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Plasmid Miniprep for 2022

Forked from Plasmid Miniprep

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Protocol status: Working
We use this protocol and it's working

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Abstract

Plasmid extraction and purification, based on Vazyme Mini-prep Kit manual.

Troubleshooting

1 Pellet bacteria culture in a 1.6mL centrifugation tube at 13,000rpm for 1 minute. Decant supernatant. The pellet in the tube can be stored in -20 freezer if time not enough for the following steps.

2 Add 250uL of resuspension solution (P1 with RNase) and vortex. After adding RNase into the P1, P1 is stored in 4 degree refrigerator.

3 Add 250uL of lysis solution (P2) and invert gently 4-6 times. P2 contains NaOH, and must keep tightly closed.

4 Add 350uL of neutralization solution (P3) and invert gently 4-6 times. Do not vortex, which will break bacteria genomic DNA and genomic DNA fragments will contaminate purified plasmids.

5 Spin at 13,000rpm for 10 minutes, at 4 dgree.

6 Take the clear supernatant, without any white protein cloud. Bind the DNA to the column by decanting.

7 Centrifuge at 13,300 rpm for 1 minute.
00:01:00

8 Wash the column with 600uL of wash solution (PW2) and centrifuge for 1 minute. Remind adding pure ethanol into PW2 as indicated on the bottle.
00:01:00

9 Discard the flow-through, wash again with 600uL of PW2, and centrifuge for 1 minute. Discard the flow-through and centrifuge the column again for 2 minutes.
00:03:00

3m

10 Keep the cap open and wait until no ethanol smell. Move the column into a new centrifugation tube. Elute the purified DNA with 50uL of elution buffer (TE; 10 mM Tris-Cl, 1 mM EDTA). Then, centrifuge for 1 minutes. Collect the flow-through, which is the plasmid DNA. Because pET28 based is low-copy plasmid, pre-warm TE to 50 degree helps the elution.
00:01:00