Project: Structural studies of the catalytic domain of SETDB1 protein

Experiment: Expression test and purification of SETDB1 catalytic domain constructs' for structural studies by X-ray crystallography.

Aim: In the present section of this study, we focused on the development of the efficient bacterial expression systems to produce large amounts of soluble SETDB1 catalytic domain for structural studies. This report involves a summary of expression test results of different fragments of SETDB1 and purification of various fusion proteins.

Methods

Transformation of plasmid DNA

15 µL of *Escherichia coli* (*E. coli*) BL21 competent cell suspension was placed on ice and then thawed. 0.5 µL of plasmid DNA (>200 ng/µL) was added to the tube and incubated on ice for 20 minutes. Later, the tube was heated for 45 seconds at 42 °C and immediately put in the icebox for 3 to 5 minutes to create a thermal shock. 150 µL of Lysogeny Broth (LB) medium with no antibiotic was added to the tube and incubated at 37 °C for 45-60 minutes. Lastly, the culture was placed on LB agar plate with the corresponding antibiotic and incubated overnight at 37 °C.

Table 1 Details of the vectors used in this study listed. Please visit https://www.thesgc.org/reagents/vectors for more details.

Vector code	Antibiotic resistance	N-terminal fusion sequence	Affinity Column
pET28-MHL	Kanamycin (50 µg/mL	6x-His followed by a tobacco etch virus (TEV) cleavage site	Ni-NTA
pET28GST-LIC	Kanamycin (50 µg/mL)	217 amino acid GST-tag protein followed by a 6x-His followed and a thrombin cleavage site	GST
pET28-MKH8SUMO	Kanamycin (50 µg/mL)	N-terminal fusion tag containing 8x-His followed by a thrombin cleavage site, SUMO, and a TEV cleavage site	Ni-NTA

Expression of recombinant proteins

A single colony was inoculated and grown overnight in 50 mL of LB medium supplemented with antibiotic(s) based on different plasmids, see Table 1 for antibiotic selection markers. 30 mL of overnight culture was used to inoculate ~1.8 liter of TB medium. The culture was grown at 37 °C until reaching OD600 = ~2.0 (approximately 5-6 hours). Cultures were cooled to 14 °C before IPTG induction and 0.5mM of IPTG added to induce protein expression and then cultures were grown at 14 °C for an additional 16 hours. The cells were harvested by centrifugation at 6.000 rpm for 10 minutes.

Cell disruption by ultrasonication

The cell pellet from 1.8 L of culture was suspended in 200 mL lysis buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 10% glycerol). Phenylmethylsulfonyl fluoride (PMSF) was included at a final concentration of 1 mM. The resuspended cells were lysed by tip sonication for around 10 minutes (5 seconds on, 7 seconds off).

Purification of soluble proteins by Ni^{+2}-NTA\GST affinity column

The cell lysate obtained from sonication step was centrifuged for 1 hour at 14,000 rpm at 4 °C. 200 mL of clarified cell lysate was added to beads (Ni-NTA or GST beads) and incubated at 4 °C on shaker 1 to 2 hours. Clarified lysate with beads was loaded on the column. The column was washed with washing buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 25 mM imidazole and later 50 mM Imidazole for Ni-NTA affinity purification). The protein was eluted with elution buffer (250 mM Tris-HCl pH 8.0, 300 mM NaCl, 300 mM

imidazole). For GST tag purification, beads were washed with lysis buffer around 10 CV and eluted with lysis buffer supplemented with 25 mM glutathione reduced.

Table 2 *Constructs of the catalytic domain of SETDB1 designed for E. coli expression and their test expression results listed below.*

MBD: Methyl-CpG binding domain

Batch and Plate Well	**AA start**	**AA end**	**Domain\s**	**Clone Vector**	Expression
JMC135E11	534	672	MBD	pET28-MHL	×
JMC135E12	534	675	MBD	pET28-MHL	×
JMC135F05	548	672	MBD	pET28-MHL	×
JMC135F06	548	675	MBD	pET28-MHL	×
JMC061A02	554	649	MBD	pET28GST-LIC	×
JMC135F11	556	672	MBD	pET28-MHL	×
JMC135F12	556	675	MBD	pET28-MHL	×
JMC092A04	562	669	MBD	pET28-MKH8SUMO	×
JMC125F07	569	672	MBD	pET28GST-LIC	×
JMC140D01	569	672	MBD	pET28GST-LIC	×
JMC133D04	569	672	MBD	pET28-MKH8SUMO	√
JMC135G04	569	675	MBD	pET28-MHL	×
JMC125F08	569	690	MBD	pET28GST-LIC	×
JMC133D05	569	690	MBD	pET28-MKH8SUMO	×
JMC125F09	569	730	MBD	pET28GST-LIC	×
JMC133D06	569	730	MBD	pET28-MKH8SUMO	×
JMC092A05	584	669	MBD	pET28-MKH8SUMO	×
JMC125F10	591	672	MBD	pET28GST-LIC	×
JMC133D07	591	672	MBD	pET28-MKH8SUMO	×
JMC125F11	591	690	MBD	pET28GST-LIC	×
JMC133D08	591	690	MBD	pET28-MKH8SUMO	×
JMC125F12	591	730	MBD	pET28GST-LIC	×
JMC133D09	591	730	MBD	pET28-MKH8SUMO	×
JMC092A06	597	669	MBD	pET28-MKH8SUMO	×

JMC135F01	534	889	MBD+ Pre-SET	pET28-MHL	×
JMC135F02	534	960	MBD+ Pre-SET	pET28-MHL	×
JMC135F07	548	889	MBD+ Pre-SET	pET28-MHL	×
JMC135F08	548	960	MBD+Pre-SET	pET28-MHL	×
JMC135F10	548	1291	MBD+Pre-SET	pET28-MHL	×
JMC135G01	556	889	MBD+Pre-SET	pET28-MHL	×
JMC135G02	556	960	MBD+Pre-SET	pET28-MHL	×
JMC135G05	569	889	MBD+Pre-SET	pET28-MHL	×
JMC135G06	569	960	MBD+Pre-SET	pET28-MHL	×
JMC142C07	586	753	MBD+Pre-SET	pET28-MHL	×
JMC142C08	588	753	MBD+Pre-SET	pET28-MHL	×
JMC135G03	556	1291	MBD+Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC135G07	569	1291	MBD+Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC096G02	591	1290	MBD+Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC088G10	591	1291	MBD+Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC142B10	28	109	N terminal	pET28-MHL	√
JMC142B09	29	109	N terminal	pET28-MHL	√
JMC142C01	34	110	N terminal	pET28-MHL	√
JMC142B12	37	110	N terminal	pET28-MHL	√
JMC142C02	28	449	N terminal + Triple Tudor	pET28-MHL	√
JMC142D01	785	923	Partial SET	pET28-MHL	√
JMC142C09	621	753	Partial MBD+ Partial Pre-SET	pET28-MHL	×
JMC142C10	642	753	Partial MBD+ Partial Pre-SET	pET28-MHL	×
JMC142C11	642	923	Partial MBD+ Pre-SET+ Partial SET	pET28-MHL	×
JMC142D02	1091	1291	Partial SET+ Post SET	pET28-MHL	×
JMC142C12	642	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091D06	667	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC088G11	667	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091D07	672	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E06	672	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091D08	677	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E07	677	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091D09	682	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E08	682	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091D10	687	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E09	687	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×

JMC091D11	692	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E10	692	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091D12	697	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E11	697	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091E01	702	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091E12	702	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091E02	707	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091F01	707	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091E03	712	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091F02	712	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091E04	717	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC091F03	717	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC091E05	721	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC088G12	721	1291	Pre-SET+ SET+ Post-SET	pET28-MKH8SUMO	×
JMC146C08	722	1291	Pre-SET+ SET+ Post-SET	pET28-MHL	×
JMC142D03	785	1291	SET+ Post-SET	pET28-MHL	√
JMC062G07	9	702	Triple Tudor + MBD domain	pET28GST-LIC	×

Results

Most of the constructs mapping on the C-terminal region of SETDB1 protein was not expressed in a variety of vectors as listed in *Table 2*. Only three of them (133D04, 142D01, and 142D03) were expressed which corresponds to MDB, partial SET, and partial SET+post SET, respectively. The construct 133D04 yielded a very low amount of protein, in a highly pure form after cation-exchange chromatography, as shown in Figure 1. The construct 142D01 was expressed very well; however, even after the size exclusion column step, the sample was not clean enough. More importantly, the sample appeared at higher molecular weights (around ~20kDa) than the expected size, which is 15.6kDa as shown in Figure 3. The last construct, 142D03, also showed low expression pattern and appeared at higher molecular weight than expected molecular weight. Similar to 142D01, even after size exclusion chromatography, the protein was not pure enough, as shown in Figure 3.

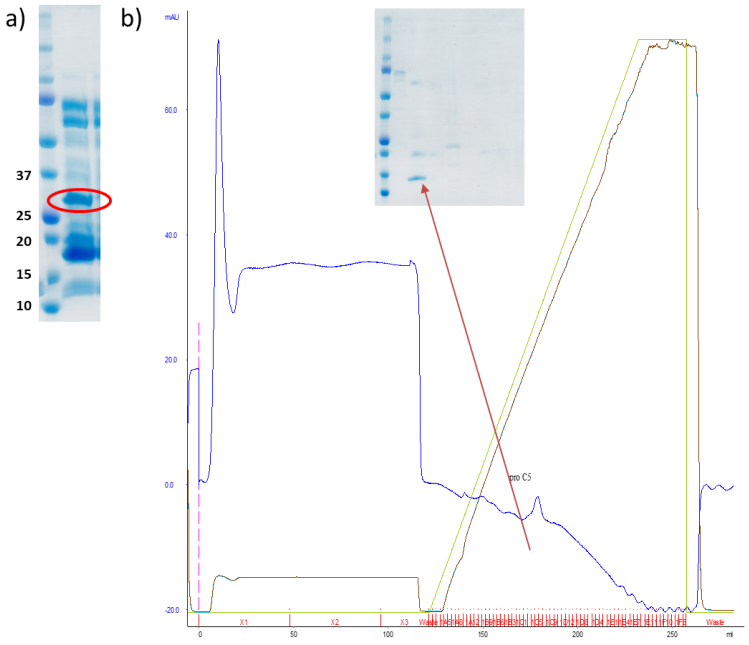

Figure 1 Large-scale purification result for JMC133D04. a) The SDS-PAGE analysis of Ni-NTA purification of JMC133D04 construct (expected MW: 27.5 kDa with SUMO tag). b) Cation exchange profile of JMC133D04 (SUMO tag cut expected MW: 10 kDa).

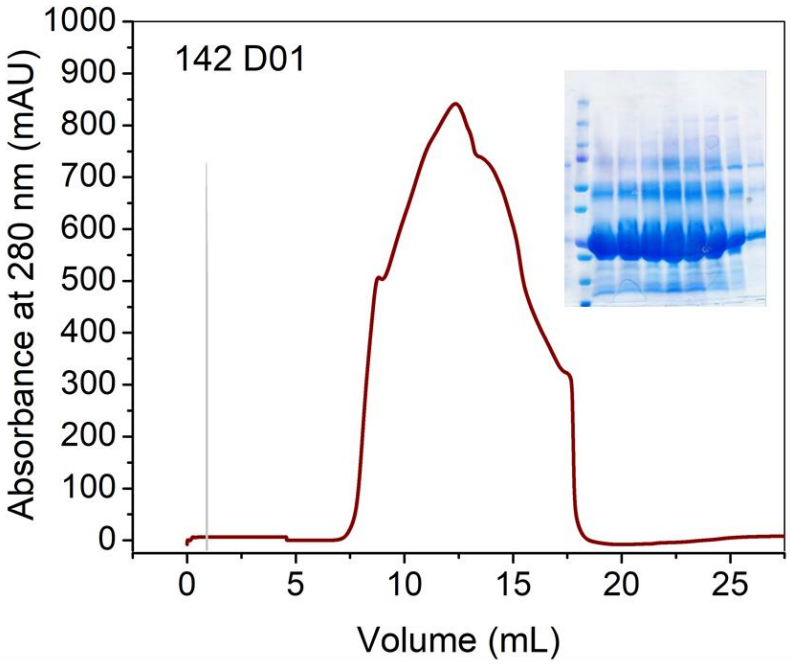

Figure 2 Large-scale purification result for JMC142D01. Size exclusion profile of JMC142D01 after Ni-NTA purification (expected MW: 15.6 kDa with his tag).

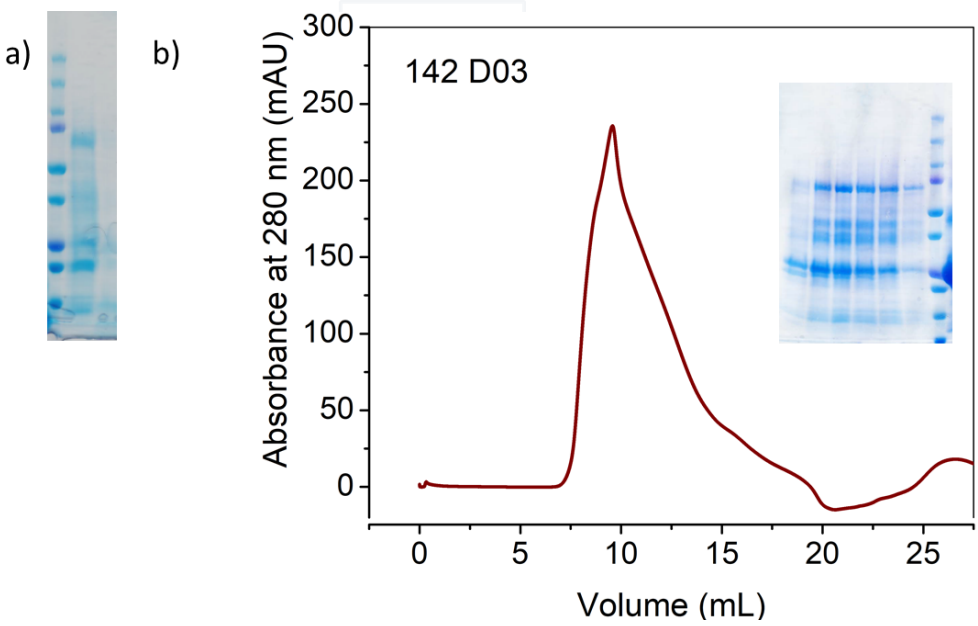

Figure 3 Large-scale purification result for JMC142D01. a) SDS-PAGE analysis of Ni-NTA purification of JMC142D03 construct (expected MW: 57.5kDa with his tag). b) Size exclusion profile of JMC142D03.