Author: Michael Burgis
Entry 1/18: Digestion of 5'UTR parts
In Project: MoChlo Recloning
No tags associated

created: 26.03.2021 16:19
updated: 26.03.2021 22:32

Digestion of E9-G10 5'UTRs MoChlo plate 1. MiniPrep 24.03.21 - entry #20 in project 'General stuff' (Laura Andreas, 25.03.2021)
Inserted 5µg of DNA, used 0,5µl of Enzyme and incubated for 2 hours at 37°C.Digestion worked except for F4: psbN 5'UTR
Bands were cut out and prepared for gel extraction

Labfolder_Table.xlsx

Labfolder_Table

	A	B	C	D	E	F	G	H	I	J
1	Plasmid	ng/µl		Plasmid	ng/µl					
2	E11	265,5		G1	306,4					
3	E12	291,7		G2	346,5					
4	F1	314,4		G3	133,7					
5	F2	381,2		G4	173,6					
6	F3	273,3		G5	297,5					
7	F4	140,3		G6	310,0					
8	F5	221,6		G7	299,9					
9	F6	227,8		G8	244,9					
10	F7	211,4		G9	294,6					
11	F8	283,7		G10	266,4					
12	F9	240,6		E9	163,3					
13	F10	285,5		E10	240,5					
14	F11	214,3								
15	F12	258,3								
16										
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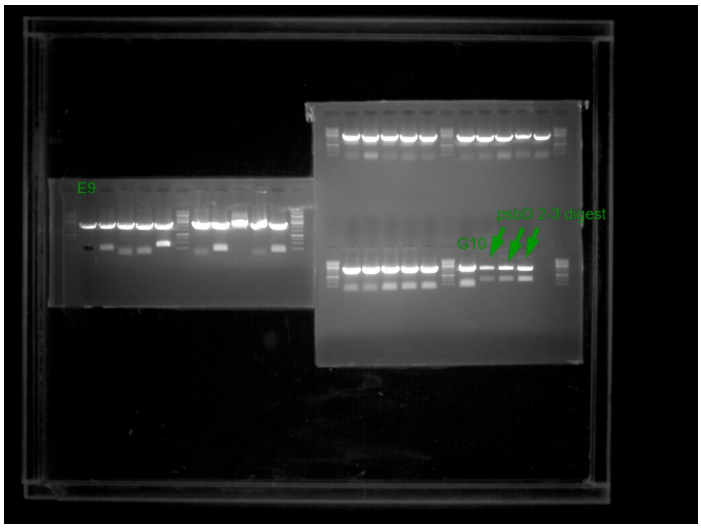
Sheet1

labfolder_table_7576625_3.xlsx

MoChlo_5'_digest.png



Author: Laura Andreas
Entry 2/18: Backbone Prom & Prom+5'UTR
In Project: MoChlo Recloning
No tags associated

created: 05.04.2021 19:42
updated: 11.04.2021 18:09

30.03. MiniPrep of Plasmids pME_Cp_0_02_001_16s_Prom_Cr (Promotor) and pME_Cp_0_2-3_004_psbA_Prom+5UTR_Cr (Promotor+5'UTR).Protocol of GE Healthcare (attached below)Changes: Lysis Buffers 7, 8, 9 used quadrupled volume. Volume distributed on 2 columns. continued as said in the Protocol. Eluted with 50µL of Elution buffer.Concentration after SpeedVac: Promotor: 638,0 ng/µL

Promotor+5'UTR: 504 ng/µL

03.04:Restriction digest with BsaI-HFv2 (Protocol attached): Use 10µg of Plasmid, distribute in two digestion recations.

Gel electrophoresis with 0,8% agarose. Upper band (backbone) cutted out (make sure to excise band as presicely as possible) and purified with Gel extraction kit.

The upper samples are the promotor, the lower one the promotor+5'UTR

NanoDrop afterwards. Graphs did not show DNA.

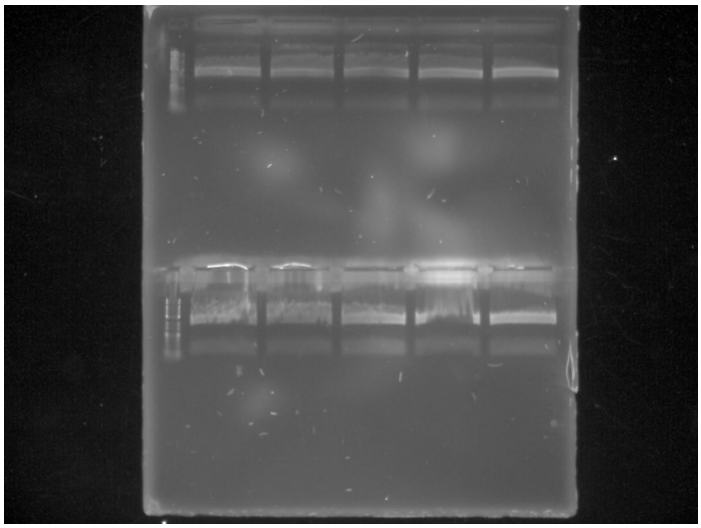
All of this must be repeated.

Mini-Prep_Protokoll_GE_Healthcare.PDF

NEBcloner_BsaI-HFv2_Restriction_digest.pdf

Gel_extration_kit_Qiagen_HB-1196-005_HB_QQ_Spin_0120_WW.pdf

2021.04.03_16-23-43.png



Author: Tristan Krause
Entry 3/18: 13.04. Recloning 3' UTR
In Project: MoChlo Recloning
With tags: toolbox

created: 13.04.2021 15:54
updated: 27.04.2021 13:27

Author: Laura Andreas
Entry 4/18: PCR for 3'UTR-Recloning
In Project: MoChlo Recloning
No tags associated

created: 18.04.2021 17:57
updated: 18.04.2021 17:57

PCR of psaC_3'UTR (B3) and TMV_3'UTR (B4). PCR was set up by hand using Q5 Mastermix (NEB). STOP-Codon was added by Primers, which was, missing in the MoChlo Collection.

Used Template DNA: Mini Prep 22.03. - entry #19 in project 'General stuff' (Tristan Krause, 22.03.2021) B3: 164ng B4:382.1 ng (calculated with wrong concentrations of Plasmids, intended to use 100ng)

Protocol PCR see below, TM 65°C (NEB TM Calculator)

Gel electrophoresis was set up with 0,8% agarose gel. 120V ran 15 min. Result see below (B3 left. B4 right). Off targets visible

Used ladder: Quantita Pro DNA Marker 100bp-10kb Biozym

Labfolder Table

	A	B	C
1		Name	Sequence
2		oRI5121_psaC_3'UTR_MoChlo_fwd	AACGTCTCGCTCGGCTTAATGATACGTTCGAGAAAAC
3		oRI5122_psaC_3'UTR_MoChlo_rev	TTCGTCTCCCTCAAGCGCATAAAAACCCGTGCTCAAA
4		oRI5123_TMV_3'UTR_MoChlo_fwd	AACGTCTCGCTCGGCTTAAATAATAAATAACGGATTG
5		oRI5124_TMV_3'UTR_MoChlo_rev	TTCGTCTCCCTCAAGCGTGGGCCCCTACCGGG
6			
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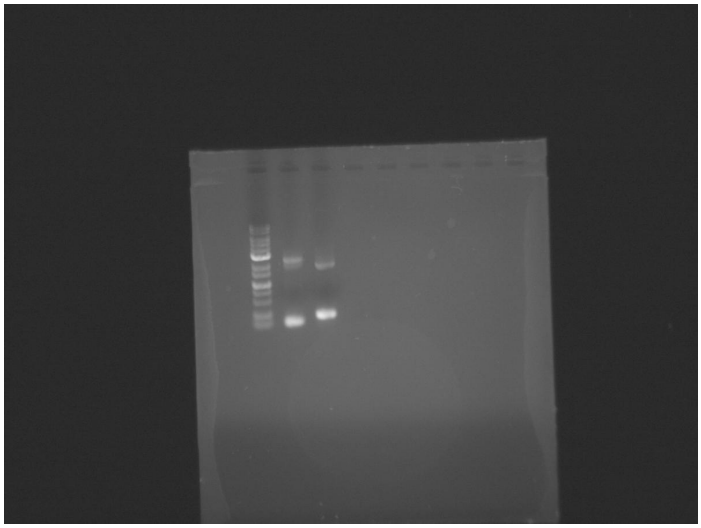
Sheet1

labfolder_table_7734197_2.xlsx

PCR_Using_Q5®_High-Fidelity_DNA_Polymerase_(M0491)___NEB.pdf

PCR_3'Recloning_17.04..Tif



Author: Laura Andreas
Entry 5/18: Inserts for Recloning
In Project: MoChlo Recloning
No tags associated

created: 18.04.2021 17:57
updated: 25.04.2021 19:48

Gel extraction of backbone after digest of pME_Cp_0_02_001_16s_Prom_Cr (Promotor) and pME_Cp_0_2-3_004_psbA_Prom+5UTR_Cr (Promotor+5'UTR) (analogous to Backbone Prom & Prom+5'UTR - entry #2 in project 'MoChlo Recloning' (Laura Andreas, 11.04.2021))

Result: Promotor: 171,9 ng/µL

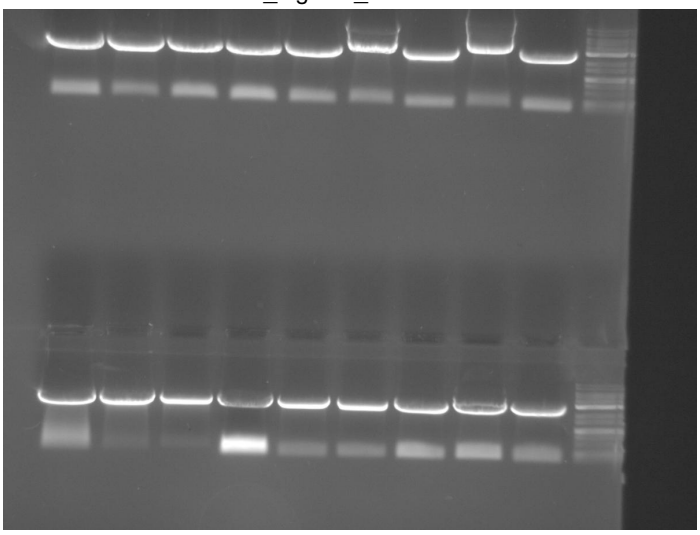
Promotor+5'UTR: 279,3 ng/µL

Digest of D3-E8. Used all of the Plasmid after SpeedVac (Miniprep 13.03 - entry #18 in project 'General stuff' (Laura Andreas, 14.03.2021)) Plasmid dissolved in 18.5µL ddH2O (1µL too much) + 2µL CutSmart Buffer +0,5µL BsaI HF V2. Incubated 1h at 37° C

Gelelectrophoresis with .8% agarose gel (result see below); D3-D11 upper row, D12-E8 lower row; lower bands(insert) cut out of gel and frozen away afterwards.

Used ladder: Quantita Pro DNA Marker 100bp-10kb Biozym

RE_digeste_D3-E8.Tif



Labfolder Table

	A	B	C	D	E	F
1	Name			Position		
2	pMK-Prrn (N. tabacum) BPrrn13			MoChlo kit - Plate1 D3		
3	pMK-Prrn (N. tabacum) BPrrn14			MoChlo kit - Plate1 D4		
4	pMK-Prrn (N. tabacum) BPrrn15			MoChlo kit - Plate1 D5		
5	pMK-Prrn (N. tabacum) BPrrn16			MoChlo kit - Plate1 D6		
6	pMK-Prrn (N. tabacum) BPrrn17			MoChlo kit - Plate1 D7		
7	pMK-Prrn (N. tabacum) BPrrn18			MoChlo kit - Plate1 D8		
8	pMK-Prrn (N. tabacum) BPrrn19			MoChlo kit - Plate1 D9		
9	pMK-Prrn (N. tabacum) BPrrn20			MoChlo kit - Plate1 D10		
10	pMK-Prrn (N. tabacum) BPrrn21			MoChlo kit - Plate1 D11		
11	pMK-Zm PclpP::5'UTR			MoChlo kit - Plate1 D12		
12	pMK-Zm PclpP::Gg10 leader			MoChlo kit - Plate1 E1		
13	pMK-PaccD::5'UTR			MoChlo kit - Plate1 E2		
14	pMK-mPrrn::RBS (3.9%)			MoChlo kit - Plate1 E3		
15	pMK-mPrrn::RBS (20%)			MoChlo kit - Plate1 E4		
16	pMK-mPrrn::RBS (46%)			MoChlo kit - Plate1 E5		
17	pMK-mPrrn::RBS (60%)			MoChlo kit - Plate1 E6		
18	pMK-mPrrn::RBS (61%)			MoChlo kit - Plate1 E7		
19	pMK-Prrn::RBS			MoChlo kit - Plate1 E8		

Sheet1

labfolder_table_7805842_3.xlsx

Author: Yasoo Morimoto
Entry 6/18: No entry title yet
In Project: MoChlo Recloning
No tags associated

created: 19.04.2021 10:43
updated: 19.04.2021 10:43

Followed protocol from this site: https://www.chlamycollection.org/methods/minipreps-of-dna-from-chlamydomonas-cultures/First protocol

Deviation: Spun down 50ml of Chlamy culture -> Multiplied the needed solutions by 2 times (amount) [eluted in 40µl still]

- Resuspend vigorously by vortexing, spin for 10 sec. and aspirate off supernatant.
- Resuspend cells in 150 ul H2O on ice and add 300 ul of SDS-EB buffer, vortex to mix.
- Extract once with 350 ul phenol/CIA(1:1) for few min by vortexing, separate phases by centrifugation for 5 min., transfer aq. phase to a new tube.
- Extract once with 300 ul CIA (24:1), transfer aq. to a new tube.
- Add 2 volumes abs. ethanol, incubate on ice for 30 min., centrifuge for 10 min., wash pellet once with 200 ul 70% ethanol.
- Dry pellet and resuspend in ca. 40 ul H2O. For Southern analysis use about 1-3 ul.

Author: Yasoo Morimoto
Entry 7/18: No entry title yet
In Project: MoChlo Recloning
With tags: toolbox

created: 19.04.2021 10:43
updated: 19.04.2021 10:51

Parts BsaI Digestion 20µL reactions, used 0,5µL enzyme

Low concentration of the plasmids makes it difficult to manage enough DNA

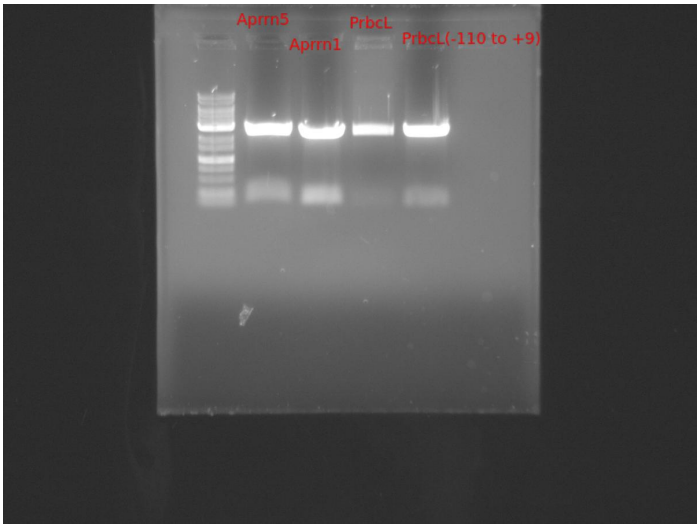
Parts digested:
* pMK-PrbcL (B10)

* pMK-PrbcL (-110 to +9) (B12)

* pMK-Prrn (N. tabacum) APrrn1 (C4)

* pMK-Prrn (N. tabacum) APrrn5 (C8)

IM002658.Tif



Author: Michael Burgis
Entry 8/18: Digestion + Ligation of MoChlo 5' parts (1)
In Project: MoChlo Recloning
No tags associated

created: 20.04.2021 19:33
updated: 21.04.2021 19:35

my date: 15.04.2021

Separation of Digestion and Ligation of the MoChlo parts to clone into Cam backbone.Used parts: E12,F1-5,F7,F10-12,G2-10Used NEBioCalculator to figure out how much DNA is needed to achieve 3(insert):1(backbone) ratio for the ligation. Calculated with 100ng/µl of backbone for further ligation.Prepared digest in 20µl final volume, used 0,5µl of BsaI.

Component	20µl Reaction
amount of DNA	see below
10X CutSmart Buffer	2µl
BsaI-HFv2	0,5µl
nuclease free water	to 20µl

Incubated at 37°C for 1 hour.Heat inactivated at 80°C for 10 minutes.

	E12	F1	F2	F3	F4	F5	F7	F10	F11	F12	G2	G3	G4	G5	G6	G7	G8	G9
ng needed for digestion	348,5	386	336	361	336	335	337	335,7	335,7	334,3	338	344,4	344,4	337,3	336,6	340	347,2	34

Used digestion directly to prepare ligation with predigested 5'UTR backbone (100ng/µl).

Component	Amount
Digest	entire digest
T4 Ligation Buffer	2µl
T4 Ligase	0,5µl
5'UTR backbone	1µl

Incubated overnight at 16°C -> Used entire ligation for transformation.

Author: Michael Burgis
Entry 9/18: Digestion + Ligation of MoChlo 5' parts (2) + CDS parts
In Project: MoChlo Recloning
No tags associated

created: 20.04.2021 19:53
updated: 21.04.2021 20:04

Separation of Digestion and Ligation of the MoChlo parts to clone into Cam backbone.Used parts: E12,F1-5,F7,F10-12,G2-10Used NEBioCalculator to figure out how much DNA is needed to achieve 3(insert):1(backbone) ratio for the ligation. Calculated with 100ng/µl of backbone for further ligation.Prepared digest in 20µl final volume, used 0,5µl of BsaI.

Component	20µl Reaction
amount of DNA	see below
10X CutSmart Buffer	2µl
BsaI-HFv2	0,5µl
nuclease free water	to 20µl

Incubated at 37°C for 1 hour.Heat inactivated at 80°C for 10 minutes.

	E9	E10	E11	F6	F8	G1	G11	G12	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	Pl. 2 A2
ng needed for digestion	366	367	346	357	349	334	338	346	338,7	348	335	344	389	365	357	337	429	430	444	559

Used digestion directly to prepare ligation with predigested 5'UTR backbone (100ng/µl) and CDS backbone (67ng/µl).

Component	Amount
Digest	entire digest
T4 Ligation Buffer	2µl
T4 Ligase	0,5µl
5'UTR backbone/CDS backbone	1µl;1,5µl

Incubated at room temperature for 1hour -> Used entire Ligation for Transformation

Author: Laura Andreas
Entry 10/18: Inserts for Recloning- Gelextraction
In Project: MoChlo Recloning
No tags associated

created: 25.04.2021 19:48
updated: 25.04.2021 19:52

Gel extraction of the gel from Inserts for Recloning - entry #5 in project 'MoChlo Recloning' (Laura Andreas, 25.04.2021)

Protocol see below.

NanoDrop showed no suitable graph for DNA (except for E3). Retry with direct ligation after digest.

Gel_extraction_Machery_Nagel_Instruction-NucleoSpin-Gel-and-PCR-Clean-up.pdf

Author: Tristan Krause
Entry 11/18: Recloning 3'UTR PCR and Gel 21.04.
In Project: MoChlo Recloning
With tags: toolbox, PCR

created: 27.04.2021 12:57
updated: 27.04.2021 13:28

due date: 21.04.2021
due date: 20.04.2021

Summary

PCR from MoChlo 3'UTR Plasmids (A12 and B1). See table below. PCR was set up by hand for rpoA 3'UTR (B1) and petD 3'UTR (A12). Added the STOP codon.

Mini Prep 22.03. - entry #19 in project 'General stuff' (Tristan Krause, 22.03.2021)

Results

There was some Backbone in the gelelectrophoresis, so I cleaned the gel. See picture below.

After Clean-Up: A12: 30,2 ng/µl B1: 25,3 ng/µl

Labfolder Table

	A	B
1	rpoA 3'UTR - Primer rv	TTCGTCTCCCTCAAGCGATTTTTAAATTGATTCAATTGTGAAATAAC
2	rpoA 3'UTR - Primer fwd	AACGTCTCGCTCGGCTTAAATCTATTGGACTTACTTAGTGAAAATAG
3	petD 3'UTR - Primer rv	TTCGTCTCCCTCAAGCGATCTATTGGACTTACTTAGTGAAAATAG
4	petD 3'UTR - Primer fwd	AACGTCTCGCTCGGCTTAAATTTTTAAATTGATTCAATTGTGAAATAAC
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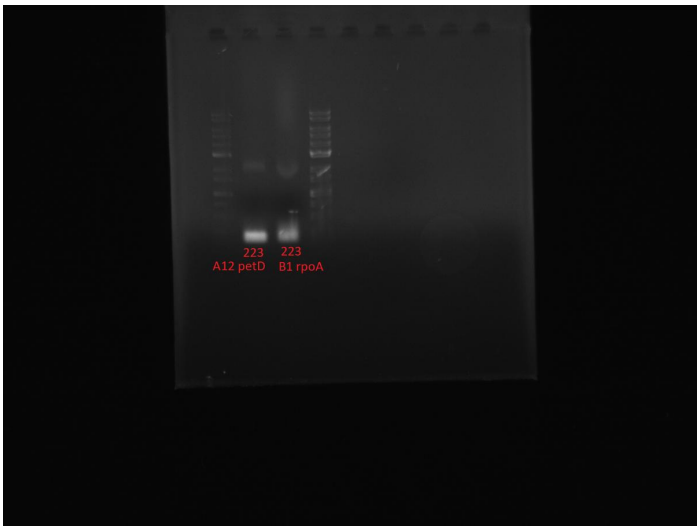
Sheet1

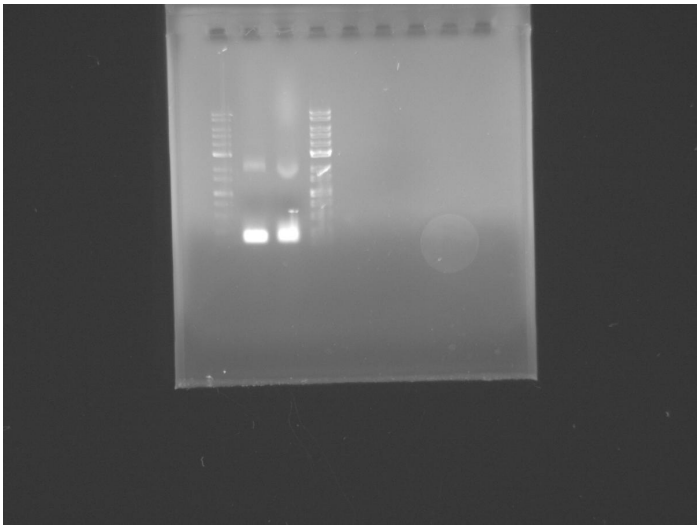
labfolder_table_7825883_2.xlsx

Instruction-NucleoSpin-Gel-and-PCR-Clean-up.pdf

IM002665_(2).tif



IM002666.Tif



Author: Laura Andreas
Entry 12/18: Ligation
In Project: MoChlo Recloning
No tags associated

created: 02.05.2021 13:10
updated: 14.05.2021 20:26

MiniPrep of D3-E8 Protocol of GE Healthcare (see below)

Digest of these Plasmids with BsaI HF V2 (NEB) Protocol see below; used quantity of Plasmid see table below. 80°C heat inactivation for 10 minutes afterwards

Ligation added Backbone (D3-D12 P16s Promotor; E1-E8 psbD Promotor+3'UTR) Backbone Prom & Prom+5'UTR - entry #2 in project 'MoChlo Recloning' (Laura Andreas, 11.04.2021)

Protocol see below. Needed quantity of DNA for inserts was calculated with http://nebiocalculator.neb.com/#!/ligation

16°C overnight protocol.

Labfolder Table

	A	B	C	D	E	F	G	H
1	Plasmid	Size Back	Mass Backbone	ng/µL Backbone	µL backbone	dilution 1:	dilution µL	Inserts
2	D3	2109	100	171,9	0,58173357	17,19	5,81733566	
3	D4	2109	100	171,9	0,58173357	17,19	5,81733566	
4	D5	2109	100	171,9	0,58173357	17,19	5,81733566	
5	D6	2109	100	171,9	0,58173357	17,19	5,81733566	
6	D7	2109	100	171,9	0,58173357	17,19	5,81733566	
7	D8	2109	100	171,9	0,58173357	17,19	5,81733566	
8	D9	2109	100	171,9	0,58173357	17,19	5,81733566	
9	D10	2109	100	171,9	0,58173357	17,19	5,81733566	
10	D11	2109	100	171,9	0,58173357	17,19	5,81733566	
11	D12	2109	100	171,9	0,58173357	17,19	5,81733566	
12	E1	2109	100	273,9	0,36509675	27,39	3,65096751	
13	E2	2109	100	273,9	0,36509675	27,39	3,65096751	
14	E3	2109	100	273,9	0,36509675	27,39	3,65096751	
15	E4	2109	100	273,9	0,36509675	27,39	3,65096751	
16	E5	2109	100	273,9	0,36509675	27,39	3,65096751	
17	E6	2109	100	273,9	0,36509675	27,39	3,65096751	
18	E7	2109	100	273,9	0,36509675	27,39	3,65096751	
19	E8	2109	100	273,9	0,36509675	27,39	3,65096751	
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Sheet1

labfolder_table_7972198_2.xlsx

Mini-Prep_Protokoll_GE_Healthcare.PDF

NEBcloner_BsaI-HFv2_Restriction_digest.pdf

Ligation_Protocol_with_T4_DNA_Ligase_(M0202)___NEB.pdf

Author: Tristan Krause
Entry 13/18: Golden Gate + Mini-Prep 3'UTR
In Project: MoChlo Recloning
No tags associated

created: 06.05.2021 15:21
updated: 06.05.2021 15:42

due date: 26.04.2021
due date: 27.04.2021
due date: 29.04.2021
due date: 03.05.2021
due date: 05.05.2021

Recloning 3'UTR PCR and Gel 21.04. - entry #11 in project 'MoChlo Recloning' (Tristan Krause, 27.04.2021)

First time Golden Gate reaction failed, after Transformation, there were only Green Colonies on the plate (done this 2 times).

After these issues, the right colonies are picked and a Mini-Prep was performed.

After Clean-Up: A12: 173,5 ng/µl B1: 171,0 ng/µl

Mini-Prep_Protokoll_GE_Healthcare.PDF

Author: Laura Andreas
Entry 14/18: Ligation and Transformation
In Project: MoChlo Recloning
No tags associated

created: 11.06.2021 11:33
updated: 11.06.2021 11:43

1st Ligation did not work so redid D3-E8 (except D12 used wrong backbone).

1. Digested D3-E8 with BsaI (Protocol see below)

2. Ligation D3-D11 used backbone of p16s-Promotor

E1-E8 with psbA-Prom::5'UTR backbone

Protocol see below; used NEB Ligation calculator

3. Transformation of D3-E8 also of B3 and B4 (3'UTR add Stop-Codon)

incubated 2h, 100µL plated on LB Agar with Chlorampenicol (400mL Agar with 400µL Cam)

Transformation worked (Agarplates look nice), Colony PCR to confirm insert of correct parts in backbone required.

NEBcloner_BsaI-HFv2_Restriction_digest.pdf

Ligation_Protocol_with_T4_DNA_Ligase_(M0202)___NEB.pdf

Transformation_&_competent_cells.pdf

Author: Laura Andreas
Entry 15/18: 3'UTR+Stop GoldenGate
In Project: MoChlo Recloning
No tags associated

created: 11.06.2021 11:43
updated: 11.06.2021 11:54

1, digested with DpnI, because off-targets were visible after PCR, incubation time 45min

2. PCR-Clean up. Protocol see below

3. GoldenGate set up. Used improved Protocol, used Universal acceptor Vector GFP as entry Vector

Transformation see Ligation and Transformation - entry #14 in project 'MoChlo Recloning' (Laura Andreas, 11.06.2021)

DpnI,_Restriction_Enzymes___D__Enzymes_-_Jena_Bioscience.pdf

Instruction-NucleoSpin-Gel-and-PCR-Clean-up.pdf

Golden_Gate_protocol.pdf

Author: Laura Andreas
Entry 16/18: ColonyPCR of D3-D11 and E1-E8
In Project: MoChlo Recloning
No tags associated

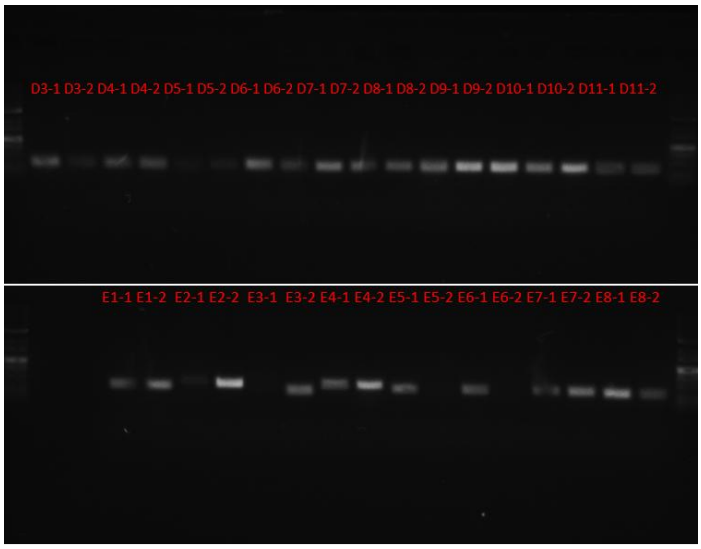
created: 26.06.2021 20:39
updated: 26.06.2021 21:02

Colony PCR of D3-D11 and E1-E8. used two colonies each, only used have a colony; Protocol see below

Had at least one Colony with insert each so the other half of each colony was but into 5mL LB-medium an cultivated at 37°

PCR_&_colony_PCR.pdf

Gel_ColonyPCR_26.06_MoChlo_Recloning_mit_Beschriftung.png



Author: Laura Andreas
created: 15.07.2021 18:42
updated: 15.07.2021 19:03
Entry 17/18: MiniPrep of D3-D11, E1-E8 and B3+4
In Project: MoChlo Recloning
No tags associated

MiniPrep of MoChlo Recloning (D3-E8) and Reclonings of 3'UTR+STOP
Protocol see below, used doubled volume for A1-A3
results see below.

Sent to sequencing afterwards

Plasmid	Ng/µL
D3	247,2
D4	226,4
D5	242,0
D6	252,9
D7	268,8
D8	291
D9	279,2
D10	285,9
D11	286,0
E1	235,5

E2	242,9
E3	247,1
E4	152,3
E5	890,1
E6	219,3
E7	308,6
E8	275,6
B3	540,7
B2	253,9

Instruction-NucleoSpin-Plasmid_Miniprep.pdf

Author: Tristan Krause
Entry 18/18: Recloning Parts A1-B7
In Project: MoChlo Recloning
No tags associated

created: 21.09.2021 14:02
updated: 21.09.2021 15:38

due date: 17.05.2021
due date: 18.05.2021
due date: 27.05.2021
due date: 28.05.2021
due date: 18.06.2021
due date: 21.06.2021
due date: 27.07.2021
due date: 28.07.2021

The Antibiotic Resistance in the Backbone of the MoChlo Parts was wrong, why they can't be used for GoldenGate Reactions.

The parts were therefore digested using BsaI and ligated with a cleaned-up backbone. The purification of the backbone of a promoter, however, took a little longer. If the part religate with the old backbone, then the bacteria with the wrong backbone should die after plating out. There were some problems in the beginning because you can't see the parts on a gel (see the first picture), just the backbone. That is why the recloning was done this way.

Instead of a test digestion, a PCR was also performed on some parts, see second picture, because the bands were not clear enough. This was done with the same primers as in the sequencing.

The purification of the backbone was also not 100% pure, as the original part of the backbone was in the plasmid at the end. This was the case in A9 and B1. From the plates of these parts, 3 colonies each were picked again, but based on the gel, it did not look like the correct parts were in them.

In the end, every part is correct, except A9 and B1, but somehow the Backbone was inverted. This isn't a huge problem, because you can still use the parts for GoldenGate reactions.

The Workflow was:

Digest Parts

Include new Backbone and ligate parts

Transformation and plate out

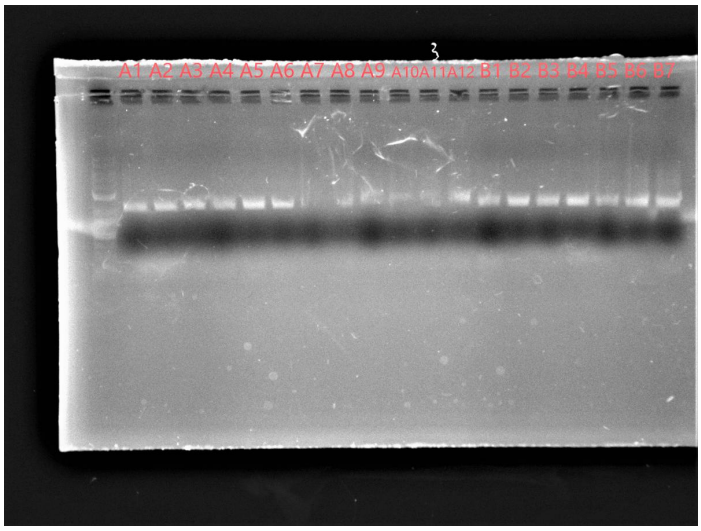
(After plating out, colony PCR would also have been performed)

Mini-Prep and measure concentration in Nanodrop

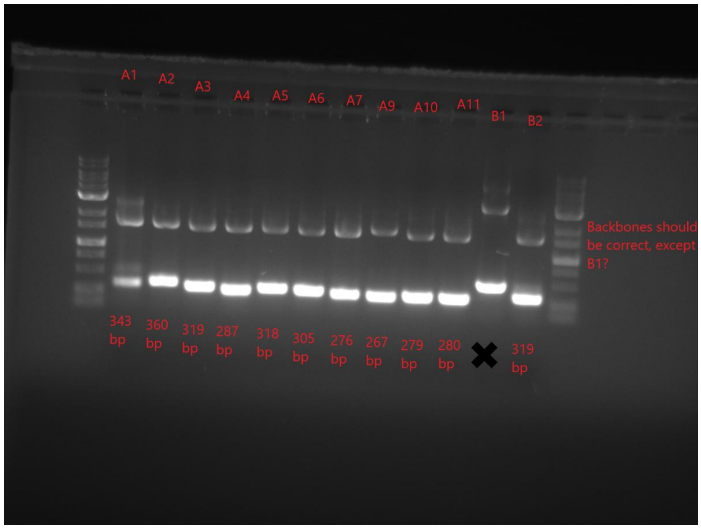
Test-Digest

Sequencing

IM002866.2.png



IM002942.png

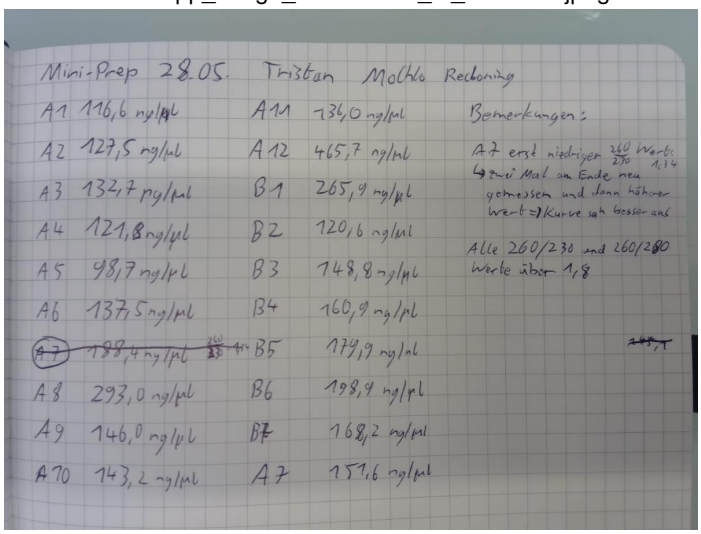


NEBcloner_BsaI-HFv2_Restriction_digest.pdf

Mini-Prep_Protokoll_GE_Healthcare.PDF

Instruction-NucleoSpin-Gel-and-PCR-Clean-up.pdf

WhatsApp_Image_2021-09-21_at_15.19.50.jpeg



WhatsApp_Image_2021-09-21_at_15.21.33.jpeg