

LB MEDIUM AND LB AGAR MEDIUM INGREDIENTS

For 1 lt LB medium,

- 10 g peptone/tryptone
- 5 g yeast extract
- 5 g NaCl

NOTE: Add 15 g agar for LB agar medium.

AGAR PLATE PREPARATION

1. Put the LB agar medium on the heater at ~150 °C and wait until it is completely dissolved.
NOTE: Open the lid of the LB agar medium a little, do not leave it on the heater unless someone else is checking it due to the risk of overheating and spilling.
2. Put the dissolved LB agar medium inside the pre-heated water bath for 45 minutes at 50°C.
3. Add appropriate antibiotics with 1:1000 ratio.
4. Pour the LB agar medium on the agar plates, approximately 20 ml for each agar plate. NOTE: Work in a sterile environment near the Bunsen Burner flame. You don't need to use a pipettor for this. Pour directly.
5. Wait until LB agar medium is solidified. NOTE: Work in a sterile environment near the Bunsen Burner flame.
6. Plates can be used immediately. Do not put hot plates into the 4°C room. Condensation will wet the plates and you will have a bad time spreading. Incubating the plates at room temperature until they are cooled down will result in better solidification.

COMPETENT CELL PREPARATION

NOTE: During competent cell preparation all steps should be performed VERY CAREFULLY in a sterile environment near the Bunsen Burner flame!

1. Incubate the cells in 5 ml LB medium overnight at 37°C.
2. Dilute the overnight culture with 1:100 ratio. (For 50 ml diluted sample get 500 µl overnight sample and mix with 49.5 ml LB medium) NOTE: If the diluted sample size is 50 ml, 50 competent cells will be prepared.
3. Incubate for ~2 hours 37°C until OD600 value is in between 0.2-0.5.
4. Split the culture into two ice-chilled falcon tubes and chill the samples on ice for 10 minutes.
5. Centrifuge the samples for 10 minutes at 3000 rpm at 4°C and remove the supernatant.
6. Resuspend the cells by pipetting with ice-chilled TSS buffer 1:10 ratio. (If diluted sample is 50 ml, add 5 ml TSS buffer) NOTE: Perform the step on ice.
7. Add 100 µl of resuspended sample to ice-chilled 1.5 ml microcentrifuge tubes. NOTE: Perform the step on ice.

TRANSFORMATION

NOTE: During transformation all steps should be performed in a sterile environment near the Bunsen Burner flame!

1. Competent cells are put on ice until they are defrosted. **NOTE: An additional competent cell should be put on ice as the control group and all procedures except plasmid addition should be performed for the control group.** NOTE: 100 μ l competent cell can be divided in two to ice-chilled micro-centrifuge tubes and one of them can be used as the control group.
2. Add 100 ng of plasmid except the control group and keep the cells on ice for 30 minutes.
3. Put the cells on the heat block for 45 seconds at 42°C, then put them back on the ice for 2 minutes.
4. Add 250 μ l LB medium.
5. Incubate the cells for 45 minutes in 37°C incubator.
6. Centrifuge the cells at 5800 g for 5 minutes.
7. Remove the supernatant (approximately 100 μ l supernatant should be left in the tube).
8. Resuspend the cells by pipetting and spread them on agar plates.
9. Incubate the agar plates overnight at 37°C.

GEL EXTRACTION

1. Weigh the 2 ml microcentrifuge tube and tare the scale.
2. Add the cut agarose gel piece into the micro-centrifuge tube and weigh the added agarose gel piece.
3. Add NT1 solution on top of the agarose gel piece. NOTE: If the weight of the gel piece is 0.4 grams, add 800 μ l NT1 according to the gel extraction kit of MN. NOTE: It is better to check the kit brand before using (for Thermo Fisher, gel to buffer ratio is 1:1)
4. Then, put the micro-centrifuge tube on the heat block for 10-15 minutes at 50°C until the agarose gel is fully dissolved. You can store this liquefied gel at 4°C for 24h.
5. Add 700 μ l of the dissolved mixture to a column and centrifuge at 12000 g for 1 minute, remove the supernatant. NOTE: If there is dissolved mixture left, repeat the step until all the dissolved mixture is passed through the column.
6. Add 700 μ l NT3 buffer and centrifuge at 12000 g for 1 minute, remove the supernatant. NOTE: Check the label on the NT3 buffer to be sure that ethanol addition is completed.
7. Repeat the 6th step.
8. Centrifuge the empty column for 1 minute to remove left ethanol. Wait for a couple of minutes to remove the ethanol completely for the column to dry.
9. Put the top of the column to a 1.5 ml microcentrifuge tube.

10. Add 20 µl 70°C pre-heated ddH₂O on the middle of the column without touching to the filter and wait for 3 minutes.
11. Centrifuge the column+micro-centrifuge tube for 4 minutes.
12. Perform nanodrop analysis with 1-2 µl of the sample.

PCR

USE NEB's PCR PROTOCOLS SPECIFIC FOR THE POLYMERASE USED.

PCR Master Mix (4X)

ddH ₂ O	66 µl
Q5 reaction buffer	20 µl
Reverse primer	5 µl
Forward primer	5 µl
dNTP mix	2 µl
Q5 polymerase	1 µl

1. The master mix is prepared in a PCR tube.
2. 25 µl of the master mix is transferred into a new PCR tube as the control group.
3. 100 ng of template DNA is added into the remaining master mix.
4. Template added master mix is aliquoted into three PCR tubes.

For PCR conditions check

<https://www.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491>.

RESTRICTION ENZYME DIGESTION

Reaction Buffer	2.5 µl
Restriction Enzyme	0.5 µl
DNA	300-400 ng of DNA for visualization * 1000 ng DNA for cloning
ddH ₂ O	Complete the reaction volume to 25 µl with the addition of ddH ₂ O.

*If the DNA amount is lower, a smaller reaction volume should be adjusted to use a lower amount of restriction enzyme.

NOTE: Check the appropriate incubation temperature, time and reaction buffer type for the specific enzyme. Time saver enzymes generally require 15mins. For detail: <https://www.neb.com/tools-and-resources/selection-charts/time-saver-qualified-restriction-enzymes>
All High-Fidelity (also some other enzymes) require CutSmart buffer.

AGAROSE GEL PREPARATION AND ELECTROPHORESIS

For 1:1 agarose gel, smallest tank;

1. Weigh 0.65 grams of agarose and mix with 65 ml TAE buffer inside an Erlenmeyer flask.
2. Heat the mixture inside the microwave oven until the agarose is melted. Place the Erlenmeyer flask on the outer diameter of the microwave plate. Wait for 2 minutes and check if the mixture becomes clear per 15 seconds.
3. After the mixture becomes clear, cool it with running water.
4. Add 0.65 μ l SYBR Safe to the melted agarose mixture. NOTE: SYBR Safe amount is depends on the amount of powder agarose you add. For instance 1.0 grams of agarose addition requires 1 μ l SYBR Safe.
5. Pour the agarose mixture on top of the prepared gel tank.
6. Place the appropriate comb.
7. Remove the bubbles with the help of a pipette tip.
8. Wait for 15-20 minutes until the agarose gel is solidified.
9. Add 6X purple loading dye to the samples and pipette gently, avoid bubble formation. (For 25 μ l sample add 5 μ l of 6X purple loading dye)
10. Load 4 μ l 2log ladder into one of the wells and load the samples.
11. Connect the plugs and run the gel until the 2log ladder is separated (With 1% gel, 20 mins at 140V, 40 mins at 120V, although size of the target fragment is important too.). Heavier fragments need more running time.

NOTE: To prepare a gel in the medium tank, use 100 ml TAE.

PCR CLEANUP

NOTE: All centrifugations should be carried out in a table-top microcentrifuge at >12000 g (10 000-14 000 rpm, depending on the rotor type).

NOTE: Instead of multiple centrifugation steps, vacuum can be used for steps: 2-3-4

1. Add NT1 solution on top of the sample. NOTE: If the sample size is 25 μ l, add 50 μ l NT1.
2. Add mixture to a column and centrifuge for 1 minute, remove the supernatant.
3. Add 700 μ l NT3 buffer and centrifuge for 1 minute, remove the supernatant. NOTE: Check the label on the NT3 buffer to be sure that ethanol addition is completed.
4. Repeat the 6th step.

5. Centrifuge the empty column for 1 minute to remove left ethanol.
6. Put the top of the column to a 1.5 ml microcentrifuge tube.
7. Put the column+microcentrifuge tube on the pre-heated heat block for 3 minutes at 70°C to remove ethanol.
8. Add 20 µl 70°C pre-heated ddH₂O on the middle of the column and wait for 3 minutes.
9. Centrifuge the column+micro-centrifuge tube for 4 minutes.
10. Perform nanodrop analysis with 1-2 µl of the sample.

GIBSON REACTION

Gibson mix	15 µl
Plasmid	50-100 ng
DNA fragment	1:1 plasmid-DNA fragment ratio, depends on their length*

After the reaction mixture is prepared the, it is incubated for 1 hour at 50°C inside the PCR machine.

NOTE: Whole mixture can be added to the competent cells during transformation.

For smaller inserts (smaller than 200 bp) use 1:5 vector:insert ratio

MINIPREP

NOTE: All centrifugations should be carried out in a table-top microcentrifuge at >12000 g (10 000-14 000 rpm, depending on the rotor type).

NOTE: For different plasmid isolation kits, different solution volumes can be applied, check the volumes for each step for each kit.

1. Centrifuge the overnight cell culture for 5-10 minutes at 8000 rpm and remove the supernatant.
2. Add 250 µl resuspension solution (P1 or A1) on top of the cell pellet and vortex until the cell pellet is dissolved.
3. Transfer the resuspended cells to a 2 ml microcentrifuge tube.
4. Add 250 µl of the lysis solution (P2 or A2) and invert the tube for 4-6 time and wait for 3 minutes. NOTE: Do not harshly mix the solution but gently invert the tube and do not incubate the mixture more than 5 minutes so as to avoid genomic DNA contamination.
5. Add 350 µl neutralization solution (N3 or A3) and mix the solution immediately by inverting the tube 6-8 times, the solution should become cloudy. NOTE: Do not harshly mix the solution but gently invert the tube.
6. Centrifuge for 10 minutes at maximum speed.
7. Transfer 700 µl of the supernatant to the column and centrifuge for 1 minute at 12000 g, remove the flow through. NOTE: Do not touch the white precipitate during the transfer of the

supernatant. NOTE: If there is remaining supernatant in the micro-centrifuge tube, repeat the step 7.

8. Add 500 µl wash buffer 1 (PB or AW)and centrifuge for 1 minute remove the flow through.

9. Add 700 µl wash buffer 2 (PE or A4)and centrifuge for 1 minute remove the flow through.

NOTE: If you have only one wash buffer, use it twice (700 ul).

10. NOTE: Check the label on the wash buffer to be sure that ethanol addition is completed.

11. Centrifuge the empty column for 1 minute at 12000 g to remove left ethanol. Wait for a couple of minutes to remove the ethanol completely for the column to dry.

12. Put the top of the column to a 1.5 ml microcentrifuge tube.

13. Add 20 µl 65°C pre-heated ddH₂O on the middle of the column without touching the filter and wait for 3 minutes. NOTE: If you are going to store the DNA for a long amount of time, elution with TE will increase shelf life because it has EDTA. However this will lower the efficiency of PCR, sequencing and other cation dependent reactions. So only use it for making stocks of your plasmid. Some other elution buffers have only Tris HCl pH 8.5 put this again might interfere with salt sensitive reactions like PCR.

14. Centrifuge the column+micro-centrifuge tube for 4 minutes at 12000 g.

15. Perform nanodrop analysis with 1-2 µl of the sample.

LIGATION

T4 DNA Ligase Buffer	2 µl
T4 DNA Ligase	1 µl
Plasmid	50 ng (0.020pmol, mass depends on length) *
Insert DNA	Usually 1:1 plasmid-insert DNA ratio, depends on their length. (1:3 molar ratio of plasmid:insert, so 0.060pmol)
ddH ₂ O	Complete the reaction volume to 20ul with the addition of ddH ₂ O.

***Useful website for ligation dna calculation**

<https://nebiocalculator.neb.com/#//ligation>

1. Gently mix the reaction by pipetting up and down and microfuge briefly.

2. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.

3. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours.
4. Heat inactivate at 65°C for 10 minutes. This isn't really necessary if you are going to perform chemical transformation.
5. Chill on ice and transform 5-10 µl of the reaction into 50 µl competent cells.

SDS GEL ELECTROPHORESIS

1. Preparation of SDS gel
2. For a 5 ml stacking gel:
 - i. ddH₂O 2.975 ml
 - ii. 0.5 M Tris-HCl, pH:8.8 1.25 ml
 - iii. 10% (w/v) SDS 0.05 ml
 - iv. Acrylamide/Bis-acrylamide (30%/0.8%) 0.67 ml
 - v. 10% (w/v) ammonium persulfate (APS) 0.05 ml
 - vi. TEMED 0.005 ml
3. For a 10 ml separating gel:

Acrylamide percentage	6%	8%	10%	%12	%15
H ₂ O	5.2 ml	4.6 ml	3.8 ml	3.2 ml	2.2 ml
1.5 M Tris-HCl, pH:8.8	2.6 ml	2.6 ml	2.6 ml	2.6 ml	2.6 ml
10%(w/v) SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	2 ml	2.6 ml	3.4 ml	4 ml	5 ml
10% (w/v) ammonium persulfate (APS)	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
TEMED	0.01 ml	0.01 ml	0.01 ml	0.01 ml	0.01 ml

1. Note: APS and TEMED are the toxic polymerizing agents and must be added right before each use in a hood.
2. Load the separating gel first and add isopropanol onto it to get rid of air bubbles. Let it solidified and discard the isopropanol by pouring. Then load the stacking gel, and immerse the comb.
3. Load 20 µl of the samples mixed with loading dye and run at 120 V for 10 mins and at 190V for 40 mins.

4. After the run we place the gel in a container with Coomassie Blue dye for staining and microwave for 15-40 seconds. The gel should be checked in between to be safe.
5. The container is put on shaker for 10 minutes then washed under water.
6. We then place the gel into another container with Destaining Buffer and it can be put on shaker overnight for the destaining. The bands are usually clearly visible the next morning.

WESTERN BLOT

1. Before setting up the cassettes we place Whatman filter papers into Transfer Buffer, and the nitrocellulose membrane into methanol first for a few minutes then into Transfer Buffer as well.
2. One of the Whatman papers is placed in the cassette and flattened with the rolling apparatus gently so there are no bubbles trapped.
3. The membrane is placed on to the first paper and the gel is placed onto the membrane. The second layer of Whatman paper goes onto the gel. After placing each layer we roll over them with the apparatus again gently to smooth out the bubbles.
4. We place this cassette inside the tank and it is set to Turbo -> Mini Gel -> Run for a 7-minute transfer.
5. While the transfer is occurring we prepare the Blocking Buffer with 5% dry milk in TBS-T. This solution can be used up to a few times so we do not discard it, keep it at -20.
6. When the transfer is over we take the membrane out with tweezers gently and the top corner is marked to know which side is up.
7. The membrane is placed inside the milk solution and incubated inside the shaker for 1-2 hours at RT.
8. Then we place the membrane carefully into the 1st Antibody solution (1:10000 in 5% dry milk solution in TBS-T) and keep it rocking on the shaker for another 1 hour at RT or O/N at +4 (cold room).
9. Remove primary Ab solution and keep it at -20 (up to 5 usages) then we put enough TBS-T to cover it, leaving it rocking for another 5 minutes. Afterwards the membrane is washed twice, first for 5 then for 10 minutes.
10. Then we expose the membrane to the 2nd Antibody (1:10000 in 5% dry milk solution in TBS-T) for 1 hour at RT, rotating.
11. Remove secondary Ab solution and keep it at -20 (up to 5 usages) then we put enough TBS-T to cover it, leaving it rocking for another 5 minutes. Afterwards the membrane is washed twice, first for 5 then for 10 minutes.
12. Then we take the image of the membrane.

13. DO IT IN DARK!!! Take 500ul A and B from ECL solution substrates and mix. Apply the solution on membrane and wait for 1 min. Remove the substrate and take the image (ASK FOR HELP).

COLUMN REGENERATION

1. Add 700 µl of 1 M HCl to previously used columns, close their lids and wait for 3 days.
2. Remove the HCl inside the column and the collection tube.
3. Centrifuge all columns for 1 minute at max speed.
4. Add 700 µl ddH₂O, centrifuge for 1 minute at max speed.
5. Repeat step 4.
6. Add 700 µl QBT, centrifuge for 1 minute at max speed.
7. Repeat step 6.
8. Repeat steps 4 and 5.
9. Centrifuge empty columns for 3 minutes at max speed.
10. Open the lids of the columns and let them dry.

INDUCTION WITH ATC FOR FLUORESCENT REPORTERS

1. The colonies are picked from the plate and put into 5 ml LB medium with 1:1000 appropriate antibiotics. Then, the culture is incubated for 16 hours at 37°C. NOTE: Also, empty competent cell type, which our plasmid is transformed, is incubated overnight and then diluted with the same way applied to other samples. For overnight culture, biological(or technical ?) replicas(3) is required to get more reliable results.
2. The overnight culture is diluted 1:50 and added 1:1000 appropriate antibiotics.
3. The diluted samples are incubated at 37°C until 0.4-0.6 OD₆₀₀ value is obtained.
4. Then, the samples are separated into two groups: induced with aTc and uninduced groups
5. 1:1000 aTc is added to the induced group.
6. Separated groups are incubated for 2.5 hours at 37°C.
7. Two samples are divided into four groups: only aTc induced, only inducing agent induced, inducing agent and aTc induced and uninduced.
8. Inducing agent is added to appropriate groups.
9. All samples are incubated at 37°C for 16 hours.
10. After the incubation, 200 µl of each sample are loaded into microcentrifuge tubes and centrifuged at 8000 g for 5 minutes.
11. The supernatant is discarded and 200 200 µl of PBS buffer are added.
12. After PBS addition the samples are centrifuged at 8000 g for 5 minutes.
13. The supernatant is discarded and 200 200 µl of PBS buffer are added.

14. After PBS addition the samples are centrifuged at 8000 g for 5 minutes.
15. Then the samples are loaded into 96-well plate.
16. OD₆₀₀ and fluorescence values are measured with plate reader.

Note: Different aTc ratios and inducing agent concentrations can be adjusted depending on the experimental setup.

For general info: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC146584/>

HEAT RELEASE

1. Overnight grown cells are centrifuged and the supernatant was discarded.
2. Cell pellet was resuspended with 1 ml 1X PBS and washed once.
3. 1X PBS was added again and cells were resuspended.
4. Resuspended cells were divided to two eppendorfs equally (1 sample and 1 control).
5. Cells were heated (except the control groups) in the heat block (or water bath) at 60°C for 5 minutes.
6. Cells were centrifuged at maximum speed for 1.5 minutes and the supernatants were collected in an M5 Plate Reader to check fluorescence intensity.

INTESTINE-LIKE BUFFER

1. 0.03g Bile Salt
2. 0.8g NaCl
3. 0.320 BSA
4. Sufficient ddH₂O to make 1 liter
5. Sufficient HCl for adjusting pH to 7.43 - ileum

MOPS MINIMAL MEDIA

A. 10x MOPS

	<u>For 50 ml</u>
1. ddH ₂ O	15 ml
2. MOPS Sodium Salt	8.372 g
3. Tricine	0.3583 g
4. 10M KOH	until pH = 7.4
5. Freshly prepared FeSO ₄	250 µl

Note: 0.028g FeSO₄ in 10 ml

6. 1.9M NH ₄ Cl	2.5 ml
7. 0.276M K ₂ SO ₄	500 µl
8. 0.2M CaCl ₂ .H ₂ O	12.5 µl
9. 2.5M MgCl ₂	105 µl
10. 5M NaCl	5ml
11. micronutrient	10 µl
12. ddH ₂ O	19.35 ml

B. MOPS Minimal Media

	<u>For 50 ml</u>
1. 10x MOPS Mixture	50 ml
2. 0.132M K ₂ HPO ₄	5 ml
3. 1mg/ml thiamine	50 µl
4. ddH ₂ O	440 ml

Notes:

1. pH = 7.2
2. Filter w/ 0.2 µm
3. Store @ +4
4. Before use, add 0.2% glucose.

MODIFIED MOPS MEDIUM

1x MOPS

	<u>For 500 ml</u>
1. ddH ₂ O	15 ml
2. MOPS Sodium Salt	8.372 g
3. Tricine	0.3583 g
4. 10M KOH	until pH = 7.4
5. Freshly prepared FeSO ₄	250 µl

Note: 0.028g FeSO₄ in 10 ml

6. 1.9M NH ₄ Cl	2.5 ml
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7.	0.276M K ₂ SO ₄	500 µl
8.	0.2M CaCl ₂ .H ₂ O	12.5 µl
9.	2.5M MgCl ₂	105 µl
10.	5M NaCl	5 ml
11.	micronutrient	10 µl
12.	ddH ₂ O	19.35 ml
13.	0.132M K ₂ HPO ₂	5 ml
14.	1 mg/µl thiamine	50 µl
15.	ddh20	440 ml

Note: Adjust pH to 8.00 with 37% HCl.

Filter w/ 0.2 µm

Store @ +4

Before use, add 0.2% glucose.

AG43 DISPLAY EXPERIMENT WITH EXTERNAL TEV PROTEASE

1. Overnight inoculated cells are centrifuged at 4000 rpm for 10 minutes.
2. Resuspend cells with desired medium (MOPS, Tev Reaction Buffer, Intestine-Like Buffer etc.) and centrifuge again at 4000 rpm for 10 minutes (Wash Step).
3. Resuspend in 3 ml of medium, split into 12 eppendorfs, 250 µl to each, including 3 replica of the medium as a blank measurement.
4. 3 replicas of cells are (t₀ cells) and 3 replicas of supernatant (t₀ supernatant) are measured at Fluorescence Plate Reader or Fluorescence Spectrometer.
5. Add TEV enzyme 1:25 to 3 replica and incubate the TEV added and not added 6 eppendorfs at 4 C for 16 hours.
6. Centrifuge the samples, and take measurement at Fluorescence Plate Reader or Fluorescence Spectrometer.

AG43 DISPLAY EXPERIMENT WITH EXTERNAL INDUCER

1. Inoculated cells are diluted 1:100 to MOPS Medium and incubated at 37 C until OD600 is between 0.38-0.42.
2. Cells with araC promoter are induced with 0.2 % concentration of L-arabinose.
3. Incubate the cells for 24 hours at 18 C.
4. After incubation, change the medium and induce the cells again with the same concentration.
5. Take measurement from supernatant at Fluorescence Plate Reader or Fluorescence Spectrometer for every 4 hours for 24 hours including the moment of induction.

IPTG INDUCTION

1. Inoculate the cells in 5 mL LB with appropriate antibiotics (1:1000) overnight.
2. Subculture the overnight grown cells as 1:100 into 10 mL of LB with %1 (w/v) glucose and add the appropriate antibiotics.
3. At mid log phase (OD_{600} : 0.4 – 0.6), add 1 mM IPTG and leave for induction at 18°C overnight. Note: To prepare %1 w/v glucose, first prepare 20% w/v glucose solution, filter it with a 0.2 μ m filter, and then dilute to 1X. For IPTG, dilute 1M (1000X) IPTG to 1X (1mM). Do not add IPTG to the un-induced control groups.
4. For heat release of the surface displayed proteins, take 5×10^8 cells (OD_{600} of 1.0 = 8×10^8 cell/mL), centrifuge and discard the supernatants. Wash with 1 mL 1X PBS.
5. Resuspend the washed cells with 250 μ L 1X PBS again. Heat the suspended cells at 60°C for 5 minutes. Centrifuge the cells at maximum speed for 5 minutes and collect 200 μ L from the each supernatant.
6. Precipitate the proteins by adding five volume of acetone (Protein volume \times 5) to the supernatant (1 mL in this case) (Acetone must be cold, store it at -20°C and do not heat before adding). Incubate overnight at -20°C.
7. Centrifuge the precipitated proteins at maximum speed at 4°C for 1 hour. Discard the and leave the pellet for air dry supernatant (Do not discard the acetone into liquid waste!!! Since it can react with bleach and release toxic gases, collect it in a beaker and leave it in a working hood). Do not over-dry the proteins.
8. Dissolve the air-dried pellets in 1X SDS-PAGE loading dye (20 μ L). Dilute the 6X loading dye by double distilled water (Prepare a master mix). Heat the proteins at 95°C for 5 minutes.

GROWTH AND FLUORESCENCE MEASUREMENT

1. Inoculate cells in LB or MOPS and grow them for 16 hours.
2. Dilute grown cells to 1:100 ratio with the medium. Add appropriate antibiotic (if any). There should be 3 replicas for each sample. Prepare also a blank medium.
3. Take 200 μ l from each replica and place to 96 well plate.
4. Place the well plate to M5 spectrophotometer.
5. Make a measurement for fluorescence. For this, choose fluorescence and adjust wavelengths to 485 nm (ex), 538 nm (em), 530 (cutoff).
6. Make a measurement for OD_{600} . For this, choose absorbance and adjust wavelength to 600 nm.
7. Put the cells to the shaker at 37 °C.

8. Repeat the steps 3,4,5 and 6 for every 30 minutes for 6 hours. Depending on the purpose of the experiment the total duration might be shorter or longer. t.