

The case study featured in this work can be replicated using the test classes in the library GitHub repository (named YeastTests), which are already configured with the appropriate parameters. To run this example within OptFlux, load the included SBML model (*iMM904_corrected_201609001170165318.xml*) into a new Optflux project and open the minimal cut set enumeration dialog. Instructions regarding this step are provided in the article. Create an environmental condition (**File** tab, **Create...** option, **Environmental condition** button) and add two constraints, namely:

- R_EX_glc_e_ with lower bound of -1.15 and upper bound of 999999
- R_ATPM_ with both lower and upper bounds of 1 (untick the **Show only drains** box so that this reaction appears)

Additionally, a set of critical reactions is also required to reduce the computation time and discard unwanted knockouts. To import this set, click on the **File** menu tab, **Import** option, and the **Critical genes/reactions...** button. Once the dialog appears, select the provided file containing critical reactions (*nontargets#[aerobic#glucose].txt* on the *SupportFiles* folder) and select the reactions option. Both the critical reactions and the environmental conditions will appear as project elements on the left of your screen.

Once the environmental condition is createThe problem inputs go as follows:

- Project: the project containing the iMM904 model reconstruction
- Select environmental conditions: select the environmental condition that you have created in the previous step
- Maximum number of modifications: the value used in this example was 9, but the results are available from size 6
- Critical reactions: Tick the **Exclude drain reactions** and **Exclude transport reactions** boxes and select the critical reaction set that was created in the previous step
- Objective configuration:
 - Biomass: R_biomass_SC5_notrace
 - Product: R_EX_succ_e_
 - Substrate: R_EX_glc_e_
- Parameter configuration: Minimum biomass value and minimum product value at 0.0001. Select the yield option.

Press OK and wait until the enumeration process is completed. This is a resource intensive process which may take several hours depending on the maximum number of modifications that are allowed. Once it is completed, a minimal cut set enumeration result will appear, from which you are able to select solutions and export them using the appropriate tools provided by OptFlux.