

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 14th-20th

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 21th-25th

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 29th - Sep 1st

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 4th - Sep 8th

1. We continued our work like making culture medium and SCRaMbLE. Finally we got 4 strains, named S1, S2, S3 and S4 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, they can still grew better than *synX*.

Sep 11th - Sep 15th

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 S1, S2, S3, S4, *synX*, 160 and 085 to see if S1, S2, S3 and S4 exactly have higher resistance to heavy ions. And obviously if they 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 18th - Sep 22th

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 25th - Sep 29th

1. After a meeting with our faculty instructor, we planed to measure the survival rate for S1. 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 200 μ L 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 9th - Oct 13th

1. This week, we did the survival rate measurement for S1 and *synX* for the second time. First, We cultivated yeast over night to make sure yeasts grow to saturation. Then, we took 200 μ L 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 S5, S6, S7 and S8 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 16th - Oct 20th

1. We did the third time of survival rate measurement for S1 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 S5 and *synX*. First, We cultivated yeast over night to make sure yeasts grow to saturation. Then, we took 200 μ L culture medium into 3mL 50mM copper ion SC culture medium and coated on YPD plate after 30min, 1h, 2h and 3h.

Oct 23th - Oct 27th

1. We counted and recorded these datas when they grow well on those plates.
2. We found that S1 still showed good tolerance to high concentration cadmium ion. But S5 grown too much that we cannot count.
3. We did the second time of survival rate measurement for S5and *synX* in 0.5M copper ion solution.

4. After 2 days, we recorded the clone number and took photos for the results.
5. 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.
6. Our team focus on writing wiki this week.