

1. Draft reconstruction

- 1| Obtain genome annotation.
- 2| Identify candidate metabolic functions.
- 3| Obtain candidate metabolic reactions.
- 4| Assemble draft reconstruction.
- 5| Collect experimental data.



2. Refinement of reconstruction

- 6| Determine and verify substrate and cofactor usage.
- 7| Obtain neutral formula for each metabolite.
- 8| Determine the charged formula.
- 9| Calculate reaction stoichiometry.
- 10| Determine reaction directionality.
- 11| Add information for gene and reaction localization.
- 12| Add subsystems information.
- 13| Verify gene–protein–reaction association.
- 14| Add metabolite identifier.
- 15| Determine and add confidence score.
- 16| Add references and notes.
- 17| Flag information from other organisms.
- 18| Repeat Steps 6 to 17 for all genes.
- 19| Add spontaneous reactions to the reconstruction.
- 20| Add extracellular and periplasmic transport reactions.
- 21| Add exchange reactions.
- 22| Add intracellular transport reactions.
- 23| Draw metabolic map (optional).
- 24–32| Determine biomass composition.
- 33| Add biomass reaction.
- 34| Add ATP-maintenance reaction (ATPM).
- 35| Add demand reactions.
- 36| Add sink reactions.
- 37| Determine growth medium requirements.

4. Network evaluation

- 43–44| Test if network is mass-and charge balanced.
- 45| Identify metabolic dead-ends.
- 46–48| Perform gap analysis.
- 49| Add missing exchange reactions to model.
- 50| Set exchange constraints for a simulation condition.
- 51–58| Test for stoichiometrically balanced cycles.
- 59| Re-compute gap list.
- 60–65| Test if biomass precursors can be produced in standard medium.
- 66| Test if biomass precursors can be produced in other growth media.
- 67–75| Test if the model can produce known secretion products.
- 76–78| Check for blocked reactions.
- 79–80| Compute single gene deletion phenotypes.
- 81–82| Test for known incapacilities of the organism.
- 83| Compare predicted physiological properties with known properties.
- 84–87| Test if the model can grow fast enough.
- 88–94| Test if the model grows too fast.



Data assembly and dissemination

- 95| Print Matlab model content.
- 96| Add gap information to the reconstruction output.

3. Conversion of reconstruction into computable format

- 38| Initialize the COBRA toolbox.
- 39| Load reconstruction into Matlab.
- 40| Verify S matrix.
- 41| Set objective function.
- 42| Set simulation constraints.

