



**Application of 12 STR markers recommended
by ISAG for the evaluation of genetic variation
in 3 Polish sheep breeds**

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List of recommended markers with primer information

Sheep STR markers

Marker	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
AME	CAGCCAAACCTCCCTCTGC	CCCGCTGGTCTTGTCTGTTGC
CSR247	GGACTTGCCAGAACTCTGCAAT	CACTGTGGTTTGTATTAGTCAGG
ETH152/D5S2	TACTCGTAGGGCAGGCTGCCTG	GAGACCTCAGGGTTGGTGATCAG
INRA005	TTCAGGCATACCCTACACCACATG	AAATATTAGCCAACCTGAAAACCTGGG
INRA006	AGGAATATCTGTATCAACCGCAGTC	CTGAGCTGGGGTGGGAGCTATAAATA
INRA023	GAGTAGAGCTACAAGATAAACTTC	TAACTACAGGGTGTTAGATGAACTC
INRA063	GACCACAAAGGGATTTGCACAAGC	AAACCACAGAAATGCTTGAAG
INRA172	CCAGGGCAGTAAAATGCATAACTG	GGCCTTGCTAGCCTCTGCAAAC
MAF065	AAAGGCCAGAGTATGCAATTAGGAG	CCACTCCTCCTGAGAATATAACATG
MAF214	AATGCAGGAGATCTGAGGCAGGGACG	GGGTGATCTTAGGGAGGTTTGGAGG
MCM042	CATCTTCAAAGAAGTCCGAAAGTG	CTTGGAATCCTTCCTAACTTTCGG
MCM527	GTCCATTGCCTCAAATCAATTC	AAACCACTTGACTACTCCCCAA
OARFCB20	GGAAAACCCCATATATACCTATAC	AAATGTGTTAAGATTCCATACATGTG

Purpose

To analyse the genetic diversity, genetic relationship and breed structure of three Polish sheep breeds and to study of the efficiency of the 11-plex STRs panel recommended by ISAG for sheep parentage testing.

Materials

A total of 503 sheep from 3 breeds in Poland:

Wielkopolska sheep (n=118)



<http://owce.bioroznorodnosc.izoo.krakow.pl/rasy>

about 20,000 individuals

Olkuska sheep (n=88)



<http://owce.bioroznorodnosc.izoo.krakow.pl/rasy>

less than 2,000 individuals

Merino sheep: Polish Merino and Old Type Polish Merino (n=297)



<http://owce.bioroznorodnosc.izoo.krakow.pl/rasy>

above 40,000 individuals

Methods

Genotyping was performed by amplifying the genomic DNA using multiplex PCR kits and fragment analysis was carried out using genetic analyzer.

STR

CSRD247
ETH152
INRA005
INRA006
INRA063
INRA172
MAF065
MAF214
McM042
McM527
OarFCB20
Amelogenine

PCR

<u>I-plex</u>	
Locus	Dye
INRA063	6-FAM
INRA006	6-FAM
MAF214	JOE
McM042	JOE
CSRD247	NED
INRA172	NED
Amelogenine	NED

<u>II-plex</u>	
Locus	Dye
MAF065	6-FAM
McM527	6-FAM
OarFCB20	JOE
ETH152	JOE
INRA005	NED

Electrophoresis and Genotyping

Genetic analysis was performed on the ABI 3130xl Sequencer and the results were analysed using GeneMapper Software 4.0.

The identified alleles were used to estimate:

H_o - observed heterozygosity

H_E - expected heterozygosity

PIC - polymorphic information content

D_N - genetic distance according to Nei (1987)

CPE - cumulative probabilities of parentage exclusion.

- CPE_1 when genotype of one parent is known

- CPE_2 two parents are known

(Fredholm i Wintero 1996, Jamieson i Taylor 1997).

CPD - combined power of discrimination (Jones 1972)

$CP_{ID(\text{theoretical})}$ - combined probability of identity (Paetkau & Strobeck 1994)

Hardy-Weinberg Equilibrium

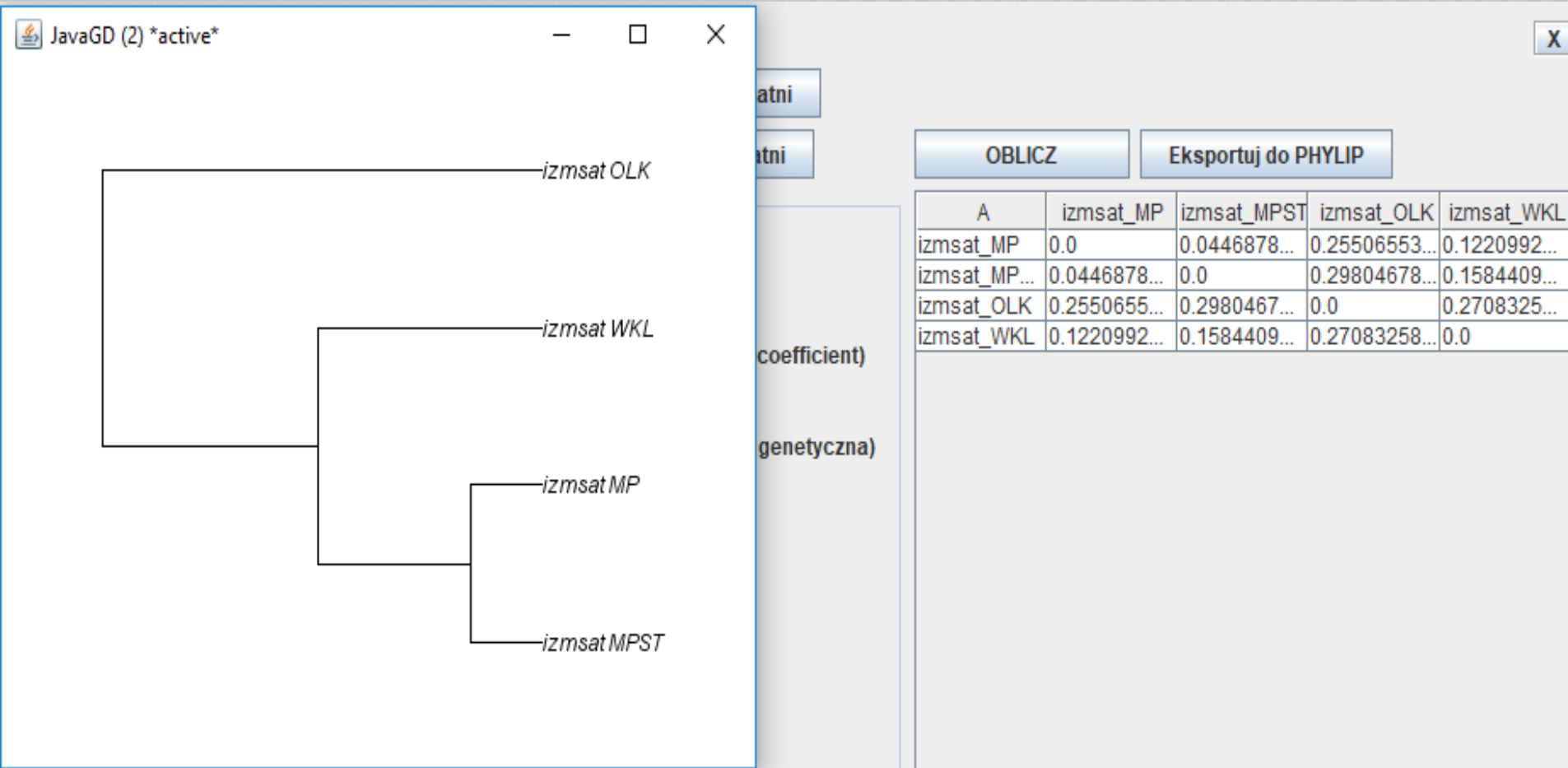
(exact test, Markov-Chain Monte Carlo)

Locus	HWE					
	Merynos n=297		Olkuska n=88		Wielkopolska n=118	
	p	std blad p-value	p	std blad p-value	p	std blad p-value
OarFCB20	0.0590	40.5508	0.1168	37.0927	0.1856	43.3838
MAF065	0.7903	3.1460	0.0543	18.0409	0.9313	12.2964
INRA63	0.0520	64.4197	0.9256	12.4834	0.8908	19.2314
INRA006	0.0572	43.5646	0.0339*	83.8072	0.9344	12.1870
McM527	0.0610	29.4778	0.5294	9.0287	0.8834	8.8858
McM042	0.2439	7.9220	0.6473	7.8113	0.5429	13.7722
INRA005	0.8177	28.2631	0.2383	18.4796	0.1984	52.7884
MAF214	0.5047	20.2618	0.0664	40.5605	0.0324*	35.0073
INRA172	0.6613	7.6669	0.5669	8.6369	0.9581	16.4865
ETH152	0.1378	9.7017	0.0523	12.4677	0.5172	5.2099
CSRD247	0.2570	18.1081	0.8211	2.9012	0.9439	11.8368

* $p \leq 0.05$

Results

genetic distance D_N according to Nei (1987)



The estimated coefficient of genetic distance based on all markers ranged from $D_N=0.122$ between Merino and Wielkopolska to $D_N=0.298$ between Merino and Olkuska sheep and $D_N=0.0447$ between Polish Merino and Old Type Polish Merino

Results

H_O - observed heterozygosity

H_E - expected heterozygosity

PIC - polymorphic information content

	Merynos n=297			Olkuska n=88			Wielkopolska n=118		
	Ho	He	PIC	Ho	He	PIC	Ho	He	PIC
OarFCB20	0.6599	0.6367	0.6037	0.8295	0.7829	0.7532	0.7458	0.7555	0.7281
MAF065	0.7071	0.6946	0.6376	0.8409	0.8110	0.7050	0.7966	0.7524	0.7085
INRA63	0.7643	0.7918	0.7646	0.7273	0.6708	0.6264	0.7712	0.7573	0.7177
INRA006	0.6599	0.6585	0.6289	0.4750	0.5378	0.5132	0.7119	0.6806	0.6509
McM527	0.7172	0.7344	0.6956	0.7173	0.6842	0.6073	0.6421	0.6841	0.6429
McM042	0.5183	0.5374	0.4927	0.6705	0.5768	0.5265	0.7458	0.7361	0.6926
INRA005	0.8010	0.8412	0.8221	0.8523	0.7980	0.7146	0.8644	0.8105	0.7860
MAF214	0.6162	0.6236	0.5624	0.7045	0.7159	0.6818	0.8305	0.7953	0.6701
INRA172	0.2391	0.2352	0.2286	0.6136	0.5874	0.5174	0.6421	0.6092	0.5713
ETH152	0.6633	0.6587	0.6039	0.6591	0.5987	0.5081	0.5169	0.5327	0.4800
CSRD247	0.7407	0.7156	0.6728	0.5795	0.5799	0.5167	0.5169	0.5188	0.4933
\bar{X}	0.6425	0.6480	0.6103	0.6972	0.6676	0.6064	0.7040	0.6942	0.6492

Probabilities of parentage exclusion – PE

(probability of pedigree confirmation)

Merynos (n=297)

<i>loci</i>	PE ₁	PE ₂
OarFCB20	0.2442	0.4247
MAF065	0.2685	0.4336
INRA63	0.4274	0.6047
INRA006	0.2662	0.4513
McM527	0.3325	0.5116
McM042	0.1543	0.3101
INRA005	0.5177	0.6849
MAF214	0.2070	0.3613
INRA172	0.0291	0.1296
ETH152	0.2390	0.4037
CSRD247	0.3149	0.4894
	0.9746	0.9987

Merynos

CPE₁ (11) = 97.46 %
 CPE₂ (11) = 99.87%

Olkuska (n=88)

<i>loci</i>	PE ₁	PE ₂
OarFCB20	0.4108	0.5886
MAF065	0.3314	0.5074
INRA63	0.2639	0.4373
INRA006	0.1668	0.3423
McM527	0.2405	0.4038
McM042	0.1763	0.3344
INRA005	0.3546	0.5368
MAF214	0.3189	0.5016
INRA172	0.1828	0.3225
ETH152	0.1515	0.2913
CSRD247	0.1713	0.3172
	0.9618	0.9978

Olkuska

CPE₁ (11) = 96.18%
 CPE₂ (11) = 99.78%

Wielkopolska (n=118)

<i>loci</i>	PE ₁	PE ₂
OarFCB20	0.3784	0.5615
MAF065	0.3392	0.5157
INRA63	0.3591	0.5353
INRA006	0.2883	0.4740
McM527	0.2805	0.4570
McM042	0.3297	0.5048
INRA005	0.4575	0.6325
MAF214	0.3007	0.4763
INRA172	0.2127	0.3870
ETH152	0.1463	0.2932
CSRD247	0.1534	0.3244
	0.9805	0.9992

Wielkopolska

CPE₁ (11) = 98.05%
 CPE₂ (11) = 99.92%

Results

Power of discrimination - PD

<i>loci</i>	Merynos n=297	Olkuska n=88	Wielkopolskan=118
OarFCB20	0.64969	0.9063	0.91037
MAF065	0.65556	0.8629	0.88725
INRA63	0.82027	0.8350	0.90003
INRA006	0.82731	0.7257	0.87216
McM527	0.80903	0.8226	0.86627
McM042	0.54044	0.7570	0.87877
INRA005	0.78609	0.8889	0.92014
MAF214	0.84686	0.8680	0.82261
INRA172	0.70166	0.7428	0.80609
ETH152	0.83833	0.6792	0.72106
CSRD247	0.73473	0.7567	0.74074
11-plex	0.9999999989	0.9999999993	0.99999999958

Results

Theoretical estimates of CP_{ID}

is the probability that 2 individuals, randomly selected, have the same genotype.

<i>loci</i>	Merynos n=297	Olkuska n=88	Wielkopolska n=118
OarFCB20	0.1650	0.07682	0.08715
MAF065	0.1503	0.10803	0.10518
INRA63	0.0705	0.15277	0.09852
INRA006	0.1462	0.23826	0.13173
McM527	0.1093	0.16965	0.14104
McM042	0.2587	0.22938	0.11312
INRA005	0.0444	0.09692	0.06039
MAF214	0.2029	0.11481	0.12627
INRA172	0.5915	0.24021	0.19062
ETH152	0.1713	0.27342	0.27107
CSRD247	0.1237	0.23966	0.25700

1.16E-11

2.06E-09

1.92E-10

PID for 11 STR was between 1.2×10^{-11} (MP) and 2.1×10^{-9} (OLK)

Random Match Probability - RMP

is the probability that randomly selected individual from reference population will have the same DNA profile as the one found in investigated cases.

What is RMP
if we use 11 STR?

To assess RMP for the 11-STR panels we used:

- Reference population included 3318 samples,
and
- DNA profile established during investigation of special case in our lab.

Results

oarfc20 a b 89 91
oarfc20 a b 127 131
maf065 a b 169 197
maf065 a b 110 132
inra63 a b 166 168
inra63 a b 87 87
inra006 a b 145 145
inra006 a b 187 189
mcm52 a b 154 154
mcm527 a b 186 190
mcm04 a b 213 237
mcm042 a b
inra005 a b
inra005 a b
maf214 a b
maf214 a b
inra172 a b
inra172 a b
eth152 a b
eth152 a b
csrd24 a b
csrd247 a b

We calculated RMP for the matching profile created for investigation of a case.

RMP

Haplotypy: RMP_seep2018 Częstość alleli - Haplotypy: RMP_seep2018 Prawd. profilu - Haplotypy: RMP_seep2018

Genotyp

Loci	Profil 1	Profil 2
oarfc20a	89	91
maf065a	127	131
inra63a	169	197
inra006a	110	132
mcm527a	166	168
mcm042a	87	87
inra005a	145	145
maf214a	187	189
inra172a	154	154
eth152a	186	190
csrd247a	213	237

Prawdopodobieństwo wystąpienia profilu : 3.3325114549048906E-13

Oblicz prawdopodobieństwo

11 STRs

The probability that a sheep selected randomly from the population will have the same profile from the certificate DNA was:

3.3×10^{-13} for 11 STRs

Conclusion

The analysed panel of 11 microsatellite markers showed a high degree of polymorphism in the *loci* of the analysed sheep breeds, which indicates that it can be used for evaluating the genetic structure of sheep.

The high combined PE and PD is indicative of the high usefulness of the tested set of markers for individual identification.



Thank you for your attention