Autor: Maurice Mager

Eintrag 1/9: promoter placeholder construct

In Projekt: Toolbox: building placeholder constructs lvl 1

Keine Tags verwendet

erstellt: 10.07.2019 17:04

aktualisiert: 17.09.2019 11:57

Autor: Maurice Mager
Eintrag 2/9: RBS placeholder construct
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

erstellt: 11.07.2019 16:27
aktualisiert: 17.09.2019 11:57

Autor: Maurice Mager
Eintrag 3/9: Ter placeholder construct
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

erstellt: 11.07.2019 16:36
aktualisiert: 17.09.2019 11:57

Autor: Marian Krämer
Eintrag 4/9: Noch kein Eintragstitel
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

erstellt: 23.08.2019 16:19
aktualisiert: 23.08.2019 16:19

Autor: Maurice Mager
erstellt: 13.09.2019 22:09
aktualisiert: 20.10.2019 20:37
Eintrag 5/9: PRO, RBS and TER spaceholder without oriT and eYFP
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

25.08

so far: transformations on spec plates resulted in huge background growth

we dont know wether its the cells or the plates but gradient agar plates showed that at 4x spec concentration in agar plates, the should be no background but colonies with the spec utex backbone part should still grow.

28.08

made agar plates with 4x spectinomycin concentration

03.09
remade the spaceholder GG with the table below and transformed into Top10
05.09

colonies were big enough to pick from

06.09

only promoter ONC were green, so those were prepped with the Macherey Nagel kit
New colonies were picked from plates

07.09

only one ONC was green again (RBS.SH.1) but i prepped all. The yield however was too low for a digest, hence they were retransformed for new ONCs.

test digesting RBS.SH.1, PRO.SH.1, PRO.SH.2 with bsmbI (see protocol)

results see gel picture no.1

lane 1: Pro SH.1

lane 2:Pro SH.2

lane 3: RBS SH.1

lane 4: generuler 1 kb ladder

Promoter spaceholder constructs were clearly correctly assembled (6 kb band for the ori part and 2 kb band for the cut out parts from 5' to 3' connector) and could be send for sequencing.

retransformation of RBS.SH.2 and TER.SH1/2 yielded green colonies, so they seem to only express the gfp on plates.

10.09

prepared ONCs of RBS.SH1/2 and TER.SH.1/2

sequencing of PRO.SH.1 didnt work but it was because johanna used the wrong primer
11.09
prepped and digested for 2h at 37°C (see table)
repeated seq of Pro.SH.1

12.09
sequencing of Pro.SH.1 looks good, rev sequencing needs to be sent away

GG spaceholder parts

	A	B	C	D	E	F	G	H	I
1	**pro**								
2	1_05	1 µl							
3	Pro SH	1 µl							
4	3_07	1 µl							
5	4_04	1 µl							
6	5_03	1 µl							
7	6_02	1 µl							
8	utex_ori 10 ng/ µl	1 µl							
9	bsaI	0,5 µl							
10	t4 Puffer	1 µl							
11	ligase	1 µl							
12									
13	**rbs**								
14	1_05	1 µl							
15	2_20	1 µl							
16	RBS SH	1 µl							
17	4_04	1 µl							
18	5_03	1 µl							
19	6_02	1 µl							
20	utex_ori 10 ng/ µl	1 µl							
21	bsaI	0,5 µl							
22	t4 Puffer	1 µl							

23	ligase	1 µl								
24										
25	**ter**									
26	1_05	1 µl								
27	2_20	1 µl								
28	3_07	1 µl								
29	4_04	1 µl								
30	TER SH	1 µl								
31	6_02	1 µl								
32	utex_ori 10 ng/ µl	1 µl								
33	bsaI	0,5 µl								
34	t4 Puffer	1 µl								
35	ligase	1 µl								
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Sheet1

gg_spaceholder_parts_4612415_2.xlsx

test digest bsmbi

	A	B	C	D	E	F	G	H	I	J
1	**component**	volume								
2	CutSmart	5 µl								
3	BsmbI	1 µl								
4	Plasmid	1 ng								
5	A.dest	to 50 µl								
6										
7										
8	digest for 2h at 37°C									
9										
10										
11										
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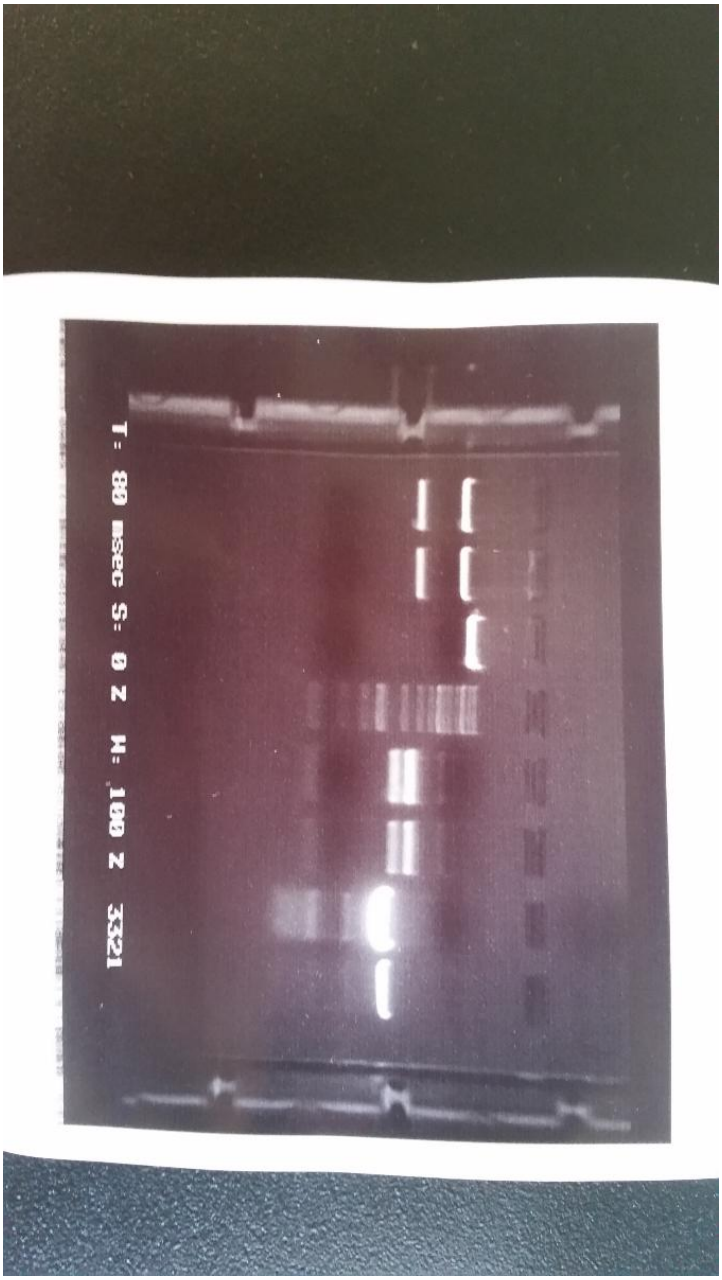
Sheet1

test_digest_bsmbi_4612415_3.xlsx

gel_no.1.jpg



digest table 11.09

	A	B	C	D	E	F	G	H	I
1	ingredients	RBSSH.1	RBSSH.2	TERSH.1	TERSH.2				
2	DNA	10	2	5	4				
3	cutsmart buffer	5	5	5	5				
4	BsmbI	1	1	1	1				
5	A.dest	34	42	39	40				
6									
7									
8	digest at 37°C for 2h								
9									
10									
11									
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20									

Sheet1

digest_table_11_4612415_5.xlsx

Autor: Maurice Mager
erstellt: 17.09.2019 10:03
aktualisiert: 01.10.2019 13:03
Eintrag 6/9: spaceholder with oriT
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

17.09
since the oriT part is correct, placeholder constructs can be assembled with this part
started golden gate (program for SH constructs, leave out the final digest) with the composition in Table 1

18.09
transformed 5 µl of GG into Top10

19.09
prepared 12 ONC of Pro.SH and 3 ONC of RBS.SH. PRO.SH colonies did not fluorescent green, just as in the construct without oriT.
RBS.SH GG only yielded in 3 presumeably positive colonies

20.09

prepped 6 Pro.SH and 3 Pro.SH ONCs

prepared bsmbI digests according to standard protocol

no correct bands

21.09
no correct bands for the next 6 preps either. golden gate needs to be repeated

1.10

repeated GG for both parts

transformed into Top10

sent 3 samples of PROSHoriT away for sequencing

sent 2 samples of RBSSHoriT away for sequencing

Labfolder Table

	A	B	C	D	E	F	G	H
1	Pro spaceholder			RBS spaceholder				
2	part	amount	actual	part	amount	actual		
3	1_05	24 ng	30	1_05	24 ng	30		
4	SH Pro Rene	37 ng	37	2_20	24 ng	28		
5	3_07	24 ng	21	RBS SH Rene	37 ng	35		
6	sfYFP	37 ng	27	sfYFP	37 ng	27		
7	5_03	24 ng	29	5_03	24 ng	29		
8	bom site	30 ng	27	bom site	30 ng	27		
9	spec UTEX ori	10 ng	12	spec UTEX ori	10 ng	12		
10	total: 7µl							
11	add 1µl of T4 Lig, 1 µl T4-Buffer, 1µl BsaI to the mix							
12								
13								
14								
15								
16								
17								
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19								
20								

Sheet1

labfolder_table_4525257_2.xlsx

Autor: Maurice Mager
erstellt: 20.09.2019 14:52
aktualisiert: 01.10.2019 17:14
Eintrag 7/9: using PRO.SH-oriT to insert promoters for PCC7942
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

23.09

PRO.SH1 without oriT is used to build promoter measurement constructs for PCC7942

PRO.SH1 is digested with BsmBI according to protocol

PRO.SH.1 is purified over an agarose gel

started 20x Lvl1,5 GG reactions with purified Pro.SH1 according to table below

used the normal Golden Gate Standard cycle protocol

24.09
Transformation into Dh5a

wrong enzyme ! needs to be repeated with BsaI

1.10.09

SPoT is done, this project is postponed

Lvl 1,5 GG

	A	B	C	D	E	F	G	H
1	23x MM							
2	ingredient	volume						
3	digested spaceholder 20 ng/ µ	23 µl						
4	T4 Buffer	23 µl						
5	BsmbI	20 µl						
6	T4Ligase	20 µl						
7	A. dest	120 µl						
8								
9	add 1 µl of 60 ng Promoter part to 9 µl of Mastermix							
10								
11								
12								
13								
14								
15								
16								
17								
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19								
20								

Sheet1

lvl_1,5_gg_4527531_2.xlsx

Lvl 0,5 GG

	A	B	C	D	E	F	G
1	23x MM						
2	ingredient	volume					
3	digested spaceholder 20 ng/ µl	23 µl					
4	T4 Buffer	23 µl					
5	BsaI	20 µl					
6	T4Ligase	20 µl					
7	A. dest	120 µl					
8							
9	add 1 µl of 60 ng Promoter part	to 9 µl of Mastermix					
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

Sheet1

lvl_0,5_gg_4527531_3.xlsx

Autor: Maurice Mager
Eintrag 8/9: inserting promoters into PRO spaceholder + oriT
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

erstellt: 01.10.2019 17:22
aktualisiert: 01.10.2019 22:48

PRO.SH+oriT will be used to insert all promoters from the Marburg Collection for measurements in UTEX2973 and PCC7942

1.10

BsaI digest according to standard protocol

Gel extraction (21,0 ng/µl)

LVL 0,5 GG according to table "LVL0,5 GG"

LVL 0,5 GG

	A	B	C	D	E	F	G
1	23x MM						
2	ingredient	volume					
3	digested SH	23 µl					
4	T4Buffer	23 µl					
5	BsaI	23 µl					
6	T4Ligase	23 µl					
7	A.dest	133 µl					
8							
9							
10							
11							
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19							
20							

Sheet1

lvl_0,5_gg_4529041_3.xlsx

Autor: Joana Esslen
erstellt: 14.10.2019 19:25
aktualisiert: 20.10.2019 21:12
Eintrag 9/9: Spaceholder (Pro/RBS) Lvl1 GG
In Projekt: Toolbox: building placeholder constructs lvl 1
Keine Tags verwendet

12.10.2019

lvl 1 Golden Gate:

1. Parts on ice, buffer on ice
2. Prepared Mastermixes in PCR-tube (out of parts)
3. Added 1 µL lvl 1 UTEX bb (c = 12.0 ng/µL)
4. Added 1 µL T4 Lig buffer
6. Enzymes in ice
7. Added 1 µL of T4 ligase
8. Added 1 µL of Bsa1
9. Pipet-mixing
10. Golden Gate program in Mastercycler (Eppendorf) (GG Spaceholder)

Mastermix **1 Pro**

name of part	c (part) / (ng/µL)	V (part) / µL
1_05	26	1.00
Pro SH	40	1.00
3_07	27	1.00
4_26	28	1.00
5_03	27	1.00
6_02	22	1.00

Mastermix **2 Pro**

name of part	c (part) /(ng/µL)	V (part) / µL
1_05	26	1.00
Pro SH	40	1.00
3_07	27	1.00
sfYFP	31	1.00
5_03	27	1.00
6_02	22	1.00

Mastermix **3 RBS**

name of part	c (part) /(ng/µL)	V (part) / µL
1_05	26	1.00
2_20	23	1.00
RBS SH	40	1.00
4_26	28	1.00
5_03	27	1.00
6_02	22	1.00

Mastermix **4 RBS**

name of part	c (part) /(ng/µL)	V (part) / µL
1_05	26	1.00
2_20	23	1.00
RBS SH	40	1.00
sfYFP	31	1.00
5_03	27	1.00
6_02	22	1.00

13.10.2019

Transformation:

1. 5 µL of the GG lvl1 in top10
2. incubation on ice for 30 minutes
3. heatshock (42°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 1h)
7. centrifugation
8. remove supernatant
9. plate on agar plate with Spec
10. in incubator 37°C

14.10.2019

1. picked 3 colonies from each plate for overnight cultures (all picked colonies were green)
2. in incubator 37°C, 210rpm

15.10.2019

nothing grew in 1_1

Plasmid DNA purification (Macherey-Nagel kit):

see protocoll; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

retrafo of 1_3

new overnight cultures of 1Pro (1_4, 1_5)

Bsmbl digest of **1_2; 2_1; 2_2; 2_3; 3_1; 3_2; 3_3; 4_1; 4_2; 4_3**

1. mixed components:

   1_2: 4.0 µL of 1_2: (253 ng/µL) + 40.0 µL of water added in one tube

   2_1: 3.0 µL of 2_1 (354 ng/µL) + 41.0 µL of water added in other tube

   2_2: 1.3 µL of 2_2 (794 ng/µL) + 42.7 µL of water added in other tube

   2_3: 1.4 µL of 2_3 (751 ng/µL) + 42.6 µL of water added in other tube

   3_1: 1.8 µL of 3_1 (585 ng/µL) + 42.2 µL of water added in other tube

   3_2: 1.9 µL of 3_2 (527 ng/µL) + 42.1 µL of water added in other tube

   3_3: 1.1 µL of 3_3 (951 ng/µL) + 42.9 µL of water added in other tube

   4_1: 1.4 µL of 4_1 (743 ng/µL) + 42.6 µL of water added in other tube

   4_2: 2.7 µL of 4_2 (373 ng/µL) + 41.3 µL of water added in other tube

   4_3 :2.9 µL of 4_3 (353 ng/µL) + 41.1 µL of water added in other tube

2. added 5.00 µL of NEBuffer and 1.00 µL of BsmbI in each tube
3. 2h, 37°C in Mastercycler
4. Agarosegel with EtBr (100 V, 45 min), see picture below

--> sent 3_3 to sequencing

--> not looking good

16.10.2019

1. picked colonies from retrafo plates **1_3** for overnight cultures
2. in incubator 37°C, 200 rpm

Plasmid DNA purification (Macherey-Nagel kit) of **1_4** and **1_5**:

see protocoll; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

-> no good concentrations

18.10.2019

Transformation:

1. 5 µL of the 2nd half of the GG lvl1 in DH5α
2. incubation on ice for 30 minutes
3. heatshock (42°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 1h)
7. centrifugation
8. remove supernatant
9. plate on agar plate with Spec
10. in incubator 37°C

19.10.2019

1. picked 3 white colonies of each plate for overnight cultures
2. in incubator 37°C, 200 rpm

20.10.2019

Plasmid DNA purification (Macherey-Nagel kit):

see protocoll; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

concentration too low for digest/sequencing

Gel1bearb.PNG