

PathwayTools Tutorial Compounds, Reactions and Pathways

Exercise 1: Creating reactions and pathways

In this exercise you will:

- A. Create an organization and curator frames and specify your username.
- B. Create new reactions in the DB (or import them from MetaCyc).
- C. Create a new pathway.

A. Creating an organization frame for your institute, a curator frame for yourself, and setting the Pathway Tools Username.

1. **Open the *H. pylori* database.** All exercises will be using that PGDB.
Under the file menu, select “Create”, then “Organization”. Type in the required info, 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. If your login name is different from your workstation login, 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.

B. Creating reactions

Please read carefully before starting your work

Table 1 (on page 3) lists the reactions to be entered.

Since reactions may already 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 1). **The message will provide the Frame-IDs of the existing reactions, printed before the actual reaction (red circle in figure 1).**

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 (see below) 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 open the exercise file from the web, and use copy and paste). 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.
4. Repeat for all other reactions. Starting with the second reaction, you will most likely see a screen similar to that in figure 1. As discussed above, copy the Frame-ID to Table 1, then click either “Import” or “Delete” depending on the case.

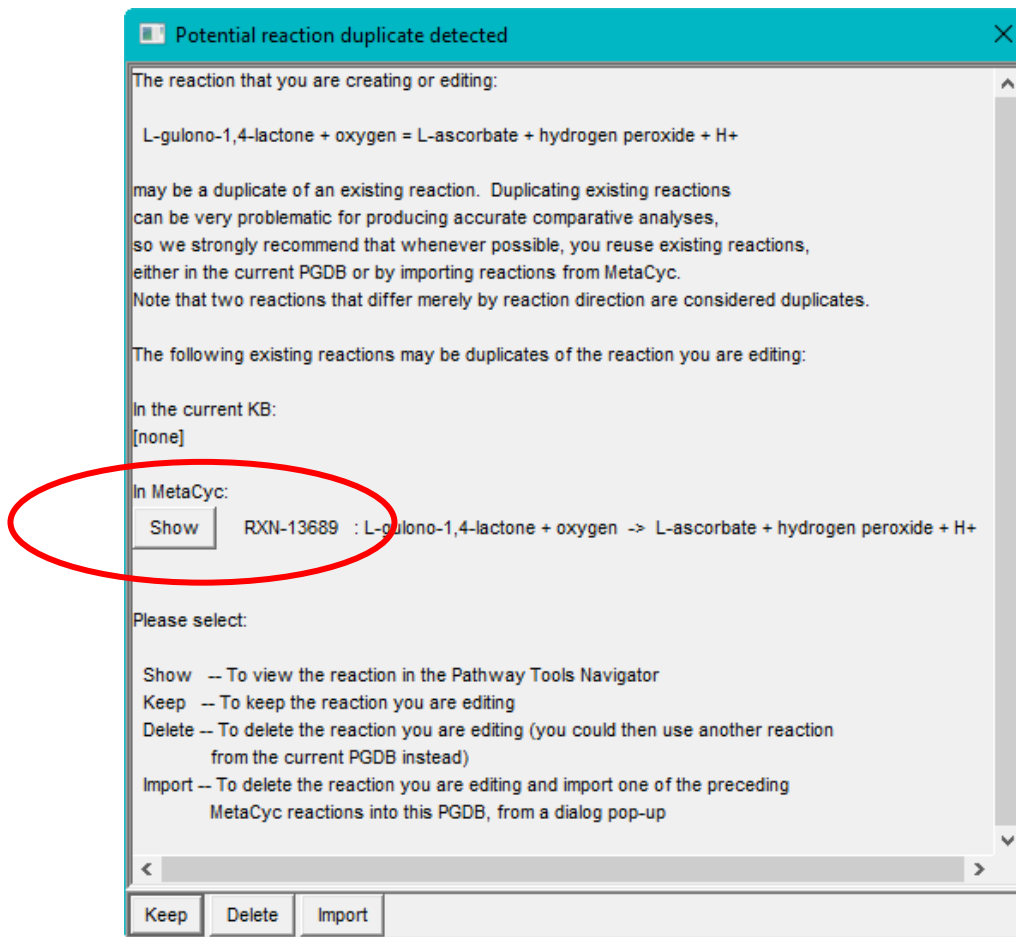


Figure 1: Duplicate Reaction dialog window

	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

C. 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:

(Pathways → Biosynthesis → Cofactor, Electron Carrier, and Vitamin Biosynthesis → Vitamin Biosynthesis → Ascorbate Biosynthesis)

Once you click OK, the **Pathway Info Editor** closes, and the **Pathway Editor** opens.

Using the Pathway Editor, add the 3 reactions that you created.

4. Select Reaction → Add Reaction(s) by Frame ID. Type in the Reaction IDs separated by white space, and click OK. You can also use “Reaction → Add Reaction(s) from History”. Once you have all three reactions, it’s a good time to choose Exit → Keep Changes and save your changes.

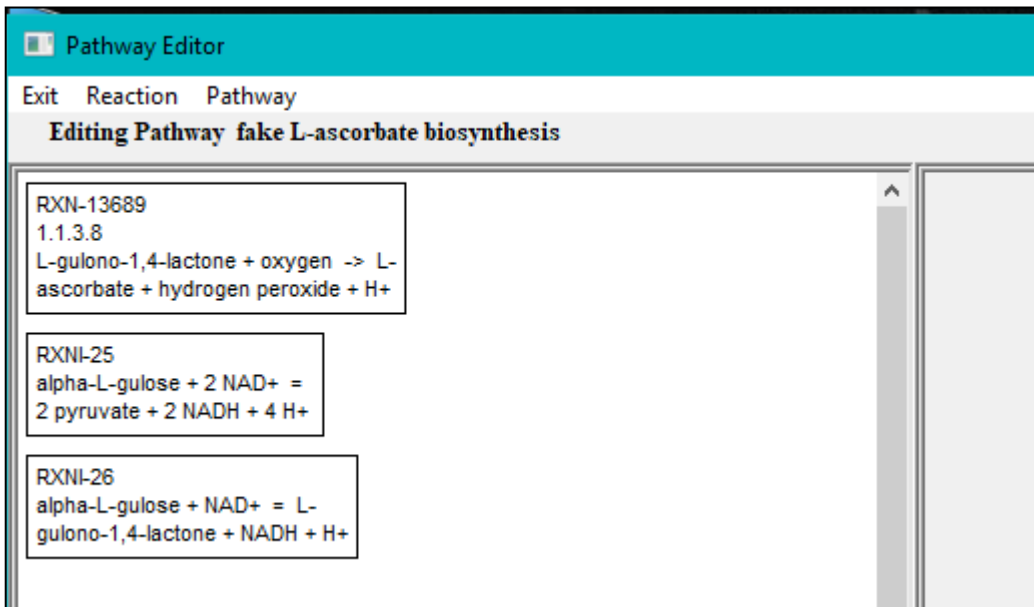


Figure 2: The Pathway Editor

5. Open the Pathway editor again (right click on the pathway name and choose “pathway editor”) and connect the reactions.

Start by clicking on the first reaction (alpha-L-gulose + 2 NAD+ = 2 pyruvate + 2 NADH + 4 H+). The reaction will become highlighted in red, and the reactions it can connect to will be highlighted in green (see figure 3).

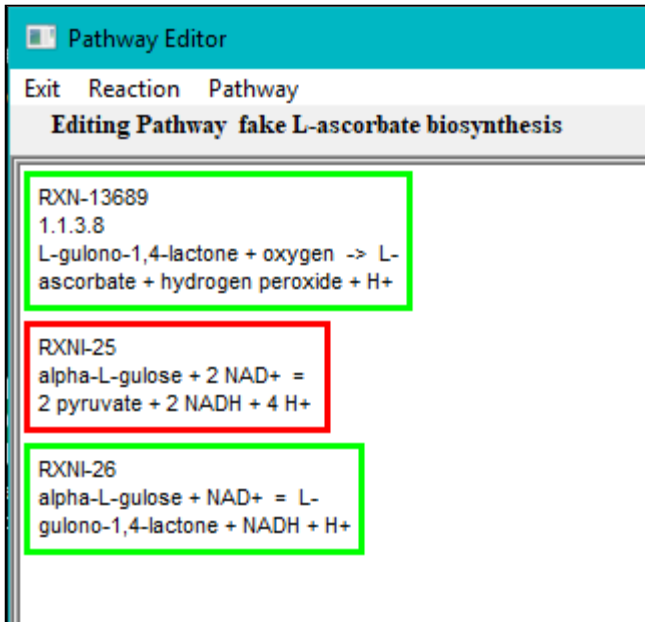


Figure 3

Click on the next reaction, and both reactions will move to the right pane. Now click on the last compound in the nascent pathway (figure 4) and repeat the process to finish the pathway. Exit, and save your changes.

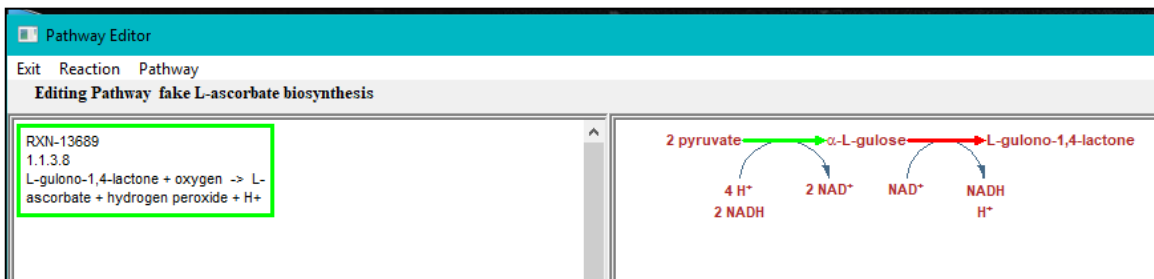


Figure 4

Adding pathway links

Add a link from the last compound of the new pathway to the pathway “pentose phosphate pathway (non-oxidative branch)” (this link makes no biological sense; we will do it just for the sake of practice).

1. Find the frame ID of the “glycolysis I (from glucose 6-phosphate)” pathway. Do it by searching for the pathway (Pathway → Search by Substring → glycolysis),

- right-clicking on its name, and selecting 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 the last compound, 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 Pentose Phosphate 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 ascorbate 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 degradation pathway in the Pathway Info Editor.
2. Type this sentence in the summary field: “This pathway is incorrect, since most of these reactions do not occur.” Click OK.
3. From the Tools menu select History → Clear.
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 “ascorbate”, place the cursor where it used to be, and click the FRAME button. It will open the history list in a 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?

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. Search for the "TCA cycle variation VIII (Helicobacter)" pathway (Pathway → Search by Substring → TCA). Open it in the Pathway Editor. Right click on "succinyl-CoA" and choose "Place this Compound at Cycle Top". Exit the Pathway Editor.