Interlab Study - Cloning and measuring of GFP and RFP

The following constructs are build for further GFP and RFP measurement:

I2020 → **GFP construct 0**
K823005 + E0240 → **GFP construct I**
K823012 + E0240 → **GFP construct II**
K823005 + K516032 in pSB3K3 → **RFP construct 0**
K823005 + K516032 → **RFP construct I**
K823012 + K516032 → **RFP construct II**

Therefore, the following **biobricks** are used:

K823005	strong promotor (J23101)
K823012	weak promotor (J23115)
K516032	RFP generator
E0240	GFP generator
I2020	GFP construct 0 and backbone pSB3K3

All biobricks are resuspended in 10 µL and 2 µL are used for transformation with heat shock.

Transformation

50 µL competent cells (XL1Blue *E. coli*) + 2 µL DNA -> incubation on ice for 20 min -> heat shock at 42°C for 60 sec -> incubation on ice for 2 min -> add 950 µL SOC-Medium -> 60 min at 37°C and 650 rpm -> plate cells on new agarplates.

Plasmid preparation of all biobricks to receive DNA material.

24.07.2014

Oli, Melanie

Restriction of both promoters (5µg DNA)with SpeI and PstI and restriction of GFP and RFP (10µg DnA) with XbaI and PstI. First restriction for 3 h and second restriction (PstI) overnight.

1,5% agarose gel for purification

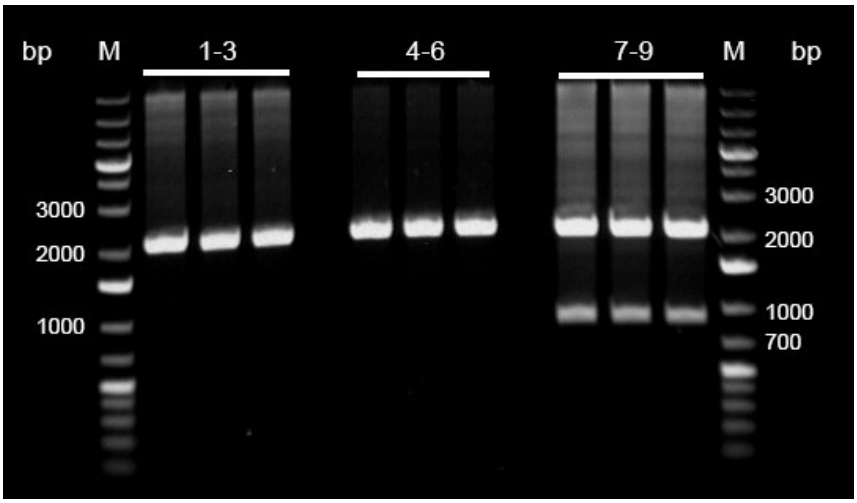

Fig 1: Gel-purification – 1-3: BBa_K823005 (Promotor) cut with Spe1+Pst1; 4-6:BBa_K823012 (Promotor) cut with Spe1+Pst1; 7-9: BBa_E0240 (GFP) cut with Xba1+Pst1

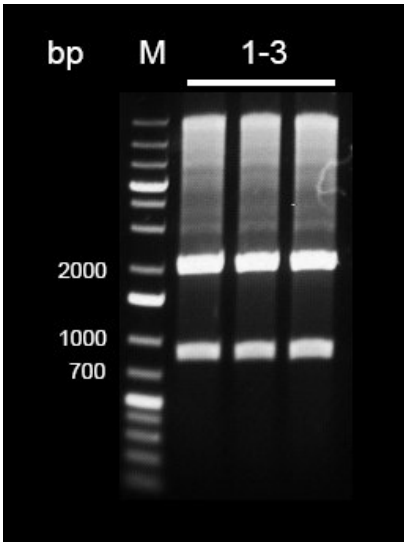

Fig 2: Gel-purification – 1-3: BBa_K516032 (RFP) cut with Xba1+Pst1

→ all biobricks show the expected band length!

Gel elution of all four samples.

Ligation:

2 µL of 1 (promoter) + 7 µL of 6 (GFP) + 1 µL 10x buffer + 0,5 µl ligase

2 µL of 1 (promoter) + 7 µL of 13 (RFP) + 1 µL 10x buffer + 0,5 µl ligase

2 µL of 10 (promoter) + 7 µL of 6 (GFP) + 1 µL 10x buffer + 0,5 µl ligase

2 µL of 10 (promoter) + 7 µL of 6 (RFP) + 1 µL 10x buffer + 0,5 µl ligase

Transformation into competent XL blue MRF' *E. coli* cells and plating on agar plates. Incubation over night at 37 °C.

25.07.2014

Only few colonies are growing on the plates… Nevertheless colony-PCR is performed with as many colonies as possible.

1,0% agarose gel electrophoresis

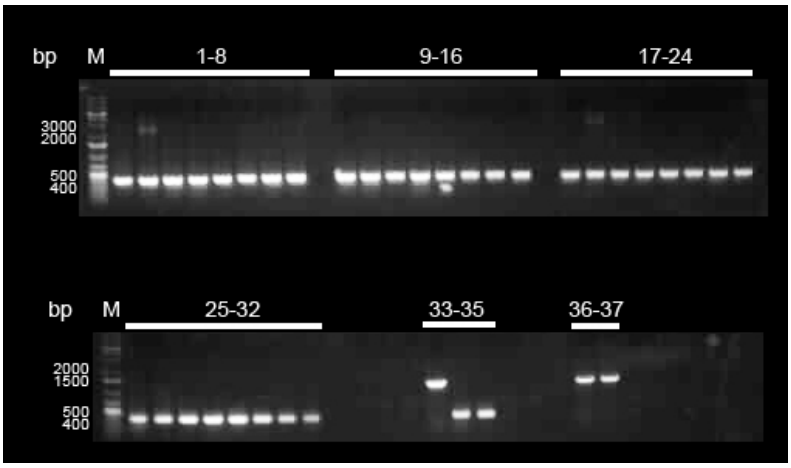

Fig 3: 1-16: RFP I (expected length: 1200 bp); **17-32**: GFP II (expected length: 1200 bp); **33-35**: RFP II (expected length: 1200 bp); **36-37**: GFP I (expected length. 1200 bp)

-> positive clones:
GFP I: clone 1 and 2
GFP II: none
RFP I: none
RFP II: clone 1

29.07.2014

Oli, Carsten

Plasmid preparation of over night cultures of positive clones.

All three samples are sent for sequencing with VF primer (primer 88)

31.07.2014

Results of sequencing:

GFP I: both clones are positive!

-> over night culture for glycerin stock is inoculated

RFP II: empty vector.

→ **GFP 0** and **GFP I** are ready. **GFP II** and **all RFP** constructs are still missing.

13.08.2014

Restriction of biobricks for construction of RFP and GFP constructs.

BBa_K823005 (strong promotor)	52µL (6,5 µg)
Cutsmart	6 µL
SpeI	2 µL
Incubation for 2h at 37 °C	
+ PstI	2 µL
+ H_2O	1 µL
+ NEB buffer 3.1	7 µL
∑	**70 µL**
Incubation for 2h at 37 °C	

BBa_K823012 (waek promotor)	52µL (4,0 µg)
Cutsmart	6 µL
SpeI	2 µL
Incubation for 2h at 37 °C	
+ PstI	2 µL
+ H_2O	1 µL
+ NEB buffer 3.1	7 µL
∑	**70 µL**
Incubation for 2h at 37 °C	

BBa_ E0240	52µL (4,5 µg)
Cutsmart	6 µL

BBa_ K516032	52µL (7,0 µg)
Cutsmart	6 µL

XbaI	2 µL
Incubation for 2h at 37 °C	
+ PstI	2 µL
+ H_2O	1 µL
+ NEB buffer 3.1	7 µL
∑	**70 µL**
Incubation for 2h at 37 °C	

XbaI	2 µL
Incubation for 2h at 37 °C	
+ PstI	2 µL
+ H_2O	1 µL
+ NEB buffer 3.1	7 µL
∑	**70 µL**
Incubation for 2h at 37 °C	

Purification of insert (RFP and GFP) via 1,5% gel electrophoresis and purification of vectors (promoters) via purification kit.

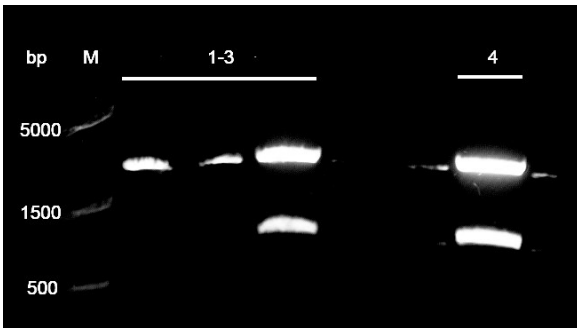

Fig 4: Gel-purification – 1: Promotor (strong); 2: Promotor (weak); 3: GFP; 4: RFP

→ all four digestions were successful. The bands of GFP and RFP (approximately 800bp) are cleaned up for further ligation.

Ligation

Insert (GFP/RFP)	7 µL
Vector (promotor stong/weak)	2 µL
T4 DNA Ligase	0,5 µL
T4 Ligase Buffer	1 µL
∑	**10,5 µL**

Ligation over night at room temperature.

14.08.2014

Transformation of ligation into competent XL1 blue mrf' E. coli cells.

15.08.2014

Colony PCR with primer 88 and 89, extension time: 2 min

1,0% agarose gel electrophoresis:

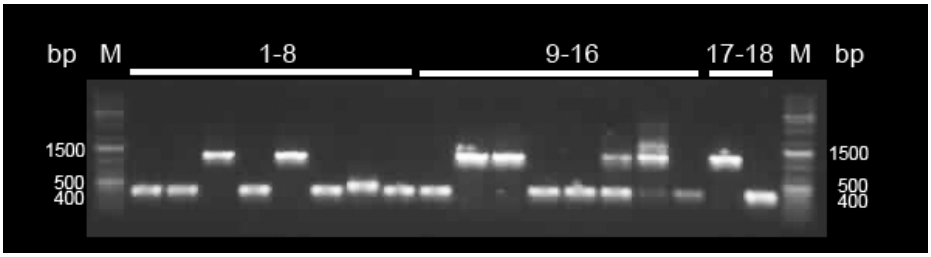

Fig 5: Colony PCR – 1-18: GFP+II 1-18 (expected length: 1200 bp)

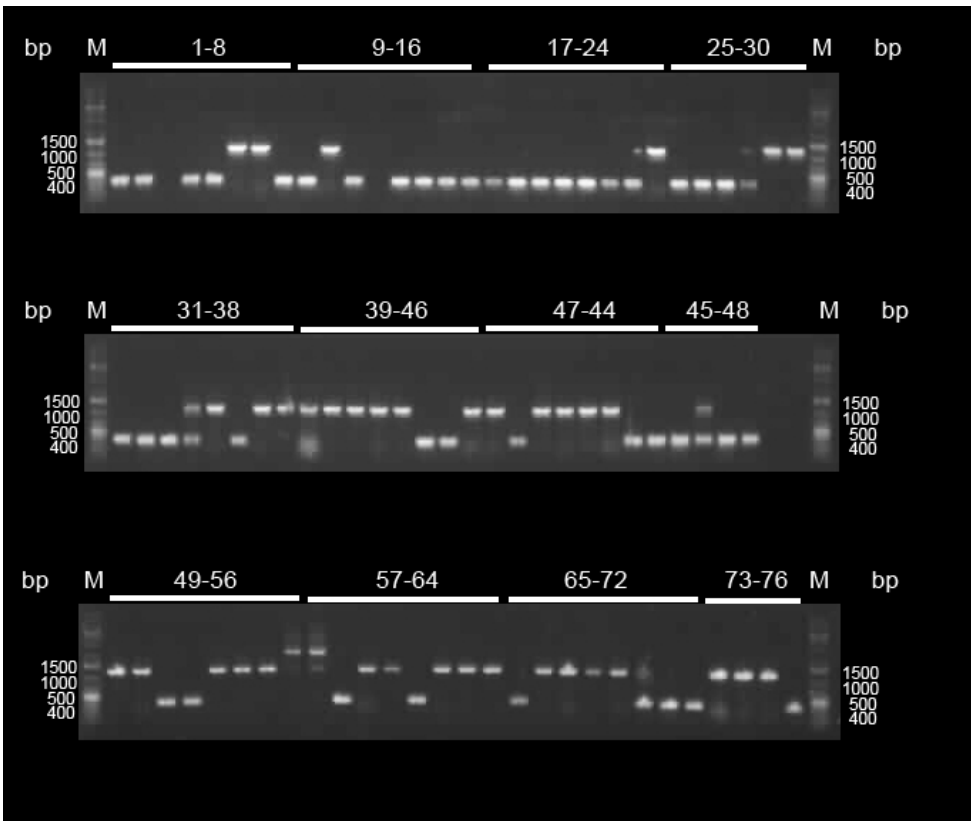

Fig 6: Colony PCR – 1-30: GFP+II 19-48 (expected length: 1200 bp); 31-58: RFP+II (expected length: 1200 bp); 59-86: RFP+I (expected length: 1200 bp)

→ all three constructs (GFP II, RFP I and RFP II) show positive clones.

The following clones are picked for over night cultures and further plasmid preparation and DNA sequencing:

GFP II	Clone 3 and 5
RFP I	Clone 1 and 2
RFP II	Clone 7 and 8

18.08.2014

Plasmid preparation of over night cultures and sending for sequencing.

21.08.2014

All six clones of the three constructs are positive without any mutations.

The only construct missing is **RFP 0**!

Constructs of the RFP 0 construct:

RFP I is digested the with EcoRI and SpeI and GFP 0 is digested with EcoRI and SpeI to receive the pSB3K3 backbone.

GFP 0	30 µL (3,0 µg)
Cutsmart	4 µL
SpeI	2 µL
EcoRI	2 µL
+ H_2O	2 µL
∑	**40 µL**
Incubation over night at 37 °C	

RFP I	30 µL (4,5 µg)
Cutsmart	4 µL
SpeI	2 µL
EcoRI	2 µL
+ H_2O	2 µL
∑	**40 µL**
Incubation over night at 37 °C	

Inoculation of over night cultures in LB medium of all five constructs for first measuring attempt.

22.08.2014

Measuring of GFP 0, I, II and RFP I and II.

Purification of insert and vector via 1,5% gel electrophoresis:

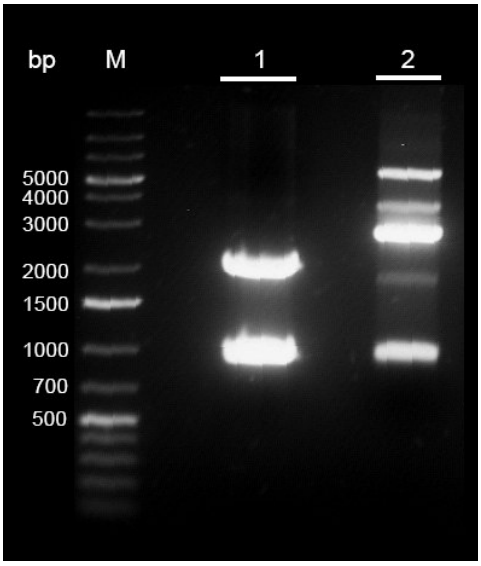

Fig 7: Gel-purification – **1**: RFP1 (insert, expected length: 850bp) cut with EcoRI + SpeI; **2**: GFP0 (vector, expected length: 2750bp) cut with EcoRI + SpeI

→ both digestions were successful. The bands are cleaned up for further ligation.

Ligation

Insert (GFP/RFP)	7 µL
Vector (promotor stong/weak)	2 µL
T4 DNA Ligase	0,5 µL
T4 Ligase Buffer	1 µL
∑	**10,5 µL**

Ligation over night at room temperature.

25.08.2014

<u>Colonoy PCR</u>

of RFP 0 construct that were cultivated over night

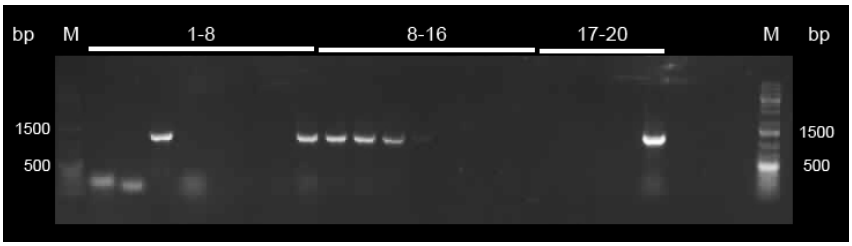

Fig 8: 1-20 RFP 0 (exp. Length: 1200 bp)

→ clones 3, 8-11, maybe 12 and 20 are positive

02.09.2014

Oli, Rüdiger

RFP 0 is measured.