

Supplementary file 4. Stoichiometric model for *P. pastoris*

Methanol Metabolism

1. MET => FORM
2. FORM => FOR + NADH
3. FOR => NADH + CO₂
4. XU5P + FORM + ATP => ADP + GAP + DHA
5. GAP => G3P
6. DHA => G3P

Glycolysis and Gluconeogenesis Pathways

7. GLC + ATP => G6P + ADP
8. G6P <=> F6P
9. F6P + ATP => 2G3P + ADP
10. 2 G3P => F6P + Pi
11. G3P + ADP + Pi => 3PG + ATP + NADH
12. 3PG + ATP + NADH => G3P + ADP + Pi
13. 3PG <=> Pep
14. Pep + ADP => Pyr + ATP
15. Pyr => ACCoA_{mit} + CO₂ + NADH_{mit}
16. Pyr => ACCoA_{cyt} + CO₂ + NADH_{cyt}
17. Pyr + CO₂ + ADP => Oaa + ATP

Pentose Phosphate Pathway

18. G6P + 2NADP => RU5P + 2 NADPH + CO₂

19. $\text{RU5P} \rightleftharpoons \text{R5P}$
20. $\text{RU5P} \rightleftharpoons \text{XU5P}$
21. $\text{R5P} + \text{XU5P} \rightleftharpoons \text{S7P} + \text{G3P}$
22. $\text{S7P} + \text{G3P} \rightleftharpoons \text{F6P} + \text{E4P}$
23. $\text{XU5P} + \text{E4P} \rightleftharpoons \text{F6P} + \text{G3P}$

TCA cycle

24. $\text{ACCoA}_{\text{mit}} + \text{Oaa} \Rightarrow \text{CIT}$
25. $\text{CIT} \Rightarrow \text{ICIT}$
26. $\text{ICIT} \Rightarrow \text{AKG} + \text{CO}_2 + \text{NADH}_{\text{mit}}$
27. $\text{AKG} \Rightarrow \text{SUCCoA} + \text{CO}_2 + \text{NADH}_{\text{mit}}$
28. $\text{SUCCoA} + \text{Pi} + \text{ADP} \Rightarrow \text{SUC} + \text{ATP}$
29. $\text{SUC} + \text{ATP} \Rightarrow \text{SUCCoA} + \text{ADP} + \text{Pi}$
30. $\text{SUC} \Rightarrow \text{FUM} + \text{FADH}_2$
31. $\text{FUM} \rightleftharpoons \text{MAL}$
32. $\text{MAL} \Rightarrow \text{Oaa} + \text{NADH}_{\text{mit}}$

Biosynthesis of amino acids

Serine Family

33. $3\text{PG} + \text{Glu} \Rightarrow \text{Ser} + \text{AKG} + \text{NADH} + \text{Pi}$
34. $\text{Ser} + \text{THF} \Rightarrow \text{Gly} + \text{MetTHF}$
35. $\text{Ser} + \text{ACCoA} + \text{H}_2\text{S} \Rightarrow \text{Cys}$

Alanine Family

36. $\text{Pyr} + \text{NADPH} \Rightarrow \text{Ala} + \text{NADP}$

37. Pyr + Glu => AKG

38. 2 Pyr + NADPH_{mit} => Kval + CO₂

39. Kval + Glu => Val + AKG

40. Kval + ACCoA_{mit} + Glu => Leu + AKG + NADH + CO₂

Histidine Family

41. R5P + ATP => PRPP + AMP

42. PRPP + ATP + Gln => His + AKG + Pi + 2 NADH

Aspartic Family

43. OAA + Glu => Asp + AKG

44. Asp + Gln + ATP => Asn + Glu + AMP

45. Asp + ATP + 2 NADPH => Ser + ADP + Pi

46. Thr + NADPH_{mit} + Glu + Pyr => Ile + AKG + NH₄ + CO₂

47. ACCoA + Ser + H₂S + MTHF => Met + THF

Aromatic Family

48. 2 Pep + E4P + ATP + NADPH => CHOR + ADP + 4 Pi

49. CHOR + Glu => Phe + AKG + CO₂

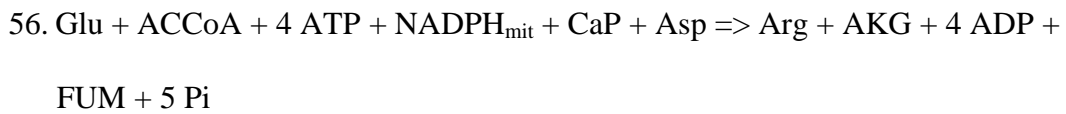
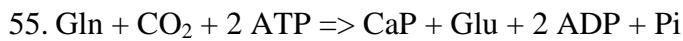
50. CHOR + Glu => Tyr + AKG + NADH + CO₂

51. CHOR + Gln + PRPP + Ser => Trp + Glu + Pyr + G3P + CO₂

Glutamic Family

52. AKG + NH₄ + NADPH => Glu

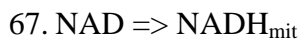
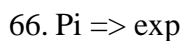
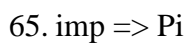
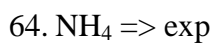
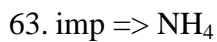
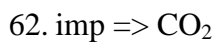
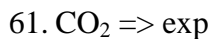
53. Glu + ATP + NH₄ => Gln + ADP + Pi



Biosynthesis and Interconversion of One-carbon Units



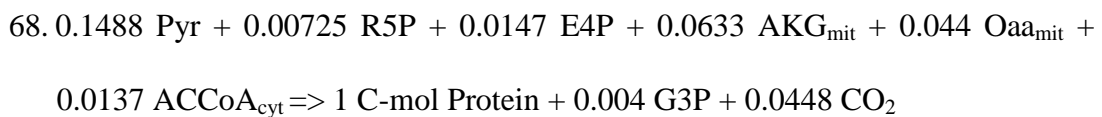
Transport Reactions



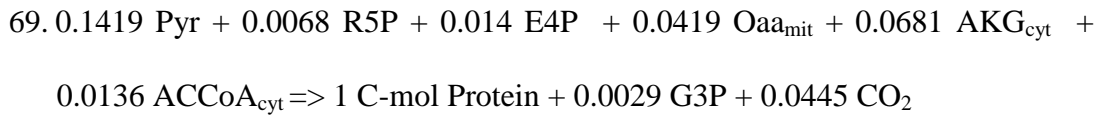
Biomass Synthesis

Protein synthesis

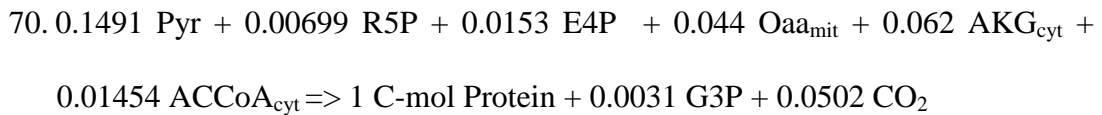
X-33 control strain



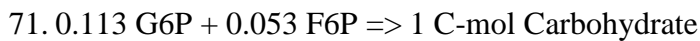
X-33 ROL 1-copy strain



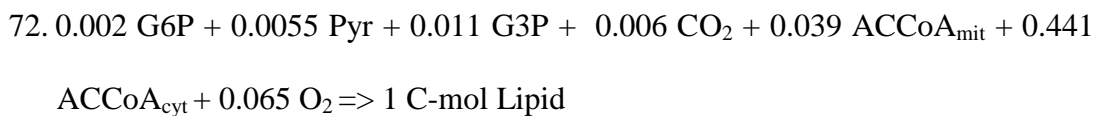
X-33 ROL 2-copy strain



Carbohydrate Synthesis



Lipids Synthesis



RNA Synthesis



DNA Synthesis



ROL Synthesis

