

This SCRaMbLE experiment consists of screening strains, dilution assay and survival rate measurement. The experiment of this part started at mid August and lasted for about 11 weeks.

## August

### Aug 14<sup>th</sup>-20<sup>th</sup>

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1. We have saw a lot of literatures to find the threshold of yeast's resistance to heavy metals like copper ion and Cadmium ion. The results are 3mM and 0.5mM.
2. Transform the plasmid with CRE into synX strain and get three strains, 079 and 160 with ura tag, 085 with His tag.
3. We made copper ion YPD culture medium: 3mM, 4mM, 5mM, 6mM, 8mM, 10mM, 13mM, 15mM. However, we found that yeast can grow well even in 15mM YPD culture medium. We hypothesized that the protein in the culture medium was complexed with copper ions. So we planed to change to SC culture medium.
4. We contacted the teacher of inorganic chemistry in Tianjin university and we borrowed 3g cadmium ion for our cadmium ion resistance experiment.

### Aug 21<sup>th</sup>-25<sup>th</sup>

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1. We made copper ion SC culture medium: 3mM, 4mM, 4.5mM, 5mM, 5.5mM, 6mM, 6.5mM, 7mM.  
cadmium ion SC culture medium: 0.01mM, 0.05mM, 0.1mM, 0.15mM, 0.2mM.
2. We use SCRaMbLE to screen strains with good resistance to copper ions and cadmium ion on a series of plates with copper ion and cadmium ion concentration gradient.
3. There are some strains that can grow on 4.5mM copper ion SC culture medium and 0.1mM cadmium ion SC culture medium. Every time we found these strains we would save them and do SCRaMbLE on them.

## September

### Aug 29<sup>th</sup> - Sep 1<sup>st</sup>

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1. We made copper ion SC culture medium: 4.8mM, 5mM, 5.5mM, 6mM.  
cadmium ion SC culture medium: 0.1mM, 0.12mM, 0.14mM, 0.15mM, 0.18mM.
2. We streaked those yeasts with higher resistance phenotype on higher copper ion and cadmium

ion concentration plate while we still used SCRaMbLE to screen strains with good resistance to copper ions and cadmium ion on a series of plates with copper ion and cadmium ion concentration gradient.

### **Sep 4<sup>th</sup> - Sep 8<sup>th</sup>**

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1. We continued our work like making culture medium and SCRaMbLE. Finally we got two strains, named 160A and 085A respectively, could grow on 0.15mM cadmium ion culture medium.
2. We streaked them and synX once again on 0.15mM cadmium ion culture medium. Fortunately, 160A and 085A can still grew better than synX.

### **Sep 11<sup>th</sup> - Sep 15<sup>th</sup>**

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1. We diluted yeast to  $10^{-1}$ 、 $10^{-2}$ 、 $10^{-3}$ 、 $10^{-4}$ 、 $10^{-5}$  and made dilution assay on 0.14mM cadmium ion SC culture medium for 160A, 085A, synX, 160 and 085 to see if 160A and 085A exactly have higher resistance to heavy ions. And obviously if 160A and 085A grow better than other blanks, the answer is YES.
2. We did many times to ensure the accuracy of the experiment and some time the plates were bacterial contamination of course.

### **Sep 18<sup>th</sup> - Sep 22<sup>th</sup>**

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1. We continued our work like making culture medium and SCRaMbLE. And we got a strain that could grow on 4.8mM copper ion SC culture medium.
2. We were very excited to do dilution spot plate for it but unfortunately we found that it did not grow better than synX. Which means we still need to do SCRaMbLE.

### **Sep 25<sup>th</sup> - Sep 29<sup>th</sup>**

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1. After a meeting with our instructing teacher, we planed to measure the survival rate for 160A and 085A. Because only in this way could we get quantitative datas.
2. First, We cultivated yeast over night to make sure yeasts grow to saturation. Then, we took

100µL culture medium into 3mL 5mM cadmium ion SC culture medium and coated on YPD plate after 10min, 30min, 1h and 2h.

3. After two days, when these yeasts were growing well in these plates, we would counted them and wrote down. What's more, we also took photos for them as our experiment results.

## October

### Oct 9<sup>th</sup>- Oct 13<sup>th</sup>

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1. This week, we did the survival rate measurement for 160A and 085A for the second time. First, We cultivated yeast over night to make sure yeasts grow to saturation. Then, we took 100µL culture medium into 3mL 5mM cadmium ion SC culture medium and coated on YPD plate after 10min, 30min, 1h and 2h.
2. Meanwhile, we were still screening yeast having higher resistance to copper ion. We cleaned up our our plates in the incubator and we had new found. 4 plates, 4mM, 4.5mM and two of 4.8mM copper ion SC culture medium respectively, had some strains on them and their form are pointy which was similar with those had resistance to cadmium ion SC culture medium.
3. We saved them named 160A, 160B, 160C and 085A and made dilution assay on 4.8mM copper ion SC culture medium for them, synX, 160 and 085. The result was what we've been looking forward to. They grow better than other blanks.

### Oct 16<sup>th</sup>- Oct 20<sup>th</sup>

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1. We did the third time of survival rate measurement for 160A and 085A which can resistant to cadmium ion.
2. We did another time dilution assay for all the strains we have screened to get a perfect photo.
3. We did the survival rate measurement for 160A, 160B, 160C and 085A. First, We cultivated yeast over night to make sure yeasts grow to saturation. Then, we took 100µL culture medium into 3mL 50mM copper ion SC culture medium and coated on YPD plate after 30min, 1h, 2h and 3h.
4. We counted and recorded these datas after two days they grow well on those plates.

## **Oct 23<sup>th</sup> - Oct 27<sup>th</sup>**

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1. Our experiment was near the end. We did other two times of survival rate measurement for 160A, 160B, 160C and 085A. And we took photos for the results.
2. We analyzed the experimental results and it turned out that SCRaMBLE really makes sense. We are very excited that we have some positive results after nearly 3 months hard work.
3. Our team focus on writing wiki this week.