Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4828271

SGC Construct ID: ZBTB33A-c002

GenBank GI number: gi|5803229

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Expressed sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^SMESRKL
ISATDIQYSGSLLNSLNEQRGHGLFCDVT
VIVEDRKFRAHKNILSASSTYFHQLFSVA
GQVVELSFIRAEIFAEILNYIYSSKIVRV
RSDLLDELIKSGQLLGVKFIA**A**LGVPL

^ TEV cleave site
The construct contains the mutation E115A (shown bold and underlined)

Amplified construct DNA sequence:
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGAGAGTAGAAAACTG
ATTTCTGCTACAGACATTCAGTACTCTGG
CAGTCTGCTGAACTCCTTGAATGAGCAAC
GTGGCCATGGACTCTTCTGTGATGTTACC
GTTATTGTGGAAGACCGAAAATTCCGGGC
TCACAAGAATATTCTTTCAGCTTCTAGTA
CCTACTTCCATCAGCTCTTCTCTGTTGCT
GGGCAAGTTGTTGAACTGAGCTTTATAAG
AGCAGAGATCTTTGCAGAAATTCTCAATT
ATATCTATAGTTCTAAAATTGTTCGTGTT
AGATCAGATTTGCTTGATGAGTTAATTAA
ATCAGGGCAGTTATTAGGAGTGAAATTTA
TAGCAGCGCTTGGTGTCCCATTGTGACAG
TAAAGGTGGATACGGATCCGAA

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: Cleavable N-terminal His6 tag.

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Growth medium, induction protocol: 5ml from a 50 ml overnight culture containing 50 µg/ml kanamycin, 34 µg/ml chloramphenicol was used to inoculate each of four 1 litre cultures of LB containing 50 µg/ml kanamycin, 34µg/ml chloramphenicol. Cultures were grown at 37°C until the OD_{600} reached ~0.5 then the temperature was adjusted to 18°C. Expression was induced overnight using 0.5 mM IPTG at an OD_{600} of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. **Binding buffer**: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5mM TCEP, 1mM PMSF added to the lysate. Cells were lysed using sonication. The lysate was centrifuged at 16,500 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, then washed with 20 ml binding buffer prior to loading the sample.

Buffers: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol

Procedure: Supernatant was applied by gravity flow, followed by a wash with 100 ml binding buffer. The column flow-through was collected.

Column 2: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol.

Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted. 10 mM DTT was added for overnight storage.

Enzymatic treatment : The N-terminal His tag was cleaved by treatment with TEV protease

Column 3: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad

Buffers: 50 mM HEPES, pH 7.5; 150 mM NaCl, 0.5 mM TCEP

Procedure: ZBTB33 was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES, pH 7.5; 150 mM NaCl, 0.5 mM TCEP using an ÄKTAexpress system.

Column 3: Ni-affinity. Ni-sepharose (Amersham), 1 ml of 50% slurry in a Bio-rad poly-prep column, washed with binding buffer.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole , 5% Glycerol.

Procedure: Gel filtration fractions containing ZBTB33 were pooled and loaded by gravity flow on the Ni-sepharose column. After loading a further 4 ml of binding buffer was added and the full flow through was collected. The column was then washed with 5 ml wash buffer at gravity flow. Finally, 6 ml of elution buffer was added. Flow through, wash and elution fractions were analysed by SDS PAGE. The TEV-cleaved ZBTB33 protein was mainly found in the wash fraction. 10 mM DTT was added for overnight storage.

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 13506.8 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 5.5 mg/ml using an Amicon 3 kDa cut-off concentrator.

Crystallization: Crystals grown at 4°C in 1ml sitting drops from a 2:1 ratio of protein to reservoir solution (20% PEG3350, 0.2M $(NH_4)_2H$(citrate) pH 5.0).

Data Collection: Crystals were cryo-protected using the well solution supplemented with 20% ethylene glycol and flash frozen in liquid nitrogen.
X-ray source: Diffraction data were collected from a single crystal on a Rigaku FR-E SuperBright at a single wavelength of 1.5Å.
Resolution: The structure was solved by molecular replacement and refined to 1.70 Å.