CTAB-based DNA extraction for citrus

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ABSTRACT

CTAB-based protocols are used for genomic DNA extraction from many kinds of plant species. However, the protocols can't necessarily completely remove contamination of polysaccharide and RNA in extracted genomic DNA solution. Especially, citrus leaves generally contain high polysaccharide. This protocol is a simple and efficient method for extracting genomic DNA for citrus without contamination of polysaccharide and RNA.

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CTAB, DNA extraction, citrus, High-salt precipitation solution

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Buffer preparation

1

- 2×CTAB solution: 2% (w/v) CTAB, 100mMTris-HCl pH8.0, 1.4M NaCl, 20mM EDTA pH8.0 [1]
- High-salt precipitation solution: 1.2M NaCl , 0.8M Sodium citrate [2]

- 10 mg/ml RNase (Nippon gene)
- Chloroform
- Isopropanol
- 70% Ethanol
- TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Reference

1. Allen GC, Flores-Vergara MA, Krasynanski S et al. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat Protoc 2006;1:2320–5.
2. Chomczynski P, Mackey K. Modification of the TRI Reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. Biotechniques 1995;19:492–5.

Homogenization and cell lysis

2 Preheat 2×CTAB solution to 60 °C in water bath. Add [M] 2 % (v/v) of 2-Mercaptoethanol to the 2×CTAB solution just before use.

3 Homogenize 100 mg of fresh leaf in liquid Nitrogen. Add 800 µL of 2×CTAB solution and completely suspend homogenized leaf. Transfer the suspended solution to 2 ml tube.

4 Add 4 µL of [M] 10 mg/mL RNase, mix by inversion, and incubate at 37 °C for 00:15:00 (In order to prevent RNase contamination in laboratory, RNase treatment is conducted before denaturing proteins by chloroform).

5 Incubate at 56 °C for 00:30:00 inverting the tube once every 00:10:00 .

Chloroform extraction

6 Add 300 µL of Chloroform and mix gently with tube rotator for 00:15:00 .

7 13000 rpm, 25°C, 00:10:00

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8 Transfer supernatant carefully to new 2 ml tube.

9 Repeat step 6 and 7

Precipitation and wash of DNA pellet

10 Transfer 600 µL of supernatant to new 1.5 ml tube, add 300 µL of High-salt precipitation solution, and mix by inversion.

11 Add 300 µL of Isopropanol and mix by inversion.

12 15000 rpm, 25°C, 00:10:00

13 Discard and remove supernatant (DNA pellet is often transparent).

14 Add 1000 µL of 70% ethanol and mix by inversion 10 times to wash salts.

15 15000 rpm, 25°C, 00:05:00

16 Completely remove supernatant and dry up DNA pellet in air.

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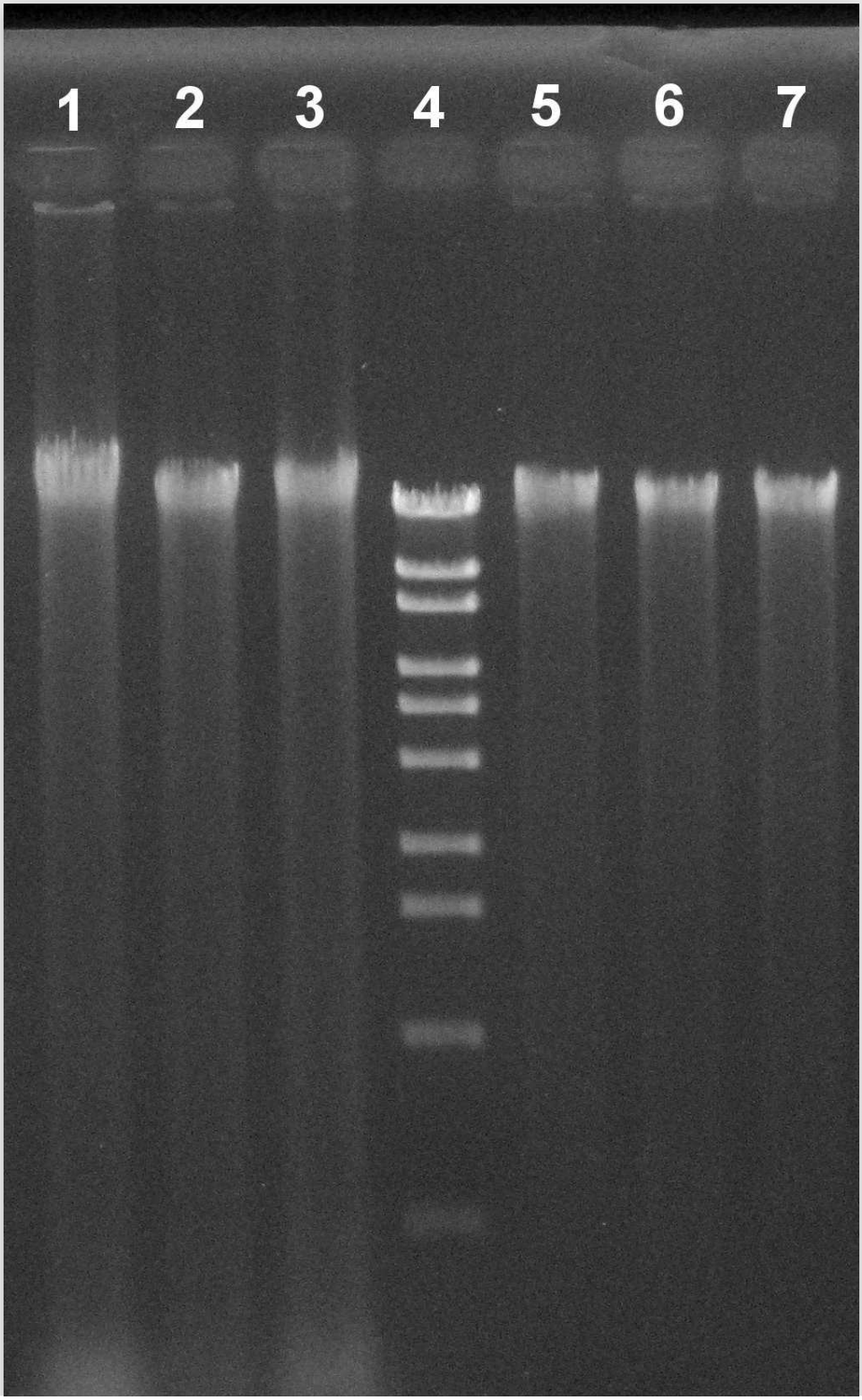
17 Dissolve pallet in 100 µL of TE buffer.

Result of gel electrophoresis

18



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0.8% agarose gel electrophoresis
Lane 1-3: Genomic DNA extracted by isopropanol precipitation .
Lane 4: size marker
Lane 4-6: Genomic DNA extracted by isopropanol precipitation with high salt precipitation solution.
All genomic DNA is a citrus cultivar, 'Kiyomi'.

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