Creating scented concerts with a heat-inducible promoter and fragrance-producing genes

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Humans readily indulge their senses: we make fine cuisine for its taste, we listen to a variety of music for their pleasing sounds, and we travel to view the beautiful scenery. However, hardly any form of entertainment involves scent. Our team proposes a revolutionary form of entertainment to fill this gap: a scented concert that combines sweet musical tones with pleasant fragrances, leaving the audiences with a profound experience. Using synthetic biology, we propose a design for a set of scented violin strings that produce distinct fragrances. The production of scents will be triggered by the friction of the bow moving across the strings. Four devices (BBa_M50438, BBa_K395600, BBa_K727007, and BBa_K1322231) responsible for encoding molecules associated with the scents of pine, apple, rose, and sandalwood have all been expressed in *Escherichia coli*. Our design will put the grpE promoter in front of each gene responsible for the four scents. GrpE promoter has been shown to positively control the heat shock response in *E. coli* cells at the transcriptional level. Using this promoter, we will activate the scent genes through the heat caused by the bow's friction on the violin strings. Our constructs include the grpE promoter (BBa_K338001), the strong ribosome binding site (BBa_B0030), four fragrance-producing genes, and the double terminator (BBa_B0015) in the plasmid backbone pOGG005. These parts will be cloned within the lacZ gene on the plasmid using XbaI and XmaI restriction sites. Using the blue-white screening, we will select the *E. coli* transformed with the correct recombinant plasmid associated with the LacZ gene's disruption. Before each performance, the violinists can spray our genetically engineered *E. coli* cells with four distinct scents onto each respective violin string. As the bow moves across the strings, the produced scents will transform the musical experience for all listeners, providing an unprecedented multisensory experience.

Keywords: Scent, fragrance, heat shock promoter, music, concert, violin, friction, bowed string

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Watch a video introduction by the authors at https://youtu.be/r2jWH3XFAQo

Background

As one of human's irreplaceable senses, the sense of olfactory is largely undervalued in most cultures despite providing huge psychological and physical benefits. For example, fragrances provide strong cues for detailed past memories (Chu & Downes, 2002). With this special trait, scent is proven to be effective in the form of aromatherapy when applied to patients with Dementia or Alzheimer's Disease (Jimbo et al., 2009). In daily lives, scent has the potential of becoming the most popular source of entertainment, as it can directly influence people's memories and therefore leave the audience with some memorable experiences.

In our society, the value of scent remains largely unexploited. Scent is often treated as a condiment instead of a focal point: when entering a high-end clothing store, we can often smell the perfume that serves to elevate our shopping experience. Before attending an important meeting, people will spray perfume onto themselves and brush their teeth to avoid bad breath. In either case, scent, though crucial, is not the main focus of any part of our lives. Intending to fill in this gap, our team developed the idea of the scented concert--a revolutionary form of entertainment that aims to fully exploit the potential of fragrances and combine that with music.

Further investigation shows us that the combination of auditory and olfactory senses is particularly favorable for entertainment. Experiments by Professor Seo and Hummel (2011) from the University of Dresden Medical School have shown that there's a clear link between the pleasure one felt toward an odor and the extent to which one enjoyed the preceding sound. As for our scented concert, this result indicates that the pleasure the audience will take from the fragrances will be boosted by the music they hear, resulting in a magnified multisensory experience that no current entertainment provides.

Another possible benefit of the scented concert is that it might be able to induce artificial synesthesia between sounds and scents. Researchers have shown that in general, people with synesthesia achieved better memory scores than the control group, especially in memories related to their connected senses (Tierney, 2019). Thus, musicians and perfumers may find the scented concert especially beneficial to their careers, so as the general public who might benefit from the improved memory.

To implement our idea, we decided to utilize the power of synthetic biology. Many pieces of research and experiments focusing on the use of biotechnology in the fragrance industry have been conducted by scholars. In a review written by several researchers from the Macquarie University, the authors claimed that "as already shown with the development of a hoppy yeast, many of the strategies employed and discussed [in the review] could easily be used to engineer aroma development in strains involved in the fermented beverage industry" (Wyk et al., 2018). The use of genetically engineered yeast to generate scents in the wine industry is just one example of many real-world applications of synthetic biology in the production of fragrances. According to Lux Research, "accelerating advances in our understanding of synthetic biology can unlock novel methods to produce flavor and fragrance chemicals that avoid [the drawbacks of conventional methods] altogether" (Lux Research, 2015). The drawbacks, as Lux claimed, are the "suffer from high cost, low sustainability, and high risk of supply chain disruption."

In fact, several scented concerts have been held before by different artists. However, none of them attempted to set up a repeatable standard for this new artistic format. In 2004, an instrument known as "Olfactiano" (shown in Figure 1), designed by Belgian artist Peter de Cupere, was for the first time being played in a scent sonata. The instrument can release scents corresponding to the keys--similar to the ones on a regular piano--that are being pressed. Although the concert was successfully held, it was too costly and technically difficult to be copied in other places.

Figure 1. *The Olfactiano designed by Peter de Cupere (Frank, 2014).*

Nonetheless, synthetic biology provides a less costly and more sustainable alternative to realize our idea. We can reach the desired effect by transforming fragrance-producing genes into well-researched organisms such as *Escherichia coli,* which will be programmed to release fragrances as the music starts to play. Since *E. coli* can undergo binary fission and easily multiply in size over a few hours, scented concerts can be performed simultaneously across the world with little cost being added per show.

With these clear advantages, we are convinced that synthetic biology will provide the best solution to achieve our goal. The following sections of our paper will discuss the specific design we have from the perspective of system, device, and parts.

Systems Level

The system of our project consists of the natural heat shock that exists in most concerts and genetically edited *E. coli* that will be sprayed onto string instruments right before each performance. In the article, "Bowed String Simulation Using a Thermal Friction Model", Woodhouse from Cambridge University analyzed the heat generated when a bow moves across strings (Woodhouse, 2003). His simulation result shown in Figure 2 suggested that when a string is bowed, therefore releasing heat into the surrounding space, the temperature above ambient varies between 17 and 31 degrees Celsius (Woodhouse, 2003). This shift in temperature indicated the presence of heat shock when string instruments such as violin are played by bows.

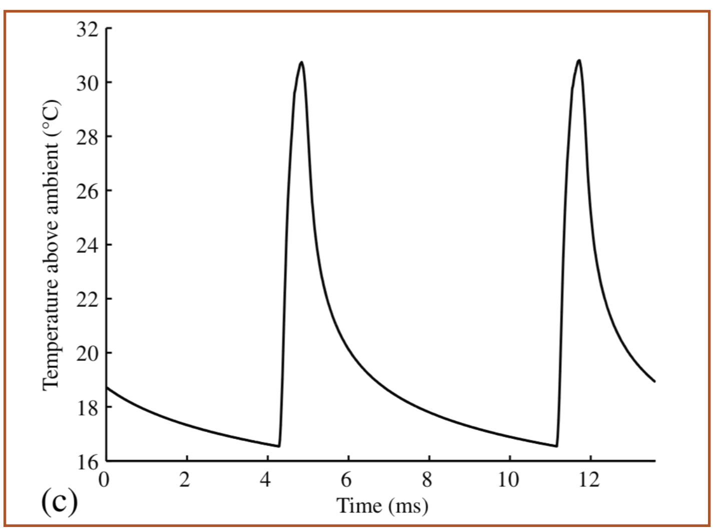

Figure 2. *The graph shows the temperature variation when strings are bowed during the simulation. The temperature shifts frequently between 17 and 31°C (Woodhouse, 2003).*

Taking advantage of the natural heat shock generated by string instruments during a concert, our system then includes synthetically engineered *E. coli* which can recognize the heat shock and activate the fragrance-producing genes contained in their transformed plasmids. The design will make sure that the production of fragrance and the performance of music will be generally in sync. Only when the *E. coli* are sprayed onto the string instruments and heat is produced from the bowed strings could the fragrance-producing genes be transcribed. The process is indicated in Figure 3. To ensure a high survival rate of *E. coli* when they depart from their cultures during the performances, they should be sprayed onto the strings immediately before each concert. Also, for the system to function properly, performers have to make sure that there isn't any significant temperature difference between the *E. coli* cultures and the performance site.

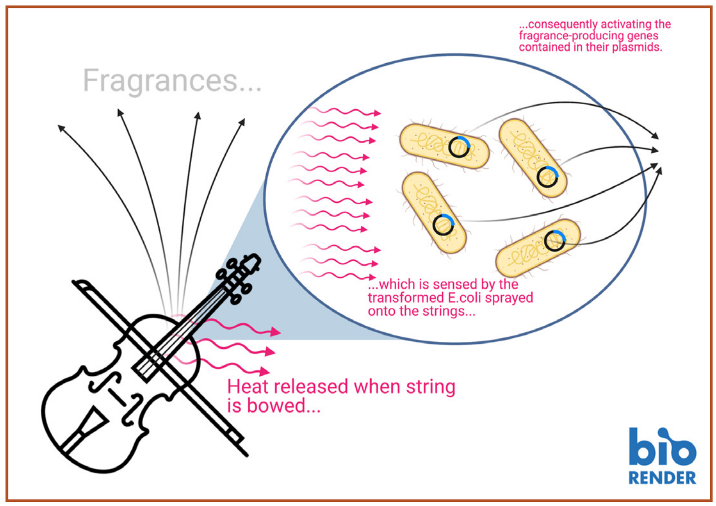

Figure 3. *The picture shows how our design works at the system level.*

Device Level

Plasmids containing four distinct fragrance-producing genes (FPGs) will be transformed into *E. coli* separately. We wish to match each string on a violin with a distinct aroma, therefore associating musical notes with specific scents. We identified four genes on the iGEM registry encoding for beta-Pinene synthase (BBa_M50438), MpAAT1 enzyme (BBa_K395600), Geraniol (BBa_K727007), and Santalene Synthase (BBa_K1322231), which can produce the scent of pine, apple, rose, and sandalwood respectively. As discussed earlier, we will insert a heat-shock inducible promoter known as the grpE promoter (BBa_K338001) before the FPGs so that the scents can be released in sync with the music. The promoter, like other heat-shock inducible promoters in *E. coli*, regulates the σ-32 factor. σ-32 controls heat shock response by directing RNA polymerase to transcribe heat shock proteins, which are synthesized when the temperature rises (Nonaka et al., 2006). To increase the efficiency and accuracy of the translation of the genes, a strong ribosome binding site (BBa_B0030) will be placed in front of the FPGs. Finally, the double terminator rrnBT1-T7TE will be assembled after the FPGs. This terminator is the most commonly used terminator according to the iGEM registry and seems to be reliable (Shetty, 2003). Due to the lack of plasmids containing the aforementioned parts, we decided to synthesize them manually with their complete sequences that can be found on the iGEM registry. Since *E. coli* is one of the most studied bacteria and is commonly used in synthetic biology, it will be an ideal chassis for the purpose of our project. All of our parts are either designed to be transformed into *E. coli* or proven to be expressed in *E. coli* (Matsubara, 2010; Orhan, 2012; Schreiner, 2018; Shilts, 2014) . The way in which our FPGs produce fragrances is summarized in Figure 4.

The first FPG, shown in Figure 4.1, codes for beta-Pinene synthase, an enzyme that converts GPP (geranyl diphosphate) into beta-Pinene (Schreiner, 2018). Beta-Pinene is a molecule that produces pine fragrance. In *E. coli,* Mevalonate (MVA) and methylerythritol phosphate (MEP) pathway naturally produce GPP from dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), allowing the device to function (Zhou et al., 2015). Figure 4.2 demonstrates the mechanism of the apple FPG, which codes for the enzyme MpAAT1, which catalyzes the production of Butyl acetate and 2-Methylbutyl acetate from butanol and 2-Methyl-1-butanol (Matsubara, 2010). Both Butyl acetate and 2-Methylbutyl acetate can release apple scents. Acetyl CoA, the substrate of MpAAT1 enzyme, naturally exists in *E. coli* cells. Thus, in order to produce apple fragrance, butanol and 2-methyl-1-butanol should be applied to the *E.coli* culture containing the transformed apple FPG before performances. The third FPG, depicted in Figure 4.3, includes genes that code for GPP synthase and geraniol synthase (Orhan, 2012). GPP synthase will catalyze the production of GPP from IPP and DMAPP as discussed above, and geraniol synthase will produce geraniol from GPP, which is a chemical that has a rose fragrance (Orhan, 2012). The sandalwood FPG, shown in Figure 4.4, translates into alpha-Santalene synthase, an enzyme that converts Farnesyl pyrophosphate (FPP) into (+)-alpha-Santalene (Shilts, 2014). FPP is produced in *E. coli* as an intermediate in several pathways, including MVA and MEP (Shilts, 2014). (+)-alpha-Santalene, on the other hand, is a sesquiterpene (a kind of compound) that releases sandalwood scents.

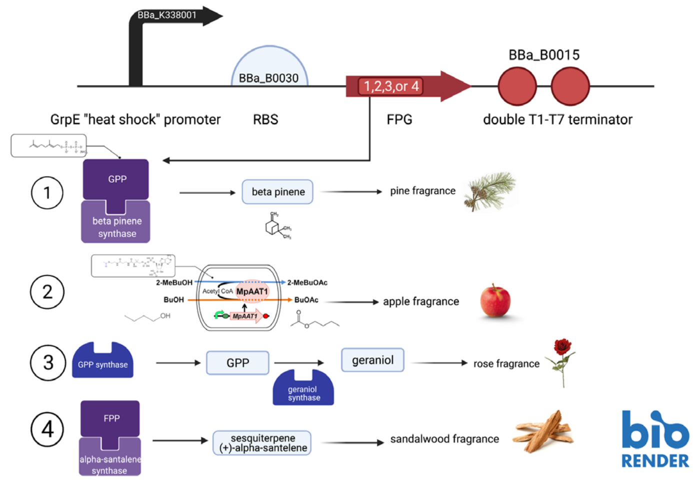

Figure 4. This figure shows the procedures through which each FPG produces its corresponding scent.

Parts Level

Following the procedures depicted in Figure 5, we will assemble the parts mentioned above into the vector backbone pL1V-Lv1-ColE1-amp, a high-copy empty backbone used for *E. coli* transformation (Geddes et al., 2019). All FPGs except for the rose FPG will be cloned into the plasmid through the XbaI restriction site. The rose FPG, on the other hand, will be cloned using the XmaI restriction enzyme due to the existence of XbaI restriction sites within the gene. As both XbaI and XmaI restriction sites are located inside the LacZ gene within the plasmid, the correctly transformed plasmid will contain disrupted LacZ genes. Thus, after the transformation, blue-white screening can be applied to identify the successfully transformed *E. coli*, which will appear white under screening.

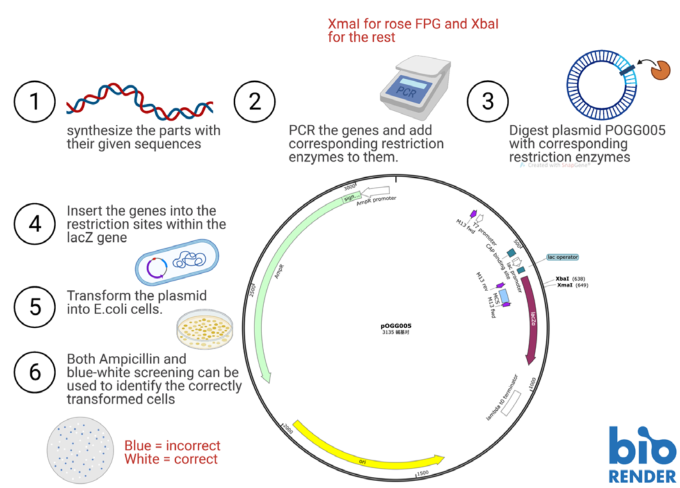

Figure 5: The picture describes each step that needs to be performed in order to obtain desired E. coli cells.

Safety

Generally, there should not be any safety concerns with our proposed experimental design. We will be working with the DH5 alpha strain, which is a non-pathogenic *E. coli* strain. All procedures will be conducted in a Biosafety Level 1 laboratory (BSL-1) environment. However, we recognize the possibility of physical contact and inhalation of *E. coli* cells due to their complete exposure during the performance. Although little or no health issues should result from this, we recommend all performers and audiences who sit close to the stage to wear masks, gloves, and eye goggles during the performance. All participants, both the musicians and the audience, should wash their hands with soap thoroughly after the concert. Meanwhile, the instruments being sprayed with *E. coli* cells should be cleaned thoroughly following appropriate procedures.

Discussions

Although the design should theoretically work, several problems might impede us from achieving the desired result. First and foremost, there have not been any previous experiences with the four fragrance-producing genes that can prove their effectiveness, nor sufficient records of their usages on the iGEM registry. Compared to the FPGs, all other parts including the heat shock promoter, the RBS, and the double terminator are much more frequently used and reliable.

A second major issue is whether the amount of heat shock generated by the friction between violin bow and strings is enough to activate the grpE promoter, and whether the amount of fragrance produced is able to disseminate through the entire concert hall. An experiment with the grpE promoter fused to Green Fluorescent Protein (GFP) revealed that after applying heat shock to *E. coli* cells at 42°C for 0 (control), 5, and 10 minutes respectively, only the group that received 10 minutes of continuous heat shock gained a significant increase in GFP production, while the 5-minute group showed little difference from the control group (2010 Caltech iGEM Team, 2010). Nevertheless, another experiment with the gprE promoter suggested an increase in transcriptions activated by the heat shock promoter as temperature increases (2010 Caltech iGEM Team, 2010).

The experiments cast suspicion on the assumption that the heat generated by the bowed strings during the concert can immediately lead to a noticeable amount of fragrance production. Further research and experiments are required to determine if there will be a significant time lag between the onset of music and the production of scents and whether the temporary pauses of heat production during the performances would have significant effects on the expression of FPGs. On-site investigations will be conducted to measure the strength and distinctiveness of the fragrances produced and how well they spread across the room.

Another concern is the survival rate of the *E.coli* cells without the support of nutrients and an optimal temperature from their cultures during the performances. According to a study conducted by the Spanish Ministry of Science and Innovation, after leaving two groups of *E.coli* cells in nutrient-deprived environments for 21 days at 4 °C and 20 °C respectively, the absence of nutrients had no effect on cell viability and integrity, though their culturability declined faster at a higher temperature (Orruño et al., 2017). Nevertheless, at the end of 3 days, almost all cells (>90%) experimented at both high and low temperature levels remained both culturable and viable (Orruño et al., 2017). Hence, we are confident that our *E. coli* will be able to persist during a scented concert even without the support of their cultures.

Besides the practicality of our idea, we also identified some potential defects with our specific design. First, it is unclear whether the act of spraying *E. coli* onto the violin strings can cause damage to the strings, the bow, or the violin itself. According to Marshall (2015) , excess humidity can affect the texture of the bow and thus hurt its performance during the concert. Therefore, it is reasonable to believe that the *E. coli* cells, sprayed onto the strings as liquid solutions, will interfere with the musical quality of the instruments. In the long term, the enduring existence of moisture on the instruments might also shorten their longevity. However, we will need to analyze the exact side effects of the presence of *E.coli* on the strings by comparing the instruments used in the scented concerts with the ones used in regular concerts. If severe side effects are identified, we will develop other methods that enable our device to work properly on the strings while causing no or minor damage to the instruments.

Another problem is that our chassis, *E. coli*, is notorious for its bad smell due to the reaction of the tryptophanase enzyme that produces indole, a smelly chemical (*Remove the Bad Smell, 2014*). The bright side is that several teams have already developed mechanisms to eliminate their bad smells (*Remove the Bad Smell, 2014*). In addition, this is not a serious setback considering all the advantages of working with *E. coli*--an accessible, safe, and well-known organism.

Next Steps

The next step of our project is to synthesize such a device and test it in a controlled environmental setting. This might be the best way to find solutions to the concerns stated in the previous section. We also encourage other individuals and groups to develop further research and experiments based on our model. Bearing all the challenges and concerns in mind, we still believe that our project contributes an innovative idea and approach to create an avant-garde. By further modifying our design and testing it with experiments simultaneously, if we do eventually conquer the aforementioned challenges, our scented concert will transform the musical experience for all listeners, providing an unprecedented multisensory enjoyment.

Author Contributions

B.O. assisted in the preliminary research for this project. G.W. contributed the original idea and identified all necessary parts for the device. G.W. also did all the writings and research for all sections of the paper.

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