***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**

Martin R. Webb

The Francis Crick Institute, London

mwebb2@mrw.org.uk

**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

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

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

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

4381 ggtatggctt cattcagctc cggttcccaa cgatcaaggc gagttacatg atcccccatg

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.