

SUPPLEMENTARY INFORMATION SI3

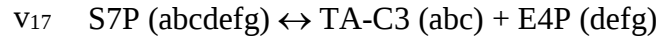
Metabolic network model used for ^{13}C -MFA for *E. coli* $\Delta tpiA$ case study

Glycolysis

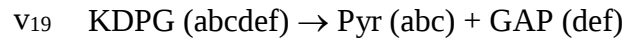
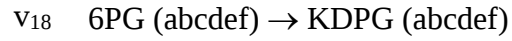
- v₁ Gluc.ext (abcdef) + PEP (ghi) $\rightarrow$ G6P (abcdef) + Pyr (ghi)
- v₂ G6P (abcdef) $\leftrightarrow$ F6P (abcdef)
- v₃ F6P (abcdef) + ATP $\rightarrow$ FBP (abcdef)
- v₄ FBP (abcdef) $\leftrightarrow$ DHAP (cba) + GAP (def)
- v₅ DHAP (abc) $\leftrightarrow$ GAP (abc)
- v₆ GAP (abc) $\leftrightarrow$ 3PG (abc) + ATP + NADH
- v₇ 3PG (abc) $\leftrightarrow$ PEP (abc)
- v₈ PEP (abc) $\leftrightarrow$ Pyr (abc) + ATP

Pentose Phosphate Pathway

- v₉ G6P (abcdef) $\rightarrow$ 6PG (abcdef) + NADPH
- v₁₀ 6PG (abcdef) $\rightarrow$ Ru5P (bcdef) + CO₂ (a) + NADPH
- v₁₁ Ru5P (abcde) $\leftrightarrow$ X5P (abcde)
- v₁₂ Ru5P (abcde) $\leftrightarrow$ R5P (abcde)
- v₁₃ X5P (abcde) $\leftrightarrow$ TK-C2 (ab) + GAP (cde)
- v₁₄ F6P (abcdef) $\leftrightarrow$ TK-C2 (ab) + E4P (cdef)
- v₁₅ S7P (abcdefg) $\leftrightarrow$ TK-C2 (ab) + R5P (cdefg)
- v₁₆ F6P (abcdef) $\leftrightarrow$ TA-C3 (abc) + GAP (def)



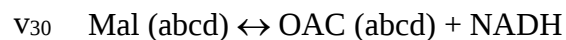
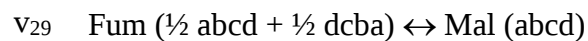
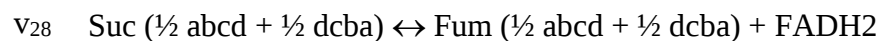
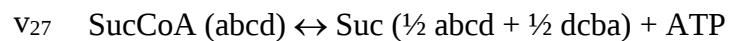
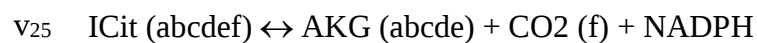
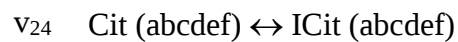
Entner-Doudoroff Pathway



Methylglyoxal Pathway



TCA Cycle



Glyoxylate Shunt

v₃₁ ICit (abcdef) $\leftrightarrow$ Glyox (ab) + Suc ($\frac{1}{2}$ edcf + $\frac{1}{2}$ fcde)

v₃₂ Glyox (ab) + AcCoA (cd) $\rightarrow$ Mal (abdc)

Amphibolic Reactions

v₃₃ Mal (abcd) $\rightarrow$ Pyr (abc) + CO₂ (d) + NADPH

v₃₄ Mal (abcd) $\rightarrow$ Pyr (abc) + CO₂ (d) + NADH

v₃₅ PEP (abc) + CO₂ (d) $\rightarrow$ OAC (abcd)

v₃₆ OAC (abcd) + ATP $\rightarrow$ PEP (abc) + CO₂ (d)

Mixed Acid Fermentation

v₃₇ Pyr (abc) + NADH $\leftrightarrow$ Lact (abc)

v₃₈ AcCoA (ab) $\leftrightarrow$ Ac (ab) + ATP

Amino Acid Biosynthesis

v₃₉ AKG (abcde) + NADPH + NH₃ $\rightarrow$ Glu (abcde)

v₄₀ Glu (abcde) + ATP + NH₃ $\rightarrow$ Gln (abcde)

v₄₁ Glu (abcde) + ATP + 2 NADPH $\rightarrow$ Pro (abcde)

v₄₂ Glu (abcde) + CO₂ (f) + Gln (ghijk) + Asp (lmno) + AcCoA (pq) + 5 ATP +
NADPH $\rightarrow$ Arg (abcdef) + AKG (ghijk) + Fum (lmno) + Ac (pq)

v₄₃ OAC (abcd) + Glu (efghi) $\rightarrow$ Asp (abcd) + AKG (efghi)

v₄₄ Asp (abcd) + 2 ATP + NH₃ $\rightarrow$ Asn (abcd)

v₄₅ Pyr (abc) + Glu (defgh) $\rightarrow$ Ala (abc) + AKG (defgh)

- v₄₆ 3PG (abc) + Glu (defgh) → Ser (abc) + AKG (defgh) + NADH
- v₄₇ Ser (abc) ↔ Gly (ab) + MEETHF (c)
- v₄₈ Gly (ab) ↔ CO₂ (a) + MEETHF (b) + NADH + NH₃
- v₄₉ Thr (abcd) → Gly (ab) + AcCoA (cd) + NADH
- v₅₀ Ser (abc) + AcCoA (de) + 3 ATP + 4 NADPH + SO₄ → Cys (abc) + Ac (de)
- v₅₁ Asp (abcd) + Pyr (efg) + Glu (hijkl) + SucCoA (mnop) + ATP + 2 NADPH →
LL-DAP ($\frac{1}{2}$ abcdgfe + $\frac{1}{2}$ efgdcba) + AKG (hijkl) + Suc ($\frac{1}{2}$ mnop + $\frac{1}{2}$ ponm)
- v₅₂ LL-DAP ($\frac{1}{2}$ abcdefg + $\frac{1}{2}$ gfedcba) → Lys (abcdef) + CO₂ (g)
- v₅₃ Asp (abcd) + 2 ATP + 2 NADPH → Thr (abcd)
- v₅₄ Asp (abcd) + METHF (e) + Cys (fgh) + SucCoA (ijkl) + ATP + 2 NADPH →
Met (abcde) + Pyr (fgh) + Suc ($\frac{1}{2}$ ijkl + $\frac{1}{2}$ lkji) + NH₃
- v₅₅ Pyr (abc) + Pyr (def) + Glu (ghijk) + NADPH → Val (abcef) + CO₂ (d) + AKG
(ghijk)
- v₅₆ AcCoA (ab) + Pyr (cde) + Pyr (fgh) + Glu (ijklm) + NADPH →
Leu (abdghe) + CO₂ (c) + CO₂ (f) + AKG (ijklm) + NADH
- v₅₇ Thr (abcd) + Pyr (efg) + Glu (hijkl) + NADPH → Ile (abfcdg) + CO₂ (e) + AKG
(hijkl) + NH₃
- v₅₈ PEP (abc) + PEP (def) + E4P (ghij) + Glu (klmno) + ATP + NADPH →
Phe (abcefg hij) + CO₂ (d) + AKG (klmno)
- v₅₉ PEP (abc) + PEP (def) + E4P (ghij) + Glu (klmno) + ATP + NADPH →
Tyr (abcefg hij) + CO₂ (d) + AKG (klmno) + NADH

v60 Ser (abc) + R5P (defgh) + PEP (ijk) + E4P (lmno) + PEP (pqr) + Gln (stuvw) + 3
ATP + NADPH → Trp (abcdklmnoj) + CO₂ (i) + GAP (fgh) + Pyr (pqr) + Glu
(stuvw)

v61 R5P (abcde) + FTHF (f) + Gln (ghijk) + Asp (lmno) + 5 ATP → His (edcbaf) + AKG
(ghijk) + Fum (lmno) + 2 NADH

One-Carbon Metabolism

v62 MEETHF (a) + NADH → METHF (a)

v63 MEETHF (a) → FTHF (a) + NADPH

Oxidative Phosphorylation

v64 NADH + $\frac{1}{2}$ O₂ → 1.5 ATP

v65 FADH₂ + $\frac{1}{2}$ O₂ → 0.5 ATP

Transhydrogenase

v66 NADH ↔ NADPH

ATP Hydrolysis

v67 ATP → ADP

Transport

v68 Ac (ab) → Ac.ext (ab)

v₆₉ CO₂ (a) → CO₂.ext (a)

v₇₀ O₂.ext → O₂

v₇₁ NH₃.ext → NH₃

v₇₂ SO₄.ext → SO₄

v₇₃ Pi.ext → Pi

Dilution reactions

v₇₄ CO₂.atm (a) + CO₂ (b) → CO₂ (a) + CO₂.sink (b)

Biomass Formation (strain-specific)

v₇₅ Ala + Arg + Asn + Asp + Cys + Glu + Gln + Gly + His + Ile + Leu + Lys + Met +
Phe + Pro + Ser + Thr + Trp + Tyr + Val + G6P + F6P + R5P + GAP + 3PG + PEP +
Pyr + AcCoA + AKG + OAC + MEETHF + ATP + NADPH → Biomass + NADH

**strain-specific coefficients are used based on the measured biomass composition*