iGEM MIT_MAHE
Product Development Experiments Handbook

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1. Introduction

The **Inoculum development process** is a stepwise and gradual increase in scaling-up the volume of inoculum to the desired level. The initial inoculum and the media formulation are the most important step to determine the efficiency of any product development process. Scale up from lab scale to industrial scale requires many changes to be made to both. Changes to the inoculum significantly effects the entire operation be it by lack of cell growth or loss of integrity etc.

Since our product is the organism itself, we need to test various properties before we can implement it on an industrial level. Examining the tolerance of bacteria to pH and temperature, effects of different inoculum percentage and ages on growth curve, calculating the doubling time and plasmid loss rates, etc are very important to obtain a final inoculum of appropriate properties.

Nitrogen is used for the anabolic synthesis of nitrogen-containing cellular substances, such as amino acids, purines, DNA, and RNA. The amount of nitrogen in media greatly effects the growth of the bacteria. During media formulation, different nitrogen content and sources can be used and optimum concentration can be determined.

2. General Protocols

Note: For safety instructions of all the experiments included in general protocols and experimentation refer to iGEM MIT_MAHE safety handbook.

A. Preparation of media

i. Preparation of terrific broth

Aim: To prepare a media for cell culture.

Principle:

Terrific broth is an enriched media used for cell culture. It uses glycerol as the carbon source.

Materials required:

Yeast extract (yeastolate), glycerol, weigh dishes/paper, Balance, Potassium Phosphate (0.17M monobasic, 0.72M dibasic), Media bottle, autoclave.

Procedure:

Component	Amount	Unit
Yeast extract	24	g
Glycerol	10	mL
Potassium phosphate (0.17M monobasic, 0.72 M dibasic)	100	mL

Broth - final volume 1000mL, Autoclave volume - 900mL

- Add approximately 80% of the final volume of dH_2O to a sterile media bottle
- Add 24g yeast extract, 10mL glycerol to the dH_2O.
- Seal the cap of the media bottle and shake until the components are completely dissolved.
- Transfer the solution to a sterile volumetric flask and adjust the volume to the autoclave volume (i.e. total volume – potassium phosphate volume) using dH_2O, ensure that it is well mixed, then return it to the media bottle.
- Apply autoclave tape to the bottle and autoclave on liquids cycle.
- Begin warming a bath to 55°C.

- Allow solution to cool to 55°C in water bath.
- Add the calculated amount (100mL) of potassium phosphate solution.
- If adding selectable components, add them at this stage.
- Store media at 4°C.

Precautions:

- Ensure that the solution is always well mixed.

ii. Preparation of Terrific Broth agar

Aim: To prepare a media for cell culture.

Principle:

Terrific broth is an enriched media used for cell culture. It uses glycerol as the carbon source.

Materials required:

Yeast extract (yeastolate), glycerol, weigh dishes/paper, Media bottle, Potassium Phosphate (0.17M monobasic, 0.72M dibasic), Agar autoclave, Balance.

Procedure:

Component	Amount	Unit
yeast extract	24	g
glycerol	10	mL
potassium phosphate (0.17M monobasic, 0.72 M dibasic)	100	mL
bacto agar	15	g

Solid Medium: (final vol. 1000mL)

- Add approximately 80% of the final volume of dH_2O to a sterile media bottle
- Add 24g yeast extract, 10mL glycerol to the dH_2O.
- Seal the cap of the media bottle and shake until the components are completely dissolved.
- Transfer the solution to a sterile volumetric flask and adjust the volume to the autoclave volume (i.e. total volume – potassium phosphate volume) using dH_2O, ensure that it is well mixed, then return it to the media bottle.
- Add appropriate amount (15g) of agar at this point. It does not need to dissolve at this stage.
- Apply autoclave tape to the bottle and autoclave on liquids cycle.
- Begin warming a bath to 55°C. Also label approximately 1 plate per 15mL of solution.
- Allow solution to cool to 55°C in water bath.
- Add the calculated amount (100mL) of potassium phosphate solution.
- If adding selectable components, add them at this stage.
- Pour approximately 15mL into each labelled petri dish and allow to cool to room temperature.
- Once the media has gelled, replace the dishes inverted into their respective sleeve.
- Store media at 4°C.

Precautions:

- Ensure that the solution is always well mixed.
- When pouring agar media to petri dish, ensure that the entire bottom of the dish is covered.
- Solid media should always be stored/incubated in inverted position.

Protocol Source: Terrific Broth Agar

B. Polymerase Chain Reaction (PCR)

Aim: To conduct a Polymerase Chain Reaction of the DNA fragments (parts) before the Gibson Assembly Reaction.

Principle:

The principle of Polymerase Chain Reaction (PCR) is based on the temperature variations of heating and cooling-thermocycling reaction divided into three steps:

Denaturation: The hydrogen bonds between the nitrogen bases are broken and the double-stranded DNA is broken to single-stranded DNA at a high temperature. This occurs at 98°C.

Annealing: The primer (short RNA sequence) attaches to the DNA (complementary to it). The primer provides a site for the initiation of synthesis. This occurs at 60-72°C.

Extension: DNA polymerase uses the 3' end of the primer and starts DNA synthesis by adding nucleotides to the growing DNA strand. This occurs at 72°C.

These steps make up 1 cycle and each cycle is repeated for about 25-30 times resulting in the amplification of the desired DNA. This results in an exponential increase/amplification of DNA.

Materials required:

Micropipette, PCR tubes, Phusion HF DNA Polymerase, dNTP mix (10mM) (deoxyribonucleotide triphosphate), Nuclease-free Water, Insert DNA (template DNA), 10µM forward primers, 10µM reverse primers, Vortex, Ice bath, Thermal cycler, Tips, Gloves, and 5X Phusion HF Buffer.

Procedure:

Note: Maintain aseptic conditions throughout.

PCR primers used to amplify the Bio-Brick Parts:

Primers	Description	Sequence
VR	Reverse primer for sequencing/amplifying Bio-Brick parts (VR)	attaccgcctttgagtgagc (R)
VF2	Forward primer for sequencing/amplifying Bio-Brick parts (VF2)	tgccacctgacgtctaagaa (F)

Reaction preparation:

- Prepare a Master Mix with 10µL of 5x Phusion HF buffer, 5µL of 10mM dNTP and 2.5µL of forward and reverse primers each.
- Aliquot 8µL of the Master Mix into each PCR tube. Prepare Master Mix for 5 PCR tubes.
- Mix 2.5µL of the template DNA in 10µL of nuclease-free water.
- Take 1µL of the sample in each PCR tube with the help of a micropipette.
- Mix 2.5µL of Phusion HF DNA polymerase in 10µL of nuclease-free water, taking 1µL of the sample in each PCR tube.
- Add 15µL of nuclease-free water into each tube. Hence the total volume in each tube comes up to be 25µL.
- Mix the contents gently with the help of a vortex.

Thermocycler:

Initial Denaturation	98°C	30 seconds	1 cycle
Amplification	98°C. Primer Tm (60-70°C). 72°C	10 seconds 20 seconds 30 seconds per kb	25-30 cycles
Final Extension	72°C	5 minutes	1 cycle
Hold	4°C	-	1 cycle

Precautions:

- Proportions of different reagents should be carefully monitored to get the desired output.
- Handle with gloves and maintain aseptic conditions to prevent contamination by unwanted DNA.

Protocol source: Polymerase chain reaction

C. Construction of plasmids

i) Restriction digestion of the Bio-Bricks using XbaI and SpeI and Agarose Gel Electrophoresis

Aim: To perform the restriction digestion (double digestion) of the amplified Bio-Bricks with XbaI & SpeI enzymes and visualization in Agarose Gel Electrophoresis.

Principle:

Digesting a DNA substrate with two restriction endonucleases simultaneously is called double digestion. These restriction enzymes are nucleases that cleave the sugar-phosphate backbone of DNA, in a specific site called restriction site to generate a smaller set of fragments. These restriction sites are palindromic sequences. The enzyme usually cuts within the molecule and are hence called restriction endonucleases. These enzymes act as a defense system in bacteria by cleaving foreign DNA.

Restriction sites:

XbaI: 5'…T | CTAG A…3'

3'…A GATC | T…5'

SpeI: 5'…A | CTAGT…3'

3'…TGATC | A…5'

Materials required:

Distilled water, DNA (Bio-Bricks), Control DNA, 2X Assay buffer, Restriction enzymes (XbaI, SpeI), 0.5mL Microfuge tubes (vials), Micropipettes and tips, Dry bath incubator, Vortex, Crushed ice, Beakers, Gel loading buffer and dye, Conical flask, Measuring cylinder and Staining tray.

Procedure:

- Place the vials containing restriction enzyme (XbaI and SpeI) on ice.
- Thaw the vials containing substrate (DNA) and assay buffer.
- Prepare the reaction mixture using the following constituents.

Reaction mix (XbaI and SpeI double digestion) (10 µL):

DNA (substrate)	0.5-1µg
2X assay buffer	2µL
XbaI	1µL
SpeI	1µL
Distilled water	Rest

- Gently mix by tapping. Briefly spin for a few seconds.
- Incubate the vial at 37°C for 1 hour on a dry bath.
- Meanwhile, prepare agarose gel for electrophoresis.
- After an hour, incubate at room temperature for 10 minutes.
- Add 5 µL of gel loading buffer to vials.
- Load the digested samples, 10 µL of Control DNA, and 5 µL of the gel loading dye; note down the order of loading.
- Electrophorese the samples at 50-100 V for 1-2 hours.
- Using UV illuminator visualize the gel.

Precautions:

- Make sure that the restriction enzyme does not exceed more than 10% of the total reaction volume, otherwise, the glycerol and the EDTA in the enzyme storage buffer may inhibit the digestion process.

Note: Other restriction sites that can be used are EcoR1, Pst1, and Not1 (Bio-Brick Compatibility).

Protocol source: Restriction digestion
Agarose gel electrophoresis: Protocol
For more info on Bio-Brick Compatibility refer here.

ii) Agarose Gel Electrophoresis

Aim: To visualize and analyze the restriction digested fragments.

Principle:

DNA is a negatively charged molecule at neutral pH. Gel electrophoresis apparatus is used to separate charged molecules under the influence of a constant current. The DNA molecules migrate towards the positive charge with the separation depending on the molecular size of DNA, the strength of the electric field applied, hydrophobicity of the DNA, the concentration of agarose, ionic strength of the buffer, the temperature of the buffer, and conformity of the DNA.

The agarose gel is a linear polymer extracted from seaweed. It (purified powder) is insoluble in water and buffers at room temperature but dissolves on boiling. On pouring into a mold and cooling, it undergoes polymerization acting like a molecular sieve. The DNA moves through this on the application of current. Pore size depends on the concentration of agarose used.

Small fragments can pass through the pores easily whereas the larger ones find it harder to do so and separation occurs. A visible tracking dye is applied to determine the progress of the DNA through the gel, and Serva DNA stain G is used for visualizing the DNA in the transilluminator.

Materials required:

Agarose, Micropipettes and tips, Beakers, Distilled water, 2.5X Gel loading buffer, Gel loading dye (bromophenol blue), 6X Staining dye (Serva DNA stain G), TAE 10X Buffer, Heating apparatus (microwave oven), Gel electrophoresis apparatus (Basic Unit, Gel casting tray, Gel running tray, Comb, Comb stand, Electrodes, and Power cord) and UV transilluminator.

Procedure:

Gel preparation:

- Dilute the appropriate amount of 50X TAE buffer and prepare 1X TAE.
- Prepare 1% agarose by weighing 0.5g of agarose and adding it to 50mL of 1X TAE.
- Boil the agarose in the microwave till it dissolves completely and a clear solution is obtained.
- Add 1 µL of Serva DNA stain G (staining dye) to the clear agarose solution.
- Meanwhile, place the combs of electrophoresis set approximately 2 cm away from the cathode (black).

- Pour the agarose + Serva DNA stain G solution in the central part of the tank when the temperature reaches approximately 60°C. Make sure no air bubbles are generated. The thickness of the gel should be around 0.5 to 0.9 cm.
- Keep the gel undisturbed at room temperature for the agarose to solidify.
- Pour 1 X TAE buffer into the gel tank till the buffer level stands at 0.5 to 0.8 cm above the gel surface.
- Gently lift the combs, ensuring that wells remain intact and no air bubbles are formed.

Ladder & samples:

- Mix 2 µL of the control DNA with 2 µL loading dye (bromophenol blue) and 1 µL Ultra-pure water for the preparation of the ladder.
- Mix 10 µL of PCR samples with 2 µL loading dye.

Electrophoresis conditions:

- Move the solidified gel to the electrophoresis set up and connect the wires appropriately (Red (anode), Black (cathode)).
- Load the samples (and ladder) in each well carefully and run it for 90 minutes at 100V.
- Visualize the sample in UV transilluminator.

Precautions:

- The comb/well side should face the cathode (Black).
- Make sure there aren't any air bubbles when the agarose is in its liquid form.

Protocol source: Agarose gel electrophoresis

iii) Gibson Assembly Protocol (E5510)

Aim: To ligate and assemble one or more inserts into a vector to create a circular vector containing the inserts of interest.

Principle:

Gibson assembly is a molecular cloning technique used to ligate fragments of DNA and is named after its creator Daniel G. Gibson.

Three enzymatic activities: exonuclease, polymerase and ligase are carried out in isothermal conditions. The 5' exonuclease generates long overhangs by which the 5' end sequences are chewed back. This exposes the complementary sequence for annealing to which the primer attaches followed by filling of gaps of the annealed single-stranded regions by the polymerase. The nicks of the annealed and filled-in gaps are then sealed and ligated (covalently linked) by DNA ligase.

Materials required:

Milli-Q water (dH2O), Bucket with ice, PCR tubes, gBlock(s), Linearized vector, 2X Gibson Assembly Master Mix (NEB), Thermal cycler, Fiber-free tissues, NanoDrop spectrophotometer, labels (Cryo-tags), Pipettes and tips, Pre-prepared primers.

Procedure:

Design primers to amplify fragments (and/or vector) with appropriate overlaps.

- Determine the concentration of fragments using agarose gel electrophoresis, a Nanodrop™ instrument, or another method.

Nanodrop protocol:

- Start the NanoDrop spectrophotometer and select DNA measurement as 'Nucleic Acid' in the NanoDrop menu.
- Clean the surface of the NanoDrop with dH2O and a fibre-free tissue.
- Perform calibration and blank measurement by entering one drop of 2 µL autoclaved dH2O.
- Clean the surface again with dH2O and a fibre-free tissue, place 2 µL per sample on the NanoDrop and measure the concentration. Note the concentration on appropriate labels (Cryo-tags).

- Calculate how much insert is needed for the reaction.
- NEB recommendations:
    - When 1-3 inserts are being assembled, a total of 0.02 – 0.5 pmol of DNA fragments is needed.
    - When 4-6 inserts are being assembled, a total of 0.2-1.0 pmol of DNA fragments is needed.

Formula:

pmol= (weight in nanogram) × 1000/number of bp ×650 da

Online Tools: NEBioCalculator

(***Note:*** Yields will be best when the different inserts are present in equimolar concentrations.)

- Prepare an icebox, put every material on ice.

Set up the following reaction in a PCR tube on ice:

Assembly type	**2-3 Fragment Assembly (vector + 1-2 inserts)**	**4-6 Fragment Assembly (vector + 3-5 inserts)**	**Positive Control****
Total Amount of Fragments	0.02–0.5 pmols* (x µL)	0.2–1 pmols* (x µL)	10 µL
2X Gibson Assembly Master Mix	10 µL	10 µL	10 µL
Milli-Q water	10-XµL	10-XµL	0
Total Volume	20 µL***	20 µL***	20 µL***

* Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if the size is less than 200 bps. The total volume of unpurified PCR fragments in the Gibson Assembly reaction should not exceed 20%.

** Control reagents are provided for 5 experiments.

*** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.

- Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.
- Following incubation, store samples on ice or at –20°C for subsequent transformation.
- Transform competent cells with 2µL of the assembly reaction, following the transformation protocol.

Precautions:

- Reaction time less than 15 minutes are generally not recommended. Extended incubation time (up to 4 hours) has been shown to improve assembly efficiencies in some cases. Do not incubate the reaction overnight.
- The efficiency of assembly decreases as the number or length of inserts increases. Hence use the optimal amount only.
- Inserts to be assembled should not have a stable single-stranded DNA secondary structure, such as a hairpin or a stem-loop, or repeated sequences, as this would directly compete with the required single-stranded annealing/priming of neighboring assembly fragments.

D. Competent cell preparation

Aim: To prepare fresh competent cells of *Escherichia coli* Nissle 1917.

Principle:

Competent cells are cells that have altered cell walls that allow DNA to easily pass through. The most widely used method to artificially induce competency in cells is the calcium chloride heat shock method. The exact mechanism of how this process works is still unknown. One of the hypothesis states that the calcium ions act as a cation bridge between the negatively charged phosphorylated lipid in lipopolysaccharide (LPS) of the cell membrane and the phosphate backbone of DNA. The ice-cold $CaCl_2$ solution facilitates the binding of DNA to the surface of the cell which enters after a short period of heat shock. Competent cells are then identified by selection or screening markers such as drug resistance (Chloramphenicol and Kanamycin in our case) etc.

Materials required:

Petri plates with TB, TB liquid medium, Wild type *Escherichia coli* Nissle 1917, Incubator, Laminar flow chamber, Autoclave, Micropipette, tips, Sterile loop, 500mL flask, Shaker incubator, Ice, $CaCl_2$ solution, Glycerol solution, Centrifuge and Micro-centrifuge tubes.

Procedure:

Note: All the steps should be performed aseptically using sterile equipment and experimental conditions.

- Culture the wild type *Escherichia coli* Nissle 1917 on TB plate overnight (Do not add the antibiotic).
- Using a sterile loop inoculate one colony from the TB plate into 2 mL TB liquid medium. Shake at 37°C overnight (Do not add antibiotic).
- Inoculate 1 mL overnight cell culture into 100 mL TB medium (in a 500 mL flask).
- Shake vigorously at 37°C to OD600 ~0.25-0.3.
- Chill the culture on ice for 15 min. Also, make sure the 0.1M $CaCl_2$ solution and 0.1M $CaCl_2$ plus 15% glycerol are on ice.
- Centrifuge the cells for 10 min at 5000*g at 4°C.
- Discard the medium and resuspend the cell pellet in 30-40 mL cold 0.1M $CaCl_2$. Keep the cells on ice for 30 min.

- Centrifuge the cells as above.
- Remove the supernatant and resuspend the cell pellet in 6 mL 0.1 M $CaCl_2$ solution plus 15% glycerol.
- Pipette 0.4-0.5 mL of the cell suspension into sterile 1.5 mL micro-centrifuge tubes

Storage:

- Prepare a glycerol solution (65% glycerol, 0.1 M $MgSO_4$, 0.025 M Tris/HCl pH 8).
- Store in 1 mL aliquots at -80°C.

Precautions:

- Stock freezing can decrease the transformation efficiency, hence if possible, prepare fresh competent cells.

E. Transformation

Aim: To introduce the composite Bio-Bricks into the competent *Escherichia coli* Nissle 1917 (or any other bacteria).

Principle:

Genetic alteration of a cell due to direct uptake and incorporation of genetic material from its surroundings is called Transformation. It occurs through the cell membrane. It is a key step in molecular cloning. Transformation occurs in nature due to conditions such as starvation, cell density, etc. It can also be induced in the lab. The bacteria then produce multiple copies of foreign DNA which can be utilized according to the application.

Materials required:

Competent cells (*Escherichia coli* Nissle 1917*)*, Plasmid DNA, Competent cells, Sterile TB broth, TB agar plate (supplemented with chloramphenicol and Kanamycin), Microcentrifuge tubes, L-rod, Ice bath, Dry bath or water bath, Incubator with shaker and Laminar airflow chamber.

Procedure:

Note: All the steps should be performed aseptically using sterile equipment and experiment conditions.

Use media without antibiotics.

- If the competent cells were stored, take it out of -80°C and thaw on ice (approximately 20-30 mins).
- Keep TB agar plates ready (Incubate at 37°C (optional)).
- Add 1-5 µL of plasmid DNA and 20 -50 µL of competent cells into a microfuge tube and gently mix by flicking.
- Incubate the tube on ice for 20-30 mins.
- Place the tube (1/2 to 2/3 of it) in a 42°C water bath or a dry bath for 30-60 seconds (heat shock treatment).
- Put the tubes back on the ice for 2 min.
- Add 250-1,000 µL TB broth (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 45 min.
- Plate the transformed bacteria onto a 10 cm TB agar plate containing the appropriate antibiotic (Chloramphenicol and/or Kanamycin).
- Incubate plates at 37°C overnight.

Precautions:

- If the culture volume is high, centrifuge and gently collect and resuspend the cells in a smaller volume of TB broth so that there isn't too much of this media on the plates.
- If too much media is present, the bacteria diffuse through the liquid and will not grow colonies. Invert the plates after the liquid is absorbed.
- Avoid the thawing of cells before use.
- The cells should not be kept on ice for longer than 3 hours.
- The cells that have been on ice should not be used again.

F. Calibration

Aim: To calibrate instruments to ensure accurate reading

Principle:

Instrument calibration is an essential stage in most measurement procedures. It is a set of operations that establish the relationship between the output of the measurement system (e.g., the response of an instrument) and the accepted values of the calibration standards (e.g., the amount of analyte present).

Materials:

Spectrophotometer, fluorometer, 187 µg FITC, 10mL 1xPBS (phosphate buffered saline), cuvettes.

Procedure:

GFP:

Note:

- By measuring these in all standard modes in your plate reader or fluorimeter, you will generate a standard curve of fluorescence for FITC concentration.
- You will be able to use this to correct your cell-based readings to an equivalent fluorescein concentration.
- You will then be able to convert this into a concentration of GFP.

Prepare the FITC stock solution:

- Spin down FITC stock tube to make sure pellet is at the bottom of tube.
- Prepare 10x FITC stock solution by resuspending FITC in 1 mL of 1xPBS.
- Incubate the solution at 42°C for 4 hours.
- Dilute the 10x FITC stock solution in half with 1xPBS to make a 5x FITC solution and resulting concentration of FITC stock solution 2.5 µM.

[***Note:*** it is important that the FITC is properly dissolved. To check this after the incubation period, pipette up and down – if any particulates are visible in the pipette tip continue to incubate overnight.]

Note: Use 2.0mL tubes for cuvette dilutions, and then transfer 1.0mL into your cuvettes.

- Add 1 mL of PBS into tubes 2-11.
- Add 2.0 mL of FITC 5x stock solution tube 1.
- Transfer 1.0 mL of FITC stock solution tube 1 into tube 2.
- Mix tube 2 by pipetting up and down 3x and transfer 1 mL into tube 3…
- Mix tube 3 by pipetting up and down 3x and transfer 1 mL into tube 4…
- Mix tube 4 by pipetting up and down 3x and transfer 1 mL into tube 5…
- Mix tube 5 by pipetting up and down 3x and transfer 1 mL into tube 6…
- Mix tube 6 by pipetting up and down 3x and transfer 1 mL into tube 7…
- Mix tube 7 by pipetting up and down 3x and transfer 1 mL into tube 8…
- Mix tube 8 by pipetting up and down 3x and transfer 1 mL into tube 9…
- Mix tube 9 by pipetting up and down 3x and transfer 1 mL into tube 10…
- Mix tube 10 by pipetting up and down 3x and transfer 1 mL into tube 11…
- Mix tube 11 by pipetting up and down 3x and transfer 1 mL into liquid waste

Measurement:

- Measure the plate (or cuvettes) in your plate reader (or fluorimeter).
- Setup the machine with the standard GFP settings (filters or excitation and emission wavelengths) that you will use when measuring your cells (if you change them you will not be able to use this standard curve).
- Repeat the measurement several times with different settings. You will then have a series of standard curves to choose from. Most important it is necessary to use several settings that affect the sensitivity (principally gain and/or slit width).

OD_{600}:

- Calibration curve can be determined by comparing measured OD_{600} to expected OD_{600}.
- Determine the expected OD_{600} by counting cell number using an alternative technique (for example microscope slide method).
- Convert to OD_{600} using the rule of thumb that 1 OD_{600} = 8 x 108 cells/mL for *Escherichia coli*.

Note: Clumping together of cells will also affect readings, so the medium they are suspended in will also make a difference.

Protocol source: GFP OD600

3. Experimentation

A. Preliminary requirements:

Aim: To ensure same initial cell conditions before each experiment.

Materials required:

TB agar, 37° Incubator, 20% glycerol stock solution (glycerol + distilled water), terrific broth medium, -80 freezer, transformed cells.

Procedure:

- Using a sterile loop inoculate TB liquid medium with transformed bacterium and grow at 37° ± 1°C for overnight.
- Dilute pure glycerol in distilled water to create a 50% glycerol solution.
- After you have bacterial growth, add 500 µL of the overnight culture to 500 µL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.
- Maintain the mixture in –80 freezer.

Results:

- The stock solution of bacteria is prepared.

B. Inoculum percentage:

Aim: To determine the optimum inoculum percentage to be used.

Principle:

The main objective of inoculum development for unicellular bacterial fermentations is to produce an active inoculum, which will give as short a lag phase as possible in subsequent culture. A long lag phase is disadvantageous in that not only time is wasted but also medium constituents are consumed in maintaining a viable culture prior to growth. The length of the lag phase is affected by the size of the inoculum and its physiological condition with mid-exponential cultures tending to reduce the length of the lag phase [7].

Materials required:

Glycerol stock solution, TB broth, Incubator, Erlenmeyer flasks, pH probe, spectrophotometer, centrifuge.

Procedure:

- Revive the culture was revived from a glycerol vial in 15 mL TB liquid medium and incubate at 37° ± 1°C.
- Inoculate the culture in five Erlenmeyer flasks with the TB liquid medium at the following concentrations (v/v): 1.0%, 2.5%, 5.0%, 7.5% and 10.0%.
- Incubate the flasks at 37° ± 1°C for 20 h under anaerobic conditions.
- At every 4-h interval, check the samples from each flask for pH and OD600.
- Plot graphs of Inoculum age vs pH and inoculum %age vs OD600.

Results:

- The optimum inoculum percentage can be determined.

[7] Stanbury et al., 2017.

Transformation: protocol TB broth: protocol

C. Doubling time:

Aim: To determine the doubling time of *Escherichia coli* Nissle 1917.

Principle:

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. The time taken by the bacteria to double in number during a specified time period is known as the generation time or doubling time (DT). Generation time varies widely across organisms and is an important factor in the life cycle, life history and evolution of organisms. The determination of generation time can also be used to estimate the number of cycles and generations of bacterial growth required in a bioreactor.

Materials:

12-18h terrific broth culture of *Escherichia coli* Nissle 1917, terrific broth, laminar hood, Orbital incubator shaker, 250 mL conical flasks, 15 mL test tubes, Glassware marker, 1.0 and 0.2 mL sterile disposable tips, Micropipettes.

Procedure:

- An over-night culture of *Escherichia coli* Nissle 1917 is used to inoculate 100 mL of nutrient broth in a 250 mL conical flask at 1% level.
- The flask containing culture was incubated in an orbital shaker at 37°C, 180rpm.
- Aliquots of the culture were taken aseptically at regular intervals and the turbidity was measured in a spectrophotometer at 600 nm using nutrient broth as blank.
- Optical density of the samples at 600nm was recorded till 24h of growth
- The $O.D_{600}$ values as a function of time were plotted in a semi-log paper to generate the growth curve
- The generation time of the bacteria can be determined by extrapolation from the growth curve
- Plot the growth curve and calculate the generation time from the curve.
- The biomass concentration in different samples is obtained by use of calibration curve obtained earlier.
- A graph is plotted between biomass concentrations vs. time.
- Linear part of the graph, which is exponential phase of growth, is taken for specific growth calculation.

Observation and Results:

- In all cases the estimated DT in the wild is greater than that of the bacterium in the laboratory.
- *Escherichia coli* Nissle 1917 can double every 20 min in the laboratory, but we estimate that it only doubles every 15 h in the wild.

Incubation time (h)	**Optical Density (600 nm)**
0	
1.0	
2.0	
3.0	
4.0	
5.0	
6.0	
8.0	
12.0	
18.0	
24.0	

D. Inoculum age

Aim: To determine the optimum inoculum age of the bacteria.

Principle:

Age of the inoculum is an important parameter which contributes to the optimal efficiency of the bioreactor.

Materials required:

Glycerol stock solution, TB broth, Incubator, Erlenmeyer flasks, pH probe, spectrophotometer, centrifuge.

Procedure:

- Revive the culture from a glycerol vial in 15 mL TB liquid medium and incubate at 37° ± 1°C for different log hours of growth, namely 6-, 10- and 15-h.
- At the different log hours, transfer the optimum amount of inoculum to Erlenmeyer flasks containing the TB liquid medium.
- Incubate at 37° ± 1°C for 24 h.
- At every 4-h interval, check each sample for pH and OD600.
- Plot graphs of Inoculum age vs pH and inoculum age vs OD600.

Result:

- The optimum inoculum age can be determined.

Transformation: protocol

TB broth: protocol

Calibration: Protocol

E. Loss of plasmid stability

Aim: To determine the rate of loss of plasmid stability due to genetic burden.

Principle:

Low-copy-number plasmids risk plasmid loss during cell division. This will cause our probiotics to lose its therapeutic ability. Hence, we need to calculate the plasmid loss rate so that we can modify the manufacturing process to generate an optimal generation time for the therapeutics effect [6] [9].

Materials:

Overnight culture of *Escherichia coli* Nissle 1917 containing GFP + plasmid, Eppedorf tubes, liquid TB+ chloramphenicol and kanamycin, Micropipette and tips.

Procedure:

- For ten replicates add 1uL of liquid overnight culture to a labelled Eppendorf tube (1, 2, 3, 4, 5... 10).
- Make a 1:1000 dilution in TB + chloramphenicol and Kanamycin for each aliquot of culture in order to allow 10 generations grow in each cycle. (Add 999 µL of TB chloramphenicol to all tubes).
- Put all tubes on incubation/growth in 37° C for 24 hours.
- Measure expression of GFP in each tube by comparing the colour intensity of the tubes. Rank by: ++ strong color; + weak color; -, colorless. Plate all cultures with high color intensity on TB + chloramphenicol and Kanamycin agar plates.
- Take 1 µL of each tube/culture and make a 1:1000 dilution in TB + chloramphenicol and Kanamycin again in a new Eppendorf tube and put all tubes on incubation for 37° C in 24 hours. After incubation, measure the color intensity in each tube again and rank in the same way as before.
- Repeat the procedure 4 times in order to get an assay spanning over 40 generation.

Result:

- The plasmid loss rate can be determined.

[6]Lau B. T. et al., 2013 [9] Sengupta M. et al, 2011

Transformation: protocol TB broth: protocol

Calibration: Protocol

F. Effect of temperature

Aim: To determine the effect of temperature on bacteria.

Principle:

Each species of microbe has a particular temperature at which it grows best. At this temperature all aspects of the cell metabolism function at their optimum values, the cell is able to rapidly increase in size and divide.

Materials required:

Glycerol stock solution, TB broth, Incubator, Erlenmeyer flasks, pH probe, spectrophotometer, incubator.

Procedure:

- Revive the culture from a glycerol vial in 15 mL TB liquid medium.
- Inoculate the cells at optimum %age at the optimum age in five Erlenmeyer flasks containing the growth medium.
- Incubate the flasks at 30° ± 1°C, 34° ± 1°C, 37° ± 1°C, 38° ± 1°C and 40° ± 1°C for 24 h under anaerobic conditions.
- At every 4-h interval, check the samples for pH and OD600 and plot a graph of temp vs pH and OD vs pH.

Result:

- The optimal temperature can be determined.

G. Effect of pH

Aim: To determine the optimal pH for growth of bacteria.

Principle:

Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn, promotes changes in the folding of the molecule, promoting denaturation and destroying activity. The optimum growth pH is the most favourable pH for the growth of an organism.

Materials required:

Glycerol stock solution, TB broth, Incubator, Erlenmeyer flasks, pH probe, spectrophotometer, Incubator.

Procedure:

- Revive the culture from a glycerol vial in 15 mL TB liquid medium.
- Inoculate the cells at optimum %age at the optimum age in five Erlenmeyer flasks containing the growth medium.
- Adjust the pH of the growth medium for each of these tubes to 3.0, 5.0, 6.0, 7.0 and 9.0 prior to sterilization, using 0.1N sodium hydroxide (NaOH) or 1N orthophosphoric acid (OPA).
- Incubate the tubes at 37° ± 1°C for 24 h under anaerobic conditions.
- At every 4-h interval, check each tube for pH and OD600.
- Plot graph of OD600 vs pH.

Result:

- The optimal pH can be determined.

H. Establishment of growth curve of the strain

Aim: To determine the growth curve of the strain at determined optimal conditions and to calculate cell density.

Principle: To ensure that the bacterium has the most suitable growth curve at these conditions, we need to study and assess its growth curve by plotting a graph of OD600 vs time.

Materials required:

Plated bacterial strain of interest, Terrific broth media, Graphing software, Non-selective agar plates (~20), Permanent marker, Spreaders, 2 mL Eppendorf tubes, Vortex, UV-VIS spectrophotometer, Cuvettes.

Procedure:

- Revive the culture from a glycerol vial in 15 mL Terrific broth and incubated at 37° ± 1°C for 24h.
- Inoculate 1% (v/v) seed inoculum (6-h-old) to TB.
- Dilute the culture of bacteria 1/200 into fresh TB and shake at 30 °C for about 3 h (until OD600 ~1.0)

Note: Do not overgrow the bacteria. The turbidity of the culture should be within the linear range of the UV-VIS instrument.

- Dilute the freshly grown culture 4:1, 3:2, 2:3, 1:4, 1:9 (Culture: Media) into fresh TB media and measure the OD600 of each sample (including the original sample where OD~1).
- Dilute each of the six samples to 10^{-2} by vortexing 10 µL of sample with 990 µL of TB in a 2 mL tube; dilute the samples to 10^{-4} by vortexing 10µL of the 10^{-2} dilutions into 990 µL of TB; plate 100 µL of the 10^{-4} dilution of each sample onto a labeled non-selective agar plate.
- Dilute the solutions further by vortexing 100 µL of the 10^{-4} dilutions into 900 µL TB (to give 10^{-5}); repeat this procedure using 100 µL of the 10^{-5} dilutions into 900 µL TB (to give 10^{-6}); plate 100 µL of the resulting 10^{-5} and 10^{-6} dilutions onto non-selective agar plates.
- Incubate the plates overnight at 30 °C and count the resulting colonies.

Note: Assuming six samples are plated at three different dilutions (10^{-4}, 10^{-5}, and 10^{-6}), there should be 18 plates total.

- Calculate the CFU/mL for each of the original six samples using the equation:

$$\frac{CFU}{mL} = \frac{Colonies\ per\ plate}{Dilution\ \times Volume\ plated}$$

For example, 44 colonies from 100 µL of a 10^{-4} dilution would correspond to 4.4×10^6 CFU/mL. Colony counts are significant if there are between 30 and 300 colonies on a plate.

- Graph data with CFU/mL on the y-axis and OD600 on the x-axis, setting the intercept equal to zero; add a linear trendline and record the resulting equation

Result:

- The growth rate and CFU/mL at optimal conditions can be determined.

I. Estimation of amount of nitrogen in the sample

i. Spectrophotometric method

Aim: To measure the total nitrogen (TN) using spectrophotometric method.

Principle:

This method of spectrophotometry is based on colorimetric principles, measuring nitrogen after a persulfate oxidation of nitrogen compounds in alkaline conditions to nitrate, reduction step, and addition of chemicals reacting with the N-containing molecule [2]. Nitrate formed during the oxidation is reduced to nitrite here with an acidic vanadium chloride reagent, which is simultaneously added with amines forming a coloured compound, an azo dye, that is detectable spectrophotometrically.

Total dissolved nitrogen (TDN) can be determined after filtering the sample, which excludes the particulate organic carbon (PON). TDN can be measured together with dissolved organic carbon (DOC) using high-temperature (>700°C) catalytic oxidation [5] [8].

Materials required:

Boric acid, Concentrated hydrochloric acid (37%), Disodium ethylenediaminetetraacetate dihydrate (e.g., Titriplex® III), N-(1-naphtyl)-ethylenediamine dihydrochloride (NED), Potassium peroxodisulfate with low nitrogen content (e.g., Merck 1.05092), Sodium hydroxide, Sulfanilamide, Vanadium chloride, Ultrapure water, 25 mL reaction flasks with screw caps, 10 mL reaction tubes with screw caps, 50 mL graduated cylinders, 50–1000 mL volumetric flasks, 100–1000 mL storage bottles, Spatulas, Adjustable pipettes and pipette tips, 1 cm plastic cuvettes or flow-through cuvette, Analytical balance, Autoclave, Oven (capable of being maintained at 45°C), Refrigerator and Spectrophotometer.

Procedure:

- Prepare 0.075M sodium hydroxide solution by dissolving 3 g sodium hydroxide in 1000 mL of ultrapure water such that the amount of NaOH in the solution is adequate for neutralization of the 2 mol of H+ formed per mol of persulfate.
- Prepare oxidizing reagent by dissolving 10 g potassium peroxodisulfate and 6 g boric acid in 1 L of 0.075M sodium hydroxide solution. Store in a brown glass bottle in a refrigerator (stable for several weeks).

- Prepare sulfanilamide solution (nitrite reagent I) by dissolving 1.0 g sulfanilamide in a mixture of 85 mL ultrapure water and 14.5 mL concentrated HCl. Store in a glass bottle at room temperature.
- Prepare NED solution (nitrite reagent II) by dissolving 0.07 g NED in 100 mL ultrapure water. Store in a brown glass bottle in a refrigerator (stable about 1 month).
- Prepare vanadium chloride solution by dissolving 1.6 g vanadium chloride in a mixture of 170 mL ultrapure water and 16.8 mL concentrated HCl, and then add ultrapure water to a final total volume of 200 mL. Store in glass bottle in a refrigerator (stable about 1 month).
- Prepare TN mix reagent by mixing 200 mL vanadium chloride solution with 40 mL sulfanilamide solution and 40 mL amine solution. Store in a refrigerator (prolongs stability).
- Prepare total nitrogen standard 140 µg/mL N (10 µmol/mL N) by dissolving 0.1862 g Na_2-EDTA in ultrapure water, and then add ultrapure water to a final total volume of 100 mL. Store in a glass bottle in a refrigerator (stable for several months).
- On the day of analyses, prepare working solutions of total nitrogen (0–600 µg/L) for calibration by diluting the standard stock solution (previous step) with ultrapure water.
- Pipette 5 mL of each calibration standard and 5 mL of ultrapure water for blanks to separate reaction flasks respectively. Add 5 mL oxidizing reagent solution to each flask and close the flask. Mix by swirling, autoclave for 30 min, and allow them to cool to room temperature.
- Prepare spectrophotometer and pipette 0.5 mL of the oxidized standards and blanks to reaction tubes. Add 3 mL TN mix reagent to each tube, and close the tubes. Mix well by swirling, incubate for 1 h at 45°C, and allow to cool to room temperature.
- Measure the absorbance of blanks and standards with a spectrophotometer in a 1 cm cuvette (or flow-through cuvette) at 545 nm for a calibration curve.
- Collect the samples in glass bottles or polyethylene bottles, and analyse the samples immediately after collection. The sample can be stored in a glass bottle in a refrigerator for a day.
- For analysing the sample, pipette 5 mL of sample (or diluted sample) to reaction flasks.
- Add 5 mL oxidizing reagent to each flask and close the flasks. Add 5 mL oxidizing reagent to three empty flasks for reagent blanks. Mix by swirling, autoclave for 30 min, and allow to cool to room temperature.
- Add 5 mL ultrapure water to the reagent blanks, close the flasks, and mix well.
- Perform manual analyses with a spectrophotometer along with ultrapure water blanks in the run.
- For manual analyses, pipette 0.5 mL of the oxidized samples and reagent blanks to reaction tubes. Add 3 mL TN mix reagent to each tube and close the tubes. Mix well by swirling, incubate 1 h at 45°C, and allow to cool to room temperature.
- Measure the absorbance of the reagent blanks and samples with a spectrophotometer in a 1 cm cuvette (or flow-through cuvette) at 545 nm, and calculate the total nitrogen

concentration using the calibration curve by plotting measured absorbance versus standard concentrations.

Expected observation:

- Starting measurement with sample run with ultrapure water blank(s) and reference materials should verify the proper operation of the instrument.
- Insertion of standards in the sequence tests the accuracy and drift during the analytical runs.
- The slope of the calibration curve determines the concentration of nitrate in sample after persulfate digestion of nitrogen compounds.

Expected result:

- There should be no influence by varying salt contents on the final result.
- The slope of the linear regression of the calibration curve (measured absorbance versus standard concentrations) determines the nitrate concentration and hence the total nitrogen concentration in the sample.

[2] K. Grasshoff et al., 1999 [5] J. Koistinen et al., 2020 [8] Bernhard Schnetger and Carola Lehners, 2014

ii. Kjeldahl method

Aim: To determine the nitrogen content using Kjeldahl's method.

Principle:

The basis of the Kjeldahl method is digestion of the sample with sulfuric acid in the presence of catalysts. Organic nitrogen is reduced to ammonium sulphate, which is distilled in the presence of sodium hydroxide, liberating ammonia gas. The distillate is collected into boric acid solution, and the borate anions formed are titrated with standardized hydrochloric acid solution.

The milliequivalents of acid required for titration are used to calculate the nitrogen content in the sample. However, this method has the disadvantages of using corrosive and/or toxic chemicals with consequent waste production and risk to human health, long analysis time, and multiple steps providing many opportunities for error. Inorganic forms of nitrogen, such as nitrates and nitrites, are not measured by the Kjeldahl method if they are not adequately reduced during digestion [4].

Materials required:

Sulfuric acid (93% - 98%; N-free), Mercuric oxide(HgO) or metallic mercury, Potassium sulphate (or anhydrous sodium sulphate), Salicylic acid, Sulphide or thiosulphate solution, Sodium hydroxide, Hydrochloric acid or sulphuric acid standard solution (0.5N or 0.1N), Sodium hydroxide standard solution (0.1N), Digestion flask, Heating device, Paraffin, Distilled water, Zinc granules, Condenser, Methyl red indicator.

Procedure:

- Place weighed sample (0.7 – 2.2 g) in digestion flask and add 0.7 g HgO or 0.65 g metallic Hg, 15 g powered potassium sulphate or anhydrous sodium sulphate and 25 mL sulphuric acid.
- Place the flask in an inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing).
- Boil briskly until the solution becomes clear and then equal to or more than 30 min longer.
- Cool the solution and add 200 mL distilled water. After the solution cools down to less than 25°C, add 25 mL sulphide or thiosulphate solution and mix to precipitate Hg.
- Add few zinc granules to prevent bumping, tilt flask and add layer of sodium hydroxide without agitation.
- Immediately connect the flask to the distributing bulb on condenser, with the tip of the condenser immersed in standard acid and 5-7 drops indicator in the receiver.

- Rotate flask to mix the contents thoroughly, then heat until all NH_3 has distillated.
- Remove the receiver, wash the tip of the condenser and titrate excess standard acid in distillate with standard NaOH solution.
- Correct for blank determination on reagents.

Formula used:

% N = [(mL std acid x normality of acid) – (mL std NaOH x normality of NaOH)] x 1.4007/g sample

Expected observation:

- The total nitrogen content for nitrate-free samples is determined using the Kjeldahl method.

Expected result:

- The detection limit for Kjeldahl method is higher compared to Dumas method.
- The total nitrogen value measured is lower compared to Dumas combustion method.

[4] S. Jung et al., 2003

4. Glossary

Term	Definition	Ref
2X Assay buffer	Buffer is a solution that resists changing its pH level even when acidic or basic components are added to it. For assays, this makes it very useful for maintaining a desired pH (often physiological pH, but not always) even during extended or multi-step experiments.	Link
Agarose	A linear polysaccharide made up from alternating D-galactose and 3,6-anhydro-α-L-galactopyranose residues joined by α-(1→3)- and β-(1→4)-linkages.	Link
Agarose gel electrophoresis	Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar.	Link
Agitation	Agitation is the brisk stirring or disturbance of a liquid.	Link
Amplification	The production of multiple copies of a sequence of DNA. Repeated copying of a piece of DNA.	Link
Anaerobic conditions	Anaerobic conditions occur when the uptake or disappearance of oxygen is greater than its production by photosynthesis or diffusion by physical transport from the surrounding environment.	Link
Analyte	An analyte is a chemical substance or chemical constituent that is of interest in the analytical procedure.	Link
Anhydrous	A substance is anhydrous if it contains no water.	Link
Annealing	The process of two strands of DNA rejoining is called annealing.	Link
Antibiotics	Antibiotics are medicines that help stop infections caused by bacteria. They do this by killing the bacteria or by keeping them from copying themselves or reproducing. The word antibiotic means “against life.” Any drug that kills germs in your body is technically an antibiotic.	Link
Aseptic	Asepsis or aseptic means the absence of germs, such as bacteria, viruses, and other microorganisms that can cause disease or contamination.	Link

Autoclave	An autoclave is a machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure/temperature.	Link
Bio-Brick	Bio-Brick parts are DNA sequences which conform to a restriction-enzyme assembly standard.	Link
Bio-Brick compatibility	The assembly standard enables two groups of synthetic biologists in different parts of the world to re-use a Bio-Brick part without going through the whole cycle of design and manipulation.	Link
Bromophenol blue	Bromophenol blue is used as a pH indicator, an electrophoretic color marker, and a dye.	Link
Calibration	In measurement technology, calibration is the comparison of measurement values delivered by a device under test with those of a calibration standard of known accuracy.	Link
Catalyst	A catalyst is a substance that can be added to a reaction to increase the reaction rate without getting consumed in the process. Catalysts typically speed up a reaction by reducing the activation energy or changing the reaction mechanism.	Link
Cell culture	Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment. After the cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions which vary for each cell type.	Link
Cell suspension	A cell suspension or suspension culture is a type of cell culture in which single cells or small aggregates of cells are allowed to function and multiply in an agitated growth medium, thus forming a suspension.	Link
Centrifuge	A centrifuge is a device that uses centrifugal force to separate component parts of a liquid or fluid. This is achieved by spinning the fluid at high speed within a container, thereby separating fluids of different densities or liquids from solids.	Link
Chloramphenicol	Chloramphenicol is an antibiotic useful for the treatment of a number of bacterial infections.	Link

Cleaving	A reaction that severs one of the covalent sugar-phosphate linkages between NUCLEOTIDES that compose the sugar phosphate backbone of DNA. It is catalyzed enzymatically, chemically or by radiation. Cleavage may be exonucleolytic - removing the end nucleotide, or endonucleolytic - splitting the strand in two.	Link
Competent cells	Cell competence refers to a cell's ability to take up foreign (extracellular) DNA from its surrounding environment.	Link
Composite Bio-Brick	Composite Bio-Brick is an assembly of larger synthetic biological circuits from individual parts and combinations of parts with defined functions to construct new biological systems.	Link
Control DNA	Control DNAs are powerful tools in the investigation of genomic DNA methylation and epigenomic research. They can be utilized in many protocols where they can serve as comparisons to sample DNA.	Link
Denaturation	Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent, etc.	Link
dNTP	dNTP stands for deoxyribonucleotide triphosphate. Each dNTP is made up of a phosphate group, a deoxyribose sugar and a nitrogenous base. There are four different dNTPs and can be split into two groups: the purines and the pyrimidines.	Link
Doubling time	Doubling time (DT) or generation time is the time taken by the bacteria to double in number during a specified time period.	Link
EDTA	Ethylenediaminetetraacetic acid, also known by several other names, is a chemical used for both industrial and medical purposes.	Link
Enzyme	An enzyme is a biological catalyst which is not destroyed during the reaction and is almost always a protein. It speeds up the rate of a specific chemical reaction in the cell.	Link
Equimolar	The definition of equimolar is having the same amount of moles.	Link

Erlenmeyer flask	An Erlenmeyer flask, also known as a conical flask or a titration flask, is a type of laboratory flask which features a flat bottom, a conical body, and a cylindrical neck. It is named after the German chemist Emil Erlenmeyer, who created it in 1860.	Link
Escherichia coli DH5alpha	*DH5-Alpha* Cells are *Escherichia coli* cells engineered to maximize transformation efficiency.	Link
Escherichia coli Nissle 1917	*Escherichia coli* strain Nissle 1917 is a Gram-negative microorganism with probiotic properties that has been successfully used for the treatment of intestinal inflammation, especially in patients suffering from ulcerative colitis.	Link
Exponential	An exponential rate of increase becomes quicker and quicker as the thing that increases becomes larger.	Link
Extension	In PCR, the extension step, also referred to as the elongation step, is the PCR step in which Taq polymerase adds nucleotides to the annealed primer.	Link
FITC	Fluorescein isothiocyanate (FITC) is the derivative of fluorescein with an isothiocyanate reactive group, rendering it reactive towards amine and sulfhydryl groups commonly found in biomolecules and used in wide-ranging applications including flow cytometry.	Link
Fluorescence	Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.	Link
Fluorometer	A fluorometer or fluorimeter is a device used to measure parameters of visible spectrum fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light.	Link
Frothing	A mass of bubbles in or on a liquid; foam.	Link
Gel loading buffer	DNA gel loading buffer is intended to be mixed with samples containing DNA in order to facilitate loading of the samples into the wells of horizontal and vertical agarose and polyacrylamide.	Link
Genetic burden	In synthetic biology, the term "metabolic burden" describes the influence of heterologously expressed genes on the distribution availability of resources in the host cell.	Link

Gibson assembly	Gibson Assembly is a molecular cloning method which allows for the joining of multiple DNA fragments in a single, isothermal reaction.	Link
Growth curve	A growth curve is an empirical model to visually represent the growth of bacteria over time.	Link
Initiation	Initiation is the beginning of transcription. It occurs when the enzyme RNA polymerase binds to a region of a gene called the promoter.	Link
Inoculum	Cells used in an inoculation, such as cells added to start a culture.	Link
Lag phase	During lag phase in bacterial growth, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.	Link
Laminar flow chamber	A laminar flow chamber or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user.	Link
LB Agar	LB agar is LB broth that contains agar and is typically prepared in petri dishes.	Link
LB Broth	Lysogeny broth is a nutritionally rich medium primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended LB to stand for lysogeny broth, but LB has also come to colloquially mean Luria broth, Lennox broth, or Luria–Bertani medium.	Link
Ligase	Ligase is an enzyme that can catalyze the joining of two large molecules by forming a new chemical bond, usually with accompanying hydrolysis of a small pendant chemical group on one of the larger molecules or the enzyme catalyzing the linking together of two compounds.	Link
Lipopolysaccharide	Lipopolysaccharides (LPS), also known as endotoxins, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria.	Link

Molecular cloning	Molecular cloning is a set of techniques used to insert recombinant DNA from a prokaryotic or eukaryotic source into a replicating vehicle such as plasmids or viral vectors.	Link
NanoDrop spectrophotometer	The NanoDrop Spectrophotometer from NanoDrop Technologies is designed for measuring nucleic acid concentrations in sample volumes of one microliter.	Link
Nitrogen bases	A nitrogenous base, or nitrogen-containing base, is an organic molecule with a nitrogen atom that has the chemical properties of a base.	Link
Oxidation	Oxidation is the loss of electrons during a reaction by a molecule, atom or ion.	Link
pH	pH is a scale used to specify the acidity or basicity of an aqueous solution.	Link
Phosphorylated	Phosphorylation of a molecule is the attachment of a phosphoryl group. Together with its counterpart, dephosphorylation, it is critical for many cellular processes in biology.	Link
Physiological	Physiology is the branch of biology relating to the function of organs and organ systems, and how they work within the body to respond to challenges.	Link
Plasmid	A plasmid is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently.	Link
Polymerase	A polymerase is an enzyme that synthesizes long chains of polymers or nucleic acids. DNA polymerase and RNA polymerase are used to assemble DNA and RNA molecules, respectively, by copying a DNA template strand using base-pairing interactions or RNA by half ladder replication.	Link
Polymerase chain reaction	Polymerase chain reaction is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.	Link
Polymerization	Polymerization is a process through which a large number of monomer molecules react together to form a polymer.	Link
Primers	A primer is a short single-stranded nucleic acid utilized by all living organisms in the initiation of DNA synthesis.	Link

Probiotic	Probiotics are live microorganisms promoted with claims that they provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are considered generally safe to consume but may cause bacteria-host interactions and unwanted side effects in rare cases.	Link
Reagents	A reagent is a substance or compound added to a system to cause a chemical reaction or added to test if a reaction occurs.	Link
Restriction digestion	Restriction digestion also called restriction endonuclease is a process used in molecular biology in which DNA is cut at specific sites, dictated by the surrounding DNA sequence.	Link
Restriction endonuclease	A restriction enzyme, restriction endonuclease, or restrictase is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites. Restriction enzymes are one class of the broader endonuclease group of enzymes.	Link
Serva DNA stain G	Serva DNA Stain G is a safer alternative to traditional ethidium bromide stain for detecting nucleic acids in agarose gels.	Link
Spectrophotometer	A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.	Link
Staining tray	Staining Trays are designed specifically for incubation and storage of immunohistochemistry slides.	Link
Sterile loop	An inoculation loop, also called a smear loop, inoculation wand or microstreaker, is a simple tool used mainly by microbiologists to pick up and transfer a small sample from a culture of microorganisms, e.g. for streaking on a culture plate.	Link
Stock solution	A stock solution is a concentrated solution that will be diluted to some lower concentration for actual use. Stock solutions are used to save preparation time, conserve materials, reduce storage space, and improve the accuracy with which working lower concentration solutions are prepared.	Link
Supernatant	The soluble liquid reaction of a sample after Centrifugation or precipitation of insoluble solids.	Link

TAE buffer	TAE buffer is a buffer solution containing a mixture of Tris base, acetic acid and EDTA.	Link
Template DNA	A template strand is the term that refers to the strand used by DNA polymerase or RNA polymerase to attach complementary bases during DNA replication or RNA transcription, respectively; either molecule moves down the strand in the 3' to 5' direction, and at each subsequent base, it adds the complement of the current DNA base to the growing nucleic acid strand (which is thus created in the 5' to 3' direction).	Link
Terrific broth	Terrific Broth (TB) is an enriched medium designed for high density bacterial culturing. TB contains slightly more tryptone and more than four times the amount of yeast extract than the standard LB media. Thus, TB may be used as an alternative to achieve much greater yields of recombinant *E. coli* in protein expression or plasmid purification.	Link
Thermocycling	The use of a thermocycler to amplify DNA segments.	Link
Titration	Titration is a common laboratory method of quantitative chemical analysis to determine the concentration of an identified analyte.	Link
Toxic chemicals	Toxic chemical is any substance which may be harmful to the environment or hazardous to your health if inhaled, ingested or absorbed through the skin.	Link
Transformation	Transformation is the active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information.	Link
Vector	In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed. A vector containing foreign DNA is termed recombinant DNA.	Link
Vortexer	A vortex mixer or vortexer is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an electric motor with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center. As the motor runs the rubber piece oscillates rapidly in a circular motion. When a test tube or other appropriate container is pressed into the rubber cup the motion is transmitted to the liquid inside and a vortex is created.	Link

Yeast extract	Yeast extracts consist of the cell contents of yeast without the cell walls; they are used as food additives or flavorings, or as nutrients for bacterial culture media.	Link
Wild type	Wild type refers to the phenotype of the typical form of a species as it occurs in nature.	Link

5. References

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Bacterial transformation

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- https://www.researchgate.net/publication/338885590_Bioprocess_development_for_enhanced_production_of_probiotic_Bifidobacterium_bifidum

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