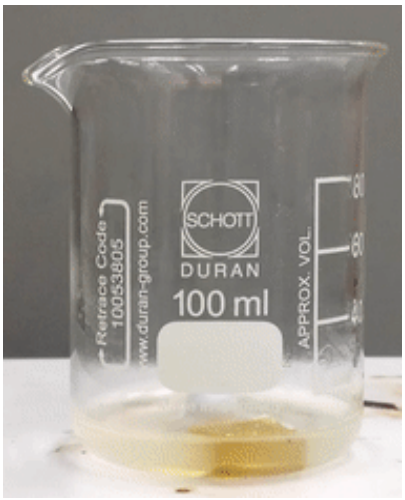
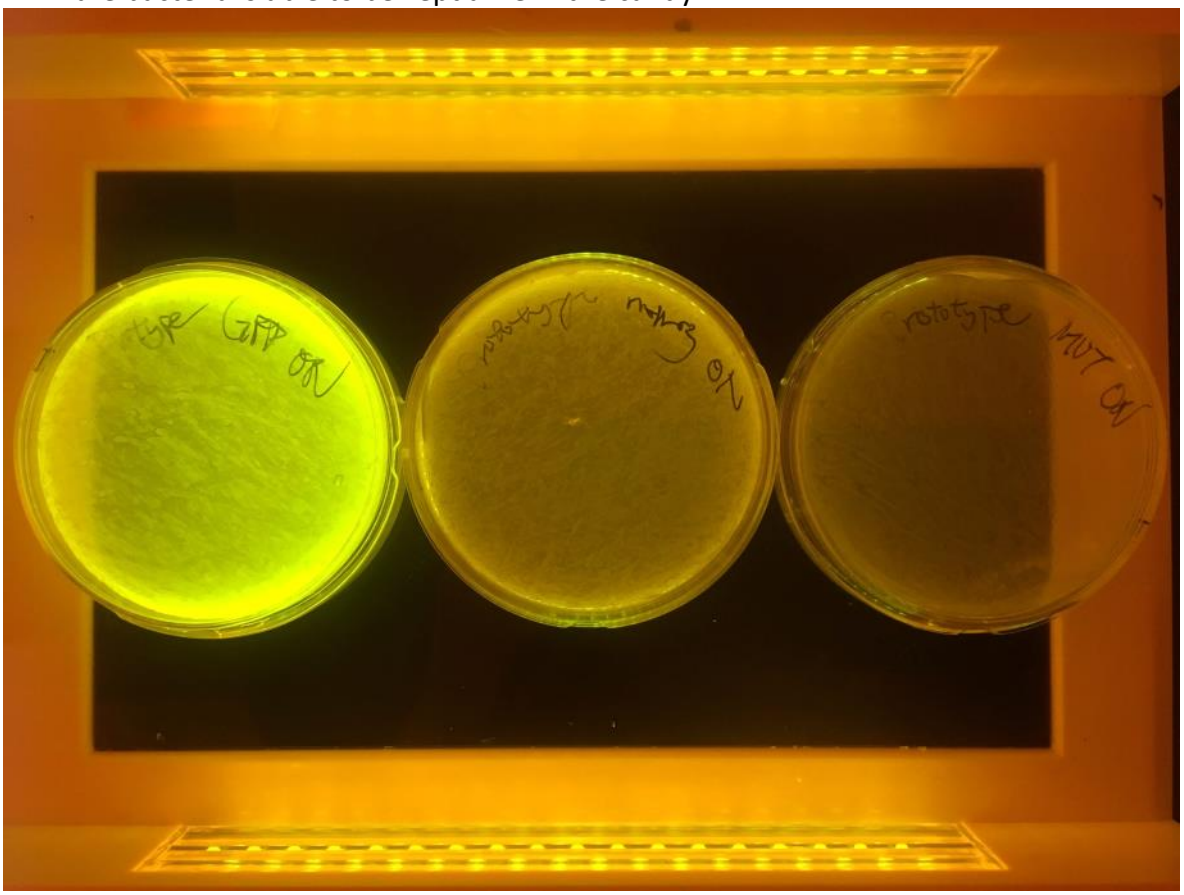
Applied Design

2018-10-11

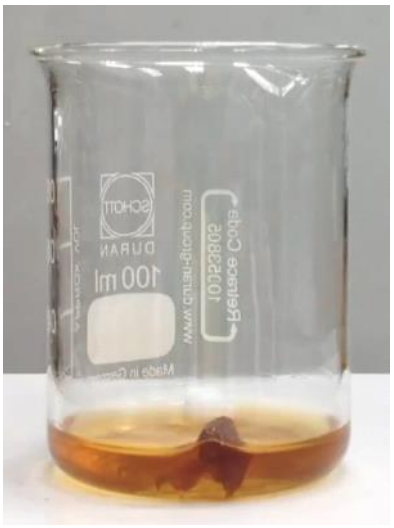
1. We tested that the candy takes around 60 min to melt a gel at 37º in artificial saliva.



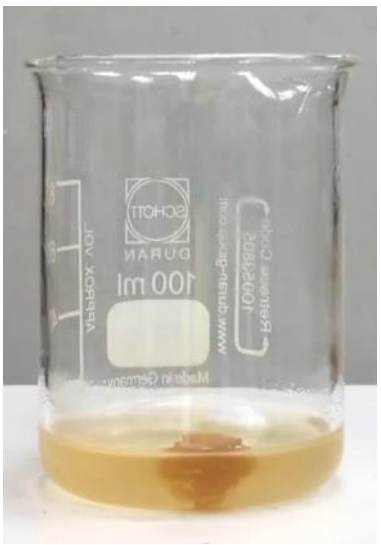
2. Checked plating results from 10/10
    a. Result: GFP glows but the negative control has bacteria as well. However, this shows are bacteria is able to be kept alive in the candy.
3. 

3. We also tested how long it takes to melt Nin Jom and Ricola candy in artificial saliva at 37 degress Celsius. The Nin Jom took 54 minutes while Ricola took 63 minutes

- Ricola



- Nin Jom



2018-10-10

1. Functional test of the candy (check functional test notebook)
    a. The candy was first dissolved in 37 degrees water.
    b. The solution was centrifuged and decanted to remove the sugar solution.
    c. The cell pellet was treated with lysis buffer to start the functional test preparation. (For more information, check the protocols notebook)
        i. The results are documented in the functional test lab notebook
2. We also plated gels to see if the bacteria in the candy is able to survive over-night.
    a. The candy was first dissolve in 37 degrees water.
    b. The solution was centrifuged and decanted to remove the sugar solution
    c. The cell pellet was resuspended in 50 uL water and plated on CM plates
    d. Centrifuge overnight at 37 degrees

2018-10-09

1. Checked results from 10/8
2. GFP glows significantly more than negative control

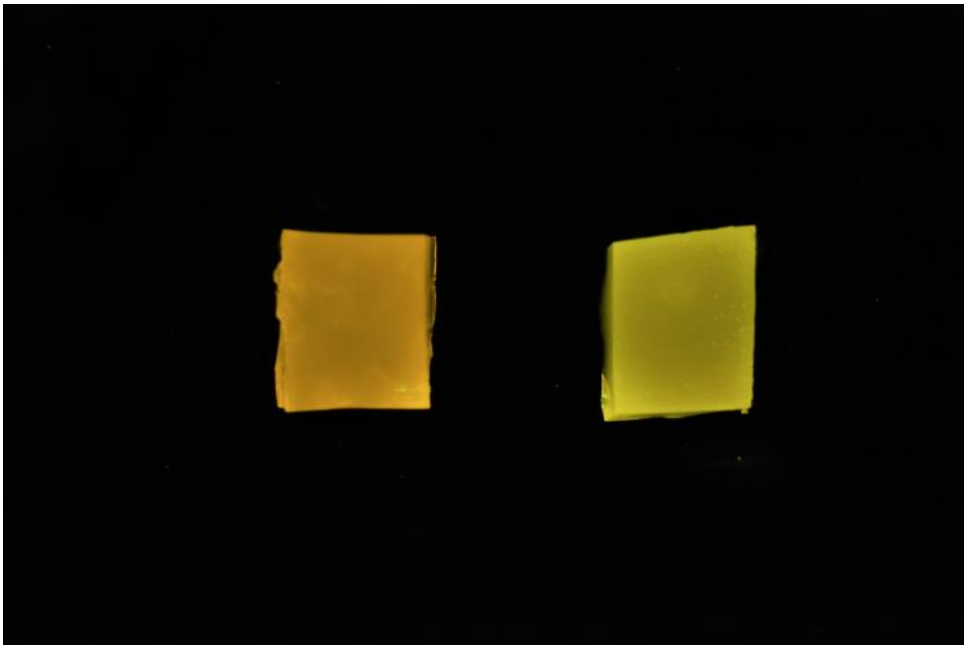
Results:

2018-10-08

1. Made new batch of jello with GFP, BOB, BOB mut
    - We followed the protocol is from 10/3

2018-10-04

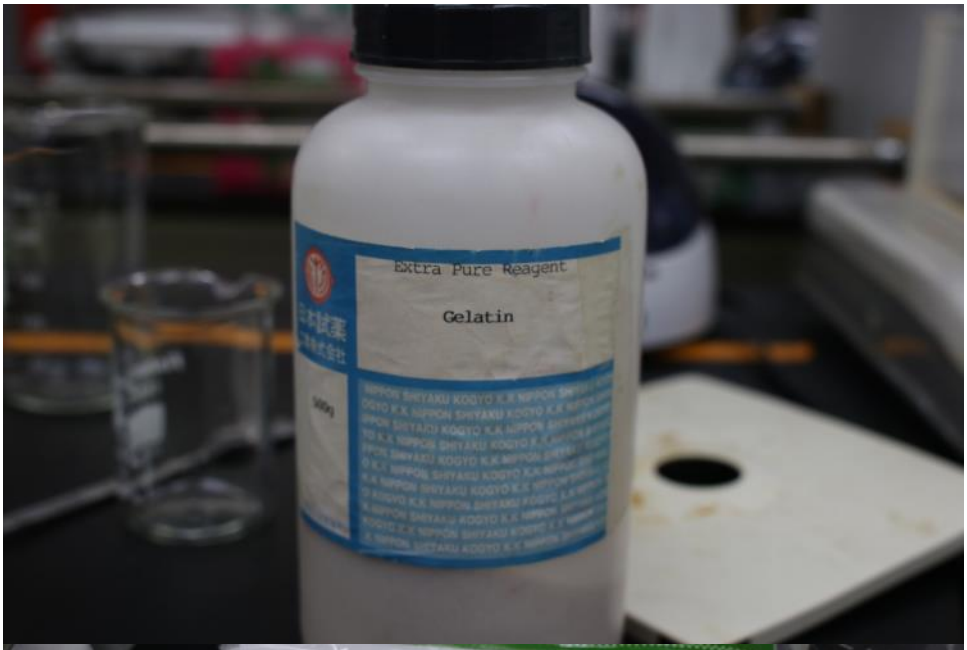
1. Checked results from 10/3
2. GFP glows brighter than negative control.

3. 

2018-10-03

1. We made engineered E. coli Nissle 1917 jello candies
2. Protocol
    a. Grow 10mL of Nissle overnight
    b. Spin down Nissle, resuspend in water, and decant
    c. Place a beaker/pan on a hot plate set at around 70 degrees
    d. Add 120 mL water, 30mL gelatin, 30mL sugar
    e. Heat and stir until solutes melt
    f. Remove from hot plate
    g. Use a temperature probe to measure temperature
    h. When the temperature cools down to 55~60 degree, mix with Nissle (resuspended in small amount of water)
    i. Aliquot 3mL of solution into each dent of cooling tray
    j. Let sit overnight

Ingredients:


Extra Pure Reagent
Gelatin


優冰糖
YASATO
結晶冰糖
RockSugar
Use for deluxe beverages and cuisine
600g

Set up:

2018-10-02

Tested approach #1: hard candies

1. Add 1 cup sugar, 1/2 cup corn syrup, some water to a pan
2. Heat in a pan and remove from heat when it reaches 300F
3. Allow candy to cool down until it reaches 55º
4. Result:
   *This test showed us that making a hard probiotic using this method is ineffective. As the hard structure does not allow us to mix our probiotics into the solution. Also, since we burned the candy, we could not see the GFP well enough.
   *We plan to use gelatin next time, in order to allow us to mix probiotics much more easily into the candy solution at 60 degrees celsius.

Ingredients

Set up:

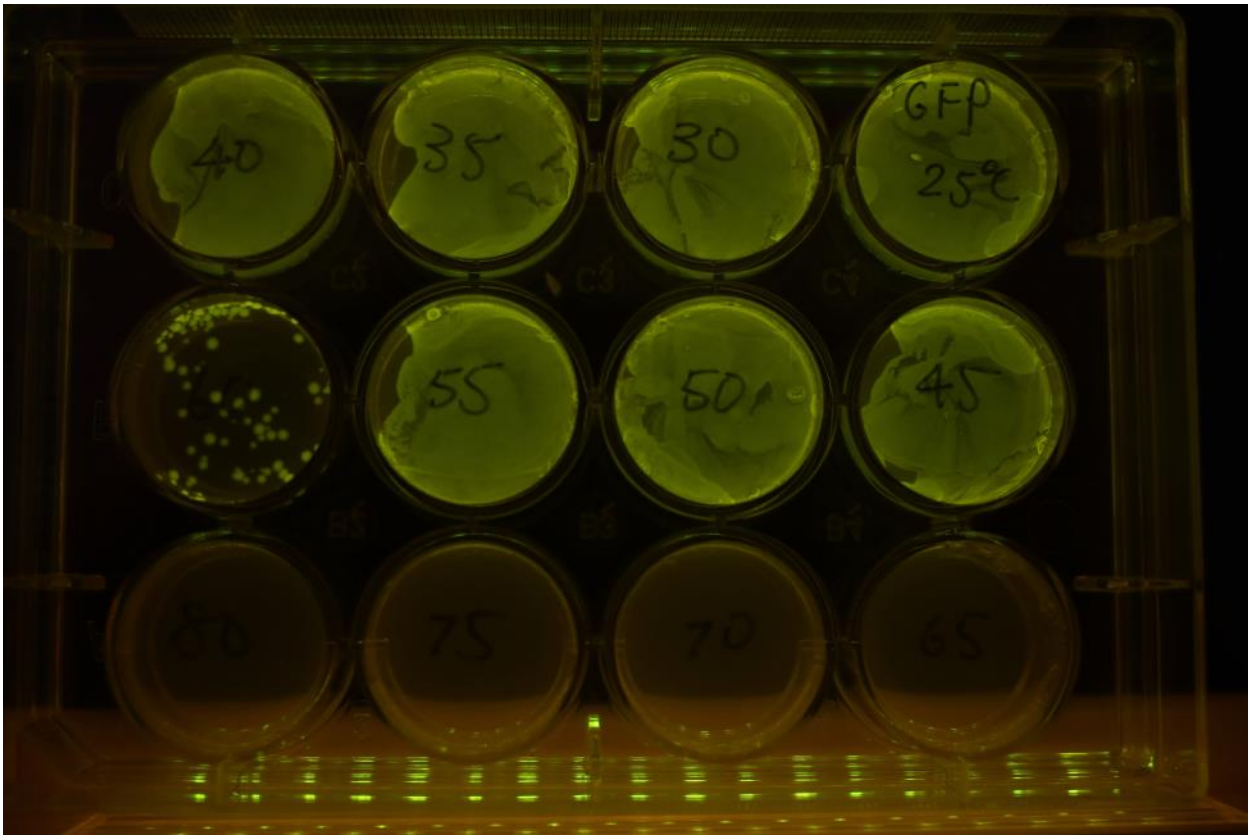

CAUTION
HOT TOP
Heat

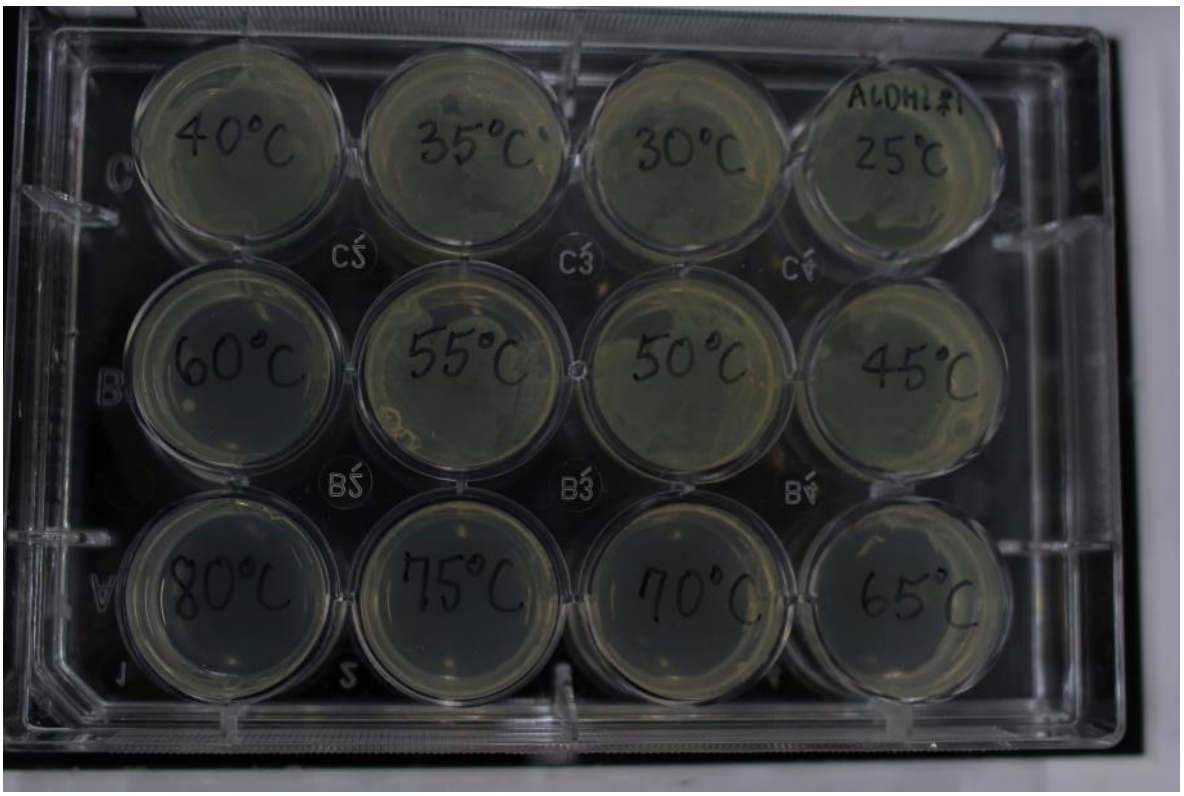

CAUTION
HOT TOP

Results:

2018-09-30

1. Checked results from 9/29
2. Only bacteria below temperatures of 60 degrees Celsius grew on the plates. In addition, all the bacteria on the plates that grew glowed, showing that protein is still functional under 60 degrees Celsius. The temperature thresholf for both Nissle and GFP is determined to be 60º.

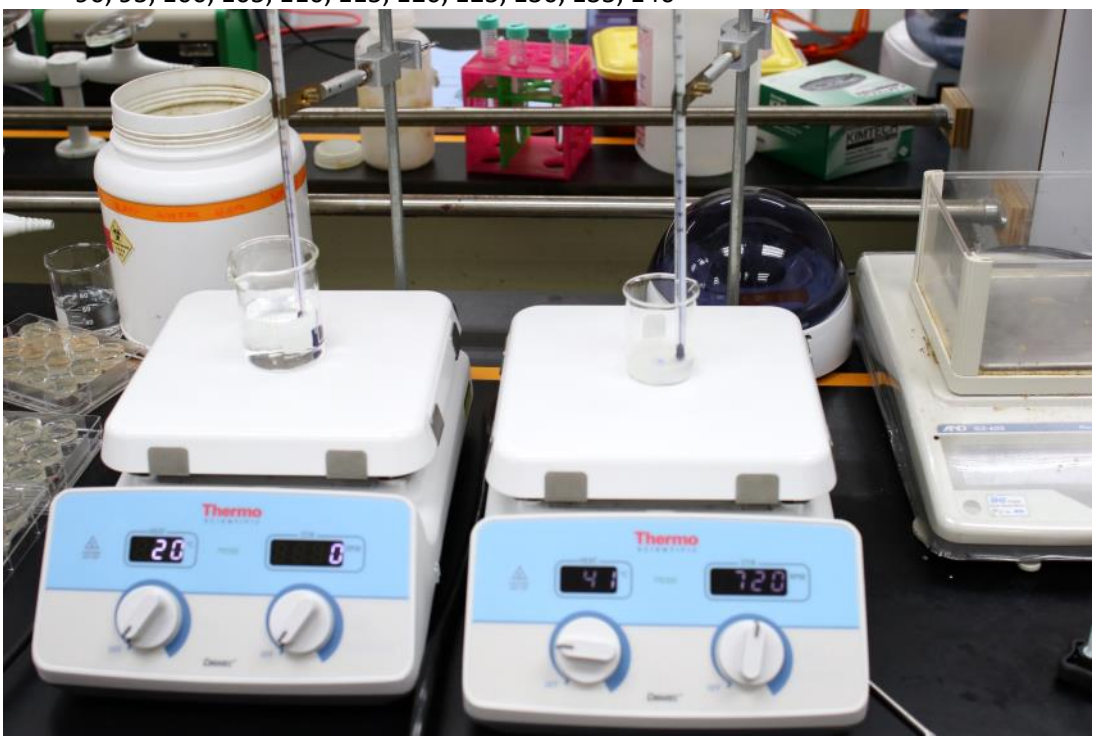
3. 

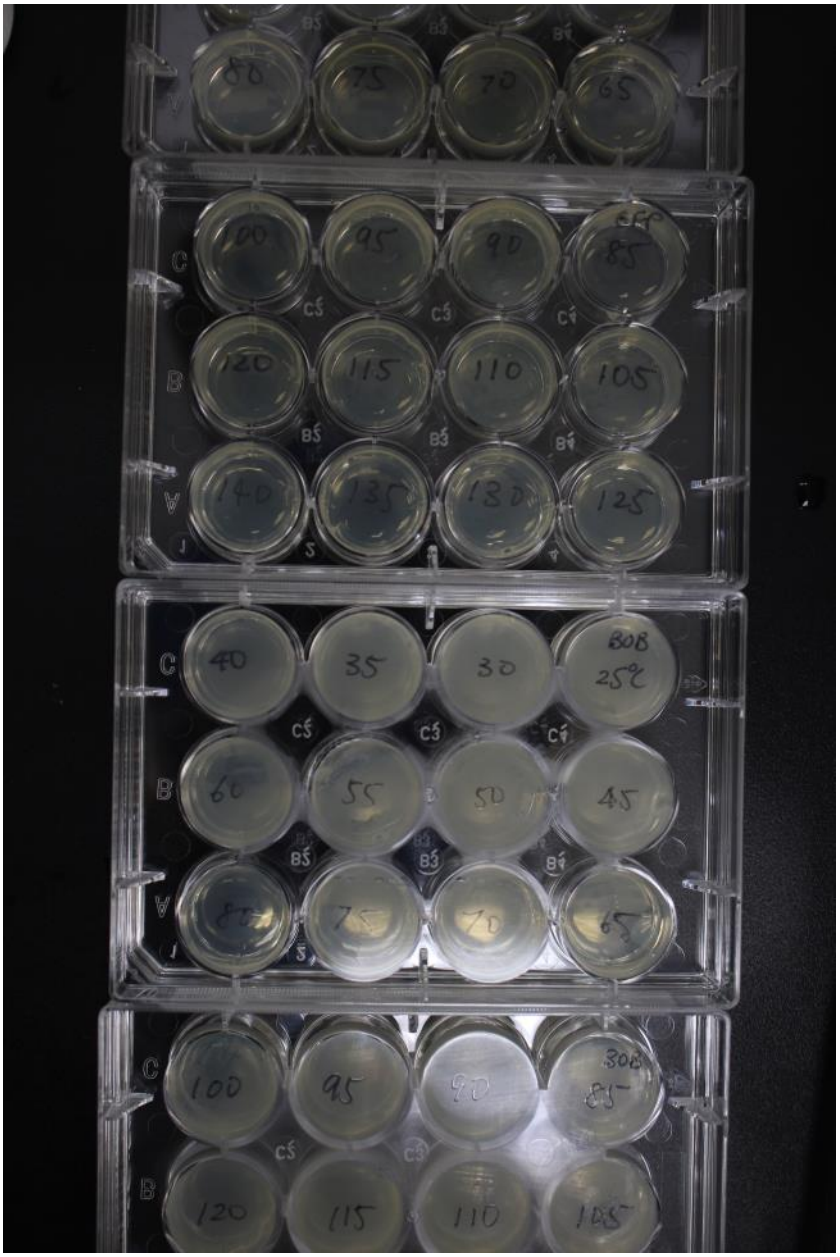
4. 

2018-09-29

1. Tested the max temperature for growth of nissle and production of GFP
    a. Grow 20mL LC of Nissle and GFP overnight

b. Spin down the cultures
c. Resuspend and gently pipet in 20 mL of water
d. Spin down and decant
e. Repeat step 3 and 4 to wash twice
f. Resuspend in 10mL water
g. Pour the mixtures into two small beakers and place on hot plate
h. Place a stirrer in the beakers
i. Set the temperature to 150 degree
j. Place a thermometer in the beaker and secure with a gripper attached to a pole
k. When the thermometer reaches 25 degree, take 10uL of culture out of the beaker and place one of the 12-wheels
l. Repeat step 11 for the following temperatures: 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140

2. 

3. 

2018-09-28

1. We ran functional tests with purified ALDH2*1 and ALDH2*2, with elution buffer as the negative control, at 37 degrees Celsius in artificial saliva
    a. We used the same functional tests protocol as before
    b. For more information of the results, check out our Functional test notebook
- Our results showed that our purified enzymes are able to significantly metabolize NAD+, even better than that at 25 degrees.

2018-09-27

1. We ran a functional test with our E.coli Nissle 1917 at 37 degrees Celsius in artificial saliva
    a. For more information of the protocols, check out our Protocol notebook
    b. For more information of the results, check out our Functional test notebook
- We summed up our results from the 3 E. coli Nissle 1917 functional tests. Our results showed that our modified E. coli Nissle 1917 is able to significantly metabolize NAD+

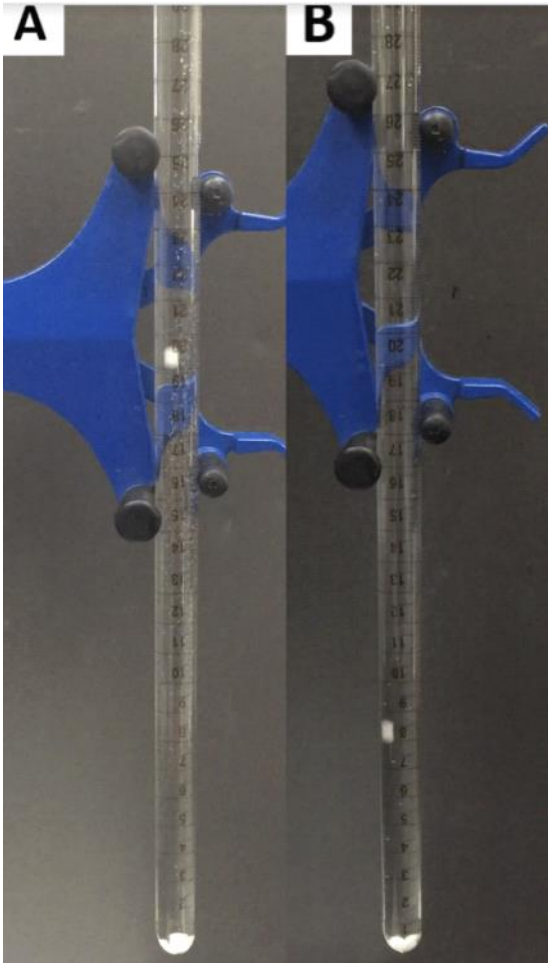
2018-09-25

1. We ran a functional test with our E.coli Nissle 1917 at 37 degrees Celsius in artificial saliva
    a. For more information of the protocols, check out our Protocol notebook
    b. For more information of the results, check out our Functional test notebook

2018-09-21

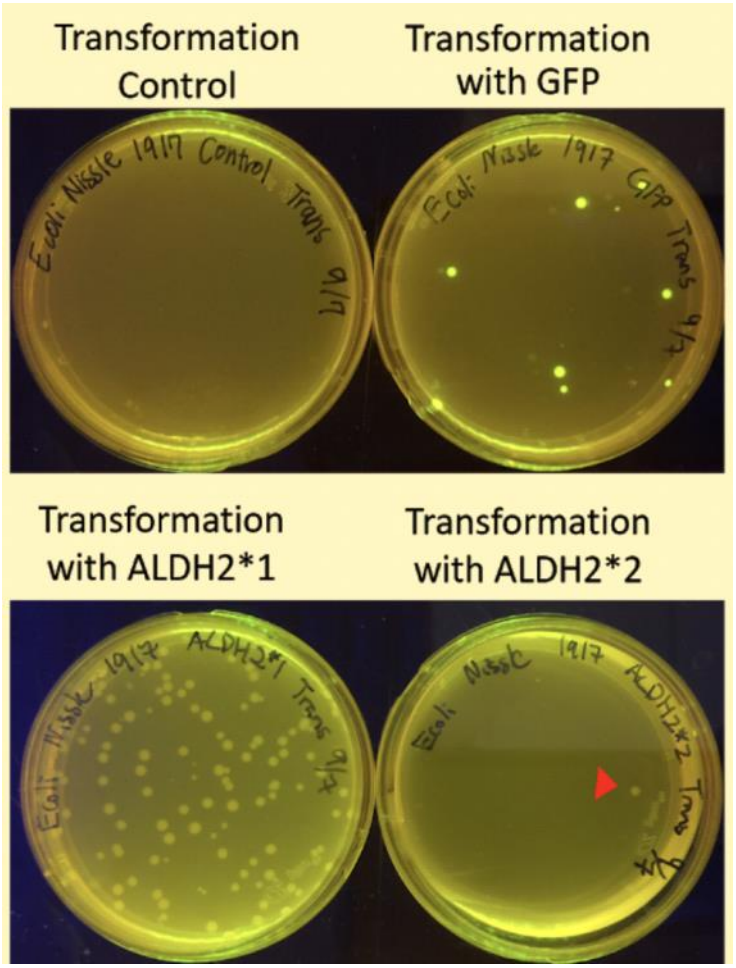
1. We ran a functional test with our E.coli Nissle 1917 at 37 degrees Celsius in artificial saliva
    a. For more information of the protocols, check out our Protocol notebook
    b. For more information of the results, check out our Functional test notebook

2018-09-08

1. We conducted a ball drop test (check protocol notebook for more information) We made artificial saliva that matches the literature values of viscosity. We determined that the best recipe for artificial saliva is by adding 6.5g OralBalance Moisturizing gel in 50mL of water.
2. 

Setup: long glass test tube, beads, clamp, OralBalance Moisturizing Gel

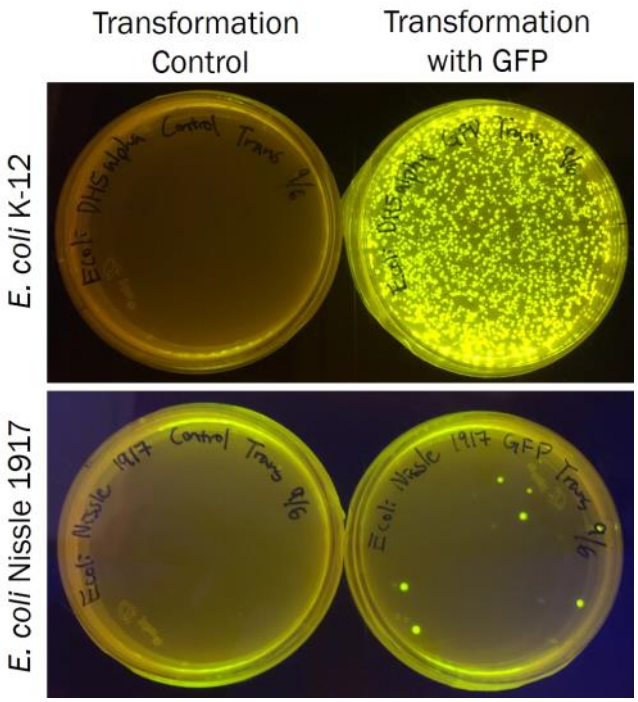
2018-09-06

1. Checked plates from 9/5: ALDH2*1 and ALDH2*2 both grew. The negative control did not have any colonies. The positive control GFP grew, showing that our E. coli Nissle 1917 transformation with ALDH2 was successful!
2. 

2018-09-05

1. Transformation of Nissle (ALDH2*2 and ALDH2*1)
a. Perform steps with open samples by the flame or in the hood
    - Thaw competent cells on ice (~ 50 uL in each tube)
    - Add 5 uL of Ligation reaction to competent cells
        - Mix by gently swirling.
        - Leave on ice ~60 min
    - Heat shock at 42 °C for 2 min
    - Put tubes on ice for 2 min
    - Add 500 ml LB [no antibiotics] and shake at 37 °C for ~45 min
    - Centrifuge at 12,000 rpm for 1 min.
        - Gently pour out supernatant
        - Re-suspend pellet in remaining supernatant
    - Plate ALL on LB + [antibiotic] agar plates
        - Incubate at 37 °C overnight

2018-09-04

1. Checked plates from 9/3
2. Result: GFP grew for both dh5 and Nissle, while negative control (no plasmid) did not grow for both strains. This shows that we are able to transform Nissle
3. 

2018-09-03

1. Made DH5 E. coli incompetent by taking DH5 E. coli competent cells through a transformation cycle
    - Thaw DH5 competent cells on ice (~ 50 uL in each tube)
    - Add 5 uL of to competent cells
        - Mix by gently swirling. DO NOT PIPETTE OR VORTEX
        - Leave on ice ~5 min
    - Heat shock at 42 °C for 30 sec
    - Put tubes on ice for 2 min
    - Add 1 ml LB [no antibiotics] and shake at 37 °C for ~45 min

2. Made Nissle and DH5 from previous step competent and stored them at –80 degree (protocl below)
3. Transformation of GFP into Nissle and DH5 (protocol below)

***E. coli* Calcium Chloride competent cell protocol**

1. Inoculate a single colony into 5mL Lb in 50mL falcon tube. Grow O/N @ 37°C.
2. Use 1mL to inoculate 100mL of LB in 250mL bottle the next morning.
3. Shake @ 37°C for 1.5-3hrs.

Or

1. Inoculate a single colony into 25mL LB in a 250 mL bottle in the morning.
2. Shake @ 37°C for 4-6 hrs.

Then....

1. Put the cells on ice for 10 mins (keep cold form now on).
2. Collect the cells by centrifugation in the big centrifugue for 3 mins @6krpm
3. Decant supernatant and gently resuspend on 10 mL cold 0.1M CaCl (cells are susceptible to mechanical disruption, so treat them nicely).
4. Incubate on ice x 20 mins
5. Centrifuge as in 2
6. Discard supernatant and gently resuspend on 5mL cold 0.1MCaCl/15%Glycerol
7. Dispense in microtubes (300µL/tube). Freeze in -80°C.

Transformation of Ca++ competent cells

1. Put 1µL of circular plasmid or all of a ligation reaction of plasmid DNA in a microtube. Gently add ~100µL of competent cells. Do NO DNA control tube with cells and no DNA.
2. Incubate for 30 mins on ice.
3. Heat shock for 2 mins @ 42°C. Put back on ice.
4. Add 900 µL of LB to tubes. Incubate @ 37°C for 30 mins.
5. Plate 100-1000 µL of the cells in LBAmp or LBCarb (100µg/ml) plates. Plate 100 µL of the NO DNA control in a blood plate (to check for quality of cells). Grow O/N. U can save the rest in the cold room or freeze with 15% of Gly in case u get no colonies (v. unlikely).
6. If you need a lot of colonies or the ligation is of low efficiency, centrifuge the transformation for 1 min @ 8krpm, discard 900 µL of supernatant, resuspend on the 100 µL left and plate the whole lot.

Source: NYU Medical Facility

2018-09-02

1. Plates from 9/1 grew
2. Made LC for transformation

2018-09-01

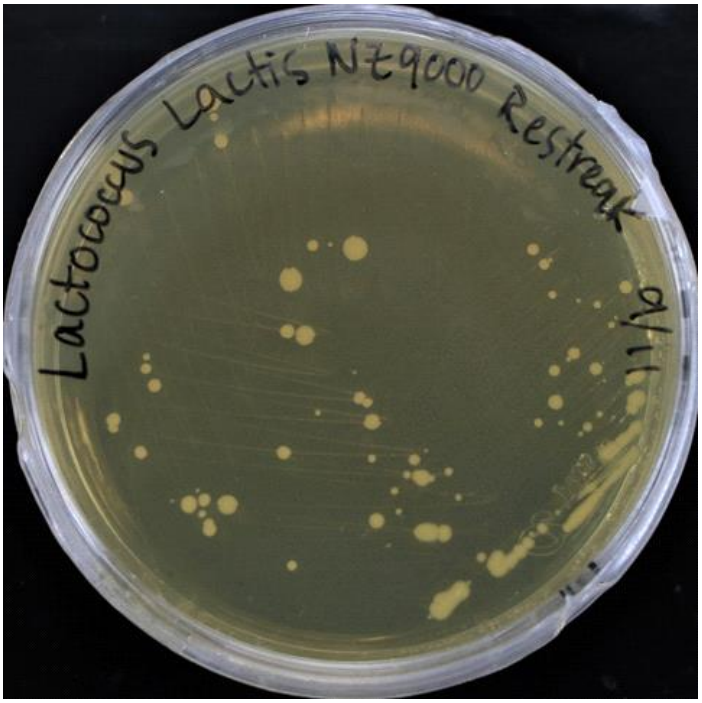
1. Received E. coli Nissle 1917 from mutaflor
2. Restreaked onto CM LB plates

2018-08-25

1. check results from 8/21: none of the plates grew, the electroporation was unsuccessful :'(

2018-08-23

1. We miniprepped the ALDH2*1 and GFP in pNZ8008 vector
2. The restreak plates from two days ago yesterday grew

a. 

3. Made L. Lactis competent (refer to the protocols notebook)
4. Transformed GFP and ALDH2*1 in the NICE vectors into NICE L. Lactic strains (protocol below) by utilizing electroporation

Transformation Protocol **Protocol No. 4308 915.519 – 12/2001**

Microorganism	*Lactococcus lactis* MG1363
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pGK12)
Growth medium	Complex medium with 1% glycine
Washing solution	0.5 M sucrose, 10% glycerol
Electroporation solution	0.5 M sucrose, 10% glycerol
Outgrowth medium	Ice-cold complex medium with 0.5 M sucrose, 20 mM $MgCl_2$, 2 mM $CaCl_2$
Cuvette	1 mm gap width
Reference	Dr. Horst Neve • Bundesanstalt für Milchforschung • Institut für Mikrobiologie Hermann-Weigmann Str. 1 • D-24103 Kiel • Phone +49 431 6091 • Fax +49 431 609222

Making electrocompetent cells:

1. Grow cells overnight at 30 °C to an $O.D._{620}$ of 0.7.
2. Wash twice with ice-cold washing solution.
3. Resuspend cells in 1/100 volume of electroporation solution. Keep on ice.

Electroporation of cells:

1. Add 0.25 µg plasmid DNA (in water) to 100 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

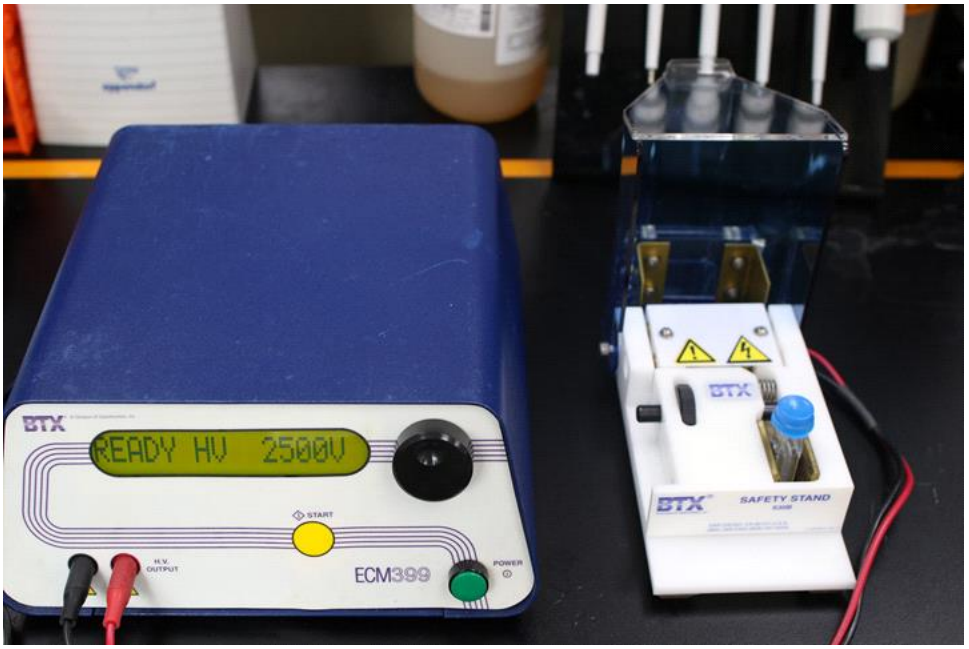
Mode	Prokaryotes "O"
Voltage (V)	2,000 V
Time constant (τ)	5 ms

4. Add 1 ml ice-cold complex medium, incubate 2 hours at 30 °C.
5. Plate diluted cells on selective chloramphenicol plates. Incubate 2 days at 30 °C.

Expected results:

Transformation efficiency up to 1.4 x 10^6 transformants/µg of DNA.

Source: Eppendorf

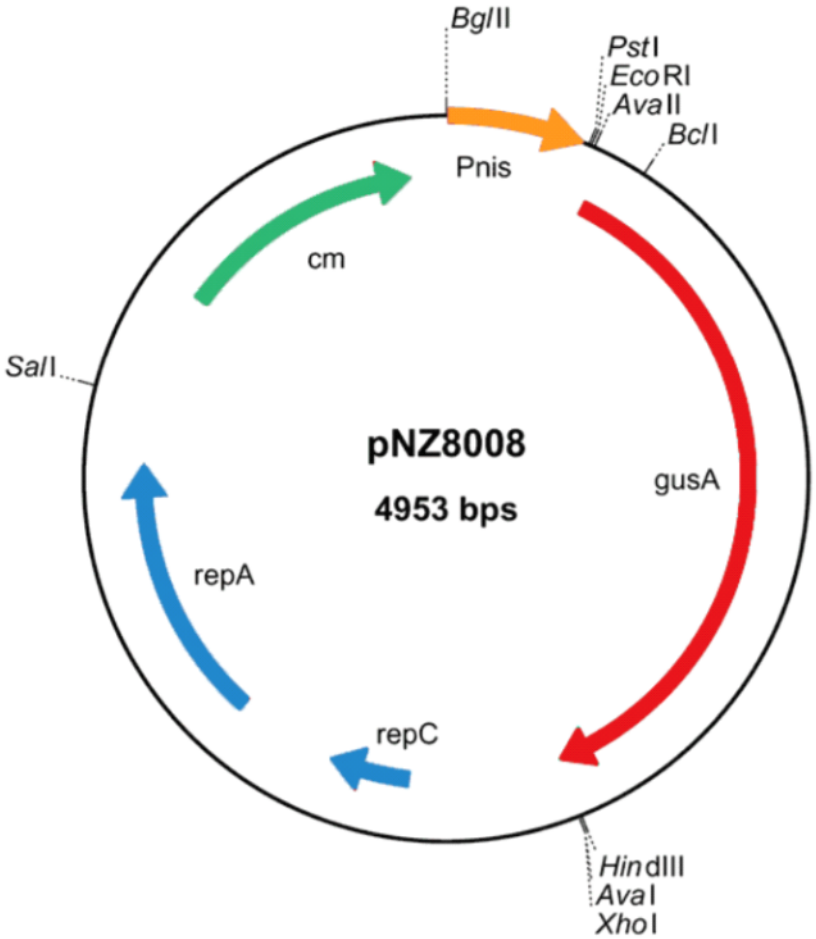


2018-08-22

1. PCR checked GFP+pNZ8008 and ALDH2*1+pNZ8008
2. We made LC of the ALDH2*1 and GFP in pNZ8008 vector E. coli

2018-08-21

1. Started digested our ALDH2*1 and GFP and NICE pNZ8008 vector at E and P restriction sites



2. Performed ligation and transformation

2018-08-20

1. We received the NICE L. Lactis system (backbone and bacteria) from NCHU
2. Restreaked L. Lactis on M17 plates and made LC for cloning

2018-06-19

1. Functional tests w/ sonicator and increased concentration
2. MRS plates (FAIL)
    a. Add more agar next time!!
    b. No more MRS powder left
3. Sent term and bob aldh2 for sequencing
4. Lacto Electroporation
    a. Looks very promising
    b. Pellet was white, indicating living lactobacillus

2018-06-08

Checking lactobacillus transformation success

1. 1 treatment seems to grow

a. Lactobacillus casei + glycine
b. Reculture by suspending 100 uL in 9.9 mL of MRS (cm resistant)

Conclusion:

Why didn't cells grow?

1. Electro transformation failed
2. Left the LC after electro transformation out for too long
    a. Must one-shot

2018-06-07

Plating and making antibiotic resistant MRS LC

1. Made new chloramphenicol resistant MRS plates
2. Made new chloramphenicol resistant MRS broth
3. Plate all lactobacillus products on chloramphenicol resistant MRS plates
4. Culture all lactobacillus with chloramphenicol resistant MRS broth

2018-06-06

Electroporation

1. Calculate the volume of plasmid DNA needed
    a. 200ng needed per transformation
    b. 6/6:
        i. Take 1 uL if use 101.7 ng/uL DNA
        ii. Take 0.8 uL if use 126.7 mg/uL DNA
2. Mix with 100 uL of cell suspension
3. Voltage setting to 2000 V
4. After electroporation, add 900 uL recovery medium (0.5 M Sucrose MRS broth)
5. Fill LC to to 4 mL with MRS broth (without antibiotic resistance)
6. Incubate at 37 C overnight without shaking

2018-06-05

Pretreatment of Lactobacillus casei

1. Measure OD at 600nm
    a. Take 800uL from each LC and transferred them into the micro-volume cuvettes
    b. Prepare a blank (800mL of MRS, MRS + 1% glycine, or MRS + 0.9 NaCl)
    c. Change the mode to time base on the spectrophotometer
    d. Set the wavelength to 600 nm
    e. Calibrate/ warm-up the spectrophotometer with the blank solution
    f. Take absorbance of the samples
        i. * Found to be around 0.3 abs

Pretreatment

1. Spin culture down in 15mL tubes at 4500rpm for ~7mins until the supernatant is clear
    a. Discard supernatant
2. Resuspend pellet in ~500uL of MRS
3. Add 900uL of cold sterile water

    a. Pipet or stir to mix
4. Transfer to micro centrifuge tubes
5. Let sit on ice for 30 minutes
6. Spin culture down at 12000rpm for 2~3 minutes
    a. Discard supernatant
7. Wash in 1mL of cold sterile 0.5M sucrose
8. Spin culture down at 12000rpm for 2~3 minutes
    a. Discard supernatant
9. Add 100 uL of cold sterile 0.5M sucrose

2018-06-04

Prepping cell culture

1. Grow LC
    a. 2mL original LC + 8mL MRS (PEG)
    b. 2mL original LC + 8mL MRS (sucrose + MRS)
    c. 2mL original LC + 8mL MRS with 1.0% glycine
    d. 2mL original LC + 8mL MRS with 0.9M NaCl
    e. 2mL original LC + 8mL MRS (for growth)
2. Place on shaking incubate overnight

2018-06-02

Result from 5/30: none of the plates grew, going to try pretreatment with water rather than lithium acetate

2018-05-30

PCR + Electroporation of Lactobacillus Casei

1. Transform bob & chromo protein
2. Electroporation at NYMU
3. **<u>Lactobacillus/Lactococcus Electroporation</u>**
   Pretreatment (WITH LITHUM ACETATE)
    a. **Take out solutions from –20 C**
    b. Spin culture down in 15mL tubes at 4500rpm for ~7mins until the supernatant is clear
        i. (in hood) Pipet out supernatant until it reaches the 600uL mark
        ii. Resuspend cell pellet
    c. Add 750 uL of lithium acetate solution (200mM lithium acetate, 1.2M sucrose, 20mM Tris, pH 7.5) and 150uL of 100mM DTT solutions
        i. Solution has to be cold and filter sterilized
    d. Transfer to micro centrifuge tubes
    e. Let sit on ice for 30 minutes
    f. Spin culture down at 12000rpm for 2~3 minutes
        i. Discard supernatant
    g. Wash in 1mL of cold sterile 30% PEG solution
    h. Spin culture down at 12000rpm for 2~3 minutes
        i. Discard supernatant

i. Add 300 uL of cold sterile 30% PEG Solution
1.

Electroporation

1. Calculate the volume of plasmid DNA needed
    a. 200ng needed per transformation
        i. Maybe increase?
    b. 6/13
        i. 2 uL for 133 ng/uL
2. Mix with 300 uL of cell suspension
3. Voltage setting to 2000 V
4. After electroporation, IMMEDIATELY add 900 uL recovery medium (0.5 M Sucrose MRS broth)
5. Incubate at 37 C for 4 hr
6. Preheat MRS plates 1 hr before plating
7. Then plate on MRS plate
    a. 2 plate depending on assituation

Transformation

- Centrifuge tubes
- Carefully discard supernatant with pipet
- Wash with MRS + cm broth
- Centrifuge & discard
- Add MRS + cm broth to yield around 300uL cell suspension
- Resuspend
- Take 2 x 100uL to plate two plates
- Take the rest 100uL and add 10mL MRS + cm broth
- Shake overnight

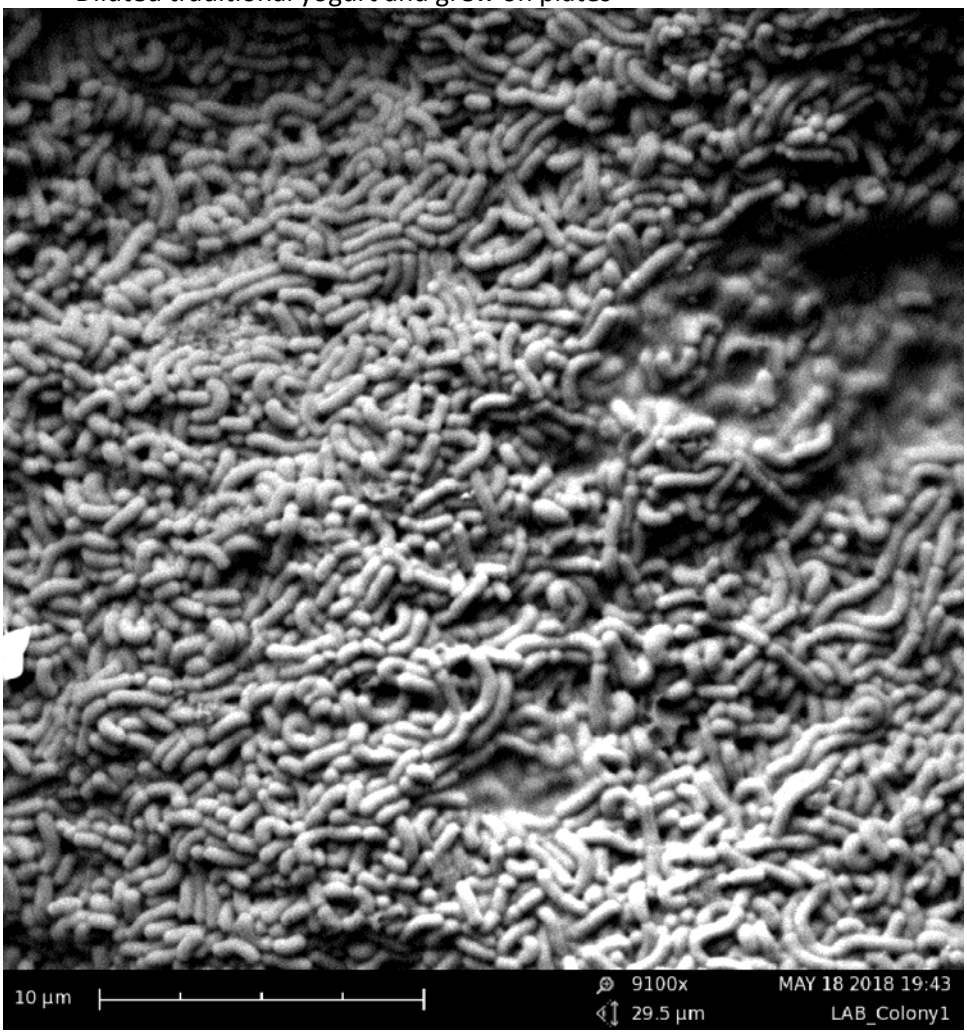
2018-05-30

1. **Prepping cell culture for lactobacillus casei**
    1. Grow LC
        a. 2mL original LC + 8mL MRS (PEG)
        b. 2mL original LC + 8mL MRS (sucrose + MRS)
        c. 2mL original LC + 8mL MRS with 1.0% glycine
        d. 2mL original LC + 8mL MRS with 0.9M NaCl
        e. 2mL original LC + 8mL MRS (for growth)
    2. Place on stirrer overnight
    3. Measure OD at 600nm
        a. Take 800uL from each LC and transferred them into the micro-volume cuvettes
        b. Prepare a blank (800mL of MRS, MRS + 1% glycine, or MRS + 0.9 NaCl)
        c. Change the mode to time base on the spectrophotometer
        d. Set the wavelength to 600 nm
        e. Calibrate/ warm-up the spectrophotometer with the blank solution
        f. Take absorbance of the samples
        g. Make sure the value lands around 0.6~1.2

2018-05-25

- made MRS plates and MRS + cm plates

2018-04-24

- Took high quality pictures of lactobacillus w/SEM
- Made B0015 ALDH2 LC (for xtractor lysis buffer test)
- Ran Protein gel for B0015 ALDH2
- Made new batch of DNPH
- DNPH test w/ lower concentrations (0, 50, 100, 150, 200)
- Diluted traditional yogurt and grew on plates
- 

- Restreak grown trad. Yogurt colonies