# PROTOCOL FOR:

Simple, inexpensive and RNase-free purification of plasmid DNA by fractional precipitation with isopropanol

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## LEGEND

***ATTENTION***

\* ***HINT***

***REST***

# REAGENTS AND MATERIALS

# Reagents

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemicals** | **Abbreviation** | **Manufacturer** | **Catalog Number** |
| Ethylenediaminetetraacetic acid disodium salt dihydrate | EDTA-Na**2** | Sigma-Aldrich | E9884 |
| Isopropanol (2-propanol) |  | Honeywell Riedel-de Haën | 24137 |
| Potassium acetate | KOAc | Sigma-Aldrich | 236497 |
| Sodium hydroxide | NaOH | Sigma-Aldrich | S8045 |
| Sodium dodecyl sulfate | SDS | Sigma-Aldrich | 436143 |
| Tris base, Tris(hydroxymethyl)aminomethane | Tris | Sigma-Aldrich | T1503 |

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| **Solutions** | **Abbreviation** | **Ingredients** |
| Alkaline lysis solution I | Sol I | 25 mM Tris-HCl, pH 7.5, 10 mM EDTA-Na**2**, pH 8.0 |
| Alkaline lysis solution II | Sol II | 0.1N NaOH, 1% SDS |
| Alkaline lysis solution III | Sol III | 3M KOAc, pH 5.5 |
| 70% Ethanol |  | Ethanol/water (v/v 70/30) |
| TE |  | 10 mM Tris-HCl, pH 7.5, 1 mM EDTA-Na**2** |

# Materials

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| --- | --- |
| **Pipettes, tubes and tips** | **Manufacturer (product code)** |
| PIPETMAN P1000, 100-1000 µL | Gilson (F144059M) |
| PIPETMAN P200, 20-200 µL | Gilson (FA10005M) |
| 1.5 mL Microcentrifuge Tubes | Axygen (MCT-150-C) |
| 200 µl Yellow Reference Tips | Axygen (TR-222-Y) |
| 1000 µl Blue Pipet Tips | Axygen (T-1000-B) |

# PROCEDURE

The following protocol is a modification of alkaline lysis procedure for plasmid minipreparation described in {1] and is based on classical works of Birnboim and Doly 1979 [2] and Ish-Horowicz and Burke 1981 [3]. Schematic illustration of the protocol is shown in Fig. 1 (see below).

All centrifugation steps are carried out at room temperature

Steps (1-12):

(1) Transfer 1.4 ml overnight bacterial culture into a 1.5 ml microcentrifuge tube. Centrifuge at maximum speed (12,100 x g) for 1 min to pellet bacteria.

(2) Remove the supernatant by aspiration and resuspend the pellet in 200 µl of Solution I by vigorous vortexing. To facilitate resuspension use pipette tip.

(3) Add 400 µl of Solution II. Mix end-over-end by hand 5-6 times and place on ice for 2-3 min. Do not vortex.

(4) Add 300 µl of ice-cold Solution III and mix by inverting the tube several times.  Note the complete and uniform formation of white pellet. Let stand on ice for 2-3 min.

(5) Centrifuge at maximum speed for 5 min.

(6) Transfer 875 µl of the supernatant to a new tube containing 290 µl of isopropanol (0.33 volume) and mix.

(7) Centrifuge at max speed for 5 min.

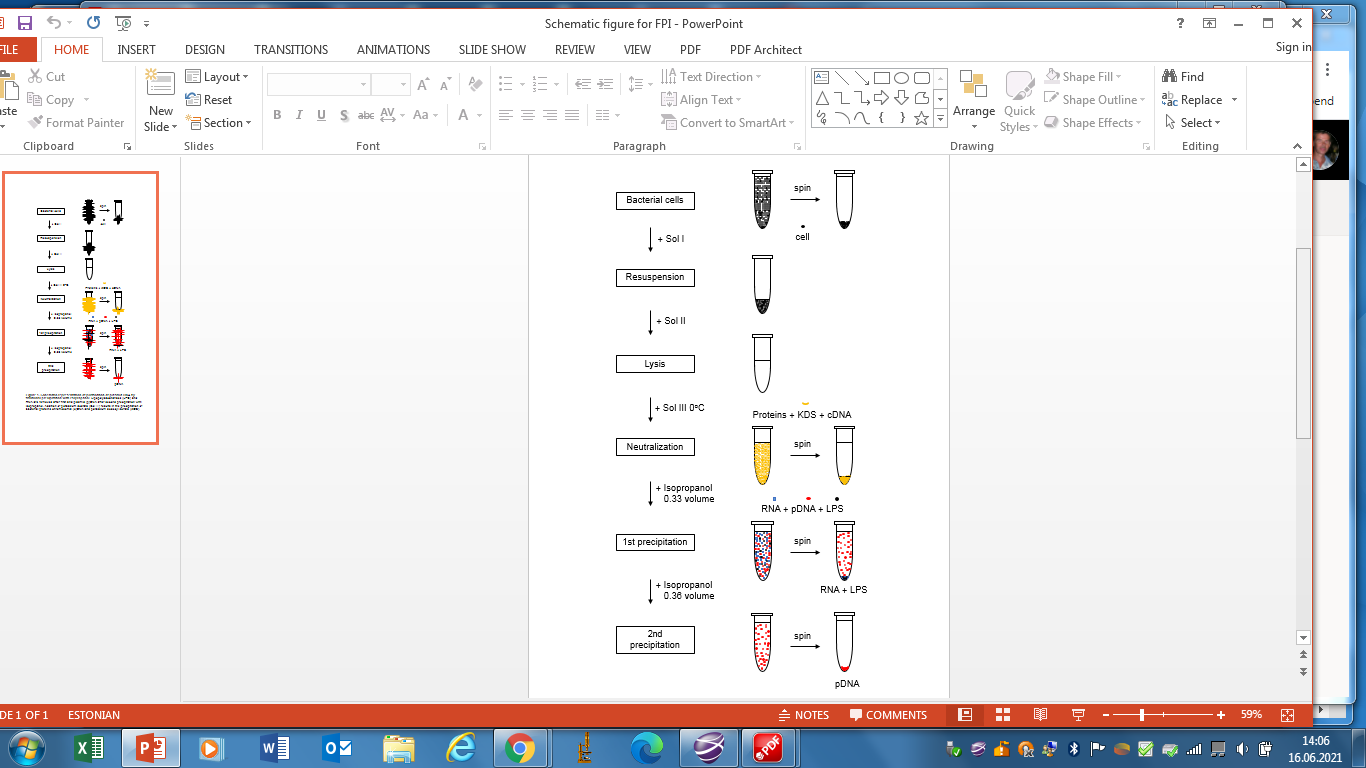
(8) Carefully remove the supernatant (1150 µl) and transfer to a new tube containing 30 µl of isopropanol (0.36 volumes of the initial solution) and mix immediately. \*Avoid touching the pellet, containing RNA, bacterial DNA and polysaccharides.

(9) ****Centrifuge at maximum speed for 8 min.

(10) Remove most of the supernatant by aspiration, quick spin and very carefully remove the remaining 100-150 µl portion with a pipette. Avoid touching the transparent pellet, normally containing 10-20 µg of plasmid DNA.

(11) Wash the pellet with 200-400 µl of 70% Ethanol, centrifuge and remove supernatant completely from the opposite side of the pellet.

(12) Dissolve the pellet in 50 µl TE and analyse about 1/50 of plasmid DNA by agarose gel electrophoresis using appropriate standards.



**Figure 1**. **Schematic representation of purification of plasmid DNA by fractional precipitation with isopropanol.** Lipopolysaccharides (LPS) and RNA are removed after first and plasmid (p)DNA after second precipitation with isopropanol. Addition of potassium acetate (Sol III) results in the precipitation of bacterial proteins, chromosomal (c)DNA and potassium dodecyl sulfate (KDS).

# EQUIPMENT

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| **Equipment** | **Manufacturer** |
| MiniSpin personal microcentrifuge | Eppendorf |
| Icebox containing wet ice |  |

# TROUBLESHOOTING

To guarantee rapid mixing, and avoid oversaturation and/or precipitation at the interphase between alcohol and aqueous solution (Steps 6 and 8), it is important to add the aqueous solution to the isopropanol, not vice versa. This is opposite to traditional precipitation with alcohol where the latter is added to the aqueous solution. Care must be taken in washing the pellet with 70% Ethanol (Step 11).

# REFERENCES

[1] Green MR, Sambrook J. Preparation of Plasmid DNA by Alkaline Lysis with Sodium Dodecyl Sulfate: Minipreps. *Cold Spring Harb Protoc*. 10, 911-916 (2016).

[2] Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*. 7(6), 1513-1523 (1979).

[3] Ish-Horowicz D, Burke JF. Rapid and efficient cosmid cloning. *Nucleic Acids Res*. 9(13), 2989-2998 (1981).