**Supplemental file 1**

Natural Transformation, Protein Expression, and Cryoconservation of the Filamentous Cyanobacterium *Phormidium lacuna*

Cloning of vectors for transformation of *P. lacuna*

For pAK1, pAK2 and pAK3, the inserts of pBK47, pBK54, and pBK55 were amplified by PCR, respectively, and the PCR products were cloned into pFN1. Plasmids and primers are given in the tables below. Cloning and PCR were performed according to standard procedures1. The sequence of pMH1 was generated by DNA synthesis and cloned into pUC19.

**List of transformation vectors**

|  |  |  |  |
| --- | --- | --- | --- |
| **Vector** | **Purpose** | **Size [bp]** | **References** |
| pFN1 | *P. lacuna* knock out of *chwA*, KanR resistance | 6,400 | 2 |
| pBK47 | *sfGFP* expression in *Synechococcus* sp. PCC 7002, *cpc560* promoter | 6,301 | 3 |
| pBK54 | *sfGFP* expression in *Synechococcus* sp. PCC 7002, A2813 promoter | 6,244 | 3 |
| pBK55 | *sfGFP* expression in *Synechococcus* sp. PCC 7002, psbA2s promoter | 5,949 | 3 |
| pAK1 | *sfGFP* expression in *P. lacuna*, *cpc560* promoter | 7,697 | this work |
| pAK2 | *sfGFP* expression in *P. lacuna,* A2813 promoter | 7,640 | this work |
| pAK3 | *sfGFP* expression in *P. lacuna* , *psbA2s* promoter | 7,345 | this work |
| PMH1 | *sfGFP* expression in *P. lacuna* | 6,078 | this work |

**List of primers used for cloning of pAK1, pAK2, and pAK3** and for detection of integrated DNA in *P. lacuna*: Primer names contain “fwd” forward primers and “rev” for reverse primers. Restriction sites are indicated by the name of the restriction enzyme, followed by “site”. The last column shows the vectors for which the primers were designed. Vector that are used as template are given in parentheses.

|  |  |  |  |
| --- | --- | --- | --- |
| **primer** | **purpose** | **sequence** | **(template) and target** |
| AK1fwd | cloning, amplification of *psbA2s* / *sfGFP*, NheI site | ATGCGTGCTAGCCAGGTAAAC | (pBK55), pAK3 |
| AK21fwd | cloninng, amplification of A2813 / *sfGFP*, NheI site | ATGCGTGCTAGCCCGATTTAAG | (pBK54), pAK2 |
| AK5fwd | cloning, amplification of *cpc560* / *sfGFP* , NheI site | ATGCGTGCTAGCACCTGTAGA | (pBK47), pAK1 |
| AK6rev | cloning, amplification of *psbA2s*, *A2813*, *CPC560* and*sfGFP,* PacI site | GTGGTGTTAATTAAGTATGCTCTTCTGCTCCTGCAG | (pBK47, pBK54, pBK55) pAK1  pAK2  pAK3 |
| AK3fwd | cloning, amplification of pFN1 (without KanR) for integration DNA into chwA homologous sites, PacI site | GTGGTGTTAATTAAGGCTGACATAGAGTTTGCCTCG | (pFN1)  pAK1  pAK2  pAK3 |
| AK4rev | cloning, amplification of pFN1 (without KanR) for integration DNA into chwA homologous sites, NheI site | GTGGTGGCTAGCGCCTCGCAAACTTTGCTTTGC | pAK1  pAK2  pAK3 |
| AK9fwd | detection, outer PCR *chwA* | ATCGACATCCCAATCCTCTGC | pAK1  pAK2  pAK3 |
| AK10rev | detection, outer PCR *chwA* | CCTCGCACTCGTTTGGC | pAK1  pAK2  pAK3 |
| F25fwd | detection, inner PCR *chwA* | GGTCTAGGTGAGGCAATCC | pAK1  pAK2  pAK3 |
| F28rev | detection, inner PCR *chwA* | ACCTGATTTGTTTATATCTGACGC | pAK1  pAK2  pAK3 |
| MH1fwd | detection,, outer PCR *cpcB-cpcA* | GTTCGTCCATCATGGCTCAG | pMH1 |
| MH2rev | detection, outer PCR *cpcB-cpcA* | CGACGGAATCGACATAGGAGTC | pMH1 |
| MH3fwd | detection, inner PCR *cpcB-cpcA* | CGAGAATCACTCAGGTGAAGAG | pMH1 |
| MH4rev | detection, inner PCR *cpcB-cpcA* | CGTAGTCGAGGTAGGAGTTGG | pMH1 |
| F29 fwd | detection, KanR | CTATGACCATGATTACGAATTC CC | any |
| F30 rev | detection, KanR | AAGCCGTTTCTGTAATGAAGG | any |
| cpc560\_pBK47\_fwd (A23) | detection *cpc560* (and sfGFP) | GCTGTGGTTCCCTAGGC | any |
| GFP\_pBK47\_rev (A24) | detection (*cpc560*) and  GFP | CAAGAAGGACCATGTGGTC | any |

1 Sambrook, J., Russell, D. W. *Molecular Cloning. A Laboratory Manual. 3rd edition.*, Cold Spring Harbor Laboratory Press (2001).

2 Nies, F., Mielke, M., Pochert, J., Lamparter, T. Natural transformation of the filamentous cyanobacterium *Phormidium lacuna*. *PLoS One.* **15** (6), e0234440 (2020).

3 Kachel, B., Mack, M. Engineering of *Synechococcus* sp. strain PCC 7002 for the photoautotrophic production of light-sensitive riboflavin (vitamin B2). *Metabolic Engineering.* **62**, 275–286 (2020).

**Sequence of pMH1 in gb format**

LOCUS Exported 6078 bp DNA circular SYN 14-DEC-2021

DEFINITION Standard E. coli vector with a multiple cloning site (MCS) for DNA

cloning. The MCS is reversed in pUC18.

ACCESSION .

VERSION .

KEYWORDS puc19\_cpcB\_sfGFP

SOURCE synthetic DNA construct

ORGANISM synthetic DNA construct

REFERENCE 1 (bases 1 to 6078)

AUTHORS .

TITLE Direct Submission

JOURNAL Exported Tuesday, Dec 14, 2021 from SnapGene Viewer 5.3.0

https://www.snapgene.com

COMMENT See also GenBank accession L09137.

FEATURES Location/Qualifiers

source 1..6078

/organism="synthetic DNA construct"

/mol\_type="other DNA"

promoter 96..200

/gene="bla"

/label=AmpR promoter

CDS 201..1061

/codon\_start=1

/gene="bla"

/product="beta-lactamase"

/label=AmpR

/note="confers resistance to ampicillin, carbenicillin, and

related antibiotics"

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYI

ELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYS

PVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRW

EPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSA

LPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGAS

LIKHW"

rep\_origin 1232..1820

/direction=RIGHT

/label=ori

/note="high-copy-number ColE1/pMB1/pBR322/pUC origin of

replication"

promoter 2144..2174

/label=lac promoter

/note="promoter for the E. coli lac operon"

protein\_bind 2182..2198

/label=lac operator

/bound\_moiety="lac repressor encoded by lacI"

/note="The lac repressor binds to the lac operator

toinhibit transcription in E. coli. This inhibition can be

relieved by adding lactose or

isopropyl-beta-D-thiogalactopyranoside (IPTG)."

primer\_bind 2206..2222

/label=M13 rev

/note="common sequencing primer, one of multiple similar

variants"

misc\_feature 2265..2598

/label=cpcB-Promoter

misc\_feature 2599..3117

/label=cpcB

misc\_feature 3118..3130

/label=Shine-Dalgarno-Sequenz

misc\_feature 3131..3847

/label=sfGFP

primer\_bind 3154..3173

/label=GFP\_Primer

misc\_feature 3848..5063

/label=KanR

primer\_bind complement(4818..4838)

/label=F30

3'UTR 5064..5165

/label=Terminator

misc\_feature 5166..5654

/label=cpcA

primer\_bind complement(5684..5700)

/label=M13 fwd

/note="common sequencing primer, one of multiple similar

variants"

ORIGIN

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121 tctaaataca ttcaaatatg tatccgctca tgagacaata accctgataa atgcttcaat

181 aatattgaaa aaggaagagt atgagtattc aacatttccg tgtcgccctt attccctttt

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