***S. aureus* PcrA – His-tagged**

**Plasmid**

pET22B\_SA\_PcrA

**Usage**

The plasmid is used to express *Staphylococcus aureus* PcrA, a DNA helicase, with N-terminal His-tag and Gly-Gly linker.

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**Contact**

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**Protein sequence**

MHHHHHHGGM NALLNHMNTE QSEAVKTTEG PLLIMAGAGS GKTRVLTHRI AYLLDEKDVS

PYNVLAITFT NKAAREMKER VQKLVGDQAE VIWMSTFHSM CVRILRRDAD RIGIERNFTI

IDPTDQKSVV KDVLKNENID SKKFEPRMFI GAISNLKNEL KTPADAQKEA TDYHSQMVAT

VYSGYQRQLS RNEALDFDDL IMTTINLFER VPEVLEYYQN KFQYIHVDEY QDTNKAQYTL

VKLLASKFKN LCVVGDSDQS IYGWRGADIQ NILSFEXDYP EANTIFLEQN YRSTKTILNA

ANEVIKNNSE RKPKGLWTAN TNGEXIHYYE AMTERDEAEF VIREIMKHQR NGXXYQXXAI

LYRTNXQSRV LEKTFMK SN MPYTMVGGQK FYDRKEIKDL LSYLRIIANS NDDISLQRII NVPKRGVGPS SVEKVQNYAL QNNISMFDAL GEADFIGLSK KVTQECLNFY ELIQSLIKEQ EFLEIHEIVD EVLQKSGYRE MLERENTLES RSRLENIDEF MSVPKDYEEN TPLEEQSLIN FLTDLSLVAD IDEADTENGV TLMTMHSAKG LEFPIVFIMG MEESLFPHIR AIKSEDDHEM QEERRICYVA ITRAEEVLYI THATSRMLFG RPQSNMPSRF LKEIPESLLE NHSSGKRQTI QPKAKPFAKR GFSQRTTSTK KQVLSSDWNV GDKVMHKAWG EGMVSNVNEK NGSIELDIIF KSQGPKRLLA QFAPIEKKED

**Gene sequence**

ATGCATCATCACCATCACCACGGCGGCATGAATGCACTGCTGAATCACATGAATACCGAACAGAGCGAAGCAGTTAAAACCACCGAAGGTCCGCTGCTGATTATGGCAGGCGCAGGTAGCGGTAAAACCCGTGTTCTGACCCATCGTATTGCATATCTGCTGGATGAAAAAGATGTGAGCCCGTATAATGTTCTGGCAATTACCTTTACCAATAAAGCAGCCCGTGAAATGAAAGAACGTGTTCAGAAACTGGTTGGTGATCAGGCCGAAGTTATTTGGATGAGCACCTTTCATAGCATGTGTGTTCGTATTCTGCGTCGTGATGCAGATCGTATTGGTATTGAACGCAACTTTACCATTATTGATCCGACCGATCAGAAAAGCGTTGTTAAAGATGTGCTGAAAAACGAGAACATCGATAGCAAAAAATTCGAGCCTCGCATGTTTATTGGTGCCATTAGCAATCTGAAAAATGAACTGAAAACACCGGCAGATGCACAGAAAGAAGCAACCGATTATCATAGCCAGATGGTTGCCACCGTTTATAGCGGTTATCAGCGTCAGCTGAGCCGTAATGAAGCACTGGATTTTGATGATCTGATTATGACCACCATCAACCTGTTTGAACGTGTGCCGGAAGTTCTGGAATATTATCAGAACAAATTCCAGTATATCCATGTGGATGAGTATCAGGATACAAACAAAGCACAGTATACCCTGGTTAAACTGCTGGCAAGCAAATTCAAAAATCTGTGCGTTGTTGGTGATAGCGATCAGAGCATTTATGGTTGGCGTGGTGCAGATATTCAGAACATTCTGAGCTTCGAAnAAGATTATCCGGAAGCCAATACCATCTTTCTGGAACAGAATTATCGTAGCACCAAAACCATTCTGAATGCAGCCAATGAAGTGATCAAAAATAACAGCGAACGTAAACCGAAAGGTCTGTGGACCGCAAATACCAATGGTGaAnAAATCCACTATTATGAGGCAATGACCGAACGTGATGAAGCCGAATTtGTTATTcGCGAAATCATGAAACATCAGCGCAACGGCAAnAnaTACCAGGanntGGCAATTCTgtaTcGTACcaaTGnncagaGCCgtgtGCTGGAAaaaaCCTTtatgAAaATGATCGCAAAGAAATCAAAGACCTGCTGAGCTATCTGCGCATTATTGCAAATAGCAACGATGATATTAGCCTGCAGCGTATTATCAATGTTCCGAAACGTGGTGTTGGTCCGAGCAGCGTTGAAAAAGTTCAGAATTATGCACTGCAGAACAACATCAGCATGTTTGATGCACTGGGTGAAGCAGATTTCATTGGTCTGAGCAAAAAAGTTACCCAAGAATGCCTGAACTTCTATGAACTGATTCAGAGCCTGATCAAAGAACAAGAATTCCTGGAAATCCATGAGATCGTTGATGAAGTGCTGCAGAAAAGTGGTTATCGTGAAATGCTGGAACGTGAAAATACCCTGGAAAGCCGTAGCCGTCTGGAAAATATTGATGAATTTATGAGCGTGCCGAAAGATTATGAAGAAAATACACCTCTGGAAGAACAGTCCCTGATTAACTTTCTGACCGATCTGAGCCTGGTTGCAGATATCGATGAAGCGGATACCGAAAATGGTGTTACCCTGATGACCATGCATAGCGCAAAAGGTCTGGAATTTCCGATTGTGTTTATTATGGGTATGGAAGAAAGCCTGTTTCCGCATATTCGTGCAATTAAAAGCGAAGATGATCACGAGATGCAAGAAGAACGTCGTATTTGTTATGTTGCAATTACCCGTGCAGAAGAGGTTCTGTATATCACCCATGCAACCAGCCGTATGCTGTTTGGTCGTCCGCAGAGTAATATGCCGAGCCGTTTTCTGAAAGAAATTCCGGAAAGCCTGCTGGAAAATCATAGCAGCGGTAAACGTCAGACCATTCAGCCGAAAGCAAAACCGTTTGCAAAACGTGGTTTTAGCCAGCGTACCACCAGTACCAAAAAACAGGTTCTGAGCAGCGATTGGAATGTGGGTGATAAAGTGATGCATAAAGCATGGGGTGAAGGTATGGTTAGCAATGTGAATGAAAAAAACGGCAGCATCGAACTGGACATCATCTTTAAAAGCCAGGGTCCGAAACGGCTGCTGGCACAGTTTGCACCGATTGAAAAAAAAGAGGACTAAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCAC

His-tag

**Insert position:** PcrA insertion using Nde and BamH1 sites on pET22B

**Pet22b vector sequence**

**T7 primer sequence** 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

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 2101 tgcacgccct cgctcaagcc ttcgtcactg gtcccgccac caaacgtttc ggcgagaagc

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 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

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 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

 3061 agatgcgtaa ggagaaaata ccgcatcagg cgctcttccg cttcctcgct cactgactcg

 3121 ctgcgctcgg tcgttcggct gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg

 3181 ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg ccagcaaaag

 3241 gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg cccccctgac

 3301 gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg actataaaga

 3361 taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac cctgccgctt

 3421 accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca tagctcacgc

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 3541 cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc caacccggta

 3601 agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag agcgaggtat

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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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 4441 ttgtgcaaaa aagcggttag ctccttcggt cctccgatcg ttgtcagaag taagttggcc

 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

 4981 aaacaaatag gggttccgcg cacatttccc cgaaaagtgc cacctgaaat tgtaaacgtt

 5041 aatattttgt taaaattcgc gttaaatttt tgttaaatca gctcattttt taaccaatag

 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

**Protocol for preparation of protein**

1. The pET22B\_SA\_PcrA was transformed into B834 (DE3) pLysS E. coli and plated onto ampicillin plates.
2. A starter culture of 100 ml, of Luria-Bertani broth (LB), with ampicillin (100 µg/ml), was inoculated with single colonies, from the plates. These were grown overnight, at 37 °C, on shaker, at 225 rpm. The large scale growth used 4L of LB, in split between four 3L flasks, at 25 °C. A 1/100 dilution of the overnight culture was made up in each 1L, of LB with ampicillin, in 3 litre flasks. The cells were grown to and OD595 0.5.
3. PcrA expression was induced with 1 mM IPTG, and grown for a further 24 h, at 25 °C, shaking at 225 rpm. After incubation, the culture was transferred into 4 x L centrifuge bottles 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. Cells were stored at -80 °C.
4. The PcrA was purified in a number of steps. The resuspended cells were thawed and diluted 1:1, with *S. aureus* dilution buffer (200 mM NaCl and 50 mM Tris·HCl pH7.5, containing a dissolved protease inhibitor tablet (Complete, Santa Cruz biotechnology). The cells were sonicated at 40%, for 2 X 30 s (54 Joules) bursts, on ice and spun at 13200 rpm, at 4 °C, for 25 min, using 45 Ti rotor (Beckman Coulter). The pH of the supernatant was adjusted to 7.5.
5. The solution was loaded onto a 1 ml HisTrap column (GE healthcare), equilibrated in HisTrap column buffer; 50 mM Tris·HCl (pH 7.5), 200 mM NaCl. A flow rate of 1 ml/min, using an AKTA FPLC (GE healthcare), at 4 ˚C, was used. The column was then washed, in 50 ml of the Histrap column buffer, used in the loading, followed by 50 ml the same buffer, with 10 mM Imidazole (pH adjusted to 7.5) added in *S. aureus* elution buffer. The PcrA was eluted by a linear imidazole gradient, from 10 mM to 250 mM, over 20 ml.
6. PcrA eluted at ~35-100 mM Imidazole and its presence was confirmed by SDS-PAGE and spectroscopy 260nm:280nm ratio. The fractions containing the least DNA, with only a single protein band on a gel 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.
7. The PcrA protein was quantified, using an extinction coefficient of 66490 M-1cm-1 at 280 nm (from amino acid sequence). Protein was stored in small aliquots at -80 °C.