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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Foreword

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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.

5.2 SSR primer sequences

See Table 4.

Table 4 — SSR primer sequences

No.	SSR	Forward primer sequence (5'---3')	Reverse primer sequence (5'---3')
1	umc1147	GAGAAACCATCGACCCTTCCTAAC	TTCCTATGGTACAGTTCTCCCTCG
2	phi109275	CGGTTCATGCTAGCTCTGC	GTTGTGGCTGTGGTGGTG
3	phi427913	CAAAAGCTAGTCGGGGTCA	ATTGTTCGATGACACACTACGC
4	umc1885	TATACCAGCATCAGGTCTCGTCG	GTAGAGTGACCGTGCTGTAGCAGA
5	phi064	CCGAATTGAAATAGCTGCGAGAACCT	ACAATGAACGGTGGTTATCAACACGC
6	phi96100	AGGAGGACCCCAACTCCTG	TTGCACGAGCCATCGTAT
7	phi083	CAAACATCAGCCAGAGACAAGGAC	ATTCATCGACGCGTCACAGTCTACT
8	umc1448	ATCCTCTCATCTTTAGGTCCACCG	CATATACAGTCTCTTCTGGCTGCTCA
9	phi102228	ATTCCGACGCAATCAACA	TTCATCTCCTCCAGGAGCCTT
10	umc1489	TTAATAGCTACCCGCAACCAAGAA	CTGAGCCACAGTACGTTGCTGTT
11	umc1117	AATTCTAGTCCTGGGTCGGAACTC	CGTGGCCGTCGAGTCTACTACT
12	umc1329	CCTCTCACATCTCCTCTCCCCT	GTGTCGCTGTAGGTCTCCGTCTT
13	phi093	AGTGCGTCAGCTTCATCGCCTACAAG	AGGCCATGCATGCTTGCAACAATGGATACA
14	umc1180	GAAGCCCCTTGAAATGAATGAAC	CGACGTACGTATAGACTCGCTCAG
15	nc130	GCACATGAAGATCCTGCTGA	TGTGGATGACGGTGATGC
16	umc1478	GAAGCTTCTCCTCTCGCGTCTC	CAGTCCCAGACCCTAGCTCAGTC
17	umc1792	CATGGGACAGCAAGAGACACAG	ACCTTCATCACCTGCAACTACGAC
18	umc1153	CAGCATCTATAGCTTGCTTGCATT	TGGGTTTTGTTTGTTTGTTTGTTG
19	umc1143	GACACTAGCAATGTTCAAAACCCC	CGTGGTGGGATGCTATCCTTT
20	phi423796	CACTACTCGATCTGAACCACCA	CGCTCTGTGAATTTGCTAGCTC
21	umc1133	ATTCGATCTAGGGTTTGGGTTCAG	GATGCAGTAGCATGCTGGATGTAG
22	phi123	GGAGACGAGGTGCTACTTCTTCAA	TGTGGCTGAGGCTAGGAATCTC
23	phi089	GAATTGGGAACCAGACCACCCAA	ATTTCCATGGACCATGCCTCGTG
24	umc1545	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA
25	umc1134	AAAACTAACAGGCAGCAGACCAAC	ATCAGCAAGTGACTGAATTCCTCC
26	phi116	GCATACGGCCATGGATGGGA	TCCCTGCCGGGACTCCTG
27	umc1304	CATGCAGCTCTCCAAATTAAATCC	GCCAACTAGAACTACTGCTGCTCC
28	phi233376	CCGGCAGTCGATTACTCC	CGAGACCAAGAGAACCCTCA
29	bnlg1782	CGATGCTCCGCTAGGAATAG	TGTGTTGGAAATTGACCCAA
30	phi015	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG
31	phi032	CTCCAGCAAGTGATGCGTGAC	GACACCCGGATCAATGATGGAAC
32	bnlg1129	GAGAGTATGCTACTCGCCGC	GACGAGTTTGGAGTGCCATT
33	umc1319	TGAGAGCCACCTTCTTGAGCTACT	TTCCTTGAAGGCGAAGGTAGGTAT
34	phi050	TAACATGCCAGACACATACGGACAG	ATGGCTCTAGCGAAGCGTAGAG
35	phi084	AGAAGGAATCCGATCCATCCAAGC	CACCCGTACTTGAGGAAAACCC
36	umc1061	AGCAGGAGTACCCATGAAAGTCC	TATCACAGCACGAAGCGATAGATG

5.3 Observed SSR profiles of maize lines

Molecular profiles of 10 maize lines are reported as estimated fragment sizes in bp for each of 36 SSR markers. For a given SSR in a line, a single fragment size indicates that the marker is monomorphic within the line while two fragment sizes indicate that the marker is polymorphic within the line.

Table 5 — SSR profiles observed for 10 maize lines during intralaboratory validation

No.	SSR				Name of the line						
		A632	A641	CM7	EP1	F7	W64A	B73	CO255	F2	MO17
1	umc1147	86	86	86	84	72	72	84	84	72	72
2	phi109275	137	126	126/137	121/126	126	137	137	126	121	121
3	phi427913	124	130	130	130/133	133	130	130	133	133	130
4	umc1885	138	136	136	138	136	138	138	138	138	138
5	phi064	79	91	107	75	107/110	85	99	107	107	79
6	phi96100	278	278	278	294	294	288	278	288	294	287
7	phi083	123	123	123	125/131	129	129	125	123	132	125
8	umc1448	161	157	148	161	148	148	157	148	148	148
9	phi102228	130	126	122	122	130	126	122	122	122	130
10	umc1489	123	123	123	123	131	123	123	123	123	131
11	umc1117	127	133	126	127	127	133	133	127	127	127
12	umc1329	74	83	83	77/83	83	83	83	83	77	83
13	phi093	281	281	286	286	286	282	288	281	286	281
14	umc1180	99/102	99	99	102	99	99	99	102	102	102
15	nc130	144	144	141/144	141	141	144	141	141	141	144
16	umc1478	135	134	134	139	139	141	135	139	134	135
17	umc1792	125	119	123	116	115	116	115	115	115	116
18	umc1153	103/106	106	103	101/111	111	106	106	111	111	103
19	umc1143	80	80	74	71	71	80	80	71	71	76
20	phi423796	130	130	130	125/130	130	137	130	130	125	130
21	umc1133	98	98	101	91/105	91	98	98	105	101	91
22	phi123	147	141	147	141	141	145	141	141	141	141
23	phi089	82	81	90	90	90	90	81	90	90	82
24	umc1545	81	70	81/83	70/81	70	70	79	81	85	83
25	umc1134	84	84	81	81/88	81	84	81	81	81	88
26	phi116	173	173	152	162	162	169	169	152	162	173
27	umc1304	136	136	131	131	131	131	136	136	131	131
28	phi233376	144	152	144	149/152	140	144	140	140	144	144
29	bnlg1782	230	219	219	226	221	219	219	226	219	219
30	phi015	97	97	97	84	97	82	103	97	97	97
31	phi032	232	232	238	232	232	232	232	232	232	238
32	bnlg1129	194/196	194	190	196/198	187	179	194	194	190	179
33	umc1319	115	124	115	121	121	121	115	121	115	115

NOTE According to the repeated pattern of a SSR marker (di-, tri-, or tetranucleotide), allele sizes were mainly observed as expected. For few cases, discrepancies between expected and observed allele sizes were noticed and validated during the intralaboratory study.