**High-Efficiency expression and secretion of human FGF21 in *Bacillus subtilis* by intercalation of a mini-cistron cassette and combinatorial optimization of cell regulatory components**

Dandan Li1,2,#, Gang Fu2,3,#, Ran Tu2, Zhaoxia Jin1\* and Dawei Zhang2,3\*

1School of Biological Engineering, Dalian Polytechnic University, Dalian 116034, People’s Republic of China.

2Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, People’s Republic of China.

3Key Laboratory of Systems Microbial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, People’s Republic of China.

# DL and GF are equally contributed to this work.

\* Corresponding author: Zhaoxia Jin, E-mail address: [jinzx2018@163.com](mailto:jinzx2018@163.com);

Dawei Zhang, E-mail address: zhang\_dw@tib.cas.cn.

**Additional file 6: Table S2.** All primers used in this study.

|  |  |
| --- | --- |
| Primers | Sequence (5’-3’) |
| rhFGF21.F | catccgattccggatagcag |
| rhFGF21.R | ttaatgatgatgatgatgatggctcg |
| rhFGF21.F2 | taaTAAGGAGGatttacatatgaaatacctattgcctacggcagc |
| rhFGF21.R2 | gctatgcgagccatcatcatcatcatcattaa |
| pMATE-vector.F | agaagtctcgttccgacagttgg |
| pMATE-vector.R | aattattccccctagctaattttcgtTTAATTATAAATTAAG |
| pMA5-vector.F | catatgagttatgcagtttgtagaatgc |
| pMA5-vector.R | gtgtgctctgcgaggctg |
| pMATEF-vector.F | catccgattccggatagcagcc |
| pMATEF-vector.R | TTTAACTTAATTTATAATTAAacgaaaattagctagggggaataatt |
| p*malA*.F | cgcGGATCCCTTTTGTCCCCTGCCTTTTCT |
| p*malA*.R | cggGGTACCATGACGACCTCCTTGATAAatTTTACAATTCCATTTA |
| CIS1.F | TAAacgatgaaatacctattgcctacggcagc |
| CIS1.R | ttcttctcctttacTcATaattattccccctagctaattttcgtTT |  |  |
| CIS2.F | cttgtatcatgaaatacctattgcctacggcagc |  |  |
| CIS2.R | GTTTTTGGCGTCTTCCATaattattccccctagctaattttcgtTT |  |  |
| CIS3.F | atctgttcaatgaaatacctattgcctacggcagc |  |  |
| CIS3.R | GAATGATTTTTTCTTCATaattattccccctagctaattttcgtTT |  |  |
| CIS4.F | aggttttatatgaaatacctattgcctacggcagc |  |  |
| CIS4.R | ataaataccatatttcataattattccccctagctaattttcgtTT |  |  |
| CIS5.F | aTAAGGAGGatttacatatgaaatacctattgcctacggcagc |  |  |
| CIS5.R | TTTGTTATTGTCTGCCATaattattccccctagctaattttcgtTT |  |  |
| CIS6.F | ataaggaggaataaatgaaatacctattgcctacggcagc |  |  |
| CIS6.R | ttaatcgatacataattattccccctagctaattttcgtTT |  |  |
| CIS7.F | ggatgattgaatgaaatacctattgcctacggcagc |  |  |
| CIS7.R | tccttgaacataattattccccctagctaattttcgtTT |  |  |
| SP*pelB*.F | atgaaatacctattgcctacggcagc |  |  |
| SP*pelB*.R | gctcaacccgcaatggcc |  |  |
| SP*phoD*.F | atggcatacgacagtcgttttgatgaa |  |  |
| SP*phoD*.R | ggttggggcctttgaagtaaatgct |  |  |
| SP*pel*.F | ATGAAAAAAGTGATGTTAGCTACGGCTTT |  |  |
| SP*pel*.R | GCTGGCGCGAACGCA |  |  |
| SP*ywbN*.F | atgagcgatgaacagaaaaagccag |  |  |
| SP*ywbN*.R | gggcagccgttgcg |  |  |
| SP*lipA*.F | ATGAAATTTGTAAAAAGAAGGATCATTGCACTTGT |  |  |
| SP*lipA*.R | CGTCAGCAAAAGCCGCT |  |  |
| SP*protA*.F | ATGAAAAAGAAAAACATTTATTCAATTCGTAAACTAGGTGT |  |  |
| SP*protA*.R | CGTAACACCTGCTGCAAATGCT |  |  |  |
| SP*ywmC*.F | atgaagaaaagattttcactgatcatgatgacagg |  |  |  |
| SP*ywmC*.R | ctttttggattaacttcacctgcttttgca |  |  |  |
| SP*dacB*.F | ATGCGCATTTTCAAAAAAGCAGTATTCG |  |  |  |
| SP*dacB*.R | ACCGTAAATGTGAATACAGCACATGCT |  |  |  |
| SP*nprE*.F | ATGGGTTTAGGTAAGAAATTGTCTGTTGCT |  |  |  |
| SP*nprE*.R | GCCTGCCAGGTGTTCAGGCT |  |  |  |
| SP*yddT*.F | ATGAGAAAGAAAAGAGTTATTACTTGTGTTATGGCT |  |  |  |
| SP*yddT*.R | TTTACCTGCAGGTTACGCTTCTGCA |  |  |  |
| SP*yoqm*.F | ATGAAATTAAGAAAAGTATTGACTGGTTCTGTT |  |  |  |
| SP *yoqm*.R | TTCTGCTTCTCCTGCATTCGCT |  |  |  |
| SP*yvce*.F | ATGAGAAAGAGTTTAATTACACTTGGTTTGGC |  |  |  |
| SP*yvce*.R | CATTTACAAGTAAAACTGCATCGGCG |  |  |  |
| BlsecA.F | atgcttggaattttaaataaagtgtttgat |  |  |  |
| BlsecA.R | ttattctgttcttccgcagcagtttttata |  |  |  |
| CsaA.F | TTATCCGATTTTTGTGCCGTTTGGG |  |  |  |
| CsaA.R | ATGGCAGTTATTGATGACTTTGAGAA |  |  |  |
| DnaK.F | TTATTTTTTGTTTTGGTCGTCGTTTACTTCTT |  |  |  |
| DnaK.R | GTGAGTAAAGTTATCGGAATCGACTTAGGAAC |  |  |  |
| Ffh.F | ATGGCATTTGAAGGATTAGCCGAC |  |  |  |
| Ffh.R | TTACATAAAAGGTAGCTTAAACCCTTTTTTCTT |  |  |  |
| Ftsy.F | ATGAGCTTTTTTAAAAAATTAAAAGAGAAAATCACAAAAC |  |  |  |
| Ftsy.R | TTAATCGTCGGCTTTTTCCACTAAATCTGAAA |  |  |  |
| GroESL.F | TTGTTAAAGCCATTAGGTGATCGCG |  |  |  |
| GroESL.R | TTACATCATTCCACCCATACCGCCC |  |  |  |
| PrsA.F | TTATTTAGAATTGCTTGAAGATGAAGAAGTGCTG |  |  |  |
| PrsA.R | ATGAAGAAAATCGCAATAGCAGC |  |  |  |
| QssecA.F | TCAAACATTTTATTTAAAATTCCAAGCAT |  |  |  |
| QssceA.R | CCGCTTCGTGAGTATCAAATGGAATAG |  |  |  |
| Scr.F | CGTTTGGGTCCTGCGCAAT |  |  |  |
| Scr.R | AGGGTTGCCTGGGCCGAGC |  |  |  |
| YrdF.F | CTACTCTATTATTATTTTAAATTCGTCCTTTAGTTCTTCT |  |  |  |
| YrdF.R | ATGAGAAAAATAATAATAGATGGAAGAGACTTTGAAAAT |  |  |  |
| Hbs.f | TTATTTTCCGGCAACTGCGTCTT |  |  |  |
| Hbs.r | ATGAACAAAACAGAACTTATCAATGCGGTT |  |  |  |
| Pgrac.F | AAAGGAGGTAAGGATCACTAGAAAATTTTTTAAA |  |  |  |
| Pgrac.F | TTCCTCCTTTaattggtgttggttgttgt |  |  |  |
| PDF.F | TAATAACCGGGCAGGCCATGTCT |  |  |  |
| PDF.R | CCAGTGCAGGAGCTCTTAGCATATTATG |  |  |  |
| UP.F | AATCTCGGCAATGAAAGAAGCGG |  |  |  |
| UP.R | TTTCATCCCCCTTTTTCAACATGCT |  |  |  |
| DN.F | GTGGAAAAAAAGCTGCCGTCATT |  |  |  |
| DN.R | CCGACAGCTTTGTTGTGCTGATA |  |  |  |
| Cm.F | TCTTCAACTAAAGCACCCATTAGTTCAACAAA |  |  |  |
| Cm.R | TTATTCATTCAGTTTTCGTGCGGACTGG |  |  |  |
| araR.F | ATGTTTTCTTACAAAGAACGCTGTGATAT |  |  |  |
| araR.R | TTATTCATTCAGTTTTCGTGCGGACT |  |  |  |
| G1.F | ATGAGGAAAAAAACGAAAAACAGACTCATCA |  |  |  |
| G1.R | TTAATTTTCTGTGTTCATATTAAGTTTTCCATTCGC |  |  |  |
| G2.F | ATGAAAAACATGTCTTGCAAACTTGT |  |  |  |
| G2.R | TTACTTTTTCGGTTTGACGGCGTTCAG |  |  |  |
| G3.F | ATGAAACGCAGAAAATTCAGCTCGG |  |  |  |
| G3.R | TTACTTTTCAACAACAACTTTTGCTTCCT |  |  |  |
| G4.F | ATGAAATTAGTTCCAAGATTCAGAAAACAATGGT |  |  |  |
| G4.R | TTAGACGGAGTCTTTTTTGCTTTTGCC |  |  |  |
| G5.F | TTGCGCAACTTGACCAAGAC |  |  |  |
| G5.R | TCATAGAATGCCGACAGCCTCATACG |  |  |  |
| G6.F | TTGAAAAAGGGGATCATTCGCTTTCTG |  |  |  |
| G6.R | CTTATTCAACAGTGAAAGGTTCTTCGGTCAAA |  |  |  |
| UP2.F | CTTGGAAGGCGAGACGGTTGG |  |  |  |
| UP2.R | GATTCATCTCCTTTTTCTATGATGTTTGATATATATATCG |  |  |  |
| DN2.F | CCAAAAACCTTTAAGATTTGCATTCCA |  |  |  |
| DN2.R | TAGCCCATCATTGGGATATGAAGCCC |  |  |  |
| UP3.F | AAAAAGGCATAAGCAGTTTTTTATCGG |  |  |  |
| UP3.R | GTTATCCCTCCTGCAAAATAATGAATCT |  |  |  |
| DN3.F  DN3.R | CCAAAAAGCGGTGCTCGATGC  TATAAAGCTCGCCATTGTAAATAATGGTGTACGTAT |  |  |  |
| UP4.F | TTGCGACGGATGAATTGCTGAAGCT |  |  |  |
| UP4.R | TTTGTCATCTCCCTCCTTTAGTGTCA |  |  |  |
| DN4.F | TAAGACGGAGTCTTTTTTTATTTCGTTTTTAAGAA |  |  |  |
| DN4.R | GAGGAAAAAGATGAAAAAGCATATCAGCATGC |  |  |  |
| UP5.F | TCATTGACCGATACATGGAGGAACTAAATGCA |  |  |  |
| UP5.R | AACACCACATCCTTCCTATTTTGGAAT |  |  |  |
| DN5.F | GCAAACAAAAACAGTCAGGACACAGAG |  |  |  |
| DN5.R | ATTTTGGCAAATTAATATGATATAGTTGGCTTAACT |  |  |  |
| UP6.F | TCCACTTTATTCATAGGGATGAGATCATTTTTCTT |  |  |  |
| UP6.R | TGTGTTTCCCCCTTTGTATTTAGAAAAAATGTGA |  |  |  |
| DN6.F | AAAAAGCCCTGCCGATTCGG |  |  |  |
| DN6.R | CAGAAACGGCCTTACAGCAAACC |  |  |  |