Molecular Biology

Entry Clone Accession:
Entry Clone Source: Invitrogen
SGC Construct ID: MTHFRA-c042
Protein Region: D38-N644
Vector: pFB-CT10HF-LIC
Tag: C-TEV;C-10HIS;C-FLAG
Host: DH10Bac

Sequence (with tag(s)):
MDPERHERLREKMRRRLESGDKWFSLEFFPPRTAEGAVNLISRFDRMAAGGPLYIDVTW
HPAGDPGSDKETSSMMIASTAVNYCGLETILHMTCCRQRLEEITGHLHKAKQLGLKNIM
ALRGDPIGDQWEEEEGGFNYAVDLVKHIRSEFGDYFDICVAGYPKGHPEAGSFEADLKHL
KEKVSAGADFIITQLFFEADTFFRFVKACTDMGITCPIVPGIFPIQGYHSLRQLVKLSKLEV
PQEIKDVIEPIKDNDAAIRNYGIELAVSLCQELLASGLVPGLHFYTLNREMATTEVLKRLG
MWTEDPRRPLPWALSAHPKRREEDVRPIFWASRPKSYIYRTQEWDEFPNGRWGNSSSPAF
GELKDYYLFYLKSKSPKEELLKMWGEELTSEASVFEVFVLYLSGEPNRNGHKVTCLPWN
DEPLAAETSLLKEELLRVNRQGILTINSQPNINGKPSSDPIVGWGPSGGYVFQKAYLEFFTS
RETAEALLQVLKKYELRVNYHLVNVKGENITNAPELQPNAVTWGIFPGREIIQPTVVDPV
SFMFWKDEAFALWIEQWGKLYEEESPSRTIIQYIHDNYFLVNLVDNDFPLDNCLWQVVED
TLELLNAENLYFQSHHHHHHHHHHDYKDDDDK
Sequence after tag cleavage:
MDPERHERLREKMRRRLESGDKWFSLEFFPPRTAEGAVNLISRFDRMAAGGPLYIDVTW
HPAGDPGSDKETSSMMIASTAVNYCGLETILHMTCCRQRLEEITGHLHKAKQLGLKNIM
ALRGDPIGDQWEEEEGGFNYAVDLVKHIRSEFGDYFDICVAGYPKGHPEAGSFEADLKHL
KEKVSAGADFIITQLFFEADTFFRFVKACTDMGITCPIVPGIFPIQGYHSLRQLVKLSKLEV
PQEIKDVIEPIKDNDAAIRNYGIELAVSLCQELLASGLVPGLHFYTLNREMATTEVLKRLG
MWTEDPRRPLPWALSAHPKRREEDVRPIFWASRPKSYIYRTQEWDEFPNGRWGNSSSPAF
GELKDYYLFYLKSKSPKEELLKMWGEELTSEASVFEVFVLYLSGEPNRNGHKVTCLPWN
DEPLAAETSLLKEELLRVNRQGILTINSQPNINGKPSSDPIVGWGPSGGYVFQKAYLEFFTS
RETAEALLQVLKKYELRVNYHLVNVKGENITNAPELQPNAVTWGIFPGREIIQPTVVDPV
SFMFWKDEAFALWIEQWGKLYEEESPSRTIIQYIHDNYFLVNLVDNDFPLDNCLWQVVED
TLELLNAENLYFQ
DNA Sequence:
TTAAGAAGGAGATATACTATGGACCCTGAGCGGCATGAGAGACTCCGGGAGAAGATG
AGGCGGCGATTGGAATCTGGTGACAAGTGGTTCTCCCTGGAATTCTTCCCTCCTCGAA
CTGCTGAGGGAGCTGTCAATCTCATCTCAAGGTTTGACCGGATGGCAGCAGGTGGCC
CCCTCTACATAGACGTGACCTGGCACCCAGCAGGTGACCCTGGCTCAGACAAGGAGA
CCTCCTCCATGATGATCGCCAGCACCGCCGTGAACTACTGTGGCCTGGAGACCATCCT
GCACATGACCTGCTGCCGTCAGCGCCTGGAGGAGATCACGGGCCATCTGCACAAAGC
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GCCTCCACTTCTACACCCTCAACCGCGAGATGGCTACCACAGAGGTGCTGAAGCGCC
TGGGGATGTGGACTGAGGACCCCAGGCGTCCCCTACCCTGGGCTCTCAGTGCCCACC
CCAAGCGCCGAGAGGAAGATGTACGTCCCATCTTCTGGGCCTCCAGACCAAAGAGTT

ACATCTACCGTACCCAGGAGTGGGACGAGTTCCCTAACGGCCGCTGGGGCAATTCCTC
TTCCCCTGCCTTTGGGGAGCTGAAGGACTACTACCTCTTCTACCTGAAGAGCAAGTCC
CCCAAGGAGGAGCTGCTGAAGATGTGGGGGGAGGAGCTGACCAGTGAAGCAAGTGT
CTTTGAAGTCTTTGTTCTTTACCTCTCGGGAGAACCAAACCGGAATGGTCACAAAGTG
ACTTGCCTGCCCTGGAACGATGAGCCCCTGGCGGCTGAGACCAGCCTGCTGAAGGAG
GAGCTGCTGCGGGTGAACCGCCAGGGCATCCTCACCATCAACTCACAGCCCAACATC
AACGGGAAGCCGTCCTCCGACCCCATCGTGGGCTGGGGCCCCAGCGGGGGCTATGTC
TTCCAGAAGGCCTACTTAGAGTTTTTCACTTCCCGCGAGACAGCGGAAGCACTTCTGC
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AAAACATCACCAATGCCCCTGAACTGCAGCCGAATGCTGTCACTTGGGGCATCTTCCC
TGGGCGAGAGATCATCCAGCCCACCGTAGTGGATCCCGTCAGCTTCATGTTCTGGAAG
GACGAGGCCTTTGCCCTGTGGATTGAGCAGTGGGGAAAGCTGTATGAGGAGGAGTCC
CCGTCCCGCACCATCATCCAGTACATCCACGACAACTACTTCCTGGTCAACCTGGTGG
ACAATGACTTCCCACTGGACAACTGCCTCTGGCAGGTGGTGGAAGACACATTGGAGC
TTCTCAACGCAGAGAACCTCTACTTCCAATCGCACCATCATCACCATCACCATCACCA
CCATGATTACAAGGATGACGACGATAAGTGAGGATCC

Protein Expression

Procedure: Protein was expressed in insect cells in 6L Sf9 media (ThermoFisher), infected with 3mL/L P2 virus.

Protein Purification

Procedure: Protein was purified by affinity (Ni-NTA, Qiagen) and size-exclusion (Superdex 200) chromatography, followed by cleavage of the C-terminal tag by His-tagged tobacco etch virus protease (1:20 mass ratio) overnight at 4°C and re-passage over Ni-NTA resin. Selenomethionine(SeMet)-derivatized proteins were expressed using SelenoMethionine Medium Complete (Molecular Dimensions) and purified as above.

Concentration: 21.0 mg/ml
Mass-spec Verification: yes

Structure Determination

Crystallization: SeMet-derivatized and native *Hs*MTHFR$_{38\text{-}644}$ were concentrated to 15-20 mg/ml, and crystals were grown by siting drop vapour diffusion at 20°C, in mother liquor containing 0.1M Na citrate tribasic, 22.5% PEG4K, 5% 2-propanol.
Data Collection: *Beamline:* Dmnd I04-1; *Resolution:* 2.5 Å
Data Processing: X-ray diffraction data were collected at the Diamond Light Source and processed using XIA2. The *Hs*MTHFR$_{38\text{-}644}$ structure was solved by selenium multi-wavelength anomalous diffraction phasing using autoSHARP, and subjected to automated building with BUCCANEER.