Plasmid microextraction

Materials:

· Axyprep plasmid DNA miniprep Kit（Axygen，catalog no.AP-MN-P-250）
· bacteria solution
· ddH2O

Procedure:

1. Heat ddH2O to 55 ℃ with metal temperature homogenizer.
2. Take 4 ml of the overnight culture broth in LB medium，centrifuge at 12000 rpm for 1 minute, and discard the supernatant.
3. Add 250ul Buffer S1 to suspend bacterial precipitation.
4. Add 250ul buffer S2, gently turn up and down for above 30 second, until the bacteria form transparent bacterial solution.
5. Add 350ul buffer S3, gently and thoroughly invert up and down to mix 6-8 times, and centrifuge at 12000 rpm for 10minutes.
6. Absorb the supernatant, transfer it to the preparation tube, centrifuge at 12000 rpm for 1minute, and discard the filtrate.
7. Add 500ul buffer W1 to the preparation tube，centrifuge at 12000 rpm for 1minute, and discard the filtrate.
8. Add 700ul buffer W2 to the preparation tube，centrifuge at 12000 rpm for 1minute, and discard the filtrate.
9. Repeat step 8 once.
10. centrifuge at 12000 rpm for 1minute.
11. Place the centrifuge tube at 55 ℃ to volatilize ethanol for 10minutes.
12. Transfer the preparation tube to a new centrifuge tube, add 30-50ul ddH2O to the center of the preparation tube, and stand at room temperature for 1min，centrifuge at 12000 rpm for 1minute.
13. Suck the filtrate into the preparation tube，centrifuge at 12000 rpm for 1minute.
14. Save plasmid in - 20 ℃.

Notes

1. RNase A was all added to buffer S1 and stored at 4 ° C before first use.
2. Before using for the first time, buffer W2 concentrate add the indicated volume of absolute ethanol.
3. Avoid violent shaking during the experiment.