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High Molecular Weight DNA Extraction from Cannabis sativa for Long-Read Sequencing V.1

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We are still developing and optimizing this protocol

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Abstract

This protocol describes a method for isolating high molecular weight (HMW) genomic DNA from Cannabis sativa plant tissue suitable for long-read sequencing platforms. The protocol uses nuclei isolation to minimize nuclease contamination and mechanical homogenization with the Precellys Evolution system. Expected yield: ~4 µg HMW DNA per gram of young shoot tissue.

Guidelines

- Nuclei isolation is critical to remove nucleases from vacuoles
- Protocol optimized for long-read sequencing of highly duplicated genes (e.g., cannabinoid synthases)
- Work performed under Resolution 1164 of 2021, Ministry of Justice, Republic of Colombia

Materials

Equipment
- Precellys Evolution homogenizer (Bertin Technologies)
- Microcentrifuge (refrigerated, capable of 12,000g)
- Centrifuge (capable of 1,500g, refrigerated to 4°C)
- Heat block or water bath (65°C)
- Vortex mixer
- Liquid nitrogen container

Consumables
- For 1g tissue: 6× reinforced 2mL tubes (P000943-LYSK0-A) OR 1× 7mL tube (P000944-LYSK0-A)
- 2.8mm stainless steel beads (P000925-LYSK0-A)
- 60 µm and 30 µm cell strainers/filters
- Disposable pipettes and wide-bore pipette tips
- 1.5-2 mL microcentrifuge tubes
- Plastic inoculation loop or p200 tip

Reagents
- **Nuclei Isolation Buffer (NIB)****
  - Prepare according to Circulomics Nuclei Isolation Protocol
  - Store at 4°C
- **Homogenization Buffer (HB)****
  - Prepare 1L of 1× solution (as per Edwards 1991)
  - Contains PVP (polyvinylpyrrolidone) and BME (β-mercaptoethanol)
  - Store at 4°C
- **Edward's Lysis Buffer****
  - 200 mM Tris-HCl pH 7.5
  - 250 mM NaCl
  - 25 mM EDTA
  - 0.5% SDS
- **Other Reagents****
  - Proteinase K
  - 1M KCl
  - RNase (DNase-free)
  - Chloroform ($CHCl_3$)
  - Saturated NaCl solution
  - 95% ethanol and 70% ethanol
  - EB buffer (10 mM Tris-HCl pH 8.0, no EDTA)
  - Liquid nitrogen

Troubleshooting

Safety warnings

⚠️ **Chloroform**: Use in fume hood. Toxic and potentially carcinogenic.**
⚠️ **Liquid nitrogen**: Wear cryogenic gloves and face protection. Can cause severe cold burns.**
⚠️ **β-mercaptoethanol (BME)**: Toxic. Use in fume hood with appropriate PPE.**

PART 1: Nuclei Isolation

1 Select young shoot tips (preferred tissue type)

Optional: Incubate plants in darkness for 3 days prior to extraction to reduce carbohydrate content

0.2 Divide 1g fresh tissue among six 2mL tubes with 2.8mm metal beads, OR use one 7mL tube with 2.8mm metal beads

0.3 Snap-freeze tissue in liquid nitrogen

0.4 Homogenize frozen samples using Precellys Evolution at 6,200 RPM for 5 seconds
Tip: Keep samples frozen until homogenization to minimize nuclease activity.

0.5 Add 1.6 mL Nuclei Isolation Buffer (NIB) to each tube and resuspend thoroughly

Note: Buffer will likely freeze on contact with frozen sample

0.6 Incubate with gentle shaking at room temperature for 15 minutes

0.7 Filter sample sequentially through 60 µm filter, then 30 µm filter into new 2 mL tubes

0.8 Pellet nuclei by centrifugation at 1,500g for 1 minute at 4°C. Remove supernatant.

0.9 Resuspend pellet in 1 mL ice-cold NIB by pipetting

0.10 Repeat steps 4-5 until supernatant is absolutely clear (typically 2-5 washes)

0.11 Resuspend pellet in 1 mL ice-cold Homogenization Buffer (HB) and centrifuge at 1,500g for 1 minute at 4°C

0.12 Remove HB and either proceed to DNA purification or store nuclei pellet at -80°C
Checkpoint: Nuclei pellet should be free of green pigmentation and supernatant should be clear

PART 2: DNA Purification

0.13 Resuspend nuclei pellet in 30 µL Proteinase K and vortex strongly for 20 seconds

Critical: Ensure thorough resuspension so each nucleus is surrounded by proteinase

0.14 Add 400 µL Edward's buffer and vortex briefly to mix

Important: Beyond this point DNA is fragile. Use wide-bore tips and handle gently.

0.15 Incubate at 65°C for 30 minutes to 2 hours

0.16 Pellet cellular debris at 12,000g for 5 minutes

0.17 Transfer 400 µL supernatant to new tube, avoiding pellet

0.18 Add 200 µL of 1M KCl, mix by gentle inversion, and incubate at room temperature for 5 minutes

0.19 Centrifuge at 12,000g for 5 minutes and transfer 500 µL supernatant to new tube

0.20 Add 2.5 µL RNase, mix by inversion, and incubate at room temperature for 5 minutes

0.21 Add 500 µL chloroform and incubate at room temperature for 10 minutes with occasional inversion (perform in fume hood)

0.22 Separate phases at 5,000g for 1 minute

0.23 Transfer 400 µL aqueous (upper) layer to new tube. Do not disturb precipitate at interphase.

0.24 Add 200 µL saturated NaCl and mix by gentle inversion

0.25 Add 600 µL 95% ethanol and mix by inversion. DNA gel should become visible.

0.26 Hook out DNA using plastic inoculation loop or p200 tip and transfer to tube with 1 mL 70% ethanol

0.27 Incubate at room temperature for 10 minutes

0.28 Hook DNA into another tube with 1 mL fresh 70% ethanol

0.29 Centrifuge at 1,000g for 1 minute

0.30 Remove ethanol and air dry DNA gel at room temperature for 30 minutes (gel should appear clear)

0.31 Resuspend in 100 µL EB buffer (10 mM Tris-HCl pH 8.0, no EDTA)

0.32 Incubate at room temperature overnight to allow DNA to relax before quality control
Final Checkpoint: DNA should be in solution and appear viscous

Expected Results

1 Yield: ~4 µg HMW DNA per 1g young Cannabis sativa shoots
Quality Metrics:
- 260/280 ratio: ~1.8 (indicating pure DNA)
- 260/230 ratio: e2.0 (indicating removal of contaminants)
DNA Integrity: Should show high molecular weight fragments (3e50 kb) suitable for Oxford Nanopore and PacBio sequencing

2 Troubleshooting

Problem	Possible Cause	Solution
Low yield	Insufficient tissue/poor quality	Use younger shoot tips; increase starting material
Green contamination	Chloroplast contamination	Reduce centrifugation speed; increase wash steps
Degraded DNA	Nuclease activity	Work quickly; keep samples cold; ensure buffers are fresh
Poor 260/230 ratio	Polysaccharide/ polyphenol contamination	Increase wash steps; ensure dark incubation before harvest
DNA won't resuspend	Over-drying	Reduce air-drying time; incubate longer at RT to rehydrate

Protocol references

33. Circulomics Nuclei Isolation Protocol (Circulomics, Baltimore, MD, USA)
34. Edwards K, Johnstone C, Thompson C. (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research 19(6):1349

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