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

Amplified construct sequence:

CCATGGGCCACCATCATCATCATCATTCTT
CTGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGCTTGTACCATCCAAA
AGGCAGAAGCACTTGACGGGGCTCATTTGA
TGCAGATCCTCTGGTATGATGAGGAAGAGT
CTCTCTACCCAGCTGTATGGTTGAGAGACA
ACTGTCCGTGCTCTGATTGCTACCTGGATT
CTGCAAAAGCACGGAAACTTCTAGTGGAAG
CTCTTGATGTGAACATTGGAATTAAAGGCT
TGATATTTGACAGAAAAAAGGTGTACATCA
CATGGCCCGATGAGCATTACAGTGAATTCC
AGGCTGATTGGCTGAAGAAAAGATGCTTTT
CCAAGCAGGCCAGAGCAAAGCTCCAAAGAG
AATTGTTTTTTCCAGAATGCCAATACTGGG
GCTCAGAGCTCCAGCTACCCACTTTGGATT
TTGAAGATGTTTTAAGATATGATGAACATG
CATACAAGTGGCTCTCCACCCTCAAGAAAG
TAGGCATAGTAAGACTCACCGGAGCATCTG
ACAAACCAGGAGAAGTTTCAAAACTTGGGA
AAAGGATGGGTTTCCTCTATCTCACATTTT
ATGGACATACTTGGCAAGTGCAAGACAAAA
TCGATGCAAACAATGTGGCTTACACAACTG
GGAAGCTAAGCTTTCACACTGATTATCCAG
CCCTCCATCATCCACCTGGGGTTCAGCTTC
TTCACTGCATAAAGCAAACAGTCACAGGGG
GTGATTCAGAAATTGTAGATGGGTTTAATG
TGTGCCAAAAACTAAAGAAAAATAATCCTC
AGGCATTCCAGATTTTGTCCTCTACCTTTG
TGGACTTTACAGACATTGGAGTGGATTACT
GTGATTTTTCTGTACAATCAAAACATAAAA
TTATAGAGTTAGATGATAAAGGCCAAGTGG
TTCGCATCAACTTCAATAACGCAACTAGGG
ACACAATATTTGATGTACCTGTTGAAAGAG
TTCAGCCTTTTTATGCTGCTCTGAAGGAGT
TTGTTGACCTCATGAACAGCAAAGAATCCA
AGTTTACCTTCAAGATGAATCCAGGTGATG
TGATTACTTTTGATAACTGGCGCTTACTTC
ATGGCCGACGTAGCTATGAAGCAGGAACTG
AGATATCCCGCCATCTAGAAGGAGCTTATG
CTGACTGGGATGTGGTCATGTCAAGGCTTC
GTATCTTAAGGCAGAGGGTGGAGAATGGAA
ACTGACAGTAAAGGTGGATACGGATCCGAA
TTCGAGCTCCGTCGACAAGCTT

Final protein sequence (tag sequence in lowercase)

mghhhhhhssgvdlgtenlyfqsmACTIQK
AEALDGAHLMQILWYDEEESLYPAVWLRDN
CPCSDCYLDSAKARKLLVEALDVNIGIKGL
IFDRKKVYITWPDEHYSEFQADWLKKRCFS
KQARAKLQRELFFPECQYWGSELQLPTLDF

EDVLRYDEHAYKWLSTLKKVGIVRLTGASD
KPGEVSKLGKRMGFLYLTFYGHTWQVQDKI
DANNVAYTTGKLSFHTDYPALHHPPGVQLL
HCIKQTVTGGDSEIVDGFNVCQKLKKNNPQ
AFQILSSTFVDFTDIGVDYCDFSVQSKHKI
IELDDKGQVVRINFNNATRDTIFDVPVERV
QPFYAALKEFVDLMNSKESKFTFKMNPGDV
ITFDNWRLLHGRRSYEAGTEISRHLEGAYA
DWDVVMSRLRILRQRVENGN

Tags and additions: N-terminal, TEV cleavable hexahistidine tag.

Host: *Trichoplusia Ni* (High five)

Expression:
High five cells were grown in Sf900II medium supplemented with 1% FCS at 27°C. Cells were infected at a density of $2x10^6$/ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/1l of cell culture). 72 hours post-infection the cultures were collected and centrifuged for 30min at 2000rpm. The cell pellet was resuspended in cold PBS and centrifugation was repeated. The pellet was resuspended in lysis buffer (pellet from 1l in 25ml of buffer) (50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml)) and frozen at -80°C till purification.

Extraction: Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml).
The thawed cellular lysates were supplemented with Igepal CA 630 (Fluka) to a final concentration of 0.1%, and benzonase (Novagen) (25u/10ml of lysate). Cells were broken using a Dounce homogeniser, followed by centrifugation for 45 min at 20.000rpm at 4°C.

Purification:
Step 1: Ni-affinity, Ni-Sepharose - (GE Healthcare) purification in batch
Step 2: Superdex 200Column , HiPrep 16/60 (Amersham)
Step 3: Ion exchange - 5ml HiTrap Q Sepharose
Buffers:
Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Washing buffer: 50mM HEPES pH 7.5, 500mM NaCl, 10mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, step gradient of imidazole 20mM, 40mM, 60mM, 80mM, 100mM 250mM Imidazole, 0.5mM TCEP
GF buffer: 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
IEX buffers: Buffer A: 25mM Tris pH 8.0; Buffer B: 25mM Tris pH 8.0, 1M NaCl
Procedure: The cell extract was incubated with Ni-Sepharose (0.250ml of resin/lysate from 1l of culture) during 1h at 4°C with gentle rotation. The resin was centrifuged at 1000g for 5min at 4°C, and washed 4x with 50ml of washing buffer. Resin was loaded on the gravity column and protein was eluted in 20ml elution fractions. Protein fractions were analysed by SDS-PAGE. Metal was stripped by incubation with EDTA (final concentration 1mM) o/n at 4°C. Protein was concentrated using Amicon Ultra-15 concentrators with 30kDa cut-off, and purified on a gel filtration column (Superdex 200) on an Akta Express System. BBOX1A containing fractions were combined and His-tag was removed by TEV (o/n digestion at 4°C. Protein was diluted with 25mM Tris buffer pH 8.0 to a concentration of NaCl of 50 mM. The protein was loaded onto a HiTrap Q Sepharose column, and eluted with a NaCl gradient (50-300mM). Fractions containing protein were analysed by SDS-PAGE.

Concentration and buffer exchange:
Using Amicon Ultra-15 concentrators with 30kDa cutoff, the sample was concentrated to 20mg/ml. Concentrations were determined from the absorbance at 280 nm using a NanoDrop spectrophotometer.

Mass spectrometry characterization: The calculated mass of the construct was 44802 Da, and the observed mass (ESI-MS) was 44803Da.

Protein concentration: The final fraction was concentrated to 30.9 mg/ml using a 10kDa MW CO spin concentrator (measured by 280 nm absorbance). Fresh (unfrozen) protein was used for crystallization.

Crystallisation: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. The protein (20mg/ml) was supplemented with *N*-oxalylglycine (8 mM) and either substrate GBB or substrate analogue THP (4 mM) prior to crystallization. Sitting drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 0.2 M Ammonium citrate pH 7.0, 20% PEG 3350, 6% diamine hexan and 10mM $ZnSO_4$. Crystals were flash-cooled in liquid nitrogen with 25 % glycerol as cryoprotectant.

Data collection: Resolution: 2.0 Å; **X-ray source:** Swiss Light source (SLS), beamline X-10.