YAN Xiaowei's Notebook

07.01—07.07

• Positive transformation of the parts which may be used in our project from 2011 Distribution.

07.08—07.16

• Primary construction of the part about c-di-GMP responsive ribozyme.

At first, assembly the c-di-GMP responsive ribozyme with GFP using OE-PCR (pBAD+c-di-GMP ribozyme+GFP+ter B0015). Because of the delayed primers, the problems of DNA purification kit and gel extraction kit, the ignorance of the question on fidelity of Taq enzyme, these experiments have been done many times, so they're finished a little late. Whatever, WT、M6、M8 and GFP have been OK now.

• The growth curve determination of DH5α in media of LB.

07.17—07.24

• Assembly the c-di-GMP responsive ribozyme (WT/M6/M8) with GFP using OE-PCR successfully.

• Some comparisons between different kits.

07.25—07.31

• Violacein

Separation of individual genes in the violacein metabolic pathway ——vioA/B/D/E.

The primers of vioC seem to have a prefer annealing ability with a particular sequence within vioC.

Testing and optimizing the protocols of PCR.

08.01—08.14

• c-di-GMP ribozyme construction.

Because the GFP fused with c-di-GMP responsive ribozyme (WT) couldn't be ligated with pBAD and terminator B0015 for some reason. I have tried another approach——Gibson assembly and finally the sequence has been correct.

08.15—09.05 with the help of GONG Yan

• With the help of GONG Yan, we construct c-di-GMP responsive ribozyme (M6/M8) regulatory system (pBAD+c-di-GMP M6/M8+GFP+ter B0015) using Gibson assembly.

• Construction of a new theophylline responsive ribozyme regulatory system.(pBAD+RBS+theo Parental/theo Th2P6/theo B11+GFP+ter)

NOTE:

Theo Th2P6 is a reconstructed Group I intron with an anti-theophylline aptamer.

Theo Parental is the original Group I intron.

Theo B11 is another reconstructed Group I intron which shows no activity in vivo.

09.06—10.04

• Help GONG Yan constructing some parts of bistable switch. (inserting theo N8-3/1G1 into both

BS1C3 and BS1C3 with 1.20/2.5)

• Constructing the part of vioC (ter B0015+pBAD+theophylline riboswitch N8-3/1G1+vioC+ter B0015).

Because the product of vioC isn't sufficient for digesting of restriction enzyme, I insert the vioC into pUC18 first. However, it didn't work. Finally, I tried another approach----Gibson assembly.

• Characterization of c-di-GMP ribozyme and engineered theophylline ribozyme. Collecting experiment datas.