



Benchling

An Electronic Laboratory Notebook

Guide to use in Biochemistry 426 at UMass Amherst

(~ 10 min to complete this guide)

***Note:** Not all features in this guide will be used in all sections of 426

Before completing this guide,
review [“Benchling Basics”](#) or be familiar with Benchling

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Biochemistry 426

- Each student laboratory group is assigned a “Project” folder
- Students add entries to Benchling for each experiment
 - Benchling Notebook Entries are automatically saved every few seconds
 - Faculty and TAs (“Admins”) have access to all projects for grading
 - Feedback may be given in the Notes section where you entered your data
- Protocols can be found in a labeled Folder or Project
- Tools used in Blochem 426 experiments
 - Amino acid sequence alignment
 - DNA sequence alignment
 - Primer design
 - Virtual digests
 - Virtual gel electrophoresis
 - Cloning

Amino Acid Sequence Alignment

Amino Acid Sequence Alignment

What is it?

- Compare Amino Acid Sequences to identify regions of similarity between sequences of interest
- Find information about conserved regions that may have an important role in the protein's structure or function

Amino Acid Sequence Alignment

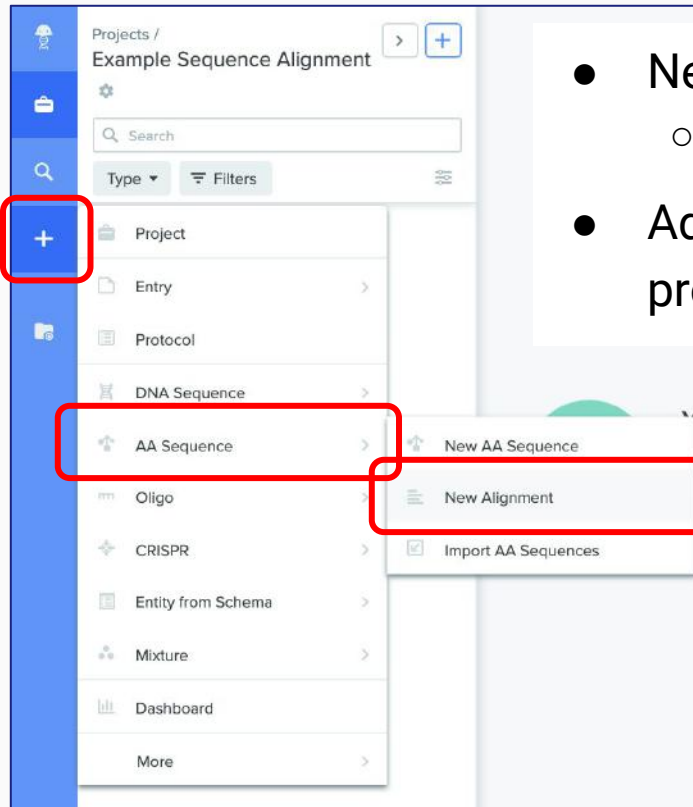
Uploading the protein sequences

- First, add the **Amino Acid Sequences to Benchling**
 - + → AA Sequence → New AA Sequence
- Import a file from your computer, or import from a database using the protein accession number

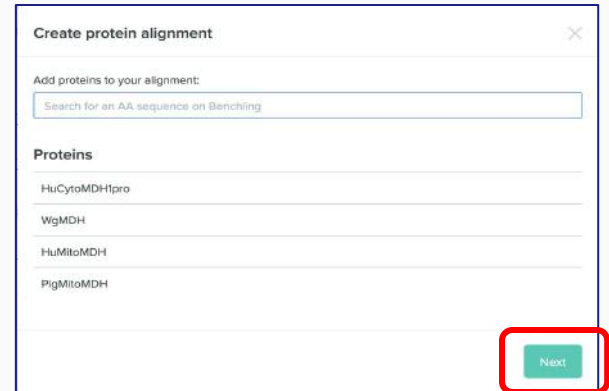
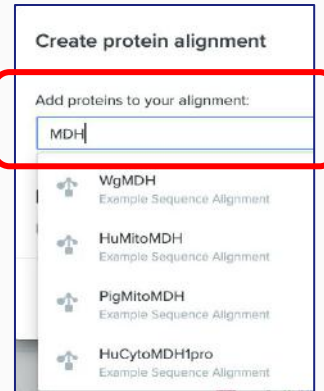
The screenshot displays the Benchling interface for 'Example Sequence Alignment'. The left sidebar contains a navigation menu with a '+' icon highlighted in a red box. The main content area shows a list of items with 'AA Sequence' selected, and a dropdown menu is open, showing 'New AA Sequence' highlighted in a red box. Below this, a modal window is open, showing 'Upload files' and 'Import from database' buttons highlighted in red boxes. The modal also displays the project name 'EXAMPLE SEQUENCE ALIGNMENT' and a 'Drag and drop to upload or Choose files' area. At the bottom right of the modal, the 'Import' button is highlighted in a red box.

Amino Acid Sequence Alignment

Creating the Alignment



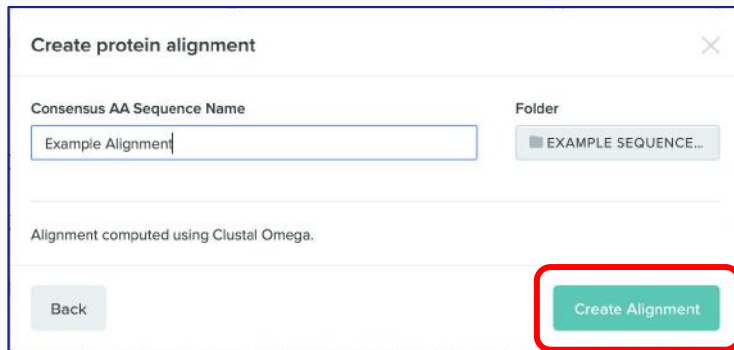
- Next, **create a New Alignment**
 - + → AA Sequence → New Alignment
- Add proteins to be aligned by searching for the proteins you have added to Benchling



Amino Acid Sequence Alignment

Creating the Alignment - cont'd

- Name your Protein Alignment and add it to the desired project folder
 - Click **“Create Alignment”**
 - Alignments are computed using the web program Clustal Omega
- A notification indicating the alignment was successfully created will appear in the bottom left corner.
 - Click **“Open”** to open the alignment in a new tab



Create protein alignment

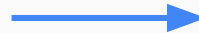
Consensus AA Sequence Name
Example Alignment

Folder
EXAMPLE SEQUENCE...

Alignment computed using Clustal Omega.

Back

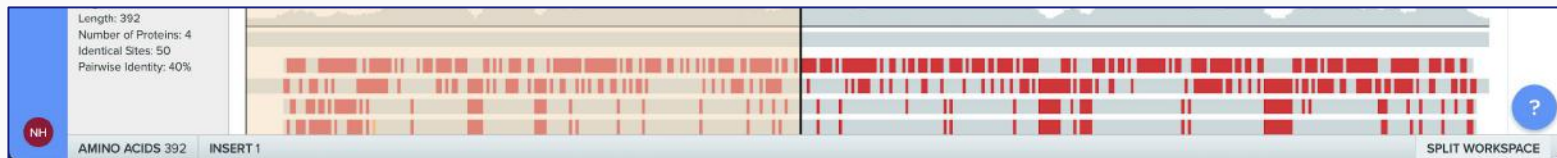
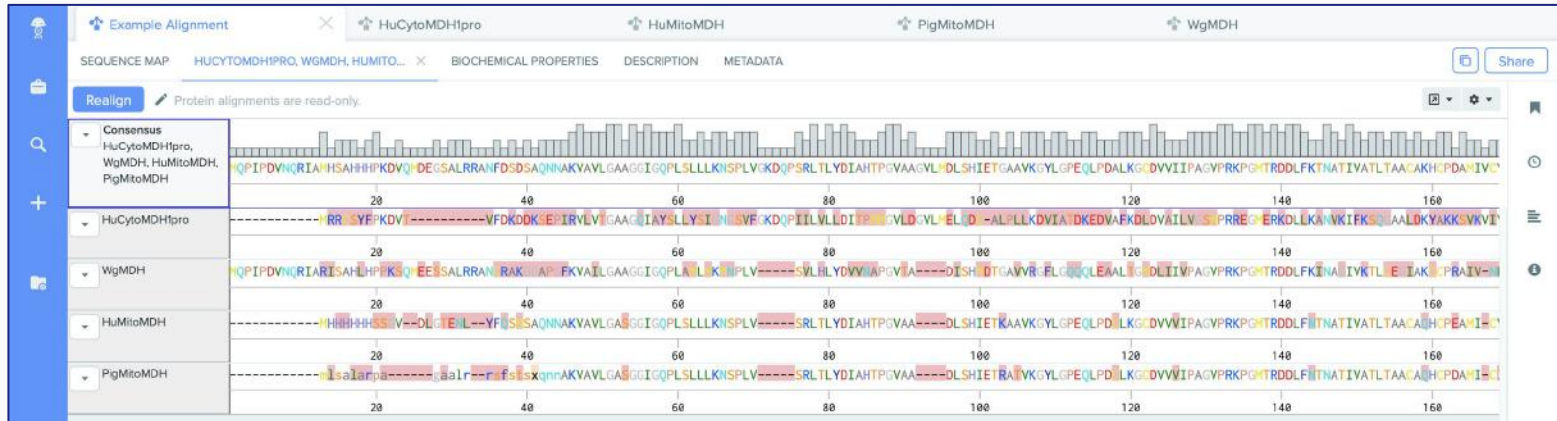
Create Alignment



Amino Acid Sequence Alignment

Viewing your alignment

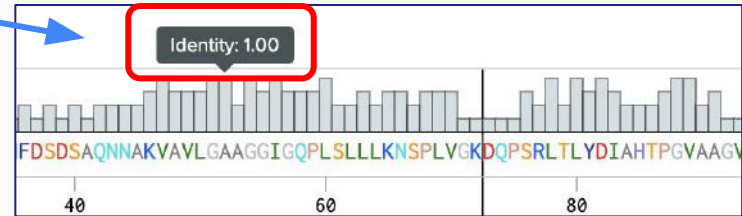
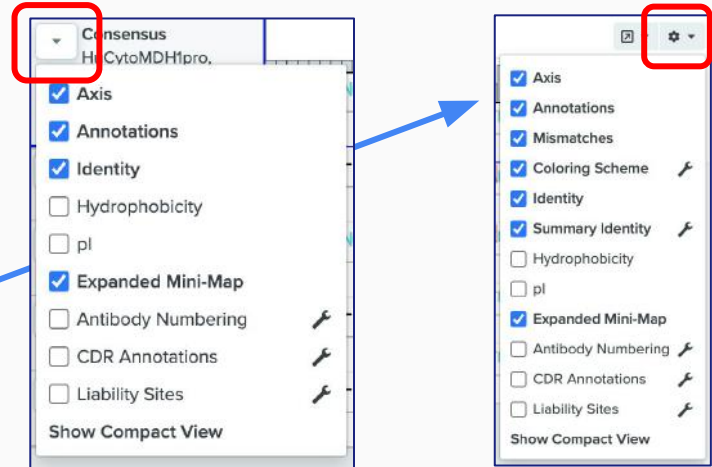
- Your alignment will look something like this
 - Each AA has a distinct color
 - Red highlighted regions = mismatches



Amino Acid Sequence Alignment

Interpreting your alignment

- Toggle features on and off for individual AA Sequences using the drop-down arrow next to the sequence name
- Toggle features on and off for the whole alignment using the gear icon at the top right corner
- Identity is shown at the top of the alignment
 - 1.00 = the same amino acid is found in that same position in each sequence



For more:

<https://www.benchling.com/2016/12/14/introducing-protein-alignments-on-benchling/>

DNA Sequence Alignment

DNA Sequence Alignment

Uploading the DNA sequences

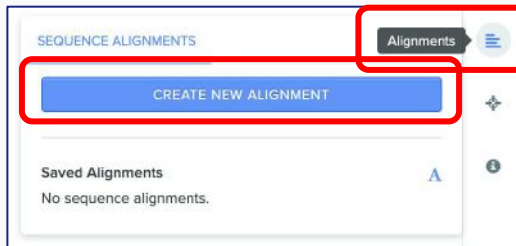
- **First, add your DNA Sequences to Benchling**
 - + → DNA Sequence → New DNA Sequence
- Import a file from your computer, import from a database, or input the raw sequence (copy and paste)

The image shows two screenshots from the Benchling web application. The left screenshot displays the main interface with a red box around a '+' icon in the top-left corner and another red box around the 'DNA Sequence' menu item in the left sidebar. A blue arrow points from the 'DNA Sequence' menu to the right screenshot. The right screenshot shows the 'New DNA Sequence' dialog box, which is also outlined in red. This dialog has four tabs: 'Convert Files', 'Search External Databases', 'Select Chromosomal Region', and 'Input Raw Sequence'. The 'Input Raw Sequence' tab is selected. It contains a text input field for 'Entity Name*' with the value 'Example Sequence', a 'Topology' dropdown menu set to 'Linear', and a 'Folder' dropdown menu set to 'Example Sequence Alignment'. A green 'Create' button is at the bottom right, and a grey 'Close' button is at the bottom right of the dialog.

DNA Sequence Alignment

Creating a new alignment

- Next, **create a new alignment** using the alignments tab on the right panel of the sequence
- Choose a sequence to align with a desired template sequence
 - Choose from existing Benchling files, upload a file with the sequence, or paste in the raw sequence



Alignment

Template Alignment. The results will be attached as an alignment on the template sequence. Deselect the template below to switch to a consensus alignment.

Upload sequence and trace files (.ab1 or .ftv), or choose an existing Benchling sequence.

CHOOSE FILE(S) or Search for a sequence

Alternatively, enter bases directly to add a raw sequence to the alignment.

Name Bases ADD

Sequences

HuMDH1 Template

Algorithm

MAFFT (faster, less precise, can reverse sequences) ADJUST PARAMS

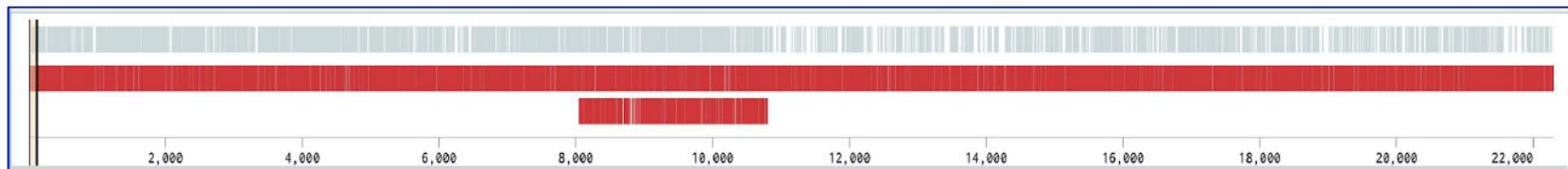
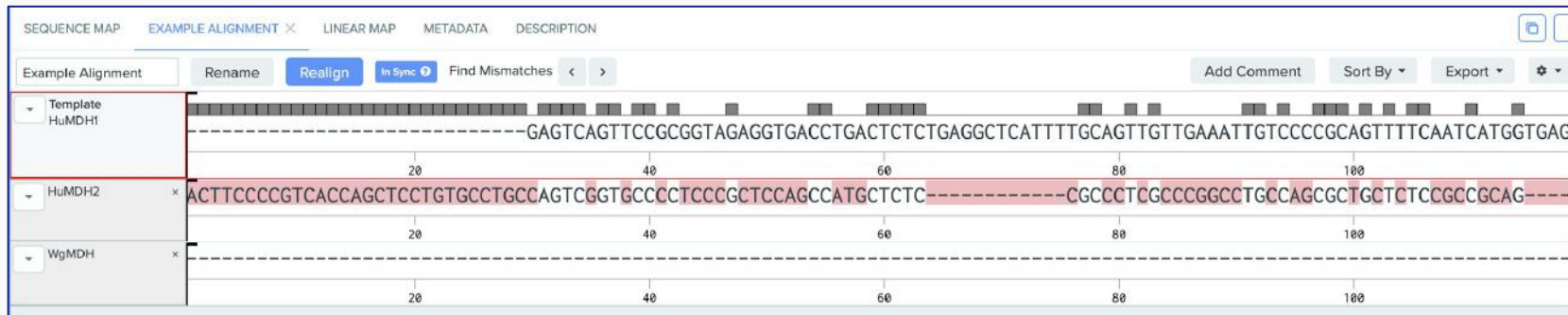
Alignments performed via MAFFT v7 (Katoh, Standley 2013)

CREATE ALIGNMENT CANCEL

DNA Sequence Alignment

Viewing the alignment

- Your alignment should look like this:
 - The red highlighted bases indicate mismatches from the template
 - This may be a different base or an insertion/deletion



DNA Sequence Alignment

Viewing the alignment - cont'd

The screenshot shows the settings for the 'HuMDH2' sequence. The 'HuMDH2' name is highlighted with a red box. A blue arrow points from the 'HuMDH2' name to the settings menu. The settings menu is open, showing the following options:

- Annotations
- Translations
- Amino Acid Indices
- Primers
- Alignment Axis
- Sequence Axis
- DNA

At the bottom of the settings menu, there is a 'Show Compact View' button.

- Toggle features on and off for individual DNA Sequences using the **drop-down arrow** next to the sequence name
- Toggle features on and off for the whole alignment using the **gear icon** at the top right

The screenshot shows the global settings menu for the alignment. The gear icon at the top right is highlighted with a red box. A blue arrow points from the gear icon to the settings menu. The settings menu is open, showing the following options:

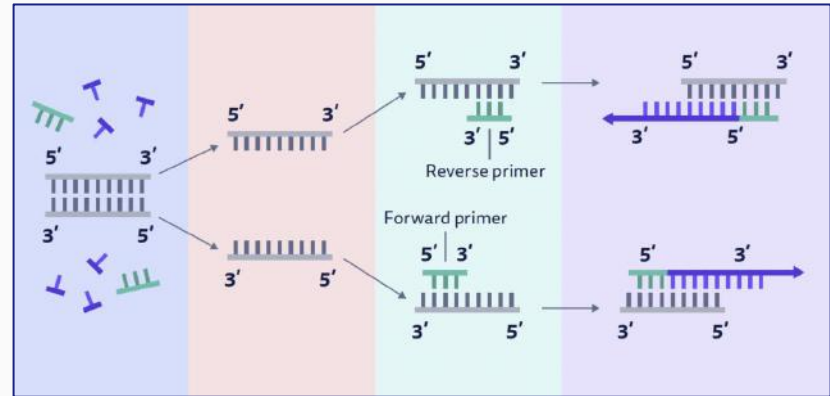
- Annotations
- Translations
- Amino Acid Indices
- Reading Frames
- Primers
- Alignment Axis
- Sequence Axis
- DNA
- Trace
- Trace Quality
- Votes
- Comments
- Expanded Mini-Map

At the bottom of the settings menu, there is a 'Show Compact View' button.

Primer Design

Primer Design

- Primers are short single-stranded nucleotide sequences used to provide a starting point for DNA synthesis
 - Used in Polymerase Chain Reaction, DNA Sequencing, Cloning Reactions, etc.
- Primers should flank the region of DNA you would like to amplify
 - **Forward** Primer: anneals to the template strand
 - **Reverse** Primer: anneals to the complementary strand
 - Each bind on opposite ends of the sequence being amplified with their **3'** ends pointing toward each other

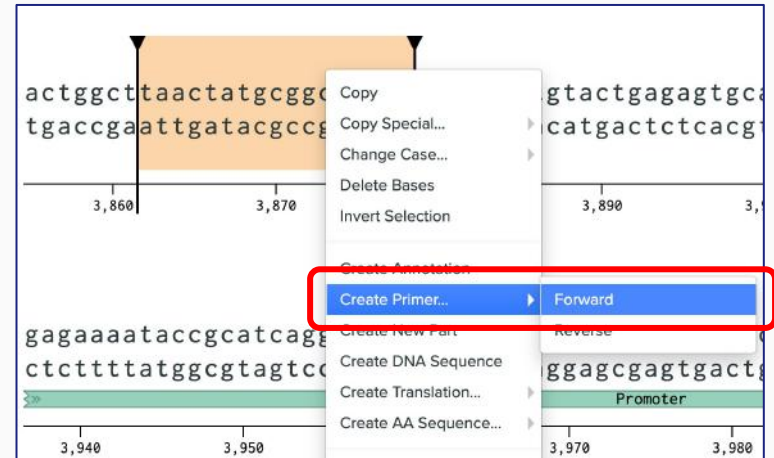
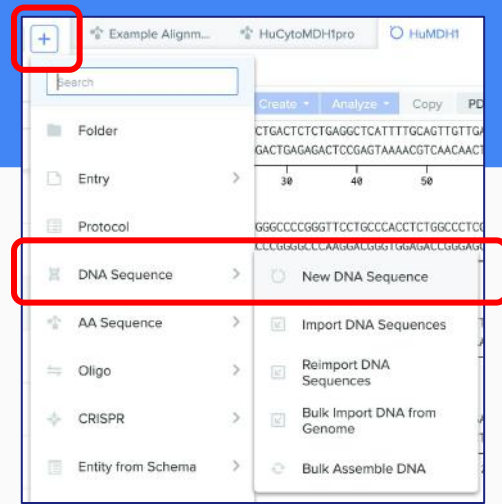


Primer Design

In Benchling, you can add annotations for the primers you designed and save their sequences

1. Add/open your DNA sequence
2. Highlight the desired primer region in your sequence
 - Note: primer region depends on your experimental goal, check with TAs if you are unsure of the sequence
3. Right click and select **Create Primer**
 - Select Forward or Reverse

For more: <https://www.benchling.com/primers/>



Primer Design

- In the **Design section**:
 - View primer details: direction, sequence, and location
- In the **Verify section**:
 - View information: melting temperature (T_m), GC Content, and length

Design

Strand Forward Reverse

Bases 5' GGATCC **taactatgcgcatcag** 3'

3' Location

Overhang

Cut Site BamHI

Use the dropdown above to look up restriction sites.

Verify at °C

T_m 	47.9°C
GC Content	47.06%
Length	23 bp
Min ΔG Homodimer	-9.5 kcal All Structures
Min ΔG Monomer	0 kcal All Structures

Primer Design

- To create a primer pair, choose **Primer Pair** from the drop down menu at the top of the **Design** tab
- If you have Benchling in **SPLIT WORKSPACE** mode, you can select the region of the sequence you want the **second primer** to span, and click **set from selection** in the top right
- Lastly, name your primers and save them to the desired folder



Primer Design

Another way to add primers to your Benchling

1. Open your DNA sequence
2. Click on the Primers tool on the toolbar located on the right side and select **Create Primers**
 - Then, click **Manual**
3. The same “Design and Verify” window from the previous slide will pop up with no fields filled in
4. Paste your primer sequences into the “Bases” field, then save
 - Benchling will automatically generate the information in the “Verify” section



The screenshot shows the 'Design' window in Benchling. The 'Strand' is set to 'Forward'. The 'Bases' field is empty. The '3' Location' is 3532. The 'Overhang' is 0. The 'Cut Site' is set to 'Enzyme'. The 'Verify' section shows 'Check Secondary Structure' at 37 °C. The 'Tm', 'GC Content', and 'Length' fields are empty.

T _m	--
GC Content	--
Length	0 bp

- 1) Restriction Enzyme Digest**
followed by
- 2) Gel Electrophoresis**

1) Restriction Enzyme Digest

Open DNA Sequence (Linear or Plasmid)

1. Click Digest Scissors (top right)
2. Search for and select Restriction Enzymes
3. **Run Digest**
4. Analyze Digest
5. Click Virtual Digest tab for gel results (next slide)

SEQUENCE MAP LINEAR MAP PLASMID DIGEST **5** VIRTUAL DIGEST METADATA DESCRIPTION

Digest Save

Enzymes	Cuts	Temp.	11	2.1	3.1	4/CS
CspCI	2	37°C	10	100	10	100

4

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
735	769	35	CspCI	3'	CspCI	3'
770	4365	3596	CspCI	3'	CspCI	3'
4366	4400	35	CspCI	3'	CspCI	3'
4401	734	4362	CspCI	3'	CspCI	3'

NEW DIGEST SAVED DIGESTS **1**

Enzyme CspCI

11(N)CAANNNNNGTGG(N)12
13(N)GTTNNNNNCACC(N)16

NEB

Use HF ?

Link: NEB

Inactivation: 65°C

Incubation: 37°C

Activity:

1.1	2.1	3.1	4/CS
10	100	10	100

Isos.: None

Jump to Cut Site:

735

4401

2

Name	Cuts	Selected	Color
ClalI	2	<input type="checkbox"/>	CspCI
CviAII	31	<input type="checkbox"/>	
CviKI-1	146	<input type="checkbox"/>	
CviQI	12	<input type="checkbox"/>	

Find Enzyme Clear Selected

Search by name or number of cuts

Show enzymes that cut

anywhere in the sequence

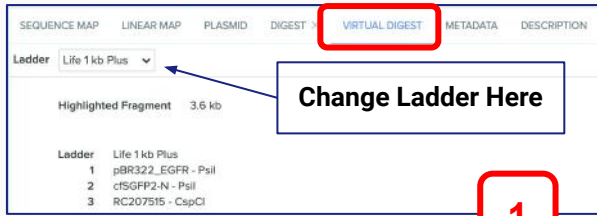
Highlight enzymes with compatible sticky ends

3 RUN DIGEST

1

For more: help.benchling.com/en/articles/4577136-gel-electrophoresis-and-restriction-enzymes

2) Gel Electrophoresis



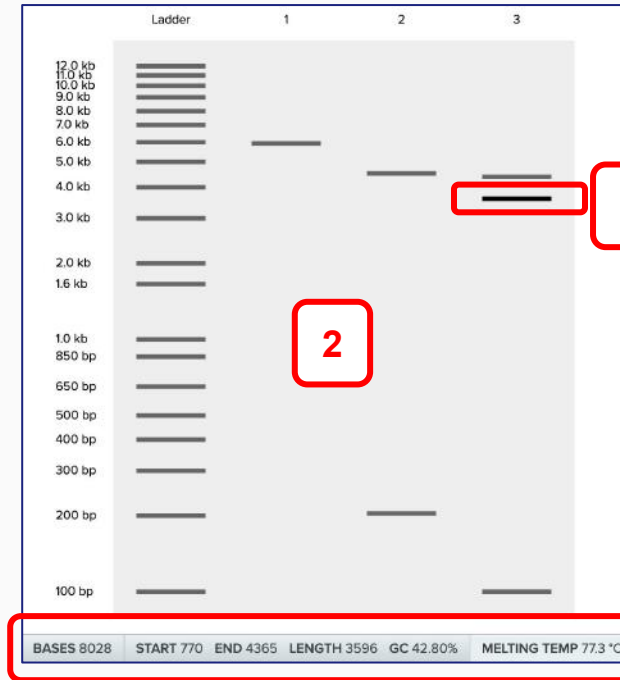
1. Virtual Digest

2. View Simulated Gel

3. Select an individual band to:

A. Copy the DNA sequence

B. Analyze the sequence properties



Click the sequence type you would like to copy.

DNA Sequence	DNA Reverse Complement
<pre>CTCACGGGATTTCCAAGTCTCCACCCCA TTGACGTCAATGGGAGTTTGTTTGGCAC CAAAATCAACGGGACTTCCAAMATGTCG TAATAACCCCGCCCGTTGACGCAATGG GGGTAGGGGTGACGGTGGAGGTCTAT</pre>	<pre>GGCAACTCCAGGCGCAGGAGGCCTG GGGAGGGGTACAGGGATGCCACCCGGGA TCTGTTACGAAACAGCTATGACCCGGCC CGGCCGTTAAACCTTATGTCGTATCC TTGTAATCCAGGATATCATTGCTGCCAG</pre>

AA Translation

AA Translation	Reverse Translation
<pre>LTGISKSPPH*RWQEFVLPKSTGLSKM **PRPVDANGR*ACTVGLYKQSSFSEPS EFCNTHYRAAGNSSTGSGTEEICRRDRH GVSNDTEETNSYPF*HNPFTQTPWG*MV S*NSAL*CHSGCSKEPCDRGLHRQAFDRN</pre>	<pre>IFQGQERHWGGVTMPGICSGNSYDRG RPFKPYRRHPCNPYHLLPDPLLR*VSR AAAYA*PFP*TPDYRPHDCLWPRSLDTA RSMGELAGLLATQQNSHRAFSGAGGTWT SERASQEI SR*SHQLFHP*ASDGT SKMLS</pre>

RNA Sequence

RNA Sequence	RNA Reverse Complement
<pre>CUCACGGGGAUUUCAAGUCCACCCCA UUGACGUCUAAUGGGAGUUUGUUUGGCAC CAAAUACACGGGACUUUCAAAUUGUCG UAAUAACCCCGCCGUGAGCAAUUGG GGGUAGGGCGUGUACGGUGGAGGUCUUA</pre>	<pre>GGCAACUCCAGGCGCAGGAGGCACUG GGGAGGGGUCACAGGGAUCCACCCGGGA UCUGUUCAGGAAACAGCUAUGCCCGCC CGGCCGUUUAACCUUAUCGUCGUCAUCC UUGUAUCCAGGAUAUUAUUGUGCCAG</pre>

Cloning

Cloning

- **Cloning** is the process of creating recombinant plasmids by adding DNA fragments to vector backbones for a wide range of applications.
- Tools such as Serial Cloner and NEBuilder can be used to virtually assemble plasmids, but this can also be done in Benchling with simple copying and pasting!

Cloning

Benchling Cloning Basic Steps:

- Select the desired region of DNA you want to insert into a plasmid by highlighting and copy the sequence (Ctrl/Cmd+C or Right Click+Copy)
 - If you are selecting a specific region between two enzyme cut sites, select both cut sites while holding Shift
- Paste (Ctrl/Cmd+V) the DNA sequence into the desired location
 - If between two cut sites on the target DNA, select the cut sites while holding Shift and paste -- your copied bases will replace the existing sequence

For more: <https://help.benchling.com/en/articles/671055-clone-by-copy-and-paste>

Cloning: Example

- If you wanted to insert a DNA sequence at a specific location in a plasmid, you would first click on that location

The screenshot displays a bioinformatics software interface for cloning. On the left, a 'SEQUENCE MAP' shows a DNA sequence with a 'HindIII' restriction site highlighted by a red box. The circular map on the right, titled 'Copy of pRFHUE_MCS_moxGFP 9449 bp', shows various restriction sites, with the 'HindIII' site also highlighted by a red box. The interface includes a 'LINEAR MAP' tab and a 'DESCRIPTION' tab. The bottom of the interface shows 'BASES 9449' and 'INSERT 8998'.

- In this case, the desired location is a cut site for the restriction enzyme HindIII
- The DNA fragment being cloned into the plasmid will replace this cut site

Cloning: Example

- Next, you would **delete** the restriction cut site, **copy** the bases of the desired insertion sequence, and **paste** it at the site:

The screenshot displays a bioinformatics software interface with two main panels. The left panel, titled 'SEQUENCE MAP', shows a linear DNA sequence with various features highlighted. A red box highlights a specific region of the sequence, including a 'stop codon' and an 'intron' within a 'moxGFP' gene. The right panel, titled 'LINEAR MAP', shows a circular plasmid map for 'Copy of pRFHUE_MCS_moxGFP' (9773 bp). A red box highlights a 'stop codon' and an 'intron' on the plasmid map, corresponding to the highlighted region in the sequence map. The plasmid map also shows various restriction enzyme sites and other features like 'ori', 'pMB1', and 'pUC19'.

Cloning: Example

- You can **annotate** your cloned sequence by using the Annotation Tool on the side panel OR by right-clicking the sequence and selecting “Create Annotation”

The screenshot displays a bioinformatics software interface. On the left, a 'SEQUENCE MAP' shows a DNA sequence with various features like 'gfpA Intron' and 'moxGFP' highlighted. A circular plasmid map is shown in the center, with restriction sites like 'SalI', 'ApoI', 'PspOMI', 'AflIII', 'PstAI, ZraI, AatII', and 'RsrII' marked. On the right, an 'ANNOTATIONS' panel is open, showing a table of existing annotations and a 'New annotation' form. The 'New annotation' form has fields for Name, Annotation type, Position (8698-9025), Color, and Strand (Forward). A red box highlights the 'Annotations' panel and the 'New annotation' form.

Filter visible by type	Name	Location	Length
<input checked="" type="checkbox"/>	M13 fwd	33-49	17
<input checked="" type="checkbox"/>	trfA	435-1583	1149
<input checked="" type="checkbox"/>	KanR	1882-2676	795
<input checked="" type="checkbox"/>	oriV	2958-3589	632
<input checked="" type="checkbox"/>	RB T-DNA repeat	3728-3752	25
<input checked="" type="checkbox"/>	pBRrevBam	4090-4109	20

New annotation form fields:
Name: [Annotation name]
Annotation type: [Annotation type]
Position: 8698 - 9025
Color: [Blue]
Strand: Forward

The screenshot shows a close-up of a DNA sequence map. A context menu is open over the sequence, listing various actions. The 'Create Annotation' option is highlighted with a red box. The sequence map shows 'moxGFP' and 'gfpA Intron' features.

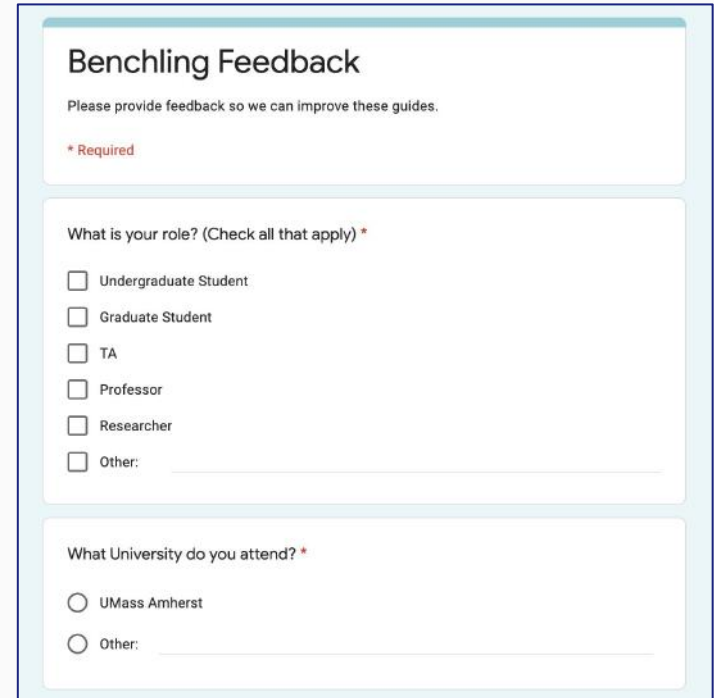
- Copy
- Copy Special...
- Change Case...
- Delete Bases
- Invert Selection
- Create Annotation**
- Create Primer...
- Create New Part
- Create DNA Sequence
- Create Translation... gactaactgttg
- Create AA Sequence... ctgatigacaacg
- Run Primer3
- Oligo Analysis... 8,970
- NCBI BLAST
- Analyze As Translation TTCAGTGTCCA
- Optimize Codons AAGTACAGTT

Questions or Comments?

Fill out our **Google Form** to provide feedback:
forms.gle/92Q9vp6jYzSB8Z9j6

Benchling **FAQ:**

docs.google.com/document/d/1DkSHnZ6FX8L4fORjmYm_omzotnJHzjTa587kfqtSiT0/edit?usp=sharing



Benchling Feedback

Please provide feedback so we can improve these guides.

* Required

What is your role? (Check all that apply) *

- Undergraduate Student
- Graduate Student
- TA
- Professor
- Researcher
- Other: _____

What University do you attend? *

- UMass Amherst
- Other: _____