**Overview**

Vika-vox system is used in our project in order to switch the expression from ***RFP*** to β-carotene, as a characterization of our ***Mating Switcher.*** In this way, we can easily visualize the function of our switcher through its color, as well as measure its efficiency and error rate.

Vika-vox system mainly consists of vox sites and reporting parts. At first the expression of RFP will be activated and the expression of β-carotene will be inhibited so that we can detect red fluorescence when vika enzymes doesn’t exist in Saccharomyces cerevisiae. After the expression of vika enzymes, with the deletion of ***RFP*** and terminators flanked by ***vox*** locus, β-carotene expresses and the strains take on an orange color. This is the whole characterization process of mating ***Mating Switcher***.

**Theoretical Background**

**Vika-vox system**

**Experiment Design**

**1.****Construction of** **vox-ura3-vox System**

We use synthetic chromosomeⅤof *Saccharomyces cerevisiae* to load our device, which is a single-celled organism called ***a***. First of all, we use PCR to amplify basic parts including TEF promotor, ura3 gene, ura3-terminator and β-carotene gene. Among them, ura3 gene and ura3-terminator are flanked by ***vox*** locus. Then we use overlap PCR to combine these parts together. The next step is transform this composite part into Saccharomyces cerevisiae. We screen for the correctly transformed cell by using the Sc-Ura plate. For the purpose of verifying desired strain ***TVUVC***, we use colony PCR to amplify the ***TEF promoter-vox-ura3*** gene and ***ura3 terminator-vox-β-carotene*** gene. The length of the strip was observed by agarose gel electrophoresis

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**2.Construction of vox-RFP-vox System**

This system has a great similarity to the ***vox-ura3-vox*** system above. Therefore, it is easy to construct because we only need to change the ura3 gene to the ***RFP*** gene. We use PCR to amplify five basic prats including TEF promotor, ***RFP*** gene, Adh1 terminator, ura3-terminator and β-carotene gene. Among them, ***RFP*** gene and ura3-terminator are flanked by ***vox*** locus. Then we use overlap PCR to combine these parts together. After that we use the lithium acetate conversion method to transfer this composite part into ***TVUVC***. We screen for the correctly transformed cell by using the 5-FOA plate. This part will integrate into chromosomeⅤ by homologous recombination, and we will get another desired strain called ***TVRVC***.

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**3.** **Verification of RFP in the TVRVC**

The verification of ***RFP*** is carried out by using colony PCR to amplify the ***TEF promoter-vox-RFP*** gene and ***ura3 terminator-vox-β-carotene*** gene. We can observe the length of 1122bp band and 1391bp band by agarose gel electrophoresis, which determine the existence of ***vox*** sites and ***RFP*** gene. Then we can detect the red fluorescence.

**3. Method of Red Fluorescence Assay**

We used a variant of the mCherry red fluorescent protein (RFP). The variant sequence was codon-optimized for the expression in Saccharomyces cerevisiae as yeast-enhanced mRFP (yEmRFP) and can combine fluorescence and a purple visible phenotype. Unfortunately, the RFP can’t be directly observed by bare eyes, we decided to use the Fluorescence spectrophotometer and use OD600 to determination cell concentration. Meanwhile, we will observe using fluorescence microscopy for fluorescent proteins expression. The red color can be observed if yEmRFP is expressed.

**4.Construction of vika System**

We use a common expression vector plasmid, pRS416, to load vika part. First of all, we use corresponding restriction endonuclease *Sal1 and Not1* to cut plasmid pRS416 and plasmid pRS415-vika, a gift from Y.J lab, and then use T4 DNA ligase to link them together, we can obtain the complete device we want. Finally, we transform this device into BY4742 by the lithium acetate conversion method, and we screen for the correctly transformed cell by using the Sc-Ura plate. *BY4742* is a single-celled organism called ***α***.

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**5. The characterization of *Mating Switcher***

The *Saccharomyces cerevisiae* called ***TVRVC*** is a single-celled organism called ***a.*** At first, we cultivate pRS416-vikain Sc-Ura medium without glucose for three hours. To induce the expression of vika, they will culture to saturation in Sc medium with raffinose and galactose for twelve hours. After that, vika recombinase are induced to express and we make α***-***pRS416-vika cell and ***a-TVRVC*** cellmate in YPD medium for eight hours. Two types cells are fused and form diploid yeasts, in which vika recombinase bind with vox locus, and then delete RFP gene and Adh1 terminator flanked by vox sites. After the Mating Switcher, β-carotene expresses and he color of cell will transform from white to orange. At last we smear bacteria solution on Sc-Ura-Leu plate to screen for the correctly mating cell. We can judge the existence of vika recombinase by the color of the colony, and obtain the efficiency of mating.

**6. Culture and Expression Condition of Saccharomyces cerevisiae in this experiment**

Traditional YPD culture medium (22g/L glucose, 20g/L peptone, 10g/L yeast extracts) is used by us. Sc-Ura solid culture medium (22g/L glucose, 6.7g/L yeast nitrogen base, 1.224g/L nutrient deficiency mixture without Ura, His, Leu and Trp, 20g/L agar powder, 5mg/L Trp, His and Leu) is used to screen for correctly transformed cell. 5-FOA solid culture medium (22g/L glucose, 6.7g/L yeast nitrogen base, 1.224g/L dropout, 20g/L agar powder, 1ml/L His, Trip, Leu and 2.5ml/L Ura) is used to screen for correctly transformed cell. Sc medium with raffinose and galactose culture medium (20g/L raffinose, 6.7g/L yeast nitrogen base, 1.224g/L nutrient deficiency mixture without Ura, His, Leu and Trp, 20g/L agar powder, 10x galactose, 5mg/L Trp, His and Leu) is used to induce to express vika recombinase. Sc-Ura-Leu solid culture medium(22g/L glucose, 6.7g/L yeast nitrogen base, 1.224g/L nutrient deficiency mixture without Ura, His, Leu and Trp, 20g/L agar powder, 5mg/L Trp and His) is used to screen for the correctly mating cell. All the cells are cultured in 5mL medium at 30℃ with shaking speed of 220rpm.

**Expected Results**

In our design, Mating Switcher is a means of gene regulation. We can transform from one functional system to another system through this switch conveniently. To show the function of Mating Switcher more intuitively, we construct this RFP system to be a characterization.

First, we expect to observe red fluorescent protein