Molecular Biology

Entry Clone Accession: IMAGE:3048375
Entry Clone Source: MGC
SGC Construct ID: DCLRE1AA-c082
Protein Region: T700-Y1040
Vector: pFB-LIC-Bse. This is a baculovirus transfer vector (Bac-to-bac), with N-terminal 6 His tag followed by a TEV cleavage site
Host: DH10Bac

Sequence (with tag(s)):
MGHHHHHHSSGVDLGTENLYFQSMTCPFYKKIPGTGFTVDAFQYGVVEGCTAYFLTHFH
SDHYAGLSKHFTFPVYCSEITGNLLKNKLHVQEQYIHPLPLDTECIVNGVKVVLLDANHC
PGAVMILFYLPNGTVILHTGDFRADPSMERSLLADQKVHMLYLDTTYCSPEYTFPSQQEV
IRFAINTAFEAVTLNPHALVVCGTYSIGKEKVFLAIADVLGSKVGMSQEKYKTLQCLNIPEI
NSLITTDMCSSLVHLLPMMQINFKGLQSHLKKCGGKYNQILAFRPTGWTHSNKFTRIADV
IPQTKGNISIYGIPYSEHSSYLEMKRFVQWLKPQKIIPTVNVGTWKSRSTMEKYFREWKL
EAGY
Sequence after tag cleavage:
SMTCPFYKKIPGTGFTVDAFQYGVVEGCTAYFLTHFHSDHYAGLSKHFTFPVYCSEITGN
LLKNKLHVQEQYIHPLPLDTECIVNGVKVVLLDANHCPGAVMILFYLPNGTVILHTGDFR
ADPSMERSLLADQKVHMLYLDTTYCSPEYTFPSQQEVIRFAINTAFEAVTLNPHALVVCG
TYSIGKEKVFLAIADVLGSKVGMSQEKYKTLQCLNIPEINSLITTDMCSSLVHLLPMMQIN
FKGLQSHLKKCGGKYNQILAFRPTGWTHSNKFTRIADVIPQTKGNISIYGIPYSEHSSYLE
MKRFVQWLKPQKIIPTVNVGTWKSRSTMEKYFREWKLEAGY
DNA Sequence:
CCATGGGCCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTG
TACTTCCAATCCATGACATGTCCATTCTATAAGAAAATACCTGGAACCGGCTTTACAGT
TGATGCCTTTCAGTATGGCGTGGTTGAAGGTTGCACAGCCTATTTTCTCACACATTTTC
ATTCTGATCATTATGCTGGATTGTCTAAACACTTCACATTTCCAGTTTATTGTAGTGAGA
TAACTGGCAATTTGTTGAAGAACAAGCTTCATGTGCAAGAACAATATATTCACCCATT
GCCACTGGACACTGAATGTATTGTGAATGGTGTCAAAGTTGTTTTGCTTGATGCCAAT
CACTGTCCAGGTGCTGTCATGATCCTCTTTTATCTTCCTAATGGTACTGTCATATTACAC
ACGGGAGACTTCAGAGCAGATCCCAGCATGGAACGTTCTCTTCTTGCGGACCAGAAA
GTCCATATGCTGTACTTAGATACCACATATTGTAGCCCAGAATACACCTTTCCATCTCAG
CAAGAGGTTATCCGGTTTGCCATCAACACTGCCTTTGAGGCTGTAACTCTAAACCCAC
ATGCTCTTGTTGTCTGTGGCACTTACTCTATTGGAAAAGAGAAAGTCTTCCTAGCCATT
GCTGATGTTTTAGGTTCAAAAGTGGGCATGTCCCAGGAAAAATATAAAACTCTACAGT
GCCTCAATATACCAGAAATTAATTCACTCATCACTACCGACATGTGCAGTTCATTGGTT
CACCTTCTCCCAATGATGCAAATTAATTTTAAGGGCTTACAGAGTCATTTGAAGAAGT
GTGGTGGGAAATACAATCAGATTTTGGCATTTCGACCTACAGGATGGACACACTCTAA
CAAGTTCACTAGAATAGCAGATGTTATTCCCCAGACCAAAGGAAACATTTCAATATATG
GAATTCCTTACAGTGAACACAGCAGCTACCTAGAAATGAAGCGCTTTGTCCAGTGGCT
GAAGCCCCAGAAAATCATACCTACTGTAAATGTGGGCACCTGGAAATCTAGGAGCAC
AATGGAGAAATATTTTAGAGAGTGGAAATTGGAAGCTGGATATTGACAGTAAAGGTGG
ATACGGATCCGAATTCGAGCTCCGTCGACAAGCTT

Protein Expression

Medium: SF900II
Antibiotics: Ampicillin
Procedure: Baculoviruses were generated by recombination in *E. coli* DH10Bac (Life

Technologies) followed by transfection and two rounds of amplification in SF9 cells. DCLRE1A was expressed in 1-L cultures of SF9 cells in 4-L shaker flasks at 27°C, infected at 2 × 10^6 cells/ml with 3 ml of virus, and incubated for further 70 h. The cells were collected by centrifugation, suspended in 15 ml/l of lysis buffer (50 mM HEPES, pH 7.5, 0.5 M NaCl, 5% v/v glycerol, 10 mM imidazole, and 1 mM TCEP) and frozen at −80°C.

Protein Purification

Procedure: Cells were thawed, 3–5 volumes of lysis buffer were added, and the cells were disrupted by sonication. The lysate was centrifuged for 30 min at 40 000 × g, and the clear supernatant was collected. The clarified cell lysate was loaded on a 5-ml Histrap FF column. The column was washed with 20 volumes of wash buffer (lysis buffer with 30 mM imidazole), and the protein was recovered with elution buffer (lysis buffer with 300 mM imidazole). The eluted protein was combined with His10-tagged TEV protease (1/10 w/w) in a dialysis tubing, and digestion of the tag was performed overnight at 6°C while dialysing against 4 l of wash buffer. The material was then passed through a 1-ml HisTrap column to remove contaminating proteins and remaining TEV protease. The column was developed with a 40-ml gradient from wash buffer to elution buffer, and all fractions were analyzed by SDS-PAGE.

The DCLRE1A containing fractions from the second IMAC column were combined, concentrated to <4 ml using a centrifugal concentrator (MWCO = 10 kDa), and loaded on a Superdex S200 HR 16/60 column equilibrated with GF buffer (10 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM Tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol) at 1.2 ml/min.

The protein was confirmed by ESI-TOF intact mass spectrometry (Predicted: 38836.2 observed: 38836.3)

Structure Determination

Crystallization: Protein crystallization was performed by vapour diffusion in sitting drops at 4°. A protein solution at 8-10 mg/mL was mixed at with a 1:2 ratio of protein to crystallization solution containing 0.20M Sodium Flouride, 0.1M Bis-Tris Propane pH 8.5, 20.0% PEG 3350, 10.0% Ethylene Glycol. Crystals were transferred to a cryo protectant solution consisting of well solution supplemented with an additional 20 % Ethylene Glycol before being loop mounted and plunged directly into a pool of liquid nitrogen.

Data Collection: Data was collected to 2.19Å resolution at Diamond Light Scource beamline I04-1, and processed using XDS.
Data Processing: The structure was solved by molecular replacement using the program MOLREP and PDBid 4B87 as a search model. Refinement was performed using PHENIX REFINE to a final Rfactor = 17.9%, Rfree = 20.1%.