Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: MAT2BB-c018

GenBank GI number: gi|33519455

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

Amplified construct sequence:

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CTTAAGAAGGAGATATACTATGAACC
GTCGCGTTCTGGTTACCGGTGCTACT
GGTCTGCTGGGTCGTGCAGTACACAA
GGAGTTTCAGCAGAATAACTGGCACG
CTGTAGGTTGCGGTTTTCGTCGTGCG
CGTCCAAAATTCGAGCAAGTAAACCT
GCTGGACTCTAACGCTGTTCACCACA
TCATCCACGATTTCCAGCCGCACGTT
ATTGTGCATTGCGCTGCTGAACGTCG
TCCTGACGTTGTAGAAAACCAGCCAG
ATGCTGCCTCCCAACTGAACGTTGAT
GCGTCTGGTAACCTGGCCAAGGAAGC
TGCTGCAGTAGGCGCATTTCTGATCT
ATATCAGCTCCGATTACGTTTTCGAC
GGTACCAACCCGCCTTATCGTGAGGA
AGACATCCCGGCTCCACTGAACCTGT
ACGGTAAGACGAAGCTGGATGGCGAA
AAAGCGGTGCTGGAAAATAACCTGGG
TGCTGCAGTTCTGCGTATCCCGATCC
TGTATGGTGAAGTCGAAAAACTGGAA
GAATCCGCGGTTACTGTTATGTTCGA
CAAGGTCCAGTTCTCCAATAAGTCTG
CAAACATGGACCATTGGCAACAGCGC
TTCCCGACTCACGTTAAAGATGTGGC
TACGGTTTGCCGCCAACTGGCAGAGA
AGCGTATGCTGGACCCGAGCATCAAG
GGTACTTTCCATTGGAGCGGCAACGA
ACAGATGACGAAATACGAAATGGCAT
GTGCGATTGCCGACGCATTCAACCTG
CCATCTTCTCACCTGCGTCCGATCAC
CGATTCCCCAGTGCTGGGTGCACAAC
GTCCACGTAATGCGCAACTGGACTGT
TCTAAACTGGAAACTCTGGGTATCGG
TCAGCGCACTCCATTTCGTATCGGTA
TTAAGGAGTCTCTGTGGCCGTTCCTG
ATCGATAAACGTTGGCGTCAGACTGT
GTTCCATGCAGAGAACCTCTACTTCC
AATCGCACCATCATCACCACCATGAT
TACAAGGATGACGACGATAAGTGAGG
ATCC
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Final protein sequence (Tag sequence in lowercase):

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MNRRVLVTGATGLLGRAVHKEFQQNN
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WHAVGCGFRRARPKFEQVNLLDSNAV
HHIIHDFQPHVIVHCAAERRPDVVEN
QPDAASQLNVDASGNLAKEAAAVGAF
LIYISSDYVFDGTNPPYREEDIPAPL
NLYGKTKLDGEKAVLENNLGAAVLRI
PILYGEVEKLEESAVTVMFDKVQFSN
KSANMDHWQQRFPTHVKDVATVCRQL
AEKRMLDPSIKGTFHWSGNEQMTKYE
MACAIADAFNLPSSHLRPITDSPVLG
AQRPRNAQLDCSKLETLGIGQRTPFR
IGIKESLWPFLIDKRWRQTVFHaenl
yfqshhhhhhdykddddk

Tags and additions: C-terminal His-tag with TEV protease cleavage site

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

Growth medium, induction protocol: 10µl of a glycerol stock was inoculated into 5ml of LB medium (supplemented with 50µg/ml Kanamycin, 34µg/ml Chloramphenicol) and cultured at 37°C o/n in a shaking incubator (275 rpm). Next evening the o/n starter culture was used to inoculate 100ml of LB medium and was grown at 37°C (200 rpm) o/n. Next morning at an OD_{600} of 2.2 the culture was harvested and the cell pellet was washed twice with M9 minimal medium (Molecular Dimensions Ltd). The cells were resuspended and used to inoculate 1 liter of prewarmed minimal medium. Methionine synthesis was suppressed by addition of leucine, isoleucine and valine (dissolved as 50mg/l for each aa) and lysine, threonine, and phenylalanine (100mg/l of each aa). Selenomethionine was added to a concentration of 25mg/l, and at an OD_{600} of 1.0 cells were induced by supplementation with 1 mM IPTG. Cells were grown overnight at 18°C, collected by centrifugation and stored frozen until further use.
Lysis buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5 mM Imidazole; 5% Glycerol; Complete® protease inhibitors (Roche, 1 tbl/50ml).
Extraction buffer, extraction method: Frozen pellets were thawed and resuspended in a total volume of 30-40ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000rpm for 45 minutes.

Column 1: Ni-Sepharose 6 Fast Flow

Column 1 Buffers:
Lysis buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP; 5 mM Imidazole.
Wash buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP; 30 mM Imidazole.
Elution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP; 250 mM Imidazole.

Column 1 Procedure: The column was packed with 2ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20ml of binding buffer and then 20ml of washing buffer. The protein was eluted with 10ml of elution buffer.

Column 2: Superdex S200 16/60 HiLoad (GE/Amersham)

Column 2 Buffer: 10 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP.

Column 2 Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 1.0ml/min on an ÄKTA Purifier system. Eluted protein was collected in 1ml fractions.
Subtilisin treatmeant of MAT2B protein: Around 18mg of MAT2B protein at a concentration of 8.9mg/ml was treated with 0.6mg of subtilisin for 40 minutes at room temperature. After 40 minutes, reaction was stopped by adding 20µl of 0.5 M PMSF. Later sample was loaded on gel filtration column to remove PMSF.
Column 3: Superdex S200 16/60 HiLoad (GE Amersham)
Column 3 Buffer: 10 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP.
Column 3 Procedure: The subtilisin-treated protein was loaded on the gel filtration column in GF buffer at 1.0ml/min on an ÄKTA Purifier system. Eluted protein was collected in 1ml fractions.
Mass spectrometry characterization: The truncated mass of selenomethionine labelled MAT2B (subtilisin treated) was 24675Da, as determined by ESI-TOF MS.
Protein concentration: Protein was concentrated to 16mg/ml using an Vivaspin 10k cut-off concentrator and stored at -80°C.
Crystallisation: Crystals were grown by vapour diffusion in sitting drops at 20°C. A sitting drop consisting of 100nl protein and 50nl well solution was equilibrated against well solution containing 0.1 M Hepes pH 7.5 and 1.5 M Lithium sulphate. The crystals were mounted directly from the drop using 25% glycerol as a cryoprotectant and flash-cooled in liquid nitrogen.
Data collection: Resolution: 2.20Å. **Phasing:** Synchrotron Diamond Light Source beamline I04, single wavelength.