Author: Daniel Marchal
created: 10.04.2018 16:42
updated: 10.04.2018 16:54
Entry 1/214: Retrafo of mcr-plasmids
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation

Retransformation in *E.Coli* (mcr CA, mcr C-term; mcr N-Term, mcr ST)

1. add 1µl of plasmid into competent cells (50µl *E.coli* Dh5α)

2. incubate 5 min on ice

3. heatshock at 42°C for 60 sec.

4. incubate 2 min on ice

5. add 800 µl LB

6. incubate 30 min at 37°C (shaking)

7. spread out on plates with antibiotics (ampicillin)

8. incubate over night at 37°C

Author: Daniel Marchal
Entry 2/214: No entry title yet
In Project: ERBsen
No tags associated

created: 11.04.2018 08:23
updated: 11.04.2018 08:40

Author: Daniel Marchal
created: 11.04.2018 08:27
updated: 05.09.2018 15:41

Entry 3/214: Preparation of chemocompetent E. coli cells
In Project: ERBsen
With tags: competent, heat shock, CaCl2, chemocompetent, Competent cells, E. coli

Materials

- 250ml LB medium (autoclaved)
- 50ml TfBI (0.22µm filter sterilized, store at 4°C)
  - 30mM KAc (2,94g/L)
  - 50mM $MnCl_2$ (9,9g/L) (add after autoclave)
  - 100mM KCl (7,45g/L)
  - 10mM$CaCl_2$ (1,11g/L)
  - 15% v/v Glycerol
- 15ml TfBII (0.22µm filter sterilized without MOPS, add filter sterilized MOPS stock fresh, store at 4°C)
  - 10mM MOPS (10,46 g/50ml for 1M stock)
  - 75mM CaCl2 (8,32g/L)
  - 10mM KCl (0,74g/L)
  - 15% Glycerol
  - pH 7
- Autoclaved Erlenmeyers
- 37°C shaking incubator
- Pre-cooled centrifuge (suitable for 50ml falcons)
- Pre-cooled sterile Eppendorfs (ice)

Method

1. grow 50ml overnight culture in LB medium
2. transfer approximately 10ml cells to 250ml TYM medium
3. grow cells to midlog phase (OD_{600} = 0.5 - 0.6)
4. cool cells on ice (keep cells cold from now on)
5. centrifuge 15min, 3500g, 4°C (tubes have to be autoclaved first)
6. discard supernatant
7. resuspend cells in 50ml cold TfB1 on ice
8. centrifuge 15min, 3500g, 4°C, discard supernatant
9. resuspend cells in cold TfB2 on ice to an theoretical OD of 10 (app. 15ml)
10. make aliquots (50µl), freeze in liquid nitrogen and store at -80°C

Notes

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

Comments

- oN culture inoculated from Cryostock E. coli NEB Turbo
- from oN culture 250ml LB were inoculated with 5ml preculture at 8:15 AM and incubated at 30°C shaking (see step 2)
- OD600(9:15)=0.109
- OD600(10:15)=0.356
- OD600(11:00)=0.670 → harvested in step 5
- in step 9 15ml were used

Author: Daniel Marchal
Entry 4/214: Vn preculture for cryostock
In Project: ERBsen
With tags: Cryostock

created: 11.04.2018 08:28
updated: 11.04.2018 08:44

1. 5ml LB 2.5 inoculated from Glystock (Bangelab, -80°C)
2. Incubate 5 hours at 37°C while shaking
3. streak out from undiluted and 1:50 diluted preculture on LB 2.5 plate without antibiotics
4. Inoculate 150ml BHI for competent Vn cells (Preparation of chemocompetent Vn cells (Weinstock) - entry #7 in project 'ERBsen' (Daniel Marchal, 11.04.2018)
5. Before harvesting the cells take two 800µl samples and add 200µl Glycerol
6. Mix well and freeze at -80°C

Author: Daniel Marchal
Entry 5/214: Colorcode for LB-plates
In Project: ERBsen
With tags: colorcode

created: 11.04.2018 08:28
updated: 27.09.2018 08:46

	Stock [mg/ml]	Color	Solvent	LB	LBv2
Ampicillin	100	black	H_2O	|	|| |
Kanamycin	50	green	H_2O	|	|| |
Chloramphenicol	34	blue	100% EtOH	|	|| |
Tetracyclin	10	red	70% EtOH	|	|| |
Gentamycin	15	red	H_2O	||	|| ||
Streptomycin	20	blue	H_2O	||	|| ||
Spectinomycin	/	green	H_2O	||	|| ||
no antibiotic		black	H_2O	||	|| ||

Author: Daniel Marchal
Entry 6/214: Media preparation
In Project: ERBsen
With tags: Stock, media

created: 11.04.2018 08:28
updated: 11.04.2018 08:28

v2 salts 10x:

2.4 M NaCl (119.22 g/l)

42 mM KCl (3.13 g/l)

231.4 mM $MgCl_2$ (47.04 g/l from hexahydrate)

100mM $MgCl_2$

20.32 g/l $MgCl_2$ x 6 H_2O

100mM $CaCl_2$

14.7 g/l $CaCl_2$ x 2 H_2O

LB 2.5

25 g/l LB-medium (Luria/Miller)

15 g/l NaCl

LB 2.5 agar

25 g/l LB-medium (Luria/Miller)

15 g/l NaCl

15 g/l Agar

BHI v2

37 g/l BHI

ad 900 ml H_2O

after autoclaving supplement with 100 ml v2 salt 10x

BHI v2 agar

37 g/l BHI

15 g/l Agar

ad 900 ml H_2O

after autoclaving supplement with 100 ml v2 salt 10x

Electroporation Buffer for Weinstock electrocompetent cells

680 mM Sucrose (232.8 g/l)

7 mM K_2HPO_4 (1.219 g/l)

adjust to pH 7.0

Author: Daniel Marchal
created: 11.04.2018 08:37
updated: 11.04.2018 15:40
Entry 7/214: Preparation of chemocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: competent, chemocompetent, weinstock

Reagents

150ml BHI + v2 salts

1.5ml storage buffer

100ml $MgCl_2$ [100mM]

100ml $CaCl_2$ [100mM]

15ml $MnCl_2$ [550mM]

15ml KCl [1M]

15ml PIPES [100mM]

120µl spec. DMSO

Recipes

BHI + v2 salts

37g/l brain heart infusion broth

204mM NaCl

4.2mM KCl

23.14mM $MgCl_2$

Storage buffer

55mM $MnCl_2$

15mM $CaCl_2$

250mM KCl

10mM Pipes

7% spec. DMSO

Preparation of chemically competent cells

On the day of competent cell preparation, 150 mL of BHI + v2 salts is inoculated directly from a glycerol stock of *V. natriegens* (carrying a deletion of the chromosomal Dns endonuclease) and incubated in a baffled flask at 30 °C with agitation at 200 r.p.m. to an OD_{600} of 0.4 (~2 h). All subsequent steps are performed quickly at room temperature. The culture is split into three 50-mL conical tubes, and the cells are pelleted by centrifugation at 3,000 × *g* for 5 min. The supernatant is carefully removed, and each pellet is gently suspended with 5 mL 100 mM $MgCl_2$. The cells from all three conical tubes are consolidated into two 50-mL conical tubes, the volume in each tube is brought up to 30 mL with 100 mM $MgCl_2$, and the tubes are mixed by gentle inversion. Cells are pelleted by centrifugation at 3,000 × *g* for 4 min. The pellets are each suspended in 5 mL 100 mM $CaCl_2$, consolidated into one tube, and the volume brought up to 30 ml with additional 100 mM $CaCl_2$. The tube is gently mixed by inversion and then incubated at room temperature for 20 min. Following the incubation, cells are pelleted by centrifugation at 3,000 × *g* for 4 min. The supernatant is removed, and the cells are resuspended in ~1.5 mL transformation storage buffer (a modified version of the buffer of Inoue containing DMSO): 55 mM $MnCl_2$, 15 mM $CaCl_2$, 250 mM KCl, 10 mM PIPES (from 0.5 M, pH 6.7, stock), 7% (v/v) spec grade DMSO (where the DMSO is added after cells are suspended in the other buffer components). The cells are then aliquoted into chilled tubes, frozen in a dry ice bath, and stored at –80 °C until use.

Comments

- cell culture inocculated from LB plate (platet out one day before from Cryostock) at 08:15 AM
- OD600(9:15)=0.001
- OD600(10:15)=0.029
- OD600(11:00)=0.129
- OD600(12:00)=1.440 → OD is very high, I will nevertheless try to get competent cells from it
- instead of dry ice I used liquid nitrogen
- the 20min incubation step was a little bit longer
- the used DMSO is not spec grade and not sterilized

Author: Daniel Marchal
Entry 8/214: Retrafo of pYTK into Vn + Ec
In Project: ERBsen
No tags associated

created: 13.04.2018 12:55
updated: 13.04.2018 12:55

Updated: 12.04.2018

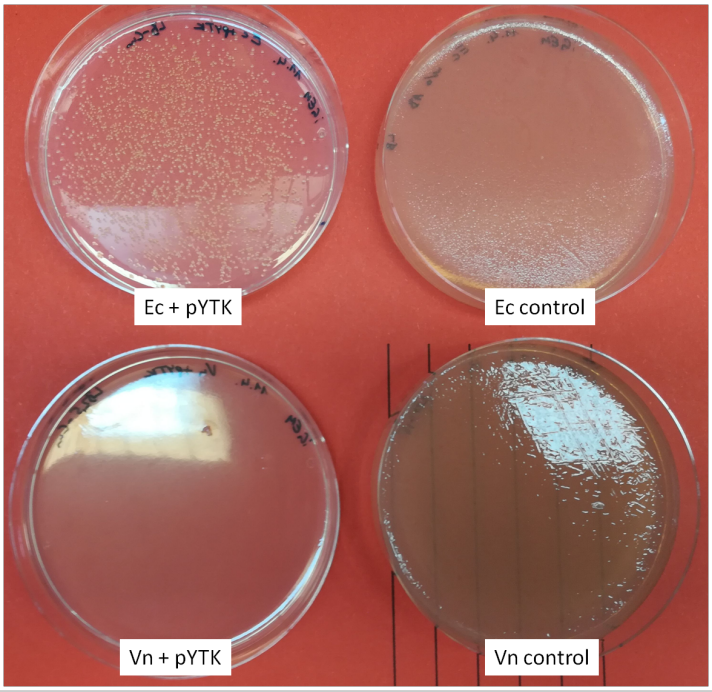
Procedure

1. Thaw 2 aliquots of competent E. coli NEB Turbo and 3 aliquots of competent V. natriegens on ice
2. Add 2µl pYTK [380ng] into one aliquot of Ec and Vn
3. 30min ice
4. 45sec 42°C
5. 90sec ice
6. Add 1ml prewarmed BHIv2/LB
7. 2h shaking at 30°C/37°C
8. Plate out on LB2.5/LB with and without Cm (50µl for Ec and pellet for Vn)
9. Incubate oN at 30°C/37°C

Results

- Ec + pYTK shows viable colonies with green color (pYTK encodes *gfp*) → Ec cells are competent !
- Vn control shows lawn → Vn cells are viable
- Vn + pYTK shows no colonies → Vn cells aren't competent. Cells will be discarded and new ones will be prepared

Results.png



Author: Daniel Marchal
created: 13.04.2018 12:56
updated: 13.04.2018 16:24
Entry 9/214: Preparation of media for pH tolerance assay
In Project: ERBsen
No tags associated

1M KH_2PO_4 100ml → 13,61g

1M K_2HPO_4 100ml → 17,42g

1M Na-Acetate 100ml → 13,61g Trihydrate

1M Acetic acid 100ml → 60,05g (liquid)

1M Glycine 100ml → 7,51g

KH_2PO_4, K_2HPO_4 and Na-Acetate were autoclaved, Glycine was sterilfiltrated and Acetic acid is still sterile

Author: Daniel Marchal
created: 13.04.2018 16:41
updated: 13.04.2018 16:42
Entry 10/214: Preparation of chemocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: chemocompetent, competent, weinstock

Reagents

150ml BHI + v2 salts

1.5ml storage buffer

100ml $MgCl_2$ [100mM]

100ml $CaCl_2$ [100mM]

15ml $MnCl_2$ [550mM]

15ml KCl [1M]

15ml PIPES [100mM]

120µl spec. DMSO

Recipes

BHI + v2 salts

37g/l brain heart infusion broth

204mM NaCl

4.2mM KCl

23.14mM $MgCl_2$

Storage buffer

55mM $MnCl_2$

15mM $CaCl_2$

250mM KCl

10mM Pipes

7% spec. DMSO

Preparation of chemically competent cells

On the day of competent cell preparation, 150 mL of BHI + v2 salts is inoculated directly from a glycerol stock of *V. natriegens* (carrying a deletion of the chromosomal Dns endonuclease) and incubated in a baffled flask at 30 °C with agitation at 200 r.p.m. to an OD_{600} of 0.4 (~2 h). All subsequent steps are performed quickly at room temperature. The culture is split into three 50-mL conical tubes, and the cells are pelleted by centrifugation at 3,000 × *g* for 5 min. The supernatant is carefully removed, and each pellet is gently suspended with 5 mL 100 mM $MgCl_2$. The cells from all three conical tubes are consolidated into two 50-mL conical tubes, the volume in each tube is brought up to 30 mL with 100 mM $MgCl_2$, and the tubes are mixed by gentle inversion. Cells are pelleted by centrifugation at 3,000 × *g* for 4 min. The pellets are each suspended in 5 mL 100 mM $CaCl_2$, consolidated into one tube, and the volume brought up to 30 ml with additional 100 mM $CaCl_2$. The tube is gently mixed by inversion and then incubated at room temperature for 20 min. Following the incubation, cells are pelleted by centrifugation at 3,000 × *g* for 4 min. The supernatant is removed, and the cells are resuspended in ~1.5 mL transformation storage buffer (a modified version of the buffer of Inoue containing DMSO): 55 mM $MnCl_2$, 15 mM $CaCl_2$, 250 mM KCl, 10 mM PIPES (from 0.5 M, pH 6.7, stock), 7% (v/v) spec grade DMSO (where the DMSO is added after cells are suspended in the other buffer components). The cells are then aliquoted into chilled tubes, frozen in a dry ice bath, and stored at –80 °C until use.

Comments:

- cells inoculated from cryostock
- cells harvested at OD600=0.69
- Liquid N_2 used instead of dry ice

Author: Daniel Marchal
Entry 11/214: pH tolerance assay for Vn
In Project: ERBsen
With tags: pH, tolerance, V. natriegens

created: 17.04.2018 14:52
updated: 18.04.2018 10:19

Preparation of media for pH tolerance assay - entry #9 in project 'ERBsen' (Daniel Marchal, 13.04.2018)

Procedure:

- Inoculate precultur of BHIv2 with V. natriegens and incubate oN at 30°C
- Prepare buffer solutions for each pH value in the table
- Measure pH with pH paper (sterile!)
- Prepare 5ml flasks with 4ml BHI + 1mlbuffer (see table) for pH2 – pH9
- Measure pH with pH paper (sterile!)
- Inoculate flasks with 10µl preculture
- Mix well and incubate at 37°C shaking
- Take samples after 15min and 6h and plate out 50µl on LB2.5 plates (for pH6-8 use 1:100 dilution)
  - for samples 7.25, 8, 8.5 and 9 after 6h make a 1:100 dilution and plate it out
- Incubate plates at 37°C oN and determine CFUs

All stock solutions should be at the same molarity (volumens refer to an buffer volume of 50ml):

Measured pH values in BHI-tubes:

2,5

3

4

4

4,5

4,75

5

6

6,25

6,75

7,25

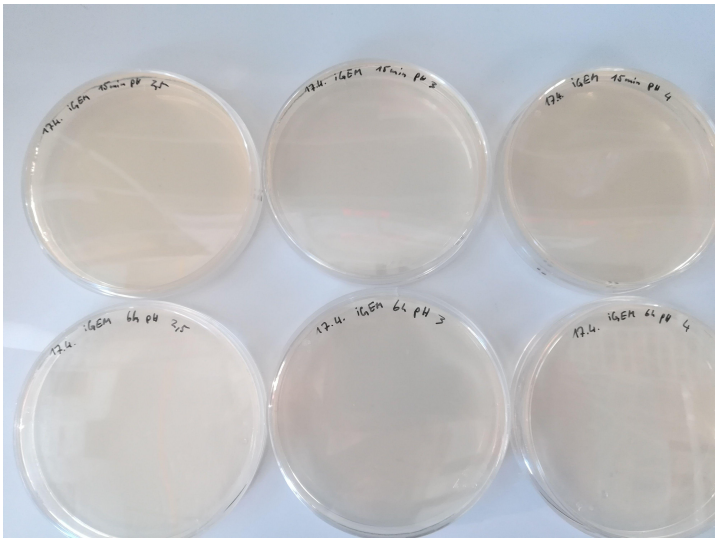
8

8,5

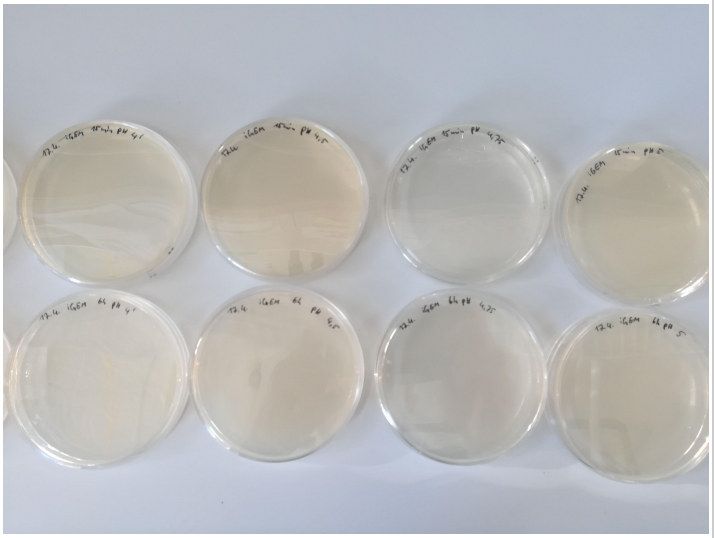
9

pH	Acid	V(Acid-Stock)	Base	V(Base-Stock)
2.4	HCl	32,40	Glycine	50,00
3	HCl	11,40	Glycine	50,00
3.5	Acetic acid	2,60	Sodium acetate	47,40
4	Acetic acid	7,40	Sodium acetate	42,60
4.5	Acetic acid	17,73	Sodium acetate	32,27
5	Acetic acid	31,74	Sodium acetate	18,26
5.5	Acetic acid	42,30	Sodium acetate	7,70
6	KH_2PO_4	2,90	K_2HPO_4	47,10
6.5	KH_2PO_4	8,16	K_2HPO_4	41,84
7	KH_2PO_4	19,07	K_2HPO_4	30,93
7.5	KH_2PO_4	33,05	K_2HPO_4	16,95
8	KH_2PO_4	43,02	K_2HPO_4	6,98
8.5	Glycine	50,00	NaOH	4,00
9	Glycine	50,00	NaOH	8,80
9.5	Glycine	50,00	NaOH	22,40

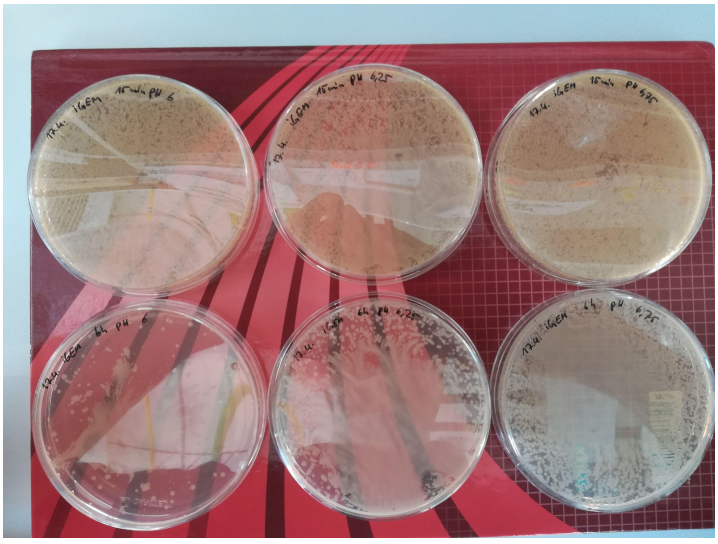
2.5 / 3.0 / 4.0.jpg



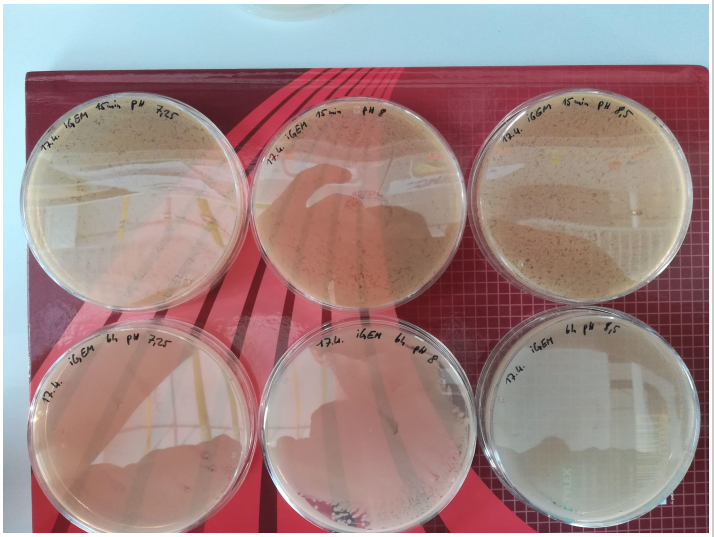
4.0 / 4.5 / 4.75 / 5.0.jpg



6.0 / 6.25 / 6.75.jpg



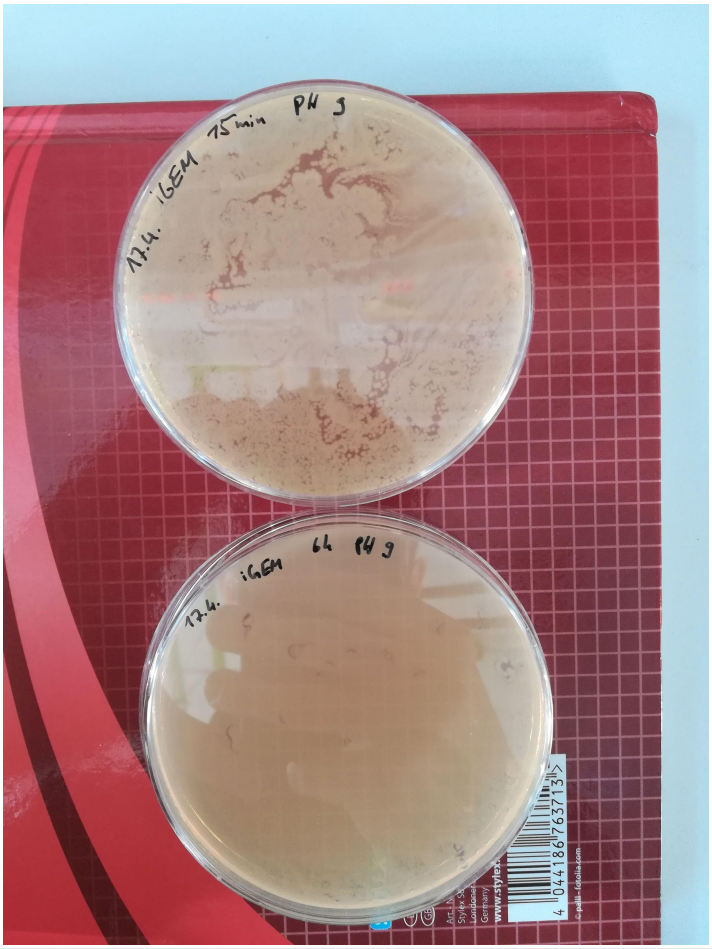
7.25 / 8.0 / 8.5.jpg



Results

- pH 5 or lower shows no viable colonies
- pH 6 - pH 6.75 shows a slightly decreased viability
- pH 7.25 - pH 9 shows high viability
- seawater has a pH of 8.4 so our results fit to the environmental growth conditions of Vn
- the assay will be repeated for pH 9 - pH 14

9.0.jpg



Author: Daniel Marchal
Entry 12/214: Growth assay for Vn at 30 degree
In Project: ERBsen
With tags: growth, V. natriegens, E. coli

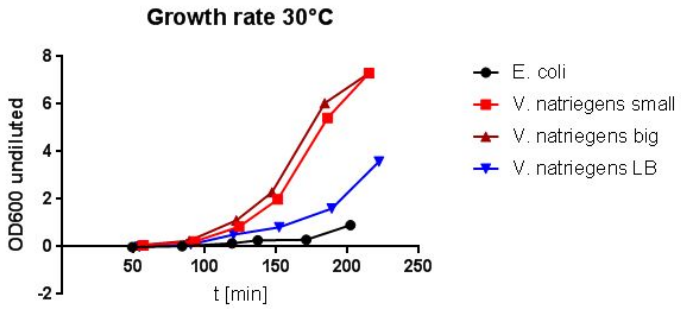
created: 17.04.2018 16:06
updated: 17.04.2018 16:18

Procedure

1. Prepare pre- and maincultures (see excel file)
2. Inoculate precultures of Ec NEB Turbo (LB) and Vn (BHIv2)
3. Incubate oN at 37°C (Ec) / 30°C (Vn)
4. Measure OD600 and inoculate prewarmed main cultures to ODs of ~0.05
5. Measure OD600 of main cultures and incubate at 37°C (Ec) or 30°C (Vn)
6. Measure OD600 every 25-60min

Growth_assay_30_degree.xlsx

growth_assay_30_degree.jpg



Results

- Vn grows faster than Ec
- Vn in BHIv2 grows faster than in LB2.5
- Flask size doesn't play a role for growth of Vn
- growth rate of Vn (Vn-small between t=150 and t=215): μ=0,026 min^{-1}
- doubling time of Vn (Vn-small between t=150 and t=215): T=26,57 min

Author: Daniel Marchal
Entry 13/214: Media preparation
In Project: ERBsen
With tags: BHI, ocean salt, sucrose

created: 18.04.2018 11:03
updated: 18.04.2018 15:47

BHI-O

37g BHI in 900ml H_2O

after autoclaving add 100ml 10xOceanSalt

5xOceanSalt (500ml)

150g/l Ocean salt

2M Sucrose 100ml

68,46g ad 100ml H_2O

Regeneration buffer 15ml

5.1ml 2M Sucrose

555mg BHI

1.5ml v2 salts

ad 15ml H_2O

Author: Daniel Marchal
Entry 14/214: Retrafo of pYTK into Vn
In Project: ERBsen
With tags: transformation, V. natriegens, retrafo, retransformation

created: 18.04.2018 11:12
updated: 19.04.2018 08:07

Procedure

1. Thaw 3 pellets of chemocompetent *V. natriegens* on ice
2. Inoculate one pellet with 1µl plasmid (pYTK, Cm, 219ng/µl)
3. Incubate 30min on ice
4. Heat shock 45sec at 42°C
5. Incubate 1.5min on ice
6. Add 1ml prewarmed BHIv2
7. Incubate 2h at 30°C while shaking
8. Plate out the pellet on LB2.5 + Cm

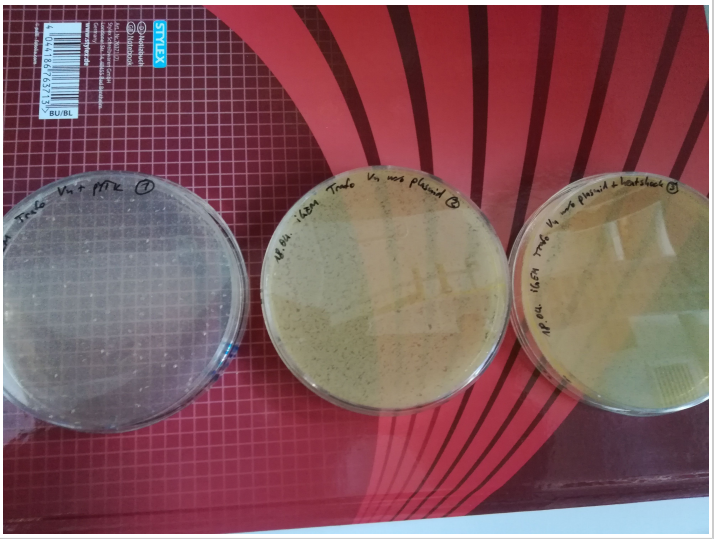
Samples

1	pYTK
2	Control without plasmid
3	Control without plasmid + heat shock

Results

- both control strains show lawn of cells → prepared cells are viable
- Sample 1 (with pYTK on LB2,5+Cm) shows a very high amount of small dots which look like colonies → seems that prepared cells are competent!
- next steps: enrichment of these cells, Miniprep, control digestion

0419_retrafo_pYTK.jpg



Author: Daniel Marchal
Entry 15/214: Growth assay for Vn at 37 degree
In Project: ERBsen
With tags: growth, V. natriegens, E. coli

created: 18.04.2018 16:58
updated: 18.04.2018 17:09

Procedure

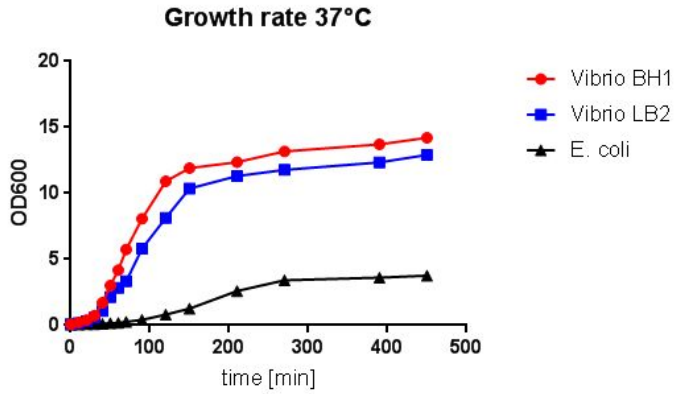
1. Prepare pre- and maincultures (preculture: 100ml flask with 10ml BHIv2/LB) main culture: 250ml flask with 40ml BHIv2/LB2,5 /LB)
2. Inoculate oN cultures and incubate at 30°C
3. Measure OD600 of the precultures
4. Inoculate maincultures of Ec NEB Turbo (LB) and Vn (BHIv2) to an OD of 0.05
5. Measure OD600 of main cultures and incubate at 37°C while shaking (~180rpm)
6. Measure OD600 every 10min

Results

- Vn grows faster than Ec
- Vn in BHIv2 grows faster than in LB2.5 at exponential phase, but reaches nearly the same OD at stationary phase
- doubling time of Vn (Vn-BH1 between t=0 and 10): T=7.11 min
- doubling time of Ec (between t=30 and t=40): T=19.10 min

Growth_assay_37_degree.xlsx

Growth_assay_37_degree.jpg



Author: Daniel Marchal
created: 19.04.2018 12:29
updated: 19.04.2018 14:06
Entry 16/214: Preparation of electrocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: competent, V. natriegens, electroporation, weinstock, electrocompetent

Materials

260-510ml BHI + v2 salts

110ml Electroporation buffer

liquid nitrogen

Recipes

BHI + v2 salts

37g/l brain heart infusion broth

204mM NaCl

4.2mM KCl

23.14mM $MgCl_2$

Electroporation buffer

680mM sucrose

7mM K_2HPO_4

pH7

Procedure

10 mL BHI + v2 salts is inoculated with V. natriegens andincubated overnight at 30 °C with agitation at 200 r.p.m. On thefollowing day, 250–500 mL of the same growth medium is inoculated with the overnight culture at a dilution of 1:100 to 1:200(overnight culture /fresh medium). The culture is grown at 37 °Cin a baffled flask with shaking at 200 r.p.m. until an OD600 of 0.5.The culture is then split into two chilled 250-mL centrifuge bottlesand incubated on ice for 15 min. The cells are pelleted at 6,500r.p.m. in a Beckman JA-14 centrifuge rotor for 20 min at 4 °C.The supernatant is carefully decanted and the cell pellets are gently resuspended in 5–10 mL of electroporation buffer (680 mMsucrose, 7 mM K2HPO4, pH 7). The suspensions are transferred to a centrifuge tube, and the tube is filled to top (~35 mL)with additional electroporation buffer and inverted several timesto mix. The cells are centrifuged down at 6,750 r. p.m. for 15min at 4 °C in a JA-17 rotor. The supernatant is decanted with apipette. The wash is repeated two times for a total of three washes.After the final wash, the cells are gently resuspended in residualelectroporation buffer. The volume is adjusted with additionalelectroporation buffer to bring the final OD600 to 16. Cells arealiquoted into chilled tubes, frozen in a dry ice bath and storedat -80 °C until use.

Comments

- Mainculture inoculated with 1:200 dilution of preculture (1.25ml) → OD600=0.120
- Cells harvested at OD600=0.55
- Cells harvested in 50ml Falcons (4°C/10min/4000rpm)
- Cells washed with 28ml electroporation buffer instead of 35ml
- To reach final OD of 16 cells were resuspendet in 5ml electroporation buffer
- Aliquots were made with 50µl volume

Author: Daniel Marchal
Entry 17/214: pH tolerance assay for Vn (2)
In Project: ERBsen
No tags associated

created: 19.04.2018 14:06
updated: 24.04.2018 10:03

pH tolerance assay for Vn - entry #11 in project 'ERBsen' (Daniel Marchal, 18.04.2018)

Procedure

1. Inoculate precultur of BHIv2 with V. natriegens and incubate oN at 30°C
2. Prepare media with 50% BHI + 50% Glycine 1M, adjust pH with NaOH (pH 6 / 6.5 / 7 / 7.5 / 8 / 8.5 / 9 / 9.5 / 10 / 11 / 12)
3. Sterilfiltrate the media (the pH meter contaminated the samples)
4. Inoculate flasks with 10µl preculture
5. Mix well and incubate at 37°C shaking
6. Take samples after 15min and 6h and make 1:10,000 dilutions
7. plate out 50µl on LB2.5 plates
8. Incubate plates at 37°C oN and determine CFUs
9. Check if pH in media is still in appropriate range

Results

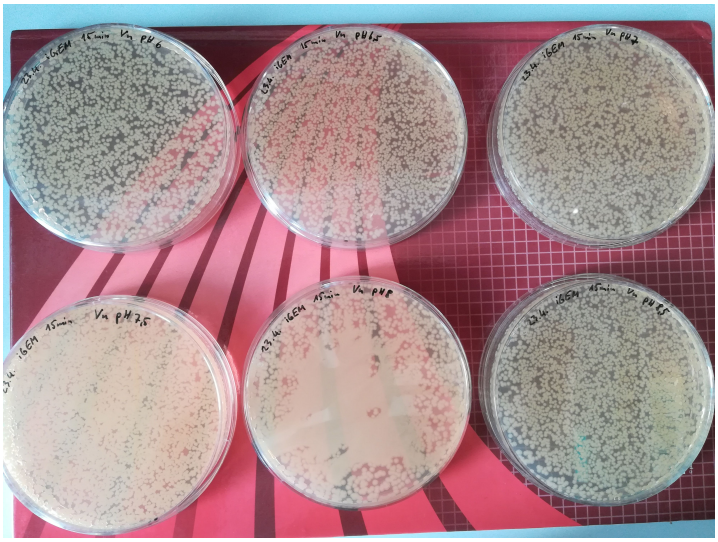
- the plates after 5h show no viable cells → dilution was probably too high
- pH 6 - 9.5 after 15 min shows viable colonies (pH6 2952 colonies, pH 9.5 3304 colonies) with maximum at pH 7.5 + pH8
- pH 10 after 15 min shows no viable cells → Vn is tolerant until pH 9.5
- last pH tolerance assay showed unaffected growth at pH9 what is consistent with these results
- last pH tolerance assay showed decreased viability below pH 7 what couldn't be observed in these results

Comments

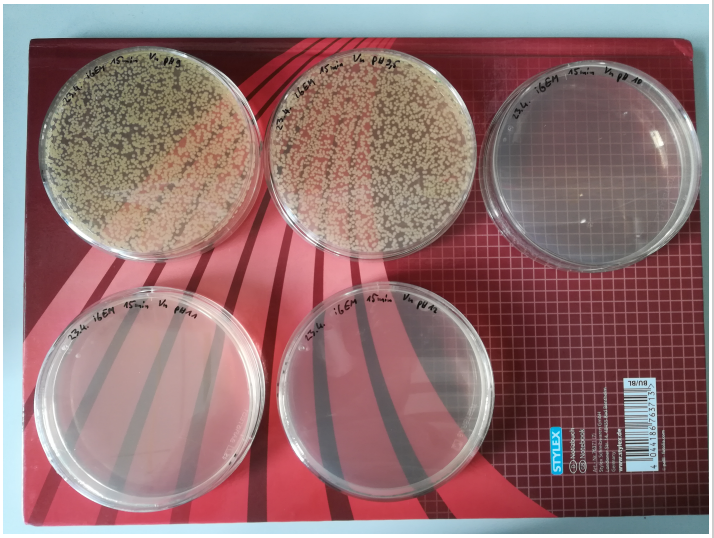
- for media preparation a stock of 70ml BHIv2 + 70ml 1M Glycine was prepared and with HCl/NaOH the pH altered, every time when a target pH was reached 10ml were aliquoted and sterilfiltrated
- while adjusting the pH the medium got unclear → not sure if contamination (but it must be a fast growing organism!) or salt was the reason. After sterilfiltration the media were clear. To ensure that the media are sterile, after sterilfiltration but before inoculation the media were incubated at room temperature for 1 hour to see if the media gets unclear again.
- by accident from the 15min samples just a 1:100 dilution was plated out

0424_pH_assay_3.jpg

0424_pH_assay_2.jpg



0424_pH_assay_1.jpg



Author: Daniel Marchal
Entry 18/214: Colorcode
In Project: ERBsen
With tags: colorcode

created: 19.04.2018 14:32
updated: 10.05.2018 12:55

Colorcode for -80°C boxes

pink: chemocompetent Vn

yellow: chemocompetent Ec

blue: electrocompetent Vn

green: electrocompetent Vmax

white: cryostocks

Colorcode for DNA

red: PCR fragments

yellow: Primer 10µM

blue: Plasmids isolated from Vn/Vmax

green: Plasmids isolated from Ec

white: cryostocks

Author: Daniel Marchal

created: 19.04.2018 15:41
updated: 19.04.2018 15:51

Entry 19/214: Retrafo of pYTK into Vn (Weinstock electroporation)
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

Procedure

1. thaw aliquots of electrocompetent Vn on ice
2. add plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 700-900 V (depending on the strain), 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM $MgCl_2$), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 1-2h at 30-37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 30-37°C

Author: Daniel Marchal
created: 25.04.2018 10:05
updated: 26.04.2018 13:12

Entry 20/214: Enrichment and isolation of pYTK + pEntry from Vn
In Project: ERBsen
With tags: miniprep

Yesterday, Vn was transformed with pYTK and pEntry via heat shock transformation and today the plates show lawn with green smear. We assume that the antibiotic solution was too old so the plates didn't have any antibiotics. Nevertheless the green smear shall be picked and enriched so that we can check today in the evening via miniprep if the cells contain plasmids.

Procedure

1. prepare 4 tubes with following annotations:
    1. Vn + pYTK
    2. Vn + pYTK + Cm
    3. Vn + pEntry
    4. Vn + pEntry + Cm
2. add 5ml BHIv2 into the tubes and if required 5µl Cm [2mg/ml] (fresh prepared)
3. Inoculate with smear of pYTK or pEntry
4. Incubate over day at 37°C shaking
5. Make miniprep

Result

- no plasmid DNA
- cell pellet was greenish
- → maybe the miniprep wasn't sufficient. The cells still contain DNases which are inhibited by RNA so the use of a RNase-free resuspension buffer could increase miniprep efficiency. Enrichment and Miniprep will be repeated tomorrow

Author: Daniel Marchal
Entry 21/214: Cryostocks of Vmax
In Project: ERBsen
With tags: Vmax, Cryostock

created: 25.04.2018 16:16
updated: 25.04.2018 16:20

Procedure

1. Streak out Vmax from Cryostock (AG Bremer) on LB
2. Inoculate 5ml oN culture with BHIv2 with big stripe from the plate
3. Incubate oN at 37°C shaking
4. Inoculate main cultur (BHIv2, 10ml in 100ml flask) and incubate at 37°C until OD600 ~0,5-1
5. Mix 900µl cell suspension with 100µl glycerol and freeze at -80°C

Author: Daniel Marchal
created: 26.04.2018 11:23
updated: 26.04.2018 14:06
Entry 22/214: Preparation of electrocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: competent, electrocompetent, V. natriegens, weinstock, electroporation

Materials

260-510ml BHI + v2 salts

110ml Electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)

liquid nitrogen

Recipes

BHI + v2 salts

37g/l brain heart infusion broth

204mM NaCl

4.2mM KCl

23.14mM $MgCl_2$

Electroporation buffer

680mM sucrose

7mM K_2HPO_4

pH7

Procedure

1. 10mL BHIv2 is inoculated with V. natriegens and incubated oN at 30 °C with agitation at 200 r.p.m.
2. 250–500 mL of the same growth medium is inoculated with the overnight culture at a dilution of 1:100 to 1:200 (overnight culture/fresh medium)
3. The culture is grown at 37 °C in a baffled flask with shaking at 200 r.p.m. until an OD600 of 0.5 is reached
4. The culture is then split into five to ten chilled 50-mL falcons and incubated on ice for 15 min
5. The cells are pelleted at 4,000 r.p.m. in a Beckman JA-14 centrifuge rotor for 10 min at 4 °C
6. The supernatant is carefully decanted and the cell pellets are gently resuspended in 5 mL of cooled electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)
7. The suspensions are pooled in one tube and the tube is filled up to 35 mL electroporation buffer and inverted several times to mix
8. The cells are centrifuged down at 4,000 r.p.m. for 15min at 4 °C in a JA-17 rotor
9. The supernatant is decanted with a pipette
10. The wash is repeated two times for a total of three washes
11. the cells are gently resuspended in residual electroporation buffer
12. The volume is adjusted with additional electroporation buffer to bring the final OD600 to 16 (~5ml)
13. Cells are aliquoted into chilled tubes (50µl), frozen in liquid nitrogen and stored at -80 °C until use

Comments

- Mainculture inoculated with 1:100 dilution of preculture (2.5ml)
- Cells harvested at OD600 = 0.623
- In step 12 5ml buffer were added

Author: Daniel Marchal
created: 26.04.2018 13:12
updated: 29.04.2018 13:18

Entry 23/214: Enrichment and isolation of pYTK + pEntry from Vn
In Project: ERBsen
With tags: miniprep

Yesterday the Miniprep from pYTK + pEntry out of electroporated Vn cells wasn't sufficient. To validate if the usage of RNase-free resuspension buffer enables plasmid isolation the enrichment and miniprep shall be repeated.

Procedure

1. prepare 3 tubes with following annotations:
    1. Vn + pYTK + Cm
    2. Vn + pEntry + Cm
    3. Vn without plasmid + Cm
2. add 5ml BHIv2 into the tubes and add 5µl Cm [2mg/ml]
3. Inoculate with smear of pYTK, pEntry or WT
4. Incubate over day at 37°C shaking
5. Make miniprep with H_2O instead of Buffer A1

Result

- c(pYTK)=148ng/µl
- c(pEntry)=0ng/µl

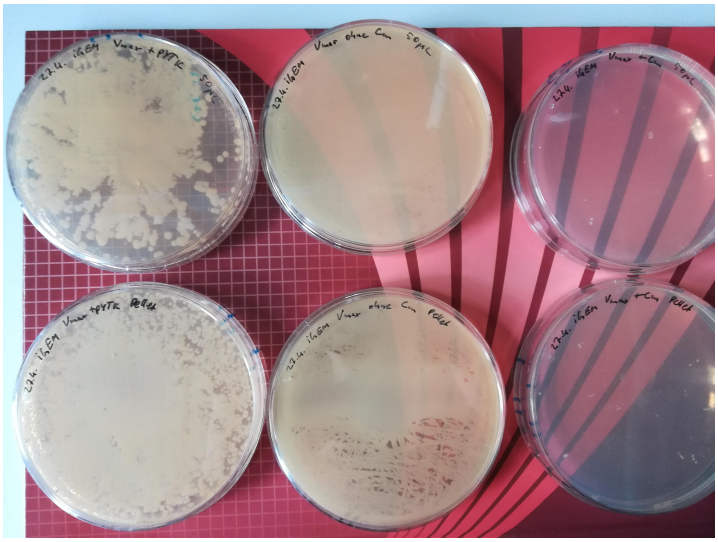
Author: Daniel Marchal
created: 29.04.2018 13:19
updated: 29.04.2018 13:48

Entry 24/214: Retrafo of pYTK into Vmax (electroporation)
In Project: ERBsen
With tags: electroporation, retrafo, electrocompetent, retransformation, V. natriegens, weinstock

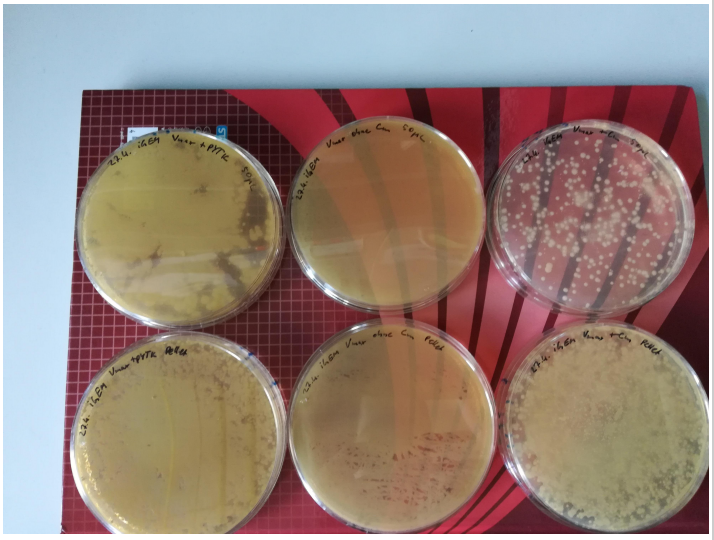
Procedure

1. thaw 3 aliquots of electrocompetent Vmax on ice
    1. Vmax + pYTK on LB2,5+Cm
    2. Vmax without plasmid on LB2,5
    3. Vmax without plasmid on LB2.5+Cm
2. add plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 700-900 V (depending on the strain), 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 1-2h at 30-37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 30°C

over night at 30°C.jpg



over night at 30°C + 24h 20°C.jpg



Results

- Control without Cm shown lawn control with Cm shows just few colonies → as expected but Cm concentration (2µg/ml) could be too low, I will repeat it with 4 and 8µg/ml Cm
- Trafo was sufficient! Miniprep and restriction digest is planned
- After one further day at RT the controlplates with Cm got more colonies → Vmax grows better at RT?? Too low Cm concentration?? Cm degrades at RT??

Author: Daniel Marchal
created: 29.04.2018 13:48
updated: 29.04.2018 14:06
Entry 25/214: Enrichment and isolation of pYTK from Vmax
In Project: ERBsen
With tags: M9, media, pH

Yesterday, Vn was transformed with pYTK and pEntry via heat shock transformation and today the plates show lawn with green smear. We assume that the antibiotic solution was too old so the plates didn't have any antibiotics. Nevertheless the green smear shall be picked and enriched so that we can check today in the evening via miniprep if the cells contain plasmids.

Procedure

1. prepare 2 tubes
2. add 5ml BHIv2 into the tubes and 5µl Cm [2mg/ml] (fresh prepared)
3. Inoculate with smear of pYTK or pEntry
4. Incubate over day at 37°C shaking
5. Make miniprep
    1. One times with H_2O for resuspension of cells
    2. One times with buffer A1 for resuspension of cells

Result

- pYTK(A1): c=19ng/µl
- pYTK(H_2O): c=99ng/µl

Author: Daniel Marchal

created: 30.04.2018 14:13
updated: 01.05.2018 13:57

Entry 26/214: Retrafo of pYTK into Vn + Vmax

In Project: ERBsen

With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

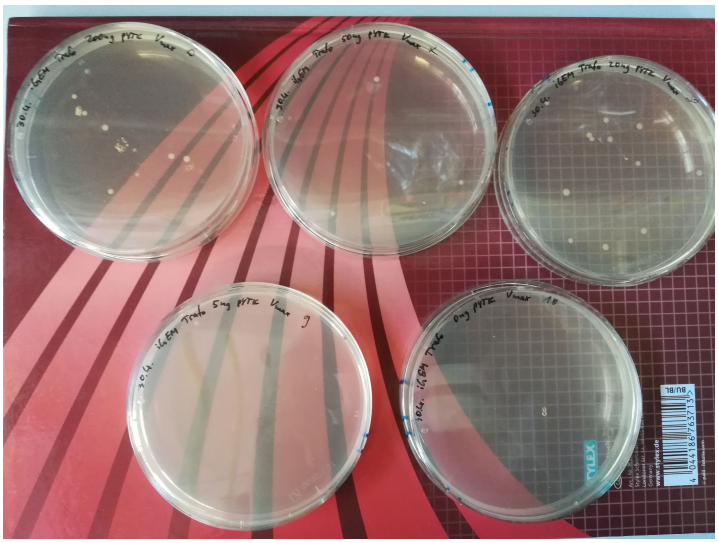
Procedure

1. thaw 6 aliquots of electrocompetent Vmax/Vn on ice

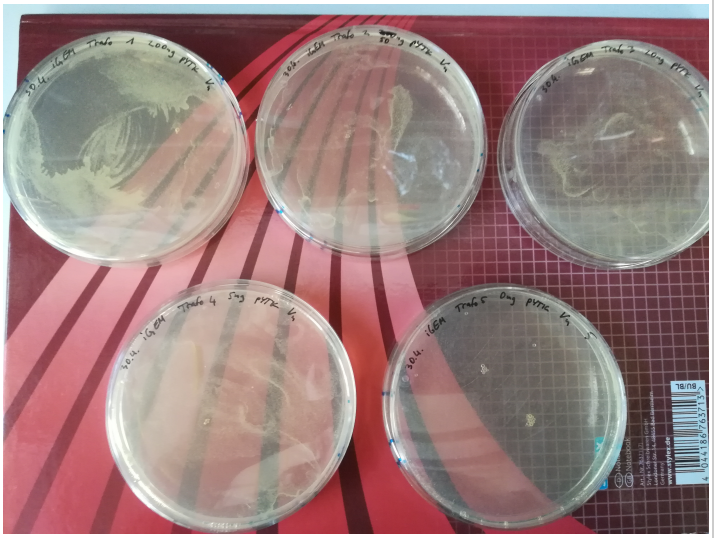
Sample	Strain	c(pYTK) [ng/µl]	c(Cm) in LB2.5-plates [µg/ml]
1	Vn	200	2
2	Vn	50	2
3	Vn	20	2
4	Vn	5	2
5	Vn	0	2
6	Vmax	200	2
7	Vmax	50	2
8	Vmax	20	2
9	Vmax	5	2
10	Vmax	0	2

3. add plasmid DNA into the aliquot
4. transfer suspension into an electroporation cuvette (0.1cm gap size)
5. electroporate with following parameters: 800 V (depending on the strain), 25 µF, 200 Ω
6. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
7. Incubate 2h at 30°C while shaking
8. Plate out on selection plates
9. Incubate oN at 30°C

0501_retrafo_pYTK_1.jpg



0501_retrafo_pYTK_2.jpg



Results

- The Vmax colonies aren't green and higher amounts of plasmid DNA didn't increas CFUs → failed
- Too much colonies on Vn plates → failed
- We will repeat it tomorrow

Author: Daniel Marchal

created: 01.05.2018 10:13
updated: 02.05.2018 10:37

Entry 27/214: Retrafo of pTrc_McrCa into Vmax and pYTK into Ec

In Project: ERBsen

With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

Procedure

1. thaw aliquots of electrocompetent Vn on ice

2.	1	Ec (heat shock trafo)	pYTK (from Ec)	200ng	LB+Cm 34µg/ml
	2	Vmax	pYTK (from Vn)	150ng	LB2.5+Cm 2µg/ml
	3	Vmax	pTrc_McrCa	67ng	LB2.5+Amp 25µg/ml / LB2.5+Amp 50µg/ml
	4	Vmax	-	-	LB2.5 / LB2.5+Cm 2µg /ml / LB2.5+Amp 50µg /ml

3. add plasmid DNA into the aliquot
4. transfer suspension into an electroporation cuvette (0.1cm gap size)
5. electroporate with following parameters: 800 V (depending on the strain), 25 µF, 200 Ω
6. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
7. Incubate 2h at 37°C while shaking
8. Plate out on selection plates
9. Incubate oN at 37°C

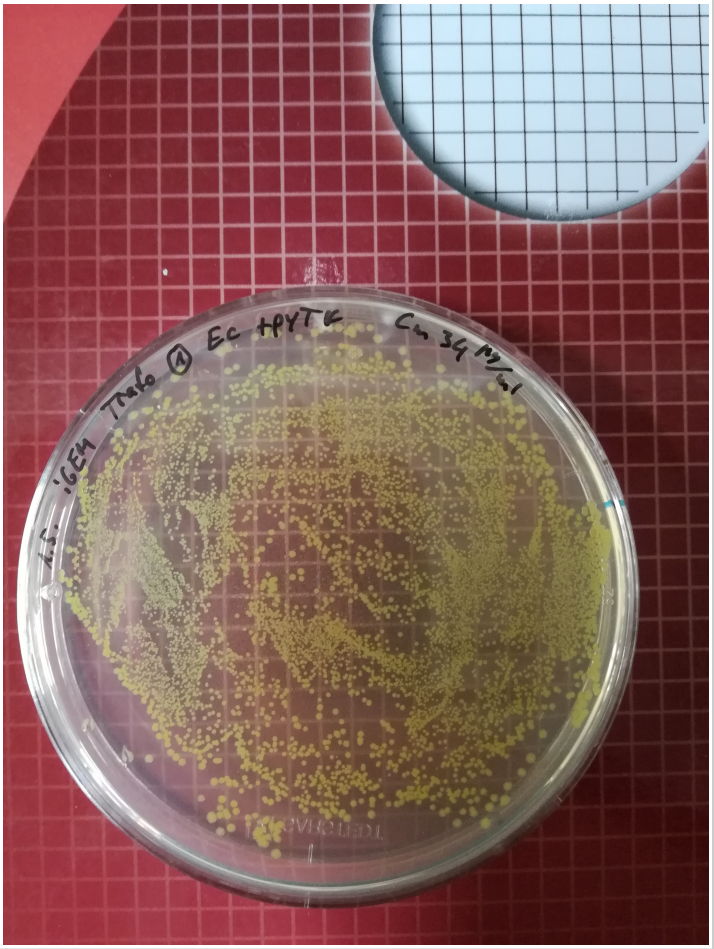
Comments

- we had no LB2,5+Amp plates so we used LB2.5 plates and added 25-50µg Amp to diffuse
  - 25µg/ml: 6.25µl Amp + 43.75µl H_2O
  - 50µg/ml: 12.5µl Amp + 37.5µl H_2O

Results

- Ec trafo was good, colonies shine greenish → 3 colonies picked for plasmid isolation and glystock
- All Vmax plates with antibiotic show no colonies and the control without antibiotic shows 200 colonies what is verly low. We assume that the cell density of the competent cells is very low and therefore the transformations don't work. We will repeat the preparation and pay attention to resuspend the pellet completely before making aliquots

0502_retrafe_pYTK.jpg



Author: Daniel Marchal
created: 03.05.2018 13:08
updated: 03.05.2018 13:25

Entry 28/214: Retrafo of pEntry, pAcc into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2.

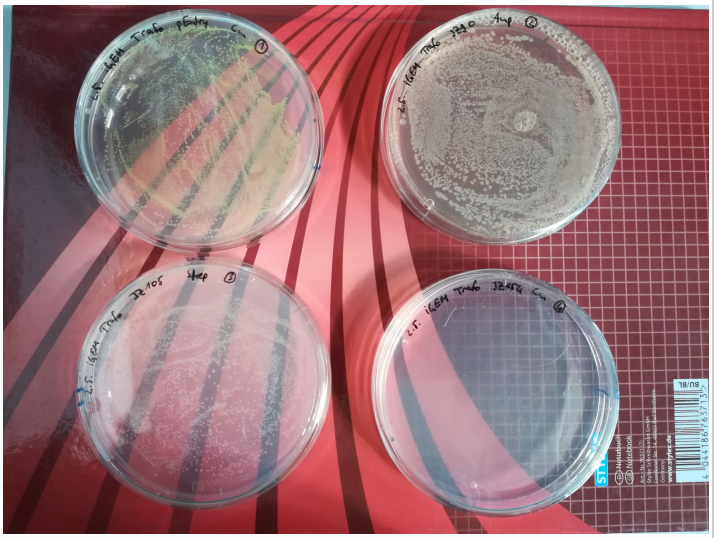
Vector	Insert	Resistance	Box in -80°C freezer
pEntry	lvl0 entry vector	Cm	-
JZ90	Acc from S. coelicolor	Amp	box 16, locus 66
JZ105	Pcc* from M. extorquens	Strep	box 16, locus 79
JZ147	Acc from Synechococcus	Cm	box 17, locus 35

3. add 1µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Results

- pEntryshows green colonies → 3 colonies picked for miniprep and glystock
- JZ90 + JZ105 shows colonies → 1 colonie picked for miniprep and glystock
- JZ147 shows no colonies → plate will be further incubated and trafo repeated

IMG_20180503_102602.jpg



Author: Daniel Marchal
Entry 29/214: Restriction digest of pYTK
In Project: ERBsen
With tags: StyI, restriction, digest

created: 03.05.2018 13:33
updated: 04.05.2018 12:44

In last days several minipreps of pYTK were made, some from Ec, some from Vn, some with RNase and some without. These plasmids will be analysedusing a restriction digest.

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

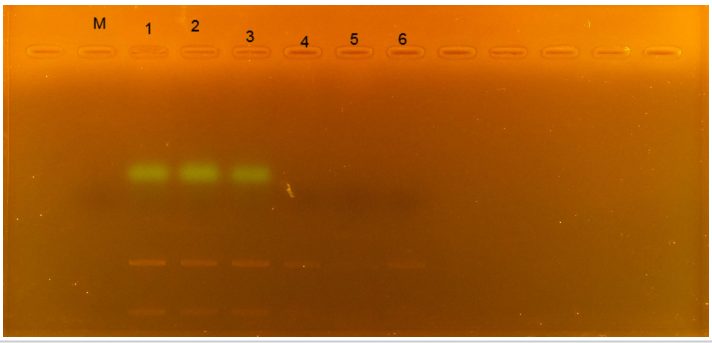
Sample	**Master mix (7x)**
1µl DNA	-
0.2µl StyI	1.4µl StyI
1µl CutSmart Buffer	7µl CutSmart Buffer
7.8µl H_2O	54.6µl H_2O

Number	**c(pYTK)**	**Source**
1	49	Ec
2	56	Ec
3	46	Ec
4	99	Vmax (with H_2O instead of buffer A1)
5	149	?
6	19	Vmax

Results

- no marker detectable
- Sample 1, 2 and 3 show two clear bands → pure plasmids
- Sample 5 don't show any bands → no plasmid in
- Sample 4 and 6 show slight bands → just small amount of plasmid → waste

0503_Restriction_Digest.JPG



Author: Daniel Marchal

created: 03.05.2018 13:33
updated: 03.05.2018 13:35

Entry 30/214: Retrafo of JZ147 + JZ154 into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, JZ147, JZ154

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance	Box in -80°C freezer
JZ154	Acc+BirA from E. coli	Cm	box 17, locus 42
JZ147	Acc from Synechococcus	Cm	box 17, locus 35

3. add 1µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal
created: 03.05.2018 17:44
updated: 04.05.2018 11:56

Entry 31/214: Enrichment and isolation of pEntry, JZ90, JZ105 from Ec
In Project: ERBsen
With tags: pEntry, JZ105, JZ90, miniprep

Yesterday a retrafo of pEntry, JZ90, JZ105 and JZ147 were made. The first three were successful so a plasmid isolation and glystocks shall be made

Procedure

1. prepare 5 tubes with following annotations:
    1. Ec + pEntry + Cm
    2. Ec + pEntry + Cm
    3. Ec + pEntry + Cm
    4. Ec + JZ90 + Amp
    5. Ec + JZ105
2. add 5ml LB into the tubes and if required antibiotics
3. Inoculate with colony of trafo plate
4. Incubate over day at 37°C shaking
5. Make miniprep and glystock

Result

- 30ng/µl in all samples

Author: Daniel Marchal
created: 04.05.2018 09:45
updated: 04.05.2018 11:53
Entry 33/214: Preparation of electrocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: competent, electrocompetent, V. natriegens, weinstock, electroporation

Materials

260-510ml BHI + v2 salts

110ml Electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)

liquid nitrogen

Recipes

BHI + v2 salts

37g/l brain heart infusion broth

204mM NaCl

4.2mM KCl

23.14mM $MgCl_2$

Electroporation buffer

680mM sucrose

7mM K_2HPO_4

pH7

Procedure

1. 10mL BHIv2 is inoculated with V. natriegens and incubated oN at 30 °C with agitation at 200 r.p.m.
2. 250–500 mL of the same growth medium is inoculated with the overnight culture at a dilution of 1:100 to 1:200 (overnight culture/fresh medium)
3. The culture is grown at 37 °C in a baffled flask with shaking at 200 r.p.m. until an OD600 of 0.5 is reached
4. The culture is then split into five to ten chilled 50-mL falcons and incubated on ice for 15 min
5. The cells are pelleted at 4,000 r.p.m. in a Beckman JA-14 centrifuge rotor for 10 min at 4 °C
6. The supernatant is carefully decanted and the cell pellets are gently resuspended in 5–10 mL of electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)
7. The suspensions are pooled in one tube and the tube is filled up to 35 mL electroporation buffer and inverted several times to mix
8. The cells are centrifuged down at 4,000 r.p.m. for 15min at 4 °C in a JA-17 rotor
9. The supernatant is decanted with a pipette
10. The wash is repeated two times for a total of three washes
11. the cells are gently resuspended in residual electroporation buffer
12. The volume is adjusted with additional electroporation buffer to bring the final OD600 to 16 (~1.5ml)
13. Cells are aliquoted into chilled tubes (50µl), frozen in dry ice and stored at -80 °C until use

Comments

- Mainculture inoculated with 200µl dilution of preculture
- Cells harvested at OD600=0.5
- To check if cells are competent, a trafo was made with cells which weren't cooled in dry ice

Author: Daniel Marchal
created: 04.05.2018 11:57
updated: 05.05.2018 11:03
Entry 34/214: Retrafo of pYTK, pEntry, JZ90 into Vmax (electroporation)
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

Procedure

1. thaw 10 aliquots from the todays competence preparation of electrocompetent Vn on ice
2. add 100ng plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 700-900 V (depending on the strain), 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 37°C

Sample	Plasmid	Resistance
1	pYTK	Cm
2	pEntry	Cm
3	MCR_ST	Amp 10µg/ml
4	MCR_c-term	Amp
5	MCR_n-term	Amp
6	MCR-CA	Amp
7	JZ90	Amp
8	Control	Amp
9	Control	Cm
10	Control	-

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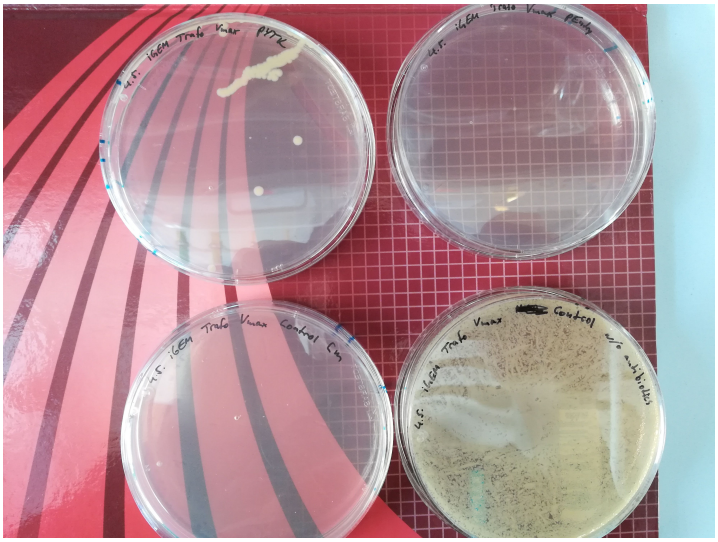

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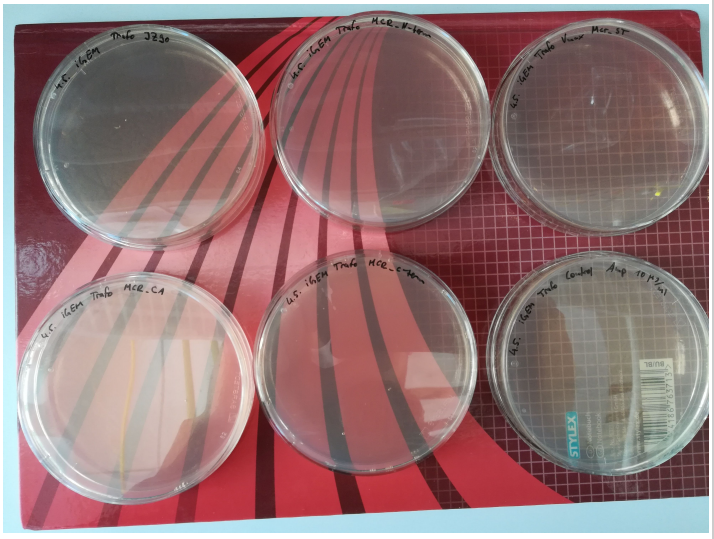


Author: Daniel Marchal
Entry 35/214: Preparation of electrocompetent Vmax cells (Lee)
In Project: ERBsen
With tags: competent, electrocompetent, Vmax, Lee, Sorbitol

created: 10.05.2018 10:59
updated: 10.05.2018 12:05

Material

- 1M Sorbitol 20ml
- LB2.5 300ml
- Dry ice

Procedure

1. Inoculate 10ml LB2.5 with Vmax from cryostock and incubate oN at 37°C and 200 rpm
2. On the following day pellet 2.5 ml for 1 min at 20,000 rcf, discard supernatant
3. Resuspend in 500µl LB2.5 and inoculate a main culture with 250ml LB2.5 (→ 1:100 dilution)
4. Incubate at 37°C and 225 rpm until OD~0.4 is reached (~1 hour)
5. Pellet at 3500 rpm for 5 min at 4°C and wash in 1ml cold 1M sorbitol
6. Pellet at 20,000 rcf for 1 min at 4°C
7. Repeat washing steps for a total of three times
8. Resuspend the final pellet in 250µl 1M sorbitol
9. Make 50µl aliquots in chilled tubes and freeze them in dry ice
10. Store aliquots at -80°C

Author: Daniel Marchal
created: 10.05.2018 12:05
updated: 10.05.2018 12:19
Entry 36/214: Retrafo of pYTK + pEntry into Vmax (Lee)
In Project: ERBsen
With tags: electrocompetent, electroporation, Lee, PYTK

To check if the Vmax cells from the lee-competence protocol (Retrafo of pYTK + pEntry into Vmax (Lee) - entry #36 in project 'ERBsen' (Daniel Marchal, 10.05.2018)) are competent, a trafo will be made with pYTK from Ec, pEntry from Ec, pYTK from Vmax and pYTK from Vmax (1:10).

Procedure

1. thaw 4 aliquots from the todays competence preparation of electrocompetent Vmax on ice
2. add 100ng plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 400 V, 25 µF, 1 kΩ
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 37°C

Sample	Plasmid	Resistance
1	pYTK (Ec)	Cm
2	pEntry (Ec)	Cm
3	pYTK (Vmax)	Cm
4	pYTK (Vmax 1:10)	Cm
5	Control	Cm
6	Control	-

Author: Daniel Marchal
created: 28.05.2018 16:24
updated: 28.05.2018 16:46

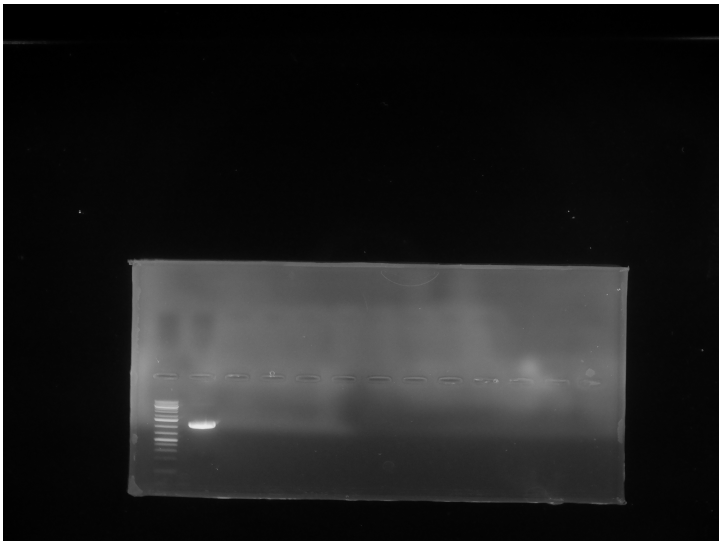
Entry 37/214: PCR control gel MCRSt
In Project: ERBsen
No tags associated

A PCR for MCR from Sulfolobus tokodaii was made and PCR efficiency was tested via gel electrophoresis.

DNA Ladder 1kb

Expected size 1kb

0528_PCR_MCRSt.JPG



Author: Daniel Marchal
created: 28.05.2018 19:32
updated: 28.05.2018 19:42
Entry 38/214: Lvl0 GoldenGate AccCg+McrSt
In Project: ERBsen
No tags associated

Procedure:

1. Dilute AccBC, AccDts1, BirA to 50ng/µl in H_2O
2. Dilute pEntry to 10ng/µl in H_2O
3. Mix GoldenGate Reaction (see Table)
4. Incubate in Cycler (Program see Table)
5. store at 4°C

Reagent	**Volume**
AccBC/AccDts1/BirA/McrSt	0.5 µl
pEntry	0.5 µl
T4 Ligase Buffer	1 µl
T4 Ligase	0.5 µl
BsmBI	0.5 µl
H_2O	ad 10µl

42°C	2 min	Repeat 25x
16°C	5 min	Repeat 25x
60°C	10 min	
80°C	10 min	

Author: Daniel Marchal
created: 30.05.2018 16:05
updated: 31.05.2018 11:46
Entry 39/214: Miniprep AccCgpEntry + McrStpEntry
In Project: ERBsen
No tags associated

Miniprep was made with the QIAprep Spin Miniprep Kit and the integrated manual.

Results:

McrStpEntry 1	72 ng/µl
McrStpEntry 2	56 ng/µl
McrStpEntry 3	51 ng/µl
McrStpEntry 4	60 ng/µl
AccBCpEntry 1	76 ng/µl
AccBCpEntry 2	40 ng/µl
AccBCpEntry 3	92 ng/µl
AccBCpEntry 4	49 ng/µl
AccDpEntry 1	82 ng/µl
AccDpEntry 2	55 ng/µl
AccDpEntry 3	81 ng/µl
AccDpEntry 4	61 ng/µl
BirApEntry 1	80 ng/µl
BirApEntry 2	72 ng/µl
BirApEntry 3	70 ng/µl
BirApEntry 4	58 ng/µl

Author: Daniel Marchal
created: 30.05.2018 16:06
updated: 30.05.2018 16:12

Entry 40/214: Ec Trafo AccCgpEntry + McrStpEntry
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, McrSt, AccBC, AccD, BirA

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance	Box in -80°C freezer
AccBCpEntry	AccBC from C. glutamicum codonoptimized	Cm	-
AccDpEntry	AccD from C. glutamicum codonoptimized	Cm	box 16, locus 66
BirApEntry	BirA from C. glutamicum codonoptimized	Cm	box 16, locus 79
McrStpEntry	Mcr from S. tokodaii	Cm	box 17, locus 35

3. add 10µl of plasmid (after GoldenGate cloning)
4. incubate 5 min on ice
5. heat shock at 42°C for 45 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Results

- All plates show 500-1000 colonies, most of them shining → false-positive
- nonshining colonies ~ 2% → just 2% of all colonies are successfully cloned
- from each plate 4 colonies will be picked and enriched for miniprep+digest

Author: Daniel Marchal
created: 30.05.2018 16:12
updated: 30.05.2018 16:18
Entry 41/214: Enrichment Ec with AccCgpEntry + McrStpEntry
In Project: ERBsen
No tags associated

Ec Trafo AccCgpEntry + McrStpEntry - entry #40 in project 'ERBsen' (Daniel Marchal, 30.05.2018)

Procedure

1. prepare 17 tubes with 5ml LB + 5µl Cm [34mg/µl]
2. inoculate from each trafoplate4 tubes and one control with Ec WT
3. Incubate over day at 37°C shaking

Author: Daniel Marchal
created: 01.06.2018 16:21
updated: 02.06.2018 12:41
Entry 42/214: Restriction digest of piGEM2105-piGEM2108
In Project: ERBsen
With tags: BsaI, restriction, digest, piGEM2105, piGEM2106, piGEM2107, piGEM2108

In last days GoldenGate Assembly of Lvl 0 CDS parts of AccBC, AccD, BirA and McrSt were made and transformed into Ec. From each vector 4 cultures were enriched and the plasmids isolated. Now they shall be digested to check correct cloning.

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

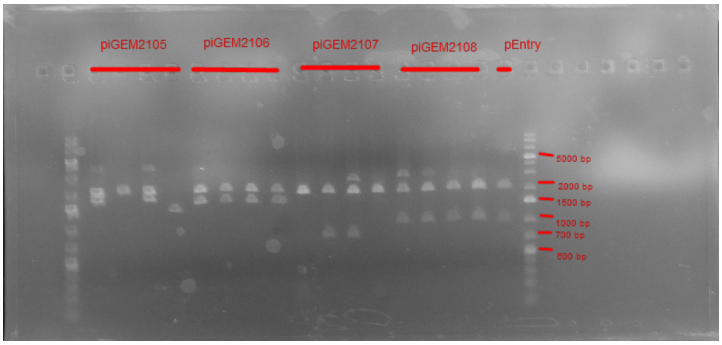
Sample	**Master mix (18x)**
1µl DNA	-
0.2µl BsaI	3.6µl BsaI
1µl CutSmart Buffer	18µl CutSmart Buffer
7.8µl H_2O	140.4µl H_2O

Vector	Insert	Expected fragment length
piGEM2105 1	AccBC	1774bp, 2105bp
piGEM2105 2	AccBC	1774bp, 2105bp
piGEM2105 3	AccBC	1774bp, 2105bp
piGEM2105 4	AccBC	1774bp, 2105bp
piGEM2106 1	AccD	1630bp, 2105bp
piGEM2106 2	AccD	1630bp, 2105bp
piGEM2106 3	AccD	1630bp, 2105bp
piGEM2106 4	AccD	1630bp, 2105bp
piGEM2107 1	BirA	808bp, 2105bp
piGEM2107 2	BirA	808bp, 2105bp
piGEM2107 3	BirA	808bp, 2105bp
piGEM2107 4	BirA	808bp, 2105bp
piGEM2108 1	McrSt	?
piGEM2108 2	McrSt	?
piGEM2108 3	McrSt	?
piGEM2108 4	McrSt	?
pEntry (Control)	(Control)	914bp, 2105bp

Results

- From piGEM2105 (AccBC) plasmid 1 and 3 looks good
- From piGEM2106 (AccD) all plasmids look good
- From piGEM2107 (BirA) plasmid 2 and 3 looks good
- piGEM2108 (McrSt) looks exactly like the control, so another enzyme has to be used or the vector have to be sequenced
- pEntry shows the expected bands
- Next step: sequencing of one sample per plasmid

0602_Restriction_digest.jpg



Author: Daniel Marchal

created: 02.06.2018 12:51
updated: 02.06.2018 12:53

Entry 43/214: Trafo of McrCapEntry into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, McrCa

Procedure

1. thaw 1 aliquots of Ec NEB Turbo on ice
2. add 5µl of McrCapEntry
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB + Cm
9. incubate oN at 37°C

Author: Daniel Marchal

created: 04.06.2018 13:17
updated: 04.06.2018 13:18

Entry 44/214: Retrafo of JZ147 into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, JZ147

Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 1µl of plasmid (JZ147 - Acc from Synechococcus elongatus, Box 17 locus 35)
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB + Cm
9. incubate oN at 37°C

Author: Daniel Marchal
Entry 45/214: Sequencing of piGEM2105-piGEM2108
In Project: ERBsen
With tags: piGEM2105, piGEM2106, piGEM2107, piGEM2108

created: 04.06.2018 13:23
updated: 18.06.2018 12:23

Samples:

Vektor	Eppi	Label	Primer
piGEM2105 3 (AccBC Lvl 0)	1	AGB0039943	Seq 3
piGEM2105 3 (AccBC Lvl 0)	2	AGB0039944	Seq 4
piGEM2106 1 (AccD Lvl 0)	3	AGB0039945	Seq 3
piGEM2106 1 (AccD Lvl 0)	4	AGB0039946	Seq 4
piGEM2107 2 (BirA Lvl 0)	5	AGB0039947	Seq 3
piGEM2107 2 (BirA Lvl 0)	6	AGB0039948	Seq 4
piGEM2108 1 (McrSt Lvl 0)	7	AGB0039949	Seq 3
piGEM2108 1 (McrSt Lvl 0)	8	AGB0039950	Seq 4

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order 11104443046):

- piGEM2105 is correct
- piGEM2106_for was dirty and will be resequenced from the company, reverse looks fine
- piGEM2107 is correct
- piGEM2108 couldn't be analyzed because I have no plasmid map. But it will be done tomorrow

Author: Daniel Marchal
created: 04.06.2018 13:39
updated: 04.06.2018 13:41
Entry 46/214: Media preparation
In Project: ERBsen
With tags: LB, v2, LBv2

2x 400ml LBv2-Agar prepared (10g LB + 6g Agar + 40ml 10x-V2 + 360ml H_2O)

Author: Daniel Marchal

created: 05.06.2018 08:38
updated: 05.06.2018 16:28

Entry 47/214: Retrafo of piGEM2105-piGEM2108
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, piGEM2105, piGEM2106, piGEM2107, piGEM2108, Lvl 0 plasmids

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance
piGEM2105	AccBC	Cm
piGEM2106	AccD	Cm
piGEM2107	BirA	Cm
piGEM2108	McrSt	Cm

3. add 1µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Since the sequences of piGEM2105 - piGEM2108 are correct, the vectors shall be retranformed into Ec to make cryostocks and minipreps

Author: Daniel Marchal
created: 05.06.2018 14:03
updated: 06.06.2018 13:33
Entry 48/214: Enrichment and isolation of MoClo plasmids
In Project: ERBsen
No tags associated

To build Lvl 1 plasmids we need the Lvl 0 parts from the part collection team

Procedure

1. prepare 4 tubes with following annotations:
2.

Plasmid	Part	Insert
piGEM1011_LVL0_1_5'Connector Dummy	5'-Connector	5'-Connector
piGEM1012_LVL0_6_3'Connector Dummy	3'-Connector	3'-Connector
piGEM1008_LVL0_3_B0034	RBS	B0034
piGEM1010_LVL0_3_B0032	RBS	B0032
piGEM1013_LVL0_3_B0031	RBS	B0031
piGEM1016_LVL0_3_B0030	RBS	B0030
piGEM1035_LVL0_5_B0015	Terminator	B0015
piGEM1036_LVL0_7_ColE1	Ori	ColE1
piGEM1037_LVL0_7_pMB1	Ori	pMB1
piGEM1056_LVL0_8_Kan(pSB3K3) in 1005	Resistenz	Kan(pSB3K3) in 1005
piGEM1057_LVL0_8_Kan(pSB3K3) in 1006	Resistenz	Kan(pSB3K3) in 1006

3. add 5ml LB and 5µl Cm [34mg/ml] or Kan [50mg/ml] into the tubes (fresh prepared)
4. Inoculate with Ec + plasmid from cryostock of Graumann lab
5. Incubate over day at 37°C shaking
6. Make miniprep + cryostocks

Result

- piGEM1008, piGEM1016, piGEM1056 and piGEM1057 weren't found, we have to enrich them on another day

Author: Daniel Marchal
created: 06.06.2018 08:38
updated: 13.07.2018 09:21
Entry 49/214: Enrichment and isolation of MoClo plasmids (2)
In Project: ERBsen
No tags associated

To build Lvl 1 plasmids we need the Lvl 0 parts from the part collection team

Procedure

1. prepare 4 tubes with following annotations:
2.

Plasmid	Part	Insert
piGEM1007_LVL0_2_J23100	Promotor	J23100
piGEM1017_LVL0_2_J23101	Promotor	J23101
piGEM1018_LVL0_2_J23102	Promotor	J23102
piGEM1019_LVL0_2_J23103	Promotor	J23103
piGEM1009_LVL0_2_J23104	Promotor	J23104
piGEM1020_LVL0_2_J23105	Promotor	J23105
piGEM1014_LVL0_2_J23106	Promotor	J23106
piGEM1021_LVL0_2_J23107	Promotor	J23107
piGEM1022_LVL0_2_J23108	Promotor	J23108
piGEM1023_LVL0_2_J23109	Promotor	J23109
piGEM1024_LVL0_2_J23110	Promotor	J23110
piGEM1027_LVL0_2_J23114	Promotor	J23114
piGEM1015_LVL0_2_J23115	Promotor	J23115
piGEM1028_LVL0_2_J23116	Promotor	J23116
piGEM1029_LVL0_2_J23117	Promotor	J23117
piGEM1030_LVL0_2_J23118	Promotor	J23118
piGEM1031_LVL0_2_J23119	Promotor	J23119

3. add 5ml LB and 5µl Cm [34mg/ml] into the tubes
4. Inoculate with Ec + plasmid from cryostock of Graumann lab
5. Incubate over day at 37°C shaking
6. Make miniprep + cryostocks

Result

Plasmid
Concentration [ng/µl]piGEM1007_LVL0_2_J23100
49piGEM1017_LVL0_2_J23101
99piGEM1018_LVL0_2_J23102
61piGEM1019_LVL0_2_J23103
70piGEM1009_LVL0_2_J23104
53piGEM1020_LVL0_2_J23105
54piGEM1014_LVL0_2_J23106
39piGEM1021_LVL0_2_J23107
47piGEM1022_LVL0_2_J23108
65piGEM1023_LVL0_2_J23109
60piGEM1024_LVL0_2_J23110
100piGEM1027_LVL0_2_J23114
-piGEM1015_LVL0_2_J23115
60piGEM1028_LVL0_2_J23116
28piGEM1029_LVL0_2_J23117
64piGEM1030_LVL0_2_J23118
55piGEM1031_LVL0_2_J23119
70

Miniprep for piGEM1027 will be repeated

Author: Daniel Marchal
created: 06.06.2018 13:31
updated: 08.06.2018 13:43

Entry 50/214: Enrichment of JZ147, piGEM205, piGEM206, piGEM207
In Project: ERBsen
No tags associated

Procedure

1. prepare 4 tubes with following annotations:

Plasmid	Strain
JZ147	Ec
piGEM2105	Ec
piGEM2106	Ec
piGEM2107	Ec

1. add 5ml LB and 5µl Cm [34mg/ml] into the tubes
2. Inoculate with Ec + plasmid
3. Incubate over night at 37°C shaking
4. Make miniprep + cryostocks

Result of Miniprep:

Plasmid	Concentration
piGEM2105 1	61
piGEM2105 2	64
piGEM2105 3	64
piGEM2106 1	52
piGEM2106 2	57
piGEM2106 3	56
piGEM2107 1	51
piGEM2107 2	59
piGEM2107 3	44

Author: Daniel Marchal
created: 06.06.2018 13:31
updated: 09.06.2018 14:51
Entry 51/214: Enrichment and isolation of MoClo Plasmids (3)
In Project: ERBsen
No tags associated

The plasmids from "Enrichment and isolation of MoClo plasmids" were wrongly labeled, so the enrichment has to be repeated

Procedure

1. prepare 4 tubes with following annotations:

Plasmid	**Part**	**Insert**
piGEM1011_LVL0_1_5'Connector Dummy	5'-Connector	5'-Connector
piGEM1012_LVL0_6_3'Connector Dummy	3'-Connector	3'-Connector
piGEM1008_LVL0_3_B0034	RBS	B0034
piGEM1010_LVL0_3_B0032	RBS	B0032
piGEM1013_LVL0_3_B0031	RBS	B0031
piGEM1016_LVL0_3_B0030	RBS	B0030
piGEM1035_LVL0_5_B0015	Terminator	B0015
piGEM1036_LVL0_7_ColE1	Ori	ColE1
piGEM1037_LVL0_7_pMB1	Ori	pMB1
piGEM1056_LVL0_8_Kan(pSB3K3) in 1005	Resistenz	Kan(pSB3K3) in 1005
piGEM1057_LVL0_8_Kan(pSB3K3) in 1006	Resistenz	Kan(pSB3K3) in 1006
piGEM1025_LVL0_2_J23111	Promotor	J2311
piGEM1027_LVL0_2_J23114	Promotor	J23114

2. add 5ml LB and 5µl Cm [34mg/ml] or Kan [50mg/ml] into the tubes (fresh prepared)
3. Inoculate with Ec + plasmid from cryostock of Graumann lab
4. Incubate over day at 37°C shaking
5. Make miniprep + cryostocks

Result

piGEM1010	71 ng/µl
piGEM1011	90 ng/µl
piGEM1012	86 ng/µl
piGEM1013	65 ng/µl
piGEM1025	65 ng/µl
piGEM1027	96 ng/µl
piGEM1035	93 ng/µl
piGEM1036	81 ng/µl
piGEM1037	115 ng/µl

- piGEM1008, piGEM1016, piGEM1056, piGEM1057 fehlen immernoch

Author: Daniel Marchal
created: 15.06.2018 08:43
updated: 15.06.2018 08:46
Entry 52/214: Enrichment and isolation of piGEM1056 & piGEM1057
In Project: ERBsen
No tags associated

Procedure

1. prepare 2 tubes with following annotations:
    1. Ec + piGEM1056
    2. Ec + piGEM1057
2. add 5ml LB into the tubes and if required 5µl Kan [50mg/ml] (fresh prepared)
3. Inoculate from part collection cryostock
4. Incubate over day at 37°C shaking
5. Make miniprep and own cryostocks

Result

- piGEM1056 27ng/µl
- piGEM1057 39ng/µl

Author: Daniel Marchal
Entry 53/214: Construction of LVL0 Streptag parts
In Project: ERBsen
With tags: Lvl 0 plasmids, annealing, Golden Gate, transformation

created: 15.06.2018 08:47
updated: 15.06.2018 08:55

Annealing:

Set up Annealing reaction in 1,5 mL microcentrifuge tube

fwd Oligo	1,5 µL (10 µM)
rev Oligo	1,5 µL (10 µM)
T4 ligase buffer	5 µL (10x)
ddH_20	42 µL

Incubate in heatblock for 10 min at 85°C

Turn off heatblock and allow samples to remain in the heatblock for slow cooling to room temperature.

Proceed with next step or freeze annealed oligos for long term storage.

oiGEM2002	streptag 4x part extract
oiGEM2003	streptag 4x part
oiGEM2004	streptag 5a part extract
oiGEM2005	streptag 5a part

Golden Gate Reaction

add following reagents to your annealing mix:

Entry Vector	50 - 70 ng
T7-Ligase (NEB)	1 µL
BsmBI (NEB)	1 µL
T4-Ligas Buffer	1 µL
ddH_20	Ad 10 µL

Start Golden Gate Reaction in Thermocycler

Digest	42°C	2 min
Ligation	16°C	5 min
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Author: Daniel Marchal
created: 18.06.2018 12:17
updated: 18.06.2018 12:22
Entry 54/214: Enrichment and Miniprep of Lvl0-Streptags
In Project: ERBsen
No tags associated

From the goldendate cloning of Streptags into pEntry, 4 tubes of Lb+Cm were inocculated from each plasmid

Procedure

1. prepare 8 tubes with following annotations:
    1. Ec + piGEM
    2. Ec + piGEM
    3. Ec + piGEM
    4. Ec + piGEM
    5. Ec + piGEM
    6. Ec + piGEM
    7. Ec + piGEM
    8. Ec + piGEM
2. add 5ml LB into the tubes and 5µl Cm [2mg/ml] (fresh prepared)
3. Inoculate from transformation plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

- about half of the transformation plates show green colonies (negativ) and die other half is white, indication a correct insertion of streptag-parts
- Plasmid concentrations are between 80 and 100 ng/µl
- from 4X and 5A a sample for sequencing was prepared

Author: Daniel Marchal
created: 18.06.2018 12:23
updated: 19.06.2018 17:07
Entry 55/214: Sequencing of Streptag-Plasmids (piGEM2109 & piGEM2110)
In Project: ERBsen
With tags: sequencing, piGEM2109, piGEM2110

Samples:

Vektor	**Eppi**	**Label**	**Primer**
piGEM2109 (Streptag 5A in LVL 0)	5A	AIM0030005	Seq 3
piGEM2110 (Streptag 4X in LVL 0)	4X	AIM0030006	Seq 3

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results:

- both plasmids are correct!
- Tomorrow I will retransform them into Ec to isolate more plasmid

Author: Daniel Marchal
Entry 56/214: Retrafo of JZ147 into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

created: 18.06.2018 13:47
updated: 18.06.2018 13:49

Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 1µl of plasmid (JZ147 - Acc from Synechococcus elongatus, Box 17 locus 35)
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB + Cm
9. incubate oN at 37°C

Author: Daniel Marchal

created: 19.06.2018 09:53
updated: 19.06.2018 09:58

Entry 57/214: Overview cut sites in JZ plasmids
In Project: ERBsen
No tags associated

Plasmid	Insert	BsaI	BsmBI	EcoRI	NotI	PstI	SpeI	XbaI	Total
JZ90	AccSc	1	0	0	1	3	0	0	5
JZ105	Pcc*	1	2	2	2	2	0	0	9
JZ147	AccSe	2	2	0	0	2	1	0	7
JZ154	AccBirAEc	0	1	1	1	0	0	0	3

Author: Daniel Marchal
created: 19.06.2018 14:26
updated: 19.06.2018 14:33
Entry 58/214: Construction of piGEM2108 (McrSt Lvl 0)
In Project: ERBsen
With tags: Lvl 0 plasmids, annealing, Golden Gate, transformation, piGEM2108

The plasmid of piGEM2108 got lost, so the GoldenGate assembly has to be repeated. As insert the PCR fragment of McrSt was used

Procedure:

Mix the following reagents:

Insert (McrSt PCR)	70 ng (1µl)
Entry Vector	14 ng (1:5 ratio to insert) (1µl 1:5)
T7-Ligase (NEB)	0,5 µL
BsmBI (NEB)	0,5 µL
T4-Ligas Buffer	1 µL
ddH_20	Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (30 cycles)
Ligation	16°C	5 min (30 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Author: Daniel Marchal
Entry 59/214: Construction of BirA Lvl 1
In Project: ERBsen
With tags: Lvl 1 plasmids, Golden Gate, transformation, piGEM2109

created: 19.06.2018 14:40
updated: 19.06.2018 17:05

Total bullshit because 4x part is missing !!!

Golden Gate Reaction:

add following reagents to your annealing mix:

5' Connector	piGEM1011	70 ng
Promotor	piGEM1007	70 ng
RBS	piGEM1010	70 ng
CDS	piGEM2107	70 ng
Terminator	piGEM1035	70 ng
3' Connector	piGEM1012	70 ng
Resistance	piGEM1056	70 ng
Ori	piGEM1036	70 ng
T7-Ligase (NEB)		0,5 µL
BsaI (NEB)		0,5 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (30 cycles)
Ligation	16°C	5 min (30 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Author: Daniel Marchal
Entry 60/214: Preparation of LB2,5 + Kan Agar
In Project: ERBsen
No tags associated

created: 19.06.2018 15:13
updated: 19.06.2018 15:16

Procedure:

1. prepare 400 ml LB 2,5-Agar (Lennox medium supplemented with 1,5% NaCl)
2. autoclave
3. add 2 ml Kan [50 mg/µl] to get a final concentration of 250 µg/ml
4. pour plates

Author: Daniel Marchal

created: 19.06.2018 17:00
updated: 19.06.2018 17:03

Entry 61/214: Trafo of piGEM2108, piGEM2109 & piGEM2110 into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, piGEM2108, piGEM2109, JZ105, piGEM2110

Procedure

1. thaw 3 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance
piGEM2108	McrSt	Cm
piGEM2109	5' Streptag	Cm
piGEM2110	3' Streptag	Cm

3. add 1µl of piGEM2109 or 1µl of piGEM2110 or 10µl from piGEM2109 Golden Gate
4. incubate 10 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 10 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal

created: 21.06.2018 11:32
updated: 21.06.2018 12:39

Entry 62/214: Enrichment and isolation of piGEM2108, piGEM2109, piGEM2110 & JZ147 from Ec

In Project: ERBsen

With tags: miniprep, JZ147, piGEM2108, piGEM2109, piGEM2110

Procedure

1. prepare 7 tubes with following annotations:
    1. Ec + piGEM2108
    2. Ec + piGEM2108
    3. Ec + piGEM2108
    4. Ec + piGEM2108
    5. Ec + piGEM2109
    6. Ec + piGEM2110
    7. Ec + JZ147
2. add 5ml LB into the tubes and 5µl Cm [2mg/ml] (fresh prepared)
3. Inoculate from transformation plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2108 a	64 ng/µl
piGEM2108 b	56 ng/µl
piGEM2108 c	66 ng/µl
piGEM2108 d	62 ng/µl
piGEM2109	46 ng/µl
piGEM2110	44 ng/µl
JZ147	206 ng/µl

Author: Daniel Marchal
created: 21.06.2018 12:05
updated: 21.06.2018 15:22
Entry 63/214: Preparation of electrocompetent Vn cells (Weinstock)
In Project: ERBsen
With tags: competent, electrocompetent, V. natriegens, weinstock, electroporation

Materials

260-510ml BHI + v2 salts

110ml Electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)

liquid nitrogen

Recipes

LBv2

400ml LB medium supplemented with 40ml 10xV2-salts

Electroporation buffer

680mM sucrose

7mM K_2HPO_4

pH7

Procedure

1. 10mL LBv2 is inoculated with V. natriegens and incubated oN at 37 °C with agitation at 200 r.p.m.
2. 250 mL of the same growth medium is inoculated with the overnight culture at a dilution of 1:100 (2,5 ml overnight culture /fresh medium)
3. The culture is grown at 37 °C in a baffled flask with shaking at 200 r.p.m. until an OD600 of 0.5 is reached
4. The culture is then split into five chilled 50-mL falcons and incubated on ice for 15 min
5. The cells are pelleted at 4,000 r.p.m. in a Beckman JA-14 centrifuge rotor for 10 min at 4 °C
6. The supernatant is carefully decanted and the cell pellets are gently resuspended in 5–10 mL of electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7)
7. The suspensions are pooled in one tube and the tube is filled up to 35 mL electroporation buffer and inverted several times to mix
8. The cells are centrifuged down at 4,000 r.p.m. for 15min at 4 °C in a JA-17 rotor
9. The supernatant is decanted with a pipette
10. The wash is repeated two times for a total of three washes
11. the cells are gently resuspended in residual electroporation buffer
12. Cells are aliquoted into chilled tubes (50µl), frozen in liquid nitrogen and stored at -80 °C until use

Comments

- Inoculated at 10 a.m. with 2.5ml preculture → OD = 0.076
- Cells harvested at 12:10 a.m. → OD = 0.537

Author: Daniel Marchal
created: 21.06.2018 15:22
updated: 25.06.2018 16:39
Entry 64/214: Retrafo of pYTK into Vn to check competence
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

Today competent Vn cells were prepared and shall now be tested via transformation of pYTK.

Procedure

1. thaw 3 aliquots of electrocompetent Vn on ice
2. add plasmid DNA into the aliquot
    1. Vn control without electroporation and without antibiotics
    2. Vn + pYTK (150ng) with chloramphenicol
    3. Vn + pYTK (150ng) without chloramphenicol
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 700-900 V (depending on the strain), 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM $MgCl_2$), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 37°C

Result:

- Control shows lawn
- Vn + pYTK without Cm shows ~500-1000 colonies, none of them are green
- Vn + pYTK on Cm shows no colonies → trafo didn't work
- Since the trafo didn't work I will repeat it on monday

Author: Daniel Marchal
created: 25.06.2018 13:39
updated: 25.06.2018 17:15

Entry 65/214: PCR amplification of Acc variants for Gibson assembly
In Project: ERBsen
With tags: PCR, PYTK, JZ154, JZ90, JZ105, JZ147

The Erblab provides 4 JZ plasmids with Acc variants. Unfortunately they aren't usable for MoClo because of several restriction sites (JZ90: BsaI, NotI, PstI / JZ105: BsaI, BsmBI, EcoRI, NotI, PstI / JZ147: BsaI, BsmBI, PstI, SpeI / JZ154: BsmBI, EcoRI, NotI). Therefore a PCR shall be made to amplify the acc inserts an then a Gibson assembly can be made to integrate them into pYTK. Afterwards they can be tested and if one of them shows a better activity then the Acc from C. glutamicum, then this variant can be synthetisized with optimized codon-usage.

Procedure:

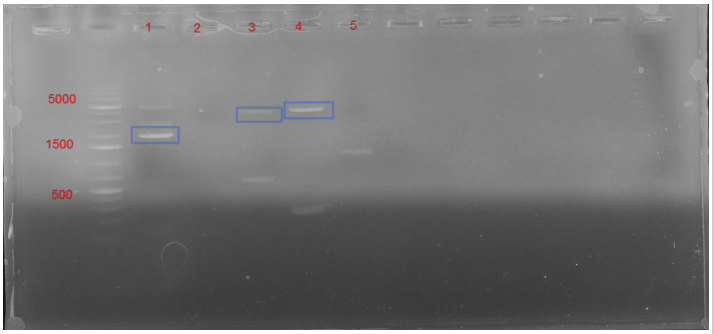
1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	pYTK	piGEM2100	piGEM2101
2	JZ90	piGEM2102	piGEM2103
3	JZ105	piGEM2104	piGEM2105
4	JZ147	piGEM2106	piGEM2107
5	JZ154	piGEM2108	piGEM2109

Mastermix (6x)	Sample
60 µl buffer	10 µl buffer
6 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
9 µl DMSO	1,5 µl DMSO
3 µl Q5 polymerase	0,5 µl Q5 polymerase
186 µl H_2O	31 µl H_2O (ad 50 µl)

Results:

0622_PCR_for_Gibson.JPG



expected lengths:

1	1959 bp
2	3511 bp
3	3837 bp
4	4006 bp
5	5238 bp

- sample 1 is correct but has more bands → gel elution
- sample 2 has no bands → must be repeated
- sample 3 is correct but has more bands → gel elution
- sample 4 is correct but has more bands → gel elution
- sample 5 has the wrond band → must be repeated

Author: Daniel Marchal
Entry 66/214: Construction of BirA Lvl 1
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation

created: 25.06.2018 16:33
updated: 06.07.2018 13:18

To test the functionality of BirA, 3 variants of lvl 1 BirA vectors will be made with strong / medium / weak promotor.

Golden Gate Reaction:

add following reagents to your annealing mix:

Streptag	piGEM2109	70 ng
5' Connector	piGEM1011	70 ng
Promotor	piGEM1007 (strong) piGEM1014 (medium) piGEM 1024 (weak)	70 ng
RBS	piGEM1008	70 ng
CDS	piGEM2107	70 ng
Terminator	piGEM1035	70 ng
3' Connector	piGEM1012	70 ng
Resistance	piGEM1057	70 ng
Ori	piGEM1036	70 ng
T7-Ligase (NEB)		0,5 µL
BsaI (NEB)		0,5 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (30 cycles)
Ligation	16°C	5 min (30 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Author: Daniel Marchal
Entry 67/214: Retrafo of pYTK into Vn to check competence (2)
In Project: ERBsen
No tags associated

created: 25.06.2018 17:18
updated: 26.06.2018 18:09

Since the last trafo didn't work it shall be repeated.

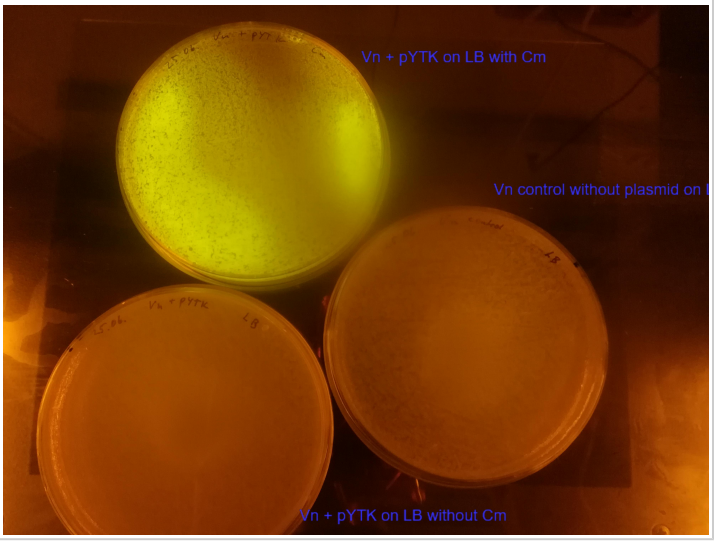
Procedure:

Retrafo of pYTK into Vn to check competence - entry #64 in project 'ERBsen' (Daniel Marchal, 25.06.2018)

Results:

- all plates look like expected
- since the plate on the top shines green, the trafo was sufficient and the cells must be competent :)

IMG_20180626_160801.jpg



Author: Daniel Marchal
created: 26.06.2018 18:11
updated: 04.07.2018 08:54

Entry 68/214: PCR amplification of Acc variants for Gibson assembly (2)
In Project: ERBsen
With tags: PCR, JZ154, JZ90

Since the PCR of JZ90 and JZ154 didn't work, these two PCRs will be repeated.

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	pYTK	piGEM2100	piGEM2101
2	JZ90	piGEM2102	piGEM2103
3	JZ105	piGEM2104	piGEM2105
4	JZ147	piGEM2106	piGEM2107
5	JZ154	piGEM2108	piGEM2109

Mastermix (6x)	Sample
60 µl buffer	10 µl buffer
6 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
9 µl DMSO	1,5 µl DMSO
3 µl Q5 polymerase	0,5 µl Q5 polymerase
186 µl H_2O	31 µl H_2O (ad 50 µl)

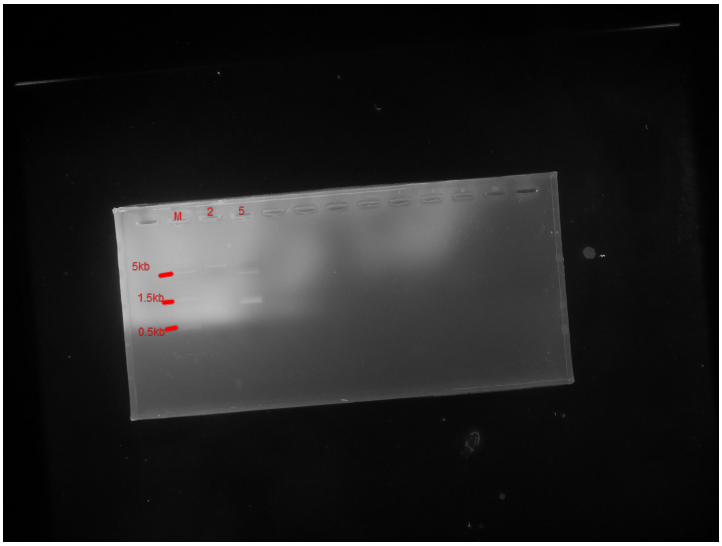
Results:

expected lengths:

1	1959 bp
2	3511 bp
3	3837 bp
4	4006 bp
5	5238 bp

- unfortunately we have agains no bands for sample 2 and the wrong bands for sample 5. One reason could be that the elongation time was too short (1.5 min). We will repeat the PCR with another Q5 buffer (enhanced GC) and an elongation time of 3.5 min.

P1020712.JPG



Author: Daniel Marchal
created: 27.06.2018 09:29
updated: 06.07.2018 13:15

Entry 69/214: Trafo of Acc Lvl1 into Ec
In Project: ERBsen
With tags: transformation, e.coli

Procedure

1. thaw 3 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance
Acc_Lvl1_strong	BirA Lvl 1 strong promotor	Kan
Acc_Lvl1_middle	BirA Lvl 1 middle promotor	Kan
Acc_Lvl1_weak	BirA Lvl 1 weak promotor	Kan

3. add 10µl of GoldenGate reaction
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Results:

The trafos were sufficient, all plate have ~500-1000 colonies but nearly all of them are shining green, presumably because of the kan-part, which can also exist as sole vector without the other MoClo parts. To get single colonies we will streak them out and then enrich single, white colonies.

Update 02.07.:

The isolated cells are all green, so it seems that we have no positive clones. We will repeat the golden gate assembly with a lower amount of the resistance part to prevent a overgrowth of resistance lvl 0 parts.

Author: Daniel Marchal
created: 27.06.2018 16:58
updated: 28.06.2018 18:14
Entry 70/214: Activity assay for native Acc from Vn
In Project: ERBsen
With tags: Acc, enzyme activity, activity, assay, cell extract

Since the growth rate of Vn is very high, we assume, that the native Acc must have a naturally high activity. To validate it we will perform an activity assay with cell extract by adding Mcr, NADPH and Acetyl-CoA and measuring the NADPH/NADP+ conversion. The procedure is adapted from Pascal.

Procedure:

1. Prepare MOPS Buffer (Low salt as standard buffer and high salt because of Vibrios higher salt preference)
2. Prepare a tube with 10ml LB2,5 and inoculate from Vn cryostock
3. Incubate over night at 37°C shaking
4. Prepare a 1000ml flask with 500ml LB2,5 and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture and incubate at 37°C shaking
6. Stop incubating when OD_{600}=2-3
7. Harvest the cells in 500ml centrifugation bottles (each bottle with 250ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
8. Let the cells splitted in two fractions (one for low salt conditions and one for high salt conditions, labels as LS & HS)
9. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 15ml Falcon.
10. Add 400µl 10xProtease-Inhibitor-Cocktail (provided)
11. Fill the tubes up to 4ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS Low salt

50mM MOPS/KOH

150mM NaCl

pH 7,8

MOPS High salt

50mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor Cocktail

12. Use the frenchpress to break the cells at 900 psi
    1. lever on "down" and wheel on high pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
13. If the solution is clear, the cell breakage was successful
14. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
15. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
16. For the enzyme assay use the software "Cary UV" with the program "kinetics"
17. Mix 40-229µl of your cell lysate together with MOPS buffer, $MgCl_2$, NADPH, ATP, $KHCO_3$ and MCR_Ca and measure slope (background)
18. Add Acetyl-CoA to start the reaction and again measure slope to calculate specific activity (see excel sheet)
19. If there is enzyme activity you can make a bradford to normalize your results
20. As a positive control you can add Pcc_Me
21. To store the cell lysate add 300µl glycerol and store at -20°C

2018_06_28_ACC_Vn.ods

Results:

- Cells harvested at OD_{600}=2.2
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used
- Centrifugation bottle weights: 74.05g/75.19g
- Cell weights: LS=1.39g / HS=1.23g
- There was no significant activity detectable. We will prepare a sample for HPLC to see if malonyl-CoA is formed when acetyl-CoA is added. HPLC is more sensitive but doesn't show kinetics

Author: Daniel Marchal
Entry 71/214: Preparation of samples for HPLC to check malonyl-CoA availability
In Project: ERBsen
No tags associated

created: 28.06.2018 17:55
updated: 28.06.2018 18:14

Activity assay for native Acc from Vn - entry #70 in project 'ERBsen' (Daniel Marchal, 28.06.2018)

Since the activity assay for native Acc from Vn showed no results, a HPLC will be made. We will prepare samples and then Nina will make a HPLC to screen for Malonyl-CoA

Procedure:

1. Make a reaction mixture according to the following table

Volume	**Substance**	**Stock c**	**Endconcentration**
255µl	Cell lysate (low salt)	~1.4 mg/ml (reference from E. coli)	~1.2mg/ml
10	Acetyl-CoA	66.3 mM	2.21 mM
10	MgCl2	200 mM	6.67 mM
10	ATP	100 mM	3.33 mM
15	KHCO3	1 M	50 mM

2. Incubate 30min at 37°C shaking
3. add 33µl Formic acid (endconcentration: 10% v/v) and mix well (air bubbles will form indicating the forming CO2)
4. Centrifuge 15 minutes at 4°C and 17,000g (max speed)
5. Transfer supernatant into new eppi and repeat centrifugation step
6. Transfer 100µl into a HPLC tube and store on ice until use, do the same for an 1:10 dilution in H_2O

Author: Daniel Marchal
created: 04.07.2018 08:54
updated: 04.07.2018 10:24
Entry 72/214: PCR amplification of Acc variants for Gibson assembly (3)
In Project: ERBsen
With tags: PCR, PYTK, JZ154, JZ90, JZ105, JZ147

The Erblab provides 4 JZ plasmids with Acc variants. Unfortunately they aren't usable for MoClo because of several restriction sites (JZ90: BsaI, NotI, PstI / JZ105: BsaI, BsmBI, EcoRI, NotI, PstI / JZ147: BsaI, BsmBI, PstI, SpeI / JZ154: BsmBI, EcoRI, NotI). Therefore a PCR shall be made to amplify the acc inserts an then a Gibson assembly can be made to integrate them into pYTK. Afterwards they can be tested and if one of them shows a better activity then the Acc from C. glutamicum, then this variant can be synthetisized with optimized codon-usage.

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	pYTK	piGEM2100	piGEM2101
2	JZ90	piGEM2102	piGEM2103
3	JZ105	piGEM2104	piGEM2105
4	JZ147	piGEM2106	piGEM2107
5	JZ154	piGEM2108	piGEM2109

Mastermix (6x)	Sample
60 µl buffer	10 µl buffer
6 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
9 µl DMSO	1,5 µl DMSO
3 µl Q5 polymerase	0,5 µl Q5 polymerase
186 µl H_2O	31 µl H_2O (ad 50 µl)

Program:

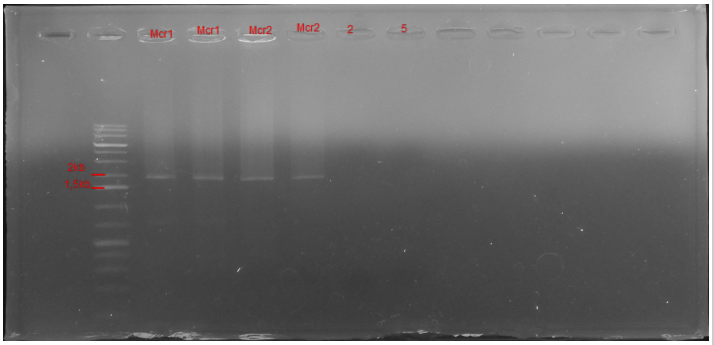
94°C	3min	
94°C	20s	25x
60°C	1min	25x
72°C	3.5min	25x
72°C	5min	
4°C	infinite	

Results:

expected lengths:

1	1959 bp
2	3511 bp
3	3837 bp
4	4006 bp
5	5238 bp

0704_PCR_for_Gibson_+_Mcr.jpg



- The Pcr of part 2 and 5 shows no bands, so the PCR must be repeated
- Additionaly the PCR of codonoptimized Mcr Parts was put on the gel and shows a band at 2kb what was expected. These Mcr Parts can now be fused to come to the fulllength gene

Author: Daniel Marchal

created: 04.07.2018 14:25
updated: 10.07.2018 20:41

Entry 73/214: Enrichment and isolation of piGEM1008, 1016, 1070, 1071, 1075, 1076, 1077, 1078, 1079, 1080

In Project: ERBsen

With tags: piGEM1008, piGEM1016, piGEM1076, piGEM1079, piGEM1078, piGEM1077, piGEM1070, piGEM1080, piGEM1075, piGEM1071

Procedure

1. prepare 10 tubes with following annotations:
    1. Ec + piGEM1008
    2. Ec + piGEM1016
    3. Ec + piGEM1070
    4. Ec + piGEM1071
    5. Ec + piGEM1075
    6. Ec + piGEM1076
    7. Ec + piGEM1077
    8. Ec + piGEM1078
    9. Ec + piGEM1079
    10. Ec + piGEM1080
2. add 5ml LB into the tubes and 5µl Cm [2mg/ml] (fresh prepared)
3. Inoculate from Grauman labs cryostock
4. Incubate over day at 37°C shaking
5. Make miniprep and cryostock

Result

piGEM1008	RBS (B0034)	60 ng/µl
piGEM1016	RBS (B0030)	44 ng/µl
piGEM1070	3'con1	50 ng/µl
piGEM1071	3'con2	40 ng/µl
piGEM1075	5'con1	75 ng/µl
piGEM1076	5'con2	71 ng/µl
piGEM1077	5'con3	49 ng/µl
piGEM1078	5'con4	55 ng/µl
piGEM1079	5'con5	39 ng/µl
piGEM1080	3'con5	45 ng/µl

Author: Daniel Marchal
created: 05.07.2018 14:06
updated: 05.07.2018 17:29

Entry 74/214: PCR amplification of Acc variants for Gibson assembly (4) + Restriction digest of JZ90 and JZ154
In Project: ERBsen
With tags: PCR, restriction, JZ154, JZ90, HindIII

Since die other PCRs didn't work, we will repeat it again and in parallel will male a restriction digest of JZ90 and JZ154 to see if they are really the correct plasmids.

Procedure PCR:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Procedure restriction digest:

1. Prepare 12µl H_2O + 1.5µl 10xFD-buffer prestained + 1µl JZ90/JZ154 + 0.5µl HindIII
2. Incubate 30min at 37°C
3. Run gel

Sample	DNA template	primer for	primer rev
2	JZ90	piGEM2102	piGEM2103
5	JZ154	piGEM2108	piGEM2109

Sample
10 µl Q5 buffer
1 µl dNTPs
2,5 µl primer for
2,5 µl primer rev
3 µl DNA
1,5 µl DMSO
0,5 µl Q5 polymerase
29 µl H_2O (ad 50 µl)

Results:

expected lengths:

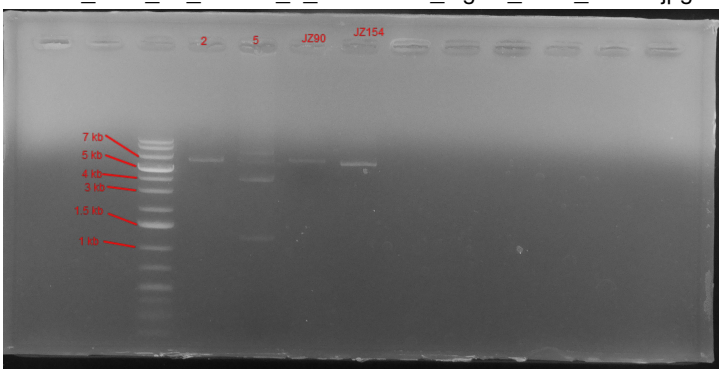
2	3511 bp
5	5238 bp
JZ90	6179 bp
JZ154	6294 bp + 3820 bp

Program:

94°C	3min	
94°C	20s	25x
55°C	30sec	25x
72°C	3.5min	25x
72°C	5min	
4°C	infinite	

- Sample 2 shows a band, but it has the wrong size (~5kb). Maybe its the JZ90 template.
- Sample 5 shows also the wrong bands, but there could be a slight band in the correct position, but even if its the right fragment, die amount is too low.
- JZ90 shows a band with the expected size, but it is difficult to say, if it was cleaved by HindIII. Nevertheless there is plasmid (hopefully the correct ones^^)
- JZ154 has a size of 10.1 kb, the gel shows a band with a size of 5-7kb what would fit to the expectations but there is no smaller band with 3.8kb detectable, so maybe the vector is the wrong one.
- We will sequence the plasmids to see if they are really the correct ones

0705_PCR_for_Gibson_+_Restriction_digest_JZ90_JZ154.jpg



Author: Daniel Marchal
Entry 75/214: DpnI digestion & Gel extraction for Gibson assembly
In Project: ERBsen
With tags: Gel extraction, DpnI

created: 06.07.2018 10:47
updated: 12.07.2018 11:21

MN_NucleoSpin_Gel_and_PCR_Clean-Up.pdf

Procedure DpnI digestion:

1. Mix all your PCR (49µl) with 6µl prestained FD-buffer, 3µl H_2O and 2µl FD-DpnI
2. Mix carefully and incubate over night at 37°C
3. Make a gel extraction

Procedure Gel extraction:

1. Load the whole sample on a 1% agarose gel and run at 100V for 33min
2. Cut the right bands out and follow the protocoll of "MN NucleoSpin Gel and PCR Clean-Up" (page 19-20 in the file)
    1. we used 600µl NTI buffer
    2. we eluted in 20µl H_2O
3. Determine concentrations using Nanodrop

Results:

c(pYTK backbone)	44 ng/µl
c(AccSe)	22 ng/µl (but peak was at 230nm so probably it is 0 ng/µl)
c(PccMe)	0 ng/µl

Since the DNA concentrations are too low, we have to repeat all PCRs at a larger scale

Author: Daniel Marchal
created: 10.07.2018 15:11
updated: 29.09.2018 13:40

Entry 76/214: Preparation of M63 minimal medium
In Project: ERBsen
With tags: M63, minimal medium

For enzyme assays cells have to be cultivated on minimal medium. It was already shown that Vibrio grows on M63 minimal medium with maltose mal operon of V. natriegens - entry #1 in project 'Protein Interaction Strain' (Memduha Muratoglu, 10.07.2018), so we will prepare the same medium just with glucose instead of maltose.

Procedure:

1. Prepare a 5x M63 stock, fill to 900ml with water, adjust to pH=7 with KOH and autoclave
2. Prepare a Glucose stock
3. Mix 200ml 5xM63 stock together with 100ml 10xV2 stock, glucose (final concentration 0.5-2%) and water to a final volume of 1L
4.

	Substrate	**Final concentration**
10g	$(NH_4)_2SO_4$	75 mM
68g	KH_2PO_4	500 mM
2.5mg	$FeSO_4$ x 7 H_2O	0.00025 %
5mg	Hydrothiamine	0.0005 %
ad 900ml	H_2O	-

Author: Daniel Marchal
Entry 77/214: PCR amplification of Acc variants for Gibson assembly (5)
In Project: ERBsen
With tags: PCR, PYTK, JZ154, JZ90, JZ105, JZ147

created: 10.07.2018 15:33
updated: 11.07.2018 15:48

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	pYTK	piGEM2100	piGEM2101
1	pYTK	piGEM2100	piGEM2101
1	pYTK	piGEM2100	piGEM2101
1	pYTK	piGEM2100	piGEM2101
3	JZ105	piGEM2104	piGEM2105
3	JZ105	piGEM2104	piGEM2105
4	JZ147	piGEM2106	piGEM2107
4	JZ147	piGEM2106	piGEM2107

Mastermix (9x)	Sample
90 µl buffer	10 µl buffer
9 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	2 µl DNA
13.5 µl DMSO	1,5 µl DMSO
4.5 µl Q5 polymerase	0,5 µl Q5 polymerase
270 µl H_2O	30 µl H_2O (ad 50 µl)

Results:

expected lengths:

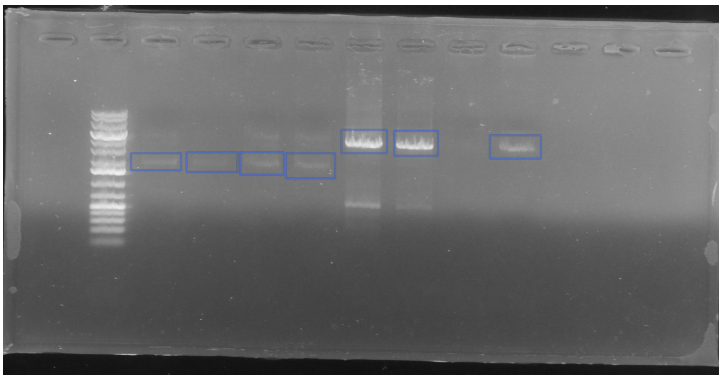
1	1959 bp
3	3837 bp
4	4006 bp

- sample 1 is correct but has very pale bands → repeat the PCR!
- sample 3 is correct but has more bands → gel elution
- sample 4 shows a correct band without unwanted bands → PCR purification

Program:

94°C	3min	
94°C	20s	25x
55°C	30sec	25x
72°C	3.5min	25x
72°C	5min	
4°C	infinite	

0710_PCR_for_Gibson.jpg



Author: Daniel Marchal
Entry 78/214: PCR amplification of Acc variants for Gibson assembly (6)
In Project: ERBsen
With tags: PCR, PYTK, JZ154, JZ147

created: 11.07.2018 15:48
updated: 11.07.2018 15:59

The Erblab provides 4 JZ plasmids with Acc variants. Unfortunately they aren't usable for MoClo because of several restriction sites (JZ90: BsaI, NotI, PstI / JZ105: BsaI, BsmBI, EcoRI, NotI, PstI / JZ147: BsaI, BsmBI, PstI, SpeI / JZ154: BsmBI, EcoRI, NotI). Therefore a PCR shall be made to amplify the acc inserts an then a Gibson assembly can be made to integrate them into pYTK. Afterwards they can be tested and if one of them shows a better activity then the Acc from C. glutamicum, then this variant can be synthetisized with optimized codon-usage.

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	pYTK	piGEM2100	piGEM2101
4	JZ147	piGEM2106	piGEM2107
5	JZ154	piGEM2108	piGEM2109

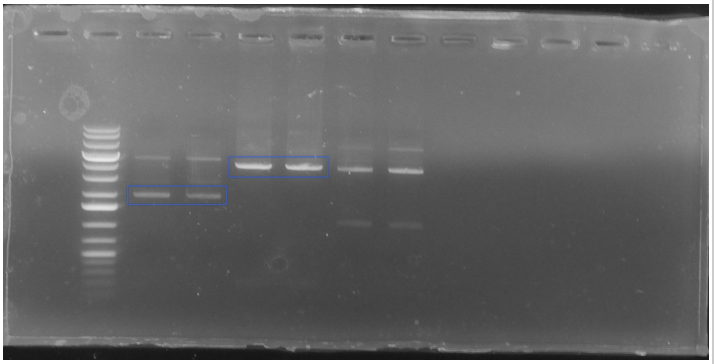
Mastermix (7x)	Sample
70 µl buffer	10 µl buffer
7 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
10.5 µl DMSO	1,5 µl DMSO
3.5 µl Q5 polymerase	0,5 µl Q5 polymerase
217 µl H_2O	31 µl H_2O (ad 50 µl)

Results:

expected lengths:

1	1959 bp
4	4006 bp
5	5238 bp

0711_PCR_for_Gibson.jpg



- sample 1 is correct but has more bands → gel elution
- sample 4 is correct → PCR purification
- sample 5 has the wrond bands, but it was additionally a sequencing made revealing a wrong template plasmid → wait until we know where is the correct plasmid

Author: Daniel Marchal
Entry 79/214: DpnI digest for Gibson assembly
In Project: ERBsen
With tags: DpnI, PYTK, JZ105, JZ147

created: 11.07.2018 16:30
updated: 11.07.2018 16:36

Procedure:

1. Pool all sufficient PCR aliquots of each sample (100µl)
2. add 12µl FD-buffer, 6µl H_2O and 2µl FD-DpnI
3. incubate 60min at 37°C
4. Store at -20°C until PCR purification/gel elution

Author: Daniel Marchal
Entry 80/214: Gel extraction for Gibson assembly
In Project: ERBsen
With tags: PYTK, JZ105, JZ147, Gel extraction

created: 12.07.2018 11:20
updated: 12.07.2018 14:16

For gibson assembly, a gel extraction must be made for pYTK and JZ105 (PccMe) because there were several bands in the PCR gel. JZ147 (AccSe) can easily be PCR purified because it was a clear band.

Procedure:

1. Load the whole sample of pYTK and PccMe on a 1% agarose gel and run at 100V for 33min
2. Cut the right bands out and follow the protocoll of "MN NucleoSpin Gel and PCR Clean-Up" (page 19-20 in the file)
    1. we used 600µl NTI buffer
    2. we eluted in 20µl H_2O
3. Make a PCR purification for AccSe
4. Determine concentrations using Nanodrop

MN_NucleoSpin_Gel_and_PCR_Clean-Up.pdf

Result:

pYTK: 47 ng/µl

PccMe: 60 ng/µl

AccSe: 122 ng/µl

Author: Daniel Marchal

created: 12.07.2018 14:20
updated: 16.07.2018 08:58

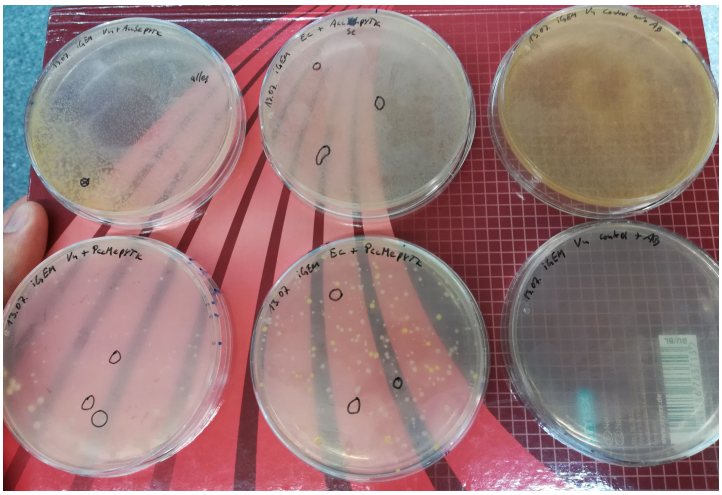
Entry 81/214: Gibson assembly for AccSe and PccMe and trafo into Ec and Vn
In Project: ERBsen
With tags: gibson cloning, AccSe, PccMe, JZ105, JZ147

Procedure:

1. Set up the following reaction on ice (0.2pmol DNA, Insert:Backbone = 3:1)
2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.
3. Transform 10µl into Ec and 10µl into Vn
    1. Ec trafo: 10µl sample / 2h regeneration / plating out on LB+Cm
    2. Vn trafo: 10µl sample / 900mV / plating out on LB2,5+Cm

							Pipettierschema	
Fragment	**bp**	**ng /µl**	**pmol/µl**	**µl for 0,2 pmol**				
Backbone	1959	47	0,072703	2,750936			**AccSe**	**PccMe**
AccSe	4006	122	0,092286	2,16718		Fragment [µl]	2,16718	4,2207
PccMe	3837	60	0,047386	4,2207		Backbone [µl]	2,750936	2,750936
						Gibson Mastermix 2x [µl]	10	10
						H_2O [µl]	5,081884	3,028364
						Total volume [µl]	20	20

0716_gibson_assembly.jpg



Results:

- Control with antibiotic: no colonies as expected
- Control w/o antibiotic: lawn as expected
- Vn + AccSepYTK: there are a lot of colonies, all are white exept of one colony → picking of 3 colonies
- Vn + PccMepYTK: there are several colonies with different sizes, most of them are white → picking of 3 colonies of different sizes
- Ec + AccSepYTK: there are a lot of colonies, all are white → picking of 3 colonies
- Ec + PccMepYTK: there are several colonies, most of them are white → picking of 3 colonies of different sizes
- in total 12 colonies will be picked and enriched in LB+Cm/LBv2+Cm. Then a miniprep and control digest can be made. A sequencing will confirm the correct assembly of the plasmids.

Author: Daniel Marchal
created: 12.07.2018 15:40
updated: 23.07.2018 12:34
Entry 82/214: Sequencing of JZ147, JZ90 and JZ154
In Project: ERBsen
No tags associated

Since the PCRs of JZ90 and JZ154 didn't work, Pascal ordered a sequencing of them. The result for JZ154 was, that it is definitely the plasmid JZ147 and not JZ154, so maybe we switched the samples. To confirm that JZ147 is really the correct plasmid a sequencing shall be made.

Procedure:

- 6µl JZ147 [200ng/µl] + 6µl H_2O + 2µl Primer "pNS3SeqfII10"
- Label number: AIM0030075

Result JZ147:

The sequence of JZ147 is definitely the correct one, so we can continue working with that plasmid!

Result JZ90:

The sequence was wrong, I got a new plasmid with the correct plasmid and will retransform it.

Author: Daniel Marchal

created: 13.07.2018 15:48

updated: 13.07.2018 16:24

Entry 83/214: Lvl 1 GoldenGate of piGEM2111_LVL1_HisAccBC

In Project: ERBsen

With tags: Golden Gate, Lvl 1 plasmids, transformation, piGEM2109

Golden Gate Reaction:

add following reagents to your annealing mix:

Vector:	**piGEM2111_LVL1_HisAccBC**		
Part/Reagent	**Plasmid**	**Conc. [ng/µl]**	**Volume for 75ng [µl]**
1	piGEM1075	75	1,00
2	piGEM1007	49	1,53
3	piGEM1008	60	1,25
4x	4xHisPart*	180	0,42
4y	piGEM2105	61	1,23
5	piGEM1035	93	0,81
6	piGEM1070	50	1,50
7	piGEM1036	81	0,93
8	piGEM1057	39	0,64
BsaI			1,00
T7 Ligase			1,00
T4 Ligase Buffer			1,00
H2O			0,00

Start Golden Gate Reaction in Thermocycler:

Digest	37°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	60 min
Inactivation	80°C	10 min

*4xHisTag is not sequenced yet, so there might be the case, that its sequence is wrong and the reaction fails.

Author: Daniel Marchal

created: 16.07.2018 08:48
updated: 16.07.2018 11:41

Entry 84/214: Enrichment and isolation of piGEM2116_AccSe_pYTK + piGEM2115_PccMe_pYTK in Ec + Vn

In Project: ERBsen

With tags: Enrichment, miniprep, AccSe, PccMe, piGEM2116, piGEM2115

Procedure

1. prepare 12 tubes with following annotations:
    1. Vn + AccSepYTK 1
    2. Vn + AccSepYTK 2
    3. Vn + AccSepYTK 3
    4. Vn + PccMepYTK 1
    5. Vn + PccMepYTK 2
    6. Vn + PccMepYTK 3
    7. Ec + AccSepYTK 1
    8. Ec + AccSepYTK 2
    9. Ec + AccSepYTK 3
    10. Ec + PccMepYTK 1
    11. Ec + PccMepYTK 2
    12. Ec + PccMepYTK 3
2. add 5ml LB/LBv2 into the tubes and 5µl Cm [34ng/µl] / [2mg/ml] (fresh prepared)
3. Inoculate from gibson plates
4. Incubate over two days at 37°C shaking
5. Make miniprep

Result

Vn + AccSepYTK 1	35 ng/µl
Vn + AccSepYTK 2	45 ng/µl
Vn + AccSepYTK 3	40 ng/µl
Vn + PccMepYTK 1	27 ng/µl
Vn + PccMepYTK 2	24 ng/µl
Vn + PccMepYTK 3	30 ng/µl
Ec + AccSepYTK 1	112 ng/µl
Ec + AccSepYTK 2	90 ng/µl
Ec + AccSepYTK 3	65 ng/µl
Ec + PccMepYTK 1	120 ng/µl
Ec + PccMepYTK 2	39 ng/µl
Ec + PccMepYTK 3	140 ng/µl

Author: Daniel Marchal
created: 16.07.2018 08:53
updated: 16.07.2018 15:34

Entry 85/214: Trafo of piGEM2111_LVL1_HisAccBC into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 5µl of lvl1 Golden Gate (Lvl 1 GoldenGate of piGEM2111_LVL1_HisAccBC - entry #83 in project 'ERBsen' (Daniel Marchal, 13.07.2018))
3. incubate 15 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 5 min on ice
6. add 800µl LB
7. incubate 1h at 37°C shaking
8. spread out on LB selection plates
9. incubate oN at 37°C

Results:

- there are up to 1000 colonies on the plate but all of them are green. Probably the resistance part has to be digested before the cloning procedure.

Author: Daniel Marchal
created: 16.07.2018 13:32
updated: 19.07.2018 12:16
Entry 86/214: Restriction digest of piGEM2115 & piGEM2116
In Project: ERBsen
With tags: piGEM2115, restriction, digest, piGEM2116

To check which plasmids of piGEM2115 & piGEM2116 are correct a restriction digest shall be made.

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

Sample	**Master mix (8x)**
4µl DNA	-
0.2µl FD-EcoRI/FD-NcoI	1.6 µl FD-EcoRI/FD-NcoI
1µl FD-buffer prestained	7µl FD-buffer prestained
4.8µl H_2O	38.4 µl H_2O

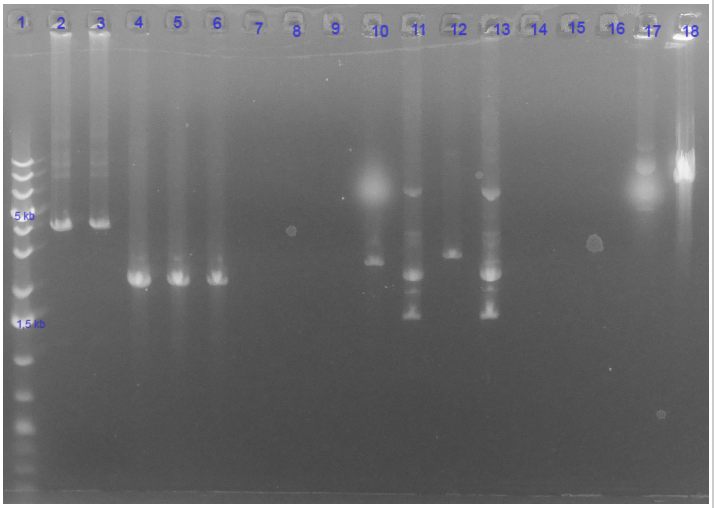
Results

Plasmid	**Enzyme**	**Expected length**
piGEM2115_PccMe_pYTK from Ec	EcoRI	2168 + 3564 bp
piGEM2115_PccMe_pYTK from Vn	EcoRI	2168 + 3564 bp
piGEM2116_AccSe_pYTK from Ec	NcoI	1172 + 2319 + 2410 bp
piGEM2116_AccSe_pYTK from Vn	NcoI	1172 + 2319 + 2410 bp
pYTK	EcoRI	2676 (circular)
pYTK	NcoI	2676 (linearized)
JZ147 (AccSe)	NcoI	1171 + 1172 + 6525 bp

- JZ105 (PccMe) can't be in the samples because it has the wrong resistance cassette
- lane 4,5 & 6 with AccSe from Ec show a large band at~2.3kb and a slight band at ~2.6kb. These could be 2 of 3 expected bands, but then the 1172bp band is missing. The band is definitely too short to be pYTK (compare to lane 10) and it can also not be JZ147 (compare to lane 18). We will sequence one of these samples.
- lane 7,8 & 9 shows no bands, maybe the concentration of plasmid is too short or there is no plasmid. We will waste the samples and hope, that the ones from E. coli are correct.
- lane 10 and lane 18 are controls for NcoI and lane 17 is a control for EcoRI
- lane 11 and 13 show several bands, including 2168bp and 3564bp. Maybe there is also a second plasmid or undigestes plasmid inside the sample. We will sequence on of these samples.
- lane 12 has a band at ~2.5-3kb, maybe it is just pYTK
- lane 14.15 & 16 show no bands, maybe the concentration of plasmid is too short or there is no plasmid. We will waste the samples and hope, that the ones from E. coli are correct.

1	Marker 1kb plus ladder
2	PCR (other protocol)
3	PCR (other protocol)
4	piGEM2116_AccSe_pYTK from Ec 1 (NcoI)
5	piGEM2116_AccSe_pYTK from Ec 2 (NcoI)
6	piGEM2116_AccSe_pYTK from Ec 3 (NcoI)
7	piGEM2116_AccSe_pYTK from Vn 1 (NcoI)
8	piGEM2116_AccSe_pYTK from Vn 2 (NcoI)
9	piGEM2116_AccSe_pYTK from Vn 3 (NcoI)
10	pYTK (NcoI)
11	piGEM2115_PccMe_pYTK from Ec 1 (EcoRI)
12	piGEM2115_PccMe_pYTK from Ec 2 (EcoRI)
13	piGEM2115_PccMe_pYTK from Ec 3 (EcoRI)
14	piGEM2115_PccMe_pYTK from Vn 1 (EcoRI)
15	piGEM2115_PccMe_pYTK from Vn 2 (EcoRI)
16	piGEM2115_PccMe_pYTK from Vn 3 (EcoRI)
17	pYTK (EcoRI)
18	JZ147 (NcoI)

0717_Restriction_digest_+_PCR.jpg



Author: Daniel Marchal
Entry 87/214: PCR amplification of JZ154 for Gibson assembly
In Project: ERBsen
With tags: PCR, PYTK, JZ154, JZ90, JZ105, JZ147

created: 16.07.2018 16:20
updated: 18.07.2018 10:58

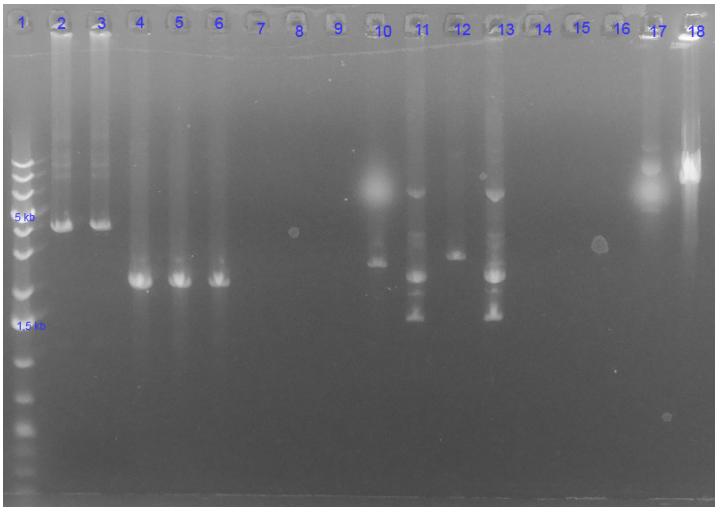
Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

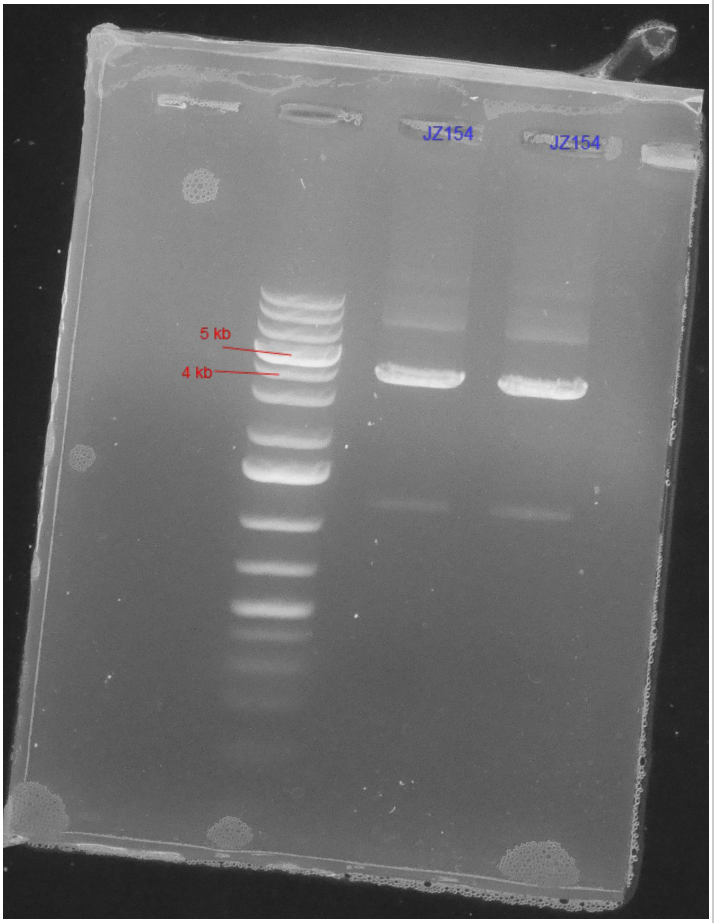
Sample	DNA template	primer for	primer rev
5	JZ154	piGEM2108	piGEM2109

Mastermix (2x)	Sample
20 µl buffer	10 µl buffer
2 µl dNTPs	1 µl dNTPs
5 µl primer for	2,5 µl primer for
5 µl primer rev	2,5 µl primer rev
2 µl DNA	1 µl DNA
3 µl DMSO	1,5 µl DMSO
1 µl Q5 polymerase	0,5 µl Q5 polymerase
62 µl H_2O	31 µl H_2O (ad 50 µl)

0717_Restriction_digest_+_PCR.jpg-with-annotations.png



0718_PCR_for_Gibson.jpg



Results:

expected length: 5238 bp

- Left gel: both samples show a large band at 4-5kb and two smaller bands at 10kb and 20kb. Unfortunatelly the large band is too short, so it is probably the wrong one. Maybe it's the coiled JZ154 plasmid. We will repeat the PCR with 20ng of template per 50µl approach
- right gel: there are again large bands at 4-5kb and smaller bands at 10kb and 1.2kb. We have no explanation for that, but maybe it is the right band. We will continue with DpnI digest, gel elution and gibson assembly

Author: Daniel Marchal

created: 17.07.2018 10:15
updated: 17.07.2018 10:20

Entry 88/214: Retrafo of piGEM1057 into Ec + Miniprep

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, piGEM1057, JZ147

Procedure Trafo:

1. thaw one aliquot of Ec NEB Turbo on ice
2. add 1µl of plasmid piGEM1057
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (Cm 34µg/ml)
9. incubate oN at 37°C

Procedure Enrichment and Miniprep:

1. prepare one tube with the annotation 'Ec + piGEM1057'
2. add 5ml LB into the tubes and 5µl Cm [34ng/µl] (fresh prepared)
3. Inoculate from trafo plate
4. Incubate over two days at 37°C shaking
5. Make miniprep

Author: Daniel Marchal
created: 17.07.2018 11:46
updated: 18.07.2018 14:01

Entry 89/214: Sequencing of piGEM2115 & piGEM2116
In Project: ERBsen
With tags: sequencing, piGEM2115, piGEM2116

Samples:

Vektor	Eppi	Label	Primer
piGEM2115_PccMe_pYTK from Ec 3	1	AIM0030089	oiGEM0031
piGEM2115_PccMe_pYTK from Ec 3	2	AIM0030090	oiGEM0032
piGEM2116_AccSe_pYTK from Ec 1	3	AIM0030091	oiGEM0031
piGEM2116_AccSe_pYTK from Ec 1	4	AIM0030092	oiGEM0032

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order):

- Primer oiGEM0032 binds in the resistance cassette, what is absolutely useless for us
- PccMe is wrong, the backbone fragment was recircularized, so that after promotor and rbs there comes directly the terminator. Maybe the ligase worked faster than the exonuclease and polymerase. We will pick more colonies from the gibson plate and repeat the restriction digest, maybe there is another colony which looks better.
- AccSe is wrong, after promotor and rbs there comes half of the first gene and then directly the terminator. We have no explanation for that phenomenon but will continue the same way as for PccMe, by picking more colonies and digest their plasmids.

Author: Daniel Marchal
created: 19.07.2018 10:50
updated: 20.07.2018 09:57

Entry 90/214: Gibson assembly for AccEc and trafo into Ec
In Project: ERBsen
With tags: AccEc, gibson cloning, piGEM2117

Procedure:

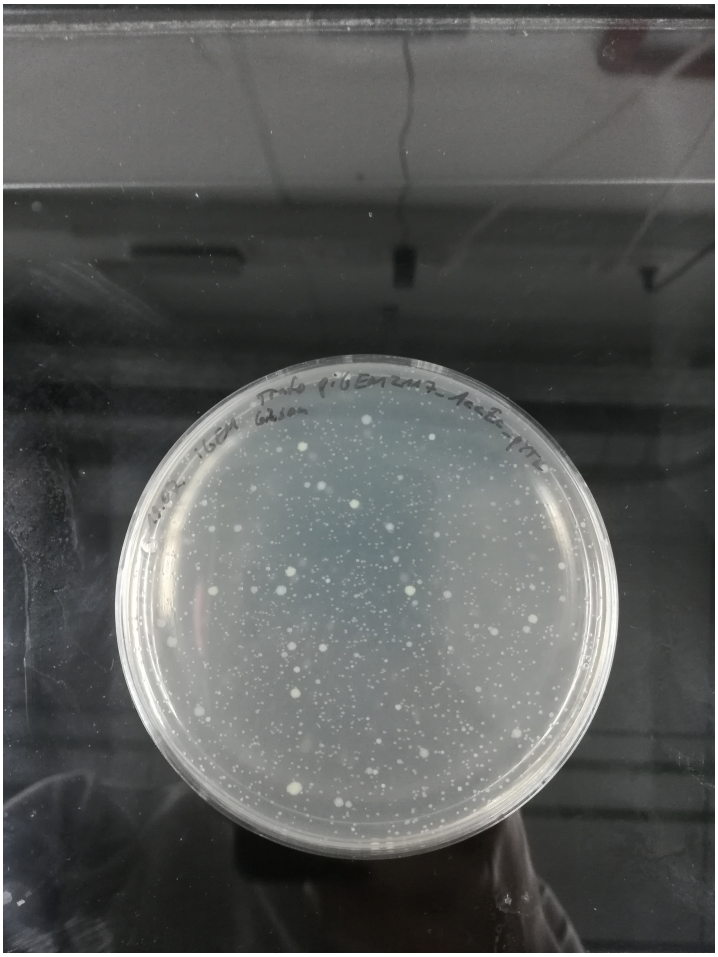
1. Set up the following reaction on ice (0.2pmol DNA, Insert:Backbone = 5:1)
2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.
3. Transform 10µl into Ec, store the rest at -20°C
    1. Ec trafo: 10µl sample / 2h regeneration / plating out on LB+Cm

						Pipettierschema	
Fragment	**bp**	**ng /µl**	**pmol /µl**	**µl for 0,2 pmol**		Gibson Mastermix 2x [µl]	10
Backbone	1959	47	0,07	2,75		H_2O [µl]	7.86
AccEc	5238	211	0,12	1,64		Fragment [µl]	1,64
						Backbone [µl]	0,5
						Total volume [µl]	20

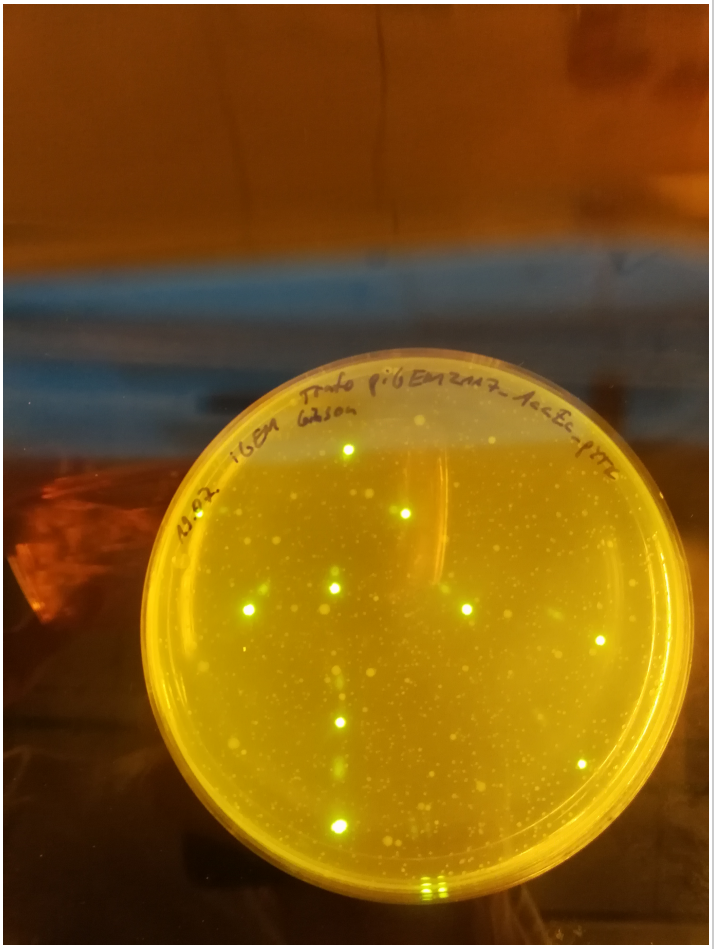
Results:

There are many colonies on the gibson plate. Some of them are green (wrong) and some are larger than the others. We will pick 4 large colonies and 4 small colonies and enrich them in LB+Kan

0720_gibson_assembly_2.jpg



0720_gibson_assembly.jpg



Author: Daniel Marchal
Entry 91/214: Gel extraction for Gibson assembly
In Project: ERBsen
With tags: JZ154, Gel extraction

created: 19.07.2018 11:32
updated: 19.07.2018 17:22

For gibson assembly, a gel extraction must be made for JZ154 (AccEc) because there were several bands in the PCR gel.

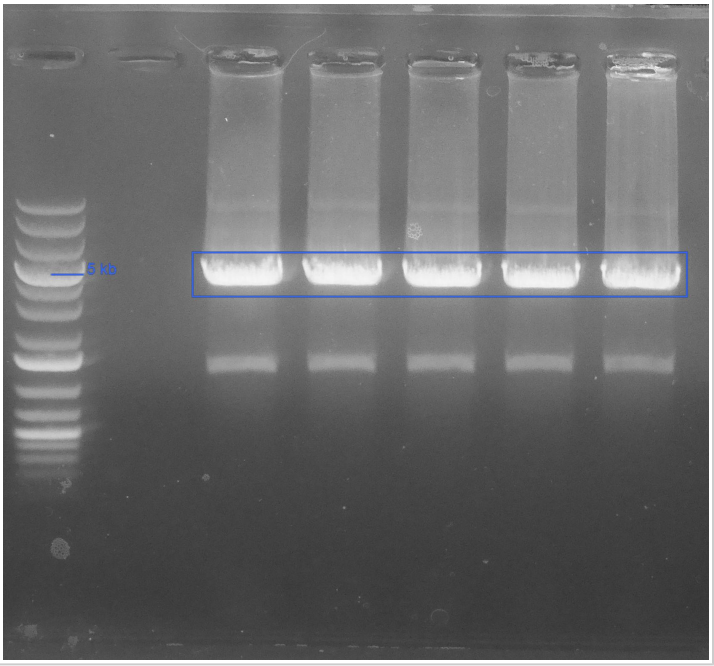
Procedure:

1. Load the whole sample on a 0.8% agarose gel and run at 100V for 33min
2. Cut the correct bands out and follow the protocoll of "MN NucleoSpin Gel and PCR Clean-Up" (page 19-20 in the file)
    1. we used 600µl NTI buffer
    2. we eluted in 20µl H_2O
3. Determine concentrations using Nanodrop

MN_NucleoSpin_Gel_and_PCR_Clean-Up.pdf

Result: 211 ng/µl

0719_PCR_elution.jpg



Author: Daniel Marchal
created: 19.07.2018 11:50
updated: 19.07.2018 12:24
Entry 92/214: Enrichment and miniprep of more Gibson colonies
In Project: ERBsen
With tags: AccSe, PccMe, piGEM2115, piGEM2116

Since the sequencing and restriction digest of the last gibson colonies didn't lead to a good result, we want to pick 10 further colonies und see if some of them look like correct plasmids.

Procedure Enrichment & Miniprep

1. prepare 10 tubes with following annotations:
    1. Ec + piGEM2115_PccMe_pYTK 1
    2. Ec + piGEM2115_PccMe_pYTK 2
    3. Ec + piGEM2115_PccMe_pYTK 3
    4. Ec + piGEM2115_PccMe_pYTK 4
    5. Ec + piGEM2115_PccMe_pYTK 5
    6. Ec + piGEM2116_AccSe_pYTK 1
    7. Ec + piGEM2116_AccSe_pYTK 2
    8. Ec + piGEM2116_AccSe_pYTK 3
    9. Ec + piGEM2116_AccSe_pYTK 4
    10. Ec + piGEM2116_AccSe_pYTK 5
2. add 5ml LBv2 into the tubes and 5µl Cm [34 mg/ml] (fresh prepared)
3. Inoculate from gibson plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

Ec + piGEM2115_PccMe_pYTK 1	147 ng/µl
Ec + piGEM2115_PccMe_pYTK 2	97 ng/µl
Ec + piGEM2115_PccMe_pYTK 3	91 ng/µl
Ec + piGEM2115_PccMe_pYTK 4	16 ng/µl
Ec + piGEM2115_PccMe_pYTK 5	22 ng/µl
Ec + piGEM2116_AccSe_pYTK 1	0 ng/µl
Ec + piGEM2116_AccSe_pYTK 2	82 ng/µl
Ec + piGEM2116_AccSe_pYTK 3	80 ng/µl
Ec + piGEM2116_AccSe_pYTK 4	78 ng/µl
Ec + piGEM2116_AccSe_pYTK 5	80 ng/µl

Author: Daniel Marchal

created: 19.07.2018 12:09
updated: 20.07.2018 08:46

Entry 93/214: Restriction digest of piGEM2115 & piGEM2116 (2)
In Project: ERBsen
With tags: restriction, digest, EcoRI, NcoI, piGEM2115, piGEM2116

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 60min at 37°C
4. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

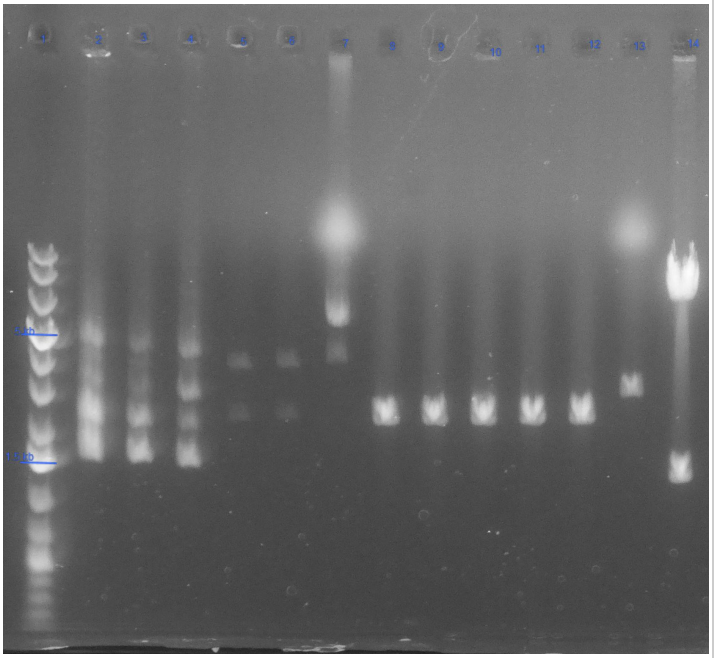
Sample	**Master mix (7x)**
4µl DNA	-
0.2µl FD-EcoRI/FD-NcoI	1,4µl FD-EcoRI/FD-NcoI
1µl FD-Buffer prestained	7µl FD-Buffer prestained
4.8µl H_2O	33,6µl H_2O

Lane	Sample	Enzyme	Expected fragments
1	1kb plus DNA ladder	-	-
2	piGEM2115_PccMe_pYTK 1	EcoRI	2168 + 3564 bp
3	piGEM2115_PccMe_pYTK 2	EcoRI	2168 + 3564 bp
4	piGEM2115_PccMe_pYTK 3	EcoRI	2168 + 3564 bp
5	piGEM2115_PccMe_pYTK 4	EcoRI	2168 + 3564 bp
6	piGEM2115_PccMe_pYTK 5	EcoRI	2168 + 3564 bp
7	pYTK	EcoRI	2676 (circular)
8	piGEM2116_AccSe_pYTK 1	NcoI	1172 + 2319 + 2410 bp
9	piGEM2116_AccSe_pYTK 2	NcoI	1172 + 2319 + 2410 bp
10	piGEM2116_AccSe_pYTK 3	NcoI	1172 + 2319 + 2410 bp
11	piGEM2116_AccSe_pYTK 4	NcoI	1172 + 2319 + 2410 bp
12	piGEM2116_AccSe_pYTK 5	NcoI	1172 + 2319 + 2410 bp
13	pYTK	NcoI	2676 (linearized)
14	JZ147	NcoI	1171 + 1172 + 6525 bp

Results

- JZ105 (PccMe) can't be in the samples because it has the wrong resistance cassette
- Lane 2, 3 & 4 don't show the expected fragment lengths. They have several bands, looking like a religated pYTK-fragment which has several coiled-states. Tomorrow we will look into the computer and see if the lengths fit to a religation
- Lane 5 & 6 could have the expected lengths, but the bands seem to be a little bit higher. Nevertheless we will make a sequencing of one of them tomorrow.
- Lane 7, 13 & 14 are controls
- Lane 8 - 12 have just one band at 2 - 2.5kb or two bands which are close to each other. Probably it is the linearized pYTK backbone with a size of 1959bp. It seems that the content of pYTK backbone in the reaction mix is too high or the ligase activity is too high. Maybe one should start the reaction without the ligase and after a certain time (when all fragments annealed to each other) the ligase is added.

0719_Restriction_digest.jpg



Author: Daniel Marchal
created: 20.07.2018 09:16
updated: 20.07.2018 14:33
Entry 94/214: Predigestion of piGEM1057 for Lvl 1 golden gate
In Project: ERBsen
With tags: piGEM1057, BsaI, digestion

Since all approaches of lvl 1 cloning led to a lawn of piGEm1057 bearing colonies, a predigestion is needed where the resistance part is digested with BsaI and then gel eluted.

Procedure:

1. Mix 60.5µl piGEM1057 with 2.5µl BsaI and 7µl Cutsmart
2. Incubate 3h at 37°C
3. Add 15µl 6xLoadingDye
4. Make a gel extraction (1187bp) and finally a nanodrop

Result:

c(piGEM1057_kan part) = 33 ng/µl

Author: Daniel Marchal
created: 20.07.2018 09:58
updated: 23.07.2018 12:22
Entry 95/214: Enrichment and isolation of putative piGEM2117_AccEc_pYTK
In Project: ERBsen
With tags: Enrichment, miniprep, piGEM2117, AccEc

To check if the colonies from the gibson plate bear the correct plasmid, 8 colonies have to be enriched and their plasmids isolated and digested.

Procedure

1. prepare 8 tubes with following annotations:
    1. Ec + piGEM2117_AccEc_pYTK 1
    2. Ec + piGEM2117_AccEc_pYTK 2
    3. Ec + piGEM2117_AccEc_pYTK 3
    4. Ec + piGEM2117_AccEc_pYTK 4
    5. Ec + piGEM2117_AccEc_pYTK 5
    6. Ec + piGEM2117_AccEc_pYTK 6
    7. Ec + piGEM2117_AccEc_pYTK 7
    8. Ec + piGEM2117_AccEc_pYTK 8
2. add 5ml LB into the tubes and 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from gibson plate
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2117_AccEc_pYTK 1	69 ng/µl
piGEM2117_AccEc_pYTK 2	209 ng/µl
piGEM2117_AccEc_pYTK 3	67 ng/µl
piGEM2117_AccEc_pYTK 4	45 ng/µl
piGEM2117_AccEc_pYTK 5	94 ng/µl
piGEM2117_AccEc_pYTK 6	154 ng/µl
piGEM2117_AccEc_pYTK 7	136 ng/µl
piGEM2117_AccEc_pYTK 8	130 ng/µl

Author: Daniel Marchal
created: 20.07.2018 10:23
updated: 23.07.2018 12:23
Entry 96/214: Retrafo of put. pIGEM2115_PccMe_pYTK 5 for sequencing
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, piGEM2115, PccMe

In the last restriction digest it was shown, that sample 5 of the putative piGEM2115 plasmid could be a correct ones because of the fragment lengths in the gel. Unfortunately the concentration is too low for a sequencing (22ng/µl) so a retrafo has to be made.

Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 5µl of plasmid "putative piGEM2115_PccMe_pYTK 5" (see Restriction digest of piGEM2115 & piGEM2116 (2) - entry #93 in project 'ERBsen' (Daniel Marchal, 20.07.2018))
3. incubate 10 min on ice
4. heat shock at 42°C for 45 sec
5. incubate 5 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates
9. incubate oN at 37°C

Result:

c(piGEM2115_PccMe_pYTK 5) = 84 ng/µl

Author: Daniel Marchal
Entry 97/214: Gibson assembly of piGEM2114_AccSc_pYTK & piGEM2116_AccSe_pYTK
In Project: ERBsen
With tags: piGEM2114, piGEM2116, gibson cloning

created: 23.07.2018 14:13
updated: 25.07.2018 16:28

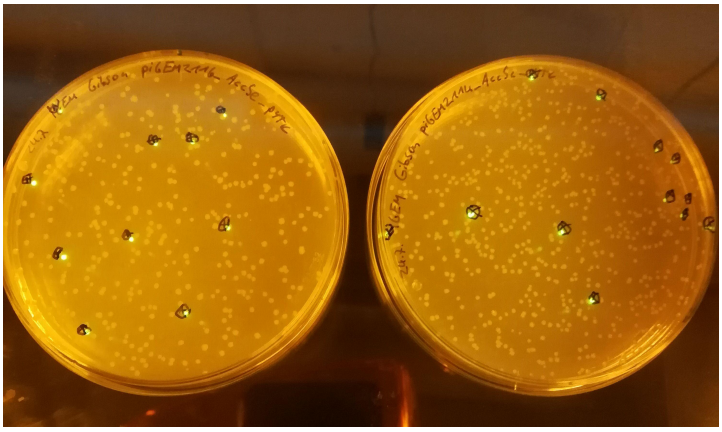
Procedure:

1. Set up the following reaction on ice (0.2pmol DNA, Insert:Backbone = 5:1)
2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.
3. Transform 10µl into Ec, store the rest at -20°C
    1. Ec trafo: 10µl sample / 2h regeneration / plating out on LB+Cm

Fragment	bp	ng/µl	pmol/µl	µl for 0,2 pmol
Backbone	1959	47	0,072703	2,75093617
AccSe	4006	122	0,092286	2,167180328
AccSc	3511	182	0,157082	1,27321978

	AccSc	AccSe
Fragment [µl]	1,27321978	2,167180328
Backbone [µl]	0,5	0,5
Gibson Mastermix 2x [µl]	10	10
H_2O [µl]	8,22678022	7,332819672
Total volume [µl]	20	20

Result.jpg



Author: Daniel Marchal
created: 23.07.2018 14:13
updated: 23.07.2018 16:22

Entry 98/214: Restriction digest of piGEM2117
In Project: ERBsen
With tags: StyI, restriction, digest, HindIII, EcoRV, piGEM2117

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate 60min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1% gel with 1 droplets of EtBr; GeneRuler 1kb plus; 135V, 20min)

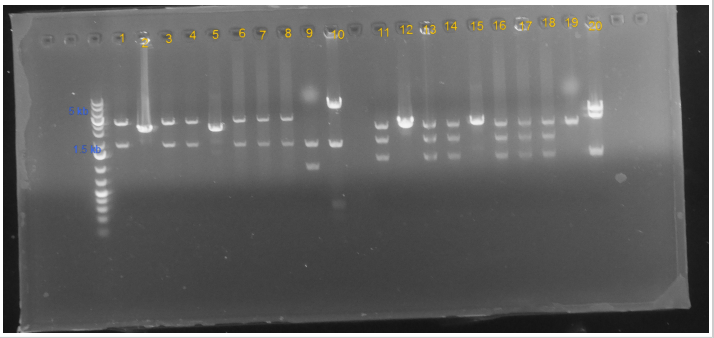
Sample	**Master mix (11x)**	**Master mix (11x)**
4µl DNA	-	-
0.5µl of each enzyme	5.5µl HF-StyI	5.5µl HF-HindIII + 5.5µl HF-EcoRV
1µl CutSmart Buffer	11µl CutSmart Buffer	11µl CutSmart Buffer
ad 10µl H_2O	49.5µl H_2O	44µl H_2O

Number	Plasmid	Enzyme	Expected fragments
1	piGEM2117_AccEc_pYTK 1	StyI	1555, 1773, 3805 bp
2	piGEM2117_AccEc_pYTK 2	StyI	1555, 1773, 3805 bp
3	piGEM2117_AccEc_pYTK 3	StyI	1555, 1773, 3805 bp
4	piGEM2117_AccEc_pYTK 4	StyI	1555, 1773, 3805 bp
5	piGEM2117_AccEc_pYTK 5	StyI	1555, 1773, 3805 bp
6	piGEM2117_AccEc_pYTK 6	StyI	1555, 1773, 3805 bp
7	piGEM2117_AccEc_pYTK 7	StyI	1555, 1773, 3805 bp
8	piGEM2117_AccEc_pYTK 8	StyI	1555, 1773, 3805 bp
9	pYTK	StyI	943, 1733 bp
10	JZ154	StyI	45, 207, 316, 327, 1697, 3717, 3805 bp
11	piGEM2117_AccEc_pYTK 1	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
12	piGEM2117_AccEc_pYTK 2	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
13	piGEM2117_AccEc_pYTK 3	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
14	piGEM2117_AccEc_pYTK 4	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
15	piGEM2117_AccEc_pYTK 5	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
16	piGEM2117_AccEc_pYTK 6	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
17	piGEM2117_AccEc_pYTK 7	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
18	piGEM2117_AccEc_pYTK 8	HindIII + EcoRV	1159, 1462, 1851, 2661 bp
19	pYTK	HindIII + EcoRV	2676 bp (linearized)
20	JZ154	HindIII + EcoRV	1159, 2661, 2957, 3337 bp

Results

- the controls with pYTK and JZ154 look like expected
- Sample piGEM2117_AccEc_pYTK 2 and piGEM2117_AccEc_pYTK 5 are wrong! In the Hind/EcoRV digest it looks like pYTK
- all other samples show a good fragment pattern, we will sequence one of them

0723_Restriction_digest.jpg



Author: Daniel Marchal
created: 23.07.2018 16:44
updated: 24.07.2018 16:33
Entry 99/214: Retrafo of JZ90 into Ec
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90

Since the previously used JZ90 had the wrong sequence, another stock plasmid was sequenced and the sequence was correct. Therefore this plasmid shall be retransformed into Ec to get an own plasmid stock.

Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 0.5µl of plasmid (JZ90)
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB + Amp
9. incubate oN at 37°C

Author: Daniel Marchal
Entry 100/214: PCR amplification of JZ90 & JZ147 for Gibson assembly
In Project: ERBsen
With tags: PCR, JZ90, JZ147

created: 23.07.2018 18:03
updated: 24.07.2018 16:32

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
2	JZ90	piGEM2102	piGEM2103
4	JZ147	piGEM2106	piGEM2107

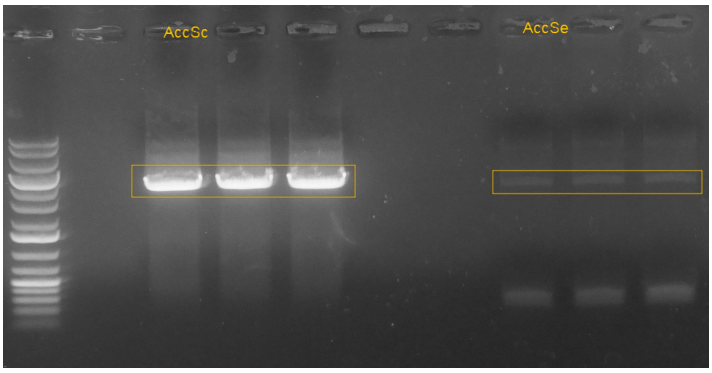
Sample
10 µl buffer
1 µl dNTPs
2,5 µl primer for
2,5 µl primer rev
0.25 µl DNA
1,5 µl DMSO
0,5 µl Q5 polymerase
31.75 µl H_2O (ad 50 µl)

Results:

expected lengths:

2	3511 bp
4	4006 bp

0724_PCR_for_Gibson_and_Gel_extraction.jpg



- AccSc shows a bright band, but is too big. We will however try the gibson reaction.
- AccSe has correct bands but they are really light, so we will waste the samples and use the purified ones from the last time

Author: Daniel Marchal
Entry 101/214: Gel extraction of AccSc for Gibson assembly
In Project: ERBsen
With tags: PYTK, JZ90, JZ147, Gel extraction, AccSe, AccSc

created: 24.07.2018 12:30
updated: 24.07.2018 16:11

For gibson assembly, a gel extraction must be made for AccSc

MN_NucleoSpin_Gel_and_PCR_Clean-Up.pdf

Procedure:

1. Load the whole sample on a 0.8% agarose gel and run at 130V for 20min
2. Cut the right bands out and follow the protocoll of "MN NucleoSpin Gel and PCR Clean-Up" (page 19-20 in the file)
    1. we used 600µl NTI buffer
    2. we eluted in 20µl H_2O
3. Determine concentrations using Nanodrop

Result:

c(AccSc)=182ng/µl

Author: Daniel Marchal
created: 25.07.2018 15:22
updated: 26.07.2018 14:30

Entry 102/214: List of sequencing primers for piGEM2114-piGEM2117 (Gibson plasmids)
In Project: ERBsen
With tags: list, sequencing, piGEM2114, piGEM2115, piGEM2116, piGEM2117

Plasmid	**Primer for**	**Primer rev**
piGEM2114_AccSc_pYTK	oiGEM1031 CT-Sc_for01 scar_seq1 (alternative but with a little gap: BC-Sc_for01)	CT-Sc_rev01 oiGEM1018
piGEM2115_PccMe_pYTK	oiGEM1031 PCC_seq2 II PCC_seq3 II PCC_seq4 PCC_seq5 PCC_seq6 PCC_seq7	oiGEM1018
piGEM2116_AccSe_pYTK	oiGEM1031 lavS_mut_HindIII (alternative but with little gap: LavS_seq_for)	pNS3_MCS_rev (alternative: ptrc_gib_rev) oiGEM1018
piGEM2117_AccEc_pYTK	oiGEM1031 bcarb_ec_seq1 bcarb_Ec_seq2 accA_Ec_seq1 pNS3_seq_for	pNS3_MCS_rev (alternative: ptrc_gib_rev) oiGEM1018

Author: Daniel Marchal

created: 25.07.2018 16:00
updated: 26.07.2018 16:50

Entry 103/214: Colony PCR of gibson plates with putative piGEM2114 & piGEM2116

In Project: ERBsen

With tags: PCR, colony PCR, cPCR, piGEM2114, piGEM2116

To check, if the gibson assembly of piGEM2114_AccSc_pYTK and piGEM2116_AccSe_pYTK was sufficient we could again enrich some colonies, make a miniprep and finally a restriction digest. To analyse the cells more rapid, we will do a colony PCR to check, if the correct fragment length is reached.

Procedure:

1. Prepare mastermix
2. Aliqupt 25µl into 16 Eppis, labeled from 1-8 and A-H
3. pick half of a colony and inocculateit into one aliquot, streak the other half of the colony on LB+Cm out
4. Start PCR program with initial 10min at 98°C
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
1	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
2	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
3	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
4	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
5	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
6	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
7	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
8	piGEM2114_ AccSc_pYTK	oiGEM1031	oiGEM1018
A	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
B	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
C	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
D	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
E	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
F	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
G	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018
H	piGEM2116_ AccSe_pYTK	oiGEM1031	oiGEM1018

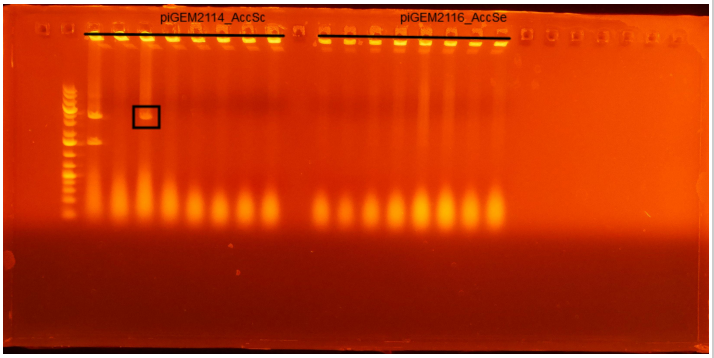
Mastermix (16x)	Sample
8µl Primer for	0.5µl Primer for
8µl Primer rev	0.5µl Primer rev
100µl TaqMastermix2x	12.5µl TaqMastermix2x
184µl H_2O	ad 25µl H_2O

Results:

expected lengths:

piGEM2114_AccSc_pYTK	3781 bp
piGEM2114_AccSc_pYTK	4276 bp
pYTK	1051 bp
JZ90	-
JZ147	-

0726_cPCR.jpg



- Sample 3 of piGEM2114 looks good and has the expected fragment length
- piGEM2116 has unfortunatelly no bands

Author: Daniel Marchal
created: 25.07.2018 16:19
updated: 25.07.2018 16:21

Entry 104/214: Retrafo of JZ90 and piGEM1085
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, piGEM1085

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2.

Vector	Insert	Resistance	Box in -80°C freezer
piGEM1085_4xHis	4x-His-Part	Cm	-
JZ90	Acc from S. coelicolor	Amp	box 16, locus 66

3. add 0.2µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal
created: 25.07.2018 16:30
updated: 25.07.2018 16:32
Entry 105/214: Sequencing of piGEM2115 & piGEM2117
In Project: ERBsen
With tags: piGEM2115, piGEM2117

Samples:

Vektor	Eppi	Primer
piGEM2115_PccMe_pYTK	1	oiGEM1031
piGEM2117_AccEc_pYTK	2	oiGEM1031

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order 11104443046):

- piGEM2105 is correct
- piGEM2106_for was dirty and will be resequenced from the company, reverse looks fine
- piGEM2107 is correct
- piGEM2108 couldn't be analyzed because I have no plasmid map. But it will be done tomorrow

Author: Daniel Marchal

created: 26.07.2018 10:44
updated: 26.07.2018 12:41

Entry 106/214: Retrafo of put. piGEM2115_PccMe & put. piGEM2117_AccEc for sequencing

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, pEntry, piGEM2115, piGEM2117, JZ147

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2. add 0.5µl of plasmid
3. incubate 5 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates
9. incubate oN at 37°C

Author: Daniel Marchal

created: 27.07.2018 10:27
updated: 28.07.2018 15:43

Entry 107/214: Enrichment and isolation of putative piGEM2114, piGEM2115, piGEM2117 & AccBirASe

In Project: ERBsen

With tags: piGEM2114, piGEM2115, piGEM2117, AccBirASe

Procedure

1. prepare 11 tubes with following annotations:
    1. Ec + piGEM2114_AccSc_pYTK
    2. Ec + piGEM2114_AccSc_pYTK
    3. Ec + piGEM2114_AccSc_pYTK
    4. Ec + piGEM2115_PccMe_pYTK
    5. Ec + piGEM2115_PccMe_pYTK
    6. Ec + piGEM2115_PccMe_pYTK
    7. Ec + piGEM2117_AccEc_pYTK
    8. Ec + piGEM2117_AccEc_pYTK
    9. Ec + piGEM2117_AccEc_pYTK
    10. Ec + AccBirASe
    11. Ec + piGEM1085
2. add 5ml LB into the tubes and 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate with plasmid DNA
4. Incubate over nightat 37°C shaking
5. Make miniprep and pool the identical samples

Result

piGEM2114_AccSc_pYTK	460 ng/µl
piGEM2115_PccMe_pYTK	49 ng/µl
piGEM2117_AccEc_pYTK	116 ng/µl
AccBirASe	381 ng/µl
piGEM1085	53 ng/µl

Author: Daniel Marchal
Entry 108/214: Sequencing of piGEM2114-AccSc_pYTK
In Project: ERBsen
With tags: piGEM2105, piGEM2114

created: 27.07.2018 11:26
updated: 30.07.2018 09:59

Samples:

Vektor	Eppi	Label	Primer
piGEM2114_AccSc_pYTK	14_1_oiGEM1031	AIM0030104	oiGEM1031
piGEM2114_AccSc_pYTK	14_2_CT_Sc_for01	AIM0030105	CT_Sc_for01
piGEM2114_AccSc_pYTK	14_3_CT_Sc_rev01	AIM0030106	CT_Sc_rev01
piGEM2114_AccSc_pYTK	14_4_scar_seq1	AIM0030107	scar_seq1
piGEM2114_AccSc_pYTK	14_5_oiGEM1018	AIM0030108	oiGEM1018

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order 11104443046):

- there is a 8bp insertion in the insert, leading to a frameshift so the plasmid is waste. We must repeat the PCR and gibson reaction.

Author: Daniel Marchal
created: 28.07.2018 14:41
updated: 01.08.2018 15:16
Entry 109/214: Trafo of piGEM2114, piGEM2115, piGEM2117, AccBirASe into Vn
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock, piGEM2114, piGEM2115, piGEM2117, AccBirASe

Procedure

1. thaw aliquots of electrocompetent Vn on ice
2. add plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 950 V, 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 30-37°C while shaking
7. Plate out on selection plates (LB2.5 + 2µg/ml Cm)
8. Incubate oN at 37°C

A colony PCR revealed, that there weren't any plasmids in the cells. Therefore the trafo will be repeated with controls

Author: Daniel Marchal
Entry 110/214: Lvl 1 GoldenGate of piGEM2111_LVL1_HisAccBC (2)
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation, piGEM2109

created: 28.07.2018 15:23
updated: 30.07.2018 10:00

Golden Gate Reaction:

add following reagents to your annealing mix:

Vector:	piGEM2111_LVL1_HisAccBC		
Part/Reagent	Plasmid	Conc. [ng/µl]	Volume for 75ng
1	piGEM1075	75	1,00
2	piGEM1007	49	1,53
3	piGEM1008	60	1,25
4x	piGEM1085	53	1,42
4y	piGEM2105	61	1,23
5	piGEM1035	93	0,81
6	piGEM1070	50	1,50
7	piGEM1036	81	0,93
8	piGEM1057	39	0,64
BsaI			1,00
T7 Ligase			1,00
T4 Ligase Buffer			1,00
H2O			0,00

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (50 cycles)
Ligation	16°C	5 min (50 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

After the Golden Gate Assembly 5µl of the mixture were transformed into E. coli and plated out on LB + Kan.

Results:

there are 6 colonies on the plate, none of them shining green. We will pick each colonie, isolate their plasmids and do a restriction digest to confirm the correctness of the plasmid.

Author: Daniel Marchal
Entry 111/214: Sequencing of piGEM2115_PccMe & piGEM2117_AccEc
In Project: ERBsen
With tags: sequencing, piGEM2115, piGEM2117

created: 30.07.2018 10:56
updated: 31.07.2018 12:17

Samples:

Vektor	Eppi	Label	Primer
piGEM2115_PccMe_pYTK	15_1	AIM0030166	piGEM1031
piGEM2115_PccMe_pYTK	15_2	AIM0030167	PCC_seq2_II
piGEM2115_PccMe_pYTK	15_3	AIM0030168	PCC_seq3_II
piGEM2115_PccMe_pYTK	15_4	AIM0030169	PCC_seq4
piGEM2117_AccEc_pYTK	17_1	AIM0030170	piGEM1031
piGEM2117_AccEc_pYTK	17_2	AIM0030171	bcarb_ec_seq1
piGEM2117_AccEc_pYTK	17_3	AIM0030172	bcarb_ec_seq2
piGEM2117_AccEc_pYTK	17_4	AIM0030173	accA_ec_seq1
piGEM2117_AccEc_pYTK	17_5	AIM0030174	pNS3_seq_for
piGEM2117_AccEc_pYTK	17_6	AIM0030175	pNS3_MCS_mut_rev
piGEM2117_AccEc_pYTK	17_7	AIM0030176	piGEM1018

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order 11104549388):

- In this sequencing just the first half of the piGEM2115 vector was sequenced because we hadn't enough plasmid. But the sequenced part looks perfect!
- piGEM2117 is also correct

Author: Daniel Marchal

created: 30.07.2018 11:02
updated: 01.08.2018 15:15

Entry 112/214: Enrichment and isolation of put. piGEM2111 from GoGate Lvl1
In Project: ERBsen
With tags: miniprep, piGEM2111

The golden gate reaction of piGEM2111 showed 6 colonies, which shall be enriched and miniprepped to make a restriction digest.

Procedure

1. prepare 6 tubes with following annotations:
    1. Ec + piGEM2111_1
    2. Ec + piGEM2111_2
    3. Ec + piGEM2111_3
    4. Ec + piGEM2111_4
    5. Ec + piGEM2111_5
    6. Ec + piGEM2111_6
2. add 5ml LB into the tubes and 5µl Kan [50mg/ml] (fresh prepared)
3. Inoculate from GoldenGate Trafo plate
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

- Miniprep was sufficient, now a restriction digest can be made

Author: Daniel Marchal

created: 30.07.2018 12:53
updated: 31.07.2018 12:17

Entry 113/214: Preparation of low-OD cell extract from V. natriegens with piGEM2115 /piGEM2117/AccBirASe for activity assays

In Project: ERBsen

With tags: Acc, piGEM2117, piGEM2115, assay, cell extract, AccBirASe

Procedure:

1. Prepare MOPS Buffer
2. Prepare 3 tubes with 5ml LB2,5 and inoculate from Vn+piGEM2115/2117/AccBirASe
3. Incubate over night at 37°C shaking
4. Prepare 500ml flasks with 250ml Suc-MM and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture and incubate at 37°C shaking
6. Stop incubating when OD_{600}=2-3
7. Harvest the cells in 500ml centrifugation bottles (each bottle with 250ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
8. Let the cells splitted in two fractions (one for low salt conditions and one for high salt conditions, labels as LS & HS)
9. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 15ml Falcon.
10. Add 400µl 10xProtease-Inhibitor-Cocktail (provided)
11. Fill the tubes up to 4ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS Low salt

50mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor_Cocktail

1 droplet solved in 10ml H_2O

Suc-MM

150ml 5xMM

75ml 10xV2-salts

2ml 2M Sucrose (→2% Sucrose)

524ml H_2O

12. Use the frenchpress to break the cells at 900 psi
    1. lever on "down" and wheel on high pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
13. If the solution is clear, the cell breakage was successful
14. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
15. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
16. For storage of cell extract add 300µl glycerole and freeze at -20°C

Results:

- Cells harvested at OD_{600}=1.3
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used, for ultracentrifugation the Thermo Fisher T-1270 Rotor was used
- Cell weights: PccMe: 1.02g, AccBirAEc: 1.19g, AccBirASe: 1.22g

Author: Daniel Marchal

created: 31.07.2018 12:17
updated: 01.08.2018 08:58

Entry 114/214: Preparation of high-OD cell extract from V. natriegens with piGEM2115 /piGEM2117/AccBirASe and enzyme activity assay

In Project: ERBsen

With tags: Acc, enzyme activity, activity, assay, cell extract

To validate enzyme activity of acc variants we will perform an activity assay with cell extract by adding Mcr, NADPH and Acetyl-CoA and measuring the NADPH/NADP+ conversion. The procedure is adapted from Pascal.

Procedure:

1. Prepare MOPS Buffer (Low salt as standard buffer and high salt because of Vibrios higher salt preference)
2. Use the 500ml flasks from the previous cell cultivation and add 250ml Suc-MM
3. Induce the AccBirASe cells with 1µM IPTG and incubate over night at 37°C shaking
4. Harvest the cells in 500ml centrifugation bottles (each bottle with 250ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
5. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 15ml Falcon.
6. Add 400µl 10xProtease-Inhibitor-Cocktail (provided)
7. Fill the tubes up to 4ml with buffer (rule of thumb: per gramm cells add 3ml buffer)
8. Use the frenchpress to break the cells at 900 psi
    1. lever on "down" and wheel on high pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw

Reagents:

MOPS (fresh prepared)

50mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor_Cocktail

1 droplet solved in 10ml H_2O

Suc-MM

150ml 5xMM

75ml 10xV2-salts

2ml 2M Sucrose (→2% Sucrose)

524ml H_2O

9. If the solution is clear, the cell breakage was successful
10. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
11. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
12. For the enzyme assay use the software "Cary UV" with the program "kinetics"
13. Mix 40-229µl of your cell lysate together with MOPS buffer, $MgCl_2$, NADPH, ATP, $KHCO_3$ (fresh prepared) and MCR_Ca and measure slope (background)
14. Add Acetyl-CoA to start the reaction and again measure slope to calculate specific activity (see excel sheet)
15. If there is enzyme activity you can make a bradford to normalize your results
16. As a positive control you can add Pcc_Me
17. To store the cell lysate add 300µl glycerol and store at -20°C

2018_07_31_PccMe_AccEc_AccSe.xlsx

Results:

- Cells harvested at OD_{600}=2.48
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used and for ultracentrifugation Thermo Fisher T-1270
- Cell weights: ~2g
- There was no significant activity detectable. Unfortunately the colony PCR of Vn with plasmids revealed, that there are no plasmids, so we just measured the WT strain. Nevertheless we can assume that there is no difference between complex medium and minimal medium in regard to native acc activity. In both cases there was no significant activity.

Author: Daniel Marchal
created: 01.08.2018 15:16
updated: 08.08.2018 13:29

Entry 115/214: Restriction digest of mcr1_pjet, mcr2_pjet & piGEM2111
In Project: ERBsen
With tags: piGEM2111, restriction, digest

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate 90min at 37°C
4. If required mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 20min)

Sample	**Master mix (6.5x)**	**Master mix (10.5x)**
4µl DNA	-	-
0.2µl per enzyme	1.3µl HindIII-FD	2.1µl EcoRV-HF + 2.1µl XbaI
1µl 10xBuffer	6.5µl FD-buffer prestained	10.5µl CutSmart Buffer
ad 10µl H_2O	31.2µl H_2O	31.2µl H_2O

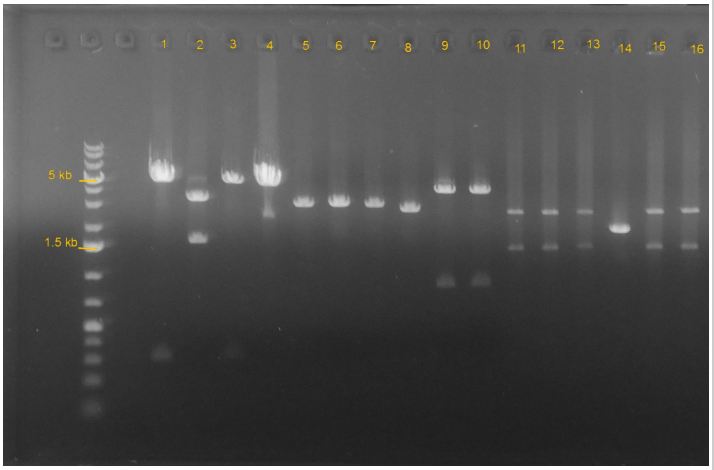
Number	Plasmid	Expected fragment length
1	mcr1_pjet 1	1579 bp, 3260 bp
2	mcr1_pjet 2	1579 bp, 3260 bp
3	mcr1_pjet 3	1579 bp, 3260 bp
4	mcr1_pjet 4	1579 bp, 3260 bp
5	mcr1_pjet 5	1579 bp, 3260 bp
6	mcr2_pjet 1	1011 bp, 3793 bp
7	mcr2_pjet 2	1011 bp, 3793 bp
8	mcr2_pjet 3	1011 bp, 3793 bp
9	mcr2_pjet 4	1011 bp, 3793 bp
10	mcr2_pjet 5	1011 bp, 3793 bp
11	piGEM2111 1	1345bp, 2590 bp
12	piGEM2111 2	1345bp, 2590 bp
13	piGEM2111 3	1345bp, 2590 bp
14	piGEM2111 4	1345bp, 2590 bp
15	piGEM2111 5	1345bp, 2590 bp
16	piGEM2111 6	1345bp, 2590 bp
not tested	pjet	2968 bp

Results

- Sample 2 looks good, sample 1, 3, 4, 5 are wrong → sequencing of sample 2
- Sample 9 and 10 look good, sample 6, 7, 8 are wrong → sequencing of sample 9
- Sample 11, 12, 13, 15 and 16 look good, sample 14 is wrong → sequencing of sample 11
- The good looking samples were nanodroped:

mcr1_pjet 2	169
mcr2_pjet 4	151
mcr2_pjet 5	121
piGEM2111 1	106
piGEM2111 2	104
piGEM2111 3	114
piGEM2111 5	139
piGEM2111 6	124

0801_Restriction_digest.jpg



Author: Daniel Marchal

created: 01.08.2018 15:27
updated: 02.08.2018 12:33

Entry 116/214: Retrafo of piGEM2115_PccMe, piGEM2117_AccEc and AccBirASe into Vn

In Project: ERBsen

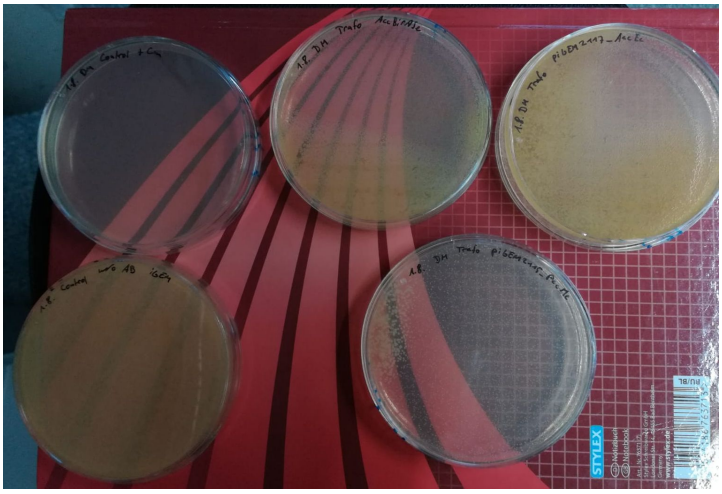
With tags: transformation, e.coli, retrafo, retransformation, V. natriegens, weinstock, electroporation, AccBirASe, AccEc, piGEM2117, piGEM2115, PccMe

Procedure

1. thaw 6 aliquots of electrocompetent Vn on ice
2. add ~50ng plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 910 V, 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates (LB2.5 + 2µg/ml Cm)
8. Incubate oN at 37°C

1	piGEM2115_PccMe	LB + Cm [2µg/ml]
2	piGEM2117_AccEc	LB + Cm [2µg/ml]
3	AccBirASe	LB + Cm [2µg/ml]
4	-	LB + Cm [2µg/ml]
5	-	LB

Trafo.jpg



Outlook:

There are too many colonies on the plate so we will first isolate single colonies and then do a colony PCR to ensure correct plasmid possession

Author: Daniel Marchal

created: 02.08.2018 10:02
updated: 02.08.2018 12:31

Entry 117/214: Enrichment and isolation of piGEM2115_PccMe, piGEM2117_AccEc and AccBirASe

In Project: ERBsen

With tags: Enrichment, miniprep, piGEM2117, piGEM2115, AccBirASe

Procedure

1. prepare 6 tubes with following annotations:
    1. Ec + piGEM2115_PccMe
    2. Ec + piGEM2115_PccMe
    3. Ec + piGEM2117_AccEc
    4. Ec + piGEM2117_AccEc
    5. Ec + AccBirASe
    6. Ec + AccBirASe
2. add 5ml LB into the tubes and 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from cryostock
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2115_PccMe	62 ng/µl
piGEM2117_AccEc	45 ng/µl
AccBirASe	37 ng/µl

Author: Daniel Marchal
created: 02.08.2018 10:19
updated: 07.08.2018 14:16

Entry 118/214: Sequencing of piGEM2115(2), piGEM2111, mcr1pjet, mcr2pjet
In Project: ERBsen
With tags: piGEM2105, piGEM2106, piGEM2107, piGEM2108

Samples:

Vektor	Eppi	Label	Primer
piGEM2115_PccMe_pYTK	15_5	AIM0030177	Pcc_seq5
piGEM2115_PccMe_pYTK	15_6	AIM0030178	Pcc_seq6
piGEM2115_PccMe_pYTK	15_7	AIM0030179	Pcc_seq7
piGEM2115_PccMe_pYTK	15_8	AIM0030180	Pcc_seq8
piGEM2111_LVL1_AccBC	2111	AIM0030181	oiGEM2501
mcr1pjet	mcr1_for	AIM0030182	pJET_seq_for
mcr1pjet	mcr1_rev	AIM0030183	pJET_seq_rev
mcr2pjet	mcr1_for	AIM0030184	pJET_seq_for
mcr2pjet	mcr2_rev	AIM0030185	pJET_seq_rev

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Comments:

from piGEM2111 we used sample 1, from mcr1pjet we used sample 1b, from mcr2pjet we used sample 2d

Results (Order 11104556949):

- The sequencing of piGEM2111 failed so it will be repeated
- piGEM2115 has the correct sequence with 1 exception, a GAG → AAG conversion (Glu→Lys) which could be horrible for the enzyme so we have to repeat it
- The sequence of mcr1pjet looks fine
- mcr2pjet seems to have a point mutation CCT→CCC (Pro→Pro) but it doesn't matter, the rest is correct

Author: Daniel Marchal

created: 03.08.2018 10:09
updated: 03.08.2018 15:58

Entry 119/214: Colony PCR of Vn trafos with piGEM2115, piGEM2117, AccBirASe
In Project: ERBsen
With tags: PCR, cPCR, piGEM2115, piGEM2117, AccBirASe

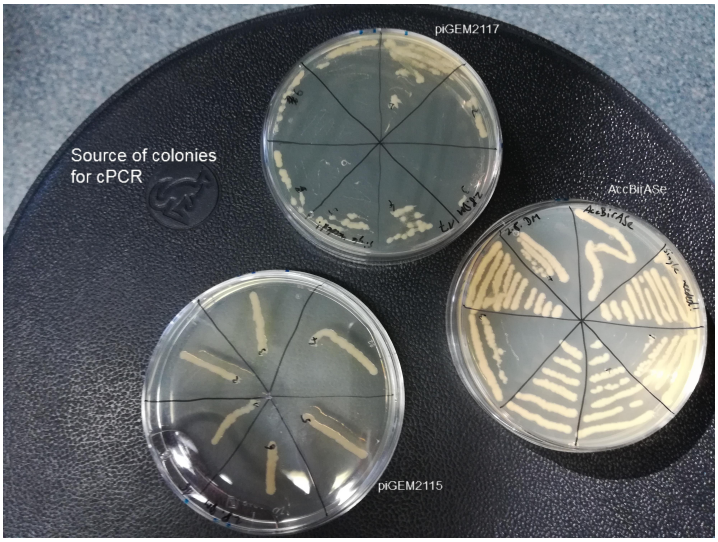
Procedure:

1. Prepare mastermix
2. Aliqupt 25µl into pcr tubes
3. Pick colonies and inocculate them into the tubes
4. Start PCR program with initial 10min at 98°C
5. Run a gel

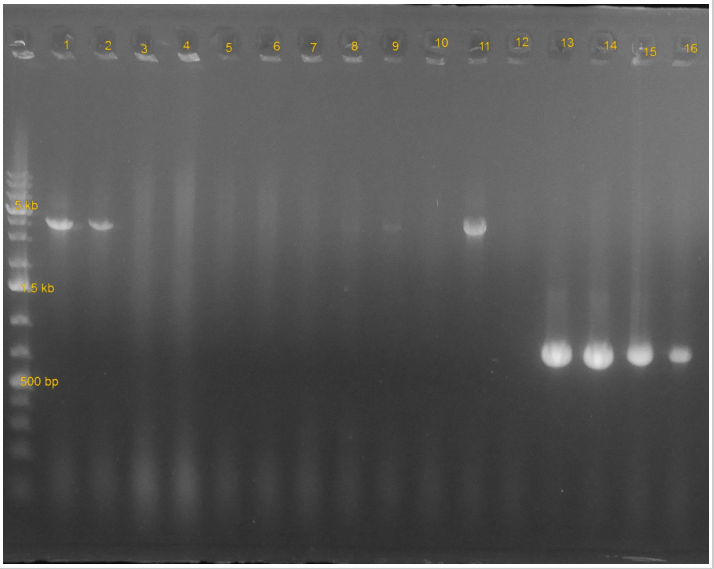
Sample	Mastermix (4.5x [AccBirASe])	Mastermix (12.5x [piGEM2115 /piGEM2117])
12.5µl 2x-Mastermix	56.25µl 2x-Mastermix	156.25µl 2x-Mastermix
0.5µl Primer_for	2.25µl birA_Se_mut_for	18.75µl oiGEM1031
0.5µl Primer_rev	2.25µl birA_Se_rev	18.75µl oiGEM1018
11.5µl H_2O	51.75µl H_2O	143.75 H_2O

Sample	DNA template	Expected fragment length	Result
1	piGEM2115 1	4107 bp	Correct
2	piGEM2115 2	4107 bp	Correct
3	piGEM2115 3	4107 bp	No band detectable
4	piGEM2115 4	4107 bp	No band detectable
5	piGEM2115 5	4107 bp	No band detectable
6	piGEM2115 6	4107 bp	No band detectable
7	piGEM2117 1	5508 bp	No band detectable
8	piGEM2117 2	5508 bp	No band detectable
9	piGEM2117 3	5508 bp	No band detectable
10	piGEM2117 4	5508 bp	No band detectable
11	piGEM2117 5	5508 bp	Correct
12	piGEM2117 6	5508 bp	No band detectable
13	AccBirASe 1	866 bp	Correct
14	AccBirASe 2	866 bp	Correct
15	AccBirASe 3	866 bp	Correct
16	AccBirASe 4	866 bp	Correct

0803_cPCR.jpg



0803_cPCR.jpg



Author: Daniel Marchal

created: 06.08.2018 13:00
updated: 07.08.2018 14:16

Entry 120/214: Retrafo of piGEM2111, mcr1pjet, mcr2pjet into Ec

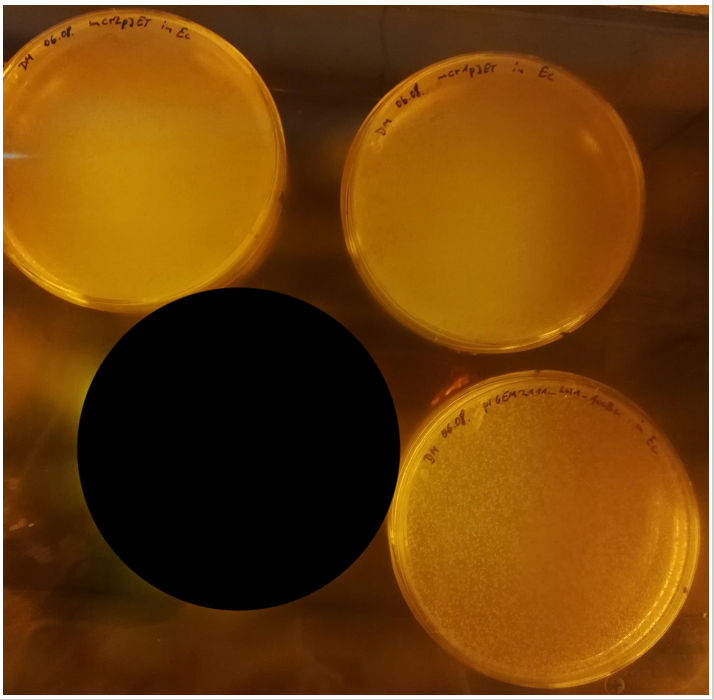
In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, piGEM2111, mcr1pJET, mcr2pJET

Procedure

1. thaw 3 aliquots of Ec NEB Turbo on ice
2. add 1µl of plasmid (2111/mcr1pJET/mcr2pJET)
3. incubate 10 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 2 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (2111 on LB+Kan, mcrpJET on LB+Amp)
9. incubate oN at 37°C

0807_retrafo_pYTK+mcrpJET+2111.jpg



Author: Daniel Marchal
created: 06.08.2018 13:03
updated: 08.08.2018 11:01

Entry 121/214: Retrafo of pYTK into Vn
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

For the acc activity assay we use plasmids with an J72163 GlpT promotor, which is only activ under starvation conditions. Therefore we need a pYTK harbouring strain, which indicates the starvation stage by its fluorescence. When the maincultur with pYTK shines green we can harvest the main cultures with acc-plasmids.

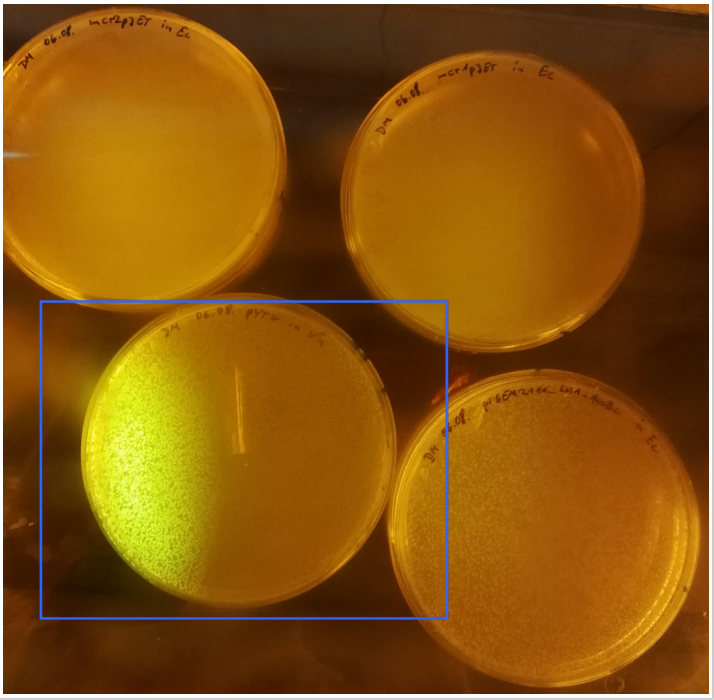
Procedure

1. thaw an aliquot of electrocompetent Vn on ice
2. add 1µl pYTK plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 950 V, 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates (Cm)
8. Incubate oN at 37°C

Result:

The plate has an area with dense colonies and less dense colonies. The dense area shines green what fits to the expectation that the promotor for gfp is only activ under starvation conditions. The trafo was sufficient

0807_retrafo_pYTK+mcrpJET+2111.jpg



Author: Daniel Marchal
created: 06.08.2018 13:16
updated: 06.08.2018 13:21
Entry 122/214: Lvl1 GoldenGate of piGEM2112 & piGEM2113
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation, piGEM2112, piGEM2113

Golden Gate Reaction:

add following reagents to your annealing mix:

4x-Tag	piGEM1085	
5' Connector	piGEM1076 / 1077	70 ng
Promotor	piGEM1007	70 ng
RBS	piGEM1008	70 ng
CDS	piGEM2106 / 2107	70 ng
Terminator	piGEM1035	70 ng
3' Connector	piGEM1071 / 1080	70 ng
Resistance	piGEM1057 (digested)	70 ng
Ori	piGEM1036	70 ng
T7-Ligase (NEB)		0,5 µL
BsaI (NEB)		0,5 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

Author: Daniel Marchal

created: 07.08.2018 14:17
updated: 09.08.2018 18:01

Entry 123/214: Enrichment and isolation of piGEM2111, mcr1pJET, mcr2pJET, pYTK from Ec/Vn

In Project: ERBsen

With tags: PYTK, Enrichment, miniprep, piGEM2111, mcr1pJET, mcr2pJET

Since we have consumed most of the plasmid amount for sequencing them we need a new miniprep. The Vn culture with pYTK doesn't have to be miniprepped, just enriched for the enzyme assay tomorrow

Procedure

1. prepare 3 tubes with following annotations:
    1. Vn + pYTK [Cm]
    2. Ec + piGEM2111 [Kan]
    3. Ec + mcr1pJET [Amp]
2. add 5ml BHIv2 or LB into the tubes and 5µl of antibiotic
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2111 60ng/µl

mcr1pJET 74ng/µl

Author: Daniel Marchal

created: 07.08.2018 14:21
updated: 09.08.2018 18:04

Entry 124/214: Trafo of piGEM2112 + piGEM2113 from GoGate into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2. add 6µl of golden gate reaction
3. incubate 15 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (LB+Kan)
9. incubate oN at 37°C

Author: Daniel Marchal
created: 08.08.2018 09:39
updated: 22.08.2018 17:43

Entry 125/214: Activity assay for AccBirAEc, AccBirASe & PccMe in Vn
In Project: ERBsen
With tags: Acc, enzyme activity, activity, assay, cell extract, AccBirASe, AccEc, PccMe

Procedure:

1. Prepare MOPS Buffer (Low salt as standard buffer and high salt because of Vibrios higher salt preference)
2. Prepare 4 tubes with 5ml LB2,5 and inoculate from cryostocks (Vn with piGEM2115, piGEM2117, AccBirASe, pYTK)
3. Incubate over night at 37°C shaking
4. Prepare a 1000ml flask with 500ml LB2,5 and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture and incubate at 37°C shaking
6. When OD=0.6 add
7. Stop incubating when the flask with Vn+pYTK shines green (OD_{600}=2-3)
8. Harvest the cells in 500ml centrifugation bottles (each bottle with 250ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
9. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 15ml Falcon.
10. Add 400µl 10xProtease-Inhibitor-Cocktail
11. Fill the tubes up to 4ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS

50mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor_Cocktail

12. Use the frenchpress to break the cells at 900 psi
    1. lever on "down" and wheel on high pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
13. If the solution is clear, the cell breakage was successful
14. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
15. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
16. For the enzyme assay use the software "Cary UV" with the program "kinetics"
17. Mix 40-229µl of your cell lysate together with MOPS buffer, $MgCl_2$, NADPH, ATP, $KHCO_3$ and MCR_Ca and measure slope (background)
18. Add Acetyl-CoA to start the reaction and again measure slope to calculate specific activity (see excel sheet)
19. If there is enzyme activity you can make a bradford to normalize your results
20. As a positive control you can add Pcc_Me
21. To store the cell lysate add 300µl glycerol and store at -20°C

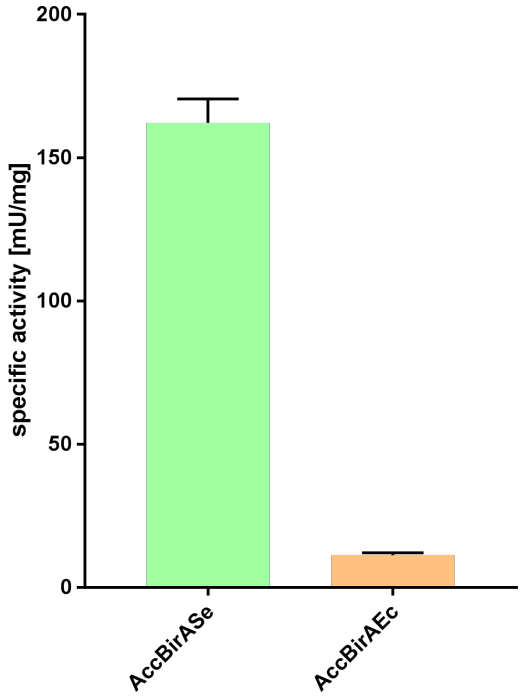
Results:

- Cells inocculated at 9:00
- 10:15 OD=0.20
- Cells harvested after 24h
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used
- Centrifugation bottle weights:

AccBirAEc	75.04g (before)	78.35g (after)
AccBirASe	74.62g (before)	77.00g (after)
PccMe	74.68g (before)	78.87g (after)

- Cell weights: AccBirAEc 3.31g, AccBirASe 2.38g, PccMe 4,19g
- AccBirASe with an IPTG inducible promotor has a specific activity of 162 mU/mg protein. The protein concentration is 28mg/ml
- PccMe has no detectable activity presumably because of the mutation it bears (Glu→Lys)
- EccBirAEc with a starvation promotor has a specific activity of 11 mU/mg protein what is much lower than for AccBirASe. But we can't say if it is due to the enzyme or due to the different promotor.

2018_08_09_-_AccBirASe_,_AccBirAEc_(piGEM2117).png



Enzyme activity of Acetyl-CoA Carboxylase in V. natriegens

2018_08_09_PccMe_AccEc_AccSe.xlsx

Results.xlsx

Author: Daniel Marchal

created: 08.08.2018 13:26
updated: 09.08.2018 18:10

Entry 126/214: Enrichment and isolation of put. piGEM2112 and piGEM2113 from GoGate Lvl1

In Project: ERBsen

With tags: Enrichment, miniprep, piGEM2112, piGEM2113

To check if the golden gate plasmids are correct, the plasmids must be isolated and a restriction digest performed

Procedure

1. prepare 10 tubes with following annotations:
    1. Ec + piGEM2112_LVL1_AccD 1
    2. Ec + piGEM2112_LVL1_AccD 2
    3. Ec + piGEM2112_LVL1_AccD 3
    4. Ec + piGEM2112_LVL1_AccD 4
    5. Ec + piGEM2112_LVL1_AccD 5
    6. Ec + piGEM2113_LVL1_BirA
    7. Ec + piGEM2113_LVL1_BirA
    8. Ec + piGEM2113_LVL1_BirA
    9. Ec + piGEM2113_LVL1_BirA
    10. Ec + piGEM2113_LVL1_BirA
2. add 5ml LB into the tubes and 5µl Kan [34mg/ml] (fresh prepared)
3. Inoculate from GoGate trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2112_LVL1_AccD 1	90ng/µl
piGEM2112_LVL1_AccD 2	91
piGEM2112_LVL1_AccD 3	77
piGEM2112_LVL1_AccD 4	67
piGEM2112_LVL1_AccD 5	74
piGEM2113_LVL1_BirA 1	71
piGEM2113_LVL1_BirA 2	97
piGEM2113_LVL1_BirA 3	37
piGEM2113_LVL1_BirA 4	80
piGEM2113_LVL1_BirA 5	81

Author: Daniel Marchal
created: 08.08.2018 16:14
updated: 13.08.2018 21:07

Entry 127/214: PCR for Aqua Cloning of piGEM2105, piGEM2106, piGEM2107
In Project: ERBsen
With tags: PCR, piGEM2105, piGEM2106, piGEM2107

The codonoptimized parts for AccBC, AccD and BirA from C. glutamicum are designed to have a 5'-Tag. Since we also want to have tagfree versions, aqua cloning is needed to introduce a point mutation changing the overhang from 4y to 4. First a PCR will be made, then the fragments are transformed into V. natriegens.

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 5 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (1µl amplificate + 1µl 6xLoading Dye + 4µl H_2O)

Sample	DNA template	primer for	primer rev
AccBC_right	piGEM2105_ LVL1_AccBC	oiGEM2110	oiGEM2113
AccBC_left	piGEM2105_ LVL1_AccBC	oiGEM2112	oiGEM2111
AccD_right	piGEM2106_ LVL1_AccD	oiGEM2114	oiGEM2113
AccD_left	piGEM2106_ LVL1_AccD	oiGEM2112	oiGEM2115
BirA_right	piGEM2107_ LVL1_BirA	oiGEM2116	oiGEM2113
BirA_left	piGEM2107_ LVL1_BirA	oiGEM2112	oiGEM2117

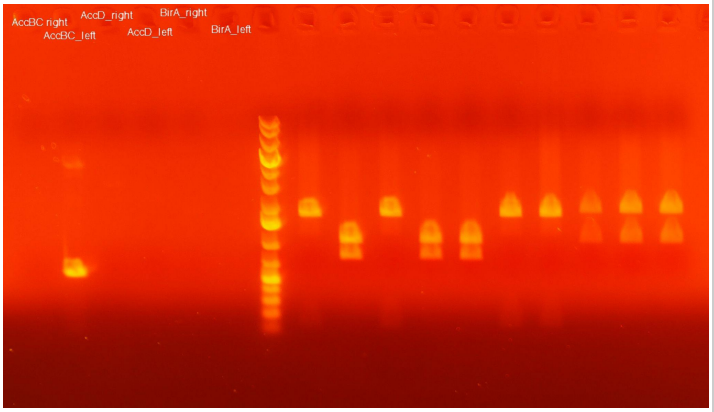
Mastermix (7x)	Sample
70 µl buffer	10 µl buffer
7 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
10.5 µl DMSO	1,5 µl DMSO
3.5 µl Q5 polymerase	0,5 µl Q5 polymerase
217 µl H_2O	31 µl H_2O (ad 50 µl)

Results:

expected lengths:

AccBC_right	3307 bp
AccBC_left	616 bp
AccD_right	3166 bp
AccD_left	617 bp
BirA_right	2339 bp
BirA_left	607 bp

0810_aqua_PCR_+_restriction_digest.jpg



- unfortunately just one sample has a band, so the PCR has to be repeated

Author: Daniel Marchal
Entry 128/214: Restriction digest of put. piGEM2112 & piGEM2113
In Project: ERBsen
With tags: piGEM2112, restriction, digest, piGEM2113

created: 09.08.2018 18:57
updated: 10.08.2018 12:10

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate over night at 37°C
4. add 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 20min)

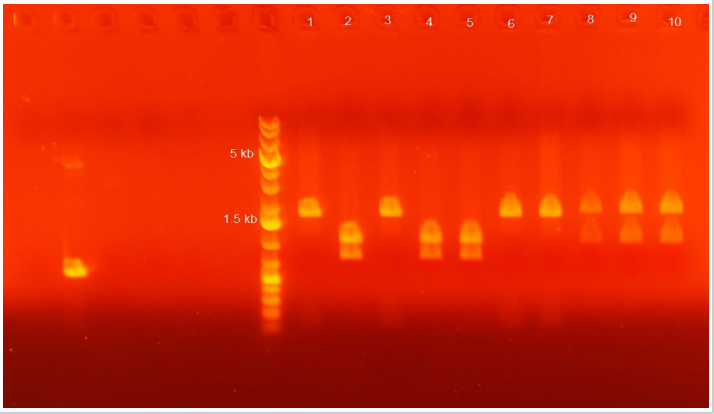
Sample	Master mix (11x)
4µl DNA	-
0.2µl BspHI	2,2µl BspHI
1µl CutSmart Buffer	11µl CutSmart Buffer
4.8µl H_2O	52,8µl H_2O

Number	Plasmid	Expected fragment lengths
1	piGEM2112 1	1100bp + 2691bp
2	piGEM2112 2	1100bp + 2691bp
3	piGEM2112 3	1100bp + 2691bp
4	piGEM2112 4	1100bp + 2691bp
5	piGEM2112 5	1100bp + 2691bp
6	piGEM2113 1	1100bp + 1869bp
7	piGEM2113 2	1100bp + 1869bp
8	piGEM2113 3	1100bp + 1869bp
9	piGEM2113 4	1100bp + 1869bp
10	piGEM2113 5	1100bp + 1869bp

Results

- for piGEM2112 no sample has the expected fragment lengths
- for piGEM2113 the last 3 samples look correct, we will sequence one of them

0810_aqua_PCR_+_restriction_digest.jpg



Author: Daniel Marchal

created: 11.08.2018 14:35
updated: 11.08.2018 15:25

Entry 129/214: Bradford assay with cell extract of Vn WT, PccMe_pointmutation, AccBirAEc, AccBirASe

In Project: ERBsen

With tags: Bradford, assay

Procedure:

1. Make dilutions of a 1mg/ml BSA solutions (see scheme)
2. Make dilutions of your samples (cell extract from Vn wt and Vn bearing plasmids for PccMe_pointmutation, AccBirAEc, AccBirASe)
3. mix 100µl sample with 900µl bradford reagent
4. Incubate 10min at room temperature
5. Measure absorption at 595nm and determine proteinconcentration

Dilution scheme for BSA stock solutions:

0 µg/ml → 0µl BSA [1mg/ml] + 1000µl H_2O

20 µg/ml → 20µl BSA [1mg/ml] + 800µl H_2O

40 µg/ml → 40µl BSA [1mg/ml] + 600µl H_2O

60 µg/ml → 60µl BSA [1mg/ml] + 400µl H_2O

80 µg/ml → 80µl BSA [1mg/ml] + 200µl H_2O

100 µg/ml → 1000µl BSA [1mg/ml] + 0µl H_2O

Dilution scheme for cell extract:

1:10 → 100µl cell extract + 900µl H_2O

1:100 → 100µl 1:10 dilution + 900µl H_2O

1:1000 → 100µl 1:100 dilution + 900µl H_2O

1:2000 → 500µl 1:1000 dilution + 500H_2O

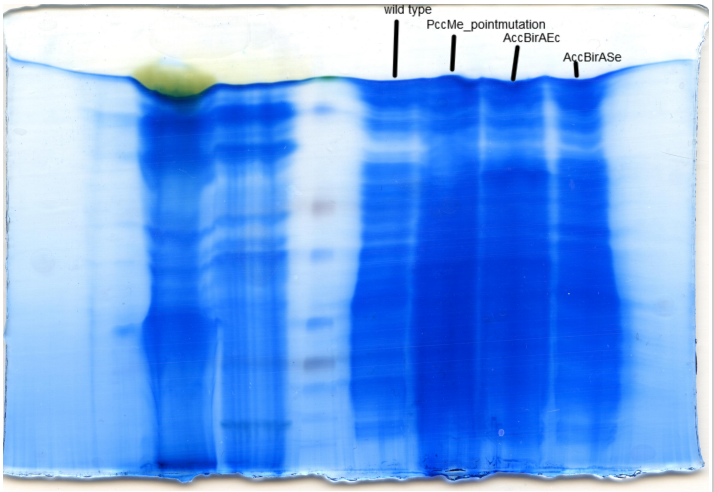
1:3000 → 300µl 1:1000 dilution + 600µl H_2O

Results:

- Cell extract from Vn wild type (harvested at OD=2,2) has 8.6 mg/ml protein
- Cell extract from Vn with PccMe_pointputation (harvested after 24 hours) has 65.6 mg/ml protein
- Cell extract from Vn with AccBirAEc (harvested after 24 hours) has 56.6 mg/ml protein
- Cell extract from Vn with AccBirASe (harvested after 24 hours) has 28.0 mg/ml protein
- It seems that the starvation promotor (glpT) of piGEM2115 and piGEM2117 is stronger than the lac promotor of AccBirASe or its ori is better
- Additionaly a sds-page was made to check if there are detectable bands for the acc subunits. Unfortunately the protein concentration was too high so the gel will be repeated

piGEM2115,_piGEM2117,_AccBirASe.xlsx

20180809_luise_extracts006.jpg



Author: Daniel Marchal
Entry 130/214: PCR for Aqua Cloning of piGEM2105, piGEM2106, piGEM2107 (2)
In Project: ERBsen
With tags: PCR, piGEM2105, piGEM2106, piGEM2107

created: 13.08.2018 21:02
updated: 16.08.2018 16:33

See PCR for Aqua Cloning of piGEM2105, piGEM2106, piGEM2107 - entry #127 in project 'ERBsen' (Daniel Marchal, 13.08.2018)

Results:

it worked

Author: Daniel Marchal
Entry 131/214: Lvl 1 GoldenGate of piGEM2112_LVL1_AccD (2)
In Project: ERBsen
With tags: Lvl 1 plasmids, piGEM2112, Golden Gate

created: 13.08.2018 21:08
updated: 15.08.2018 10:23

See Lvl1 GoldenGate of piGEM2112 & piGEM2113 - entry #122 in project 'ERBsen' (Daniel Marchal, 06.08.2018)

For piGEM2112 the restriction digest revealed no correct plasmids, so the GoldenGate must be repeated.

Result:

The trafo plate has two colonies which were picked and enriched to do a restriction digest

Author: Daniel Marchal

created: 13.08.2018 21:14
updated: 22.08.2018 17:48

Entry 132/214: Chemical synthesis of acetyl-CoA and purification via HPLC/MS
In Project: ERBsen
With tags: acetyl-CoA, HPLC, LC/MS

Procedure synthesis:

1. Prepare 5ml 0.5M $NAHCO_3$/CoA Solution and cool it on ice
2. Add 45µl acetic anhydride and stirr on ice for 20min
3. To confirm complete CoA consumption take 5µl and mix it with 45µl Ellmanns reagent (DTNB). If the solution gets yellow there is still free CoA and if not then you can proceed
4. Add ~200µl formic acid until pH=3 (be careful because a lot of CO_2 gets free)
5. Use HPLC/MS to purify acetyl-CoA

The protocol was adapted from Peter et al., 2016 (A chemo-enzymatic road map to the synthesis of coA esters)

Procedure HPLC:

1. Prepare 5L 25mM ammonium formate pH4.2 and link it to the HPLC. Check if the waste bottle is empty.
2. Open the Software "OpenLAB CDS". A short window for the "Agilent activ splitter" opens also. Do not close this window or the system gets an error.
3. Precool the DL sampler to 4°C. Click "On" for each device to turning them on.
4. The line system is stored with 10% MeOH/90% ammonium formate and the column with 80% MeOH/20% ammoium formate. So you have to slowly increase the MeOH content up to 80% before you can connect the column to the line. While washing the system check if there are bubbles. Flow 20ml/min.
5. Connect the column without inserting bubbles into the system.
6. Decrease MeOH content to 10% slowly.
7. Adjust the parameters of the splitter to a volume of 300µl and a ratio of 1000:1
8. Open the protocol "Preparative_acetyl_coA_pH4.2" and test it with 500µl H_2O.
9. Start the protocol ("Run control" → "start run"). The MS signal should be lower than 200.000, if that is not the case then something is dirty. The DAD signal should give a peak at the beginning and the ending but not in between.
10. If the water control was okay, start with 500µl sample.
11. After the run check the ms signals for purity and look in which tubes the pure substrate are. Collect them in a 250ml bottle which was washed with ddH_2O.
12. Refill the fraction collector and reset it (right click on "Fraction collector" → "reset fraction collector")
13. Precool the lyophile and prepare a vacuum
14. When the purification is finished click on "light out" and on "off" (red button)

Reagents:

5L 25mM Ammonium Formate pH4.2

Weigh 7.88g Ammonium formate and dissolve in nearly 5L H_2O

Adjust pH with formic acid to pH4.2

Fill to 5L with H_2O

Filter and degase the schottbottle

5ml 0.5M $NAHCO_3$/CoA Solution

210.0mg $NaHCO_3$

200mg Na_3CoA

ad 5ml H_2O

Acetic anhydride

Formic acid

Ellmanns reagent

Author: Daniel Marchal
created: 15.08.2018 15:02
updated: 15.08.2018 15:25
Entry 133/214: Enrichment and isolation of piGEM2112
In Project: ERBsen
With tags: piGEM2112, Enrichment, miniprep

Procedure

1. prepare 4 tubes with following annotations:
    1. Ec + piGEM2112
    2. Ec + piGEM2112
2. add 5ml LBinto the tubes and Kan [50mg/ml] (fresh prepared)
3. Inoculate with smear of pYTK or pEntry
4. Incubate over day at 37°C shaking
5. Make miniprep

Result

piGEM2112_1 54ng/µl

piGEM2112_2 43ng/µl

Author: Daniel Marchal
created: 15.08.2018 19:17
updated: 20.08.2018 11:41
Entry 134/214: Restriction digest of piGEM1048 and put. piGEM2112
In Project: ERBsen
With tags: piGEM2112, restriction, digest, piGEM1048

For a lvl2 cloning we have to predigest the resistance part with bsaI and elute it from a gel. The put. piGEM2112 plasmids have to be digested to check their correctness

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

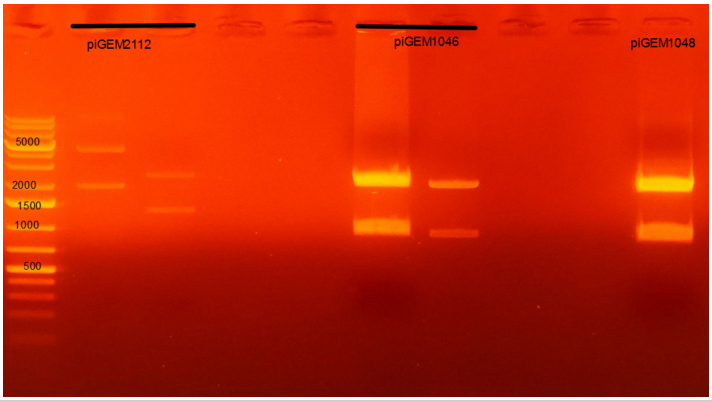
piGEM2112_1	**piGEM2112_2**	**Res-part 5_2**
3µl DNA	3µl DNA	20µl DNA
0.2µl BspHI	0.2µl BspHI	1.4µl BsaI
1µl CutSmart Buffer	1µl CutSmart Buffer	3µl CutSmart Buffer
5.8µl H_2O	5.8µl H_2O	5.6µl H_2O

Sample	**Expected fragments**
piGEM2112_1	1100bp + 2691bp
piGEM2112_2	1100bp + 2691bp
piGEM1048	1118bp + 1979bp

Results

- piGEM2112_1 is wrong
- piGEM2112_2 is correct
- piGEM1048 has the expected fragments, the smaller one was extracted

0820_gel_extraction_1046+1048.jpg



Author: Daniel Marchal
Entry 135/214: Restriction digest of piGEM1046 for Lvl2 GoGate
In Project: ERBsen
With tags: piGEM1046, restriction, digest

created: 20.08.2018 08:49
updated: 20.08.2018 11:41

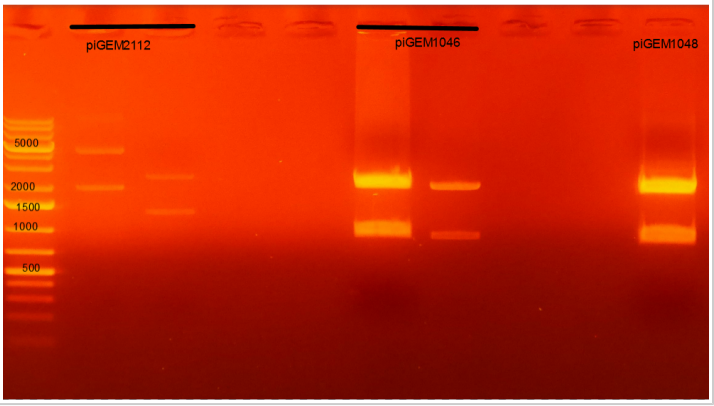
Procedure

1. Mix 30µl plasmid DNA with 4µl Cutsmart Buffer, 5µl H_2O and 1µl BsaI
2. incubate over night at 37°C
3. add 8µl Loading Dye
4. run gel (0.8% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 20min) and make gel elution

Results

- Expected length: 929bp + 2109bp
- piGEM1046 has the correct fragments, the smaller ones were extracted

0820_gel_extraction_1046+1048.jpg-with-annotations.png



Author: Daniel Marchal

created: 20.08.2018 08:52
updated: 20.08.2018 08:57

Entry 136/214: Enrichment and isolation of put. piGEM2100, 2101, 2102, pEmatB, 1046

In Project: ERBsen

With tags: M9, media, pH

Procedure

1. prepare 4 tubes with following annotations:
    1. Vn + piGEM2100
    2. Vn + piGEM2100
    3. Vn + piGEM2101
    4. Vn + piGEM2101
    5. Vn + piGEM2102
    6. Vn + piGEM2102
    7. Ec + piGEM1046
    8. Ec + pEmatB
2. add 5ml LB or LB2.5 into the tubes and 5µl Cm [34mg/ml / 2mg/ml] (fresh prepared)
3. Inoculate from aquacloning/trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

- piGEM2100, piGEM2101, piGEM2102 have high concentrations (700-800 ng/µl) due to the presence of RNA (we used H_2O as resuspension buffer because the remaining RNA inhibits DNase activity)
- pEmatB 160ng/µl

Author: Daniel Marchal
created: 22.08.2018 15:32
updated: 22.08.2018 15:42

Entry 137/214: Lvl 2 GoldenGate of piGEM2103_LVL2_AccBirA-nHis
In Project: ERBsen
With tags: Golden Gate, Level 2

Golden Gate Reaction:

add following reagents to your annealing mix:

transcriptional unit 1 - AccBc	piGEM2111	70 ng
transcriptional unit 2 - AccD	piGEM2112	70 ng
transcriptional unit 3 - BirA	piGEM2113	70 ng
Resistance	piGEM1048	70 ng
Ori	piGEM1046	70 ng
T7-Ligase (NEB)		1 µL
BsaI (NEB)		1 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

Author: Daniel Marchal

created: 22.08.2018 16:32
updated: 22.08.2018 16:35

Entry 138/214: Trafo of piGEM2103_LVL2_AccBirA-nHis (Lvl2 GoGate) and aqua plasmids into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, JZ90, piGEM2103, JZ147, piGEM2100, piGEM2101, piGEM2102

Procedure

1. thaw 6 aliquots of Ec NEB Turbo on ice
2.

Vector	**Resistance**
piGEM2103	Cm
piGEM2100	Cm
piGEM2101_1	Cm
piGEM2101_2	Cm
piGEM2102_1	Cm
piGEM2102_2	Cm

3. add 1µl of plasmid or 5µl of GoGate mix
4. incubate 10 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 10 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal

created: 22.08.2018 16:35
updated: 26.08.2018 18:24

Entry 139/214: Enrichment and isolation of put. piGEM2103, 2100, 2101, 2102

In Project: ERBsen

With tags: M9, media, pH

Procedure

1. prepare 6 tubes with following annotations:
    1. Ec + piGEM2103
    2. Ec + piGEM2100
    3. Ec + piGEM2101_1
    4. Ec + piGEM2101_2
    5. Ec + piGEM2102_1
    6. Ec + piGEM2102_2
2. add 5ml LB into the tubes and 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

- concentrations weren't measured because the sequencing showd no correct plasmids

Author: Daniel Marchal
Entry 140/214: Sequencing of piGEM2100, piGEM2101_1, piGEM2102_1
In Project: ERBsen
With tags: piGEM2105, piGEM2106, piGEM2107, piGEM2108

created: 22.08.2018 16:42
updated: 24.08.2018 18:40

Samples:

Vektor	Eppi	Label	Primer
piGEM2100	2100_seq3	AIM0030140	Seq 3
piGEM2101_1	2101_1_seq3	AIM0030141	Seq 3
piGEM2102_1	2102_1_seq3	AIM0030142	Seq 3

Procedure:

1200ng DNA

2µl Primer

ad 15µl H_2O

Results (Order 11104588240):

- all samples are wrong or couldn't be sequenced because of too much RNA

Author: Daniel Marchal
created: 22.08.2018 16:45
updated: 31.08.2018 13:53
Entry 141/214: SDS-PAGE for Vn wt, Vn + Mcr, Vn + AccBirAEc
In Project: ERBsen
With tags: SDS-PAGE, McrCa, AccBirAEc

Procedure:

1. Mix 5µl Sample with 10µl H_2O and 5µl 4xLoadingDye (see following list of samples)
    1. X
    2. PageRuler Plus
    3. V. natriegens wild type uninduced
    4. V. natriegens wild type induced
    5. V. natriegens + McrCa (pTrc-McrCa) uninduced
    6. V. natriegens + McrCa (pTrc-McrCa) induced
    7. V. natriegens + AccBirAEc (JZ154) induced
    8. V. natriegens + AccBirAEc (JZ154) after french pressing
    9. V. natriegens + AccBirAEc (JZ154) cell lysate
    10. X
2. Incubate 10min at 99°C
3. Spin down the droplets from the lid
4. Put the SDS-Gel into the device and sink the wells
5. Load the gel with sample and run at 120V for 45-90min
6. Wash 15min with H_2O and at least 4h in stain solution
7. Incubate over night in destain solution

Reagents:

stain solution

GelCode Blue Safe Protein Stain

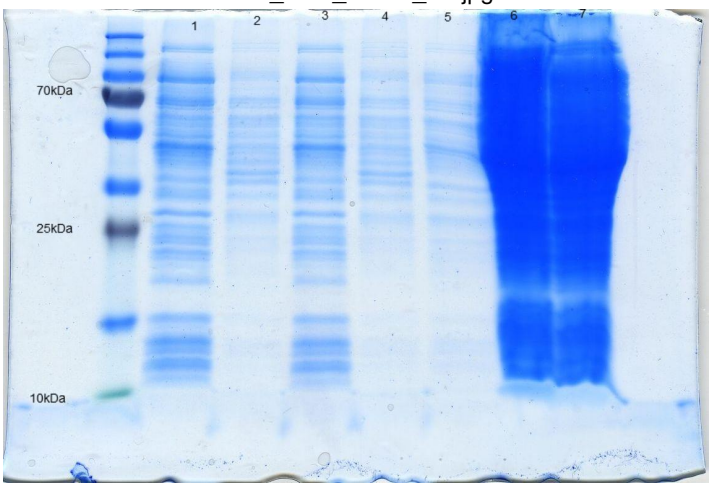
destain solution

50% (v/v) MeOH

10% (v/v) Acetic acid

Results:

0826_SDS_PAGE_Vn.jpg



Number	Sample	Expected protein	mass	detected?
1	V. natriegens wild type uninduced	-		
2	V. natriegens wild type induced	-		
3	V. natriegens + McrCa (pTrc-McrCa) uninduced	McrCa	132kDa	no
4	V. natriegens + McrCa (pTrc-McrCa) induced	McrCa	132kDa	yes
5	V. natriegens + AccBirAEc (JZ154) induced	Bccp, bcarb, AccA, AccBeta, BirA	16.1kDa, 49.33kDa, 35.22kDa, 33.33kDa, 35,81kDa	no
6	V. natriegens + AccBirAEc (JZ154) after frenchpressing	AccA, AccB, AccC, AccD, BirA	16.1kDa, 49.33kDa, 35.22kDa, 33.33kDa, 35,81kDa	no
7	V. natriegens + AccBirAEc (JZ154) cell lysate	AccA, AccB, AccC, AccD, BirA	16.1kDa, 49.33kDa, 35.22kDa, 33.33kDa, 35,81kDa	no

The McrCa is detectable but the band is weak indicating, that the protein concentration is low. This observation makes the calculation of a specific activity hard because we can't calculate the exact amount of McrCa. Nevertheless it is good, that we could see a band. The low expression is due to the pTrc promoter which is weak in comparison to a T7 promoter.

The AccEc was not detectable indicating that there was no protein in the cells. Since the band isn't in lane 5 with induced cells it seems that the plasmid got lost and not that a problem in cell lysis would be the reason. We will repeat it.

Author: Daniel Marchal
created: 22.08.2018 17:03
updated: 22.08.2018 17:32
Entry 142/214: Activity assay for AccBirAEc (JZ154) in Vn
In Project: ERBsen
With tags: Acc, enzyme activity, activity, assay, cell extract, AccBirAEc, JZ154

Procedure:

1. Prepare MOPS Buffer
2. Prepare a tube with 10ml LBv2 and inoculate from Vn cryostock in the morning
3. Incubate over day at 37°C shaking
4. Prepare a 1000ml flask with 500ml LBv2 and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture in the afternoon and incubate at 37°C shaking
6. When OD_{600}=0.4-0.6 induce the plasmids with 50µM IPTG (50µl of 0.5M IPTG) and incubate over night
7. Harvest the cells in 1L centrifugation bottles (each bottle with 500ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
8. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 50ml Falcon.
9. Add 1.2ml 10xProtease-Inhibitor-Cocktail
10. Fill the tubes up to 12ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS buffer

200mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor_Cocktail

11. Use the frenchpress to break the cells at 900 psi and middle pressure if you use the small device or at 1200 psi and high pressure if you use the large device
    1. lever on "down" and rotate the wheel to increase pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
12. If the solution is clear, the cell breakage was successful
13. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
14. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
15. For the enzyme assay use the software "Cary UV" with the program "kinetics"
16. Mix 40-229µl of your cell lysate together with MOPS buffer, $MgCl_2$, NADPH, ATP, $KHCO_3$ and MCR_Ca and measure slope (background)
17. Add Acetyl-CoA to start the reaction and again measure slope to calculate specific activity (see excel sheet)
18. If there is enzyme activity you can make a bradford to normalize your results
19. As a positive control you can add Pcc_Me
20. To store the cell lysate add 300µl glycerol and store at -20°C

2018_08_21_-_AccBirAEc_(JZ154).xlsx

Results:

- Cells harvested at OD_{600}=8
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used
- Cell weight: 6g
- There was no activity detectable. To check if the protein is in the cell extract we will do a SDS-PAGE with cells after induction, cells after french pressing and cell extract. A colony PCR wasn't made

Author: Daniel Marchal
created: 22.08.2018 17:13
updated: 22.08.2018 17:39
Entry 143/214: Activity assay for McrCa (pTrc-McrCa) in Vn
In Project: ERBsen
With tags: Acc, enzyme activity, activity, assay, cell extract, McrCa

Procedure:

1. Prepare MOPS Buffer
2. Prepare a tube with 10ml LBv2 and inoculate from Trafo plates in the morning
3. Incubate over day at 37°C shaking
4. Prepare a 1000ml flask with 500ml LBv2 and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture in the afternoon and incubate at 37°C shaking
6. When OD_{600}=0.4-0.6 induce the plasmids with 50µM IPTG (50µl of 0.5M IPTG) and incubate over night
7. Harvest the cells in 1L centrifugation bottles (each bottle with 500ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
8. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 50ml Falcon.
9. Add 10xProtease-Inhibitor-Cocktail (rule of thumb for the overall volume: per gramm cells add 3ml buffer)
10. Fill the tubes up to 12ml with buffer

Reagents:

MOPS

200mM MOPS/KOH

150mM NaCl

pH 7,8

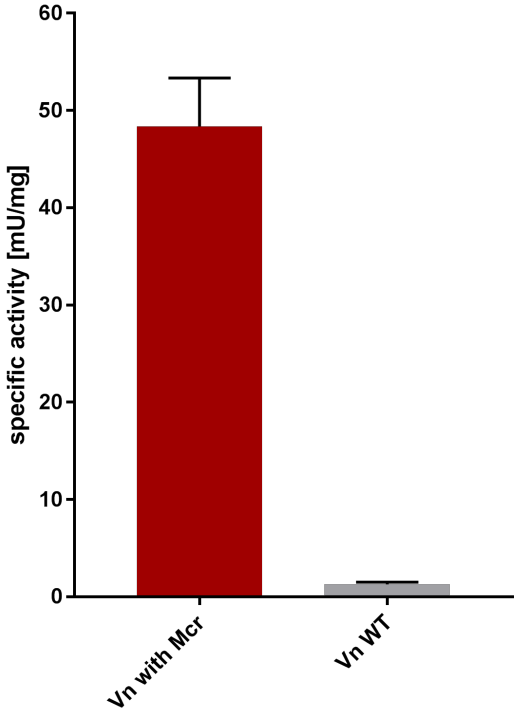
10xProtease-Inhibitor_Cocktail

11. Use the frenchpress to break the cells at 900 psi and middle pressure if you use the small device or at 1200 psi and high pressure if you use the large device
    1. lever on "down" and rotate the wheel to increase pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
12. If the solution is clear, the cell breakage was successful
13. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
14. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
15. For the enzyme assay use the software "Cary UV" with the program "kinetics"
16. Mix Acetyl-CoA together with MOPS buffer, $MgCl_2$, NADPH, ATP, $KHCO_3$ and purified PccMe_D407I and incubate 10min. In this time the Pcc will convert most of the acetyl-coA into malonyl-CoA.
17. Measure slope (background)
18. Add 40-229µl of your cell lysate to start the reaction and again measure slope to calculate specific activity (see excel sheet)
19. If there is enzyme activity you can make a bradford to normalize your results
20. As a positive control you could add purified McrCa
21. To store the cell lysate add 300µl glycerol and store at -20°C

Results:

- Cells harvested at OD_{600}=8
- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used
- Cell weights: 6g
- Wild type cell lysate has an activity of 1.28 mU/mg protein, lysate with McrCa has an activity of 48 mU/mg what is significantly higher than the wild type indicating that the enzyme is functional. A bradford revealed a protein concentration of 19mg/ml in Vn+Mcr

2018_08_17_-_Vibrio_lysate_-_McrCa_(pTrc-McrCa).png



Enzyme activity of malonyl-CoA reduction in V. natriegens

2018_08_17_-_Vibrio_lysate_-_McrCa_(pTrc-McrCa).xlsx

Results.xlsx

Author: Daniel Marchal

created: 22.08.2018 17:44
updated: 22.08.2018 17:55

Entry 144/214: Synthesis of Malonyl-CoA via MatB ligation and HPLC purification
In Project: ERBsen
With tags: MatB, HPLC, Malonyl-CoA

Procedure synthesis:

1. Prepare 13ml Ammoniumhydrogencarbonate buffer and add 200mg CoA, 132mg malonic acid, 704mg ATP
2. Add 5mM purified MatB and incubate at 30°C
3. To confirm complete CoA consumption take 5µl and mix it with 45µl Ellmanns reagent (DTNB). If the solution gets yellow there is still free CoA and if not then you can proceed
4. Add formic acid until pH=3 (be careful because a lot of CO_2 gets free)
5. Use HPLC/MS to purify malonyl-CoA

The protocol was adapted from Peter et al., 2016 (A chemo-enzymatic road map to the synthesis of coA esters)

Procedure HPLC:

1. Prepare 5L 25mM ammonium formate pH4.2 and link it to the HPLC. Check if the waste bottle is empty.
2. Open the Software "OpenLAB CDS". A short window for the "Agilent activ splitter" opens also. Do not close this window or the system gets an error.
3. Precool the DL sampler to 4°C. Click "On" for each device to turning them on.
4. The line system is stored with 10% MeOH/90% ammonium formate and the column with 80% MeOH/20% ammoium formate. So you have to slowly increase the MeOH content up to 80% before you can connect the column to the line. While washing the system check if there are bubbles. Flow 20ml/min.
5. Connect the column without inserting bubbles into the system.
6. Decrease MeOH content to 10% slowly.
7. Adjust the parameters of the splitter to a volume of 300µl and a ratio of 1000:1
8. Open the protocol "Preparative_acetyl_coA_pH4.2" and test it with 500µl H_2O.
9. Start the protocol ("Run control" → "start run"). The MS signal should be lower than 200.000, if that is not the case then something is dirty. The DAD signal should give a peak at the beginning and the ending but not in between.
10. If the water control was okay, start with 500µl sample.
11. After the run check the ms signals for purity and look in which tubes the pure substrate are. Collect them in a 250ml bottle which was washed with ddH_2O.
12. Refill the fraction collector and reset it (right click on "Fraction collector" → "reset fraction collector")
13. Precool the lyophile and prepare a vacuum
14. When the purification is finished click on "light out" and on "off" (red button)

Reagents:

5L 25mM Ammonium Formate pH4.2

Weigh 7.88g Ammonium formate and dissolve in nearly 5L H_2O

Adjust pH with formic acid to pH4.2

Fill to 5L with H_2O

Filter and degase the schottbottle

13 mL Ammoniumhydrogencarbonate buffer

200mM NH4CO3

15mM MgCl2

pH 6.8 (with HCl)

ad 15ml H_2O

Malonic acid

Formic acid

Ellmanns reagent

CoA

ATP

Author: Daniel Marchal
Entry 145/214: Restriction digest of piGEM2103_LVL2_AccBirA-nHis
In Project: ERBsen
With tags: piGEM2103, restriction, digest

created: 24.08.2018 17:19
updated: 30.08.2018 15:20

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate oN at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1% gel with 5µl of EtBr; GeneRuler 1kb plus; 135V, 25min)

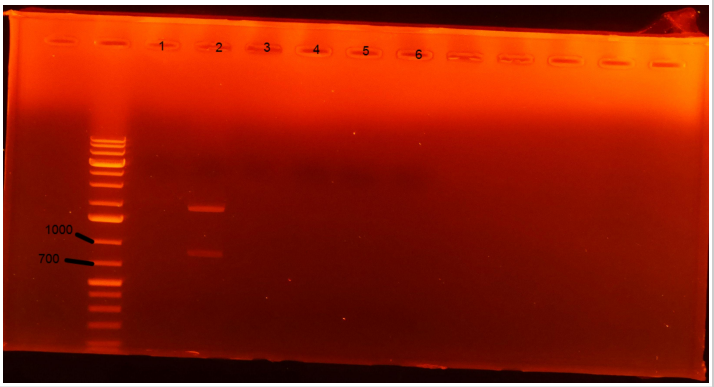
Sample	Master mix (7x)
4µl DNA	-
0.2µl XhoI	1.4µl XhoI
1µl CutSmart Buffer	7µl CutSmart Buffer
4.8µl H_2O	33.6µl H_2O

Plasmid	Expected fragment length	Correct?
piGEM2103_1	892bp + 6052bp	no
piGEM2103_2	892bp + 6052bp	no
piGEM2103_3	892bp + 6052bp	no
piGEM2103_4	892bp + 6052bp	no
piGEM2103_5	892bp + 6052bp	no
piGEM2103_6	892bp + 6052bp	no

Results

- None of the samples is correct
- Plasmid 2 has the expected 892bp band but the bigger band is too small indicating that just one of the transcriptional units was transfered

0824_Restriction_digest_2103.jpg



Author: Daniel Marchal
created: 24.08.2018 18:39
updated: 24.08.2018 18:39
Entry 146/214: DpnI digest of Aqua PCR
In Project: ERBsen
With tags: DpnI, aqua

Procedure:

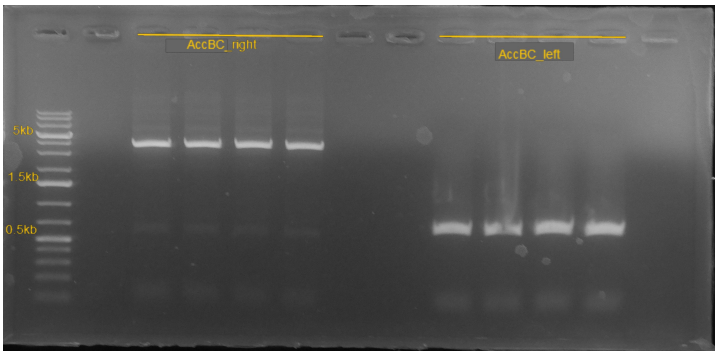
1. Pool all sufficient PCR aliquots of each sample (100µl)
2. add 12µl FD-buffer, 6µl H_2O and 2µl FD-DpnI
3. incubate 60min at 37°C
4. Store at -20°C until PCR purification/gel elution

Author: Daniel Marchal
Entry 147/214: PCR for Aqua Cloning of piGEM2105, piGEM2106, piGEM2107 (3)
In Project: ERBsen
With tags: aqua, piGEM2100, piGEM2101, piGEM2102

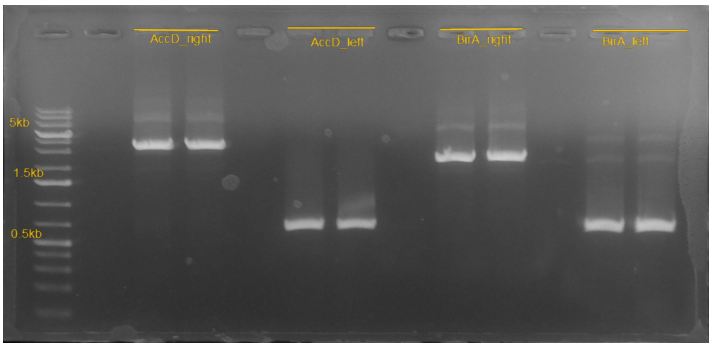
created: 24.08.2018 18:39
updated: 28.08.2018 13:28

See PCR for Aqua Cloning of piGEM2105, piGEM2106, piGEM2107 - entry #127 in project 'ERBsen' (Daniel Marchal, 13.08.2018)

0828_Gel_extraction_1.jpg



0828_Gel_extraction_2.jpg



Results:

expected lengths:

AccBC_right	3307 bp
AccBC_left	616 bp
AccD_right	3166 bp
AccD_left	617 bp
BirA_right	2339 bp
BirA_left	607 bp

- All samples show the expected fragment, which was cutted out in an gel extraction

Author: Daniel Marchal
created: 27.08.2018 15:48
updated: 27.08.2018 15:49
Entry 148/214: Retrafo of JZ54 into Vn for AccBirAEc production
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock, JZ154

Procedure

1. thaw one aliquot of electrocompetent Vn on ice
2. add 1µl plasmid DNA into the aliquot
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 950 V (depending on the strain), 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 37°C while shaking
7. Plate out on selection plates
8. Incubate oN at 37°C

Author: Daniel Marchal
Entry 149/214: Lvl 2 GoldenGate of piGEM2103_LVL2_AccBirA-nHis (2)
In Project: ERBsen
With tags: Level 2, lvl 2 plasmids, piGEM2103

created: 27.08.2018 18:10
updated: 29.08.2018 16:12

See Lvl 2 GoldenGate of piGEM2103_LVL2_AccBirA-nHis - entry #137 in project 'ERBsen' (Daniel Marchal, 22.08.2018)

Afterwards a transformation mit 5µl in E. coli was made

Author: Daniel Marchal
created: 28.08.2018 10:47
updated: 28.08.2018 14:24

Entry 150/214: Gel extraction for for aqua coning of 2100, 2101, 2102
In Project: ERBsen
With tags: piGEM2100, piGEM2101, piGEM2102, Gel extraction

Procedure:

1. Load the whole sample on a 1% agarose gel and run at 130V for 28min
2. Cut the right bands out and follow the protocoll of "MN NucleoSpin Gel and PCR Clean-Up" (page 19-20 in the file)
    1. we used 600µl NTI buffer
    2. we eluted in 20µl H_2O
3. Determine concentrations using Nanodrop

Results:

AccBC_right	45ng/µl
AccBC_left	85ng/µl
AccD_right	48ng/µl
AccD_left	126ng/µl
BirA_right	86ng/µl
BirA_left	130ng/µl

MN_NucleoSpin_Gel_and_PCR_Clean-Up.pdf

Author: Daniel Marchal
created: 29.08.2018 16:10
updated: 31.08.2018 12:00
Entry 151/214: Enrichment and isolation of put. piGEM2103 (2)
In Project: ERBsen
With tags: Miniprep, Enrichment, piGEM2103

Procedure

1. prepare 10 tubes with following annotations:
    1. Ec + piGEM2103_LVL2_AccBirA-nHis 1
    2. Ec + piGEM2103_LVL2_AccBirA-nHis 2
    3. Ec + piGEM2103_LVL2_AccBirA-nHis 3
    4. Ec + piGEM2103_LVL2_AccBirA-nHis 4
    5. Ec + piGEM2103_LVL2_AccBirA-nHis 5
    6. Ec + piGEM2103_LVL2_AccBirA-nHis 6
    7. Ec + piGEM2103_LVL2_AccBirA-nHis 7
    8. Ec + piGEM2103_LVL2_AccBirA-nHis 8
    9. Ec + piGEM2103_LVL2_AccBirA-nHis 9
    10. Ec + piGEM2103_LVL2_AccBirA-nHis 10
2. add 5ml LB + 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from trafo plate
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

- no plasmids showd the correct restriction pattern therefore the concentrations weren't determined and the samples were discarded

Author: Daniel Marchal
created: 29.08.2018 16:44
updated: 30.08.2018 12:53

Entry 152/214: Gibson assembly of piGEM2100, 2101, 2102
In Project: ERBsen
With tags: gibson cloning, piGEM2100, piGEM2101, piGEM2102

Since aqua cloning didn't work for piGEM2100, 2102, 2102 we will in parallel do the cloning via gibson assembly

Procedure:

1. Set up the following reaction on ice (0.2pmol DNA, all fragments equimolar)
2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.
3. Transform 10µl into Ec, store the rest at -20°C
    1. Ec trafo: 2µl sample / 2h regeneration / plating out on LB+Cm

Fragment	bp	ng/µl	pmol/µl	µl for 0,5 pmol	µl for 0,2 pmol
AccBC_right	3307	45	0,041235	12,12566667	4,850266667
AccBC_left	616	85	0,418142	1,195764706	0,478305882
AccD_right	3166	48	0,045943	10,883125	4,35325
AccD_left	617	126	0,61883	0,80797619	0,323190476
BirA_right	2339	86	0,111418	4,487616279	1,795046512
BirA_left	607	130	0,648994	0,770423077	0,308169231

	piGEM2100	piGEM2102	piGEM2102
Fragment [µl]	4,85	4,35	4,49
Backbone [µl]	0,48	0,32	0,77
Gibson Mastermix 2x [µl]	10	10	10
Total volume [µl]	15	15	15

Author: Daniel Marchal
Entry 153/214: cPCR of Vn + JZ154
In Project: ERBsen
With tags: PCR, cPCR, JZ154

created: 30.08.2018 12:48
updated: 31.08.2018 11:58

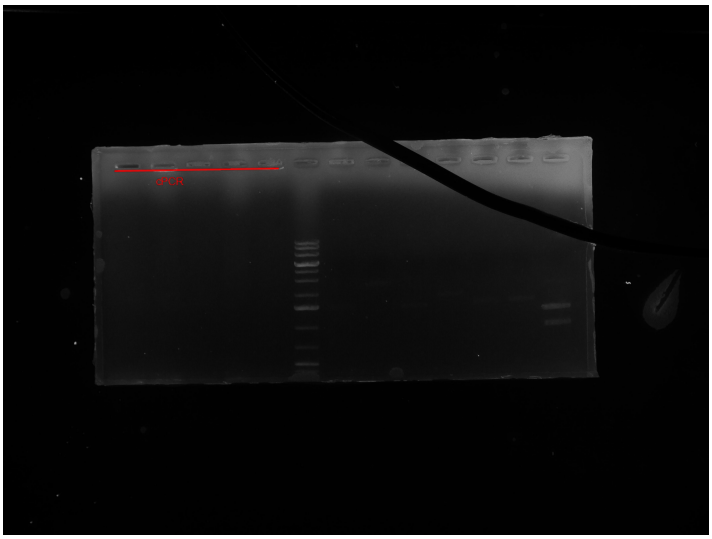
Procedure:

1. Prepare mastermix
2. Aliqupt 25µl into pcr tubes
3. Pick colonies and inocculate them into the tubes
4. Start PCR program with initial 10min at 98°C
5. Run a gel

Sample	Mastermix (6x)
12.5µl 2x-Mastermix	75µl 2x-Mastermix
0.5µl Primer_for (pNS3_seq_for)	3µl Primer_for
0.5µl Primer_rev (oiGEM2109)	3µl Primer_rev
11.5µl H_2O	69µl H_2O

Sample	DNA template	Expected fragment length	Result
1	piGEM2115 1	4107 bp	No band detectable
2	piGEM2115 2	4107 bp	No band detectable
3	piGEM2115 3	4107 bp	No band detectable
4	piGEM2115 4	4107 bp	No band detectable
5	piGEM2115 5	4107 bp	No band detectable

P1030084.JPG



Author: Daniel Marchal
created: 30.08.2018 12:55
updated: 31.08.2018 16:54
Entry 154/214: Enrichment and isolation of pTE16b for Ald cloning
In Project: ERBsen
With tags: Enrichment, Miniprep, pTE16b

Procedure

1. prepare 2 tubes with following annotations:
    1. Ec + pTE16b
    2. Ec + pTE16b
2. add 5ml LB + 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from trafo plate
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

49ng/µl

45ng/µl

Author: Daniel Marchal
Entry 155/214: Restriction digest of piGEM2103_LVL2_AccBirA-nHis (2)
In Project: ERBsen
With tags: restriction, digest, piGEM2103

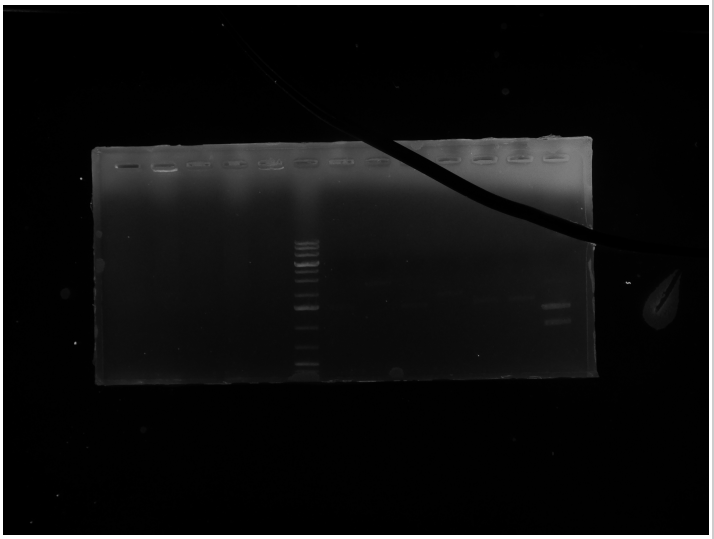
created: 30.08.2018 16:37
updated: 03.09.2018 13:56

See Restriction digest of piGEM2103_LVL2_AccBirA-nHis - entry #145 in project 'ERBsen' (Daniel Marchal, 30.08.2018)

Result:

Plasmid	Expected fragment length	Correct?
piGEM2103_1	892bp + 6052bp	No
piGEM2103_2	892bp + 6052bp	No
piGEM2103_3	892bp + 6052bp	No
piGEM2103_4	892bp + 6052bp	No
piGEM2103_5	892bp + 6052bp	No
piGEM2103_6	892bp + 6052bp	No
piGEM2103_7	892bp + 6052bp	No
piGEM2103_8	892bp + 6052bp	No
piGEM2103_9	892bp + 6052bp	No
piGEM2103_10	892bp + 6052bp	No

P1030084.JPG



Author: Daniel Marchal
created: 01.09.2018 11:20
updated: 04.09.2018 09:34
Entry 156/214: Enrichment and isolation of put. piGEM2100, 2101, 2102
In Project: ERBsen
With tags: Miniprep

To confirm correctness of piGEM2100, 2101 and 2102 3 colonies of each gibson trafo plate will be pickedm, enriched and their plasmids isolated. Afterwards they can be sequenced.

Procedure

1. prepare 9 tubes with following annotations:
    1. Ec + piGEM2100 1
    2. Ec + piGEM2100 2
    3. Ec + piGEM2100 3
    4. Ec + piGEM2101 1
    5. Ec + piGEM2101 2
    6. Ec + piGEM2101 3
    7. Ec + piGEM2102 1
    8. Ec + piGEM2102 2
    9. Ec + piGEM2102 3
2. add 5ml LB + 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from trafo plate
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

2100_1 60ng/µl

2100_2 63ng/µl

2100_3 82ng/µl

2101_1 60ng/µl

2101_2 52ng/µl

2101_3 63ng/µl

2102_1 69ng/µl

2102_2 82ng/µl

2102_3 77ng/µl

The sequencing showd, that each plasmid is correct!

Author: Daniel Marchal

created: 03.09.2018 07:22
updated: 03.09.2018 13:56

Entry 157/214: Trafo of GoGate piGEM2000, 2001, 2002 into Ec

In Project: ERBsen

With tags: piGEM2002, piGEM2000, e.coli, transformation, piGEM2001

Procedure

1. thaw 3 aliquots of Ec NEB Turbo on ice
    1. piGEM2000_LVL0_4_Mcr
    2. piGEM2001_LVL0_4_McrC
    3. piGEM2002_LVL0_4_McrN
2. add 5µl of golden gate mix
3. incubate 20 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates
9. incubate over day at 37°C

Author: Daniel Marchal
created: 03.09.2018 07:26
updated: 05.09.2018 15:43
Entry 158/214: Enrichment and isolation of put. Ald_pET16b
In Project: ERBsen
With tags: Miniprep

Procedure

1. prepare 6 tubes with following annotations:
    1. Ec + Ald_pET16b 1
    2. Ec + Ald_pET16b 2
    3. Ec + Ald_pET16b 3
    4. Ec + Ald_pET16b 4
    5. Ec + Ald_pET16b 5
    6. Ec + Ald_pET16b 6
2. add 5ml LB + 5µl Amp [100mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over day at 37°C shaking
5. Make miniprep

Author: Daniel Marchal
created: 04.09.2018 09:40
updated: 04.09.2018 09:44

Entry 159/214: Retrafo of piGEM1048, 2100, 2101, 2102
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, piGEM1048, piGEM2100, piGEM2101, piGEM2102

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2.

Vector	Resistance
piGEM1048_LVL0_8_Cam	Cm
piGEM2100_LVL0_4_AccBC	Cm
piGEM2101_LVL0_4_AccD	Cm
piGEM2102_LVL0_4_BirA	Cm

3. add 0.5µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal

created: 04.09.2018 09:57
updated: 04.09.2018 12:11

Entry 160/214: Enrichment and isolation of piGEM1036, 1048, 1057 for lvl1 and lvl2 digestion

In Project: ERBsen

With tags: Miniprep

Procedure

1. prepare 3 100ml buffled flasks with following annotations:
    1. Ec + piGEM1036_LVL0_7_ColE1
    2. Ec + piGEM1048_LVL0_8_Cam
    3. Ec + piGEM1057_LVL0_8_Kan
2. add 13ml LB + 13µl Kan [50mg/ml] / Cm [34mg/ml] (fresh prepared)
3. Inoculate from cryostock
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM1036 233ng/µl in 90µl

piGEM1057 218ng/µl in 90µl

Author: Daniel Marchal

created: 04.09.2018 10:13
updated: 04.09.2018 12:23

Entry 161/214: Restriction digest of piGEM1036 + piGEM1057 for LVL1 Golden Gate
In Project: ERBsen
With tags: restriction, digest, BsaI, piGEM1036, piGEM1057

Sample	Mastermix (17x)
7µl DNA (1500ng)	-
0.2µl BsaI	3.4µl BsaI
1µl 10xCutSmart Buffer	17µl 10xCutSmart Buffer
1.8µl H_2O	30,6µl H_2O

Author: Daniel Marchal
created: 05.09.2018 15:40
updated: 07.09.2018 10:15
Entry 162/214: Preparation of chemocompetent E. coli cells
In Project: ERBsen
With tags: CaCl2, chemocompetent, competent, Competent cells, E. coli, e.coli

Materials

- 250ml LB medium (autoclaved)
- 50ml TfBI (0.22µm filter sterilized, store at 4°C)
  - 30mM KAc (2,94g/L)
  - 50mM $MnCl_2$ (9,9g/L) (add after autoclave)
  - 100mM KCl (7,45g/L)
  - 10mM$CaCl_2$ (1,11g/L)
  - 15% v/v Glycerol
- 15ml TfBII (0.22µm filter sterilized without MOPS, add filter sterilized MOPS stock fresh, store at 4°C)
  - 10mM MOPS (10,46 g/50ml for 1M stock)
  - 75mM CaCl2 (8,32g/L)
  - 10mM KCl (0,74g/L)
  - 15% Glycerol
  - pH 7
- Autoclaved Erlenmeyers
- 37°C shaking incubator
- Pre-cooled centrifuge (suitable for 50ml falcons)
- Pre-cooled sterile Eppendorfs (ice)

Method

1. grow 50ml overnight culture in LB medium
2. transfer approximately 10ml cells to 250ml TYM medium
3. grow cells to midlog phase (OD_{600} = 0.5 - 0.6)
4. cool cells on ice (keep cells cold from now on)
5. centrifuge 15min, 3500g, 4°C (tubes have to be autoclaved first)
6. discard supernatant
7. resuspend cells in 50ml cold TfB1 on ice
8. centrifuge 15min, 3500g, 4°C, discard supernatant
9. resuspend cells in cold TfB2 on ice to an theoretical OD of 10 (app. 15ml)
10. make aliquots (50µl), freeze in liquid nitrogen and store at -80°C

Comments

- oN culture inoculated from Cryostock E. coli NEB Turbo
- from oN culture 250ml LB were inoculated with 10ml preculture at 3:30 PM and incubated at 30°C shaking (see step 2)
- cells harvested at OD=0.5
- in step 9 15ml were used

Result:

A test trafo with 25ng/µl and 0.1ng/µl pYTk was made and the plate with the lower concentration has ~400 colonies indicating that the cells are very competent

Author: Daniel Marchal
Entry 163/214: Lvl 1 GoldenGate of piGEM2145-2162
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation

created: 07.09.2018 15:58
updated: 09.09.2018 07:40

Golden Gate Reaction:

prepare 18 reactions according to the excel file

Start Golden Gate Reaction in Thermocycler:

Digest	37°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

GoGate_LVL_1_for_Echo.xlsx

Author: Daniel Marchal
created: 07.09.2018 16:01
updated: 07.09.2018 16:05

Entry 164/214: Trafo of Lvl 1 Golden Gate piGEM2145-2162
In Project: ERBsen
With tags: transformation, e.coli

Procedure

1. thaw 18 aliquots of Ec NEB Turbo on ice
2.

Vector	Resistance
piGEM2145	Cm
piGEM2146	Cm
piGEM2147	Cm
piGEM2148	Cm
piGEM2149	Cm
piGEM2150	Cm
piGEM2151	Cm
piGEM2152	Cm
piGEM2153	Cm
piGEM2154	Cm
piGEM2155	Cm
piGEM2156	Cm
piGEM2157	Cm
piGEM2158	Cm
piGEM2159	Cm
piGEM2160	Cm
piGEM2161	Cm
piGEM2162	Cm

3. add 1µl of plasmid
4. incubate 30 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 10 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Author: Daniel Marchal
created: 08.09.2018 15:33
updated: 10.09.2018 20:01

Entry 165/214: Restriction digest of Lvl 1 Golden Gate piGEM2145-2162
In Project: ERBsen
With tags: restriction, digest

Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

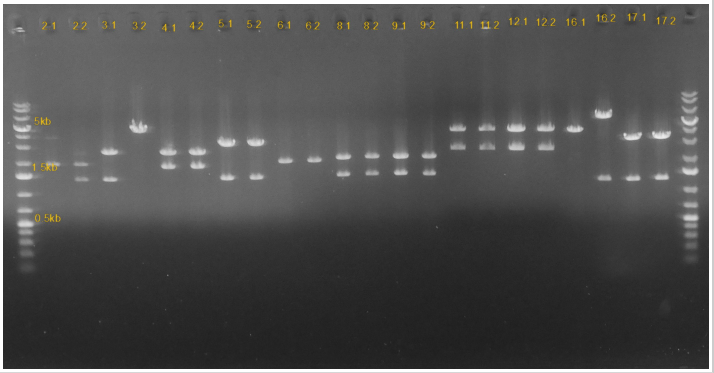
Sample	**Hind Master mix (19x)**	**EcoRV Master mix (5x)**
4µl DNA	-	-
0.2µl Enzyme	3.8µl FD-HindIII	1µl EcoRV-HF
1µl Buffer	19µl FD-Buffer prestained	5µl CutSmart Buffer
4.8µl H_2O	91.2µl H_2O	24µl H_2O

Number	Enzyme	Fragments	Correct?
2.1	HindIII	2,4 + 1,8 kb	no
2.2	HindIII	2,4 + 1,8 kb	no
3.1	HindIII	3,0 + 1,3 kb	yes
3.2	HindIII	3,0 + 1,3 kb	no
4.1	HindIII	2,4 + 1,8 kb	yes
4.2	HindIII	2,4 + 1,8 kb	yes
5.1	HindIII	3,0 + 1,3 kb	yes
5.2	HindIII	3,0 + 1,3 kb	yes
6.1	HindIII	2,4 + 1,8 kb	no
6.2	HindIII	2,4 + 1,8 kb	no
8.1	HindIII	2,0 + 1,3 kb	yes
8.2	HindIII	2,0 + 1,3 kb	yes
9.1	HindIII	2,0 + 1,3 kb	yes
9.2	HindIII	2,0 + 1,3 kb	yes
11.1	EcoRV	3,8 + 2,3 kb	yes
11.2	EcoRV	3,8 + 2,3 kb	yes
12.1	EcoRV	3,8 + 2,3 kb	yes
12.2	EcoRV	3,8 + 2,3 kb	yes
16.1	HindIII	3,3 + 1,3 kb	no
16.2	HindIII	3,3 + 1,3 kb	no
17.1	HindIII	3,3 + 1,3 kb	yes
17.2	HindIII	3,3 + 1,3 kb	yes

Results

- Sample 3, 4, 5, 8, 9, 11, 12 and 17 are correct
- Sample 1, 2, 6, 7, 10, 13, 14, 15, 16, 18 must be repeated

0908_Restriction_digest_Lvl_1.jpg



Author: Daniel Marchal

created: 08.09.2018 16:30

updated: 08.09.2018 16:31

Entry 166/214: Miniprep, digestion and gelelution of piGEM1048 for Lvl2 Golden Gate

In Project: ERBsen

With tags: Level 2, piGEM1048

Author: Daniel Marchal
Entry 167/214: Lvl 1 GoldenGate and trafo of piGEM2145-2162 (2)
In Project: ERBsen
With tags: level 1, Golden Gate

created: 09.09.2018 07:40
updated: 09.09.2018 07:43

See Lvl 1 GoldenGate of piGEM2145-2162 - entry #163 in project 'ERBsen' (Daniel Marchal, 09.09.2018) for the protocol.

See excel file for the pipeting scheme.

See Trafo of Lvl 1 Golden Gate piGEM2145-2162 - entry #164 in project 'ERBsen' (Daniel Marchal, 07.09.2018) for trafo protocol.

GoGate_LVL_1_for_Echo_(Versuch_2).xlsx

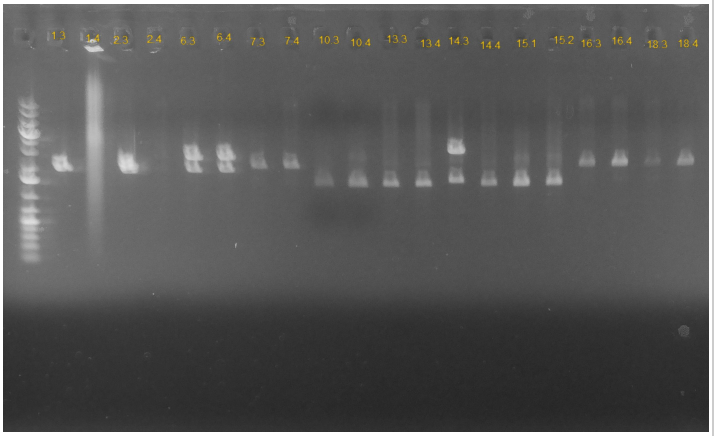
Author: Daniel Marchal
Entry 169/214: Restriction digest of some lvl2 TU4 plasmids
In Project: ERBsen
No tags associated

created: 10.09.2018 20:00
updated: 19.09.2018 17:27

Restriction digest of Lvl 1 Golden Gate piGEM2145-2162 - entry #165 in project 'ERBsen' (Daniel Marchal, 10.09.2018)

Number	Enzyme	Fragments	Correct?
1.3	HindIII	3.0 + 1.3	no
1.4	HindIII	3.0 + 1.3	no
2.3	HindIII	2.4 + 1.8	maybe
2.4	HindIII	2.4 + 1.8	no
6.3	HindIII	2.4 + 1.8	yes
6.4	HindIII	2.4 + 1.8	yes
7.3	HindIII	2.0 + 1.3	no
7.4	HindIII	2.0 + 1.3	no
10.3	EcoRV	3.8 + 1.3	no
10.4	EcoRV	3.8 + 1.3	no
13.3	MunI	2.8 + 1.3	no
13.4	MunI	2.8 + 1.3	no
14.3	MunI	2.8 + 1.3	yes
14.4	MunI	2.8 + 1.3	no
15.1	MunI	2.8 + 1.3	no
15.2	MunI	2.8 + 1.3	no
16.3	HindIII	3.3 + 1.3	no
16.4	HindIII	3.3 + 1.3	no
18.3	HindIII	3.3 + 1.3	no
18.4	HindIII	3.3 + 1.3	no

1008_Restriction_digest_Lvl_1.jpg



Author: Daniel Marchal
created: 11.09.2018 21:17
updated: 11.09.2018 21:25

Entry 170/214: Lvl 2 GoldenGate of 5TU plasmids with Echo
In Project: ERBsen
With tags: Golden Gate, Level 2, Echo, 5TU

The Golden Gates were pipeted with an Echo 525. We are not sure, in which direction the destination plate was placed in the device, maybe A1 is on Position H12.

	Well										
36 plasmids	A1	piGEM2147	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	A2	piGEM2147	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	A3	piGEM2147	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
	A4	piGEM2147	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	A5	piGEM2147	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	A6	piGEM2147	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
TU1	A7	piGEM2147	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2147	A8	piGEM2147	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2149	A9	piGEM2147	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2111	A10	piGEM2147	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	A11	piGEM2147	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	A12	piGEM2147	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
	B1	piGEM2149	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	B2	piGEM2149	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	B3	piGEM2149	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
	B4	piGEM2149	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	B5	piGEM2149	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
TU2	B6	piGEM2149	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2146	B7	piGEM2149	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2148	B8	piGEM2149	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2150	B9	piGEM2149	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2112	B10	piGEM2149	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	B11	piGEM2149	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	B12	piGEM2149	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC

	C1	piGEM2111	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	C2	piGEM2111	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	C3	piGEM2111	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
TU3	C4	piGEM2111	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2152	C5	piGEM2111	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2153	C6	piGEM2111	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2113	C7	piGEM2111	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
	C8	piGEM2111	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	C9	piGEM2111	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
TU4	C10	piGEM2111	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2158	McrN	piGEM2161	McrC
piGEM2158	C11	piGEM2111	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2158	McrN	piGEM2161	McrC
	C12	piGEM2111	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2158	McrN	piGEM2161	McrC
TU5_end											
piGEM2161											

Echo_Plan_-_LVL2_-_Reduzierte_Form.xlsx

Echo_Picking_Metabolic_5TU.csv

Author: Daniel Marchal
created: 11.09.2018 21:21
updated: 11.09.2018 21:24

Entry 171/214: Lvl 2 GoldenGate of 4TU plasmids with Echo
In Project: ERBsen
With tags: Golden Gate, Level 2, Echo, 4TU

The Golden Gates were pipeted with an Echo 525. We are not sure, in which direction the destination plate was placed in the device, maybe A1 is on Position H12.

	Well								
72 plasmids	A1	piGEM2147	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2155	Mcr
	A2	piGEM2147	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2156	Mcr
	A3	piGEM2147	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2155	Mcr
	A4	piGEM2147	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2156	Mcr
	A5	piGEM2147	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2155	Mcr
	A6	piGEM2147	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2156	Mcr
	A7	piGEM2147	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2155	Mcr
	A8	piGEM2147	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2156	Mcr
	A9	piGEM2147	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2155	Mcr
	A10	piGEM2147	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2156	Mcr
	A11	piGEM2147	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2155	Mcr
	A12	piGEM2147	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2156	Mcr
	B1	piGEM2147	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2155	Mcr
	B2	piGEM2147	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2156	Mcr
TU1	B3	piGEM2147	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2155	Mcr
piGEM2147	B4	piGEM2147	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2156	Mcr
piGEM2149	B5	piGEM2147	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2155	Mcr
piGEM2111	B6	piGEM2147	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2156	Mcr
	B7	piGEM2147	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2155	Mcr
	B8	piGEM2147	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2156	Mcr
	B9	piGEM2147	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2155	Mcr
	B10	piGEM2147	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2156	Mcr
	B11	piGEM2147	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2155	Mcr
	B12	piGEM2147	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2156	Mcr

	C1	piGEM2149	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2155	Mcr
TU2	C2	piGEM2149	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2156	Mcr
piGEM2146	C3	piGEM2149	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2155	Mcr
piGEM2148	C4	piGEM2149	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2156	Mcr
piGEM2150	C5	piGEM2149	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2155	Mcr
piGEM2112	C6	piGEM2149	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2156	Mcr
	C7	piGEM2149	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2155	Mcr
	C8	piGEM2149	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2156	Mcr
	C9	piGEM2149	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2155	Mcr
	C10	piGEM2149	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2156	Mcr
	C11	piGEM2149	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2155	Mcr
TU3	C12	piGEM2149	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2156	Mcr
piGEM2152	D1	piGEM2149	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2155	Mcr
piGEM2153	D2	piGEM2149	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2156	Mcr
piGEM2113	D3	piGEM2149	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2155	Mcr
	D4	piGEM2149	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2156	Mcr
	D5	piGEM2149	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2155	Mcr
TU4_end	D6	piGEM2149	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2156	Mcr
piGEM2155	D7	piGEM2149	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2155	Mcr
piGEM2156	D8	piGEM2149	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2156	Mcr
	D9	piGEM2149	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2155	Mcr
	D10	piGEM2149	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2156	Mcr
	D11	piGEM2149	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2155	Mcr
	D12	piGEM2149	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2156	Mcr
	E1	piGEM2111	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2155	Mcr
	E2	piGEM2111	AccBC	piGEM2146	AccD	piGEM2152	BirA	piGEM2156	Mcr
	E3	piGEM2111	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2155	Mcr
	E4	piGEM2111	AccBC	piGEM2146	AccD	piGEM2153	BirA	piGEM2156	Mcr
	E5	piGEM2111	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2155	Mcr
	E6	piGEM2111	AccBC	piGEM2146	AccD	piGEM2113	BirA	piGEM2156	Mcr

	E7	piGEM2111	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2155	Mcr
	E8	piGEM2111	AccBC	piGEM2148	AccD	piGEM2152	BirA	piGEM2156	Mcr
	E9	piGEM2111	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2155	Mcr
	E10	piGEM2111	AccBC	piGEM2148	AccD	piGEM2153	BirA	piGEM2156	Mcr
	E11	piGEM2111	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2155	Mcr
	E12	piGEM2111	AccBC	piGEM2148	AccD	piGEM2113	BirA	piGEM2156	Mcr
	F1	piGEM2111	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2155	Mcr
	F2	piGEM2111	AccBC	piGEM2150	AccD	piGEM2152	BirA	piGEM2156	Mcr
	F3	piGEM2111	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2155	Mcr
	F4	piGEM2111	AccBC	piGEM2150	AccD	piGEM2153	BirA	piGEM2156	Mcr
	F5	piGEM2111	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2155	Mcr
	F6	piGEM2111	AccBC	piGEM2150	AccD	piGEM2113	BirA	piGEM2156	Mcr
	F7	piGEM2111	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2155	Mcr
	F8	piGEM2111	AccBC	piGEM2112	AccD	piGEM2152	BirA	piGEM2156	Mcr
	F9	piGEM2111	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2155	Mcr
	F10	piGEM2111	AccBC	piGEM2112	AccD	piGEM2153	BirA	piGEM2156	Mcr
	F11	piGEM2111	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2155	Mcr
	F12	piGEM2111	AccBC	piGEM2112	AccD	piGEM2113	BirA	piGEM2156	Mcr

Echo_Plan_-_LVL2_-_Reduzierte_Form.xlsx

Echo_Picking_Metabolic_4TU.csv

Author: Daniel Marchal

created: 14.09.2018 11:23
updated: 17.09.2018 14:13

Entry 172/214: Enrichment of some lvl2 TU4 plasmids for test digest

In Project: ERBsen

With tags: Miniprep

Procedure

1. prepare 12 tubes with following annotations:
    1. Ec + 2.1
    2. Ec + 2.2
    3. Ec + 3.1
    4. Ec + 3.2
    5. Ec + 4.1
    6. Ec + 4.2
    7. Ec + 6.1
    8. Ec + 6.2
    9. Ec + 67.1
    10. Ec + 67.2
    11. Ec + 72.1
    12. Ec + 72.2
2. add 5ml LB + 5µl Cm [34mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep and test digest

Result

2.1 47ng/µl

2.2 60ng/µl

3.1 51ng/µl

3.2 61ng/µl

4.1 44ng/µl

4.2 37ng/µl

6.1 56ng/µl

67.2 56ng/µl

72.1 56ng/µl

72,2 57ng/µl

Author: Daniel Marchal
Entry 173/214: Restriction digest of some lvl2 TU4 plasmids
In Project: ERBsen
With tags: digest, restriction

created: 17.09.2018 11:32
updated: 19.09.2018 10:37

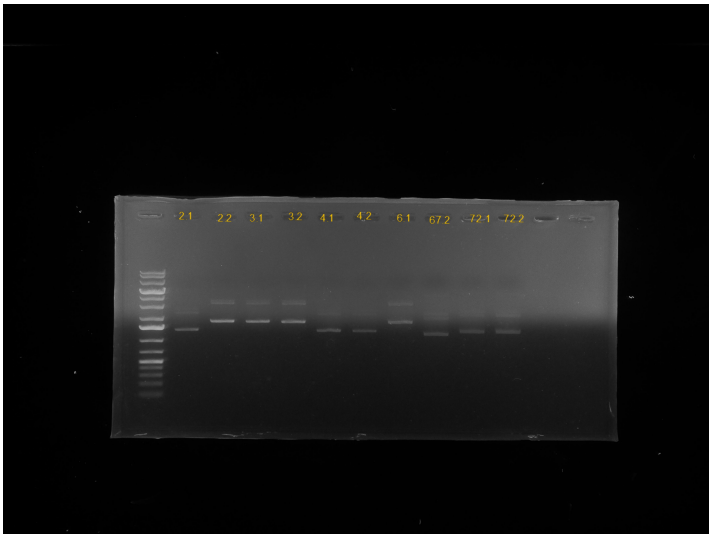
Procedure

1. Make master mix (see table)
2. Aliquot 9µl master mix into eppis, add 1µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (1.1% gel with 2 droplets of EtBr; GeneRuler 1kb plus; 135V, 45min)

Sample	Master mix MunI (7x)	Master mix NcoI/MunI (1x)	Master mix HindIII (4x)
4µl DNA	-	-	-
0.2µl Enzyme	1.4µl MunI	0.2µl NcoI + 0.2µl MunI	0.8µl HindIII
1µl FD-Buffer prestained	7µl FD-Buffer prestained	1µl FD-Buffer prestained	4µl FD-Buffer prestained
4.8µl H_2O	54.6µl H_2O	4.8µl H_2O	19.2µl H_2O

Number	c(pYTK)	Expected fragment length	Correct?
2.1	47	1340 + 4055 + 6551 bp	no
2.2	60	1340 + 4055 + 6551 bp	no
3.1	51	1340 + 4055 + 6551 bp	no
3.2	61	1340 + 4055 + 6551 bp	no
4.1	44	1340 + 4055 + 6551 bp	no
4.2	37	1340 + 4055 + 6551 bp	no
6.1	56	1451 + 1842 + 3990 bp	maybe
67.2	56	649 + 1340 + 2534 + 6643 bp	no
72.1	56	1449 + 1451 + 3604 bp	no
72.2	57	1449 + 1451 + 3604 bp	no

P1030158.JPG



Results

-

Author: Daniel Marchal

created: 19.09.2018 10:37
updated: 20.09.2018 08:16

Entry 174/214: Enrichment and miniprep of Echo Lvl1 plasmids for test digest

In Project: ERBsen

With tags: Miniprep, Echo, 1TU

Procedure

1. prepare 63 tubes with following annotations:
    1. Ec + C2
    2. Ec + C3
    3. Ec + C4
    4. Ec + C5
    5. Ec + C6
    6. Ec + C7
    7. Ec + C8
    8. Ec + C9
    9. Ec + C10
    10. Ec + C11
    11. Ec + C12
    12. Ec + D1
    13. Ec + D2
    14. Ec + D3
    15. Ec + D4
    16. Ec + D5
    17. Ec + D6
    18. Ec + D7
    19. Ec + D8
    20. Ec + D9
    21. Ec + D10
    22. Ec + D11
    23. Ec + D12
    24. Ec + E1
    25. Ec + E2
    26. Ec + E3
    27. Ec + E4
    28. Ec + E5
    29. Ec + E6
    30. Ec + E7
    31. Ec + E8
    32. Ec + E10
    33. Ec + E11
    34. Ec + E12
    35. Ec + F2
    36. Ec + F3
    37. Ec + F5
    38. Ec + F6
    39. Ec + F7
    40. Ec + F8

41. Ec + F9
42. Ec + F10
43. Ec + F11
44. Ec + F12
45. Ec + G2
46. Ec + G4
47. Ec + G5
48. Ec + G6
49. Ec + G7
50. Ec + G8
51. Ec + G10
52. Ec + G11
53. Ec + G12
54. Ec + H1
55. Ec + H2
56. Ec + H3
57. Ec + H4
58. Ec + H6
59. Ec + H7
60. Ec + H8
61. Ec + H9
62. Ec + H10
63. Ec + H11

2. add 5ml LB + 5µl Kan [50mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

The prepps didn't work :(

Maybe because we used a new miniprep kit from zymo. We will repeat the minipreps for few plates and compare the new kit with the old one.

Author: Daniel Marchal
created: 19.09.2018 17:27
updated: 20.09.2018 19:27
Entry 175/214: ____Overexpression of Matriptase C122S and cell lysis
In Project: ERBsen
With tags: french press, matriptase, cell extract

Procedure:

1. Prepare MOPS Buffer
2. Prepare two tube with 5ml LBv2+Carb [200µg/ml] and inoculate from Vn trafoplates with matriptase plasmid
3. Incubate over day at 37°C shaking
4. Prepare two 1000ml flask with 500ml LBv2+Carb and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture in the afternoon and incubate at 37°C shaking
6. When OD_{600}=0.4-0.6 induce the plasmids with 1mM IPTG (1ml of 0.5M IPTG) and incubate over night at 24°C
7. Harvest the cells in 1L centrifugation bottles (each bottle with 500ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
8. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 50ml Falcon.
9. Add 1.2ml 10xProtease-Inhibitor-Cocktail
10. Fill the tubes up to 12ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS Low salt

50mM MOPS/KOH

150mM NaCl

pH 7,8

10xProtease-Inhibitor_Cocktail

11. Use the frenchpress to break the cells at 900 psi and middle pressure if you use the small device or at 1200 psi and high pressure if you use the large device
    1. lever on "down" and rotate the wheel to increase pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
12. If the solution is clear, the cell breakage was successful
13. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
14. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
15. Run SDS-Gel

Results:

- Cells induced at OD=0.55
- Matriptase I had 8g cell mass, Matriptase II had 6g cells

Author: Daniel Marchal

Entry 176/214: Restriction digest and gel extraction of piGEM1036 + piGEM1048 for Lvl2 Golden Gates

In Project: ERBsen

With tags: StyI, restriction, digest

created: 19.09.2018 19:21
updated: 19.09.2018 19:54

Procedure

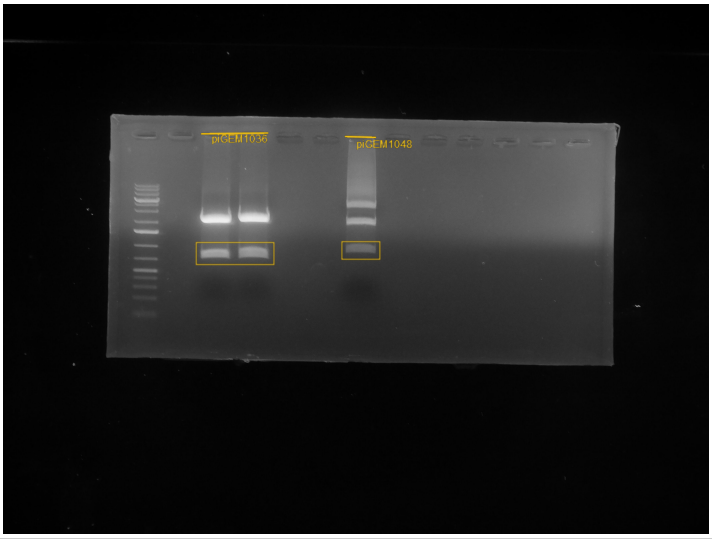
1. Mix DNA with Enzyme, Buffer and water
2. incubate 4h at 37°C
3. add 10µl 6xLoading Dye
4. run gel (1% gel with 5µl droplets of EtBr; GeneRuler 1kb plus; 135V, 28min)
5. make gel extraction

piGEM1036	piGEM1036	piGEM1048
10µl DNA	10µl DNA	10µl DNA
1µl BsaI	1µl BsaI	1µl BsaI
5µl CutSmart Buffer	5µl CutSmart Buffer	5µl CutSmart Buffer
4µl H_2O	4µl H_2O	4µl H_2O

Results

- Expected fragments: res part (piGEM1048) → 1114bp; ori part (piGEM1036) → 721bp

P1030176.JPG



Author: Daniel Marchal

created: 20.09.2018 08:07
updated: 21.09.2018 15:00

Entry 177/214: Trafo of LVL2 Golden Gates (TU4 & TU5) into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2.

Vector	**Resistance**
LVL2_TU4	Cm
LVL2_TU5	Cm

3. add 5µl of Golden Gate reaction
4. incubate 30 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 10 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate over day at 37°C
11. Pick colonies after 12h

Results:

Ec+LVL2_TU4 shows just one colony

Ec+LVL2_TU5 shows 33 colonies

We will make cPCR to check, if there are correct plasmids.

Author: Daniel Marchal
Entry 178/214: Lvl 2 GoldenGate of LVL2_TU4 & LVL2_TU5
In Project: ERBsen
With tags: Golden Gate, Level 2

created: 20.09.2018 08:09
updated: 20.09.2018 08:15

Golden Gate Reaction:

add following reagents to your annealing mix:

	LVL2_TU4_mmww	**LVL2_TU5_mmwmm**	
transcriptional unit 1 - AccBc	piGEM2147	piGEM2147	70 ng
transcriptional unit 2 - AccD	piGEM2148	piGEM2148	70 ng
transcriptional unit 3 - BirA	piGEM2153	piGEM2153	70 ng
transcriptional unit 4 - Mcr/McrN	piGEM2156	piGEM2158	
transcriptional unit 5 - McrC	-	piGEM2161	
Resistance	piGEM1048 digested	piGEM1048 digested	70 ng
Ori	piGEM1046 digested	piGEM1046 digested	70 ng
T7-Ligase (NEB)			1 µL
Esp3I (NEB)			0.5 µL
T4-Ligas Buffer			1 µL
ddH_20			Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	37°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

Author: Daniel Marchal
Entry 179/214: Lvl 1 GoldenGate of piGEM2244-2247
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation

created: 20.09.2018 17:36
updated: 20.09.2018 19:21

Golden Gate Reaction:

GoGate_LVL_1_piGEM21.xlsx

Prepare Golden Gate Reaction from the template in the excel file.
The yellow highlighted reagents were mixed in a mastermix (5x).

Start Golden Gate Reaction in Thermocycler:

Digest	37°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Author: Daniel Marchal
Entry 180/214: cPCR of Echo TU4 samples 8, 10, 49, 51 and 67
In Project: ERBsen
With tags: PCR, cPCR, Echo, 4TU

created: 20.09.2018 19:21
updated: 21.09.2018 14:54

Procedure:

1. Prepare mastermix
2. Aliquot 25µl into pcr tubes
3. Pick colonies in accordance to the table and inocculate them into the tubes
4. Start PCR program with initial 10min at 98°C
5. Run a gel

Sample	Mastermix (AccBC+AccD+BirA 28x)	Mastermix (Mcr+Ori)
6.25µl 2x-Mastermix	175µl 2x-Mastermix	175µl 2x-Mastermix
0.25µl Primer_for	7µl oiGEM2134_cPCR_accBC_for	7µl fw mcr without strep 4er part
0.25µl Primer_rev	7µl oiGEM2135_cPCR_birA_rev	7µl oiGEM2136_cPCR_colEI_rev
5.75µl H_2O	161µl H_2O	161µl H_2O

Sample	DNA template	Primer	Expected fragment length	Result
1	8_LVL2_TU4 1	AccBC+AccD+BirA	3787 bp	No
2	8_LVL2_TU4 2	AccBC+AccD+BirA	3787 bp	No
3	8_LVL2_TU4 3	AccBC+AccD+BirA	3787 bp	No
4	8_LVL2_TU4 4	AccBC+AccD+BirA	3787 bp	No
5	8_LVL2_TU4 5	AccBC+AccD+BirA	3787 bp	No
6	10_LVL2_TU4 1	AccBC+AccD+BirA	3788 bp	No
7	10_LVL2_TU4 2	AccBC+AccD+BirA	3788 bp	No
8	49_LVL2_TU4 1	AccBC+AccD+BirA	3789 bp	No
9	49_LVL2_TU4 2	AccBC+AccD+BirA	3789 bp	No
10	49_LVL2_TU4 3	AccBC+AccD+BirA	3789 bp	No
11	49_LVL2_TU4 4	AccBC+AccD+BirA	3789 bp	No
12	49_LVL2_TU4 5	AccBC+AccD+BirA	3789 bp	No
13	51_LVL2_TU4 1	AccBC+AccD+BirA	3790 bp	No
14	51_LVL2_TU4 2	AccBC+AccD+BirA	3790 bp	No
15	51_LVL2_TU4 3	AccBC+AccD+BirA	3790 bp	No
16	51_LVL2_TU4 4	AccBC+AccD+BirA	3790 bp	No

17	51_LVL2_TU4 5	AccBC+AccD+BirA	3790 bp	No
18	67_LVL2_TU4 1	AccBC+AccD+BirA	3396 bp	No
19	67_LVL2_TU4 2	AccBC+AccD+BirA	3396 bp	No
20	67_LVL2_TU4 3	AccBC+AccD+BirA	3396 bp	No
21	67_LVL2_TU4 4	AccBC+AccD+BirA	3396 bp	No
22	67_LVL2_TU4 5	AccBC+AccD+BirA	3396 bp	No
23	67_LVL2_TU4 6	AccBC+AccD+BirA	3396 bp	No
24	67_LVL2_TU4 7	AccBC+AccD+BirA	3396 bp	No
25	8_LVL2_TU4 1	Mcr+Ori	3904 bp	No
26	8_LVL2_TU4 2	Mcr+Ori	3904 bp	No
27	8_LVL2_TU4 3	Mcr+Ori	3904 bp	No
28	8_LVL2_TU4 4	Mcr+Ori	3904 bp	No
29	8_LVL2_TU4 5	Mcr+Ori	3904 bp	No
30	10_LVL2_TU4 1	Mcr+Ori	3904 bp	No
31	10_LVL2_TU4 2	Mcr+Ori	3904 bp	No
32	49_LVL2_TU4 1	Mcr+Ori	3904 bp	No
33	49_LVL2_TU4 2	Mcr+Ori	3904 bp	No
34	49_LVL2_TU4 3	Mcr+Ori	3904 bp	No
35	49_LVL2_TU4 4	Mcr+Ori	3904 bp	No
36	49_LVL2_TU4 5	Mcr+Ori	3904 bp	No
37	51_LVL2_TU4 1	Mcr+Ori	3904 bp	No
38	51_LVL2_TU4 2	Mcr+Ori	3904 bp	No
39	51_LVL2_TU4 3	Mcr+Ori	3904 bp	No
40	51_LVL2_TU4 4	Mcr+Ori	3904 bp	No
41	51_LVL2_TU4 5	Mcr+Ori	3904 bp	No
42	67_LVL2_TU4 1	Mcr+Ori	3904 bp	No
43	67_LVL2_TU4 2	Mcr+Ori	3904 bp	No
44	67_LVL2_TU4 3	Mcr+Ori	3904 bp	No
45	67_LVL2_TU4 4	Mcr+Ori	3904 bp	No
46	67_LVL2_TU4 5	Mcr+Ori	3904 bp	No

47	67_LVL2_TU4 6	Mcr+Ori	3904 bp	No
48	67_LVL2_TU4 7	Mcr+Ori	3904 bp	No

Results:

None of the samples showd bands but it is unclear, if the plasmids are wrong or if the pcr went wrong. We will repeat the cPCR for a small number of colonies.

Author: Daniel Marchal
Entry 181/214: SDS-Page of Matriptase for testing protein solubility
In Project: ERBsen
With tags: SDS-PAGE, matriptase

created: 21.09.2018 16:05
updated: 28.09.2018 13:01

Procedure:

1. Mix 5µl Sample with 10µl H_2O and 5µl 4xLoadingDye (see following list of samples)
    1. Matriptase before induction
    2. Matriptase after induction
    3. Matriptase after french press
    4. Matriptase cell lysate
    5. Matriptase before induction 1:10
    6. Matriptase after induction 1:10
    7. Matriptase after french press 1:10
    8. Matriptase cell lysate 1:10
2. Incubate 10min at 99°C
3. Spin down the droplets from the lid
4. Put the SDS-Gel into the device and sink the wells
5. Load the gel with sample and run at 120V for 45-90min
6. Wash 15min with H_2O and at least 4h in stain solution
7. Incubate over night in destain solution

Reagents:

stain solution

GelCode Blue Safe Protein Stain

destain solution

50% (v/v) MeOH

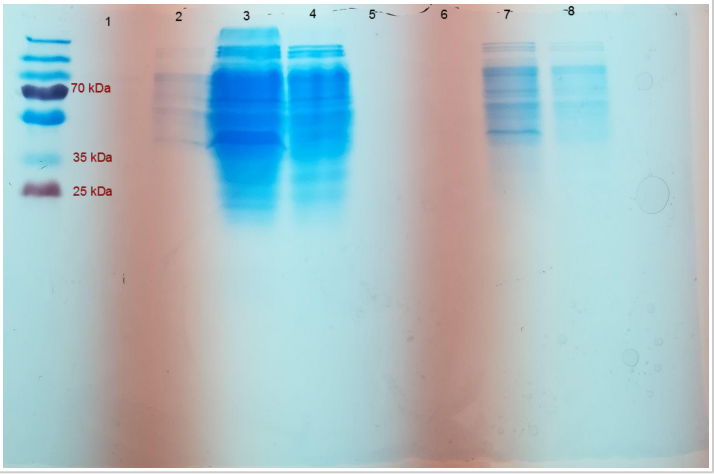
10% (v/v) Acetic acid

Results:

Expected protein mass: 27.53 kDa

For any reason there are no visible bands for samples before and after induction. Maybe the incubation time at 99°C was too short. Next time I will increase it to 20min. There is no significant band in the range of 27 kDa indicating, that no protein was expressed. We will repeat the experiment.

0928_SDS-PAGE_Matriptase.jpg



Author: Daniel Marchal
Entry 182/214: cPCR of handmade TU4 and TU5 plasmids
In Project: ERBsen
With tags: PCR, cPCR, piGEM2115, piGEM2117, AccBirASe

created: 24.09.2018 21:18
updated: 27.09.2018 11:15

Procedure:

1. Prepare mastermix
2. Aliqupt 12.5µl into pcr tubes
3. Pick colonies and inocculate them into the tubes
4. Start PCR program with initial 20min at 98°C
5. Run a gel

Sample	Mastermix (8x AccBC+AccD+BirA)	Mastermix (8x Mcr+Ori)
6.25µl 2x-Mastermix	50µl 2x-Mastermix	50µl 2x-Mastermix
0.25µl Primer_for	2µl oiGEM2134_cPCR_accBC_for	2µl fw mcr without strep 4er part
0.25µl Primer_rev	2µl oiGEM2135_cPCR_birA_rev	2µl oiGEM2136_cPCR_colEI_rev
5.75µl H_2O	46µl H_2O	46µl H_2O

Sample	DNA template	Primer	Expected fragment length	Correct?
1	TU4	AccBC+AccD+BirA	3788 bp	No
2	TU5_1	AccBC+AccD+BirA	3788 bp	No
3	TU5_2	AccBC+AccD+BirA	3788 bp	No
4	TU5_3	AccBC+AccD+BirA	3788 bp	No
5	TU5_4	AccBC+AccD+BirA	3788 bp	No
6	TU5_5	AccBC+AccD+BirA	3788 bp	No
7	TU5_6	AccBC+AccD+BirA	3788 bp	No
8	TU4	Mcr+Ori	3904 bp	No
9	TU5_1	Mcr+Ori	4520 bp	No
10	TU5_2	Mcr+Ori	4520 bp	No
11	TU5_3	Mcr+Ori	4520 bp	No
12	TU5_4	Mcr+Ori	4520 bp	No
13	TU5_5	Mcr+Ori	4520 bp	No
14	TU5_6	Mcr+Ori	4520 bp	No

Result:

no bands → everything wrong

Author: Daniel Marchal
created: 26.09.2018 13:11
updated: 27.09.2018 11:15

Entry 183/214: Retrafo Matriptase C122S for integrated Human Practices
In Project: ERBsen
With tags: matriptase, retrafo

1. Thaw one eppi of competent Vn cells
2. Add 50ng plasmid (Matriptase C122S)
3. Electroporate at 950V (we used the electroporator from our lab)
4. Add 500µl recovery medium
5. Incubate 2h at 37°C while shaking
6. Plate out on LBv2+Carb

Result:

The trafo didn't work, we will repeat it with the electroporator from another lab.

Author: Daniel Marchal
created: 27.09.2018 11:09
updated: 27.09.2018 11:14
Entry 184/214: Lvl 1 GoldenGate of piGEM2250_LVL1_AccD_middle_pos2_end
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation, piGEM2250

We want to build a lvl2 vector with accBC and accD. For that we need a lvl1 plasmid with a 5'con2 and 3'con5_end for accD

Golden Gate Reaction:

add following reagents to your annealing mix:

5' Connector	piGEM1066	70 ng
Promotor	piGEM1015	70 ng
RBS	piGEM1010	70 ng
CDS	piGEM2101	70 ng
Terminator	piGEM1035	70 ng
3' Connector	piGEM1080	70 ng
Resistance	piGEM1057 digested	25 ng
Ori	piGEM1036	70 ng
T7-Ligase (NEB)		1 µL
BsaI (NEB)		0,5 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

Author: Daniel Marchal
Entry 185/214: Lvl 1 GoldenGate of Mcr with p15a promoter
In Project: ERBsen
With tags: Golden Gate, Lvl 1 plasmids, transformation

created: 27.09.2018 22:38
updated: 02.10.2018 10:06

Golden Gate Reaction:

add following reagents to your annealing mix:

5' Connector	piGEM1068	70 ng
Promotor	piGEM1015	70 ng
RBS	piGEM1010	70 ng
CDS	piGEM2000	70 ng
Terminator	piGEM1035	70 ng
3' Connector	piGEM1080	70 ng
Resistance	piGEM1057 digested	70 ng
Ori	piGEM1046 (p15a ori)	70 ng
T7-Ligase (NEB)		1 µL
BsaI (NEB)		0,5 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	30 min
Inactivation	80°C	10 min

Author: Daniel Marchal

created: 27.09.2018 22:41
updated: 28.09.2018 12:49

Entry 186/214: Trafo of Lvl1 Goldengate piGEM2250_LVL1_AccD_middle_pos2_end

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2. add 5µl of GoldenGate Reaction
3. incubate 30 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (Kan)
9. incubate oN at 37°C

Author: Daniel Marchal
created: 28.09.2018 10:58
updated: 29.09.2018 19:00
Entry 187/214: TSS trafo of pYTK into Vn
In Project: ERBsen
With tags: TSS, PEG, trafo, V. natriegens

To test a new transformation method in V. natriegens, this protocol will be tested.

Procedure:

1. Inoculate target strain from a single colony in LB and grow till the culture is slightly turbid (OD600 0.1-0.3)
2. Chill on ice for 10 min
3. Add equal volume of ice cold 2xTSS (see below)
4. Vortex thoroughly but avoid warming up the cells
5. Incubate for 30 min up to several hours (whatever is convenient). Even overnight storage works (I tested that - Tobi).
6. add 1µl plasmid DNA (should be at least 10ng) to 1ml of competent cells in a pre-chilled Eppendorf tube and vortex briefly
7. Incubate on ice for 30min up to several hours (increasing incubation time enhances transformation efficiency!!!)
8. If selecting for ampicillin, plate immediately on selective plates, if selecting for any other resistance incubate at room temp or 30-37°C for 1 hour (phenotypic expression).
9. Before plating, cells can be concentrated by a 1 min spin in a benchtop centrifuge, remove supernatant and resuspend in the supernatant leftover by vortexing the tube.

Reagents:

2xTSS (Transformation and Storage Solution)

in 50ml ddH2O dissolve:

0.8g Bacto-Tryptone

0.5g Yeast extract

0.5g NaCl

20g PEG8000

ad 10ml 1M MgSO4

ad 10 ml DMSO

adjust pH to 6.5 (should already have that pH)

fill to 100ml

add 11 ml 10xV2 salts

filter sterilize through a 0.2µm filter

store at 4°C

We inoculated the culture directly from cryostock

We used 1µl pYTK and 5µl Golden Gate reaction of piGEM2251_LVL1_Mcr_middle_pos4_end_p15a

Results:

Unfortunately, there were no colonies on the plates, so the trafo didn't work

Author: Daniel Marchal
created: 28.09.2018 12:46
updated: 28.09.2018 12:48
Entry 188/214: Retrafo of Matriptase C122S into Vn
In Project: ERBsen
With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Since the last 3 approaches of transform the matriptase C122S plasmid into V. natriegens failed, a last try will be made with 150ng of DNA.

Procedure

1. thaw one aliquot of Vn on ice
2. add 1.8µl of plasmid (150ng)
3. electroporate at 950V
4. add immediatly 500µl Recovery medium
5. incubate 2h at 37°C shaking
6. spread out on LB selection plates
7. incubate oN at 37°C

Author: Daniel Marchal

created: 28.09.2018 12:50
updated: 29.09.2018 19:00

Entry 189/214: Trafo of Lvl1 Goldengate piGEM2251_LVL1_Mcr_middle_pos4_end_p15a into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw an aliquots of Ec NEB Turbo on ice
2. add 5µl of Golden Gate reaction
3. incubate 30 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates
9. incubate oN at 37°C

Author: Daniel Marchal
Entry 190/214: Lvl 1 GoldenGate of piGEM2250_LVL1_AccD_middle_pos2_end & piGEM2251_Mcr_middle_pos4_end_p15a
In Project: ERBsen
With tags: LVL1, piGEM2251, piGEM2250, Golden Gate

created: 29.09.2018 19:01
updated: 02.10.2018 10:06

Since, the last golden gates didn't work we will repeat it.

Protocol for piGEM2250_LVL1_AccD_middle_pos2_end see Lvl 1 GoldenGate of piGEM2250_LVL1_AccD_middle_pos2_end - entry #184 in project 'ERBsen' (Daniel Marchal, 27.09.2018)

Protocol for piGEM2251_LVL1_Mcr_middle_pos4_end_p15a seeLvl 1 GoldenGate of Mcr with p15a promoter - entry #185 in project 'ERBsen' (Daniel Marchal, 27.09.2018)

Author: Daniel Marchal

created: 29.09.2018 19:04
updated: 30.09.2018 22:42

Entry 191/214: Retrafo of pYTK, pTrc_McrCa, Matriptase_C122S & piGEM1047_p15a into Ec

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, pEntry, JZ90, JZ105, JZ147, JZ154

Procedure

1. thaw 4 aliquots of Ec NEB Turbo on ice
2.

Vector	**Resistance**
pYTK	Cm
pTrc_McrCa	Amp
Matriptase_C122S	Amp
piGEM1047_LVL0_7_p15a	Cm

3. add 1µl of plasmid
4. incubate 5 min on ice
5. heat shock at 42°C for 60 sec
6. incubate 2 min on ice
7. add 800µl LB
8. incubate 2h at 37°C shaking
9. spread out on LB selection plates
10. incubate oN at 37°C

Trafo worked, we picked colonies for miniprep.

Author: Daniel Marchal
created: 29.09.2018 19:06
updated: 02.10.2018 09:59
Entry 192/214: Enrichment and miniprep of piGEM2250 & piGEM2251
In Project: ERBsen
With tags: Miniprep, Enrichment, piGEM2250, piGEM2251

Procedure

1. prepare 4 tubes with following annotations:
    1. Ec + piGEM2250_LVL1_AccD_middle_pos2_end 1
    2. Ec + piGEM2250_LVL1_AccD_middle_pos2_end 2
    3. Ec + piGEM2251_LVL1_Mcr_middle_pos4_end_p15a
2. add 5ml LB + 5µl Kan [50mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate 12h at 37°C shaking
5. Make miniprep

Result

- piGEM2250 1 ~20ng/µl
- piGEM2250 2 0ng/µl
- piGEM2251 ~20ng/µl

Author: Daniel Marchal

created: 30.09.2018 22:42
updated: 02.10.2018 10:01

Entry 193/214: Enrichment and miniprep of pYTK, pTrc_McrCa, Matriptase_C122S & piGEM1047

In Project: ERBsen

With tags: Miniprep, PYTK, matriptase, piGEM1047

Procedure

1. prepare 4 tubes with following annotations:
    1. Ec + pYTK (Cm)
    2. Ec + pTrc_McrCa (Amp)
    3. Ec + Matriptase_C122S (Amp)
    4. Ec + piGEM1047
2. add 5ml LB + antibiotic (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

pYTK	390ng/µl
pTrc_McrCa	170ng/µl
Matriptase_C122S	108ng/µl
piGEM1046	481ng/µl

Author: Daniel Marchal
created: 30.09.2018 22:45
updated: 01.10.2018 08:41

Entry 194/214: Trafo of Lvl 1 GoldenGate piGEM2250 & piGEM2251

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, piGEM2250, piGEM2251

Procedure

1. thaw 2 aliquots of Ec NEB Turbo on ice
2. add 5µl of golden gate reaction
3. incubate 30 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (Cm)
9. incubate oN at 37°C

Results:

piGEM2250_AccD_LVL1_middle_pos2_end 30 white colonies, 1 green colony

piGEM2251_Mcr_LVL1_middle_pos4_end_p15a no colonies

from piGEM2250, we will pick 4 colonies and make a restriction digest

for piGEM2251 the golden gate has to be repeated

Author: Daniel Marchal
created: 02.10.2018 09:22
updated: 02.10.2018 11:59

Entry 195/214: Restriction digest of put. piGEM2250_AccD_LVL1_middle_pos2_end
In Project: ERBsen
With tags: piGEM2250, restriction, digest

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate 60min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye if necesarry
5. run gel (0.8% gel with 5µl of EtBr; GeneRuler 1kb plus; 135V, 25min)

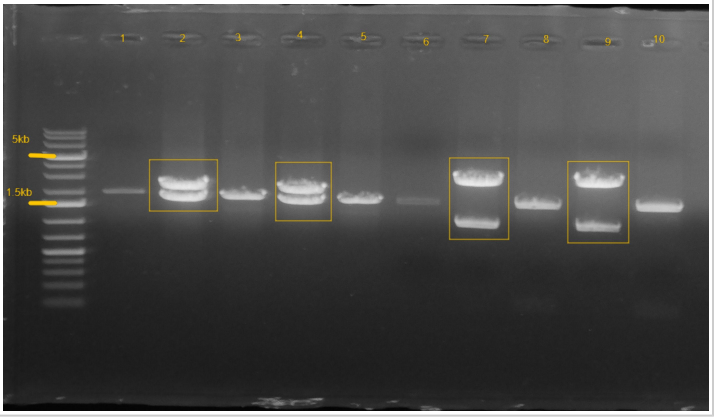
Sample	Master mix (6x)	Master mix (6x)
4µl DNA	-	-
0.2µl Enzyme	1.2µl HindIII	1.2µl BspHI
1µl CutSmart Buffer	6µl FD-buffer	6µl CutSmart Buffer
4.8µl H_2O	28.8µl H_2O	28.8µl H_2O

Number	Plasmid	Expected size	Correct?
1	piGEM2250 1 + HindIII	1757 + 2425 bp	No
2	piGEM2250 2 + HindIII	1757 + 2425 bp	Yes
3	piGEM2250 3 + HindIII	1757 + 2425 bp	No
4	piGEM2250 4 + HindIII	1757 + 2425 bp	Yes
5	piGEM2250 5 + HindIII	1757 + 2425 bp	No
6	piGEM2250 1 + BspHI	1100 + 3082 bp	No
7	piGEM2250 2 + BspHI	1100 + 3082 bp	Yes
8	piGEM2250 3 + BspHI	1100 + 3082 bp	No
9	piGEM2250 4 + BspHI	1100 + 3082 bp	Yes
10	piGEM2250 5 + BspHI	1100 + 3082 bp	No

Results

- Sample piGEM2250 2 and 4 are correct
- The next step will be to make a lvl 2 goldengate, so that we get a plasmid with accBC and accD

Zwischenablage01.jpg



Author: Daniel Marchal
Entry 196/214: Lvl 1 GoldenGate of iGEM2251_Mcr_middle_pos4_end_p15a
In Project: ERBsen
With tags: piGEM2251, Golden Gate

created: 02.10.2018 10:02
updated: 02.10.2018 10:06

Lvl 1 GoldenGate of Mcr with p15a promoter - entry #185 in project 'ERBsen' (Daniel Marchal, 02.10.2018)

Author: Daniel Marchal
Entry 197/214: Trafo of Lvl1 Goldengate piGEM2251_LVL1_Mcr_middle_pos4_end_p15a into Ec (2)
In Project: ERBsen
No tags associated

created: 02.10.2018 10:07
updated: 03.10.2018 13:49

Trafo of Lvl1 Goldengate piGEM2251_LVL1_Mcr_middle_pos4_end_p15a into Ec - entry #189 in project 'ERBsen' (Daniel Marchal, 29.09.2018)

The plate has 3 white colonies. We picked them and will do a control digest to verify plasmid correctness.

Author: Daniel Marchal
created: 02.10.2018 12:41
updated: 03.10.2018 13:48
Entry 198/214: Lvl 2 GoldenGate of piGEM2252_LVL2_AccBCD_middle
In Project: ERBsen
With tags: Golden Gate, Level 2, piGEM2252

Golden Gate Reaction:

add following reagents to your annealing mix:

transcriptional unit 1 - AccBc	piGEM2147	70 ng
transcriptional unit 2 - AccD	piGEM2250	70 ng
Resistance	piGEM1048 digested with bsaI	70 ng
Ori	piGEM1036 digested with bsaI	70 ng
T7-Ligase (NEB)		1 µL
BsaI (NEB)		1 µL
T4-Ligas Buffer		1 µL
ddH_20		Ad 10 µL

Start Golden Gate Reaction in Thermocycler:

Digest	42°C	2 min (60 cycles)
Ligation	16°C	5 min (60 cycles)
Final Digest	60°C	20 min
Inactivation	80°C	10 min

Author: Daniel Marchal
Entry 199/214: Trafo of Lvl 2 Goldengate piGEM2252
In Project: ERBsen
With tags: transformation, e.coli, pEntry

created: 03.10.2018 13:50
updated: 03.10.2018 13:57

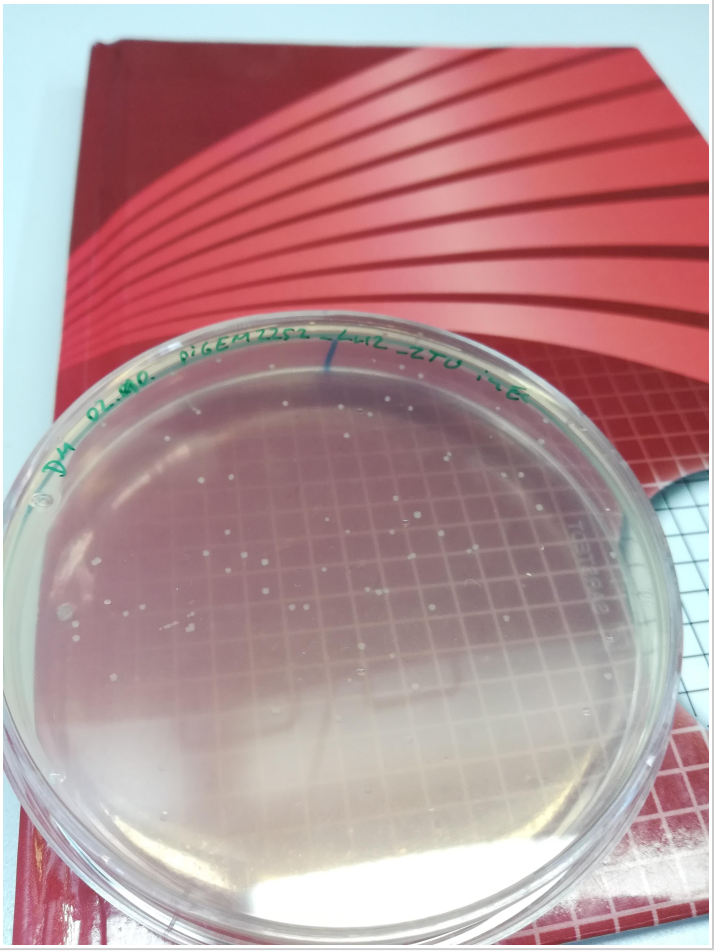
Procedure

1. thaw one aliquots of Ec NEB Turbo on ice
2. add 5µl of goldfen gate reaction
3. incubate 30 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates (Cm)
9. incubate oN at 37°C

Results:

Roughly 100 colonies on the plate, each colony is white. We picked 6 colonies for test digest.

Result



Author: Daniel Marchal
created: 03.10.2018 13:54
updated: 04.10.2018 12:49

Entry 200/214: Enrichment and isolation of put.
piGEM2251_LVL1_Mcr_middle_pos4_end_p15a &
piGEM2252_LVL2_AccBCD_middle
In Project: ERBsen
With tags: Miniprep, piGEM2251, piGEM2252

Procedure

1. prepare 9 tubes with following annotations:
    1. Ec + piGEM2251 1
    2. Ec + piGEM2251 2
    3. Ec + piGEM2251 3
    4. Ec + piGEM2252 1
    5. Ec + piGEM2252 2
    6. Ec + piGEM2252 3
    7. Ec + piGEM2252 4
    8. Ec + piGEM2252 5
    9. Ec + piGEM2252 6
2. add 5ml LB + 5µl Cm [34mg/ml] or Kan [50mg/µl] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2251 1 87ng/µl

piGEM2251 2 89ng/µl

piGEM2251 3 108ng/µl

piGEM2252 1 257ng/µl

piGEM2252 2 237ng/µl

piGEM2252 3 256ng/µl

piGEM2252 4 198ng/µl

piGEM2252 5 214ng/µl

piGEM2252 6 230ng/µl

Author: Daniel Marchal
created: 04.10.2018 12:24
updated: 06.10.2018 16:32

Entry 201/214: Restriction digest of put. piGEM2252_LVL2_AccBCD_middle
In Project: ERBsen
With tags: piGEM2252, restriction, digest

Procedure

1. Make master mix (see table)
2. Aliquot 7µl master mix into eppis, add 3µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye if required
5. run gel (0.8% gel with 5µl of EtBr; GeneRuler 1kb plus; 135V, 24min)

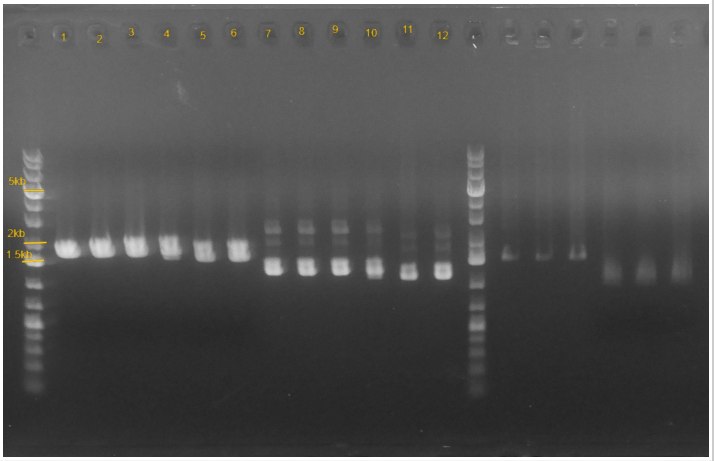
Sample	**Master mix (7x)**	**Master mix (7x)**
3µl DNA	-	-
0.2µl Enzyme	1.4µl EcoRV	1.4µl HindIII FD
1µl CutSmart Buffer	7µl CutSmart Buffer	7µl FD buffer prestained
5.8µl H_2O	40.6µl H_2O	40.6µl H_2O

Number	**Sample**	**Enzyme**	**Expected fragments**	**Correct?**
1	piGEM2252 1	EcoRV	2551 + 3691 bp	unsure
2	piGEM2252 2	EcoRV	2551 + 3691 bp	unsure
3	piGEM2252 3	EcoRV	2551 + 3691 bp	unsure
4	piGEM2252 4	EcoRV	2551 + 3691 bp	unsure
5	piGEM2252 5	EcoRV	2551 + 3691 bp	unsure
6	piGEM2252 6	EcoRV	2551 + 3691 bp	unsure
7	piGEM2252 1	HindIII	1840 + 4402 bp	unsure
8	piGEM2252 2	HindIII	1840 + 4402 bp	unsure
9	piGEM2252 3	HindIII	1840 + 4402 bp	unsure
10	piGEM2252 4	HindIII	1840 + 4402 bp	unsure
11	piGEM2252 5	HindIII	1840 + 4402 bp	unsure
12	piGEM2252 6	HindIII	1840 + 4402 bp	unsure

Results

Its not clear, if the plasmids are correct. We don't get the expected fragment pattern but we have no other explanations for the fragments. To clearify is, we sent one sample for sequencing.

1004_Restriction_Digest_2251_+_2252.jpg



Author: Daniel Marchal
created: 04.10.2018 13:19
updated: 11.10.2018 14:19

Entry 202/214: Restriction digest of put.
piGEM2251_LVL1_Mcr_middle_pos4_end_p15a
In Project: ERBsen
With tags: restriction, digest, piGEM2251

Procedure

1. Make master mix (see table)
2. Aliquot 7µl master mix into eppis, add 3µl plasmid DNA
3. incubate 30min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye if required
5. run gel (0.8% gel with 5µl of EtBr; GeneRuler 1kb plus; 135V, 24min)

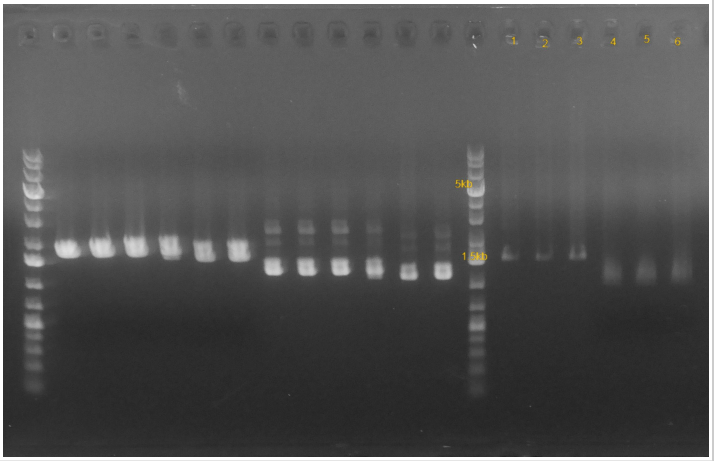
Sample	Master mix (4x)	Master mix (4x)
3µl DNA	-	-
0.2µl ENzyme	0.8µl MunI FD	1.4µl BspHI
1µl FD buffer prestained	4µl FD buffer prestained	4µl CutSmart Buffer
5.8µl H2O	23.2µl H2O	23.2µl H2O

Number	Sample	Enzyme	Expected fragments	Correct?
1	piGEM2251 1	MunI FD	1340 + 5069 bp	unsure
2	piGEM2251 2	MunI FD	1340 + 5069 bp	unsure
3	piGEM2251 3	MunI FD	1340 + 5069 bp	unsure
4	piGEM2251 1	BspHI	1117 + 5292 bp	unsure
5	piGEM2251 2	BspHI	1117 + 5292 bp	unsure
6	piGEM2251 3	BspHI	1117 + 5292 bp	unsure

Results

Its not clear, if the plasmids are correct. We don't get the expected fragment pattern but we have no other explanations for the fragments. To clearify is, we sent one sample for sequencing.

1004_Restriction_Digest_2251_+_2252.jpg



Author: Daniel Marchal

created: 05.10.2018 13:17
updated: 05.10.2018 13:19

Entry 203/214: Golden Gate of piGEM2251_LVL1_Mcr_middle_pos4_end_p15a & piGEM2252_LVL2_AccBCD

In Project: ERBsen

No tags associated

Since each approach of assembling piGEM2251 & piGEM2252 went wrong, we repeated the golden gates but added 20fmol instead of 70ng per part

Author: Daniel Marchal

created: 05.10.2018 13:19
updated: 06.10.2018 16:32

Entry 204/214: Trafo of put. piGEM2251 & 2252

In Project: ERBsen

With tags: transformation, e.coli, retrafo, retransformation, piGEM2252, piGEM2251

Procedure

1. thaw two aliquots of Ec NEB Turbo on ic
2. add 5µl of golden gate reaction
3. incubate 30 min on ice
4. heat shock at 42°C for 60 sec
5. incubate 10 min on ice
6. add 800µl LB
7. incubate 2h at 37°C shaking
8. spread out on LB selection plates
9. incubate oN at 37°C

Results:

It worked, we got ~30 colonies per plate. No green colonies. We picked 5 colonies and enriched them into LB + Kan/Cm

Author: Daniel Marchal
Entry 205/214: Pcr for construction of pAccMcr & pET16b_Ald_L38K
In Project: ERBsen
With tags: PCR, pAccMcr, pET16b_Ald_L38K

created: 06.10.2018 16:33
updated: 07.10.2018 11:45

Procedure:

1. Prepare mastermix
2. Aliqupt 44µl into 6 PCR tubes
3. Add DNA template and primer to the tubes (see primer table)
4. Start PCR program
5. Make control gel (3µl amplificate + 1µl 6xLoading Dye + 2µl H_2O)

Sample	DNA template	primer for	primer rev
Acc_Backbone	pNS3_Acc_BirA_Se	oiGEM2139_pNS3pTrc_for	oiGEM2140_pNS3pTrc_rev
Acc_Backbone	pNS3_Acc_BirA_Se	oiGEM2139_pNS3pTrc_for	oiGEM2140_pNS3pTrc_rev
Mcr_Insert	pTrc_Mcr_Ca	oiGEM2141_pTrcpNS3_for	oiGEM2142_pTrcpNS3_rev
Mcr_Insert	pTrc_Mcr_Ca	oiGEM2141_pTrcpNS3_for	oiGEM2142_pTrcpNS3_rev
pET16b_Backbone	pET16b	oiGEM2143_fw_backbone	oiGEM2144_rv_backbone
pET16b_Backbone	pET16b	oiGEM2143_fw_backbone	oiGEM2144_rv_backbone

Mastermix (7x)	Sample
70 µl buffer	10 µl buffer
7 µl dNTPs	1 µl dNTPs
-	2,5 µl primer for
-	2,5 µl primer rev
-	1 µl DNA
10.5 µl DMSO	1,5 µl DMSO
3.5 µl Q5 polymerase	0,5 µl Q5 polymerase
217 µl H_2O	31 µl H_2O (ad 50 µl)

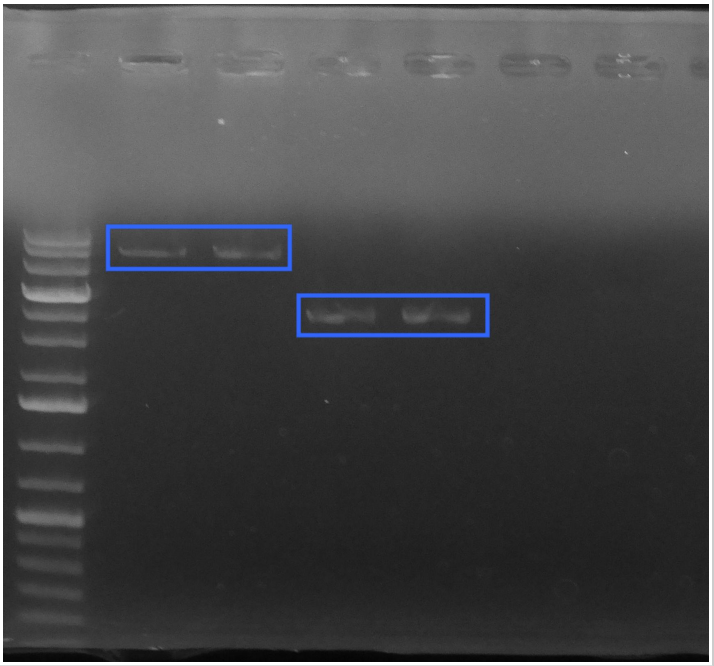
Results:

expected lengths:

1	9988 bp
2	9988 bp
3	4173 bp
4	4173 bp
5	5706 bp
6	5706 bp

- sample 1, 2, 3 & 4 look good
- sample 5 & 6 didn't work, we will repeat the PCR

1006_PCR.jpg



Author: Daniel Marchal
Entry 206/214: Gibson cloning of pAccMcr
In Project: ERBsen
With tags: pAccMcr, gibson cloning

created: 07.10.2018 13:35
updated: 08.10.2018 20:40

Procedure:

1. Use Mcr_Insert from the eluted PCR as "Fragment" and Acc_Backbone as "Backbone"
2. Mix pipeting scheme
3. Incubate 60min at 50°C
4. make trafo in Ec NEB Turbo (4µl, plating out on LB+Cm)

Fragment	**bp**	**ng/µl**	**pmol/µl**
Acc_Backbone	9988	10	0,003034
Mcr_Insert	4173	20	0,014523

Pipettierschema	
Fragment [µl]	6
Backbone [µl]	4
Gibson Mastermix 2x [µl]	10
H_2O [µl]	0
Total volume [µl]	20

Results:

The trafo worked, there are 28 colonies. We picked 11 colonies for test digests.

Author: Daniel Marchal
Entry 207/214: New restriction digest of put. piGEM2251 & piGEM2252
In Project: ERBsen
With tags: piGEM2251, piGEM2252

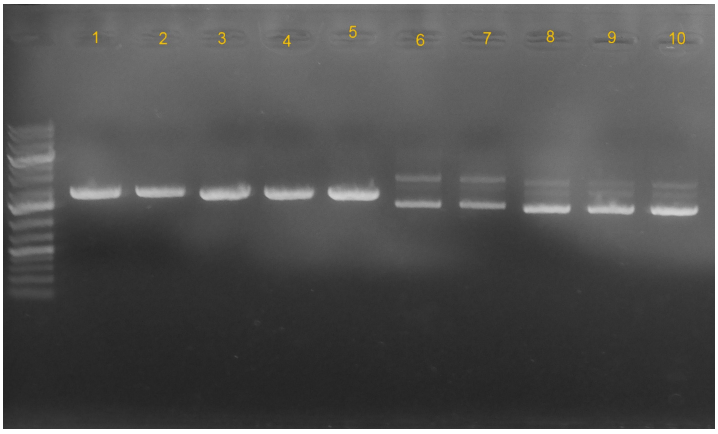
created: 07.10.2018 13:55
updated: 08.10.2018 20:40

See Restriction digest of put. piGEM2251_LVL1_Mcr_middle_pos4_end_p15a - entry #202 in project 'ERBsen' (Daniel Marchal, 06.10.2018) for 2251 protocol

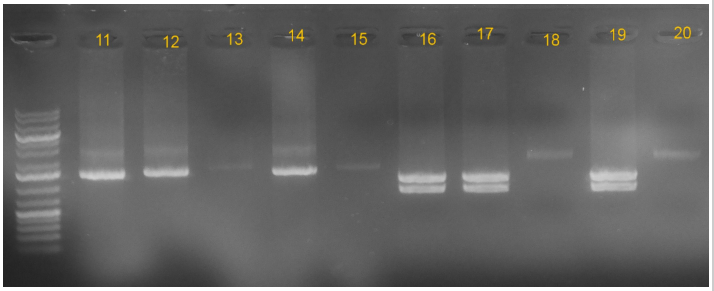
See Restriction digest of put. piGEM2252_LVL2_AccBCD_middle - entry #201 in project 'ERBsen' (Daniel Marchal, 06.10.2018) for 2252 protocol

Sample	Plasmid	Enzyme	Expected fragments	Correct?
1	piGEM2251 1	EcoRV	2551 + 3691 bp	yes
2	piGEM2251 2	EcoRV	2551 + 3691 bp	yes
3	piGEM2251 3	EcoRV	2551 + 3691 bp	yes
4	piGEM2251 4	EcoRV	2551 + 3691 bp	yes
5	piGEM2251 5	EcoRV	2551 + 3691 bp	yes
6	piGEM2251 1	HindIII	1840 + 4402 bp	yes
7	piGEM2251 2	HindIII	1840 + 4402 bp	yes
8	piGEM2251 3	HindIII	1840 + 4402 bp	yes
9	piGEM2251 4	HindIII	1840 + 4402 bp	yes
10	piGEM2251 5	HindIII	1840 + 4402 bp	yes
11	piGEM2252 1	MunI	1340 + 5069 bp	no
12	piGEM2252 2	MunI	1340 + 5069 bp	no
13	piGEM2252 3	MunI	1340 + 5069 bp	no
14	piGEM2252 4	MunI	1340 + 5069 bp	no
15	piGEM2252 5	MunI	1340 + 5069 bp	no
16	piGEM2252 1	BspHI	1117 + 5292 bp	no
17	piGEM2252 2	BspHI	1117 + 5292 bp	no
18	piGEM2252 3	BspHI	1117 + 5292 bp	no
19	piGEM2252 4	BspHI	1117 + 5292 bp	no
20	piGEM2252 5	BspHI	1117 + 5292 bp	no

1007_Acc_digest.jpg



1007_piGEM2251_digest.jpg



Results:

All samples of piGEM2252 look good, we will sent one sample to sequencing. All samples of piGEM2251 look wrong, we will have to repeat the cloning. We sent sample 1 rfom piGEM2252 to sequencing.

Author: Daniel Marchal

created: 08.10.2018 20:40
updated: 13.10.2018 16:44

Entry 208/214: Restriction digest and gel extraction of piGEM1046 for Lvl2 Golden Gates

In Project: ERBsen

With tags: restriction, digest

Procedure

1. Make reaction mix
2. incubate 4h at 37°C
3. load onto a gel and make gel extraction

Sample
20.5µl DNA
2µl BsaI-HFv2
2.5µl CutSmart Buffer

Results

- c= 10 ng/µl

Author: Daniel Marchal
created: 08.10.2018 20:53
updated: 10.10.2018 23:17
Entry 209/214: Retrafo of AccBirAEc, AccBirASe, piGEM2252 into Vn
In Project: ERBsen
With tags: electrocompetent, electroporation, retrafo, retransformation, V. natriegens, weinstock

Procedure

1. thaw 3 aliquots of electrocompetent Vn on ice
2. add 50ng plasmid DNA into the aliquots
3. transfer suspension into an electroporation cuvette (0.1cm gap size)
4. electroporate with following parameters: 950 V, 25 µF, 200 Ω
5. Add 500µl recovery medium (BHI supplemented with v2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2), and 680 mM sucrose) and transfer into a 1.5ml tube.
6. Incubate 2h at 30-37°C while shaking
7. Plate out on selection plates (Cam)
8. Incubate oN at 37°C

Results:

All trafos worked, we picked 2 colonies for proteinexpression

Author: Daniel Marchal
Entry 210/214: Golden Gate of piGEM2253 & piGEM2251
In Project: ERBsen
No tags associated

created: 08.10.2018 20:55
updated: 10.10.2018 23:26

piGEM2253_Lvl2_AccBCD_middle_p15a	
piGEM2147	0.67µl
piGEM2250	0.52µl
piGEM1048 digested	1.14µl
piGEM1046 digested	µl
T7 ligase	1µl
BsaI	0.5µl
T4 buffer	1µl

piGEM2251_LVL1_Mcr_middle_p15a	
piGEM1068	1.04µl
piGEM1015	1.17µl
piGEM1010	0.99µl
piGEM2000	0.42µl
piGEM1080	1.56µl
piGEM1035	0.75µl
piGEM1046	0.86µl
piGEM1057 digested	0.36µl
GoGate buffer	2µl
GoGate mix	1µl
H2O	9,85µl

Results:

Both trafos worked, we will pick 5 colonies from each plate

Author: Daniel Marchal

created: 10.10.2018 23:17
updated: 11.10.2018 13:11

Entry 211/214: ____Overexpression of AccEc, AccSe, AccCg in V. natriegens for SDS-PAGE

In Project: ERBsen

With tags: Overexpression, AccBirAEc, AccBirASe, AccCg

Procedure:

1. Prepare six 150ml flasks with 20ml LB+Cm
2. Pick two colonies from each trafo plate (AccEc/AccSe/AccCg) and inoculate the flasks
3. Incubate at 37°C shaking
4. From AccCg take 1ml samples when OD=0.2/1.5/8/14
5. Take a 1ml sample when OD=0.4 - 0.6 and induce with 500µM IPTG
6. Incubate at 24°C shaking
7. 16h after induction take again a 1ml sample
8. Centrifuge all samples, discard supernatant and dilute cells to an OD of 10
9. Run SDS-PAGE

Author: Daniel Marchal
created: 10.10.2018 23:25
updated: 11.10.2018 13:36
Entry 212/214: Enrichment and isolation of put. piGEM2251 & piGEM2253
In Project: ERBsen
With tags: Miniprep, Enrichment, piGEM2251, piGEM2253

Golden Gate of piGEM2253 & piGEM2251 - entry #210 in project 'ERBsen' (Daniel Marchal, 10.10.2018)

Procedure

1. prepare 10 25ml flasks with following annotations:
    1. Ec + piGEM2251_LVL1_Mcr_middle_p15a 1
    2. Ec + piGEM2251_LVL1_Mcr_middle_p15a 2
    3. Ec + piGEM2251_LVL1_Mcr_middle_p15a 3
    4. Ec + piGEM2251_LVL1_Mcr_middle_p15a 4
    5. Ec + piGEM2251_LVL1_Mcr_middle_p15a 5
    6. Ec + piGEM2253_LVL2_Acc_middle_p15a 1
    7. Ec + piGEM2253_LVL2_Acc_middle_p15a 2
    8. Ec + piGEM2253_LVL2_Acc_middle_p15a 3
    9. Ec + piGEM2253_LVL2_Acc_middle_p15a 4
    10. Ec + piGEM2253_LVL2_Acc_middle_p15a 5
2. add 10ml LB + 5µl Cm [34mg/ml]/Kan[50mg/ml] (fresh prepared)
3. Inoculate from trafo plates
4. Incubate over night at 37°C shaking
5. Make miniprep

Result

piGEM2251 1	38ng/µl
piGEM2251 2	60ng/µl
piGEM2251 3	76ng/µl
piGEM2251 4	51ng/µl
piGEM2251 5	44ng/µl
piGEM2253 1	120ng/µl
piGEM2253 2	154ng/µl
piGEM2253 3	178ng/µl
piGEM2253 4	171ng/µl
piGEM2253 5	176ng/µl

Author: Daniel Marchal
created: 11.10.2018 13:37
updated: 11.10.2018 20:31

Entry 213/214: Restriction digest of put. piGEM2251 + piGEM2253
In Project: ERBsen
With tags: piGEM2252, restriction, digest, piGEM2253

Procedure

1. Make master mix (see table)
2. Aliquot 6µl master mix into eppis, add 4µl plasmid DNA
3. incubate 60min at 37°C
4. mix 10µl sample with 2µl 6xLoading Dye
5. run gel (0.8% gel with 5µl droplets of EtBr; GeneRuler 1kb plus; 135V, 25min)

Sample	**Master mix (6x) 2251**	**Master mix (6x) 2253**
4µl DNA	-	-
0.2µl Enzyme	1.2µl FD MunI	1.2µl FD HindIII
1µl CutSmart Buffer	6µl FD buffer	6µl FD buffer
4.8µl H_2O	28.8µl H_2O	28.8µl H_2O

Number	**Sample**	**Enzyme**	**Expected fragments**	**Correct?**
1	piGEM2251 1	FD MunI	1340 + 5069 bp	No
2	piGEM2251 2	FD MunI	1340 + 5069 bp	No
3	piGEM2251 3	FD MunI	1340 + 5069 bp	No
4	piGEM2251 4	FD MunI	1340 + 5069 bp	No
5	piGEM2251 5	FD MunI	1340 + 5069 bp	No
6	piGEM2253 1	FD HindIII	1840 + 4606 bp	Yes
7	piGEM2253 2	FD HindIII	1840 + 4606 bp	Yes
8	piGEM2253 3	FD HindIII	1840 + 4606 bp	Yes
9	piGEM2253 4	FD HindIII	1840 + 4606 bp	Yes
10	piGEM2253 5	FD HindIII	1840 + 4606 bp	Yes

Results

-

Author: Daniel Marchal
created: 13.10.2018 16:11
updated: 13.10.2018 16:38
Entry 214/214: Activity assay of pAccBCD_Mcr
In Project: ERBsen
With tags: Acc, enzyme activity, activity, assay, cell extract, model prediction

To validate if the model predicted pathway works, we will overproduce cells harboring pAccBCD_Mcr and break them. With the cell extract we make an activity assay

Procedure:

1. Prepare MOPS Buffer
2. Prepare a tube with 10ml LBv2 and inoculate from Vn + pAccBCD_Mcr plates in the morning
3. Incubate over day at 37°C shaking
4. Prepare a 1000ml flask with 500ml LBv2 and prewarm it at 37°C
5. Inoculate mainculture with 1ml preculture in the afternoon and incubate at 37°C shaking
6. Harvest the cells in 1L centrifugation bottles (each bottle with 500ml) at 8000g/12min/4°C. Weigh the bottles before and after harvesting to estimate the cell weight. It is needed to dilute them in the right amount of buffer
7. Resuspend the cells with a 5ml glas pipet in 2ml MOPS buffer und pipet them into a 50ml Falcon.
8. Add 1.2ml 10xProtease-Inhibitor-Cocktail
9. Fill the tubes up to 12ml with buffer (rule of thumb: per gramm cells add 3ml buffer)

Reagents:

MOPS

200mM MOPS/KOH

150mM NaCl

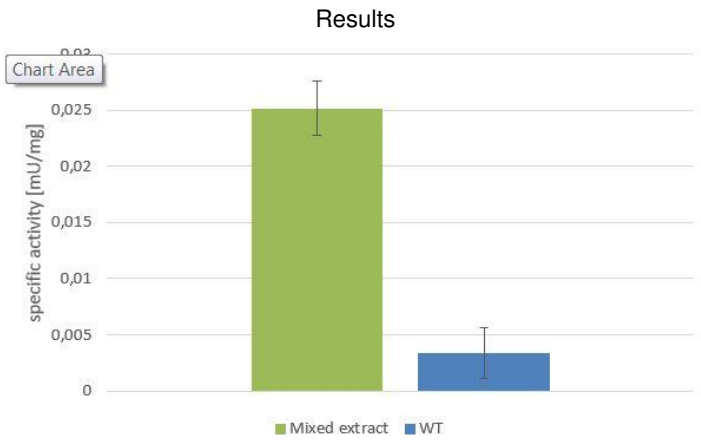
pH 7,8

10xProtease-Inhibitor_Cocktail

10. Use the frenchpress to break the cells at 900 psi and middle pressure if you use the small device or at 1200 psi and high pressure if you use the large device
    1. lever on "down" and rotate the wheel to increase pressure → the area goes down
    2. Clean the french press device (the thing where the suspension is filled in) and grease the seals with oil
    3. Close the screw, raise the lever to the top, remove the bottom part, fill in the suspension, push the lever until the suspension reaches the screw and close the device with the bottom part
    4. Position the device without calling up a collision
    5. wheel to lowest pressure, lever on middle, turn the wheel until the are starts raising. Turn carefully until 900 psi are reached
    6. Fix a cannula to the pipe and hold a new, cooled tube under the cannula
    7. Open the screw, be careful that the solution is just dropping not rinsing and that the pressure oscillates as few as possible around 900psi
    8. When finished clean all parts of the device with ethanol and water and let them dry. If necesarry replace the ball at the tip of the screw
11. If the solution is clear, the cell breakage was successful
12. Pellet the cell fragments via ultracentrifugation at 100,000g /45min/4°C
13. Sterilfiltrate the solution with an orange filter (0,45µm pore diameter)
14. For the enzyme assay use the software "Cary UV" with the program "kinetics"
15. Mix 40-229µl of your cell lysate together with MOPS buffer, $MgCl_2$, NADPH, ATP and $KHCO_3$ and measure slope (background)
16. Add Acetyl-CoA to start the reaction and again measure slope to calculate specific activity (see excel sheet)
17. If there is enzyme activity you can make a bradford to normalize your results
18. As a positive control you can add purified Pcc_Me and/or McrCa
19. To store the cell lysate add 300µl glycerol and store at -20°C

Results:

- For harvesting the cells, the rotor Beckman Coulter JLA-10.500 was used
- Centrifugation bottle weights: 74.05g/75.19g
- Cell weights: LS=6.39g



Results_pAccBCD_Mcr.xlsx

Calculations_pAccBCD_Mcr.xlsx