Supplementary Material (ESI) for Chemical Communications

A short-chain dehydrogenase/reductase from *Vibrio vulnificus* with both blue fluorescence and oxidoreductase activity

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Supplementary Information.

1. The codon optimized BFP gene sequence was produced from DNAworks [1] using the amino acid sequence from NCBI (accession number: AAG41118). The program designed the 34 oligonucleotides to be used in gene synthesis. The DNAworks output is given below:

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PROTEIN SEQUENCE, WITH 239 RESIDUES
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   1 MKKLVVITGASSGIGEAIARRFSEEGHPLLLLARRVERLEALNLPNTLCAQVDVTDKNTF
  61 DAAITRAEKIYGPADVLVNNAGVMLLGQIDTQEANEWQRMFDVNVLGLLNGMQAVLAPMK
 121 ARNSGTIINISSIAGKKTFPDHAAYCGTKFAVHAISENVREEVAASNVRVTTIAPGAVET
 181 ELLSHTTSQQIKDGYDAWKVDMGGVLAADDVARAVLFAYQQPQNVCIREIALAPTKQQP
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 The DNA sequence #   1 is:
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   1 ATGAAGAAACTGGTTGTCATCACCGGCGCAAGCTCTGGCATTGGCGAAGCCATCGCGCGT
  61 CGCTTTAGCGAGGAAGGCCATCCATTACTGCTGCTGGCACGCCGTGTTGAGCGCTTAGAA
 121 GCCCTGAATCTGCCAAACACCCTGTGCGCCCAAGTCGACGTCACCGACAAGAACACGTTC
 181 GACGCAGCCATCACCCGCGCAGAGAAGATTTACGGCCCGGCAGACGTGCTGGTGAATAAC
 241 GCGGGCGTCATGCTGTTAGGCCAAATCGACACCCAGGAAGCGAACGAGTGGCAGCGCATG
 301 TTTGACGTGAACGTGCTGGGTCTGTTAAACGGTATGCAGGCGGTCTTAGCACCGATGAAG
 361 GCGCGCAACAGCGGCACCATCATTAACATCAGCAGCATCGCAGGCAAGAAGACGTTCCCG
 421 GATCACGCGGCGTACTGTGGCACCAAATTCGCGGTGCACGCGATTTCTGAAAATGTCCGC
 481 GAAGAGGTCGCGGCCTCTAATGTTCGCGTGACGACGATTGCACCAGGCGCAGTCGAAACC
 541 GAGCTGCTGAGCCACACGACCAGCCAACAAATCAAGGACGGCTACGACGCATGGAAGGTC
 601 GACATGGGTGGTGTGCTGGCCGCAGACGATGTCGCCCGTGCCGTTCTGTTTGCGTACCAA
 661 CAGCCGCAAAACGTGTGCATCCGTGAGATCGCGCTGGCGCCGACCAAGCAGCAACCATGA
 721
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        34 oligonucleotides need to be synthesized
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  1 ATGAAGAAACTGGTTGTCATCACCGGCGCAAG  32
  2 ATGGCTTCGCCAATGCCAGAGCTTGCGCCGGTGATGACAA  40
  3 TGGCATTGGCGAAGCCATCGCGCGTCGCTTTAGCGAGGAA  40
  4 CAGCAGCAGTAATGGATGGCCTTCCTCGCTAAAGCGACGC  40
  5 GGCCATCCATTACTGCTGCTGGCACGCCGTGTTGAGCGCT  40
  6 TGTTTGGCAGATTCAGGGCTTCTAAGCGCTCAACACGGCG  40
  7 TAGAAGCCCTGAATCTGCCAAACACCCTGTGCGCCCAAGT  40
  8 AACGTGTTCTTGTCGGTGACGTCGACTTGGGCGCACAGGG  40
  9 CGTCACCGACAAGAACACGTTCGACGCAGCCATCACCCGC  40
 10 TGCCGGGCCGTAAATCTTCTCTGCGCGGGTGATGGCTGCG  40
 11 GAAGATTTACGGCCCGGCAGACGTGCTGGTGAATAACGCG  40
 12 GGCCTAACAGCATGACGCCCGCGTTATTCACCAGCACGTC  40
 13 GGCGTCATGCTGTTAGGCCAAATCGACACCCAGGAAGCGA  40
 14 AACATGCGCTGCCACTCGTTCGCTTCCTGGGTGTCGATTT  40
 15 CGAGTGGCAGCGCATGTTTGACGTGAACGTGCTGGGTCTG  40
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16 GACCGCCTGCATACCGTTTAACAGACCCAGCACGTTCACG  40
17 TTAAACGGTATGCAGGCGGTCTTAGCACCGATGAAGGCGC  40
18 GTTAATGATGGTGCCGCTGTTGCGCGCCTTCATCGGTGCT  40
19 CAACAGCGGCACCATCATTAACATCAGCAGCATCGCAGGC  40
20 GCGTGATCCGGGAACGTCTTCTTGCCTGCGATGCTGCTGA  40
21 GACGTTCCCGGATCACGCGGCGTACTGTGGCACCAAATTC  40
22 AGAAATCGCGTGCACCGCGAATTTGGTGCCACAGTACGCC  40
23 GCGGTGCACGCGATTTCTGAAAATGTCCGCGAAGAGGTCG  40
24 ACGCGAACATTAGAGGCCGCGACCTCTTCGCGGACATTTT  40
25 CGGCCTCTAATGTTCGCGTGACGACGATTGCACCAGGCGC  40
26 GGCTCAGCAGCTCGGTTTCGACTGCGCCTGGTGCAATCGT  40
27 GAAACCGAGCTGCTGAGCCACACGACCAGCCAACAAATCA  40
28 CATGCGTCGTAGCCGTCCTTGATTTGTTGGCTGGTCGTGT  40
29 GGACGGCTACGACGCATGGAAGGTCGACATGGGTGGTGTG  40
30 GGCGACATCGTCTGCGGCCAGCACACCACCCATGTCGACC  40
31 CCGCAGACGATGTCGCCCGTGCCGTTCTGTTTGCGTACCA  40
32 TGCACACGTTTTGCGGCTGTTGGTACGCAAACAGAACGGC  40
33 AGCCGCAAAACGTGTGCATCCGTGAGATCGCGCTGGCGCC  40
34 TCATGGTTGCTGCTTGGTCGGCGCCAGCGCGA  32
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2. The gene was synthesized according to the procedure in Stemmer [2]. Briefly, the oligonucleotides mixed in equal molar ratio and diluted to 250 nm in a 50 ul PCR reaction. A hot start program with preincubation of all components except for the Pfu polymerase was employed with an annealing temperature of 55°C and an extension time of 30 seconds. Ten microliters of this PCR product was used in a second PCR reaction with the terminal primers to amplify the full length product.

3. The point mutations were incorporated via overlap extension PCR using the following oligonucleotides (5' to 3'):

bfpe40ks cgccgtgttgagcgcttaaaagccctgaatctgccaaacacc
bfpe40kas ggtgtttggcagattcagggcttttaagcgctcaacacggcg

bfp7677s cgcagagaagatttacggcccggcagacgcgatcgtgaataacgcgggcg
bfp7677as cgcccgcgttattcacgatcgcgtctgccgggccgtaaatcttctctgcg

bfpv83ms gtgaataacgcgggcatgatgctgttaggccaaatcg
bfpv83mas cgatttggcctaacagcatcatgcccgcgttattcac

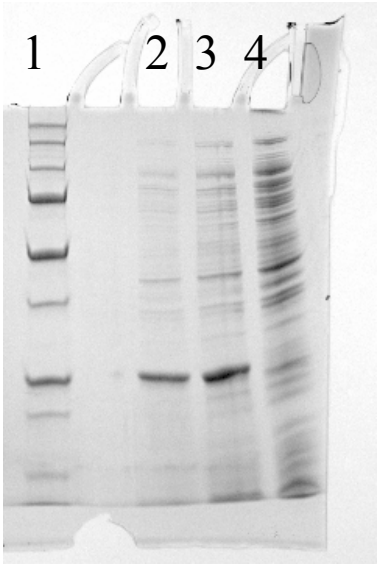
bfps124cs cgatgaaggcgcgcaactgcggcaccatcattaacatcagc
bfps124cas gctgatgttaatgatggtgccgcagttgcgcgccttcatcg

bfp176179s cgcgtgacgacgattgcaccaagcgcagtcaaaaccgagctgctgagccacacg
bfp176179as cgtgtggctcagcagctcggttttgactgcgcttggtgcaatcgtcgtcacgcg

BFP-7 was synthesized from 6 fragments in one step and BFP-2 from 2 fragments in one step using the cyling program outlined above.

4. The BFP variants were overexpressed in *E. coli* BL21 cells using 0.1 mM IPTG at room temperature for 4 hours. The cells were harvested by centrifugation, lysed by sonication, centrifuged again to remove cell debris and the BFP protein was purified by immobilized metal affinity chromatography on Ni-NTA resin (Qiagen, Valencia, CA). The purity of the proteins was assessed by SDS-PAGE using a 12% tris-glycine gel.

SDS-PAGE gel (12%) of cell lysate of BFP wild-type, double and heptuple mutant



1. Ladder
2. 15µL of wild-type
3. 15µL of double mutant (BFP-2)
4. 15µL of 7-mutant (BFP-7)

All proteins diluted to about 1000 µg/mL

References

1. D. Hoover and J. Lubkowski, *Nucleic Acids Res*, 2002, **30**, e43.
2. W. Stemmer, A. Crameri, K. Ha, T. Brennan and H. Heyneker, *Gene*, 1995, **164**, 49-53.