Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: TAF1LA-c061

GenBank GI number: gi|24429572

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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGCAGGTGGCCTTTAGC
TTTATCCTGGATAATATCGTGACCCAGAA
AATGATGGCGGTGCCGGATAGCTGGCCGT
TTCATCATCCGGTTAACAAAAAATTTGTT
CCGGATTATTATAAAATGATTGTGAATCC
GGTTGATCTGGAAACCATCCGTAAAAATA
TTAGCAAACATAAATATCAGAGCCGCGAA
AGCTTTCTGGATGATGTGAACCTGATTCT
GGCCAACAGCGTTAAATATAACGGTCCGG
AAAGCCAGTATACCAAAACCGCGCAGGAA
ATTGTGAATATTTGCTATCAGACCATTAC
CGAATATGATGAACATCTGACCCAGCTGG
AAAAAGATATTTGCACCGCGAAAGAAGCG
GCGCTGGAAGAAGCCGAACTGGAAAGCCT
GGATTGACAGTAAAGGTGGATACGGATCC
GAA
```

Tags and additions: Cleavable N-terminal His6 tag.

Final protein sequence:

```
mhhhhhhssgvdlgtenlyfq^sMQVAFS
FILDNIVTQKMMAVPDSWPFHHPVNKKFV
PDYYKMIVNPVDLETIRKNISKHKYQSRE
SFLDDVNLILANSVKYNGPESQYTKTAQE
IVNICYQTITEYDEHLTQLEKDICTAKEA
ALEEAELESLD

^ 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 0.5 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: 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 Imidazole, (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.
Column 2: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad
Buffers: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol
Procedure: TAF1L 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.
Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 18000 for this construct as predicted from the sequence of this protein.
Protein concentration: Protein was concentrated to 10.8 mg/ml using an Amicon 3 kDa cut-off concentrator.
Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 1:2 ratio of protein to reservoir solution containing 15 % PEG3350, 0.17 M $(NH_4)_2SO_4$, 15 % glycerol.
Data Collection: Crystals were cryo-protected using the well solution supplemented by 20 % ethylene glycole 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 2.06 Å; **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.