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Modified Phenol Chloroform Genomic DNA Extraction Protocol from the Christner Lab (University of Florida) V.2

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Lead author S. Barnes did not create protocol but is submitting with permission of author N. Bryan.

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Christner Lab protocol reference:

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Phenol Chloroform Extraction , Genomic DNA Extraction

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Lysis Step:

10% SDS
TE Buffer (1x) - diluted with Molecular Bio Water
Proteinase K (20 mg/mL) - in Molecular Bio Water
Lysozyme (10 mg/mL) - in Molecular Bio Water

Phenol Chloroform Extraction Step:

Phenol Chloroform Isoamyl alcohol (25:24:1)
Chloroform Isoamyl alcohol (24:1)
3 - 2 mL microcentrifuge tubes per sample

Ethanol Precipitation Step:

Ice cold 100% Ethanol
3M Sodium Acetate pH 5.2
70% Ethanol - diluted with Molecular Bio Water
Molecular Bio Water

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**

Filter with 0.2 um PES filter before use:
10% SDS
1x TE Buffer
100% Ethanol
70% Ethanol
All Molecular Bio Water used as reagent or diluent

Take precaution when working with Phenol Chloroform Isoamyl Alcohol and Chloroform Isoamyl Alcohol.
Use eye protection and work in highly ventilated space like fume hood.

Phenol Chloroform Isoamyl Alcohol
H301 Toxic if swallowed. H312 + H332 Harmful in contact with skin or if inhaled. H314 Causes severe skin burns and eye damage. H336 May cause drowsiness or dizziness. H341 Suspected of causing genetic defects. H351 Suspected of causing cancer. H361

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Suspected of damaging fertility or the unborn child. H372 Causes damage to organs (Liver, Kidney) through prolonged or repeated exposure if swallowed. H373 May cause damage to organs (Nervous system, Kidney, Liver, Skin) through prolonged or repeated exposure.

Chloroform Isoamyl Alcohol
H227 Combustible liquid. H302 Harmful if swallowed. H315 Causes skin irritation. H318 Causes serious eye damage. H331 Toxic if inhaled. H336 May cause drowsiness or dizziness. H351 Suspected of causing cancer. H361 Suspected of damaging fertility or the unborn child. H372 Causes damage to organs (Liver, Kidney) through prolonged or repeated exposure if swallowed.

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Lysis 5h

1 Start with filtered cells OR spin to get pellet from liquid culture.

1.1 Pipette off supernatant, if any.

2 Add 380 uL 1x TE Buffer + 20 uL Lysozyme to sample.

3 Incubate for 1 h at 37˚C.

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4 Add 60 uL SDS + 30 uL Proteinase K + 110 uL 1x TE Buffer (total volume = 600 uL) to sample.

5 Incubate for 2 h at 37˚C.

6 Vortex tube thoroughly.

7 Incubate at 37˚C for 1 h , or until samples are clear.

8 Incubate at 65˚C for 30 min to inactivate Proteinase K.

9 Stop here and store at -20˚C or move to extraction.

Phenol Chloroform Extraction 1h

10 Add equal volume (600 uL) of Phenol Chloroform Isoamyl alcohol (P/C/I) to sample.

10.1 Invert samples thoroughly before centrifuge step.

11 Spin at 10,640 rcf for 10 min.

11.1 White interface should be visible.

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12 Transfer aqueous phase (top layer) to new tube.

12.1 Transfer approx. 500 - 600 uL in 100 uL increments.

12.2 Do not disturb interface.

13 Add equal volume of Chloroform Isoamyl alcohol (C/I) to sample.

13.1 Approx. 500 uL

13.2 Invert sample thoroughly before centrifuge step.

14 Spin at 10,640 rcf for 10 min.

14.1 White interface should be visible.

15 Transfer aqueous phase (top layer) to new tube.

15.1 Do not disturb interface.

15.2 Approx. 300 - 500 uL

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16 Store at -20˚C or move to ethanol precipitation.

Ethanol Precipitation 2h

17 Add 2 volumes of ice cold 100% Ethanol to sample.

17.1 E.g. Have 50 uL of DNA solution, add 100 uL Ethanol.

18 Add 0.1 volumes of 3M Sodium Acetate pH 5.2 to sample.

18.1 E.g. Have 50 uL DNA solution, add 5 uL Sodium Acetate.

19 Incubate at -20˚C for at least 1 hr.

19.1 Cold incubation for a longer period of time (e.g. over night) may lead to higher yield.

20 Spin at 13,000 rcf for 30 min at 4˚C .

20.1 Should see small white pellet.

If tube is placed hinge-side outward, the pellet should be found below the hinge and almost to the bottom.

21 Pipette off supernatant.

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22 Gently add 150 uL of 70% ethanol to remove salts.

22.1 Do not dislodge pellet.

Keeping the samples on ice or in cold tube racks will help pellet stay in place.

23 Spin at 13,000 rcf for 10 min at 4˚C .

24 Pipette off supernatant.

25 Spin at 13,000 rcf for 1 min to bring down any excess ethanol.

26 Pipette off any remaining liquid.

27 Dry pellet for 5 min MAX.

27.1 Any longer makes resuspending difficult.

28 Resuspend pellet in 50 uL Molecular Bio water.

28.1 Use 1- 3 uL of DNA to quantify using Qubit HS dsDNA assay kit.

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29 Store at -20˚C .

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