

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

SN/T 4877.13-2019

**DNA barcoding screening method - Part 13: Quarantine
Potyviruses**

**基因条形码筛查方法 第13部分：检疫性马铃薯Y病毒属病毒
(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.

This section was drafted. Beijing Customs of the People's Republic of China, Qingdao Customs of the People's Republic of China.

1 Scope

This section specifies the RNA extraction, gene amplification, and sequence analysis of quarantine potato prion virus gene barcode screening methods.

Comparison analysis and result determination.

This section applies to the screening of quarantine viruses in the potato prion genus in host plants.

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.

SN/t 2122 quarantine sampling of inbound and outbound plants and plant products.

3 Terms and definitions

The following terms and definitions apply to this document.

DNA barcode refers to a standard, sufficiently mutated, easily amplified, and relatively short DNA that can represent the species in the organism.

Fragment.

4 Basic Information of Potato Y Virus

Chinese name. Potato Prion See Appendix A for details.

5 Method principle

Design and Synthesis of Degenerate Primers Based on Conserved Regions in the Genome Sequence of Potato Prion Virus Shape-code screening method; sequence determination of PCR products, and determination of virus types by sequence analysis. So potato prion Molecular biological characteristics are the main basis for the development of this detection and identification method.

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6.1 Apparatus and equipment

Grinding instrument, microbalance (sensitivity. 0.01g), PCR instrument, electrophoresis instrument, horizontal electrophoresis tank, gel imager, high-speed refrigerated desktop centrifuge Machine, water bath or constant temperature incubator, pH meter, adjustable pipettes of various ranges (1000 µL, 200 µL, 100 µL, 20 µL, 10 µL, 2 µL), etc.

6.2 Reagents

See Appendix B for reagents.

7.1 Sampling inspection

Sampling and sampling shall be carried out in accordance with the method given by Sn/T 2122.

7.2 RT-PCR method

According to the method given in Appendix B, the general RT-PCR test is performed. Use known virus isolates as positive controls and healthy plant tissues as Negative control, and set a blank control.

7.3 Sequence determination and analysis

After the PCR product is recovered, it can be cloned, sequenced, or directly sequenced (the sequence determination can be completed by a professional biological company) to make). The determined nucleotide sequence and translated amino acid sequence were compared with the known potato prion virus sequence (see Appendix C) Line comparison. Adjacent method (or NJ algorithm of MEGA6) is used to construct phylogenetic tree.

8 Result judgment

8.1

If the test result of the RT-PCR method is negative, it is determined that no potato prion virus is detected.

8.2

If the result of the RT-PCR method is positive, perform sequence determination and analysis.

a) If the translated amino acid sequence has the highest homology with the known amino acid sequence of the potato prion virus and is greater than 80%, then judge A potato prion virus was identified (specific virus types need to be identified by other methods).

b) If the translated amino acid sequence has less than 80% homology with the known sequence of the potato prion virus.

--- If phylogenetic analysis indicates clustering with the potato prion virus, a potato prion disease is determined to be detected Poison (specific virus types need to be further identified by other methods);

Note: The detected virus may belong to a known virus whose genome sequence has not been determined or a new virus species that has not been reported.

--- If phylogenetic analysis indicates that they are not clustered with the potato prion virus, it is determined that no potato prion virus has been detected is a virus.

c) If the determined genomic sequence is related to plant virus genes in PPV, PVA, PPV and BBMV in the potato prion genus the group sequence has the highest homology, so it is determined to screen for quarantine plant virus. It needs to be developed according to the specific virus quarantine identification standard method.

The line finally identified the species.

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9 Results Record

Record sample information and test data, including sample source, plant species, sampling time and place, test time, method and results, and Signed by personnel. In RT-PCR gel electrophoresis detection, electrophoresis photos should be saved, and sequencing reports should be saved in the sequencing results.

10 Sample preservation

The samples of the potato prion genus should be properly stored in an ultra-low temperature refrigerator, or stored at low temperature after lyophilization, and registered and labeled.

For review.

SN/T 487.7.13-201919 ?????, ????, ????,?

Appendix A

(Informative appendix) Potato Y Virus Basic Information A. Morphology of virion the virus particles of the potato prion gene are linear particles, usually about 680-900 nm in length, 12-15 nm in diameter, and a spiral symmetrical structure.

The pitch is about 3.4nm. The viral coat protein of this genus is composed of a polypeptide with a molecular weight of 28-38 kDa, accounting for 95% of the total viral particles.

(Figure A.1).

Figure A. 1 Mitochondrial structure of potato Y virus

(Http. //viral_one.expas.org/al_by_spices/50.html) A. 2 Virus genome structure and function the potato prion virus is a single-split positive single-stranded RNA virus with a total length of about 10 Kb, covalently linked at the 5 ' end of the RNA genome.

Take a protein, called VPg (viral protein, genome-linked), about 24kDa, with a polly (A) tail at the 3 ' end, 5 ' and 3 ' There is an untranslated region (UTR) at each end; the first open