

**Supporting Information 3: Description of 19 primer pairs selected for PCR optimization.**

Name	Sequence	DS					Expected fragment length (M13 excluded)														Location (Gene)	Genotype	Seq
		L <sub>prim</sub>	2 <sup>nd</sup>	3 <sup>rd</sup>	%GC	T <sub>m</sub>	T <sub>init</sub>	Hsa	Ptr	Mmu	Rno	Cfa	Fca	Bta	Sar	Eeu	Ete	Meu	Oan				
C1-2125	F=TCAGRGAAGCTTGAAGA	22	1	-	50.00	60.24	59	133	133	143	137	131	145	133	139	135	?	?	276	3'-UTR (ZNF238)			
	R=AGRCTGCTTTAGACCGTACC	21	1	-	52.38	59.80																	
C2-1218	F=GGAYAAGAACAAAGAYGACCA	21	2	-	47.62	60.14	60	281	276	295	285	272	273	267	316	<b>266</b>	266	?	248	3'-UTR (VSLN1)	Y	Y	
	R=TGAMATTCTCATGCACACRAGT	19	2	-	40.91	59.22																	
C2-6868	F=CTTCCTCCAGAAGCCTCCTT	20	-	-	55.00	59.95	59	233	233	273	240	<b>264</b>	?	233	255	234	276	?	357	3'-UTR (MEIS1)	Y	Y	
	R=TGGTGRGTAATGAATTAGACATGC	25	1	-	36.00	59.47																	
C2-1915	F=TCTTCTTCTTAATCACAATTCAGYC	25	1	-	36.00	60.14	59	174	160	197	176	184	?	173	228	172	?	<b>201</b>	223	Intron (ZEB2)	Y		
	R=CAAACAGCSMCAAACACC	18	2	-	55.56	59.94																	
C3-1615	F=TGCTGTCTGGTAGRAGGGCT	20	1	-	55.00	60.01	59	233	231	236	235	244	244	233	233	223	221	?	?	Intron (ZBTB20)			
	R=TTTCTGCTCYYGATGTT	18	2	-	50.00	59.17																	
C4-1514	F=GGCATGTAWGTGGTTTTTGAAC	23	1	-	39.13	60.16	58	282	283	314	275	291	304	278	<b>322</b>	322	<b>280</b>	?	225	Intron (LCORL)	Y	Y	
	R=GATCTGGAAACACTGAAGACKC	21	1	-	52.38	59.87																	
C5-1211	F=ACKTGGCAGCAGACCCTGT	19	1	-	57.89	60.88	59	269	269	280	280	258	<b>266</b>	260	259	261	263	?	260	3'-UTR (ZNF608)			
	R=TGGATCACATGGRAAGC	18	1	-	55.56	60.43																	
C6-5453	F=TTAGTMAAACCATTTGCGACY	21	2	-	42.86	59.99	59	275	271	261	<b>259</b>	266	273	<b>252</b>	?	268	?	<b>244</b>	273	5'-UTR (NRN1)			
	R=TGGGCGTGRGGCTGWRCT	18	3	-	61.11	60.43																	
C6-1112	F=AATTGCTGYGCTAATTACAACAT	23	1	-	34.78	60.06	59	153	151	160	162	151	186	147	?	155	144	<b>150</b>	146	IGR (MRDS1)	Y		
	R=GACTTCTCCCAWGGCATVA	19	1	1	52.63	59.57																	
C9-1918	F=GCCTTGCCAYGACCAYTT	18	2	-	50.00	59.46	59	302	313	303	296	301	<b>330</b>	307	?	311	323	?	293	IGR (PBX3)	Y	Y	
	R=GCTCYSYCGCTCAATTTAATT	20	2	-	45.00	60.41																	
C11-1417	F=GGCAARAGACACTAGAGVARTG	22	2	1	50.00	59.15	59	207	218	228	225	179	203	197	?	204	184	<b>228</b>	?	3'-UTR (NCAM1)			
	R=RGYCMAGACGGTCCCAA	18	3	-	55.56	59.02																	
C13-7574	F=AATCAATATGGAAGGAGCGGT	21	-	-	45.00	59.81	59	253	255	275	303	254	257	254	253	247	267	<b>327</b>	265	Intron (BX647243)			
	R=HGCTTTTGTTCAGATGGT	20	1	1	42.86	60.11																	
C14-2527	F=GGATGCTGAAATGACAGGC	19	-	-	52.63	59.19	59	249	251	236	266	262	?	267	245	?	294	?	?	IGR (FOXG1B)			
	R=GGTCTTCACACAGGGRA	18	1	-	61.11	59.42																	
C14-9692	F=TTAAGTGATTTTGTAAATGGTCCG	24	-	-	33.33	59.35	59	234	227	234	239	215	<b>222</b>	209	?	?	239	<b>205</b>	210	IGR (BC043585)	Y		
	R=YACCTCCAACAACCTTCCGTAAAT	23	1	-	43.48	59.44																	
C15-3531	F=CCAAGAGGGRTTATTTATGGC	21	1	-	47.62	59.69	59	228	226	298	224	240	256	244	265	223	?	<b>239</b>	195	Intron (BMF)	Y		
	R=TGATTTGACACTGKGARGG	19	2	-	52.63	59.46																	
C17-4243	F=TGCMKAGGTTGCAGAATA	18	2	-	50.00	58.89	59	311	313	311	317	307	?	306	314	<b>305</b>	325	?	299	Intron (HOXB3)	Y	Y	
	R=TRTCACATTTACGACAATARCATTA	26	2	-	30.77	59.87																	
C19-3338	F=GCTGTCCACCRAGATTACCC	20	1	-	55.00	60.52	59	206	204	197	232	260	?	<b>196</b>	226	?	197	<b>170</b>	?	IGR (ZNF536)			
	R=GCATYCCTGCCTTTRACTAA	20	2	-	50.00	60.21																	
C20-3430	F=GGAAGAATGGCGTAATGCTG	20	-	-	50.00	60.61	59	185	167	201	<b>177</b>	180	<b>173</b>	162	207	184	<b>177</b>	<b>211</b>	?	Intron (GDF5)			
	R=CGTTTCCAATTCYAGATT	19	1	-	47.37	59.92																	
CX-4344	F=GCCTGATTGATATGMCTGS	21	2	-	47.62	59.55	59	212	212	216	210	202	196	204	204	226	241	?	214	5'-UTR (NDP)			
	R=AAAGAGGTCYGYTACTTCCA	21	3	-	47.62	59.98																	

F: forward primer; R: reverse primer; L<sub>prim</sub>: Primer length; DS: number of secondary (W, S, M, K, R and Y) and tertiary (B, D, H and V) degenerate sites; %GC: G+C content; T<sub>m</sub>: melting temperature; T<sub>init</sub>: initial temperature (see methods); Hsa: human, Ptr: chimpanzee; Mmu: mouse, Rno: rat, Cfa: dog, Fca: cat, Bta: cow, Sar: shrew, Eeu: hedgehog, Ete: tenrec, Meu: tammar wallaby, Oan: platypus; Genotype: indicates if the locus has been genotyped; Seq: indicates if the locus has been sequenced. Expected lengths were calculated based on the 17-WA, or directly from trace files using MegaBLAST (<http://www.ncbi.nlm.nih.gov/BLAST/mmtrace.shtml>) when applicable, i.e. when the sequence is absent in the 17-WA and for tammar wallaby, which is not included in the 17-WA.