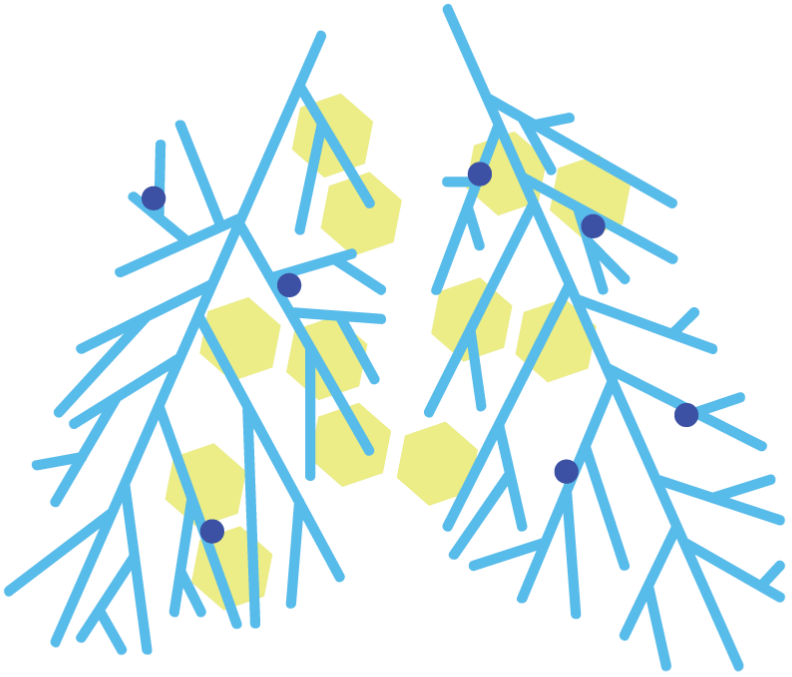


PROLUNG

DEGRADATION

EXPRESSION

LAB BOOK 3

iGEM Stockholm

Expression of enzymes from plasmids received after literature study

Background

Continued attempt to express enzymes from plasmids received from different research groups.

In the protocol we used for SDS-PAGE it was stated that a concentration of 2µg/µL is needed for SDS-PAGE which made our results from the Nanodrop seem very low. After discussions with an advisor we decided to continue with SDS-PAGE even with the low concentrations we had since 2µg/µL is a very high concentration and protein measurements with Nanodrop can't be trusted. At this point we had started to redo the whole process of protein expression with focus on only Sialidase (from transformation to SDS-PAGE) and added some of the new Sialidase samples (Sia A1-A3, Sia B1-B2 and Sia No) to the gels with the old samples.

As advised from supervisors we will only work with the BL21(DE3) strain going forward.

Protein expression

Aim
To grow the bacteria transformed with the plasmid containing Sialidase at three different OD values where each will be induced with three different concentrations of IPTG. See table below for the setup of this experiment.

Cultivation of BL21(DE3) with transformed Au54			
IPTG (mM)	**OD**		
0,1	0,6	0,8	1,0
0,5	0,6	0,8	1,0
1,0	0,6	0,8	1,0

Procedure
The protocol was used with no modifications.

The volume of six of the ten cultures were 10 mL and four of them had the volume of 25 mL. Induction was made over night in room temperature.

Results
The table shows the relationship of how much each culture was cultivated and with what concentration each culture was induced.

E-flask number	Volume (mL)	OD	IPTG (mM)	Induced
A1	10	0,6	0,5	2017-07-10
A2	10	0,4	1	2017-07-10
A3	10	0,4	0,5	2017-07-10
B1	10	0,6	1	2017-07-10
B2	10	0,2	1	2017-07-10
B3	25	0,4	0,1	2017-07-11
C1	25	1,2	0,1	2017-07-11
C2	25	0,1	0,1	2017-07-11
C3	25	0,3	0,5	2017-07-11
Control	10	1,0	-	-

Sonication

Aim
To lyse the cells used for the expression of Sialidase to get access to the enzyme for purification with IMAC.

Procedure
The protocol was used with no modifications. Only samples A1-A3, B1-B2 and the control were sonicated since the rest of the cultures had not reached the aimed OD value. Before sonication of these samples the supernatant was removed to be tested on SDS-PAGE with the old samples as a test to see if enzymes are secreted by the bacteria.

SDS-PAGE and staining

Aim
To visualize the expressed enzymes and make sure the correct protein has been expressed by comparing the size to the ladder on the SDS-PAGE gel.

Procedure

The protocol for SDS-PAGE used with some modifications.

1. Protein samples and loading buffer were mixed at a ratio of 24 µL of protein sample and 6 µL of loading buffer in a fume hood. Loading buffer was prepared by staff at floor 2.
2. A pre-casted gel was used (Mini-Protean TGX from BIORAD).

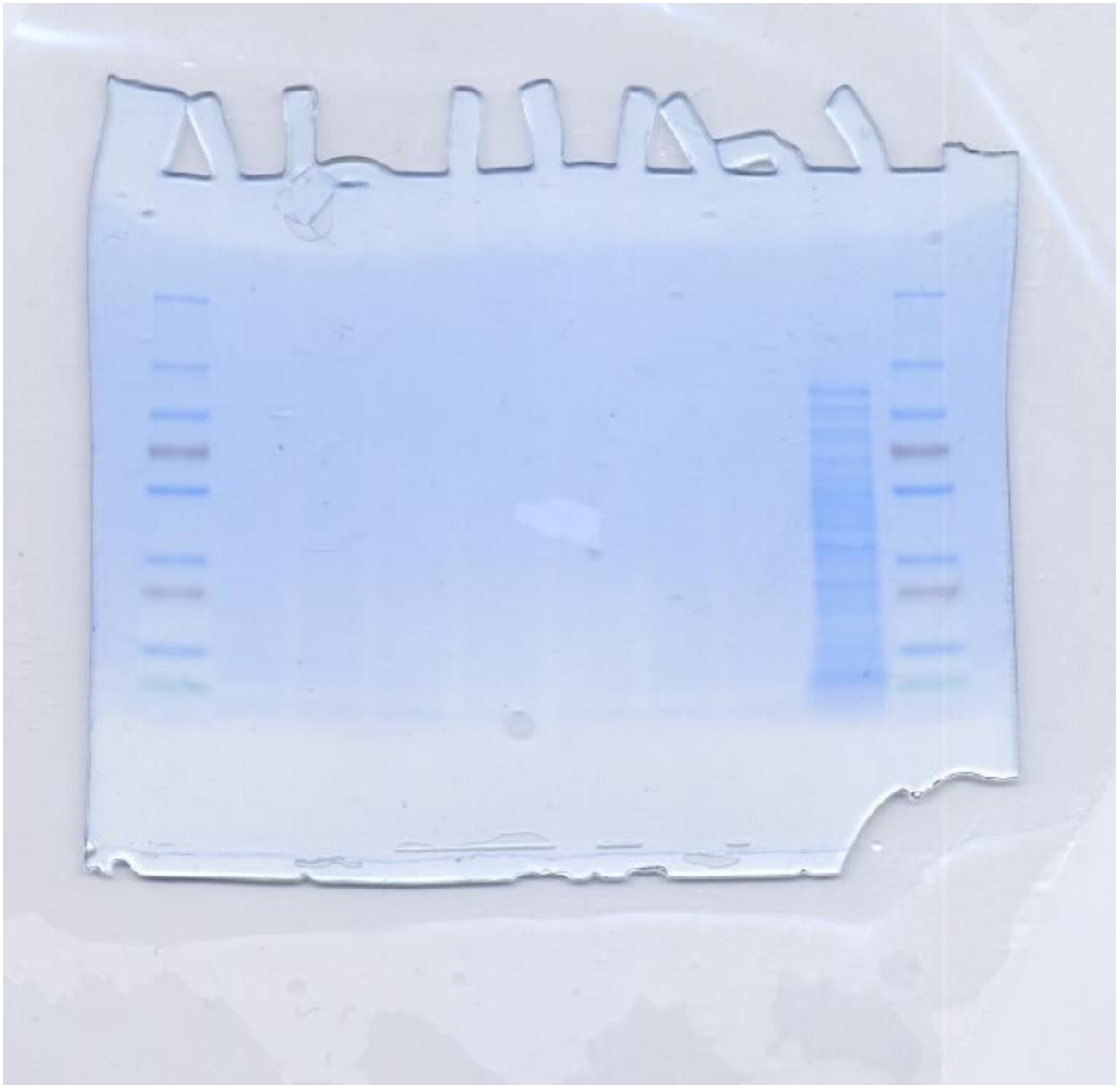
The samples that were run on SDS-PAGE were the first attempt to express Sialidase, Endo-β-Galatosidase, Metalloprotease and the two different Sulfatases. In addition the supernatant of the new attempt to express Sialidase (A1-A3, B1-B2 and the control) was also run on this SDS-PAGE gel.

The gels were stained using the protocol for SimplyBlue SafeStain.

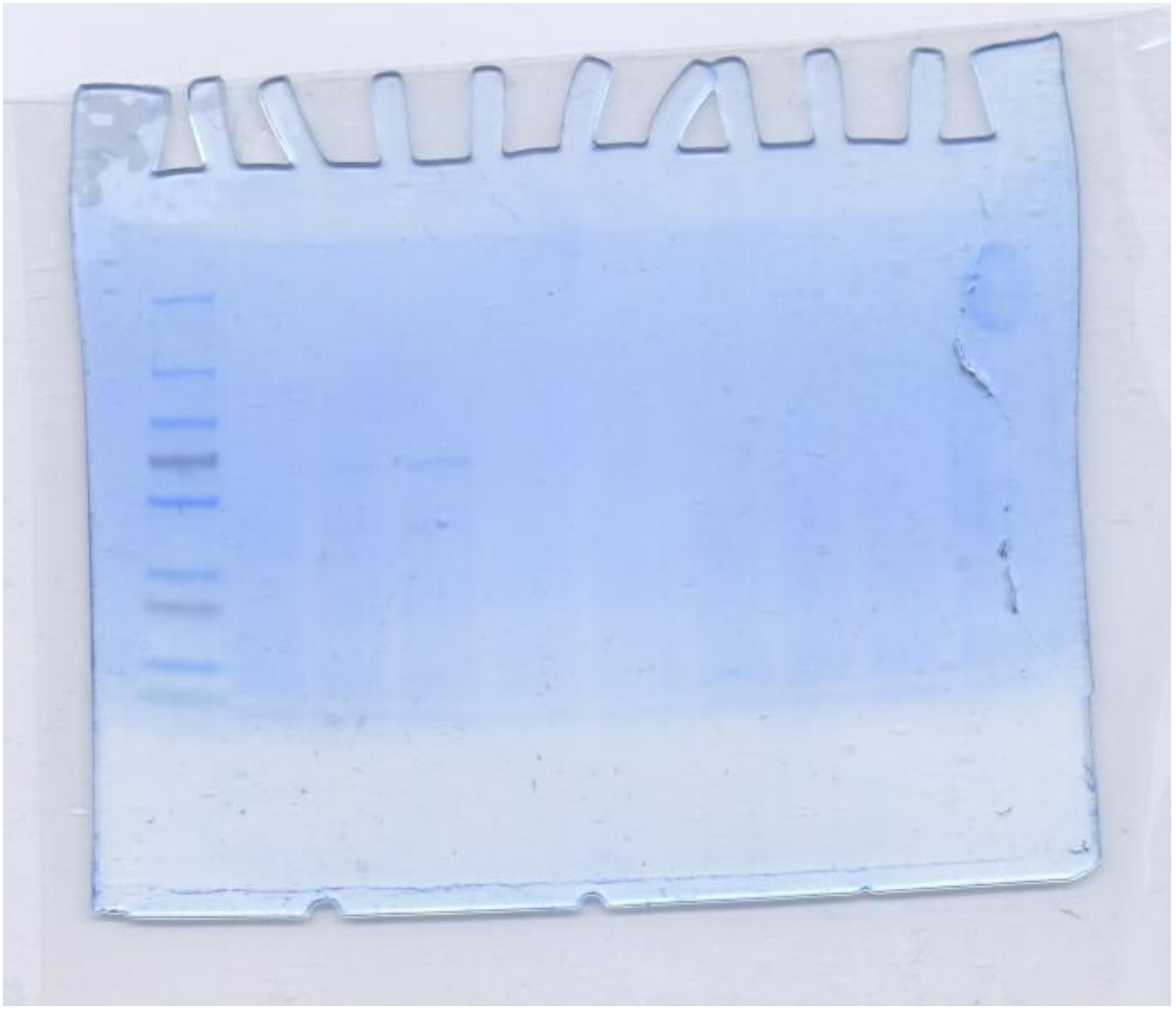
Results

The samples were organized in the wells of the gels as follows. The results of the SDS-PAGE can be seen in images below.

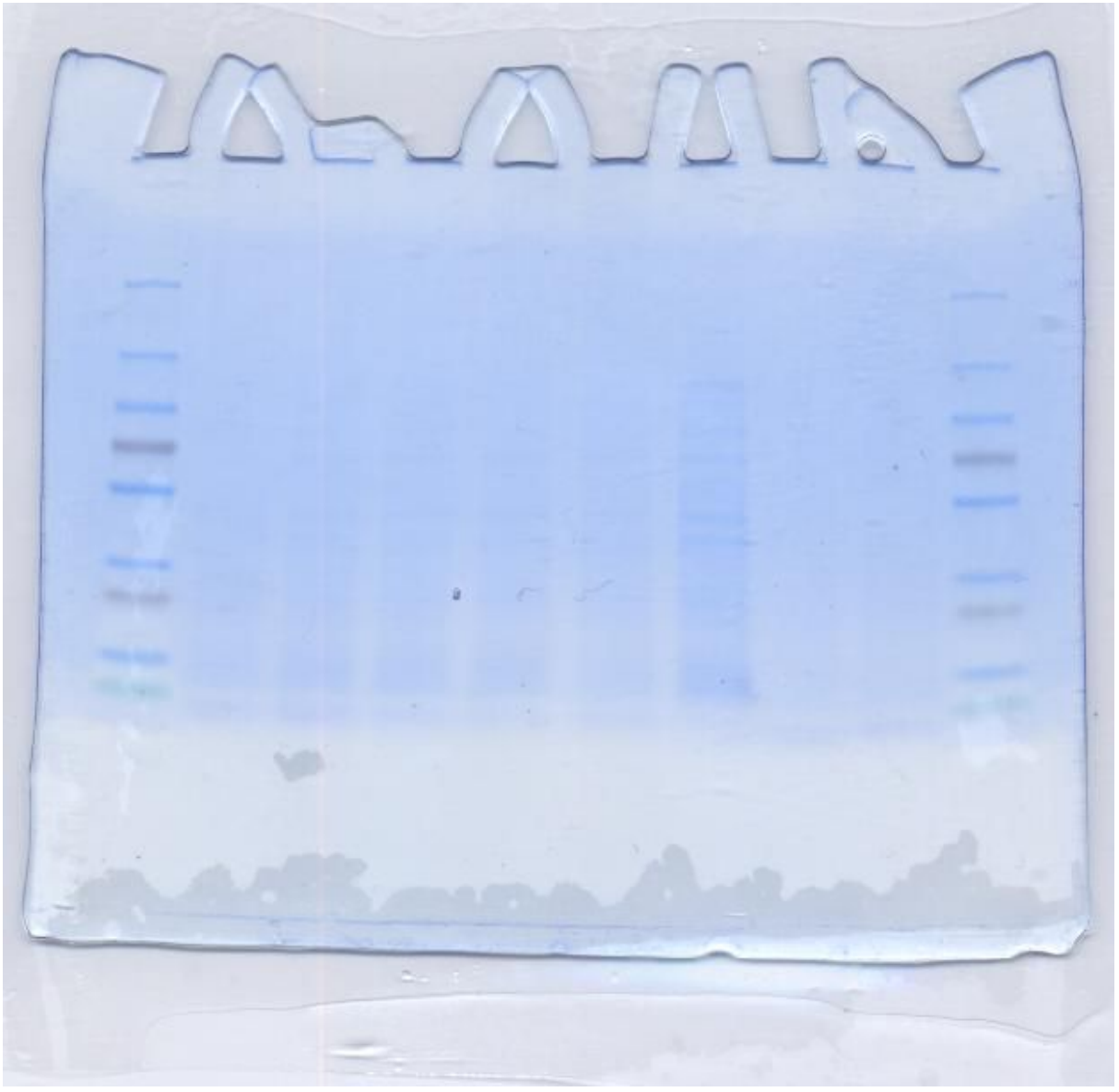
	1	2	3	4	5	6	7	8	9	10
Gel A	L	S:1	S:2	S:3	S:4	S:5	Su1: 1	Su1: 2	EBG	L
Gel B	L	Su4: 1	Su4: 2	Su4: 3	Su4: 4	Su4: 5	M1	M2	M3	M4
Gel C	L	M5		Sia No	Sia A1	Sia A2	Sia A3	Sia B1	Sia B2	L



Gel A.



Gel B.



Gel C.

IMAC purification

Aim

To purify the Sialidase samples A1-A3, B1-B2 and the control based on the containing Histag.

Procedure

The protocol was used with no changes. The colons used for the IMAC purification was one nickel colon with colon volume of 3,2 mL and three cobalt colons, each with colon volumes of 1,2 mL. The protein samples was eluted to five fractions each.

Sonication

Aim
To lyse the cells used for the expression of Sialidase to get access to the enzyme for purification with IMAC.

Procedure
The protocol was used with no modifications. The rest of the samples (B3, C1-C3) in the second attempt to express Sialidase were sonicated.

IMAC purification

Aim
To purify the Sialidase samples B3, C1-C3 and the control based on the containing Histag.

Procedure
The protocol was used with no changes. The colons used for the IMAC purification was one nickel colon with colon volume of 3,2 mL and three cobalt colons, each with colon volumes of 1,2 mL. The protein samples was eluted to five fractions each.

SDS-PAGE and staining

Aim
To visualize the expressed Sialidase samples and make sure the correct protein has been expressed by comparing the size to the ladder on the SDS-PAGE gel.

Procedure
The protocol for SDS-PAGE used with some modifications.

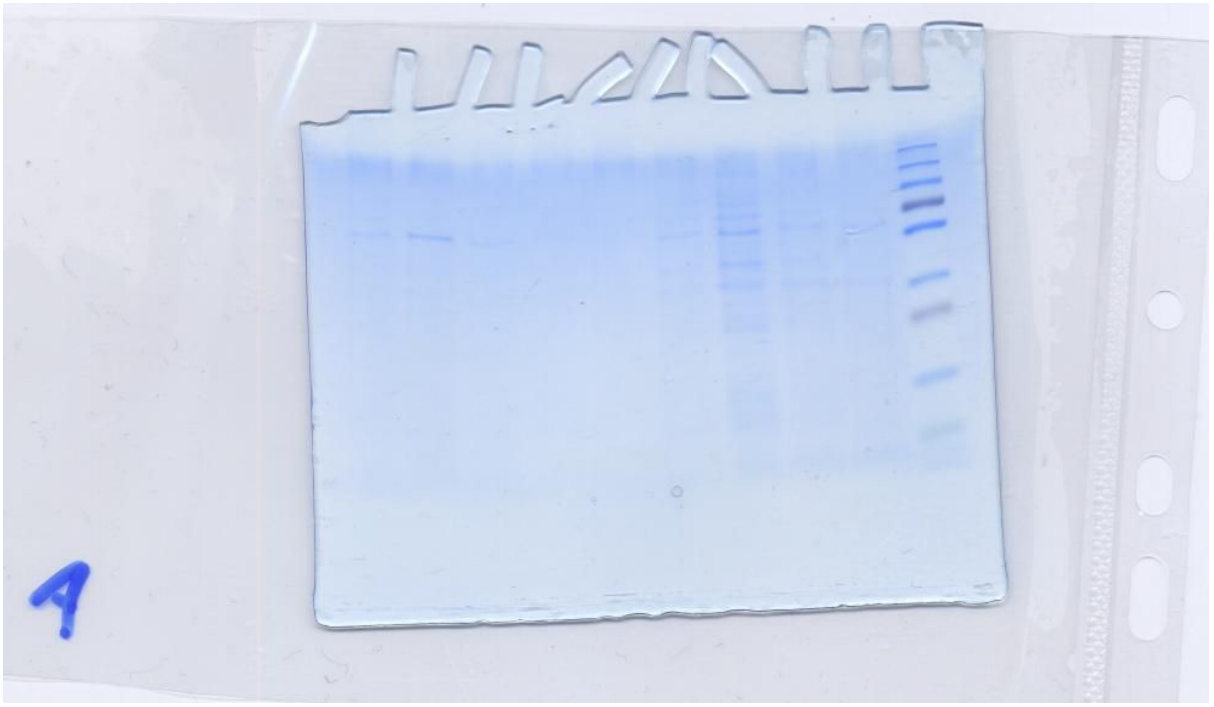
3. Protein samples and loading buffer were mixed at a ratio of 24 µL of protein sample and 6 µL of loading buffer in a fume hood. Loading buffer was prepared by staff at floor 2.
4. A pre-casted gel was used (Mini-Protean TGX from BIORAD).

The samples that were run on SDS-PAGE were the IMAC purified A1-A3, B1-B2 and C1-C3. B3 and the control were not run because lack of space of the gels. These samples were run later on SDS-PAGE gel.

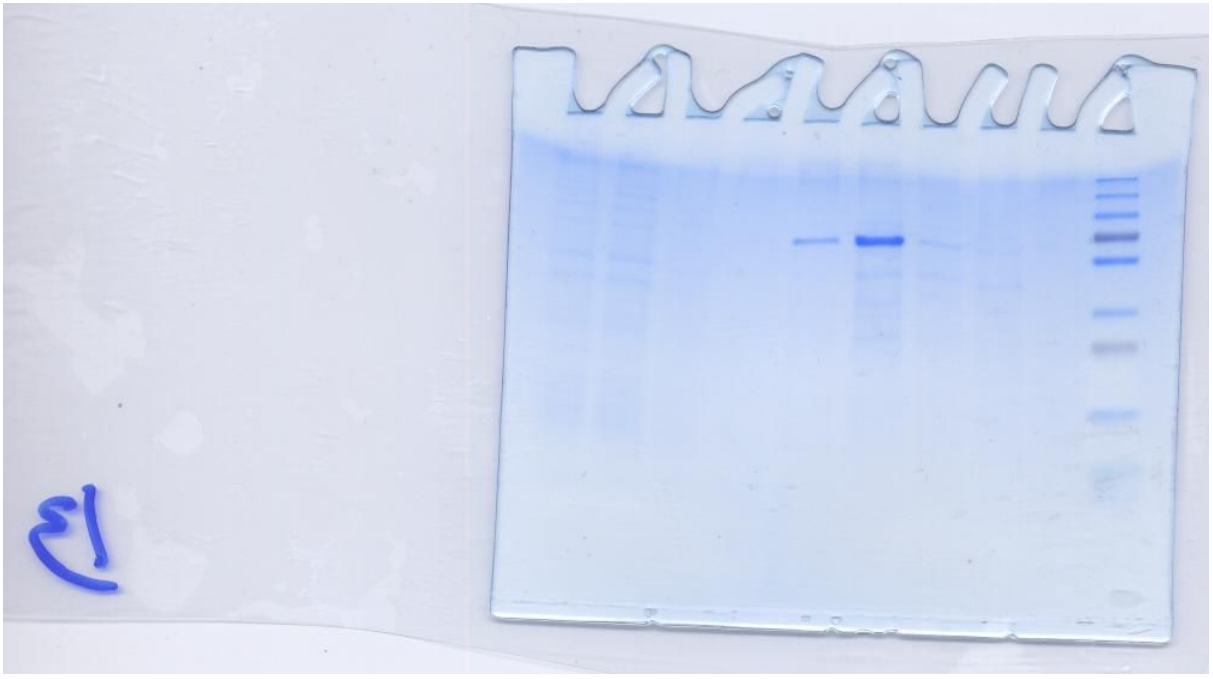
The gels were stained using the protocol for SimplyBlue SafeStain.

Results

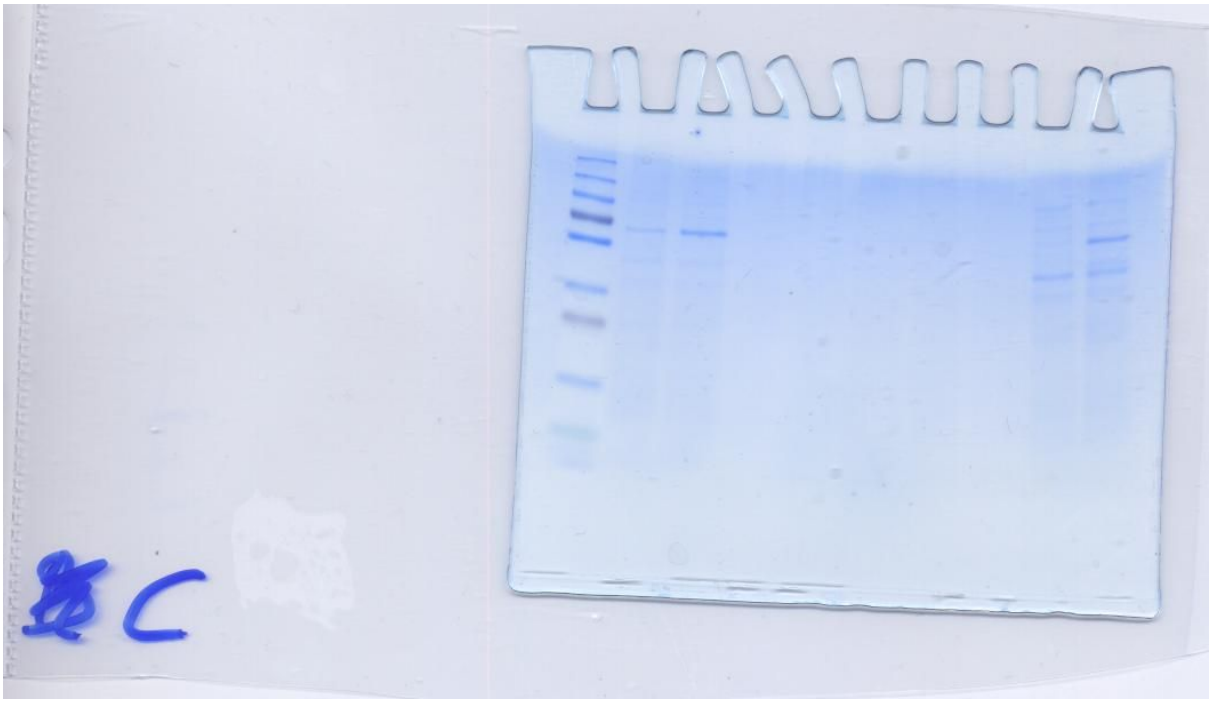
Gel A									
1	2	3	4	5	6	7	8	9	10
Ladder	A1:1	A1:2	A1:3	A1:4	A1:5	A2:1	A2:2	A2:3	A2:4



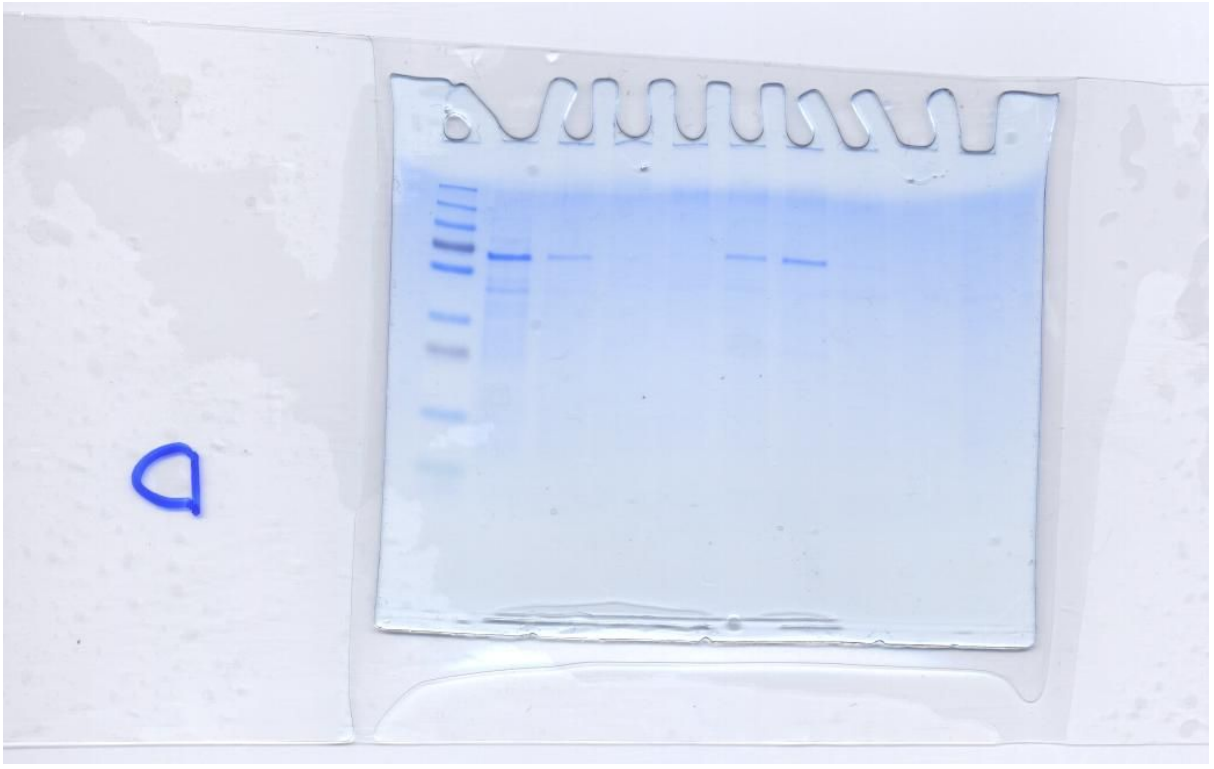
Gel B									
1	2	3	4	5	6	7	8	9	10
Ladder	A2:5	A3:1	A3:2	A3:3	A3:4	A3:5	B1:1	B1:2	B1:3



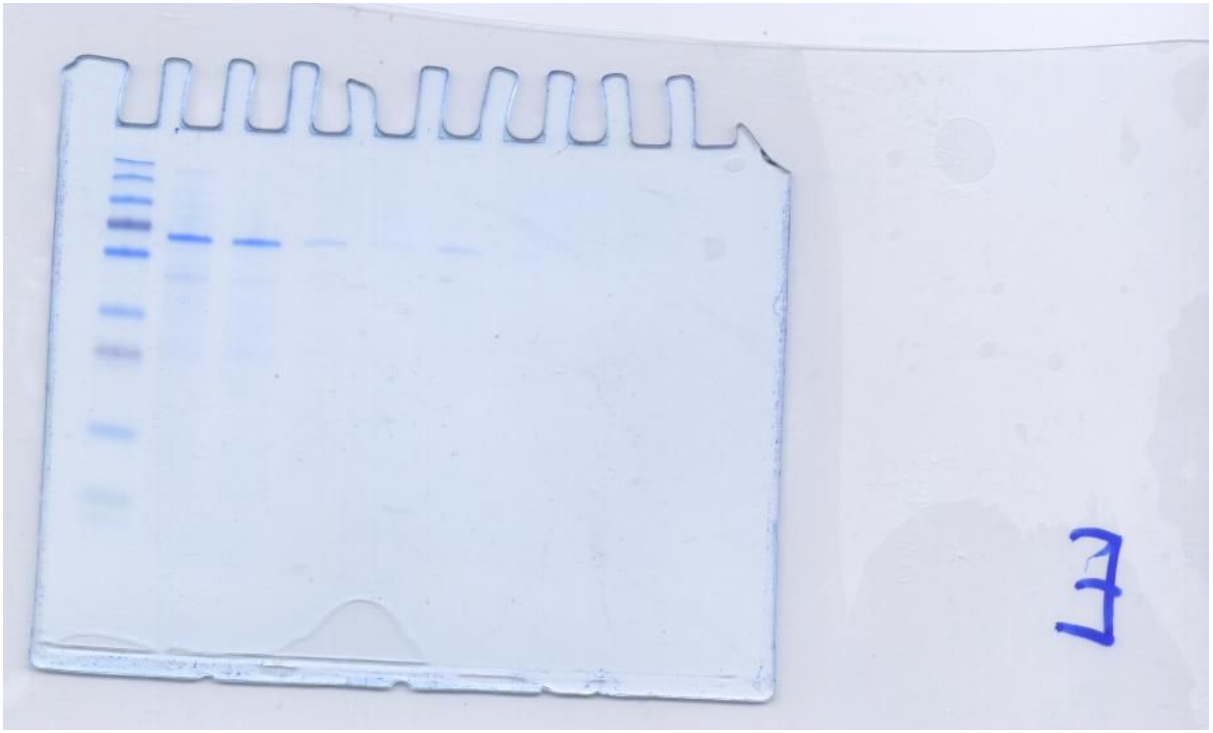
Gel C									
1	2	3	4	5	6	7	8	9	10
Ladder	B1:4	B1:5	B2:1	B2:2	B2:3	B2:4	B2:5	C1:1	C1:2



Gel D									
1	2	3	4	5	6	7	8	9	10
Ladder	C1:3	C1:4	C1:5	C2:1	C2:2	C2:3	C2:4	C2:5	C3:1



Gel E									
1	2	3	4	5	6	7	8	9	10
Ladder	C3:2	C3:3	Bad sample	Bad sample	C3:4	C3:5			



Assembly of Sialidase in gBlock with backbone

Aim
To put the gBlock with Sialidase, prefix and suffix into a backbone.

Procedure
Digestion
The backbone pSB1C3 provided in the iGEM kit and the gBlock containing Sialidase were digested using the following digestion mastermix.

Digestion mastermix for five runs
2 µl Tango Buffer
1.1 µl EcoRI
1.1 µl PstI
15.8 µl H_2O

A total of 4 µl of the mastermix was mixed with a total of 100 ng of DNA and incubated at 37 °C for 60 minutes. Heat kill was performed at 80 °C for 20 minutes.

Ligation
A service was used to calculate how much vector DNA should be added to insert DNA for a ratio of 1:3 moles.

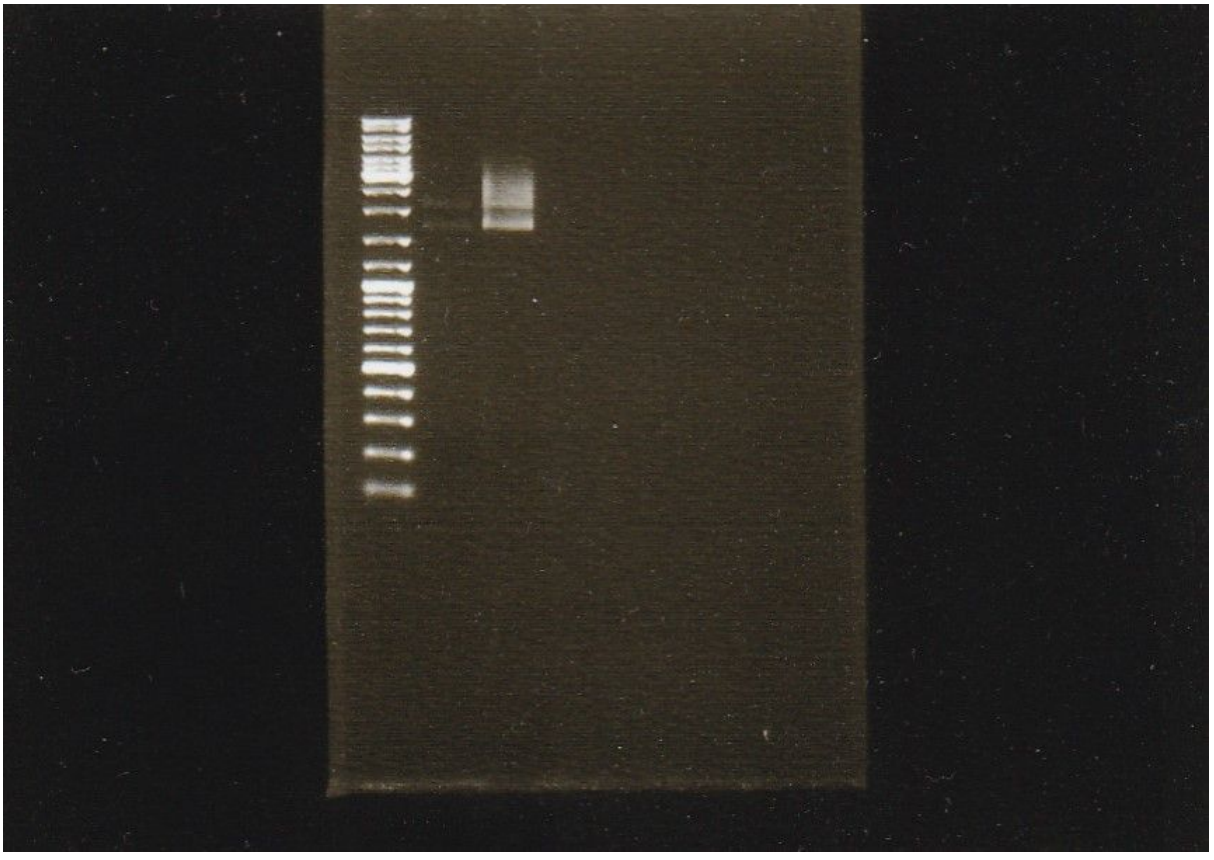
Plasmid backbone
2070 bp
25 ng

Sialidase gBlock sequence
1673 bp
60,6 ng

The backbone was mixed with 1 µl of T4 DNA ligase buffer and 0.5 µl T4 DNA ligase. Water was added to 10 µl. The mix was incubated at room temperature for 1 hour and heat killed at 65 °C for 10 minutes.

The ligated product of the gBlock and pSB1C3 was digested with EcoRI and PstI and visualized on a gel to make sure the ligation had succeeded.

Results
Both lanes contain digestion of the ligated product.



Transformation of ligated product

Aim

To transform Top10 and BL21(DE3) cells with the ligated product of Sialidase and pSB1C3 backbone. The Top10 cells to be used to make stocks of the plasmid and the BL21(DE3) to express the enzyme.

Procedure

The protocol for transformation was used with no modifications except 25 µl of bacteria used instead of 50 µl.

Results

Successful transformations.