

# Notebook

20180910–20181025

After a summer vacation, we finally started our project! We intend to use the light control system to control the assembly of T7 RNA polymerase to achieve real-time quantification of proteins. In the first month, generally, we were relaxed. We looked up the relevant literature and synthesized the sequence of goals we needed. Everything is ready!

20181026–20181202

We divided into four groups to build different vectors, and we quickly built a simple vector like PLac-GFP and Ptet-RFP. We think that the god of luck will always care for us, however, the fact is not what we think...

20181202–20190131

Continuous digestion, ligation, transformation... But the sequencing results are always wrong. After repeated disappointments, we still have not given up easily. We constantly adjust the experimental scheme, use seamless cloning, and design primers many times, but our plasmids are still not successfully constructed. The only happy thing is that the light control system is built! The Spring Festival is coming, we hope that the new year will have good luck!

20190220–20190331

Time is getting tighter, but we still have no success. Too frustrating!

20190401–20190428

After the discussion, everyone made a major decision - change the project. Fortunately, we still have a long preparation time. After brainstorming again and again, we will do biological test strips about phenylketonuria!

20190429–20190601

Everyone regained their confidence and began looking for documentation and screening promoters. After we decided to use the two promoters, tyrP and aroF, we started the synthesis sequence. Before the experiment began, we constructed a preliminary mathematical model to verify the feasibility of the project.

20190602–20190630

We first constructed two vectors, P<sub>tyrP</sub>-GFP and P<sub>aroF</sub>-RFP. Still enzymatically cut, connected, transformed... Finally, we have transformed it! Send them to the sequencing, waiting for the results, and succeeded! so happy! Hope everything goes well afterwards.

20190701–20190725

Summer vacation is coming, but happiness has nothing to do with us. Only plasmid, transformation, PCR... We sent the TyrR sequence to the Huada gene for synthesis, but eventually the company failed to synthesize. Later, after several experiments, it was found that TyrR was over-expressed due to the high intensity of J23119, which was toxic to cells.

We decided to change the experimental plan. The HP part started to move, everything started from scratch, and even a little excited!

20190726-20190831

After discovering the problem, we decided to replace the promoter with Plac. After the replacement of the promoter, the process was not smooth, we carried out a round of transformation, colony PCR, sequencing, repeated failures, repeated adjustment programs, we finally succeeded on the eve of the school! Happy want to cry! One month before the start of the school, the nights of one-on-one reply slipped away during the summer vacation, and we quickly completed most of HP's content.

20190901-20190928

First of all, it is really sad to pay homage to the summer vacation that we did not have. After the experimental data came out, we further improved the mathematical model. After the summer vacation, we returned to calm. We began to sort out HP's bits and pieces and found that we still have a lot of things to do.

20190930-20191007

We constantly measure experimental data overnight. The results of the experiment finally came out. Although it was not particularly ideal, it even indicated that our system could not work according to our ideas, but finally got the result.