Author: Memduha Muratoglu
Entry 46/46: Cotransformation of T7 cassettes
In Project: Protein Expression Strain
No tags associated

created: 11.10.2018 23:22
updated: 11.10.2018 23:28

Strains containing *tfoX* plasmids (SAD1306, WT+piGEM3002) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG (WT+piGEM3002 was induced with anhydrotetracyclin 100µM) in a culture tube. Grow at 30 °C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG (or 100µM anhydrotetracyclin). Invert gently to mix. Don't forget a control without DNA!!!!
4. Add 50 ng of DNA (dns cassette and 200ng of T7 cassette and lon cassette, invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

did not work!, repeated by Daniel Bauersachs!

Author: Memduha Muratoglu
Entry 45/46: Natural Transformation for T7 strain
In Project: Protein Expression Strain
No tags associated

created: 11.10.2018 23:11
updated: 11.10.2018 23:23

Strains containing *tfoX* plasmids (SAD1306,WT+piGEM3002) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG (WT+piGEM3002 was induced with anhydrotetracyclin 100µM) in a culture tube. Grow at 30 ° C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG (or 100µM anhydrotetracyclin). Invert gently to mix. Don't forget a control without DNA!!!!
4. Add 50 ng of DNA (dns cassette and 200ng of T7 cassette and lon cassette, invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

did not work!

Author: Memduha Muratoglu
Entry 44/46: Phusion PCR for T7 int cassette
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

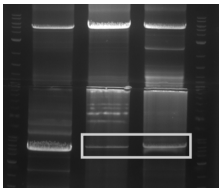
created: 11.10.2018 20:58
updated: 11.10.2018 21:10

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM59 (DanielB)
2.5 µl	Primer rev oiGEM58(DanielB)
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	upstream,T7,downstream
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7_cassette_int.PNG



expected size of 6600bp was cutted out and purified by gelextraction

Author: Memduha Muratoglu
Entry 43/46: fragmetns for T7 casssette in int19
In Project: Protein Expression Strain
With tags: T7, dns

created: 06.10.2018 14:23
updated: 06.10.2018 14:34

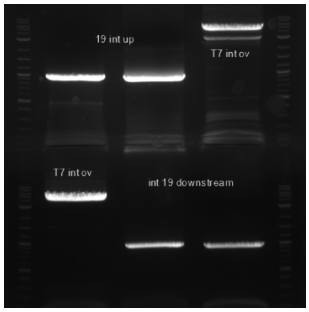
my date: 05.10.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3059(DanielB)//63//65
2.5 µl	Primer rev oiGEM3062//64//58(DanielB)
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	60 ng T7 fragment, 10ng dns downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7_int.PNG



expected sizes: upstream: 1000bp, T7: 4600bp, downstream 1000bp. fragments were purified by gelextraction

Author: Memduha Muratoglu
Entry 42/46: Fusion PCR dns cassette 1000bp
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

created: 06.10.2018 14:16
updated: 06.10.2018 14:28

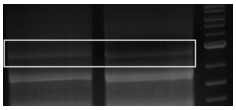
my date: 05.10.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3054
2.5 µl	Primer rev oiGEM3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	60 ng T7 fragment, 10ng dns downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x30
65/63/61 °C (10 cycles each temperature)	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

dns_cassette_1000bp.png



fragment with expected size of ~2900bp was cut out and purified by gelextraction.

Author: Memduha Muratoglu
Entry 41/46: Fusion PCRs dnsup+cm / dnsdown+cm
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

created: 06.10.2018 13:54
updated: 06.10.2018 14:27

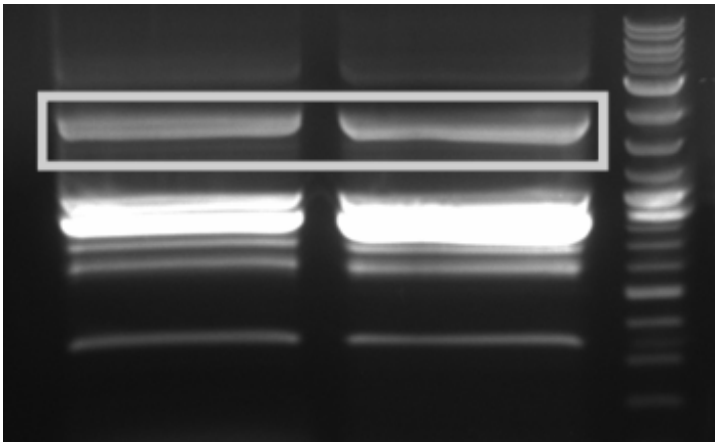
my date: 05.10.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3054/ 3010
2.5 µl	Primer rev oiGEM3009/3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	dns up/ dns down /cm fragments
ad 50 µl	H2O

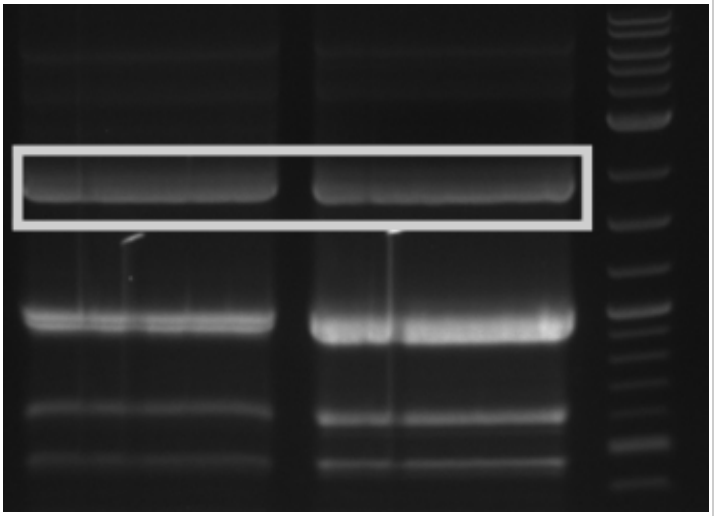
Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

fusion_pcr_dnsup+cm.PNG



fusion_pcr_dnsdown+cm.PNG



expected size was in both cases 1900bp. fragments were purified by gelextraction.

Author: Memduha Muratoglu
Entry 40/46: Amplification of dns downstream-frt 1000bp
In Project: Protein Expression Strain
No tags associated

created: 06.10.2018 13:48
updated: 06.10.2018 14:25

my date: 04.10.2018

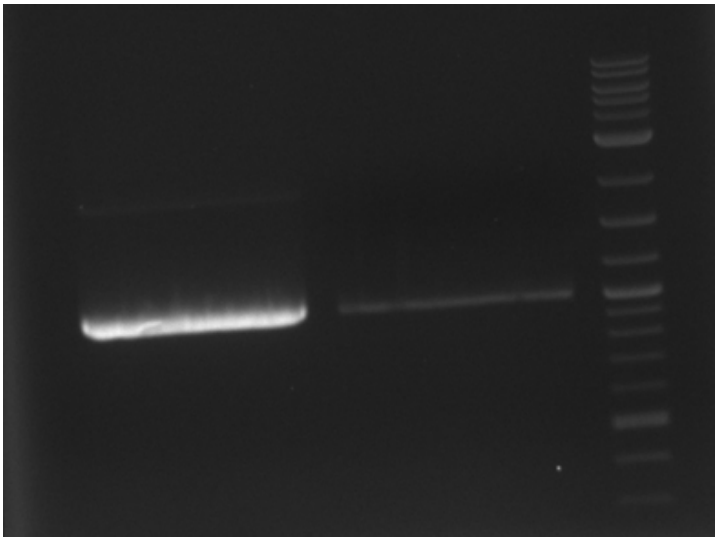
Fragments with homologues overlapping sequences of can be joined by fusion PCR as shown in the figure.

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3034
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	gDNA V. natriegens
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

dns_downstream_1000_bp_frt.PNG



Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	NucleoTrap®: Excise DNA fragment / Solubilize gel slice NucleoTraP®CR: Adjust binding conditions	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	Bind DNA	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	Wash silica matrix	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	Dry silica matrix	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	Elute DNA	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

1000 bp fragment was extraced by gelextraction.

Author: Memduha Muratoglu
Entry 39/46: T7 fusion PCR performed by Steffi Lindow
In Project: Protein Expression Strain
With tags: T7, fusion pcr

created: 05.10.2018 16:30
updated: 05.10.2018 16:32

my date: 03.10.2018

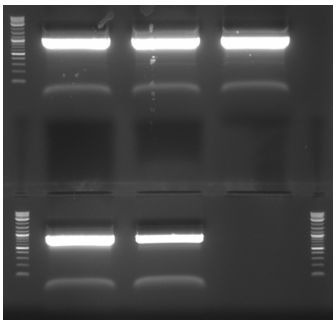
Amount	Compound
10 µl	Phusion buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3054
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	fragments
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

Expected size was 7600 bp. Did not work!

T7_steffi.PNG



Author: Memduha Muratoglu
Entry 38/46: Fusion PCR T7
In Project: Protein Expression Strain
With tags: T7, fusion pcr

created: 05.10.2018 16:20
updated: 05.10.2018 16:24

my date: 02.10.2018

Fragments with homologues overlapping sequences of can be joined by fusion PCR as shown in the figure.

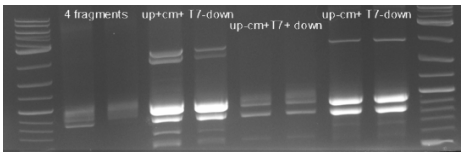
Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3054
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase
x µl	fragments
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

expected size was 7600 bp. Did not work

Fusion_T7_renes_ansatz.PNG



Author: Memduha Muratoglu
Entry 37/46: Fusion PCR T7 Hotstart
In Project: Protein Expression Strain
With tags: T7, fusion pcr

created: 05.10.2018 16:15
updated: 05.10.2018 16:20

my date: 01.10.2018

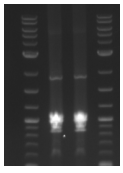
Fusion PCR for T7-down + up-cm

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3054
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Q5 Hotstart Polymerase
x µl	fragments
ad 50 µl	H2O

Programm

98 °C	3 min	
98 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

full_T7_hotstart.PNG



size of 7600bp was expected. Did not work!

Author: Memduha Muratoglu
Entry 36/46: Fusion PCRs
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

created: 01.10.2018 21:10
updated: 01.10.2018 21:18

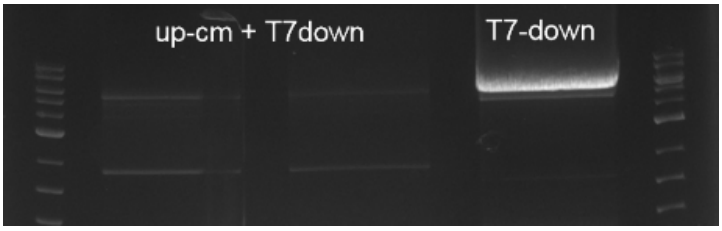
my date: 30.10.2018

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3018 // 3054
2.5 µl	Primer rev oiGEM303055
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase Hotstart!!!!
1 µl	60 ng T7 fragment, 10ng dns downstream fragment // upstream-cm, T7 downstream
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7down_and_fusion.PNG



expected size for full cassette: 7600bp, exprected size fp T7 down: 5600bp. was cutted out and purified.

Author: Memduha Muratoglu
Entry 35/46: Fusion PCRs
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

created: 01.10.2018 21:02
updated: 01.10.2018 21:19

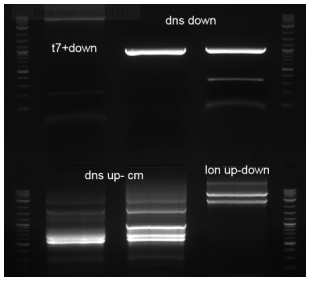
my date: 30.09.2018

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd
2.5 µl	Primer rev
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase Hot start!
1 µl	DNA fragments
ad 50 µl	H2O

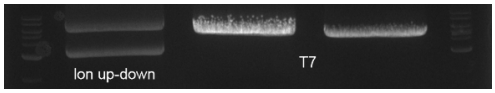
Programm

98 °C	3 min	
98 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7etc.PNG



t7etc2.PNG



Expected sizes:

T7-down: 5600bp

dns down: 1000bp

dns up-cm: 2000bp

lon-updown: 6000bp

T7: 4600 bp

fragments were cutted out and purified by gelextraction

Author: Memduha Muratoglu
Entry 34/46: Fusion of lon up and lon down
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

created: 01.10.2018 20:24
updated: 01.10.2018 20:26

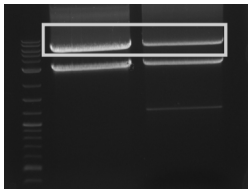
my date: 29.09.2018

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3050
2.5 µl	Primer rev oiGEM3053
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase
1 µl	upstream and downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

lon_fusion.PNG



expected size of 6000bp was cutted out and purified by gelextraction.

Author: Memduha Muratoglu
Entry 33/46: PXR amplification of lon up and down
In Project: Protein Expression Strain
With tags: lon, protease

created: 01.10.2018 20:16
updated: 01.10.2018 20:26

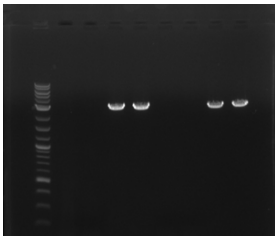
my date: 29.09.2018

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3050 /5052
2,5 µl	Primer rev oiGEM 3051 / 5053
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase
gDNA V. natiregens µl	fragments
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

Lon_upstream_downstream.PNG



expected size of 3000 bp was cutted out and purified by gelextraction

Author: Memduha Muratoglu
created: 01.10.2018 20:15
updated: 01.10.2018 20:15
Entry 32/46: T7 downstream fusion PCR
In Project: Protein Expression Strain
With tags: t7-down
my date: 29.09.2018

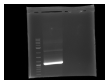
Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3018
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	fragments
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

expected size was 5600 bp

immernoch_T7_down.png



Author: Memduha Muratoglu
Entry 31/46: Phusion PCR T7-downstream 1000
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

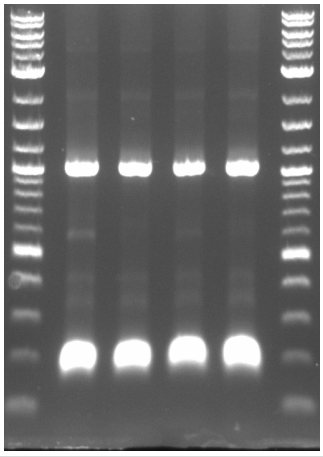
created: 27.09.2018 11:15
updated: 27.09.2018 11:16

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3018
2.5 µl	Primer rev oiGEM3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	60 ng T7 fragment, 10ng dns downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7_down.PNG



Author: Memduha Muratoglu
Entry 30/46: new dns downstream fragment 1000bp
In Project: Protein Expression Strain
No tags associated

created: 27.09.2018 11:12
updated: 01.10.2018 18:17

my date: 22.09.2018

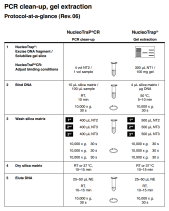
Fragments with homologues overlapping sequences of can be joined by fusion PCR as shown in the figure.

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3020
2,5 µl	Primer rev oiGEM 3055
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	fragments
ad 50 µl	H2O

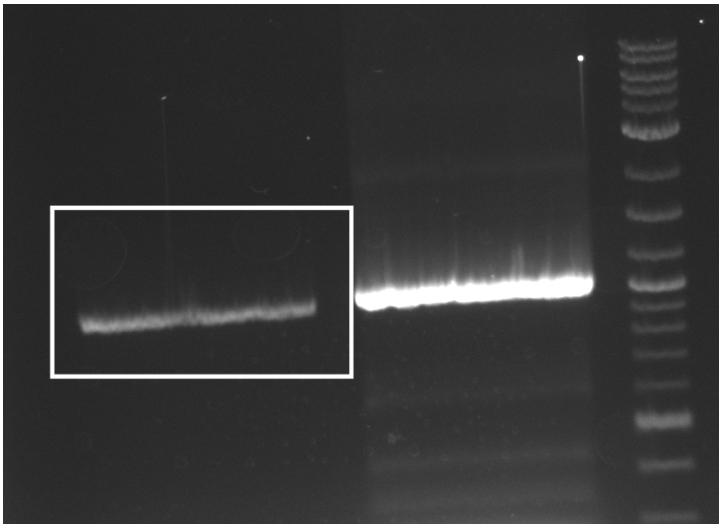
Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

Gel_extraction_protocol.PNG



dns_downstream_100bp.PNG



1000 bp fragment was extraced by gelextraction.

Author: Memduha Muratoglu
Entry 29/46: New dns upstream fragment
In Project: Protein Expression Strain
With tags: T7

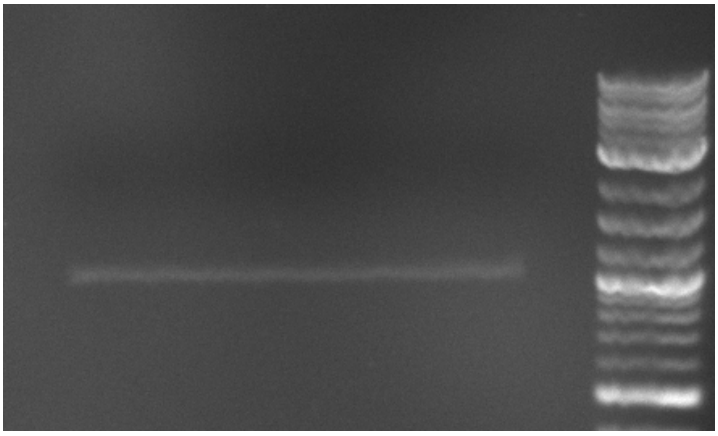
created: 27.09.2018 11:00
updated: 27.09.2018 11:03

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3054
2.5 µl	Primer rev oiGEM3033
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	50 ng gDNA *V. natriegens*
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

upstream1000.PNG



Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	**NucleoTrap®: Excise DNA fragment / Solubilize gel slice** **NucleoTraP®CR: Adjust binding conditions**	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	**Bind DNA**	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	**Wash silica matrix**	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	**Dry silica matrix**	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	**Elute DNA**	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

PCR was ok. expected size was 1000bp.

Author: Memduha Muratoglu
Entry 28/46: Fusion of up-cm and T7-down
In Project: Protein Expression Strain
With tags: T7, dns replacement with T7

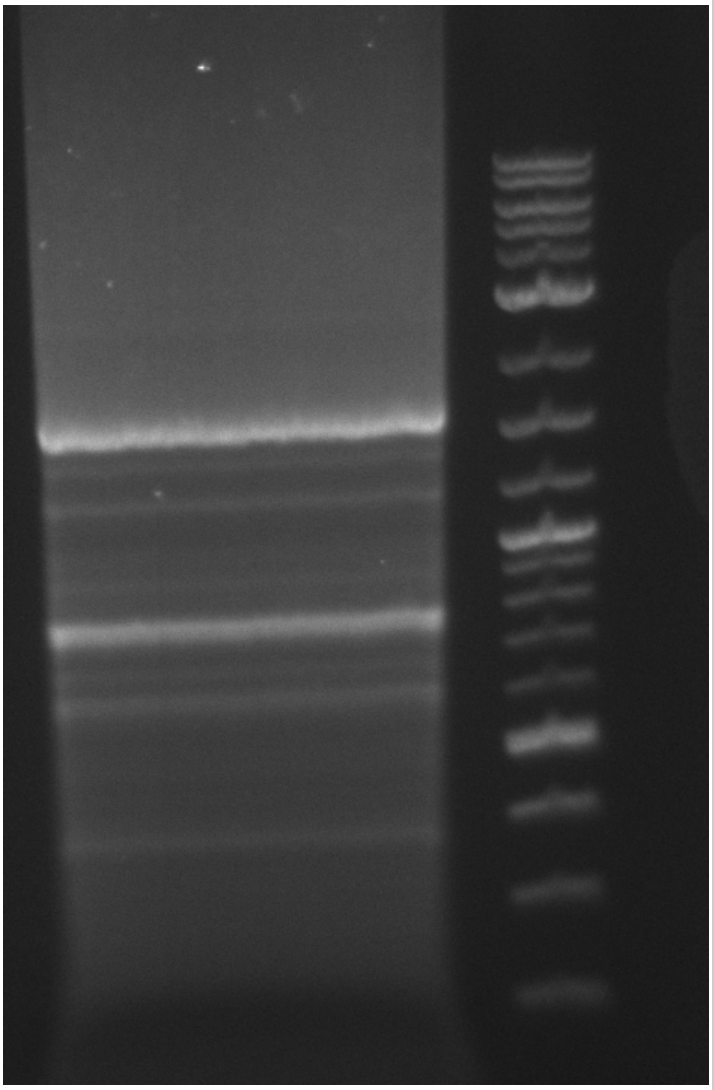
created: 27.09.2018 01:47
updated: 27.09.2018 01:50

my date: 25.09.2018

Fragments with homologues overlapping sequences of can be joined by fusion PCR as shown in the figure.

Amount	Compound
10 µl	Q5 Buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3005
2,5 µl	Primer rev oiGEM 3008
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	fragments
ad 50 µl	H2O

fusion_full.PNG



Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

Expected size was 5600 bp. PCR did not work.

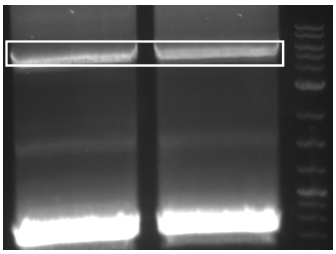
Author: Memduha Muratoglu
Entry 27/46: T7- down phusion PCR
In Project: Protein Expression Strain
With tags: t7-down

created: 27.09.2018 01:45
updated: 27.09.2018 01:45

my date: 25.09.2018

Q5 PCR was repeated as in Phusion PCR of T7 downstream - entry #4 in project 'Example project' (Memduha Muratoglu, 24.09.2018)

t7-down_phusion_repeated.PNG



Author: Memduha Muratoglu
Entry 26/46: Amplification of dns-up and T7-down
In Project: Protein Expression Strain
With tags: dns, T7, fusion pcr

created: 27.09.2018 01:45
updated: 27.09.2018 01:45

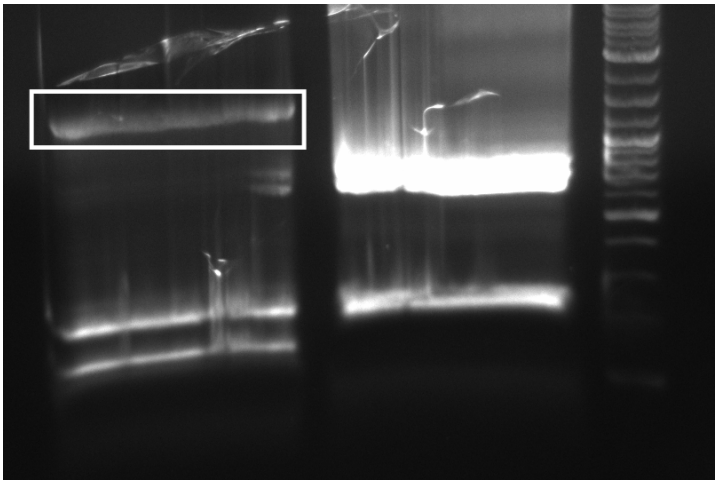
my date: 24.09.2018

Amount	Compound
10 µl	Q5 buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3005 /oiGEM3018
2.5 µl	Primer rev oiGEM3009 /oiGEM3021
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

amplification_of_upcm_T7down.PNG



Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	**NucleoTrap®: Excise DNA fragment / Solubilize gel slice** **NucleoTraP®CR: Adjust binding conditions**	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	**Bind DNA**	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	**Wash silica matrix**	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	**Dry silica matrix**	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	**Elute DNA**	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

amplification of T7-down did not work

Author: Memduha Muratoglu
Entry 25/46: amplification of dns downstream fragment 700bp
In Project: Protein Expression Strain
No tags associated

created: 27.09.2018 01:45
updated: 27.09.2018 01:45

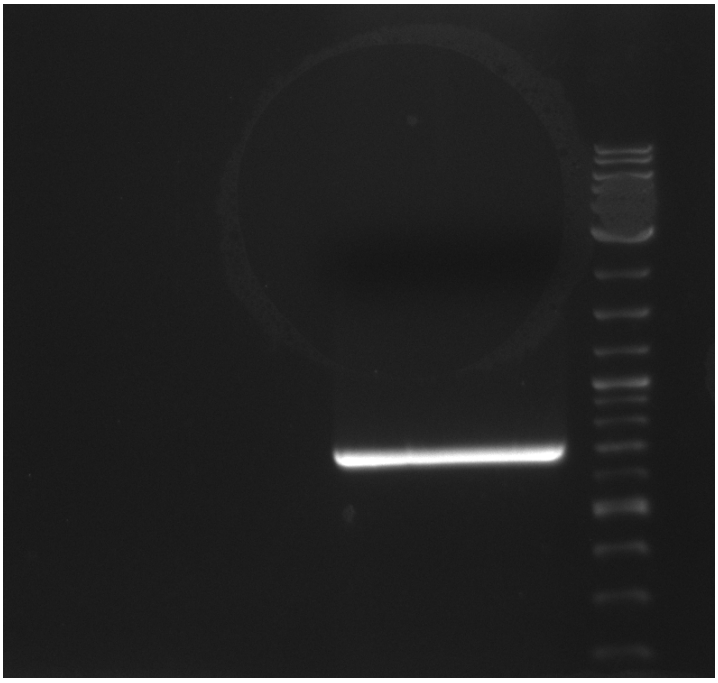
my date: 23.09.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3020
2,5 µl	Primer rev oiGEM 3021
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
x µl	fragments
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

dns_down_700.PNG



Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	**NucleoTrap®: Excise DNA fragment / Solubilize gel slice** **NucleoTraP®CR: Adjust binding conditions**	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	**Bind DNA**	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	**Wash silica matrix**	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	**Dry silica matrix**	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	**Elute DNA**	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

7000 bp fragment was extraced by gelextraction.

Author: Memduha Muratoglu
Entry 24/46: Phusion PCR of T7 downstream
In Project: Protein Expression Strain
No tags associated

created: 27.09.2018 01:44
updated: 27.09.2018 01:44

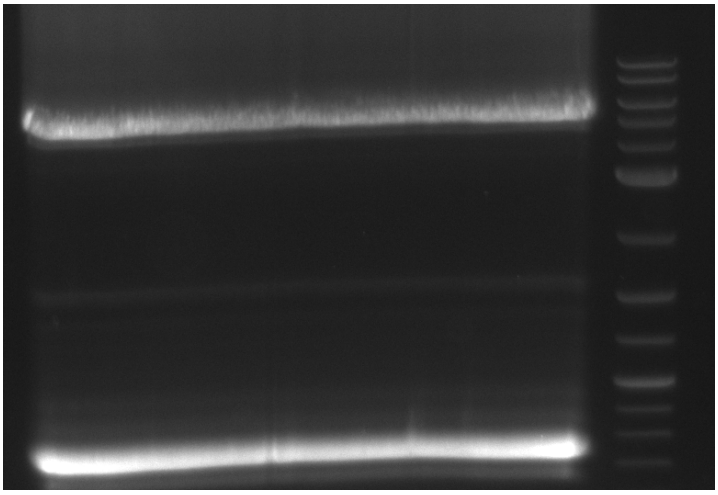
my date: 23.09.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2,5 µl	Primer fwd oiGEM 3018
2,5 µl	Primer rev oiGEM 3021
10 µl	PCR Enhancer
0.5 µl	Q5 Polymerase
x µl	fragments : 300ng T7 fragment and 40ng downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7dns_down_correct.PNG



Gel_extraction_protocol.PNG

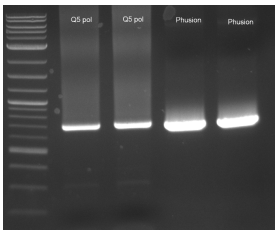
PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	NucleoTrap®: Excise DNA fragment / Solubilize gel slice NucleoTraP®CR: Adjust binding conditions	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	Bind DNA	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	Wash silica matrix	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	Dry silica matrix	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	Elute DNA	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

expected size of 5300 bp seems to be correct. but to be sure, after gelextraction a PCR for only downstreamfragment was performed on this DNA.

T7_downstream_test_pcr.PNG



Test PCR shows, that T7-downstream fragment is correct!

Author: Memduha Muratoglu
Entry 23/46: T7 + dns downstream phusion PCR
In Project: Protein Expression Strain
With tags: T7, dns, fusion pcr

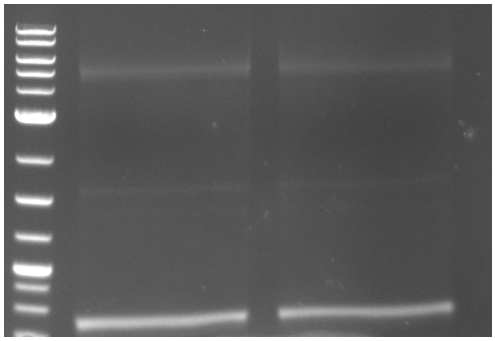
created: 21.09.2018 18:19
updated: 24.09.2018 21:07

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3018
2.5 µl	Primer rev oiGEM3021
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	60 ng T7 fragment, 10ng dns downstream fragment
ad 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7_dns_down_fragment1.PNG



PCR did not work. Expected size was 5300 bp

Author: Memduha Muratoglu
Entry 22/46: Amplification of T7 fragment
In Project: Protein Expression Strain
With tags: T7 fragment, PCR

created: 21.09.2018 17:47
updated: 11.10.2018 18:27

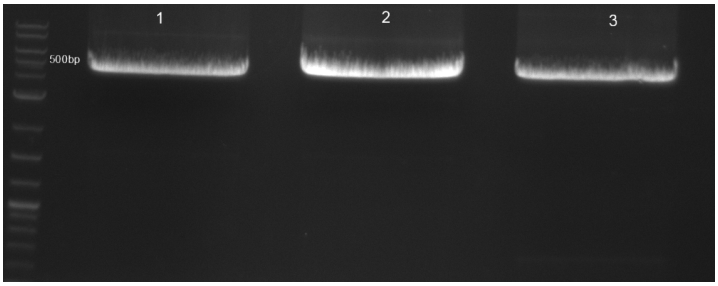
my date: 20.09.2018

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2.5 µl	Primer fwd oiGEM3018
2.5 µl	Primer rev oiGEM3019
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	gDNA 50ng BL21
ad. 50 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

T7_new_fragments_20.09.PNG



Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up		NucleoTrap® Gel extraction
1	**NucleoTrap®: Excise DNA fragment / Solubilize gel slice** **NucleoTraP®CR: Adjust binding conditions**	4 vol NT2 / 1 vol sample		300 µL NT1 / 100 mg gel
2	**Bind DNA**	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	**Wash silica matrix**	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	**Dry silica matrix**	RT or 37 °C, 10–15 min		RT or 37 °C 10–15 min
5	**Elute DNA**	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s		25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

Three samples were loaded on 1% agarose gel expected size of 4600 bp was correct for each of the samples.

Fragments were cutted out and purified by gelextraction.

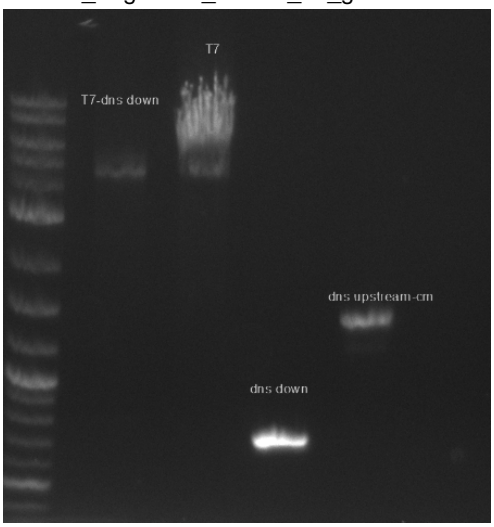
Author: Memduha Muratoglu
Entry 21/46: Test of T7 fragments
In Project: Protein Expression Strain
With tags: T7

created: 21.09.2018 17:39
updated: 21.09.2018 17:45

my date: 19.09.2018

Because T7 clones were negative, Phusion PCRs were repeated and did not work. To test if the single PCR products show the right size, each PCR product was loaded on 1% agarose gel.

t7_fragments_loaded_on_gel.PNG



T7-dns down does not show the expected size of 5300 bp. Additionally the T7 fragments does not seem to be pure. T7 fragment needs to be amplified again.

Author: Jana Jung
Entry 20/46: T7 Test PCR
In Project: Protein Expression Strain
No tags associated

created: 16.09.2018 17:54
updated: 16.09.2018 18:18

PCR

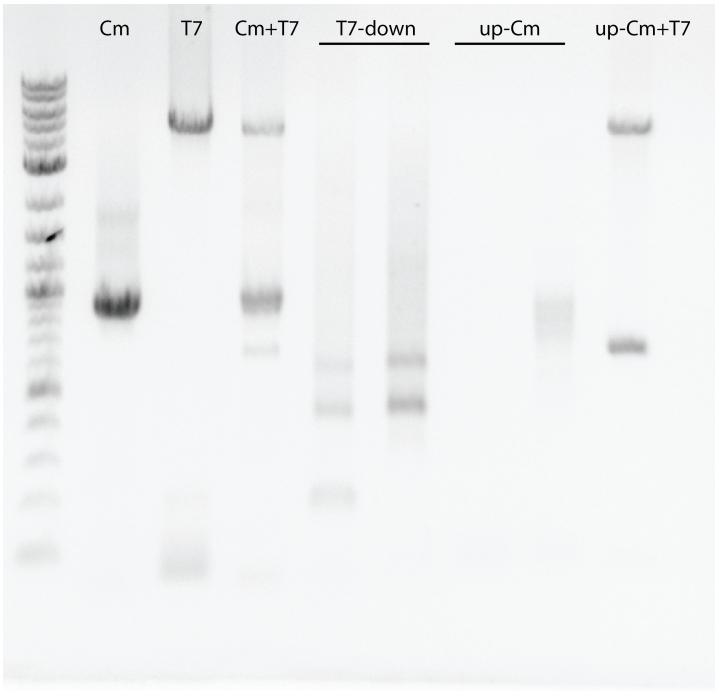
Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
Primer forward	1,25 µl
Primer reverse	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	1,5µl

PCR Programm

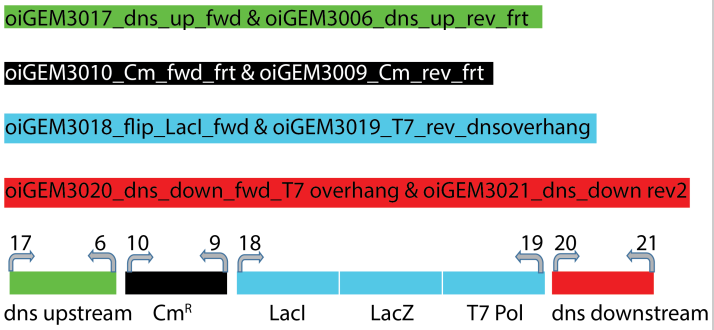
Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	30x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	5min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

	Cm	T7	Cm + T7	T7-down
Primer forward	oiGEM3010_Cm_fwd_frt	oiGEM3018_flip_LacI_fwd	oiGEM3010_Cm_fwd_frt	oiGEM3018_flip_LacI_fv
Primer reverse	oiGEM3009_Cm_rev_frt	oiGEM3019_T7_rev_dnsoverhang	oiGEM3019_T7_rev_dnsoverhang	oiGEM3021_dns_down rev2
DNA	Cm-Fragment	T7-Fragment	Cm-Fragment & T7-Fragment	T7-down-Fragment

180916_T7_Cm_up_down.png



T7_Konstruktion_PCR_mit_Primern.png



Author: Jana Jung
Entry 19/46: Fusions-PCR T7 and Cm
In Project: Protein Expression Strain
With tags: T7, fusion pcr, Protein Expression, Cm

created: 13.09.2018 21:15
updated: 16.09.2018 18:18

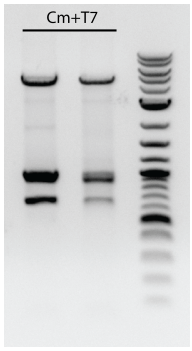
Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3010_Cm_fwd_frt)	1,25 µl
Primer reverse (oiGEM3019_T7_rev_dnsoverhang)	1,25 µl
$dNTP_3$	1 µl
T7 Fragment	0,5 µl
Cm Fragment	0,5 µl
Polymerase	0,5µl

PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	5min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

Expected fragment size: 5640bp

180914_Fusions-PCR_Cm+T7.png



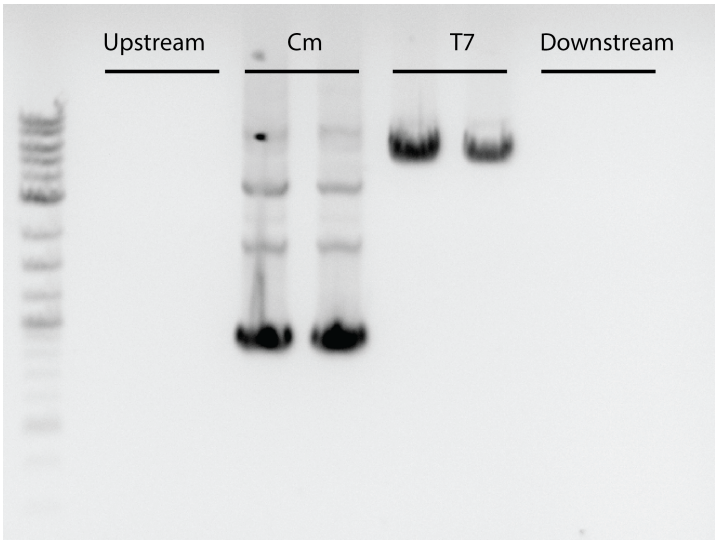
Author: Jana Jung
Entry 18/46: Amplification of T7 casette, up down dns and Cm
In Project: Protein Expression Strain
With tags: Upstream, Cm, T7, Downstream, Protein Expression

created: 13.09.2018 15:25
updated: 13.09.2018 21:15

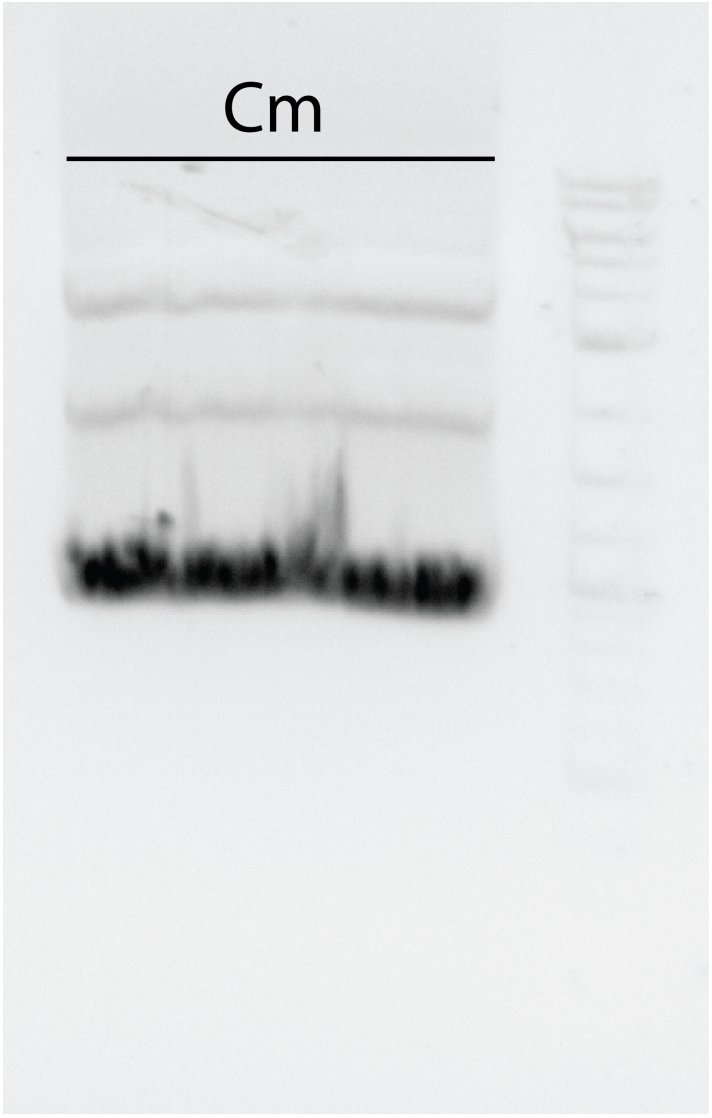
Substanz	**Menge**
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward	1,25 µl
Primer reverse	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	0,5µl

	Primer forward	**Primer reverse**	**DNA**	**Fragment größe**
Upstream	oiGEM3017_dns_up_fwd	oiGEM3006_dns_up_rev_frt	*V. natriegens* gDNA	700bp
Cm	oiGEM3010_Cm_fwd_frt	oiGEM3009_Cm_rev_frt	pYTK	940bp
T7	oiGEM3018_flip_LacI_fwd	oiGEM3019_T7_rev_dnsoverhang	*E. coli* BH21 D3 gNDA	4700bp
Downstream	oiGEM3020_dns_down_fwd_T7 overhang	oiGEM3021_dns_down rev2	V. natriegens gDNA	700bp

180913_Up_Cm_T7_down.png



180913_Cm_für_T7.png



The PCR approach for the T7 fragment was purified and the batch for the chloramphenicol cassette was gel purified.

Author: Jana Jung
Entry 17/46: Fusion PCR T7 Fragment
In Project: Protein Expression Strain
With tags: T7, Protein Expression, fusion pcr

created: 11.09.2018 22:16
updated: 12.09.2018 19:02

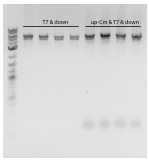
1. PCR dns up + Cm & T7-Fragment & dns down

Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3017_dns_up_fwd)	1,25 µl
Primer reverse (oiGEM3021_dns_down rev2)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	0,5µl

2. PCR T7-Fragment & dns down

Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3018_flip_LacI_fwd)	1,25 µl
Primer reverse (oiGEM3021_dns_down rev2)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	0,5µl

180912_T7_Fusions_PCR.png



Author: Jana Jung
Entry 16/46: natural Transformation with T7
In Project: Protein Expression Strain
No tags associated

created: 11.09.2018 16:48
updated: 11.09.2018 16:49

Strains containing *tfoX* plasmids (SAD1306, SAD1495, SAD1313) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG in a culture tube. Grow at 30 °C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG. Invert gently to mix. Don't forget a control without tDNA!!!!
4. Add 50 ng of tDNA (T7 Fragment) and invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

Author: Jana Jung
created: 10.09.2018 15:48
updated: 10.09.2018 15:49

Entry 15/46: natural Transformation T7, dns and pYTK in SAD1306
In Project: Protein Expression Strain
No tags associated

Strains containing *tfoX* plasmids (SAD1306, SAD1495, SAD1313) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG in a culture tube. Grow at 30 °C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG. Invert gently to mix. Don't forget a control without tDNA!!!!
4. Add 50 ng of tDNA and invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

No colonies have grown on the plate.

Author: Jana Jung
created: 10.09.2018 15:46
updated: 10.09.2018 15:47

Entry 14/46: natural Transformation T7 in SAD1306
In Project: Protein Expression Strain
No tags associated

Strains containing *tfoX* plasmids (SAD1306, SAD1495, SAD1313) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG in a culture tube. Grow at 30 °C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG. Invert gently to mix. Don't forget a control without tDNA!!!!
4. Add 50 ng of tDNA (T7 Fragment) and invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

No colonies have grown on the plate.

Author: Franziska Müller
Entry 13/46: No entry title yet
In Project: Protein Expression Strain
No tags associated

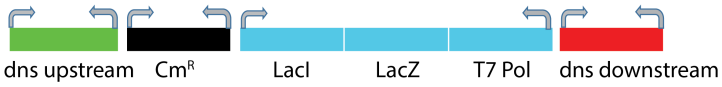
created: 05.09.2018 10:53
updated: 05.09.2018 13:18

T7_Konstruktion_PCR.png

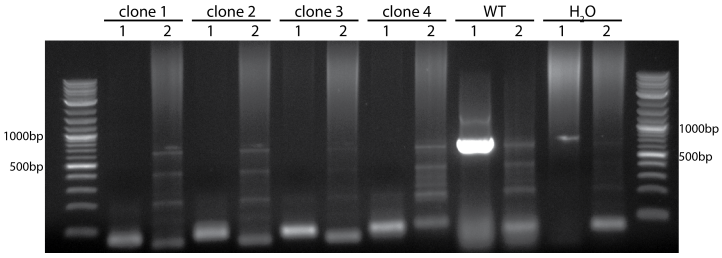
1 = oiGEM3017_dns_up_fwd & oiGEM3006_dns_up_rev_frt

2 = oiGEM3018_flip_LacI_fwd & oiGEM3019_T7_rev_dnsoverhang

3 = oiGEM3020_dns_down_fwd_T7 overhang & oiGEM3021_dns_down rev2



1 % agarose gel loaded with 7 µl of PCR



Author: Franziska Müller

created: 04.09.2018 11:01
updated: 04.09.2018 13:35

Entry 11/46: Test PCR of possible T7 integration clones
In Project: Protein Expression Strain
With tags: test pcr, T7, sad1306, protein expression strain, chloramphenicol resistance, dns

Test PCR on possible clones with integrated T7 was repeated again.

Primers:

1. oiGEM3018_flip_LacI_fwd + oiGEM3019_T7_rev_dnsover hang (T7 fragment)
2. oiGEM3017_dns_up_fwd + oiGEM3009_Cm_rev_frt (dns upstream fragment + Cm^R fragment)
3. oiGEM3010_Cm_fwd_frt + oiGEM3009_Cm_rev_frt (Cm^R f ragment)

Estimated fragment sizes:

1) 468 bp (if integrated)

2) 1442 bp (if integrated)

3) 942 bp (if integrated)

Preparation of DNA from colony

1. colony is resupended in 20 ml H_2O
2. samples are boiled at 95 °C for 10 min
3. sample is centrifuged at 13.000 rpm for 1 min

Reaction Mix

Amount	Compound
10 µl	2xTaq reaction mix
2 µl	DMSO
1 µl	Primer 1
1 µl	Primer 2
4 µl	H_2O
2 µl	DNA

PCR program

95 °C	3 min	
95 °C	30 sec	35 x
60 °C	30 sec	
72 °C	1 kbp per 1 min	
72 °C	10 min	
4 °C	∞	

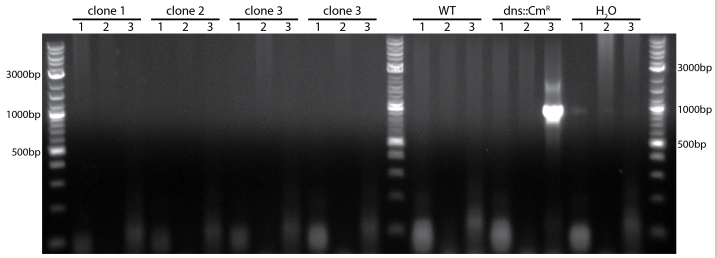
T7_Konstruktion_PCR.png

1 = oiGEM3017_dns_up_fwd & oiGEM3006_dns_up_rev_frt

2 = oiGEM3018_flip_LacI_fwd & oiGEM3019_T7_rev_dnsoverhang

3 = oiGEM3020_dns_down_fwd_T7 overhang
& oiGEM3021_dns_down rev2

1 % agarose gel loaded with 5 µl of PCR



Only the PCR amplifying the Cm^R resistance worked. And it only worked in the dns deletion strain which was used as a control.

Natural transformation gets repeated since we can't amplify fragments in the possible deletion candidates.

Author: Memduha Muratoglu
Entry 10/46: Test PCR 3 T7 strains
In Project: Protein Expression Strain
With tags: T7, test pcr, dns replacement with T7

created: 02.09.2018 16:19
updated: 02.09.2018 16:26

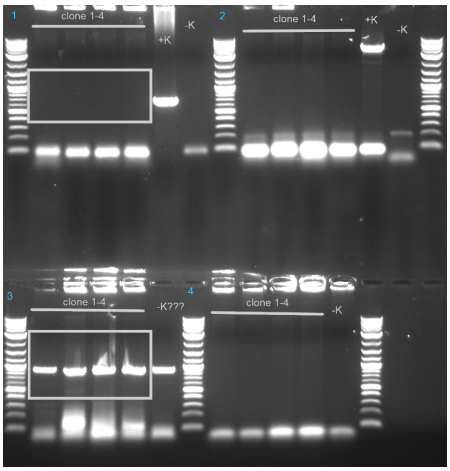
my date: 28.08.2018
my date: 31.08.2018

Amount	Compound
5 µl	Phusion GC buffer
0,5 µl	dNTPs
0.12 µl	Primer (1) fwd dns_fwd_NcoI / (2) oiGEM3018 // (3) oiGEM3010/ (4) oiGEM3006
0.12 µl	Primer (1) rev dns_rev_BamHI / (2) oiGEM3019 // (3) oiGEM3009/ (4) oiGEM3021
5 µl	PCR Enhancer
0.25 µl	Phusion Polymerase
ad. 25 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

Test_PCr_T7_3.PNG



Expected sizes 1: 700 bp in WT (should be negative in T7 strain :)); 2: ~5000bp; 3: 900bp, 4: 7000bp

Author: Memduha Muratoglu
Entry 9/46: Test PCR for T7 strain 2
In Project: Protein Expression Strain
With tags: T7, test pcr, dns replacement with T7

created: 02.09.2018 15:06
updated: 02.09.2018

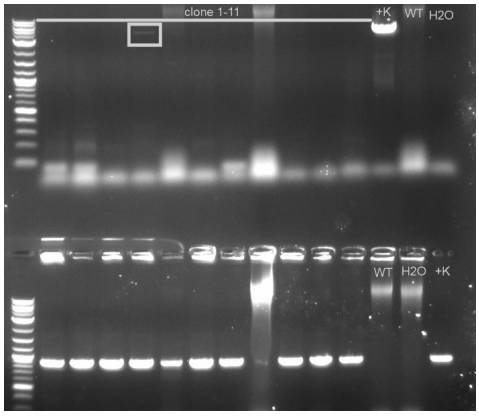
my date: 28.08.2018

Amount	Compound
5 µl	Phusion GC buffer
0,5 µl	dNTPs
0.12 µl	Primer fwd oiGEM3018 / oiGEM3010
0.12 µl	Primer rev oiGEM3019 /oiGEM3009
5 µl	PCR Enhancer
0.25 µl	Phusion Polymerase
ad. 25 µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per 60 sec	
72 °C	5 min	
4 °C	hold	

test_pcr_t7_2.PNG



expected size for first PCR was ~5000bp (T7 fragment),

expected size for second PCR was ~900bp (Cm fragment),

samples of clone 4 showed right fragments in both PCRs. First 4 clones were restreaked and will be tested again!

Author: Memduha Muratoglu
created: 02.09.2018 14:58
updated: 02.09.2018 15:02
Entry 8/46: Test PCR for T7 strain 1
In Project: Protein Expression Strain
my date: 27.08.2018
With tags: T7, test pcr, dns replacement with T7

colony is directly streaked into PCR cup.

Reaction Mix

Amount	Compound
12,5 µl	2xTaq reaction mix
2 µl	DMSO
0,25 µl	Primer 1 piGEM 3010 / dns_fwd_NcoI
0,25 µl	Primer 2 piGEM 3019 / dns_rev_BamHI
ad. 25 µl	H_2O

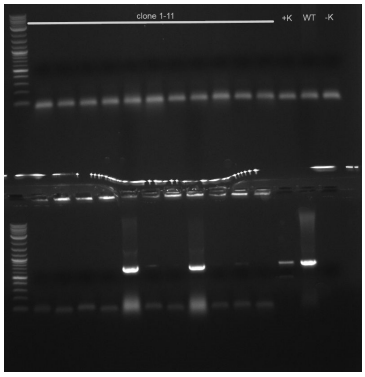
PCR program

95 °C	3 min	
95 °C	30 sec	35 x
60 °C	30 sec	
72 °C	1 kbp per 1 min	
72 °C	10 min	
4 °C	∞	

first PCR was to amplify chloramphenicol resistance and T7 fragment. Exprected size was ~5500bp

second PCR was to test, if dns is replaced by T7 fragment and should be negative. Otherwise ~700bp fragment is expected.

test_pcr_t7_1.PNG



first PCR did not work. second PCR shows some clones were no dns fragment could be amplified.

Author: Franziska Müller
created: 27.08.2018 14:32
updated: 04.09.2018 12:15

Entry 7/46: Test PCR of possible T7 integration clones
In Project: Protein Expression Strain
With tags: T7, protein expression strain, sad1306, test pcr

8 clones of Natural Transformation for Integration of T7 Polymerase unit - entry #5 in project 'Protein Expression Strain' (Franziska Müller, 27.08.2018) were tested for integration via test PCR.

Primers:

1) oiGEM3017 + oiGEM3008

2) oiGEM3019 + oiGEM3018

Estimated fragment sizes:

1) 6700bp

2) 4700bp

Preparation of DNA from colony

1. colony is resupended in 20 ml H_2O
2. samples are boiled at 95 °C for 10 min
3. sample is centrifuged at 13.000 rpm for 1 min

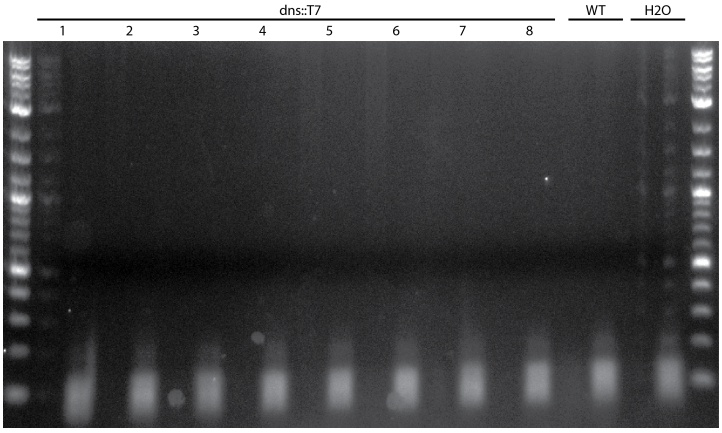
Reaction Mix

Amount	Compound
10 µl	2xTaq reaction mix
2 µl	DMSO
1 µl	Primer 1
1 µl	Primer 2
4 µl	H_2O
2 µl	DNA

PCR program

95 °C	3 min	
95 °C	30 sec	35 x
60 °C	30 sec	
72 °C	1 kbp per 1 min	
72 °C	10 min	
4 °C	∞	

20180827_testpcr_T7integration.png



PCR did not work

Author: Memduha Muratoglu

Entry 6/46: Alignment of Proteases

In Project: Protein Expression Strain

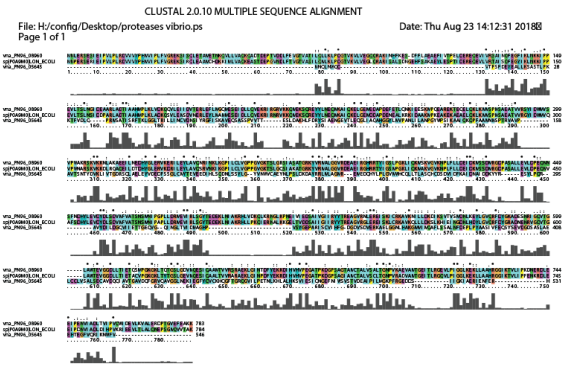
With tags: protein expression strain, alignment, lon, protease

created: 23.08.2018 14:25

updated: 02.09.2018 18:06

conserved lon Protease was found in *V. natriegens* with high homology to lon protease of *E. coli*

proteases_alignment.PNG



Author: Franziska Müller
created: 23.08.2018 11:14
updated: 27.08.2018 10:02
Entry 5/46: Natural Transformation for Integration of T7 Polymerase unit
In Project: Protein Expression Strain
With tags: natural transformation, dns, T7, chloramphenicol resistance, sad1306, SAD1495, protein expression strain

Strains containing *tfoX* plasmids (SAD1306, SAD1495) from the MuGENT for *V. natriegens* paper were kindly provided by Thorsten Waldminghaus. Google Drive Strain list

Both strains were transformed using the fragment that should integrate into the dns site and insert the T7 polymerase.

Additionally two controls without any plasmid were prepared.

Selection on LBv2-Chloramphenicol

1. Inoculate 3 ml of LBv2 + 100 µg/ml Carbenicillin + 100 µM IPTG in a culture tube. Grow at 30 °C for 12-18 hrs.
2. OD_{600nm} should be 7 - 10.
3. For each transformation reaction, take 3.5 µl of the ON culture and dilute into 350 µl of 2 XIO + 100 µM IPTG. Invert gently to mix. Don't forget a control without tDNA!!!!
4. Add 50 ng of tDNA and invert gently to mix.
5. Incubate reactions at 30 °C statically for 4-6 hours.
6. Add 1 ml LBv2 and outgrow at 30 °C shaking for 1-2 hours
7. Plate for selection

Materials

LBv2

400 ml	LB
32 ml	5M NaCl
3.4 ml	1M KCl
18.5 ml	1M $MgCl_2$

2XIO

28 g/l Instant ocean sea salts

Transformation DNA (= tDNA)

selected product that replaces gene of interest or a neutral locus

Homology on each side of the mutation: 0.5 kb - 3kb

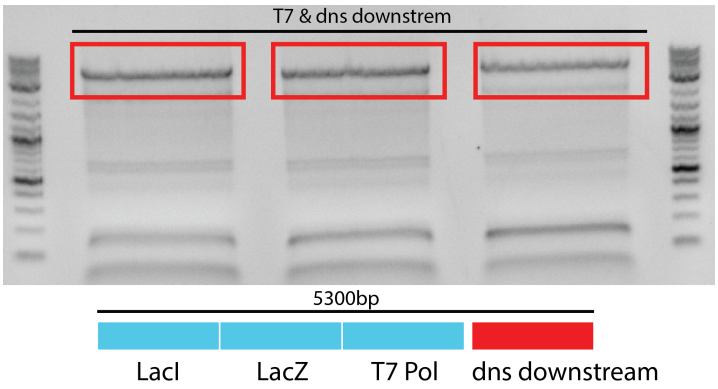
Plates of SAD1306 transformed with T/ integration casette showed colonies on plate.
24 colonies were restreakes on LBv2-Cml agar plates.

Author: Jana Jung
Entry 4/46: Amplification of T7 casette
In Project: Protein Expression Strain
With tags: Protein Expression, T7, fusion pcr

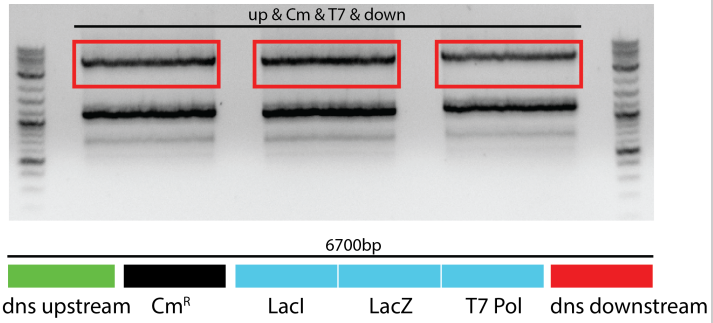
created: 22.08.2018 16:30
updated: 22.08.2018 16:34

PCR approach and programm see Memduhas entry "Amplification of T7 casette".

180822_T7_Fusins_PCR.png



180822_Fusions_PCR_up&Cm&T7&down.png



The fragments were cut out und purified.

Author: Memduha Muratoglu
Entry 3/46: amplification of T7 casette
In Project: Protein Expression Strain
No tags associated

created: 21.08.2018 15:30
updated: 26.09.2018 02:00

PCR

1. upstream dns

Substanz	**Menge**
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
Primer forward (oiGEM3017_dns_up_fwd)	2,5 µl
Primer reverse (oiGEM3006_dns_up_rev_frt)	2,5 µl
$dNTP_3$	1 µl
gDNA (*V. natriegens*)	1 µl
Polymerase	0,5µl

2. LacI / LacZ / T7 Pol

Substanz	**Menge**
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
Primer forward (oiGEM3018_flip_LacI_fwd)	2,5 µl
Primer reverse (oiGEM3019_T7_rev_dnsoverhang)	2,5 µl
$dNTP_3$	1 µl
gDNA (*E. coli BL21*)	1 µl
Polymerase	1,5µl

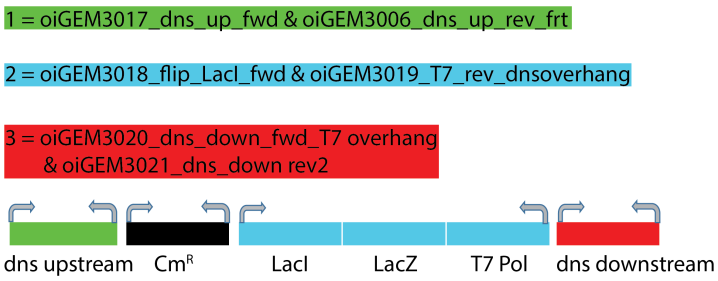
PCR Programm

Temperatur	Time	Cycles
96°C	3 min	
96°C	30 s	35x
65°C	30 s	
72°C	(1 min/kb)	
72°C	10 min	
4°C	∞	

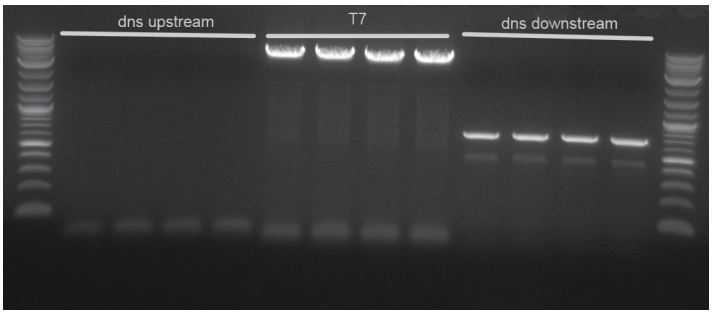
3. downstream dns

Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
Primer forward (oiGEM3020_dns_down_fwd_T7 overhang)	2,5 µl
Primer reverse (oiGEM3021_dns_down rev2)	2,5 µl
$dNTP_3$	1 µl
´gDNA (*V. natriegens*)	1 µl
Polymerase	0,5µl

T7_Konstruktion_PCR.png



dns_up,_t7,_dns_down_pcr.PNG



Expected size for Upstream fragment: 700bp; T7 Fragment: ~4600bp, dns downstream fragment: 700bp
PCRs were loaded to an 1% agarose gel.

T7 and downstream PCRs were pooled and downstream PCR was loaded to an 1% agarose gel for gelextraction. Both products were purified and used for phusion PCR.

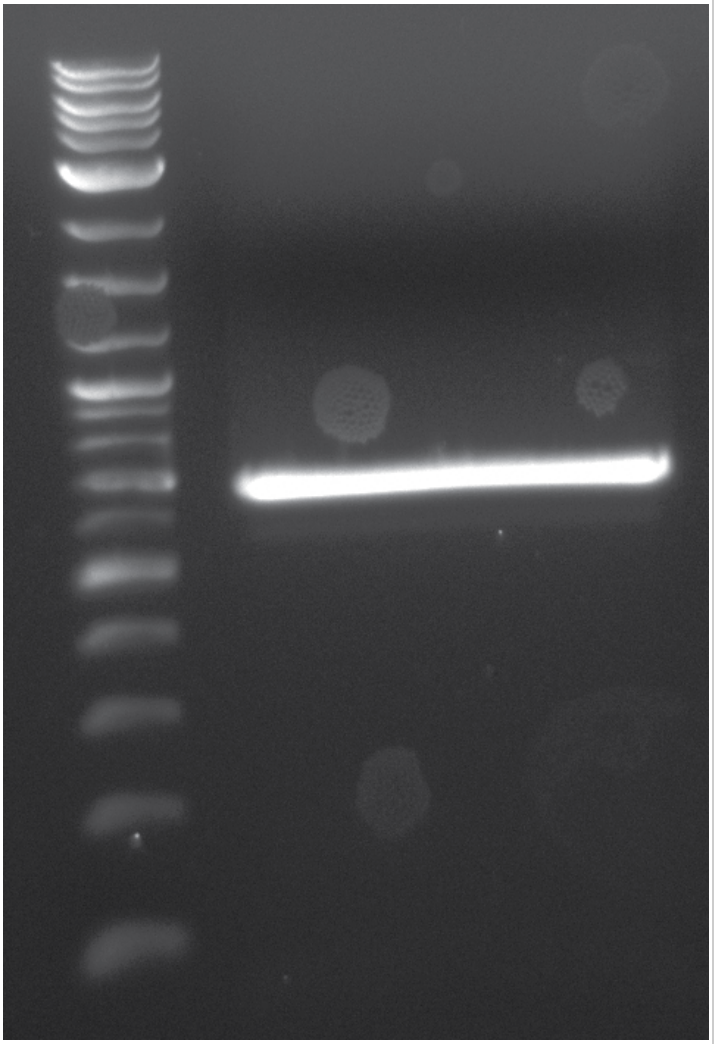
Gel_extraction_protocol.PNG

PCR clean-up, gel extraction

Protocol-at-a-glance (Rev. 06)

		NucleoTraP®CR PCR clean-up	NucleoTrap® Gel extraction
1	NucleoTrap®: Excise DNA fragment / Solubilize gel slice NucleoTraP®CR: Adjust binding conditions	4 vol NT2 / 1 vol sample	300 µL NT1 / 100 mg gel
2	Bind DNA	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s	4 µL silica matrix / µg DNA 50 °C, 5–10 min 10,000 x *g*, 30 s
3	Wash silica matrix	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s	1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4	Dry silica matrix	RT or 37 °C, 10–15 min	RT or 37 °C 10–15 min
5	Elute DNA	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s	25–50 µL NE RT, 10–15 min 10,000 x *g*, 30 s

dns_downstream_210818.PNG



Using those purified fragments and the upstream-chloramphenicol fragments (also used for dns deletion casette) phusion PCRS were performed.

Amount	Compound
10 µl	Phusion GC buffer
1 µl	dNTPs
2,5 µl	Primer fwd
2.5 µl	Primer rev
10 µl	PCR Enhancer
0.5 µl	Phusion Polymerase
1 µl	fragments: T7 & dns downstream // upstreamdns-cm & T7 & downstream dns
ad. 50µl	H2O

Programm

95 °C	3 min	
95 °C	30 sec	x35
60 °C	30 sec	
72 °C	1000 bp per min	
72 °C	5 min	
4 °C	hold	

Expected size: 5300 bp // 6300 bp

Author: Jana Jung
Entry 2/46: PCR T7 Strain
In Project: Protein Expression Strain
No tags associated

created: 20.08.2018 10:51
updated: 04.09.2018 17:22

PCR

1. upstream dns

Substanz	**Menge**
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3017_dns_up_fwd)	1,25 µl
Primer reverse (oiGEM3006_dns_up_rev_frt)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA (*V. natriegens*)	1 µl
Polymerase	1,5µl

PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	3 min	
96°C	Denaturierung	30 s	40x
65°C	Primerhybridisierung	30 s	
72°C	Elongation	5min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

2. LacI / LacZ / T7 Pol

Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3018_flip_LacI_fwd)	1,25 µl
Primer reverse (oiGEM3019_T7_rev_dnsoverhang)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA (*E. coli BL21*)	1 µl
Polymerase	1,5µl

3. downstream dns

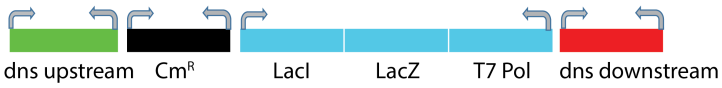
Substanz	Menge
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3020_dns_down_fwd_T7 overhang)	1,25 µl
Primer reverse (oiGEM3021_dns_down rev2)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA (*V. natriegens*)	1 µl
Polymerase	1,5µl

T7_Konstruktion_PCR.png

1 = oiGEM3017_dns_up_fwd & oiGEM3006_dns_up_rev_frt

2 = oiGEM3018_flip_LacI_fwd & oiGEM3019_T7_rev_dnsoverhang

3 = oiGEM3020_dns_down_fwd_T7 overhang
& oiGEM3021_dns_down rev2



Author: Memduha Muratoglu
Entry 1/46: preparation of genomig DNA from E.coli BL21
In Project: Protein Expression Strain
No tags associated

created: 15.08.2018 16:50
updated: 15.08.2018 17:10

my date: 13.08.2018

Lysis

- 5ml BL21 culture centrifugation (10min/RT/4700rpm)
- diltute 2µl of 50mg/ml Proteinase K in 300µl $H_2O$and 300µl T+C Lysis Solution.
- Resuspend cellpellet in 600µl of the solution and incubate for 15min at 65°C
- Incubate 5 min on ice
- add 2µl of 5mg/ml RNaseA and mix up
- incubate 30min at 37°C
- incubate 5min on ice.

Precipitation of total DNA

- Add 360µl MPC Protein Precipitation Reagent to 600µl of lysate
- Vortexe for 10 sec
- centrifugation (10min/10.000rcf/4°C)
- transfer supernatant to new eppendorf cup
- add 1000µl cold Isopropanol
- inverte 30-40X
- centrifugation (4°C/10min)
- discard supernatant
- rinse 2x with 2ml 75% Ethanol
- resuspend DNA in 50µl EB Buffer