TECHNICAL REPORT

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Molecular biomarker analysis — SSR analysis of maize

Analyse moléculaire de biomarqueurs — Méthode d'analyse SSR sur le maïs

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Contents

Page

Foreword

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

Introduction

Varietal identification testing requires high-quality markers which are able to provide reproducible data using a variety of equipment, chemistries, and reagents. Accordingly, this Technical Report only addresses specific amplification methods for maize.

The aims of this Technical Report are to provide a list of simple sequence repeat (SSR) markers and methods of analysis for maize. The SSR marker set has been validated through an intralaboratory study at GEVES (Laboratoire BioGEVES, Domaine du Magneraud, BP.52, 17700 SURGERES). Properties and sequences of these SSR markers are publicly available on the website www.maizegdb.org.

This Technical Report is linked to ISO 13495, which lists the different steps toward method validation and defined acceptance criteria.

Molecular biomarker analysis — SSR analysis of maize

1 Scope

The methods and SSR markers included in this Technical Report are suitable for applications such as testing hybrid conformity, molecular fingerprinting of varieties, and checking variety identity.

2 Principle

Simple sequence repeat (SSR) analysis is based on the amplification and visualization of the polymorphism caused by variation in the number of repeats in a sequence motif that is two to five base pairs in length also known as a microsatellite. SSR analysis consists of the following steps:

a) sample preparation;

b) DNA extraction;

c) PCR amplification;

d) separation;

e) detection of the PCR products.

3 Consumables and equipment

— 96-well or 384-well microplate

— PCR reagents [(DNA polymerase), buffer, $MgCl_2$, dNTP, primers, etc.]

— capillary electrophoresis reagents

— mixer/grinding mill

— microplate centrifuge

— adjustable volume micropipettes

— micro-centrifuge for microtubes

— capillary electrophoresis system with fluorescence detection

— thermocycler

4 Procedure

4.1 Sample preparation

For each sample, either individual seeds or seed mixes depending on the context are ground using a suitable mill (such as an IKA A10 or a Retsch MM301).

4.2 DNA extraction and quantification

a) Obtain an aliquot of each homogenously ground sample. The amount required will depend upon the extraction protocol employed.

b) Extract DNA using in house protocol or equivalent.

NOTE Collaborative study has been carried out with QIAGEN DNeasy®[1)] 96 Plant Kit.

c) The laboratory validates that the quantity of DNA extracted is appropriate to ensure a reliable result.

4.3 PCR amplification

Conditions optimised for ABI 9700 thermocycler.

a) Mix preparation for simplex PCR (see Table 1).

Table 1 — Mix preparation for simplex PCR

	Concentration	Volume for 1X
H_2O		3,125 µL
Buffer 10X	1 X	1 µL
dNTP (10 mM)	125 µM	0,125 µL
$MgCl_2$ (25 mM)	3 mM	1,2 µL
DNA polymerase (5 U/µL)	0,25 U	0,05 µL
Forward primer (10 µM)	0,25 µM	0,25 µL
Reverse primer (10 µM)	0,25 µM	0,25 µL
Vol 1X mix		**6 µL**
DNA (2,5 ng/µL)		4 µL
Final PCR vol		**10 µL**

b) Amplification conditions (see Table 2).

A touchdown (TD) program is used. The hybridization temperature is lowered from 64 °C to 55 °C in decrements of 1 °C per cycle.

Table 2 — Amplification conditions

	10 cycles			30 cycles				
94 °C	94 °C	TD		94 °C				
10:00	0:30		72 °C	0:30		72 °C	72 °C	
		64 °C	0:30		55 °C	0:30	10:00	10 °C
		0:30			0:30			∞

NOTE Units for times in Table 2 are “minutes:seconds”.

5 List of SSR-based maize markers validated through a GEVES intralaboratory study

5.1 Characteristics of the SSRs

Data obtained with a 3130 Genetic Analyser (Applied Biosystems) (see Table 3).

1) QIAGEN DNeasy is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table 3 — Characteristics of the SSRs

No.	SSR	Bin/Chromosome	Number of alleles recorded	range of estimated allele sizes (bp)[a]	Nei's diversity index[b]
1	umc1147	1	4	61–86	0,69
2	phi109275	1	6	121–137	0,60
3	phi427913	1,01	5	119–133	0,49
4	umc1885	1,1	3	136–142	0,63
5	phi064	1,11	8	75–110	0,78
6	phi96100	2	4	275–294	0,74
7	phi083	2,04	6	123–136	0,76
8	umc1448	2,04	5	137–161	0,77
9	phi102228	3,04	6	122–133	0,54
10	umc1489	3,07	4	123–135	0,51
11	umc1117	4,04	3	122–135	0,67
12	umc1329	4,06	4	74–92	0,63
13	phi093	4,08	7	281–294	0,63
14	umc1180	4,1	2	99–102	0,47
15	nc130	5	5	139–148	0,48
16	umc1478	5,01	4	134–144	0,62
17	umc1792	5,08	5	115–134	0,74
18	umc1153	5,09	5	101–113	0,71
19	umc1143	6	5	71–82	0,66
20	phi423796	6,01	5	125–137	0,53
21	umc1133	6,01	3	91–105	0,66
22	phi123	6,07	4	141–147	0,66
23	phi089	6,08	4	81–91	0,34
24	umc1545	7	6	70–85	0,75
25	umc1134	7,03	4	75–88	0,61
26	phi116	7,06	4	152–173	0,70
27	umc1304	8,02	2	131–136	0,50
28	phi233376	8,03	6	140–159	0,68
29	bnlg1782	8,05	7	219–236	0,73
30	phi015	8,08	7	82–103	0,45
31	phi032	9,04	3	232–239	0,53
32	bnlg1129	9,08	5	179–202	0,72
33	umc1319	10,01	4	115–124	0,65
34	phi050	10,03	3	82–88	0,61
35	phi084	10,04	2	154–157	0,50
36	umc1061	10,06	8	97–107	0,46

[a] Allele sizes observed at the GEVES and illustrative data.

[b] Nei's diversity index was calculated based on several hundred maize lines already analysed at the GEVES.