Jun 15, 2018

DNA EXTRACTION USING PHENOL-CHLOROFORM

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

Santiago Montero-Mendieta[1]

[1]Doñana 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**

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

Created: June 15, 2018

Last Modified: June 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_2O	99 ml (depends of SDS's volume)

★ **Proteinase K (20mg/ml):** Dilute 10 mg of proteinase K in 0,5 ml of ddH_2O. [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_2O up to 100 ml. Use it within 1 month.
Alternatively, **NaAc 3M**: 40,8 g of NaAc is dissolved in H_2O 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_2O. [store at -20ºC]

Materials

MATERIALS

- EDTA
- Ethanol 100%
- Phenol **Merck MilliporeSigma (Sigma-Aldrich)**
- Proteinase K **Thermo Fisher Scientific** **Catalog #**EO0491
- NaCl **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**53014
- Tris-HCl (Tris-Hydrochloride), 100gm **Promega** **Catalog #**H5121
- SDS **Bio Basic Inc.** **Catalog #**SB0485.SIZE.500g
- double distilled water (ddH2O)
- Phenol-chloroform-isoamyl alcohol 25:24:1 (PCI) **Invitrogen - Thermo Fisher** **Catalog #**15593049
- Ethanol 70%

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 56ºC (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 (4ºC) 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 (4ºC) 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 (4ºC) 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 -20ºC) to each tube E.g.: 800 µl to 400 µl of supernatant. Mix it softly (turn the tubes upside down).

16 Leave at -20ºC overnight (or at least 5-6 hours).DAY3:

DAY 3

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

18 Pour off ethanol.

19 Add 1000 µl ice cold ethanol 70%.

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

21 Pour off ethanol.

22 Centrifuge for 5 min (4ºC) 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.