

Eau d'E coli: A synthetic biology approach to reprogramming bacterial odor

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Abstract

The underlying goal of synthetic biology is to make the design, construction, and characterization of engineered biological systems easier. Here, we evaluate whether synthetic biology approaches can support the process of metabolic engineering. As a model problem, we chose to reprogram the odor of *Escherichia coli*. We first designed and produced a bacterial chassis with reduced fecal odor. Then, by applying fundamental engineering principles such as abstraction and standardization, we (1) implemented wintergreen and banana odorant generators that use exogenously supplied precursors, (2) developed transcriptional control devices for exponential and stationary phase protein production, and (3) combined a stationary phase transcriptional control device with a banana odorant generator to produce banana odor in a growth phase-dependent manner. Our results also confirm that the enzymes that produce odorants can serve as reporters of gene expression, complementing existing genetically-encoded reporters such as β -galactosidase, fluorescent proteins, and luciferases.

1 Introduction

Metabolic engineers have demonstrated successful construction of novel biosynthetic pathways in industrial microorganisms for the purpose of producing commercially useful compounds [1]. However, such engineering feats require huge investments of labor, time, and capital by world-renowned genetic engineers. Typically, such large resource investments are only justified when the product is overwhelmingly compelling from an industrial or medical perspective.

The underlying goal of synthetic biology is to make the design, construction, and characterization of engineered biological systems easier. A proposed approach advocates applying fundamental engineering principles such as abstraction, standardization, and decoupling to the substrate of biology and the process of biological engineering [2]. However, the relevance of principles from classical engineering disciplines to biological engineering has not yet been fully explored.

We examined how the ideas of abstraction and standardization can be applied to metabolic engineering problems. Abstraction is an approach for managing complexity by hiding unnecessary detail [2, 3]. An abstraction hierarchy is organized around a set of functional layers (Fig. 1). For synthetic biology, the lowest abstraction layer is currently defined at the level of primary nucleic acid sequences. Moving up one level in the abstraction hierarchy, parts are defined as nucleic acid sequences that encode basic biological functions such as a transcriptional promoter or enzyme coding sequence [4]; parts are specified by their innate biological function(s), while details regarding nucleic acid sequence remain hidden. At the next level in the abstraction hierarchy, devices are defined as human-defined functions that can be realized via a combination of one or more parts. Devices are specified in terms of their inputs and outputs, with details regarding all underlying parts hidden. Different classes of devices have different types of inputs and outputs. For example, transcriptional devices

either receive transcriptional input(s), produce transcriptional output(s), or both. As a second example, biosynthetic devices convert one or more chemical precursor inputs to one or more chemical product outputs. At the topmost layer of the abstraction hierarchy, engineered biological systems are defined as combinations of devices that produce more powerful behaviors, such as a tumor-killing microbe [5]. Systems are specified by their overarching behavior, while details regarding the input/output relationships of the component devices remain hidden. By using an abstraction hierarchy, the behavior of an engineered biological system can be implemented as a combination of devices, each device can be defined in terms of its component parts, and each part can be specified by its primary nucleic acid sequence.

The utility of the abstraction hierarchy depends on standards that define how components in each layer of the hierarchy are combined and shared across layers. For example, standards for physical composition specify how parts physically connect; Knight developed the BioBrick standard for physical composition of genetic parts (T Knight, 2003, <http://hdl.handle.net/1721.1/21168>). Using the BioBrick standard, the synthetic biology community has developed a collection of genetic parts in the Registry of Standard Biological Parts (Registry). All parts in the Registry can be readily assembled using the BioBrick assembly standard. As a second example, standards for functional composition specify how device inputs and outputs are functionally connected. A proposed signal standard for transcriptional devices is the rate at which RNA polymerases move past a particular point on a strand of nucleic acid, Polymerases Per Second (PoPS, B Canton *et al.*, submitted) [6]. By using the PoPS signal standard, transcriptional devices can have one or more PoPS inputs, one or more PoPS outputs, or both. Thus, PoPS defines a standard, common signal carrier for transcriptional devices. In practice, while the goal of functional composition standards is to ensure that devices work reliably and predictably when used in combination, only a few parts in the Registry and elsewhere have yet been shown to function reliably in combination [7, 8]. Nevertheless, the modularity afforded by abstraction and standardiza-

tion already enables parts and devices to be used as “off-the-shelf” components that can be independently tested, optimized for function as necessary, and improved over time.

As a model problem for exploring the relevance of synthetic biology to metabolic engineering, we chose to reprogram the odor of *Escherichia coli*. Odorants are volatile chemicals that have an odor or smell detectable by the human olfactory system [9]. For example, most flowers produce a complex array of odorants to generate a pleasant smell [10]. In contrast, cultures of most laboratory strains of *E. coli* have a fecal odor. To reprogram bacterial odor, we had the option of eliminating natural odorants from *E. coli*, adding novel odorants to *E. coli*, or both. Eliminating natural odorants from *E. coli* requires modifying existing cellular biosynthetic pathways. Adding novel odorants to *E. coli* involves engineering new biosynthetic pathways for chemical production.

2 Results

2.1 Identifying an odor-free chassis

We named our project to reprogram bacterial odor, Eau d’E coli. The first challenge in the project was to ensure that the natural, fecal odor of *E. coli* did not overpower our engineered odors. Indole was suggested to be the primary contributor to the fecal odor of *E. coli* (E Pichersky, personal communication, 2006). We confirmed that indole is the primary odorant produced by *E. coli* by noting that LB Lennox medium supplemented with indole at a concentration comparable to that produced in LB cultures of *E. coli* strain MG1655 ($\sim 300 \mu\text{M}$) smelled similar to typical *E. coli* laboratory cultures [11]. Indole is a product of the tryptophanase enzyme encoded by the *tnaA* gene of the *tna* operon in *E. coli* [12]. Mutations to the *tna* operon can reduce indole levels [13]. We tested four *E. coli* strains carrying mutations in the *tnaA* gene as potential odor-free chassis for the Eau d’E coli project: YYC912, JC12337, MEB61, and MB408 [14, 15, 16]. We verified that

E. coli strain YYC912 does not produce indole initially through smell tests and later by gas chromatography analysis (SI Fig. 5). Thus, we selected *E. coli* strain YYC912 as an odor-free chassis for Eau d’E coli.

2.2 Engineering bacteria to smell like wintergreen and banana

We constructed two biosynthetic devices for odorant production in *E. coli* (Fig. 1B). The wintergreen odorant generator (BBa_J45120) is based on the *S*-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase I (*BSMT1*) gene from *Petunia × hybrida* [17, 18]; it catalyzes the conversion of the precursor salicylic acid to the odorant methyl salicylate, which has a wintergreen odor. The banana odorant generator (BBa_J45200) is based on the *Saccharomyces cerevisiae* alcohol acetyltransferase I (*ATF1*) gene [19, 20]; it catalyzes the conversion of the precursor isoamyl alcohol to the odorant isoamyl acetate, which has a banana odor. Each biosynthetic device is made up of two transcriptional devices: a transcription source and an odorant enzyme generator. Transcription sources, such as promoters, produce a transcriptional signal output. Odorant enzyme generators take as input a transcriptional signal and produce as output an enzyme that catalyzes production of an odorant from a chemical precursor. All transcriptional devices in this work use PoPS as a common signal carrier.

To confirm that our biosynthetic devices produced the correct odorants we analyzed the *E. coli* cultures for odorant production by gas chromatography. The wintergreen odorant generator (BBa_J45120) produced high levels of methyl salicylate when the precursor salicylic acid was added to the culture medium (Table 1, SI Fig. 6). The cellular chassis, *E. coli* strain TOP10, did not produce methyl salicylate in the presence of exogenous salicylic acid, demonstrating that our biosynthetic device was indeed responsible for methyl salicylate production. Similarly, the banana odorant generator (BBa_J45200) produced high levels of isoamyl acetate when the precursor isoamyl alcohol was added to the culture medium,

whereas the cellular chassis did not (Table 1, SI Fig. 7).

A blind smell test demonstrated that we had successfully reprogrammed the odor of bacteria. Smell test participants distinguished between cultures producing wintergreen odorant, banana odorant, or the natural fecal odorant of *E. coli*. Of the 116 respondents, 64% were able to correctly identify the culture producing methyl salicylate through its wintergreen odor, 87% were able to correctly identify the culture producing isoamyl acetate through its banana odor, and 86% were able to correctly identify the laboratory *E. coli* strain TOP10 through its fecal odor (Fig. 2). Both the wintergreen and banana odorant generators were propagated in the odor-free chassis for the smell test. Based on the survey results, humans can smell the odorant produced by both odorant generators (Pearson’s chi-square test yields $p < 0.01$).

2.3 Engineering growth-dependent transcriptional control devices

We sought to extend the initial Eau d’E coli system by developing and demonstrating that odorant production could be regulated and, in turn, used as a genetically encoded reporter of cell state. Specifically, we sought to engineer *E. coli* to produce one odorant during exponential growth and a different odorant during stationary phase. We use the terms exponential growth and stationary phase practically; we define exponential growth as the period of culture during which cells are growing and dividing and stationary phase as the subsequent period during which cells undergo little or no growth. In our smell tests of the constitutive odorant generators, the culture producing banana odorant had a stronger odor than the culture producing wintergreen odorant. Thus, we opted to design a system to produce wintergreen odorant only during exponential growth and, as the culture transitions to stationary phase, wintergreen odorant production should plateau or decrease, while banana odorant production begins. We predicted that batch cultures of such cells would initially smell like wintergreen, and then the banana odor would overpower any residual wintergreen

odor.

We considered different designs for exponential and stationary phase regulation of odorant production. All designs focused on using transcriptional control devices to regulate the odorant enzyme generators and thus odorant production. Several *E. coli* promoters that are primarily active in stationary phase have been previously characterized [21, 22]. We evaluated stationary phase promoters that control transcription of *rpoS* and *osmY* in *E. coli* as potential transcriptional control devices [23, 24, 25]. Preliminary tests demonstrated that only the *osmY* promoter met our requirements for control device function: it produced a low GFP synthesis rate during exponential growth and a high GFP synthesis rate in stationary phase (Fig. 3). Thus, we selected the *osmY* promoter as our stationary phase transcriptional control device (BBa_J45992).

Since we had already engineered a stationary phase transcriptional control device (BBa_J45992) and we had access to an “off-the-shelf” working transcriptional inverter (BBa_Q04401) from the Registry [26], we opted to construct an exponential phase transcriptional device by combining the *osmY* promoter and transcriptional inverter (an inverter is a device that converts a HIGH input signal to a LOW output signal and vice versa). The resulting composite exponential phase device (BBa_J45994) worked well when tested: the device only produced a high GFP synthesis rate in exponential phase (Fig. 3). As expected, the timing of the exponential and stationary phase devices were well-coordinated, with the GFP synthesis rate of the exponential phase device decreasing just as the GFP synthesis rate of the stationary phase device increases.

2.4 Growth-dependent regulation of odorant production

To enable growth-dependent regulation of odorant production, we used the engineered exponential and stationary phase control devices to control the wintergreen and banana odorant enzyme generators, respectively. To evaluate the function of the constitutive, exponential,

and stationary phase odorant generators, we quantified the odorant production of cultures at different cell densities using gas chromatography. The exponential phase wintergreen odorant generator (BBa_J45181) produced methyl salicylate, but its methyl salicylate levels were indistinguishable from the constitutive device during stationary phase (SI Fig. 8). In contrast, the stationary phase banana odorant generator (BBa_J45250) worked as designed: the composite device produced little isoamyl acetate at low cell densities and more isoamyl acetate in stationary phase (Fig. 4). As a comparative control, the constitutive banana odorant generator (BBa_J45200) produced isoamyl acetate across all cell densities.

There are two possible reasons why we were unable to regulate wintergreen odorant production in a growth-dependent fashion (SI Fig. 8). First, assuming that the control devices regulated the BSMT1 generator similarly to the GFP test device, then the experimental results suggest that enzyme concentration was not rate-limiting in methyl salicylate production. Instead, the substrate salicylic acid or the cofactor *S*-adenosyl-L-methionine may have been limiting. Although a rough estimate suggests that at most $\sim 10\%$ of the exogenously supplied precursor was consumed in the assay, the intracellular concentration of salicylic acid could be limiting. Alternatively, the constitutive and exponential phase transcriptional control devices may regulate the BSMT1 generator differently than the GFP test device. Either both control devices maintained BSMT1 expression in stationary phase, or both devices turned off BSMT1 expression. In other words, functional composition of the transcriptional control devices with the BSMT1 generator failed. We cannot definitively exclude either explanation based on the data.

3 Discussion

There are five successes from our work that are worth noting. First, we identified a bacterial chassis for odorant production that is free of the natural, fecal odor of most *E. coli*

strains. The odor-free chassis is useful for ensuring that the natural odor of *E. coli* does not overpower any engineered odors. Second, we implemented wintergreen and banana odorant generators that use exogenously supplied precursors. Blind smell tests demonstrated that most people can smell the wintergreen and banana odorants produced in culture. Third, we successfully engineered exponential and stationary phase transcriptional control devices. The exponential and stationary phase control devices can be combined with a GFP generator in order to produce regulated, growth-dependent protein production. Fourth, we combined the stationary phase transcriptional control device with the banana odorant enzyme generator to regulate banana odorant production. Finally, taken together, our results demonstrate that odorant-producing enzymes can serve as genetically-encoded reporters of gene expression. Odor-based reporters complement existing optically-based reporters such as β -galactosidase, fluorescent proteins, and luciferases. Furthermore, odor-based reporters may prove useful in situations in which direct culture sampling and measurement is difficult, such as industrial fermentation where off-gas analysis by gas chromatography is already common [27].

In contrast to most metabolic engineering whose goal is to produce a particular chemical at a target yield as defined by a specific application [1], the goal of Eau d'E coli was to engineer bacterial odorants to change the odor of cultures in a regulated fashion. Thus, we were able to choose which odorants to produce based on a preliminary evaluation of different candidate odorants (see *SI Text* for details). Moreover, since the human olfactory system is quite sensitive, production of small amounts of odorant can be sufficient to smell and therefore constitute successful implementation of our engineered system [28]. Nevertheless, the Eau d'E coli project enabled us to explore how synthetic biology principles might best be applied to metabolic engineering.

3.1 Use of abstraction to organize system function

In metabolic engineering, most engineered biosynthetic systems are built from *ad hoc* collections of genetic components that can be assembled, tested, and used in screens or selections if necessary. In this work, we instead used abstraction to systematically compile overall system function, reprogramming bacterial odor, into two biosynthetic devices. The biosynthetic devices were in turn compiled to transcriptional devices, and then BioBrick standard biological parts, and finally nucleic acid sequences. Thus, abstraction provides an approach for systematically mapping high-level system behavior, such as reprogramming bacterial odor, to low-level primary sequence data. As a result, abstraction allows biological engineers to cope with the complexity of engineering multi-component synthetic biological systems; as the number of components in engineered biological systems increases from a few dozen parts to hundreds of interacting devices, abstraction will become even more important.

3.2 Standards for physical composition make system construction easier and faster

Standards that support the physical composition of genetic parts make construction of many-component, engineered biological systems, including metabolic engineering projects, both easier and faster. For example, in the Eau d'E coli project, we used the BioBrick standard for physical composition of genetic parts. Our use of the BioBrick physical composition standard offered four advantages over classical molecular cloning approaches. First, our use of a uniform part assembly procedure reduced the learning curve associated with system construction. Making construction easier was critical for our team of novice biological engineers to begin construction of Eau d'E coli devices quickly despite limited prior research experience. Second, standardization of the assembly procedure tends to make the device and system construction process more reliable since the same reagents and protocols are used

at each stage. Third, since our system was constructed of BioBrick parts, we could readily reuse preexisting parts from the Registry in our system design. For example, we reused a promoter, ribosome binding site, transcriptional terminator, GFP generator, and transcriptional inverter from the Registry (BBa_R0040, BBa_B0032, BBa_B0015, BBa_E0840, and BBa_Q04401). Each reused part could be readily combined “off-the-shelf” with our newly constructed parts because all parts adhered to the BioBrick assembly standard. Our reuse of parts resulted in significant time and effort savings since we did not have to develop parts *de novo* or redesign reused parts. Fourth, the parts that encode growth-dependent transcriptional regulation and odorant production are now freely available to the community via the Registry for reuse and improvement (SI Table 3).

3.3 Additional work on standards for functional composition is needed

Functional composition builds upon physical composition: parts must not only be readily connected but should also function as expected. Just as standards for physical composition ensure that any two parts that adhere to a physical composition standard can be readily combined, standards for functional composition ensure that any two parts or devices function as expected when combined. Today, we lack adequate standards to ensure reliable functional composition; the only proposed standard for functional composition is the use of PoPS as a common signal carrier for transcriptional devices. The PoPS standard ensures that the output(s) of one transcriptional device can be connected to the input(s) of another PoPS-based device. Additional standards, such as prescribed PoPS signal ranges that ensure transcriptional device signal levels are well-matched, are needed so that devices can be developed to meet proscribed functional specifications, and evaluated for the reliability of their use in combination.

In the absence of sufficient standards for functional composition, we relied on trial-and-error in building the Eau d’E coli system. Such *ad hoc* approaches can yield success. For example, to construct an exponential phase transcriptional control device, we combined a stationary phase promoter with an “off-the-shelf” transcriptional inverter. Both devices were independently characterized, and we successfully combined them to demonstrate growth-phase dependent GFP production (Fig. 3). Similarly, to construct a stationary phase banana odorant generator, we combined a stationary phase PoPS source with the banana odorant enzyme generator. Again, both devices had been independently characterized and behaved as expected when combined (Fig. 4). Nevertheless, functional composition of transcriptional devices remains challenging. For example, when we combined the exponential phase control device with the wintergreen odorant enzyme generator, the resulting composite device produced methyl salicylate during exponential phase, but we did not measure lower methyl salicylate levels in stationary phase as compared to the constitutive wintergreen odorant generator (SI Fig. 8).

Metabolic engineering depends on functional composition of not only transcriptional devices but also biosynthetic devices. For instance, the Eau d’E coli system could be further extended to produce odorants from endogenous cellular metabolites rather than supplied exogenous precursors. Such an extension would require additional biosynthetic devices that convert natural cellular metabolites to the odorant precursors salicylic acid and isoamyl alcohol. In fact, salicylate production from the cellular metabolite chorismate has already been shown in *E. coli* [29]. (Salicylate is the anion of salicylic acid; at intracellular pH, salicylic acid is primarily in its anion form.) Thus, we could construct biosynthetic device(s) that catalyze the conversion of chorismate to salicylate. By combining the salicylate generator with the wintergreen odorant generator, we could construct a complete wintergreen odorant biosynthetic system based on methyl salicylate production from cellular metabolites (SI Fig. 9A). In the case of banana odor, although *E. coli* isoamyl alcohol production has

not been previously reported to our knowledge, a yeast biosynthetic pathway for isoamyl alcohol production from leucine is known [30, 31, 32, 33]. Thus, we might similarly engineer biosynthetic device(s) that catalyze the conversion of leucine to isoamyl alcohol and again combine the device(s) with the banana odorant generator to make a complete banana odorant biosynthetic system (SI Fig. 9B). The challenge in functional composition of biosynthetic devices is therefore the classical metabolic engineering challenge of matching or maximizing flux through the set of devices [1]. It is interesting to speculate whether it would be useful or even possible to define a common signal carrier, such as flux, for biosynthetic devices. Could we then set standards for minimum and maximum fluxes through biosynthetic devices? Similarly, could biological engineers construct libraries of catabolic devices that degrade any number of feedstocks to a small set of core metabolites as well as libraries of anabolic devices that can convert those core metabolites to any number of useful chemicals? Although such steps pose great technical challenges, they also hold promise for further reducing the work needed to develop engineered biosynthetic systems.

Synthetic biology approaches are clearly not now sufficiently advanced to replace classical metabolic engineering techniques. Nevertheless, fundamental engineering principles, such as abstraction and standardization, can already enable rapid prototyping of many-component, biosynthetic systems. One benchmark for the power of synthetic biology approaches is the annual international Genetically Engineered Machines (iGEM) competition. In iGEM, teams of undergraduate students engineer synthetic biological systems of their own design. In fact, we reprogrammed bacterial odor as a part of the 2006 competition. The iGEM students are largely novice biological engineers, many of whom have little or no prior biological research experience. Using the BioBrick physical composition standard, the teams reuse standard genetic parts from the Registry and design any new parts needed for their engineered biological system. As new technical standards are developed, iGEM provides a convenient framework to further examine whether these standards make the process of engineering bi-

ology easier. We propose that synthetic biology approaches, when combined with classical metabolic engineering techniques, have the potential to dramatically reduce the resources needed to engineer biosynthetic systems. In particular, synthetic biology may expand access to metabolic engineering from a small number of expert labs and companies to a broader base of novice biological engineers.

4 Materials and Methods

4.1 Part, device, and system construction

All genetic parts used in this work were designed and assembled according to the BioBrick assembly standard unless otherwise specified (T Knight, 2003, <http://hdl.handle.net/1721.1/21168>) [34]. See *SI Text* for details.

4.2 Analysis of odorant production

See *SI Text* for details. Briefly, cultures of cells containing appropriate devices supplemented with the appropriate precursor were extracted with heptane and analyzed by gas chromatography. For growth phase-dependent devices, cultures were sampled periodically during growth, the culture optical density at 600nm measured, and then similarly analyzed for odorant production.

4.3 Testing function of transcriptional control devices

See *SI Text* for details. Briefly, each of the transcriptional control devices were assembled with a GFP reporter. The change in fluorescence over time of cultures containing each device plus reporter were analyzed using a multi-well fluorimeter.

5 Abbreviations

PoPS, RNA polymerases per second; BSMT1, *S*-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase I from *Petunia × hybrida*; ATF1 - alcohol acetyltransferase I from *Saccharomyces cerevisiae*

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Figure 1: The abstraction hierarchy used in Eau d'E coli. (A) Systems are defined by their overarching behavior, such as reprogramming bacterial odor. (B) A biosynthetic device, such as a wintergreen or banana odorant generator, takes one or more chemical inputs and produces one or more chemical outputs. Biosynthetic devices are made up of two transcriptional devices: a transcription source and a biosynthetic enzyme generator. A transcription source is a device that produces a transcriptional output signal. A biosynthetic enzyme generator takes as input a transcriptional signal and produces as output an enzyme, such as BSMT1 or ATF1, that catalyzes the conversion of a precursor to a product. (C) Biological parts are nucleic acid sequences that encode basic biological functions. A transcription source can be made up of a single part such as a promoter. An odorant enzyme generator is made up of three parts: a ribosome binding site (RBS), an enzyme coding sequence, and a transcriptional terminator (stop). (D) In synthetic biology, the lowest layer of the abstraction hierarchy is nucleic acid sequence.

Figure 2: During a blind smell test at the 2006 iGEM Jamboree, participants smelled cultures containing the wintergreen odorant generator, the banana odorant generator, and *E. coli* strain TOP10. Participants were asked to characterize each culture as smelling like wintergreen (green bars), banana (yellow bars), or the natural fecal odor of *E. coli* (brown bars). Based on the survey results, people can smell the odorant from both odorant generators (Pearson's chi-square test yields $p < 0.01$). For the smell test, the odor-free chassis (*E. coli* strain YYC912) was used for the wintergreen and banana odorant generators.

Figure 3: To test and verify function of the constitutive, stationary phase and exponential phase transcriptional control devices, each control device was assembled with the GFP generator (BBa_E0840), and the fluorescence of *E. coli* cultures with each device was monitored over time. A plot of the change in fluorescence per unit time (normalized GFP synthesis rate) versus the cell density (OD600nm) for each device is shown. The constitutive transcriptional control device produced a high GFP synthesis rate irrespective of cell density. The stationary phase transcriptional control device produced a low initial GFP synthesis rate which increased with culture cell density. The exponential phase transcriptional control device produced an initially high GFP synthesis rate which decreased as cell density increased. Data shown are averages of triplicate measurements of cultures grown from three individual colonies of each device. Error bars are the 95% confidence interval of the mean of the three independent cultures.

Figure 4: To demonstrate growth phase-dependent banana odorant production, isoamyl acetate concentrations of cultures of the constitutive and stationary phase banana odorant generators were measured at different cell densities (OD_{600nm}). The stationary phase banana odorant generator produced very little isoamyl acetate at low cell densities but its isoamyl acetate production increased with cell density. By comparison, the constitutive banana odorant generator produced more isoamyl acetate at lower cell densities than the stationary phase banana odorant generator. We conducted three independent experiments (days 1-3). To aid visual comparison of the two odorant generators, an empirical fit to the data for each device is shown.

Table 1: Odorant generators produce appropriate chemicals

<i>Description</i>	<i>Precursor</i>	<i>Retention time</i>	<i>Peak abundance</i>
Wintergreen odorant generator (BBa_J45120)	+	11.471	1.0×10^8
Methyl salicylate standard	–	11.473	3.3×10^4
<i>E. coli</i> strain TOP10	n/a	11.461	2.1×10^8
Banana odorant generator (BBa_J45200)	+	11.347	2.2×10^4
Isoamyl acetate standard	–	4.456	4.7×10^8
<i>E. coli</i> strain TOP10	n/a	4.400	3.6×10^6
	+	4.388	9.6×10^7
	+	4.432	8.0×10^5