Evaluating the resolution power of new microsatellites for species identification and stock delimitation in the Cape hakes *Merluccius paradoxus* and *M. capensis* (Teleostei: Merlucciidae)

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Abstract

The utility of 15 new and 17 previously published microsatellite markers was evaluated for species identification and stock delimitation in the deep-water hake *Merluccius paradoxus* and the shallow-water hake *Merluccius capensis*. A total of 14 microsatellites was polymorphic in *M. paradoxus* and 10 in *M. capensis*. Two markers could individually discriminate the species using Bayesian clustering methods and a statistical power analysis showed that the set of markers for each species is likely to detect subtle genetic differentiation ($F_{ST} < 0.006$), which will be valuable to delimit and characterise genetic stocks.

Key words: Bayesian methods; cross-species amplification; genetic markers; genomic library; power analysis

Both the shallow-water hake *Merluccius capensis* Castelnau, 1861 and the deep-water hake *M. paradoxus* Franca, 1960 are targeted by a valuable demersal fishery along the west coasts of Southern Africa (>100 million USD annually; Butterworth & Rademeyer, 2005), but the intensification of exploitation over recent decades caused a resource decline (Payne & Punt, 1995). Due to their morphological similarity and overlapping distribution, the two species are not distinguished in the commercial landings records (von der Heyden et al., 2007b) and they are combined into geographic managements units, namely Namibia, west coast and south coast of South Africa (Butterworth & Rademeyer, 2005). Previous genetic surveys successfully distinguished the two species (Grant *et al.,* 1987; von der Heyden et al., 2007b; Garcia-Vazquez *et al.*, 2012) and detected population differentiation (Grant *et al.,* 1987; von der Heyden *et al.,* 2007a) using mtDNA markers and allozymes. Since these markers are inadequate to draw final conclusions regarding stock delimitation, highly informative markers such as microsatellites are necessary (Selkoe *et al.,* 2006).

To provide reliable genetic markers for species identification and stock delimitation in the two Cape hake species, the resolution power of newly developed and previously published microsatellites is assessed. The development of *de novo* microsatellite markers is presented. The discriminating power of each microsatellite for the correct identification of species was evaluated. A simulation approach was used to assess the robustness of each set of markers in detecting subtle genetic differentiation.

Total genomic DNA was isolated using the DNeasy tissue extraction kit (Qiagen, www.qiagen.com). A partial genomic library enriched using two sets of four tetranucleotide repeat probes (TATC/AGCA/GCGA/CAGC and GATA/GTCT/GAAA/ACGT) was generated for *M. paradoxus* following Zane *et al.* (2002). A total of 585 clones was selected and sequenced and Msatcommander 0.8.2 (Faircloth, 2008) was used to identify 213 sequences containing repeats and design 141 primers after exclusion of duplicates (Table SI, Supporting Information). A total of 20 loci was chosen for further analyses based on quality/length of the repeats (*e.g.* containing perfect repeats). Additional microsatellites were tested from: Moran *et al.* (1999, five markers), Seibert & Ruzzante (2006, four), D'Amato *et al.* (1999, six) and Rico *et al.* (1997, two).

A total of 15 markers with consistent PCR amplification on 2% agarose gels stained with GelRedTM Acid stain (Biotium, www.biotium.com) were selected. PCRs were prepared with 25-100 ng of genomic DNA, 1X PCR buffer, 0.4U Supertherm *Taq* polymerase (Southern Cross Biotechnologies, http://za.w393.com/27566370205), 1.5 mM $MgCl_2$, 1 pmol forward and reverse primers (Inqaba Biotec, www.inqababiotec.co.za) and 0.2 mM dNTPs (Promega, www.promega.com) in a final volume of 10 µL. The PCR cycling conditions were: 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 45 sec at 72°C and a final elongation step of 20 min at 72°C. Polymorphism was assessed in 32 adults of each species collected within the same location in 2005 (von der Heyden *et al.,* 2007a). The genotyping was performed by combining the Quantitect Multiplex PCR kit (Qiagen, www.qiagen.com) and four markers fluorescently labelled using the G5 dye set (NED, VIC, PET or FAM) from Applied Biosystems

Table I. Characterization of microsatellite markers for the deep-water hake *Merluccius paradoxus*. The "Fluo" column refers to the fluorescent labels; F and R refer to the forward and reverse primers; *A*: number of alleles; H_K and H_O: unbiased expected and observed heterozygosity; F_{IS}: inbreeding coefficient assessing Hardy–Weinberg equilibrium (*HWE*) within samples; "*" indicates markers showing a significant deficit of heterozygotes (departure from *HWE*) and potential presence of null alleles according to Micro-Checker.

Locus	Fluo	Repeat motif (5'–3')		Primer sequences (5'–3')	Size (bp)	*A*	H_K	H_O	F_{IS}
MP0051	NED	$(GGCT)_8$	F	GTT GGA CCA ACA CCG GGA TG	176-220	8	0.590	0.531	0.101^{ns}
			R	ACG CTC CTG TGG ACC AGA TG					
MP0318	FAM	$(ACAG)_5$	F	TTG TCC GGC CAT GGT GAC AG	129-165	9	0.641	0.531	0.174^{ns}
			R	AGG CCT AGC ATG CCA AGA AG					
MP0374	PET	$(CTGT)_5$	F	GCT TGG TGT GGC TAT GAC TC	89-105	4	0.441	0.406	0.081^{ns}
			R	CAA CCT CGT CTG TCC TTT GG					
MP8450	VIC	$(AGAT)_{16}$	F	AGA GTT AGC GCA CAG TCC AC	194-274	16	0.917	0.812	0.115^{ns}
			R	TGT CCT GTC TAC CCG TTG TC					
MP8477	VIC	$(AG)_6$...$(CTGT)_8$	F	TTG GGA CGG TCC CAG GAA AG	99-171	14	0.891	0.812	0.089^{ns}
			R	GAA GTG ACC GTT GGC CCA TC					
MP8478	VIC	$(ACAG)_8$...$(GT)_9$...$(GT)_6$...$(GT)_6$	F	AGA ACA ATG CAG CGG CTA AC	188-224	12	0.885	0.906	-0.024^{ns}
			R	AGC CAC AGT CCA CAT TCA TC					

MP8494	NED	(CTGT)$_5$	F	TGA TGA TGA TGC GGA TGG AG	290-326	10	0.690	0.687	0.004^{ns}
			R	GTG GCT TGA TGA TGT GAA CC					
MP9102	NED	(ACAG)$_{16}$	F	GTA CAT ACG CAC GCA GAC AG	242-334	25	0.967	1.000	-0.034^{ns}
			R	TGT GTC TTG GGA CAG AGT TG					
MP9117	PET	(AGAT)$_{11}$	F	GTA AAC ACA GGT GGG AAA GG	273-321	14	0.916	0.875	0.046^{ns}
			R	GGT CTG CAA CAC AAC ACA AG					
MP9131	PET	(AGAT)$_5$	F	CGG GAG GGT TAA CAG TTA TG	293-317	7	0.569	0.406	0.289*
			R	GTG TCC ACC CAA GAT GTA TG					
Mmerhk3b	FAM	(GT)$_{-}$	F	TAG ATT ACC CGC TAC TGG	182-202	5	0.441	0.437	0.009^{ns}
			R	GGC TTC AGG AAC AGA ATG					
Mmerhk20	PET	(GT)$_{13}$	F	CAG AGC CCT GCA AAG GAG AC	214-282	20	0.931	0.937	-0.007^{ns}
			R	CTA CCG CAC TGG ACT GTG AG					
Mmerhk29	VIC	(TG)$_{12}$	F	GTT GCC AGT GGT TAC G	134-162	10	0.880	0.594	0.329*
			R	TCC AGC TAG ACC TTG TG					
Mmer110-8	NED	(CA)$_{12}$	F	CAT GGT CGG GGA AAT AGA GGG	113-141	14	0.918	0.656	0.288*
			R	CTA CCA ACG GAT GAG CCA AAC					

(www.appliedbiosystems.com). The PCR products were then electrophoresed along with the GeneScan-500 LIZ size standard on an ABI 3100 (Applied Biosystems, www.appliedbiosystems.com) and alleles scored using GeneMarker 1.80 (SoftGenetics, www.softgenetics.com).

Genetic parameters and significance tests were performed using Genetix 4.05 (Belkhir *et al.*, 2004) and included unbiased expected heterozygosity (H_K; Nei, 1978), observed heterozygosity (H_O), number of alleles per locus (A), Hardy-Weinberg (F_{IS}) and linkage (LD) disequilibrium

Table II. Characterization of the 10 polymorphic microsatellite markers for the shallow-water hake *Merluccius capensis*. A: number of alleles; H_E and H_O: expected and observed heterozygosity; F_{IS}: inbreeding coefficient assessing Hardy–Weinberg equilibrium within samples. “*” indicate markers showing significant deficit of heterozygotes (departure from HWE) and potential presence of null alleles according to Micro-Checker.

Locus	Size (bp)	A	H_K	H_O	F_{IS}
MP0051	192-212	5	0.306	0.344	-0.127^{ns}
MP0318	129-149	5	0.310	0.281	0.095^{ns}
MP0374	83-87	2	0.146	0.156	-0.069^{ns}
MP8450	215-275	12	0.870	0.812	0.067^{ns}
MP8478	192-228	10	0.824	0.969	-0.179^{ns}
MP8494	308-320	4	0.305	0.125	0.593*
Mmerhk3b	321-325	3	0.588	0.562	0.044^{ns}
Mmerhk20	215-259	19	0.917	0.844	0.081^{ns}
Mmerhk29	151-183	14	0.877	0.344	0.612*
Mmerhk34b	118-166	18	0.924	0.750	0.190*

(Table I). A total of 14 polymorphic microsatellites (10 *de novo*) were obtained for the deep-water hake (A = 4–25; H_K = 0.441–0.967; Table I) and 10 (six *de novo*) for the shallow-water hake (A = 2–19; H_K = 0.146–0.924; Table II). No LD was observed among markers. Micro-Checker 2.2.3 (Van Oosterhout et al., 2004) detected null alleles in markers showing departure from Hardy-Weinberg equilibrium in the deep-water hake (*MP9131*, *Mmerhk29*, *Mmer110-8*) and the shallow-water hake (*MP8494*, *Mmerhk29*, *Mmerhk34b*). However, this departure could be linked to genetic subdivision in the samples rather than to the presence of null alleles (e.g. Hoareau *et al.*, 2009).

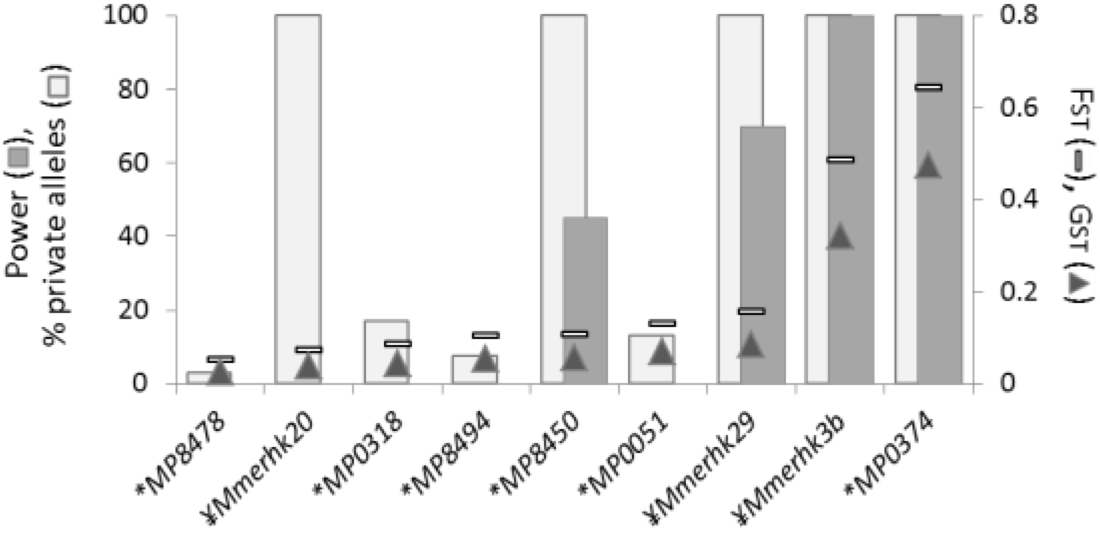

Figure 1. Power of each locus to distinguish between *Merluccius paradoxus* (N = 32) and *M. capensis* (N = 32), their associated F_{ST} and G_{ST} values and cumulated frequency of private alleles observed between the two species. The markers, ranked according to their F_{ST} values, are those giving consistent amplification in both species. The power refers to the percentage of cluster analysis runs implemented in Structure v2.3.1 for which the locus can discriminate the species. ¥ denotes previously published markers and * *de novo* markers.

The resolution power of each locus in distinguishing the two species was evaluated using *F*-statistics and a clustering method. F_{ST} (in Genetix) and G_{ST} (in GenAlEx 6.5; Peakall & Smouse, 2012) were estimated for each locus giving consistent amplification in both species (Fig. 1). The clustering method implemented in Structure 2.3.1 (Pritchard *et al.*, 2000) was applied per locus (no admixture, correlated allele frequency) using 10^6 MCMC replicates following a burn-in period of 10^5. The most likely number of clusters (K) was determined following Evanno *et al.* (2005) over 20 runs for each K = 1–4. The markers *MP0374* and *Mmerhk3b* showed high genetic differentiation ($F_{ST} \geq 0.25$; $G_{ST} \geq 0.15$) with 100% private alleles and could individually assign each genotype to the correct species with high accuracy (Fig. 1; Fig. 2). All the other markers cannot individually discriminate the species even though some show 100% private alleles.

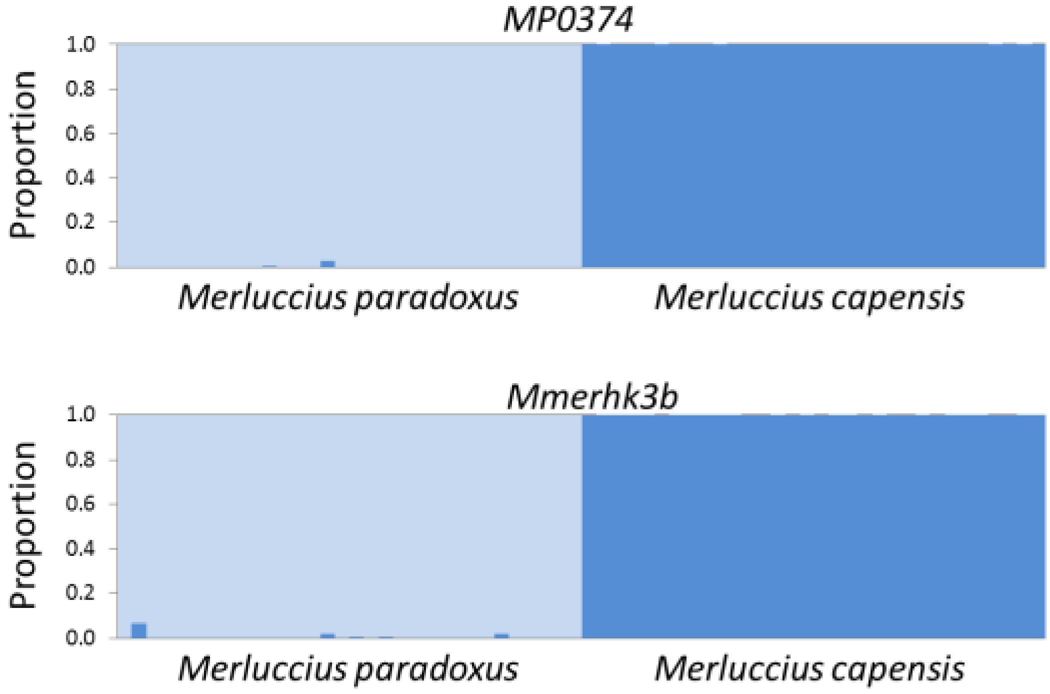

Figure 2. Bayesian cluster analyses illustrating the distinction between samples of *Merluccius paradoxus* (n=32) and *M. capensis* (n=32) at the markers *MP0374* and *Mmerhk3b*. These two markers have the highest F_{ST} values and can identify the species.

The effect of directional/balancing selection on the discriminating power of the microsatellites was investigated using LOSITAN (Antao *et al.*, 2008). The program compares observed F_{ST} values to 10^6 coalescent simulations obtained under mutation–drift equilibrium and a stepwise mutation model. Variation at several loci deviated significantly from neutral expectations but only F_{ST} values of *MP0374* and *Mmerhk3b* were found above the 99% confidence interval (Fig. 3), suggesting directional selection. Considering the long divergence of the species (Grant & Leslie, 2001), the patterns of selection are likely ancient as already observed in other taxa (Narum *et al.*, 2008). Therefore, should divergent populations be included, the two markers will still accurately distinguish the Cape hake species.

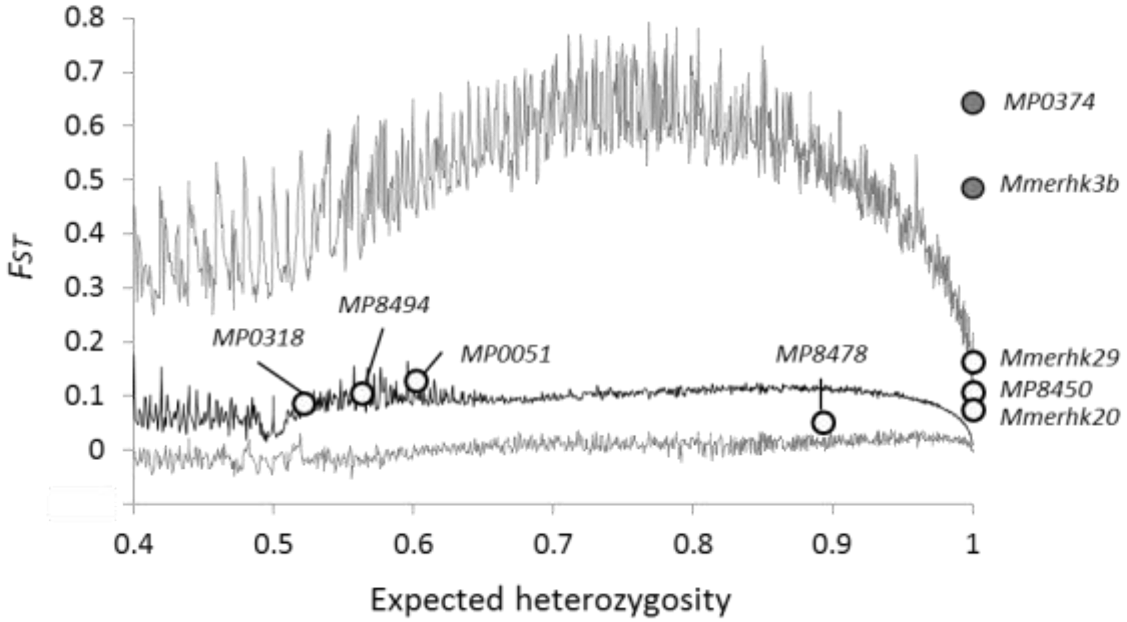

Figure 3. Results of LOSITAN analysis of the two Cape hakes *M. paradoxus* and *M. capensis* for the detection of microsatellites deviating from a model of neutral evolution. The average and 99% confidence limits (three trendlines) of F_{ST} are obtained from simulations under a stepwise mutation model using the weighted mean F_{ST} assumed to be neutral. The circles represent the F_{ST} calculated for each microsatellite marker; the dark grey circles illustrate the markers under directional selection.

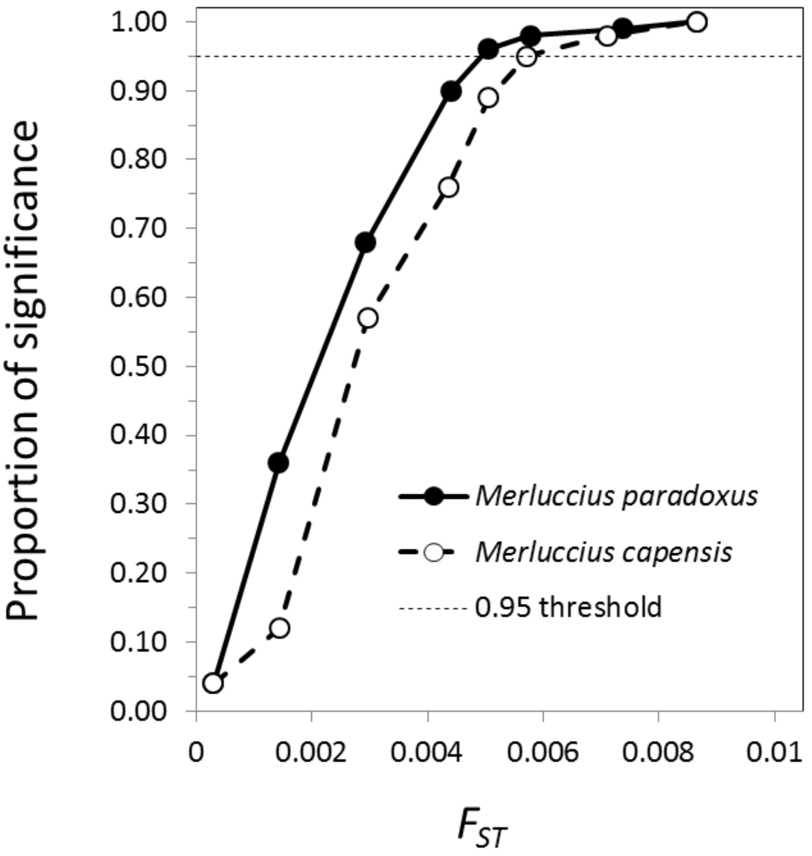

Figure 4. Power analysis of the microsatellite dataset for *Merluccius paradoxus* (14 markers) and *M. capensis* (10 markers) for different F_{ST} values and sampling 50 individuals from each population. The minimum level of genetic differentiation that can be detected with 95% statistical power (resolution of the markers) is $F_{ST} = 0.0049$ for *M. paradoxus*, $F_{ST} = 0.0057$ for *M. capensis*.

To evaluate whether the microsatellites are suitable to delimit genetic stocks, the resolution power of each set of markers was assessed using POWSIM 4 (Ryman & Palm, 2006). The test relies on the detection of significant values of F_{ST} estimated at different time points since population divergence under the Wright–Fisher model. For each run, the program randomly assigns alleles among the simulated divergent populations and randomly samples genotypes to estimate F_{ST} values and their significance (Chi-square tests). The parameter sets were $5x10^5$

individuals and 50 samples for each population and 10^3 simulations. The results show that the microsatellite sets are able to detect low genetic differentiation ($F_{ST} < 0.006$) in both deep-water and shallow-water hake (Fig. 4). These F_{ST} values fall within the threshold over which clustering methods can distinguish populations ($F_{ST} = 0.005–0.007$; Waples & Gaggiotti, 2006), and should therefore detect subtle genetic differentiation and demographic independence.

The present study describes molecular tools and results that are relevant for the management of the Cape hakes. First, the two markers with high discriminating power will be important for species identification in several fields including management aspects (distribution area, landings, unintentional harvest, *etc.*), legal actions (*e.g.* poaching, traceability) or ecological surveys (especially ichthyoplankton). Secondly, the sets of microsatellite markers will be used to further investigate the currently recognized management units of Cape hakes. Finally, considering the high level of transferability of microsatellites within (Reid *et al.*, 2012) or across fish families (Carreras-Carbonell *et al.*, 2008), the new markers provide a potential resource for genetic studies of other species within the Gadiformes.

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References

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A. & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on a *F(st)*-outlier method. *BMC Bioinformatics* **9**.

Butterworth, D. S. & Rademeyer, R. A. (2005). Sustainable management initiatives for the Southern African hake fisheries over recent years. *Bulletin of Marine Science* **76**, 287-319.

Carreras-Carbonell, J., Macpherson, E. & Pascual, M. (2008). Utility of pairwise mtDNA genetic distances for predicting cross-species microsatellite amplification and polymorphism success in fishes. *Conservation Genetics* **9**, 181-190.

D'Amato, M. E., Lunt, D. H. & Carvalho, G. R. (1999). Microsatellite markers for the hake *Macruronus magellanicus* amplify other gadoid fish. *Molecular Ecology* **8**, 1086-1088.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.

Faircloth, B. C. (2008). MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* **8**, 92-94.

Garcia-Vazquez, E., Machado-Schiaffino, G., Campo, D. & Juanes, F. (2012). Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks. *Fisheries Research* **114**, 52-55.

Grant, W. S. & Leslie, R. W. (2001). Inter-ocean dispersal is an important mechanism in the zoogeography of hakes (Pisces : *Merluccius spp.*). *Journal of Biogeography* **28**, 699-721.

Grant, W. S., Leslie, R. W. & Becker, I. I. (1987). Genetic stock structure of the Southern African hakes *Merluccius capensis* and *Merluccius paradoxus*. *Marine Ecology Progress Series* **41**, 9-20.

Moran, P., Lundy, C., Rico, C. & Hewitt, G. M. (1999). Isolation and characterization of microsatellite loci in European hake, *Merlucius merlucius* (Merlucidae, Teleostei). *Molecular Ecology* **8**, 1357-1358.

Narum, S. R., Banks, M., Beacham, T. D., Bellinger, M. R., Campbell, M. R., Dekoning, J., Elz, A., Guthrie, C. M., Kozfkay, C., Miller, K. M., Moran, P., Phillips, R., Seeb, L. W., Smith, C. T., Warheit, K., Young, S. F. & Garza, J. C. (2008). Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. *Molecular Ecology* **17**, 3464-3477.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-590.

Payne, A. I. L. & Punt, A. E. (1995). Biology and Fisheries of South African Cape hakes (*M. capensis* and *M. paradoxus*). In Hake: Biology, Fisheries and Markets (Alheit, J. & Pitcher, T. J., eds.), pp. 15-47. London: Chapman & Hall.

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**, 2537-2539.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

Reid, K., Hoareau, T. B. & Bloomer, P. (2012). High-throughput microsatellite marker development in two sparid species and verification of their transferability in the family Sparidae. *Molecular Ecology Resources* **12**, 740-752.

Rico, C., Ibrahim, K. M., Rico, I. & Hewitt, G. M. (1997). Stock composition in North Atlantic populations of whiting using microsatellite markers. *Journal of Fish Biology* **51**, 462-475.

Ryman, N. & Palm, S. (2006). POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* **6**, 600-602.

Seibert, J. & Ruzzante, D. E. (2006). Isolation and characterization of eight microsatellite loci for white hake (*Urophycis tenuis*). *Molecular Ecology Notes* **6**, 924-926.

Selkoe, K. A. & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* **9**, 615-629.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

von der Heyden, S., Lipinski, M. R. & Matthee, C. A. (2007a). Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* **42**, 517-527.

von der Heyden, S., Lipinski, M. R. & Matthee, C. A. (2007b). Species-specific genetic markers for identification of early life-history stages of Cape hakes, *Merluccius capensis* and *Merluccius paradoxus* in the southern Benguela Current. *Journal of Fish Biology* **70**, 262-268.

Waples, R. S. & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15**, 1419-1439.

Zane, L., Bargelloni, L. & Patarnello, T. (2002). Strategies for microsatellite isolation: a review. *Molecular Ecology* **11**, 1-16.

Electronic References

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. (2004). GENETIX, logiciel sous WindowsTM pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université Montpellier II, Montpellier (France). http://www.univ-montp2.fr/~genetix/genetix.htm.

Supplementary material

Table S1. Details of *Merluccius paradoxus* sequences containing at least four repeats and with sufficient flanking region for primer design generated from the microsatellite library; TM refers to the annealing temperature of the primers.

CLONE	REPEAT MOTIF	PRIMER_LEFT	TM	GC CONTENT	PRIMER_RIGHT	TM	GC CONTENT	AMPLICON (BP)	START	ENDS
M7958	(AGAT)12	CACCCAAATGGAGGTGCTG	59	58	AAGGTGTCAGCAAGGGAAG	58	53	249	155	203
M8418	(ACAG)5…(AGGC)4	CAGACATGCAGACAGACGG	59	58	ACCGGACCATCTCAACAGC	60	58	185	114	134
M8424	(CTGT)4	AATAACCACCTCGCTCACG	58	53	GGTCTCACCTCGGTAGTGC	60	63	500	670	686
M8426	(CTTT)13	GGATGGGGAACTGACCACC	60	63	CATGTTTGCACACCGCCTC	61	58	204	162	214
M8428	(AG)21…(ACAG)20…(ACAG)4…(ACAG)10	AGAGAGAGAGAGAGAGAGAGAGAG	60	50	CTGTCGGCCCGTCCATC	61	71	250	2	44
M8429	(AC)7	CCCGTTTACCCGGAGGTC	60	67	GTCCATCGGCCTGTCTCTC	60	63	231	42	56
M8432	(AGGC)4	ACAATGATAACCTCAAACCTGC	58	41	AGAGCTGCTTGCCCTCTTG	61	58	237	70	86
M8438	(GT)13…(GT)17	TGTGTGTGTGTGTGTGTGTG	60	50	AGCCTGGGACTGGGAGG	61	71	136	109	135
M8439	(ACAG)4	AAGGCAGACAGACAGGTGG	60	58	CTAGTCTCGGATCGCTGGG	60	63	177	121	137
M8441	(ACAG)4	AAGGCAGACAGACAGGTGG	60	58	CTAGTCTCGGATCGCTGGG	60	63	177	122	138
M8446	(ACAG)5	AAACCTTGCATCGACACCC	59	53	GGCCTAGCATGCCAAGAAG	59	58	163	131	151
M8449	(AGAT)5…(AGAT)6…(AGAT)4	TGCACCTTTCACCTGGGAG	60	58	TTGGACCCTGAATGTCCCC	60	58	200	224	244
M8450	(AGAT)16	GTTAGCGCACAGTCCACAG	60	58	CTTCCTGTCCTGTCTACCCG	60	60	236	258	322
M8453	(ACAG)4…(ACAG)8	GATGTCAGTCATGTGACCCC	59	55	TCTGTCTGTCTGTCTGTCTGTC	60	50	219	102	118
M8454	(CT)6…(AGG)4	GGATTTCAGCCGACTGTGC	60	58	ATGCTGAAGCTCCCTCCTC	60	58	187	256	268
M8457	(GT)8…(GT)6	AGGATACGACGGTTAGGCG	60	58	TGCACACACACACACACAC	60	53	246	488	504
M8458	(AGGC)4…(ACAG)4	TGTGTCTTGGGCATTCGAG	59	53	TCTGTCTGTCTGTCTGTCTGC	60	52	152	151	167
M8460	(CT)10…(CTGT)11	TGTCGCTCTCTGTCTGTCTG	60	55	CCTGCCAGAGTGAAAATCT	60	48	129	90	110

					AAGC					
M8461	(AG)9	GACTCCGGGTACAGTCAGC	60	63	ACACACGGCAACACTGATAAC	60	48	211	537	555
M8466	(ACAG)4…(ACAG)4	GACAGACCAATAGGACGACAATC	60	48	CGCATCTGCATCCATCTTCC	60	55	249	56	72
M8469	(AC)8…(AC)6…(CT)21	CTCTCTCGCTCTCGCTCTC	60	63	GCCATTTAGCTTGCGGGTC	60	58	164	164	180
M8470	(CT)20…(CT)6	GCGCCAATGTACAGCCAC	60	61	AGAGAGAGAGAGAGAGAGAGAGAG	60	50	154	163	203
M8472	(ACAG)5…(ACAG)5	ACAGACAGACAGCCAGACAG	60	55	ACTGTTAGCACCATTAGCACG	59	48	150	32	52
M8474	(ACAG)4…(ACAG)5…(AGAT)6…(ACAG)5…(AGAT)4…(ACAG)4	GAGACAGCGAGAGCGAGAG	60	63	TCTGTCTACCTACCTACCTATCTG	58	46	266	147	163
M8477	(ACAG)8…(CT)6	CTTGGAGGAGTGTGGGGAG	60	63	GGGTTGCTGGATGTTGGAC	59	58	201	75	107
M8478	(GT)6…(GT)6…(GT)9…(ACAG)8	GGGTAGAACAATGCAGCGG	60	58	CGAGCCACAGTCCACATTC	59	58	191	328	340
M8479	(ATT)4	ATGAGGTCACGCCCACTTC	60	58	TTACCTGCCTGCCTGTCTG	60	58	227	156	168
M8480	(GT)7…(AAAG)10…(ACAG)22	AGTGTGTGTTTGTGTAAGGGG	59	48	TCTGTCTGTCTGTCTGTCTGTC	60	50	153	273	287
M8481	(AG)6…(ACAG)15	AGGGAGAGAGACAGAGAGAGAG	60	55	GGAAAGTGGCGTGAAGACG	60	58	211	9	21
M8482	(AG)6	AGGTCTGAGAGCCAGGAAG	59	58	TGACCCTCAGGAAAGGTCAAC	60	52	165	63	75
M8483	(CT)8…(GT)7	TATTGCAGACATCCACGGC	59	53	GCAACAGGAAGACAGCGAG	60	58	224	262	278
M8484	(ACAG)5…(AGAT)17…(ACAG)8	GATAGATAGAGACAGACAGACAGAC	58	44	ACGCACATGCACACATACAC	60	50	241	100	120
M8487	(AG)11…(AG)6…(ACAG)18	GAGAGAGAGAGAGAGAGAGAGAG	58	52	CTTCCCACAAAGCCCAACG	60	58	179	3	25
M8489	(CTTT)4…(CTGT)16…(CTTT)10	TGTCTGTCTGTCTGTCTGTCTG	60	50	AGGAAAGGAAAGCGTGAGAG	58	50	175	276	292
M8494	(ACAG)5	ACACAGACAGACAGGCAGG	60	58	GCGAGGGTAAATTCCGAGG	59	58	243	211	231
M8495	(ACAG)5…(ACAG)5	ACAGGCAGACAGATAAAACGG	59	48	GGCGGCACTCAAGTTCAAG	60	58	220	54	74
M8497	(CTTT)13…(CTTT)13…(CTTT)10	TATCCGAGCCCAAACTGGC	61	58	ACCAAAGGGAATAAGAGGGG	57	50	243	239	291
M8502	(ACAG)5…(GT)8	GGGTGTGTGTGTGTGTGTG	60	58	CGACACCTGCGTTAAGTGC	60	58	185	83	103
M8504	(CTTT)5…(AAT)4	TCTCTTTCTTTCATTCTTCCC	57	38	GTGCACACACAAAGTGGTC	58	53	179	72	92

		TTC								
M8505	(CT)14…(CT)7	CACATTCCCGTACTGGCTC	59	58	AACGCACCAGCCAATGAAC	60	53	375	392	420
M8508	(AAG)4	GCACGGTTCACTTGGGTTC	60	58	TTGCCTCATCTGGGCCTTG	61	58	152	223	235
M8511	(AGAT)6…(ACAG)13…(AGAT)16	GTGGACGTAACTGAAAGCCTC	60	52	ATCTGTCTGTCTGTCTGTCTGTC	60	48	150	65	89
M8727	(AGCC)6…(CAGT)4	CAGTCAGTCAGCCACCCAG	61	63	CCTGAGGCGAAGGGTTAGG	60	63	246	334	358
M9100	(CT)15…(CTGT)4	TGTCTGTCTGTCTGTCTGTCTC	60	50	CCACTCCCGTGTCACATTAC	59	55	166	137	167
M9101	(CT)6	AGATTTACTCATCCGCTCCCC	60	52	ACAGACAGACAGCCAGACAG	60	55	193	50	62
M9102	(ACAG)16	TGATAAAGGAGCATTCCACGG	59	48	CAGCATAGCAAGAGATACTGC	57	48	238	241	305
M9117	(AGAT)11	AGGTGGGAAAGGCTGCAC	61	61	ATCAGCGGAAAGGCTGAGG	61	58	195	75	119
M9119	(AGCC)6	TCCTGTGGTACGAGCAAGC	60	58	TCTGGCTGTATGTCGGTCTG	60	55	244	168	192
M9121	(AGAT)10…(ACAG)8…(AGAT)4	AGACAGACAGACAGACAGACAG	60	50	TCGATTTGTGTGGGATGGAC	59	50	188	135	175
M9122	(CTGT)4…(AGCC)4…(AGCC)8	CTCAGAAAACCAGGCGTCC	59	58	AGACATAGAGCTGGCTGGC	60	58	224	495	511
M9129	(ACAG)9…(AGAT)21…(AGAT)5	AGACAGACAGACAGACAGACAG	60	50	GCCCAGGACATACGAATGATG	60	52	250	37	73
M9130	(ACAG)15…(AGAT)23…(AG)9…(AG)9…(ACAG)4…(ACAG)7	AGACAGACAGACAGACAGACAG	60	50	TGGGAACAGGTGGGAGTTG	60	58	385	202	262
M9131	(AGAT)5	TGGACAGATCAGCCTACCG	59	58	GAAGGAAAGATCAGCCCGC	59	58	233	126	146
M9133	(CT)10…(CT)6…(CT)11…(CT)6	CTGTCTCTGTCTCTCTCTCTCTC	59	52	ATCAGCGGGTTCAGTGCTC	61	58	156	104	124
M9347	(CT)18…(CTGT)5	TCCGAAGGTCGATTTCTCCC	60	55	TGTATGTGTGTGTGCGTGC	60	53	208	161	197
M9349	(CTTT)23	GCGGTGTTTACCCACAAGG	60	58	GCAGCCATGAGGAAGGAAG	59	58	203	259	351
M9350	(AGAT)4…(ACAG)11…(AGAT)8…(AGAT)14…(ACAG)13…(AGAT)4	AGACAGACAGACAGACAGACAG	60	50	AAGCGTCCACCAAATGACC	59	53	277	36	52
M9351	(ACAG)9…(ACAG)5…(ACAG)4…(ACAG)4	AGCGACACCCTATACGCTAC	60	55	TCCTTCCTTCCTGTCTGTCTG	60	52	247	72	108
M9352	(CT)15…(CT)10…(CT)7…(GT)14	TCAAGAAGCGGCCTGGAAG	61	58	AGAGAGTCGTCGCTCCAAC	60	58	250	307	337

M9354	(AGAT)13…(AGAT)4…(CT)8…(AGAT)6	TGGACGGATGGACAGATGG	60	58	TGTGAAACGTGTCCTGTGG	59	53	242	149	201
M9355	(GT)8	AGCAGTTGGGAGACAGGTG	60	58	CAGATGGCTGAATGACGGC	60	58	241	192	208
M9356	(CT)8…(CT)6…(CT)6…(CT)6…(GT)10	GACGCCGGTAAAACCAGAC	60	58	GACAGGGAGACAGAGAGAGAC	59	57	250	474	490
M9358	(CT)7…(CT)6…(CT)9…(CT)8…(CT)6…(CT)9…(CT)6…(CT)6	TGTCTCTCTCTCTCCCTCTCTC	60	55	ATTGTGTACGCCAACCTGC	59	53	232	238	252
M9359	(CTTT)5…(CTTT)13…(CTTT)7…(CTTT)9…(CTTT)5	TCTTTCCTTCTTTCCCTCTTTCC	59	43	CGATCGTTCGTTATGGTAGTCG	60	50	100	247	267
M9363	(ACAG)4…(AGGC)6…(ACAG)4…(AGGC)4…(ACAG)5	CAGACGGACGGATGGACG	61	67	TCTGTCTGCCTGCCTGTC	60	61	284	88	104
M9364	(GT)25…(GT)8	CTCAGAGTCAGGAGAGAGCC	59	60	CACACACACACACACACGG	60	58	226	299	349
M9366	(ACAG)5…(ACAG)22…(ACAG)5…(ACAG)8	ACAGACAGACAGACAGGCAG	60	55	TGAAACACGCATTCAGCCG	60	53	243	43	63
M9367	(ACAG)5…(ACAG)22…(ACAG)5…(ACAG)8	TTGATCGTCTCTCCATCCTG	57	50	TCCTGTCTGTCCGTCTGTC	59	58	243	43	63
M9370	(CTTT)4	ACAACACTTTAGCAGCACG	57	47	GAATGAACGAAAGAAAGAACGAAAG	58	36	248	67	83
M9373	(GT)7…(GT)8…(GT)11…(GT)6…(GT)6…(GT)7	TGTGTGTGTGTGTGTGCG	60	56	CACAAGTGCCATCCAGCTC	60	58	192	204	218
M9375	(AC)9…(ACAG)10	GGGGTGACCACATCCATCC	61	63	TCCACATTCGTCCATCTCTCC	60	52	160	298	316
M9378	(GT)26	AAGCACGTTCACCGTACCC	61	58	CACCCTCTCCCACACACAC	61	63	220	172	224
M9379	(ACAG)6…(ACAG)5…(AGAT)16…(ACAG)4	ACAGACAGGCAGACAGACAG	60	55	CGAGCGAGTGACGCAATAC	60	58	249	60	84
M9380	(GT)10…(GT)8	AGACCTCTTAACGACCGGC	60	58	ACCCACTCTCAGCTTGACC	60	58	230	86	106
M9381	(ACAG)8…(GT)9…(ACAG)20…(CTT)9	ATGCAAACATCAGCCCTCC	59	53	ACGCACACACATACTTGGC	59	53	242	117	149
M9383	(ACAG)6	GGATAAGCCAGCAGTTTTAGTTTC	59	42	TTACCTGTCTGCCTGCCTG	60	58	206	120	144
M9386	(ACAG)22	CATTTACATGCTTCCTGACTGC	59	45	CTGATTGGTGCAGATAATGGC	58	48	168	49	137
M9388	(ACAG)5	CAGACAGACAGACATCAGCAC	59	52	CACCGGACAGCACAAAGTC	60	58	245	240	260
M9389	(AC)21…(CT)28	CCGCTGAGTGTCGAGAGAG	60	63	ACGCAAGTACGTTTTGGGTC	60	50	242	66	108
M9391	(GCCT)4…(GT)8	AGCTTGGGGAAAGAATCCG	58	53	AAAACGGCCCAATGTGGAG	59	53	168	139	155

M9392	(GT)15	TACACCCCGACCTACCGTC	61	63	AATAAGACCCGCGCTCCTC	60	58	160	59	89
MP005	(CT)6…(CT)6…(CT)6	ATGATTGGTCGGTCTCTCTC	57	50	GACAGAAAGAGAGCTTAGAGGC	59	50	178	134	146
MP021	(ACAG)18…(AGAT)13…(AGAT)4…(AGAT)6	AGACAGACAGACAGACAGACAG	60	50	ACATTCACCCCAGGCACTC	60	58	221	33	105
MP024	(AC)12	GAGTAGCTCTGCCTTCCCC	60	63	GTGTTTGTGAGTGTTTTGTCTG	57	41	167	224	248
MP031	(GCT)4	GTAGGTCTAGCACGGCTCC	60	63	GCCAAAGCCGAGTGGAAAG	60	58	184	778	790
MP032	(AGAT)16	AGATAGATAGACAGACAGACCGAC	59	46	GCATTTGTCAGTAGAACGTAAAAC	58	38	215	102	166
MP038	(GCCT)4…(CAGT)6	AGCCAGTCAGTCAGTCAGC	60	58	TTCCATCGCTGCCAAAAGC	60	53	150	118	134
MP039	(AGAT)10	CCATTTTCCAGTCAAAGTCTGC	59	45	GTACCAACATTGCCCACGG	60	58	246	155	195
MP042	(AGAT)8…(AGAT)15…(ACAG)8…(ACAG)10…(ACAG)6…(ACAG)5	AGACAGACAGACAGACAGACAG	60	50	CCAACACAGGACCCCTCC	60	67	292	193	225
MP044	(AGC)4	TAGACCAGCAGACCAGCAG	59	58	TAACCGGAGCTGATGACGG	60	58	200	296	308
MP045	(AGCC)5	TCCTGTGGTACGAGCAAGC	60	58	GACGCCAGAGACCAAAACG	60	58	224	171	191
MP046	(CT)11	TGCTGCACTACCCTGCTAC	60	58	CGGTGCAAATACTGGGCTG	60	58	171	223	245
MP051	(AGCC)8	GCCTATACGATCCCCACGG	60	63	CCTCCAACCTCTCTGCCTG	60	63	178	163	195
MP052	(CT)14	GGAGCTGGCTGGGATGG	60	71	TCCCATCATTAGAAACATAGAGAGG	58	40	120	1243	1271
MP053	(CAGT)4…(CAGT)4…(CAGT)4…(CAGT)4…(CAGT)4…(CAGT)4	TGGGTGAATGAAGCAACAGC	60	50	TGACTGACTGGATGTATAACTGAC	59	42	489	544	560
MP055	(ACAG)4	GAACCCAGAACCTTTCGGC	59	58	GGACGCCTAGGACTGTCTG	60	63	207	494	510
MP059	(AGCC)5…(AGCC)6…(ACAG)4	CAGCCAGCCAGTGAGTGAG	61	63	TGCCTGTCCGTCTGTCTTC	60	58	233	135	155
MP060	(AGAT)27	TGCAGACATATGGGGAGGG	59	58	CCTCTATGACCAGCTCGGG	60	63	238	213	321
MP062	(GT)8…(GT)10	AACCTGTGGAGTGGAGCTG	60	58	TCCCACATTTCCCAGGAGC	60	58	248	460	476
MP065	(ACAG)4…(ACAG)5…(AGAT)7…(ACAG)4	ACAGACAGACAGGCAGACAG	60	55	AGGCTAGACACACATGAGGC	60	55	284	171	187
MP066	(AGCC)6…(ACAG)5	ACTCTCTGTGGTTGCCGTG	61	58	TCTCAGATCTTCGCGAGCC	60	58	200	144	168
MP067	(AGCC)5	TGGAGGGGTCTCCAGTCTC	60	63	GTCTGTTGGGCTTGCTGAC	60	58	156	409	429

MP068	(ATCC)4…(AC)6	GACCAACGTAGCTGGAAAGG	59	55	ATGATGCGACTCCTCCACC	60	58	197	167	183
MP071	(AGCC)8…(AGCC)5…(CAGT)5	AAAGGCACAGGACCCAGAG	60	58	TCCGAGTGAGTCAACAGCG	60	58	231	127	159
MP073	(GCT)4	CTAGGTCTAGCACGGCTCC	60	63	GCCAAAGCCGAGTCGAAAG	60	58	186	172	184
MP074	(AGAT)5…(AGAT)17	ACGCTAGAGAGAGGAGAGGG	60	60	CCAGTTGGCATTCACACCG	60	58	245	166	186
MP075	(GCT)4	GTAGGTCTAGCACGGCTCC	60	63	GCCAAAGCCGAGTCGAAAG	60	58	186	377	389
MP076	(AGAT)12…(ACAG)12…(AGAT)6…(ACAG)5…(ACAG)4…(AGAT)5…(ACAG)5…(AGAT)4	GGCGCCACTAGATTAGGAAG	59	55	CTGCCTGTCTACCTTTCTGC	59	55	390	118	166
MP078	(AC)6	AGTCTCAGTGGACAGCACC	60	58	AACTAGCCAGCCACTAGCC	60	58	212	257	269
MP079	(ACAG)9…(ACAG)4…(ACAG)5…(AGAT)6…(AGAT)12…(ACAG)15	AGATGGATAGACAGATTGATGGATAG	58	38	GACTTCCTTGGACCCCTGC	61	63	335	131	167
MP080	(GT)48	ACCATCATCGGTATATATGGGC	58	45	ATGGCTTTTAGTAGCCGAATG	57	43	250	433	529
MP081	(ACAG)4	CGGAGACGGAAGGGGAAAC	61	63	CATTGTCTGTCTGGCTCGC	60	58	235	286	302
MP082	(ATCC)7	GGTATGCTTTCCAACCCCTG	59	55	AGCGGTCCACTCACTTCAC	60	58	250	148	176
MP084	(AG)7	CGTATGTTTCAGCGGCACC	60	58	CTCTGCCTAAAACGCGCAC	61	58	207	245	259
MP085	(AG)23…(AG)9…(AG)9	GAGAGAGAGAGAGAGCGAGAC	59	57	GCAGATGATTGGGTAATAACAAAAGG	60	38	358	305	351
MP088	(CCT)4	ATGCATTGGCAAGAGCAGG	60	53	TTTGCCCATTTCAGCCACG	60	53	212	243	255
MP091	(CT)11…(CT)11…(AGCC)6	GCCTCCTCCAGCCTCTAAC	60	63	TTCAGTGCGCCGACAAAAG	60	53	177	222	244
MP092	(GGT)4	AGATAGCAGTAGGTGGTGCG	60	55	ACCAGTCACACAGTCAGCC	60	58	201	273	285
MP097	(ACAG)5…(ACAG)8…(ACAG)4	AGGGACAGACAGACAGACAG	59	55	GGCGACTGAATTTCCCCTC	59	58	177	25	45
MP100	(CT)13…(CTGT)4	TGGGAAGCATGATCACAAATC	57	43	TGCAGAGAGAGAGAGAGAGAG	58	52	286	294	320
MP105	(CT)6…(CT)9…(CT)13	TCCAGGAGGCTGCATTGAC	60	58	GCCGTCTCTCTCTTTCTGTG	59	55	237	176	188
MP106	(GT)6…(CT)8	GCCCCTCGCTCTCTGTATG	60	63	TATGGAAAGCCAGTCGGGG	60	58	221	185	197
MP117	(CT)6…(CT)6…(CT)7	GCCCACTGTTGTCATAGCG	60	58	ACAGGGAGGGAGAGACAGG	60	63	240	581	593

MP119	(CT)10	TGTCTCTCTCTCTGTCTTATGTCTC	60	44	GTGGGTTTTATAGCCCTCACATC	60	48	193	350	370
MP127	(CT)14…(CT)6	TGTCTCTGTCTGCCTCTCAC	60	55	ATAGCCCTCACTCGACCCC	61	63	230	337	365
MP128	(ACAG)14…(AGAT)17	AGACAGACAGACAGACAGACAG	60	50	ACAATTTGCATTTGTCAGTAGAACG	60	36	190	43	99
MP129	(GT)12	CTGTGTGAAGGGTTGTGGC	60	58	CGAGCCTGCTTTTCGGATG	60	58	151	370	394
MP136	(CTTT)20…(CT)7…(CT)7…(CT)7	ACTTGGTACTCTCTCTCCCC	58	55	CGGCGTGTGTACTTTACTTTTC	59	45	247	78	158
MP137	(ACAG)6	CATTGATTGCTCTGCCGCC	61	58	TGTCTGTCTGGTGGTCTGTC	60	55	177	132	156
MP142	(ACAG)5…(ACAG)4	GGGTCACGTTGTGCATGTC	60	58	GCCTGTCTCTGCCTGTCTC	61	63	249	194	214
MP144	(GT)8…(GT)9	GCCCAATACAGCAACCGTC	60	58	TCATAGACACACACACACACAC	59	45	224	372	388
MP145	(AG)7	CGACTCGCTTTTCAGACAGAC	60	52	AGCTGGATTCTGCTCCGTG	61	58	185	200	214
MP151	(GT)8…(GT)9	GGCTTTCTTATCGGCTGCG	60	58	CCAACCCTCCCACAAACAC	59	58	234	347	363
MP168	(CT)9…(CT)6	CTCCCTCTCTCTGTGTGCC	60	63	CCGGATGTGAGTGGGTGAG	61	63	223	1003	1021
MP175	(ACAG)5	ACCTTCTCCGTTCCTGTGTC	60	55	CTCTCCGCTCTCTACCTCTC	59	60	229	224	244
MP179	(AGAT)4…(ACAG)4…(ACAG)6	CACATCCATCTCATCCTGTGC	60	52	TCTGTCTGTCTGTCTGTCTGTC	60	50	222	131	147
MP180	(AC)6…(GT)12…(GT)7…(GT)6	AGCATCGCTCCATTACCCC	60	58	ACACACACACACACACACAC	60	50	226	111	123
MP183	(ACAG)5…(ACAG)5	ACCTTCTCCGTTCCTGTGTC	60	55	CTCTCCGCTCTCTACCTCTC	59	60	228	219	239
MP184	(AG)7…(AG)6…(AG)6…(ACAG)5…(AG)9…(AG)6…(ACAG)5…(AG)6…(ACAG)4…(AG)6	GAGAGACAGAGAGAGAGAGACAG	59	52	CACCTCCAGCGGGTCAG	60	71	398	33	47
MP188	(AC)11…(GT)21…(GT)6	AAGCATCGCTCCATACCCC	60	58	ACACACACACACACACACAC	60	50	220	97	119
MP191	(AGGC)7	GTCGGAGCTTGAGGAGGAG	60	63	CTGGATGCAGGTGAGACCC	61	63	194	76	104