

# 09. (September) 2019

**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Sarah Brajkovic

TUESDAY, 10/9/2019

**Restriction Digest:** see protocol

Double Digestion of gBlock "fluc\_TEV cleavage site\_6xHis".

Double Restriction Digest		
	A	B
1	Component	50 µl Reaction
2	gBlock (IDT)	25 µl
3	10X CutSmart Buffer	5 µl
4	NdeI	1 µl
5	NheI-HF	1 µl
6	ddH <sub>2</sub> O	18 µl

Table3		
	A	B
1	Component	50 µl Reaction
2	gBlock (IDT)	15 µl
3	10X CutSmart Buffer	5 µl
4	NdeI	1 µl
5	BmtI-HF	1 µl
6	ddH <sub>2</sub> O	28 µl

**Incubate at 37°C for 1 hour.**

**Restriction Digest:** see protocol

Double Digestion of plasmid backbone "pET151" (for bacterial overexpression).

Table1			^
	A	B	
1	<b>Component</b>	<b>50 µl Reaction</b>	
2	pET151 (100ng/µl)	7 µl	
3	10X CutSmart Buffer	5 µl	
4	NdeI	1 µl	
5	NheI-HF	1 µl	
6	ddH <sub>2</sub> O	36 µl	

Table2			^
	A	B	
1	<b>Component</b>	<b>50 µl Reaction</b>	
2	pET151 (50ng/µl)	20 µl	
3	10X CutSmart Buffer	5 µl	
4	NdeI	1 µl	
5	BmtI-HF	1 µl	
6	ddH <sub>2</sub> O	23 µl	

**Incubate at 37°C for 1 hour.**

**Gel extraction kit:** see protocol

**T4 DNA Ligase Ligation:** see protocol

Calculations (3:1)					^
	A	B	C	D	
1		<b>bp</b>	<b>mass</b>	<b>concentration</b>	
2	<b>insert</b>	1650 bp	37.24 ng		
3	<b>vector</b>	5750 bp	50 ng		

fluc 27

52 43 --> 50ng (= 1.5 uL) + 45pg (= 1,5 uL)

53 15 --> 50ng (= 4 uL) + 45pg (= 1,5 uL)

155 31 --> 50ng (= 2 uL)+ 45pg(= 1.5 uL)

T4 DNA Ligase Ligation					
	A	B	C	D	E
1	Component	20 µl reaction	52	53	155
2	T4 DNA Ligase Buffer (10X)	2 µl	2	2	2
3	Vector DNA	1.5 µl	1.5	4	2
4	Insert DNA	1.4 µl	1.5	1.5	1.5
5	T4 DNA Ligase	1 µl	1	1	1
6	ddH <sub>2</sub> O	14 µl	14	11.5	13.5

Incubate at RT for 10 min. Heat Inactivation at 65°C for 10 min.

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SUNDAY, 15/9/2019

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**Protein Expression (fluc):** see protocol

Transformation: pET151\_fluc\_TEV\_6xHis (C1 #2) in chemocompetent *E.coli* BL21(DE3).

Pre-culture: 100 ml LB<sub>0</sub>+Amp were inoculated with 250 µl of transformed *E.coli* BL21(DE3).

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MONDAY, 16/9/2019

**Protein Expression (fluc):** see protocol

Main culture: 500 ml LB<sub>0</sub>+Amp were inoculated with 10 ml of pre-culture medium (1:50).

Induction: 0.5 mM IPTG, at OD<sub>600</sub> = 0.6 - 0.8

Conditions: RT vs. 37°C / Expression Test: 0 h, 1 h, 2 h, 3 h, o/n (18 h at 22°C = RT)

37°C: incubate 3 hours post induction

RT: incubate o/n

**Expression:**

fluc Expression (37°C)		
	Time after Induction	OD600
1	0 h	
2	1 h	
3	2 h	
4	3 h	

fluc Expression (RT)		
	Time after Induction	OD600
1	0 h	
2	1 h	
3	2 h	
4	3 h	
5	o/n	

**Harvesting (fluc, 37°C):**

Harvesting of the cell pellet by centrifugation (10 min, 10'000 rpm, 4°C).

Cell pellet is stored at -20°C.

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TUESDAY, 17/9/2019

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## Harvesting (fluc, RT):

Of the cell pellet by centrifugation (10 min, 10'000 rpm, 4°C).

## Purification (fluc):

**Lysis Buffer:** 1 x PBS + 0.5 mg/ml Lysozyme + Protease Inhibitor (1:100)

- a. Resuspend Pellet in 20 ml Lysis Buffer.
- b. Incubate for 30 min on ice, stirring.
- c. Sonication: short pulses (5-10 sec) with pauses (10-30 sec)
- d. Centrifugation: 12'000 rpm, 30 min, 4°C
- e. Incubate Supernatant with 1:10 ml of clean His-Beads.
- f. Wash Buffer: 1 x PBS, 20 mM Imidazole
- g. Elution Buffer: 1 x PBS, 500 mM Imidazole
- h. Buffer exchange / Removal of Imidazole: size of fluc: 62 kDa -> centricon cut-off with 30 kDa

### **Amicon® Ultra Method for Concentration, Desalting or Buffer Exchange**

1. Pre-rinse device with MilliQ Water.
2. Add the sample to the reservoir of the centrifugal device. *If the sample is smaller than the maximum volume, it can be diluted up to the maximum volume before the first centrifugation step. This will help increase salt removal.*
3. Centrifuge at 4,000 × g maximum for approximately 10 minutes
4. Remove the initial filtrate from the filtrate tube and set aside.
5. Add enough 1 x PBS to the device to bring the sample volume up to 15 ml.
6. Centrifuge again.
7. Set aside the filtrate.
8. Recover the concentrated and buffer-exchanged sample.

NOTE: Both of the filtrates should be retained until the concentrated sample has been analyzed!

- i. Check A280 with Nanodrop. Extinction Coefficient: 41050, MW: 62318.82 kDa

# 10. (October) 2019

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**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Annika Elimelech

MONDAY, 14/10/2019

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- preculture of FLuc p52\_2 in 50mL Amp LB
- shake at 240rpm 37°C overnight
- Susanne seeded 60 wells of a 96-well plate; HEK293T-cells

# 10. (October) 2019

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**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Annika Elimelech

TUESDAY, 15/10/2019

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- transferred preculture into 200mL Amp LB
  - growth at 37°C until OD 0.6-0.8
  - induction with 500µM IPTG
  - expression at 37°C for 3h
  - cell harvesting at 6000xg for 20min at 4°C
  - stored pellet at -20°C overnight
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- preculture of FLuc p52\_2 in 50mL Amp LB
  - shake at 240rpm 37°C overnight
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- Johanna transfected the HEK293T-cells with V15 (fluc)

# 10. (October) 2019

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**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Annika Elimelech

WEDNESDAY, 16/10/2019

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- transferred preculture into 4 x 150mL Amp LB
- growth at 37°C until OD 0.6-0.8
- induction with 1μM, 250μM, 500μM or 1 mM IPTG respectively
- expression o/n at RT

# 10. (October) 2019

**Project:** iGEM\_Munich2019 Shared Project

**Authors:** Annika Elimelech

THURSDAY, 17/10/2019

- fluc-assay with different substrate-dilutions and luciferin-dilutions was performed

Luciferin Assay							
	A	B	C	D	E	F	G
1		2	3	4	5	6	
2	B	0	4 mM	4 mM	4 mM	4 mM	
3	C	0	2 mM	2 mM	2 mM	2 mM	
4	D	0	1 mM	1 mM	1 mM	1 mM	
5	E	0	500 µM	500 µM	500 µM	500 µM	
6	F	0	250 µM	250 µM	250 µM	250 µM	
7	G	0	1:2	1:2	1:2	1:2	

Assay Substrate					
	A	B	C	D	E
1		8	9	10	11
2	B	1:12.5	1:12.5	1:12.5	1:12.5
3	C	1:25	1:25	1:25	1:25
4	D	1:50	1:50	1:50	1:50
5	E	1:100	1:100	1:100	1:100
6	F	1:200	1:200	1:200	1:200
7	G	undiluted	undiluted	undiluted	undiluted

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FRIDAY, 18/10/2019

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- Sarah analysed the data from the fluc-assay
  - result: the activity of the luciferase is dependent on the concentration of the substrate