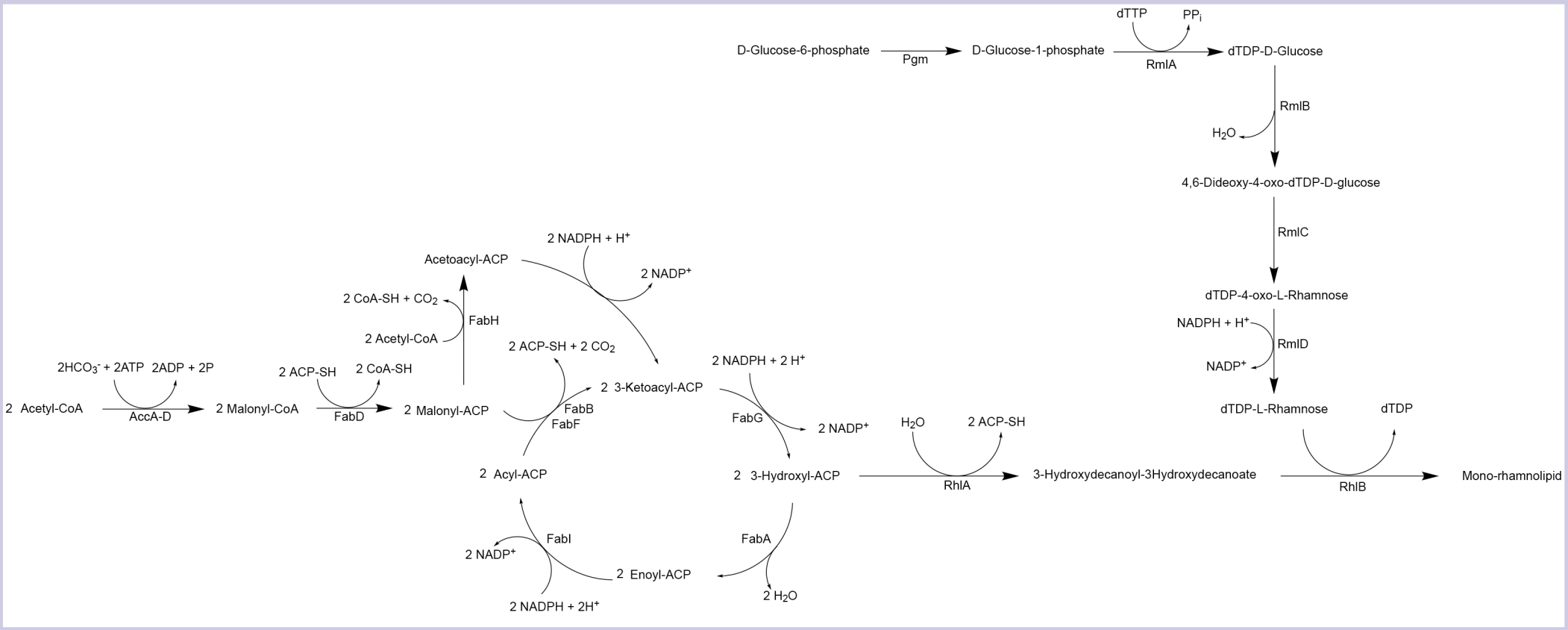
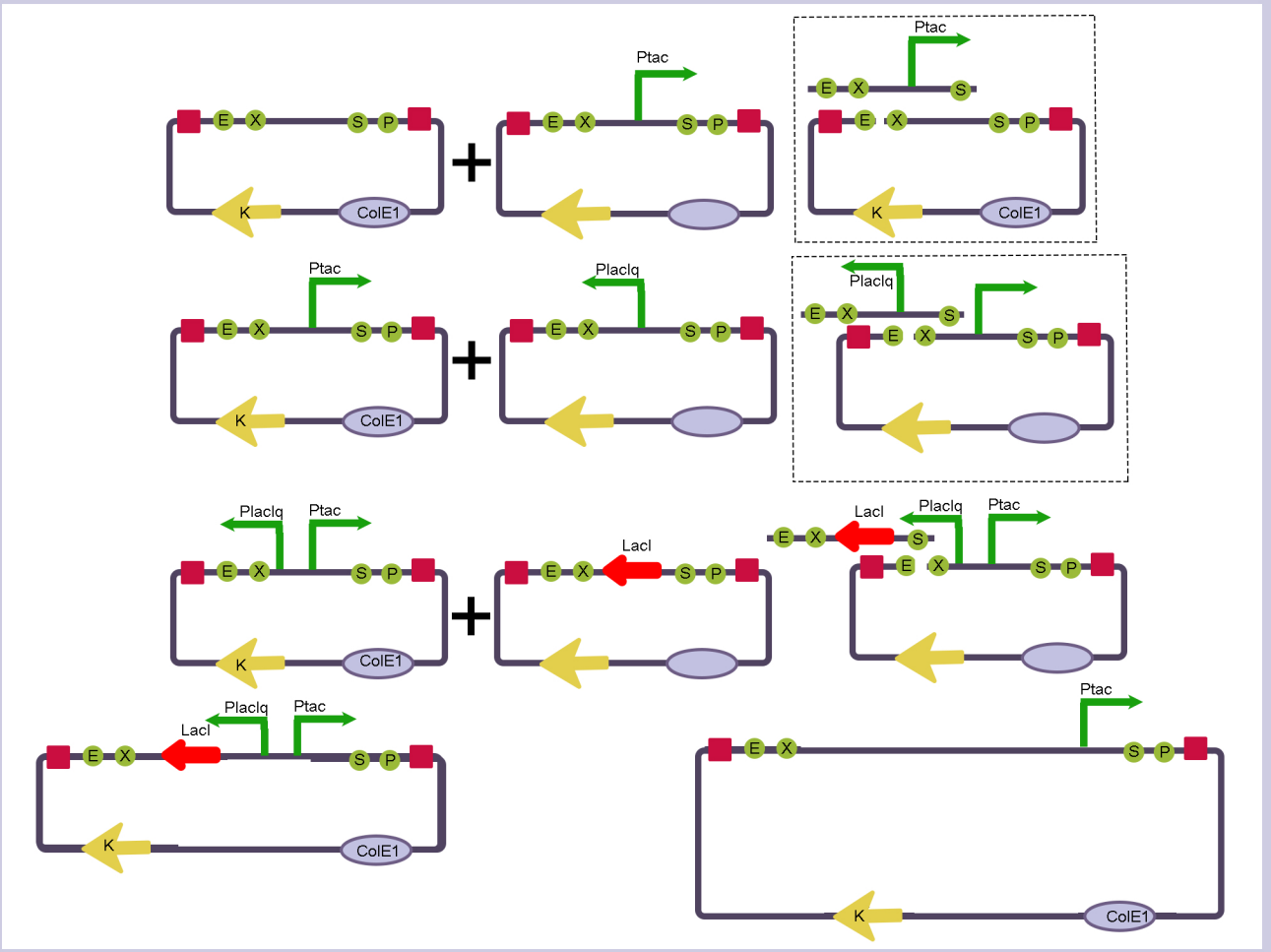


We used a pre-existing metabolic pathways of P.putida [1,2] and P.aeruginosa [3] to predict how proteins RhlA and Rhlb, encoded by genes rhlA and rhlB respectively, are inserted into P.putida metabolic pathways. As can be seen from the diagram, there are two main pathways directly involved in the production of rhamnolipid: rhamnose pathway and fatty acid de nova synthesis. Fatty acid synthesis produce substrate betta-hydroxyacyl-ACP for RhlA, which turns it into betta-D-(betta-D- hydroxyalkanoyloxy)alkanoic acid (HAA), precursor for RhlB. Rhamnose pathway leads to the production of dTDP-L-rhamnose, substrate for RhlB, which reacts with HAA to produce mono-rhamnolipid. Alpha-D-glucose-6-phosphate comes from central carbon metabolism. From the diagram it also can be seen that there are 3 steps where NADPH is involved, 1 in the production of the substrate for RhlA, second in the production of the substrate for RhlB, and third in the fatty acid synthesis cycle. These substrates are key factor in the rhamnolipid production and their availability is expected to be limited in non-natural RL producers such as P. putida, thus the gene nadE for NAD synthetase was incorporated for overproduction of NAD/NADH. This extra supply would ensure that enough substrate will be produced, increasing the theoretical yield of rhamnolipid production.



1. Bahia, F.M., de Almeida, G.C., de Andrade, L.P. et al. Rhamnolipids production from sucrose by engineered Saccharomyces cerevisiae. Sci Rep 8, 2905 (2018). https://doi.org/10.1038/s41598-018-21230-2
2. Tiso, Till & Sabelhaus, Petra & Behrens, Beate & Wittgens, Andreas & Rosenau, Frank & Hayen, Heiko & Blank, Lars. (2016). Creating metabolic demand as an engineering strategy in Pseudomonas putida – Rhamnolipid