

## Flux Scanning based on Enforced Objective Function

The Flux Scanning based on Enforced Objective Function (FSEOF) method is a technique employed to identify genetic modifications that can enhance the production of a desired metabolite in a target organism.

### Overview of FSEOF

#### 1. Objective:

- The main goal of FSEOF is to identify gene knockout or overexpression targets that can increase the flux towards a desired product in a metabolic network.

#### 2. Methodology:

- **Step 1:** Set the objective as maximizing the production of the desired metabolite in the FBA model. This is done by modifying the objective function to include the desired metabolite.
- **Step 2:** Constrain the production of the target compound from 5% to 95% of the value calculated previously, using the growth rate as objective function
- **Step 3:** Identify reactions whose flux increased/decreased consistently.
- **Step 4:** Filter the reactions identified with flux variability analysis simulations. Consider reactions that satisfy:  $v_{maxj} - v_{minj} > 0$
- **Step 5:** For each reaction, perform an overexpression simulation (e.g., 1.2x) using an appropriate method (e.g., MOMA). Determine the phenotypic evaluation function, defined as:

$$i. \quad f_{ph} = \frac{v_{carotenoid,OE}}{v_{carotenoid,WT}} \cdot \frac{v_{biomass,OE}}{v_{biomass,WT}},$$

where  $v_{carotenoid}$  are the production rates of the target carotenoid,  $v_{biomass}$  are the growth rates. OE and WT design for overexpression and wild-type, respectively.