

## PathwayTools 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.

- **Please only modify the *H. pylori* database.**

**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 “HP1580” 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.

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

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

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 and move to the “Subunits” tab. Modify the name for the subunit to “ascorbate hydratase monomer”. Click OK and save.

Specify Protein Subunit Structure

Protein: H. pylori predicted coding region HP1580

Macromolecule Type: protein complex Number of distinct subunits: 1

Specific Class(es), if any:

e.g. A homotetramer counts as 1 gene product, not 4 -- the number supplied here should match the number of subunits supplied below.

For a complex of complexes, check the "Complex?" box below for each subunit that is a complex, and enter the number of distinct subunits and the components for each. The coefficient can be omitted if it is not known. The Status column below tells if a protein already exists or will be created.

Subunit	Complex?	Gene or #Subunits	Coefficient	Status
H. pylori predicted coding region HP1580	<input checked="" type="checkbox"/>	Gene: HP1580	2	Already exists (edit name to create a new object)

OK Cancel

**Figure 1**

### **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 HP1587 and HP1588, 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).

Specify Protein Subunit Structure

Macromolecule Type:  Number of distinct subunits:

Specific Class(es), if any:

e.g. A homotetramer counts as 1 gene product, not 4 -- the number supplied here should match the number of subunits supplied below.  
 For a complex of complexes, check the "Complex?" box below for each subunit that is a complex, and enter the number of distinct subunits and the components for each. The coefficient can be omitted if it is not known. The Status column below tells if a protein already exists or will be created.

Subunit	Complex?	Gene or #Subunits	Coefficient	Status
<input type="text" value="conserved hypothetical protein"/>	<input type="checkbox"/>	Gene: <input type="text" value="HP1587"/>	<input type="text" value="2"/>	Already exists (edit name to create a new object)
<input type="text" value="conserved hypothetical protein"/>	<input type="checkbox"/>	Gene: <input type="text" value="HP1588"/>	<input type="text" value="2"/>	Already exists (edit name to create a new object)

OK Cancel

**Figure 2**

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

Choose protein

Find protein by name or ID:

OR

OK Cancel

**Figure 3**

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 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”. Click OK, and you should now see the following diagram:

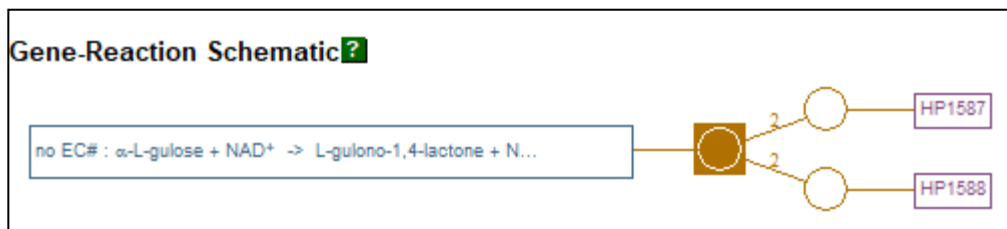


Figure 4

**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 purified protein” 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.

**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, click out of the field, wait for the system to fill it in, then click OK to exit the editor

Edit Publication Data for PUB-BONIN00

PubMed ID:  AGRICOLA ID:  DOI ID:

Title:

Authors (surname first):
 

1.	<input type="text" value="Bonin CP"/>	2.	<input type="text" value="Reiter WD"/>
3.	<input type="text"/>	4.	<input type="text"/>
5.	<input type="text"/>	6.	<input type="text"/>

Source:  Year:

URL:

**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).

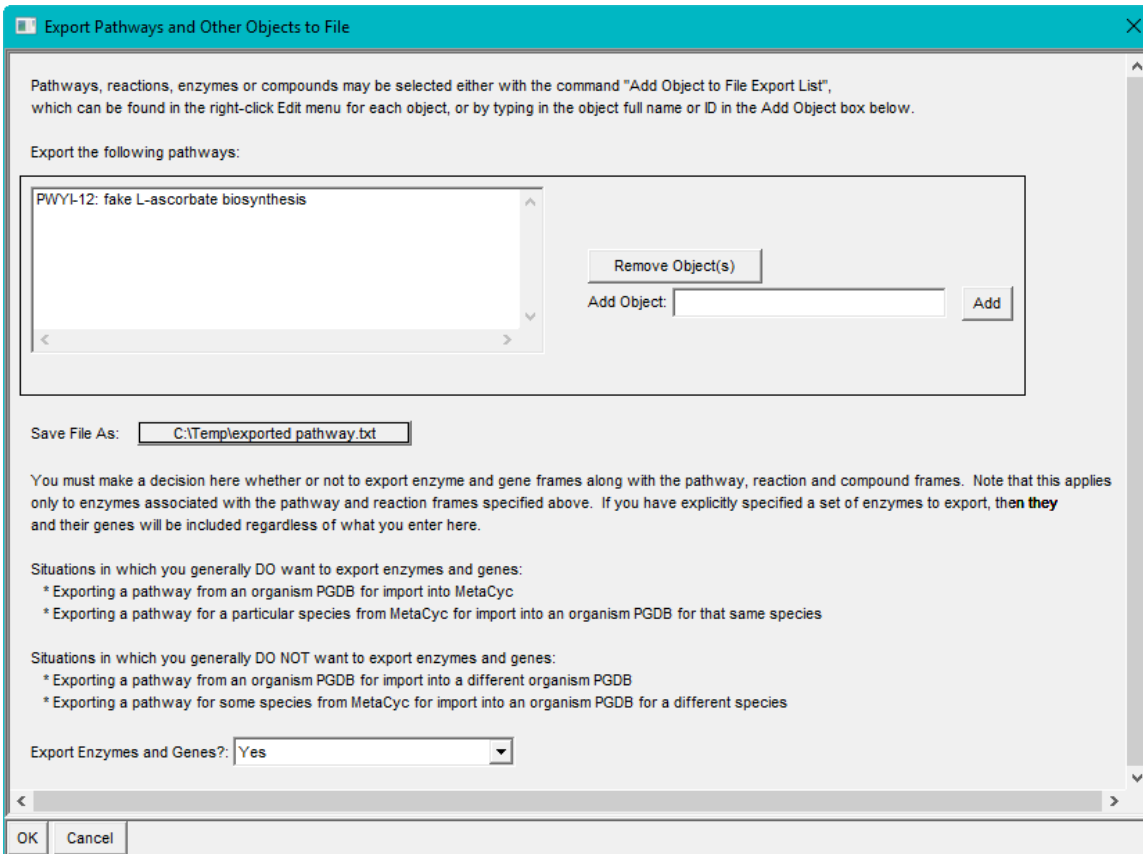


Figure 6