Nuclease

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 of pSB1C3-Nuc **2**

2 PCR purification of pSB1C3-Nuc **4**

1 Colony Picking of pSB1C3-Nuc

Responsible

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Protocols used

- Colony Picking
- PCR
- Collecting BioBrick from Distribution

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 for later purification and making glycerol stocks.

4 colonies were picked for pSBIC3-Nuc, two were used to start a PCR and a two were used to make a liquid culture. The liquid culture was incubated at 37°C , 180 rpm. The PCR was run with primers VF2 and VR (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 μl were used to make glycerol stocks (250 μl bacteria + 250 μl 50% glyercol) The remaining 175 μl 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 .

Working Stock VF2 primer	18 μl
Working Stock VR primer	18 μl
10x PCR Buffer	90 μl
Taq DNA Polymerase	4.5 μl
dNTP mix	18 μl
Autoclaved ddH_2O	751.5 μl

Sample Calculation

For the PCR, a mastermix for 18 samples was created (final volume: 1800 μl). For a tube of 900 μl Master Mix (two tubers were used) the reagents under 'Experimental Data' were added together.

Results and Conclusions

The Nuc samples had the anticipated size of 900 base pairs; the Nuc BioBrick BBa_K729004 had 570 bp and the primers had appoximately 250 bp . The results from the gel were considered reliable.

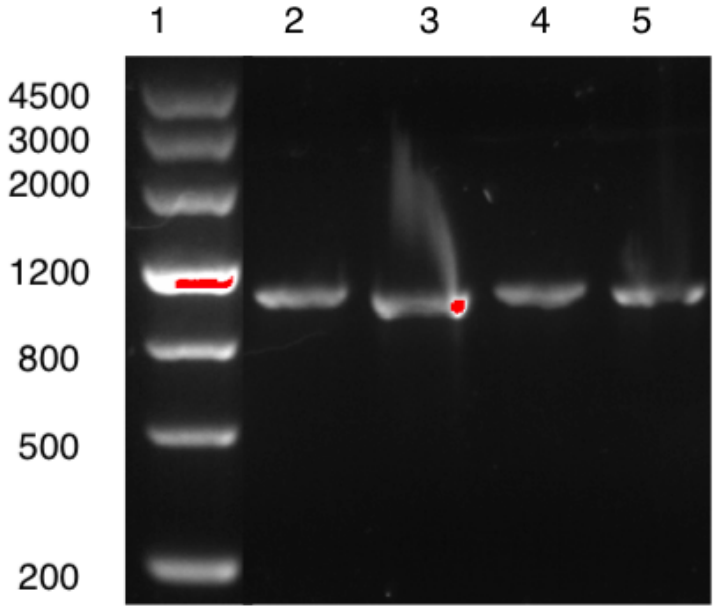

Figure 1: (1) Ladder (2) Nuc (3) Nuc (4) Nuc (5) Nuc

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 will be available.

2 PCR purification of pSB1C3-Nuc

Responsible

Aman Mebrahtu, Reskandi Rudjito, Bethel Tesfai Embaie

Protocols used

- PCR Purification Protocol

Modifications to protocols

- Used 50 μL of DNA sample which was too little, and resulted in a to low concentration.
- Reduced elution buffer (EB) to 30 μL to increase final DNA concentration

Experimental Set Up

Description: The aim was to purify PCR product from the ordered Nuc BioBrick (BBa_K729004), and prepare it for sequencing.

Prepared HCL 1M
Made Sodium Acetate Solution 3M, pH 5
Purified Nuc1, Nuc2, with 50 uL elution buffer

Sample Calculation

Following reactions were prepared for the experiment.

Table 1: HCL, Sodium Acetate, Forward Primer

Reaction	**Volume**	**Calculation**
HCL 1 M	100 ml	12.8 ml HCl 25% + 87.2 ml dH_2O
Sodium Acetate 3M	~15 ml	4.1 g of sodium acetate + 10 ml of dH_2O Adjust pH to 5, used around 8 ml of HCl 1M
Forward Primer 5μM	50 μl	25 μL of 10μM into 25 μl sterilized dH_2O

Results and Conclusions

After PCR, the nanodrop showed the following concentration of the Nuc1 and Nuc2 samples:

Table 2: Concentrations of Nuc

Sample	Concentration (ng/μL)	Ready for Sequencing
Nuc1	29.2	Yes
Nuc2	21.6	Yes

Discussion and Troubleshooting

Nuc 1, Nuc 2, can be sent for sequencing (after being mixed with 5μM forward primer).