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Development of SNP for the deep-sea fish blue ling, *Molva dypterygia* (Pennant, 1784) from ddRAD sequencing data

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ABSTRACT

Blue ling is a deep-water species that has been severely fished upon in to coastal and offshore fisheries since the early seventies, thus causing the collapse of the populations in the last two decades. Genetic information is scarce in this species, and molecular markers are therefore needed to provide advice both for management and for rebuilding the stocks.

A suite of 103 SNP markers was identified from ddRADsequencing data. From those, 81 were organized in three multiplex reactions, and tested on 150 individuals from three different sampling locations. Good-quality amplification products were successfully obtained from 70 of the markers.

All SNP loci were biallelic, with averaged H_e per locus ranging between 0.101 and 0.500.

Keywords: blue ling, *Molva dipterygia*, SNP, ddRAD, management advice, stock rebuilding, fisheries depletion

Blue ling (*Molva dipterygia*) are distributed across the Northeast Atlantic (Large et al. 2010); inhabiting depths between 200 and 1500 m (Gordon & Hunter 1994). This species spawns in very dense aggregations throughout its entire geographic range (Magnússon et al. 1997; Large et al. 2010). Blue ling stocks in the NE Atlantic were moderately exploited until the 1960s and 1970s. Subsequently, more intensive fishing across its range, mainly in the spawning grounds led to the collapse of the populations during the late 1990ies (Large et al. 2010). Different regulations were enacted to rebuild the stocks, with varying success (ICES 2018).

The genetic population structure of this species has barely been investigated (Reiss et al. 2009), and, hence, genetic information is needed to develop appropriate management strategies to rebuild the fishing stocks.

Genomic DNA was extracted from sixteen blue ling ethanol-preserved fin samples (eight from Faroe Islands and eight from Norwegian offshore - Eggakanten) using the Qiagen DNeasy Blood & Tissue Kit. A ddRAD library for SNP discovery was constructed following Manousaki et al. (2016) and employed a *Sbf* I - *Sph* I restriction enzyme combination. The library was sequenced as part of an Illumina MiSeq run (v2 chemistry, 160 base paired end reads). Stacks software v1.27 (Catchen et al. 2013) was used to demultiplex samples and identify / call genotypes (de novo assembly; key STACKS parameters m=6, M=2, n=1). A total of 956 polymorphic RAD loci containing one or two SNPs and scored for at least 12 of the 16 samples. Initially a subset of 103 polymorphic RADtags was identified for Sequenom MassARRAY individual SNP assay (i.e., minor allele frequency > 0.1; > 35 bases available flanking DNA either side of SNP, absence of repetitive DNA close to the SNP). Three multiplex assays were successfully designed for 81 of these loci; which were then test screened on 150 individuals collected in three different sampling sites (i.e. Eggakanten, Yrkefjorden and Faroe Islands). After purging bad quality markers, a set of 70 SNPs loci were retained for further analyses.

Observed (H_o) and expected heterozygosity (H_e) as well as the inbreeding coefficient (F_{IS}) were computed with GenAlEx (Peakall & Smouse 2006). The genotype distribution of each locus was compared with the expected Hardy-Weinberg distribution (HWE) as was the departure from Linkage Disequilibrium (LD), using the program GENEPOP 7 (Rousset 2008). Analyses were conducted both at population level and pooling the 150 individuals.

In the pooled set of individuals, deviations from Hardy Weinberg expectations were detected at $P<0.05$ at two loci (Mdy_015 and Mdy_038) whereas loci Mdy_021, Mdy_027, Mdy_041 showed deviations at $P<0.001$ that remained significant after Bonferroni sequential correction. The individual analyses conducted on the population basis showed that 7.6% of the tests departed from HWE; percentage that dropped to half after correction for multiple tests. Loci Mdy_021 and Mdy_41 were out of HWE in the three sampling sites and continued to be so after Bonferroni correction. Departures from LD were detected in 5% of tests dealing with pooled data (0.1% after multiple test correction) and in 4.4% across the three populations (0.05% after correction). Observed heterozygosity per locus (H_o) ranged between 0.107 and 0.947 (**Table 1**), with averaged (±SE) over all loci in the pooled set of individuals of 0.362 ± 0.017; whereas H_e ranged from 0.101 to 0.500, with an average of 0.354 ± 0.014. The range of the inbreeding coefficient (F_{IS}) per locus was -0.896 to 0.152, with an average of -0.018 ± 0.018. Locus Mdy_41, showing H_o=0.947, was heterozygote CT for 94.7% of the individuals.

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CONFLICTS OF INTEREST

The authors declare that they do not have conflicts of interests.

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Table 1.- Summary table for the 70 SNP markers distributed in three multiplex reactions. Sequence and **[SNP]**, number de individuals tested (N), minor allele frequency (MAF); observed (H_o) and expected (H_e) heterozygosity; inbreeding coefficient (F_{IS}); and probability for Hardy-Weinberg equilibrium tests (ns = non-significant, *P<0.05, **P<0.01, ***P<0.001).

M	SNP_ID	Sequence	Alleles	N	MAF	Ho	He	F_{IS}	HWEP
M1	Mdy_001	CATGCAGTCAGGACACAGCAATGGAAAGATGCTCTTCTTAAAATCGGAATATTGCAAAGCTGTTCGACATGATTTATGTTGAGCTGTATTGACCATA**[C/T]**CATAACAGCATGTTAAATCATGAGGTGACCRTCCATT	C/T	150	0.403	0.540	0.481	-0.122	ns
M1	Mdy_009	CATGCTGAGCAGCGGGGAGAAGGTCAAGGTGGACCTGCACCCCCTCAGTGGCTGACCCGGGAAGAGAGCTCTGGTCTACATGGTCCCATGGCAGCGTCC**[C/T]**GGGGCACACGGCCATCAGCTCCACGAGAACCCCCC	C/T	150	0.460	0.453	0.497	0.087	ns
M1	Mdy_015	TGCAGGGGGTCGTTCTCCAGGTCAAAGGTCAGGAAGGTCAAGGTGAT**[A/G]**TCCATGCGGGGCGGGGCCATGATCATATAGGAGCACTCCAGATTGTGGGGGTACTTGTCCGGGAACCCCGGGGAATCGATCATCCCG	A/G	150	0.410	0.580	0.484	-0.199	*
M1	Mdy_020	CATGCACAGCACAACTGCGTGAGGTAAAACCATTGATGTAGAGAACACATTGAAATAGATG**[G/T]**AGACAGGAGGTTATGATAGTTTATTCCTAATACAAAGTGGCTGCACTAAAACCAACCTGTAACCACCTTCATT	G/T	150	0.487	0.480	0.500	0.039	ns
M1	Mdy_023	CATGCTGAAGTAGGATCGAGTCATCACGTTACATAAAGAAGGCTGTCATTAGTCTTAACATAGAC**[A/C]**ATATACCTGCGTTCCCTTATACATGGATTSGGGCTTTACCGAAGAACTCATATGTGAATCAACATATAT	A/C	150	0.253	0.400	0.378	-0.057	ns
M1	Mdy_025	TGCAGGAGAACTCCGAGGACGACAAGAAGCGAGGCCGGTCGACGGACAGCGAGGTCAGCCAGGTGAGGGAGAGCTGCAGAACCTCCTCCTG**[C/T]**GGAGGAGGAGGTCCATGTTCTCCTCTKCCTCCCCGTGAAGGAT	C/T	150	0.220	0.293	0.343	0.145	ns
M1	Mdy_027	TGCAGGTGGAGGGCTGAGGGGGGCTGCGTTCAACGGCACAC**[A/G]**GTGTTGTTGTAATGAGGGAGGGGGCGTGGCGACGCCAGGCACAGCCTCACTCTGCGTGAAATACCGCAGTACCGCCGGAACTAATGTGCTTTG	A/G	150	0.433	0.627	0.491	-0.276	***
M1	Mdy_029	TGCAGGTTCACTGCGGCCATTGGCTGTTCGAACCCAGATTCTTT**[C/G]**GGCTGGGGAGGCGAAACACCTCGAAACTAGACTATACTGACTGTTGCTGTGATCCAGGGTTTGAGTCCTCCATCTTACCATGACGTGGAA	C/G	150	0.383	0.487	0.473	-0.029	ns
M1	Mdy_031	TGCAGGACGAGCAGCCGTACGGTTACATACCGCAGTTACCAGGTTACGCTTCATTTAGC**[C/G]**TTTACATGGTCACCAAGGTTACCAGGTCACTGTCAGTTAAGTGCTGTCTTCATTTACAACCTTACAATAGAACCA	C/G	150	0.177	0.287	0.291	0.015	ns
M1	Mdy_032	CATGCAGATTCTGGAGTCTTCAACACTAATTGGCAAACATTGACAGACAGCGCAGTGTTACTGTCAAATGCCG**[A/C]**CCAATTACCAGAGGTTGTTCCTTTTTGTAATCTTCCAATAGAATGCCTTAATTAGCATTTT	A/C	150	0.413	0.507	0.485	-0.045	ns
M1	Mdy_034	TGCAGGAGAGCTTTGTTTGCCTTCCATAGGCTTAGCTTGAGGGGTCGCCTGAGAAGCTGCACTCAACCGAGACATAAAGATC**[A/G]**TCGTTTTTGTTCTCGTGGTGTACACCTTTAAGTAGAGGTATGGGTGATACCA	A/G	150	0.283	0.407	0.406	-0.001	ns
M1	Mdy_035	CATGCATTTGTTTCGGTGGTGTTTACATATGAATGAAGTCAAACTGTCTGAATGTGTATATGTATGTGTTGCATGGCGGTGCA**[C/T]**GTAATTTAAAGCTAGAGTAGGCAATTTATTTTAGAAGAATTCTTTGTCATA	C/T	150	0.337	0.447	0.447	0.000	ns
M1	Mdy_045	CATGCAGCCATACATGCCACCTATTCTCCTTGAAAGGCGTCCGTCGCTTTTATTGTGATTGTCGAACTATTTTGTAAAAACAGTAAAGGTCTTA**[A/C]**TGGGGCGTTGATTGCCTTTCAATATCAGTCAGTGCATGAA	A/C	150	0.403	0.513	0.481	-0.067	ns
M1	Mdy_050	CATGCTAACACTGTTACAGCAGACAGAGGGAGAGAATGTGCTTGGTTTTCTAGTGATAATGTCAGTCTGATAAT**[A/G]**TTACATAGCGCATTCATTTTGCTGTTTGGTTTTCRTAGGCTTTTCTGTAAAGAAAAGTTA	A/G	150	0.247	0.360	0.372	0.031	ns
M1	Mdy_056	TGCAGGGGCTCAGGCCTCAGCACGATCCGCTCCCCGTAGGAGATGCGCTCAACAGTGGTGCCGGGTTCTGCGAATATAAA**[C/T]**GAATATACGCACGCAGATTGGGCAGGTTAGTGGGGGGTGTAGTTGCGTCAAGAT	C/T	150	0.300	0.400	0.420	0.048	ns
M1	Mdy_067	CATGCTGAACTTTAAAGGGGTAATTGCTCAAATTGCCATGAGATTTGCACTTC**[A/G]**CTTAGTGCTCTGTTACGTAAAAAAAAAAGAAGAAGAAAACGACTGATGGTCTATTCGGACCCAGAAAGCAAATTAGATTGA	A/G	150	0.097	0.180	0.175	-0.031	ns
M1	Mdy_068	TGCAGGTTGCCGCGGGAAACACACCCCAACAACCAATCACGGACAAGGGCCAGGGGCGGAGTAGCCCGTGAAATACAAGA**[C/T]**ATCCAGAGGGGCCGATCCCTGACAAGTTACACTGTAAGAAAGAGTGCCTATATG	C/T	150	0.127	0.213	0.221	0.036	ns
M1	Mdy_070	TGCAGGCCCCTCCCCCTCATCCACCTGCCTCCTCCCAGGAGCATCAGGCTCCGTGGCTGGCCAGTGTTTGATCTGTGCAGGTA**[C/T]**CTCTTCTCATAGCCTGTCCTGGGCTTACAAACGGAGGTTAGAGGGCTAGCT	C/T	150	0.177	0.247	0.291	0.152	ns
M1	Mdy_078	TGCAGGTGTCTTACACTGGGTAATATTTCCTAGCAGTAAACATATTAATGCTGGAGCCCCTGTCAGGCTGTCCCTGCTGCCTCAGTAAAACACTTCCAG**[C/T]**CATAATCAGAATGTYAAACCTGGCACAGAGCAGGG	C/T	150	0.077	0.140	0.142	0.011	ns
M1	Mdy_080	TGCAGGATATTTCTATGCTAATACAGTTCATCAGTATGGGCAATGAAGGACGGCTCCAGCATTTAGCCAGAAACTGAAC**[A/G]**TGCTGTTGGAATGTTGGACCATGGACGTTTTCTACTTGTACTTTAAAGGGCTAGT	A/G	150	0.133	0.213	0.231	0.077	ns
M1	Mdy_085	TGCAGGAAAGGGCAGCAGGTGGATCCGGCTCTGGGGTGGACCGGGAGTCTCAGCTGGTCGAGCGAGGAGCTCCCTATGCCCGGACAGTGACCC**[A/G]**TGTGTTGGAGCGGTAAGGCAGGAAGGCCTGGTAACATTCTC	A/G	150	0.187	0.307	0.304	-0.010	ns
M1	Mdy_086	CATGCACGATGTGACACTTCCACTGCAGGCTTCACTTGAGTTTGAAATTGGATTTTTCTCCACAGACTAGTTATGTTTTCAG**[A/C]**GAAAGAAATCATTGGGCTGATGGTTTTTACYCTTTAGACACTTGACCATCCC	A/C	149	0.117	0.221	0.207	-0.068	ns
M1	Mdy_088	CATGCACCTGAGGGCAGYCTATTATTGGTTATGCATTTGGATCAATGAACCGAACCACACCATGC**[G/T]**TAGTCAGCGTTTGACTTTAGTGCATAACAGACTTCCTTTATTTAGGCAAAACGTTATTAATCCAAAAAC	G/T	150	0.090	0.180	0.164	-0.099	ns
M1	Mdy_096	TGCAGGCAGACCGCGTACACTGGGCGTCGAAACCCAGTACCTTTGGCTGGGAGTCAAACACCCTATAGCCACTGCACTTAACCACT**[A/G]**CACTGCTTGGTTACCTTACTATATTCTTTAGAAAACAAAATGCATGTG	A/G	150	0.053	0.107	0.101	-0.056	ns
M1	Mdy_098	CATGCTCCTTTGCTTAAGAGAAGCCCCATTTCTTCCTCACTCGTTTGTAAGATACG**[C/T]**GTTTTGTCTGAAAGTGGTGCGAAAGCCCTTTTCAAAGGTACTTTGTTTTAGGCTGAGACWATAGTTGGGCCAACATAA	C/T	150	0.187	0.280	0.304	0.078	ns
M2	Mdy_002	TGCAGGTAGACGTCGGCCACGCTGGCCAGGTCCATGTCCAGCGCCCTGCAGCCCGCCACGGCCTCAA**[A/C]**CCACGTCAGGTTCCGCATCAACAGCTGCACCGTGTGATTGTTGAAGTGGAGGAGCTCCGTCGGAGCC	A/C	150	0.393	0.480	0.477	-0.006	ns
M2	Mdy_012	TGCAGGACCAGGTCAACTTTGAATTATACCTCTGCACTGAGCCGCCGTACTAGTAGGTACATCATTGGCTCT**[A/C]**TCCGGTGCTTGCATGTATTCTTCCGCTTCAGTGATTCTGAGCTTCRTCTGCCAAATTGTTTC	A/C	150	0.350	0.447	0.455	0.018	ns
M2	Mdy_019	CATGCAGCCCCAGCCACAGTCAGTGGTGAGACTGGATCCCTCCAGCTGGGGGAACTCTCTCCTGTAGGTCAGCCACAGCCTGGAGACGAACGCTCC**[A/G]**TGGAAACGCTCTACCTCGTCTGAAATAGAGAGAGAAAT	A/G	150	0.330	0.393	0.442	0.111	ns
M2	Mdy_026	TGCAGGATGGTTCGCGACAGCGCAAAGAGAGGAAGAAGACGGTCTCCTTCAGCAGCATGCCCACGGAGAAGAAGATCAGCAG**[C/T]**GCCAGCGACTGCATAAACGCCATGGTGGATGGCTCGGAGCTGAAGAAGGTGC	C/T	150	0.423	0.447	0.488	0.085	ns
M2	Mdy_028	TGCAGGCGGTCTCTGTTTTGAACAGGTCCCGGTTAGTTTGAAATAAACGCC**[A/T]**TTTACTGATGTTTTTGTGTACACATGACATTCTTGCTTTGGCACAAGGACAGCGGCCCTGAGTGCCCGAGTGAGCTGTGTGTC	A/T	149	0.460	0.477	0.497	0.041	ns
M2	Mdy_030	TGCAGGATGAGGAGATGCTGTGGATTCCAGTTGGACTGGCGGCCTTTACGCTTCTGTGCAGGGGATATGTCATCCATCTC**[A/C]**TCCTGCGTGGCACCCTCAATGTCCGGGCGTTCACACAGGCTGGTGGGTGTGGAG	A/C	150	0.467	0.453	0.498	0.089	ns
M2	Mdy_037	TGCAGGATGTTTGCTTCACATTTGTGAGGAATTAGTTTCATTTTCATAATTTTGCTGTAGTTCCTTGGGAAACAAATGAACTGAATTACACAAGAA**[C/T]**GAAATAATGACCAAATAATGATACAGTGGACTTTCTTT	C/T	150	0.297	0.367	0.417	0.121	ns
M2	Mdy_042	TGCAGGTTAAAAACATTGTGCTTAGCGCAAACATGACCATGGTGAATAATTGGTTCAAATGATCTGAATTGACTTGTCAT**[C/T]**AGGAATGCCTTGAGAGTGCAAAAGTTGCTAAACCAACACATGCTAGCGATGTAT	C/T	150	0.243	0.367	0.368	0.004	ns
M2	Mdy_046	CATGCTTCAGCAGGATGCTCATTTATCTGCATCTAAAAATATATATGTATTCAAATTCACGGGCAGTCGGATTATCTTTGGATAAAATAACCATCA**[G/T]**TGCTAAAAATGACAMGTGCCTACATGTCAAAATGGGAT	G/T	150	0.317	0.420	0.433	0.030	ns

M2	Mdy_048	TGCAGGGTTAAACCATCGTCACAGAAGTGTGATTATTCATTT**[C/T]**GCTTAATCCCACTATGAAGAATGGAGCCTGATTAAATGAACGTAAGGATATTTATTGATTCCTGTGTCCAATTGGAAAATCTATTACATTTA	C/T	150	0.447	0.467	0.494	0.056	ns
M2	Mdy_049	TGCAGGCGCTTGTGTCACCGCTAAACATTCAGAGGGGGCCAGCAGGAGCTGCTCCTCCTACCTCCTCGGCTGCCGCCTC**[C/T]**GACAGGAGCCCCTCCCTCTGAGCGCAGGTGACGTGGAAGTAGGAGCGGCACATGC	C/T	150	0.277	0.340	0.400	0.151	ns
M2	Mdy_051	TGCAGGGTAAGAAAATAATGACCTTCAGCCCAAAGCAGATTCCCCGGTTAGATTATCTACTCTGATATTT**[C/G]**TTTTATTTGTGAGTAAAGTTGACATGGGGTTTTTCTTCCGGGTGCAGCTGTCTGATGCAACACG	C/G	150	0.113	0.213	0.201	-0.061	ns
M2	Mdy_054	TGCAGGTATCGACCCTAATGTGCTCAGACTGGAGCTTGCAGATCTGTGGCACAATAAAGAGTCGACCGAGGTGAGTCAGAATGACGCAGA**[G/T]**GGTCAATCATGTCAAGATGTGTTTGATTCTCATCCTGCATCAAA	G/T	150	0.190	0.287	0.308	0.069	ns
M2	Mdy_059	TGCAGGACTCCTGCACCAAGCTGCAGACGCTCAACACCCAGCTACAGGTGAGCCCACGCGCTGTTACACACAAAGCTGCGGCT**[A/T]**ACTGAAGAGTGTATGCGTGGCTGGAACACAGCTAGCACGTAGCTATTACTG	A/T	150	0.250	0.367	0.375	0.022	ns
M2	Mdy_063	TGCAGGAAGCCCACCTCCCTCAGACTCTTGTGGGAGTTCAGCAGATTCTAGACCGAGAGGCGTGCATGACATCTGC**[A/G]**TCAAAACCTAGATCTTACGAGCGCCCTAATGCATGTTAATAACATTACTTTATTGTGA	A/G	150	0.240	0.400	0.365	-0.096	ns
M2	Mdy_064	CATGCTAAATGCTTCCCGAGCATTTTAATTTTCCGATACTTGCGGAACGTGGCACACACACTAAT**[G/T]**CCATGAAATCACCGTACATCCAAGCGATTGAATTTAAAGATTTACGGCAACAGTCCAGTGGGTTTTGTT	G/T	150	0.127	0.240	0.221	-0.085	ns
M2	Mdy_073	CATGCTTAACATAGATGCTTATATACTGCATATGCACACAGTACCGTACATAAATACATATGTGTTCATACAGACACATG**[A/C]**AGTTTGGAGTGTGAGAGCAGAGCTGGGCCAGGACACAGTAAGTGGGTCAGAGTC	A/C	150	0.170	0.247	0.282	0.126	ns
M2	Mdy_074	TGCAGGTCAGGTCACGCTCATGGGTGAAAGGCCACAGCACCACTGAACTTCAGCTACACGGG**[C/T]**GTCCCTGAGACCCGTTTGCTTATCTTTGGGACAGTCGTTTGGAAAGAACTAGAGTAAAAGGAACKTGTTTGG	C/T	150	0.143	0.260	0.246	-0.059	ns
M2	Mdy_077	CATGCCTGAAACCCCGAAAAAACCCAGGTATAAATTAGGCTTT**[C/T]**GATGTTTTATTGATTCAAACCACAATGTGACGAGGGCCAACAGCATCCATTGTTCCTRTCCTGTAACGACCTCCTAATGAACCACCACGCC	C/T	150	0.263	0.353	0.388	0.089	ns
M2	Mdy_087	CATGCCTGTATATGTAATTAGGTCATCATTTATTTAGTTTTATTTCACTGGCATATTTACTCAGTTCCCTTAAGGTGGTTTTCTTAGGGAAAGC**[A/G]**CCACTGTAGTGTAAGAACGTTCTGCTTTCCACAGCTAGAA	A/G	150	0.113	0.227	0.201	-0.128	ns
M2	Mdy_094	CATGCAGAGTCAGCTAGAAATACAATACATGCCCACAGATGATTTGCATTGCA**[C/T]**TGCCAGAGTGTCTTATGATGGACAATTAGCAAGGTTTGAGGAAGTTTAGTTWAAAAAAAAAAGGTTATATAACATCCATGT	C/T	150	0.743	0.353	0.382	0.074	ns
M2	Mdy_099	CATGCAGCACCACGTGTAAGGAGCACGTTTGCAGTGCATGCTGGGA**[A/C]**TTGTAGTGCCGTGCATCGCTGGCGATGCAGGCCAAAATTGATTGAAAACTCAAATGATAGATTGGATGGAATTGGTCCAAGGTCGGGA	A/C	150	0.127	0.213	0.221	0.036	ns
M2	Mdy_101	TGCAGGAGCCCTTTGCACCGGTCAACTCCTGGCTAACCAGGGGCCGGAGAAAACCAGAACGGTCCTACTTCAACCGGC**[A/G]**TGGATTACACTCACATCAACCAACATTAAMATATAGAGAAGAAACCCAACCCAATT	A/G	150	0.253	0.333	0.378	0.119	ns
M3	Mdy_004	TGCAGGAAATGTATTTTGTTGGATGCATCATGTTTTGCCAAAAAGGACACACCACAAGAGATGTGTGCGCCC**[C/T]**GCAACCATACTAAAAACTTAAATTTAAAAAATGCCCAACGTCTGTTTCCAATTAGCGGTCGT	C/T	150	0.380	0.467	0.471	0.010	ns
M3	Mdy_006	TGCAGGGGACCCATCCAATCAACTCCAGTTCCACAGGTTAGTCTCCCCAAACAGCCGTGTTTCTGGGAAAG**[A/C]**AACACAAACATTAACWTGATGCATAACAATGGGTTGGGACACACAAAGAGACTTAGTTGGCAC	A/C	150	0.483	0.553	0.499	-0.108	ns
M3	Mdy_010	TGCAGGTCAAATGTTGCTTCTTATAAAAAATKGGTGTGTATGTATGTGTGGCTTTCATAGGTCTGTTT**[A/G]**ATTTTGTTCCAAAAACTACTTTAGGAAGTTAATCAGCGATTATTACGAGTTTGGTCGTTATTTATG	A/G	150	0.477	0.447	0.499	0.105	ns
M3	Mdy_018	TGCAGGCTACCCTGTAGGCATTCTTGAGTAAGATGTCCAACACCTAGATGACC**[A/G]**TGGCACATCTCAAAATTCCTGGTACGTCCCTTAAAAGTAACAATGTTCAACTCATTACTTATGTTGTCTTGGCTTAGGTGT	A/G	150	0.433	0.520	0.491	-0.059	ns
M3	Mdy_021	CATGCTGTAATTACAGTATTGTTGAAAGGTCTGTTATTTGATTTTTGTGTTGGTTGAAGGG[**C/T]**TCATTTAAMTTTTGATTAAAATAGTGTATCAGTAAACTAAATAGGATGTGGTGCTCTTGCTCTGTTCTTTGAA	C/T	149	0.322	0.644	0.437	-0.475	***
M3	Mdy_024	TGCAGGCCTGTGTACCTAAATGAACAAAAATAAATCCTCCAGTCCCAGGAGATTTGTGCRGGTCTGTTGATGATAGAATGTTCTCGTGTATATT**[A/G]**GTGATGATGACCCATGTGTGTGACACATTGTCACAATATT	A/G	150	0.207	0.307	0.328	0.065	ns
M3	Mdy_038	TGCAGGCTGTGGGATCCCTCGGGGGTCCAAACGCTCTTCTCTCACAGACC**[C/T]**GCGGTCTGAGTCAGCGCCGACGCCGAGGACCGACCAGATGAAGTCTGAAGTGGGCGGTGGCATACTTGTGATTTGTGAAAATGT	C/T	150	0.480	0.587	0.499	-0.175	*
M3	Mdy_039	TGCAGGGGGAATCACTGCTGACTGACGAGAGATAGGAAGTCCAGGAGATGG**[C/G]**GGAGAGGGAAAAGGACAGGAGAGAGGTGGAATTAAGACGTATAAGATGGCCGGGTGGGAGATATGGTTAAGACAATTATTATA	C/G	150	0.307	0.440	0.425	-0.035	ns
M3	Mdy_041	TGCAGGGACACAGCAGAGACCTTATGCTCTGGAAGGGCACTGCAGACGTGCGG**[C/T]**GTTTGAGTTTAAAGGTGCAGTACGTAACGTTGACCGGAGGGTTCAATACGGTCCTGCAGACGTGCGGTGTTTGAGTTTAAA	C/T	150	0.480	0.947	0.499	-0.896	***
M3	Mdy_047	CATGCTAAACGTCTTTCATGGCTGTGCTGTTGTCAAAATGGTGGGTTATTGTTTGGTCCATGTTC**[A/T]**ATCTCCTTATAACATATCTGTCTTATCTGTGTTGGCAGCTGGACTTCCTGATATGTCCATGTCGGCGCT	A/T	150	0.433	0.480	0.491	0.023	ns
M3	Mdy_052	TGCAGGTAGGATATTATAGAGCTGGGTAATCATTGTAAGTCTTTTCAATAGGAACTGGAAGGGGGTTTGTTGGATATGTGGATCTTGAAGATAAAGAGG**[A/G]**ACGAATCGTACTGGACAATGCAACATCAGACGCCC	A/G	150	0.210	0.327	0.332	0.015	ns
M3	Mdy_058	TGCAGGTGGGTTGGGGTACGTTGTTGGTGGTGGTGGTGAGGGTGTTTGGGGTTGTGTGAGGAACACTT**[A/G]**GGCAGTGCATTCATCTTAATCTGCGAGAAATAAAGATGTGTAAATTTACTTTTAAAGCTTTTGCAC	A/G	150	0.137	0.233	0.236	0.011	ns
M3	Mdy_060	TGCAGGTAAGATACAAGGTCGACCATTGAAGGCAAAATAATTATTTTGTGTCATGAAATTAACCACATTCTCAGTATT**[C/T]**CTTGTTCATTGGTTACCATAGATCTTTTACTGTGTACAAACTCTATCACAGCCCAT	C/T	150	0.127	0.213	0.221	0.036	ns
M3	Mdy_061	TGCAGGTACTCACACTATGGGTTAGCACTGCGGCTAGCGCTGTTGCTAACCACGTCATGGGTTTAAACCGACTGTT**[C/T]**TGTGAGTTTGTATGTTCTCCGTGTTCATGTGGGTATCCTCCYATACCAAAGACATACA	C/T	150	0.207	0.293	0.328	0.105	ns
M3	Mdy_069	CATGCTTCAAACAAACAATATTCCCCCTCCTGATTTGAAACCAAGTTCAACGCCTCCATACCAAACCAACTCAATCGGGCGAAACCATTGACCGCGTAG**[A/G]**GGGGGGGGGRGAAACAGGGAACCCCGCCGCTGAAC	A/G	150	0.183	0.287	0.299	0.043	ns
M3	Mdy_071	TGCAGGTCCGTCACTGGCCCCCACTGGATCTTATAACCTCTAAGGCACTCCATTTTCCACACGGACTAAACGCGTA**[C/T]**GAGCCGGTGTTTATTTGCGGGGTAATACCCACCGCGTTAATGGCAGGTGACCTTCACG	C/T	150	0.100	0.200	0.180	-0.111	ns
M3	Mdy_075	TGCAGGTTAAAAGACCGCATTCTAAAAGATAATTTTGTACCCTGAGTACAGAGATACAAATAAGATGAAAG**[G/T]**ATCACCATCTACACCAACAAGAAAAATCCCAAAATGTTAACCGAATTCACGGTCCAAATCTCA	G/T	150	0.130	0.247	0.226	-0.090	ns
M3	Mdy_081	CATGCAAGCTCCTTTCTTCTGTCAGTGACTTTGGTCAGGTTGGCATGGAAATGGACATCACCCTGGGTTTAGTACACAGCAGCATCTGAGAAAG**[A/T]**GAACAGCAAATCAAATATGAAATGTCTTACTTTGCAACCT	A/T	149	0.114	0.228	0.202	-0.129	ns
M3	Mdy_083	CATGCAGGCAGATAGAAAGTAGAACAAAAGAGTCTGTTCACTGCTTTTAGTTTCTGAGAGACT**[C/G]**GTGGATGATGATTCATAACATCACAATGCTAATACCAAAAAAGAGACGTTGCTCCCCCACAATCTTAAAAC	C/G	150	0.107	0.187	0.191	0.021	ns
M3	Mdy_084	TGCAGGGTACCAGCCCTCCCTGGACCCCCTGACACCTCCAATCATTCTCAGACCCACCGTGACATT**[C/T]**GTATCGCATTCGTTCAGAAAGGCCTTCCAGGTTGCTGGACGCTGAGAACGGCTGCGTATTGCTRGCCG	C/T	150	0.100	0.187	0.180	-0.037	ns
M3	Mdy_089	CATGCTGCGATGTTACGCAACGACCACACGGCCGTGAATCAGCAGCCAC**[C/T]**GCCAGACGTTATGGAGGCTTAGCGGACACAACACATGGTCCAATCGTGACAACACTACTGGGTTCCCAAAAAAACGAAATGAGGT	C/T	150	0.170	0.300	0.282	-0.063	ns
M3	Mdy_092	CATGCATTTCACTTGCTGAAGACAAAACTAAAGGCAGAAAGACCCACGAACAAACAGCAACTGAAGAC**[A/C]**RCTGCAGTAAAGGCCTGGCAAAGCATCACAAAGGAGGAAACCGAGCATTTGGTGATGTCCATGGGT	A/C	150	0.120	0.200	0.211	0.053	ns