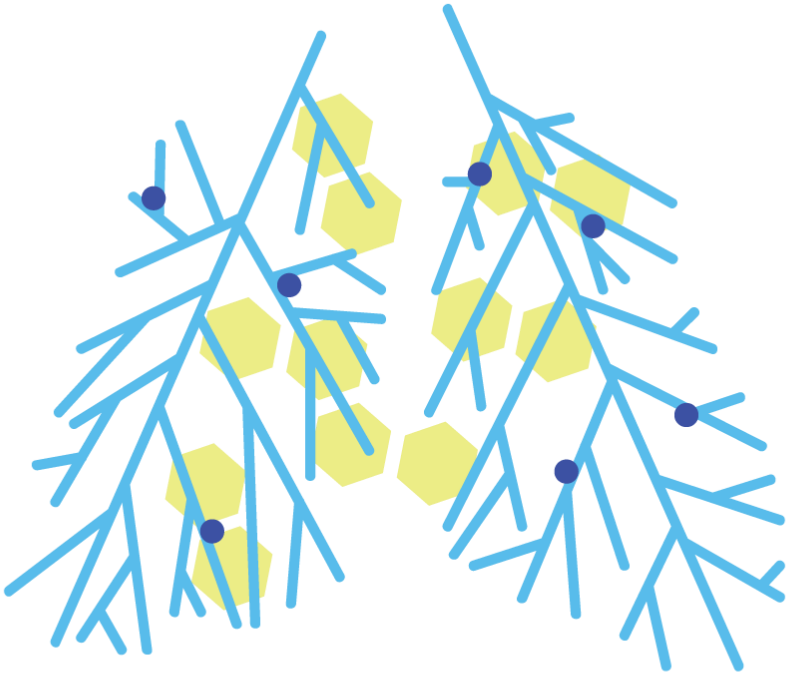


PROLUNG

DEGRADATION

SECRETION

LAB BOOK 2

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 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, gel electrophoresis and nanodrop were used with no modification.

Sialidase gBlock Digestion

Add components to a clean tube in the order shown:

1. 4 µL sialidase gblock DNA (concentration 244.3 ng/µL)
2. 2 µL 10x buffer
3. 1 µL XbaI
4. 1 µL SpeI
5. 12 µL sterile water
6. Incubate the reaction at digestion temperature (usually 37°C) for 1 hour.
7. Stop the digestion by heat inactivation (65°C for 15 minutes)
8. PCR purification

Secretion System Digestion

Add components to a clean tube in the order shown:

1. 14 µL Backbone DNA (concentration 69.6 ng/µL)
2. 2 µL 10x buffer
3. 1 µL XbaI
4. 2 µL sterile water
5. Incubate the reaction at digestion temperature (usually 37°C) for 1 hour.
6. Stop the digestion by heat inactivation (65°C for 15 minutes)

7. PCR purification

PCR purification
Device vs Insert= 1:3
Conc. Sialidase insert= 5.2ng/ul, 16 ul, 84 ng
Conc. Secretion device = 70 ng/ul, 0.4ul, 28 ng
18.4µl+1ul+1ul

Device vs Insert=1:1
Conc. Sialidase insert= 5.2ng/ul, 4 µl
Conc. Secretion device = 70 ng/ul, 0.3 ul, 21ng
6.3ul+ 1ul+12.7ul

Assembly of Sialidase gBlock with secretion system device(BBa_K1166002)

Aim

The gBlock contains sialidase with a Histag and has to be inserted into secretion device before the sialidase can be secreted into supernatant.

Ligation

1. Set up the following reaction in a microcentrifuge tube on ice.
2. *(*T4 DNA Ligase should be added last. *Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.)* Use NEBioCalculator to calculate molar ratios.

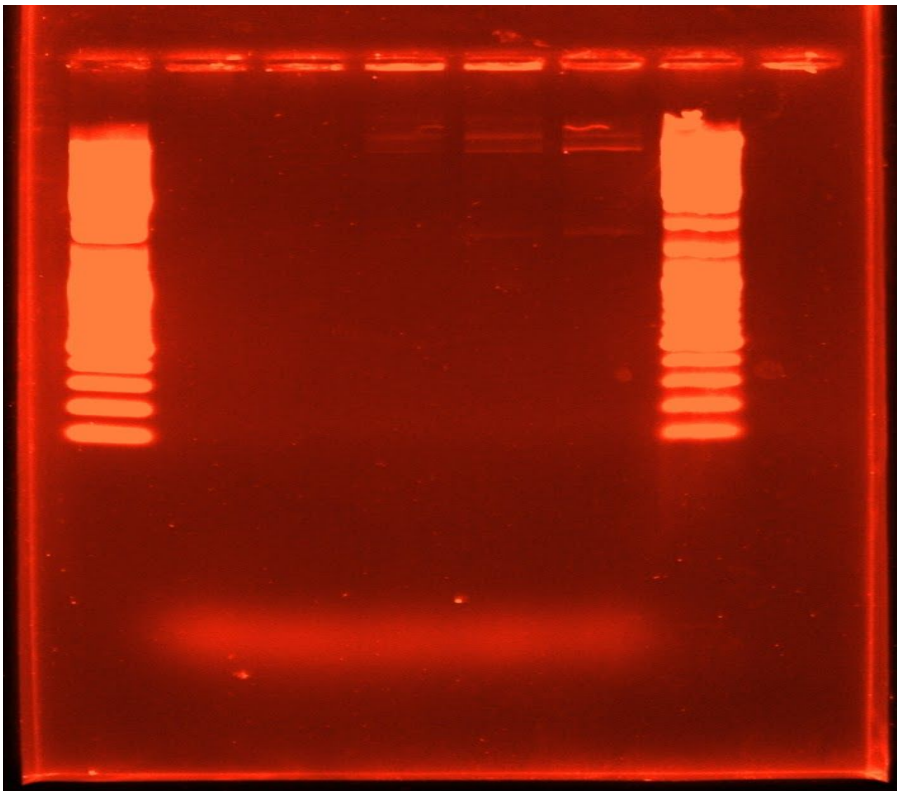
COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
Secretion system Vector DNA (4 kb)	1ul
Sialidase Insert DNA (1 kb)	3ul

Nuclease-free water	to 20 µl
T4 DNA Ligase	1 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. Heat inactivate at 65°C for 10 minutes.
6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

Results

The ligation was unsuccessful. Treated sample were all negative. In the negative control, the digested secretion system plasmid was ligated with undigested sialidase gBlock PCR product. The amount of secretion system plasmid was 50 ng in each sample. And the amount of sialidase insert was 50ng/ 150ng/ 500ng, in ratio 1:1, 1:3, 1:10.



M 1:1 1:3 N: 1:1 N:1:3 N: 1:10 M

Secretion system plasmid : Sialidase = 1:1, 1:3

N: Negative control

Discussion

Digest only one restriction site in the secretion device is improper for assembling gblock into the device. We suggest digest two restriction sites in the secretion device for the next attempt.