

## Pathway Tools Editing Tutorial

### Exercise 2: Assigning enzymes and creating protein complexes

In this exercise you will:

1. Assign enzymatic activities to proteins
2. Define protein complexes
3. Create a publication frame
4. Export a pathway to a file

Disclaimer: In this exercise we will create enzyme monomers and complexes that are completely fictitious. The enzymatic activities described are not based on real data.

**The PGDB to be used for this exercise is an editable version of the *Arthrospira platensis* NIES-39 PGDB that is included with the software. Instructions for creating an editable version of that PGDB are included in exercise 1 (Creating reactions and pathways).**

**Open the L-ascorbate biosynthesis pathway (created in the last exercise) in the Navigator.**

#### **Assigning enzymatic activity to an existing protein.**

Right click on the first reaction in the pathway and choose “Edit → Create/Add Enzyme”. Type in “NIES39\_RS34785” and click OK.

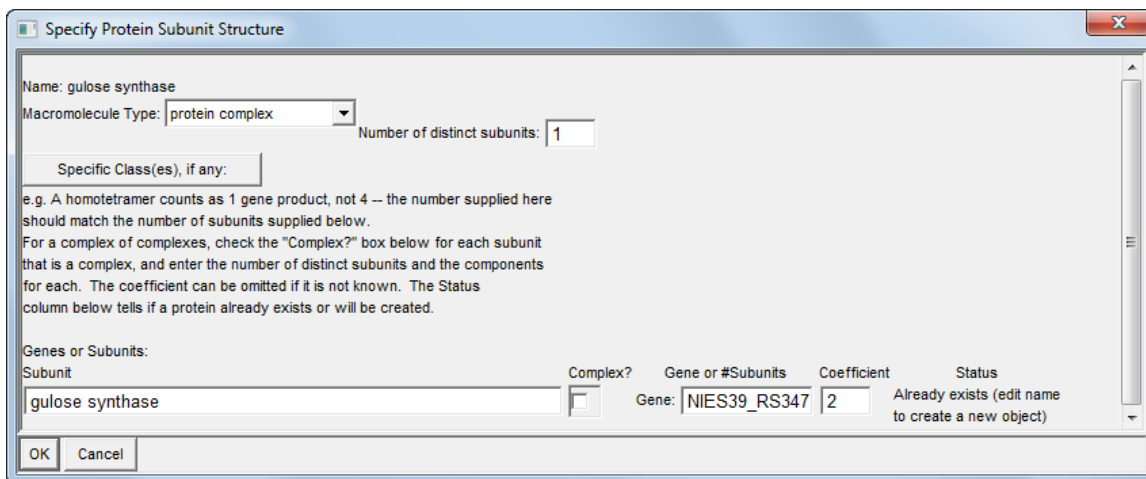
The Protein Editor opens up. Click on the “Enzymatic Activity” tab and type “gulose synthase”. Change the reaction direction to “Physiol Right to Left”.

Familiarize yourself with the different fields of this editor.

Close the editor. Note that specifying an activity name does not change the protein name. Right-click the protein, select “Synonym Editor”, type “gulose synthase” in a new line, then click outside that field. A “Make this the Common Name” button appears – click it, then OK.

#### **Converting an existing protein to a complex.**

First, we will convert the enzyme you just assigned to a homo-dimer.



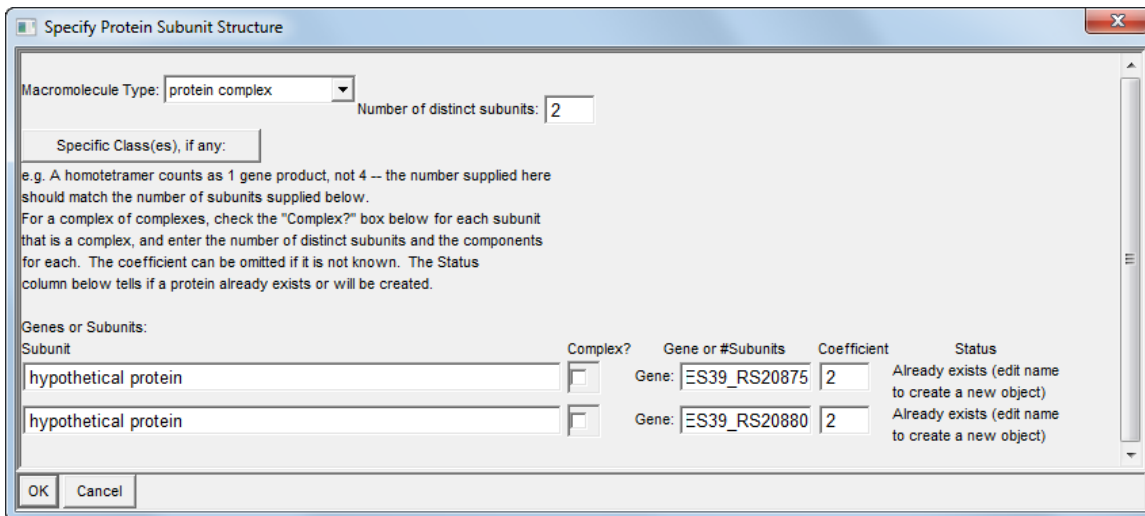
**Figure 1**

Right-click on the protein and choose Edit → Protein Subunit Structure Editor. Click on “polypeptide” (in the button next to “Macromolecule Type”) and change the selection to “protein complex”. Leave the “Number of distinct subunits” as 1 and type a coefficient of 2 for the subunit (see Figure 1). Click OK.

Now open the new protein complex in the Protein Editor. Click on the “Edit Enzyme Name” button at the top – note that it is blank. Type “gulose synthase” and click OK. Now move to the “Subunits” tab. Modify the name for the subunit to “gulose synthase monomer”. Click OK and save.

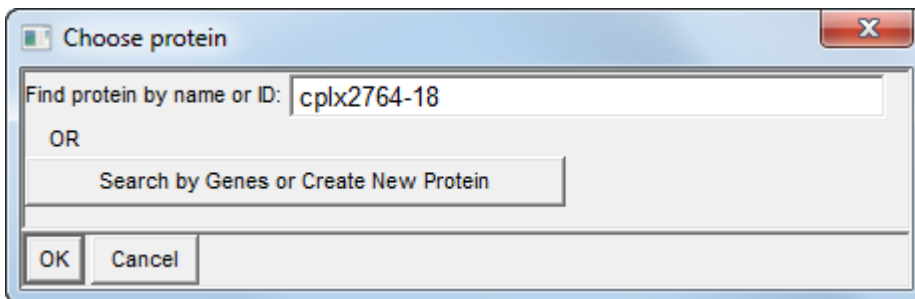
### **Defining a new protein complex and assigning activity to it.**

Now we will create a new complex, made out of two existing gene products. Right click on the second reaction in the pathway and choose “Edit → Create/Add Enzyme”. Click the “Search by Genes or Create New Protein” button. Specify “protein complex”. Change the number of subunits to 2, and type in the gene names NIES39\_RS20875 and NIES39\_RS20880, respectively. After typing in a gene name, click outside the text field to have the system process your input. Add coefficients of 2 to both subunits (see Figure 2).



**Figure 1**

When you click OK, the system assigns an ID for the new complex (Figure 3). Sometimes it takes a few seconds for the system to assign the ID – **don't click any button until the name shows up!**



**Figure 2**

When you click OK this time, the Protein Editor for the new protein opens up. Move to the “Enzymatic Activity” tab and type “L-gulose lactonase” in the “Enzyme activity name” field.

Move to the first tab (the one called “Complex”). Click the button “Edit Enzyme Name” and type the name “L-gulose lactonase I”. **Sometimes enzyme name and enzymatic activity name are NOT the same!**

**Adding names to genes and subunits.** Still in the Protein Editor, move to the “Subunits” tab, and change the subunit names to “L-gulose lactonase &alpha; subunit” and “L-gulose lactonase &beta; subunit”, respectively. Move to the gene

tab and name the genes *IgIA* and *IgIB*, respectively. Click OK, and you should now see the following diagram:

#### Gene-Reaction Schematic?

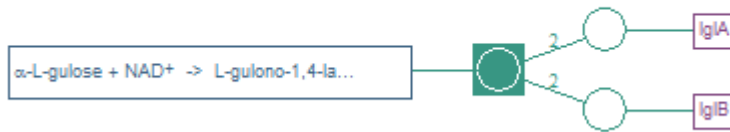


Figure 4

**Adding evidence codes and creating publication frames.** Open the complex in the Protein Editor, move to the “Enzymatic Activity” tab, and add the evidence code “Assay of protein purified to homogeneity” by clicking on the Evidence Code button below the enzymatic activity name field. Type the PubMed ID “17906139” in the citation box next to the evidence code button. Close the editor – the system will automatically retrieve the citation information from PubMed and store it in the database. Note a Flask icon at the upper right corner of the enzymatic activity section. This marks an experimental evidence code. Click on it to see more details.

**Citations without PubMed ID.** Open the editor, add another evidence code as you please, and type Bonin00 in the citation box next to it. As soon as you click out of that box, a window will pop up to alert you that publication Bonin00 was not found. Click “Search or Create Publication Frame”. When the Publication Editor appears (Figure 6) you have two options: If a DOI number exists, you can paste it in the DOI field, click out of that field, and the system will retrieve the reference automatically. If a DOI number does not exist, you need to enter the data manually. For this exercise, enter **10.1046/j.1365-313x.2000.00698.x** into the DOI field (see Figure 5), click out of the field, wait for the system to fill it in, then click OK to exit the editor. Be careful when you paste in the DOI – every little error, like a space before or after the text string, will interfere with the download.

Edit Publication Data for PUB-BONIN00

PubMed ID:  AGRICOLA ID:  DOI ID: 10.1046/j.1365-313x.2000.00698.x

Title: A bifunctional epimerase-reductase acts downstream of the MUR1 gene product and

Authors (surname first): 1.  Bonin CP 2.  Reiter WD  
3.  4.   
5.  6.

Source:  The Plant Journal 21(5);445-454 Year:  2000

URL:  http://doi.wiley.com/10.1046/j.1365-313x.2000.00698.x

OK Cancel

**Figure 5**

Back at the Protein Editor, click OK. Go back to the pathway page. Notice that now this enzyme shows up in darker color than the other one. This indicates a presence of an experimental evidence code for this activity.

**6. Exporting pathways to a file.** Right-click the pathway name and select “Edit → Add Object to File Export List”. Then click “File → Export → Selected Objects to Lisp-Format File”. You can see that the pathway is already listed. Click the “Enter File” button and specify the path and a file name for the exported file. Set the “Export Enzymes and Genes?” option to Yes (an option you would want if you were exporting the pathway for inclusion in MetaCyc).

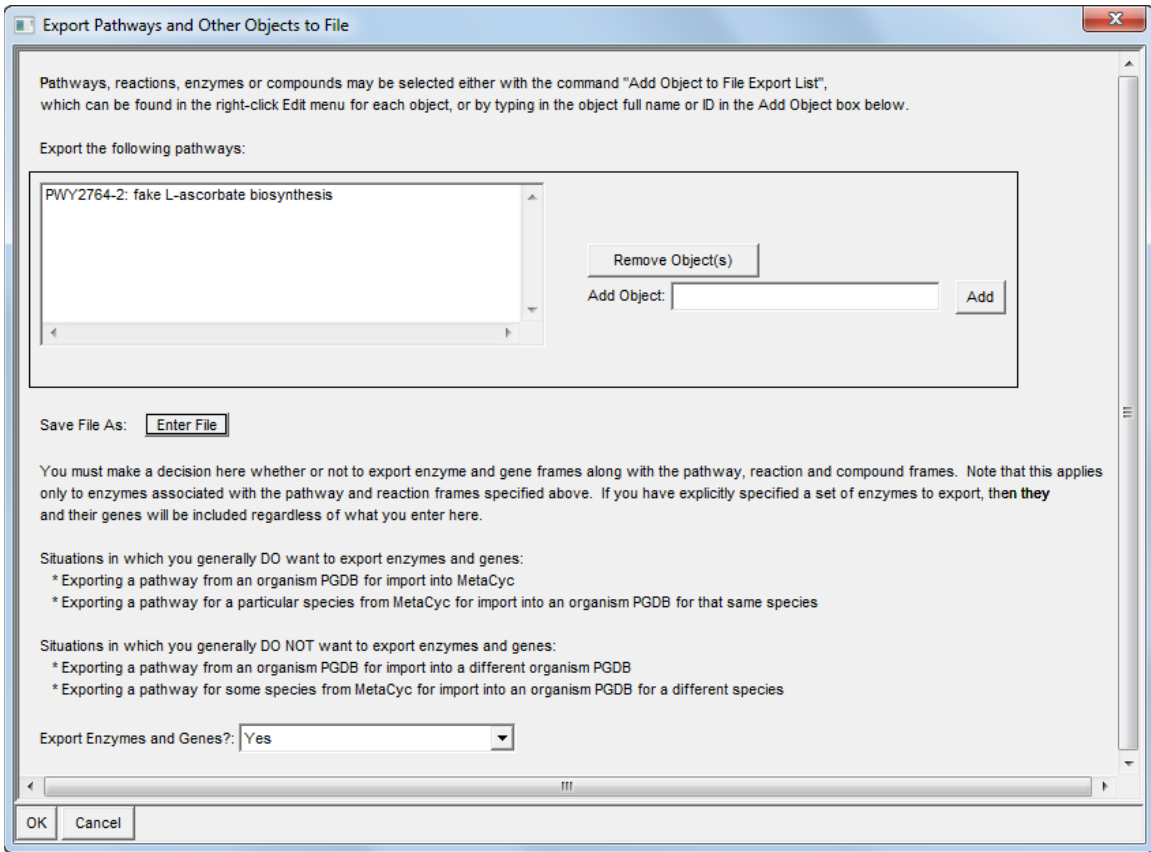


Figure 6