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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGAACGTTACCCTGCTGATC
CAGGAACTGATCCACAATCTGTTCGTGAGC
GTGATGAGCCACCAGGATGATGAAGGTCGC
TGCTATAGCGATAGCCTGGCGGAAATCCCG
GCGGTTGATCCGAATTTTCCGAACAAACCG
CCGCTGACCTTTGATATCATTCGCAAAAAC
GTTGAAAACAACCGTTATCGTCGTCTGGAT
CTGTTTCAGGAACACATGTTTGAAGTGCTG
GAACGTGCGCGTCGTATGAACCGTACCGAT
AGCGAAATCTATGAAGATGCGGTGGAACTG
CAGCAGTTCTTTATTAAAATTCGCGATGAA
CTGTGCAAAAATGGTGAAATCCTGCTGAGC
CCGGCCCTGAGCTATACCACCAAACATCTG
CATAATGATGTTGAAAAAGAACGTAAAGAA
AAACTGCCGAAAGAAATTGAAGAAGATTGA
CAGTAAAGGTGGATACGGATCCGAA
```

Final protein sequence (tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq^sMNVTLLI
QELIHNLFVSVMSHQDDEGRCYSDSLAEIP
AVDPNFPNKPPLTFDIIRKNVENNRYRRLD
LFQEHMFEVLERARRMNRTDSEIYEDAVEL
QQFFIKIRDELCKNGEILLSPALSYTTKHL
HNDVEKERKEKLPKEIEED
^ TEV cleave site
```

Tags and additions: Cleavable N-terminal His6 tag.

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

Growth medium, induction protocol: 5 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin/34 µg/ml chloramphenicol were used to inoculate each of two 1 litre cultures of LB containing 50 µg/ml kanamycin/34 µg/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 0.9. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen.
Binding buffer: 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5 % glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP, 1 mM 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.

Column 1 Buffers: 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol

Column 1 Procedure: The supernatant was applied by gravity flow, followed by a wash with 50 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.

Column 2 Buffers: **Binding buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 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 (step elution).
Column 2 Procedure: The flow-through from column 1 was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 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 S200 16/60 HiLoad
Column 3 Buffers: 25 mM HEPES, pH 7.5; 300 mM NaCl, 0.5 mM TCEP
Column 3 Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 25 mM HEPES, pH 7.5; 300 mM NaCl, 0.5 mM TCEP using an ÄKTAexpress system.
Column 4: Ni-affinity. Ni-sepharose (Amersham), 2 ml of 50% slurry in a Bio-rad poly-prep column, washed with binding buffer.
Column 4 Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% Glycerol. **Wash buffer:** 25 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM TCEP. **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole , 5% Glycerol
Column 4 Procedure: Gel filtration fractions containing the protein 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 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 17447.8 for this construct as predicted from the sequence of this protein.
Protein concentration: Protein was concentrated to 15.8 mg/ml using an Amicon 3 kDa cut-off concentrator.
Crystallization: Crystals were grown at 4 °C in 150 nl sitting drops from a 1:2 ratio of protein to reservoir solution containing 0.01 M $ZnCl_2$; 0.1 M Tris pH 8.0; 20 % PEG 6K; 10% EtGly.
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 Diamond beamline IO3 at a single wavelength of 0.9763 Å and the structure was refined to 1.79 Å. **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.