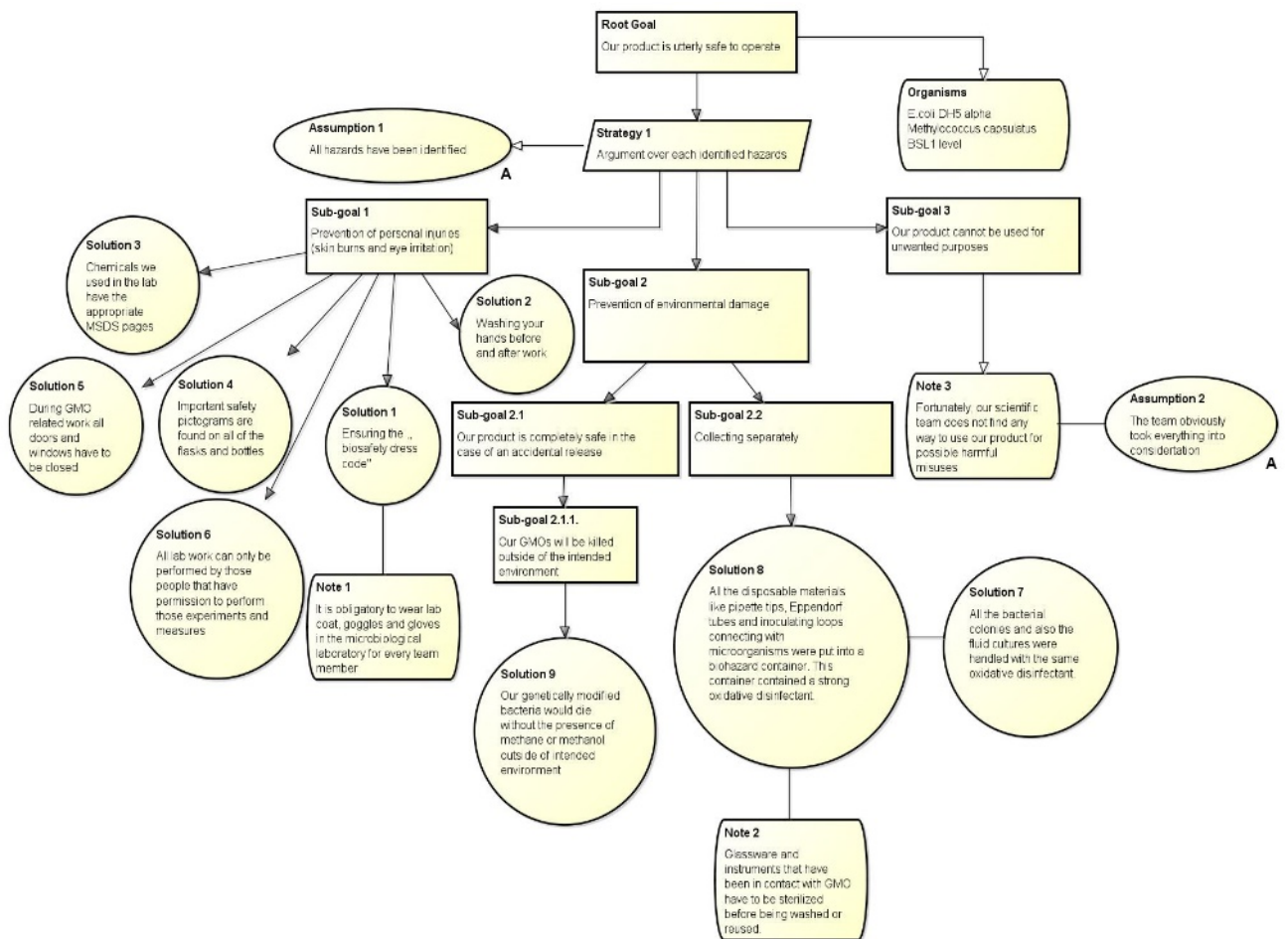


# Biosafety

For the Methungeny research team, the security and safety measures of our project have had the highest priority. We considered among others how we can prevent personal injuries, environmental risks and potential unwanted purposes. When planning and conducting experiments, our scientific team try to evaluate all potential risks to human health and the environment and take all necessary precautionary measures.

## Productive collaboration concerning Biosafety:

We collaborated with the UNbraska-Lincoln team concerning biosafety. They designed a new idea called Safety Cases to make researches safer during the iGEM competitions. Safety Case makes it possible to logically break down and analyse safety concerns. We helped the UNL team with practical suggestions how to make this mindmap clearer and more detailed, and we also gave them some tips regarding personal and environmental safety. For our great pleasure, they completed their biosafety page with several new ideas advised by our team. As the result of the collaboration with UNL team, we also enriched with very useful and helpful inspirations, and we decided to try their Safety Case what you can see below. This mindmap provides the opportunity to track easily the safety protocols which are followed by Methungeny team.



During our experiments, we used different bacterial strains, moreover, synthetic promoter, three types of plasmid, and Lactate-dehydrogenase (LDH) gene.

















| <b>Bacteria strain</b>                         | <b>Institute</b> | <b>Product Number</b> | <b>Biosafety level</b> |
|------------------------------------------------|------------------|-----------------------|------------------------|
| <i>Escherichia coli</i><br><i>DH5-alpha</i>    | <i>NEB®</i>      | <i>C2987</i>          | <i>1</i>               |
| <i>Escherichia coli</i><br><i>JM109</i>        | <i>NEB®</i>      | <i>E4107</i>          | <i>1</i>               |
| <i>Escherichia coli</i><br><i>JM109 λpir</i>   | <i>DSMZ</i>      | <i>26091</i>          | <i>1</i>               |
| <i>Escherichia coli</i><br><i>K12 HB101</i>    | <i>DSMZ</i>      | <i>1607</i>           | <i>1</i>               |
| <i>Methylococcus</i><br><i>capsulatus Bath</i> | <i>ATCC®</i>     | <i>33009</i>          | <i>1</i>               |

| <b>Synthetic parts</b>                                                 | <b>Institute</b>                                     | <b>Plasmid in which they were sent</b> |
|------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------|
| <b>Promoter from</b><br><b>Methylococcus capsulatus</b><br><b>Bath</b> | Invitrogen                                           | pMA-RQ                                 |
| <b>Lactate-dehydrogenase gene</b><br><b>from Bacillus coagulans</b>    | Invitrogen                                           | pMA-RQ                                 |
| <b>pMHE5 plasmid</b>                                                   | Department of Biology at<br>the University of Szeged | ----                                   |
| <b>pMHE7 plasmid</b>                                                   | Department of Biology at<br>the University of Szeged | ----                                   |
| <b>pDSK5 plasmid</b>                                                   | Department of Biology at<br>the University of Szeged | ----                                   |

|                    | <b>Sequence</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>p.p.r.o.m.o</b> | ATGAAAAAGGTCAATCGTATTGCAAGTGGTGGAAACGGGTGCAAGTGGTACAAGTTACTGCTACGCCATGATTAATCAGGGTGTGCAGAAAGAGCTTGTTTAATC<br>GATATTAACGAAGCAAAGCAGAAGGGGAAGCCACGGTCTGAACACGGCTGCCATTTGCGCCTACGCCACCCGCGTTTGGAAAGGCGATTATTCCGATTG<br>CGGCACTGCCGATCTTGTGTGCTTACGGCAGGTTCCCGCAAACCGGGCGAAACAAGGCTTGATCTTGTGCCAAAAACGCAAAAATTTTAAAGGCATGAT<br>TAAGAGCATCATGGACAGCGGCTTTAACGGGATTTTTCTGTTGCCAGCAACCCGGTTGACATTTGACATATGTAACCTGGAAAGAGTCCGGCCTGCCGAAAGA<br>ACATGTTATCGGTTCCGGGCACAGTGTGACTCCGCGCGTCTCCGCAACTCTTTAAGCGCCACTTCGGAATTGACCCGCGCAATGTCCATGCCGCAATTATCGGC<br>GAACACGGGCGACACGGAACCTTCCGGTTTGGAGCCATACAACGATCGGTTATGACACCATTGAAAAGCTATCTGCAAAAAGGGAACCATGACCAAAAAACATTAGAT<br>CACAGAGGAGGTTCTTAACACTACGGCTGGGACGTAGCCGCGAGATGCTTGTGCGCCGCTTAAGACCCTGTCTAGTACAGCCGCGCAATG<br>CCTTATTGGGGAGCTTCCGGCGAACTTTTTGAGAGCTTACGAGCGACCTTTTGGAGGTGCCGCCCAAGGCGAGCTTACGGGGCCTAGG<br>CAGCCCTTATAGAGGGACAGTGTCTGCGGCTAGCTCCGTTTATTGCTAGAAAAGGGCCCTCGTTCGCGGGAACCTAGACGCCAAAGCG<br>GACTTGGAG |

**Material Safety Data Sheets:**

| <b>Materials</b> | <b>Exclamation</b> | <b>Environment</b> | <b>Corrosion</b> | <b>Health hazards</b> |
|------------------|--------------------|--------------------|------------------|-----------------------|
|------------------|--------------------|--------------------|------------------|-----------------------|

|                                     | <i>mark</i>                                                                         |                                                                                   |                                                                                   |                                                                                      |                                                                                      |
|-------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <i>LB medium</i>                    | ----                                                                                | ----                                                                              | ----                                                                              | ----                                                                                 | ----                                                                                 |
| <i>NMS medium</i>                   |    |  |  |   | ----                                                                                 |
| <i>SOC medium</i>                   |    | ----                                                                              | ----                                                                              | ----                                                                                 | ----                                                                                 |
| <i>AMS medium</i>                   |    |  | ----                                                                              |   | ----                                                                                 |
| <i>GeneJET Gel Extraction Kit</i>   |    | ----                                                                              | ----                                                                              | ----                                                                                 | ----                                                                                 |
| <i>GeneJET Plasmid Miniprep Kit</i> |    | ----                                                                              |  | ----                                                                                 | ----                                                                                 |
| <i>Lactate assay Kit</i>            |    | ----                                                                              | ----                                                                              | ----                                                                                 | ----                                                                                 |
| <i>Ethidium bromide</i>             |   | ----                                                                              | ----                                                                              |  |  |
| <i>Agarose</i>                      |  | ----                                                                              | ----                                                                              | ----                                                                                 | ----                                                                                 |

**Personal safety:**

Before we started our laboratory work, we participated in a course held by our mentor. The aim of this lecture was to let members know that scientific members have to be responsible and careful while working on synthetic biology experiments. All lab work can only be performed by those people that have permission to do those experiments and measures.

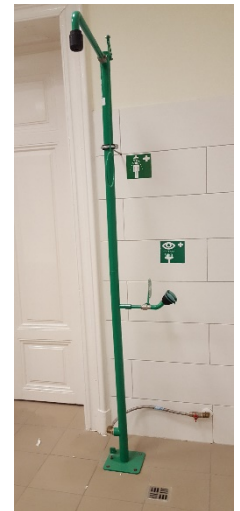
All participants have to wear latex gloves, safety goggles and fire resistant lab coats (“Biosafety dress code”) in our microbiological labs in order to prevent skin burns or eye damage. Gloves are dropped into a container, and of course, lab coats are worn only in the appropriate laboratories.



During GMO related work all doors and windows have to be closed in our lab. Before and after performing lab works, all members have to wash their hands.



Furthermore, the important safety pictograms are found on all of the flasks and bottles at the same time. In this way, it is possible to reduce the probability of misunderstanding that would lead unpredictable consequences. In case of an unwanted accident, we have eye-wash and shower stations in our lab.



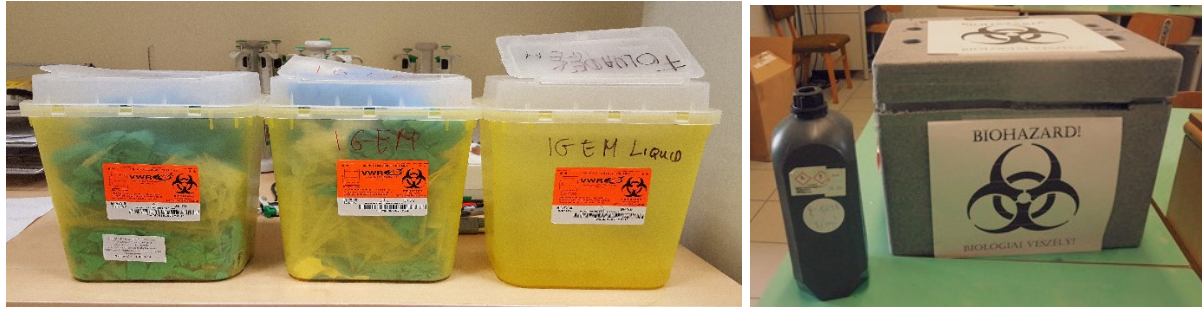
While working under ultraviolet light, we always used UV protection sunglasses in order to prevent any eye damage caused by UV rays.

Working in the laboratory, we always take care of the appropriate sterilization process. Our team use an autoclave as a container. It is filled by different objects connecting with microorganisms in order to sterilize and totally sealed them. Objects are handled at 120 °C and 2 bar pressure. As a result, high temperature destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins.



### ***Environmental risks:***

All the disposable materials like pipette tips, petri dishes with growth, Eppendorf tubes, Pasteur pipettes, pipette tips and inoculating loops connecting with microorganisms are put into a biohazard container. In this container, there is a strong oxidative disinfectant. In the cases of BSL1 materials, all the bacterial colonies and also the fluid cultures were handled with the same chlorine-containing disinfectant. Finally, these containers filled by hazardous materials were transported by an officially approved company which will continue the safety measures according to the strict Hungarian Regulation.



It is relevant to mention that we use non-pathogenic *Escherichia coli* strains and *Methylococcus capsulatus* bacteria, belonging only into BSL1 level. We also use the LDH gene from *Bacillus coagulans*, also BSL1 level. Nevertheless, we have a considerable caution to prevent any spreading of used living organisms, since these will be S1 genetically modified organisms. Our genetically modified organisms would die outside in the intended environment, without the presence of methane or methanol sources.

***Unwanted purposes:***

In our days, the prevention of harmful misuse of GMO has a great importance. That is why, we continuously pay attention on the possible harmful misuse of our research. Fortunately, we have not been able to find any aspect of our work which can be used to potential unwanted purposes.