**Supplement**

**Supplementary Table 1.** BASIC- compatible primers used in this study (BASIC prefix and suffix sequences in bold)

|  |  |  |  |
| --- | --- | --- | --- |
| **DNA part** | **Forward primer (prefix)** | **Reverse primer (suffix)** | **source** |
| 5’-NS (neutral site slr0168) | **TCTGGTGGGTCTCTGTCC**CCTTTGACAACAATGTGGCCTGG  | **CGATAGGTCTCCCGAGCC**CCATATAAATCCCCGCCACTGTTATTTTG  | *Synechocystis* 6803 genomic DNA |
| 3’-NS (neutral site slr0168) | **TCTGGTGGGTCTCTGTCC**AGACCAAGCCCAATTTCGTTTGC  | **CGATAGGTCTCCCGAGCC**GCTAAACCCACCTCTTGCCCAATG  | *Synechocystis* 6803 genomic DNA |
| PA1lacO1 | **TCTGGTGGGTCTCTGTCC**GACACCATCGAATGGTGCAAAACC  | **CGATAGGTCTCCCGAGCC**AATTGTTATCCGCTCACAATTGAATCTAAGTATC | pDF-lac (Guererro et al, 2012) |
| eYFP | **TCTGGTGGGTCTCTGTCC**ATGGTGAGCAAGGGCGAGGAG  | **CGATAGGTCTCCCGAGCC**TTACTTGTACAGCTCGTCCATGCCG  | vector provided by Dr. P. Armshaw, UL, Limerick, Ireland |
| SpecR | **TCTGGTGGGTCTCTGTCC**ATTCTCACCAATAAAAAACGCCCG  | **CGATAGGTCTCCCGAGCC**ATGACATGTTTTTTTGGGGTACAGTCTATG | pDF-lac (Guerrero et al., 2012)  |
| Z. mobilis  pyruvate decarboxylase (pdc) | **TCTGGTGGGTCTCTGTCC**ATGAGTTATACTGTCGGTACCTATTTAGCGG  | **CGATAGGTCTCCCGAGCC**CTAGAGGAGCTTGTTAACAGGCTTACGG  | pPSBA2EtoHKan provided by Dr. P. Armshaw, UL, Limerick, Ireland |
| alcohol dehydrogenase -Synechocystis sp. PCC 6803 slr1192 (adh) | **TCTGGTGGGTCTCTGTCC**ATGATTAAAGCCTACGCTGCCCTG  | **CGATAGGTCTCCCGAGCC**CTAATTTTTACTATGGCTGAGCACTACCCG  | pPSBA2EtoHKan provided by Dr. P. Armshaw, UL, Limerick, Ireland |
| pdc mutagenesis primers | GCGGCTTGTCCAGATTGGCCTCAAGCATCACTTCGCAG | CTGCGAAGTGATGCTTGAGGCCAATCTGGACAAGCCGC | IDT |
| *E. coli* pyruvate kinase (pykF) | **TCTGGTGGGTCTCTGTCC**ATGAAAAAGACCAAAATTGTTTGCACCATC | **CGATAGGTCTCCCGAGCC**TTACAGGACGTGAACAGATGCGG | *E. coli* genomic DNA |
| bifunctional fructose-1,6-bisphosphatase/sedoheptulose-1,7-bisphosphatase, *slr2094 (bibp)* | **TCTGGTGGGTCTCTGTCC**GTGGACAGCACCCTCGGTTTAG | **CGATAGGTCTCCCGAGCC**TTAATGCAGTTGGATTACTTTGGGGc | *Synechocystis* 6803 genomic DNA |

**Supplementary Table 2**. Ribosome binding site sequences used in this study. The capitalized nucleotides refer to the spacer sequence and are not part of the RBS.

|  |  |  |  |
| --- | --- | --- | --- |
| **RBS** | **RBS sequence** | **BioBrick™ part number** | **Additional information** |
| **RBS A** | AtcacacaggacTA | BBa\_B0033 |  |
| **RBS B** | AaagaggggaaaTA | BBa\_B0064 |  |
| **RBS C** | AaagaggagaaaTA  | BBa\_B0034 |  |
| **RBS D** | AtcacaaggaggTA | - | *E. coli* consensus Shine-Dalgarno sequence |
| **RBS E** | AttagtggaggtTA  | - | RBS\* in Heidorn et al., 2011 |

**Supplementary Table 3. Plasmid DNA constructed in this study.**

|  |  |  |
| --- | --- | --- |
| **Strain** | **Plasmid(s)** | **Genotype** |
|  | CB1 | pMB1, AmpR, PA1lacO1 promoter, GFP placeholder, slr0168 integration site, SpecR |
|  | NB2 | pMB1, AmpR, Ptrc promoter, GFP placeholder, slr1395 integration site, ChlR |
| RBSA\_eYFP | CB1\_RBSA\_eYFP | CB1, RBS A, eYFP |
| RBSB\_eYFP | CB1\_RBSB\_eYFP | CB1, RBS B, eYFP |
| RBSC\_eYFP | CB1\_RBSC\_eYFP | CB1, RBS C, eYFP |
| RBSD\_eYFP | CB1\_RBSD\_eYFP | CB1, RBS D, eYFP |
| RBSE\_eYFP | CB1\_RBSE\_eYFP | CB1, RBS E, eYFP |
| AA | CB1\_RBSA\_pdc\_RBSA\_adh | CB1, RBS A, pdc, RBS A, adh |
| AB | CB1\_RBSA\_pdc\_RBSB\_adh | CB1, RBS A, pdc, RBS B, adh |
| AC | CB1\_RBSA\_pdc\_RBSC\_adh | CB1, RBS A, pdc, RBS C, adh |
| AD | CB1\_RBSA\_pdc\_RBSD\_adh | CB1, RBS A, pdc, RBS D, adh |
| AE | CB1\_RBSA\_pdc\_RBSE\_adh | CB1, RBS A, pdc, RBS E, adh |
| BA | CB1\_RBSB\_pdc\_RBSA\_adh | CB1, RBS B, pdc, RBS A, adh |
| BB | CB1\_RBSB\_pdc\_RBSB\_adh | CB1, RBS B, pdc, RBS B, adh |
| BC | CB1\_RBSB\_pdc\_RBSC\_adh | CB1, RBS B, pdc, RBS C, adh |
| BD | CB1\_RBSB\_pdc\_RBSD\_adh | CB1, RBS B, pdc, RBS D, adh |
| BE | CB1\_RBSB\_pdc\_RBSE\_adh | CB1, RBS B, pdc, RBS E, adh |
| CA | CB1\_RBSC\_pdc\_RBSA\_adh | CB1, RBS C, pdc, RBS A, adh |
| CB | CB1\_RBSC\_pdc\_RBSB\_adh | CB1, RBS C, pdc, RBS B, adh |
| CC | CB1\_RBSC\_pdc\_RBSC\_adh | CB1, RBS C, pdc, RBS C, adh |
| CD | CB1\_RBSC\_pdc\_RBSD\_adh | CB1, RBS C, pdc, RBS D, adh |
| CE | CB1\_RBSC\_pdc\_RBSE\_adh | CB1, RBS C, pdc, RBS E, adh |
| DB | CB1\_RBSD\_pdc\_RBSB\_adh | CB1, RBS D, pdc, RBS B, adh |
| DC | CB1\_RBSD\_pdc\_RBSC\_adh | CB1, RBS D, pdc, RBS C, adh |
| DD | CB1\_RBSD\_pdc\_RBSD\_adh | CB1, RBS D, pdc, RBS D, adh |
| DE | CB1\_RBSD\_pdc\_RBSE\_adh | CB1, RBS D, pdc, RBS E, adh |
| EA | CB1\_RBSE\_pdc\_RBSA\_adh | CB1, RBS E, pdc, RBS A, adh |
| EB | CB1\_RBSE\_pdc\_RBSB\_adh | CB1, RBS E, pdc, RBS B, adh |
| EC | CB1\_RBSE\_pdc\_RBSC\_adh | CB1, RBS E, pdc, RBS C, adh |
| ED | CB1\_RBSE\_pdc\_RBSD\_adh | CB1, RBS E, pdc, RBS D, adh |
| EE | CB1\_RBSE\_pdc\_RBSE\_adh | CB1, RBS E, pdc, RBS E, adh |
| A  | CB1\_RBSA\_pdc | CB1, RBS A, pdc |
| B  | CB1\_RBSB\_pdc | CB1, RBS B, pdc |
| C  | CB1\_RBSC\_pdc | CB1, RBS C, pdc |
| D  | CB1\_RBSD\_pdc | CB1, RBS D, pdc |
| E  | CB1\_RBSE\_pdc | CB1, RBS E, pdc |
| EE\_RBS A PYK | CB1\_RBSE\_pdc\_RBSE\_adh\_RBSA\_pyk | CB1, RBS E, pdc, RBS E, adh, RBSA, pyk |
| EE\_RBS D PYK | CB1\_RBSE\_pdc\_RBSE\_adh \_RBSD\_pyk | CB1, RBS E, pdc, RBS E, adh, RBSD, pyk |
| EE\_RBS E PYK | CB1\_RBSE\_pdc\_RBSE\_adh \_RBSE\_pyk | CB1, RBS E, pdc, RBS E, adh, RBSE, pyk |
| EE\_RBS A BiBP | CB1\_RBSE\_pdc\_RBSE\_adh\_RBSA\_bibp | CB1, RBS E, pdc, RBS E, adh, RBSA, bibp |
| EE\_RBS B BiBP | CB1\_RBSE\_pdc\_RBSE\_adh\_RBSB\_bibp | CB1, RBS E, pdc, RBS E, adh, RBSB, bibp |
| EE\_RBS C BiBP | CB1\_RBSE\_pdc\_RBSE\_adh\_RBSC\_bibp | CB1, RBS E, pdc, RBS E, adh, RBSC, bibp |
| EE\_RBS D BiBP | CB1\_RBSE\_pdc\_RBSE\_adh\_RBSD\_bibp | CB1, RBS E, pdc, RBS E, adh, RBSD, bibp |
| EE\_AA | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSA\_pyk\_RBSA\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS A, pyk, RBS A, bibp |
| EE\_AB | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSA\_pyk\_RBSB\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS A, pyk, RBS B, bibp |
| EE\_AD | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSA\_pyk\_RBSD\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS A, pyk, RBS D, bibp |
| EE\_BB | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSB\_pyk\_RBSB\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS B, pyk, RBS B, bibp |
| EE\_DA | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSD\_pyk\_RBSA\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS D, pyk, RBS A, bibp |
| EE\_DD | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSD\_pyk\_RBSD\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS D, pyk, RBS D, bibp |
| EE\_DE | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSD\_pyk\_RBSE\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS D, pyk, RBS E, bibp |
| EE\_EA | CB1\_RBSE\_pdc\_RBSE\_adhNB2\_RBSE\_pyk\_RBSA\_bibp | CB1, RBS E, pdc, RBS E, adh, NB2, RBS E, pyk, RBS A, bibp |

**Supplementary Table 4. Signature peptides and transtions for SRM Mass spectroscopy.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein** | **Peptide** | **Mass info** | **Retention time** |
| pdc | LVQIGLK.+2y6.light | 385.8 / 657.4 | 12,2 |
| pdc | LVQIGLK.+2y5.light | 385.8 / 558.4 | 12,2 |
| pdc | LVQIGLK.+2y4.light | 385.8 / 430.3 | 12,2 |
| pdc | VAVLVGSK.+2y6.light | 386.8 / 602.4 | 8,8  |
| pdc | VAVLVGSK.+2y5.light | 386.8 / 503.3 | 8,8 |
| pdc | VAVLVGSK.+2y4.light | 386.8 / 390.2 | 8,8 |
| pdc | TGALDFFK.+2y6.light | 449.7 / 740.4 | 17 |
| pdc | TGALDFFK.+2y5.light | 449.7 / 669.4 | 17 |
| pdc | TGALDFFK.+2y4.light | 449.7 / 556.3 | 17 |
| pdc | TGGELAEAIK.+2y8.light | 494.8 / 830.5 | 9,7 |
| pdc | TGGELAEAIK.+2y6.light | 494.8 / 644.4 | 9,7 |
| pdc | TGGELAEAIK.+2y5.light | 494.8 / 531.3 | 9,7 |
| adh | AWGC[CAM]EVTAFTSSAR.+2y10.light | 771.9 / 1068.5 | 15,1 |
| adh | AWGC[CAM]EVTAFTSSAR.+2y9.light | 771.9 / 939.5 | 15,1 |
| adh | AWGC[CAM]EVTAFTSSAR.+2y8.light | 771.9 / 840.4 | 15,1 |
| adh | FDYIISTVNLK.+2y6.light | 656.9 / 661.4 | 18,5 |
| adh | FDYIISTVNLK.+2y10.light | 656.9 / 1165.6 | 18,5 |
| adh | FDYIISTVNLK.+2y9.light | 656.9 / 1050.6 | 18,5 |
| adh | FDYIISTVNLK.+2y8.light | 656.9 / 887.6 | 18,5 |
| adh | FDYIISTVNLK.+2y7.light | 656.9 / 774.5 | 18,5 |
| atpB | VIDLLTPYR.+2y7.light | 545.3 / 877.5 | 17,7 |
| atpB | VIDLLTPYR.+2y6.light | 545.3 / 762.5 | 17,7 |
| atpB | VIDLLTPYR.+2y5.light | 545.3 / 649.4 | 17,7 |
| atpB | YVSLADTIK.+2y8.light | 505.3 / 846.5 | 13,3 |
| atpB | YVSLADTIK.+2y7.light | 505.3 / 747.4 | 13,3 |
| atpB | YVSLADTIK.+2y6.light | 505.3 / 660.4 | 13,3 |
| atpB | YVSLADTIK.+2y5.light | 505.3 / 547.3 | 13,3 |
| **collision** |  |  |  |