DNA EXTRACTION USING PHENOL-CHLOROFORM

Santiago Montero-Mendieta

1Doñana Biological Station (Seville, Spain)

DOI

dx.doi.org/10.17504/protocols.io.q2cdyaw

PROTOCOL CITATION

Santiago Montero-Mendieta 2018. DNA EXTRACTION USING PHENOL-CHLOROFORM. protocols.io

https://dx.doi.org/10.17504/protocols.io.q2cdyaw

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jun 15, 2018

LAST MODIFIED

Jun 15, 2018

PROTOCOL INTEGER ID

13092

Citation: Santiago Montero-Mendieta (06/15/2018). DNA EXTRACTION USING PHENOL-CHLOROFORM. https://dx.doi.org/10.17504/protocols.io.q2cdyaw

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
GUIDELINES

REAGENT PREPARATION

★ Laird’s buffer (100ml) (adjust pH to 8.5) [store at room temperature]

- Tris: 1.21 g
- EDTA-NA$_2$: 0.19 g
- NaCl: 1.17 g
- SDS: 0.2% (= 1 ml if 20% SDS stock is used)
- ddH$_2$O: 99 ml (depends of SDS’s volume)

★ Proteinase K (20mg/ml): Dilute 10 mg of proteinase K in 0.5 ml of ddH$_2$O. [store at -20ºC]

★ Phenol: stock concentration

★ P/C/I, Phenol-Chloroform-Isoamyl (25:24:1) [store at 4ºC]

To prepare a 250 ml solution:
- Phenol: 125 ml
- Chloroform: 120 ml
- Isoamyl: 5 ml

★ C/I, Chloroform-Isoamyl (24:1) [store at 4ºC]

To prepare a 250 ml solution:
- Chloroform: 240 ml
- Isoamyl: 10 ml

★ NaCl 5M: 29.24 g of NaCl is dissolved in H$_2$O up to 100 ml. Use it within 1 month. Alternatively, NaAc 3M: 40.8 g of NaAc is dissolved in H$_2$O up to 100 ml. [store at RT]

★ Ethanol 100% or alternatively: propanol. [store at room temperature]

★ Ethanol 70%: To prepare 50 ml: 36.5 ml Ethanol 100% + 13.5 ml ddH$_2$O. [store at -20ºC]
MATERIALS

**EDTA** Contributed by users

**Ethanol 100%** Contributed by users

**Phenol** Sigma Aldrich

**Proteinase K** Thermo Fisher Scientific Catalog #E00491

**NaCl** Sigma Aldrich Catalog #53014

**Tris-HCl (Tris-Hydrochloride)**, 100gm Promega Catalog #H5121

**SDS** Bio Basic Inc. Catalog #SB0485.SiZE.500g

**double distilled water (ddH2O)** Contributed by users

**Phenol-chloroform-isooamyl alcohol 25:24:1 (PCI)** Invitrogen - Thermo Fisher Catalog #15593049

**Ethanol 70%** Contributed by users

---

**DAY 1**

1. Add 500 µl Laird’s buffer in 1.5 ml eppendorf tube, one for each sample.

2. Cut 10-30 µg of muscle tissue and put it in the tube with Laird’s buffer.

3. Add 20 µl Proteinase K (20 mg/ml) to each tube

4. Incubate overnight in movement at 56°C (or at least 4 hours). *If your samples are not completely solved, add more Proteinase K and incubate for longer time.DAY 2:

5. Add 500 µl Phenol to each tube and shake heavily during 10 min.

6. Centrifuge for 10 min (4ºC) at 13000 rpm.

7. Pipette and keep all of the supernatant (top layer) into a new 1,5 eppendorf tube.

---

**Citation:** Santiago Montero-Mendieta (06/15/2018). DNA EXTRACTION USING PHENOL-CHLOROFORM. [https://dx.doi.org/10.17504/protocols.io.q2cdyw](https://dx.doi.org/10.17504/protocols.io.q2cdyw)

This is an open access protocol distributed under the terms of the *Creative Commons Attribution License* ([https://creativecommons.org/licenses/by/4.0/](https://creativecommons.org/licenses/by/4.0/)), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
8 Add 500 µl Phenol-Chloroform-Isoamyl and shake heavily during 10 min.

9 Centrifuge for 10 min (4°C) at 13000 rpm.

10 Pipette and keep all of the supernatant (top layer) into a new 1,5 eppendorf tube.

11 Add 500 µl Chloroform-Isoamyl and shake heavily during 10 min.

12 Centrifuge for 10 min (4°C) at 13000 rpm.

13 Pipette and keep all of the supernatant (top layer) into a new 1,5 eppendorf tube. Be careful not to get anything of the down layer.

14 Add 0.1 volumes of 3M NaAC to each tube and mix it softly (do not use vortex). E.g.: 40 µl to 400 µl of supernatant.

15 Add 2 volumes of ice cold 95-100% ethanol (previously stored at -20°C) to each tube E.g.: 800 µl to 400 µl of supernatant. Mix it softly (turn the tubes upside down).

16 Leave at -20°C overnight (or at least 5-6 hours). DAY 3:

17 Centrifuge for 30 min (4°C) at 13000 rpm.

18 Pour off ethanol.

19 Add 1000 µl ice cold ethanol 70%.

20 Centrifuge for 15 min (4°C) at 13000 rpm.

---

Citation: Santiago Montero-Mendieta (06/15/2018). DNA EXTRACTION USING PHENOL-CHLOROFORM. https://dx.doi.org/10.17504/protocols.io.q2cdyw

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
21 Pour off ethanol.

22 Centrifuge for 5 min (4°C) at 13000 rpm.

23 Pour off residual ethanol.

24 Dry pellet completely by leaving the tube open at room temperature.

25 Dissolve pellet in 100 µl ddH2O.