Pathway Tools Editing Tutorial

Exercise 1: Creating reactions and pathways

In this exercise you will:

A. Make an editable copy of a PGDB
B. Create organization and curator frames and configure your installation to use your curator frame.
C. Create new reactions in the DB (or import them from MetaCyc).
D. Create a new pathway.

A. Make an editable copy of a PGDB

The 25.5 distribution of Pathway Tools includes three built-in databases - MetaCyc, EcoCyc, and a tier 3 PGDB for the cyanobacterium *Arthrospira platensis* NIES-39. Built-in databases cannot be edited. You may have created your own editable PGDB, but you would not want to use it for performing these exercises. For this exercise we will generate an editable copy of the *A. platensis* PGDB. Once created, that PGDB could be edited freely. Do not be afraid to experiment. You can always delete this PGDB and create a new copy.

To create an editable copy:

1. In Pathway Tools, select the *A. platensis* PGDB.
2. From the File menu select Save PGDB as….
3. Type any text string in the box "New PGDB Id" and click outside the box. The software will fill the rest of the fields automatically (see figure 1). Click OK.
4. The software will create an editable copy of the PGDB. When it is done, you can click on the Home button to see the available databases. There will be two databases for *A. platensis.* If you didn't rename the new PGDB, you can still distinguish between the two by looking at the Source column. The original one is labeled as Built-In or BioCyc, while the new one is labeled as User (figure 2).

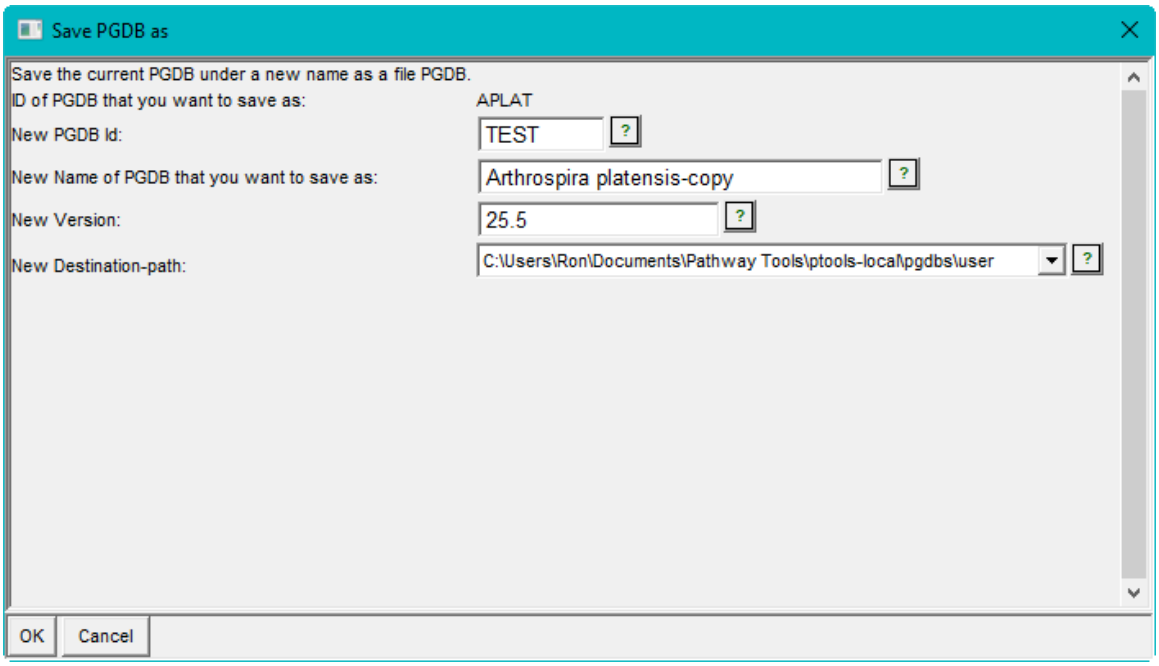

Figure 1

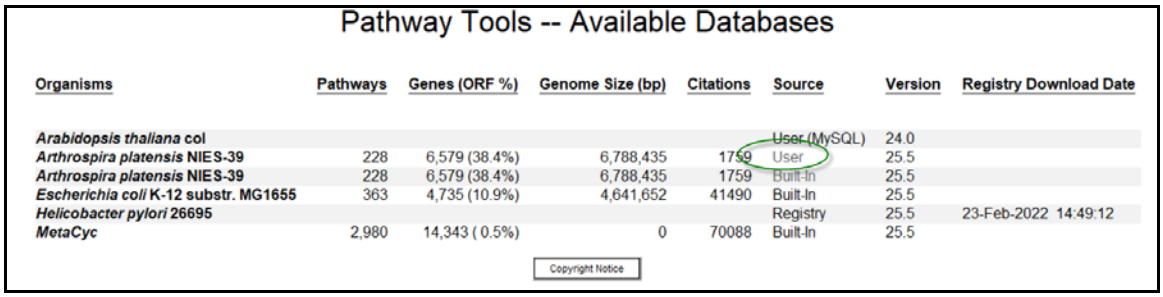

Figure 2

B. Creating an organization frame for your institution, a curator frame for yourself, and configuring Pathway Tools to recognize you.

1. **Open the new database** (all exercises will be using that PGDB).

Under the file menu, select “Create”, then “Organization”. Type in the relevant info for your institution, click OK and save.

2. Repeat, this time selecting “Create” → Curator”. Type in your first and last name and **a login name**. Click on the “Select/Change” button next to “Affiliations”, select the organization you just specified, click OK and save.
3. Select Tools → Preferences → UserID and type in **the login name** you just specified above. Click OK.

From now on the system will know to assign credit to you and your organization automatically whenever you create a new object in the database.

C. Creating reactions

In this section we will create three reactions which we will use later to construct a pathway. While entering the reactions you will encounter the reaction duplicate checker. At the end of this section, you will have three reaction frame IDs that you will use later to construct a pathway.

Please read carefully before starting your work

Table 1 (on page 5) lists the reactions to be entered.
Since new reactions may already be present in MetaCyc or in the current DB, after entering the reaction in the Reaction Editor the Reaction Duplicate Checker may ask if you want to delete the new reaction, keep it, or import the MetaCyc reaction instead (see figure 3). The message will provide the Frame-IDs of the existing reactions, printed before the actual reaction (green circle in figure 3).
If the reaction already exists in the DB, choose “**delete**” – you don’t want to create a duplicate of an existing reaction.
If the reaction is present in MetaCyc only, choose “**import” – this will import it into the database and delete the reaction you just entered.**
Either way, before you click on a button write the frame ID of the reaction in table 1. You will need these ID numbers in the next step.

1. Select “New” from the Reaction menu.

2. When the reaction editor opens, type the first reaction into the field “quick entry of equation”. (To save time, you can simply copy from this document and paste into the editor). When you are done, click outside of the field. The system will recognize the names and create the reaction.
3. Click OK to exit the editor. Right-click the new reaction’s object-handler (where either an EC number is displayed or it says “no EC number assigned”) and select Show → Show frame name. The reaction’s ID will be printed in the Lisp console. Copy it into Table 1 below.
4. Repeat for the other reactions. When entering the third reaction the duplicate checker will pop up a window as shown in figure 3. Click on the “Show” button to display the MetaCyc reaction. Use Show → Show frame name to copy the frame ID of the MetaCyc reaction into Table 1, then click the “Import” button.

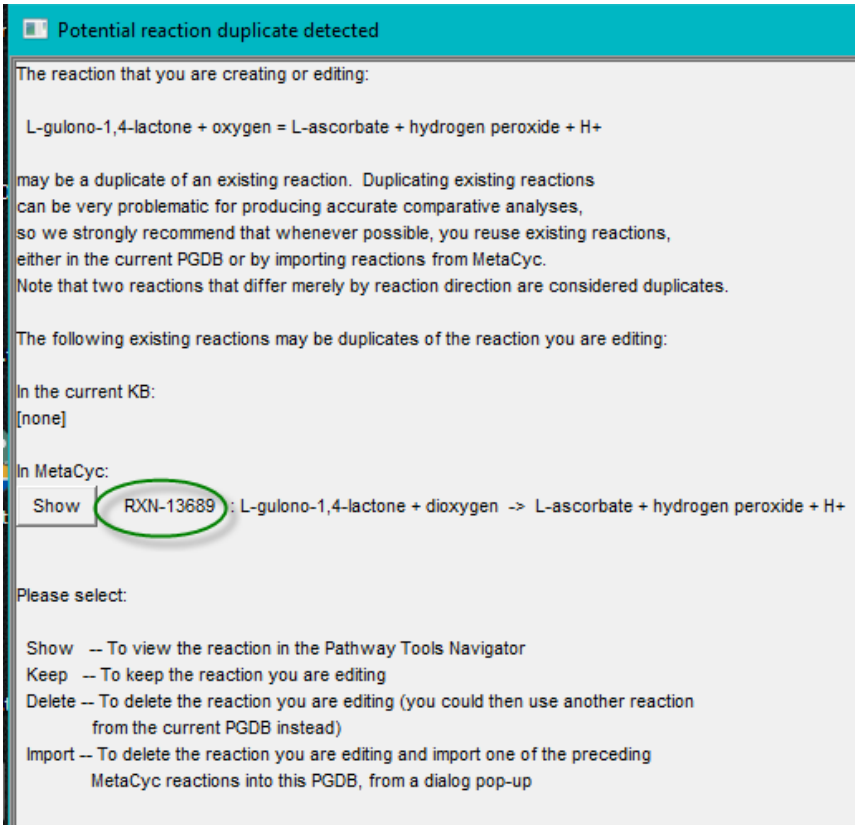

Figure 3: Duplicate Reaction dialog window

	Reaction	Frame ID
1	alpha-L-gulose + 2 NAD+ = 2 pyruvate + 2 NADH + 4 H+	
2	alpha-L-gulose + NAD = L-gulono-1,4-lactone + NADH + H+	
3	L-gulono-1,4-lactone + O2 = L-ascorbate + hydrogen peroxide + H+	

Table 1

D. Creating a new pathway

1. Select “New Metabolic Pathway” from the Pathway menu. The **Pathway Info Editor** will open up.

2. Enter the common Name: “fake L-ascorbate biosynthesis”.

3. Assign a class: Click on the Class button, then select “Ascorbate Biosynthesis” (Pathways → Biosynthesis → Cofactor, Electron Carrier, and Vitamin Biosynthesis → Vitamin Biosynthesis → Ascorbate Biosynthesis)

4. Click OK, the **Pathway Info Editor will** close, and the **Pathway Editor** will open.

5. Select Reaction → Add Reaction(s) by Frame ID. Type in the Reaction IDs separated by white space (or one on each line) and click OK. Once you have all three reactions, it’s a good time to choose Exit → Keep Changes and save your changes.

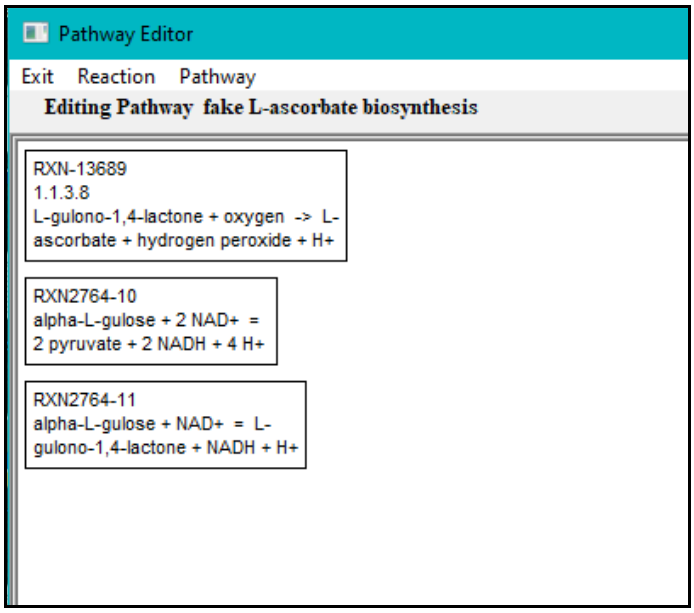

Figure 4

6. Open the Pathway editor again (right click on the pathway name and choose Edit → Pathway Editor) and connect the reactions as follows:.
Start by right-clicking on the first pathway's reaction (the one with pyruvate) and selecting "choose main compounds for reaction". Select pyruvate for the main reactant and alpha-L-gulose for the main product and click OK. Now click on the reaction with the left mouse button. The reaction will become highlighted in red, and the reactions it can connect to will be highlighted in green (see figure 5).

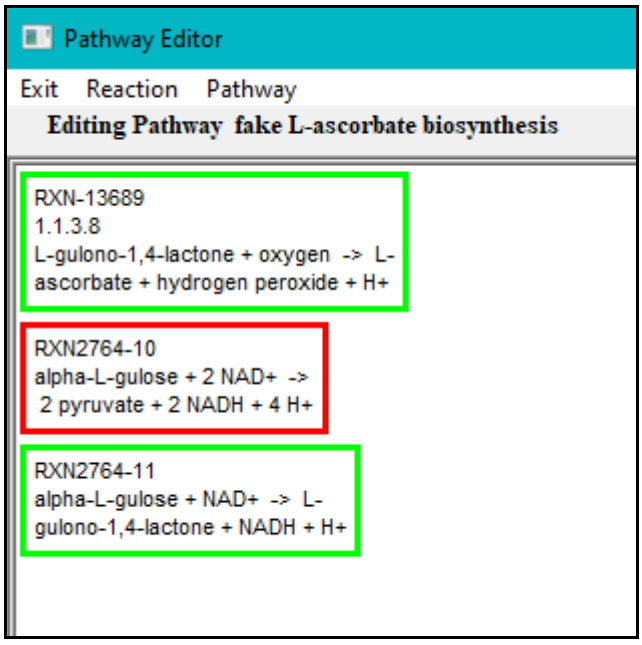

Figure 5

Click on the next reaction in the pathway, and both reactions will move to the right pane. Now click on the last compound in the nascent pathway (figure 6) and repeat the process to finish the pathway. Exit, and save your changes.

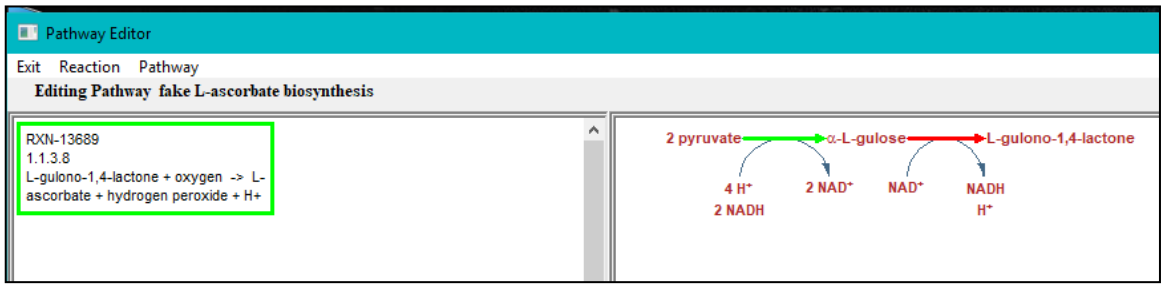

Figure 6

Adding pathway links

Now we will add links connecting the glycolysis pathway (source of pyruvate) to the new pathway.

1. Find the glycolysis pathway (Pathway → Search by Substring → glycolysis). Right-click on the pathway's name and select Show → Show frame name. The frame name will be printed in the lisp console. Write it down or copy to the clipboard.
2. Open the new pathway you created in the Pathway Editor, right-click pyruvate, and choose "Add Link from/to Pathway".
3. Type the Frame ID and click OK. If the link appears in the wrong direction, right-click on it and select the correct direction. Exit the editor and save changes.
4. Repeat step 1, but this time find the frame ID of the new pathway.
5. Back in the Navigator, click on the link that you created to move to the glycolysis pathway.
6. Open this pathway in the pathway editor and add a reciprocal link to the new ascorbate degradation pathway that you created. Save your work.

Adding evidence codes

1. Open the new ascorbate biosynthesis pathway in the Pathway Info Editor.
2. Click on the "Evidence code" button and select "inferred from direct assay".
3. Type the Pubmed ID number "11741871" in the citation box next to it and click OK.

Adding commentary and using internal hyperlinks

1. Open the new ascorbate biosynthesis pathway in the Pathway Info Editor.
2. Type this sentence in the summary field: "This L-ascorbate biosynthesis pathway is incorrect, since most of these reactions do not occur in this organism." Click OK.

3. From the Tools menu select History → Clear. The History list keeps all of the objects you visited. By selecting this command you are resetting the list.
4. Click on the compound L-ascorbate (this adds the L-ascorbate frame to the history list), then navigate back to the pathway.
5. Open the Pathway Info Editor again, delete the word “L-ascorbate”, place the cursor where it used to be, and click the FRAME button. It will open the history list in a small window. Locate L-ascorbate and click on it. The software inserts the text “|FRAME: ASCORBATE|” into the text. Click OK.

Do you see the difference? Now the word “ascorbate” in the pathway summary is a hyperlink to the compound.

Don’t forget to save your changes.

Cyclic pathways

When editing cyclic pathways it is possible to specify which compound should be at the top. Often this can help in a better-looking diagram. Search for the “ppGpp metabolism” pathway” (Pathway → Search by Substring → ppgpp). Open it in the Pathway Editor. Right click on “GDP” and choose “Place this Compound at Cycle Top”. Exit the Pathway Editor.