2011 Rihm iGEM Team

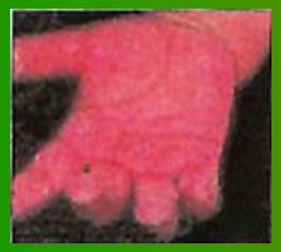
Chris Thompson, Lucas Ruff, Brent Poling, and Austin Frazier,

Here at Greenfield-Central High School, we have identified and isolated a gene in Saccharomyces cerevisiae, commonly known as yeast, which promotes the expression of genes when in the presence of cadmium and copper, a gene known as CUP-1. After extracting CUP-1, we assembled the promoter into a BioBrick format for submission to the iGEM database. We then continued to insert the gene into a high-copy plasmid, along with a gene coding for red fluorescent protein. Our plasmid was then ligased into a yeast strand. This allows us to detect the presence of cadmium or copper in an aqueous solution. Because cadmium presents significant health detriment when in drinking supply, we feel that this strain of yeast can provide for effective and easily available testing of these heavy metals in water.

1. Our Problem

Cadmium pollution is a huge problem in China, America, and many countries in Africa. This cadmium pollution is caused by many factors, such as industrial waste dumping by companies, landfills where batteries and computers leak into the environment, and also cadmium mining operations. May products such as certain lines of Miley Cyrus jewelry and McDonald's novelty glasses have been recalled due to high levels of cadmium contamination. Cadmium has a direct effect on the body, especially when its toxic fumes are inhaled. Some of the effects are chills, fever, pneumonia-like symptoms, and in extreme cases, cancer and renal failure. So our group thought that there needs to be a more efficient and cost-effective means of testing water sources and other aqueous solutions for cadmium contamination. Testing applications exist, but are in limited supply and very expensive.
So we researched for a way to effectively test for cadmium in an aqueous environment. We quickly came upon Cup1, a promoter that helps yeast sustain itself in environments that contain a high amount of heavy metals. We also learned about Red Fluorescent Protein (RFP), which produces a protein that fluoresces red. Using these two parts, we decided that we could make a plasmid that recognizes cadmium, and then fluoresces red when it is present in the environment.

A picture of a waterway in China that has been severely contaminated with cadmium.



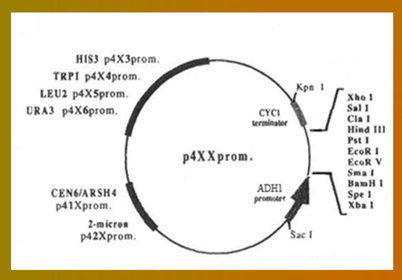
A picture of the effects of cadmium on skin.

2. Cup-1 and Red Fluorescent Protein

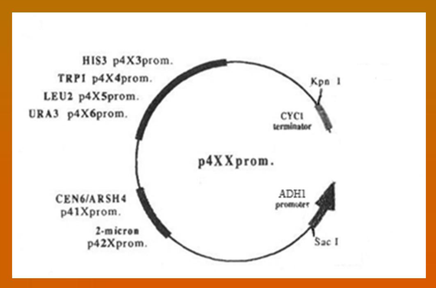
A promoter is a part of DNA that allows for the transcription of a particular gene. The promoter that we decided to use was Cup-1, a promoter that is already present in yeast. Cup-1 helps yeast to cope with different heavy metals, such as cadmium, in its environment. This is a result of the detoxification of the cadmium, to allow cells to have greater resistance against it. Cup1 detoxifies it by chelating the cadmium, effectively quarantining it from the rest of the cell. Since Cup-1 is already present within yeast, all we have to do is extract the DNA sequence from yeast DNA. In addition to Cup-1, we are also using red fluorescent protein This is a segment of DNA that produces a protein in yeast that fluoresces red when the promoter senses the presence of cadmium. Fluorescence is defined as light that is given off when exposed to radioactive energy at a higher energy level. Ultraviolet light causes the electrons in the substance to become excited and transition into a higher energy level. Upon returning to the lower energy level, the electron releases energy in the form of light, which in this case is red. The light emitted is based on the difference of where the electron was and where it returns to. In this case the emitted light is 700 nanometers long, which is in the red light range.

3. Plasmid

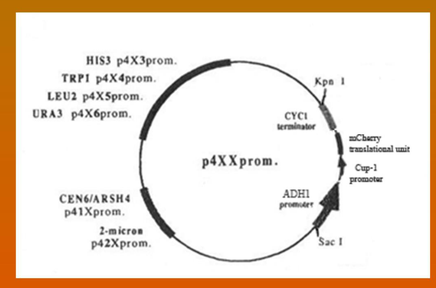
The plasmid we chose to use is the ADH p416 plasmid, with a uracil marker, created by Dominik Mumberg, Rolf Muller, and Martin Funk.



This is how plasmid p416 is arranged.



This is after we cut the plasmid with enzymes.



This is after we perform Gibson and insert our parts.

4. How To Use

Our product is designed for use at home and by major relief organizations such as the Red Cross.

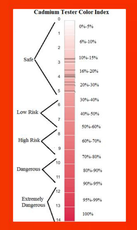
Here are the steps that a potential customer would follow to test their water:

1. Receive pieces of filter paper that have suspended yeast.

2. Re-suspend yeast in provided nutrient broth.

3. Pour growing yeast into the desired water sample.

4. Wait 48 hours for full effect, then compare to provided index to find the precise cadmium concentration.



Other Potential Uses:

- Urine Tester
- Food Tester
- Paint Tester
- ...And more!