

# 2011 Warren Central iGEM Team

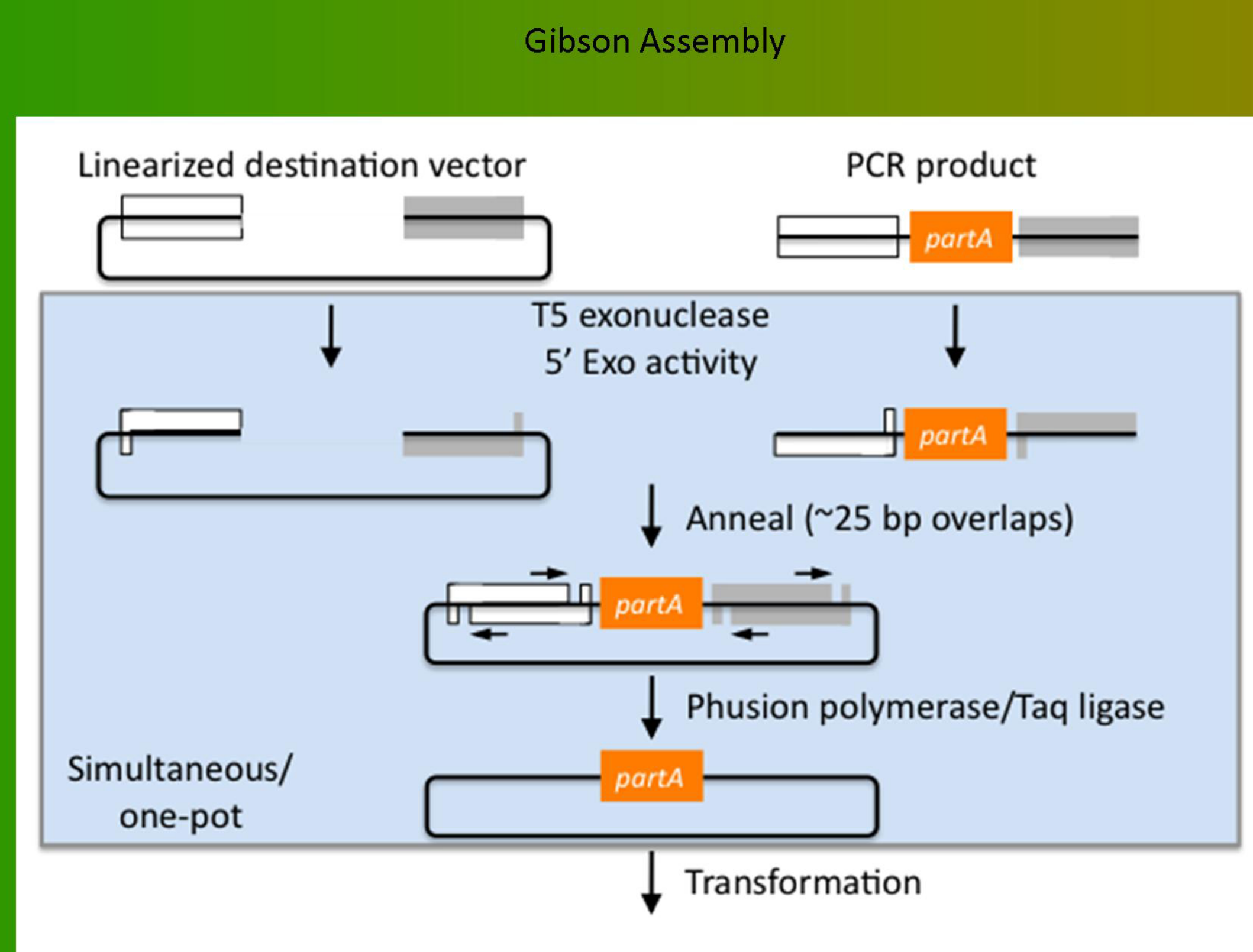


## 1. Our Problem

There are many metals that can be found in water that are dangerous to people or even the environment in high concentrations. With the insertion of plasmids our goal is to use yeast promoters that sense different metals and fluorescent protein genes to detect these metals in water so people can see if it needs to be treated. Our objective this year is to make a part that combines RFP with ADH1 using Gibson assembly. This will eliminate a step in plasmid modification.

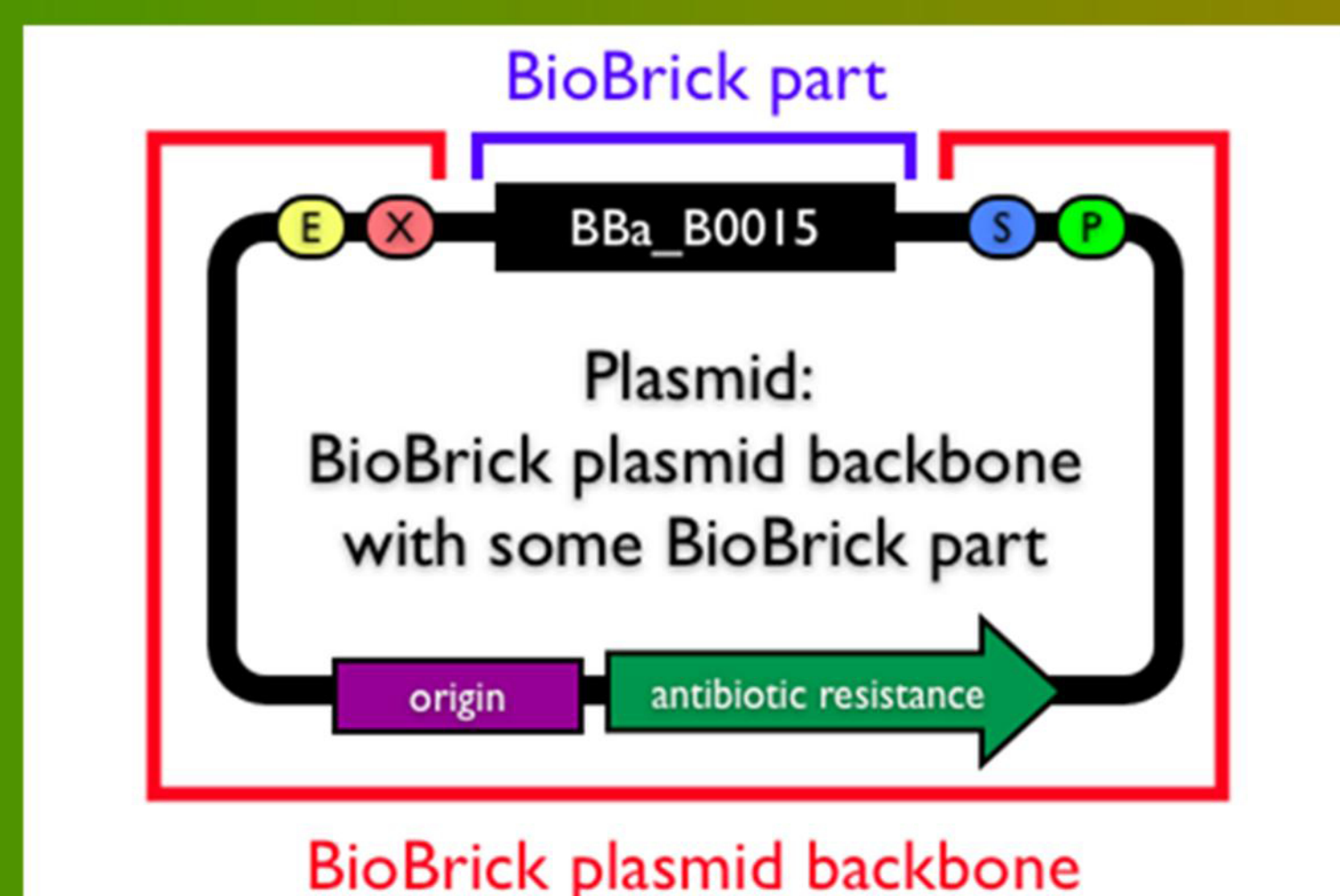
## 2. Why Yeast and Gibson Assembly?

Yeast, also known as *Saccharomyces cerevisiae*, has many of the different promoters necessary for the expression of the metal detection genes we hope to insert and can take in plasmids like bacteria while being safer to use than bacteria. Gibson assembly's use of exonuclease, phusion polymerase, and ligase make it much less likely to leave nicks and mistakes in the DNA than restriction enzymes. Gibson assembly is also a one step reaction that allows a higher efficiency than other methods.



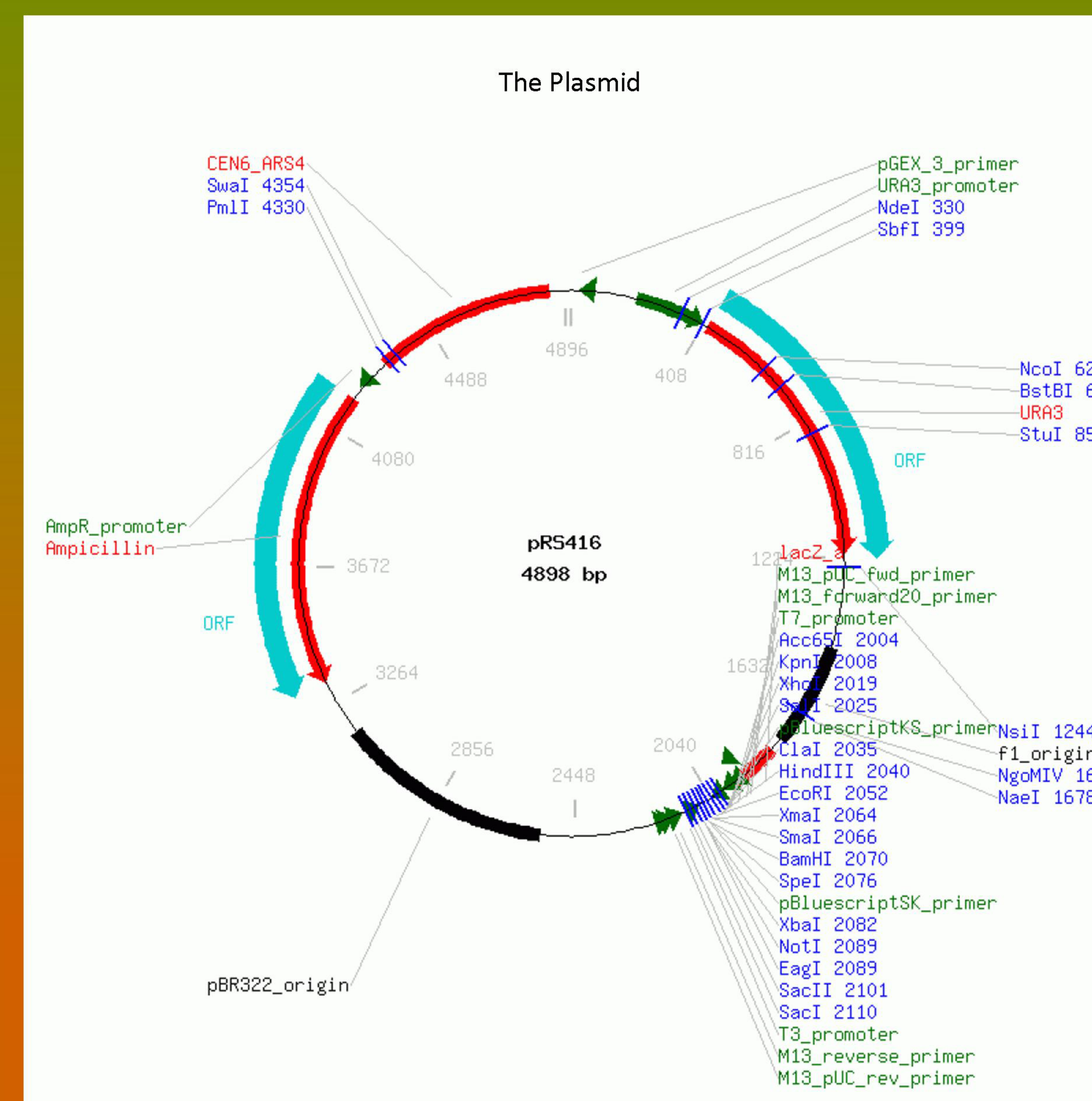
## 3. Primer Design

Our first forward primer will contain an overhang with base pairing complimentary to the plasmid to attach the biobrick to it, two restriction enzyme sites (EcoR1 and Xba1, for cutting the sequence out), the sequence for red fluorescent protein (RFP) and a forward primer for creating a new strand. The second forward primer will contain a primer to construct a new strand that will bridge the RFP and the ADH1 terminator; this sequence will be complimentary to the end of the RFP sequence and the beginning of the ADH1 terminator sequence. The reverse primer will contain another overhang to connect the biobrick to the plasmid, the other two restriction enzyme sites, the sequence for the ADH1 terminator, and a primer for constructing a new strand.



## 4. Process

First the RFP and the ADH1 terminator has to be extracted from E. Coli, purified, and amplified; a gel is run to insure it has been successfully extracted. Next the biobrick is constructed and put into the multi-cloning site of the plasmid using Gibson assembly. Then the yeast will be made to take in the plasmid through transformation. To make sure the yeast have the plasmid we use the Ura3 selection marker and different LB plates; if the yeast has taken in the plasmid then it can grow without uracil present on the plates because it will make its own.



## 5. Results

Our results were inconclusive; there was no bacterial growth on the plates until 4 days later. When we cut the multi-cloning site on the plasmid we only used the EcoR1 restriction enzyme but we designed our biobrick to be inserted with cuts using the Kpn1 and Sac1 enzymes. It's possible that after the EcoR1 made its cuts, the DNA recombined and the Amp resistance made its way into a small amount small amount of bacteria that took more time to grow. Also, our primer design was flawed. The forward primer that was responsible for ligating RFP to ADH1 was not made complementary to the upper 3 prime strand.