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Introduction

This List of *Descriptors for Genetic Markers Technologies* was originally developed by Dr Carmen de Vicente, Dr Thomas Metz and Ms Adriana Alercia in an effort attempt to define community standards for documenting information about genetic markers. This document is targeted to researchers using genetic marker technologies to generate and exchange genetic marker data that are standardized and replicable.

This initial proposed set of descriptors was reviewed widely by international experts from national research institutions, universities and CGIAR centres, and their comments and contributions were included through several iterations of the document. This first official version of the list is now being published by IPGRI to encourage application of the descriptors to current research projects and to stimulate further refinement of the standards.

This List of Descriptors defines an initial minimum set of information that is needed to describe a genetic marker technology. This List provides descriptions of content, and coding schemes that can assist in the computerized data exchange. It is realized that users may want to implement modifications and/or additions to meet specific needs. As long as these modifications allow for an easy conversion to the format proposed below, this type of data can be exchanged worldwide in a consistent manner.

It is anticipated that future refinements of the standards may evolve towards documentation comparable to the MIAME standards for microarray experiments and associated encoding formats (i.e. the XML-based MAGE-ML implementation of MIAME).¹ Future versions may also incorporate refinements based on the methodology of biological ontology research community,² for example, assignment of a term accession identifier to each descriptor definition, and placement of the terms into a structured ontology.

The standardization of such information should facilitate the development of data exchange encoding formats (i.e. an XML DTD) for information on markers and the creation of a global registry containing a full and accurate inventory of species-specific reference markers already published.

Special thanks are due to Dr Richard Bruskiewich of IRRI for the comprehensive advice given during the revision process.

Comments and suggestions are welcome and should be addressed to: Adriana Alercia <<u>a.alercia@cgiar.org</u>>.

¹Brazma *et al*., 2001 ²Gene Ontology Consortium, 2000

1. Marker identifiers and names

Universal marker identifier

It is proposed that marker systems be registered with the global community of genetic mapping databases and be assigned a permanent *Universal marker identifier* (UMI), an accession identifier similar in concept to international public sequence database (Genbank/EMBL/DDBJ) accession identifiers. The UMI will serve as a unique identifier of a marker system and should never be re-assigned

Canonical marker name

Record the canonical name as the first published or most authoritative name of the marker insofar possible.

Below are presented two options to generate new canonical marker names:

(i) A marker could be given a descriptive canonical marker name based upon standard protocols specific to a particular class of marker: RFLP, AFLP, etc. Letters could be used to identify either: a DNA library, a series of a commercial kit, primer sequences, or the selective bases and enzyme combination.

(ii) Alternatively, the canonical marker name could consist of the following: [Function][Lab Designator][Species][Type of marker][serial # of clone] e.g. Xipam001 A marker of unknown function (X), developed by ICRISAT-Patancheru (ip) for Arachis (a), and a microsatellite marker (m), followed by serial # of clone Where different marker types could be identified as follows: microsatellite =m, aflp=a, rapd=r, rflp=f, snp=s, scars=c, sts=t, est=e

When more than one genetic locus is identified by a particular marker, then it should be followed by a full stop and a (single-digit) serial number indicating the order in which they were identified: e.g., Xipam001.1 and Xipam001.2

Accession number

(i.e. Genebank, Herbarium, University)

3

2. Taxonomy

Where feasible, the NCBI taxonomy <u>http://www.ncbi.nlm.nih.gov/Taxonomy/</u> accession identifier will be used to specify the species or general taxon of organism targeted by the marker system and full taxonomic details of the target species should be recorded as stated below.

Strains, cultivars or subspecies should also be qualified by their SINGER or comparable germplasm accession identifier

Genus

Genus name for taxon. Initial uppercase letter required.

> Species

Specific epithet portion of the scientific name in lowercase letters. Following abbreviation is allowed: 'sp.' for undetermined species or generic group of samples from the same genus

* Species authority

Provide the species authority

Subtaxa

Any additional taxonomic identifier. The following abbreviations are allowed: 'subsp.' (for subspecies); 'convar.' (for convariety); 'var.' (for variety); 'f.' (for form).

* Subtaxa authority

Provide the subtaxa authority

3. Markers nature and types

> Protein-based markers

Isozymes Seed storage proteins Total soluble proteins

> Metabolic based markers

Polyphenol profile Flavonoids Carbohydrates Oils Secondary products

DNA-based markers

Hybridization based

RFLP

Polymerase chain reaction-based

RAPD SSR, STR, STMS or microsatellite AFLP CAPS EST Inter-SSR SNPs SCAR PCR-sequencing Other (specify in descriptor **7. Remarks**)

> Phenotype based markers

4. Experimental conditions

Provide information on reliable and proven protocols (that is laboratory recipes for extraction, generation of markers and interpretation) with references, as appropriate

> Protein based markers

4.1 Isozymes

* Canonical marker name

In the case of isozymes, the marker identifier should refer to the International codes for Enzyme Nomenclature (IUBMB). For example, PGM (phosphoglucomutase)

* Sample extraction method

Indicate the tissue and the recipe for the extraction buffer used

* Marker sample separation

- Electrophoresis
 - Gel composition
 - Buffer composition
 - Running conditions
 - Voltage
 - o Current
 - Time of run
 - Other methods of separation Specify in descriptor 7. Remarks

* Identification process

Staining

Indicate the recipe for the staining solutions and conditions for incubation

• Other staining solutions

Specify in descriptor 7. Remarks

4.2 Seed storage proteins

4.3 Total soluble proteins

> DNA-based markers

4.4 RFLP

* Canonical marker name

See instructions under *Section 1*.

* DNA extraction method

Indicate the protocol used or/the reference where the protocol was published

Total

Chloroplastic

Mitochondrial

***** DNA probe and origin

Indicate the name of the probe, if used, and the Laboratory, which obtained it

* Genome location

Nuclear Chloroplastic Mitochondrial

* Restriction enzyme

Indicate the restriction enzyme used to digest the DNA

* Electrophoresis

Gel composition

Running conditions

- Voltage
- o Current
- o Time of run

* Visualization

Indicate experimental procedures for detection of markers

Radioactive

Enumerate radioisotopes and their activities

 P^{33}

 $P^{\scriptscriptstyle 32}$

 S^{35}

Other (specify in descriptor 7. Remarks)

Non-radioactive

Fluorescence Chemiluminescence Other (specify in descriptor **7. Remarks**)

4.5 RAPD ³

* Canonical marker name

See instructions in *Section 1.*

* DNA extraction method

Indicate the protocol used or/the reference where the protocol was published

* Primer sequence

List primer sequence and commercial provider

³Be aware that this technology is not highly reproducible.

* PCR reaction (composition)

Specify $MgCl_2$ and/or primer concentrations and any other reagent, in addition to standard components

* PCR conditions

Thermocycler

Indicate the brand name and model

• Initial denaturation

- o Temperature [°C]
- o Duration [s]

• Amplification conditions

- Number of cycles
- Denaturation
 - Temperature [°C]
 - o Duration [s]

• Annealing

- Temperature [°C]
- \circ Duration [s]

• Elongation

- Temperature [°C]
- o Duration [s]

• Terminal elongation

- Temperature [°C]
- o Duration [s]

* Electrophoresis

Gel composition

Running conditions

- Voltage
- o Current
- Time of run

* Visualization

Indicate experimental procedures for detection of markers.

Detection

Ethidium bromide Other (specify in descriptor *7. Remarks*)

4.6 Microsatellites

***** Canonical marker name

See instructions under *Section 1.*

* Publication of primers

Provide original reference

* DNA extraction method

Indicate the protocol used or/the reference where the protocol was published

* Primer sequences

List primer sequences

* Genomic location

Nuclear Chloroplastic Mitochondrial

* Primers' origin

Indicate the Research Laboratory which designed them

* PCR reaction (composition)

Specify $MgCl_2$ and/or primer concentrations and any other important reagent, in addition to standard components

* PCR conditions

Initial denaturation

- Temperature [°C]
- o Duration [s]

• Touchdown amplification conditions

Touchdown amplification conditions (only if using touchdown program)

• Number of cycles

• Denaturation

- Temperature [°C]
- o Duration [s]

• Annealing

- Starting temperature [°C]
- $\circ \quad \Delta T \ [^{\circ}C]$
- o Duration [s]

• Elongation

- Temperature [°C]
- o Duration [s]

Amplification conditions

- Number of cycles
- Denaturation
 - Temperature [°C]
 - o Duration [s]

• Annealing

- Temperature [°C]
- o Duration [s]

• Elongation

- o Temperature [°C]
- o Duration [s]

Terminal elongation

- o Temperature [°C]
- o Duration [s]

* Electrophoresis

• Gel composition

• Running conditions

- Voltage
- o Current
- Time of run

* Visualization

Indicate experimental procedures to visualize the markers

Radioactive

 P^{33} P^{32} S^{35}

Other (specify in descriptor 7. Remarks)

Non-radioactive

Ethidium bromide Silver staining Fluorescence Other (specify in descriptor **7. Remarks**)

4.7 AFLP

* Canonical marker name

See instructions under *Section 1.*

***** DNA extraction method

Indicate the protocol used or/the reference where the protocol was published

* Enzyme combinations

Indicate the restriction enzymes used to digest the DNA

* Adaptors

Indicate adaptors for the restriction sites

* Primer sequences

List primer sequences

* PCR reaction (composition)

Specify $MgCl_2$ and/or primer concentrations and any other important reagent for both amplifications in addition to standard components

Pre-selective Selective

* PCR conditions

- Pre-selective
 - Initial denaturation
 - o Temperature [°C]
 - o Duration [s]

- Amplification conditions
 - Number of cycles
 - Denaturation
 - o Temperature [°C]
 - \circ Duration [s]
 - Annealing
 - o Temperature [°C]
 - o Duration [s]
 - Elongation
 - Temperature [°C]
 - o Duration [s]

• Terminal elongation

- o Temperature [°C]
- o Duration [s]
- ♦ Selective

• Initial denaturation

- o Temperature [°C]
- o Duration [s]

• Amplification conditions

- Number of cycles
- Denaturation
 - Temperature [°C]
 - o Duration [s]

• Annealing

- Temperature [°C]
- \circ Duration [s]

• Elongation

- Temperature [°C]
- o Duration [s]

• Terminal elongation

- Temperature [°C]
- o Duration [s]

* Electrophoresis

- Gel composition
- Buffer composition
- Running conditions
 - Voltage
 - o Current
 - $\circ \quad \text{Time of run} \\$

* Visualization

Indicate experimental procedures to visualize the markers

Radioactive

Enumerate radioisotopes and their activities

- $P^{\scriptscriptstyle 33}$
- $P^{\scriptscriptstyle 32}$

 S^{35}

Other (specify in descriptor 7. Remarks)

Non-radioactive

Silver staining

Fluorescence

Other (specify in descriptor 7. Remarks)

4.8 CAPS

* Canonical marker name

See instructions under Section 1.

* DNA extraction method

Indicate the protocol used, or the reference where the protocol was published

* Primer sequence

List primer sequences

* Genome location

Nuclear Chloroplastic Mitochondrial

* PCR conditions

Thermocycler

Indicate the brand name and model

Initial denaturation

- Temperature [°C]
- Duration [s]

Amplification conditions

- Number of cycles
- Denaturation
 - Temperature [°C]
 - o Duration [s]

• Annealing

- Temperature [°C]
- o Duration [s]

• Elongation

- o Temperature [°C]
- \circ Duration [s]

* Restriction enzyme

Indicate the restriction enzyme used to digest the amplified DNA fragment

* Electrophoresis

Gel composition

• Running conditions

- o Voltage
- o Current
- $\circ \quad \ \ Time \ of \ run$

* Visualization

Indicate experimental procedures to visualize the markers

• Detection

Ethidium bromide Silver staining Other (specify in descriptor **7. Remarks**)

5.Interpretation of markers

> Genotypes used as reference standards

List genotypes used as reference standards

> Protein based markers

* Isozymes

- Genetic control of the isoenzymatic system (Number of genes)
- Number of alleles per gene
- Molecular structure of the isozyme
 Monomeric
 - Dimeric
 - Other (specify in descriptor 7. Remarks)
- * Seed storage proteins
- * Total soluble proteins

> Metabolic based markers

DNA based markers

* Fragment size-marker used

Indicate the fragment size-marker used (i.e. Lambda)

* Predicted product length

Indicate the range length in which the marker may provide informative bands

* Number of bands or alleles obtained

Report the number of unique bands or alleles obtained for the genotypes used as reference standards

* Dominance

Indicate if the marker is co-dominant or dominant

Dominant Co-dominant

> Phenotype based markers

6. Use of the results

Indicate in which aspects the specific marker can be used:

Fingerprinting (e.g. identification of duplicates)

Measure of diversity and/or genetic distance

Taxonomic classification

Identification of abiotic and biotic stresses susceptibilities

Map location

Linking markers with traits

Measure of the mating patterns (out crossing rate and gene flow)

Other (specify in descriptor 7. Remarks)

7. Remarks

The remarks field is used to add notes or to elaborate on descriptors listed as 'Other. Prefix remarks with the field name they refer to and a colon. Separate remarks referring to different fields are separated by semicolons without space.

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Acronyms

AFLP	Amplified fragment length polymorphism
CAPS	Cleaved amplified polymorphic sequence
EST	Expressed sequence tag
Inter-SSR	Inter-simple sequence repeat
PCR	Polymerase chain reaction-based
RFLP	Restriction Fragment length polymorphism
RAPD	Random amplified polymorphic DNA)
SCAR	Sequence-characterized amplified region
SNPs	Single nucleotide polymorphism
SSR or microsatellite	Simple sequence repeat
STR	Simple tandem repeat
STMS	Sequence-tagged microsatellite site

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