DNA EXTRACTION USING PHENOL-CHLOROFORM

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Abstract

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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

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

Protocol

DAY 1

Step 1.

Add 500 µl Laird's buffer in 1,5 ml eppendorf tube, one for each sample.

DAY 1

Step 2.

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

DAY 1

Step 3.

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

DAY 1

Step 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

Step 5.

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

DAY 2

Step 6.

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

DAY 2

Step 7.

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

DAY 2

Step 8.

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

DAY 2

Step 9.

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

DAY 2

Step 10.

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

DAY 2

Step 11.

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

DAY 2

Step 12.

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

DAY 2

Step 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.

DAY 2

Step 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.

DAY 2

Step 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).

DAY 2

Step 16.

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

DAY 3

Step 17.

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

DAY 3

Step 18.

Pour off ethanol.

DAY 3

Step 19.

Add 1000 µl ice cold ethanol 70%.

DAY 3

Step 20.

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

DAY 3

Step 21.

Pour off ethanol.

DAY 3

Step 22.
Centrifuge for 5 min (4ºC) at 13000 rpm.

DAY 3

Step 23.
Pour off residual ethanol.

DAY 3

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

DAY 3

Step 25.
Dissolve pellet in 100 µl ddH2O.