

## Exercises for the Introduction to GenoCAD (Version 1, 2013)

**You can choose to do these using the questions as your only guide—or see the following pages for the step-by-step solutions.**

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1) Obtain the coding sequence of the mOrange fluorescent protein from GenBank by visiting [bit.ly/mOrange](http://bit.ly/mOrange). Use this sequence to create a new part in a new library named mOrange.

*Skills: external sequence sources; custom part creation*

2) Enhance your project “Training Set” with a second library, and move parts from the training library to a new project library. Use the “My Cart” mechanism to obtain and copy items to your new library.

*Skills: library creation and part sharing with “My Cart”*

3) Use the new Orange protein part and the training library parts you added to create a design. When the design is complete, export the sequence first as a FASTA formatted file, and also as a GenBank formatted record style.

*Skills: design of constructs, exporting constructs*

**You will need to register and log in to accomplish these steps.**

**Note that during the introductory tutorial we import a new library that is editable to store your custom parts. If you have not already done so, you need to have a library prepared to complete these steps. The Training Set Library is available from figshare:**

<http://dx.doi.org/10.6084/m9.figshare.153827>

**Introduction to GenoCAD Exercises, version 1.  
Correspond to the data available in September 2013**

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## Step-by-Step checklists for the GenoCAD exercises

1) Obtain the coding sequence of the mOrange fluorescent protein from GenBank by visiting [bit.ly/mOrange](http://bit.ly/mOrange). Use this sequence to create a new part in a new library named mOrange.

*This relies on items completed during the tutorial. You need an editable project library to proceed.*

Step	Action	✓
1	Go to <a href="http://www.genocad.org">http://www.genocad.org</a>	
2	Log in to the system with your ID and password if you aren't already logged in. If you have been working with GenoCAD, <b>access the main home page</b> by clicking the GenoCAD logo at the top.	
3	From the main homepage, <b>click the "Browse Parts" button</b> to access the libraries and parts features.	
4	On the Parts page, <b>click the "My Parts" tab</b> .	
5	On the My Parts tab, <b>click the "New Part" button</b> .	
6	In a new window, obtain a sequence from NCBI that we will use for our coding sequence. Type this URL in the address bar: <b><a href="http://bit.ly/mOrange">http://bit.ly/mOrange</a></b>	
7	A new page should provide you with the FASTA-formatted sequence corresponding to this record: <b>Synthetic construct monomeric orange fluorescent protein gene, complete cds</b>	
8	<b>Copy</b> the part of the sequence record that is <b>only nucleotide sequence</b> . <i>Do not take the description line.</i>	
9	Take the copied sequence back to your GenoCAD window. <b>Paste the sequence into the "sequence" textbox</b> on the Edit Part interface.	
10	Give the part a helpful <b>short name in the "Part Name" box</b> . I'll use <b>Ex1_mOrange</b> .	
11	Return to the NCBI page and copy the description line. Return to the GenoCAD interface and <b>paste that into the Description box</b> .	
12	In the Grammar drop-down menu, <b>choose the editable grammar</b> that is available. If you did the tutorial steps it will be "Training Set E. Coli Grammar".	
13	If you did the tutorial steps, the library " <b>Training Library</b> " will be available to you. <b>Select that</b> in the "Add to Library" box.	
14	This new part is a gene coding sequence. <b>In the Category box select "Gene (GEN)"</b> .	
15	In the center <b>click the arrow that points to the right</b> to add that category to the right side box.	
16	<b>Click the Save button</b> to save this part.	
17	<b>Examine the "My parts" tab area</b> . A new gene item should be listed. Click "Gene (GEN)" to see the details on the right.	

- 2) Enhance your project “Training Set” with a second library, and move parts from the training library to a new project library. Use the “My Cart” mechanism to obtain and copy items to your new library.

*This is a continuation of exercise 1, and assumes you have completed that series of steps.*

Step	Action	✓
1	<b>Click the “Libraries” tab</b> on the Parts interface page.	
2	<b>Click the “New Library” button</b> to create a new project library.	
3	In the new dialog box, <b>select the grammar from the list: “Training Set E. Coli grammar”</b> .	
4	<b>Name the new library:</b> My Orange Protein. <b>Add a bit of description such as:</b> <i>My fluorescent orange protein construct parts from the training exercises.</i> <b>Save.</b> When this is done you will have 2 project libraries in your Training Set Grammar area.	
5	<b>Click on the “My Orange Protein” library</b> to examine the current parts. By default the structural delimiters appear, but no other parts are yet available. We’ll add parts from the training library to this one next.	
6	<b>Select the “Training library”</b> in the “Training Set E. coli Grammar” group.	
7	On the right, note that all of the library parts will be listed over 2 pages in a table. <b>Choose Show “All” entries</b> from the menu at the top of the table. This should re-load the table so that all of the library parts are on one page now.	
8	Let’s choose some items to bring over to our Orange Protein project. Select the checkboxes next to these items: <b>In Gene (GEN): choose the mOrange part</b> <b>In Promoter (PRO): choose the T7 Consensus promoter</b> <b>In Ribosome Binding Site (RBS) choose RBS A</b> <b>In Terminator (TER): choose T7 Terminator</b>	
9	Above the table, <b>click the button that says “Add Selected to My Cart”</b> .	
10	<b>Look on the left at the tabs.</b> The “My Cart” tab should indicate that you now have items copied to that set, with a number indicated. This should include the items we had selected.	
11	<b>Click the “My Cart” tab.</b>	
12	<b>Click the “Training Set E. Coli Grammar” heading.</b> The parts should be listed on the right.	
13	<b>Choose the uppermost checkbox</b> in the table row with the column names to select all the items in the table. Ensure that all the items are selected.	
14	<b>In the –Select Library- menu, choose “My Orange Protein” library.</b>	
15	<b>Click the “Remove from My Cart...” checkbox</b> to empty the cart.	
16	<b>Click the “Copy to Library” button.</b>	
17	<b>Return to the Libraries tab. Click the “My Orange Protein” folder.</b> All of the parts for this project should be available to you now. Your mOrange coding sequence should also be in the Gene area.	
<i>This is only one way to create a new project library. You can create them from scratch or from other existing projects in that grammar. Or you can do both.</i>		

- 3) Use the new Orange protein part and the training library parts you added to create a design. When the design is complete, export the sequence first as a FASTA formatted file, and also as a GenBank formatted record style.

*This is a continuation of exercises 1 and 2, and assumes you have completed those steps.*

Step	Action	✓
1	From the navigation tabs, <b>select the Step 2: Design link</b> to access the Design interface.	
2	On the Design page, select the Grammar and Library to use for the construct. <b>Choose “Training Set E. Coli Grammar” in the first menu. Then choose “My Orange Protein” in the second menu.</b>	
3	On the lower section, the parent rule structure will be shown as Step 1. You have the choice of building a design with 1 or 2 expression cassettes in a plasmid, or just a plain cassette. For this example, <b>choose the +cas box</b> to design 1 cassette.	
4	The graphical representation should show the expression cassette. The list below shows the next choices. <b>Click the +pct box to access the rule components.</b>	
5	Because there is only one promoter in our library, it is automatically selected. In the CIS cistron area, you can choose to have either 2 cistrons or the ribosome binding site + gene option. <b>Choose the +rbgn option.</b>	
6	Because we have only RBS and only one gene in this library, those will be automatically put in place.	
7	Our last choice is the terminator. Here we can have two terminators, or just one. For our purpose one is fine. <b>Click the T7 terminator.</b>	
8	The interface should now alert you that your design is complete. <b>Choose “Save Design” to keep it in your designs list. Give it a name and brief description.</b>	
9	<b>Click “Save”</b> to store the design.	
10	Orange buttons on the upper right will offer you a couple of ways to download this construct. <b>Click the “Download &gt;” button</b> to generate a text file of the sequence. A dialog box will let you save it and open it.	
11	Back on the GenoCAD interface, <b>click the “GenBank &gt;” button.</b> Again a dialog box will let you save and open the resulting document. Download and <b>open this file.</b> <i>(To see the correct formatting, you may need to use WordPad on PC, or a Mac word processing tool.)</i>	
12	The GenBank formatted record of your design will contain details about the design. The parts’ names and descriptions will be listed with the sequence range they occupy. The complete sequence will be provided at the bottom.	

*This formatted record is a handy way to have details of your construct with the annotations about the parts from the parts records. Keep this in mind when curating the details of your parts as you create them.*