

Exercises for the Advanced 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.

- 1) Create a new part for the HisTag category, and use that to fix the invalid RFP design.

Skills: add custom part; edit an existing construct; re-check validation status

- 2) During the tutorial, we create rules that allowed users to select HisTags either before or after the gene. In this exercise, create a new rule that offers HisTag features on both sides of a gene part. Finally, create a design that uses this new rule, and examine this design in GenBank format.

Skills: creating a new rule; completing a new design; examine a GenBank formatted file

- 3) Modify the grammar you added during the Advanced Tutorial so it can be used to design Cassettes (i.e., Promoter RBS Gene Terminator), and use the Test functionality to validate the modified grammar.

Skills: Develop Grammar rules on your own, verify your rules hierarchy.

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

Note that during the introductory tutorial we imported a new library that is editable to store your custom parts. We continue to use this in the Advanced tutorial. If you have not already done so, you need to have a library prepared to complete these steps. The Training

Set Grammar (with Library) is available from figshare:

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

In the Advanced Tutorial, we added a new grammar, but did not add any categories or rules; you will need this grammar for the third exercise.

**Advanced GenoCAD Exercises, version 1.
Corresponds to the data available in August 2013**

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Step-by-Step checklists for the GenoCAD exercises

- 1) Create a new part for the HisTag category, and use that to fix the invalid RFP design.

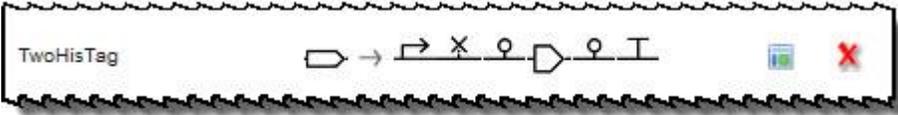
This relies on items completed during the introductory and advanced tutorials.

Step	Action	✓
1	Go to http://www.genocad.org	
2	Log into the system with your ID and password if you aren't already logged in. If you have been working with GenoCAD, access the main home page by clicking a GenoCAD logo at the top.	
3	From the main homepage, click the "Browse Parts" button to access the libraries and parts features.	
4	On the Libraries page, click the "Fluors" library .	
5	On the right, click the "Add New Part" button .	
6	In the new window, create a short part name: N-histag In the sequence area, type the sequence of this new part. It will carry a starting methionine (ATG) and then 6 histidine residues (CAT): ATGCATCATCATCATCAT	
7	Provide a brief description of the new part, something like: A 6-histidine tag suited for the N terminal part of an expression construct.	
8	In the Category box, select the "6-histidine residue tag for expression" and move it to the right-hand box.	
9	Click the "Save" button.	
10	Return to the "Available Designs" page by choosing the "Available Designs" folder at the top of the page.	
11	Click the "View" icon for the "Copy of RFP Construct" to examine the design details.	
12	Click the Step 1 box on the left. This lets you begin to design anew.	
13	As before, choose "cas". Then choose PCT- .	
14	In the CIS column, choose the "rbgn" rule . Note that it now carries the HisTag we created, in the location we edited for this rule.	
15	We have only one N-HisTag part, so ensure that is selected . Now choose the RFP gene part . Select any other parts that need to be re-selected.	
16	Click "Save Design". In the new window click "Save".	
17	Return to the "Available Designs" page by clicking the designs folder.	
18	Determine the validation status of this design now.	

The "Copy of RFP construct" should be repaired now, and conform to the grammar. So your status should be the green checkmark indicating a "valid" design.

2) During the tutorial, we create rules that allowed users to select HisTags either before or after the gene. In this exercise, create a new rule that offers HisTag features on both sides of a gene part. Finally, create a design that uses this new rule, and examine this design in GenBank format.

This is a continuation of other work and assumes you have completed all the prior steps.

Step	Action	✓
1	Click the “Parts” tab.	
2	Click the “Grammars” sub-tab to access the Training Set grammar.	
3	On the Grammars tab, select the “Training Set E. Coli Grammar” item.	
4	Click the “Manage Grammar” button.	
5	On the Manage Grammar page, click the “Expression Cassette (CAS)” category.	
6	Next click the “Add Rule” button.	
7	Name the new rule: TwoHisTag	
8	Create a new rule by dragging categories from the left to the lower box on the right.	
9	Add to the box the items in this order: PRO → RBS → HisTag → GEN → HisTag → TER If they don't set in the right order, drag them to the right places before saving.	
10	Click “Save”.	
11	Back on the Manage Grammar page, your new rule should be in place. It should look like this: 	
12	Now we'll use this to create a design. Click the “Design” tab.	
13	Select the “Training Set E. Coli Grammar” and “Fluors” library from the menus at the top.	
14	Click the CAS rule in step 1.	
15	Click the TwoHisTag child rule in step 2.	
16	Most of your design will be assembled because there is only 1 choice for the parts. But now choose the Cyan fluorescent protein (eCFP) as the GEN part.	
17	Select the single terminator.	
18	GenoCAD should indicate that your sequence is complete. Let's save it as CFP Construct by clicking the “Save Design” button. Provide a short description, and save that.	
19	Obtain the GenBank formatted file by clicking the GenBank button.	
<i>A brand new rule was created, and we used it in a new design. GenBank formatted output can be obtained to use in other tools.</i>		

3) Modify the grammar you added during the Advanced Tutorial so it can be used to design Cassettes (i.e., Promoter RBS Gene Terminator), and use the Test functionality to validate the modified grammar.

Step	Action	✓															
1	Click the Parts tab, then the Grammars sub-tab.																
2	Select your new, blank grammar and click on the Manage Grammar button.																
3	Click on the New Category button, and in the popup, enter “ TP ” for Letter , “ Transcription Unit ” for Description , leave GenBank Qualifier blank, and choose an icon for Icon . Click the Save button to save the category.																
4	Repeat Step 3 to add the following four categories (choose appropriate icons): <table border="1" data-bbox="228 810 1386 995"> <thead> <tr> <th>Letter</th> <th>Description</th> <th>GenBank Qualifier</th> </tr> </thead> <tbody> <tr> <td>PRO</td> <td>Promoter</td> <td>promoter</td> </tr> <tr> <td>RBS</td> <td>Ribosome Binding Site</td> <td>RBS</td> </tr> <tr> <td>CDS</td> <td>Coding Sequence</td> <td>CDS</td> </tr> <tr> <td>TER</td> <td>Terminator</td> <td>terminator</td> </tr> </tbody> </table>	Letter	Description	GenBank Qualifier	PRO	Promoter	promoter	RBS	Ribosome Binding Site	RBS	CDS	Coding Sequence	CDS	TER	Terminator	terminator	
Letter	Description	GenBank Qualifier															
PRO	Promoter	promoter															
RBS	Ribosome Binding Site	RBS															
CDS	Coding Sequence	CDS															
TER	Terminator	terminator															
5	When you have finished adding these five categories, click on category S and click on Add Rule on the right side of the screen.																
6	In the Add Rule screen, type “TP” in the Code field, make sure that the Transform From is set to Start/Transcription unit (S) , and drag Transcription Unit (TP) from the left side of the screen to the Transform To field . Click Save . Notice that S is now a Rewritable Category because it has a child rule, and TP is a Terminal Category because it has a parent rule but no child rules.																
7	Click on TP . Click on the Add Rule button to add the two rules below: <table border="1" data-bbox="228 1335 1393 1520"> <thead> <tr> <th>Code</th> <th>Transform From</th> <th>Transform To</th> </tr> </thead> <tbody> <tr> <td>revTP</td> <td>S</td> <td>[TP] (Brackets represent Opening and Closing Reverse Complement Delimiters, respectively.)</td> </tr> <tr> <td>PRCT</td> <td>TP</td> <td>PRO RBS CDS TER</td> </tr> </tbody> </table> Notice that TP is now a Rewritable Category, and Opening Reverse Complement Delimiter (I), Closing Reverse Complement Delimiter (I), PRO, RBS, CDS, and TER are Terminal Categories.	Code	Transform From	Transform To	revTP	S	[TP] (Brackets represent Opening and Closing Reverse Complement Delimiters, respectively.)	PRCT	TP	PRO RBS CDS TER							
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8	Test your grammar by clicking on the Test button on the right side of the screen. Notice that you can test the different rules of the grammar by clicking on the different categories, just like building a Design in the Design screen. To start over, click on the Start Over button . When you are done parsing your grammar to verify the layout, click on Done .																
<p><i>This is just a simple example of what can be done when creating grammars. Even as your grammar becomes more complex, you can use the Test button to validate your work.</i></p>																	