Hi! I am Miss Blue Vanilla, CEO of the Fragrance Factory; pleased to meet you!
I belong to the ‘Essencia coli’, a class of fragrance producing bacteria. My special asset is vanillin, the main component of vanilla odour. To keep the concentration of this vanillin at a constant level, I’m equipped with a feedback control system. My vanillin synthesis is initiated by irradiation with blue light. The preferred concentration of vanillin can be modulated using the intensity of blue light. At the same time I measure the amount of vanillin outside the cell by means of a vanillin receptor and control its production to maintain the set point. My design is universal in nature and has therefore potential benefits in different areas. Other flavour and odour producing bacteria for example can easily adopt this mechanism. In fact, any application that requires a constant concentration of a molecular substance is possible.

Please take a tour of my factory below!

**Essencia coli**
The Fragrance Factory

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### Vanillin Production

**What & Why:** Vanillin production is controlled by the key/antikey balance such that the vanillin concentration reaches the desired level set by the blue light intensity.

**In the lab:** We constructed two devices. The first converts tyrosine into ferulic acid via Sam8, Sam5 and ComT. The other one produces vanillin from ferulic acid via Fcs and Ech (A, 4, 5, 6).

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### Blue Light Receptor

**What & Why:** The desired concentration of vanillin is set by blue light. The Blue Light Receptor controls the amount of key, which controls vanillin production.

- The Blue Light Receptor is a blue light responsive promoter.
- Blue light responsiveness is mediated by two proteins: YcgE and YcgF.
- YcgE binds to the promoter with its MerR-like domain and blocks transcription of the downstream gene.
- Since this domain is also needed for DNA binding, the promoter repression is relieved and the key is transcribed.

**In the lab:** We characterized the Blue Light Receptor, see post-it.

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### (Anti)Key/Lock control

**What & Why:** The (anti)key/lock system is the core of our feedback control system. It receives signals from the Blue Light Receptor and the Vanillin Receptor. The feedback system regulates the vanillin production at the post-transcriptional level.

- In the absence of blue light, no key is produced and translation of the enzymes, necessary for vanillin synthesis, is blocked by the lock.
- After blue light activates the production of the key, the key opens the lock and thereby initiates vanillin production.
- When the vanillin receptor senses vanillin outside the cell, the antitype is transcribed.
- The antitype will basepair with the key, thereby forming dsRNA, and thus inhibiting the key’s functioning.
- A higher production of key leads to a higher production of vanillin.
- More detection of vanillin leads to a higher antitype production.
- More antitype can bind more key and in this way lower the vanillin production.

**In the lab:** We contributed the lock and key BioBricks.

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### Vanillin receptor

**What & Why:** To ensure that the vanillin concentration is constant, Essencia coli has to produce less vanillin when there is an additional source of vanillin (e.g. in a perfume store), or more when the vanillin is quickly removed (e.g. open window). The vanillin receptor signals to the (anti)key/lock control system.

- The vanillin receptor is based on a two component signal transduction system of Agrobacterium tumefaciens.
- VirA senses the vanillin concentration.
- Upon binding of vanillin, VirA autophosphorylates and in turn activates VirG by phosphorylation.
- The activated VirG interacts with the Vir-box of the virR promoter region, leading to transcription of the antitype.
- The alpha subunit of RNA polymerase (RpoA) is necessary for making the VirA/VirG system work in E. coli.

**In the lab:** We contributed VirG, VirA and the virR promoter region BioBricks.

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### How can we improve our design?

**What & Why:** We translated Essencia coli into ordinary differential equations (Simbiology) to check whether the system is performing as intended and to optimize the control system.

The system controller is represented in a block diagram, whereby each block represents an essential part of the system. This allows us to perform a theoretical analysis of the control system.

**Dry lab conclusions:**
- The most important conclusion is that a steady state error occurs, i.e. the vanillin concentration will never reach exactly the wanted level.
- We can minimize this error by increasing the sensitivity of the feedback system through changing the number of vanillin receptors. After a disturbance in the vanillin concentration, the system with the highest number of vanillin receptors recovers the best.

**References:**