Phenol/chloroform genomic DNA extraction-1.5 ml Eppendorf tube.

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Abstract

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Before start

You will need to make the 20 mL of Lysis Buffer prior to running this protocol.

Protocol

Step 1.

In a centrifuge, spin cells down in a 50 mL conical tube at maximum speed for 20 minutes at 4º C.

NOTES
Ashley Humphrey 11 Sep 2017

Maximum speeds vary, adjust according to individual availability.

Step 2.

Per cell pellet, add lysis buffer in multiples of 700 µL per pellet.

AMOUNT
700 µl Additional info:

PROTOCOL

. Lysis Buffer for Phenol/ chloroform genomic DNA extraction (makes 20 mL of buffer)

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NOTES
Ashley Humphrey 17 Aug 2017

For example, 1.4 mL, 2.1 mL, etc., depending on pellet volume.

Step 2.1.

1 M Tris-Cl

AMOUNT
200 µl Additional info: pH 8.0

Step 2.2.

0.5 M EDTA

AMOUNT
4 µl Additional info: pH 8.0

Step 2.3.

SDS

AMOUNT
1 g Additional info:

Step 3.

Pass sample through a 1 mL or a 5 mL syringe fitted with a 21 gauge needle, twenty times.

Step 4.

Spin cells down again for 20 minutes at maximum speed at 4º C, to pellet unlysed cellular material.

NOTES
Ashley Humphrey 11 Sep 2017

Maximum speeds vary, adjust according to individual availability.

Step 5.

Split the supernatant from each conical tube into 700 µL aliquots and place into 1.5 mL Eppendorf tubes.

NOTES
Daniel Richter 15 Sep 2017

If desired, retain mostly bacterial unlysed pellets for later use.

Step 6.

Add 3.5 µL RNAse A per tube (4 mg/ml, final concentration 20 ng/µl).

AMOUNT
3.5 µl Additional info: RNAse A

Step 7.

Incubate for 5 minutes at room temperature on a nutator.

Step 8.

Add 7 µL of Proteinase K (at 10 mg/mL, final concentration of 100 ng/µL).

AMOUNT
7 µl Additional info: Proteinase K

Step 9.

Incubate at 50º C for 3 hours, swirling occasionally.

Step 10.

Cool to room temperature.

Step 11.

Add 700 µL of phenol:chloroform:isoamyl alcohol at a pH of 8.0, per tube.

AMOUNT
700 µl Additional info: per tube

NOTES
Ashley Humphrey 27 Jun 2017

All phenol:chloroform steps should be performed in the chemical hood.

Step 12.

In hood, shake vigorously to mix.

Step 13.

In centrifuge, spin for 10 minutes at maximum speed, at room temperature.

Step 14.

Transfer aqueous phase (upper) into a new tube.

Step 15.

Repeat phenol:chloroform:isoamyl extraction (steps 11-14 above) until no protein remains at interphase.

NOTES
Ashley Humphrey 27 Jun 2017

This can take 3-4 times.

Step 16.

Add an equal volume of chloroform:isoamyl alcohol (no phenol), and shake vigorously to mix.

Step 17.

Spin for 10 minutes at maximum speed, at room temperature.

Step 18.

Remove aqueous phase (upper), combining into a single tube with room for 3X the volume of sample.

NOTES
Ashley Humphrey 27 Jun 2017

Can use a 1.5 mL Eppendorf tube or a 15 mL conical.

Step 19.

Add 1/20 of the volume of 10 M ammonium acetate, for a final concentration of 0.5 M.

Step 20.

Add 1/3 µL of GlycoBlue per 100 µL of aqueous phase.(at 15 mg/mL, final concentration 50 ng/µL)

Step 21.

Add 2x the volume of 100% EtOH.

Step 22.

Invert 5-10 times to precipitate DNA.

Step 23.

Precipitate several hours, or overnight at -20º C.

Step 24.

Spin at maximum speed at room temperature for 20 minutes.

Step 25.

Pour off supernatant.

Step 26.

Add 1 mL or 10 mL of 100% EtOH to wash pellet.

NOTES

Ashley Humphrey 11 Sep 2017

Volume of EtOH to use depends on whether the pellet is in a 1.5 mL or 15 mL tube.

Step 27.

Wash on nutator for 5 minutes at room temperature.

Step 28.

Spin 5-20 mintues at maximum speed.

Step 29.

Repeat 100% EtOH wash, for a total of 2-3 washes.

Step 30.

Pour off supernatant.

Step 31.

Add 1 mL or 10 mL of 70% EtOH, depending on tube size, to wash pellet.

Step 32.

Wash on nutator for 5 minutes at room temperature.

Step 33.

Spin 5-20 minutes at maximum speed.

Step 34.

Pour off supernatant.

Step 35.

Repeat 70% EtOH wash for a total of 2-3 washes.

NOTES

Ashley Humphrey 11 Sep 2017

Volume of EtOH to use depends on whether the pellet is in a 1.5 mL or 15 mL tube.

Step 36.

Remove supernatant, dry pellet for 5-10 minutes, do not over dry.

Step 37.

Re-suspend overnight in a small volume (50-500 µL) of DNAse-free water.

Warnings

Do all Phenol and Chloroform procedures in a fume hood.