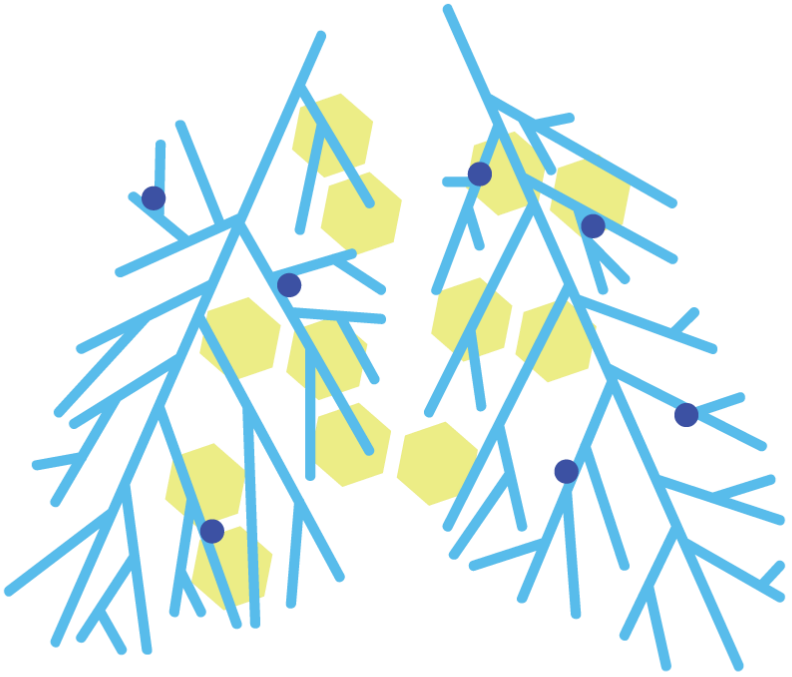

PROLUNG

DEGRADATION

EXPRESSION

LAB BOOK 9

iGEM Stockholm

Investigating the control of the expression of source Sialidase - Third attempt

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.

The two previous attempts to get a blank negative control failed. In the first attempt an old cobalt colon was used but pulsed with twice the volume of imidazole than stated in the protocol to make sure the contaminants were washed away. The negative control was still contaminated.

In the second try a completely new colon was packed and the experiment is repeated but the negative control was not only contaminated but it also seemed it had not been purified at all. The reason for this is unknown.

A third attempt will be made and a new colon will be packed again.

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. A new colon was packed and used. The negative control was purified on the colon before the positive control to make sure the colon would not contaminated the negative 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