**Supplementary File 1**

**1.1 Table of corresponding primers used.**

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| --- | --- |
| **Primer**  | **Sequence (5’→ 3’)**  |
| lctBCD\_pET21a\_for  | tttaagaaggagatatacatATGAAAATACTGGTATGTATCAAAC  |
| lctBCD\_pET21a\_rev  | acggagctcgaattcggatcCTACATCTGACAGACTTTTTTC  |
| pet21a\_for  | GATCCGAATTCGAGCTCC |
| pet21a\_rev  | ATGTATATCTCCTTCTTAAAGTTAAAC |
| lctBCD\_pMTL84211\_fwd  | ACCGCTATTGATGTTGCTCCGACGGAAGCCATTCTTCAGGAAGG  |
| lctBCD\_pMTL84211\_rev  | TCCAGCGTTTGTAGCTGCGTCACCAATAACCGCTATTTTATTTTCAATCGTGATG  |
| pMTL84211\_fwd  | AGTTAGGGAATGTTACTTTGTAGTG  |
| pMTL84211\_rev  | GGATCCGTCCTCCCTTTAAATTTAAC  |
| ∆Fe/S\_for  | gccaaaatggccaaaatggccCTTAAAAAAGGACCTGAAGGGG  |
| ∆Fe/S\_rev  | AGCTGCCGTGACTTCCAG  |
| ∆Fe/S-arm\_for  | GACAAAAGCTTATATCGG |
| ∆Fe/S-arm\_rev  | CATGATTTTTTCCTTTCTAGCC |
| ΔR205\_for  | AGTTCAGATTgcaCCAGCTTTTG  |
| ΔR205\_rev  | AAATCGGTGTTTTCTTTCATTTC |
| ΔD189\_for  | ACTTACCGCTggaTGTACCATTTTGGAAATG  |
| ΔD189\_rev  | CCGGTTCGATAACGAGCC |
| Δβ-FAD\_for  | aggagcaggagcaGAAATGGCTGAATTTTTGGG  |
| Δβ-FAD\_rev  | gctcctgctcctgcGGTGGTTTGTTTACCGCA  |
| ΔNAD\_for  | aggcgcaGCCGATGTGGTAGCGACC  |
| ΔNAD\_rev  | gcttttgcATCCGATAATAAACAACCCTCATCAG  |
| ΔSPT\_for  | gcaggagcaCAGGTGGAACGAATTTTCC  |
| ΔSPT\_rev  | TCCGCTCAGTCCATATTTTTTTTC  |
| Strep-Tag\_for  | ccacagttcgagaagTAAGATGAATTATAAAAAAGTGGAAGC  |
| Strep-Tag\_rev  | gtgggaccatgctgaAACGACCATCCTTTCCGG  |

Plasmids generated with exchanges in EtfA are pET21a\_lctBCD[∆Fe/S](C41A, C44A, C47A), pET21a\_lctBCD[∆Fe/S-arm](∆2A-65I), pMTL84211\_lctBCD[∆Fe/S](C41A, C44A, C47A), pMTL84211\_lctBCD[∆Fe/S-arm](∆2A-65I), pET21a\_lctBCD[ΔR205](R205A) and pET21a\_lctBCD[ΔD189](D189A). Plasmids generated with exchanges in EtfB are pET21a\_lctBCD[Δb-FAD](D122A, D124A, T125G, Q127G, V128A, P130A), pET21a\_lctBCD[ΔNAD](R87A, F89A, G91A) and pET21a\_lctBCD[ΔSPT](S223A, P224G, T225A).

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**1.2 Cloning of *pET21a\_lctBC-StrepD*.** For the production of Ldh-EtfAB-Strep in *E. coli* the construct *pET21a\_lctBC-StrepD* was cloned (**A**) Therefore, *pET21a* backbone, including a T7-promotor, was amplified using corresponding primers pET21a\_for (1) and pET21a\_rev (2) via PCR (size: 5406 bp) (**B**) *LctBCD* was amplified from genomic DNA of *A. woodii* via PCR, using lctBCD\_pET21a\_for (3) and lctBCD\_pET21a\_rev (4) primers (size: 3537 bp) (**C**) Amplified *lctBCD* and *pET21a* backbone were fused via Gibson Assembly and transformed in *E. coli* HB101. Afterwards, plasmids where isolated and a sequence encoding for a Strep-tag was introduced at the 3’-end of the gene *lctC* by using corresponding primers Strep-Tag\_for (5) and Strep-Tag\_rev (6) (size: 8903 bp) (**D**) The resulting *pET21a\_lctBC-StrepD* was digested with *Hind*III (E). The resulting sizes were 6393 bp and 2510 bp. M, Gene Ruler 1 kb DNA ladder.

**Source data 1.** Source data for Supplementary File 1-II