Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: PB1A-c014

GenBank GI number: gi|30794372

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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGAGCCCGGCGTATCTG
AAAGAAATTCTGGAACAGCTGCTGGAAGC
CATCGTTGTTGCGACCAATCCGAGCGGTC
GTCTGATCAGCGAACTGTTTCAGAAACTG
CCGAGCAAAGTGCAGTATCCGGATTATTA
TGCGATCATTAAAGAACCGATTGATCTGA
AAACCATTGCCCAGCGTATCCAGAACGGT
AGCTATAAAAGCATCCACGCCATGGCGAA
AGATATTGATCTGCTGGCGAAAAATGCGA
AAACCTATAATGAACCGGGTAGCCAGGTG
TTTAAAGATGCGAATAGCATTAAAAAAAT
TTTTTATATGAAAAAAGCGGAAATCGAAC
ACCATGAATGACAGTAAAGGTGGATACGG
ATCCGAA
```

Tags and additions: Cleavable N-terminal His6 tag.

Final protein sequence:

```
mhhhhhhssgvdlgtenlyfq^sMSPAYL
KEILEQLLEAIVVATNPSGRLISELFQKL
PSKVQYPDYYAIIKEPIDLKTIAQRIQNG
SYKSIHAMAKDIDLLAKNAKTYNEPGSQV
FKDANSIKKIFYMKKAEIEHHE

^ TEV cleave site
```

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

Growth medium, induction protocol: 10 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol were used to inoculate each of two 1 litre cultures of TB containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol. Cultures were grown at 37°C until the OD_{600} reached ~2.5then the temperature was adjusted to 18°C. Expression was induced overnight using 0.1 mM IPTG at an OD_{600} of 3.0. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. **Binding buffer**: 50 mM HEPES pH 7.5; 500 mM NaCl; 10 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: 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 mM imidazole; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazol , (step elution).

Procedure: Supernatant 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.

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

Column 2: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad

Buffers: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol

Procedure: PB1 was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 500mM NaCl, 5% glycerol using an ÄKTAexpress system.

Column 3: Ni-affinity. Ni-sepharose (Amersham), 2 ml of 50% slurry in a Bio-rad poly-prep column, washed with binding buffer.

Buffers: Binding buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Wash buffer:** 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole.

Procedure: Gel filtration fractions containing PB1 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 PB1protein 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 13213 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 9.8 mg/ml using an Amicon 3 kDa cut-off concentrator.

Crystallization: Crystals were grown at 4°C in 600 nl sitting drops from a 1:1 & 1:2 ratio of protein to reservoir solution containing 0.01 M $ZnCl_2$; 15 w/v PEG6000; 10 v/v ethylene glycol; 5.5pH MES.

Data Collection: Crystals were cryo-protected using the well solution supplemented with 25% 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 1.5 Å and the structure was refined to 1.63 Å; **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.