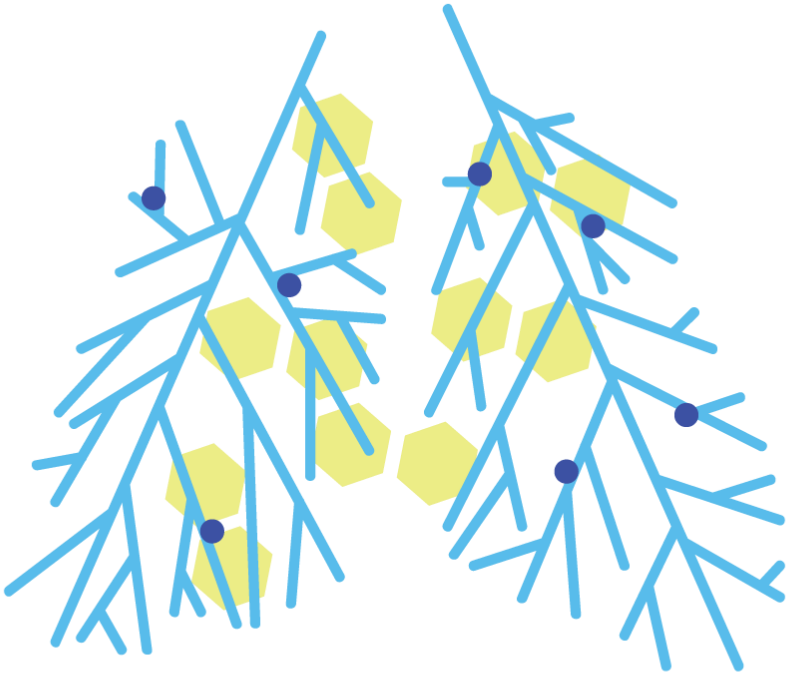


PROLUNG

DEGRADATION

EXPRESSION

LAB BOOK 7

iGEM Stockholm

Investigating the control of the expression of source Sialidase

Background

When expressing Sialidase from the source plasmid the negative control was contaminated. The theory was that the IMAC colon had been contaminated from the samples. The expression of the Sialidase from the source plasmid is done again to attempt to get a blank negative control.

Transformation of BL21(DE3) with source Sialidase

Aim

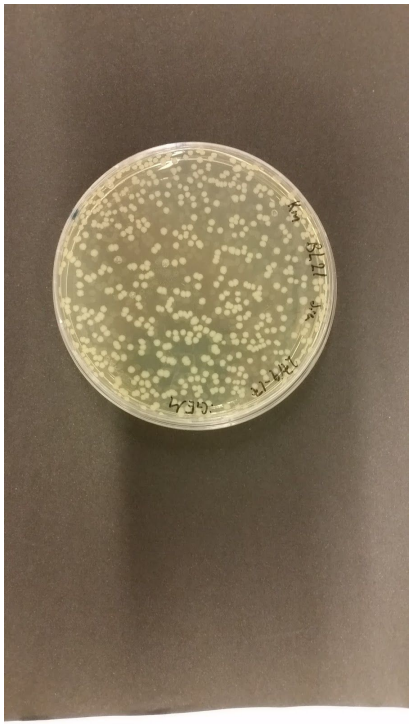
To transform BL21(DE3) cells with the source plasmid of Sialidase. The colonies will be use to cultivate and express Sialidase.

Procedure

The protocol for transformation was used with no modifications except 25 µl of bacteria used.

Results

Colonies grew on the agar plate.



Cultivation and expression of BL21(DE3) with source Sialidase

Aim

Cultivation of BL21(DE3) with source plasmid of Sialidase to use as positive and negative control. Also expression of Sialidase.

Procedure

Two flasks with 10 ml LB cultivated. Final concentration of 50 µl/ml of kanamycin added to both flasks.

Expression of the positive control with 0,5 mM IPTG at room temperature and overnight.

Sonication of BL21(DE3) with source Sialidase and IMAC purification

Aim

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

Procedure

Protocol for sonication and IMAC purification used without any modifications except that the colon was pulsed with imidazole with twice the colon volume than stated in the protocol to make sure the contaminants are washed away. The negative control was purified on the colon before the positive control.

Colon with cobalt matrix used with a colon volume of 1.2 ml.

SDS-PAGE of Sialidase expressed from source plasmid

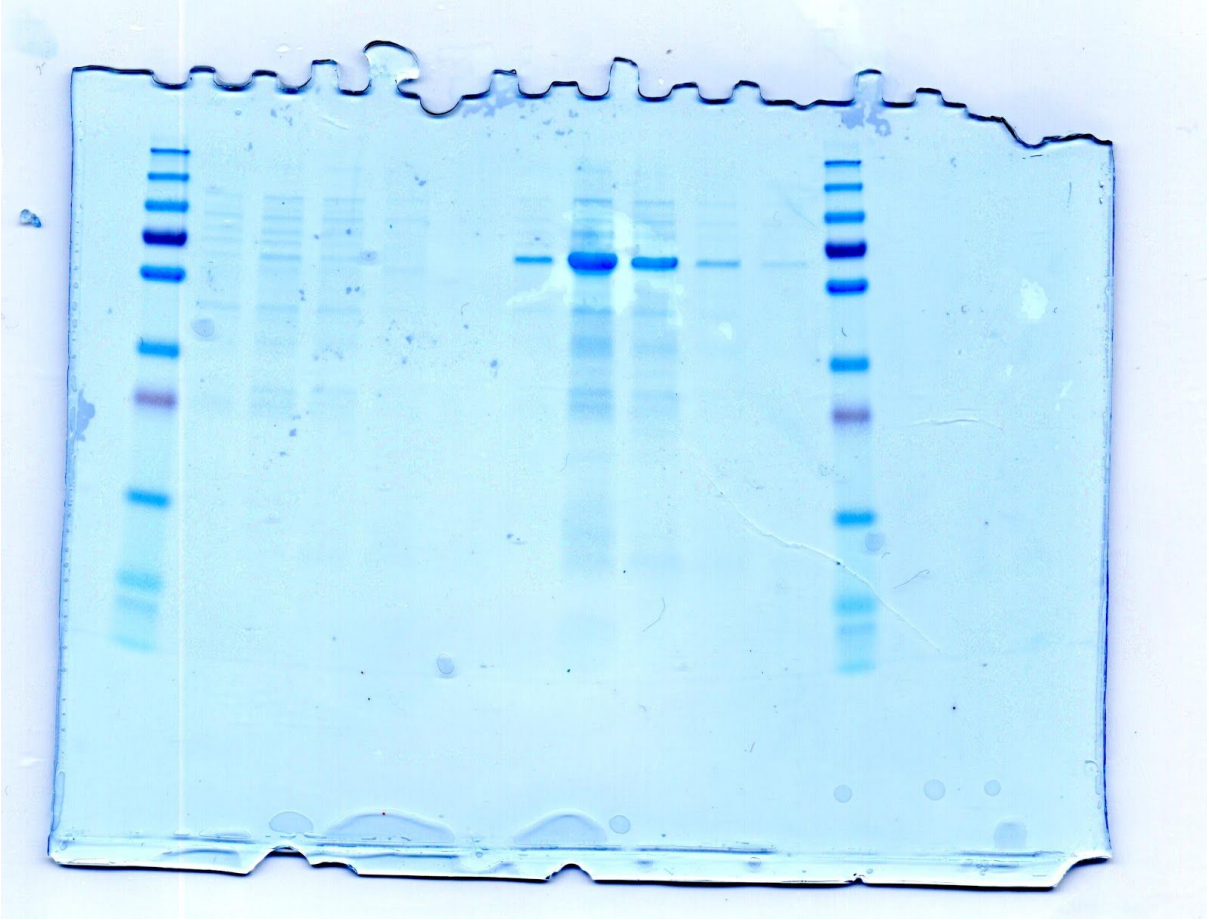
Aim

To visualize the positive and negative control of the expressed Sialidase from the plasmid source to control if the negative control is blank.

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



Cultivation and expression of BL21(DE3) with source Sialidase - Second attempt

Aim

Cultivation of BL21(DE3) with source plasmid of Sialidase to use as positive and negative control. Also expression of Sialidase.

Procedure

Two flasks with 10 ml LB cultivated. Final concentration of 50 µl/ml of kanamycin added to both flasks.

Expression of the positive control with 0.5 mM IPTG at room temperature and overnight.

Sonication of BL21(DE3) with source Sialidase and IMAC purification

Aim

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

Procedure

Protocol for sonication and IMAC purification used without any modifications. A completely new colon was packed to make sure it did not contain any contaminants. The negative control was purified on the colon before the positive control.

Colon with cobalt matrix used with a colon volume of 1.2 ml.