JUN 15, 2018

DNA EXTRACTION USING PHENOL-CHLOROFORM

Santiago Montero-Mendieta¹

¹Doñana Biological Station (Seville, Spain)

CONSEVOL

Santiago Montero-Mendieta

Doñana Biological Station (CSIC)

OPEN ACCESS

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

Protocol status: Working
We use this protocol and it's working

Created: Jun 15, 2018

Last Modified: Jun 15, 2018

PROTOCOL integer ID:
13092

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 ddH₂O. [store at -20°C]

★ **Phenol**: stock concentration

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

To prepare a 250 ml solution:

Phenol	125 ml
Chloroform	120 ml
Isoamyl	5 ml

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

To prepare a 250 ml solution:

Chloroform	240 ml
Isoamyl	10 ml

★ **NaCl 5M**: 29,24 g of NaCl is dissolved in H₂O up to 100 ml. Use it within 1 month.
Alternatively, **NaAc 3M**: 40,8 g of NaAc is dissolved in H₂O 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 ddH₂O. [store at -20°C]

MATERIALS

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-isoamyl 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:

DAY 2

- 5 Add 500 µl Phenol to each tube and shake heavily during 10 min.
- 6 Centrifuge for 10 min (4°C) at 13000 rpm.
- 7 Pipette and keep all of the supernatant (top layer) into a new 1,5 eppendorf tube.
- 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).DAY3:

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.

- 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.