Lysostaphin

Week 3

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

To obtain a construct that could be transcribed into a fully functional protein, a promoter must be placed prior to the lysostaphin coding sequence. To accomplish this, we have chosen to place a T7 promoter by means of 3A assembly with pSB1K3 as the new backbone.

1 Digestion of Lys, T7 and Kanamycin Backbone pSB1K3 for 3A assembly (Trial 1)

Responsible

Aman Mebrahtu, Oscar Frisell, Sigrun Stulz, Bethel Tesfai Embaie

Protocols used

- 3A Assembly
- Gel Purification

Experimental Set Up

All three components consisting of the T7 promoter (BBa K525998), the trucated lysostaphin (BBa K748002) and the kanamycin backbone (pSB1K3) were digested with the correct restriction enzymes, then ligated to form a 3A assembled construct of pSB1K3-T7-Lys.

Table 1: Concentrations of the samples used for digestion.

Sample	Concentration [ng/μl]
T7 (2)	93.2
Lys (1)	89.1
pSB1K3	25.0

Table 2: Digestions performed on each sample.

Sample	Digestions
T7 (2)	Digested with EcoRI and SpeI
Lys (1)	Digested with XbaI and PstI
pSB1K3	Digested with EcoRI and PstI

To minimize pipetting errors, we decided to make master mixes for all enzymes XbaI, PstI, EcoRI and SpeI. The composition of the master mixes are the same for all and it is stated in the table below.

Table 3: Composition of Enzyme Master Mixes

Sample	Volume [μl]
10X Cutsmart Buffer	0.8
Sterilized water	6.8
Enzyme	0.4

The following tables show the reaction mix for all digestions with the appropriate enzyme mixes.

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (1)$$

Table 4: Composition of single digestion reaction

Sample	Volume [μl]
DNA sample	100ng (according to calculation)
Enzyme MasterMix	2
10x Cutsmart Buffer	0.6
Sterilized water	up to 8μl

Table 5: Composition of double digestion reaction

Sample	Volume [μl]
DNA sample	100ng (according to calculation)
Enzyme MasterMix 1	2
Enzyme MasterMix 2	2
10x Cutsmart Buffer	0.4
Sterilized water	up to 8μl

Results and Conclusions

Unsuccessful digestion of Lysostaphin as seen in the gel below with bands of 1000-1200 bp size for all Lys samples.
Backbone pSB1K3 migrates at right distance according to its size. Gel purification of digested product was therefore performed resulting in a concentration of 12.8 ng/μl.
T7 PCR product was purified resulting in a concentration of 35.8 ng/μl.

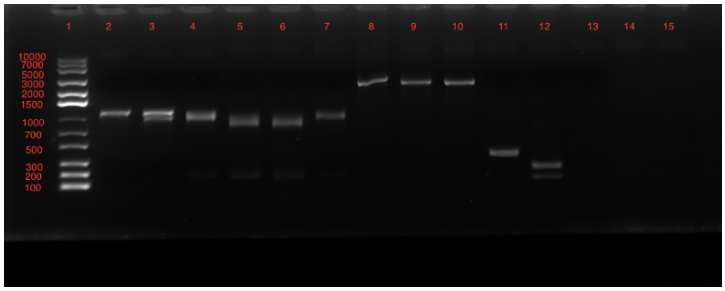

Figure 1: Gel of digested products.

Table 6: Gel labelling

Well	Label
1	DNA ladder
2	Lys negative control
3	Lys single digestion (XbaI)
4	Lys single digestion (PstI)
5	Lys double digestion
6	Lys double digestion
7	Lys double digestion
8	pSB1K3 single digestion (EcoRI and PstI)
9	pSB1K3 double digestion
10	pSB1K3 double digestion
11	T7 negative control
12	T7 double digestion (EcoRI and SpeI)

Discussion and Troubleshooting

Lysostaphin was unsuccessfully digested, which may have been due to poor sample handling or pippetting error with enzyme master mix that was prepared. The digestion was repeated in the following experiment but care was taken with reaction mix, once the enzymes were added.
Successful digestion and gel purification of pSB1K3 which is ready for use in the 3A assembly ligation step.
Further T7 digestion needed to be performed for ligation.

2 Digestion of Lysostaphin (Trial 2)

Responsible

Aman Mebrahtu, Oscar Frisell, Sigrun Stulz, Bethel Tesfai Embaie

Protocols used

- 3A Assembly

Experimental Set up

T7 promoter and Lysostaphin insert were digested for the 3A assembled construct: pSB1K3-T7-Lys.

Table 7: Concentrations of the samples used for digestion.

Sample	Concentration [ng/μl]
T7 (2)	35.8
Lys (1)	107.0

Table 8: Digestions performed on each sample.

Sample	Digestions
T7 (2)	Digested with EcoRI and SpeI
Lys (1)	Digested with XbaI and PstI

Master mixes were prepared for all enzymes XbaI, PstI, EcoRI and SpeI. The composition of the master mixes are the same for all and it is stated in the table below.

Table 9: Composition of Enzyme Master Mixes

Sample	Volume [μl]
10X Cutsmart Buffer	0.8
Sterilized water	6.8
Enzyme	0.4

The following tables show the reaction mix for all digestions with the appropriate enzyme mixes.

Table 10: Composition of single digestion reaction

Sample	Volume [μl]
DNA sample	100ng (according to calculation)
Enzyme MasterMix	2
10x Cutsmart Buffer	0.6
Sterilized water	up to 8μl

Table 11: Composition of double digestion reaction

Sample	Volume [μl]
DNA sample	100ng (according to calculation)
Enzyme MasterMix 1	2
Enzyme MasterMix 2	2
10x Cutsmart Buffer	0.4
Sterilized water	up to 8μl

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \tag{2}$$

Results and Conclusions

Gel 1: ran for too long; lost digested Lysostaphin.
Gel 2: repeated gel with remaining T7 digestion products as shown in the figure below.

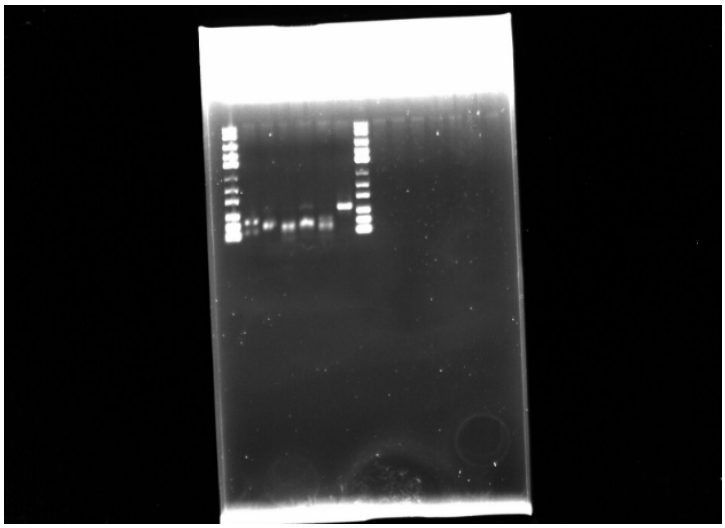

Figure 2: Gel 2 of remaining T7 digested product.

Table 12: Gel labelling

Well	**Label**
1	DNA ladder
2	T7 single digestion (EcoRI)
3	T7 single digestion (SpeI)
4	T7 double digestion
5	T7 double digestion
6	T7 double digestion
7	T7 negative control
8	Ladder

Discussion and Troubleshooting

The Lysostaphin digested product was lost in the gel that ran for too long, so lysostaphin needs to be re-digested.
T7 was successfully digested as seen in the single digestions and the similar sized bands of double digestion. It can therefore be used for ligation in 3A assembly.