

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 4876.7-2019

DNA barcode method Part 7: Poinsettia subgenus

DNA条形码方法 第7部分：一品红亚属

(English Translation)

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Foreword

This section is drafted in accordance with the rules given in GB/T 1.1-2009.

Please note that some elements of this document may involve patents. Publication of this document the agency is not responsible for identifying these patents.

This section is proposed and managed by the General Administration of Customs of the People's Republic of China.

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SN/T 4876 "DNA Barcoding Method" is divided into 7 parts.

- Part 1. Quarantine milk termites;
- Part 2. Quarantine broken eye *Monochamus*;
- Part 3. Quarantine volume moth;
- Part 4. Quarantine Sorghum
- Part 5. *Datura*;
- Part 6. *Pleurotus*
- Part 7. *Poinsettia* subgenus.

This section is part 7 of SN/T 4876.

DNA Barcoding Methods Part 7. Subgenus *Poinsettia*

1 Scope

This section specifies the DNA barcode detection method for common species of *Euphorbia poinsettia* subgenus.

This section applies to the determination, comparison analysis and judgment of DNA barcodes of common species of *Euphorbia poinsettia* subgenus.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB/T 3214 quarantine identification method

3 Terms and definitions

The following terms and definitions apply to this document.

DNA barcodes are short, standardized fragments of genes used to distinguish between different species. This fragment has obvious legacy among species Mutation and differentiation and easy PCR amplification.

The sequence of the sequence from 5' to 3' is as follows. external transcription spacer (ETS), 18S gene, internal transcription Spacer 1 (ITS1), 5.8S gene, internally transcribed spacer 2 (ITS2), 28S gene, and intergenic sequence spark, IGS). The 18S, 5.8S, and 28S genomic sequences in ribosomal RNA tend to be conserved in most organisms, and Change is small, while the internal transcription spacers ITS1 and ITS2 are non-coding regions, they have less selection pressure, relatively large changes, and Enough to provide the heritable traits required for a detailed systematic analysis. The amplified region selected by this standard includes 5.8S partial sequence, ITS2 the full sequence and the 28S partial sequence were amplified with a total length of about 449 bp, of which the length of the tooth split ETS2 was 219 bp.

Chloroplast NADH dehydrogenase complex F subunit gene
ChloroplastNADhyderogensubunitF, position in the small single copy region of the chloroplast genome, it is located at the end of the hydrophobic arm of the thylakoid NDH complex. Hypothetical chloroplast NADH 727bp.

4 Basic Information of the Subgenus Poinsettia

Euphorbia is one of the most genus in the angiosperms, with about 2,000 species, which are found all over the world, among which Africa and Central and South America are more common.

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Mayfield (1997) classified the Euphorbia poinsettia subgenus. There are about 24 species of this subgenus in the world.

Halberd, David Euphorbia and Orangutan are harmful weeds, and poinsettia and orangutan are garden plants. See GB/T 3214 for detailed information.

5 Method principle

Identification.

6.1 Apparatus and equipment

PCR instrument, electronic balance (sensitivity. 0.01g), freeze-mix mill, electrophoresis instrument, gel imaging system, high-speed refrigerated centrifuge, dissection Mirror, vortex shaker, refrigerator, water bath, sequencer, autoclave, adjustable micropipette (2 μ L, 10 μ L, 20 μ L, 100 μ L, 200 μ L, 1000 μ L) and corresponding tips, centrifuge tubes, PCR tubes, etc.

6.2 Main reagents

Unless otherwise specified, all reagents are analytical grade or biochemical reagents.

Plant Genome Extraction Kit, Ultrapure Water, DNA Marker, Agarose, Nucleic Acid Gel Dye, Liquid Nitrogen, Deoxyribonucleoside Triphosphate (dNTPs), Ta enzyme, 10 x PCR buffer, 10 x loading buffer, 50 x TAE buffer, primers (see Appendix B.1). CTAB method Extraction reagents (see Appendix A.1).

7.1 Processing of samples

See GB/T 3142 for sample identification. Plant material that needs to be extracted from DNA (1-2 seeds, silica gel dried leaves are 10mg), put into a 2mL round bottom centrifuge tube, add 2 steel balls to each tube, add liquid nitrogen and grind for 3min using a frozen mixing mill (30 times/second).

7.2 DNA preparation

Total plant DNA was extracted using a commercial plant genome extraction kit or a modified CTAB method. improved refer to Appendix A for the CTB method. 2.

7.3 DNA barcode gene fragment amplification

See Appendix B for conditions.

7.4 Detection of PCR products

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7.5 Sequencing and Sequence Processing

45 μ L of the remaining PCR amplification product was taken and separated by 1.5% agarose gel electrophoresis. The target fragment was purified using DNA product purification kit and sequenced. Sequencing primers are the same as PCR amplification primers.

Sequencing results were edited using sequence splicing software, peak maps were compared, sequence directions were determined, sequencing primer sequences were removed, and sequencing was performed at both ends Sequences with mass Q value 8