

 Search

Type: Location ▾

Folder ▾

Filters

Group by ▾

Save

Clear

< > 1-50 of 1159 items 

<input type="checkbox"/> Barcode	Name	Location	Modified	Schema
4C012	4C EE&SB fridge transient storage	4°C Fridge	09/08/2018	4°C Fridge
4C002	4C Fridge 00271	4°C Fridge	09/08/2018	4°C Fridge
4C009	4C Fridge 01223	4°C Fridge	09/08/2018	4°C Fridge
4C001	4C Fridge 01233	4°C Fridge	09/08/2018	4°C Fridge
4C014	4C Fridge 01871	4°C Fridge	09/08/2018	4°C Fridge
4C015	4C Fridge Aaron	4°C Fridge	15/04/2021	4°C Fridge
4C016	4C Fridge Adam	BioInnovati...	15/04/2021	4°C Fridge
4C005	4C Fridge ANALYTICS	4°C Fridge	19/11/2018	4°C Fridge
4C011	4C Fridge CFB00266	4°C Fridge	09/08/2018	4°C Fridge
CFB01478	4C Fridge CFB01478	4°C Fridge	19/11/2018	4°C Fridge
CFB01653	4C Fridge CFB01653	4°C Fridge	09/08/2018	4°C Fridge
4C003	4C Fridge DSP1	4°C Fridge	09/08/2018	4°C Fridge

**Reach out when struggling with the platform:**

BRIGHT Benchling support  
[lims\\_support@bright.dtu.dk](mailto:lims_support@bright.dtu.dk)

**Access Benchling:**

[bright.benchling.com](https://bright.benchling.com)



(login with DTU credentials)

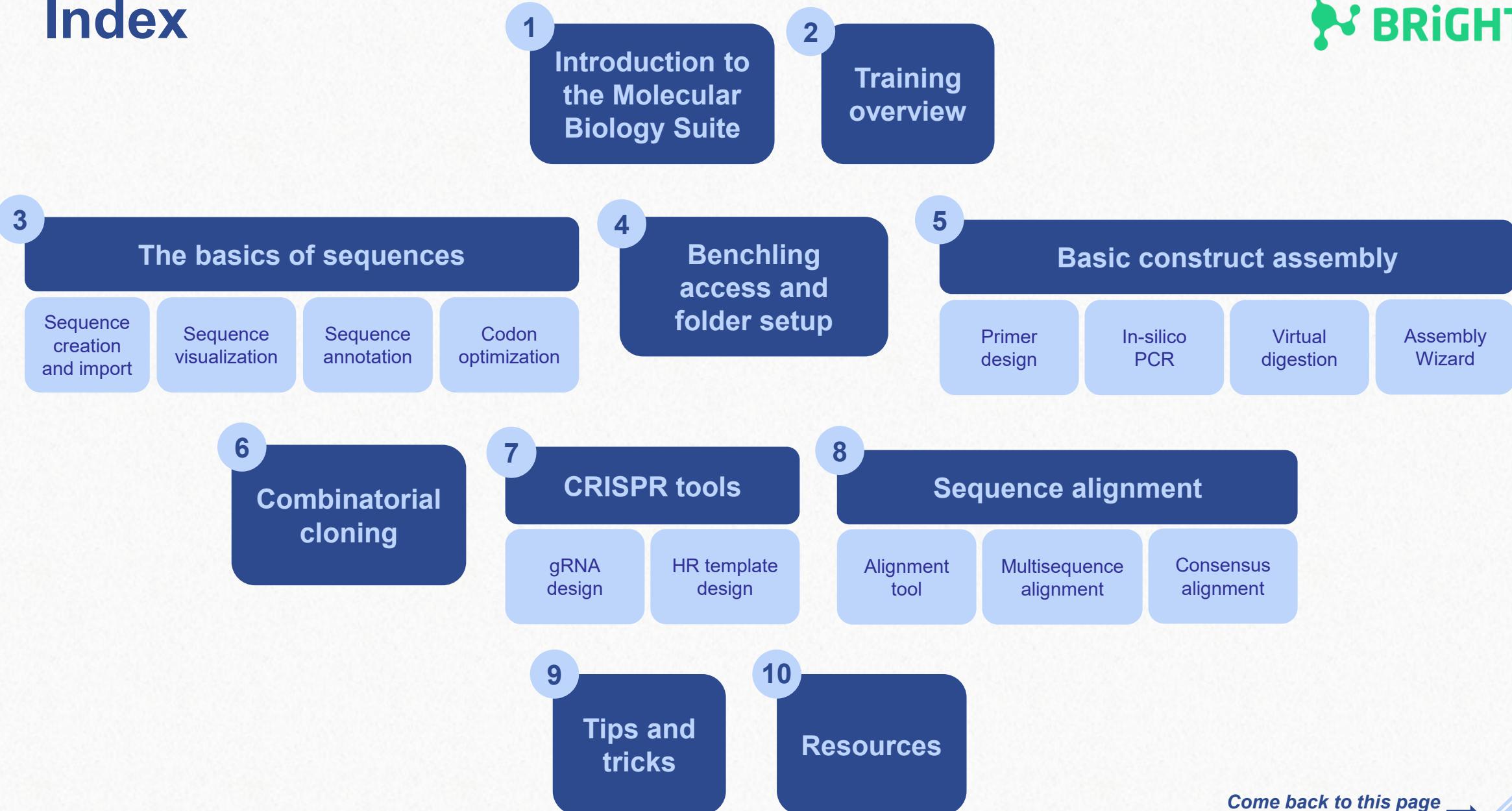
**Additional resources:**

[LIMS Help guides](#)



[Benchling Help Center: Molecular Biology](#)

# Index



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

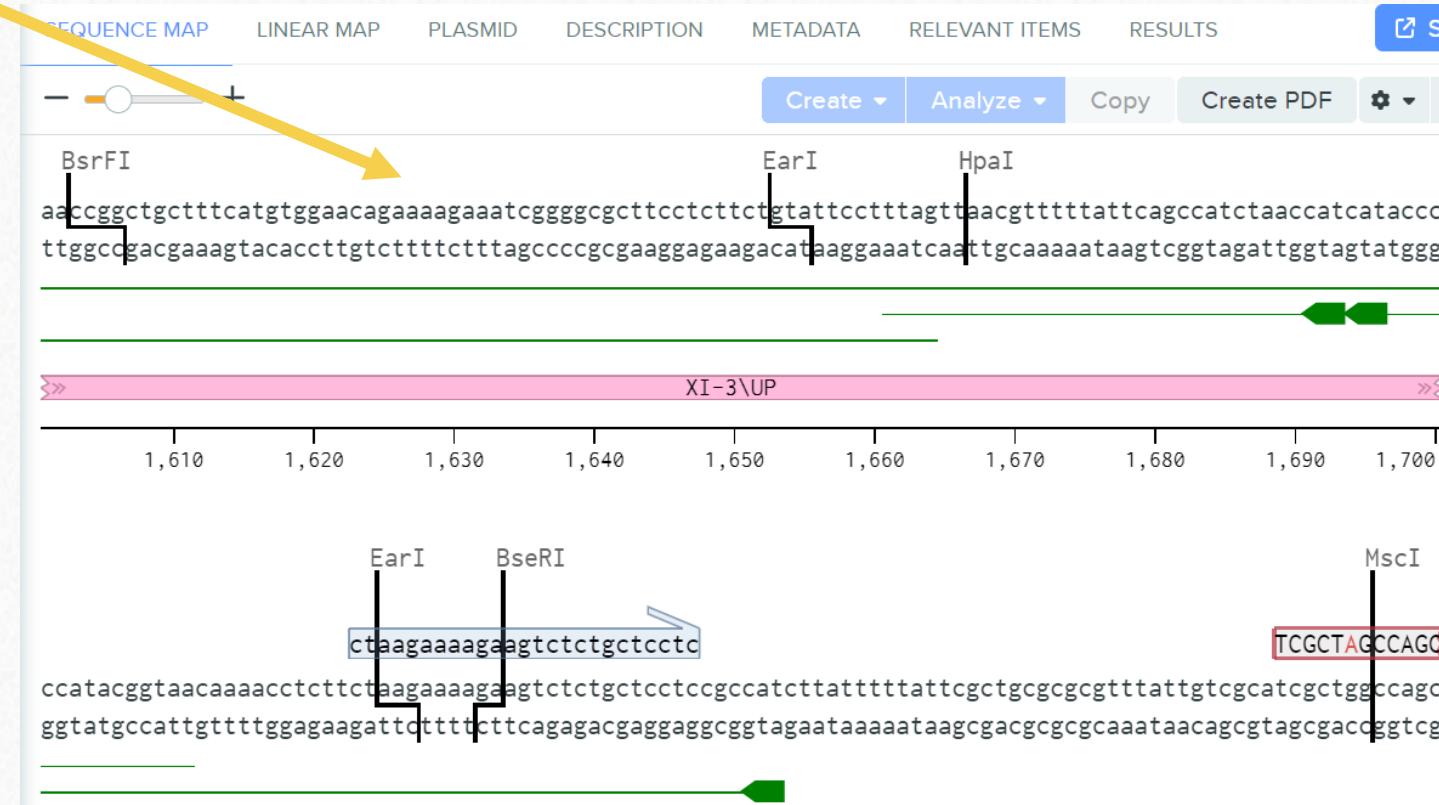
## CRISPR

- ✓ 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

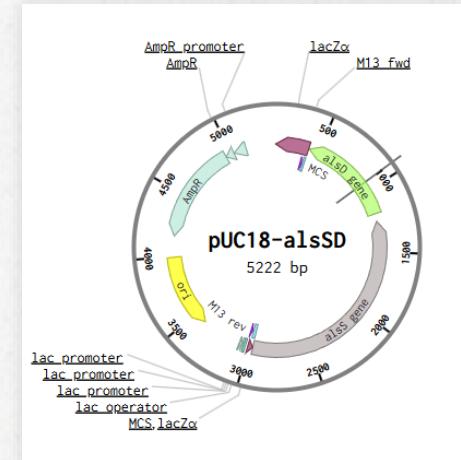
Information (topology, tags)

## 2. Training overview



# Training goals:

The basics of...



This interface shows a search results page for constructs. The search term is "AF\_medium\_copy\_(pET)\_ori/KanR". Three constructs are listed, each represented by a circular map and labeled with its name and a small preview icon. Each construct has 8 associated primers.

- 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
- AF\_medium\_copy\_(pET)\_ori/KanR-T5 promoter-RBS B0030-alsS-rmB T1 terminator

- 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](http://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

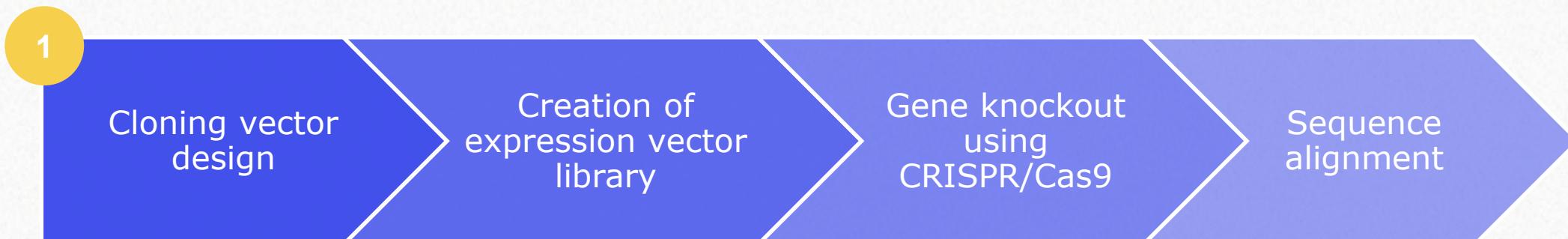
Chan-Hsiang Hsu, Sefli Sri Wahyu Effendi, Wan-Wen Ting, Yu-Hsiu Li, I-Son Ng \*

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

[Link to article](#)

# Today's work example:

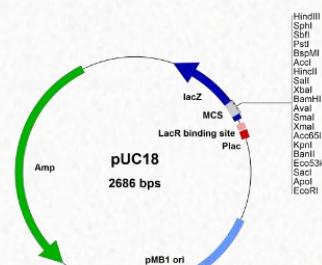
Hypothetical scenario: **Production of acetoin in *E. coli***



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



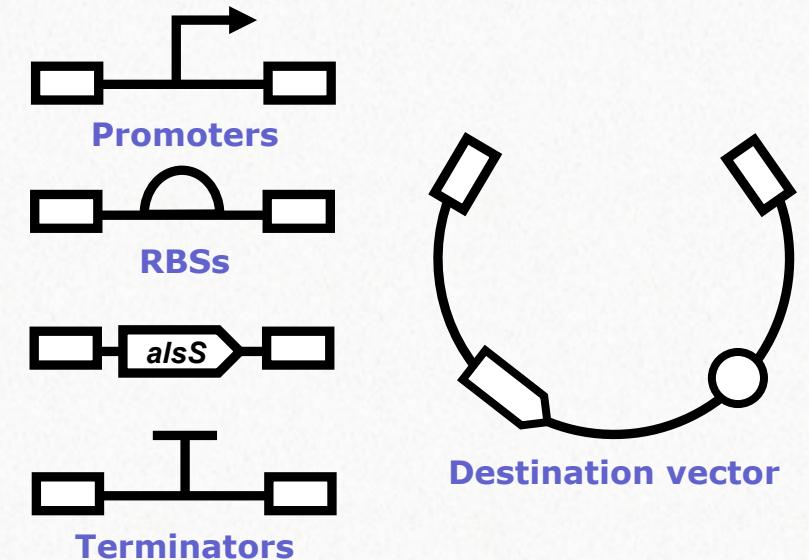
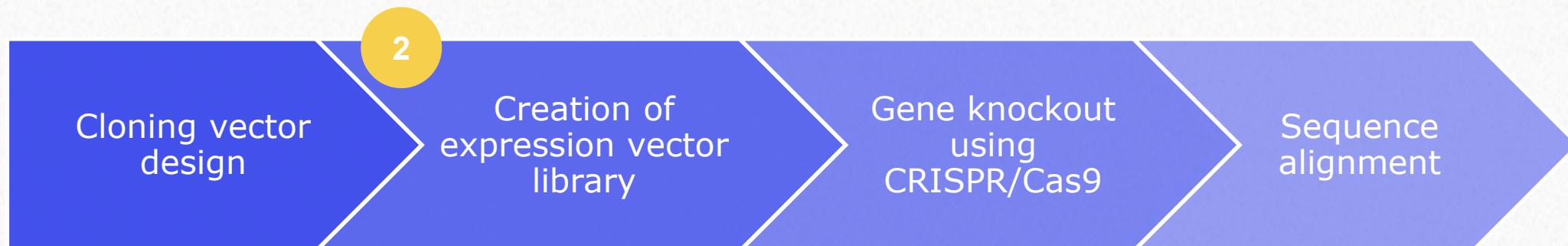
**pUC18** cloning vector



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

# Today's work example:

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



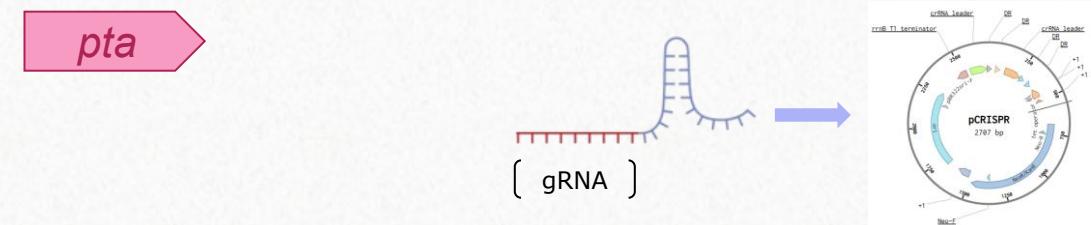
✓ Combinatorial cloning:  
**Golden Gate**

# Today's work example:

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

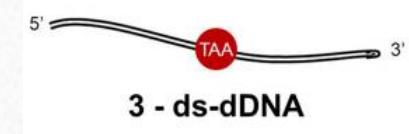


**Target:** *pta* in *E. coli*    **gRNA** design + assembly into pCRISPR



- ✓ gRNA design
- ✓ HR template design

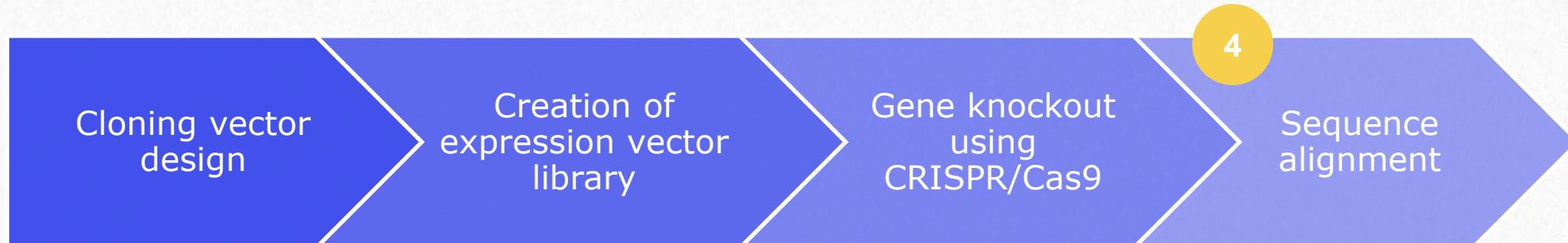
**HR template** design to KO *pta*



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

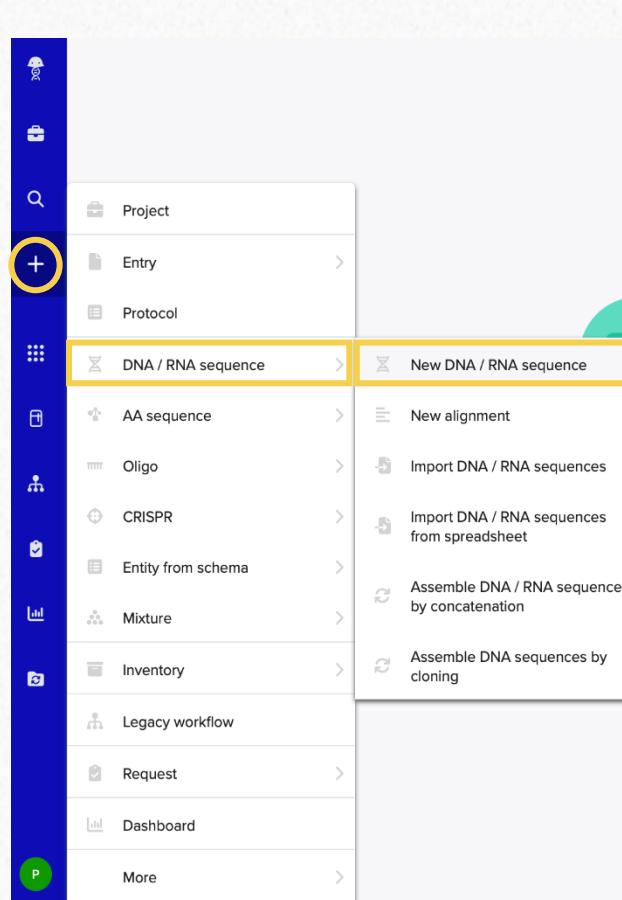
## 3.1 Sequence creation and import



# 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

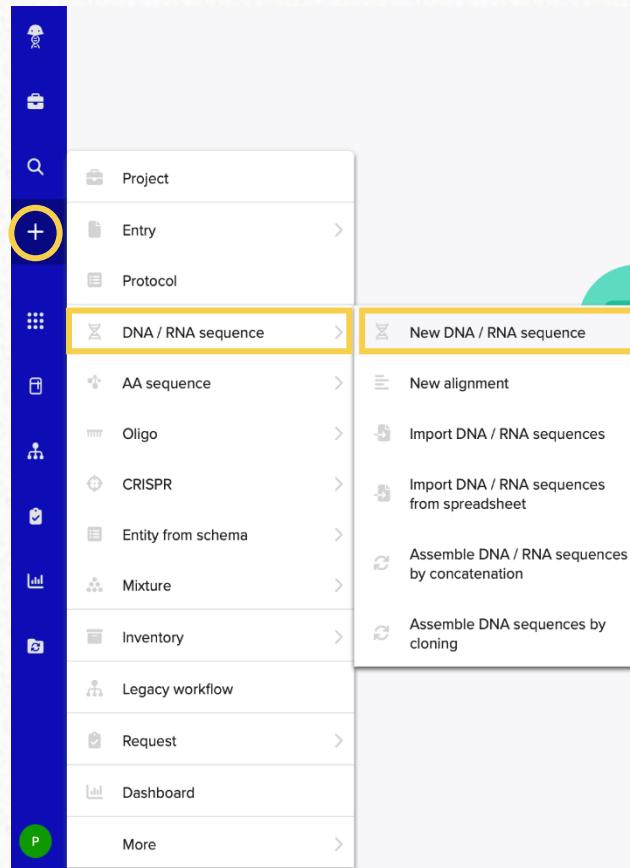
**i** You can leave the **Bases** field **empty** and add your sequence later. This can be useful if you wish to copy and paste a sequence with its annotations.

**Close** **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

This is a screenshot of the 'Create DNA / RNA sequence' dialog. The 'IMPORT FROM DATABASE' tab is active and highlighted with a yellow box. In the 'Sequence' input field, the accession number 'Y11520' is entered. To the right of the input field is a 'Search' button, which is also highlighted with a yellow box. Below the input field, there is a note: 'Import multiple sequences at once by entering space-separated or comma-separated accession numbers.' A list of example searches is provided, including URLs for Addgene, gene names, NCBI accessions, Ensembl IDs, Registry IDs, and JBEI Public Registry entries.

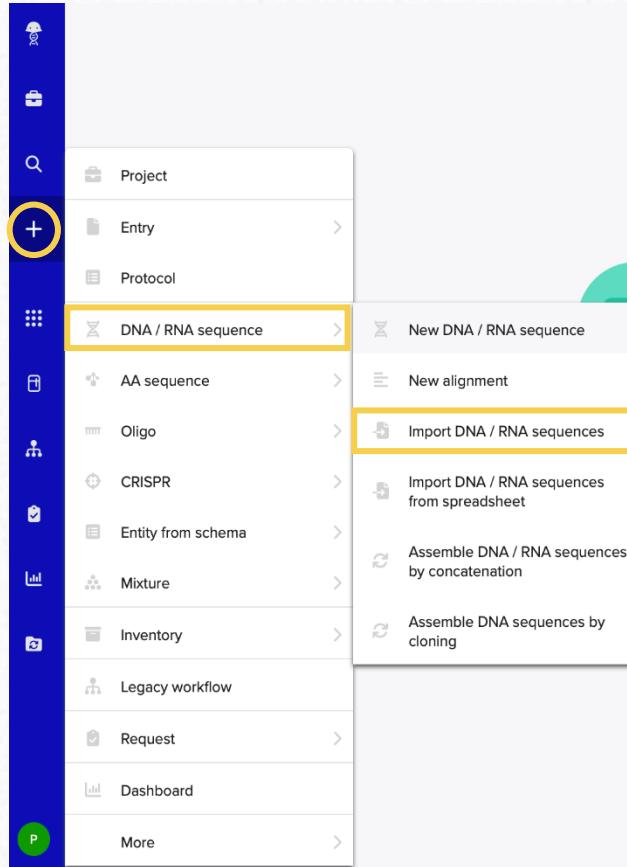
- 3 If the ID is valid, Benchling will show you the gene's description. You can set its schema and import it.

This screenshot shows the 'Create DNA / RNA sequence' dialog after the accession 'Y11520' has been searched. The 'Sequence' field now contains 'Y11520'. The 'Entry' field shows 'Y11520', 'Database' shows 'NCBI Nucleotide (Genbank)', and 'Length' shows '3544'. The 'Description' field contains 'Pseudomonas sp. vdh gene and ORF2'. On the right, a 'SCHEMAS' dropdown menu is open, showing options like 'DNA Fragment' (which is highlighted with a yellow box), 'Gene', 'gRNA', 'Marker', 'Origin of Replication', 'Plasmid', 'Primer', 'Promoter', 'Tag', and 'Terminator'. At the bottom right of the dialog 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.

**Create DNA / RNA sequence**

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

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

Nucleotide type\* **DNA** RNA

Project folder **Mia**

Drag and drop files to upload or **Choose a file**

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

GFP.dna **UPLOAD DONE** OPEN SEQUENCE - UPLOADED TO MIA  
 GFP - linear DNA creator Clontech (TaK...  
accession L09136 marker AmpR organism Escherichia coli

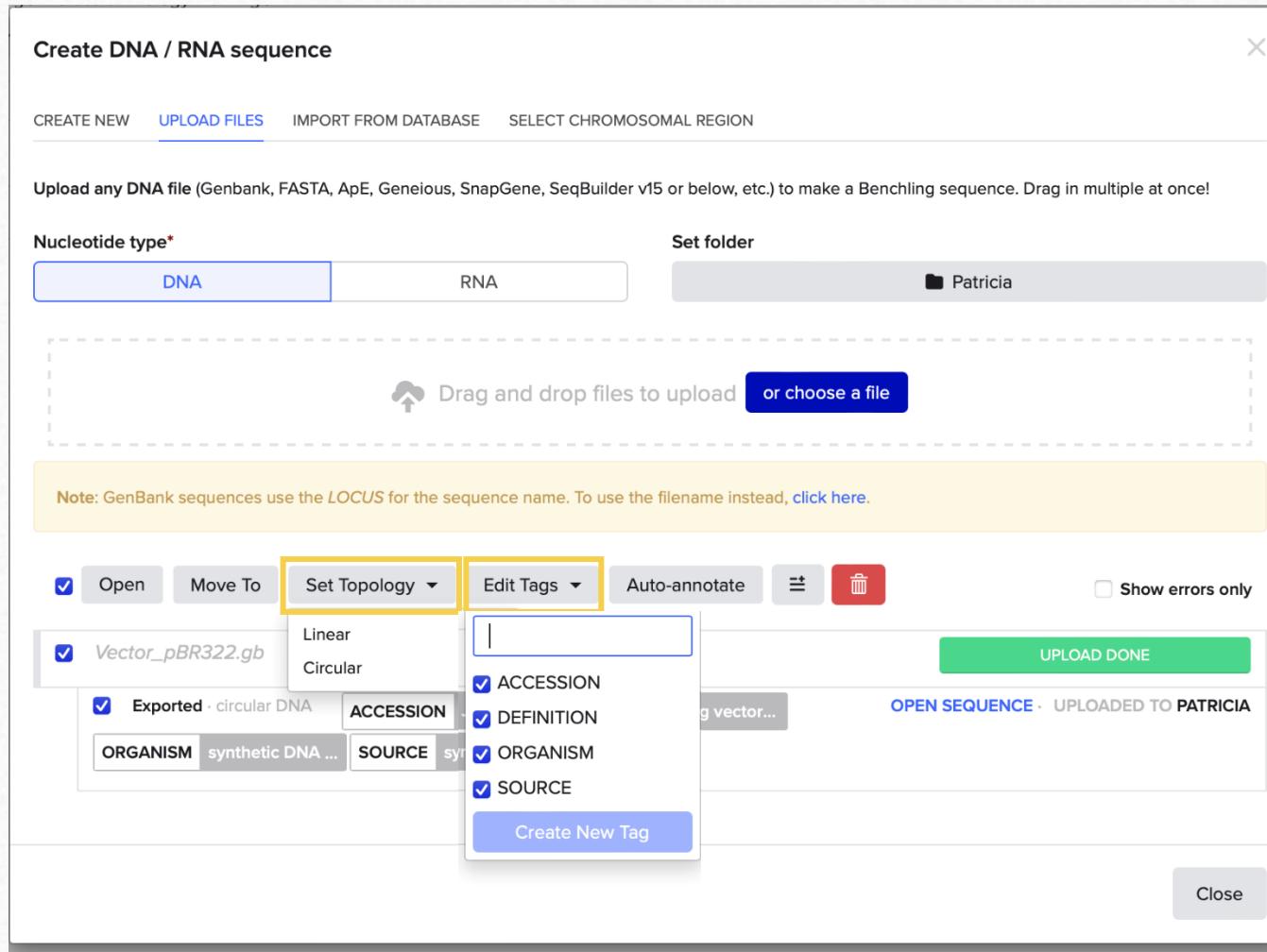
pUC18.dna **UPLOAD DONE** OPEN SEQUENCE - UPLOADED TO MIA  
 pUC18 - circular DNA ref pmid:2985470 accession L09136 marker AmpR organism Escherichia coli

- 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*



The screenshot shows the 'Create DNA / RNA sequence' interface. At the top, there are four tabs: 'CREATE NEW', 'UPLOAD FILES' (which is highlighted in blue), 'IMPORT FROM DATABASE', and 'SELECT CHROMOSOMAL REGION'. Below the tabs, a note says: 'Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!'. There are two radio buttons for 'Nucleotide type': 'DNA' (selected) and 'RNA'. A 'Set folder' button shows a folder named 'Patricia'. Below these are upload options: 'Drag and drop files to upload' and 'or choose a file'. A note below says: 'Note: GenBank sequences use the LOCUS for the sequence name. To use the filename instead, click here.' In the bottom right, there's a 'UPLOAD DONE' button and a message: 'OPEN SEQUENCE · UPLOADED TO PATRICIA'. On the left, there are buttons for 'Open', 'Move To', 'Set Topology' (which is highlighted with a yellow box), 'Edit Tags' (also highlighted with a yellow box), 'Auto-annotate', and 'Show errors only'. Under 'Set Topology', it says 'Linear' and 'Circular'. Under 'Edit Tags', it lists 'ACCESSION', 'DEFINITION', 'ORGANISM', and 'SOURCE', each with a checked checkbox. There's also a 'Create New Tag' button. A 'Vector\_pBR322.gb' file is listed under 'Exported · circular DNA'. The bottom right corner has a 'Close' button.

When uploading a sequence, it is possible to:

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

# Create and import a sequence

*How to import sequences from a file*

**Create DNA / RNA sequence**

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

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

Nucleotide type\*    **DNA**    RNA

Set folder    Patricia

Drag and drop files to upload    or choose a file

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

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

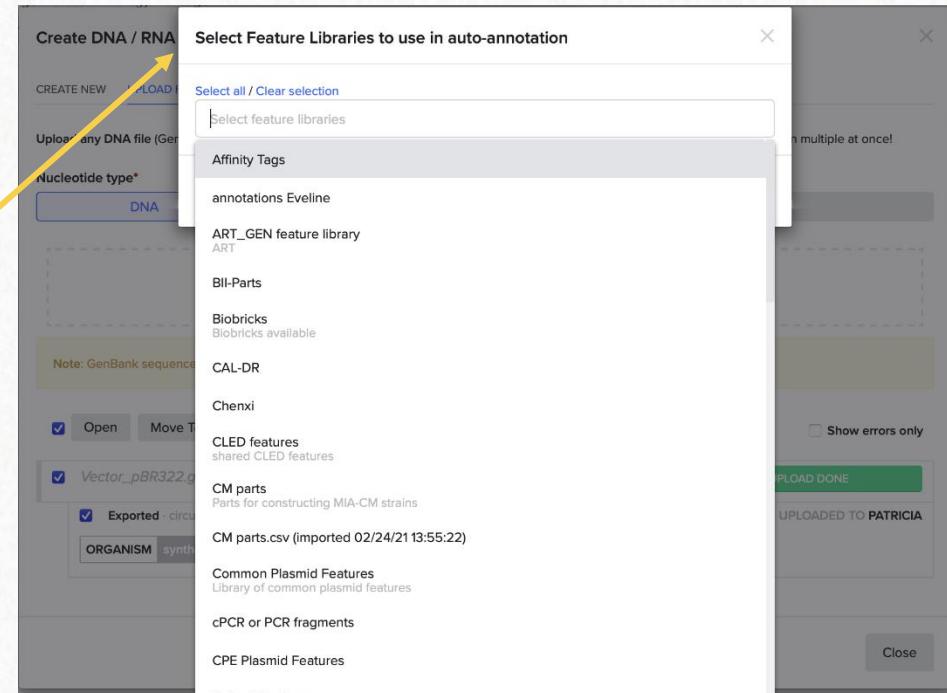
Vector\_pBR322.gb

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

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

UPLOAD DONE

Close



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

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

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

## Import of sequences from a file

The screenshot shows the 'Create DNA / RNA sequence' interface. At the top, there are tabs for 'CREATE NEW', 'UPLOAD FILES' (which is selected), 'IMPORT FROM DATABASE', and 'SELECT CHROMOSOMAL REGION'. Below this, a note says: 'Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder v15 or below, etc.) to make a Benchling sequence. Drag in multiple at once!' A 'Nucleotide type\*' dropdown is set to 'DNA'. A 'Set folder' button is shown above a folder list containing 'Patricia'. A central area has a 'Drag and drop files to upload' field and a 'choose a file' button. Below this is a note: 'Note: GenBank sequences use the LOCUS for the sequence name. To use the filename instead, click here.' A toolbar at the bottom includes 'Open', 'Move To', 'Set Topology', 'Edit Tags', 'Auto-annotate' (with a three-dot menu icon highlighted by a yellow box), and 'Upload Done'. A sequence entry for 'Vector\_pBR322.gb' is shown with details: 'Exported · circular DNA', 'ACCESSION J01749', 'DEFINITION Cloning vector...', 'ORGANISM synthetic DNA ...', and 'SOURCE synthetic DNA ...'. An 'OPEN SEQUENCE - UPLOADED TO PATRICIA' link is also present.

The screenshot shows a 'Create DNA / RNA' dialog box with a 'New worklist' tab selected. A modal window titled 'Add items to entity worklist' is open, showing a 'Worklist Name\*' input field with 'Project\_training' and a 'Selected items' section with 'Exported'. A 'Add items to worklist' button is at the bottom right. The background shows a list of uploaded sequences, including 'Vector\_pBR322.gb' with its details: 'Exported · circular DNA', 'ACCESSION J01749', 'DEFINITION Cloning vector...', 'ORGANISM synthetic DNA ...', and 'SOURCE synthetic DNA ...'. A 'Close' button is at the bottom right of the modal.

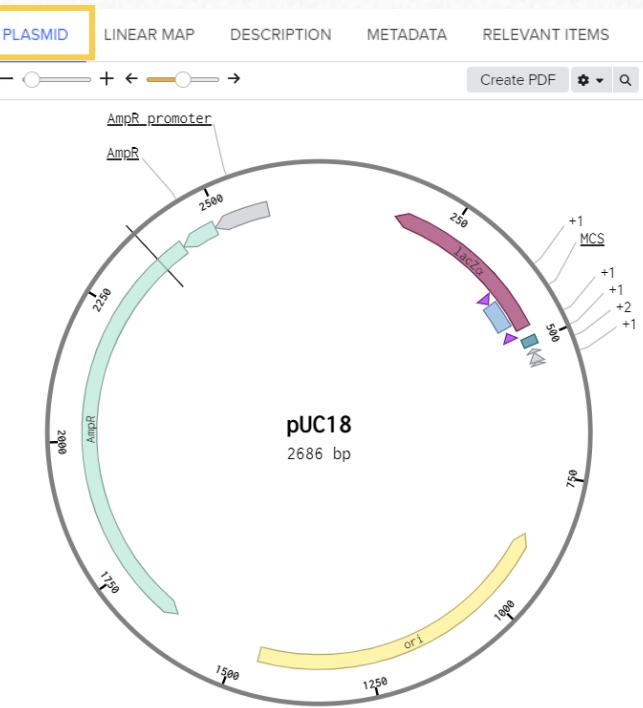
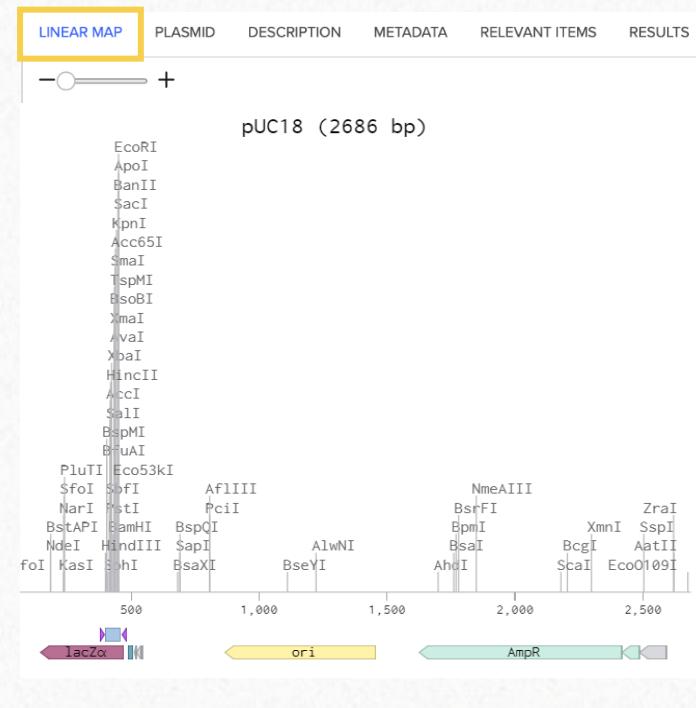
**i** You can also **create worklists or add to existing ones** to find your currently used entities faster.

# 3. The basics of sequences

## 3.2 Sequence visualization



## **Different viewing options:**



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

# View, annotate and edit your sequences

*Different viewing options:*

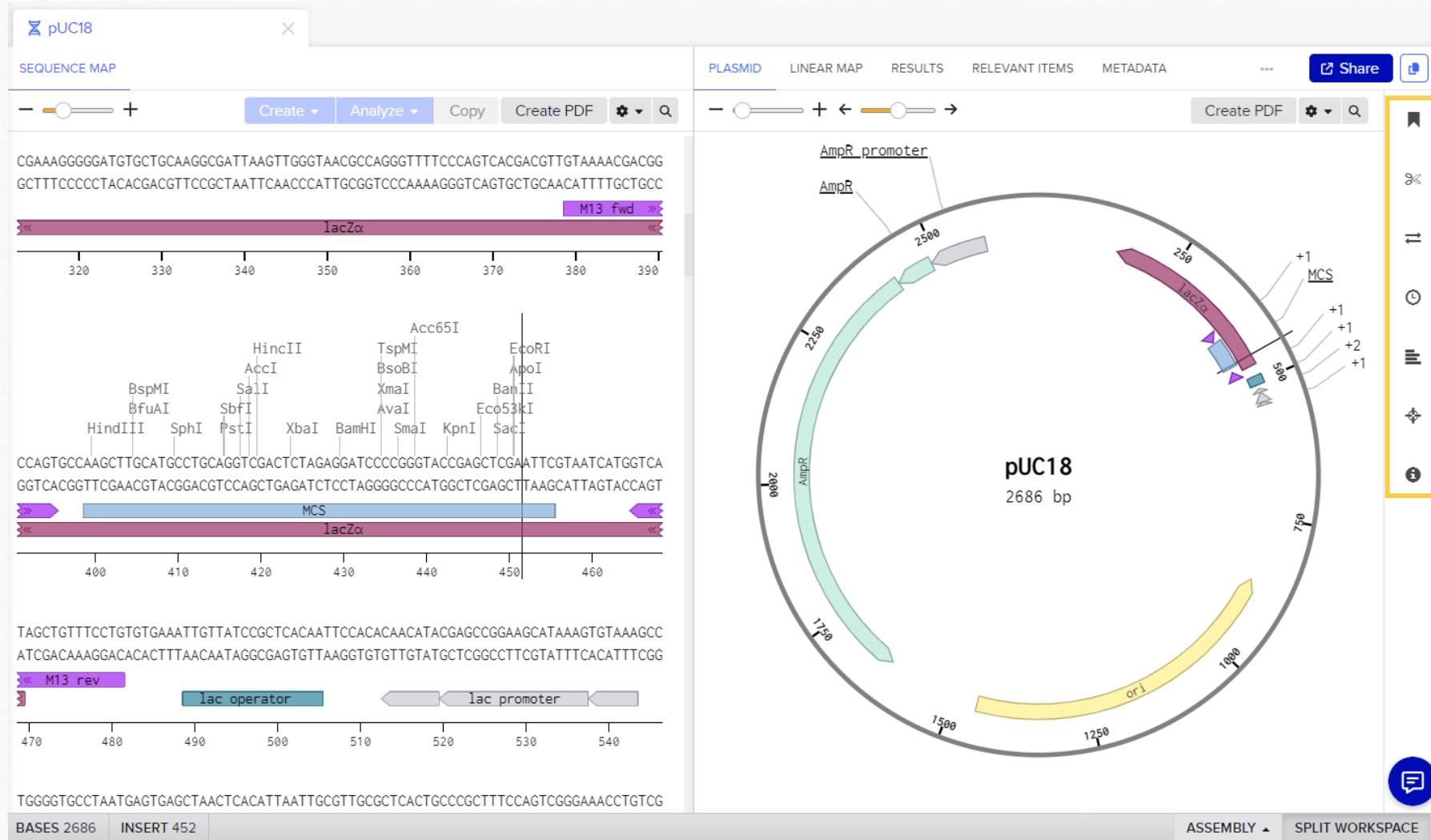


**PRO TIP:**

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

# View, annotate and edit your sequences

## Sequence navigation:

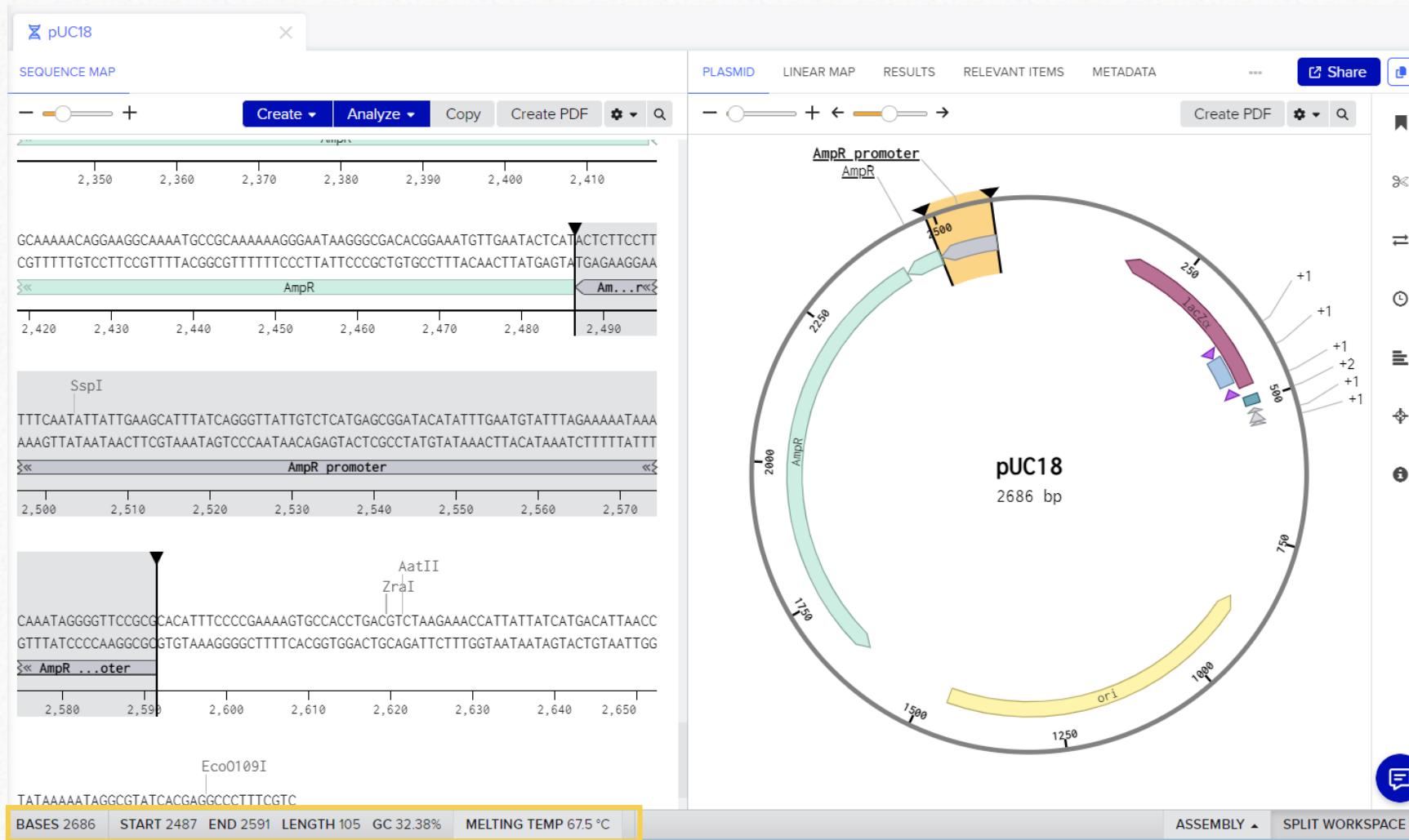


## Functionalities

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

# View, annotate and edit your sequences

## Sequence navigation:



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



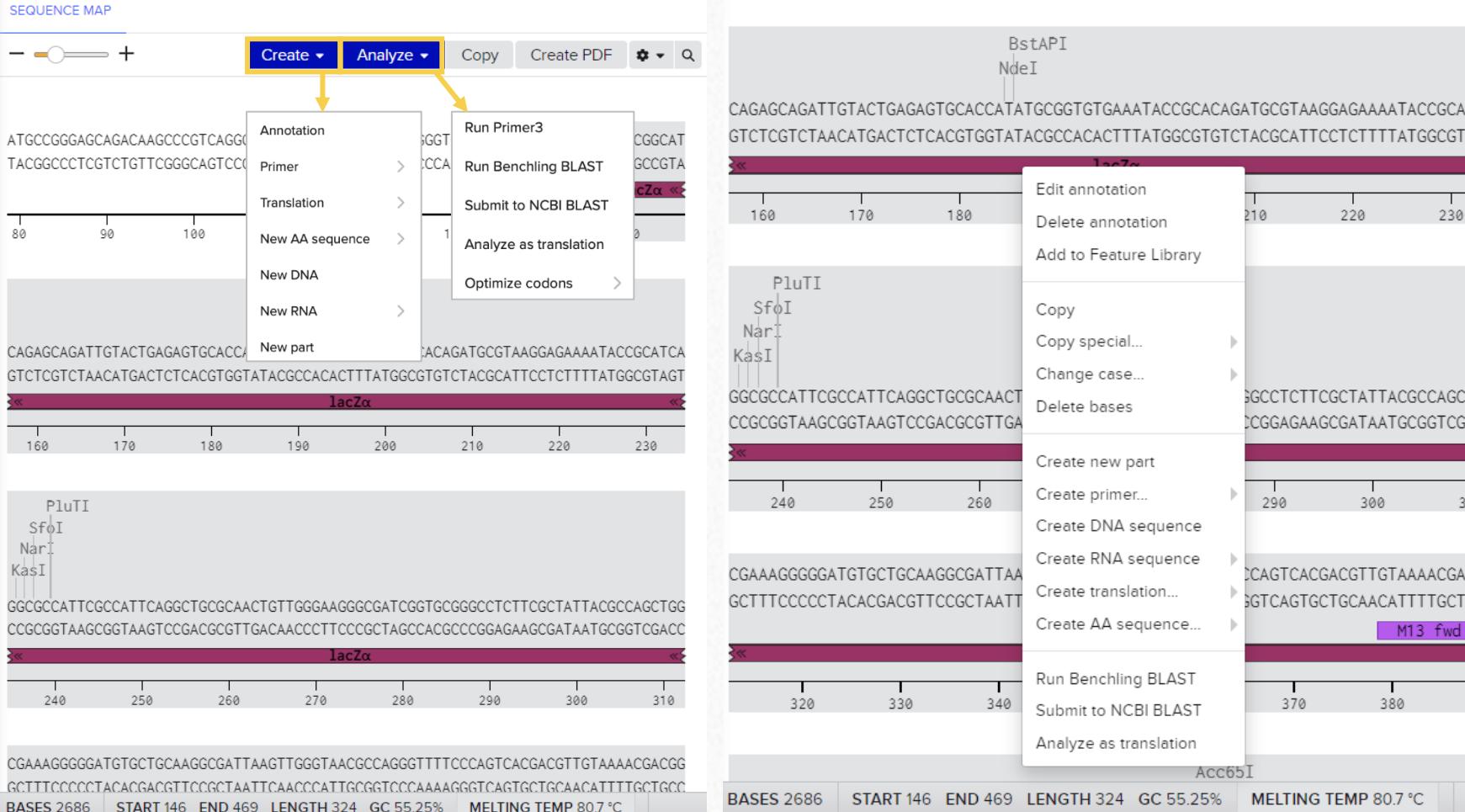
### PRO TIP:

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

# View, annotate and edit your sequences

## Sequence navigation:

SEQUENCE MAP



The screenshot shows a sequence map with several DNA fragments. 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', 'Primer', 'Translation', 'New AA sequence', 'New DNA', 'New RNA', 'New part', 'Run Primer3', 'Run Benchling BLAST', 'Submit to NCBI BLAST', 'Analyze as translation', and 'Optimize codons'. Another context menu is open over a different fragment, showing options such as 'Edit annotation', 'Delete annotation', 'Add to Feature Library', 'Copy', 'Copy special...', 'Change case...', 'Delete bases', 'Create new part', 'Create primer...', 'Create DNA sequence', 'Create RNA sequence', 'Create translation...', 'Create AA sequence...', 'Run Benchling BLAST', 'Submit to NCBI BLAST', and 'Analyze as translation'. The sequence map displays base numbers (e.g., 80, 160, 240) and GC content percentages (e.g., 55.25%).

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



### PRO TIP:

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

# 3. The basics of sequences

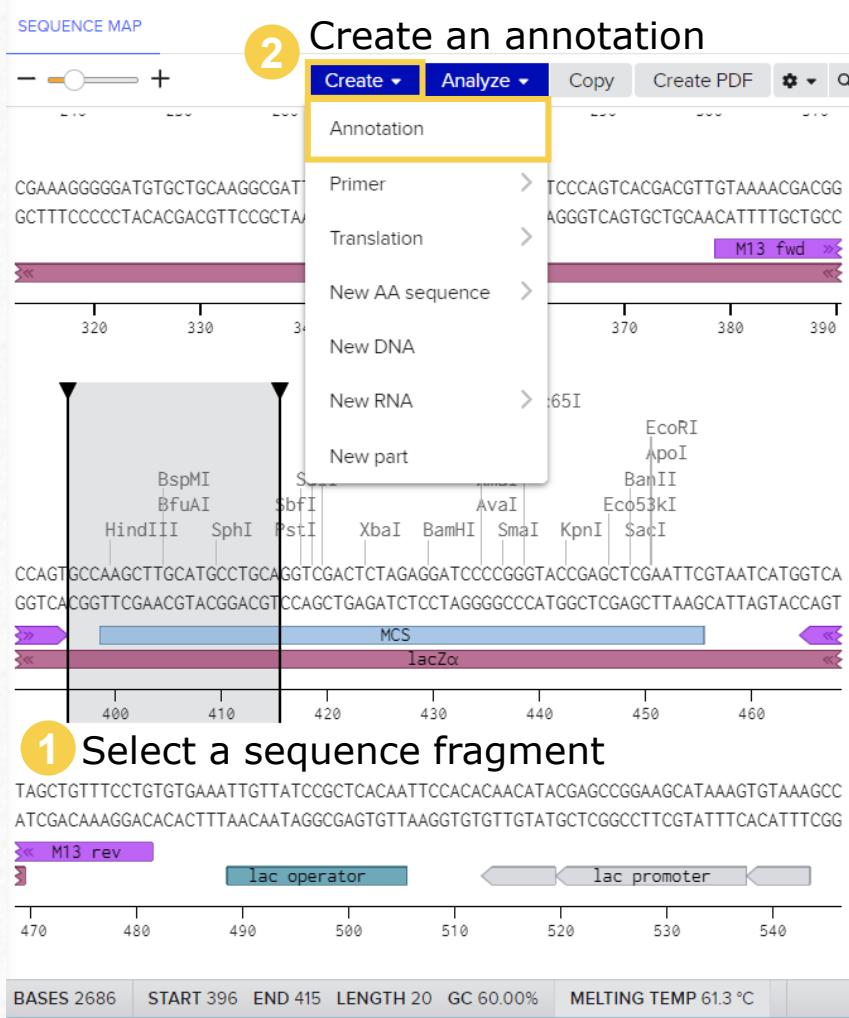
## 3.3 Sequence annotation



# View, annotate and edit your sequences

## Sequence annotations

**1 Select a sequence fragment**



SEQUENCE MAP

Annotation menu (highlighted):

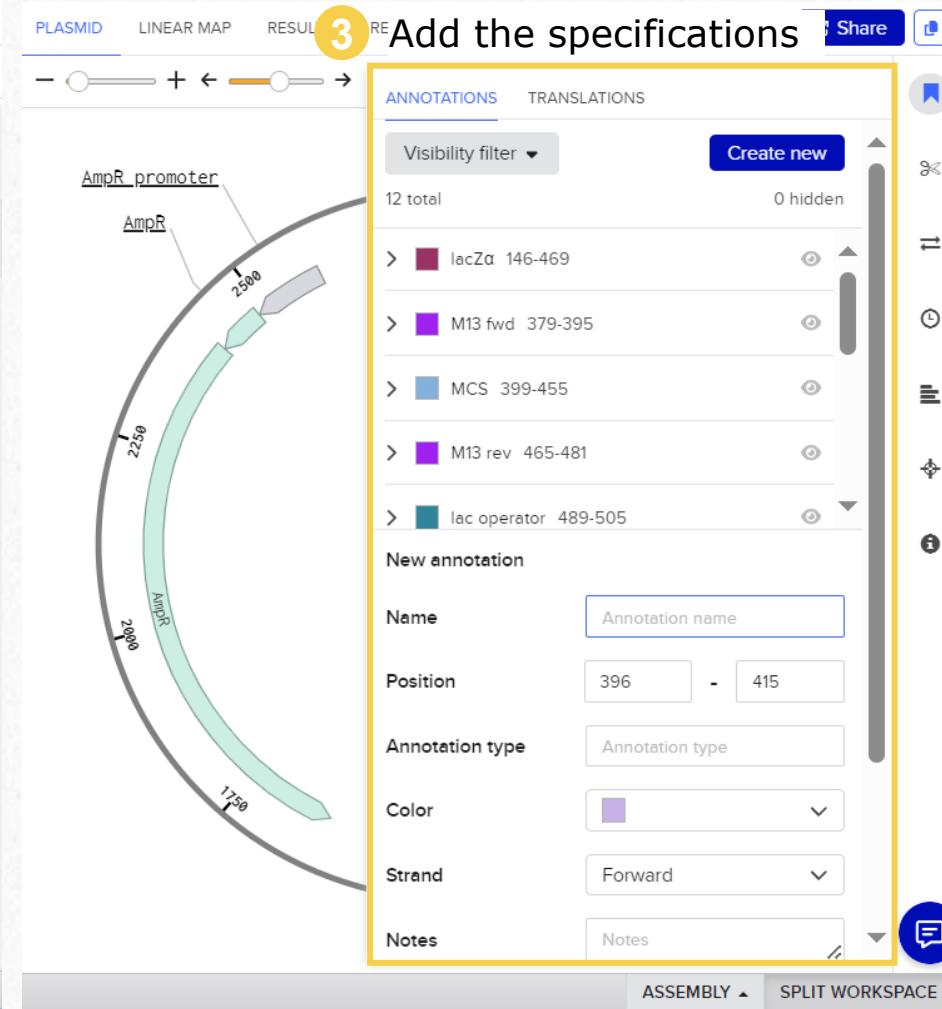
- Create
- Analyze
- Annotation
- Primer
- Translation
- New AA sequence
- New DNA
- New RNA
- New part

Sequence details:

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

**2 Create an annotation**

**3 Add the specifications**



PLASMID LINEAR MAP RESULT RE Share

Annotations (List):

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

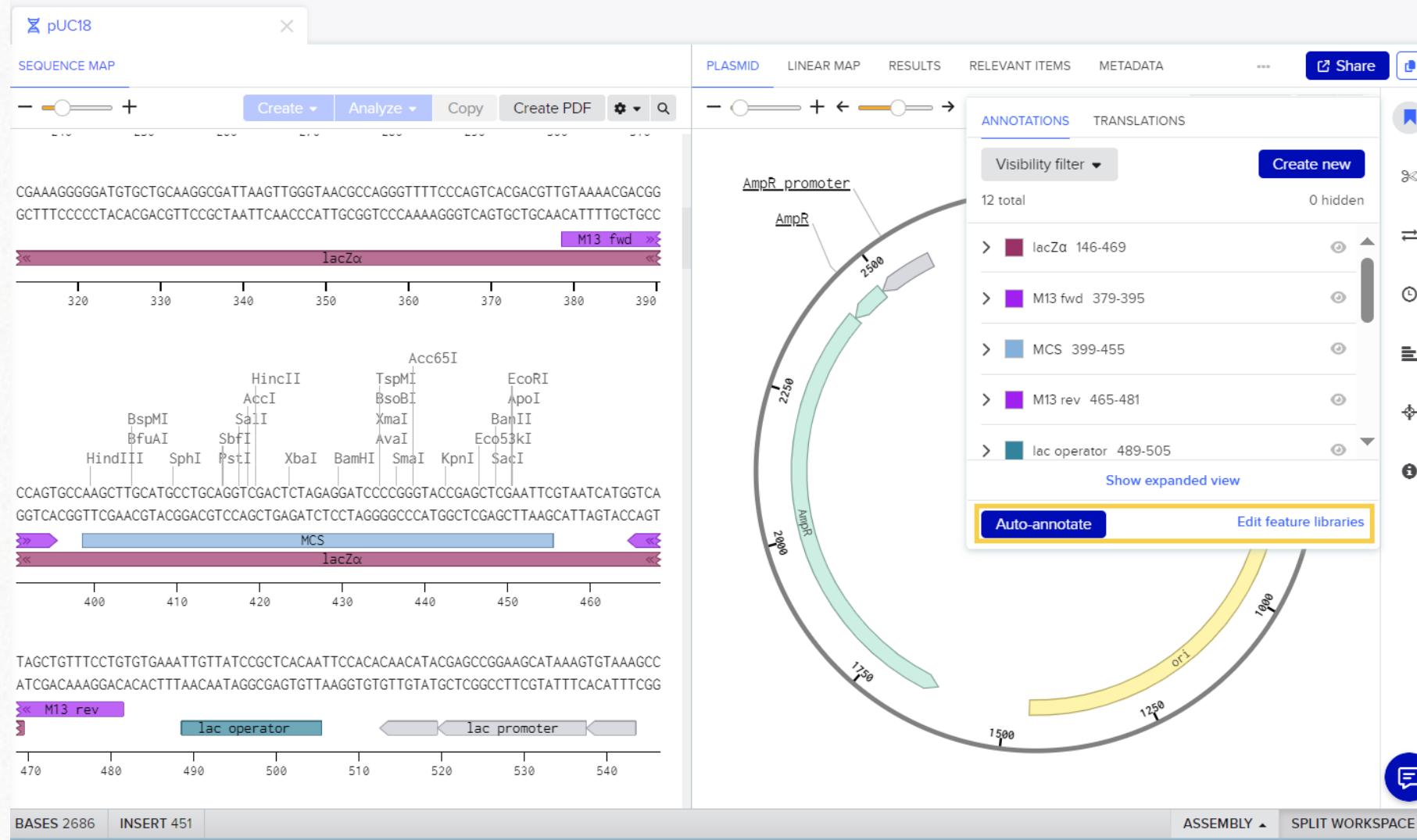
New annotation fields:

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

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

# View, annotate and edit your sequences

## Sequence annotations



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

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

# 3. The basics of sequences

## 3.4 Codon optimization



# View, annotate and edit your sequences

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

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

The screenshot shows the BRIGHT software interface with several panels:

- SEQUENCE MAP**: Shows DNA sequence fragments (e.g., ORF2 CDS, repeat\_region, source) with their positions (1,540 to 1,800). A yellow circle labeled **3** points to the **Create** dropdown menu.
- LINER MAP**: Shows restriction enzyme sites (AvrII, DrdI, PspXI, EcoNI, BpuMI, ApaI, BsrGI, FspI, Bsu36I, KfII, HpaI, AleI, BsaI, BspHI, XbaI, HindIII, BamHI, PfoI, SexAI, XbaI) and genes (ORF2 gene, vdh gene) with their positions (500 to 1,500).
- DESCRIPTION**, **METADATA**, **RELEVANT ITEMS**, **RESULTS**: Standard software tabs.
- Annotations** and **Translations**: Sub-tabs under the main panel.
- CREATE TRANSLATION**: A modal window where a new translation is being created:
  - Name**: vdh translation (highlighted with a yellow circle labeled **4**)
  - Position**: 1786 - 3242
  - # of AA's**: 485
  - Genetic code**: Standard
  - Color**: Blue
  - Strand**: Forward
  - Notes**: (empty)
- BASES 3544 START 1786 END 3242 LENGTH 1457 GC 58.48% MELTING TEMP 83.5 °C**: Sequence statistics at the bottom.

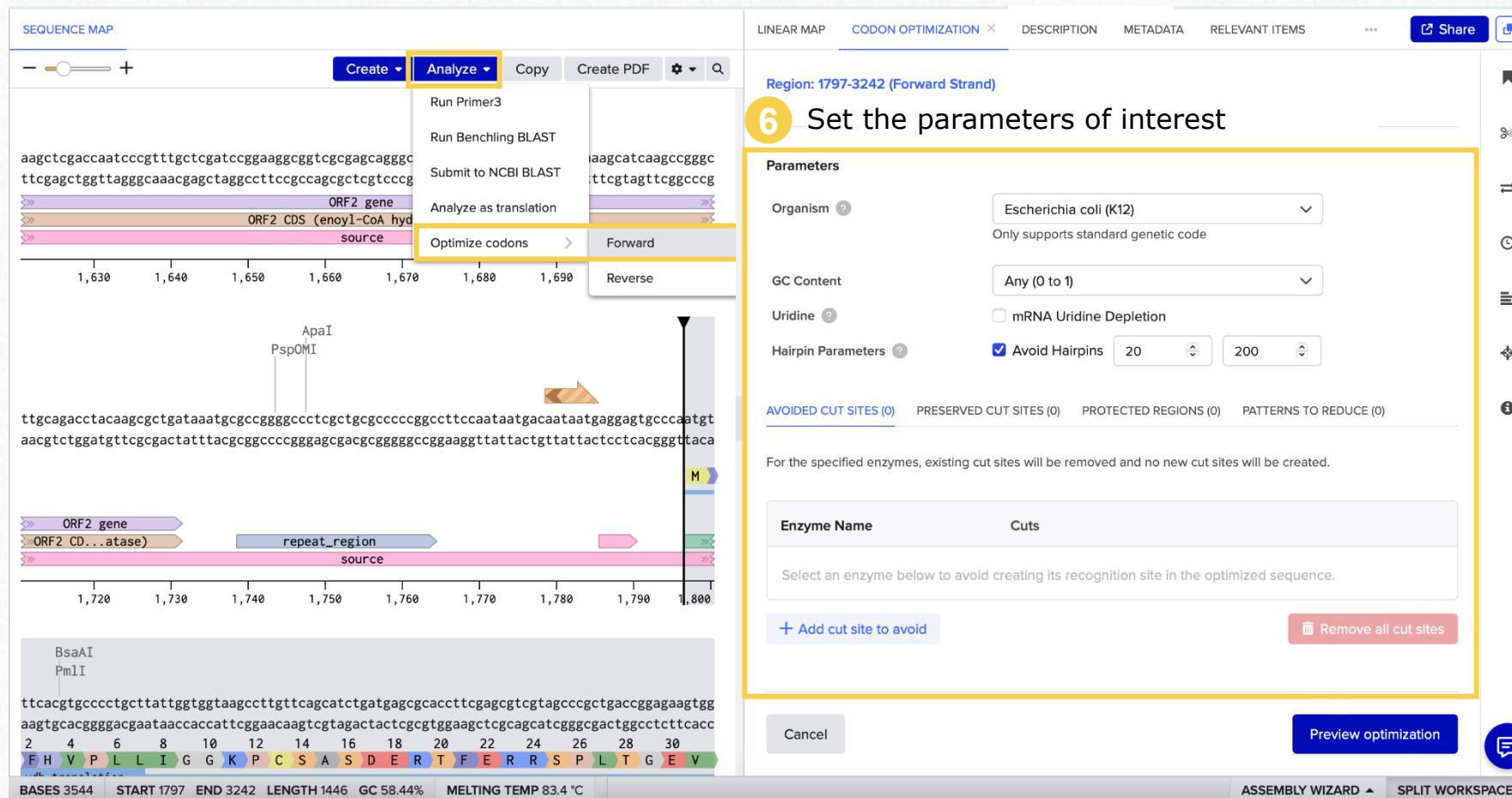
✓ Before codon optimization, the DNA sequence must be translated

ⓘ 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 BRIGHT software interface. On the left, there is a Sequence Map view with two main regions labeled "ORF2 gene" and "repeat\_region". The top region has enzymes ApaI and PspOMI indicated. The bottom region has enzymes BsaAI and PmlI indicated. A context menu is open over the top region, with the "Optimize codons" option highlighted. To the right of the menu is the "CODON OPTIMIZATION" tab, which is active. The "Region: 1797-3242 (Forward Strand)" is specified. A yellow box highlights the "Parameters" section. Inside this box, the "Organism" dropdown is set to "Escherichia coli (K12)". Under "GC Content", the dropdown is set to "Any (0 to 1)". Under "Hairpin Parameters", the checkbox "Avoid Hairpins" is checked, with values 20 and 200. Below this, a table lists enzymes and their avoidance status:

Enzyme Name	Cuts
BsaAI	Select an enzyme below to avoid creating its recognition site in the optimized sequence.
PmlI	

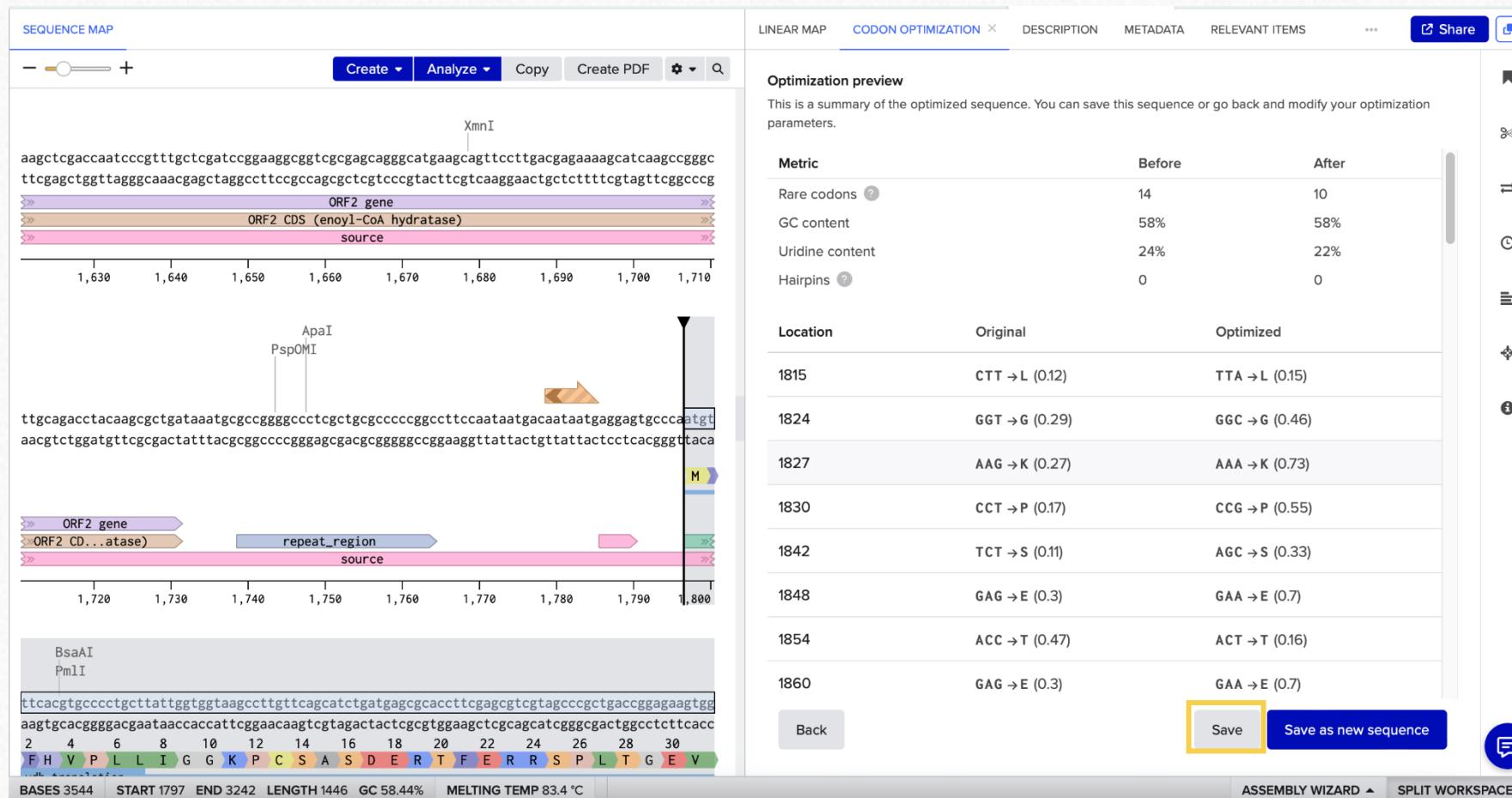
At the bottom of the "Parameters" section, there are "Cancel" and "Preview optimization" buttons, along with a message icon.

At the very bottom of the interface, there are buttons for "ASSEMBLY WIZARD" and "SPLIT WORKSPACE".

# 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



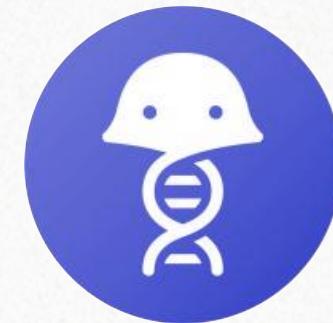


## ***LET'S MOVE TO BENCHLING TO START THE HANDS-ON!***

**Access Benchling:**

[bright.benchling.com](https://bright.benchling.com)

(login with DTU credentials)



# Create a training folder to work in

The screenshot shows the Benchling application interface. On the left, there is a vertical toolbar with various icons. A yellow circle with the number 1 is placed over the icon for creating a new item. The main workspace shows a list of existing projects and a dropdown menu. A yellow circle with the number 2 is placed over the 'Molecular Biology Training' project in the list. Another yellow circle with the number 3 is placed over the 'Folder' option in the dropdown menu. To the right, a modal window titled 'Create folder' is open. It has fields for 'Name\*' (containing 'Your name'), 'Location\*' (set to 'Molecular Biology Training'), and a 'Description' field. A yellow circle with the number 4 is placed over the 'Create' button at the bottom right of the modal.

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 X Clear

1-2 of 2 items, including items in subfolders

Name	Starred	Owner	Modified	Review Process
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 3

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

Copy 4

**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 workspace interface. At the top, there's a navigation bar with 'Projects / Biosustain Training / \*Molecular Biology Training /' and a search bar. Below the search bar, the title 'Training Files [Results]' is highlighted with a yellow box. To the right of the title are buttons for 'Saved Searches' and navigation arrows. Below the title, there are search and filter options: 'Search' with a magnifying glass icon, 'Type' with a dropdown arrow, and 'Filters' with a gear icon. A message '1-4 of 4 items' is displayed above a table. The table has columns for 'Name', 'Starred', 'Owner', 'Modified', 'Review Proces...', and 'Description'. The data rows are:

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

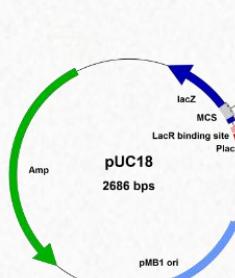
Gene knockout using CRISPR/Cas9

Sequence alignment

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



**pUC18** cloning vector

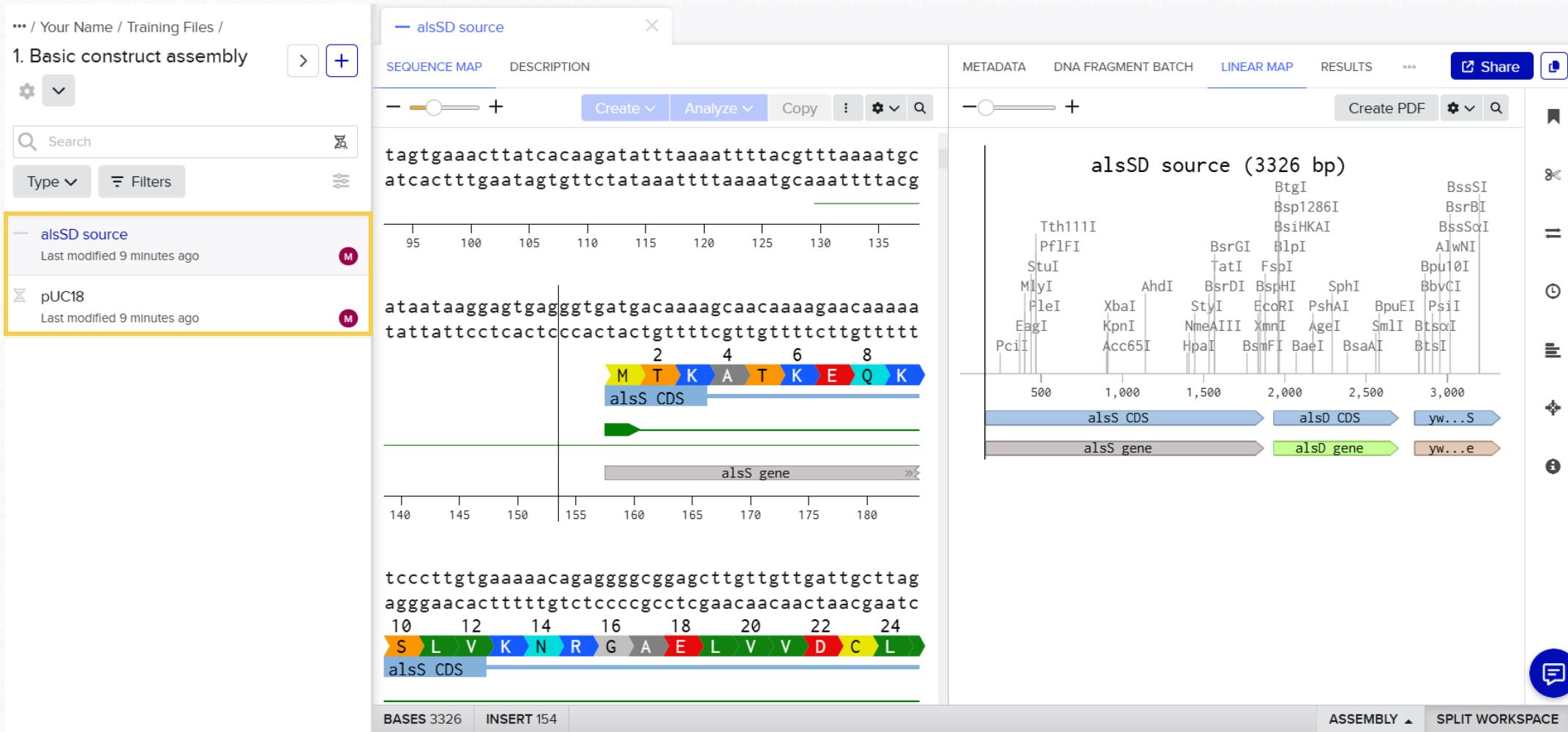


- ✓ Primer design
- ✓ *In silico* PCR
- ✓ Virtual digestion
- ✓ RE-based cloning

#### 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 BRIGHT software interface with two main panels. The left panel displays the 'alsSD source' sequence map, showing DNA sequence fragments and their assembly. The right panel displays the 'alsSD source (3326 bp)' linear map, showing restriction enzyme cleavage sites and gene predictions. Key features include a search bar, filters, and a 'Share' button.

**alsSD source Sequence Map:**

- SEQUENCE MAP tab is active.
- DESCRIPTION tab shows the sequence: tagtgaaacttatcacaagatattaaaattttacgtttaaatgc atcactttgaatagtgttctataaattttaaaatgcaaattttacg.
- Linear scale from 95 to 135.
- Sequence fragments are shown with labels: alsS CDS (blue), alsS gene (grey), and alsD CDS (blue).
- Amino acid sequence below: S-L-V-K-N-R-G-A-E-L-V-V-D-C-L.
- Numbered positions 2, 4, 6, 8 are indicated above the alsS CDS.
- Base count: BASES 3326, Insert count: INSERT 154.

**alsSD source Linear Map:**

- LINEAR MAP tab is active.
- alsSD source (3326 bp) is shown.
- Restriction enzymes listed on the left: Tth111I, Pfl1FI, StuI, MlyI, PfeI, EagI, PciI, XbaI, KpnI, Acc65I, HpaI, NmeAIII, BsrGI, AhdI, BsrDI, StyI, BspHI, EcoRI, BsmFI, BaeI, PsfI, AgeI, SmlI, BsaAI, BpuEI, BbvCI, PsfI, BtsI, BtsII, Bsp1286I, BsiHKAI, BlpI, FspI, SphI, PsfI, Bpu10I, AlwNI, BssSI, BsrBI, BssSXI, BbvCI, PsfI, BtsI, BtsII, Bsp1286I, BsiHKAI, BlpI, FspI, SphI, PsfI, Bpu10I, AlwNI, BssSI, BsrBI, BssSXI, BbvCI, PsfI, BtsI, BtsII.
- Genes: alsS CDS, alsD CDS, yw...S, alsS gene, alsD gene, yw...e.
- Base scale: 500, 1,000, 1,500, 2,000, 2,500, 3,000.

# 5. Basic construct assembly

## 5.1 Primer design

### 5.1.1 Manual primer design

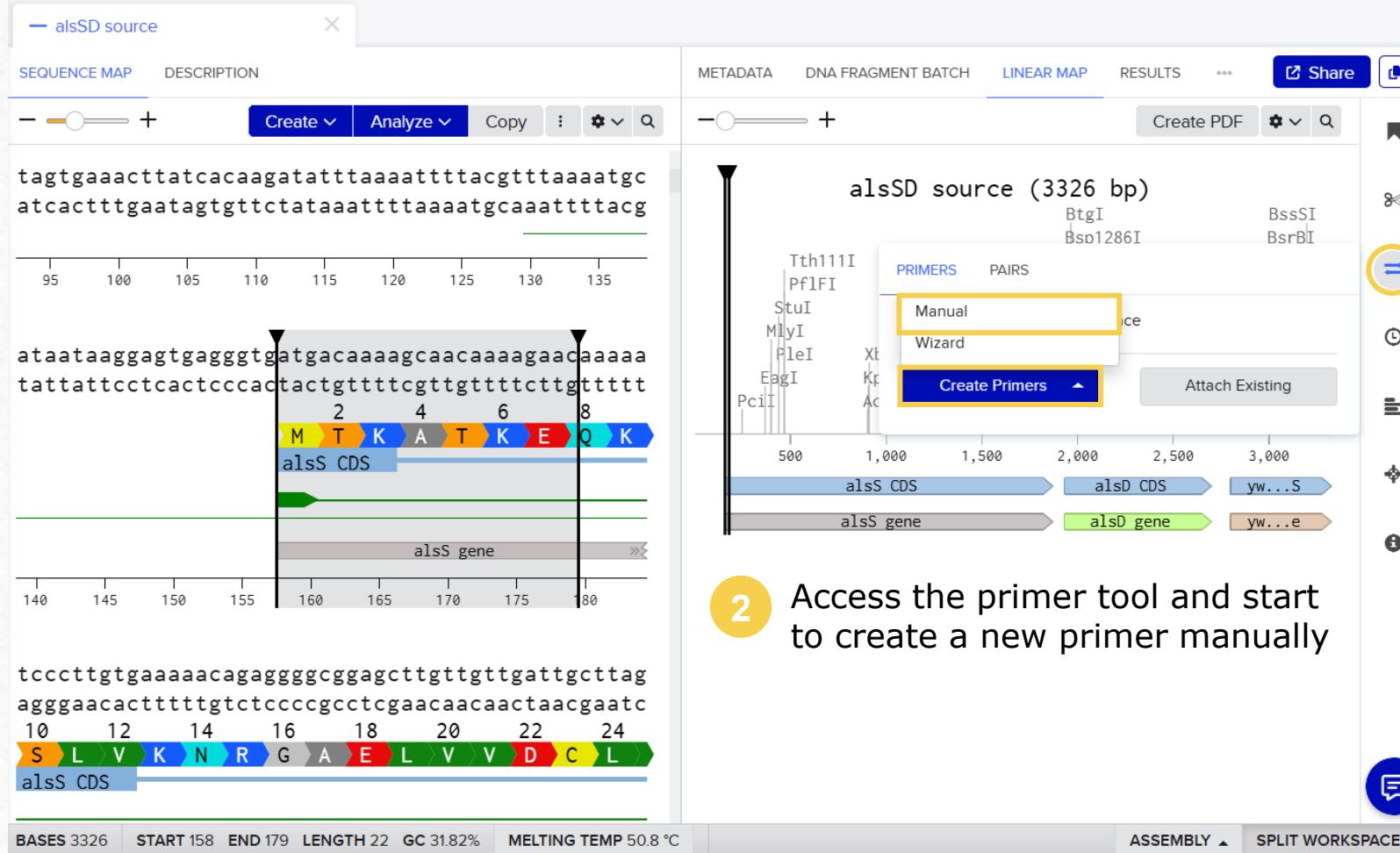


# Construct design

## Manual primer creation

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

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



The screenshot shows two main panels of the Benchling software.

**Left Panel (Sequence Map):** Displays the *alsSD source* sequence map. It includes a sequence map view with a green line indicating the reading frame, a linear map view showing restriction sites (Tth111I, Pfl1FI, StuI, MlyI, PleI, EagI, PciI, XbaI, KpnI, AccI), and a detailed sequence view for two regions of the *alsS* gene. The top region shows a CDS from position 116 to 165 with codons MTAKATKEQK. The bottom region shows a CDS from position 158 to 179 with codons SLVKRNRAEELVVDCL. Below these are sequence details: **BASES 3326**, **START 158**, **END 179**, **LENGTH 22**, **GC 31.82%**, and **MELTING TEMP 50.8 °C**.

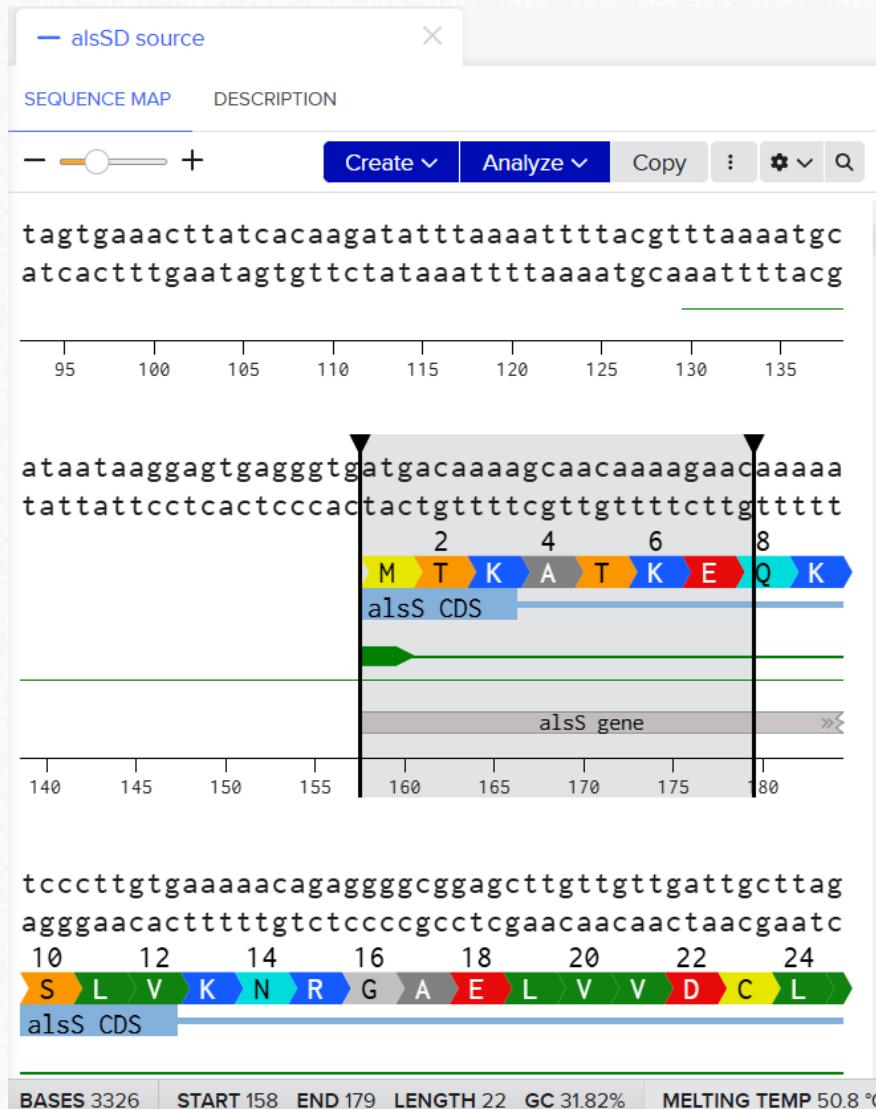
**Right Panel (Primer Creation):** Shows the *alsSD source (3326 bp)* in the Linear Map view. A context menu is open over the *alsS CDS* region, with the "PRIMERS" tab selected. The "Manual" option is highlighted with a yellow box and a blue arrow pointing to the "Create Primers" button. Other options include "Wizard" and "Attach Existing". The panel also displays restriction enzymes **BtgI** and **Bsp1286I** located upstream of the *alsS* gene.

- 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: AanI Use the dropdown above to look up restriction sites.

Verify Check Secondary Structure at 50 °C

T<sub>m</sub> -

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 agtt 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

**Verify**

	alsSD-fwd	alsSD-rev
T <sub>m</sub>	50.8°C	53.4°C
GC Content	31.82%	40.91%
Length	28 bp	28 bp
Product Size	2554 bp	
T <sub>m</sub> Diff.	+2.57°C	

**Save**

Name	alsSD-fwd	alsSD-rev
------	-----------	-----------

Save To: 1. Basic construct assembly

**Save Primer Pair**

✓ 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

LINEAR MAP DESIGN PRIMER **ALL STRUCTURES X** DESCRIPTION METADATA ... Share

Primer Pair ▾ Jump to Primer ▾ Set from Selection ▾

Overhang 0 bp 0 bp

Cut Site Aanl

Use the dropdown above to look up restriction sites.

Verify

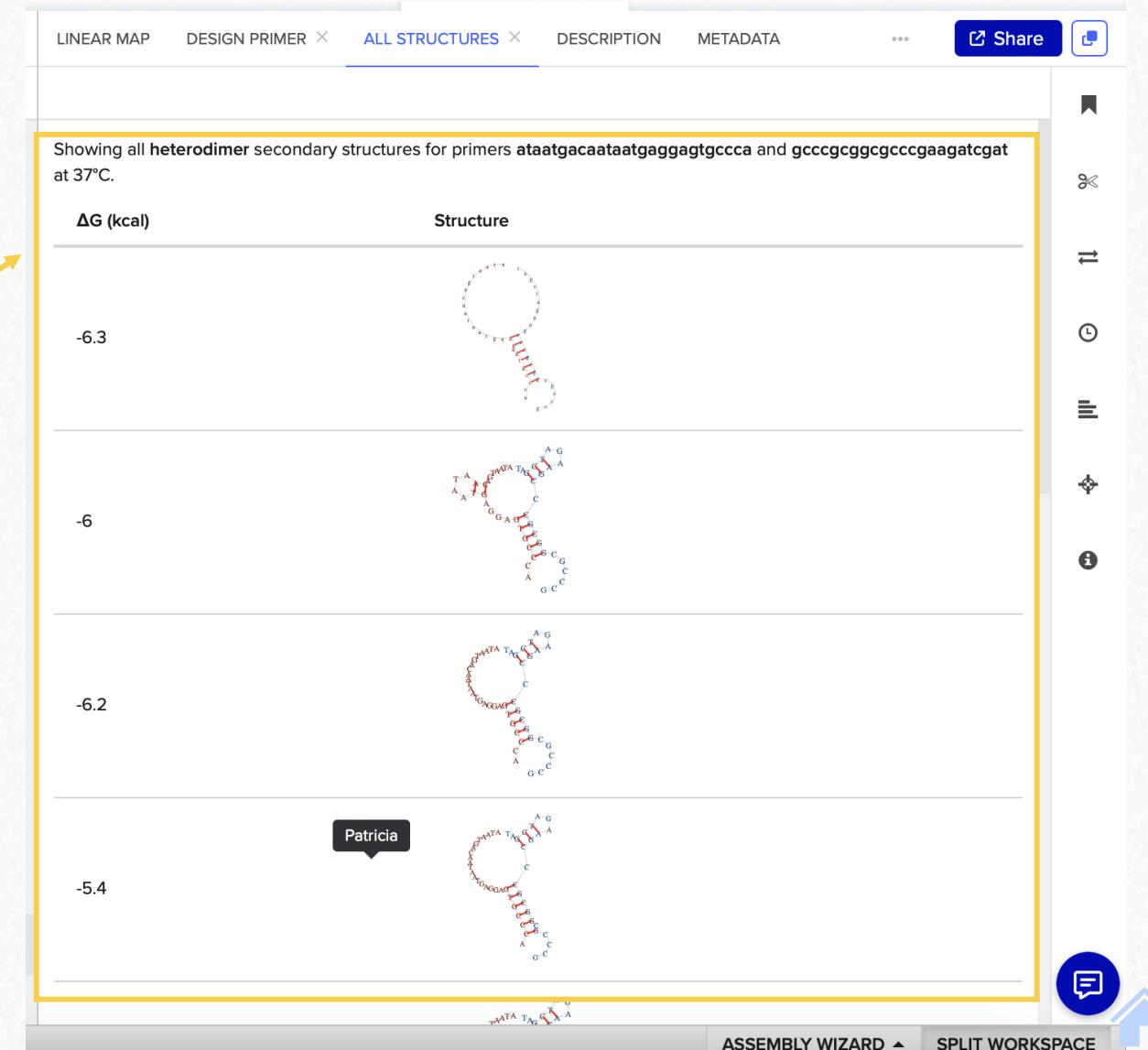
T <sub>m</sub>	56.1°C	69.8°C
GC Content	38.46%	73.91%
Length	26 bp	23 bp
Min ΔG Homodimer	-3.3 kcal	All Structures
Min ΔG Monomer	-0.1 kcal	All Structures
-2.5 kcal	All Structures	
Product Size	1495 bp	
T <sub>m</sub> Diff.	+13.77°C	
Min ΔG Heterodimer	-6.3 kcal	All Structures

Patricia

Save

Name fwd\_vdh rev\_vdh

ASSEMBLY WIZARD ▾ SPLIT WORKSPACE



# 5. Basic construct assembly

## 5.1 Primer design

### 5.1.2 Primer wizard

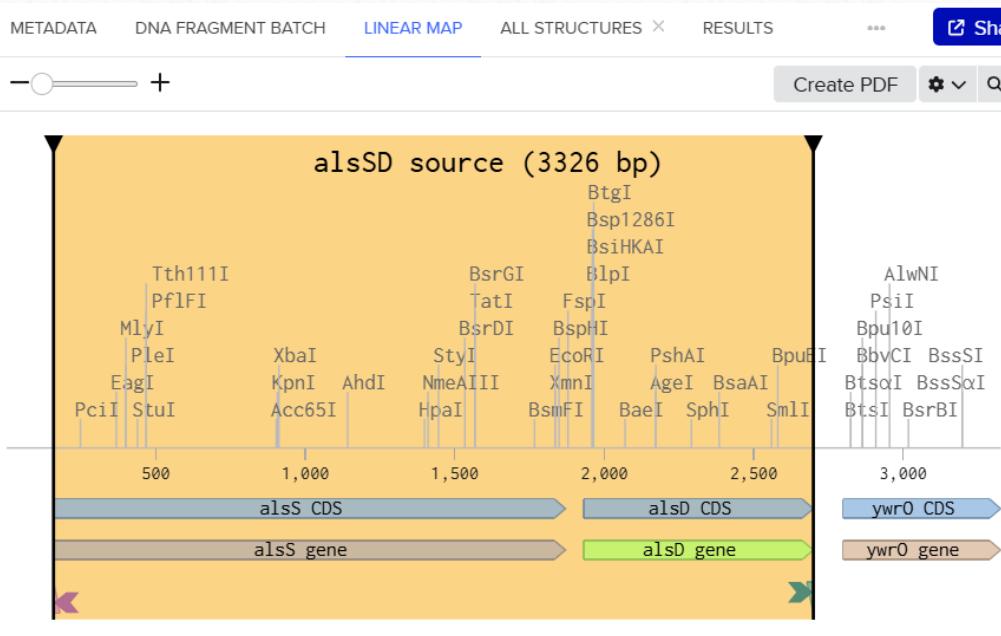


# Construct design

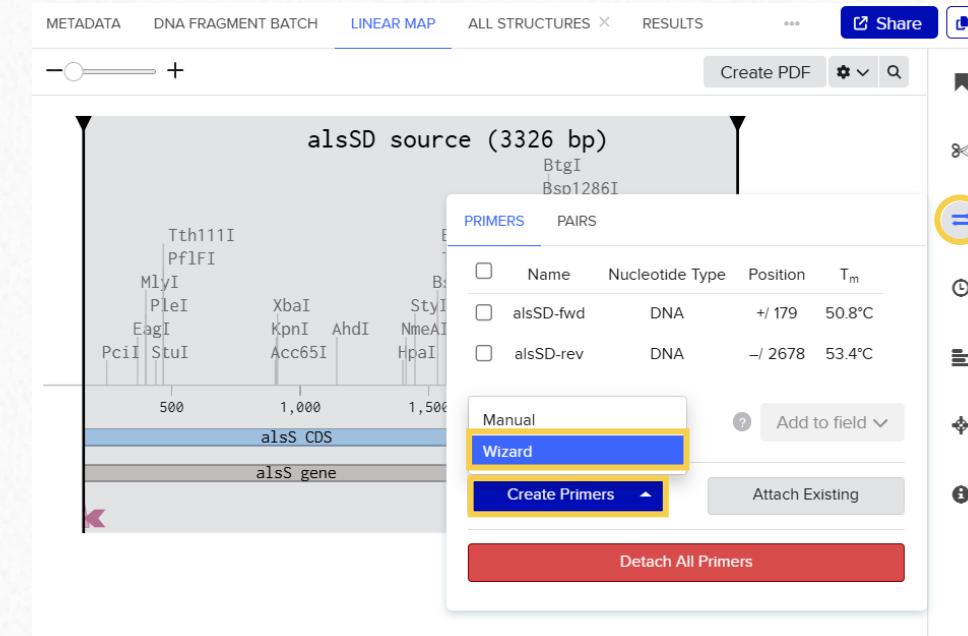
## Automatic primer creation – Primer Wizard

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

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



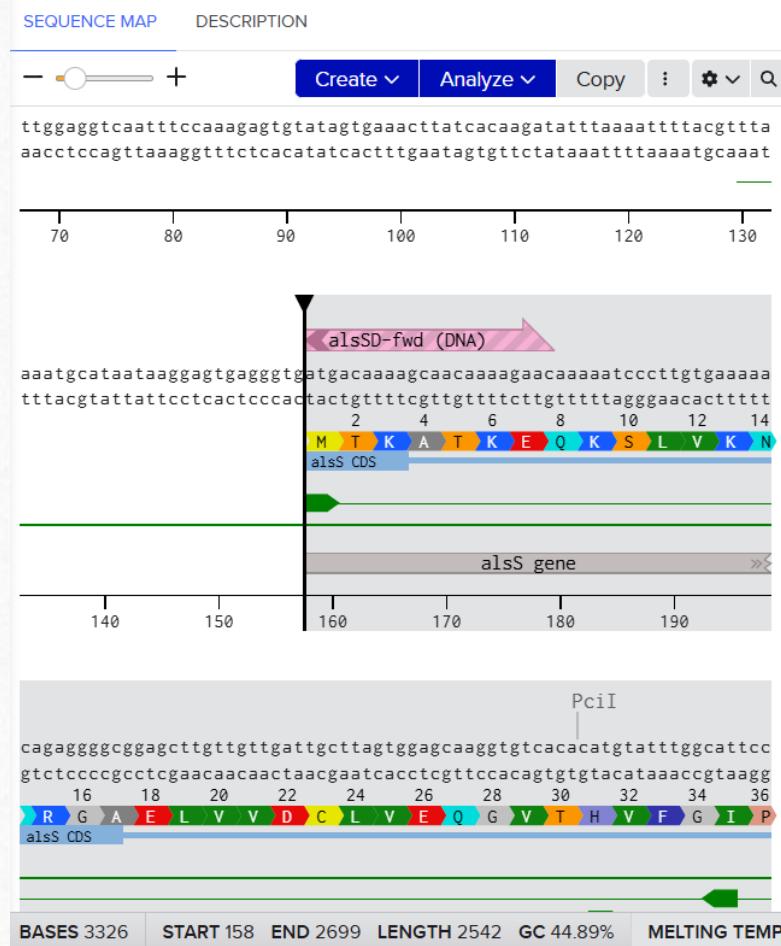
### 2 Access the primer tool and select *wizard*



**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**

**5 Generate Primers**

**4 Use your selected sequence**

The screenshot shows the **PRIMER WIZARD** interface. The **Task** dropdown is set to **PCR**. In the **Region** section, the **Target** range is set from 158 to 2699, and the **Use selection** button is highlighted. In the **Primer** section, the following parameters are set:

	Min	Opt	Max
GC%	30	50	65
T <sub>m</sub>	45	62	65
Size	15	22	31
3' GC clamp	0		

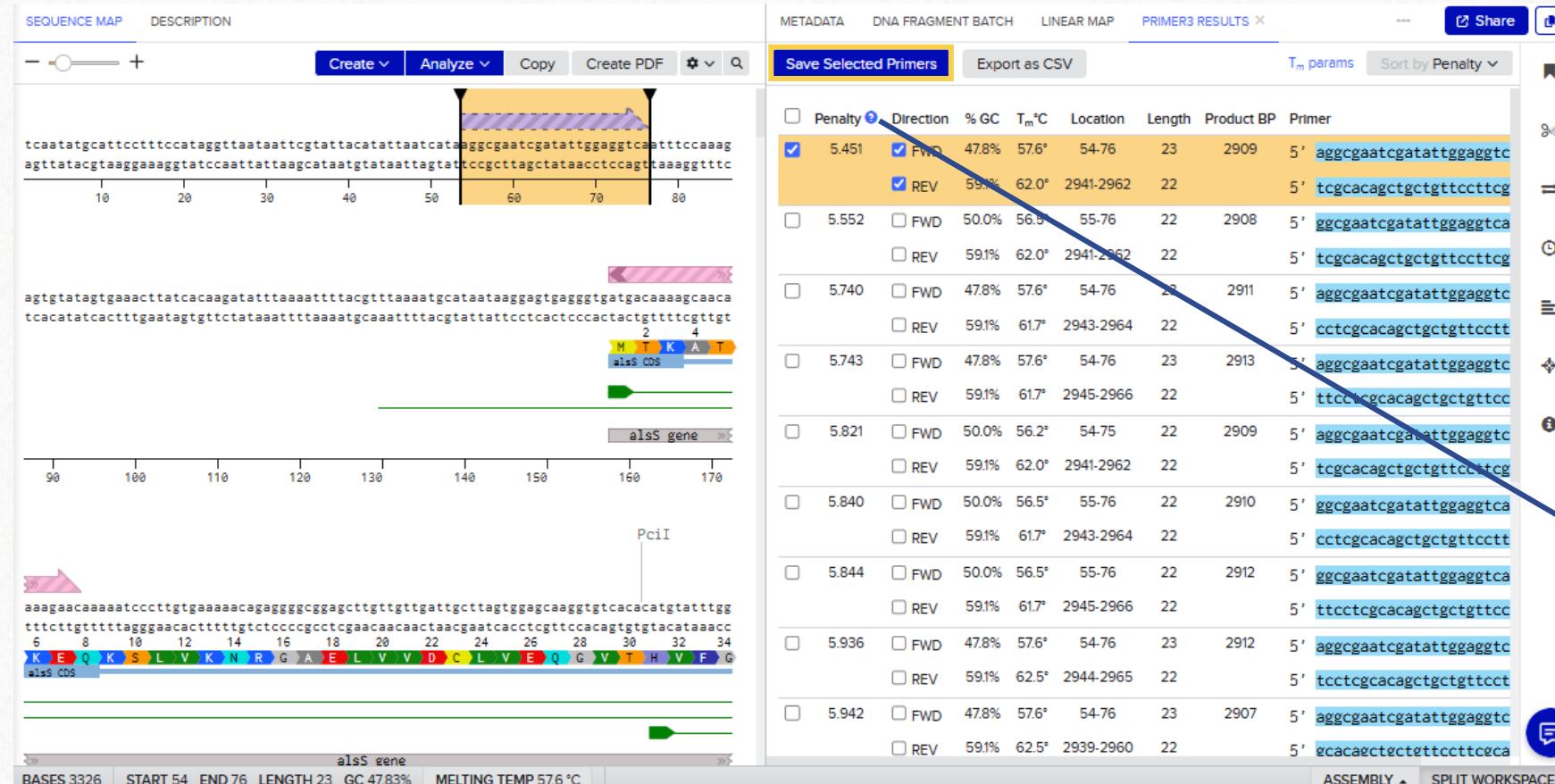
- ✓ Primer Wizard allows for different sequencing tasks
- ✓ Primer Wizard is powered by Primer3

 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 interface with two main panels. On the left, the 'SEQUENCE MAP' panel displays three DNA sequences: 'alsS gene', 'PciI', and 'alsS CDS'. The 'alsS gene' sequence has a blue shaded region from position 50 to 70. The 'PciI' sequence has a pink shaded region from position 140 to 160. The 'alsS CDS' sequence has a blue shaded region from position 2 to 4. On the right, the 'PRIMER3 RESULTS' panel lists 12 primer pairs with their details:

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

At the bottom of the results panel, there are buttons for 'ASSEMBLY' and 'SPLIT WORKSPACE'.

✓ 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 Bioworkspace interface for construct design. On the left, a linear map of the *alsSD source* (3326 bp) is displayed, showing various restriction sites (BtgI, Bsp1286I, BssSsI, Tth11I, StuI, MlyI, PstI, EagI, PciI, PflFI, 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, resulting in a 2554 bp product size with a  $T_m$  difference of +2.6°C. Step 1 (yellow circle) points to the copy icon in the toolbar. Step 2 (yellow circle) points to the 'PAIRS' tab in the primer list. Step 3 (yellow circle) points to the primer information table. Step 4 (yellow circle) points to the 'Create PCR Product' button. Step 5 (yellow circle) points to the 'Copy' button in the context menu of the 'alsSD source' panel, which lists options for copying primer bases, annotations, translations, and descriptions.

- ✓ 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)

GGATCCatgacaaaagcaacaaaagaacaaaaatccctgtgaaaaacagagggcgaggctgt  
CCTAGGtactttcgttctttaggaacacttttgcctccgcctcgaaaca

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

alsS gene

10 20 30 40 50 60

PciI

tgttgattgttagggagcaagggtcacacatgtattggcattccagggtcaaaaattgatg  
acaactaacgaatcacctcggtccacagtgtacataaccgttaaggccacgttttaactac

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

**LINEAR MAP**

alsSD source [158-2699] (2554 bp)

BtgI  
Bsp1286I  
BsiHKAI  
Tth111I  
StuI  
EagI  
PciI  
BstYI  
MlyI  
BamHI  
PleI  
BseYI  
Acc65I

PvuI  
SspI  
XbaI  
KpnI  
AhdI

BsrGI  
TatI  
BsrDI  
StyI  
NmeAIII  
HpaI  
FokI  
NdeI  
BtsCI  
PvuII  
BlpI  
Agel  
BsaAI

FspI  
BspHI  
EcoRI  
PshAI  
BpuEI  
BaeI  
SphI  
AccI

SmlI  
SalI

500 1,000 1,500 2,000 2,500

alsS CDS

alsD CDS

alsS gene

alsD gene

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

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

# 5. Basic construct assembly

## 5.3 Virtual digestion

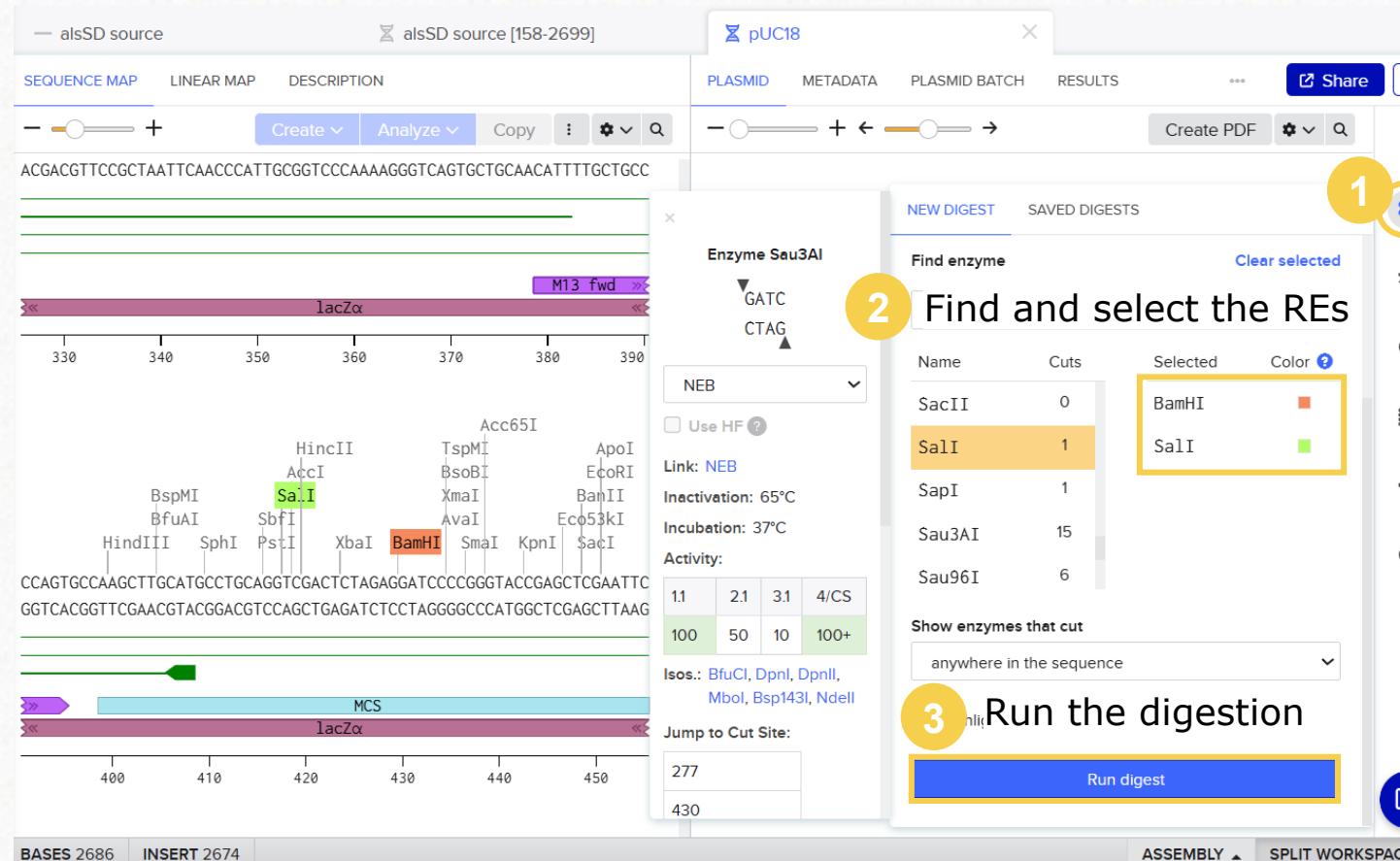


# Construct design

## 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 fwd primer, and the Multiple Cloning Site (MCS). Various restriction enzymes are marked along the sequence, including **BamHI** and **SalI**. The main window shows a digestion setup for **Sau3AI** (GATC site). A yellow circle labeled **1** points to the search bar where **Sau3AI** is entered. A yellow circle labeled **2** points to the enzyme selection table where **BamHI** and **SalI** are selected. A yellow circle labeled **3** points to the **Run digest** button.

Name	Cuts	Selected	Color
SacII	0		
<b>SalI</b>	1	<input checked="" type="checkbox"/>	<span style="background-color: #ffcc00;">■</span>
SapI	1		
Sau3AI	15		
Sau96I	6		

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

# Construct design

## Virtual digestion

The screenshot shows the NEBcutter software interface. On the left, a linear map of a DNA sequence is displayed with various restriction sites labeled. A specific site for **NcoI** is highlighted with a yellow arrow. A tooltip for this site shows the sequence **CCATGG**. A dropdown menu for **NEB** is open, showing enzyme lists like **Deduplicated commercial** and **Single cutters**. The **Find enzyme** search bar contains **nc**, and a list of enzymes is shown, including **HincII** (4 cuts), **NciI** (10 cuts), and **NcoI** (1 cut). The **NcoI** entry is highlighted with a yellow box and has a blue info icon with a callout pointing to a list of selection options: **anywhere in the sequence**, **✓ in the current selection**, **only in the current selection**, and **anywhere except the current selection**.

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

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

# Construct design

## Virtual digestion

### Digestion of the backbone

The screenshot shows the BRIGHT software interface for a virtual digestion. At the top, there are tabs for PLASMID, DIGEST (which is selected), VIRTUAL DIGEST, and METADATA. Below the tabs, there's a 'Share' button and a 'Save' button with a yellow circle containing the number '4' above it, indicating pending saves. A dropdown menu says 'Save the digestion' with a 'Use HF' checkbox checked. To the right of the save button are icons for bookmarking, sharing, and other functions. The main area displays two rows of enzyme digestions:

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

Below this, another table provides detailed cut information:

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

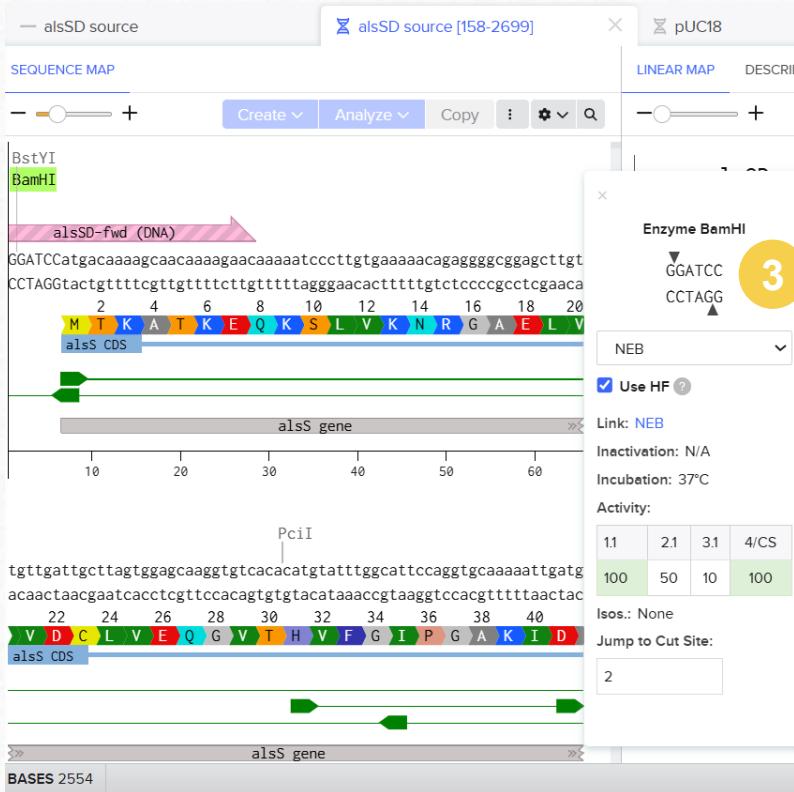
- ✓ A saved digestion will allow you to easily find the fragments you need to work with for the assembly

# Construct design

## Virtual digestion

### Digestion of the insert

- 1 Open the amplified *alsSD* sequence



alsSD source [158-2699]

SEQUENCE MAP

BstYI  
BamHI

alsSD-fwd (DNA)

GGATCCatgacaaaaggcaaaaaaaatccctgtgaaaaacagagggcgagcttg  
CCTAGTactttcgttctgttttaggaaacttttgtctccgcctcgaaaca

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

alsS gene

PciI

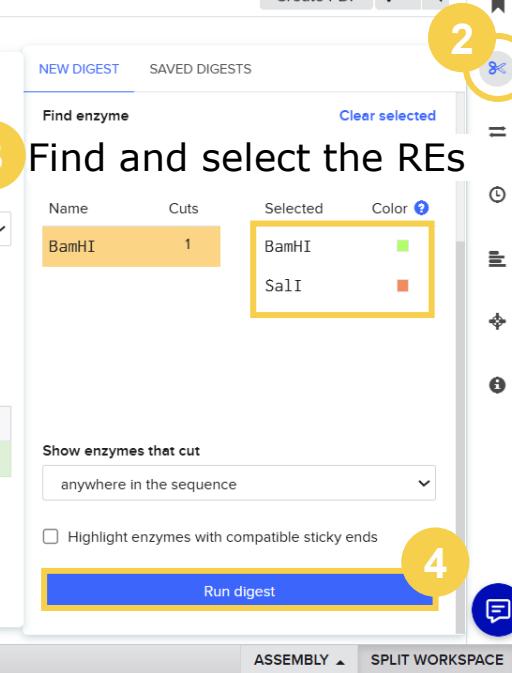
tgttattgttagtggaggcaagggttcacatgtatggcattccagggtgaaaaattgtat  
acaactaacaaatcacatcggttccacagtgtgtacataaaccgtaaaggccacgttttaacta

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

- 3 Find and select the REs



Enzyme BamHI

Find enzyme

Clear selected

NEB

Use HF

Link: NEB

Inactivation: N/A

Incubation: 37°C

Activity:

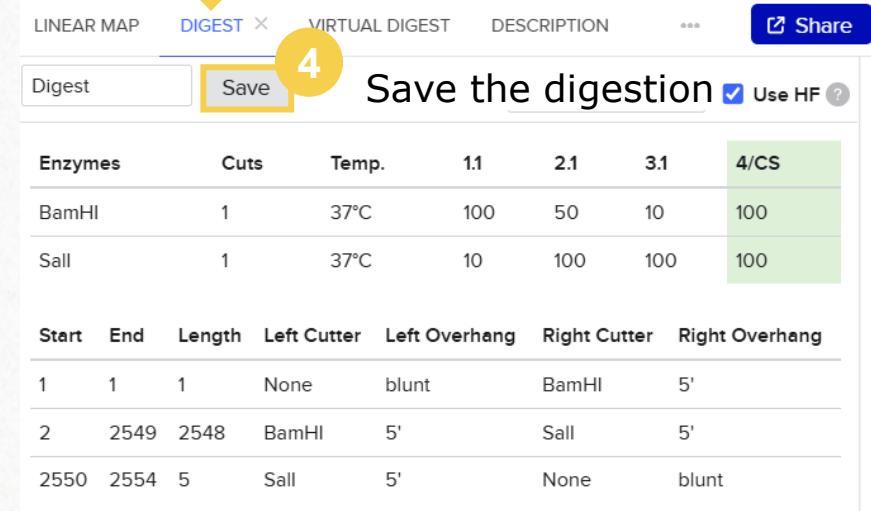
Name	Cuts	Selected	Color
BamHI	1	<input checked="" type="checkbox"/>	green
SalI		<input type="checkbox"/>	red

Show enzymes that cut: anywhere in the sequence

Highlight enzymes with compatible sticky ends

Run digest

The digest tab will open



LINEAR MAP DIGEST VIRTUAL DIGEST DESCRIPTION ... Share

Digest Save 4

Enzymes Cuts Temp. 1.1 2.1 3.1 4/CS

BamHI	1	37°C	100	50	10	100
SalI	1	37°C	10	100	100	100

Start End Length Left Cutter Left Overhang Right Cutter Right Overhang

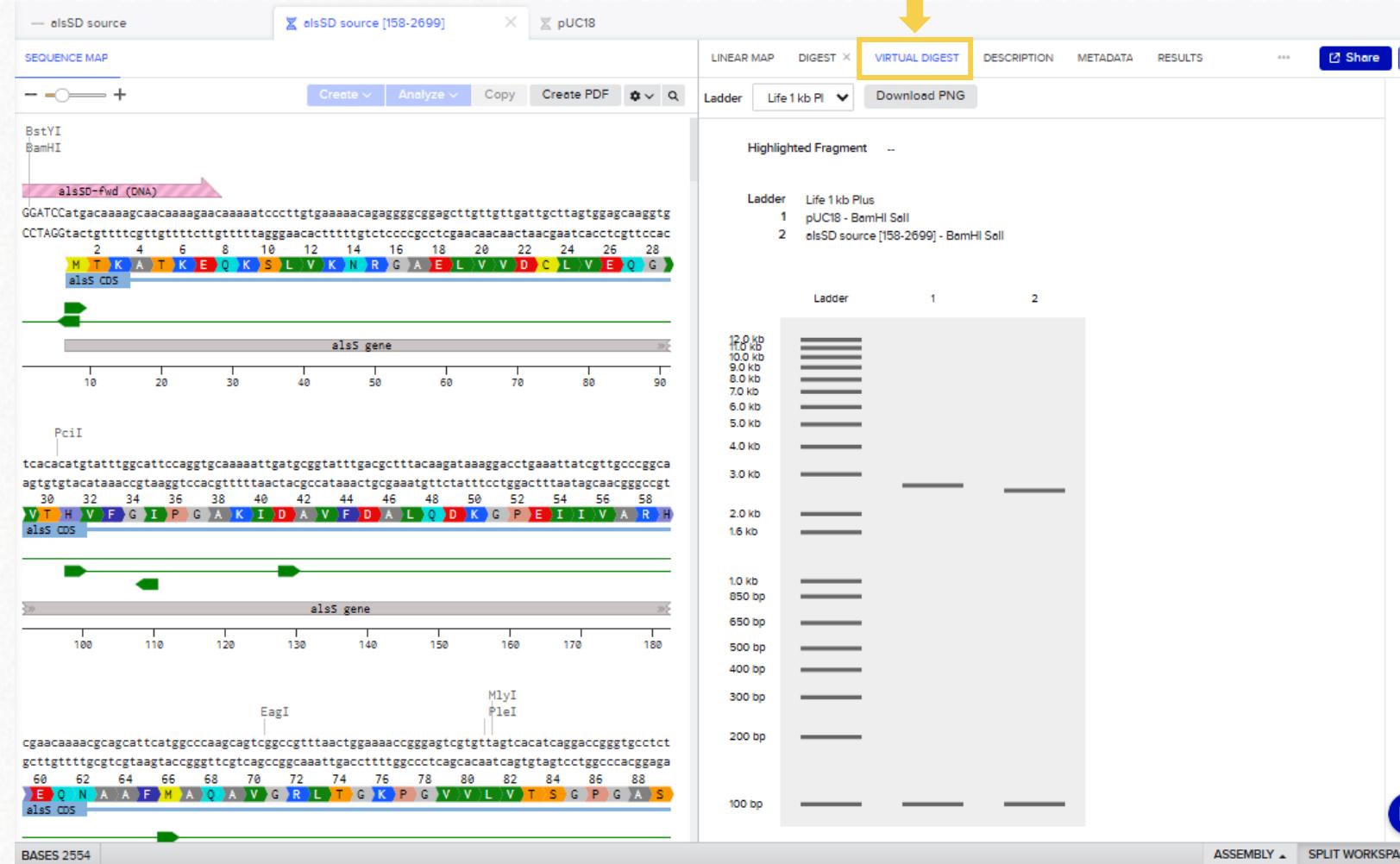
1	1	1	None	blunt	BamHI	5'
2	2549	2548	BamHI	5'	SalI	5'
2550	2554	5	SalI	5'	None	blunt

- 4 Run the digestion

# Construct design

## Virtual digestion

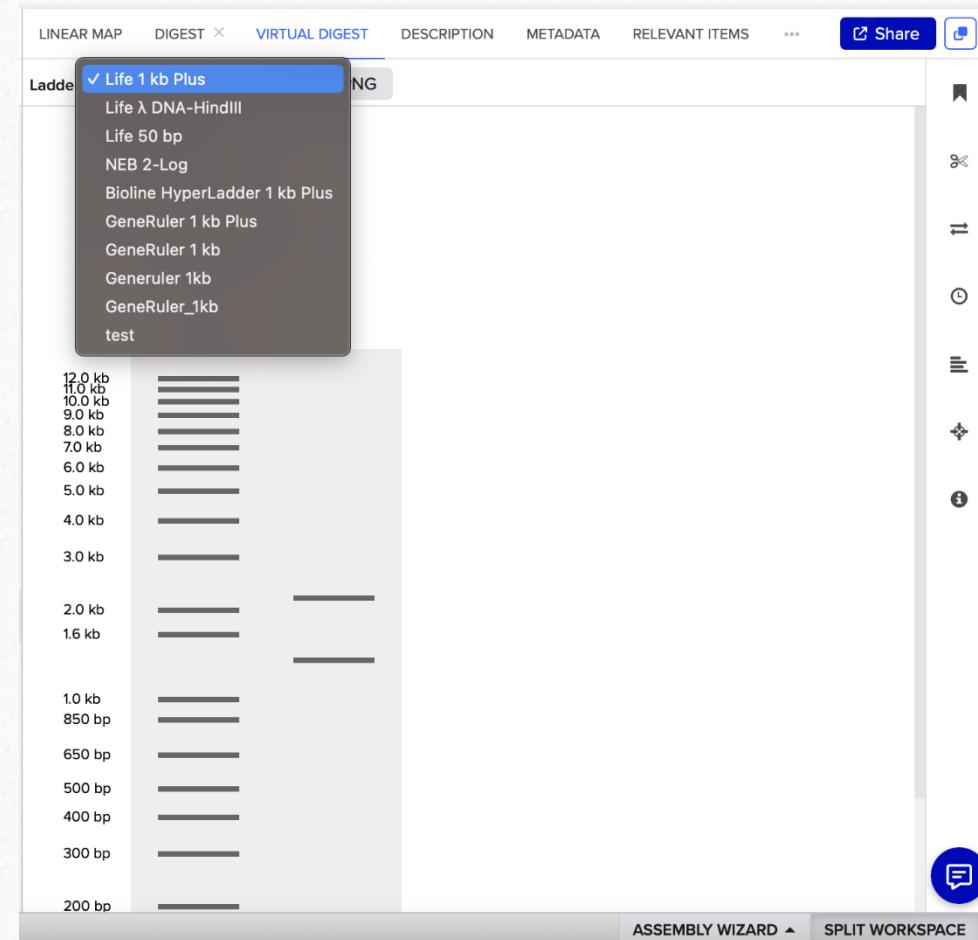
### Gel visualization



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

# Construct design

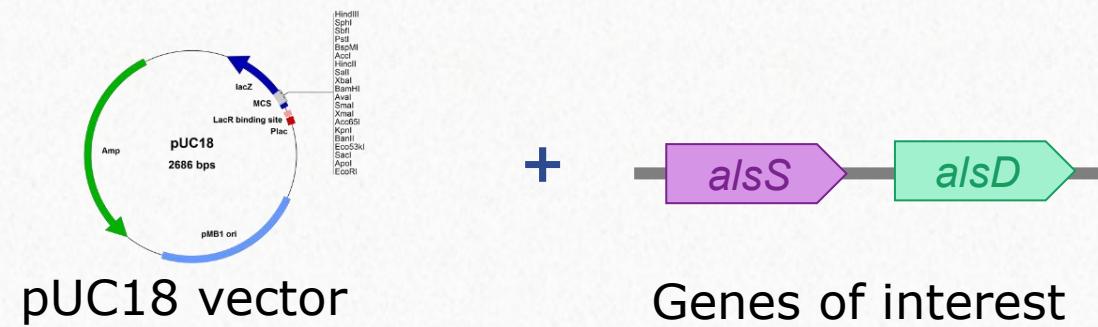
## Virtual digestion



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

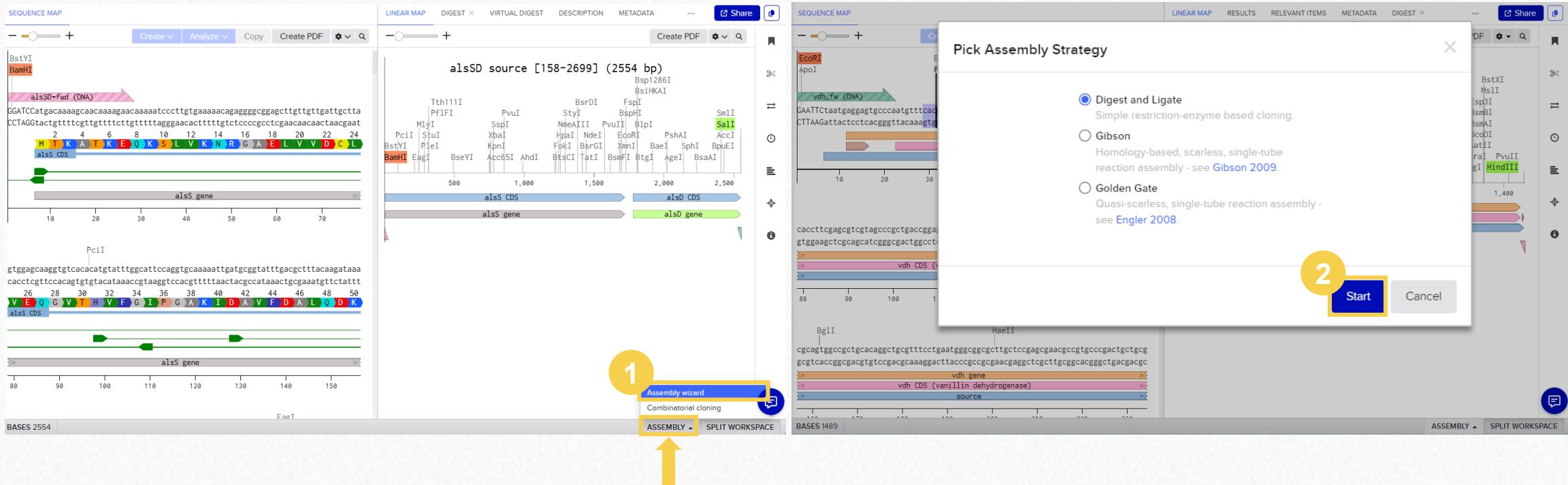
## 5. Basic construct assembly

### 5.4 Assembly Wizard



# Construct Assembly

## Assembly Wizard



**1** Assembly wizard  
Combinatorial cloning  
**ASSEMBLY ▾**

**2** Start Cancel

Pick Assembly Strategy

- 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](#).

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 BRIGHT Assembly Wizard interface. On the left, a circular plasmid map of pUC18 is displayed, showing various restriction sites and features: AmpR promoter (2500), AmpR (2250), RSPD (2000), lacZ (1750), lacZ (1500), lacZ (1250), lacZ (1000), ori (1000), M13 fwd MCS (500), +3 lac\_promoter, and M13 rev (65). The total size is 2686 bp. Above the map, tabs for 'PLASMID', 'SEQUENCE MAP', 'LINEAR MAP', and 'DESCRIPTION' are visible, along with a 'Create PDF' button. To the right of the map is a 'DIGEST' tab panel containing a table of enzyme digestions:

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

Below the digest table are two rows of restriction site information:

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

At the bottom of the interface, there are two main sections: 'SET FRAGMENT' and 'OVERALL ASSEMBLY'. The 'SET FRAGMENT' section contains a diagram of a backbone with an insert, labeled 'Backbone' and 'Insert', with 'BASES 2686' and 'INSERT 693'. The 'OVERALL ASSEMBLY' section has a text input field 'pUC18-alsSD' with a delete icon, and a blue 'Assemble' button. A yellow arrow points from the text 'This will remain open even if you go from one file to another' to the 'SET FRAGMENT' section. Another yellow arrow points from the text 'Name your construct' to the 'pUC18-alsSD' input field.

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

# Construct Assembly

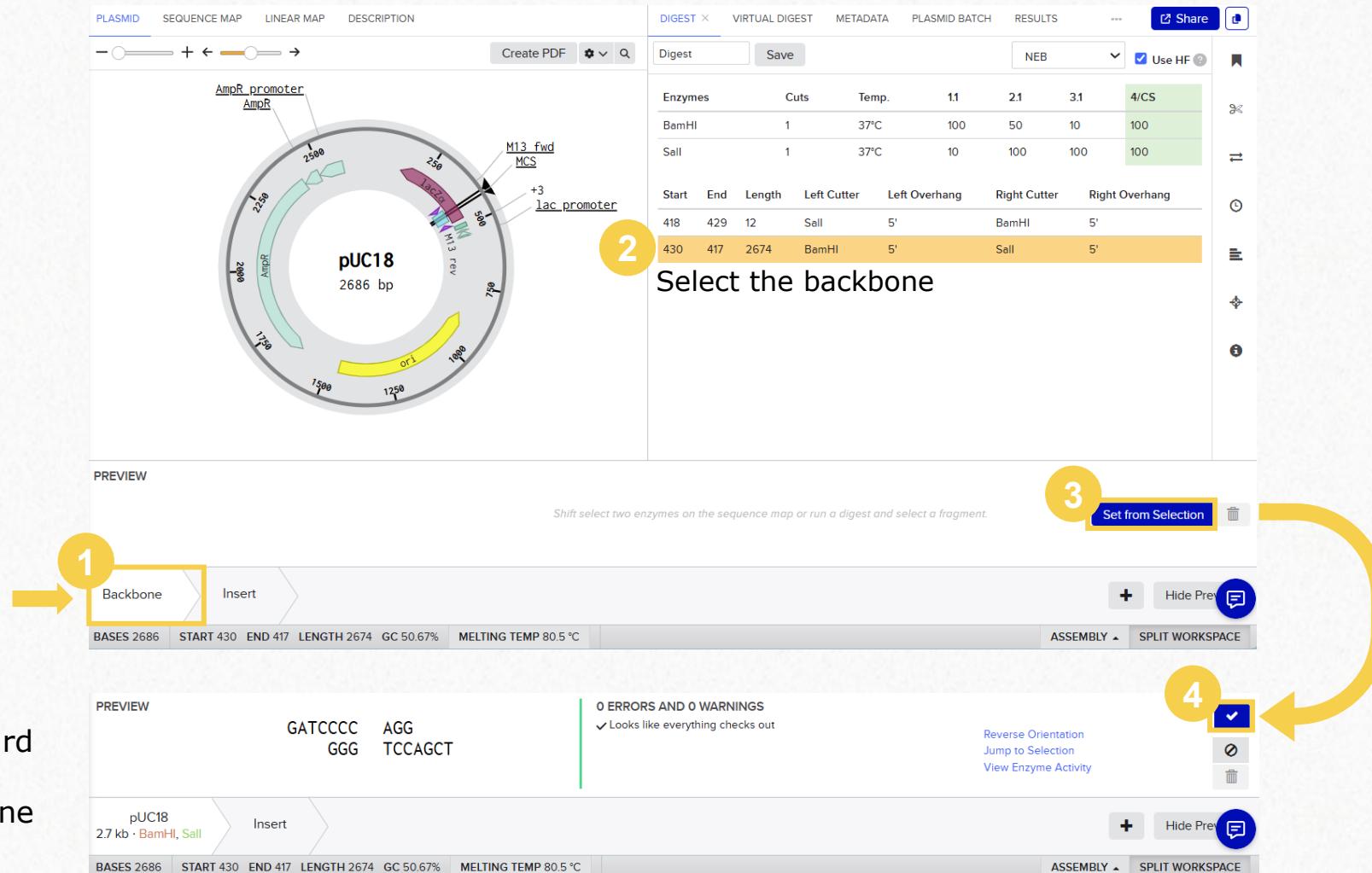
## Digest and Ligate: Add the backbone

**1** Backbone

**2** Select the backbone

**3** Set from Selection

**4** ✓



The screenshot shows the BRIGHT Assembly Wizard interface. At the top, there's a navigation bar with tabs like PLASMID, SEQUENCE MAP, LINEAR MAP, and DESCRIPTION. Below this is a circular sequence map of the pUC18 plasmid (2686 bp), showing various restriction sites and promoters: AmpR promoter, AmpR, M13 fwd MCS, +3 lac promoter, M13 rev, and ori. A yellow arrow labeled '1' points to the 'Backbone' button in the preview section. The preview section also shows the sequence GATCCCC GGG AGG TCCAGCT and a note: '0 ERRORS AND 0 WARNINGS' with a checkmark. In the center, a table lists enzymes and their digest conditions:

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'

On the right side, there are buttons for 'DIGEST X', 'VIRTUAL DIGEST', 'METADATA', 'PLASMID BATCH', 'RESULTS', 'Share', and a 'DIGEST' button. A large yellow arrow labeled '2' points to the 'Select the backbone' step. Another yellow arrow labeled '3' points to the 'Set from Selection' button. A final yellow arrow labeled '4' points to a checked checkbox in the bottom right corner of the preview area.

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

# Construct Assembly

## Digest and Ligate: Add the insert

The screenshot shows the BRIGHT Assembly Wizard interface for constructing a genetic construct. The main window is divided into several panels:

- SEQUENCE MAP**: Shows the *alsSD source* [158-2699] (2554 bp) with restriction enzyme sites (Bsp1286I, BsiHKA1, SmlI, SalI, etc.) and gene regions (*alsS CDS*, *alsD CDS*, *alsS gene*, *alsD gene*). A yellow circle labeled **2** highlights the *SalI* site.
- LINER MAP**: Below the sequence map, it shows the linear sequence with restriction sites and gene locations.
- DIGEST X**: A table for digesting the construct with NEB enzymes. It includes columns for Enzymes, Cuts, Temp., and conditions (1.1, 2.1, 3.1, 4/CS). The *BamHI* and *SalI* rows are highlighted in green.
- RESULTS**: A table showing the results of the digestions, including Start, End, Length, Left Cutter, Left Overhang, Right Cutter, and Right Overhang.
- PREVIEW**: A sequence preview showing the restriction sites and the insertion site for the *pUC18* vector (2.7 kb). The *SalI* site is indicated by a yellow box labeled **1**. An arrow points from the **Insert** box to the *SalI* site.
- Buttons and Tools**: Includes "Create PDF", "Share", "Save", "Set from Selection", and "Hide Prev".

**Select the insert**

**1** **2** **3**

# Construct Assembly

## Digest and Ligate: Check for compatibility

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

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

**Top Preview Panel:**

- PREVIEW:** Shows the assembly of pUC18 (2.7 kb · BamHI, Sall) and alsSD source [158-2699] (2.5 kb · BamHI, Sall). The backbone sequence is AGGGATCC and the insert sequence is TCCAGCT.
- Sticky Ends:** The backbone has a left sticky end (AGG) and a right sticky end (TCC). The insert has a left sticky end (TCC) and a right sticky end (GAT).
- Status:** 2 ERRORS AND 0 WARNINGS
  - The left sticky end does not match.
  - The right sticky end does not match.
- Actions:** A yellow circle labeled "1" points to a "Reverse Orientation" button. Other buttons include a checked checkbox, an uncheckable checkbox, and a trash bin.

**Bottom Preview Panel:**

- PREVIEW:** Shows the same backbone and insert sequences, but with different orientations. The backbone now has a left sticky end (TCG) and a right sticky end (AGG). The insert has a left sticky end (GAA) and a right sticky end (CTAG).
- Sticky Ends:** The backbone has a left sticky end (TCG) and a right sticky end (AGG). The insert has a left sticky end (GAA) and a right sticky end (CTAG).
- Status:** 0 ERRORS AND 0 WARNINGS
  - Looks like everything checks out
- Actions:** A yellow circle labeled "2" points to a checked checkbox. Other buttons include an uncheckable checkbox and a trash bin.

A large yellow arrow points from the top panel to the bottom panel, indicating the result of the orientation adjustment.

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

# Construct Assembly

## Digest and Ligate: Assemble

**SET FRAGMENT**  
Select an assembly fragment below.

**OVERALL ASSEMBLY**  
✓ Looks like everything checks out

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

**SEQUENCE MAP**

LINEAR MAP PLASMID DESCRIPTION METADATA RELEVANT ITEMS Share

**Linear Map:** pUC18-alsSD (5222 bp)

Key features labeled on the map include:  
 - AmpR AmpR\_promoter (green arrow at ~4500)  
 - lacZα (purple arrow at ~4800)  
 - MCS (blue box at ~4900)  
 - alsS gene (green arrow at ~5000)  
 - alsD gene (green arrow at ~5200)  
 - M13 fwd (green arrow at ~5300)  
 - M13 rev (green arrow at ~5400)  
 - lac\_promoter (green arrow at ~3000)  
 - lac\_promoter (green arrow at ~2500)  
 - MCS\_lacZα (+1) (green arrow at ~3500)

**Sequence Map:** Shows DNA sequence fragments for regions 320-390, 400-460, 470-540, and 500-590. Restriction sites AccI, SalI, SbfI, and MCS are indicated.

**Bottom Navigation:** BASES 5222, INSERT 710, ASSEMBLY ▾, SPLIT WORKSPACE

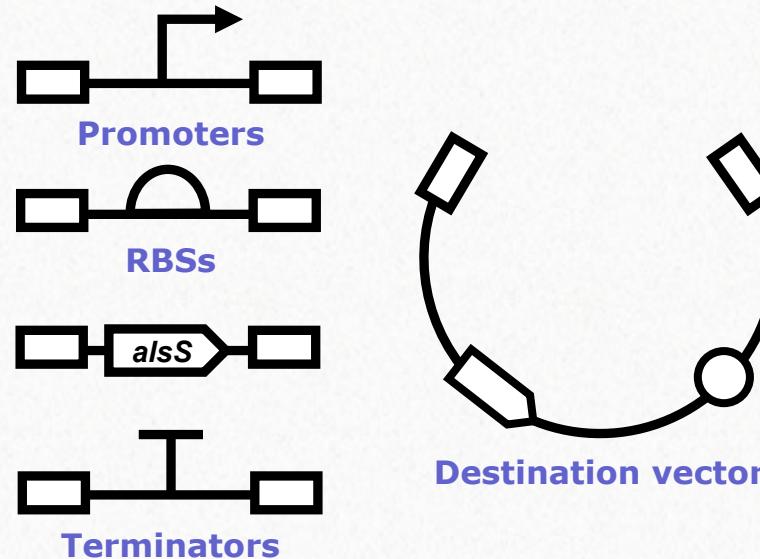
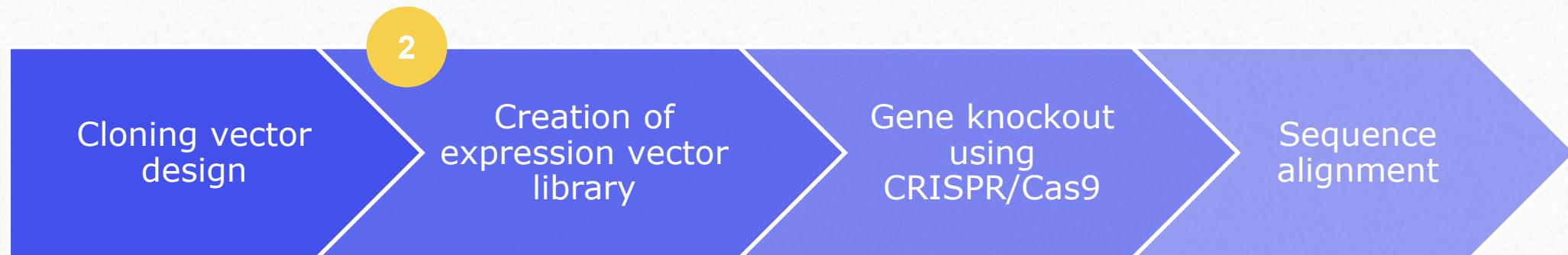
- ✓ You will be asked to choose a folder to save the construct in

- ✓ 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

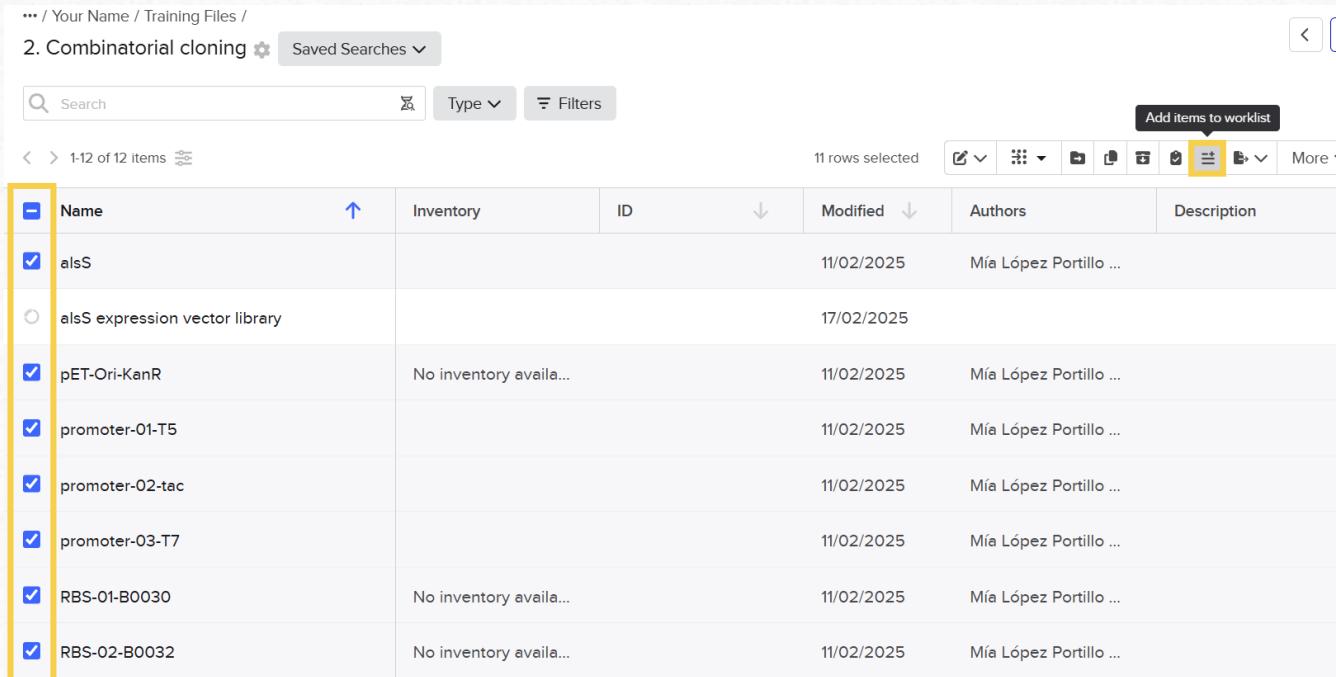
You will need the files in the ***Combinatorial cloning*** subfolder.

# Construct Assembly

## Worklist creation

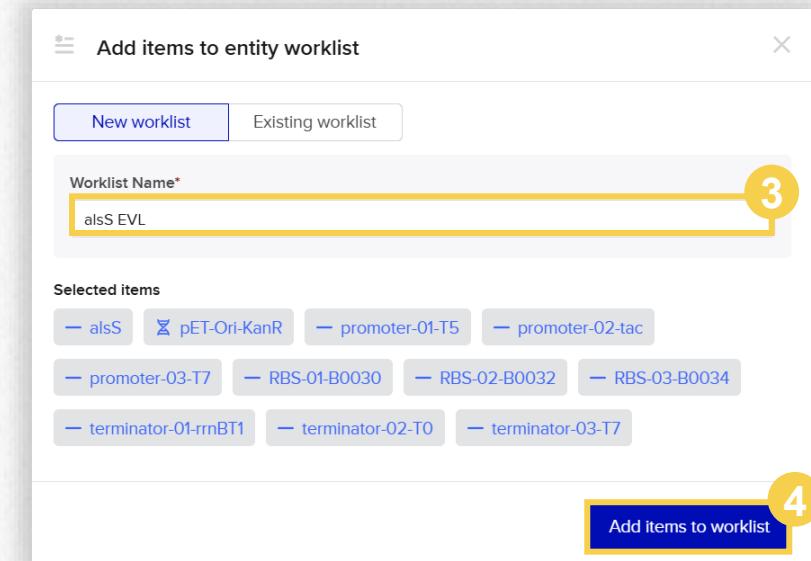
### PRO TIP:

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



The screenshot shows a table of 12 items from the 'Combinatorial cloning' folder. The first item, 'alsS', is selected. The 'Add items to worklist' button is highlighted.

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 ...	



The dialog box shows the 'New worklist' tab selected. A yellow box highlights the 'Worklist Name\*' input field containing 'alsS EVL'. Another yellow box highlights the 'Add items to worklist' button at the bottom right.

New worklist

Existing worklist

Worklist Name\*

alsS EVL

Selected items

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

Add items to worklist

Worklist 'alsS EVL' was created with 11 items

1 Select the sequence files in the **Combinatorial cloning** folder

2 Select "Add items to worklist"

# Construct Assembly

## Combinatorial Cloning Tool

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

It allows you to work with several cloning methods:

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

The screenshot shows the Combinatorial Cloning Tool interface. On the left, a sidebar has icons for search, add, edit, and delete. The main area displays a 'Golden Gate assembly' record under 'OVERVIEW'. It shows 'Bins & Spacers (3)': BIN 1 (Backbone, 1 fragment), BIN 2 (Promoter, 3 fragments), and BIN 3 (Gene, 8 fragments). An arrow points from these bins to a box labeled 'Constructs' containing '24 constructs'. Below this is a table of 'Fragments' with columns: Sequence, Bin, Start, End, Length, Orientation, Type IIS enzyme, and Frag. The table lists 12 fragments, each associated with a backbone, promoter, or gene. At the bottom is a table of 'Constructs' with columns: Name, Backbone, Overhang, Promoter, Overhang, and Gene. The first four constructs are listed. To the right, a 'CONSTRUCTS' section shows a grid of 12 circular construct visualizations, each with a label like 'backbone-promoter001-gene001' and a note 'No associated primers'. A 'SPLIT WORKSPACE' button is at the bottom right.

# Construct Assembly

## Combinatorial Cloning Tool: How to access it

... / Your Name / Training Files /

2. Combinatorial cloning

**1** 

alsS

SEQUENCE MAP

Search Create Analyze Copy Create PDF Share

DESCRIPTION METADATA RESULTS LINEAR MAP RELEVANT ITEMS

alsS (1713 bp)

MmeI MscI SmaI TspMI ScaI KpnI Acc65I BsgI RsrII BsrGI StyI XmnI AgeI AfeI BsaHI PstI XbaI BtsI BtsNI BspMI FokI BspCNI BtgZI BspMI EcoRI BspPHI PyuII

BssS $\alpha$ I BssSI MboII SacII EciI

aaatccctcgtaagaaccggggggggagctgggttggatttttagttggag  
tttagggcacttcttggccccccctcgaccaacacctaacaatcacctc

10 12 14 16 18 20 22 24 26  
K S L V K N R G A E L V V D C L V E

30 40 50 60 70

alsS gene

PstI

gctgcaggataaaggccct  
cgcgacgtcttattccggga

46 48 50 52  
A L Q D K G P

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

BssS $\alpha$ I BssSI PstI BtsI BtsNI BspGI AlwNI BsmFI Bari BfaI AhDI BstXI DdeI

FseI NaeI SfiI NgOMIV PaqCI BspMI BspCNI BfuaI BtsCI BtgZI BspMI BspCNI BspPHI PyuII

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

alsS CDS

alsS gene

Assemble DNA sequences by cloning

Assemble sequences and oligos by concatenation

Assembly CRISPR Entity from schema Mixture More

BASES 1713

ASSEMBLY ▾ SPLIT WORKSPACE

**2**

**3**

# Construct Assembly

## Combinatorial Cloning Tool: Configuration

The screenshot shows the Combinatorial Cloning Tool interface. On the left, there's a sequence map for the *alsS* gene, which is 1713 bp long. The gene has a CDS from position 24 to 28. On the right, a detailed view of the *alsS* CDS is shown, listing restriction enzymes like ScaI, KpnI, Acc65I, BsgI, FseI, NaeI, SfiI, BsrDI, AlwNI, BsmFI, BsrDI, BsmFI, BarI, BfaI, NgOMIV, and BfaI. A blue box highlights the *alsS* CDS and gene regions.

**Assemble DNA Dialog:**

- Name\***: alsS expression vector library (highlighted by a yellow circle)
- Number of fragment bins\***: 5 (highlighted by a yellow circle)
- Cloning method**: Golden Gate (highlighted by a yellow circle)
- Type IIS Restriction Enzyme**: BsAI (highlighted by a yellow circle)
- Save** button (highlighted by a yellow circle)

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

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

# Construct Assembly

## Combinatorial Cloning Tool: Full view

The screenshot shows the Combinatorial Cloning Tool interface for the 'alsS' library. The top navigation bar includes tabs for METADATA, OVERVIEW (which is selected), and CONSTRUCTS. A search bar at the top left contains the text 'alsS'. A dropdown menu next to it is set to 'alsS expression vector library'. On the far left, there is a vertical sidebar with various icons.

The main workspace is titled 'alsS expression vector library' and 'GOLDEN GATE'. It features a 'Bins & Spacers (5)' section with five bins labeled BIN 1 through BIN 5. Each bin has a delete icon and a more options icon. Below each bin is a dropdown menu set to 'Use existing cut sites'. Each bin also has a '0 fragments' button with a plus sign. An arrow points from the bins to a 'Constructs' section which currently displays '0 constructs'.

Below the bins is a 'Fragments' section with a table:

Sequence	Bin	Start	End	Length	Orientation	Type IIS enzyme	Fragment production method	Status
1					Forward	Bsal	Use existing cut sites	

At the bottom is a 'Constructs' section with a table:

Name	Backbone	Overhang	Insert 1	Overhang	Insert 2	Overhang	Insert 3	Overhang	Insert 4
1									

Buttons for 'Provide feedback' and 'Assemble' are located in the top right of the main workspace. A 'Status' dropdown, 'Edit fragments' button, and other controls are also present in the fragment table header.

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

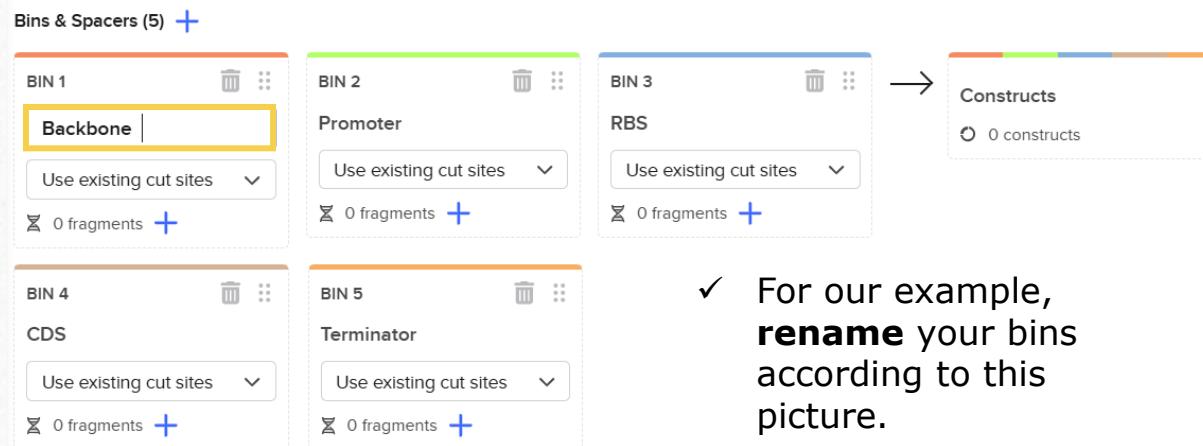
**i** All added fragments will show up here  
(You can change some configurations)

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

# Construct Assembly

## Combinatorial Cloning Tool: Bins and spacers

**i** You can rename the bins for better organization.



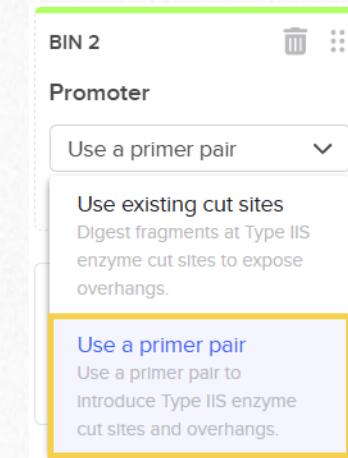
Bins & Spacers (5) +

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

Constructs  
0 constructs

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

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



**BIN 2**

Promoter

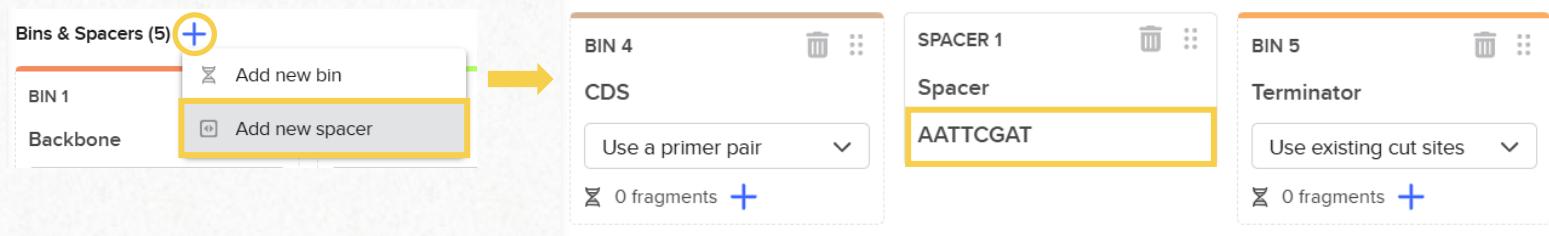
Use a primer pair

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

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

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

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



Bins & Spacers (5) +

Add new bin

**BIN 1** Backbone | Add new spacer

**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' section with 6 items. A yellow circle highlights the '+' button at the top right. Below it, 'BIN 1' is selected, and 'Backbone' is highlighted with a yellow box. A dropdown menu is open, showing 'Open sequences' and 'Search for sequences' options. At the bottom, there are buttons for 'Use existing cut sites' and 'Add from worklist'. The 'Add from worklist' button is also highlighted with a yellow box.

This is a modal dialog titled 'Add from worklist'. It contains a search bar with 'alsS EVL' and a list of items. 'alsS' is collapsed, and 'pET-Ori-KanR' is expanded, showing its sequence details. A yellow box highlights the 'pET-Ori-KanR' item. Other items like 'promoter-01-T5' are also listed. At the bottom are 'Cancel' and 'Next' buttons.

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

This screenshot shows the 'Add fragment(s)' dialog. It displays a circular map of the pET-Ori-KanR backbone with two BsaI restriction sites. The region between them is highlighted with a blue bar labeled 'medium copy (pET) ori/KanR'. Below the map, the sequence is shown with markers for 'bla' (beta-lactamase gene), 'ori' (origin of replication), and 'BsaI' sites. The 'Add' button at the bottom right is highlighted with a yellow box.

- ✓ 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.

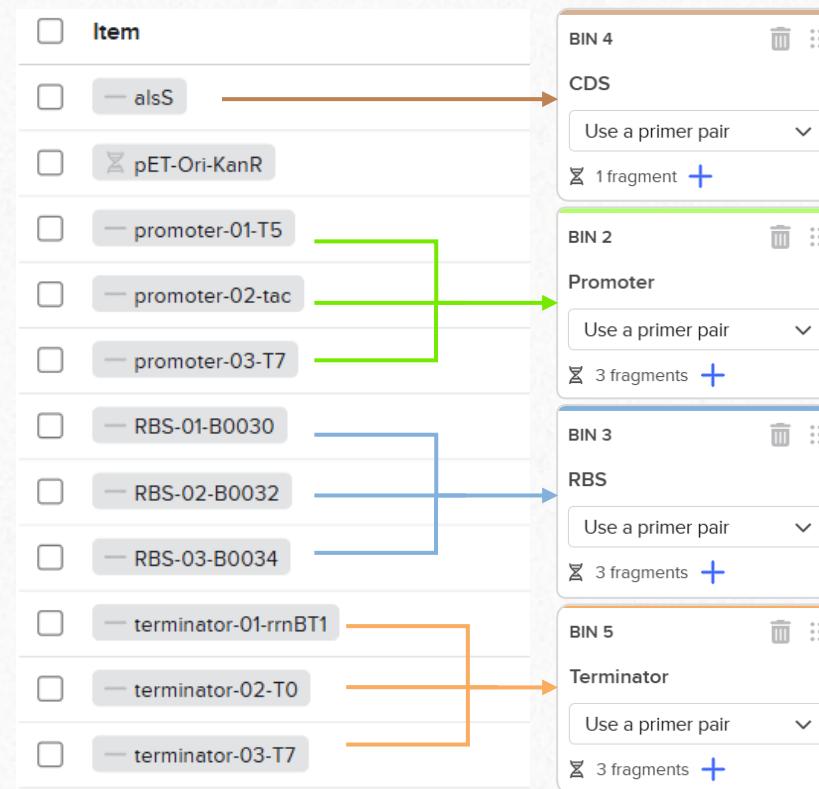
# Construct Assembly

**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.

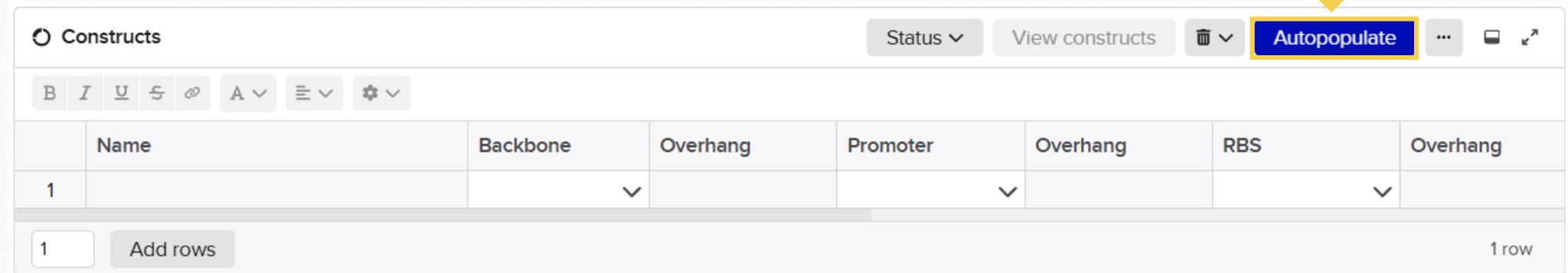
Fragments												Status	Edit fragments	Delete	...	Close
	Sequence	Bin	Start	End	Length	Orientation	Type IIS enzyme	Fragment production method	Preferred 5' primer	Preferred 3' primer	Status					
1	pET-Ori-KanR	Backbone	28	3327	3300	Forward	Bsal	Use existing cut sites			Looks good					
2	promoter-01-T5	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.



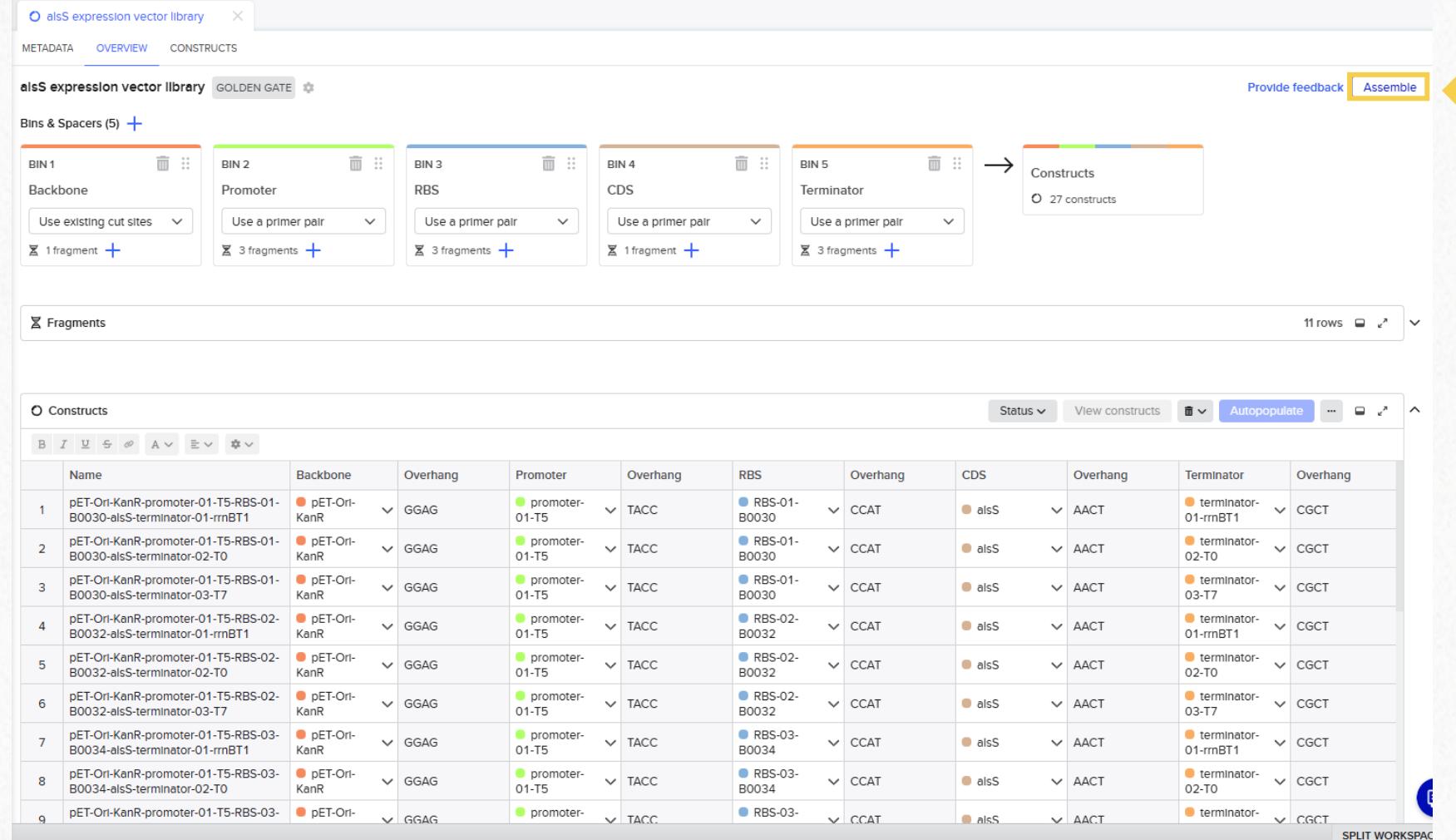
Constructs							
	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang
1							
1	Add rows						1 row

- ✓ 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.



	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 with a green checkmark).
  - Sub-options: 'Save fragments' and 'Save primers'.
  - Instructions: 'Add constructs to a folder and optionally set a schema'.
    - 'Set location\*' dropdown: 'Mia'.
    - 'Set schema' dropdown: 'Plasmid'.
  - Checkboxes:
    - 'Add constructs to a worklist'
  - Buttons: 'Cancel' and 'Next' (highlighted with a yellow arrow).

**Step 2:** 'Save constructs' (highlighted with a green checkmark).
  - Instructions: 'Saving fragments is optional.' and 'Create DNA Sequences to represent amplified fragments' (with an info icon).
  - Buttons: 'Back' and 'Next' (highlighted with a yellow arrow).

**Step 3:** 'Save constructs' (highlighted with a green checkmark).
  - Instructions: 'Saving primers is optional.' and 'Create DNA Oligos to represent newly designed primers' (with an info icon).
  - Buttons: 'Back' and 'Assemble' (highlighted with a yellow arrow).

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

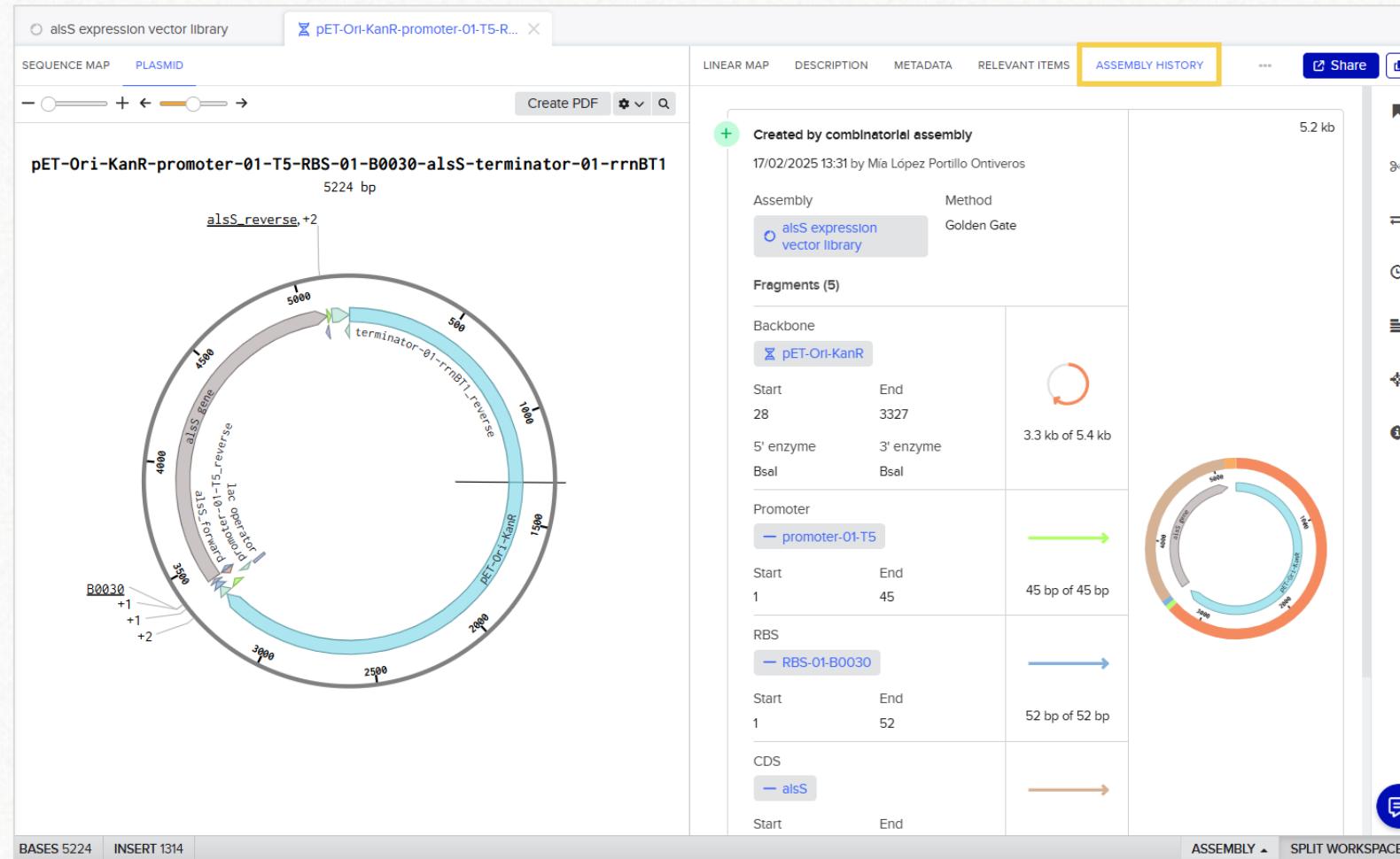
**i** If you choose not to create the primer files, you will still be able to find them later.



# Construct Assembly

## Golden Gate: Results

- ✓ 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.



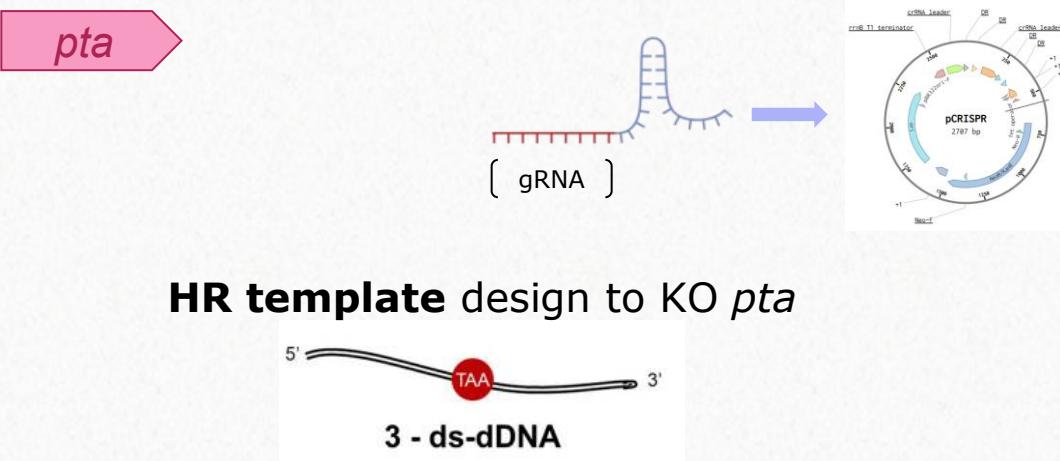
# 7. CRISPR tools



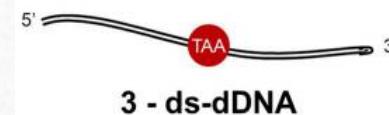
This is the third part of the *hands-on* example.



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



**HR template** design to KO *pta*



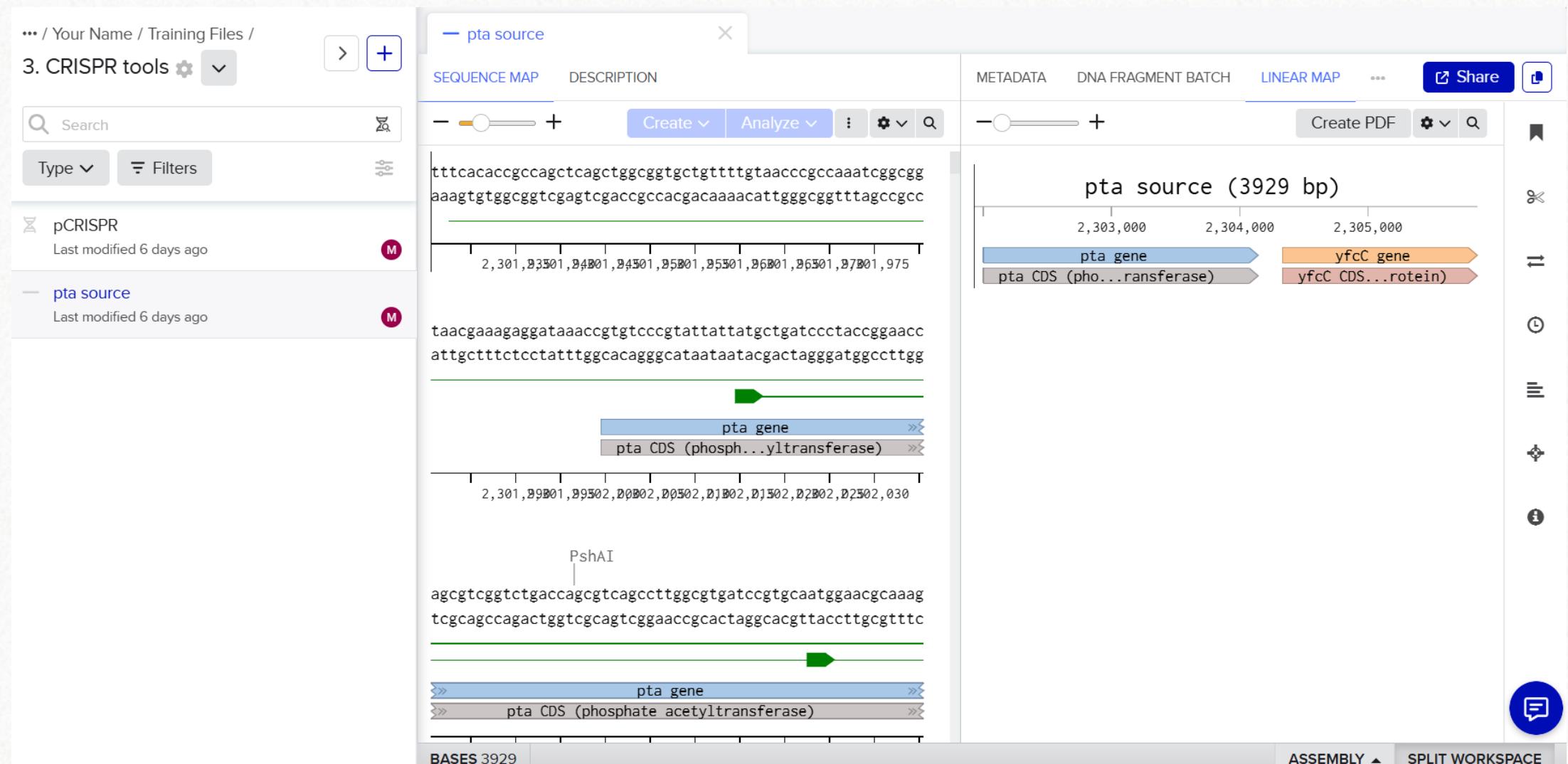
- ✓ gRNA design
- ✓ HR template design

#### 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>

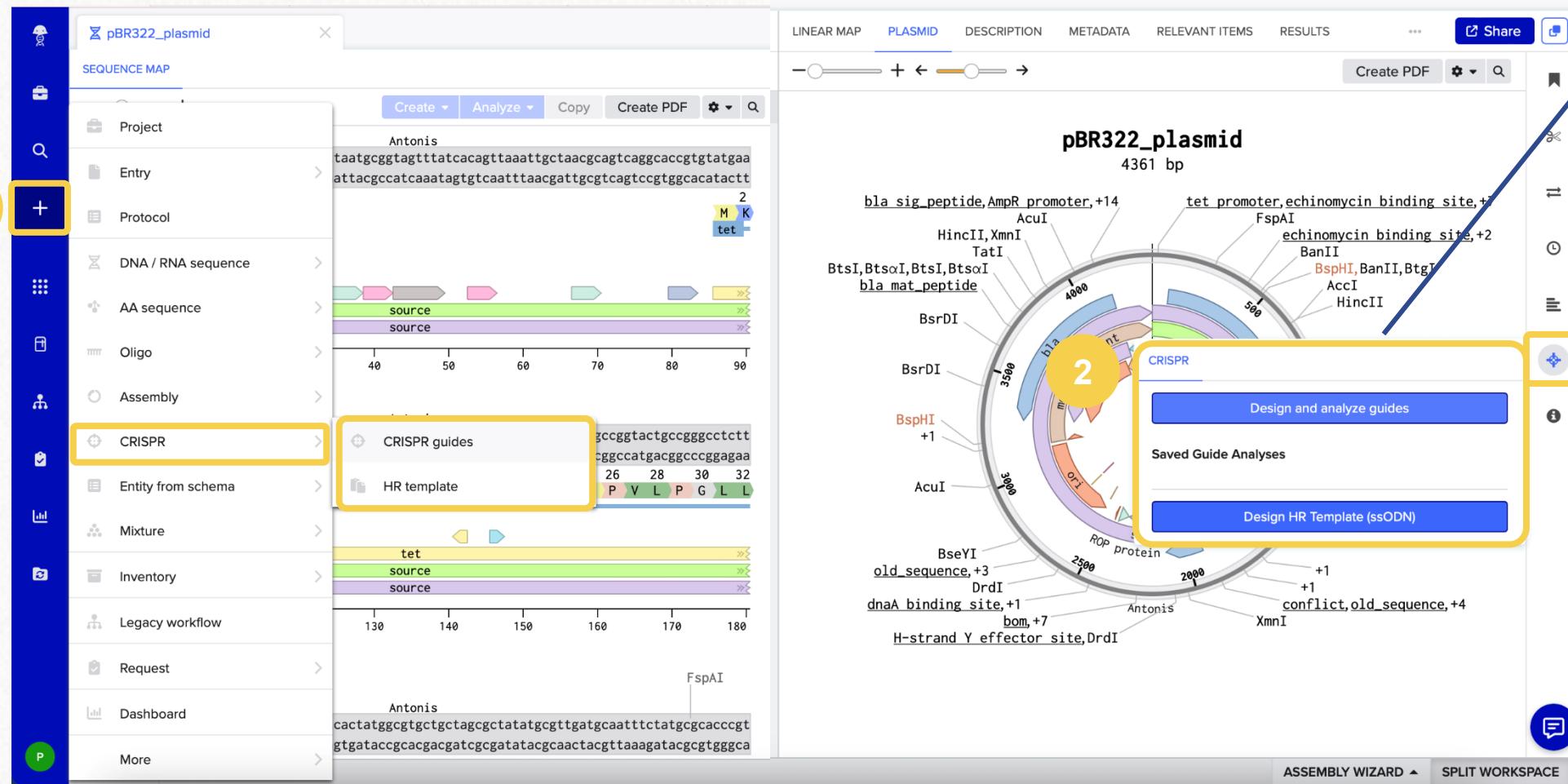
You will need the files in the **CRISPR tools** subfolder.



# 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 project management and sequence analysis options. A yellow circle labeled '1' highlights the '+ New Project' button at the top of the sidebar. Below it, the 'CRISPR' option under 'DNA / RNA sequence' is highlighted with a yellow box, and the 'CRISPR guides' and 'HR template' sub-options under it are also highlighted with a yellow box.

The main workspace displays a circular 'LINEAR MAP' of a 'pBR322\_plasmid' with a size of '4361 bp'. The map shows various restriction enzyme sites (e.g., HincII, XbaI, KpnI) and genetic features like the 'bla' gene, 'tet' gene, and 'ori' (origin of replication). A yellow circle labeled '2' highlights the 'CRISPR' section of the workspace, which contains buttons for 'Design and analyze guides' and 'Design HR Template (ssODN)'. An arrow points from this section to a callout box containing the text: 'By default, Benchling will use the open sequence as to design the gRNA on'.

# CRISPR tools

## Tool overview

**Design CRISPR Guides: Guide parameters**

**Design Type**

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

**Guide Length**: 20

**Genome**: GRCm38 (mm10, Mus musculus)

**PAM**: NGG (SpCas9, 3' side)

Save these as my default

**Custom PAM** dropdown menu:

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

**SEQUENCE MAP** showing genomic regions for Brca2 and Brca2-201 Exon 1 across three genomic tracks.

**RESULTS** panel:

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

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

# 7. CRISPR tools

## 7.1 gRNA design



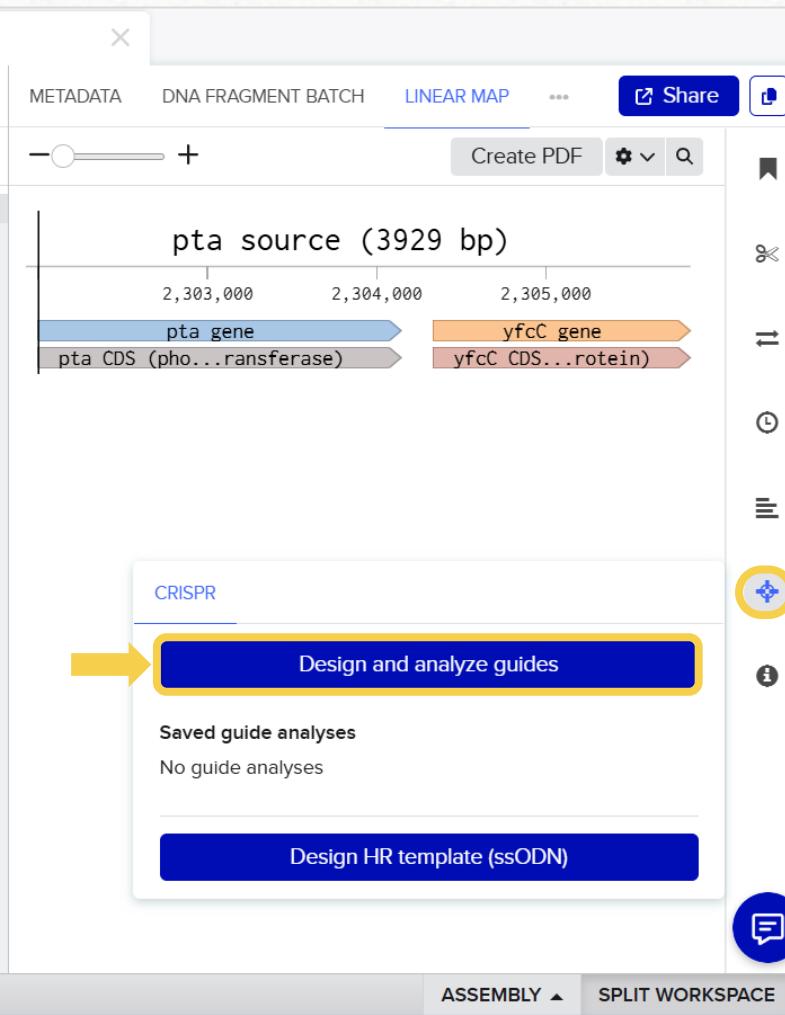
# CRISPR tools

## gRNA design

1 Open the **pta source** file.



2 Access the **gRNA design** menu.



## gRNA design

3

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

4

Click **Finish** and continue.

Design CRISPR guides: Guide parameters

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

Guide length

Genome

PAM

Show advanced settings

Save these as my default CRISPR settings

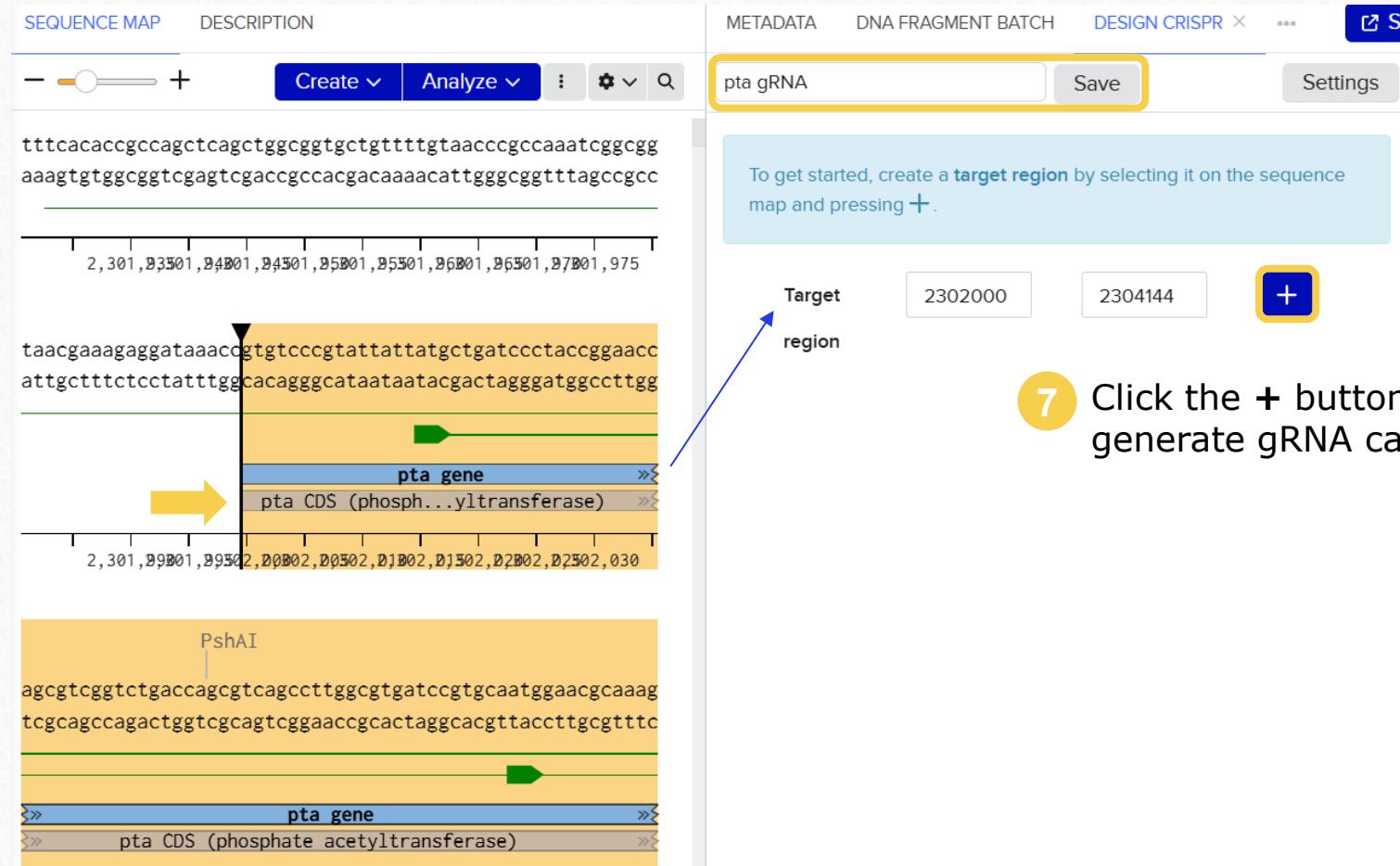
Finish

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

# CRISPR tools

## gRNA design

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



The screenshot shows the CRISPR tools interface with two main panels: 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 vertical bar and a yellow arrow pointing to the "pta gene" annotation.
- Annotations:** "pta gene" and "pta CDS (phosph...yltransferase)" are shown above the sequence, with the CDS being the target region.
- Bottom View:** Shows the same sequence with the "pta gene" and "pta CDS" annotations again, along with a "PshAI" restriction site and its corresponding sequence.

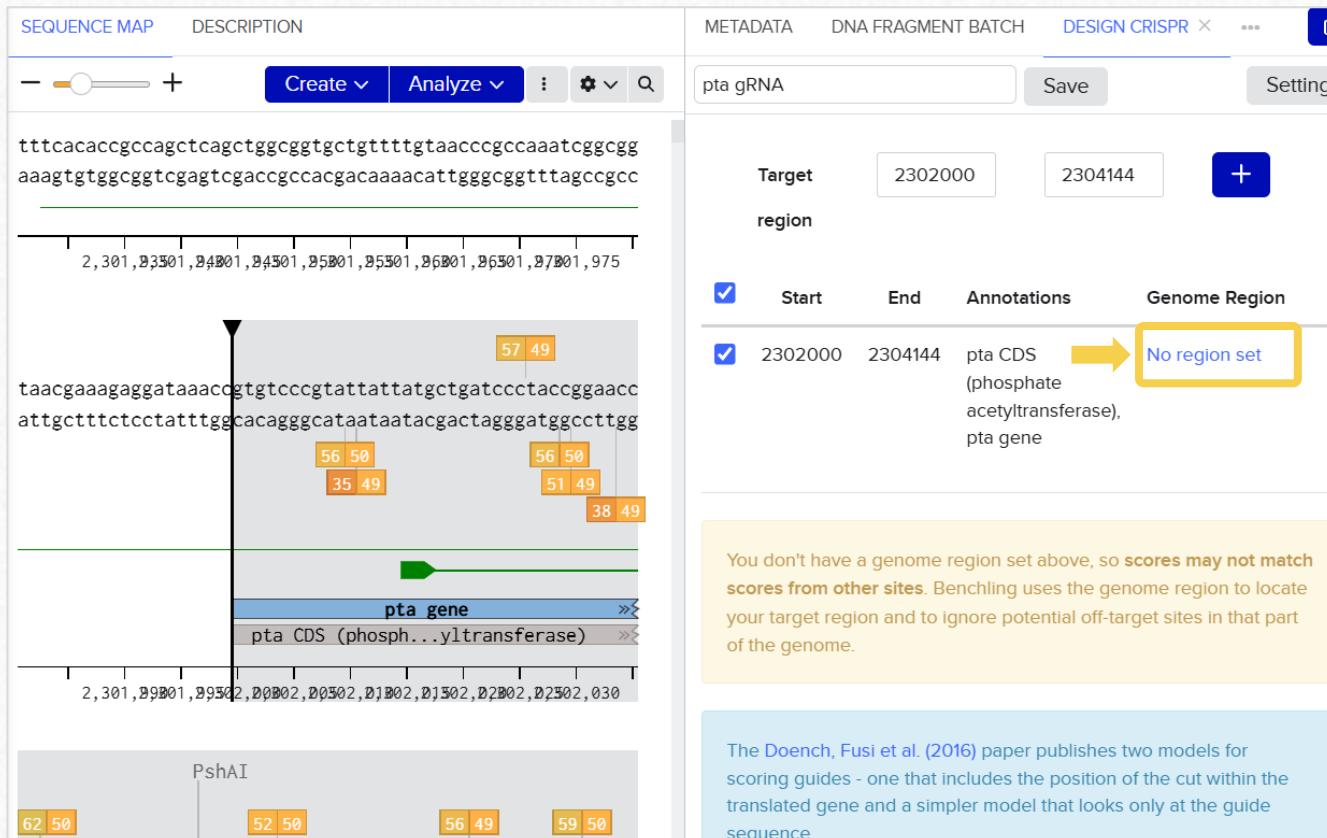
**Design CRISPR Tab:**

- Metadata:** pta gRNA, Save, Settings.
- Target Region:** A blue arrow points from the "Target region" label to the highlighted target region in the sequence map.
- Target Region Input:** 2302000, 2304144, + (the + button is highlighted with a yellow box).
- Instructions:** To get started, create a target region by selecting it on the sequence map and pressing +.
- Step 7:** Click the + button to generate gRNA candidates.

# CRISPR tools

## gRNA design

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



The screenshot shows the CRISPR tools interface. On the left, the "SEQUENCE MAP" tab displays a DNA sequence with several potential target sites highlighted by orange boxes with coordinates (e.g., 57 49, 56 50, 35 49, 56 50, 51 49, 38 49). A gene annotation for "pta gene" is shown with its corresponding CDS. On the right, the "DESIGN CRISPR" tab shows two targets selected: 2302000 and 2304144. In the "Genome Region" section, a message states "No region set". Below it, a note explains that without a genome region, scores may not match those from other sites. A callout box at the bottom provides information about scoring models from the Doench, Fusi et al. (2016) paper.

**Genome region**

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

None

Chromosome 2302000 | 2304144

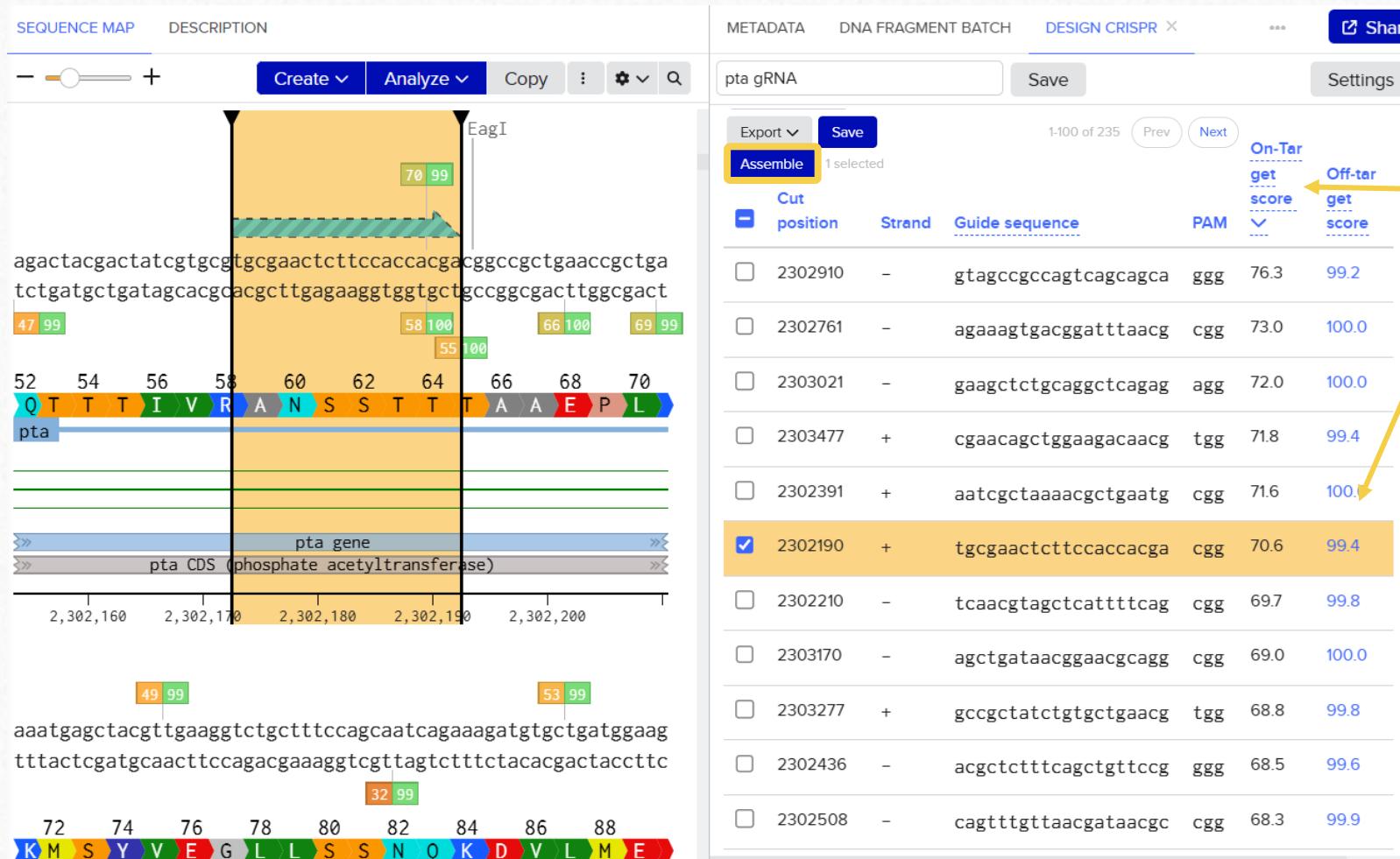
Find genome matches !

**Set genome region** Cancel

# CRISPR tools

## gRNA design

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



The screenshot shows the Benchling CRISPR tool interface. On the left, there is a "SEQUENCE MAP" tab displaying a DNA sequence with various restriction sites (EagI, KpnI, PstI, etc.) and a protein sequence below it. The protein sequence is labeled "pta" and shows the amino acid sequence: QTTTITIVRANSSTTAAEPL. A blue bar indicates the "pta gene" and a grey bar indicates the "pta CDS (phosphate acetyltransferase)". Below the sequence map, a portion of the DNA sequence is shown: aaat gagctacgttgaaggctgtcttccagcaatcagaagatgtgtatggaaat ttactcgatgcacttccagacaaaggctgttagtcttctacacgactacccttc. On the right, there is a "DESIGN CRISPR" tab with a table listing 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, with the highest value being 99.4. The 23rd row, which corresponds to the gRNA shown in the sequence map, has a checked checkbox and is highlighted in yellow. The "Save" button is located at the top right of the table.

Cut position	Strand	Guide sequence	PAM	On-Tar get score	Off-tar get score
2302910	-	gtagccgccagtca	ggc	76.3	99.2
2302761	-	agaaaagtgacggat	tac	73.0	100.0
2303021	-	gaagctctgcaggctc	agg	72.0	100.0
2303477	+	cgaacagctggaagaca	acg	71.8	99.4
2302391	+	aatcgctaaaacgctga	atg	71.6	100.0
2302190	+	tgcgaaactttccaccac	gta	70.6	99.4
2302210	-	tcaacgtagctcat	ttc	69.7	99.8
2303170	-	agctgataacggAACgc	agg	69.0	100.0
2303277	+	gccgctatctgtgtac	tg	68.8	99.8
2302436	-	acgcctttcagctgttcc	g	68.5	99.6
2302508	-	cagtttgttaacgataac	gc	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 on the right. The 'pta source' file is also highlighted with a yellow arrow. 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 has the following settings:

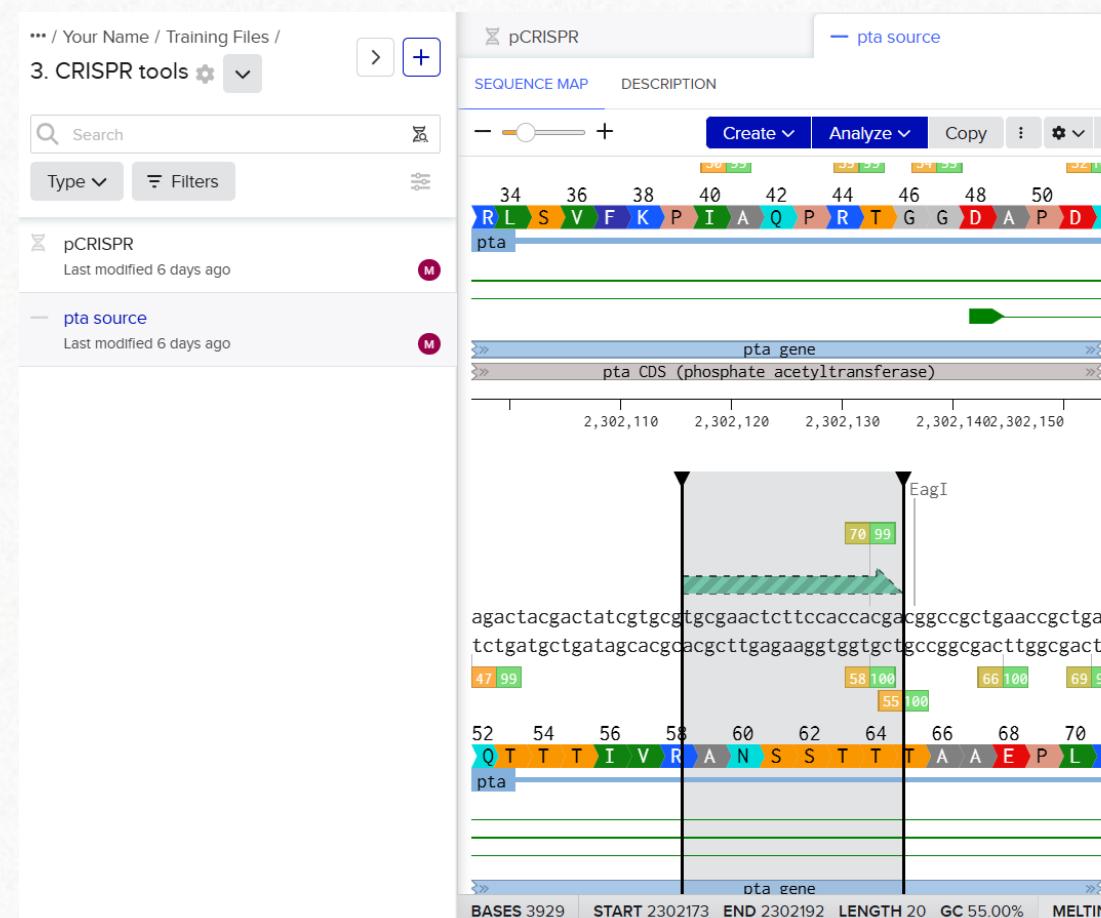
- Select Expression Vector:**
  - Vector Source:** The 'Choose a plasmid from your Benchling folders' option is selected (radio button highlighted).
  - Search field:** 'Search for a sequence in the file browser and drag it here'.
- Assembly Method:** Type IIS Cloning
- Insertion Region:** Start and End fields are empty.
- Buttons:** Next, a blue message icon, ASSEMBLY ▲, and SPLIT WORKSPACE.

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

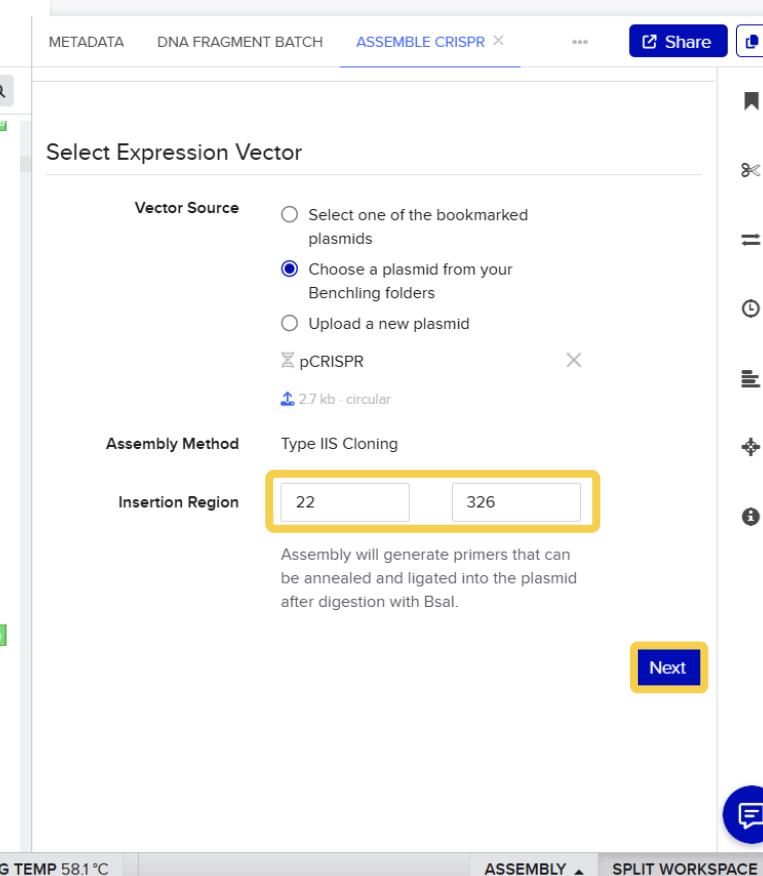
# CRISPR tools

## gRNA design

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



14 Click **Next**.



The figure shows the 'ASSEMBLE CRISPR' step in Benchling. The 'Insertion Region' field is highlighted with a yellow box, showing values 22 and 326. A note below states: "Assembly will generate primers that can be annealed and ligated into the plasmid after digestion with Bsal." A blue 'Next' button is visible at the bottom right.

 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 Assemble CRISPR.

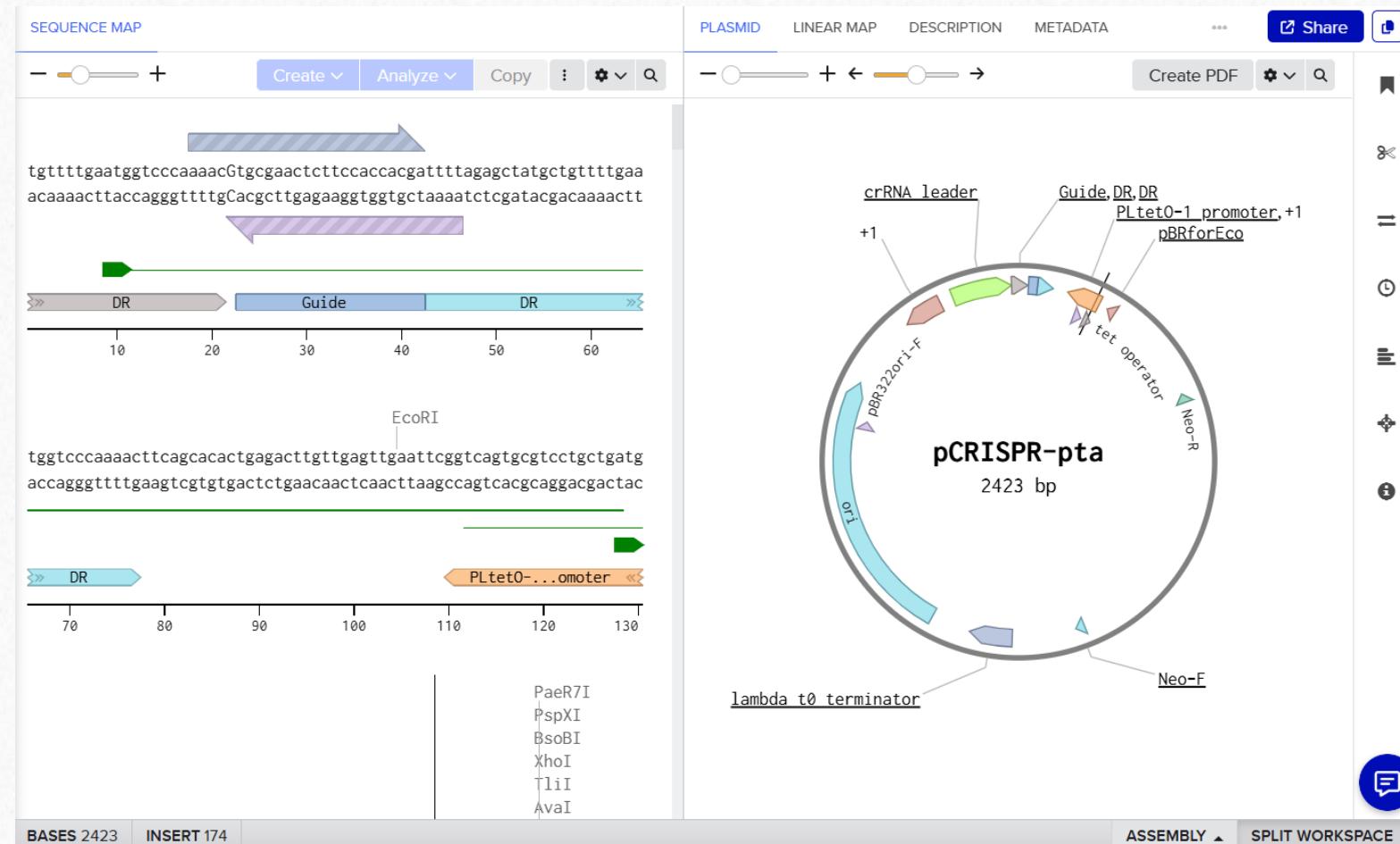
**Sequence Map:** This section displays two DNA sequence regions. The top region is labeled "pta" and shows a restriction site for "EagI". The bottom region is labeled "pta gene" and "pta CDS (phosphate acetyltransferase)". Both regions show nucleotide positions from 52 to 70 above the sequence and amino acid translations below. A blue bar at the bottom indicates the coding strand.

**Assemble CRISPR:** This section is titled "Finalize Assemblies". It includes fields for "Assembly Name" (pCRISPR-ptt) and "Guide Sequence" (tgcgaaactttccaccacga). A "Folder" dropdown is set to "3. CRISPR tools". At the bottom right 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

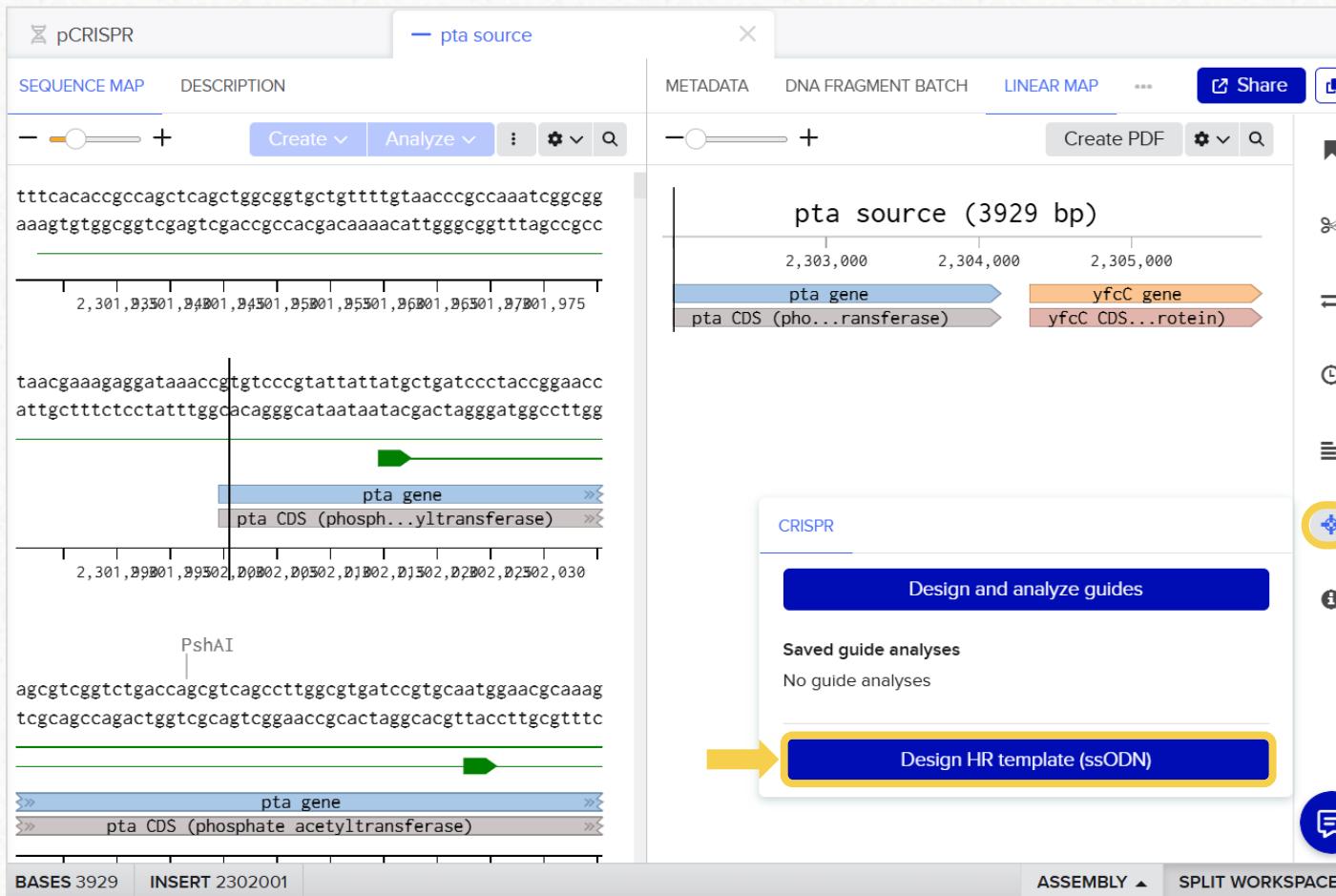
## 7.2 HR template design



# CRISPR tools

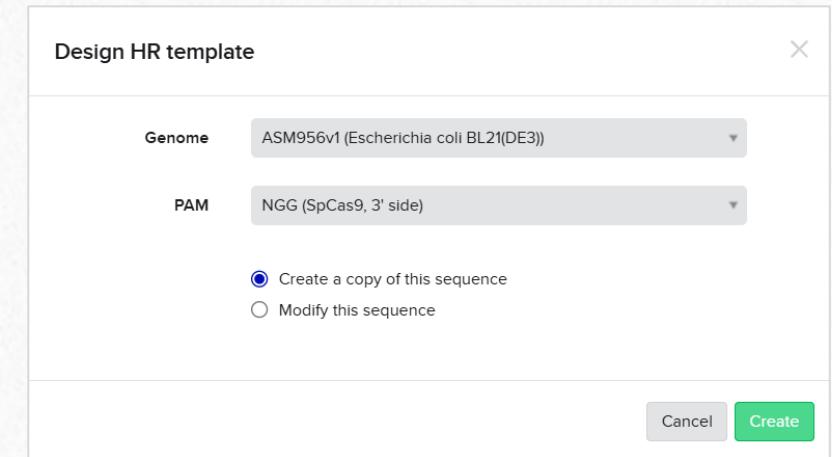
## HR template design

- 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, which is highlighted in blue.

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



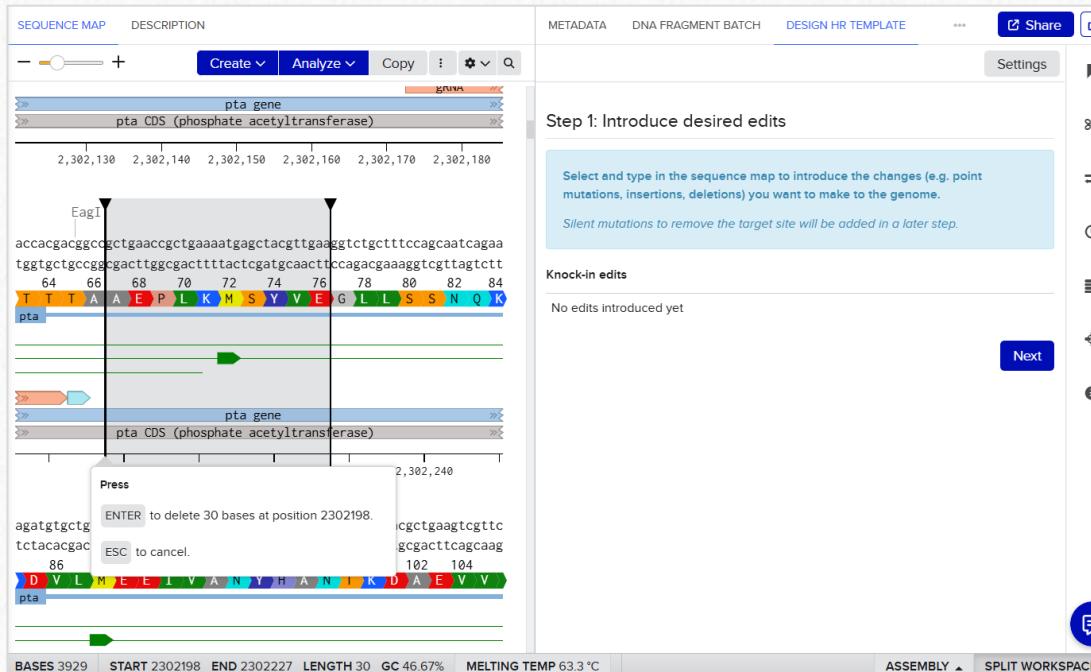
The dialog box for 'Design HR template' is shown. It has fields for 'Genome' (ASM956v1 (Escherichia coli BL21(DE3))), 'PAM' (NGG (SpCas9, 3' side)), and two radio button options: 'Create a copy of this sequence' (selected) and 'Modify this sequence'. At the bottom are 'Cancel' and 'Create' buttons.

# CRISPR tools

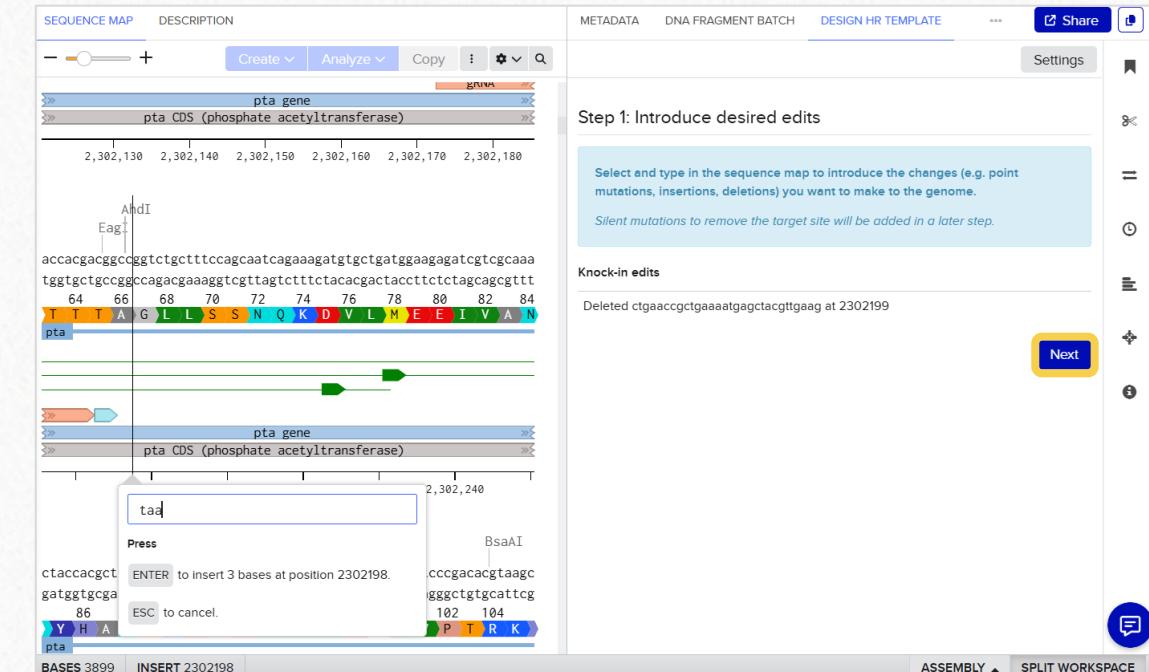
## HR template design

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

- 4 Delete 30 nt as shown.



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



- 6 Click **Next**.

## **HR template design**

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

SEQUENCE MAP DESCRIPTION METADATA DNA FRAGMENT BATCH DESIGN HR TEMPLATE ...  Share

Editing disabled because no mutations have been selected.

Copy Create PDF

cagcctggcgtatccgtcaatggaaacgcacaaggcggttcgtctgagcgtttcaaaccatacg  
gtcggAACCGactaggcacgttacccgtcggttccgcaagcagactcgcaaaagtggatagc  
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

ctcagccgcgtaccggggcatgcggccgatcagactacgactatcgctgcgtgcgaactttcc  
gagtcggccatggccaccgctacggggctagtcgtatgtatagcgcacgcttgagaagg  
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

accacgacggcctaaggctgtcttccagcaatcagaagatgtgctgtatggaaagatcgatcg  
Settings

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

2302098 - 2302297  Reset to default

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

Knock-in edits

Deleted gctgaaccgctgaaaatgagctacgt at 2302198

Deleted g at 2302199

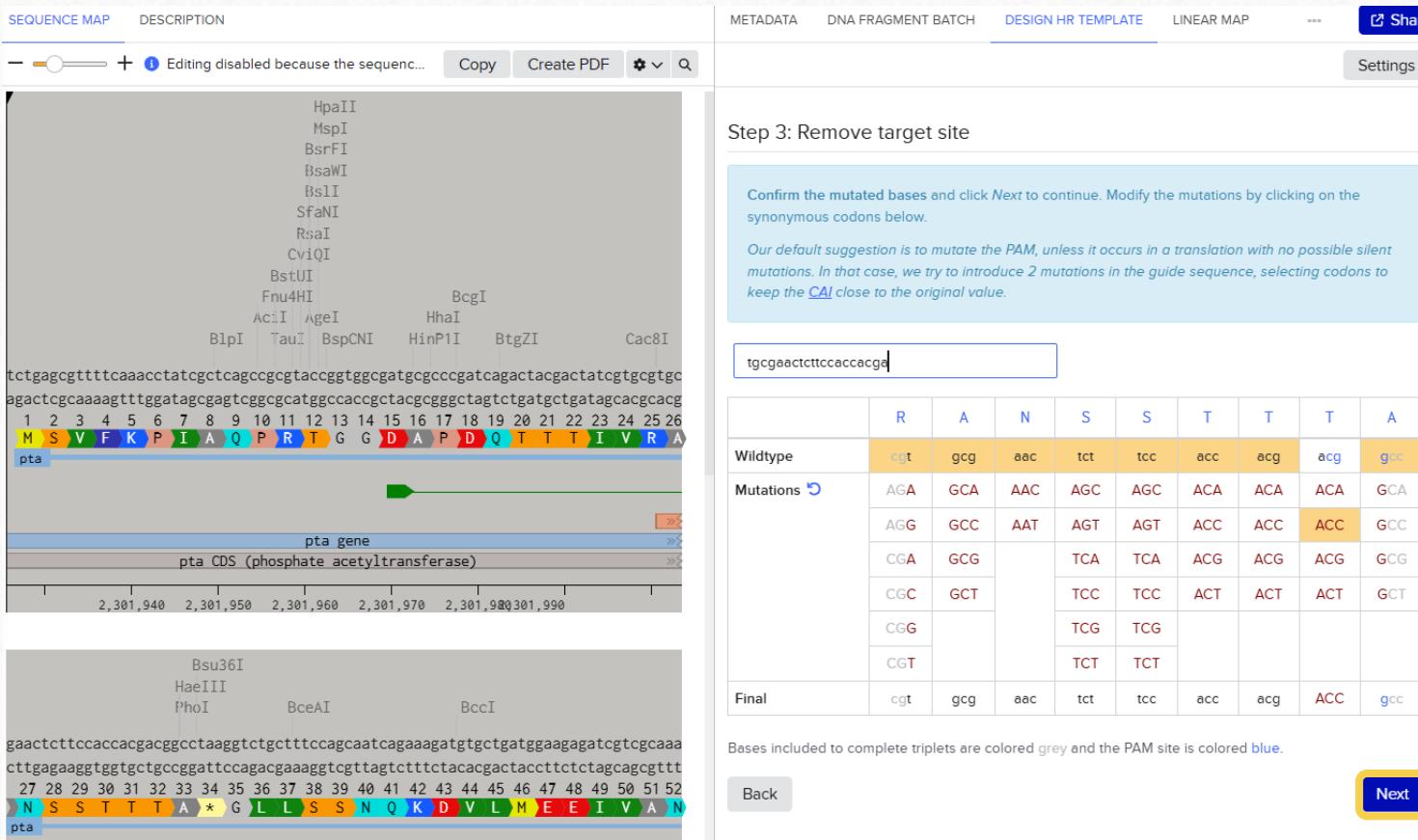
Back  Next

**7** Click **Next.**

# CRISPR tools

## HR template design

- Paste the gRNA sequence: tgcgaaactttccaccacga



**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.

	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.

- 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.

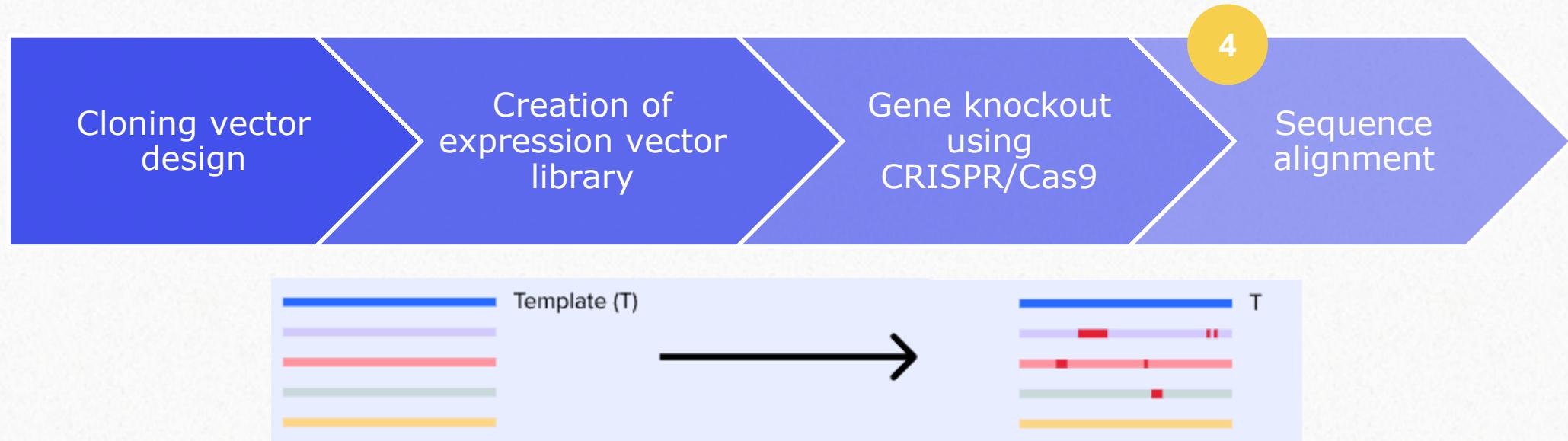
To design a template for the same knock-in edits but with a different guide, [click here](#).

- ✓ 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.

... / Your Name / Training Files /

### 4. Sequence alignments

- FW-seq-1 Last modified 6 days ago
- MID-seq-1 Last modified 6 days ago
- plasmid-seq Last modified 6 days ago
- pSEVA6311-phaC-pct540 Last modified 6 days ago

**pSEVA6311-phaC-pct540**

PLASMID DESCRIPTION METADATA PLASMID BATCH SEQUENCE MAP ... Share

Search

Search Type Filters

PLASMID MAP

**pSEVA6311-phaC-pct540** 7955 bp

Propionate-CoA transferase

phaC1 (MBEL6-19)

chnR-PchnB-GA-R

+2 +1 +1

T<sub>E</sub>

EcoRV EcoRV

oriT

rep gene

misMatches: 0

PmeI SphI NdeI +3 +8

BASES 7955 INSERT 1180

SEQUENCE MAP

tgtatctcagggtgcattgtgtcattgttccgtatatacgcttcataagcca  
acatagagtcccacgtAACACAGTAACAAAGGCACTATATCGAAGAGTATTCCGT  
106 108 110 112 114 116 118 120 122  
V S Q G A L C H L F R D I A S H K P  
7642-1261

Propionate-CoA transferase

5 10 15 20 25 30 35 40 45 50 55

ggcgtattttacaaaggtaggtatcggtactttcattgaccccagaaatggcgccg  
ccgcataaatgtttccatccatagccatgaaagttaactgggtctttaccgcgc  
124 126 128 130 132 134 136 138 140  
G V F T K V G I G T F I D P R N G G  
7642-1261

Propionate-CoA transferase

60 65 70 75 80 85 90 95 100 105 110

gtaaagtaaatgatattaccaaaaagaaatattgtgaattggtagagattaagg  
catttcatttactataatgtttcttataacaacttaaccatcttaattccc

ASSEMBLY ▲ SPLIT WORKSPACE

# 8. Sequence alignments

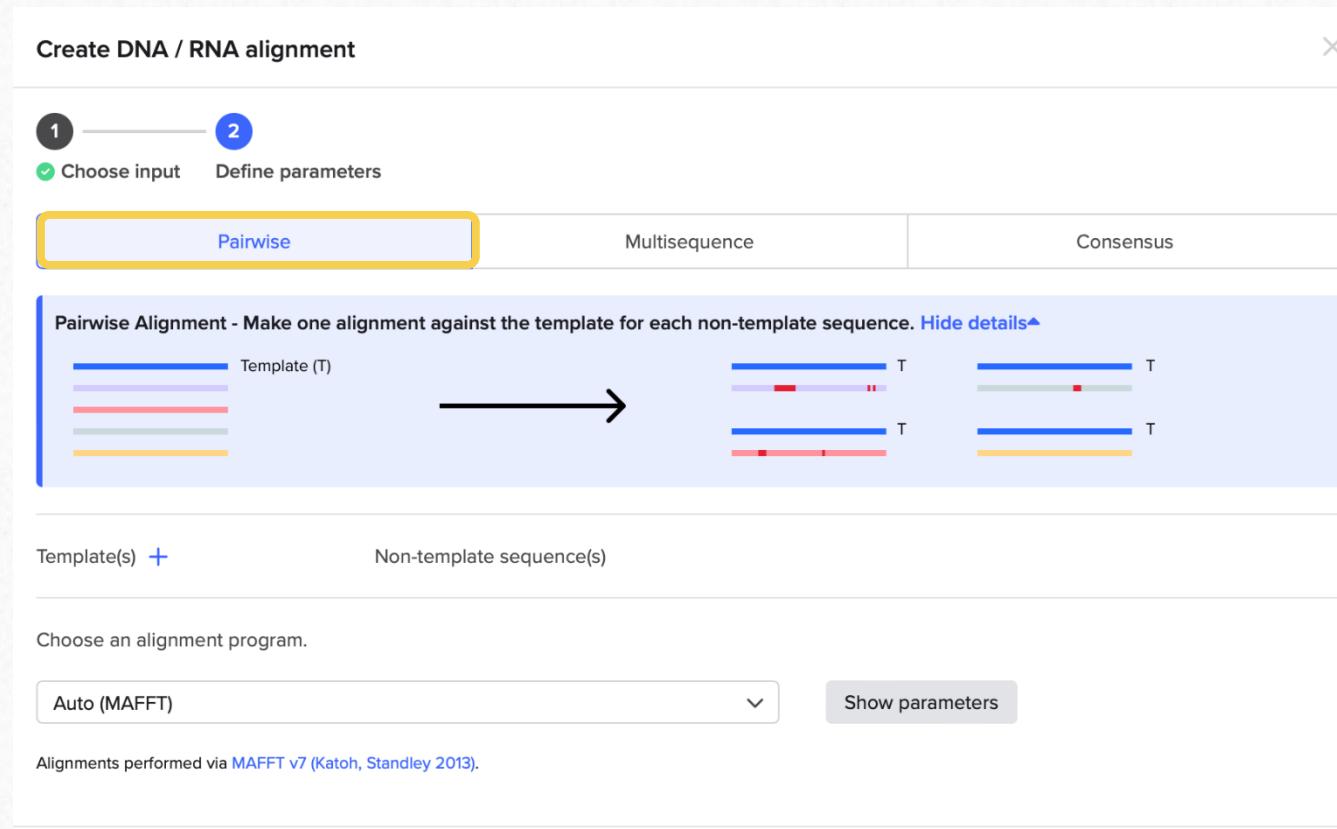
## 8.1 Alignment tool



# 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:



Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

Pairwise Alignment - Make one alignment against the template for each non-template sequence. [Hide details](#)

Template (T)

Non-template sequence(s)

Choose an alignment program.

Auto (MAFFT)

Show parameters

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

1

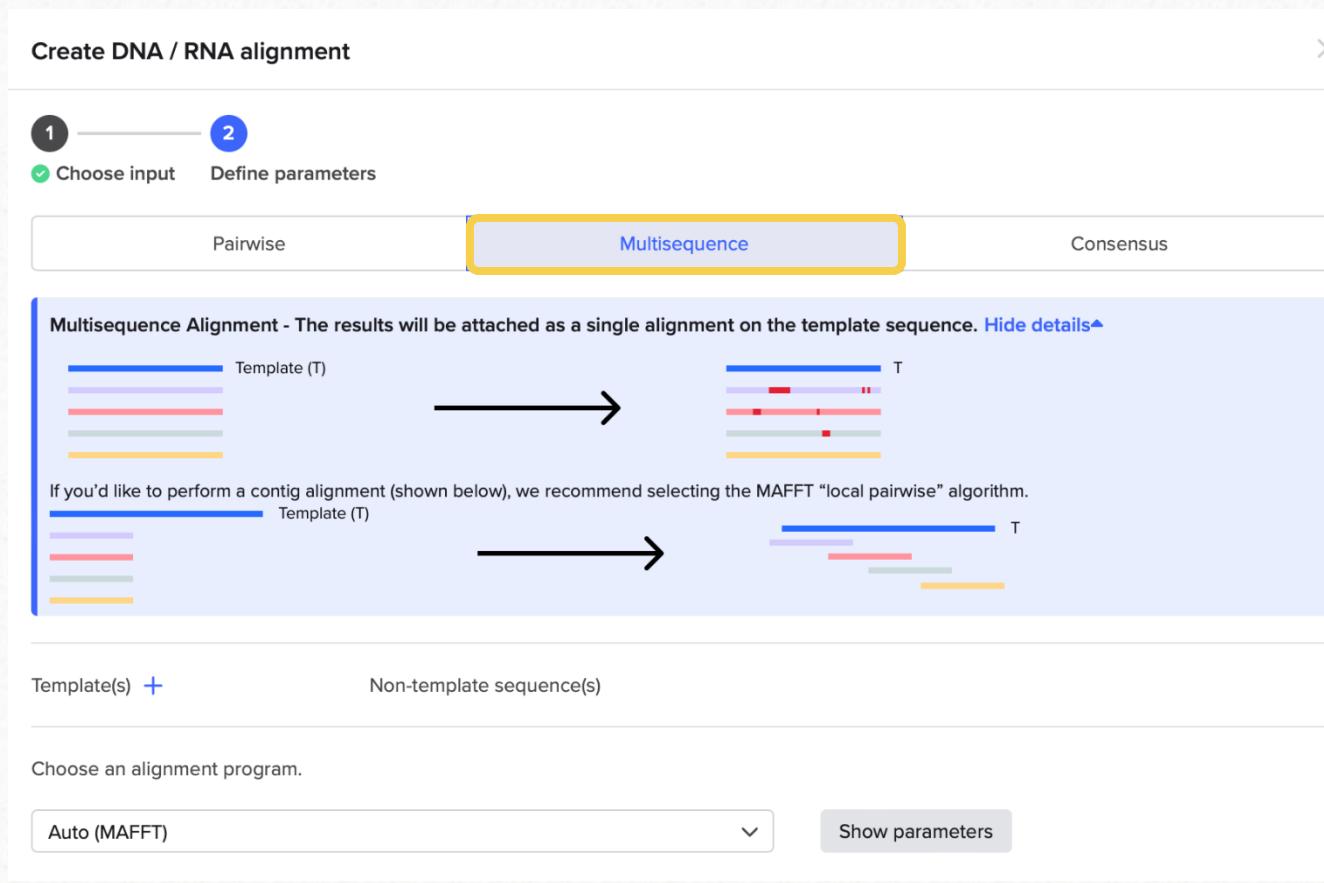
### Pairwise alignment:

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

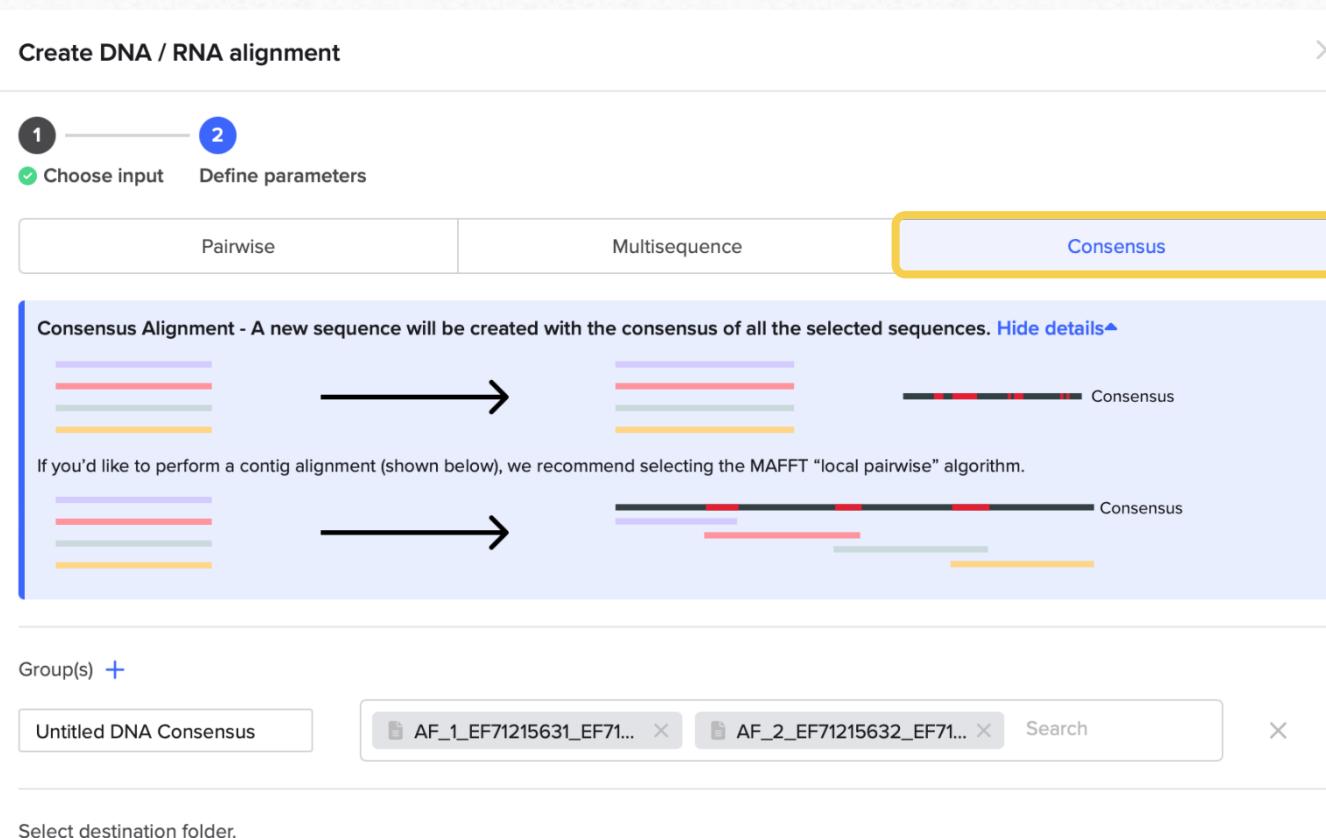
### Multisequence alignment:

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:



Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

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

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

Group(s) +

Untitled DNA Consensus AF\_1\_EF71215631\_EF71... AF\_2\_EF71215632\_EF71... Search

Select destination folder.

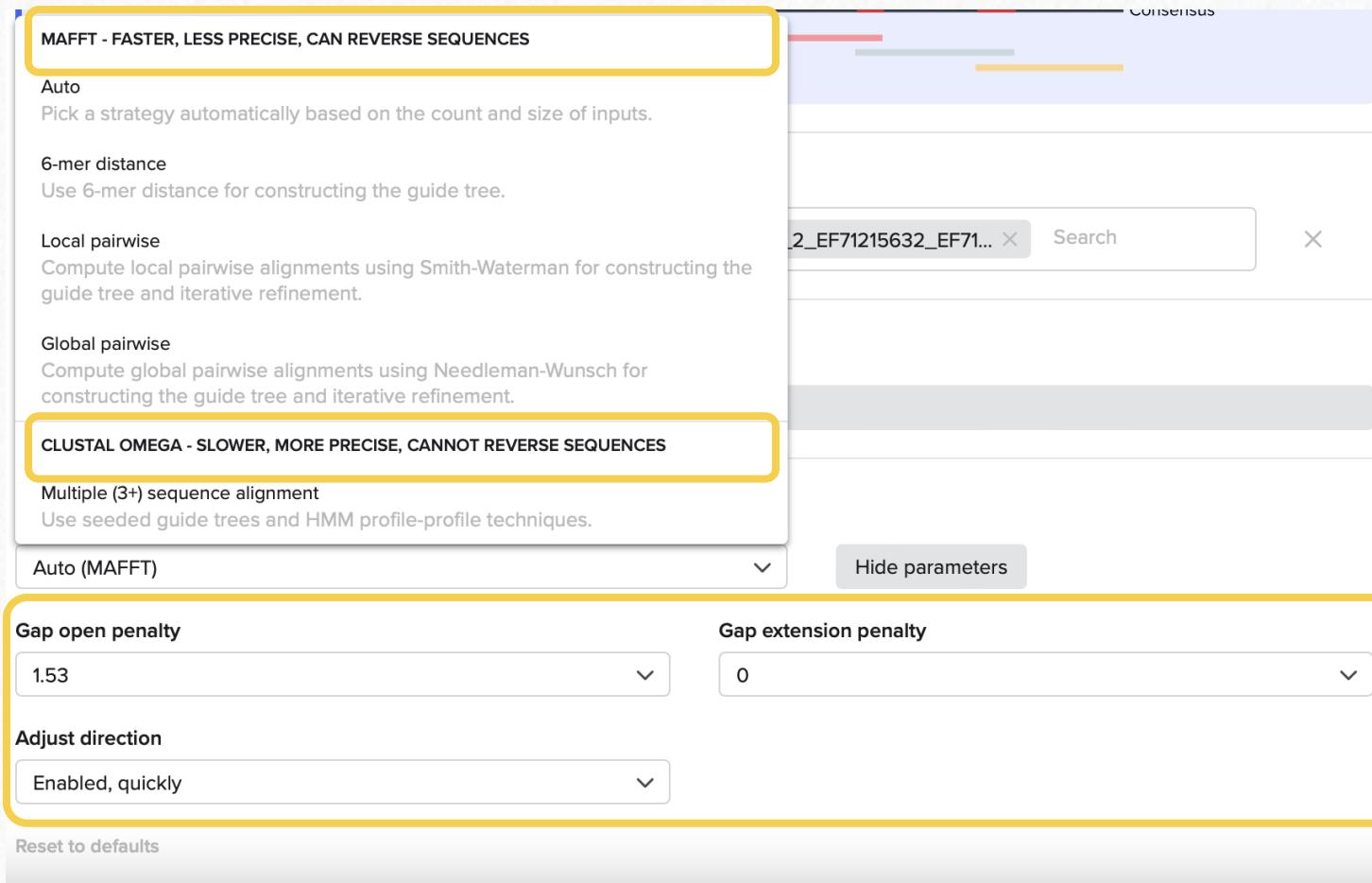
3

### Consensus alignment:

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

# Alignment creation

## Alignment tool overview



The screenshot shows the Alignment tool interface. On the left, there's a sidebar with various icons. The main area has two sections: 'MAFFT - FASTER, LESS PRECISE, CAN REVERSE SEQUENCES' and 'CLUSTAL OMEGA - SLOWER, MORE PRECISE, CANNOT REVERSE SEQUENCES'. The 'MAFFT' section contains options: 'Auto', '6-mer distance', 'Local pairwise', and 'Global pairwise'. The 'CLUSTAL OMEGA' section contains 'Multiple (3+) sequence alignment' and 'Auto (MAFFT)'. Below these are parameters: '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 at the bottom. To the right, a sequence alignment interface shows a consensus sequence with colored bars above it, and a search bar with the text '2\_EF71215632\_EF71...'. A 'Hide parameters' button is also present.

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

# 8. Sequence alignments

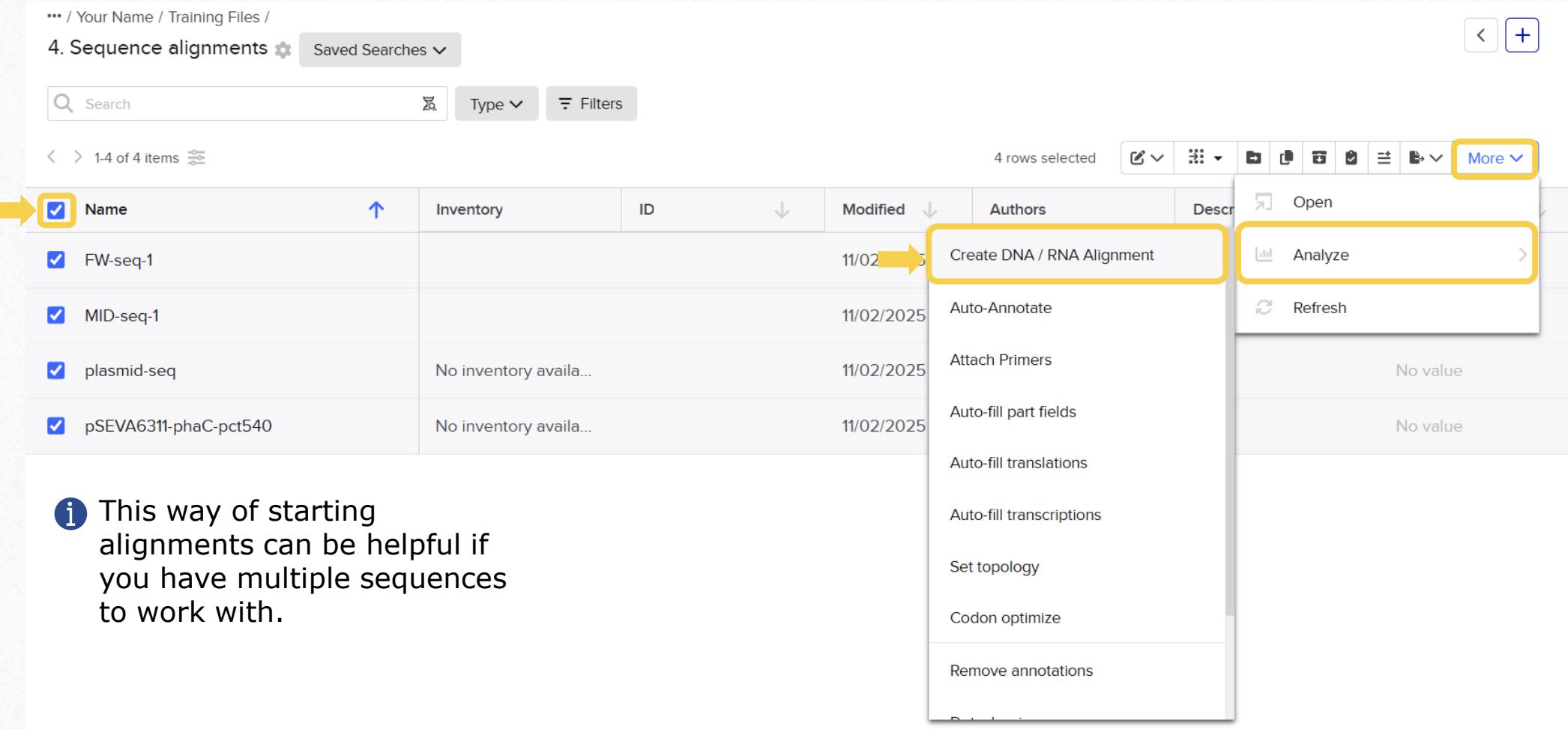
## 8.2 Multisequence alignment



# Alignment creation

## Multisequence alignment

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



The screenshot shows a software interface for managing sequence alignments. At the top, there's a navigation bar with '... / Your Name / Training Files /' and a dropdown for 'Saved Searches'. Below it is a search bar, a type filter, and a filters button. The main area displays a table with four rows, each representing a sequence alignment. The columns are 'Name', 'Inventory', 'ID', 'Modified', 'Authors', and 'Description'. The 'Name' column has a checked checkbox. The 'Modified' column shows dates like '11/02/2025'. The 'More' button in the toolbar is highlighted with a yellow arrow. A context menu is open over the last row, listing options: 'Create DNA / RNA Alignment' (highlighted with a yellow box), 'Analyze', 'Auto-annotate', 'Attach Primers', 'Auto-fill part fields', 'Auto-fill translations', 'Auto-fill transcriptions', 'Set topology', 'Codon optimize', and 'Remove annotations'. The 'Create DNA / RNA Alignment' option is the primary target of the yellow arrow.

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

 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 Choose input 2 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 Search

plasmid-seq Search

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) Show parameters

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

Back Create Alignment

- 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.

The screenshot shows a circular map of the pSEVA6311-phaC-pct540 plasmid. Key features labeled include:

- Propionate-CoA transferase gene
- T<sub>θ</sub> promoter
- EcoRV restriction site
- oriT origin of replication
- rep gene
- chnR gene
- P<sub>S1-1</sub> promoter
- P<sub>neo</sub> promoter
- NdeI restriction site
- TGCA motif present in xyL-regulated promoters
- chnR-PchmB-G4-R cassette
- phaC1(NBEL6-19) cassette

The map also indicates various start sites (+1, +2, +3, +4, +5, +6, +7, +8) and mismatch counts (e.g., 0, 1, 2, 4).

On the right, the "SEQUENCE ALIGNMENTS" panel displays a sequence alignment interface. It includes:

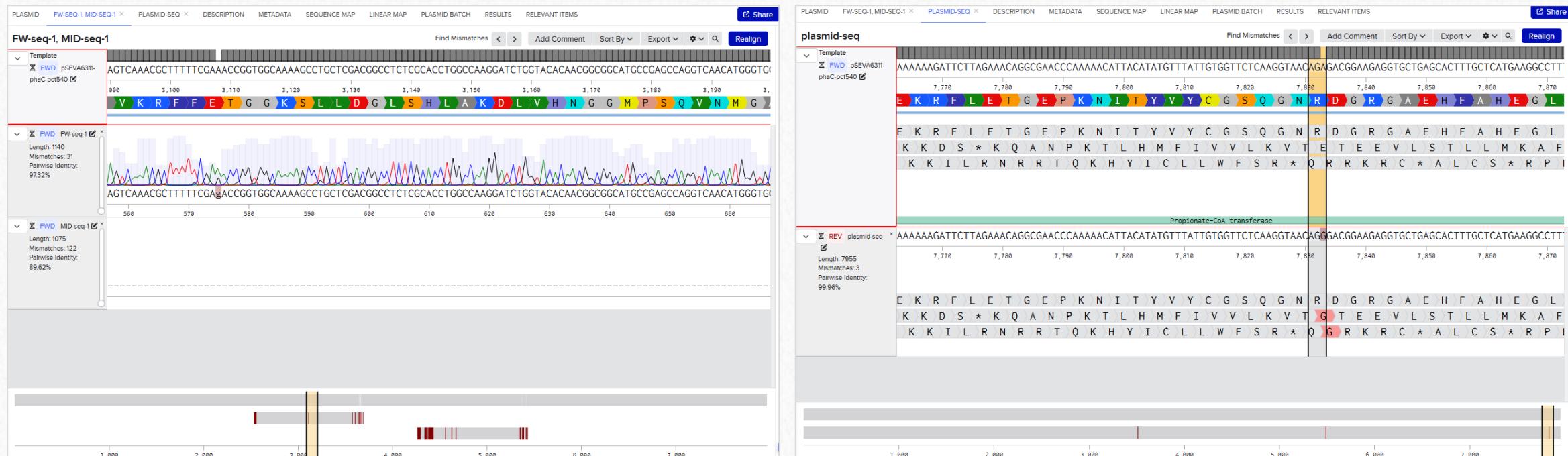
- A "Create New Alignment" button.
- A "Saved Alignments" section containing:
  - FW-seq-1, MID-seq-1 (date: 17/02/2025 18:55)
  - plasmid-seq (date: 17/02/2025 18:55)

- 7 Open the alignments.

# Alignment creation

## Multisequence alignment

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



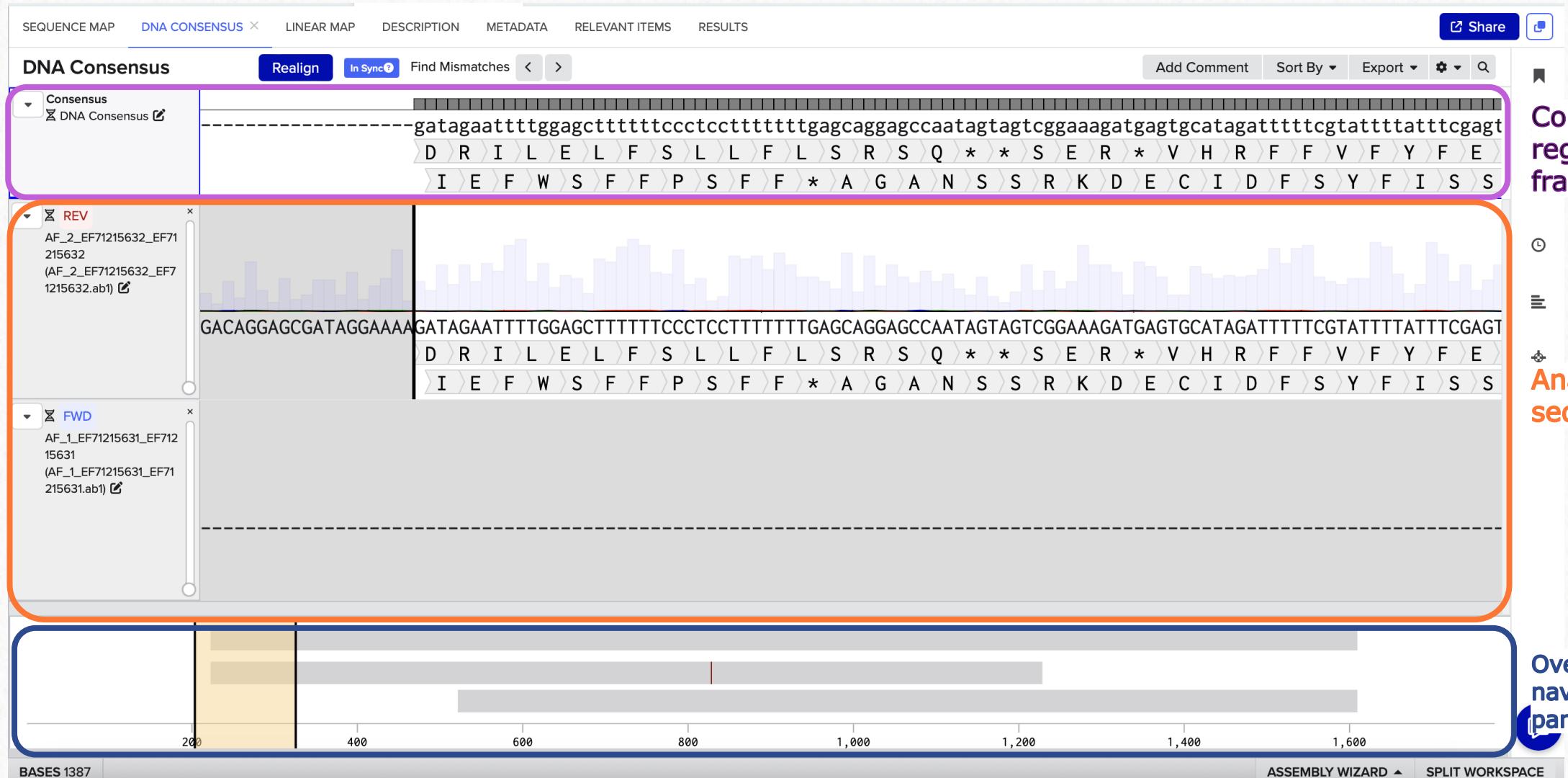
# 8. Sequence alignments

## 8.3 Consensus alignment



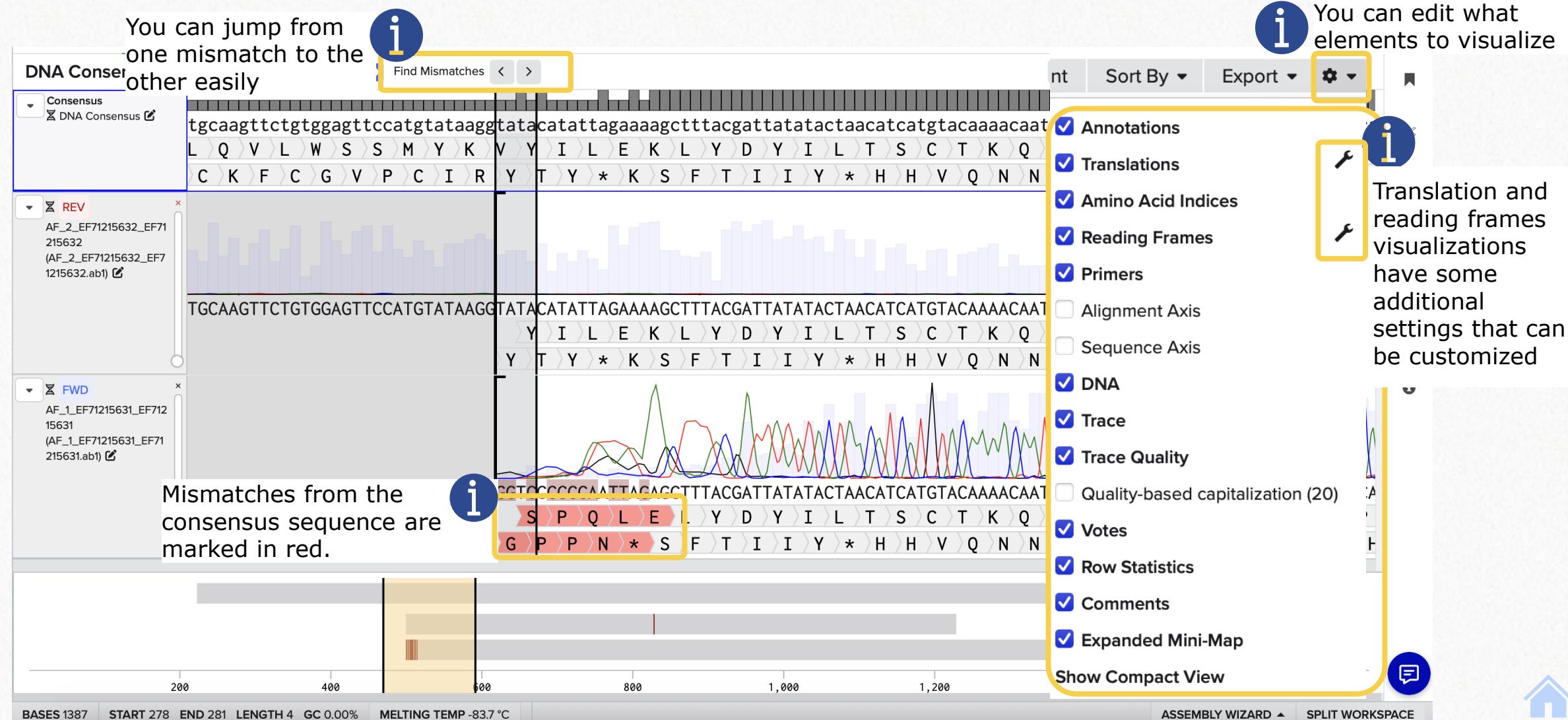
# Alignment creation

## Consensus alignment navigation



# Alignment creation

## Consensus alignment navigation



# 9. Tips and tricks



# Tips and tricks

## Overview:

- You can work in bulk using the expanded view of the workspace
- Re-indexing of sequences when creating alignments.
- Benchling [trouble-shooting articles](#) and [Help center](#) offers many resources, frequently asked questions and articles that can help you



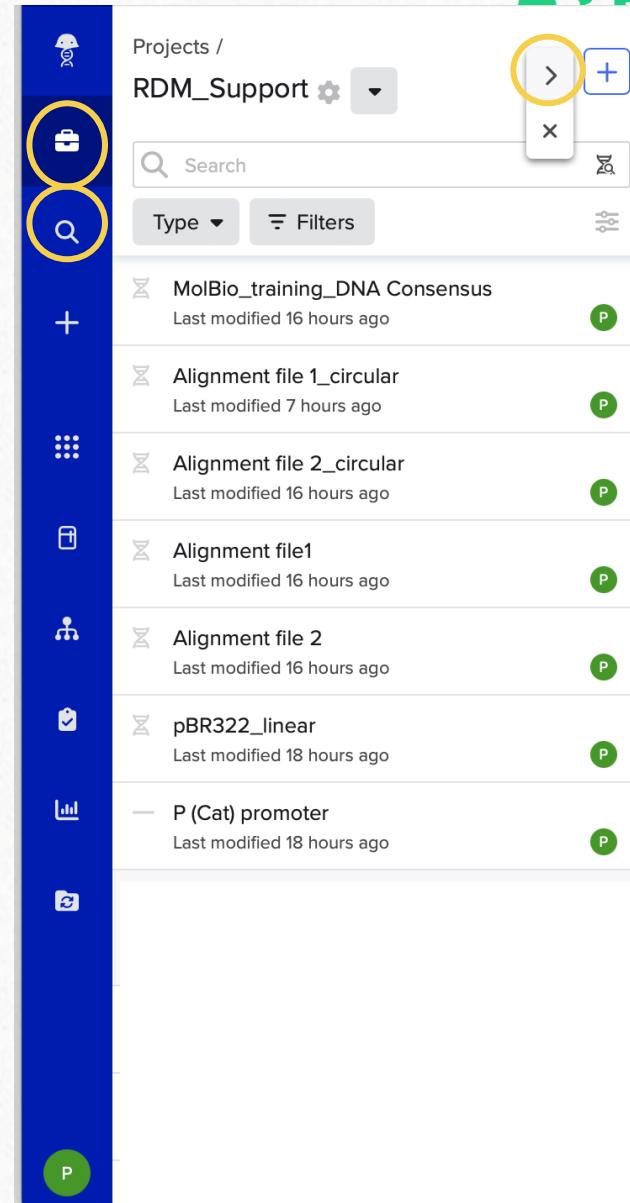
# Tips and tricks

## 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

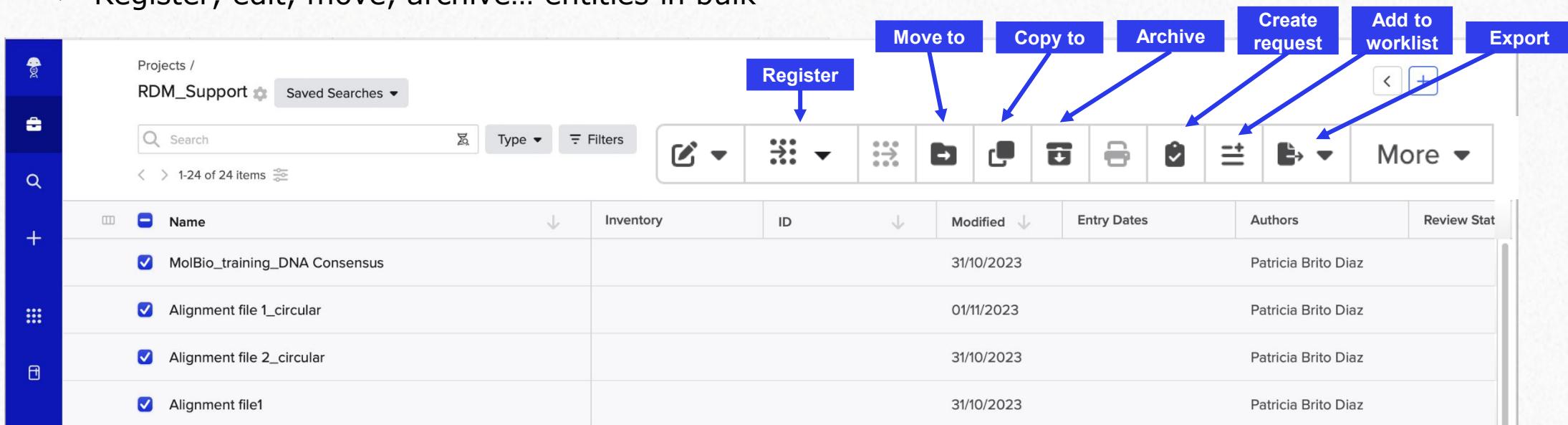
P (Cat) promoter  
Last modified 18 hours ago

# Tips and tricks

## Work in bulk using the expanded view

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

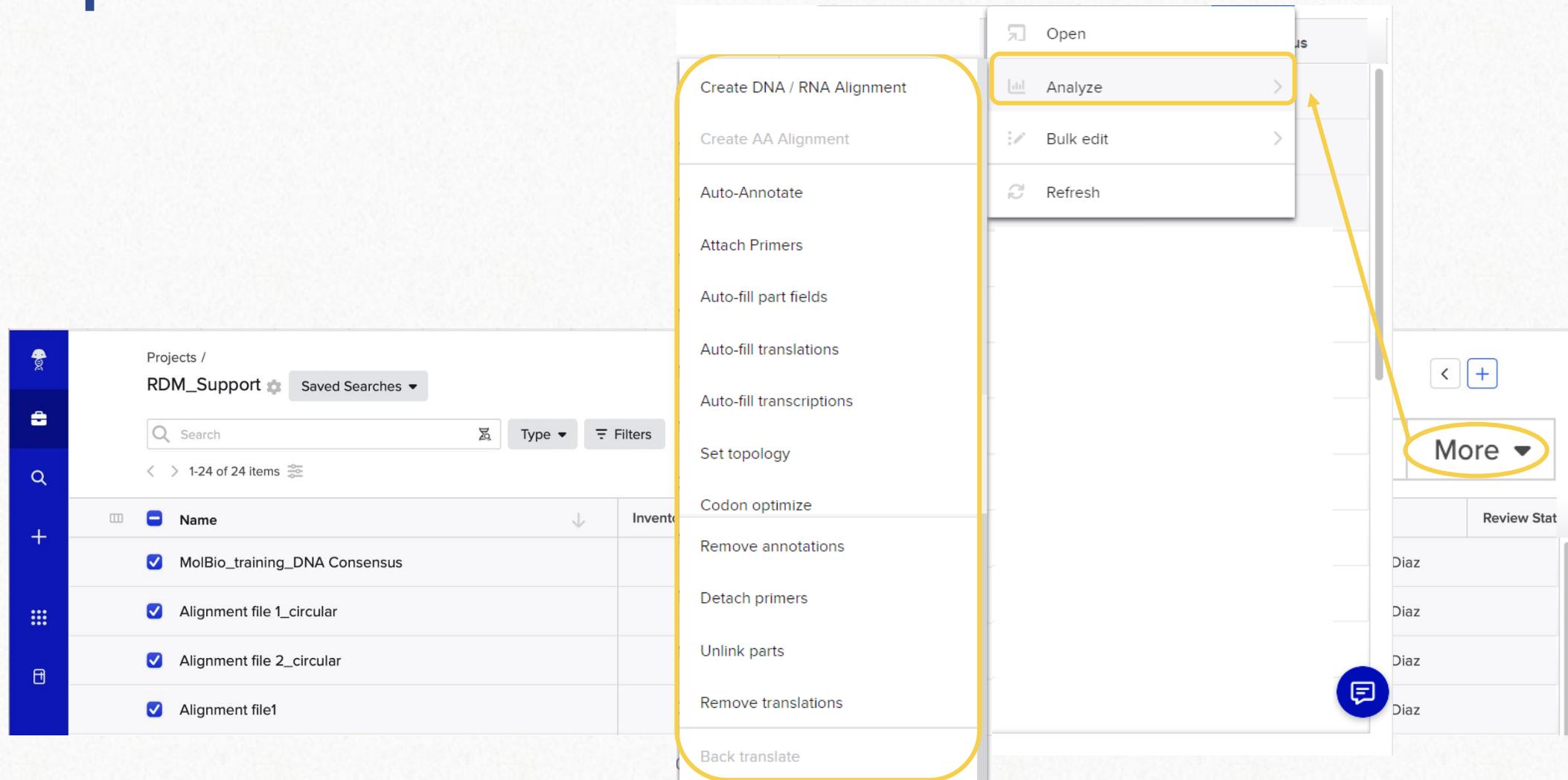
- ✓ Register, edit, move, archive... entities in bulk



The screenshot shows the RDM Support workspace interface. On the left, there's a vertical sidebar with icons for Home, Projects, Search, and Add. The main area has a header with 'Projects / RDM\_Support' and 'Saved Searches'. Below is a search bar and filter options. The central part is a table titled '1-24 of 24 items' with columns: Name, Inventory, ID, Modified, Entry Dates, Authors, and Review Stat. The 'Name' column is expanded, showing five items with checkboxes. Above the table is a toolbar 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.

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

# Tips and tricks



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

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

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

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

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

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

# Tips and tricks

## Autoindexing when creating alignments

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

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



# Tips and tricks

## Autoindexing when creating alignments

2 **Realign DNA / RNA**

1 Choose Input 2 Define parameters

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

Drag and drop to upload or Choose files

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type\*

DNA RNA

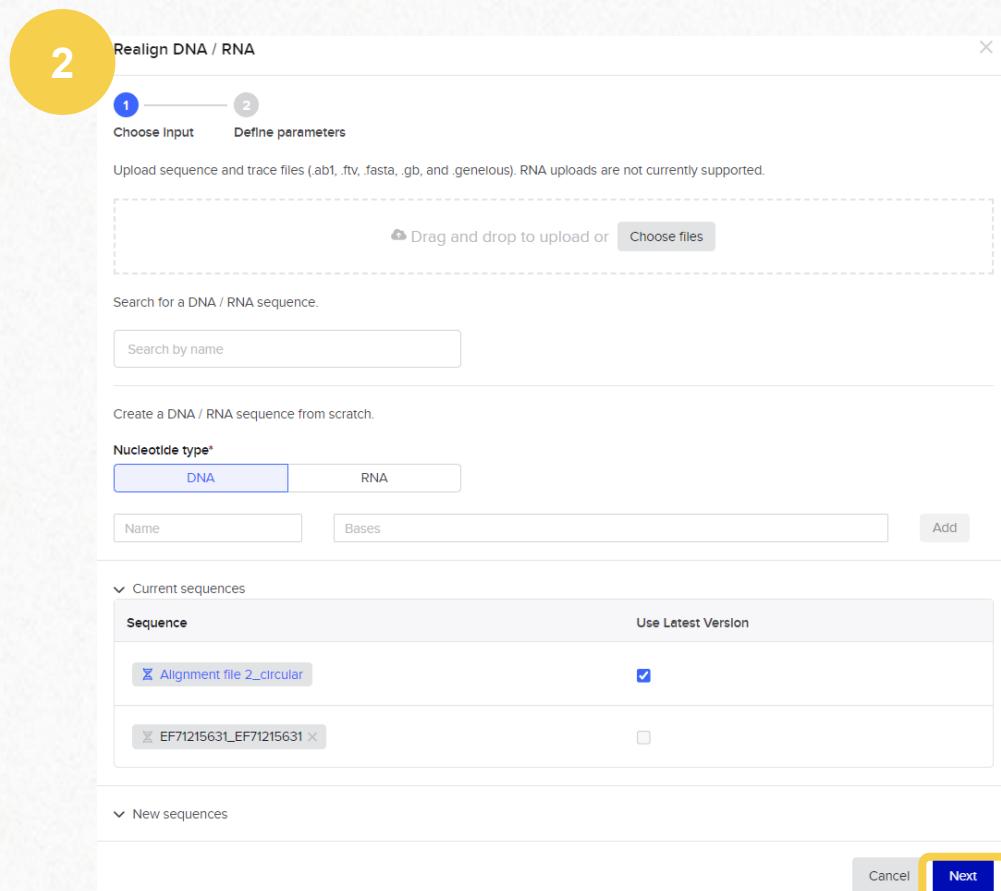
Name Bases Add

Current sequences

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

New sequences

Cancel Next



3 **Realign DNA / RNA**

1 Choose Input 2 Define parameters

Pairwise Multisequence Consensus

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

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

Template(s) Non-template sequence(s)

Alignment file 2\_circular EF71215631\_EF71215631 Search

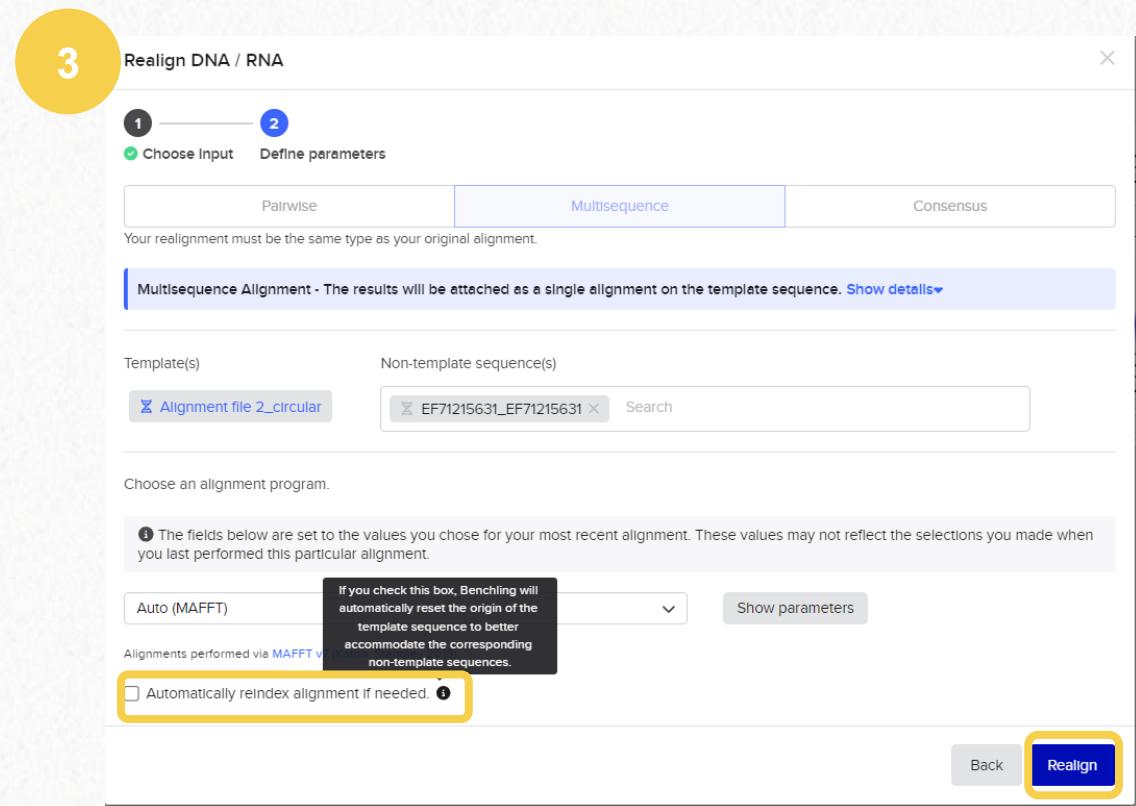
Choose an alignment program.

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

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

Automatically reindex alignment if needed.

Back Realign



# Tips and tricks

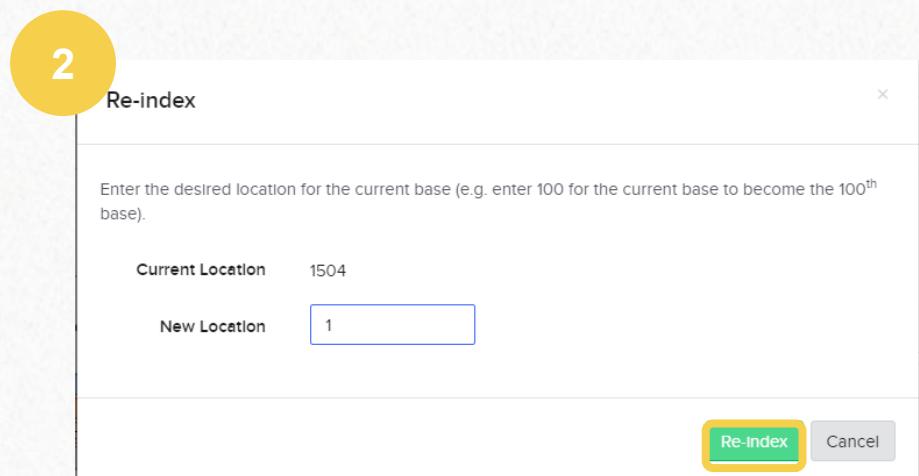
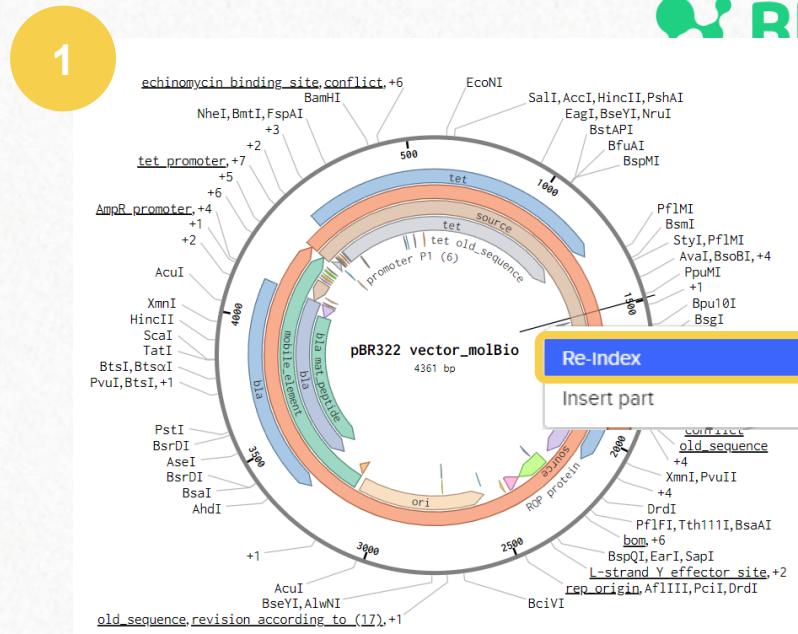
## Autoindexing when creating alignments

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

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

### Pro TIP:

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



# 10. Resources





# Questions?



Contact [lims\\_support@bright.dtu.dk](mailto:lims_support@bright.dtu.dk)

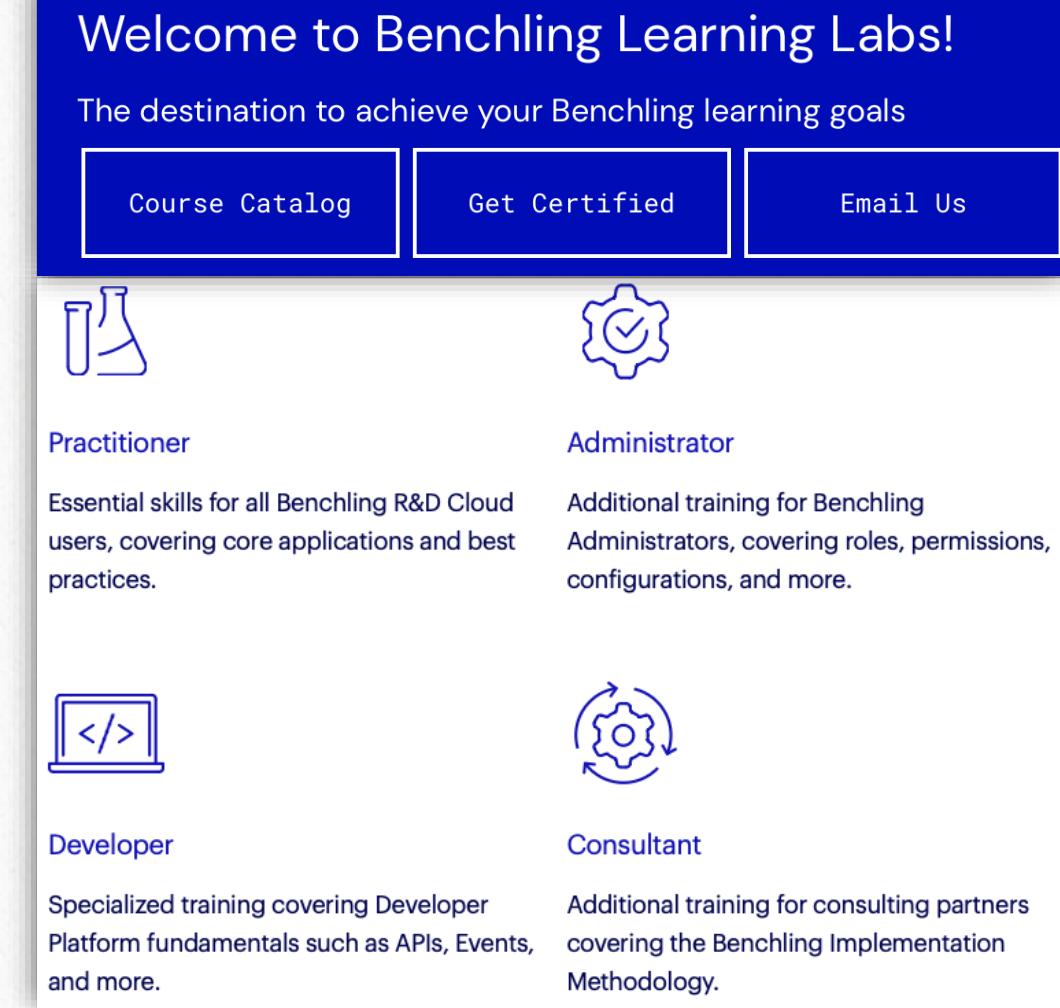
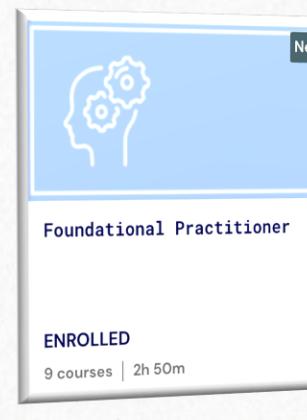


# More resources

## 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>

