Panels of markers for parentage verification tested at the 2001/02 ISAG Comparison test

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SHEEP

Nineteen microsatellites, 15 of which combined into two multiplexes, were tested:

Multiplex 1: **CSRD0247 - HSC – INRA0063 – MAF0214 – OarAE0129 – OarCP0049 – OarFCB0011 – OarFCB0304**

(proposed by LGS, Cremona, Italy - e-mail: **lgspz@memex.it**)

Multiplex 2: **D5S2 - INRA0005 – INRA0023 – MAF0065 – McM0527 – OarFCB0020 – SPS0113**

(proposed by LGS, Cremona, Italy - e-mail: **lgspz@memex.it**)

Other markers: **BM1258 - BM1329 - BM1818 – INRA132**

(proposed by G. Erhardt, Giessen, Germany - e-mail: **Georg.Erhardt@agrar.uni-giesse.de**)

At the 2001/02 Comparison Test three to seven laboratories (according to the marker) sent the results back, while some other laboratories tested the samples for the microsatellites they routinely use. Therefore, due to this heterogeneity in the output, the comparison gave poor information. Concerning the markers listed above, there was a good agreement among the laboratories for most of them, with the exception of MAF214 and OarAE129, for which considerable discrepancy was observed.

At the 2002/03 Comparison Test multiplex 1 and 2 were tested by all the participating laboratories. Some problems were observed for multiplex I: in many samples it was difficult to see the peak of the second allele for OarCP49 and MAF 214; FCB11 showed a shadow band (137); OarAE129 showed a low degree of polymorphism and so it could be deleted from multiplex I. As for multiplex II, only the amplification of FCB20 gave problems in some samples.

Multiplex 1

Locus	Primer sequences	Dye
CSRD0247	GGA CTT GCC AGA ACT CTG CAA T CAC TGT GGT TTG TAT TAG TCA GG	HEX
HSC	CTG CCA ATG CAG AGA CAC AAG A GTC TGT CTC CTG TCT TGT CAT C	6'FAM
INRA0063	GAC CAC AAA GGG ATT TGC ACA AGC AAA CCA CAG AAA TGC TTG GAA G	6'FAM
MAF0214	AAT GCA GGA GAT CTG AGG CAG GGA CG GGG TGA TCT TAG GGA GGT TTT GGA GG	TET
OarAE0129	AAT CCA GTG TGT GAA AGA CTA ATC CAG GTA GAT CAA GAT ATA GAA TAT TTT TCA ACA CC	TET
OarCP0049	CAG ACA CGG CTT AGC AAC TAA ACG C GTG GGG ATG AAT ATT CCT TCA TAA GG	HEX
OarFCB0011	GCA AGC AGG TTC TTT ACC ACT AGC ACC GGC CTG AAC TCA CAA GTT GAT ATA TCT ATC AC	6'FAM
OarFCB0304	CCC TAG GAG CTT TCA ATA AAG AAT CGG CGC TGC TGT CAA CTG GGT CAG GG	HEX

Multiplex 2

Locus	Primer sequences	Dye
D5S2	TAC TCG TAG GGC AGG CTG CCT G GAG ACC TCA GGG TTG GTG ATC AG	6'FAM
INRA0005	TTC AGG CAT ACC CTA CAC CAC ATG AAA TAT TAG CCA ACT GAA AAC TGG G	HEX
INRA0023	GAG TAG AGC TAC AAG ATA AAC TTC TAA CTA CAG GGT GTT AGA TGA ACT C	TET
MAF0065	AAA GGC CAG AGT ATG CAA TTA GGA G CCA CTC CTC CTG AGA ATA TAA CAT G	TET
McM0527	GTC CAT TGC CTC AAA TCA ATT C AAA CCA CTT GAC TAC TCC CCA A	HEX
OarFCB0020	GGA AAA CCC CCA TAT ATA CCT ATA C AAA TGT GTT TAA GAT TCC ATA CAT GTG	6'FAM
SPS0113	AAA GTG ACA CAA CAG CTT CTC CAG AAC GAG TGT CCT AGT TTG GCT GTG	6'FAM

Other markers

Locus	Primer sequences	Dye
BM1258	GTA TGT ATT TTT CCC ACC CTG C GAG TCA GAC ATG ACT GAG CCT G	Cy
BM1329	TTG TTT AGG CAA GTC CAA AGT C AAC ACC GCA GCT TCA TCC	Cy
BM1818	AGCTGG GAA TAT AAC CAA AGG AGT GCT TTC AAG GTC CAT GC	Cy
INRA0132	AAC ATT TCA GCT GAT GGT GGC TTC TGT TTT GAG TGG TAA GCT G	Cy

PCR CONDITIONS

Multiplex 1

DNA	100 ng
Buffer 10X	1 µl
$MgCl_2$ (25 mM)	1,2 µl
DNTPs (10 mM)	0,4 µl
Taq Gold (5U/ µl)	0,4 µl
Primers (10 µM):	
CSRD247	0,4 + 0,4 µl
HSC	0,3 + 0,3 µl
INRA0063	0,2 + 0,2 µl
MAF0214	0,5 + 0,5 µl
OarAE0129	0,2 + 0,2 µl
OarCP0049	0,05 + 0,05 µl
OarFCB0011	0,15 + 0,15 µl
OarFCB0304	0,3 + 0,3 µl
In a total volume of	10 µl

Amplification programme

95°C x 12’ 31 x (95°C x 20” - 63°C x 1’ - 72°C x 1’

Multiplex 2

DNA	100 ng
Buffer 10X	1 µl
$MgCl_2$ (25 mM)	1,2 µl
DNTPs (10 mM)	0,4 µl
Taq Gold (5U/ µl)	0,4 µl
Primers (10 µM):	
D5S2	0,2 + 0,2 µl
INRA0005	0,3 + 0,3 µl
INRA0023	1,0 + 1,0 µl
MAF0065	0,4 + 0,4 µl
McM0527	0,2 + 0,2 µl
OarFCB0020	0,4 + 0,4 µl
SPS0113	0,1 + 0,1 µl
In a total volume of	10 µl

Amplification programme

95°C x 10’ 31 x (94°C x 30” - 55°C x 30" - 72°C x 1’

Other markers

BM1258 $MgCl_2$ 1.5 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

BM1329 $MgCl_2$ 1,5 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

BM1818 $MgCl_2$ 1,5 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 56°C x 1’ - 72°C x 1’) 72°C x 5’

INRA0132 $MgCl_2$ 1,5 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

Microsatellite characteristics

Locus	Size
CSRD0247	209 - 261
HSC	267 - 301
INRA0063	169 - 207
MAF0214	181 - 265
OarAE0129	135 - 165
OarCP0049	82 - 140
OarFCB0011	122 - 148
OarFCB0304	148 - 190
D5S2	190 - 210
INRA0005	120 - 180
INRA0023	201 - 219
MAF0065	121 - 159
McM0527	165 - 179
OarFCB0020	92 - 118
SPS0113	130 - 158
BM1258	100 - 128
BM1329	145 - 161
BM1818	258 - 270
INRA0132	152 – 172

Additional information on the markers can be found at: http://www.thearkdb.org/

In the following tables data on seven Italian sheep breeds are reported (Di Stasio and coworkers) in order to give additional information on the markers

Data on seven Italian sheep breeds

Locus	allele size (bp)	total n. obs. alleles	n. alleles/breed
CSRD247	209-261	20	6-13
HSC	267-295	14	8-10
MAF0214	181-259	12	3-8
OarAE0129	135-153	7	3-5
OarCP0049	84-140	18	6-13
OarFCB0011	122-148	14	6-10
OarFCB0304	150-188	13	6-11
D5S2	190-204	7	3-6
INRA0005	128-180	14	6-11
INRA0023	198-266	12	7-10
MAF0065	121-159	12	3-9
McM0527	149-179	10	4-8
OarFCB0020	92-112	11	6-9
SPS0113	130-158	12	3-8

Alleles found in seven Italian sheep breeds

Locus	Alleles
CSRD0247	209, 213, 215, 217, 219, 221, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 249, 255, 257, 261
D5S2	190, 192, 194, 196, 198, 200, 204
HSC	267, 269, 271, 273, 275, 277, 279, 281, 283, 287, 289, 291, 293, 295
INRA0005	128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 154, 180
INRA0023	198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 226
MAF0214	181, 185, 187, 189, 219, 221, 227, 249, 251, 255, 257, 259
MAF0065	121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 159
McM0527	149, 153, 165, 167, 169, 171, 173, 175, 177, 179
OarAE0129	135, 137, 139, 147, 149, 151, 153
OarCP0049	84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 122, 136, 140
OarFCB011	122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148
OarFCB0020	92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112
OarFCB0304	150, 162, 164, 166, 168, 170, 172, 174, 178, 180, 184, 186, 188
SPS0113	130, 134, 138, 140, 142, 144, 146, 148, 150, 152, 154, 158

Number of observed and effective alleles in seven Italian sheep breeds

Loci	Biellese		Langhe		Frabosana		Garessina		Sambucana		Savoiarda		Tacola	
CSRD0247	11	7.3	10	3.4	11	6.7	6	2.1	13	6.9	8	3.6	11	5.8
D5S2	6	2.9	3	1.3	5	2.9	5	2.7	4	2.6	5	1.9	4	3.1
HSC	10	6.9	10	6.8	10	8.7	9	4.7	11	7.8	8	5.0	8	6.3
INRA0005	9	8.8	6	4.0	10	5.9	7	3.5	10	7.0	8	5.5	11	8.4
INRA0023	10	5.7	7	5.0	9	7.6	9	3.7	9	5.8	8	4.6	9	6.0
MAF0214	8	2.9	8	2.4	5	2.7	5	2.7	8	2.7	3	2.7	6	3.1
MAF0065	9	4.2	6	4.0	8	4.1	3	1.5	7	4.2	6	1.8	7	4.7
McM0527	8	4.4	5	3.9	6	4.2	4	1.7	6	3.9	7	3.8	7	4.4
OarAE0129	3	3.0	3	1.8	5	3.8	5	2.2	3	2.4	3	2.2	3	3.0
OarCP0049	13	2.9	9	3.8	13	5.2	6	2.4	12	3.6	7	5.1	9	2.4
OarFBC0011	8	4.4	6	5.2	7	4.6	6	2.7	6	5.0	8	5.4	10	5.4
OarFCB0020	7	5.1	6	5.3	7	4.8	9	6.1	8	3.6	6	4.2	8	4.7
OarFCB0304	11	3.6	7	4.7	7	3.3	6	2.7	7	2.3	7	4.0	8	3.2
SPS0113	5	1.9	8	5.0	5	3.5	7	3.8	8	3.2	3	1.6	6	3.3
mean	**8.4**	**4.6**	**6.7**	**4.0**	**7.7**	**4.8**	**6.2**	**3.0**	**8.0**	**4.3**	**6.2**	**3.6**	**7.6**	**4.5**

Observed and expected heterozygosity in seven Italian sheep breeds

Loci	Biellese		Langhe		Frabosana		Garessina		Sambucana		Savoiarda		Tacola	
CSRD0247	.83	.86	.78	.71	.75	.85	.58	.54	.78	.86	.90	.73	.88	.83
D5S2	.74	.66	.23	.28	.55	.66	.63	.64	.52	.62	.52	.49	.65	.68
HSC	.80	.86	.86	.85	.85	.89	.88	.79	.90	.82	.81	.80	.92	.84
INRA0005	.90	.89	.67	.75	.75	.83	.57	.72	.42	.86	.81	.82	.92	.88
INRA0023	.65	.83	.73	.80	.88	.87	.74	.73	.70	.83	.65	.79	.80	.83
MAF0214	.65	.67	.50	.59	.58	.63	.63	.63	.62	.64	.57	.64	.54	.68
MAF0065	.69	.76	.62	.75	.67	.76	.42	.35	.75	.77	.45	.46	.81	.79
McM0527	.42	.77	.26	.75	.40	.77	.35	.41	.58	.75	.31	.74	.42	.77
OarAE0129	.46	.67	.38	.47	.48	.74	.28	.55	.44	.59	.38	.56	.31	.67
OarCP0049	.65	.66	.68	.74	.80	.81	.67	.59	.75	.72	.95	.80	.62	.59
OarFBC0011	.68	.78	.62	.81	.85	.78	.71	.64	.74	.80	.76	.82	.65	.82
OarFCB0020	.78	.81	.75	.81	.68	.79	.83	.84	.62	.73	.71	.76	.69	.79
OarFCB0304	.78	.72	.73	.79	.58	.70	.79	.63	.46	.57	.71	.75	.69	.69
SPS0113	.48	.49	.82	.80	.80	.72	.88	.74	.75	.69	.38	.38	.65	.70
mean	**.68**	**.74**	**.62**	**.71**	**.69**	**.77**	**.64**	**.64**	**.66**	**.74**	**.64**	**.68**	**.68**	**.76**

GOATS

Eighteen microsatellites, 14 of which combined into two multiplexes, were tested:

Multiplex 1: **HSC – ILST0019 - INRA0005 – INRA0063 – MAF0065 – SRCRSP0005 – SRCRSP0008 – SRCRSP0024**

(proposed by LGS, Cremona, Italy - e-mail: **lgspz@memex.it**)

Multiplex 2: **CSRD0247 – ILSTS0087 - INRA0023 – McM0527 – OarFCB0020 – SRCRSP0023**

(proposed by LGS, Cremona, Italy - e-mail: **lgspz@memex.it**)

Other markers: **BM1258 - BM1329 - BM1818 – INRA0132**

(proposed by G. Erhardt, Giessen, Germany e-mail: **Georg.Erhardt@agrar.uni-giesse.de**)

At the 2001/02 Comparison Test three to seven laboratories (according to the marker) sent the results back, while some other laboratories tested the samples for the microsatellites they routinely use. Therefore, due to this heterogeneity in the output, the comparison gave poor information. Concerning the markers listed above, there was a good agreement among the laboratories for most of them, with the exception of MAF0065, for which considerable discrepancy was observed.

At the 2002/03 Comparison Test multiplex 1 and 2 were tested and no problems were reported.

Multiplex 1

Locus	Primer sequences	Dye
HSC	CTG CCA ATG CAG AGA CAC AAG A GTC TGT CTC CTG TCT TGT CAT C	6'FAM
ILST019	AGG GAC CTC ATG TAG AAG C ACT TTT GGA CCC TGT AGT GC	HEX
INRA0005	TTC AGG CAT ACC CTA CAC CAC ATG AAA TAT TAG CCA ACT GAA AAC TGG G	HEX
INRA0063	GAC CAC AAA GGG ATT TGC ACA AGC AAA CCA CAG AAA TGC TTG GAA G	6'FAM
SRCRSP0005	GGA CTC TAC CAA CTG AGC TAC AAG TGA AAT GAA GCT AAA GCA ATG C	TET
SRCRSP0008	TGC GGT CTG GTT CTG ATT TCA C CCT GCA TGA GAA AGT CGA TGC TTA G	TET
SRCRSP0024	AGC AAG AAG TGT CCA CTG ACA G TCT AGG TCC ATC TGT GTT ATT GC	6'FAM

Multiplex 2

Locus	Primer sequences	Dye
CSRD0247	GGA CTT GCC AGA ACT CTG CAA T CAC TGT GGT TTG TAT TAG TCA GG	HEX
ILST0087	AGC AGA CAT GAT GAC TCA GC CTG CCT CTT TTC TTG AGA G	6'FAM
INRA0023	GAG TAG AGC TAC AAG ATA AAC TTC TAA CTA CAG GGT GTT AGA TGA ACT	TET
McM0527	GTC CAT TGC CTC AAA TCA ATT C AAA CCA CTT GAC TAC TCC CCA A	HEX
OarFCB0020	GGA AAA CCC CCA TAT ATA CCT ATA C AAA TGT GTT TAA GAT TCC ATA CAT GTG	TET
SRCRSP0023	TGA ACG GGT AAA GAT GTG TGT TTT TAA TGG CTG AGT AG	6'FAM

Other markers

Locus	Primer sequences	Dye
BM1258	GTA TGT ATT TTT CCC ACC CTG C GAG TCA GAC ATG ACT GAG CCT GM	Cy
BM1329	TTG TTT AGG CAA GTC CAA AGT C AAC ACC GCA GCT TCA TCC	Cy
BM1818	AGCTGG GAA TAT AAC CAA AGG AGT GCT TTC AAG GTC CAT GC	Cy
INRA0132	AAC ATT TCA GCT GAT GGT GGC TTC TGT TTT GAG TGG TAA GCT G	Cy

PCR CONDITIONS

Multiplex 1

DNA	100 ng
Buffer 10X	1 µl
$MgCl_2$ (25 mM)	1,6 µl
DNTPs (10 mM)	0,2 µl
Taq Gold (5U/ µl)	0,4 µl
Primers (10 µM):	
HSC	0,3 + 0,3 µl
ILST0019	0,2 + 0,2 µl
INRA0005	0,1 + 0,1 µl
INRA0063	0,2 + 0,2 µl
MAF0065	0,3 + 0,3 µl
SRCRSP0005	0,5 + 0,5 µl
SRCRSP0008	0,2 + 0,2 µl
SRCRSP0024	0,4 + 0,4 µl
In a total volume of	10 µl

Amplification programme

95°C x 4’ 31 x (94°C x 30” - 55°C x 30” - 72°C x 1’

Multiplex 2

DNA	100 ng
Buffer 10X	1 µl
$MgCl_2$ (25 mM)	1,2 µl
DNTPs (10 mM)	0,4 µl
Taq Gold (5U/ µl)	0,4 µl
Primers (10 µM):	
CSRD0247	0,15 + 0,15 µl
ILSTS0087	0,08 + 0,08 µl
INRA0023	1,2 + 1,2 µl
McM0527	0,1 + 0,1 µl
OarFCB0020	0,25 + 0,25 µl
SRCRSP0023	0,25 + 0,25 µl
In a total volume of	10 µl

Amplification programme

95°C x 10’ 31 x (94°C x 30” - 55°C x 30" - 72°C x 1’

Other markers

BM1258 $MgCl_2$ 2,0 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

BM1329 $MgCl_2$ 1,5 mM

Amplification programme
94°C x 1,5’ 34 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

BM1818 $MgCl_2$ 3,0 mM

Amplification programme
94°C x 1,5’ 34 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

INRA0132 $MgCl_2$ 2,0 mM

Amplification programme
94°C x 1,5’ 30 x (94°C x 1’ - 58°C x 1’ - 72°C x 1’) 72°C x 5’

Microsatellite characteristics

Locus	Size
HSC	271 - 304
ILST19	144 - 158
INRA0005	118 - 126
INRA0063	171 - 181
MAF0065	119 - 157
SRCRSP0005	158 - 180
SRCRSP0008	209 - 235
SRCRSP0024	139 - 175
CSRD0247	221 - 247
ILST0087	137 - 155
INRA0023	197 - 215
McM0527	152 - 168
OarFCB0020	093 - 117
SRCRSP0023	85 - 123
BM1258	110 - 120
BM1329	145 - 161
BM1818	258 - 270
INRA0132	152 – 172

Additional information on the markers can be found at:
http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE=goat

In the following tables data on two Italian goat breeds are reported (Di Stasio and coworkers) in order to give additional information on the markers

Data on two Italian goat breeds

Locus	allele size (bp)	total n. obs. alleles	n. alleles/breed
HSCA	274-304	12	9-10
ILSTS0019	144-156	6	4-6
INRA0005	118-124	4	4
INRA0063	171-181	6	4-6
MAF0065	119-157	12	8-11
SRCRSP0024	155-175	9	5-9
SRCRSP0005	159-171	6	6
SRCRSP0008	218-240	7	5-6
CSRD0247	233-247	7	5-6
ILSTS0087	139-153	8	6-8
INRA0023	197-215	9	7
McM0527	152-168	6	4-6
OarFCB0020	95-117	8	5-6
SRCRSP0023	87-123	15	12

Ms markers and observed alleles in two Italian goat breeds

Locus	Alleles
CSRD0247	233, 235, 237, 241, 243, 245, 247
HSC	274, 276, 278, 282, 284, 286, 288, 290, 292, 298, 300, 304
ILSTS0019	144, 148, 150, 152, 154, 156
ILSTS0087	139, 141, 143, 145, 147, 149, 151, 153
INRA0005	118, 120, 122, 124
INRA0023	197, 199, 201, 205, 207, 209, 211, 213, 215
INRA0063	171, 173, 175, 177, 179, 181
MAF0065	119, 121, 123, 125, 127, 131, 133, 135, 137, 153, 155, 157
McM0527	152, 154, 162, 164, 166, 168
OarFCB0020	95, 97, 99, 101, 105, 107, 109, 117
SRCRSP0023	87, 89, 95, 99, 101, 103, 105, 107, 109, 111, 115, 117, 119, 121, 123
SRCRSP0024	155, 157, 159, 161, 163, 165, 167, 171, 175
SRCRSP0005	159, 161, 165, 167, 169, 171
SRCRSP0008	218, 220, 222, 224, 236, 238, 240

Number of observed and effective alleles in two Italian goat breeds

Loci	Roccaverano		Vallesana	
CSRD0247	5	3.3	6	4.1
HSC	10	5.8	9	5.8
ILSTS0019	6	4.1	4	3.8
ILSTS0087	8	5.9	6	4.1
INRA0005	4	1.7	4	1.4
INRA0023	7	3.3	7	4.3
INRA0063	6	2.7	4	1.9
MAF0065	11	5.6	8	5.3
McM0527	6	3.9	4	2.7
OarFCB0020	5	2.9	6	2.8
SRCRSP0023	12	6.0	12	6.7
SRCRSP0024	9	3.0	5	3.4
SRCRSP0005	6	4.2	6	3.0
SRCRSP0008	6	3.1	5	2.3
mean	**7.2**	**4.0**	**6.1**	**3.7**

Observed and expected heterozygosity in two Italian goat breeds

Loci	Roccaverano		Vallesana	
CSRD0247	.81	.71	.70	.77
HSC	.97	.84	.70	.84
ILSTS0019	.73	.77	.83	.75
ILSTS0087	.89	.85	.70	.77
INRA0005	.43	.42	.23	.27
INRA0023	.77	.71	.70	.78
INRA0063	.66	.65	.57	.48
MAF0065	.87	.84	1.0	.82
McM0527	.71	.76	.60	.64
OarFCB0020	.64	.66	.70	.66
SRCRSP0023	.82	.85	.77	.87
SRCRSP0024	.44	.68	.70	.72
SRCRSP0005	.79	.77	.63	.68
SRCRSP0008	.66	.69	.50	.57
mean	**.73**	**.73**	**.67**	**.69**