

<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	15/04/2021	4°C Fridge
4C009	4C Fridge 01223	4°C Fridge	15/04/2021	4°C Fridge
4C001	4C Fridge 1233	4°C Fridge	15/04/2021	4°C Fridge
4C014	4C Fridge 01871	4°C Fridge	15/04/2021	4°C Fridge
4C015	4C Fridge Aaron	4°C Fridge	15/04/2021	4°C Fridge
4C016	4C Fridge Adam	4°C Fridge	15/04/2021	4°C Fridge
4C005	4C Fridge ANALYTICS	4°C Fridge	15/04/2021	4°C Fridge
4C011	4C Fridge CFB00266	4°C Fridge	15/04/2021	4°C Fridge
CFB01478	4C Fridge CFB01478	4°C Fridge	15/04/2021	4°C Fridge
CFB01653	4C Fridge CFB01653	4°C Fridge	15/04/2021	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](#)

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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

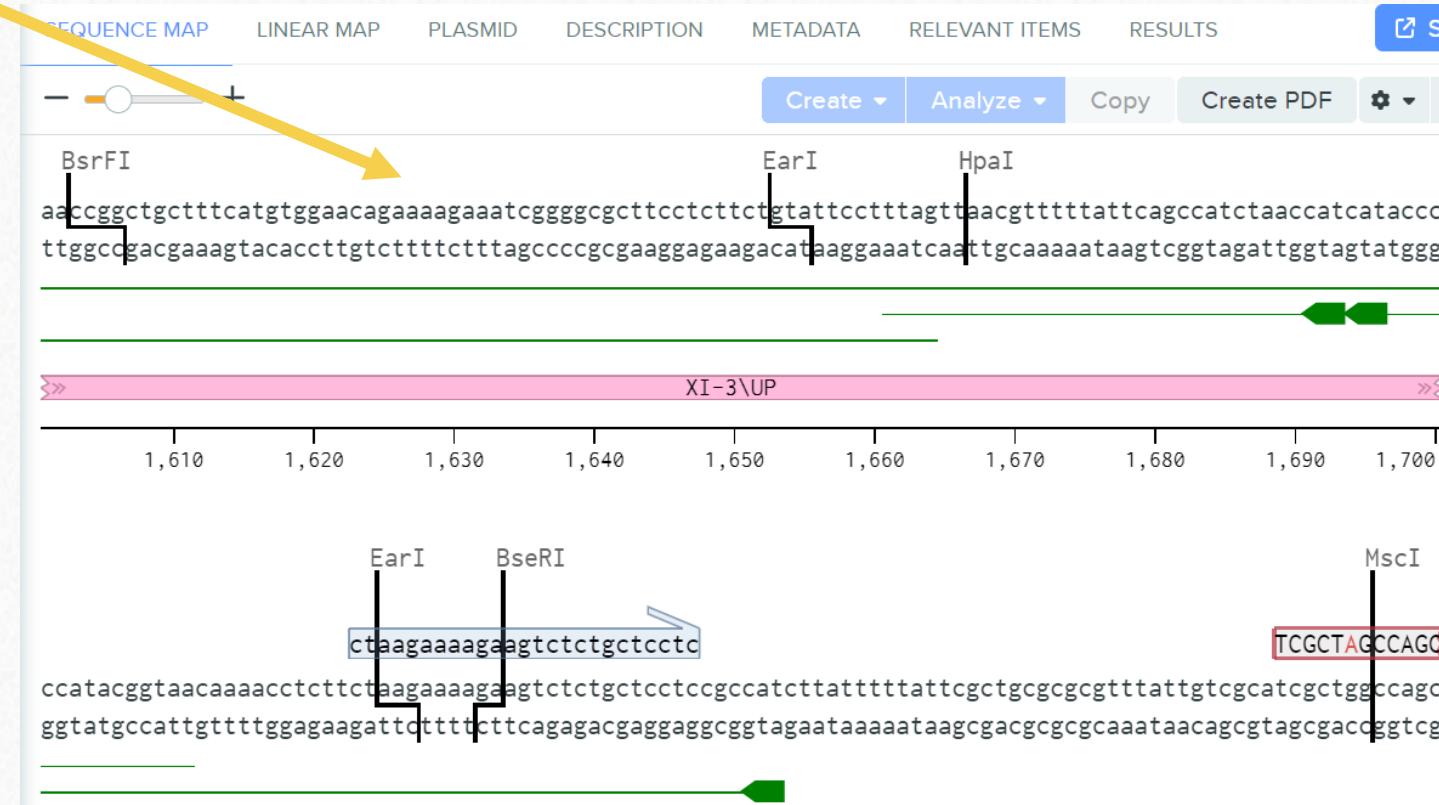
CRISPR

- ✓ 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

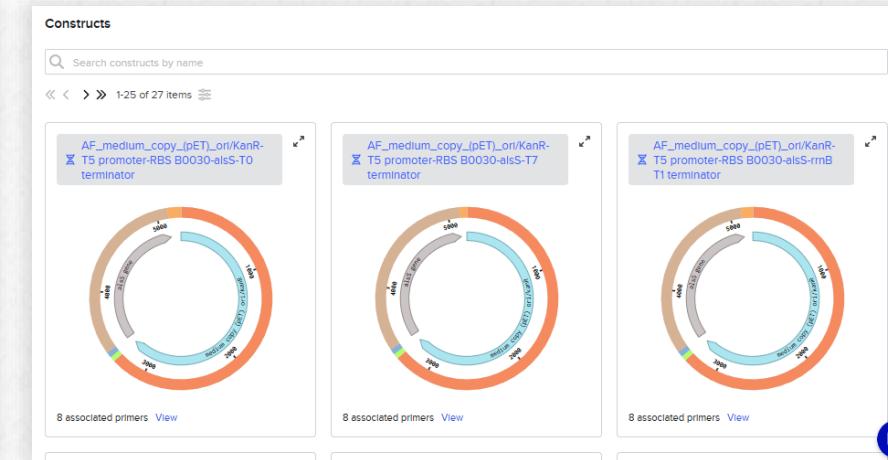
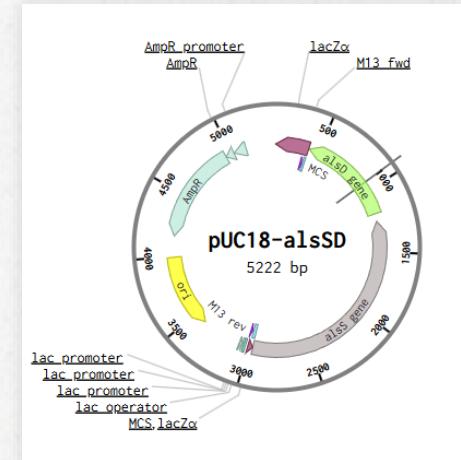
Information (topology, tags)

2. Training overview



Training goals:

The basics of...



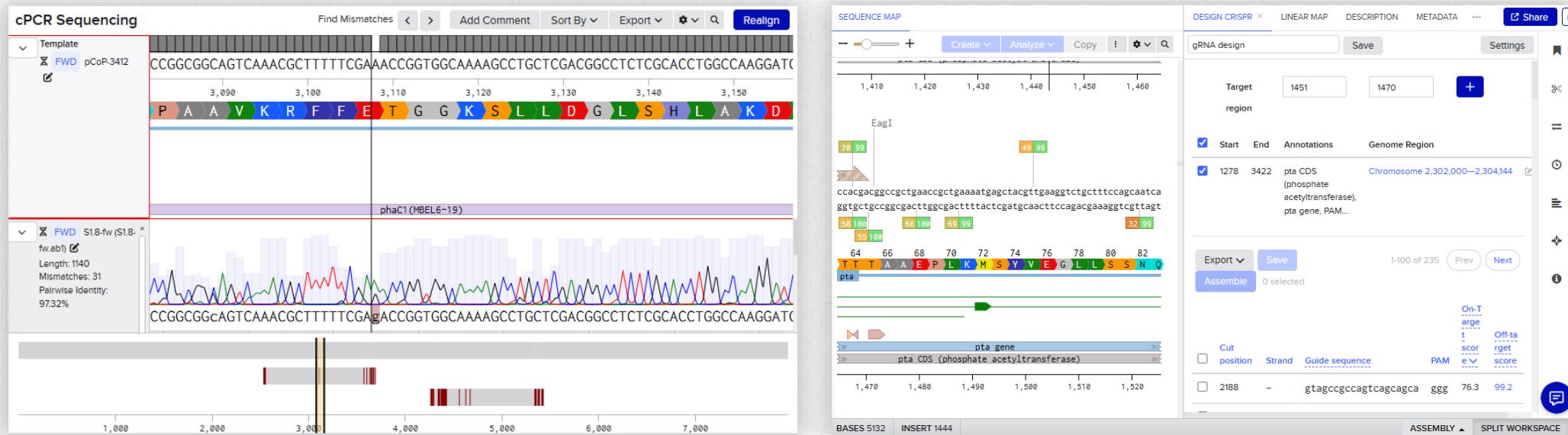
The 'Constructs' search interface shows three examples of assembled constructs. Each example is a circular diagram with a grey outer ring and an orange inner ring, representing a plasmid. The constructs are labeled: AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-T7 terminator, AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-T7 terminator, and AF_medium_copy_(pET)_ori/KanR-T5 promoter-RBS B0030-alsS-rmB T1 terminator. Each construct has a 'View' button below it.

- 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

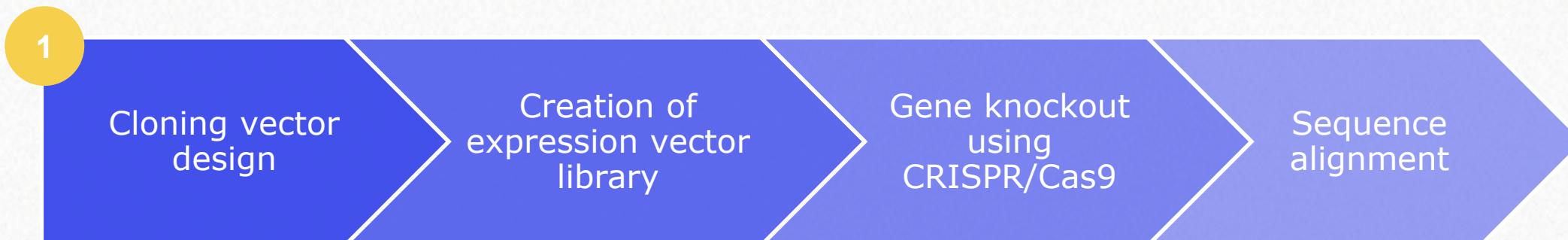
Chan-Hsiang Hsu, Sefli Sri Wahyu Effendi, Wan-Wen Ting, Yu-Hsiu Li, I-Son Ng *

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

[Link to article](#)

Today's work example:

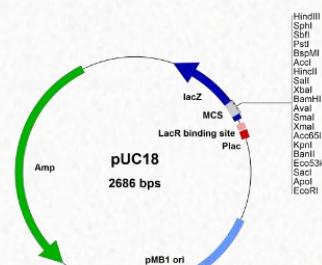
Hypothetical scenario: **Production of acetoin in *E. coli***



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



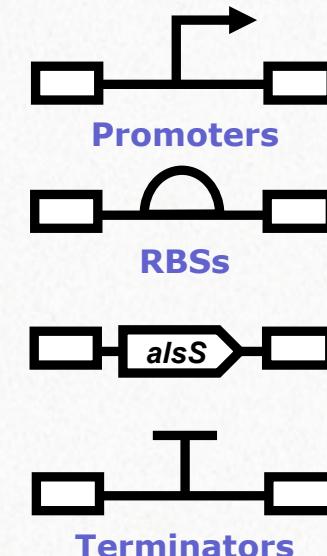
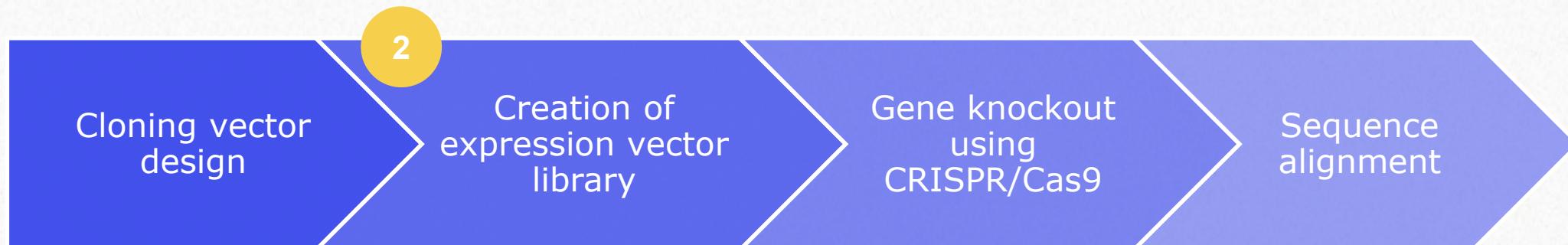
pUC18 cloning vector



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

Today's work example:

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



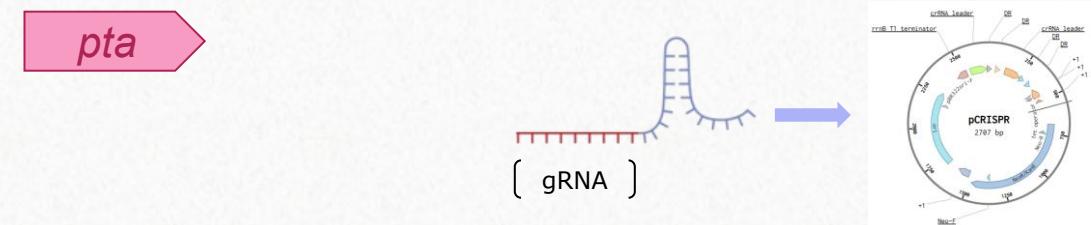
- ✓ Combinatorial cloning:
Golden Gate

Today's work example:

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

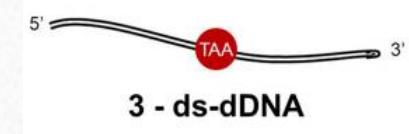


Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR



- ✓ gRNA design
- ✓ HR template design

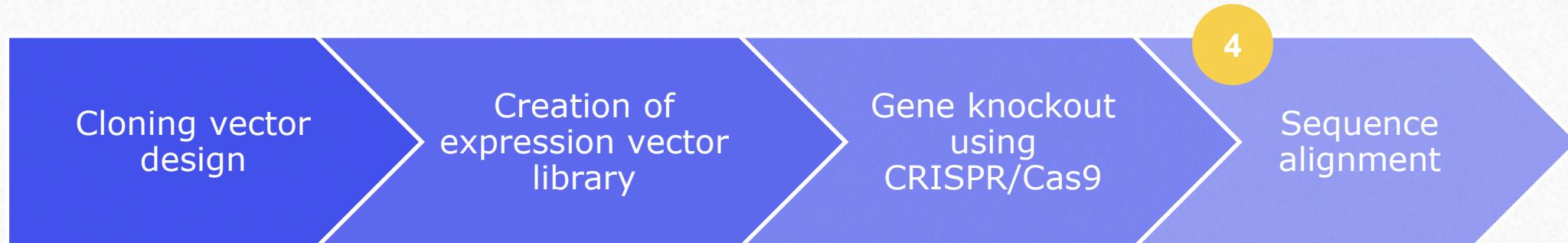
HR template design to KO *pta*



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

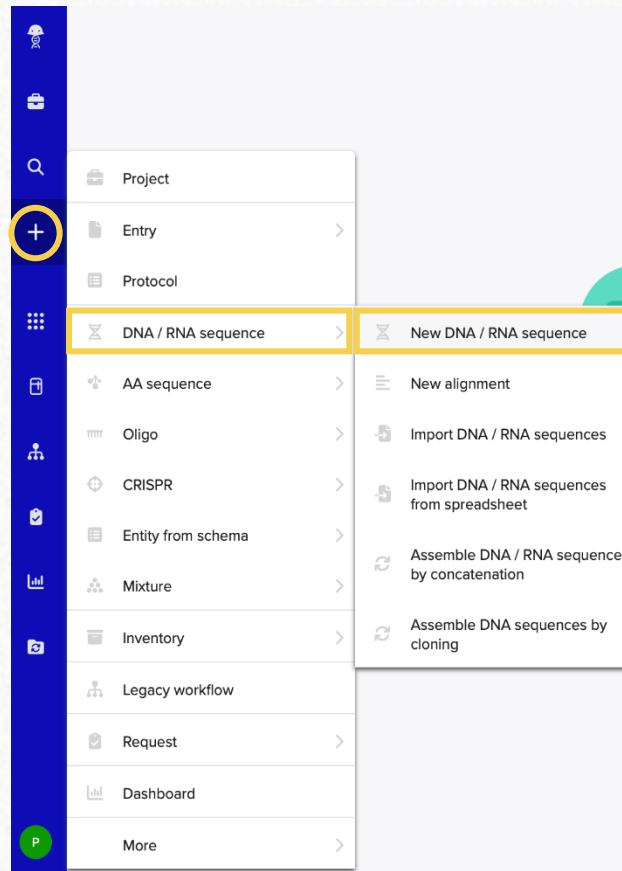
3.1 Sequence creation and import



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

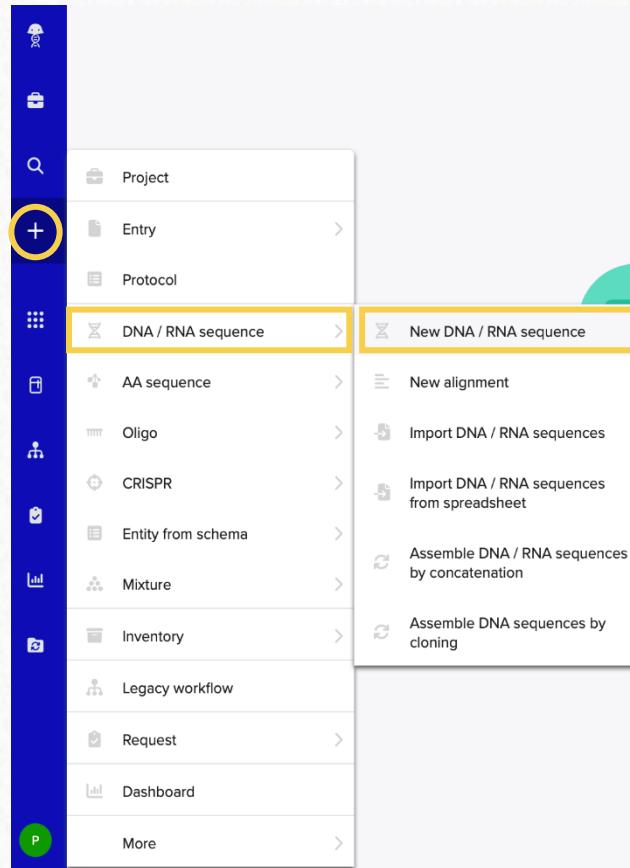
i You can leave the **Bases** field **empty** and add your sequence later. This can be useful if you wish to copy and paste a sequence with its annotations.

Create

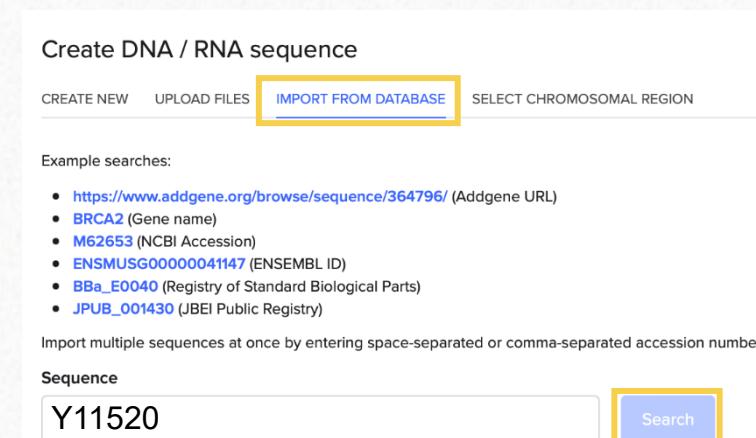
Create and import a sequence

How to import of sequences from a database

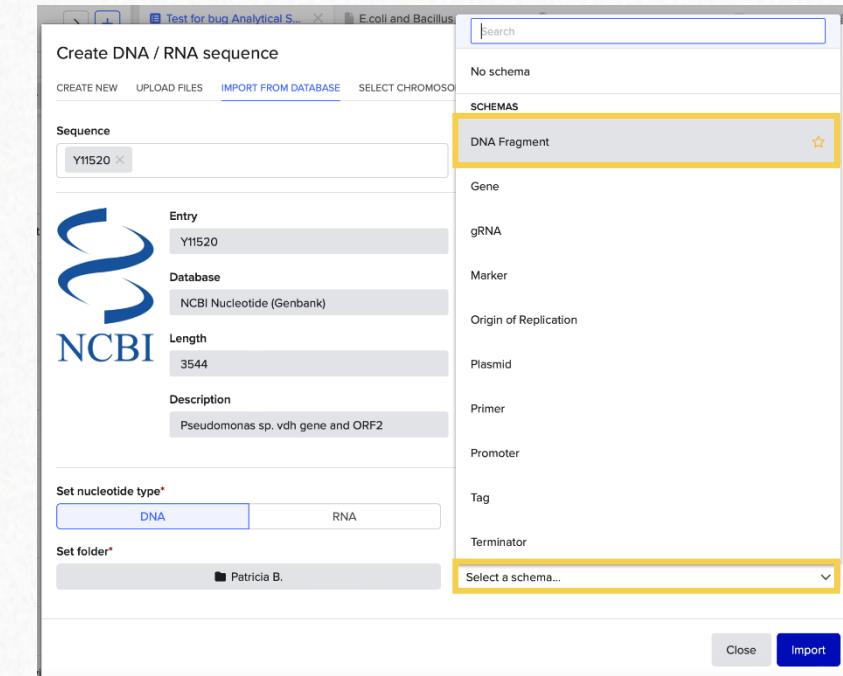
1 Create a new DNA sequence



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 box. At the top, there are tabs: CREATE NEW, UPLOAD FILES, IMPORT FROM DATABASE (which is highlighted with a yellow box), and SELECT CHROMOSOMAL REGION. Below the tabs, there is a section titled 'Example searches:' with a list of items: '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 'JPUB_001430 (JBEI Public Registry)'. Below this is a 'Sequence' input field containing 'Y11520' and a 'Search' button. A note below says 'Import multiple sequences at once by entering space-separated or comma-separated accession numbers.'

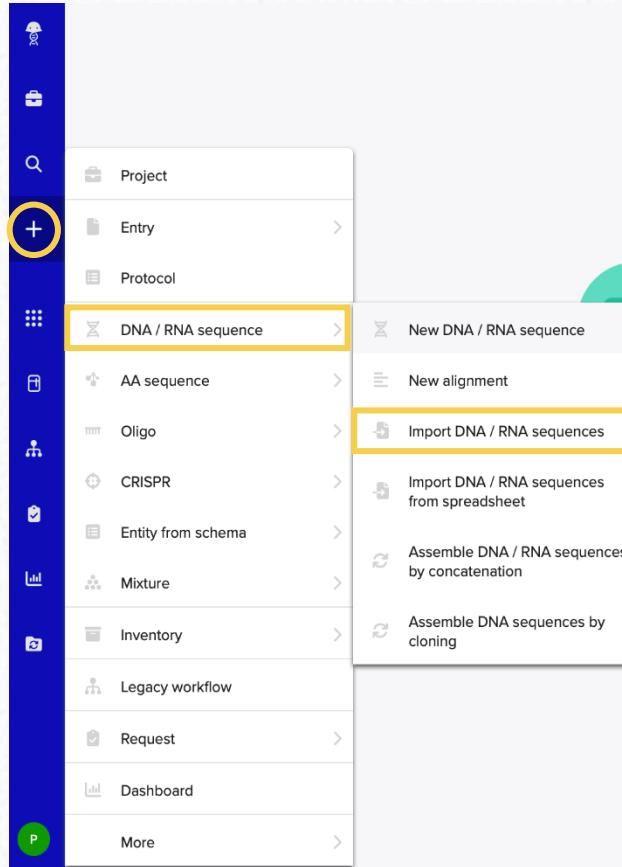
3 If the ID is valid, Benchling will show you the gene's description. You can set its schema and import it.

A screenshot of the 'Create DNA / RNA sequence' dialog box showing the results of the search. 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*' dropdown, 'Patricia B.' is listed. On the right, a sidebar titled 'SCHEMAS' shows a list of options: No schema (selected), DNA Fragment (highlighted with a yellow box), Gene, gRNA, Marker, Origin of Replication, Plasmid, Primer, Promoter, Tag, and Terminator. At the bottom right are 'Close' and 'Import' buttons.

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.

The screenshot shows the 'Create DNA / RNA sequence' dialog box. The 'UPLOAD FILES' tab is selected. The 'Nucleotide type*' dropdown is set to 'DNA'. In the 'Project folder' section, 'Mia' is selected. Below, there's a dashed box for dragging files or a 'Choose a file' button. Two files are listed: 'GFP.dna' and 'pUC18.dna', both with 'UPLOAD DONE' status. The bottom right has a 'Close' button.

- 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](#).

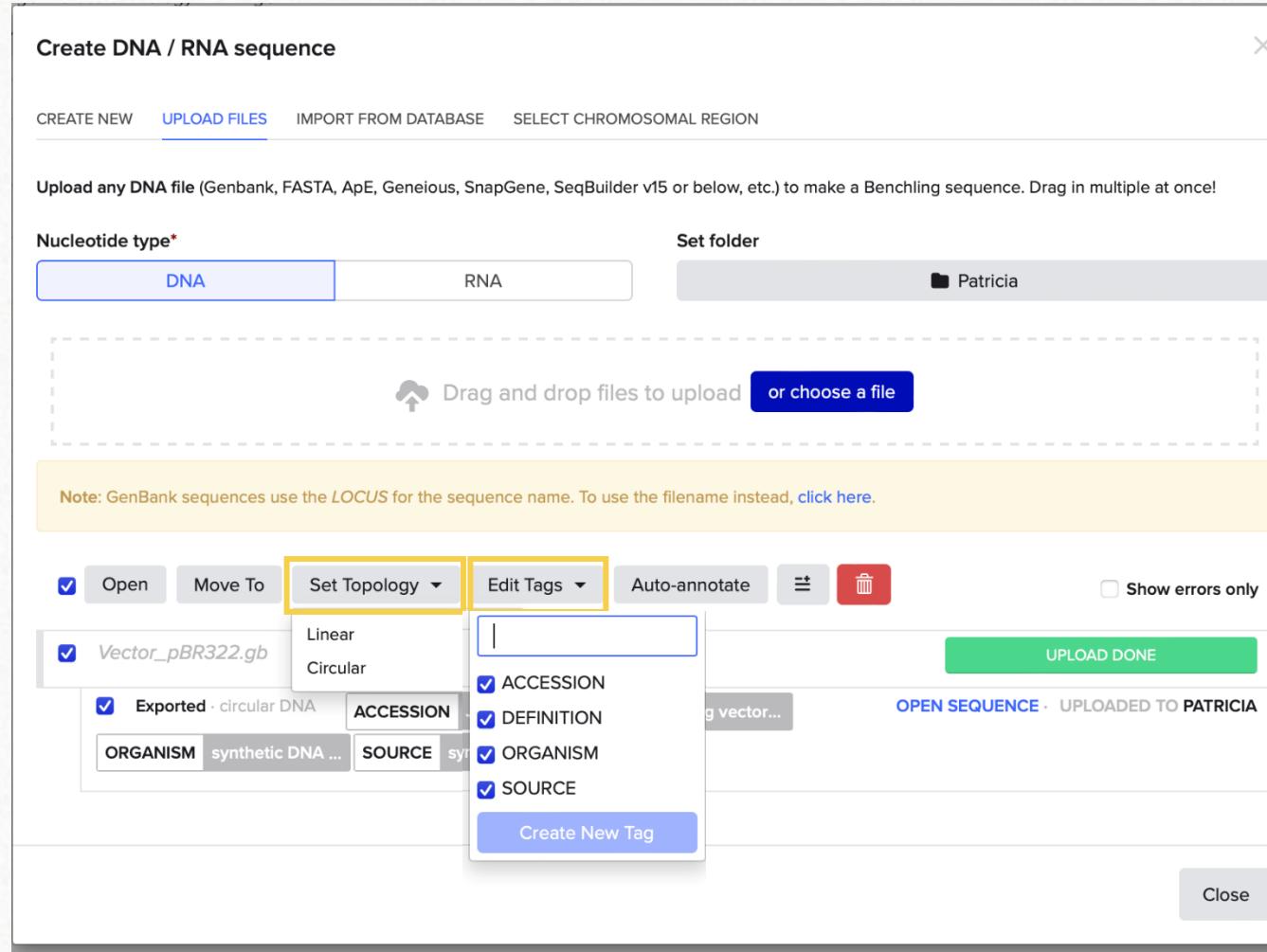
Open Move To **Set Topology** Edit Tags Auto-annotate Show errors only

Vector_pBR322.gb Set Topology Linear Circular Exported - circular DNA ACCESSION ORGANISM SOURCE Create New Tag

ACCESSION DEFINITION ORGANISM SOURCE

UPLOAD DONE OPEN SEQUENCE · UPLOADED TO PATRICIA

Close



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

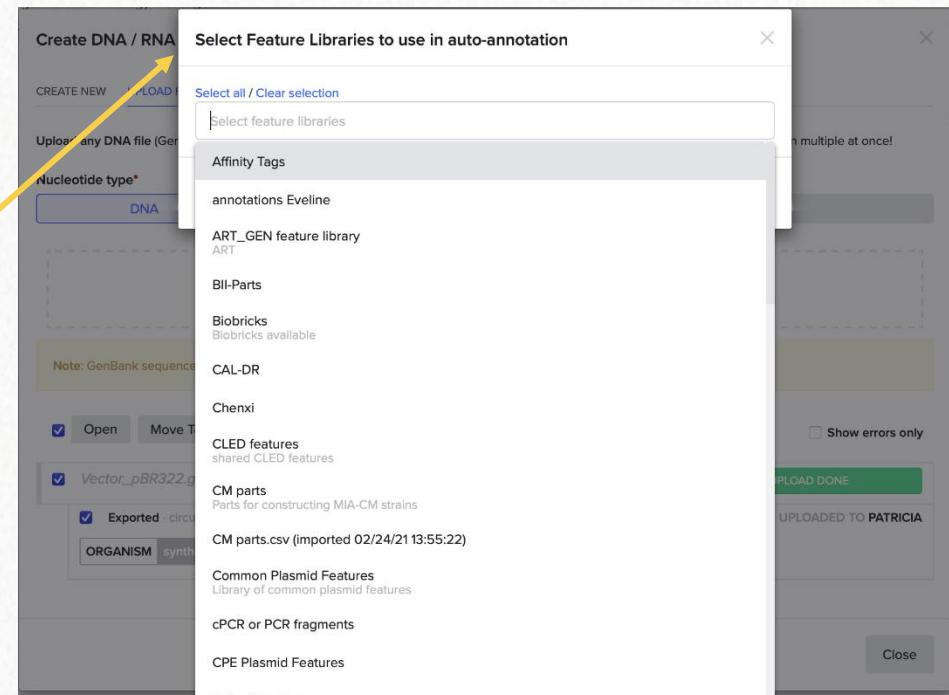
Vector_pBR322.gb

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

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](#).

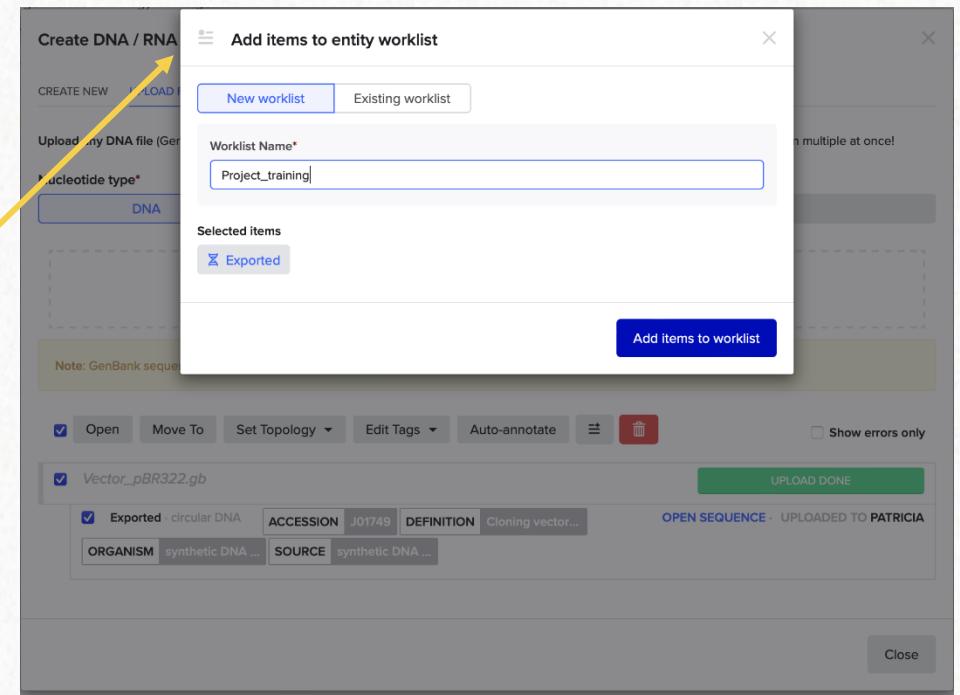
Open Move To Set Topology Edit Tags Auto-annotate  

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



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

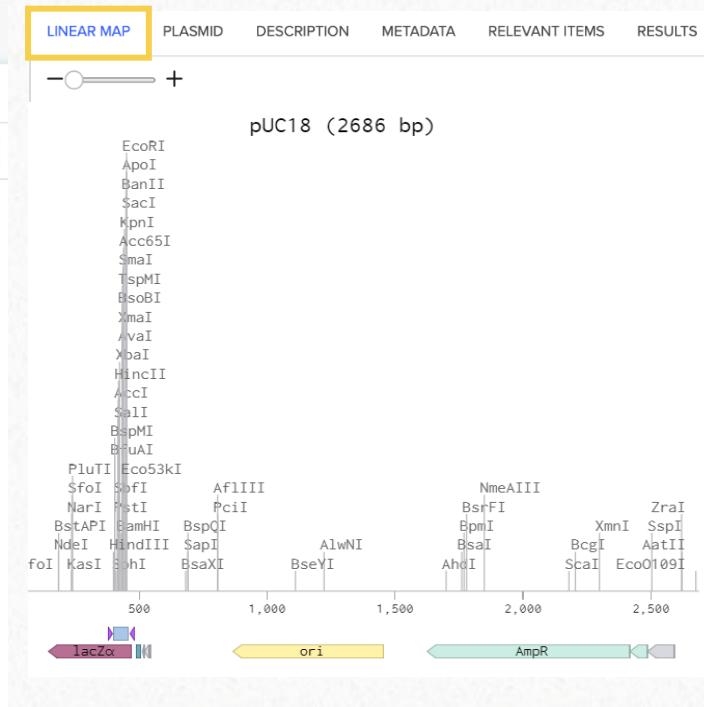
3. The basics of sequences

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:

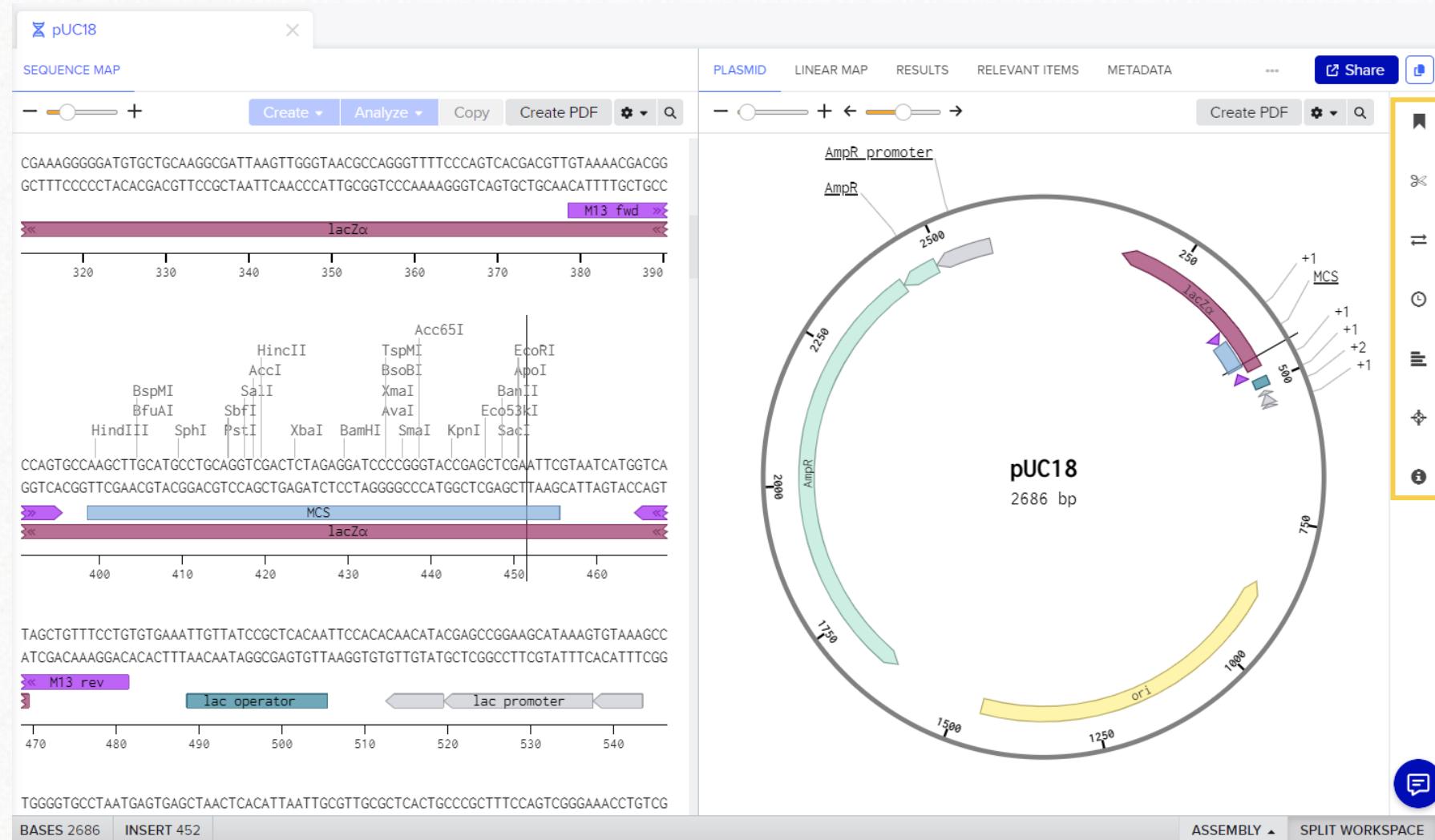


PRO TIP:

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

View, annotate and edit your sequences

Sequence navigation:

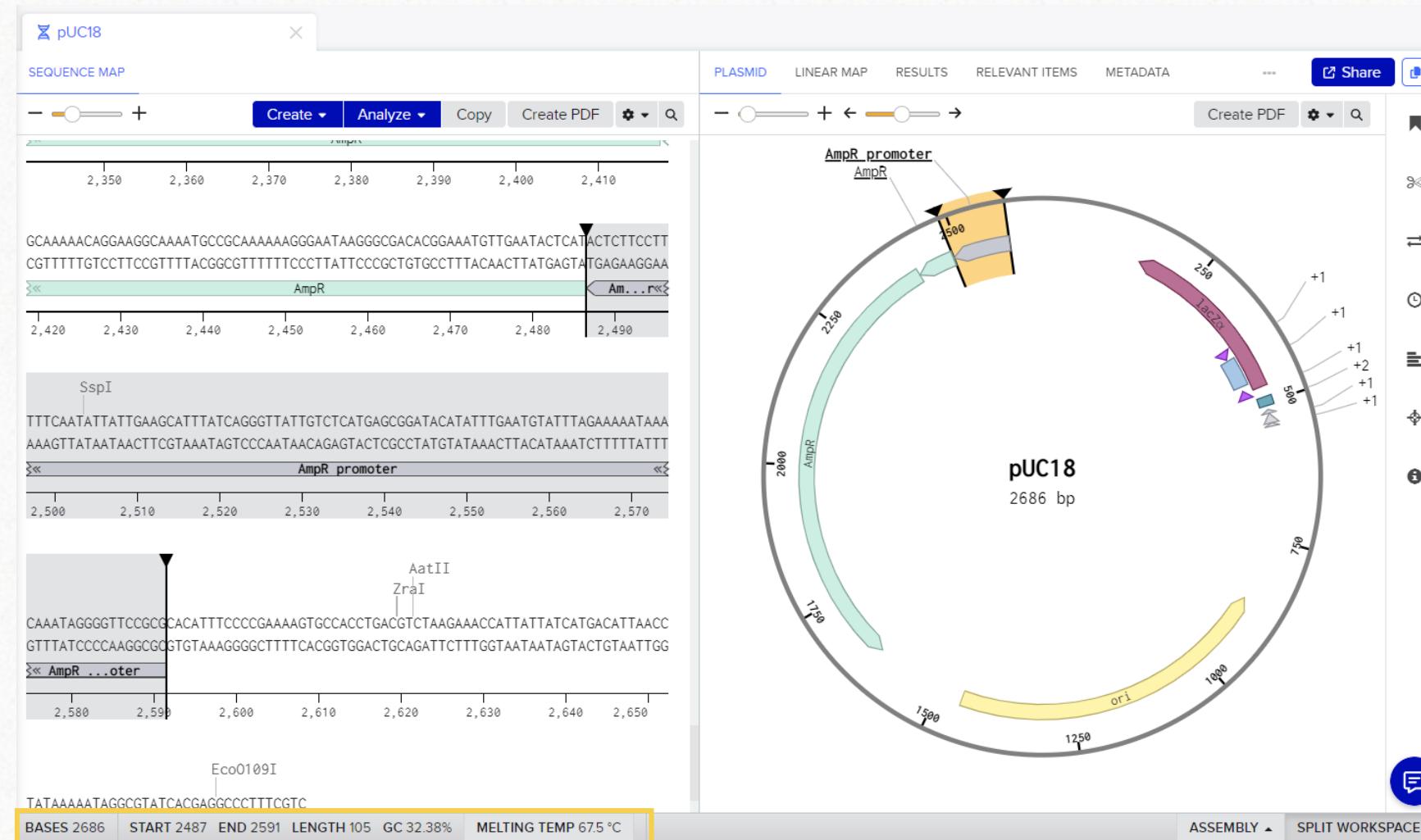


Functionalities

- 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:

The screenshot shows a sequence viewer interface with a "SEQUENCE MAP" tab selected. At the top, there are buttons for "Create" and "Analyze". A context menu is open over a sequence fragment, with "Analyze" highlighted. The menu includes options like "Annotation", "Run Primer3", "Run Benchling BLAST", "Submit to NCBI BLAST", "Analyze as translation", and "Optimize codons". Below the menu, the sequence is shown with several restriction enzyme sites (BstAPI, NdeI, PluTI, SfoI, NarI, KasI) and a "lacZα" marker. The sequence is divided into fragments with labels like "M13 fwd" and "Acc65I". At the bottom, sequence statistics are provided: BASES 2686, START 146, END 469, LENGTH 324, GC 55.25%, and 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

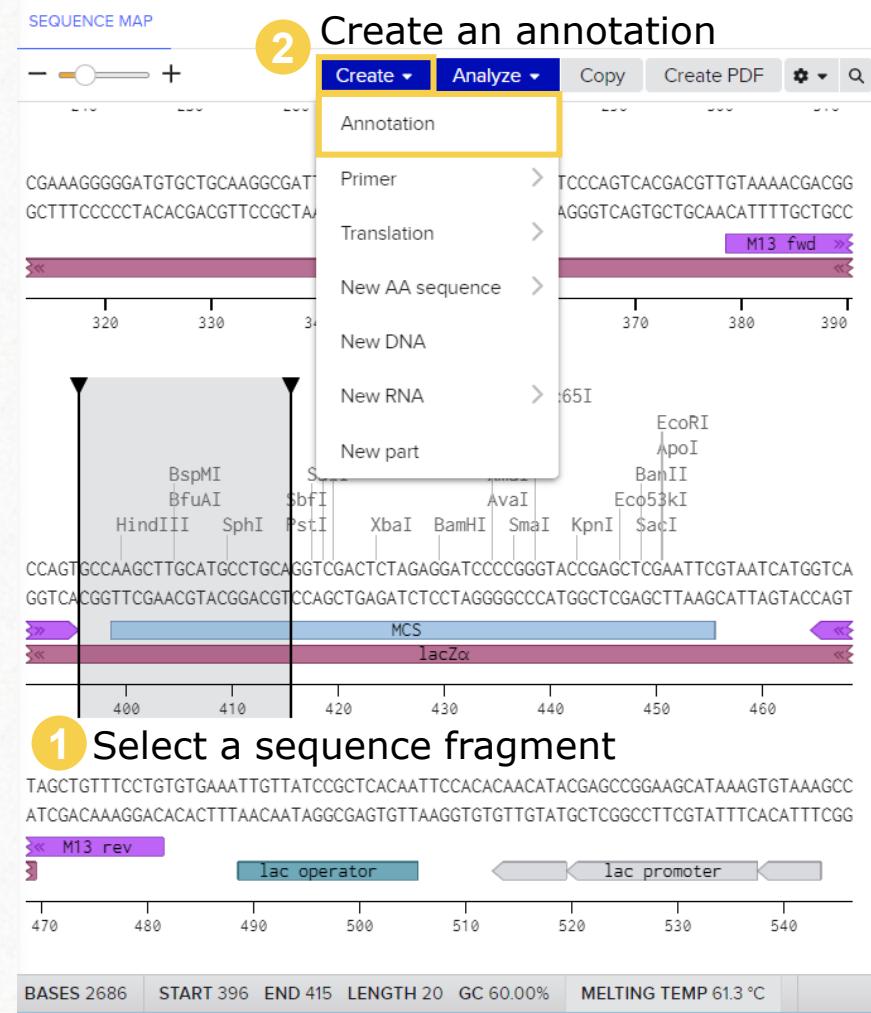


View, annotate and edit your sequences

Sequence annotations

1 Select a sequence fragment

SEQUENCE MAP



Bases: 2686 | Start: 396 | End: 415 | Length: 20 | GC: 60.00% | Melting Temp: 61.3 °C

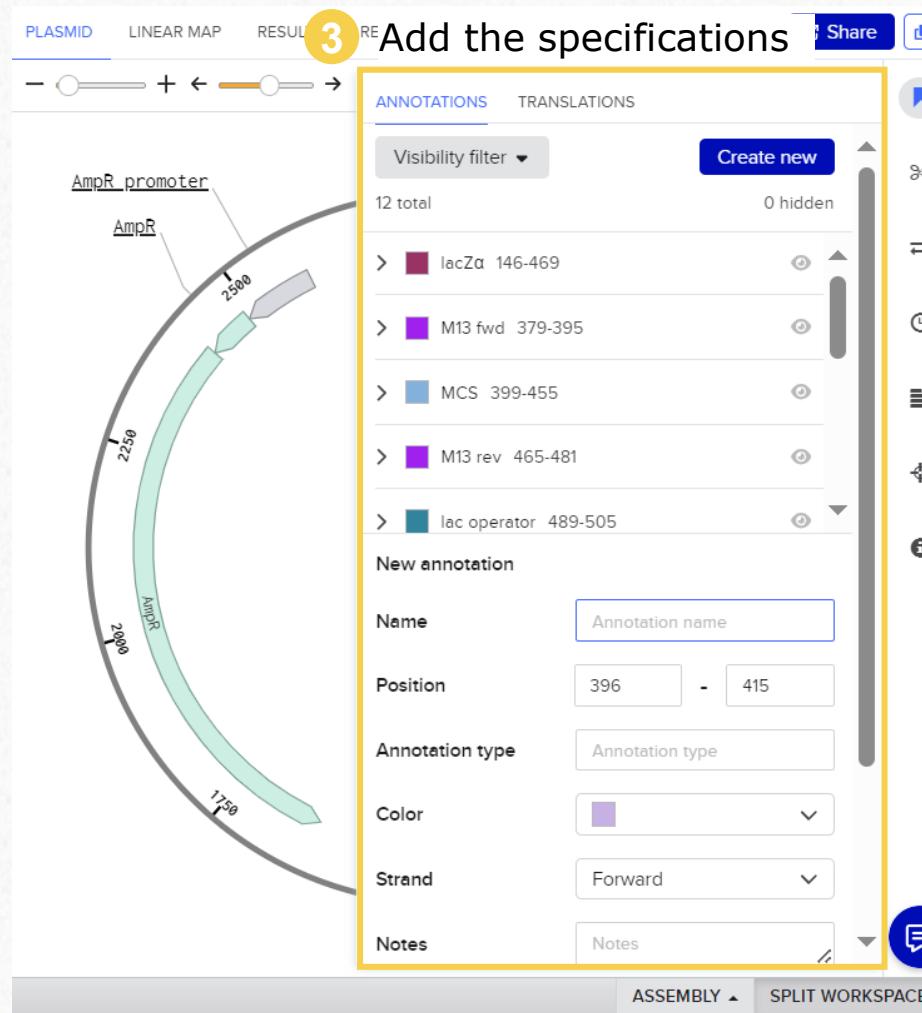
2 Create an annotation

Create ▾ Analyze ▾

- Annotation (highlighted)
- Primer
- Translation
- New AA sequence
- New DNA
- New RNA
- New part

3 Add the specifications

PLASMID LINEAR MAP RESULT Share



Annotations Translations

Visibility filter Create new

12 total 0 hidden

- AmpR promoter
- AmpR
- 250°
- 225°
- 200°
- 175°
- 150°
- 125°
- 100°
- 75°
- 50°
- 25°

New annotation

Name: Annotation name

Position: 396 - 415

Annotation type: Annotation type

Color: Purple

Strand: Forward

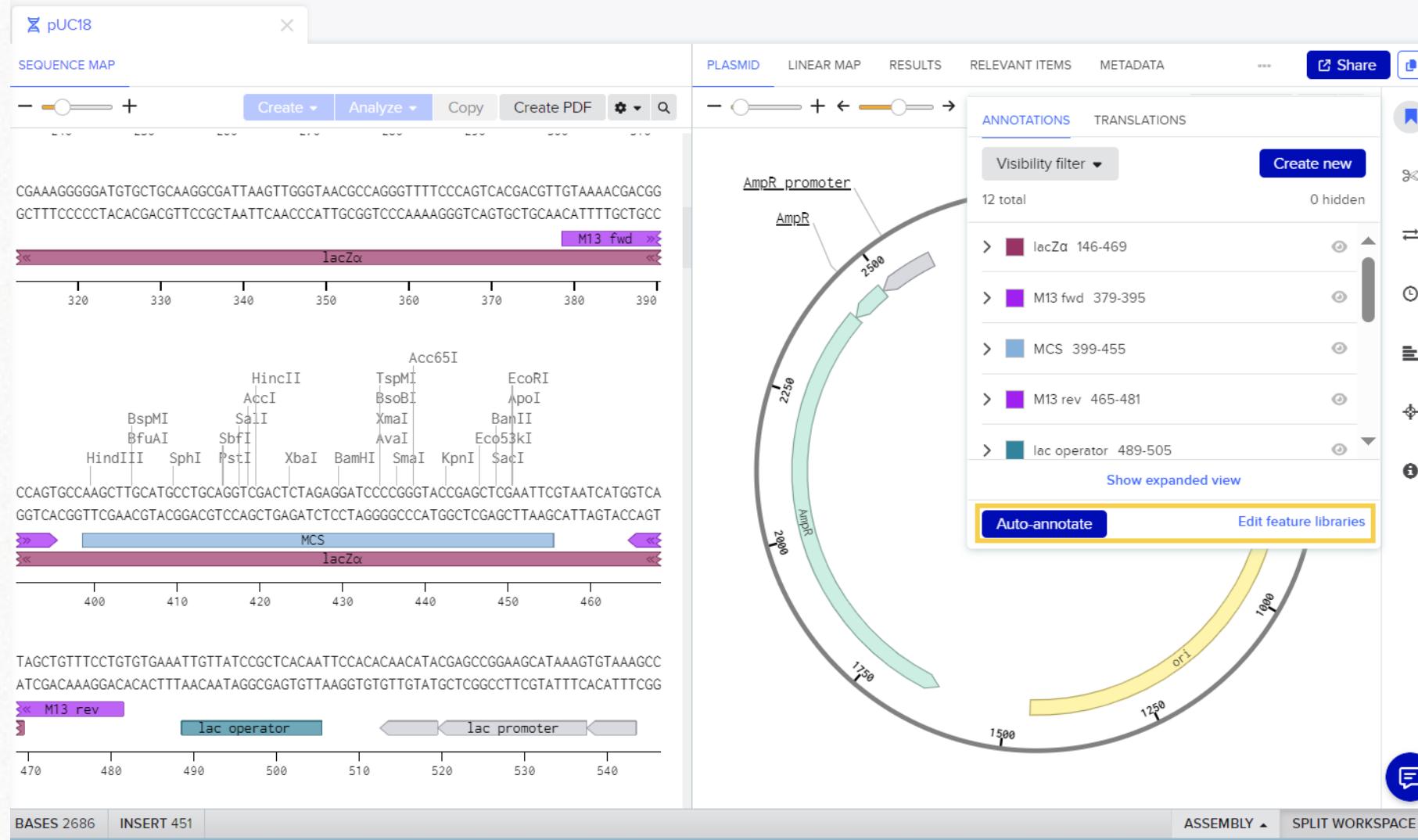
Notes: Notes

ASSEMBLY ▾ SPLIT WORKSPACE

- ✓ 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

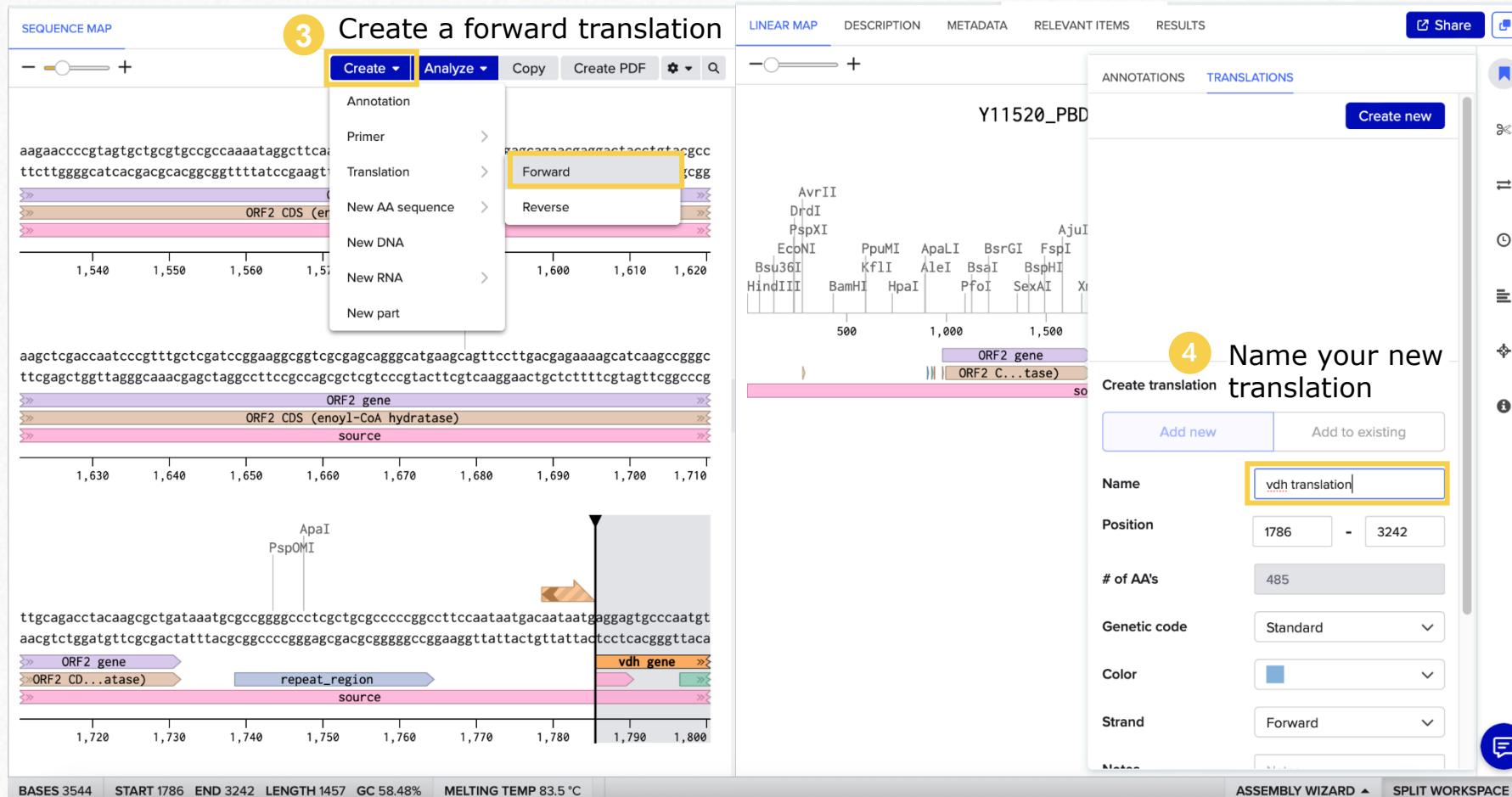
3.4 Codon optimization



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 starting at position 1,600. The bottom strand has an ORF2 gene and a repeat region. A linear map on the right shows restriction sites like AvrII, DrdI, PspXI, EcoNI, PpuMI, ApaI, BsrGI, FspI, Bsu36I, KfII, HpaI, AleI, BsAI, BspHI, HindIII, BamHI, and XbaI. Annotations include 'Y11520_PBD', 'ORF2 gene', 'repeat_region', and 'vdh gene'. A central panel shows the sequence from 1,540 to 1,800. A context menu is open at position 1,600, with options like 'Create', 'Analyze', 'Copy', 'Create PDF', etc. Step 3 highlights the 'Create' option. Step 4 highlights the 'Create new' button in the 'Create translation' dialog, which is used to name the new vdh translation.

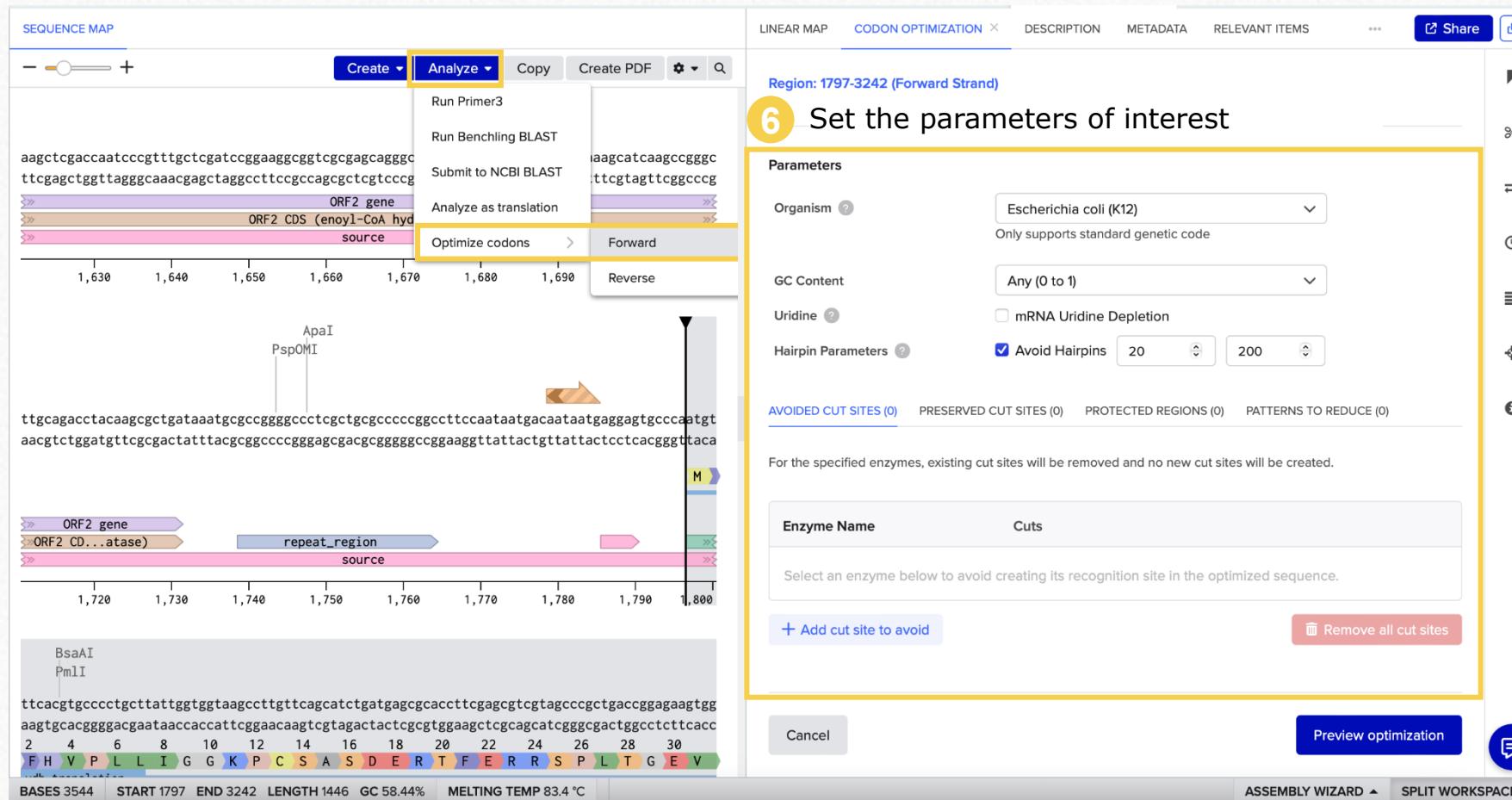
✓ Before codon optimization, the DNA sequence must be translated

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

5 Select the newly created translation and codon optimize it



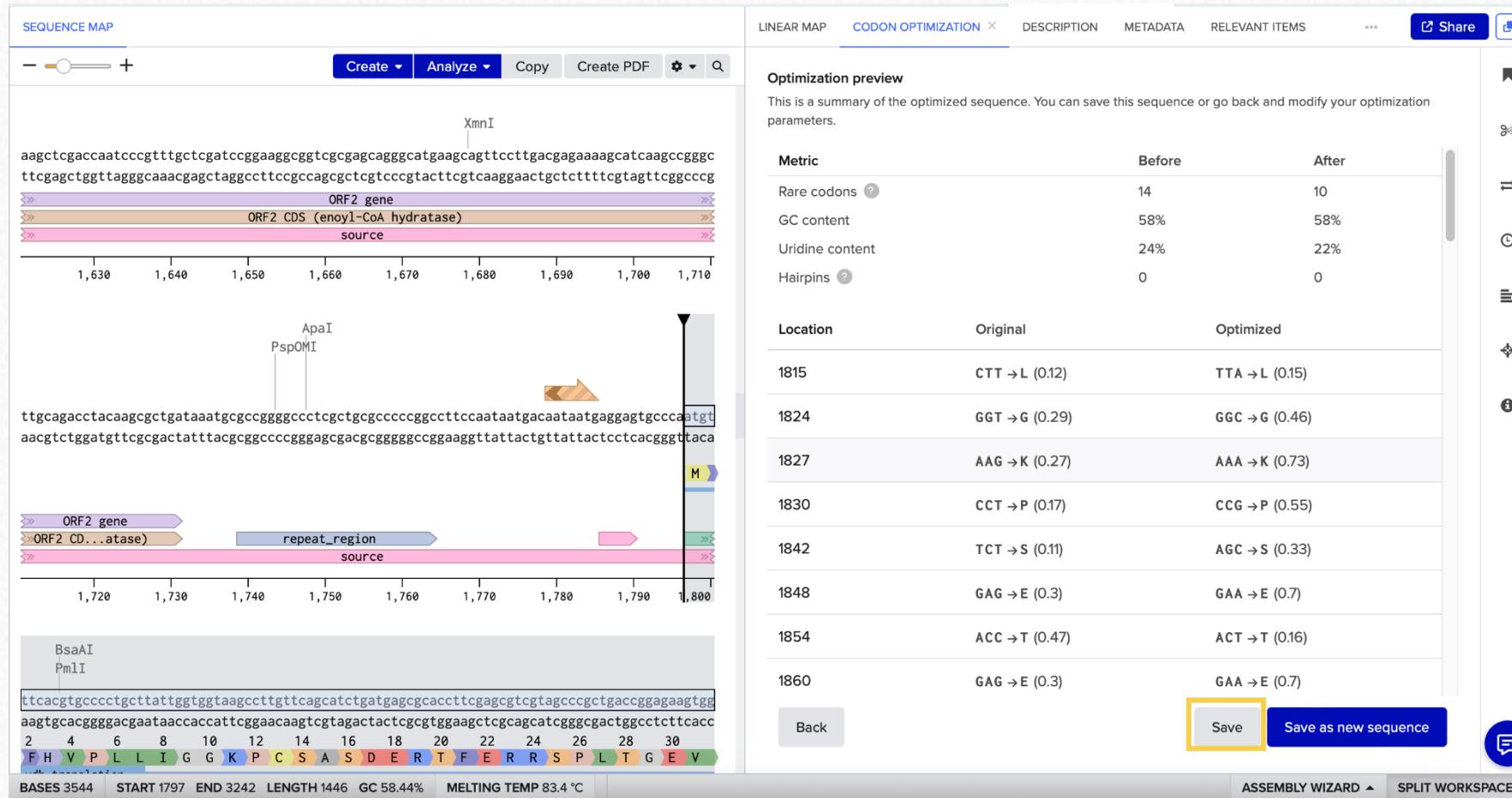
The screenshot shows the Bioworkshop software interface. On the left, there are two sequence maps. The top map shows a region from 1,630 to 1,690, highlighting an ORF2 gene, ORF2 CDS (enoyl-CoA hydrolase), and a source region. It includes restriction sites for ApaI and PspOMI. The bottom map shows a region from 1,720 to 1,800, highlighting an ORF2 gene, ORF2 CDS (atase), a repeat_region, and a source region. It includes restriction sites for BsaAI and PmlI. The middle section is titled "CODON OPTIMIZATION" and shows a dropdown menu with options like "Run Primer3", "Run Benchling BLAST", "Submit to NCBI BLAST", "Analyze as translation", "Optimize codons", and "Forward" or "Reverse". A yellow circle labeled "6 Set the parameters of interest" highlights the "Optimize codons" option. The right side of the interface shows the "Parameters" panel with fields for "Organism" (Escherichia coli K12), "GC Content" (Any 0 to 1), "Uridine" (mRNA Uridine Depletion checked), and "Hairpin Parameters" (Avoid Hairpins checked). Below this are sections for "AVOIDED CUT SITES (0)", "PRESERVED CUT SITES (0)", "PROTECTED REGIONS (0)", and "PATTERNS TO REDUCE (0)". A note states: "For the specified enzymes, existing cut sites will be removed and no new cut sites will be created." At the bottom are buttons for "Cancel", "Preview optimization", and a message icon.

- ✓ 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

Location* Molecular Biology Training

Description

Create

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

Copy the *Training Files* folder into your own

Projects / Biosustain Training / Molecular Biology Training Saved Searches

Search Type: Folder, Entry, Dataset 1 filter Save Clear

1-2 of 2 items, including items in subfolders

Name	Starred	Owner	Modified	Review Process
<input checked="" type="checkbox"/> Training Files	★	DTU Biosustain	03/02/2025	
Your Name	★	DTU Biosustain	03/02/2025	

Copy To...

Item is currently in: Molecular Biology Training

Projects

Filter...

- Biosustain Training biosustain
 - Ester
 - Inventory
 - Joana
 - Molecular Biology Training
 - Your Name

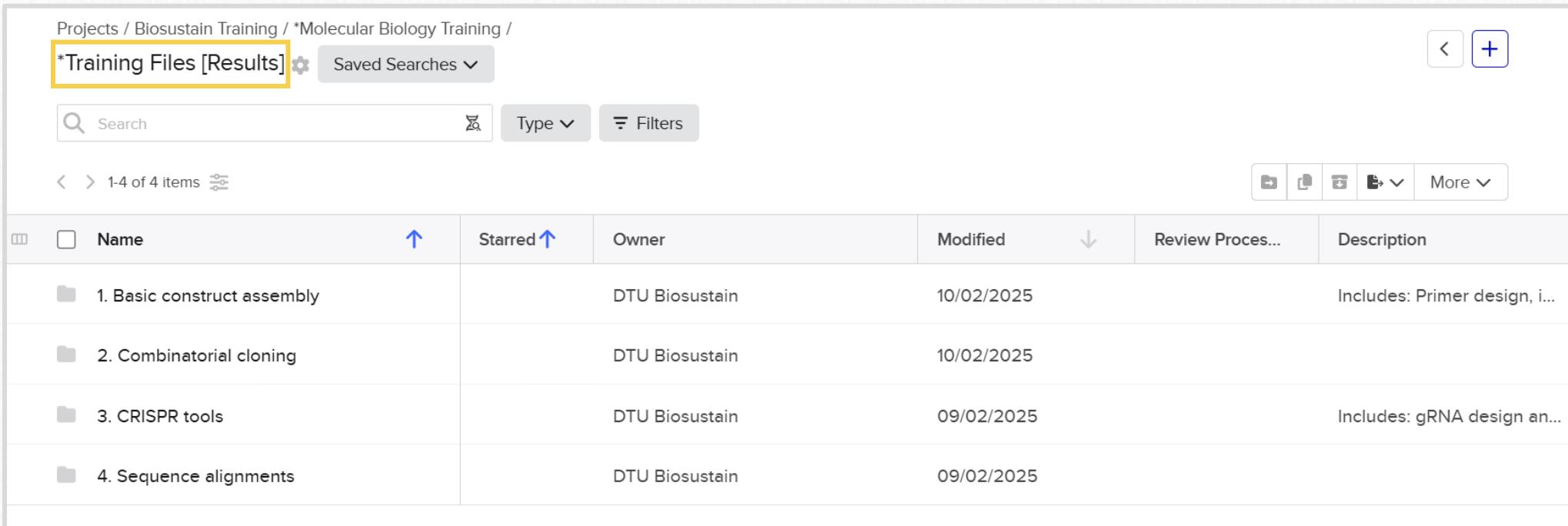
Create new folder (biosustain / Biosustain Training / Molecular Biology Training / Your Name)

Copy

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 tools. 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

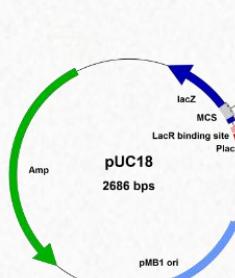
Gene knockout using CRISPR/Cas9

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 sequence maps:

- alsSD source:** A sequence map showing the DNA sequence of the alsSD construct. It includes a sequence map view with a ruler from 95 to 135, a linear map view with a ruler from 140 to 180, and a detailed view of the alsS CDS region (codons 2-8: MTKATAKEQK) and alsS gene. The alsD CDS and alsD gene are also indicated. Restriction enzyme sites for Tth11I, Pfl1FI, StuI, MlyI, PfeI, EagI, PciI, XbaI, KpnI, Acc65I, HpaI, NmeAIII, SphI, EcoRI, BsrDI, BspHI, PsfI, BsrGI, TatI, Bsp1286I, BsiHKAI, BlpI, FspI, BpuEI, PsfI, BbvCI, AlwNI, BssSI, BsrBI, BssSII, and BtsI are marked along the construct.
- pUC18:** A sequence map showing the pUC18 vector sequence. It includes a sequence map view with a ruler from 140 to 180, and a detailed view of the alsS CDS region (codons 10-24: SLVKNRGAELEVVDCL). The alsS gene and alsD gene are also indicated.

At the bottom, the status bar shows "BASES 3326" and "INSERT 154". Navigation buttons include "ASSEMBLY ▲" and "SPLIT WORKSPACE".

5. Basic construct assembly

5.1 Primer design

5.1.1 Manual primer design

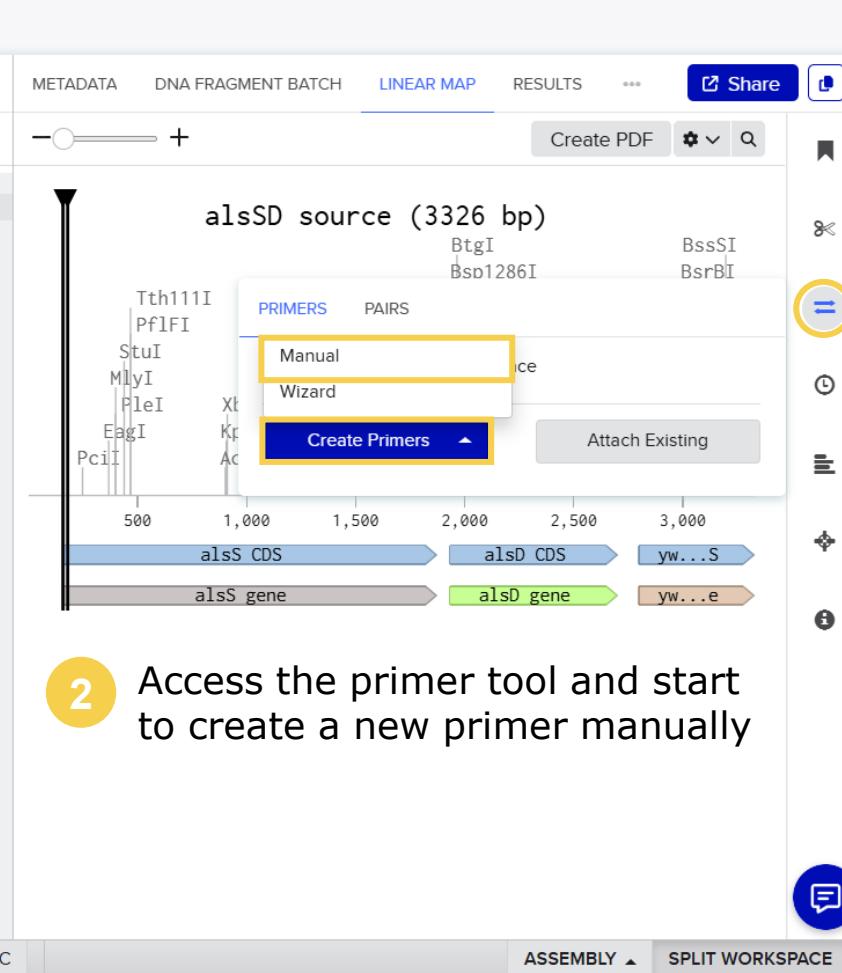
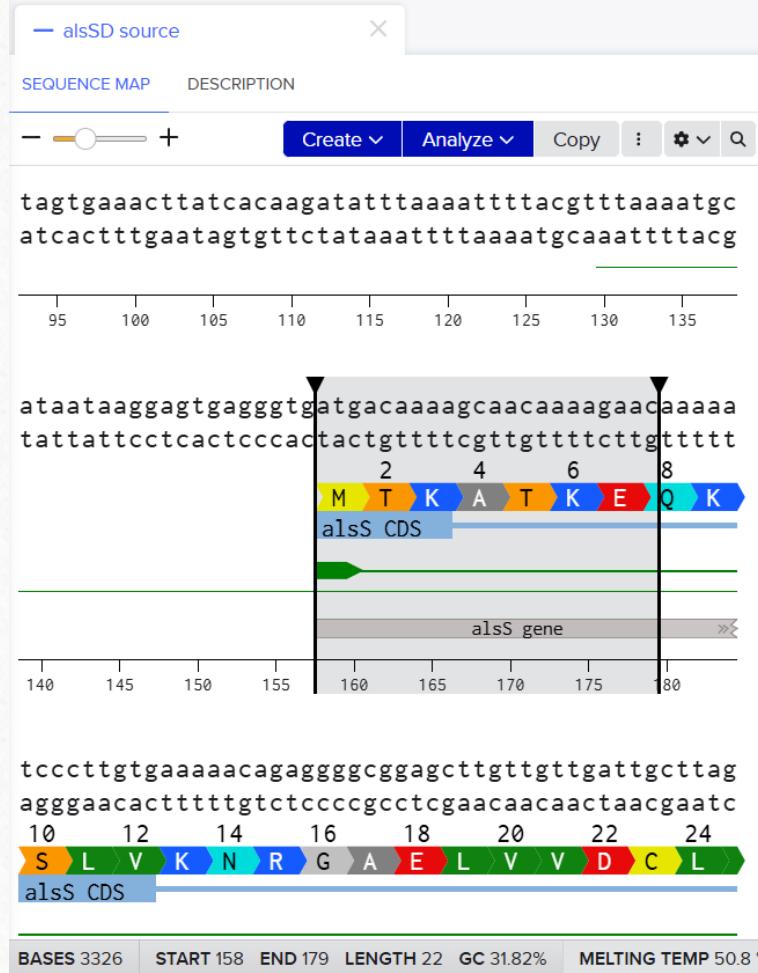


Construct design

Manual primer creation

Scenario: Creating primers to add restriction sites to *alsSD*

- 1 Select ~ 22 bases at the start of *alsS*

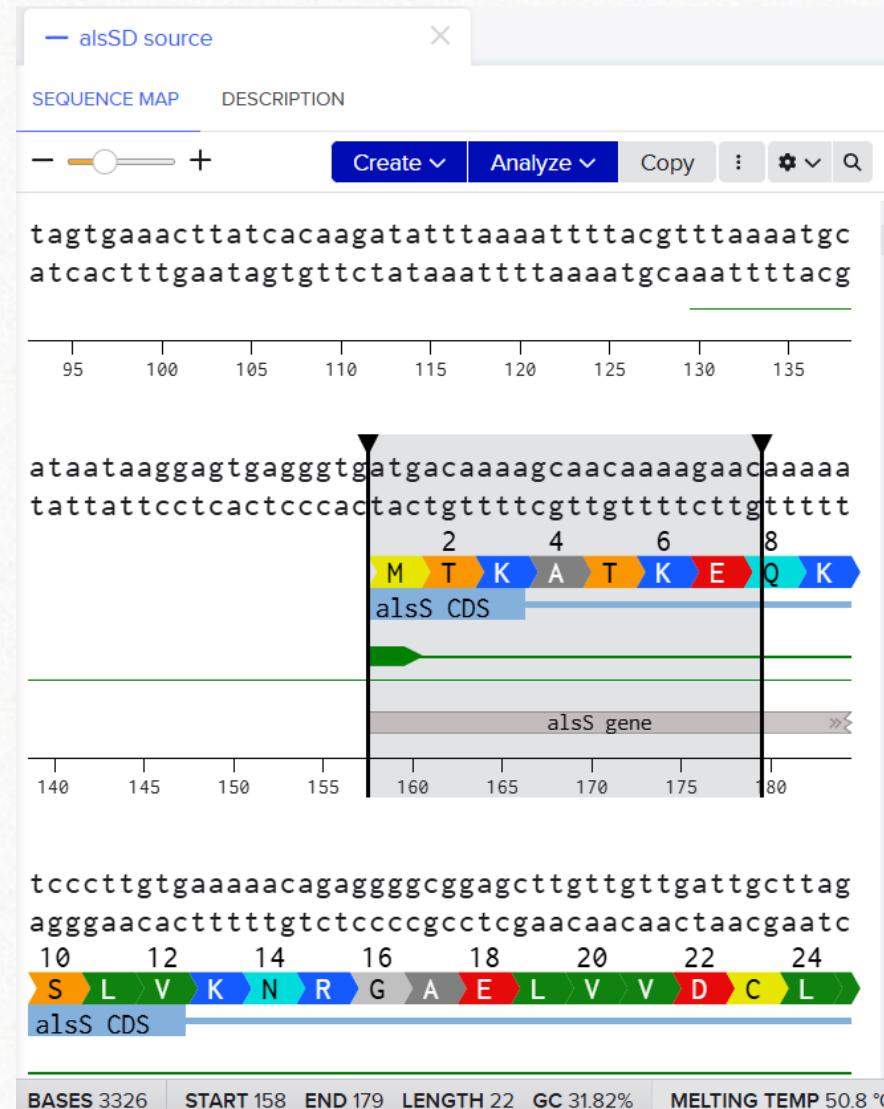


- 1 You can also attach **already existing** primers to your sequence if the entities are uploaded on Benchling

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

Construct design

Manual primer creation



3 Select primer pair creation

METADATA DNA FRAGMENT BATCH DESIGN PRIMER X Share

Primer Pair Jump to Primer Set from Selection

Single Primer Primer Pair

Strand Forward Reverse

Bases 5' 3' 5'

Primer must be at least 6 bp. Primer must be at least 6 bp.

3' Location 1 1
Overhang 0 bp 0 bp
Cut Site Aanl

Use the dropdown above to look up restriction sites.

Verify Check Secondary Structure at 50 °C

T_m GC Content

ASSEMBLY SPLIT WORKSPACE

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

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' GGATCCatgacaaaagca acaaaagaac 3'	5' ttattcagggttccttc agt 3'
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' GGATCCatgacaaaagca acaaaagaac 3'	5' GTCGACttattcagggttc tccttcagtt 3'
3' Location	179	2678
Overhang	6	6
Cut Site	Sall	GTCGAC

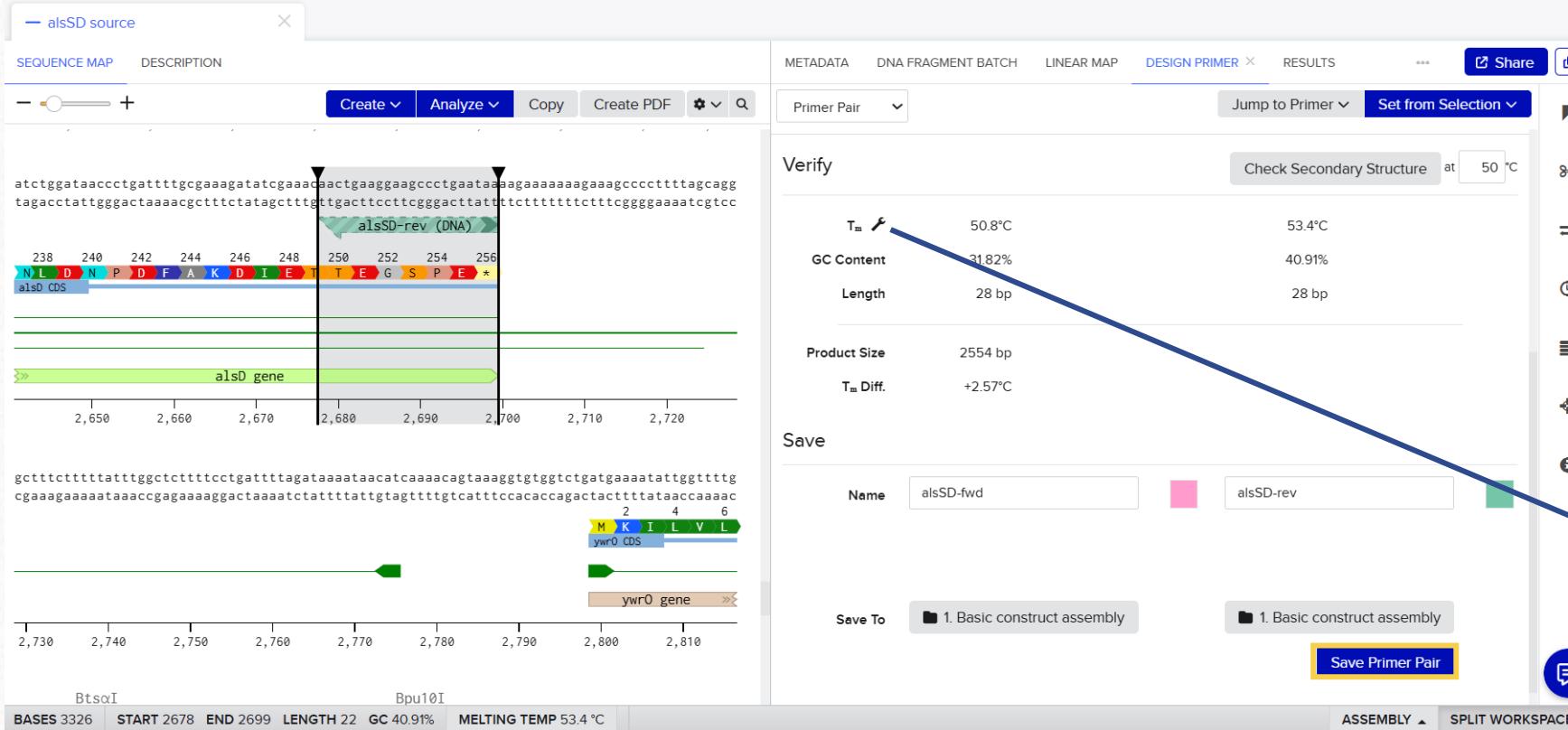
Use the dropdown above to look up restriction sites.

- 8 Repeat the process to add a **SalI** 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

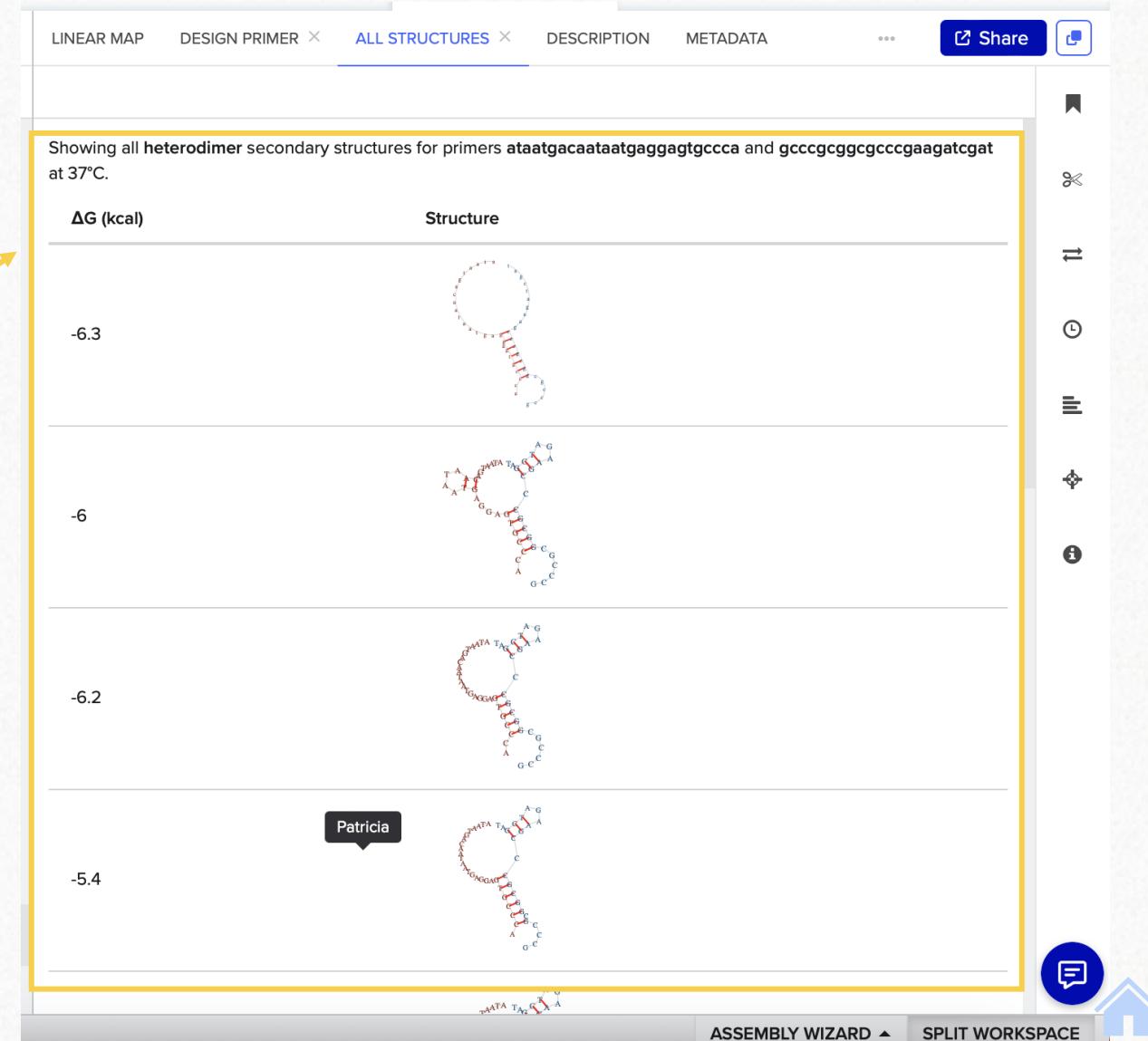
PRO TIP: You can adjust the default parameters for thermodynamic calculations

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 input fields for Primer Pair, Overhang (0 bp), Cut Site (Aanl), and dropdowns for Jump to Primer and Set from Selection. 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 checks at 37 °C. The bottom section for saving includes fields for Name (fwd_vdh and rev_vdh) and color-coded boxes for fwd and rev primers. Navigation buttons for ASSEMBLY WIZARD and SPLIT WORKSPACE are at the bottom.



5. Basic construct assembly

5.1 Primer design

5.1.2 Primer wizard

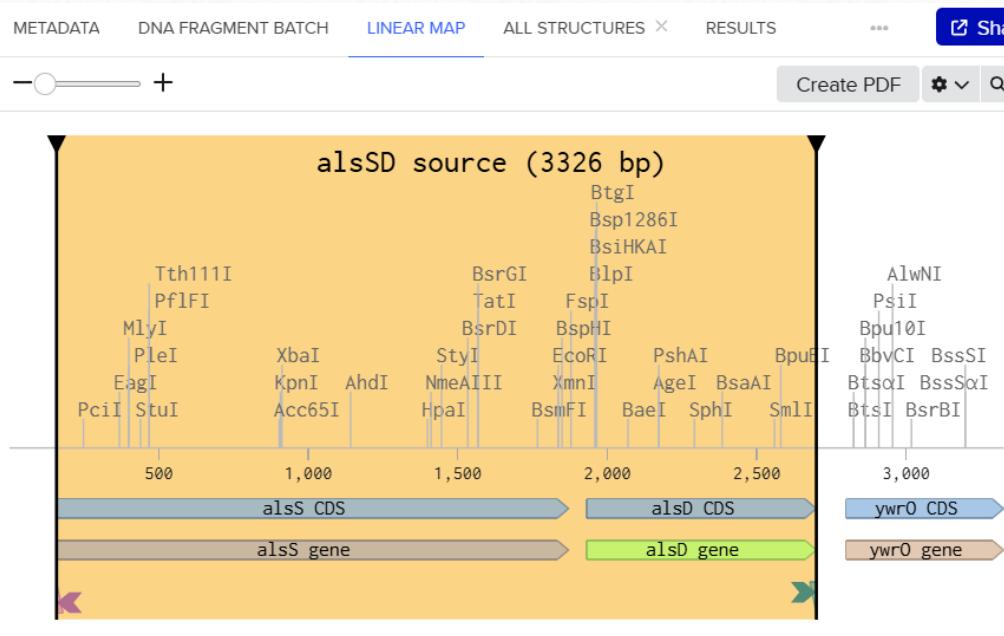


Construct design

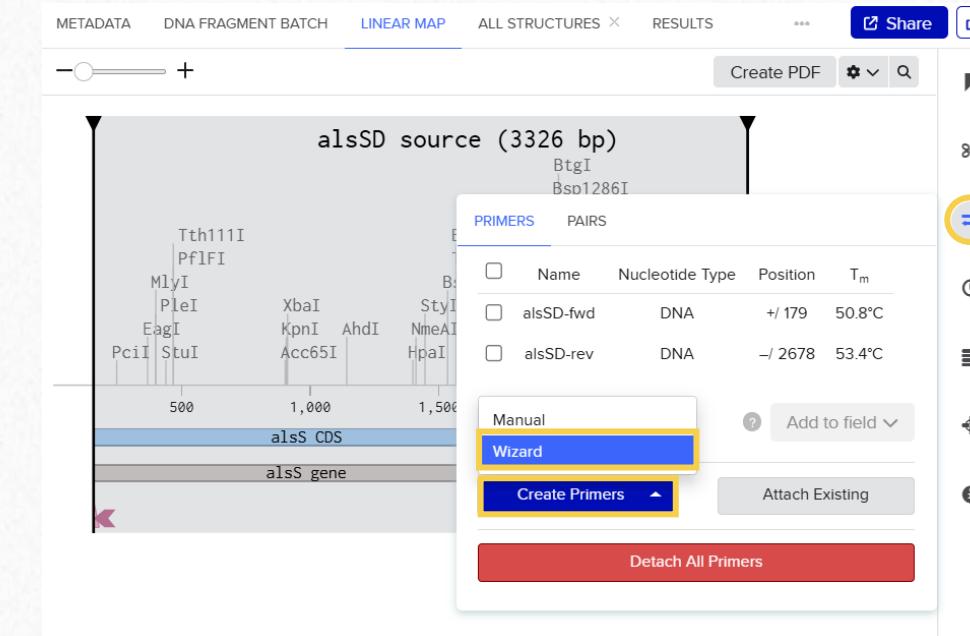
Automatic primer creation – Primer Wizard

- ✓ 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*



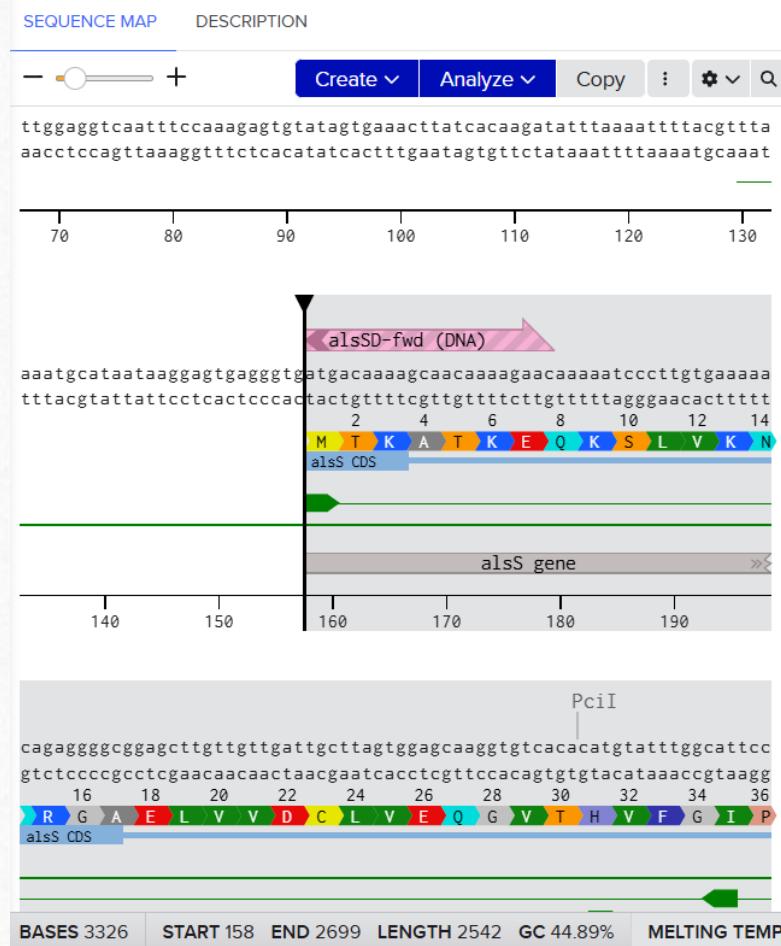
2 Access the primer tool and select *wizard*



i 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 Share ⚙️

Task PCR T_m Reset params

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

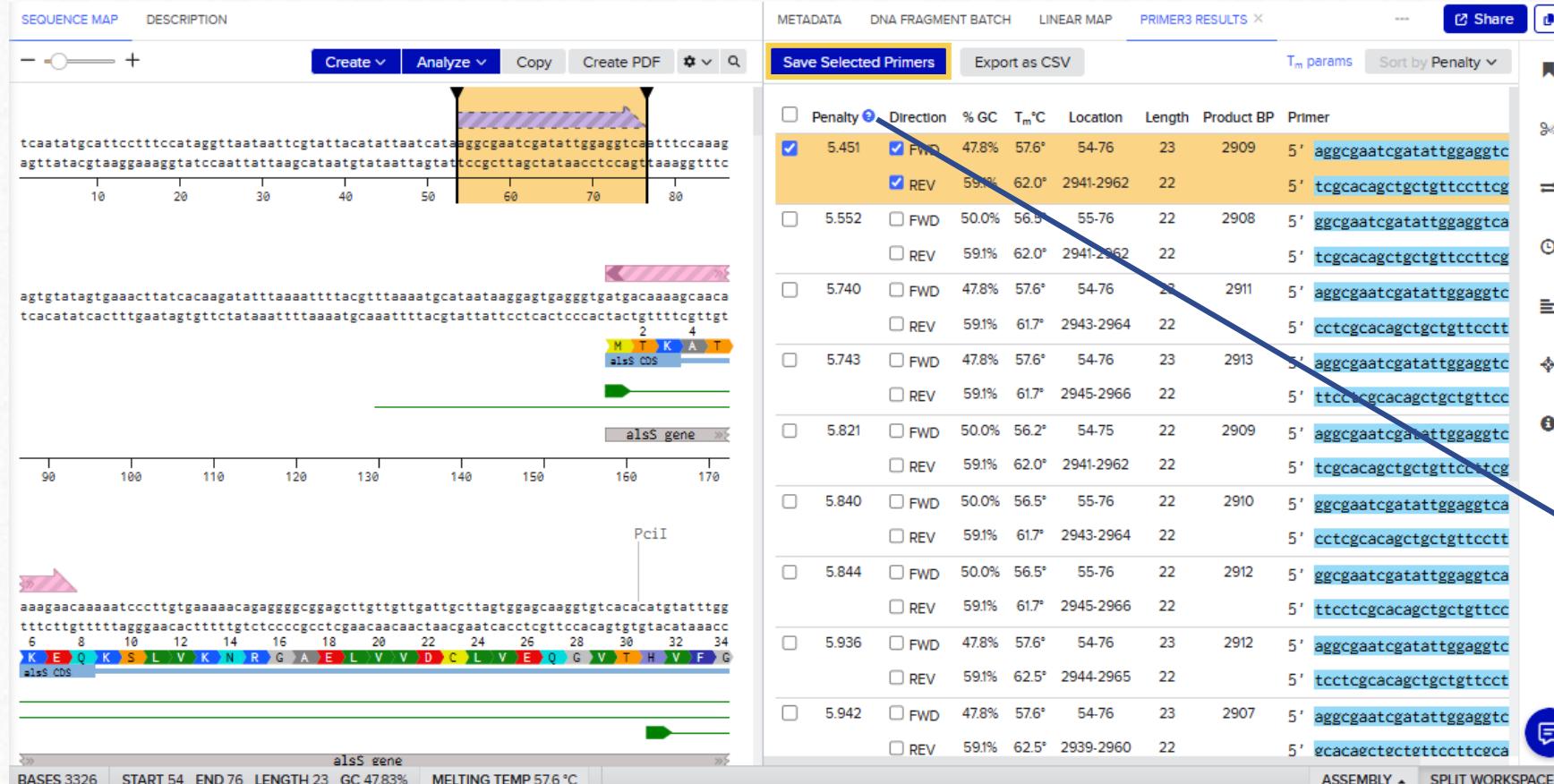
- ✓ Primer Wizard allows for different sequencing tasks
- ✓ Primer Wizard is powered by Primer3

 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 displayed: BASES 3326, START 54, END 76, LENGTH 23, GC 47.83%, and MELTING TEMP 57.6 °C.

The right side of the interface shows a table titled "PRIMER3 RESULTS" with the following columns: Penalty, Direction, % GC, T_m °C, Location, Length, Product BP, and Primer. The table lists 12 primer pairs, each with a checkbox for "Penalty". The first primer pair is selected (checked). A blue arrow points from the "Save Selected Primers" button at the top of the table to the "Sort by Penalty" button at the bottom right of the table.

Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
5.451	FWD	47.8%	57.6°	54-76	23	2909	5' aggccaatcgatattggaggtc
	REV	59%	62.0°	2941-2962	22		5' tcgcacagctgctgtccctcg
5.552	FWD	50.0%	56.5°	55-76	22	2908	5' ggcgaatcgatattggaggtc
	REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgtccctcg
5.740	FWD	47.8%	57.6°	54-76	23	2911	5' aggccaatcgatattggaggtc
	REV	59.1%	61.7°	2943-2964	22		5' cctcgcacagctgctgtccct
5.743	FWD	47.8%	57.6°	54-76	23	2913	5' aggccaatcgatattggaggtc
	REV	59.1%	61.7°	2945-2966	22		5' ttccgcacagctgctgtcc
5.821	FWD	50.0%	56.2°	54-75	22	2909	5' aggccaatcgatattggaggtc
	REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgtccctcg
5.840	FWD	50.0%	56.5°	55-76	22	2910	5' ggcgaatcgatattggaggtc
	REV	59.1%	61.7°	2943-2964	22		5' cctcgcacagctgctgtcc
5.844	FWD	50.0%	56.5°	55-76	22	2912	5' ggcgaatcgatattggaggtc
	REV	59.1%	61.7°	2945-2966	22		5' ttccgcacagctgctgtcc
5.936	FWD	47.8%	57.6°	54-76	23	2912	5' aggccaatcgatattggaggtc
	REV	59.1%	62.5°	2944-2965	22		5' tcctcgcacagctgctgtcc
5.942	FWD	47.8%	57.6°	54-76	23	2907	5' aggccaatcgatattggaggtc
	REV	59.1%	62.5°	2939-2960	22		5' gcacaactcttcccttceca

✓ 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

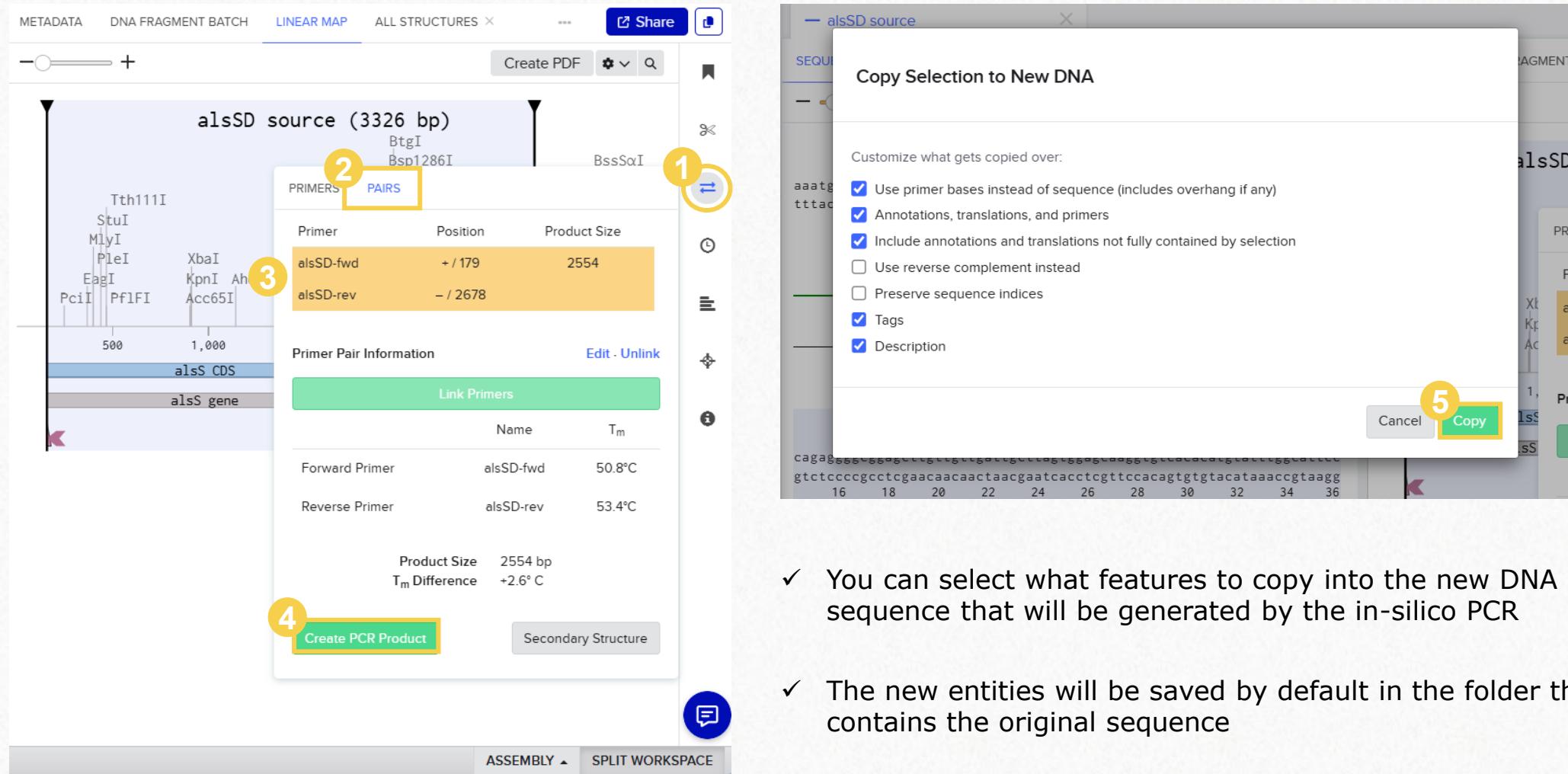
5.2 *In-silico* PCR



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, PstI, EagI, PciI, Pf1FI, XbaI, 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. The right panel shows the "Copy Selection to New DNA" dialog, where options like "Use primer bases instead of sequence (includes overhang if any)" and "Annotations, translations, and primers" are checked. The "Copy" button is highlighted with a yellow circle labeled "5".

Linear Map View:

- 1: Copy icon (highlighted)
- 2: PRIMERS tab (highlighted)
- 3: Primer pair selection (highlighted)
- 4: Create PCR Product button (highlighted)

Copy Selection to New DNA Dialog:

- 1: Copy button (highlighted)

Product Summary:

Forward Primer	Name	T_m
<i>alsSD-fwd</i>	50.8°C	
Reverse Primer	Name	T_m
<i>alsSD-rev</i>	53.4°C	

Product Size: 2554 bp
 T_m Difference: +2.6°C

- ✓ 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

SEQUENCE MAP

BstYI
BamHI

alsSD-fwd (DNA)

GGATCCatgaaaaagcaacaaaagaacaaaaatcccttgtaaaaaacagaggggcgagcttgt
CCTAGGtactgtttcggtttctgttttaggaacacttttgcctcccccgcctcgaaca

M T K A T K E Q K S L V K N R G A E L V
alsS CDS

alsS gene

PciI

tgttgattgcttagggagcaagggttcacacatgtattggcattccagggtgcaaaaattgatg
acaactaacgaatcacctcggtccacagtgtgtacataaaccgtaaagggtccacgttttaactac

V D C L V E Q G V T H V F G I P G A K I D
alsS CDS

alsS gene

BASES 2554

LINEAR MAP DESCRIPTION METADATA RESULTS ... Share Create PDF ⚙️ 🔍

alsSD source [158-2699] (2554 bp)

BstYI BamHI

Tth111I PciI

EagI PflFI

SstI MlyI PleI

PvuI XbaI BseYI

SspI KonI Acc65I

HpaI FokI BtsCI

NmeAIII AhdI

BsrGI TatI BsrDI

StyI HpaI NdeI

BspHI EcoRI BsmFI

FspI Bsp1286I BspII

SmlI BsiHKAI BpuEI

Bsp1286I BsiHKAI BpuEI

alsS CDS alsD CDS

alsS gene alsD gene

ASSEMBLY ▾ SPLIT WORKSPACE

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

5. Basic construct assembly

5.3 Virtual digestion

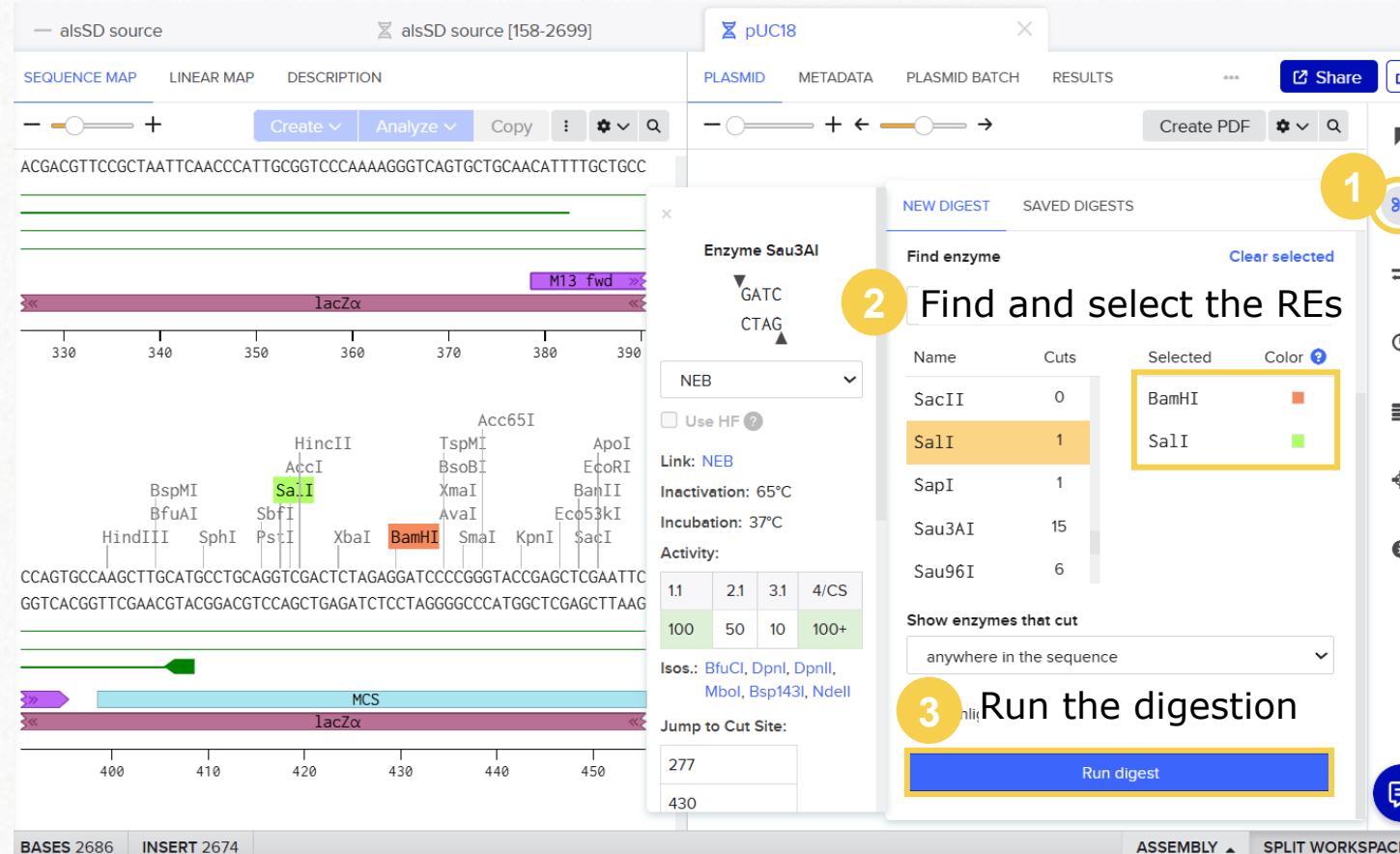


Construct design

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. On the left, the pUC18 sequence map is displayed, showing the *lacZα* gene, M13 forward primer (M13 fwd), and the Multiple Cloning Site (MCS). Various restriction enzymes are marked along the sequence, including **Sa.I**, **BamHI**, and **HincII**. The sequence is shown in two regions: bases 330-390 and 400-450.

In the center, a dialog box titled "Enzyme Sau3AI" is open. It shows the restriction site GATC and its reverse complement CTAG. The "NEB" dropdown is selected. The "Activity:" section shows values for different enzymes: 11, 2.1, 3.1, 4/CS; 100, 50, 10, 100+.

On the right, a "Find and select the REs" dialog is open. It lists enzymes with their cut counts and allows selection by color. **BamHI** and **Sa.I** are selected (highlighted in yellow).

Three numbered steps are overlaid on the interface:

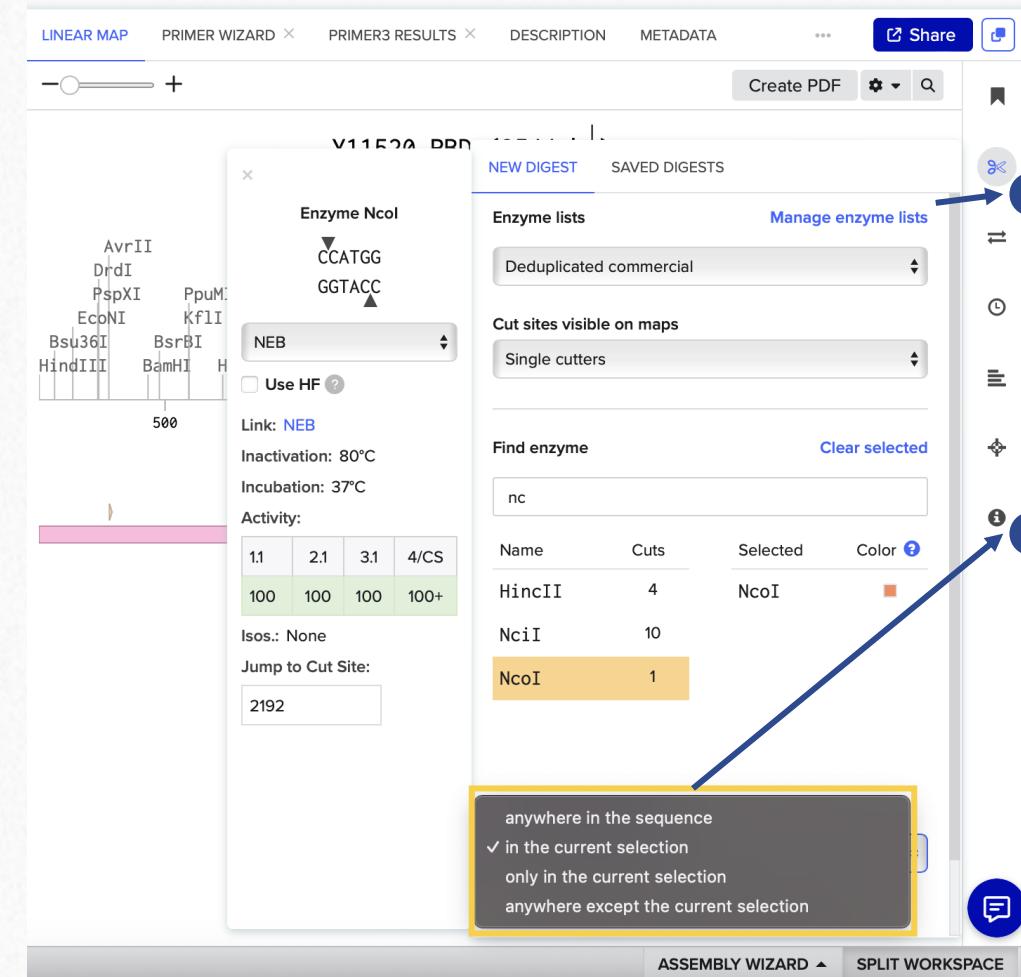
- 1 Click the "Find enzyme" search icon.
- 2 Find and select the REs: **BamHI** and **Sa.I**.
- 3 Run the digestion.

At the bottom, buttons for "Run digest" and "SPLIT WORKSPACE" are visible.

- ✓ The REs selected for this example are **BamHI** and **Sa.I**, 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, specifically for the enzyme NcoI. The menu displays the restriction site CCATGG and GGTACC, along with options for NEB enzymes and HF enzymes. Below the menu, a table shows activity levels for different enzymes: 1.1, 2.1, 3.1, and 4/CS, with values 100, 100, 100, and 100+ respectively. A 'Find enzyme' search bar contains 'nc'. On the right side of the interface, there is a sidebar titled 'Enzyme lists' which includes a dropdown for 'Deduplicated commercial' and a section for 'Cut sites visible on maps' set to 'Single cutters'. A 'Find enzyme' search bar also exists here. A tooltip at the bottom of the sidebar provides instructions for selecting enzyme lists based on the current selection.

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

ASSEMBLY WIZARD ▾ SPLIT WORKSPACE

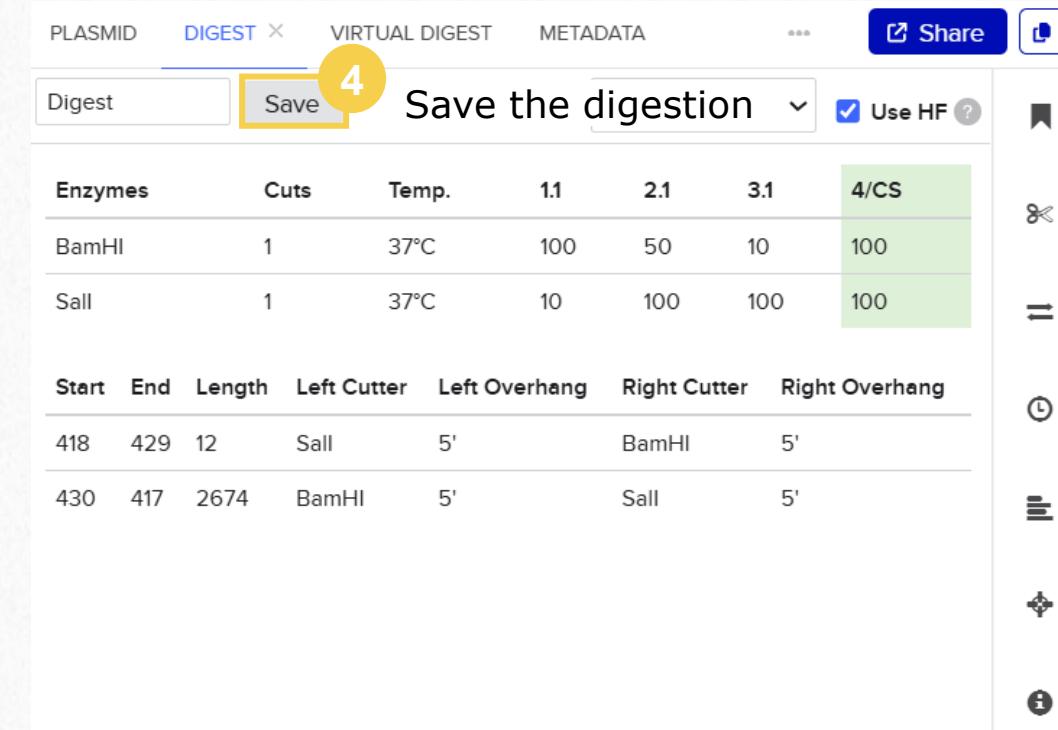
PRO TIP: The enzyme lists available can be managed, similarly to the features libraries and are shared within the Center.

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 'Virtual Digest' tab of a bioinformatics tool. At the top, there are tabs for 'PLASMID', 'DIGEST X', 'VIRTUAL DIGEST' (which is selected), and 'METADATA'. Below the tabs is a navigation bar with 'Digest' (selected), 'Save' (highlighted with a yellow circle containing the number 4), 'Share', and a download icon. A dropdown menu says 'Save the digestion' with a 'Use HF' checkbox checked. To the right are icons for bookmarking, sharing, and other functions.

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

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'

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

Virtual digestion

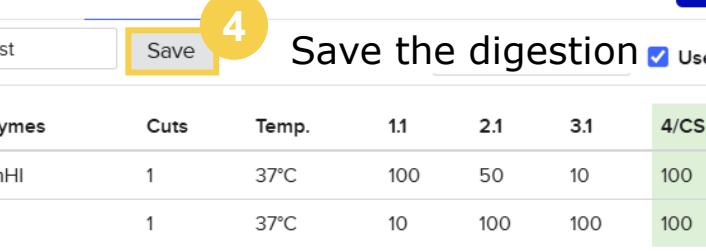
Digestion of the insert

1 Open the amplified *alsSD* sequence

3 Find and select the REs

Run the digestion

The digest tab will open



4 Save the digestion Use HF ?

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

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

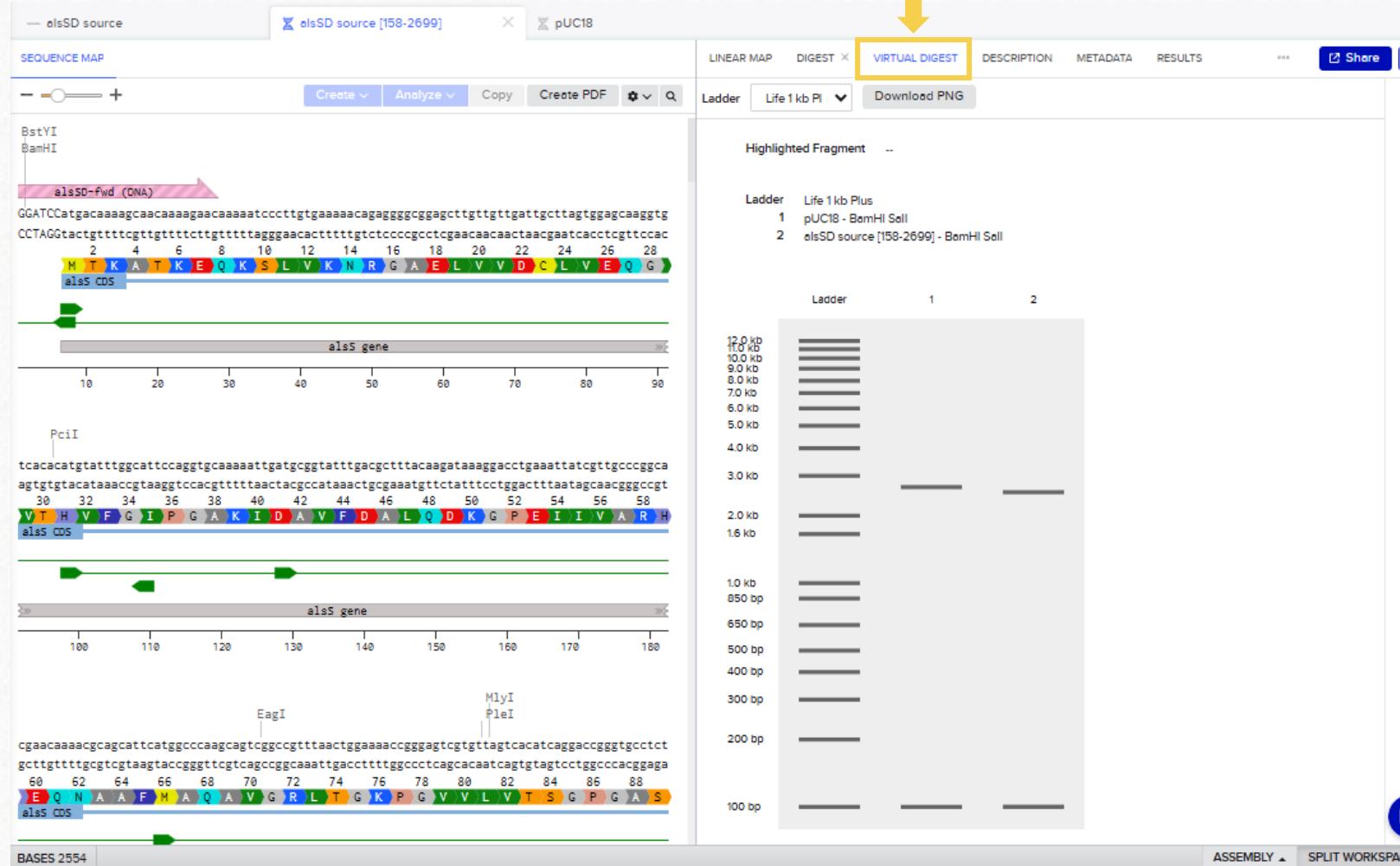
The digest tab will open

Save the digestion		<input checked="" type="checkbox"/> Use HF	?
Temp.	1.1	2.1	3.1
37°C	100	50	10
37°C	10	100	100
itter	Left Overhang	Right Cutter	Right Overhang
	blunt	BamHI	5'
	5'	Sall	5'
	5'	None	blunt

Construct design

Virtual digestion

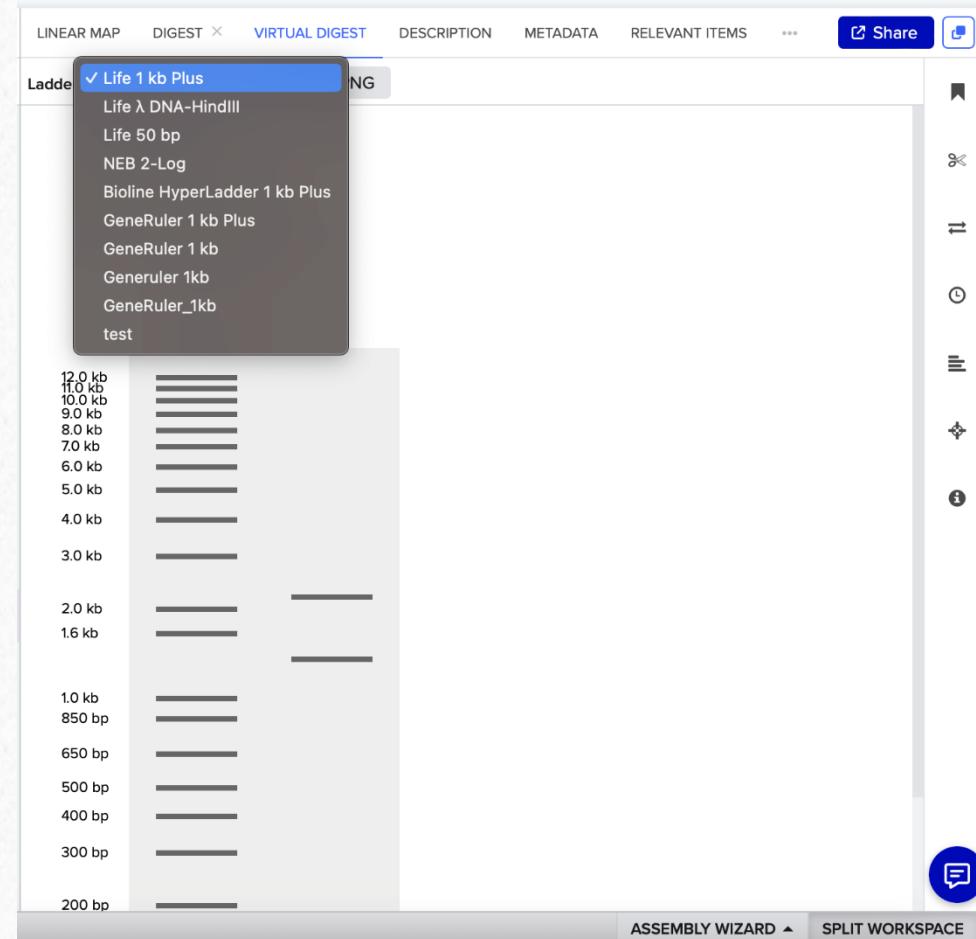
Gel visualization



- ✓ 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

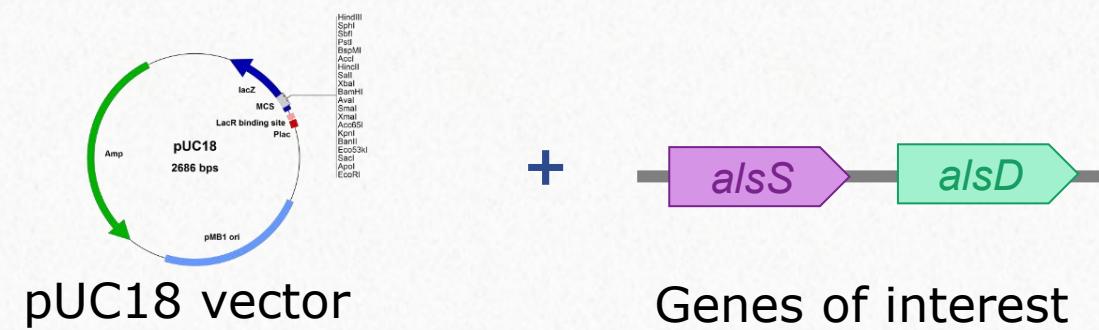
Virtual digestion



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

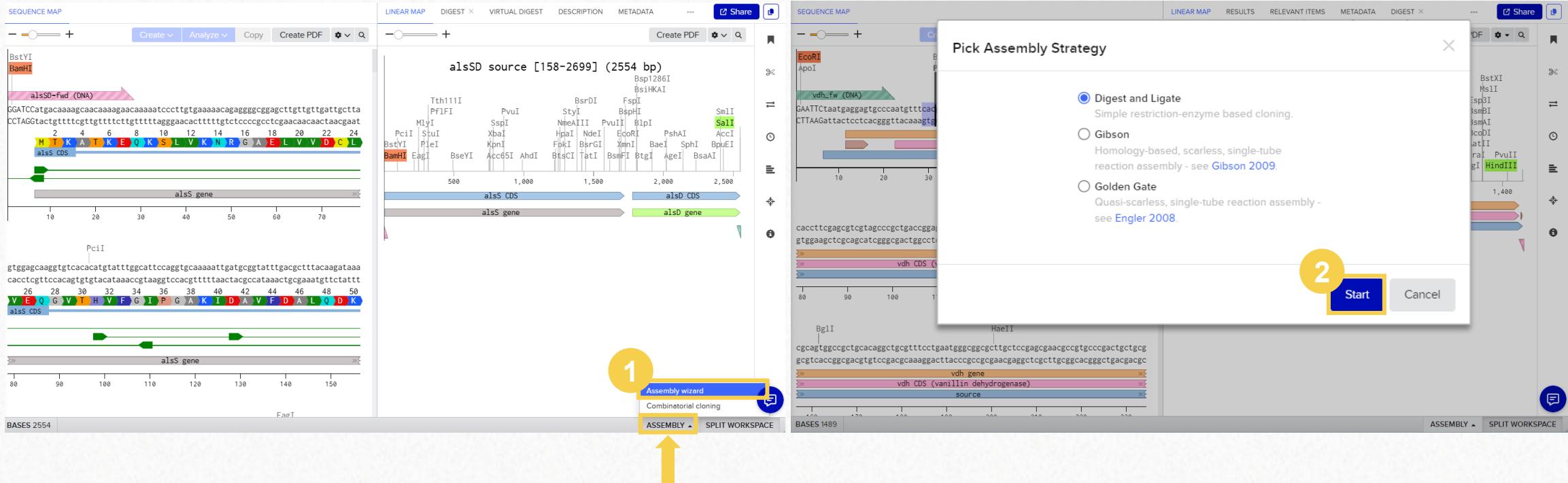
5. Basic construct assembly

5.4 Assembly Wizard



Construct Assembly

Assembly Wizard



The screenshot shows the QIIME Assembly Wizard interface. On the left, there are two sequence maps: one for the *alsS* gene (2554 bp) and one for the *alsD* gene (1489 bp). The *alsS* map includes restriction enzyme sites for BstYI and BamHI, and a sequencing primer labeled alsS-fwd (DNA). The *alsD* map includes sites for Tth11I, PflI, PvuI, StyI, BsrDI, FspI, Bsp126I, BsiHKAI, SmaI, SalI, MspI, HpaI, NdeI, PvuII, BpuI, AccI, KpnI, XbaI, BsrGI, XbaII, BaeI, SphI, BpuII, BstYI, PstI, BseYI, Acc65I, AhdI, BtaCI, TbtI, BsmFI, BtgI, AgeI, BsaAI, BpuII, BglI, and HaeII. Below the maps are protein sequence alignments and amino acid translations for the *alsS* and *alsD* genes.

In the center, a "Pick Assembly Strategy" dialog box is open, showing three options:

- 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](#).

A large yellow arrow points from the "ASSEMBLY" tab at the bottom of the main window up to the "ASSEMBLY" tab in the dialog box, indicating that the dialog is part of the main assembly 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 Assembly Wizard interface with the following components:

- Plasmid Map:** A circular diagram of pUC18 with various restriction sites and features labeled: AmpR_promoter, AmpR, M13_fwd, MCS, lacZ, +3 lac_promoter, ori, and various bp markers (e.g., 2500, 2250, 2000, 1750, 1500, 1250, 1000).
- Digest Table:** A table showing enzyme digestions. The table includes columns for Enzymes, Cuts, Temp., and concentrations (1.1, 2.1, 3.1). It lists BamHI and Sall digestions.
- Overall Assembly:** A summary section indicating the backbone or insert is unset. It shows the construct name "pUC18-alsSD" and an "Assemble" button.
- Bottom Navigation:** Buttons for "Backbone" and "Insert", and tabs for "BASES 2686" and "INSERT 693".

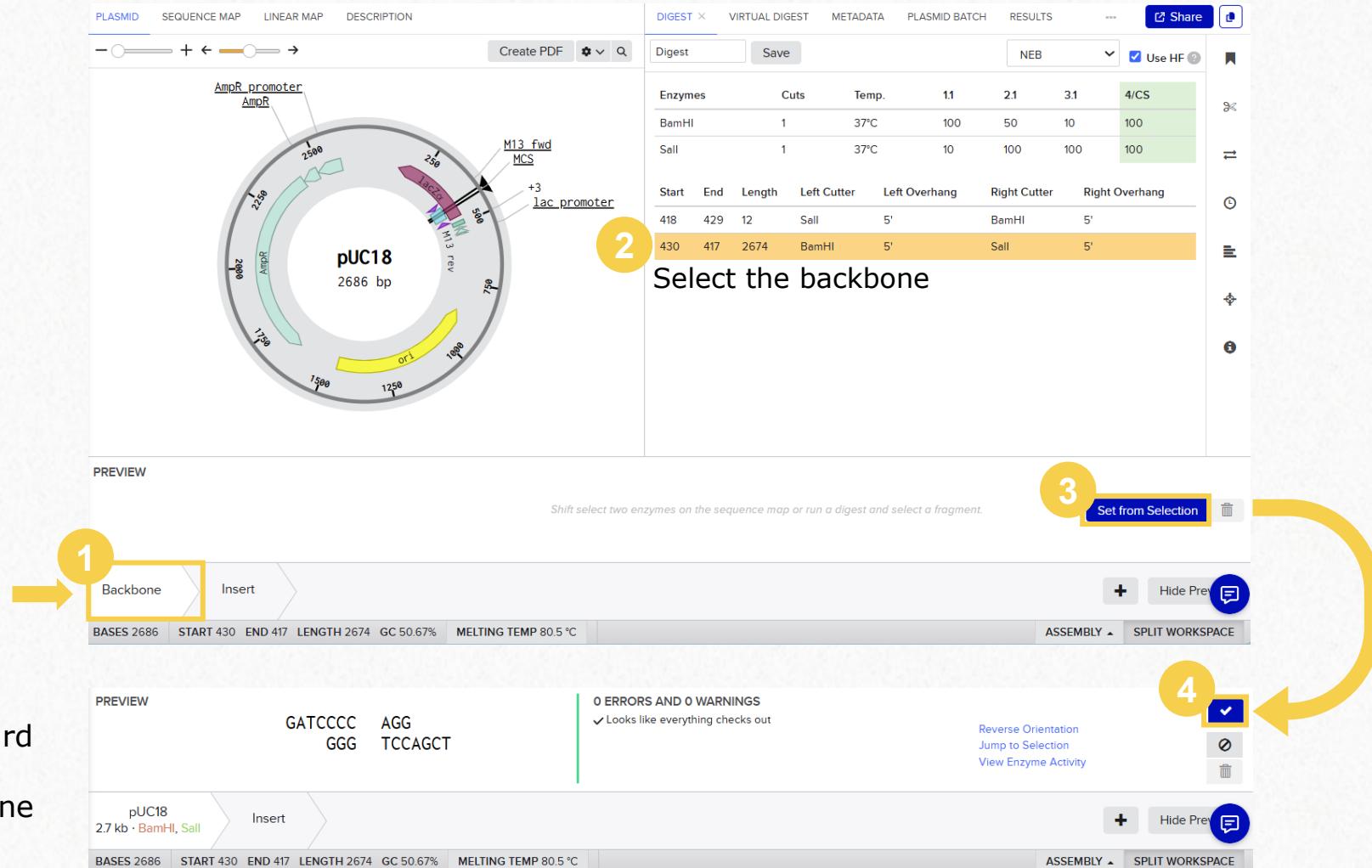
A yellow box highlights the "Overall Assembly" section, and a yellow arrow points to it from the left. Another yellow arrow points to the "pUC18-alsSD" input field with the text "Name your construct".

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

Name your construct

Construct Assembly

Digest and Ligate: Add the backbone



The screenshot illustrates the Assembly Wizard process for adding a backbone to a plasmid construct:

- 1 Backbone**: The user has selected the "Backbone" option in the "PREVIEW" section.
- 2 Select the backbone**: The user has selected the "BamHI" and "Sall" enzymes in the "DIGEST" table.
- 3 Set from Selection**: The user has clicked the "Set from Selection" button to apply the enzyme digest to the backbone.
- 4 Preview**: The user has checked the preview checkbox to view the resulting digested ends of the backbone.

PLASMID Sequence Map Linear Map Description

DIGEST

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
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'

PREVIEW

Shift select two enzymes on the sequence map or run a digest and select a fragment.

BASES 2686 START 430 END 417 LENGTH 2674 GC 50.67% MELTING TEMP 80.5 °C

ASSEMBLY ▾ SPLIT WORKSPACE

0 ERRORS AND 0 WARNINGS

GATCCCC AGG
GGG TCCAGCT

Reverse Orientation
Jump to Selection
View Enzyme Activity

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 (Top Left):** Shows the **alsSD source [158-2699] (2554 bp)**. It displays restriction sites for numerous enzymes, with **BamHI** and **SalI** specifically highlighted in red and green boxes respectively. A yellow circle labeled **2** points to the **alsS CDS** and **alsD CDS** regions.
- DIGEST (Top Right):** A table for digesting the **alsSD source** with **BamHI** and **SalI**. The table includes columns for Enzymes, Cuts, Temp., and NEB values. The **Use HF** checkbox is checked.
- PREVIEW (Bottom):** Shows a sequence fragment with the **pUC18** vector (2.7 kb) on the left and the **Insert** sequence in the center. The sequence for the **Insert** is shown as **AGG TCCAGCT GATCCCC GGG**. A yellow arrow points upwards from the preview area towards the sequence map.

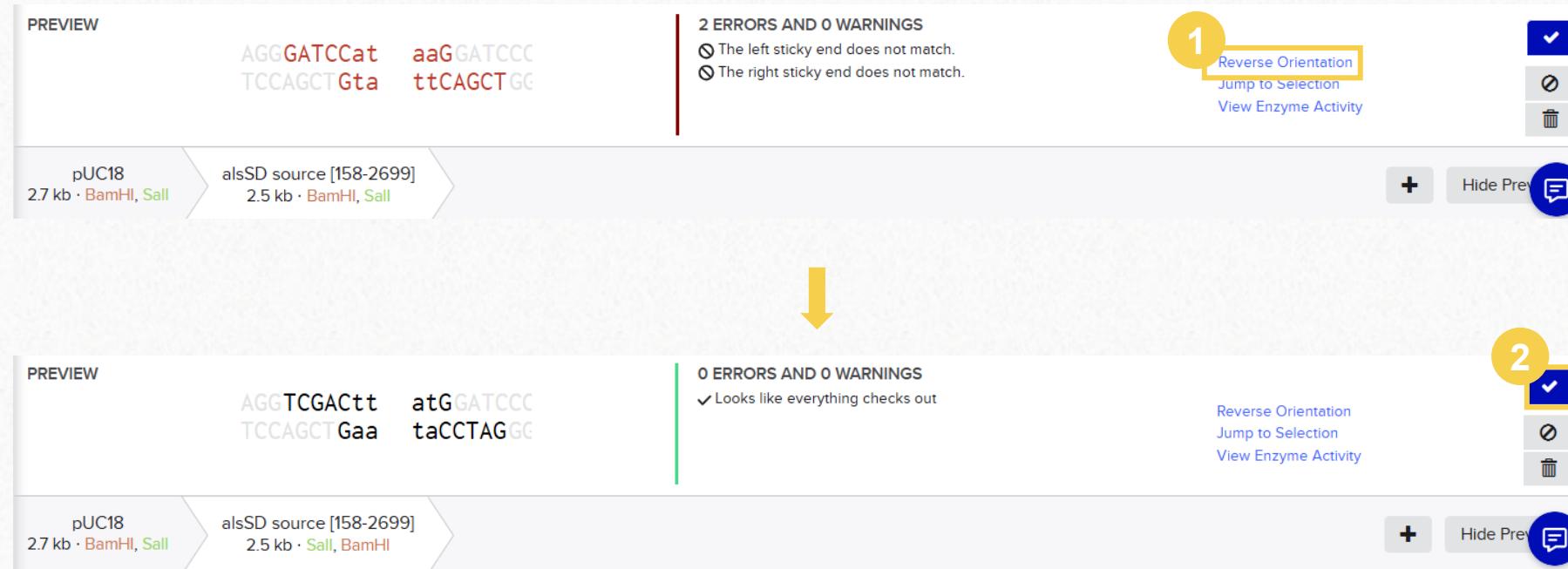
Select the insert

Set from Selection

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!



PREVIEW

AGG GATCCat aaGGATCCC
TCCAGCT Gta ttCAGCT GC

pUC18 2.7 kb · BamHI, Sall alsSD source [158-2699] 2.5 kb · BamHI, Sall

2 ERRORS AND 0 WARNINGS

- The left sticky end does not match.
- The right sticky end does not match.

1 Reverse Orientation
Jump to Selection
View Enzyme Activity

PREVIEW

AGG TCGACTt atGGATCCC
TCCAGCT Gaa taCCTAGGC

pUC18 2.7 kb · BamHI, Sall alsSD source [158-2699] 2.5 kb · Sall, BamHI

0 ERRORS AND 0 WARNINGS

- Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

2 Reverse Orientation
Jump to Selection
View Enzyme Activity

- ✓ 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, Sall) 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'. Below these are buttons for 'pUC18-alsSD' (trash, Assemble, plus, hide preview), with a yellow circle containing the number '1' over the 'Assemble' button. A large yellow arrow points down from the overall assembly area to the 'SEQUENCE MAP' and 'LINEAR MAP' sections.

SEQUENCE MAP

This panel shows two sequence maps. The top map shows restriction sites AccI, SalI, SbfI, and MCS (multiple cloning site) with the 'lacZα' gene. The bottom map shows the 'alsD gene'. The x-axis ranges from 320 to 460 for the top map and 400 to 540 for the bottom map. The y-axis shows DNA sequence segments.

LINEAR MAP

This panel shows a circular map of the construct 'pUC18-alsSD' with a size of 5222 bp. Key features include the 'AmpR_promoter' (green), 'lacZα' gene (purple), 'MCS' (blue), 'M13_fwd' (grey), 'M13_rev' (yellow), and 'lacS gene' (grey). The map is oriented with 'Linear Map' at the top and 'Plasmid' at the bottom. The x-axis ranges from 100 to 1000.

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

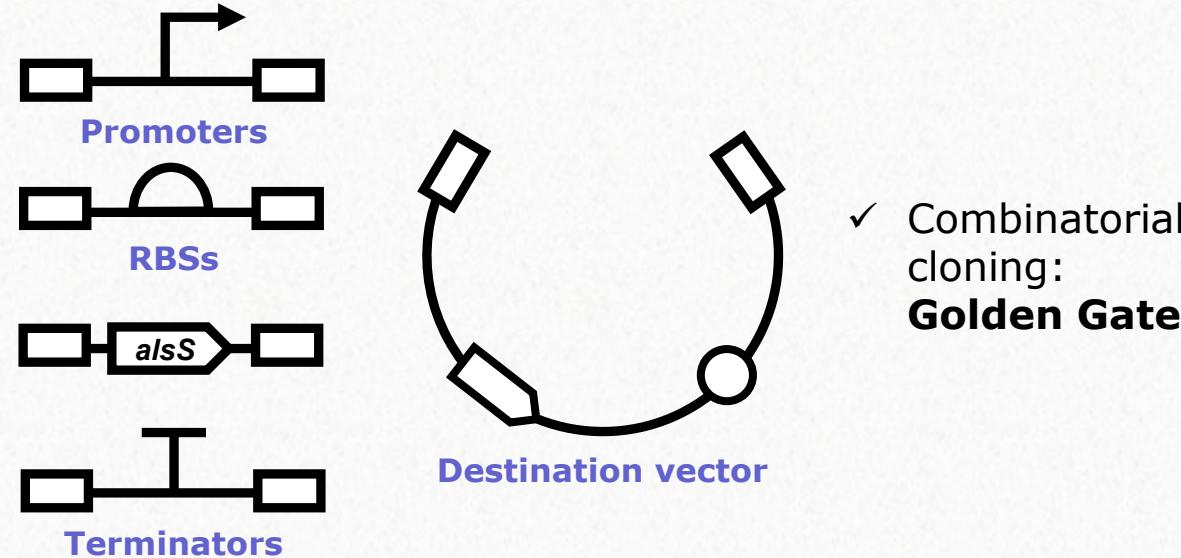
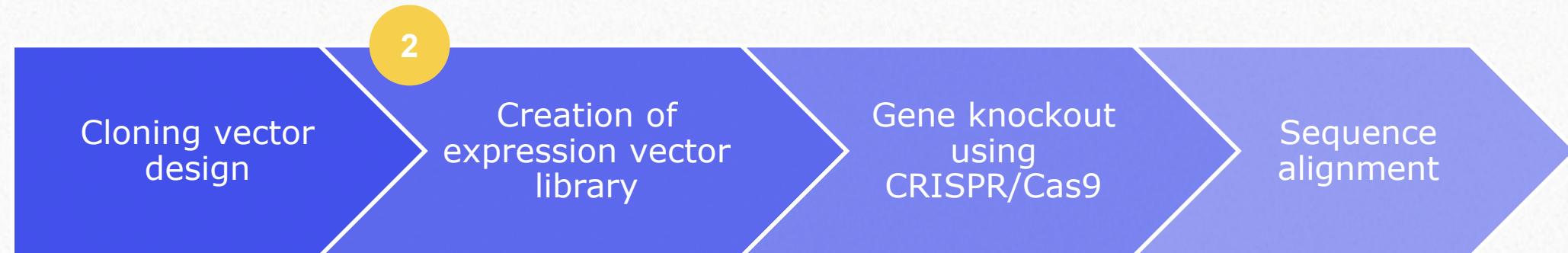
- ✓ You will be asked to choose a folder to save the construct in

- ✓ 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

SEQUENCE MAP

alsS

Create Analyze Copy Create PDF Share

DESCRIPTION METADATA RESULTS LINEAR MAP RELEVANT ITEMS

Search Type Filters

alsS Last modified 3 hours ago

pET-Ori-KanR Last modified 3 hours ago

promoter-01-T5 Last modified 3 hours ago

promoter-02-tac Last modified 3 hours ago

promoter-03-T7 Last modified 3 hours ago

RBS-01-B0030 Last modified 3 hours ago

RBS-02-B0032 Last modified 3 hours ago

RBS-03-B0034 Last modified 3 hours ago

terminator-01-rnnBT1 Last modified 3 hours ago

terminator-02-T0 Last modified 3 hours ago

Sequence Map showing restriction enzyme sites (BssSaiI, BssSI, MboII, SacII, EcoII) and the alsS gene (CDS). The sequence is: atgacc...tagtttgcgtggttctgttttagggacacttctggccccccctcgaccaacacctaacaatcacctc. The alsS CDS is highlighted in blue, spanning from position 9 to 26. The alsS gene is shown below the CDS. A zoomed-in view shows positions 10-70 with restriction sites NspI and PstI.

alsS (1713 bp)

Create PDF Share

DESCRIPTION METADATA RESULTS LINEAR MAP RELEVANT ITEMS

MmeI MscI SmaI TspMI ScAI XbaI KpnI Acc65I Xmni AgeI Afel StyI PpuMI EcoRI BspPHI Pvui

PstI XmaI BsaHI BsgI RsrII BsrGI

EciI BtsI FseI PaqCI BspMI FokI BtgZI BspCNI DdeI BstXI AhDI BsmFI BarI BfaI BtsCI NgoMIV

NspI BtsNI BsrDI BsrXI BtsCI BspCNI BspPHI PvuII

MboII BtsI Pf1MI AlwNI BsmFI BfuaI BtsCI DdeI BstXI AhDI BsmFI BarI BfaI BtsCI NgoMIV

SacII BtsNI Pf1MI BsrDI BsrXI BtsCI BspCNI BspPHI PvuII

BssSaiI BssSI PspGI BsmFI BfuaI BtsCI DdeI BstXI AhDI BsmFI BarI BfaI BtsCI NgoMIV

BssSI PspGI BsmFI BfuaI BtsCI DdeI BstXI AhDI BsmFI BarI BfaI BtsCI NgoMIV

200 400 600 800 1,000 1,200 1,400 1,600

alsS CDS alsS gene

28 30 32 34 36 38 40 42 44 46 48 50 52

Q G V T H V F G I P G A K I D A V F D A L Q D K G P

alsS CDS

alsS gene

80 90 100 110 120 130 140 150

BstNI BtsI RsaHT

PstI GT PF1MT

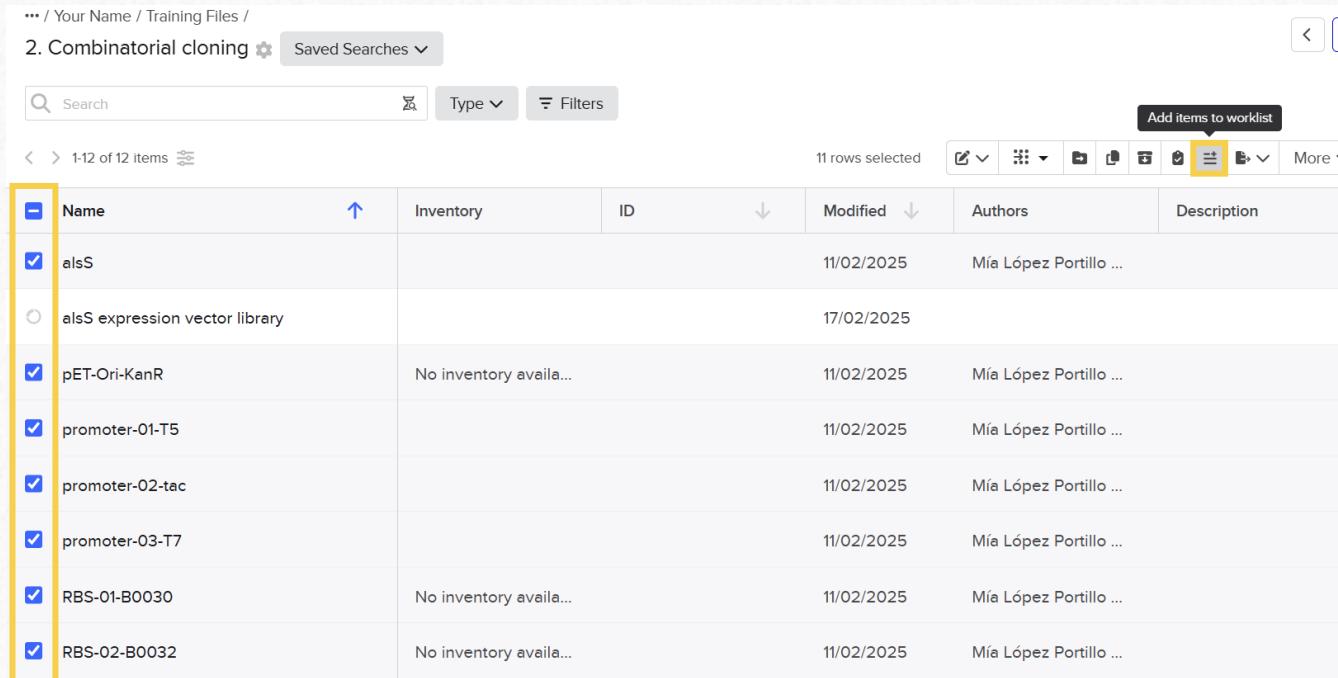
BASES 1713 ASSEMBLY ▾ SPLIT WORKSPACE

Construct Assembly

Worklist creation

PRO TIP:

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



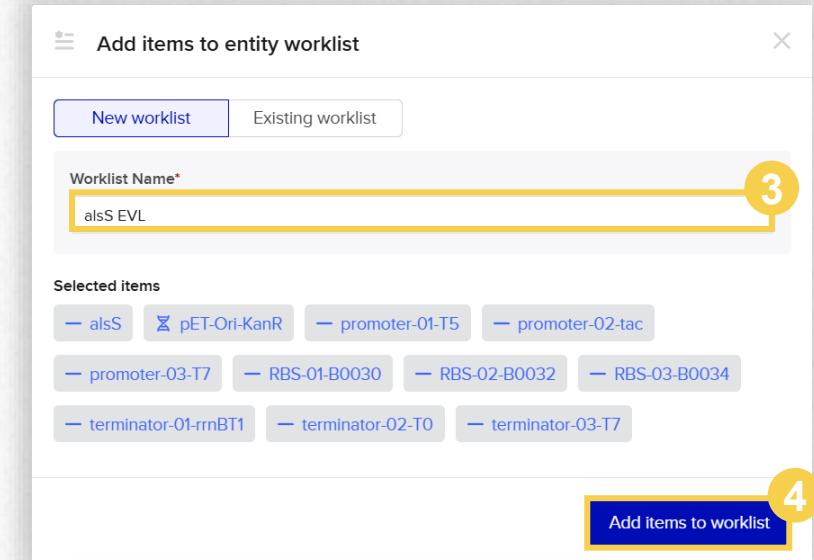
... / Your Name / Training Files /
2. Combinatorial cloning Saved Searches

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 3

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 4

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 its DNA sequence, amino acid sequence, and restriction enzyme sites. A dropdown menu is open at the bottom of this panel, showing three assembly options: 'Assembly', 'CRISPR', and 'Entity from schema'. The 'Assembly' option is highlighted with a yellow circle and number 2. A sub-menu for 'Assembly' is open, showing 'Assemble DNA sequences by cloning' and 'Assemble sequences and oligos by concatenation'. The 'Assemble DNA sequences by cloning' option is highlighted with a yellow circle and number 3. The right side of the interface shows a detailed linear map of the 'alsS' construct, including its CDS and gene regions, and a list of relevant enzymes.

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

alsS

SEQUENCE MAP

Search Create Analyze Copy Create PDF Share

Description Metadata Results Linear Map Relevant Items

alsS (1713 bp)

alsS CDS

alsS gene

Assemble DNA sequences by cloning

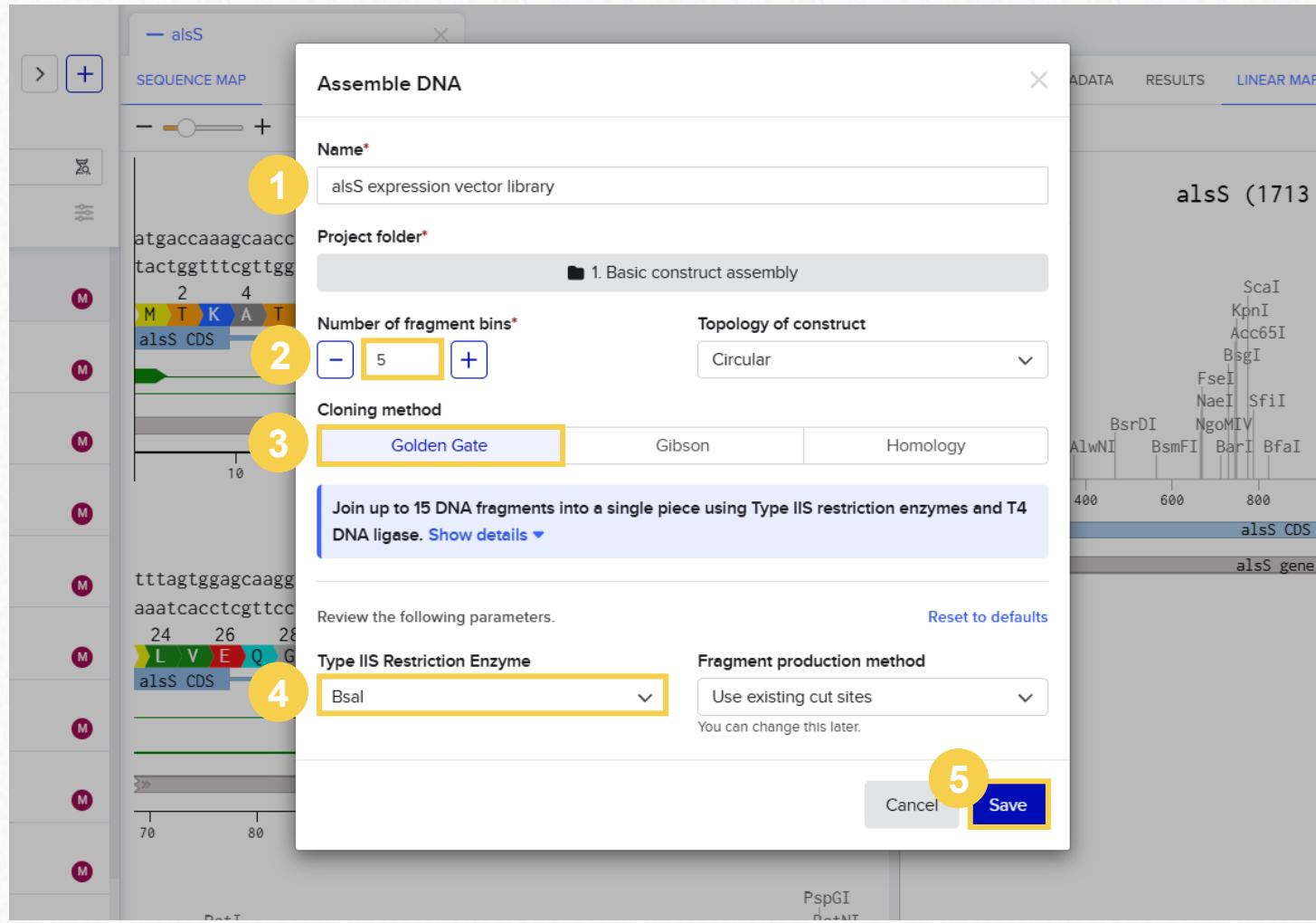
Assemble sequences and oligos by concatenation

BASES 1713

ASSEMBLY ▾ SPLIT WORKSPACE

Construct Assembly

Combinatorial Cloning Tool: Configuration



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 'alsS' project in the 'OVERVIEW' tab of the Combinatorial Cloning Tool. The interface is divided into several sections:

- Bins & Spacers (5):** A row of five bins labeled BIN 1 through BIN 5. Each bin has a delete icon, a settings icon, and a dropdown menu set to "Use existing cut sites". Each bin also has a "0 fragments" button with a plus sign.
- Constructs:** A dashed box labeled "Constructs" containing "0 constructs".
- Fragments:** A table with columns: Sequence, Bin, Start, End, Length, Orientation, Type IIS enzyme, Fragment production method, and Status. One row is present: Sequence 1, Bin 1, Start, End, Length, Orientation Forward, Type IIS enzyme BsAl, Fragment production method Use existing cut sites, and Status.
- Constructs:** A table with columns: Name, Backbone, Overhang, Insert 1, Overhang, Insert 2, Overhang, Insert 3, Overhang, and Insert 4. One row is present: Name 1, Backbone, Overhang, Insert 1, Overhang, Insert 2, Overhang, Insert 3, Overhang, and Insert 4.

Buttons at the top right include "Provide feedback" and "Assemble". On the left, there are icons for search, add, and other tools.

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

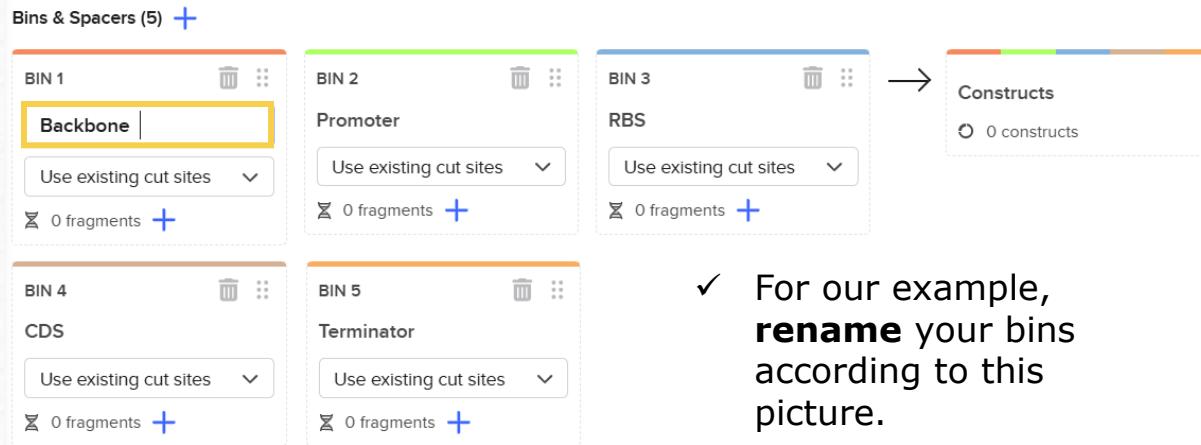
i All added fragments will show up here
(You can change some configurations)

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.



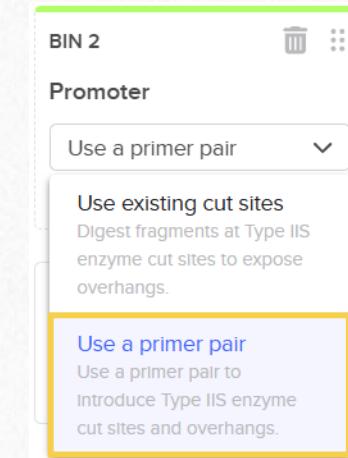
Bins & Spacers (5) +

- 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

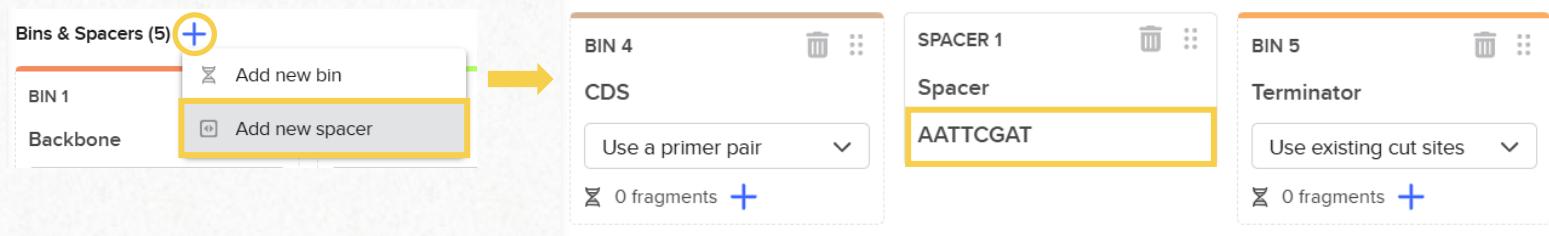
Use a primer pair

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
Add new spacer

BIN 4 CDS | Use a primer pair 0 fragments +

SPACER 1 Spacer | AATTCGAT

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 (6)' section. A dropdown menu is open over 'BIN 1' with the option 'Backbone' highlighted. Below it, the 'Add from worklist' option is also highlighted with a yellow box.

Add from worklist

- alsS EVL
- 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 for the 'pET-Ori-KanR' backbone. The sequence is shown as a circular map with two BsaI restriction sites. The region between them is selected. The 'Add' button is highlighted with a yellow box.

Add fragment(s)

pET-Ori-KanR

Start: 28, End: 3327, Orientation: Forward

BsaI

3.3 kb of 5.4 kb

View: Linear map

(5420 bp)

medium copy (pET) ori/KanR

pET-Ori-KanR

bla

ori

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.

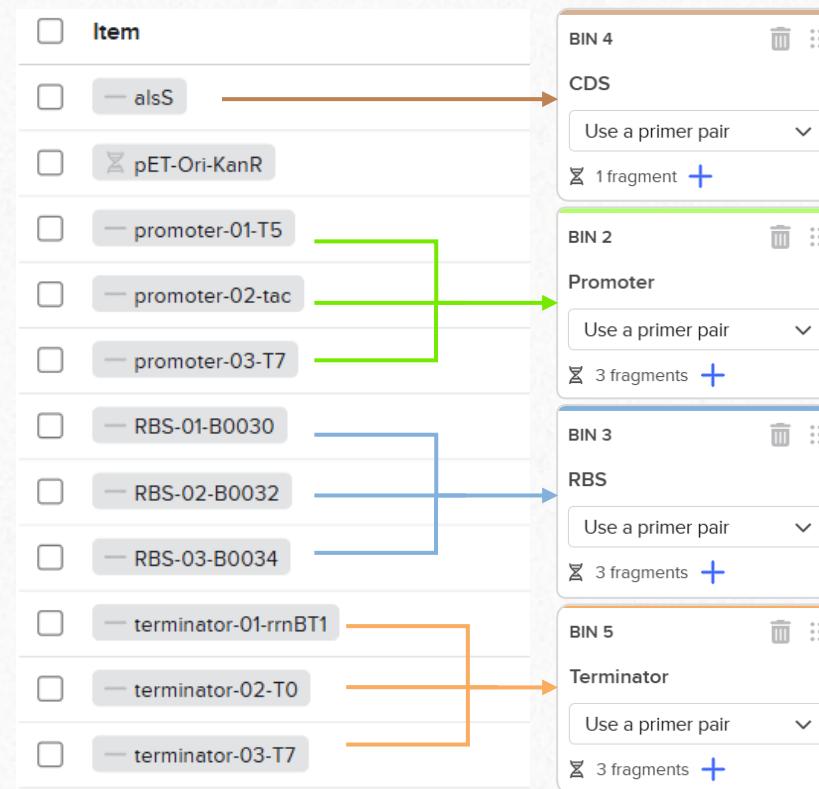
Construct Assembly

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.



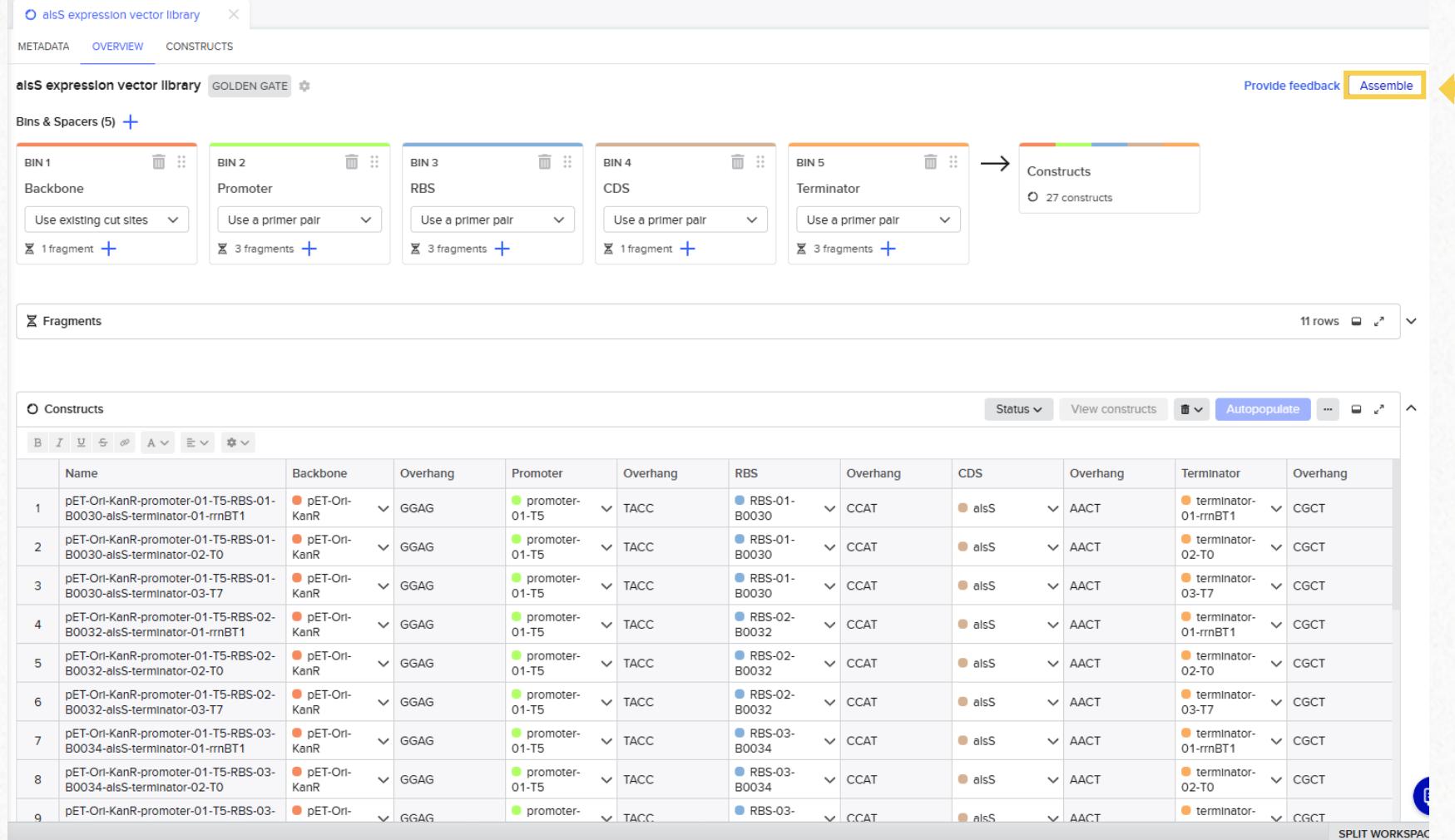
Constructs							
	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang
1		v		v		v	
1	Add rows						1 row

- ✓ 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 table titled "Constructs" with columns for Name, Backbone, Overhang, Promoter, Overhang, RBS, Overhang, CDS, Overhang, Terminator, and Overhang. The table lists 9 rows of construct details, such as pET-Ori-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-01-rnBT1. At the bottom right of the workspace, there are buttons for "Provide feedback" and "Assemble", with a yellow arrow pointing to the "Assemble" button.

	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 screenshot shows three sequential steps in the 'Assemble DNA' process:

- Step 1:** 'Save constructs' (highlighted in yellow), 'Save fragments', 'Save primers'. A note says 'Add constructs to a folder and optionally set a schema'. Options include 'Set location*' (Mia) and 'Set schema' (Plasmid). A checkbox 'Add constructs to a worklist' is present.
- Step 2:** 'Save constructs' (green checkmark), 'Save fragments' (blue circle), 'Save primers'. A note says 'Saving fragments is optional.' and a checkbox 'Create DNA Sequences to represent amplified fragments' is shown.
- Step 3:** 'Save constructs' (green checkmark), 'Save fragments' (green checkmark), 'Save primers' (blue circle). A note says 'Saving primers is optional.' and a checkbox 'Create DNA Oligos to represent newly designed primers' is shown.

In all steps, the 'Next' button is highlighted in yellow, except for the final step where the 'Assemble' button is highlighted in yellow.

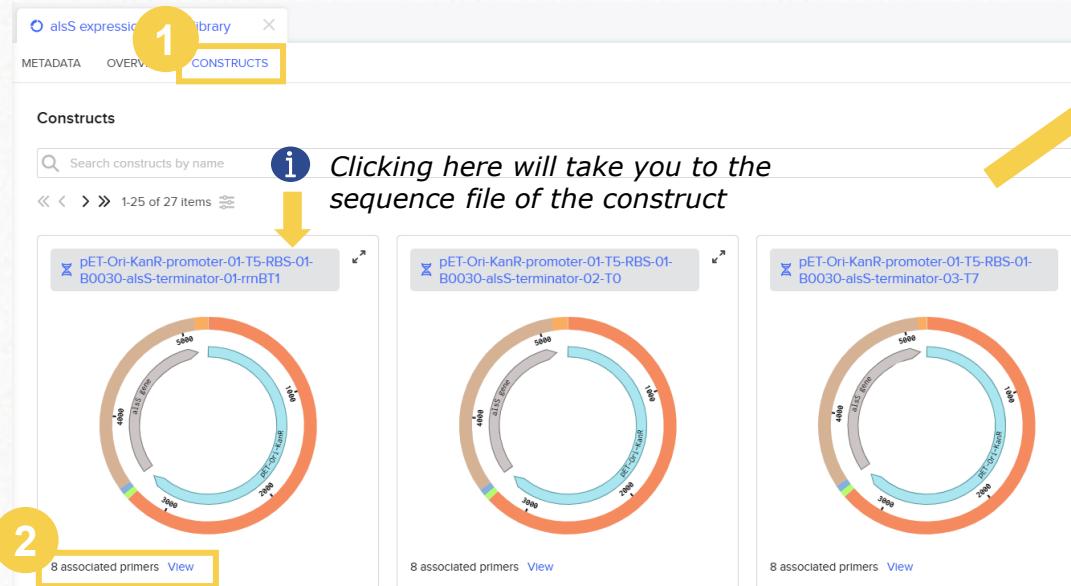
i You can choose whether to create files for every primer and related amplicon.

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

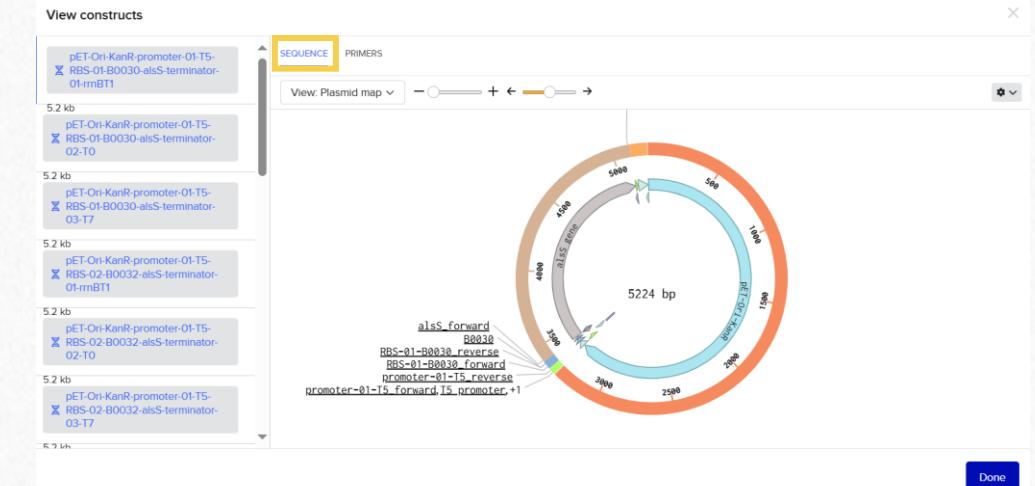
View constructs

SEQUENCE PRIMERS

Fragment	Orientation	Action	Primer	Bases	T _m whole (°C)
promoter-01-T5	5' primer	Design new primer	promoter-01-T5_forward	TTTCATGG... 44 bp	62.22
promoter-01-T5	3' primer	Design new primer	promoter-01-T5_reverse	TTTCATGG... 40 bp	62.08
RBS-01-B0030	5' primer	Design new primer	RBS-01-B0030_forward	TTTCATGG... 45 bp	59.16
RBS-01-B0030	3' primer	Design new primer	RBS-01-B0030_reverse	TTTCATGG... 44 bp	59.93
alsS	5' primer	Design new primer	alsS_forward	TTTCATGG... 34 bp	65.81
alsS	3' primer	Design new primer	alsS_reverse	TTTCATGG... 34 bp	63.86
terminator-01-rmBT1	5' primer	Design new primer	terminator-01-rmBT1_forward	TTTCATGG... 33 bp	69.64
terminator-01-rmBT1	3' primer	Design new primer	terminator-01-rmBT1_reverse	TTTCATGG... 40 bp	68.77

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

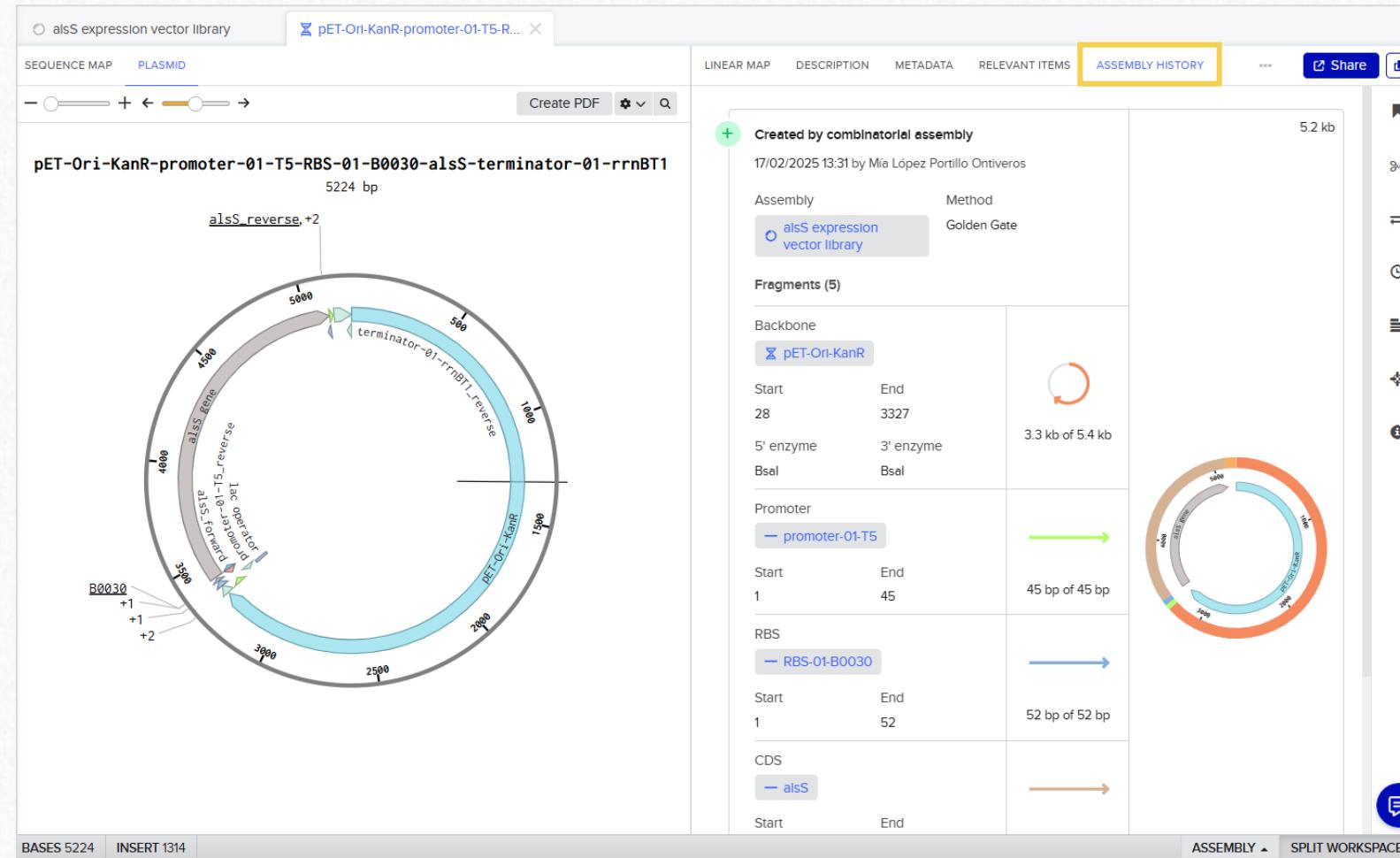
Sequence view



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.

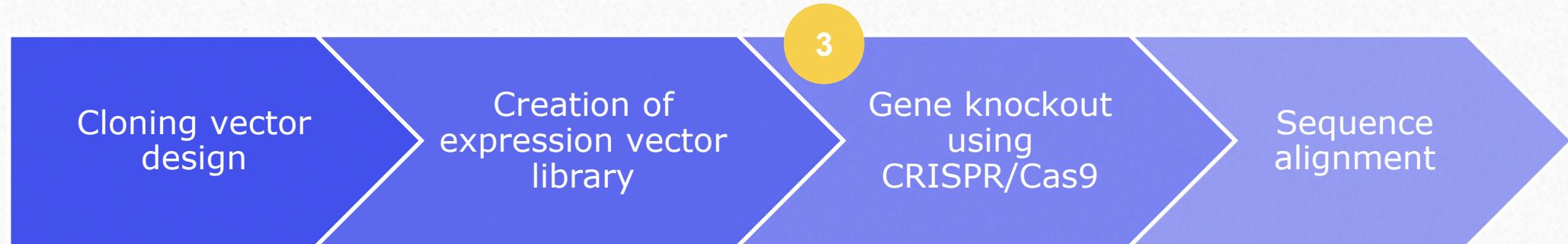


7. CRISPR tools

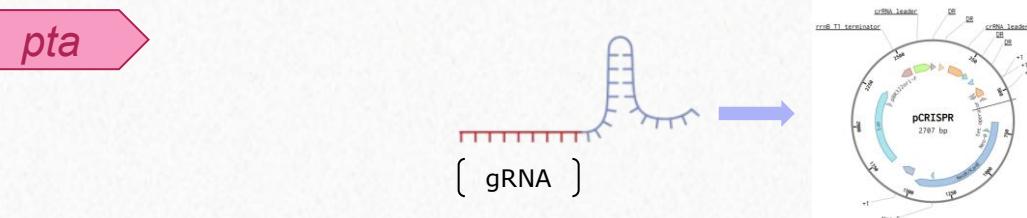




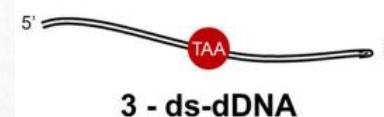
This is the third part of the *hands-on* example.



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



HR template design to KO *pta*



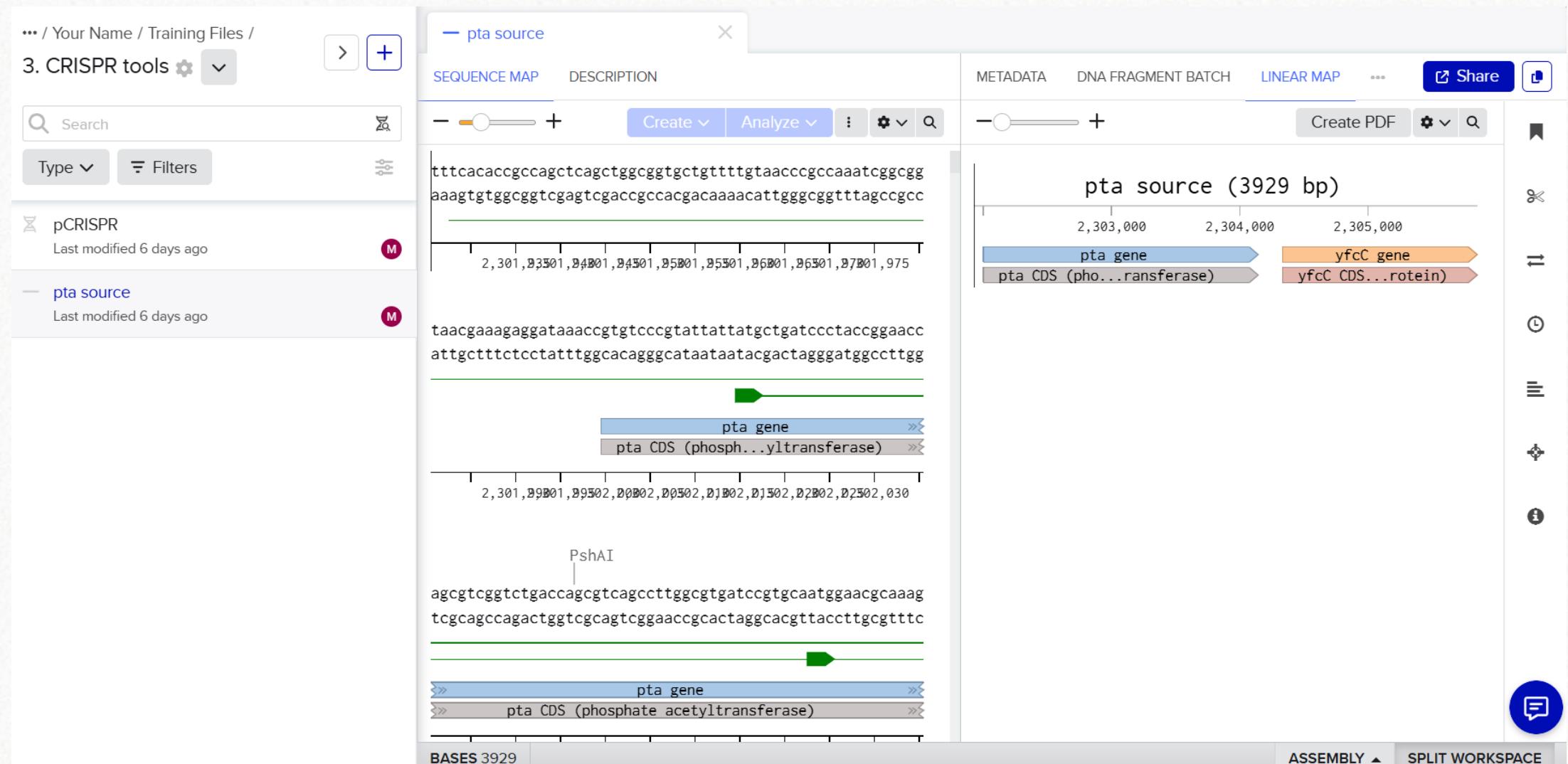
- ✓ gRNA design
- ✓ HR template design

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*. *Protocol*, 8(2), e2688. <https://doi.org/10.21769/BioProtoc.2688>

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



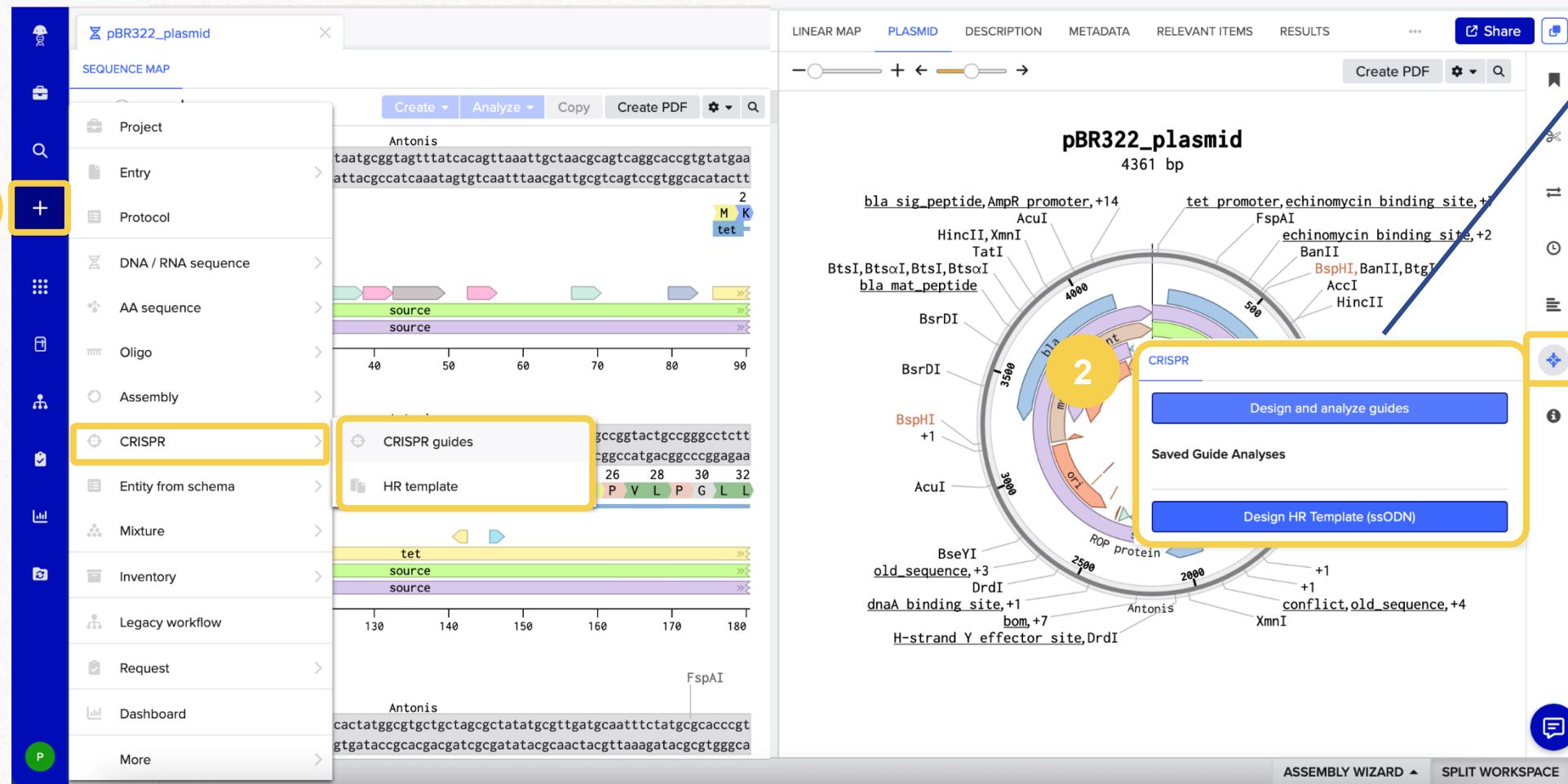
The screenshot shows the CRISPR tools interface with the following details:

- 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...ransferase)" is highlighted in grey.
 - PshAI:** A sequence with one gene: "pta gene" (blue arrow) and "pta CDS (phosphate acetyltransferase)" (grey arrow).
- Bottom Status Bar:** Shows "BASES 3929", "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:

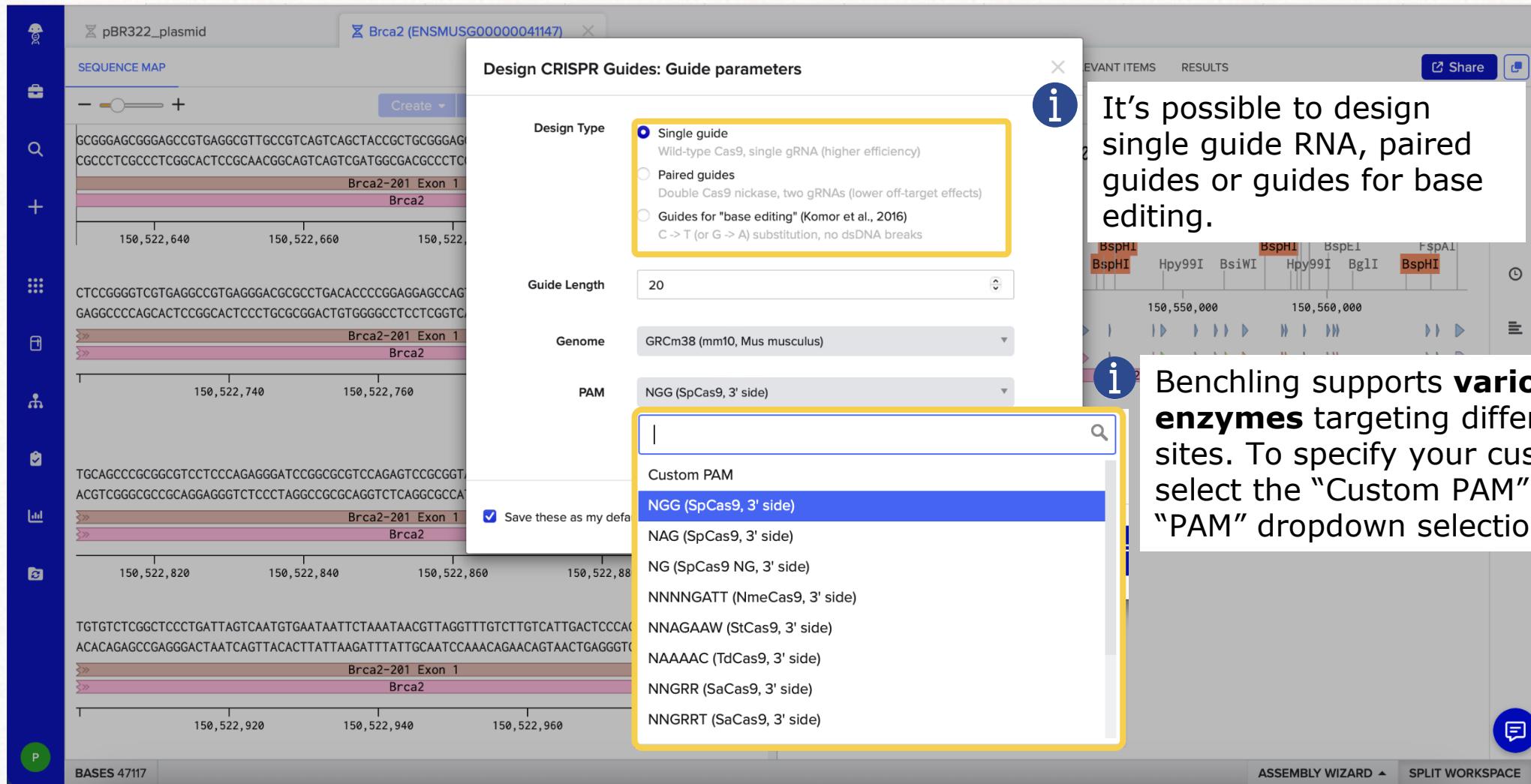


The screenshot shows the Benchling software interface. On the left, there is a sidebar with various tools and a main workspace. In the main workspace, a plasmid named "pBR322_plasmid" is shown as a circular map with restriction sites and gene regions. A yellow circle labeled "1" highlights the "+" button in the sidebar, which is used to add new entries. Another yellow circle labeled "2" highlights the "CRISPR" section in the sidebar, which is expanded to show "CRISPR guides" and "HR template". A yellow box surrounds the "CRISPR guides" and "HR template" sections. A blue arrow points from the text on the right to the "CRISPR" section in the sidebar.

By default, Benchling will use the open sequence as to design the gRNA on

CRISPR tools

Tool overview



The screenshot shows the Benchling CRISPR tool interface. On the left, a sequence map of the *Brca2* gene is displayed across four exons. The top exon is labeled "Brca2-201 Exon 1" and the bottom exon is labeled "Brca2". The genome version is GRCm38 (mm10, *Mus musculus*). A "Design CRISPR Guides: Guide parameters" dialog is open in the center. The "Design Type" section has three options: "Single guide" (selected), "Paired guides", and "Guides for 'base editing' (Komor et al., 2016) C > T (or G > A) substitution, no dsDNA breaks". The "Guide Length" is set to 20. The "PAM" dropdown is set to "NGG (SpCas9, 3' side)". A search bar below the dropdown shows "NGG (SpCas9, 3' side)" highlighted. A checkbox "Save these as my default" is checked. The "SEQUENCE MAP" tab is selected. In the top right, there are tabs for "EVENT ITEMS" and "RESULTS". A "Share" button and a "Print" icon are also present.

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: 20

Genome: GRCm38 (mm10, *Mus musculus*)

PAM: NGG (SpCas9, 3' side)

Save these as my default

SEQUENCE MAP

EVENT ITEMS **RESULTS**

Share

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



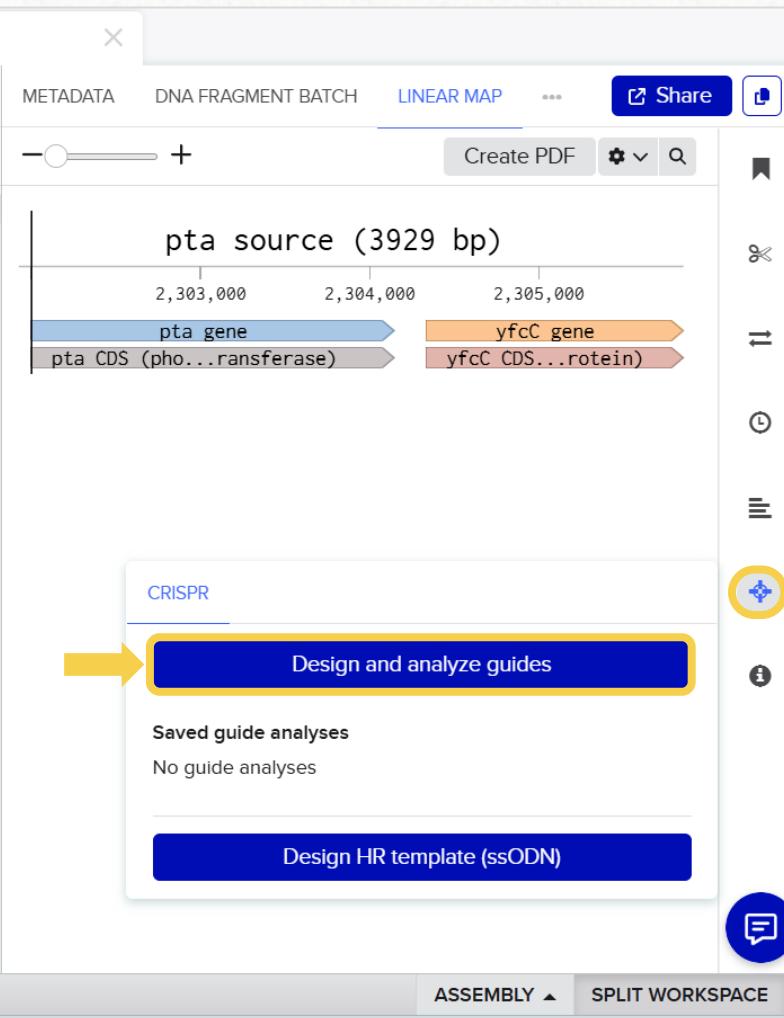
CRISPR tools

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: a Sequence Map on the left and a Design CRISPR tab on the right.

Sequence Map Panel:

- Top Bar:** SEQUENCE MAP, DESCRIPTION, Create, Analyze, Settings, Save.
- Sequence View:** Shows DNA sequence with a target region highlighted by a black bracket. A yellow arrow points from the text "Click the + button to generate gRNA candidates." to this bracket.
- Annotations:** pta gene, pta CDS (phosph...yltransferase).
- Coordinates:** 2,301, 23501, 24001, 24501, 25001, 25501, 26001, 26501, 27001, 975 at the top; 2,301, 23901, 239502, 24002, 240502, 241002, 241502, 242002, 242502, 2430 at the bottom.
- PshAI Site:** PshAI is indicated above a restriction enzyme site.

Design CRISPR Tab:

- Metadata:** pta gRNA, Save, Settings.
- Target Region:** To get started, create a target region by selecting it on the sequence map and pressing +. A blue arrow points from the text "Click the + button to generate gRNA candidates." to the "+" button.
- Target Region Input:** 2302000, 2304144, +.
- Buttons:** + (highlighted with a yellow box), -.

CRISPR tools

gRNA design

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

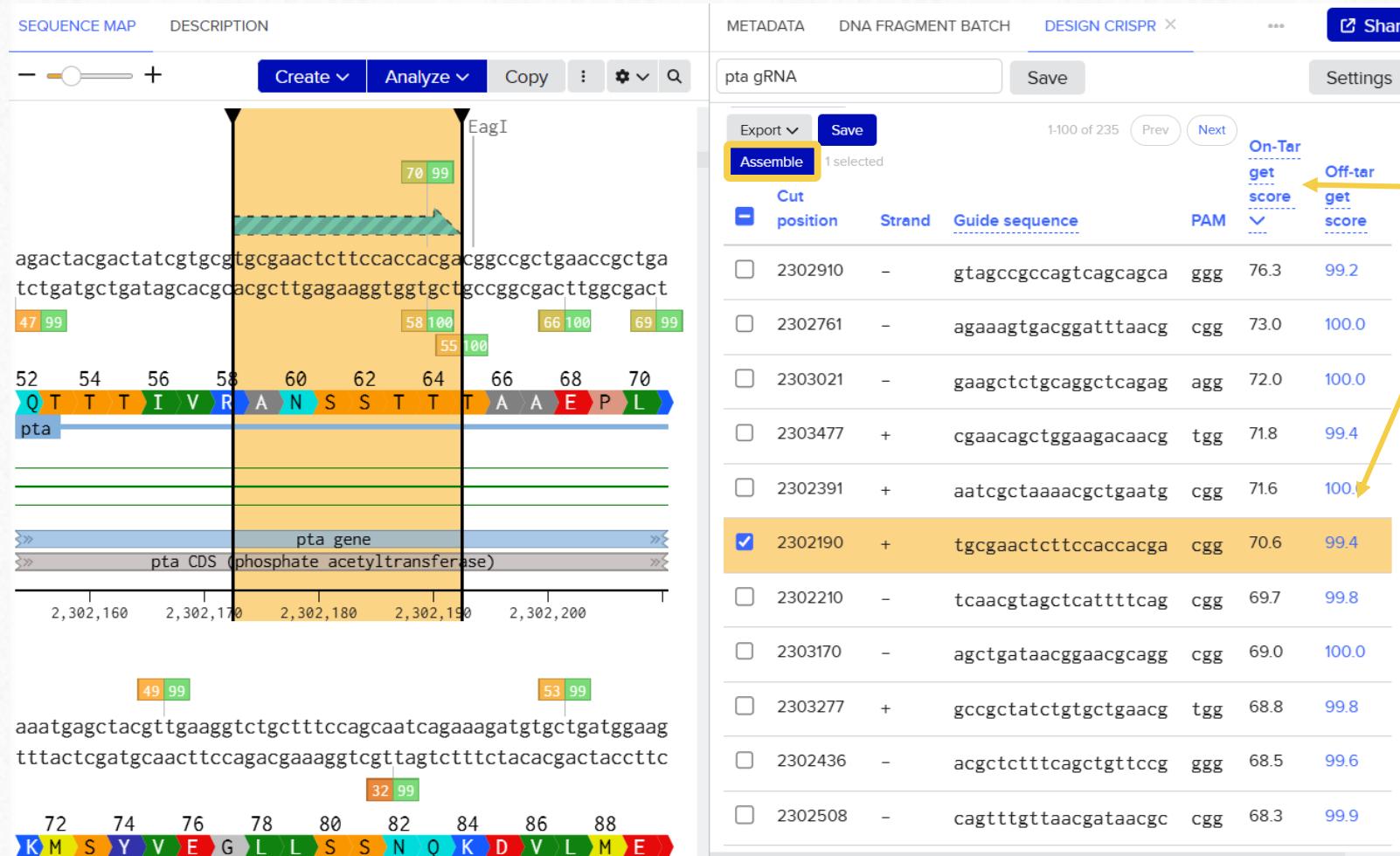
The screenshot shows the CRISPR tools interface with the following components:

- SEQUENCE MAP**: A sequence map showing a target DNA sequence with several potential gRNA binding sites marked by orange boxes with coordinates (e.g., 57 49, 56 50, 35 49, 56 50, 51 49, 38 49).
- DESCRIPTION**: A detailed description of the target gene, "pta gene", which encodes "pta CDS (phosphate acetyltransferase)".
- METADATA**: Fields for "Target" (2302000, 2304144) and "region".
- DESIGN CRISPR**: A panel showing the selected target regions and a message: "No region set".
- Genome region**: A modal dialog with the following content:
 - Setting a genome region will remove it from off-target analysis.
 - Options: None (radio button), Chromosome (radio button, selected), and a range input field showing "2302000" to "2304144" (both highlighted with a yellow box).
 - Buttons: "Find genome matches" (with a warning icon), "Set genome region" (highlighted with a yellow box), and "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, a "SEQUENCE MAP" panel displays a DNA sequence with a restriction site (EagI) at position 2,302,190. A protein sequence for "pta" is shown below the DNA, with amino acid positions 52 to 70 labeled. Two gene regions are highlighted: "pta gene" and "pta CDS (phosphate acetyltransferase)". Below the sequence map, a portion of the sequence is shown with mutations at positions 49, 53, and 32. On the right, a "DESIGN CRISPR" panel lists 235 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. The 11th row, corresponding to the gRNA at position 2302190, is highlighted with a blue checkmark and a yellow background. This row has a "PAM" sequence of "cg", an "On-Tar get score" of 70.6, and an "Off-tar get score" of 99.4.

Cut position	Strand	Guide sequence	PAM	On-Tar get score	Off-tar get score
2302910	-	gtagccgccagtca	ggc	76.3	99.2
2302761	-	agaaaagtgcggat	cgg	73.0	100.0
2303021	-	gaagctctgcaggct	agg	72.0	100.0
2303477	+	cgaacagctgaa	aac	71.8	99.4
2302391	+	aatcgctaaaacgct	atg	71.6	100.0
2302190	+	tgcgaaactttccacc	cg	70.6	99.4
2302210	-	tcaacgtagctcat	ttc	69.7	99.8
2303170	-	agctgataacgg	acgc	69.0	100.0
2303277	+	gccgctatctgtgt	aa	68.8	99.8
2302436	-	acgcctttcagctt	cc	68.5	99.6
2302508	-	cagtttgttaacgata	acgc	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.

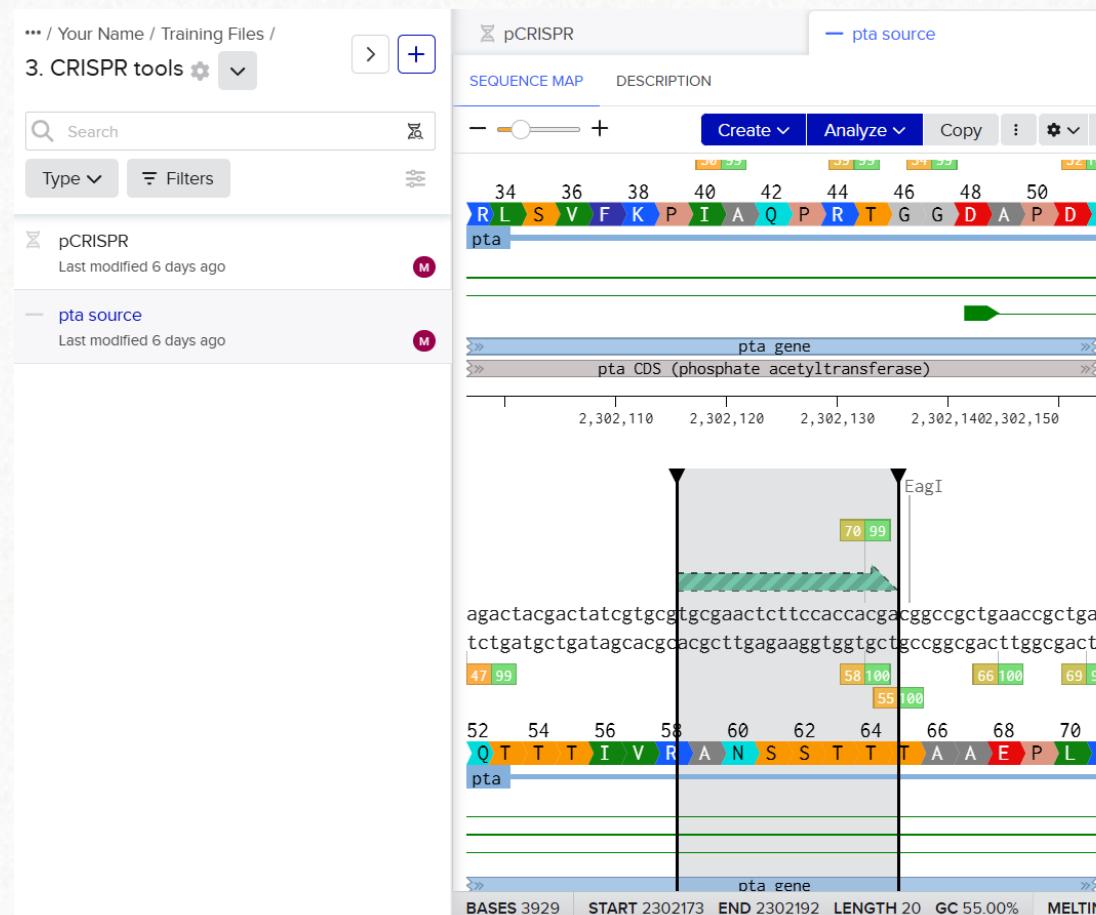
The screenshot shows the CRISPR tools interface. On the left, a sidebar lists files: 'pCRISPR' and 'pta source'. A yellow arrow points from the 'pCRISPR' file to the 'ASSEMBLE CRISPR' dialog box. The main area displays a sequence map of the 'pta' gene, showing amino acid sequences and restriction enzyme sites like EagI. The 'ASSEMBLE CRISPR' dialog box is open, showing the 'Select Expression Vector' section. The 'Vector Source' dropdown is set to 'Choose a plasmid from your Benchling folders', which is highlighted with a yellow box and arrow. Below it are options for 'Select one of the bookmarked plasmids' and 'Upload a new plasmid'. A dashed box indicates where to search for a sequence in the file browser and drag it here. The 'Assembly Method' is set to 'Type IIS Cloning', and the 'Insertion Region' fields are empty. A 'Next' button is visible at the bottom right of the dialog box.

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

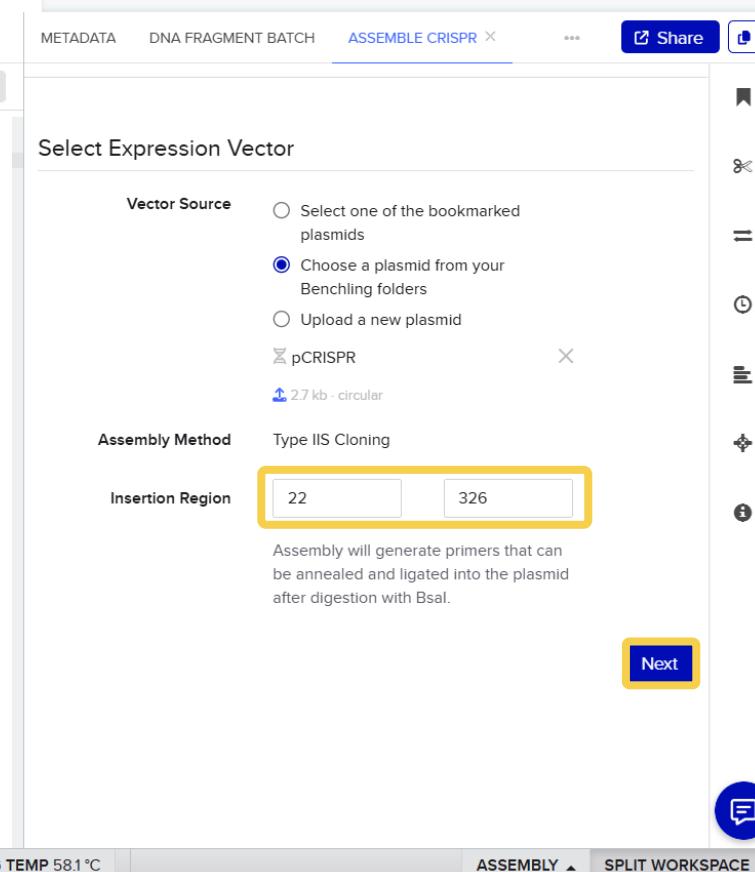
CRISPR tools

gRNA design

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



14 Click **Next**.



The figure shows the 'ASSEMBLE CRISPR' step in Benchling. The 'Vector Source' section has 'Choose a plasmid from your Benchling folders' selected. The 'Assembly Method' is set to 'Type IIS Cloning'. The 'Insertion Region' field is highlighted with a yellow box, showing values 22 and 326. A note below says: 'Assembly will generate primers that can be annealed and ligated into the plasmid after digestion with Bsal.' A 'Next' button is visible.

 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.

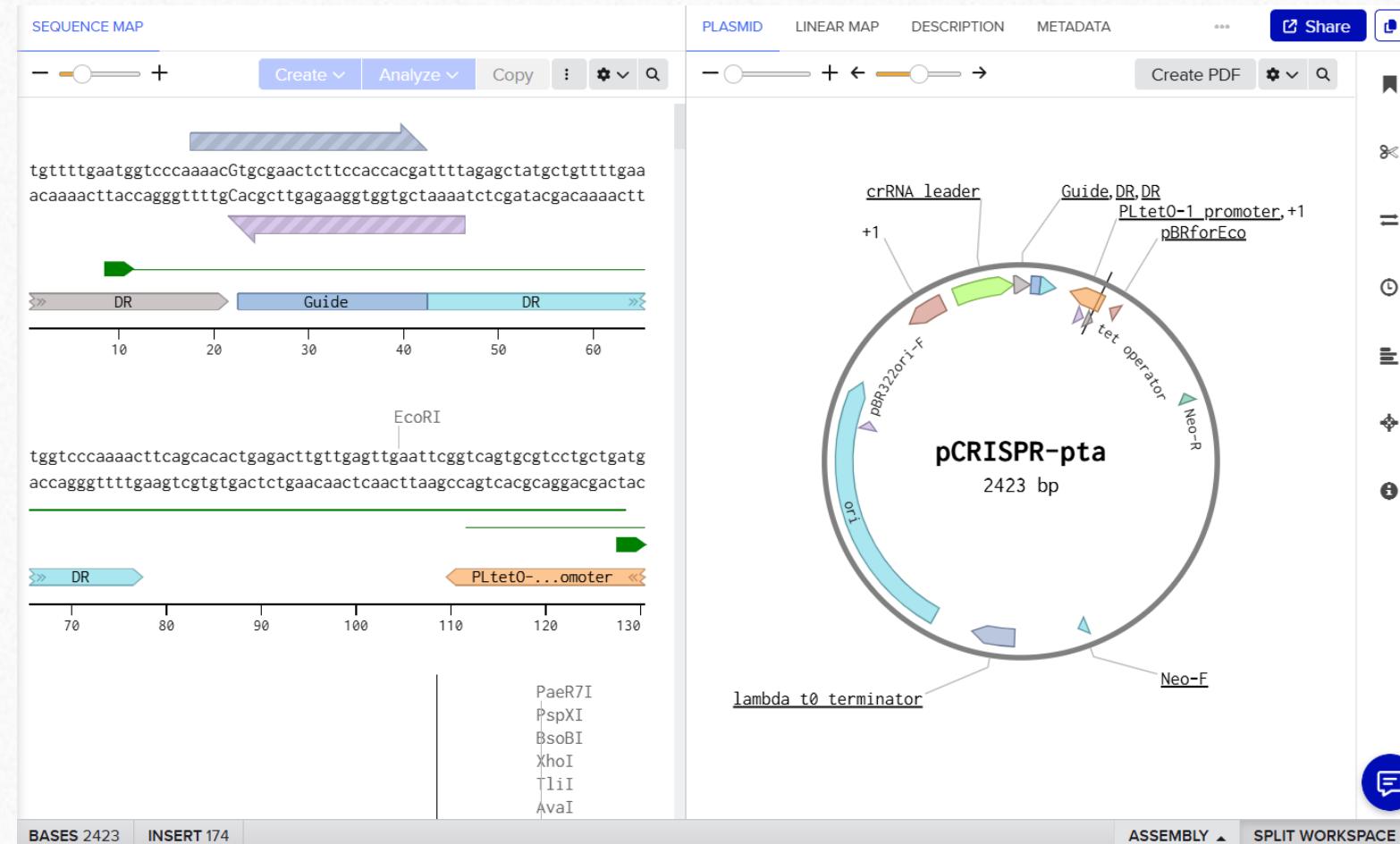
Sequence Map: This section displays two DNA sequence regions. The top region is labeled "pta" and shows a restriction site for "EagI" at position 66. Below the sequence, the "pta gene" and "pta CDS (phosphate acetyltransferase)" are indicated. The bottom region also shows the "pta" gene and CDS. Both regions have coordinate markers from 2,302,160 to 2,302,200.

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 large 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 two panels of the pCRISPR software. The left panel displays the 'pta source' sequence map with three main regions: 'pta gene' (blue arrow), 'pta CDS (phosph...yltransferase)' (grey arrow), and 'yfcC gene' (orange arrow). The right panel shows the 'Design HR template' menu, which includes fields for 'Genome' (ASM956v1 (Escherichia coli BL21(DE3))), 'PAM' (NGG (SpCas9, 3' side)), and options for 'Create a copy of this sequence' (selected) or 'Modify this sequence'. A yellow arrow points to the 'Design HR template (ssODN)' button at the bottom of the menu.

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

A detailed view of the 'Design HR template' dialog box. It shows the genome set to 'ASM956v1 (Escherichia coli BL21(DE3))' and the PAM sequence set to 'NGG (SpCas9, 3' side)'. There are 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.



SEQUENCE MAP DESCRIPTION

pta gene

EagI

pta CDS (phosphate acetyltransferase)

2,302,130 2,302,140 2,302,150 2,302,160 2,302,170 2,302,180

accacgacggccgtgaaccgctgaaaatgagctacgttgaaggctgtctgtttccaggcaatcagaaatgggtctgcggccactggcactttactgtgcaactccagacaaaggtcgttagtt

64 66 68 70 72 74 76 78 80 82 84

T T T A A E P L K M S Y V E G L L S S N Q K

pta

pta gene

pta CDS (phosphate acetyltransferase)

2,302,240

agatgtgctg
tctcacacac
86 D V L M E I V A N Y H A N I K D A E V V

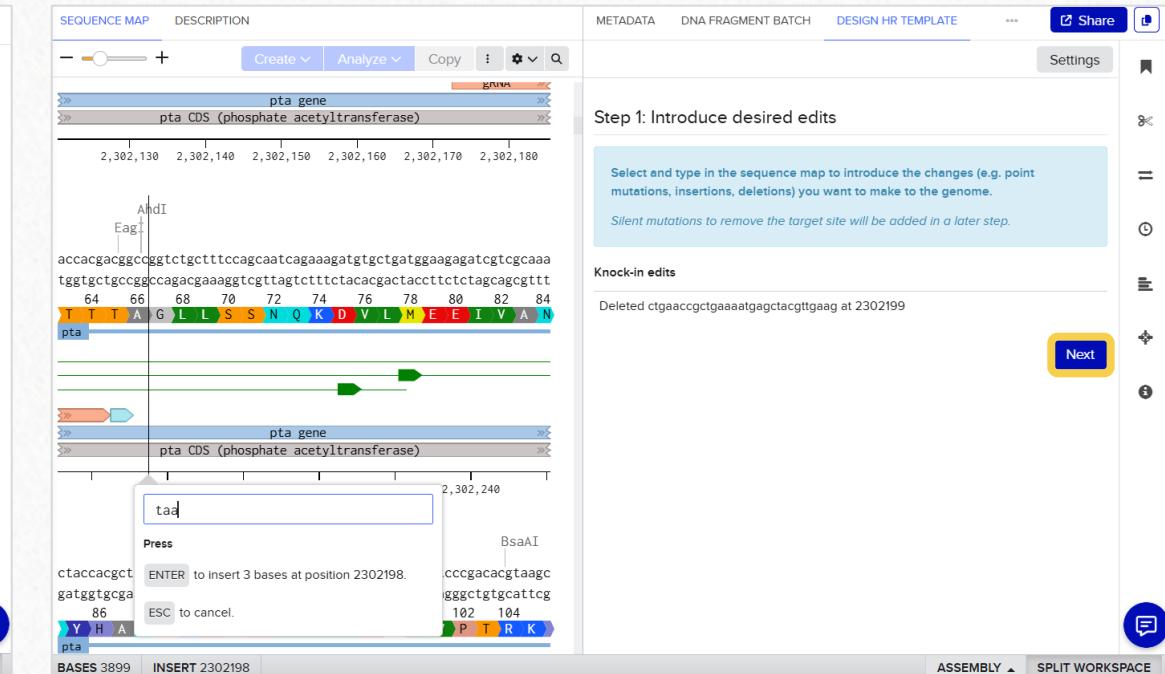
Press ENTER to delete 30 bases at position 2302198.
ESC to cancel.

102 104

BASES 3929 START 2302198 END 2302227 LENGTH 30 GC 46.67% MELTING TEMP 63.3 °C

ASSEMBLY SPLIT WORKSPACE

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



SEQUENCE MAP DESCRIPTION

pta gene

EagI

pta CDS (phosphate acetyltransferase)

2,302,130 2,302,140 2,302,150 2,302,160 2,302,170 2,302,180

accacgacggccgtctttccaggcaatcagaaaggatgtctgtatggaaaggatcgctgcggaaatgggtctgcggccactggcactttactgtgcaactccagacaaaggtcgttagtt

64 66 68 70 72 74 76 78 80 82 84

T T T A G L S S N Q K D V L M E I V A N

pta

pta gene

pta CDS (phosphate acetyltransferase)

2,302,240

taa

Press ENTER to insert 3 bases at position 2302198.
ESC to cancel.

102 104

BsaAI

ctaccacgct gatggtgca
86 Y H A

ccgcacacgtaaac
gggttgtgtcattcg
102 104 P T R K

BASES 3899 INSERT 2302198

ASSEMBLY SPLIT WORKSPACE

- 6** Click **Next**.

CRISPR tools

HR template design

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

SEQUENCE MAP DESCRIPTION

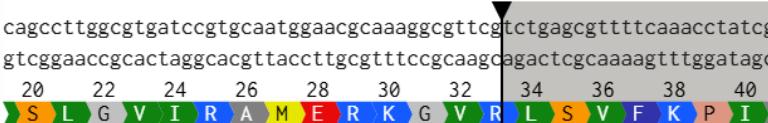
- Editing disabled because of mutations Copy Create PDF ⚙️ 🔍

```
caggctggcgtatccgtcaatggAACGAAAGCGTTCTGAGCGTTTCAAACCTATCG  
gtcgAACCGCACTAGGCACGTACCTGCGTTCCGAAGCAGACTCGAAAAGTTGGATAGC
```

20 22 24 26 28 30 32 34 36 38 40

S L G V I R A M E R K G V R L S V F K P I

pta



pta gene

pta CDS (phosphate acetyltransferase)

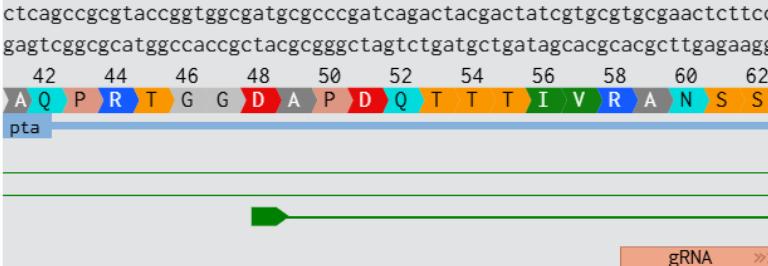
2,302,070 2,302,080 2,302,090 2,302,100 2,302,110

```
ctcagccgcgtaccgggtggcgatgcgcggcatcactacgtactatcgatcgacttcc  
gagtcggcgcatggccaccgctacggggtagtctgtatgcgtatgcacgcacgttgagaagg
```

42 44 46 48 50 52 54 56 58 60 62

A Q P R T G G D A P D Q T T T I V R A N S S

pta



pta gene

pta CDS (phosphate acetyltransferase)

2,302,130 2,302,140 2,302,150 2,302,160 2,302,170 2,302,180

METADATA DNA FRAGMENT BATCH DESIGN HR TEMPLATE ... Share

Step 2: Adjust HR arms

Adjust the region to use as the HR template by clicking and dragging the ends of the selection on the sequence map.

A 200 bp region around your mutations has already been selected. At least 50 bp on each side flanking the mutations is recommended.

Template region

- Reset to default

Template Length: 200 bp
 Left arm length: 100 bp
 Right arm length: 99 bp

Knock-in edits

Deleted gctgaaccgctaaaaatgagctacgt at 2302198
 Deleted g at 2302199

Back
Next

7 Click **Next**.

CRISPR tools

HR template design

8 Paste the gRNA sequence: tgcgaaactttccaccacga

The screenshot shows the CRISPR tools interface. The top navigation bar has tabs for Sequence Map, Description, Metadata, DNA Fragment Batch, DESIGN HR TEMPLATE (selected), Linear Map, Share, and Settings. Below the tabs, a note says "Editing disabled because the sequence is being used in a template". There are buttons for Copy, Create PDF, and a dropdown menu.

Step 3: Remove target site

Confirm the mutated bases and click **Next** to continue. Modify the mutations by clicking on the synonymous codons below.

Our default suggestion is to mutate the PAM, unless it occurs in a translation with no possible silent mutations. In that case, we try to introduce 2 mutations in the guide sequence, selecting codons to keep the **CAI** close to the original value.

tgcgaaactttccaccacga

	R	A	N	S	S	T	T	T	A
Wildtype	cgt	gcg	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	gcg	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.

9 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](#).

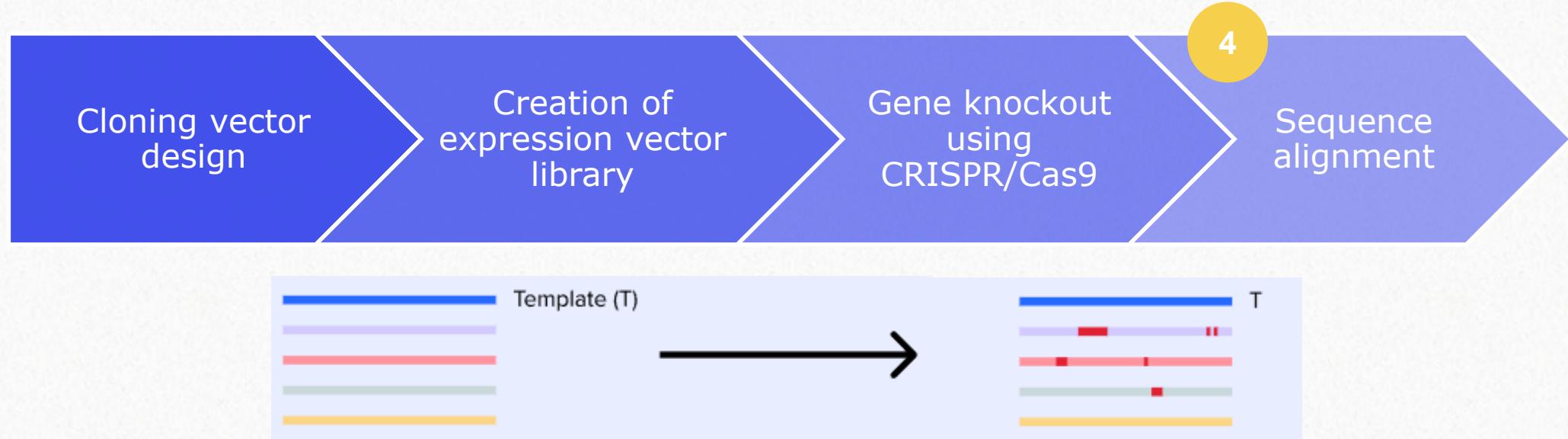
- ✓ After the design process, you can copy the resulting HR template and paste it onto a new DNA sequence file to save it.

8. Sequence alignments





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



✓ Multisequence alignment

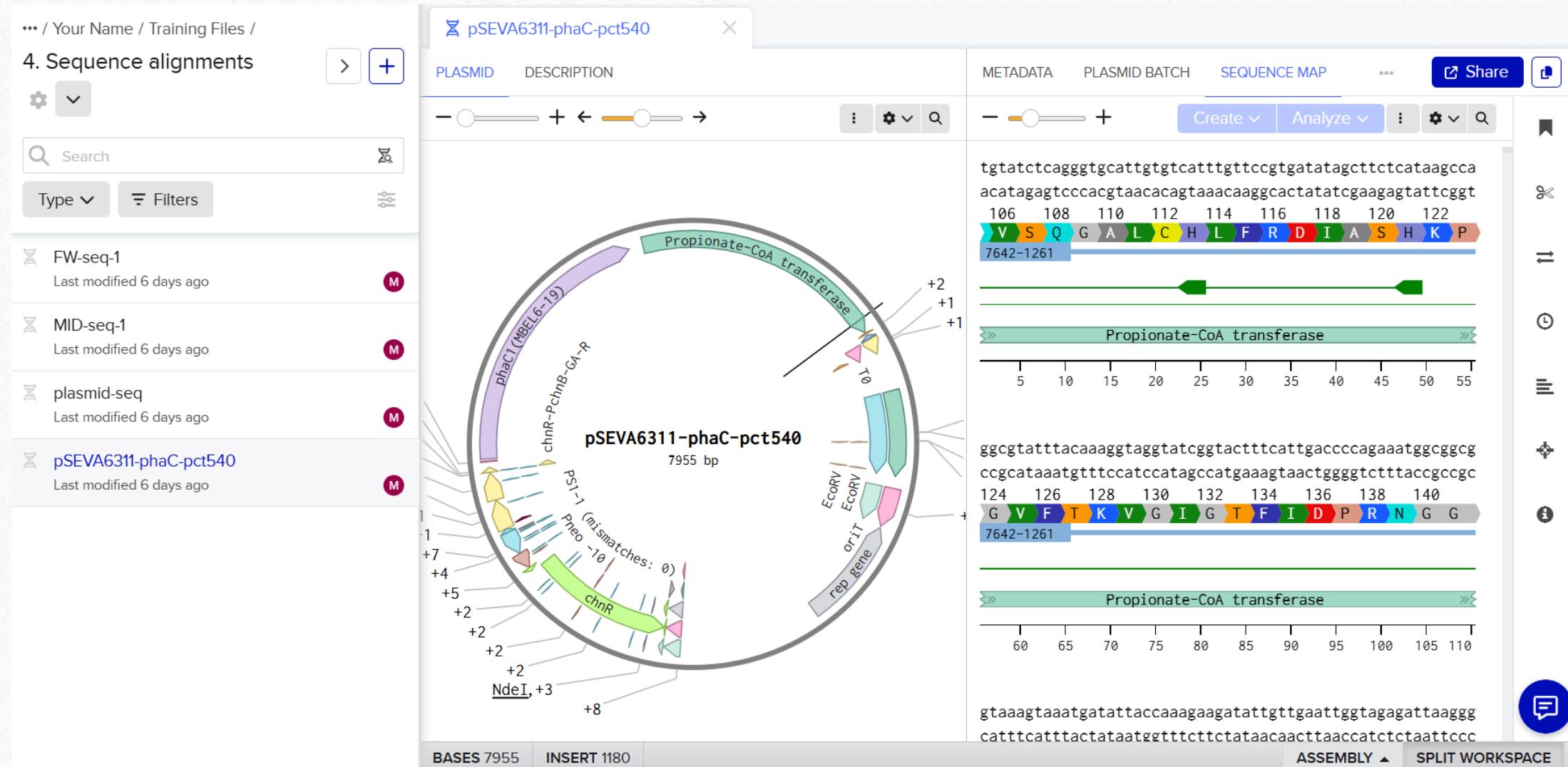
Bonus: How to do consensus alignments

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.



The screenshot shows the QIIME 2 interface for sequence alignments. On the left, a sidebar lists training files: FW-seq-1, MID-seq-1, plasmid-seq, and pSEVA6311-phaC-pct540. The main area displays a circular sequence map for the plasmid pSEVA6311-phaC-pct540 (7955 bp). The map highlights several genes: phaC1 (MBEL6-19), chnR-PchmB-GA-R, Propionate-CoA transferase, EcoRV, EcoRV, orIT, rep gene, and chnR. Insertions and deletions are indicated by arrows and numbers (+1, +2, +3, etc.). Below the map, sequence statistics are shown: BASES 7955 and INSERT 1180. To the right, two sequence alignments are presented for the Propionate-CoA transferase gene. The top alignment covers positions 7642-1261, and the bottom alignment covers positions 60-110. Both alignments show the DNA sequence with corresponding amino acid translations below. The alignments include various restriction enzyme sites (NdeI, PmeI, SphI, KpnI, EcoRI, EcoRV) and their locations relative to the insertion points.

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:

A screenshot of the 'Create DNA / RNA alignment' interface in Benchling. The interface has two main steps: 'Choose input' (step 1) and 'Define parameters' (step 2). Step 1 is completed, indicated by a green checkmark. Step 2 is active, indicated by a blue circle with the number 2. A yellow box highlights the 'Pairwise' tab, which is currently selected. Below the tabs, a diagram illustrates the 'Pairwise Alignment' process: a single 'Template (T)' sequence is aligned against multiple 'Non-template sequence(s)'. The alignment results show matches (blue bars) and mismatches (red bars). Below the diagram, there are input fields for 'Template(s)' and 'Non-template sequence(s)'. A dropdown menu allows choosing an alignment program, with 'Auto (MAFFT)' selected. At the bottom, a note states 'Alignments performed via MAFFT v7 (Katoh, Standley 2013)'.

1

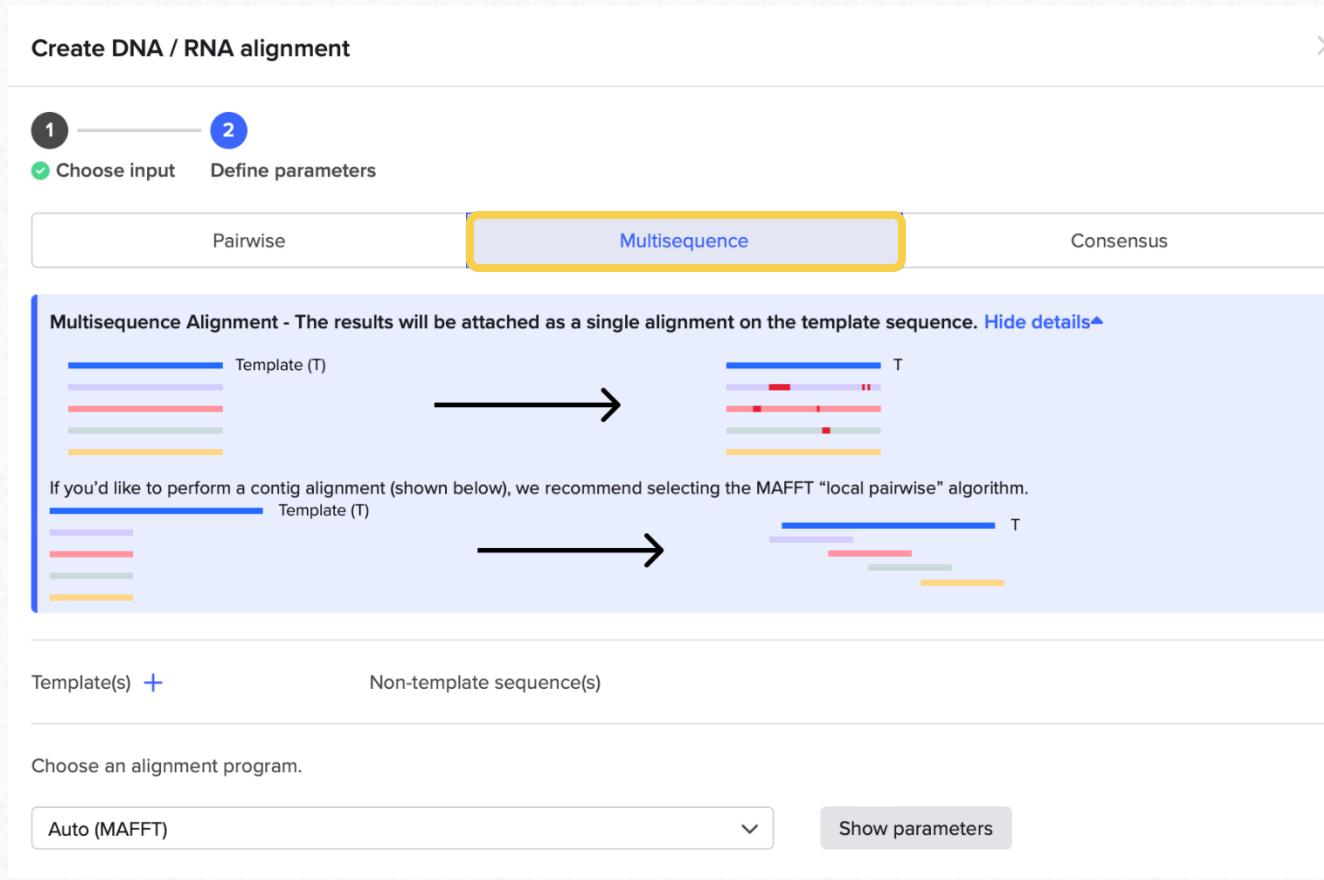
Pairwise alignment:

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:



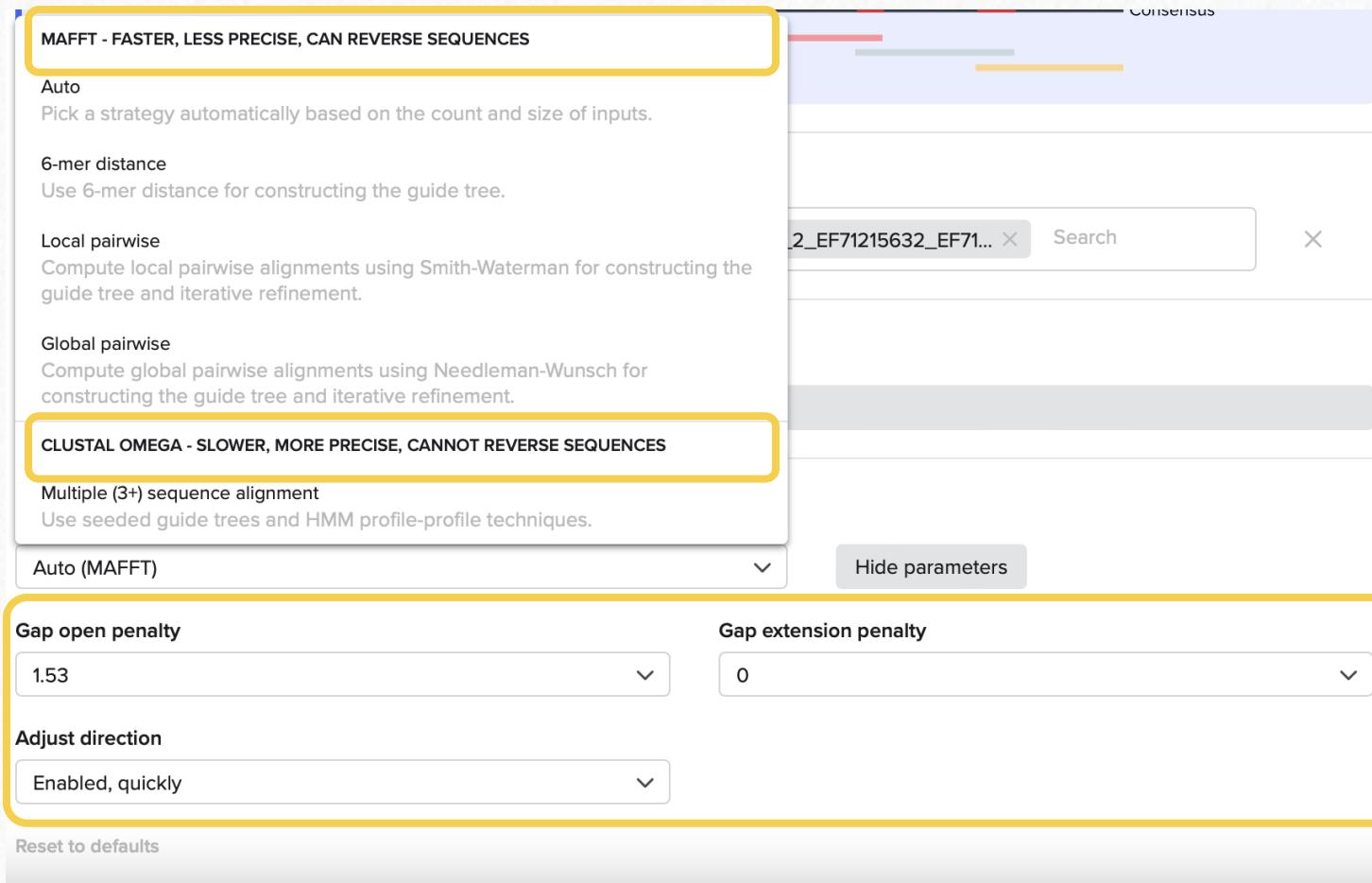
3

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, there's 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.

... / Your Name / Training Files /
4. Sequence alignments Saved Searches

Search Type Filters 1-4 of 4 items

4 rows selected More

Name	Inventory	ID	Modified	Authors	Description
FW-seq-1			11/02/2025		Create DNA / RNA Alignment
MID-seq-1			11/02/2025		Analyze
plasmid-seq	No inventory availa...		11/02/2025		Auto-Announce
pSEVA6311-phaC-pct540	No inventory availa...		11/02/2025		Attach Primers

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)  

pSEVA6311-phaC-pct540

Non-template sequence(s) 

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)

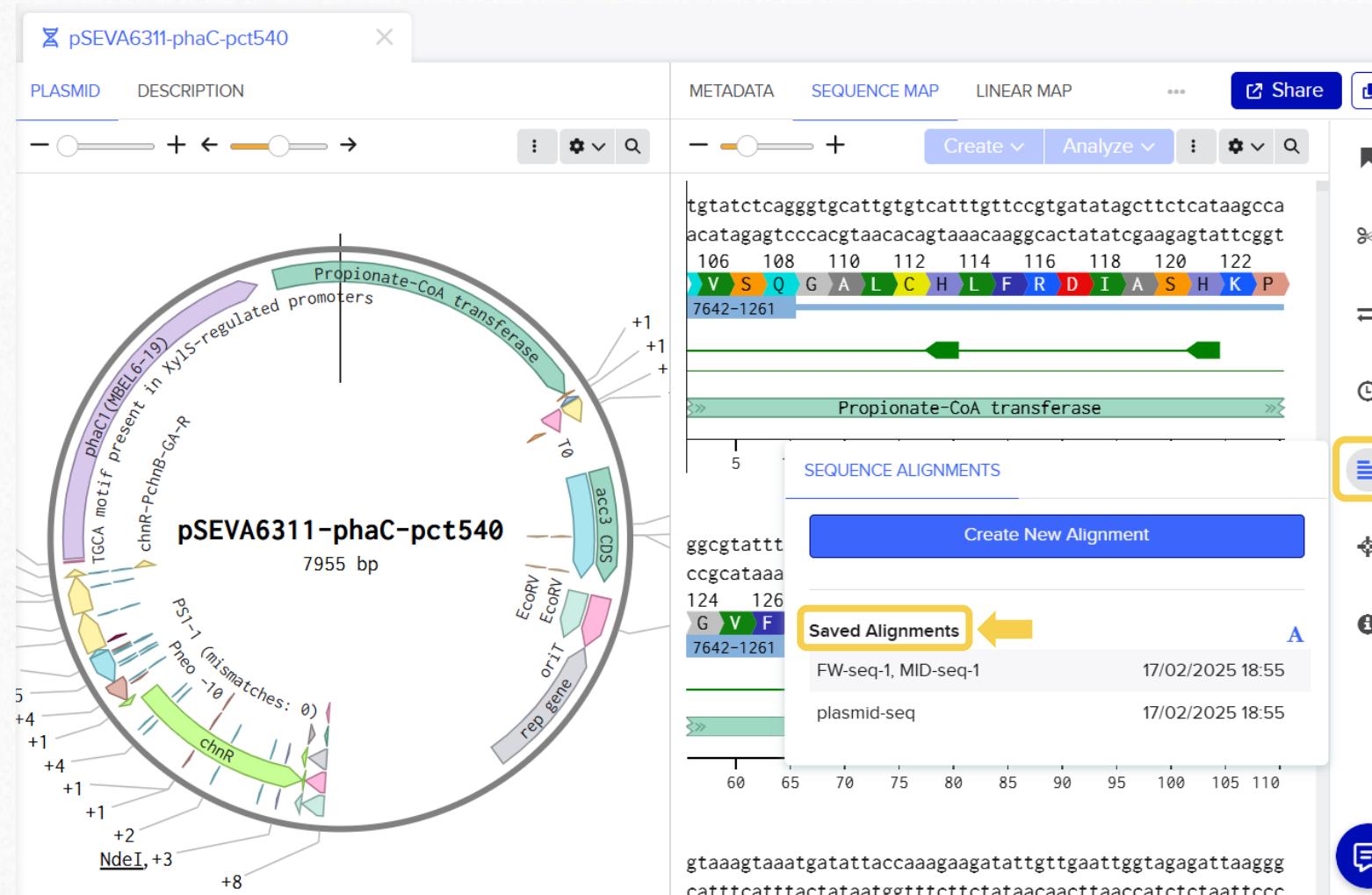
Alignments performed via MAFFT v7 (Katoh, Standley 2013).

- 5 Create the alignments.

Alignment creation

Multisequence alignment

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

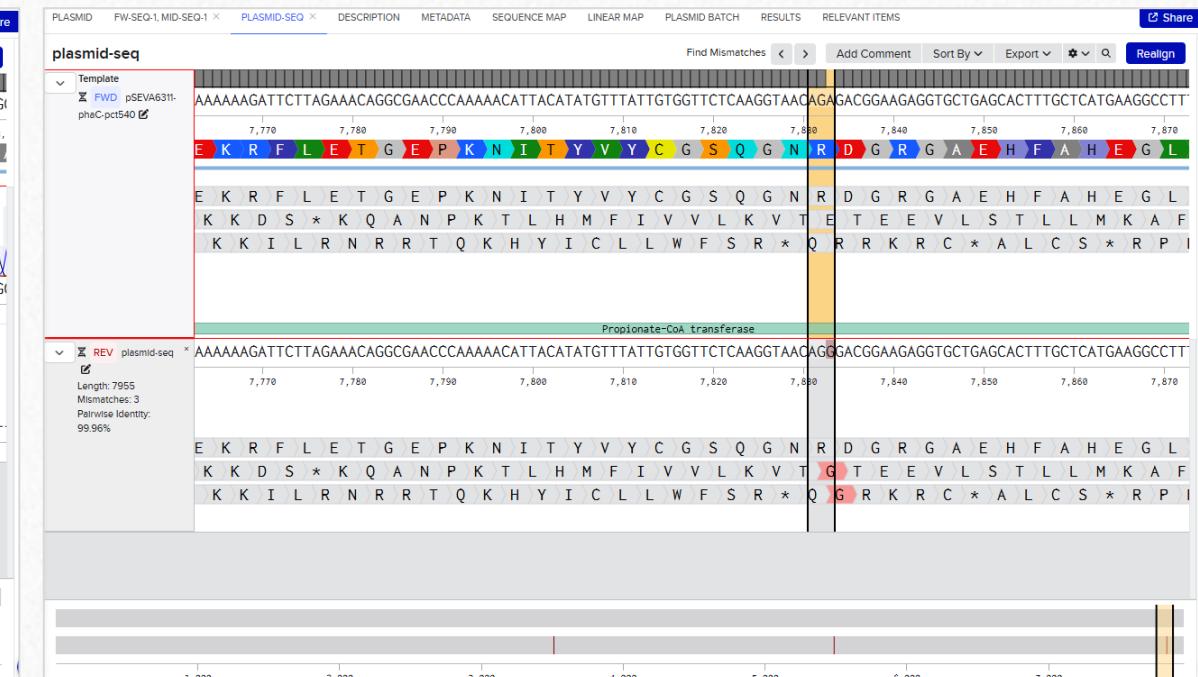
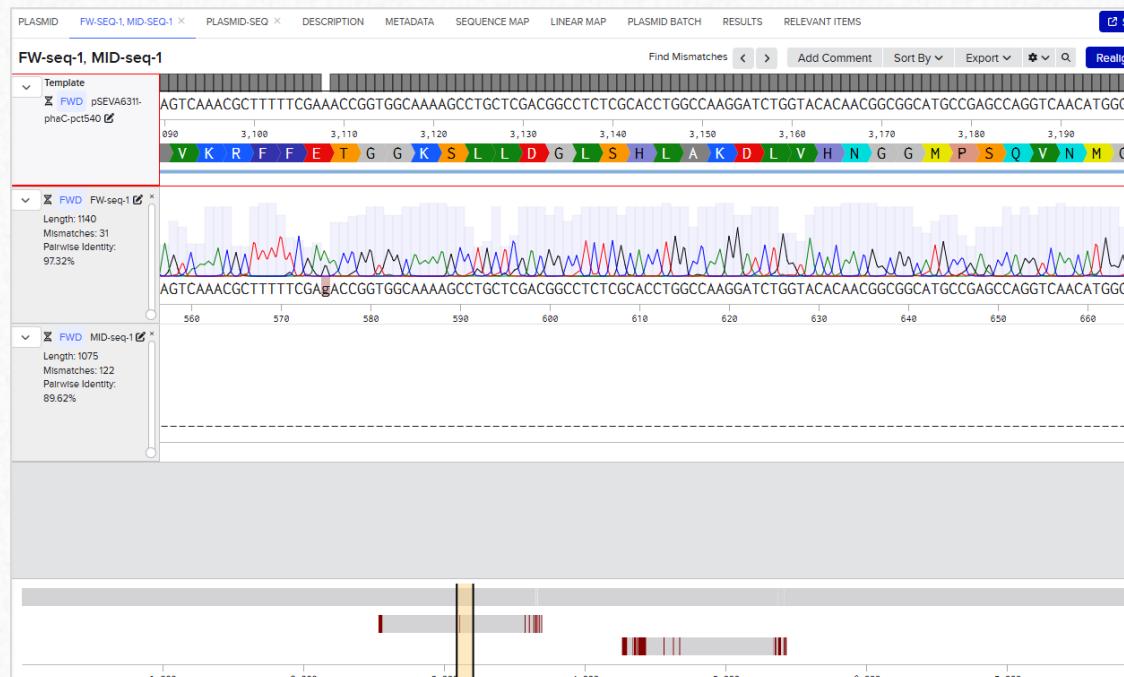


- 7 Open the alignments.

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.



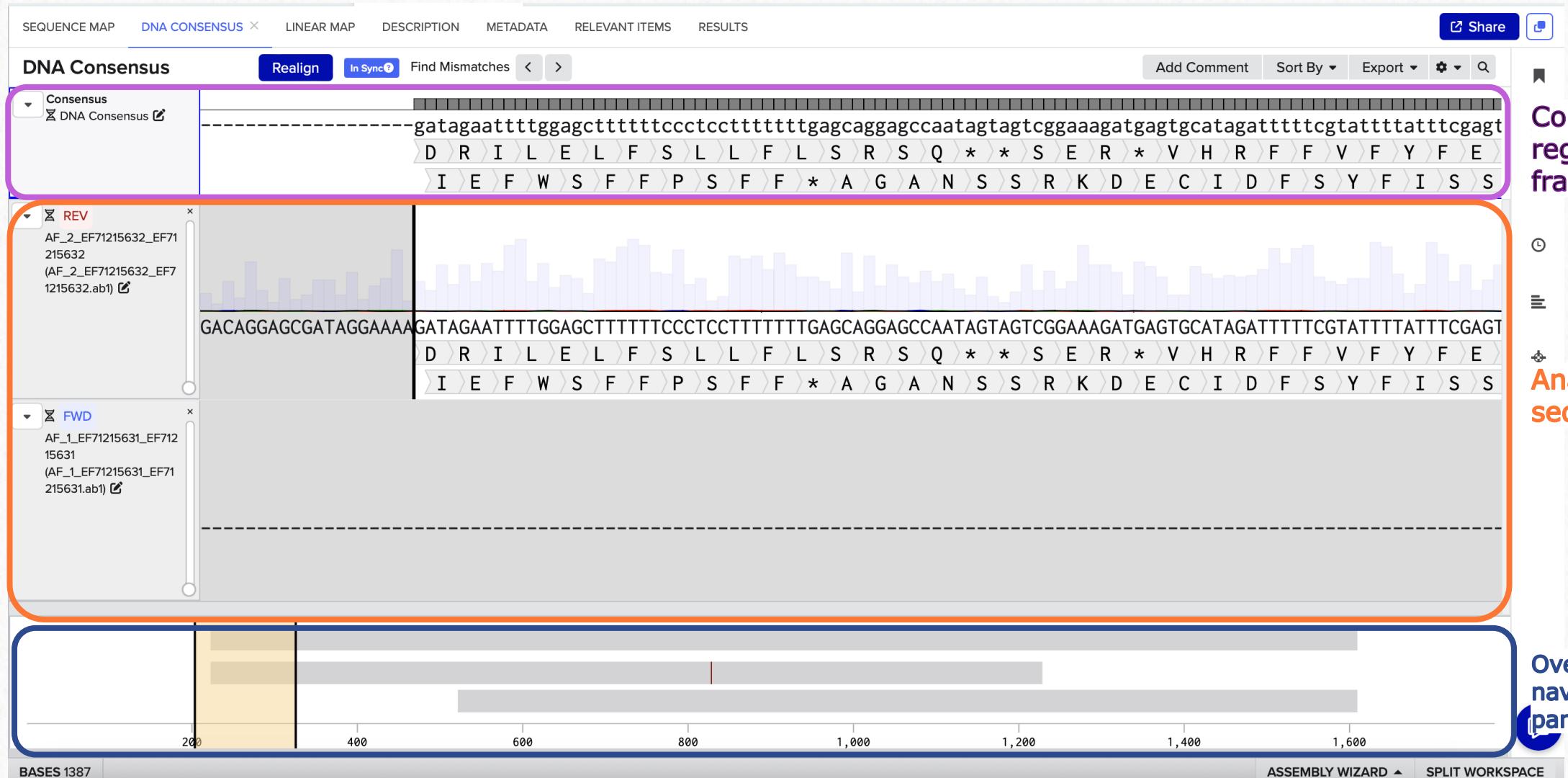
8. Sequence alignments

8.3 Consensus alignment



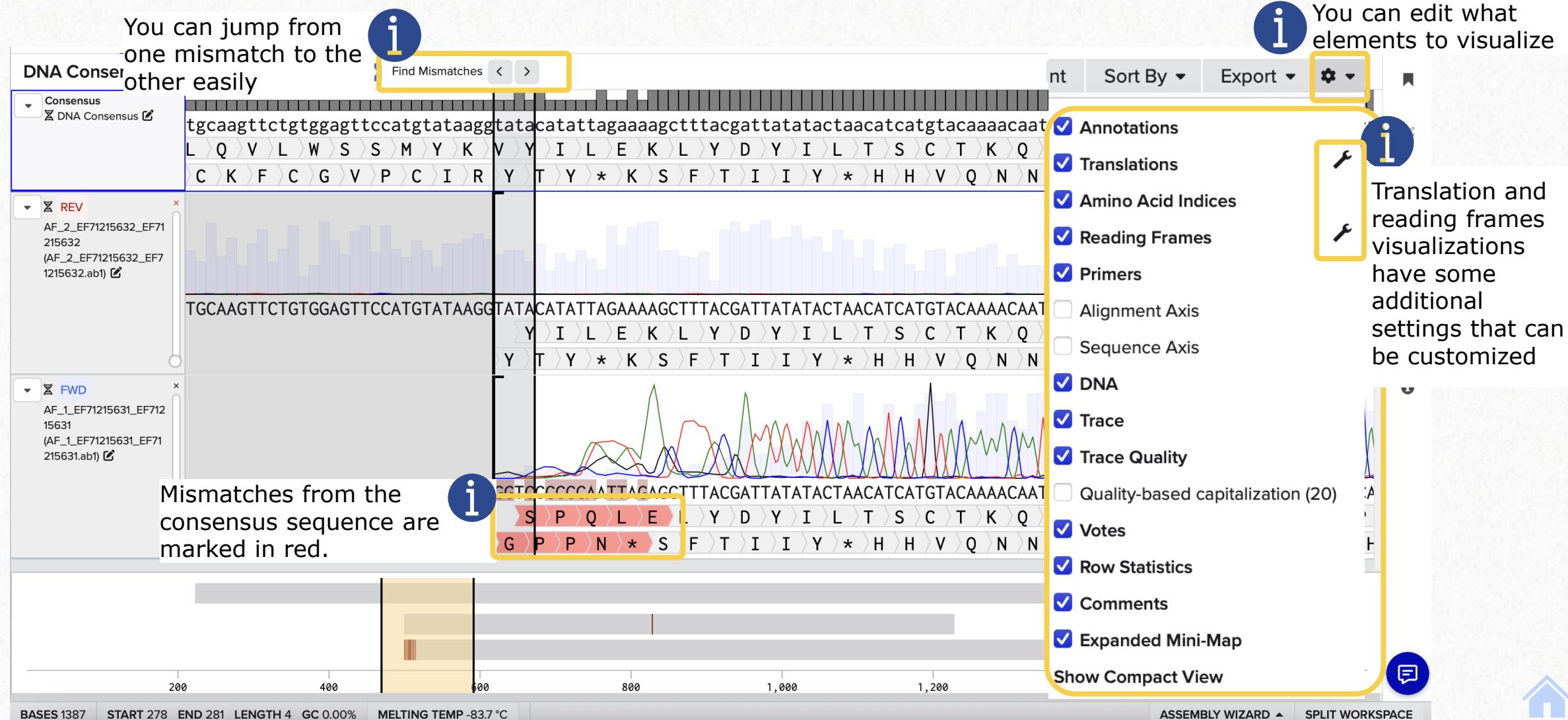
Alignment creation

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](#)



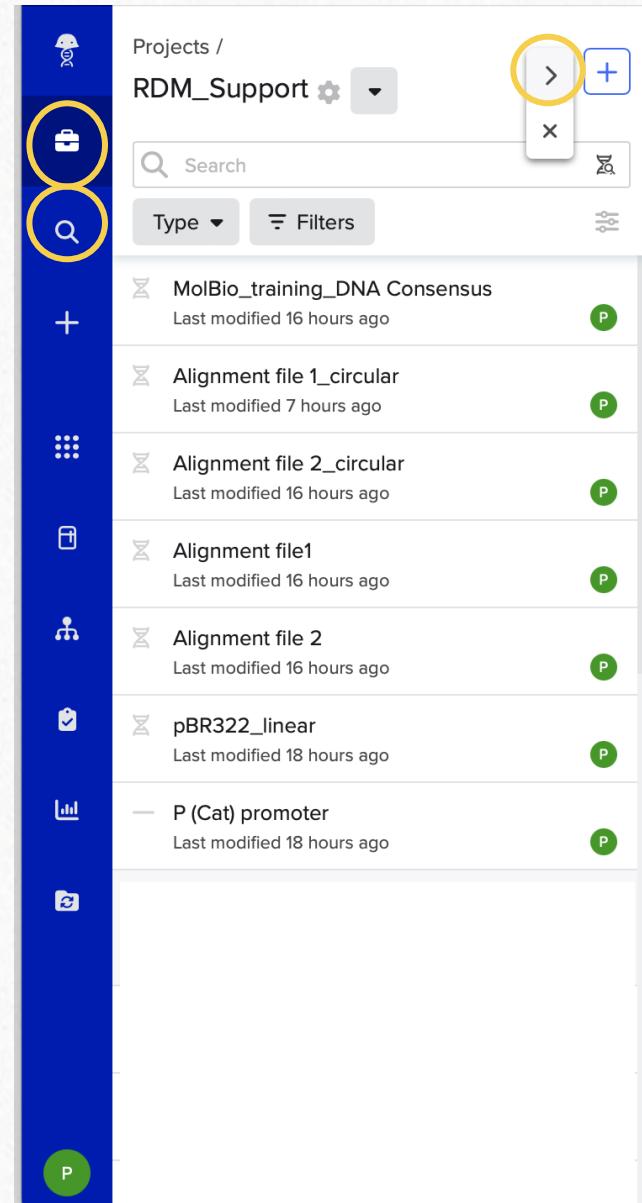
Tips and tricks

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

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

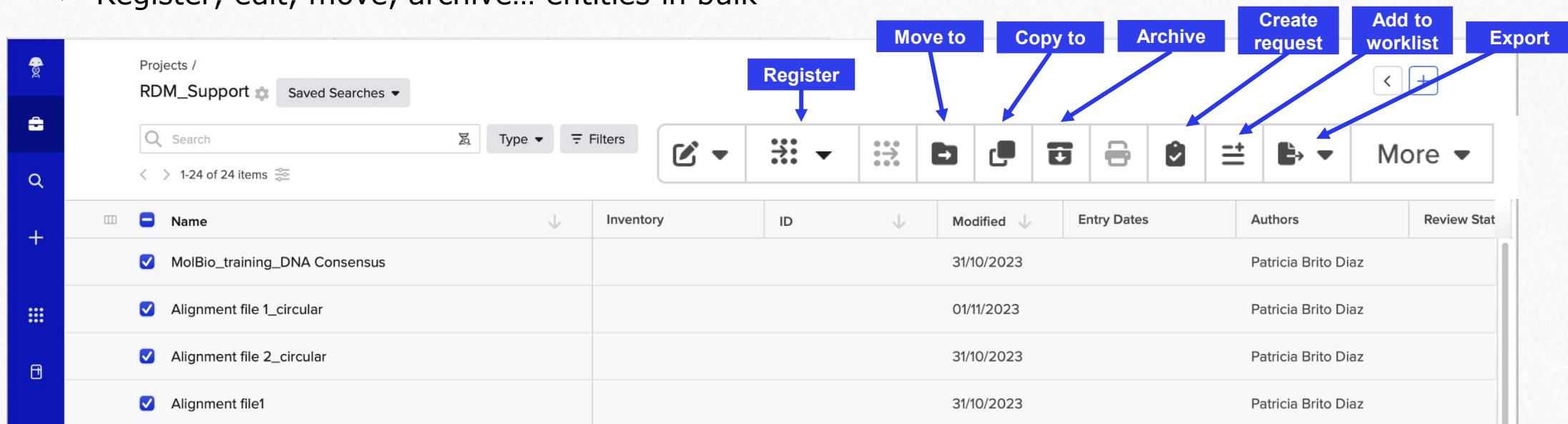


Tips and tricks

Work in bulk using the expanded view

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

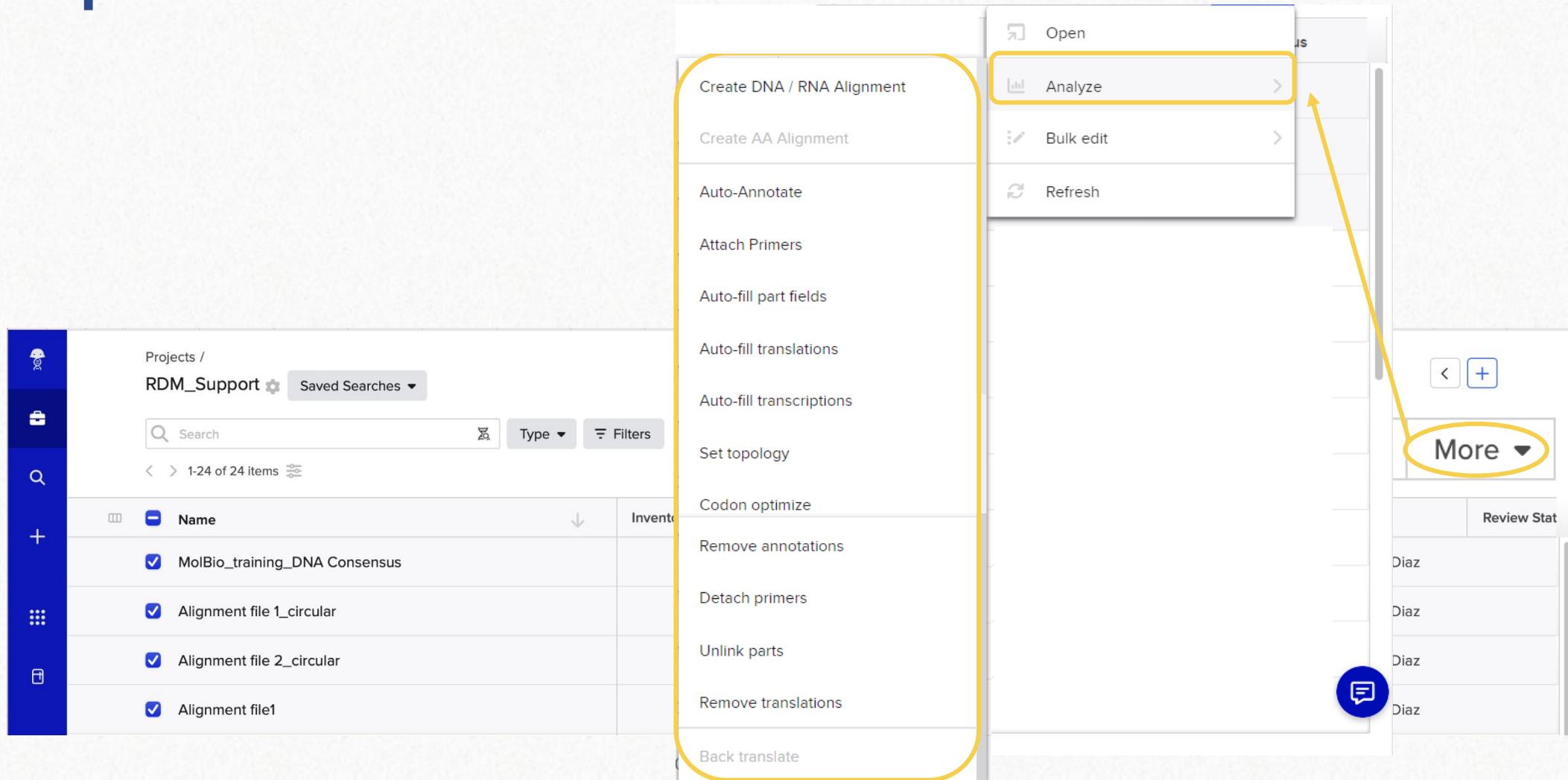
- ✓ Register, edit, move, archive... entities in bulk



The screenshot shows a workspace interface with a sidebar on the left containing icons for Projects, RDM_Support, Saved Searches, Search, Type, Filters, and a More dropdown. The main area displays a list of 24 items under the project 'RDM_Support'. The items are: MolBio_training_DNA Consensus, Alignment file 1_circular, Alignment file 2_circular, and Alignment file1. The interface includes a toolbar at the top with buttons for Register, Move to, Copy to, Archive, Create request, Add to worklist, and Export. Blue arrows point from each of these buttons to their corresponding icons in the toolbar. The table has columns for Name, Inventory, ID, Modified, Entry Dates, Authors, and Review Stat. The 'Modified' column is sorted in descending order.

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 for managing biological projects. On the left, a sidebar provides navigation icons for Home, Projects, Search, and Add. The main area displays a list of items under the project 'RDM_Support'. A context menu is open over one of the items, highlighting the 'Analyze' option. To the right, a 'More' dropdown menu is also highlighted, containing options like 'Review Stat', 'Diaz', and a message icon.

- Open
- Analyze
- Bulk edit
- Refresh

- Create DNA / RNA Alignment
- Create AA Alignment
- 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

More ▾

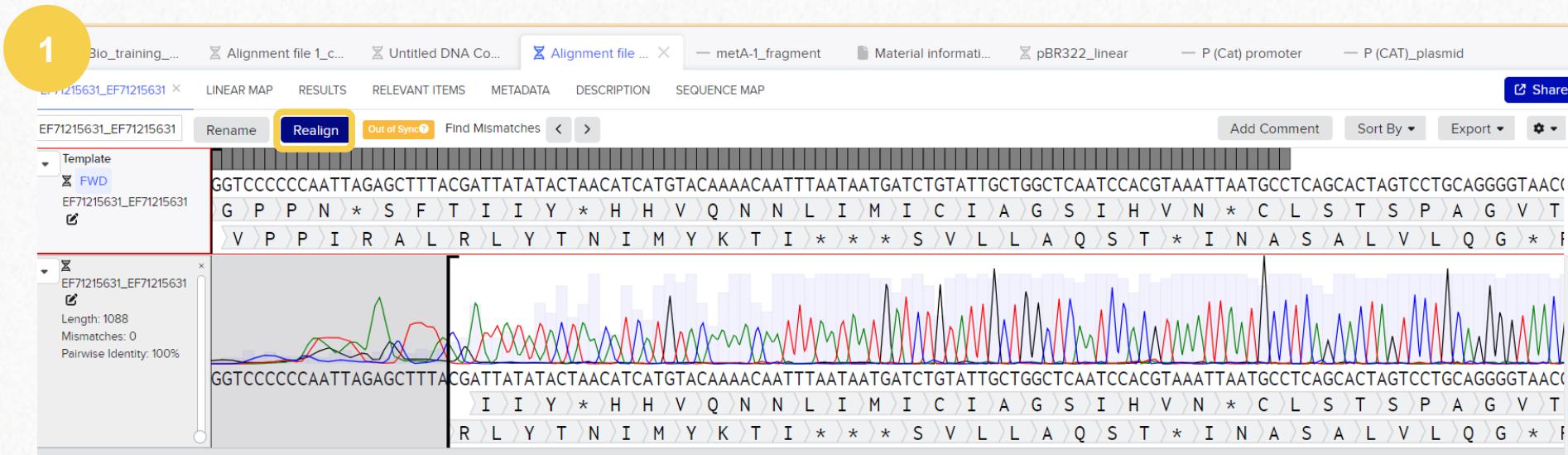
Review Stat
Diaz
Diaz
Diaz
Diaz

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

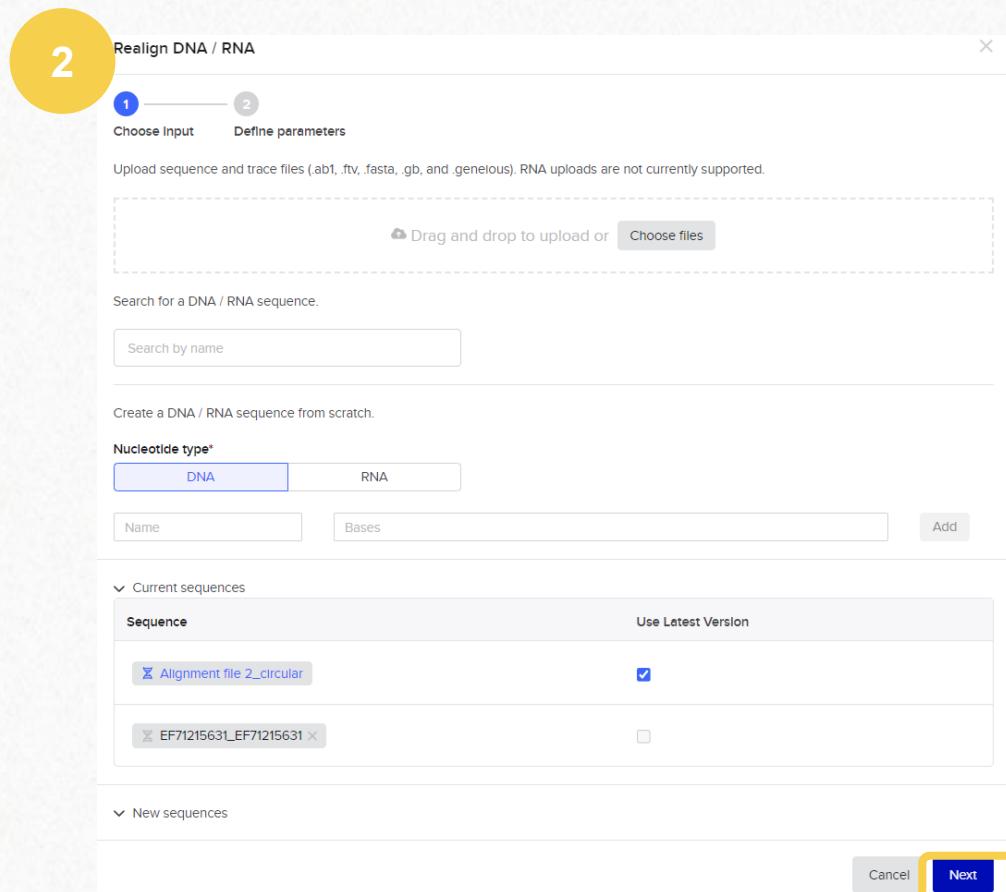
Name Bases Add

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

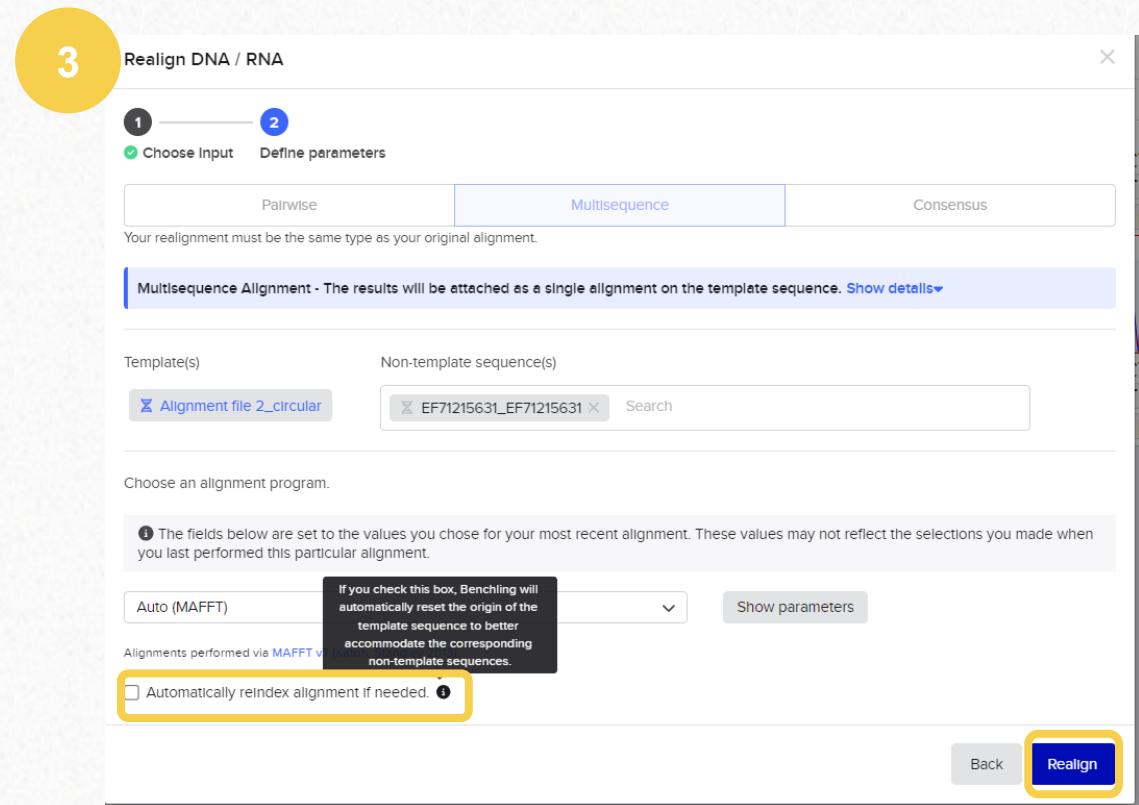
Choose an alignment program.

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

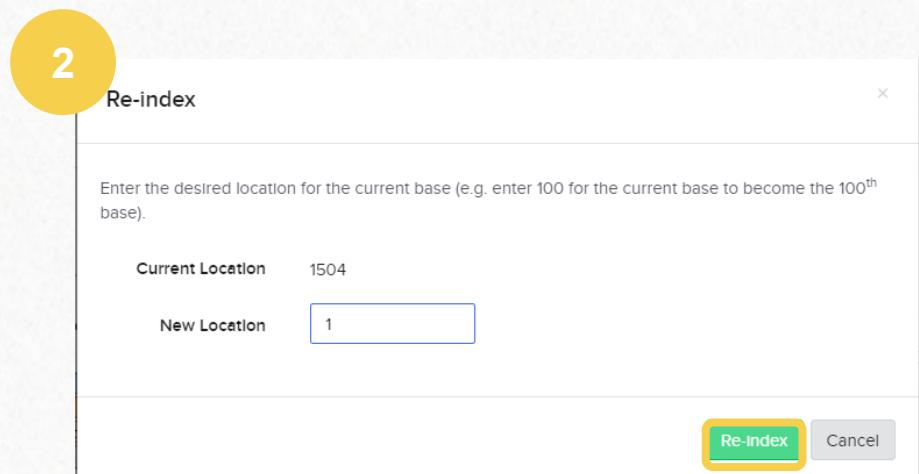
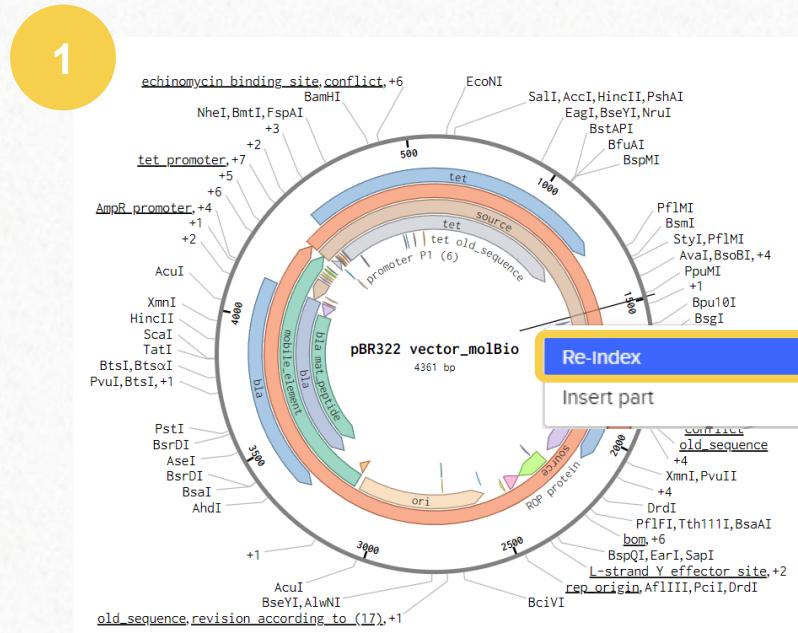
Autoindexing when creating alignments

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.

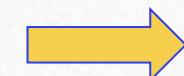


10. Resources





Questions?



Contact lims_support@biosustain.dtu.dk

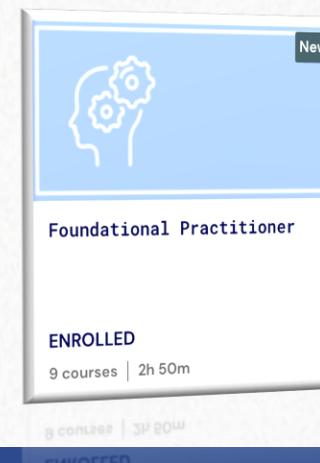


More resources

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

Essential skills for all Benchling R&D Cloud users, covering core applications and best practices.



Administrator

Additional training for Benchling Administrators, covering roles, permissions, configurations, and more.



Developer

Specialized training covering Developer Platform fundamentals such as APIs, Events, and more.



Consultant

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>

