Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5194423

SGC Construct ID: LNX1A-c062

GenBank GI number: gi|14249128

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

Amplified construct sequence:
ATGCACCATCATCATCATCATTCTTCTGG
TGTAGATCTGGGTACCGAGAACCTGTACT
TCCAATCCATGCATGAGAAGGTGGTAAAT
ATCCAAAAAGACCCCGGTGAATCTCTCGG
CATGACCGTCGCAGGGGGAGCATCACATA
GAGAATGGGATTTGCCTATCTATGTCATC
AGTGTTGAGCCCGGAGGAGTCATAAGCAG
AGATGGAAGAATAAAAACAGGTGACATTT
TGTTGAATGTGGATGGGGTCGAACTGACA
GAGGTCAGCCGGAGTGAGGCAGTGGCATT
ATTGAAAAGAACATCATCCTCGATAGTAC
TCAAAGCTTTGGAAGTCAAAGAGGGTAGC
ATCGTTTGA

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

Final protein sequence (His408 to Glu498):
mhhhhhhssgvdlgtenlyfqsMHEKVVN
IQKDPGESLGMTVAGGASHREWDLPIYVI
SVEPGGVISRDGRIKTGDILLNVDGVELT
EVSRSEAVALLKRTSSSIVLKALEVKE**GS**
IV

The N-terminal residues, mhhhhhhssgvdlgtenlyfqsM, derive from the vector.
The C-terminal residues, **GSIV**, are an addition to promote crystallisation.

Host:BL21(DE3)-R3-pRARE2 (previously known as Rosetta)

Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock preparation: A number of colonies from the transformation were used to inoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression: A glycerol stock was used to inoculate 10 ml of LB 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 1L of TB media containing 50 µg/ml kanamycin. When the OD_{600} was approximately 1.0, the temperature was reduced to 25°C and the cells were induced by the addition of IPTG. The expression was continued overnight.

Cell harvest: Cells were spun at 5000rpm for 20 mins and the pellets frozen.

Purification: Cell Lysis: The cells were resuspended in an equal volume of 2x Lysis Buffer. The resuspended cell pellet was lysed by high-pressure homogenization. PEI (polyethyleneimine) was added to a final concentration of 0.15 % and the cell debris and precipitated DNA were spun down (17000 rpm, JA17 rotor, 30 min). **Lysis Buffer:** 50 mM Potassium Phosphate pH 8.0, 500 mM NaCl, 10% Glycerol, 0.5 mM TCEP, 1:1000 dilution of Novagen Protease Inhibitor Cocktail VII.

Column 1: HisTrap FF 5 ml (IMAC).
Column 1 Buffers: Binding Buffer: 50 mM Potassium Phosphate pH 8.0, 500 mM NaCl, 10% Glycerol, 0.5 mM TCEP; **Wash Buffer:** 50 mM Potassium Phosphate pH 8.0, 500 mM NaCl, 10% Glycerol, 0.5 mM TCEP, 30 mM Imidazole; **Elution Buffer:** 50 mM Potassium Phosphate pH 8.0, 500 mM NaCl, 10% Glycerol, 0.5 mM TCEP, 250 mM Imidazole.
Column 2: S75 16/60 Gel Filtration.
Column 2 Buffers: GF Buffer: 10 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 0.5 mM DTT
Column 1 and 2 Procedure: The protein was purified through the two columns using an automated method on an AktaExpress system.
Concentration: The pooled fractions from Column 2 were concentrated to 64 mg/ml (measured by 280 nm absorbance), distributed into aliquots and frozen at -80°C.
Mass spectrometry characterisation: Measured: 12805; **Expected:** 12806.
Crystallisation: Crystals grew from a mixture of protein and precipitant solution (0.8 M Potassium Sodium Phosphate pH 6.0, 1% PEG 3350), using the vapour diffusion method.
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 20% Ethylene glycol, and then flash frozen in liquid nitrogen. **Resolution:** 1.75 Å; Data was collected at the Swiss Light Source, beamline X10.