Isolation of DNA from phage lysate

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Abstract

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Materials

Quant-iT™ PicoGreen® dsDNA Assay Kit P11496 by Life Technologies

RNase A 19101 by Qiagen

Protocol

Preparation of phage lysate

Step 1.
Put phage lysate in plastic centrifuge bottle and add RNase (for 100 ml add 10 µl, for 150 ml add 15 µl, etc.)

REAGENTS
RNase A 19101 by Qiagen

ANNOTATIONS
Natalie Solonenko 13 Feb 2018

Not entirely necessary, depending on your application. Your choice!

Preparation of phage lysate

Step 2.
Incubate 30 min. at room temperature

DURATION
00:30:00

Preparation of phage lysate

Step 3.
Add NaCl to a final concentration of 1M (for 100 ml add 6.5 g NaCl)

AMOUNT
7 g Additional info:

REAGENTS
Sodium chloride View by P212121

Preparation of phage lysate

Step 4.

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Incubate for 1 hour on ice

DURATION
01:00:00

NOTES
VERVE Team 17 Jun 2015
can be left overnight if necessary

Preparation of phage lysate

Step 5.
Centrifuge for 10 min. at 11,000xg (8,300 rpm on GSA rotor)

DURATION
00:10:00

Preparation of phage lysate

Step 6.
Transfer supernatant to a new bottle

Preparation of phage lysate

Step 7.
Add PEG 8000 at 100g/l (for 100 ml add 10 g PEG) and shake to mix

Preparation of phage lysate

Step 8.
Incubate 1 hour on ice

DURATION
01:00:00

Preparation of phage lysate

Step 9.
Centrifuge for 10 min. at 10,000xg (7,835 rpm on GSA rotor)

DURATION
00:10:00

Preparation of phage lysate

Step 10.
Carefully pour out the supernatant to discard

Preparation of phage lysate

Step 11.
Set the bottle upside down on paper towels/Kimwipes to drain remaining liquid

Preparation of phage lysate

Step 12.
Rinse the inside of the bottle twice with SM buffer (total volume of 1-2 ml)

AMOUNT
2 ml Additional info:

Preparation of phage lysate

Step 13.
Collect in 2 ml microcentrifuge tubes (1 ml per bottle)

DNA isolation using Wizard Prep

Step 14.
Shake resin to resuspend and heat TE to 80°C

DNA isolation using Wizard Prep

Step 15.

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Add 1 ml resin to each 2 ml tube

DNA isolation using Wizard Prep

Step 16.

Mix by inversion

DNA isolation using Wizard Prep

Step 17.

Attach column to 3 ml syringe and push sample through 1 ml at a time

NOTES

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If column gets clogged, switch to a new one

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Remove column before pulling out plunger to add more

DNA isolation using Wizard Prep

Step 18.

Push 2 ml of 80% isopropanol through each column 1 ml at a time

AMOUNT

2 ml Additional info:

DNA isolation using Wizard Prep

Step 19.

Place column in original 2 ml tube

DNA isolation using Wizard Prep

Step 20.

Centrifuge 2 min at 10,000xg to remove excess isopropanol

DURATION

00:02:00

NOTES

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Cut the caps off the 2 ml tubes to cover tops of columns

DNA isolation using Wizard Prep

Step 21.

Place column in new 1.5 ml microcentrifuge tube

DNA isolation using Wizard Prep

Step 22.

Add 100 µl warm TE to elute

AMOUNT

100 µl Additional info:

DNA isolation using Wizard Prep

Step 23.

Briefly vortex

DNA isolation using Wizard Prep

Step 24.

Centrifuge for 30 sec. at 10,000xg

DURATION

00:00:30

NOTES

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Check to see if 100 µl came through the column- if not, repeat centrifugation until most of the TE comes through

DNA isolation using Wizard Prep

Step 25.

Check concentration of isolated DNA using NanoDrop.

DNA isolation using Wizard Prep

Step 26.

Use Quant IT DNA quantification to validate NanoDrop readings

REAGENTS

Quant-iT™ PicoGreen® dsDNA Assay Kit P11496 by Life Technologies

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