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El-cheep-o miniprep

Forked from El-cheep-o bacterial DNA isolation / miniprep

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1 Works for me dx.doi.org/10.17504/protocols.io.peudjew

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ABSTRACT

This is a protocol for cheep miniprep isolation. Be aware that the DNA quality might not be sufficient to use this protocol for cloning purposes. It works very well for the digestion analysis though.

BEFORE STARTING

Prepare following buffers and keep P3 at 4C:

P1: into 250 ml H2O
EDTA 0.093g 1mM
TRIS 1.515g 50mM(pH 8.0)
AUTOCLAVE

P2: into 250 ml H2O
NaOH 2g 0.2N
SDS 2.5g 1%
AUTOCLAVE

P3: into 250 ml H2O
K-acetate 73.63g
Acetic Acid 27.5ml
pH 5.5
AUTOCLAVE

1 ·Spin 3ml of the overnight culture to pellet cells (1.5-ml for time) @13000 rpm for 5 min

2 ·Remove the supernatant

3 ·Add 250 ml of sol P1+Rnase 5ml and vortex to resuspend cells

4 ·Take 15 min @ room temperature

5 ·Add 250 ml of sol P2 and mix gently

6 ·Take 5 min @ room temperature

7 ·Add 350 ml of sol P3 and mix gently

8 ·Leave 10 min on ice

9 ·Spin @ the max speed for 10 min

10 ·Take 750 ml of the supernatant and put in a new eppendorf tube and add in this tube 750 ml of isopropanol

11 ·Mix well

12 ·Spin for 30 min @ 14000 rpm

13 ·Remove the supernatant

14 ·Spin again for other 10 min

15 ·Take @ 37°C for 10 min

16 ·Add 50 ml of H2O

17 ·Store @ -20°C