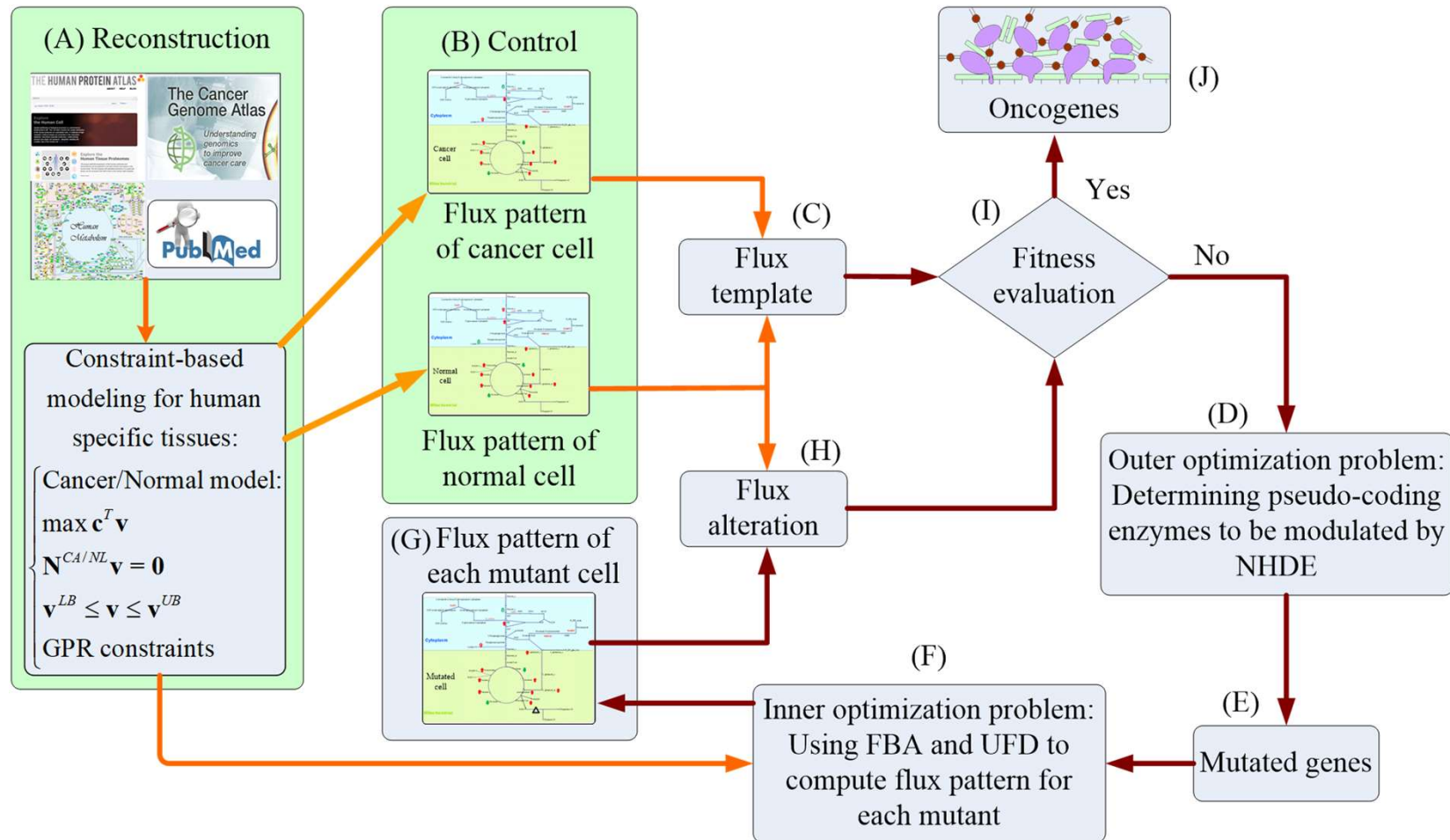# Numerical Example for Computing Template, Similarity Ratio and LFC

Flowchart of the in silico experiment for inferring oncogenes

- (A) Reconstruct the cancer and normal models. (B) Compute the flux distributions of cancer and normal models. (C) Build the flux template according to the flux distributions of cancer and normal models. (D)–(J) Simulation of a wet lab experiment for determining oncogenes. Orange arrows indicate the building processes of the flux template that acted as the control in the oncogene inference problem. Red arrows present the mutant schemes for formulating the tri-level oncogene inference problem.

Example: Template, Similarity Ratio and LFC

- We use a numerical example to illustrate the fitness evaluation in Step (D) of the above flowchart. The fitness is applied for the NHDE algorithm to infer oncogenes, iteratively.

- Suppose that a toy metabolic network consisted of 5 metabolites, and their synthesis rates for the normal model and cancer model. Both rates are then applied to build the flux template, which it serves as the control for the fitness evaluation.

- Suppose that the NHDE algorithm is selected three mutant genes, and their flux distributions as follows:

Synthesis rate for each metabolite:

$$r_m = \sum_{m_i \in \Omega^c} \left(\sum_{N_{ij} > 0, j} N_{ij} v_{f,j} - \sum_{N_{ij} < 0, j} N_{ij} v_{b,j} \right), m \in \Omega^m$$

			Flux Pattern		
	Normal	Cancer	Mutant 1	Mutant 2	Mutant 3
Metabolite 1	1	4	2.24	2.1	3.9
Metabolite 2	0.5	1.5	0.76	4	1.5
Metabolite 3	1	0.7	0.93	0.4	1.07
Metabolite 4	0.5	0.25	0.44	1.1	0.5
Metabolite 5	1	1.5	1.04	1.35	1.48

- Using the above table, the log2 fold changes (LFC$_m$) for the template and each mutant are computed as follows:

$LFC_m^T = \log_2\left(r_{m,\text{cancer}}/r_{m,\text{normal}}\right)$		$LFC_m = \log_2\left(r_{m,\text{mutant}}/r_{m,\text{normal}}\right)$		
	Template	Mutant 1	Mutant 2	Mutant 3
Metabolite 1	2	1.1635	1.0704	1.9635
Metabolite 2	1.585	0.6041	3	1.585
Metabolite 3	-0.5146	-0.1047	-1.3219	0.0976
Metabolite 4	-1	-0.1844	1.1375	0.0
Metabolite 5	0.585	0.0566	1.8074	0.5656

- The lower and upper bounds the template for evaluating the membership functions

Lower and upper bounds of each membership function:

$$LFC_m^{CABL,LB} = \begin{cases} LFC_m^T/4, & \text{if } LFC_m \geq 0 \\ 4\times LFC_m^T, & \text{if } LFC_m < 0 \end{cases}$$

$$LFC_m^{CABL,UB} = \begin{cases} 4\times LFC_m^T, & \text{if } LFC_m \geq 0 \\ LFC_m^T/4, & \text{if } LFC_m < 0 \end{cases}$$

	$LFC_m^T\_{LB}$	$LFC_m^T\_{UB}$
Metabolite 1	0.5	8
Metabolite 2	0.3962	6.3399
Metabolite 3	-2.0583	-0.1286
Metabolite 4	-4	-0.25
Metabolite 5	0.1462	2.3399

- Suppose that we set 3% of the tolerance for increase/decrease, i.e. $tol_+ = 0.0426$ and $tol_- = -0.0439$

- The similarity indicator, μ_m, and similarity ratio, SR are computed as follows:

Similarity indicator:

$$\mu_m^M = \begin{cases} 1, & \text{if } LFC_M^{MUBL} > tol_+ \text{ and } LFC_M^{CABL} > tol_+ \\ -1, & \text{if } LFC_M^{MUBL} < tol_- \text{ and } LFC_M^{CABL} < tol_- \\ 0, & \text{otherwise} \end{cases}$$

	Similarity indicator, μ_m		
	Mutant 1	Mutant 2	Mutant 3
Metabolite 1	1	1	1
Metabolite 2	1	1	1
Metabolite 3	-1	-1	0
Metabolite 4	-1	0	0
Metabolite 5	1	1	1
$SR = \sum_{m=1}^{5} |\mu_m| \Big/ 5$	1.0	0.8	0.6

- The membership grades are evaluated by the left and right membership function. Both functions are defined and calculated as follows:

Left membership function:

$$\eta_m^L(LFC_m^{MUBL}) = \frac{LFC_m^{MUBL} - LFC_m^{CABL,LB}}{LFC_m^{CABL} - LFC_m^{CABL,LB}}$$

Right membership function:

$$\eta_m^R(LFC_m^{MUBL}) = \frac{LFC_m^{CABL,UB} - LFC_m^{MUBL}}{LFC_m^{CABL,UB} - LFC_m^{CABL}}$$
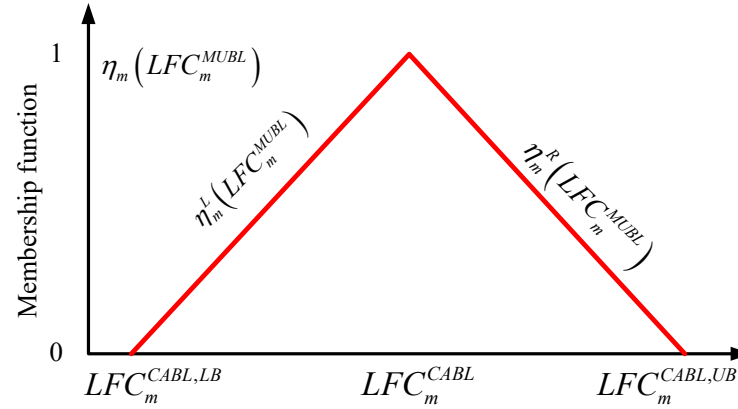
Component	Mutant 1		Mutant 2		Mutant 3	
	$\eta_m^L(LFC_m^{MUBL})$	$\eta_m^R(LFC_m^{MUBL})$	$\eta_m^L(LFC_m^{MUBL})$	$\eta_m^R(LFC_m^{MUBL})$	$\eta_m^L(LFC_m^{MUBL})$	$\eta_m^R(LFC_m^{MUBL})$
Metabolite 1	0.4423	1.1394	0.3803	1.1549	0.9756	1.0061
Metabolite 2	0.1748	1.2063	2.1904	0.7024	1	1
Metabolite 3	1.2655	-0.062	0.477	3.092	1.3966	-0.5863
Metabolite 4	1.2719	-0.0874	1.7125	-1.85	1.3333	-0.3333
Metabolite 5	-0.2044	1.3011	3.7863	0.3034	0.9559	1.011

- Using the definition of the left and right membership function, the membership grades and the fitness for each mutant are obtained as follows:

Fuzzy equal membership function:

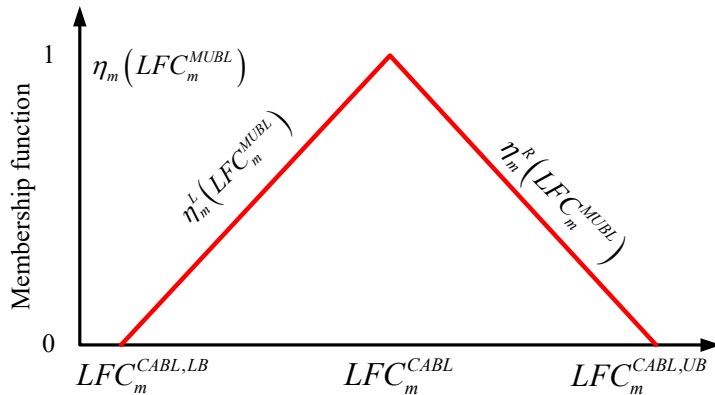$$\eta_m\left(LFC_m^{MUBL}\right) = \max\left\{\min\left(\eta_m^L, \eta_m^R, 1\right), 0\right\}$$

Mean for all metabolites :

$$\eta_E = \sum_{m=1}^{5} \eta_m / 5$$

The fitness for each mutant:

$$\eta_D = \left[\left(SR + \eta_E\right)/2 + \min\left\{SR, \eta_E\right\}\right]/2$$

where $SR = \eta_S$

	Mutant 1	Mutant 2	Mutant 3
Metabolite 1	0.4423	0.3803	0.9756
Metabolite 2	0.1748	0.7024	1
Metabolite 3	0	0.477	0
Metabolite 4	0	0	0
Metabolite 5	0	0.3034	0.9559
η_E	0.1234	0.3726	0.5863
$\eta_S = SR^T$	1.0	0.8	0.6
η_D	0.3426	0.4795	0.5897

- The fitnesses for all mutants are then provided for the NHDE algorithm for generating the next mutated genes from Step (D)-(I), iteratively, towards achieving oncogenes.