Week1(8.1-8.7): -10 region mutant construction of NdmB^{Q289T}

Starting this week, we used molecular means to optimise the promoter of the Q289T mutant for the purpose of increasing PX production.

(A) Construction of the -10 region mutant of NdmB^{Q289T}:

1.PCR, cut glue recovery

Design the parsimonious primers, use pYB1s-ndmB (J23107 as promoter) as template, PCR to obtain the desired fragment, and the rest of the procedure is the same as the previous construction process.

2. Connections

Three fragments are connected using the golden gate method.

3. Transformation

The procedure is the same as the previous build process.

4. Washing and extraction of plasmids

The procedure is the same as before.

(ii) Screening for mutants in the -10 region of NdmB^{Q289T}:

1. Transformation

The extracted plasmid was transferred into BW25113 receptor cells, coated with plates, and cultured inverted for 12h until monoclonal growth.

2. Induction

The monoclones were picked into deep-well plates (800 μL of ZY + arabinose + IPTG + S50), and the first 4 wells were spotted with unmutated strains and induced at 25°C for 18h.

3. Whole-cell catalysis

The deep-well plate was centrifuged at 4200 rpm for 10 min, the supernatant was discarded, 400 μL of 1XM9 + 0.5 mM caffeine (equivalent to doubling the bacterial concentrate) was added, and placed at 25°C for 18 h of whole-cell catalysis.

4. Screening

After centrifuging the deep-well plate bacterial solution, 200 μL of supernatant was taken into a 96-well plate and 10 μL of supplement was added. (Contains 5052, calcium chloride, magnesium chloride, trace, inducer and antibiotic)
Add 10 μL of induced pYB1s-ndmDCEA strain to it (since pYB1s-ndmDCEA strain may grow

slowly and need to be delayed for induction, the 96-well plate sealed with the added supernatant will be stored in a sequencing bag at 4°C until DCEA induction is completed).

5. Enzyme marker test

The growth curves of the different mutants were examined under an enzyme labeller, and those that grew well were the strains that could produce PX.

Week2-3 (8.8-8.21): -10 region mutant screening of $NdmB^{Q289T}$

(i) Screening for mutants in the -10 region of $NdmB^{Q289T}$

1. Liquid-phase detection

The mutant data obtained last week were analysed and 20 groups of mutant organisms with OD values greater than 1 after the assay were selected, samples were prepared, and PX yield assays were performed using the liquid phase.

2. Sequencing analysis

Several of the 20 sets of mutants with higher PX production were sent for sequencing and the results were awaited.