## Supplementary Table 8 - Plasmids

**Table 8A gRNA plasmids**

|  |  |  |  |
| --- | --- | --- | --- |
| Plasmid | Relevant characteristics | Primer(s) used for gRNA | Source |
| pMEL10 | 2μm ampR *KlURA3* gRNA-*CAN1*.Y | N.A. | Mans*, et al.* 9 |
| pROS10 | 2μm ampR *URA3* gRNA*-CAN1.Y* gRNA*-ADE2.Y* | N.A. | Mans*, et al.* 9 |
| pROS11 | 2μm ampR *amdSYM* gRNA*-CAN1*.Y gRNA*-ADE2*.Y | N.A. | Mans*, et al.* 9 |
| pROS12 | 2μm ampR *hphNT1* gRNA*-CAN1.Y* gRNA*-ADE2.Y* | N.A. | Mans*, et al.* 9 |
| pROS13 | 2μm ampR *kanMX* gRNA-*CAN1*.Y gRNA*-ADE2*.Y | N.A. | Mans*, et al.* 9 |
| pUDR286 | 2µm ampR *URA3* gRNA*-TKL2* gRNA*-SOL4* | 9508 & 9503 | Ref CCM BioRXiv |
| pUDR400 | 2µm ampR *hphNT1* gRNA*-mTurquoise* | 12911 & 12912 | This study |
| pUDR406 | 2µm ampR *URA3* gRNA*-ARO10* gRNA*-PDC5/6* | 13614 & 7246 | This study |
| pUDR413 | 2µm ampR *kanMX* gRNA*- RECYCLE SinLoG* | N.A. | Boonekamp*, et al.* 1 |
| pUDR426 | 2µm ampR *KanMX* gRNA*-spHIS5 (2x)* | 10641 | This study |
| pUDR546 | 2µm ampR *hphNT1* gRNA*-URA3* gRNA*-HIS3* | 14756 & 8314 | This study |
| pUDR590 | 2µm ampR *amdS* gRNA*-NQM1* gRNA*-GND2* | 12569 &7231 | This study |
| pUDR700 | 2µm ampR *KanMX* gRNA*-GND1* gRNA*-RKI1* | 16895 & 16897 | This study |
| pUDR701 | 2µm ampR *KanMX* gRNA*-TAL1 (2x)* | 16909 | This study |
| pUDR702 | 2µm ampR *hphNT1* gRNA*-RPE1* gRNA*-TKL1* | 16903 & 16905 | This study |
| pUDR703 | 2µm ampR *hphNT1* gRNA*-ZWF1* gRNA*-SOL3* | 8564 & 16889 | This study |
| pUDR756 | 2µm ampR *hphNT1* gRNA-*RKI1\_WM (2x)* | 17613 | This study |
| pUDR765 | 2µm ampR *hphNT1* gRNA*-Chunk16AB (2x)* | 17868 | This study |
| pUDR771 | 2µm ampR *hphNT1* gRNA*-CAN1* gRNA-*X2* | 6008 & 10866 | This study |
| pUDR772 | 2µm ampR *kanMX* gRNA*-YPRCtau3* gRNA-*SPR3* | 12985 & 12034 | This study |
| pUDR780 | 2µm ampR *hphNT1* gRNA*-Chunk7BC* gRNA-*Chunk15CD)* | 18226 & 18277 | This study |
| pUDR781 | 2µm ampR *kanMX* gRNA*-shrN* gRNA-*Chunk9CD)* | 18228 & 18299 | This study |

**Table 8B in-house golden gate part plasmids**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant characteristics | Source |
| pUD565 | camR *GFP* entry vector | Boonekamp*, et al.* 1 |
| pYTK012 | camR *pHHF2* | Lee*, et al.* 2 |
| pYTK013 | camR *pTEF1* | Lee*, et al.* 2 |
| pYTK015 | camR *pHHF1* | Lee*, et al.* 2 |
| pYTK016 | camR *pHTB2* | Lee*, et al.* 2 |
| pYTK017 | camR *pRPL18B* | Lee*, et al.* 2 |
| pGGKp038 | camR *tTEF2* | Hassing*, et al.* 10 |
| pGGKp039 | camR *tTEF1* | Hassing*, et al.* 10 |
| pGGKp062 | kanR *pSkADH1* | Hassing*, et al.* 10 |
| pGGKp063 | kanR *pSkTDH3* | Hassing*, et al.* 10 |
| pGGkp074 | camR *pSePDC1* | Hassing*, et al.* 10 |
| pGGKp075 | camR *pSeFBA1* | Hassing*, et al.* 10 |
| pGGkp095 | camR *pSeGPM1* | Hassing*, et al.* 10 |
| pGGKp113 | camR *tADH3* | Hassing*, et al.* 10 |
| pGGKp119 | camR *coEcaroG*(p150L) | Hassing*, et al.* 10 |
| pGGKp120 | camR *coEcaroB* | Hassing*, et al.* 10 |
| pGGKp121 | camR *coEcaroD* | Hassing*, et al.* 10 |
| pGGKp122 | camR *coEcaroE* | Hassing*, et al.* 10 |
| pGGKp123 | camR *coEcaroL* | Hassing*, et al.* 10 |
| pGGKp124 | camR *coEcaroA* | Hassing*, et al.* 10 |
| pGGKp125 | camR *coEcaroC* | Hassing*, et al.* 10 |
| pGGKp126 | camR *coEcpheAT326P* | Hassing*, et al.* 10 |
| pGGKp182 | camR *tCYC1* | This study |
| pGGKp327 | camR *coRcTAL1* | This study |

**Table 8C Part plasmids subcloned by GeneArt in entry vector pUD565**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant characteristics | Source |
| pGGKp131 | camR *AtPAL1* | GeneArt |
| pGGKp135 | camR *coEctyrAM53I A354V* | GeneArt |  |  |
| pGGKp245 | ampR *coEctyrB* | GeneArt |
| pUD262 | camR *pCWP2* | GeneArt |
| pUD819 | camR *tACO1* | GeneArt |
| pUD826 | camR *tCIT1* | GeneArt |
| pUD829 | camR *tDIC1* | GeneArt |
| pUD832 | camR *tIDH2* | GeneArt |
| pUD836 | camR *tFUM1* | GeneArt |
| pUD841 | camR *tGDB1* | GeneArt |
| pUD842 | camR *tGLC3* | GeneArt |
| pUD844 | camR *tTAL1* | GeneArt |
| pUD845 | camR *tGPD2* | GeneArt |
| pUD846 | camR *tGPH1* | GeneArt |
| pUD848 | camR *tGSY2* | GeneArt |
| pUD850 | camR *tSDH2* | GeneArt |
| pUD851 | camR *tSDH3* | GeneArt |
| pUD852 | camR *tSDH4* | GeneArt |
| pUD858 | camR *tLAT1* | GeneArt |
| pUD862 | camR *tMDH1* | GeneArt |
| pUD873 | camR *tPGM2* | GeneArt |
| pUD875 | camR *tSOL3* | GeneArt |
| pUD876 | camR *tRKI1* | GeneArt |
| pUD879 | camR *tGND1* | GeneArt |
| pUD880 | camR *tSOL4* | GeneArt |
| pUD881 | camR *tRPE1* | GeneArt |
| pUD882 | camR *tTKL1* | GeneArt |
| pUD884 | camR *tZWF1* | GeneArt |
| pUD911 | camR *pTAL1* | GeneArt |
| pUD933 | camR *pSOL3* | GeneArt |
| pUD934 | camR *pRKI1* | GeneArt |
| pUD944 | camR *pGND1* | GeneArt |
| pUD946 | camR *pRPE1* | GeneArt |
| pUD947 | camR *pTKL1* | GeneArt |
| pUD949 | camR *pZWF1* | GeneArt |

**Table 8D Part plasmids ordered from GeneArt and subcloned in house in entry vector pUD565**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant characteristics | Source |
| pUD1038 | ampR *ZWF1 \** | GeneArt |
| pUD1039 | ampR *TKL1 \** | GeneArt |
| pUD1040 | kanR *TAL1 \** | GeneArt |
| pUD1041 | ampR *SOL3 \** | GeneArt |
| pUD1042 | ampR *RPE1 \** | GeneArt |
| pUD1043 | ampR *RKI1 \** | GeneArt |
| pUD1044 | kanR *GND1 \** | GeneArt |
| pGGKp245 | Amp *coEctyrB* | GeneArt |
| pGGKp247 | camR *ZWF1 \** | This study |
| pGGKp248 | camR *TKL1 \** | This study |
| pGGKp249 | camR *TAL1 \** | This study |
| pGGKp250 | camR *SOL3 \** | This study |
| pGGKp251 | camR *RPE1 \** | This study |
| pGGKp252 | camR *RKI1 \** | This study |
| pGGKp253 | camR *GND1 \** | This study |
| pGGKp293 | camR *coEctyrB* | This study |

**Table 8E Part plasmids made in house by PCR**

**\***For *pSEPYK1* and initially the *coAtANS* no correct *E.coli* part plasmid transformant was found and expression cassettes were thus assembled with PCR fragments with yeast toolkit flanks.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Plasmid | Relevant characteristics | Template | Primers | Source |
| pUD257 | camR *pRPP0* | CEN.PK113-7D genomic DNA | 16294 & 16295 | This study |
| pUD258 | camR *pRPL3* | CEN.PK113-7D genomic DNA | 16300 & 16301 | This study |
| pUD259 | camR *pRPL8A* | CEN.PK113-7D genomic DNA | 16298 & 16299 | This study |
| pUD260 | camR *pRPL10* | CEN.PK113-7D genomic DNA | 16296 & 16297 | This study |
| pUD261 | camR *pRPL25* | CEN.PK113-7D genomic DNA | 16292 & 16293 | This study |
| pGGKp324 | camR *AtCHI1* | pUDI065 | 17829 & 17830 | This study |
| pGGKp325 | camR *coAtCHS3* | pUDE185 | 17827 & 17828 & 17834 & 17835 | This study |
| pGGKp326 | camR *coAtC4H* | pUDE172 | 17823 & 17824 | This study |
| pGGKp328 | camR *coGhDFR* | Genomic DNA PATW076 11 | 17872 & 17873 | This study |
| pGGKp329 | camR *coAt3GT* | Genomic DNA PATW076 11 | 17876 & 17877 | This study |
| pGGKp330 | camR *pSePGK1* | pUDI102 | 9413 & 9414 | This study |
| pGGKp331 | camR *pSeENO2* | *Saccharomyces eubayanus* genomic DNA | 9743 & 9744 | This study |
| pGGKp332 | CamR *coAtF3H* | Genomic DNA PATW076 | 17870 & 17871 | This study |
| pGGKp340 | camR *coAtANS* | Genomic DNA PATW076 | 17874 & 17875 | This study |
| PCR fragment\* | *coAtANS* | Genomic DNA PATW076 | 17874 & 17875 | This study |
| PCR fragment\* | *pSePYK1* | pUDI129 | 10610 & 10611 | This study |

**Table 8F in house expression plasmids described in other studies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Plasmid | | Relevant characteristics | | Source |
| pGGKd005 | | CEN6/ARS4 ampR *hphNT1 GFP* | | Hassing*, et al.* 10 |
| pGGKd012 | CEN6/ARS4 *cloNATR GFP* | | Boonekamp*, et al.* 1 | |
| pUDC212 | | CEN6/ARS4 *ampR natNT2 pFBA1-FBA1\*-tFBA1* | | Boonekamp*, et al.* 1 |
| pUDC213 | | CEN6/ARS4 *ampR natNT2 pPGM1-PGM1\*-tPGM1* | | Boonekamp*, et al.* 1 |
| pUDC214 | | CEN6/ARS4 *ampR natNT2 pHXK2-HXK2\*-tHXK2* | | Boonekamp*, et al.* 1 |
| pUDC215 | | CEN6/ARS4 *ampR natNT2 pPDC1-PDC1\*-tPDC1* | | Boonekamp*, et al.* 1 |
| pUDC216 | | CEN6/ARS4 *ampR natNT2 pPFK1-PFK1\*-tPFK1* | | Boonekamp*, et al.* 1 |
| pUDC217 | | CEN6/ARS4 *ampR natNT2 pPFK2-PFK2\*-tPFK2* | | Boonekamp*, et al.* 1 |
| pUDC219 | | CEN6/ARS4 *ampR natNT2 pPGK1-PGK1\*-tPGK1* | | Boonekamp*, et al.* 1 |
| pUDC220 | | CEN6/ARS4 *ampR natNT2 pPYK1-PYK1\*-tPYK1* | | Boonekamp*, et al.* 1 |
| pUDC222 | | CEN6/ARS4 *ampR natNT2 pTPI1-TPI1\*-tTPI1* | | Boonekamp*, et al.* 1 |
| pUDC229 | | CEN6/ARS4 *ampR natNT2 pADH1-ADH1\*-tADH1* | | Boonekamp*, et al.* 1 |
| pUDC230 | | CEN6/ARS4 *ampR natNT2 pTDH3-TDH3\*-tTDH3* | | Boonekamp*, et al.* 1 |
| pUDC231 | | CEN6/ARS4 *ampR natNT2 pENO2-ENO2\*-tENO2* | | Boonekamp*, et al.* 1 |
| pUDC232 | | CEN6/ARS4 *ampR natNT2 pPGI1-PGI1\*-tPGI1* | | Boonekamp*, et al.* 1 |

**Table 8G Expression plasmids constructed in this study**

**\***For *pSEPYK1* and initially *coAtANS* no correct *E.coli* part plasmid transformant was found and expression cassettes were thus assembled with PCR fragments with yeast toolkit flanks  
\*\* the ORF of pUDC357 turned out to be mutated after sanger sequencing.

|  |  |  |  |
| --- | --- | --- | --- |
| Plasmid | Relevant characteristics | Parts used | Source |
| pUDC275 | CEN6/ARS4 ampR *natNT2 pZWF1-ZWF1\*-tZWF1* | pGGKd012, pUD949, pGGKp247, pUD884 | This study |
| pUDC276 | CEN6/ARS4 ampR *natNT2 pTKL1-TKL1\*-tTKL1* | pGGKd012, pUD947, pGGKp248, pUD882 | This study |
| pUDC277 | CEN6/ARS4 ampR *natNT2 pGND1-GND1\*-tGND1* | pGGKd012, pUD911, pGGKp253, pUD844 | This study |
| pUDC278 | CEN6/ARS4 ampR *natNT2 pRKI1-RKI1\*-tRKI1* | pGGKd012, pUD933, pGGKp252, pUD875 | This study |
| pUDC279 | CEN6/ARS4 ampR *natNT2 pTAL1-TAL1\*-tTAL1* | pGGKd012, pUD946, pGGKp249, PUD881 | This study |
| pUDC280 | CEN6/ARS4 ampR *natNT2 pRPE1-RPE1\*-tRPE1* | pGGKd012, pUD934, pGGKp251, pUD876 | This study |
| pUDC281 | CEN6/ARS4 ampR *natNT2 pSOL3-SOL3\*-tSOL3* | pGGKd012, pUD944, pGGKp250, pUD879 | This study |
| pUDC293 | CEN6/ARS4 ampR *natNT2 pRPL3-coEcaroA-tSOL4* | pGGKd012, pUD258, pGGKp124, pUD880 | This study |
| pUDC296 | CEN6/ARS4 ampR *natNT2 pRPL25-coEcaroD*-*tGPH1* | pGGKd012, pUD261, pGGKp121, pUD846 | This study |
| pUDC297 | CEN6/ARS4 ampR *natNT2 pRPP0-coEcaroE*-*tCYC1* | pGGKd012, pUD257, pGGKp122, pGGKp182 | This study |
| pUDC298 | CEN6/ARS4 ampR *natNT2 pHHF1-coEcaroG*(p150L)-*tTEF1* | pGGKd012, pYTK015, pGGKp119, pGGKp039 | This study |
| pUDC299 | CEN6/ARS4 ampR *natNT2 pHTB2-coEcaroL*-*tPGM2* | pGGKd012, pYTK016, pGGKp123, pUD873 | This study |
| pUDC301 | CEN6/ARS4 ampR *natNT2 pRPL10-coEctyrAM53I A354V*-*tGDB1* | pGGKd012, pUD260, pGGKp135, pUD841 | This study |
| pUDC302 | CEN6/ARS4 ampR *natNT2 pCWP2-coEctyrB*-*tGLC3* | pGGKd012, pUD262, pGGKp293, pUD842 | This study |
| pUDC294 | CEN6/ARS4 ampR *natNT2 pHHF2-coEcaroB*-*tTEF2* | pGGKd012, pYTK012, pGGKp120, pGGKp038 | This study |
| pUDC295 | CEN6/ARS4 ampR *natNT2 pRPL8A-coEcaroC*-*tGPD2* | pGGKd012, pUD259, pGGKp125, pUD845 | This study |
| pUDC300 | CEN6/ARS4 ampR *natNT2 pRPL18B-coEcpheAT326P*-*tGSY2* | pGGKd012, pYTK017, pGGKp126, pUD848 | This study |
| pUDC349 | CEN6/ARS4 ampR *natNT2 pSePDC1-AtPAL1-tLAT1* | pGGKd012, pGGkp074, pGGKp131, pUD858 | This study |
| pUDC350 | CEN6/ARS4 ampR *natNT2 pSeGPM1-coRcTAL1-tCIT1* | pGGKd012, pGGkp095, pGGKp327, pUD826 | This study |
| pUDC352 | CEN6/ARS4 ampR *natNT2 pTEF1-coAtCHS3-tMDH1* | pGGKd012, pYTK013, pGGKp325, pUD862 | This study |
| pUDC353 | CEN6/ARS4 ampR *natNT2 pSkADH1-AtCHI-tSDH4* | pGGKd012, pGGKp062, pGGKp324, pUD852 | This study |
| pUDC354 | CEN6/ARS4 ampR *natNT2 pSeFBA1-coAtC4H-tADH3* | pGGKd012, pGGKp075, pGGKp326, pGGKp113 | This study |
| pUDC355 | CEN6/ARS4 ampR *natNT2 pSkTDH3-coAtF3H-tSDH3* | pGGKd012, pGGKp063, pGGKp332, pUD851 | This study |
| pUDC356 | CEN6/ARS4 ampR *natNT2 pSePGK1-coGhDFR-tACO1* | pGGKd012, PGGKp330, pGGKp328, pUD819 | This study |
| pUDC357\*\* | CEN6/ARS4 ampR *natNT2 pSeENO2-coAtANS-tFUM1* | pGGKd012, pGGKp331, *coAtANS* PCR fragment\*, pUD836 | This study |
| pUDC358 | CEN6/ARS4 ampR *natNT2 pSePYK1-coAt3GT-tDIC* | pGGKd012, *coAt3GT* PCR fragment \*, pGGKp329, pUD829 | This study |
| pUDC398 | CEN6/ARS4 ampR *natNT2 pSeENO2-coAtANS-tFUM1* | pGGKd012, pGGKp331, pGGKp340, pUD836 | This study |

**Table 8H Expression plasmids made by Gibson assembly in this study**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant characteristics | Source |
| pUDC348 | CEN6/ARS4 ampR *natNT2 pRPS3-coAtCPR1-tIDH2* | This study |
| pUDC351 | CEN6/ARS4 ampR *natNT2 pSeTPI1-At4CL3-tSDH2* | This study |

|  |  |  |
| --- | --- | --- |
| Part | Source | Primers |
| pUDC348 | | |
| *pRPS3* | CEN.PK113-7D genomic DNA | 17811 &17812 |
| *coAtCPR1* | pUDE172 | 17813 & 17814 |
| *tIDH2* | pUD832 | 17815 & 17816 |
| Backbone | pGGKd012 | 12377 & 12378 |
| pUDC351 | | |
| *pSeTPI1* | pUDI116 | 17817, 17818 |
| *At4CL3* | pUDI065 | 17819, 17820 |
| *tSDH2* | pUD850 | 17821, 17822 |
| Backbone | pGGKd012 | 12377, 12378 |

**Table 8I Other plasmids**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant characteristics | Source |
| pUDI065 | Integration plasmid *LEU2 pTDH3-AtCHI1-tCYC1 pTPI-AtCHS3-tADH pTEF-At4CL3-tTEF PYK2*(1-710) | Koopman*, et al.* 12 |
| pUDI102 | *pSePGK1-mRuby2-tENO2* | Boonekamp*, et al.* 5 |
| pUDI116 | *pSeTPI1-mRuby2-tENO2* | Boonekamp*, et al.* 5 |
| pUDI129 | *pSePYK1-mRuby2-tENO2* | Boonekamp*, et al.* 5 |
| pUDE172 | *CEN6/ARS4, URA3, pTDH3-AtPAL1-tCYC1, pTPI-coC4H-tADH, pPGI-coCPR1-tPGI* | Koopman*, et al.* 12 |
| pUDE185 | 2 μm *HIS3 pTDH3-coCHS3-tCYC1* | Koopman*, et al.* 12 |
| pLM092 | CEN6/ARS4, ampR, *HIS3*, 5’*URA3*-*ACT1*intron[Tess-I-SceI-Tess]-3’*URA3* | Mitchell and Boeke 13 |
| pUDC191 | CEN6/ARS4, ampR, *URA3, pCCW12-mRuby2-tENO1* | Postma*, et al.* 8 |
| pUDC192 | CEN6/ARS4, ampR, *URA3, pTEF2-mTurquoise2-tSSA1* | Postma*, et al.* 8 |