Gene Designer by DNA 2.0 Tutorial

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Table of Contents

Getting Started

-Go to https://www.dna20.com

-Under the tab **Scientific Resources**, select **Gene Designer**

-Click on the **Download Now** icon

-Create an Account

-Click on the **Install Now** icon

-An activation key will be sent to your email address.

-Once you have it installed and activated, open Gene Designer

Library Explorer

The Library Explorer should automatically pop up when you open Gene Designer. It is a floating icon.
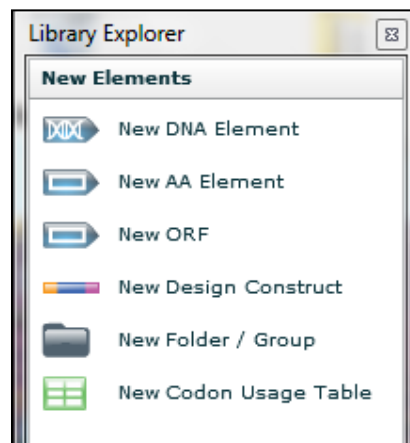
New Elements

Under the New Elements tab you have the options to add:

- New DNA Element
- New AA Element
- New ORF
- New Design Construct
- New Folder/Group
- New Codon Usage Table

Add New DNA Element:

1. Click and drag icon from Library Explorer onto desired window pane
2. Name your new DNA Element
3. Pick a color for your DNA
4. Input the DNA sequence (GATC)
5. Select Orientation
6. Add any notes
7. Click OK

Add New AA Element:

1. Click and drag icon from Library Explorer onto desired window pane
2. Name your new Amino Acid Element
3. Pick a color for your AA sequence
4. Input the AA sequence (GAVLIPMFYWSTCNQKHRDE)
5. Select Orientation
6. Add any notes
7. Click OK

Add New ORF Element:

1. Click and drag icon from Library Explorer onto desired window pane
2. Name your new Open Reading Frame Element
3. Pick a color for your ORF
4. Input the DNA sequence (GATC)
5. Select which frame to start reading
6. The corresponding AA sequence will appear
7. Select whether or not to keep the original DNA sequence
8. Select Orientation
9. Add any notes
10. Click OK

Add New Construct:

1. Click and drag icon from Library Explorer onto desired window pane
2. Name your new construct
3. Choose if you want it to be circular or linear
4. Add any notes
5. Click OK
6. Your new construct will appear in a new window pane.

Add New Folder/Group:

1. Click and drag icon from Library Explorer onto desired window pane
2. Name your new folder
3. Choose a color
4. Click OK
5. Your new folder will appear under the construct you placed it in.

Design Toolbox

The design toolbox has built-in DNA elements available for your projects.

Prokaryotic Regulatory Elements

>Transcription

>Promoters
>Operators
>Terminators

>Translation

>RBS

>Replication

Eukaryotic Regulatory Elements

>Transcription

>Enhancers
>Promoters
>Terminators
>Polyadenylation signal sequence

>Translation

>5'UTR
>Initiation Context
>IRES

>Replication
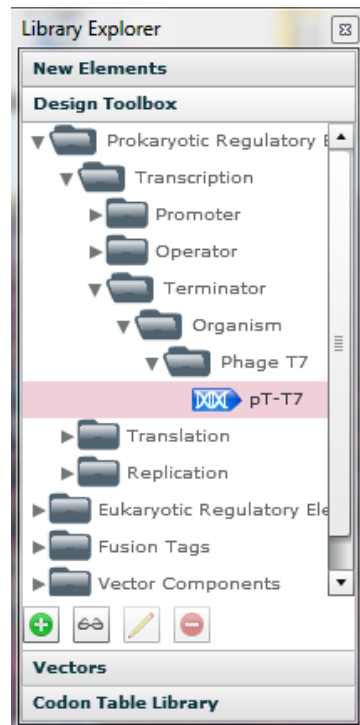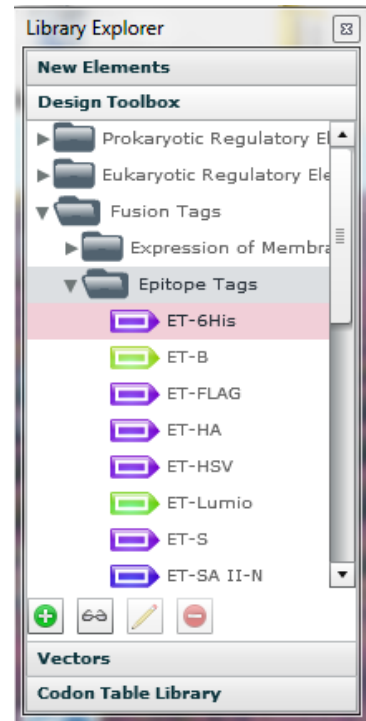
Fusion Tags

> Expression of Membrane Proteins (N-terminal Fusion Tags)
> Epitope Tags
> Solubility and Purification Tags
> Protease Cleavage Sites
> Secretion Signals
> Imaging TAGS

Vector Components

> Regulatory Proteins
> Antibiotic Resistance Markers
> Counter-Selectable Markers

Vectors

Under the Vectors tab in the Library Explorer, built-in vectors are available for your use.

pT7-SNAP

pT7-SNAP is an *E. coli* expression plasmid encoding SNAP26b, a SNAP-tag protein, which can be expressed under the T7 promoter. This plasmid is intended to be used for the expression of SNAP-tag protein fusions for tighter control of protein expression. It can also be used as a control plasmid expressing the SNAP-tag alone. The fusion partner should be cloned as a fusion to the C-Terminus of the SNAP-tag.

pMCPm™

pMCPm™ is a mammalian expression plasmid encoding MCP, an MCP-tag polypeptide, which is expressed under the CMV promoter. This plasmid is intended for the cloning and expression of MCP-tag protein fusions in mammalian cells. pMCPm contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the C-Terminus of the MCP-tag and an appropriate signal peptide to the N-Terminus of the MCP-tag.
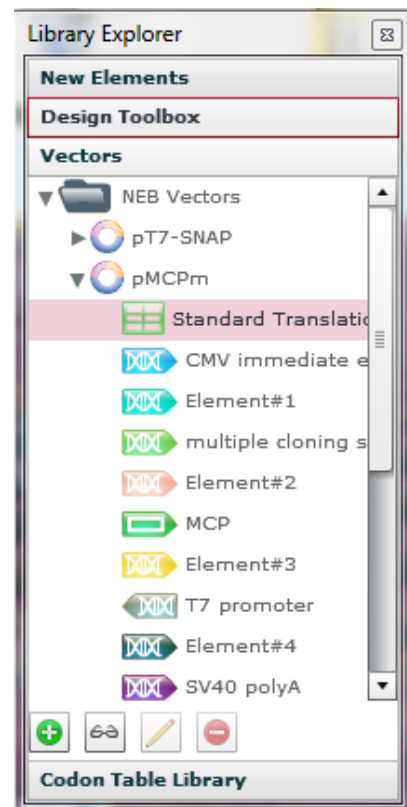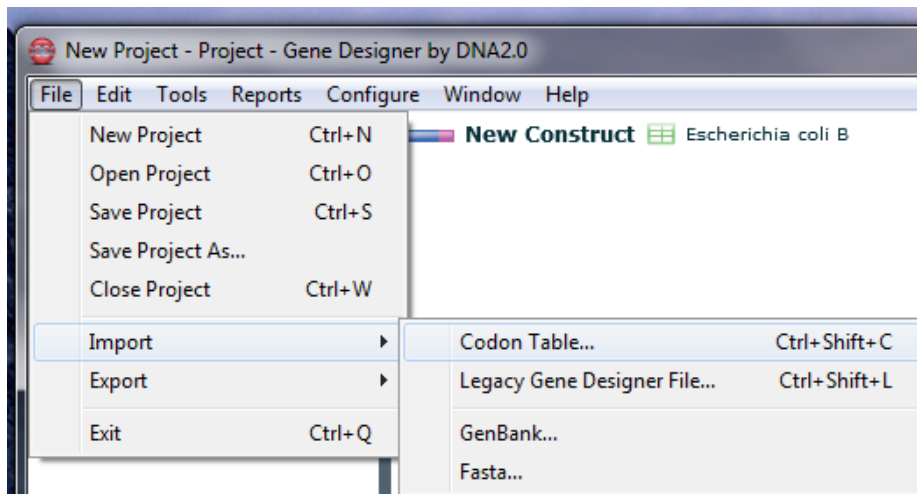
Codon Table Library

The Codon Table Library shows you the organisms that have a codon table in Gene Designer. Gene Designer comes installed with *Escherichia coli B,* and *E. coli_*CII. Any other desired organism codon table must be imported to Gene Designer.
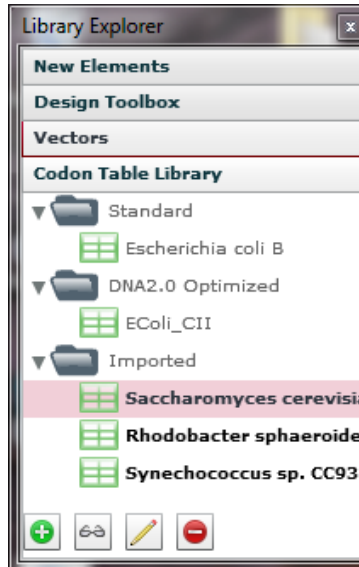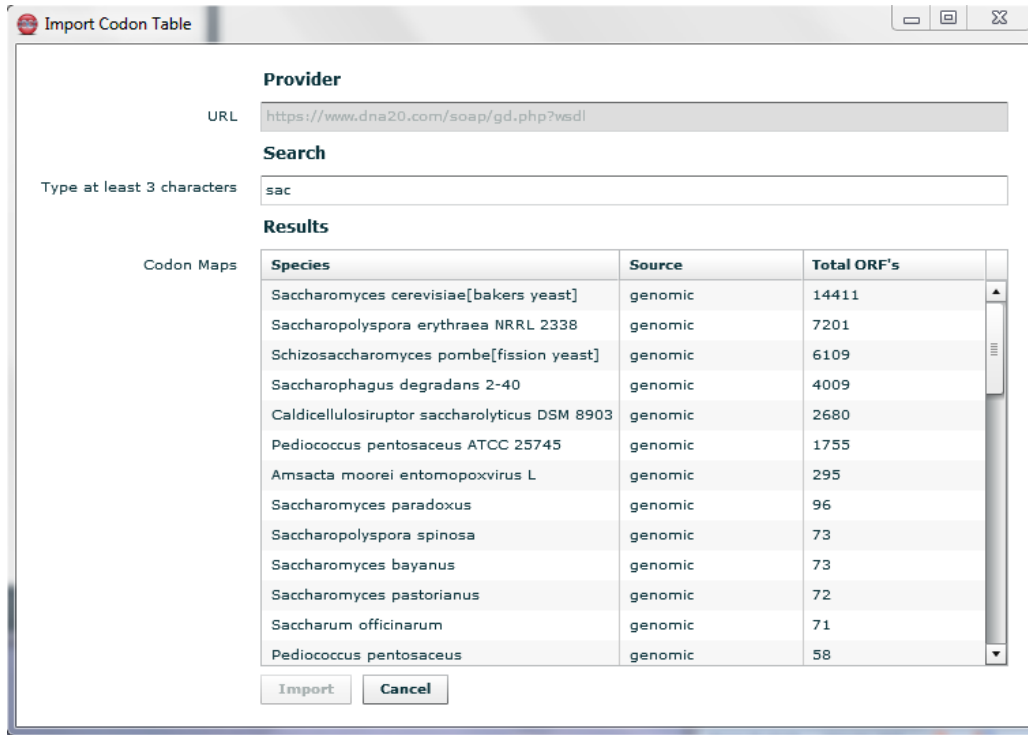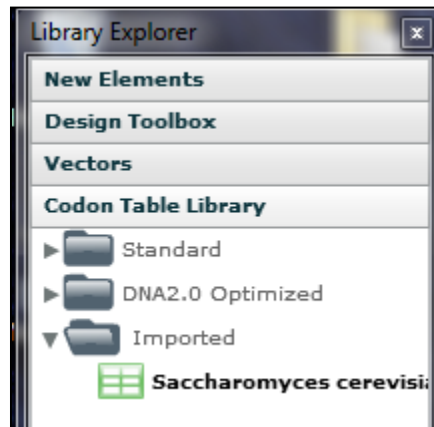

Importing a New Codon Table

Go to:

>File > Import > Codon Table

In the Search box of the new window, start typing the name of the desired organism. Possible species will appear in the Results table.


Select desired species and click **Import.** This will put the new codon information under the imported section of the codon library.

Codon Optimization

One of the most useful features of Gene Designer is the ability to optimize the codons in a protein for a specific organism.

With the desired protein in the main window, you can optimize the codons by clicking the organism button located on the bottom right.
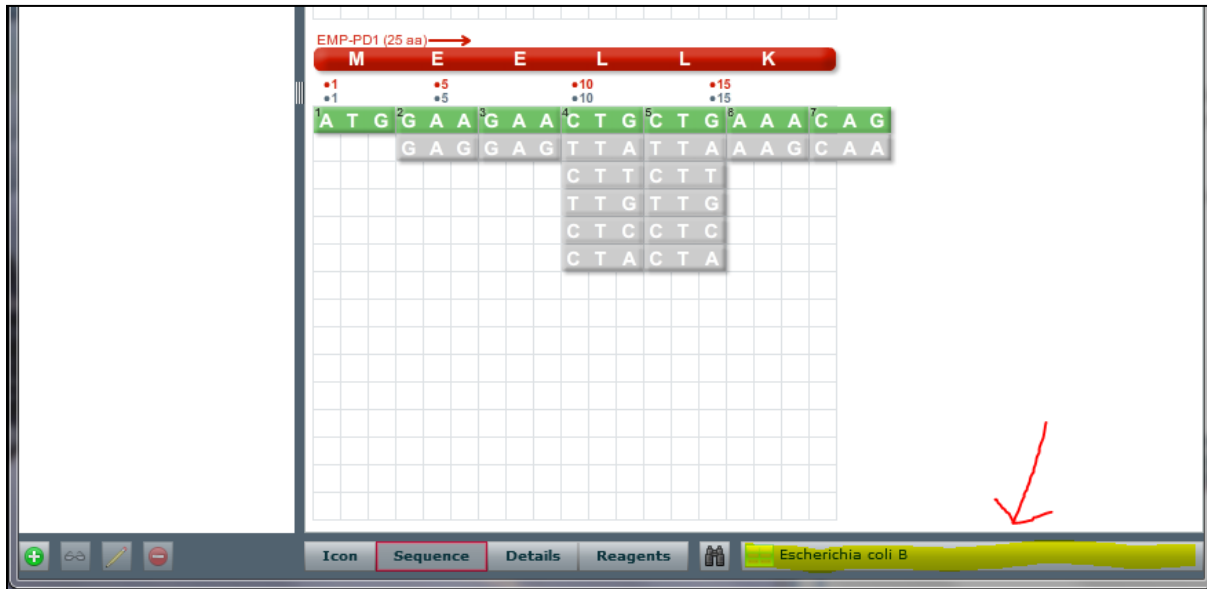
The window below will appear and then you choose which organism you want to use and click **Set.**
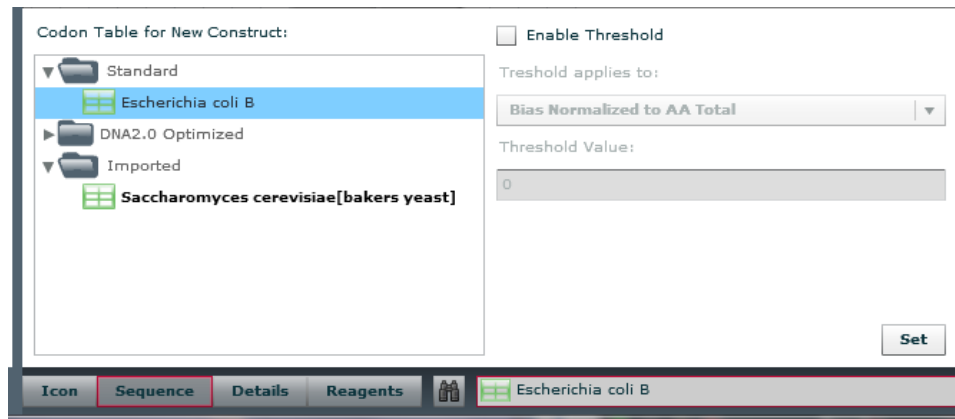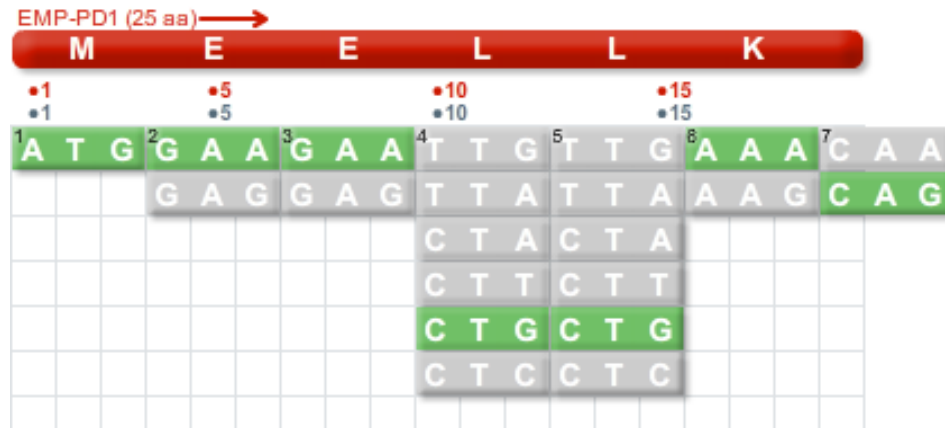
The codons that work best in your selected organism will be highlighted in green as seen below.
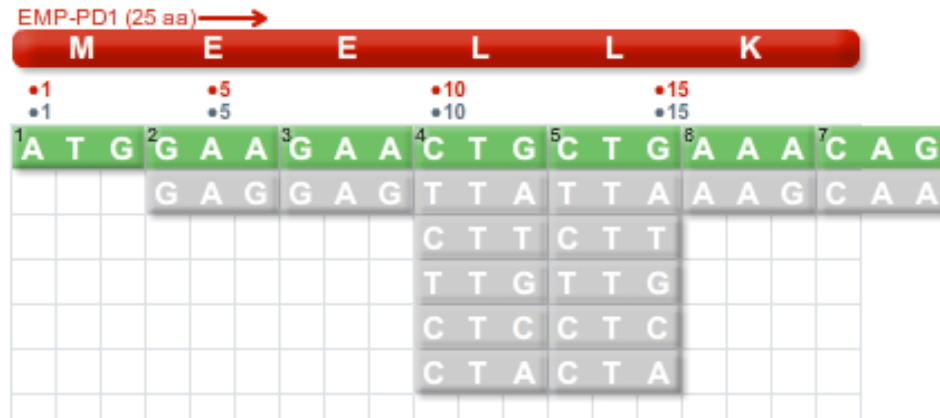
Protein's codon optimized for *E. coli.*

Protein's codon optimized for *S. cerevisiae.*

The highlighted codons still code for the same amino acid needed in your protein, but it is using a codon that has a higher success rate in your desired organism. This tool is extremely useful because you can convert existing genes from one organism into viable genes for another organism.

Find Panel

The Find Panel is a useful tool that is opened by clicking the **binoculars** next to the **Reagants** tab.
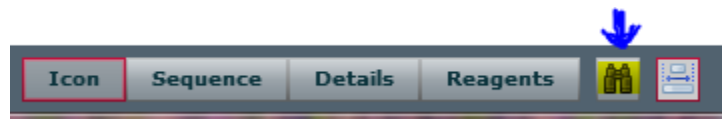
With the Find Panel, you can search your DNA sequence for motifs, restriction sites, and open reading frames.

Motifs

Under the **Motifs** tab, you can search the entire DNA construct for a specific DNA or AA sequence. Just input the sequence of interest, select whether it is a DNA or AA sequence and click **Find.** The results will be displayed below.

Restriction Sites

Under the **Restriction Sites** tab, you can search for the location of multiple restriction sites. Hold **CTRL** while selecting the desired restriction sites. Then click **Find** and it will display the locations of those selected restriction sites.

ORF's

Under the **ORF's** tab, you can search for open reading frames within your project.

Icon View

The icon view allows you to see all the DNA elements in your construct as icons. You view the icons as proportional or as non-proportional icons as seen below.
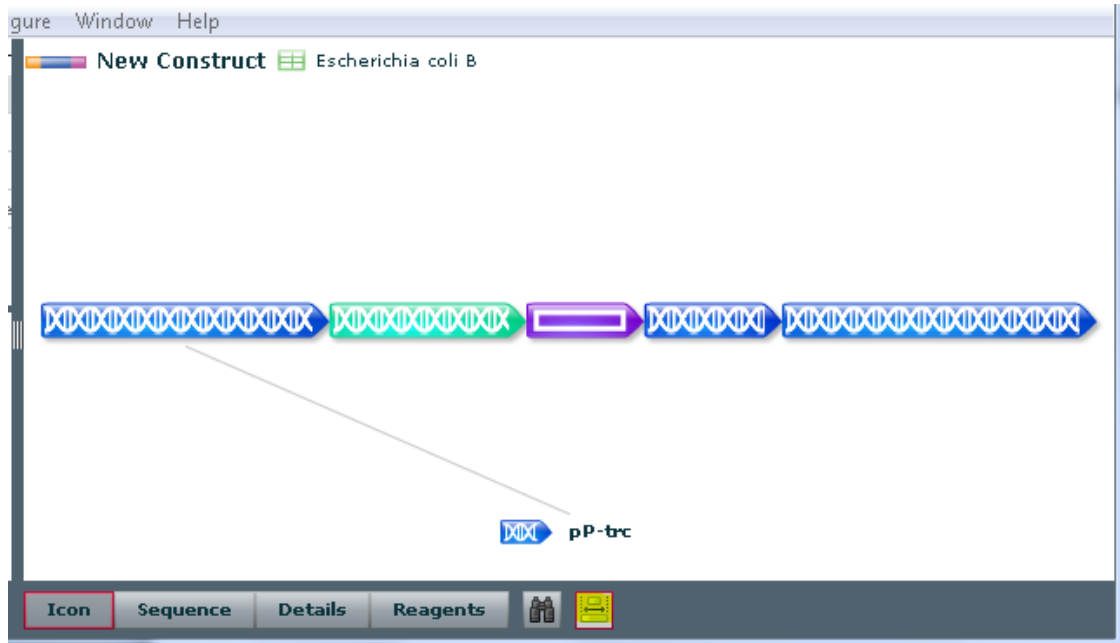

Button to toggle back and forth between proportional and non-proportional view
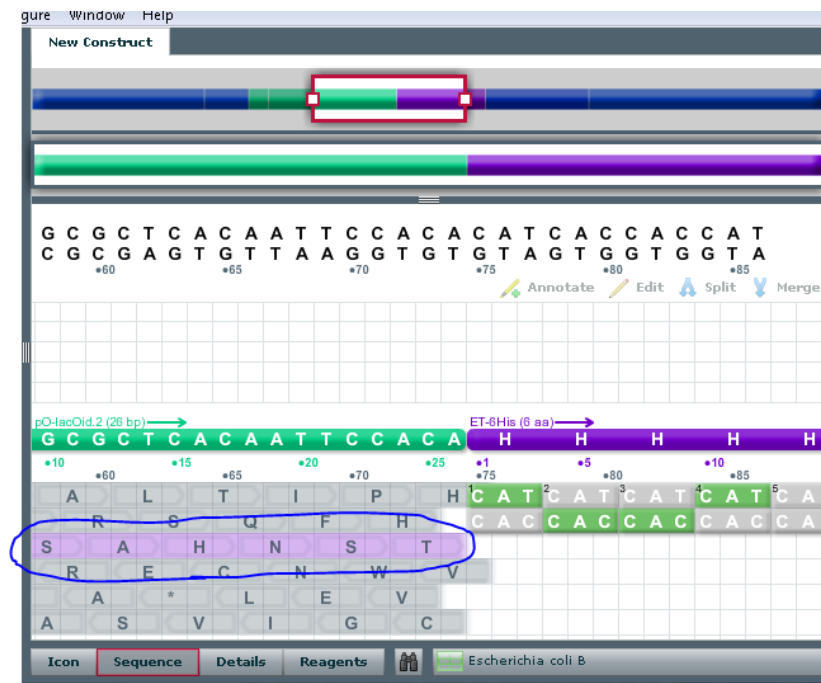
Sequence View

The sequence view allows you to see the DNA and/or AA sequences. The red box shown below can be dragged to highlight the specific area of the DNA you are interested in. The corresponding sequence appears below.
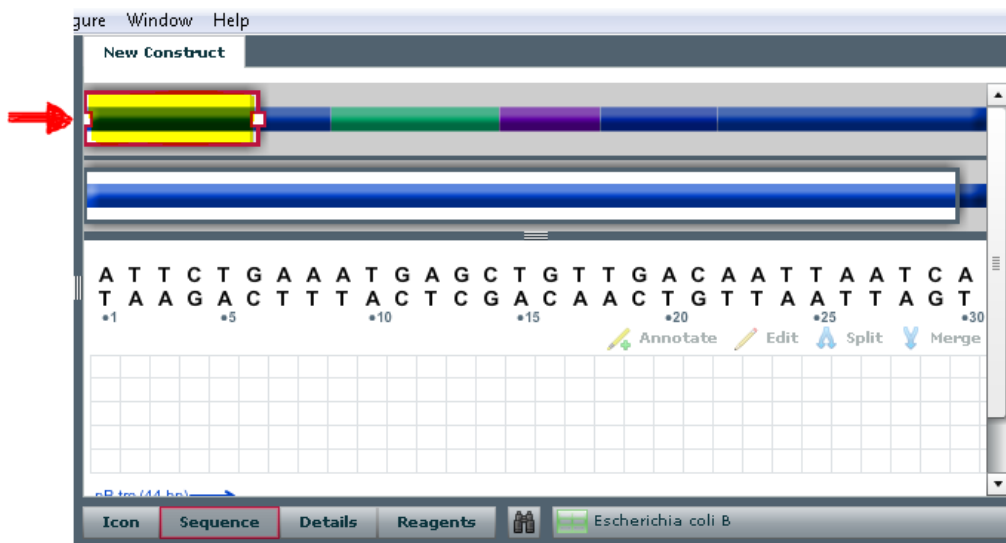
You can highlight specific nucleotides as shown below. You can highlight a single strand or a double strand. Once highlighted you have the options to annotate, edit, split or merge that section of DNA. You CANNOT edit a highlighted sequence that overlaps DNA elements in your construct. It will also calculate the Tm value for your highlighted sequence as seen below.
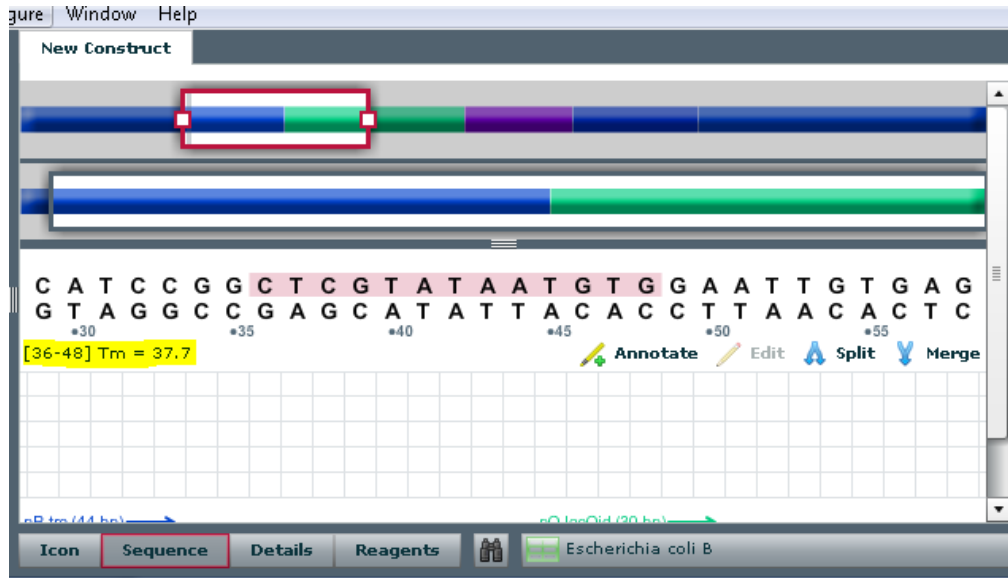

Beneath the DNA sequence is the Amino Acid sequence. If the DNA sequence is given, it shows the corresponding Amino Acid sequence which CANNOT be changed.

If the Amino Acid sequence is given, then it displays all possible codons for that specific amino acid. You can the select which codons to use which is when the codon optimization becomes useful.
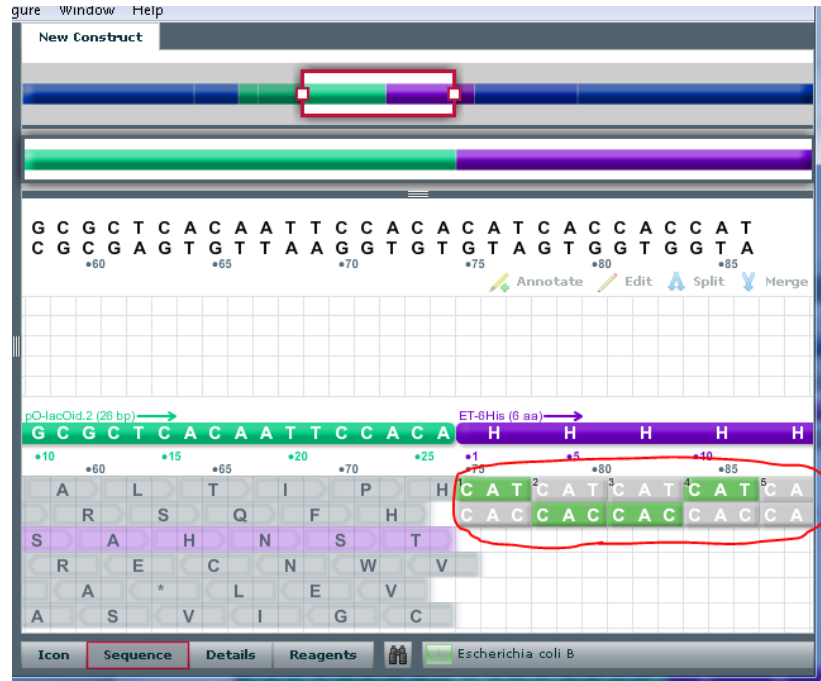
For Vectors, the sequence view is slightly different. The entire vector is shown as an icon in the upper-left corner (see below). You move the wedge to highlight a specific region of the vector. The highlighted region will appear in the window to the right. Next, you can zoom in at a higher level by moving the box (circled in red below) to highlight the sequence you wish to analyze.
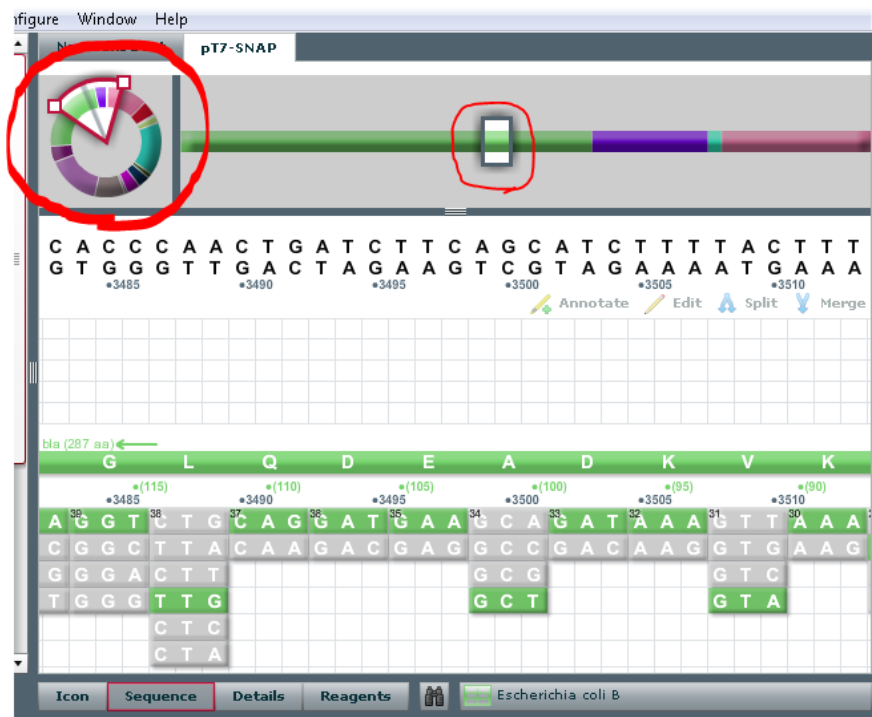
Designing

In the left window pane, you can select one of your DNA elements. To see the details on the selected element, click the [icon] button. To edit the details, click [icon]. To delete the selected element, click [icon].

To rearrange DNA elements in your construct, go to the icon view and click and drag icons to the desired location.


Tools

Back Translate

This tool allows you to back translate amino acids into specific codons. This is another tool that ultimately does the same thing as Codon Optimization.

The Back Translate tool requires you to select a backtranslation profile. You can add, edit, or view backtranslation profiles from the **Configure** menu.

Tip: Only use this tool if you want to change the algorithm Gene Designer uses to optimize codons for a specific organism.
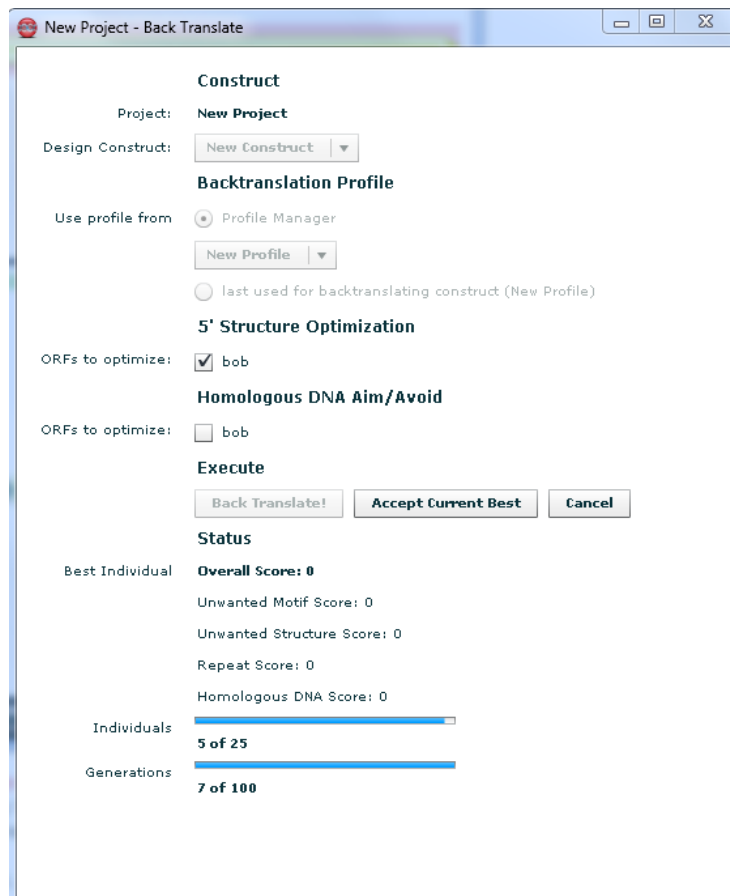
Cloning Tool

The Cloning Tool is a valuable tool that allows you to do *in silico* cloning.

Steps for Cloning:

1. Select **Cloning Tool** under the **Tool** menu. A new window called **Cloning Tool** will open.
2. Drag an element from your project into the upper left corner of the **Cloning Tool** window
3. In the lower left corner, select the restriction sites you wish to use. Hold **Ctrl** in order to select multiple sites.
4. Once all desired restriction sites are highlighted, click **"Choose."** The restriction sites you selected will appear in the **Cuts:** window pane.
5. Select the **Use Cut** check box if you want to use the cut. The DNA element resulting from the cut will appear next to the DNA element you cut.
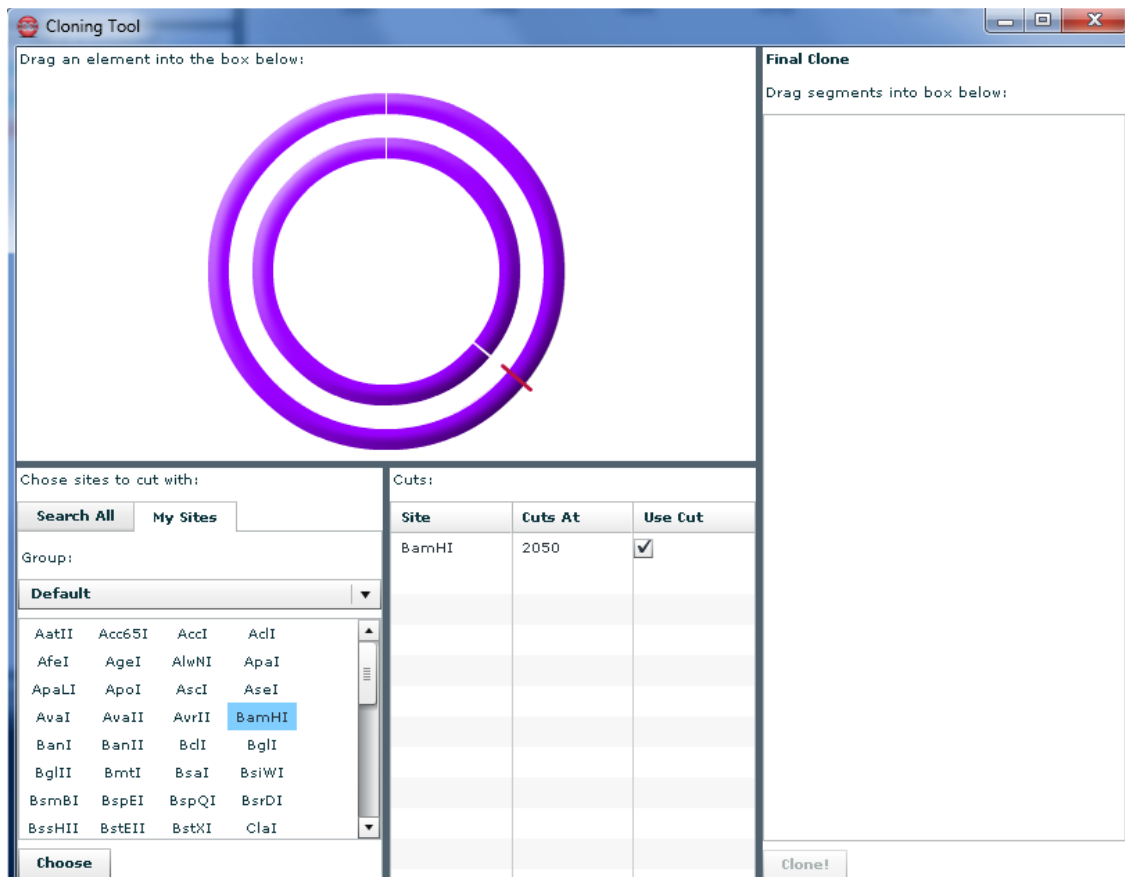
6. If you wish to use this new DNA element in the cloning, click and drag it to the **Final Clone** window pane to the right.
7. You can re-cut your original DNA element as many times and in as many ways as you desire, but only drag cut elements that will be used in the Final Clone to the **Final Clone** pane. Otherwise the cloning will not work and you will have to start over.

8. Once you are finished with your first original DNA element, you can click and drag a new DNA element from your Project window into your Cloning window. Repeat Steps 2-7 until you have all desired parts for your cloning.
9. In the **Final Clone** pane, you can choose the order of ligation by dragging the DNA elements into the correct position. Ligations will start at the top of the list
10. Once all is in order, click **"Clone!"** and your finished product will appear.
11. You can drag your newly cloned DNA into your project window pane.

Design sequencing oligos

After selecting part of the DNA sequence in the Sequence View, you can use this tool to design an oligo for that sequence.

First select if you want the **Best Oligo for the Region**, a **Single Stand Oligo**, or a **Double Strand Oligo**. Next, edit the size, Tm, and GC % you want in the oligo under the **Design Parameters**. Then click **Create.**

Order gene/Request quote

Use this tool to request a quote on the cost of having DNA 2.0 synthesize your DNA.

Configure

Restriction Sites

Here you can create groups of restriction sites used in your project. For example, you can create a BioBrick group that consists of the four major restriction sites employed in BioBricks as seen below.

Then when you are searching for Restriction sites in the **Find** panel, you can select the group to be "BioBrick" and then only the restriction sites that you are interested in appear as seen below.

Back Translation Profiles

The **Backtranslation Profile** allows you to edit the algorithm and codon table used in the back translation. You can change the codon usage table by dragging an imported codon table from the **Library Explorer** into the box labeled Codon Usage.

Motifs

Here you can add Motifs to the library that are significant to your project.

Default Codon Table

Here you can change the default codon table to the desired organism used for you project.