

ICS 65.020
CCS B 60

LY

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

LY/T 3191-2020

Technical regulations of DNA barcodes in woody species

林木DNA条形码构建技术规程

(English Translation)

Issued on: March 30, 2020

Implemented on: October 1, 2020

Issued by: State Forestry and Grassland Administration

Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Terms and definitions	
3.1 DNA barcodes	
3.2 Primer	
3.3 Polymerase Chain Reaction (PCR)	
3.4 DNA sequence DNA sequence	
3.5 DNA sequencing DNA sequencing technology	
3.6 Phylogeny	
3.7 Phylogenetic Analysis Using Parsimony (and Other Methods) (PAUP)	
3.8 Cyberinfrastructure for Phylogenetic Research (CIPRES)	
3.9 Molecular Evolutionary Genetics Analysis (MEGA)	
3.10 Phylotools R package for DNA barcode phylogeny	
4 Sample collection strategy	
5 Selection criteria for core DNA barcode fragments	
6 DNA extraction technology and preservation method	
7 PCR amplification and sequencing	
7.3 Sequence determination strategy	
8 Sequence editing method	
9 Method of phylogenetic relationship construction	
9.1 Unweighted paired arithmetic average method	
9.2 Least-squares (LS)	
9.3 Neighbor-joining (NJ)	
9.4 Maximum parsimony (MP)	
9.5 Maximum likelihood (ML)	
9.6 Bayesian Inference	

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed by the State Forestry and Grassland Administration.

This standard is under the jurisdiction of the National Forest Seed Standardization Technical Committee (SAC/TC115).

Drafting organizations of this standard. Institute of Tropical Forestry, Chinese Academy of Forestry, Hunan Academy of Forestry, South China Plants, Chinese Academy of Sciences Garden, Institute of Botany, Chinese Academy of Sciences, College of Forestry, Jiangxi Agricultural University.

1 Scope

This standard specifies the sample collection strategy in the construction of forest tree DNA barcodes, the selection criteria for core DNA barcode fragments, and DNA extraction Technical requirements for technology and preservation, sequence determination and editing methods, and phylogenetic relationship construction.

This standard applies to the construction of forest tree DNA barcodes.

2 Normative references

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

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

SN/T 4625-2016 DNA barcode screening and quality requirements SN/T 4626-2016 DNA barcode species identification operating procedures SN/T 4714-2016 DNA barcode database technical specifications

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1 DNA barcodes

It is a short universal DNA sequence screened from mitochondria, chloroplasts, or nuclear genome regions, in order to compare existing organisms in species Fast and accurate identification and identification at the level.

3.2 Primer

It is a small piece of single-stranded DNA or RNA, which serves as the starting point for DNA replication. During the nucleic acid synthesis reaction, it enters as each polynucleotide chain.

A polynucleotide chain that functions as the starting point for line extension.

3.3 Polymerase Chain Reaction (PCR)

A method for enzymatically synthesizing specific DNA fragments in vitro, which consists of several steps of high-temperature denaturation, low-temperature annealing, and suitable temperature extension.

Cycles,, so that the target DNA can be rapidly amplified, with the characteristics of strong specificity, high sensitivity, simple operation and time-saving.

3.4 DNA sequence DNA sequence

The sequence of nucleotides in a polynucleotide chain. The only possible letters are A, C, G and T, which represent the four nucleosides that make up DNA.

Acids-adenine, cytosine, guanine, thymine; arranged together without gaps, such as the sequence AAAGTCTGAC.

3.5 DNA sequencing DNA sequencing technology

The determination of the sequence of nucleotides in a DNA molecule is to determine the sequence of A, T, G, and C that make up the DNA molecule.

3.6 Phylogeny

The origin, evolution history and genetic relationship of biological organisms over time.

3.7 Phylogenetic Analysis Using Parsimony (and Other Methods) (PAUP)

A software used to construct evolutionary trees (phylogenetic trees) and carry out related tests, including many molecular evolution models and methods, which can be beneficial. Analyze molecular data (DNA, protein sequence), morphological data and other types of data using maximum likelihood method, parsimonious method, distance method, etc.

3.8 Cyberinfrastructure for Phylogenetic Research (CIPRES)

A remote supercomputer platform designed and opened for scientific research institutions and researchers, which can meet the operation requirements of huge amounts of data, and can greatly this saves time and cost and improves the reliability of the calculation results.

3.9 Molecular Evolutionary Genetics Analysis (MEGA)

A convenient and powerful free open source software for molecular evolution genetic analysis, which can complement other online public supercomputing platforms.

3.10 Phylotools R package for DNA barcode phylogeny

A software that can automatically arrange and compare large-scale DNA sequences, and can construct a super matrix based on multiple barcode fragments to obtain the phylogenetic relationship at the community level. The phylogenetic tree transformed by the non-parametric rate smoothing NPRS method (r8s) can be used for the corresponding community Phylogenetic analysis. In addition, according to the actual situation, it is possible to increase the comparison software such as MAFFT and other algorithms with faster running speed to obtain more accurate analysis results.

4 Sample collection strategy

4.1

Collection site. Clean mature leaves are best. Under special circumstances, materials such as branches, roots and bark phloem can be collected; avoid collecting leaves that are bitten by insects and have pathogens.

4.2

Sample weight. 5-10 pieces of broad-leaved tree leaves, 5-10 grams of thin pinnate compound leaves or conifer leaves; enough for DNA extraction.

4.3

Number of samples. Common species are 3-5 individuals from different populations, and rare species should be collected as much as possible; to avoid false identification in samples Set species.

4.4

Sample number. Combine the species number and tree grade in the sample plot for numbering; when there are multiple individuals, they need to be distinguished; for example, camphor tree-1 (tree grade), Camphor tree-2 (tree grade). Record the collection time in detail.

4.5

Sample preservation. Put the material directly in the sealed bag and pour clean silica gel into the sealed bag; or put the material in an empty tea bag, no direct contact with silica gel to ensure that the material is not contaminated. If the material has high water content, pay attention to timely replacement of silica gel to ensure the quality of the material;

Under normal circumstances, the color of silica gel is dark blue. If it absorbs more water, the color of silica gel will change to light purple red. At this time, it needs to be replaced with a new one. When conditions permit, for some leaf materials that are not easy to extract DNA samples can be stored in cold storage.