# Oneonta iGEM 2024: pHish and CHIPs

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## Background and Inspiration

- The CHIPS and Science Act of 2022 has secured up to $6.1 billion dollars for investment in microprocessor manufacturing in NYS (1), the goal of which is to increase production capacity15. to meet ever increasing demand (2).
- Money from the act will be used to expand the production capacity of an existing fabricator (fab) in Malta, NY, and the construction of a new plant in Clay, NY (3). The anticipated increase will require increased resources, including workforce, energy requirements, and water demand.


**Figure 1:** US President Joe Biden, US Senate Majority Leader Chuck Schumer, and New York Governor Kathy Hochul, look at a 3D rendering of a future Micron factory in Syracuse, New York on October 27, 2022. Image credit: Mandel Ngan | AFP | Getty Images

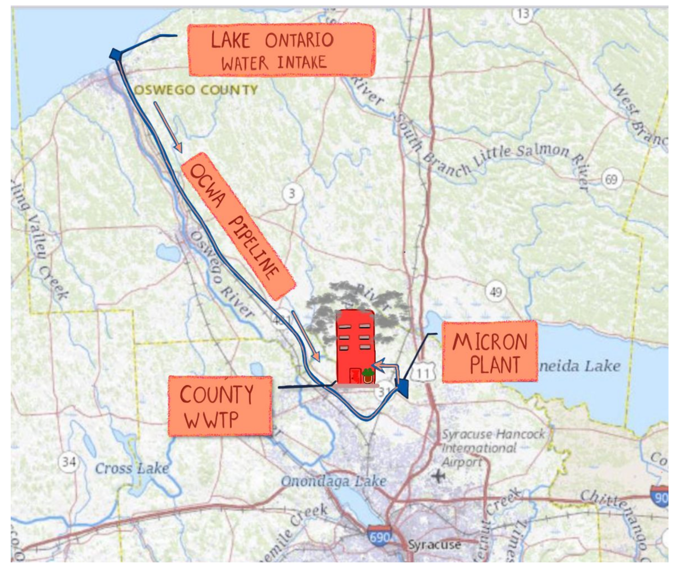
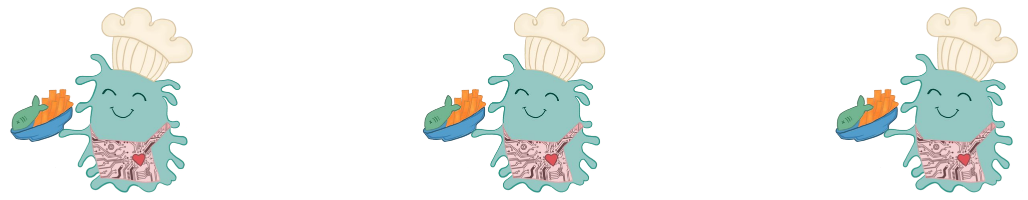



**Figure 2:** Map indicating the site of the new Micron fab, showing the water supply and wastewater effluent locations.

- Water is a critical resource for the manufacturing of microprocessors, being used as both a cooling agent and for cleaning during the complex fabrication process, which uses photo and chemical lithography (4)
- The new fab in Clay may require up to 48 million gallons of water per day, sources of which include Lake Ontario, the Onondaga County Water Authority, and recycling of wastewater (5, Figure 2).
- Prior to recycling or release, wastewater from fabs must undergo extensive purification to remove chemical and physical contaminants, as well as adjustment of pH, which varies at different points in the manufacturing process (6).
- In interviews with staff at NY CREATES (Albany NanoTech Complex), we learned that pH adjustment is technically challenging at the scale of a fab, requiring rapid detection and adjustment to neutralize wastewater at multiple points In the process.
- To neutralize acidic wastewater (low pH), base must be added; if the wastewater is too basic (high pH), acid must be added. Molecules that can help maintain a stable pH within a solution are called buffers.



## Concept

- The goal of pHish and CHIPS is to use synthetic biology to help make microprocessor manufacturing more sustainable.  Our strategy involves building a pH-sensitive wastewater system using synthetic biology. In the presence of acidic or alkaline conditions,  our genetic circuits will “turn on” and allow for the production and release of buffers to neutralize the water (Figure 3).

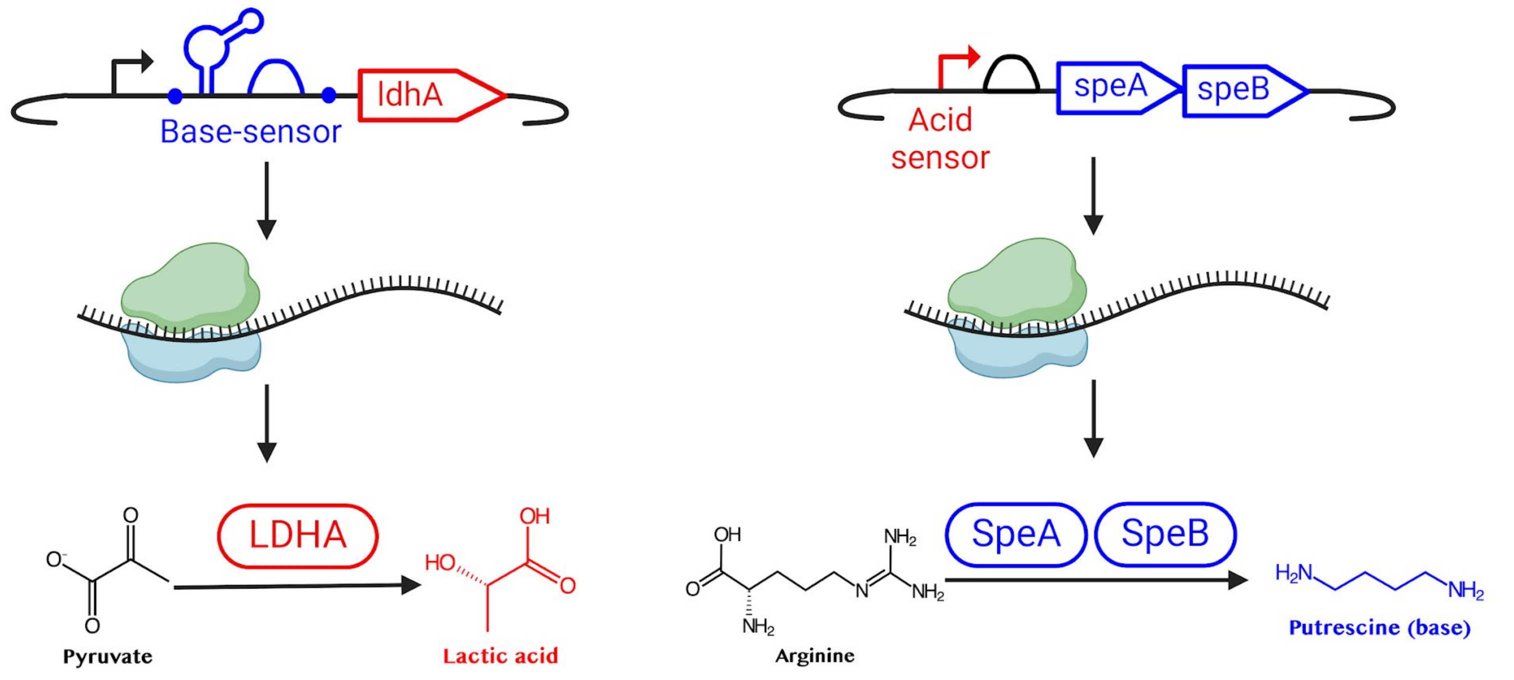



Figure 3: Schematic Design of the pH-sensing and adjusting genetic constructs and their function. Construction of genetic devices with a pH sensing component, can be used to drive the expression of acid producing (ldhA), or base producing (speA and speB) genes. These genes, when transcribed and translated, will catalyze chemical reactions that will produce acidic (Lactic acid), or base (Putricine)

## Conclusions

- We successfully optimized a base sensing riboswitch that is activated at pH values above 7.5.
- An acid sensor was successfully cloned and its pH sensing properties verified. The sensor is activated at pH values below 6.0.

## Future Work

- Complete construction of base sensing acid producing circuit replacing BFP with Lactate dehydrogenase gene.
- Complete construction of acid sensing base producing circuit replacing RFP with Putrescine.
- Perform a test of the acid- and base-producing functions of the new circuits.
- Create an implementation plan for how our design can be utilized in an industrial bioreactor located in-line with wastewater treatment at the microprocessor manufacturing facility.

### References

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## Results

- Circuits that sense acidic or basic pH and produce a fluorescent reporter gene were constructed via DNA synthesis and standard restriction enzyme assembly. Successful cloning was verified by colony PCR (Figure 4A) and DNA sequencing (not shown).
- Bacterial cultures containing a genetic circuit with a blue fluorescent protein (BFP) reporter gene regulated by an alkaline-sensitive riboswitch or a red fluorescent protein (RFP) regulated by an acid-sensitive promoter were grown in LB media buffered to specific pH values. Changes in reporter protein fluorescence were measured over a 6-hour period to assess how pH influenced gene expression.

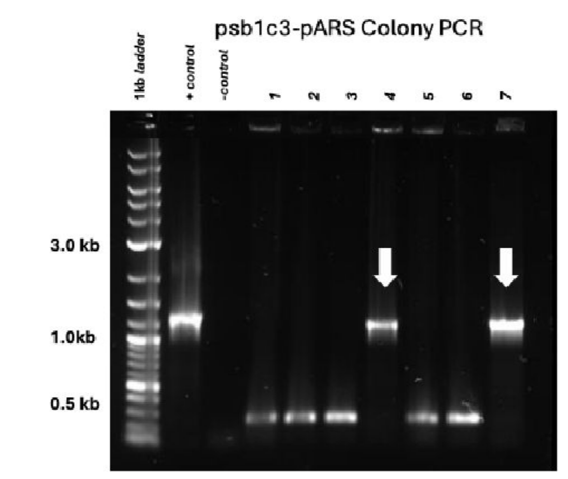



**Figure 4A:** Agarose gel of colony PCR reaction demonstrating successful cloning of the acid-sensing circuit. White arrow indicates insert of the desired size.

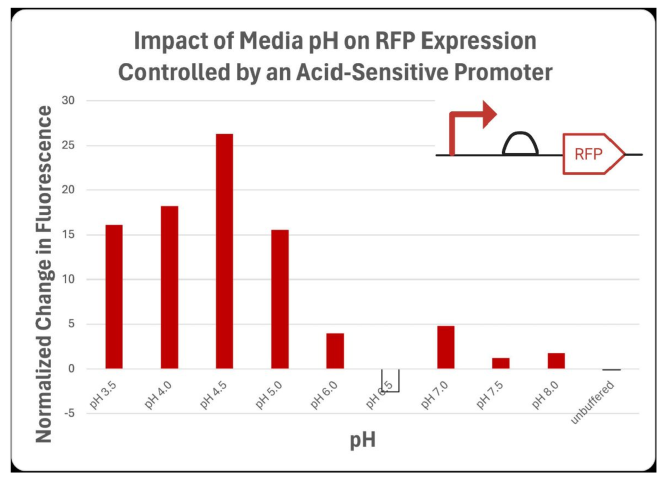



**Figure 4B:** Growth study demonstrating acid induction of a reporter protein. Normalized change in fluorescence is graphed based on the pH of the LB buffered media. The fluorescence peaked at pH 4.5 and at 7.5  the fluorescence was the lowest in buffered media.

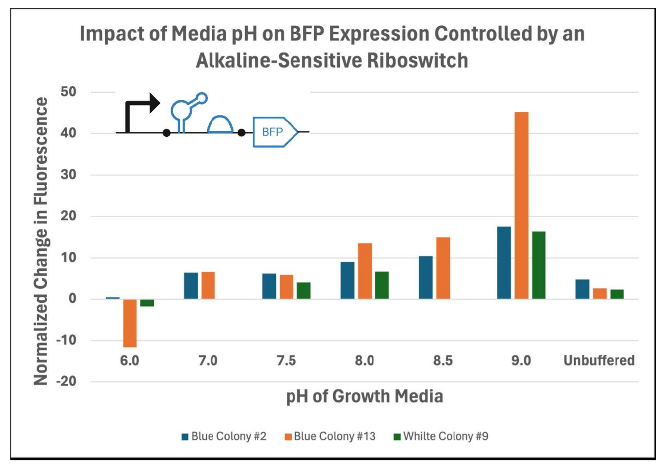
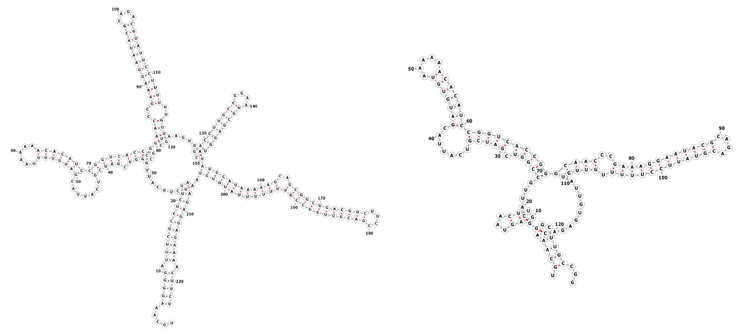



**Figure 5A:**  Impact of Media pH on BFP Expression Controlled by an Alkaline-Sensitive Riboswitch. Bars represent fluorescence at different pH values normalized by cell density.



PREmR34 PREm13

**Figure 5B:**  Diagram depicting the differences in secondary structure between two base-sensing riboswitches. The optimized base sensor is depicted on the right. Structures were predicted using the RNAFold server.

- Significant changes in RFP fluorescence were observed below pH 6, verifying acid-sensitivity of the circuit. (Figure 4B)
- The greatest BFP fluorescence was observed at pH 9.0, suggesting the riboswitch activates expression optimally under alkaline conditions. Gene expression was greatly reduced in acidic media. Of the colonies tested, Colony #13 (PREm13) was most responsive to basic pH. (Figure 5A)
- Sequencing of PREm13 identified 3 deletions in the riboswitch, which significantly altered the RNA structure.  (Figure 5B, sequences not shown)