Autor: Michael Burgis

erstellt: 02.04.2019 13:02
aktualisiert: 20.10.2019 18:33

Eintrag 1/15: inoculation of the first PCC7942 culture
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

Inoculation from old culture: volume (100µl)

OD (730nm) after inoculation: 0,264

OD (730nm) the next day: 1,054

Autor: Michael Burgis

erstellt: 02.04.2019 13:04
aktualisiert: 20.10.2019 18:23

Eintrag 2/15: first transformation with PCC7942
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

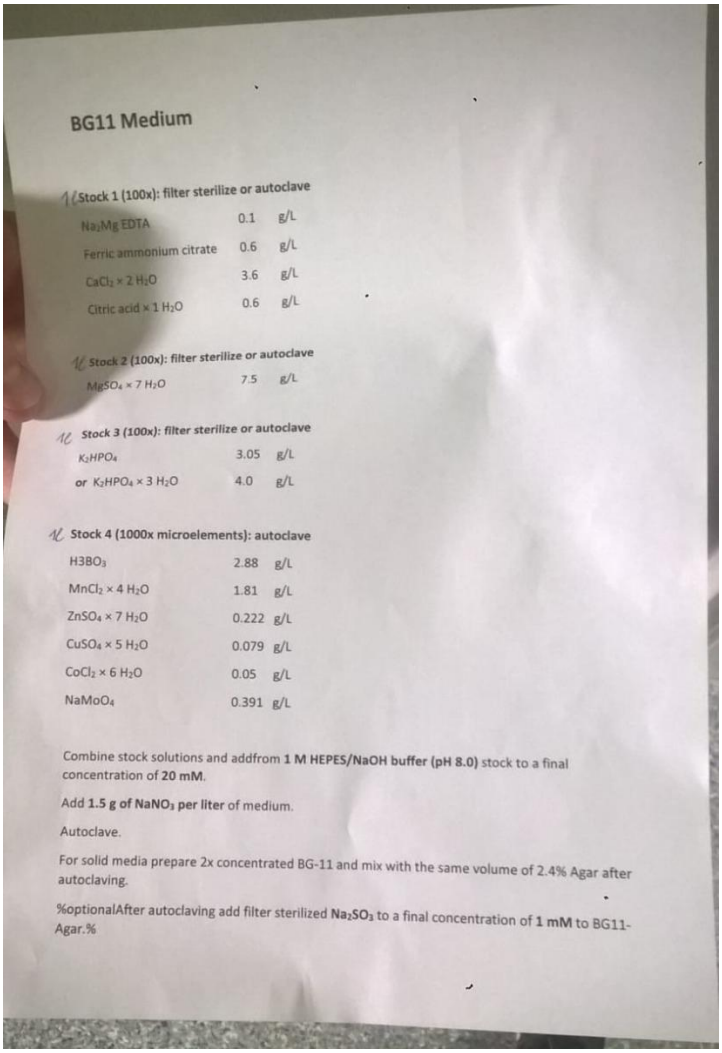
Transformation of PCC 7942 with plasmid PAM4787:
-took 2ml of culture (OD:1,054)
-centrifuge for 2 min (4000g)
-resuspend in 400µl BG11
-pipette approx. 1000ng of plasmid onto the cells: In our case 3µl of 400ng/µl and 4µl of 300ng/µl
-mix by gently inverting the tube
-wrap in aluminum foil and put in Percival (light chamber ~70µmolphotons/m²s)

-inoculation of 4 flasks with transformants (50ml), 4 plates with transformants
-flask were put in light shaker approx. 300µE
-BG11 agar plates were put in light chamber approx. 70µE

Autor: Michael Burgis
Eintrag 3/15: recipe of BG11 media (Erblab)
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

erstellt: 02.04.2019 13:17
aktualisiert: 20.10.2019 18:25

BG11_Medium.jpeg



BG11 Medium

1 L **Stock 1 (100x): filter sterilize or autoclave**

Na_2Mg EDTA	0.1	g/L
Ferric ammonium citrate	0.6	g/L
$CaCl_2 \times 2\ H_2O$	3.6	g/L
Citric acid $\times 1\ H_2O$	0.6	g/L

1 L **Stock 2 (100x): filter sterilize or autoclave**

$MgSO_4 \times 7\ H_2O$	7.5	g/L

1 L **Stock 3 (100x): filter sterilize or autoclave**

K_2HPO_4	3.05	g/L
or $K_2HPO_4 \times 3\ H_2O$	4.0	g/L

1 L **Stock 4 (1000x microelements): autoclave**

$H3BO_3$	2.88	g/L
$MnCl_2 \times 4\ H_2O$	1.81	g/L
$ZnSO_4 \times 7\ H_2O$	0.222	g/L
$CuSO_4 \times 5\ H_2O$	0.079	g/L
$CoCl_2 \times 6\ H_2O$	0.05	g/L
$NaMoO_4$	0.391	g/L

Combine stock solutions and addfrom **1 M HEPES/NaOH buffer (pH 8.0)** stock to a final concentration of **20 mM**.

Add **1.5 g of $NaNO_3$ per liter** of medium.

Autoclave.

For solid media prepare 2x concentrated BG-11 and mix with the same volume of 2.4% Agar after autoclaving.

%optionalAfter autoclaving add filter sterilized **Na_2SO_3** to a final concentration of **1 mM** to BG11-Agar.%

$Na_2S_2O_3$ (Sodium thiosulfate) -> crucial for nice plates, cause it reduces hydrogen peroxide that comes from autoclaving.

Autor: Michael Burgis
Eintrag 4/15: Übertragung der ersten Travos
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

erstellt: 02.04.2019 13:24
aktualisiert: 04.04.2019 13:41

-Zugabe der Travos (erster Eintrag) in jeweils einmal Flüssig und Festmedium (BG11) pro Ansatz (4 Ansätze) = 4 Kolben + 4 Agarplatten

-Ausplattieren der Trafos auf Agarplatten mit Rührkugeln

-Zugabe der Travos auf 50ml Flüssigmedium

-Inkubation der Platten im Lichtschrank

-Inkubation der Kolben im Lichtschüttler

Autor: Jonas Freudigmann

Eintrag 5/15: Chemically competent E.Coli

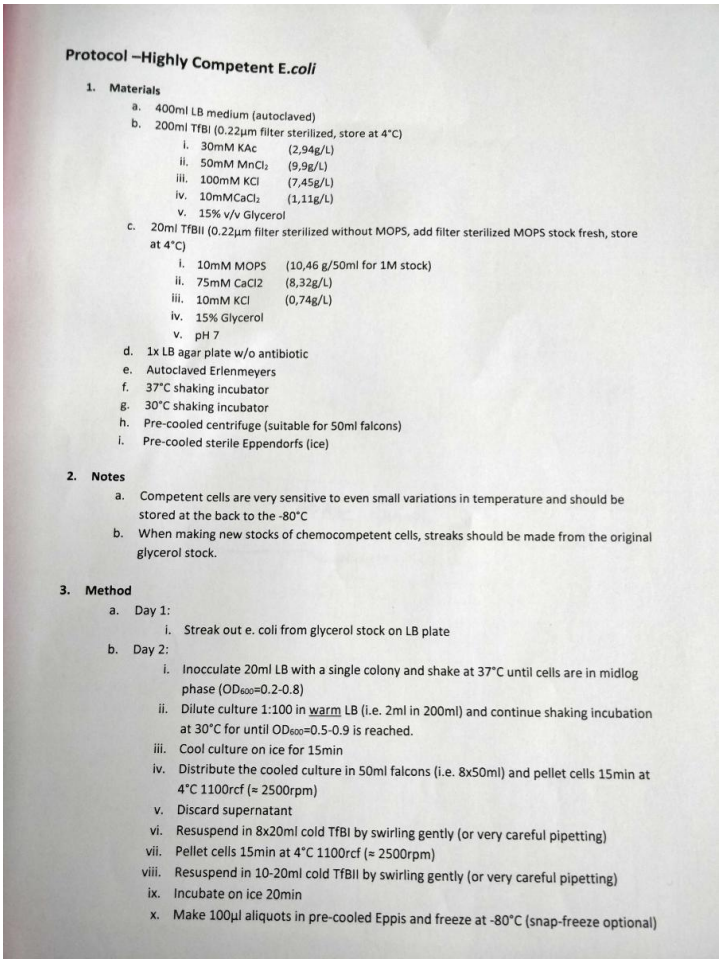
In Projekt: Generell_Strain Engineering

Mit Tags: competent, chemically, chemisch, kompetent, cells, Zellen

erstellt: 05.04.2019 16:10

aktualisiert: 03.10.2019 14:42

IMG_20190405_161126.jpg



Protocol –Highly Competent E.*coli*

1. **Materials**
   a. 400ml LB medium (autoclaved)
   b. 200ml TfBI (0.22µm filter sterilized, store at 4°C)
      i. 30mM KAc (2,94g/L)
      ii. 50mM $MnCl_2$ (9,9g/L)
      iii. 100mM KCl (7,45g/L)
      iv. 10mM$CaCl_2$ (1,11g/L)
      v. 15% v/v Glycerol
   c. 20ml TfBII (0.22µm filter sterilized without MOPS, add filter sterilized MOPS stock fresh, store at 4°C)
      i. 10mM MOPS (10,46 g/50ml for 1M stock)
      ii. 75mM CaCl2 (8,32g/L)
      iii. 10mM KCl (0,74g/L)
      iv. 15% Glycerol
      v. pH 7
   d. 1x LB agar plate w/o antibiotic
   e. Autoclaved Erlenmeyers
   f. 37°C shaking incubator
   g. 30°C shaking incubator
   h. Pre-cooled centrifuge (suitable for 50ml falcons)
   i. Pre-cooled sterile Eppendorfs (ice)

2. **Notes**
   a. Competent cells are very sensitive to even small variations in temperature and should be stored at the back to the -80°C
   b. When making new stocks of chemocompetent cells, streaks should be made from the original glycerol stock.

3. **Method**
   a. Day 1:
      i. Streak out e. coli from glycerol stock on LB plate
   b. Day 2:
      i. Inocculate 20ml LB with a single colony and shake at 37°C until cells are in midlog phase (OD_{600}=0.2-0.8)
      ii. Dilute culture 1:100 in warm LB (i.e. 2ml in 200ml) and continue shaking incubation at 30°C for until OD_{600}=0.5-0.9 is reached.
      iii. Cool culture on ice for 15min
      iv. Distribute the cooled culture in 50ml falcons (i.e. 8x50ml) and pellet cells 15min at 4°C 1100rcf (≈ 2500rpm)
      v. Discard supernatant
      vi. Resuspend in 8x20ml cold TfBI by swirling gently (or very careful pipetting)
      vii. Pellet cells 15min at 4°C 1100rcf (≈ 2500rpm)
      viii. Resuspend in 10-20ml cold TfBII by swirling gently (or very careful pipetting)
      ix. Incubate on ice 20min
      x. Make 100µl aliquots in pre-cooled Eppis and freeze at -80°C (snap-freeze optional)

Autor: Vinca Seiler
erstellt: 07.04.2019 16:11
aktualisiert: 20.10.2019 19:57
Eintrag 6/15: Noch kein Eintragstitel
In Projekt: Generell_Strain Engineering
Mit Tags: Travo E.Coli, PAMs 5187, pams 5188, pams 4787

Transformation of pAMS 5187 / 5188 / 4787 (from Pascal)
→ Chemical Kompetent Cells throw on ICE from -80°C
→ after 30 min mix with euch Plasmid (1µl)
→ 42°C for 30 sec
→ 5min on ice
→ add 0.5 ml SOB-Media
→ 2h in 37°C
→ Plate on Plates

Plasmid	Konz. ng/ul	Antibiotikares.
5187	241	spec
5188	210	spec
4787	410	spec

Picking Colonies at next day
→ in 10ml LB +spec

Autor: Michael Burgis
Eintrag 7/15: first E.coli Trafo
In Projekt: Generell_Strain Engineering
Mit Tags: Trafo E.Coli, pUC19, PilN

erstellt: 08.04.2019 17:03
aktualisiert: 20.10.2019 18:29

Trafo of plasmid pUC 19 und PilN

-used amount Plasmid: pUC 19 (2µl - 125ng/µl), PilN (1µl - 232 ng/µl)

Resistance: amp for pUC19, chloramp for PilN
protocol:

Transformation_of_chemically_competent_cells.docx

Autor: Vinca Seiler
erstellt: 11.04.2019 14:31
aktualisiert: 19.04.2019 15:43
Eintrag 8/15: Plasmid Concentration messurement
In Projekt: Generell_Strain Engineering
Datum: 10.04.2019
Mit Tags: Plasmid Prep, PilN, pams 4787, PAMs 5187, pams 5188

Plasmidisolation via Kit (NucleoSpin Plasmid from Machery-nagel)

→ Elution with EA-Buffer 50ul

Plasmid	Number	Amount of Plasmid [ng /ul]	260/280	260/230
PilN	1	121,1	1,84	2,20
pilN	2	131,5	1,83	2,12
pAM 4787	A	153,0	1,84	2,11
pAM 4787	B	111,7	1,83	2,13
pAM 4787	C	184,5	1,86	2,14
pAM 5788	5	136,0	1,84	2,09
pAM 5788	6	120,8	1,82	2,11
pAM 5788	8	140,1	1,83	2,01
pAM 5787	A / B	0,5 / 0,3	0,76 / 0,64	2,53 / -4,41

⇒ New isolation of pAM 5787 necessary

→ also new travo / new competent cells?

Autor: Vinca Seiler
Eintrag 9/15: Plate new UTEX
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

erstellt: 26.05.2019 03:14
aktualisiert: 26.05.2019 17:00

Replate UTEX WT on new BG11-Plates
→ 100 ul and 50ul

Replate previous Transformations of pilN_NSII_cm
→ pick colonies and dilut in 100ul
→ plate 100 ul

Autor: Vinca Seiler

erstellt: 26.05.2019 16:49
aktualisiert: 26.05.2019 17:01

Eintrag 10/15: Restreak Synecco on BG11- Plates and reinoculate liquid cultures
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

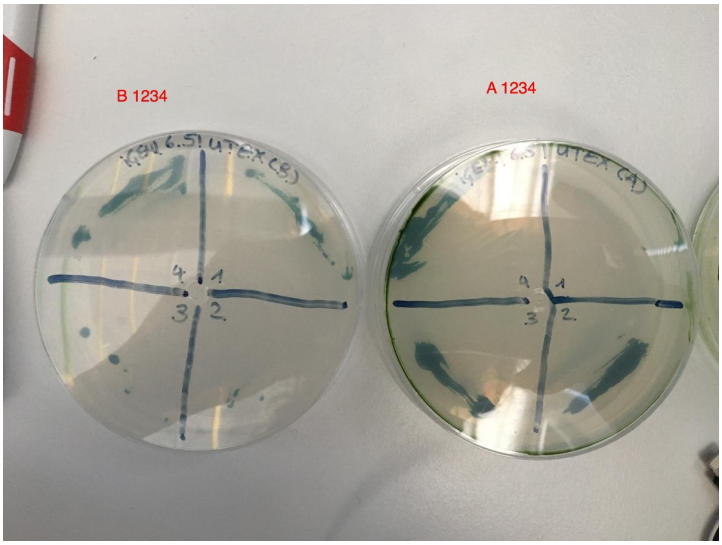
Restreak (old) UTEX on BG11
→ from 6.5 A 1234 and B 1234

Inoculate Synecchococcus PCC 7942
→ Pick colonies and solve in 25 ml liquid BG11

Inoculate new UTEX
→ 100 ul 1:10 from liquid culture and solve in 50ml liquid BG11

→ put in Incubator with 41 °C + 300 uE + 5% CO_2

IMG_9107.jpg



Autor: Vinca Seiler

erstellt: 26.05.2019 17:02

aktualisiert: 26.05.2019 17:03

Eintrag 11/15: Inoculate UTEX with (?) pilN-NSII-cm

In Projekt: Generell_Strain Engineering

Keine Tags verwendet

Autor: Jonas Freudigmann

Eintrag 12/15: Saving private synthesis

In Projekt: Generell_Strain Engineering

Keine Tags verwendet

erstellt: 26.07.2019 18:56

aktualisiert: 26.07.2019 21:55

23.07. CloneJET PCR Cloning

to save our synthesis of the ori (Synthesis 1-3) and Michaels synthesis of the split gRNA (split 1-4) we clone the fragments into the pJET 1.2/blunt Cloning Vector (50ng/ul) from Thermo Scientific provided in their CloneJET PCR Cloning Kit. For an exact protocol see the pdf below.

Fragment	concentration (ng/ul)	volume pipetted in the mix
Synthesis 1	41,8	2 µL
Synthesis 2	95,8	1 µL
Synthesis 3	36	2 µL
split 1	28,7	2 µL
split 2	9,2	1 µL
split 3	8,3	1 µL
split 4	10,4	1 µL

Set up the ligation reaction on ice:

Component	volume
2X Reaction Buffer	10 µL
Non-purified PCR product or purified PCR product/other blunt-end DNA fragment	1 µL 0.15 pmol ends
pJET1.2/blunt Cloning Vector (50 ng/µL)	1 µL (0.05 pmol ends)
Water, nuclease-free	up to 19 µL
DNA Blunting Enzyme	1 µL
Total volume	20 µL

Vortex briefly and centrifuge for 3–5 s.

Incubate the ligation mixture at room temperature (22°C) for 60 min.

Transform 5ul in Top10 competent cells via Transformation chemically competent cells - entry #8 in project 'pANS Domestizierung' (Jonas Freudigmann, 29.05.2019)

25.07.

still no growth on Split 4 plates.

Picked 2 colonies each from every other plate (Split 1-3 & Synth. 1-3) and inoculated in 10ml LB+amp(100mg/ml)

26.07.

prepped cultures:

Fragment	concentration (ng/ul)
pJET Synthesis 1.1	603
pJET Synthesis 1.2	510
pJET Synthesis 2.1	519,8
pJET Synthesis 2.2	542
pJET Synthesis 3.1	202,8
pJET Synthesis 3.2	541,7
pJET split 1.1	624,9
pJET split 1.2	650,8
pJET split 2.1	614,2
pJET split 2.2	481,5
pJET split 3.1	625,9
pJET split 3.2	426,9

Test digest w/ BglII

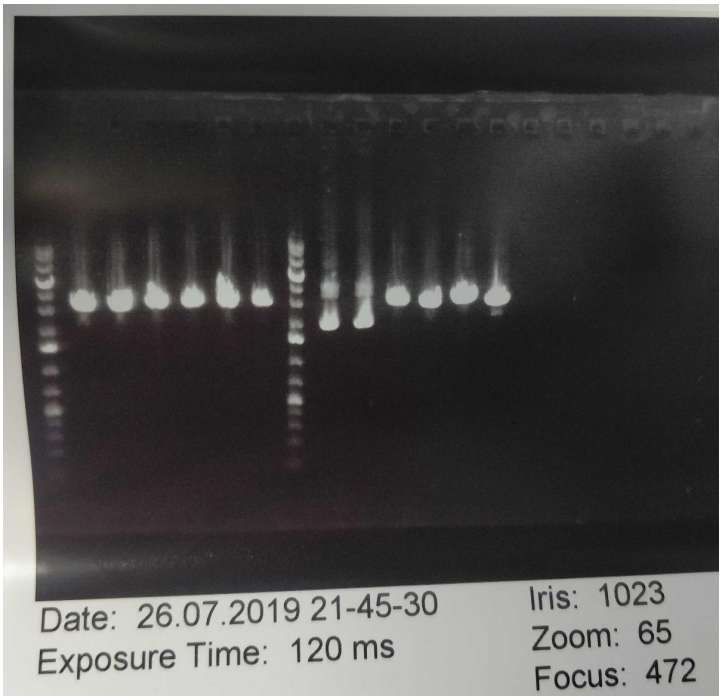
for each 10ul reaction: 1ul FD BglII (Thermo Scientific) + 1ul FD Buffer + 2ul Plasmid + 6ul ddH2O

just pJET Synthesis 3.1 w/ 8ul Plasmid and no ddH2O, due to low concentration.

incubated at 37°C for 1h.

As shown below on the gel it did not work.

kumamamakothermo.jpg



Autor: Jonas Freudigmann
Eintrag 13/15: Strain sequencing
In Projekt: Generell_Strain Engineering
Keine Tags verwendet

erstellt: 13.08.2019 17:28
aktualisiert: 02.09.2019 14:26

Genome prep.

Cultures to prep: UDAR WT, UTEX WT, PCC7942, UDAR 5188+spec

cultured in 100ml BG11 in 500ml flasks

2x 50ml of each culture spinned down for 20min at 4000g.

	UDAR	UDAR-2	UTEX	UTEX-2	PCC	PCC-2	5188	5188-2
pellet weight	0,54g	0,49g	0,48g	0,52g	0,61g	0,53g	0,53g	0,53g

UDAR-2, UTEX, PCC-2 and 5188-2 were chosen for the genomic DNA prep after the Macherey-Nagel NucleoSpin Microbial DNA Kit. It is stated that ~40mg of dry weight pellet should be used, so 1ml (instead of 100µl) of BE Buffer was added, the pellet resuspended and 100µl of the suspension used for the protocol provided in the kit.

	UDAR	UTEX	PCC 7942	UDAR 5188
concentration [ng/µl]	192,1	216,7	123,7	179
260/280	1,85	1,85	1,83	1,84
260/230	1,70	1,58	1,46	1,52

PCR to test

→ assembling all reaction components on ice

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Q5 Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	< 1,000 ng
Q5 High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
5X Q5 High GC Enhancer (optional)	(5 µl)	(10 µl)	(1X)
Nuclease-Free Water	to 25 µl	to 50 µl	

Used amount:
→ 8 50µl reactions

Used Template:
→ UDAR, UTEX, PCC & 5188 genomes

Used Primers:
→ o_iGEM_1_604_nblArepr_f & o_iGEM_1_605_nblArepr_r

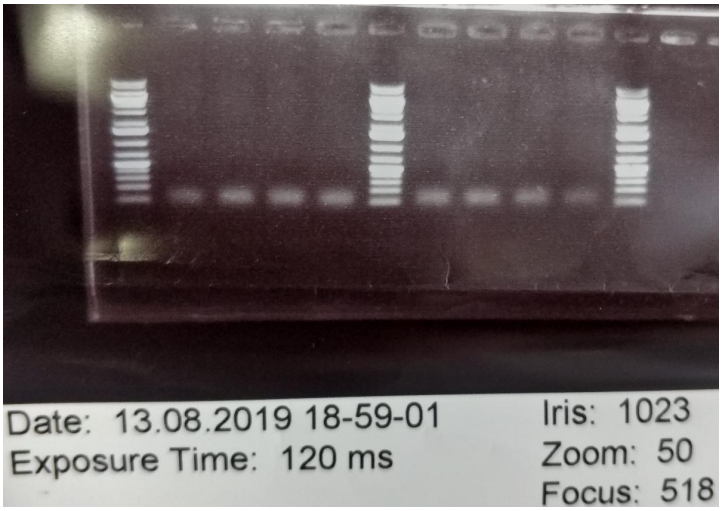
Thermocycling Conditions

STEP	**TEMP**	**TIME**
Initial Denaturation	98 °C	30 seconds
25 Cycles	98 °C *59 / 60 °C 72 °C	10 seconds 20 seconds 25 seconds
Final Extension	72 °C	2 minutes
Hold	4–10 °C	

Put on gel as follows: UDAR 59°C, UDAR 60°C, UTEX 59°C, UTEX 60°C, PCC 59°C, PCC 60°C, 5188 59°C, 5188 60°C

bad results, see image below. Maybe due to primers.

Gel PCR NblA.jpg

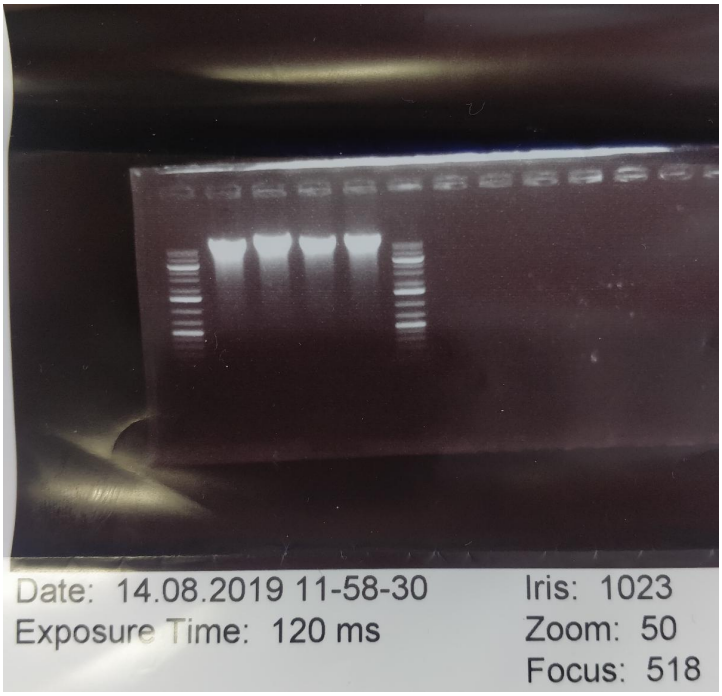


New test:

The extracted DNA was put on a gel directly as follows: UDAR, UTEX, PCC, 5188

Looks like genomic DNA; see below.

genomic DNA on gel.jpg



Autor: Jonas Freudigmann

erstellt: 03.10.2019 14:08
aktualisiert: 03.10.2019 15:56

Eintrag 14/15: Quick electroporation protocol for E.coli
In Projekt: Generell_Strain Engineering
Mit Tags: water, wash, electroporation, electro, competent cells, Transformation, competent, kompetent, Elektroporation

This quick electroporation protocol was explained to me by Dr. Alberto Sánchez-Pascuala Jerez from the research group of Prof. Dr. Tobias J. Erb in the Max-Planck-Institute for terrestrial microbiology in Marburg. It was adapted by me and might not be exactly how he does it.

Quick electroporation of *E.coli*

Optional:

1. Restreak cells from glycerol stock on LB.
2. Inoculate a single colony in liquid LB.

Standard:

1. Inoculate your culture at **OD_{600}=0,05** from an overnight culture.

   I use 50ml, but less probably works well too.

2. Grow the culture until an **OD_{600}=0,5** is reached.

   If the culture reaches OD_{600}=~0,6 it can still be used, but I would recommend reinoculating if it reaches higher values.

3. Directly put the **culture on ice** for 10-15min.
4. Transfer the culture into a falcon and spin down in a cooled centrifuge at **2500rpm, 4°C** for **10-15min**.

   Heraeus™ Multifuge™ X1 is used for higher volumes; I think you could go up to 4000g and shorter time, dependig on what centrifuge and culture volume you use.

5. **Wash** the cells **two to three times** in ddH_2O (or other sterile water).

   I typically wash the first time in 25ml and afterwards in 10ml.

6. Resuspend in a few ml water, depending on how many aliquots you want and how big the pellet is.

   I use 0,5 - 2ml water.

7. Make 50µl - **100µl aliquots**.
8. **Add DNA** to your aliquot on ice.
9. Transfer cell/DNA mix **into** an **electroporation cuvette** on ice.

   I use Gene Pulser®/MicroPulser™ Electroporation Cuvettes (green cap) from Bio-Rad.

10. **Wipe the cuvette** with a paper towel to remove any liquid that might cause an arc and place it in the electroporation chamber.

11. **Electroporate** the sample, directly **add recovery medium** and **transfer** the cells **into a reaction tube**.

    I use the following settings: 2500V, 25µF, 200Ω, 2mm

    I add 500µl SOB medium.

12. **Incubate** the cells at 37°C and 250rpm for 1h (amp resistance) or 2h (other resistances; kan, cam, spec..)

13. **Plate** and incubate at 37°C over night.

Autor: Michael Burgis
Eintrag 15/15: new BG11 agarose plates (James Golden)
In Projekt: Generell_Strain Engineering
Mit Tags: James Golden, Plates, BG11 plates, UTEX2973, PCC7942

erstellt: 13.10.2019 16:23
aktualisiert: 14.10.2019 11:01

Standard BG11 recipe for plates found in entry (Ansetzen der Medien)
Slight deviation:
100mM Sodium thiosulfate pentahydrate 500ml have been made to add to 2xBG11 to a final concentration of 2mM
also used 1,4% Agarose biozym instead of Agar Agar

Made 7 plates á 30ml (100ml 2xBG11 + 100ml 1,4% Agarose)