iGEM MIT_MAHE

SAFETY HANDBOOK

Acknowledgements

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1. Safety of Composite BioBrick 1 and 2

Safety Sheet of Standard Biological Parts

Part number/ name	Species name (including strain)	How did you acquire it?	Risk Group	Risk Group Source	Disease risk to humans?	Disease or other risks to the environment
Composite BioBrick 1						
Constitutive Promoter (J23100)	No natural comparator	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
MerR+ RBS (K346001)	*Shigella flexneri*	DNA Synthesis - IDT company	Risk Group 2	Link	Shigellosis	The presence of these in the environment can be taken as an indicator of faecal contamination etc., which can lead to the risk of disease outbreaks.
PmerT (K346002)	*Shigella flexneri*	DNA Synthesis - IDT company	Risk Group 2	Link	Shigellosis	The presence of these in the environment can be taken as an indicator of faecal contamination etc. which can lead to the risk of disease outbreaks.
MerP (K1420003)	*Serratia marcescens*	DNA Synthesis - IDT company	Risk Group 2	Link	Respiratory tract infections, urinary tract infections (UTI), septicemia, meningitis, pneumonia, conjunctivitis wound, and eye infections	If this organism is released into the environment can cause multiple diseases in silkworm and other insects. It can also contaminate the water bodies - causing a reduction of the dissolved oxygen concentration and causing the death of aquatic life.

MerT (K1420005)	*Serratia marcescens*	DNA Synthesis - IDT company	Risk Group 2	Link	Respiratory tract infections, urinary tract infections (UTI), septicemia, meningitis, pneumonia, conjunctivitis wound, and eye infections	If this organism is released into the environment can cause multiple diseases in silkworm and other insects. It can also contaminate the water bodies - causing a reduction of the dissolved oxygen concentration and causing the death of aquatic life.
MerC +RBS (K346088)	No natural comparator	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
RBS with MerE (K1471002)	No natural comparator	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
MerA (K1420001)	*Serratia marcescens*	DNA Synthesis - IDT company	Risk Group 2	Link	Respiratory tract infections, urinary tract infections (UTI), septicemia, meningitis, pneumonia, conjunctivitis wound, and eye infections	If this organism is released into the environment can cause multiple diseases in silkworm and other insects. It can also contaminate the water bodies - causing a reduction of the dissolved oxygen concentration and causing the death of aquatic life.

Mer B (K1420002)	*Serratia marcescens*	DNA Synthesis - IDT company	Risk Group 2	Link	Respiratory tract infections, urinary tract infections (UTI), septicemia, meningitis, pneumonia, conjunctivitis wound, and eye infections	If this organism is released into the environment can cause multiple diseases in silkworm and other insects. It can also contaminate the water bodies - causing a reduction of the dissolved oxygen concentration and causing the death of aquatic life.
Sox R (K554003)	*Escherichia coli K-12*	DNA Synthesis - IDT company	Risk Group 1	Link	N/A	N/A
RBS (B0034)	*Escherichia coli K-12*	DNA Synthesis - IDT company	Risk Group 1	Link	N/A	N/A
SYFP2(K864100)	*Aequorea victoria*	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
Double Terminator(B0015)	*Escherichia coli K-12*	DNA Synthesis - IDT company	Risk Group 1	Link	N/A	N/A
Composite BioBrick 2						
SoxS promoter (K554000)	*Escherichia coli K-12*	DNA Synthesis - IDT company	Risk Group 1	Link	N/A	N/A
Hemolysin B -(K554007)	*Escherichia coli O157 H7 strain EDL 933*	DNA Synthesis - IDT company	Risk Group 2	Link	Hemorrhagic colitis, urinary infection, etc	This can contaminate the water and hence reduce the dissolved oxygen concentration and harm the aquatic life.
Hemolysin D -(K554008)	*Escherichia coli O157 H7 strain EDL 933*	DNA Synthesis - IDT company	Risk Group 2	Link	Hemorrhagic colitis, urinary infection, etc	This can contaminate the water and hence reduce the dissolved oxygen concentration and harm the aquatic life.

TolC (K554009)	*Escherichia coli O157 H7 strain EDL 933*	DNA Synthesis - IDT company	Risk Group 2	Link	Hemorrhagic colitis, urinary infection, etc	This can contaminate the water and hence reduce the dissolved oxygen concentration and harm the aquatic life.
IL-10 (K554004)	Human (Th1 cell)	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
HlyA (K554002)	*Escherichia coli O157 H7 strain EDL 933*	DNA Synthesis - IDT company	Risk Group 2	Link	Hemorrhagic colitis, urinary infection, etc	This can contaminate the water and hence reduce the dissolved oxygen concentration and harm the aquatic life.
GFP (E0040)	*Aequorea victoria*	DNA Synthesis - IDT company	N/A	Link	N/A	N/A
Chassis						
	*Escherichia coli (*DH5-Alpha strain*)*	Ordered from New England BioLabs	Risk Group 1	Link	N/A	N/A
	*Escherichia coli (*Nissle 1917 strain*)*	Ordered from New England BioLabs	Risk Group 1	Link	N/A	N/A

2. Material Safety Data Sheet

As a responsible iGEM team, we consider it good lab practice to keep a **'Material Safety Data Sheet' (MSDS)** for all the reagents we use in our experiments.
Given below are the MSDS for the reagents used in the protocols given in the wiki:

1. Chloramphenicol
2. Terrific Broth
3. Nuclease Free water
4. Glycerol
5. Ethanol
6. Boric acid
7. LB Broth
8. Disodium ethylenediaminetetraacetate dihydrate
9. dNTP mix (10mM)
10. N-(1-naphtyl)-ethylenediamine dihydrochloride
11. Phusion HF DNA Polymerase
12. Potassium peroxodisulfate with low nitrogen
13. 5x Phusion HF Buffer
14. Sodium hydroxide
15. Distilled Water
16. Sulfanilamide
17. Gel loading buffer
18. Vanadium chloride
19. Gel loading dye
20. Sulfuric acid (93% - 98%; N-free)
21. 2.5x Gel loading buffer
22. Mercuric oxide(HgO) or metallic mercury
23. Gel loading dye (bromophenol blue)
24. Potassium sulphate (or anhydrous sodium sulphate)
25. 6x staining dye (Serva DNA stain G)
26. Salicylic acid
27. TAE 10x buffer
28. Sulphide or thiosulphate solution
29. 2x Gibson Assembly Master mix (NEB)
30. Ethylmercury chloride
31. gBlock(s)
32. Hydrochloric acid or sulphuric acid standard solution (0.5N or 0.1N)
33. LB Agar
34. Sodium hydroxide standard solution (0.1N)
35. Calcium chloride (Cacl2)
36. Paraffin
37. Glycerol
38. Zinc granules
39. Tris buffered saline
40. Methyl red indicator
41. EDTA
42. Guar Gum
43. Deionized water
44. CMC
45. NP-40
46. Bile Salts
47. L (+)-Cysteine hydrochloride solution
48. NaHCO3
49. Sodium citrate solution
50. HPMC
51. Sulphuric acid (2M)
52. NaCl

53. Cupric sulphate
54. Sodium alginate
55. Potassium bromide
56. Gellan Gum
57. 4M. Hydrochloric acid
58. FITC
59. 4M. Mercuric chloride
60. DMSO
61. Benzene
62. MTT
63. Toluene
64. TN Buffer (0.15M NaCl +10mM Tris-HCl)
65. Methylmercury chloride
66. Potassium Phosphate
67. Agar
68. Kanamycin
69. Yeast extract

3. Risk Assessment of General Protocols.

The protocol for this experiment is given in the protocol handbooks:

Composite BioBrick 1	*Composite BioBrick 2*
Product development experiments	SAUL

A. Preparation of Media

i. Preparation of Luria-Bertani broth (LB broth) (1L) (And terrific broth)

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here.

MSDS for the reagents used is given in the MSDS section.

i. Chloramphenicol
ii. Kanamycin
iii. Nuclease free water
iv. Ethanol
v. Yeast extract
vi. Potassium Phosphate

General Risks:

There are no major risks associated with this technique and the risk is to future experiments through contamination of media. To avoid this the media should be autoclaved to sterilize before storage.

For safety precautions, refer here

ii. Preparation of Luria-Bertani agar (LB agar) (1L) (& Terrific broth agar)

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. Chloramphenicol
ii. Kanamycin
iii. Nuclease free water
iv. LB Broth
v. TB Broth
vi. Glycerol
vii. Potassium phosphate
viii. Agar

General Risks:

There are no major risks associated with this technique and the risk is to future experiments through contamination of media. To avoid this the media should be autoclaved to sterilize before storage.

For safety precautions, refer here

B. Polymerase Chain Reaction

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. dNTP mix (10mM)
ii. Phusion HF DNA Polymerase
iii. 5x Phusion HF Buffer
iv. Nuclease free water

General Risks:

Heat – The high temperatures used in this technique can lead to skin burns.

For safety precautions, refer here

C. Construction of plasmids

i. Restriction digestion of the BioBricks using XbaI and SpeI and Agarose Gel Electrophoresis

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

Restriction digestion

i. Distilled Water
ii. 2x assay buffer
iii. Gel loading buffer
iv. Gel loading dye

Agarose Gel Electrophoresis

v. Distilled Water
vi. 2.5x Gel loading buffer
vii. Gel loading dye (bromophenol blue)
viii. 6x staining dye (Serva DNA stain G)
ix. TAE 10x buffer

General Risks:

Gel preparation – The TAE buffer and Agarose are not dangerous but to be effectively mixed they need to be heated, usually in a microwave. To fully dissolve the agarose, the mixture must be heated to a temperature which is too hot to touch.

There is also a chance that the mixture could superheat and this becomes extremely dangerous. The mixture may not appear to be boiling when taken out of the microwave but can boil violently when swirled.

For safety precautions, refer here

ii. Gibson assembly Protocol (E5510)

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. Milli Q water
ii. 2x Gibson Assembly Master Mix (NEB)
iii. gBlock(s)

General Risks:

Cold – It is entirely optional to make up the master mix and store it at -20°C until use. This temperature however can cause freeze burns to the skin with prolonged exposure.

For safety precautions, refer here

D. Competent cell preparation

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. LB Agar
ii. LB broth
iii. Calcium chloride ($CaCl_2$)
iv. Glycerol
v. TB Agar
vi. TB broth

General Risks:

- *Bacteria* **–** *Escherichia coli* Nissle 1917 and *Escherichia coli* DH5alpha are non-pathogenic strains. It reduces the risks that are associated with using a violent pathogen. *Escherichia coli* Nissle 1917 is responsible for producing fitness factors called 'colicins' that are toxic to other *Escherichia coli* strains. In our case, it would be an advantage as we want our bacteria to compete while it stays in the gut. Furthermore, its ability to form biofilms increases its long-term persistence in the gut.

- *Infection* **–** In this experiment, the risk of infection is less as non-pathogenic strains are used.

- *Disposal* - Unused bacteria and other materials/reagents that have come in contact with the bacteria should be disposed of in the respective waste bins in the laboratory.

- *Cold* - The extreme cold used to store the competent cells while using can cause freeze burns.

For safety precautions, refer here

E. Transformation

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. LB Agar
ii. LB broth
iii. Chloramphenicol
iv. Kanamycin
v. TB Broth
vi. TB agar

General Risks:

- *Bacteria* – *Escherichia* coli Nissle 1917 and *Escherichia coli* DH5alpha are non-pathogenic strains. It reduces the risks that are associated with using a violent pathogen. *Escherichia coli* Nissle 1917 is responsible for producing fitness factors called 'colicins' that are toxic to other *Escherichia coli* strains. In our case, it would be an advantage as we want our bacteria to compete while it stays in the gut. Furthermore, its ability to form biofilms increases its long-term persistence in the gut.

- *Infection* – In this experiment, the risk of infection is less as non-pathogenic strains are used.

- *Disposal* - Unused bacteria and other materials/reagents that have come in contact with the bacteria should be disposed of in the respective waste bins in the laboratory.

For safety precautions, refer here

F. Cell lysis using Sonication

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. MQ grade water
ii. Lysis buffer
    a. Tris-buffered saline
    b. EDTA
    c. Deionized water
    d. NP-40

General Risks:

- The first hazard is the hearing damage caused by high-frequency sound.
- The second hazard is the generation of aerosols from the sonication process.
- Sonication probe can shatter if misused potentially causing injuries.

For safety precautions, refer here

G. Method for the determination of methylmercury by Gas Chromatography and Calibration

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS used for this protocol is given here

MSDS for the reagents used is given in the MSDS section.

i. L (+) Cysteine hydrochloride solution
ii. Sodium citrate solution
iii. Sulphuric Acid
iv. Cupric Sulphate
v. Potassium bromide
vi. 4M. Hydrochloric Acid
vii. 4M. Mercuric Chloride
viii. Benzene
ix. Toluene
x. Methylmercury Chloride
xi. Ethylmercury Chloride
xii. FITC

General Risks:

- Gas chromatography requires handling compressed gases and flammable and toxic chemicals.

For safety precautions, refer here

4. Methylmercury Handling and Safety

Methylmercury Risks:

All forms of mercury, both organic and inorganic are highly toxic. It can cause serious effects even in small amounts. The effects of exposure may not be noticed until long after serious damage has been done. Mercury poisoning can result from inhalation, ingestion, and injection or absorption through the skin. Chronic exposure may lead to teratogenic and systemic effects.

Possible Symptoms include

- On ingestion; pain, vomiting, and severe cases may result in loss of life within a few hours due to peripheral vascular collapse secondary to fluid and electrolyte loss.
- Lesions of mouthparts, haemorrhagic inflammation of the colon, and kidney failure may also occur.
- In case of contact with eyes, tearing or conjunctival redness may be observed. Slight abrasive damage may also result.
- Entry into the bloodstream through open wounds may produce systemic injury with harmful effects. Skin must be examined carefully, and appropriate gloves must be worn before using the chemical.
- Inhalation of fumes or dust generated by the material may prove to be severely toxic.
- Long-term exposure to high dust concentrations may cause changes in lung function i.e. pneumoconiosis; caused by particles less than 0.5 microns penetrating and remaining in the lung. The prime symptom is breathlessness; lung shadows show on X-ray.
- **Mercury can easily cross the placenta and cause birth defects**.
- Chronic exposure results in excess saliva production, loss of appetite, stomach upset, vague abdominal discomfort, and mild diarrhoea.
- Chronic mercury poisoning mainly shows itself through effects on the nervous system, especially the CNS. There may be tremors involving various body parts like fingers and cheeks. Motor control may be impaired, and behavioural changes may be observed.
- Other symptoms include constant metallic taste, gum inflammation, and loosening of teeth. Uncommon symptoms may include acrodynia.

Methylmercury chloride (and mercury) safety (how to handle):

- All forms of mercury (inorganic and organic) are toxic. Avoid keeping liquid mercury near a heat source. It may generate enough toxic vapours.
- All experiments involving any form of mercury must be performed under a fume hood.
- Avoid dust formation or breathing in dust.
- Chemical goggles/face shield, appropriate gloves, lab coat with full sleeves, full-length pants, and closed toe shoes must be worn at all times.

Glove choices:

- Elemental mercury: Silver Shield®, Responder®, Tychem® BR/LV, Tychem® SL, Tychem TK; double layer, **Neoprene/Nitrile disposable gloves can also be used.**
    a. Mercuric chloride: Tychem BR/LV, Tychem SL, Tychem TK
    b. Methyl mercury: Nitrile (8 mil) over Silver Shield®
- The laboratory must be equipped with an **emergency shower, eyewash station, and a first aid kit.**

Mercury spill-kits

Mercury spill-kits (which are commercially available) must be readily available in the lab for immediate use in case of any spillage.

- They usually include gloves, sponges impregnated with a material to absorb mercury, the absorbent powder that reacts with mercury to form a harmless amalgam, and plastic bags for disposal. Some kits may include a small handheld pump.
- Make your own spill kit using: disposable gloves, disposable shoe covers (double plastic bags will work), index card or rubber squeegee/mop, disposable syringe, or vacuum trap flask fitted with tubing and a Pasteur pipet, inactivating solutions and/or powders.

Disposal:

Never put mercury-contaminated material in regular waste. Furthermore, mercury waste should be placed in a **chemically compatible container with a sealed lid** separated from other chemical waste. Mercury waste is treated differently than other types of hazardous waste. All waste containers must be labelled with a hazardous waste label with the full chemical name written out.

Disposal Instructions:

In USA:

EPA Waste Number & Descriptions:

General Product Information Toxicity characteristic:

- Use EPA hazardous waste number D009 (waste code E) if this substance, in solid waste, produces an extract containing greater than 0.2 mg/L of mercury.
- All waste must be handled in accordance with local, state, and federal regulations. Puncture containers to prevent re-use and bury at an authorized landfill. Each user must refer to laws operating in their area. In some areas, certain wastes must be tracked.
- A Hierarchy of Controls seems to be common - the user should investigate: Reduction, Reuse, Recycling, Disposal (if all else fails).
- This material may be recycled if unused, or if it has not been contaminated to make it unsuitable for its intended use. Shelf life considerations should also be applied in making decisions of this type. Note that properties of a material may change in use, and recycling or reuse may not always be appropriate.
- DO NOT allow wash water from cleaning equipment to enter drains. Collect all wash water for treatment before disposal. Recycle wherever possible. Consult manufacturer for recycling options.

Dispose of by:

- Burial in a licensed land-fill or Incineration in a licensed apparatus (after admixture with suitable combustible material) Decontaminate empty containers.
- Observe all label safeguards until containers are cleaned and destroyed.

In India:

- Mercury waste falls under the category of 'Hazardous Waste' as defined under scheduled II of the Hazardous Waste (Management, Handling and Transboundary Movement) Rules, 2008 and amendments made thereof.
- According to Hazardous Waste (Management, Handling & Transboundary Movement) Rules, 2008, any hazardous waste including mercury bearing waste is permitted to store on-site only for a period of 90 days unless otherwise extended by the concerned State Pollution Control Board (SPCB) /Pollution Control Committee (PCC) to the generator of any such waste.

Disposal through Hazardous Waste Treatment Storage and Disposal Facility (TSDF):

- Waste containing residual mercury and not economically viable for recovery needs to be disposed of through a nearby authorized Common Hazardous Waste Treatment, Storage and Disposal Facility (TSDF) following the manifest as stipulated under the HW (M, H & TM) Rules, 2008.
- Final disposal of such waste in a secured landfill must take place after ensuring pre-treatment by stabilization technique meeting the requirements of criteria for final disposal of hazardous waste in a secured landfill as suggested under the CPCB guidelines.

5. Paraquat Handling and Safety

In the USA: Paraquat is categorized under 'restricted use' by the US Environmental Protection Agency. Only those with a license are allowed to use this chemical. A blue dye and sharp odor are added as a warning, and another agent is added to induce vomiting in case of ingestion.

In India: CIBRC has categorized paraquat dichloride as highly toxic.

Paraquat risks:

- Paraquat is highly toxic. Accidental ingestion can be fatal, and there is no antidote. It can be corrosive to the skin and eyes.
- Paraquat poisoning may occur through the skin that is not intact. The impact of the poisoning depends on the duration and amount of exposure, along with the affected person's health at the time.
- It causes toxic chemical reactions to occur across various parts of the body, especially in the lungs, kidneys, and liver.
- Inhalation of paraquat could lead to severe lung damage. Cells in the lung selectively accumulate paraquat.
- Long-term effects of paraquat poisoning include damage to the lungs, esophageal strictures, kidney and/or heart failure.
- Large amounts of paraquat can result in acute kidney failure, liver failure, muscle weakness, respiratory failure, which may lead to eventual death.

For more info (USA), contact:

i. Regional poison control center: 1-800-222-1222
ii. Centers for Disease Control and Prevention
    a. Public Response Hotline (CDC)
        i. 800-CDC-INFO
        ii. 888-232-6348 (TTY)
    b. E-mail inquiries: cdcinfo@cdc.gov
iii. Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH), Pocket Guide to Chemical Hazards.

How to handle paraquat:

- Wear protective clothing, including **gloves, safety glasses, respiratory equipment, full-sleeved lab coat, full-length pants, and closed-toe shoes.**
- Gloves must be inspected before usage. After use, gloves must be disposed of according to good laboratory practices.
- If swallowed /in contact with skin, wash the mouth/skin with plenty of water and immediately call a doctor/poison center.
- If inhaled, remove person to fresh air and ensure that the area is well ventilated, and the person is kept comfortable for breathing. Immediately call a doctor/poison center.

Gloves:

- The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.
  - Full contact/ Splash contact
  - Material: Nitrile rubber
  - Minimum layer thickness: 0.11 mm
  - Breakthrough time: 480 min
- If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the **CE approved gloves**.
- This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers.
- It should not be construed as offering an approval for any specific use scenario.

Disposal:

- Offer surplus and non-recyclable solutions to a licensed disposal company. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.
- Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself.

Disposal in India:

- Do not pour it down the drain or evaporate it in a fume hood. Do not mix it with any other waste. Avoid any sort of chemical waste accumulation.
- **Store it in compatible containers with an unbroken screw-top lid**. Store these within a secondary container in case the first container breaks or leaks.
- Must be disposed of by special means: suitable incineration and chemical treatment, in accordance with local regulations.
- The hazardous waste can be disposed of at a captive treatment facility installed by the individual waste generators or at Common Hazardous Waste Treatment, Storage, and Disposal Facilities (TSDFs).
- There are 40 Common Hazardous Waste Treatment, Storage, and Disposal Facilities (TSDFs) available in 17 States/UTs.

6. Experimentation Safety and Risk Assessment

I. Composite BioBrick 1

Handbook: iGEM MIT_MAHE composite BioBrick 1

H. Preliminary Experiment

Experiment 1:

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Luria-Bertani + Mercury (LM) medium
v. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for methylmercury chloride handling and safety.

***Note**:* In this experiment low concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

Experiment 2:

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Luria-Bertani + Mercury (LM) medium
v. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for methylmercury chloride handling and safety.

Note: In this experiment high concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

I. Characterization of MerR

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Luria-Bertani + Mercury (LM) medium
v. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for experimental risk assessment of the preliminary experiment.
- Refer here for risk assessment of the Gas Chromatography method.
- Refer here for methylmercury chloride handling and safety.

For safety precautions, refer here

J. Characterization of Methylmercury Transport system

Experiment 1 – 5 (Inclusive of all 5 experiments):

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Luria-Bertani + Mercury (LM) medium
v. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB agar preparation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for experimental risk assessment of the preliminary experiment.
- Refer here for risk assessment of the Gas Chromatography method.
- Refer here for methylmercury chloride handling and safety.

For safety precautions, refer here

K. Characterization of MerA and MerB

Experiment 1 and 2:

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB agar
ii. Chloramphenicol
iii. Methylmercury Chloride

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB agar.
- Refer here for methylmercury chloride handling and safety.

For safety precautions, refer here

MTT assay:

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) stock solution
v. DMSO(Dimethyl Sulphoxide)

General Risks:

- MTT reagent can cause skin irritation and eye irritation. Hence it is a must to wash skin with soap and plenty of water for at least 15 minutes. Remove contaminated clothing. Furthermore, hold eyelids apart and flush eyes with plenty of water for 15 minutes.
- May cause respiratory irritation. Hence it is a must to get fresh air as soon as possible and receive medical supervision immediately.

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation.
- Refer here for methylmercury chloride handling and safety.

For safety precautions, refer here

L. Working of Composite BioBrick 1

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Luria-Bertani + Mercury (LM) medium
v. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB agar.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for experimental risk assessment of the preliminary experiment.
- Refer here for risk assessment of Gas Chromatography method.
- Refer here for risk assessment of the Sonication protocol.
- Refer here for methylmercury chloride handling and safety.

For safety precautions, refer here

II. COMPOSITE BIOBRICK 2

Handbook: iGEM MIT_MAHE Composite BioBrick 2 handbook

M. Characterization of SoxR and SoxS

Experiment 1-3:

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Paraquat (Methyl viologen dichloride hydrate)
iv. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for paraquat handling and safety.

For safety precautions, refer here

N. Characterization of IL-10 transport system

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for risk assessment of the Sonication protocol.

Note: Methylmercury chloride nor paraquat is utilized for this experiment making it safer to perform.

For safety precautions, refer here

O. Working of Composite BioBrick 2

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Methylmercury Chloride
iv. Paraquat
v. Luria-Bertani medium
vi. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for experimental risk assessment of the preliminary experiment.
- Refer here for methylmercury chloride handling and safety.
- Refer here for paraquat handling and safety.

For safety precautions, refer here

P. Measurement of the growth rate of *Escherichia coli* Nissle 1917

Experiment 1 -2 (Both Oxidative stress and different pH conditions):

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. Chloramphenicol
iii. Paraquat
iv. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB broth preparation protocol.
- Refer here for paraquat handling and safety.

For safety precautions, refer here

Q. Safety of *Escherichia coli* Nissle 1917

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. LB Broth
ii. LB agar
iii. Chloramphenicol
iv. TN Buffer (0.15M NaCl +10mM Tris-HCl)

Procedure Risks:

- Refer here for risk assessment of transformation protocol.
- Refer here for risk assessment of LB agar preparation protocol.

For safety precautions, refer here

III. Product development experiments

Handbook: iGEM MIT_MAHE Product development experiments handbook.

A. Preliminary experiment

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions

MSDS for the reagents used is given in the MSDS section.

i. Glycerol
ii. Terrific Broth

Reagents safety:

- TB agar and TB medium– The principle hazards from this concentrate are skin and eye contact. Ensure good ventilation.
- Wear personal protective equipment, such as a lab coat, gloves and eye protection. Do not get into eyes, on skin or clothing. Washing and eye wash facilities should be available in the work area.
- Prevent the formation of aerosols. Do not breathe in vapours or dust from dried-up buffer solution. Do not ingest.

Procedure risks and safety

- While maintaining the mixture at -80*C freezer ensure protective equipment is worn to prevent damage to skin.
- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of TB agar preparation protocol

***Note**:* In this experiment low concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

B. Inoculum percentage

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Glycerol
ii. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of TB agar preparation protocol

Note*:* In this experiment low concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

C. Doubling time

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of TB agar preparation protocol

***Note**:* In this experiment low concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

D. Inoculum age

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Take adequate measures to prevent leakage of sample while incubating in an orbital shaker at 37°C, 180 rpm.
- Wear personal protective equipment while measuring turbidity using a spectrophotometer.
- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of TB agar preparation protocol

***Note**:* In this experiment low concentrations of methylmercury chloride are used. Accordingly, the safety aspects must be considered.

For safety precautions, refer here

E. Loss of plasmid stability

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth
ii. Chloramphenicol
iii. Kanamycin

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Ensure to plate all cultures with high colour intensity on TB + chloramphenicol and Kanamycin agar plates under laminar flow chamber.
- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of TB agar preparation protocol

For safety precautions, refer here

F. Effect of temperature

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of Transformation.

For safety precautions, refer here

G. Effect of pH

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of Transformation.

For safety precautions, refer here

H. Establishment of growth curve of the strain

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth

Reagents safety:

TB broth - The TB buffer concentrate should not be released into the environment. If it enters drains, it should be washed away (diluted) with plenty of water.

Procedure risks and safety

- Refer here for risk assessment of TB broth preparation protocol.
- Refer here for risk assessment of Transformation.

For safety precautions, refer here

I. Estimation of amount of nitrogen in the sample

i. Kjeldahl's method

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth
ii. Sulfuric acid (93% - 98%; N-free)
iii. Mercuric oxide(HgO) or metallic mercury
iv. Potassium sulphate (or anhydrous sodium sulphate)
v. Salicylic acid
vi. Sulphide or thiosulphate solution
vii. Sodium hydroxide
viii. Hydrochloric acid or sulphuric acid standard solution (0.5N or 0.1N)
ix. Sodium hydroxide standard solution (0.1N)

Reagents safety:

- ***Mercuric Oxide*** - Use only under a chemical fume hood. Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location. Wear self-contained breathing apparatus and protective suit. Evacuate personnel to safe areas. Avoid dust formation. Do not get in eyes, on skin, or on clothing.

- ***Concentrated HCl***- Ventilate area of leak or spill. Isolate hazard area and keep unnecessary and unprotected personnel away from the area of the leak or spill. Keep upwind. Keep out of low areas. Wear appropriate personal protective equipment as specified in the Exposure Control and Personal Protection Section 8. Avoid contact with eyes, skin, and clothing.

Procedure risks and safety:

- Ensure lab coats and gloves are worn while handling the chemicals used for the experiment.
- Ensure to prevent leakage of sample while vortexing or incubating in a shaker

ii. Spectrophotometric method

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Terrific Broth
ii. Boric acid
iii. Concentrated hydrochloric acid (37%)
iv. Disodium ethylenediaminetetraacetate dihydrate (e.g., Titriplex® III)
v. N-(1-naphtyl)-ethylenediamine dihydrochloride (NED)
vi. Potassium peroxodisulfate with low nitrogen content (e.g., Merck 1.05092)
vii. Sodium hydroxide
viii. Sulfanilamide
ix. Vanadium chloride

Reagents safety:

- ***Boric Acid*** – If in contact with skin, immediately flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention. Wash clothing before reuse. Thoroughly clean shoes before reuse.

- ***Concentrated HCl*** - Ventilate area of leak or spill. Isolate hazard area and keep unnecessary and unprotected personnel away from the area of the leak or spill. Keep upwind. Keep out of low areas. Wear appropriate personal protective equipment as specified in the Exposure Control and Personal Protection Section 8. Avoid contact with eyes, skin, and clothing.

- ***Disodium ethylnediaminetetraacetate*** - Provide good ventilation in process area to prevent formation of vapor. Avoid breathing dust. Use only outdoors or in a well-ventilated area. Hygiene measures: Do not eat, drink or smoke when using this product. Wash exposed skin thoroughly after handling.

- ***Pottasium peroxodisulfate*** – Disposal - Inorganic peroxides and oxidants as well as bromine and iodine should be rendered harmless by reduction with acidic sodium thiosulfate solution slightly soluble oxidants should be collected separately.

- ***Vanadium chloride*** - Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.

Procedure risks and safety :

- Ensure lab coats and gloves are worn while handling the chemicals used for the experiment.
- Ensure to prevent leakage of sample while vortexing or incubating in a shaker.

IV. Product development

Identification of Worst case in cleaning validation:

It is extremely difficult to carry out cleaning validation of all products manufactured in the facility. Hence products are selected on the basis of worst case. Worst case refers the condition when it is difficult to clean the residues of the manufactured product from equipment surface.

The parameters involved in considering the worst case identification in pharmaceutical cleaning validation are as follows:

1. Potency of product
2. Solubility of API in water
3. Maximum Allowable Carryover (MACO)
4. Toxicity of API
5. Concentration of API
6. Contact surface area
7. Product excipients
8. Manufacturing process

1. ***Potency of product*** –
    - The products having low quality of API are highly potent whereas the products of low quantity API are low potent products. The highly potent products are more active and a low amount of those can cause an effect on the user.
    - Consider for example a product containing 1mg API is more potent than the product containing 10mg API. This means that small quantity of API is effective. Hence highly potent product is considered as worst case in cleaning validation.

2. ***Solubility of API in water*** **-** This plays a major role in the cleaning of equipment. Insoluble API shall be hard to clean than the freely soluble one. Hence solubility of API is an important factor in cleaning validation. A product that contains less soluble API is considered as worst case.

3. ***Maximum Allowable Carryover (MACO)*** –Lowest MACO is considered as worst case for cleaning validation.

4. ***Toxicity of API*** -It is determined by the LD 50 value. API with higher LD is less toxic than the one with a lower LD value because a very low quantity of it is toxic than the other one.

5. ***Concentration of API*** – Residues of product on equipment having more concentration of API will contaminate the next product more than the product with low API concentration.

6. ***Contact surface area*** - Product manufactured on big equipment shall be considered worst case.

7. ***Product excipients*** – Products containing excipients that support microbial growth such as starch, lactose, sucrose, gelatin, etc. are considered as worst case in general pharma drugs. However, since our product is the microbe itself, the excipients which are allergic to the consumers are considered as worst case.

8. ***Manufacturing process*** – Contamination by other bacteria or plasmid loss would be considered worst case.

A. Powder design and development

i. Upstream processing and Bioprocessing

Since the volume of the microorganisms present in bioreactors is much larger, extreme care should be taken to prevent the contamination of the bioreactor itself and also the surrounding environment.
There is a possibility of liquid spills and aerosol formation for some of the bacteria which are used. However, the bacteria we use is *E. Coli* Nissle 1917 which is a risk 1 non-pathogenic organism which does not have the capability to form aerosols.

The proximity of large volumes of liquid and mains electrical equipment (eg, from heaters, aerators, sensors, etc) presents a hazard. Commercially-designed equipment from a reliable source should prevent access to live conductors.

In case of any gas production, the gases must be vented out to prevent high pressure build up as well as any igniting substance should be kept away.

Steam sterilisation may be impossible if the bioreactor is too large to fit into an autoclave, so chemical disinfection will be necessary. Some possible disinfectants are hazardous.

Typical control measures to reduce risk

- Use only risk free non-pathogenic organisms.
- Do not seal bioreactors but prevent contamination due to external microorganisms.
- Sterilise all equipment before and after use and sterilise the any by-products or discards before disposal.
- Keep electrical leads tidy and site mains equipment as far away from the reactor as possible.
- Wear appropriate protective equipment and train to know the importance of hygiene.

Maintenance and quality of the equipment is integral to ensure safe product development:

Fermenter

Challenges associated with designing large-scale biochemical production facilities are almost similar to those related to the design of facilities producing biopharmaceuticals. The genetically modified microorganisms used in both types of facilities are, often by design, not very robust; thus, they often find it difficult to compete against microorganisms occurring in nature (in general, highly specialized breeding in a microorganism tends to reduce its viability overall). This is also a safety consideration.

Since novel microorganisms are created, in case it exhibits some unforeseen undesirable traits, it should not be able to outcompete the wild type organisms. Therefore, preventing contamination of the bioreactor/ fermenter systems is of paramount importance in both biochemical and biopharmaceutical facilities. In biopharmaceutical plants, this is

accomplished by incorporating extensive clean-in-place (CIP) and sterilize-in-place (SIP) systems, and using components and equipment that lend themselves to being cleaned and sterilized in place.

Prevention of microbial contamination:

The quality and safety of the manufactured product should be a key concern in order to ensure the client's safety.

Plant hygiene

- Consideration of building requirements by manufacturers should be done. For example: Windows should be sealed properly to prevent microbial contamination or dust from being collected.
- The sinks used in the production area should be of stainless steel to prevent rusting that may contaminate the pharmaceutical product that is being manufactured. Utensils used and transfer lines in manufacturing should be made up of stainless steel only.
- Air filtration ad air change rates should be set and done regularly.
- In case of contamination in one area, the plant should have a HVAC system to remove and prevent contaminants from spreading to other areas.
- Ventilation dampers should also be positioned for maintenance purpose away from the production area.

Personnel hygiene:

- Every person working in the production area should maintain high-level hygiene.
- The people handling the production should be trained on the importance of hygiene and should wear protective clothing such as hair cover, overshoes, over garments, beard covers, etc. to ensure prevention of contamination.
- They must avoid direct contact with the product or an equipment which comes in contact with the product.

Cleaning:

- Cleaning agents should be of a suitable grade to minimize any health risks. Cleaning and disinfection should be done regularly.
- The equipment for cleaning should not have direct contact with the pharmaceutical product.
- The cleaning practice should be validated.

iii. Downstream processing

Pharmaceutical clean room:

- According to the standard operating procedure, the sterile pharmaceutical manufacturing clean room must be cleaned after a fixed amount of time.
- The importance of maintaining and cleaning the area periodically should not be neglected.
- If sanitation is done using disinfectants then it is advised that two or more disinfectants should be used.
- Additionally, sometimes disinfectants have no effect on some organisms called resistant organisms.
- To counter this, a procedure should be developed to identify these organisms and a suitable effective disinfectant should be used for sanitation.
- Validation of the sanitation procedure and disinfectants used is necessary.
- When disinfectants are used to clean equipment, a cleaning validation is done for the removal of disinfectant to ensure that no residue of the disinfectant remains on the equipment after cleaning.
- Sterile disinfectants and detergents should be employed to sanitize the Class A and Class B area of sterile manufacturing. To sterilize disinfectants, they must be filtered through a 0.2 micron membrane filter in sterile conditions.
- Also, the hold time period of diluted disinfectants should be validated.
- It is advisable to use different disinfectants in rotation.
- It is important that the disinfectants utilized should have sporadic activity because many disinfectants do not have sporadic activity.
- The effectiveness of these disinfectants should be validated.
- To sanitize the air from the sterile area, fumigation is used as it reduces the microbial contamination in the air.
- As with other sanitation procedures, fumigation should also be validated to achieve better results.
- The area must be de-fumigated after fumigation. An example of a fumigant is Hydrogen peroxide solution with silver chloride.
- It is available in the market under different brand names and it is used in the fogger for fumigating the controlled areas.

Centrifuge:

Table top:

Over time centrifuge rotors show signs of metal fatigue as a result of the powerful mechanical forces to which they are subjected. Hence manufacturing guidelines should be followed regarding when to de-rate (permanently lower the speed) and when to retire the centrifuge rotors.

Common causes of centrifuge malfunctions include:

- Failure to place the lid on the rotor.
- Failure to properly secure the rotor lid.
- Failure to properly secure the rotor to the drive.
- Overloading the rotor's maximum mass.
- Running swinging bucket rotor with missing buckets.
- Buckets hooked incorrectly and unable to swing freely.
- Improper balancing of centrifuge tubes.
- Utilization of centrifuge tubes that are not rated for the correct speed.

Centrifuge malfunction:

- Turn off the centrifuge and do not attempt to open the lid.
- Contact your supervisor or administrative assistant to determine who to call for centrifuge repairs.
- If there is evidence of leakage or tube damage when centrifuging infectious materials,
- Close the lid immediately, allow aerosols to settle, and plan the disinfection procedure.
- All centrifugation shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If any spills or leakage are apparent in the centrifuge the rotor should be cleaned with a mild detergent, rinsed thoroughly with distilled water, and allowed to air dry completely. Do not use abrasive or corrosive materials to clean the rotor as this can cause damage to the rotor.

Recommended safe procedures for centrifugation:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet.
- Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity.
- The maximum capacity for centrifuge tubes is 3/4 full.
- Always cap tubes before spinning. Use screw cap tubes.
- Place all tubes in safety buckets or sealed rotors when centrifuging infectious materials.
- Open safety buckets or rotors in a biological safety cabinet.
- Never exceed safe rotor speed.

Industrial centrifuge:

- To prevent corrosion, do not expose aluminum rotor components to strong acids or bases, alkaline lab detergents, or salts or heavy metals (e.g. cesium, lead, silver or mercury).
- Check that the centrifuge chamber, drive spindle, and tapered mounting surface of the rotor are clean and free of scratches or burns.

- Damaged rotors must not be used. If the rotor is dropped, it should be taken out of service immediately and sent to the supplier for examination.
- Wipe drive surfaces prior to installing the rotor.
- Make sure rotor, tubes and spindle are dry and that the rotor is properly seated and secured to the drive hub.
- Note: Do not operate the centrifuge without the appropriate rotor cover securely fitted with seals in place.
- If the temperature of the chamber is below room temperature, pre-cool the rotor to the lower temperature before securing the rotor (this would minimize the chance of it seizing to the tapered spindle).
- It is recommended that a log book be kept for the centrifuge in order to determine the life and maintenance schedule of the rotor. High speed rotor heads are prone to metal fatigue. Do not exceed the design mass for the maximum speed of the rotor. Failure to observe the precaution can result in dangerous and expensive rotor disintegration.

Pelletizer

Regular pelletizer maintenance is the best way to prevent wear or component malfunctions. It is also a great way to detect potential component issues early, before more serious issues occur.

- Regular lubrication procedures on the bearings, reducer, and motor is recommended. As each component may vary based on custom specifications, refer to the appropriate section of your disc pelletizer manual to follow recommended manufacturer lubrication procedures.
- Material can tend to build up on several places on the pelletizer.
- The most common places include: the discharge chute, pelletizer plows, pelletizer pan rim, and pelletizer feed chute.
- Material buildup can impede material flow and can reduce the pelletizer's efficiency. Also, significant buildup can cause more problems down the line if a large piece of buildup breaks free and travels down the process line.

The spray system requires periodic inspection and cleaning as necessary. The spray tip may become blocked if not checked and cleaned regularly. Any irregularity with the spray pattern of the nozzle will indicate if a block has occurred. Maintenance recommendations from the spray system manufacturer should be followed as outlined in the disc pelletizer manual.

Freeze dryer

Given the freeze dryer might be under vacuum or the condenser might be very cold, caution should be taken when working in and around this unit. Do not rapidly vent the drum. Wear safety glasses.

Prepare the Freeze Dryer

- The condenser should be dry and free of water (liquid or solid) before starting a new session. With a dry condenser basin and the drain hose removed, make sure the unit has power (power switch on back) and turn on the condenser by pressing the 'condenser' button.
- While you are waiting for the temperature to drop below -40 °C, decide if you are using the drum or the tree.
- If you are going to use the drum, it's supposed to be made sure that the metal shelves are spaced appropriately for your samples.
- If you are using the tree, place the bottom acrylic plate specific for the tree on top of the condenser basin then place the tree with white o-ring on top of the plate.

Miller

Milling results in a powder with defined particle size and density. When handling use appropriate personal protective equipment like mask, gloves, etc.

B. Process design

Empty capsule manufacturing and Capsule filling process

MSDS for the reagents used is given in the MSDS section.

i. HPMC
ii. Gellan gum
iii. NaCl
iv. Sodium Alginate

Automated capsule filler

- If tablets or capsules are crushed and/or broken, avoid breathing dust and avoid contact with eyes, skin, and clothing.
- When handling, use appropriate personal protective equipment.
- Wash thoroughly after handling. Releases to the environment should be avoided.
- Review and implement appropriate technical and procedural wastewater and waste disposal measures to prevent occupational exposure or environmental releases.
- Potential points of process emissions of this material to the atmosphere should be controlled with dust collectors, HEPA filtration systems or other equivalent controls. Store as directed by product packaging.

Controlled area in sterile pharmaceutical manufacturing

A controlled area is an enclosed environment which has flawless control over particulate contamination. To be precise, the areas have a controlled contamination level which is specified regarding the number of particles for every cubic meter for a specified particle size. These areas are constructed in a manner so as to maintain impeccable humidity, temperature and pressure control to minimise the generation, introduction, and retention of particulate matter inside the rooms.

These rooms are used for sterile pharmaceutical manufacturing. The controlled areas ensure low levels of environmental pollutants like dust, aerosol particles, airborne microbes and chemical vapours. They help the sterile pharmaceutical industry to manufacture products which are free of particulate and microbial contamination. The sterile controlled rooms used in pharmaceutical industries are clean rooms fitted with HEPA filters, and dehumidifier systems to allow preparation of pharmaceutical products in a moisture free and contamination free environment. The usage of these rooms in sterile product manufacturing has grown tremendously.

Objectives of using controlled areas in sterile pharmaceutical manufacturing

In this industry the core objective of using controlled rooms is preventing contamination and ensuring that the preparations are completely sterile. These areas have become a crucial quality assurance step for every drug company. These rooms can easily develop and retain undesirable electrostatic charges as they are made of poor conductors. Hence the surfaces are cleaned and controlled in a manner that the charges can be conducted away as soon as they form.

Sections and classification of controlled area in SPM

The sterile pharmaceutical controlled area is divided into two sections: general and critical area. The general area is the section of the rooms where existence of contaminants does not affect the sterility of products. It should however be properly cleaned and controlled to avoid transfer of contaminants into the critical area. The critical area is the section around the production point. Any contaminants may gain direct access to the preparations. It is usually protected using localized laminar flow workstations and clean benches.

Ensuring complete sterilisation

In order to manufacture completely sterile products various stages of product processing such as component preparation filling in product preparation should be performed in separate sections of the area. So these manufacturing operations should be divided into stage-wise sterilization and terminal sterilization.

To ensure complete stage-wise sterilization the control room should allow for at-rest and in-operation states. At-rest state means the production equipment in the classified room operates without the presence of any personnel. In-operation state means that the manufacturing processes are run by a specified ie limited number of personnel.

There are four different levels that a sterile pharmaceutical company should grade its control production: A B C and D. Grade A should be the zone for high risk operations like filling zone and zone for making aseptic connections this zone should have laminar airflow systems with homogeneous airspeeds. Grade B is the background environment for grade A that allows for aseptic preparation and filling. Grade C and D are classified areas that handle the less critical stages of SPM.

Assigning Manufacturing Date and Expiry Date:
It should be assigned as per the date of dispensing.

During Processing:

- Should be processed strictly as per BMR.
- Operators should wear protective accessories like gloves, masks and muffs.
- Equipment should have status label with signature of pharmacist.
- Operators and pharmacists should check the area and equipment cleanliness before starting manufacturing process.
- Environmental conditions like Relative humidity, Temperature should be maintained.

- Pharmacist should personally monitor all critical operations like Dry Mixing, Drying, etc.
- SOP should be followed properly.
- Any adverse incidence or spillage should be immediately informed to superiors.
- All safety instructions should be strictly followed.

D. Packaging

Blister punching machine

Blister packaging machines are electromechanical equipment, consisting of stationary and moving parts which help to seal products in preformed cavities. The blister packs employ the form-fill-seal procedure, where there're rolls of flat sheet that have pharmaceutical items packed in them.

It's closed at the manufacturing part, thus remains tamper resistant. A vacuum shaped plastic covering is positioned around the mold and thus the item can comfortably fit into the covering.

Capsule loading machine

Reduced cleaning requirements are another important benefit. In setting up a typical encapsulation machine, there are a number of product contact parts that have to be cleaned and verified, swabbed after dosing to show residual API left on the instrument.

The drug substance can be stored in a standard jar or vial with the dosing head screwed on top. The whole vessel with dispensing head can be stored in the refrigerator if necessary. If the drug product is moisture-sensitive or oxygen-sensitive, this concept reduces the exposure of the substance to the ambient environment. It also minimizes any substance loss due to transfer from one storage or dispensing vessel to another. The design of the dosing head design offers flexibility for dispensing material with various characteristics.

Lidding operation

The lidding material will be aluminum push through foil with the paper on top. A layer of heat seal coating will be added to the region where lidding material will be attached to the blisters to form a good seal. Hence personal protective equipment must be used.

E. Recycling and waste management

Pharmaceutical waste management forms an integral part in pharmaceutical industries. These wastes are found in different forms mainly as strips, expired products, manufacturing wastes etc. It comes from various sectors in the health care system including pharmaceutical developers and manufactures, hospitals, individual physicians and all those who are involved in the health care system.

Pharmaceutical waste is further classified in two categories:

- **Hazardous waste**
  Wastes that are dangerous or potentially harmful to human health or the environment are known as hazardous wastes. These can be liquids, solids, contained gases, or sludges. Pharmaceutical wastes come under listed hazardous wastes since they contain commercial chemical products.

- **Non-hazardous waste**
  Materials in this category are considered to present no significant hazardous properties i.e. the hazardous components are below the threshold for causing harm to human health. This non-hazardous state is subject to change and the addition or removal of specific items from the waste stream may significantly alter the management options available.

Pharmaceutical waste disposal includes various tools for effective management of wastes as follows

- **Incineration**
  Incineration is an effective method used for disposal of wastes, in which solid organic wastes are subjected to combustion so as to convert them into residue and gaseous products. It is recognized as a practical method of disposing of certain hazardous waste materials (such as biological medical waste). However, it is a controversial method of waste disposal, due to issues such as emission of gaseous pollutants.

- **Chemical disinfection**
  Chemical disinfection is most suitable for treating liquid wastes such as blood, urine, stools, or health care facility sewage. Addition of strong oxidants-like chlorine compounds, ammonium salts, aldehydes, or phenol compounds-kills or inactivates pathogens in the BMW depending on the extent and duration of contact between the disinfectant and the BMW. However, microbiological cultures, mutilated sharps, or shredded solids can also be treated by chemical disinfection.

- **Deep burial**
  The BMW Rules require that human anatomical and animal wastes in cities with population less than 500,000 and in rural areas be disposed of by deep burial, which should be pre- pared by digging a pit or trench of about 2 meters deep in an area that is not prone to flooding or erosion, and where the soil is relatively impermeable, there

are no inhabitants or shallow wells in the vicinity, and the risk to surface water contamination is remote. The pit should be half-filled with the BMW, and then covered with lime within 50 cm of the surface, before filling the rest of the pit with soil. On each occasion when BMW is added to the pit, a layer of 10 cm of soil should be added to cover the waste.

- **Secure land filling**
  Secure land filling involves disposal of solid BMWs at a landfill designed and operated to receive hazardous wastes. The BMW Rules require disposal of discarded medicines, cytotoxic drugs, solid chemical wastes, and incineration ash in secured landfills. Disposing of waste in a landfill involves burying the waste in abandoned or unused quarries, mining voids or borrow pits. Many landfills also have landfill gas extraction systems installed to extract the landfill gas. Gas is pumped out of the landfill using perforated pipes and flared off or burnt in a gas engine to generate electricity.

- **Waste immobilization: encapsulation**
  Encapsulation involves immobilizing the pharmaceuticals in a solid block within a plastic or steel drum, which is cleaned prior to use and did not contain any explosive or hazardous material previously. They are filled to 75% capacity with solid and semi-solid pharmaceuticals, and the remaining space is filled with cement or cement/lime mixture, plastic foam or bituminous sand and water in 15:15:5 (by weight). The sealed drums should be placed at the base of a landfill and covered with fresh municipal solid waste.

- **Waste immobilization: Inertization**
  Inertization is a variant of encapsulation and involves removing the packaging materials, paper, cardboard and plastic, from the pharmaceuticals. The pharmaceuticals are then ground and a mix of water, cement and lime added to form a homogenous paste. The paste is then transported in the liquid state by concrete mixer truck to a landfill, decanted into the normal urban waste and then sets as a solid mass dispersed within the municipal solid waste.

- **Sewer**
  Some liquid pharmaceuticals, e.g. syrups and intravenous (IV) fluids, can be diluted with water and flushed into the sewers in small quantities over a period of time without serious public health or environmental affect.

The following strategies can be used to manage the hazardous waste that reduces the waste materials generated

- **Waste minimization**
  An important method of waste management is the prevention of waste material being created, also known as waste reduction.

- **Reuse**
  Re-use means the use of a product on more than one occasion, either for the same purpose or for a different purpose, without the need for reprocessing. Re-use avoids discarding a material to a waste stream when its initial use has concluded.

- **Recycling**
  Recycling involves the treatment or reprocessing of a discarded waste material to make it suitable for subsequent reuse either for its original form or for other purposes.

V. Systematic Analyser of Underlying Limitations

i. Mechanical and experimentation safety

Aim: To assess the safety aspect of the experiment and follow the appropriate precautions.

MSDS for the reagents used is given in the MSDS section.

i. Guar gum
ii. Bile salts
iii. Hydrochloric acid 0.1N and 1N
iv. $NaHCO_3$
v. Methylmercury
vi. Paraquat

Mechanical safety:

- Isolation of all electronic components from any conducting media has to be ensured. All wiring connections should be insulated properly, and care must be taken to avoid spilling or leakage of chemicals or fluids onto the motors.
- Refer here for methylmercury handling.
- Refer here for Paraquat handling.
- The dialysis tube to be discarded after every use of methylmercury.
- To prevent leakage into the incubator, the entire set up should be placed in a closed air tight container.
- All of the components used are checked for temperature and pH resistance.

For safety precautions, refer here

7. Safety Precautions

Heat:

1. Handle hot autoclaved equipment with heatproof gloves.
2. Autoclave the test tubes along with the cotton plug before pouring the broth.
3. Do not overheat agar mixtures in the microwave as they will bubble and overflow.
4. Heat it gradually, in small bursts, and be careful when swirling the mixture at arm's length after each round. Use appropriate gloves if needed.
5. Care must be taken while using the thermocycler and while transferring samples. The use of tongs or heatproof gloves is recommended for carrying out this experiment.

Cold:

1. When handling reagents taken out of the freezer, use gloves to avoid cold burns.

Laminar Flow Chamber:

2. Ensure that the laboratory is well ventilated. Avoid intake of any gas vapours. Use respiratory equipment when handling hazardous chemicals.
3. The laminar flow chamber must be sterilized using UV light for 30 minutes priorly. The chamber and hands should be cleaned with 70% ethanol to prevent any contamination.
4. Perform all steps in sterile conditions under laminar flow chamber.
5. UV light is damaging to the eyes and skin and is a carcinogen. When switched on, avoid nearby area to minimise exposure. Ensure that the UV light is switched off when working under the laminar flow chamber.

Electrophoresis:

6. If electrophoresis buffer is spilled or leaks from the gel box, stop the run and clean up the benchtop immediately.
7. Switch off all power and unplug the leads before opening the gel chamber lid or reaching inside the gel chamber.

Reagents:

1. Handle $CaCl_2$ with gloves as it is hazardous to skin, eyes, and the respiratory tract and may also cause burns.
2. Gloves and Lab coats are always to be worn while working with bacteria and handling paraquat. Hands are to be washed regularly.
8. Avoid contact of any reagents with the skin. In case of contact, rinse thoroughly under running water.
9. In case of any accidental spills, handle with gloves. Do not let the product enter the drain.

Sonication:

1. Do not use the sonicator unless an experienced user, preferably the equipment custodian, has given you a thorough demonstration of how to use it correctly and safely.

2. During sonication, earphone type sound mufflers should be worn to prevent any damage to the eardrums. Do not sonicate in a room with people not wearing appropriate ear protection.
3. If possible, use the sonicator in a sound-proof cabinet/room. Close doors when sonicating.
4. Use the **CORRECT SONICATION PROBE TIP** for the instrument and power rating you are using. Consult the equipment custodian if in doubt.

Gas Chromatography:

1. Perform periodic visual inspections and pressure leak tests of the sampling system plumbing, fittings, and valves. Also perform radioactive leak test on ECDs every 6 months for sources of 50MBq (1.35 mCi) or greater.
2. Follow the manufacturer's instructions when installing columns. Glass or fused capillary columns are fragile: handle them with care and wear safety glasses to protect eyes from flying particles while handling, cutting, or installing them.
3. Turn off and allow heated areas such as the oven, inlet, and detector, as well as connected hardware, to cool down before touching them.
4. To avoid electrical shock, turn off the instrument and disconnect the power cord at its receptacle whenever the access panel is removed.
5. Turn off the hydrogen gas supply at its source when changing columns or servicing the instrument.

Clean-up and Disposal:

1. After performing the experiment, clean up the area carefully using appropriate gloves (and respiratory protection if needed) and store separately for disposal. Do not mix it with other waste.
2. Dispose of the agar and plates appropriately. Do not spill any chemicals down the drain.

When handling paraquat or any form of mercury, follow necessary safety protocols. Use appropriate gloves, respiratory equipment and follow clothing guidelines.

8. General Disposal Techniques:

Used Agar and Broth disposal (without paraquat or methylmercury):

- Autoclave the used plates and broth at 121 degrees C at 1.05 kg/cm2 (15 psi) for 30 minutes and kill off all the bacteria. Then dispose into the sink or a disposable bag. (No other material should be autoclaved with this batch)
- Discard into appropriate dustbins.

Autoclave protocol:

- Place bag to be autoclaved into a shallow pan or tub.
- Affix a small piece of autoclave indicator tape or test strip to the outside of the bag.
- Place the pan and bag inside the autoclave. Do not overload the autoclave. There should be at least 2 inches of space around each waste bag on all sides to allow access to surfaces by steam.
- No other materials should be autoclaved together with waste in the same load.
- Run the autoclave at a chamber temperature of 121°C for 60 minutes* using a dry cycle run.
- When the cycle has been completed, verify that the autoclave chamber and ambient pressure are the same. The chamber may now be opened, and the waste bag removed. Wear autoclave gloves when handling hot items.
- Also use caution when opening the autoclave door, as a small amount of hot steam may be released when opening the chamber.

For more information refer to autoclave handling.

9. Biosafety and Biosecurity of our Project

iGEM MIT_MAHE firmly understands and believes in the **importance of safety and security** while working in the laboratory. We have made sure to take into consideration every safety aspect while designing the protocols for our desired experiments.

General issues:

Researcher safety:

- Our entire project requires a **Biosafety Level 1 (BSL-1)** laboratory. This is because our project only deals with Biosafety level 1 bacteria (*Escherichia coli* DH5alpha and *Escherichia coli* Nissle 1917 and therefore we consider our project to be harmless to researchers in this aspect. **None** of our experiments are expected to **increase the pathogenicity** of the bacteria.
- *Escherichia coli* Nissle 1917 is responsible for producing fitness factors called 'colicins' that are toxic to other *Escherichia coli* strains. In our case, it would be an advantage as we want our bacteria to compete while it stays in the gut. Furthermore, its ability to form biofilms increases its long-term persistence in the gut.
- As far as lab safety goes for the researcher, **PPE (personal protective equipment)** will be worn at all times while experimenting. This includes lab-coats, gas masks, and gloves.

  Note: **Specialized gloves** will be worn while handling methylmercury and paraquat as they are highly toxic. Details regarding the safety of methylmercury and paraquat have been discussed above.

- All laboratory equipment and bacterial cultures are to be decontaminated by **autoclaving**.
- A **risk assessment** for each experiment has been given. **Material Safety Data Sheet** (MSDS) for various reagents have also been listed.

Public Safety:

- There is no threat to public safety as we are using non-pathogenic strains.

Environmental Safety*:*

- *Escherichia coli* Nissle 1917 is highly characterized and studied upon. To maintain safety, we will not be disclosing any important information which could lead to issues. Our project has ensured that there is **no threat to environmental safety** as we are using **non-pathogenic and highly studied strains strains**.

Biosafety is a very important aspect to be considered after having brainstormed for a project idea. While working with a **Genetically Modified Organism (GMO),** considerations have to be made for preventing the uncontrolled release of the genetically modified organism into the environment.

Note: We are not currently implementing these strategies. However, it is important to keep all of these aspects in mind to prevent the spread of the GMO into the environment.

iGEM MIT_MAHE will follow nation guidelines for the surveys we conduct.

Biosafety guidelines followed:

We are a team from India that will follow the National Biosafety regulations while handling methylmercury. However, the Material Safety Sheets mentioned above follow safety procedures that are common across the globe. Methylmercury and Paraquat handling and safety have been compiled along with all the above-mentioned guidelines and hence will work well within the law.

Biosafety and biosecurity of our project:

Introduction:
iGEM competition encourages its participants to consider the safety aspects of their projects diligently. Coming up with a set of engineered and procedural measures to ensure the safe use of valuable biological materials.
iGEM MIT_MAHE will be using organisms from Risk Group 1. However, we are aware that these seemingly harmless organisms have the potential to cause harm due to the modified BioBricks which would be potentially adding new characteristics and capabilities. This could be accidentally leading to the production of harmful substances or increasing their potential of survival or mutations.

Safety organizations of India and US:

In India:

1. **Recombinant DNA Advisory Committee (RDAC)**
    a. Review developments in Biotechnology at national and international levels.
    b. Shall recommend suitable and appropriate safety regulations for India in recombinant research, use and applications from time to time.
    c. Evolve long-term policy for research and development in Recombinant DNA research.

2. **Institutional Biosafety Committee (IBSC)**
    a. Institutions handling risk-inherent microorganisms or GE organisms shall prepare, with the assistance of the Institutional Biosafety Committee (IBSC), on-site emergency plan and update from time to time according to the

manuals/guidelines of the RCGM and make available as required copies to the District Level Committee/State Biotechnology Co-ordination Committee and the Genetic Engineering Appraisal Committee.

3. **Review Committee on Genetic Manipulation (RCGM)**
    a. This committee functions from the Department of Biotechnology to monitor the safety-related aspect in respect of on-going research projects or activities involving hazardous microorganisms, GE organisms and cells, and products thereof.
    b. The RCGM includes representatives of:
        i. Department of Biotechnology
        ii. Indian Council of Medical Research
        iii. Indian Council of Agricultural Research

4. **Genetic Appraisal Committee**
    a. To appraise activities involving large scale use of hazardous microorganisms GE organisms or cells in research and industrial production from the environmental angle.
    b. To appraise proposals relating to release of GE organisms and products into the environment including experimental field trials.

5. **State Biotechnology Co-ordination Committee (SBSC)**
    a. To inspect, investigate, and to take punitive action in case of violations of statutory provisions through the State Pollution Control Board (SPCB) or the Directorate of Health etc.
    b. To review periodically the safety and control measures established at various institutions handling GE organisms.
    c. To act as nodal agency at the State level to assess the damage, if any, due to release of GE organisms and to take on-site control measures.

In USA:

1. Proposals concerning research involving genetically modified organisms and even double stranded DNA itself are reviewed by the ORA-IBC (Office of Research Administration - Institutional Biosafety Committee).

2. National Academics (Fink-commission) and National Science Advisory Board for Biosecurity (NSABB) - Deal with the dual use research of concern and the potential misuse of information created in life sciences.

Potential Biosafety issues of our project:
Team Bielefeld-CeBiTec 2015 proposed a detailed analysis of biosecurity, in detail the dual use issue, with respect to the iGEM competition in which they have mentioned several aspects and questions to be considered.

Can you imagine any malevolent use of the knowledge and sequences published on your team's wiki? Could the knowledge you provide be used for the creation of products or organisms that pose a danger to humans or the environment?

Our project involves the release of an anti-inflammatory cytokine (IL-10) whose release is regulated by a control mechanism involving SoxS and SoxR genes. The SoxR gene is also regulated to be released only in the presence of methylmercury using the MerR-PmerT mercury responsive genes.

Selective mutation of any of these control mechanisms could lead to release of IL-10. This could lead to reduced immune response to pathogens. IL-10 also down regulates transcription and secretion of IL-1β, IL-6, IL-8, TNF-α, and G-CSF by activated monocytes and macro- phages. This could lead to cancer [3]. IL-10 upregulates ICAM-1 within neural tissues. This promotes massive macrophage influx, demyelination due to inflammation and subsequent loss of neural tissue, resulting in muscle weakness and paralysis [2].

How were your concerns addressed in your project?

The probiotic capsule can have an activating mechanism which will not be disclosed to anyone without proper documentation/procedures that ensure biosecurity.

Do you work with any sequences or toxins or plant/animal origin that might be a threat to health of humanity or environment?

The sequences used are of non-toxic substances which would not harm the environment or humanity. However as mentioned above, there could be a potential misuse of anti-inflammatory substances.

Who will use our product? If your product is successful, who will receive benefits and who will be harmed?

Our product if successful will be used by people who are at risk for methylmercury poisoning. we have made sure to not use any allergens or preservatives which could potentially harm consumers.

Can your chosen host be aerosoled?

Our project uses *Escherichia coli* Nissle 1917 as the host. This is a non-pathogenic strain which cannot be aerosoled.

Does this BioBrick produce any product that can regulate the immune system in animals or humans?

BioBrick 2 produces IL-10 under certain circumstances but the amount will not be significant enough to cause any harm to animals or humans, unless misused (as mentioned above).

Is the chassis in anyway pathogenic in animals or humans or towards plants?

No, the bacterial strains *Escherichia coli* Nissle 1917 and DH5alpha are non-pathogenic. The nonpathogenic *E. coli strain* Nissle 1917 (EcN) is a widely employed probiotic strain, and several in vivo studies have demonstrated its promising probiotic activity in humans and animals, including the treatment of acute, chronic, or frequent recurring diarrhea and inflammatory bowel disease [2].

WHO also has a self-assessment questionnaire to ensure good quality, ethical research activities are conducted in safe and secure facilities. Some of the important question relevant to biosecurity and biosafety aspects have been answered below:

Pillar 2: Ethics	
Questions:	**Answers**
Research is subject to a risk assessment that includes the societal impact of the research.	There is a strong cultural pattern of fish consumption among the coastal people and among the people residing in the plains of India. Other sources of mercury exposure include mining, hospital wastes, etc. Thus, many people would be benefitted from the research and would be harmed by misuse. Hence, we would minimize the risks due to dual use by taking appropriate measures as mentioned.
Research is subject to a risk assessment that includes potential environmental impact.	*Escherichia coli* Nissle 1917 is highly characterized and studied upon. To maintain safety, we will not be disclosing any important information. Our project has ensured that there is no threat to public safety as we are using non-pathogenic strains.
Potential for misuse of the research is considered at all stages and appropriate action taken if necessary	Yes. Mechanism of activation of the probiotic would not be disclosed unless a valid proof is given. At all stages, adequate research was done, and safety was ensured.
Researchers know how to assess whether the risk outweighs the benefit of continuing with their research or activities	Yes. Adequate precautions and risk assessments are done to mitigate the risk. Researchers have not only extensively listed down the risks but also have provided a solution to the same.
Researchers are aware of and informed about national and international conventions, laws and regulations related to their research	Yes. Researchers are aware of the different laws and regulations related to the project of research. Laws and Regulations are also documented for future reference.
National legislation and policy relevant to the life sciences provides protection against the misuse of science	Yes. Policies relevant to our project of research have provided protection against the misuse of science.

Pillar 3: BIOSAFETY AND LABORATORY BIOSECURITY	
Researchers have somewhere to turn to get competent advice if they have safety or security questions relating to their research.	Yes. We have followed aspects of safety as per the guidelines of iGEM safety and security committee. We also have taken advice from our PI and other professors from our institute.
National legislation/regulation exists that sets safety and security practices and procedures for laboratories	Yes. There are regulations that sets safety and security practices and procedures related to our project of research for laboratories. The team will be following National guidelines and Regulations during the entire course of research.
An assessment of the risk associated with research activities is conducted	Yes. The team has documented a Safety Handbook that covers various safety aspects and a detailed risk assessment for each experiment.
Risk assessments are able to identify requirements for risk reduction measures including the level of containment required	Yes. The Safety Handbook covers not only the risk reduction measures but also the level of containment required for each stage of the research that will be conducted.
Legislation/regulations exist to address hazardous waste disposal	Yes. The Safety Handbook covers not only the risk reduction measures but also the level of containment required for each stage of the research that will be conducted.
Legislation/regulations regarding hazardous waste disposal are followed	Yes. The team will strictly follow the regulations pertaining to hazardous waste disposal.
Valuable biological material is safely and securely stored	Yes. The team will ensure that the valuable biological material is safely and securely stored for future use.

10. 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 the most effective way of separating a mixed population of macromolecules such as DNA fragments or proteins of varying sizes ranging from 100 bp to 25 kb, in a matrix of agarose of varying sizes ranging from 100 bp to 25 kb.	Link
Agitation	Agitation is the brisk stirring or disturbance of a liquid.	Link
Allergen	An allergen is any substance (antigen), most often eaten or inhaled, that is recognized by the immune system and causes an allergic reaction.	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

Antibiotic resistance	Antibiotic resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow.	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
Antidote	Antidotes are agents that negate the effect of a poison or toxin, mediating its effect either by preventing the absorption of the toxin, by binding and neutralizing the poison, antagonizing its end-organ effect, or by inhibition of conversion of the toxin to more toxic metabolites.	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	Autoclaves are also known as steam sterilizers, is a machine used to carry out industrial and scientific processes (for example, to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel) requiring elevated temperature and pressure in relation to ambient pressure/temperature that uses steam under pressure.	Link
BioBrick	BioBrick parts are DNA sequences which conform to a restriction-enzyme assembly standard.	Link
BioBrick compatibility	The assembly standard enables two groups of synthetic biologists in different parts of the world to re-use a BioBrick part without going through the whole cycle of design and manipulation.	Link
Biofilms	A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix.	Link
Biomass	Biomass refers to the mass of living organisms, including plants, animals, and microorganisms or, from a biochemical perspective, cellulose, lignin, sugars, fats, and proteins.	Link

Bioreactor	Bioreactor is defined as a vessel that carries out a biological reaction and is used to culture aerobic cells for conducting cellular or enzymatic immobilization.	Link
Biosafety	Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety is used to protect from harmful incidents.	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
Capsule	Capsules include medication that's enclosed in an outer shell. This outer shell is broken down in the digestive tract and the medication is absorbed into the bloodstream and then distributed and metabolized in much the same way as medication from a tablet.	Link
Carcinogen	A carcinogen is an agent with the capacity to cause cancer in humans which works by interacting with a cell's DNA and inducing genetic mutations.	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 lysis	Cell lysis or cellular disruption is a method in which the outer boundary or cell membrane is broken down or destroyed in order to release inter-cellular materials such as DNA, RNA, protein or organelles from a cell.	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
Clinical Trial	A clinical trial is a type of research that studies a test or treatment given to people. Clinical trials study how safe and helpful tests and treatments are. When found to be safe and helpful, they may become tomorrow's standard of care.	Link
Cold burns	Cold burns or frostbite is a condition in which skin and the tissue just below the skin freezes.	Link
Competent cells	Cell competence refers to a cell's ability to take up foreign (extracellular) DNA from its surrounding environment. The cells with such ability are known as competent cells.	Link
Composite BioBrick	Composite BioBrick 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
Cryopreservation	It is the process of cooling and storing cells, tissues, or organs at very low temperatures to maintain their viability.	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
Endospore	An endospore is a dormant, tough, non-reproductive structure produced by a small number of bacteria from the Firmicute family. The primary function of most endospores is to ensure the survival of a bacterium through periods of environmental stress.	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
EPA waste number	EPA waste number, issued either by the U. S. Environmental Protection Agency or by DTSC, identifies each handler of hazardous waste on hazardous waste manifests or other paperwork and enables regulators to track the waste from its origin to final disposal.	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	*Escherichia coli* DH5alpha *is o*ne of the most common laboratory *E*. *coli* strains used to maintain and amplify small plasmid DNA.	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
Excipient	Excipients are inert pharmaceutical ingredients that are used in product formulations.	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
Free burn	Free burn (of an electric arc) refers to continuous heat transfer to the anode.	Link
Frothing	A mass of bubbles in or on a liquid; foam.	Link
Gas chromatography	Gas chromatography is a method of separating the components of mixtures of gases and vapors without decomposition and is widely applied in analytical chemistry.	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

Genetic drift	Genetic drift describes random fluctuations in the numbers of gene variants in a population. Genetic drift takes place when the occurrence of variant forms of a gene, called alleles, increases and decreases by chance over time.	Link
Genetically Modified Organism (GMO)	Genetically modified organisms (GMOs) are organisms whose genetic material has been artificially modified in order to favor the expression of desired physiological traits or the generation of desired biological products.	Link
Gibson assembly	Gibson assembly is a robust exonuclease-based method to assemble multiple DNA fragments seamlessly in the correct order.	Link
Growth curve	A growth curve is an empirical model to visually represent the growth of bacteria over time.	Link
Incinerator	Incinerator is a device used in a waste treatment technology and involves the combustion of waste materials at high temperatures.	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
Inoculum	A small amount of material containing bacteria, viruses, or other microorganisms that is used to start a culture.	Link
Kill switch	Kill switch is defined as an artificial system that result in cell death under certain conditions.	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 containing agar which is a nutritionally rich mixture, allowing the growth of most microorganisms.	Link
LB Broth	Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab.	Link
Lidding material	Lidding materials and products have removable or hinged covers and are placed on the top of a container to secure its contents.	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
Lipids	Lipids are molecules that contain hydrocarbons and make up the building blocks of the structure and function of living cells. Examples of lipids include fats, oils, waxes, certain vitamins (such as A, D, E and K), hormones and most of the cell membrane that is not made up of protein.	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
Material safety data sheet (MSDS)	A Material Safety Data Sheet (MSDS) is a document that contains information on the potential hazards (health, fire, reactivity and environmental) and how to work safely with the chemical product. It is an essential starting point for the development of a complete health and safety program.	Link
Mercury poisoning	Mercury poisoning is a type of metal poisoning due to exposure to mercury causing symptoms like muscle weakness, poor coordination, numbness in the hands and feet, skin rashes, anxiety, memory problems, trouble speaking, trouble hearing, or trouble seeing.	Link
Metabolite	A metabolite refers to any substance involved in metabolism. It is often regarded as the immediate by-product of a metabolic process. However, some references consider those involved in a metabolic reaction (not necessarily a by-product) as a metabolite.	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
Oxidative stress	Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.	Link
Paraquat	Paraquat or N, N'-dimethyl-4,4'-bipyridinium dichloride, also known as Methyl Viologen, is a toxic organic compound that is widely used as an herbicide (plant killer), primarily for weed and grass control.	Link
Pathogen	A pathogen is defined as an organism causing disease to its host, with the severity of the disease symptoms referred to as virulence.	Link
Pathogen	A pathogen is defined as an organism causing disease to its host, with the severity of the disease symptoms referred to as virulence.	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 (PCR) is a revolutionary method developed by Kary Mullis in the 1980s to rapidly amplify very small sample of specific DNA to millions or billions of copies.	Link
Polymerization	Polymerization is a process through which a large number of monomer molecules react together to form a polymer.	Link
PPE	Personal protective equipment (PPE) is protective clothing, helmets, goggles, or other garments or equipment designed to protect the wearer's body from injury or infection. The hazards addressed by protective equipment include physical, electrical, heat, chemicals, biohazards, and airborne particulate matter.	Link
Pre-treatment	Pretreatment allows us to hydrolyze biomass with mechanical, thermal and chemical processes. It is often the first step of a process and lets us carry out subsequent processes.	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
Probiotic	Probiotics are live microorganisms that are intended to have health benefits when consumed or applied to the body. They can be found in yogurt and other fermented foods, dietary supplements, and beauty products.	Link
Promoter	A promoter is a sequence of DNA needed to turn a gene on or off. The process of transcription is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule.	Link

Reagent	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
Recombinant	Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.	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
Risk assessment	Risk assessment is the combined effort of identifying and analyzing potential events that may negatively impact individuals and/or the environment; and making judgments "on the tolerability of the risk on the basis of a risk analysis" while considering influencing factors.	Link
Risk group	Risk Groups are classifications that describe the relative hazard posed by infectious agents or toxins in the laboratory.	Link
Selectable marker	Selectable marker genes are conditionally dominant genes that confer an ability to grow in the presence of applied selective agents that are normally toxic to plant cells or inhibitory to plant growth, such as antibiotics and herbicides.	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
Sonication	Sonication is the act of applying sound energy to agitate particles in a sample, for various purposes such as the extraction of multiple compounds from plants, microalgae and seaweeds. Ultrasonic frequencies are usually used, leading to the process also being known as ultrasonication or ultra-sonication.	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
Sterilized	Sterilized means made free from all forms of life and other biological agents like prions present in a specific surface, object or fluid.	Link
Stock solution	A stock solution is a concentrated solution of known, accurate concentrations 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
Strain	A strain is a genetic variant, a subtype or a culture within a biological species.	Link
Strains	A strain is a genetic variant, a subtype or a culture within a biological species.	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
Viscosity	Viscosity is a measure of a fluid's resistance to flow. It describes the internal friction of a moving fluid. For liquids, it corresponds to the informal concept of "thickness": for example, syrup has a higher viscosity than water.	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
Wild type	Wild type refers to the phenotype of the typical form of a species as it occurs in nature.	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

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