**2B chimera PcrA**

**Plasmid**

pET22B\_PcrA\_2Bchimera

**Protein**

2B chimera PcrA: *B. stearothermophilus* sequence with *S. aureus* 2B domain

**Depositor**

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**Protein sequence**

MNFLSEQLLA HLNKEQQEAV RTTEGPLLIM AGAGSGKTRV LTHRIAYLMA EKHVAPWNIL

AITFTNKAAR EMRERVQSLL GGAAEDVWIS TFHSMCVRIL RRDIDRIGIN RNFSILDPTD

QLSVMKTILK EKNIDPKKFE PRTILGTISA AKNELLPPEQ FAKRASTYYE KVVSDVYQEY

QQRLLRNHSL DFDDLIMTTI QLFDRVPDVL HYYQYKFQYI HIDEYQDTNR AQYTLVKKLA

ERFQNICAVG DADQSIYRWR GADIQNILSF ERDYPNAKVI LLEQNYRSTK RILQAANEVI

EHNVNRKPKR LWTENPEGKP ILYYEAMNEA DEAQFVAGRI REAVERGERR YRDFAVLYRT

NAQSRVMEEM LLKANIPYQI VGGQKFYDRK EIKDLLSYLR IIANSNDDIS LQRIINVPKR

GVGPSSVEKV QNYALQNNIS MFDALGEADF IGLSKKVTQE CLNFYELIQS LIKEQEFLEI

HEIVDEVLQK SGYREMLERE NTLESRSRLE NIDEFMSVPK DYEENTPLEE QSLINFLTDL

SLVADIDEAN GTEQAAEGDA VMLMTLHAAK GLEFPVVFLI GMEEGIFPHN RSLEDDDEME

EERRLAYVGI TRAEEELVLT SAQMRTLFGN IQMNPPSRFL NEIPAHLLET ASRRQAGASR

PAVSRPQASG AVGSWKVGDR ANHRKWGIGT VVSVRGGGDD QELDIAFPSP IGIKRLLAKF

 APIEKV

**Gene sequence**

GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAACTTTCTGAGCGAACAGCTGCTGGCACATCTGAATAAAGAACAGCAAGAAGCAGTTCGTACCACCGAAGGTCCGCTGCTGATTATGGCAGGCGCAGGTAGCGGTAAAACCCGTGTTCTGACCCATCGTATTGCATATCTGATGGCAGAAAAACATGTTGCACCGTGGAATATTCTGGCAATTACCTTTACCAATAAAGCCGCACGTGAAATGCGTGAACGTGTTCAGAGCCTGCTGGGTGGTGCAGCAGAAGATGTTTGGATTAGCACCTTTCATAGCATGTGTGTTCGTATTCTGCGTCGTGATATTGATCGCATTGGTATTAATCGCAACTTTAGCATTCTGGACCCGACCGATCAGCTGAGCGTTATGAAAACCATTCTGAAAGAAAAAAACATCGATCCGAAAAAATTCGAGCCTCGTACCATTCTGGGCACCATTAGCGCAGCAAAAAATGAACTGCTGCCTCCGGAACAGTTTGCAAAACGTGCAAGCACCTATTATGAAAAAGTTGTGTCCGATGTGTACCAAGAATATCAGCAACGTCTGCTGCGTAATCATAGCCTGGATTTTGATGATCTGATTATGACCACCATCCAGCTGTTTGATCGTGTTCCGGATGTTCTGCATTATTATCAGTACAAATTCCAGTACATCCACATCGATGAATATCAGGATACCAATCGTGCACAGTATACCCTGGTTAAAAAACTGGCAGAACGCTTTCAGAACATTTGTGCAGTTGGTGATGCAGATCAGAGCATTTATCGTTGGCGTGGTGCAGATATTCAAAACATTCTGAGCTTTGAACGCGATTATCCGAATGCCAAAGTTATTCTGCTGGAACAGAATTATCGTAGCACCAAACGCATTCTGCAGGCAGCAAATGAAGTGATTGAACATAATGTGAACCGTAAACCGAAACGTCTGTGGACCGAAAATCCGGAAGGTAAACCGATTCTGTATTATGAAGCAATGAACGAAGCAGATGAAGCACAGTTTGTTGCAGGTCGTATTCGTGAAGCAGTTGAACGTGGTGAACGTCGTTATCGTGATTTTGCAGTTCTGTATCGTACCAATGCACAGAGCCGTGTTATGGAAGAAATGCTGCTGAAAGCAAATATCCCGTATCAGATTGTTGGTGGCCAGAAATTCTATGATCGCAAAGAAATTAAAGATCTGCTGAGCTATCTGCGCATTATTGCCAATAGCAATGATGATATTAGCCTGCAGCGTATTATCAATGTTCCGAAACGTGGTGTTGGTCCGAGCAGCGTTGAAAAAGTGCAGAATTATGCCCTGCAGAACAACATCAGCATGTTTGATGCACTGGGTGAAGCAGATTTCATTGGTCTGAGCAAAAAAGTTACCCAAGAATGCCTGAACTTCTATGAACTGATTCAGTCCCTGATCAAAGAACAAGAATTCCTGGAAATCCATGAGATCGTTGATGAAGTGCTGCAGAAAAGCGGTTATCGCGAAATGCTGGAACGTGAAAATACCCTGGAAAGCCGTAGCCGTCTGGAAAATATTGATGAATTTATGAGCGTGCCGAAAGATTATGAAGAAAATACACCGCTGGAAGAACAGAGCCTGATCAATTTTCTGACCGATCTGAGCCTGGTTGCAGATATCGATGAAGCAAATGGCACCGAACAGGCAGCCGAAGGTGATGCCGTTATGCTGATGACCCTGCATGCCGCAAAAGGTCTGGAATTTCCGGTTGTGTTTCTGATTGGCATGGAAGAAGGTATTTTTCCGCATAATCGTTCCCTGGAAGATGATGATGAAATGGAAGAGGAACGTCGTCTGGCCTATGTTGGTATTACCCGTGCAGAAGAAGAACTGGTTCTGACCAGCGCACAGATGCGTACCCTGTTTGGTAACATTCAGATGAATCCGCCTAGCCGTTTTCTGAATGAAATTCCTGCACATCTGCTGGAAACCGCAAGCCGTCGTCAGGCTGGTGCCAGCCGTCCGGCAGTTAGTCGTCCGCAGGCAAGCGGTGCCGTTGGTAGCTGGAAAGTGGGTGATCGTGCAAATCATCGTAAATGGGGTATTGGCACCGTTGTTAGCGTTCGTGGTGGCGGTGATGATCAAGAACTGGATATTGCATTTCCGAGCCCGATTGGCATTAAACGCCTGCTGGCAAAATTTGCACCGATCGAAAAAGTGTAAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCAC

**Gene source**

*B. stearothermophilus* , with the amino acids in positions between 381 and 547( 2B domain) substituted with *S. aureus* 2B domain amino acids.

Synthesised by GeneArt

**Plasmid**

pET22B

**Insert position:** PcrA 199-2384 insertion using Nde and BamH1

**Plasmid sequence**

 **Pet22b vector sequence**

**T7 primer sequence (MWG)** TAA TAC GAC TCA CTA TAG GG

**Reverse compliment T7** CCC TAT AGT GAG TCG TAT TA

**T7 ter sequence** CTA GTT ATT GCT CAG CGG T

 1 atccggatat agttcctcct ttcagcaaaa aacccctcaa gacccgttta gaggccccaa

 61 ggggttatg**c tagttattgc** **tcagcggt**gg cagcagccaa ctcagcttcc tttcgggctt

 121 tgttagcagc cggatctcag tggtggtggt ggtggtgctc gagtgcggcc gcaagcttgt

 181 cgacggagct cgaattcgga tccgaattaa ttccgatatc catggccatc gccggctggg

 241 cagcgaggag cagcagacca gcagcagcgg tcggcagcag gtatttcata tgtatatctc

 301 cttcttaaag ttaaacaaaa ttatttctag aggggaattg ttatccgctc acaattc**ccc**

 361 **tatagtgagt cgtatta**att tcgcgggatc gagatctcga tcctctacgc cggacgcatc

 421 gtggccggca tcaccggcgc cacaggtgcg gttgctggcg cctatatcgc cgacatcacc

 481 gatggggaag atcgggctcg ccacttcggg ctcatgagcg cttgtttcgg cgtgggtatg

 541 gtggcaggcc ccgtggccgg gggactgttg ggcgccatct ccttgcatgc accattcctt

 601 gcggcggcgg tgctcaacgg cctcaaccta ctactgggct gcttcctaat gcaggagtcg

 661 cataagggag agcgtcgaga tcccggacac catcgaatgg cgcaaaacct ttcgcggtat

 721 ggcatgatag cgcccggaag agagtcaatt cagggtggtg aatgtgaaac cagtaacgtt

 781 atacgatgtc gcagagtatg ccggtgtctc ttatcagacc gtttcccgcg tggtgaacca

 841 ggccagccac gtttctgcga aaacgcggga aaaagtggaa gcggcgatgg cggagctgaa

 901 ttacattccc aaccgcgtgg cacaacaact ggcgggcaaa cagtcgttgc tgattggcgt

 961 tgccacctcc agtctggccc tgcacgcgcc gtcgcaaatt gtcgcggcga ttaaatctcg

 1021 cgccgatcaa ctgggtgcca gcgtggtggt gtcgatggta gaacgaagcg gcgtcgaagc

 1081 ctgtaaagcg gcggtgcaca atcttctcgc gcaacgcgtc agtgggctga tcattaacta

 1141 tccgctggat gaccaggatg ccattgctgt ggaagctgcc tgcactaatg ttccggcgtt

 1201 atttcttgat gtctctgacc agacacccat caacagtatt attttctccc atgaagacgg

 1261 tacgcgactg ggcgtggagc atctggtcgc attgggtcac cagcaaatcg cgctgttagc

 1321 gggcccatta agttctgtct cggcgcgtct gcgtctggct ggctggcata aatatctcac

 1381 tcgcaatcaa attcagccga tagcggaacg ggaaggcgac tggagtgcca tgtccggttt

 1441 tcaacaaacc atgcaaatgc tgaatgaggg catcgttccc actgcgatgc tggttgccaa

 1501 cgatcagatg gcgctgggcg caatgcgcgc cattaccgag tccgggctgc gcgttggtgc

 1561 ggatatctcg gtagtgggat acgacgatac cgaagacagc tcatgttata tcccgccgtt

 1621 aaccaccatc aaacaggatt ttcgcctgct ggggcaaacc agcgtggacc gcttgctgca

 1681 actctctcag ggccaggcgg tgaagggcaa tcagctgttg cccgtctcac tggtgaaaag

 1741 aaaaaccacc ctggcgccca atacgcaaac cgcctctccc cgcgcgttgg ccgattcatt

 1801 aatgcagctg gcacgacagg tttcccgact ggaaagcggg cagtgagcgc aacgcaatta

 1861 atgtaagtta gctcactcat taggcaccgg gatctcgacc gatgcccttg agagccttca

 1921 acccagtcag ctccttccgg tgggcgcggg gcatgactat cgtcgccgca cttatgactg

 1981 tcttctttat catgcaactc gtaggacagg tgccggcagc gctctgggtc attttcggcg

 2041 aggaccgctt tcgctggagc gcgacgatga tcggcctgtc gcttgcggta ttcggaatct

 2101 tgcacgccct cgctcaagcc ttcgtcactg gtcccgccac caaacgtttc ggcgagaagc

 2161 aggccattat cgccggcatg gcggccccac gggtgcgcat gatcgtgctc ctgtcgttga

 2221 ggacccggct aggctggcgg ggttgcctta ctggttagca gaatgaatca ccgatacgcg

 2281 agcgaacgtg aagcgactgc tgctgcaaaa cgtctgcgac ctgagcaaca acatgaatgg

 2341 tcttcggttt ccgtgtttcg taaagtctgg aaacgcggaa gtcagcgccc tgcaccatta

 2401 tgttccggat ctgcatcgca ggatgctgct ggctaccctg tggaacacct acatctgtat

 2461 taacgaagcg ctggcattga ccctgagtga tttttctctg gtcccgccgc atccataccg

 2521 ccagttgttt accctcacaa cgttccagta accgggcatg ttcatcatca gtaacccgta

 2581 tcgtgagcat cctctctcgt ttcatcggta tcattacccc catgaacaga aatccccctt

 2641 acacggaggc atcagtgacc aaacaggaaa aaaccgccct taacatggcc cgctttatca

 2701 gaagccagac attaacgctt ctggagaaac tcaacgagct ggacgcggat gaacaggcag

 2761 acatctgtga atcgcttcac gaccacgctg atgagcttta ccgcagctgc ctcgcgcgtt

 2821 tcggtgatga cggtgaaaac ctctgacaca tgcagctccc ggagacggtc acagcttgtc

 2881 tgtaagcgga tgccgggagc agacaagccc gtcagggcgc gtcagcgggt gttggcgggt

 2941 gtcggggcgc agccatgacc cagtcacgta gcgatagcgg agtgtatact ggcttaacta

 3001 tgcggcatca gagcagattg tactgagagt gcaccatata tgcggtgtga aataccgcac

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 3121 ctgcgctcgg tcgttcggct gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg

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 3301 gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg actataaaga

 3361 taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac cctgccgctt

 3421 accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca tagctcacgc

 3481 tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtgt gcacgaaccc

 3541 cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc caacccggta

 3601 agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag agcgaggtat

 3661 gtaggcggtg ctacagagtt cttgaagtgg tggcctaact acggctacac tagaaggaca

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 4081 tttcgttcat ccatagttgc ctgactcccc gtcgtgtaga taactacgat acgggagggc

 4141 ttaccatctg gccccagtgc tgcaatgata ccgcgagacc cacgctcacc ggctccagat

 4201 ttatcagcaa taaaccagcc agccggaagg gccgagcgca gaagtggtcc tgcaacttta

 4261 tccgcctcca tccagtctat taattgttgc cgggaagcta gagtaagtag ttcgccagtt

 4321 aatagtttgc gcaacgttgt tgccattgct gcaggcatcg tggtgtcacg ctcgtcgttt

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 4501 gcagtgttat cactcatggt tatggcagca ctgcataatt ctcttactgt catgccatcc

 4561 gtaagatgct tttctgtgac tggtgagtac tcaaccaagt cattctgaga atagtgtatg

 4621 cggcgaccga gttgctcttg cccggcgtca atacgggata ataccgcgcc acatagcaga

 4681 actttaaaag tgctcatcat tggaaaacgt tcttcggggc gaaaactctc aaggatctta

 4741 ccgctgttga gatccagttc gatgtaaccc actcgtgcac ccaactgatc ttcagcatct

 4801 tttactttca ccagcgtttc tgggtgagca aaaacaggaa ggcaaaatgc cgcaaaaaag

 4861 ggaataaggg cgacacggaa atgttgaata ctcatactct tcctttttca atattattga

 4921 agcatttatc agggttattg tctcatgagc ggatacatat ttgaatgtat ttagaaaaat

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 5101 gccgaaatcg gcaaaatccc ttataaatca aaagaataga ccgagatagg gttgagtgtt

 5161 gttccagttt ggaacaagag tccactatta aagaacgtgg actccaacgt caaagggcga

 5221 aaaaccgtct atcagggcga tggcccacta cgtgaaccat caccctaatc aagttttttg

 5281 gggtcgaggt gccgtaaagc actaaatcgg aaccctaaag ggagcccccg atttagagct

 5341 tgacggggaa agccggcgaa cgtggcgaga aaggaaggga agaaagcgaa aggagcgggc

 5401 gctagggcgc tggcaagtgt agcggtcacg ctgcgcgtaa ccaccacacc cgccgcgctt

 5461 aatgcgccgc tacagggcgc gtcccattcg cca

**Publication**

To be published

**Protocol for prep of protein**

1. The transformed B834 (DE3) cells, containing the pET22b-2BPcrA, are used to inoculate a starter culture, of 100 ml of Luria-Bertani broth (LB), with ampicillin (100 µg/ml). These were grown overnight, at 37 °C, on a shaker, at 225 rpm. The protein expression used 8 X 500 ml cultures. A 1/100 dilution of the overnight culture was made up in each 500 ml of LB, with ampicillin, in 2 L flasks. The cells were grown until they reached an OD595 0.5. PcrA expression was induced with 1 mM IPTG, and grown for 5 h, at 25 ° C, shaking at 225 rpm. The culture was transferred into 4 x L centrifuge bottles, after incubation, and cells were centrifuged, at 4 °C, at 4000 rpm, in JS 4.2 swing bucket rotor (Beckman Coulter), for 30 min. The culture pellets were resuspended, into 20 ml of PcrA resuspension buffer containing; 50 mM Tris·HCl (pH 7.5), 2 mM, EDTA, 1 mM DTT, 200 mM NaCl, and 10 % (w/v) sucrose. Then the cells were stored at -80 °C.
2. The resuspended cells were thawed and a dissolved protease inhibitor tablet (Complete, Santa Cruz biotechnology) added. The cells were lysed, using a probe sonicator, with burst of 4 x 20 s (80 Joules), on ice, then the cells were spun, at 13200 rpm, at 4 °C, for 20 min, using 45 Ti rotor (Beckman Coulter). The pellet was discarded. Saturated (NH4)2SO4, at a volume of 0.7 times that of the supernatant, was added gradually to the supernatant form centrifugation, with stirring at room temperature, to precipitate the PcrA. This was centrifuged at 13 200 rpm, at 4 °C, for 20 min, this time discarding the supernatant, the pellet was resuspended into 20 ml, of PcrA low salt pellet buffer containing; 50 mM Tris·HCl (pH 7.5), 2 mM, EDTA, 1 mM DTT and 100 mM NaCl. Its conductivity was adjusted to match the re-suspension buffer, by addition of PcrA adjustment buffer containing; 50 mM Tris·HCl (pH 7.5), 2 mM EDTA, 1 mM DTT.
3. The conductivity adjusted sample was loaded onto a 20 ml heparin sepharose column (GE healthcare), equilibrated in PcrA column buffer; 50 mM Tris·HCl (pH 7.5), 2 mM EDTA, 1 mM DTT, and 100 mM NaCl, using an AKTA FPLC (GE healthcare), at 4 ˚C, with a flow rate of 1 ml/min. The PcrA was eluted, with a NaCl gradient, from 100 mM NaCl to 700 mM NaCl, over 150 ml. PcrA eluted at approximately, 300-400 mM NaCl, across four fractions. The purity of the protein was confirmed by SDS-PAGE gel and those fractions with just a single protein band were pooled and concentrated, using a 20 ml, 10000 MWCO, Vivaspin concentrator (Millipore), by centrifugation, at 4000 RPM, at 4 ˚C, in a JS 4.2 swing bucket rotor. The absorbance ratio of 260 nm:280 nm showed a large amount of DNA present in the final pool of protein, therefore the protein was then gel filtered to remove the DNA.
4. A Superdex 200 column was washed overnight, in a gel filtration buffer containing; 500 mM NaCl, with 1 mM NaN3, at a flow rate of 0.2 ml/min, for 12 hours. A 2 ml aliquot of the pooled protein was loaded onto the gel filtration column; the column was then washed, with 40 ml, of buffer at a flow rate of 0.5 ml/min. The sample was eluted at a flow of 0.5 ml/min, with 2 ml fractions being collected over 120 ml. The DNA eluted first, at 45 – 64 ml, the protein eluted at 75 – 84 ml, this was confirmed on agarose and SDS-PAGE gels, the 260:280 nm spectrometry showed that there was little DNA contamination remaining in the sample. The molar extinction coefficient of 71865 M-1cm-1 at 280 nm (from amino acid sequence), was used to calculate the concentration of the PcrA protein. PcrA was stored in aliquots at -80 ˚C.