Detecting High Levels of Radiation with the fwYellow Pigment in Engineered Spiderwort

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Toxic levels of radiation cause many health issues. Individuals exposed to high concentrations of radon, a natural, radioactive gas found in dirt, for five to twenty-five years are at significant risk for developing lung cancer. Symptoms associated with two to three years of exposure to high radon levels include difficulty breathing, persistent coughing, and chest pains. The risk of childhood cancer increases with those living near nuclear power plants emitting high radiation. This project involves genetically altering the houseplant *Tradescantia pallida*, commonly known as spiderwort, to change color when exposed to high concentrations of radiation—effectively alerting those around to evacuate and treat the high concentration of radiation appropriately. In 1992, scientists proved that radiation damages *T. pallida*'s DNA. The proposed design adds a specific operator sequence operator site called a Cheo box to the promoter region of the *recA* promoter. DNA damage caused by radiation activates the *recA* promoter. Adding an extra Cheo box in the promoter region of *recA* will increase the responsiveness to radiation. We will insert this altered promoter into plasmid pCGN1547 which contains a fwYellow pigment gene. This pigment will be produced under the control of the *recA* promoter, causing the plant to appear yellow when presented with radiation-induced DNA damage and thus warning the houseplant owner of high radiation levels through a visible signal.

Keywords: Radiation, detection, *Tradescantia pallida*, plants

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Watch a video introduction by the authors at https://youtu.be/xelSY3MKd-M

Background

Radiation causes many health issues (CDC, 2021). An average United States (US) resident is expected to be exposed to no more than 3 millisieverts (mSv), a measure of absorption and dose, of radiation each year (Boyles, 2009). Toxic levels of radiation, 20 mSv or higher, increase the risk of cancer (Nguyen & Wu, 2011). Two to three years of exposure to high concentrations of radon (4 picocuries per liter (pCi/L), a measure of radioactivity) can cause difficulty breathing, persistent coughing, and chest pains (EPA, 2018; Simon, 2019). People may develop lung cancer when exposed to high amounts of radon for five to twenty-five years. Radon is one example of harmful radiation. A 1980-2003 case study revealed an increased risk of childhood cancer in those living near nuclear power plants (Spix *et al.*, 2008). People may develop Acute Radiation Syndrome (ARS), a condition that occurs when the high radiation dose penetrates internal organs, after exposure to high concentrations of radiation (CDC, 2018). ARS causes nausea, vomiting, headaches, diarrhea, and seizures. Exposure to dangerous levels of radiation is common and often undetected. Approximately 21,000 people die from lung cancer caused by radon each year (EPA, 2022). However, awareness of radiation and the need for testing is not well known. While tests for radiation concentration are available, many people do not think about purchasing and using them. While at-home radiation tests can precisely detect radiation exposure, these tests tend to be expensive, and access is limited (Mirion, 2022). This genetically modified houseplant will allow constant, sustainable, and visible radiation detection.

This project genetically alters the houseplant *Tradescantia pallida*, commonly known as spiderwort, to express yellow pigment in its leaves when exposed to radiation. Therefore, effectively alerting people of the presence of radiation in their environment. The goal of this project is to provide a simplified way of testing radiation with accessible houseplants by using synthetic biology to modify the plants' genes. This plant will alert people to harmful radiation in doctor's offices, homes, and other buildings, encouraging them to take the proper health precautions.

Plants from the genus *Tradescantia* are common houseplants and botanic testers for ionic radiation and mutagenicity (Hicks-Hamblin, 2021; Ichikawa, 1992). Ichikawa's study shows that the stamen hair cells, organelles found in spiderwort leaves, can detect radiation in small quantities (Ichikawa, 1981). An experiment from 2002 found that 0.2 Gy (the equivalent of 200 mSv) of radon radiation per day for twelve weeks causes mild single-stranded DNA breaks in *Tradescantia* plants (Tavera *et al.*, 2002). This behavior demonstrates this plant genus will react to radiation exposure: the crux of this project. *T. pallida* will be used because it is a successful biosensor: its cells mutationally change immediately and significantly after radiation exposure (*Serra*, 2011).

To deliver the plasmid to the cells of the plant, we will use a gene gun. We will load the plasmid, attached to microscopic gold particles, into the gene gun to transfect the plant cells. The gun releases a shock wave that projects the plasmid into the plant tissue. We chose the plasmid backbone pCGN1547 because it can integrate into the DNA of plants and *E. coli* (Lee & Gelvin, 2008). The plasmid will contain the *recA* promoter with an additional Cheo box, the fwYellow pigment gene, the iGEM part B0030 ribosomal binding site, and the tNOS terminator; these components will be incorporated into the plant's nuclear DNA. The proposed design adds a specific operator sequence operator site called a Cheo box to the promoter region of the *recA* promoter. Radiation causes single-strand breaks in DNA which activate the *recA* promoter (Wang *et al.*, 2019) (*Figure 1)*. The Cheo box is a specific operator sequence (SOS) found in the promoter region of *recA*. An experiment proved that inserting an additional Cheo box in the promoter region of *recA* will increase the responsiveness to radiation (Nuyts *et al.*, 2020). This design heightens the responsiveness of the *recA* promoter to radiation with the addition of a Cheo box.

The *recA* promoter sequence (with an additional Cheo box, increasing reactiveness to radiation) inserted into *T. pallida* will respond to the DNA damage caused by radiation, inducing the expression of the yellow pigment fwYellow. The yellow color will provide a vivid contrast to the purple leaves of the plant. The B0030 ribosome binding site that precedes the pigment is strong, meaning that the device will accurately produce high amounts of mRNA and protein production (parts.igem.org, 2022). Once triggered, this pigment will cause the plant leaves to turn yellow, indicating that the plant's surroundings contain unhealthy amounts of radiation (*Figure 2)*. This device enables individuals to visualize high radiation levels, alerting them to treat the unhealthy concentration before adverse health effects set in. This plant provides continuous monitoring for radiation and is easy to propagate (Irisandco.com, 2022), meaning that it has a simple reproduction process (Andrychowicz, 2022). Therefore, this plant can be cost-effectively generated in a sustainable manner. Not only is the plant a nice addition to homes and offices, but it is also an effective method for keeping people safe in their environments.

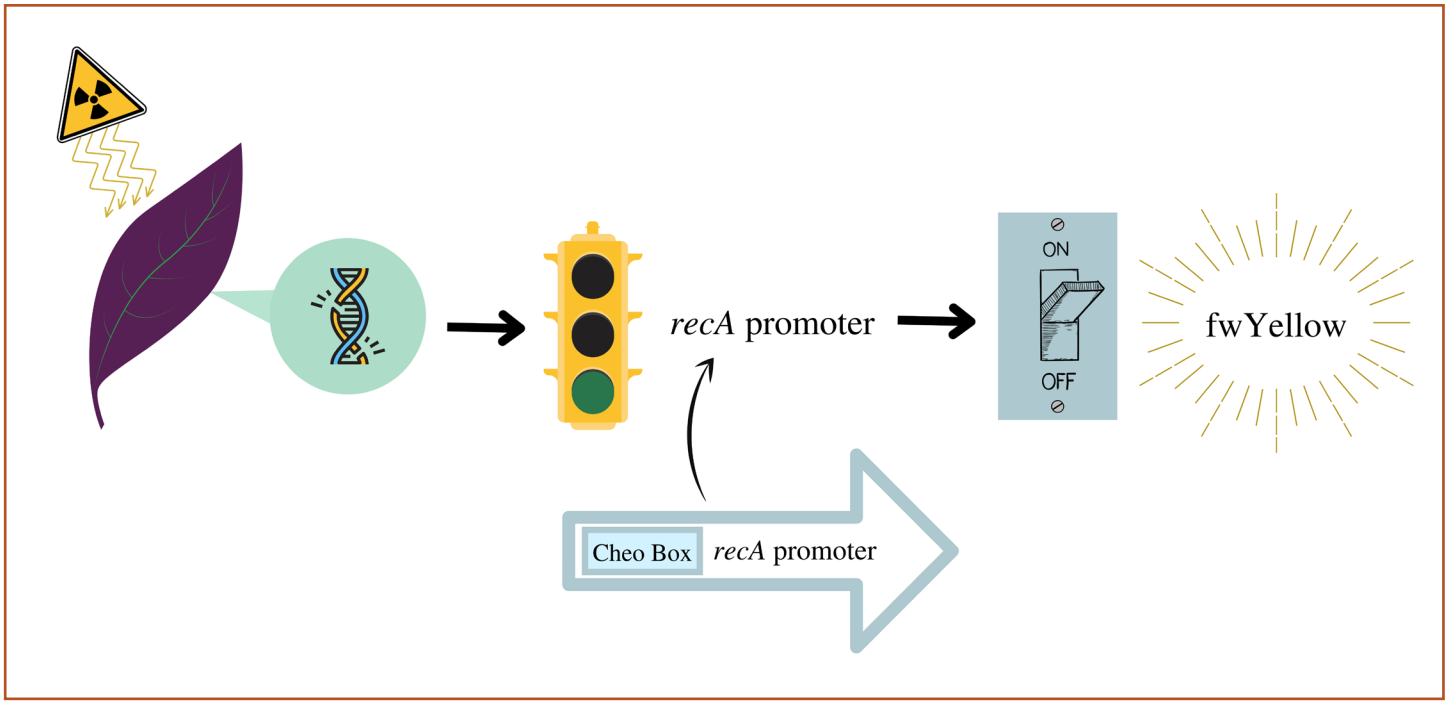

Figure 1. *As the genetically modified houseplant is exposed to radiation, the plant will receive DNA damage, resulting in single-strand breaks of the DNA. The recA promoter will respond to these single-strand breaks, effectively signaling the recA promoter to begin transcription of the fwYellow pigment.*

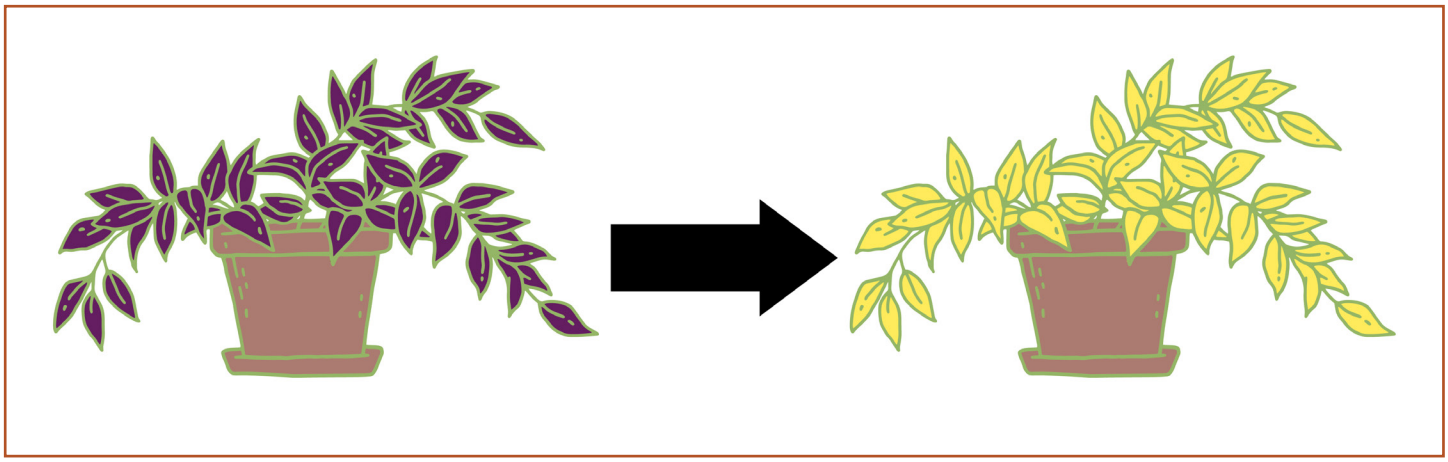

Figure 2. *After implementing the design, the modified houseplant will turn yellow when exposed to radiation.*

Systems Level

We will implement the device in *E. coli* in order to test the production of the fwYellow pigment. The *recA* promoter has a well-characterized response to radiation within *E. coli* constructs (Nuyts *et al.*, 2020). Once we have successfully tested this construct in the bacteria, we will insert it into a plant, *T. pallida*. We chose the plant *T. pallida* because it exhibits a significant quantity of cell alterations immediately after radiation exposure (Ibrahim *et al.*, 2012). *T. pallida*, genetically altered to contain the fwYellow pigment under the control of the promoter that responds to radiation, will react to high levels of radiation that cause single-strand breaks in the plant's DNA. The DNA breaks trigger the *recA* promoter (with an added Cheo box that will increase reactiveness to radiation) to produce the fwYellow pigment throughout the plant (Nuyts *et al.*, 2020). We plan to insert the plasmid with a Bio-Rad Helios gene gun. The plasmid will be attached to gold particles and loaded into the gene gun. The gene gun will release a shockwave that will project the gold particles through the plant tissue,

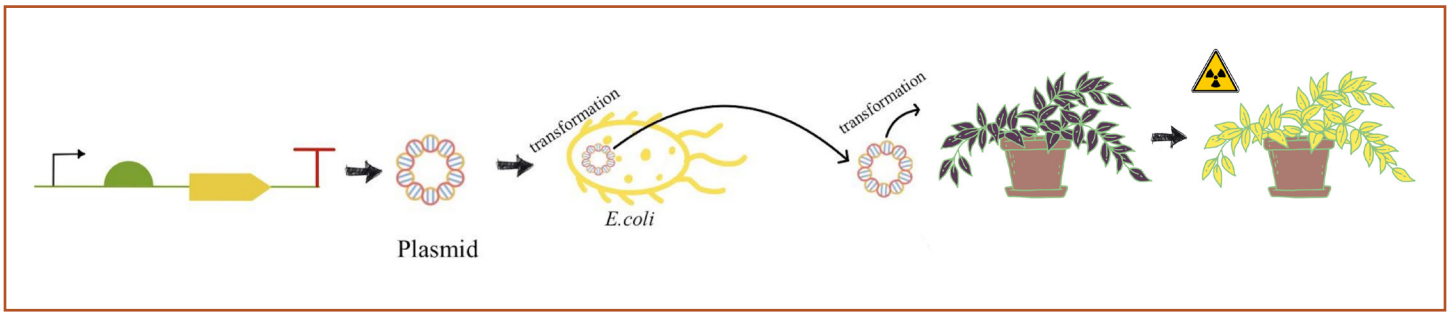

Figure 3. *We will insert the selected components into the plasmid backbone pCGN1547 before transforming the plasmid into E. coli to test the pigment's visibility. Once successfully tested, we will transform the plasmid into Tradescantia pallida so that it will turn yellow during radon exposure.*

embedding the plasmid into the plant (Bio-Rad, Helios® Gene Gun System) (*Figure 3)*. This system serves as a constant, sustainable, and visible detection mechanism for alerting people to harmful radiation in offices, homes, and other buildings.

Device Level

The chosen plasmid backbone, pCGN1547, will contain a *recA* promoter with an added Cheo box, the iGEM part B0030 ribosomal binding site, an fwYellow pigment gene, and a tNOS terminator. The plasmid contains a ColE1 origin for fast replication in *E. coli* and a pRi origin for replication within plants (Lee & Gelvin, 2008). The selectable markers within the plasmid are gentamicin resistance for *E. coli* and kanamycin resistance for plant cells. The pCGN1547 plasmid effectively replicates in plants and *E. coli*. The function of the system is for the *Tradescantia pallida* to turn yellow with the fwYellow pigment gene after radiation exposure.

Parts Level

The design of this project uses the *recA* promoter with an additional Cheo box to detect radiation. The *recA* promoter responds to single-strand breaks in DNA, the type of breaks caused by radiation exposure (Wang *et al.*, 2019). Studies show that an additional Cheo box in the *recA* promoter increases responsiveness to radiation, indicating that this altered promoter will respond to radiation exposure (Nuyts *et al.*, 2020). This promoter will be more sensitive in detecting radiation due to the Cheo box, allowing the promoter to activate fwYellow chromoprotein production in the presence of radiation.

The fwYellow chromoprotein gene does not include the sequence for a ribosome binding site (RBS), a sequence necessary for transcription initiation. We will insert an RBS, iGEM part B0030, into the DNA sequence upstream of the fwYellow gene sequence. The B0030 RBS is strong, ensuring that the device accurately produces high amounts of mRNA and proteins (parts.igem.org, 2022).

The fwYellow gene encodes a chromoprotein, a color visible to the naked eye on the plant's purple leaves, allowing people to see that radiation is present.

The tNOS terminator concludes the sequence, stopping the production of fwYellow. tNOS is compatible with plants, making it an excellent choice for a successful terminator (Holden *et al.*, 2009) (*Figure 4)*.

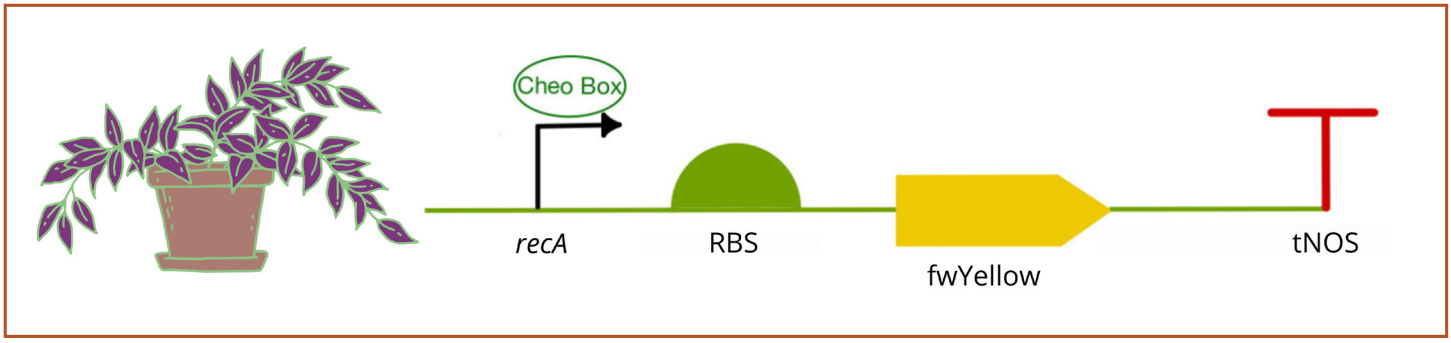

Figure 4. *The plasmid will consist of the recA promoter with an additional Cheo box, BBa_B0030 ribosome binding site, fwYellow gene, and tNOS terminator.*

Safety

In this experiment, we will insert a plasmid containing the *recA* promoter with an additional Cheo box and a gene for fwYellow pigment production into the plant *T. pallida* to serve as a visible form of detection for high concentrations of radiation. These plants can contain mildly toxic sap within the stem that causes minor discomfort when ingested (Haydn, 2008). The sap may cause minor skin irritation but is not deadly. While working with *Tradescantia*, gloves will be worn to reduce skin contact. In households and offices, the plant should be kept in areas that are inaccessible to small children and pets. The toxicity does not pose a significant hazard, as the plant's stem must break to expose the sap.

While using a gene gun to insert the plasmid, individuals should avoid immersing the gun in liquid or autoclaving it. A gene gun can be cleaned with a cloth and mild soap. Although the Helios Gene gun has a trigger button that is time-activated by a safety interlock switch, it is still possible to have accidental or unintended discharge. Do not point the gene gun at people. If you are exposed to a gene gun emission, contact your supervisor immediately.

This experiment contains a testing procedure that requires radiation at harmful levels. It is crucial to safely regard and contain those procedures in a laboratory setting. While in a radioactive laboratory, one should avoid working with any unprotected cuts or breaks in the skin—particularly in the hands or the forearms (CDC, 2021). Individuals should plan to minimize time spent handling radioactivity and distance themselves from the source of radiation. Radioactive materials should be contained in a defined work area. Testing of the product will begin with alpha radiation. Alpha particles do not penetrate skin and can be blocked by paper. This process will take place in a ventilation hood, ensuring the prevention of the inhalation of these particles. Scientists will use appropriate lab coats, goggles, and gloves, therefore minimizing any safety risks.

Discussions

If testing the device in *E. coli* and *T. pallida* to produce fwYellow pigment yields the desired results, the houseplants could be sold on the market as radiation detection products. The project initially looked at houseplants detecting radon in dwelling places. The team ultimately decided to expand its focus to different types of radiation because radon is difficult to target specifically.

We will need to consult experiments on the production of the fwYellow pigment in the system in bacteria. We will then insert the pigment's gene, through the plasmid, into *T. pallida*. The plant will contain the fwYellow pigment under the control of the *recA* promoter that responds to radiation (Wang *et al.*, 2019). High levels of radiation cause single-strand break in the plant's DNA which will trigger the *recA* promoter containing an added Cheo box for increased reactiveness to radiation (Nuyts *et al.*, 2020). This reaction will prompt the production of the fwYellow pigment within the plant. The project will use a gene gun to insert the plasmid into *T. pallida*.

This houseplant serves as an easy means for radiation detection. It is cheap, aesthetically appealing, and provides a constant, sustainable, and visible detection mechanism for alerting people to harmful radiation in offices, homes, and other buildings. Exploring the genetic modification of houseplants for identifying high radiation levels could help future scientists continue the development of similar processes for detecting other harmful materials.

Next Steps

This team plans on using a gene gun to insert the device into *T. pallida.* Once we transform the plant, we plan on growing the plant in a pot containing kanamycin, the biosensor inserted to see if the transformation is successful. The plasmid was successfully inserted into the plant if the plant grows in the presence of this antibiotic. After determining if the plant contains the device, we will grow the genetically modified *T. pallida* in the same pot as an unaltered *T. pallida.* By growing both plants in the same conditions, we can test how the design functions and how the altered plant differs from the unaltered plants when exposed to radiation. This experiment will determine the levels of radiation exposure and the time necessary for the yellow pigment to become visible. In addition to determining the color difference of the altered and unaltered plants when exposed to radiation, the team will conduct an experiment to determine the color differences of *T. pallida* when the plant is overwatered, underwatered, or exposed to radiation. By growing the altered and unaltered plant in the same conditions, our group will discover the color differences that correlate to certain growing conditions. Similarly, we will place the altered plant in an area frequently exposed to radiation and another altered plant in an area with limited radiation exposure, testing the sensitivity of the system. In addition to experiments regarding the visibility of the pigment, we will also need to test how long the yellow pigment remains visible in the plant after radiation exposure. If the altered plant does not produce the fwYellow pigment in the presence of radiation, we will replace the *recA* promoter with a promoter naturally

found in plants that respond to radiation, such as the P1 promoter (Rius *et al.*, 2012).

Author Contributions

E.B. created the initial idea and began the early research process. All authors contributed to the background research and the writing and proofreading of the paper. D.Y. was the principal designer of the images and graphics for the project.

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