July 2025

Phase 1 | 7.8 - 7.15

In this phase, we conducted theoretical and practical training in molecular biology. We engaged in the preliminary test for the extracellular targeted cleavage of *mecA* gene. We discussed and designed the sequences of primers of *mecA* gene. Meanwhile, we aimed to obtain the *mecA* gene through double-enzyme digestion and gel extraction to conduct the preliminary test.

1) We drafted the protocol for the extracellular targeted cleavage of *mecA* gene.
2) The restriction enzymes and the *mecA* plasmid were received.
3) We digested the *mecA* plasmid with restriction enzymes EcoRI and HindIII. DNA electrophoresis analysis was used to observe the results of enzyme digestion. Unfortunately, the plasmids were not cleaved as we expected.
4) We retried the enzyme digestion of the *mecA* plasmid by extending the reaction duration and adjusting the amount of restriction enzymes. This time, part of the plasmids was correctly cleaved.
5) We extracted the targeted DNA band from the gel. As a result, the purity of the extracted product was extremely low.

Phase 2 | 7.16 - 7.19

In this phase, we further refined the design of the primers of *mecA* and made some improvements on enzyme digestion.

1) We digested the *mecA* plasmid with appropriate amounts of restriction enzymes EcoRI and HindIII.
2) DNA electrophoresis analysis was used to observe the result of enzyme digestion. This time, all the plasmids were correctly cleaved.

3) We extracted the targeted DNA band from the gel. Unfortunately, the purity of the extracted product stayed extremely low.
4) The primers for *mecA* gene were received.
5) We used low-fidelity enzymes to test the thermocycling conditions for PCR. DNA electrophoresis analysis was used to identify the PCR products. As a result, the *mecA* gene has been successfully amplified by PCR under certain thermocycling conditions.

Phase 3 | 7.20 - 7.24

In this phase, we amplified *mecA* gene by PCR and incubated *mecA* gene with sgRNA-Δ*mecA*/Cas9 complex to test whether sgRNA-Δ*mecA*/Cas9 complex could successfully cleave *mecA* gene.

1) The high-fidelity enzyme was received.
2) We used high-fidelity enzyme to amplify *mecA* gene by PCR. DNA electrophoresis analysis was used to identify the PCR products.
3) We incubated the purified PCR products with sgRNA-Δ*mecA*/Cas9 complex. DNA electrophoresis analysis was used to determine whether sgRNA-Δ*mecA*/Cas9 complex correctly cleaved *mecA* gene. Under UV light, we found that a large amount of DNA remained in the loading wells.
4) We retried the incubation of *mecA* gene and sgRNA-Δ*mecA*/Cas9 complex. We added proteinase K to the incubated products. DNA electrophoresis analysis was used to determine whether sgRNA-Δ*mecA*/Cas9 complex correctly cleaved *mecA* gene. This time, sgRNA-Δ*mecA*/Cas9 complex successfully cleaved the *mecA* gene as we expected.