Author: Jana Jung

Entry 20/20: Amplification dns up down and lacZ up down lacOperon

In Project: Cloning Strain

No tags associated

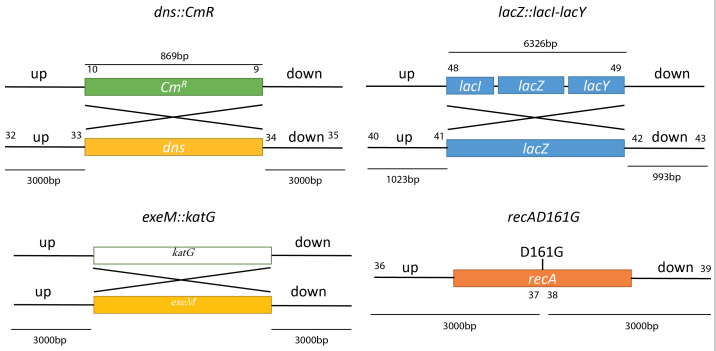
created: 08.10.2018 13:24

updated: 10.10.2018 02:28

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment	1 µl
Polymerase	0,5µl

VibriClone_Design2.png



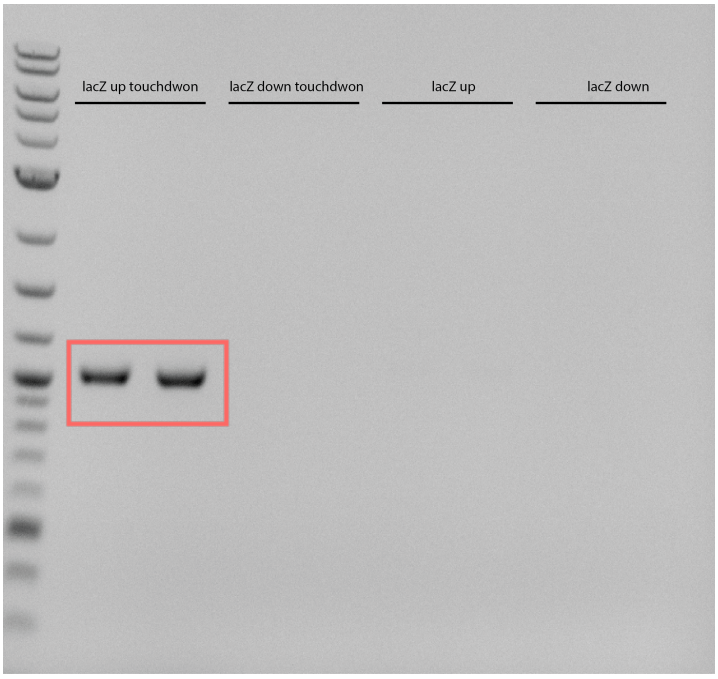
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	30s/kb	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

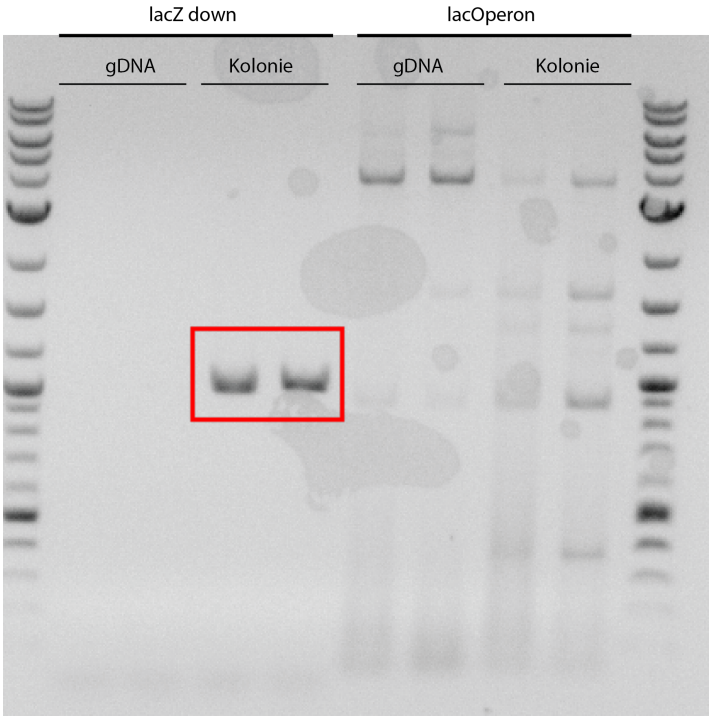
Primer

See the picture

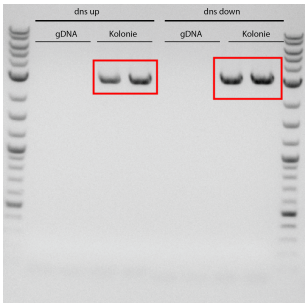
181007_up_down_lacZ.png



181008_lacZ_down_LacOperon.png

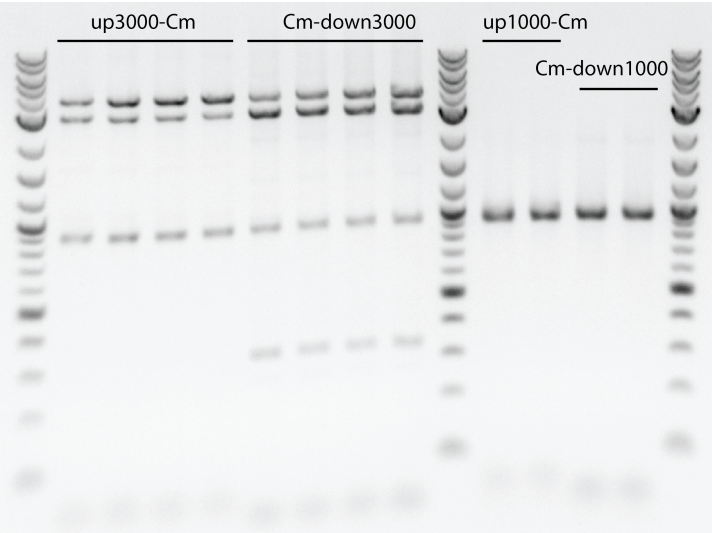


181008_dns_up_down.png

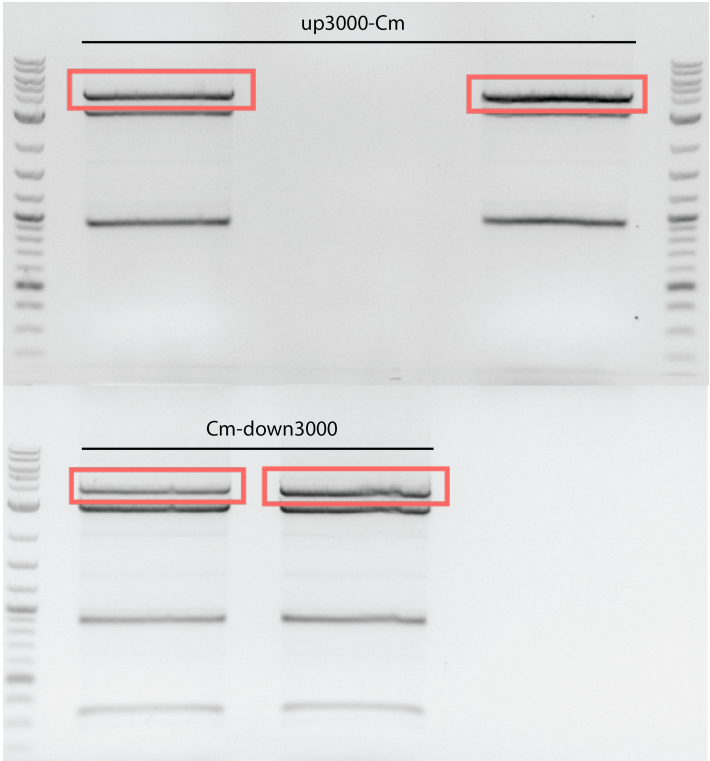


The PCR approaches are purifed and a Fusion-PCR will be done.

181010_up-Cm_Cm-down_3000_1000.png



181010_up-Cm_Cm-down.png



Expected size: ≈ 4000bp

Author: Jana Jung
Entry 19/20: No entry title yet
In Project: Cloning Strain
No tags associated

created: 07.10.2018 14:39
updated: 08.10.2018 13:36

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment	1 µl
Polymerase	0,5µl

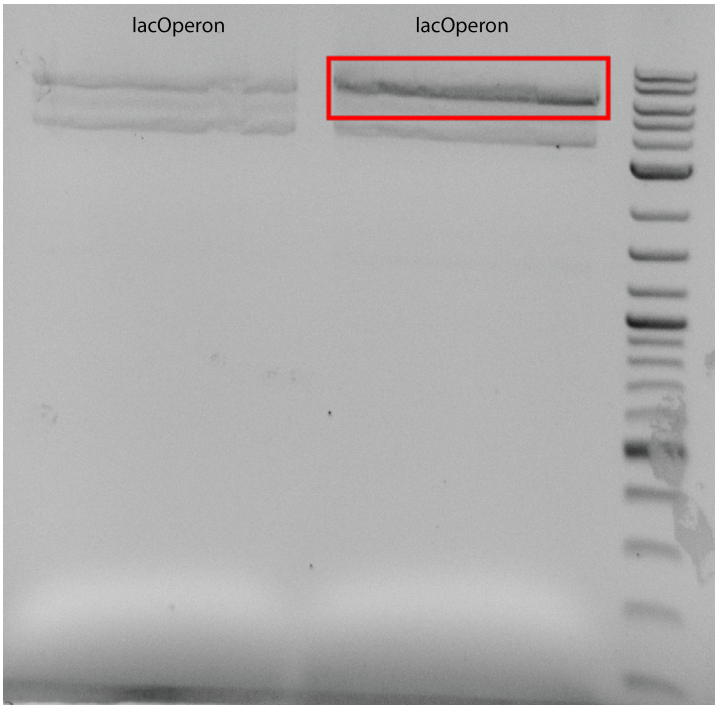
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	4min (30s/kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

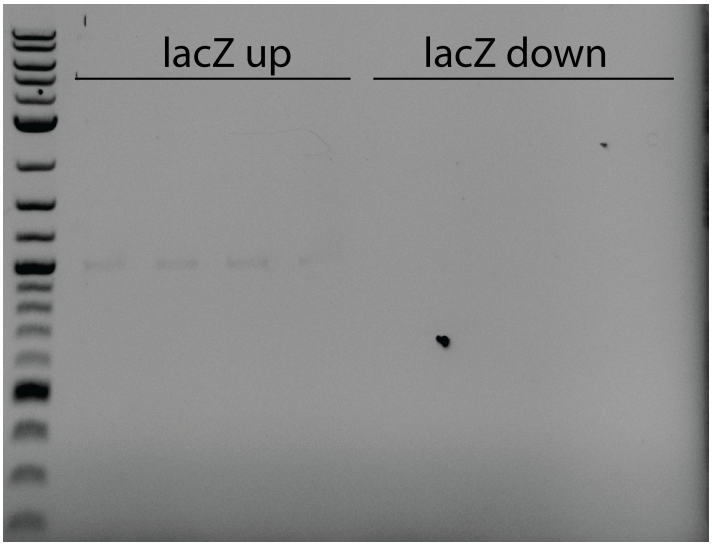
Primer

See the picture

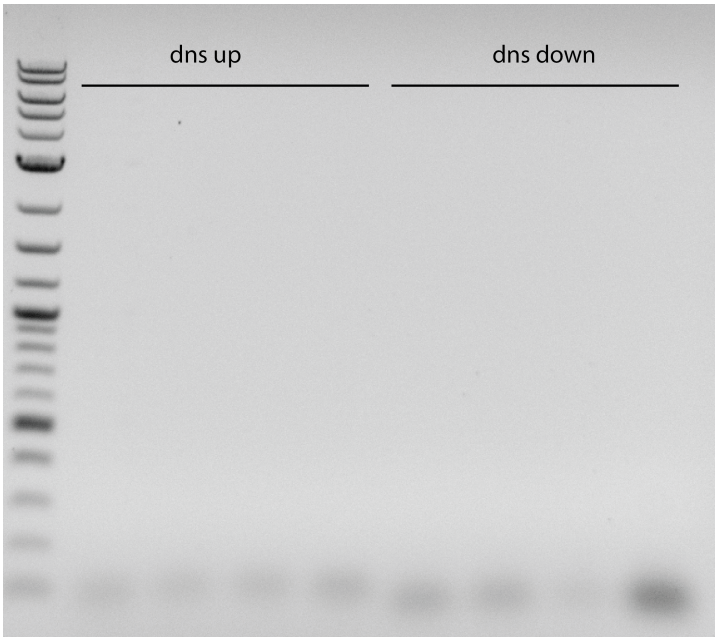
181007_lacOperon.png



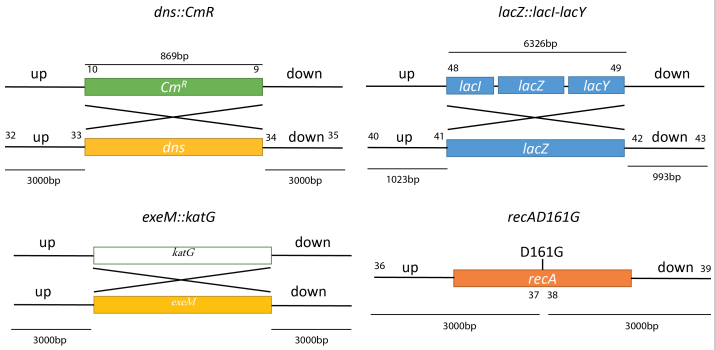
181006_lacZ_up_down.png



181007_up_down_dns.png



VibriClone_Design2.png



Author: Jana Jung
Entry 18/20: Fusions PCR dns and lacOperon
In Project: Cloning Strain
No tags associated

created: 04.10.2018 22:00
updated: 04.10.2018 22:32

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment	1 µl
Polymerase	0,5µl

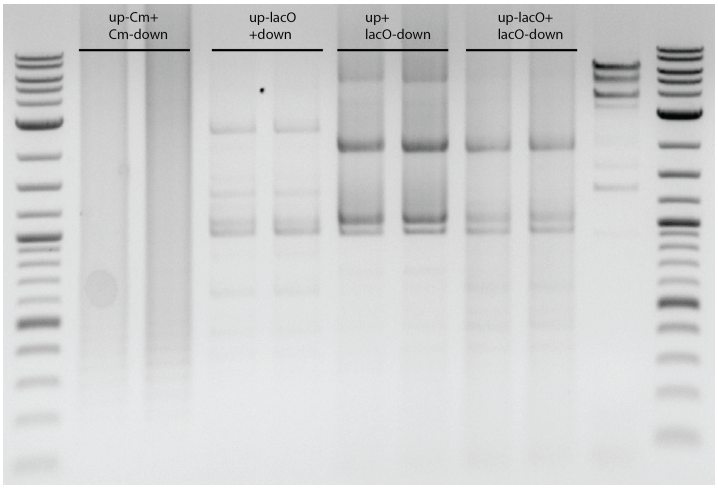
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	4min (30s/kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

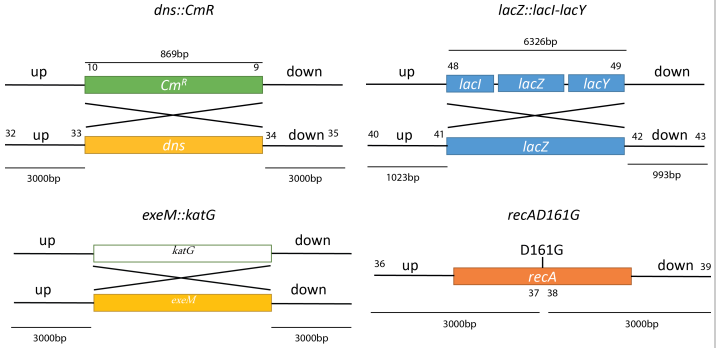
Primer

See the picture

181004_dns_kassette_lac_Operon.png



VibriClone_Design2.png



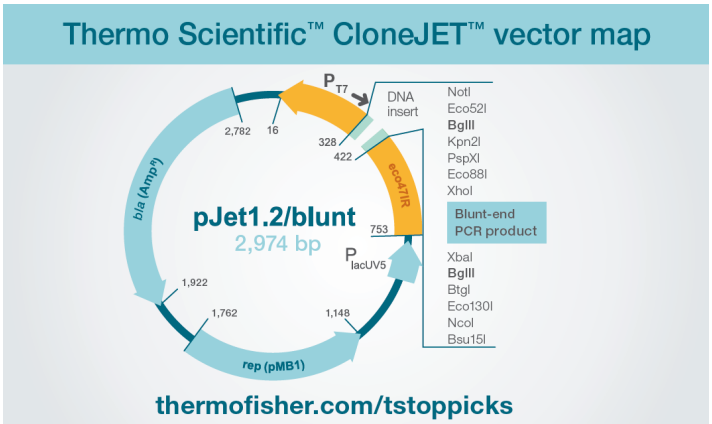
Author: Jana Jung
Entry 17/20: Lac-Operon
In Project: Cloning Strain
No tags associated

created: 04.10.2018 13:17
updated: 04.10.2018 13:26

pjet Liagtion

Substance	Amount
Ligase buffer	2µl
Ligase	1µl
pjet Vector	0,5µl
Fragment (lacZ up - lac Operon - lacZ down 01.10.18)	4µl
H_2O	12µl

pjet.png



Transformation of E. coli

- Allow competent cells to melt on ice

- Under sterile conditions, 10 µl of the ligation are added to the competent cells

- 2min on ice- Heat shock: 1min 40sec at 42 ° C

- 5min on ice

- Add 1ml LB

- 1 h on the shaker at 37 ° C

- Centrifugation (1min / 11.000rpm / RT)

- Remove supernatant

- Resuspend in 40µl LB

- Plate on LB + antibiotic (Ampicilin)

- Incubate at 37 ° C

The grown colonies are inoculated and the next day a plasmid preparation is performed.

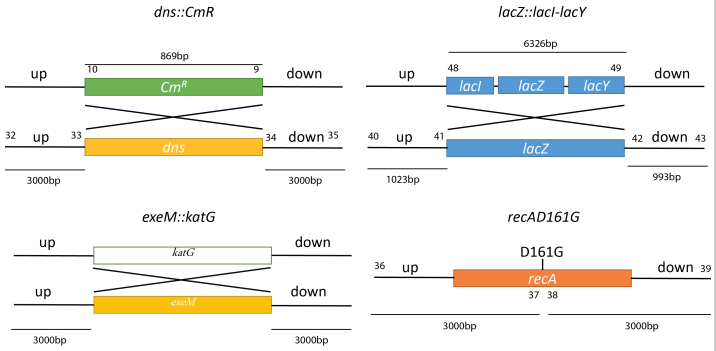
Author: Jana Jung
Entry 16/20: No entry title yet
In Project: Cloning Strain
No tags associated

created: 03.10.2018 13:40
updated: 04.10.2018 22:31

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment	1 µl
Polymerase	0,5µl

VibriClone_Design2.png



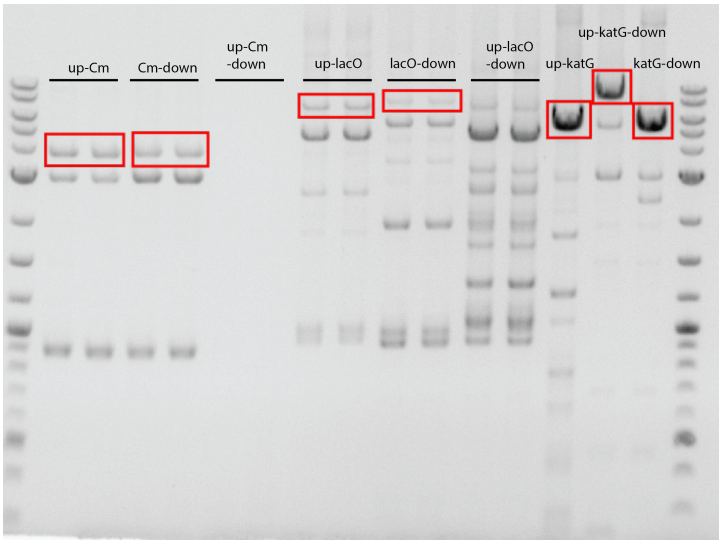
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	4min (30s /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

Primer

See the picture

181003_Fusion_dns_kasette,_lacOperon,_exeM--katG.png



The circled approach were mixed together and reapplied to gel purification.

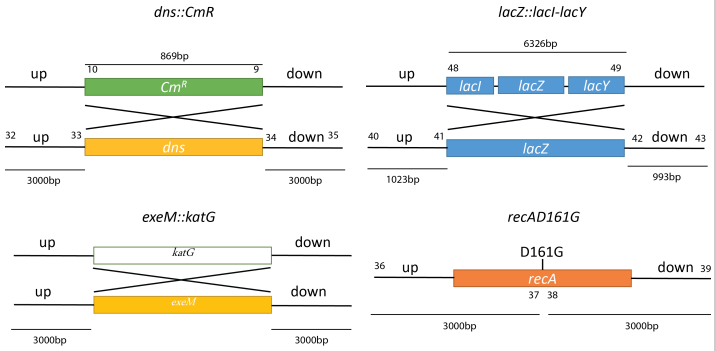
Author: Jana Jung
Entry 15/20: Amplification of dns, katG and lac Fragments
In Project: Cloning Strain
No tags associated

created: 02.10.2018 13:07
updated: 03.10.2018 13:40

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment	1 µl
Polymerase	0,5µl

VibriClone_Design2.png



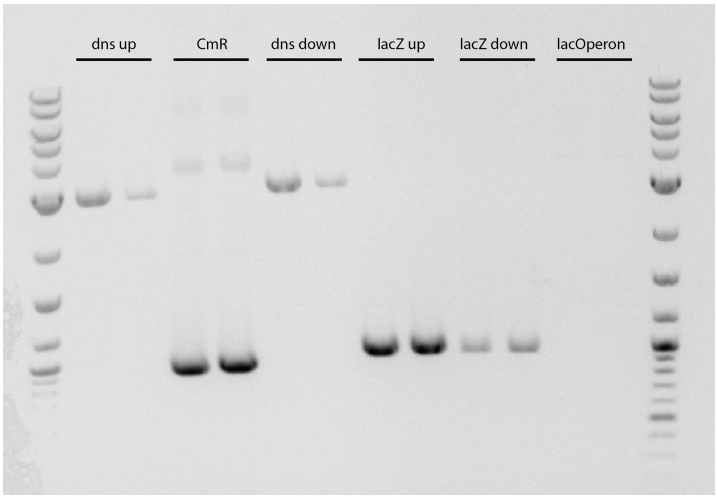
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	4min (30s /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

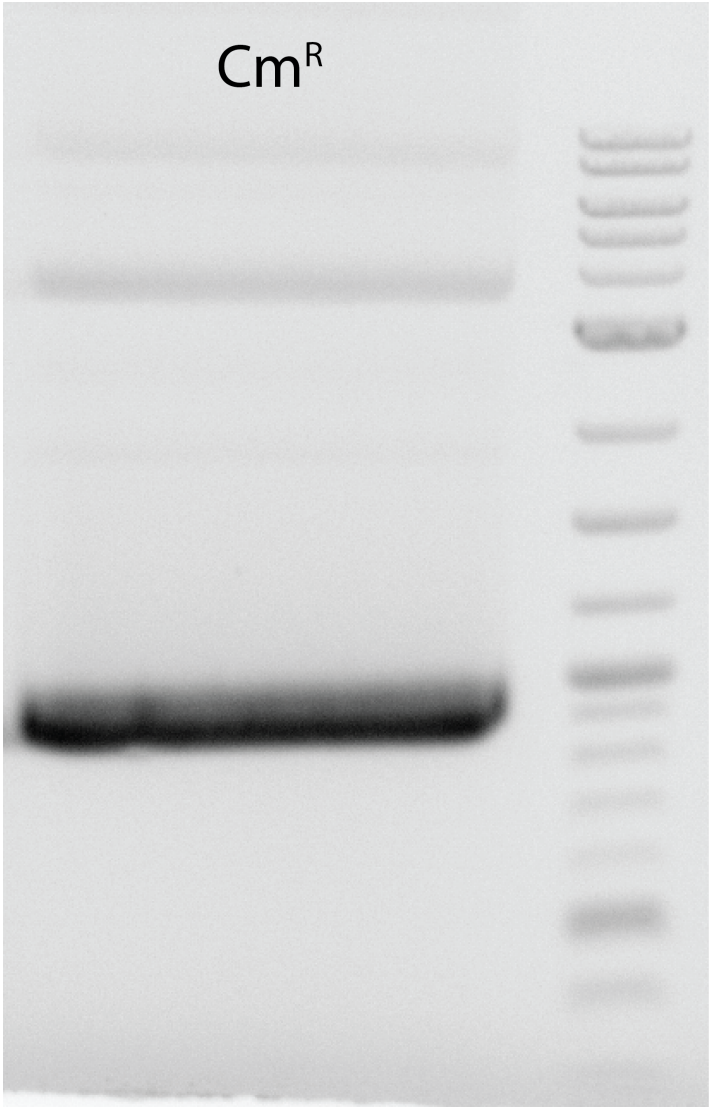
Primer

See the picture

181002_dns_lac_Fragmente.png

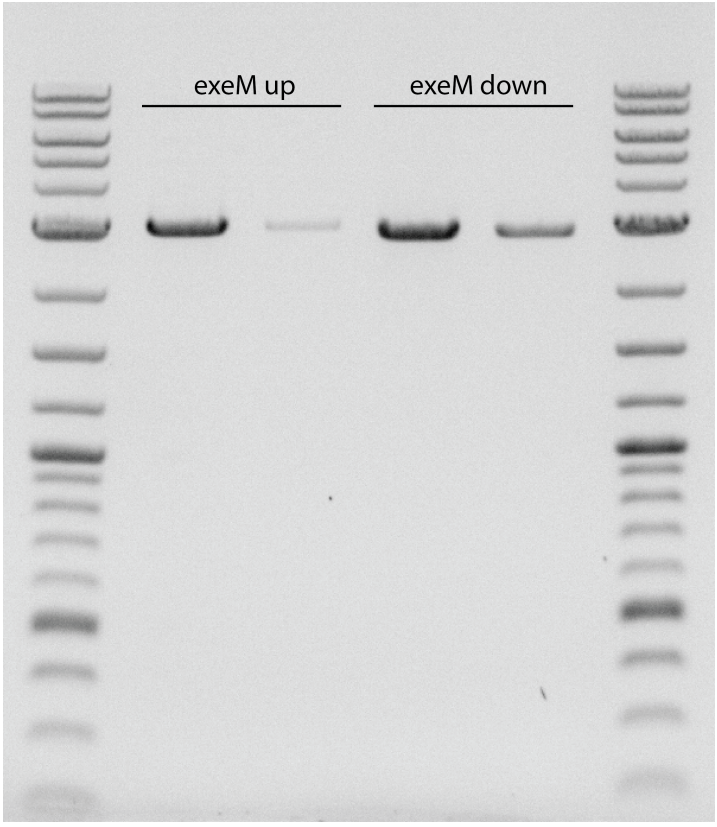


181002_CmR.png



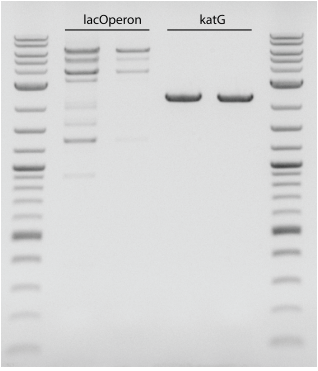
dns up, dns down, lacZ up and lacZ down are purified and Cm is purified from the gel. The PCR from the lacOperon is made again.

181002_exeM_up_down.png



The PCR approaches are purified

181002_lacO_katG.png



Author: Jana Jung
Entry 14/20: Fusions PCR lac Operon
In Project: Cloning Strain
No tags associated

created: 01.10.2018 01:10
updated: 01.10.2018 14:43

PCR Approach

Substance	Amount
H_2O	31µl
Q5 Reactionpuffer	10 µl
DMSO	1,5 µl
Primer forward	2,5 µl
Primer reverse	2,5 µl
$dNTP_3$	1 µl
Fragment 1	1 µl
Fragment 2	1 µl
Polymerase	0,5µl

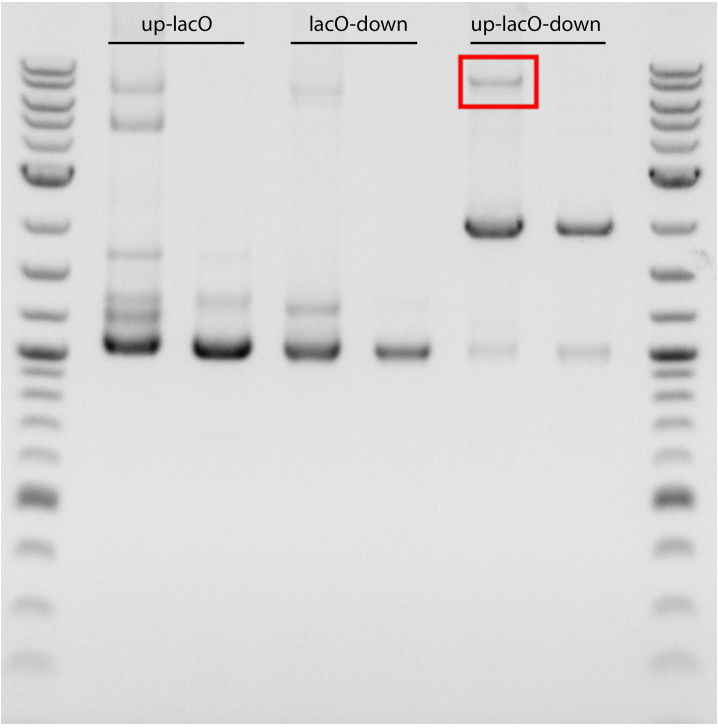
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	2 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	4min (30s/kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

Primer

	upstream+lac Operon	downstream + lac Operon	upstream+lac Operon + downstream
Primer forward	oiGEM3040_lacZ_up_fwd	oiGEM3048_lac_operon_fwd	oiGEM3040_lacZ_up_fwd
Primer reverse	oiGEM3049_lac_operon_rev	oiGEM3043_lacZ_down_rev	oiGEM3043_lacZ_down_rev

181001_lac_Operon_up-lacO_lacO-down_up-lacO-down.png



Author: Jana Jung
Entry 13/20: Fusions PCR dns cassette
In Project: Cloning Strain
No tags associated

created: 30.09.2018 22:39
updated: 01.10.2018 01:18

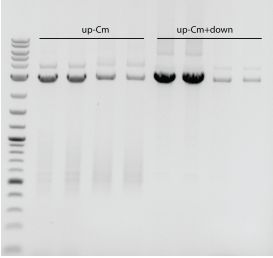
PCR Approach

Substance	Amount
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	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	6min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180930_Cm-up_up-Cm+down.png



Author: Jana Jung
Entry 12/20: No entry title yet
In Project: Cloning Strain
No tags associated

created: 30.09.2018 22:29
updated: 30.09.2018 22:40

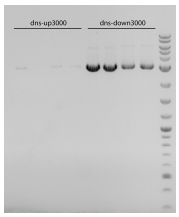
PCR Approach

Substance	Amount
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

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	3min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180930_dns-up_dns-down.png



Author: Jana Jung
Entry 11/20: Fusions PCR dns
In Project: Cloning Strain
No tags associated

created: 28.09.2018 17:37
updated: 30.09.2018 22:30

PCR Approach

Substance	Amount
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

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	6min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

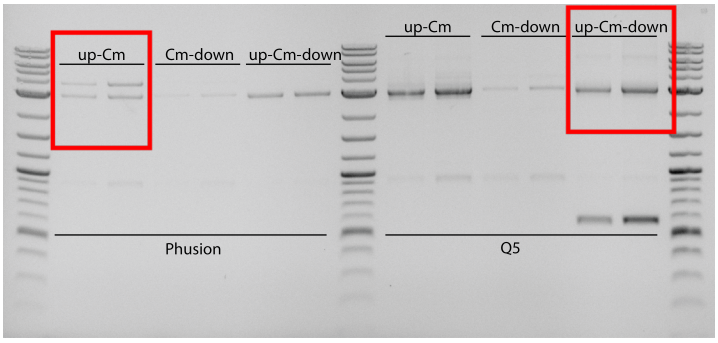
Primer

	upstream+Cm	upstream+Cm+downstream	Cm+downstream
Primer forward	oiGEM3032_dns_up_fwd_3000	oiGEM3032_dns_up_fwd_3000	oiGEM3010_Cm_fwd_frt
Primer reverse	oiGEM3009_Cm_rev_frt	oiGEM3035_dns_down_rev_3000	oiGEM3035_dns_down_rev_3000

DNA

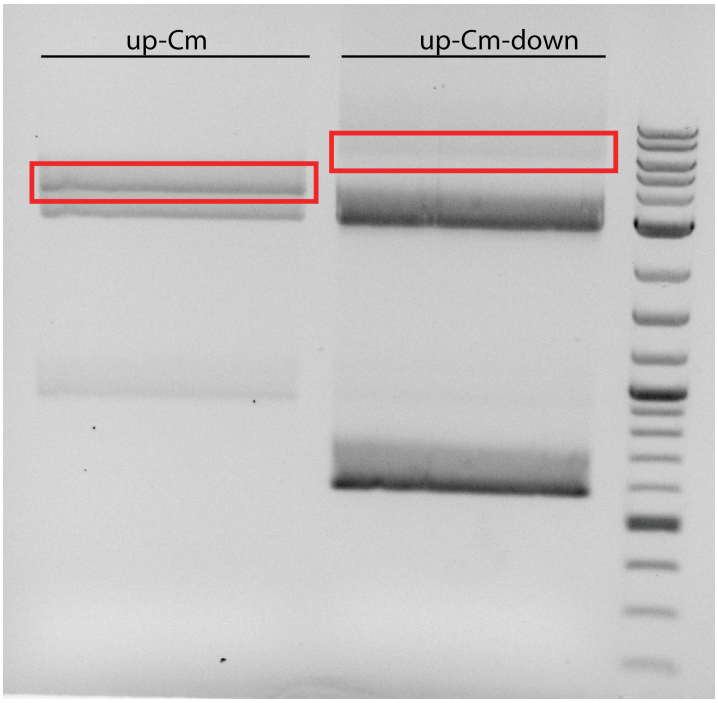
	Upstream	CmR	Downstream
1	50ng	15,89ng	50ng
2	100ng	31,7ng	100ng

180929_dns_up-Cm_up-Cm-down_Cm-down.png



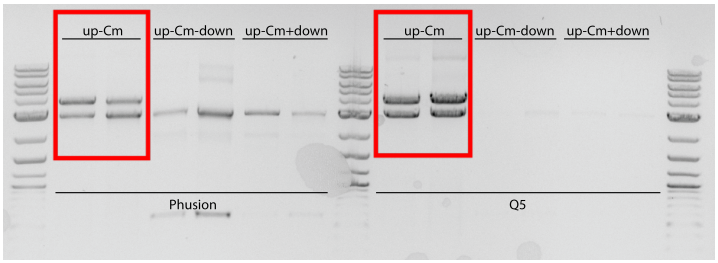
The circled approach were mixed together and reapplied to gel clean.

180929_dns_up-Cm_up-Cm-down_ausschneiden.png



´The bands were cut out and purified. The product was used for a new PCR.

180929_dns_up-Cm_up-Cm-down.png



The circled approach were mixed together and reapplied to gel clean.

Author: Jana Jung
Entry 10/20: Fusions PCR lac Operon
In Project: Cloning Strain
No tags associated

created: 27.09.2018 23:15
updated: 28.09.2018 00:11

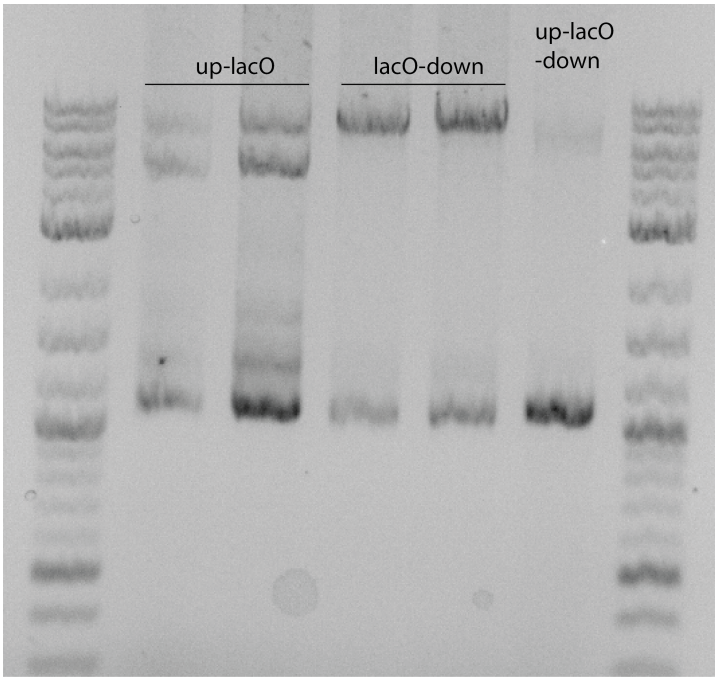
PCR Approach

Substance	Amount
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward up-lacO: oiGEM3040_lacZ_up_fwd lacO-down: oiGEM3048_lac_operon_fwd up-lacO-down: oiGEM3040_lacZ_up_fwd	1,25 µl
Primer reverse up-lacO: oiGEM3049_lac_operon_rev lacO-down: oiGEM3043_lacZ_down_rev up-lacO-down: oiGEM3043_lacZ_down_rev	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	0,5µl

PCR Programm

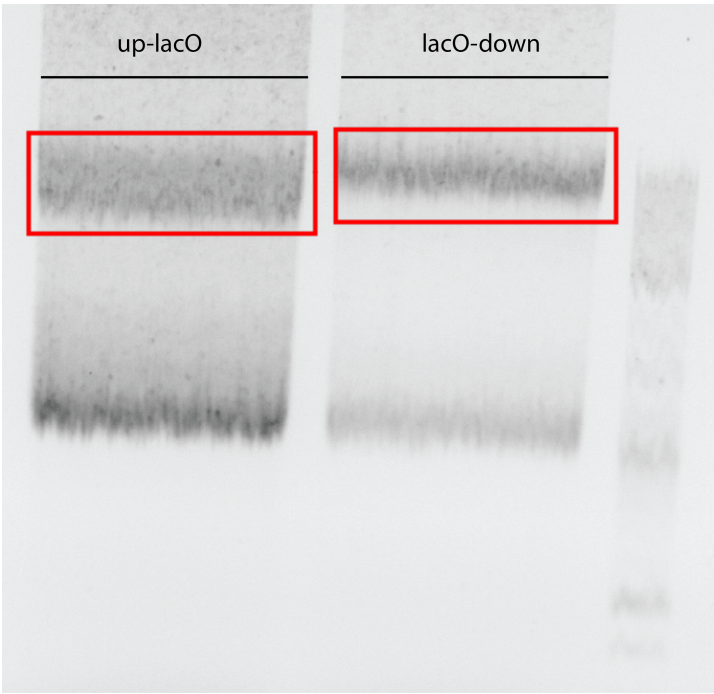
Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	8min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180927_lacO-up_lacO-down.png



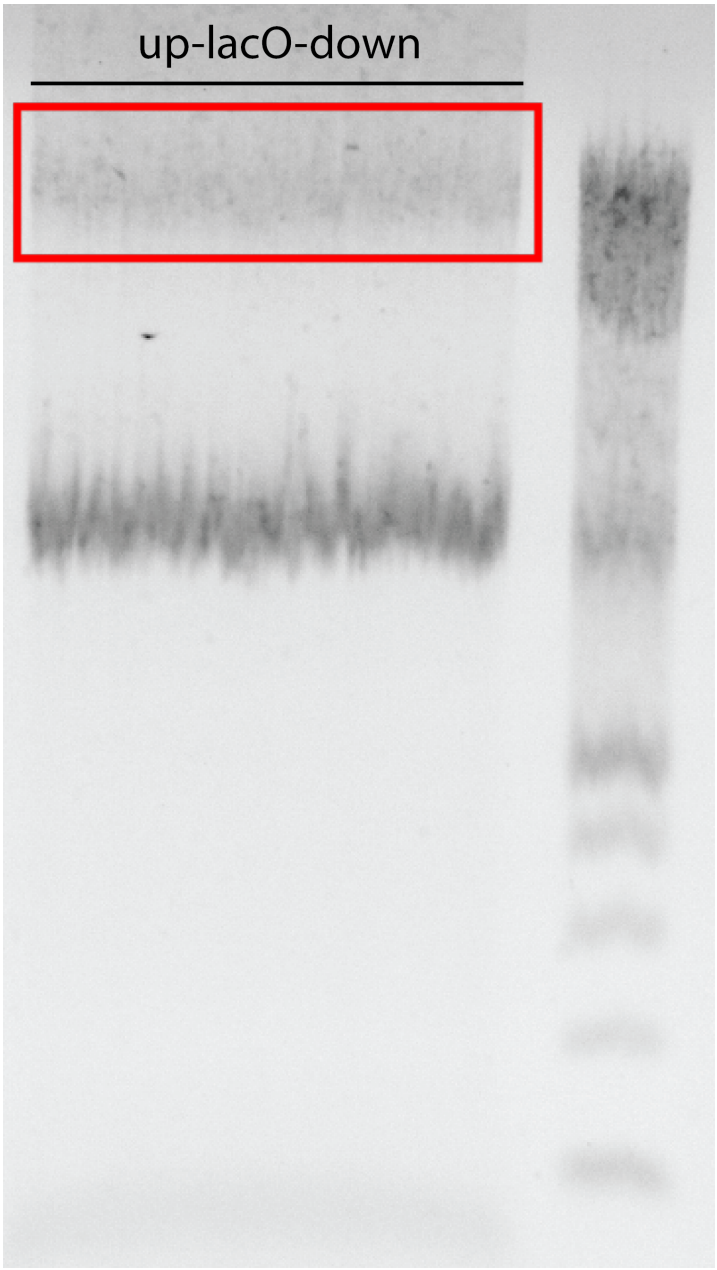
The entire PCR approach is applied to excise the bands.

180927_lacO-up_lacO-down_ausschneiden.png



Although the ladder is not easily recognizable, the visible band is cut out. After gel purification, a PCR is done on it.

180927_Fusions_PCR_up-lacO-down.png



Although the ladder is not easily recognizable, the visible band is cut out. After gel purification, a PCR is done on it.

Author: Jana Jung
Entry 9/20: Fusions PCR dns
In Project: Cloning Strain
No tags associated

created: 27.09.2018 00:38
updated: 27.09.2018 23:14

PCR Approach

Substance	Amount
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3032_dns_up_fwd_3000)	1,25 µl
Primer reverse (oiGEM3033_dns_up_rev_frt_3000)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 µl
Polymerase	0,5µl

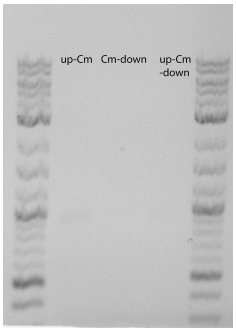
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	20x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	3min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

Primer

	upstream+Cm	upstream+Cm+downstream	Cm+downstream
Primer forward	oiGEM3032_dns_up_fwd_3000	oiGEM3032_dns_up_fwd_3000	oiGEM3010_Cm_fwd_frt
Primer reverse	oiGEM3009_Cm_rev_frt	oiGEM3035_dns_down_rev_3000	oiGEM3035_dns_down_rev_3000

180927_dns-up_dns-down.png



Author: Jana Jung
Entry 8/20: Amplification of upstream dns
In Project: Cloning Strain
No tags associated

created: 26.09.2018 22:32
updated: 27.09.2018 00:38

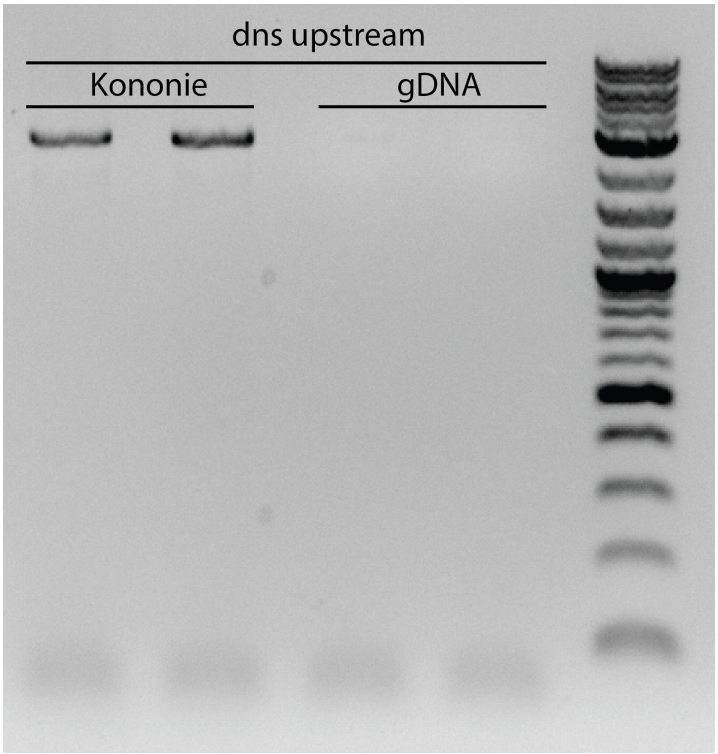
PCR Approach

Substance	Amount
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward (oiGEM3032_dns_up_fwd_3000)	1,25 µl
Primer reverse (oiGEM3033_dns_up_rev_frt_3000)	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 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	3min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180926_dns_upstream_3000.png



The PCR is cleaned up.

Author: Jana Jung
Entry 7/20: Fusion PCR Lac Operon with up and down from lacZ
In Project: Cloning Strain
No tags associated

created: 26.09.2018 22:30
updated: 27.09.2018 01:04

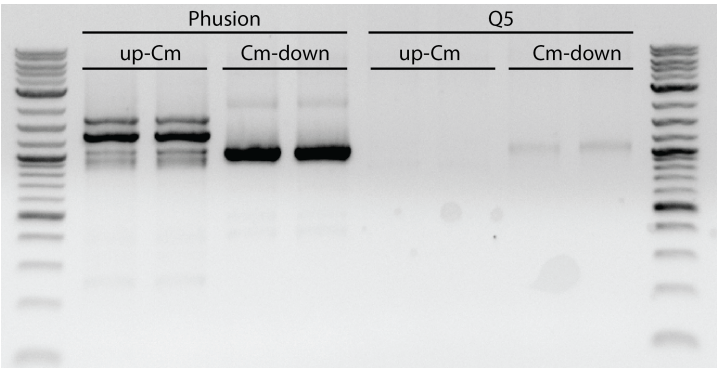
PCR Approach

Substance	Amount
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
DMSO	5 µl
Primer forward up-lacO: oiGEM3040_lacZ_up_fwd lacO-down: oiGEM3048_lac_operon_fwd	1,25 µl
Primer reverse up-lacO: oiGEM3049_lac_operon_rev lacO-down: oiGEM3043_lacZ_down_rev	1,25 µl
$dNTP_3$	1 µl
Genomische DNA	1 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	7min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180925_Fusion_PCR_LacOperon-up_and_-down.png



The PCR is repeated.

Author: Jana Jung
Entry 6/20: Fusions PCR Lac Operon und ddns
In Project: Cloning Strain
No tags associated

created: 25.09.2018 23:25
updated: 27.09.2018 00:29

PCR Approach

Substance	Amount
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	1,5µl

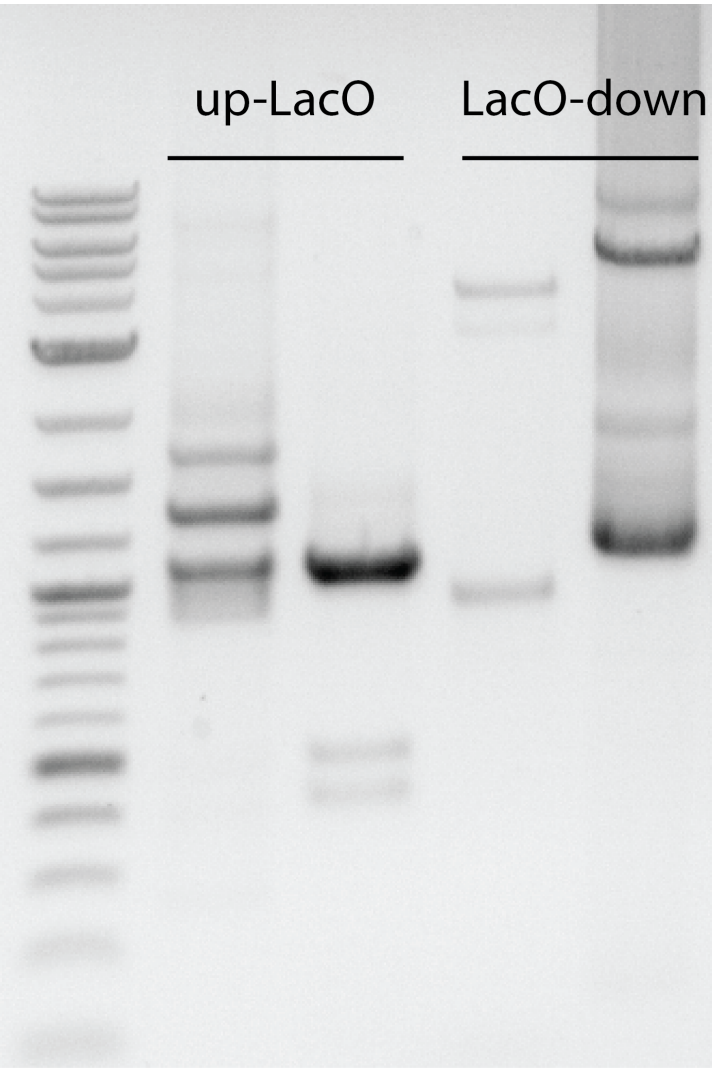
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	30x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	7min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

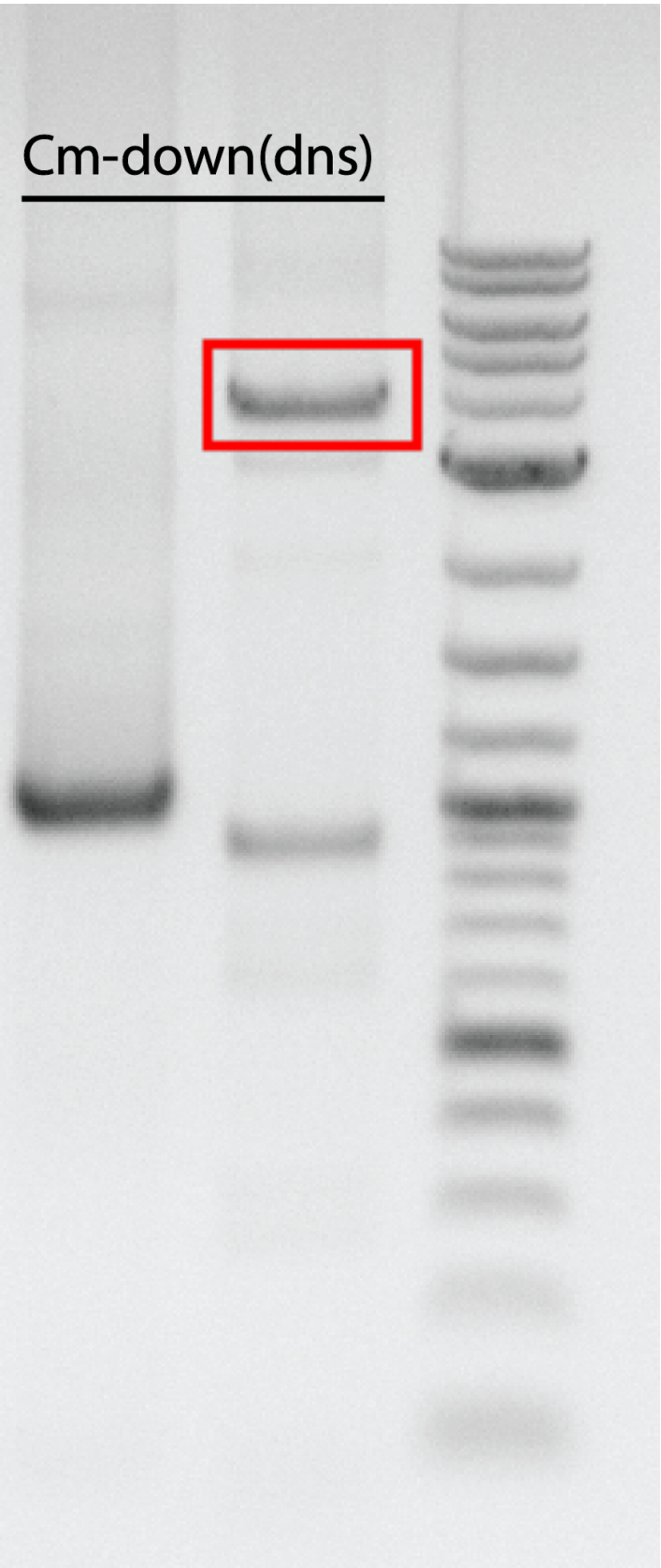
Primer

	up(lacZ)-lacO	lacO-down(lacZ)	Cm-down(dns)
Primer forward	oiGEM3040_lacZ_up_fwd	oiGEM3048_lac_operon_fwd	oiGEM3010_Cm_fwd_frt
Primer reverse	oiGEM3049_lac_operon_rev	oiGEM3043_lacZ_down_rev	oiGEM3035_dns_down_rev_3000

180925_Fusions_PCR_LacO-up_LacO-down.png



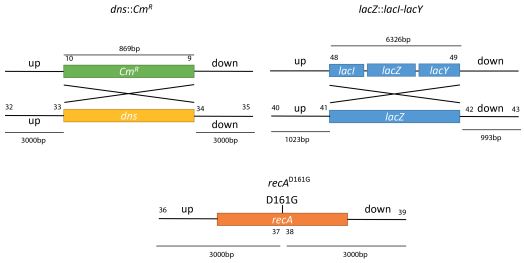
180925_Fusion_Cm-dnsdown.png



The PCR is repeated.

The band was cut out.

VibriClone_Design.png



Author: Jana Jung
Entry 5/20: Amplification of lac operon
In Project: Cloning Strain
With tags: lac operon

created: 24.09.2018 23:38
updated: 27.09.2018 00:37

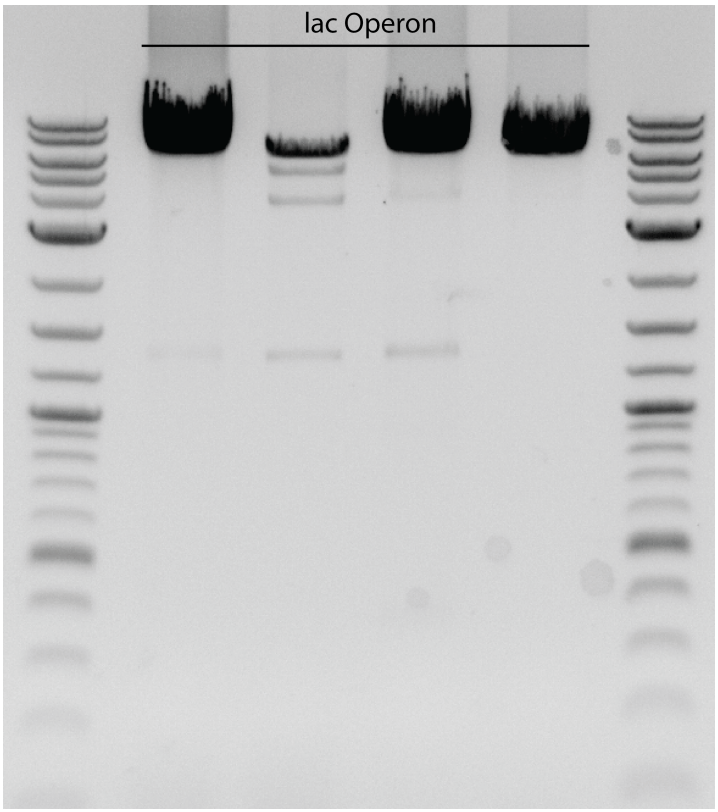
PCR Approach

Substance	Amount
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	1,5µl

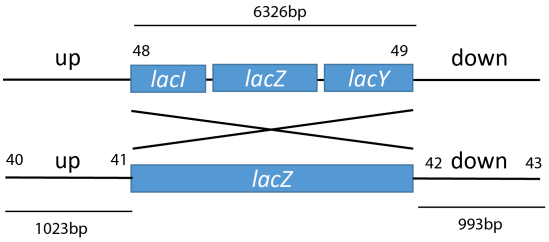
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	30x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	6min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180924_lac_operon.png

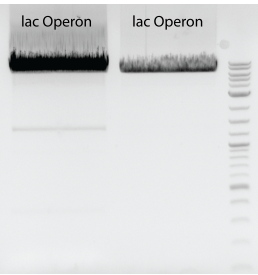


lac_Operon_Design.png



lacZ::lacI-lacY

180925_lacOperon_ausschneiden.png



Author: Jana Jung
Entry 4/20: Fusions PCR of recA
In Project: Cloning Strain
No tags associated

created: 22.09.2018 22:24
updated: 23.09.2018 12:43

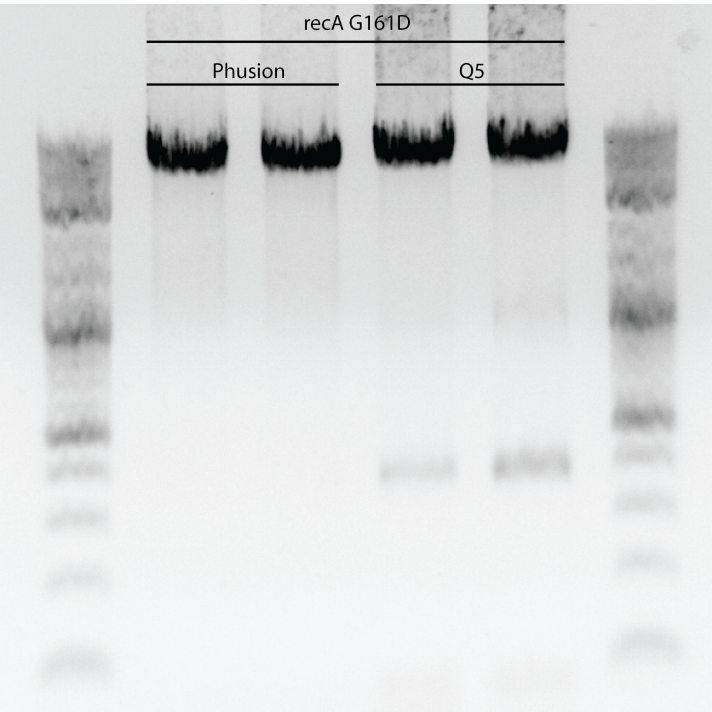
PCR-Approach

Substance	Amount
H_2O	20µl
Enhancer	10 µl
Buffer GC	10 µl
Primer forward oiGEM3036_recA_up_fwd	1,25 µl
Primer reverse oiGEM3039_recA_down_rev	1,25 µl
$dNTP_3$	1 µl
recA up	0,5 µl
recA down	0,5µl
Polymerase (1. Phusion, 2. Q5)	0,5µl

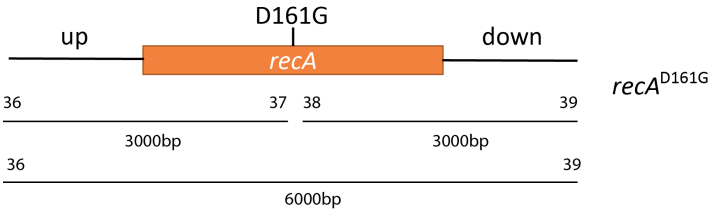
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	30x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	6min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

180923_recA_Fusions_PCR.png



recA_Design.png



Because the marker is not clearly visible the Gel is repeated.

Author: Jana Jung
Entry 3/20: Amplification dns recA and lacZ
In Project: Cloning Strain
No tags associated

created: 22.09.2018 21:13
updated: 22.09.2018 22:51

PCR-Approach

Substance	Amount
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	0,5µl

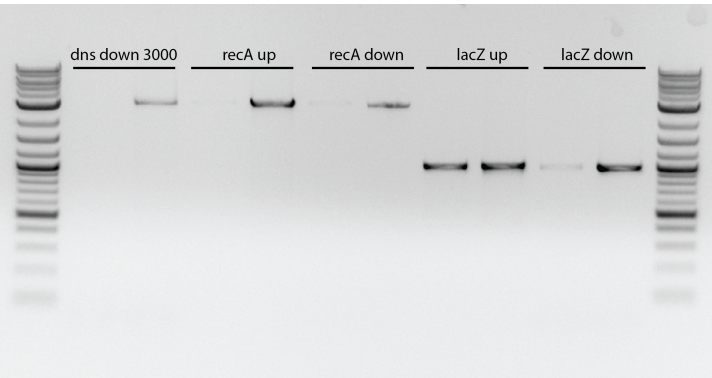
PCR Programm

Temperatur	Step	Time	Cycles
96°C	Initiale Denaturierung	10 min	
96°C	Denaturierung	30 s	30x
58°C	Primerhybridisierung	30 s	
72°C	Elongation	3min (1 min /kb)	
72°C	Finale Elongation	10 min	
4°C	Halten	∞	

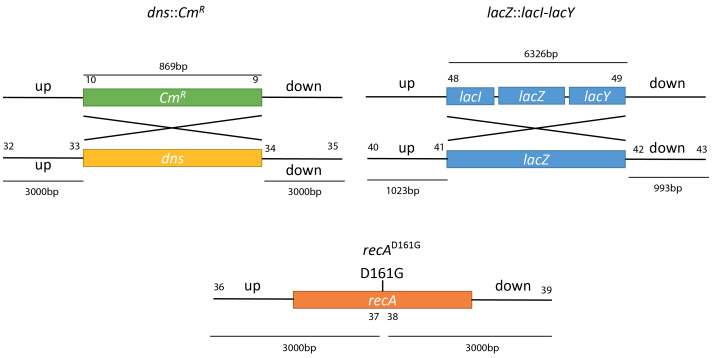
Primer

	dns down 3000	recA up	recA down	lacZ up
forward	oiGEM3034_dns_down_fwd_frt_3000	oiGEM3036_recA_up_fwd	oiGEM3038_recA_down_fwd	oiGEM3040_lacZ_up_fw
reverse	oiGEM3035_dns_down_rev_3000	oiGEM3037_recA_up_rev	oiGEM3039_recA_down_rev	oiGEM3041_lacZ_up_re

180922_VibriClone_Fragmente.png



VibriClone_Design.png



Author: Franziska Müller
created: 21.06.2018 14:26
updated: 25.06.2018 14:25
Entry 1/20: Investigation of possible deletions that are important for a cloning strain
In Project: Cloning Strain
With tags: cloning strain, mcrBD, mrr, mcrA, recA, hsdRMS, hsdR, hsdM

recA

- =catalyzes crossing over events, activator of SOS response in *E. coli*
- deletion is beneftitial because plasmid stability is increased

Homologue found in *V. natriegens:*

Bioinformatics RecA in V. natriegens

If *recA* is going to be deleted it would be done as the last deletion in the cloning strain, because deletion of *recA* results in less efficient homologous recombination.

mcrA, mcrBD* or *mrr

- mutations in these genes prevent methylated DNA from other organisms from being recognized as foreign
- beneficial because it allows cloning of genomic DNA or methylated cDNA

mrr

- only c-terminal domain found in *V. natriegens*
- found in other Vibrionales
- contains coaD domain
  - phosphopantetheine adenylyltransferase domain
  - condensation ATP to phosphopantein
- was not looked further into
- Bioinformatics mrr

mcrA

- only c-terminal domain found in *V. natriegens*
- with HNHc endonuclease superfamily domain
- found in other Vibrionales
- Bioinformatics mcrA

mcrBD

- no homologue to *mcrC* from *E. coli* found in *V. natriegens*

hsdRMS

hsdR

- The EcoKI enzyme recognizes 5'-AACN$_6$GTGC-3'. Subunit R is required for both nuclease and ATPase activities, but not for modification.
- Type I restriction and modification enzymes are complex, multifunctional systems which require ATP, S-adenosyl methionine and magnesium as cofactors and, in addition to their endonucleolytic and methylase activities, are potent DNA-dependent ATPases.
- Endonucleolytic cleavage of DNA to give random double-stranded fragments with terminal 5'-phosphates; ATP is simultaneously hydrolyzed.
- Bioinformatics hsdR

hsdM

- The M and S subunits together form a methyltransferase (MTase) that methylates two adenine residues in complementary strands of a bipartite DNA recognition sequence. In the presence of the R subunit the complex can also act as an endonuclease, binding to the same target sequence but cutting the DNA some distance from this site. Whether the DNA is cut or modified depends on the methylation state of the target sequence. When the target site is unmodified, the DNA is cut. When the target site is hemimethylated, the complex acts as a maintenance MTase modifying the DNA so that both strands become methylated. The EcoKI enzyme recognizes 5'-AACN$_6$GTGC-3'.
- S-adenosyl-L-methionine + adenine in DNA = S-adenosyl-L-homocysteine + N-6-methyladenine in DNA.
- Bioinformatics hsdM