Week 1 - Electroporation

Project: iGEM2021
Authors: Iris Noordermeer
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TUESDAY, 13/7/2021

Project: Lab Notebook
Authors: Quint van Loosen and Hoda Ekhlasi
Created at: 2021-07-13

Goal

- Preparations for the Electroporation
    - Culture bacterial strains
    - Make LB medium

Material

Consumables (media, antibiotics, etc.)

- LB broth
- LB medium
- Ampicilline
- Chloramphenicol
- 98% glycerol

Bacterial strains

- DH5α
- pKIKOarsBKm
- pBAM1
- PSIJ8
- pGRG25

Equipment

- Autoclave
- Incubator

Protocols

- 25g LB broth, filled up to 1L with water (non demi-Q was used per accident)
- Autoclave
- 250 ml of LB medium was poured in a 1L flask
- It was autoclaved
- After cooling down 1 ml of DHFalpha bacteria were pipetted into the flask
- Flask was placed at 37C at 300rpm overnight
- 98% Glycerol was diluted to 500ml of 10%
- Autoclaved
- Placed in fridge at 4 °C

Together with Hoda:

- 5ml of sterilized LB medium (above) was pipetted into 25ml tubes.

- Bacteria of pKIKOarsBKm, pBAM1, PSIJ8 and pGRG25 were stipped with an autoclaved toothpick, which was dropped into the tube.
- 5 ul antibiotics was added to each tube
- pKIKOarsBKm: Chloramphenicol and Ampicillin (5 ul each)
- pBAM1: Chloramphenicol and Ampicillin (5 ul each)
- PSIJ8 and pGRG25: Ampicillin
- Tubes were labelled and went into the 30C incubator at 250rpm

WEDNESDAY, 14/7/2021

Electrocompetent cells - Electroporation (2)

Project: Lab Notebook
Authors: Quint van Loosen
Created at: 2021-07-14

Goals

- Make electrocompetent cells

Materials

- 10% glycerol
- Liquid nitrogen

Protocol

- Liquid bacterial culture requires an OD600 between 0,5-0,7
- Put liquid culture on ice for 15m
- Then divide the culture (250 ml) over 6 50ml tubes that had been cooled in the fridge.
- Centrifuge the tubes at 5000rpm for 10 minutes at 4 °C.
- Discard supernatant
- 20,83 ml 10% glycerol was put in each tube for a total of 125ml glycerol. (this might be an error, 250 might be needed).
- Dissolve the pellet by pipetting up and down.
- The tubes were centrifuged at 5000rpm for 10 minutes at 4 °C.
- Discard supernatant
- Resuspend cells in 20,83ml 10% glycerol and centrifuged at 5000rpm for 10 minutes.
- Discard supernatant.
- 1ml 10% glycerol was added to each of the 6 tubes. Cells were resuspended by pipetting up and down.
- 50ul of electrocompetent cells was pipetted into Eppendorf tubes.
- Tubes containing electrocompetent cells were snap-frozen in liquid nitrogen and stored at -80C.

Results

- Of last night’s culture had an OD600 of 1,5.
- After futher dilution with 10x sterile LB medium and 1,5H growth in 37 °C the OD600 was 0,514.

Extra

Of the cultures made at 13-07 pKIKOarsBKm and pBAM1 the plates were wrongly labelled. And the wrong antibiotics were added resulting in dead cells. They need Kanamycin in stead of Chloramphenicol.
The protocol was done again for the two strains. The right antibiotics (Kanamycin and Ampicillin) were added.
The 30C 250rpm incubater had broken. So they were placed on the shelve at RT to wait for allowance to use upstairs incubator.

THURSDAY, 15/7/2021

Electroporation (3)

Project: Lab Notebook
Authors: Quint van Loosen
Created at: 2021-07-15

Goal

- Make glycerol stocks of pKIKOarsBKm and pBAM1
- Electroporation

Materials

- 20% glycerol
- ThermoScientific GeneJET plasmid miniprep kit
- pSB1C3 plasmid
- DH5α cells
- chloramphenicol
- LB agar medium

Protocol

Make glycerol stocks of pKIKOarsBKM and pBAM1

- Pipetting 0.4ml cell culture and 0.8ml 20% glycerol into sterile Eppendorf tubes
- Resuspend by pipeting up and down
- Transfer the cells to the original cell culture tube
- Centrifuge the cells 2 times at 1500 rpm to create a pellet
- Discard supernatant
- Cells resuspended according to ThermoScientific GeneJET plasmid Miniprep kit

Check the electrocompetent cells

- thaw pSB1C3 isolated plasmids (Chloramphenicol resistance)
- Thaw 4x 50 ul electrocompetent DH5-alpha cells on ice (one tube appeared to be empty so we only thawed 3)
- Turn on electroporator and set to 1.0, 1.5 and 2.0 kv , 200 ohms and 25 µF.

Recovery LB was not placed in 37°C water bath.

- Place 3 microcentrifuge tubes and 3 mm-electroporation cuvettes on ice.
- Pipet up and down to mix and add 50 µl to new microcentrifuge tubes.
- Add 1 µl of a 348 ng/ul pSB1C3 isolated plasmid DNA solution to the cells in the microcentrifuge tube.
- Transfer the DNA-cell mixture to the cold cuvette, wipe water from the exterior of the cuvette and place it in the electroporation module, and press pulse (don’t hold the button down).
- Immediately add 975 µl LB, mix by pipetting up and down once and transfer to microcentrifuge tubes.
- Rotate in the 37°C incubator for 1 h.
- Plate two chloramphenicol plates:
  25ul antibiotic stock was added to 250ml LB+agar medium when it had cooled down enough to be touched for 10s.

Results

- pKIKOarsBKM and pBAM1 had grown overnight.

Extra

Making glycerol stocks

- Per accident too many 20% glycerol stocks were made of pKIKOarsBKm. To retrieve cells, Eppendorf tubes were spun at 8000 rpm for 10 minutes.
  Glycerol was removed via pipet, 0.4ml LB medium was added and pellet containing cells was resuspended

Check electrocompetent cells

- Note: for the 1kv electroporation it looked like the buttons were not pressed long enough.
- Note: after pipetting from the cuvettes it looked like some was still inside, this was mixed and pipetted into another microcentrifuge tube labelled ‘mixed kv’.
  50ul, 100ul and the rest of the tube containing transformed bacteria were pipetted onto plates containing Chloramphenicol.
  Of the mixed kv bacteria, not enough ul remained to also do 100ul, so just 50ul and the rest were divided onto two plates.

FRIDAY, 16/7/2021

Electroporation (4)

Project: Lab Notebook
Authors: Quint van Loosen
Created at: 2021-07-16

The DH5-alpha-pSB1C3 transformed cells that had incubated overnight on plates were dead.
The electroporation with pSB1C3 was done again.

Goal

- Reperform the electroporation

Materials

- Culturing plates
- LB medium

Protocol

- Electrocompetent cells were plated on plates with and without antibiotics
- As a control also not electrocompetent cells were plated out

Extra

- On the second batch we found that some cuvets had different sized slits. This has an effect on electroporation

The Eppendorf tubes with markings:
Last 2kv and first 1.5 kv were from the same batch of competent cells as per accident that Eppendorf tube had double the amount of competent cells. To compensate 2ul in stead of 1ul of plasmid DNA was added.
Plates were poured
Of the 0kv, (not electroporated) 50ul was per accident pipetted onto a plate containing Kanamycin (should be no antibiotic).
So it was done again on a plate without antibiotic, resulting in less remaining cell culture for ‘the rest’ plate.

Week 2 - Electroporation

Project: iGEM2021
Authors: Iris Noordermeer
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TUESDAY, 20/7/2021

Electroporation (5)

Project: Lab Notebook
Authors: Quint van Loosen
Created at: 2021-07-20

Goal

- Make new glycerol stocks

Materials

- 10% glycerol
- Ice
- LB Medium
- Centrifuge

Protocol

- Of the plates liquid cultures were inoculated in LB medium
- Culture was incubated at 37 °C 250rpm for 3 hours and 15 minutes.
- Place cultures on ice for 15 minutes. From this point on the cultures must be kept ice cold.
- Pour each 250 ml culture into chilled centrifuge bottles.
- Centrifuge at 5000 rpm for 10 min at 4 °C.
- Discard supernatant
- Add 250 ml of glycerol to each of the centrifuge bottles and completely suspend the cells by pipetting up and down.
- Centrifuge at 5000 rpm for 10 min.
- Discard supernatant
- Completely suspend the cells in 250 ml glycerol and re-centrifuge.
- Pour off the supernatant and suspend the cells in 1ml glycerol
- At this point, you can electroporate or freeze the cells away. To freeze, add 100 microliters of the culture to microcentrifuge tubes on ice. Once you have used all of the cultures, transfer the tubes to dry ice for 10 minutes. Once the cultures are frozen, transfer them to a -80°C freezer. The cultures should be good for >6 months.

Results

- Plates had grown cultures over the weekend.
- The OD600 of the liquid cultures was 0.700

Week 2 - Transformation with electroporation

Project: iGEM2021
Authors: Iris Noordermeer
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WEDNESDAY, 21/7/2021

Project: Lab Notebook
Authors: Chanel Naar, Quint van Loosen
Created at: 2021-07-21

Goal

- Make competent E.coli cells and transform them with electroporation with 1, 2, and 3 plasmids at the same time

Materials

Consumables (media, antibiotics, etc.)

- LB media
- Glycerol
- Plasmids (pJUMP46-2ABF(GFP), pJUMP49-2E(sfGFP), pKIKOarsBKm, pSIJ8)
- Agar plates
- Green, yellow, pink and orange antibiotics

Bacterial strains

- DH5α

Equipment

- Electroporation machine

Protocol

Make competent cells

- Make a 1L flasks containing 250 ml each of LB medium pre-warmed to 37°C.
- Add two drops of the overnight culture to the flask
- Shake at 37°C and 250 rpm until the cultures reach an OD600 of 0.7.
- Be sure to turn on centrifuge and cool rotor to 4°C well in advance of harvesting cells. Be sure to place 500ml of 10% glycerol on ice well in advance of harvesting cells

- Place cultures on ice for 15 minutes. From this point on the cultures must be kept ice cold.
- Pour each 250 ml culture into chilled centrifuge bottles.
- Centrifuge at 3000 rpm for 10 min at 4C.
- Pour off the supernatant

- Add 250 ml of glycerol to each of the centrifuge bottles and completely suspend the cells by pipetting up and down.
- Centrifuge at 3000 rpm for 10 min.
- Pour off the supernatant

- Completely suspend the cells in 250 ml glycerol and re-centrifuge.
- Pour off the supernatant and suspend the cells in 1ml glycerol

- At this point you can electroporate or freeze the cells away. To freeze, add 100 microliters of the culture to microcentrifuge tubes on ice. Once you have used all of the culture, transfer the tubes to dry ice for 10 minutes. Once the cultures are frozen, transfer them to a -80°C freezer. The cultures should be good for >6 months.

Electroporation with RFP, Kiko and helper plasmid

- Thaw pSB1C3 isolated plasmids (Chloramphenicol resistance)
- Thaw 4x 100 ul electrocompetent DH5-alpha cells on ice
- Turn on electroporator and set to 1.7 , 200 ohms and 25 µF.
- Place recovery LB in 37°C water bath.
- Pre-warm LB-Choramphenicol plates at 37°C.
- Place 4 microcentrifuge tubes and 4 mm-electroporation cuvettes on ice.
- Flick the tubes containing cells a few times to mix and add 100 µl to new microcentrifuge tubes.
- Add 1 µl (if concentration between 50 and 100 ng/ul) of the isolated plasmid DNA solution to the cells in the microcentrifuge tube.
- Transfer the DNA-cell mixture to the cold cuvette, tap on countertop 2X, wipe water from exterior of cuvette and place in the electroporation module and press pulse (don’t hold the button down).
- Immediately (very fastly done) add 975 µl of 37°C LB, mix by pipetting up and down once and transfer to a 15 ml tube.
- Rotate in the 37°C incubator for 1 h.

- Dilute 10 µl cells into 990 µl LB and plate 100 µl. (1000-fold dilution)
- Dilute 100 µl cells into 900 µl LB and plate 100 µl. (100-fold dilution)
- Dilute 10 ul cells into 90ul LB and plate 100ul (10-fold dilution)
- Plate 100 ul (non-diluted)
- Incubate overnight at 37°C.

Report

Helper plasmid (pSIJ8) is thermosensitive so should be kept at 30 °C and not 37 °C.

PSBIC3 (348.2 ng/ul) - didn´t work possibly due to arcking because of too much DNA
pJUMP46-2ABF(GFP) (105.3 ng/ul) - did work (plate was grown full)
pJump49-2E 54 (sfGFP) (46.8 ng/ul) - did work (plate was grown full)
pKIKOarsBKm (109.7 ng/ul) - didn´t work, possibly because of the E.coli strain
pSIJ8 (17.9 ng/ul) - didn´t work as it was grown at 37 °C instead of 30 °C

--> So plasmid concentration should be between 50 and 100 ng/ul

THURSDAY, 22/7/2021

Transformation with electroporation (2)

Project: Lab Notebook
Authors: Chanel Naar, Quint van Loosen
Created at: 2021-07-22

Goal

- Reperform electroporation of PSIJ8 and pSBIC3 plasmids

Material

Consumables (media, antibiotics, etc.)

- LB media

- Glycerol
- Plasmids (pSBIC3, pSIJ8)
- Agar plates
- Green and yellow antibiotics

Bacterial strains

- DH5a

Equipment

- Electroporation machine

Protocol

The electroporation of the pSIJ8 plasmid was tried again, the same protocol (2ul of the plasmid due to low concentration) was done but this time the max of 30 °C was taken into account.
The electroporation of the pSBIC3 plasmid was tried again, this time a 3 fold dilution was made to put in around 100 ng of plasmid instead of 300 ng.

Result

Did not work, nothing grew on the plates

FRIDAY, 23/7/2021

Transformation with electroporation (3)

Project: Lab Notebook
Authors: Chanel Naar, Quint van Loosen
Created at: 2021-07-23

Goal

- Reperform electroporation of PSIJ8 and pSBIC3 plasmids

Material

Consumables (media, antibiotics, etc.)

- LB media
- Glycerol
- Plasmids (pSBIC3, pSIJ8)
- Agar plates
- Green and yellow antibiotics

Bacterial strains

- DH5a

Equipment

- Electroporation machine

Protocol

The electroporation was tried again with the same protocol, but with 2.5 (as an article stated works for ecoli for them).
Used the electrocompetent cells of the first batch: QvL 14-07-2021

PSBIC3 (dilution used: 116 ng/ul)

pJUMP46-2ABF(GFP) (105.3 ng/ul)

pJump49-2E 54 (sfGFP) (46.8 ng/ul)

pSIJ8 (17.9 ng/ul, so 2 ul used) - was grown at 30 °C

Result

Did not work, nothing grew on the plates

Week 3 - Transformation with electroporation

Project: iGEM2021
Authors: Iris Noordermeer
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TUESDAY, 27/7/2021

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-07-27

Goals:

- Make new electro competent E.coli cells to transform with electroporation with 1 and 2 plasmids at the same time

Material

Consumables (media, antibiotics, etc.)

- LB media
- Glycerol
- Plasmids (pKIKOarsBKm, pSIJ8)
- Agar plates
- Green and yellow antibiotics

Bacterial strains

- DH5a

Equipment

- Electroporation machine

Protocol

Competent cells - Protocol from Ivo

Day 1
Streak out frozen glycerol stock of bacterial cells (DH5 alpha with PSIJ8 plasmid) onto a LC plate (no antibiotics). Grow plate overnight at 30°C.

Day 2
Morning: select a single colony of E.coli from the LC plate, inoculate a 10ml starter culture of LC and grow at 30°C in a shaker
Afternoon: Inoculate 500ml of LC medium with 10ml starter culture and grow at 20°C at 200 rpm for 36 hours (start morning day 4).

Day 3
Materials for next day overnight at 4°C or 2 hours before use in -20°C

10% Glycerol (2L)
50ml Falcon conical tubes (x8)
1.5ml Eppendorf tubes (1 jar)

Day 4

- When the OD600 reaches 0.4 -0.8 put the cells on ice. Chill the culture for 15 minutes.
- Pour the culture in pre-chilled 50ml Falcon tubes and spin down for 10´ at 4000 rpm at 4°C (cold room).
- Decant the supernatant and add the remaining culture equally in each Falcon tube (for 500ml culture, 12.5 ml in each tube). Add 10% Glycerol until 50ml. Spin down for 10´ at 4000 rpm at 4°C (cold room).
- Decant the supernatant and re-suspend each pellet in 25ml ice cold 10% v/v Glycerol. Add 25ml of re-suspended pellet to other tubes (8 to 4 tubes). Spin down for 10´ at 4000 rpm at 4°C (cold room).
- Decant the supernatant and re-suspend each pellet in 25ml ice cold 10% v/v Glycerol. Add 25ml of re-suspended pellet to other tubes (4 to 2 tubes). Spin down for 10´ at 4000 rpm at 4°C (cold room).
- Decant the supernatant and re-suspend each pellet in 50ml ice cold 10% v/v Glycerol. Spin down for 10´ at 4000 rpm at 4°C (cold room).
- Carefully remove supernatant and resuspend pellet in 1ml ice cold 10% Glycerol. (OD600 of resuspended cells should be between 200-250)
- Aliquot in 1.5ml Eppendorf tubes and snap freeze with liquid nitrogen. Store at -80°C

N.B. Work on ice at all time and use pre-chilled equipment

Electroporation

- Thaw isolated plasmids
- Thaw 100 ul electrocompetent DH5-alpha cells on ice
- Turn on electroporator and set to 1.4 Volt , 200 ohms and 25 µF.
- Place recovery LB in 37°C water bath.
- Pre-warm LB-Choramphenicol plates at 37°C.
- Place 4 microcentrifuge tubes and 4 mm-electroporation cuvettes on ice.
- Flick the tubes containing cells a few times to mix and add 100 µl to new microcentrifuge tubes.
- Add 1 µl (if concentration between 50 and 100 ng/ul) of the isolated plasmid DNA solution to the cells in the microcentrifuge tube.
- Transfer the DNA-cell mixture to the cold cuvette, tap on countertop 2X, wipe water from exterior of cuvette and place in the electroporation module and press pulse (don’t hold the button down).
- Immediately (very fastly done) add 975 µl of 37°C LB, mix by pipetting up and down once and transfer to a 15 ml tube.
- Rotate in the 37°C incubator for 1 h.

- Plate 100 ul (non-diluted)
- Incubate overnight at 37°C.

WEDNESDAY, 28/7/2021

Transformation with electroporation (2)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-07-28

Goal

- Measure if the OD600 reached 0.4 -0.8 of the DH5 alpha with PSIJ8 plasmid.

Protocol

From the (DH5alpha PSIJ8 plasmid) starting culture a 150 ml culture was made and grown to the right OD600 at 30°C.

Result

Unfortunately at 5 o´clock the OD600 was still too low, so was diluted and grown overnight

THURSDAY, 29/7/2021

Transformation with electroporation (3)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-07-29

Goal

- Measure if the OD600 reached 0.4 -0.8 of the DH5α with PSIJ8 plasmid.
- If so, competent cells can be made.

Protocol

Culture was too full, so diluted and left to grown again for 3.5 hours.

Competent cell protocol was followed.

FRIDAY, 30/7/2021

Transformation with electroporation (4)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-07-30

Goal

- Electroporate the competent cells DH5α with pSIJ8.

Protocol

The electroporation protocol described on 2021-07-27 was followed.

Competent cells were electroporated and plated out the electroporated DH5α and grow the cultures over the weekend.

Week 4 - Check heat instability of pSIJ8 plasmid

Project: iGEM2021
Authors: Iris Noordermeer
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TUESDAY, 3/8/2021

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-03

Goal

- Determine the heat instability of the pSIJ8 plasmid, does it actually lose its plasmid when heated

Material

Consumables (media, antibiotics, etc.)

- Agar plates
- Ampicillin

Bacterial strains

- DH5α competent cells - transformed with heatshock with the pSIJ8 plasmid

Equipment

- Incubator

Protocol

Some colonies were picked from the heatshocked plate and diluted down to give it space to grow.

- A ampicillin antibiotic plate - to see if the bacteria would die --> no that does not work as you select for the ones that have not lost it!
- Two non-antibiotic plates - to put one on 30°C and one on 37 °C overnight (on thursday, first they should grow)

WEDNESDAY, 4/8/2021

Check heat instabililty of pSIJ8 plasmid (2)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-04

Goal

- Check if the plates of 2021-8-04 grew colonies.

Result

The plates grew colonies, so now one was put on 30 °C and one on 37°C overnight.

THURSDAY, 5/8/2021

Check heat instabililty of pSIJ8 plasmid (2)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-05

Goal

- Picking colonies that have grown and strike out on new agar plates.

Protocol

From both the 30°C plate and the 37°C plate one colony was picked and put on an ampicillin antibiotic plate and one on a plate without antibiotics.

FRIDAY, 6/8/2021

Check heat instabililty of pSIJ8 plasmid (3)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-06

You would expect that the colonies picked from the 37°C plate would not have the plasmid anymore, so would grow on a plate without antibiotics but not on a plate with the ampicillin antibiotic.
The 30°C is the control, should still have the plasmid so should grown on both.

Goal

- Picking colonies that have grown and strike out on new agar plates.

Protocol

--> Result: [insert result tomorrow]

How to proceed: If the 37°C plate with the ampicillin antibiotic still has colonies then grow a plate with no antibiotics with new colonies striked on it from a plate from the 30°C over the weekend on 37°C. Maybe it needs more time to loose the plasmid.

If the 37°C plate with green antibiotic is empty, then the heat instability is checked, so done!

--- **written by Sebas** ---
The plates all had bacteria growing on them, so they will be grown over the weekend again. After the weekend, the strain grown at 37 °C without antibiotics should be replated on antibiotic plates to check for loss of plasmid.
Additionally, I put in liquid cultures over the weekend, 2 at 30 °C, 2 at 37 °C, both once with and once without antibiotics. This is because I expect that it will be easier for bacteria that lost their plasmid to outcompete other plasmid carriers in liquid culture, since the culture is completely mixed and can grow faster.
After the weekend, these liquid cultures should be plated out in a dilution series (100 uL, 10 uL, 1 uL, 0.1 uL, 0.01 uL, 0.001 uL) , on LB with and without antibiotics to screen for the percentage of bacteria with intact plasmids in them. This is in my opinion the best way to do it, but we can discuss this on Tuesday again. I will plate them for you on Monday anyway.

Edit: oops, the liquid cultures at 30 °C accidentally grew at 37 °C for about 1.5 hours.

WEDNESDAY, 11/8/2021

Check heat instabililty of pSIJ8 plasmid (4)

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-11

Goal

- Plate with bacteria grown at 30°C overnight without antibiotics and a plate that was grown at 37°C without antibiotics.

Result

IMG_3302.jpg

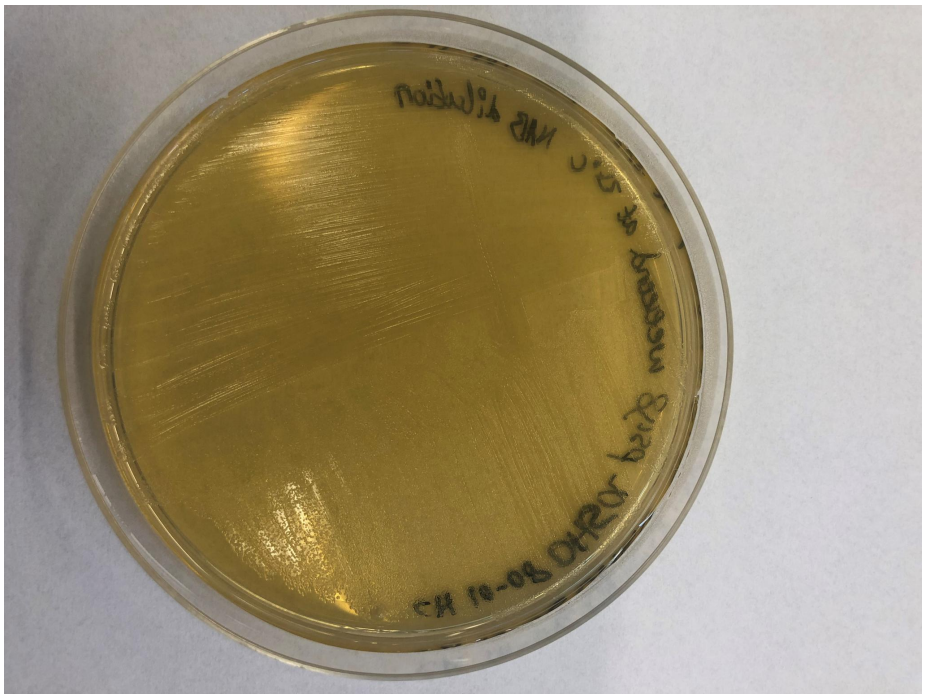
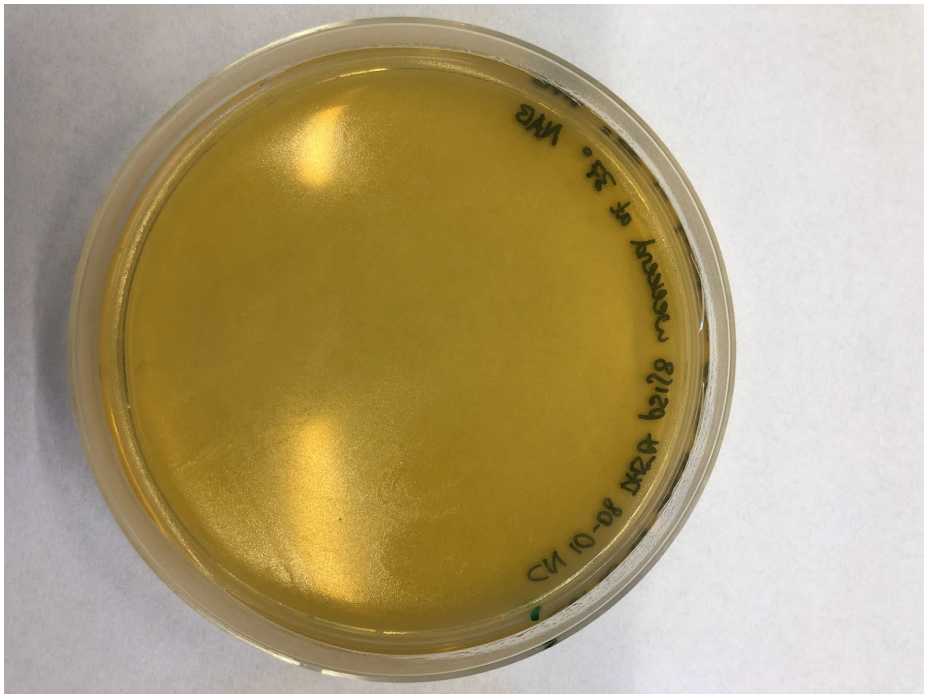


image.png



Has lost the plasmid after a weekend at 37°C

Week 4 - Heat shock for genome integration

Project: iGEM2021
Authors: Iris Noordermeer
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MONDAY, 2/8/2021

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-02

Goal

- Transform E.coli cells with heat shock with 1 and 2 plasmids at the same time

Materials

Consumables (media, antibiotics, etc.)

- LB media
- Plasmids (pKIKOarsBKm, pSIJ8)
- Agar plates
- Green and yellow antibiotics

Bacterial strains

- DH5α competent cells

Equipment

- Heat block

Protocols

Heat shock protocol

1. Switch on the water bath and set temperature at 42 °C. Also turn on the heat/shaking-block and set up to 37 °C
2. Thaw 50 ul competent cells on ice
3. Add 1 µL of each plasmid into 20 µL bacteria. Mix well. Make sure you work near the Bunsen burner flame
4. Leave the cells on ice for 5 minutes
5. Heat-shock the cells for 30 seconds (exactly!) at 42°C
6. Return the cells directly to ice for 2 minutes
7. Incubate for 60 minutes at 37 °C
8. Plate on LB agarplates with appropriate antibiotics
9. Incubate overnight at 37^0C

Result

Helper plasmid (pSIJ8) has grown, had a very full plate.

TUESDAY, 3/8/2021

Heat shock for genome integration

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-03

Goal

- Transform E.coli cells with heat shock with 1 and 2 plasmids at the same time

Materials

Consumables (media, antibiotics, etc.)

- LB media
- Plasmids (pKIKOarsBKm, pSIJ8)
- Agar plates
- Green and yellow antibiotics

Bacterial strains

- E.col competent cells

Equipment

- Microscope

Protocol

pSIJ8 plate was diluted it down to make sure nice colonies can grow on it and checked under the microscope and it was E.coli.
Standard transformation protocol was used.

Result

The pKIKOarsBKm however did not work, no colonies were grown on either the single or cotransformation of pKIKO (and pSIJ8).
Probably due to the fact that the DH5α strain cannot amplify the ori of the pKIKOarsBKm.

--> Put the DH5α strain in culture.

Week 5 - Make arabinose-DH5alfa electrocompetent cells

Project: iGEM2021
Authors: Iris Noordermeer
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THURSDAY, 12/8/2021

Project: Lab Notebook
Authors: Chanel Naar
Created at: 2021-08-12

Goal

- Transform DH5α E.coli cells with electroporation with 1 and 2 plasmids at the same time

Material

Consumables (media, antibiotics, etc.)

- LB media
- Plasmids (pKIKOarsBKm, pSIJ8)
- Agar plates
- Arabinose
- Ampicillin
- Kanamycin

Bacterial strains

- DH5α

Equipment

- Electroporation machine

Protocol

Make the arabinose media:

In the article they used: 10mM so 0.01 M (mol per litre)
0.01 * 150.13 = 1.5013 gram per litre

So for 25mL = 1.5013 * 0.025 = 0.375325 gram

Stock 10% so 100 gram per litre, so 0.1 gram per mililitre
So then need: 0.375 ml

Week 5 - PSIJ8 heat inactivation

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:37:53 AM +0000
Entry Last Modified: 2021-10-06 11:41:44 AM +0000
Export Generated On: 2021-10-11 04:18:23 PM +0000

MONDAY, 9/8/2021

Project: Lab Notebook
Authors: Quint van Loosen
Created at: 2021-08-09

Goal

- Heat inactivate the PSIJ8 that is heatshocked into DH5α

Material

Consumables (media, antibiotics, etc.)

- PSIJ8 plasmid
- Ampicillin
- Liquid LB medium
- LB agar medium

Bacterial strain

- DH5α

Equipment

- Toothpick
- Vortex
- Agar plates
- Trigalski spatula
- Incubator

Protocol

The plates from Chanel and Sebas containing PSIJ8 heat-shocked into DH5α that had been grown on 30 °C, 37 °C and with and without ampicillin, as well on plates as in 5ml LB in the incubator rotating.

The plates were plated on ampicillin antitibiotic plates to be able to see whether they have lost the plasmid.
A single colony was pricked with a toothpick, put into an eppendorf tube, 1 ml LB was added and vortexed, then poured onto a plate containing ampicillin. The cells were spread out using trigalski spatula. The plates were dried near a bunsen burner before put in the incubator at 37 °C.

The liquid cell solution, 100 ul of solution was added to 5ml LB medium + ampicillin in a 15ml tube. Then placed back into the incubator at 37 °C + 200rpm.

Week 6 - Checking genome integration

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-04 02:16:52 PM +0000
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MONDAY, 16/8/2021

Goal

- Check if Top10 competent cells were transformed with 2 plasmids at the same time and if the pKIKO is integrated after arabinose stimulation

Material

Consumables (media, antibiotics, etc.)

- LB media
- Plasmids (pKIKOarsBKm, pSIJ8)
- Agar plates
- Arabinose
- Ampicillin
- Kanamycin

Bacterial strains

- Top10 competent cells

Equipment

- Heat block

Protocol

Cells from the grown culture (with Kanamycin in the media) were grown on non-antibiotic plates over the weekend.

Some colonies were taken from these plates and were replated on 1x:

- No antibiotic plate
- Ampicillin plate
- Kanamycin plate
- Ampicillin and Kanamycin plate

This was done for both the plate incubated over the weekend at 30°C and the one on 37°C.