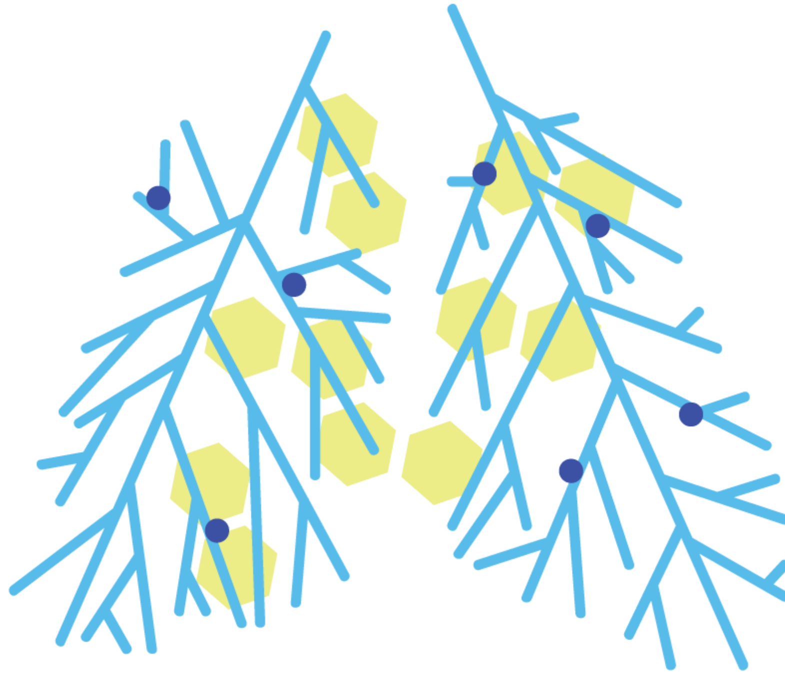


# PROLUNG

# *DEGRADATION*

## SECRETION

## LAB BOOK 3

iGEM Stockholm

# Digestion of Sialidase gBlock and digests secretion system device(BBa_K1166002)

### Background

The team designed the gBlock for the sialidase enzyme. It includes prefix, histag, insert, stop codon and suffix.

### Aim

Digest the Sialidase gBlock by cutting the restriction sites XbaI and SpeI. Digest the plasmid backbone EcoR1 and XbaI. This step is to prepare for the further experiment to assembly sialidase gblock into the secretion device(BBa_K1166002).

### Procedure

Protocols for PCR purification, digestion, gel electrophoresis and nanodrop were used with no modification.

# Assembly of sialidase gBlock w/o stop codons with secretion device

### Aim

The gBlock contains sialidase w/o stop codons with a histag and has to be inserted into pSB1C3 before it can be expressed.

### Procedure

DNA Ligation Sialidase insert w/o stop codons + Secretion system biobrick plasmid

Protocol (DNA molar ratio: Secretion device vs sialidase = 1:1)
Set up the following reaction in a microcentrifuge tube on ice. (T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert.)

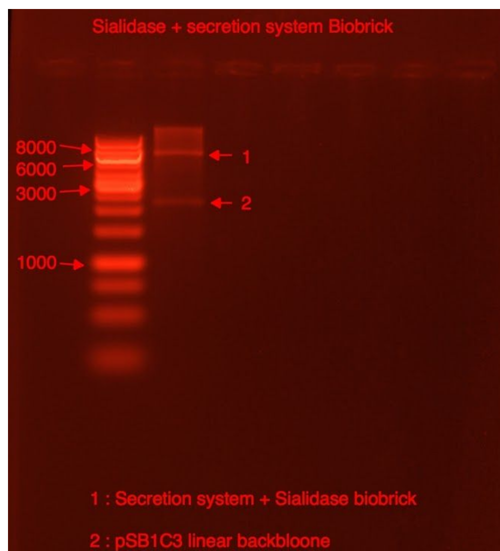
| COMPONENT | 20 µl REACTION |
| --- | --- |
| 10X T4 DNA Ligase Buffer* | 2 µl |
| Vector DNA (25.4ng/ul) | 25 ng (1ul) |
| Insert DNA (10ng/ul) | 25 ng (2.5ul) |
| T4 DNA Ligase | 1 µl |
| Nuclease-free water | 13.5 µl |

1. The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.
2. Gently mix the reaction by pipetting up and down, and microfuge briefly.
3. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
4. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours (*alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation*).
5. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

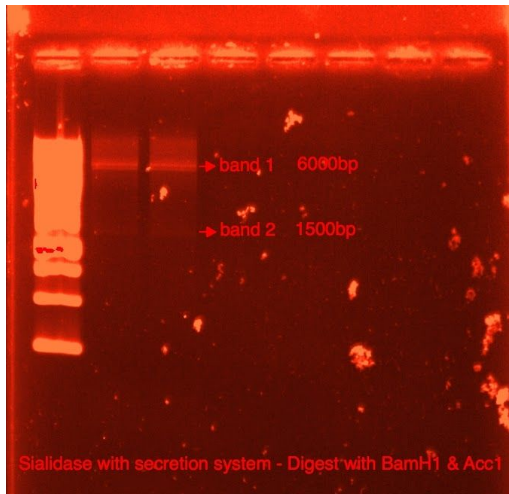
**Protocol (DNA molar ratio: Secretion device vs sialidase = 1:3)**
Set up the following reaction in a microcentrifuge tube on ice. (T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert.) Ligation steps are the same as above.

| COMPONENT | 20 µl REACTION |
| --- | --- |
| 10X T4 DNA Ligase Buffer* | 2 µl |
| Vector DNA (25.4ng/ul) | 25 ng (1ul) |
| Insert DNA (10ng/ul) | 75 ng (7.5ul) |
| T4 DNA Ligase | 1 µl |
| Nuclease-free water | 8.5 µl |

## Results



Digestion of DNA ligate



Digestion of DNA ligate on unique restriction sites from secretion system device

### Discussion

We successfully ligated secretion device with sialidase. Sample 0, 1 and 2 were all confirming the correct size of DNA on gel.

# Transformation of sialidase gBlock w/o stop codons with secretion device

### Aim

To transform bacteria with the plasmids in Top10 cells. The goal is to express and confirm if the ligation was succeeded.

### Procedure

Protocols for cultivation and transformation were used with no modification.

### Results

Sialidase cloning into secretion system plasmid vector without any sialidase DNA insert or with sialidase DNA insert from E. coli (T4 Ligase) at 22 °C .

A: Transformation with sialidase DNA insert,

B: Transformation with sialidase DNA insert,

C: Transformation without sialidase DNA insert,

D: Transformation with existing biobrick insert GFP.