SDS-PAGE

Introduction

The protocol is used to check if the bacteria can really produce the protein we want by observing its band on the page, which indicates the size of the product.

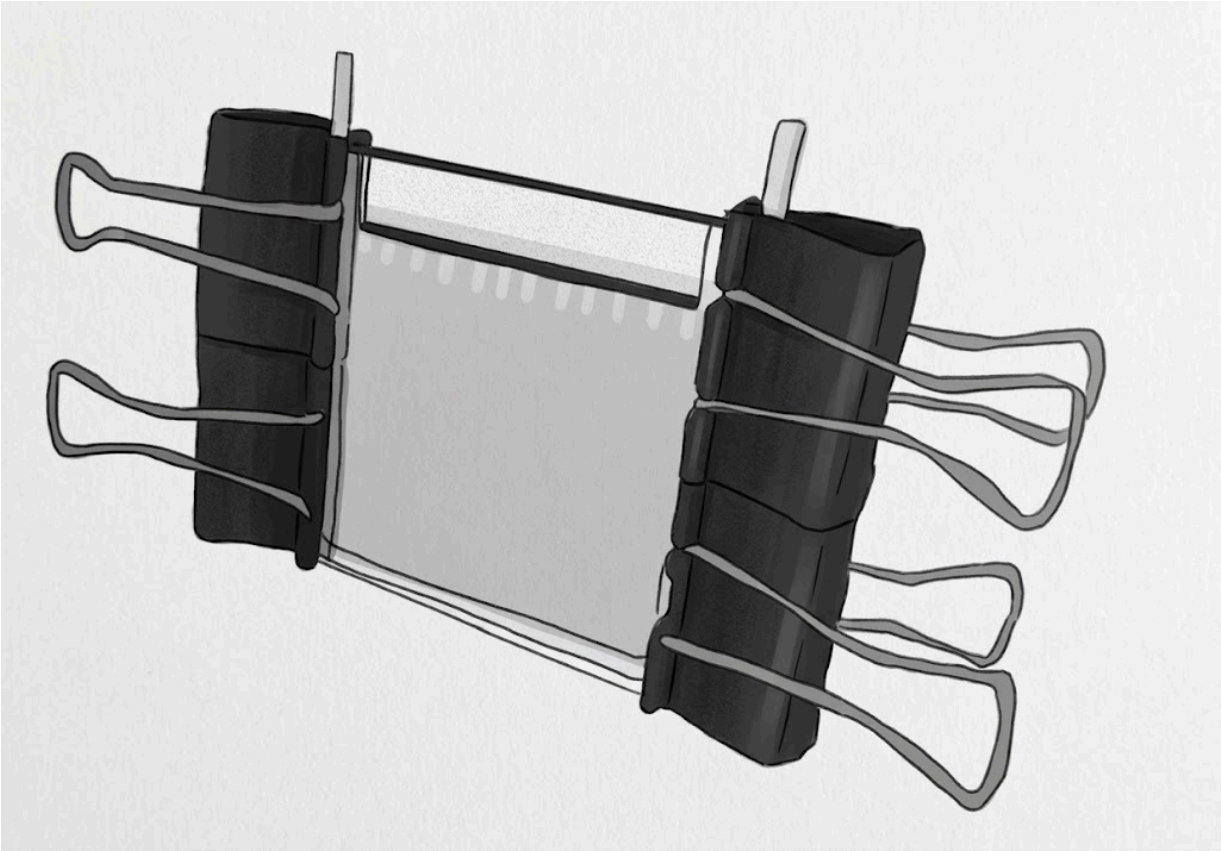
Materials

- 12.5% Separation gel
- Stacking gel
- 10X Tank buffer
- 1X Tank buffer + SDS
- sample

Procedure

Operation

1. Take 50ul O/N (over night) culture and 5mL LB, incubate for 6 hours at 37°C.
2. Measure OD value
3. Take 1 mL culture to centrifuge at 12000rpm for 2 min, then remove the supernatant.
4. Add 100uL SDS buffer (Sample buffer) to suspend the pellet.
5. Heat the sample at 100°C for 5 min.
6. Set up for gel preparation as the picture below (the inner side of it are grooves, and it is sealed by a rubber strip).

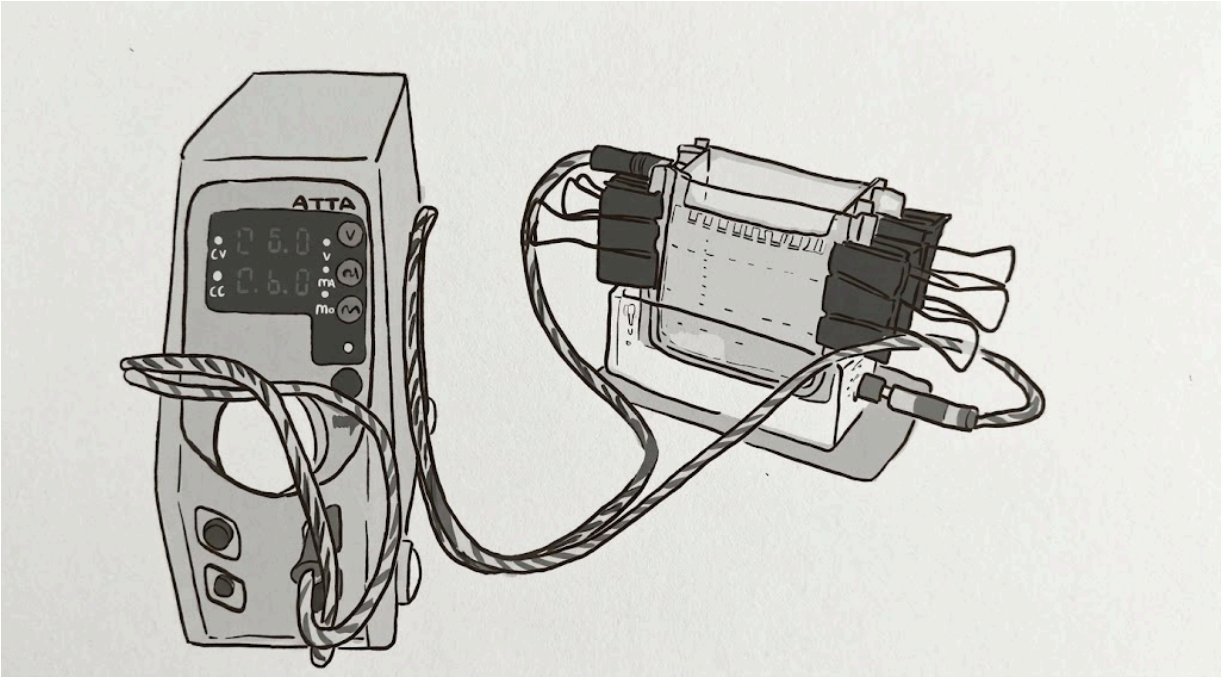


7. Add the separation gel, then add alcohol, wait at least one hour.

8. Pour out the alcohol, add stacking gel.

9. Insert the comb, then wait at least on hour.

10. Set up the device as the picture below (the grooves of stacking gel should be the inner side of tank), then add tank buffer in both of the tanks (make sure the buffer contacts with the gel).



11. Add marker, samples.

12. Set the machine for 80V, 1 hour, then start the machine.

13. Set the machine for 120V, 1 hour, then start the machine.

14. Remove the stacking gel, then dye the separation gel for at least 30 mins.

15. Read the result, then check the size according to the marker you load.