Chronic Kidney Disease (CKD), which is characterized by alterations in kidney functions and structure, affects millions of people worldwide and a large portion of them is unaware of it. Absence of symptoms in early stages leads to a late diagnosis when patients need dialysis or even transplants. Currently, CKD is diagnosed by measuring creatinine levels in blood, which are only detectable at late stages of renal dysfunction and are also sensitive to factors such as diet, gender, ethnicity, age, muscle mass. We report the development of a biosensor that can diagnose CKD in its early stages, identifying a biomarker named Cystatin C. Using a cell surface biotinase as detector, a qPore-sensing system as transducer and a fluorescent response mechanism, we develop a genetic circuit that establishes a threshold, differentiating concentration ranges of Cystatin C. We envisioned it as a fast, simple and reliable tool for CKD screening and diagnosis.

Cystatin C (Cys C) is an inhibitor of cysteine proteases that has 120 residues and is produced by all nucleated cells. It is an excellent biomarker for renal dysfunction, specially for CKD, due to its constant rate in the blood and its independence of the variables (diet, gender, ethnicity, age, muscle mass, and others), varying with changes in the glomerular filtration rate (GFR).

More specifically, Cys C levels rise when GFR decreases and falls when the GFR increases. In order to work with a system capable of detecting an inhibitor like Cys C, we looked for an enzyme that has its activity inhibited specifically by the Cys C so we could measure the Cys C levels through alteration in the enzyme activity.

Our detection system will work on the Gram-positive bacteria B. subtilis. There are two reasons why we chose this chassis: 1) Our detection system needs the peptidoglycan wall of the bacteria to be properly attached; 2) The B. subtilis have the ability to form spores, which greatly improves its storage capabilities.

After construction of the circuit we will integrate it using plasmids pOR110 or pOR111, both with the amyE integration sequences.

### References


### Policy and Practices

Synthetic Biology is a emerging science in constant development. Our team prepared a survey to analyze the opinion of the academic community, which play an important role in the scientific knowledge dissemination. We have achieved 503 participants from different institutions and universities over the world!

The majority of people came from biological sciences, in agreement we can see that most of the participants have already heard at least once of Synthetic Biology!

Interestingly 73% answered that there were no conflict between SynBio and their religious values.

But this did not mean they neglect the creation of a code of conduct concerning biotechnological matter. 60% of the participants were in favor of this code of conduct (3). Much more than just a code, most of the people agree that controlling strategies are crucial to be implemented (4).

### Conclusions and Future Directions

The main achievement we had during the development of the "Kidney Sensing" was the confirmation that expression barriers can be artifically created.

After completion of the circuit construction, we plan to first insert it in the biobrick subtilis genome followed by an assay with known Cys C / Cat S, to validate the circuit with the GFP expression and IPTG gradient, then we can calibrate the threshold for proper kidney function diagnosis. Furthermore, we plan to test it in real blood samples, and finally use it in combination with the microfluidic device.

Regarding our Policy and Practices project, we came to the conclusion that the majority of the academic community is in favor of creating a regulation for the development of genetically modified organisms.