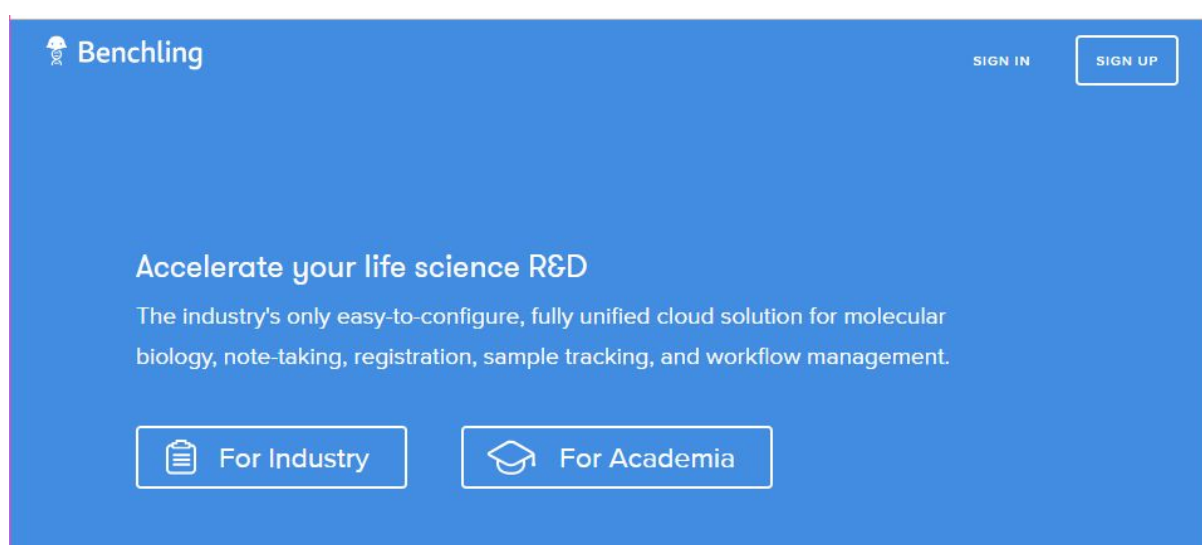


Benchling Guidebook

The Calgary iGEM team utilizes Benchling as a tool to keep all of our DNA constructs and parts organized. Here's how to get started:

1. Setting up Benchling

Benchling is a free online tool for visualizing DNA parts and constructs. There's no need to download anything onto your computer to use it. You should have received an invitation to sign up and join the iGEM Calgary Workspace through your UCalgary email address, but if you did not, just go to the website (<https://benchling.com/>), and click on the "sign up" button.



2. Your Benchling Workspace

When you initially log into Benchling, it will take you to a page that looks like this:

Welcome to Benchling

Benchling is an intelligent research platform with tools for note-taking, molecular biology and CRISPR.

Lab Notebook



A notebook built for researchers.

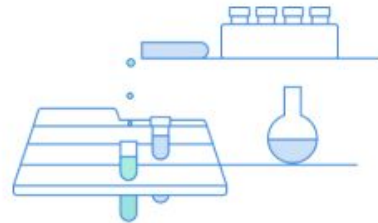
Plan multi-day experiments

Easily reference all data

Get PDF exports, fast

[Take Notes](#)

Molecular Bio & CRISPR



10+ biology tools, one interface.

Design primers, align DNA, and more

Design CRISPR gRNAs in seconds

Import from SnapGene, Geneious, etc

[Start Cloning](#)

Don't worry about which one you click; either way, you'll end up being forced to create a workspace and then you will be taken to the main benchling interface, which will look something like this:

The screenshot shows the Benchling software interface. On the left is a blue sidebar with a navigation menu. At the top of the sidebar is a jellyfish icon, followed by the date 'January 28'. Below this are icons for a folder, a grid, a document, a plus sign, and a search icon. The 'Getting Started' section lists five tasks: 'Create your first item', 'Take a tour of Benchling', 'Create or join an organization', 'Verify your email address', and 'Set a recovery email address'. The 'Recently Opened' section lists five documents: 'Sample Sequence' (2m), 'Gibson Assembly* Protocol (E5510)' (2m), 'Cloning custom sgRNAs into Zhang lab plasmids' (2m), 'Example Entry' (3m), and 'Example Title' (3m). The main workspace on the right has a top bar with tabs for 'Gibson Assembl...', 'Sample Sequence', and 'Example Entry'. Below the tabs are sections for 'ADD PROTOCOL', 'NOTES', and 'METADATA'. A rich text editor toolbar is visible with options for text formatting and alignment. The main content area is titled 'Example Entry' and contains the following text:

TUESDAY, 1/29

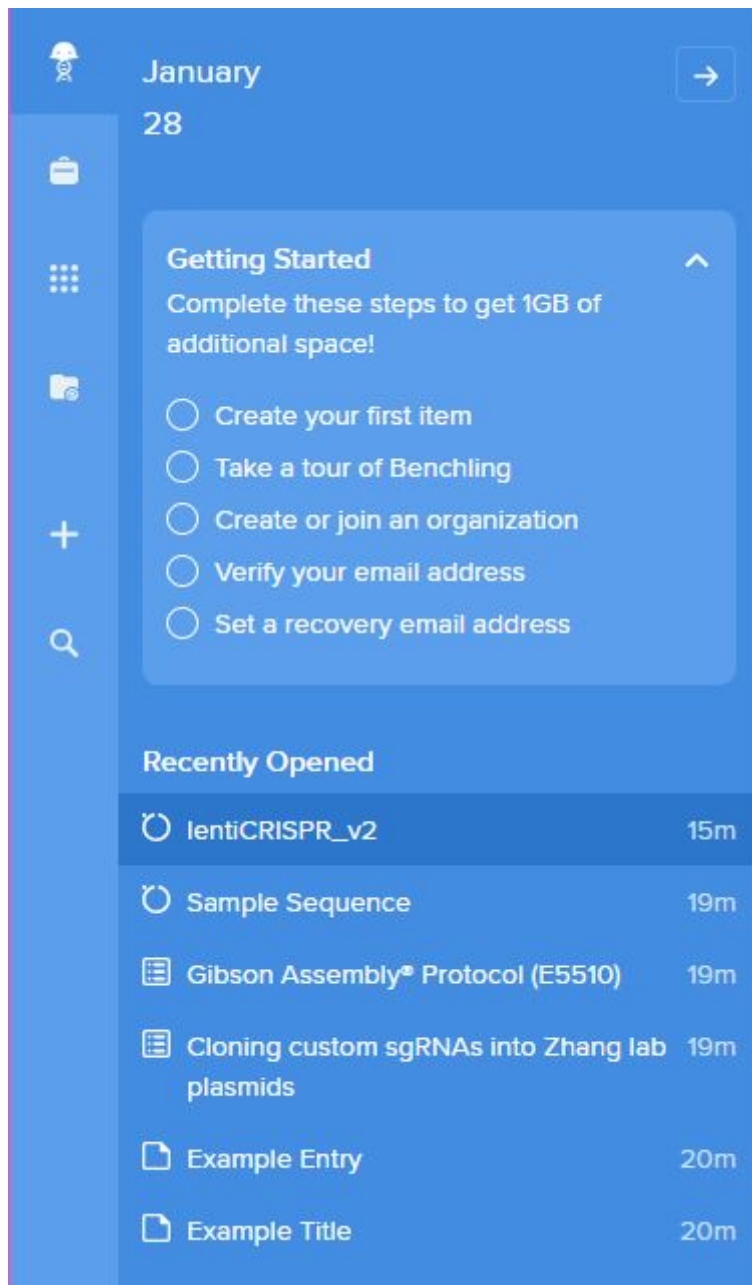
Here's a sample experiment to show you how notebook entries work.

Use **tables with formulas** to make a master mix calculation. **Edit the number of reactions** to see it in action!

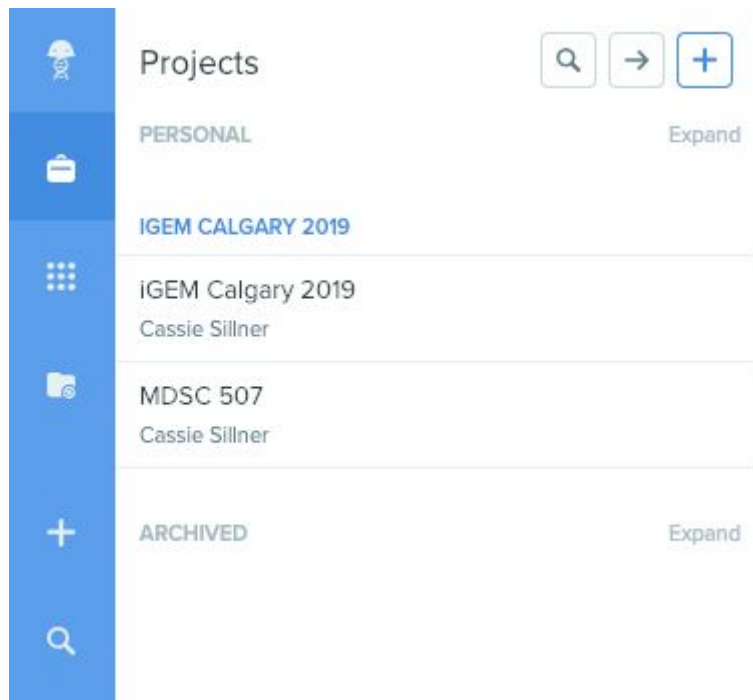
Table1

| | A | B | C |
|---|----------------------------|------------------|-----------------|
| 1 | Number of Reactions | 80 | |
| 2 | Component | Amount (uL/tube) | Master Mix (uL) |
| 3 | Water | 16 | 1280 |
| 4 | 10x Buffer | 2 | 160 |
| 5 | Enzyme | 1 | 80 |
| 6 | Master Mix Total | 19 | 1520 |
| 7 | Template DNA | 1 | |
| 8 | Reaction Total | 20 | |

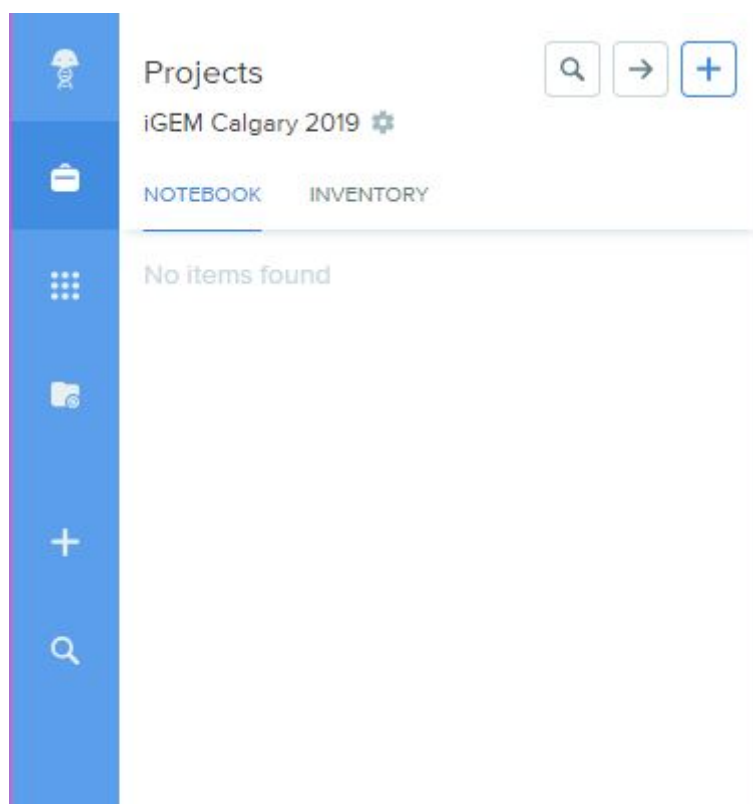
When you click on the jellyfish icon at the top left of your page, it will take you to a list of your recently opened documents.



When you click on the briefcase icon underneath the jellyfish icon, you will be able to access your projects and workspaces. If you click on the word “Project” at the top of the page, you will most likely only see the example project you created when you first logged on to benchling. Worry not! Once you are added to the iGEM workspace, it will show up as one of your projects on this screen.



When you click on the iGEM Calgary 2019 folder, you will be taken to a page that looks like this:

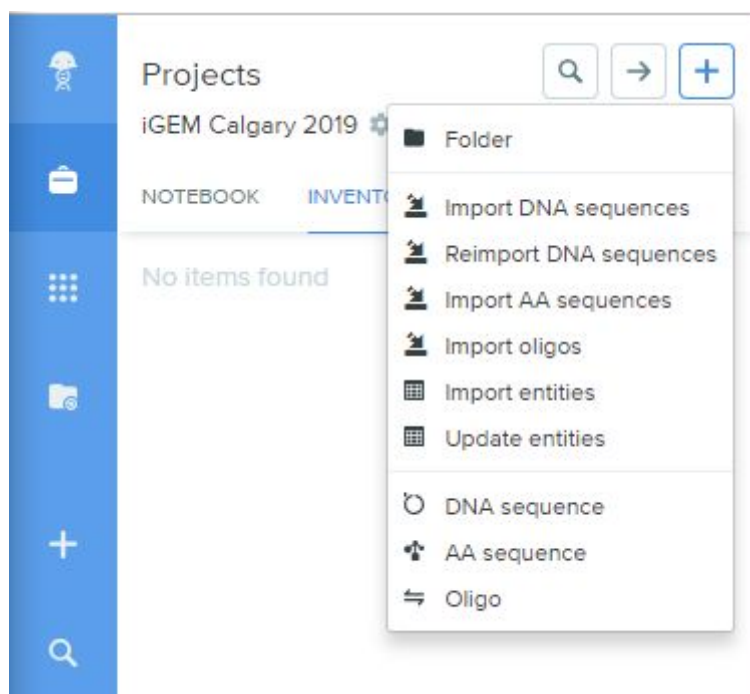


Don't worry about the bioregistry or the external data icons that are also located on the left navigation bar. For the purposes of the Calgary iGEM project, these tools are not necessary, so we won't talk about them here.

3. Creating New Entries

Because we will be keeping amazingly detailed lab notebooks by hand in the blue notebooks, we also will not be using the laboratory notebook feature that benchling offers for our lab notes. Instead, we will heavily be using the DNA construct design feature.

To get started, click on "Inventory", then click on the plus-sign icon at the top of the left-hand window.



Here, you have the option of importing DNA, amino acid (AA), or oligonucleotide sequences from elsewhere, or to manually input these sequences onto Benchling. Often times, we use DNA sequences (such as plasmid backbone sequences, pre-made AddGene plasmids, etc) that are already designed and constructed by others. In this case, it is easiest to import these sequences rather than to type each letter in yourself.

When you click "Import DNA sequences," this pop-up window will appear:

The screenshot shows a web interface with four tabs at the top: 'Convert Files', 'Search External Databases', 'Select Chromosomal Region', and 'Input Raw Sequence'. The 'Search External Databases' tab is active. Below the tabs, a text instruction reads: 'Upload any DNA file (Genbank, FASTA, ApE, Geneious, SnapGene, SeqBuilder, etc.) to make a Benchling sequence. Drag in multiple at once!'. Below this is a light blue box containing the text 'Add sequences to' followed by a dropdown menu showing 'IGEM CALGARY 2019'. Below that is a dashed rectangular box containing a cloud upload icon, the text 'Drag and drop files to upload', and a green button labeled 'OR CHOOSE A FILE'. In the bottom right corner of the interface is a 'CLOSE' button.

You have the option of uploading a file that contains a DNA sequence (available for download from other bioinformatics tools such as GenBank, FASTA, etc.), finding your sequence online via keyword or item number search, or selecting your chromosomal region of choice. The benefit of importing your sequence from external databases (rather than pasting in the raw sequence) is that any annotations that already exist within these databases will automatically be imported with the sequence.

If you're lazy like me, the search function is usually the easiest to use. For example, I searched for iGEM's standardized plasmid backbone, pSB1C3

Convert Files

Search External Databases


Select Chromosomal Region

Input Raw Sequence

Example searches:

- www.addgene.org/browse/sequence/59271/ (Addgene URL)
- BRCA2 (Gene Name)
- M62653 (NCBI Accession)
- ENSMUSG00000041147 (ENSEMBL ID)
- BBa_E0040 (Registry of Standard Biological Parts)
- JPUB_001431 (JBEI Public Registry)

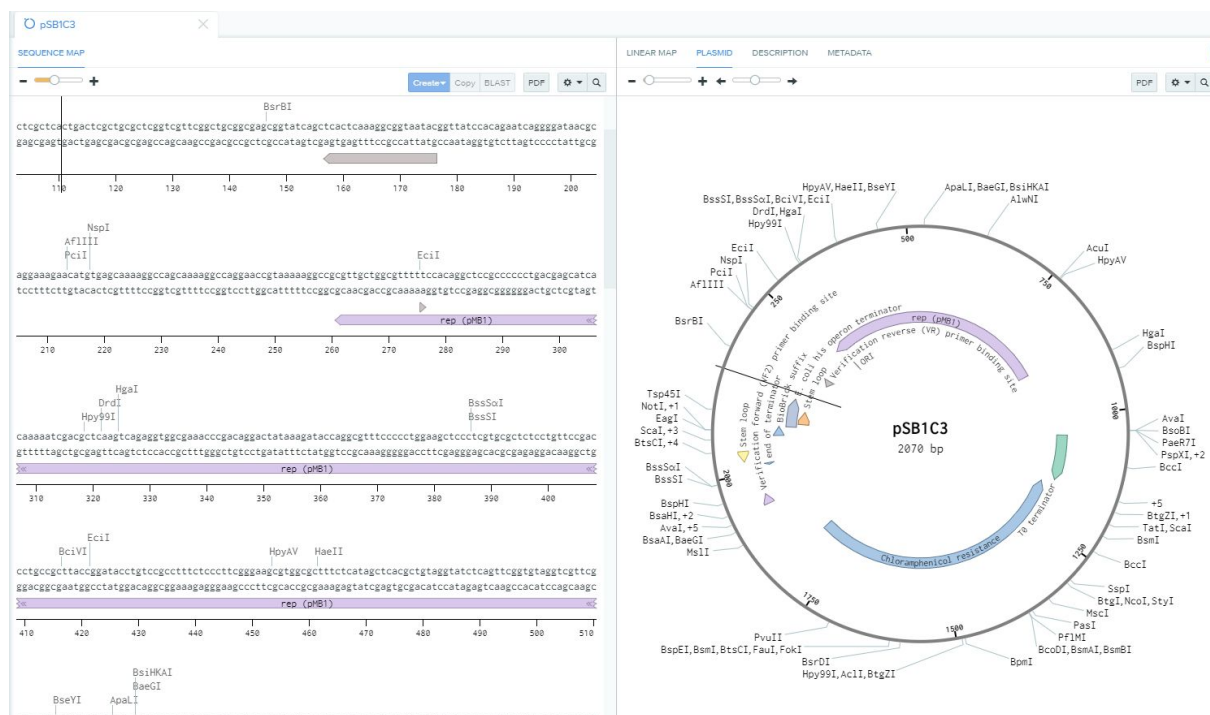
Import multiple sequences at once by entering space-separated or comma-separated accession numbers.



| | | | |
|-------------|---------------------------------------|-----------|--|
| Entry | pSB1C3 | Import As | <input type="text" value="pSB1C3"/> |
| Database | Registry of Standard Biological Parts | Save To | <input type="button" value="IGEM CALGARY 2019"/> <input type="button" value="IMPORT"/> |
| Length | 2070 | | |
| Description | High copy BioBrick assembly plasmid | | |

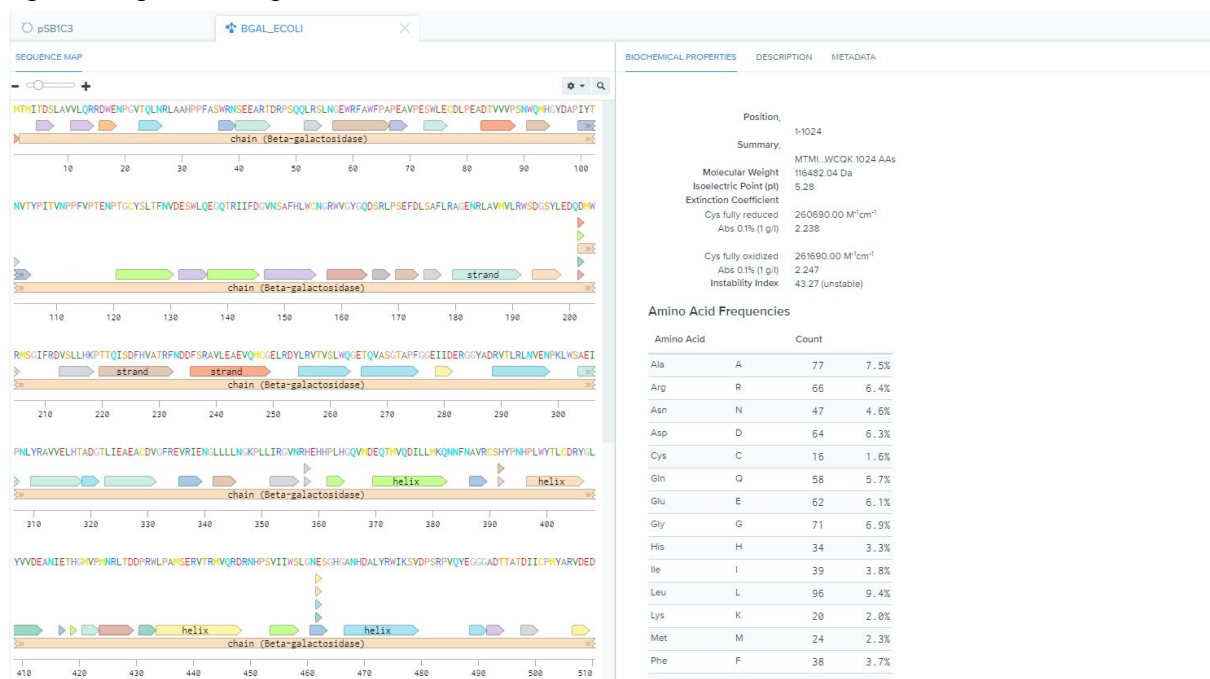
Once you have found your sequence of interest, click “Import” and your DNA sequence will be successfully uploaded onto your benchling workspace.

Once successfully uploaded, your DNA sequence should look like this:



Importing protein sequences or oligonucleotide sequences also works in the same way as DNA sequences - you have the option of uploading a file containing sequence information, or you can search online databases for sequence information.

Uploaded protein sequences look like this:



Uploaded oligonucleotide sequences look like this:

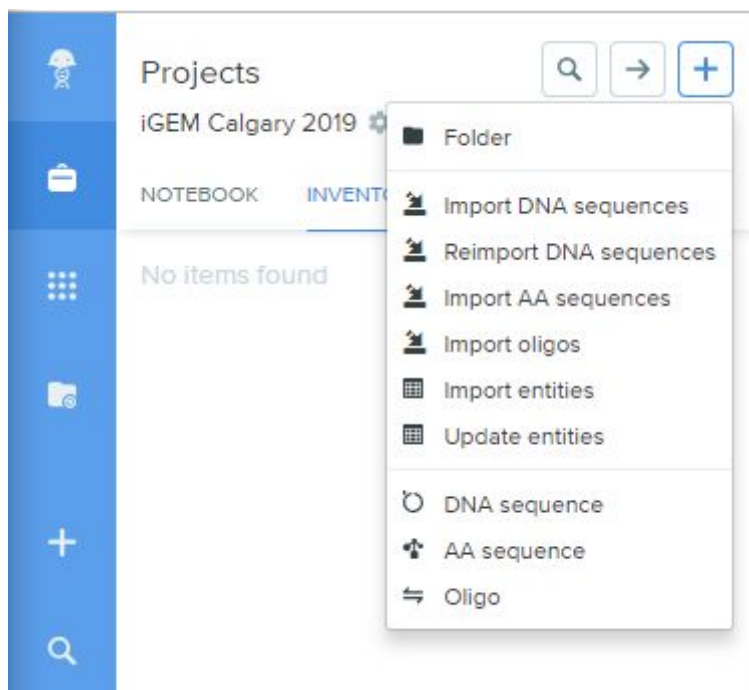
If you find the viewer too crowded to work with, there are two handy buttons that can simplify things.

The 'Split Workspace' button indicated by the blue arrow reverses the screen splitting so that you are only looking at one representation of the sequence at a time. The button indicated by the pink arrow collapses the navigation sidebar on the left so that you have more room to work.



You can change between representations of the sequence using the toolbar at the top, which includes 'Sequence Map', 'Linear Map', 'Plasmid', 'Description', and 'Metadata'.

If you are designing a part mostly from scratch, click on one of the bottom three options available from the drop-down menu that comes from the plus sign.



Indicate whether you want your DNA to be circular (like a plasmid) or linear (like a eukaryotic chromosome)

Convert Files

Search External Databases

Select Chromosomal Region

Input Raw Sequence

Name

Untitled Sequence

Folder

IGEM CALGARY 2019

Topology

Circular

Circular

Linear

CREATE

CLOSE

It will take you to a page like this, where you can manually type in your sequences.

Untitled Sequence

pSB1C3

BGAL_ECOLI

Sample Primer 1

SEQUENCE MAP

Untitled Sequence

+

+

Create

Copy

BLAST

PDF

LINEAR MAP

PLASMID

DESCRIPTION

METADATA

Type or paste bases here

+

+

+

+

+

+

Untitled Sequence

0 bp

The 'Description' tab should be used to keep track of the source (journal article, website, etc.) of the sequence and any other important features for all non-registry sequences.

SEQUENCE MAP

DESIGN PRIMER

LINEAR MAP

DESCRIPTION

METADATA

Insert

H

B

I

U

A

x₂

x²

More

Enter text here

4. Annotating your Entries

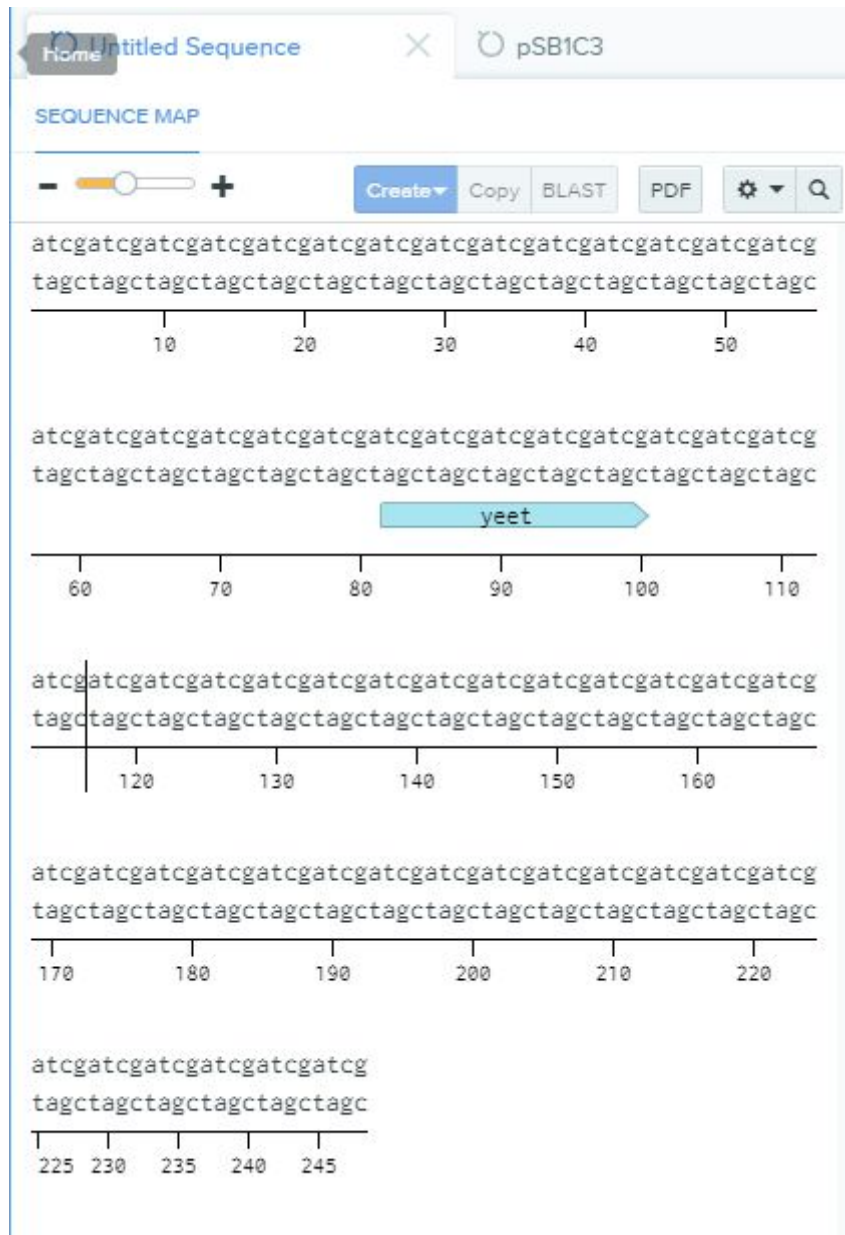
Once you have your sequences uploaded onto Benchling, you can annotate specific parts of a sequence to indicate their name or function. This is especially important for sequences you made manually; make sure to annotate them well so that anybody else could understand why your sequence is built the way it is.

In order to annotate your sequence, highlight the sequence you want to annotate and click on the annotation icon on the right sidebar.

[illegible]

You can type in the name of your annotation as well as indicate its color, type, and determine the directionality of your annotation. Click Save Annotation to add it to your sequence.

An annotated sequence looks like this:



This feature works on DNA sequences, amino acid sequences, and oligonucleotide sequences.

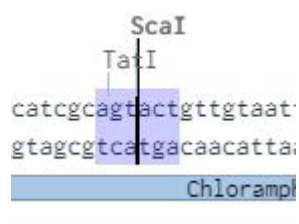
5. Digesting Sequences

If you want to check if your sequence contains unwanted restriction sites, or if you want to know how long your sequence will be after a restriction digest, you can use the Digests tool to run mock digests of your DNA or oligonucleotide sequences.

If your sequence contains restriction enzyme sequences, they will already be annotated directly onto your sequence. You can hover over them to determine whether or not the restriction enzyme has a blunt-end or sticky-end cut.



Sticky end cut



Blunt end cut

You can also click on the scissors icon on the right hand menu to access the restriction enzyme menu.

NEW DIGEST

SAVED DIGESTS

Enzyme Lists

Manage Enzyme Lists

Deduplicated Commercial

Cut Sites Visible on Maps

Single and Double Cutters

Find Enzyme

Clear Selected

Search by name or number of cuts

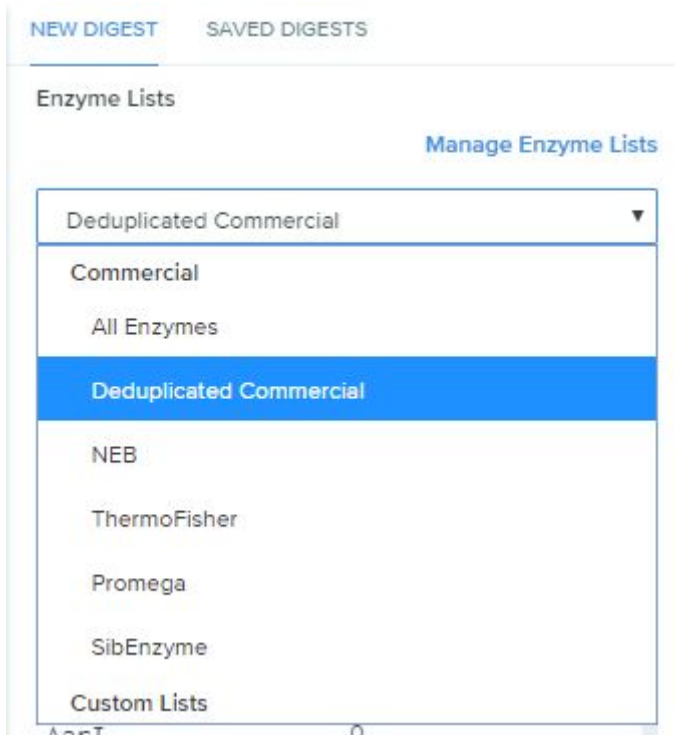
| Name | Cuts |
|----------|---------|
| AarI | 0 |
| AatII | 1 |
| Acc65I | 0 |
| AccI | 0 |
| AciI | 19 |
| Selected | Color ? |

Show enzymes that cut

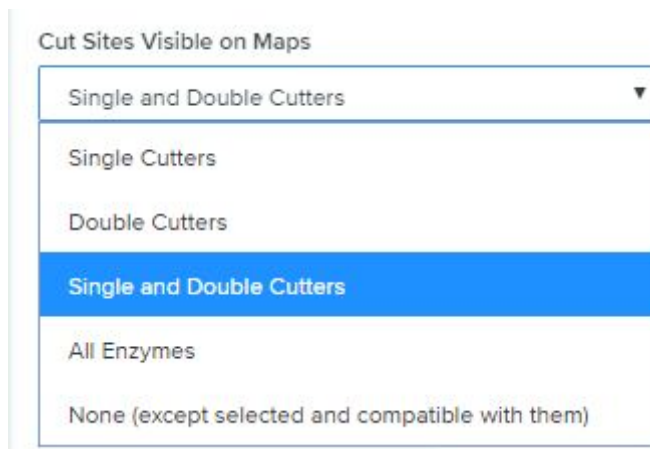
anywhere in the sequence

☐ Highlight enzymes with compatible sticky ends

The Enzyme Lists menu refers to databases of functional restriction enzymes. You can choose to only look at restriction enzymes manufactured by certain manufacturers, such as NEB or ThermoFisher. This may be useful to us because we cannot perform experiments with restriction enzymes that aren't commercially available to us.



You can also change which enzymes are visible on your sequence map.



You can also search for specific restriction enzymes. Benchling will tell you how many times this restriction enzyme will cut on your part. This is useful because when you are digest-confirming a plasmid, you don't want your part to be broken apart into many tiny fragments; instead, you want to find an enzyme that cuts twice on your plasmid so it will form two large linear pieces of DNA.

Find Enzyme Clear Selected

Spei

| Name | Cuts |
|-------|------|
| BspEI | 1 |
| FspEI | 231 |
| SpeI | 1 |

Selected
Color ?

If you click on a restriction enzyme under the “Find Enzyme” menu, you can select that enzyme to be used in a mock digest of your part. Once you have selected your restriction enzymes of interest, click “run digest” at the bottom of the page.

Selected
Color ?

| | |
|------|--|
| SpeI | ■ |
| XbaI | ■ |

Show enzymes that cut

anywhere in the sequence
▼

☐ Highlight enzymes with compatible sticky ends

RUN DIGEST

This will bring you to the Digests page, which shows you the temperature and buffers at which your enzymes are active. It also shows you the length of fragments you will have upon cutting your DNA.

[LINEAR MAP](#)
[PLASMID](#)
[DIGEST X](#)
[VIRTUAL DIGEST](#)
...

Digest
 NEB ▼ ☐ Use HF ?

| Enzymes | Cuts | Temp. | 1.1 | 2.1 | 3.1 | 4/CS |
|---------|------|-------|-----|-----|-----|------|
| SpeI | 1 | 37°C | 75 | 100 | 25 | 100 |
| XbaI | 1 | 37°C | 10 | 100 | 75 | 100 |

| Start | End | Length | Left Cutter | Left Overhang | Right Cutter | Right Overhang |
|-------|------|--------|-------------|---------------|--------------|----------------|
| 3 | 2064 | 2062 | SpeI | 5' | XbaI | 5' |
| 2065 | 2 | 8 | XbaI | 5' | SpeI | 5' |

6. Primer Design

If you want to create primers for PCR and/or sequencing, the first step is to identify the sequence that you want to amplify. Determine your primer parameters as per the following rules:

- Primers should be 18-24 nucleotides in length (excluding any overhangs) with a primer pair not differing by >3 nucleotides
- T_m values of primer pairs should not differ by >5°C
- The GC content should be between 40-60% with no long runs of bases anywhere in the sequence
- There must be a G or C at the 3' end of each primer
- Primers must not form secondary structure (hairpins) or primer-dimers, especially at the 3' end

Primers can be generated using the Primer Wizard tool or manually. Either way, be sure to analyze these sequences using [IDT's OligoAnalyzer 3.1 tool](#) (see OligoAnalyzer Guide) so that your designed primers will be effective. The primer analysis tools within Benchling are adequate, but it doesn't hurt to double check and get an idea of the secondary structures that may be forming.

If using the **Wizard tool**, click on the primer icon in the right-hand sidebar. Click on 'Create Primers' and select 'Wizard'.

PRIMERS PAIRS

| Name | Position | T _m |
|----------------------------------|----------|----------------|
| No primers attached to sequence. | | |

Manual
Wizard
 CREATE PRIMERS ▲

LINK PRIMERS
 ATTACH EXISTING

This will open up a new tab called the 'Primer Wizard', which looks like this:

SEQUENCE MAP **PRIMER WIZARD** × LINEAR MAP DESCRIPTION METADATA

Generate Primers

Task: PCR Tm Params Reset

Region

Target: Start: End: USE SELECTION Show Advanced

Primer

| | Min | Opt | Max |
|----------------|-----|-----|-----|
| GC% | 40 | 50 | 60 |
| T _m | 55 | 62 | 65 |
| Size | 18 | 22 | 24 |
| 3' GC Clamp | 1 | | |

Amplicon

Result Generation

Results: 10

Fill in all parameters as desired (or leave as default). When complete, click on 'Generate Primers' in the top right hand corner. It will open another tab called 'Primer3 Results', which looks this:

| SEQUENCE MAP PRIMER WIZARD × PRIMER3 RESULTS × LINEAR MAP DESCRIPTION METADATA | | | | | | | | |
|--|---------|-----------|-------|-------------------|----------|--------|------------|-------------------------------|
| Save Selected Primers T_m params Sort by Penalty | | | | | | | | |
| <input type="checkbox"/> | Penalty | Direction | % GC | T _m °C | Location | Length | Product BP | Primer |
| <input type="checkbox"/> | 0.100 | FWD | 59.1% | 58.1° | 43-64 | 22 | 767 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 58.1° | 788-809 | 22 | | 5' ACCCATCCACTTCGAGACAAGG 3' |
| <input type="checkbox"/> | 0.367 | FWD | 59.1% | 58.1° | 43-64 | 22 | 723 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 57.8° | 744-765 | 22 | | 5' AGGTAGAGTCAGCTTCAGTGGC 3' |
| <input type="checkbox"/> | 0.890 | FWD | 54.5% | 57.3° | 50-71 | 22 | 760 | 5' CCGATCTTCGTCTCCAATTCGG 3' |
| | | REV | 54.5% | 58.1° | 788-809 | 22 | | 5' ACCCATCCACTTCGAGACAAGG 3' |
| <input type="checkbox"/> | 0.920 | FWD | 59.1% | 58.1° | 43-64 | 22 | 879 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 57.4° | 900-921 | 22 | | 5' CGTTTCCTTCTCCACGTGTACC 3' |
| <input type="checkbox"/> | 0.968 | FWD | 59.1% | 58.1° | 43-64 | 22 | 827 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 59.0° | 848-869 | 22 | | 5' AATCGAGGGAGCACCTTCACGG 3' |
| <input type="checkbox"/> | 1.156 | FWD | 54.5% | 57.3° | 50-71 | 22 | 716 | 5' CCGATCTTCGTCTCCAATTCGG 3' |
| | | REV | 54.5% | 57.8° | 744-765 | 22 | | 5' AGGTAGAGTCAGCTTCAGTGGC 3' |
| <input type="checkbox"/> | 1.167 | FWD | 56.5% | 58.2° | 37-59 | 23 | 773 | 5' CCATCTCCTTCTCCGATCTTCG 3' |
| | | REV | 54.5% | 58.1° | 788-809 | 22 | | 5' ACCCATCCACTTCGAGACAAGG 3' |
| <input type="checkbox"/> | 1.175 | FWD | 59.1% | 58.1° | 43-64 | 22 | 885 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 57.2° | 906-927 | 22 | | 5' CACTCTCGTTTCCTTCTCCACG 3' |
| <input type="checkbox"/> | 1.234 | FWD | 59.1% | 58.1° | 43-64 | 22 | 812 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 59.3° | 833-854 | 22 | | 5' TTCACGGGCCATCTCGAAAAGG 3' |
| <input type="checkbox"/> | 1.254 | FWD | 59.1% | 58.1° | 43-64 | 22 | 886 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 52.2% | 58.2° | 906-928 | 23 | | 5' TCACTCTCGTTTCCTTCTCCACG 3' |

You can select your primer pair from this list as per your needs. Keep in mind, the lower the Primer3 Penalty score (column on left-most side), the less secondary structure and primer dimers are formed and the better the primer pair. If you have found a pair of primers that you wish to use, check off the box on the left hand side, then select 'Save Selected Primers' in the top left hand corner.

| Save Selected Primers T_m params Sort by Penalty | | | | | | | | |
|---|---------|-----------|-------|-------------------|----------|--------|------------|------------------------------|
| <input type="checkbox"/> | Penalty | Direction | % GC | T _m °C | Location | Length | Product BP | Primer |
| <input checked="" type="checkbox"/> | 0.100 | FWD | 59.1% | 58.1° | 43-64 | 22 | 767 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |
| | | REV | 54.5% | 58.1° | 788-809 | 22 | | 5' ACCCATCCACTTCGAGACAAGG 3' |
| <input type="checkbox"/> | 0.367 | FWD | 59.1% | 58.1° | 43-64 | 22 | 723 | 5' CCTTCCTCCGATCTTCGTCTCC 3' |

A popup window will allow you to choose the folder in which to save them, but they will be saved initially with generic names. You can edit these names by right-clicking on the primer annotations and selecting 'Edit Primer', or by renaming them in the left-hand navigation sidebar. Primer annotations appear like this:



The primer pair will already be linked, and further analysis can be performed on the sequence as described below.

If creating the primers **manually**, highlight the sequence where your forward primer will bind. Right click and select ‘Create Primer’ > ‘Forward’ from the menu. This will open a new window (as below).

For now, check that the drop down menu in the top left corner says ‘Single Primer.’

The following step pertains only to overhang PCR primers:

If designing an overhang PCR primer, you must now type out the sequence of the overhang at the 5’ end of your primer sequence in the ‘Bases’ field, and indicate how many nucleotides are overhanging. You can use the ‘Cut Site’ tool to look up the sequence of a restriction enzyme recognition site to be included in the overhang sequence.

Design

Strand ☒ Forward ☐ Reverse

Bases 5' ataGACGTCtttctctctctc

3' Location

Overhang

Cut Site

Use the dropdown above to look up restriction sites.

Fill in the name of the primer at the bottom under the header 'Save' and be sure that you are saving to the correct folder. You can also change the annotation colour by clicking on the coloured square box.

Save

Name

Save To

Click 'Save Primer' when you're finished. The 'Primer Design' window will close.

Highlight the sequence where your reverse primer will bind. Right click and select 'Create Primer' > 'Reverse' from the menu. This will open the primer design window again. Repeat all of the same steps as for the forward primer, but don't save yet. In the top left hand corner, select 'Primer Pair'. The window will change slightly.

SEQUENCE MAP DESIGN PRIMER

Single Primer

✓ Primer Pair

Design

| Strand | Forward | Reverse |
|-------------------------------|-----------------------------------|--|
| Bases | 5' <input type="text"/> 3' | 5' <input type="text" value="cttgatgggaatg"/> 3' |
| Primer must be at least 6 bp. | | |
| 3' Location | <input type="text" value="1"/> | <input type="text" value="1503"/> |
| Overhang | <input type="text" value="0 bp"/> | <input type="text" value="0 bp"/> |
| Cut Site | <input type="text" value="AANI"/> | <input type="text"/> |

Use the dropdown above to look up restriction sites.

Paste the sequence of the forward primer that you have already designed in the 'Bases' box for the forward strand. It will suggest to you the primer that you have already designed. Click 'Use'. You will have to manually add the 3' location of the forward primer so that it becomes highlighted in green as the reverse primer sequence is below. You can also add a 5' overhang to the reverse primer at this point if desired in the same manner as previously described.

Design

| Strand | Forward | Reverse |
|---|---|--|
| Bases | 5' <input type="text" value="ttcttctctctctc"/> 3' | 5' <input type="text" value="cttgatgggaatg"/> 3' |
| Saved primers exist with the same bases: | | |
| Forward Primer for Rad Unknown Sequence | | <input type="button" value="USE"/> |
| 3' Location | <input type="text" value="1"/> | <input type="text" value="1503"/> |
| Overhang | <input type="text" value="0"/> | <input type="text" value="0"/> |
| <p>Ignore this warning for now</p> <p>Primer bases lying off of the sequence cannot be more than the overhang. Using overhang size of 14 instead.</p> | | |
| Cut Site | <input type="text" value="AANI"/> | <input type="text"/> |

Use the dropdown above to look up restriction sites.

Add an appropriate name for the reverse primer, choose the correct folder, and click on 'Save Primer Pair'.

Save

| | | |
|---|--|--|
| Name | <input type="text" value="Forward Primer for Rad Unknown Sequence"/> | <input type="text" value="Reverse Primer for Rad Unknown Sequence"/> |
| Save To | <input type="text" value="MDSC 507"/> | <input type="text" value="MDSC 507"/> |
| <input type="button" value="SAVE PRIMER PAIR"/> | | |

Following these steps, your primers should appear as a linked pair that can be used for further analysis. In the right-hand sidebar, select the primer icon. From this menu, you can further

analyze your primer pair, or create a sequence of the PCR product that would be generated using such a primer pair.

PRIMERS PAIRS

| Primer | Position | Product Size |
|---|----------|--------------|
| Forward Primer for Rad Unknown Sequence | + / 37 | 1496 |
| Reverse Primer for Rad Unknown Sequence | - / 1506 | |

Primer Pair Information [Edit · Unlink](#)

LINK PRIMERS

| | Name | T _m |
|----------------|---|----------------|
| Forward Primer | Forward Primer for Rad Unknown Sequence | 38.0°C |
| Reverse Primer | Reverse Primer for Rad Unknown Sequence | 38.9°C |

Product Size 1496 bp
T_m Difference +0.9° C

CREATE PCR PRODUCT SECONDARY STRUCTURE

9. General tips and tl;dr

Make sure that every single DNA sequence used in our project this summer makes it onto benchling. This is a great way for us to be able to cross-references our different DNA parts to make sure they are compatible with one another.

Practice makes perfect when it comes to using this tool. There are so many features in the software that even a guide document as long as this cannot cover them all. Feel free to experiment and learn about as many features as you can!

We should aim to keep the shared workspace well-organized so that sequences are easy to find.

Benchling saves in real-time as long as you are connected to the internet, so you do not have to worry about losing sequences.

Feel free to DM us (Sara, Cassie, or Chris) if you have any questions :)