MOL231: Protein expression of ErbB3-binding protein 1 tagged with MBP or GB1

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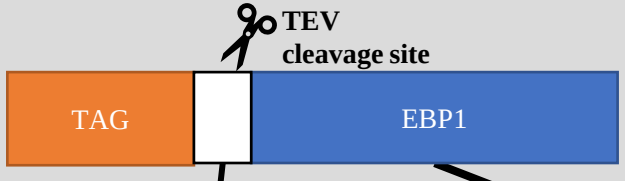
Introduction

ErbB3-binding protein 1 (EBP1) is a multifunctional and highly conserved protein located in cytoplasm and nucleolus. It is involved in cell proliferation and survival, protein synthesis and regulation of gene expression [1, 2].

EBP1 structure

Figure 1. Cryo-EM structure of EBP1. Made by Andrea P. Morovicz on Pymol with the PBD id pdb:6sxo.



Aim

- Generate the TEV protease to cleave off tag
- Produce pure EBP1-product for futrue use

Methods

1. Subcloning of EBP1 into pETGB1-1a/pETMBP-1a vectors
2. Transformation into BL21DE3 cells
3. Expression and purification of TEV and MBP- and GB1-tagged EBP1-proteins

Results

Expression and purification of TEV

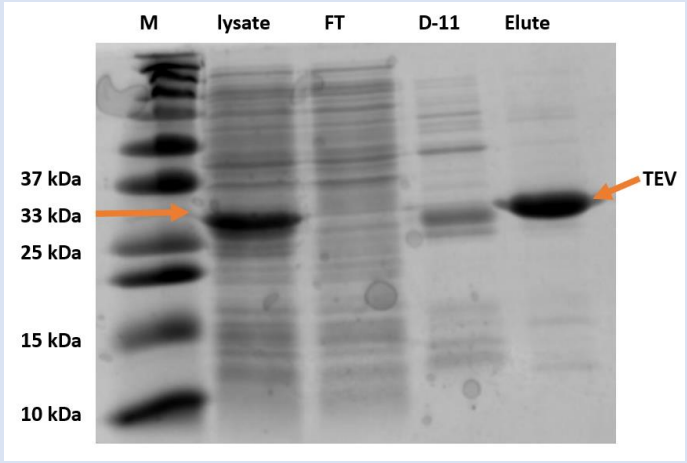


Figure 2. Expression and purification of TEV. TEV is a high sequence specific protease used to cleave proteins. SDS-PAGE of lysate and flow though (FT), His-trap (fraction D11) and His-trap (fraction E).

Cloning of EBP1 into GBP1 and MBP vectors

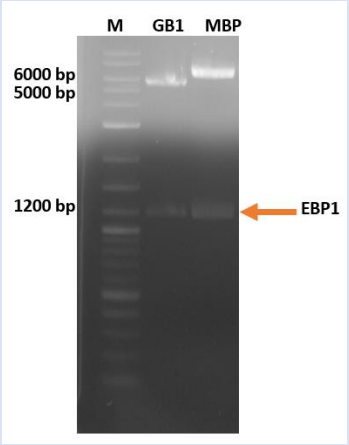


Figure 3. Cloning of EBP1 into GBP1 and MBP vectors. Agarose gel visualizing restriction digestion of mini-prep products from the cloning of EBP1 into BamHI/NcoI digested vectors

Expression of GB1 and MBP tagged EBP1

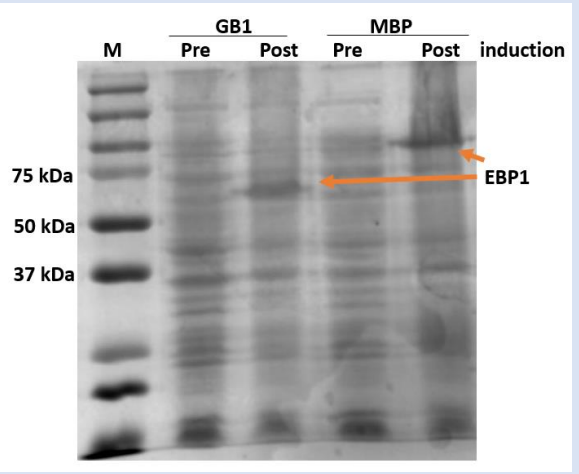


Figure 4. Protein expression of GB1-EBP1 and MBP-EBP1. SDS-PAGE from bacterial lysates before (pre) and after (post) induction with 1mM IPTG.

REFERENCES

1. Stevenson BW, Gorman MA, Koach J, Cheung BB, Marshall GM, Parker MW, Holien JK. A structural view of PA2G4 isoforms with opposing functions in cancer. J Biol Chem. 2020 Nov 20;295(47):16100-16112. doi: 10.1074/jbc.REV120.014293. Epub 2020 Sep 20. PMID: 32952126; PMCID: PMC7681029.
2. Hwang, I., Ko, H.R. & Ahn, JY. The roles of multifunctional protein ErbB3 binding protein 1 (EBP1) isoforms from development to disease. Exp Mol Med 52, 1039–1047 (2020).

Conclusion

The cloning of EBP1 into the vectors MBP and GB1 and production of TEV was sucessfull.

Future prospects

- Study the thermostability of untagged EBP1 WT
- Produce constructs of EBP1 tumour mutants and compare their biophysical & biochemical properties to the WT

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