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GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
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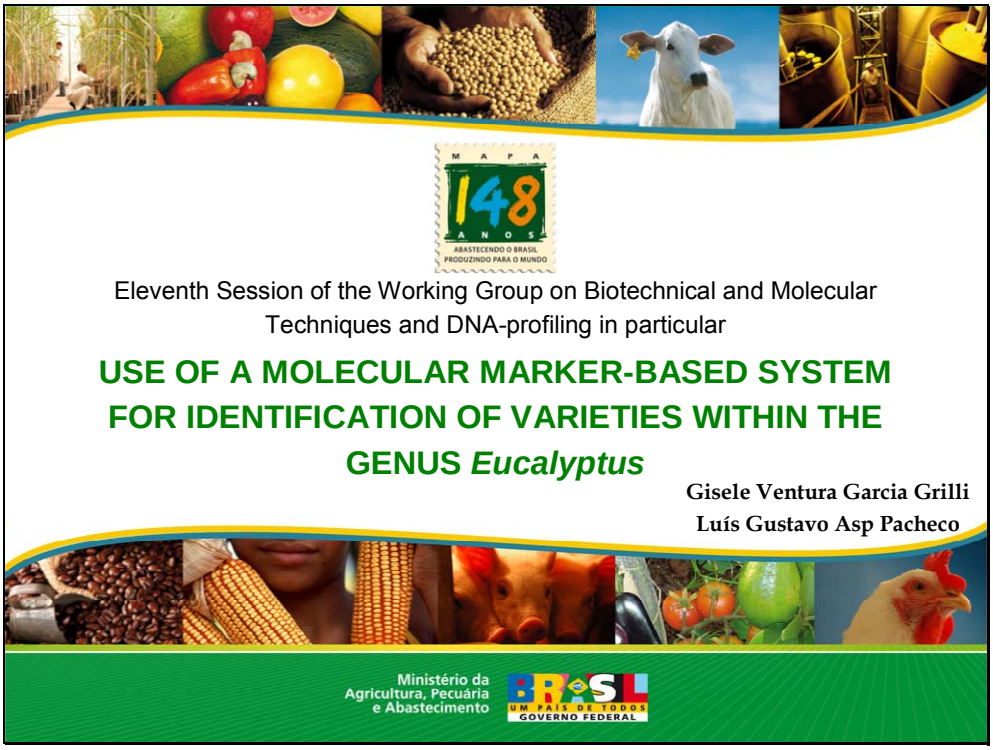
USE OF A MOLECULAR MARKER-BASED SYSTEM FOR IDENTIFICATION OF VARIETIES WITHIN THE GENUS *EUCALYPTUS*

Document prepared by experts from Brazil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ABASTECENDO O BRASIL
PRODUZINDO PARA O MUNDO
Eleventh Session of the Working Group on Biotechnical and Molecular Techniques and DNA-profiling in particular
USE OF A MOLECULAR MARKER-BASED SYSTEM FOR IDENTIFICATION OF VARIETIES WITHIN THE GENUS Eucalyptus
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UM PAÍS DE TODOS
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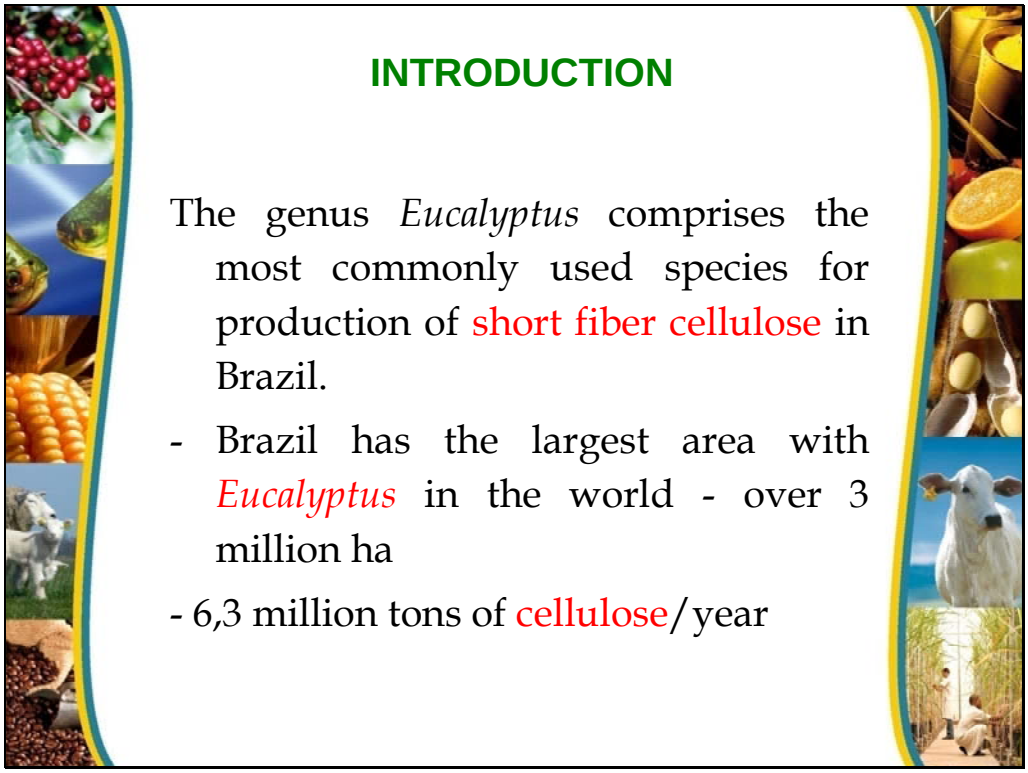
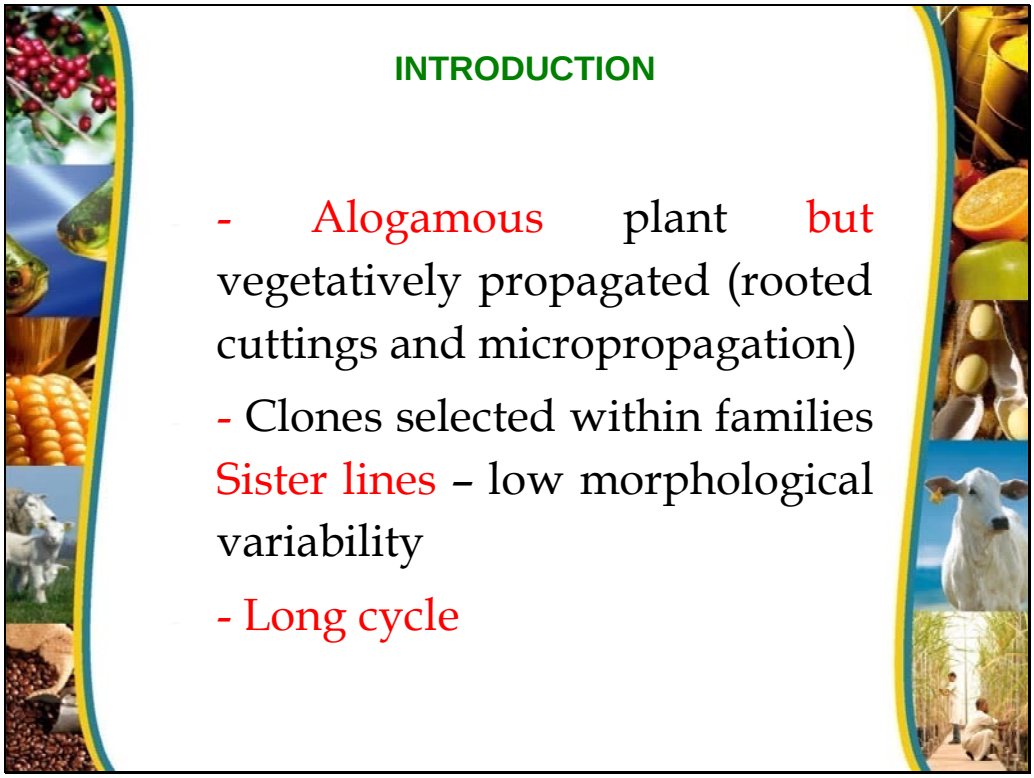
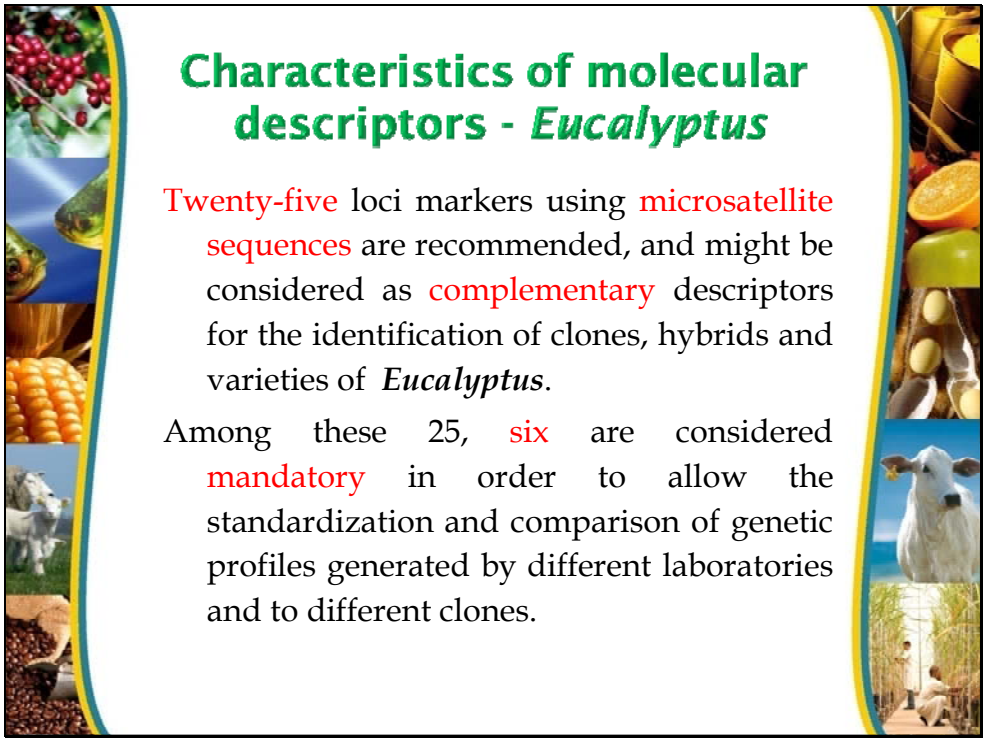

INTRODUCTION
The genus Eucalyptus comprises the most commonly used species for production of short fiber cellulose in Brazil.
- Brazil has the largest area with Eucalyptus in the world - over 3 million ha
- 6,3 million tons of cellulose/year


INTRODUCTION
- Alogamous plant but vegetatively propagated (rooted cuttings and micropropagation)
- Clones selected within families
Sister lines – low morphological variability
- Long cycle


Characteristics of molecular descriptors - Eucalyptus
Twenty-five loci markers using microsatellite sequences are recommended, and might be considered as complementary descriptors for the identification of clones, hybrids and varieties of Eucalyptus.
Among these 25, six are considered mandatory in order to allow the standardization and comparison of genetic profiles generated by different laboratories and to different clones.

SSR Markers - *Eucalyptus*

Loci	Allele size (base pairs)	Sequence 5'-3' of direct primer	Sequence 5'-3'of reverse primer	Linkage Group
Embra01	100-145	gatagaactttcctatttgatcg	gtaggatttgatgtctgcaa	8
Embra02	103-148	cgtgacaccaggacattac	acaaatgcaaattcaaatga	11
Embra05	78-142	atgctggtccaactaagatt	tgagcctaaaagcccaac	5
Embra06	120-170	agagaattgctcttcatgga	gaaaagtctgcaaagtctgc	1
Embra10	110-152	gtaaagacatagtgaagacattcc	agacagtacgttctctagctc	10
Embra11	123-165	gcttagaatttgcctaaacc	gtaaaatccatgggcaag	1
Embra12	104-162	aggatttgtggggcaagt	gttcccattttcatgtcc	1
Embra15	90-125	tttgttggatgaggactt	caacatgttctccgaaaag	8
Embra16	110-165	caacgttccccttttcttc	atgttaggccaaacccag	1
Embra17	120-170	aggatactcgtgagagaagc	gtagatctgttctgcatgttg	9
Embra19	55-145	gacggttgatttcctgatt	gtggtgctctctcctct	4
Embra23	118-145	ggttgtttcatcttttccatg	agcgaaggcaatgtgttt	10
Embra26	112-200	cccacaacaaaaggaaag	agaggtgttcgattcaattc	11
Embra27	100-170	ataaccacaccaatctgca	tatagctcgaacgctcaac	2
Embra28	180-300	caagacatgcatttcgtagt	actcttgatgtgacgagaca	6
Embra34	100-160	tcaaaaccctctctctcat	aataaacatttttctttgaacaga	3
Embra37	115-165	cacctctccaaactacacaa	ctcctctctcttcaccattc	5
Embra42	115-170	gagtaaaaattggttttgagtg	cctctttttcattttgtctt	7
Embra44	205-225	ggggtttgttctgcttag	caaaagagttcagctgtg	4
Embra46	90-130	gaagtcatcatctgtagattgc	acccattattctttgtgagc	7
Embra49	125-195	attattggttcatattgaaaacc	agatagagattgagtgagaccc	3
Embra51	95-200	gatgcattccttttttcc	cattctcttgcatctggac	6
Embra58	140-245	caccaactggtactatgaggat	ttggcttagggtagaacact	9
Embra63	175-230	catctggagatcgaggaa	gagagaaggatcatgcca	2
Embra72	118-170	ctggtcaacgtccgaaag	atgctgcagagggcataa	10

Reliability

The molecular characterization of these loci have been already published in the literature and were validated in a ring test in several laboratories around the world:

Scotland - 1;

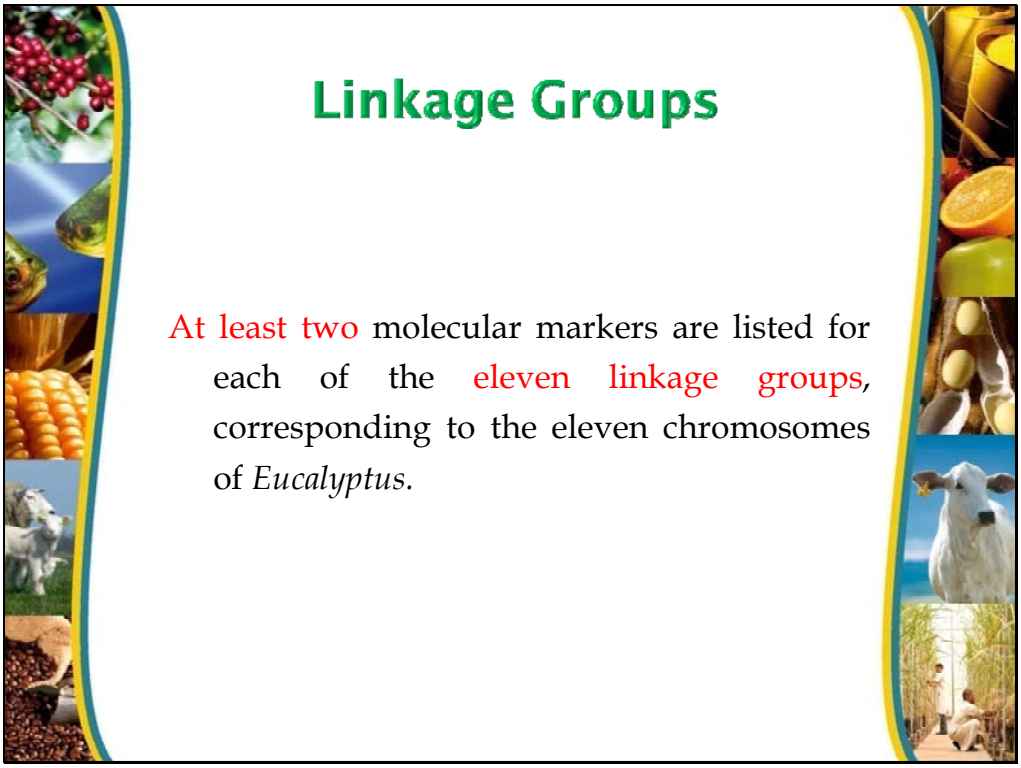
Brazil -3;

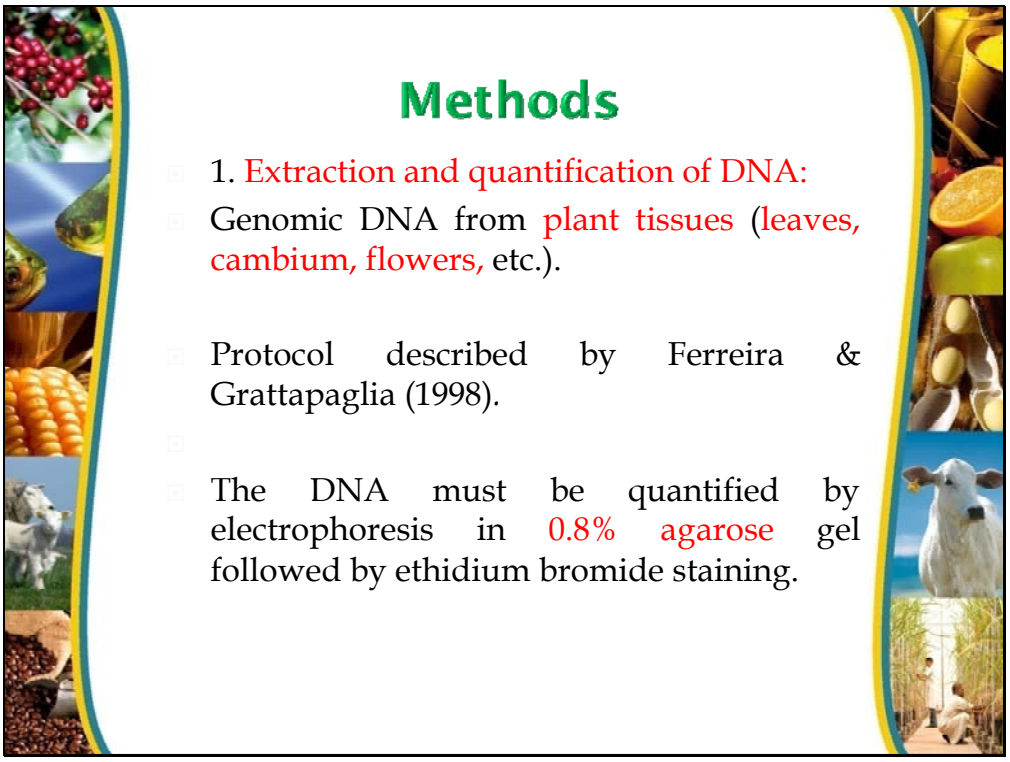
South Africa - 1;

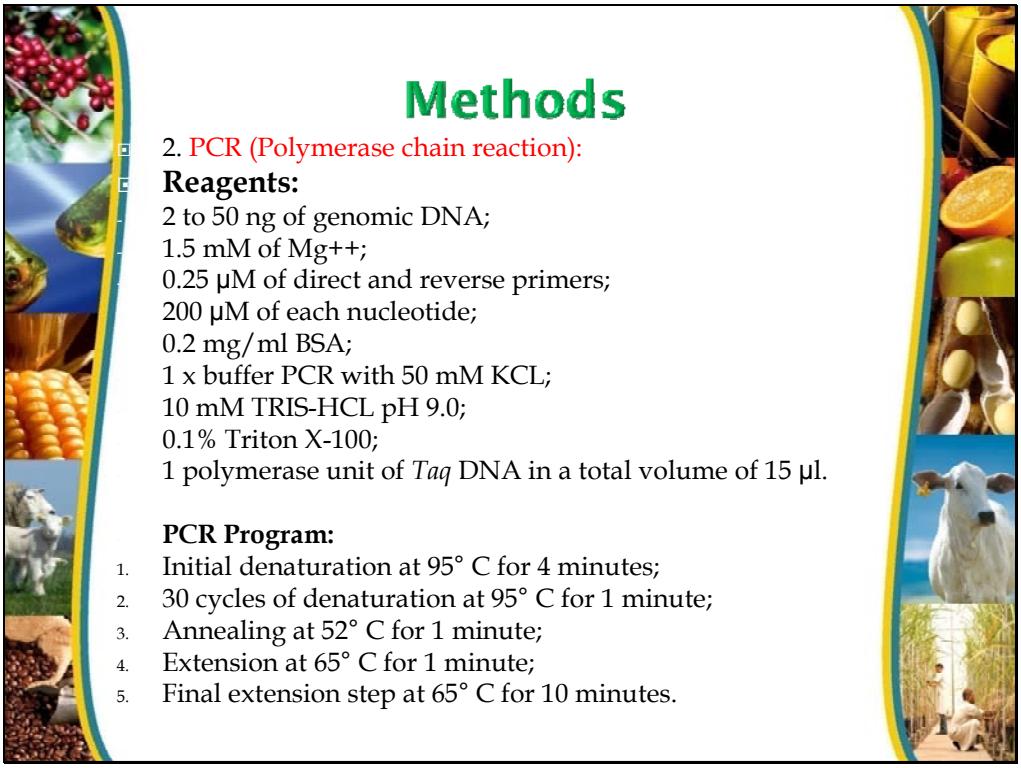
Portugal - 1;

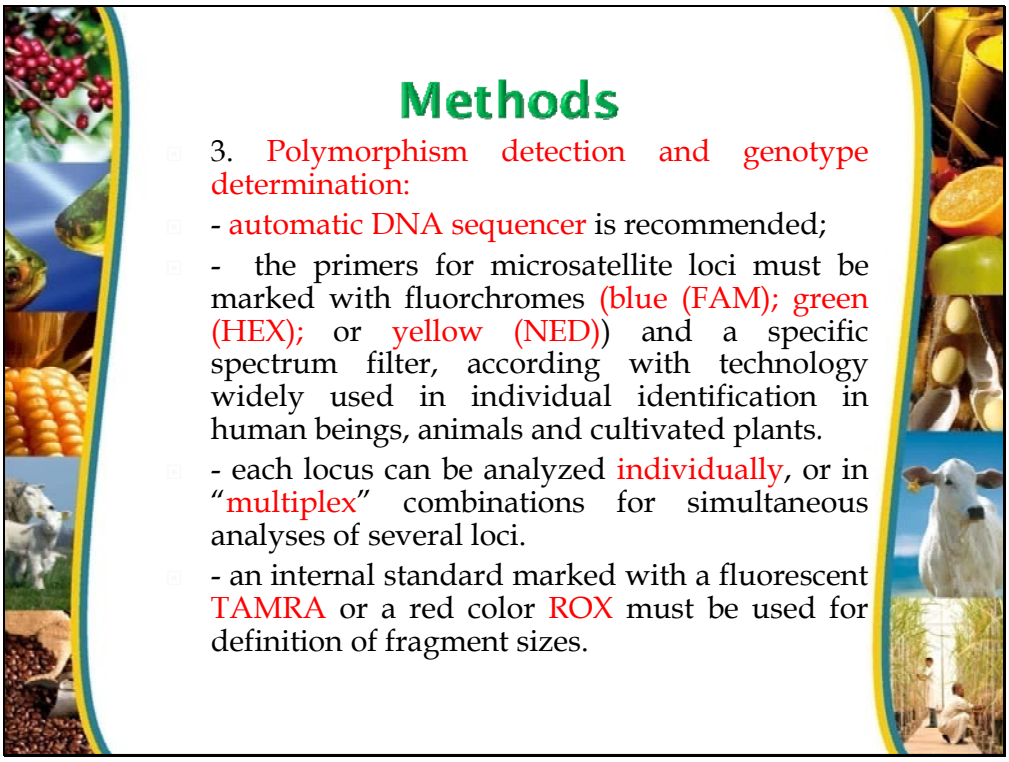
Australia – 2;

Argentina - 1.


Linkage Groups
At least two molecular markers are listed for each of the eleven linkage groups, corresponding to the eleven chromosomes of *Eucalyptus*.


Methods
1. Extraction and quantification of DNA:
Genomic DNA from plant tissues (leaves, cambium, flowers, etc.).
Protocol described by Ferreira & Grattapaglia (1998).
The DNA must be quantified by electrophoresis in 0.8% agarose gel followed by ethidium bromide staining.


Methods
2. PCR (Polymerase chain reaction):
Reagents:
2 to 50 ng of genomic DNA;
1.5 mM of Mg++;
0.25 µM of direct and reverse primers;
200 µM of each nucleotide;
0.2 mg/ml BSA;
1 x buffer PCR with 50 mM KCL;
10 mM TRIS-HCL pH 9.0;
0.1% Triton X-100;
1 polymerase unit of Taq DNA in a total volume of 15 µl.
PCR Program:
1. Initial denaturation at 95° C for 4 minutes;
2. 30 cycles of denaturation at 95° C for 1 minute;
3. Annealing at 52° C for 1 minute;
4. Extension at 65° C for 1 minute;
5. Final extension step at 65° C for 10 minutes.


Methods
3. Polymorphism detection and genotype determination:
- automatic DNA sequencer is recommended;
- the primers for microsatellite loci must be marked with fluorchromes (blue (FAM); green (HEX); or yellow (NED)) and a specific spectrum filter, according with technology widely used in individual identification in human beings, animals and cultivated plants.
- each locus can be analyzed individually, or in "multiplex" combinations for simultaneous analyses of several loci.
- an internal standard marked with a fluorescent TAMRA or a red color ROX must be used for definition of fragment sizes.

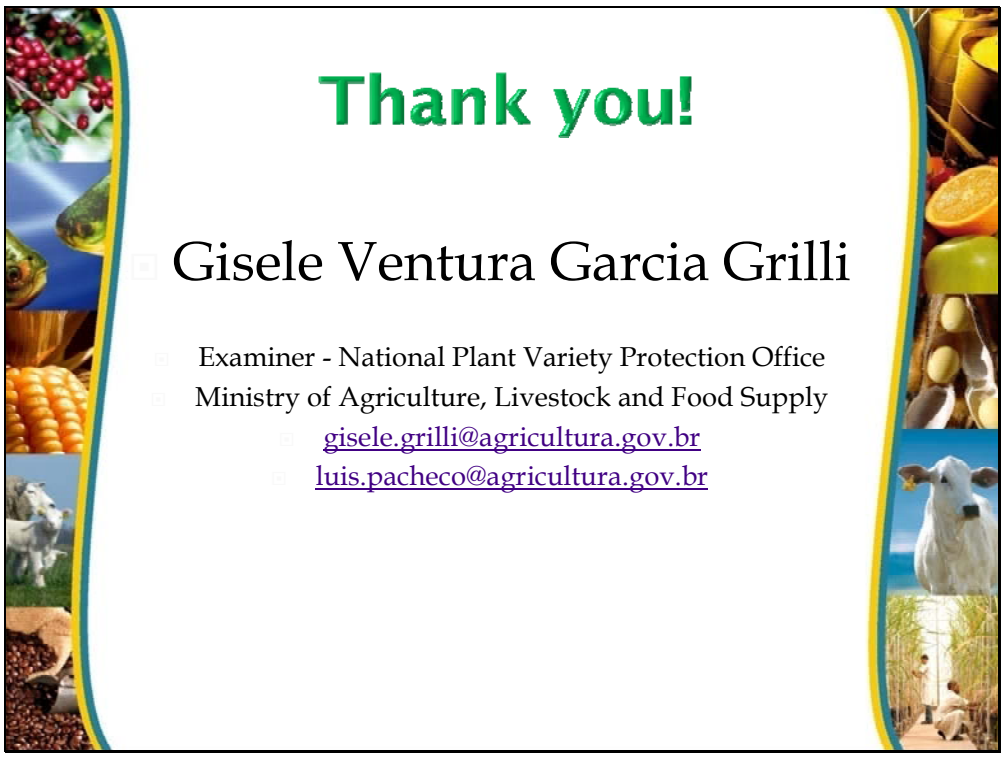
Methods

4. Interpretation of results

- for each of the analyzed descriptor loci, the observed genotype should be identified and registered.
- the alleles will be visualized as peaks in the electropherogram and will be identified by their size in base pairs.
- genotypes should be described with the alleles identified in number of base pairs.
- the analysis should include, as control check, the DNA of a well characterized *Eucalyptus* clone, to be identified by the laboratory, to be used for comparison of allele size in base pairs among laboratories or between different experiments within the same laboratory.

Database Model

		Genotypes in pairs of bases							
Loci EMBRA		**2**		**28**		**11**		**15**	
Sample	**Number**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
DF-EG112	1	108.42	113.12	190.52	209.15	133.73	133.73	117.92	142.22
DF-EG113	2	108.30	113.12	190.39	209.13	133.68	133.68	117.92	142.32
DF-EG114	3	108.30	**124.54**	190.53	**221.26**	**117.73**	**12459**	**126.07**	**138.22**
DF-EG115	4	108.30	113.00	190.41	209.13	133.69	133.69	117.69	142.11
Control Check	C	124.43	145.13	197.06	221.31	117.40	119.30	125.92	136.16



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