
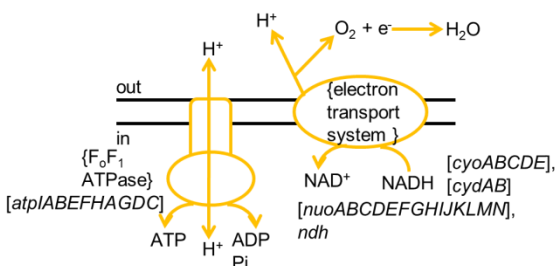
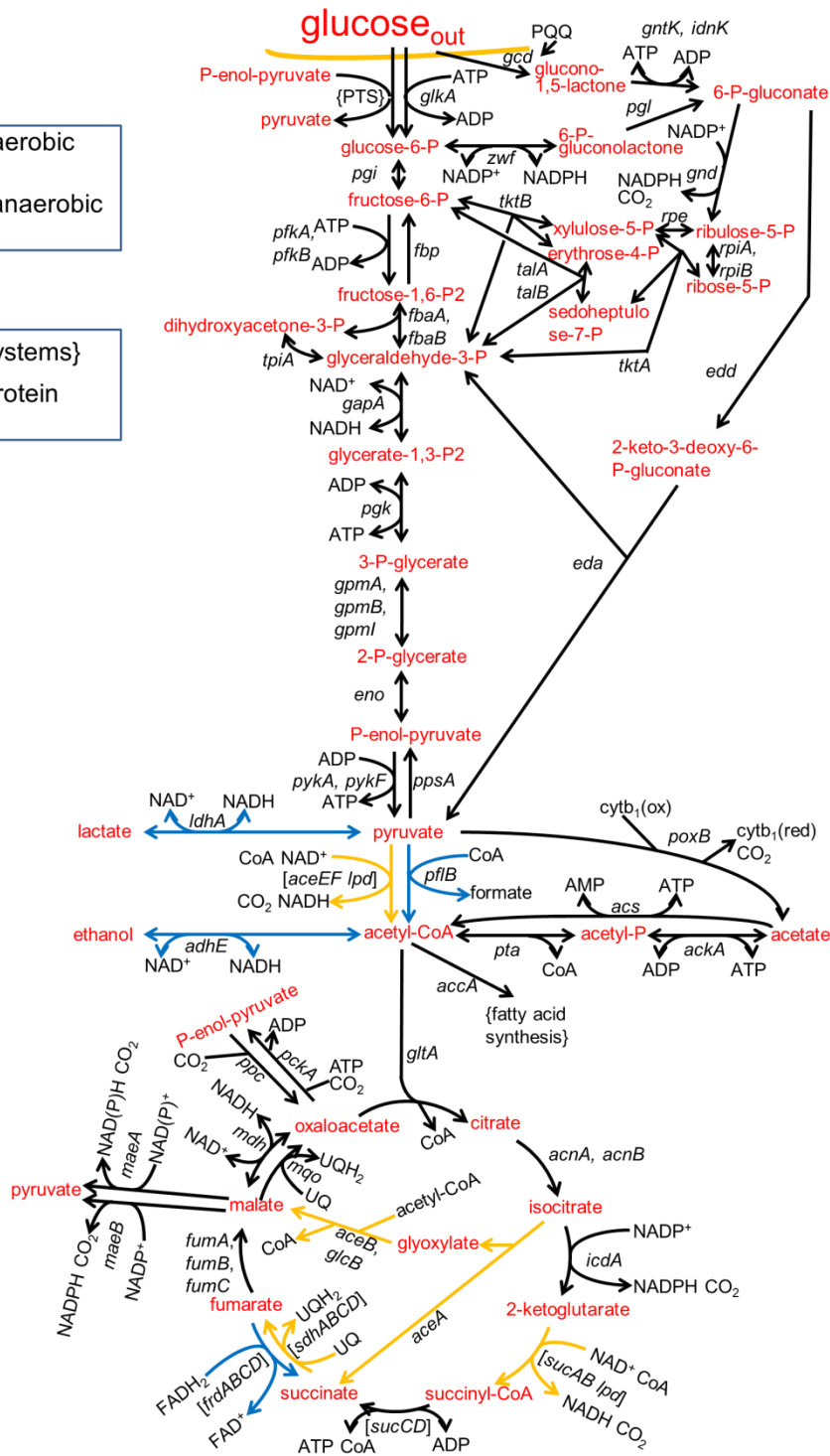
 work under aerobic conditions  
 work under anaerobic conditions

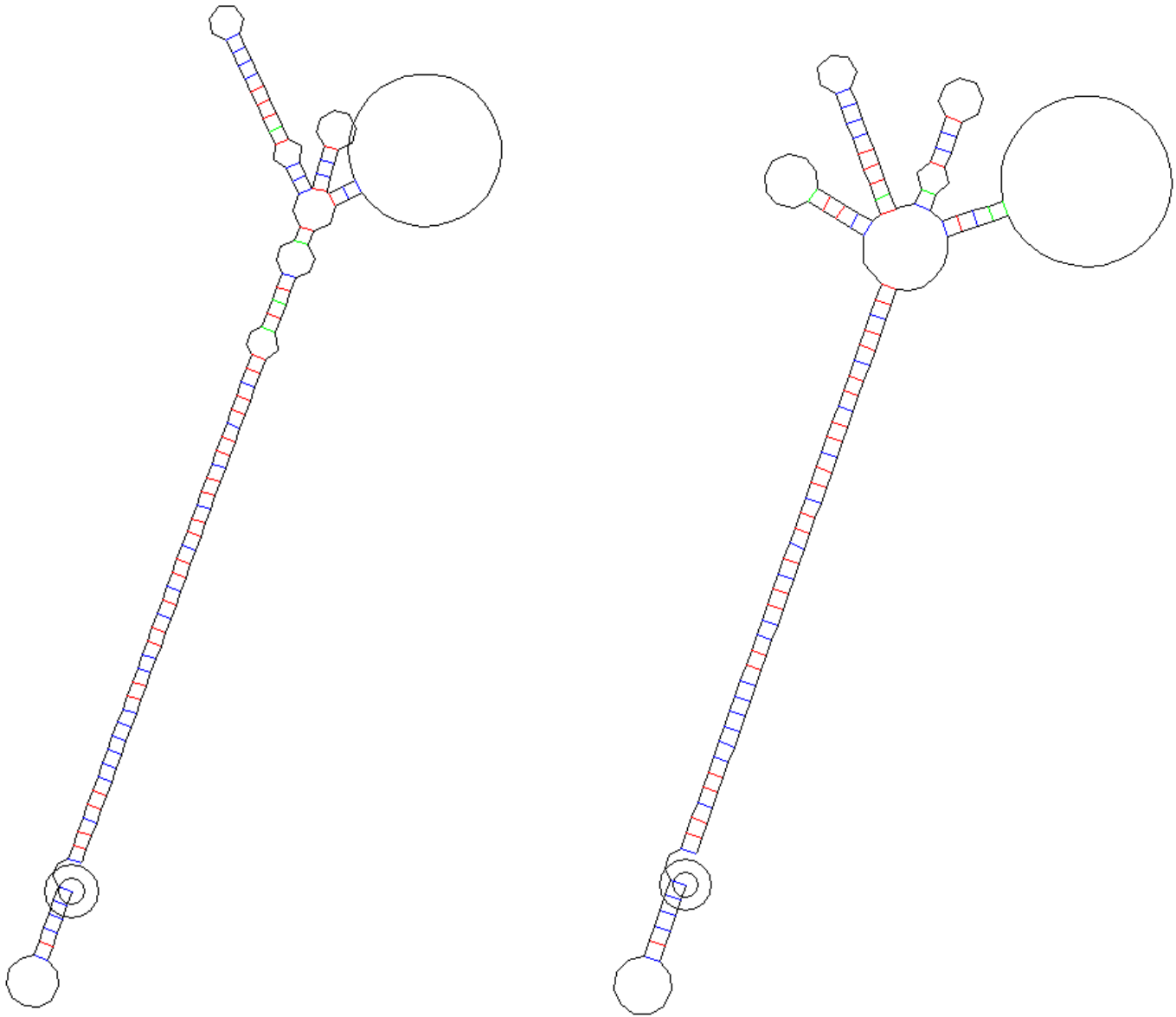
{multi-reaction systems}  
 [reactions with protein complexes]



### transcriptional regulators

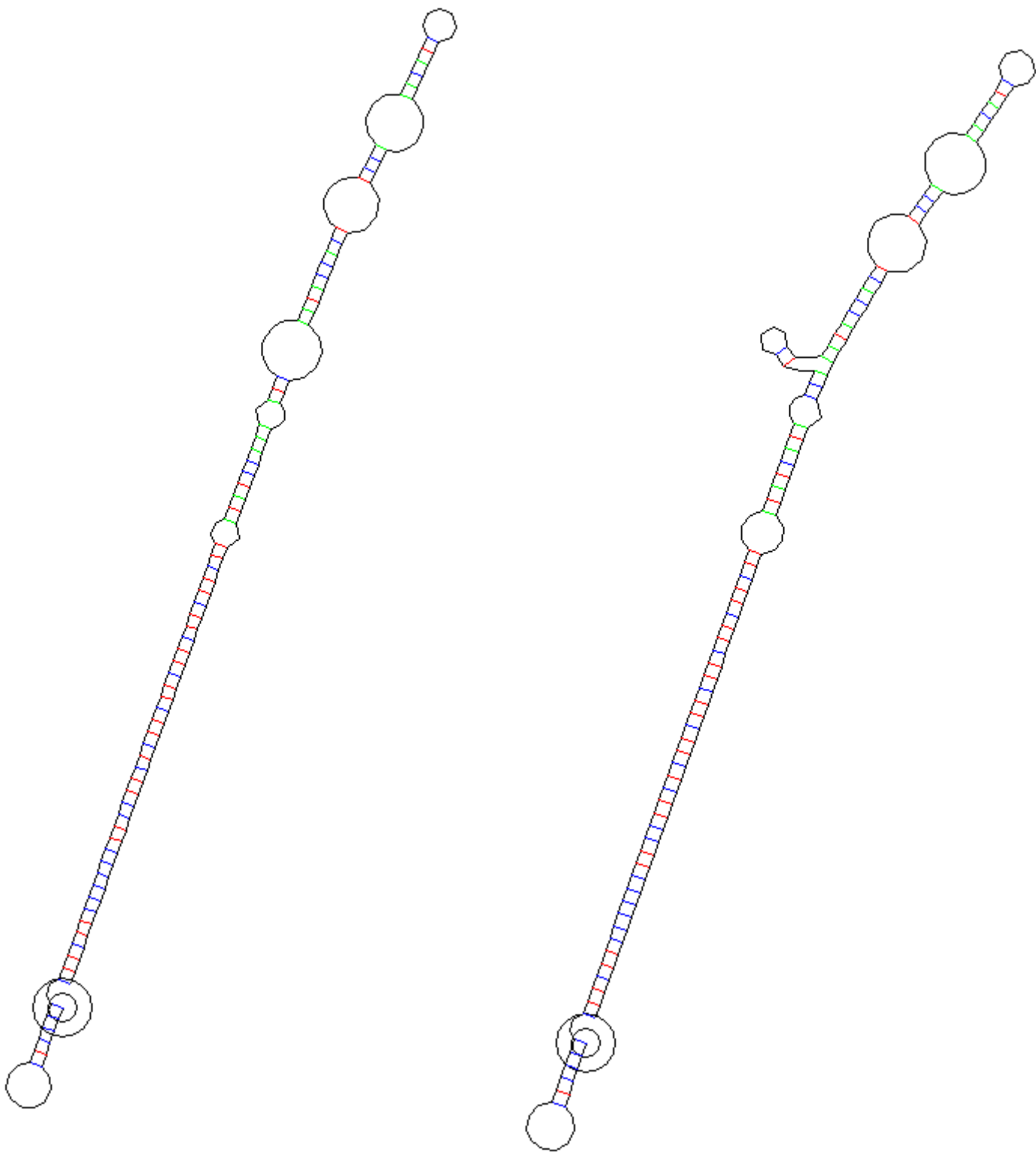
- arcA* (negative regulator of genes in aerobic pathways)
  - *gltA acnA icdA lpd sucABCDEF sdhCDAB fumAC mdh aceBAK aceEF cyoABCDE*
  - + *pfkB cydAB*
- fnr* (global regulator of anaerobic growth)
  - *acnA icdA sdhCDAB fumAC cyoABCDE cydAB ndh*
  - + *pfkB fumB frdABCD*
- cra* (negative regulator for glycolysis and positive regulator for gluconeogenesis)
  - *pfkA pykF edd eda ptsHI acnB*
  - + *fbp pps aceA pckA acnA icdA cydAB*
- crp* (cAMP receptor protein, key regulator of carbon catabolite repression)
- mhc* (negative regulator of sugar permease genes)

Nakashima et al., Supplementary Fig. S1.  
 Detailed map of the central carbon metabolism.



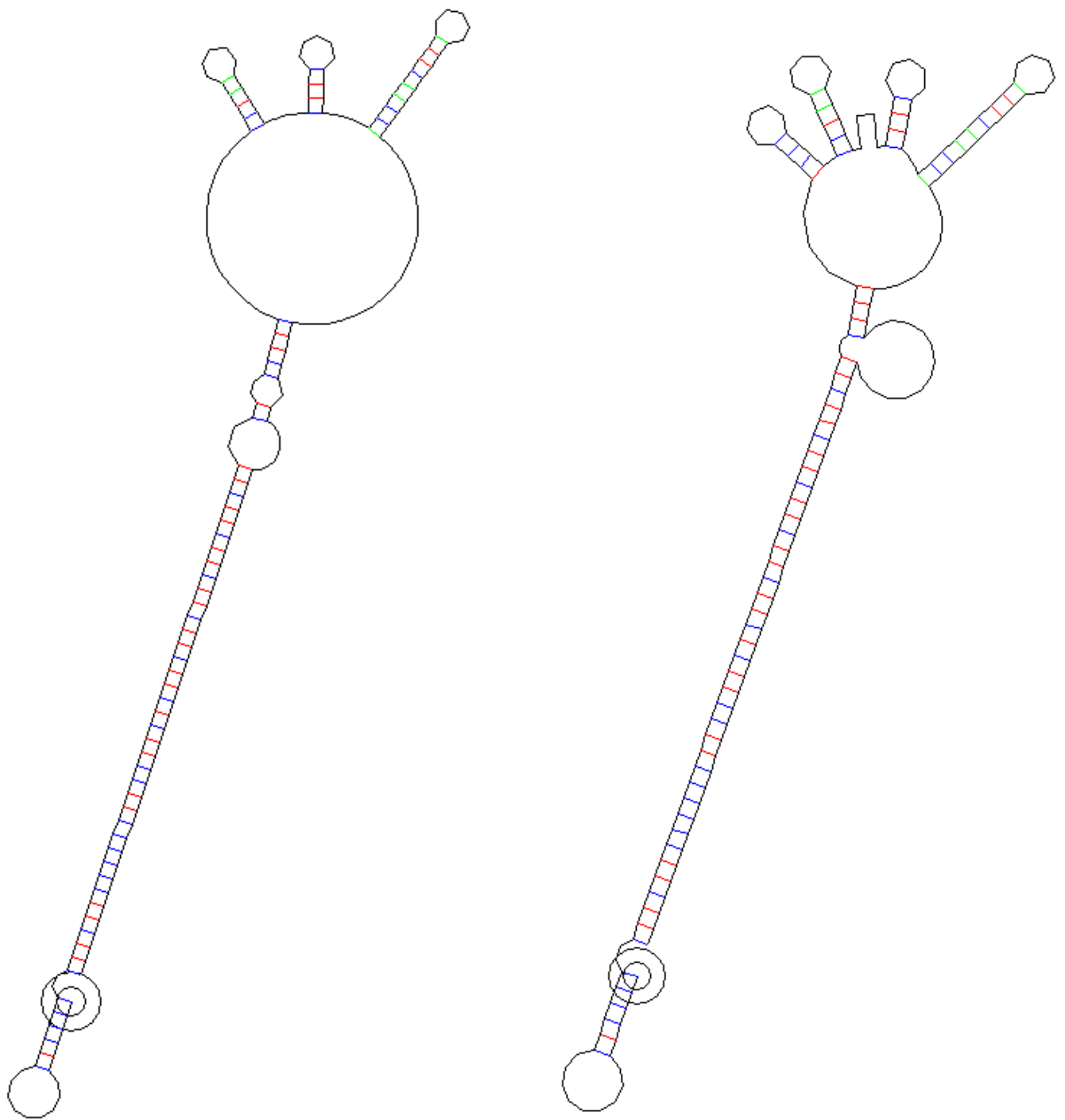
Nakashima et al., Supplementary Fig. S2.

Structures of the *sucC* PTasRNAs that are expressed from pSM80 (left, good silencing efficacy) and pSM59 (right, poor silencing efficacy). The RNA 5'-termini are marked with double circles. The RNA structure was predicted using the STAR program (Abrahams J.P., van den Berg M., van Batenburg E. & Pleij C.W.A. (1990): Prediction of RNA secondary structure, including pseudoknotting, by computer simulation. *Nucleic Acids Res.* 18, 3035-3044.).



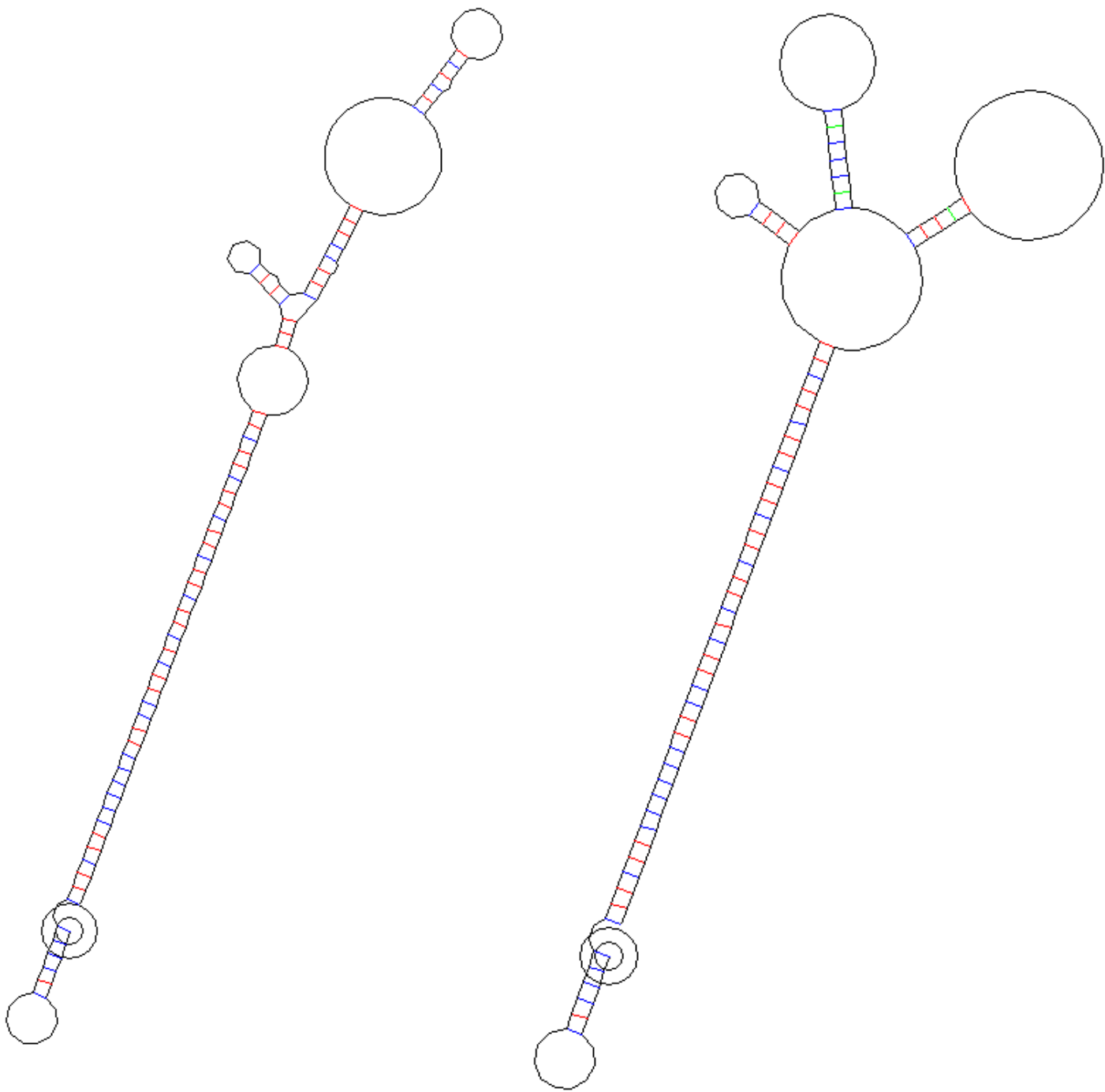
Nakashima et al., Supplementary Fig. S3.

Structures of the *acs* PTasRNAs that are expressed from pSM75 (left, good silencing efficacy) and pSM33 (right, poor silencing efficacy).



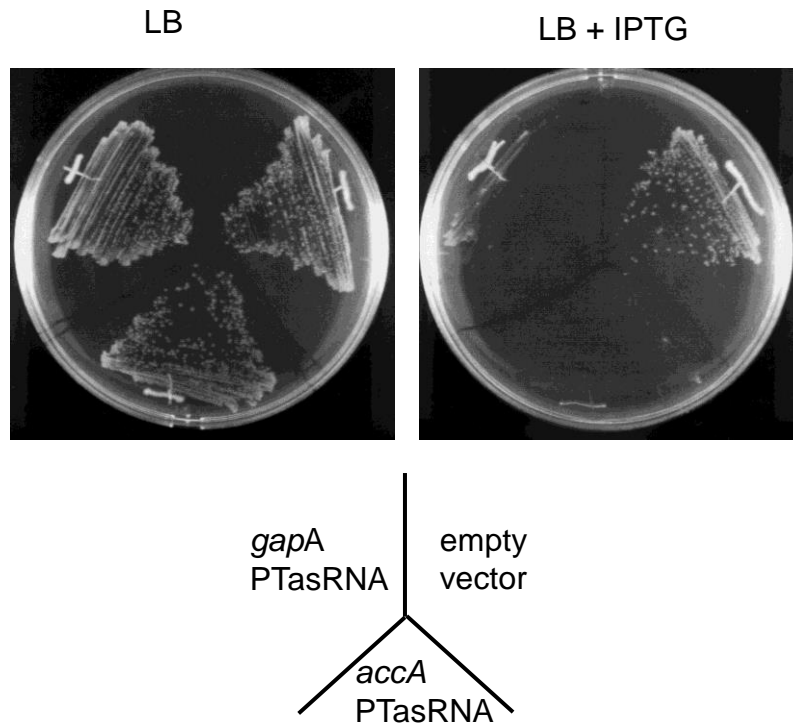
Nakashima et al., Supplementary Fig. S4.

Structures of the *arcA* PTasRNAs that are expressed from pHN1801 (left, good silencing efficacy) and pHN1316 (right, poor silencing efficacy).



Nakashima et al., Supplementary Fig. S5.

Structures of the *cyoA* PTasRNAs that are expressed from pHN1807 (left, good silencing efficacy) and pSM64 (right, poor silencing efficacy).



Nakashima et al., Supplementary Fig. S6.

Silencing of *gapA* and *accA*, growth essential genes.  
Transformants with the PTasRNA vectors for these genes were  
streaked onto the LB and LB+IPTG plates.

## Supplementary TABLE S1

### 71 vectors that express PTasRNAs with sufficient silencing efficacy.

| Gene name   | Forward oligonucleotide                       | Reverse oligonucleotide                          | Vector name constructed |
|-------------|---|--|-------------------------|
| <i>accA</i> | sSN1454<br>aactcgagtttgactaatacaggaatac<br>ta | sSN1455<br>cccatggctaaccgcagtcagag<br>aatcga     | pSM20                   |
| <i>aceA</i> | sSN1584<br>ccctcgagcgtaaaccaccacataact<br>atg | sMO66<br>ctccatgggcgagtaatgccttccc<br>aacgc      | pSM83                   |
| <i>aceB</i> | sMO67<br>atctcgagttcacagtggggaagtttc<br>gg    | sMO68<br>ttccatggctcgccatacggccttgt<br>gaaa      | pSM60                   |
| <i>aceE</i> | sSN1218<br>ggctcgagaaaactcaacgttattagat<br>ag | sSN1219<br>tcccatggagccagtcgcgagtttc<br>gat      | pSM8                    |
| <i>ackA</i> | sSN17<br>aactcgagctacgctctatggctccctg<br>ac   | sSN18<br>caccatggctactcttaccatttactg<br>catc     | pSM27                   |
| <i>acnA</i> | sMO51<br>tgctcgagttatctttaatattcacctga        | sMO52<br>agccatggtaagttttatctttggcct<br>gca      | pSM52                   |
| <i>acnB</i> | sMO53<br>cactcgagcgtgaagagaatcgctg<br>ccgc    | sMO54<br>cccatggtttgcatccaggggtttg<br>ggcg       | pSM53                   |
| <i>acs</i>  | sMO101<br>aactcgagatacccctacatttaacgctt<br>a  | sMO102<br>cgccatgggatgttggcaggaatg<br>gtgtgt     | pSM75                   |
| <i>adhE</i> | sSN1261<br>tactcgagcagatgatttactaaaaaag<br>tt | sSN1262<br>caccatgggcttttttacacgctcta<br>cga     | pHN1319                 |
| <i>arcA</i> | sSN11013<br>ttctcgagtctgtttcgatttagttggca     | sSN1274<br>gccccatggcttcgaaaatacttttca<br>acgtgt | pHN1801                 |
| <i>atpF</i> | sSN1263<br>ttctcgagcaatatcagaacgttaacta       | sSN1264<br>aaccatggccatacgtacttcatgc             | pHN1313                 |

|             |  |  |         |
|-------------|--|--|---------|
| <i>cra</i>  | aatag<br>sMO95<br>tactcgagaccagtacaatggctatggt<br>tt | agaac<br>sSN1669<br>ttccatggatagcttgcaagtggccg<br>cgac | pHN1836 |
| <i>cydA</i> | sSN1444<br>tactcgagttcactctcggagtcttcag<br>c         | sSN1445<br>ggccatggcaaactgtaagecgca<br>cagttc          | pSM28   |
| <i>cyoA</i> | sMO91<br>cactcgagaaacgccaccagatcccc<br>tgga          | sSN11016<br>atccatggcctgcaaataatgacaa<br>ccatc         | pHN1807 |
| <i>eda</i>  | sMO29<br>ttctcgagacaaatttgtaatcaggcga<br>ga          | sMO30<br>atccatggtacaaccgggcccgtg<br>gtcagg            | pSM43   |
| <i>edd</i>  | sMO107<br>cactcgagctctgaatgaaacgcgttgt<br>ga         | sMO108<br>gccccatggtcaatgattcgatttgta<br>cgc           | pSM77   |
| <i>eno</i>  | sSN1502<br>acctcgagtacgcgttgttctggagt<br>t           | sSN1503<br>taccatgggagtcgatgatttcacg<br>accg           | pSM25   |
| <i>fbaA</i> | sSN11009<br>agctcgaggagtataaggcccagcga<br>taca       | sSN1501<br>acccatggttttacgaaatcaaaaat<br>cttagacatg    | pHN1825 |
| <i>fbaB</i> | sMO3<br>aactcgaggaaaaactcgcccgtaac<br>gacc           | sMO4<br>ttccatggcaactgcgaatatctgt<br>catt              | pSM31   |
| <i>fbp</i>  | sSN1508<br>ttctcgagattgttgcggtcgctttactcc            | sSN1509<br>gtccatggagaaaactcgtgctgct<br>tttcg          | pSM23   |
| <i>fnr</i>  | sSN1436<br>tactcgagacaaatatcaattacggctt<br>gagcag    | sMO90<br>tgccatggacaaccgccagactga<br>atgcgc            | pSM78   |
| <i>frdA</i> | sSN1430<br>gtctcgagaccgtaacttcaggtactta<br>c         | sSN1431<br>taccatggcaagatcggcttgaaaag<br>gtttg         | pSM12   |
| <i>fumA</i> | sMO57<br>ggctcgaggaacaccgccagagc                     | sMO58<br>ttccatggtaataactcagtatcatcttt                 | pSM56   |



|             |                                |                            |         |
|-------------|--------------------------------|----------------------------|---------|
|             | ataac                          | tt                         |         |
| <i>fumB</i> | sMO59                          | sMO60                      | pSM62   |
|             | cactcgagaataacaatacagagtta     | gtccatggaagtgagtagatagtatt |         |
|             | cag                            | cggt                       |         |
| <i>fumC</i> | sMO61                          | sMO116                     | pSM81   |
|             | aactcgagagctaaaagttgcttaacg    | ggccatggaatcgccccatcgaa    |         |
|             | aaa                            | tctttt                     |         |
| <i>gapA</i> | sSN1372                        | sSN1373                    | pHN1505 |
|             | ttctcgaggcaaccttttattcactaaca  | caccatggaaaacaatgcgaccga   |         |
|             | a                              | tacggc                     |         |
| <i>gcd</i>  | sSN1269                        | sSN1270                    | pHN1321 |
|             | atctcgagctactaaaatattaatgaatt  | caccatgggctgttagcgtgacga   |         |
|             | gaaatggtg                      | gtaatc                     |         |
| <i>glcB</i> | sMO117                         | sMO118                     | pSM70   |
|             | ttctcgagataagcgaaaacgaggag     | cgccatggcttcttcatccacaaaa  |         |
|             | ataa                           | cgttt                      |         |
| <i>glkA</i> | sSN1588                        | sSN1497                    | pHN1821 |
|             | ttctcgagccccaggtatttacagtgt    | tgccatggtgcccccacatcacc    |         |
|             | ga                             | gactaa                     |         |
| <i>gltA</i> | sSN1267                        | sSN1268                    | pHN1320 |
|             | gtctcgaggcaaatttaagttccggcag   | accatggttcaacagctgtatccc   |         |
|             | tc                             | cgttg                      |         |
| <i>gnd</i>  | sMO31                          | sMO32                      | pSM44   |
|             | agctcgagagcattcagcgcggtgatc    | aaccatggcttctgatgttgagcgc  |         |
|             | aca                            | aaggt                      |         |
| <i>gntK</i> | sSN1662                        | sSN11008                   | pHN1831 |
|             | ttctcgagttaattcttgtattgtgggggc | cgccatggcactggcgaccgcag    |         |
|             |                                | at ttgcc                   |         |
| <i>gpmA</i> | sMO13                          | sMO14                      | pSM35   |
|             | gtctcgagtaagcattgctgttgcttctgt | cgccatggcgaaccagaaccagct   |         |
|             | c                              | tagtta                     |         |
| <i>gpmB</i> | sMO15                          | sMO16                      | pSM36   |
|             | tcctcgagttaacgttacagcagtatac   | gtccatgggctgtcagactggccct  |         |
|             | gg                             | gaata                      |         |
| <i>gpmI</i> | sMO103                         | sMO104                     | pSM76   |
|             | ggctcgagtatcgattcagctgtagtaa   | taccatggcagaatcaccagtacca  |         |

|             |                               |                             |         |
|-------------|-------------------------------|-----------------------------|---------|
|             | aa                            | taggt                       |         |
| <i>icdA</i> | sSN1446                       | sSN1447                     | pSM16   |
|             | tactcgaggacgcaaacgcatatgcaa   | tgccatggaacaactactttactttcc |         |
|             | cgt                           | attcacctc                   |         |
| <i>idnK</i> | sMO23                         | sMO24                       | pSM40   |
|             | gcctcgaggcgtagtatcgagcaggt    | aaccatggttttaccactccctgaaa  |         |
|             | aag                           | cgcc                        |         |
| <i>ldhA</i> | sSN1259                       | sSN1260                     | pHN1318 |
|             | atctcgagtagcttaaatgtgattcaaca | gccccatggactcgttcacctgttgc  |         |
|             | tcactg                        | aggta                       |         |
| <i>maeA</i> | sSN1321                       | sSN1322                     | pHN1503 |
|             | aactcgagacgataaaagccccccag    | gccccatgggatataaagcgaacgc   |         |
|             | ggat                          | tgtttt                      |         |
| <i>maeB</i> | sSN1323                       | sSN1324                     | pHN1504 |
|             | ggctcgagccccacacactttatttgtga | atccatggaaatcaagtgcactttgt  |         |
|             | ac                            | ttta                        |         |
| <i>mdh</i>  | sSN1428                       | sSN1429                     | pSM11   |
|             | tactcgagtttatcaatataataaggagt | gccccatgggttttaacagtagtgca  |         |
|             | ttaggatgaaa                   | agcgcct                     |         |
| <i>mlc</i>  | sMO99                         | sMO100                      | pSM67   |
|             | tgctcgagcatagcctacagattatttcg | tgccatggtttatttgatcaatgtgcc |         |
|             | g                             | cag                         |         |
| <i>mgo</i>  | sSN1450                       | sSN11018                    | pHN1816 |
|             | tactcgagtgttaccgttgatgccgcgc  | cgccatgggagaagagcatggca     |         |
|             | aa                            | gtcactt                     |         |
| <i>ndh</i>  | sSN11019                      | sSN1670                     | pHN1854 |
|             | ccctcgagtaattaagagctatgtaata  | ttccatggcgtcccagcttatgcccc  |         |
|             | a                             | agct                        |         |
| <i>nuoA</i> | sSN1424                       | sSN1425                     | pSM9    |
|             | aactcgagcgtccatctgccgtgaaga   | gtccatggc gatgacttcagtggat  |         |
|             | gca                           | gttga                       |         |
| <i>pckA</i> | sMO63                         | sMO64                       | pHN2155 |
|             | gactcgagtcgcggttaacacccccaa   | aaccatggagttcttgcgggggtcaa  |         |
|             | aaa                           | accat                       |         |
| <i>pfkA</i> | sSN1442                       | sSN1443                     | pSM15   |
|             | acctcgagcacattcgttggatcacttc  | gccccatggccaacacaccgattttct |         |

|             |   |  |         |
|-------------|---|--|---------|
| <i>pfkB</i> | ga<br>sMO7<br>tcctcgagttaatttcttcaactttccgctg       | taatcatgac<br>sMO8<br>1. gaccatgggcgcaagtgca<br>acgtatagat | pSM30   |
| <i>pflB</i> | sSN1216<br>tgctcgagtacgcagtaaataaaaaatc<br>ca       | sSN1217<br>accatggtaaaacctcccagget<br>gtgg                 | pHN1312 |
| <i>pgi</i>  | sSN1498<br>ttctcgagactggcgctacaatcttcca<br>aa       | sSN1499<br>ctccatggcagcggctctcggttga<br>ttgat              | pSM22   |
| <i>pgk</i>  | sMO11<br>gactcgagcagcgtctgcaaaacttta<br>ga          | sMO12<br>ttccatggtacgttcagatccgcac<br>ggata                | pSM34   |
| <i>pgl</i>  | sMO25<br>tactcgagcattcaccgcaaaagcga<br>cta          | sMO26<br>ctccatgggctggcgatataaactg<br>tttgc                | pSM41   |
| <i>poxB</i> | sMO97<br>ccctcgaggtcagatgaactaaactgt<br>ta          | sMO98<br>gcccattggcccctgccgattcgagt<br>gtttt               | pSM66   |
| <i>ppc</i>  | sSN1672<br>ttctcgagctttataaaagacgacgaaa<br>agcaaagc | sSN1673<br>ttccatggcgttcaagaatgtgttctc<br>cca              | pHN1858 |
| <i>ppsA</i> | sSN1235<br>tactcgagcagaaatgtgtttctcaaac<br>cg       | sSN1236<br>caccatggtctacatcattcatgccg<br>agtt              | pHN1297 |
| <i>pta</i>  | sSN1193<br>ccctcgaggcggtaacgaaagaggat<br>aaac       | sSN1194<br>ttccatggcacggatcacgccaag<br>gctg                | pHN1260 |
| <i>ptsG</i> | sSN1486<br>acctcgaggctctcccccttggccacg<br>c         | sSN1487<br>caccatggatttaccgaccttttga<br>ggtt               | pSM21   |
| <i>pykA</i> | sMO19<br>cgctcgagcggcgttatttcattcggatt<br>t         | sMO20<br>taccatggtaacgattttgttctcg<br>aag                  | pSM38   |
| <i>pykF</i> | sSN1448   | sSN1449  | pSM17   |

|             |                               |                               |         |
|-------------|-------------------------------|-------------------------------|---------|
|             | tctcagagtgcgccagaaagcaagtt    | gtccatggccgatggtgcaaacia      |         |
|             | tct                           | ttttg                         |         |
| <i>rpe</i>  | sMO111                        | sMO112                        | pSM79   |
|             | aactcgagtctcaaggagaagcggat    | caccatgggctgccagggttttgc      |         |
|             | gaaa                          | ggtat                         |         |
| <i>rpiA</i> | sMO33                         | sMO34                         | pSM45   |
|             | ttctcgagtataatgcgtgtgaaatttc  | aaccatggtgccgggctgaacata      |         |
|             | atacc                         | ctgaag                        |         |
| <i>rpiB</i> | sMO35                         | sMO110                        | pHN1827 |
|             | cactcgagtttttgattgtgaagttttgc | atccatggctatttcatgttttaaatg   |         |
|             | acgga                         | aa                            |         |
| <i>sdhC</i> | sSN1452                       | sSN1453                       | pSM19   |
|             | gtctcgaggtaacagaaagttaacctct  | ttccatggtctttgtttttcacatttctt |         |
|             | gt                            | atcatga                       |         |
| <i>sucA</i> | sSN1265                       | sSN1266                       | pHN1314 |
|             | ctctcgagtgaaccccgccacgcacat   | caccatggtaagaagagtccaacc      |         |
|             | cac                           | aggctt                        |         |
| <i>sucC</i> | sMO55                         | sMO114                        | pSM80   |
|             | atctcgagtctacggtttaaaagataac  | cgccatgggtaagccatagcgggc      |         |
|             | ga                            | aaaaag                        |         |
| <i>talA</i> | sMO45                         | sMO46                         | pSM49   |
|             | aactcgagcactcatctaactttactt   | gccccatggactcaatcgcgctg       |         |
|             | t                             | tctgc                         |         |
| <i>talB</i> | sMO47                         | sMO48                         | pSM50   |
|             | atctcgagggcagaccggttacatccc   | tcccatggtgatacagcttcattgc     |         |
|             | cct                           | cgcg                          |         |
| <i>tktA</i> | sMO41                         | sMO42                         | pSM47   |
|             | atctcgagcaaattttccggcgtagccc  | agccatggcaagctctttacgtgag     |         |
|             | aa                            | gacat                         |         |
| <i>tktB</i> | sMO43                         | sMO44                         | pSM48   |
|             | tactcgagccacggagtgttatatgtcc  | cgccatgggatgaccagagttggc      |         |
|             | cg                            | ttttg                         |         |
| <i>tpiA</i> | sMO9                          | sMO10                         | pSM32   |
|             | ctctcgagcggggcgccatcttccttt   | tgccatggctgccgttcagttccag     |         |
|             | at                            | ttac                          |         |

*zwf*

sSN1271

sSN1272

pHN1315

atctcgagtataccctggcttaagtacc

gcccattggtcgccttcgcgcccga

gg

aatga

---

**Supplementary TABLE S2**

**Vectors that express PTasRNAs with insufficient silencing efficacy.**

| Gene name   | Forward oligonucleotide                             | Reverse oligonucleotide                            | Vector name constructed | result of validation (% reduction) <sup>a)</sup> |
|-------------|---|--|-------------------------|--|
| <i>aceA</i> | sMO65<br>aactcgagacataactatgg<br>agcatctgca         | sMO66<br>ctccatgggcgagtaatg<br>ccttccaacgc         | pSM59                   | 6 ± 3 (protein)                                  |
| <i>acs</i>  | sMO5<br>ccctcgagcctacatttaac<br>gcttatgcca          | sMO6<br>ggccatggctcgcgatgtt<br>ggcaggaatgg         | pSM33                   | 5 ± 23 (mRNA)                                    |
| <i>arcA</i> | sSN1273<br>ctctcgagatttagttggcaa<br>ttaggtagca      | sSN1274<br>gcccattggcttcgaaaata<br>ctttcaacgtgt    | pHN1316                 | 8 ± 13 (mRNA)                                    |
| <i>arcA</i> | sSN1273<br>ctctcgagatttagttggcaa<br>ttaggtagca      | sMO124<br>ccccatggaaaataacttttc<br>aacgtgttc       | pHN1799                 | -44 (mRNA)                                       |
| <i>arcA</i> | sMO123<br>acctcgagtttcgatttagttg<br>gcaattta        | sSN1274<br>gcccattggcttcgaaaata<br>ctttcaacgtgt    | pHN1800                 | 0.3 (mRNA)                                       |
| <i>cra</i>  | sSN1293<br>cactcgagtacaatggctat<br>ggttttacatttt    | sSN1294<br>tgccatggctccgcgacac<br>tcccgccag        | pHN1350                 | 47± 23 (mRNA)                                    |
| <i>cra</i>  | sSN1668<br>gcctcgagatgtaataatgaatt<br>taaccataaccag | sMO96<br>tgccatgggacactcccg<br>ccagccgagcga        | pHN1834                 | 58 (mRNA)  |
| <i>cra</i>  | sSN1668<br>gcctcgagatgtaataatgaatt<br>taaccataaccag | sSN1294<br>tgccatggctccgcgacac<br>tcccgccag        | pHN1835                 | 43 (mRNA)  |
| <i>cyoA</i> | sSN1438<br>ttctcgagccaccagatccc<br>gtggaattga       | sSN1439<br>ccccatggcaatacagtg<br>cctgcaataatgacaac | pSM64                   | 33 ± 21 (mRNA)                                   |
| <i>cyoA</i> | sSN1438<br>ttctcgagccaccagatccc<br>gtggaattga       | sSN11016<br>atccatggcctgcaataa<br>tgacaaccatc      | pHN1806                 | -86 (mRNA)                                       |
| <i>cyoA</i> | sSN11015  | sSN1439  | pHN1808                 | 51 (mRNA)  |

|             |                        |                     |         |                          |
|-------------|------------------------|---------------------|---------|--------------------------|
|             | gcctcgagactttaacgcc    | cccatggcaatacagtg   |         |                          |
|             | accagatccc             | cctgcaataatgacaac   |         |                          |
| <i>edd</i>  | sMO27                  | sMO28               | pSM42   | 38 ± 15 (mRNA)           |
|             | atctcgagaatgaaacgcgt   | ctccatggaacgtcaatg  |         |                          |
|             | tgtgaatcat             | attcgattgt          |         |                          |
| <i>fbaA</i> | sSN1500                | sSN1501             | pSM24   | 29 (mRNA)                |
|             | ggctcgagcgaacgcaga     | acccatggtttacgaaat  |         |                          |
|             | cgcagcacatg            | caaaaatcttagacatg   |         |                          |
| <i>fnr</i>  | sSN1436                | sSN1437             | pSM13   | -34 ± 41 (mRNA)          |
|             | tactcgagacaaatatcaatt  | ggccatggatagcacaac  |         |                          |
|             | acggcttgagcag          | cgccagactgaa        |         |                          |
| <i>fumC</i> | sMO61                  | sMO62               | pSM57   | -88 ± 111                |
|             | aactcgagagctaaaagttg   | ctccatggacatcaatcgc |         | (mRNA)                   |
|             | cttaacgaaa             | cccatcgaat          |         |                          |
| <i>fumC</i> | sMO115                 | sMO116              | pSM57u  | 38 (mRNA)                |
|             | tactcgagaagtgagctaaa   | ggccatggaatcgcccc   |         |                          |
|             | agttgcttaa             | atcgaatctttt        |         |                          |
| <i>gntK</i> | sMO21                  | sMO22               | pSM39   | -751 ± 232 <sup>b)</sup> |
|             | atctcgagatattgcttctgat | aaccatggatcatgggt   |         | (mRNA)                   |
|             | attgtccg               | agtcgtgctca         |         |                          |
| <i>gntK</i> | sSN1662                | sSN1663             | pHN1832 | 57 (mRNA)                |
|             | ttctcgagtaattcttgattg  | aaccatggcatgaagttg  |         |                          |
|             | tgggggc                | atgcgccactt         |         |                          |
| <i>gpmI</i> | sMO17                  | sMO18               | pSM37   | 32 ± 16 (mRNA)           |
|             | ttctcgagattcagctgtagt  | agccatggccatccagaa  |         |                          |
|             | aaaattacg              | tcaccagtacca        |         |                          |
| <i>mgo</i>  | sSN1450                | sSN1451             | pSM18   | 54 ± 6 (mRNA)            |
|             | tactcgagtgtaccgttgat   | ggccatggagaagagca   |         |                          |
|             | gccgcgcaa              | tggcagtcacttt       |         |                          |
| <i>ndh</i>  | sSN1426                | sSN1427             | pSM10   | -147 ± 37(mRNA)          |
|             | atctcgagctatgtaataac   | cgccatggacaatcacia  |         |                          |
|             | cattaattaacaattgg      | tcttttcaatggcgtagtc |         |                          |
|             |                        | a                   |         |                          |
| <i>ndh</i>  | sSN11019               | sSN11020            | pHN1789 | 33 (mRNA)                |
|             | ccctcgagtaattaagagct   | caccatggccgccgaca   |         |                          |
|             | atgtaataa              | atcacaatctttt       |         |                          |

|             |  |   |         |                     |  |
|-------------|--|---|---------|---------------------|--|
| <i>ndh</i>  | sSN1426<br>atctcgagctatgtaataac<br>cattaattaacaattgg | sMO126<br>cccatggcacaatctttt<br>caatggcgta                | pHN1803 | 51 (mRNA)           |  |
| <i>ndh</i>  | sMO125<br>tgctcgagaagagctatggt<br>aataaccatt         | sSN1427<br>cgccatggacaatcacia<br>tcttttcaatggcgtagtc<br>a | pHN1804 | 32 (mRNA)           |  |
| <i>ndh</i>  | sSN11019<br>ccctcgagtaattaagagct<br>atgtaataa        | sSN1427<br>cgccatggacaatcacia<br>tcttttcaatggcgtagtc<br>a | pHN1805 | 49 (mRNA)           |  |
| <i>ndh</i>  | sMO125<br>tgctcgagaagagctatggt<br>aataaccatt         | sSN11020<br>caccatggccgcccagaca<br>atcacaatctttt          | pHN1852 | 26 (mRNA)           |  |
| <i>ndh</i>  | sMO125<br>tgctcgagaagagctatggt<br>aataaccatt         | sSN1670<br>ttccatggcgcccagctt<br>atgccccagct              | pHN1853 | 58 (mRNA)           |  |
| <i>poxB</i> | sMO1<br>tcctcgagatgaactaaact<br>gttaccgtt            | sMO2<br>caccatggttcaccctg<br>ccgattcgagt                  | pSM29   | -125 ± 18<br>(mRNA) |  |
| <i>ppc</i>  | sSN11011<br>cgctcgagaaagcccagac<br>atattcgcgcc       | sSN11012<br>tcccatggcactttgccga<br>gcatactgaca            | pHN1783 | 22 (mRNA)           |  |
| <i>ppc</i>  | sSN1257<br>tactcgagccaatgcgacgt<br>gaaggataca        | sMO122<br>tgccatggcccagcacttt<br>gccgagcatac              | pHN1795 | 34 (mRNA)           |  |
| <i>ppc</i>  | sMO121<br>agctcgagccgagcatattc<br>gcgccaatgc         | sSN1258<br>gccccatggccgagcatac<br>tgacattactac            | pHN1796 | 48 (mRNA)           |  |
| <i>ppc</i>  | sMO121<br>agctcgagccgagcatattc<br>gcgccaatgc         | sSN11012<br>tcccatggcactttgccga<br>gcatactgaca            | pHN1797 | 23 (mRNA)           |  |
| <i>ppc</i>  | sSN11011<br>cgctcgagaaagcccagac<br>atattcgcgcc       | sSN1258<br>gccccatggccgagcatac<br>tgacattactac            | pHN1798 | 19 (mRNA)           |  |
| <i>ppc</i>  | sSN1666  | sSN1667   | pHN1840 | 53 (mRNA)           |  |



|             |  |   |         |                |
|-------------|--|---|---------|----------------|
|             | aactcgagctatttattcgttt<br>gctgaagc     | caccatggaatattgttcgt<br>tcatattaccccagaca |         |                |
| <i>rpe</i>  | sMO49                                  | sMO50                                     | pSM51   | 58 ± 2 (mRNA)  |
|             | aactcgagaggagaagcg<br>gatgaaacagta     | gaccatggcgccagctgc<br>cagggcctttgc        |         |                |
| <i>rpiB</i> | sMO35                                  | sMO36                                     | pSM46   | 15 ± 35 (mRNA) |
|             | cactcgagtttttgattgtga<br>agttttgcacgga | acccatggtgccactattt<br>catgttttaaaatgaa   |         |                |
| <i>rpiB</i> | sMO109                                 | sSN11006                                  | pHN1828 | 59 (mRNA)      |
|             | aactcgagaaatgtttttgat<br>tgtgaagt      | cccatggtcatgttttaa<br>atgaaaccga          |         |                |
| <i>sucC</i> | sMO55                                  | sMO56                                     | pSM55   | 39 ± 16 (mRNA) |
|             | atctcgagtctacggtttaa<br>agataacga      | cccatggtgctggtgaag<br>ccatagcgggca        |         |                |

a) Numbers indicate reduction (%) of mRNA amounts (mRNA) or enzymatic activities (protein) as compared to control cells which carry an empty vector (pHN1257). The minus values indicate increase in mRNA amount.

b) The asRNA seems to silence the adjacent gene, *gntR* which encodes a transcriptional repressor for *gntK*.

**Supplementary TABLE S3****Oligonucleotides used in real-time quantitative RT-PCR assays and in construction for gene disruption vectors.**

| Gene name   | Forward oligonucleotide | Reverse oligonucleotide | Used for     |
|-------------|-------------------------|-------------------------|--------------|
| <i>accA</i> | accART1                 | accART2                 | quantitative |
|             | agagcgtggtcagtctgaagc   | ccacctcaccgataaccgta    | RT-PCR       |
| <i>aceA</i> | aceART1                 | aceART2                 | quantitative |
|             | cctggctctggtgtgaaacctc  | ggttttctgccagtgaacg     | RT-PCR       |
| <i>aceB</i> | aceBRT1                 | aceBRT2                 | quantitative |
|             | gaactggctttcacaaggcc    | tgtggcgtaaaatgcgtcac    | RT-PCR       |
| <i>aceE</i> | aceERT1                 | aceERT2                 | quantitative |
|             | ctgactcaggagcagctggat   | cagaattccggcatcagtttc   | RT-PCR       |
| <i>ackA</i> | ackART1                 | ackART2                 | quantitative |
|             | cagcgaagcgtcaactttat    | gtatacttttcgccccgtgt    | RT-PCR       |
| <i>acnA</i> | acnAqpcrF               | acnAqpcrR               | quantitative |
|             | aaaacgtacgcctggaaatg    | cacactgctttgccgagata    | RT-PCR       |
| <i>acnB</i> | acnBqpcrF               | acnBqpcrR               | quantitative |
|             | ccgttgagaagaaaggcaag    | aaaggcctgctcaacttca     | RT-PCR       |
| <i>acs</i>  | acsRT1                  | acsRT2                  | quantitative |
|             | cccggtaatgtgccattaa     | gtacgatcgccgtttcttg     | RT-PCR       |
| <i>adhE</i> | adhEqpcrF               | adhEqpcrR               | quantitative |
|             | cctgactctgggtgtggtt     | ctcgcttagcaacggtttc     | RT-PCR       |
| <i>arcA</i> | arcART1                 | arcART2                 | quantitative |
|             | atcacaaaccgttcaacc      | acgctacgacgttcttcgct    | RT-PCR       |
| <i>atpF</i> | atpFRT1                 | atpFRT2                 | quantitative |
|             | gtaatcatcgagcaggcgaac   | ctgggccacgattttagtacg   | RT-PCR       |
| <i>cra</i>  | craRT1                  | craRT2                  | quantitative |
|             | tcttgtgatccccgatctgg    | agcaggcaatcagcagttga    | RT-PCR       |
| <i>cydA</i> | cydART1                 | cydART2                 | quantitative |
|             | ctggcaatcgaaggctgat     | ccagccaggtgacacacata    | RT-PCR       |
| <i>cyoA</i> | cyoRT1                  | cyoRT2                  | quantitative |
|             | ggcattgctaccgtgaatga    | agacgcggaatgaagaagga    | RT-PCR       |
| <i>eda</i>  | edaqpcrF                | edaqpcrR                | quantitative |
|             | ctgaaagctgctaccgaagg    | agccgggaagaattgaact     | RT-PCR       |
| <i>edd</i>  | eddRT1                  | eddRT2                  | quantitative |

|             |                       |                       |              |
|-------------|-----------------------|-----------------------|--------------|
|             | gtaccgctgatggcacgtct  | gctttgagcagttcacgcac  | RT-PCR       |
| <i>eno</i>  | enoRT1                | enoRT2                | quantitative |
|             | aacgcaatcctggctgtatc  | ttcagttcagcgatgtgctc  | RT-PCR       |
| <i>fbaA</i> | fbaAqpcrF             | fbaAqpcrR             | quantitative |
|             | cgggtaacgtggttctgact  | ccaccgtggaatacgaagtt  | RT-PCR       |
| <i>fbaB</i> | fbaBRT1               | fbaBRT2               | quantitative |
|             | gtacaacaccgggcgtctg   | gcgggttagcagcaaatgaa  | RT-PCR       |
| <i>fbp</i>  | fbpqqpcrF             | fbpqqpcrR             | quantitative |
|             | aaaaactgaaagccgcactg  | cacagccttcaaagacgaca  | RT-PCR       |
| <i>fnr</i>  | fnrRT1                | fnrRT2                | quantitative |
|             | cgctgtttaaggctggtgat  | gtcgcctgctaaatggaaac  | RT-PCR       |
| <i>frdA</i> | frdART1               | frdART2               | quantitative |
|             | tctctcaggccttctggcac  | tttttctcggcaggtgac    | RT-PCR       |
| <i>fumA</i> | fumART1               | fumART2               | quantitative |
|             | atgtcgatcaactgcaagcg  | gaagccgccgtgtttttac   | RT-PCR       |
| <i>fumB</i> | fumBRT1               | fumBRT2               | quantitative |
|             | gtaccctcggctactgcagcc | agcgcttgctaactgacgg   | RT-PCR       |
| <i>fumC</i> | fumCRT1               | fumCRT2               | quantitative |
|             | ccctaacgacgacgtgaaca  | gaggaatgagttgcttgccg  | RT-PCR       |
| <i>gapA</i> | gapRT1                | gapRT2                | quantitative |
|             | ccgctactcagaaaaccgttg | tttagcagcaccggtagagga | RT-PCR       |
| <i>gcd</i>  | gcdRT1                | gcdRT2                | quantitative |
|             | tctggtcttctcggcatct   | ctaatacagcagtgcgaccac | RT-PCR       |
| <i>glcB</i> | glcBRT1               | glcBRT2               | quantitative |
|             | ctccagcacagtttgcggtt  | attggcatcgatttgcagct  | RT-PCR       |
| <i>glkA</i> | glkqpcrF              | glkqpcrR              | quantitative |
|             | cggtcattcgcgtttatctt  | attgagaacgccaggtatg   | RT-PCR       |
| <i>gltA</i> | gltAqpcrF             | gltAqpcrR             | quantitative |
|             | atgattctttccgctgatg   | cccagctctttcagcacttc  | RT-PCR       |
| <i>gnd</i>  | gndRT1                | gndRT2                | quantitative |
|             | ggcgctgaaaggtccttctat | gttcaccgtcttcagctacgg | RT-PCR       |
| <i>gntK</i> | gntKRT1               | gntKRT2               | quantitative |
|             | gaaccactgaatgacgacga  | agaacagacgatcagcgaca  | RT-PCR       |
| <i>gpmA</i> | gpmART1               | gpmART2               | quantitative |
|             | aggcgtaagcgaagcaaaag  | gggtatggatagcgcgttc   | RT-PCR       |
| <i>gpmB</i> | gpmBRT1               | gpmBRT2               | quantitative |

|             |                       |                       |              |
|-------------|-----------------------|-----------------------|--------------|
|             | ggtattgcactgggatgcct  | taatccacgcgcgaaataga  | RT-PCR       |
| <i>gpmI</i> | gpmIRT1               | gpmIRT2               | quantitative |
|             | gactggcgcggtagataaag  | gccatgatgtgatcttcgtg  | RT-PCR       |
| <i>icdA</i> | icdRT1                | icdRT2                | quantitative |
|             | cggaagacatttatgcgggta | ttcttcacccccatctctca  | RT-PCR       |
| <i>idnK</i> | idnKRT1               | idnKRT2               | quantitative |
|             | ccccatgttcatttcctctg  | aacgctaccggcataaaatg  | RT-PCR       |
| <i>ldhA</i> | ldhRT1                | ldhRT2                | quantitative |
|             | tgctggaagagctgaaaaagc | tcataggctggaacacggact | RT-PCR       |
| <i>maeA</i> | sfcRT1                | sfcRT2                | quantitative |
|             | ataaaggcagtgccctcagca | tgctcgttccgcttgttctt  | RT-PCR       |
| <i>maeB</i> | maeBRT1               | maeBRT2               | quantitative |
|             | tggtttgcgattcaaaaggc  | gagggtacgtttgccgtcat  | RT-PCR       |
| <i>mdh</i>  | mdhRT1                | mdhRT2                | quantitative |
|             | accgggtaacaccacagttg  | cctttcagttccgcaacaaag | RT-PCR       |
| <i>mlc</i>  | mlcRT1                | mlcRT2                | quantitative |
|             | ggtccagtctcgcgtatcga  | tcttgcaccaggtgtgcttc  | RT-PCR       |
| <i>mqo</i>  | mqoRT1                | mqoRT2                | quantitative |
|             | gtcgccattaacgaagcatt  | gtcctatgcggaacggat    | RT-PCR       |
| <i>ndh</i>  | ndhRT1                | ndhRT2                | quantitative |
|             | gtcgatcgtaaccacagcca  | gcatgggccagatagctcaa  | RT-PCR       |
| <i>nuoA</i> | nuoqpcrF              | nuoqpcrR              | quantitative |
|             | gaaaaacgtgccgtttgaat  | gaagaacatggccaccagat  | RT-PCR       |
| <i>pckA</i> | pckART1               | pckART2               | quantitative |
|             | cattatcgacgccatcctcaa | taggtgttacgcggatcgaga | RT-PCR       |
| <i>pfkA</i> | pfkART1               | pfkART2               | quantitative |
|             | ccgctttgatttcgtttacca | gcttcaccatcacctctcac  | RT-PCR       |
| <i>pfkB</i> | pfkBRT1               | pfkBRT2               | quantitative |
|             | gttggcggatgaaaatgtcc  | aacgatactgctcaccgcttg | RT-PCR       |
| <i>pflB</i> | pflBRT1               | pflBRT2               | quantitative |
|             | taccaccgaacgggataaga  | gctggctacatcaacaagca  | RT-PCR       |
| <i>pgi</i>  | pgiRT1                | pgiRT2                | quantitative |
|             | atttccggtgagtgaaaggt  | ggtttttgtacggacgcagag | RT-PCR       |
| <i>pgk</i>  | pgkqpcrF              | pgkqpcrR              | quantitative |
|             | gtctgatgttcgcgtagcaa  | ggatctgctcgtcagctttc  | RT-PCR       |
| <i>pgl</i>  | pglRT1                | pglRT2                | quantitative |

|             |                       |                      |              |
|-------------|-----------------------|----------------------|--------------|
|             | agtccgacgcatattccac   | ccatctccagacgcgttac  | RT-PCR       |
| <i>poxB</i> | poxBRT1               | poxBRT2              | quantitative |
|             | aaaagccgatcgcaagttc   | ggtgaatggctttctcgctc | RT-PCR       |
| <i>ppc</i>  | sSN1243               | sSN1244              | quantitative |
|             | ctgagcgaagacacatcaa   | gtaatttcggtgggtgagc  | RT-PCR       |
| <i>ppsA</i> | sSN1245               | sSN1246              | quantitative |
|             | cagcaggaaaccttctcaa   | taacctggtgcacacgata  | RT-PCR       |
| <i>pta</i>  | ptaRT1                | ptaRT2               | quantitative |
|             | cctgccggtatttatggtgaa | cgatacgttcgtgatcgtaa | RT-PCR       |
| <i>ptsG</i> | ptaGRT1               | ptsGRT2              | quantitative |
|             | actgcgggtaaactgtctgg  | tcataataccgccactttcg | RT-PCR       |
| <i>pykA</i> | pykART1               | pykART2              | quantitative |
|             | cgttaccacgtaggccag    | gcgagccgtgagaaaagttc | RT-PCR       |
| <i>pykF</i> | pykFRT1               | pykFRT2              | quantitative |
|             | tgtctggtgaatccgcaaaa  | ctcgagacggctgttcatca | RT-PCR       |
| <i>rpe</i>  | rpeRT1                | rpeRT2               | quantitative |
|             | gccaatggtgctgaaatcct  | cagcgaatcaggcacaatg  | RT-PCR       |
| <i>rpiA</i> | rpiART1               | rpiART2              | quantitative |
|             | gatgggcggcacttcagtat  | gccttcattgtaccgagcg  | RT-PCR       |
| <i>rpiB</i> | rpiBRT1               | rpiBRT2              | quantitative |
|             | gagaggttgatggcgggatt  | aggttcgctacagacgaccg | RT-PCR       |
| <i>sdhC</i> | sdhCRT1               | sdhCRT2              | quantitative |
|             | tgatcacctttgttcagtgg  | gtgatacggcagagcgtaag | RT-PCR       |
| <i>sucA</i> | sucAqpcrF             | sucAqpcrR            | quantitative |
|             | gataaagtggccgatctgga  | aatgaaccgacgttgaagg  | RT-PCR       |
| <i>sucC</i> | sucCRT1               | sucCRT2              | quantitative |
|             | ccaaaatcttcatgggcctg  | gcaaatcagatgccctgtt  | RT-PCR       |
| <i>talA</i> | talART1               | talART2              | quantitative |
|             | ctcaaaatcgtaccggctcg  | tacaagtccaccagatggcg | RT-PCR       |
| <i>talB</i> | talBRT1               | talBRT2              | quantitative |
|             | accgtagtggccgacactg   | ggaatctgcgctgcgtaag  | RT-PCR       |
| <i>tktA</i> | tktART1               | tktART2              | quantitative |
|             | agtctgtggcgtgatttcc   | agatcagcatggagccgtg  | RT-PCR       |
| <i>tktB</i> | tktBRT1               | tktBRT2              | quantitative |
|             | cccgaaaagaccttgccaat  | aatatcagccatgccatcg  | RT-PCR       |
| <i>tpiA</i> | tpiART1               | tpiART2              | quantitative |

|             |   |                                 |                                |              |
|-------------|---|---------------------------------|--------------------------------|--------------|
|             |   | agaagtttgcgcacgtcagat           | tgccatacaggttcgtaagc           | RT-PCR       |
| <i>zwf</i>  |   | zwfRT1                          | zwfRT2                         | quantitative |
|             |   | ccaggtttaccgtatcgacca           | tctgccacggtaatctcaaca          | RT-PCR       |
| 16          | S | 16S1                            | 16S2                           | quantitative |
| <i>rRNA</i> |   | ttgctcattgacgttaccg             | acgccagtaattccgatta            | RT-PCR       |
| <i>cra</i>  |   | cra1                            | cra2                           | gene         |
|             |   | cttgatgcatttttagcatcgattcgc     | aaactcgagtctgcaggcaatcagcagt   | disruption   |
|             |   |                                 | tgataaccccgttgccg              | vector       |
| <i>cra</i>  |   | cra3                            | cra4                           | gene         |
|             |   | aaactcgagtactagtggtcaacgtcacc   | aaaccatggtgcaaaccatttgaccggc   | disruption   |
|             |   | gcgatgtcgcagag                  | agcgttc                        | vector       |
| <i>pflB</i> |   | pflB1                           | pflB2                          | gene         |
|             |   | atactgcagcaaccactggcacaggcaca   | gttctagacgtttacttcattctgccagtc | disruption   |
|             |   |                                 |                                | vector       |
| <i>pflB</i> |   | pflB3                           | pflB4                          | gene         |
|             |   | actctagaacagcagcaggacgttattac   | ttccatggatctcgtcgttcattctgttga | disruption   |
|             |   |                                 |                                | vector       |
| <i>ppsA</i> |   | ppsA1                           | ppsA2                          | gene         |
|             |   | aaactgcagtttcatctcttgtgtagcggg  | aaactcgagtatgcataaatgcgctggtt  | disruption   |
|             |   | gc                              | tacgccgctttggtc                | vector       |
| <i>ppsA</i> |   | ppsA3                           | ppsA4                          | gene         |
|             |   | aaactcgagtactagttcctcgaatatttcg | aaaccatggaagatattttattgcagcatc | disruption   |
|             |   | acggcttctcaa                    | ctaaaattcac                    | vector       |
| <i>sucC</i> |   | sucC1                           | sucC2                          | gene         |
|             |   | aaactgcagcatggcggtttctacgccgc   | aaactcgagtatgcatttcactaccacg   | disruption   |
|             |   | gcgg                            | gaccggcaccgattt                | vector       |
| <i>sucC</i> |   | sucC3                           | sucC4                          | gene         |
|             |   | atctcgagtctacggtttaaaagataacga  | aaaccatggtaacgtgctctttgatgtac  | disruption   |
|             |   |                                 | gcagc                          | vector       |
| <i>pykF</i> |   | pykF1                           | pykF2                          | gene         |
|             |   | aaactgcagatcgcgcatttccagcacia   | aaactcgagtatgcatggtgcggatttcc  | disruption   |
|             |   | ctttac                          | ggaccttgggtatc                 | vector       |
| <i>pykF</i> |   | pykF3                           | pykF4                          | gene         |
|             |   | aaactcgagtactagtagtaataacttccc  | aaaccatggtcaacttttagcgttcagagt | disruption   |
|             |   | ggatgccaccatc                   | ctg                            | vector       |
| <i>cydA</i> |   | cydA1                           | cydA2                          | gene         |

|             |                                |                              |            |
|-------------|--------------------------------|------------------------------|------------|
|             | aaactgcagtgaccaaaaaagaattaag   | aaactcgagtatgcatggtcagaccggt | disruption |
|             | gtcaac                         | agccacaccagag                | vector     |
| <i>cydA</i> | cydA3                          | cydA4                        | gene       |
|             | aaactcgagtactagtgtcatccgcaaccg | aaaccatggagcggtagtcaaaaccga  | disruption |
|             | cattggcgagaaaa                 | ccggacg                      | vector     |

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**Supplementary TABLE S4**

**HPLC analysis of metabolites and glucose consumption following silencing.**

| Gene name   | HPLC retention time (min) | Compound name <sup>a)</sup> | Fold change <sup>b)</sup> | glucose consumed (g•L <sup>-1</sup> ) <sup>c)</sup> |
|-------------|---------------------------|-----------------------------|---------------------------|---|
| <i>accA</i> | -                         | glucose                     | -                         | 17  |
|             | 9.64                      | ?                           | 0.95                      |   |
|             | 10.85                     | pyruvate                    | 10                        |   |
|             | 12.21                     | ?                           | 1.6                       |   |
|             | 14.16                     | ?                           | 1.0                       |   |
|             | 16.52                     | fumarate                    | 1.0                       |   |
|             | 17.91                     | acetate                     | 1.1                       |   |
| <i>aceA</i> | -                         | glucose                     | -                         | 16  |
|             | 9.62                      | ?                           | 1.5                       |   |
|             | 12.21                     | ?                           | 1.7                       |   |
|             | 14.61                     | ?                           | 0.67                      |   |
|             | 16.55                     | fumarate                    | 1.3                       |   |
|             | 17.92                     | acetate                     | 0.9                       |   |
| <i>aceB</i> | -                         | glucose                     | -                         | 16  |
|             | 9.62                      | ?                           | 2.1                       |   |
|             | 12.23                     | ?                           | 1.5                       |   |
|             | 14.19                     | ?                           | 0.67                      |   |
|             | 16.53                     | fumarate                    | 0.73                      |   |
|             | 17.93                     | acetate                     | 0.74                      |   |
| <i>aceE</i> | -                         | glucose                     | -                         | 16  |
|             | 9.65                      | ?                           | 0.65                      |   |
|             | 10.93                     | pyruvate                    | 427                       |   |
|             | 14.16                     | ?                           | 0.62                      |   |
|             | 16.52                     | fumarate                    | 0.46                      |   |
| <i>ackA</i> | -                         | glucose                     | -                         | 17  |
|             | 9.64                      | ?                           | 0.64                      |   |
|             | 10.87                     | pyruvate                    | 2.7                       |   |
|             | 14.17                     | ?                           | 0.73                      |   |
|             | 16.52                     | fumarate                    | 0.71                      |   |



|             |       |          |       |    |
|-------------|-------|----------|-------|----|
|             | 17.91 | acetate  | 0.91  |    |
| <i>acnA</i> | -     | glucose  | -     | 15 |
|             | 9.59  | ?        | 3.3   |    |
|             | 12.19 | ?        | 4.0   |    |
|             | 14.16 | ?        | 0.65  |    |
|             | 16.48 | fumarate | 0.9   |    |
|             | 17.89 | acetate  | 0.75  |    |
| <i>acnB</i> | -     | glucose  | -     | 17 |
|             | 9.59  | ?        | 1.2   |    |
|             | 12.17 | ?        | 0.97  |    |
|             | 14.16 | ?        | 0.9   |    |
|             | 16.47 | fumarate | 1.3   |    |
|             | 17.88 | acetate  | 0.89  |    |
| <i>acs</i>  | -     | glucose  | -     | 14 |
|             | 9.64  | ?        | 0.89  |    |
|             | 12.2  | ?        | 2.4   |    |
|             | 14.16 | ?        | 0.97  |    |
|             | 16.55 | fumarate | 1.0   |    |
|             | 17.89 | acetate  | -0.84 |    |
| <i>adhE</i> | -     | glucose  | -     | 15 |
|             | 9.64  | ?        | 0.79  |    |
|             | 10.87 | pyruvate | 1.7   |    |
|             | 12.2  | ?        | 0.94  |    |
|             | 14.17 | ?        | 9.8   |    |
|             | 16.55 | fumarate | 1.7   |    |
|             | 17.89 | acetate  | 1.1   |    |
| <i>arcA</i> | -     | glucose  | -     | 12 |
|             | 9.65  | ?        | 6.1   |    |
|             | 12.23 | ?        | 11    |    |
|             | 17.92 | acetate  | 0.69  |    |
| <i>atpF</i> | -     | glucose  | -     | 15 |
|             | 9.65  | ?        | 0.52  |    |
|             | 10.87 | pyruvate | 6.0   |    |
|             | 14.17 | ?        | 0.89  |    |
|             | 16.56 | fumarate | 0.63  |    |
|             | 17.92 | acetate  | 1.2   |    |

|             |            |          |         |    |
|-------------|------------|----------|---------|----|
| <i>cra</i>  | -          | glucose  | -       | 17 |
|             | 9.64       | ?        | 0.74    |    |
|             | 10.85      | pyruvate | 10      |    |
|             | 12.2       | ?        | 1.6     |    |
|             | 14.16      | ?        | 0.96    |    |
|             | 16.52      | fumarate | 0.86    |    |
|             | 17.91      | acetate  | 1.2     |    |
| <i>cydA</i> | -          | glucose  | -       | 24 |
|             | 9.64       | ?        | 0.84    |    |
|             | 10.85      | pyruvate | 36      |    |
|             | 12.23      | ?        | 1.0     |    |
|             | 14.16      | ?        | 1.3     |    |
|             | 16.53      | fumarate | 1.5     |    |
|             | 17.91      | acetate  | 1.8     |    |
| <i>cyoA</i> | -          | glucose  | -       | 19 |
|             | 9.63       | ?        | 0.76    |    |
|             | 10.85      | pyruvate | 9.0     |    |
|             | 14.17      | ?        | 1       |    |
|             | 16.53      | fumarate | 0.95    |    |
|             | 17.91      | acetate  | 1.2     |    |
|             | <i>eda</i> | -        | glucose |    |
| 9.65        |            | ?        | 1.2     |    |
| 10.89       |            | pyruvate | 1.1     |    |
| 12.21       |            | ?        | 0.82    |    |
| 14.17       |            | ?        | 0.88    |    |
| 16.56       |            | fumarate | 0.92    |    |
| 17.92       |            | acetate  | 0.8     |    |
| <i>edd</i>  | -          | glucose  | -       | 15 |
|             | 9.64       | ?        | 0.95    |    |
|             | 14.17      | ?        | 0.89    |    |
|             | 16.55      | fumarate | 0.79    |    |
|             | 17.92      | acetate  | 0.82    |    |
| <i>eno</i>  | -          | glucose  | -       | 15 |
|             | 9.65       | ?        | 1.1     |    |
|             | 14.17      | ?        | 0.90    |    |
|             | 16.55      | fumarate | 0.85    |    |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 17.92 | acetate  | 0.85 |    |
| <i>fbaA</i> | -     | glucose  | -    | 9  |
|             | 9.66  | ?        | 0.82 |    |
|             | 11.07 | pyruvate | 0.48 |    |
|             | 14.19 | ?        | 0.93 |    |
|             | 16.57 | fumarate | 0.19 |    |
|             | 17.92 | acetate  | 0.21 |    |
| <i>fbaB</i> | -     | glucose  | -    | 15 |
|             | 9.64  | ?        | 0.76 |    |
|             | 14.17 | ?        | 0.86 |    |
|             | 16.55 | fumarate | 0.68 |    |
|             | 17.92 | acetate  | 0.84 |    |
| <i>fbp</i>  | -     | glucose  | -    | 17 |
|             | 9.64  | ?        | 0.70 |    |
|             | 10.89 | pyruvate | 0.65 |    |
|             | 14.19 | ?        | 0.91 |    |
|             | 16.53 | fumarate | 0.79 |    |
|             | 17.91 | acetate  | 0.99 |    |
| <i>fnr</i>  | -     | glucose  | -    | 16 |
|             | 9.64  | ?        | 0.59 |    |
|             | 10.88 | pyruvate | 0.99 |    |
|             | 12.23 | ?        | 0.78 |    |
|             | 14.17 | ?        | 0.92 |    |
|             | 16.55 | fumarate | 0.99 |    |
|             | 17.91 | acetate  | 0.89 |    |
| <i>frdA</i> | -     | glucose  | -    | 17 |
|             | 9.63  | ?        | 0.35 |    |
|             | 10.96 | pyruvate | 1.6  |    |
|             | 14.17 | ?        | 1.0  |    |
|             | 16.51 | fumarate | 0.69 |    |
|             | 17.92 | acetate  | 1.1  |    |
| <i>fumA</i> | -     | glucose  | -    | 16 |
|             | 9.6   | ?        | 0.92 |    |
|             | 10.84 | pyruvate | 0.32 |    |
|             | 12.19 | ?        | 0.99 |    |
|             | 14.16 | ?        | 0.89 |    |

|             |       |          |       |    |
|-------------|-------|----------|-------|----|
|             | 16.51 | fumarate | 0.80  |    |
|             | 17.89 | acetate  | 0.88  |    |
| <i>fumB</i> | -     | glucose  | -     | 17 |
|             | 9.6   | ?        | 0.83  |    |
|             | 10.81 | pyruvate | 1.9   |    |
|             | 12.2  | ?        | 0.87  |    |
|             | 14.19 | ?        | 0.9   |    |
|             | 16.52 | fumarate | 1.1   |    |
|             | 17.89 | acetate  | 1.0   |    |
| <i>fumC</i> | -     | glucose  | -     | 17 |
|             | 9.61  | ?        | 1.1   |    |
|             | 10.83 | pyruvate | 5.4   |    |
|             | 12.21 | ?        | 1.7   |    |
|             | 14.17 | ?        | 0.92  |    |
|             | 16.53 | fumarate | 1.4   |    |
|             | 17.92 | acetate  | 1.1   |    |
| <i>gapA</i> | -     | glucose  | -     | 12 |
|             | 9.65  | ?        | 0.87  |    |
|             | 14.19 | ?        | 0.87  |    |
|             | 16.53 | fumarate | 0.030 |    |
|             | 17.89 | acetate  | 0.32  |    |
| <i>gcd</i>  | -     | glucose  | -     | 16 |
|             | 9.64  | ?        | 0.78  |    |
|             | 10.85 | pyruvate | 1.6   |    |
|             | 12.24 | ?        | 0.83  |    |
|             | 14.16 | ?        | 0.98  |    |
|             | 16.55 | fumarate | 0.83  |    |
|             | 17.91 | acetate  | 1.1   |    |
| <i>glcB</i> | -     | glucose  | -     | 17 |
|             | 9.61  | ?        | 1.1   |    |
|             | 10.85 | pyruvate | 1.0   |    |
|             | 12.2  | ?        | 0.83  |    |
|             | 14.16 | ?        | 0.87  |    |
|             | 16.55 | fumarate | 1.0   |    |
|             | 17.93 | acetate  | 0.89  |    |
| <i>glkA</i> | -     | glucose  | -     | 15 |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 9.64  | ?        | 0.72 |    |
|             | 14.17 | ?        | 0.95 |    |
|             | 16.55 | fumarate | 0.82 |    |
|             | 17.91 | acetate  | 0.95 |    |
| <i>gltA</i> | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 0.87 |    |
|             | 10.85 | pyruvate | 15   |    |
|             | 12.20 | ?        | 2.0  |    |
|             | 14.17 | ?        | 1.2  |    |
|             | 16.52 | fumarate | 0.71 |    |
|             | 17.91 | acetate  | 1.6  |    |
| <i>gnd</i>  | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 0.81 |    |
|             | 10.88 | pyruvate | 0.66 |    |
|             | 12.23 | ?        | 0.8  |    |
|             | 14.2  | ?        | 0.9  |    |
|             | 16.57 | fumarate | 0.94 |    |
|             | 17.92 | acetate  | 1.0  |    |
| <i>gntK</i> | -     | glucose  | -    | 15 |
|             | 9.65  | ?        | 0.60 |    |
|             | 14.17 | ?        | 0.87 |    |
|             | 16.53 | fumarate | 0.70 |    |
|             | 17.91 | acetate  | 0.86 |    |
| <i>gpmA</i> | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 1.3  |    |
|             | 14.17 | ?        | 0.89 |    |
|             | 16.55 | fumarate | 0.83 |    |
|             | 17.91 | acetate  | 0.89 |    |
| <i>gpmB</i> | -     | glucose  | -    | 17 |
|             | 9.64  | ?        | 0.52 |    |
|             | 10.85 | pyruvate | 1.7  |    |
|             | 12.21 | ?        | 1.4  |    |
|             | 14.19 | ?        | 0.91 |    |
|             | 16.52 | fumarate | 0.86 |    |
|             | 17.91 | acetate  | 0.81 |    |
| <i>gpmI</i> | -     | glucose  | -    | 17 |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 9.64  | ?        | 0.60 |    |
|             | 10.85 | pyruvate | 3.0  |    |
|             | 12.21 | ?        | 1.7  |    |
|             | 14.16 | ?        | 1.0  |    |
|             | 16.53 | fumarate | 1.2  |    |
|             | 17.91 | acetate  | 1.1  |    |
| <i>icdA</i> | -     | glucose  | -    | 10 |
|             | 9.65  | ?        | 0.53 |    |
|             | 11.07 | pyruvate | 0.58 |    |
|             | 12.2  | ?        | 0.53 |    |
|             | 14.19 | ?        | 0.93 |    |
|             | 17.93 | acetate  | 0.36 |    |
| <i>idnK</i> | -     | glucose  | -    | 11 |
|             | 9.65  | ?        | 1.9  |    |
|             | 12.21 | ?        | 1.2  |    |
|             | 14.19 | ?        | 0.67 |    |
|             | 17.89 | acetate  | 0.36 |    |
| <i>ldhA</i> | -     | glucose  | -    | 15 |
|             | 9.65  | ?        | 1.1  |    |
|             | 10.89 | pyruvate | 0.54 |    |
|             | 12.2  | ?        | 1.1  |    |
|             | 14.17 | ?        | 0.94 |    |
|             | 16.53 | fumarate | 0.97 |    |
|             | 17.91 | acetate  | 0.90 |    |
| <i>maeA</i> | -     | glucose  | -    | 18 |
|             | 9.63  | ?        | 0.67 |    |
|             | 10.87 | pyruvate | 3.3  |    |
|             | 14.17 | ?        | 0.98 |    |
|             | 16.53 | fumarate | 0.82 |    |
|             | 17.91 | acetate  | 1.2  |    |
| <i>maeB</i> | -     | glucose  | -    | 21 |
|             | 9.63  | ?        | 0.71 |    |
|             | 10.85 | pyruvate | 4.9  |    |
|             | 12.24 | ?        | 1.6  |    |
|             | 14.16 | ?        | 1.0  |    |
|             | 16.52 | fumarate | 1.1  |    |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 17.92 | acetate  | 1.1  |    |
| <i>mdh</i>  | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 1.4  |    |
|             | 10.85 | pyruvate | 2.3  |    |
|             | 12.21 | ?        | 1.0  |    |
|             | 14.16 | ?        | 0.96 |    |
|             | 16.53 | fumarate | 0.98 |    |
|             | 17.91 | acetate  | 1.1  |    |
| <i>mlc</i>  | -     | glucose  | -    | 16 |
|             | 9.62  | ?        | 1.7  |    |
|             | 10.85 | pyruvate | 4.1  |    |
|             | 12.21 | ?        | 1.3  |    |
|             | 14.19 | ?        | 0.65 |    |
|             | 16.53 | fumarate | 0.72 |    |
|             | 17.93 | acetate  | 0.81 |    |
| <i>mgo</i>  | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 0.97 |    |
|             | 14.15 | ?        | 1.2  |    |
|             | 16.55 | fumarate | 1.6  |    |
|             | 17.92 | acetate  | 0.95 |    |
| <i>ndh</i>  | -     | glucose  | -    | 18 |
|             | 9.63  | ?        | 0.43 |    |
|             | 10.85 | pyruvate | 9.2  |    |
|             | 14.19 | ?        | 0.92 |    |
|             | 16.51 | fumarate | 0.58 |    |
|             | 17.92 | acetate  | 1.2  |    |
| <i>nuoA</i> | -     | glucose  | -    | 16 |
|             | 9.64  | ?        | 1.6  |    |
|             | 14.17 | ?        | 0.71 |    |
|             | 16.55 | fumarate | 0.83 |    |
|             | 17.91 | acetate  | 0.81 |    |
| <i>pckA</i> | -     | glucose  | -    | 12 |
|             | 9.59  | ?        | 1.0  |    |
|             | 10.80 | pyruvate | 0.19 |    |
|             | 14.21 | ?        | 0.76 |    |
|             | 16.59 | fumarate | 0.92 |    |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 17.95 | acetate  | 0.82 |    |
| <i>pfkA</i> | -     | glucose  | -    | 18 |
|             | 9.63  | ?        | 0.58 |    |
|             | 14.17 | ?        | 0.92 |    |
|             | 16.53 | fumarate | 0.45 |    |
|             | 17.92 | acetate  | 0.39 |    |
| <i>pfkB</i> | -     | glucose  | -    | 16 |
|             | 9.64  | ?        | 0.92 |    |
|             | 10.89 | pyruvate | 0.37 |    |
|             | 14.19 | ?        | 0.96 |    |
|             | 16.53 | fumarate | 0.86 |    |
|             | 17.91 | acetate  | 0.99 |    |
| <i>pflB</i> | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 1.1  |    |
|             | 10.85 | pyruvate | 9.9  |    |
|             | 12.21 | ?        | 1.8  |    |
|             | 14.15 | ?        | 1.2  |    |
|             | 16.52 | fumarate | 0.92 |    |
|             | 17.91 | acetate  | 1.2  |    |
| <i>pgi</i>  | -     | glucose  | -    | 16 |
|             | 9.63  | ?        | 0.51 |    |
|             | 12.2  | ?        | 0.91 |    |
|             | 14.16 | ?        | 0.51 |    |
|             | 16.53 | fumarate | 0.53 |    |
|             | 17.89 | acetate  | 0.46 |    |
| <i>pgk</i>  | -     | glucose  | -    | 15 |
|             | 9.65  | ?        | 1.6  |    |
|             | 14.19 | ?        | 0.71 |    |
|             | 16.53 | fumarate | 0.54 |    |
|             | 17.92 | acetate  | 0.72 |    |
| <i>pgl</i>  | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 1.1  |    |
|             | 14.19 | ?        | 1.0  |    |
|             | 16.56 | fumarate | 1.3  |    |
|             | 17.93 | acetate  | 0.96 |    |
| <i>poxB</i> | -     | glucose  | -    | 15 |



|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 9.64  | ?        | 0.89 |    |
|             | 10.85 | pyruvate | 0.95 |    |
|             | 12.2  | ?        | 0.82 |    |
|             | 14.17 | ?        | 0.96 |    |
|             | 16.52 | fumarate | 0.74 |    |
|             | 17.89 | acetate  | 1.1  |    |
| <i>ppc</i>  | -     | glucose  | -    | 13 |
|             | 9.65  | ?        | 4.0  | -  |
|             | 10.87 | pyruvate | 39   | -  |
|             | 14.19 | ?        | 1.1  |    |
|             | 16.55 | fumarate | 0.2  |    |
|             | 17.91 | acetate  | 0.91 |    |
| <i>ppsA</i> | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 2.7  | -  |
|             | 10.85 | pyruvate | 13   | -  |
|             | 14.19 | ?        | 1.2  | -  |
|             | 16.53 | fumarate | 0.66 | -  |
|             | 17.91 | acetate  | 1.0  | -  |
| <i>pta</i>  | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 0.60 |    |
|             | 10.92 | pyruvate | 5.2  |    |
|             | 12.20 | ?        | 7.5  |    |
|             | 14.17 | ?        | 1.1  |    |
|             | 16.55 | fumarate | 1.1  |    |
|             | 17.92 | acetate  | 1.0  |    |
| <i>ptsG</i> | -     | glucose  | -    | 13 |
|             | 9.63  | ?        | 0.41 |    |
|             | 12.2  | ?        | 1.7  |    |
|             | 14.17 | ?        | 1.0  |    |
|             | 16.49 | fumarate | 0.36 |    |
|             | 17.91 | acetate  | 0.48 |    |
| <i>pykA</i> | -     | glucose  | -    | 15 |
|             | 9.64  | ?        | 1.1  |    |
|             | 14.16 | ?        | 0.91 |    |
|             | 16.55 | fumarate | 0.75 |    |
|             | 17.91 | acetate  | 0.87 |    |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
| <i>pykF</i> | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 0.27 |    |
|             | 10.85 | pyruvate | 48   |    |
|             | 14.15 | ?        | 0.88 |    |
|             | 14.92 | ?        | 0.88 |    |
|             | 16.56 | fumarate | 1.3  |    |
|             | 17.91 | acetate  | 0.97 |    |
| <i>rpe</i>  | -     | glucose  | -    | 15 |
|             | 9.6   | ?        | 0.95 |    |
|             | 14.16 | ?        | 0.78 |    |
|             | 16.49 | fumarate | 0.83 |    |
|             | 17.88 | acetate  | 0.75 |    |
| <i>rpiA</i> | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 0.77 |    |
|             | 10.87 | pyruvate | 1.4  |    |
|             | 12.23 | ?        | 0.84 |    |
|             | 14.19 | ?        | 0.93 |    |
|             | 16.56 | fumarate | 0.89 |    |
|             | 17.92 | acetate  | 1.1  |    |
| <i>rpiB</i> | -     | glucose  | -    | 17 |
|             | 9.64  | ?        | 0.8  |    |
|             | 14.17 | ?        | 0.98 |    |
|             | 16.53 | fumarate | 0.82 |    |
|             | 17.91 | acetate  | 0.97 |    |
| <i>sdhC</i> | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 0.68 |    |
|             | 10.85 | pyruvate | 8.6  |    |
|             | 12.2  | ?        | 1.3  |    |
|             | 14.15 | ?        | 1.1  |    |
|             | 16.53 | fumarate | 0.9  |    |
| <i>sucA</i> | -     | glucose  | -    | 17 |
|             | 9.65  | ?        | 0.71 |    |
|             | 10.87 | pyruvate | 4.7  |    |
|             | 12.21 | ?        | 1.4  |    |
|             | 14.19 | ?        | 0.99 |    |

|             |       |          |      |    |
|-------------|-------|----------|------|----|
|             | 16.53 | fumarate | 0.72 |    |
|             | 17.92 | acetate  | 1.1  |    |
| <i>sucC</i> | -     | glucose  | -    | 17 |
|             | 9.59  | ?        | 0.43 |    |
|             | 10.83 | pyruvate | 35   |    |
|             | 12.21 | ?        | 0.6  |    |
|             | 14.17 | ?        | 0.99 |    |
|             | 16.51 | fumarate | 0.8  |    |
|             | 17.92 | acetate  | 1.4  |    |
| <i>talA</i> | -     | glucose  | -    | 16 |
|             | 9.63  | ?        | 0.55 |    |
|             | 14.19 | ?        | 0.7  |    |
|             | 16.55 | fumarate | 0.71 |    |
|             | 17.92 | acetate  | 0.82 |    |
| <i>talB</i> | -     | glucose  | -    | 11 |
|             | 9.66  | ?        | 0.79 |    |
|             | 12.24 | ?        | 0.96 |    |
|             | 14.19 | ?        | 1.3  |    |
|             | 16.57 | fumarate | 0.9  |    |
|             | 17.91 | acetate  | 0.99 |    |
| <i>tktA</i> | -     | glucose  | -    | 16 |
|             | 9.65  | ?        | 0.93 |    |
|             | 14.19 | ?        | 0.94 |    |
|             | 16.56 | fumarate | 1.0  |    |
|             | 17.92 | acetate  | 0.86 |    |
| <i>tktB</i> | -     | glucose  | -    | 15 |
|             | 9.65  | ?        | 0.79 |    |
|             | 14.17 | ?        | 0.72 |    |
|             | 16.53 | fumarate | 0.58 |    |
|             | 17.92 | acetate  | 0.77 |    |
| <i>tpiA</i> | -     | glucose  | -    | 15 |
|             | 9.64  | ?        | 0.79 |    |
|             | 10.93 | pyruvate | 0.7  |    |
|             | 14.17 | ?        | 0.94 |    |
|             | 16.53 | fumarate | 0.77 |    |
|             | 17.91 | acetate  | 0.99 |    |

|            |       |          |     |    |
|------------|-------|----------|-----|----|
| <i>zwf</i> | -     | glucose  | -   | 21 |
|            | 9.64  | ?        | 1.1 |    |
|            | 10.85 | pyruvate | 5.6 |    |
|            | 12.21 | ?        | 3.2 |    |
|            | 14.16 | ?        | 1.2 |    |
|            | 16.52 | fumarate | 1.2 |    |
|            | 17.92 | acetate  | 1.5 |    |

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a) Each transformant was cultured in the N2G medium containing IPTG for 24 hours, and metabolites accumulated in the medium were detected using high performance liquid chromatography. Data are shown for pyruvate, fumarate, acetate, and unknown compounds (shown with question marks) whose retention time was typically 9.65, 12.21 and 14.19 min. When no peaks for these compounds were found, data are omitted.

b) Data are presented as fold change compared to the cells containing an empty vector, pHN1257. Yellow and green highlights indicate over 10- and 5- fold increase of pyruvate.

c) Amount of consumed glucose was determined. The cells containing an empty vector, pHN1257 consumed  $18 \text{ g}\cdot\text{L}^{-1}$  of glucose under the same condition.