Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4824065

SGC Construct ID: PMP2A-c002

GenBank GI number: gi|4505909

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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGAACAAATTCCTGGGCACC
TGGAAACTTGTCTCTAGTGAGAACTTTGAC
GATTACATGAAAGCTCTGGGTGTGGGGTTA
GCCACCAGAAAACTGGGAAATTTGGCCAAA
CCCACTGTGATCATCAGCAAGAAAGGAGAT
ATTATAACTATACGAACTGAAAGTACCTTT
AAAAATACAGAAATCTCCTTCAAGCTAGGC
CAGGAATTTGAAGAAACCACAGCTGACAAT
AGAAAGACCAAGAGCATCGTAACCCTGCAG
AGAGGATCACTGAATCAAGTGCAGAGATGG
GATGGCAAAGAGACAACCATAAAGAGAAAG
CTAGTGAATGGGAAAATGGTAGCGGAATGT
AAAATGAAGGGCGTGGTGTGCACCAGAATC
TATGAGAAGGTCTAACAGTAAAGGTGGATA
CGGATCCGAA
```

Final protein sequence (tag sequence in lowercase)

```
mhhhhhhssgvdlgtenlyfqsmNKFLGTW
KLVSSENFDDYMKALGVGLATRKLGNLAKP
TVIISKKGDIITIRTESTFKNTEISFKLGQ
EFEETTADNRKTKSIVTLQRGSLNQVQRWD
GKETTIKRKLVNGKMVAECKMKGVVCTRIY
EKV
```

`mhhhhhhssgvdlgtenlyfqsm` residues originate from the vector.

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

Host: *E. coli* BL21(DE3)-R3-pRARE2

Expression: 10ul of BL21(DE3)-R3-pRARE2 glycerol stock were inoculated into 5ml of TB with 50ug/ml of kanamycin and 34ug/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 1L of TB with 50ug/ml kanamycin and incubated at 37°C, 160rpm. After the OD_{600} reached 1.0, the temperature was dropped to 18°C and 500ul of 1M IPTG was added to the final concentration of ~0.5mM. The culture was then incubated with shaking overnight at 18°C, 160rpm. The following morning the 4L culture was harvested and centrifuged for 10min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 80ml of a lysis buffer and frozen at -80°C.

Cell Extraction: Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml).

The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser followed by centrifugation for 45 min at 15.000rpm.

Purification:
Column 1: Ni-affinity, His-Trap, 1 ml (Amersham)
Column 2: Superdex 200, HiPrep 16/60 (Amersham)
Buffers: Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; **Washing buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 40mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP; **Elution buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole, 0.5mM TCEP; **GF buffer:** 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
Procedure: The cell extract was loaded on the ÄKTA Express system. The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled and characterised by the mass spectrometry.

Concentration and buffer exchange: Using Amicon Ultra-15 concentrators with 10 kDa cutoff, the sample was concentrated to 11mg/ml. Concentrations were determined from the absorbance at 280 nm using NanoDrop.

Mass spectrometry characterization: Calculated mass of the construct was 17375 Da. The exact mass was confirmed by the mass spectrometry.

Crystallisation: Crystals were grown by vapor diffusion at 4°C in 150nl sitting drops. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 3.15 M ammonium sulphate and 0.1 M sodium citrate pH 5.0. Ammonium sulphate acted as a cryo-protectant for flash-cooling of mounted crystal in liquid nitrogen.

Data collection: Resolution: 1.95 Å; **X-ray source:** Bruker in-house source