Design Brief

An *Escherichia coli*-based formaldehyde detector: an economical and effective solution for safe air monitoring*

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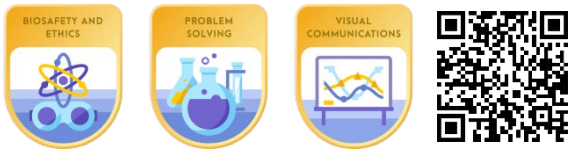
Formaldehyde is a common colorless liquid, or gas found in the production of preservatives, building materials, and vaccines. Prolonged exposure to the gas causes severe eye and respiratory irritation and increases various cancer risks. Therefore, determining formaldehyde concentration in the air and regulating it to a safe level becomes a priority for places with potential formaldehyde exposure risks. Current detection methods, including using a mechanical detector, conducting lab samples, and smelling, are often unreliable, expensive, and dangerous. Our previous design is an economical and effective detector based on the ΔfrmR strain of Escherichia coli. The detector will be yellow by default and will turn red gradually when formaldehyde is present. The depth of the color depends on the concentration of the formaldehyde. We will construct the formaldehyde-detecting plasmid based on the R0010 (pLacI)_AB plasmid backbone and transform it into cells. Using a Pfrm promoter, the detector reacts to formaldehyde at levels around 100 μM, which is the threshold for the amount of formaldehyde that a human can consume healthily. We then utilize the Plac-lacI repressor system as a genetic switch: with the presence of formaldehyde, lacI, a Plac repressor, is expressed to deactivate the yellow protein and turn the cell red. The bacteria will be cultured in lactose-rich media to ensure the constitutive expression of the yellow protein under Plac. Moreover, the frmA and frmB genes in the construct remediate formaldehyde in the solution. As the concentration of formaldehyde decreases through frmAB in the detector solution, the detector reverts to yellow, making the detector system reusable when provided with sufficient nutrients.

Keywords: Formaldehyde, formaldehyde detector, ΔfrmR strain, Escherichia coli, *Pfrm, Plac-lacI*



Formaldehyde (CH_2O) is a colorless poisonous flammable gas used extensively in preserving cadavers, insulating building materials, inactivating viruses in the production of vaccines, and many other industrial settings. Despite its wide applications, exposure to formaldehyde may cause severe eye and respiratory irritation.

Often used as a sterilant, formaldehyde kills microorganisms and is widely used to preserve organic materials such as biological specimens (National Institutes of Health, 2023). The antimicrobial and preservative properties of formaldehyde come from its ability to generate protein cross linkages by binding to amino acids like lysine and tyrosine, which stabilize the molecular

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structure of the complex (Centers for Disease Control, 2016). The protein linkages strengthen organic structures to help prevent decomposition (Facts About, 2022).

This property also makes formaldehyde an ideal insulation and adhesive agent in building materials. For example, formaldehyde is widely used in and emitted from urea-formaldehyde foam insulation (UFFI) (National Library of Medicine, 1980). UFFI, an essential bonding agent, is frequently used in the production of particleboard and plywood, commonly seen material in newly built structures, flooring, wooden toys, etc. (Environmental Protection Agency, 2022).

Numerous vaccines use small amounts of formaldehyde to inactivate the virus (such as the polio virus in the making of polio vaccines) and detoxify bacterial toxins. Viral inactivation is achieved by the alkylation of proteins and purine bases, a process of introducing one or more alkyl groups to a compound (Herrara-Rodriguez *et al.*, 2019). Formaldehyde then cross-links the viral proteins, destroying its fusion ability that connects two genes, and ultimately inactivating the virus (Food and Drug Administration, 2019).

In addition to its extensive usage, formaldehyde comes from a wide variety of sources. It could be synthetically produced by oxidizing methanol, but it is mostly generated through combustion by cars, gas stoves, and heating appliances (National Research Council (US) Committee on Toxicology, 1980). In cars, methane, ethane, and other hydrocarbons are converted into formaldehyde through the catalytic vapor phase oxidation over a metal oxide catalyst and produced by the incomplete combustion of hydrocarbons (National Library of Medicine, 1980). When methanol is put through a chemical reaction at a high temperature, it produces formaldehyde as a byproduct (Jarvis 2017).

Due to the frequent presence of formaldehyde in life, formaldehyde exposure becomes a common concern. Exposure to formaldehyde can cause irritation to the nose, eyes, skin, and throat. Formaldehyde causes irritation in the respiratory tract with a concentration as low as 0.1 ppm when inhaled. It causes eye irritation in concentrations of 0.05-0.10 ppm, equivalent to 50 to 100 μM, the concentration of formaldehyde that reaches a danger level (National Research Council (US) Committee on Toxicology, 1980). Formaldehyde is a mucous membrane and skin irritant and can cause conjunctivitis and lacrimation in the eyes and severe burns to the skin (National Research Council, 1980; *Medical Management*, 2014). Studies have shown that allergic contact dermatitis has become increasingly common since formaldehyde resins are widely used in the textile industry as an anti-wrinkle and crease-resistant component (Valdes *et al.*, 2020).

Furthermore, prolonged exposure to formaldehyde may result in severe health issues, including increased cancer risks and neurological dementia (Kou *et al*., 2022). Formaldehyde demonstrates genotoxicity, the ability to damage genetic information in cells, as it can cause increased DNA damage, micronucleus formation, sister chromatid exchanges, and chromosome aberrations (Kang *et al.*, 2021). Employees in occupations that have high exposure to formaldehyde, such as plastic manufacturers, construction workers, or even agricultural workers, have significantly higher cancer risks: up to several thousand times higher than the limit recommended by the Environmental Protection Agency (Adamović *et al.*, 2021). The risk of squamous-cell carcinomas of the nasal cavities in animals and nasopharyngeal cancers in humans increases (Adamović *et al.*, 2021). Formaldehyde is also a neurotoxin that affects movement, memory, and learning. Although formaldehyde occurs naturally, it is also formed in the process of normal metabolism in the human brain. However, exposure to high levels of formaldehyde can still severely affect metabolism, causing neurodegeneration and cognitive impairment (Tulpule & Dringen, 2013).

With its high usage and high toxicity, formaldehyde becomes a priority for places that work with this compound to closely monitor its levels. Current methods of formaldehyde detection include mechanical detection, lab sampling, and simply smelling the chemical. However, these detection methods can be inaccurate, expensive, and

hazardous, rendering them less than ideal. Laboratory testing is time-consuming and costly, and methods like spectrophotometry or gas chromatography require machinery that requires high energy demand and experienced staff (Chung, Po-Ren et al). Smelling is direct yet unreliable. Some people only start to sense the gas at 0.117 ppm. Others, like those who smoke, are more prone to experience formaldehyde's health effects as they have significantly higher thresholds (Berglund, 1992).

To address the potential shortcomings of current detectors, we aim to construct an accurate, efficient, and cost-effective formaldehyde detector. Our project aims to utilize the detection feature of *Pfrm* promoters. Using a *Pfrm* promoter, an "ideal candidate for engineering" (Rohlhill *et al.*, 2017), we will build a sensor that shows yellow when no formaldehyde is detected and turns red as formaldehyde builds up. The users can detect formaldehyde's presence on furniture or other surfaces by collecting a quick swab and inserting it in the detection solution.

We used *Pfrm* because it can perceive formaldehyde in the cell, and it is found in the naturally appearing formaldehyde-remediating frmRAB operon in *E. coli*. *FrmR* binds to the *Pfrm* promoter and inhibits its activity. The presence of formaldehyde negatively allosterically modulates the *frmR* gene, leading to the expression enzymes FrmA and FrmB, which detoxify formaldehyde (Denby *et al.*, 2016). The *E. coli* strain used in the construct is the ΔfrmR strain, which is engineered to possess a higher expression level for the genes downstream of *Pfrm* (Rohlhill *et al.*, 2017) (Figure 1).

The absence of formaldehyde will trigger the green fluorescent protein *GFP)*. Conversely, when formaldehyde is present, the promoter will activate expression of red fluorescent protein (m*RFP1), LacI, frmA, and frmB* (Figure 2). To signal the change of activation in *E. coli*, our group will employ a repressor system. The repressor system utilized in the construct is the *pLac-LacI* repressor system. *PLac* is a repressible constitutive promoter if cultured in lactose-rich media. Therefore, our design will grow the *E. coli* strain under a large supply of

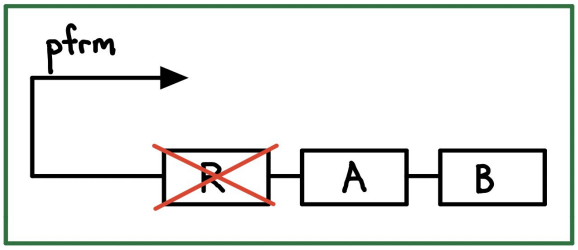

Figure 1. The pfmRAB operon in the ΔfrmR strain The original strain is composed of FrmA, FrmB, and FrmR gene. In the ΔfrmR strain, FrmR is deleted from the strain in order to maximize the expression of the detection system.

lactose, to ensure the activation of *pLac* promoter. When formaldehyde is present in the surroundings, *LacI* will be expressed and block the lactose promoter, stopping the expression of the *GFP*. The bacteriawill further demonstrate red color instead of yellow color. At this stage, we are not certain about the speed of the color switch since we don't have enough data.

To test the effectiveness of the fluorescent proteins we chose for our system, we will grow our designed *E. coli* strain in different lactose concentrations to stufy their effects on gene expression. We will prepare a series of lactose solutions with different

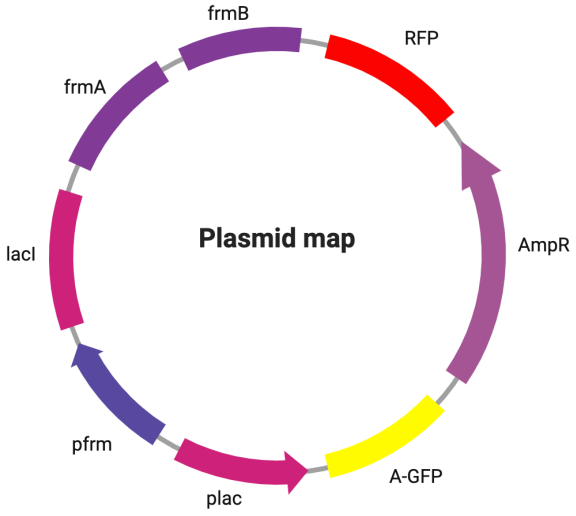

Figure 2. The modified R0010 (pLacI)_AB plasmid backbone, the Pfrm-frmR-Plac-lacI plasmid. The plasmid uses Pfrm to detect formaldehyde. FrmR inhibition is deactivated by formaldehyde, leading to frmA and frmB expression to detoxify it. Absence of formaldehyde activates GFP (green), presence activates mRFP1 (red), LacI, frmA, and frmB.

concentrations under the same exposure time. After the color change, we will quantify our results by measuring its absorbance and fluorescence. With these data, we will generate a statistical graph to find the optimization of strain under different concentrations of lactose.

To maximize the visual effect of our detector, we plan to test our design in different conditions, such as air content and type of media. With optimal expression, we will utilize the reusability based on the lactose broth since the color turns yellow after the detoxification of formaldehyde in the detection kit and improve the cost performance of this product.

Systems level

Our detector will react to formaldehyde contents inside a swabbing device as a solution. After the user collects a sample by swabbing a target surface they wish to test and inserting it into the detector solution; our system will use the *Pfrm* promoter to detect formaldehyde. If formaldehyde is absent or present at levels below 100 µM, the plasmid will express the yellow protein, GFP. If formaldehyde is present, the *pLac-LacI* repressor system will repress the originally presented yellow protein and activate the expression of red protein *mRFP1* to indicate the presence of formaldehyde. It will also activate *frmA* and *frmB*, which function to detoxify formaldehyde.

We will use the *E. coli* strain ΔfrmR. By removing *FrmR*, a repressor of *Pfrm*, from the frmRAB operon, the genes downstream of *Pfrm* can be expressed at their maximum level (Rohlhill *et al.*, 2017).

We will insert our construct onto the R0010 (pLacI)_AB plasmid backbone. The plasmid's key components include a *lac* promoter, a *lac* operator, a biobrick prefix, a biobrick suffix, and an ampicillin resistance gene. The plasmid will be transformed into *E. coli* through electroporation (Figures 2,3).

Device level

The designed plasmid contains the *frmA* gene, *frmB* gene, *LacI* gene, the ampicillin resistance gene, the red fluorescent protein *mRFP1*, *GFP* from the coral *Acropora millepora*, the *LacI* repressible constitutive promoter *pLac*, and the formaldehyde sensing promoter *Pfrm*. *Pfrm* promotes the genes *frmA*, *frmB*, *LacI*, and *mRFP1*, while *pLac* promotes the *GFP* gene. The expression of *GFP* signifies the livelihood of *E. coli* before the exposure to formaldehyde. The *mRFP1* acts as a marker protein for the genes *frmA*, *frmB*, and *LacI*, enabling visual recognition when the three genes are expressed in formaldehyde.

We will utilize the Gibson Assembly to

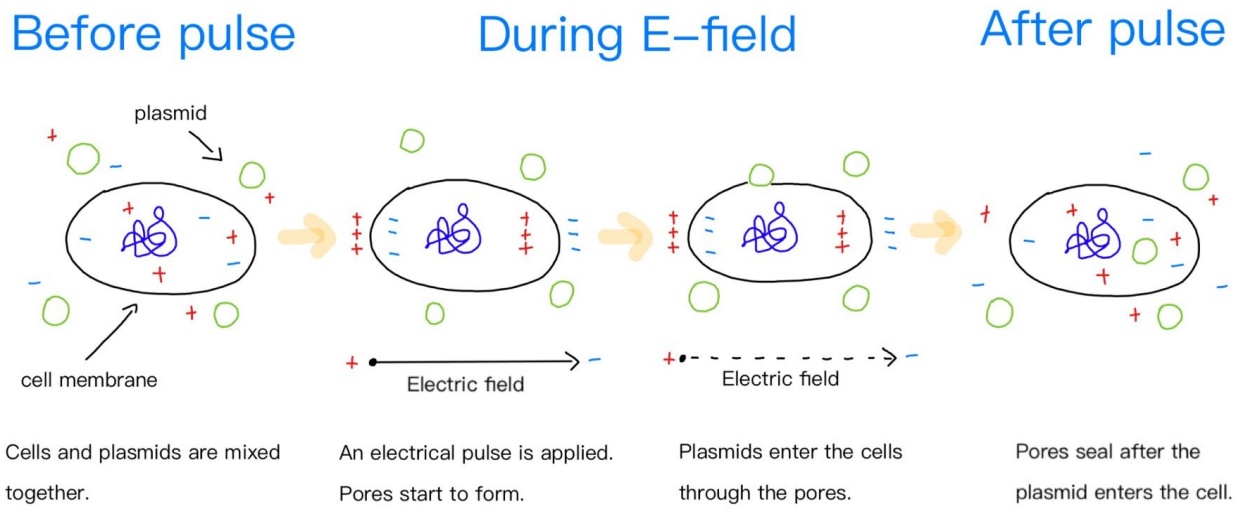

Figure 3. The process of electroporation. Electroporation is an efficient method of transfection. Electrical pulse is created in electroporation to produce temporary pores in cell membranes, allowing the plasmid to enter.

insert the desired components into our plasmid backbone. Gibson Assembly is a fast and seamless cloning method that can assemble multiple DNA fragments simultaneously, which is an ideal procedure for our multi-vector-based design. We will use inverse PCR to cut open two insertion sites for our components. For the ends of our different components to stick to each other, we will create restriction enzymes as complementary ends corresponding to their neighboring fragments in the design. We will then perform the Gibson reaction involving three enzymes: T5 exonuclease, DNA polymerase, and DNA ligase.

In the Gibson master mix containing our plasmid backbone and all the DNA components, the T5 exonuclease will cut away the five prime ends, exposing only the three prime ends. As the cut DNA fragments combine due to the overhang sequences overlapping, the DNA polymerase will fill the missing original gene content that the T5 exonuclease accidentally chewed up. Ultimately, the DNA ligase is a glue that covalently joins the different gene components firmly onto the backbone.

Parts level

Our model utilizes the *pLac-LacI* repressor system to achieve the change of color depending on the presence of formaldehyde. It consists of the *pLac* and the *LacI*. The lactose promoter, also known as the *lac* promoter, is found in *E. coli* and controls gene expression in lactose metabolism. When a large quantity of lactose is present, *pLac* activates and induces lactase synthesis, further expressing a color change through the *GFP* protein. The other vital part of this system is the *LacI*: it produces a repressor that blocks transcription of the downstream genes without lactose. When lactose is present, it binds to *LacI* and causes a conformational change that allows transcription to proceed, producing enzymes needed for lactose metabolism. In our system the lactose repressor expressed through *LacI* will act as a selection marker for the electroporation transformation.

Our group utilizes a plasmid named R0010 (pLacI)_AB (Plasmid #66004). We chose this specific plasmid since it is safe, relatively simple, and has a high copy number. In addition, the plasmid encompasses both the *pLac* and the ampicillin resistance gene (*AmpR*). The *pLac*, along with the lactose operator, has 90 bp and is situated between the restriction sites PvuII and PstI. The lactose operator binds to the *LacI* to prohibit the expression of *pLac*. The *AmpR*, a protein encoded by the ampicillin resistance gene, inactivates the β-lactam ring of antibiotics and thus creates resistance against them. *AmpR* will act as a selection marker for the electroporation transformation.We will extract *LacI* from plasmid pVER-LacI. The *LacI* gene is 1083 bp long and has an anticlockwise transcription direction. When formaldehyde is detected, the repressor produced by the *LacI* gene binds to the lactose operator and inhibits the expression of *pLac*.

To detect and remediate the formaldehyde, we employ the ΔfrmRAB strain edited by the lab that cuts out the *frmR* gene. The exclusion of the *frmR* gene maximizes the expression of the design system (Rohlhill *et al.*, 2017). *Pfrm* is central to our detector. Found in the frmRAB operon in *E. coli*, *Pfrm* is a promoter that responds to formaldehyde presence. *FrmR* binds to the *Pfrm* and inhibits its activity. Formaldehyde negatively allosterically modulates *frmR*, leading to the expression of *frmA* and *frmB* (Denby *et al.*, 2016). *FrmA* and *frmB* are the enzyme factors responsible for the detoxification of formaldehyde. The enzymes encode a formaldehyde dehydrogenase, catalyzing the oxidation of formaldehyde to a less toxic compound, formate (methanoate), which is the conjugate base of formic acid (Rohlhill *et al.*, 2017).

The fluorescent proteins we will use in our model are *GFP*, a green fluorescent protein, and *mRFP1*, a monomeric derivative of the red fluorescent protein. Both protein genes will be extracted from the plasmid pTarget: Ptaq-RFP_pLacIq-GFP. While *GFP* is clockwise and 717 bp long, *mRFP1* is anticlockwise and 678 bp long. The direction of transcription corresponds to the direction of *LacI*.

Derived from a coral species known as *Anemonia millepora*, *GFP* naturally fluoresces bright green under blue light,

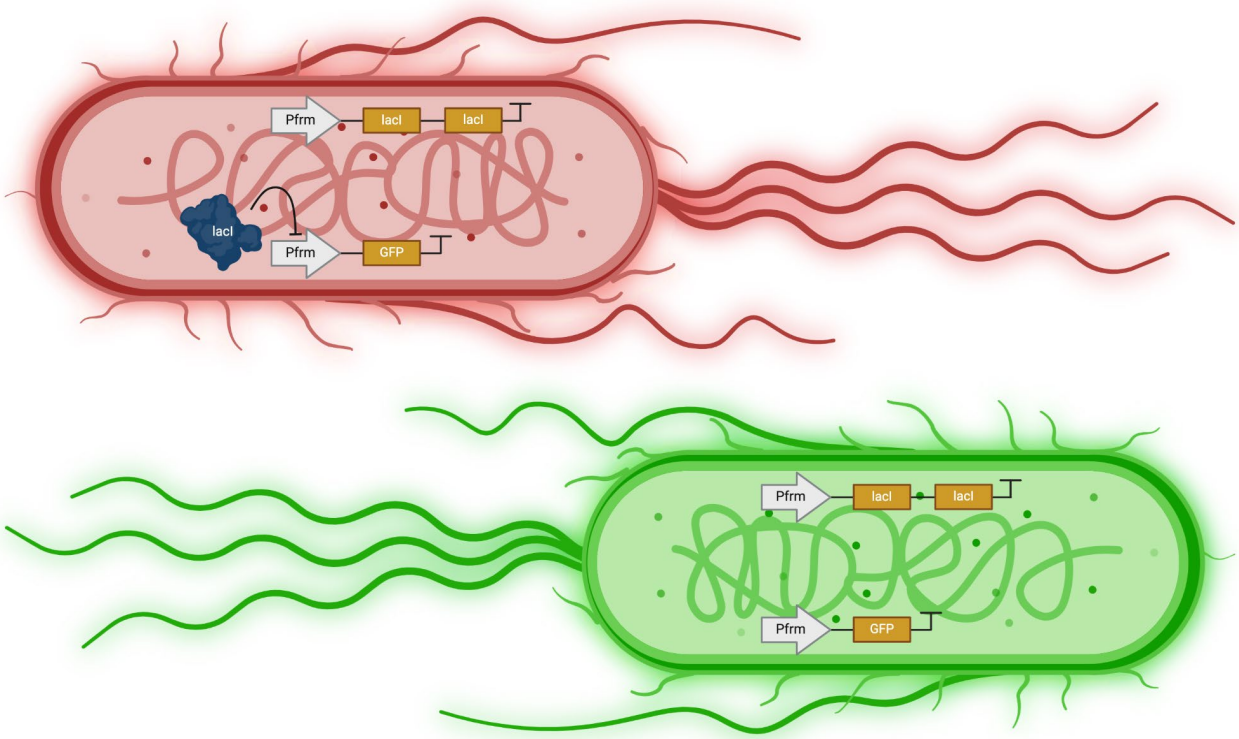

Figure 4. The activation of the system. Without the presence of formaldehyde, the plasmid will express the green pigment GFP. With the presence of formaldehyde, the pLac-lacI repressor system will repress the yellow pigment. The red pigment mRFP1 will present.

allowing the tracking and visualization of cellular structures and processes. We will use this ability to make clear and observable color changes to track the detector's status. When the detector is armed and ready to use, *GFP* will be expressed, entailing sufficient cell activities for a noticeable result should formaldehyde be detected and change color (Figure 4).

The detector displays a red pigment to indicate exposure to formaldehyde. We will use the *mRFP1*, which emits red light, to demonstrate the presence of formaldehyde in the system.

To initiate the gene expression downstream of *Pfrm* and *pLac*, we will employ BBa_J61100 from the Anderson ribosome binding site (RBS) collection as the RBS. In addition, we will use the bidirectional double terminator BBa_B0014 to ensure the expression of only the desired genes.

Safety

Formaldehyde is known to be a dangerous gas with prolonged exposure at high concentrations. There are legal exposure limits to formaldehyde depending on the duration of exposure. The short term exposure limit is an average of 2 ppm for at most 15 min. If exposed to formaldehyde for any longer, you must maintain an average of 0.75 ppm with a maximum exposure time of 8 h (California Department of Health, 2011). We can measure the amount of formaldehyde in the air using a rapid test to detect the levels. When working with formaldehyde, proper personal protective equipment (PPE) is recommended as a safety measure to prevent intake of formaldehyde (University of California, Berkeley, 2012).

We are working with *ΔFrmR*, classified as Biosafety Level 1 due to its non-pathogenic nature. Therefore, this strain poses no safety risks to human health.

Our detector is crafted with consumer safety in mind. Given the harmless nature of our *E. coli* strain, minimal precautions are needed. In case of detector fluid spillage, consumers should follow disinfection protocols utilizing isopropyl alcohol or high-concentration ethanol to eliminate any potential contamination.

Discussions

The detector has various applicability, suitable for home screening and school safety. The planned form of the swab collection kit (Figure 5) provides a portable nature that makes the detector ideal for on-site inspections in manufacturing facilities, where formaldehyde-releasing materials like adhesives and paints are commonly used. In addition to these applications, our detection system can also be utilized in other fields. In agricultural sectors, the detector can help detect formaldehyde contamination in food products, ensuring consumer safety and regulatory compliance. In laboratory settings, the swabbing detection system can serve as a tool to ensure safety protocols and quality control measures since laboratories often use various chemicals and materials that may release formaldehyde during experiments. By employing the swabbing detection system, laboratory technicians can regularly monitor workspaces, equipment, and surfaces for formaldehyde contamination. Furthermore, the detector's ability to quickly collect samples and accurately detect formaldehyde concentrations enables laboratories to identify any issue and maintain a safe environment for researchers. Ideally, our final solution will take shape as a lyophilized product that can be rehydrated when it is needed.

The detector's fast sample collection via swabbing and testing in the solution simplifies disease prevention relevant to formaldehyde exposure. The reusable design significantly cuts costs compared to conventional single-sample collection methods. Direct swabbing not only increases accuracy but also enables users to pinpoint sources of high formaldehyde concentrations.

A lactose-rich environment is necessary in order to effectively function the *pLac-LacI* system and ensure constitutive characteristics for the lactose promoter. However, low to medium concentrations could lead to insufficient *LacI* reaching the lactose promoter, resulting in the detector showing yellow colors despite formaldehyde detection. In addition, while the detector indicates the presence of formaldehyde through color changes, the correlation between the intensity of exhibited colors and the formaldehyde concentration is not fully understood. The variability in visual color perception can introduce subjectivity and inconsistency in the interpretation of color changes, highlighting the importance of standardized measurement techniques.

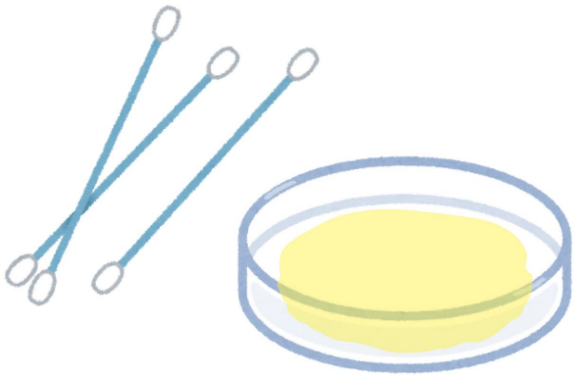

Figure 5. Swab collection kit.

Next steps

Future tests will be conducted to determine the optimal amount of lactose to be added to the detection solution in order to ensure the reusability of the system. In addition, while the detector will indicate the presence of formaldehyde through color changes, further research is important to fully understand and quantify the results of this detection method. Our group will focus on testing the colors shown in response to varying concentrations of formaldehyde and conduct quantitative analysis to establish a reliable correlation between the intensity of color changes and the concentration of formaldehyde present in the environment. Investigating the stability of color changes under different environmental conditions and over time will be essential to optimize the detector's performance and reliability.

Author contributions

L.D., B.J, J.M., E.T., G.W, and M.Y. contributed to the preliminary research of the project and submitted the article collaboratively. L.D. and E.T. continued the project, did experiments in the laboratory, and finished the paper together.

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