= SGC clone accession =
PBC007-B09

= Tag =
N-terminal His6-tag,

= Construct comments =

= Construct sequence =
MHHHHHHSSGRENLYFQGCCEHHKAMIAGLALLRNPELLLEIPLALLVVGLGGGSLPLF
VHDHFPKSCIDAVEIDPSMLE
VATQWFGFSQSDRMKVHIADGLDYIASLAGGGEARPCYDVIMFDVDSKDPTLGMSCPPP
AFVEQSFLQKVKSILTPEGVF
ILNLVCRDLGLKDSVLAGLKAVFPLLYVRRIEGEVNEILFCQLHPEQKLATPELLETAQAL
ERTLRKPGRGWDDTYVLSD
MLKTVKIV

DNA sequence has been verified by sequencing

= Vector =
pFBOH-MHL

= Expression host =
Spodoptera frugiperda

= Growth method =
Shaker

The recombinant donor vector pFBOH-MHL-METTL13 was transformed into DH10Bac E. coli cells (Invitrogen) to generate recombinant viral DNA.
Sf9 cells (Invitrogen) were transfected with Bacmid DNA using jetPRIME® transfection reagent (PolyPlus Transfection), and recombinant baculovirus particles were recovered.
The recombinant virus preparation was sequentially amplified from P1 to P3 viral stocks. Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Fisher Scientific) to a density of 4×106 cells/mL and with viability not less than 97% were infected with 10 mL of P3 viral stock for each 1 L of cell culture.
Cell culture medium was collected after 4 days of incubation on a shaker at 150 RPM and 27°C when culture viability dropped to 75-80%.

= Extraction buffers =
50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol

= Extraction procedure =
2L native cell pellet was resuspended in a total volume of 200 ml extraction buffer and the cells disrupted by sonication for
10 mins at 5" on 10" off duty cycle at 108W output power.

= Purification buffers =
Washing Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 50 mM imidazole
Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM imidazole

= Purification procedure =
The hexahistidine-tagged Mettl13 protein was isolated using Cobalt-charged TALON resin (Clontech),
followed by size exclusion chromatography Superdex 200 (26 x 60) column, pre-equilibrated with 20mM HEPES, pH 7.4, 150mM NaCl, 2mM TCEP.
The collected protein fractions belonging to a single peak were concentrated up to 10 mg/mL and added S-Adenosyl-L-homocysteine (SAH) at a 1:10 molar ratio.

= Protein stock concentration =
Concentration used for crystallization : 10.0 mg/mL

= Mass spec =
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= Functional multimerization =
Monomer

= Crystallization =
Crystallization of 5WCJ was performed in 96 well vapor diffusion sitting drop plates by mixing equal volumes of protein and reservoir solution at 20°C.
Initial hits were observed in 20% (w/v) polyethylene glycol 8000, 0.2M Ammonium Sulfate, 0.1M Sodium Cacodylate (pH 5.5).
After several seeding processes, diffracting quality crystals were grown in sitting drop vapor diffusion plates by mixing 2µl of Mettl13 with 1µl of 20% (w/v) polyethylene glycol 3350, 200mM Ammonium Chloride and 200nM seeds.
A 30% (v/v) Glycerol supplemented reservoir solution was used as cryoprotectant and cryocooled in liquid nitrogen.