Molecular Biology

Entry Clone Accession: MGC:9490 IMAGE:3922902; note this clone harbours the p.Asn314Asp polymorphism
Entry Clone Source: MGC
SGC Construct ID: GALTA-c001
Protein Region: M1-A379
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2.

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMSRSGTDPQQRQQASEADAAAATFRANDHQHIRYNP
LQDEWVLVSAHRMKRPWQGQVEPQLLKTVPRHDPLNPLCPGAIRANGEVNPQYDSTFL
FDNDFPALQPDAPSPGPSDHPLFQAKSARGVCKVMCFHPWSDVTLPLMSVPEIRAVVDA
WASVTEELGAQYPWVQIFENKGAMMGCSNPHPHCQVWASSFLPDIAQREERSQQAYKS
QHGEPLLMEYSRQELLRKERLVLTSEHWLVLVPFWATWPYQTLLLPRRHVRRLPELTPAE
RDDLASIMKKLLTKYDNLFETSFPYSMGWHGAPTGSEAGANWDHWQLHAHYYPPLLRS
ATVRKFMVGYEMLAQAQRDLTPEQAAERLRALPEVHYHLGQKDRETATIA
Sequence after tag cleavage:
SMSRSGTDPQQRQQASEADAAAATFRANDHQHIRYNPLQDEWVLVSAHRMKRPWQGQ
VEPQLLKTVPRHDPLNPLCPGAIRANGEVNPQYDSTFLFDNDFPALQPDAPSPGPSDHPLF
QAKSARGVCKVMCFHPWSDVTLPLMSVPEIRAVVDAWASVTEELGAQYPWVQIFENKG
AMMGCSNPHPHCQVWASSFLPDIAQREERSQQAYKSQHGEPLLMEYSRQELLRKERLVL
TSEHWLVLVPFWATWPYQTLLLPRRHVRRLPELTPAERDDLASIMKKLLTKYDNLFETSF
PYSMGWHGAPTGSEAGANWDHWQLHAHYYPPLLRSATVRKFMVGYEMLAQAQRDLT
PEQAAERLRALPEVHYHLGQKDRETATIA
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGTCGCGCAGTGGAACCGATCCTCAGCAACGCCAGCAGGCGTCAGA
GGCGGACGCCGCAGCAGCAACCTTCCGGGCAAACGACCATCAGCATATCCGCTACAA
CCCGCTGCAGGATGAGTGGGTGCTGGTGTCAGCTCACCGCATGAAGCGGCCCTGGCA
GGGTCAAGTGGAGCCCCAGCTTCTGAAGACAGTGCCCCGCCATGACCCTCTCAACCC
TCTGTGTCCTGGGGCCATCCGAGCCAACGGAGAGGTGAATCCCCAGTACGATAGCAC
CTTCCTGTTTGACAACGACTTCCCAGCTCTGCAGCCTGATGCCCCCAGTCCAGGACCC
AGTGATCATCCCCTTTTCCAAGCAAAGTCTGCTCGAGGAGTCTGTAAGGTCATGTGCT
TCCACCCCTGGTCGGATGTAACGCTGCCACTCATGTCGGTCCCTGAGATCCGGGCTGT
TGTTGATGCATGGGCCTCAGTCACAGAGGAGCTGGGTGCCCAGTACCCTTGGGTGCA
GATCTTTGAAAACAAAGGTGCCATGATGGGCTGTTCTAACCCCCACCCCCACTGCCAG
GTATGGGCCAGCAGTTTCCTGCCAGATATTGCCCAGCGTGAGGAGCGATCTCAGCAGG
CCTATAAGAGTCAGCATGGAGAGCCCCTGCTAATGGAGTACAGCCGCCAGGAGCTACT
CAGGAAGGAACGTCTGGTCCTAACCAGTGAGCACTGGTTAGTACTGGTCCCCTTCTG
GGCAACATGGCCCTACCAGACACTGCTGCTGCCCCGTCGGCATGTGCGGCGGCTACCT
GAGCTGACCCCTGCTGAGCGTGATGATCTAGCCTCCATCATGAAGAAGCTCTTGACCA
AGTATGACAACCTCTTTGAGACGTCCTTTCCCTACTCCATGGGCTGGCATGGGGCTCC
CACAGGATCAGAGGCTGGGGCCAACTGGGACCATTGGCAGCTGCACGCTCATTACTA
CCCTCCGCTCCTGCGCTCTGCCACTGTCCGGAAATTCATGGTTGGCTACGAAATGCTT
GCTCAGGCTCAGAGGGACCTCACCCCTGAGCAGGCTGCAGAGAGACTAAGGGCACT
TCCTGAGGTTCATTACCACCTGGGGCAGAAGGACAGGGAGACAGCAACCATCGCCTG
ACAGTAAAGGTGGATACGGATCCGAA

Protein Expression

hGALT (wt or variants) was cultured in 6 L of Terrific Broth at 37 °C, and induced with 0.1 mM IPTG overnight at 18 °C. Cell pellets were harvested, homogenized in lysis buffer (50 mM sodium phosphate pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) and centrifuged to remove insoluble material.

Protein Purification

The supernatant was purified by immobilized metal affinity (Talon resin; GE Healthcare) and size-exclusion chromatography in Superdex 200 Hi-Load 16/60 column (GE Healthcare), pre-equilibrated with buffer 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP. Purified protein was treated with His-tagged TEV protease overnight at 4 °C, and further purified by reverse Nickel affinity and size exclusion chromatography. The final purified protein was concentrated to 20 mg⁄ml, flash cooled in liquid nitrogen and stored at −80 °C.

Concentration: 20 mg/ml
Mass-spec Verification: yes

Structure Determination

Crystallization: Crystals were grown by vapour diffusion at 20 °C, from sitting drops mixing 200 nl of protein (20 mg/ml; pre-incubated with 5 m M UDP-Glc) and 100 nl of reservoir solution containing 0.2 M ammonium sulphate, 30% (w/v) PEG 8000. Crystals were cryo-protected with reservoir solution supplemented with 25% (v/v) ethylene glycol and flash-cooled in liquid nitrogen. The structures were solved by molecular replacement with PHASER, using the structure of *E. coli* GALT (1HXP) as template. Modelling and refinement were carried out using Refmac and Coot.

Data Collection: *Beamline:* Dmnd I02; *Resolution:* 1.73 Å