

Search

Search [input] Type: Location Folder Filters Group by Save X Clear

1-50 of 1159 items

Barcode	Name	Location	Modified	Schema
4C012	4C EE&SB fridge transient storage			4°C Fridge
4C002	4C Fridge 00271			4°C Fridge
4C009	4C Fridge 01223	DTU Building 110	09/08/2018	4°C Fridge
4C008	4C Fridge 01233			4°C Fridge
4C014	4C Fridge 01871			4°C Fridge
4C015	4C Fridge Aaron			4°C Fridge
4C016	4C Fridge Adam	BioInnovati...	15/04/2021	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 Building 110	19/11/2018	4°C Fridge
4C003	4C Fridge DSP1	DTU Building 110	09/08/2018	4°C Fridge

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)

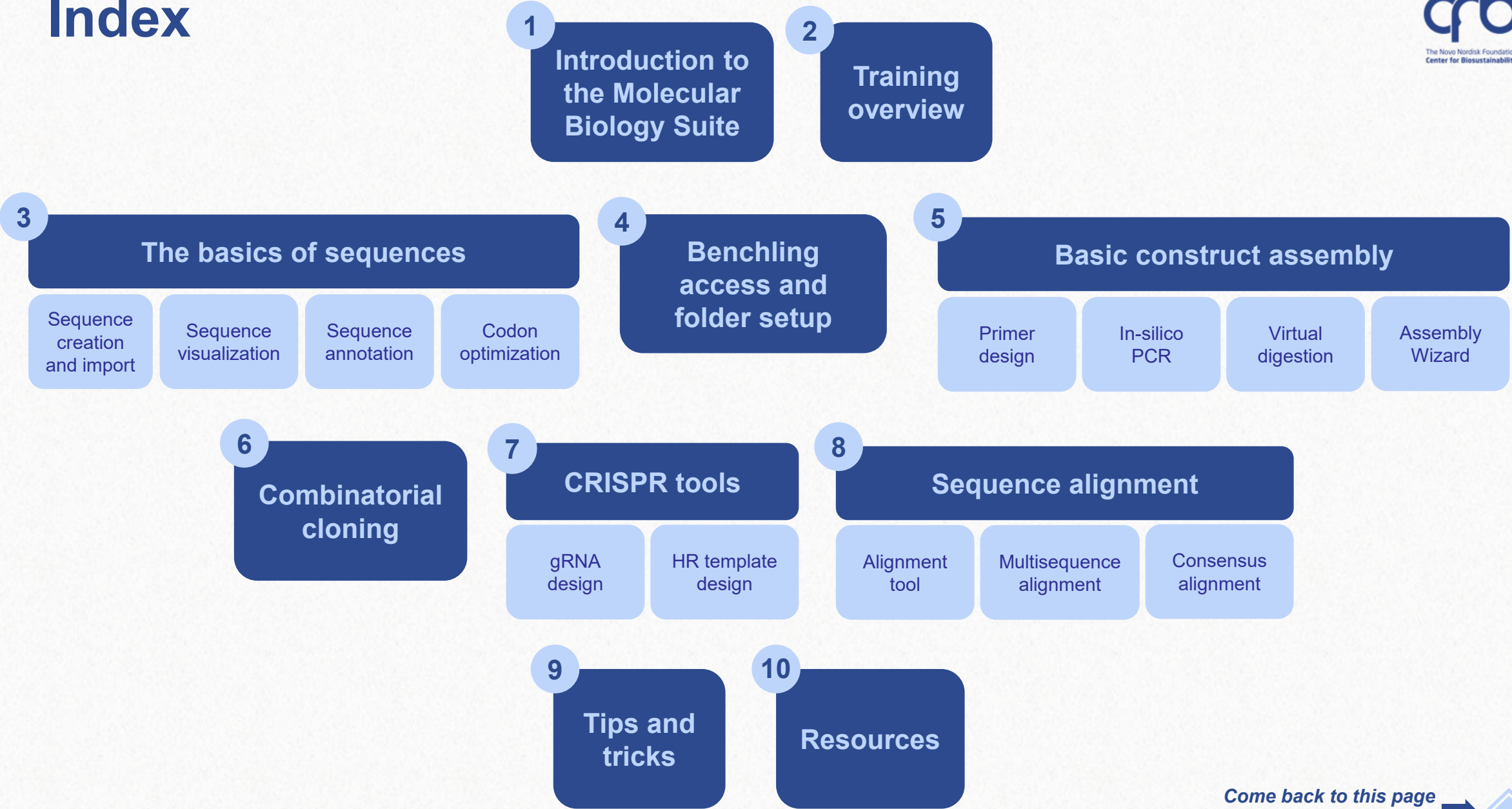


Additional resources:
[LIMS Help guides](#)
[Benchling Help Center: Molecular Biology](#)





Index



Come back to this page by clicking on the icon!

1. Introduction to the Molecular Biology Suite



Functionalities and tools overview

Sequence Alignment

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

Sequence Visualization

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

Construct Design

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

AA / Protein Analysis

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

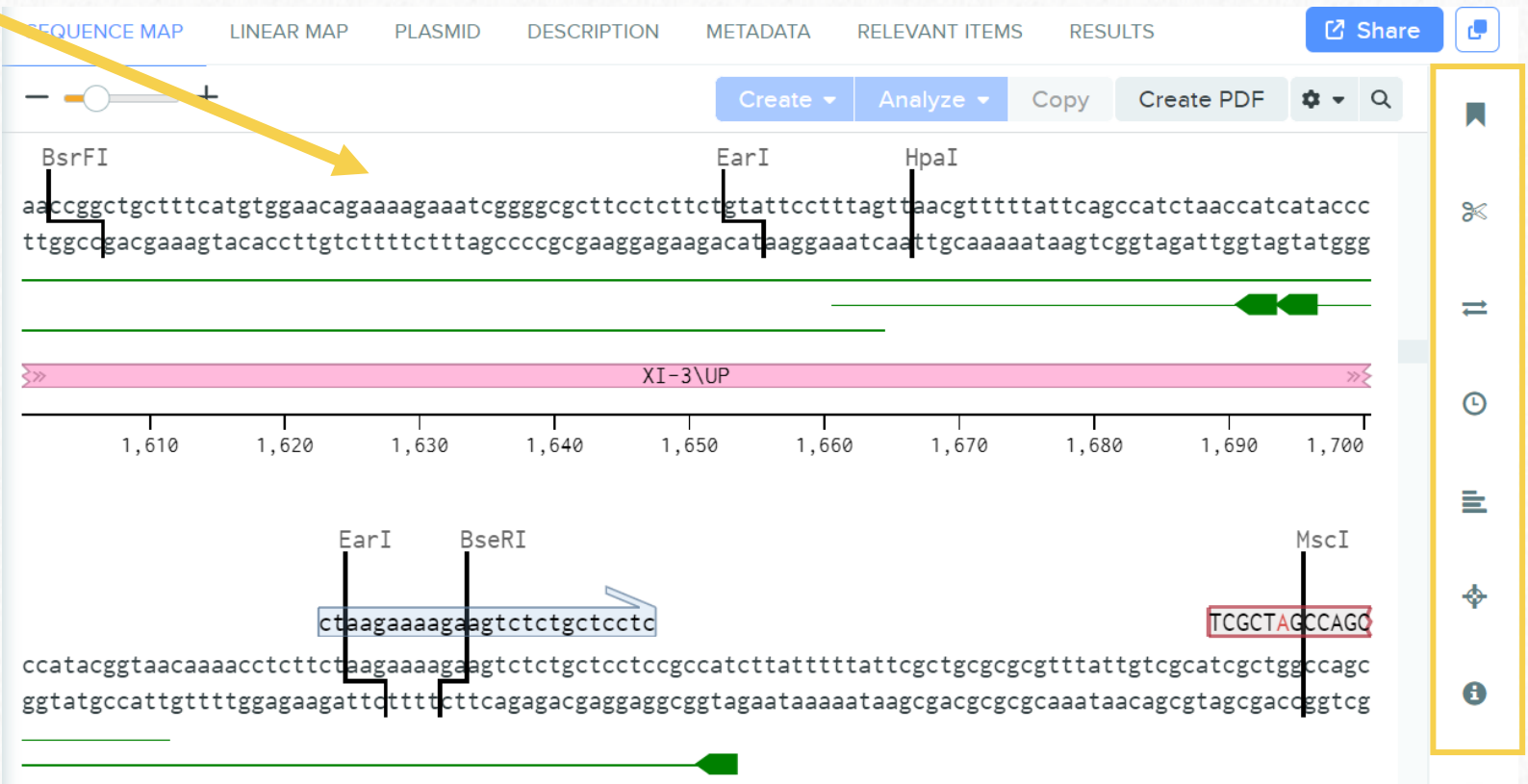
CRISPR

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

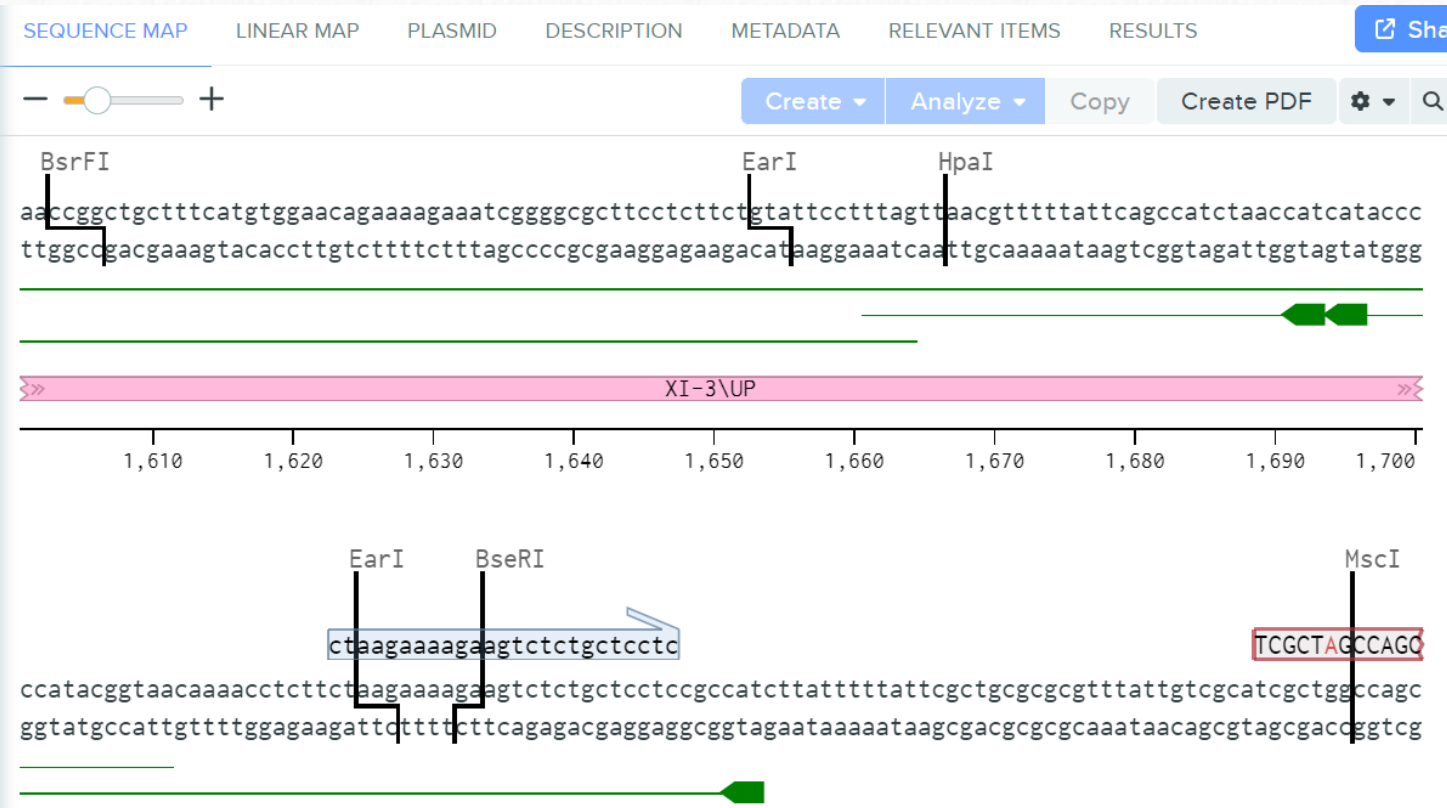
Functionalities and tools overview

Your sequence

Functionalities



Functionalities and tools overview



Features (*annotations and translations*)



Digests



Primers



History



Alignments



CRISPR



Information (*topology, tags*)

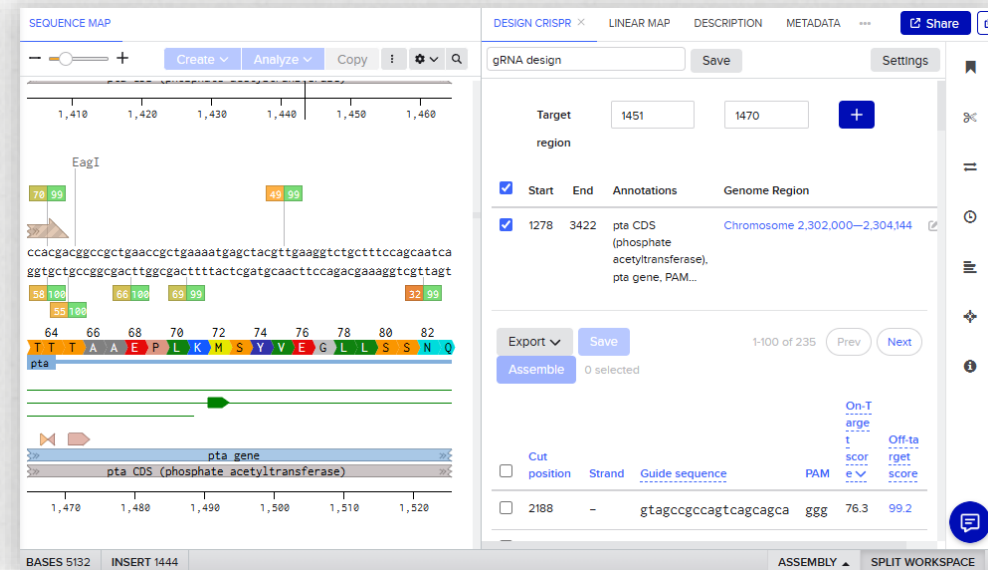
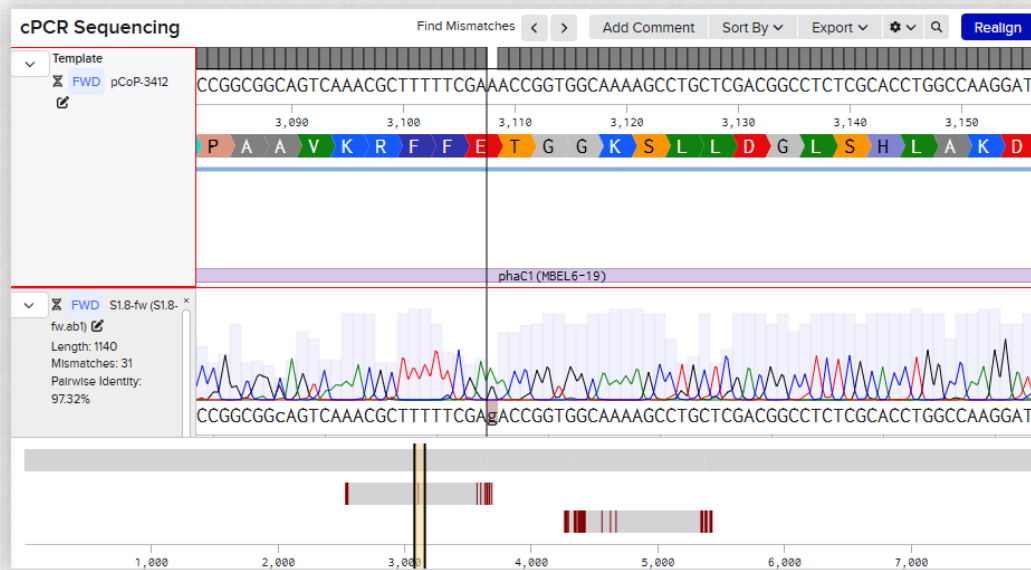


2. Training overview



Training goals:

The basics of...



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



Today's work example:

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



Scenario inspired by:

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

Contents lists available at [ScienceDirect](#)

Journal of the Taiwan Institute of Chemical Engineers

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

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

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

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

[Link to article](#)

Today's work example:

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

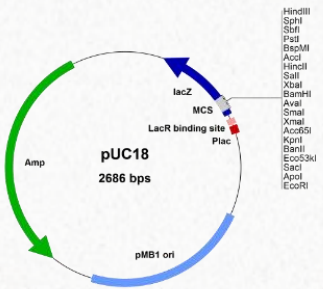
1



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



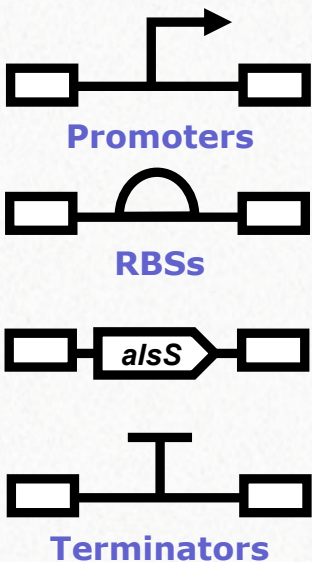
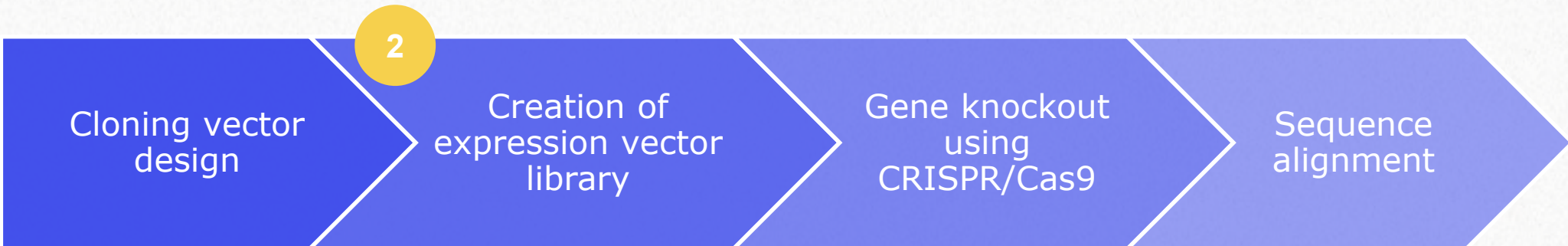
pUC18 cloning vector



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

Today's work example:

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



✓ Combinatorial cloning:
Golden Gate

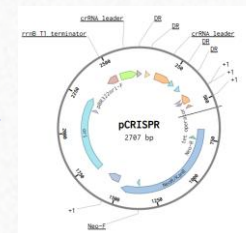
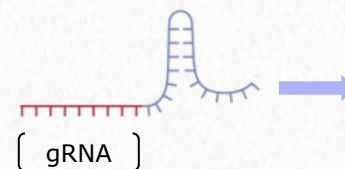
Today's work example:

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



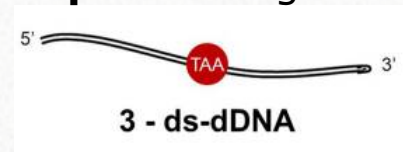
Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR

pta



- ✓ gRNA design
- ✓ HR template design

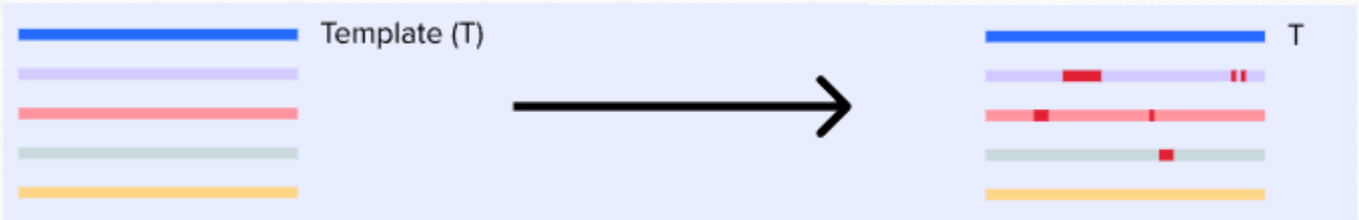
HR template design to KO *pta*



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

Today's work example:

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



✓ Multisequence alignment

3. The basics of sequences



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



3. The basics of sequences

3.1 Sequence creation and import

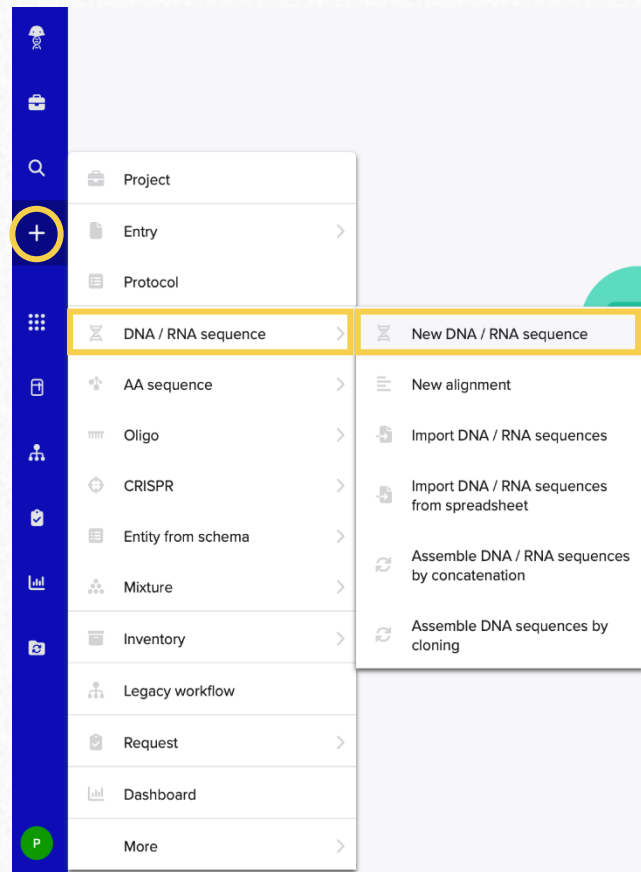




Create and import a sequence

How to create a new entity from a nucleotide sequence

- 1 Create a new DNA sequence
- 2 You can paste or write down any nucleotide sequence of your interest, and you must assign the right topology and schema.



Create DNA / RNA sequence

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

Name*

pCAT

Set nucleotide type*

DNA | RNA

Set folder*

Patricia B.

Set topology

Linear

Set schema

DNA Fragment

Bases

ggcaccgtaagaggttccaacttcaccataatgaaaca

Close

Create

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

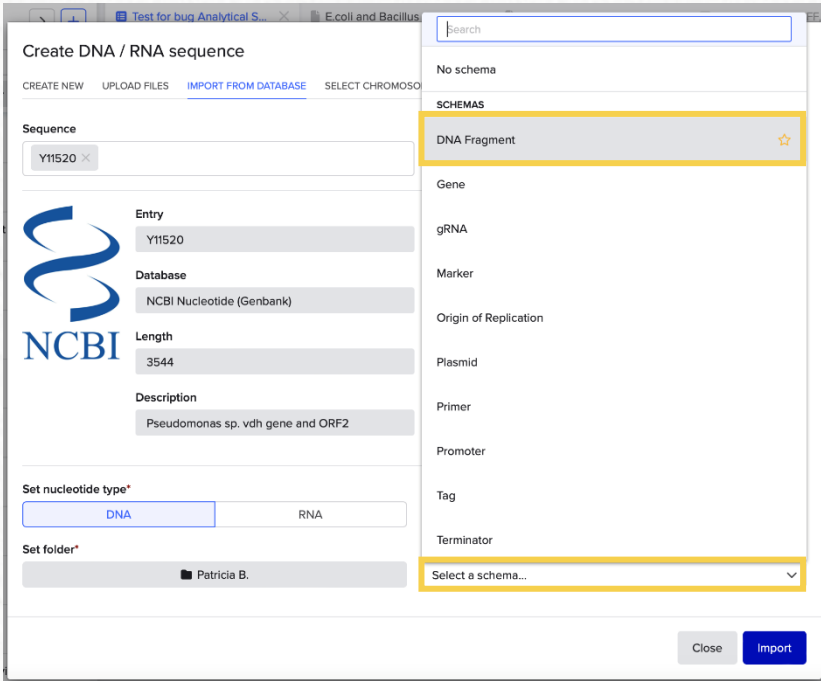
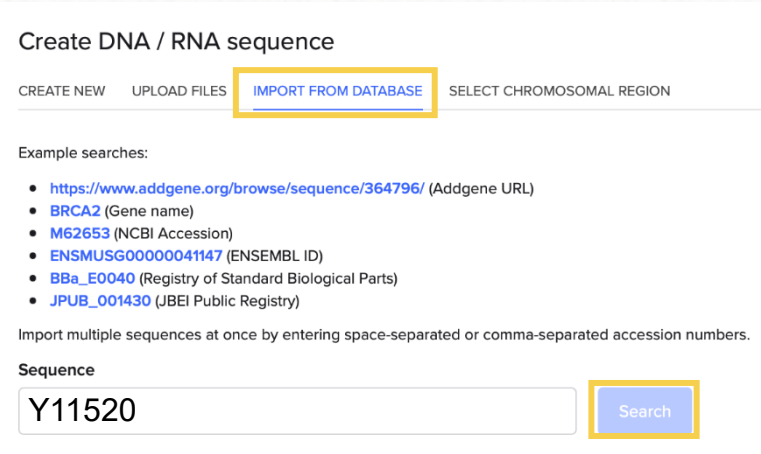
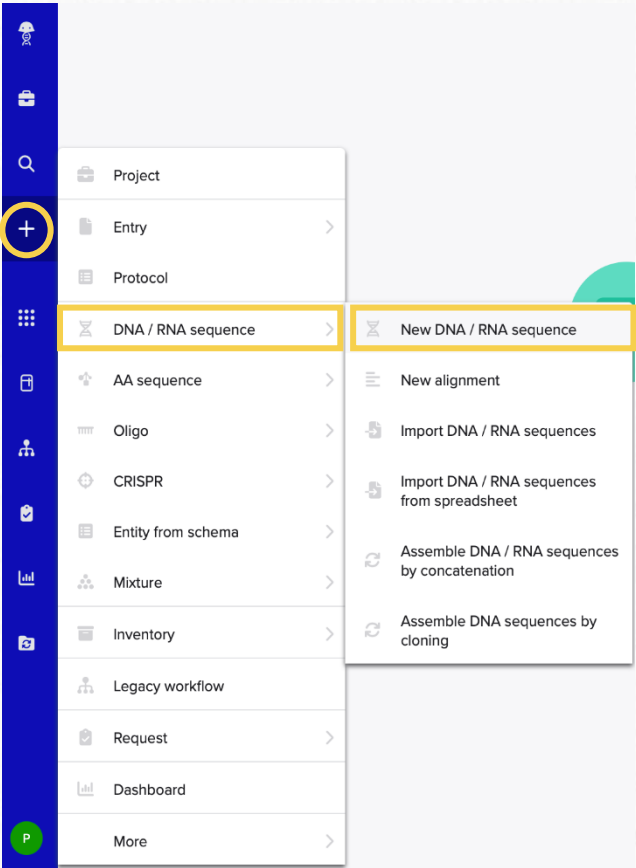




Create and import a sequence

How to import of sequences from a database

- 1
- Create a new DNA sequence
- 2
- You can write or paste a valid accession number from databases like GenBank, Addgene or the iGEM Registry
- 3
- If the ID is valid, Benchling will show you the gene's description. You can set its schema and import it.

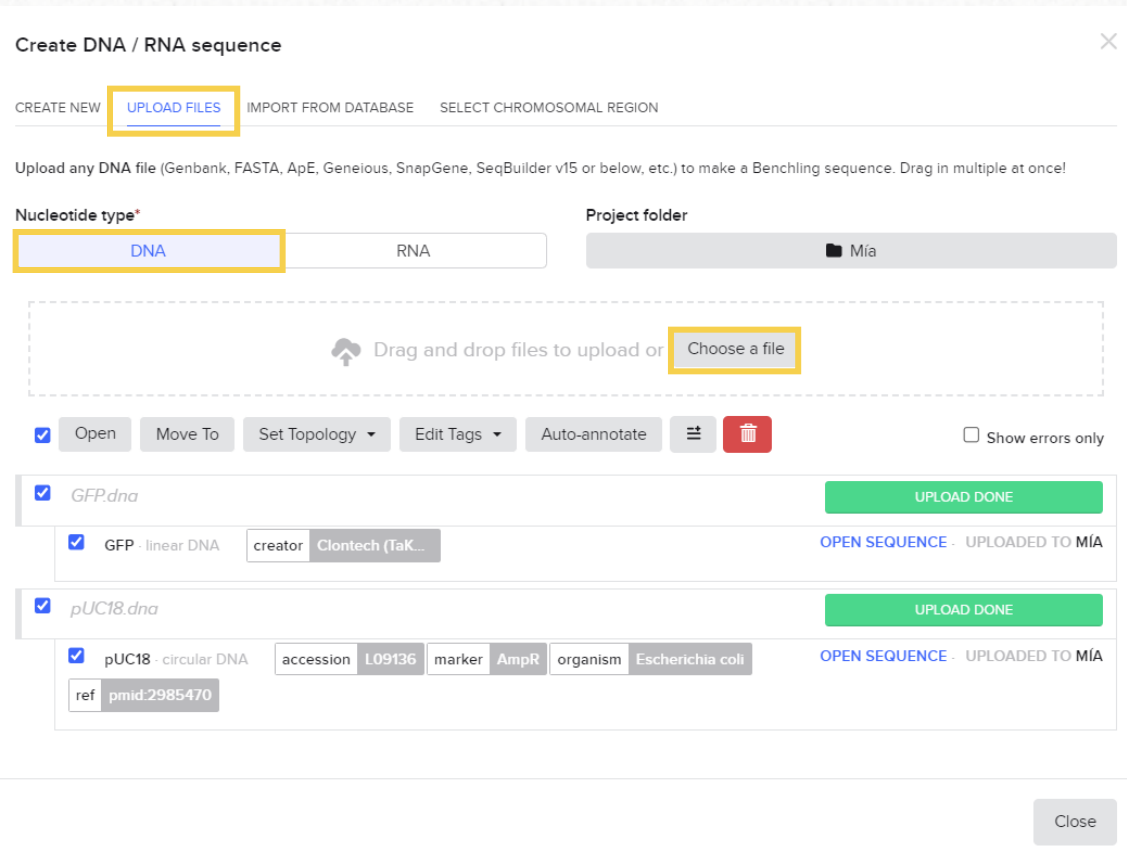
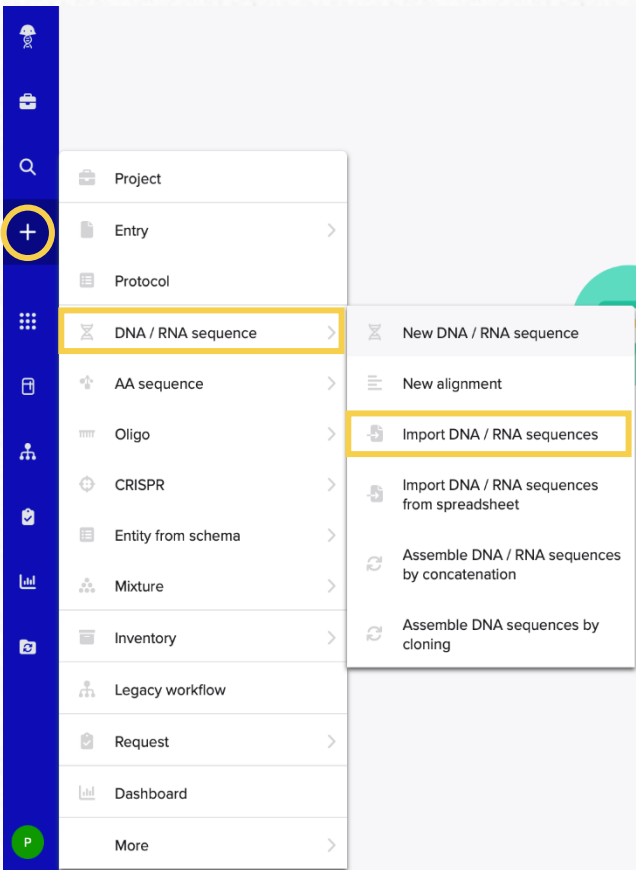




Create and import a sequence

How to import sequences from a file

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



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

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





Create and import a sequence

How to import sequences from a file

Create DNA / RNA sequence

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

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

Nucleotide type*

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

Linear

Circular

Edit Tags

ACCESSION

DEFINITION

ORGANISM

SOURCE

Create New Tag

Auto-annotate

Show errors only

☒ Vector_pBR322.gb

Exported · circular DNA

ACCESSION

ORGANISM synthetic DNA ...

SOURCE

g vector...

UPLOAD DONE

OPEN SEQUENCE · UPLOADED TO PATRICIA

Close

When uploading a sequence, it is possible to:

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



Create and import a sequence

How to import sequences from a file

Create DNA / RNA sequence

CREATE NEW

UPLOAD FILES

IMPORT FROM DATABASE

SELECT CHROMOSOMAL REGION

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

Nucleotide type*

DNA

RNA

Set folder

Patricia

Drag and drop files to upload

or choose a file

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

☒ Open

Move To

Set Topology

Edit Tags

Auto-annotate

Show errors only

Vector_pBR322.gb

UPLOAD DONE

Exported · circular DNA

ACCESSION

J01749

DEFINITION

Cloning vector...

ORGANISM

synthetic DNA ...

SOURCE

synthetic DNA ...

OPEN SEQUENCE · UPLOADED TO PATRICIA

Close

Create DNA / RNA

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

UPLOAD DONE

Exported · circular DNA

ACCESSION

J01749

DEFINITION

Cloning vector...

ORGANISM

synthetic DNA ...

SOURCE

synthetic DNA ...

OPEN SEQUENCE · UPLOADED TO PATRICIA

Close

Select Feature Libraries to use in auto-annotation

Select all / Clear selection

Select feature libraries

Affinity Tags

annotations Eveline

ART_GEN feature library

ART

BiI-Parts

Biobricks

Biobricks available

CAL-DR

Chenxi

CLED features

shared CLED features

CM parts

Parts for constructing MIA-CM strains

CM parts.csv (imported 02/24/21 13:55:22)

Common Plasmid Features

Library of common plasmid features

cPCR or PCR fragments

CPE Plasmid Features

Default Features

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

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





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

Import of sequences from a file

Create DNA / RNA sequence

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

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

Nucleotide type*

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

UPLOAD DONE

☒ Exported · circular DNA

ACCESSION

J01749

DEFINITION

Cloning vector...

OPEN SEQUENCE · UPLOADED TO PATRICIA

ORGANISM

synthetic DNA ...

SOURCE

synthetic DNA ...

Close

Create DNA / RNA

CREATE NEW LOAD

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

UPLOAD DONE

☒ Exported · circular DNA

ACCESSION

J01749

DEFINITION

Cloning vector...

OPEN SEQUENCE · UPLOADED TO PATRICIA

ORGANISM

synthetic DNA ...

SOURCE

synthetic DNA ...

Close

Add items to entity worklist

New worklist

Existing worklist

Worklist Name*

Project_training

Selected items

☒ Exported

Add items to worklist

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



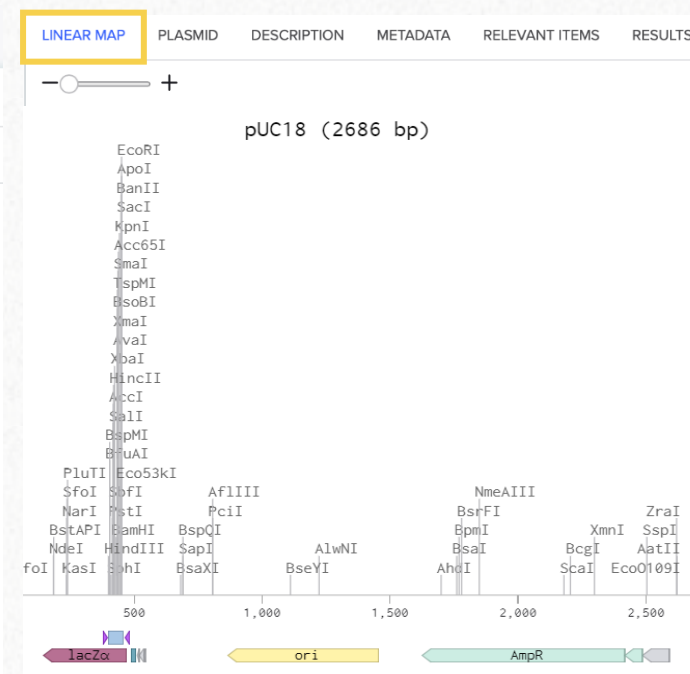
3. The basics of sequences

3.2 Sequence visualization



View, annotate and edit your sequences

Different viewing options:



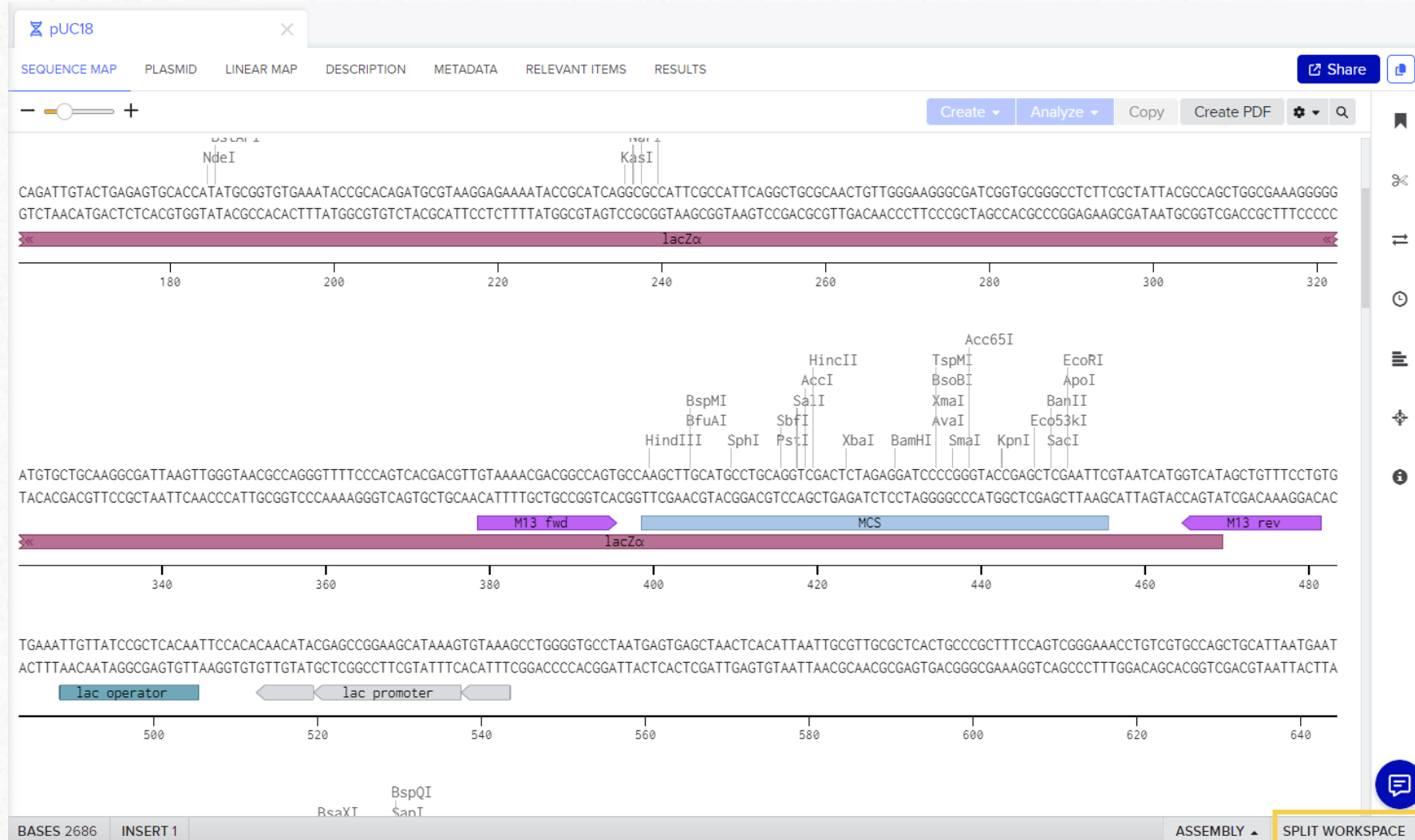
- ✓ For circular sequences, a plasmid viewing option is available
- ✓ You can click on the different elements or annotations in any of the views to select the corresponding sequence fragment



View, annotate and edit your sequences

Different viewing options:

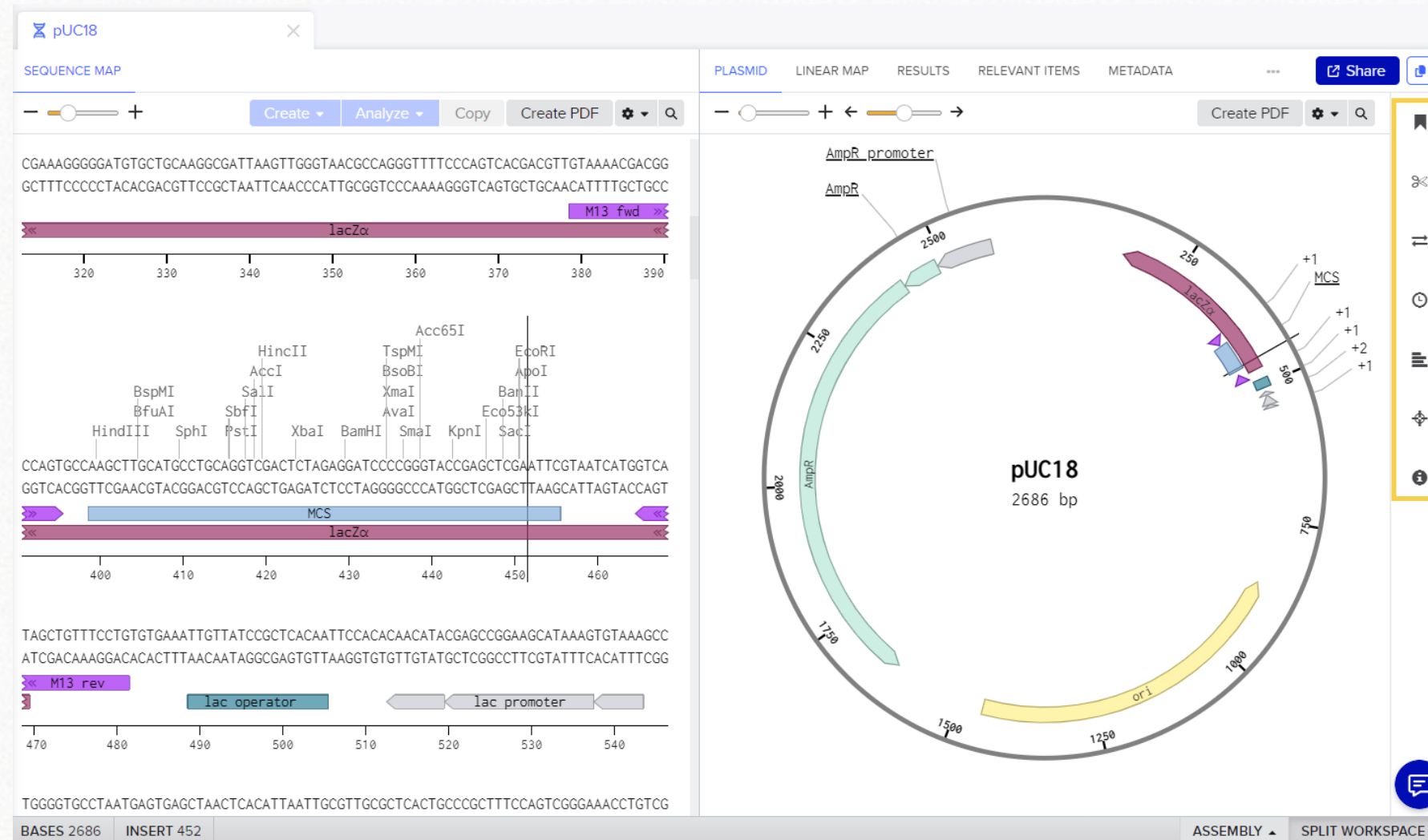
PRO TIP:
Click on “**split workspace**” to change the viewing mode to split screen/full screen



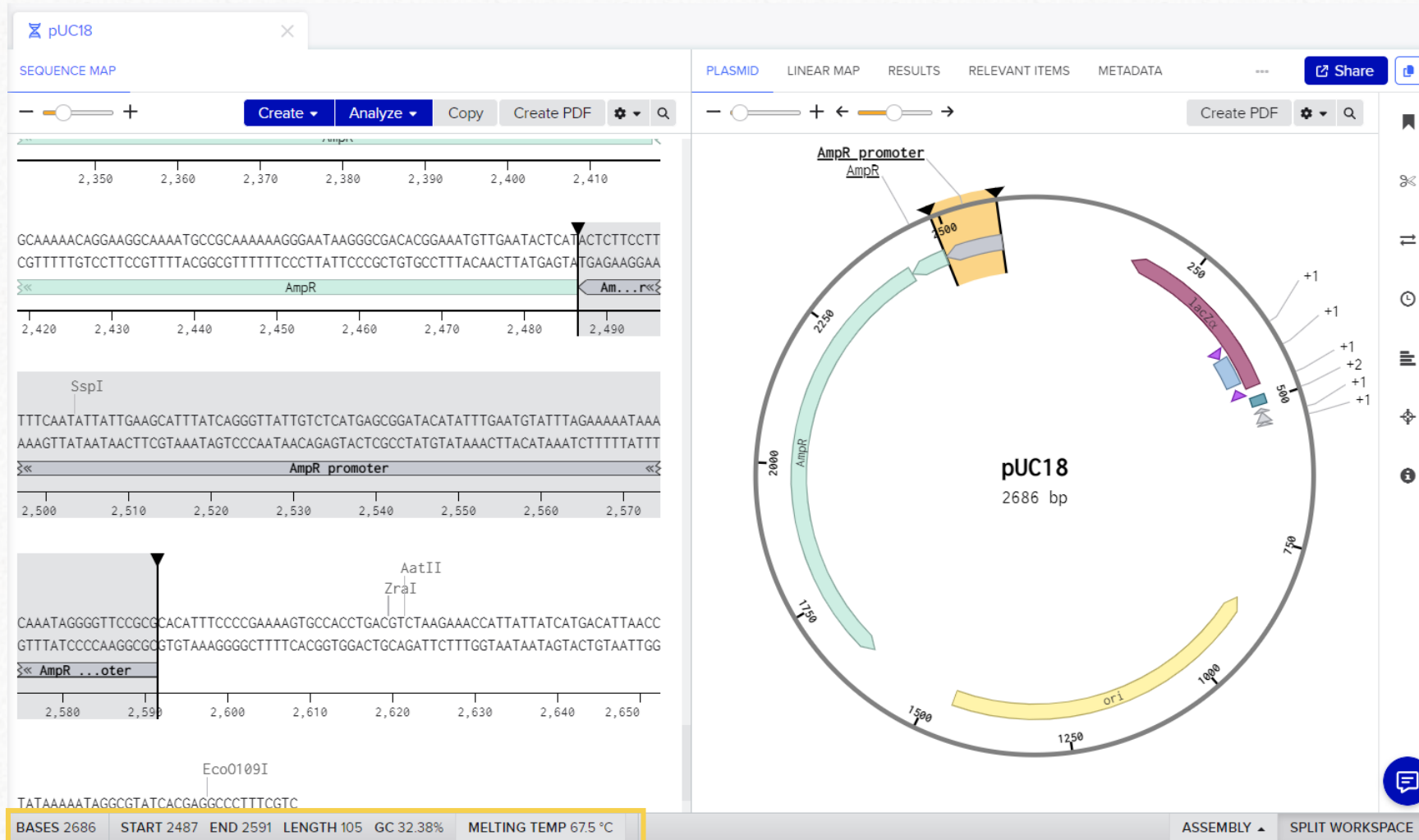
View, annotate and edit your sequences

Sequence navigation:

Functionalities



Sequence navigation:



- ✓ Click on any element or annotation in any of the views to select the corresponding sequence fragment
 - ✓ See the **electrochemical properties** of the fragment on the bottom
- PRO TIP:**
- Click on “*melting temperature*” to access the parameter settings. Different calculation algorithms are available.

View, annotate and edit your sequences

Sequence navigation:

SEQUENCE MAP

— ○ — +

Create Analyze Copy Create PDF ⚙️ 🔍

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

ATGCCGGGAGCAGACAAGCCCGTCAGG
TACGGCCCTCGTCTGTTCCGGCAGTCC

80 90 100

CAGAGCAGATTGTAAGTGCACCA
GTCTCGTCTAACATGACTCTACGTCGTTATACGCCACACTTTATGGCGTGTCTACGCATTCTCTTTATGGCGTAGT

160 170 180 190 200 210 220 230

PluTI
SfoI
NarI
KasI

GGCGCCATTGCGCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGG
CCGCGGTAAGCGGTAAGTCCGACGCGTTGACAACCTTCCCGCTAGCCACGCCCGGAGAAGCGATAATCGGGTCGACC

240 250 260 270 280 290 300 310

CGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGG
GCTTTCCCGCTACACGACGTTCCGCTAATTAACCCATTGCGGTCCCAAGGGTCAGTGCTGCAACATTTTGTGCGC

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

BstAPI
NdeI

CAGAGCAGATTGTAAGTGCACCA
GTCTCGTCTAACATGACTCTACGTCGTTATACGCCACACTTTATGGCGTGTCTACGCATTCTCTTTATGGCGTAGT

160 170 180 210 220 230

PluTI
SfoI
NarI
KasI

GGCGCCATTGCGCATTAGGCTGCGCAACT
CCGCGGTAAGCGGTAAGTCCGACGCGTTGA

240 250 260 290 300 310

CGAAAGGGGGATGTGCTGCAAGGCGATTAA
GCTTTCCCGCTACACGACGTTCCGCTAATT

320 330 340 370 380 390

Acc65I

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

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

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

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

3. The basics of sequences

3.3 Sequence annotation



View, annotate and edit your sequences

Sequence annotations

1 Select a sequence fragment

2 Create an annotation

3 Add the specifications

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

View, annotate and edit your sequences

Sequence annotations



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

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

3. The basics of sequences

3.4 Codon optimization





View, annotate and edit your sequences

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

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

3 Create a forward translation

Create Analyze Copy Create PDF

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

Forward
Reverse

4 Name your new translation

Create translation

Add new Add to existing

Name vdh translation

Position 1786 - 3242

of AA's 485

Genetic code Standard

Color

Strand Forward

SEQUENCE MAP

Y11520_PBD

AvrII
DrdI
PspXI
EcoNI
Bsu36I
HindIII
PpuMI
KflI
BamHI
ApaI
AleI
HpaI
BsrGI
BsaI
PfoI
FspI
SexAI
XbaI

ORF2 CDS (enoyl-CoA hydratase)

ORF2 gene

ORF2 CDS (enoyl-CoA hydratase)

source

ORF2 gene

ORF2 CD...atase)

repeat_region

vdh gene

vdh translation

1786 3242

485

Standard

Forward

BASES 3544 START 1786 END 3242 LENGTH 1457 GC 58.48% MELTING TEMP 83.5 °C

ASSEMBLY WIZARD SPLIT WORKSPACE

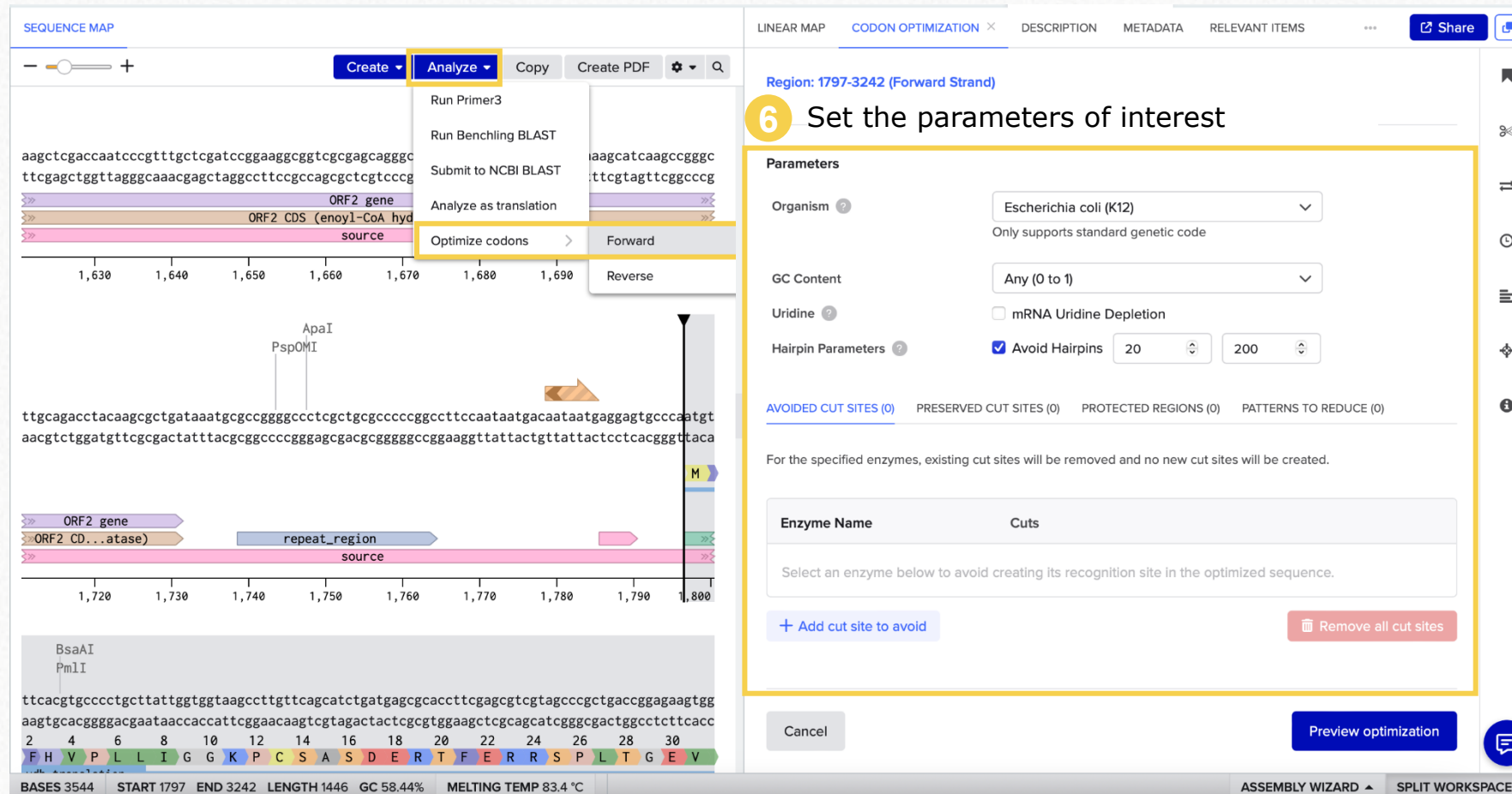
✓ Before codon optimization, the DNA sequence must be translated

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

View, annotate and edit your sequences

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

5 Select the newly created translation and codon optimize it



SEQUENCE MAP

— — — — —

Create Analyze Copy Create PDF

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

Region: 1797-3242 (Forward Strand)

6 Set the parameters of interest

Parameters

Organism
Only supports standard genetic code

GC Content

Uridine

Hairpin Parameters ☒ Avoid Hairpins

AVOIDED CUT SITES (0) **PRESERVED CUT SITES (0)** **PROTECTED REGIONS (0)** **PATTERNS TO REDUCE (0)**

For the specified enzymes, existing cut sites will be removed and no new cut sites will be created.

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

+ Add cut site to avoid Remove all cut sites

Cancel Preview optimization

BASES 3544 START 1797 END 3242 LENGTH 1446 GC 58.44% MELTING TEMP 83.4 °C

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

View, annotate and edit your sequences

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

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

SEQUENCE MAP

— ○ — +

Create Analyze Copy Create PDF ⚙️ 🔍

aaagctcgaccaatcccgtttgctcgatccggaaggcggtcgagcagggcatgaagcagttccttgacgagaaaagcatcaagccgggc
ttcgagctggtagggcaaacgagtaggccttcgccagcgctcgtccgtacttcgtcaaggaactgctcttttcgtagttcgcccg

XmnI

ORF2 gene
ORF2 CDS (enoyl-CoA hydratase)
source

1,630 1,640 1,650 1,660 1,670 1,680 1,690 1,700 1,710

ttgcagacctacaagcgtgataaatgcccggggccctcgctgcgccccggcctccaataatgacaataatgaggagtgcccaatgt
aacgtctggatgttcgcgactattacgcggcccgaggagcgacgccccggggaaggttattactgttattactcctcacgggttaca

ApaI
PspOMI

ORF2 gene
ORF2 CD...atase
repeat_region
source

1,720 1,730 1,740 1,750 1,760 1,770 1,780 1,790 1,800

BsaAI
PmII

ttcacgtgccccctgcttattggtgtaagccttgttcagcatctgatgagcgacaccttcgagcgtcgtagccgctgaccggagaagtgg
aagtgcacggggacgaataaccaccattcggaacaagtcgtagactactcgctgggaagctgcgacatcgccgactggcctcttcacc

2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
F H V P L L I G G K P C S A S D E R T F E R R S P L T G E V

BASES 3544 START 1797 END 3242 LENGTH 1446 GC 58.44% MELTING TEMP 83.4 °C

LINEAR MAP CODON OPTIMIZATION DESCRIPTION METADATA RELEVANT ITEMS ...

Optimization preview

This is a summary of the optimized sequence. You can save this sequence or go back and modify your optimization parameters.

Metric	Before	After
Rare codons	14	10
GC content	58%	58%
Uridine content	24%	22%
Hairpins	0	0

Location	Original	Optimized
1815	CTT → L (0.12)	TTA → L (0.15)
1824	GGT → G (0.29)	GGC → G (0.46)
1827	AAG → K (0.27)	AAA → K (0.73)
1830	CCT → P (0.17)	CCG → P (0.55)
1842	TCT → S (0.11)	AGC → S (0.33)
1848	GAG → E (0.3)	GAA → E (0.7)
1854	ACC → T (0.47)	ACT → T (0.16)
1860	GAG → E (0.3)	GAA → E (0.7)

Back Save Save as new sequence

ASSEMBLY WIZARD SPLIT WORKSPACE

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

4. Benchling access and folder setup



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

Access Benchling:

biosustain.benchling.com

(login with DTU credentials)





Create a training folder to work in

1

2

3

Projects / Biosustain Training /
Molecular Biology Training

Search

Type Filters

Mia
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

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

4 Create

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





Copy the *Training Files* folder into your own

Projects / Biosustain Training /
Molecular Biology Training Saved Searches

Search Type: Folder, Entry, Dataset 1 filter Save × Clear 2 Copy to...

< > 1-2 of 2 items, including items in subfolders 1 row selected

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

Copy To...

Item is currently in: Molecular Biology Training

Projects

Filter...

- ★ Biosustain Training biosustain
 - Ester
 - Inventory
 - Joana
 - ▾ Molecular Biology Training
 - ✓ Your Name 3

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

4 Copy



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



The *Training Files [Results]* folder

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

Projects / Biosustain Training / *Molecular Biology Training /

***Training Files [Results]**  Saved Searches 

 Search  Type  Filters 

 1-4 of 4 items 

    More 

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



Do not modify the contents of this folder!

5. Basic construct assembly



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

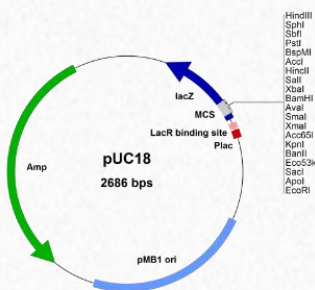
1



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



pUC18 cloning vector



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

Expected output:

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



... / Your Name / Training Files /

1. Basic construct assembly

Q Search

Type ▾ Filters

alsSD source
Last modified 9 minutes ago

pUC18
Last modified 9 minutes ago

— alsSD source

SEQUENCE MAP DESCRIPTION

— +

Create ▾ Analyze ▾ Copy ⋮ ⚙ Q

tagtgaacttatcacagatatTTTaaatTTTtagtttaaattgc
atcatttgaaatagtgttctataaatTTTaaatgcaaattttacg

95 100 105 110 115 120 125 130 135

ataataaggagtgaggggtgatgacaaaagcaacaaaagaacaaaaa
tattatttcctcactccactactgttttcgtttttcttgttttt

140 145 150 155 160 165 170 175 180

2 4 6 8

M T K A T K E Q K

alsS CDS

alsS gene

tcccttgtgaaaaacagagggcgaggcttgttgttgattgcttag
agggaacaccttttgtctccccgcctcgaacaacaactaacgaatc

10 12 14 16 18 20 22 24

S L V K N R G A E L V V D C L

alsS CDS

BASES 3326 INSERT 154

METADATA DNA FRAGMENT BATCH LINEAR MAP RESULTS ...

— +

Create PDF ⚙ Q

alsSD source (3326 bp)

Tth111I PflFI StuI MlyI PstI XbaI AhdI BsrGI BspHI SphI BssSI Bsp1286I BsiHKA I BsrBI BssSai AlwNI Bpu10I BbvCI PsiI BtsI

500 1,000 1,500 2,000 2,500 3,000

alsS CDS alsD CDS yw...S

alsS gene alsD gene yw...e

ASSEMBLY ▲ SPLIT WORKSPACE

5. Basic construct assembly

5.1 Primer design

5.1.1 Manual primer design





Construct design

Manual primer creation

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

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

alsSD source

SEQUENCE MAPDESCRIPTION

CreateAnalyzeCopy

tagtgaacttatcacaagatatttaaaattttacgtttaaatgc
atcactttgaatagtgttctataaattttaaatgcaaattttacg

95100105110115120125130135

ataataaggagtgagggtgatgacaaaagcaacaaagaacaaaa
tattattcctcactcccactactgttttcgttgttttctgtttt

2468

M T K A T K E Q K

alsS CDS

alsS gene

140145150155160165170175180

tcccttgtgaaaaacagagggcgaggcttgttgttgattgcttag
agggaacacttttgtctccccgcctcgaacaacaactaacgaatc

1012141618202224

S L V K N R G A E L V V D C L

alsS CDS

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

METADATADNA FRAGMENT BATCHLINEAR MAPRESULTS

Share

Create PDF

alsSD source (3326 bp)

Tth111IPf1FIStuIMlyIPleIEagIPciIXbaKpAc

5001,0001,5002,0002,5003,000

PRIMERSPAIRS

ManualWizard

Create Primers

Attach Existing

alsS CDSalsD CDSyw...S

alsS genealsD geneyw...e

2

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

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



Construct design

Manual primer creation

3 Select **primer pair** creation

alsSD source

SEQUENCE MAP DESCRIPTION

+

○

-

Create

Analyze

Copy

⋮

⚙

Q

tagtgaaacttatcacaagatattttaaattttacgttttaaattgc

atcactttgaatagtgttctataaatttttaaattgc

95 100 105 110 115 120 125 130 135

ataataaggagtgagggtgatgacaaaagcaacaaaagaacaaaa

tattattcctcactccactactgttttcgttgttttctgtttt

2 4 6 8

M T K A T K E Q K

alsS CDS

alsS gene

140 145 150 155 160 165 170 175 180

tccttgtgaaaaacagagggcgaggagcttgttgttgattgcttag

agggaacacttttgtctccccgcctcgaacaacaactaacgaatc

10 12 14 16 18 20 22 24

S L V K N R G A E L V V D C L

alsS CDS

BASES 3326

START 158

END 179

LENGTH 22

GC 31.82%

MELTING TEMP 50.8 °C

METADATA DNA FRAGMENT BATCH DESIGN PRIMER

Primer Pair

Single Primer

Primer Pair

Jump to Primer

Set from Selection

Strand

Forward

Reverse

Bases

5'

3'

5'

Primer must be at least 6 bp.

Primer must be at least 6 bp.

3' Location

1

1

Overhang

0 bp

0 bp

Cut Site

AanI

Use the dropdown above to look up restriction sites.

Verify

Check Secondary Structure

at 50 °C

T_m

GC Content

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

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

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

Manual primer creation

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

Strand	Forward	Reverse
Bases	5' GGATCC atgacaaaagca acaaaagaac 3'	5' ttattcagggttccttc agtt 3'
3' Location	179	2678
Overhang	6	0
Cut Site	BamHI	GGATCC

Use the dropdown above to look up restriction sites.

Strand	Forward	Reverse
Bases	5' GGATCCatgacaaaagca acaaaagaac 3'	5' GTCGAC ttattcagggttc tccttcagtt 3'
3' Location	179	2678
Overhang	6	6
Cut Site	Sall	GTCGAC

Use the dropdown above to look up restriction sites.

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



Construct design

Manual primer creation



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

alsSD source

SEQUENCE MAP DESCRIPTION

+

−

Create Analyze Copy Create PDF ⚙️ 🔍

atctggataaccctgattttgcgaaagatatcgaacaaactgaaggagccctgaataaagaaaaaagaaagcccccttttagcaggtagacctaattgggactaaaacgctttctatagctttgtgacttccttcgggacttatcttttttttttcggggaaaaatcgctcc

alsSD-rev (DNA)

238 240 242 244 246 248 250 252 254 256

N L D N P D F A K D I E T T E G S P E *

alsD CDS

alsD gene

2,650 2,660 2,670 2,680 2,690 2,700 2,710 2,720

gctttcttttttttggctcttttctgatttttagataaaataacatcaaaacagtaaaaggtgtggtctgatgaaaatattggttttcgaaaaaataaacccgagaaaaaggactaaaatctattttttagttttgtcatttccacaccagactacttttataaccaaacc

2 4 6

M K I L V L

ywr0 CDS

ywr0 gene

2,730 2,740 2,750 2,760 2,770 2,780 2,790 2,800 2,810

BtsOI Bpu10I

BASES 3326 START 2678 END 2699 LENGTH 22 GC 40.91% MELTING TEMP 53.4 °C

METADATA DNA FRAGMENT BATCH LINEAR MAP DESIGN PRIMER RESULTS

Primer Pair Jump to Primer Set from Selection

Verify

Check Secondary Structure at 50 °C

T_m

GC Content

Length

Product Size

T_m Diff.

50.8°C

53.4°C

31.82%

40.91%

28 bp

28 bp

2554 bp

+2.57°C

Save

Name

alsSD-fwd alsSD-rev

Save To

1. Basic construct assembly 1. Basic construct assembly

Save Primer Pair

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

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



Construct design

Manual primer creation

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

The screenshot shows the 'Design Primer' tab in Benchling. The 'ALL STRUCTURES' sub-tab is selected. The 'Verify' section displays the following data:

Parameter	Value	Unit
T _m	56.1°C	°C
GC Content	38.46%	%
Length	26 bp	bp
Min ΔG Homodimer	-3.3 kcal	kcal
Min ΔG Monomer	-0.1 kcal	kcal
Product Size	1495 bp	bp
T _m Diff.	+13.77°C	°C
Min ΔG Heterodimer	-6.3 kcal	kcal

The 'Check Secondary Structure' button is highlighted with a yellow box. A yellow arrow points from this button to the 'ALL STRUCTURES' sub-tab in the adjacent screenshot.

The screenshot shows the 'ALL STRUCTURES' sub-tab in Benchling. It displays a table of heterodimer secondary structures for the primers **ataatgacaataatgaggagtgccca** and **gcccgcggcgcccgaagatcgat** at 37°C.

ΔG (kcal)	Structure
-6.3	
-6	
-6.2	
-5.4	

The 'ALL STRUCTURES' sub-tab is highlighted with a yellow box. A yellow arrow points from the 'Check Secondary Structure' button in the previous screenshot to this sub-tab.

5. Basic construct assembly

5.1 Primer design

5.1.2 Primer wizard

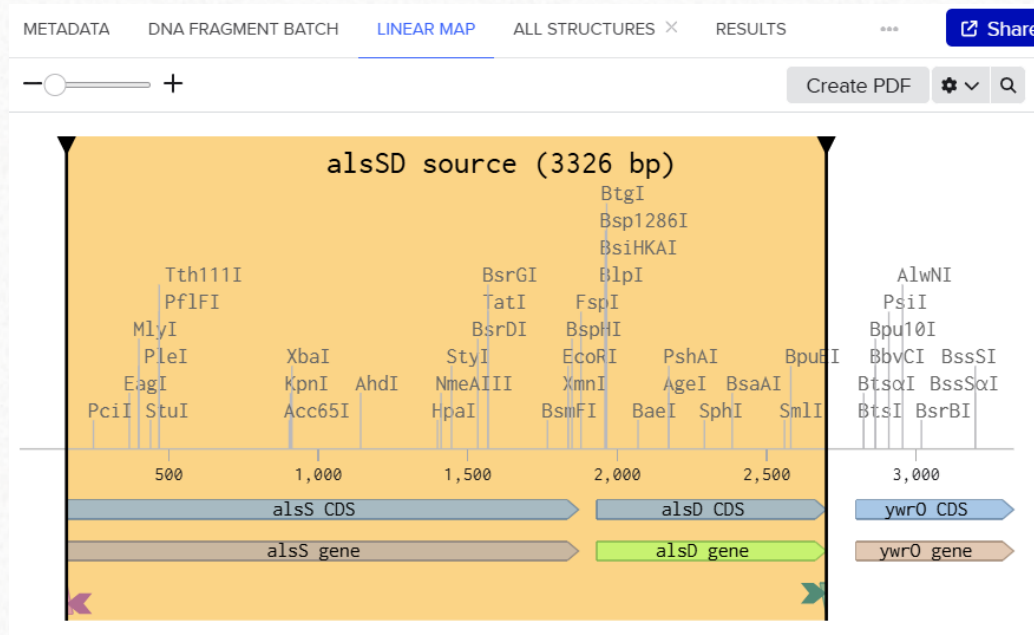


Construct design

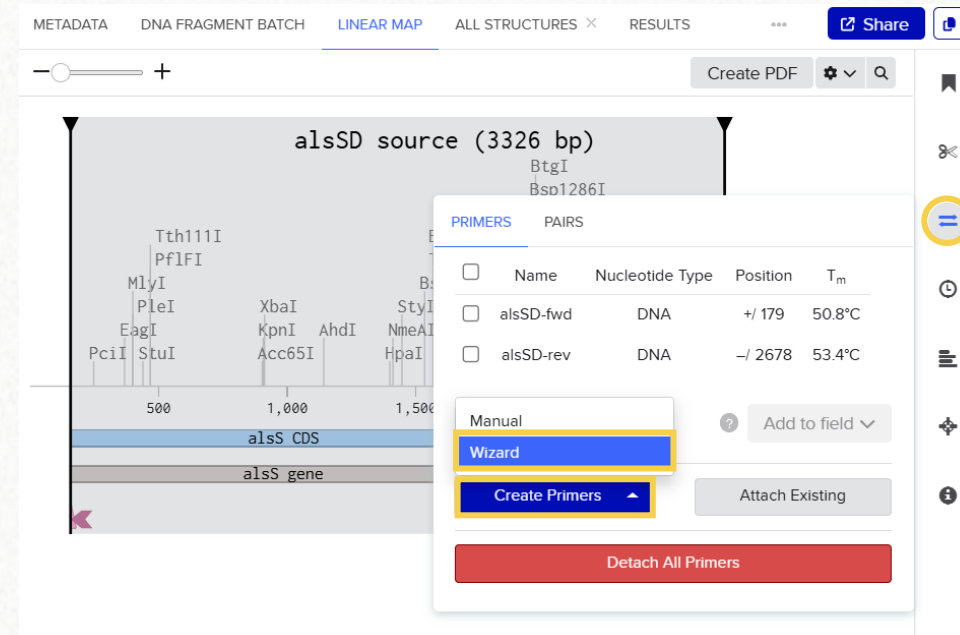
Automatic primer creation – **Primer Wizard**

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

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



2 Access the primer tool and select *wizard*



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

Construct design

Automatic primer creation – Primer Wizard

3 Select PCR as sequencing task

SEQUENCE MAP

DESCRIPTION

+

○

+

Create ▾

Analyze ▾

Copy

:

⚙

Q

ttggagggtcaatttccaaagagtgtatagtgaaccttatcacagatatataaaatttacgttta

aacctccagttaaaggtttctcacatatcactttgaatagtggtctataaatttataaatgcaaat

70

80

90

100

110

120

130

alsSD-fwd (DNA)

aaatgcataataaggagtgagggtgatgacaaaagcaaaaagaacaaaatcccttgtgaaaaa

ttacgtattattccctcactcccactactgttttcgttttctgttttagggaacactttt

2 4 6 8 10 12 14

M T K A T K E Q K S L V K N

alsS CDS

alsS gene

140

150

160

170

180

190

PciI

cagaggggaggagcttgtgtgtgattgcttagtgaggcaagggtgtcacacatgtatttggcattcc

gtctccccgcctcgaacaacaactaacgaatcacctcgttccacagtgtgtacataaacgtaagg

16 18 20 22 24 26 28 30 32 34 36

R G A E L V V D C L V E Q G V T H V F G I P

alsS CDS

BASES 3326

START 158

END 2699

LENGTH 2542

GC 44.89%

MELTING TEMP 78.1 °C

ASSEMBLY ▴

SPLIT WORKSPACE

METADATA

DNA FRAGMENT BATCH

PRIMER WIZARD ×

Share

5 Generate Primers

Task

PCR

T_m

Reset

params

Region

Target

158

2699

Use selection

Primer

Min

Opt

Max

GC%

30

50

65

T_m

45

62

65

Size

15

22

31

3' GC clamp

0

4 Use your selected sequence

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

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

Construct design

Automatic primer creation – Primer Wizard

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

The screenshot displays the Primer Wizard interface. On the left, the 'SEQUENCE MAP' tab shows a DNA sequence with a highlighted region (bases 54-76) and a gene model for 'alsS gene'. The 'DESCRIPTION' tab shows the sequence and gene model. The 'PRIMER3 RESULTS' tab on the right shows a table of generated primers. The table has columns: Penalty, Direction, % GC, T_m °C, Location, Length, Product BP, and Primer. The first two primers are selected (checked in the 'Penalty' column). A blue arrow points from the 'Penalty' column header to the first row of the table.

Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
☒ 5.451	☒ FWD	47.8%	57.6°	54-76	23	2909	5' aggcgaatcgatattggaggtc
	☒ REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgttccttcg
☐ 5.552	☐ FWD	50.0%	56.5°	55-76	22	2908	5' ggcgaaatcgatattggagggtca
	☐ REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgttccttcg
☐ 5.740	☐ FWD	47.8%	57.6°	54-76	23	2911	5' aggcgaatcgatattggaggtc
	☐ REV	59.1%	61.7°	2943-2964	22		5' cctcgacagctgctgttccttc
☐ 5.743	☐ FWD	47.8%	57.6°	54-76	23	2913	5' aggcgaatcgatattggaggtc
	☐ REV	59.1%	61.7°	2945-2966	22		5' ttctcgacagctgctgttccttc
☐ 5.821	☐ FWD	50.0%	56.2°	54-75	22	2909	5' aggcgaatcgatattggaggtc
	☐ REV	59.1%	62.0°	2941-2962	22		5' tcgcacagctgctgttccttcg
☐ 5.840	☐ FWD	50.0%	56.5°	55-76	22	2910	5' ggcgaaatcgatattggagggtca
	☐ REV	59.1%	61.7°	2943-2964	22		5' cctcgacagctgctgttccttc
☐ 5.844	☐ FWD	50.0%	56.5°	55-76	22	2912	5' ggcgaaatcgatattggagggtca
	☐ REV	59.1%	61.7°	2945-2966	22		5' ttctcgacagctgctgttccttc
☐ 5.936	☐ FWD	47.8%	57.6°	54-76	23	2912	5' aggcgaatcgatattggaggtc
	☐ REV	59.1%	62.5°	2944-2965	22		5' tcctcgacagctgctgttccttc
☐ 5.942	☐ FWD	47.8%	57.6°	54-76	23	2907	5' aggcgaatcgatattggaggtc
	☐ REV	59.1%	62.5°	2939-2960	22		5' ccacagctctcttccttcgca

At the bottom, the status bar shows: BASES 3326, START 54, END 76, LENGTH 23, GC 47.83%, MELTING TEMP 57.6 °C. The 'ASSEMBLY' and 'SPLIT WORKSPACE' buttons are also visible.

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

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

5. Basic construct assembly

5.2 *In-silico* PCR



Construct design

In-silico PCR: Create a PCR product

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

alsSD source (3326 bp)

PRIMERS **PAIRS**

Primer	Position	Product Size
alsSD-fwd	+ / 179	2554
alsSD-rev	- / 2678	

Primer Pair Information

Link Primers

	Name	T _m
Forward Primer	alsSD-fwd	50.8°C
Reverse Primer	alsSD-rev	53.4°C

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

Create 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

Cancel Copy

- ✓ 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



5. Basic construct assembly

5.3 Virtual digestion



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 displays the Benchling software interface, which is used for managing laboratory data and designing experiments. The main window shows a plasmid map for a construct named 'alsSD source' (158-2699). The map includes a sequence map, a linear map, and a description. The sequence map shows the plasmid sequence with various restriction sites and features. The linear map shows the plasmid structure with features like 'lacZα', 'MCS', and 'M13 fwd'. The description tab is currently selected, showing the plasmid's metadata, including its name, size, and a list of restriction sites. A 'NEW DIGEST' window is open, allowing the user to select enzymes and set digestion parameters. The 'Find enzyme' section shows a list of enzymes with their cut sites and a table of enzyme activities. The 'Run the digestion' button is highlighted.

SEQUENCE MAP | **LINEAR MAP** | **DESCRIPTION**

— alsSD source | alsSD source [158-2699] | pUC18

SEQUENCE MAP | **LINEAR MAP** | **DESCRIPTION**

— + | **Create** | **Analyze** | **Copy** | | |

ACGACGTTCCGCTAATTCAACCCATTGCGGTCCCAAAAGGGTCAGTGTGCAACATTTTGCTGCC

330 340 350 360 370 380 390

lacZα M13 fwd

330 340 350 360 370 380 390

CCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC
GGTCCAGGTTTCAACGTCACGACGCTCCAGCTGAGATCTCTAGGGGCCATGGCTCGAGCTTAAG

400 410 420 430 440 450

MCS lacZα

Enzyme Sau3AI

GATC
CTAG

NEB

☐ Use HF

Link: [NEB](#)

Inactivation: 65°C

Incubation: 37°C

Activity:

1:1	2:1	3:1	4:CS
100	50	10	100+

Isos.: BfuCI, DpnI, DpnII, MboI, Bsp143I, NdeI

Jump to Cut Site:

277

430

NEW DIGEST | **SAVED DIGESTS**

Find enzyme [Clear selected](#)

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

Show enzymes that cut

anywhere in the sequence

Run the digestion

Run digest

BASES 2686 | **INSERT** 2674 | **ASSEMBLY** | **SPLIT WORKSPACE**

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



The screenshot displays the 'NEW DIGEST' window in a software interface. On the left, a 'LINEAR MAP' shows a DNA sequence with various restriction enzymes mapped to it. The 'Enzyme NcoI' window is open, showing the recognition sequence 'CCATGG' and 'GGTACC'. Below this, the 'NEB' link is provided, along with inactivation and incubation temperatures. An 'Activity' table is shown with columns for different enzyme concentrations and a '4/CS' column.

1.1	2.1	3.1	4/CS
100	100	100	100+

Below the table, it states 'Isos.: None' and 'Jump to Cut Site: 2192'.

The 'Enzyme lists' section on the right shows a dropdown menu set to 'Deduplicated commercial'. Below this, a table lists enzymes and their cut sites:

Name	Cuts	Selected	Color
HincII	4	NcoI	
NciI	10		
NcoI	1		

A legend box at the bottom right explains the selection options:

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

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

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

Digestion of the backbone

PLASMID DIGEST × VIRTUAL DIGEST METADATA ... [Share](#)

Digest Save **4** Save the digestion ☒ Use HF ?

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

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

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

Digestion of the insert

1 Open the amplified *alsSD* sequence

The screenshot displays the Benchling interface for a molecular biology workflow. The main window shows a sequence map of the *alsS* gene, with a linear map and a 'NEW DIGEST' panel. The 'Find enzyme' step is highlighted, showing a table of enzymes (BamHI, SalI) with their cut sites and selected status. The 'Run digest' button is also visible.

Find and select the REs

Enzyme BamHI

GGATCC
CCTAGG

NEB

☒ Use HF ?

Link: NEB

Inactivation: N/A

Incubation: 37°C

Activity:

1.1	2.1	3.1	4/CS
100	50	10	100

Isos.: None

Jump to Cut Site:

2

Find enzyme

Clear selected

Name	Cuts	Selected	Color ?
BamHI	1	☒	Green
SalI		☐	Red

Show enzymes that cut

anywhere in the sequence

☐ Highlight enzymes with compatible sticky ends

Run digest

The digest tab will open

Save the digestion ☒ Use HF

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

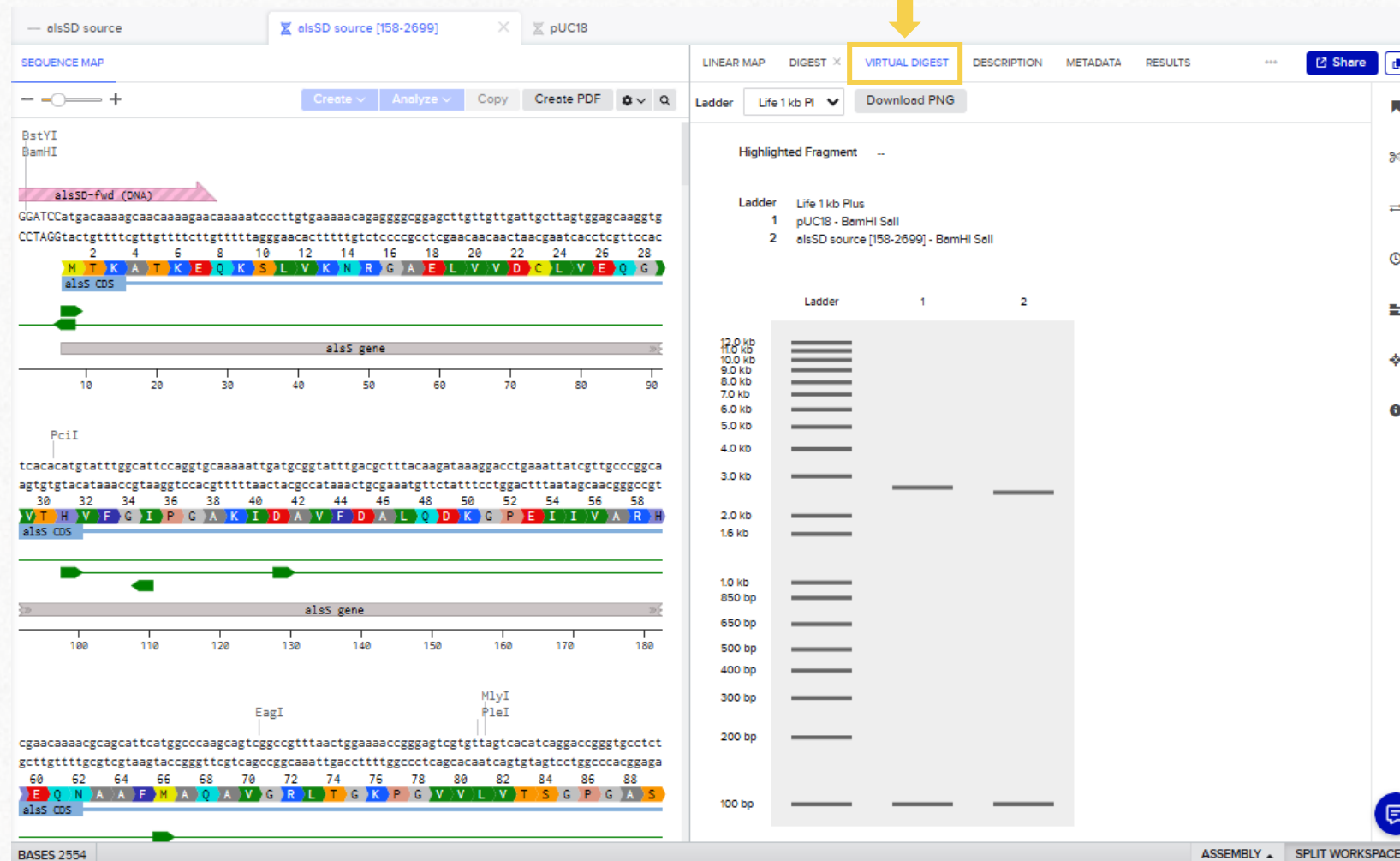
Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	BamHI	5'
2	2549	2548	BamHI	5'	Sall	5'
2550	2554	5	Sall	5'	None	blunt

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.

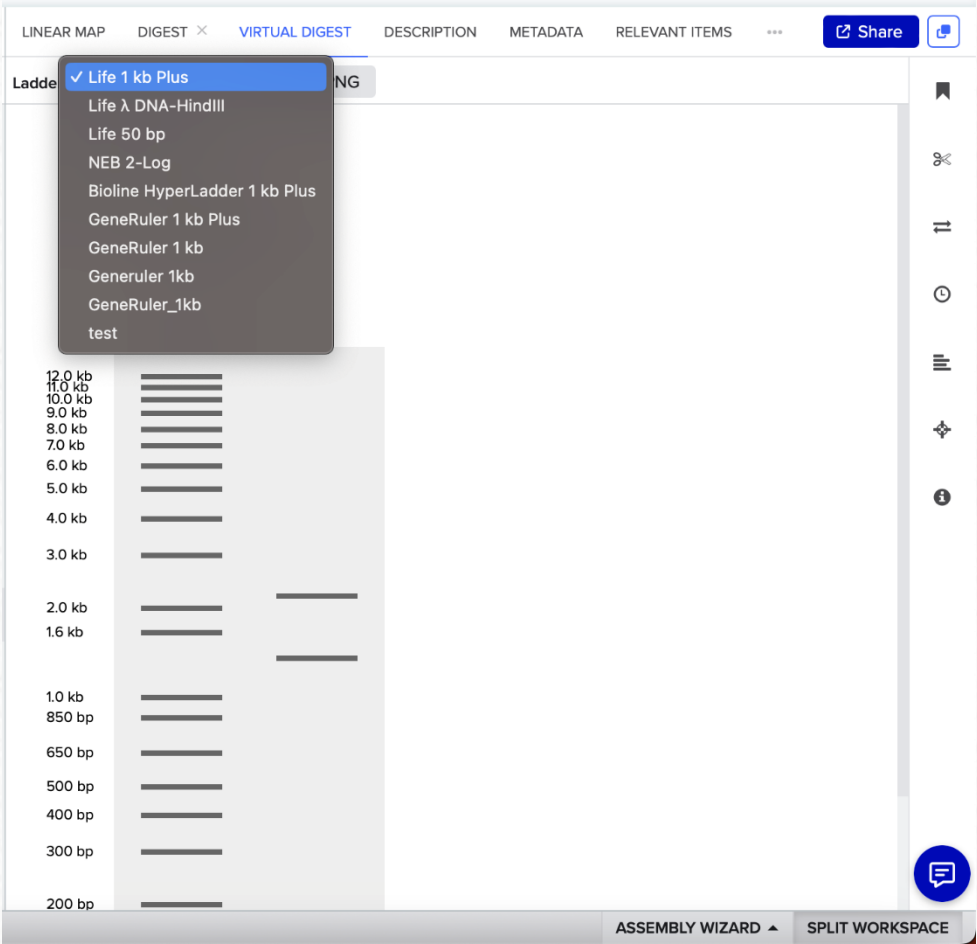
- 1st lane: **Ladder**
- 2nd lane: **Backbone**
- 3rd lane: **Insert**

✓ If you click on the bands, you can easily select the DNA sequences that correspond to the digested fragments



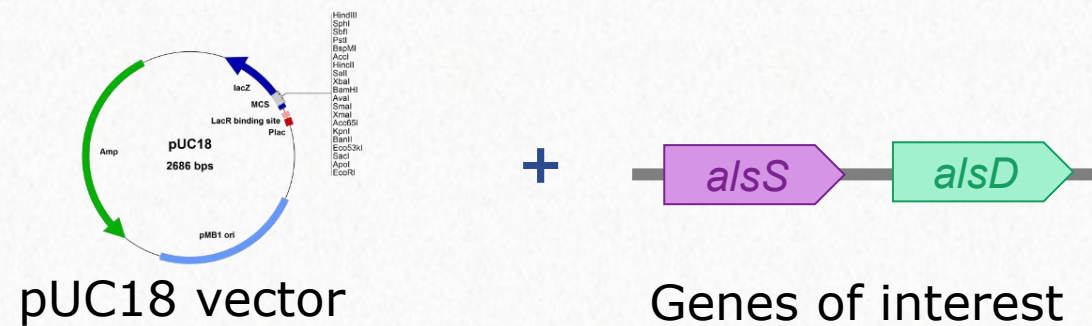
Construct design

Virtual digestion



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







Construct Assembly

Assembly Wizard

The screenshot displays the Assembly Wizard interface. On the left, a 'SEQUENCE MAP' shows the 'alsS' gene structure with exons and introns. The middle panel shows the 'alsSD source [158-2699] (2554 bp)' with various restriction enzyme sites. On the right, a 'Pick Assembly Strategy' dialog is open, listing three options: 'Digest and Ligate' (selected), 'Gibson', and 'Golden Gate'. A yellow box with the number '1' highlights the 'Assembly wizard' button in the bottom navigation bar. A yellow box with the number '2' highlights the 'Start' button in the dialog.

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

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



Digest and Ligate: Locate the Assembly Wizard work environment

The screenshot shows the Assembly Wizard interface with a circular plasmid map of pUC18 (2686 bp) and a digestion table. The plasmid map includes labels for AmpR promoter, AmpR, M13 fwd, MCS, +3, lac promoter, M13 rev, and ori. The digestion table shows enzymes BamHI and SalI with their respective cut sites and overhangs.

Enzymes	Cuts	Temp.	11	2.1	3.1	4/CS
BamHI	1	37°C	100	50	10	100
SalI	1	37°C	10	100	100	100

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

SET FRAGMENT
Select an assembly fragment below.

OVERALL ASSEMBLY
The backbone or an insert is unset.

Backbone > Insert

BASES 2686 INSERT 693

ASSEMBLY SPLIT WORKSPACE

NAME your construct

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

Digest and Ligate: Add the backbone

PLASMID SEQUENCE MAP LINEAR MAP DESCRIPTION

— ○ — + ← — ○ — → Create PDF ⚙️ 🔍

2 Select the backbone

Digest Save NEB ☒ Use HF

Enzymes	Cuts	Temp.	11	21	31	4/CS
BamHI	1	37°C	100	50	10	100
Sall	1	37°C	10	100	100	100

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

PREVIEW

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

3 Set from Selection

1 Backbone Insert

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

PREVIEW

GATCCCC AGG
GGG TCCAGCT

0 ERRORS AND 0 WARNINGS
✓ Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

pUC18 2.7 kb · BamHI, Sall Insert

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

4

✓ The Assembly Wizard shows the digested ends of the backbone



Construct Assembly

Digest and Ligate: Add the insert

SEQUENCE MAP

LINEAR MAP

— +

Create PDF

alsSD source [158-2699] (2554 bp)

Tth111I

Pf1FI

MlyI

PciI

BstYI

BamHI

EagI

BseVI

KpnI

Acc65I

AhdI

PvuI

XbaI

SspI

NmeAIII

HpaI

FokI

BtsCI

BsrDI

StyI

NdeI

BsrGI

TatI

BsmFI

XmnI

BtgI

FspI

BspHI

PvuII

B1pI

EcoRI

PshAI

BaeI

SphI

BsaAI

SmlI

SalI

AccI

BpuEI

500

1,000

1,500

2,000

2,500

alsS CDS

alsD CDS

alsS gene

alsD gene

DIGEST

VIRTUAL DIGEST

DESCRIPTION

METADATA

RESULTS

Share

Digest

Save

NEB

Use HF

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

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

Select the insert

PREVIEW

AGG

TCCAGCT

GATCCCC

GGG

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

1

pUC18

2.7 kb · BamHI, Sall

Insert

+

Hide Pre

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!

PREVIEW

AGG

GATCCat

aaG

GATCCC

TCCAGCT

Gta

ttCAGCT

GC

2 ERRORS AND 0 WARNINGS

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

1

Reverse Orientation

Jump to Selection

View Enzyme Activity

pUC18

2.7 kb · BamHI, SalI

alsSD source [158-2699]

2.5 kb · BamHI, SalI

+

Hide Pre

↓

PREVIEW

AGG

TCGACTt

atG

GATCCC

TCCAGCT

Gaa

taCCTAGGC

0 ERRORS AND 0 WARNINGS

- ✓ Looks like everything checks out

2

Reverse Orientation

Jump to Selection

View Enzyme Activity

pUC18

2.7 kb · BamHI, SalI

alsSD source [158-2699]

2.5 kb · SalI, BamHI

+

Hide Pre

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

Digest and Ligase: Assemble

SET FRAGMENT
Select an assembly fragment below.

OVERALL ASSEMBLY
✓ Looks like everything checks out

pUC18
2.7 kb • BamHI, SalI

alsSD source [158-2699]
2.5 kb • SalI, BamHI

pUC18-alsSD

Assemble

Hide Preview

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

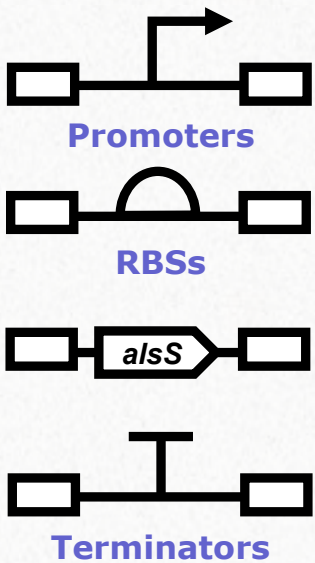


✓ The assembly is now done!

6. Combinatorial cloning: Golden Gate



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



✓ Combinatorial cloning:
Golden Gate

Expected output:

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

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

... / Your Name / Training Files /

2. Combinatorial cloning

Search

Type Filters

alsS

Last modified 3 hours ago

pET-Ori-KanR

Last modified 3 hours ago

promoter-01-T5

Last modified 3 hours ago

promoter-02-tac

Last modified 3 hours ago

promoter-03-T7

Last modified 3 hours ago

RBS-01-B0030

Last modified 3 hours ago

RBS-02-B0032

Last modified 3 hours ago

RBS-03-B0034

Last modified 3 hours ago

terminator-01-rrnBT1

Last modified 3 hours ago

terminator-02-T0

Last modified 3 hours ago

alsS

SEQUENCE MAP

atgaccaaagcaaccaaagaacaaaaatccctcgtgaagaaccgcgggcgaggctggttgtgattgttagtggag
tactgtttcgttggtttctgttttagggagcacttcttggcgccccgcctcgaccaacacctaacaatcacctc
2 4 6 8 10 12 14 16 18 20 22 24 26
M T K A T K E Q K S L V K N R G A E L V V D C L V E
alsS CDS
alsS gene

caagggtgcacgatgtatttggcattcccggtgccaaaattgatgcggtatttgacgcgtgcaggataaaggccct
gttccacagtgcgtacataaacgtaaggccacggttttaactacgccataaactgcgcgacgtcctatttccggga
28 30 32 34 36 38 40 42 44 46 48 50 52
Q G V T H V F G I P G A K I D A V F D A L Q D K G P
alsS CDS
alsS gene

Bases 1713

DESCRIPTION

METADATA

RESULTS

LINEAR MAP

RELEVANT ITEMS

Share

alsS (1713 bp)

MmeI MscI SmaI TspMI ScaI KpnI XmnI AgeI AfeI StyI PpuMI EcoRI BspHI PvuII
NspI BsaHI Acc65I BspI RsrII PaqCI FokI BtgZI BspCNI DdeI
EciI BtsOI BtsI FseI NaeI SfiI BfuAI BtsCI
MboII BtsI PflMI NcoMIV BsmFI BspGI BstNI BstXI
BssSI BssSI PspGI AlwNI BsrDI BfaI AhdI
PstI

alsS CDS

alsS gene

ASSEMBLY SPLIT WORKSPACE

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**

Golden Gate assembly

METADATA OVERVIEW

Golden Gate assembly

GOLDEN GATE

Provide feedback

This is a read-only record of a finalized assembly.

Bins & Spacers (3)

BIN 1

Backbone

Use existing cut sites

1 fragment

BIN 2

Promoter

Use existing cut sites

3 fragments

BIN 3

Gene

Use existing cut sites

8 fragments

Constructs

24 constructs

Fragments

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

Constructs

Name	Backbone	Overhang	Promoter	Overhang	Gene
backbone-promoter001-gene001	backbone	AACA	promoter001	CGAT	gene001
backbone-promoter001-gene002	backbone	AACA	promoter001	CGAT	gene002
backbone-promoter001-gene003	backbone	AACA	promoter001	CGAT	gene003
backbone-promoter001-gene004	backbone	AACA	promoter001	CGAT	gene004

CONSTRUCTS

Constructs

backbone-promoter001-gene001

backbone-promoter001-gene002

backbone-promoter001-gene003

backbone-promoter001-gene004

backbone-promoter001-gene005

backbone-promoter001-gene006

backbone-promoter001-gene007

backbone-promoter001-gene008

backbone-promoter002-gene001



Construct Assembly

Combinatorial Cloning Tool: How to access it

2. Combinatorial cloning

Search

Type Filters

alsS

Last modified 3 hours ago

pET-Ori-KanR

Last modified 3 hours ago

promoter-01-T5

Last modified 3 hours ago

promoter-02-tac

Last modified 3 hours ago

promoter-03-T7

Last modified 3 hours ago

RBS-01-B0030

Last modified 3 hours ago

RBS-02-B0032

Last modified 3 hours ago

RBS-03-B0034

Last modified 3 hours ago

terminator-01-rnBT1

Last modified 3 hours ago

terminator-02-T0

Last modified 3 hours ago

1

Folder

Entry

Protocol

DNA / RNA sequence

AA sequence

Oligo

2

Assembly

CRISPR

Entity from schema

Mixture

More

alsS

SEQUENCE MAP

Search

Create Analyze Copy Create PDF

BssSxI BssSI MboII SacII EciI

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

alsS gene

30 40 50 60 70

PstI

gctgcaggataaaggccct
cgcgacgtcctatttcggga
46 48 50 52
A L Q D K G P

alsS gene

110 120 130 140 150

BstNI BtsNI BtsI BsaHI

DESCRIPTION METADATA RESULTS LINEAR MAP RELEVANT ITEMS

alsS (1713 bp)

MmeI MscI SmaI TspMI XmnI AgeI AfeI StyI PpuMI EcoRI BspHI PvuII

NspI XmaI BsaHI ScaI KpnI Acc65I BsrII RsrII FseI NaeI SfiI PacCI BspMI FokI BtgZI BspCNI BspGI

EciI BtsI BtsNI BspGI BsmFI BsrDI BfaI AhdI BstXI DdeI

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

alsS CDS

alsS gene

BASES 1713

ASSEMBLY SPLIT WORKSPACE

Combinatorial Cloning Tool: Configuration

Assemble DNA

Name*
alsS expression vector library

Project folder*
1. Basic construct assembly

Number of fragment bins*
- 5 +

Topology of construct
Circular

Cloning method
Golden Gate Gibson Homology

Join up to 15 DNA fragments into a single piece using Type IIS restriction enzymes and T4 DNA ligase. [Show details](#)

Review the following parameters. [Reset to defaults](#)

Type IIS Restriction Enzyme
Bsal

Fragment production method
Use existing cut sites
You can change this later.

Cancel Save

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

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



Combinatorial Cloning Tool: Full view

alsS

alsS expression vector library

METADATA

OVERVIEW

CONSTRUCTS

alsS expression vector library

GOLDEN GATE

Provide feedback

Assemble

Bins & Spacers (5)

BIN 1

Backbone

Use existing cut sites

0 fragments

BIN 2

Insert 1

Use existing cut sites

0 fragments

BIN 3

Insert 2

Use existing cut sites

0 fragments

BIN 4

Insert 3

Use existing cut sites

0 fragments

BIN 5

Insert 4

Use existing cut sites

0 fragments

Constructs

0 constructs

Fragments

Status

Edit fragments

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

1Add rows

1 row

Constructs

Status

View constructs

Autopopulate

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

1Add rows

1 row

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

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

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



Construct Assembly

Combinatorial Cloning Tool: Bins and spacers

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

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

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

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

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

✓ Spacers will not be used in our example.

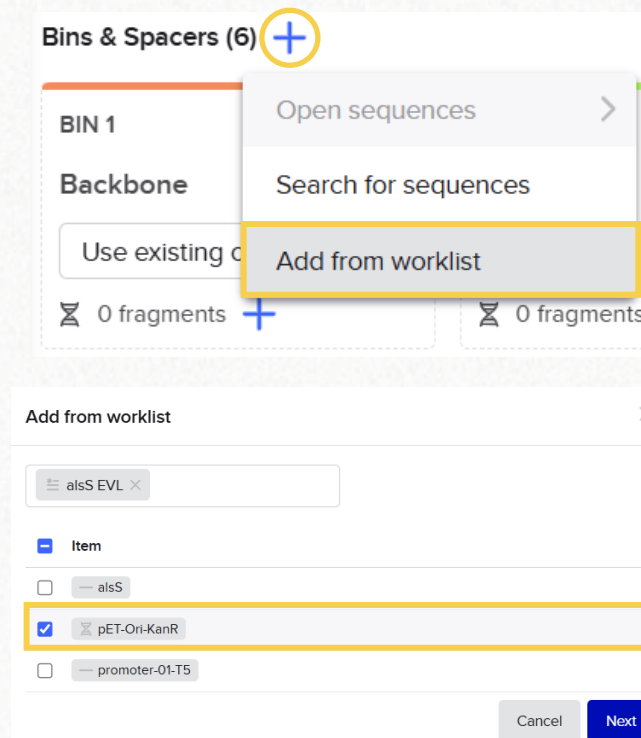


Construct Assembly

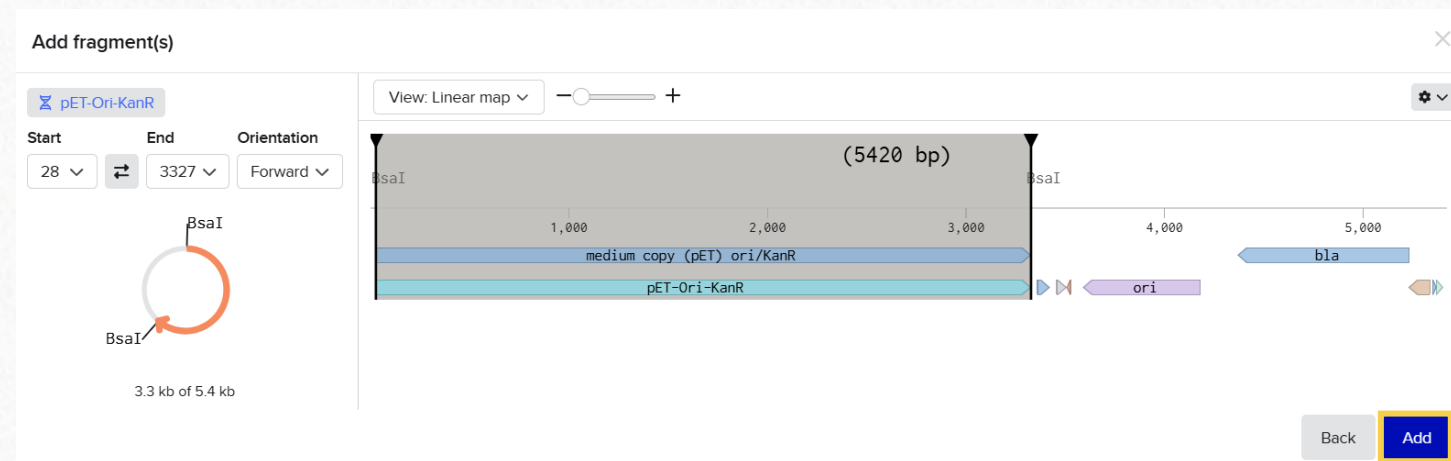
Golden Gate: Set fragments in corresponding bins

1. Backbone

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



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



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

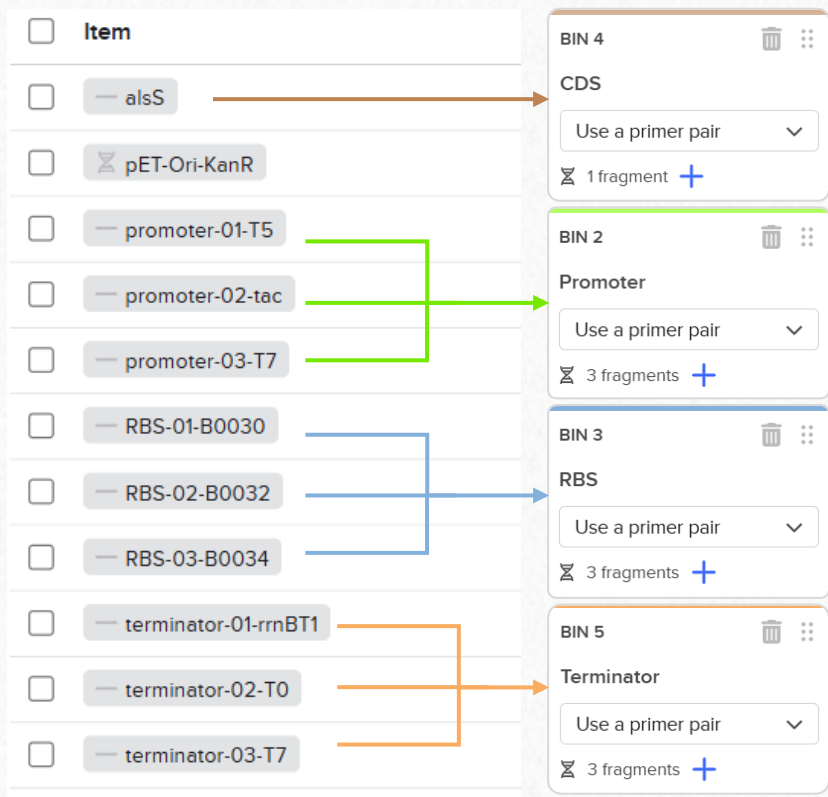


Construct Assembly

Golden Gate: Set fragments in corresponding bins

2. Inserts

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



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

Golden Gate: Verify the fragments

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

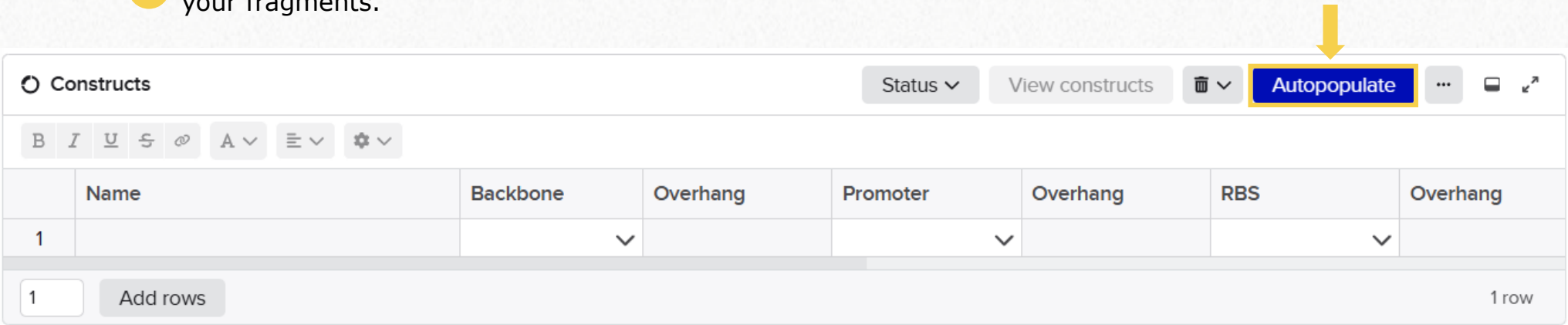
Fragments											
B I U S O A ≡ ⚙️											
	Sequence	Bin	Start	End	Length	Orientation	Type IIS enzyme	Fragment production method	Preferred 5' primer	Preferred 3' primer	Status
1	pET-Ori-KanR	Backbone	28	3327	3300	Forward	Bsal	Use existing cut sites			Looks good
2	promoter-01-T5	Promoter	1	45	45	Forward	Bsal	Use a primer pair			Looks good
3	promoter-02-tac	Promoter	1	46	46	Forward	Bsal	Use a primer pair			Looks good
4	promoter-03-T7	Promoter	1	36	36	Forward	Bsal	Use a primer pair			Looks good
5	RBS-01-B0030	RBS	1	52	52	Forward	Bsal	Use a primer pair			Looks good
6	RBS-02-B0032	RBS	1	50	50	Forward	Bsal	Use a primer pair			Looks good
7	RBS-03-B0034	RBS	1	49	49	Forward	Bsal	Use a primer pair			Looks good
8	alsS	CDS	1	1713	1713	Forward	Bsal	Use a primer pair			Looks good
9	terminator-01-rnBT1	Terminator	1	110	110	Forward	Bsal	Use a primer pair			Looks good
10	terminator-02-T0	Terminator	1	126	126	Forward	Bsal	Use a primer pair			Looks good
11	terminator-03-T7	Terminator	1	71	71	Forward	Bsal	Use a primer pair			Looks good



Construct Assembly

Golden Gate: Populate the “constructs” table

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



Constructs								Status ▾	View constructs	🗑️ ▾	Autopopulate	⋮	📄	↗️
B I U ↺ 🔗 A ▾ ☰ ▾ ⚙️ ▾														
	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang							
1		▾		▾		▾								

Add rows 1 row

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



Golden Gate: Finalize the assembly

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

alsS expression vector library

METADATA

OVERVIEW

CONSTRUCTS

alsS expression vector library

GOLDEN GATE

Provide feedback

Assemble

Bins & Spacers (5)

BIN 1

Backbone

Use existing cut sites

1 fragment

BIN 2

Promoter

Use a primer pair

3 fragments

BIN 3

RBS

Use a primer pair

3 fragments

BIN 4

CDS

Use a primer pair

1 fragment

BIN 5

Terminator

Use a primer pair

3 fragments

Constructs

27 constructs

Fragments

11 rows

Constructs

Status

View constructs

Autopopulate

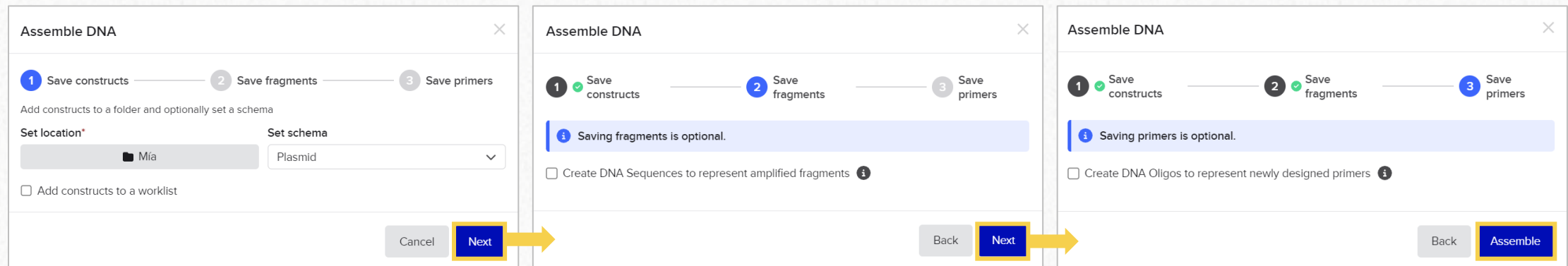
	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang	CDS	Overhang	Terminator	Overhang
1	pET-Orig-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-01-rmBT1	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-01-B0030	CCAT	alsS	AACT	terminator-01-rmBT1	CGCT
2	pET-Orig-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-02-T0	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-01-B0030	CCAT	alsS	AACT	terminator-02-T0	CGCT
3	pET-Orig-KanR-promoter-01-T5-RBS-01-B0030-alsS-terminator-03-T7	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-01-B0030	CCAT	alsS	AACT	terminator-03-T7	CGCT
4	pET-Orig-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-01-rmBT1	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-02-B0032	CCAT	alsS	AACT	terminator-01-rmBT1	CGCT
5	pET-Orig-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-02-T0	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-02-B0032	CCAT	alsS	AACT	terminator-02-T0	CGCT
6	pET-Orig-KanR-promoter-01-T5-RBS-02-B0032-alsS-terminator-03-T7	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-02-B0032	CCAT	alsS	AACT	terminator-03-T7	CGCT
7	pET-Orig-KanR-promoter-01-T5-RBS-03-B0034-alsS-terminator-01-rmBT1	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-03-B0034	CCAT	alsS	AACT	terminator-01-rmBT1	CGCT
8	pET-Orig-KanR-promoter-01-T5-RBS-03-B0034-alsS-terminator-02-T0	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-03-B0034	CCAT	alsS	AACT	terminator-02-T0	CGCT
9	pET-Orig-KanR-promoter-01-T5-RBS-03-B0034-alsS-terminator-03-T7	pET-Orig-KanR	GGAG	promoter-01-T5	TACC	RBS-03-B0034	CCAT	alsS	AACT	terminator-03-T7	CGCT

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



Construct Assembly

Golden Gate: Save the constructs and related files



The first screenshot shows the 'Assemble DNA' dialog with three steps: 1. Save constructs, 2. Save fragments, and 3. Save primers. Step 1 is active. Below the steps, there is a section 'Add constructs to a folder and optionally set a schema'. Under 'Set location*', a folder named 'Mia' is selected. Under 'Set schema', 'Plasmid' is selected in a dropdown menu. There is a checkbox 'Add constructs to a worklist' which is unchecked. At the bottom, there are 'Cancel' and 'Next' buttons. A yellow arrow points from the 'Next' button to the second screenshot.

The second screenshot shows the same dialog, but step 2 'Save fragments' is now active and marked with a green checkmark. Step 1 is now greyed out. A blue information bar appears with the text 'Saving fragments is optional.' Below it, there is a checkbox 'Create DNA Sequences to represent amplified fragments' which is unchecked. At the bottom, there are 'Back' and 'Next' buttons. A yellow arrow points from the 'Next' button to the third screenshot.

The third screenshot shows the same dialog, but step 3 'Save primers' is now active and marked with a green checkmark. Steps 1 and 2 are now greyed out. A blue information bar appears with the text 'Saving primers is optional.' Below it, there is a checkbox 'Create DNA Oligos to represent newly designed primers' which is unchecked. At the bottom, there are 'Back' and 'Assemble' buttons.

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

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

Construct Assembly

Golden Gate: Results

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

als expression library

METADATA OVERVIEW CONSTRUCTS

Constructs

Search constructs by name

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

1-25 of 27 items

2

8 associated primers View

8 associated primers View

8 associated primers View

Primer view

View constructs

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

Sequence view

View constructs

SEQUENCE PRIMERS

View: Plasmid map

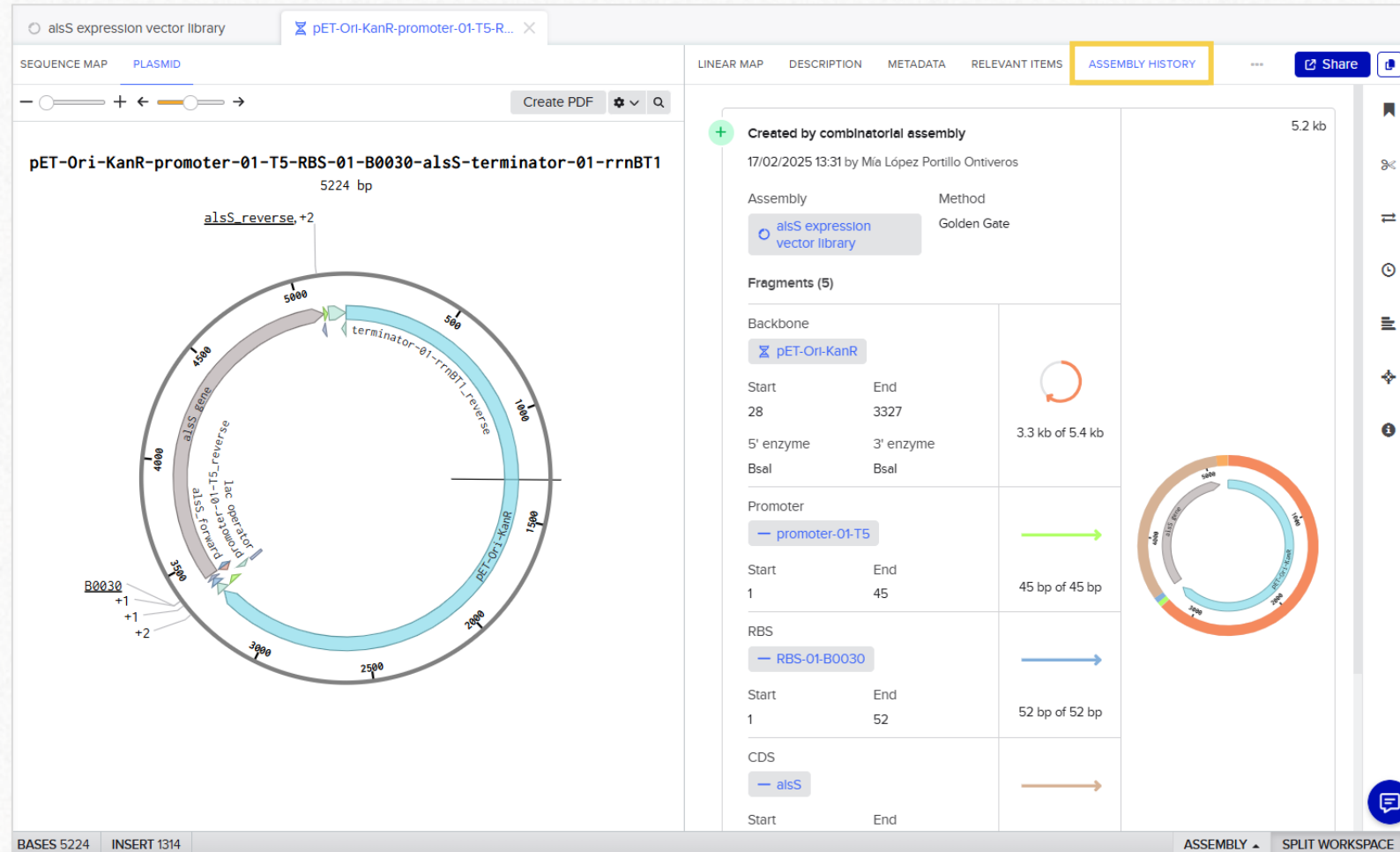
5224 bp

alsS_forward
RBS-01-B0030_reverse
RBS-01-B0030_forward
promoter-01-T5_reverse
promoter-01-T5_forward

Construct Assembly

Golden Gate: Results

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



7. CRISPR tools

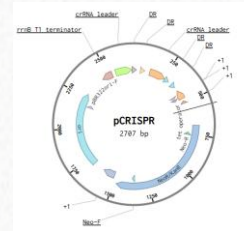
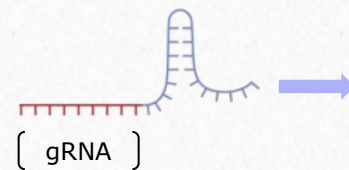


This is the third part of the *hands-on* example.

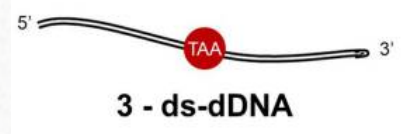


Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR

pta



HR template design to KO *pta*



- ✓ gRNA design
- ✓ HR template design

Expected output:

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

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

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

... / Your Name / Training Files /

3. CRISPR tools

Search

Type Filters

pCRISPR
Last modified 6 days ago

pta source
Last modified 6 days ago

pta source

SEQUENCE MAP DESCRIPTION

tttcacaccgccagctcagctggcggtgctgttttgaaccgccaaatcgggcggaagtggtggcggtcgagtcgaccgccacgacaaaacattggcggttagccgcc

2,301,83501,84801,84501,85801,85501,86801,86501,87801,975

taacgaaaggataaacggtgtcccggtattattatgctgatccctaccggaaccattgctttctcctatttggcacagggcataataatacgactagggatggccttgg

2,301,89801,89502,88802,88502,87802,87502,86802,86502,85802,85502,84802,84502,83802,83502,82802,82502,81802,81502,80802,80502,79802,79502,78802,78502,77802,77502,76802,76502,75802,75502,74802,74502,73802,73502,72802,72502,71802,71502,70802,70502,69802,69502,68802,68502,67802,67502,66802,66502,65802,65502,64802,64502,63802,63502,62802,62502,61802,61502,60802,60502,59802,59502,58802,58502,57802,57502,56802,56502,55802,55502,54802,54502,53802,53502,52802,52502,51802,51502,50802,50502,49802,49502,48802,48502,47802,47502,46802,46502,45802,45502,44802,44502,43802,43502,42802,42502,41802,41502,40802,40502,39802,39502,38802,38502,37802,37502,36802,36502,35802,35502,34802,34502,33802,33502,32802,32502,31802,31502,30802,30502,29802,29502,28802,28502,27802,27502,26802,26502,25802,25502,24802,24502,23802,23502,22802,22502,21802,21502,20802,20502,19802,19502,18802,18502,17802,17502,16802,16502,15802,15502,14802,14502,13802,13502,12802,12502,11802,11502,10802,10502,9802,9502,9202,8902,8602,8302,8002,7702,7402,7102,6802,6502,6202,5902,5602,5302,5002,4702,4402,4102,3802,3502,3202,2902,2602,2302,2002,1702,1402,1102,802,502,202,92

PshAI

agcgtcggtctgaccagcgtcagccttggcggtgatccgtgcaatggaacgcaaagtgcgagccagactgggtcgagtcggaaccgcactaggcacgttaccttgcgtttc

pta gene

pta CDS (phosph...yltransferase)

pta gene

pta CDS (phosphate acetyltransferase)

BASES 3929

METADATA DNA FRAGMENT BATCH LINEAR MAP

pta source (3929 bp)

2,303,000 2,304,000 2,305,000

pta gene yfcC gene

pta CDS (pho...ransferase) yfcC CDS...rotein)

ASSEMBLY SPLIT WORKSPACE

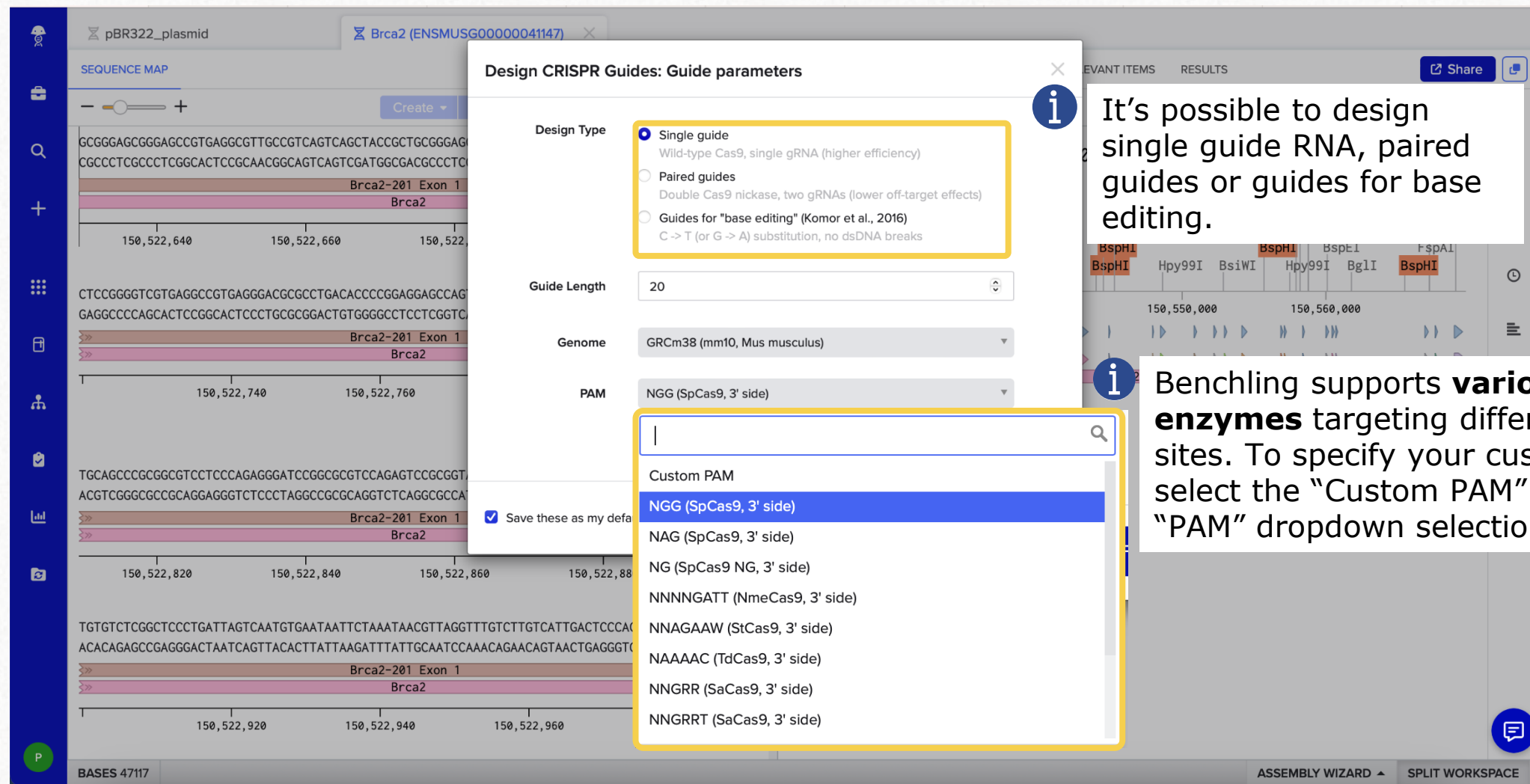
Tool overview

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

1

2

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



Design CRISPR Guides: Guide parameters

Design Type

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

Guide Length 20

Genome GRCm38 (mm10, Mus musculus)

PAM NGG (SpCas9, 3' side)

☒ Save these as my default

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

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

7. CRISPR tools

7.1 gRNA design





CRISPR tools

gRNA design

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

pCRISPR

— pta source

SEQUENCE MAP DESCRIPTION

— + Create Analyze : ⚙️ 🔍

tttcacaccgccagctcagctggcggctgttttgaacccgcaaatcgcggaagtggtggcggtcgagtcgaccgccacgacaaaacattgggcgggttagccgcc

2,301,83501,84001,84501,85001,85501,86001,86501,87001,975

taacgaaaggagataaacgtgtcccgatattattatgctgatccctaccggaaccattgtttctctatttggcacagggcataataatacactagggatggccttgg

2,301,89001,89502,90002,90502,91002,91502,92002,92502,930

PshAI

agcgtcggtctgaccagcgtcagccttggcgtgatccgtgcaatggaacgcaaagtgcagccagactggcgcagtcggaaccgcactaggcacgttaccttgcgtttc

pta gene

pta CDS (phosph...yltransferase)

pta gene

pta CDS (phosphate acetyltransferase)

BASES 3929 INSERT 2302001

METADATA DNA FRAGMENT BATCH LINEAR MAP ... Share

— + Create PDF ⚙️ 🔍

pta source (3929 bp)

2,303,000 2,304,000 2,305,000

pta gene yfcC gene

pta CDS (pho...ransferase) yfcC CDS...rotein)

CRISPR

Design and analyze guides

Saved guide analyses

No guide analyses

Design HR template (ssODN)

ASSEMBLY SPLIT WORKSPACE



- 3 Change the genome to ***E. coli* BL21(DE3)**.
- 4 Click **Finish** and continue.

Design CRISPR guides: Guide parameters

Design type

☒ Single guide
Wild-type Cas9, single gRNA (higher efficiency)

☐ Paired guides
Double Cas9 nickase, two gRNAs (lower off-target effects)

☐ Guides for "base editing" (Komor et al., 2016)
C -> T (or G -> A) substitution, no dsDNA breaks

Guide length

20

Genome

ASM956v1 (Escherichia coli BL21(DE3))

PAM

NGG (SpCas9, 3' side)

[Show advanced settings](#)

☒ Save these as my default CRISPR settings

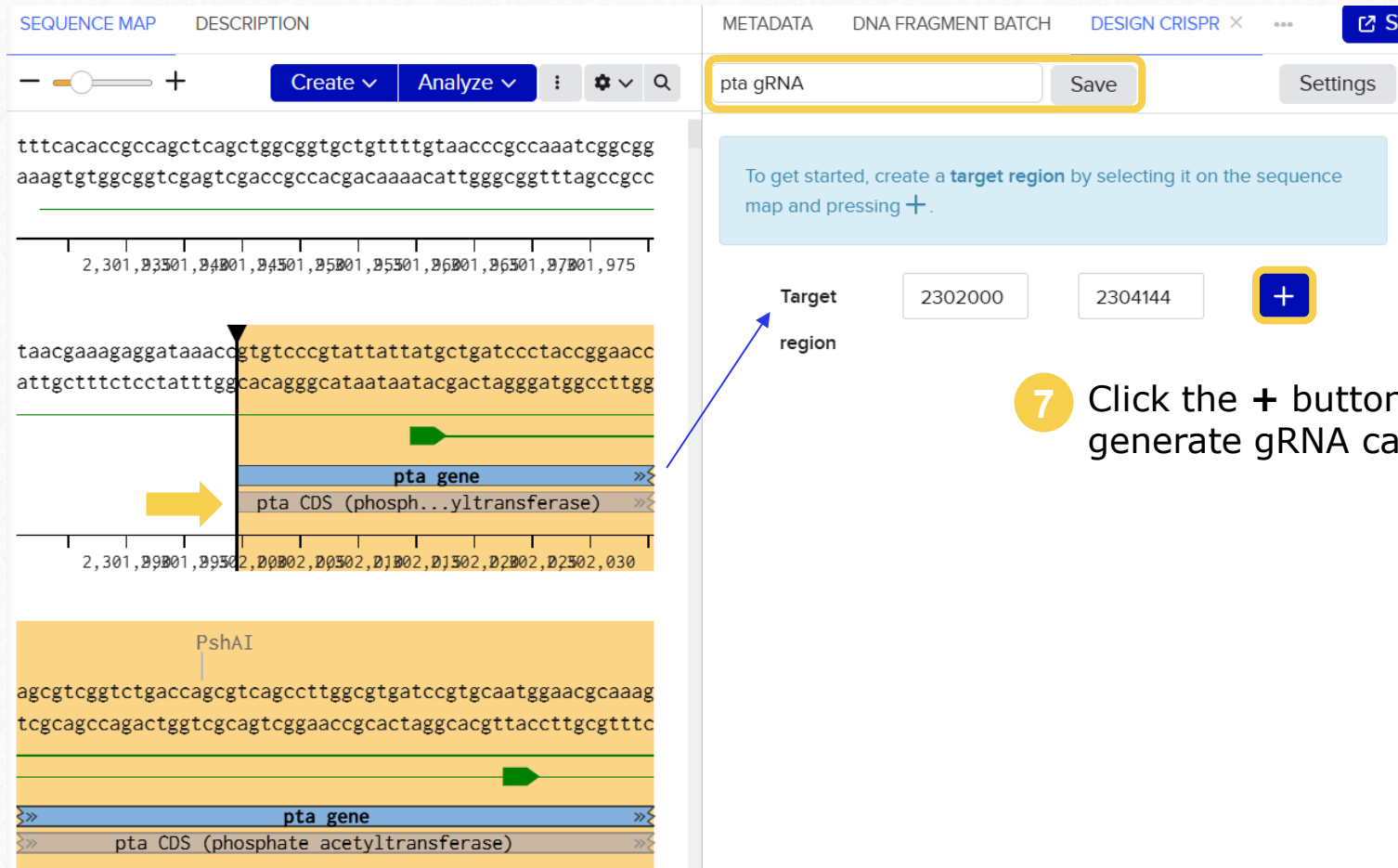
Finish

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



5 Give a name to your CRISPR design tab and save it so you can come back to it if you need to.

6 Select the **pta CDS** annotation. The target region will be set automatically.



The screenshot displays the CRISPR design tool interface. On the left, the 'SEQUENCE MAP' tab is active, showing a genomic map with a yellow arrow pointing to the 'pta CDS (phosph...yltransferase)' annotation. The 'DESCRIPTION' tab is also visible. On the right, the 'DESIGN CRISPR' tab is active, showing a text input field labeled 'pta gRNA' with a yellow border, a 'Save' button, and a 'Settings' button. Below the input field, a blue box contains the instruction: 'To get started, create a target region by selecting it on the sequence map and pressing +.' Further down, there are two input fields for 'Target region' with values '2302000' and '2304144', and a blue button with a white '+' symbol. A blue arrow points from the '+' button to the 'pta CDS' annotation in the sequence map.

7 Click the **+** button to generate gRNA candidates.

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

SEQUENCE MAP DESCRIPTION METADATA DNA FRAGMENT BATCH DESIGN CRISPR

ptg gRNA Save Settings

Target region 2302000 2304144 +

☒	Start	End	Annotations	Genome Region
☒	2302000	2304144	pta CDS (phosphate acetyltransferase), pta gene	No region set

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

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

Genome region

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

☐ None

☒ Chromosome

Find genome matches

Set genome region Cancel

CRISPR tools

gRNA design

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

The screenshot displays the Benchling gRNA design tool interface. On the left, a sequence map shows the pta gene (phosphate acetyltransferase) with a gRNA target site highlighted. The right panel shows a list of potential gRNAs with columns for position, strand, guide sequence, PAM, and on-target/off-target scores. The gRNA at position 2302190 is selected.

position	Strand	Guide sequence	PAM	On-Tar get score	Off-tar get score
☐ 2302910	-	gtagccgccagtcagcagca	ggg	76.3	99.2
☐ 2302761	-	agaaagtgcaggatttaacg	cgg	73.0	100.0
☐ 2303021	-	gaagctctgcaggctcagag	agg	72.0	100.0
☐ 2303477	+	cgaacagctggaagacaacg	tgg	71.8	99.4
☐ 2302391	+	aatcgctaaaacgctgaatg	cgg	71.6	100.0
☒ 2302190	+	tgcgaaactcttcaccacga	cgg	70.6	99.4
☐ 2302210	-	tcaacgtagctcattttcag	cgg	69.7	99.8
☐ 2303170	-	agctgataacggaacgcagg	cgg	69.0	100.0
☐ 2303277	+	gccgctatctgtgctgaacg	tgg	68.8	99.8
☐ 2302436	-	acgctctttcagctgttccg	ggg	68.5	99.6
☐ 2302508	-	cagtttgtaaagcataacgc	cgg	68.3	99.9

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

10 Select the gRNA as shown.

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

11 Click **Assemble**.

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

CRISPR tools

gRNA design

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

The screenshot shows the Benchling pCRISPR tool interface. On the left, a sidebar lists files: 'pCRISPR' (last modified 6 days ago) and 'pta source' (last modified 6 days ago). The main panel displays a sequence map of the 'pta' gene, showing the CRISPR array and the gene structure. The right panel shows the 'Select Expression Vector' section, where the option 'Choose a plasmid from your Benchling folders' is highlighted. A yellow arrow points from the 'pCRISPR' file in the sidebar to this option. Below the highlighted option, there is a dashed box with the text 'Search for a sequence in the file browser and drag it here'. The bottom of the interface shows the assembly method 'Type IIS Cloning' and the insertion region 'Start' and 'End'.

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

CRISPR tools

gRNA design

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

14 Click **Next**.

The screenshot displays the Benchling CRISPR tool interface. On the left, a sidebar shows the project structure with '3. CRISPR tools' and 'pta source'. The main workspace is divided into two panels. The left panel, titled 'pCRISPR', shows a 'SEQUENCE MAP' of the 'pta' gene. A green bar indicates the 'pta gene' and a grey bar indicates the 'pta CDS (phosphate acetyltransferase)'. A specific region is highlighted with a green bar and labeled 'pta'. The right panel, titled 'ASSEMBLE CRISPR', shows the 'Select Expression Vector' section. Under 'Vector Source', the option 'Choose a plasmid from your Benchling folders' is selected. Under 'Assembly Method', 'Type IIS Cloning' is selected. The 'Insertion Region' is set to '22' and '326'. A 'Next' button is visible at the bottom right of the assembly panel.

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

CRISPR tools

gRNA design

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

SEQUENCE MAPDESCRIPTION

+

+

Create ▾Analyze ▾Copy⋮⚙️🔍

EagI

agactacgactatcgtgcgtgcaactcttccaccacgacggcgcgtgaaccgctga

tctgatgctgatagcacgcacgttgagaaggtggtgctgccggcgacttggcgact

52545658606264666870

QTTTIVRA NSSTTTAAEPL

pta

pta gene

pta CDS (phosphate acetyltransferase)

2,302,1602,302,1702,302,1802,302,1902,302,200

aatgagctacgttgaaggtctgctttccagcaatcagaaagatgtgctgatggaag

tttactcgatgcaacttccagacgaaaggtcgttagtctttctacagactaccttc

727476788082848688

KMSYVEGLLSN QKDVLME

pta

pta gene

pta CDS (phosphate acetyltransferase)

METADATADNA FRAGMENT BATCHASSEMBLE CRISPR ✕⋮

Share

Finalize Assemblies

Assembly Name

Guide Sequence ?

pCRISPR-pta

tgcaactcttccaccacga ✕

+ Add

Folder

3. CRISPR tools

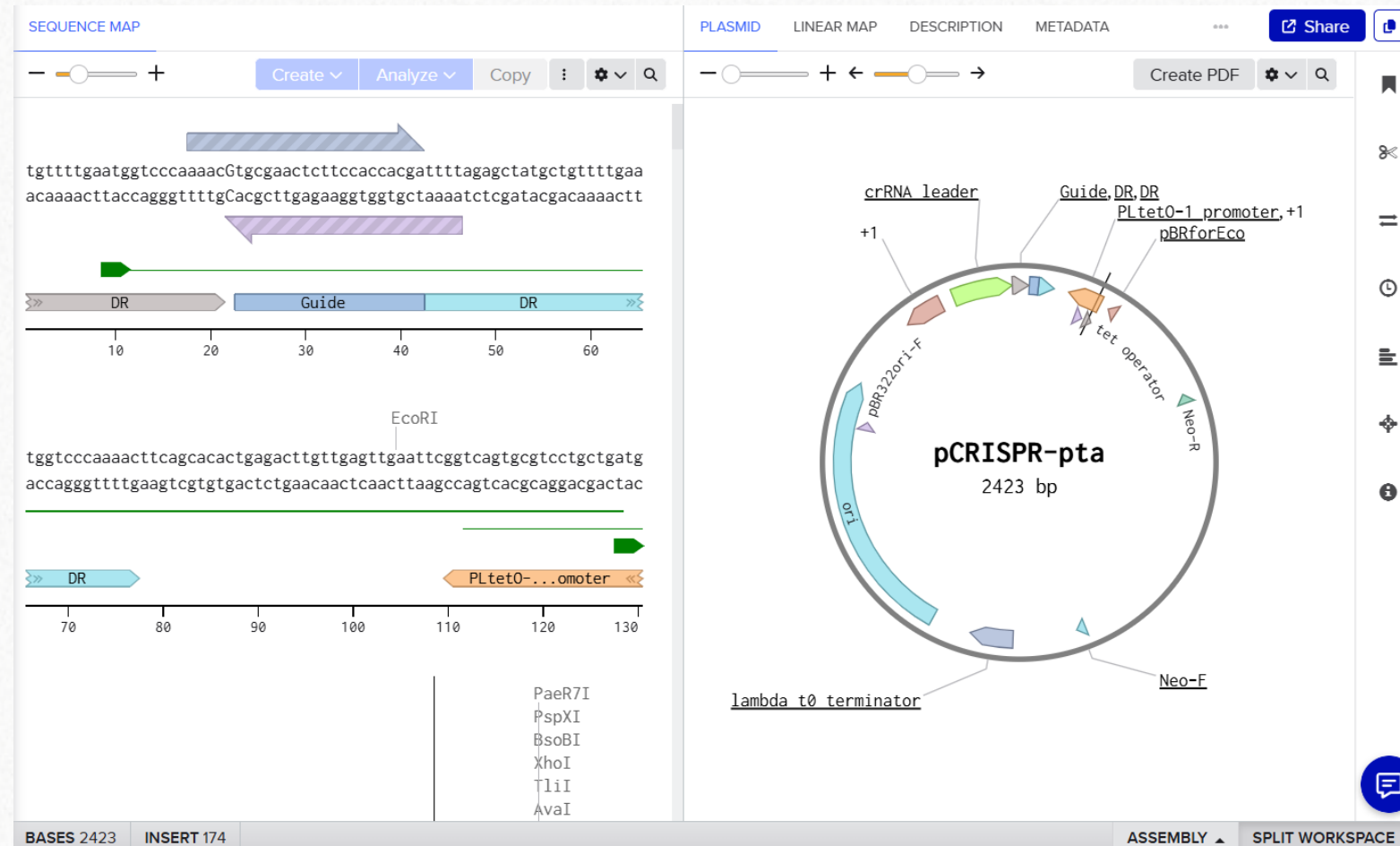
Previous

Assemble

CRISPR tools

gRNA design

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



7. CRISPR tools

7.2 HR template design



CRISPR tools

HR template design

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

The screenshot displays the pCRISPR web interface. On the left, the 'SEQUENCE MAP' view shows the 'pta source' file with its sequence and gene annotations. The 'DESCRIPTION' tab is active, showing the 'pta gene' and 'pta CDS (phosphatyltransferase)'. The 'LINEAR MAP' view on the right shows the 'pta source (3929 bp)' with annotations for 'pta gene', 'pta CDS (pho...ransferase)', 'yfcC gene', and 'yfcC CDS...rotein'. A yellow arrow points to the 'Design HR template (ssODN)' button in the 'CRISPR' section of the right panel. The 'CRISPR' section also includes a 'Design and analyze guides' button and a 'Saved guide analyses' section showing 'No guide analyses'.

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

The 'Design HR template' dialog box is shown. It contains the following fields and options:

- Genome:** ASM956v1 (Escherichia coli BL21(DE3))
- PAM:** NGG (SpCas9, 3' side)
- ☒ Create a copy of this sequence
- ☐ Modify this sequence
- Buttons:** Cancel, Create

CRISPR tools

HR template design

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

4 Delete 30 nt as shown.

SEQUENCE MAP DESCRIPTION

— — — — —

Create Analyze Copy

pt gene
pta CDS (phosphate acetyltransferase)

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

EagI

accacgacgcccgtgaaccgctgaaatgagctagcttgaagctgtctttccagcaatcagaa
tggtgtgctccggcgacttggcgacttttactgatgcaactccagacgaagctgttagtctt
64 66 68 70 72 74 76 78 80 82 84
T T T A A E P L K M S Y V E G L L S S N Q K
pta

pta gene
pta CDS (phosphate acetyltransferase)

2,302,240

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

cgctgaagtcgttc
gcgacttcagcaag
102 104
D V L M E E I V A N Y H A N S K D A E V V
pta

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

ASSEMBLY SPLIT WORKSPACE

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

SEQUENCE MAP DESCRIPTION

— — — — —

Create Analyze Copy

pt gene
pta CDS (phosphate acetyltransferase)

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

EagI

accacgacgcccgtgtctttccagcaatcagaaagatgtgctgatggaagagatcgtcgaaa
tggtgtgctccggcgacttggcgacttttactgatgcaactccagacgaagctgttagtctt
64 66 68 70 72 74 76 78 80 82 84
T T T A G L L S S N Q K D V L M E E I V A N
pta

pta gene
pta CDS (phosphate acetyltransferase)

2,302,240

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

ctaccacgct
gatgtgctga
86
Y H A
pta

cccgcacgtaagc
gggctgtgcattcg
102 104
P T R K
pta

BsAI

BASES 3899 INSERT 2302198

ASSEMBLY SPLIT WORKSPACE

6 Click **Next**.

HR template design

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

SEQUENCE MAPDESCRIPTION

Editing disabled beca...CopyCreate PDF

gagccttggcgtgatccgtgcaatggaacgcaaaggcgttcgtctgagcgttttcaaacctatcg
gtcggaaaccgactaggcacgttaccttgcgtttccgcaagcagactcgcaaaagtttgatagc
20 22 24 26 28 30 32 34 36 38 40
S L G V I R A M E R K G V R L S V F K P I
pta

pta gene
pta CDS (phosphate acetyltransferase)

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

ctcagccgcgtaccggtggcgtatgcgccgatcagactacgactatcgtgctgctgcaactcttcc
gagtcggcgcgtggccaccgctacgcgggctagtctgatgctgatagcacgcacgcttgagaagg
42 44 46 48 50 52 54 56 58 60 62
A Q P R T G G D A P D Q T T T I V R A N S S
pta

pta gene
pta CDS (phosphate acetyltransferase)
gRNA

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

accacgacggcctaaggctgctttccagcaatcagaaagatgtgctgatggaagagatcgtcgc

METADATADNA FRAGMENT BATCHDESIGN HR TEMPLATE

Share

Settings

Step 2: Adjust HR arms

Adjust the region to use as the HR template by clicking and dragging the ends of the selection on the sequence map.
A 200 bp region around your mutations has already been selected. At least 50 bp on each side flanking the mutations is recommended.

Template region

2302098 - 2302297Reset to default

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

Knock-in edits

Deleted gctgaaccgctgaaaatgagctacgt at 2302198
Deleted g at 2302199

BackNext

7.2 HR template design

107

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

9 Click **Next**.



CRISPR tools

HR template design



Step 4: Summary

Knock-in edits

Deleted gctgaaccgctgaaaatgagctacgt at 2302026

Deleted g at 2302027

Template Range	2302098 to 2302297
Guide	tgcgaaactcttccaccacga
Original Target Site	... cgt gcg aac tct tcc acc acg acg gcc ...
After Site Removal	... cgt gcg aac tct tcc acc acg ACC gcc ...

Copy the template or its reverse complement to your clipboard.

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

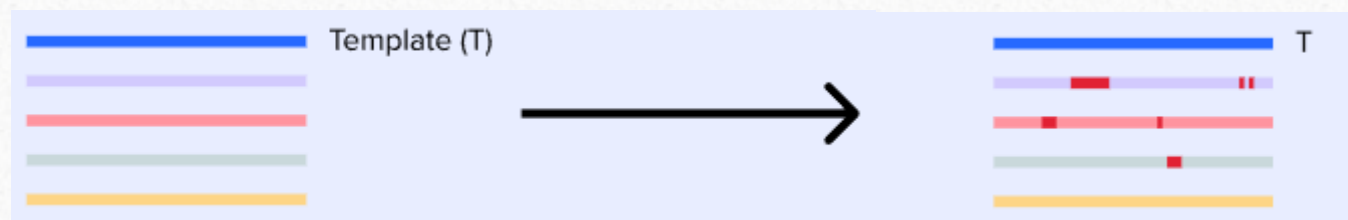
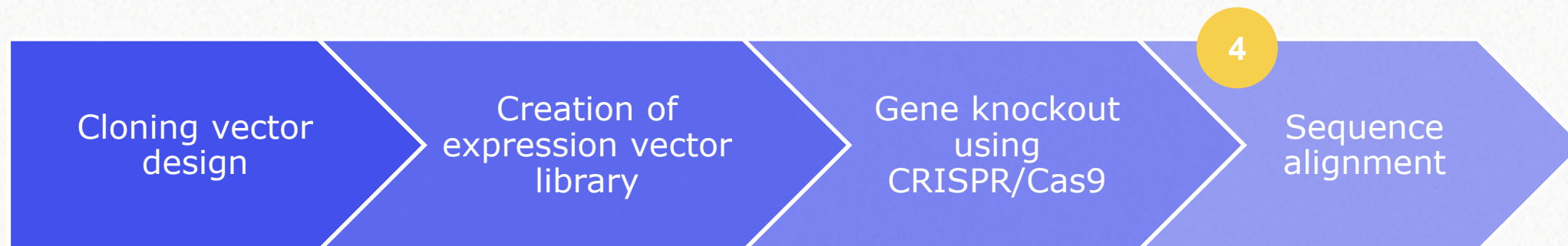
- ✓ After the design process, you can copy the resulting HR template and paste it onto a new DNA sequence file to save it.



8. Sequence alignments



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



✓ Multisequence alignment

Bonus: How to do consensus alignments

Expected output:

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

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

... / Your Name / Training Files /

4. Sequence alignments

Search

Type Filters

FW-seq-1
Last modified 6 days ago

MID-seq-1
Last modified 6 days ago

plasmid-seq
Last modified 6 days ago

pSEVA6311-phaC-pct540
Last modified 6 days ago

pSEVA6311-phaC-pct540

PLASMID DESCRIPTION

7955 bp

PSI-1 (mismatches: 0)

chnR

NdeI, +3

+8

+2

+2

+5

+4

+7

+1

+2

oriT

rep gene

EcoRV

EcoRV

phaC1 (MBEL6-19)

chnR-PchbB-GA-R

Propionate-CoA transferase

BASES 7955

INSERT 1180

METADATA PLASMID BATCH SEQUENCE MAP

Create Analyze

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

Propionate-CoA transferase

5 10 15 20 25 30 35 40 45 50 55

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

Propionate-CoA transferase

60 65 70 75 80 85 90 95 100 105 110

gtaaagtaaatgatattaccaagaagatattgtgaattgtagagattaagg
catttcattactataatgtttcttctataacaacttaaccatctctaattccc

8. Sequence alignments

112

8. Sequence alignments

8.1 Alignment tool



Alignment creation

Alignment tool overview

i In a real-life scenario, the construct sequences could be sent to sequencing. The results could then be analyzed using the **alignment tool** in Benchling.

✓ There are **three alignment options** and several alignment programs available:

Create DNA / RNA alignment

1

2

Choose input

Define parameters

Pairwise

Multisequence

Consensus

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

Template (T)

→

T

T

Template(s) +

Non-template sequence(s)

Choose an alignment program.

Auto (MAFFT)

Show parameters

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

1

Pairwise alignment:

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

8.1 Alignment tool

114

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

2

Choose input

Define parameters

Pairwise

Multisequence

Consensus

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

Template (T)

→

T

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

Template (T)

→

T

Template(s) +

Non-template sequence(s)

Choose an alignment program.

Auto (MAFFT)

Show parameters

2

Multisequence alignment:

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

8.1 Alignment tool

115

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](#)

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

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.

Auto (MAFFT)

Hide parameters

Gap open penalty

1.53

Gap extension penalty

0

Adjust direction

Enabled, quickly

Reset to defaults

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



8. Sequence alignments

8.2 Multisequence alignment



Alignment creation

Multisequence alignment

- 1 Open the **Sequence alignments** folder.
- 2 Select all the files in the folder. From the **Analyze** menu, select **Create DNA/RNA Alignment**.

... / Your Name / Training Files /

4. Sequence alignments Saved Searches

Search Type Filters

< > 1-4 of 4 items

4 rows selected More

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

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

More menu options:

- Create DNA / RNA Alignment
- Auto-Annotate
- Attach Primers
- Auto-fill part fields
- Auto-fill translations
- Auto-fill transcriptions
- Set topology
- Codon optimize
- Remove annotations

Analyze menu options:

- Open
- Analyze
- Refresh

Alignment creation

Multisequence alignment

Create DNA / RNA alignment

1

2

Choose input

Define parameters

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

Drag and drop to upload or

Choose files

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA

RNA

Name

Bases

Add

Sequences

FW-seq-1

MID-seq-1

plasmid-seq

pSEVA6311-phaC-pct540

Cancel

Next

3

Click **Next**.

8.2 Multisequence alignment

120

Alignment creation

Multisequence alignment

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

Create DNA / RNA alignment

1

2

Choose input

Define parameters

Pairwise

Multisequence

Consensus

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

Template(s)

+

pSEVA6311-phaC-pct540

pSEVA6311-phaC-pct540

Non-template sequence(s)

FW-seq-1

MID-seq-1

Search

plasmid-seq

Search

Choose an alignment program.

MAFFT

recommended for nucleotide alignments

Faster, less precise, can reverse sequences

Clustal Omega

recommended for amino acid alignments

Slower, more precise, cannot reverse sequences

Auto (MAFFT)

Show parameters

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

Back

Create Alignment

- Create the alignments.

8.2 Multisequence alignment

121

Alignment creation

Multisequence alignment

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

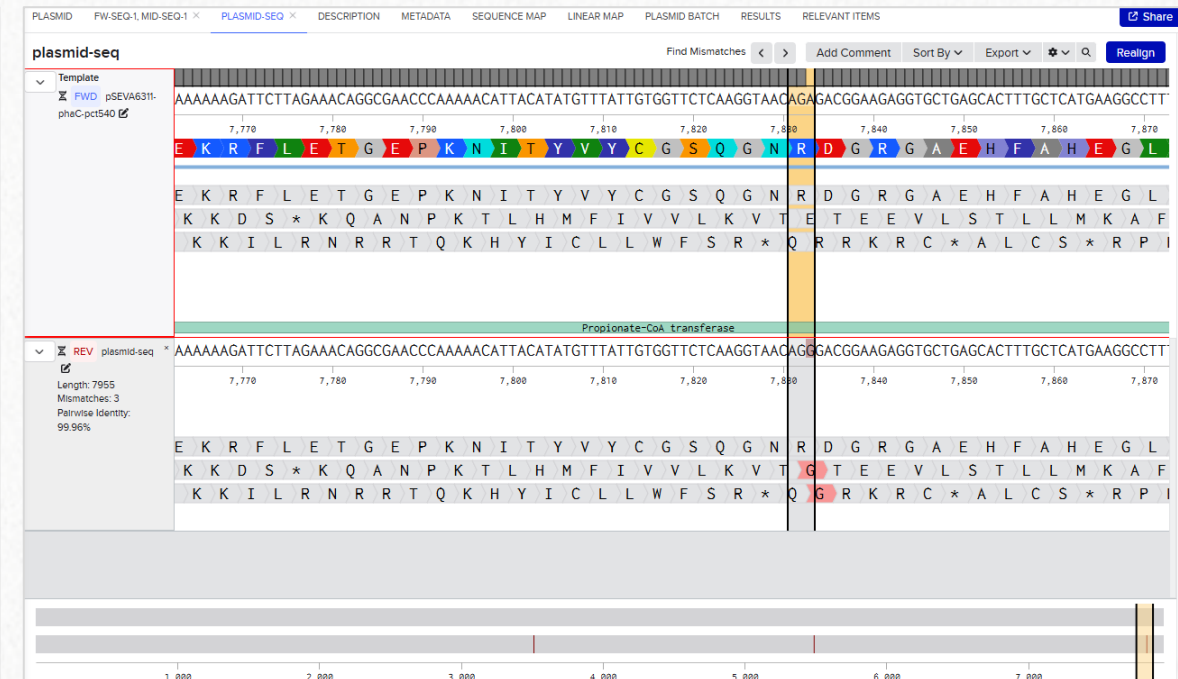
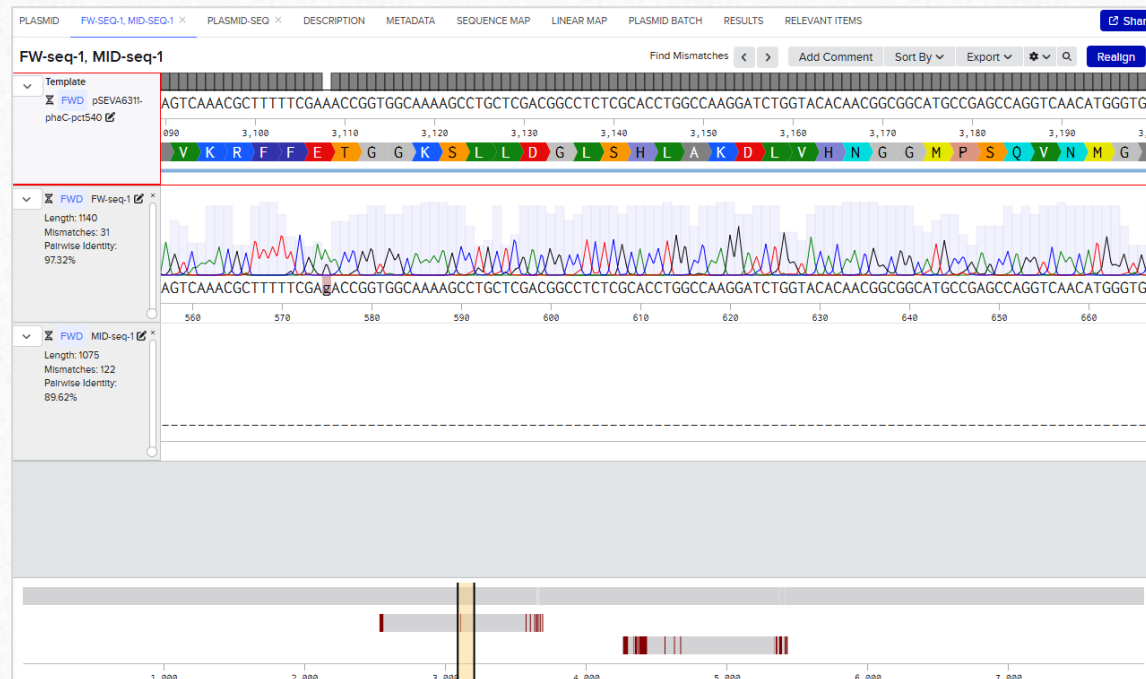
The screenshot shows the pSEVA6311-phaC-pct540 plasmid map on the left and the sequence alignment interface on the right. The plasmid map is a circular diagram with various features labeled, including the Propionate-CoA transferase gene, phaC1 (MBEL6-19), chnR-PchmB-CA-R, acc3 CDS, and chnR. The sequence alignment interface on the right displays the DNA sequence and a protein sequence (V S Q G A L C H L F R D I A S H K P). A 'SEQUENCE ALIGNMENTS' panel is open, showing a 'Create New Alignment' button and a 'Saved Alignments' section with a list of alignments: 'FW-seq-1, MID-seq-1' and 'plasmid-seq', both dated 17/02/2025 18:55. A yellow arrow points to the 'Saved Alignments' section.

- Open the alignments.

Alignment creation

Multisequence alignment

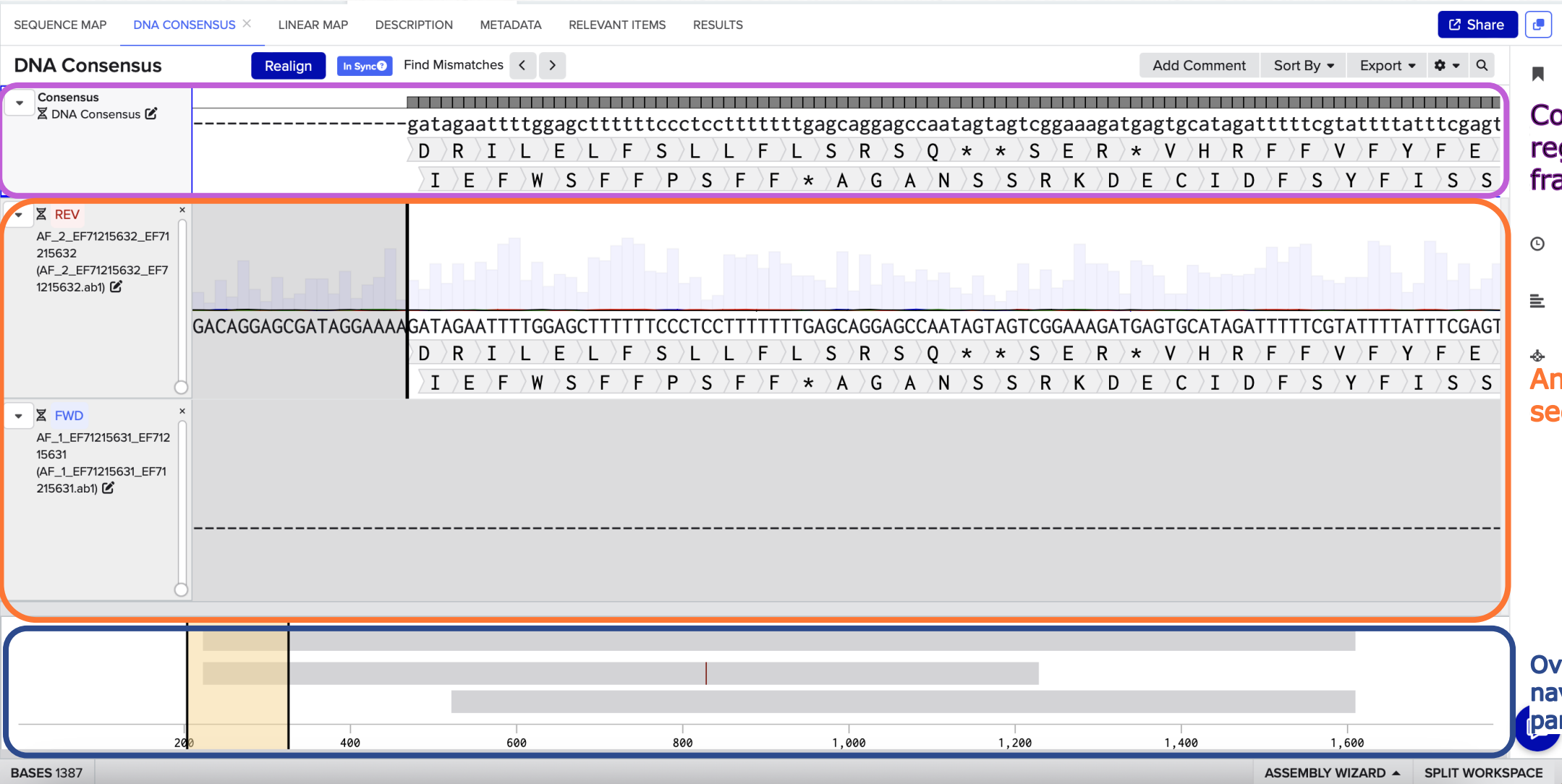
- ✓ You can now see and browse your resulting alignments.
- ✓ You may notice the first one includes trace files, which can help you assess the quality of the sequencing and assess whether the result can be considered accurate.
- ✓ The second one is a sequencing file for the whole plasmid. You can assess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



8. Sequence alignments

8.3 Consensus alignment





Consensus region fragment

Analysed sequences

Overview navigation panel

Alignment creation

Consensus alignment navigation

You can jump from one mismatch to the other easily

Find Mismatches < >

DNA Consensus

☒ DNA Consensus

Consensus: tgcaagttctgtggagttccatgtataagggtatacatattagaaaagctttacgattatataactaacatcatgtacaaaacaat

L Q V L W S S M Y K V Y I L E K L Y D Y I L T S C T K Q

C K F C G V P C I R Y T Y * K S F T I I Y * H H V Q N N

REV

AF_2_EF71215632_EF71215632 (AF_2_EF71215632_EF71215632.ab1)

FWD

AF_1_EF71215631_EF71215631 (AF_1_EF71215631_EF71215631.ab1)

Mismatches from the consensus sequence are marked in red.

S P Q L E

G P P N * S

You can edit what elements to visualize

- ☒ Annotations
- ☒ Translations
- ☒ Amino Acid Indices
- ☒ Reading Frames
- ☒ Primers
- ☐ Alignment Axis
- ☐ Sequence Axis
- ☒ DNA
- ☒ Trace
- ☒ Trace Quality
- ☐ Quality-based capitalization (20)
- ☒ Votes
- ☒ Row Statistics
- ☒ Comments
- ☒ Expanded Mini-Map

Show Compact View

nt Sort By Export

BASES 1387 START 278 END 281 LENGTH 4 GC 0.00% MELTING TEMP -83.7 °C

ASSEMBLY WIZARD SPLIT WORKSPACE

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](#)



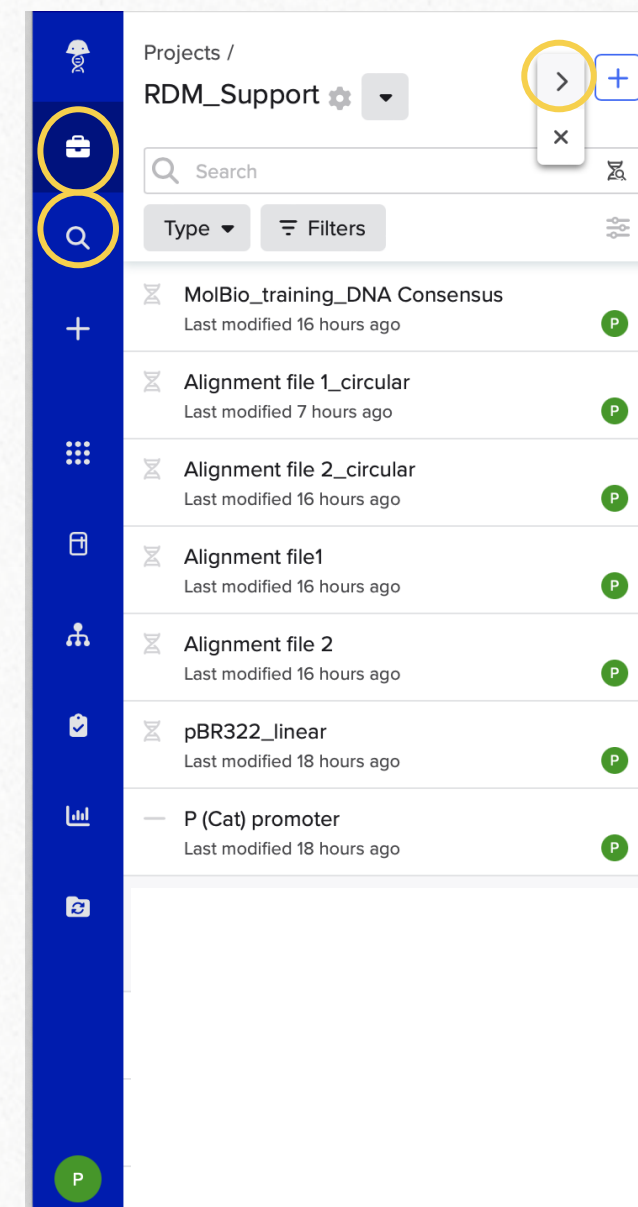
Tips and tricks

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



Tips and tricks

Work in bulk using the expanded view

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

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

The screenshot shows the RDM_Support interface in the 'expanded view'. The top navigation bar includes 'Projects / RDM_Support', a search bar, and a 'Saved Searches' dropdown. Below the navigation bar, there are buttons for 'Type' and 'Filters'. The main content area displays a table with 24 items, showing the first four rows. Each row has a checkbox for selection. Above the table, a row of bulk action buttons is visible: 'Register', 'Move to', 'Copy to', 'Archive', 'Create request', 'Add to worklist', and 'Export'. Blue arrows point from these labels to their respective buttons in the interface.

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 table of projects and a context menu. The table has columns for Name, Inventor, and Review Stat. The context menu is open over the table, showing a list of actions. A yellow box highlights the 'Analyze' action in the context menu. A yellow arrow points from the 'More' button in the table to the 'Analyze' action in the context menu.

Projects / RDM_Support Saved Searches ▼

Search Type ▼ Filters

< > 1-24 of 24 items

Name	Inventor	Review Stat
☒ MolBio_training_DNA Consensus		Diaz
☒ Alignment file 1_circular		Diaz
☒ Alignment file 2_circular		Diaz
☒ Alignment file1		Diaz

Context Menu Actions:

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

More ▼

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.

The screenshot shows the Benchling web interface for sequence alignment. At the top, there's a toolbar with various buttons. A yellow circle with the number '1' highlights the 'Realign' button. Below the toolbar, the alignment is displayed with a template sequence (GGTCCCCCAATTAGAGCTTTACGATTATATACTAACATCATGTACAAAACAATTTAATAATGATCTGTATTGCTGGCTCAATCCACGTAAATTAATGCCTCAGCACTAGTCCTGCAGGGGTAAC) and a target sequence (G P P N * S F T I I Y * H H V Q N N L I M I C I A G S I H V N * C L S T S P A G V T). Below the sequences, a chromatogram is shown. The 'Realign' button is highlighted with a yellow circle and the number 1.

Tips and tricks

Autoindexing when creating alignments

2

Realign DNA / RNA

1 2

Choose Input Define parameters

Upload sequence and trace files (.ab1, .ftv, .fasta, .gb, and .genious). 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 ☒

EF71215631_EF71215631 ☐

New sequences

Cancel Next

3

Realign DNA / RNA

1 2

Choose Input 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) Show parameters

Alignments performed via MAFFT

Automatically reindex alignment if needed. i

Back Realign

Tips and tricks

Autoindexing when creating alignments

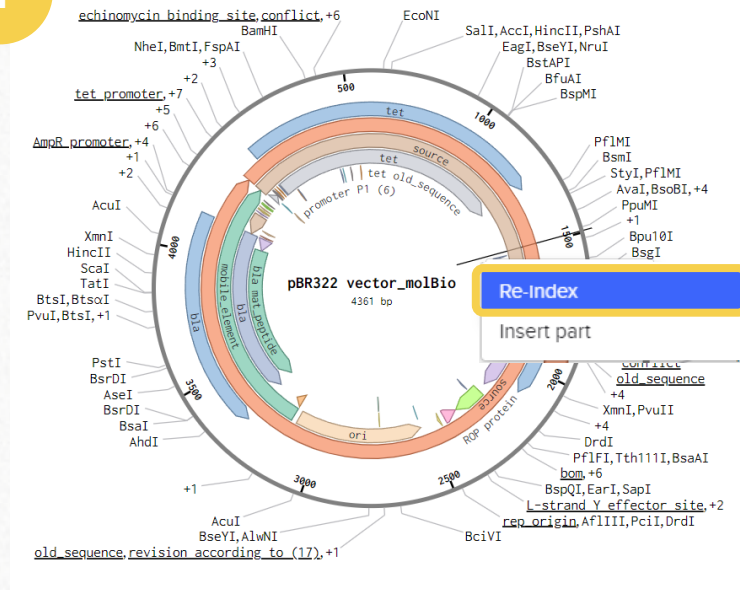
When creating an alignment of circular sequences, Benchling by default performs an **auto indexing** of these sequences.

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

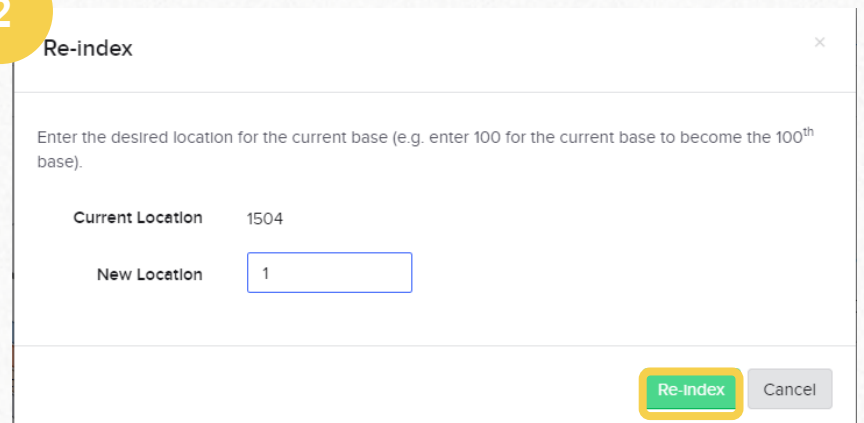
Pro TIP:

- ✓ 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.

1



2



Re-index

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

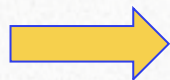
Current Location	1504
New Location	1

Re-index Cancel

10. Resources



Questions?



Contact lims_support@biosustain.dtu.dk



More resources

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>

New

Molecular Biology Tools

FREE

8 courses | 2h 45m

New

Foundational Practitioner

ENROLLED

9 courses | 2h 50m

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>

