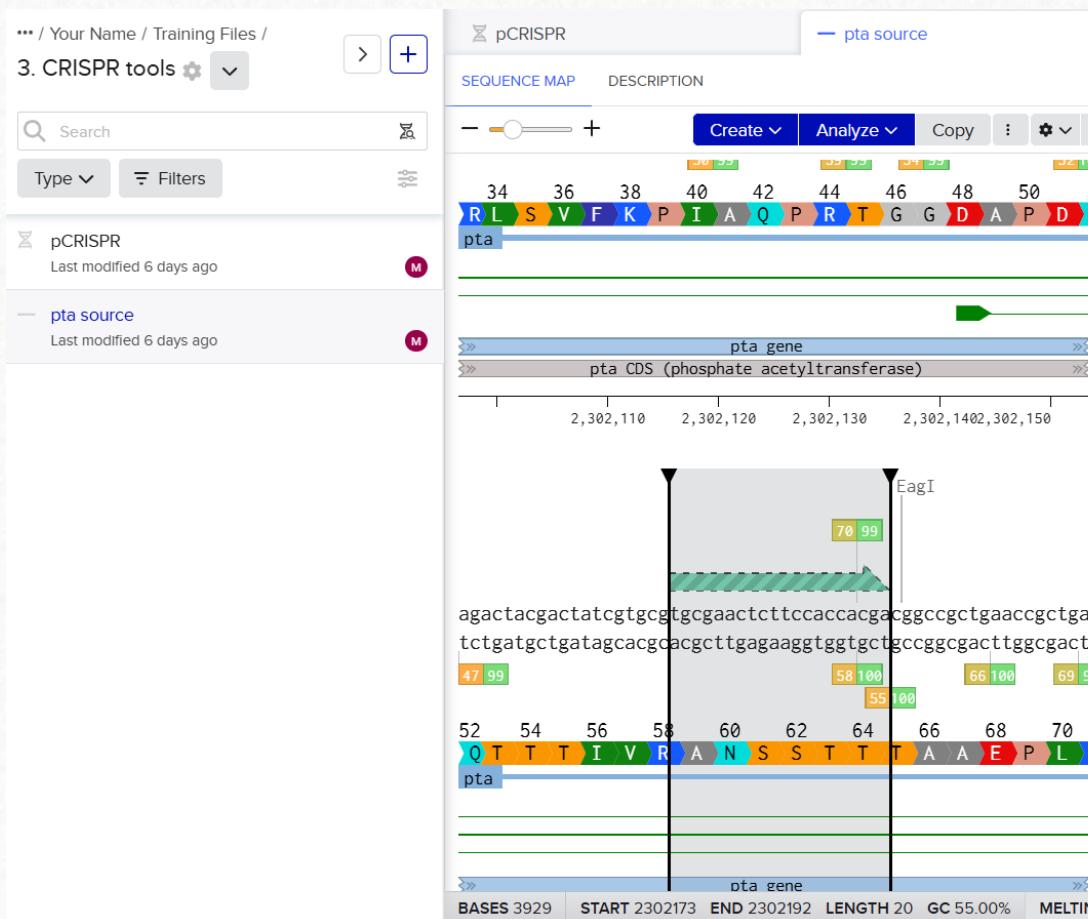
The screenshot shows the CRISPR tools interface. On the left, a sidebar lists files: 'pCRISPR' and 'pta source'. A yellow arrow points from the 'pCRISPR' file to the 'pta source' file. The main area displays a sequence map for the 'pta' gene, which encodes phosphate acetyltransferase. The gene is shown with its start and end positions (2,302,110 to 2,302,150) and GC content (55.00%). Below the sequence map, a DNA sequence is shown with various restriction sites and their coordinates (e.g., 47, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 78, 99, 100). On the right, a 'Select Expression Vector' dialog is open. It includes options for 'Vector Source' (radio button selected for 'Choose a plasmid from your Benchling folders'), 'Assembly Method' (set to 'Type IIS Cloning'), 'Insertion Region' (with 'Start' and 'End' fields), and a note to 'Select a vector.' A yellow box highlights the 'Choose a plasmid from your Benchling folders' option, and a yellow arrow points from the 'pta source' file in the sidebar to this dialog. A blue 'Next' button is visible at the bottom right of the dialog.

 The drag-and-drop option does not work in Safari.

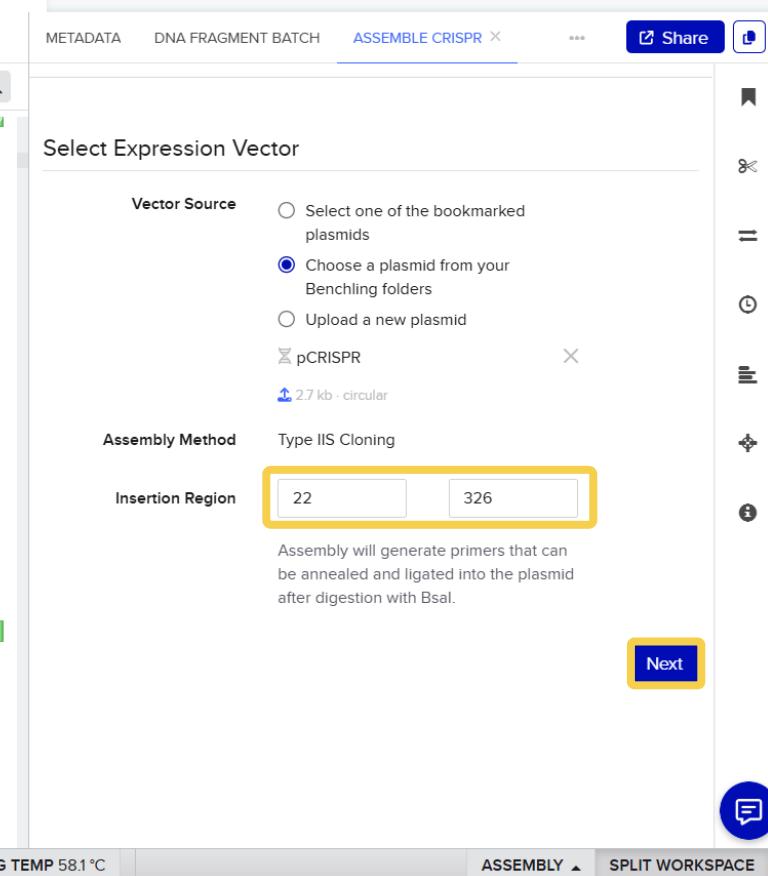
CRISPR tools

gRNA design

13 Set the **insertion region** as shown.



14 Click **Next**.



Select Expression Vector

Vector Source

- Select one of the bookmarked plasmids
- Choose a plasmid from your Benchling folders
- Upload a new plasmid

pCRISPR
2.7 kb - circular

Assembly Method: Type IIS Cloning

Insertion Region: 22 326

Assembly will generate primers that can be annealed and ligated into the plasmid after digestion with Bsal.

Next

 Benchling will look for Type IIS restriction sites in the region. Sometimes it may not work as expected; in this case, refer to [this article](#).

CRISPR tools

gRNA design

- 15 Name your assembly, choose a location to save it and click **Assemble**.

The screenshot shows the CRISPR tools interface divided into two main sections: Sequence Map and Finalize Assemblies.

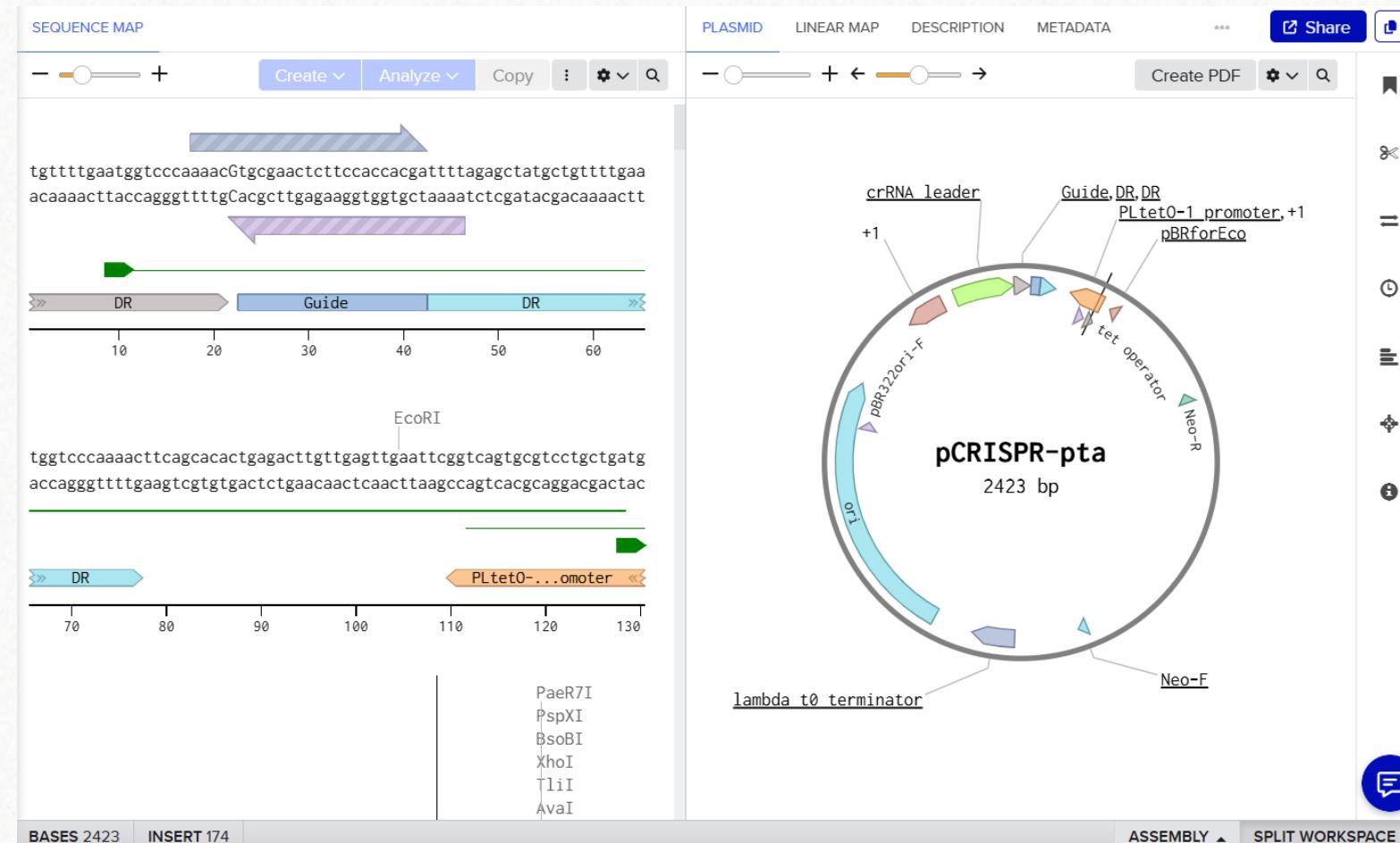
Sequence Map: This section displays two DNA sequence regions. The top region is labeled "pta" and shows a restriction site for "EagI" at position 68. Below the sequence, the "pta gene" and "pta CDS (phosphate acetyltransferase)" are indicated. The bottom region also shows the "pta" gene and CDS. Both regions have amino acid translations below them, with positions 72 through 88 shown for the bottom sequence.

Finalize Assemblies: This section allows users to name their assembly and choose a folder to save it. The "Assembly Name" field contains "pCRISPR-ptt". The "Guide Sequence" field contains "tgcgaaactttccaccacga". The "Folder" dropdown is set to "3. CRISPR tools". At the bottom right, there is a prominent blue "Assemble" button.

CRISPR tools

gRNA design

- ✓ The result will be an expression vector with your chosen gRNA and a primer pair that can be annealed and ligated into the plasmid after digestion with BsaI.
- ✓ This can also be done with multiple gRNAs at a time.



7. CRISPR tools

7.2 HR template design



CRISPR tools

HR template design

- 1 Open the **pta source** file.
- 2 Access the **HR template design** menu.

The screenshot shows the pCRISPR software interface. On the left, there is a 'SEQUENCE MAP' tab displaying a linear map of the 'pta source' sequence (3929 bp). The map highlights the 'pta gene' (phosphotransferase) and the 'yfcC gene' (protein). Below the map, the sequence is shown with its base coordinates (2,301 to 2,305,000). On the right, the 'Design HR template' menu is open, showing options for 'Genome' (ASM956v1), 'PAM' (NGG), and two radio button options: 'Create a copy of this sequence' (selected) and 'Modify this sequence'. A yellow arrow points to the 'Design HR template (ssODN)' button, which is highlighted in blue. Other buttons in the menu include 'Design and analyze guides' and 'Saved guide analyses'.

- 3 Select the option to create a copy of the sequence.

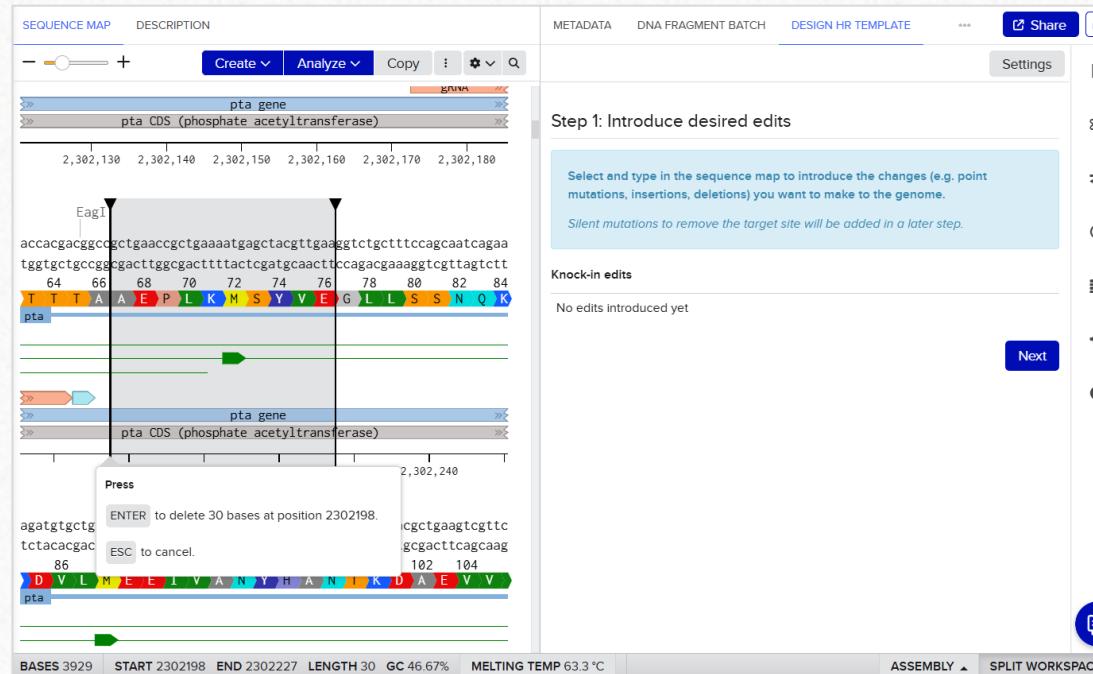
A detailed view of the 'Design HR template' dialog box. It includes fields for 'Genome' (ASM956v1), 'PAM' (NGG), and two radio button options: 'Create a copy of this sequence' (selected) and 'Modify this sequence'. At the bottom are 'Cancel' and 'Create' buttons.

CRISPR tools

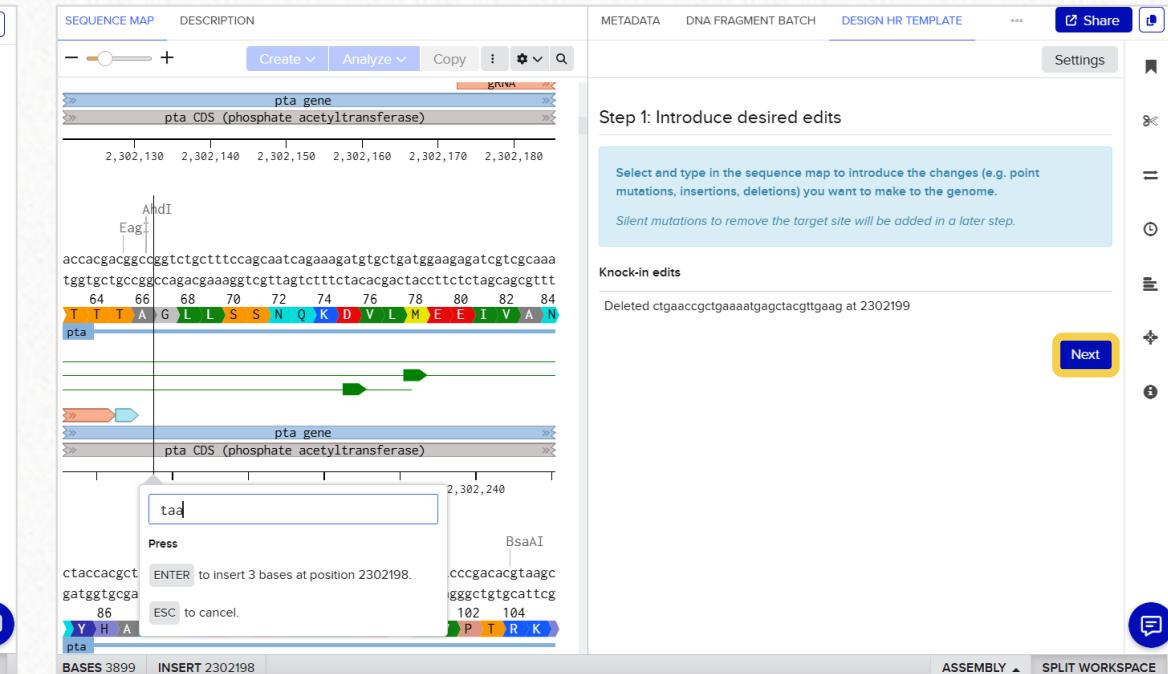
HR template design

- i** You can introduce the desired modifications to the sequence, but do not remove the gRNA region nor its PAM. Benchling will look for **both** of them. The **PAM removal** will be done by the tool at a later stage.

- 4 Delete 30 nt as shown.



- 5 Insert a stop codon **in-frame** of the *pta* CDS.

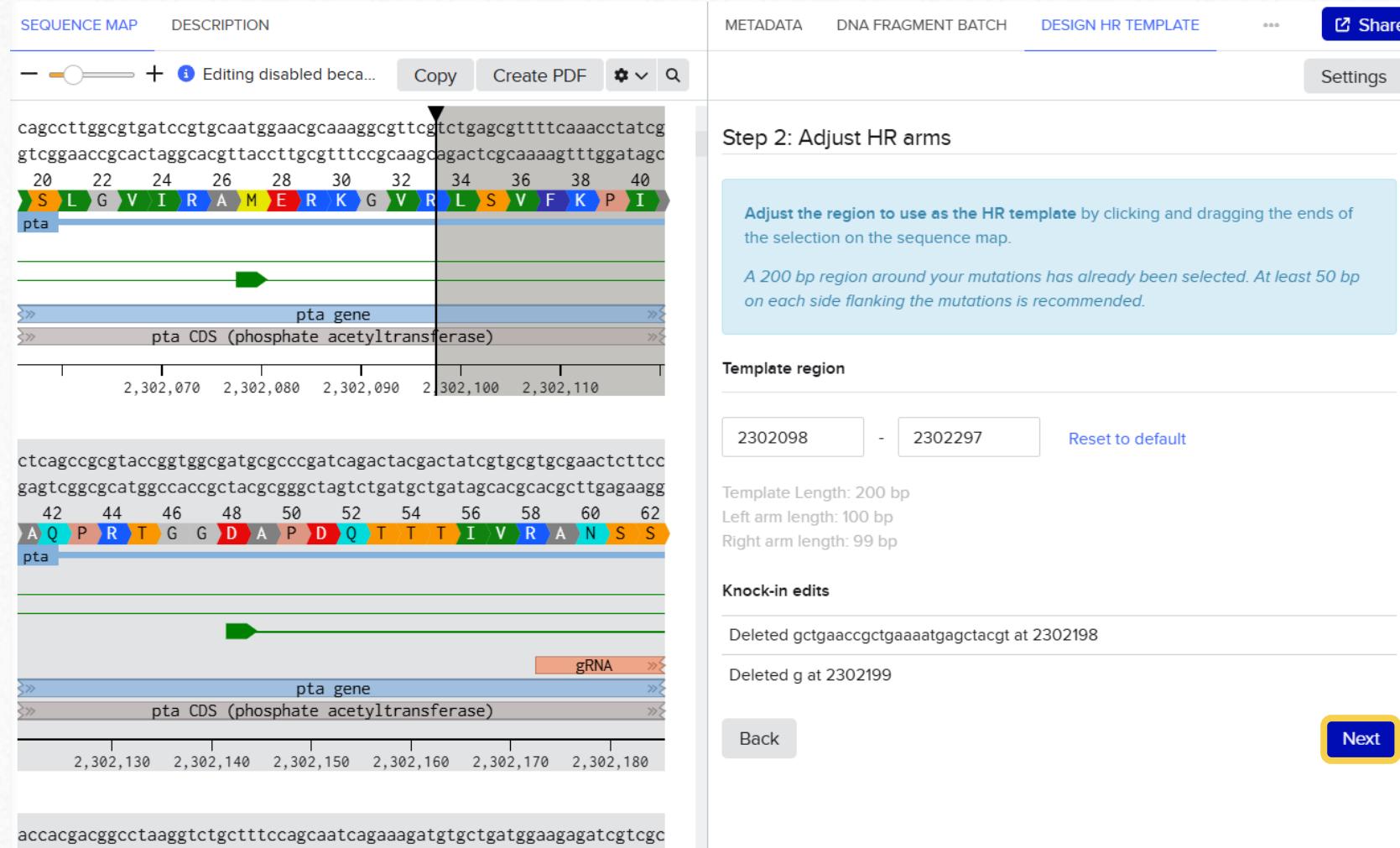


- 6 Click **Next**.

CRISPR tools

HR template design

 Benchling will select the region needed to create the HR template. You can adjust the length of the selection.



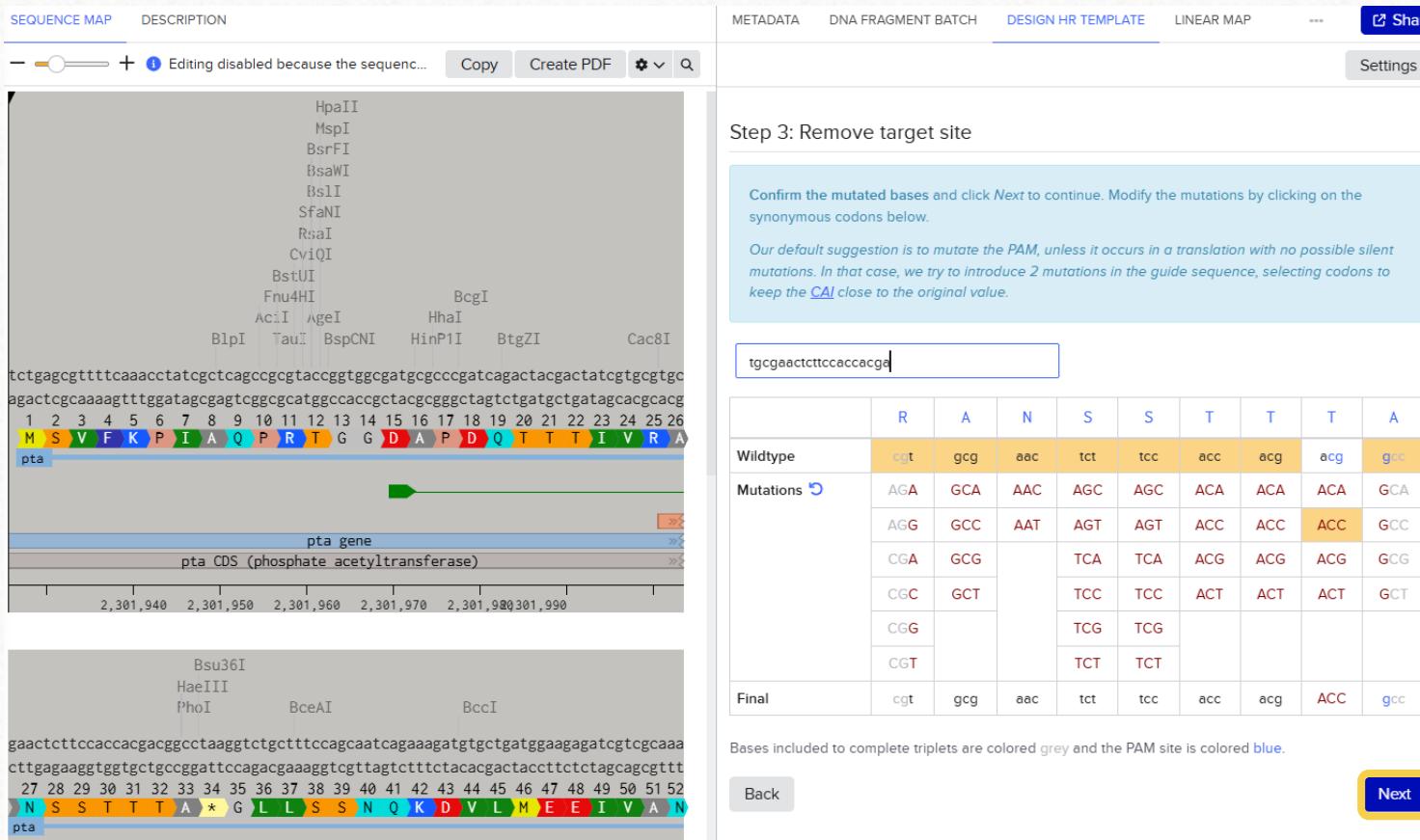
The screenshot shows two sequence maps for the pta gene (phosphate acetyltransferase) at positions 2,302,070 to 2,302,110 and 2,302,130 to 2,302,180. The top map displays mutations at positions 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40. The bottom map displays mutations at positions 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 62. Both maps show the pta gene and its CDS. A green arrow indicates the gRNA target site. The 'DESIGN HR TEMPLATE' tab is selected in the header. In the 'Step 2: Adjust HR arms' section, a note says: 'Adjust the region to use as the HR template by clicking and dragging the ends of the selection on the sequence map.' Another note says: 'A 200 bp region around your mutations has already been selected. At least 50 bp on each side flanking the mutations is recommended.' The 'Template region' section shows coordinates 2302098 to 2302297. Below it, template length is 200 bp, left arm length is 100 bp, and right arm length is 99 bp. The 'Knock-in edits' section lists deleted sequences: 'Deleted gctgaaccgctaaaaatgagctacgt at 2302198' and 'Deleted g at 2302199'. Navigation buttons include 'Back', 'Next' (highlighted in yellow), and 'Settings'.

7 Click Next.

CRISPR tools

HR template design

- Paste the gRNA sequence: tgcgaactttccaccacga



	R	A	N	S	S	T	T	T	A
Wildtype	cgt	gct	aac	tct	tcc	acc	acg	acg	gcc
Mutations	AGA	GCA	AAC	AGC	AGC	ACA	ACA	ACA	GCA
	AGG	GCC	AAT	AGT	AGT	ACC	ACC	ACC	GCC
	CGA	GCG		TCA	TCA	ACG	ACG	ACG	GCG
	CGC	GCT		TCC	TCC	ACT	ACT	ACT	GCT
	CGG			TCG	TCG				
	CGT			TCT	TCT				
Final	cgt	gct	aac	tct	tcc	acc	acg	ACC	gcc

Bases included to complete triplets are colored grey and the PAM site is colored blue.

Back **Next**

- i The PAM will be removed from the HR template to prevent the degradation of the ssODN. You can choose from several alternatives, as shown in the table.

- Click **Next**.

HR template design

Step 4: Summary

Knock-in edits

Deleted gctgaaccgctgaaaatgagctacgt at 2302026

Deleted g at 2302027

Template Range 2302098 to 2302297

Guide tgcgaaactttccaccacga

Original Target Site ... cgt gcg aac tct tcc acc acg acg
gcc ...

After Site Removal ... cgt gcg aac tct tcc acc acg ACC
gcc ...

[Copy the template](#) or its [reverse complement](#) to your clipboard.

To design a template for the same knock-in edits but with a different guide, [click here](#).

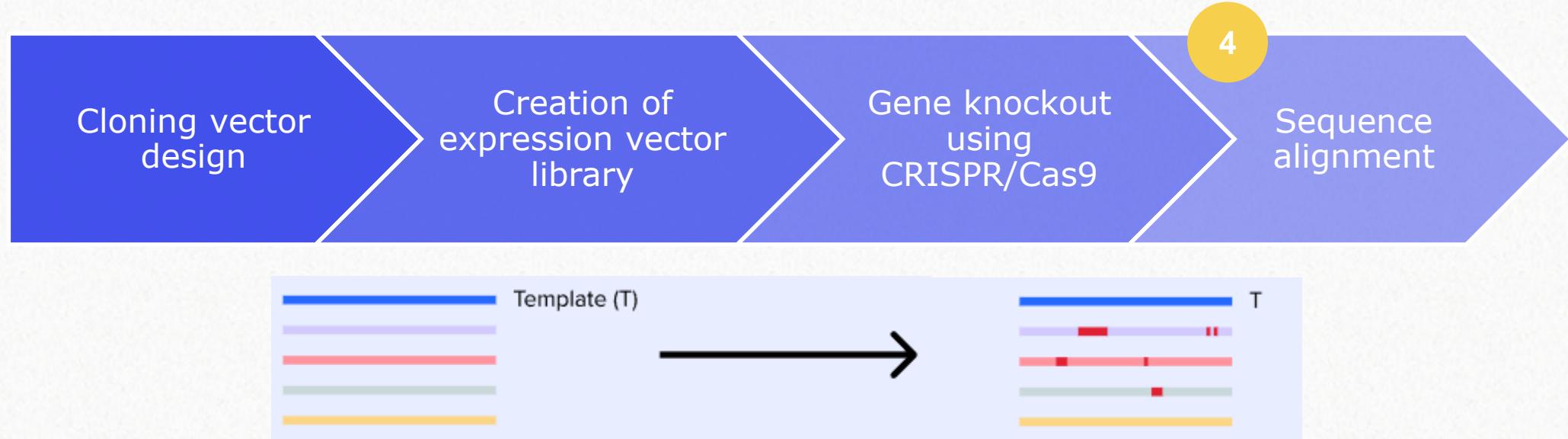
- ✓ After the design process, you can copy the resulting HR template and paste it onto a new DNA sequence file to save it.

8. Sequence alignments





This is the fourth part of the *hands-on* example.



✓ Multisequence alignment

Bonus: How to do consensus alignments

Expected output:

- Alignments using pSEVA6311-phaC-pct540 as template
 - Sanger sequencing alignments
 - Plasmid sequencing alignment



You will need the files in the ***Sequence alignments*** subfolder.

8. Sequence alignments

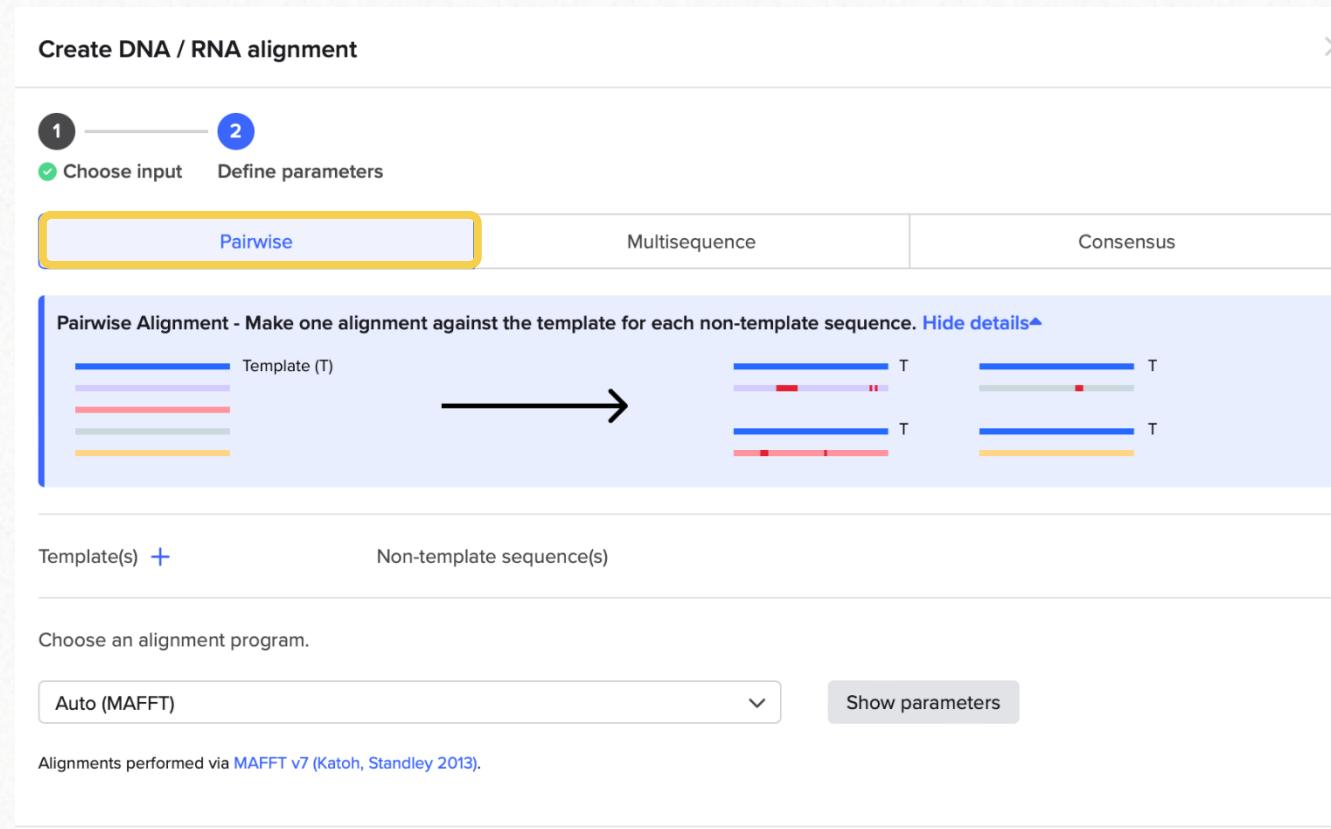
8.1 Alignment tool



Alignment creation

Alignment tool overview

- i In a real-life scenario, the construct sequences could be sent to sequencing. The results could then be analyzed using the **alignment tool** in Benchling.
 - ✓ There are **three alignment options** and several alignment programs available:



1

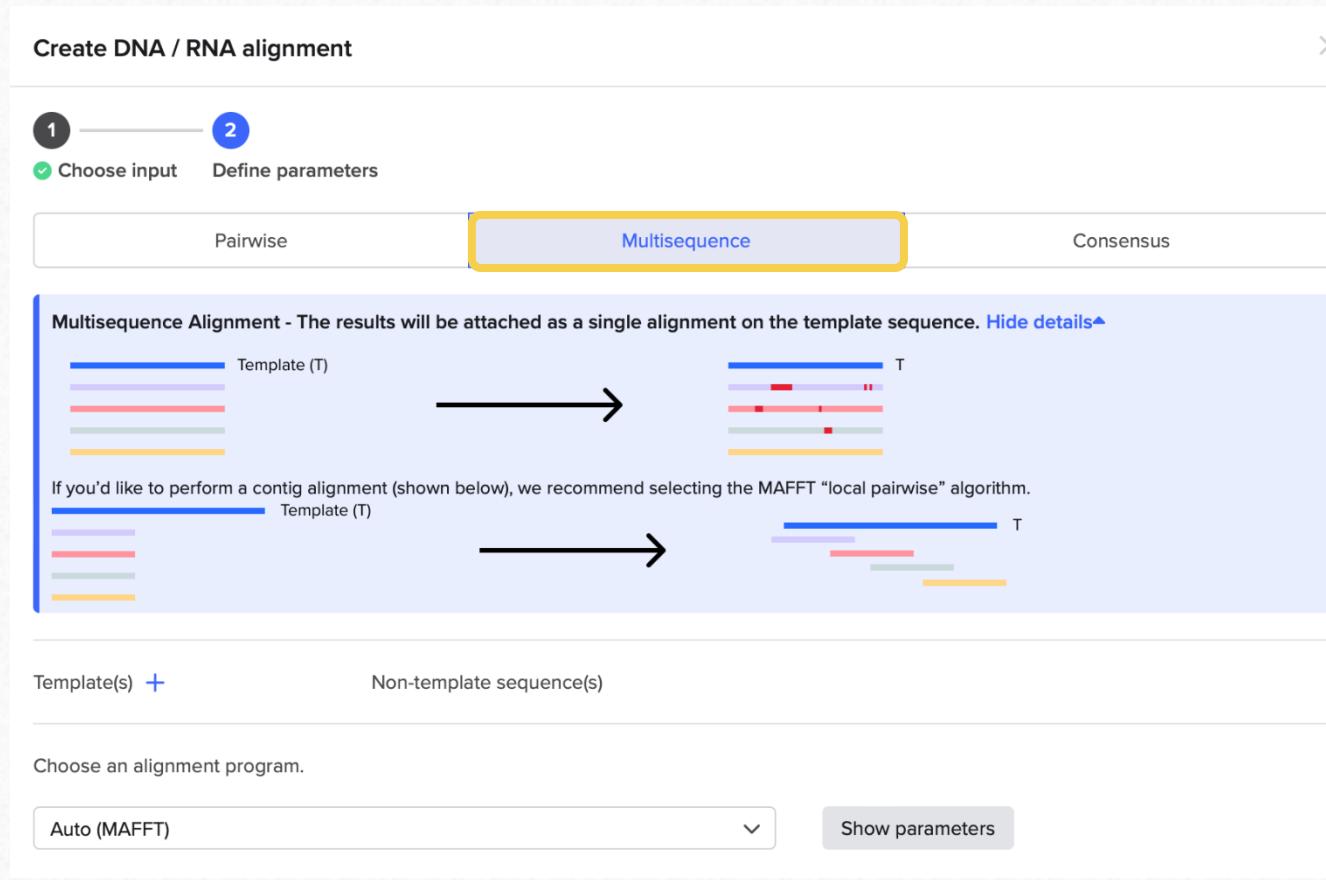
Pairwise alignment:

Sequences are compared against a template sequence, creating individual alignment files for each non-template seq.

Alignment creation

Alignment tool overview

- i In a real-live scenario, the construct sequence could be sent to sequencing. The results could then be analysed using the **alignment tool** in Benchling.
- ✓ There are **three alignment options** and several alignment programs available:



2

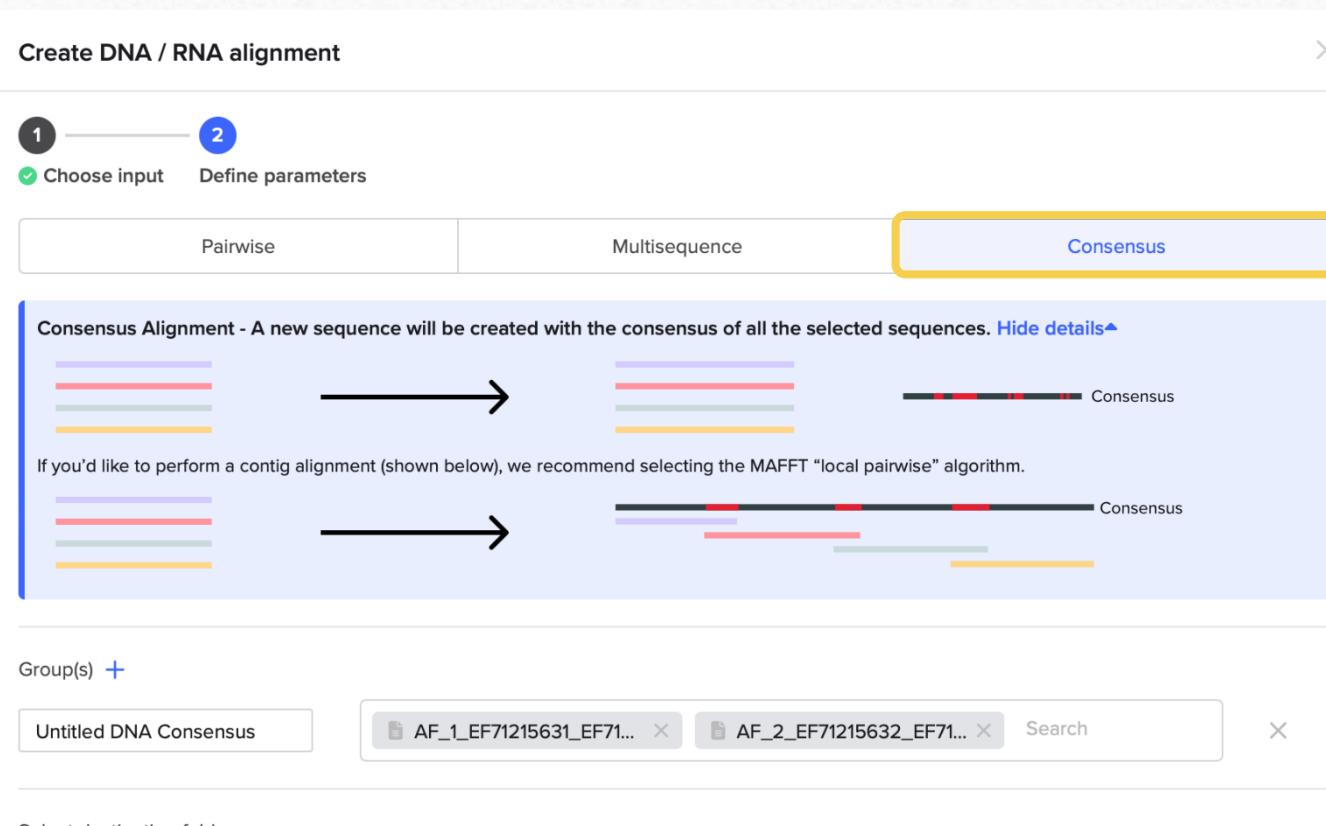
Multisequence alignment:

Multiple sequences are compared against a template sequence, creating a unique alignment file for all the non-template sequences

Alignment creation

Alignment tool overview

- i In a real-live scenario, the construct sequence could be sent to sequencing. The results could then be analysed using the **alignment tool** in Benchling.
 - ✓ There are **three alignment options** and several alignment programs available:



Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

Consensus Alignment - A new sequence will be created with the consensus of all the selected sequences. Hide details▲

If you'd like to perform a contig alignment (shown below), we recommend selecting the MAFFT "local pairwise" algorithm.

Group(s) +

Untitled DNA Consensus AF_1_EF71215631_EF71... AF_2_EF71215632_EF71... Search

Select destination folder.

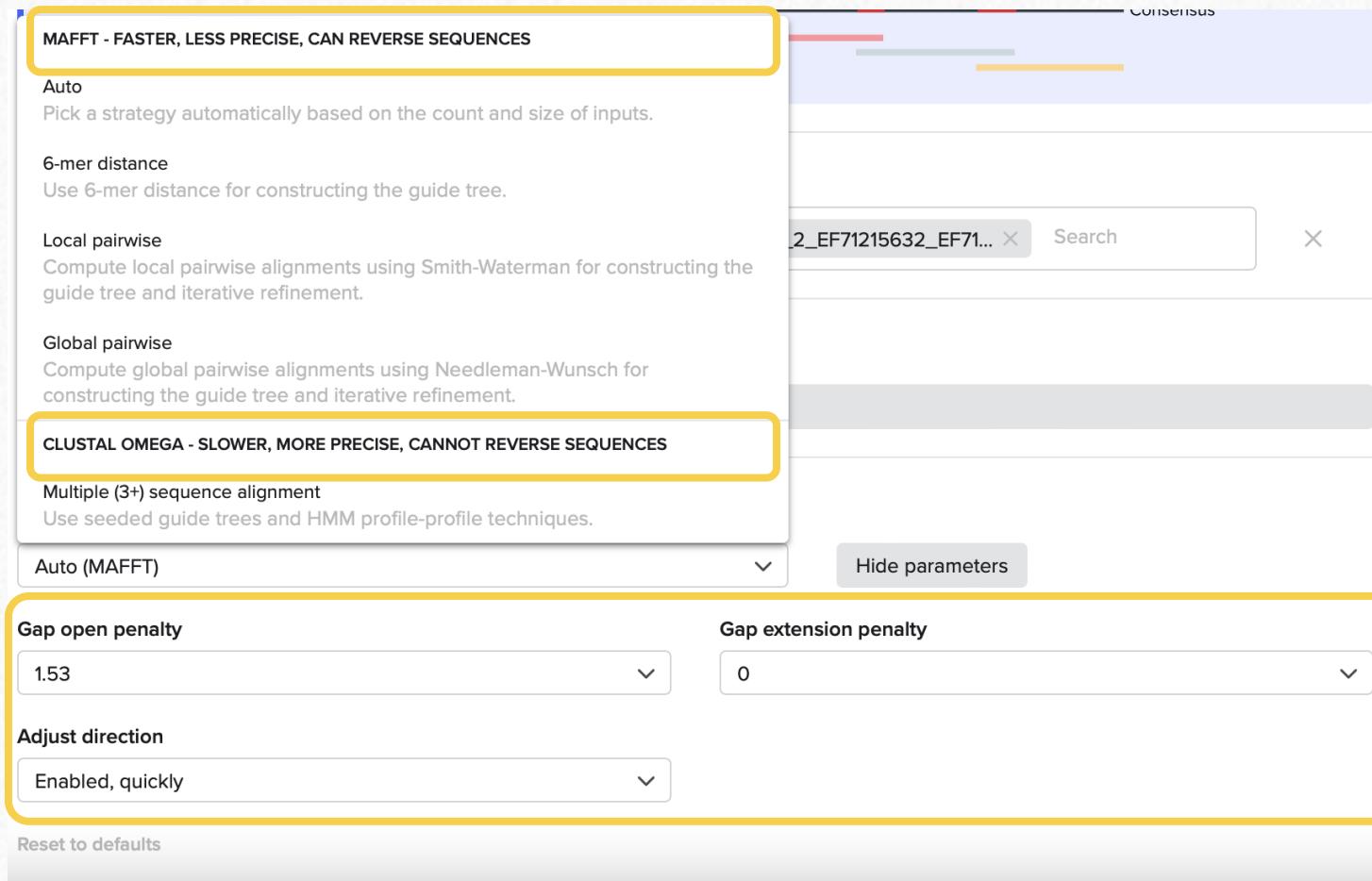
3

Consensus alignment:

Multiple sequences are compared against each other, creating a new sequence from the consensus region of all the sequences.

Alignment creation

Alignment tool overview



The screenshot shows the Alignment tool interface. On the left, there's a sidebar with various icons. The main area has two sections highlighted with yellow boxes:

- MAFFT - FASTER, LESS PRECISE, CAN REVERSE SEQUENCES**
 - Auto: Pick a strategy automatically based on the count and size of inputs.
 - 6-mer distance: Use 6-mer distance for constructing the guide tree.
 - Local pairwise: Compute local pairwise alignments using Smith-Waterman for constructing the guide tree and iterative refinement.
 - Global pairwise: Compute global pairwise alignments using Needleman-Wunsch for constructing the guide tree and iterative refinement.
- CLUSTAL OMEGA - SLOWER, MORE PRECISE, CANNOT REVERSE SEQUENCES**
 - Multiple (3+) sequence alignment: Use seeded guide trees and HMM profile-profile techniques.

Below these sections is a dropdown menu set to "Auto (MAFFT)" and a "Hide parameters" button. At the bottom, there are fields for "Gap open penalty" (set to 1.53), "Gap extension penalty" (set to 0), and "Adjust direction" (set to "Enabled, quickly"). A "Reset to defaults" button is also present.

- ✓ It's possible to choose between multiple types of **MAFFT** algorithms and **Crustal Omega** multisequence algorithm to power the alignment.
- ✓ Some of the key parameters of these can be changed as needed.

8. Sequence alignments

8.2 Multisequence alignment



Alignment creation

Multisequence alignment

- 1 Open the **Sequence alignments** folder.

The screenshot shows a software interface for managing sequence alignments. At the top, there's a navigation bar with '... / Your Name / Training Files /' and a tab labeled '4. Sequence alignments'. Below this is a search bar and some filters. The main area displays a table with four rows, each representing a sequence alignment. The columns are 'Name', 'Inventory', 'ID', 'Modified', 'Authors', and 'Description'. The 'Name' column has a checked checkbox. To the right of the table is a context menu with several options: 'Open', 'Create DNA / RNA Alignment' (which is highlighted with a yellow box), 'Analyze', 'Auto-annotate', 'Attach Primers', 'Auto-fill part fields', 'Auto-fill translations', 'Auto-fill transcriptions', 'Set topology', 'Codon optimize', and 'Remove annotations'. An information icon (i) is present in the bottom left corner of the main area.

Name	Inventory	ID	Modified	Authors	Description
FW-seq-1			11/02/2025		
MID-seq-1			11/02/2025		
plasmid-seq	No inventory availa...		11/02/2025		
pSEVA6311-phaC-pct540	No inventory availa...		11/02/2025		

i This way of starting alignments can be helpful if you have multiple sequences to work with.

Alignment creation

Multisequence alignment

Create DNA / RNA alignment

1 2

Choose input Define parameters

Upload sequence and trace files (.ab1, .ftv, .fasta, .gb, and .geneious). RNA uploads are not currently supported.

Drag and drop to upload or Choose files

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA RNA

Name Bases Add

Sequences

FW-seq-1 MID-seq-1 plasmid-seq pSEVA6311-phaC-pct540

Cancel Next

3 Click **Next**.

Alignment creation

Multisequence alignment

- 4 Configure the alignments to create two separate ones, as shown, both using **pSEVA6311-phaC-pct540** as template.

Create DNA / RNA alignment

- 1 Choose input
- 2 Define parameters

Pairwise Multisequence Consensus

Multisequence Alignment - The results will be attached as a single alignment on the template sequence. [Show details](#)

Template(s) [+](#) ← Non-template sequence(s) ↓

pSEVA6311-phaC-pct540 FW-seq-1 MID-seq-1

pSEVA6311-phaC-pct540 plasmid-seq

Choose an alignment program.

MAFFT recommended for nucleotide alignments Faster, less precise, can reverse sequences

Clustal Omega recommended for amino acid alignments Slower, more precise, cannot reverse sequences

Auto (MAFFT)

Alignments performed via MAFFT v7 (Katoh, Standley 2013).

- 5 Create the alignments.

Multisequence alignment

- 6** Go to the file you used as template and open the **Alignments** menu. You will find both alignments here.

The figure displays the circular map of the pSEVA6311-phaC-pct540 plasmid. Key features include:

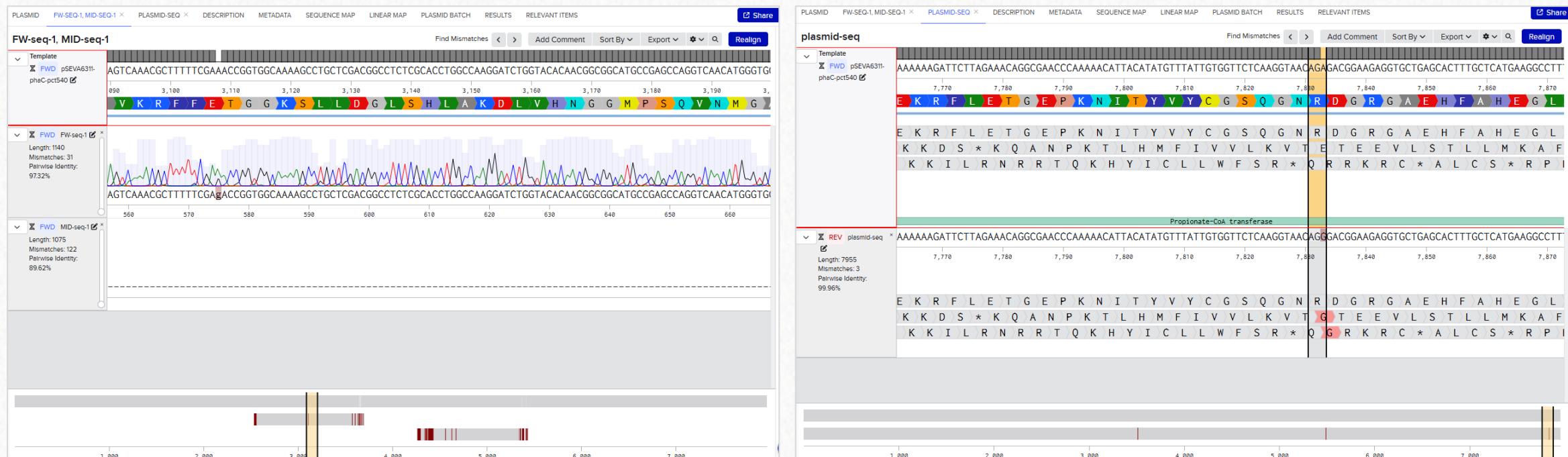
- Propionate-CoA transferase gene:** Located at the top right, with a promoter labeled "Propionate-CoA transferase" and a start site at +1.
- ChnR gene:** Located at the bottom left, with a promoter labeled "ChnR" and a start site at +1.
- Promoters:** Several promoters are indicated by arrows: "Propionate-CoA transferase" (green), "ChnR" (purple), "phaC (MBEL6-19)" (yellow), and "ChnR-PchnB-GA-R" (orange).
- Restriction sites:** EcorV sites are marked with blue triangles, and NdeI sites are marked with red triangles.
- Plasmid size:** 7955 bp.
- Sequence Map:** The right panel shows the DNA sequence from position 7642 to 1261, with amino acid translations for the Propionate-CoA transferase gene. A yellow arrow points to the "Saved Alignments" section.
- Sequence Alignments:** This section lists saved alignments for FW-seq-1, MID-seq-1, and plasmid-seq, dated 17/02/2025 18:55.

- ## 7 Open the alignments.

Alignment creation

Multisequence alignment

- ✓ You can now see and browse your resulting alignments.
- ✓ You may notice the first one includes trace files, which can help you assess the quality of the sequencing and assess whether the result can be considered accurate.
- ✓ The second one is a sequencing file for the whole plasmid. You can assess mismatches and toggle certain view options to check, for example, for amino acid changes in your CDS.



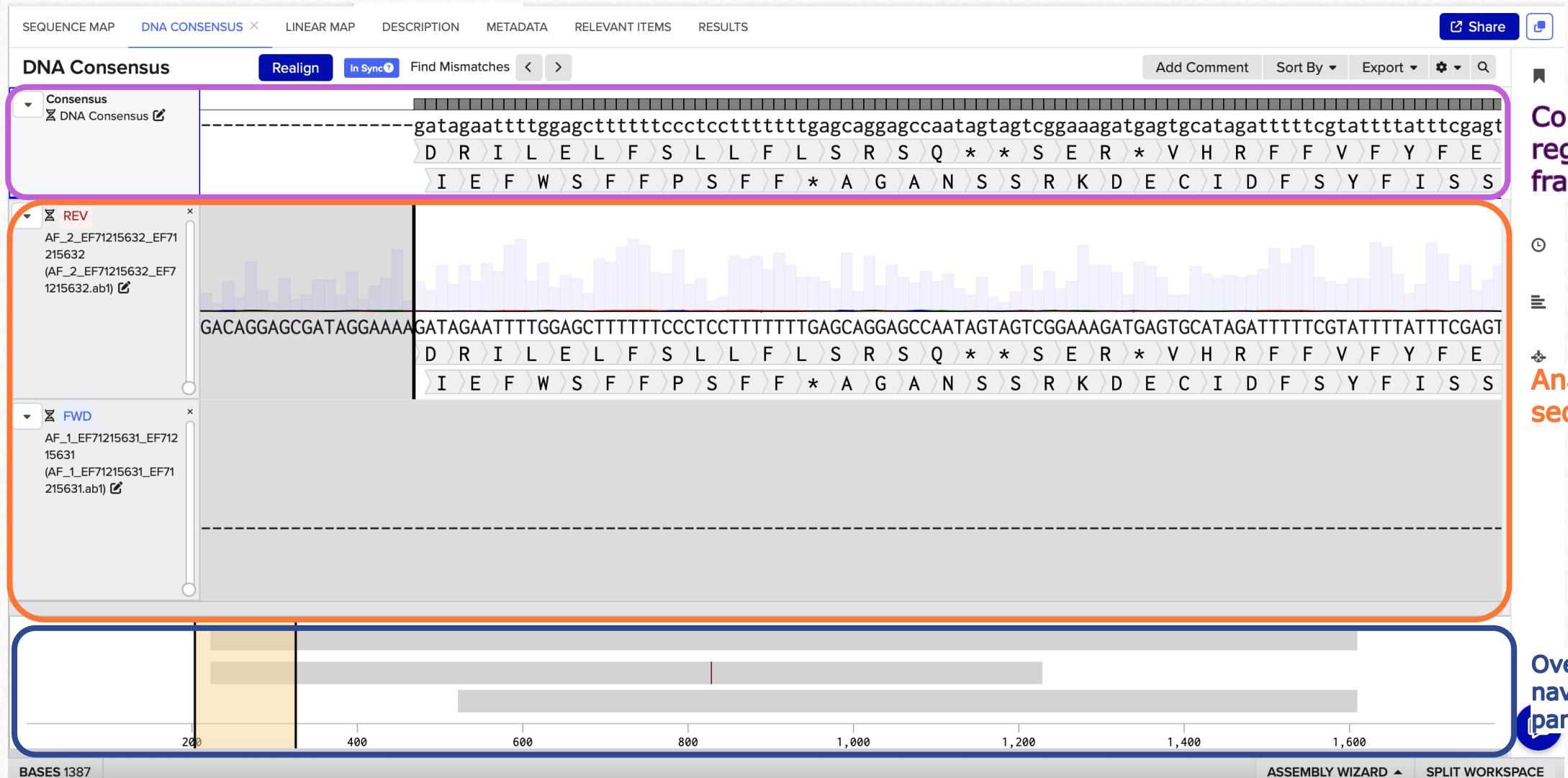
8. Sequence alignments

8.3 Consensus alignment



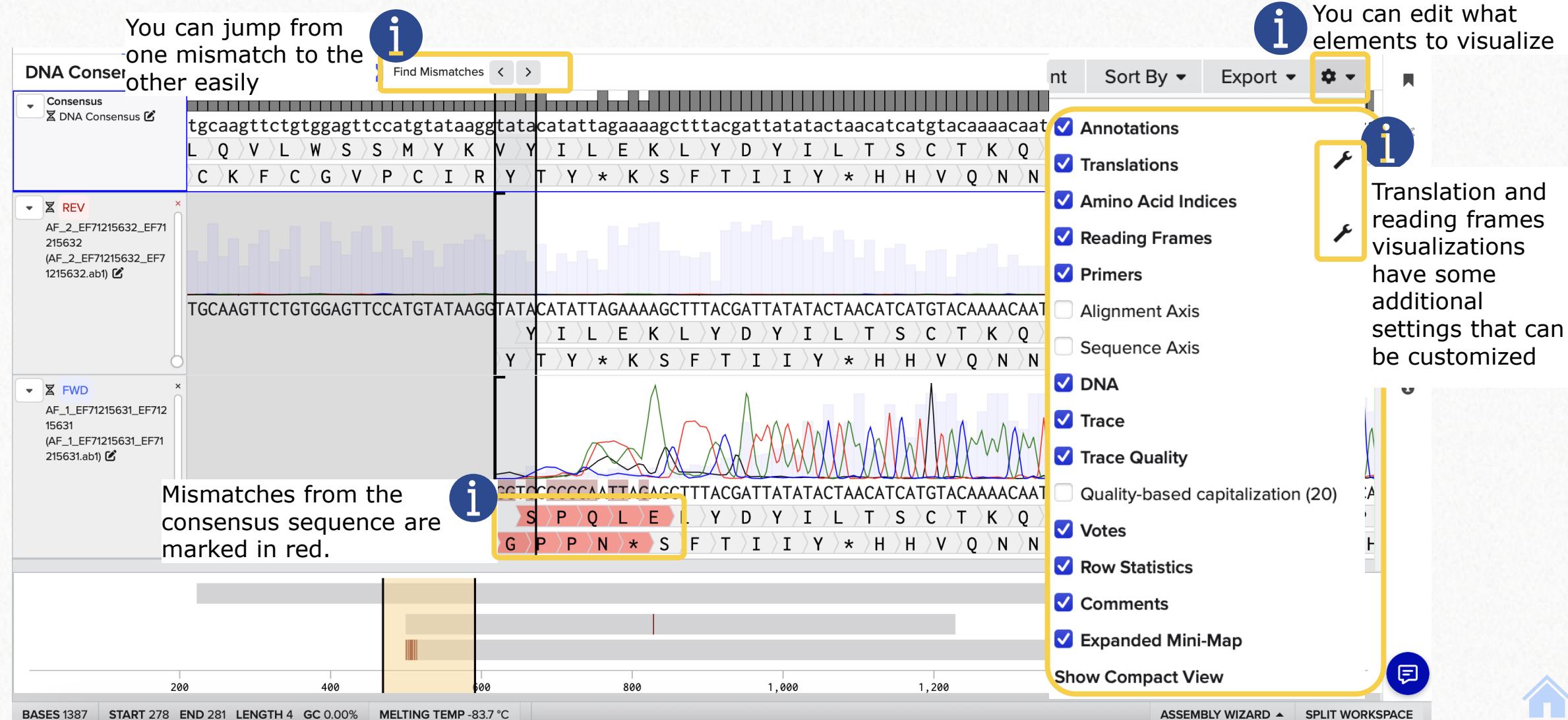
Alignment creation

Consensus alignment navigation



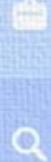
Alignment creation

Consensus alignment navigation



9. Tips and tricks





Tips and tricks

Overview:

- You can work in bulk using the expanded view of the workspace
- Re-indexing of sequences when creating alignments.
- Benchling [trouble-shooting articles](#) and [Help center](#) offers many resources, frequently asked questions and articles that can help you
 - Biosustain learning material: [Brilliant Basics: The Molecular Biology Suite - LIMS Help Guides](#)



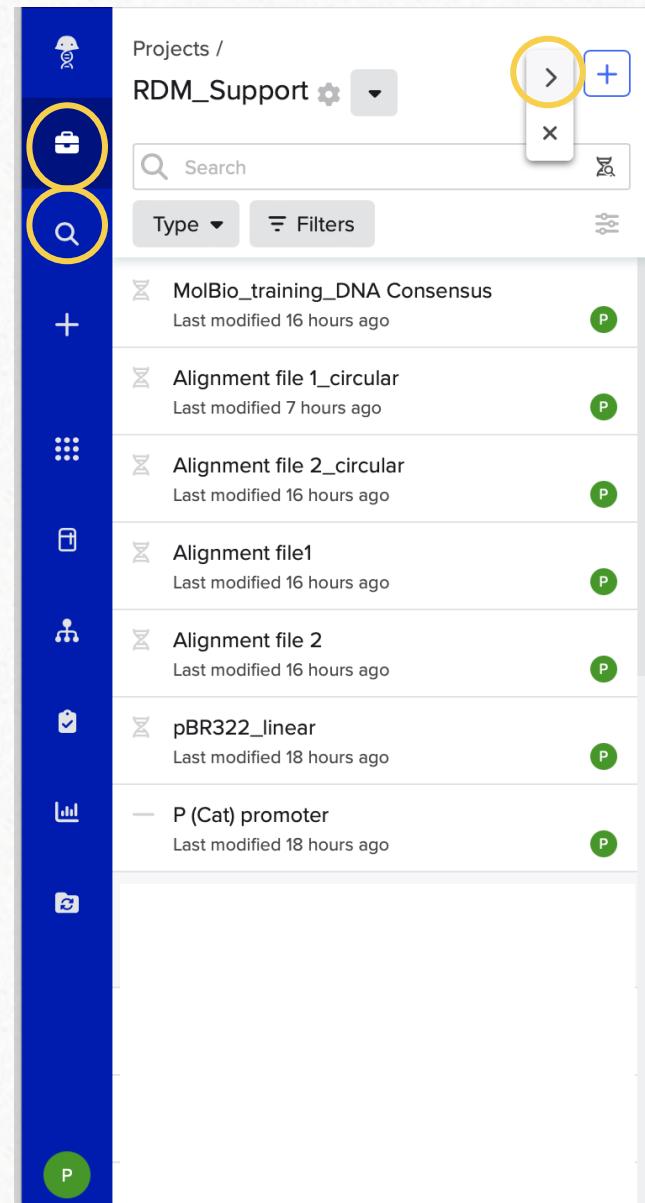
Tips and tricks

Work in bulk using the expanded view

You can use the **expanded view** of the workspace to:

- ✓ Edit, move, archive... entities in bulk
- ✓ Create Multi-sequence alignments, attach and detach primers, autofill annotations and transcriptions, auto annotate...

Pro TIP: if you access the expanded view from the search,  you will have access to all your entities, not only the ones contained in a particular project folder. Also, more filters will be available



Projects / RDM_Support

> + X

Search

Type ▾ Filters

MolBio_training_DNA Consensus
Last modified 16 hours ago

Alignment file 1_circular
Last modified 7 hours ago

Alignment file 2_circular
Last modified 16 hours ago

Alignment file1
Last modified 16 hours ago

Alignment file 2
Last modified 16 hours ago

pBR322_linear
Last modified 18 hours ago

P (Cat) promoter
Last modified 18 hours ago

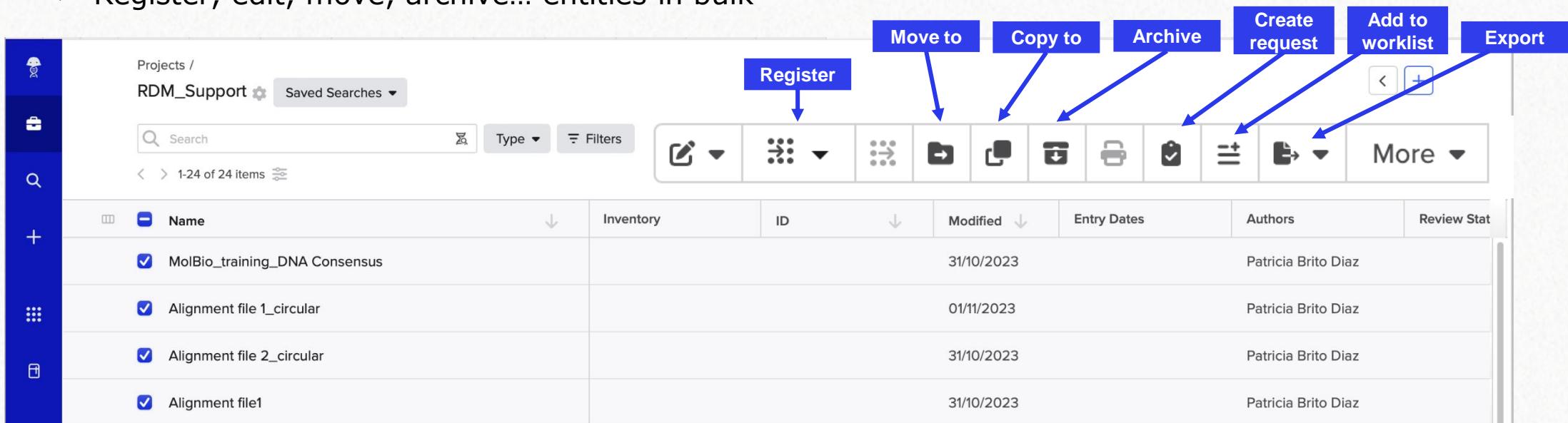


Tips and tricks

Work in bulk using the expanded view

You can use the **expanded view** of the workspace to:

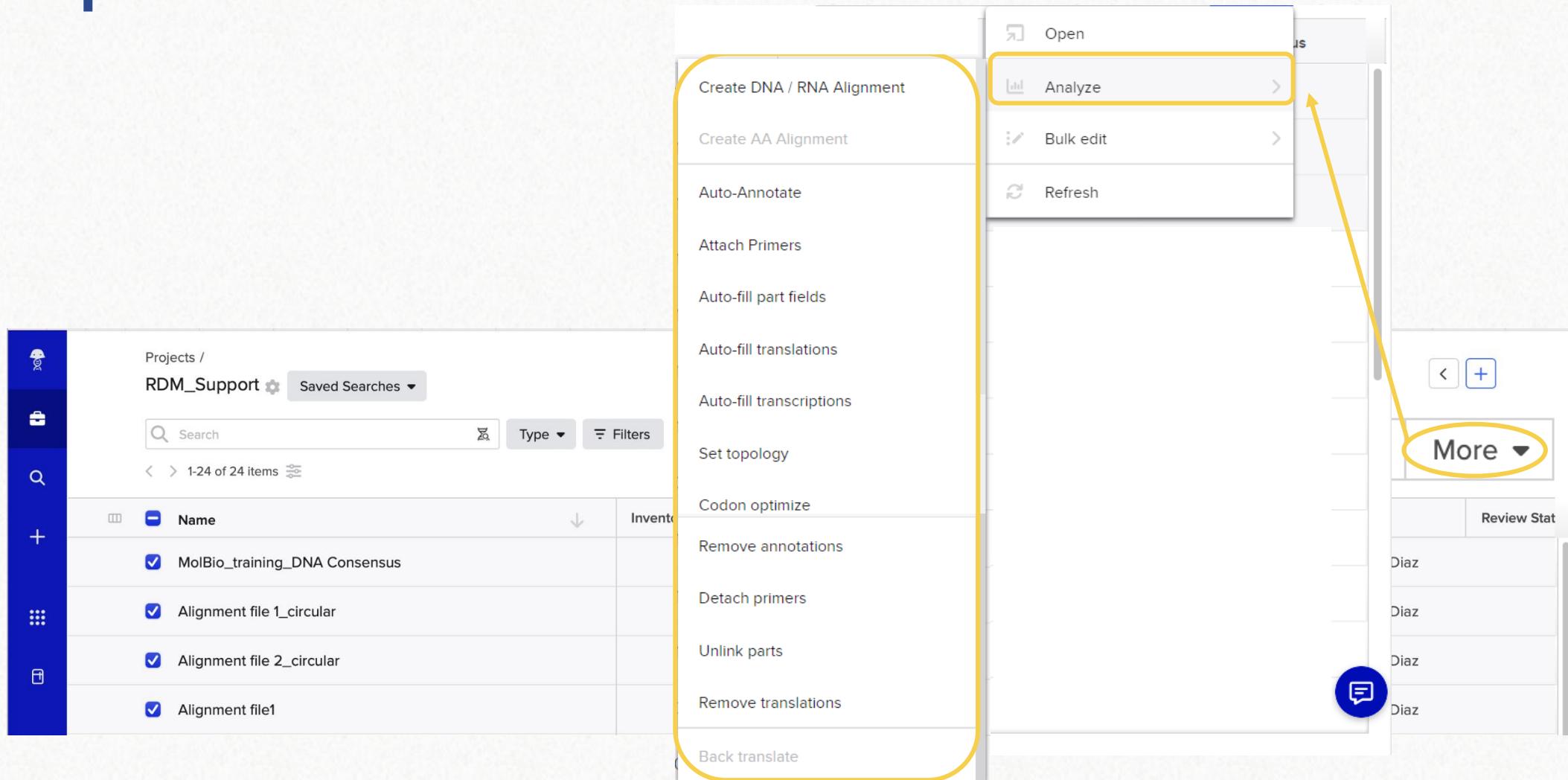
- ✓ Register, edit, move, archive... entities in bulk



The screenshot shows a workspace interface with a sidebar on the left containing icons for Projects, RDM_Support, Saved Searches, and various search/filter functions. The main area displays a list of 24 items, each with a checkbox and a name. The columns are labeled: Name, Inventory, ID, Modified, Entry Dates, Authors, and Review Stat. Above the list is a toolbar with several buttons: Register, Move to, Copy to, Archive, Create request, Add to worklist, and Export. Blue arrows point from the text labels 'Register', 'Move to', 'Copy to', 'Archive', 'Create request', 'Add to worklist', and 'Export' to their respective buttons in the toolbar.

Name	Inventory	ID	Modified	Entry Dates	Authors	Review Stat
MolBio_training_DNA Consensus			31/10/2023		Patricia Brito Diaz	
Alignment file 1_circular			01/11/2023		Patricia Brito Diaz	
Alignment file 2_circular			31/10/2023		Patricia Brito Diaz	
Alignment file1			31/10/2023		Patricia Brito Diaz	

Tips and tricks



The screenshot shows a software interface with a sidebar on the left containing various icons. The main area displays a list of items under the heading "Projects / RDM_Support". A context menu is open over the list, with several options highlighted by a yellow border:

- Create DNA / RNA Alignment
- Analyze (highlighted)
- Bulk edit
- Refresh

Below this menu, there is a long list of other options:

- Auto-Annotate
- Attach Primers
- Auto-fill part fields
- Auto-fill translations
- Auto-fill transcriptions
- Set topology
- Codon optimize
- Remove annotations
- Detach primers
- Unlink parts
- Remove translations
- Back translate

To the right of the main area, there is a "More" dropdown menu with a blue circle icon containing a white speech bubble. The dropdown contains the following items:

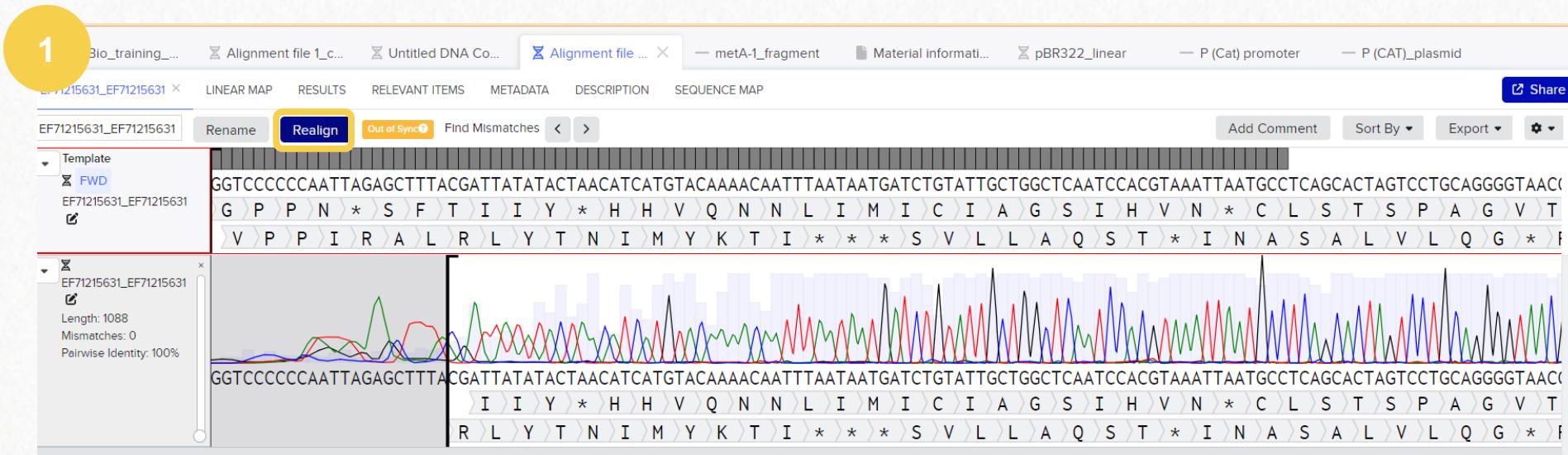
- < +
- Review Stat
- Diaz
- Diaz
- Diaz
- Diaz

Tips and tricks

Autoindexing when creating alignments

When creating an alignment of circular sequences, Benchling by default performs an **auto indexing** of these sequences.

To change this, after creating the alignment, you will have to realign the file and unmark the “automatically reindex” box.



Tips and tricks

Autoindexing when creating alignments

2 Realign DNA / RNA

1 Choose input 2 Define parameters

Upload sequence and trace files (.ab1, .ftv, fasta, .gb, and .genelous). RNA uploads are not currently supported.

Drag and drop to upload or Choose files

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA RNA

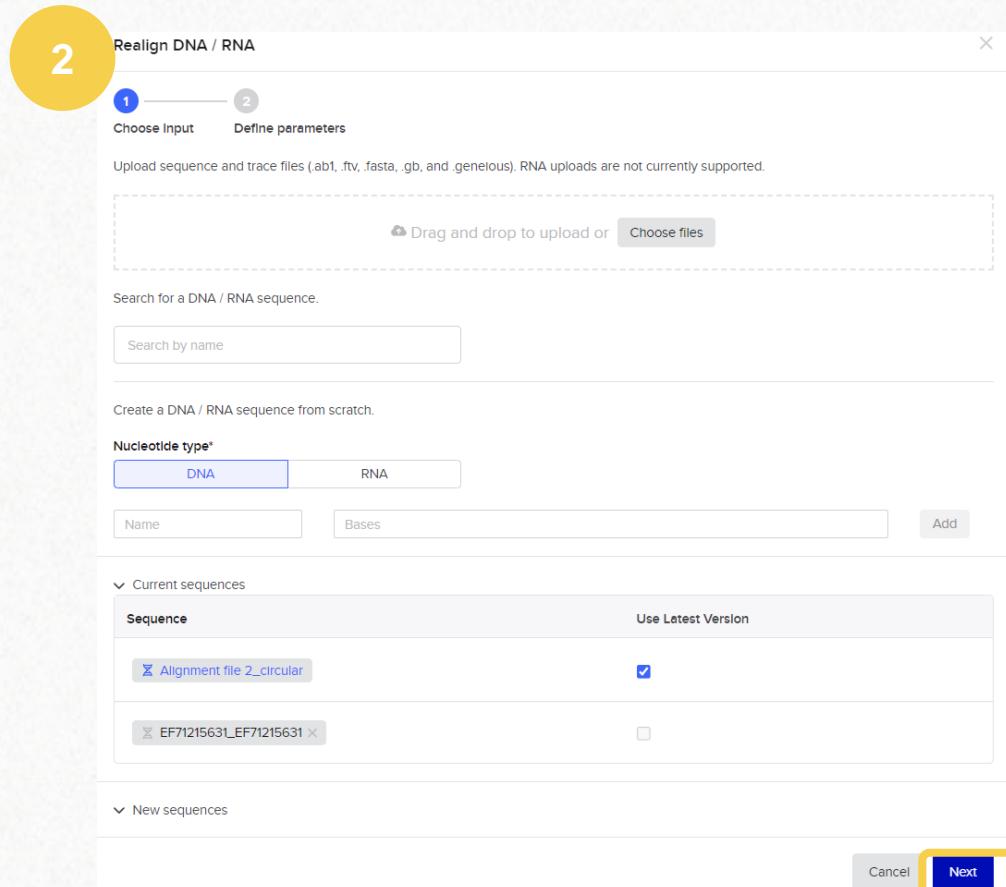
Name Bases Add

Current sequences

Sequence	Use Latest Version
Alignment file 2_circular	<input checked="" type="checkbox"/>
EF71215631_EF71215631	<input type="checkbox"/>

New sequences

Cancel Next



3 Realign DNA / RNA

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

Your realignment must be the same type as your original alignment.

Multisequence Alignment - The results will be attached as a single alignment on the template sequence. Show details▼

Template(s) Non-template sequence(s)

Alignment file 2_circular EF71215631_EF71215631 Search

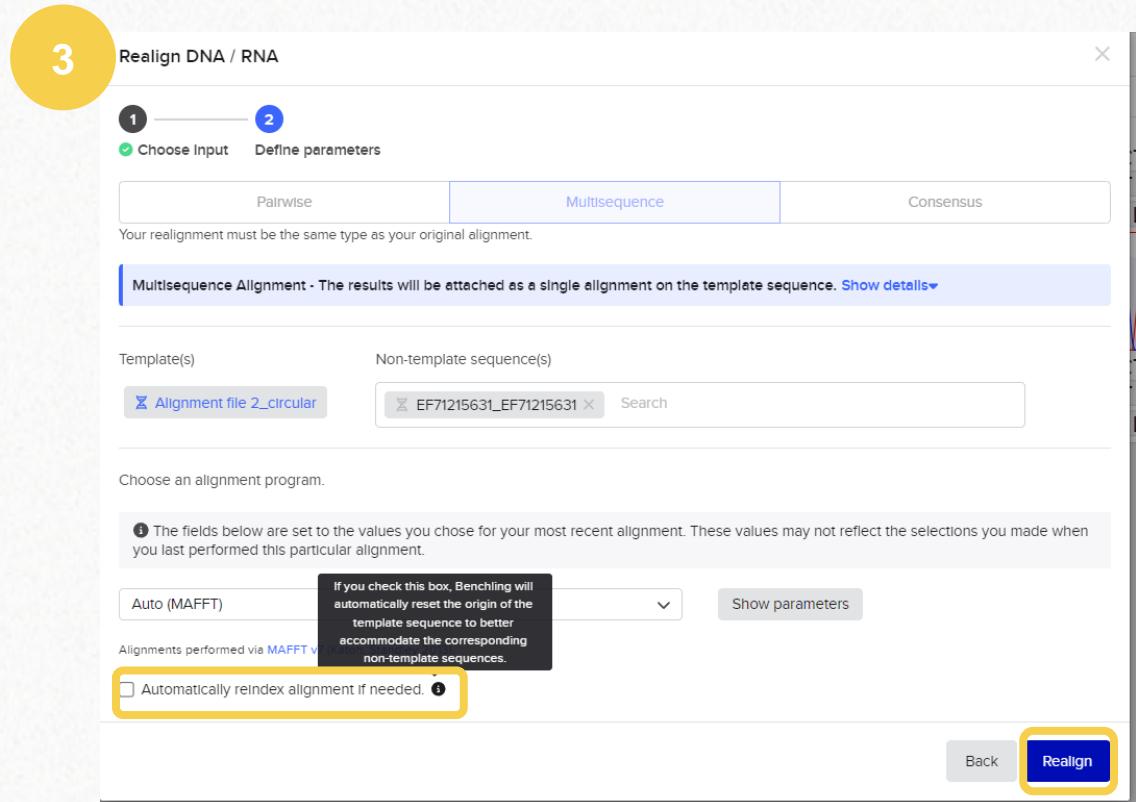
Choose an alignment program.

The fields below are set to the values you chose for your most recent alignment. These values may not reflect the selections you made when you last performed this particular alignment.

Auto (MAFFT) If you check this box, Benchling will automatically reset the origin of the template sequence to better accommodate the corresponding non-template sequences.

Automatically reindex alignment if needed.

Back Realign



Tips and tricks

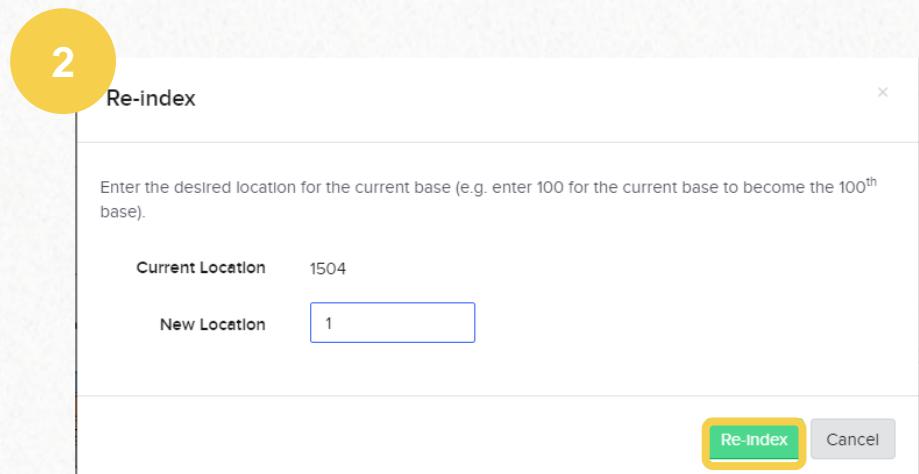
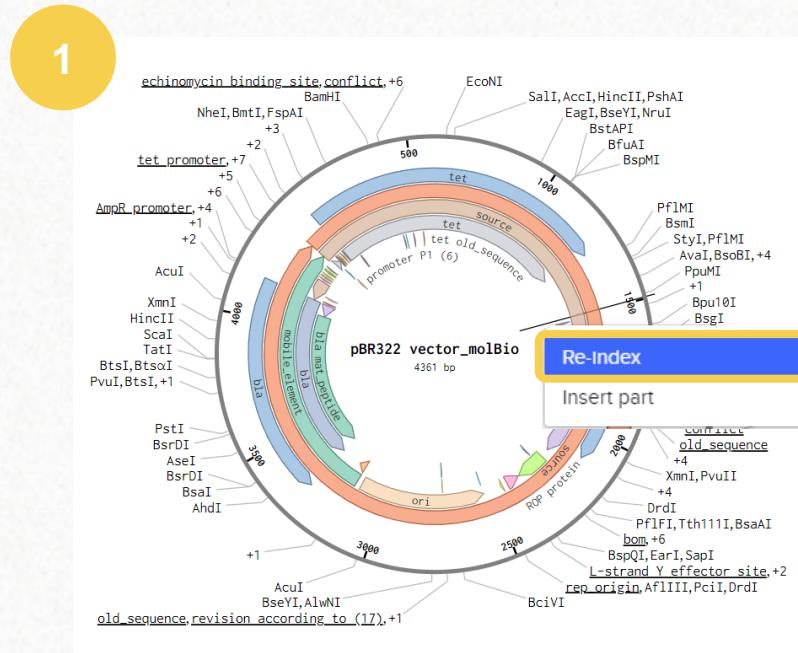
Autoindexing when creating alignments

When creating an alignment of circular sequences, Benchling by default performs an **auto indexing** of these sequences.

To change this, after creating the alignment, you will have to realign the file and unmark the “automatically reindex” box.

Pro TIP:

- ✓ You can always re-index a circular plasmid by right-clicking on any part of the sequence. For linear sequences, the index can be changed using the “information” tab on the right panel. 
- ✓ Make sure to have your sequences correctly indexed before performing an alignment to avoid further complications.



10. Resources





Questions?



Contact lims_support@biosustain.dtu.dk

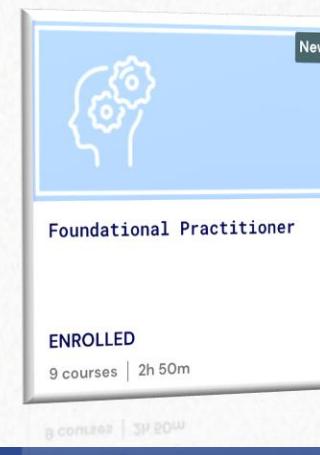


More resources

Benchling Learning Labs

Benchling provides a **learning platform** that offers role-specific courses that can be taken in a **flexible**-pace structure.

<https://www.benchling.com/learning-labs>



Welcome to Benchling Learning Labs!

The destination to achieve your Benchling learning goals

[Course Catalog](#) [Get Certified](#) [Email Us](#)

	
Practitioner	Administrator
Essential skills for all Benchling R&D Cloud users, covering core applications and best practices.	Additional training for Benchling Administrators, covering roles, permissions, configurations, and more.
	
Developer	Consultant
Specialized training covering Developer Platform fundamentals such as APIs, Events, and more.	Additional training for consulting partners covering the Benchling Implementation Methodology.

More resources

Benchling Help Center

Benchling provides some short guides on main functionalities

<https://help.benchling.com/hc/en-us>

