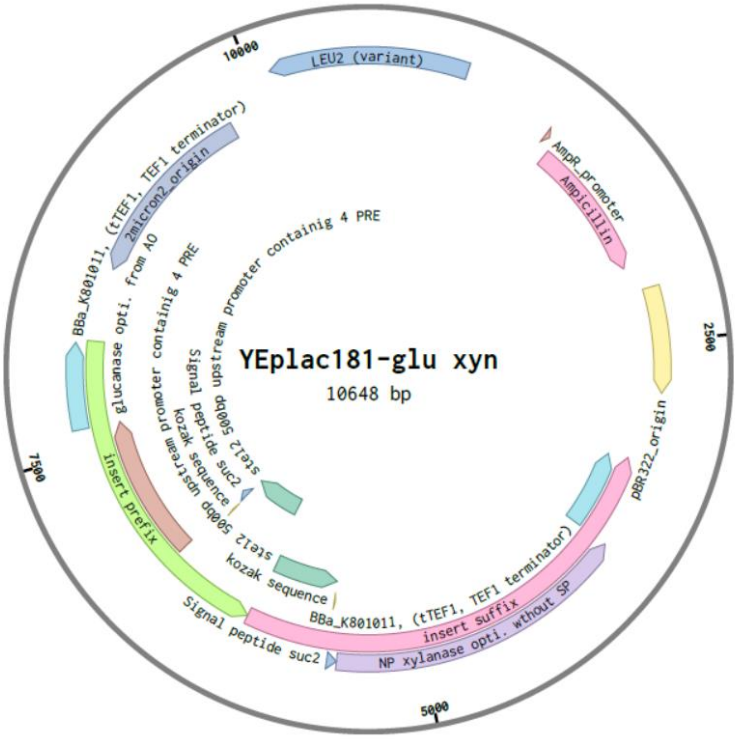
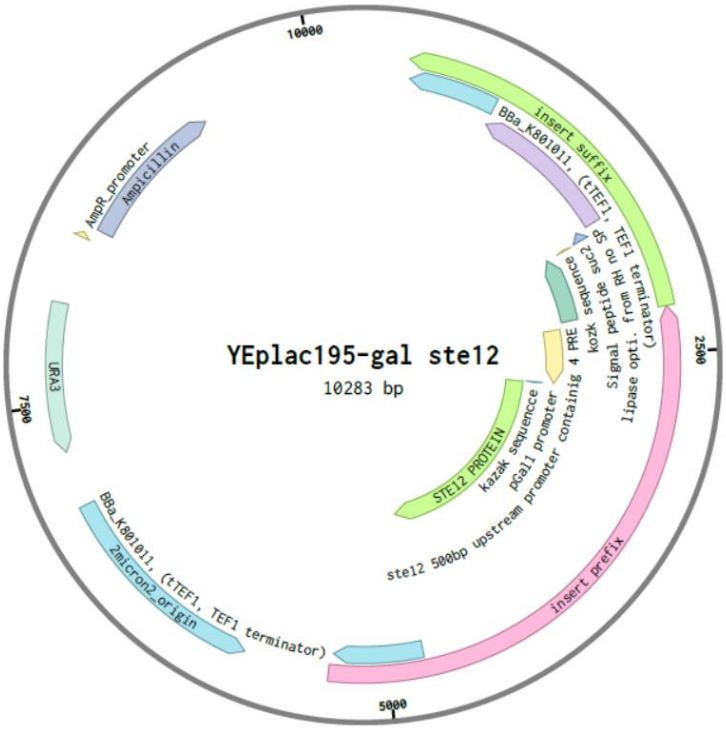
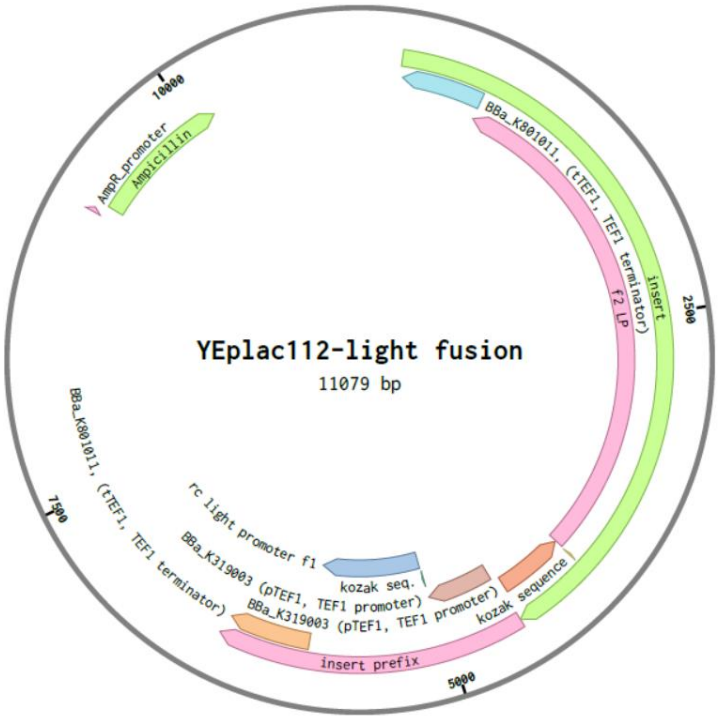
1. The plasmid construction and yeast transformation

In order to allow the yeast to produce enzymes under light stimulation, we design three major plasmids with different selection marker. The three vectors YEplac181, YEplac195, and YEplac112 are the shuttle vectors for *Escherichia coli* and *Saccharomyces cerevisiae*. The plasmid YEplac181 was constructed to generate glucanase and xylanase (BBa_K2376005). The plasmid YEplac195 was constructed to generate lipase and STE12 protein, which could increase the enzyme expression with its special feedback in yeasts (BBa_K2376006). And the plasmid YEplac112 was constructed to generate two light fusion proteins (BBa_K2376007).

After plasmid construction, we would transform them into yeast. Within different selection marker, the result shows that the transformation is successful. Because we design our enzyme as a secrete-out protein, we would then collect the medium to get the enzyme.







2. Yeast-Stain binding affinity

On the other hand, we're curious about whether yeast coated with Alcian blue can have a better binding affinity with paper. From the figure 1 you can see ink will bind to inky part but that is unstained yeast. In figure 2, stained yeast only have one colony and it is even close to the separation line of black and white. We think 1%of

stain might be too strong for the yeast to grow, so after wash and all the treatment most yeast dye. We think even without stain, yeast itself might prefer ink more than paper fiber. In the future work we will keep testing on yeast and ink's binding affinity with more different condition. Also, lowering the stain concentration to stain the yeast and see if it can bind with the ink. We hope to find a best condition that yeast will bind perfectly only on the inky part.

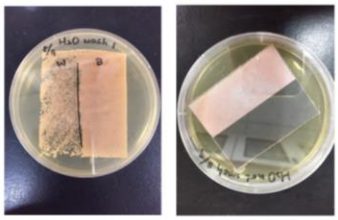


Figure 1. unstained yeast binding with ink

On the left side is the paper without ink and on the right side is paper with ink.

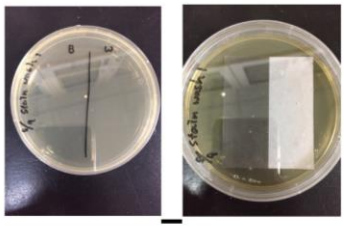


Figure 2. stained yeast binding with ink

On the right side is the paper without ink and on the left side is paper with ink.

3. Collaboration with TAS_Taipei : Floatation by E. coli biofilm

Three samples from the left to the right are the one only biofilm, the one only ink and the one including biofilm and ink. According to the picture, we can see the ink are concentrated in the bottom of 1.5 ml tube (eppendorf®) where the biofilm forms. In order to confirm the biofilm has dragged the ink, we have shake the tubes by shaker evenly. As the result below, we can see the ink in the right sample are still almost concentrated at the bottom of tube. Thereby, we speculate the biofilm will drage the inks