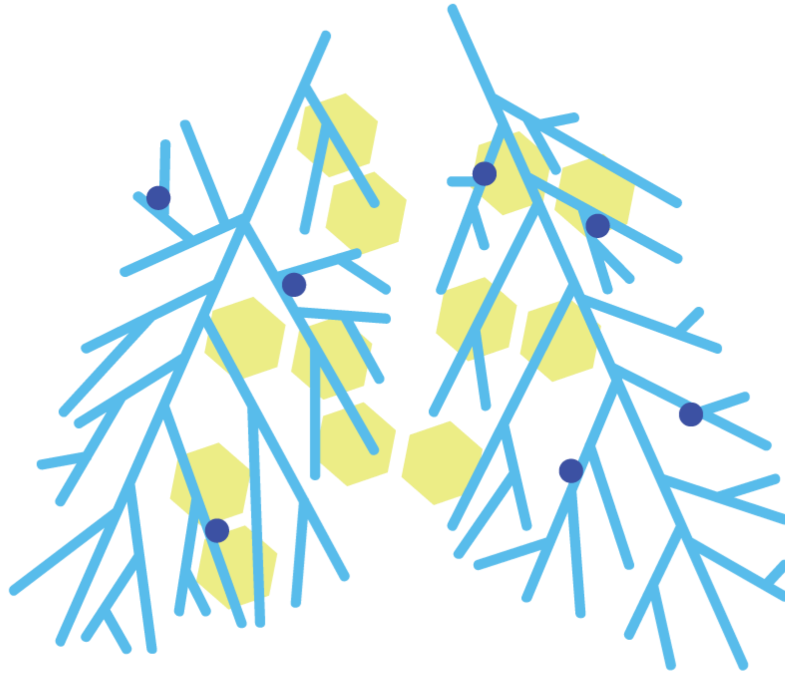


PROLUNG

DEGRADATION

EXPRESSION

LAB BOOK 6

iGEM Stockholm

Expression of Endo-β-Galactosidase from Biobrick

Background

Endo-β-Galactosidase was designed as a gBlock and inserted into pSB1C3. BL21(DE3) cells were transformed with this plasmid and cultivated to produce the enzyme.

Inducing the BL21(DE3) cells to produce Endo-β-Galactosidase

Aim
Induce the production of the enzyme with IPTG.

Procedure
The cultures were induced with IPTG of concentrations 0.1 mM to 1 mM at ODs of between 0.4 to 0.7. They were induced overnight at room temperature.

Sonication and IMAC purification

Aim
To break open the cells and purify the enzyme based on the Histag.

Procedure
Protocol for sonication and protocol for IMAC purification were used with no modifications. All six flask were sonicated and purified. The purification was made on cobalt colons with colon volumes of 1.2 ml.

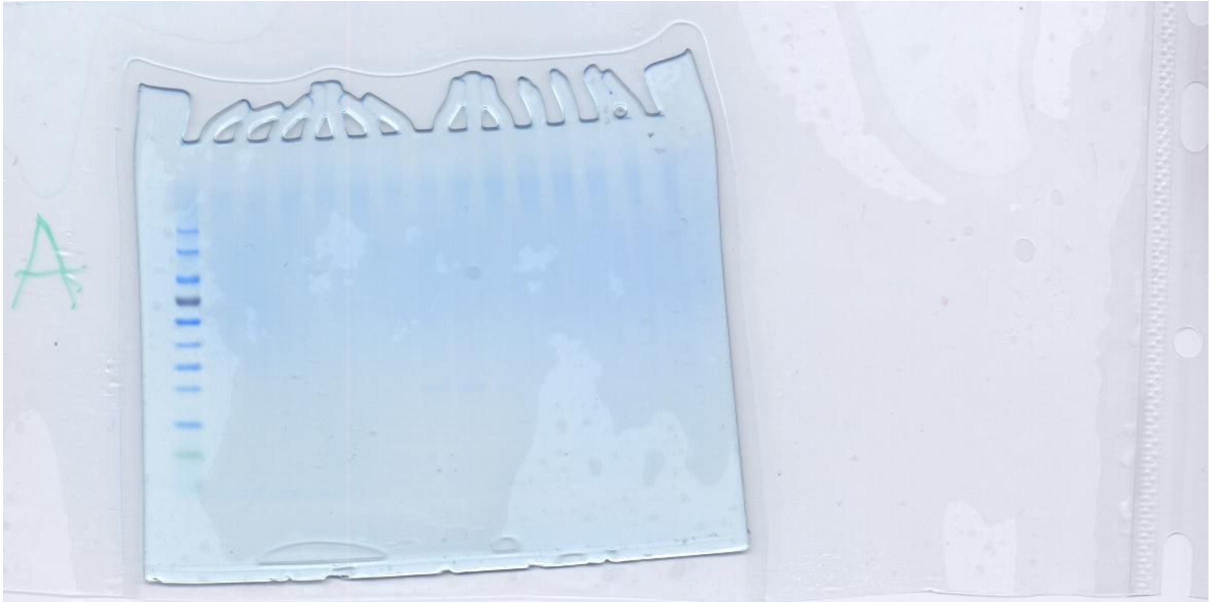
SDS-PAGE of IMAC purified samples

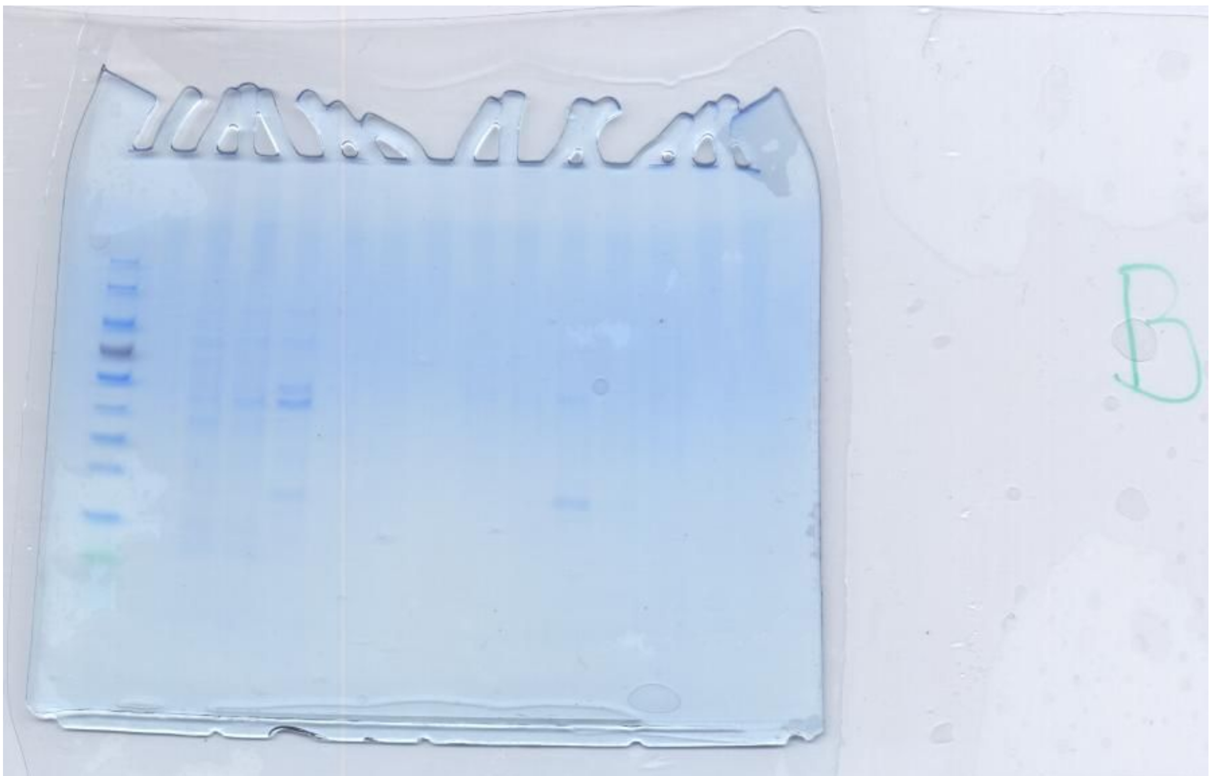
Aim
To visualize that Endo-β-Galactosidase was produced by controlling that there is a band at the correct size of the enzyme.

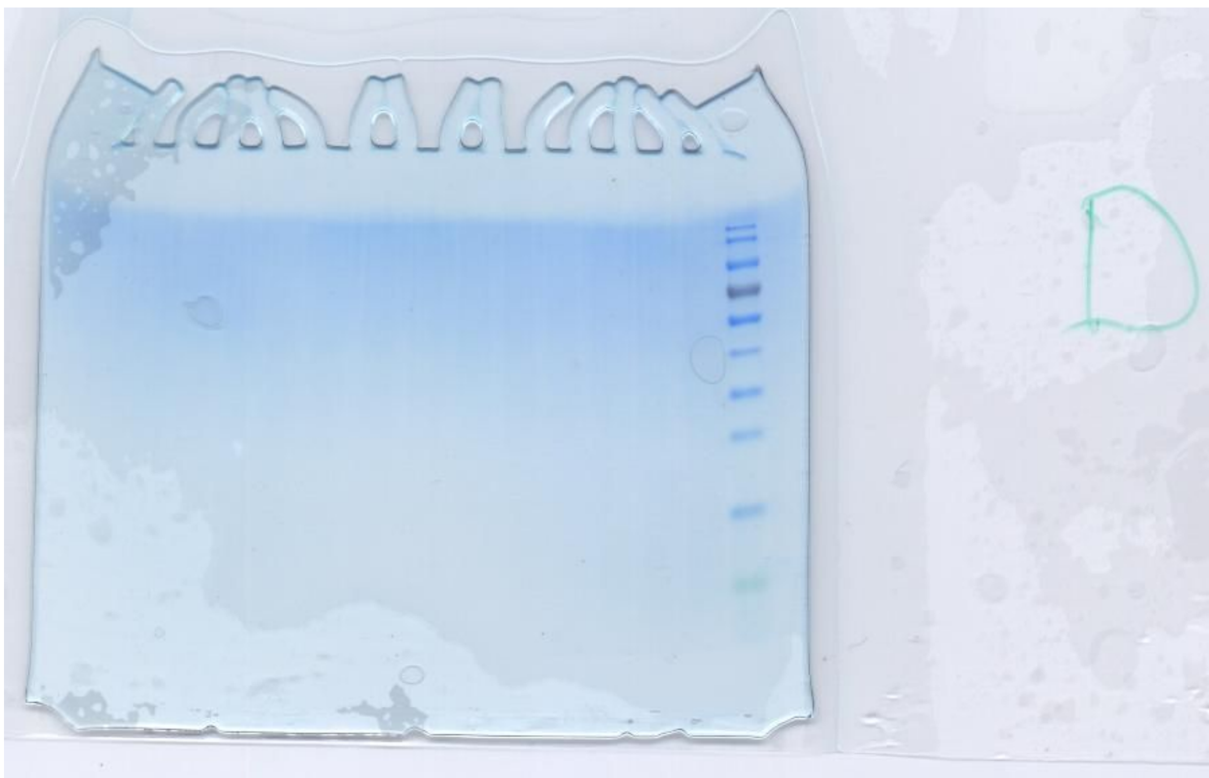
Procedure
The protocol for SDS-PAGE was used with the exception that 24 μl of sample was used with 6 μl of loading buffer. The gels were precasted gels from Biorad.

Results

Only one of the six samples had a band at the correct size.







Cultivation of BL21(DE3) transformed with pSB1C3 with Endo-β-Galactosidase and inducing with IPTG

Aim

Re-doing expression of Endo-β-Galactosidase since we were not satisfied with ambiguous results of the first try. Cultivating nine different combinations of OD

of the bacteria and IPTG concentration aiming for OD values 0.2, 0.4 and 0.6 and IPTG concentrations of 0.1 mM, 0.5 mM and 1 mM. A tenth flask will be made for the control.

<u>Procedure</u>

Ten flasks of 10 mL were cultivated where one of them were a negative control. Chloramphenicol was added in a final concentration of 20 ul/mL.

The transformed cells were taken from an agar plate that was used in the first try of expression Endo-β-Galactosidase.

The cultivations were induced with IPTG.

<u>Results</u>

Flask	IPTG concentration	IPTG volume	OD
1	0,5	10	0,466
2	0,5	10	0,9295
3	0,5	10	0,62
4	0,1	2	1,2748
5	0,5	10	1,2789
6	0,5	10	0,1909
7	1,0	20	0,5668
8	1,0	20	1,2699
9	1,5	30	1,272
10	1,0	20	0,46

Sonication and IMAC purification

<u>Aim</u>

To break open the cells and purify the Endo-β-Galactosidase based on the Histag.

Procedure

Protocols for sonication and IMAC purification used with no modifications. The samples were purified with IMAC cobalt colons with volumes of 1.2 ml.

SDS-PAGE of the IMAC purified samples

Aim

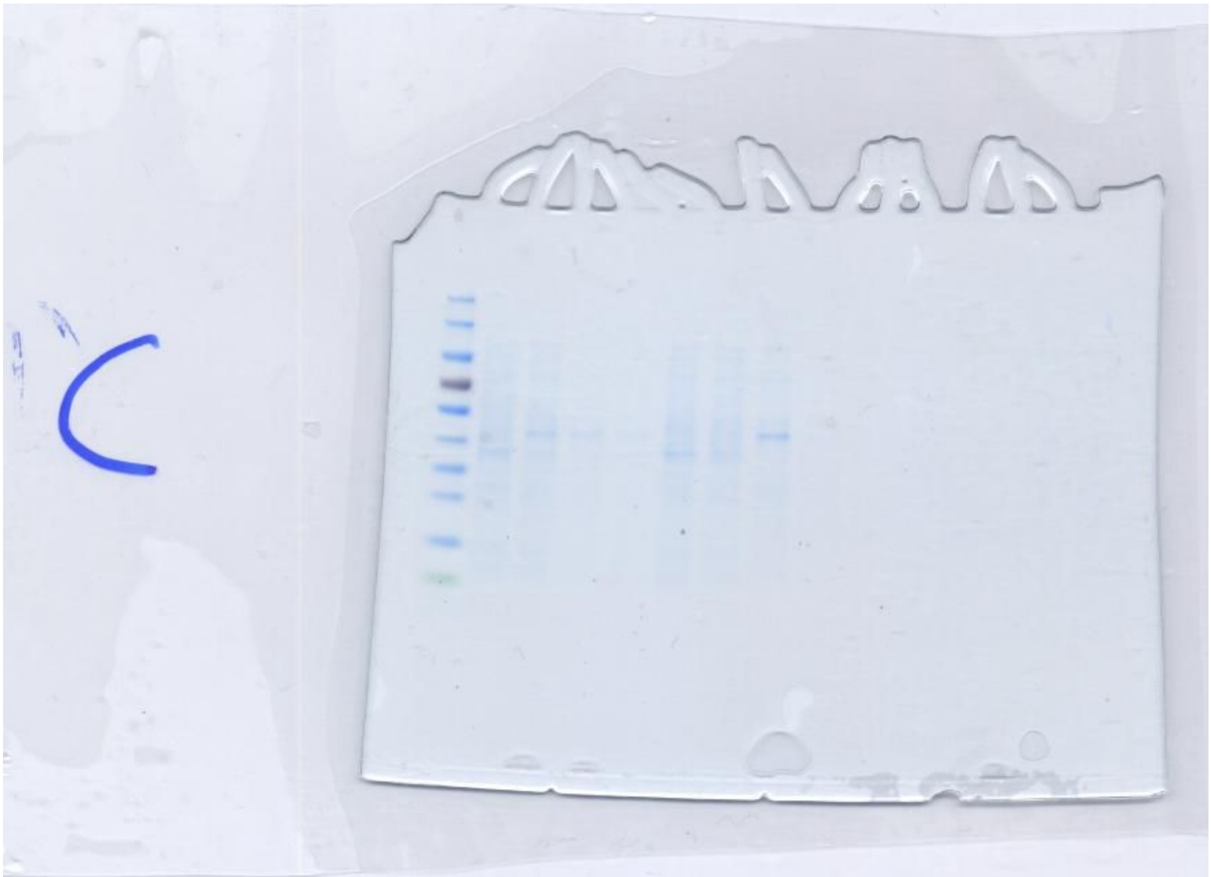
To visualize the expressed Endo-β-Galactosidase enzyme on a gel and confirm the size of the enzyme.

Procedure

The protocol for SDS-PAGE was used with the exception that 24 µl of sample was used with 6 µl of loading buffer. The gels were pre-casted gels from Biorad.

Results

B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