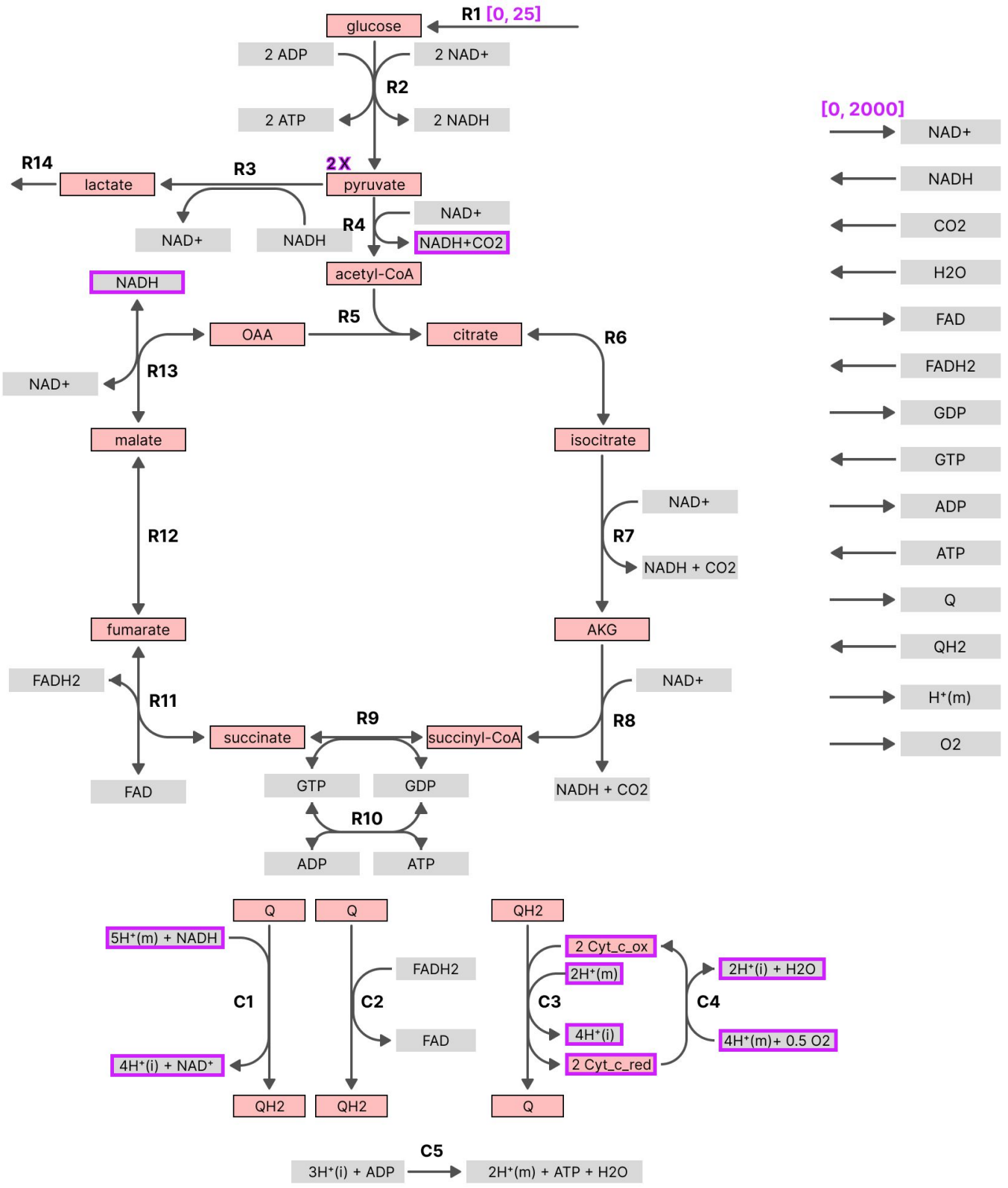
1. (30 pts) The figure below illustrates a simplified view of glycolysis, the TCA cycle, and oxidative phosphorylation (OXPHOS). Construct a metabolic model that includes the reactions and metabolites shown in the figure. Perform a parsimonious flux balance analysis (pFBA) to maximize total ATP production. *Note: You may add sink and demand reactions for the metabolites shown in grey boxes as needed. (hint: course 1's demo1)*

2. (15 pts) Conduct a flux variability analysis (FVA) on your model. Identify all reactions whose knockout results in a ≥50% reduction in the flux range of reaction C5 compared to the wild-type model. *(hint: course 1's demo2)*



3. (15 pts) Using the model constructed in Q1, gradually decrease the upper bounds of reactions C1, **C3**, **C1 and C3 (together)**, and C5 to observe their impact on lactate production. Test the following upper bounds: [0, 10, 100, 300, 500, 1000]. Plot the resulting lactate production as line graphs for each reaction. *(hint: course 1's demo2)*

4. (10 pts) Download the RNA-Seq TPM dataset from GEO accession GSE291717 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE291717). Perform a $\log_2$ (TPM + 1) transformation on the data. Then, using the textbook model, calculate the reaction activity score for each reaction. Apply maximum (max) for "OR" relationships and minimum (min) for "AND" relationships according to the GPR rules. *(hint: pipeGEM's documentation)*

5. (10 pts) For each sample, estimate the expression threshold as the 90th percentile of the $\log_2$(TPM + 1) values. *(hint: pipeGEM's documentation)*

6. (10 pts) Using the threshold obtained in Q5, apply the GIMME algorithm to reconstruct a sample-specific model for each sample. In the reconstructed models, remove all reactions that are inferred to have zero flux. Please compare their numbers of reactions, genes, and metabolites. *(hint: pipeGEM's documentation)*

7. (10 pts) Perform randomized flux sampling (100 samples) on the sample-specific models for the following four samples *(hint: course 1's demo2)*:
WT_M9-glycerol-r1
WT_M9-glycerol-r2
u-ETS-4H_M9-succinate-r1
u-ETS-4H_M9-succinate-r2

Compare the flux distributions of the objective reaction between the WT group (WT_M9-glycerol-r1 and WT_M9-glycerol-r2) and the u-ETS-4H group (u-ETS-4H_M9-succinate-r1 and u-ETS-4H_M9-succinate-r2) by drawing a violin plot.

8. (Bonus 10 pts) Implement a modified GIMME algorithm that minimizes the sum of squared differences between the flux values and the activity cutoffs. Use the data from Q4 and your new algorithm to reconstruct a sample-specific model for WT_M9-glycerol-r1. Compare its GIMME flux with the model created in Q6.

9. (Bonus 10 pts) Implement a naive pruning-based algorithm that removes low-expressed reactions as follows:

- Identify reactions whose Reaction Activity Score (rxn_score) is below the expression threshold.
- Iteratively attempt single knockouts, starting with the reaction that has the lowest rxn_score.
- After each knockout, check whether the model remains consistent (i.e., it can still generate nonzero flux through the objective reaction).
- Only permanently remove a reaction if consistency is maintained.

Apply this algorithm to reconstruct a sample-specific model for WT_M9-glycerol-r1. Finally, compare the pFBA fluxes of this model to those generated in **Q6**.