Understanding Flux Re-routing in Metabolic Networks Through an Analysis of Synthetic Lethal Pairs

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Motivation

- Robustness in metabolic systems can be attributed to the presence of multiple alternate pathways that have identical metabolic functions. This introduces metabolic redundancies.
- These redundancies show surprising variance in their distribution – some are due to gene duplication, while the others span different metabolic submodules.
- Higher-order lethals offer a straightforward method to study alternate pathways. When a single reaction that comprises a higher-order lethal is deleted, a complex rerouting of fluxes occurs in the network.
- Very little is known about how these organisms reroute their fluxes.
- Double lethals, in particular, have been classified based on the activity of individual reactions into Plastic Synthetic Lethals (PSL) and Redundant Synthetic Lethals (RSL) [1].
- The presence of two distinct reaction pair classes calls for us to analyze the cause behind the selective activation of reactions.

How do reactions that are present in different metabolic submodules, compensate for each other? Are the inactive reactions more metabolically inefficient than the active ones? What kind of reactions make up the RSL pairs, especially since they are both simultaneously active?

- Studying flux rerouting helps us to understand the complex metabolic reroutings that occur in several diseases, especially in the case of cancer.
- It will enable us in finding better therapeutic cures for diseases.

Highlights

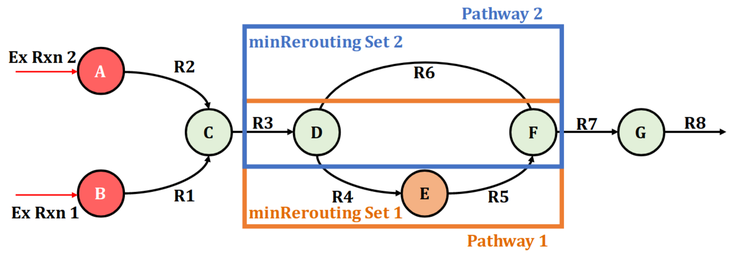
In this paper, we

- Describe a **constraint-based approach** to unravel these alternate pathways.
- Propose a **novel optimization method** that minimizes the extent of rerouting between reactions that comprise synthetic lethal pairs.
- Analyze the robustness introduced by **PSL/RSL pairs and the metabolic efficiency** of the reactions that comprise them.
- Perform a **systematic analysis of synthetic lethals** by identifying the reaction classes that make up these synthetic lethals.

Approach - minRerouting

In order to identify the minimal reaction set, we propose a novel approach, minRerouting. We use a $p-norm$ solution approach and

1. Simultaneously solve for flux distributions that satisfy the stoichiometric constraints, maximize the biomass constraint (with a slack of δ)
2. Minimize the number of reactions with varying metabolic flux values.



Approach - minRerouting

Three different minRerouting sets were obtained based on the norms employed: sparse (l_0), linear (l_1) and quadratic (l_2). The results obtained were compared across norms and models. The mathematical formalism of our algorithm is as follows:

$$\min \quad \|v_{\Delta R1} - v_{\Delta R2}\|_p \tag{1a}$$
$$s.t \;\; Sv_{\Delta R1} = 0; \quad Sv_{\Delta R2} = 0; \tag{1b}$$
$$v_{LB} \leq v_{\Delta R1} \leq v_{UB}; \quad v_{LB} \leq v_{\Delta R2} \leq v_{UB}; \tag{1c}$$
$$v_{\Delta R1,R1} = 0; \quad v_{\Delta R2,R2} = 0; \tag{1d}$$
$$v_{\Delta R1,bio} \geq (1-\gamma)v^*_{\Delta R1,bio}; \quad v_{\Delta R2,bio} \geq (1-\gamma)v^*_{\Delta R2,bio}; \tag{1e}$$

Approach - PSL/RSL Reactions

As an enhancement to the method in [1], we propose a more thorough classification approach using conditional FVA. Using the maximum and minimum possible fluxes for each reaction, the following classification approach was used:

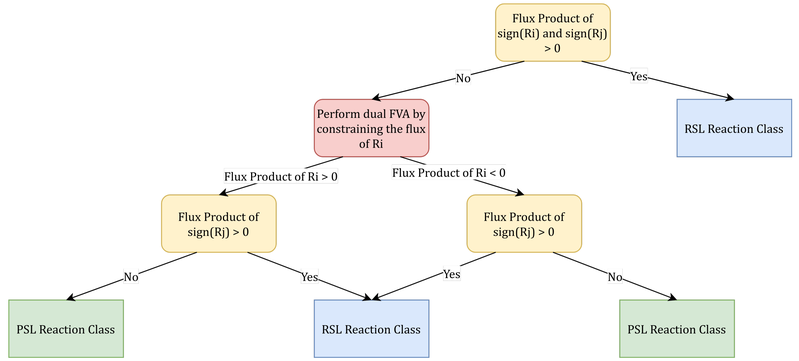


Figure 2. Flowchart depicting the classification of reaction pairs into PSL or RSL pairs.

Once the lethal pairs were classified as either PSL or RSL, each of the individual reactions were then classified based on their metabolic efficiency, using parsimonious Flux Balance Analysis (pFBA) [2]. The reaction classes returned from pFBA are - Enzymatically Less Efficient (ELE), Metabolically Less Efficient (MLE), Zero Flux, Blocked and pFBA Optimal Reactions. The pFBA class combination distribution for all the lethal pairs of an organism was obtained and analyzed.

Results

Our algorithm, minRerouting was used to identify the minimal rerouting set for 11 Genome Scale Metabolic Models (GSMMs). The main take away from the results are as follows:

- 0-norm optimization identified the minimal rerouting set of least size, followed by 1 and 2-norm.
- 0 and 1-norm optimizations resulted in approximately the same range of common minimal rerouting set size.
- While the cluster sizes returned by 2-norm optimization are much higher, the absolute flux difference between the rerouting sets is smallest for 2-norm, followed by 1 and 0-norm.

Results

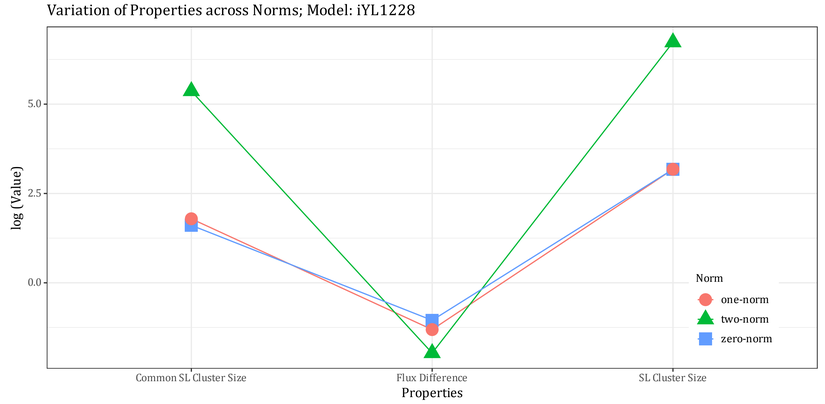


Figure 3. Variation of properties such as minRerouting Cluster Size, Common Cluster Size and Flux Difference. The values presented are in the log scale.

Our proposed approach to study the metabolic efficiencies of reaction that comprise PSL and RSL pairs was applied on all the models and we found that 60% of the models had $> 90\%$ of PSL pairs belonging to (pFBA Optimal, pFBA Optimal) pairs, and 40% models had 100% (pFBA Optimal, pFBA Optimal) pairs. As pFBA Optimal reactions are considered more metabolically active, than when compared to MLE and ELE reactions, our hypothesis was validated.

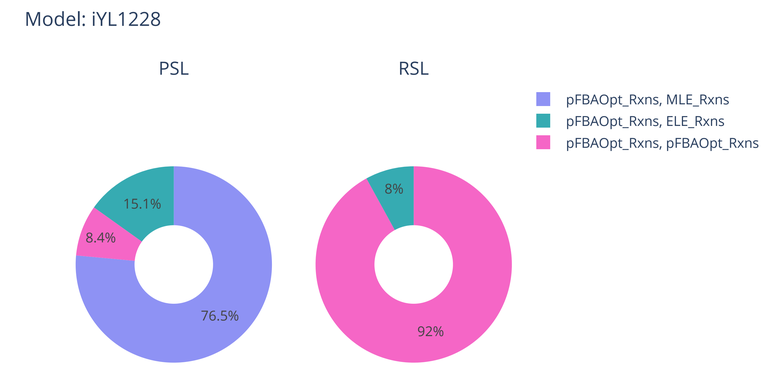


Figure 4. Reaction pair distribution between the RSL and PSL classes.

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