Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: LOC340156A-c049

GenBank GI number: gi|42657507

Vector: pFB-LIC-Bse. Details [PDF]; Sequence [FASTA] or [GenBank]

Coding DNA sequence:

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TACTTCCAATCCATGAAGAACTCCGGTGAT
CAGGCCATGGGCCACCATCATCATCATCAT
TCTTCTGGTGTAGATCTGGGTACCGAGAAC
CTGTACTTCCAATCCATGAAGAACTCCGGT
GATCAGGACTCTCGTTCAGGCCATAATGAG
GCAAAAGAAGTATGGAGTAATGCGGATTTG
ACTGAGCGCATGCCAGTGAAATCGAAGCGT
ACAAGCGCATTGGCCGTTGACATCCCTGCT
CCGCCCGCGCCATTCGATCACCGCATTGTC
ACCGCAAAACAAGGTGCCGTAAACTCCTTT
TATACTGTGTCTAAAACGGAGATCCTGGGC
GGCGGCCGTTTCGGTCAGGTTCATAAGTGC
GAGGAAACCGCTACTGGCCTGAAATTGGCA
GCCAAGATTATCAAAACACGCGGTATGAAA
GACAAAGAGGAAGTCAAGAACGAGATTTCA
GTGATGAATCAACTGGATCACGCAAACTTG
ATCCAGCTGTACGATGCATTTGAAAGTAAG
AATGATATTGTATTGGTGATGGAATATGTT
GACGGCGGTGAGCTGTTTGATCGTATCATT
GACGAATCGTACAACCTGACCGAGTTGGAT
ACTATCCTGTTCATGAAACAAATCTGTGAA
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TTGCATCTGGACCTGAAACCTGAGAACATT
TTGTGCGTCAATCGTGATGCCAAGCAGATC
AAAATTATCGACTTTGGCCTGGCGCGCCGT
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AATATCTTGGCGTGTCGTTGGGACCTTGAG
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AAAAAAAAGAACCGCGGCAGCGATGCTCAA
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ATACGGATCCGAATTCGAGCTCCGTCGACA
AGCTTGCTCAAGACTTCGTTACAAAATGAC
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Final protein sequence:
MGHHHHHHSSGVDLGTENLYFQ^SMKNSGD
QDSRSGHNEAKEVWSNADLTERMPVKSKRT
SALAVDIPAPPAPFDHRIVTAKQGAVNSFY
TVSKTEILGGGRFGQVHKCEETATGLKLAA
KIIKTRGMKDKEEVKNEISVMNQLDHANLI
QLYDAFESKNDIVLVMEYVDGGELFDRIID
ESYNLTELDTILFMKQICEGIRHMHQMYIL
HLDLKPENILCVNRDAKQIKIIDFGLARRY
KPREKLKVNFGTPEFLAPEVVNYDFVSFPT
DMWSVGVIAYMLLSGLSPFLGDNDAETLNN
ILACRWDLEDEEFQDISEEAKEFISKLLIK
EKSWRISASEALKHPWLSDHKLHSRLSAQK
KKNRGSDAQDFVTK
^ TEV cleave site

Tags and additions: Cleavable N-terminal His6 tag.

Host: SF9 insect cells

Growth medium, induction protocol:
LOC340156 was expressed in Sf-9 cells using P-III BV stock. The cells were seeded in Sf-9 cells at the density of 2 x 10^6 cells/ml. Each 2 L polycarbonate flask contained about 750 ml of the culture in Insect-Xpress medium.
The cells in each flask were infected with 7.5 ml of BV stock and incubated at 27°C for 48 hrs. After 48 hrs, cells were harvested by centrifugation at 1800 rpm for 20 min. The cell pellets were washed once with PBS and stored at -80°C for purification at later date.

Extraction buffer, extraction method:
The cell pellet from 4 L culture was removed from -80°C, thawed rapidly at 37°C and transferred on ice.
The cells were re-suspended in 3 volumes of lysis buffer (50 mM HEPES, 300 mM NaCl, 5% glycerol, pH 7.5, 1 mM TCEP, 1:1000 dilution of protease inhibitor cocktail).
The cell suspension was then sonicated (40% amplitude, pulse of 10 sec on and off for 10 sec) for 5 min and centrifuged at 16,000 rpm for 45 min.

Column 1: Ni-affinity. Ni-Sepharose (Amersham), 5 ml of 50 % slurry in 1.5 x 10 cm column, washed with binding buffer.

Procedure: The supernatant was mixed with 10 ml of 50% Ni-NTA slurry. The mixture was rotated for 1 hour at 4°C followed by loading on a column, washing with 150 ml of wash buffer-1 (50 mM HEPES, 300 mM NaCl, 5% glycerol, pH 7.5), washing with 100 ml of wash buffer-2 (50 mM HEPES, 300 mM NaCl, 5% glycerol, pH 7.5, 25 mM imidazole).
The protein was eluted 30 ml of 300 mM imidazole in buffer containing 50 mM HEPES, 300 mM NaCl, 5% glycerol, pH 7.5. Fractions of the eluates were separated on SDS-PAGE and stained with Coomassie blue.

Column 2: Hi Q-TRAP column

Buffers:
Buffer A: 50 mM HEPES, 150 mM NaCl, 5% glycerol, pH 7.5
Buffer B: 50 mM HEPES, 2 M NaCl, 5% glycerol, pH 7.5

Procedure:
The 30 ml eluate from Ni-NTA was diluted in 50 mM HEPES, 5% glycerol to achieve final

NaCl concentration of 150 mM. The resultant 60 ml protein sample was used for anion exchange chromatography using 5 ml HiTrap Q FF column.

Column 3: Superdex 75 GF column

Procedure: The fractions from anion exchange were pooled together and concentrated to 1ml using 10 spin concentrator with a 10 kDa cut off. The concentrated samples were applied onto a Superdex 75 GF column equilibrated in GF Buffer (50 mM HEPES, 300 mM NaCl, 5% glycerol, pH 7.5).

Mass spectrometry characterization: LC- ESI -MS TOF revealed the expected mass of the protein (42433) as predicted from the sequence of this protein.

Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 2M Ammonium Sulfate; 2.5w/v PEG400; in Hepes buffer (50 mM) pH 8.0.

Protein concentration: Protein was concentrated to 10 mg/ml using an Amicon 10 kDa cut-off concentrator.

Data Collection: Crystals were cryo-protected using the well solution supplemented with 2M Li_2SO_4 and flash frozen in liquid nitrogen.

X-ray source: Diffraction data were collected from a single crystal on Diamond beamline IO2 at a single wavelength of 0.9802 Å and the structure was refined to 2.8 Å.

Phasing: The structure was solved by molecular replacement using the structure of PDB 1KOB as a starting model.