# MLL5

**PDB:**2LV9

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**
**Entry Clone Source:**
**SGC Clone Accession:**
**Tag:**
**Host:**

## Construct

**Prelude:**Cloning. The DNA fragment encoding the PHD domain (residues 109-188) of human MLL5 (NP_061152,GI:91199543) was amplified by PCR and cloned into the pET28-MHL vector (GenBank, EF456735) using Infusiondry-down PCR cloning, downstream of the poly-histidine coding region.
**Sequence:**

```
MHHHHHHSSGRENLYFQG-SEDGSYGTDVTRCICGFTHDDGYMICCDKCSVWQHIDCMGIDRQHIPDTYLCERCQPRNLDKERAVLL
QRRKRENMSDGD MW= 11497.77 g/mol; the region left of the "-" sign is derived from the ve
ctor
```

**Vector:**pET28-MHL vector (GenBank, EF456735)

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and incubated overnight (18 hours) at 220 rpm at 37°C in a 125 ml flask containing 50 ml of M9 minimal media (100 uM ZnSO4, 8.55 mM NaCl, 47.6 mM Na2HPO4, 22 mM KH2PO4, 100 mM MgSO4, 2 mM biotin, 1.5 mM thiamine.HCl, 10 mM ZnSO4, and 0.1 M CaCl2) supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin. The overnight starter culture was transferred to a 2 L flask containing 1 L of M9 minimal media supplemented with supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin, and incubated at 37°C. When the OD(600) reached a value of 1.0, protein expression was induced with 100 µM isopropyl-thio-β-D-galactopyranoside and the cells were incubated overnight (15.5 hours) at 220 rpm at 15°C. Cell pellets were collected by centrifugation (7000 rpm, 20 mins) and frozen in 50 mL Falcon tubes at -80°C for storage.

## Purification

**Procedure**
The frozen cell pellet stored in a 50 ml Falcon tube obtained from 1L of culture was thawed by soaking in warm water, and resuspended in 40 mL lysis buffer (15.4 mM Tris HCl, 100 µM ZnSO4, 0.5 mM NaCl, and 15 mM imidazole, pH 8.5). The cell pellet was lysed by sonication (Branson Sonicator) on ice for 10 minutes total sonication time (10 sec pulses at half-maximal frequency with 10 second rest). The lysate was clarified by centrifugation for 20 min at 4°C. The supernatant was mixed with 2 mL of Ni2+ affinity beads per 40 mL lysate. The mixture was incubated with mixing for 20 minutes at 4°C. The lysate was spun at 2000 rpm for 6 minutes, and the supernatant was decanted. The remaining resin was resuspended and washed twice with lysis buffer, followed by two washes with with 5 mL of cold wash buffer (15.4 mM Tris HCl, 100 uM ZnSO4, 0.5 mM NaCl, and 30 mM imidazole, pH 8.5). The washed resin was transferred to a gravity filter column and further washed with 2 mL of wash buffer. Samples were eluted from the resin by exposure to 5 mL of elution buffer (15.4 mM Tris HCl, 100 uM ZnSO4, 0.5 mM NaCl, and 500 mM imidazole, pH 8.5). Buffer exchange & protein concentration. The purified protein was exchange from elution buffer into Tris-based NMR buffer (10 mM Tris HCl, 300 mM NaCl, 1 mM Benzamidine, 0.01% NaN3, 0.01 mM ZnSO4, 10 mM DTT, 10% D2O, and 90% H2O, pH 7.0) by ultracentrifugation using 5 mL concentrators with a 5,000 molecular weight cut-off (VivaSpin 2 MES) at 3000 rpm, resulting in a final volume of 300 µl (final protein concentration of 0.5 mM). The concentrated protein was transferred to a 5 mm Shigemi NMR tube.

## Extraction

**Procedure**

**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**
**NMR Spectroscopy:**A series of spectra (3D 1H-13C NOESY, 3D 1H-15N NOESY, 2D 1H-13C Constant Time HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HBHA(CO)NH, 3D (H)CCH-TOCSY, and 3D H(C)CH-TOCSY) were generated using 600MHz and 800Mhz Bruker AVANCE spectrometers. NMR data was processed and analyzed using NMRPipe, MDDGUI, Sparky, FMCGUI, TALOS, CYANA, CNS, and PSVS.
**Data Collection:**
**Data Processing:**