The MRSD web server for metabolic route search and design

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Method Article

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Abstract

The Metabolic Route Search and Design \(MRSD) server is an integrated platform for metabolic route search \(MRS) and metabolic route design \(MRD) based on the data from KEGG. MRS computes metabolic routes between the source metabolite and the product metabolite on the weighted compound transforming graph. Each graph represents a metabolic network. The output from a typical server run contains graphic illustrating metabolic routes between the appointed source and product metabolites, and the detailed information of these routes is also shown in the graph. MRD designs a route in an interactive mode according to the assigned metabolite and the users' choices. MRSD differs from other tools in providing rapid, user-friendly and versatile functions, including assistant tips and result visualization, as well as the complementary uses of MRS and MRD. Benefit from the carefully designed data format and the search algorithm, a typical search takes only a few seconds to get the result. The MRSD is freely accessible at "http://bioinfo.ustc.edu.cn/softwares/mrsd/":http://bioinfo.ustc.edu.cn/softwares/mrsd/.

Introduction

Synthetic biology is a new branch of biology and has some important and fast development. Different from traditional biology, synthetic biology construct new biological functions from biomolecular components. Synthetic biology has two complementary goals: To improve understanding of biological systems through mimicry and to produce bio-orthogonal systems with new functions. Designing and constructing new biological component and redesigning existing biological systems have great development and are benefited from the vast applications of engineered microorganisms. Computational prediction and design of metabolic pathways has numerous applications in systems biology and synthetic biology, such as the design of tracer and knockout experiments.

Equipment

A computer with Internet access and a web browser.

Procedure

This section includes three parts: the procedure of Metabolic Route Search \(MRS), Metabolic Route Design \(MRD) and the complementary usages of MRS and MRD. **Metabolic Route Search \(MRS)** Set source and product metabolites and specify search constraints 1. To search a route between two metabolites, visit the metabolic route search submodule webpage \(text to link":http://bioinfo.ustc.edu.cn/softwares/mrsd/SearchRoute.html) 2. Select represent type of metabolite, includes name, formula, KEGG ID or the InChI string. See figure in Figures section. Figure 3 representation types of the metabolites 3. Enter the source and product metabolite. See figure in Figures section. Figure 4 the source and product metabolites 4. Select the organisms in which you want to perform the metabolic route search. The type includes one organism, multiple organisms or all available organisms in KEGG.

See figure in Figures section. Figure 5 there are two options of the organisms' type: all available organisms and select. Detailed information about the options can be found in "Select the organisms" section. 5. Specify the metabolites that must be included or excluded. See figure in Figures section. Figure 6 the intermediates constraints CAUTION: The identifier used to represent the metabolites that should be included or excluded must be the same as the source and product metabolite, which means if you use name to represent the source and product metabolite, the included and excluded metabolite should use name, too. Otherwise, the system will be unable to deal with it. The user can specify a list of intermediate metabolites that should be included or excluded in the result metabolic routes. All these intermediate metabolites are separated by vertical lines. CAUTION: The number of the intermediate metabolites is not limited. Please notice that the number of the excluded metabolites has no influence on the time cost of computing, while the number of the included metabolites has remarkable influence. 6. Specify the maximum number of the result routes. The default number is ten, and you can change it as large as you want. The larger the number is, the more result routes you will get and more time you will spend. See figure in Figures section. Figure 7 the number constraint of the result routes 7. Click "continue" to check the validity of your inputs. CAUTION: The time of the search operation depends on the number of organisms that you have chosen, the size of the included metabolites and the maximum number of the result routes that you have specified. See figure in Figures section. Figure 8 Set the source and product metabolite and specify the other search constraints. Input and constraints check 8. Select if you want to display the detailed information of the provided candidates of your input metabolites. If you chose yes, then you can see the structure and the formula information of the metabolites, otherwise, these information will not be displayed. See figure in Figures section. Figure 9 select to display the details of the candidate metabolites or not CAUTION: If you chose yes, it may take a little time to load the information when you select a candidate metabolite each time. 9. Specify the source and product metabolites from the possible candidates that provided by the system. These candidates are calculated by MRS according the metabolite you input. See figure in Figures section. Figure 10 specify the source and product metabolites from the possible candidates 10. Check other constraints. 11. Submit the search task. After you have check the source and product metabolite and other constraints, then you can submit to perform this search. See figure in Figures section. Figure 11 Input and constraints check. 12. After the search process, the system provides two kinds of result: the graphic result and the detailed text result. See figure in Figures section. Figure 12 the result of the metabolic route search submodule. The first half is the graphic result, while the second half is the detailed text result. 13. Highlight the route. If you want to highlight a specific route in the graphic result, you can just click the corresponding highlight button of the route, and then the system will highlight it in the graphic result. See figure in Figures section. Figure 13 the highlight function of the MRS system **Metabolic Route Design \(MRD)** Set start metabolites and specify design constraints 1. To design a route from a metabolite, visit the metabolic route search submodule webpage \(text to link":http://bioinfo.ustc.edu.cn/softwares/mrsd/RouteDesign.html). 2. Select the direction of the metabolic route design operation. There two kinds of direction: forward and backward. See figure in Figures section. Figure 14 the direction option of the design process CAUTION: The system provides the candidate metabolites mainly based on the direction of the design operation. The result will be vastly different from one direction to another. 3. Select the representation type of the

metabolite. 4. Set the start metabolite. See figure in Figures section. Figure 15 specify the start metabolite of design process 5. Click “Continue” to check your inputs. See figure in Figures section. Figure 16 Set start metabolite and specify design constraints 6. If you just want to know how the system works, click the “Example” button, and then the system will provide an predefined start metabolite and other default constraints to start the design process. Input and constraints check 7. Select if you want to display the detailed information about the provided candidates of your inputted metabolites. 8. Check the direction of the design process. 9. Check the representation type of the metabolite. 10. Specify the start metabolites from the possible candidates that provided by the system. These candidates are calculated by MRD according to the metabolite you have inputted. See figure in Figures section. Figure 17 Check the inputs of design process. The interactive design process 11. According to the start metabolite and the design direction, the MRD system calculates and provides all possible candidates in KEGG. The MRSD system also provides the reaction information of the specified metabolite and the candidate, which can assist user to select candidate. TROUBLESHOOTING See figure in Figures section. Figure 18 Mouse over the picture of the candidate metabolite to view the details of the reactions between the specified metabolite and the candidate. 12. The user can choose any of the candidate metabolites to continue the design process recurrently. If one candidate has already existed in the previous steps, then it will be highlighted in case the user reselects it which will make a circle. See figure in Figures section. Figure 19 The MRD system will highlight the candidates that have already been selected in the previous design process. 13. If there are no candidates available or the user has found the route he wanted, the design process ends. 14. Save the design route result. In each step, you can view the result file and save it in your local computer. See figure in Figures section. Figure 20 Save the result file in your local computer CAUTION: If you save the result file in order to restart the design process in the future, please don’t modify the content of the result file, which may cause some undefined errors and unable for the MRD system to analyze the data in the file. Restart the design process from previous paused point 15. Select the previous design result file and upload it to restart the design process. Then the system will analyze the previous result file to get necessary information to continue the design process. See figure in Figures section. Figure 21 Upload the previous metabolic route design result file **Use MRD to modify the result of MRS** In “the complementary uses of MRS and MRD” section, we introduce the methods how to combine the two systems to fulfill the user’s needs. In this section, we will show the detailed protocol of the conjunctive uses of MRS and MRD. 1. Use MRS to search the metabolic routes between the specified source and product metabolite. 2. Chose the route that you want to modify, and then click “design it” button to revise this route using MRD. See figure in Figures section. Figure 22 Use the "design it" function to modify the route 3. After you click the “design it” button, the system will switch from MRS to MRD. In the MRD system, you can select the candidate metabolites that MRD provided to add step to the existing route, or you can use the “delete the last step” function to delete steps in the route and then redesign it. See figure in Figures section. Figure 23 Use the MRD system to redesign the route 4. You can save this route to the local after you get the right metabolic route.

Timing

Getting profit from the well-designed storage format of the compound transform graph and the k-shortest algorithm, it only takes few seconds to perform a typical metabolic route search. But it may still spend more time, nevertheless, when you chose to search the metabolic route in multiple organisms, specify a series of metabolites that should be included and set the number of maximum result route a high value. In MRD, the system takes only a few seconds to compute the possible candidates according to your input or selection. This procedure will be terminated only if there are no possible candidates for the current metabolite or the user have gotten the right route. So the time-consuming is highly dependent on the user other than the system.

Troubleshooting

Step 9 in MRS: Why there are no candidate metabolites for the source and product metabolites in the details check page? At present, the system only use fuzzy query to search if your input is in the database, and then provides the possible candidate metabolites for your selection. If your input metabolite does not exist in the database or even not a part of the metabolites existing in the database, the system is unable to provide any candidates metabolites. Therefore, please try to use a part of your words which may work if there are no candidates provided. Another situation, if you use the InChI string to represent the metabolite. Even if you are very sure that the metabolite exists in the database which turn out it does, but the system still provides nothing, and then try to use another representation type. Because we analyze the InChI string of the metabolites from KEGG database, and there are only a part of the metabolites have such information, that is, not all metabolites in the database have corresponding InChI string information. Step 11 in MRS: Why it shows “No route found” in the search result page? According to the fact that not all metabolite pairs are connected in the metabolic network, it is possible that there are no routes between the source and product metabolite that you have specified. Step 11 in MRD: Why it shows “No next metabolites for *****” in the metabolic design process? This message indicates that the metabolite you have selected has no successor or predecessor metabolites, which means there are no reactions that use this metabolite as reactant to produce other metabolites or vice versa. In order to continue the design process, you should go back or delete the last step to select any other candidate metabolites that the MRD system has provided. Step 15 in MRD: Why it shows “Cannot analyze the uploaded file, ********”? Please make sure that you have uploaded the right file, the MRD system can only analyze the .txt file with correct file format. Step 15 in MRD: Why it shows “No route information”, or “The design direction information has missed” or other similar error message? In order to restart the design process from the previous paused point, the MRD system should know the following information: the details of previous design result, the direction of the design and the last metabolite when the process is paused, which are the firs three sections in the design result file. Please make sure you have not modified these information in the result file, otherwise it will be unable for the MRD system to analyze it.

Anticipated Results

The result of the MRS contains two parts: the graphic result and the detailed text result. The graph visualization tool Graphviz \(text to link":http://www.graphviz.org/) is used to depict the searching results. The graphic result provides a global view of the metabolic routes between the source and product metabolite, which is clarity and simplicity for the user to find the routes which he need. The text result shows the detailed information of individual metabolic routes, which contains the steps in the route and the reactions' information in each step. The result that provided by the MRD system in each step can be divided into three parts: the candidate metabolites, the steps that have designed, and the detailed information of the previous design result. As shown in Table 1, the design result file contains four sections, and the last section is human-readable which provides the detailed information of the result route. In the result file, the reactions are separated by vertical line if there are multiple reactions in one step.

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Acknowledgements

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Figures

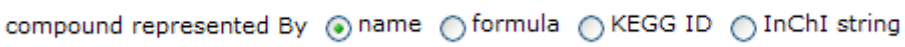


Figure 1

Figure 3 representation types of the metabolites

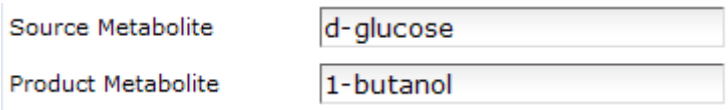


Figure 2

Figure 4 the source and product metabolites

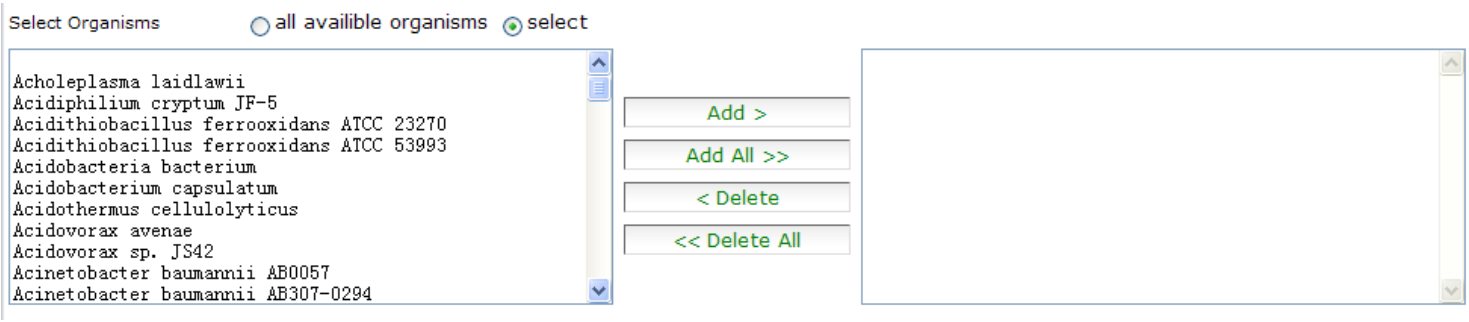


Figure 3

Figure 5 organisms type there are two options of the organisms' type: all available organisms and select. Detailed information about the options can be found in "Select the organisms" section.

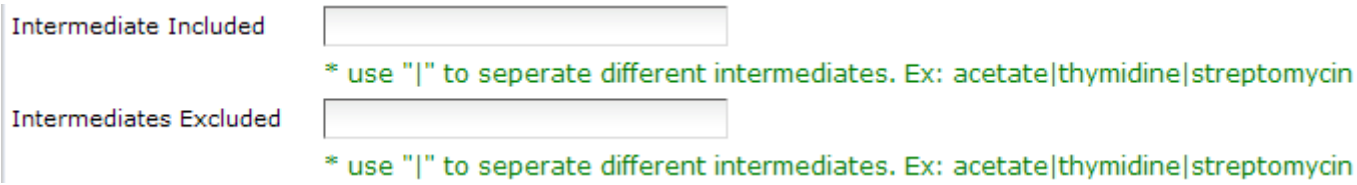


Figure 4

Figure 6 the intermediates constraints the intermediates constraints

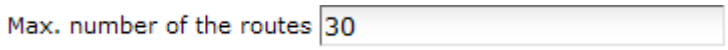


Figure 5

Figure 7 the number constraint of the result routes the number constraint of the result routes

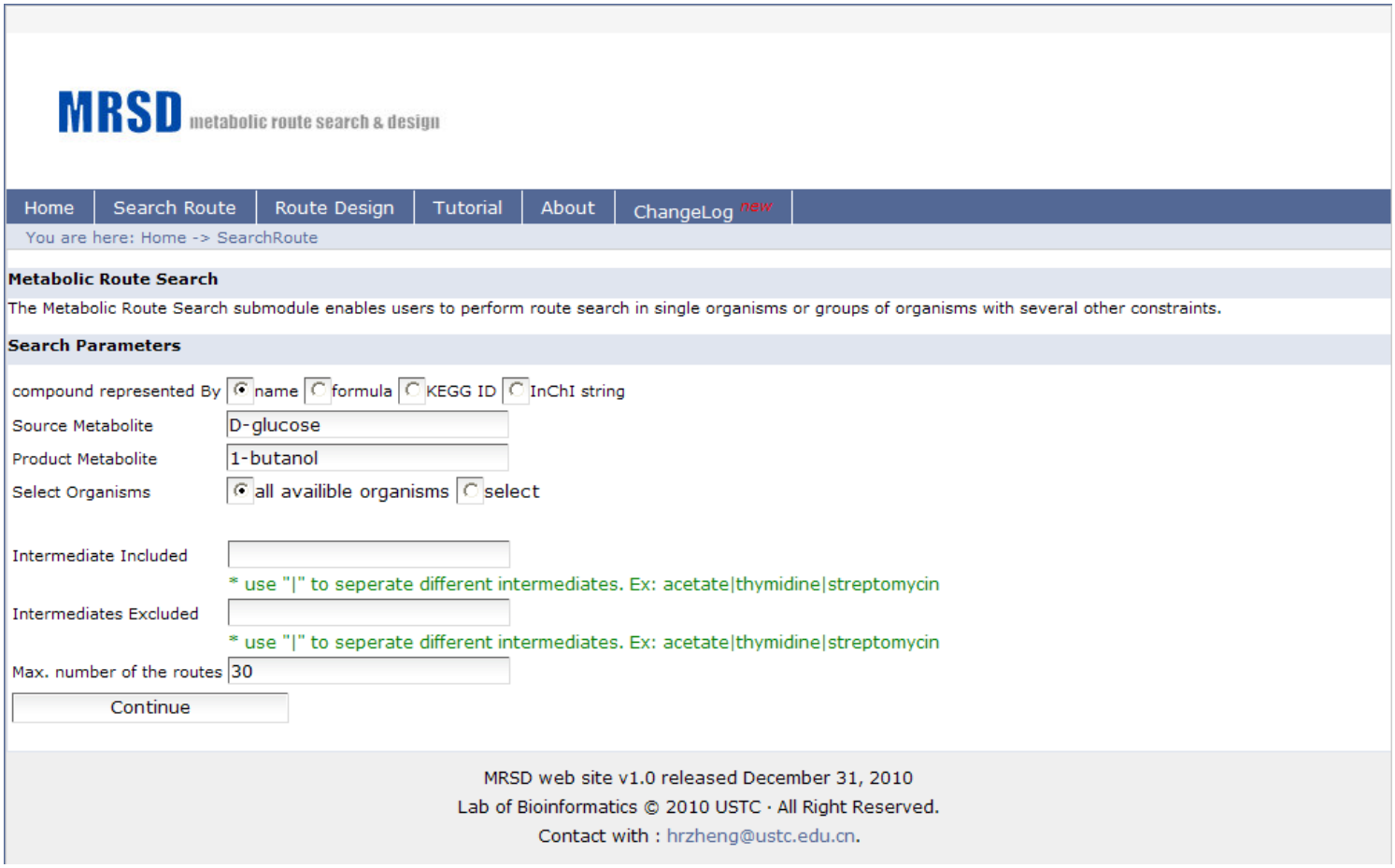


Figure 6

Figure 8 Set the source and product metabolite and specify the other search constraints Set the source and product metabolite and specify the other search constraints.

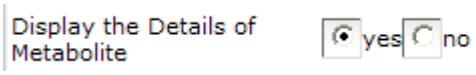


Figure 7

Figure 9 select to display the details of the candidate metabolites or not select to display the details of the candidate metabolites or not.

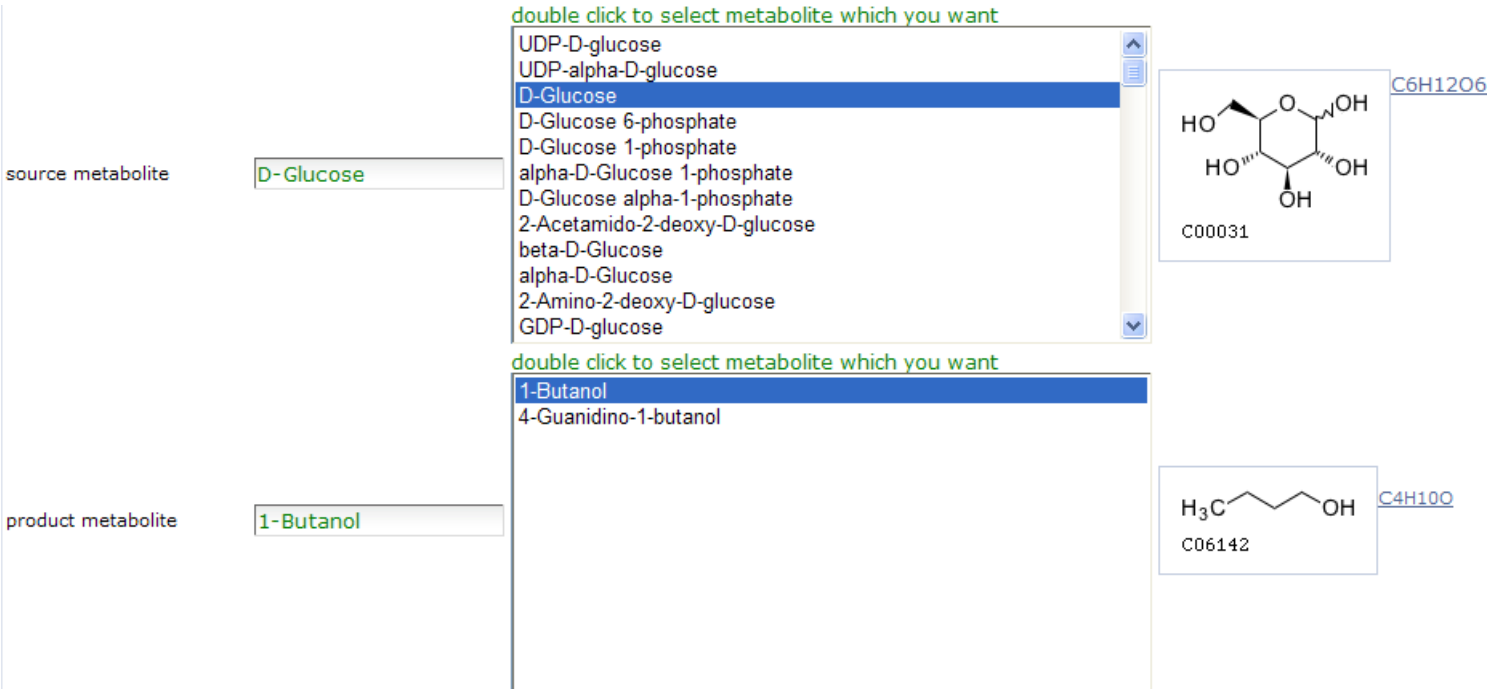


Figure 8

Figure 10 specify the source and product metabolites from the possible candidates specify the source and product metabolites from the possible candidates

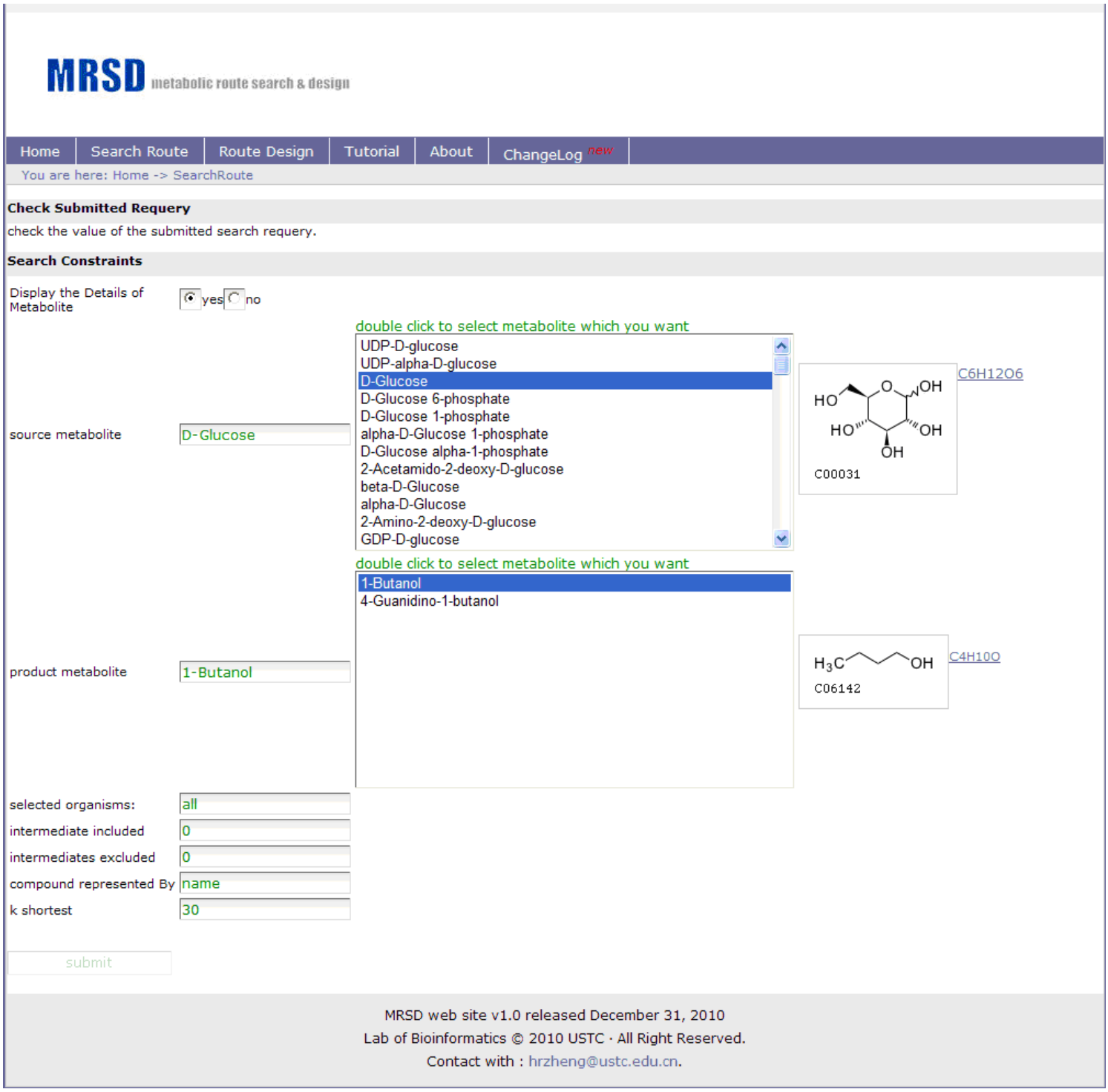


Figure 9

Figure 11 Input and constraints check Input and constraints check.

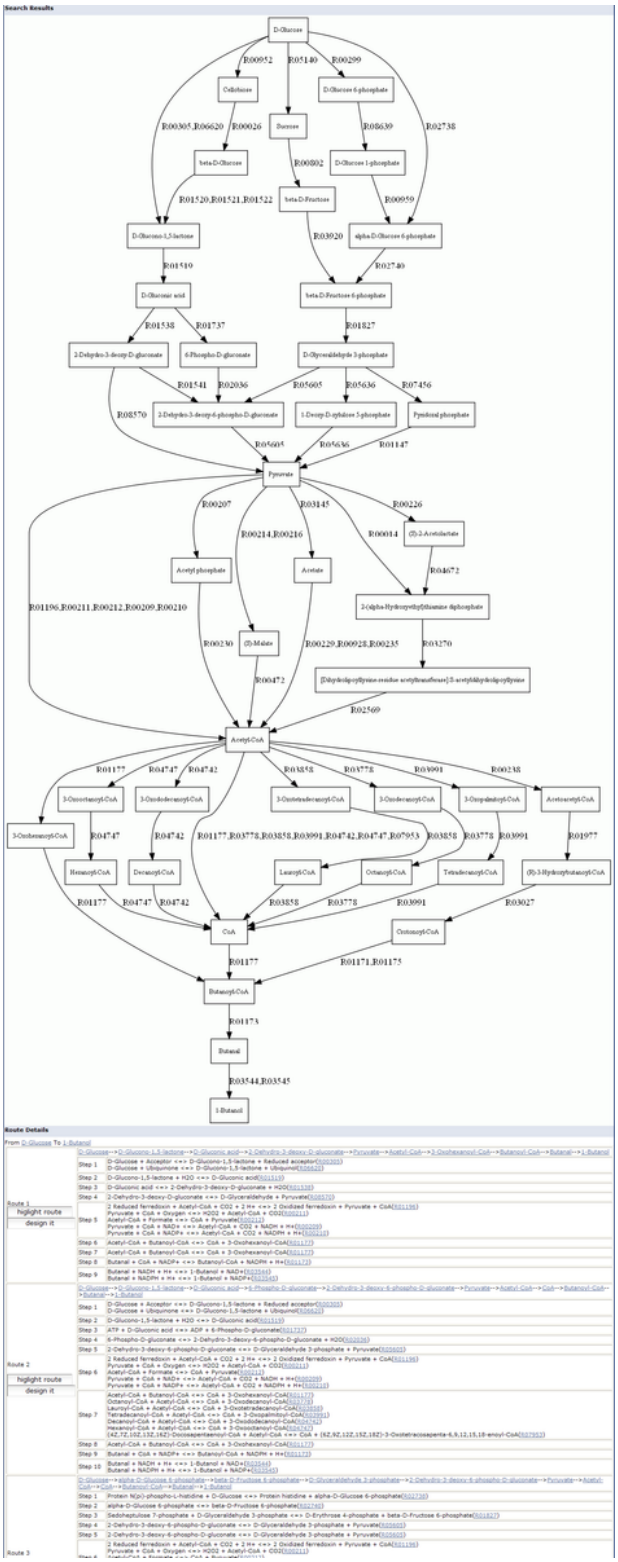


Figure 10

Figure 12 the result of the metabolic route search submodule the result of the metabolic route search submodule. The first half is the graphic result, while the second half is the detailed text result.

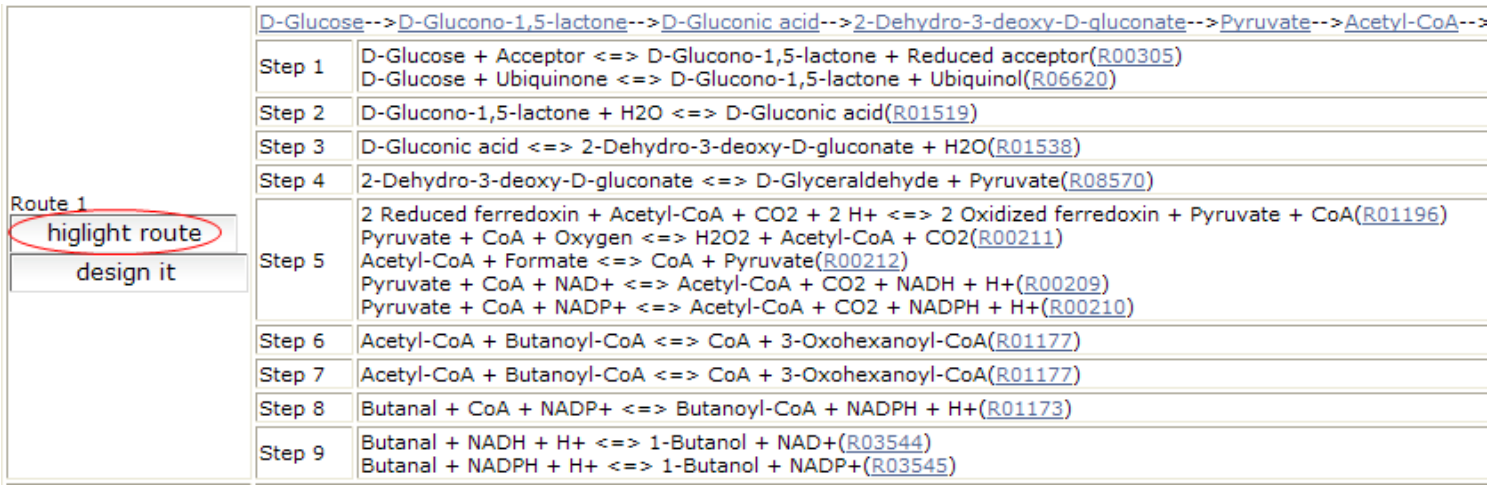


Figure 11

Figure 13 the highlight function of the MRS system the highlight function of the MRS system

Figure 12

Figure 14 the direction option of the design process the direction option of the design process

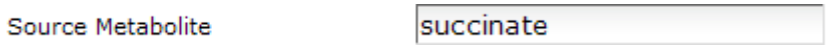


Figure 13

Figure 15 specify the start metabolite of design process

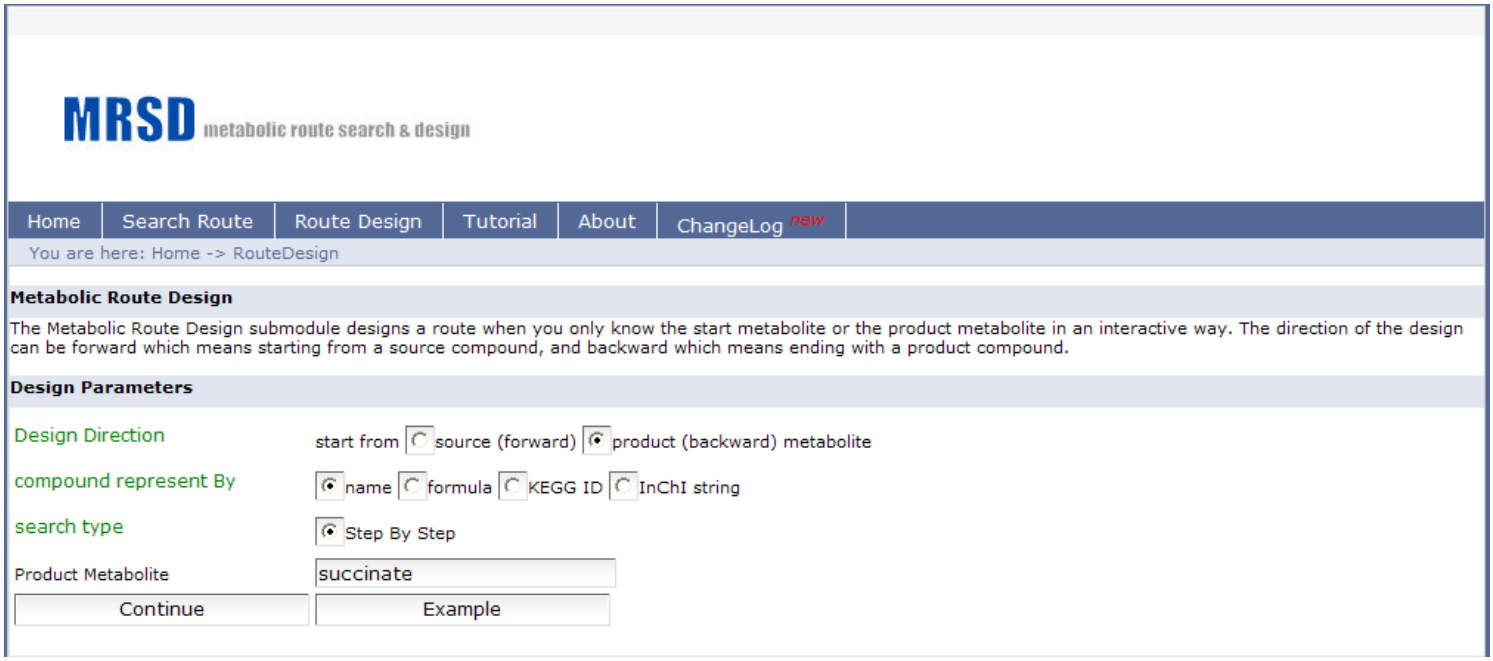


Figure 14

Figure 16 Set start metabolite and specify design constraints

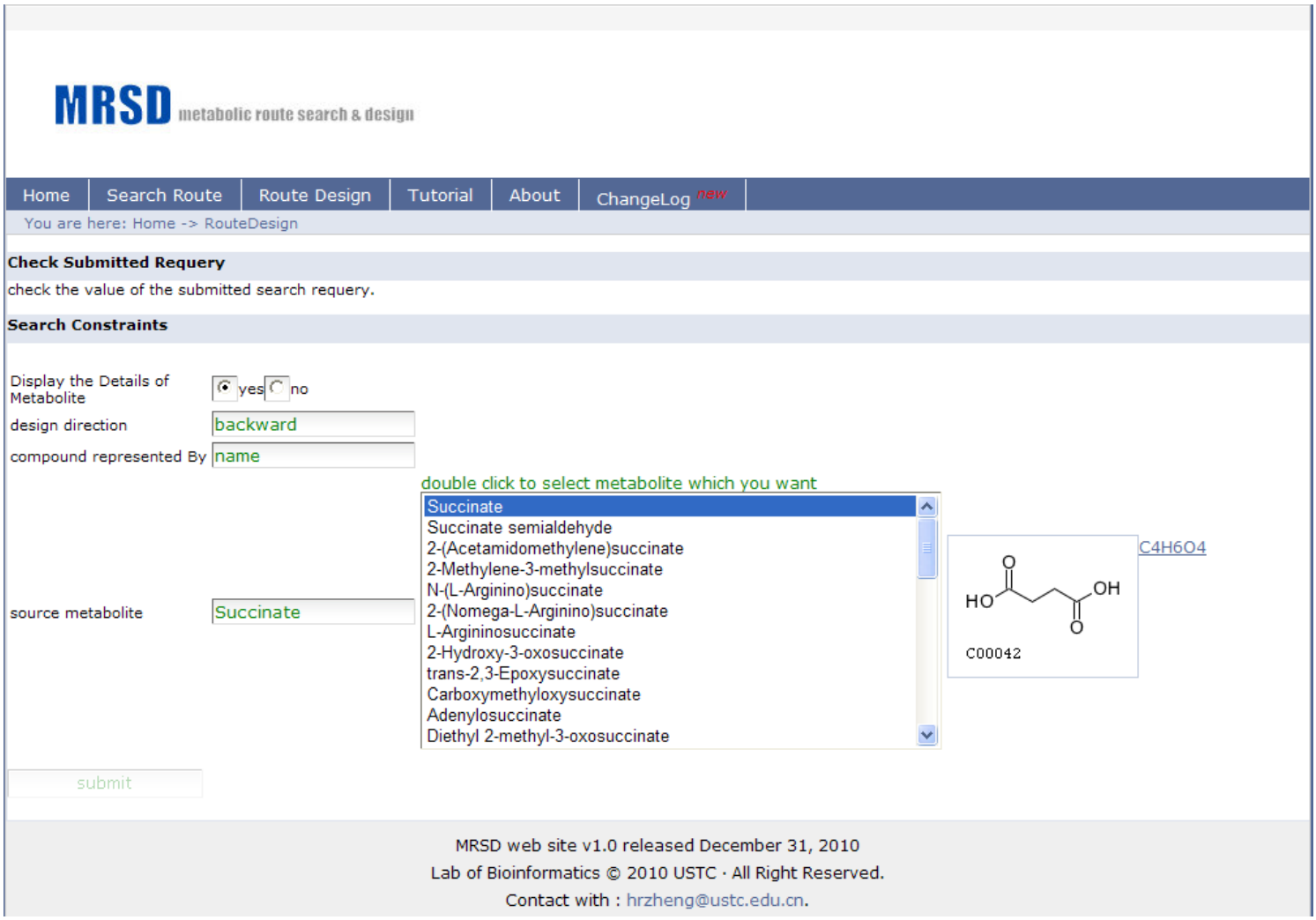


Figure 15

Figure 17 Check the inputs of design process

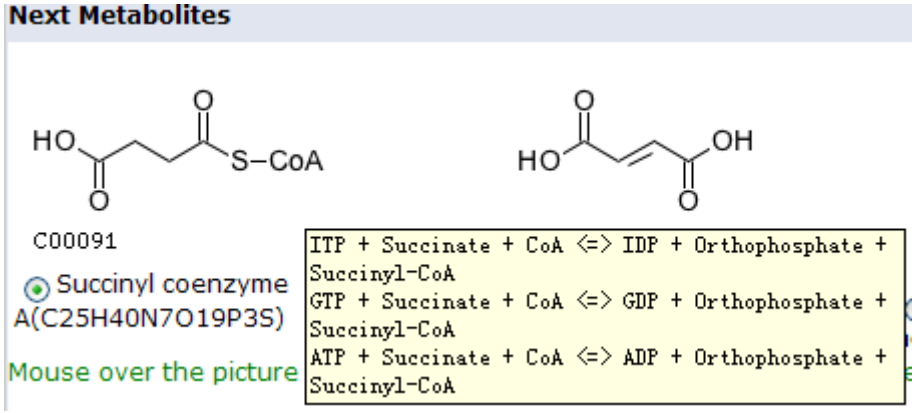


Figure 16

Figure 18 view the details of reactions Mouse over the picture of the candidate metabolite to view the details of the reactions between the specified metabolite and the candidate

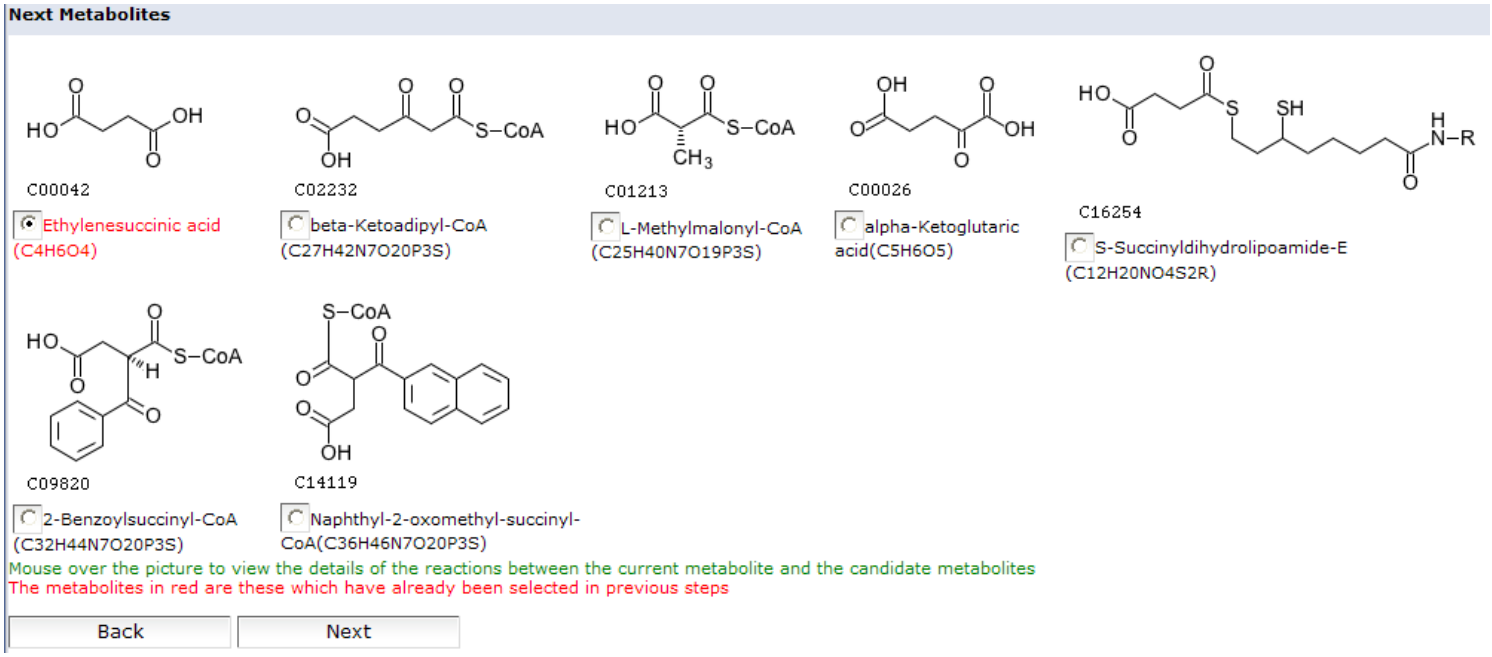


Figure 17

Figure 19 highlight the candidate The MRD system will highlight the candidates that have already been selected in the previous design process.

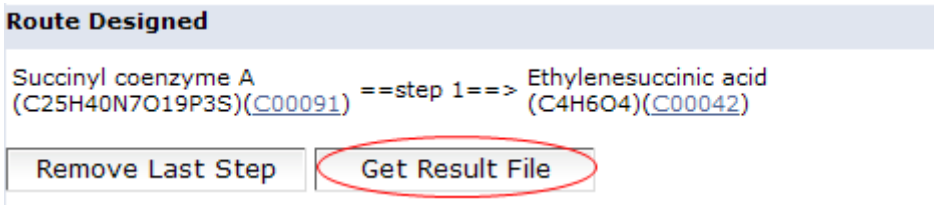


Figure 18

Figure 20 Save the result file in your local computer

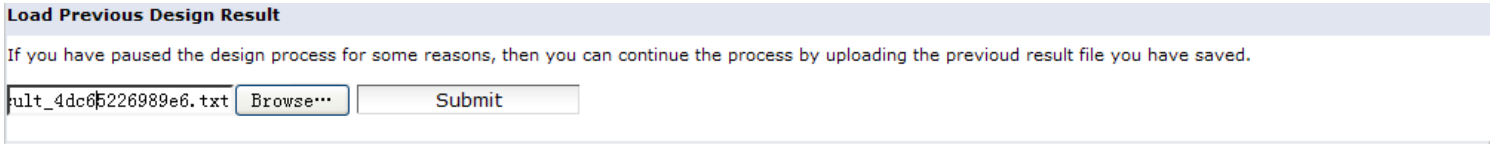
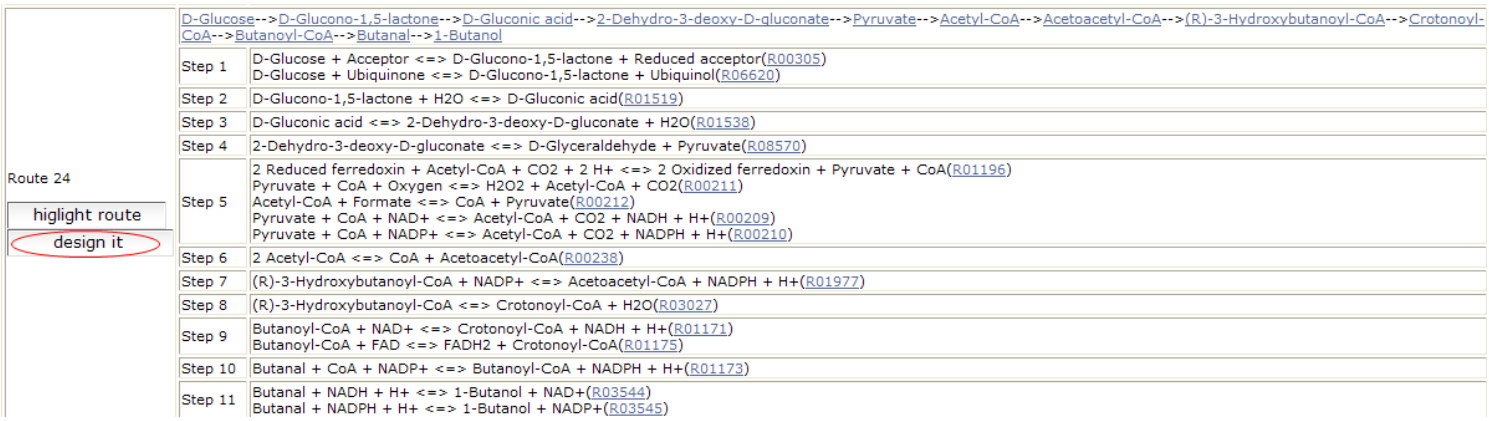


Figure 19

Figure 21 Upload the previous metabolic route design result file



Route 24

higlight route

design it

D-Glucose-->D-Glucono-1,5-lactone-->D-Gluconic acid-->2-Dehydro-3-deoxy-D-gluconate-->Pyruvate-->Acetyl-CoA-->Acetoacetyl-CoA-->(R)-3-Hydroxybutanoyl-CoA-->Crotonoyl-CoA-->Butanoyl-CoA-->Butanal-->1-Butanol

Step 1	D-Glucose + Acceptor <=> D-Glucono-1,5-lactone + Reduced acceptor(R00305) D-Glucose + Ubiquinone <=> D-Glucono-1,5-lactone + Ubiquinol(R06620)
Step 2	D-Glucono-1,5-lactone + H2O <=> D-Gluconic acid(R01519)
Step 3	D-Gluconic acid <=> 2-Dehydro-3-deoxy-D-gluconate + H2O(R01538)
Step 4	2-Dehydro-3-deoxy-D-gluconate <=> D-Glyceraldehyde + Pyruvate(R08570)
Step 5	2 Reduced ferredoxin + Acetyl-CoA + CO2 + 2 H+ <=> 2 Oxidized ferredoxin + Pyruvate + CoA(R01196) Pyruvate + CoA + Oxygen <=> H2O2 + Acetyl-CoA + CO2(R00211) Acetyl-CoA + Formate <=> CoA + Pyruvate(R00212) Pyruvate + CoA + NAD+ <=> Acetyl-CoA + CO2 + NADH + H+(R00209) Pyruvate + CoA + NADP+ <=> Acetyl-CoA + CO2 + NADPH + H+(R00210)
Step 6	2 Acetyl-CoA <=> CoA + Acetoacetyl-CoA(R00238)
Step 7	(R)-3-Hydroxybutanoyl-CoA + NADP+ <=> Acetoacetyl-CoA + NADPH + H+(R01977)
Step 8	(R)-3-Hydroxybutanoyl-CoA <=> Crotonoyl-CoA + H2O(R03027)
Step 9	Butanoyl-CoA + NAD+ <=> Crotonoyl-CoA + NADH + H+(R01171) Butanoyl-CoA + FAD <=> FADH2 + Crotonoyl-CoA(R01175)
Step 10	Butanal + CoA + NADP+ <=> Butanoyl-CoA + NADPH + H+(R01173)
Step 11	Butanal + NADH + H+ <=> 1-Butanol + NAD+(R03544) Butanal + NADPH + H+ <=> 1-Butanol + NADP+(R03545)

Figure 20

Figure 22 Use the "design it" function to modify the route

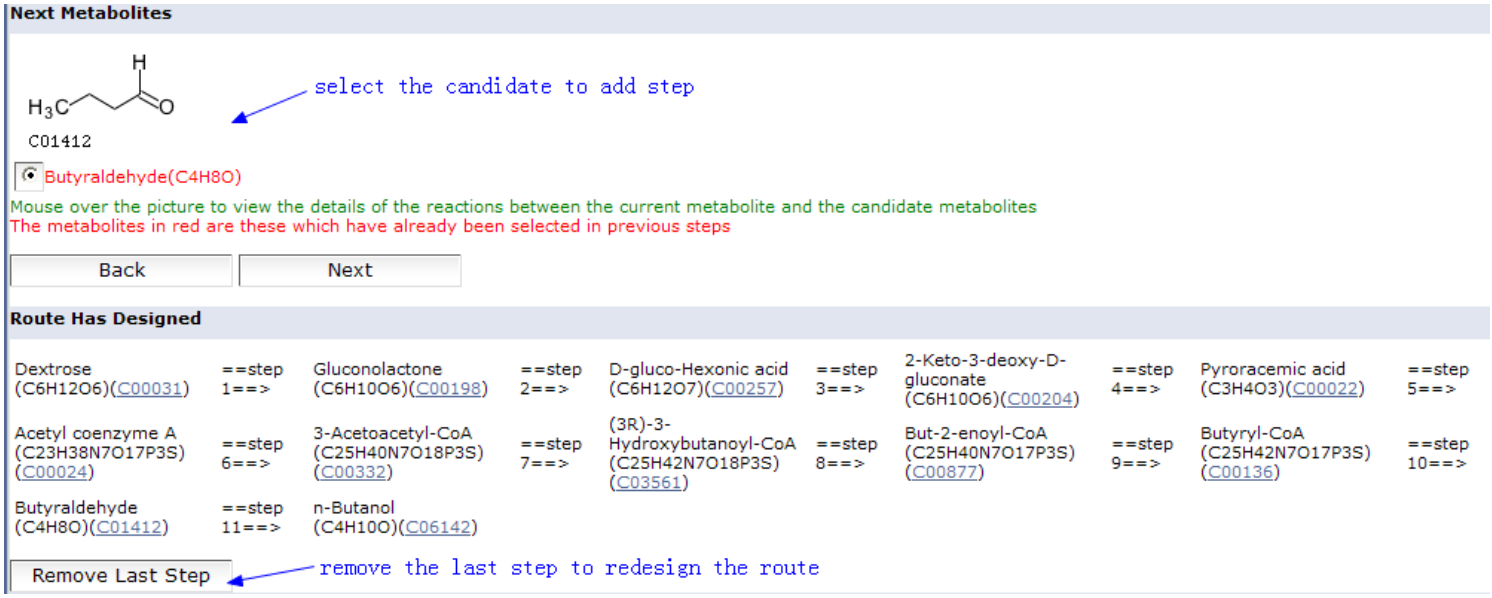


Figure 21

Figure 23 Use the MRD system to redesign the route