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Prepare gDNA for CRISPR Screen V.1

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We are still developing and optimizing this protocol

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Disclaimer

Abstract

The preparation of genomic DNA (gDNA) is a critical step for CRISPR screening applications, ensuring high-quality DNA suitable for downstream analysis such as PCR amplification and sequencing. This protocol details a streamlined method for extracting gDNA from approximately 30 million cells using QuickExtract DNA Extraction Solution. The process involves cell lysis, RNase treatment, phenol-chloroform extraction, and isopropanol precipitation to ensure the isolation of high-purity DNA. Phase Lock Gel technology is employed to enhance phase separation efficiency during organic extraction. The final DNA pellet is resuspended in EB buffer and quantified before proceeding to PCR.

Materials

Reagents and Chemicals

Quick Extract DNA Extraction Solution (Lucigen, Catalog #QE0905T)
A specialized buffer designed for rapid and efficient DNA extraction from cells. It facilitates cell lysis and initial DNA release for downstream processing.
PureLink™ RNase A (20 mg/mL) (Thermo Fisher, Catalog #12091021)
An enzyme that degrades RNA contaminants in the extracted genomic DNA, ensuring RNA-free samples for accurate downstream applications.
UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) (Thermo Fisher Scientific, Catalog #15593031)
A reagent used for organic phase separation, effectively removing proteins and other cellular contaminants from the DNA extract.
Chloroform
Used in the extraction process to enhance the separation of DNA from proteins and lipids, ensuring high-purity DNA recovery.
Phase Lock Gel Heavy 2 mL (Quantabio, Catalog #2302830)
A gel-based barrier that simplifies phenol-chloroform extraction by preventing interphase contamination and improving DNA recovery.
Ammonium Acetate (7.5 M solution)
Used in DNA precipitation to remove proteins and enhance nucleic acid purity by reducing the solubility of DNA.
Isopropanol (100%)
Facilitates DNA precipitation, allowing efficient recovery of genomic DNA from the aqueous phase. The use of 100% isopropanol ensures optimal DNA yield.
Ethanol (70%)
Used for washing the DNA pellet, removing residual salts and impurities, and replacing isopropanol for easier redissolution.
EB Buffer (10 mM Tris-Cl, pH 8.5)
A low-salt buffer used for DNA resuspension, providing stable conditions for DNA storage and downstream applications.

Equipment and Consumables

Eppendorf Thermomixer R (1.5 mL block)
- Maintains precise temperature and agitation settings for enzymatic digestion and incubation steps.

Microcentrifuge (capable of 16,000 x g spin speeds at 4C and room temperature)
Essential for phase separation, DNA precipitation, and pellet washing.
Sherlock Tube Cap (USA Scientific)
Used to securely cap tubes during heating and vortexing steps.
Pipettes and Pipette Tips
Used for precise liquid handling during all stages of DNA preparation.
1.5 mL Microcentrifuge Tubes
Used for sample processing, extraction, and DNA storage.

Protocol materials

- Quick Extract DNA Extraction Solution **Lucigen** **Catalog #**QE0905T
- PureLink™ RNase A (20 mg/mL) **Thermo Fisher** **Catalog #**12091021
- UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific** **Catalog #**15593031
- Phase Lock Gel Heavy 2 ml **Quantabio** **Catalog #**2302830

Lyse Cells in QuickExtract Buffer

1h 30m

1 Add 500 µL Quick Extract DNA Extraction Solution **Lucigen Catalog #**QE0905T to approximately 30 million cell pellet.

2 Mix by vortexing vigorously for 15 seconds.

3 Cap with Sherlock tube cap (USA Scientific). Heat in Eppendorf Thermomixer R (1.5 ml block) at 65 °C 1000 rpm for 01:00:00

1h

4 Vortex vigorously for 15 seconds and centrifuge at full speed in microcentrifuge for 5 seconds.

5 Heat at 98 °C for 5 minutes.

6 Vortex vigorously for 15 seconds and centrifuge at full speed in microcentrifuge for 5 seconds.

7 Incubate on ice for 1 minute.

8 Add 5 µL PureLink™ RNase A (20 mg/mL) **Thermo Fisher Catalog #**12091021 .
Incubate in Thermomixer at 37 °C 1000 rpm for 00:30:00

30m

9 Keep on ice.

Phenol-Chloroform Extraction

15m

10 Add 500 µL
UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific Catalog #**15593031

11 Vortex vigorously for 1 minute.

12 Centrifuge at full speed in microcentrifuge (16873 rcf, 25°C, 00:05:00) for 5 minutes. 5m

13 Prepare a phase lock tube (Phase Lock Gel Heavy 2 ml **Quantabio Catalog #**2302830) by centrifuging at 12,000 to 16,000 x g for 15 seconds.

14 In hood, carefully transfer the QuickExtract lysate into the the phase lock tube by decanting or pipetting.

15 Centrifuge 16000 x g, 25°C, 00:05:00 to separate the phases. 5m

16 Add 500 µL to upper phase. Mix thoroughly without vortexing or disrupting the Phase Lock Gel barrier.

17 Centrifuge 16000 x g, 25°C, 00:05:00 to separate the phases. 5m

18 Carefully transfer by decanting or pipetting the upper aqueous layer to a new 1.5 ml centrifuge tube.

Isopropanol precipitation 10m

19 Add ammonium acetate ([M] 7.5 Molarity (M)) to a final concentration of [M] 2.5 Molarity (M) (i.e. 1/2 volume ~ 250 µL).

20 Add 0.7X volume of Isopropanol (~ 525 µL) and vortex briefly to mix.

21 Incubate at room temperature for 00:10:00 10m

22 Centrifuge the sample immediately at 10,000–15,000 x g for 15–30 min at 4°C.

23 Carefully decant the supernatant without disturbing the pellet.

24 To wash the DNA pellet, add 1 ml of room-temperature 70% ethano. This step removes co-precipitated salts and replaces isopropanol with the more volatile ethanol, facilitating easier redissolution of the DNA.

25 Centrifuge at 10,000–15,000 x g for 5–15 min at 4°C.

26 Carefully remove the supernatant without disturbing the pellet.

27 Quick spin in microcentrifuge to bring residual Ethanol to bottom of tube.

28 Using a p200 pipette, remove residual Ethanol. Leave pellet.

29 After briefly air drying (< 2 minutes), resuspend in 100 µL EB buffer (10 mM Tris-Cl, pH 8.5).

30 Let DNA solution sit at room temperature overnight to rehydrate. Vortex well.

31 Quantify DNA and proceed to PCR.