

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

Reach out when struggling with the platform:

Biosustain Benchling support
lims_support@biosustain.dtu.dk



Access Benchling:

biosustain.benchling.com



(login with DTU credentials)

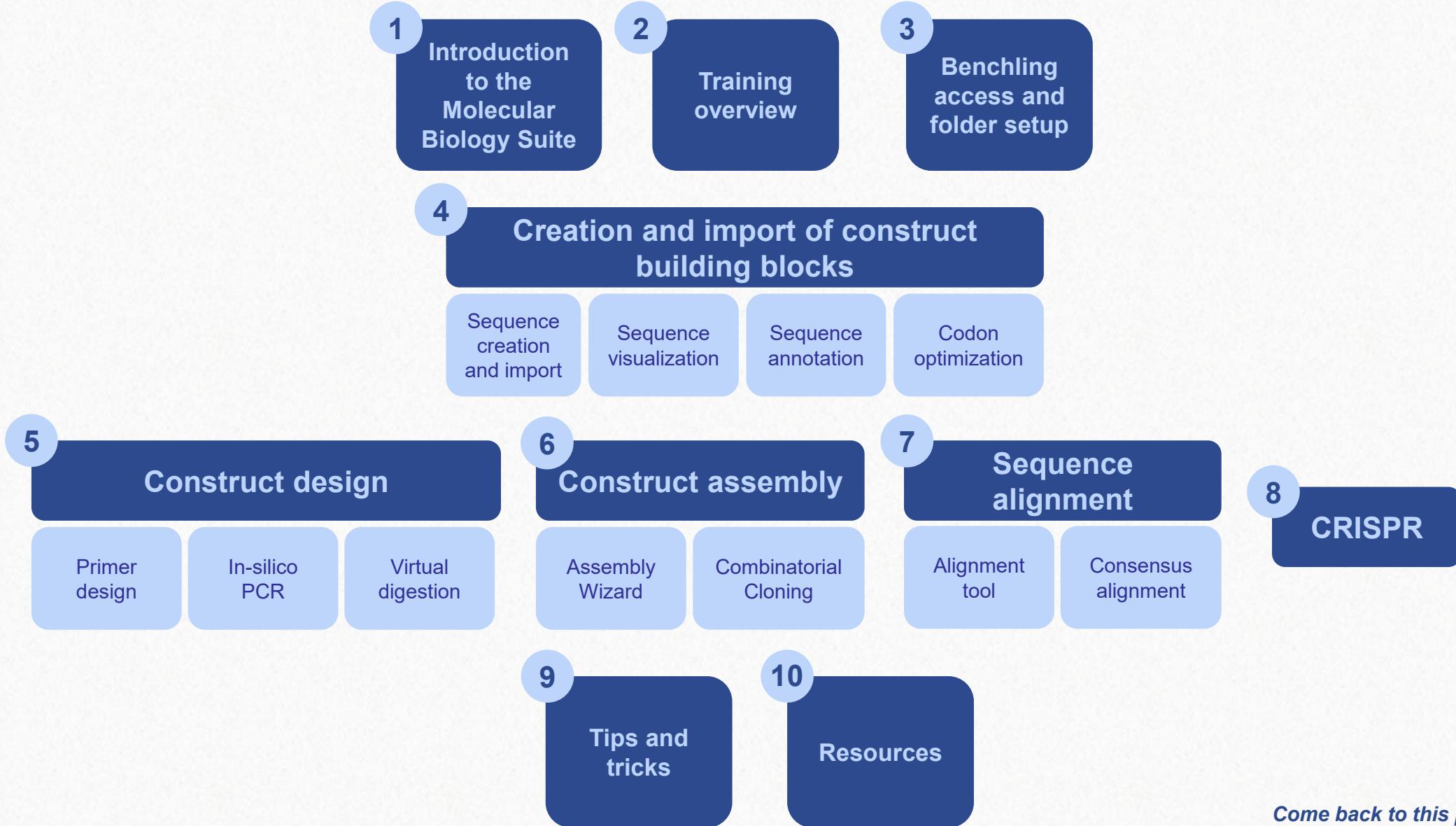
Additional resources:

[LIMS Help guides](#)



[Benchling Help Center: Molecular Biology](#)

Index



Come back to this page
by clicking on the icon!



1. Introduction to the Molecular Biology Suite

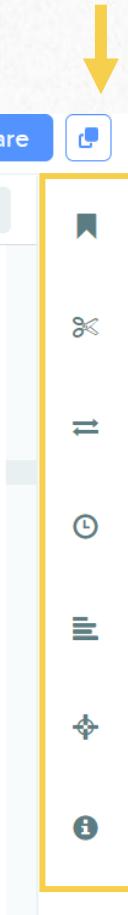


Functionalities and tools overview

Your sequence



Functionalities



Functionalities and tools overview



Features (annotations and translations)

Digests

Primers

History

Alignments

CRISPR

Information (topology, tags)

Functionalities and tools overview

Sequence Alignment

- ✓ Alignment to template
- ✓ Consensus alignment
- ✓ BLAST
- ✓ Bulk auto-alignment

Sequence Visualization

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

Construct Design

- ✓ RE-based cloning
- ✓ Golden Gate and Gibson assembly
- ✓ Bulk assembly
- ✓ Codon optimization
- ✓ Worklists integration

AA / Protein Analysis

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

CRISPR

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

Cloning

- ✓ Virtual digest visualization (PCR in-silico)
- ✓ Customizable enzyme lists

2. Training overview



Training goals:

The basics

- How to navigate the **sequence visualization** window and the workspace
- How to **create DNA/RNA sequences** in Benchling and use the different **import** options
- How to **easily design a construct** using Benchling:
 - In-silico PCR** and virtual digestion
 - Assembly wizard** – RE cloning
- How to do **high-throughput** construct design with the **Combinatorial assembly** tool
- How to **create a sequence alignment** and what are the different alignment options
- How to use the **CRISPR** tool for gRNA and HR template design

Additional knowledge

- Worklists
- Features libraries
- Access and restore an old version of a sequence



Training goals:

We will use **Benchling's Molecular Biology Tools** to work on a problem:

- ❖ *How can we synthesize vanillin in E. coli?*

This training will be based in a **start-to-end workflow**.

- ❖ Hypothetical question: *Create a plasmid to synthesize vanillin in E.coli*



Training goals:

This training will be based in a **start-to-end workflow**.

- ❖ Hypothetical question: *Create a plasmid to synthesize vanillin in E.coli*
- ❖ Steps:



Hands on:

1

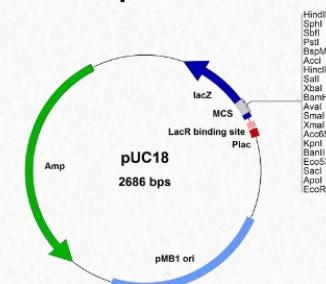


- ✓ Sequence creation and import:
 - From scratch
 - From a database
 - From a file
- ✓ Sequence visualization
- ✓ Codon optimization
- ✓ Sequence annotation

Vanillin dehydrogenase and GFP



pUC18 plasmid vector



Hands on:



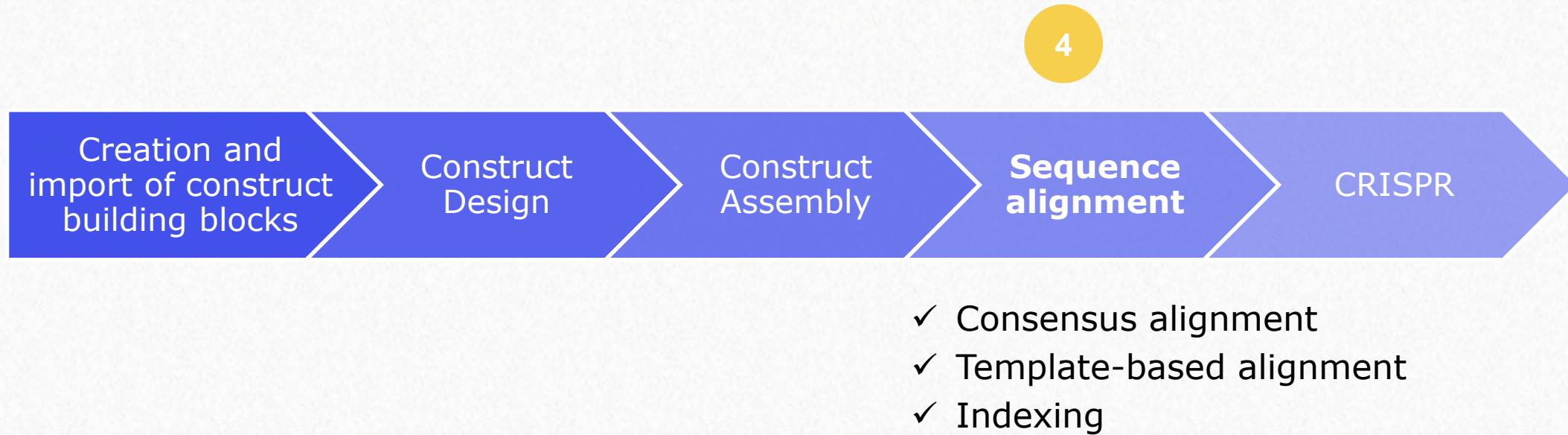
- ✓ Primer design:
 - Manual
 - Primer Wizard
- ✓ In-silico PCR
- ✓ Virtual digestion

Hands on:

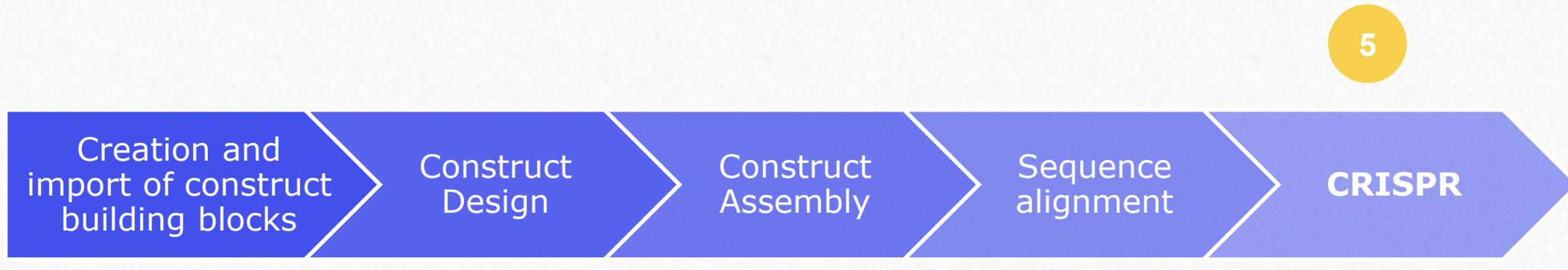


- ✓ Assembly Wizard
(RE cloning)
- ✓ Combinatorial cloning
(Gibson assembly)

Hands on:



Hands on:



- ✓ Guide RNA design

3. Benchling access and folder setup



Hands on:

Workflow example

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



Sequences and other materials needed for the training can be found in a ELN on the Biosustain Training project folder in Benchling:



Biosustain training > Molecular biology training > [Material information for molecular Biology Tool training](#)

First steps: 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' highlights the 'New' icon (a plus sign). Above it, another yellow circle with '2' highlights the 'Molecular Biology Training' folder in the navigation bar. A third yellow circle with '3' highlights the 'Folder' option in a dropdown menu. On the right, a modal window titled 'Create folder' is open, prompting the user to enter a name ('Your name') and location ('Molecular Biology Training'). A yellow circle with '4' highlights the 'Create' button.

Projects / Biosustain Training / Molecular Biology Training

Search

Type ▾ Filters

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

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

First steps: Find the ELN containing the training information

The screenshot shows the Benchling platform interface. On the left, a sidebar lists various projects and folders. A yellow circle with the number 1 highlights the folder icon. A yellow box with the number 2 highlights the 'Molecular Biology Training' folder. A yellow box with the number 3 highlights the 'Material information for the "Molecular Biology Tools" training' document. The main area displays the contents of this document, which includes:

Material information for the "Molecular Biology Tools" training

TUESDAY, 31/10/2023

In this ELN, you will find the files, links and sequences necessary to follow the training for the Molecular Biology Tool in Benchling.

Material for construct design:

- Vanillin Dehydrogenase gene from *Pseudomonas sp.* Accession number Y11520. **4** Copy this and download the files below
- To import a genome fragment that contains the coding sequence for the gene of interest (vanillin dehydrogenase), use the "import from Data Base" option.
- Plasmid backbone: pUC18** is the selected vector for this training.
Download the attached file that contains the annotated sequence of pUC18.
Create the Pladmid using the "Upload from file" functionality and rename it by adding your username (e.g., Vector_pUC18_myusername)
- GFP sequence:** Download the attached file or enter in the link to find the sequence of the GFP.

Attached files:

- pUC18.dna

SPLIT WORKSPACE

- ✓ Follow the instructions in the ELN
- ✓ Copy and download the necessary information for the next steps

4. Creation and import of construct building blocks



4. Creation and import of construct building blocks

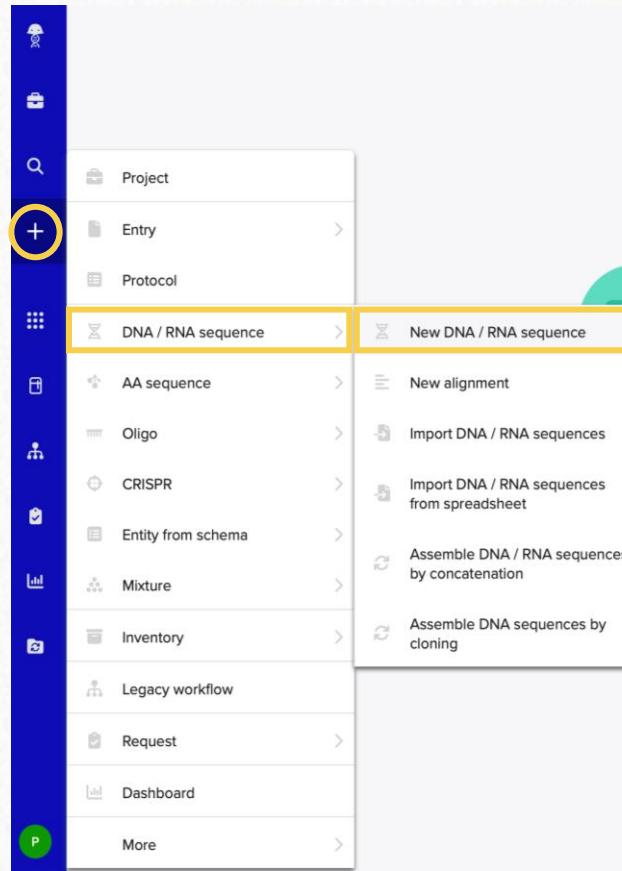
4.1 Sequence creation and import



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

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. This sequence will not be used later in this tutorial.

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

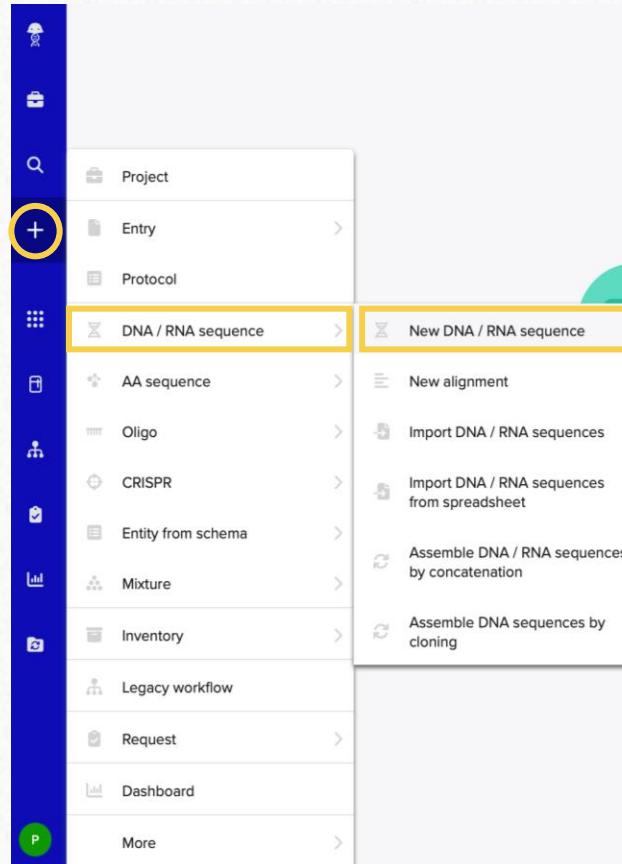
Bases
ggcacgtaaagggttccaactttcaccataatgaaaca

Close Create

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

Import of sequences from a database

- 1 Create a new DNA sequence
- 2 Paste accession number of *vdh*-containing sequence in "import from database" menu
- 3 Assign a schema to the new entity and import the sequence



Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Example searches:

- <https://www.addgene.org/browse/sequence/364796/> (Addgene URL)
- BRCA2 (Gene name)
- M62653 (NCBI Accession)
- ENSMUSG00000041147 (ENSEMBL ID)
- BBA_E0040 (Registry of Standard Biological Parts)
- JPUB_001430 (JBEI Public Registry)

Import multiple sequences at once by entering space-separated or comma-separated accession numbers.

Sequence: Y11520

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Sequence: Y11520

Entry: Y11520

Database: NCBI Nucleotide (Genbank)

Length: 3544

Description: Pseudomonas sp. vdh gene and ORF2

Set nucleotide type*: DNA RNA

Set folder*: Patricia B.

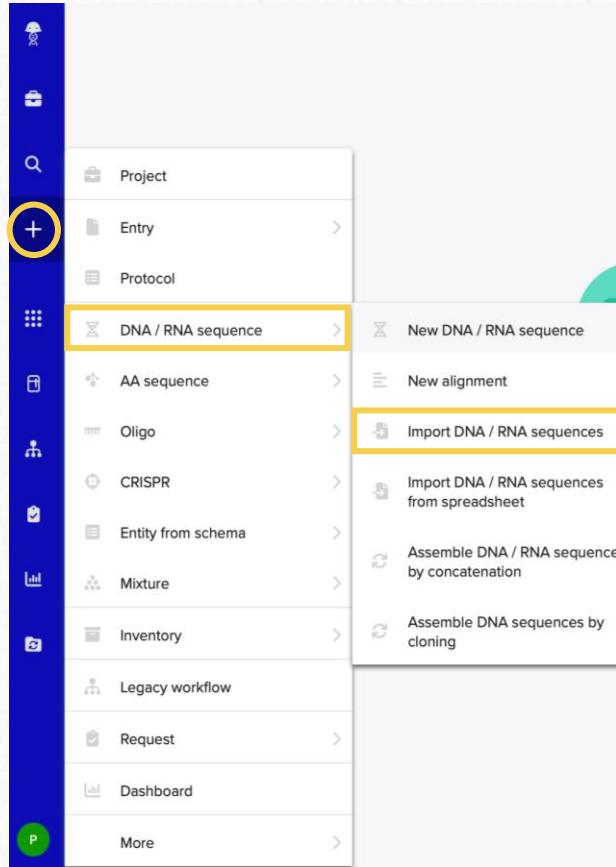
SCHEMAS

- DNA Fragment
- Gene
- gRNA
- Marker
- Origin of Replication
- Plasmid
- Primer
- Promoter
- Tag
- Terminator

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

Import of sequences from a file

- 1 Import pUC18 and GFP files



- 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 Mía

Drag and drop files to upload or

GFP.dna OPEN SEQUENCE - UPLOADED TO MÍA
 GFP - linear DNA creator Clontech (TaK...)

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

Show errors only

Remember to save everything in the correct folder!

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

Import of sequences from a file

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

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

Nucleotide type* Set folder

DNA RNA Patricia

Drag and drop files to upload or choose a file

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

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

Vector_pBR322.gb Exported · circular DNA ORGANISM synthetic DNA ... SOURCE Create New Tag

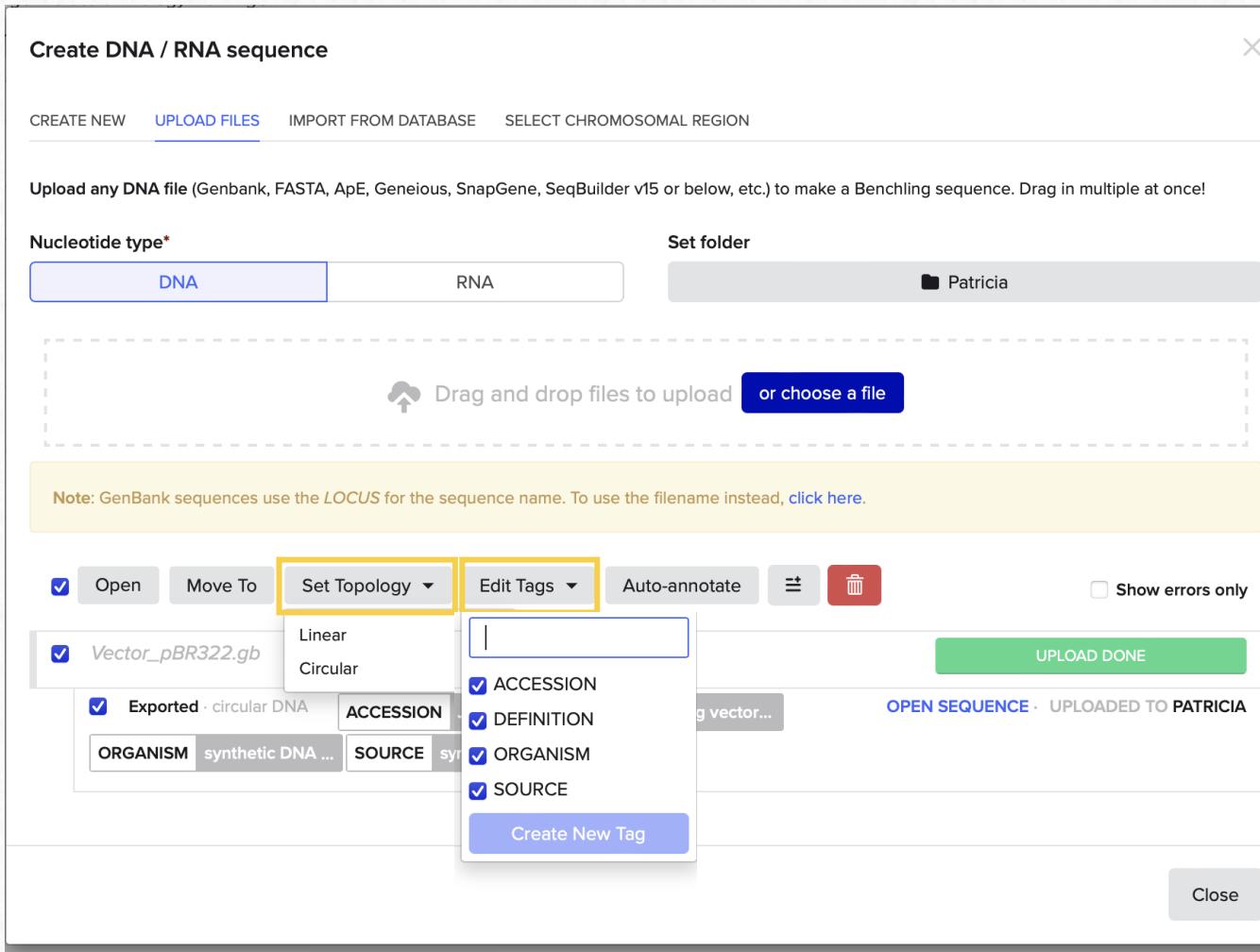
Linear Circular

ACCESSION DEFINITION ORGANISM SOURCE

g vector... OPEN SEQUENCE · UPLOADED TO PATRICIA

UPLOAD DONE

Close



When uploading a sequence, it is possible to:

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

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

Import of sequences from a file

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

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

Nucleotide type* DNA RNA

Set folder

Drag and drop files to upload or choose a file

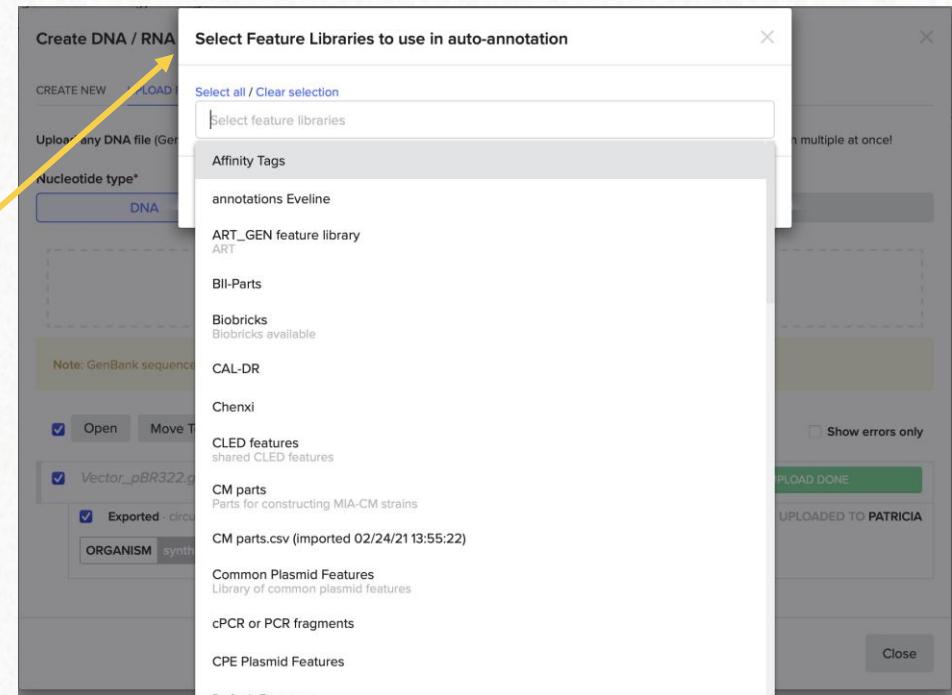
Note: GenBank sequences use the LOCUS for the sequence name. To use the filename instead, [click here](#).

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 ...



i **Auto – annotate** the sequence from an existing list of features.

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

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

Import of sequences from a file

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

The screenshot shows a 'Add items to entity worklist' dialog box. It has tabs for 'New worklist' (selected) and 'Existing worklist'. A 'Worklist Name*' input field contains 'Project_training'. Under 'Selected items', there is a list with a checked item 'Exported'. A blue 'Add items to worklist' button is at the bottom right. In the background, the main application window shows the uploaded sequence 'Vector_pBR322.gb' with its details and a 'Close' button.

i **Create worklists or add to existing ones** to make find your currently used entities faster or organize.

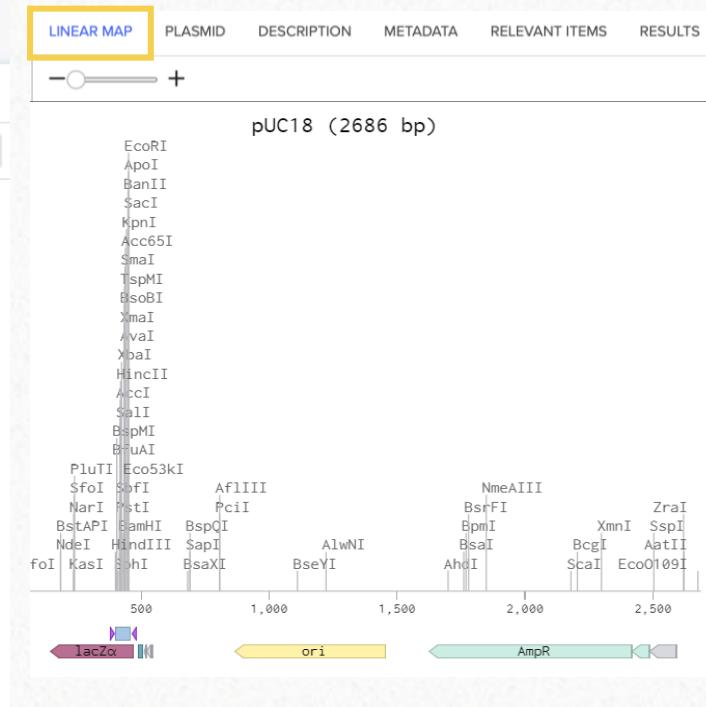
4. Creation and import of construct building blocks

4.2 Sequence visualization



View, annotate and edit your sequences

Different viewing options:



- ✓ For circular sequences, 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:

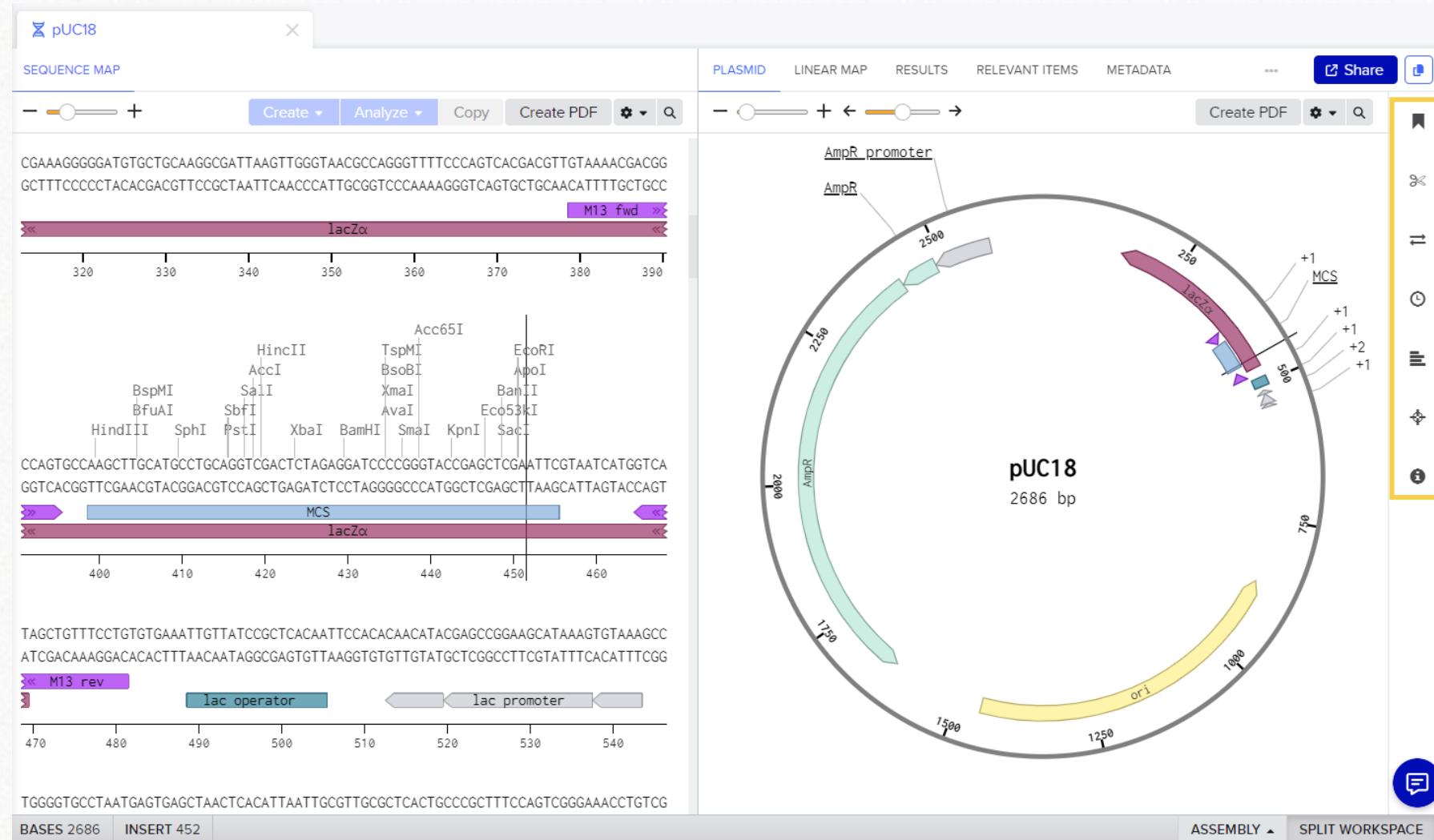


PRO TIP:

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

View, annotate and edit your sequences

Sequence navigation:

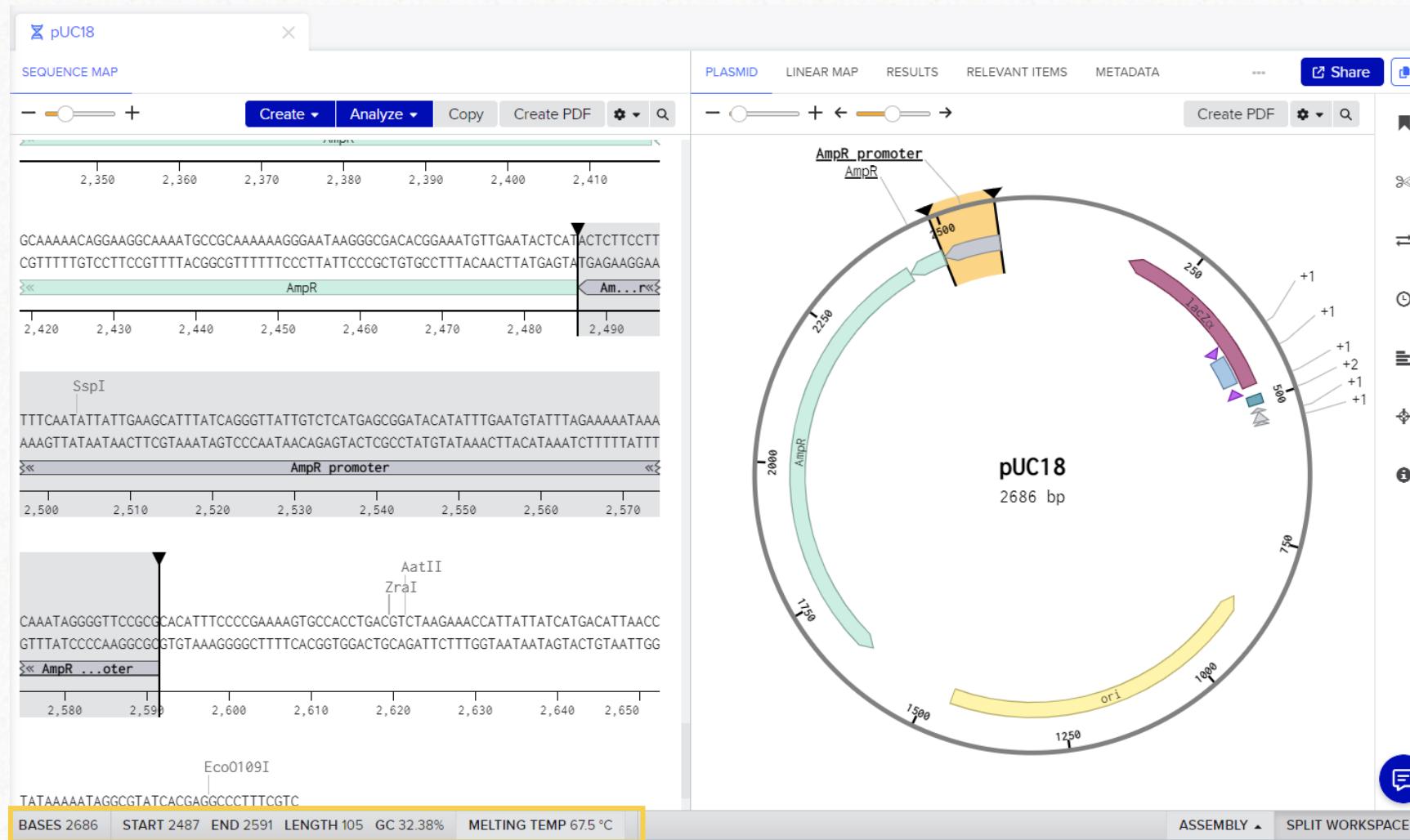


Functionality

- 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 parameters settings. Different calculation algorithms are available.

View, annotate and edit your sequences

Sequence navigation:

The screenshot shows a sequence visualization interface with the following features:

- SEQUENCE MAP:** A top-level view showing sequence fragments across a coordinate range from 80 to 390.
- Annotations:** A sidebar on the left provides options for creating and analyzing sequences, including:
 - Annotation:** Run Primer3, Run Benchling BLAST, Submit to NCBI BLAST, Analyze as translation, Optimize codons.
 - Primer:** Create primers for restriction enzymes like BstAPI and NdeI.
 - Translation:** Create translations for enzymes like PluTI, SfoI, NarI, and KasI.
 - New AA sequence:** Create amino acid sequences.
 - New DNA:** Create DNA sequences.
 - New RNA:** Create RNA sequences.
 - New part:** Create new parts.
- Sequence View:** A detailed view of a sequence fragment from position 160 to 230. It shows restriction sites (BstAPI, NdeI) and a primer binding site (lacZα). The sequence is: CAGAGCAGATTGACTGAGAGTGACCA GTCTCGTCAACATGACTCTACGTGGTACGCCACACTTATGGCGTGTACGCATTCTTTATGGCGTAGT lacZα.
- Editing Context Menu:** A context menu is open over the lacZα sequence, listing options:
 - 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... (disabled).
 - Run Benchling BLAST, Submit to NCBI BLAST, Analyze as translation.
- Sequence Statistics:** At the bottom, it shows: BASES 2686, START 146, END 469, LENGTH 324, GC 55.25%, MELTING TEMP 80.7 °C.

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



PRO TIP:

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

4. Creation and import of construct building blocks

4.3 Sequence annotation

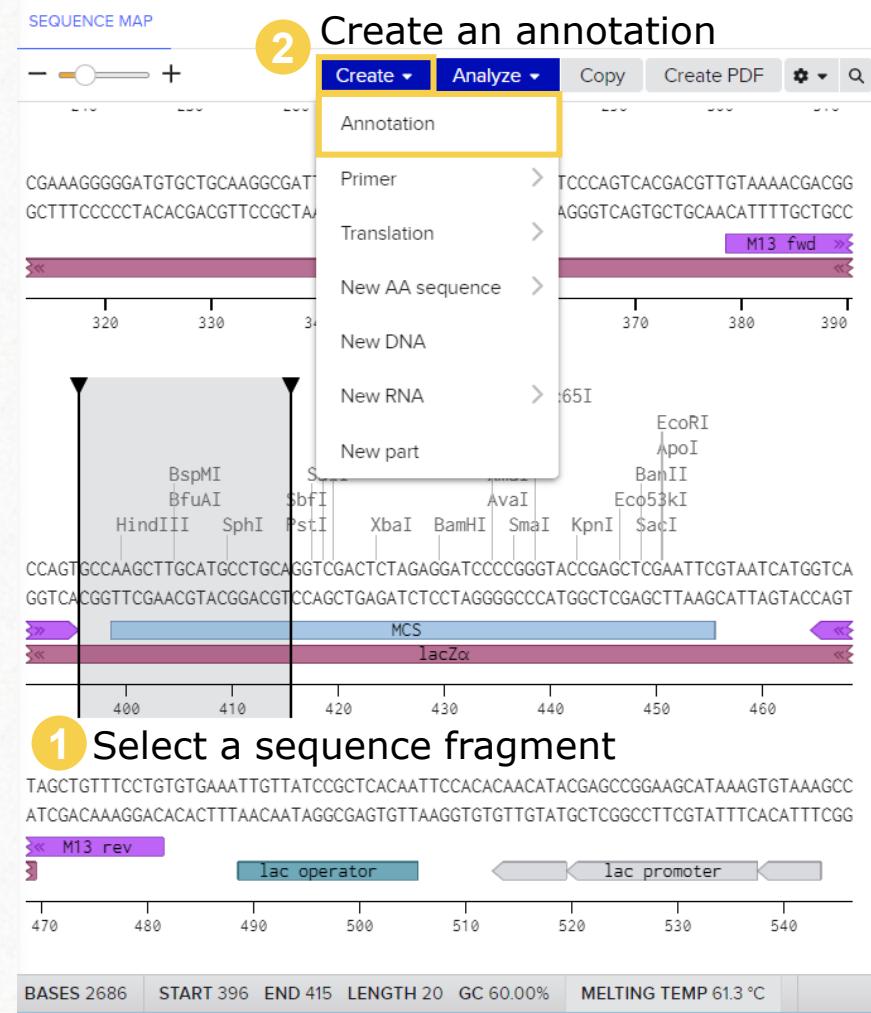


View, annotate and edit your sequences

Sequence annotations

1 Select a sequence fragment

SEQUENCE MAP



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

2 Create an annotation

Create ▾ Analyze ▾

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

3 Add the specifications

PLASMID LINEAR MAP RESULTS Share

Annotations Translations

Visibility filter Create new

AmpR promoter AmpR 2500 2250 2000 1750

12 total 0 hidden

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

New annotation

Name: Annotation name

Position: 396 - 415

Annotation type: Annotation type

Color: Purple

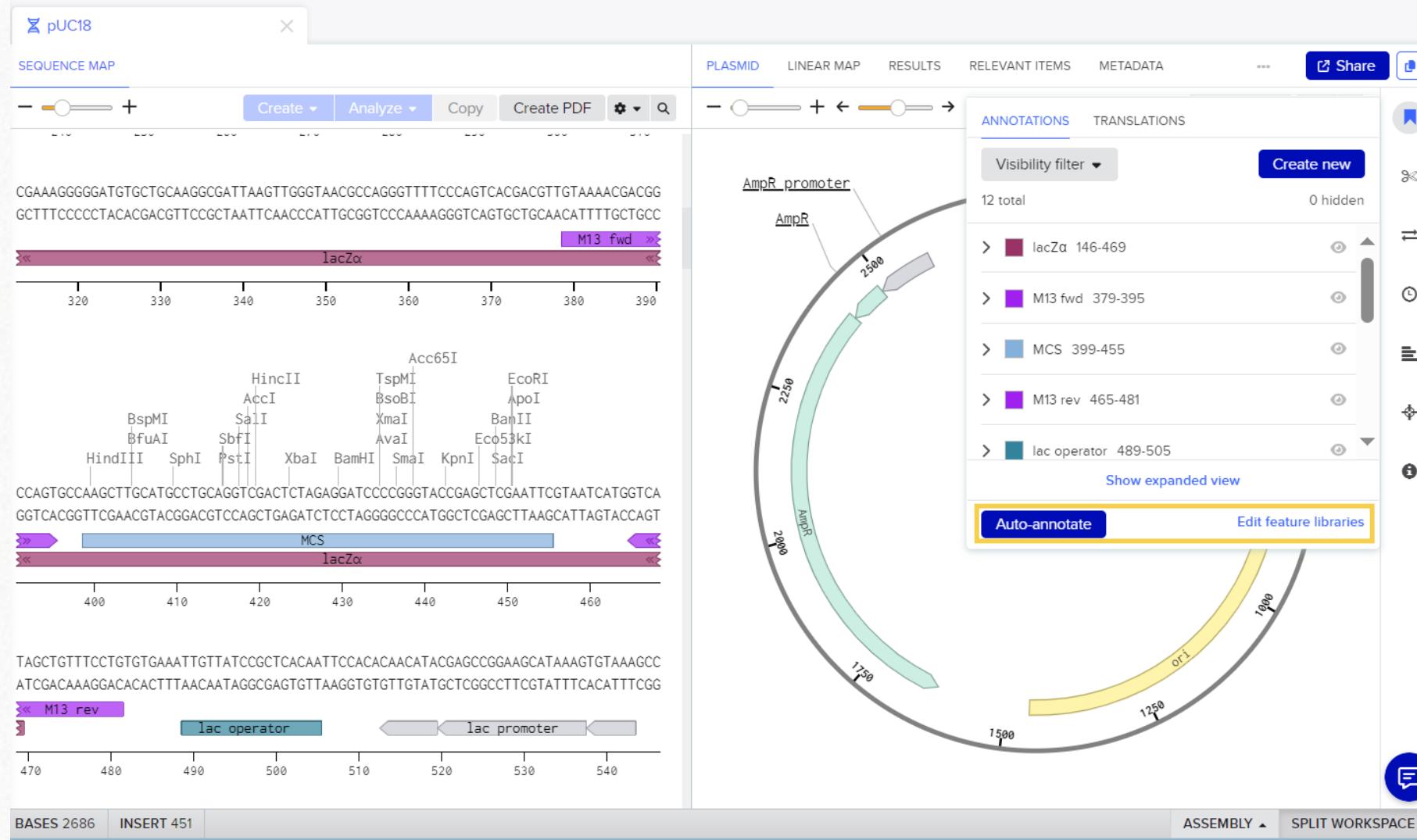
Strand: Forward

Notes: Notes

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

View, annotate and edit your sequences

Sequence annotations



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

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

4. Creation and import of construct building blocks

4.4 Codon optimization



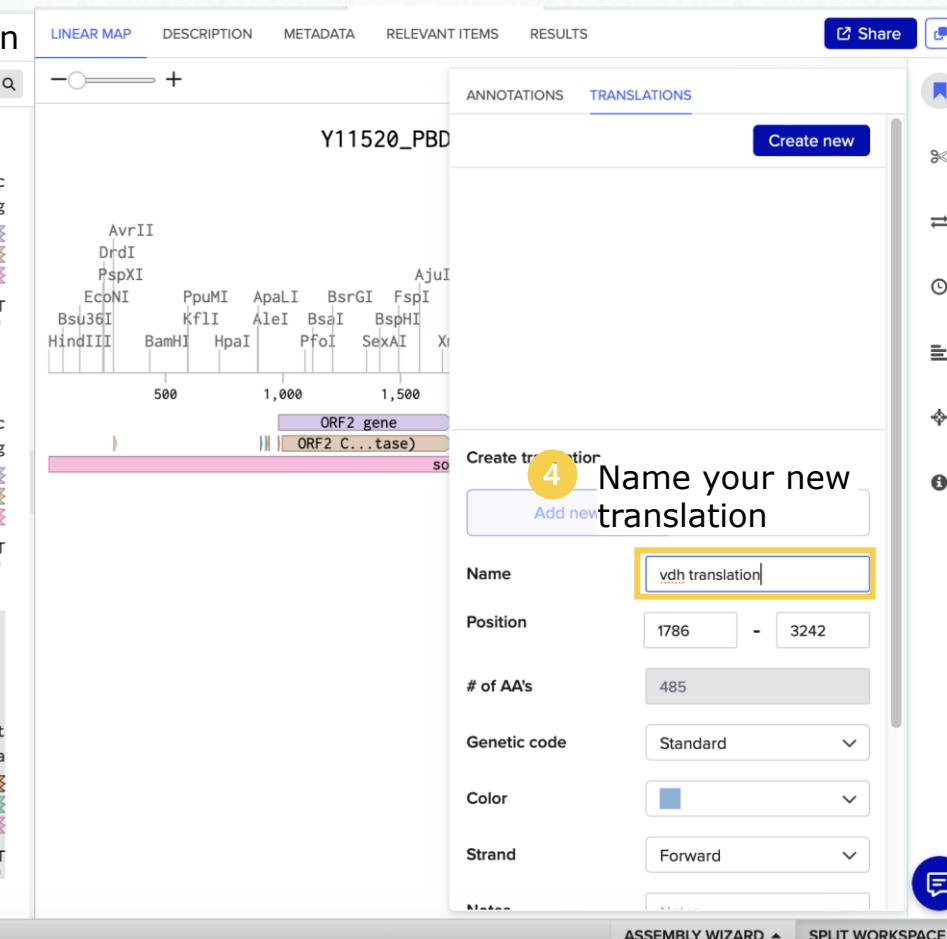
View, annotate and edit your sequences

Codon optimize the gene of interest (*vdh*) for the host (*E.coli*)

- 1 Go back to your DNA fragment (containing the gene of interest)



- 2 Select the gene of vanilin dehydrogenase (*vdh*)



- ✓ Before codon optimization, the DNA sequence must be translated

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

View, annotate and edit your sequences

Codon optimize the gene of interest (*vdh*) for the host (*E.coli*)

- Select the newly created translation and codon optimize it

The screenshot shows the Bioworkshop interface with the following components:

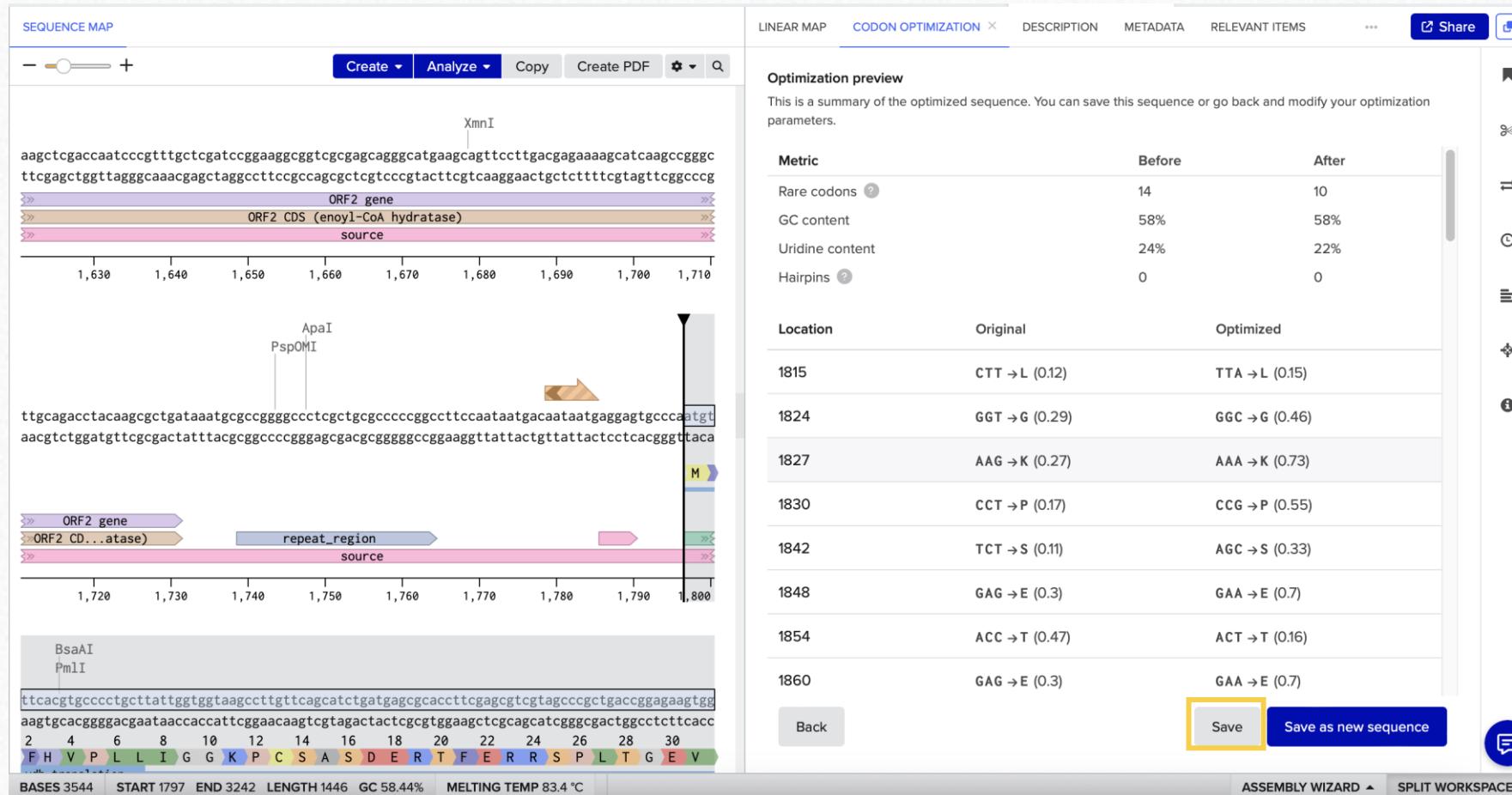
- SEQUENCE MAP:** Displays a sequence map with two main regions. The top region spans from 1,630 to 1,690, containing an **ORF2 gene** (purple) and an **ORF2 CDS (enoyl-CoA hyd)** (brown). The bottom region spans from 1,720 to 1,800, containing an **ORF2 gene** (purple), a **repeat_region** (blue), and a **source** (pink). Key restriction sites marked include ApaI, PspOMI, and BsaAI/PmlI.
- CODON OPTIMIZATION:** A context menu is open over the sequence at position 1,670, with the "Analyze" option highlighted. Other options include Run Primer3, Run Benchling BLAST, Submit to NCBI BLAST, Analyze as translation, Optimize codons (selected), Forward, and Reverse.
- Linear Map:** Shows the sequence from 1,797 to 3,242 (Forward Strand). It highlights the **ORF2 gene** (purple) and the **source** (pink).
- Parameters Panel (highlighted with a yellow box):**
 - Organism:** Escherichia coli (K12)
 - GC Content:** Any (0 to 1)
 - Uridine:** mRNA Uridine Depletion (unchecked)
 - Hairpin Parameters:** Avoid Hairpins (checked), with values 20 and 200.
 - AVOIDED CUT SITES (0):** A section for specifying enzymes to avoid.
 - Enzyme Name:** BsaAI, PmlI
 - Cuts:** Select an enzyme below to avoid creating its recognition site in the optimized sequence.
 - Add cut site to avoid:** + Add cut site to avoid
 - Remove all cut sites:** Remove all cut sites
- Buttons:** Cancel, Preview optimization, Share, and Split workspace.
- Bottom Status Bar:** BASES 3544, START 1797, END 3242, LENGTH 1446, GC 58.44%, MELTING TEMP 83.4 °C.

- When codon optimizing, it's possible to select the GC content and other details

View, annotate and edit your sequences

Codon optimize the gene of interest (*vdh*) for the host (*E.coli*)

- 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

5. Construct design



5. Construct design

5.1 Primer design

5.1.1 Manual primer design



Construct design

Manual primer creation

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

- 1 Select ~ 16 bases before and after the CDS sequence fragment

The screenshot shows the Benchling platform interface for sequence analysis. On the left, a 'SEQUENCE MAP' for pUC18 is shown with various restriction sites like ApaI, PspOMI, and BsaAI marked. On the right, a 'SEQUENCE MAP' for Y11520 is displayed, showing the 'vdh gene' and its 'vdh CDS (vanillin dehydrogenase)'. A context menu is open over the 'vdh CDS', with the 'PRIMERS' option selected. A sub-menu allows choosing between 'Manual' or 'Wizard' primer creation, with 'Manual' highlighted. A yellow circle highlights the 'Create Primers' button in the sub-menu. The bottom status bar indicates 'BASES 3544', 'START 1781', 'END 1796', 'LENGTH 16', 'GC 50.00%', 'MELTING TEMP 47.4 °C', and workspace buttons for 'ASSEMBLY' and 'SPLIT WORKSPACE'.

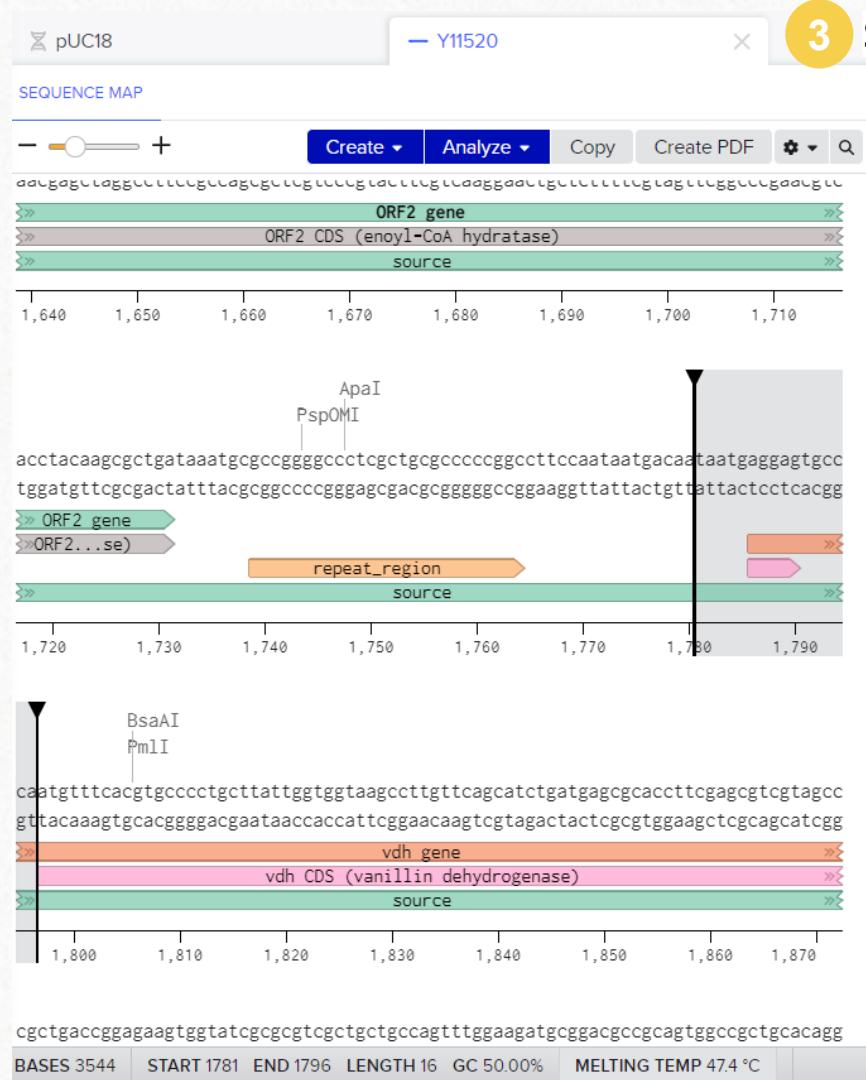
- ✓ You can also attach already existing primers to your sequence if the entities are registered on Benchling

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

Construct design

Manual primer creation

3 Select primer pair creation



The sequence map shows the pUC18 plasmid with various restriction sites and gene regions. A primer pair is being designed between positions 1720 and 1790.

4 Set the 3' selected bases as a forward

5 Set the 5' selected bases as a reverse

Verify

- T_m: --
- GC Content: --
- Length: 0 bp
- Product Size: --
- T_m Diff.: --

Check Secondary Structure at 37 °C

ASSEMBLY ▾ SPLIT WORKSPACE

- ✓ You can select the cutting sites you want to have on your primer pair and add overhangs

Construct design

Manual primer creation

- Design 7 Paste site at the beginning of the forward primer, and set the **overhang** to 6

Strand	Forward	Reverse
Bases	5' GAATTC <ins>taatgaggagt</ins> gccca	5' <ins>cgttttgcggatcgat</ins> 3'
Location	3' 1796	3' 3243
Overhang	6	0
Cut Site	EcoRI	GAATTC

Use the dropdown above to look up restriction sites.

- 6 Look up **EcoRI** restriction site

Strand	Forward	Reverse
Bases	5' GAATTC <ins>taatgaggagt</ins> gccca	5' <ins>AAGCTTcgcccgaaagat</ins> cgat
Location	3' 1796	3' 3243
Overhang	6	6
Cut Site	HindIII	AAGCTT

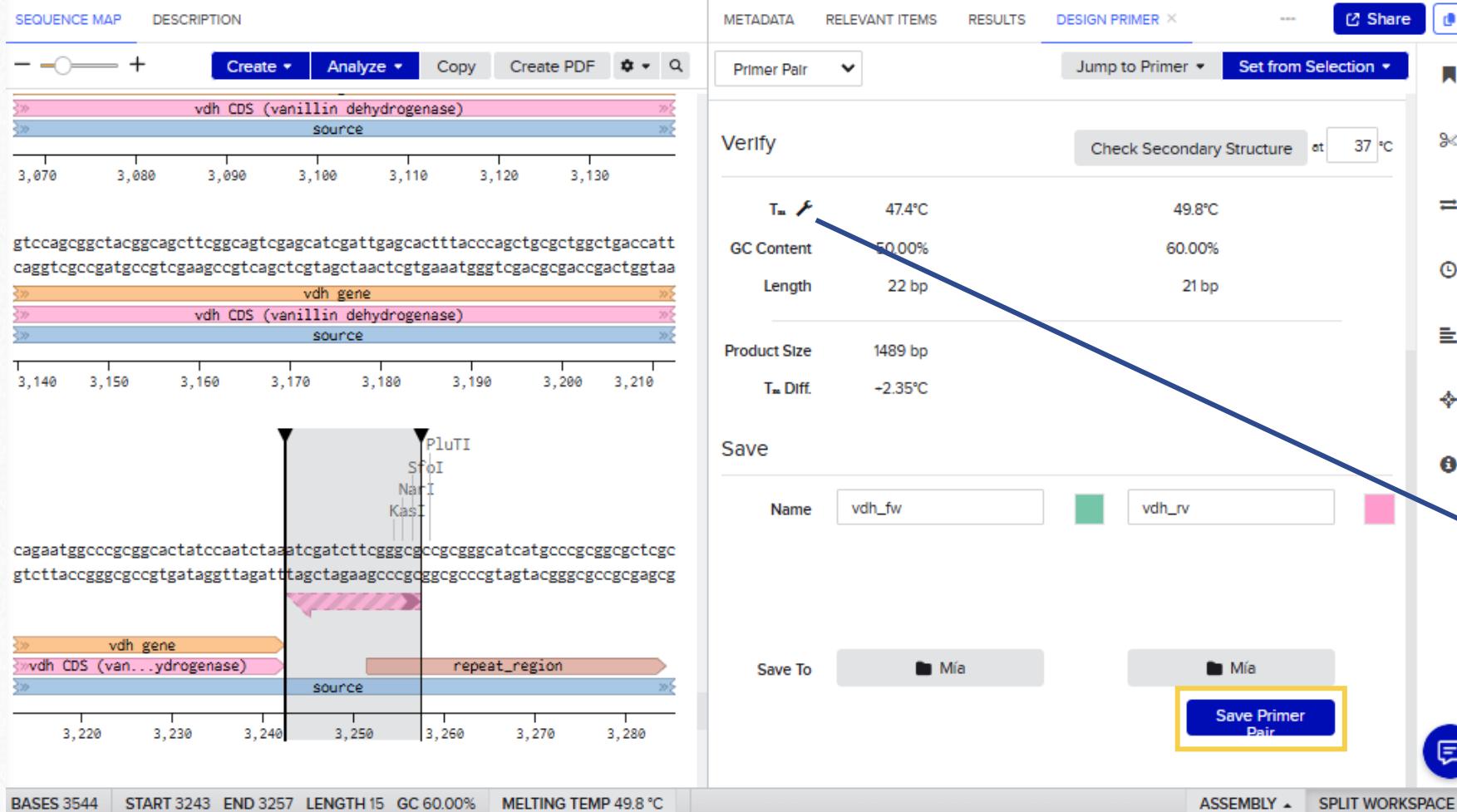
Use the dropdown above to look up restriction sites.

- 7 Repeat the process to add the **HindIII** site to the reverse primer

Construct design

Manual primer creation

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



The screenshot shows the Bioworkshop software interface for primer design. On the left, a sequence map displays the vdh CDS (vanillin dehydrogenase) gene structure across three segments: source, vdh gene, and vdh CDS (van...ydrogenase). Key restriction sites marked include PluTI, SfoI, NaiI, and KasI. A pink shaded region highlights a specific segment within the vdh CDS. The right panel, titled "DESIGN PRIMER", contains a "Verify" section with thermodynamic parameters:

	Primer Pair	Single-stranded
T _m	47.4°C	49.8°C
GC Content	50.00%	60.00%
Length	22 bp	21 bp
Product Size	1489 bp	
T _m Diff.	+2.35°C	

The "Save" section allows naming the primers: vdh_fw and vdh_rev. The "Save To" button is set to "Mía". A prominent blue arrow points from the "Save" section towards the "Save Primer Pair" button, which is highlighted with a yellow border. A blue info icon is also present near the "Save" section.

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

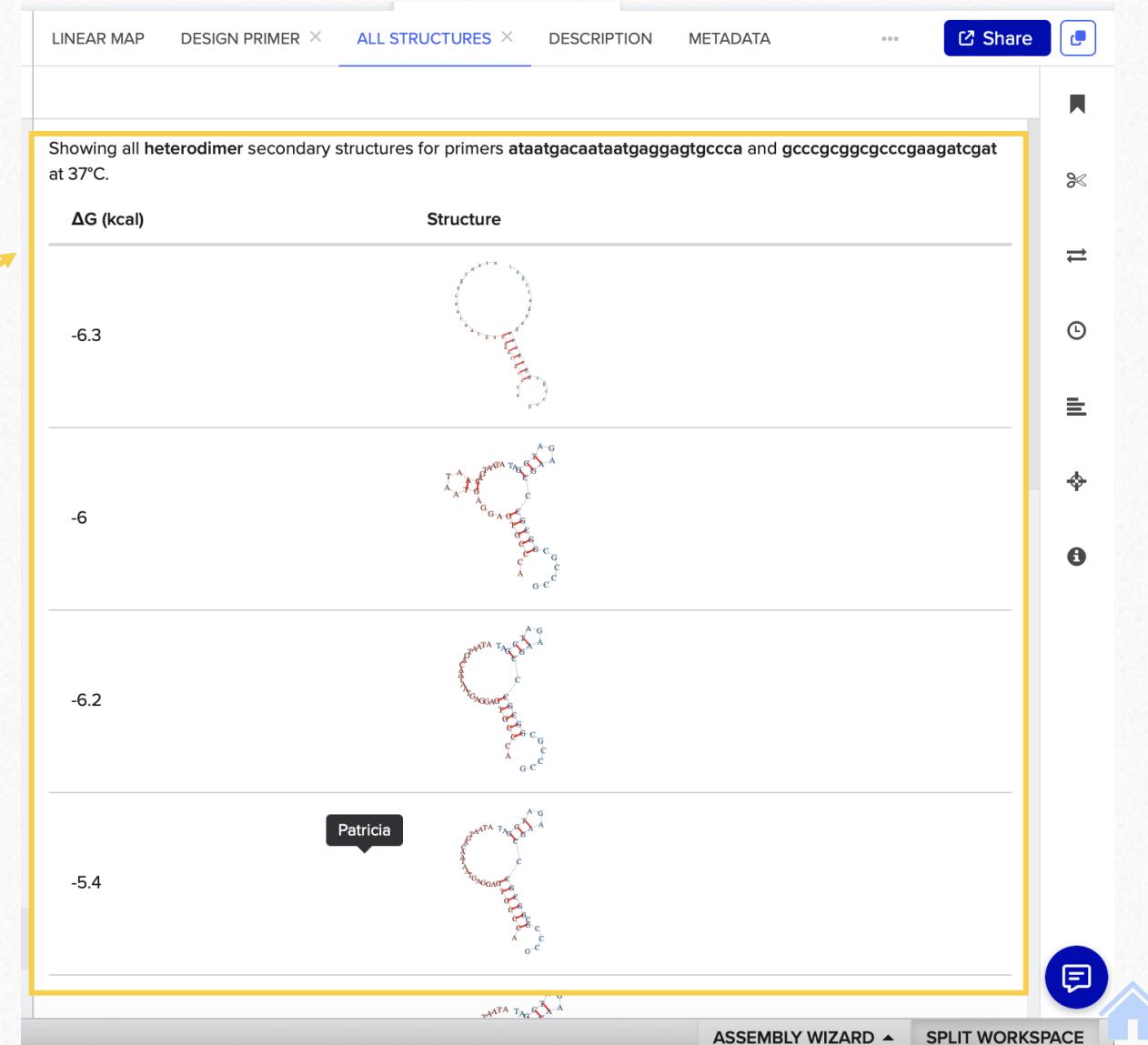
PRO TIP: You can adjust the default parameters for thermodynamic calculations

Construct design

Manual primer creation

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

The screenshot shows the Benchling software interface for primer design. The top navigation bar includes tabs for LINEAR MAP, DESIGN PRIMER (selected), ALL STRUCTURES (highlighted with a yellow box), DESCRIPTION, and METADATA. Below the tabs are input fields for Primer Pair, Overhang (0 bp), Cut Site (Aanl), and dropdowns for Jump to Primer and Set from Selection. A 'Verify' section displays various metrics: T_m (56.1°C and 69.8°C), GC Content (38.46% and 73.91%), Length (26 bp and 23 bp), Min ΔG Homodimer (-3.3 kcal and -13.8 kcal), Min ΔG Monomer (-0.1 kcal and -2.5 kcal), Product Size (1495 bp), T_m Diff. (+13.77°C), and Min ΔG Heterodimer (-6.3 kcal). A 'Check Secondary Structure' button is highlighted with a yellow box and has a tooltip indicating it's set to 37 °C. The bottom section for saving includes fields for Name (fwd_vdh and rev_vdh) and color-coded boxes for fwd_vdh (brown) and rev_vdh (purple). Navigation buttons at the bottom include ASSEMBLY WIZARD and SPLIT WORKSPACE.



5. Construct design

5.1 Primer design

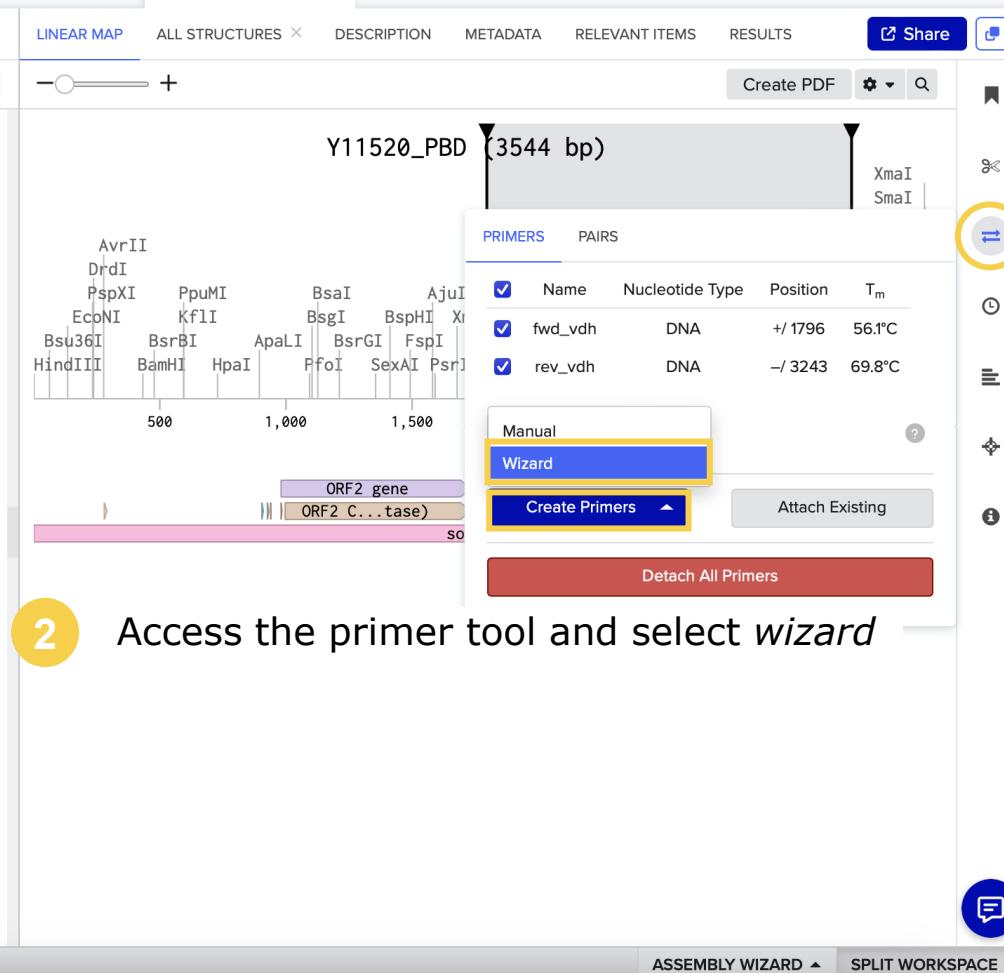
5.1.2 Primer wizard



Construct design

Automatic primer creation – Primer Wizard

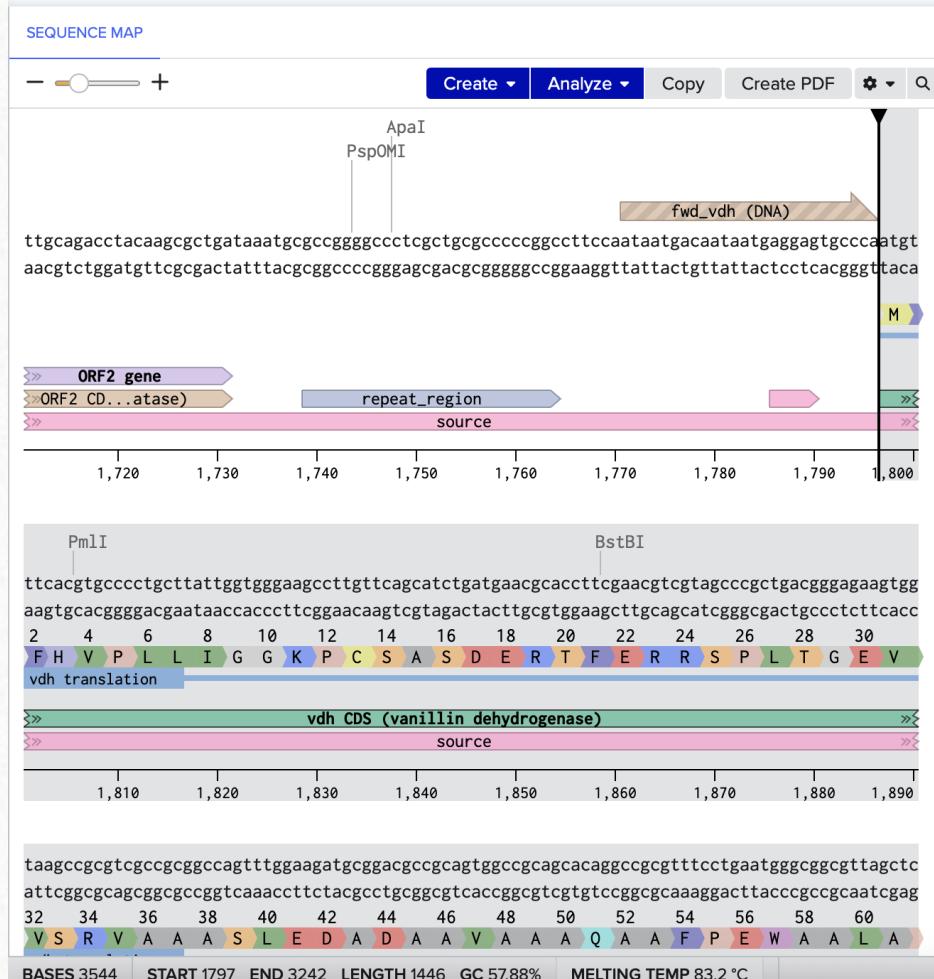
1 Select the CDS sequence of *vdh*



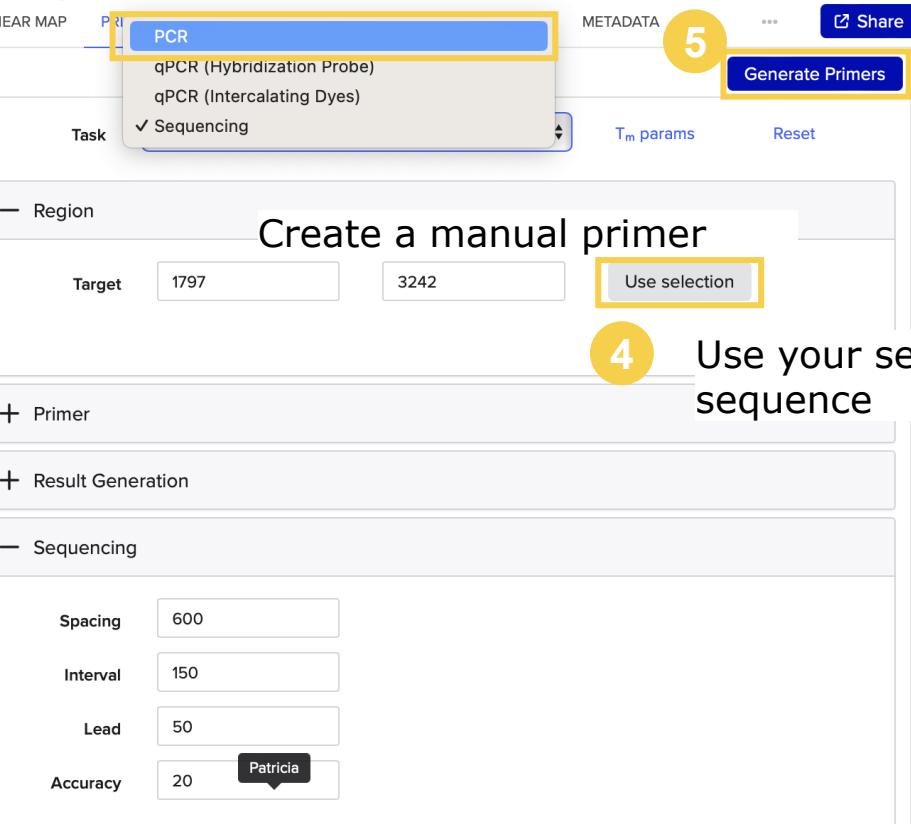
2 Access the primer tool and select wizard

Construct design

Automatic primer creation – Primer Wizard



3 Select PCR as sequencing task



5 Generate Primers

Create a manual primer

Target 1797, 3242, Use selection

Primer

Result Generation

Sequencing: Spacing 600, Interval 150, Lead 50, Accuracy 20, Name Patricia

Our primer wizard is powered by Primer3. Visit the [manual](#) for more information on the different tasks and parameters. Primer3 does not support RNA primers, so only DNA primers may be created with the Primer Wizard tool.

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

4 Use your selected sequence

i 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 Select the most appropriate primer pair and save them

The screenshot shows the QIIME 2 Primer Wizard interface. On the left, a "SEQUENCE MAP" panel displays a DNA sequence with various features: "ORF2 gene", "ORF2 CDS (enoyl-CoA hydratase)", and "source". It includes restriction site markers like XmnI and ApaI, and a primer binding site labeled "fwd_vdh (DNA)". Below the sequence map, the following statistics are shown: BASES 3544, START 1797, END 3242, LENGTH 1446, GC 57.88%, and MELTING TEMP 83.2 °C. On the right, a "PRIMER WIZARD" panel lists "PRIMER3 RESULTS" with 10 rows of primer pairs. The columns include: Penalty, Direction (FWD or REV), % GC, T_m °C, Location, Length, Product BP, and Primer sequence. The first two primer pairs are highlighted in yellow, indicating they are selected. A blue arrow points from the "Save Selected Primers" button in the top bar to the "Sort by Penalty" dropdown in the results table.

Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
63.6%	FWD	63.6%	62.0°	1464-1485	22	5'	gaaccagagtgttccgctggcc 3'
55.5%	REV	55.5%	60.7°	1757-1779	23	5'	tgtcattatttggaaaggccgggg 3'
63.6%	FWD	63.6%	62.4°	2069-2090	22	5'	cgcagcgaaaacttgtatggg 3'
63.6%	REV	63.6%	61.3°	2357-2378	22	5'	ccccagaccagcatcatggaggc 3'
59.1%	FWD	59.1%	60.2°	2661-2682	22	5'	cgtctgtttgtgacggcgtcg 3'
59.1%	REV	59.1%	62.4°	2954-2975	22	5'	ttcttcatcaccatcgccgcgc 3'
52.2%	FWD	52.2%	59.8°	3278-3300	23	5'	gcgcctgcctcattcaatctct 3'
63.6%	REV	63.6%	62.6°	3512-3533	22	5'	ggaaaggagaagaagcgccctcg 3'

✓ Is possible to select primers independently of their pair.

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

5. Construct design

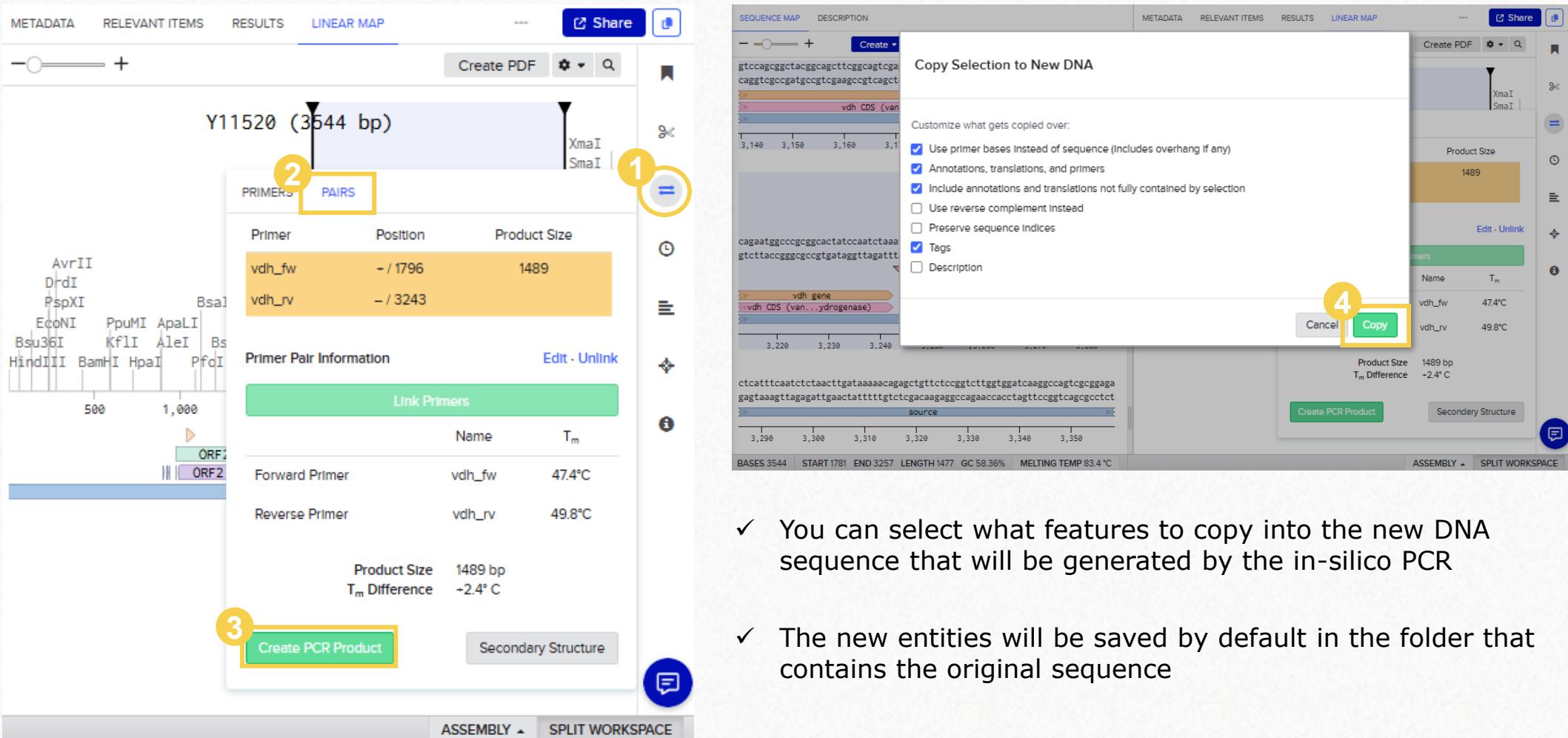
5.2 *In-silico* PCR



Construct design

In-silico PCR: Create a PCR product

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



The screenshot shows the BioEdit software interface for creating an in-silico PCR product. The main window displays a sequence map of a DNA construct (Y11520, 3544 bp) with various restriction sites (AvrII, D-dI, PspXI, EcoNI, BpuMI, ApaLI, BsaI, Bsu36I, KfI1, AleI, Bs, HindIII, BamHI, HpaI, PfdI) and two Open Reading Frames (ORF1 and ORF2). A primer pair (vdh_fw at position ~1796, vdh_rev at position ~3243) is selected, resulting in a product size of 1489 bp. The T_m difference is +2.4°C. A context menu (indicated by a yellow circle with number 1) is open over the sequence map, showing options to copy selection to new DNA. A sub-menu "Copy Selection to New DNA" (indicated by a yellow circle with number 2) is displayed, allowing customization of copied features. The "PAIRS" tab (indicated by a yellow circle with number 3) is selected in the main panel, showing primer details. A "Create PCR Product" button (indicated by a yellow circle with number 4) is visible at the bottom left. The right side of the interface shows a detailed sequence view with a zoomed-in region of the vdh gene and CDS, and a summary table of the PCR product.

Copy Selection to New DNA

Customize what gets copied over:

- Use primer bases instead of sequence (includes overhang if any)
- Annotations, translations, and primers
- Include annotations and translations not fully contained by selection
- Use reverse complement instead
- Preserve sequence Indices
- Tags
- Description

Product Size
1489

Primer Pair Information

Name	T _m
Forward Primer	47.4°C
Reverse Primer	49.8°C

Link Primers

Create PCR Product

- 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

LINEAR MAP RESULTS RELEVANT ITEMS METADATA DESCRIPTION Share

vdh_fw (DNA)

vdh gene

vdh CDS (vanillin dehydrogenase)

source

10 20 30 40 50 60 70

100 110 120 130 140

200 300 400 500 600 700 800 900 1,000 1,200 1,400

Bases 1489 Insert 37 Assembly Split Workspace

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

5. Construct design

5.3 Virtual digestion

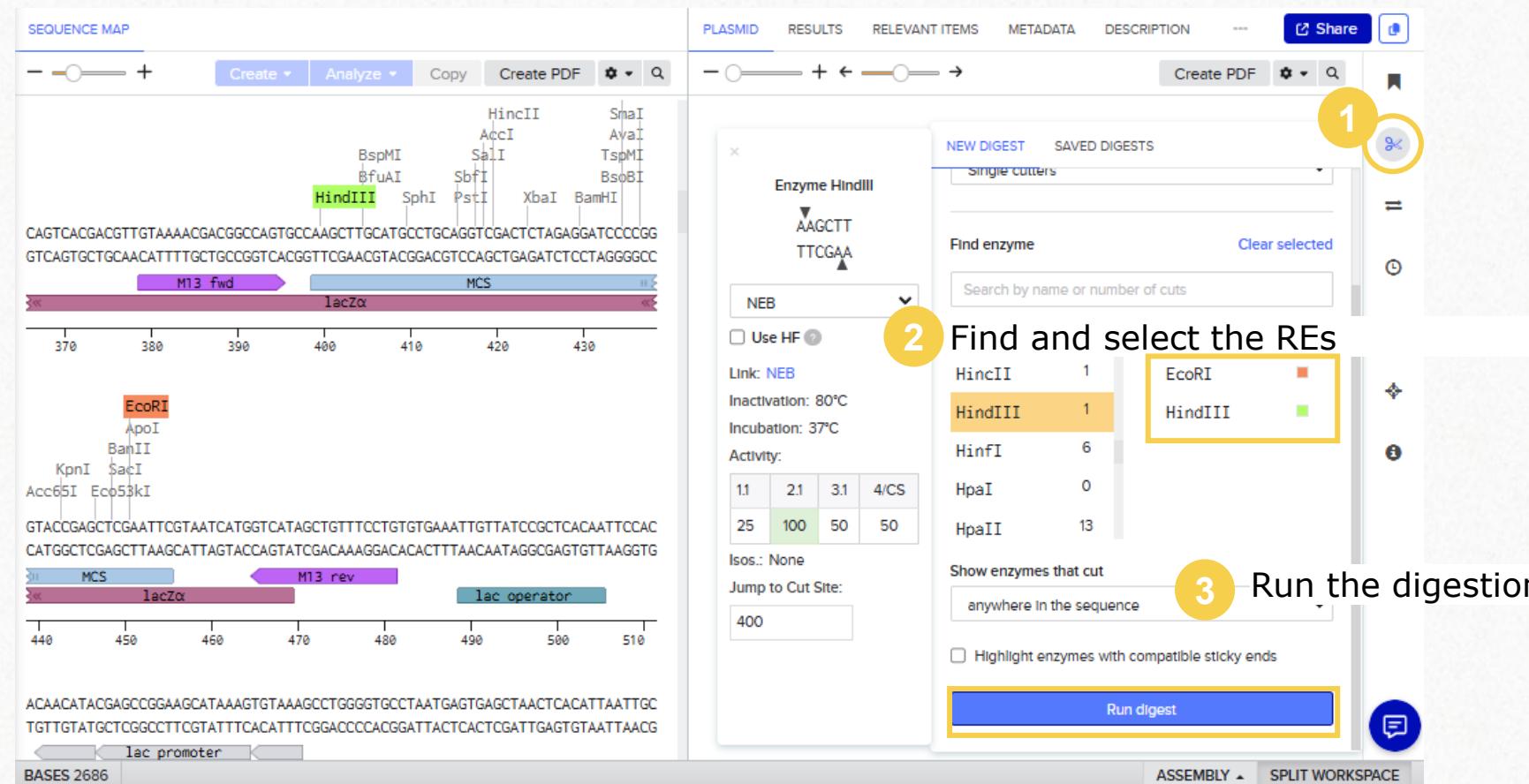


Construct design

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)



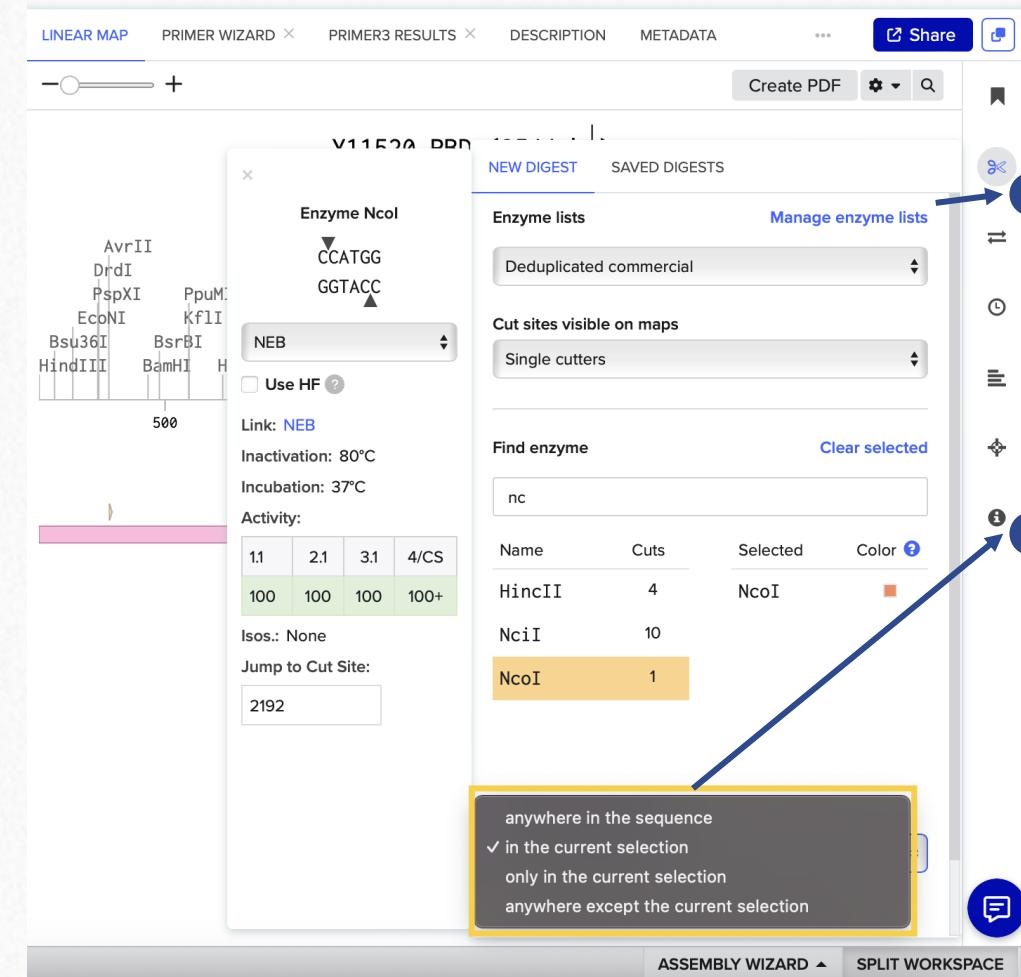
The screenshot shows the Bioworkshop software interface for construct design. On the left, the 'SEQUENCE MAP' tab displays the pUC18 backbone sequence with various restriction enzyme (RE) sites marked. Key sites include HindIII at position ~400, EcoRI at position ~450, and KpnI at position ~460. The backbone regions labeled are M13 fwd, MCS, lacZα, and lac operator. On the right, the 'PLASMID' tab is active, showing the 'NEW DIGEST' dialog. Step 1 highlights the search icon. Step 2 highlights the list of enzymes (HincII, HindIII, EcoRI, HinfI, HpaI, HpaII) with EcoRI and HindIII selected. Step 3 highlights the 'Run digest' button.

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

- ✓ You can select the most appropriate enzymes list for you and select the RE by their cutting sites

Construct design

Virtual digestion



The screenshot shows the BioEdit software interface with the 'LINEAR MAP' tab selected. A context menu is open over a sequence fragment, showing enzyme information for NcoI. The sequence 'CCATGG GGTACC' is highlighted. The enzyme list dropdown shows 'NEB' selected. Below it, activity data for NEB is listed: 1.1 (100), 2.1 (100), 3.1 (100), and 4/CS (100+). The 'Cut sites visible on maps' dropdown shows 'Single cutters'. The 'Find enzyme' search bar contains 'nc'. The results table lists enzymes: HincII (4 cuts, Selected), NciI (10 cuts), and NcoI (1 cut, highlighted with a yellow box). A tooltip at the bottom right of the table provides options: 'anywhere in the sequence', '✓ in the current selection' (with a checkmark), 'only in the current selection', and 'anywhere except the current selection'.

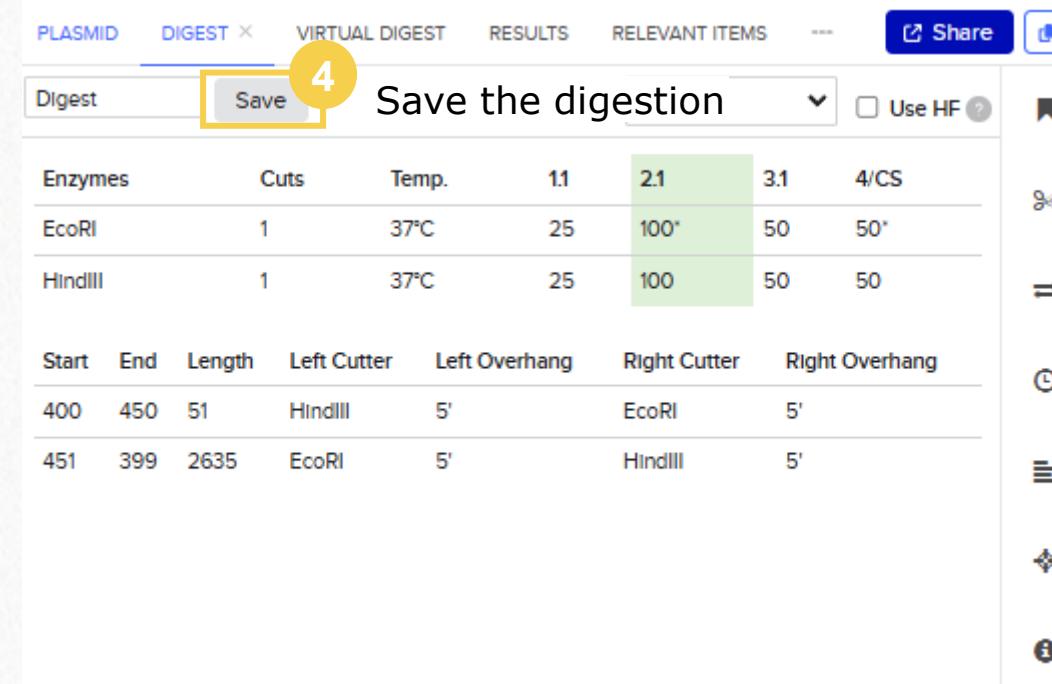
PRO TIP: The enzyme lists available can be managed, similarly to the features libraries and are shared within the Center.

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

Construct design

Virtual digestion

Digestion of the backbone



The screenshot shows the Biocutter software interface. At the top, there are tabs: PLASMID, DIGEST (which is selected), VIRTUAL DIGEST, RESULTS, RELEVANT ITEMS, and three more tabs represented by ellipses. To the right of these are 'Share' and 'Print' buttons. Below the tabs is a search bar labeled 'Save the digestion' with a dropdown arrow and a 'Use HF' checkbox. A large yellow circle with the number '4' is overlaid on the 'Save' button. To the right of the search bar is a vertical sidebar with several icons: a bookmark, a magnifying glass, a double arrow, a clock, a list, a diamond, and an information icon.

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
HindIII	1	37°C	25	100	50	50

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
400	450	51	HindIII	5'	EcoRI	5'
451	399	2635	EcoRI	5'	HindIII	5'

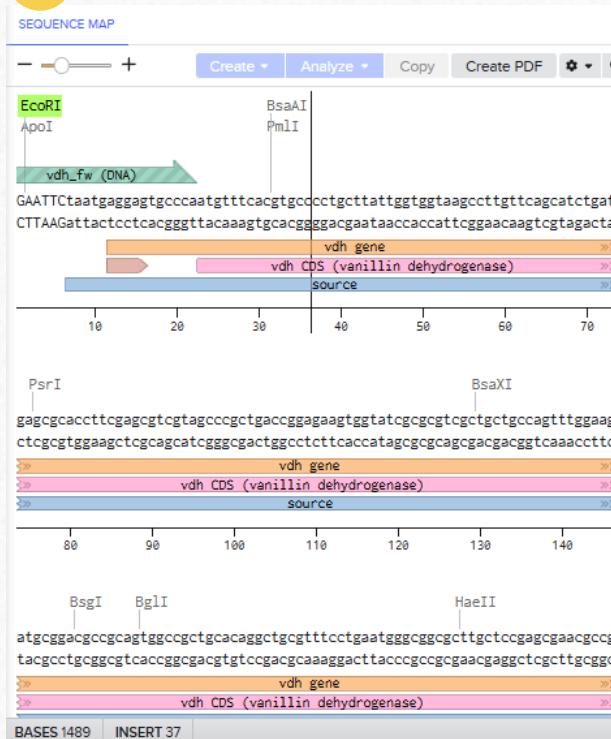
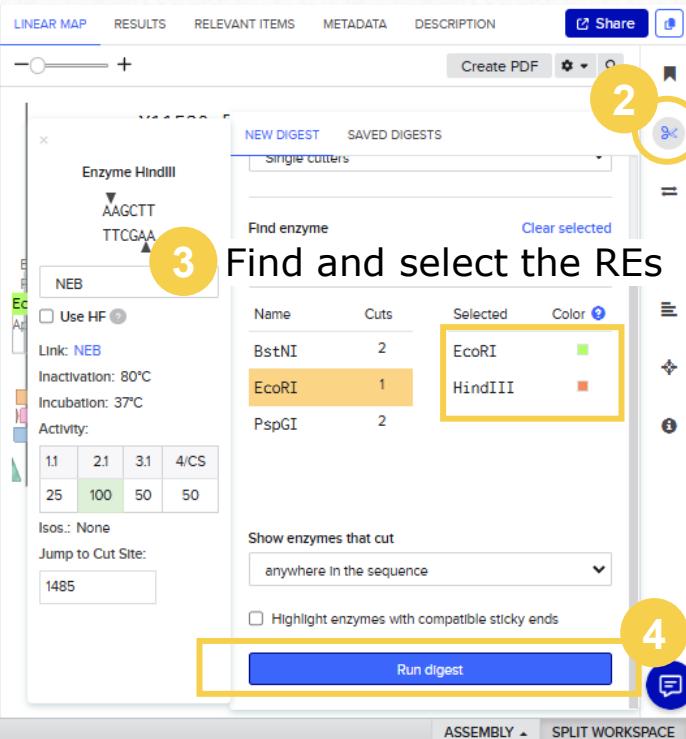
- ✓ A saved digestion will allow you to easily find the fragments you need to work with for the assembly

Construct design

Virtual digestion

Digestion of the insert

1 Open the amplified *vdh* sequence

LINEAR MAP RESULTS RELEVANT ITEMS METADATA DESCRIPTION Share

Enzyme HindIII

AAGCTT TTGAA

NEB

Link: NEB

Inactivation: 80°C

Incubation: 37°C

Activity:

Name	Cuts	Selected	Color
BstNI	2		
EcoRI	1	<input checked="" type="checkbox"/>	green
HindIII		<input type="checkbox"/>	orange
PspGI	2		

Start End Length Left Cutter Left Overhang Right Cutter Right Overhang

1 1 1 None blunt EcoRI 5'

2 1484 1483 EcoRI 5' HindIII 5'

1485 1489 5 HindIII 5' None blunt

Show enzymes that cut anywhere in the sequence

Highlight enzymes with compatible sticky ends

Run digest

ASSEMBLY SPLIT WORKSPACE



LINEAR MAP DIGEST VIRTUAL DIGEST RESULTS RELEVANT ITEMS Share

Digest Save

Enzymes Cuts Temp. 1.1 2.1 3.1 4/CS

EcoRI 1 37°C 25 100* 50 50*

HindIII 1 37°C 25 100 50 50

Start End Length Left Cutter Left Overhang Right Cutter Right Overhang

1 1 1 None blunt EcoRI 5'

2 1484 1483 EcoRI 5' HindIII 5'

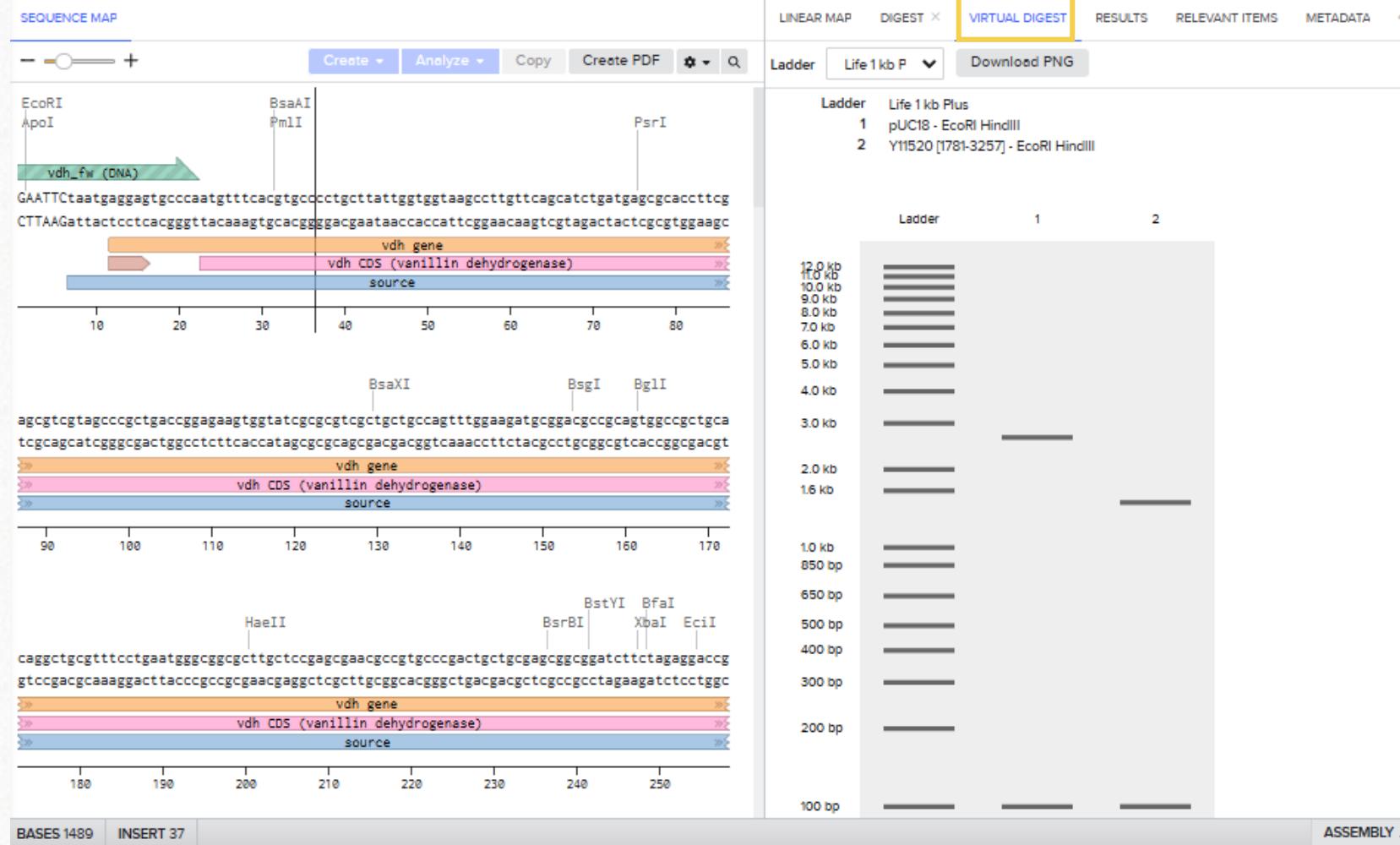
1485 1489 5 HindIII 5' None blunt

Save Use HF

Construct design

Virtual digestion

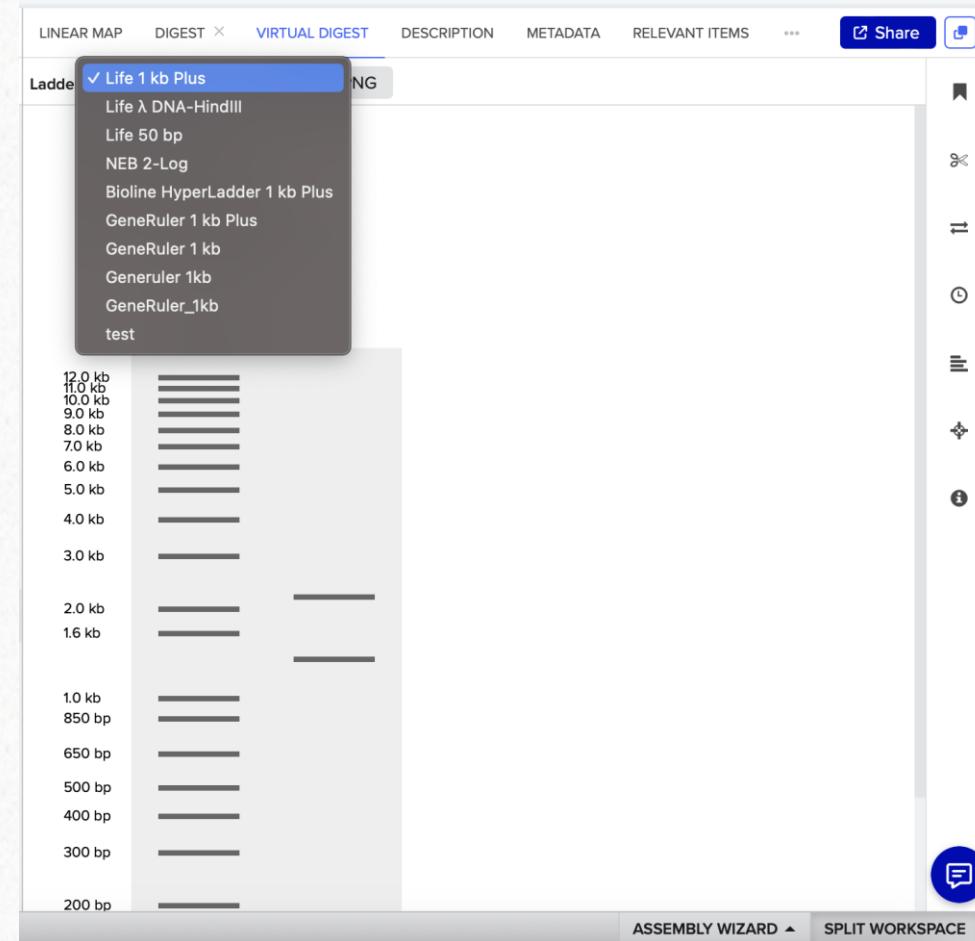
Gel visualization



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

Construct design

Virtual digestion



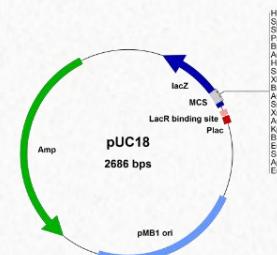
 **PRO TIP:** It's possible to choose between different ladders

6. Construct assembly



6. Construct assembly

6.1 Assembly Wizard



pUC18 plasmid vector

Plasmid backbone

+

vdh

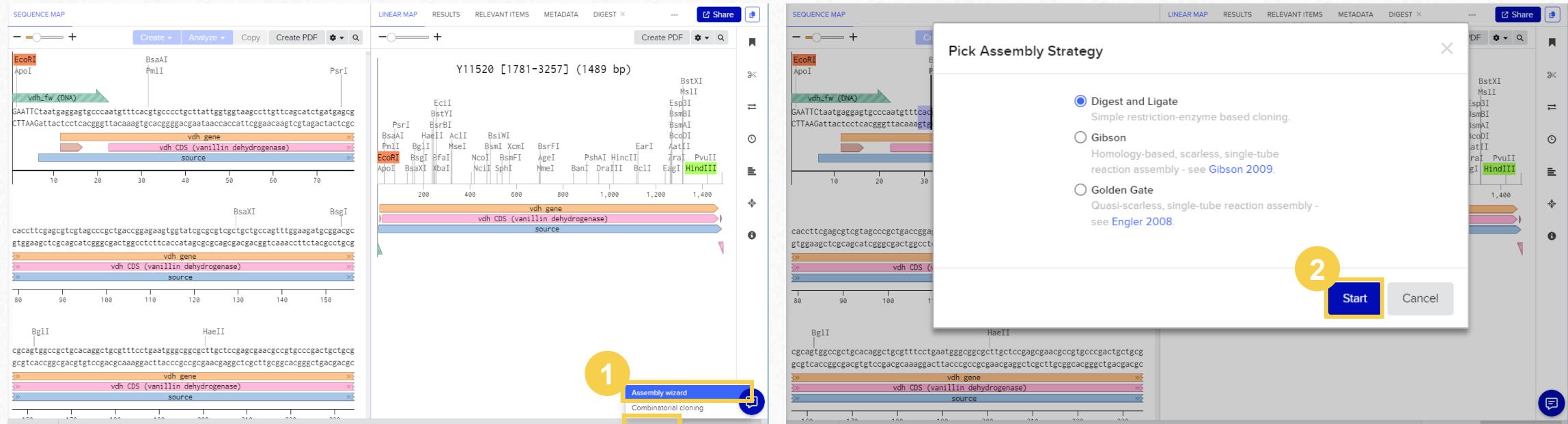
Gene of interest

Codon optimized PCR product of the originally imported DNA fragment



Construct Assembly

Assembly Wizard



The screenshot shows the Assembly Wizard interface with two sequence maps and a 'Pick Assembly Strategy' dialog.

Sequence Map 1: Shows restriction sites EcoRI, ApoI, BsaAI, PmlI, PsrI, BsaXI, BsgI, BglI, and HaeII. It includes a DNA sequence snippet and labels for 'vdh fw (DNA)', 'vdh gene', 'vdh CDS (vanillin dehydrogenase)', and 'source'.

Sequence Map 2: Shows restriction sites EcoRI, ApoI, BsaAI, BsrBI, PsrI, BsrYI, BstXI, MsI, EspBI, BsmBI, BsmAI, BcoDI, AatII, ZraI, PvuII, HindIII, BglI, BsgI, BfaI, NcoI, BsmFI, XcmI, BsrFI, AgeI, PshAI, HincII, EarI, BanI, DraIII, BclI, EagI, and HindIII. It includes a DNA sequence snippet and labels for 'vdh fw (DNA)', 'vdh gene', 'vdh CDS (vanillin dehydrogenase)', and 'source'.

Pick Assembly Strategy Dialog:

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

Buttons: 1. Assembly wizard (highlighted), 2. Start, Cancel.

Assembly Wizard allows you to use the following assembly strategies:

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

Construct Assembly

Digest and Ligate: Locate the Assembly Wizard work environment

The screenshot shows the Assembly Wizard interface for the pUC18 plasmid. The top left displays the plasmid map with various restriction sites and features labeled: AmpR promoter, AmpR, lacZα, MCS, M13 fwd, M13 rev, +2, lac promoter, and ori. The top right shows a digest table for Y11520 [1781-3257] with NEB enzymes (EcoRI, HindIII) and their conditions (Temp: 37°C, Time: 25 min). The bottom section is the 'SET FRAGMENT' workspace, which is highlighted with a yellow border. It contains tabs for 'Backbone' and 'Insert', with 'BASES 2686' and 'INSERT 724' respectively. To the right is the 'OVERALL ASSEMBLY' workspace, which is currently empty. A yellow arrow points from the text 'This will remain open even if you go from one file to another' to the bottom workspace area. Another yellow arrow points to the 'pUC18-vdh' input field in the assembly workspace, with the text 'Name your construct' next to it.

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
HindIII	1	37°C	25	100	50	50

Start End Length Left Cutter Left Overhang Right Cutter Right Overhang

400 450 51 HindIII 5' EcoRI 5'

451 399 2635 EcoRI 5' HindIII 5'

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

Name your construct

SET FRAGMENT

Select an assembly fragment below.

Backbone Insert

BASES 2686 INSERT 724

OVERALL ASSEMBLY

The backbone or an insert is unset.

pUC18-vdh

Assemble

+ Hide Prev

ASSEMBLY ▾ SPLIT WORKSPACE

Construct Assembly

Digest and Ligate: Add the backbone

PLASMID SEQUENCE MAP

1 Backbone Insert

2 Select the backbone

3 Set from Selection

4 ✓

RESULTS RELEVANT ITEMS METADATA DESCRIPTION DIGEST X Share

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
HindIII	1	37°C	25	100	50	50
	Start End Length	Left Cutter Right Cutter	Left Overhang Right Overhang			
	400 450 51	HindIII EcoRI	5' 5'			
	451 399 2635	EcoRI HindIII	5' 5'			

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

BASES 2686 START 451 END 399 LENGTH 2635 GC 50.44% MELTING TEMP 80.4 °C ASSEMBLY SPLIT WORKSPACE

AATTGCT GCA CCA GGTCGA

0 ERRORS AND 0 WARNINGS ✓ Looks like everything checks out Reverse Orientation Jump to Selection View Enzyme Activity

pUC18 2.6 kb · EcoRI, HindIII Insert

BASES 2686 START 451 END 399 LENGTH 2635 GC 50.44% MELTING TEMP 80.4 °C ASSEMBLY SPLIT WORKSPACE

Construct Assembly

Digest and Ligate: Add the insert

The screenshot shows the QFB Construct Assembly Wizard interface. The top half displays a sequence map of the plasmid Y11520 [1781-3257] (1489 bp). The map includes various restriction sites (EcoRI, BstXI, BsrBI, etc.) and a backbone construct consisting of pUC18 (2.6 kb) with EcoRI and HindIII sites, and an insert containing the vdh gene (vanillin dehydrogenase) and its CDS. The bottom half shows a preview of the resulting DNA sequence, which is a fragment of the pUC18 backbone flanked by EcoRI and HindIII sites, with the vdh gene insert in between.

RESULTS RELEVANT ITEMS METADATA DIGEST X VIRTUAL DIGEST ... Share

Digest Save NEB Use HF

Enzymes	Cuts	Temp.	11	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
HindIII	1	37°C	25	100	50	50

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	EcoRI	5'
2	1484	1483	EcoRI	5'	HindIII	5'
1485	1489	5	HindIII	5'	None	blunt

Select the backbone

PREVIEW

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

1 Insert

2.6 kb · EcoRI, HindIII

3 Set from Selection

Construct Assembly

Digest and Ligate: Check for compatibility

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

The screenshot illustrates the assembly process through two stages:

Stage 1 (Top):

- PREVIEW:** Shows the backbone (pUC18) and insert (Y11520 [1781-3257]) with their respective sizes (2.6 kb and 1.5 kb) and restriction enzymes (EcoRI, HindIII).
- Sequence View:** Displays the DNA sequences:
pUC18: CCA AATT Cta
Y11520: GGTCGA Gat
- Errors:** 2 ERRORS AND 0 WARNINGS
 - The left sticky end does not match.
 - The right sticky end does not match.
- Action Bar:** Includes a yellow circle labeled "1" over the "Reverse Orientation" button, which is highlighted with a yellow box.

Stage 2 (Bottom):

- PREVIEW:** Shows the same backbone and insert fragments.
- Sequence View:** Displays the DNA sequences after orientation adjustment:
pUC18: CCA AGCTT cg
Y11520: taGAA TCC Agc
- Errors:** 0 ERRORS AND 0 WARNINGS
 - ✓ Looks like everything checks out
- Action Bar:** Includes a yellow circle labeled "2" over the "Reverse Orientation" button, which is now checked (indicated by a blue box).

A large yellow arrow points from Stage 1 to Stage 2, indicating the progression of the assembly process.

- ✓ 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-vdh

Assemble (button highlighted with a yellow circle and number 1)

SEQUENCE MAP

LINEAR MAP (selected)

PLASMID

DESCRIPTION

METADATA

RELEVANT ITEMS

Share

Create PDF

+

Hide Pre

?

Linear Map View:

- pUC18**: 2.6 kb · EcoRI, HindIII
- Y11520 [1781-3257]**: 1.5 kb · HindIII, EcoRI

Sequence Map View:

- lacZα**: M13 fwd
- HindIII**: vdh CDS (vanillin dehydrogenase)
- BstXI**: vdh CDS (vanillin dehydrogenase)

Plasmid View:

pUC18-vdh (4118 bp)

Map features include: AmpR, AmpR_promoter, SspI, Eco0109I, PfoI, NdeI, KasI, NarI, SfoI, PluTI, MCS, LacZα, BstXI, EagI, BclI, HincII, DraIII, PshAI, AgeI, XcmI, BsmF1, +1, BsiWI, BsmI, NcoI, vdh CDS (vanillin dehydrogenase), vdh regulatory, MCS, lacZα, +7, PmII, PsfI, BsaAI, BsgI, XbaI, PsrI, BspQI, SphI, AflIII, PciI, BpuMI, NmeAIII, BpmI, BsaI, AhdI, ScaI, XmnI, 3900, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, 15000, 16000, 17000, 18000, 19000, 20000, 21000, 22000, 23000, 24000, 25000, 26000, 27000, 28000, 29000, 30000, 31000, 32000, 33000, 34000, 35000, 36000, 37000, 38000, 39000, 40000, 41000, 42000, 43000, 44000, 45000, 46000, 47000, 48000, 49000, 50000, 51000, 52000, 53000, 54000, 55000, 56000, 57000, 58000, 59000, 60000, 61000, 62000, 63000, 64000, 65000, 66000, 67000, 68000, 69000, 70000, 71000, 72000, 73000, 74000, 75000, 76000, 77000, 78000, 79000, 80000, 81000, 82000, 83000, 84000, 85000, 86000, 87000, 88000, 89000, 90000, 91000, 92000, 93000, 94000, 95000, 96000, 97000, 98000, 99000, 100000, 101000, 102000, 103000, 104000, 105000, 106000, 107000, 108000, 109000, 110000, 111000, 112000, 113000, 114000, 115000, 116000, 117000, 118000, 119000, 120000, 121000, 122000, 123000, 124000, 125000, 126000, 127000, 128000, 129000, 130000, 131000, 132000, 133000, 134000, 135000, 136000, 137000, 138000, 139000, 140000, 141000, 142000, 143000, 144000, 145000, 146000, 147000, 148000, 149000, 150000, 151000, 152000, 153000, 154000, 155000, 156000, 157000, 158000, 159000, 160000, 161000, 162000, 163000, 164000, 165000, 166000, 167000, 168000, 169000, 170000, 171000, 172000, 173000, 174000, 175000, 176000, 177000, 178000, 179000, 180000, 181000, 182000, 183000, 184000, 185000, 186000, 187000, 188000, 189000, 190000, 191000, 192000, 193000, 194000, 195000, 196000, 197000, 198000, 199000, 200000, 201000, 202000, 203000, 204000, 205000, 206000, 207000, 208000, 209000, 210000, 211000, 212000, 213000, 214000, 215000, 216000, 217000, 218000, 219000, 220000, 221000, 222000, 223000, 224000, 225000, 226000, 227000, 228000, 229000, 230000, 231000, 232000, 233000, 234000, 235000, 236000, 237000, 238000, 239000, 240000, 241000, 242000, 243000, 244000, 245000, 246000, 247000, 248000, 249000, 250000, 251000, 252000, 253000, 254000, 255000, 256000, 257000, 258000, 259000, 260000, 261000, 262000, 263000, 264000, 265000, 266000, 267000, 268000, 269000, 270000, 271000, 272000, 273000, 274000, 275000, 276000, 277000, 278000, 279000, 280000, 281000, 282000, 283000, 284000, 285000, 286000, 287000, 288000, 289000, 290000, 291000, 292000, 293000, 294000, 295000, 296000, 297000, 298000, 299000, 300000, 301000, 302000, 303000, 304000, 305000, 306000, 307000, 308000, 309000, 310000, 311000, 312000, 313000, 314000, 315000, 316000, 317000, 318000, 319000, 320000, 321000, 322000, 323000, 324000, 325000, 326000, 327000, 328000, 329000, 330000, 331000, 332000, 333000, 334000, 335000, 336000, 337000, 338000, 339000, 340000, 341000, 342000, 343000, 344000, 345000, 346000, 347000, 348000, 349000, 350000, 351000, 352000, 353000, 354000, 355000, 356000, 357000, 358000, 359000, 360000, 361000, 362000, 363000, 364000, 365000, 366000, 367000, 368000, 369000, 370000, 371000, 372000, 373000, 374000, 375000, 376000, 377000, 378000, 379000, 380000, 381000, 382000, 383000, 384000, 385000, 386000, 387000, 388000, 389000, 390000, 391000, 392000, 393000, 394000, 395000, 396000, 397000, 398000, 399000, 400000, 401000, 402000, 403000, 404000, 405000, 406000, 407000, 408000, 409000, 410000, 411000, 412000, 413000, 414000, 415000, 416000, 417000, 418000, 419000, 420000, 421000, 422000, 423000, 424000, 425000, 426000, 427000, 428000, 429000, 430000, 431000, 432000, 433000, 434000, 435000, 436000, 437000, 438000, 439000, 440000, 441000, 442000, 443000, 444000, 445000, 446000, 447000, 448000, 449000, 450000, 451000, 452000, 453000, 454000, 455000, 456000, 457000, 458000, 459000, 460000, 461000, 462000, 463000, 464000, 465000, 466000, 467000, 468000, 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594000, 595000, 596000, 597000, 598000, 599000, 600000, 601000, 602000, 603000, 604000, 605000, 606000, 607000, 608000, 609000, 610000, 611000, 612000, 613000, 614000, 615000, 616000, 617000, 618000, 619000, 620000, 621000, 622000, 623000, 624000, 625000, 626000, 627000, 628000, 629000, 630000, 631000, 632000, 633000, 634000, 635000, 636000, 637000, 638000, 639000, 640000, 641000, 642000, 643000, 644000, 645000, 646000, 647000, 648000, 649000, 650000, 651000, 652000, 653000, 654000, 655000, 656000, 657000, 658000, 659000, 660000, 661000, 662000, 663000, 664000, 665000, 666000, 667000, 668000, 669000, 670000, 671000, 672000, 673000, 674000, 675000, 676000, 677000, 678000, 679000, 680000, 681000, 682000, 683000, 684000, 685000, 686000, 687000, 688000, 689000, 690000, 691000, 692000, 693000, 694000, 695000, 696000, 697000, 698000, 699000, 700000, 701000, 702000, 703000, 704000, 705000, 706000, 707000, 708000, 709000, 710000, 711000, 712000, 713000, 714000, 715000, 716000, 717000, 718000, 719000, 720000, 721000, 722000, 723000, 724000, 725000, 726000, 727000, 728000, 729000, 730000, 731000, 732000, 733000, 734000, 735000, 736000, 737000, 738000, 739000, 740000, 741000, 742000, 743000, 744000, 745000, 746000, 747000, 748000, 749000, 750000, 751000, 752000, 753000, 754000, 755000, 756000, 757000, 758000, 759000, 760000, 761000, 762000, 763000, 764000, 765000, 766000, 767000, 768000, 769000, 770000, 771000, 772000, 773000, 774000, 775000, 776000, 777000, 778000, 779000, 780000, 781000, 782000, 783000, 784000, 785000, 786000, 787000, 788000, 789000, 790000, 791000, 792000, 793000, 794000, 795000, 796000, 797000, 798000, 799000, 800000, 801000, 802000, 803000, 804000, 805000, 806000, 807000, 808000, 809000, 810000, 811000, 812000, 813000, 814000, 815000, 816000, 817000, 818000, 819000, 820000, 821000, 822000, 823000, 824000, 825000, 826000, 827000, 828000, 829000, 830000, 831000, 832000, 833000, 834000, 835000, 836000, 837000, 838000, 839000, 840000, 841000, 842000, 843000, 844000, 845000, 846000, 847000, 848000, 849000, 850000, 851000, 852000, 853000, 854000, 855000, 856000, 857000, 858000, 859000, 860000, 861000, 862000, 863000, 864000, 865000, 866000, 867000, 868000, 869000, 870000, 871000, 872000, 873000, 874000, 875000, 876000, 877000, 878000, 879000, 880000, 881000, 882000, 883000, 884000, 885000, 886000, 887000, 888000, 889000, 890000, 891000, 892000, 893000, 894000, 895000, 896000, 897000, 898000, 899000, 900000, 901000, 902000, 903000, 904000, 905000, 906000, 907000, 908000, 909000, 910000, 911000, 912000, 913000, 914000, 915000, 916000, 917000, 918000, 919000, 920000, 921000, 922000, 923000, 924000, 925000, 926000, 927000, 928000, 929000, 930000, 931000, 932000, 933000, 934000, 935000, 936000, 937000, 938000, 939000, 940000, 941000, 942000, 943000, 944000, 945000, 946000, 947000, 948000, 949000, 950000, 951000, 952000, 953000, 954000, 955000, 956000, 957000, 958000, 959000, 960000, 961000, 962000, 963000, 964000, 965000, 966000, 967000, 968000, 969000, 970000, 971000, 972000, 973000, 974000, 975000, 976000, 977000, 978000, 979000, 980000, 981000, 982000, 983000, 984000, 985000, 986000, 987000, 988000, 989000, 990000, 991000, 992000, 993000, 994000, 995000, 996000, 997000, 998000, 999000, 1000000.

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

- ✓ The assembly is now done!

6. Construct assembly

6.2 Combinatorial Cloning



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 with various icons is visible. The main area is titled "Golden Gate assembly" under the "OVERVIEW" tab. It displays a "Bins & Spacers (3)" section where three fragments (BIN 1: Backbone, BIN 2: Promoter, BIN 3: Gene) are combined to form "Constructs" (24 constructs). Below this is a "Fragments" table listing 12 entries, each with a sequence ID, bin, start, end, length, orientation, type IIS enzyme (BsaI), and frag status. At the bottom, a "Constructs" table lists four constructs, each with a name, backbone, overhang, promoter, overhang, and gene. To the right, a grid of circular diagrams represents the constructs, each labeled with its name and showing the fragment composition and orientations. A "SPLIT WORKSPACE" button is located in the bottom right corner.

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

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

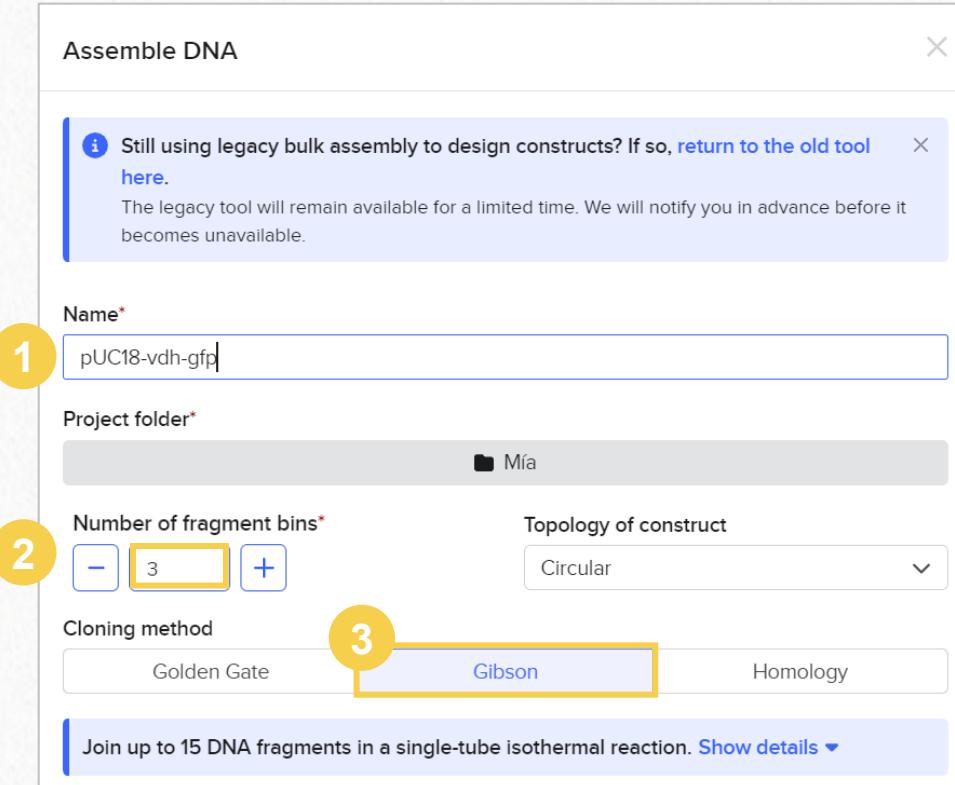
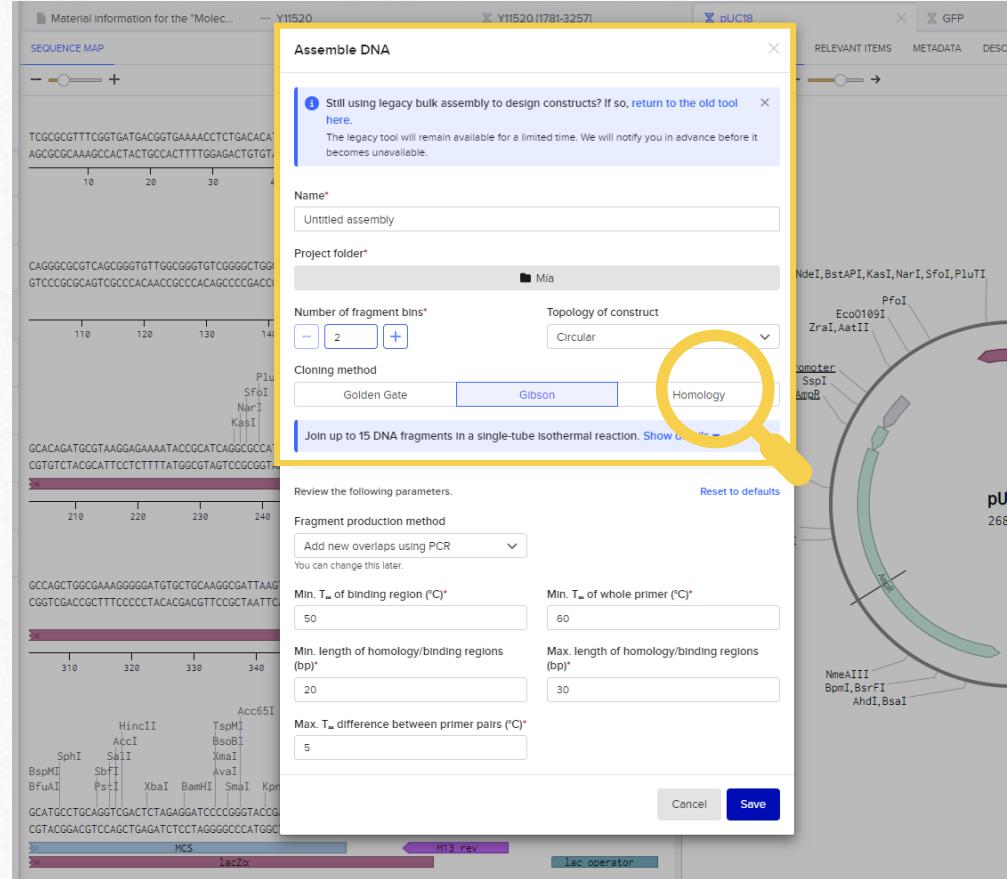
Construct Assembly

Combinatorial Cloning Tool: Opening



Construct Assembly

Combinatorial Cloning Tool: Configuration



Construct Assembly

Combinatorial Cloning Tool: Configuration

SEQUENCE MAP

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

Name*: Untitled assembly

Project folder*: M1a

Number of fragment bins*: 2

Topology of construct: Circular

Cloning method: Gibson

Join up to 15 DNA fragments in a single-tube isothermal reaction. [Show details](#)

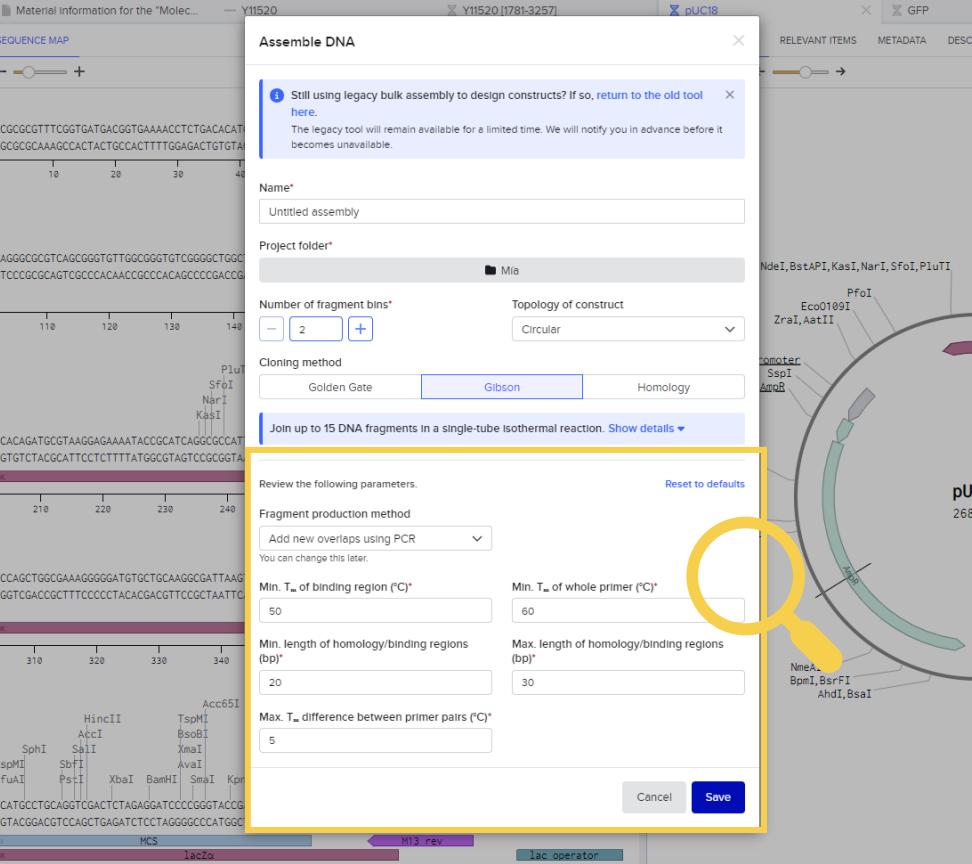
Review the following parameters.

Fragment production method: Add new overlaps using PCR

You can change this later.

Min. T_m of binding region (°C)* 50	Min. T_m of whole primer (°C)* 60
Min. length of homology/binding regions (bp)* 20	Max. length of homology/binding regions (bp)* 30
Max. T_m difference between primer pairs (°C)* 5	

Cancel Save



Review the following parameters.

Fragment production method

Add new overlaps using PCR

You can change this later.

Min. T_m of binding region (°C)* 50 **Min. T_m of whole primer (°C)*** 60

Min. length of homology/binding regions (bp)* 20 **Max. length of homology/binding regions (bp)*** 30

Max. T_m difference between primer pairs (°C)* 5

Cancel **Save** 4

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

Construct Assembly

Combinatorial Cloning Tool: Full view

The screenshot shows the Combinatorial Cloning Tool interface. At the top, there are tabs for METADATA, OVERVIEW (selected), and CONSTRUCTS. Below this, a panel for 'pUC18-vdh-gfp' shows a message about legacy bulk assembly. The main area is divided into three sections: 'Bins & Spacers (3)' containing 'BIN 1' (Backbone), 'BIN 2' (Insert 1), and 'BIN 3' (Insert 2), each with an 'Add new overlaps using PCR' button and a '0 fragments' count; an arrow points to a 'Constructs' section with '0 constructs'; 'Fragments' table showing one entry (ID 1) with sequence, bin, start, end, length, orientation, and enzymes; and a 'Constructs' table showing one entry (ID 1) with name, backbone, overlap length, and status.

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

Gibson cloning: Set fragments in corresponding bins

1. Backbone

The screenshot shows the 'Bins & Spacers' section with a list of items:

- BIN 1
- Backbone
- Add new over using PCR
- Open sequences (highlighted with a yellow circle)
- Search for sequences
- Add from worklist
- + (highlighted with a yellow circle)
- Y11520 [1781-3257] DNA sequence
- pUC18 DNA sequence (highlighted with a yellow box)
- GFP DNA sequence

1 Find and select the backbone file

3 Invert selection

The screenshot shows the 'Add fragment(s)' dialog for pUC18:

- Start: 398 (highlighted with a yellow circle)
- End: 456
- Orientation: Reverse (highlighted with a yellow box)
- Preferred 5' primer: Search by name
- Preferred 3' primer: Search by name
- View: Plasmid map

A circular plasmid map is shown with various restriction sites and markers. A blue arrow points to the Multiple Cloning Site (MCS) labeled 'M13 fwd MCS'. The text '2 Select the MCS (blue)' is overlaid on the map.

4 Reverse orientation

Cancel Add (highlighted with a yellow circle)

PRO TIP:

Open the files containing the fragments you want to work with beforehand to have quick access.

Construct Assembly

Gibson cloning: Set fragments in corresponding bins

2. Insert 1 (*vdh*)

Bins & Spacers (3) +

BIN 1	Backbone
Add new overlaps using PCR	1 fragment +

BIN 2	Insert 1
Add new overlaps using PCR	0 fragment +

BIN 3	Insert 2
Add new overlaps using PCR	0 fragments +

Open sequences > Y11520 [1781-3257] DNA sequence

Search for sequences

pUC18 DNA sequence

GFP DNA sequence

Fragments

Sequence	Bin	Start	End	Length
----------	-----	-------	-----	--------

- 1 Find and select the *vdh* file

Add fragment(s)

View: Linear map

Y11520 [1781-3257]

Start: 23 End: 1468 Orientation: Forward

Preferred 5' primer Preferred 3' primer

Search by name Search by name

(1489 bp)

vdh gene

vdh CDS (vanillin dehydrogenase)

source

1.4 kb of 1.5 kb

2 Select the *vdh* coding sequence

3 Add

Construct Assembly

Gibson cloning: Set fragments in corresponding bins

3. Insert 2 (gfp)

1 Find and select the *gfp* file

2 Select the entire sequence

3 Add

Construct Assembly

Gibson cloning: Populate the “constructs” table

- ✓ After placing all fragments in the bins, they will be visible on the **Fragments** table.
- ✓ You can verify if everything is correct before proceeding
- ✓ Afterwards, you need to click the **Autopopulate** option in the **Constructs** table.

The screenshot shows two tables in a software interface:

Fragments Table:

	Sequence	Bin	Start	End	Length	Orientation	5' enzyme	3' enzyme	Fragment production method	Preferred 5' primer	Preferred 3' primer	Stat
1	pUC18	Backbone	398	456	2629	Reverse	None selected	None selected	Add new overlaps using PCR			L
2	Y11520[1781-3257]	Insert 1	23	1468	1446	Forward	None selected	None selected	Add new overlaps using PCR			L
3	GFP	Insert 2	1	717	717	Forward	None selected	None selected	Add new overlaps using PCR			L

Constructs Table:

	Name	Backbone	Overlap length	Insert 1	Overlap length	Insert 2	Overlap length	Status
1		▼		▼		▼		

A callout box points to the **Autopopulate** button in the Constructs table toolbar, which is highlighted with a yellow arrow. The button has the text: "Create constructs involving all possible combinations of fragments."

Construct Assembly

Gibson cloning: Finalizing the assembly

The screenshot shows the 'OVERVIEW' tab of the Construct Assembly tool. At the top right, there is a yellow box around the 'Assemble' button, with a yellow arrow pointing to it from the right.

Bins & Spacers (3) +

- BIN 1**: Backbone
 - Add new overlaps using PCR
 - 1 fragment +
- BIN 2**: Insert 1
 - Add new overlaps using PCR
 - 1 fragment +
- BIN 3**: Insert 2
 - Add new overlaps using PCR
 - 1 fragment +

→ Constructs
1 construct

Fragments

	Sequence	Bin	Start	End	Length	Orientation	5' enzyme	3' enzyme	Fragment production method	Preferred 5' primer	Preferred 3' primer	Stat
1	pUC18	Backbone	398	456	2629	Reverse	None selected	None selected	Add new overlaps using PCR			L
2	Y11520[1781-3257]	Insert 1	23	1468	1446	Forward	None selected	None selected	Add new overlaps using PCR			L
3	GFP	Insert 2	1	717	717	Forward	None selected	None selected	Add new overlaps using PCR			L

Constructs

Name	Backbone	Overlap length	Insert 1	Overlap length	Insert 2	Overlap length	Status
pUC18-Y11520 [1781-3257]-GFP	pUC18	40 bp	Y11520 [1781-3257]	40 bp	GFP	40 bp	Ready to assemble

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

Construct Assembly

Gibson cloning: Save the construct and related files

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

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

Buttons at the bottom of each step are 'Cancel', 'Next' (highlighted in yellow in steps 1 and 2), 'Back' (disabled in step 3), and 'Assemble' (highlighted in yellow in step 3).

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

Gibson cloning: Results

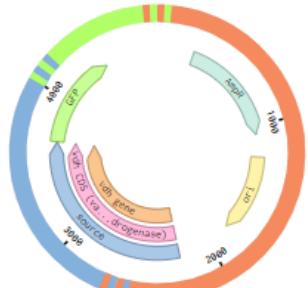
- ✓ After you finalize the assembly, you can move over to the “Constructs” tab to see the resulting constructs.
- ✓ You can view the primer information summarized in a table.

1  **CONSTRUCTS**

2  **6 associated primers [View](#)**

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

pUC18-Y11520 [1781-3257]-GFP



4600 3600 2600 1600 600

4600 GFP
3600 vdh gene (source)
2600 vanillanil dehydrogenase
1600 AmpR
600 ori

Primer view

View constructs

SEQUENCE PRIMERS						
Fragment	Orientation	Action	Primer	Bases	T _m whole (°C)	Status
1 pUC18	5' primer	Design new primer	pUC18_forward	TGGATGAA... 40 bp	66.19	OK
2 pUC18	3' primer	Design new primer	pUC18_reverse	ogcogggg... 44 bp	69.26	OK
3 Y11520[1781-3257]	5' primer	Design new primer	Y11520[1781-3257]_forward	ACAGCTAT... 40 bp	68.50	OK
4 Y11520[1781-3257]	3' primer	Design new primer	Y11520[1781-3257]_reverse	AGTTCTTC... 40 bp	64.70	OK
5 GFP	5' primer	Design new primer	GFP_forward	cgcggcac... 44 bp	64.97	OK
6 GFP	3' primer	Design new primer	GFP_reverse	GTAAAACG... 44 bp	68.32	OK

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

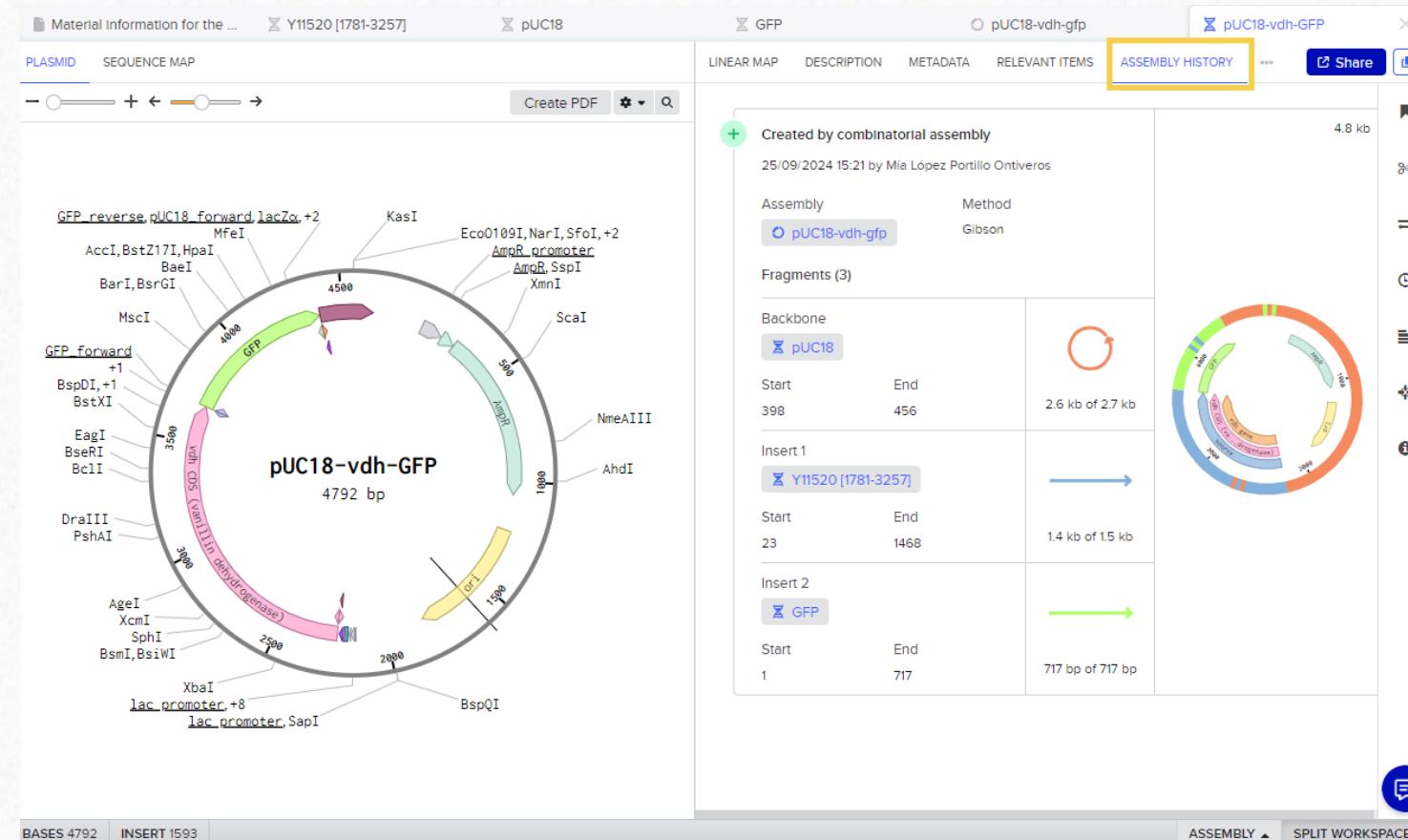
Sequence view



Construct Assembly

Gibson cloning: 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. Sequence alignment



7. Sequence alignment

7.1 Alignment tool



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:

1

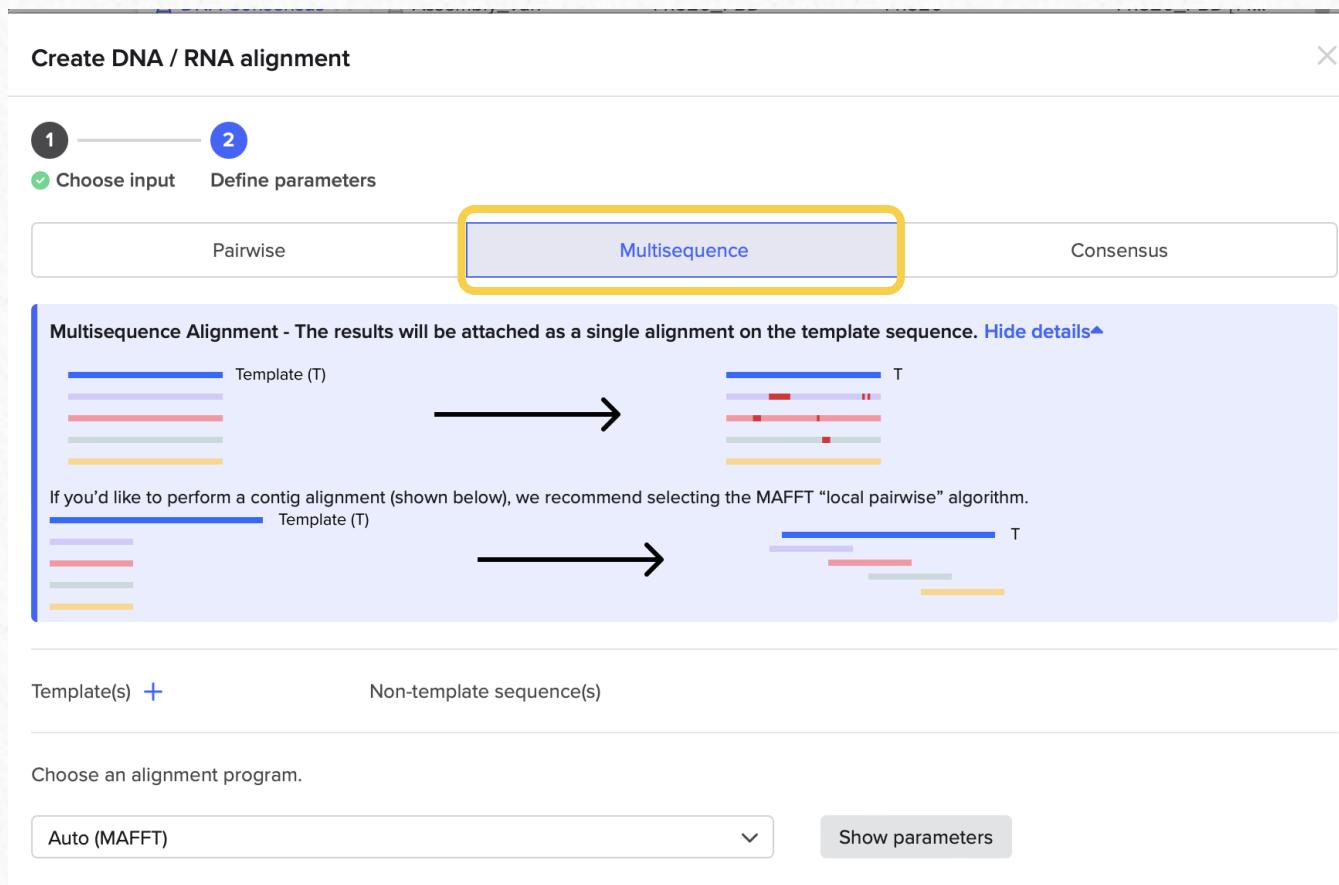
Pairwise alignment:

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:



The screenshot shows the 'Create DNA / RNA alignment' interface in Benchling. At the top, there are two steps: 'Choose input' (marked with a green checkmark) and 'Define parameters'. Below these are three tabs: 'Pairwise', 'Multisequence' (which is highlighted with a yellow box), and 'Consensus'. A large central panel titled 'Multisequence Alignment' contains two diagrams. The first diagram shows a 'Template (T)' at the top with four other colored lines (purple, red, grey, yellow) below it. An arrow points to the right, where the same four lines are shown aligned against the template, with some red and grey segments having small black dashes above them. The second diagram shows the same setup, but the template is now at the bottom, and the four lines are aligned against it, with the template having a 'T' at its end. Below these diagrams, text says: 'If you'd like to perform a contig alignment (shown below), we recommend selecting the MAFFT "local pairwise" algorithm.' At the bottom of the panel, there are sections for 'Template(s)' and 'Non-template sequence(s)', and a note to 'Choose an alignment program' with a dropdown menu set to 'Auto (MAFFT)'.

2

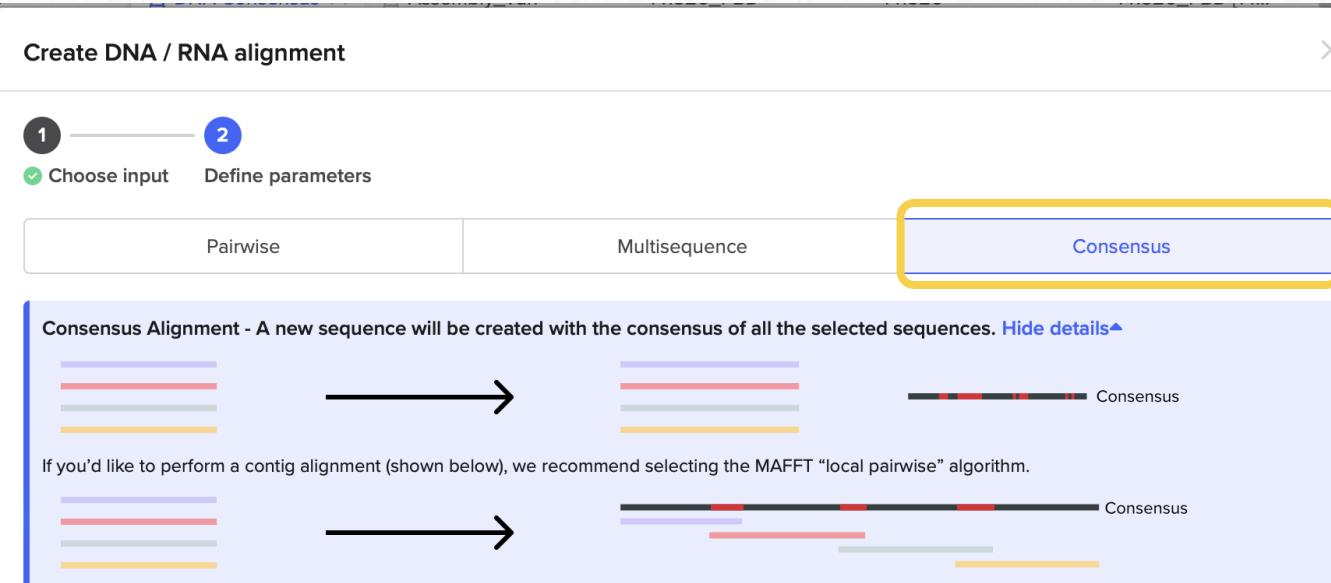
Multisequence alignment:

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

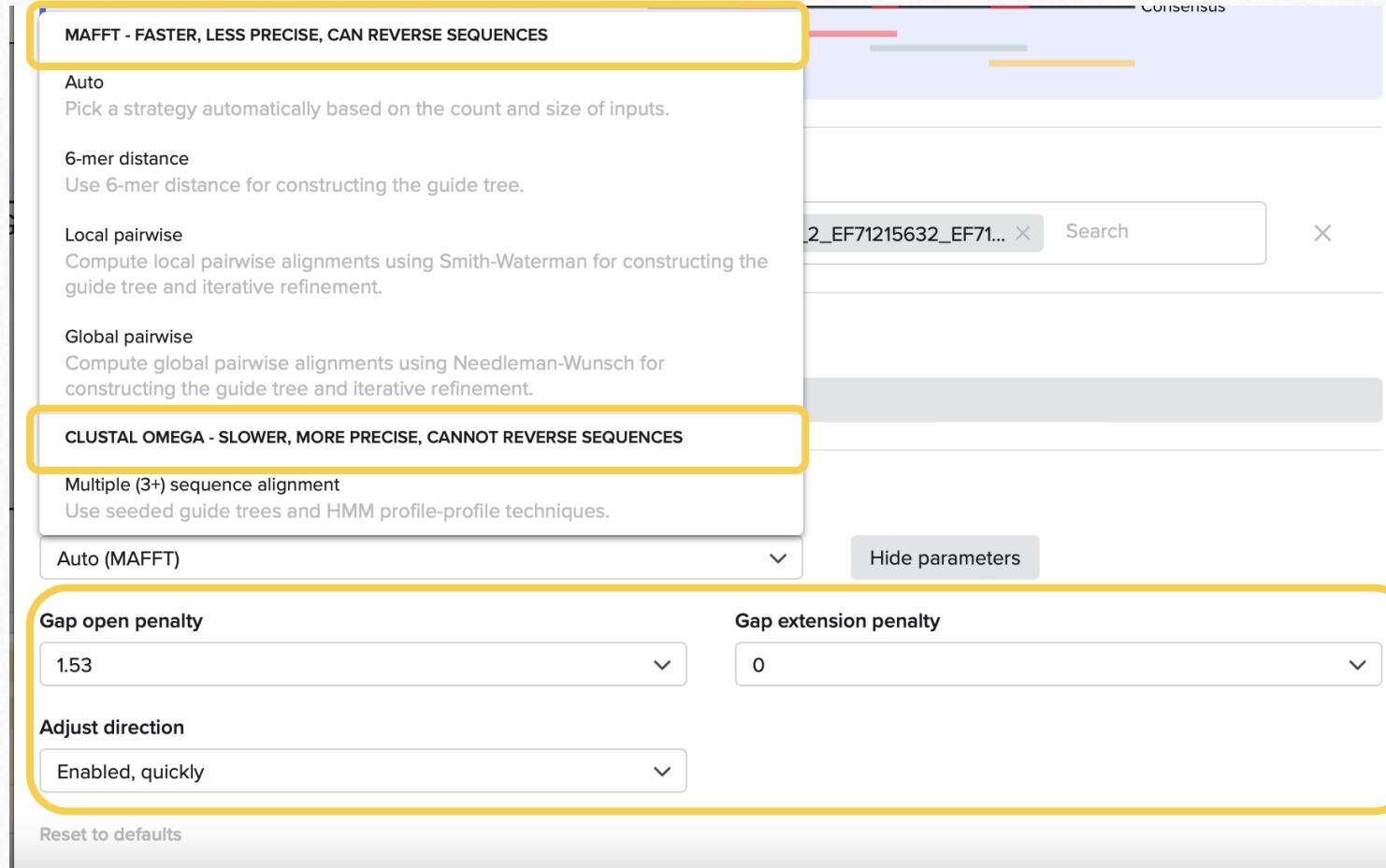
Consensus alignment:

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

Alignment creation

Alignment tool overview

- 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:



The screenshot shows the Alignment tool interface in Benchling. On the left, a sidebar lists alignment options: MAFFT (Auto, 6-mer distance, Local pairwise, Global pairwise) and Clustal Omega (Multiple sequence alignment). The MAFFT section is highlighted with a yellow box. On the right, a sequence viewer displays a consensus sequence with two input sequences: 2_EF71215632_EF71... and another sequence partially visible. Below the viewer, a search bar contains the text "Search". At the bottom, there are parameters for gap penalties: "Gap open penalty" set to 1.53, "Gap extension penalty" set to 0, and an "Adjust direction" dropdown set to "Enabled, quickly". A "Hide parameters" button is also present.

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

7. Sequence alignment

7.2 Consensus alignment



Alignment creation

Hypothetical example – Consensus alignment creation

For this training **consensus alignment** will be practiced: Instead of using the newly created sequences, for this example sample alignment files can be found in the ELN, download them.

The screenshot shows the QRB ELN interface with a sidebar on the left containing various project management and sequence analysis icons. A yellow circle with the number '1' highlights the 'Create a new alignment' button in the top right corner of the main workspace.

The main workspace displays a 'Create DNA / RNA alignment' dialog. A yellow circle with the number '2' highlights the 'Drag or select the sequences files' instruction above a 'Choose files' button. To the left of the dialog, a sequence map is shown with several DNA/RNA strands labeled with their bases (A, T, C, G) and positions (e.g., 10, 20, 100, 110, 190, 200). Below the map, a search bar says 'Search for a DNA / RNA sequence.' and a 'Search by name' input field.

A yellow circle with the number '3' highlights the 'Next' button at the bottom right of the dialog. The right side of the interface shows a vertical panel with tabs like 'Share', 'Re-open [?]', 'Self ΔG (kcal)', and a 'Value' section with a blue progress bar.

Alignment creation

Hypothetical example – Consensus alignment creation

4 Choose the consensus alignment and the MAFFT auto algorithm

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. [Show details](#)

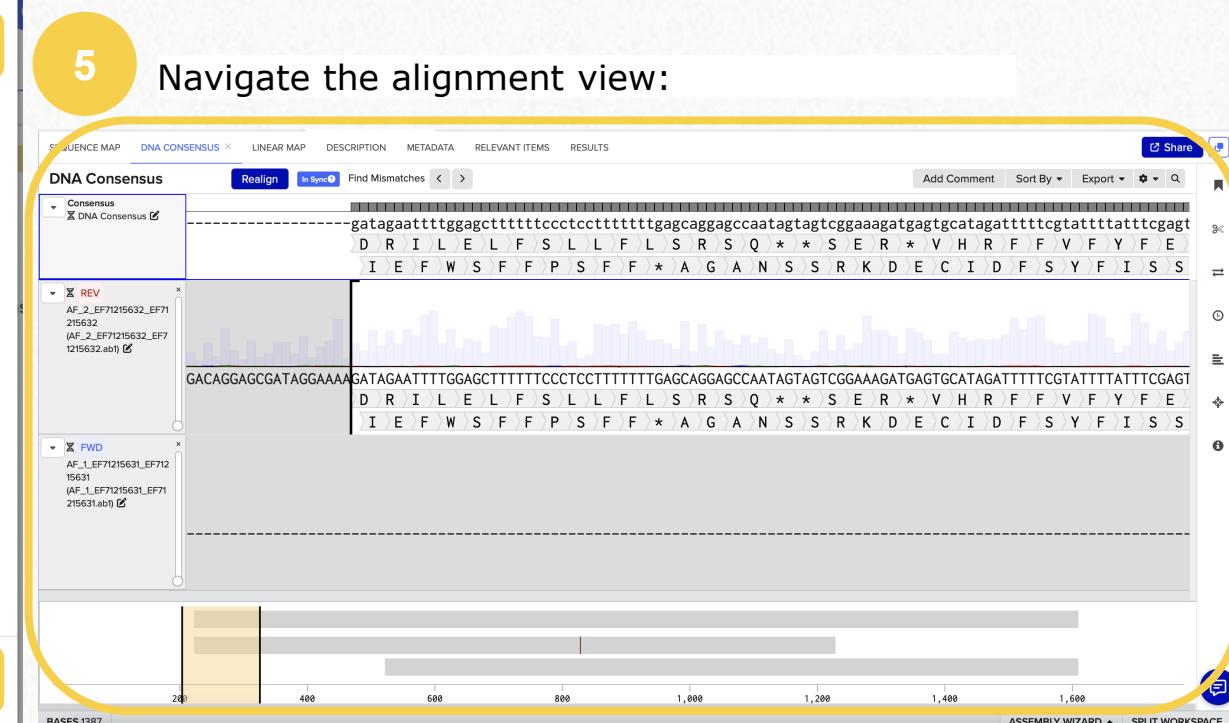
Group(s) +
Concensus_training AF_1_EF71215631_EF71... AF_2_EF71215632_EF71... Search

Select destination folder.
Patricia

Choose an alignment program.
Auto (MAFFT) Show parameters

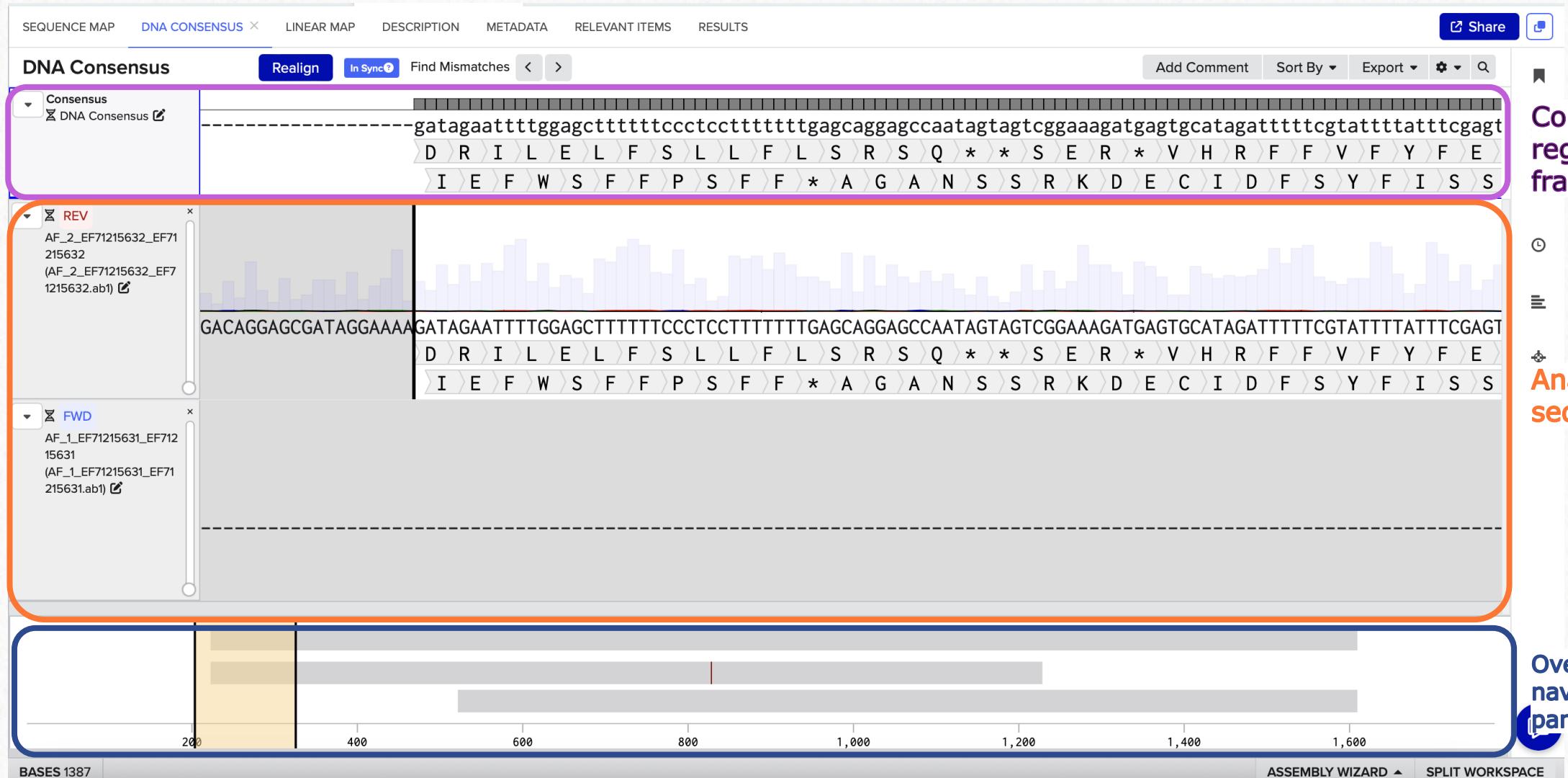
Alignments performed via MAFFT v7 (Katoh, Standley 2013).

Back **Create Alignment**



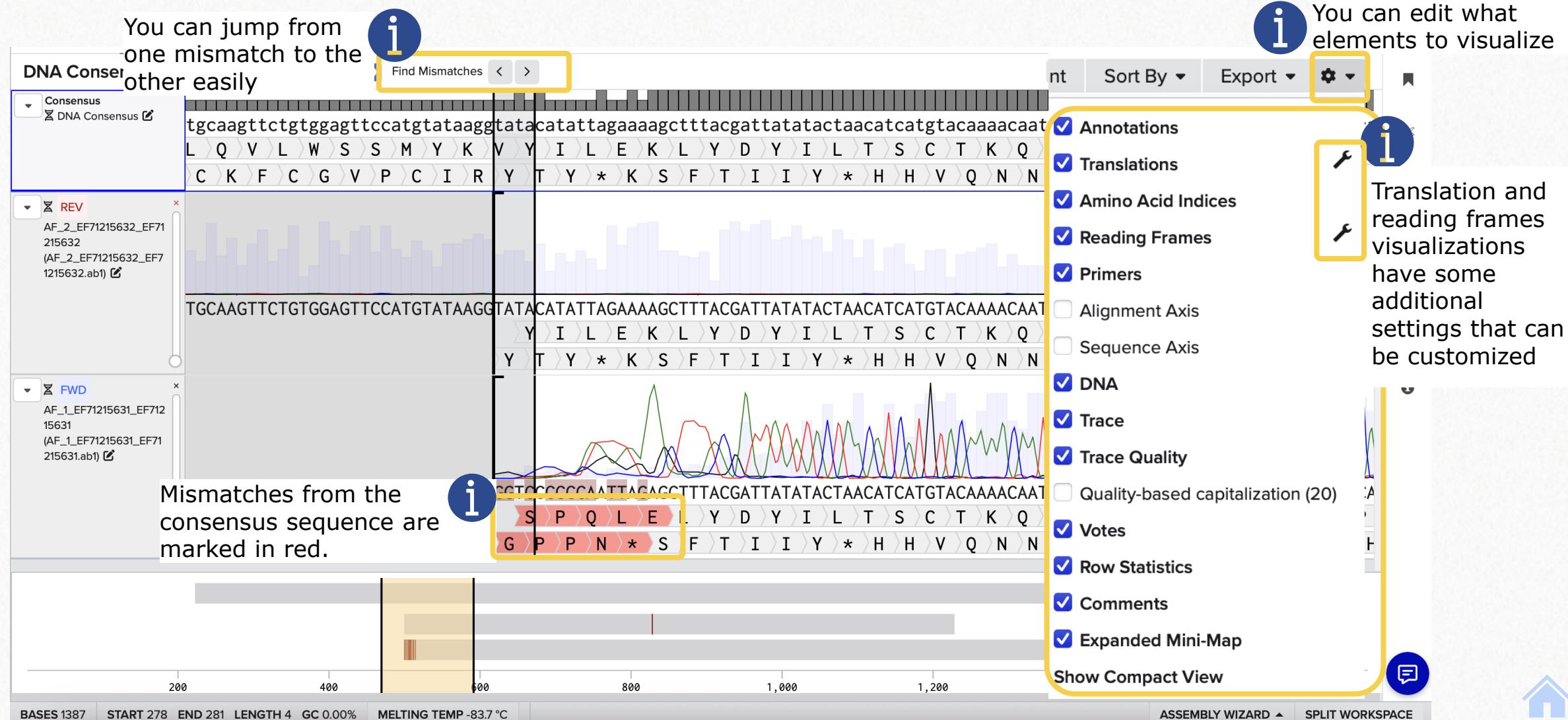
Alignment creation

Consensus alignment navigation



Alignment creation

Consensus alignment navigation



8. CRISPR



Tool overview

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



The image shows the Benchling software interface with two main sections highlighted by yellow circles and arrows.

Left Panel (1): A sidebar menu on the left with various options like Project, Entry, Protocol, etc. The "CRISPR" option is highlighted with a yellow box and a yellow circle containing the number 1.

Right Panel (2): The main workspace showing a circular "pBR322_plasmid" map with various restriction sites and gene regions labeled. A yellow box highlights the "CRISPR" section of the workspace, which contains buttons for "Design and analyze guides" and "Design HR Template (ssODN)". A yellow circle containing the number 2 points to this section.

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

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

- 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

EVANT ITEMS **RESULTS** **Share** **Help**

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.

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



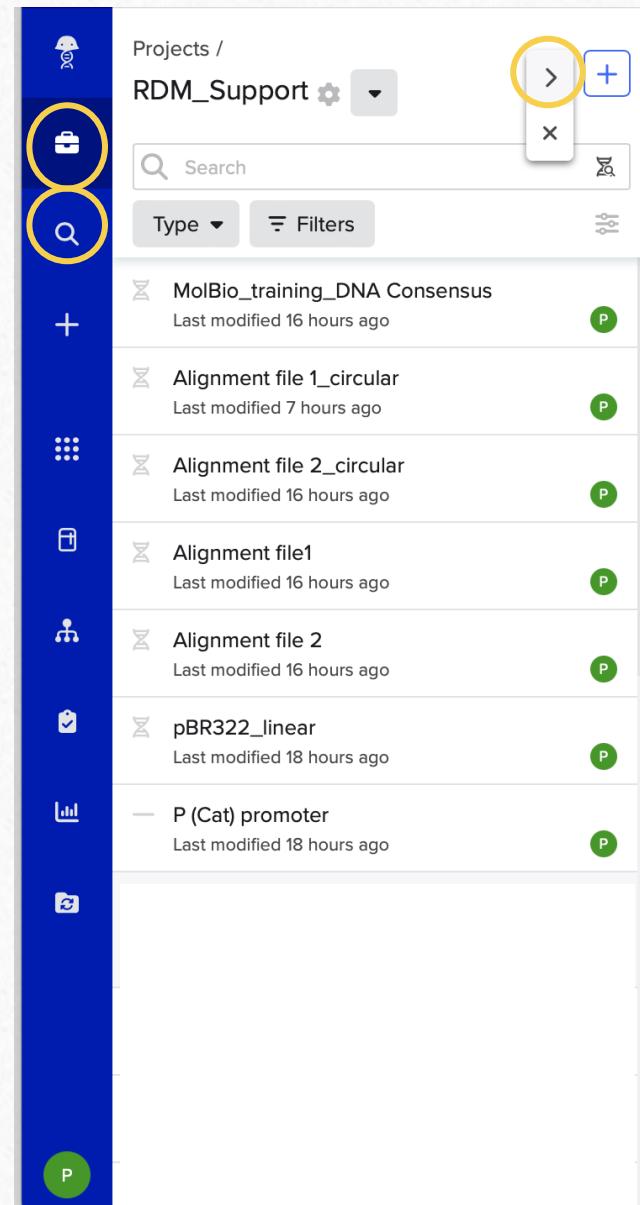
Tips and tricks

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

> + X

Search

Type Filters

MolBio_training_DNA Consensus
Last modified 16 hours ago

Alignment file 1_circular
Last modified 7 hours ago

Alignment file 2_circular
Last modified 16 hours ago

Alignment file1
Last modified 16 hours ago

Alignment file 2
Last modified 16 hours ago

pBR322_linear
Last modified 18 hours ago

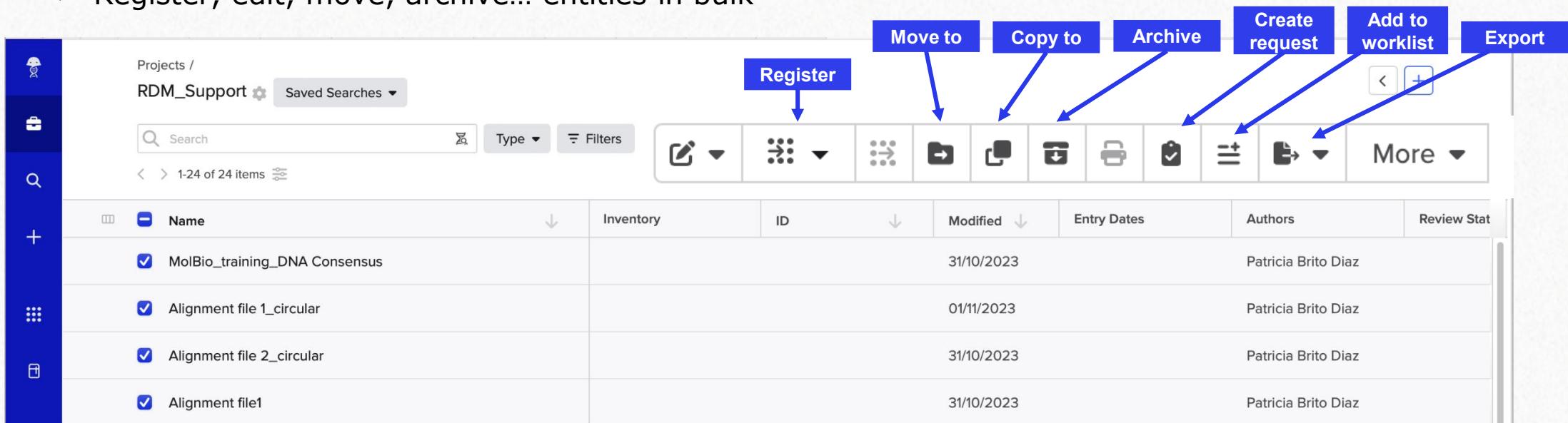
P (Cat) promoter
Last modified 18 hours ago

Tips and tricks

Work in bulk using the expanded view

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

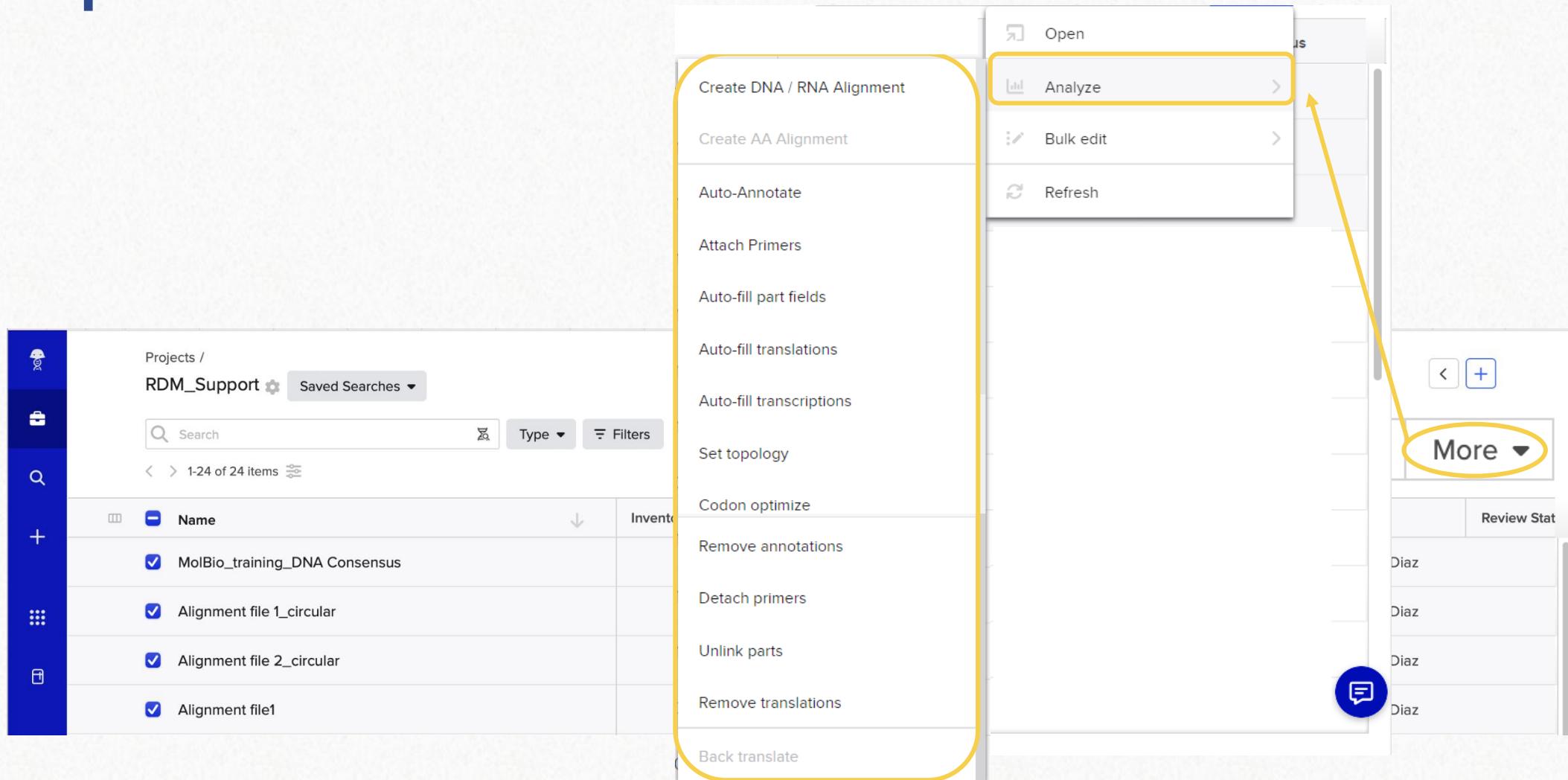
- ✓ Register, edit, move, archive... entities in bulk



The screenshot shows a workspace interface with a sidebar on the left containing icons for Projects, RDM_Support, Saved Searches, Search, Type, Filters, and a list of 1-24 of 24 items. The main area displays a table with columns: Name, Inventory, ID, Modified, Entry Dates, Authors, and Review Stat. Five items are listed, all with checkboxes checked. 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 for managing biological projects. On the left, a sidebar provides navigation icons. The main area displays a list of items under the project 'RDM_Support'. A context menu is open over one of the items, listing various actions such as 'Create DNA / RNA Alignment', 'Analyze' (which is highlighted with a yellow box), 'Bulk edit', 'Refresh', and others. To the right of the menu, a 'More' dropdown menu is also open, containing options like 'Review Stat', 'Diaz', and a message icon.

Projects / RDM_Support Saved Searches ▾

Search Type ▾ Filters

< > 1-24 of 24 items

Name	Inventor
MolBio_training_DNA Consensus	
Alignment file 1_circular	
Alignment file 2_circular	
Alignment file1	

More ▾

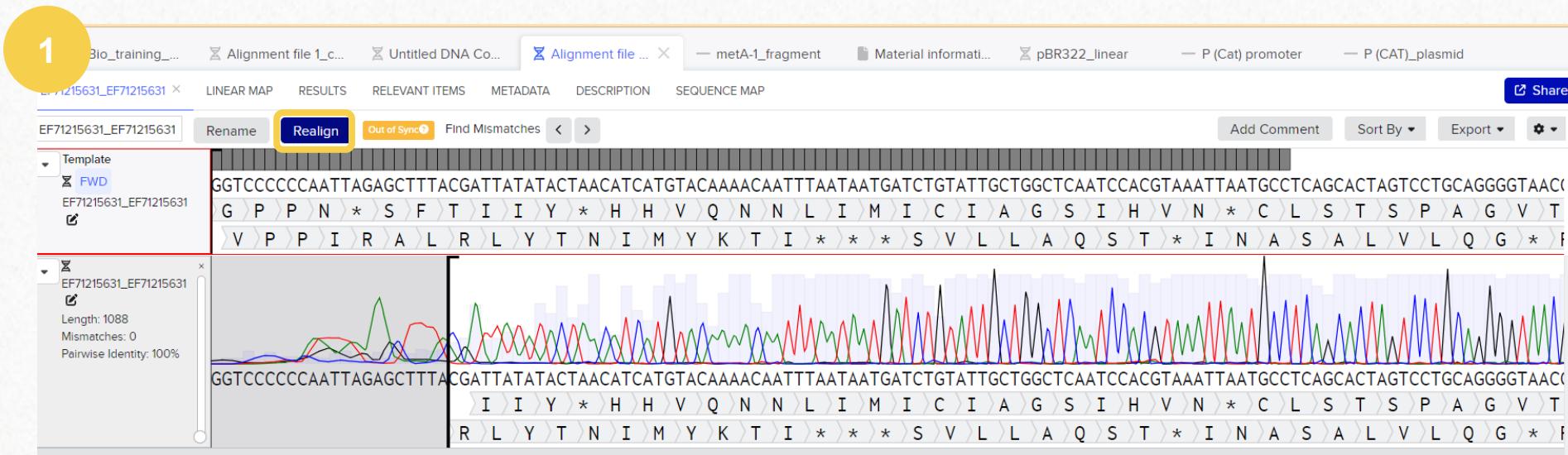
- Open
- Analyze
- Bulk edit
- Refresh
- Create DNA / RNA Alignment
- Create AA Alignment
- Auto-Annotate
- Attach Primers
- Auto-fill part fields
- Auto-fill translations
- Auto-fill transcriptions
- Set topology
- Codon optimize
- Remove annotations
- Detach primers
- Unlink parts
- Remove translations
- Back translate

Tips and tricks

Autoindexing when creating alignments

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

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



Tips and tricks

Autoindexing when creating alignments

2 Realign DNA / RNA

1 Choose input 2 Define parameters

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

Drag and drop to upload or Choose files

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA RNA

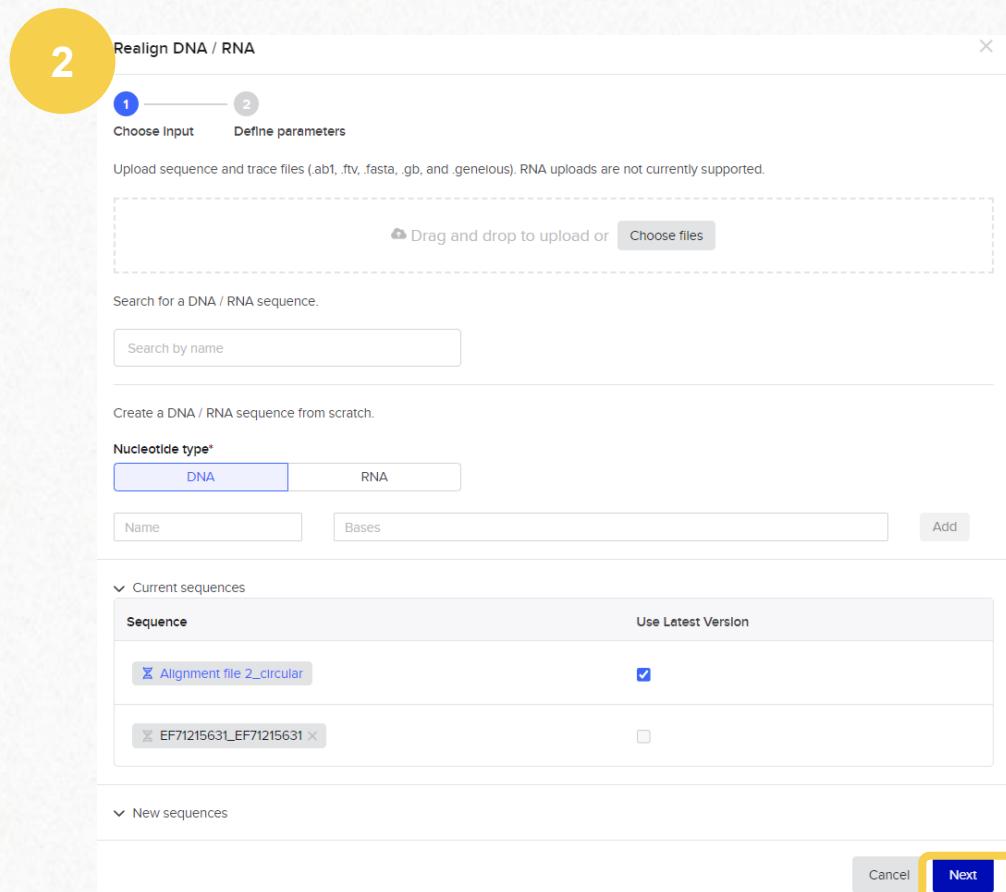
Name Bases Add

Current sequences

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

New sequences

Cancel Next



3 Realign DNA / RNA

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

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

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

Template(s) Non-template sequence(s)

Alignment file 2_circular EF71215631_EF71215631 Search

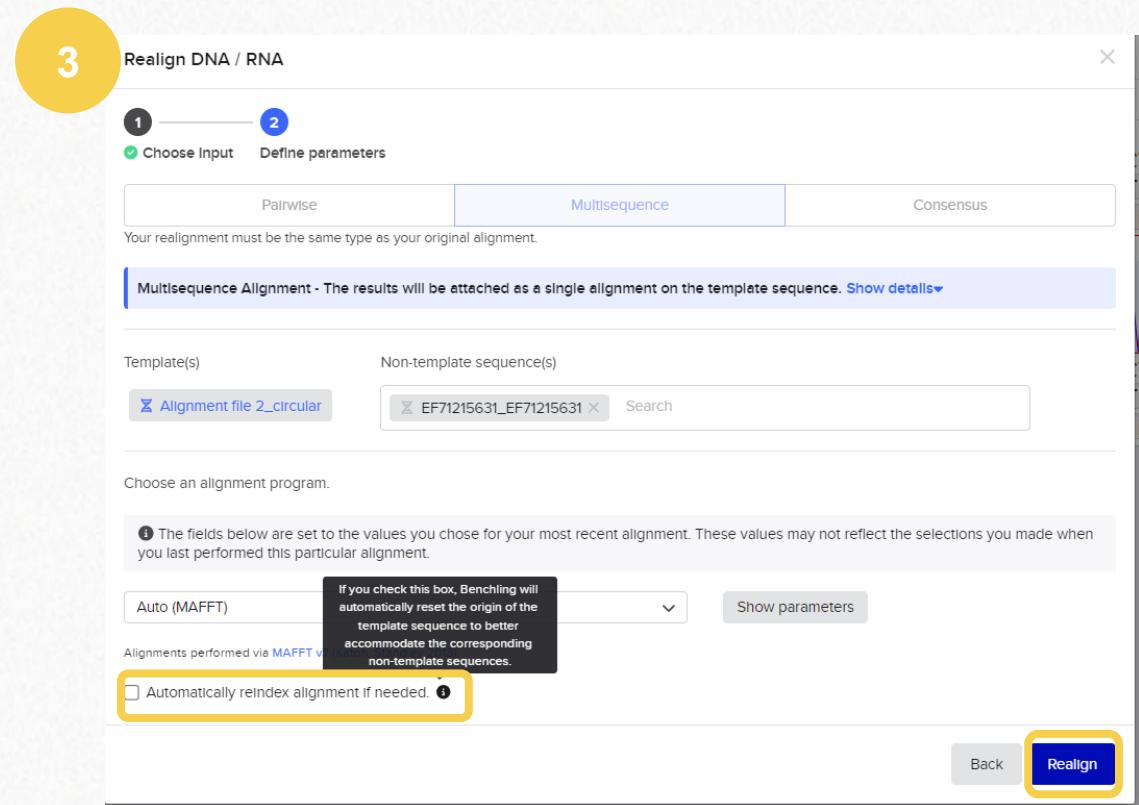
Choose an alignment program.

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

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

Automatically reindex alignment if needed.

Back Realign



Tips and tricks

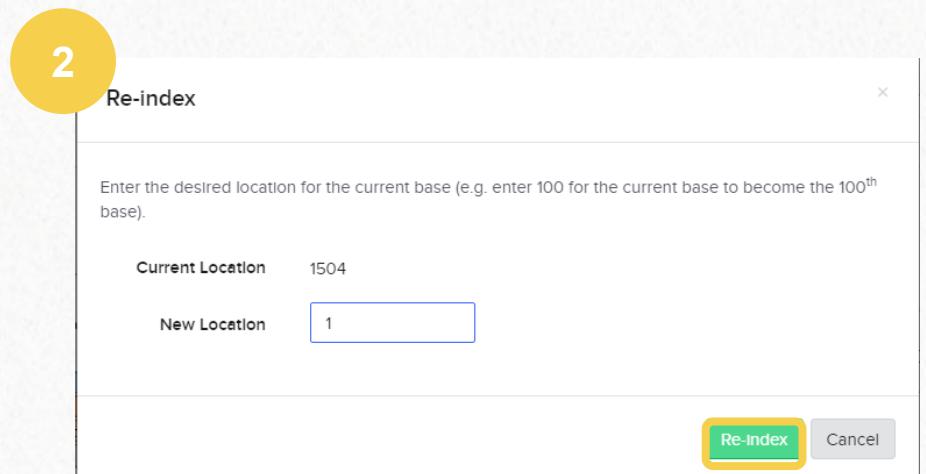
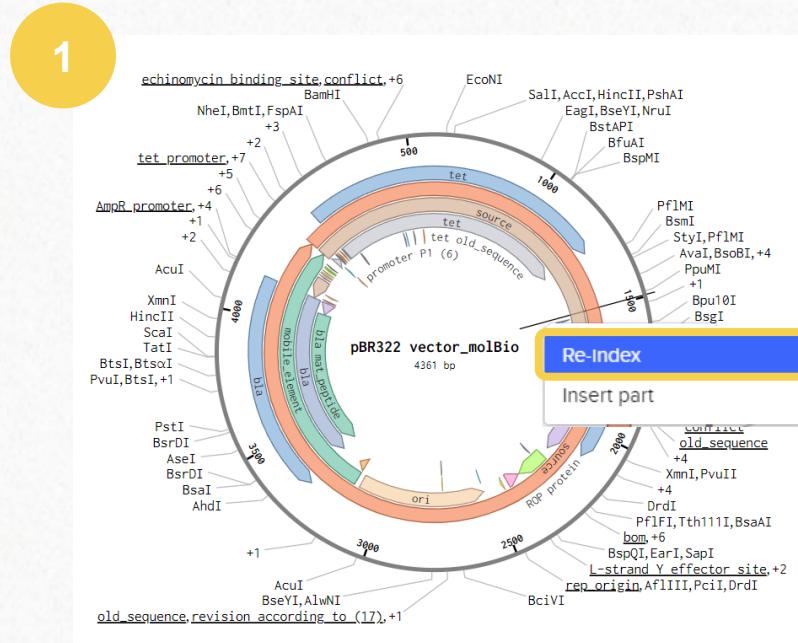
Autoindexing when creating alignments

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

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

Pro TIP:

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



10. Resources





Questions?



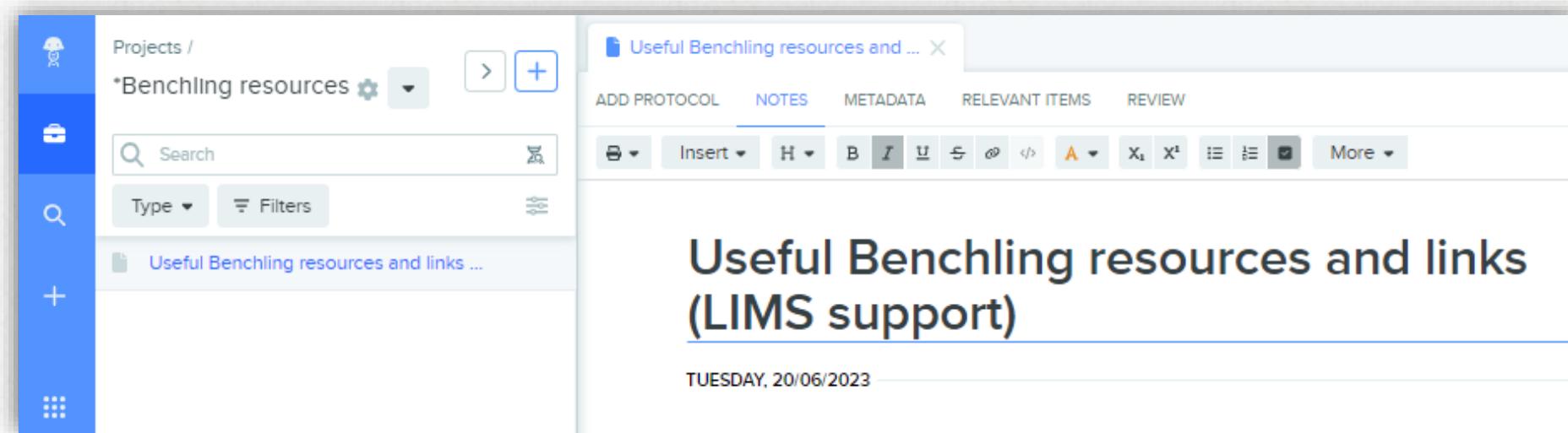
Contact lims_support@biosustain.dtu.dk



More resources

In-house resources (specific to Benchling at Biosustain)

To access them, visit the following **Notebook Entry**:



The screenshot shows a Benchling interface. On the left, there's a sidebar with icons for Projects, Search, Type, Filters, and a plus sign. The main area shows a notebook entry titled "Useful Benchling resources and ...". The entry has tabs for ADD PROTOCOL, NOTES (which is selected), METADATA, RELEVANT ITEMS, and REVIEW. Below the tabs is a toolbar with various icons. The main content area contains the text "Useful Benchling resources and links (LIMS support)" in bold, underlined black font. At the bottom, it says "TUESDAY, 20/06/2023".

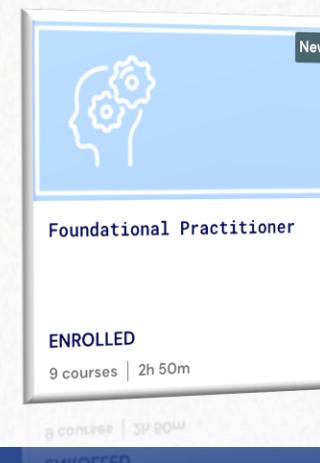
<https://biosustain.benchling.com/s/etr-axmk5ZpmZmOs4wqyjFy5?m=slm-Sh7pjB68HkjMnnXkvVhS>

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>

