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

Last Updated 04/06/21

Created by UMass Amherst Biochem and Molecular Biology Students

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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

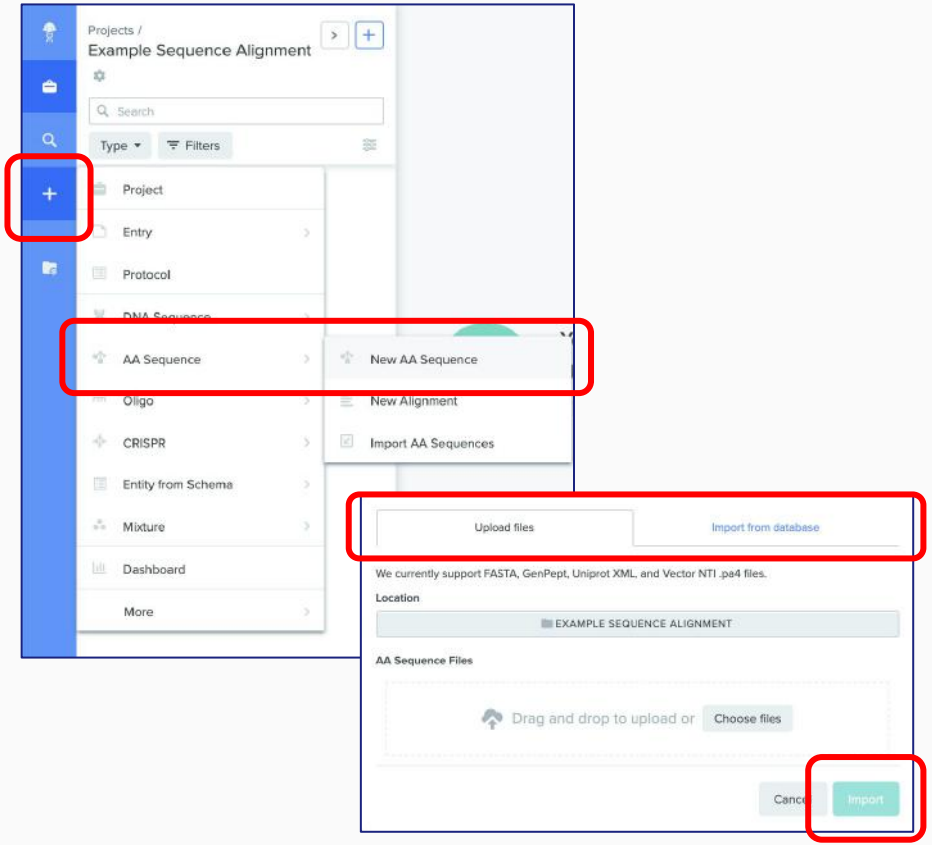
What is it?

- 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

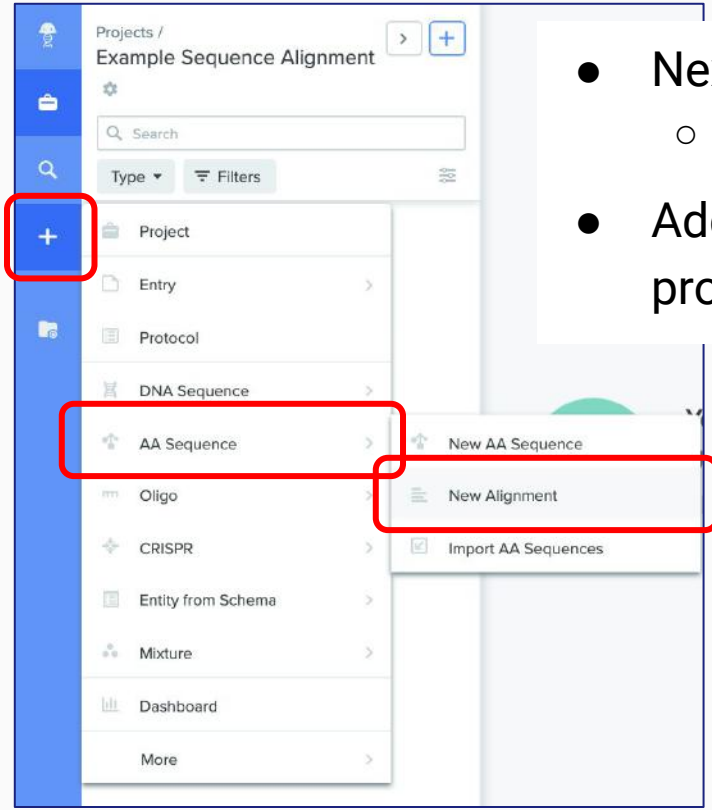
Uploading the protein sequences

- 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

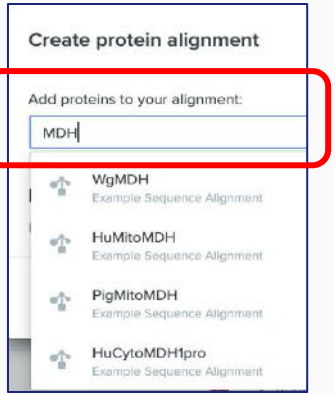


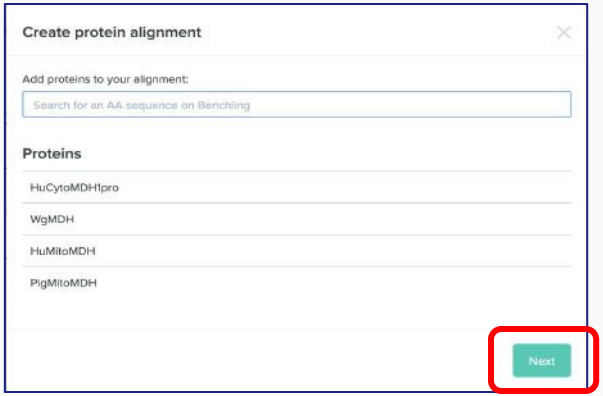
Amino Acid Sequence Alignment

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

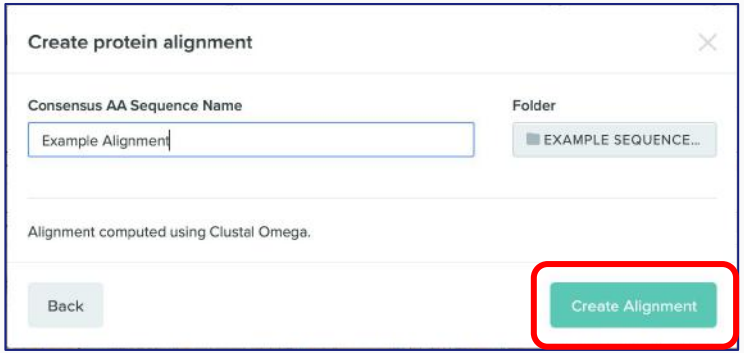




Amino Acid Sequence Alignment

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



Amino Acid Sequence Alignment

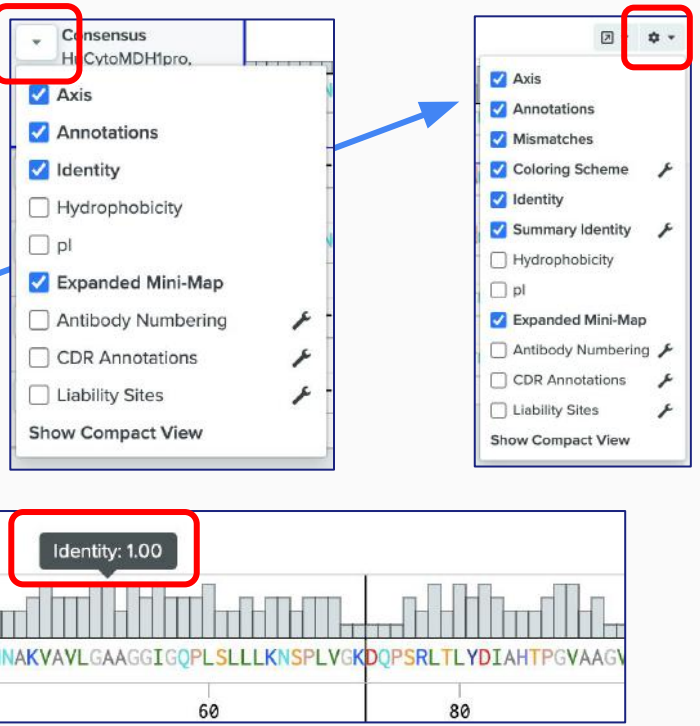
Viewing your alignment

- 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



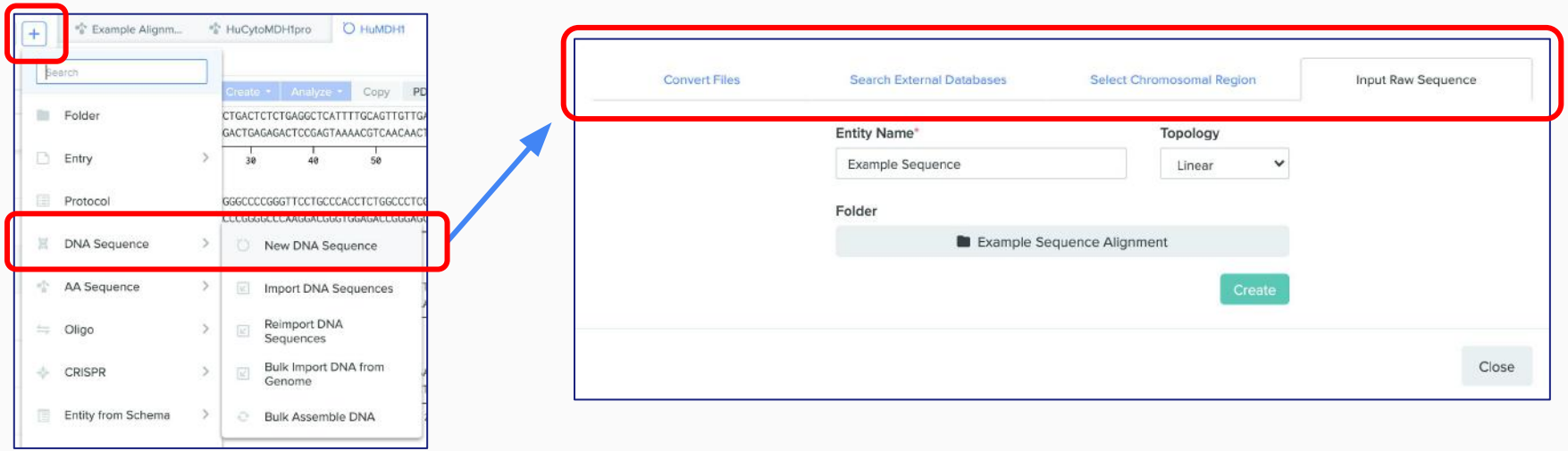
For more:
https://www.benchling.com/2016/12/14/introducing-protein-alignments-on-benchling/

DNA Sequence Alignment

DNA Sequence Alignment

Uploading the DNA sequences

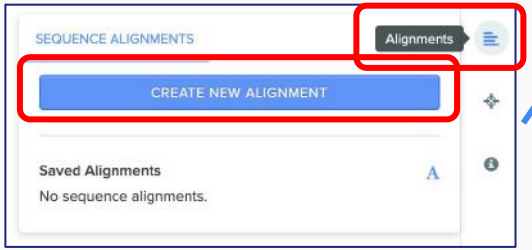
- First, **add your DNA Sequences to Benchling**
  - + → DNA Sequence → New DNA Sequence
- Import a file from your computer, import from a database, or input the raw sequence (copy and paste)

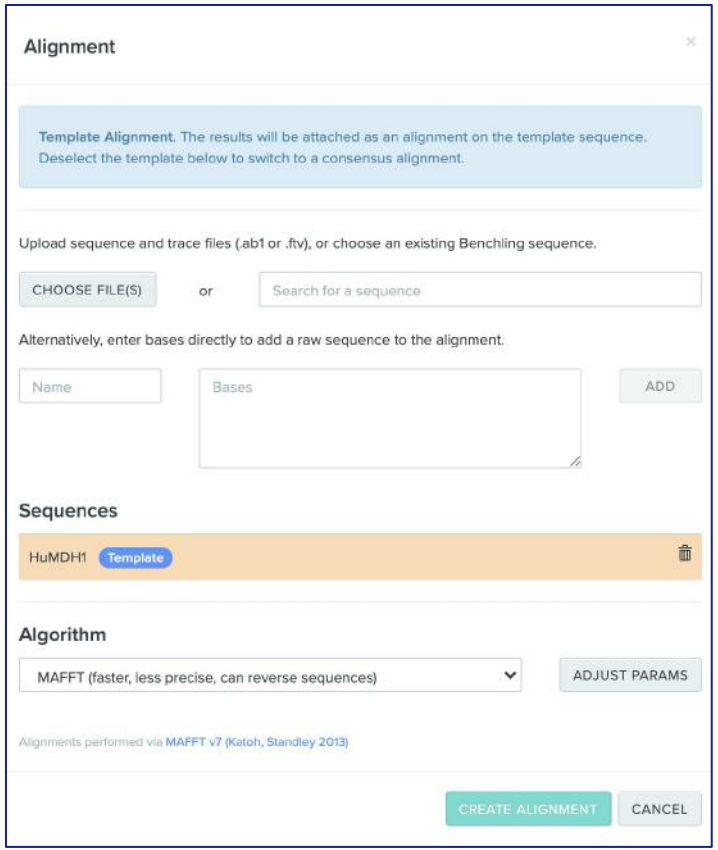


DNA Sequence Alignment

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

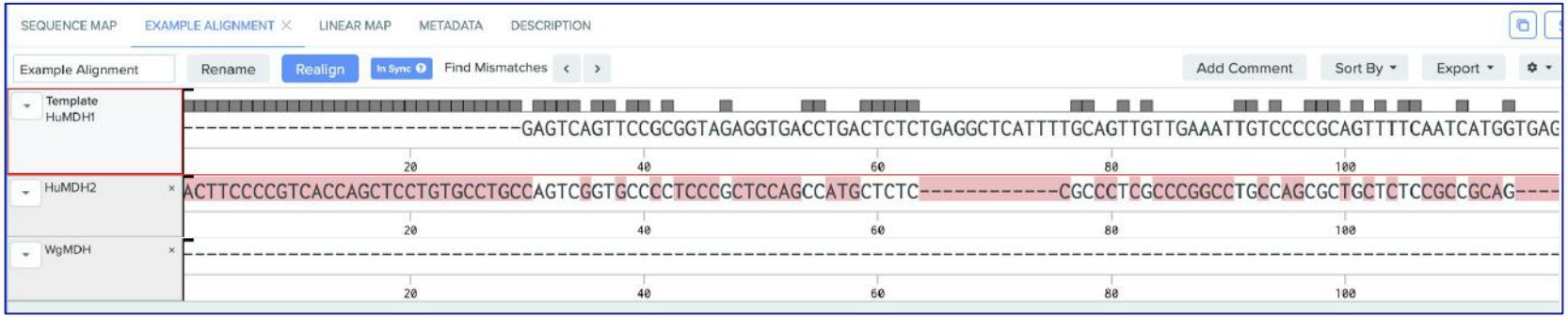


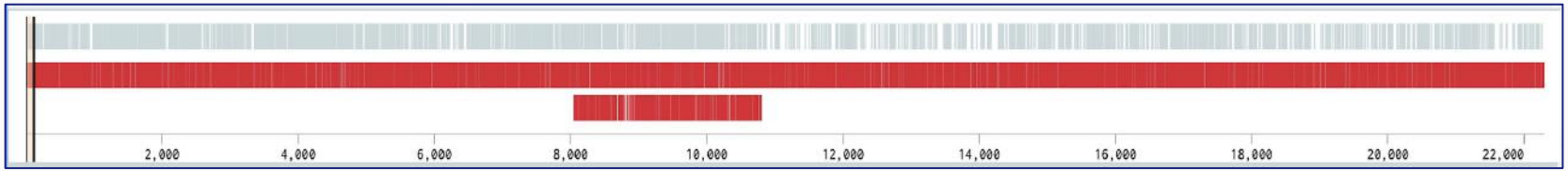


DNA Sequence Alignment

Viewing the alignment

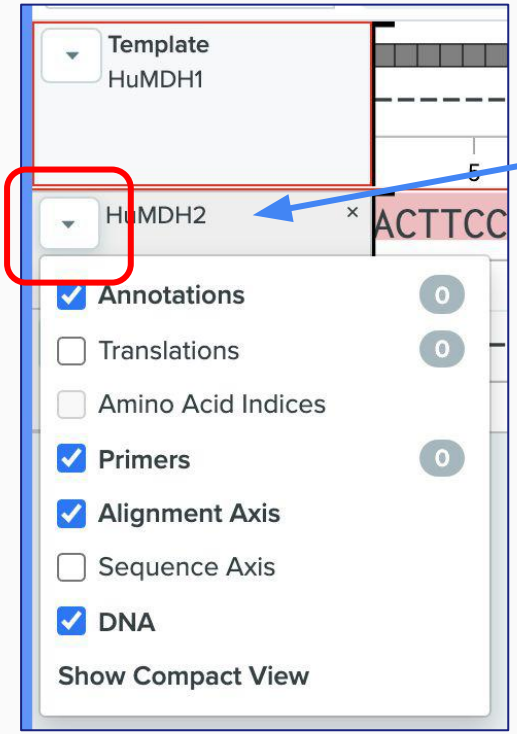
- Your alignment should look like this:
  - The red highlighted bases indicate mismatches from the template
  - This may be a different base or an insertion/deletion



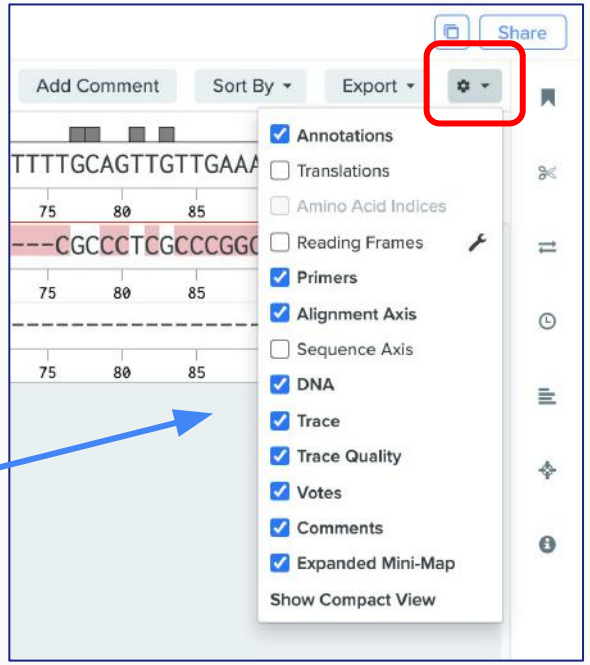


DNA Sequence Alignment

Viewing the alignment - cont'd



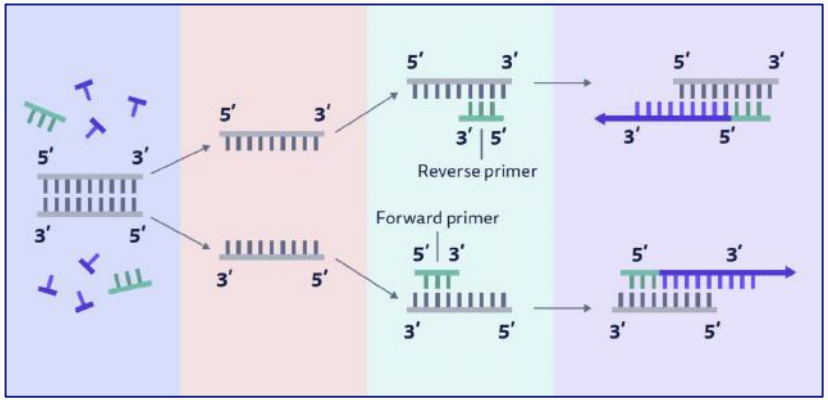
- Toggle features on and off for individual DNA Sequences using the **drop-down arrow** next to the sequence name
- Toggle features on and off for the whole alignment using the **gear icon** at the top right



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

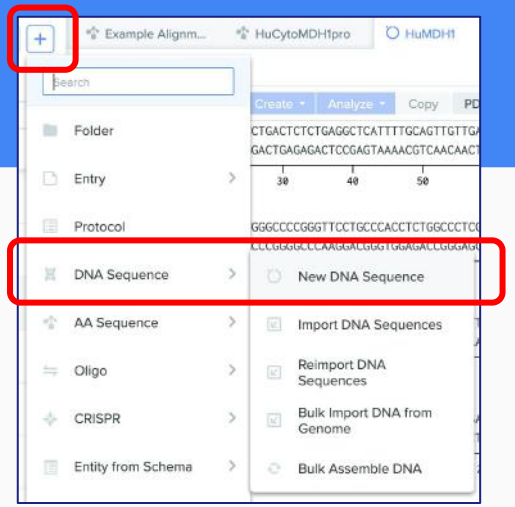


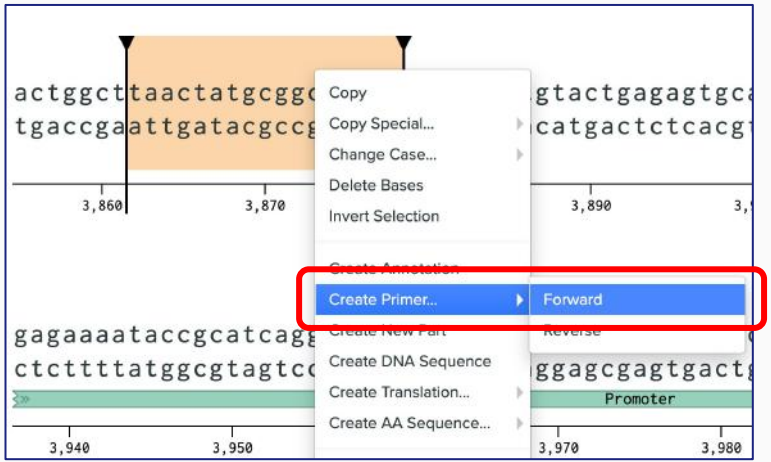
Primer Design

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

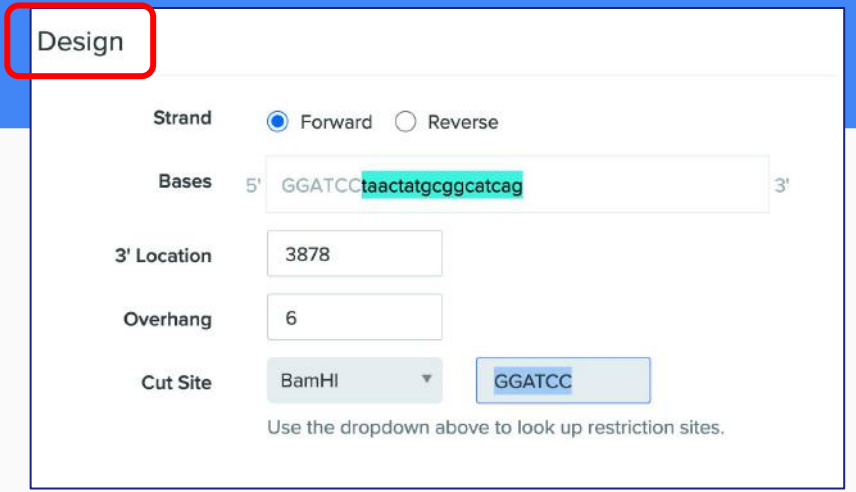
For more: https://www.benchling.com/primers/





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



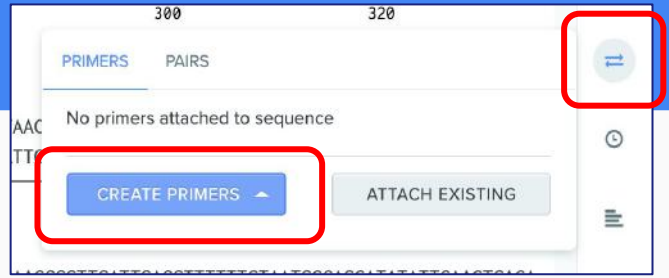
Primer Design

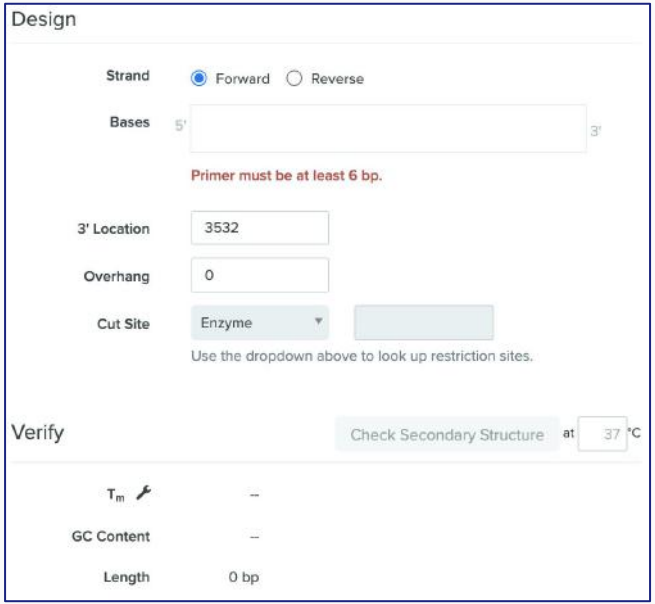
- To create a primer pair, choose **Primer Pair** from the drop down menu at the top of the **Design** tab
- If you have Benchling in SPLIT WORKSPACE mode, you can select the region of the sequence you want the **second primer** to span, and click **set from selection** in the top right
- Lastly, name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





1) Restriction Enzyme Digest

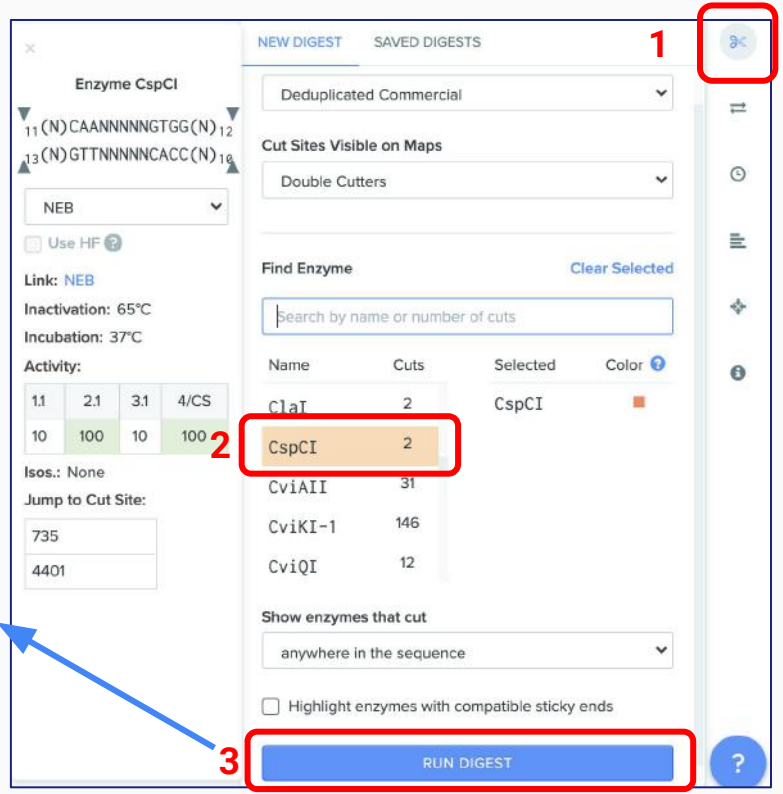
followed by

2) Gel Electrophoresis

1) Restriction Enzyme Digest

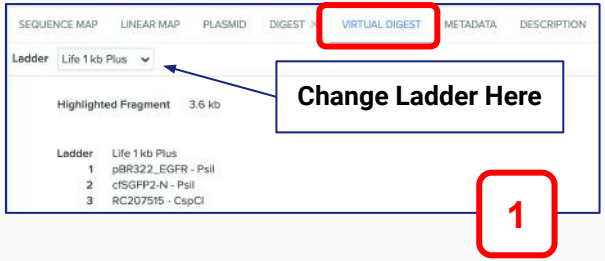
Open DNA Sequence (Linear or Plasmid)

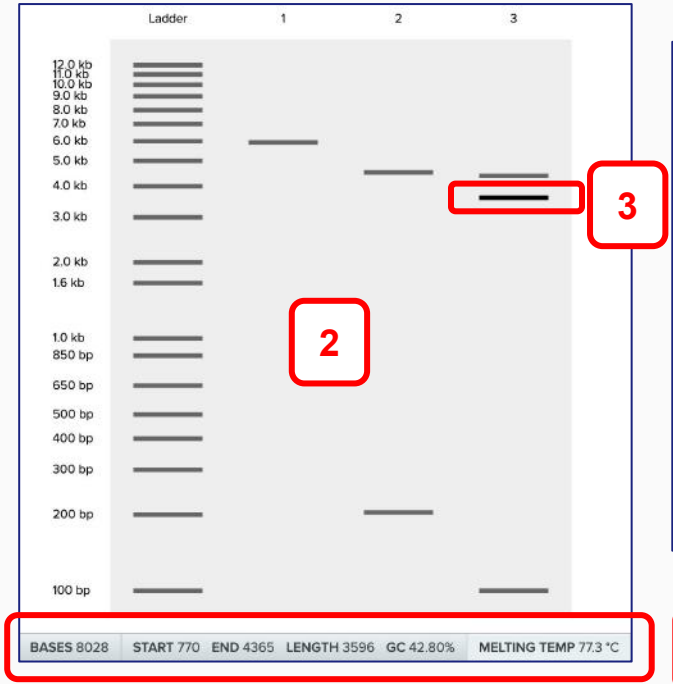
1. Click Digest Scissors (top right)
2. Search for and select Restriction Enzymes
3. **Run Digest**
4. Analyze Digest
5. Click Virtual Digest tab for gel results (next slide)



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

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

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

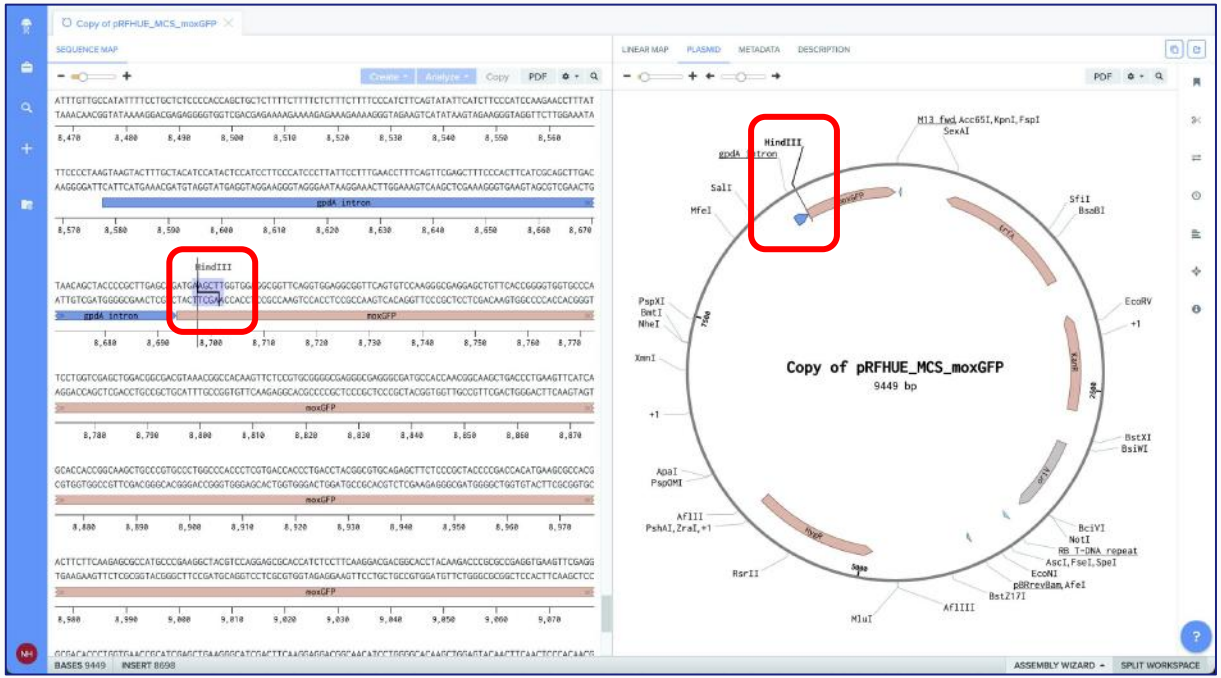
Benchling Cloning Basic Steps:

- 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



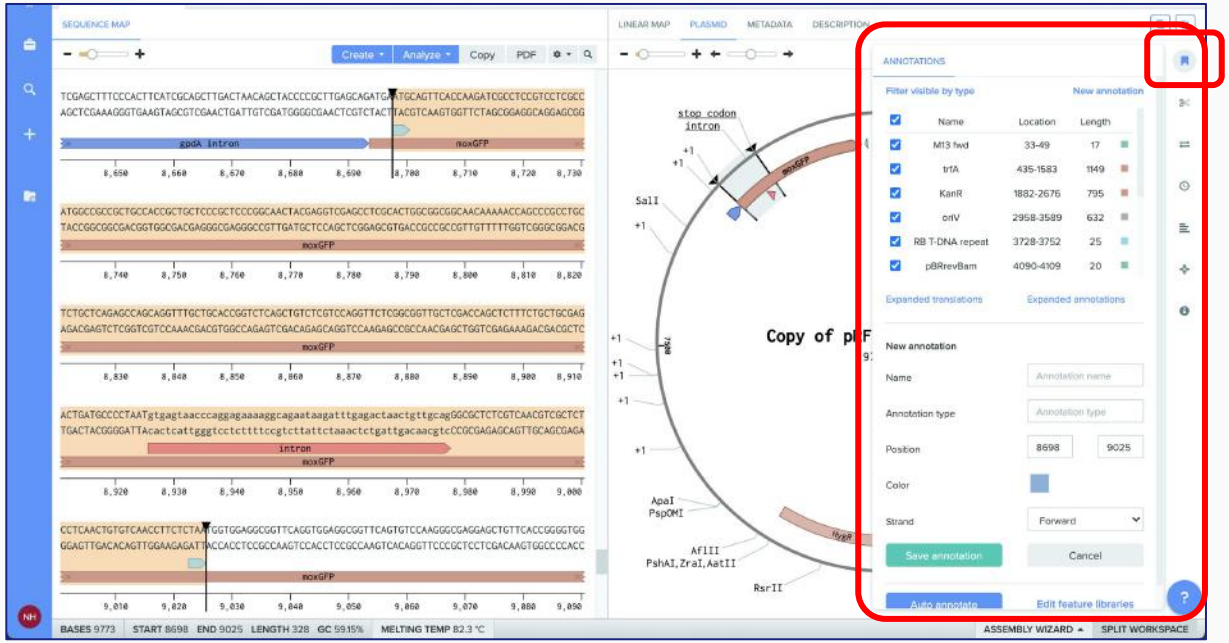
- In this case, the desired location is a cut site for the restriction enzyme HindIII
- The DNA fragment being cloned into the plasmid will replace this cut site

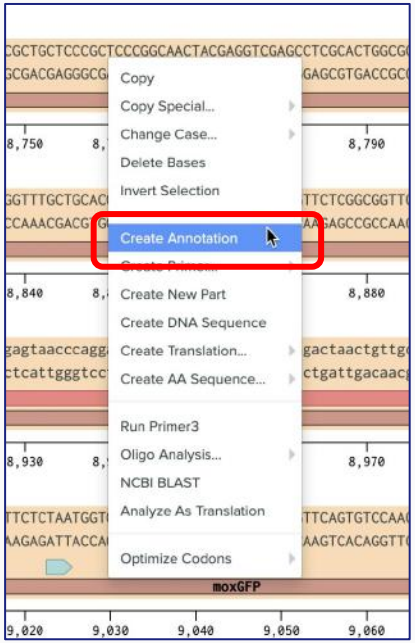
Cloning: Example

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

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”





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