

Barcode

Name

4C012

4C EE&SB fridge transient storage

4C002

4C Fridge 00271

4C009

4C Fridge 01223

4C001

4C Fridge 01233

4C014

4C Fridge 01871

4C015

4C Fridge Aaron

4C016

4C Fridge Adam

4C005

4C Fridge ANALYTICS

4C011

4C Fridge CFB00266

CFB01478

4C Fridge CFB01478

CFB01653

4C Fridge CFB01653

4C003

4C Fridge DSP1

The basics of the Molecular Biology Tool

Reach out when struggling with the platform:

Biosustain Benchling support
lims_support@biosustain.dtu.dk

**Access Benchling:**biosustain.benchling.com

(login with DTU credentials)

Additional resources:[LIMS Help guides](#)[Benchling Help Center: Molecular Biology](#)



Hands-on Benchling support

Where: Meeting Room 3rd floor (based on availability)

When: Tuesdays 13-15



Research Data Management Team

DROP-IN HOURS

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



TUESDAYS
13:00 - 15:00
3rd FLOOR

Image by Joomp on Freepik

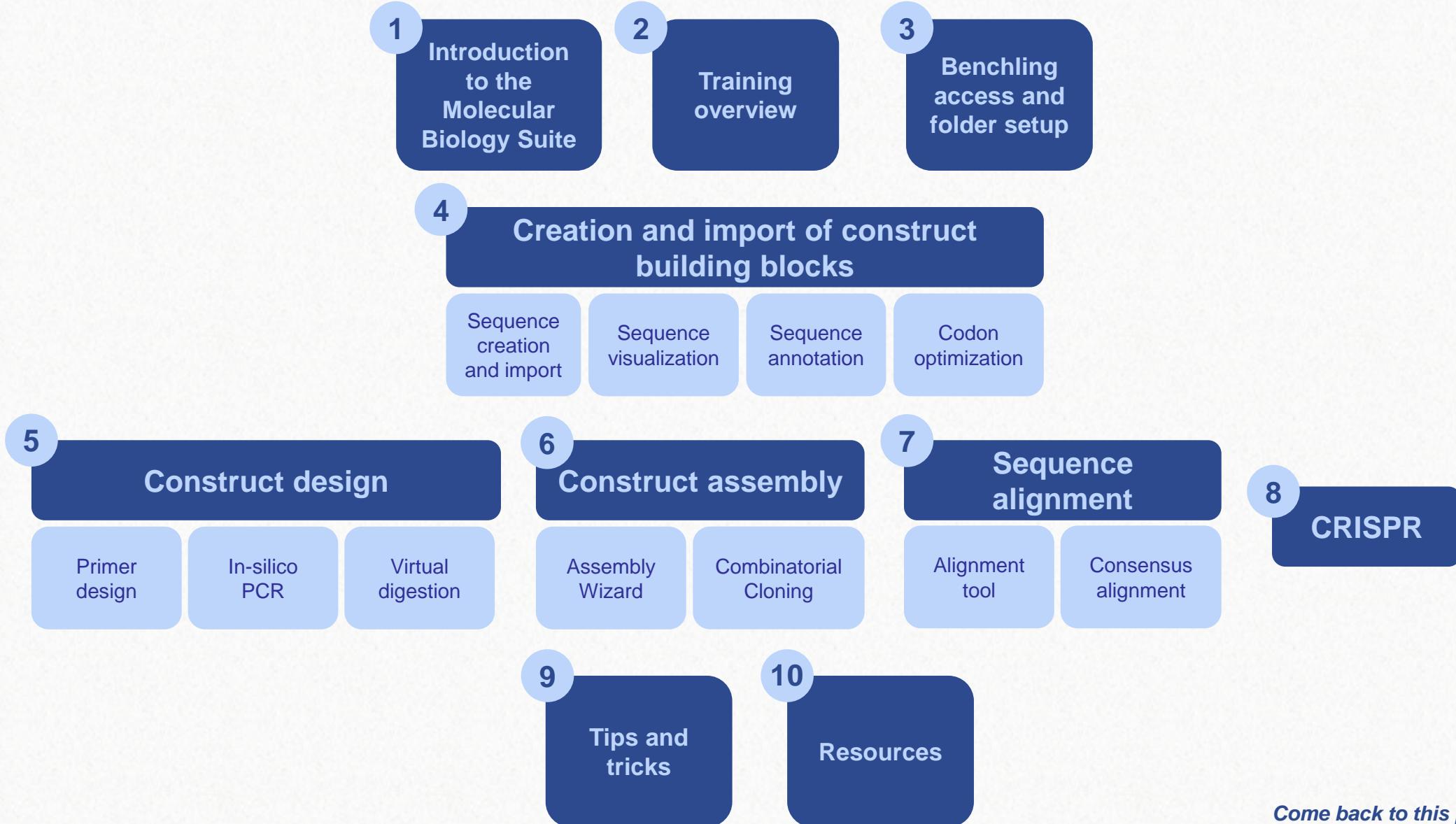


Ester Milesi
LIMS administrator



Mía López Portillo Ontiveros
RDM/LIMS support

Index



Come back to this page
by clicking on the icon!

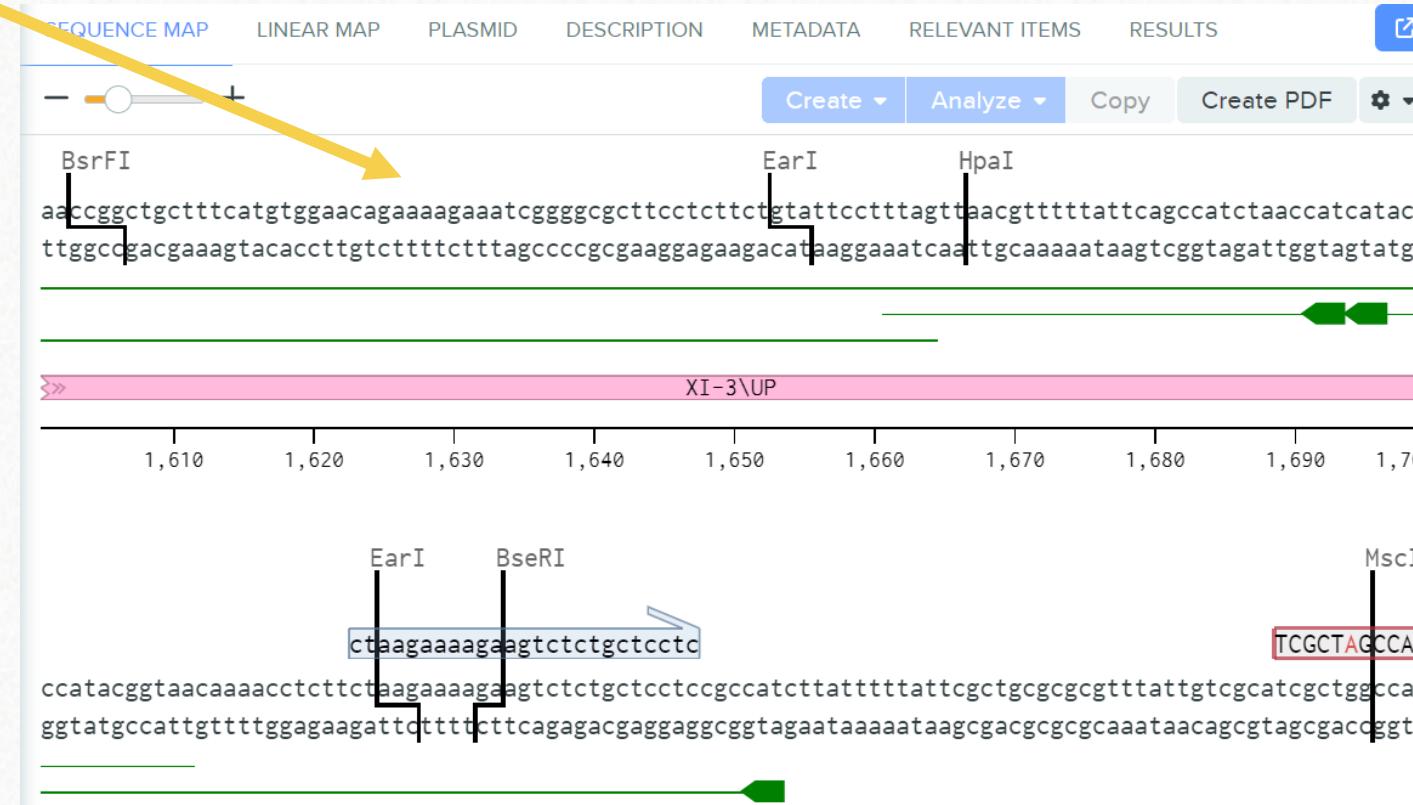


1. Introduction to the Molecular Biology Suite

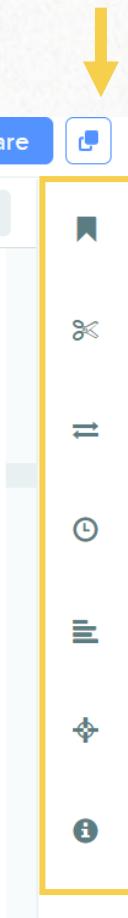


Functionalities and tools overview

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
 - Combinatorial assembly - Gibson cloning
- How to **create a sequence alignment** and what are the different alignment options

Additional knowledge

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



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

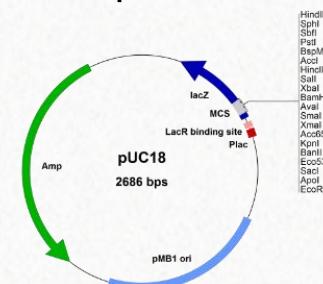


- ✓ Sequence creation and import:
 - From scratch
 - From a database
 - From a file
- ✓ Sequence visualization
- ✓ Codon optimization
- ✓ Sequence annotation

Vanillin dehydrogenase and GFP



pUC18 plasmid vector

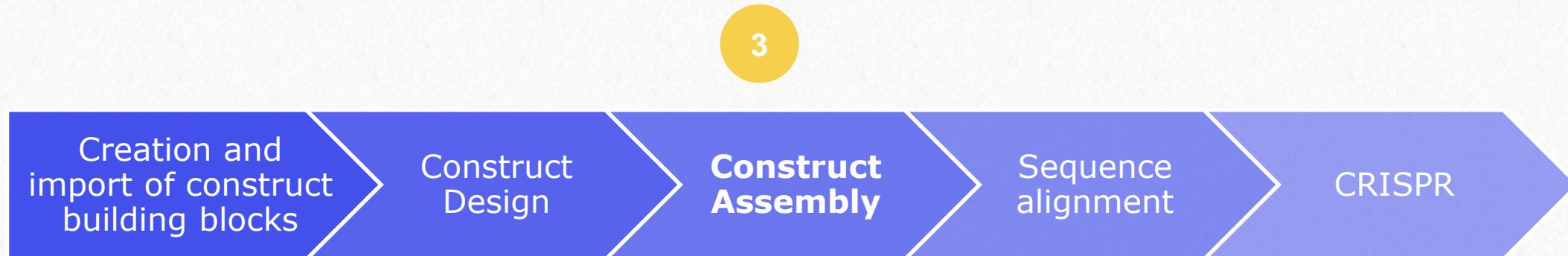


Hands on:



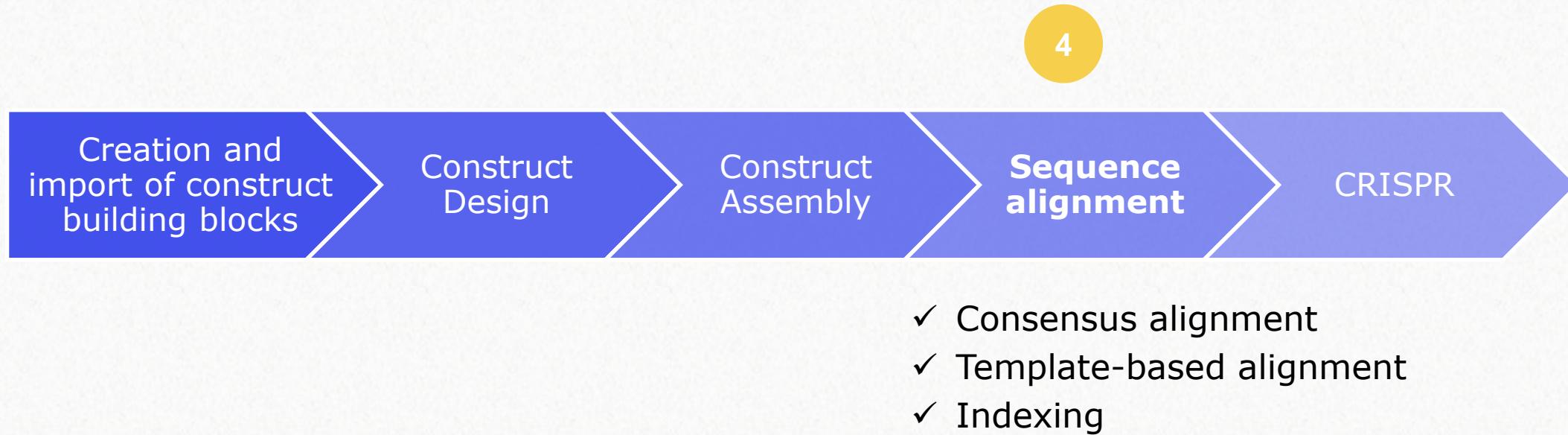
- ✓ Primer design:
 - Manual
 - Primer Wizard
- ✓ In-silico PCR
- ✓ Virtual digestion

Hands on:



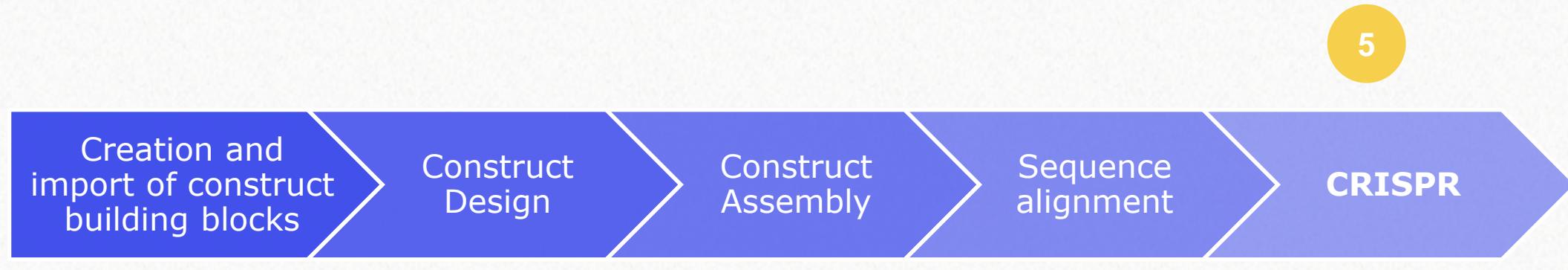
- ✓ Assembly Wizard (RE cloning)
- ✓ Combinatorial cloning (Gibson assembly)

Hands on:





Hands on:



- ✓ Guide RNA design

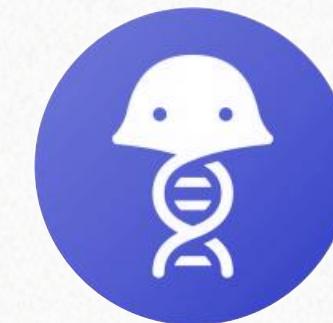
3. Benchling access and folder setup



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 is a vertical toolbar with various icons. A yellow circle with the number 1 highlights the 'New' icon (a plus sign). A yellow circle with the number 2 highlights the 'Molecular Biology Training' folder in the navigation bar. A yellow circle with the number 3 highlights the 'Folder' option in a dropdown menu. A yellow circle with the number 4 highlights the 'Create' button in a 'Create folder' dialog box.

Create folder

Name*

Location*

Description

More

4 **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, there's a sidebar with various icons and a search bar. A yellow circle labeled '1' highlights the search icon. A yellow box labeled '2' highlights the 'Molecular Biology Training' project in the list. Another yellow box labeled '3' highlights the 'Material information for the "Molecular Biology Tools" training' document. The main area shows a detailed view of this document, titled 'Material information for the "Molecular Biology Tools" training'. It includes a date (TUESDAY, 31/10/2023), a status (ELN23004871, In progress), and a note: 'In this ELN, you will find the files, links and sequences necessary to follow the training for the Molecular Biology Tool in Benchling.' Below this, it lists 'Material for construct design:' with several bullet points. One point about 'Vanillin Dehydrogenase gene from *Pseudomonas sp.*' has its accession number (Y11520) highlighted with a yellow box and a yellow circle labeled '4'. A callout bubble says 'Copy this and download the files below'. At the bottom of the document view, there's a file attachment for 'pUC18.dna' and a 'SPLIT WORKSPACE' button.

- ✓ 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

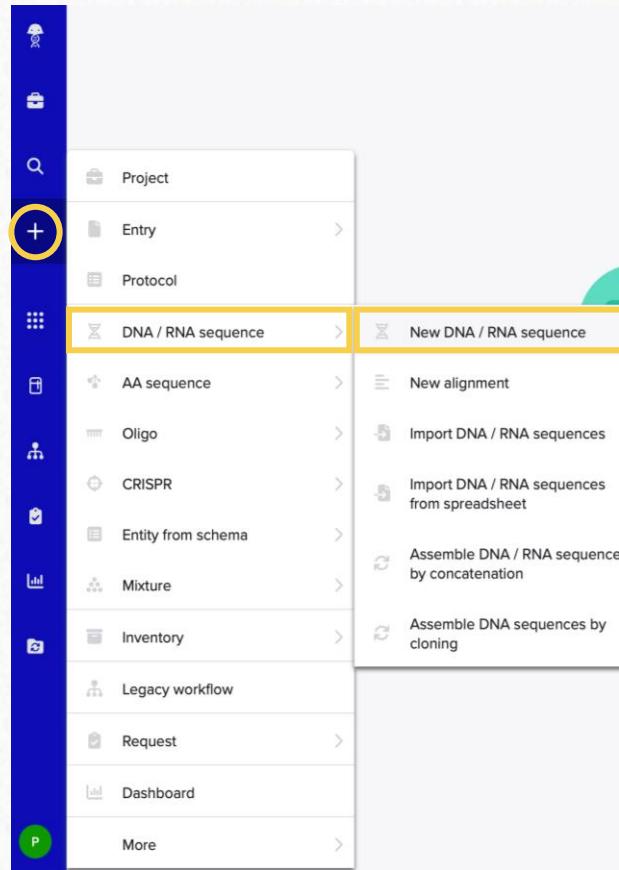
4.1 Sequence creation and import



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

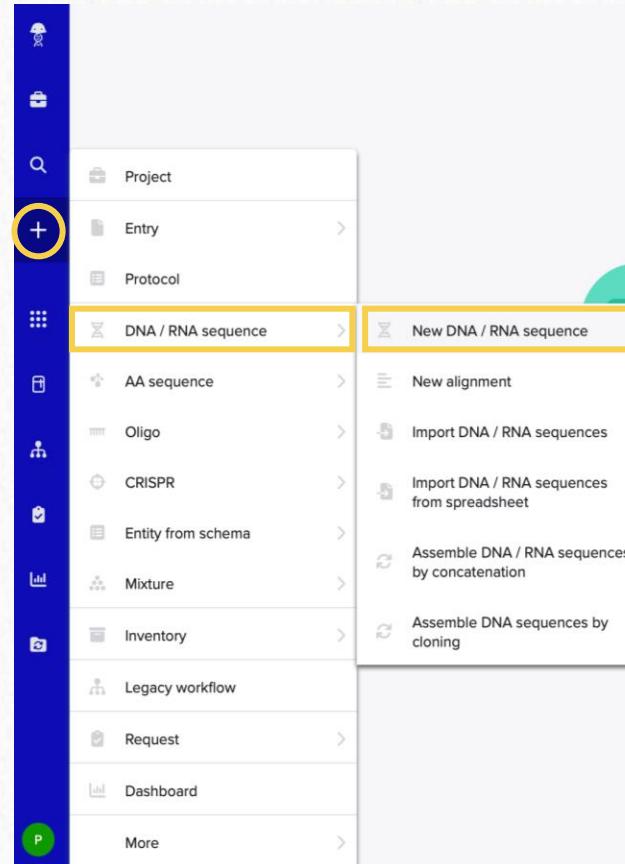
Bases
ggcacgttaagagggttccaactttcaccataatgaaaca

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

Select a schema...

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.

The screenshot shows the Benchling software interface. On the left, a sidebar menu is open, with the 'DNA / RNA sequence' option highlighted by a yellow circle. Under 'DNA / RNA sequence', the 'Import DNA / RNA sequences' option is also highlighted. The main window is titled 'Create DNA / RNA sequence' and has tabs for 'CREATE NEW', 'UPLOAD FILES' (which is selected and highlighted with a yellow box), 'IMPORT FROM DATABASE', and 'SELECT CHROMOSOMAL REGION'. The 'Nucleotide type*' dropdown is set to 'DNA' (highlighted with a yellow box). A 'Project folder' section shows a folder named 'Mia'. Below, there's a dashed box for dragging files or a 'Choose a file' button. A list of imported files includes 'GFP.dna' and 'pUC18.dna', each with its status: 'UPLOAD DONE' and 'OPEN SEQUENCE - UPLOADED TO MIA'. At the bottom right of the main window, a note says '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: syn...

Set Topology dropdown: Linear, Circular
Edit Tags dropdown: ACCESSION, DEFINITION, ORGANISM, SOURCE
Buttons: UPLOAD DONE, OPEN SEQUENCE - UPLOADED TO PATRICIA, Create New Tag

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

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

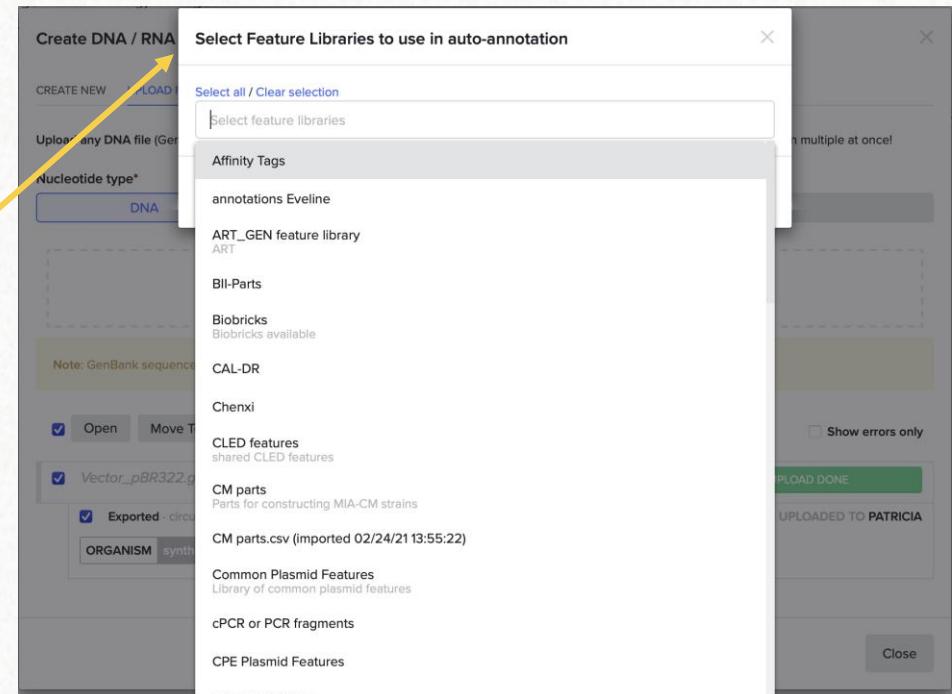
Vector_pBR322.gb

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

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

UPLOAD DONE

Close



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

Create DNA / RNA sequence

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

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

Nucleotide type* Set folder

DNA RNA Patricia

Drag and drop files to upload or choose a file

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

Open Move To Set Topology Edit Tags Auto-annotate  

Vector_pBR322.gb

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

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

UPLOAD DONE Close

Create DNA / RNA

Add items to entity worklist

New worklist Existing worklist

Worklist Name* Project_training

Selected items Exported

Add items to worklist

Open Move To Set Topology Edit Tags Auto-annotate  

Vector_pBR322.gb

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

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

UPLOAD DONE Close

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

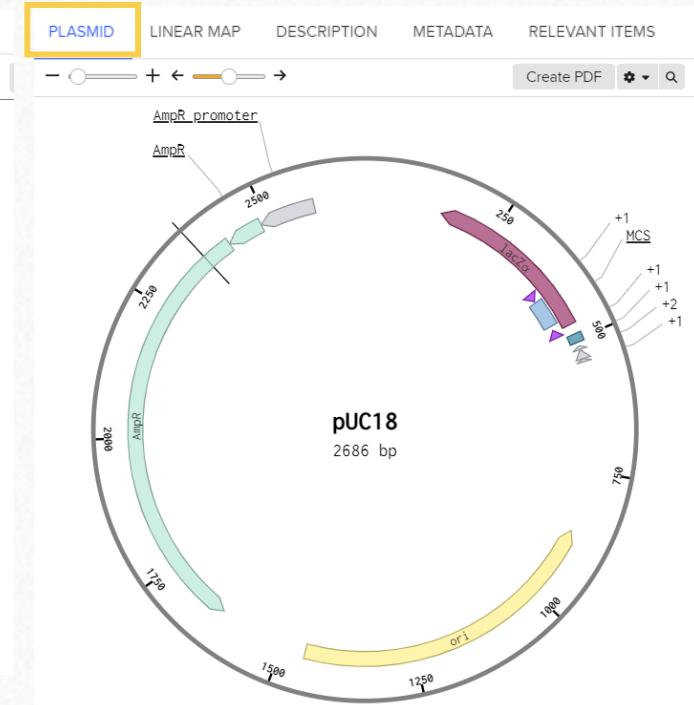
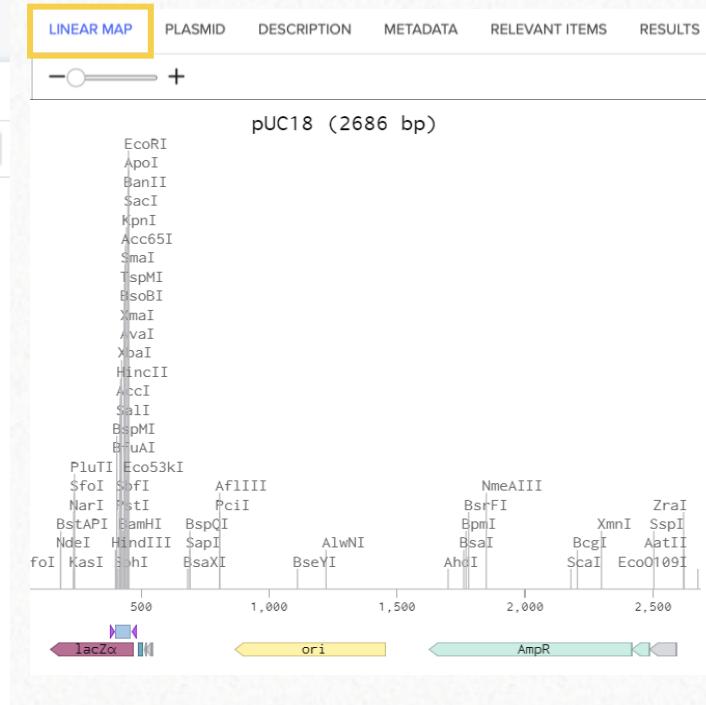
4. Creation and import of construct building blocks

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:

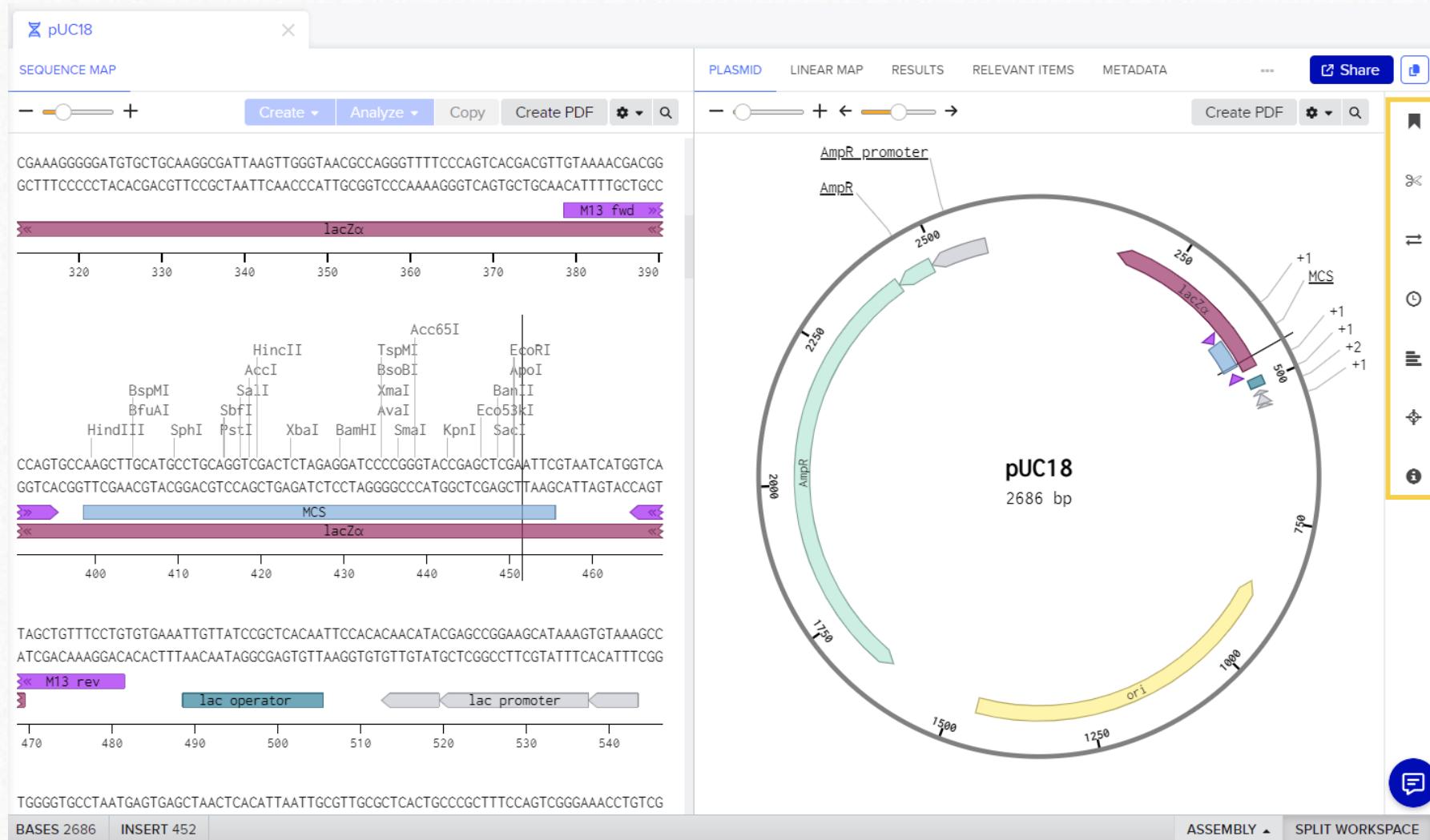


PRO TIP:

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

View, annotate and edit your sequences

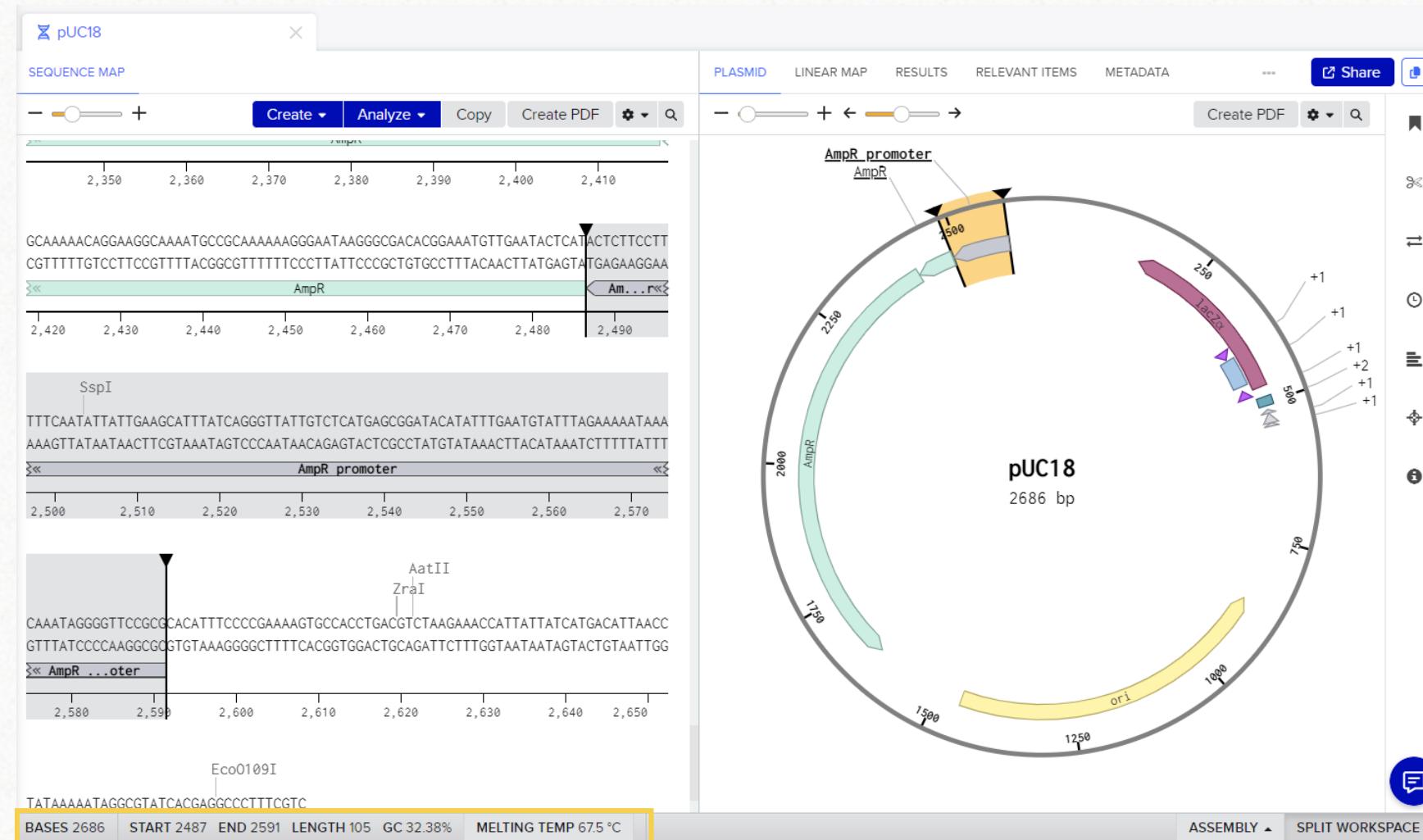
Sequence navigation:



Functionalities

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:

SEQUENCE MAP

Annotations for the sequence: lacZα

Annotations for the sequence: lacZα

Annotations for the sequence: Acc65I

CREATE AND ANALYZE

Analyze dropdown menu:

- Annotation
- Primer
- Translation
- New AA sequence
- New DNA
- New RNA
- New part
- Run Primer3
- Run Benchling BLAST
- Submit to NCBI BLAST
- Analyze as translation
- Optimize codons

RIGHT-CLICK CONTEXT MENU

For the sequence fragment BstAPI-NdeI (positions 160-230):

- 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
- Analyze as translation

For the sequence fragment M13_fwd (positions 370-390):

- 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
- Analyze as translation

- ✓ 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



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

2 Create an annotation

Annotation

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

3 Add the specifications

Annotations

AmpR promoter

Visibility filter

12 total

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

New annotation

Name: Annotation name

Position: 396 - 415

Annotation type: Annotation type

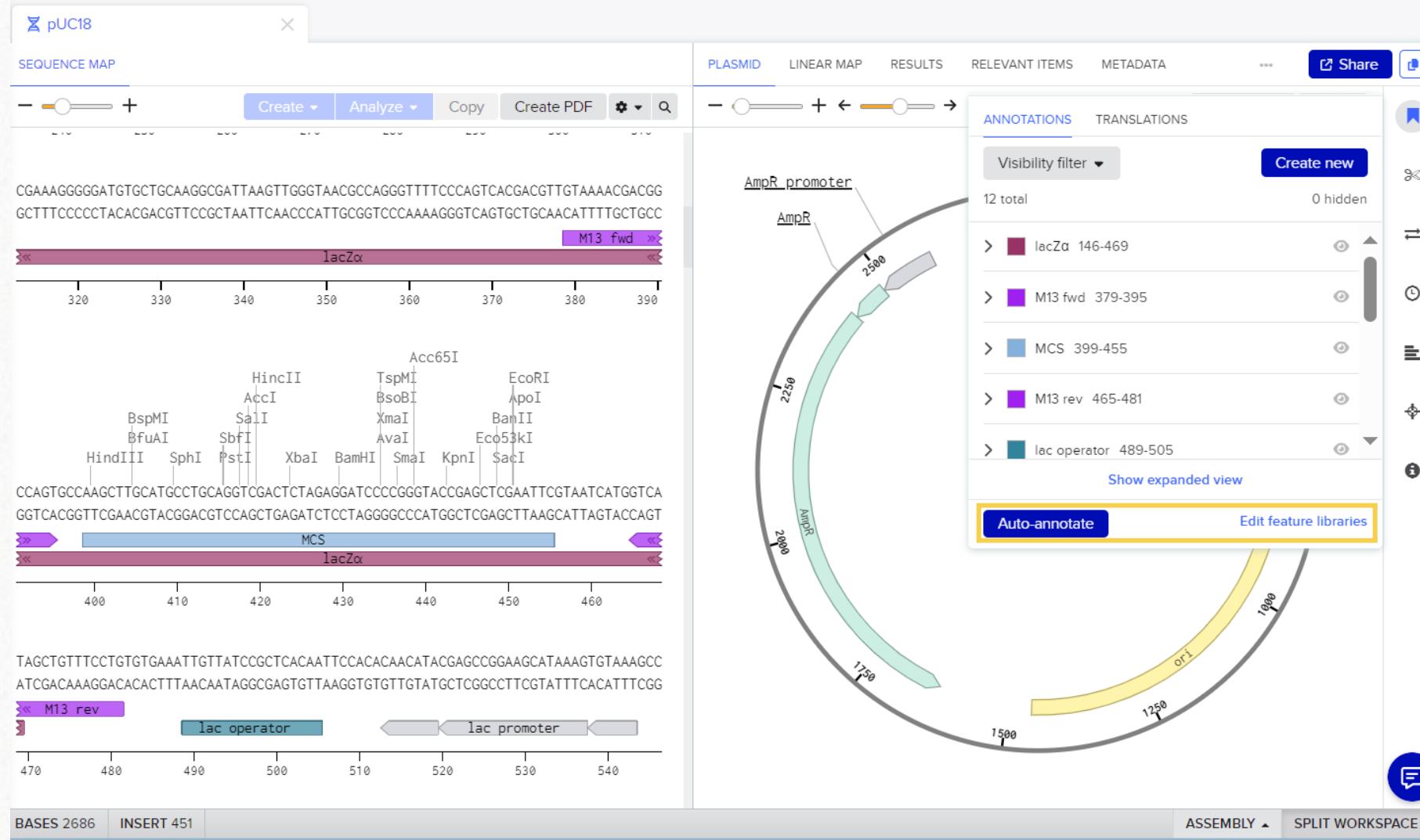
Color: Purple

Strand: Forward

Notes: Notes

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

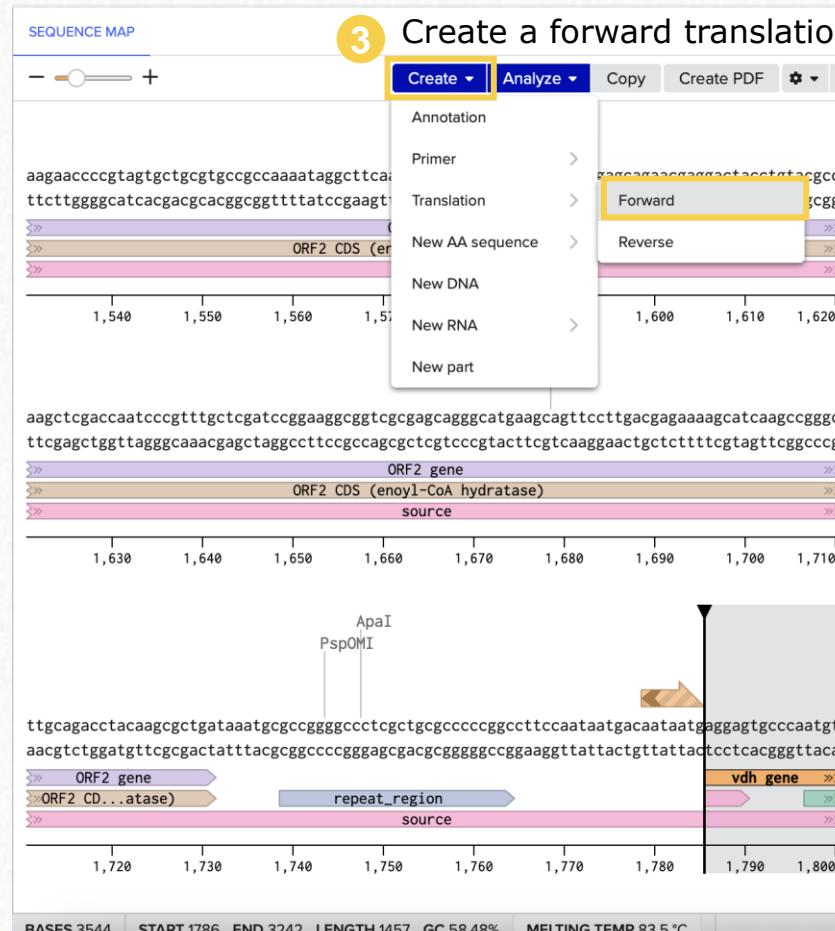
4.4 Codon optimization



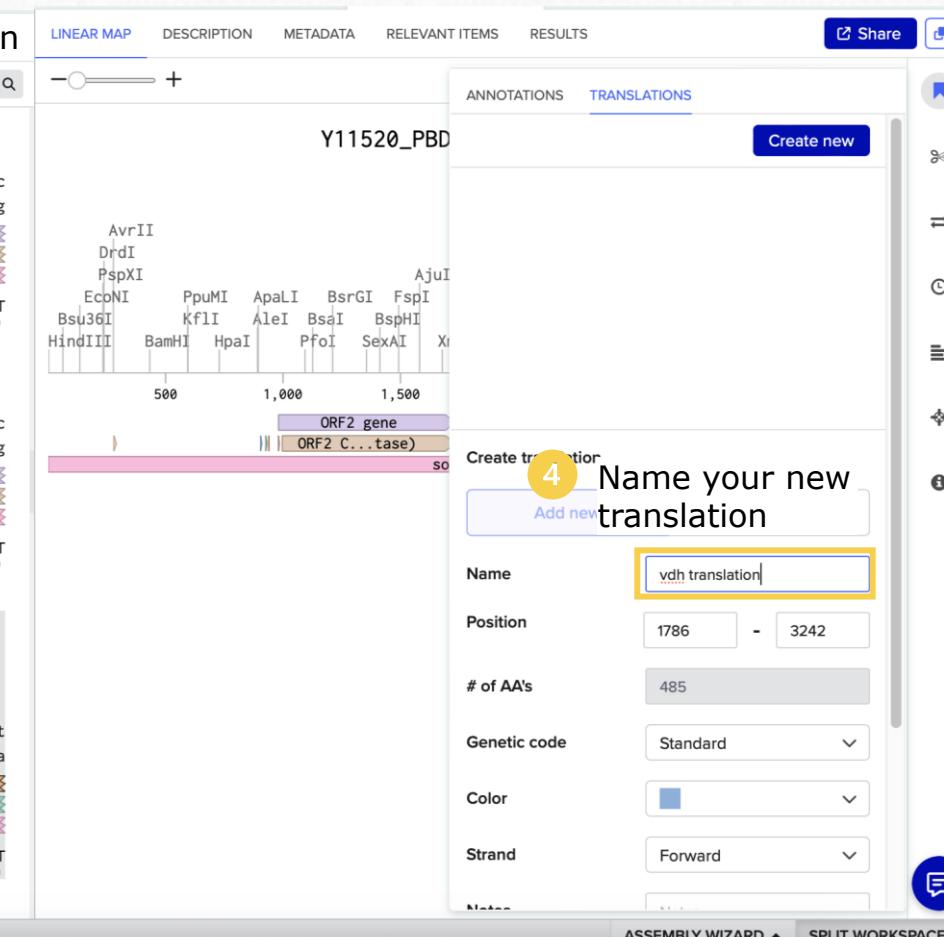
View, annotate and edit your sequences

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*)



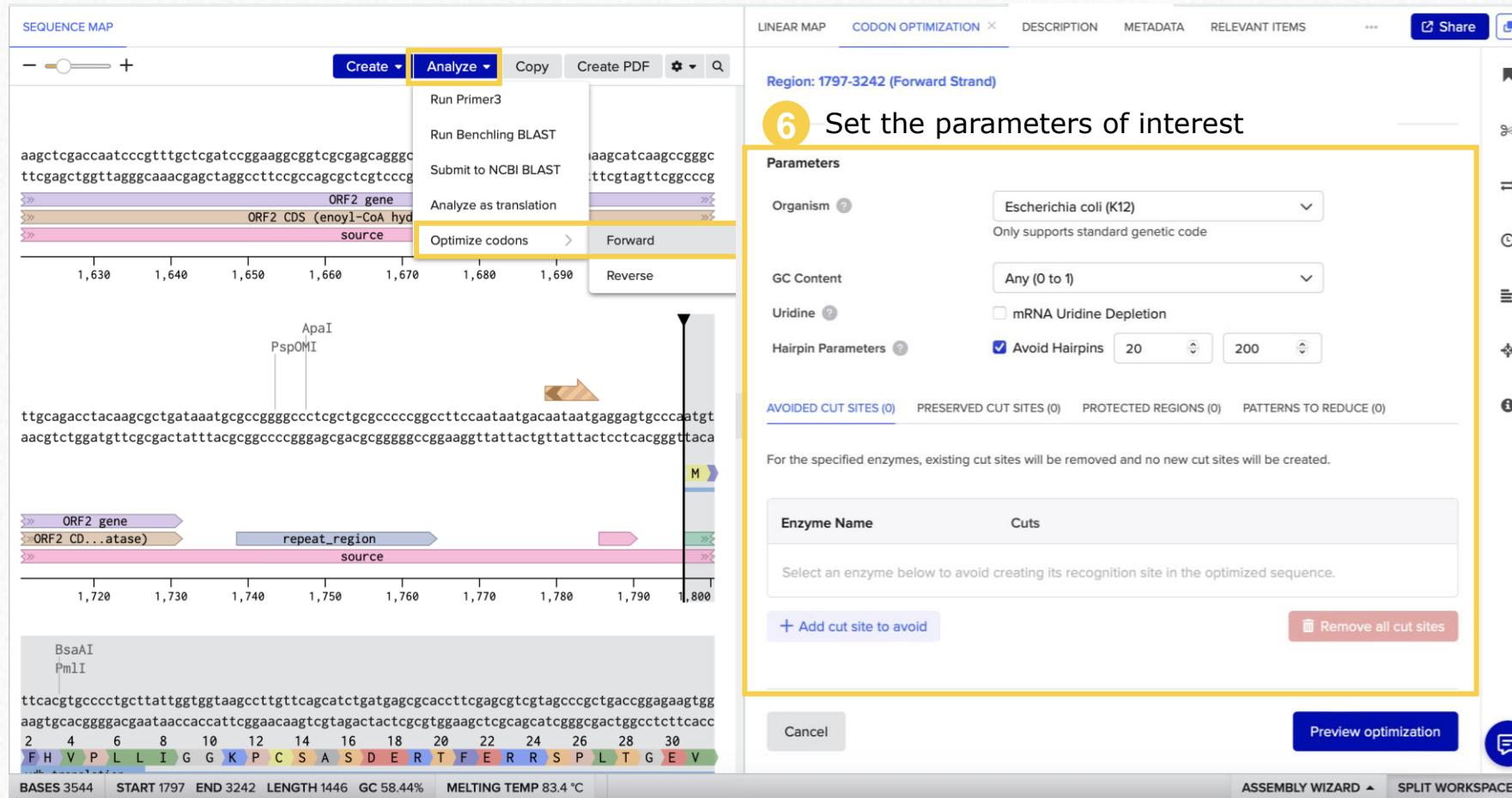
- ✓ Before codon optimization, the DNA sequence must be translated

- 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



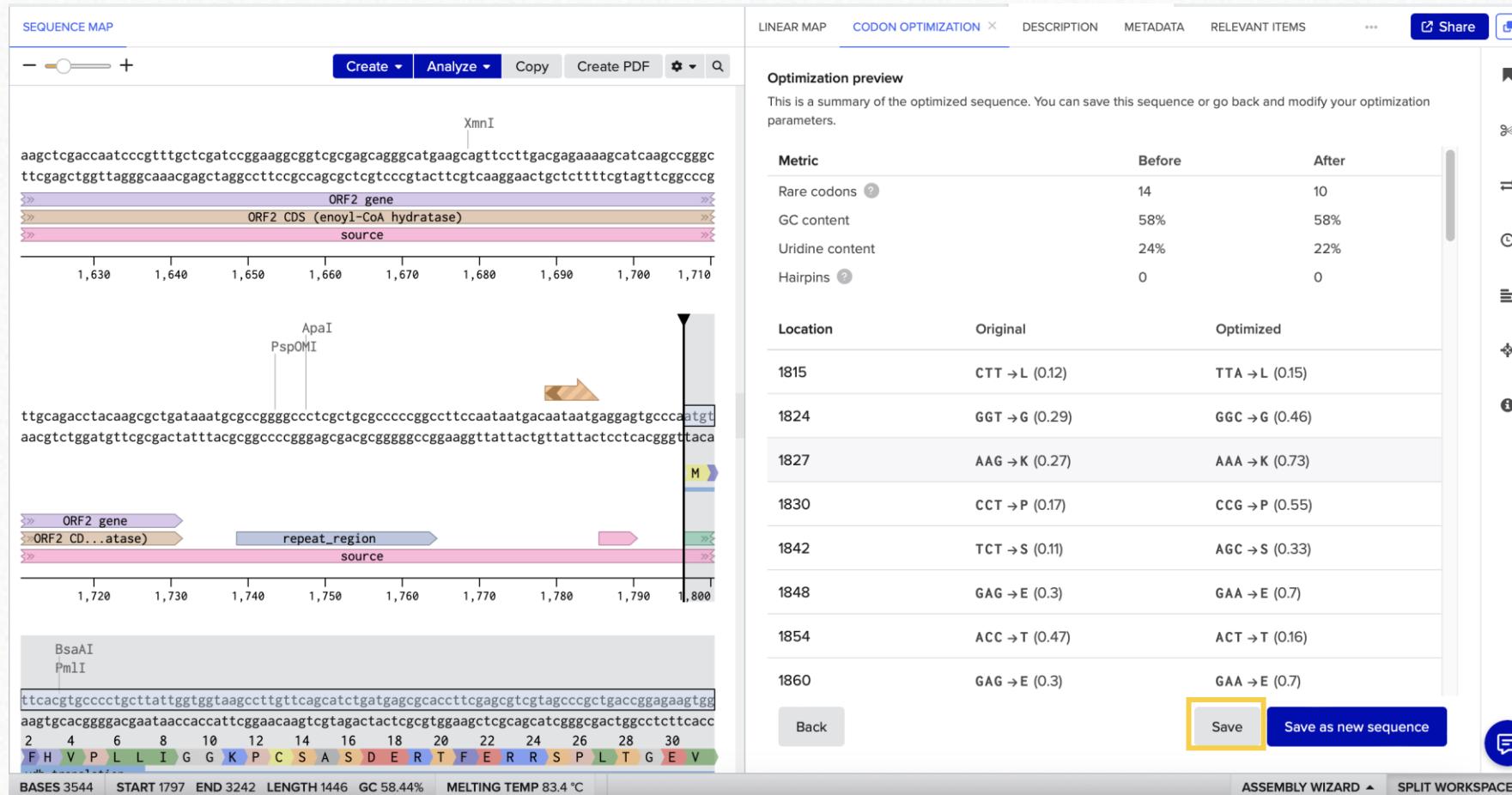
The screenshot shows the Bioworkshop interface with two main panels. On the left, a 'SEQUENCE MAP' panel displays a DNA sequence with various annotations: 'ORF2 gene', 'ORF2 CDS (enoyl-CoA hydrolase)', and 'source'. It includes restriction enzyme sites like ApaI and PspOMI, and a 'repeat_region'. The sequence is numbered from 1,630 to 1,800. On the right, a 'CODON OPTIMIZATION' panel is open, showing parameters for 'Escherichia coli (K12)'. A yellow box highlights the 'Parameters' section, which includes fields for 'GC Content' (set to 'Any (0 to 1)'), 'Uridine' (unchecked), and 'Hairpin Parameters' (checked 'Avoid Hairpins' with values 20 and 200). Below this is a section for 'AVOIDED CUT SITES (0)' where users can select enzymes to avoid. At the bottom of the optimization panel are 'Cancel', 'Preview optimization', and a message icon.

- When codon optimizing, its 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

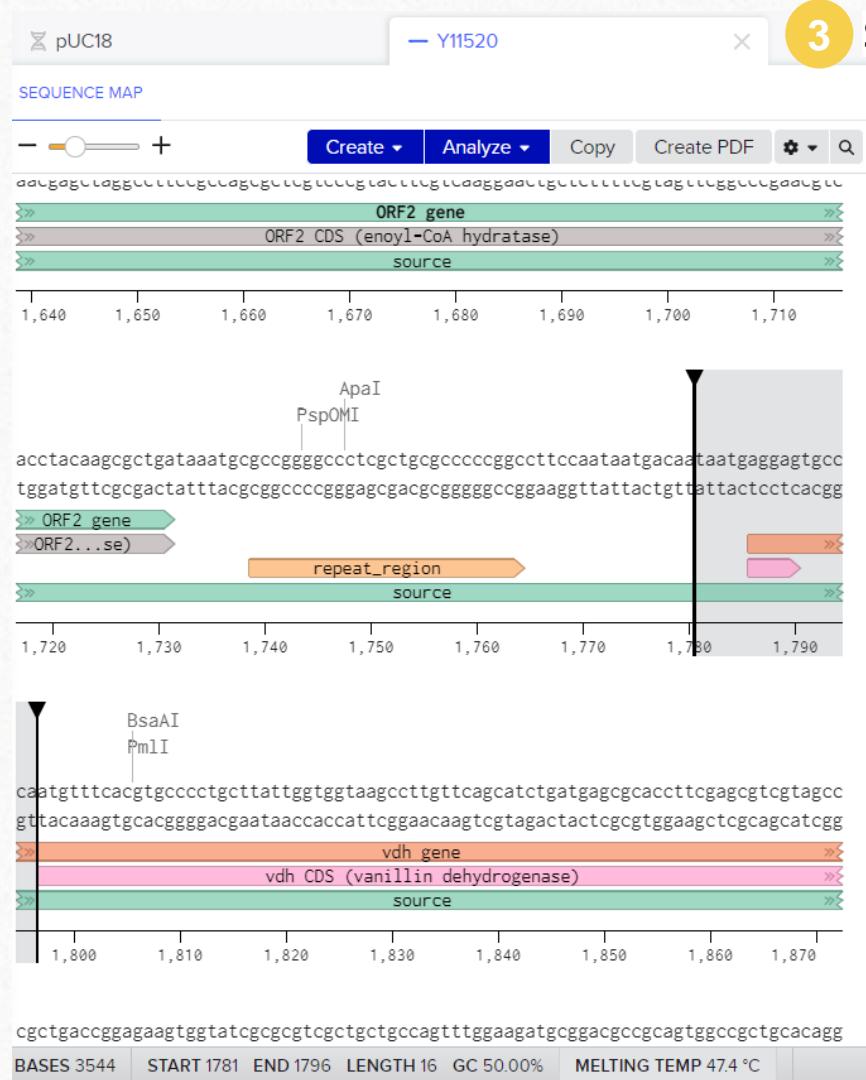
2 Access the primer tool and start to create a new primer manually

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

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 at position 1780, with the forward primer starting at 1780 and the reverse primer ending at 1780.

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

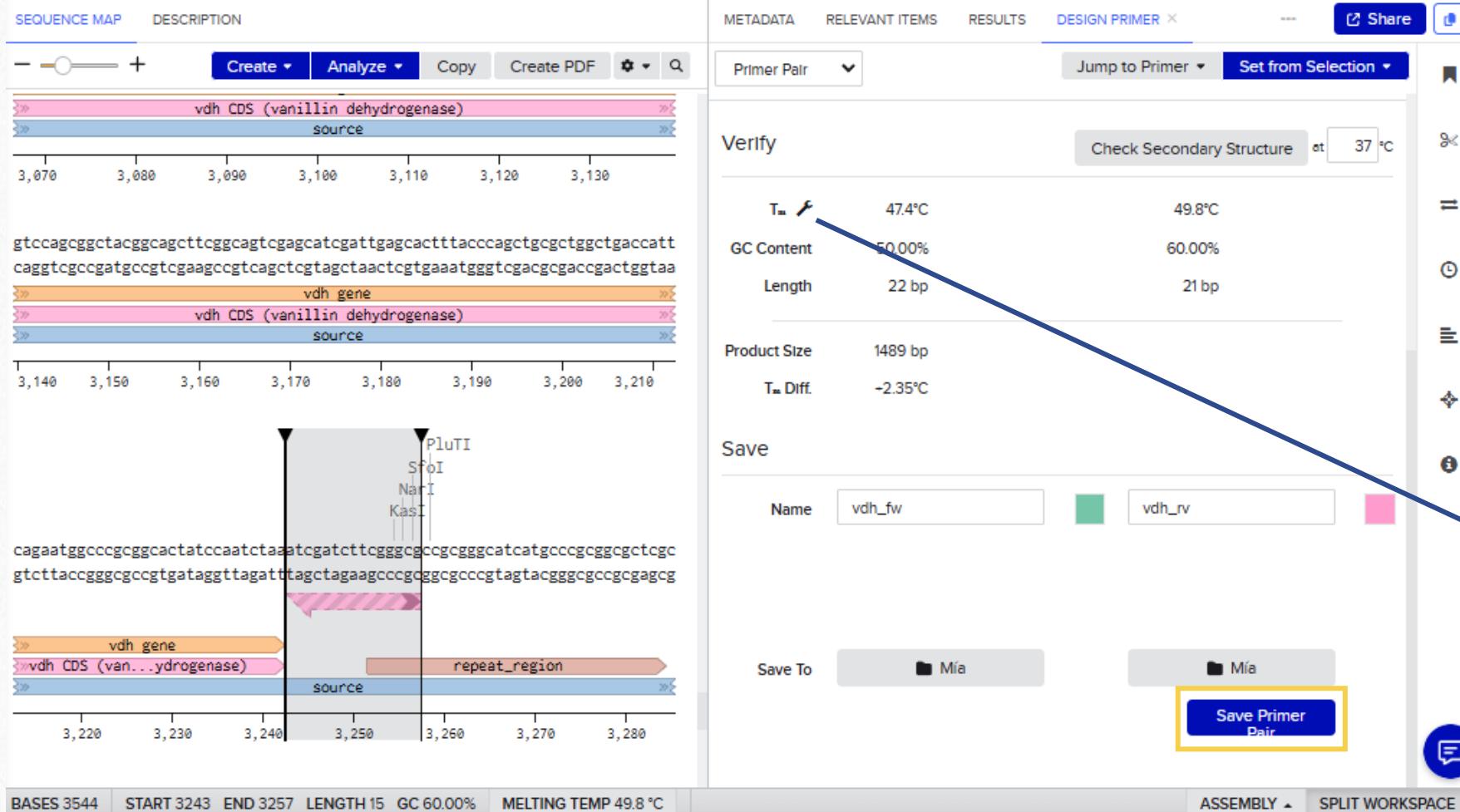
Use the dropdown above to look up restriction sites.

- 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 genomic regions: source, vdh gene, and repeat_region. The sequence is shown with various restriction sites (PluTI, SfoI, NraI, KsaI) and a poly-A tail. The primer design panel on the right shows the following parameters:

Parameter	Value	Value (Secondary Structure)
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 shows the primers named vdh_fw and vdh_rev, and a blue arrow points from the 'Save Primer Pair' button to a blue info icon (i).

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

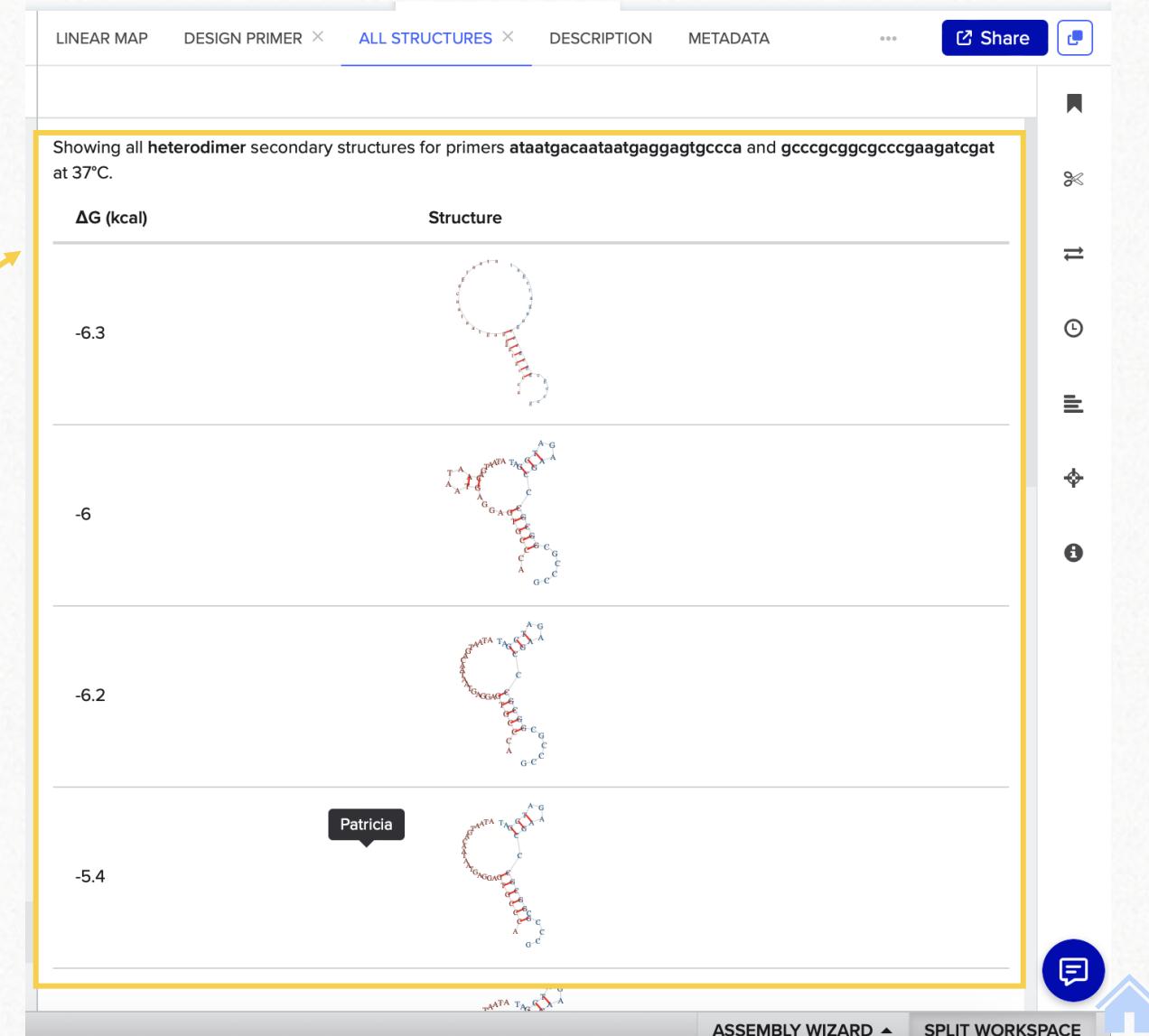
PRO TIP: You can adjust the default parameters for thermodynamic calculations

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, there are fields for Primer Pair, Overhang (0 bp), Cut Site (Aanl), and a dropdown for restriction sites. A 'Verify' section displays various metrics: T_m (56.1°C and 69.8°C), GC Content (38.46% and 73.91%), Length (26 bp and 23 bp), and ΔG values for Homodimer (-3.3 kcal and -13.8 kcal) and Monomer (-0.1 kcal and -2.5 kcal). Product Size is listed as 1495 bp. A 'Check Secondary Structure' button is highlighted with a yellow box, along with a dropdown for temperature (37 °C). The bottom section allows saving the primer with names fwd_vdh and rev_vdh.



5. Construct design

5.1 Primer design

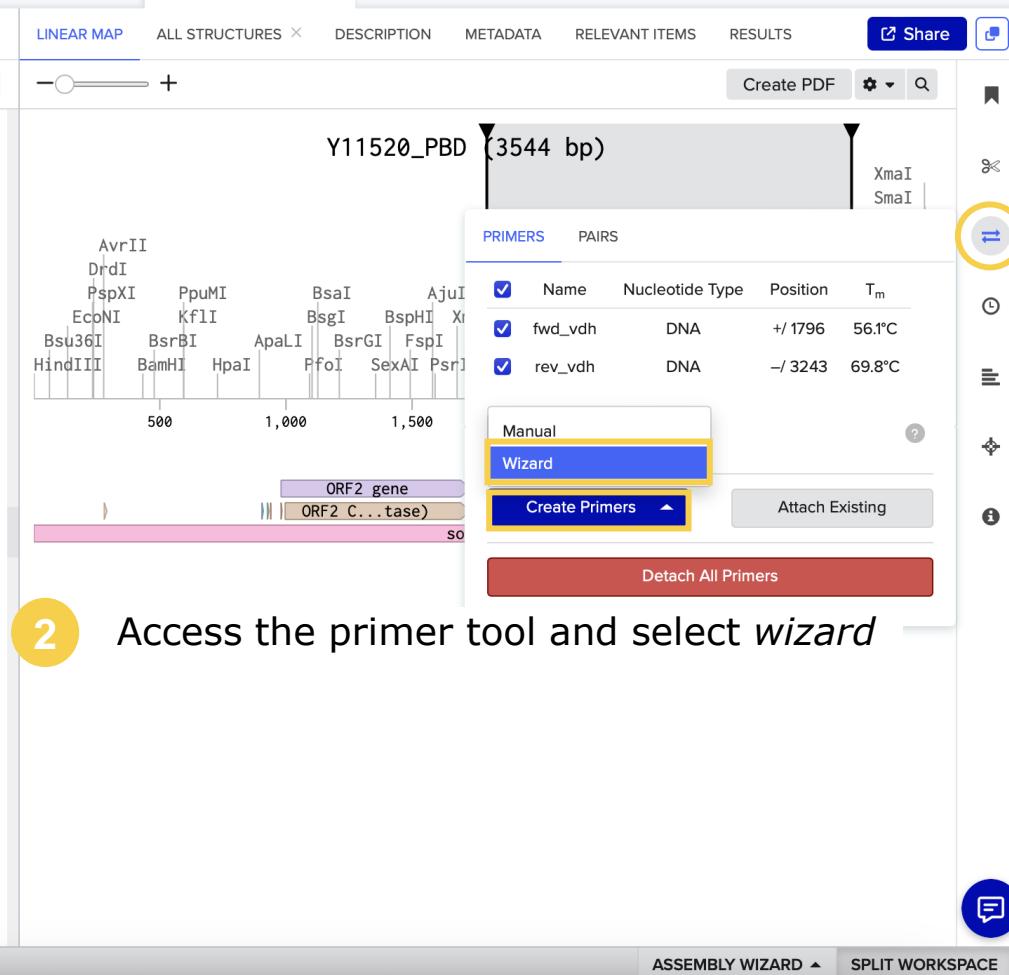
5.1.2 Primer wizard



Construct design

Automatic primer creation – Primer Wizard

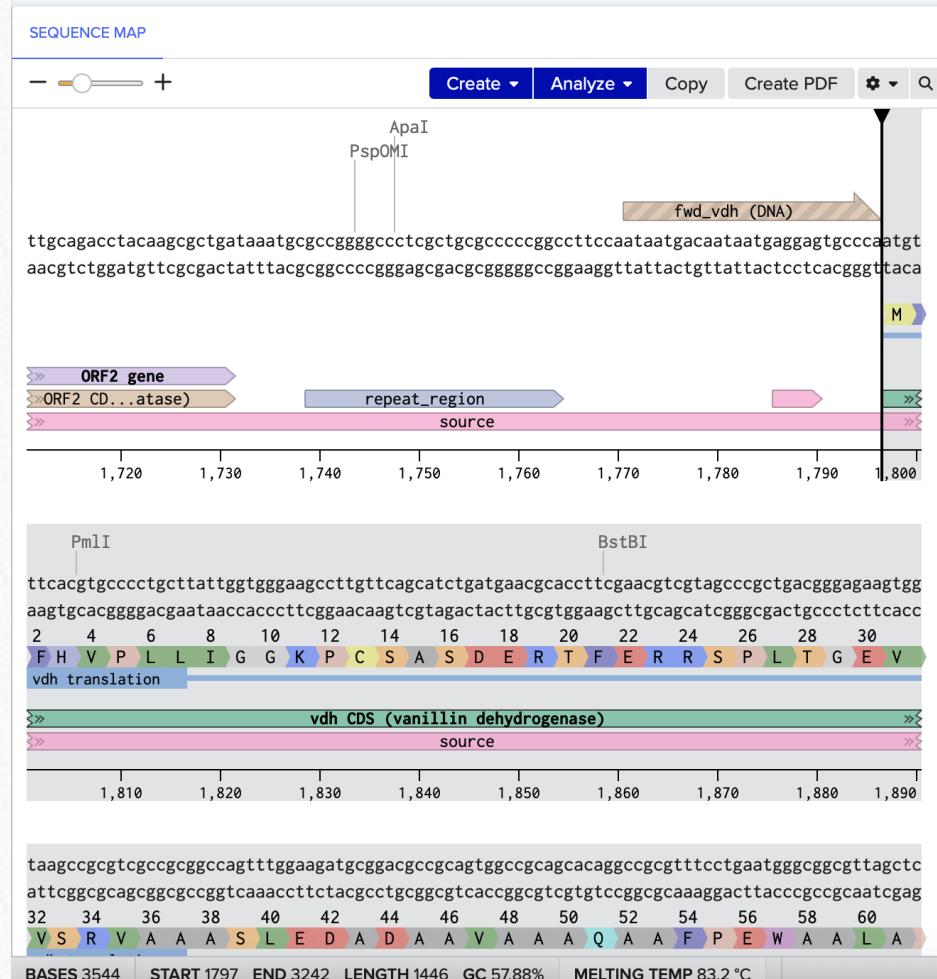
1 Select the CDS sequence of *vdh*



2 Access the primer tool and select wizard

Construct design

Automatic primer creation – Primer Wizard



Construct design

Automatic primer creation – Primer Wizard

6 Select the most appropriate primer pair and save them

The screenshot shows the Primer Wizard interface with the following components:

- SEQUENCE MAP:** A linear map of a DNA sequence. It highlights regions for "ORF2 gene", "ORF2 CDS (enoyl-CoA hydratase)", and "source". Specific restriction sites like XmnI and ApaI, along with PspOMI, are marked. A primer pair labeled "fwd_vdh (DNA)" is shown as a purple arrow pointing from position 1,660 to 1,710.
- PRIMER WIZARD:** A toolbar with "Create", "Analyze", "Copy", "Create PDF", and other options.
- PRIMER3 RESULTS:** A table showing primer pairs. The columns include: Penalty, Direction (FWD or REV), % GC, T_m °C, Location, Length, Product BP, and Primer sequence. The first primer pair (highlighted in yellow) has a Penalty of 63.6%, a T_m of 62.0°, and a sequence of 5' gaaccagagtgttccgctggcc 3'. The second primer pair has a Penalty of 55.5% and a T_m of 60.7°.

✓ 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

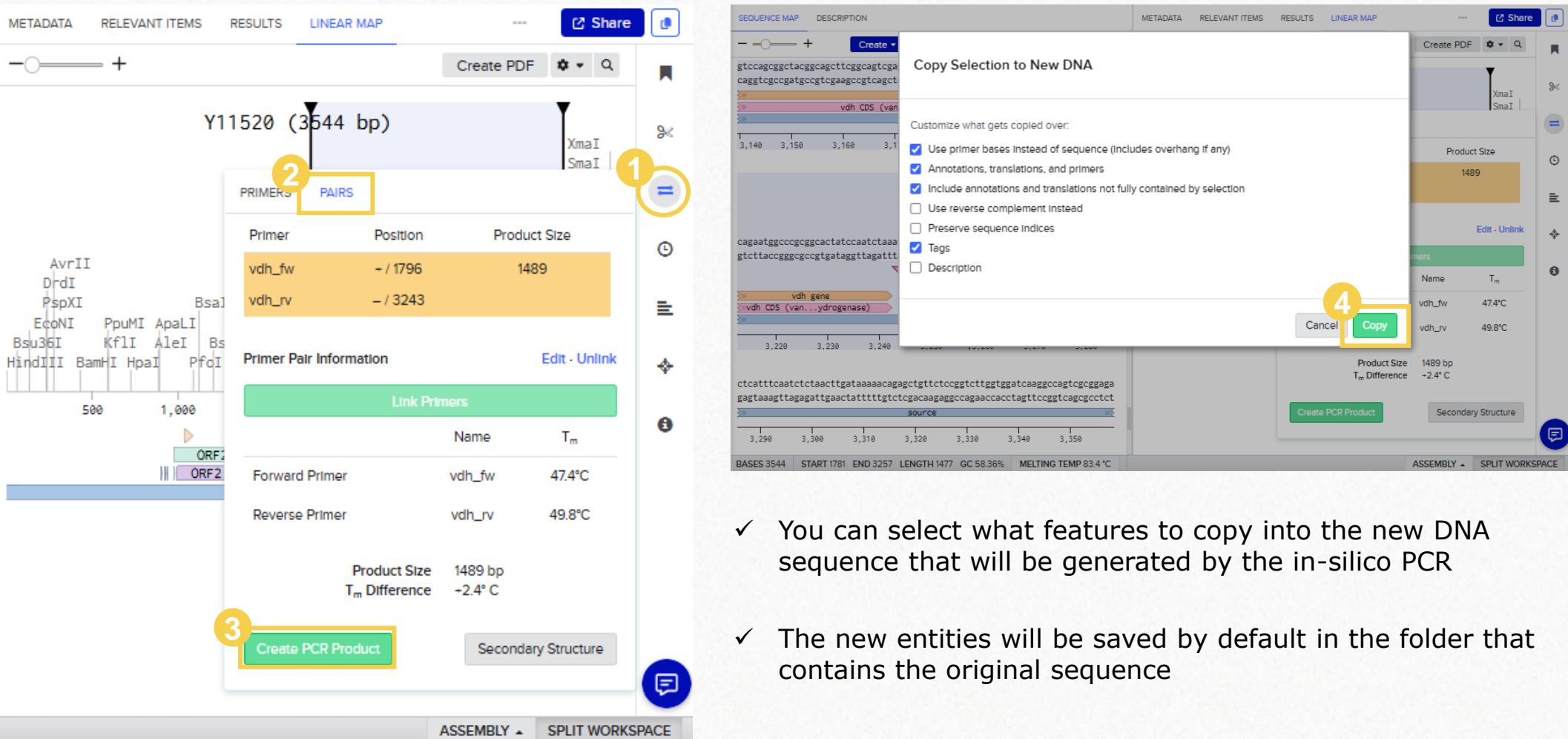
5.2 *In-silico* PCR



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 construct design. On the left, a linear map of the DNA sequence Y11520 (3544 bp) is displayed, showing various restriction sites and two open reading frames (ORFs). A primer pair is selected for PCR: vdh_fw at position ~1796 and vdh_rev at position ~3243, resulting in a product size of 1489 bp. The T_m difference is +2.4°C. A 'PAIRS' button is highlighted with a yellow circle labeled 2. On the right, a 'Copy Selection to New DNA' dialog box is open, showing customization options for what to copy over. The 'Use primer bases instead of sequence (includes overhang if any)' checkbox is checked. Other options like 'Annotations, translations, and primers' and 'Include annotations and translations not fully contained by selection' are also checked. The 'Tags' checkbox is checked. The 'Copy' button is highlighted with a yellow circle labeled 4. The background shows the sequence map with the selected primers and the resulting 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

— — + Create Analyze Copy Create PDF ⚙️ 🔎

EcoRI ApoI BsaAI PmlI

vdh_fw (DNA)

GAATTCTaataggaggatgcccaatgtttcacgtgcctctgttattgggttaaggccttggtagcatctgat CTTAAAGattactcctcacgggttacaaagtgcacggggacgaaaccaccattcggaaacaagtcgttagacta

vdh gene vdh CDS (vanillin dehydrogenase) source

10 20 30 40 50 60 70

PsRI BsaXI

gagcgcacccctcgagcgctgttagcccgctgaccggagaagtggtatcgccgtcgctgtcccgatggaaag ctccgtggaaagctcgacgatccggactggcttccaccatagcgcgcagcgacgacggtaaaccttc

vdh gene vdh CDS (vanillin dehydrogenase) source

80 90 100 110 120 130 140

BsgI BglI HaeII

atgcggacgcccacgtggccgtgcacaggctgcgtttcttgcataatggccggcgttgctccgagcgaacggc tacgcctgcggcgtaccggcgcacgtgtccgacgcaaaaggacttaccgcggcaacggaggctcgcttgcgg

vdh gene vdh CDS (vanillin dehydrogenase)

BASES 1489 INSERT 37

LINEAR MAP

— — + Create PDF ⚙️ 🔎

Y11520 [1781-3257] (1489 bp)

HindIII BstXI MsI Esp3I BsmBI BsmAI BcoDI AatII IraI PvuII

BfaI XbaI BstYI PsRI BsrBI NcoI XcmI BsaAI HaeII BsrFI BsrBI AgeI PshAI HincII EarI BclI EagI BsaXI EciI NciI SphI MmeI BanI DraIII BclI

EcoRI BsgI MseI BsmI BsmI AgeI PshAI HincII EarI BclI EagI BsaXI EciI NciI SphI MmeI BanI DraIII BclI

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

vdh gene vdh CDS (vanillin dehydrogenase) source

ASSEMBLY ▾ SPLIT WORKSPACE

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

5. Construct design

5.3 Virtual digestion

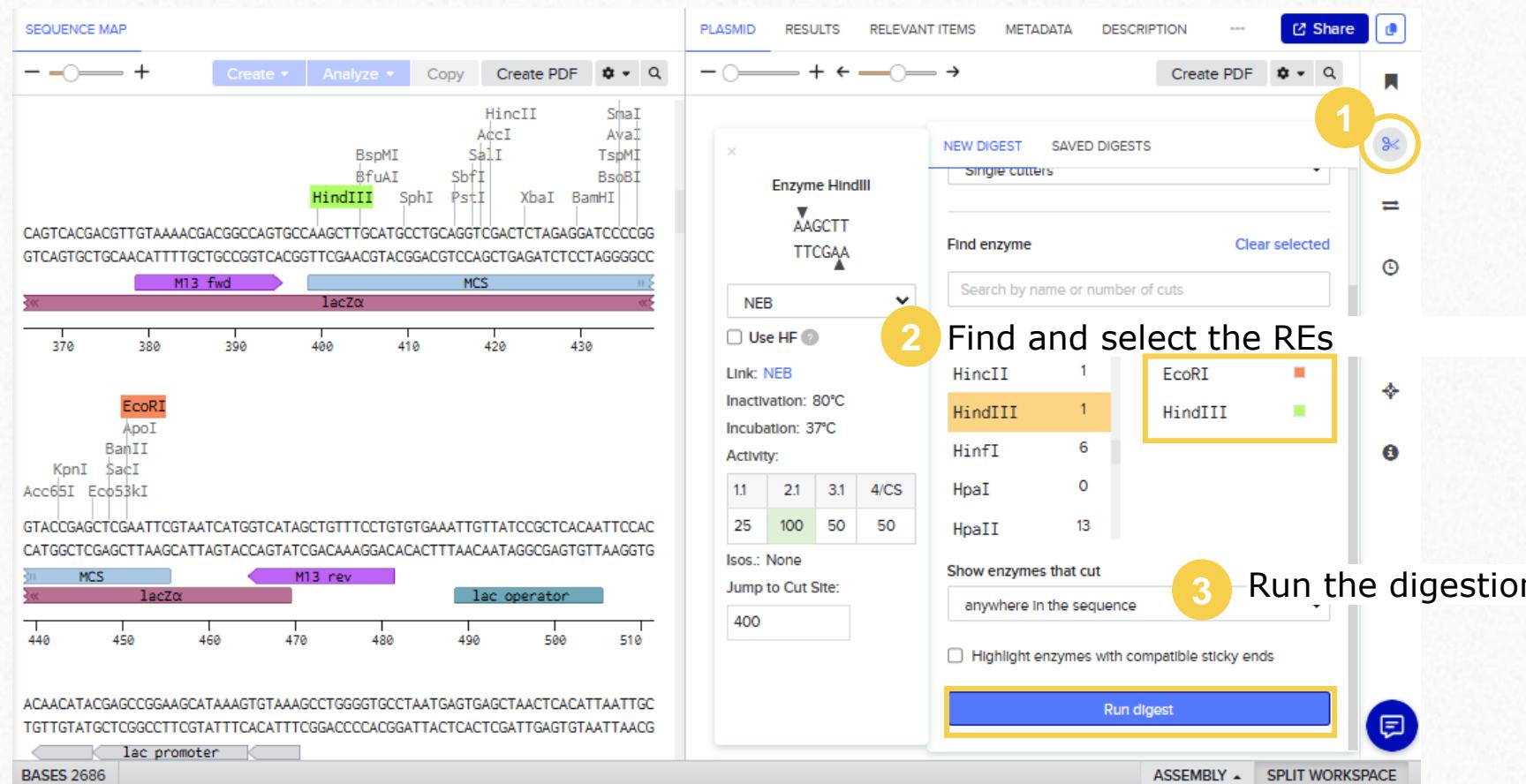


Construct design

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)



SEQUENCE MAP

PLASMID RESULTS RELEVANT ITEMS METADATA DESCRIPTION Share

Enzyme HindIII

AAGCTT
TTCAAA

NEB

Link: NEB
Inactivation: 80°C
Incubation: 37°C
Activity:
1.1 2.1 3.1 4/CS
25 100 50 50

Isos.: None
Jump to Cut Site: 400

Show enzymes that cut anywhere in the sequence

Highlight enzymes with compatible sticky ends

Run digest

1

2

3

BASES 2686

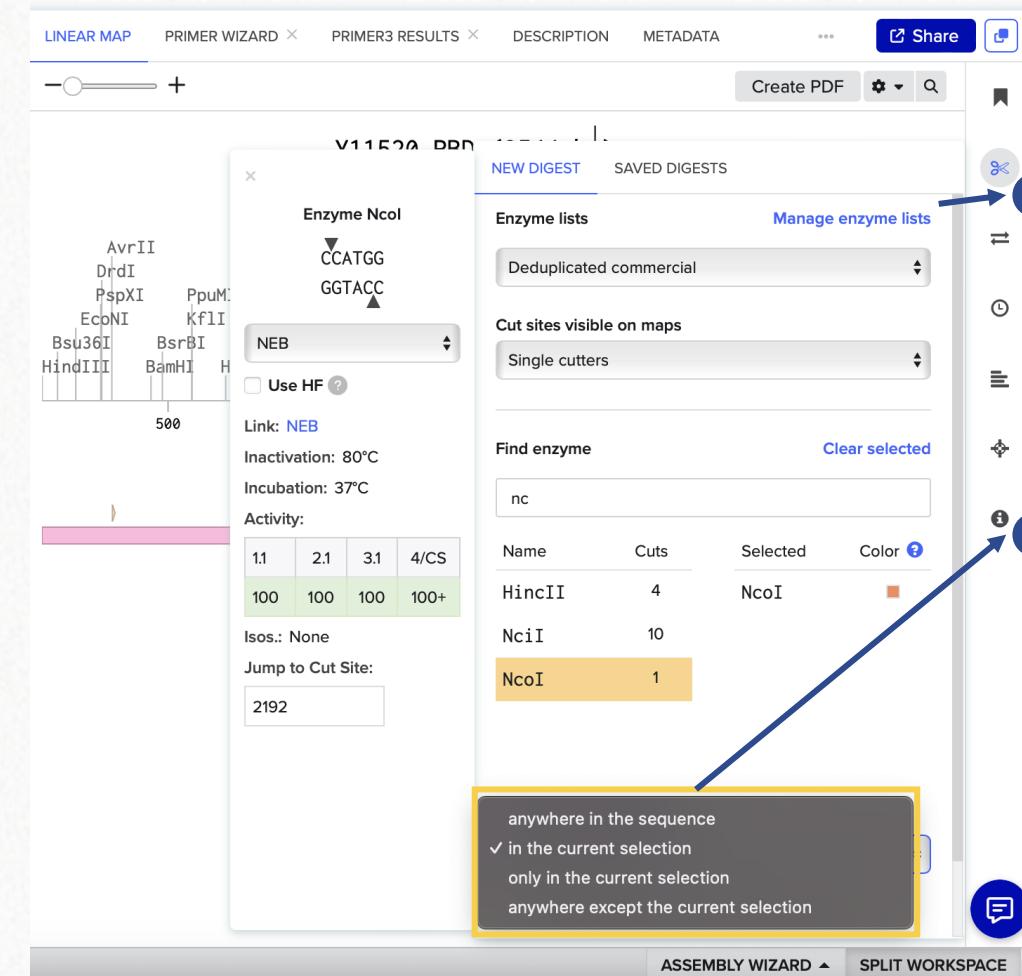
ASSEMBLY ▾ **SPLIT WORKSPACE**

- ✓ 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 Bioworkshop software interface. At the top, there are tabs for LINEAR MAP, PRIMER WIZARD, PRIMER3 RESULTS, DESCRIPTION, and METADATA. Below the tabs is a toolbar with icons for Share, Create PDF, settings, and search. A central panel displays a sequence map with various restriction sites labeled (AvrII, DrdI, PspXI, PpuM, EcoNI, Bsu36I, HindIII, BsrBI, BamHI, H). A modal dialog box for "Enzyme NcoI" is open, showing the sequence CCATGG and GGTACC, and indicating it is a NEB enzyme. It also shows activity levels for 1.1, 2.1, 3.1, and 4/CS, all at 100. A dropdown menu for "Cut sites visible on maps" is set to "Single cutters". To the right of the dialog, a sidebar titled "NEW DIGEST" lists "Enzyme lists" (Deduplicated commercial) and "Cut sites visible on maps" (Single cutters). A "Find enzyme" search bar contains "nc". A table lists enzymes: HincII (4 cuts, Selected), NciI (10 cuts), and NcoI (1 cut, highlighted in orange). A tooltip at the bottom of the sidebar provides options: anywhere in the sequence, ✓ in the current selection, only in the current selection, and anywhere except the current selection.

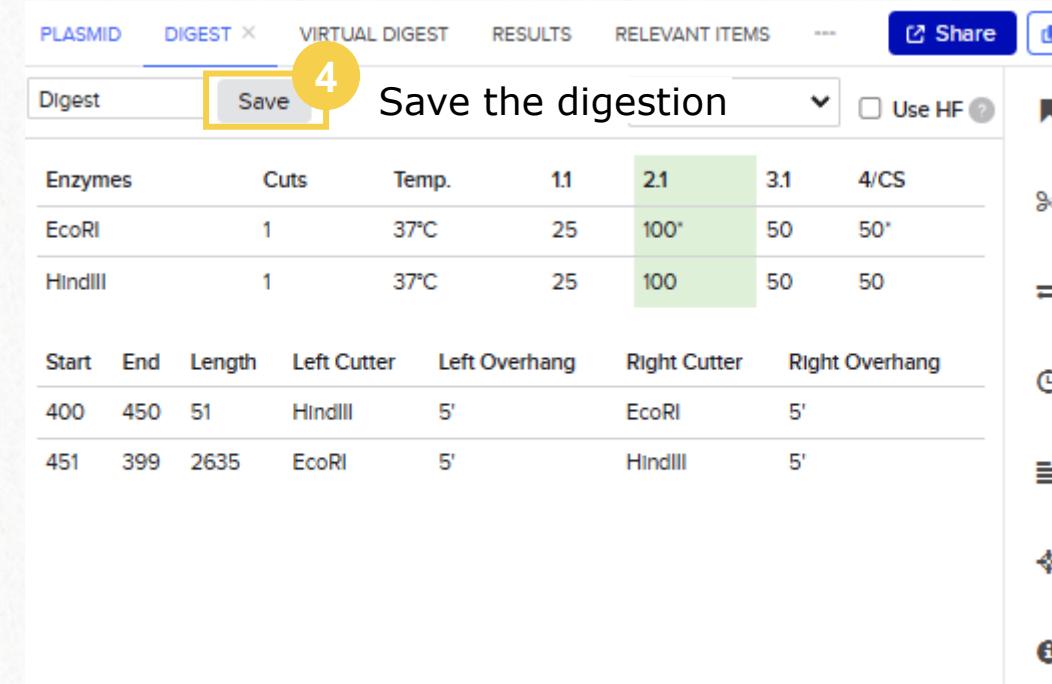
PRO TIP: The enzyme lists available can be managed, similarly to the features libraries and are shared within the Center.

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

Construct design

Virtual digestion

Digestion of the backbone



4

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

SEQUENCE MAP

The sequence map displays two genes, *vdh_fw* and *vdh*, with their respective restriction enzyme sites and gene structures.

vdh_fw (DNA) Gene:

- Enzymes:** EcoRI, ApoI, BsaAI, PmlI.
- Structure:** A green arrow labeled "vdh_fw (DNA)" points to the sequence. Below it, a blue bar represents the gene source, with an orange segment labeled "vdh gene" and a pink segment labeled "vdh CDS (vanillin dehydrogenase) source".
- Sequence:** GAATTCatatggaggatgccccaaatgtttcacgtgcggctgttattgggttaacgcgttgttcggatTTTCTTAAAGattactcctcacgggttacaaagtgcacggggacaataaccaccattcgaaacaatgtgttt

vdh Gene:

- Enzymes:** PsrI, BsaXI.
- Structure:** An orange segment labeled "vdh gene" and a pink segment labeled "vdh CDS (vanillin dehydrogenase) source".
- Sequence:** gaggcgccaccttcgagcgctgttagcccgctgacggggaaaagtggtatcgcgcgtcgtctggccaggtttctcgcgtggaaactcgccagcatcgccggcactggcttcattccatagcgcgcagcgcacgcacgttcaaa

vdh CDS (vanillin dehydrogenase) Source:

- Enzymes:** BsgI, BglI, HaeII.
- Structure:** An orange segment labeled "vdh gene" and a pink segment labeled "vdh CDS (vanillin dehydrogenase) source".
- Sequence:** atgcggccgcgcgtggccgtcacaggctgcgtttctgaatggccggcgttgccggat

BASES 1489 INSERT 37

2

3

4

Enzyme HindIII
AAGCTT
TTCTAA

NEB

Use HF

Link: NEB

Inactivation: 80°C

Incubation: 37°C

Activity:

1.1	21	3.1	4/C/S
25	100	50	50

Ios.: None

Jump to Cut Site:
1485

NEW DIGEST SAVED DIGESTS
Single cutters

Find enzyme Clear selected

Name	Cuts	Selected	Color
BstNI	2		
EcoRI	1		
HindIII			
PspGI	2		

Show enzymes that cut
anywhere In the sequence

Highlight enzymes with compatible sticky ends

Run digest

3 Find and select the REs

The digest tab will open

4

Save the digestion

4 Save the digestion

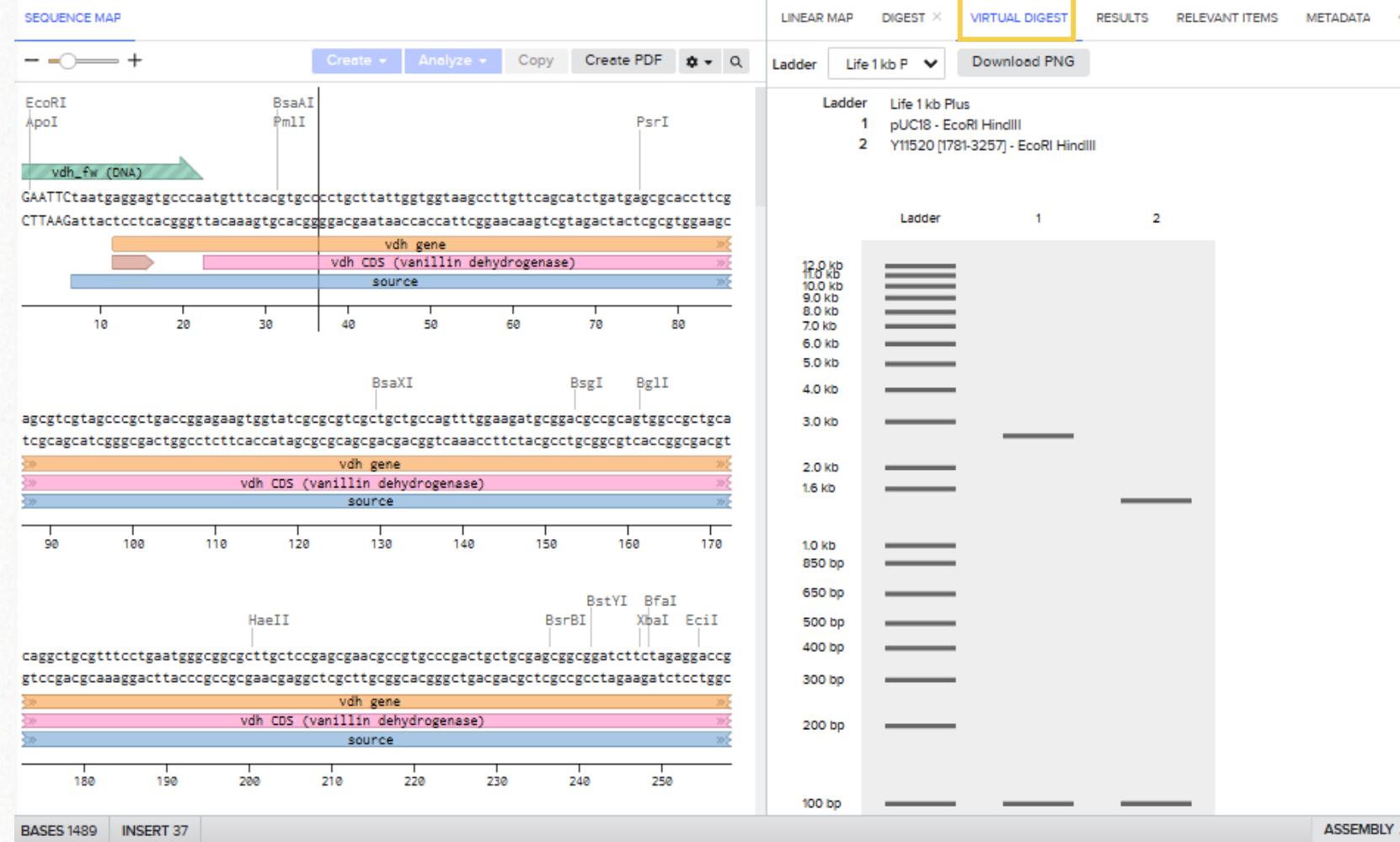
Digest		Save		Use HF		
Enzymes	Cuts	Temp.	1.1	2.1	3.1	
EcoRI	1	37°C	25	100*	50	
HindIII	1	37°C	25	100	50	
Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	EcoRI	5'
2	1484	1483	EcoRI	5'	HindIII	5'
1485	1489	5	HindIII	5'	None	blunt

Run the digestion

Construct design

Virtual digestion

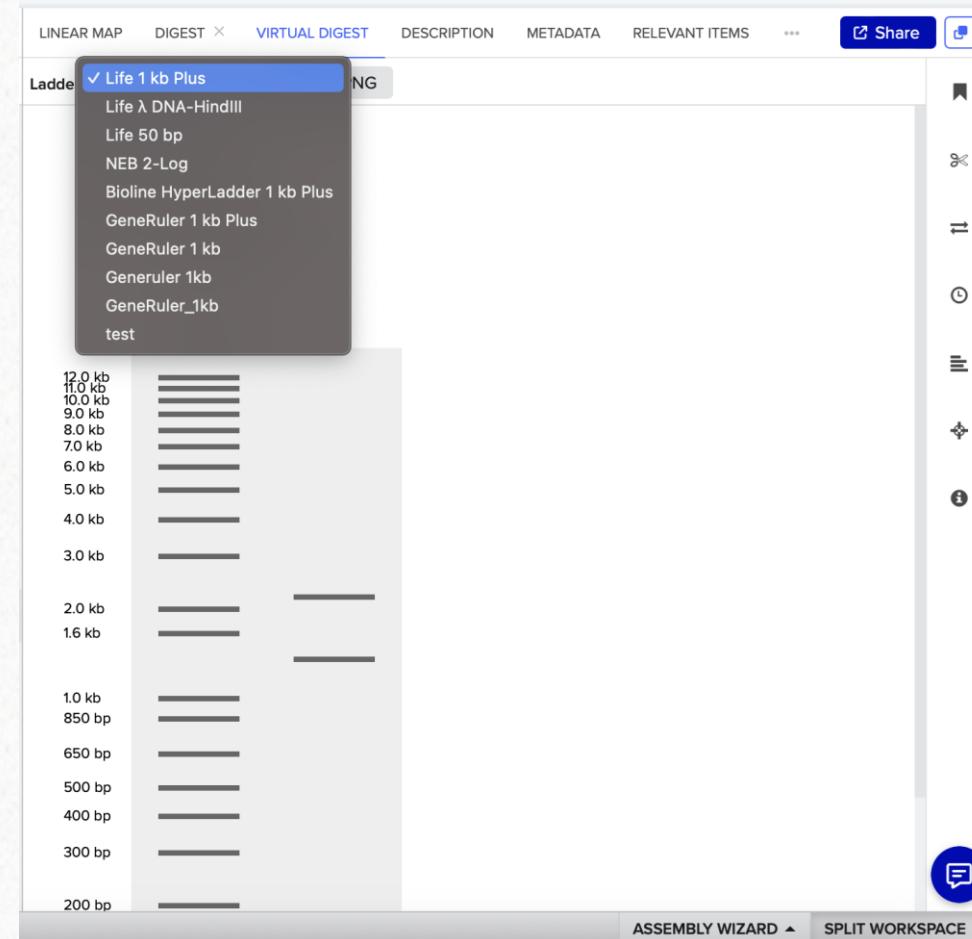
Gel visualization



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

Construct design

Virtual digestion



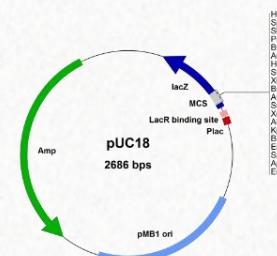
PRO TIP: It's possible to choose between different ladders

6. Construct assembly



6. Construct assembly

6.1 Assembly Wizard



pUC18 plasmid vector

Plasmid backbone

+

vdh

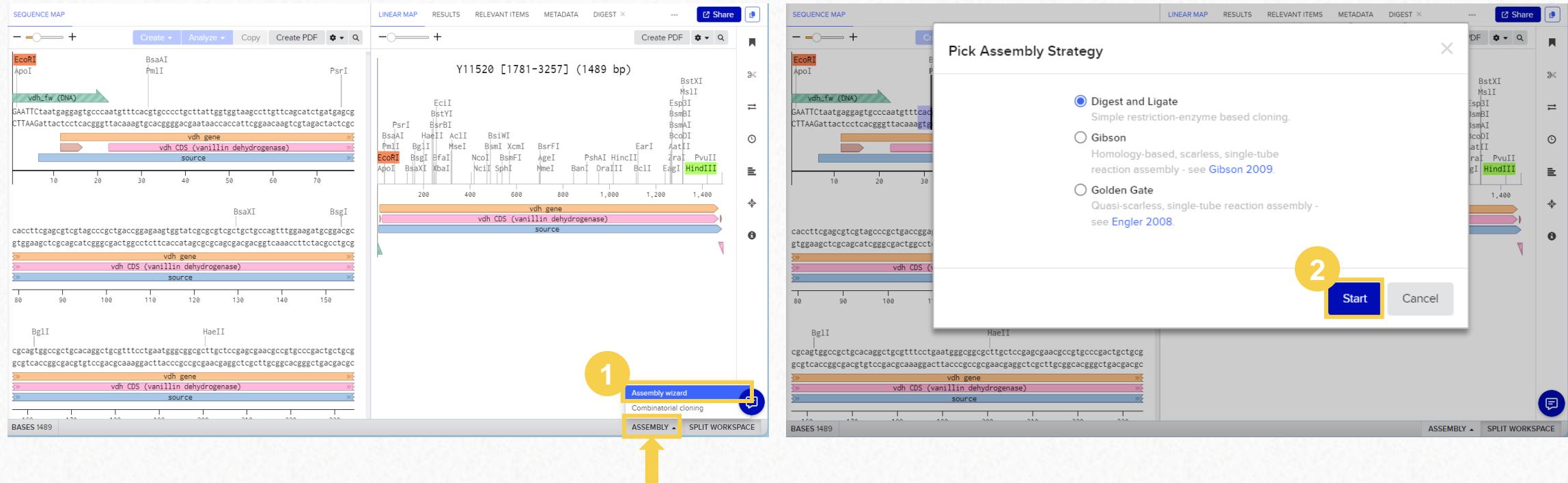
Gene of interest

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 vdh gene (vanillin dehydrogenase) and source sequence. The sequence starts with GAATTCTaatataggagtggccatgtttacgtgcggctgttattgttgttaagccgttgcacatctgtatggcg.

Sequence Map 2: Shows restriction sites EcoRI, ApoI, BsaAI, PmlI, BsrBI, PsrI, BsrI, BsrII, BsrFI, BsrVI, EarI, PshAI, HincII, ZraI, PvuII, HindIII, BglI, BsgI, BfaI, NcoI, BsmI, XcmI, SphI, NmeI, BanI, DraIII, BclI, EagI, and HindIII. It includes a vdh gene (vanillin dehydrogenase) and source sequence. The sequence starts with Y11520 [1781-3257] (1489 bp).

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, 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 QFB Assembly Wizard interface. On the left, a circular sequence map of pUC18 is displayed, showing various restriction sites and features. On the right, a table lists enzymes, cuts, temperature, and other parameters for a NEB digest. At the bottom, a yellow-highlighted section shows the 'SET FRAGMENT' and 'OVERALL ASSEMBLY' sections, where a construct named 'pUC18-vdh' is being assembled.

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

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

Name your construct

Construct Assembly

Digest and Ligate: Add the backbone

The screenshot shows the Assembly Wizard interface with four numbered steps:

- 1**: In the PREVIEW section, a yellow arrow points to the "Backbone" tab, which displays the sequence map of pUC18 with restriction sites for EcoRI and HindIII.
- 2**: In the DIGEST tab, the user has selected EcoRI and HindIII, and the resulting digest fragments are listed in the table below. A yellow circle highlights the "21" column under Enzymes.

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	
	400	450	51	HindIII	5'	EcoRI	5'
	451	399	2635	EcoRI	5'	HindIII	5'

3: In the PREVIEW section, a yellow circle highlights the "Set from Selection" button. A yellow arrow points from the "Digest" table to this button.

4: In the PREVIEW section, a yellow circle highlights the "✓" checkbox. A yellow arrow points from the "Set from Selection" button to this checkbox.

✓ The Assembly Wizard shows the digested ends of the backbone

Construct Assembly

Digest and Ligate: Add the insert

The screenshot illustrates the 'Digest and Ligate' step in the QFB Construct Assembly Wizard. The interface is divided into two main sections:

Top Section (Sequence Map):

- Linear Map:** Shows the plasmid Y11520 [1781-3257] (1489 bp) with various restriction sites labeled along the top. A yellow circle labeled **2** points to the HindIII site at position 1489.
- Sequence Map:** Below the linear map, it shows the backbone construct (pUC18, 2.6 kb) with EcoRI and HindIII sites, and the inserted gene (vdh gene, vdh CDS (vanillin dehydrogenase)).
- Digest Table:** A table showing enzyme digest parameters. The 'NEB' dropdown is selected. The table includes columns for Enzymes, Cuts, Temp., and various cutter/overhang details.

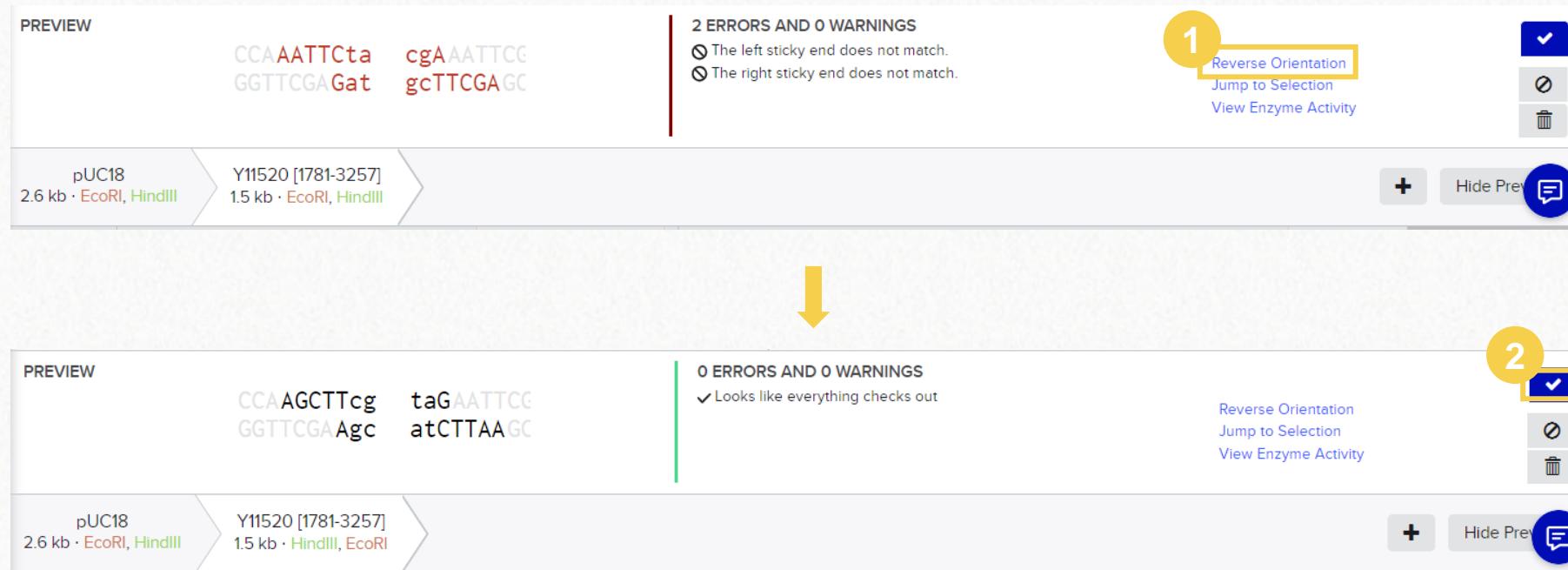
Bottom Section (Preview):

- PREVIEW:** Displays the sequence of the construct. It shows the pUC18 backbone sequence (CCA GGTCGA AATTCTGT GCA) flanking the inserted gene sequence (vdh gene).
- Instructions:** A text box states: "Shift select two enzymes on the sequence map or run a digest and select a fragment."
- Buttons:** Includes a "Set from Selection" button (yellow circle **3**) and a trash bin icon.

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 Assembly Wizard interface with two preview panels and a central status bar.

Top Preview Panel: Shows the initial assembly configuration. The backbone (pUC18) has restriction sites EcoRI and HindIII at its ends. The insert (Y11520 [1781-3257]) also has EcoRI and HindIII sites. The sticky ends are shown as CCA AATT Cta and cgA AATT CG. The status bar indicates "2 ERRORS AND 0 WARNINGS" with two error icons: "The left sticky end does not match" and "The right sticky end does not match". A yellow circle labeled "1" points to the "Reverse Orientation" button in the toolbar, which is highlighted with a yellow box.

Bottom Preview Panel: Shows the result after reversing the orientation of the backbone. The backbone now has HindIII and EcoRI sites at its ends. The insert remains the same. The sticky ends are now taG AATT CG and atCTTAA GC. The status bar indicates "0 ERRORS AND 0 WARNINGS" with a green checkmark icon and the message "Looks like everything checks out". A yellow arrow points down from the top panel to the bottom panel, indicating the progression. A yellow circle labeled "2" points to the "Reverse Orientation" button in the toolbar of the bottom panel, which is also highlighted with a yellow box.

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

Construct Assembly

Digest and Ligate: Assemble

SET FRAGMENT
Select an assembly fragment below.

OVERALL ASSEMBLY
✓ Looks like everything checks out

SEQUENCE MAP

LINER MAP **PLASMID** **DESCRIPTION** **METADATA** **RELEVANT ITEMS** **Share**

1

Assemble

Linear Map: Shows the sequence map of the construct. It includes restriction sites (HindIII, EcoRI, BstXI), promoters (AmpR, LacZα), and CDS regions (vdh CDS). The sequence is shown in three segments: pUC18 (2.6 kb), Y11520 (1.5 kb), and M13 (1.5 kb).

Plasmid Map: A circular map of the pUC18-vdh construct. It shows the 4118 bp plasmid with various restriction sites (EcoI, NdeI, SphI, KpnI, etc.), promoters (AmpR, LacZα), and CDS regions (vdh CDS). The map also indicates the insertion point of the Y11520 fragment.

Sequence Map: Detailed sequence views for the pUC18, Y11520, and M13 fragments, showing the DNA sequence, restriction sites, and gene structures (lacZα, vdh CDS).

- ✓ 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

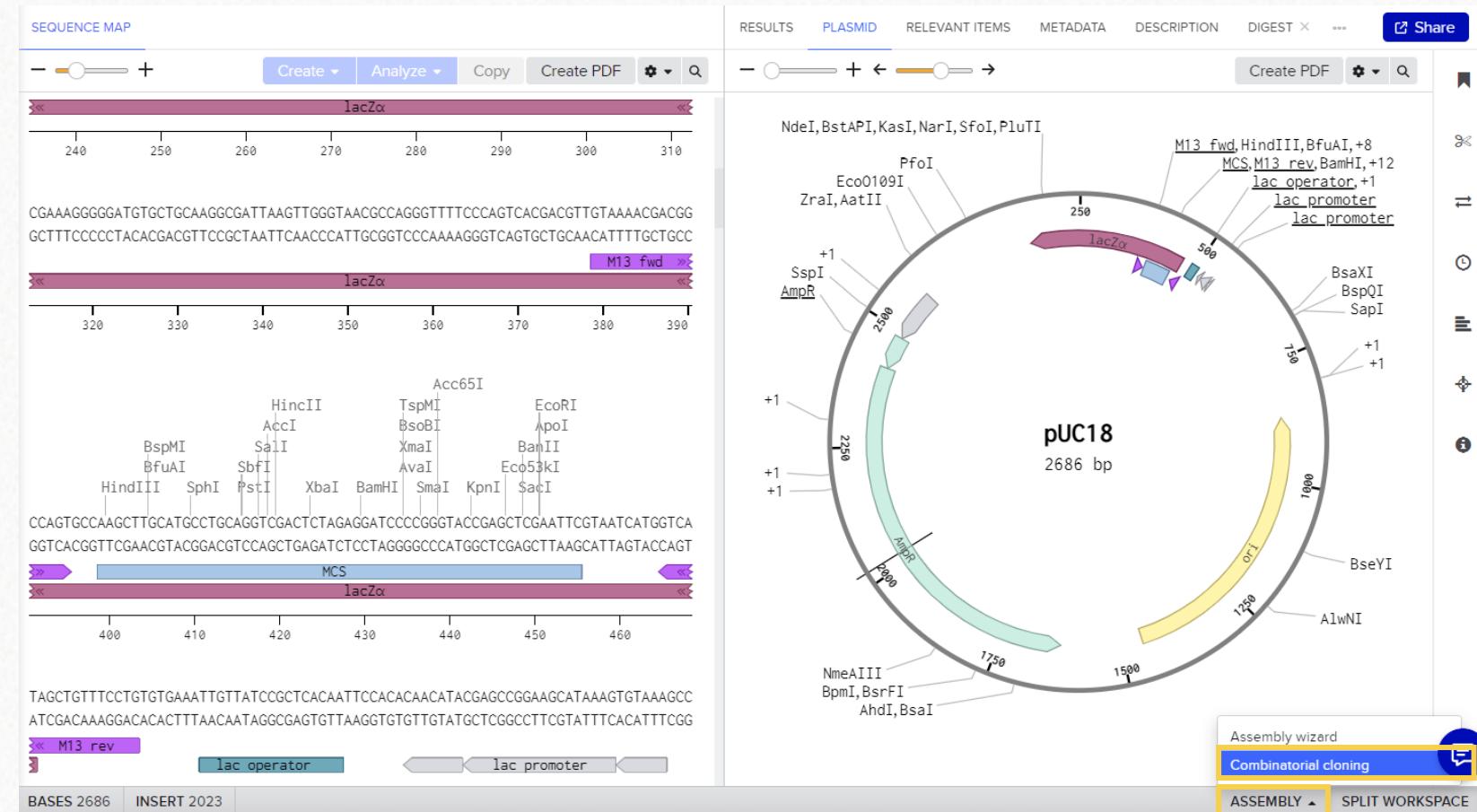
It allows you to work with several cloning methods:

- ✓ **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 displays a 'Golden Gate assembly' record under the 'OVERVIEW' tab. It shows three bins: BIN 1 (Backbone, 1 fragment), BIN 2 (Promoter, 3 fragments), and BIN 3 (Gene, 8 fragments). An arrow points from these bins to a 'Constructs' section which lists 24 constructs. Below this, a table titled 'Fragments' lists 12 entries with columns for Sequence, Bin, Start, End, Length, Orientation, Type IIS enzyme, and Frag. The last column contains a 'Use' button. At the bottom, a table titled 'Constructs' lists four entries with columns for Name, Backbone, Overhang, Promoter, Overhang, and Gene. To the right, a grid of 12 circular construct visualizations is shown, each labeled with a name like 'backbone-promoter001-gene001' and indicating 'No associated primers'. A 'SPLIT WORKSPACE' button is located at the bottom right of the visualization grid.

Construct Assembly

Combinatorial Cloning Tool: Opening



Construct Assembly

Combinatorial Cloning Tool: Configuration

The screenshot shows the Combinatorial Cloning Tool interface. On the left, there is a "SEQUENCE MAP" panel displaying DNA sequence fragments with restriction enzyme sites (SphI, SalI, XbaI, BamHI, SmaI, KpnI, BspMI, SbfI, PstI, BfuAI, HincII, AccI, TspM1, BbsI, XbaI, Avai, BpuMI, PsfI) and regions like MCS, lacZα, and lac operator. A yellow magnifying glass highlights the "Cloning method" section in the central "Assemble DNA" dialog.

Assemble DNA

- 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***: Mía
- Number of fragment bins***: 2
- Topology of construct**: Circular
- Cloning method**: Golden Gate (highlighted)
- 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
- 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**

The screenshot shows the Combinatorial Cloning Tool interface with three numbered steps highlighting specific fields:

- Name***: pUC18-vdh-gfp
- Number of fragment bins***: 3
- Cloning method**: Gibson (highlighted)

Assemble DNA

- 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***: pUC18-vdh-gfp
- Project folder***: Mía
- Number of fragment bins***: 3
- Topology of construct**: Circular
- Cloning method**: Golden Gate (highlighted)
- Join up to 15 DNA fragments in a single-tube isothermal reaction. Show details ▾**

Construct Assembly

Combinatorial Cloning Tool: Configuration

SEQUENCE MAP

TCGGCGTTCGGATGACGGTAAACCTTGACACATAGCGCGAACCACTACTGCCACTTTGGAGACTGTGAT

CAGGGCGCGTCAAGGGGTTGGCGGGTGTGGCGGGCTGGCGCCAGCGCCACAGCGCCAGCGCGACCG

TCGGCGCAGTCGGGCAACCGCCACAGCGCCAGCGCGACCG

PluI NdeI BstAPI KasI NarI SfoI PfuTI

GCACAGATGCGTAAGGGAAAAACCGCATCAAGGGCATCGTGTCTACGATCTCTCTTTATGGCGTAGTCGGCGTA

CGGTGACCCCTTCCCCTACACGACGTCGGCTAATTG

GCAGCTGGGAAGGGGATGCTGCAAGGGATAAGCGACCCCTTCCCCTACACGACGTCGGCTAATTG

HincII TspM I Acc651

SphI SalI HincII TspM I Acc651

BpuMI SbfI PstI XbaI BamHI SmaI KpnI

GCTAGCTGCAAGTGGACTCTAGAGGATCCCCGGTACCG

CGTACGGACGTCGCAAGTGGACTCTAGGGGGCATGGC

MCS lacZx lac operator

H13 rev

Assemble DNA

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

Min. T_m of binding region ($^{\circ}\text{C}$)*: 50

Min. T_m of whole primer ($^{\circ}\text{C}$)*: 60

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

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

Max. T_m difference between primer pairs ($^{\circ}\text{C}$)*: 5

RELEVANT ITEMS

Y11520 Y11520 [1781-3257] pUC18 GFP

MATERIAL INFORMATION FOR THE "MOLECULE"

SEQUENCE

SEQUENCE MAP

ASSEMBLE DNA

REVIEW THE FOLLOWING PARAMETERS

FRAGMENT PRODUCTION METHOD

MIN. T_m OF BINDING REGION ($^{\circ}\text{C}$)*

MIN. T_m OF WHOLE PRIMER ($^{\circ}\text{C}$)*

MIN. LENGTH OF HOMOLOGY/BINDING REGIONS (BP)*

MAX. LENGTH OF HOMOLOGY/BINDING REGIONS (BP)*

MAX. T_m DIFFERENCE BETWEEN PRIMER PAIRS ($^{\circ}\text{C}$)*

RESET TO DEFAULTS

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 ($^{\circ}\text{C}$)* 50

Min. T_m of whole primer ($^{\circ}\text{C}$)* 60

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

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

Max. T_m difference between primer pairs ($^{\circ}\text{C}$)* 5

Cancel **Save**

i 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' is shown with options for 'GIBSON' and 'Assemble'. A message box informs users about the legacy bulk assembly tool. 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 count of '0 fragments'; 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 sequences. A yellow circle highlights the 'BIN 1' header. Below it, 'Backbone' is selected. A yellow box highlights the 'pUC18 DNA sequence' entry. A blue circle highlights the '+' button under 'Add new over using PCR'. A yellow circle highlights the 'Open sequences' button.

1 Find and select the backbone file

PRO TIP:

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

3 Invert selection

The 'Add fragment(s)' dialog shows a list with 'pUC18' selected. A yellow circle highlights the 'Start' field (398) and the 'End' field (456). A yellow box highlights the 'Orientation' dropdown menu, which has 'Reverse' selected. A blue box highlights the 'MCS' region on the plasmid map. A yellow arrow points to the 'Add' button. A yellow circle highlights the 'Cancel' button.

2 Select the MCS (blue)

4 Reverse orientation

5

Construct Assembly

Gibson cloning: Set fragments in corresponding bins

2. Insert 1 (*vdh*)

Bins & Spacers (3) +

BIN 1	BIN 2	BIN 3
Backbone	Insert 1	Insert 2
Add new overlaps using PCR	Add new overlaps using PCR	Add new overlaps using PCR
1 fragment +	0 fragment +	0 fragments +

Open sequences >
Search for sequences
Add from worklist

Y11520 [1781-3257]
DNA sequence

pUC18
DNA sequence

GFP
DNA sequence

- 1 Find and select the *vdh* file

Add fragment(s)

View: Linear map

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

Cancel 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 the Construct Assembly software interface. On the left, there is a vertical toolbar with icons for search, add, grid, list, and other functions. The main area has two tables: the top one is the 'Fragments' table and the bottom one is the 'Constructs' table.

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 in yellow. Another callout box above it says: "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 green), 'Save fragments' (grayed out), 'Save primers' (grayed out). 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. Buttons: 'Cancel' and 'Next' (highlighted in yellow).
- Step 2:** 'Save constructs' (green), 'Save fragments' (blue), 'Save primers' (grayed out). A note says 'Saving fragments is optional.' A checkbox 'Create DNA Sequences to represent amplified fragments' is present. Buttons: 'Back' and 'Next' (highlighted in yellow).
- Step 3:** 'Save constructs' (green), 'Save fragments' (green), 'Save primers' (blue). A note says 'Saving primers is optional.' A checkbox 'Create DNA Oligos to represent newly designed primers' is present. Buttons: 'Back' and 'Assemble' (highlighted in yellow).

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

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

Construct Assembly

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

METADATA OVERVIEW **CONSTRUCTS**

Constructs

Search constructs by name

<< < > >> 1-1 of 1 item <>

pUC18-Y11520 [1781-3257]-GFP

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

2

6 associated primers [View](#)

A circular diagram of the construct pUC18-Y11520 [1781-3257]-GFP. It shows various genetic elements: GFP (green), vdh gene (purple), vanillanil dehydrogenase (orange), source (pink), and AmpR (yellow). The map includes restriction enzyme sites like EcoRI, XbaI, SphI, KpnI, PstI, BglII, SacI, SalI, and BpuMI. Molecular weight markers are indicated at 4000, 3000, 2000, and 1000 bp.

Primer view

View constructs

pUC18-Y11520 [1781-3257]-GFP

4.8 kb

SEQUENCE PRIMERS

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	green
2 pUC18	3' primer	Design new primer	pUC18_reverse	ogcgggg... 44 bp	69.26	green
3 Y11520[1781-3257]	5' primer	Design new primer	Y11520[1781-3257]_forward	ACAGCTAT... 40 bp	68.50	green
4 Y11520[1781-3257]	3' primer	Design new primer	Y11520[1781-3257]_reverse	AGTTCTTC... 40 bp	64.70	green
5 GFP	5' primer	Design new primer	GFP_forward	cgcggcac... 44 bp	64.97	green
6 GFP	3' primer	Design new primer	GFP_reverse	GTAAAACG... 44 bp	68.32	green



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

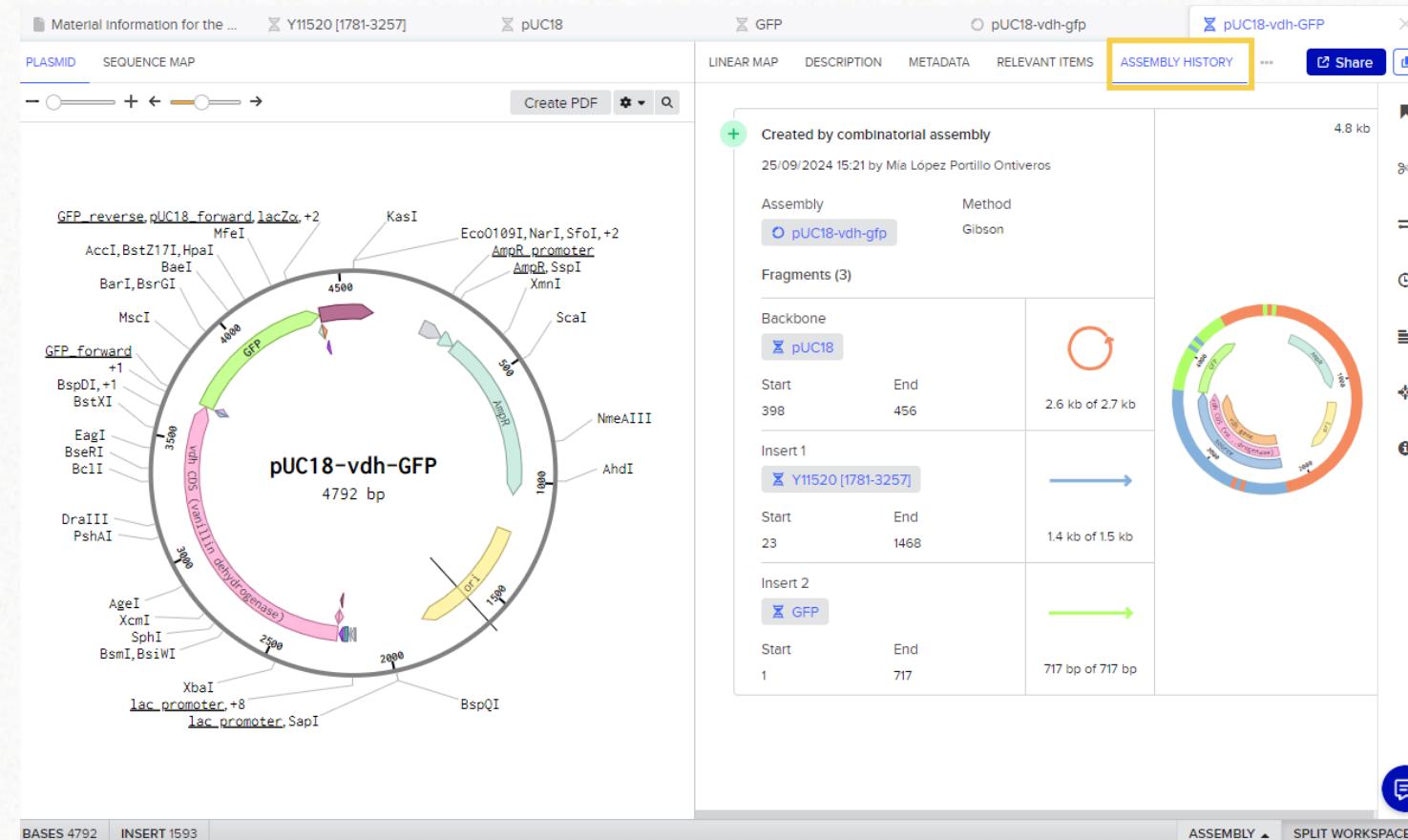


Sequence view

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:

A screenshot of the 'Create DNA / RNA alignment' interface in Benchling. The 'Pairwise' tab is selected, highlighted with a yellow border. The interface shows a template sequence (blue) and multiple non-template sequences (red, green, yellow). An arrow points from the template to the aligned sequences. Below the sequences, there are fields for 'Template(s)' and 'Non-template sequence(s)', and a dropdown for 'Choose an alignment program' set to 'Auto (MAFFT)'. A note at the bottom states 'Alignments performed via MAFFT v7 (Katoh, Standley 2013.)'.

1

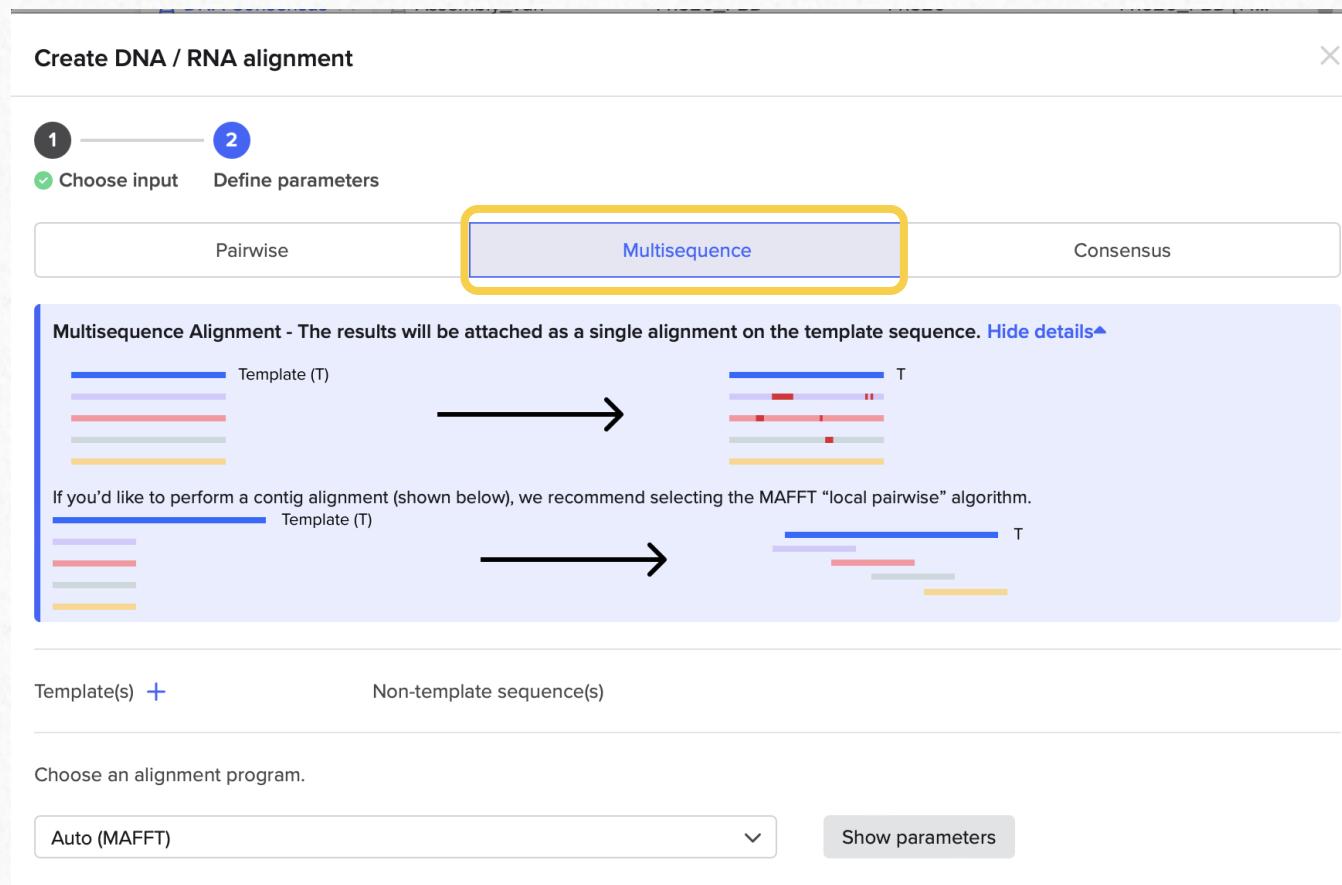
Pairwise alignment:

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

Alignment creation

Alignment tool overview

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



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)' with four colored horizontal bars (blue, purple, red, green) aligned against a template sequence with vertical dashes. The second diagram shows a 'Template (T)' with four colored horizontal bars aligned against a template sequence with gaps indicated by arrows. 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, there are fields for 'Template(s)' and 'Non-template sequence(s)', and a dropdown menu for 'Choose an alignment program' set to 'Auto (MAFFT)'.

2

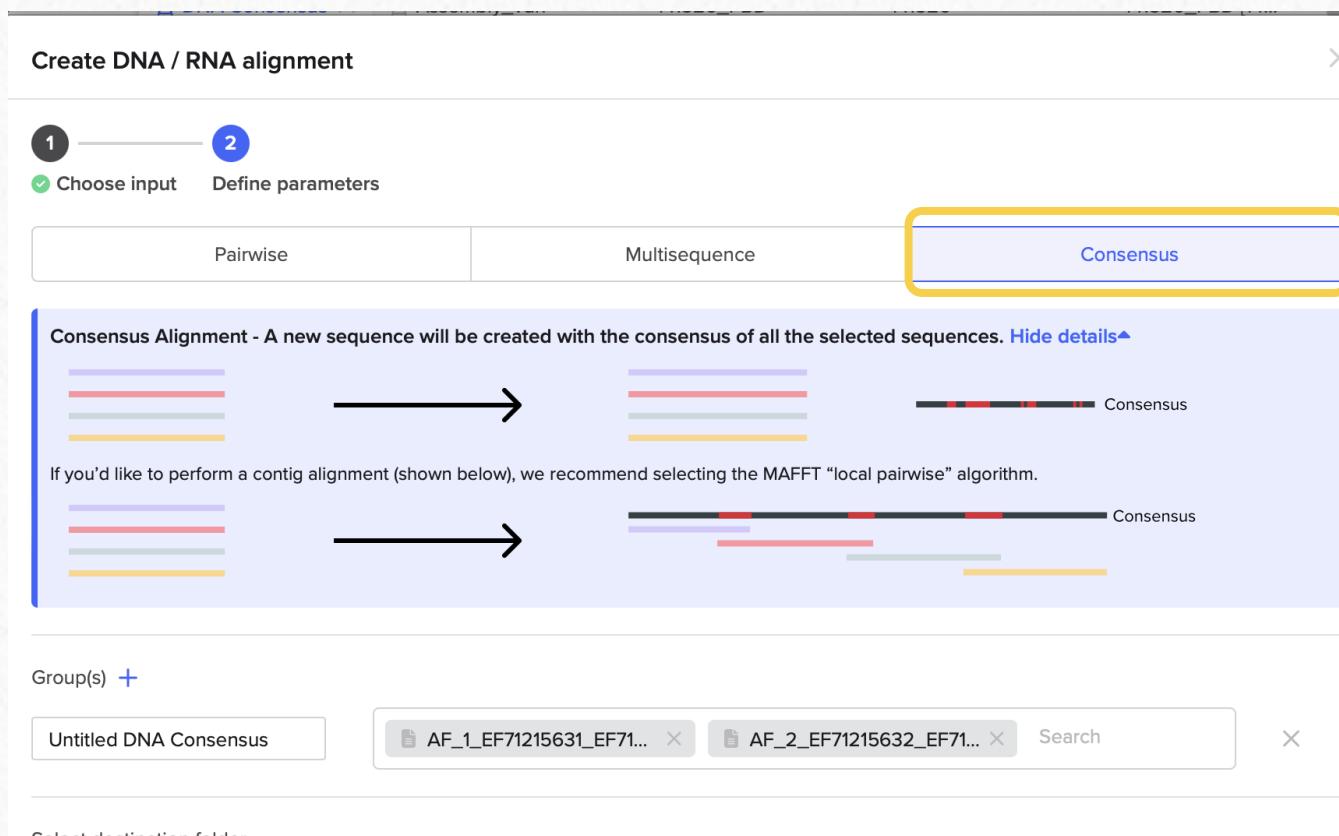
Multisequence alignment:

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:



3

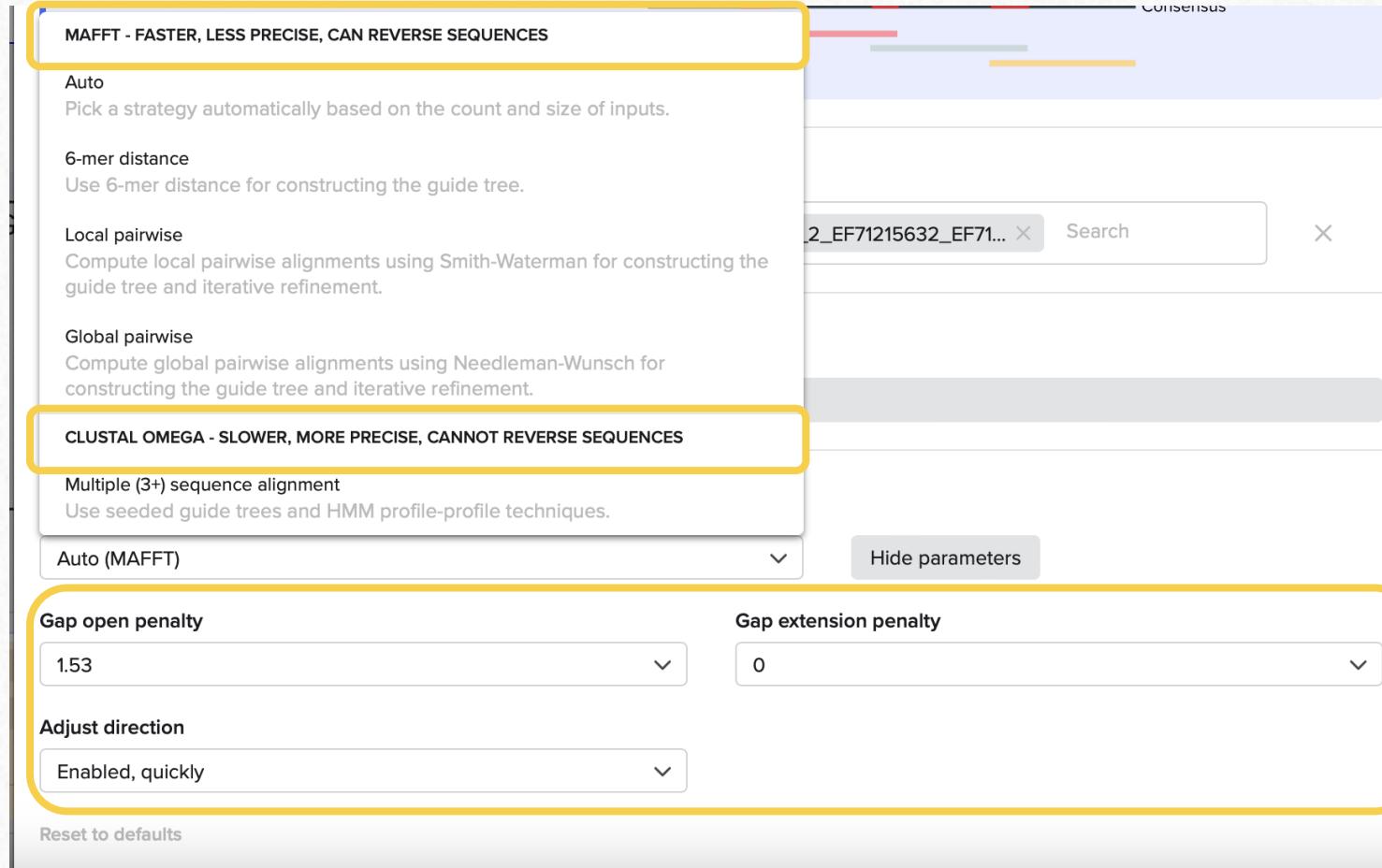
Consensus alignment:

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

Below the sidebar, the main panel shows a sequence viewer with a search bar containing "2_EF71215632_EF71...". A dropdown menu is set to "Auto (MAFFT)". A "Hide parameters" button is visible. At the bottom, there are controls for "Gap open penalty" (set to 1.53), "Gap extension penalty" (set to 0), "Adjust direction" (set to "Enabled, quickly"), and a "Reset to defaults" button.

- ✓ 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 QFB ELN interface with a sidebar on the left containing various project management icons. A yellow circle with the number 1 highlights the 'Create a new alignment' button. The main workspace shows a 'Create DNA / RNA alignment' dialog. A yellow circle with the number 2 highlights the 'Drag or select the sequences files' area, which includes a 'Choose files' button and a 'Search for a DNA / RNA sequence' input field. A yellow circle with the number 3 highlights the 'Next' button at the bottom right of the dialog. The background workspace shows a sequence map with several DNA/RNA sequences listed.

1 Create a new alignment

2 Drag or select the sequences files

3 Next

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▼

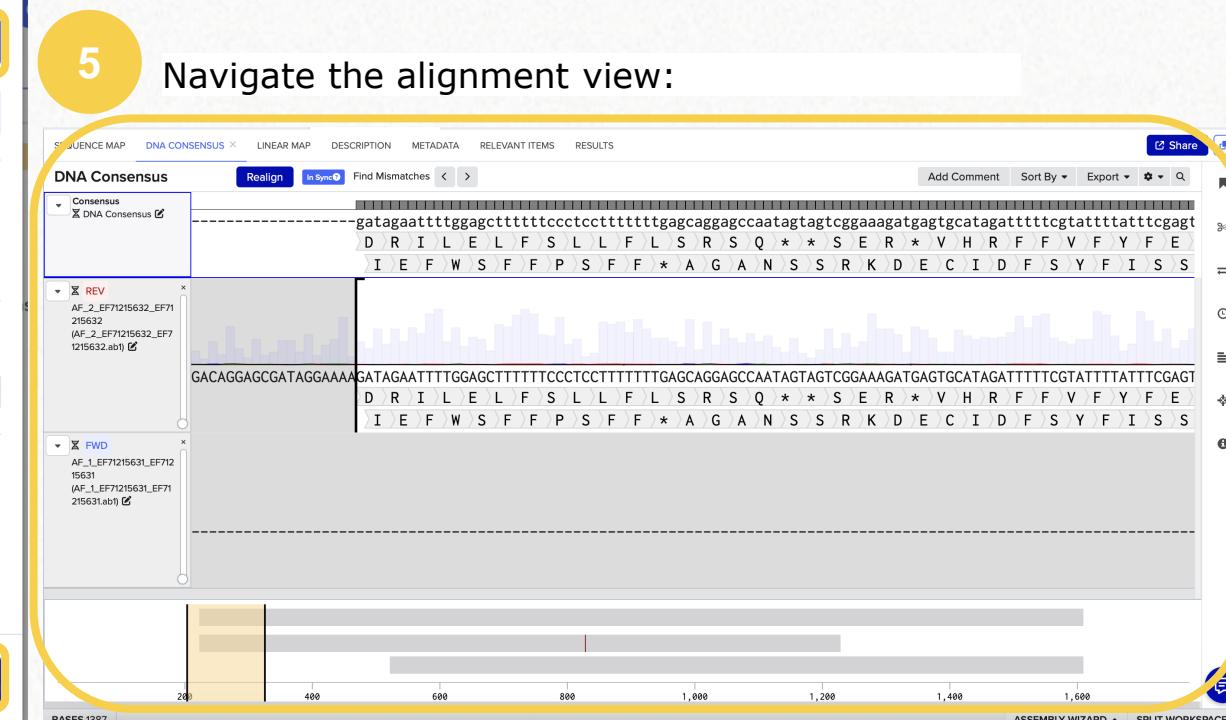
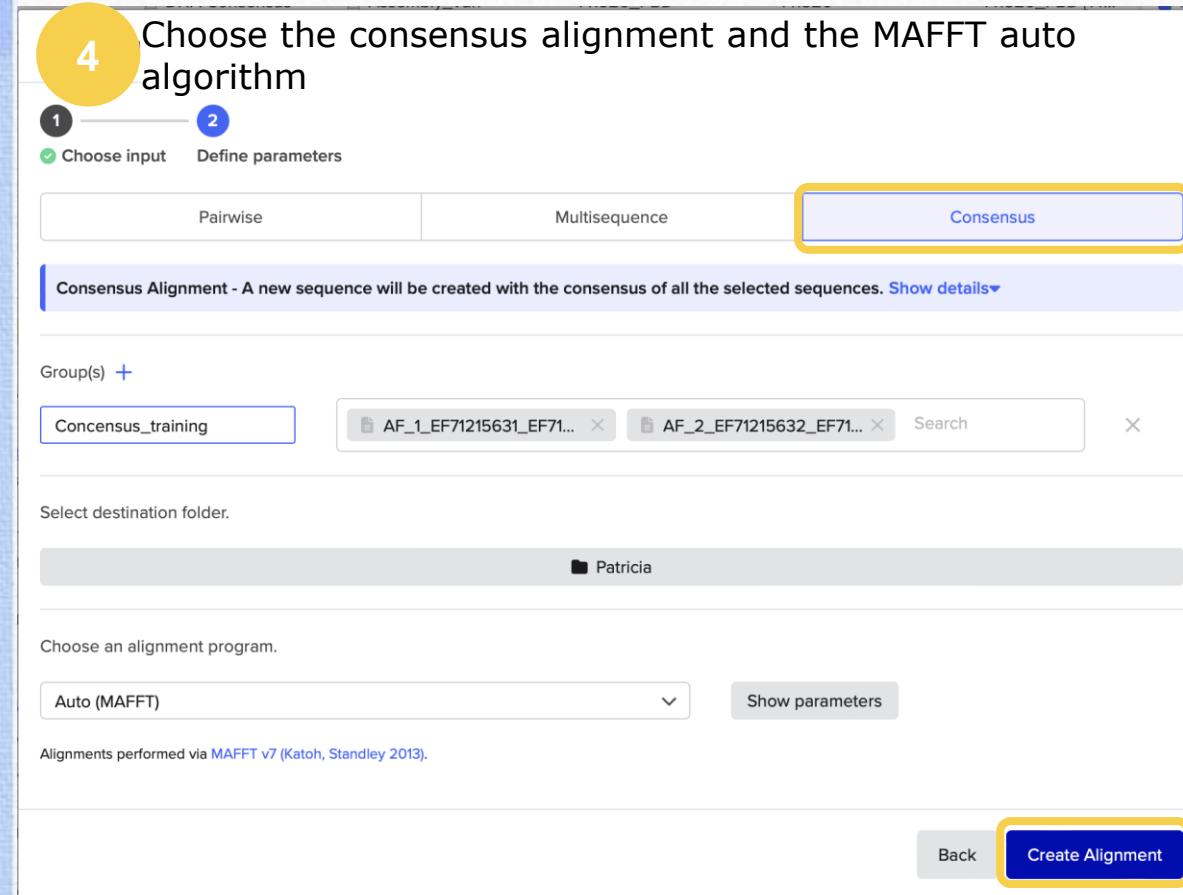
Group(s) +
Concensus_training AF_1_EF71215631_EF71... AF_2_EF71215632_EF71... Search

Select destination folder.
Patricia

Choose an alignment program.
Auto (MAFFT) Show parameters

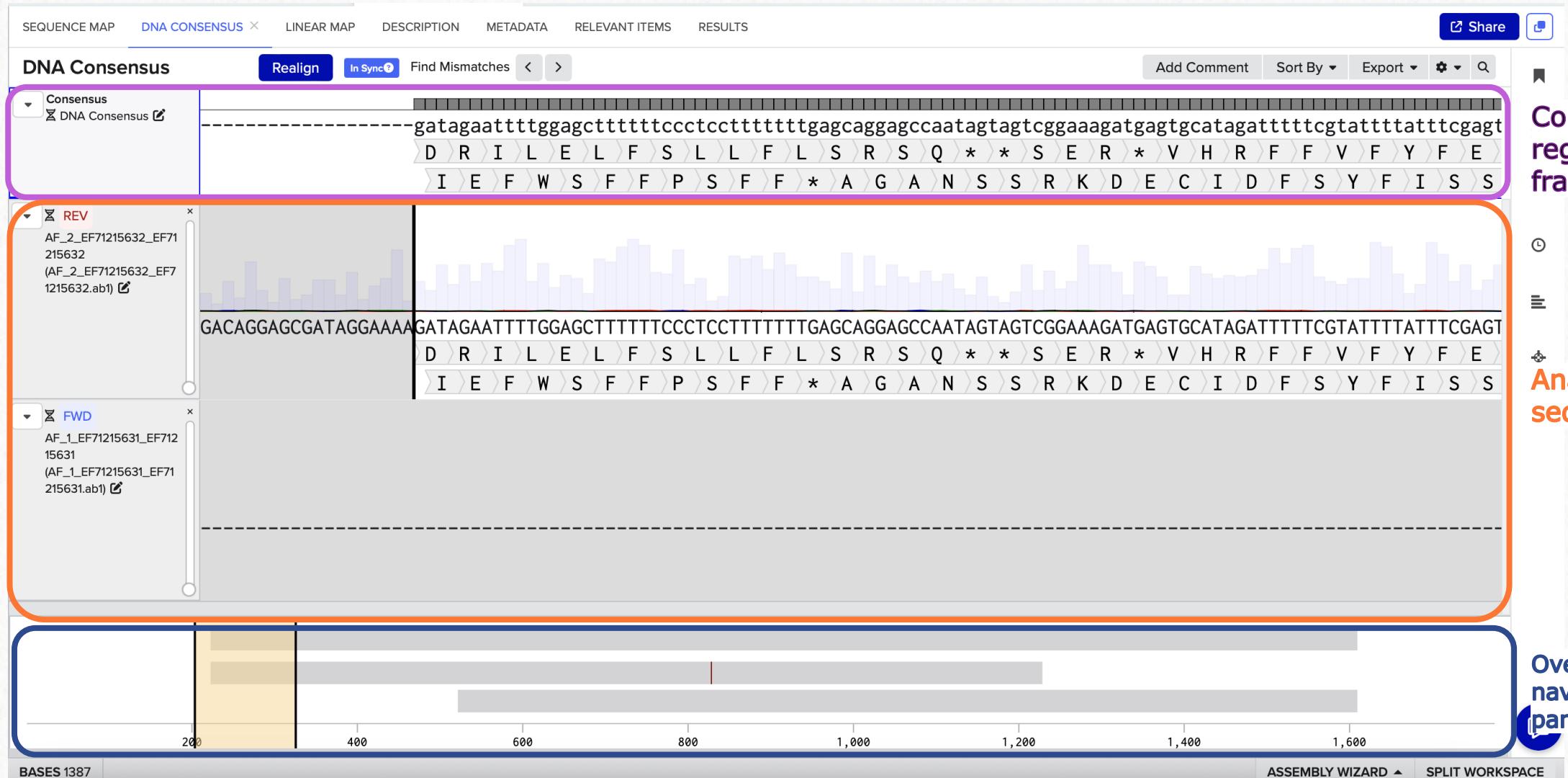
Alignments performed via MAFFT v7 (Katoh, Standley 2013).

Back Create Alignment



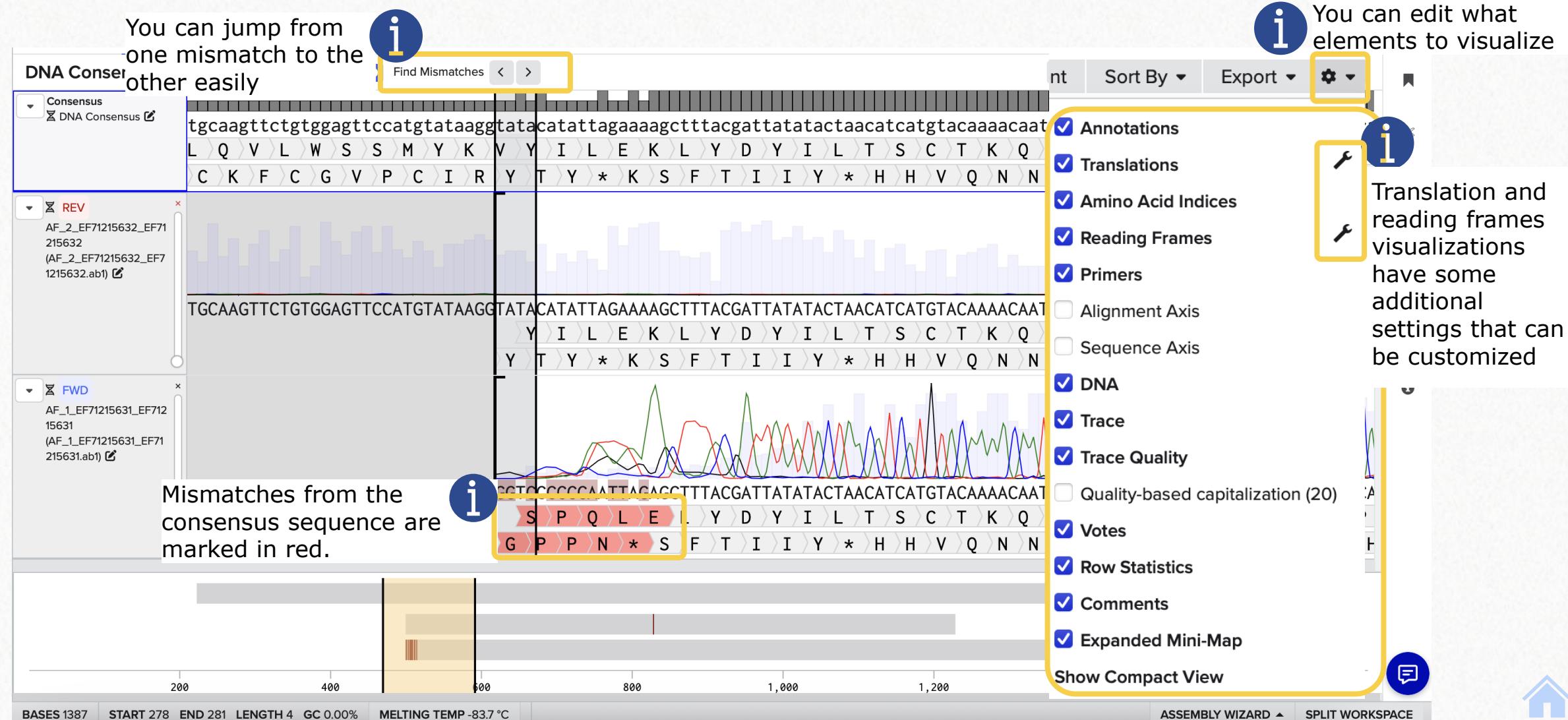
Alignment creation

Consensus alignment navigation



Alignment creation

Consensus alignment navigation

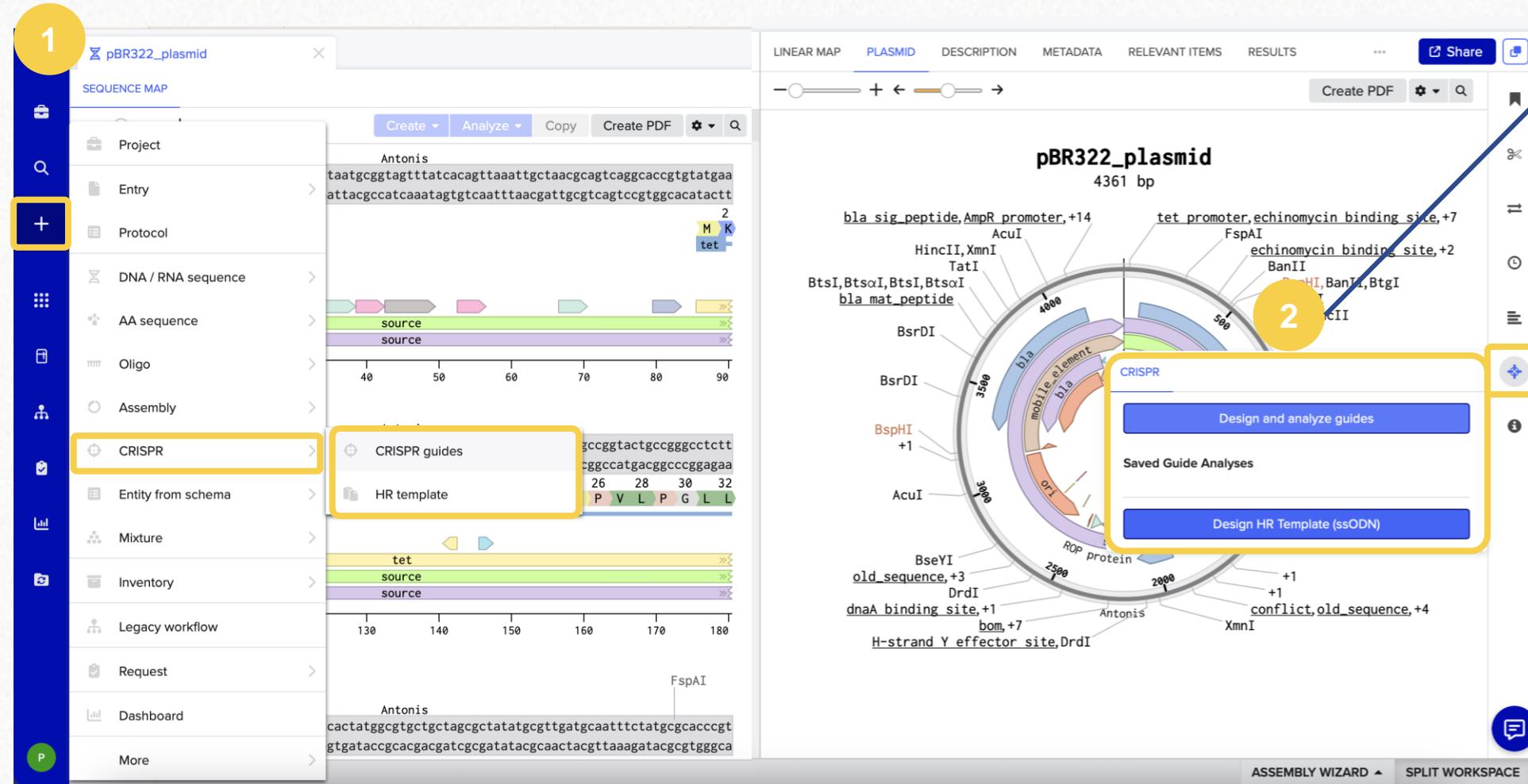


8. CRISPR



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 panels. The left panel is a sidebar with a dark blue header and a light blue footer. It features a yellow circle with the number '1' at the top, followed by a '+' button, a search icon, and a list of tools: Project, Entry, Protocol, DNA / RNA sequence, AA sequence, Oligo, Assembly, CRISPR (highlighted with a yellow box), Entity from schema, Mixture, Inventory, Legacy workflow, Request, Dashboard, and More. The right panel shows a plasmid map for 'pBR322_plasmid' with a total length of 4361 bp. The map includes various restriction sites (BsrDI, BspHI, AcuI, HincII, XbaI, TatI, FspAI, BanII, BtgI, KpnI, SacII, BtsI, BtsαI, BtsI, BtsαI) and genetic elements like 'bla sig_peptide, AmpR_promoter, +14', 'tet_promoter, echinomycin binding site, +7', 'bla_mat_peptide', 'mobile_element', 'ori', 'ROP protein', and 'Antonis' genes. A yellow circle with the number '2' highlights a callout box over the CRISPR section of the map. This callout box contains three buttons: 'Design and analyze guides', 'Saved Guide Analyses', and 'Design HR Template (ssODN)'. An arrow points from the text 'By default, Benchling will use the open sequence as to design the gRNA on' to the 'Design and analyze guides' button.

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

Brca2 (ENSMUSG00000041147)

Brca2-201 Exon 1

Brca2

150,522,640 150,522,660 150,522,

Brca2-201 Exon 1

Brca2

150,522,740 150,522,760

Brca2-201 Exon 1

Brca2

150,522,820 150,522,840 150,522,860 150,522,880

Brca2-201 Exon 1

Brca2

150,522,920 150,522,940 150,522,960

Bases: 47117

EVANT ITEMS RESULTS Share

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

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

9. Tips and tricks





Tips and tricks

Overview:

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



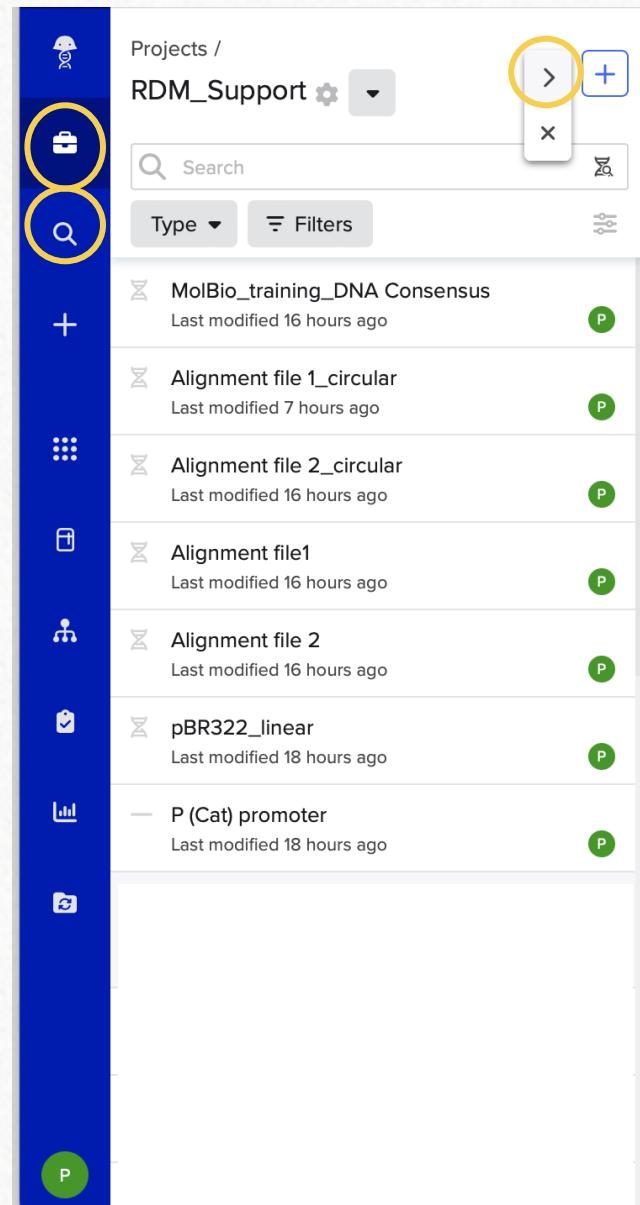
Tips and tricks

Work in bulk using the expanded view

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

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

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



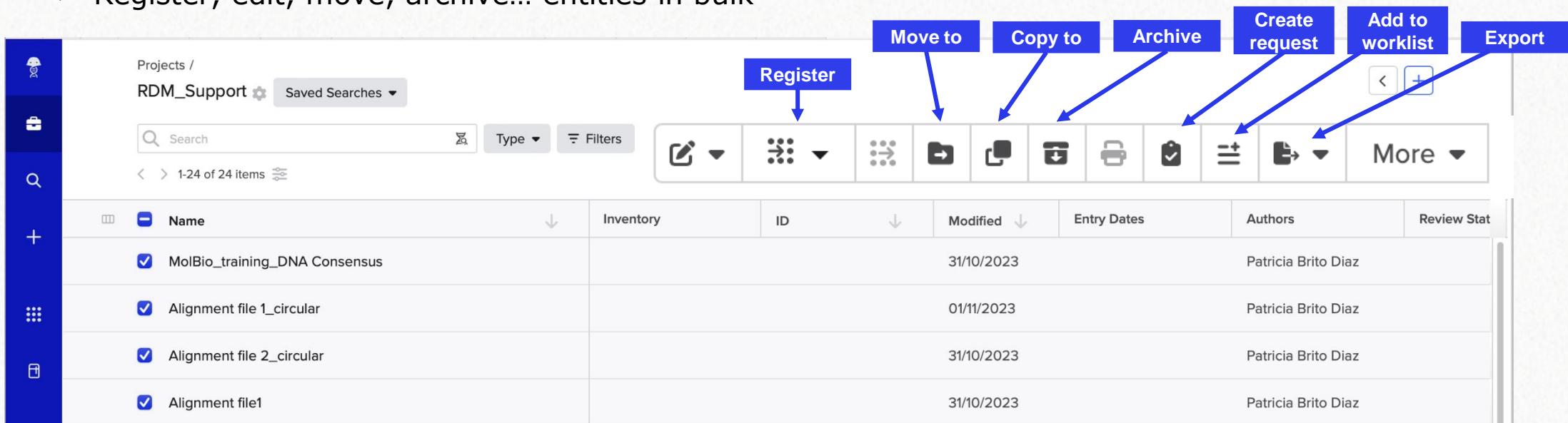
Entity	Last modified	Type
MolBio_training_DNA Consensus	16 hours ago	P
Alignment file 1_circular	7 hours ago	P
Alignment file 2_circular	16 hours ago	P
Alignment file1	16 hours ago	P
Alignment file 2	16 hours ago	P
pBR322_linear	18 hours ago	P
P (Cat) promoter	18 hours ago	P

Tips and tricks

Work in bulk using the expanded view

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

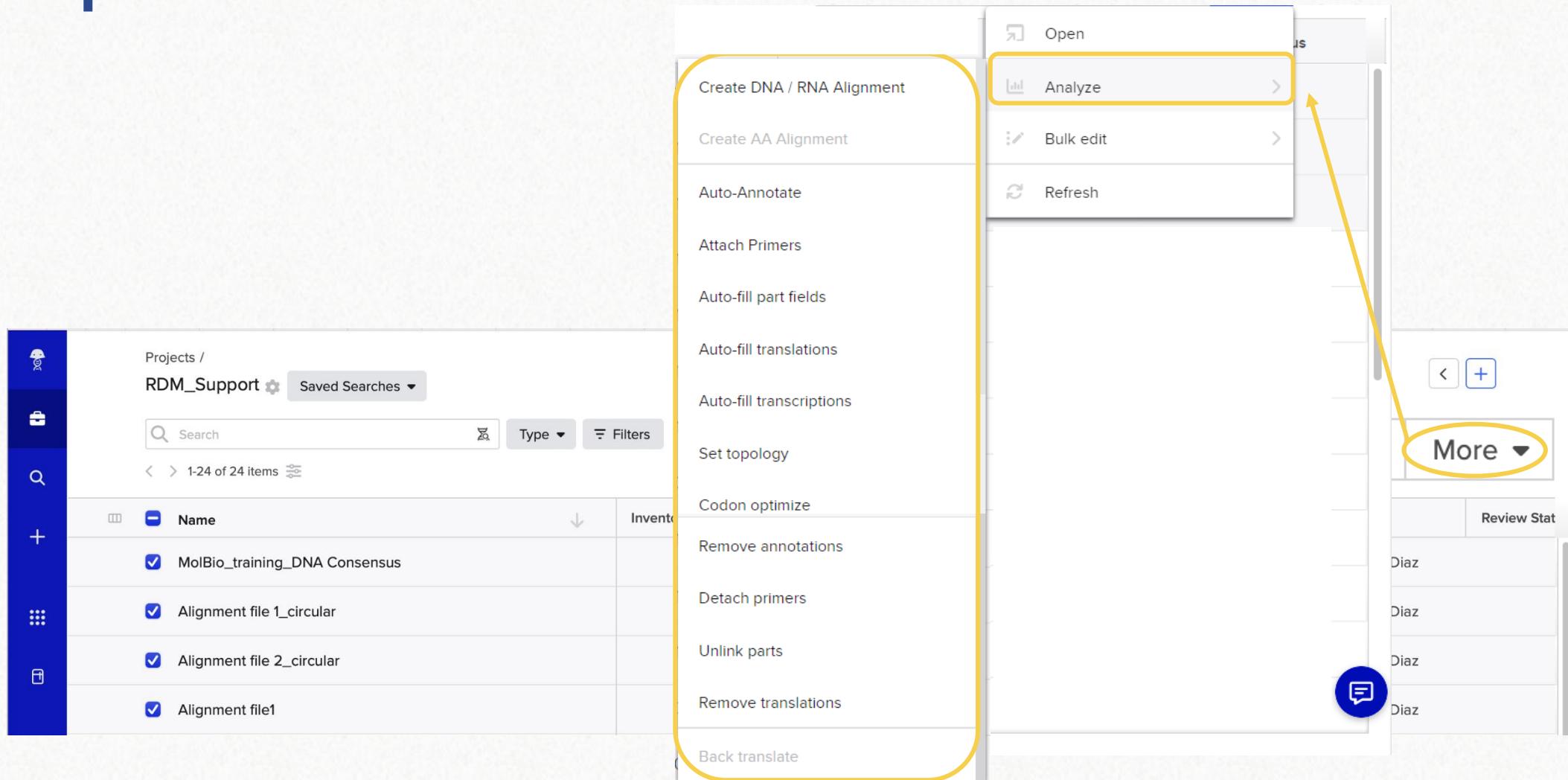
- ✓ Register, edit, move, archive... entities in bulk



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

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

Tips and tricks



The screenshot shows a software interface with a vertical toolbar on the left containing icons for projects, search, and other functions. The main area displays a list of items under the heading "Projects / RDM_Support". A context menu is open over one of the items, with several options highlighted by a yellow oval:

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

Below this menu, another yellow oval highlights the "More" button in a dropdown menu:

- Open
- Analyze (highlighted)
- Bulk edit
- Refresh

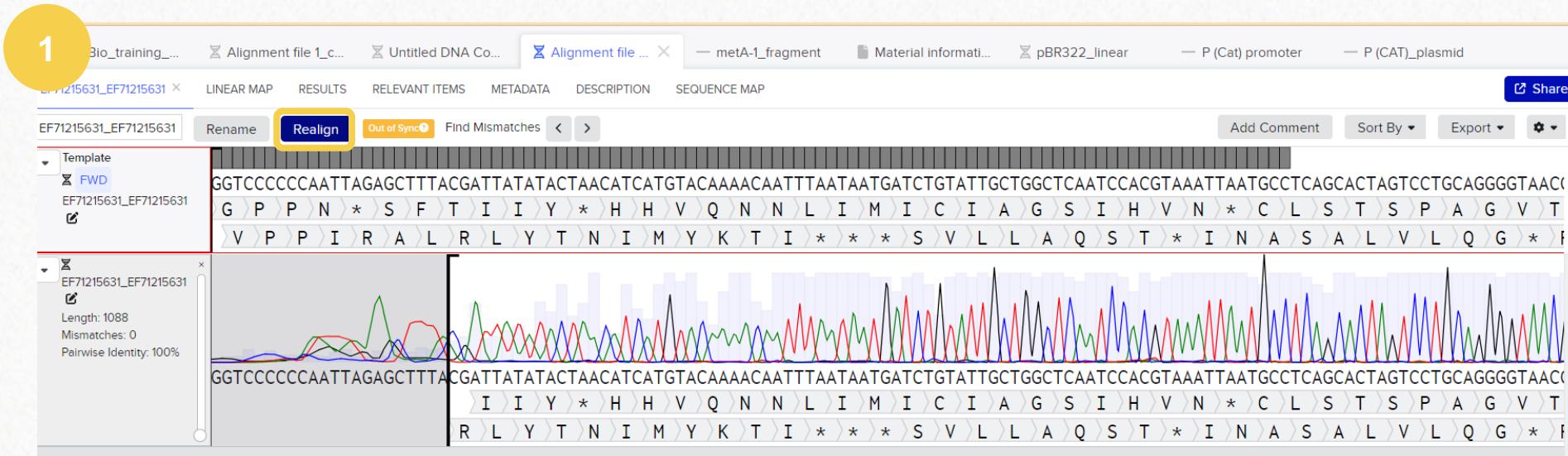
On the right side of the interface, there is a panel titled "Review Stat" showing a list of entries, each with a "Diaz" label. A blue speech bubble icon is located at the bottom right of this panel.

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

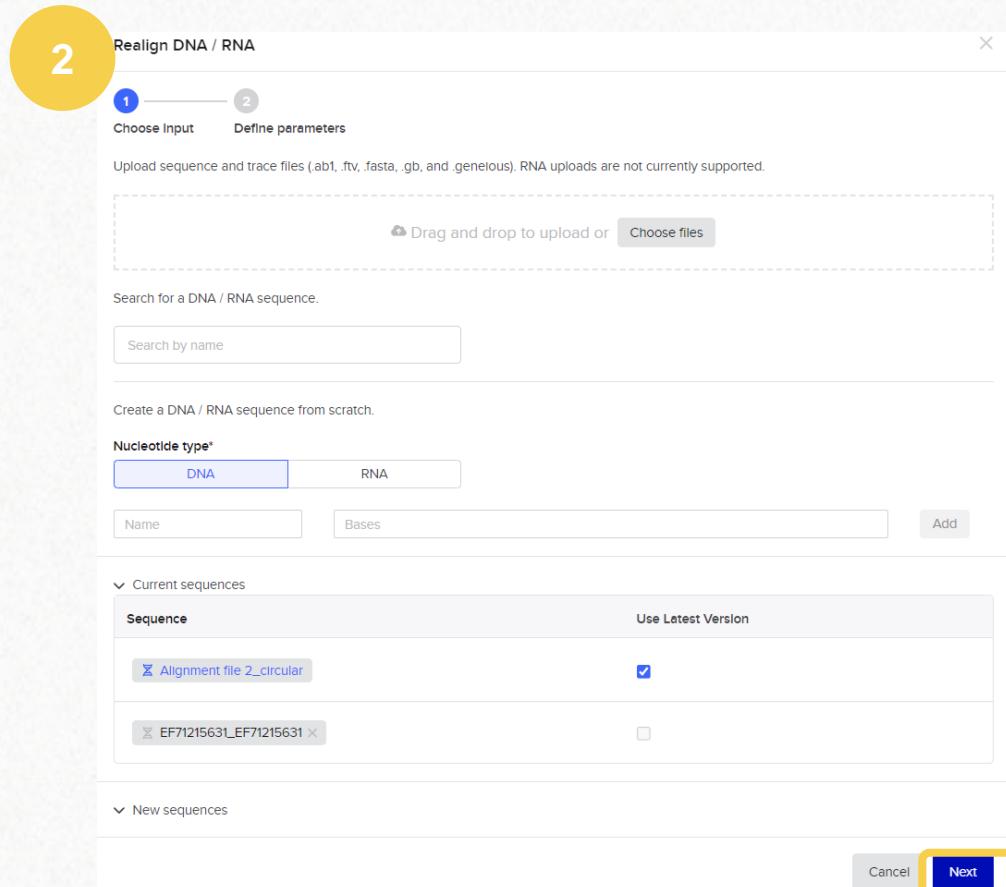
Name Bases Add

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

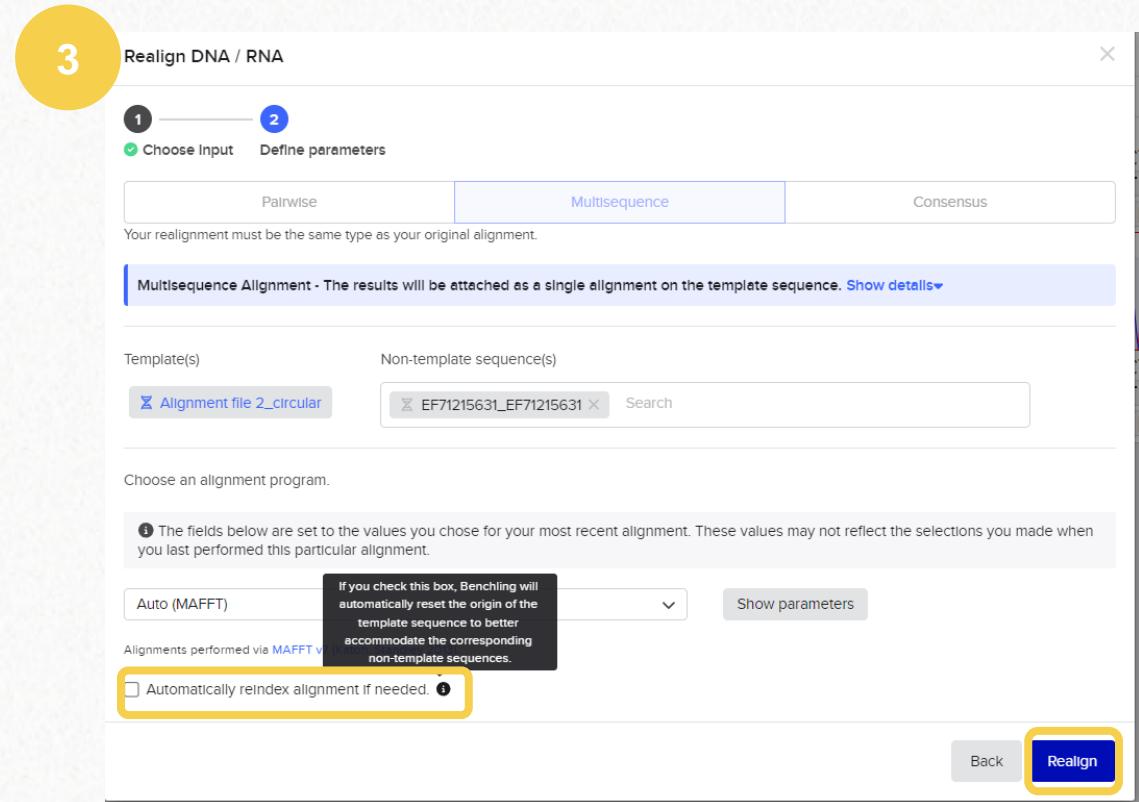
Choose an alignment program.

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

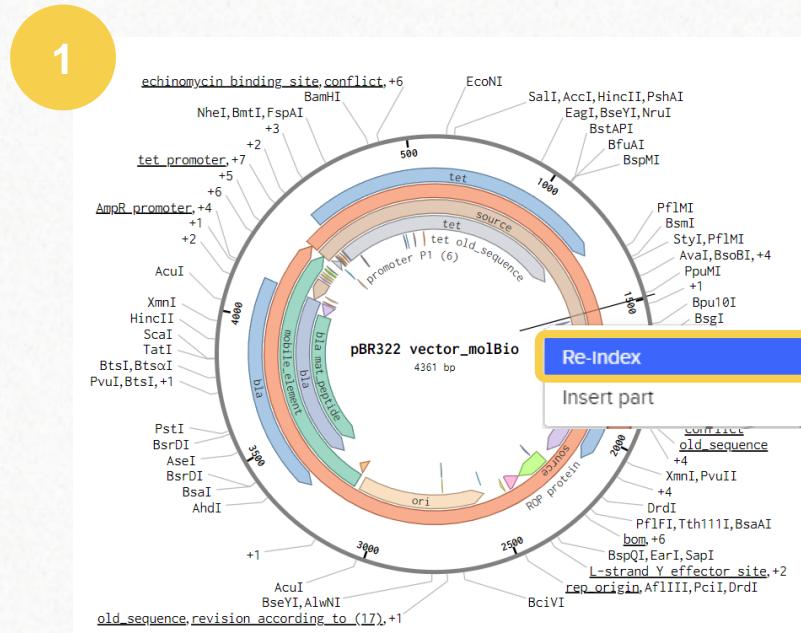
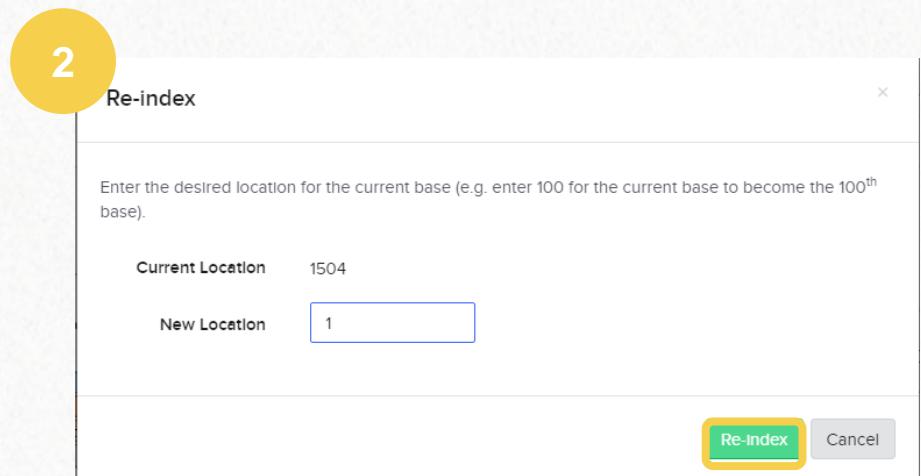
Autoindexing when creating alignments

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.

Re-index

Enter the desired location for the current base (e.g. enter 100 for the current base to become the 100th base).

Current Location 1504

New Location 1

Re-Index Cancel

10. Resources





Questions?

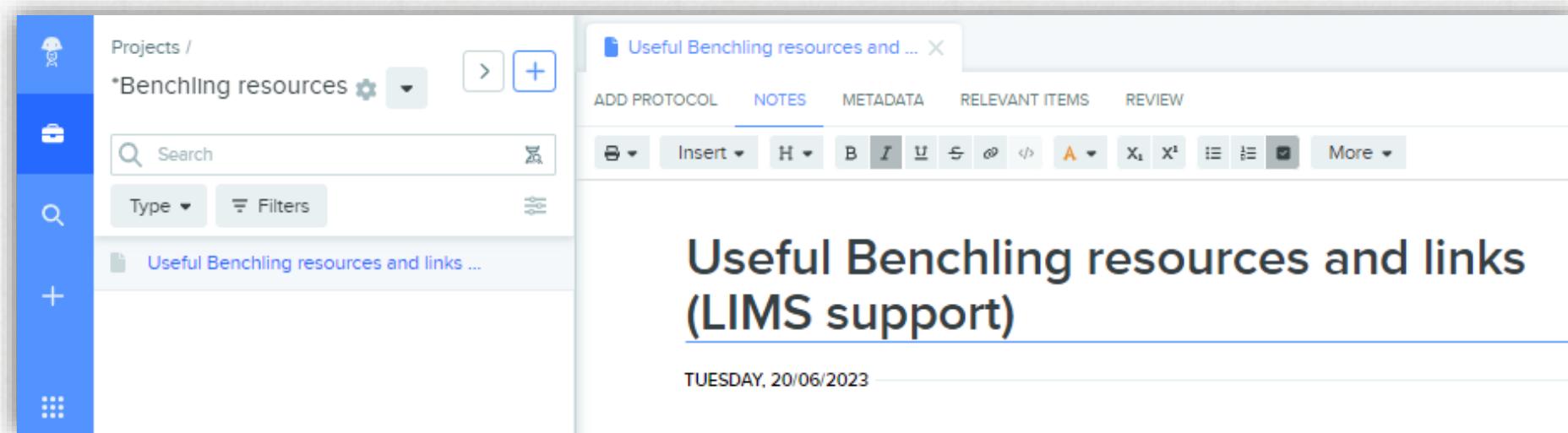


Contact lims_support@biosustain.dtu.dk

More resources

In-house resources (specific to Benchling at Biosustain)

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



The screenshot shows the Benchling software interface. On the left, there's a sidebar with icons for Projects, Notebook, and other functions. A yellow arrow points from the left margin towards the search bar in the main panel. The main panel displays a notebook entry titled "Useful Benchling resources and ...". The entry includes tabs for ADD PROTOCOL, NOTES (which is selected), METADATA, RELEVANT ITEMS, and REVIEW. Below the tabs is a toolbar with various icons for text styling and document management. The main content area contains the title "Useful Benchling resources and links (LIMS support)" and the date "TUESDAY, 20/06/2023".

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

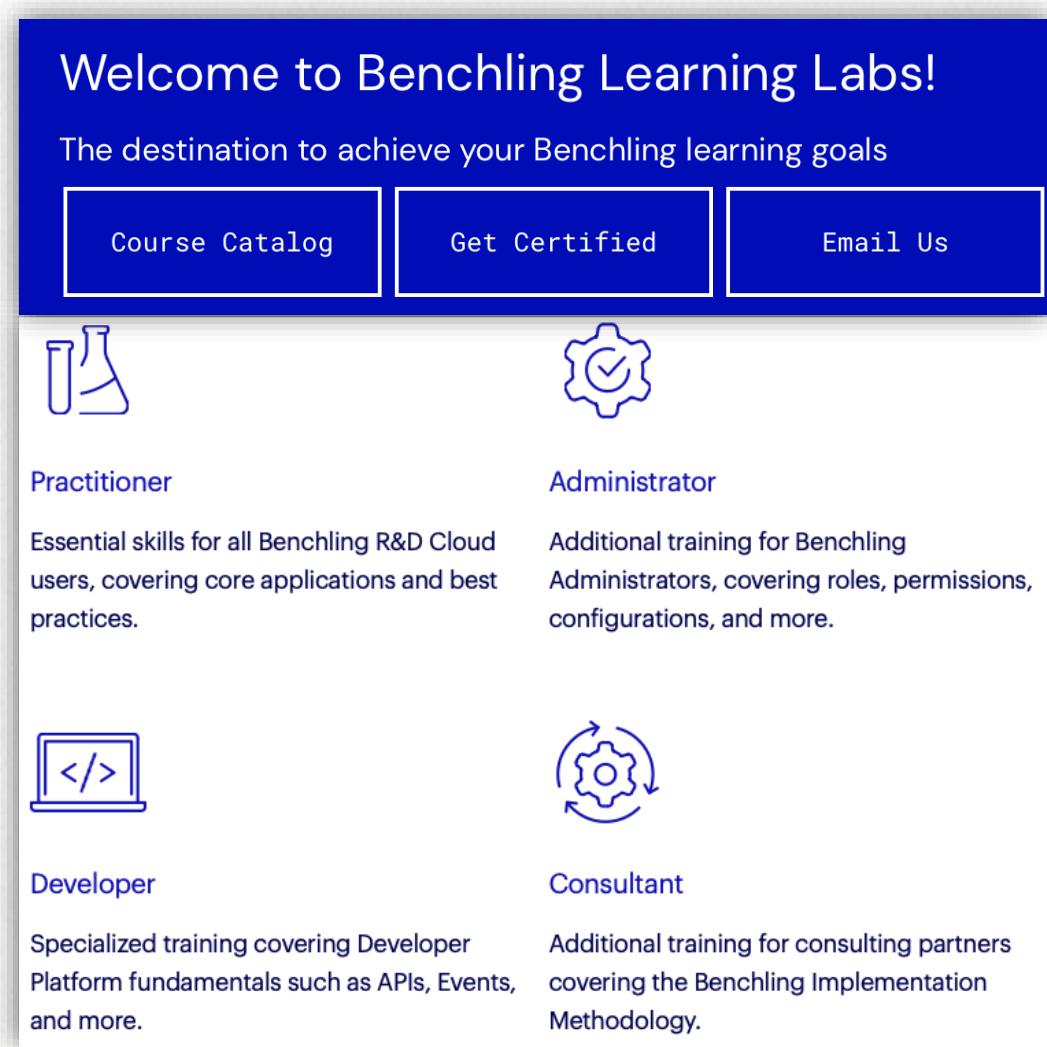
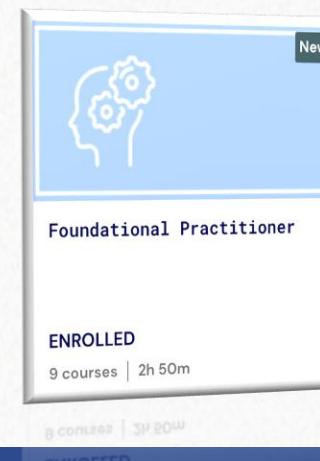


More resources

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>

