Esp

Week 1

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

1 Colony Picking and PCR Amplification of PSB1K3-EB

Responsible

Oscar Frisell, Betty Tesfai, Sigrun Stulz, Shuangjia Xue, Aman Mebrathu, Reskandi Rudjito

Protocols used

Colony Picking
PCR Protocol
Glycerol Stocks

Modifications and comments to protocols

PCR Protocol: 18 μl of both forward and reverse primers were added since they were at a concentration of 10 μM.

Experimental Set Up

Description: The aim of this experiment was to confirm the presence and construct of BioBricks ordered from iGEM Headquarters. Furthermore, the aim was to create liquid cultures for later purification and making glycerol stocks.

4 colonies were picked for pSB1K3-EB used for PCR amplification and making a liquid culture. The liquid culture was incubated at 37°C, 180 rpm. The PCR was run with primers VF2R and VF2F (both at the final concentration 0.2 μM).
The liquid cultures were grown to an OD of 0.6 (at 600nm wavelength, taking approximately 4.5 h) and 250 l were used to make glycerol stocks (250 μl bacteria + 250 μl 50% glyercol) The remaining 1.75 ml of the liquid cultures were grown for another hour, then transferred to an incubator without shaking and left there until completing their 16h incubation. On the 24.06.16 liquid cultures were spun down (15 min, 6000g), supernatant removed and pellets frozen at -20°C.

Table 1: Volumes for the PCR Master mix (9 samples) with a total volume of 900 μl. Two of these mixes were made.

Sample	Volumes [μl]
Working Stock VF2F primer	18
Working Stock VF2R primer	18
10x PCR Buffer	90
Taq DNA Polymerase	4.5
dNTP mix	18
Autoclaved ddH_2O	751.5

Results and Conclusions

All the samples had a size of approximately 1200 bp. Since the BioBrick of ESP (BBa_K531003) has the size 833 bp and the primers add approximately 250 bp during the PCR amplification this is considered a reliable result.

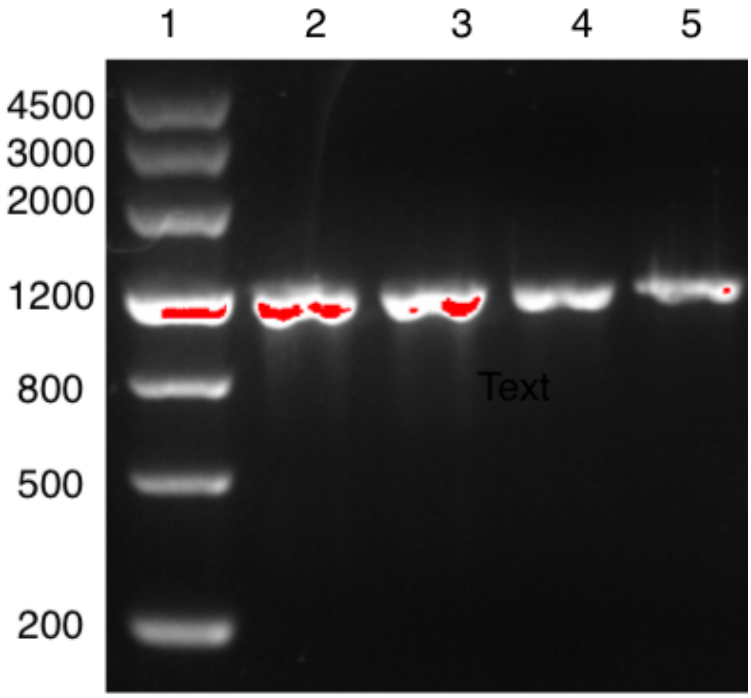

Figure 1: Gel of EB-PCR products. 1. Ladder, 2-5 EB-PCR products

Discussion and Troubleshooting

PCR products will be purified and sent for sequencing. Glycerol stocks were frozen for long-term storage and pellets from liquid cultures frozen for future purification when the results from sequencing are available.

2 PCR amplification of pSB1K3-EB

Responsible

Betty Tesfai, Maren Maanja, Sigrun Stulz

Protocols used

PCR protocol
PCR Purification

Experimental Set Up

Description: The aim of this experiment was to amplify and purify pSB1K3-EB to be able to send it for sequencing.

A total volume of 500 μl PCR Master mix was prepared (The PCR Master Mix was prepared for two EB samples labelled EB4 and EB5 and also two Defensin-samples). The primers, both with the concentration 10 μM, were diluted to 2 μM.
After the PCR amplification the samples were purified.

Results and Conclusions

Picture of gel not found.
No concentration could be measured for EB4. The sample EB5 had a concentration of 68.8 ng/μl.

Table 2: Concentration of the samples after NanoDrop measurement

Sample	Concentration ng/μl
EB 4	-
EB 5	68.7

Discussion and Troubleshooting

The sample EB5 will be send for sequencing.

3 PCR purification of pSB1K3-EB

Responsible

Aman Mebrathu, Reskandi Rudjito, Betty Tesfai

Protocols used

PCR Purification

Experimental Set Up

Description: The aim of this experiment was to purify pSB1K3-EB to prepare it for sequencing. The samples used were the PCR products from the PCR amplification 23.06.16. labelled EB1, EB2 and EB3. The purification was done with two elution methods. In the first trial EB1 and EB2 were eluted in 50 μl elution buffer and in the second trial EB1 and EB3 were eluted in 30 μl elution buffer. EB3 was re-eluted in 15 μl elution buffer.

Results and Conclusions

Table 3: Concentration of EB1 and EB2 after PCR purification and eluted in 50 μl

Trial 1, Sample	Concentration ng/μl
EB 1	15.9
EB 2	15.2

Table 4: Concentration of EB3 and EB4 after PCR purification, eluted in 30 μl elution buffer

Trial 2, Sample	Concentration ng/μl
EB 3	18.5
EB 4	33.4

Table 5: Concentration of EB2 and EB3 after re-elution in 15 μl elution buffer

Trial 3, Sample	Concentration ng/μl
EB 2	5.3
EB 3	5.6

Discussion and Troubleshooting

Only EB4 had a concentration considered high enough to be send for sequencing. A second attempt to purify EB1, EB2 and EB3 will be made.

4 PCR purification of pSB1K3-EB, second attempt

Responsible

Maren Maanja, Betty Tesfai

Protocol used

PCR Purification

Experimental Set Up

Description: The aim in this experiment was to PCR-product EB5 to prepare it for sequencing.

Results

After the PCR purification a concentration of 68.0 ng/μl was obtained.

Discussion and Troubleshooting

EB5 will be send for sequencing.

5 Transformation of pSB1K3-EC into Top10 cells

Responsible

Oskar Ohman, Shuangjia Xue

Protocol used

Collecting BioBrick DNA
Transformation

Experimental Set Up

Description: pSB1K3-EC with an estimated concentration of 2-3 ng/μl was collected from the BioBrick distribution kit. 3 μl of the sample was used for transformation into Top10 cells and a negative control was used.

Results

Approximately 100 colonies were found on the agar plate with pSB1K3-EC-transformed Top10 cells. No colonies were on the negative control.

Discussion and Troubleshooting

The transformation was considered successful and 4 colonies will be picked and PCR amplified.

6 Colony PCR and PCR purification of pSB1K3-EC

Responsible

Oskar Ohman
Shuangjia Xue

Protocol used

PCR Protocol
PCR Purification

Experimental Set Up

Description: 4 colonies from last experiment were used for colony PCR. Gel electrophoresis was run at 120 V, 30 min. DNA from 2 colonies, labelled EC,2 and EC,4, were purified. The concentration of the samples were measured with Nanodrop.

Results

Picture of gel not found.

The concentration after PCR purification of EC,2 and EC,4 were 95.6 ng/μl and 91.8 ng/μl respectively.

Discussion and Troubleshooting

The samples will be send for sequencing. If the sequencing results look good, 3A assembly with the T7-promoter will be performed.