FKBP14A (4DIP) Materials & Methods

Entry Clone Source: MGC

Entry Clone Accession: gi|4042173

SGC Construct ID: FKBP14A-c007

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

Amplified construct sequence:

```
ATGAGGCTTTTCTTGTGGAACGCGGT
CTTGACTCTGTTCGTCACTTCTTTGA
TTGGGGCTTTGATCCCTGAACCAGAA
GTGAAAATTGAAGTTCTCCAGAAGCC
ATTCATCTGCCATCGCAAGACCAAAG
GAGGGGATTTGATGTTGGTCCACTAT
GAAGGCTACTTAGAAAAGGACGGCTC
CTTATTTCACTCCACTCACAAACATA
ACAATGGTCAGCCCATTTGGTTTACC
CTGGGCATCCTGGAGGCTCTCAAAGG
TTGGGACCAGGGCTTGAAAGGAATGT
GTGTAGGAGAGAAGAGAAAGCTCATC
ATTCCTCCTGCTCTGGGCTATGGAAA
AGAAGGAAAAGGTAAAATTCCCCCAG
AAAGTACACTGATATTTAATATTGAT
CTCCTGGAGATTCGAAATGGACCAAG
ATCCCATGAATCATTCCAAGAAATGG
ATCTTAATGATGACTGGAAACTCTCT
AAAGATGAGGTTAAAGCATATTTAAA
GAAGGAGTTTGAAAAACATGGTGCGG
TGGTGAATGAAAGTCATCATGATGCT
TTGGTGGAGGATATTTTTGATAAAGA
AGATGAAGACAAAGATGGGTTTATAT
CTGCCAGAGAATTTACATATAAACAC
GATGAGTTATAG
```

Expressed sequence (Tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq^smGA
LIPEPEVKIEVLQKPFICHRKTKGGD
LMLVHYEGYLEKDGSLFHSTHKHNNG
QPIWFTLGILEALKGWDQGLKGMCVG
EKRKLIIPPALGYGKEGKGKIPPEST
LIFNIDLLEIRNGPRS

^ TEV cleavage site
```

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

Host: BL21(DE3)-R3-pRARE2

Growth Medium & Induction Protocol: A glycerol stock was used to inoculate 60 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a

37°C shaker overnight. The next day this starter culture was used to inoculate 12L of TB media (9 ml starter culture used per 1L) containing 50 µg/ml kanamycin. When the OD_{600} reached approximately 1.0 the temperature was reduced to 18°C and after a further 30 minutes the cells were induced by the addition of 0.1 mM IPTG. The expression was continued overnight.

Cell Lysis: Cell pellets were dissolved in approximately 50ml lysis buffer and broken by passing through the homogeniser (x6) at a constant pressure of 15KPa. The cell debris was pelleted at 16,000 RPM and the supernatant used for further purification.
Lysis buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole pH 7.4, 1 tablet per 50 ml protease inhibitor cocktail EDTA-free (Roche).

Column 1: Ni-NTA (5.0 ml volume in a gravity-flow column).

Column 1 Buffers:
Binding buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.4
Wash buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.4
Elution buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4

Column 1 Procedure: The clarified cell extract was incubated with 5.0 ml pre-equilibriated 50% Ni-NTA bead solution for 1 hour at 4°C with rotation after which it was passed through a glass column. The column was then washed with 50ml Binding Buffer (2 x 25ml) and 50 ml Wash Buffer (2 x25 ml). The protein was eluted with 50 ml of Elution Buffer in 5 x 5 ml fractions.

Column 2: Superdex s75 16/60 Gel Filtration

Column 2 Buffers:
Gel Filtration buffer: 10 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol

Column 2 Procedure: Wash buffer fractions 1 and 2 were pooled along with a separate pool of elution fractions 1 and 2, from the Ni-NTA column. Each pool was then concentrate to 5ml and applied directly to the GF column (pre-equilibrated in GF Buffer) at 1.0 ml/min. 1.0 ml fractions were collected. The protein eluted at a volume of between 70 ml and 100 ml for the wash fractions pool and between 40ml and 100 ml for the elution fractions pool.

Enzymatic Treatment The N-terminal His6-tag was cleaved by incubating the protein overnight with TEV protease (20°C). Cleaved protein was purified by batch binding on 1ml pre-equilibriated 50% Ni-NTA bead solution. The column was then washed with 2 ml Gel Filtration Buffer (2x1ml) and 2 ml Binding Buffer (2x1 ml). The protein was eluted with 2 ml of Elution Buffer (2x1ml).

Gel Filtration buffer: 10 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol.
Binding buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.4
Elution buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4

Concentration: To set up plates the sample was concentrated to 25 mg/ml using a 10 kDa mwco concentrator

Mass spectrometry characterization:
Observed mass: 13815.06 Da
Expected mass: 13816.2 Da

Crystallization: Crystals were grown by vapour diffusion in sitting drop at 4°C. A sitting drop consisting of 75 nl protein and 75 nl well solution was equilibrated against well solution containing 18% PEG_3350; 0.1M HEPES pH 7.2. Crystals were mounted in the presence of 20% (v/v) glycerol and flash-cooled in liquid nitrogen

Data Collection:
Resolution: 1.82 Å **X-ray source**: Diamond IO3