2A De-template and T5 conversion

Sunday, July 2, 2023

Overview

- The 28a vector, Cl2 fragment, and ADH fragment after PCR were detemplated, and then the three fragments were ligated by T5 enzyme and transferred to DH5a competent cells

Experimental equipment

- DPN1 enzyme
- rcutsmart
- 4buffer
- T5 enzyme diluted but not added with 4buffer

Experimental steps

①De-template

- First, dilute DPN1 and add 1ul DPN1 enzyme, 1ul rcutsmart buffer, and 8ul sterile water to a 1.5ml EP tube.
- The diluted DPN1 enzyme was added to 2ul, 1ul rcutsmart buffer, 2ul sterile water, and 5ulPCR product to the anti-template system.
- Put it into a PCR machine and set the parameters: 37°C for 50min, 80°C for 20min, 12°C for 2min

②T5 conversion

- Add 1.5ulCL2 fragment, 1.5ulDH fragment, and 1.0ul28a vector to the 1.5 ml EP tube

- Add 0.5 ul of diluted T5 enzyme and 0.5ul4buffer to the EP tube above in the ultra-clean bench, and immediately ice bath for 5 min
- Add competent cells to the T5 system for 70ul in an ultra-clean bench and ice bath for 30 min
- Heat excitation: water bath in a 42°C water bath for 45s, and then immediately ice bath for 2min.
- Add NZY medium 150 ul to the ultra-clean bench
- Incubate at constant temperature for 40min-60min in a shaker at 37 °C
- . After incubation, 200ul of the bacteria were coated in LK medium plates and incubated overnight at 37°C