DNA Extraction Protocol

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Abstract

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Guidelines

This protocol comes from a group of other protocols. This protocol is (2) of (4):
1. 'Large Volume Marine Cyanophage Phage Protocols'
2. 'DNA Extraction Protocol'
3. 'DNA Precipitation Protocol'
4. 'Checking DNA Concentration with Agarose Gel'

Needed:

- CsCl
- Proteinase K
- SDS
- Incubator
- Phenol
- Microfuge @ 6,000rpm
- Wide-bore pipette
- Tube
- Chloroform

Protocol

Step 1.
Create mixture of CsCl, Proteinase K, and SDS.

PROTOCOL

. CsCl purified phage lysate, Proteinase K, SDS

CONTACT: VERVE Team
Mixture
Step 1.1.
Mix 1 volume of dialyzed, CsCl purified phage lysate with 50 µg/ml Proteinase K
Mixture
Step 1.2.

Mix solution in 0.5% SDS.

Step 2.
Mix and incubate 1 hour at 56°C.

DURATION
01:00:00

Step 3.
Cool to room temperature

Step 4.
Add an equal volume of phenol and invert several times.

Step 5.
Spin 3000g (6000 rpm on microfuge), 5 minutes, room temperature.

DURATION
00:05:00

Step 6.
Carefully transfer the supernatant with a wide-bore pipette to a fresh tube.

Step 7.
Add an equal volume of phenol:choroform (1:1), invert.

Step 8.
Spin again, 3000g (6000 rpm on microfuge), 5 minutes, room temperature.

DURATION
00:05:00

Step 9.
Transfer the supernatant, as done before.

Step 10.
Add an equal volume of chloroform and invert.

Step 11.
Spin again, 3000g (6000 rpm on microfuge), 5 minutes, room temperature.

DURATION
00:05:00

Step 12.
Transfer the supernatant, as done before.

DURATION
00:05:00