

1. Draft reconstruction

- 11 Obtain genome annotation.
- 21 Identify candidate metabolic functions.
- 31 Obtain candidate metabolic reactions.
- 41 Assemble draft reconstruction.
- 51 Collect experimental data.



2. Refinement of reconstruction

- 61 Determine and verify substrate and cofactor usage.
- 71 Obtain neutral formula for each metabolite.
- 81 Determine the charged formula.
- 91 Calculate reaction stoichiometry.
- 101 Determine reaction directionality.
- 111 Add information for gene and reaction localization.
- 121 Add subsystems information.
- 131 Verify gene–protein-reaction association.
- 141 Add metabolite identifier.
- 151 Determine and add confidence score.
- 161 Add references and notes.
- 171 Flag information from other organisms.
- 181 Repeat Steps 6 to 17 for all genes.
- 191 Add spontaneous reactions to the reconstruction.
- 201 Add extracellular and periplasmic transport reactions.
- 211 Add exchange reactions.
- 221 Add intracellular transport reactions.
- 231 Draw metabolic map (optional).
- 24–321 Determine biomass composition.
- 331 Add biomass reaction.
- 341 Add ATP-maintenance reaction (ATPM).
- 351 Add demand reactions.
- 361 Add sink reactions.
- 371 Determine growth medium requirements.

Data assembly and dissemination

- 951 Print Matlab model content.
- 961 Add gap information to the reconstruction output.



4. Network evaluation

- 43–441 Test if network is mass-and charge balanced.
- 451 Identify metabolic dead-ends.
- 46–481 Perform gap analysis.
- 491 Add missing exchange reactions to model.
- 501 Set exchange constraints for a simulation condition.
- 51–581 Test for stoichiometrically balanced cycles.
- 591 Re-compute gap list.
- 60–651 Test if biomass precursors can be produced in standard medium.
- 661 Test if biomass precursors can be produced in other growth media.
- 67–751 Test if the model can produce known secretion products.
- 76–781 Check for blocked reactions.
- 79–801 Compute single gene deletion phenotypes.
- 81–821 Test for known incapabilities of the organism.
- 831 Compare predicted physiological properties with known properties.
- 84–871 Test if the model can grow fast enough.
- 88–941 Test if the model grows too fast.



3. Conversion of reconstruction into computable format

- 381 Initialize the COBRA toolbox.
- 391 Load reconstruction into Matlab.
- 401 Verify S matrix.
- 411 Set objective function.
- 421 Set simulation constraints.