

| Reactions      | <i>E. coli</i> iAF1260 growth condition |                    |           |
|----------------|---|--------------------|-----------|
|                | Aerobic                                 | Aerobic restricted | Anaerobic |
| Zero           | 1904                                    | 1900               | 1907      |
| Fixed non-zero | 355                                     | 324                | 374       |
| Variable       | 115                                     | 150                | 93        |
| Total          | 2374                                    | 2374               | 2374      |

**Table S1. Characterization of the optimal solution space with and without reversible-reaction splitting.** For the growth conditions tested, we show that the majority of reactions is fixed in the optimum. In fact, most reactions have a zero flux in the optimal solution. Only a relatively small amount of reactions is variable after optimization of the objective function.