Project: Structural studies of catalytic domain of SETDB1 protein

Experiment: Expression and solubility test of SETDB1 catalytic domain constructs

Aim: Here, we designed four constructs for SETDB1 catalytic domain for insect cell expression systems. This section includes expression test results of different fragments of SETDB1.

Methods and result

The protocols used in this study from generation of recombinant bacmid DNA to screening of high-throughput test expression for proteins are explained in more detail in BVES_protocols.docx file, available through this link https://zenodo.org/record/154611#.XiJHishKjcs.

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

Vector Name	Antibiotic resistance	N – terminal fusion sequence	Promoter
pFBOH-LIC Vector (GenBank accession EF456740)	Ampicillin and Gentamicin	MGSSHHHHHHSSGLVPRGS	Polyhedrin Promoter

Table 2 *Constructs of catalytic domain of SETDB1 designed for insect cell expression and their test expression results listed below.*

MBD: Methyl-CpG binding domain

Construct ID	AA start	AA end	Domain\s	Clone Vector	Molecular Weight (Da)	Expression
JMC01MH06	596	1290	MBD+Pre-SET+ SET+ Post-SET	pFBOH-LIC	80806	×
JMC01MH07	621	1290	MBD+Pre-SET+ SET+ Post-SET	pFBOH-LIC	77753.32	×
JMC01MH08	673	1290	MBD+Pre-SET+ SET+ Post-SET	pFBOH-LIC	71512.89	x
JMC01MH09	685	1290	MBD+Pre-SET+ SET+ Post-SET	pFBOH-LIC	69932.05	x

SDS-PAGE analysis of small-scale test expression of pFBOH-MHL constructs which are listed in Table 2 shows no expression, Figure 1.

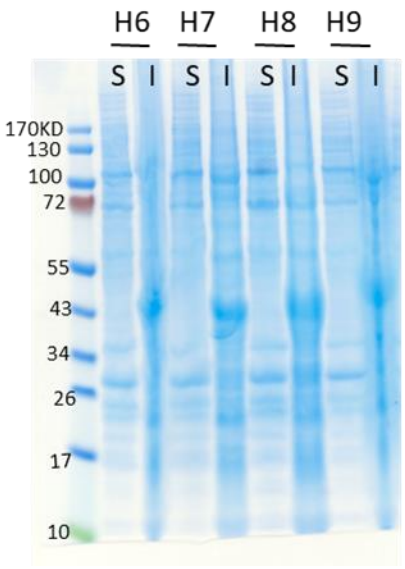

Figure 1 Small scale expression and solubility test, SDS-PAGE analysis. Sf9 cells were infected with P2 virus, harvested after four days later. S: soluble, I: insoluble (cell pellet)