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Working

El-cheep-o bacterial DNA isolation / miniprep

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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.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
RNase A	19101	Qiagen
Tris		
EDTA		
Isopropanol		
Potassium acetate	1.04820.1000	Merck Millipore
Sodium hydroxide	1064981000	Merck Millipore
SDS		Ambion
Acetic acid	695092	Sigma Aldrich

SAFETY WARNINGS

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 per time) @13000 rpm for 5 min

2 ·Remove the supernatant

3 Add 250 ul of P1 solution + Rnase 5ul and vortex to resuspend the cells

4 Incubate 15 min @ room temperature

5 Add 250 ul of P2 solution and mix gently

6 Incubate 5 min @ room temperature

7 Add 350 ul of P3 solution and mix gently

8 Leave for 10 min on ice

9 Spin @ the max speed for 10 min

10 Take 750 ul of the supernatant and put in a new eppendorf tube. Add 750 ul of isopropanol

11 Mix well

12 Spin for 30 min @ 14000 rpm

13 Remove the supernatant. Wash with 80% EtOH

14 Spin again for other 10 min

15 Dry the pellet @ room temperature for 10 min. Remove the remaining EtOH

16 Add 50 ul of sterile / MiliQ water

17 Measure the DNA concentration using NanoDrop and store the DNA @ -20°C