

Search

Search

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 stor			4°C Fridge
4C002	4C Fridge 00271			4°C Fridge
4C009	4C Fridge 01223	DTU Buildi...	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 Buildi...	19/11/2018	4°C Fridge
4C003	4C Fridge DSP1	DTU Buildi...	09/08/2018	4°C Fridge

The basics of the Molecular Biology Tools

Reach out when struggling with the platform:

BRiGHT Benchling support
lims_support@bright.dtu.dk



Access Benchling:

bright.benchling.com

(login with DTU credentials)



Additional resources:

[LIMS Help guides](#)

[Benchling Help Center: Molecular Biology](#)



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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

Functionalities

Your sequence

The screenshot displays a web-based sequence analysis tool interface. At the top, there are navigation tabs: SEQUENCE MAP (selected), LINEAR MAP, PLASMID, DESCRIPTION, METADATA, RELEVANT ITEMS, and RESULTS. To the right of these tabs are buttons for 'Share' and a clipboard icon. Below the tabs is a control bar with 'Create', 'Analyze', 'Copy', and 'Create PDF' buttons, along with a settings gear and a search icon. The main area shows a DNA sequence map with a horizontal axis from 1,610 to 1,700. The sequence is displayed in two segments. The top segment shows restriction enzyme sites: BsrFI, EarI, and HpaI. The bottom segment shows EarI, BseRI, and MscI sites. A pink bar labeled 'XI-3\UP' spans the width of the map. A blue arrow points to a specific sequence 'ctaagaaaagaagtctctgctcctc' in the bottom segment, and a red box highlights the sequence 'TCGCTAGCCAGO'. On the right side, a vertical toolbar is highlighted with a yellow box, containing icons for bookmark, copy, zoom, history, list, pan, and help.

Functionalities and tools overview



Features (*annotations and translations*)



Digests



Primers



History



Alignments



CRISPR

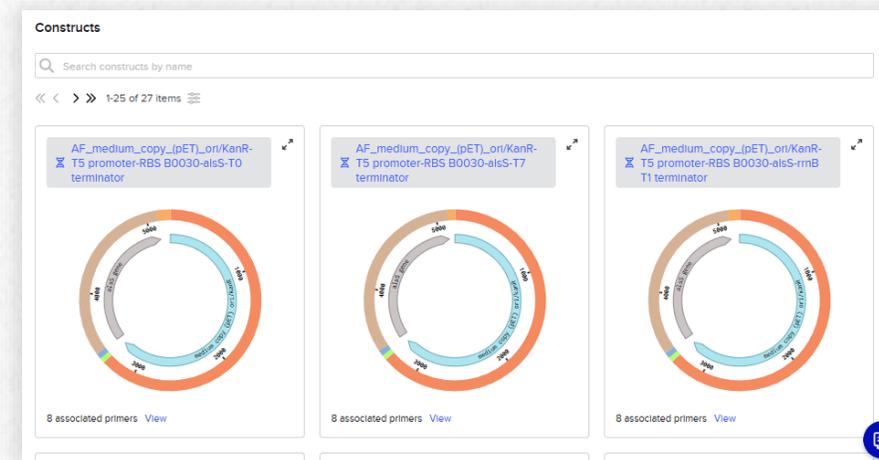
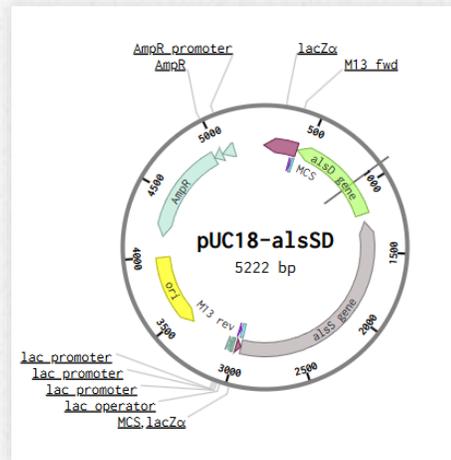


Information (*topology, tags*)

2. Training overview

Training goals:

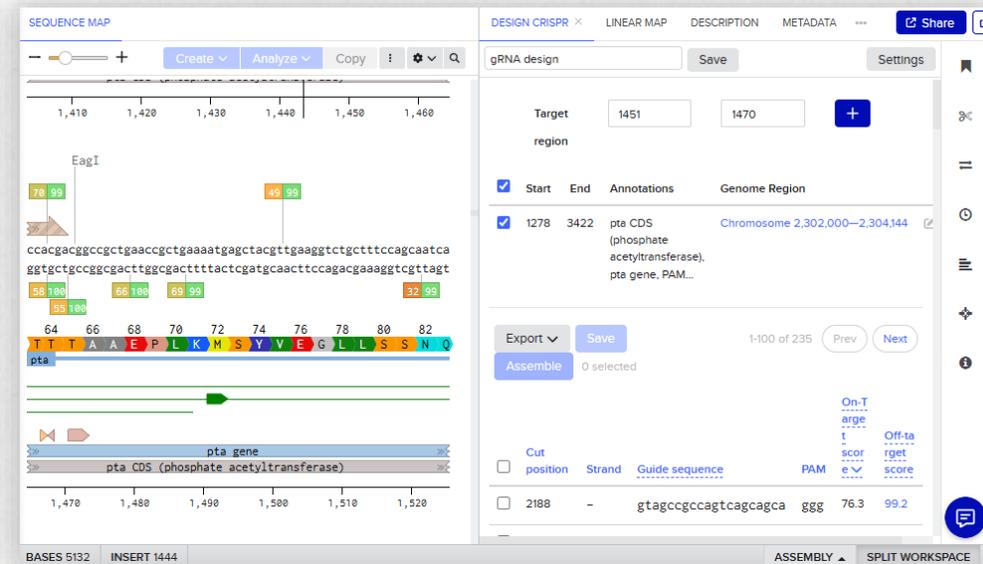
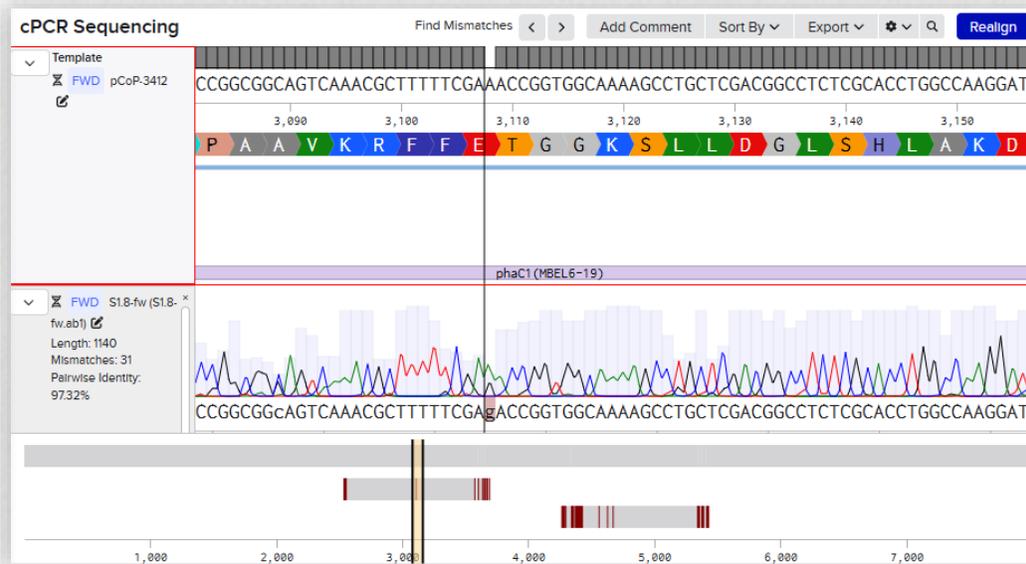
The basics of...



- How to navigate the **sequence visualization** window and the workspace
- How to **assemble** simple constructs
- How to perform bulk assemblies with the **Combinatorial Assembly** tool

Training goals:

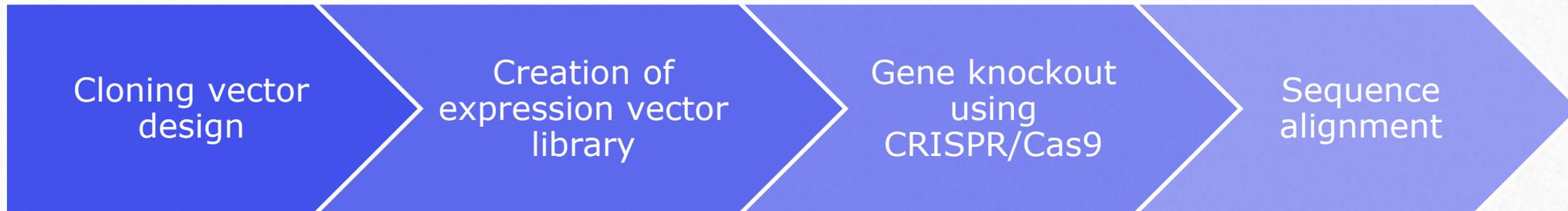
The basics of...



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

Today's work example:

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



Scenario inspired by:

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

Contents lists available at [ScienceDirect](#)



Journal of the Taiwan Institute of Chemical Engineers

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



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

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

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



[Link to article](#)

Today's work example:

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

1

Cloning vector design

Creation of expression vector library

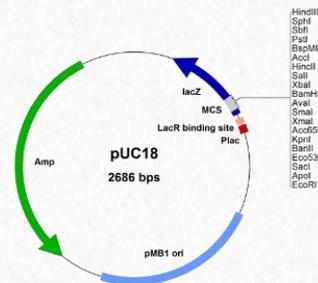
Gene knockout using CRISPR/Cas9

Sequence alignment

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



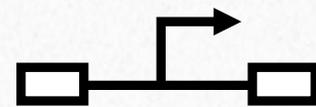
pUC18 cloning vector



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

Today's work example:

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



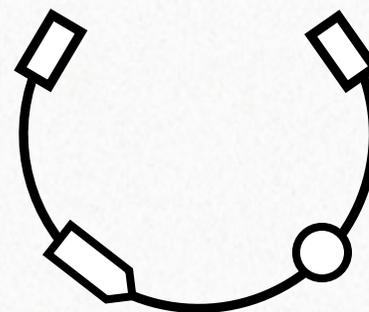
Promoters



RBSs



Terminators



Destination vector

✓ Combinatorial cloning:
Golden Gate

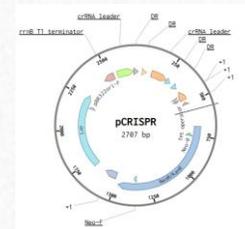
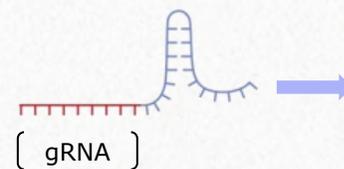
Today's work example:

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



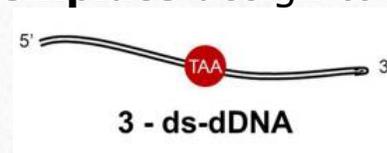
Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR

pta



- ✓ gRNA design
- ✓ HR template design

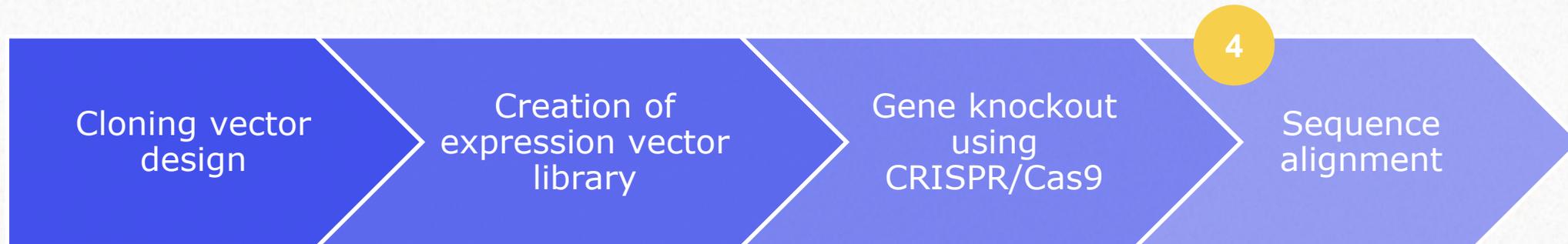
HR template design to KO *pta*



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

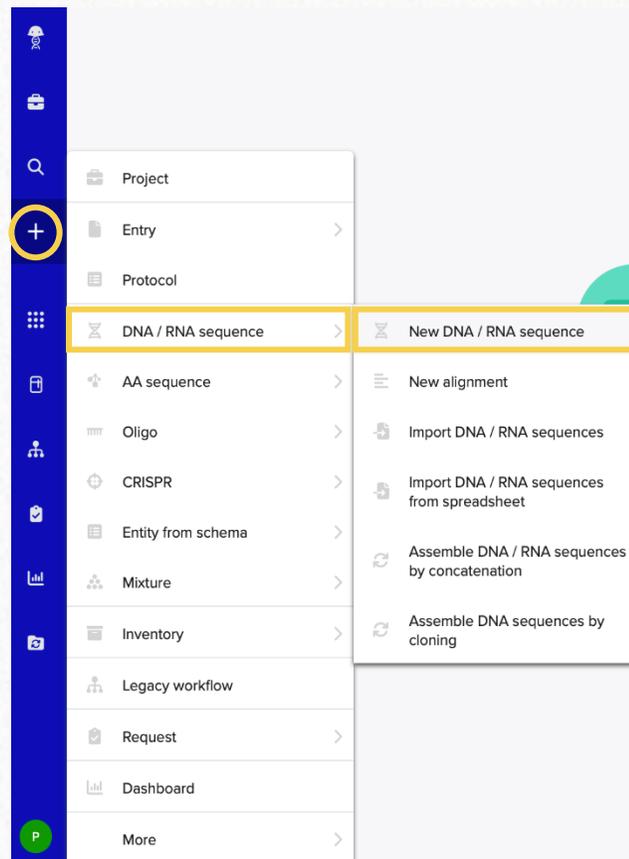
3.1 Sequence creation and import



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 topology
Linear

Set folder*
Patricia B.

Set schema
DNA Fragment

Bases
ggcacgtaagaggttccaacttcaccataatgaaaca

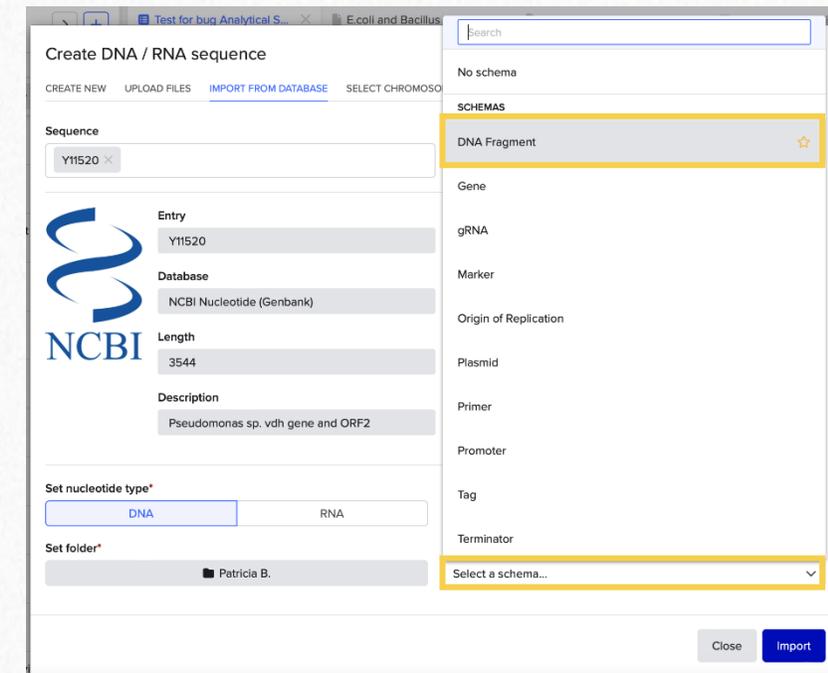
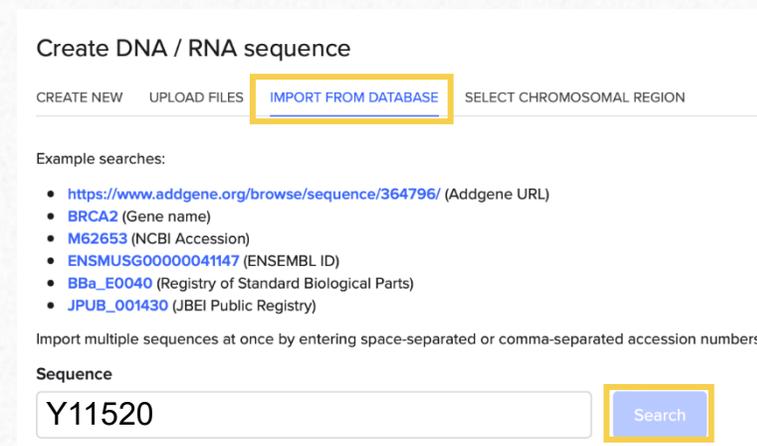
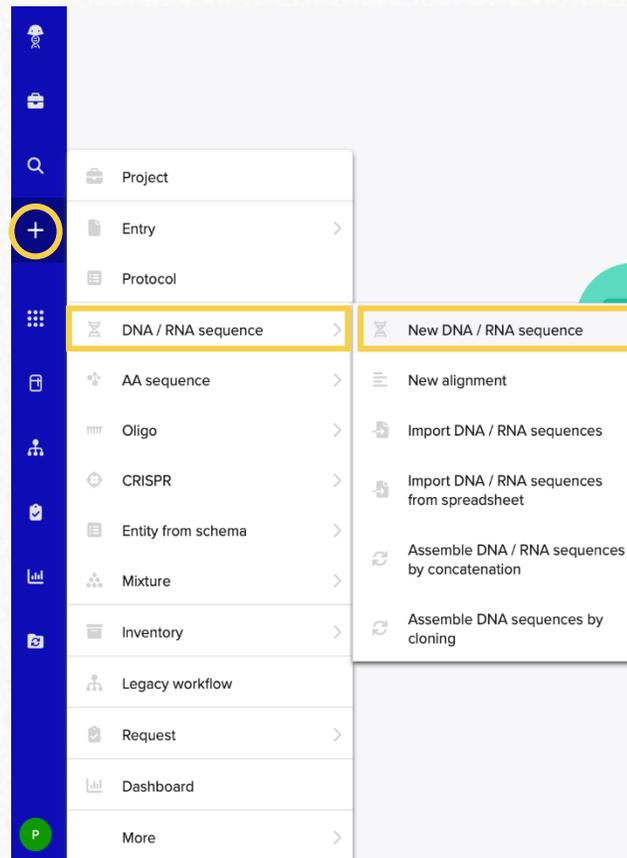
[Close](#) [Create](#)

- 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

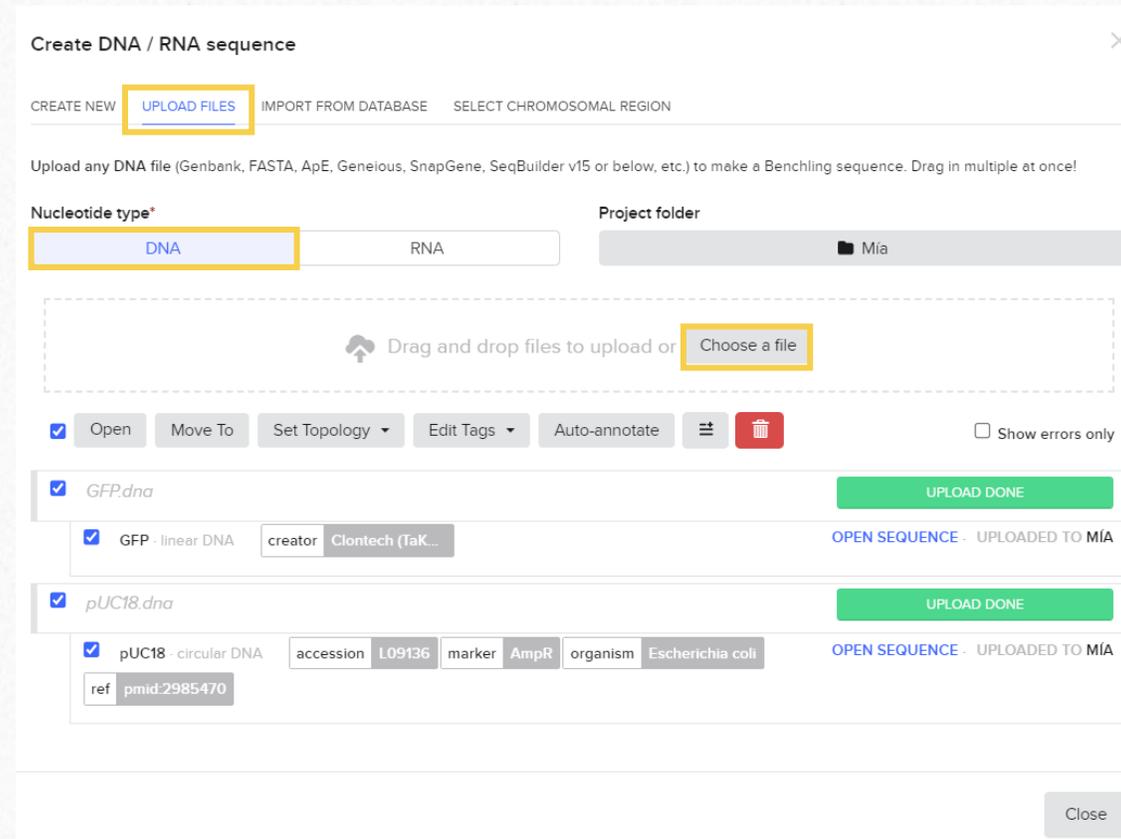
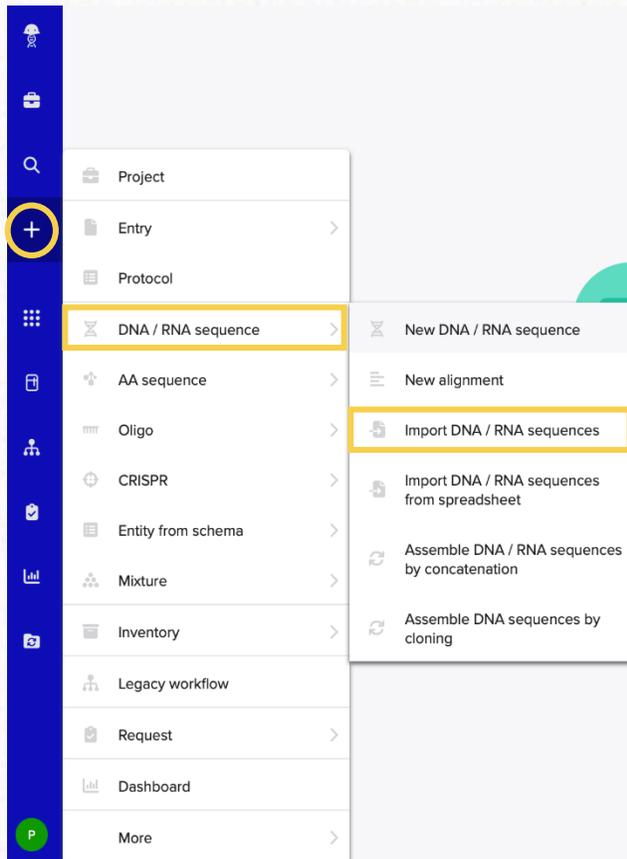
- 1 Create a new DNA sequence
- 2 You can write or paste a valid accession number from databases like GenBank, Addgene or the iGEM Registry
- 3 If the ID is valid, Benchling will show you the gene's description. You can set its schema and import it.



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

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 Auto-annotate Show errors only

Vector_pBR322.gb

Exported · circular DNA

synthetic DNA ... syn...

ACCESSION DEFINITION ORGANISM SOURCE

Create New Tag

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... [OPEN SEQUENCE](#) · UPLOADED TO PATRICIA

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

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... [OPEN SEQUENCE](#) · UPLOADED TO PATRICIA

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

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

Close

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

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

ADD ITEMS TO ENTITY WORKLIST

New worklist Existing worklist

Worklist Name*

Selected items

Exported

Add items to worklist

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 ...

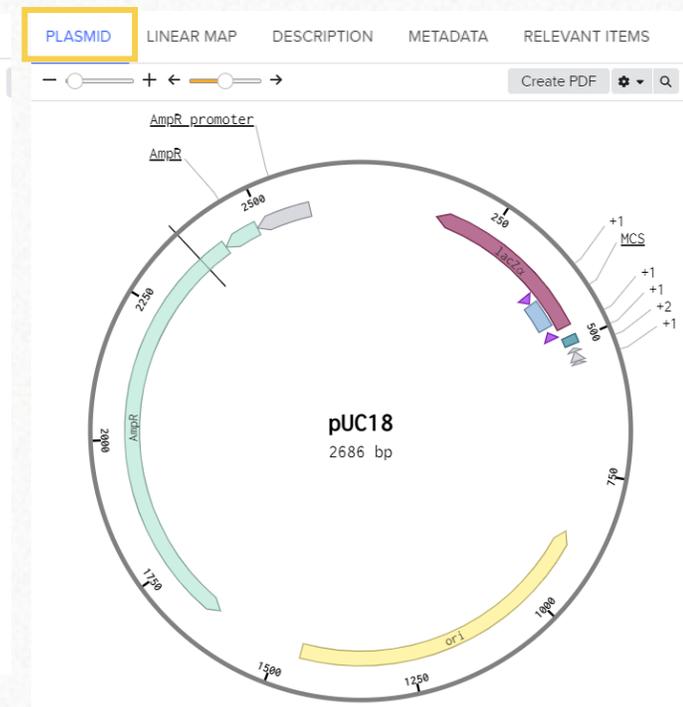
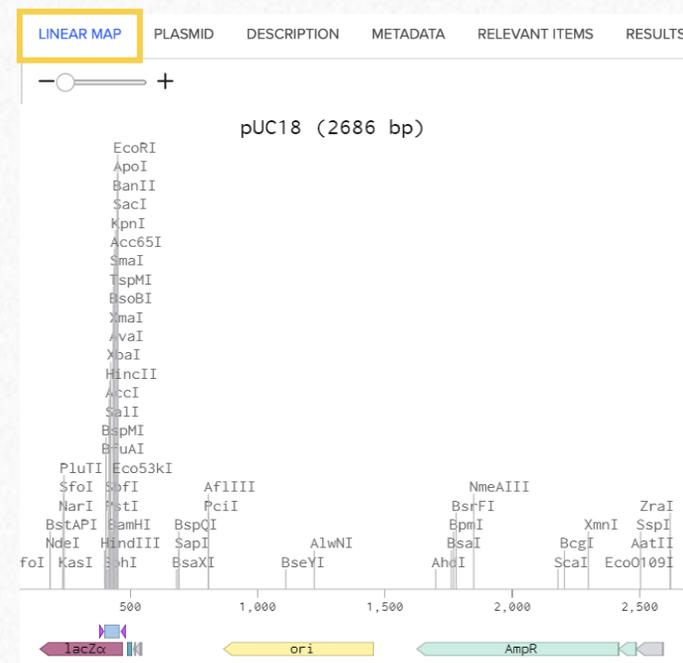
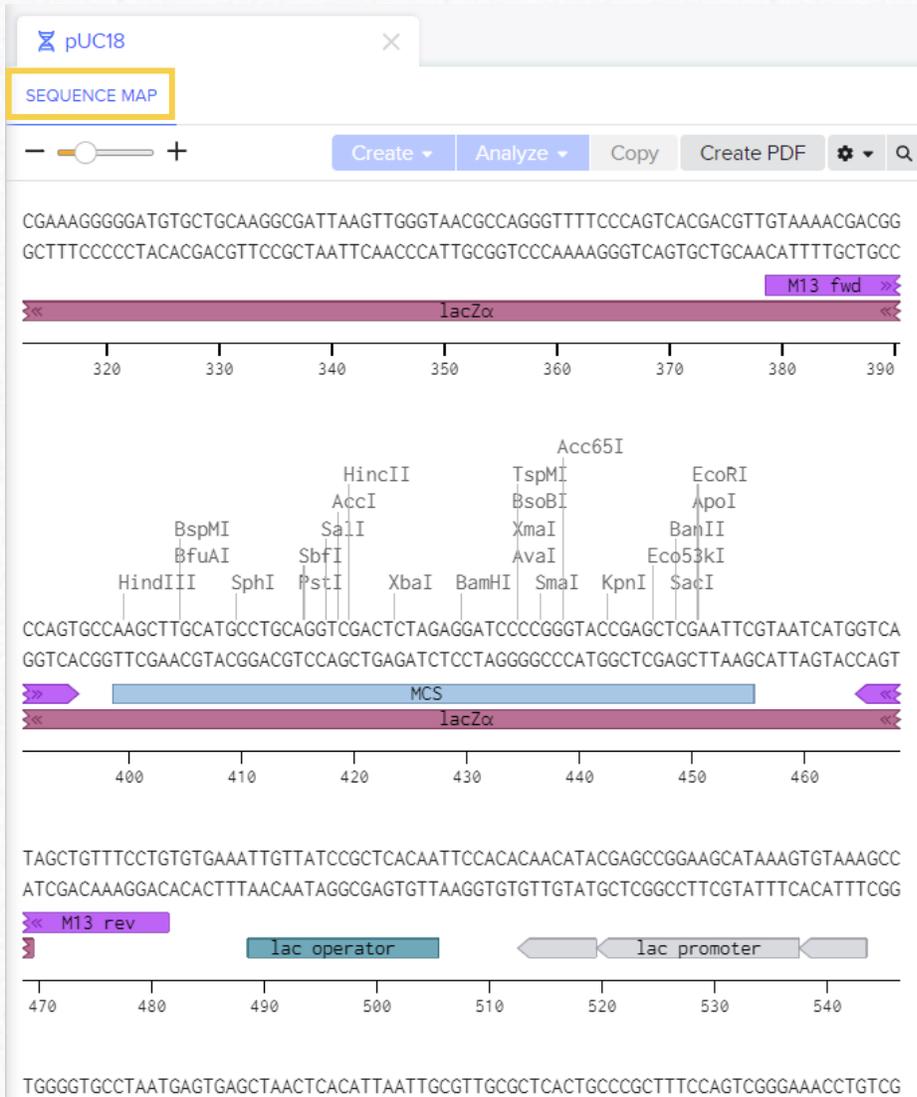
Close

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

3. The basics of sequences

3.2 Sequence visualization

Different viewing options:



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

View, annotate and edit your sequences

Different viewing options:

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



View, annotate and edit your sequences

Sequence navigation:

Functionalities

SEQUENCE MAP

PLASMID | LINEAR MAP | RESULTS | RELEVANT ITEMS | METADATA

Share

Features (annotations and translations)

Digests

Primers

History

Alignments

CRISPR

Information (topology, tags)

pUC18
2686 bp

ASSEMBLY | SPLIT WORKSPACE

View, annotate and edit your sequences

Sequence navigation:

SEQUENCE MAP

PLASMID LINEAR MAP RESULTS RELEVANT ITEMS METADATA

Share

2,350 2,360 2,370 2,380 2,390 2,400 2,410

GCAAAAACAGGAAGGC AAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCTT
CGTTTTGTCTCCGTTTTACGGCGTTTTTCCCTTATCCCGCTGTGCCCTTTACAACCTATGAGTATGAGAAGGAA

AmpR Am...

2,420 2,430 2,440 2,450 2,460 2,470 2,480 2,490

SspI

TTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCATGAGCGGATACATATTTGAATGATTTAGAAAAATAAA
AAAGTTATAAATACTTCGTAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAACTTTTTTATTT

AmpR promoter

2,500 2,510 2,520 2,530 2,540 2,550 2,560 2,570

AatII
ZraI

CAAATAGGGTTCCGCGCACATTTCCCGCAAAAAGTCCACCTGACGTCTAAGAAACCATTTATCATGACATTAACC
GTTTATCCCAAGCGCGTGTAAAGGGCTTTTACGGTGGACTGCAGATTCTTGTGAATAAATAGTACTGTAATTGG

AmpR ...oter

2,580 2,590 2,600 2,610 2,620 2,630 2,640 2,650

Eco0109I

TATAAAAATAGCGGTATCAGGAGCCCTTTCGTC

BASES 2686 START 2487 END 2591 LENGTH 105 GC 32.38% MELTING TEMP 675 °C

ASSEMBLY SPLIT WORKSPACE

pUC18
2686 bp

AmpR promoter
AmpR

lacZ

ori

+1 +1 +1 +1

✓ Click on any element or annotation in any of the views to select the corresponding sequence fragment

✓ See the **electrochemical properties** of the fragment on the bottom

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

View, annotate and edit your sequences

Sequence navigation:

The screenshot shows the 'SEQUENCE MAP' interface. At the top, there are buttons for 'Create', 'Analyze', 'Copy', and 'Create PDF'. The 'Create' and 'Analyze' buttons are highlighted with yellow boxes and arrows pointing to their respective dropdown menus. The 'Create' menu includes options like 'Annotation', 'Primer', 'Translation', 'New AA sequence', 'New DNA', 'New RNA', and 'New part'. The 'Analyze' menu includes 'Run Primer3', 'Run Benchling BLAST', 'Submit to NCBI BLAST', 'Analyze as translation', and 'Optimize codons'. Below the menus, a DNA sequence is shown with a 'lacZα' feature highlighted in red. The sequence is displayed in two segments: one from position 80 to 230 and another from 240 to 310. Restriction enzyme sites for PluTI, SfoI, NarI, and KasI are indicated above the sequence. At the bottom, a summary table provides sequence statistics:

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

The screenshot shows the sequence editor interface. A DNA sequence is displayed with a 'lacZα' feature highlighted in red. A right-click context menu is open over the sequence, showing various editing options. The menu items include: 'Edit annotation', 'Delete annotation', 'Add to Feature Library', 'Copy', 'Copy special...', 'Change case...', 'Delete bases', 'Create new part', 'Create primer...', 'Create DNA sequence', 'Create RNA sequence', 'Create translation...', 'Create AA sequence...', 'Run Benchling BLAST', 'Submit to NCBI BLAST', and 'Analyze as translation'. The sequence is shown in two segments: one from position 160 to 230 and another from 240 to 310. Restriction enzyme sites for BstAPI, NdeI, PluTI, SfoI, NarI, and KasI are indicated above the sequence. At the bottom, a summary table provides sequence statistics:

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

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

i 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

2 Create an annotation

1 Select a sequence fragment

SEQUENCE MAP

CGAAAGGGGATGTGCTGCAAGGCGAT
GCTTTCCCTACACGACGTTCCGCTA

320 330 340

M13 fwd

370 380 390

BspMI BfuAI SbfI SmaI EcoRI ApoI BanII Eco53kI

HindIII SphI PstI XbaI BamHI KpnI SacI

CCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGTACCGAGCTCGAATTCGTAATCATGGTCA
GGTCAACGGTTCGAACGTACGGAGCTCCAGCTGAGATCTCCTAGGGGCCATGGCTCGAGCTTAAGCATTAGTACCACT

MCS lacZα

400 410 420 430 440 450 460

TAGCTGTTTCCTGTGTAATTTGTTATCCGCTCACAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC
ATCGACAAAGGACACACTTAAACAATAGGGCAGGTGTTAAGGTGTGTTGATGCTCGGCCTTCGTATTCACATTTCCG

M13 rev lac operator lac promoter

470 480 490 500 510 520 530 540

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

3 Add the specifications

PLASMID LINEAR MAP RESULT

AmpR_promoter AmpR 2500

2250

2000

1750

ANNOTATIONS TRANSLATIONS

Visibility filter Create new

12 total 0 hidden

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

New annotation

Name

Position -

Annotation type

Color

Strand

Notes

ASSEMBLY SPLIT WORKSPACE

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

View, annotate and edit your sequences

Sequence annotations

The screenshot displays the BRiGHT interface for the pUC18 plasmid. It features a 'SEQUENCE MAP' on the left and a 'PLASMID' view on the right. The 'ANNOTATIONS' panel on the right lists the following features:

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

Buttons for 'Auto-annotate' and 'Edit feature libraries' are highlighted in the interface.

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

4 Name your new translation

SEQUENCE MAP

Y11520_PBD

Annotations: ORF2 CDS (er), ORF2 gene, ORF2 CDS (enoyl-CoA hydratase), source, repeat_region, vdh gene

Linear Map: AvrII, DrdI, PspXI, EcoNI, Bsu36I, HindIII, BamHI, KflI, PpuMI, HpaI, ApaI, AleI, BsaI, PfoI, BsrGI, FspI, SexAI, XbaI, AjuI

TRANSLATIONS

Create translation

Name: vdh translation

Position: 1786 - 3242

of AA's: 485

Genetic code: Standard

Color: [Blue]

Strand: Forward

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

✓ 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 mRNA Uridine Depletion

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

ASSEMBLY WIZARD SPLIT WORKSPACE

- ✓ 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

The screenshot displays the BRiGHT software interface. On the left, a 'SEQUENCE MAP' shows a DNA sequence with various annotations including restriction enzyme sites (XmnI, ApaI, PspOMI, BsaAI, PmlI), an ORF2 gene, an ORF2 CDS (enoyl-CoA hydratase), a repeat region, and a source. The sequence is shown in both raw DNA and translated amino acid format (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). On the right, the 'CODON OPTIMIZATION' tab is active, showing an 'Optimization preview' table. This table compares metrics 'Before' and 'After' optimization. Below this is a detailed table of location-specific changes, such as CTT → L (0.12) to TTA → L (0.15) at position 1815. At the bottom right, there are buttons for 'Save' and 'Save as new sequence'.

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)

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

4. Benchmarking access and folder setup

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

Access Benchling:

bright.benchling.com

(login with DTU credentials)



Create a training folder to work in

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*

Location*

Description

Create

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

Copy the *Training Files* folder into your own

Projects / Biosustain Training /
Molecular Biology Training  Saved Searches 

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

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

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

Copy To... 

Item is currently in:  Molecular Biology Training

Projects

Filter...

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

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



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 

  Type  Filters 

< > 1-4 of 4 items 

    More 

<input type="checkbox"/>	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.

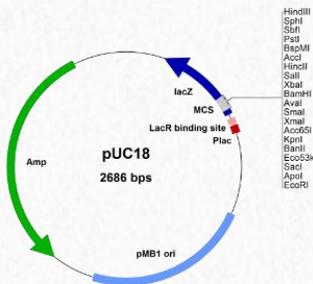
1



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 + Sall digestions for the *alsSD* PCR product and pUC18
- pUC18-*alsSD* plasmid

You will need the files in the **Basic construct assembly** subfolder.

... / Your Name / Training Files /

1. Basic construct assembly

Search

Type Filters

- alsSD source (Last modified 9 minutes ago)
- pUC18 (Last modified 9 minutes ago)

alsSD source (3326 bp)

SEQUENCE MAP DESCRIPTION

— + Create Analyze Copy

— + Create PDF

tagtgaaacttatcacaagatatTTTAAATTTTACGTTTAAATGCG
 atcactttgaatagtgTTCATAAATTTTAAATGCAAATTTACG

95 100 105 110 115 120 125 130 135

ataataaggagtgagggtgatgacaaaagcaacaaaagaacaaaa
 tattattcctcactccactactgTTTTGTTGTTTTCTGTTTT

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

tcccttgTgaaaaacagaggggCGGAGCTTGTGTTGATTGCTTAG
 agggaacactTTTTGTCTCCCCGCTCGAACAACAACCTAACGAATC

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

alsSD source (3326 bp)

METADATA DNA FRAGMENT BATCH LINEAR MAP RESULTS ...

— + Create PDF

Tth111I PflFI StuI MlyI PleI EagI PciI XbaI KpnI Acc65I AhdI BsrDI StyI NmeAIII HpaI BsrGI TatI FspI BspHI EcoRI XmnI BsmFI BaeI BspI Bsp1286I BsiHKAI BspI SphI AgeI BsaAI BtsI BssSI BsrBI BssSI 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*

The screenshot shows the BRiGHT software interface for manual primer creation. On the left, the 'alsSD source' window displays the DNA sequence with a 22-base primer highlighted in yellow and red. The primer sequence is M T K A T K E Q K. Below the sequence, the 'alsS CDS' and 'alsS gene' are shown. On the right, the 'PRIMERS' tool is open, showing a list of restriction enzymes (BtgI, Bsd1286I, BssSI, BsrBI) and a 'Manual' option selected. The 'Create Primers' button is highlighted. The bottom status bar shows the primer details: BASES 3326, START 158, END 179, LENGTH 22, GC 31.82%, MELTING TEMP 50.8 °C.

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

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

3 Select primer pair creation

alsSD source

SEQUENCE MAP DESCRIPTION

— ○ — + Create Analyze Copy ⋮ ⚙ 🔍

tagtgaaacttatcacaagatatttaaattttacgtttaaaatgcatcactttgaatagtggtctataaaattttaaaatgcaaattttacg

95 100 105 110 115 120 125 130 135

ataataaggagtgagggtgatgacaaaagcaacaaaagaacaaaaatattattcctcactcccactactgttttcggtgttttctgttttt

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

tccttgtgaaaaacagaggggaggagcttgttgttgattgcttagagggaacacttttgtctccccgctcgaacaacaactaacgaatc

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 × Share

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 AaI Use the dropdown above to look up restriction sites.

Verify Check Secondary Structure at 50 °C

T_m -- --

GC Content -- --

ASSEMBLY SPLIT WORKSPACE

- 4 Set the 3' selected bases as forward (**start of alsS**)
- 5 Set the 5' selected bases as reverse (**end of alsD**)

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

Construct design

Manual primer creation

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

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

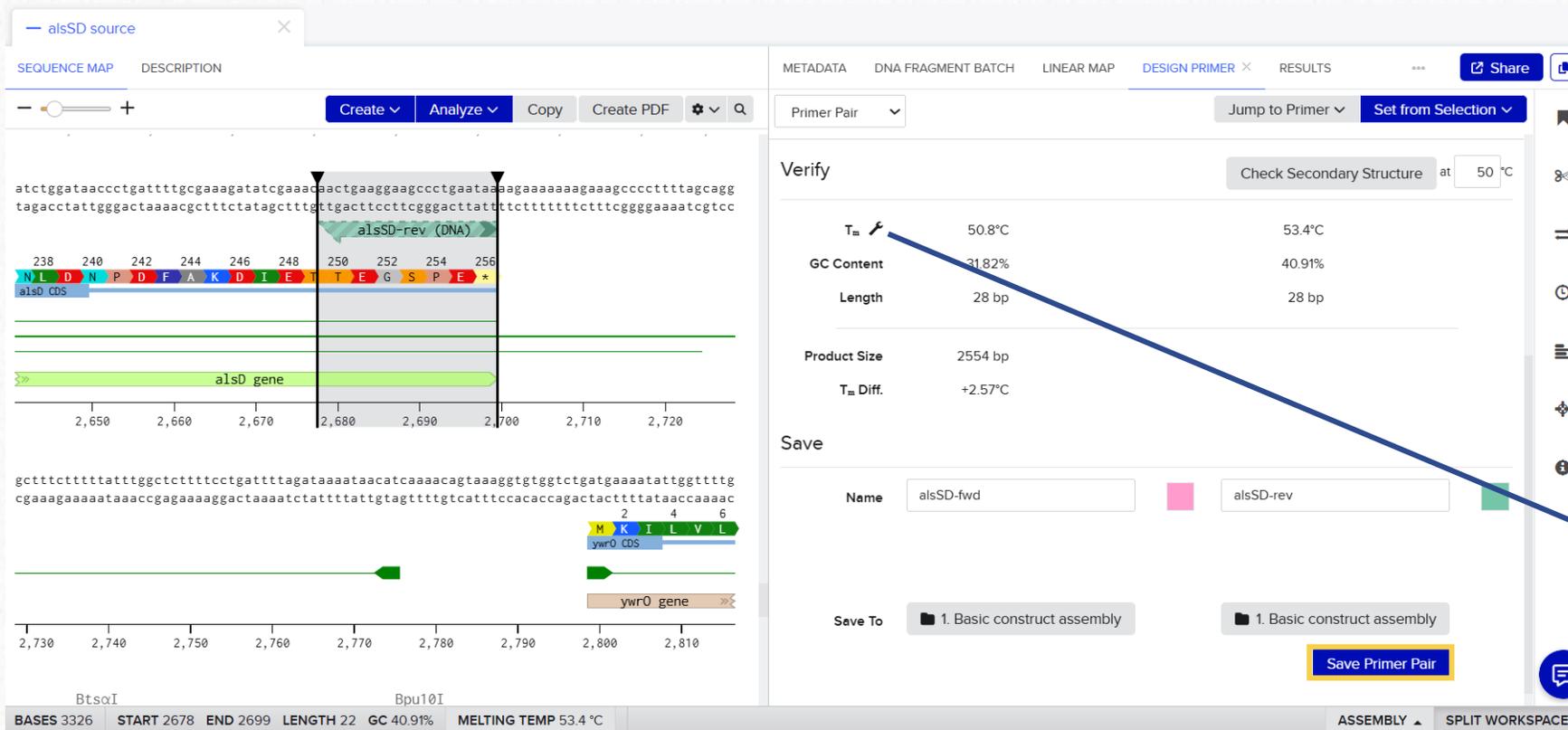
Use the dropdown above to look up restriction sites.

Strand	Forward	Reverse
Bases	5' GGATCCatgacaaaagca acaaaagaac 3'	5' GTCGACttattcagggct 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

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



The screenshot shows the BRiGHT software interface for manual primer creation. The main window displays the DNA sequence of the *alsD* gene and its coding sequence (CDS). The primer pair is defined by the following coordinates and sequence:

Primer	Coordinates	Sequence
alsD-fwd	2678-2699	atctggataaccctgattttgcgaaagatatcgaacaaactgaaggagccctgaaataaagaaaaaaaaagaaagcccccttttagcagg
alsD-rev	2699-2720	tagacctattgggactaaaaacgctttctatagctttgtgactctctcgggacttatctctttttctcttcggggaaaaatcgctcc

The design parameters for the primer pair are:

Parameter	Value
T _m	53.4°C
GC Content	40.91%
Length	28 bp
Product Size	2554 bp
T _m Diff.	+2.57°C

The primer names are `alsD-fwd` and `alsD-rev`. The save location is set to `1. Basic construct assembly`. The `Save Primer Pair` button is highlighted in yellow.

✓ 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

Manual primer creation

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

The screenshot shows the Benchling 'DESIGN PRIMER' interface. The 'ALL STRUCTURES' tab is selected. Under 'Verify', the 'Check Secondary Structure' button is highlighted with a yellow box. Below this, a table displays thermodynamic data for the primer pair.

Property	Value	Value
T_m	56.1°C	69.8°C
GC Content	38.46%	73.91%
Length	26 bp	23 bp
Min ΔG Homodimer	-3.3 kcal All Structures	-13.8 kcal All Structures
Min ΔG Monomer	-0.1 kcal All Structures	-2.5 kcal All Structures
Product Size	1495 bp	
T_m Diff.	+13.77°C	
Min ΔG Heterodimer	-6.3 kcal All Structures	

At the bottom, the primer names 'fwd_vdh' and 'rev_vdh' are visible in input fields.

This screenshot shows the 'ALL STRUCTURES' view in Benchling. It displays a table of secondary structures for the primer pair 'ataatgacaataatgaggagtgccca' and 'gcccgcggcgcccgaagatcgat' at 37°C. A yellow box highlights the table content. A yellow arrow points from the 'Check Secondary Structure' button in the previous screenshot to this table.

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

Each structure is a 3D ball-and-stick model of a DNA hairpin. A tooltip labeled 'Patricia' is visible over the -5.4 kcal structure.

5. Basic construct assembly

5.1 Primer design

5.1.2 Primer wizard

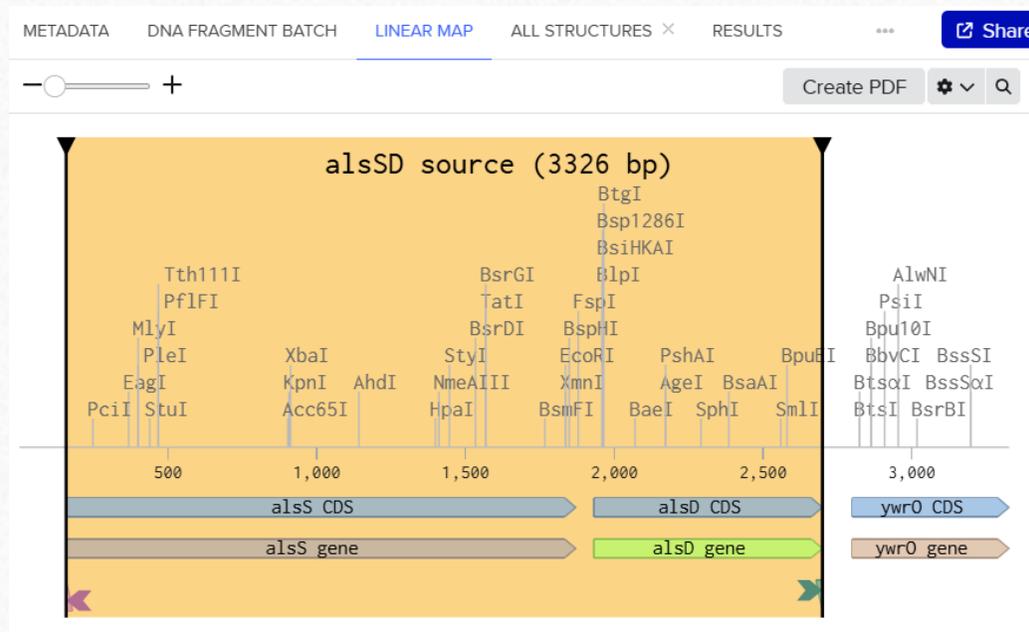


Construct design

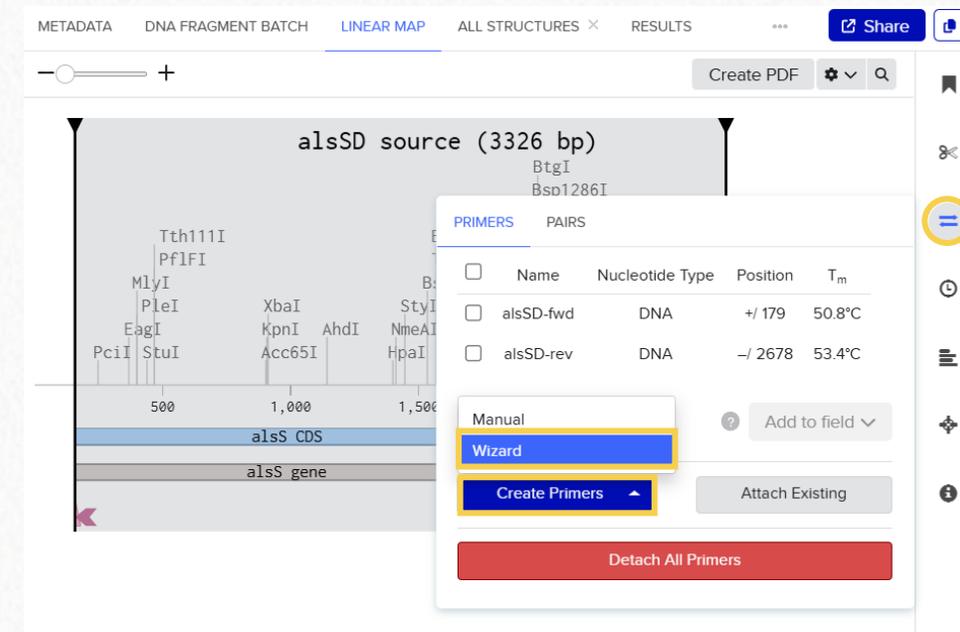
Automatic primer creation – Primer Wizard

✓ 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

Automatic primer creation – Primer Wizard

3 Select PCR as sequencing task

Task: PCR

Region: Target 158 2699 **Use selection**

	Min	Opt	Max
GC%	30	50	65
T _m	45	62	65
Size	15	22	31
3' GC clamp	0		

Primer Wizard Summary: BASES 3326 | START 158 | END 2699 | LENGTH 2542 | GC 44.89% | MELTING TEMP 78.1 °C

4 Use your selected sequence

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

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

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 'PRIMER3 RESULTS' tab in the BRiGHT software. On the left, a sequence map shows the 'alsS' gene with a PciI restriction site. The main panel shows a list of primer pairs with their respective parameters. The 'Penalty' column is highlighted, indicating that primers are sorted by their penalty score.

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

✓ 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 **SaII** restriction sites.

alsSD source (3326 bp)

PRIMERS PAIRS

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

Primer Pair Information

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

SEQUENCE MAP

— +

Create ▾ Analyze ▾ Copy ⋮ ⚙ 🔍

BstYI
BamHI

alsSD-fwd (DNA)

GGATCCatgacaaaagcaacaaaagaacaaaatcccttgtgaaaaacagagggcgagcttgt
CCTAGGtactgttttcgttgttttctgttttagggaacacttttctccccgctcgaaca

2 4 6 8 10 12 14 16 18 20

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

alsS CDS

alsS gene

10 20 30 40 50 60

PciI

tgttgattgcttagtgagcaaggtgtcacacatgtattggcattccaggtgcaaaaattgatg
acaactaacgaatcacctcgttccacagtgtgtacataaacgtaaggtccacgtttttaactac

22 24 26 28 30 32 34 36 38 40

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

alsS CDS

alsS gene

BASES 2554

LINEAR MAP DESCRIPTION METADATA RESULTS ... [Share](#)

— +

Create PDF ⚙ 🔍

alsSD source [158-2699] (2554 bp)

BtgI
Bsp1286I
BsiHKAI
BsrGI
TatI
FspI
BsrDI
StyI
BspHI
SmI
Tth111I
StuI
PvuI
NmeAIII
EcoRI
SalI
EagI
SspI
XbaI
HpaI
BsmFI
PshAI
BpuEI
PciI
PflFI
KonI
AhdI
FokI
NdeI
XmnI
BaeI
SphI
AccI
BstYI
MlyI
PleI
BseYI
Acc65I
BtsCI
PvuII
BlpI
AgeI
BsaAI

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

alsS CDS alsD CDS

alsS gene alsD gene

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

ASSEMBLY ▾ SPLIT WORKSPACE

5. Basic construct assembly

5.3 Virtual digestion

Construct design

Virtual digestion

We will run two virtual digestions to create the **compatible sticky ends** for RE-based cloning in our gene of interest and the backbone (pUC18)

Digestion of the backbone (open the pUC18 sequence)

The screenshot shows the pUC18 sequence in a bioinformatics tool. The sequence is displayed in a linear map view. The MCS (Multiple Cloning Site) region is highlighted in blue. The restriction enzyme sites are labeled with their names and recognition sequences. The 'NEW DIGEST' dialog box is open, showing a list of enzymes and their cut sites. The enzymes BamHI and SalI are selected. The 'Run digest' button is highlighted.

1 Find and select the REs

Name	Cuts	Selected	Color
SacII	0	<input type="checkbox"/>	
BamHI	1	<input checked="" type="checkbox"/>	Red
SalI	1	<input checked="" type="checkbox"/>	Green
SapI	1	<input type="checkbox"/>	
Sau3AI	15	<input type="checkbox"/>	
Sau96I	6	<input type="checkbox"/>	

2 Run the digestion

3 Run the digestion

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

Enzyme NcoI

CCATGG
GGTACC

NEB

Use HF ?

Link: [NEB](#)

Inactivation: 80°C

Incubation: 37°C

Activity:

1.1	2.1	3.1	4/CS
100	100	100	100+

Isos.: None

Jump to Cut Site:

2192

Enzyme lists Manage enzyme lists

Deduplicated commercial

Cut sites visible on maps

Single cutters

Find enzyme Clear selected

nc

Name	Cuts	Selected	Color ?
HincII	4	NcoI	
NciI	10		
NcoI	1		

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



Construct design

Virtual digestion

Digestion of the backbone

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

Digest 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

2

3 Find and select the REs

4

The digest tab will open

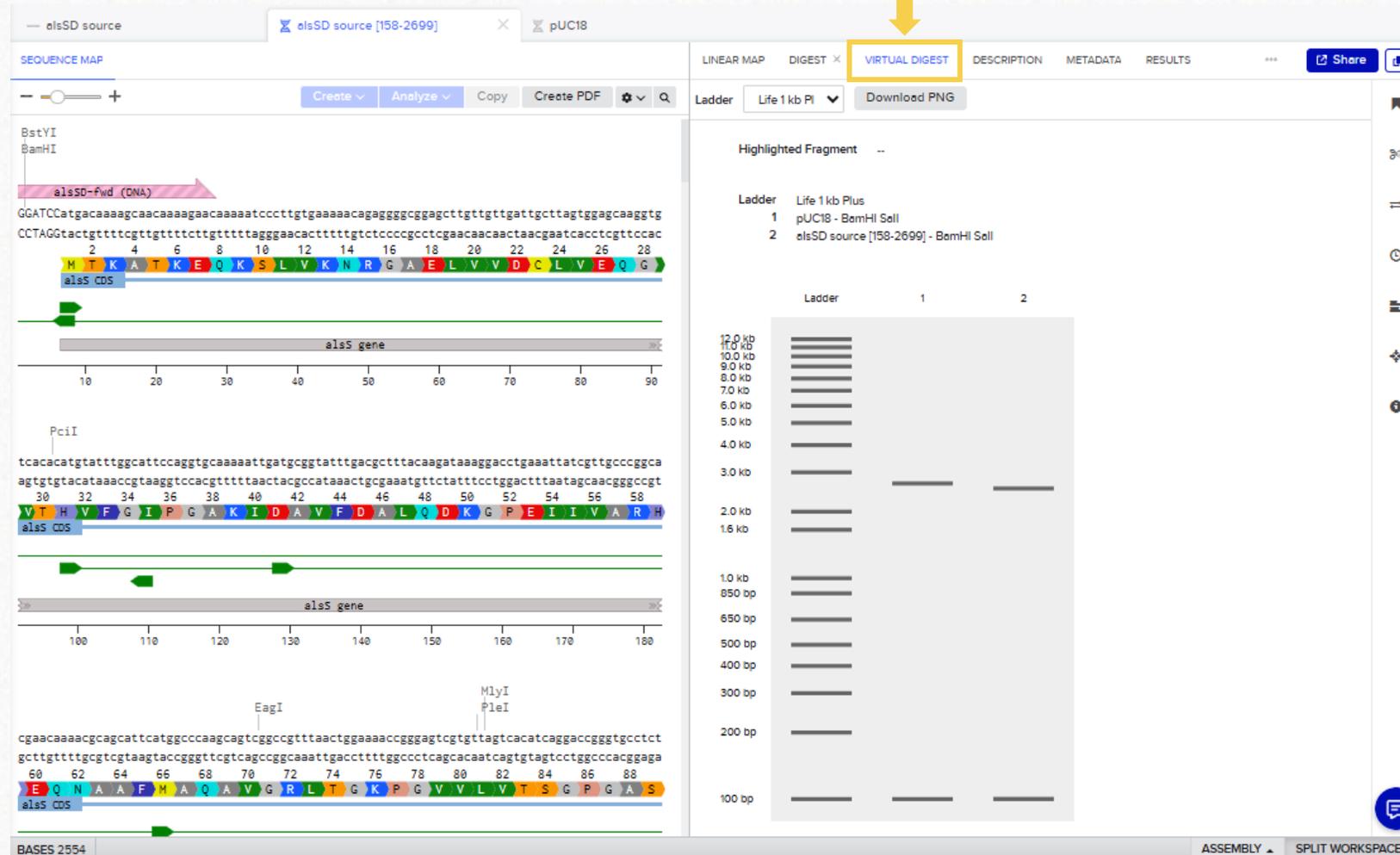
4 Save the digestion Use HF ?

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

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

Run the digestion

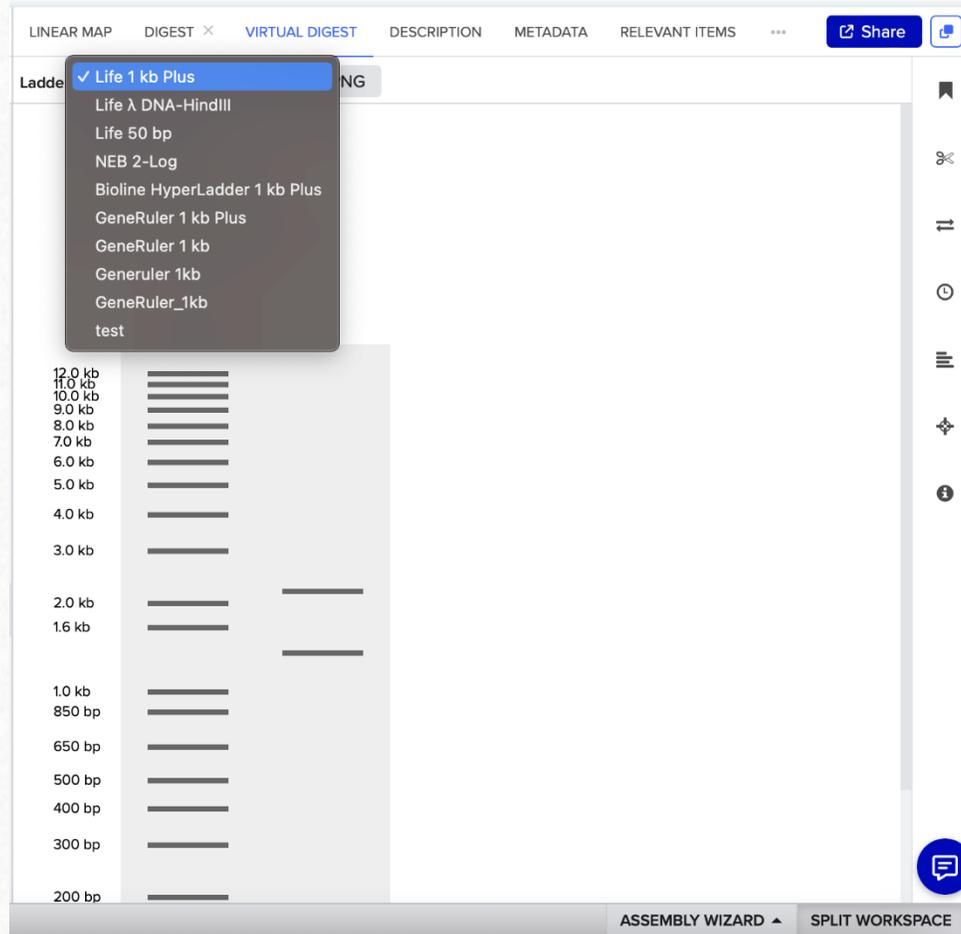
Gel visualization



The screenshot shows a web-based interface for virtual digestion. The top navigation bar includes tabs for 'LINEAR MAP', 'DIGEST', 'VIRTUAL DIGEST' (highlighted with a yellow arrow), 'DESCRIPTION', 'METADATA', and 'RESULTS'. Below the navigation, there are buttons for 'Create', 'Analyze', 'Copy', 'Create PDF', and 'Download PNG'. The main area is divided into two panels. The left panel displays a 'SEQUENCE MAP' for the 'alsSD source' and 'pUC18' constructs. It shows the DNA sequence with various restriction enzyme sites (BstVI, BamHI, PciI, EagI, MlyI, P1eI) and the resulting protein sequence (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 Q G). The right panel shows a 'Highlighted Fragment' and a simulated gel image. The gel has three lanes: 'Ladder', '1', and '2'. The 'Ladder' lane shows a range of DNA sizes from 12.0 kb down to 100 bp. Lane 1 is labeled 'pUC18 - BamHI Sell' and Lane 2 is labeled 'alsSD source [158-2699] - BamHI Sell'. Both lanes show a single prominent band at approximately 2.8 kb, corresponding to the 'alsSD' insert.

- ✓ 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



LINEAR MAP DIGEST × VIRTUAL DIGEST DESCRIPTION METADATA RELEVANT ITEMS ... Share

Ladder

- ✓ Life 1 kb Plus
- Life λ DNA-HindIII
- Life 50 bp
- NEB 2-Log
- Bioline HyperLadder 1 kb Plus
- GeneRuler 1 kb Plus
- GeneRuler 1 kb
- Generuler 1kb
- GeneRuler_1kb
- test

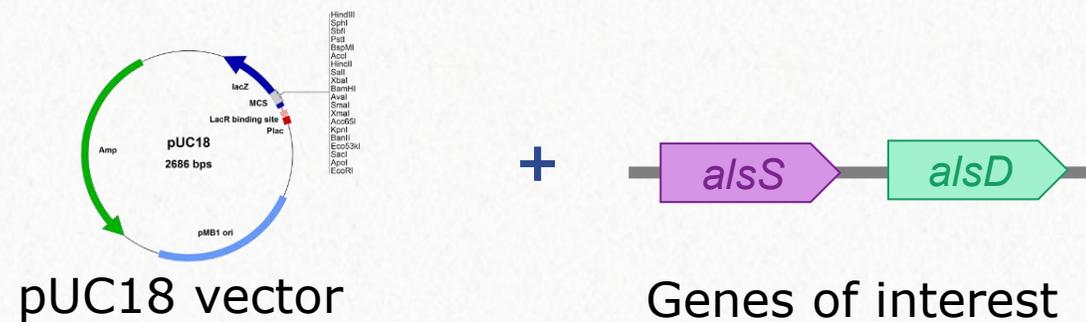
12.0 kb
11.0 kb
10.0 kb
9.0 kb
8.0 kb
7.0 kb
6.0 kb
5.0 kb
4.0 kb
3.0 kb
2.0 kb
1.6 kb
1.0 kb
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp

ASSEMBLY WIZARD SPLIT WORKSPACE

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

5. Basic construct assembly

5.4 Assembly Wizard



Assembly Wizard

The screenshot displays the Assembly Wizard interface with three panels. The left panel shows a sequence map for the *alsS* gene. The middle panel shows a linear map for the *alsSD* source (158-2699 bp) with various restriction sites and CDS regions. The right panel shows a sequence map for the *vdh* gene. A dialog box titled "Pick Assembly Strategy" is overlaid on the right panel, with a yellow circle '2' highlighting the "Start" button. The dialog offers three strategies: "Digest and Ligate" (selected), "Gibson", and "Golden Gate". A yellow circle '1' highlights the "ASSEMBLY" menu item in the bottom navigation bar.

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

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'

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

SET FRAGMENT
Select an assembly fragment below.

OVERALL ASSEMBLY
☑ The backbone or an insert is unset.

Backbone > Insert

BASES 2686 INSERT 693

pUC18-alsSD

+ Hide Prev

ASSEMBLY ▾ SPLIT WORKSPACE

← Name your construct



Digest and Ligate: Add the backbone

The screenshot shows the BRiGHT software interface for plasmid assembly. On the left, a circular plasmid map for pUC18 (2686 bp) is displayed with various features labeled: Amp^r promoter, Amp^r, M13 fwd, MCS, +3 lac promoter, M13 rev, ori, and Amp^r. On the right, a 'DIGEST' table shows the results of a virtual digest using BamHI and Sall enzymes. Below the table, a 'PREVIEW' section shows the sequence GATCCCC AGG GGG TCCAGCT and a message '0 ERRORS AND 0 WARNINGS'. At the bottom, a 'Backbone' selection step is highlighted with a yellow box and a '1' in a circle. A yellow arrow points from the 'Backbone' box to the 'Set from Selection' button in the 'PREVIEW' section, which is also highlighted with a yellow box and a '3' in a circle. Another yellow arrow points from the 'Set from Selection' button to a dropdown menu in the 'PREVIEW' section, which is highlighted with a yellow box and a '4' in a circle.

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'

1 Backbone

3 Set from Selection

4 [Dropdown]

PREVIEW

GATCCCC AGG
GGG TCCAGCT

0 ERRORS AND 0 WARNINGS
✓ Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

Backbone Insert

pUC18
2.7 kb · BamHI, Sall

ASSEMBLY SPLIT WORKSPACE

✓ The Assembly Wizard shows the digested ends of the backbone

Digest and Ligate: Add the insert

SEQUENCE MAP
LINEAR MAP

alsSD source [158-2699] (2554 bp)
Create PDF

DIGEST
VIRTUAL DIGEST
DESCRIPTION
METADATA
RESULTS
Share

Digest Save NEB Use HF

Enzymes	Cuts	Temp.	11	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.

pUC18
2.7 kb · BamHI, Sall
Insert

+ Hide Pre

Digest and Ligase: 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

AGGGATCCat aaGGATCCC
TCCAGCTGta ttCAGCTGC

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

AGGTCGACTt atGGATCCC
TCCAGCTGaa 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 Ligate: Assemble

SET FRAGMENT
Select an assembly fragment below.

OVERALL ASSEMBLY
✓ Looks like everything checks out

pUC18-alsSD **1**

Assemble

Hide Preview

pUC18
2.7 kb · BamHI, Sall

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

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

SEQUENCE MAP

LINEAR MAP PLASMID DESCRIPTION METADATA RELEVANT ITEMS

Create Analyze Copy Create PDF

320 330 340 350 360 370 380 390

CCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTtattcagggttccttcagttggttcgatattctttcgaataatca
GGTCACGGTTCGAACGTACGGACGTCAGCTGaatagctcccgaaggagtcacaagaactatagaagcgttttagt

AccI
Sall
SbfI

MCS
lacZα
alsD gene

400 410 420 430 440 450 460

gggttatccagattcgcatataaagaaatccgctgtgttcggaagctcgatcattcttttgagaatcgta
cccaataggtctaagcgttaattcttttagcgcacacaagccttcagactctaagtaaaaaactcttttagcat

alsD gene

470 480 490 500 510 520 530 540

accgtgcaatcctcaagcacatagtcaaaaacgtgctccgctgaattcgctccttcgtaagtcaggtgatag
tggcacgttagagttcgttatcagttttgacacagcggactaacgcaggaagcagttacttcacgtccactatc

BASES 5222 INSERT 710

ASSEMBLY SPLIT WORKSPACE

pUC18-alsSD
5222 bp

AmpR, AmpR_promoter
lacZα
M13 fwd
M13 rev
lac_promoter
lac_promoter
lac_promoter
MCS, lacZα, +1

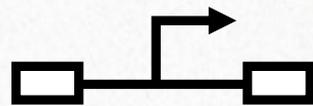
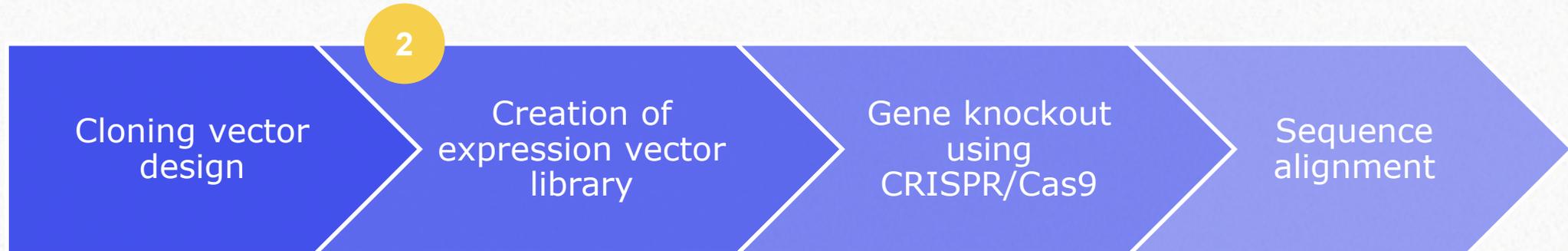
5000
4500
4000
3500
3000
2500
2000
1500
1000
500

✓ The assembly is now done!

6. Combinatorial cloning: Golden Gate



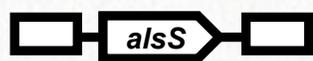
This is the second part of the *hands-on* example.



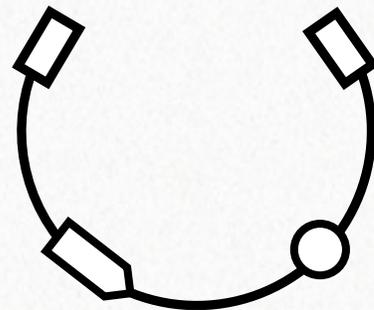
Promoters



RBSs



Terminators



Destination vector

✓ 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.

The screenshot displays a bioinformatics software interface with the following components:

- Left Panel:** A file browser showing a folder named "2. Combinatorial cloning" containing several files:
 - 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-rrmBT1 (Last modified 3 hours ago)
 - terminator-02-T0 (Last modified 3 hours ago)
- Main Panel:** A "SEQUENCE MAP" for the "alsS" gene (1713 bp).
 - Top Section:** Shows the first 26 amino acids of the protein: 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. Restriction sites BssS α I, BssSI, MboII, SacII, and EciI are indicated above the sequence.
 - Gene Model:** A green arrow indicates the "alsS CDS" (Coding Sequence) starting at position 1. A grey bar below represents the "alsS gene".
 - Bottom Section:** Shows the next 24 amino acids: 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. Restriction sites NspI and PstI are indicated above the sequence.
 - Gene Model:** A green arrow indicates the "alsS CDS" starting at position 28. A grey bar below represents the "alsS gene".
- Right Panel:** A "LINEAR MAP" for the "alsS (1713 bp)" gene.
 - Restriction Sites:** A vertical bar chart shows the positions of numerous restriction enzymes, including MmeI, MscI, SmaI, TspMI, XmaI, PstI, NspI, BsaHI, EciI, MboII, SacII, BssS α I, BssSI, BstNI, PspGI, AlwNI, BsmFI, BsrDI, BarI, BfaI, AhdI, BstXI, DdeI, PvuII, ScaI, KpnI, Acc65I, BsgI, FseI, NaeI, SfiI, NgoMIV, RsrII, PaqCI, BspMI, FokI, BtsCI, BspCNI, BspHI, XmnI, AgeI, AfeI, StyI, PpuMI, BsrGI, BtgZI, EcoRI, and BspHI.
 - Gene Model:** A blue arrow indicates the "alsS CDS" and a grey bar indicates the "alsS gene".
- Bottom Bar:** Shows "BASES 1713", "ASSEMBLY", and "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**

The screenshot displays the 'Golden Gate assembly' interface. It features a 'Bins & Spacers' section with three bins: BIN 1 (Backbone, 1 fragment), BIN 2 (Promoter, 3 fragments), and BIN 3 (Gene, 8 fragments). Below this is a 'Fragments' table with 12 rows, each representing a fragment with its sequence, bin, start/end coordinates, length, orientation, type, IIS enzyme, and a 'Frag' status.

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

At the bottom, there is a 'Constructs' table with 4 rows, showing the assembly of backbone, overhangs, promoters, and genes into specific 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

The right side of the interface shows a grid of 9 circular construct diagrams, each labeled with a unique identifier like 'backbone-promoter001-gene001'. Each diagram shows the circular arrangement of the backbone, promoter, and gene fragments. A 'SPLIT WORKSPACE' button is visible in the bottom right corner.

Combinatorial Cloning Tool: How to access it

The screenshot displays the BRiGHT software interface for a project named 'alsS'. The left sidebar shows a list of files, with 'alsS' selected. The main workspace is divided into two panes. The left pane shows the 'SEQUENCE MAP' for the 'alsS' gene, with a search bar at the top. A yellow circle with the number '1' highlights the search bar. Below the search bar, a menu is open, and a yellow circle with the number '2' highlights the 'Assembly' option. A sub-menu is also open, and a yellow circle with the number '3' highlights the 'Assemble DNA sequences by cloning' option. The right pane shows the 'LINEAR MAP' for the 'alsS (1713 bp)' gene, with various restriction enzyme sites indicated by vertical lines and labels. The bottom status bar shows 'BASES 1713' and 'ASSEMBLY'.

Combinatorial Cloning Tool: Configuration

1 Name*
alsS expression vector library

2 Project folder*
1. Basic construct assembly

3 Number of fragment bins*
- 5 +

Topology of construct
Circular

4 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

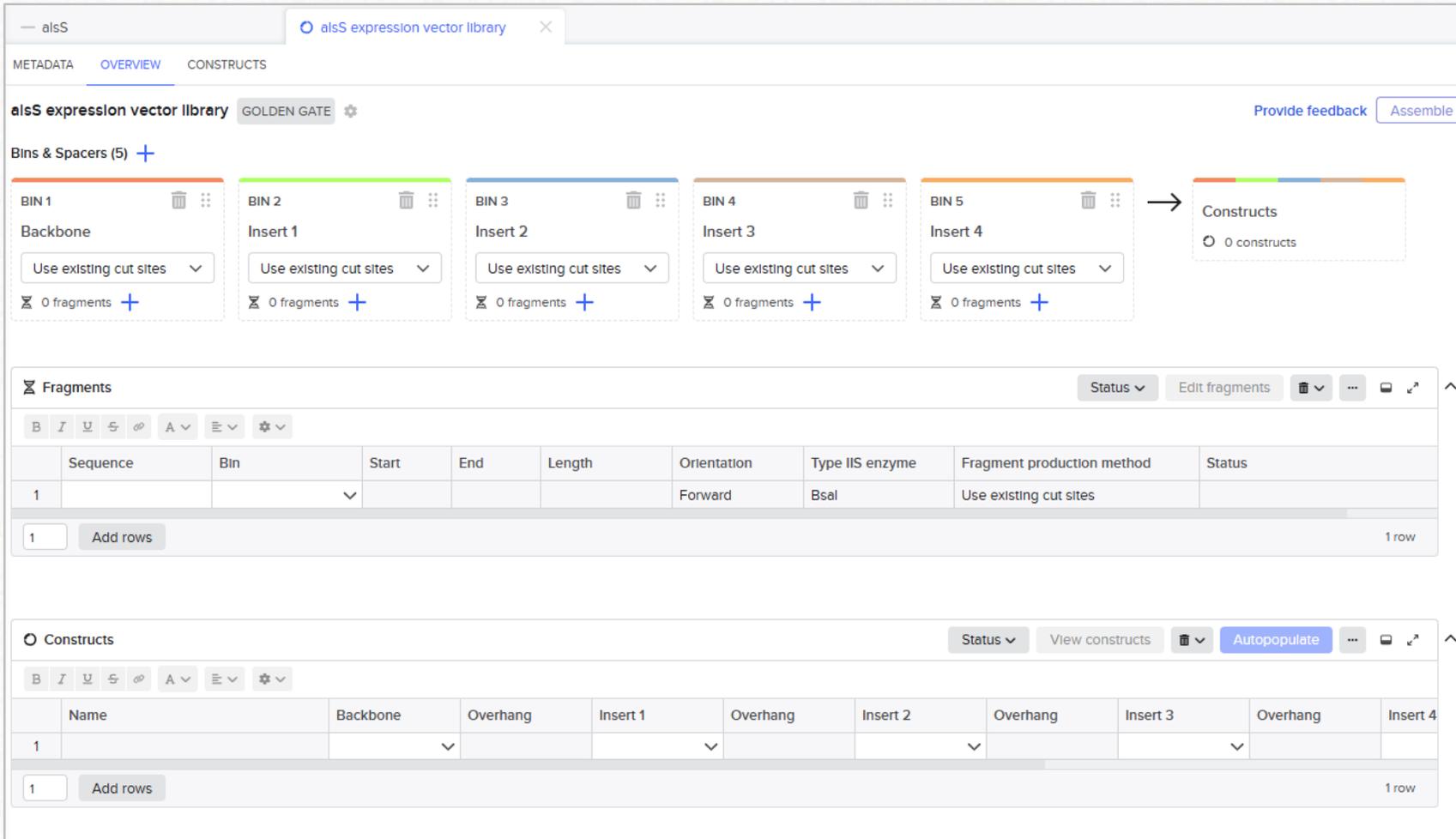
Fragment production method
Use existing cut sites
You can change this later.

5 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 expression vector library GOLDEN GATE Provide feedback Assemble

Bins & Spacers (5) +

BIN 1 Backbone 0 fragments +

BIN 2 Insert 1 0 fragments +

BIN 3 Insert 2 0 fragments +

BIN 4 Insert 3 0 fragments +

BIN 5 Insert 4 0 fragments +

Constructs 0 constructs

Fragments

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

1 row

Constructs

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

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.

Bins & Spacers (5) +

BIN 1 (Backbone) | Use existing cut sites | 0 fragments +

BIN 2 (Promoter) | Use existing cut sites | 0 fragments +

BIN 3 (RBS) | Use existing cut sites | 0 fragments +

BIN 4 (CDS) | Use existing cut sites | 0 fragments +

BIN 5 (Terminator) | Use existing cut sites | 0 fragments +

Constructs: 0 constructs

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

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

BIN 2

Promoter: Use a primer pair

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

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

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

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

Bins & Spacers (5) +

Add new bin

Add new spacer

BIN 4 (CDS) | Use a primer pair | 0 fragments +

SPACER 1 | Spacer: AATTCGAT

BIN 5 (Terminator) | Use existing cut sites | 0 fragments +

✓ Spacers will not be used in our example.

Construct Assembly

Golden Gate: Set fragments in corresponding bins

1. Backbone

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

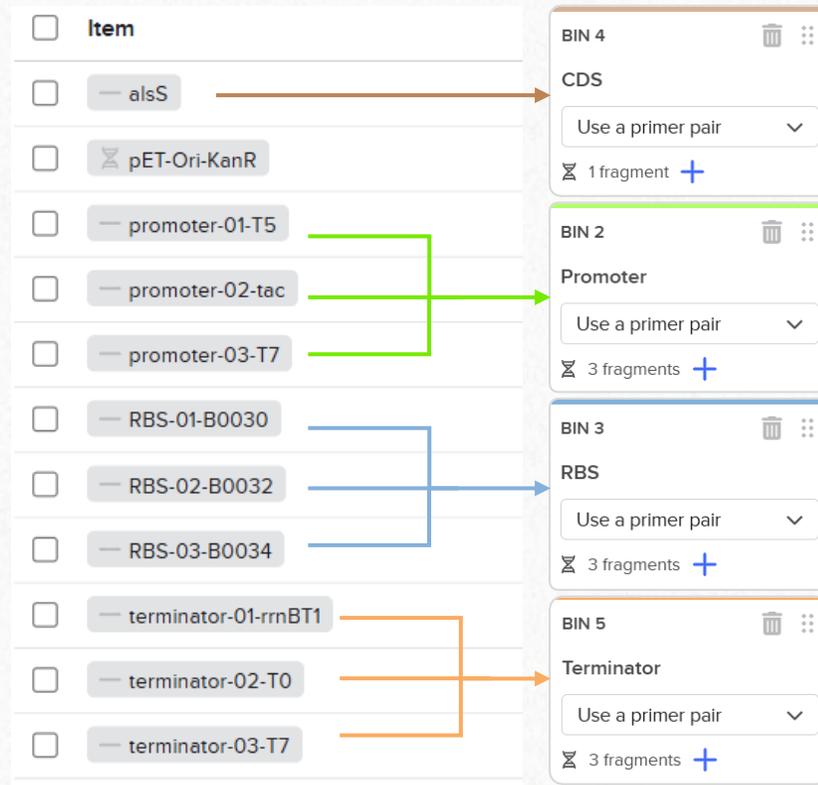
- 2 Verify the selection is correct and click "Add"

- ✓ 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.

Golden Gate: Set fragments in corresponding bins

2. Inserts

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



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

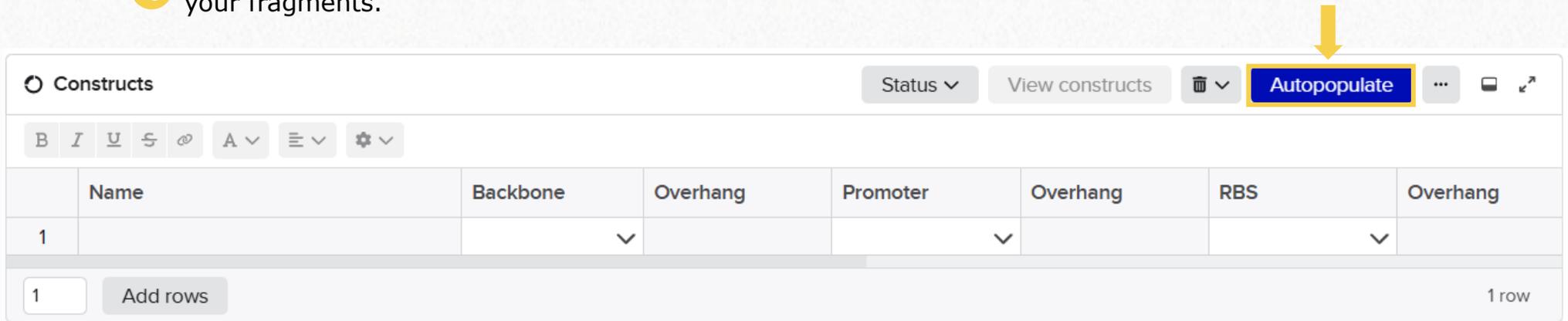
Golden Gate: Verify the fragments

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

Fragments											
Status Edit fragments 🗑️ ⋮ 📄 ✖ Close											
B I U S 🔗 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-rrnBT1	● Terminator	1	110	110	Forward	Bsal	Use a primer pair			✔ Looks good
10	 terminator-02-T0	● Terminator	1	126	126	Forward	Bsal	Use a primer pair			✔ Looks good
11	 terminator-03-T7	● Terminator	1	71	71	Forward	Bsal	Use a primer pair			✔ Looks good

Golden Gate: Populate the “constructs” table

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



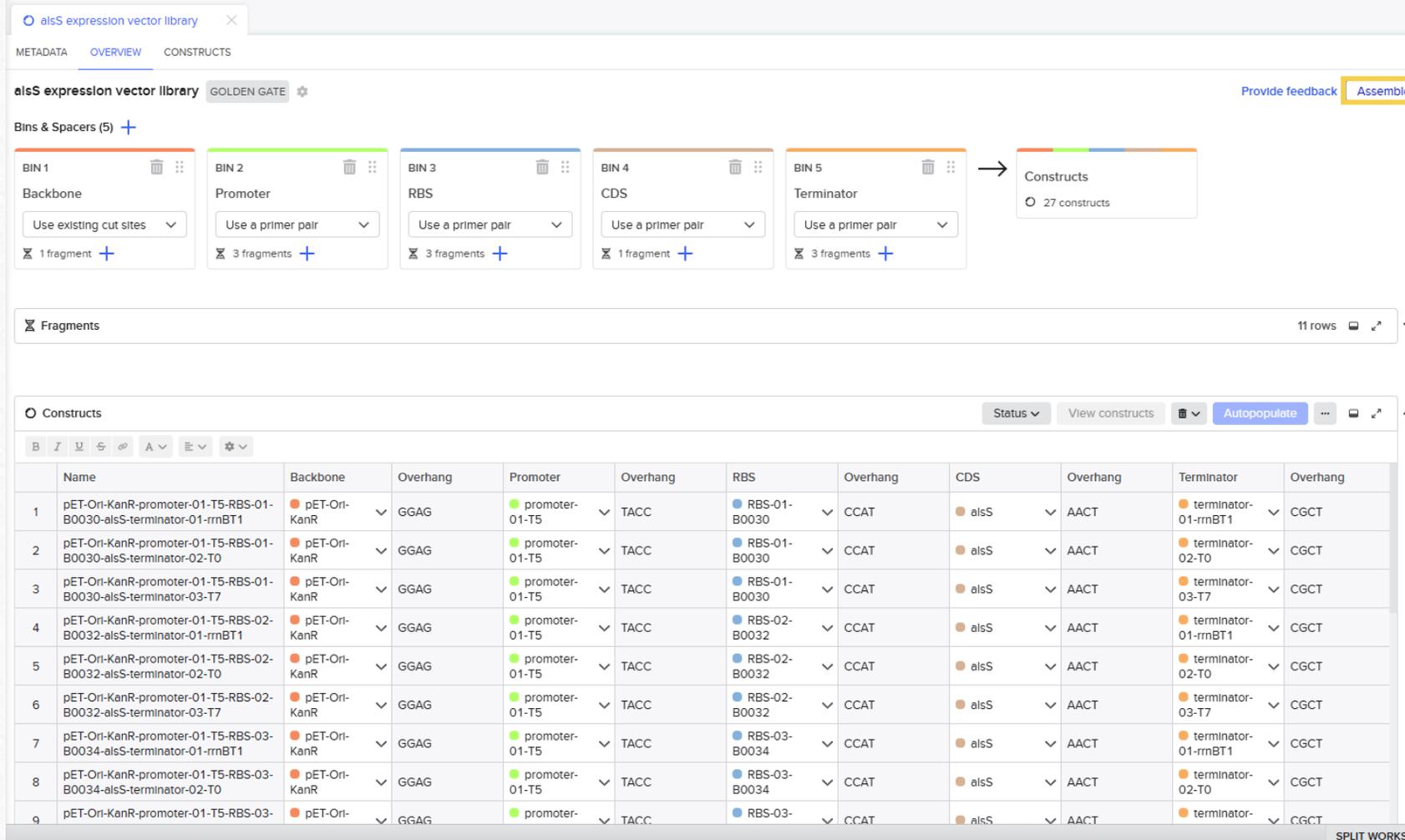
The screenshot shows the 'Constructs' table in the software interface. The table has columns for Name, Backbone, Overhang, Promoter, Overhang, RBS, and Overhang. The 'Autopopulate' button is highlighted with a yellow box and a yellow arrow pointing to it. The table currently contains one row with the number '1' in the first column. Below the table, there is a '1' in a box, an 'Add rows' button, and '1 row' on the right.

	Name	Backbone	Overhang	Promoter	Overhang	RBS	Overhang
1		▼		▼		▼	

- ✓ 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.



The screenshot shows the 'alsS expression vector library' interface. At the top, there are tabs for 'METADATA', 'OVERVIEW', and 'CONSTRUCTS'. Below this, the 'alsS expression vector library' is shown with a 'GOLDEN GATE' toggle. A yellow arrow points to the 'Assemble' button in the top right corner. The interface displays five bins (BIN 1 to BIN 5) with their respective components and options for using existing cut sites or primer pairs. Below the bins, there is a 'Fragments' section with 11 rows. At the bottom, a table titled 'Constructs' lists 9 constructs with columns for Name, Backbone, Overhang, Promoter, Overhang, RBS, Overhang, CDS, Overhang, Terminator, and Overhang.

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

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

Golden Gate: Save the constructs and related files

The image shows three sequential screenshots of the 'Assemble DNA' dialog box, illustrating the workflow for saving constructs and related files.

Step 1: Save constructs
Progress: 1 Save constructs — 2 Save fragments — 3 Save primers
Add constructs to a folder and optionally set a schema
Set location*: Mia
Set schema: Plasmid
 Add constructs to a worklist
Buttons: Cancel, Next

Step 2: Save fragments
Progress: 1 Save constructs — 2 Save fragments — 3 Save primers
Saving fragments is optional.
 Create DNA Sequences to represent amplified fragments
Buttons: Back, Next

Step 3: Save primers
Progress: 1 Save constructs — 2 Save fragments — 3 Save primers
Saving primers is optional.
 Create DNA Oligos to represent newly designed primers
Buttons: Back, Assemble

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

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

Golden Gate: Results

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

1

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

2

8 associated primers View

Primer view

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

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

Sequence view

View: Plasmid map

5224 bp

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

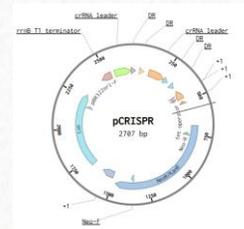
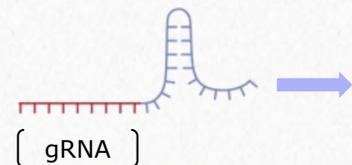
7. CRISPR tools

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

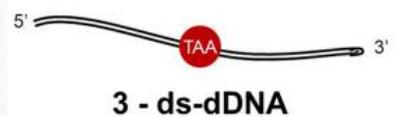


Target: *pta* in *E. coli* **gRNA** design + assembly into pCRISPR

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 *Bsa*I
- 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.

The screenshot displays the BRiGHT interface for a file named "pta source". The interface is divided into several sections:

- Left Panel:** Shows the file hierarchy: "3. CRISPR tools" with a search bar and filters. Below, it lists files: "pCRISPR" (last modified 6 days ago) and "pta source" (last modified 6 days ago).
- Top Panel:** Contains tabs for "SEQUENCE MAP" and "DESCRIPTION". It includes a zoom slider, "Create" and "Analyze" buttons, and a search icon.
- Sequence Map (Left):** Shows a DNA sequence with a green arrow indicating a CRISPR site. The sequence is:


```
tttcacaccgccagctcagctggcggtgctgttttgaaccgccaatcgggcggaagtggtggcggtcgagtcgaccgccacgacaaaacattggcggttagccgccc
```
- Sequence Map (Right):** Shows a zoomed-in view of the "pta source" (3929 bp) with a linear map. It highlights the "pta gene" (blue arrow) and "pta CDS (phosphatyltransferase)" (grey arrow). A PshAI restriction site is marked above the sequence:


```
agcgtcggtctgaccagcgtcagccttggcggtgatccgtgcaatggaacgcaaagtcgcagccagactggctgcagtcggaaccgcactaggcacgttacctgcggttc
```
- Bottom Panel:** Shows the total number of bases (3929) and options for "ASSEMBLY" and "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:

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

1

2

Tool overview

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



1 Open the **pta source** file.

2 Access the **gRNA design** menu.

The screenshot displays the pCRISPR web interface. The left panel shows the 'pta source' file with a sequence map and description. The right panel shows the 'gRNA design' menu, which is highlighted with a yellow arrow. The menu includes options for 'Design and analyze guides', 'Saved guide analyses', and 'Design HR template (ssODN)'. The interface also shows a 'CRISPR' section with a 'Design and analyze guides' button highlighted in blue.

Sequence Map (Left Panel):

```

tttcacaccgccagctcagctggcggctgttttgaaccgccaaatcggcgg
aaagtgtggcggtcgagtcgaccgccacgacaaaacattggcgggttagccgcc
2,301,93501,94001,94501,95001,95501,96001,96501,97001,975
taacgaaaggataaacctgtcccgtattattatgctgatccctaccggaacc
attgctttctctatttggcacagggcataataatcgcactagggatggccttgg
pta gene
pta CDS (phosph...yltransferase)
2,301,99001,99502,00002,00502,01002,01502,02002,02502,030
PshAI
agcgtcggcttgaccagcgtcagccttggcgtgatccgtgcaatggaacgcaaag
tcgcagccagactggcgcagtcggaaccgcactaggcacgttaccttgcgtttc
pta gene
pta CDS (phosphate acetyltransferase)

```

Linear Map (Right Panel):

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 Menu (Right Panel):

CRISPR

Design and analyze guides

Saved guide analyses
No guide analyses

Design HR template (ssODN)

Footer: BASES 3929 INSERT 2302001 ASSEMBLY SPLIT WORKSPACE

gRNA design

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

Design CRISPR guides: Guide parameters

Design type

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

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

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

Guide length

Genome

PAM

[Show advanced settings](#)

Save these as my default CRISPR settings

Finish

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

CRISPR tools

gRNA design

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

The screenshot displays the BRiGHT gRNA design tool interface. On the left, a 'SEQUENCE MAP' view shows genomic coordinates and annotations for the **pta gene** and its **CDS (phosphatyltransferase)**. A yellow arrow points to the CDS annotation. On the right, the 'DESIGN CRISPR' panel shows a text input field containing 'pta gRNA', a 'Save' button, and a 'Settings' button. Below this, a 'Target region' section shows two coordinate boxes: '2302000' and '2304144', with a blue '+' button to their right. A blue arrow points from the '+' button in the 'Target region' section to the '+' button in the 'DESIGN CRISPR' panel. A light blue instruction box above the target region reads: 'To get started, create a target region by selecting it on the sequence map and pressing +.'

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

CRISPR tools

gRNA design

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

SEQUENCE MAP DESCRIPTION

tttcacaccgagctcagctggcggtgctgttttgaaccgccaatcggcgg
aaagtgtggcgtcagctcagccacgacaaaacattggcggttagccgcc

2,301,93501,94001,94501,95001,95501,96001,96501,97001,975

taacgaaaggataaacctgtcccgtattattatgctgatccctaccggaacc
attgctttctctatttggcacagggcataataatcagactagggatggccttgg

57 49
56 50
35 49
56 50
51 49
38 49

pta gene
pta CDS (phosph...yltransferase)

2,301,99001,99502,00002,00502,01002,01502,02002,02502,030

PshAI

62 50
52 50
56 49
59 50

METADATA DNA FRAGMENT BATCH DESIGN CRISPR x ...

pta gRNA Save Settings

Target 2302000 2304144 +

region

<input checked="" type="checkbox"/>	Start	End	Annotations	Genome Region
<input checked="" type="checkbox"/>	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 v

2302000 | 2304144

Find genome matches ⚠

Set genome region Cancel

gRNA design

i Benchmarking 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 CRISPR design tool interface. On the left, a sequence map shows the DNA sequence with a highlighted region for gRNA design. The sequence is: `agactacgactatcgtgcgtgcgaactcttccaccacgacggccgctgaaccgctgatctgatgctgatagcagcagccttgagaaggtggtgctgccggcacttggcgact`. Below this, the amino acid sequence is shown: `Q T T T I V R A N S S T T T A A E P L`. The `pta` gene and `pta CDS (phosphate acetyltransferase)` are also indicated. On the right, the 'DESIGN CRISPR' tab shows a list of potential gRNAs. The table below is a representation of this list:

position	Strand	Guide sequence	PAM	On-Target score	Off-target score
<input type="checkbox"/> 2302910	-	gtagccgccagtcagcagca	ggg	76.3	99.2
<input type="checkbox"/> 2302761	-	agaaagtgcaggatttaacg	cgg	73.0	100.0
<input type="checkbox"/> 2303021	-	gaagctctgcaggctcagag	agg	72.0	100.0
<input type="checkbox"/> 2303477	+	cgaacagctggaagacaacg	tgg	71.8	99.4
<input type="checkbox"/> 2302391	+	aatcgctaaaacgctgaatg	cgg	71.6	100.0
<input checked="" type="checkbox"/> 2302190	+	tgccaactcttccaccacga	cgg	70.6	99.4
<input type="checkbox"/> 2302210	-	tcaacgtagctcattttcag	cgg	69.7	99.8
<input type="checkbox"/> 2303170	-	agctgataacggaacgcagg	cgg	69.0	100.0
<input type="checkbox"/> 2303277	+	gccgctatctgtgctgaacg	tgg	68.8	99.8
<input type="checkbox"/> 2302436	-	acgctcttcagctgttccg	ggg	68.5	99.6
<input type="checkbox"/> 2302508	-	cagtttgttaacgataacgc	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.

gRNA design

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

The screenshot displays the Benchling CRISPR tool interface. On the left, a sidebar shows a file list with 'pCRISPR' and 'pta source' files. A yellow arrow points from the 'pCRISPR' file to the 'Select Expression Vector' dialog box on the right. The dialog box has three radio button options under 'Vector Source': 'Select one of the bookmarked plasmids', 'Choose a plasmid from your Benchling folders' (which is selected and highlighted with a yellow box), and 'Upload a new plasmid'. Below these options is a dashed box containing the text 'Search for a sequence in the file browser and drag it here'. The background shows a sequence map for the 'pta' gene with a CRISPR array and a gRNA sequence being designed. The gRNA sequence is shown as 'Q T T T I V R A N S S T T T A A E P L' with a 'pta' label below it. The sequence map also shows the 'pta gene' and 'pta CDS (phosphate acetyltransferase)' with an 'EagI' restriction site indicated.

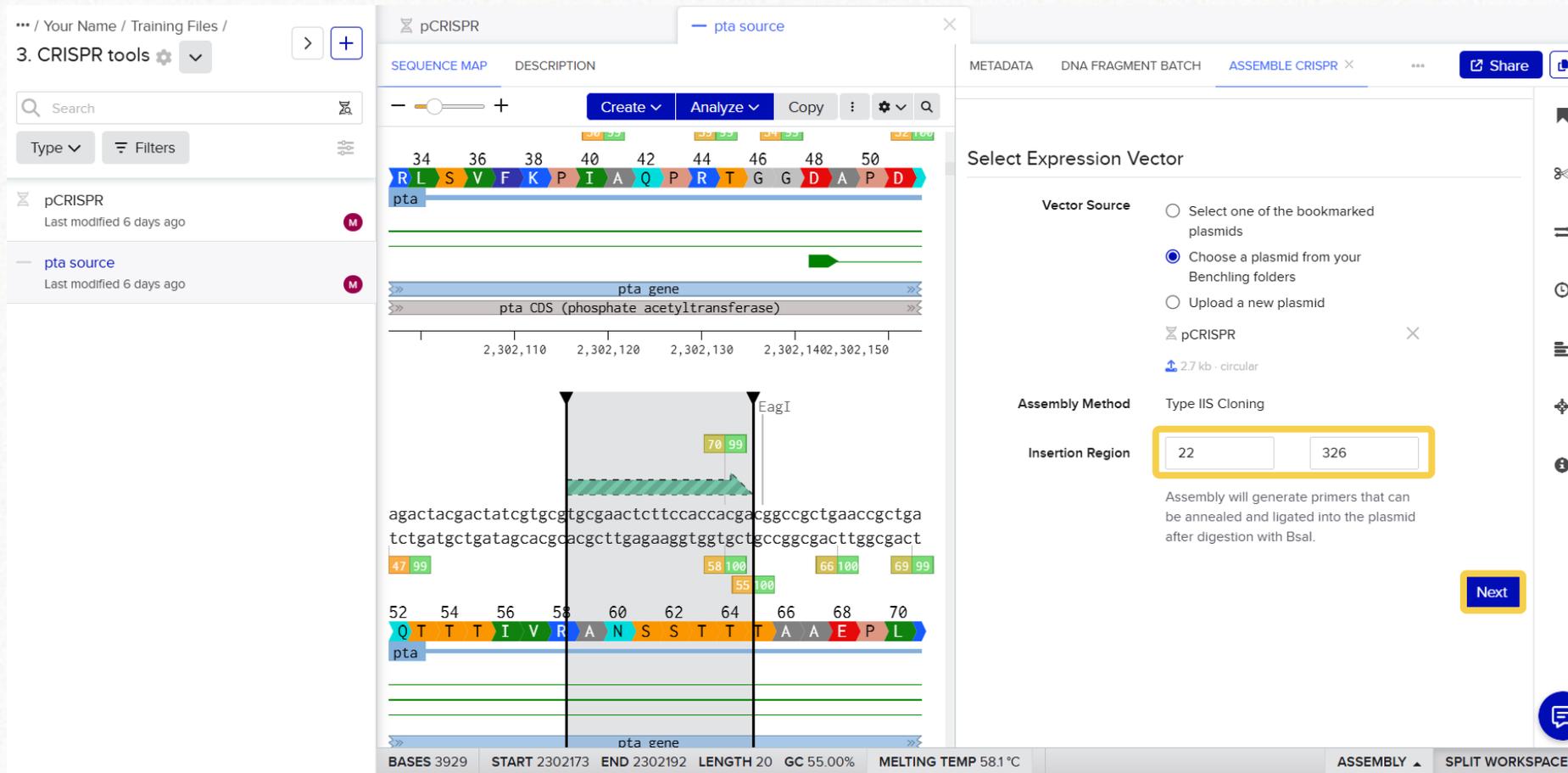
i The drag-and-drop option does not work in Safari.

CRISPR tools

gRNA design

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

14 Click **Next**.



The screenshot displays the CRISPR design tool interface. On the left, a sidebar shows the project structure with '3. CRISPR tools' selected. The main workspace is divided into two panes. The left pane, titled 'pCRISPR', shows a 'SEQUENCE MAP' for the 'pta source' gene. It displays a protein sequence from position 34 to 50 (RLS V F K P I A Q P R T G G D A P D) and a corresponding DNA sequence. A green arrow indicates the insertion site at position 70. The right pane, titled 'ASSEMBLE CRISPR', shows the 'Select Expression Vector' dialog. Under 'Vector Source', 'Choose a plasmid from your Benchling folders' is selected. Under 'Assembly Method', 'Type IIS Cloning' is chosen. The 'Insertion Region' is set to positions 22 to 326, highlighted with a yellow box. A 'Next' button is visible at the bottom right of the dialog. At the bottom of the interface, a status bar shows: BASES 3929, START 2302173, END 2302192, LENGTH 20, GC 55.00%, MELTING TEMP 58.1°C, ASSEMBLY, and SPLIT WORKSPACE.

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

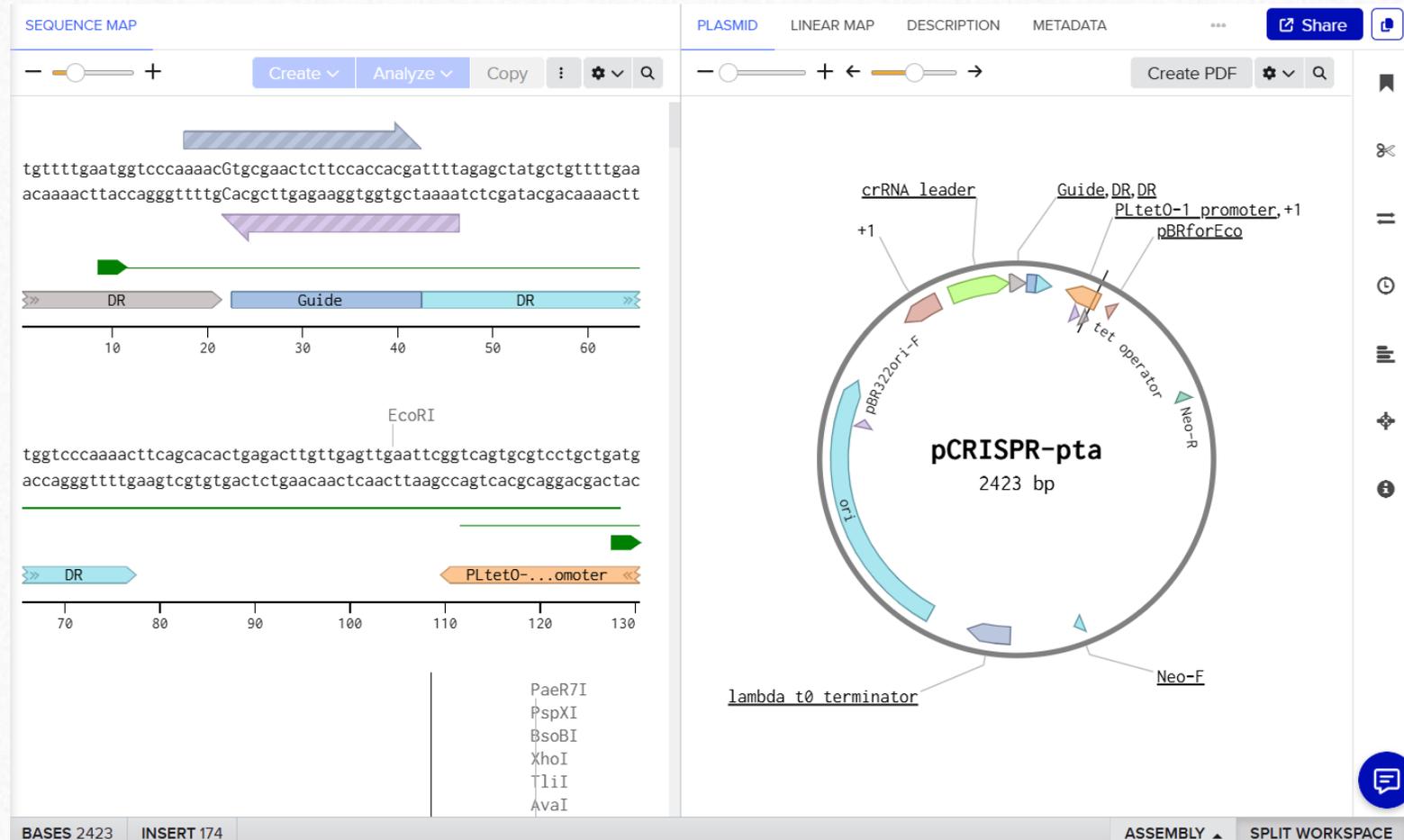
gRNA design

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

The screenshot displays the BRiGHT CRISPR tools interface. On the left, the 'SEQUENCE MAP' view shows a genomic region with a green arrow indicating an EagI restriction site. The DNA sequence is shown with a corresponding protein sequence below it, where the gRNA target sequence is highlighted in a multi-colored bar. The protein sequence is: Q T T T I V R A N S S T T T A A E P L. Below this, the 'pta' gene and its CDS (phosphate acetyltransferase) are shown. The right panel, 'Finalize Assemblies', shows the 'Assembly Name' field set to 'pCRISPR-pta' and the 'Guide Sequence' field set to 'tgcaactcttcaccacga'. The 'Folder' is set to '3. CRISPR tools'. The 'Assemble' button is highlighted in yellow.

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



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 'pta source' file is open, showing a sequence map with the following DNA sequence: `tttcacaccgccagctcagctggcggctgctgtttgtaacccgcaaatcggcggaaagtgtggcggctcagctcagccgccacgacaaaacattggcggcttagccgcc`. The map shows the 'pta gene' and 'pta CDS (phosph...yltransferase)' regions. On the right, the 'pta source (3929 bp)' is shown with a linear map highlighting the 'pta gene' and 'yfcC gene' regions. A 'CRISPR' panel is visible, containing a 'Design and analyze guides' button and a 'Design HR template (ssODN)' button, which is highlighted with a yellow arrow. The 'Design HR template' dialog box is open, showing the following options:

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

The dialog box also includes 'Cancel' and 'Create' buttons.

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

The 'Design HR template' dialog box is shown, with the following settings:

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

The dialog box includes 'Cancel' and 'Create' buttons.

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.

The screenshot shows the 'DESIGN HR TEMPLATE' interface in Benchling. The 'SEQUENCE MAP' on the left displays the 'pta gene' and 'pta CDS (phosphate acetyltransferase)'. A sequence map shows the DNA sequence with a grey box indicating a 30-base deletion at position 2302198. The sequence is: `accacgacgcccgtgaaccgctgaaatgagctacgttgaagctctgtttccagcaatcagaa`. A 'Press' dialog box is open, showing the instruction: 'ENTER to delete 30 bases at position 2302198.' and 'ESC to cancel.' The 'Knock-in edits' section is empty, and the 'Next' button is visible.

Step 1: Introduce desired edits

Select and type in the sequence map to introduce the changes (e.g. point mutations, insertions, deletions) you want to make to the genome.

Silent mutations to remove the target site will be added in a later step.

Knock-in edits

No edits introduced yet

Next

Press

ENTER to delete 30 bases at position 2302198.

ESC to cancel.

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.

The screenshot shows the 'DESIGN HR TEMPLATE' interface in Benchling. The 'SEQUENCE MAP' on the left displays the 'pta gene' and 'pta CDS (phosphate acetyltransferase)'. A sequence map shows the DNA sequence with a grey box indicating a 3-base insertion at position 2302198. The sequence is: `accacgacgcccgtctgctttccagcaatcagaaagatgctgctggaagagatcgtcgaaa`. A 'Press' dialog box is open, showing the instruction: 'ENTER to insert 3 bases at position 2302198.' and 'ESC to cancel.' The 'Knock-in edits' section shows the deletion: 'Deleted ctgaaccgctgaaatgagctacgttgaag at 2302199'. The 'Next' button is visible.

Step 1: Introduce desired edits

Select and type in the sequence map to introduce the changes (e.g. point mutations, insertions, deletions) you want to make to the genome.

Silent mutations to remove the target site will be added in a later step.

Knock-in edits

Deleted ctgaaccgctgaaatgagctacgttgaag at 2302199

Next

Press

ENTER to insert 3 bases at position 2302198.

ESC to cancel.

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 MAP DESCRIPTION

Editing disabled because... Copy Create PDF Settings

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

42 44 46 48 50 52 54 56 58 60 62

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

pta

pta gene

pta CDS (phosphate acetyltransferase)

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

accacgacggcctaaggctgctttccagcaatcagaaagatgtgctgatggaagatcgtcgc

METADATA DNA FRAGMENT BATCH DESIGN 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 - 2302297 [Reset to default](#)

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

Knock-in edits

Deleted gctgaaccgctgaaaatgagctactg at 2302198

Deleted g at 2302199

Back **Next**

7 Click **Next**.

HR template design

8 Paste the gRNA sequence: `tgcgactcttccaccacga`

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

Editing disabled because the sequenc...
Copy Create PDF
Settings

Sequence map showing restriction sites (HpaII, MspI, BsrFI, BsaWI, BslI, SfaNI, RsaI, CviQI, BstUI, Fnu4HI, BcgI, AcI, AgeI, HhaI, B1pI, TauI, BspCNI, HinP1I, BtgZI, Cac8I) and the pta gene structure. The pta gene is highlighted in blue, and the target sequence is highlighted in yellow.

Step 3: Remove target site

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

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

	R	A	N	S	S	T	T	T	A
Wildtype	cgt	gcg	aac	tct	tcc	acc	acg	acg	gcc
Mutations	AGA	GCA	AAC	AGC	AGC	ACA	ACA	ACA	GCA
	AGG	GCC	AAT	AGT	AGT	ACC	ACC	ACC	GCC
	CGA	GCG		TCA	TCA	ACG	ACG	ACG	GCG
	CGC	GCT		TCC	TCC	ACT	ACT	ACT	GCT
	CGG			TCG	TCG				
	CGT			TCT	TCT				
Final	cgt	gcg	aac	tct	tcc	acc	acg	ACC	gcc

Bases included to complete triplets are colored grey and the PAM site is colored blue.

Back
Next

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

9 Click **Next**.



HR template design

Step 4: Summary

Knock-in edits

Deleted gctgaaccgctgaaaatgagctactgt 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 ...

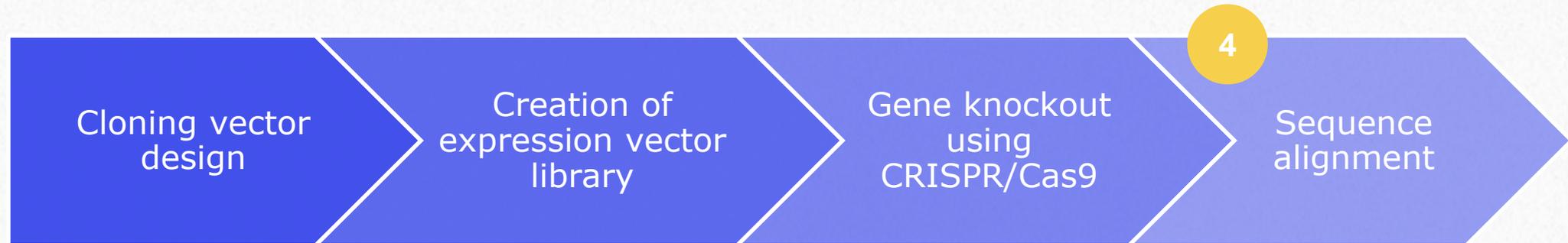
Copy the template or its reverse complement to your clipboard.

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

pSEVA6311-phaC-pct540
7955 bp

Propionate-CoA transferase
phaC1 (MBEL6-19)
chnR-PchnB-GA-R
PS1-1 (mismatches: 0)
Preo -10
chnR
NdeI, +3
oriT
rep gene
EcoRV
EcoRV

METADATA PLASMID BATCH SEQUENCE MAP

Share

Create Analyze

```

tgtatctcagggtgcattgtgtcattgttccgtgatatagcttctcataagcca
acatagagtcaccacgtaacacagtaaacaggcactatatcgaagagtattcgg
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

ggcgtatttacaaggtaggtatcggtagctttcattgacccagaaatggcggcg
ccgcataaatgtttccatccatagccatgaaagtaactgggtctttaccgccc
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

gtaaagtaatgatattaccaagaagatattgtgaattgtagagattaaggg
catttcattactataatggttcttataacaacttaaccatctctaattccc
                    
```

BASES 7955 INSERT 1180

ASSEMBLY SPLIT WORKSPACE

8. Sequence alignments

8.1 Alignment tool

Alignment creation

Alignment tool overview

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

Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise Multisequence Consensus

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

Template(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.

Alignment creation

Alignment tool overview

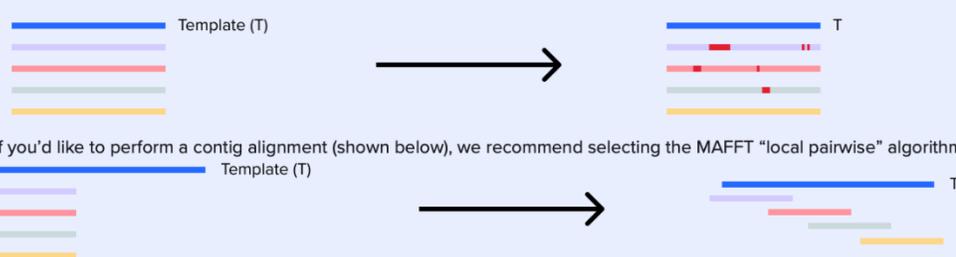
- i** In a real-live scenario, the construct sequence could be sent to sequencing. The results could then be analysed using the **alignment tool** in Benchling.
 - ✓ There are **three alignment options** and several alignment programs available:

Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise **Multisequence** Consensus

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



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

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

Alignment creation

Alignment tool overview

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

Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise Multisequence **Consensus**

Consensus Alignment - A new sequence will be created with the consensus of all the selected sequences. [Hide details](#)

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

Group(s) +

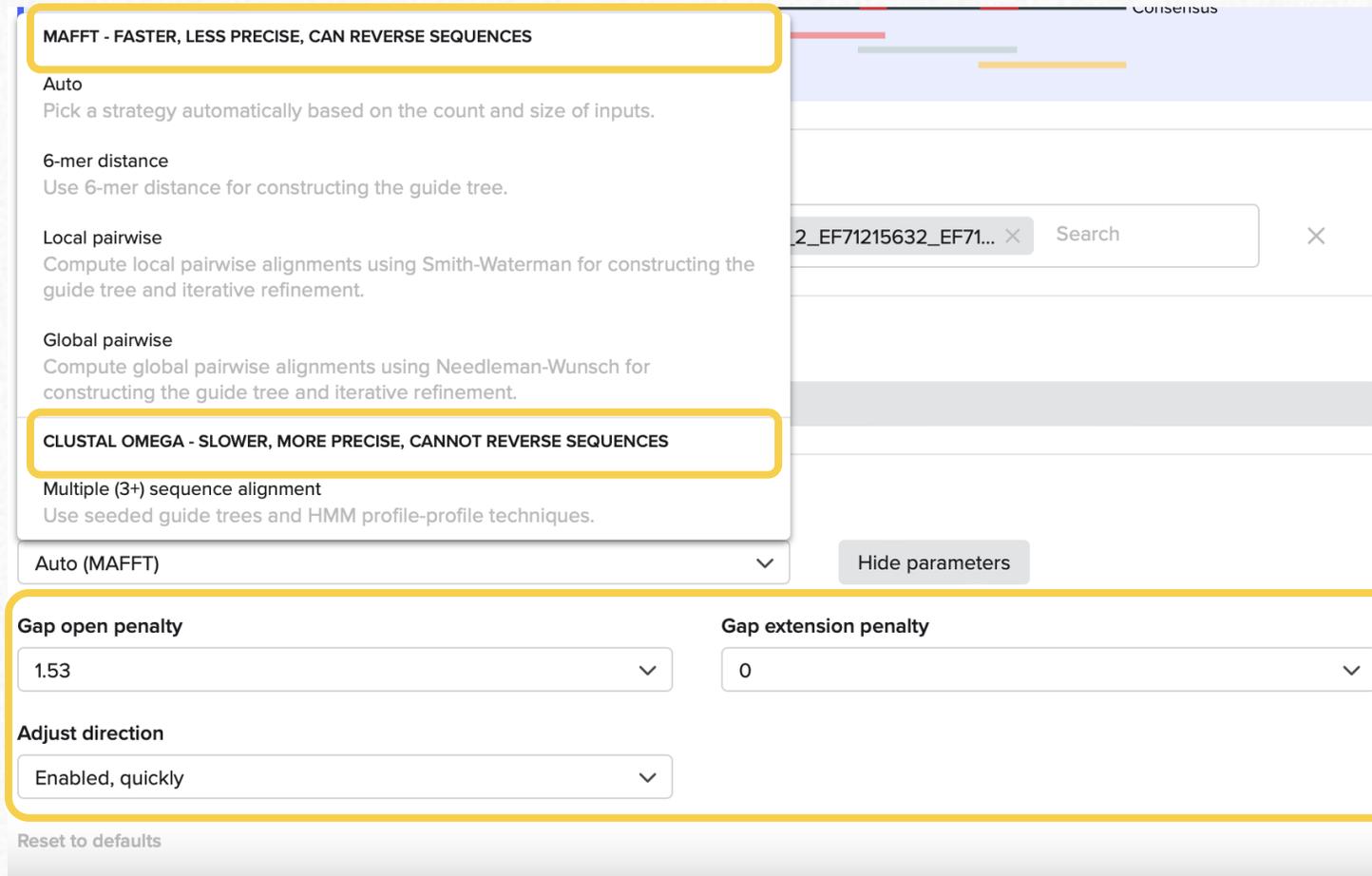
Untitled DNA Consensus AF_1_EF71215631_EF71... AF_2_EF71215632_EF71... Search

Select destination folder.

- 3 Consensus alignment:** Multiple sequences are compared against each other, creating a new sequence from the consensus region of all the sequences.

Alignment creation

Alignment tool overview



The screenshot shows the BRiGHT alignment tool interface. On the left, a sidebar contains navigation icons. The main area displays two algorithm options, each highlighted with a yellow box:

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

Below the options, a dropdown menu is set to "Auto (MAFFT)". A "Hide parameters" button is visible. The parameters section, also highlighted with a yellow box, includes:

- Gap open penalty: 1.53
- Gap extension penalty: 0
- Adjust direction: Enabled, quickly
- Reset to defaults button

In the background, a sequence alignment visualization is partially visible, showing a consensus sequence and individual sequence alignments with colored bars representing matches and gaps.

- ✓ 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**.

4. Sequence alignments Saved Searches

Search Type Filters

1-4 of 4 items 4 rows selected More

<input checked="" type="checkbox"/> Name	Inventory	ID	Modified	Authors	Descr
<input checked="" type="checkbox"/> FW-seq-1			11/02		
<input checked="" type="checkbox"/> MID-seq-1			11/02/2025		
<input checked="" type="checkbox"/> plasmid-seq	No inventory availa...		11/02/2025		No value
<input checked="" type="checkbox"/> pSEVA6311-phaC-pct540	No inventory availa...		11/02/2025		No value

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

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

Open

Analyze

Refresh

Multisequence alignment

Create DNA / RNA alignment

1 Choose input — 2 Define parameters

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

Drag and drop to upload or

Search for a DNA / RNA sequence.

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA RNA

Sequences

FW-seq-1 × MID-seq-1 × plasmid-seq × pSEVA6311-phaC-pct540 ×

3 Click **Next**.

Alignment creation

Multisequence alignment

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

Create DNA / RNA alignment

1 Choose input 2 Define parameters

Pairwise **Multisequence** Consensus

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

Template(s) **+** ← Non-template sequence(s) ↓

pSEVA6311-phaC-pct540 FW-seq-1 × MID-seq-1 × Search ×

pSEVA6311-phaC-pct540 plasmid-seq × Search ×

Choose an alignment program.

MAFFT
recommended for nucleotide alignments
Faster, less precise, can reverse sequences

Clustal Omega
recommended for amino acid alignments
Slower, more precise, cannot reverse sequences

Auto (MAFFT) Show parameters

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

Back **Create Alignment**

- 5 Create the alignments.

Alignment creation

Multisequence alignment

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

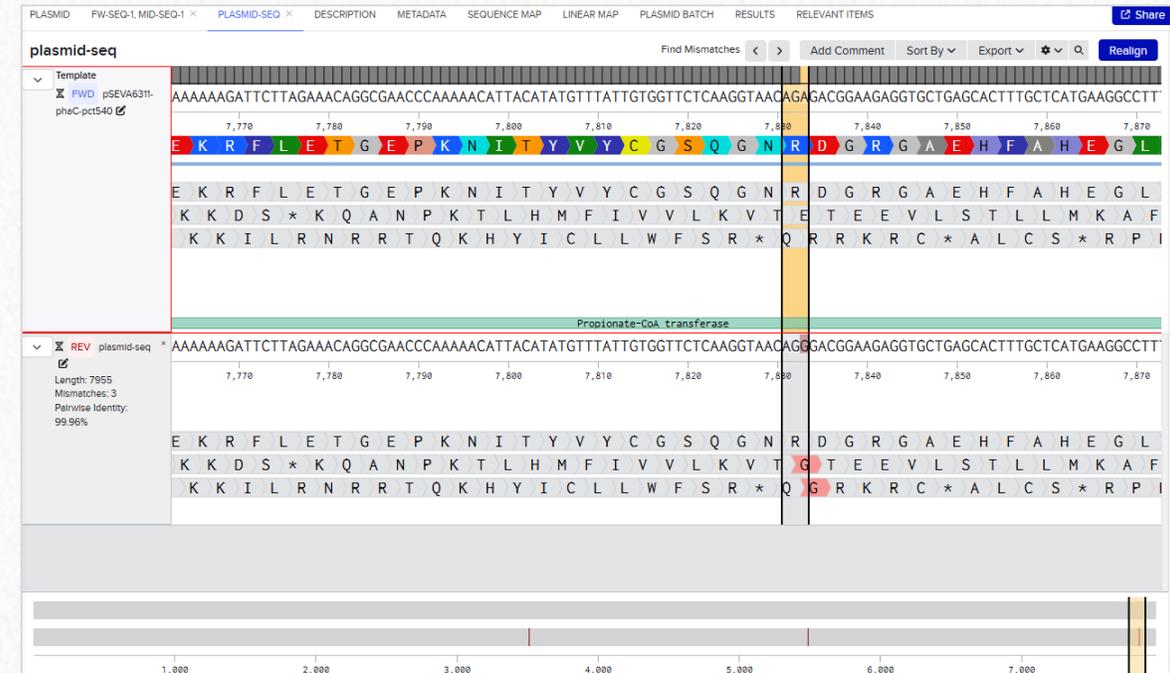
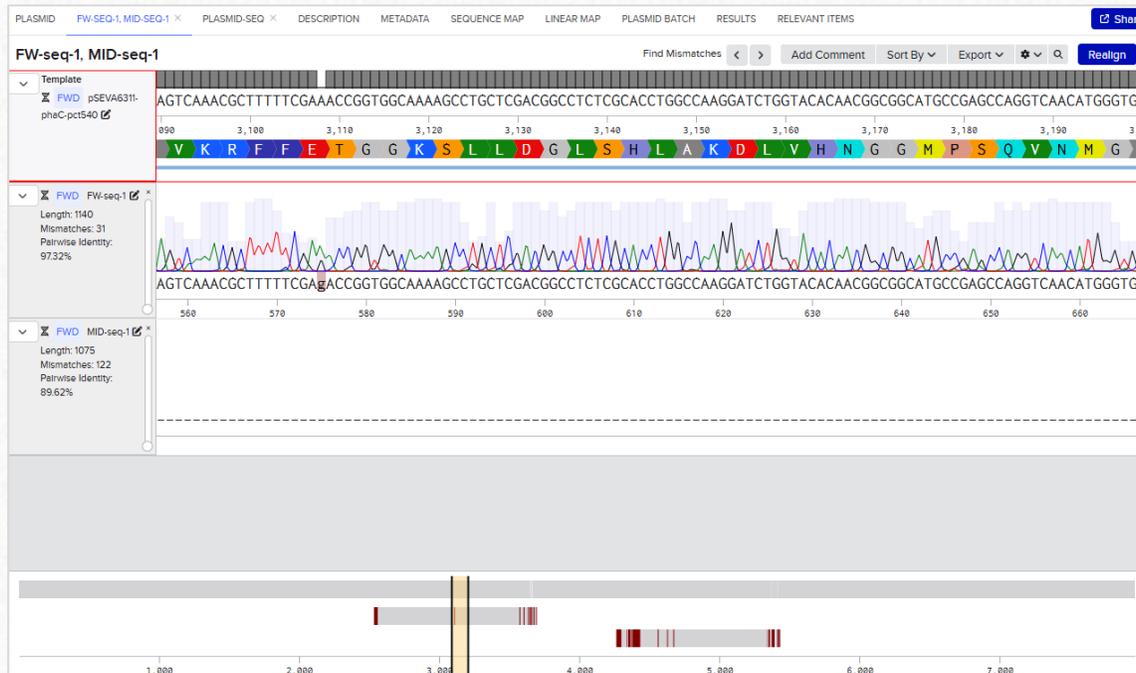
The screenshot displays the BRiGHT interface for the plasmid **pSEVA6311-phaC-pct540** (7955 bp). The left panel shows a circular plasmid map with various features including *Propionate-CoA transferase*, *phaC1 (MBEL6-19)*, *acc3* CDS, *oriT*, *rep gene*, *chnR*, and *PSI-1* (mismatches: 0). The right panel shows the sequence alignment view for the *Propionate-CoA transferase* gene. The sequence is displayed with a color-coded amino acid sequence: **V S Q G A L C H L F R D I A S H K P**. A yellow arrow points to the **Saved Alignments** section in the **SEQUENCE ALIGNMENTS** panel, which lists two alignments: **FW-seq-1, MID-seq-1** and **plasmid-seq**, both dated 17/02/2025 18:55.

- 7 Open the alignments.

Alignment creation

Multisequence alignment

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



8. Sequence alignments

8.3 Consensus alignment

Consensus alignment navigation

SEQUENCE MAP DNA CONSENSUS × LINEAR MAP DESCRIPTION METADATA RELEVANT ITEMS RESULTS Share

DNA Consensus Realign In Sync Find Mismatches < > Add Comment Sort By Export ⚙ Q

Consensus
 DNA Consensus ↗

-----gatagaattttggagctttttccctccttttttgagcaggagccaatagtagtccgaaagatgagtgcatagatttttcgtattttatttcgagt
 D R I L E L F S L L F L S R S Q * * S E R * V H R F F V F Y F E
 I E F W S F F P S F F * A G A N S S R K D E C I D F S Y F I S S

REV
 AF_2_EF71215632_EF71
 215632
 (AF_2_EF71215632_EF71215632.ab1) ↗

GACAGGAGCGATAGGAAAAGATAGAATTTTGGAGCTTTTCCCTCCTTTTGGAGCAGGAGCCAATAGTAGTCGAAAGATGAGTGCATAGATTTTTCGTATTTTATTTCGAGT
 D R I L E L F S L L F L S R S Q * * S E R * V H R F F V F Y F E
 I E F W S F F P S F F * A G A N S S R K D E C I D F S Y F I S S

FWD
 AF_1_EF71215631_EF712
 15631
 (AF_1_EF71215631_EF71215631.ab1) ↗

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

BASES 1387 ASSEMBLY WIZARD SPLIT WORKSPACE

Consensus region fragment

Analysed sequences

Overview navigation panel

You can jump from one mismatch to the other easily

Find Mismatches < >

Mismatches from the consensus sequence are marked in red.

You can edit what elements to visualize

Translation and reading frames have some additional settings that can be customized

The screenshot displays the BRiGHT software interface for consensus alignment. It features several tracks: a DNA Consensus track, a Reverse (REV) track, and a Forward (FWD) track. A 'Find Mismatches' button is located at the top. A settings panel on the right allows users to customize the visualization of various elements, including Annotations, Translations, Amino Acid Indices, Reading Frames, Primers, Alignment Axis, Sequence Axis, DNA, Trace, Trace Quality, Quality-based capitalization (20), Votes, Row Statistics, Comments, and Expanded Mini-Map. A 'Show Compact View' button is also present. The interface includes a navigation bar at the bottom with options like 'ASSEMBLY WIZARD' and '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



Tips and tricks

Work in bulk using the expanded view

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

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

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

Projects / RDM_Support

Search

Type Filters

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

Tips and tricks

Work in bulk using the expanded view

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

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

The screenshot displays the BRiGHT workspace interface. At the top, there's a navigation bar with a search bar and filters. Below it, a table lists items with columns for Name, Inventory, ID, Modified, Entry Dates, Authors, and Review Stat. The table contains four rows of data, all with checkboxes selected. Above the table, a toolbar contains various action icons. Blue callout boxes with arrows point to specific icons: 'Register' points to the expanded view icon (a 3x3 grid), 'Move to' points to the move icon (a folder with an arrow), 'Copy to' points to the copy icon (two overlapping documents), 'Archive' points to the archive icon (a document with a checkmark), 'Create request' points to the request icon (a document with a plus sign), 'Add to worklist' points to the worklist icon (a document with a plus sign), and 'Export' points to the export icon (a document with a plus sign). A 'More' dropdown menu is also visible in the toolbar.

Name	Inventory	ID	Modified	Entry Dates	Authors	Review Stat
<input checked="" type="checkbox"/> MolBio_training_DNA Consensus			31/10/2023		Patricia Brito Diaz	
<input checked="" type="checkbox"/> Alignment file 1_circular			01/11/2023		Patricia Brito Diaz	
<input checked="" type="checkbox"/> Alignment file 2_circular			31/10/2023		Patricia Brito Diaz	
<input checked="" type="checkbox"/> Alignment file1			31/10/2023		Patricia Brito Diaz	

Tips and tricks

The screenshot displays the BRiGHT interface. On the left, a vertical navigation bar contains icons for home, search, and other functions. The main content area shows a table of projects under the heading "Projects / RDM_Support". The table has columns for "Name" and "Inventory". The "Name" column contains several entries, each with a checkmark in a blue box to its left:

Name	Inventory
<input checked="" type="checkbox"/> MolBio_training_DNA Consensus	
<input checked="" type="checkbox"/> Alignment file 1_circular	
<input checked="" type="checkbox"/> Alignment file 2_circular	
<input checked="" type="checkbox"/> Alignment file1	

A context menu is overlaid on the table, listing various actions. The "Analyze" option is highlighted with a yellow box. A yellow arrow points from the "More" button in the table's header to the "Analyze" option in the context menu. The "More" button is also circled in yellow. The context menu options include:

- 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

Other options visible in the context menu include "Open", "Bulk edit", and "Refresh".

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 interface for sequence alignment. A yellow circle with the number '1' highlights the 'Realign' button in the top toolbar. The interface displays a sequence alignment between a template and a sample. The template sequence is GGTCCCCCAATTAGAGCTTTACGATTATATACTAACATCATGTACAAAACAATTTAATAATGATCTGTATTGCTGGCTCAATCCACGTAAATTAATGCCTCAGCACTAGTCCTGCAGGGTAAC. The sample sequence is 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 V P P I R A L R L Y T N I M Y K T I * * * S V L L A Q S T * I N A S A L V L Q G * F. The alignment shows a perfect match between the template and the sample. The 'Realign' button is highlighted, and the 'Out of Sync' button is also visible. The interface includes a sidebar with a template list, a main workspace with sequence and chromatogram views, and a top toolbar with various options like 'Share', 'Sort By', and 'Export'.

Tips and tricks

Autoindexing when creating alignments

2 Realign DNA / RNA

1 Choose Input 2 Define parameters

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

Drag and drop to upload or [Choose files](#)

Search for a DNA / RNA sequence.

Search by name

Create a DNA / RNA sequence from scratch.

Nucleotide type*

DNA RNA

Name Bases [Add](#)

Current sequences

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

New sequences

[Cancel](#) [Next](#)

3 Realign DNA / RNA

1 Choose Input 2 Define parameters

Pairwise Multisequence Consensus

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

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

Template(s) Non-template sequence(s)

[Alignment file 2_circular](#) [EF71215631_EF71215631](#) [Search](#)

Choose an alignment program.

i 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

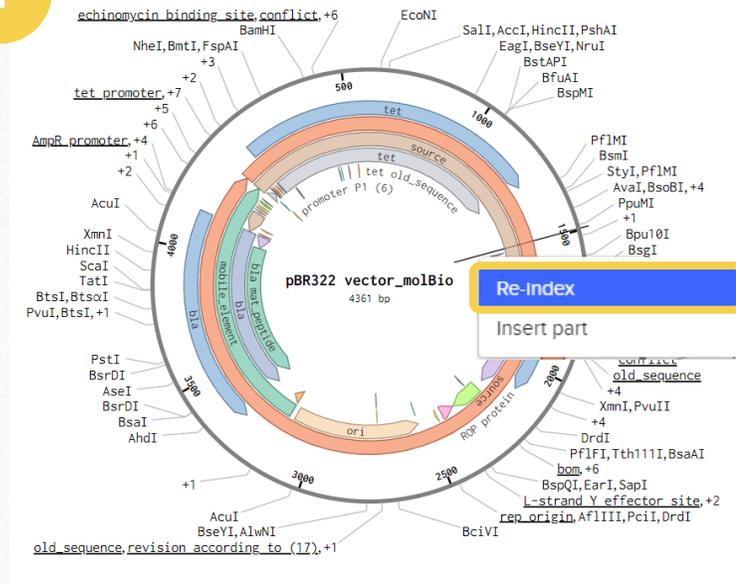
When creating an alignment of circular sequences, Benchling by default performs an **auto indexing** of these sequences.

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	<input style="width: 80%;" type="text" value="1"/>	

Re-index
Cancel

10. Resources



Questions?



Contact lims_support@bright.dtu.dk



More resources

Benchling Learning Labs

Benchling provides a **learning platform** that offers role-specific courses that can be taken in a **flexible-pace** structure.

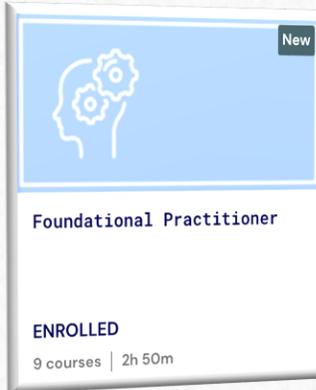
<https://www.benchling.com/learning-labs>



Molecular Biology Tools

FREE

8 courses | 2h 45m



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>

