Author: Michael Burgis
created: 01.11.2020 20:28
updated: 01.11.2020 21:14

Entry 1/25: DNA Extraction from Chlamydomonas reinhardtii
In Project: General stuff
With tags: DNA Extraction, Chlamydomonas reinhardtii, Chloroform, DNA Template

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.

Resuspended pellet in 40µl although it was very viscous -> 15390ng/µl measured on the NanodropWill resuspend later with more ddH_20

resuspended in 1ml final volume -> final concentration: 520ng/µl

Purity: $(Absorbance_{260nm}-Absorbance_{320nm})/(Absorbance_{280nm}/Absorbance_{320nm})$ = (10,069-0,023)/(4,819-0,023) = 10,046/4,796 = 2,09

TO DO: Test the DNA with a PCR to see how good it actually is

Author: Michael Burgis
created: 01.11.2020 20:29
updated: 01.11.2020 21:14
Entry 2/25: DNA Extraction from Nicotiana tabacum
In Project: General stuff
With tags: DNA Extraction, Nicotiana tabacum, DNA Template, Chloroform

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

Deviation: Put 1 plant in the blender (in 10ml TEN) -> Multiplied the needed solutions by 10 times (amount) [eluted in 200µl still]

- Resuspend vigorously by vortexing, spin for 10 sec. and aspirate off supernatant.
- Resuspend cells in 150 ul H_2O 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 H_2O. For Southern analysis use about 1-3 ul.

Resuspended pellet in 1ml -> measured concentration: 787ng/µl

Purity: $(Absorbance_{260nm}-Absorbance_{320nm})/(Absorbance_{280nm}/Absorbance_{320nm})$ = (18,108-0,194)/(9,092-0,194) = 17,914/8,898 = 2,013

TO DO: Test the DNA with a PCR to see how good it actually is

Addition: Extract twice with CIA to get rid of all P-CIA (the pellet was a little brownish)

Author: Michael Burgis
Entry 3/25: No entry title yet
In Project: General stuff
No tags associated

created: 07.11.2020 18:34
updated: 07.11.2020 18:34

Author: Michael Burgis
Entry 4/25: test PCR (Cr and Nt)
In Project: General stuff
No tags associated

created: 07.11.2020 18:34
updated: 07.11.2020 19:59

PCR_&_colony_PCR.pdf

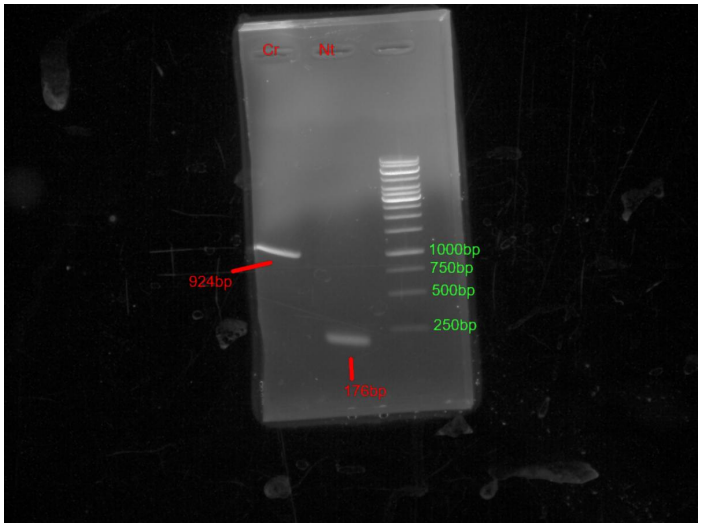
Performed a test PCR to see how well purified genomic DNA performs.

Used primer: oRI107+oRI108 [psbD 3' Homology] (for Cr genomic DNA) and rbcL 3'UTR fwd + rbcL 3'UTR rev (for Nt genomic DNA)

Expected bands: 924bp for Chlamy and 176bp for Tobacco

Used 1kb ladder Thermo

2020.11.07_17-56-19.tiff



Author: Michael Burgis
created: 07.11.2020 21:19
updated: 08.11.2020 16:33

Entry 5/25: DNA Extraction from Spinacia oleracea, Quercus robur and Chlamy (René)
In Project: General stuff
No tags associated

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

Deviation: Took leaves of Oak tree in front of Biology department, old rotten spinach from Victoria and 2 cultures of Chlamy (3269) multiplied protocol by 20x

- Added 40ml of TEN to Oak, 10ml of TEN to Spinach and 5ml TEN to Chlamy
- 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. 500 ul H2O. For Southern analysis use about 1-3 ul.
- Spinach: 487,2ng/µl 2,13;1,13
- Oak: 802ng/µl 1,37; 0,43 -> pretty unpure DNA. Tried to purify by binding it on a DNA prep column and washing it. Resulted in 5ng/µl DNA under the NanoDrop. Maybe retry by using CTAB as buffer

Author: Michael Burgis
Entry 6/25: DNA Extraction from Quercus robur and Aloe Vera
In Project: General stuff
No tags associated

created: 15.11.2020 14:57
updated: 15.11.2020 19:08

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

Deviation: Needed to resuspend Oak in a lot of TEN (10ml), used the same amount for Aloe. Multiplied the protocol by 10. Eluted in 200µl

- 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.

Oak still looks pretty dirty (try extraction with CTAB, because it removes remaining carbohydrates)

Aloe Vera was okay, but the yield was pretty low due to me loosing half of the pellet: 35ng/µl in 200µl

Author: Michael Burgis
Entry 7/25: Primer Entry
In Project: General stuff
No tags associated

created: 06.12.2020 20:32
updated: 06.12.2020 20:33

Primer list

Author: Michael Burgis
Entry 8/25: Mini Prep test: Optimization for reduced Protein withdrawal
In Project: General stuff
With tags: Mini prep, plasmid prep

created: 29.01.2021 15:23
updated: 23.02.2021 19:44

Material:

Macherey Nagel Kit: A1 buffer and columns are missing

Zymo Research Kit:

P1 buffer missing, but Midi prep available

Monarch NEB Kit: there and complete

https://international.neb.com/protocols/2015/11/20/monarch-plasmid-dna-miniprep-kit-protocol-t1010

Qiagen Miniprep Kit: there and complete

Test condition:

prolonged and shortened lysis incubation -> measurement of protein takeover

Cytiva (formerly GE Healthcare): 3 and 5 min (2ml)

NEB: 30sec and 1min (2ml)

Zymo: 2min and 3min (50ml)

Macherey Nagel: 3min and 5min (2ml)

Experiment:

Followed the exact protocol only with the lysis duration as can be seen above.

eluted in 50µl for all kits

d4200_d4201_zymopure_ii_plasmid_midiprep.pdf

Instruction-NucleoSpin-Plasmid.pdf

HB-1206-007_HB_QIAprep_Miniprep_1220_WW.pdf

Labfolder Table

	A	B	C	D	E	F	G	H	I
1		DNA-Menge in ng/µ	260/280	260/230	Proteinmenge in mg/mL				
2	MN-3	71,7	1,87	2,26	0,00				
3	MN-5	72,9	1,86	2,26	0,00				
4	GE-3	95,6	1,84	2,12	0,00				
5	GE-5	101,5	1,84	2,2	0,00				
6	NEB-30s	210,3	1,91	1,87	0,00				
7	NEB-60s	167,9	1,90	1,97	0,00				
8	Midi Zymo	126,9	1,88	2,44	0,00				
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Sheet1

labfolder_table_7303334_6.xlsx

Author: Yasoo Morimoto
Entry 9/25: No entry title yet
In Project: General stuff
With tags: toolbox, transformation

created: 08.02.2021 14:50
updated: 12.02.2021 14:24

E. coli transformation

Plasmids: CC_0_1_0007_5'Hom_trnG_Nt, pCC_0_1_0008_5'Hom_trnI_Nt, pCC_0_6_0005_3'Hom_trnfM_Nt und pCC_0_6_0006_3'Hom_trnA_Nt, with deep frozen E. coli batch

Transformation_&_competent_cells.pdf

Author: Yasoo Morimoto
Entry 10/25:
In Project: General stuff
With tags: toolbox, transformation

created: 12.02.2021 14:27
updated: 19.02.2021 18:01

E. coli transformation

Plasmids: CC_0_1_0007_5'Hom_trnG_Nt, pCC_0_1_0008_5'Hom_trnI_Nt, pCC_0_6_0005_3'Hom_trnfM_Nt und pCC_0_6_0006_3'Hom_trnA_Nt, with deep frozen E. coli batchRetry, because last transformation did not work.Plated 100µl and Rest on LB Agar Cam plates

Protocol

1. Briefly thaw chemically competent E. coli cells on ice.

2. Add ~ 100 ng of plasmid-DNA to the cells. Alternatively just use 1-2 µl for retransformations and 5µlfor freshly cloned constructs (Golden Gate reactions).

3. Incubate on ice for 30 minutes (can be skipped for retransformations).

4. Heat shock the cells at 42°C for 45 seconds (30 seconds for NEB® Turbo cells).

5. Recover on ice for 5 minutes, then add 500 µl of SOC medium to the cells. Alternatively use LB medium,but SOC is better for the cells.

6. Incubate at 37°C for 1 hour (I construct confers amp resistance) or 2 hours (if construct confers kan, cam,tet or spec resistance). Use the incubation time to prepare the plates and pre-warm them in an incubator.

7. Plate 100 µl on pre-warmed agar plates containing the corresponding antibiotic.

8. Optionally you can centrifuge for 5min at 3500 xg, resuspend the pellet in the remaining ~ 100 µl andplate it.

Author: Tristan Krause
Entry 11/25: Mini-Prep 18.02.
In Project: General stuff
No tags associated

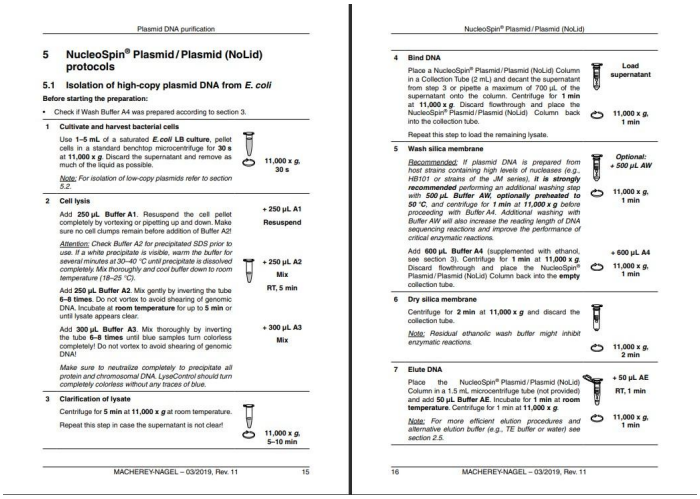
created: 20.02.2021 16:47
updated: 27.02.2021 16:28

Miniprep made with different parts from toolbox with Machery Nagel kit. See protocol page Extraction of high copy plasmid, as double amount of cells, double amount of suspension buffer, cell lysate and neutralisation buffer was added (performed twice step 4 with column).

Protocol see below. Optional step performed and buffer preheated to 50° and 70°.

Parts were from Toolbox (A1-A10) and 4 from René.

Screenshot_2021-02-20_165720.jpg



Plasmid DNA purification

5 NucleoSpin® Plasmid / Plasmid (NoLid) protocols

5.1 Isolation of high-copy plasmid DNA from *E. coli*

Before starting the preparation:

- Check if Wash Buffer A4 was prepared according to section 3.

1 Cultivate and harvest bacterial cells

Use **1–5 mL** of a saturated ***E. coli* LB culture**, pellet cells in a standard benchtop microcentrifuge for **30 s** at **11,000 x *g***. Discard the supernatant and remove as much of the liquid as possible.

Note: For isolation of low-copy plasmids refer to section 5.2.

11,000 x *g*, 30 s

2 Cell lysis

Add **250 µL Buffer A1**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

+ 250 µL A1
Resuspend

Attention: Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30–40 °C until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature (18–25 °C).

Add **250 µL Buffer A2**. Mix gently by inverting the tube **6–8 times**. Do not vortex to avoid shearing of genomic DNA. Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

+ 250 µL A2
Mix
RT, 5 min

Add **300 µL Buffer A3**. Mix thoroughly by inverting the tube **6–8 times** until blue samples turn colorless completely! Do not vortex to avoid shearing of genomic DNA!

+ 300 µL A3
Mix

Make sure to neutralize completely to precipitate all protein and chromosomal DNA. LyseControl should turn completely colorless without any traces of blue.

3 Clarification of lysate

Centrifuge for **5 min** at **11,000 x *g*** at room temperature.

Repeat this step in case the supernatant is not clear!

11,000 x *g*, 5–10 min

4 Bind DNA

Place a NucleoSpin® Plasmid / Plasmid (NoLid) Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum of 700 µL of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the NucleoSpin® Plasmid / Plasmid (NoLid) Column back into the collection tube.

Load supernatant
11,000 x *g*, 1 min

Repeat this step to load the remaining lysate.

5 Wash silica membrane

Recommended: If plasmid DNA is prepared from host strains containing high levels of nucleases (e.g., HB101 or strains of the JM series), ***it is strongly recommended*** *performing an additional washing step with* ***500 µL Buffer AW, optionally preheated to 50 °C****, and centrifuge for* ***1 min at 11,000 x g*** *before proceeding with Buffer A4. Additional washing with Buffer AW will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.*

Optional:
+ 500 µL AW
11,000 x *g*, 1 min

Add **600 µL Buffer A4** (supplemented with ethanol, see section 3). Centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the NucleoSpin® Plasmid / Plasmid (NoLid) Column back into the **empty** collection tube.

+ 600 µL A4
11,000 x *g*, 1 min

6 Dry silica membrane

Centrifuge for **2 min** at **11,000 x *g*** and discard the collection tube.

Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.

11,000 x *g*, 2 min

7 Elute DNA

Place the NucleoSpin® Plasmid / Plasmid (NoLid) Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**. Incubate for **1 min** at **room temperature**. Centrifuge for 1 min at **11,000 x *g***.

Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.5.

+ 50 µL AE
RT, 1 min
11,000 x *g*, 1 min

Author: Michael Burgis
Entry 12/25: Test digest
In Project: General stuff
No tags associated

created: 23.02.2021 19:43
updated: 24.02.2021 15:58

Test digest of several parts:Tobacco Homologies, first 10 MoChlo parts [A1-A10] (Mini-Prep 18.02. - entry #11 in project 'General stuff' (Tristan Krause, 20.02.2021)) and second batch of Cr 5'UTR parts (atpA,petD,psaB,psbA,psbC)

Digested the MoChlo parts with BsaI (only GG enzyme), and the others with NotI

Capture.PNG

Reaction setup:

Component	Volume
DNA (1µg)	xµl
10x FastDigest® Green buffer	2µl
FastDigest® enzyme	0.5µl
Add H_2O to	20µl

The mixture was incubated for 1 hour at 37°C and then separated and analyzed through agarose gel electrophoresis.

Labfolder Table

	A	B	C	D	E	F	G	H
1		Water (µl)	Amount of DNA	Green Buffer (µl)	Enzyme (µl)			conc.
2	atpA (1)	04860578	7909951394218	2	1			7
3	atpA (2)	60849136	7813391508642	2	1			5
4	petD (1)	55372283	4154462771697	2	1			6
5	petD (2)	93508315	0360649168503	2	1			4
6	psaB (1)	26272634	1646737273659	2	1			2
7	psaB (2)	16967509	6101083032491	2	1			
8	psbA (1)	67816092	8735632183908	2	1			
9	psbA (2)	63221884	3951367781155	2	1			
10	psbC (1)	07638223	9590923617769	2	1			3
11	psbC (2)	29693554	2236703064459	2	1			2
12	trnG (1)	38461539	0384615384615	2	1			
13	trnG (2)	58861885	2.963841138115	2	1			3
14	trnfM (1)	74293528	7894257064722	2	1			4
15	trnfM (2)	64557759	4762354422411	2	1			3
16	trnI (1)	72593801	2626427406199	2	1			3
17	trnI (2)	07783543	0644922164566	2	1			2
18	trnA (1)	06792946	6583932070542	2	1			3
19	trnA (2)	28798186	3446712018141	2	1			3
20	A1	28444936	6701715550636	2	1			3
21	A2	03032217	5855969677827	2	1			3
22	A3	68439928	8864315600717	2	1			3
23	A4	94397759	0140056022409	2	1			2
24	A5	86298497	4952813701503	2	1			2
25	A6	49619482	0517503805175	2	1			2
26	A7	97858099	4672021419009	2	1			2
27	A8	24506638	5880875493362	2	1			2
28	A9	49827229	5542501727713	2	1			2
29	A10	90537084	9693094629156	2	1			3
30	rbcL pSap	84984985	5015015015015	2	1			
31								
32								
33								
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35								
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38								
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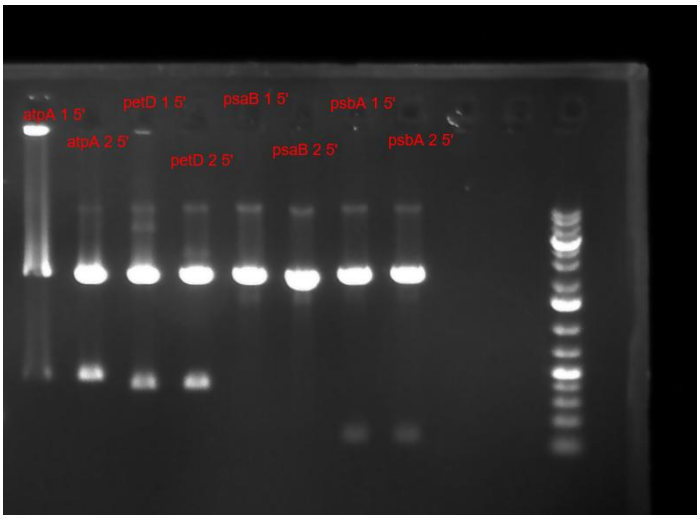
40								

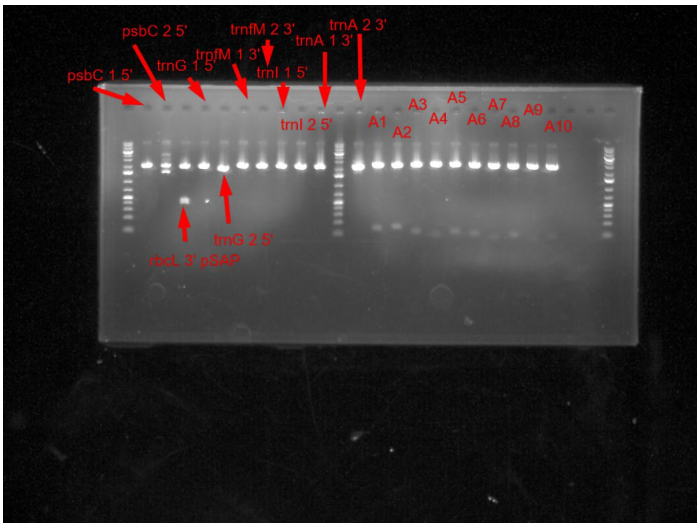
Sheet1

labfolder_table_7311832_3.xlsx

Digest_5'_(1).tiff



Testdig_5+Hom+mochlo.png



Author: Michael Burgis
Entry 13/25: PCR 2nd 5'UTR batch
In Project: General stuff
No tags associated

created: 23.02.2021 20:39
updated: 24.02.2021 13:37

Oligos: oRI5045-oRI5074 (except: oRI5059+oRI5060 [annealing] and oRI5063+oRI5064 [annealing])

PCR done with 2x Q5 MM

19µl ddH20

2,5µl Primer fwd

2,5µl Primer rev

1µl Cr Genomic template

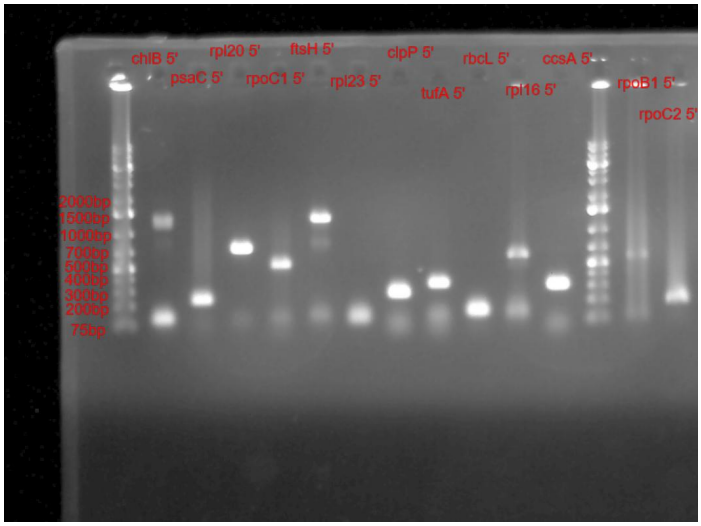
25µl 2x Q5 MM

Tm see excel sheet (used the NEB ones)

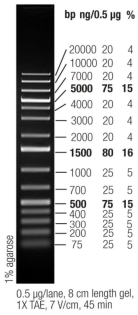
Bands look great except rpl23 5', which shows no bands and chlB 5' which band is a little too low.

Primer_Orders.xlsx

5'UTR_(2)_PCR.png



GeneRuler_1kb_Plus_DNALadder_250ug_UG.bmp-650.jpg



Author: Michael Burgis
Entry 14/25: 5' and rbcL 3'
In Project: General stuff
No tags associated

created: 24.02.2021 15:58
updated: 24.02.2021 15:59

Author: Yasoo Morimoto
created: 25.02.2021 10:13
updated: 27.02.2021 16:55

Entry 15/25: Ecoli transformation 5'UTR and t7 promoter
In Project: General stuff
With tags: toolbox, transformation

E. coli transformation

Plasmids:

chlB 5', rbcL 5', ftsH 5', rpoB1 5', psaC 5', rpl20 5', rpl16 5', clpP 5', ccsA 5', tufA 5', rpoC1 5', rpoC2 5', rpoC2, ccsA

Plated 100µl and Rest on LB Agar Cam plates

Let incubating for 2 hours before plating

Protocol

1. Briefly thaw chemically competent E. coli cells on ice.

2. Add ~ 100 ng of plasmid-DNA to the cells. Alternatively just use 1-2 µl for retransformations and 5µlfor freshly cloned constructs (Golden Gate reactions).

3. Incubate on ice for 30 minutes (can be skipped for retransformations).

4. Heat shock the cells at 42°C for 45 seconds (30 seconds for NEB® Turbo cells).

5. Recover on ice for 5 minutes, then add 500 µl of SOC medium to the cells. Alternatively use LB medium,but SOC is better for the cells.

6. Incubate at 37°C for 1 hour (I construct confers amp resistance) or 2 hours (if construct confers kan, cam,tet or spec resistance). Use the incubation time to prepare the plates and pre-warm them in an incubator.

7. Plate 100 µl on pre-warmed agar plates containing the corresponding antibiotic.

8. Optionally you can centrifuge for 15min at 3500 xg, resuspend the pellet in the remaining ~ 100 µl andplate it.

Author: Tristan Krause
Entry 16/25: Mini-Prep 27.02.
In Project: General stuff
With tags: toolbox, Mini prep

created: 27.02.2021 16:29
updated: 02.03.2021 14:15

Mini Prep of these Plasmids:

chlB 5', rbcL 5', ftsH 5', rpoB1 5', psaC 5', rpl20 5', rpl16 5', clpP 5', ccsA 5', tufA 5', rpoC1 5', rpoC2 5', rpoC2, ccsA

Compare with Entry from 25.02. Ecoli transformation 5'UTR and t7 promoter - entry #15 in project 'General stuff' (Yasoo Morimoto, 27.02.2021)

Protocol from GE Healthcare (see below) ~5mL Medium => double amount of Lysis Buffer typ 7, typ 8 and 9

103892

Author: Tristan Krause
Entry 17/25: Miniprep 03.03.
In Project: General stuff
No tags associated

created: 03.03.2021 13:54
updated: 03.03.2021 13:59

Mini Prep of these Plasmids:

A11, A12, B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10

Protocol see below, changes included there

Prep with 10ml overnight culture, Buffer preheated to 70°C

Mini-Prep_Protokoll_GE_Healthcare.PDF

Author: Laura Andreas
Entry 18/25: Miniprep 13.03
In Project: General stuff
No tags associated

created: 14.03.2021 10:06
updated: 14.03.2021 10:22

Miniprep of these Plasmids: C11, C12, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12, E1, E2, E3, E4, E5, E6, E7, E8, E9, E10

Protocol see below.
10mL highcopy E.coli culture, was spinned down the day before and frozen away over night.

Doubled volume for A1 (600µL), A2 (500µL) and A3 (550µL)
Eluted with 50µL

NanoDrop after Elution (see table below)

Labfolder Table

	A	B	C	D	E	F	G	H	I
1	Plasmid	c(DNA) ng/µL		Plasmid	c(DNA) ng/µL				
2	C11	351,0		E1	287,5				
3	C12	362,2		E2	79,2				
4	D1	319,6		E3	382,5				
5	D2	367,5		E4	77,4				
6	D3	361,0		E5	77,0				
7	D4	322,3		E6	209,0				
8	D5	363,3		E7	435,1				
9	D6	370,8		E8	224,0				
10	D7	336,3		E9	163,3				
11	D8	482,0		E10	240,5				
12	D9	139,1							
13	D10	573,6							
14	D11	184,3							
15	D12	318,5							
16									
17									
18									
19									
20									

Sheet1

labfolder_table_7451130_2.xlsx

Instruction-NucleoSpin-Plasmid_Miniprep.pdf

Author: Tristan Krause
Entry 19/25: Mini Prep 22.03.
In Project: General stuff
No tags associated

created: 22.03.2021 15:35
updated: 22.03.2021 15:50

Mini-Prep of pMK-psbA 3' A10 Pl2, pMK-rbcL 3' A11 Pl2, pMK-petD 3' A12 Pl2, pMK-rpoA 3' B1 Pl2, pMK-psaC 3' B3 Pl2, pMK-TMV 3' B4 Pl2, pMK-TYMV B5 Pl2, pMK-BMV B6 Pl2, pMK-ALMV B7 Pl2

Protocol is below

Mini-Prep_Protokoll_GE_Healthcare.PDF

Labfolder Table

	A	B	C	D	E	F	G	H	I	J
1	Plasmid	c(DNA) in	ng/µL							
2	A10	261,0								
3	A11	211,3								
4	A12	154,8								
5	B1	135,6								
6	B3	232,3								
7	B4	233,0								
8	B5	143,6								
9	B6	138,4								
10	B7	144,0								
11										
12										
13										
14										
15										
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17										
18										
19										
20										

Sheet1

labfolder_table_7522386_3.xlsx

Author: Laura Andreas
Entry 20/25: MiniPrep 24.03.21
In Project: General stuff
No tags associated

created: 25.03.2021 13:50
updated: 25.03.2021 13:57

MiniPrep of these Plasmids:E11 E12 F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 G1 G2 G3 G4 G5 G6 G7 G8 G9 G10

Protcol attached, changes included.

Elution buffer preheated to 70°C

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								
13	F10	285,5								
14	F11	214,3								
15	F12	258,3								
16										
17										
18										
19										
20										

Sheet1

labfolder_table_7559317_2.xlsx

Mini-Prep_Protokoll_GE_Healthcare.PDF

Author: Yasoo Morimoto
Entry 21/25: No entry title yet
In Project: General stuff
No tags associated

created: 24.05.2021 09:30
updated: 24.05.2021 10:34

MoChlo recloning

p16 Backbone already purified, needs speedVac and cutting

1. BsaI-HFV2 Verdau ansetzen: Menge an DNA (http://nebiocalculator.neb.com/#!/ligation); auf 20µl mit Wasser auffüllen; 2µl Cutsmart buffer; 0,5µl BsaI

2. Inkubation bei 37°C für 1Stunde

3. Heat Inactivation 80°C for 10min.

4. gesamten Ansatz für T4 Ligase Reaktion verwenden: Backbone (vorher über das Gel aufgereinigt); 2µl T4 Ligase Buffer; 0,5µl T4 Ligase

5. bei 16°C über Nacht inkubieren.

6. Transformation in E.coli.

7. Screen colonies

D1, D2, C11, C12 till step 6 done last week, this week: digestion/ligation of the remaining parts and colony PCR.

First 4 plates grew well

Author: Michael Burgis
Entry 22/25: DNA Extraction Oak, Soy
In Project: General stuff
No tags associated

created: 14.06.2021 16:25
updated: 14.06.2021 16:28

CTAB Extraction using protocol from Aboul-Maaty et al 2019. (https://doi.org/10.1186/s42269-019-0066-1)
Used fresh Oak material and previously -20°C frozen Soy biomass. (500mg)
Scaled protocol times ten to achieve more yield.
Got relatively pure Oak DNA (160ng/µl in 200µl;)
Soy yield wasn't great and curve looked bad. (110ng/µl in 200µl;)

s42269-019-0066-1.pdf

Author: Michael Burgis
Entry 23/25: DNA Extraction Tomato, Soy, Canola, Wheat
In Project: General stuff
No tags associated

created: 14.06.2021 16:28
updated: 14.06.2021 17:09

CTAB Extraction using protocol from Aboul-Maaty et al 2019. (https://doi.org/10.1186/s42269-019-0066-1)

Used fresh samples from greenhouse of canola and Wheat, fresh tomato sample from Renés plants and previously frozen -20°C soy samples.
Scaled protocol times ten to achieve more yield.
Wheat and Canola worked perfectly, soy and tomato were not ideal.

s42269-019-0066-1.pdf

Author: Michael Burgis
Entry 24/25: Primer List
In Project: General stuff
No tags associated

created: 03.07.2021 15:26
updated: 03.07.2021 15:27

Primer_Orders(1).xlsx

Primer_List

	A	B	C	D
1	**Arrived?**	**Name**	**Description**	**{richText=[{style={foreColor=#000000, fo**
2		oiGEM001	atpB 5'UTR Oak fwd	{richText=[{style={foreColor=#ff0000, font=norm
3		oiGEM002	atpB 5'UTR Oak rev	{richText=[{style={foreColor=#ff0000, font=norm
4		oiGEM003	psbA 5'UTR Oak fwd	{richText=[{style={foreColor=#ff0000, font=norm
5		oiGEM004	psbA 5'UTR Oak rev	{richText=[{style={foreColor=#ff0000, font=norm
6		oiGEM005	rbcL 5'UTR Oak fwd	{richText=[{style={foreColor=#ff0000, font=norm
7		oiGEM006	rbcL 5'UTR Oak rev	{richText=[{style={foreColor=#ff0000, font=norm
8		oiGEM007	lvl2_seq_fwd	CTTTTGCTCACATGTTCTTTCC
9		oiGEM008	lvl2_seq_rev	GCAGTTTCATTTGATGCTCG
10		oiGEM009	Spec_seq_rev	CTCGATGACGCCAACTAC
11		oiGEM010	Fluc_seq_rev	GGATGCCCGCAAAATCAG
12		oiGEM011	Fluc_seq_fwd	GTACCATCTTCAAGAGGATAGAAAG
13		oiGEM012	T7_5'_impro_fwd	{richText=[{style={foreColor=#ff0000, font=norm
14		oiGEM013	T7_5'_impro_rev	{richText=[{style={foreColor=#ff0000, font=norm
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oiGEM Primer | oMCB Primer | oRI Primer

primer_list_8382731_3.xlsx

Author: Michael Burgis
created: 17.08.2021 15:51
updated: 17.08.2021 15:54
Entry 25/25: Paris Bettencourt plate setup + growth curve
In Project: General stuff
No tags associated

All 18 constructs shall be measured in a 384 well plate black with transparent bottom.
This includes 1_02, J23101, RB0029-0036+RBS Dummy, sfGFP and sfGFP + cat Tag, TB0015, 6_02, OColEI, Akan mScarlet.

The plate setup can be found in the following schematic.