

# Supporting Information

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## SI Methods

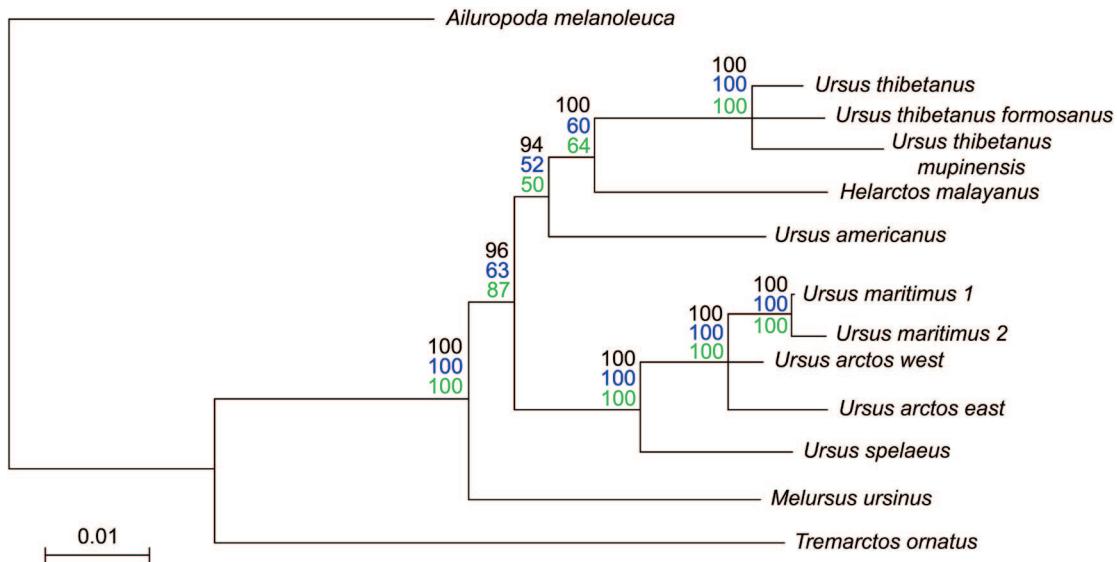
**Amino Acid Racemization Analysis.** Because it has been reported that archeological samples containing authentic ancient DNA display a specific pattern of amino acid racemization (1), we measured the D/L ratio for aspartic acid and alanine. Amino acids were derivatized as described in ref. 2, resolved by high-pressure liquid chromatography (HPLC), using a HyPURITY C18 column (Thermo Electron), and measured through fluorescence detection. The HPLC elution gradient consisted of a mixture of 50 mM sodium acetate and methanol (gradient, 0–10 min: 0% methanol; 10–11 min: 0–30% methanol; 11–30 min: 30% methanol). The extent of racemization caused by the experimental procedure was measured using commercial BSA, and yielded D/L ratios of 0.02 and <0.01 for aspartic acid and alanine, respectively. For the Chauvet cave bear *Ursus* sample, we obtained D/L ratios of 0.06 (aspartic acid) and 0.01 (alanine). This pattern is consistent with that described for a series of

archeological samples from which ancient DNA could be recovered (1).

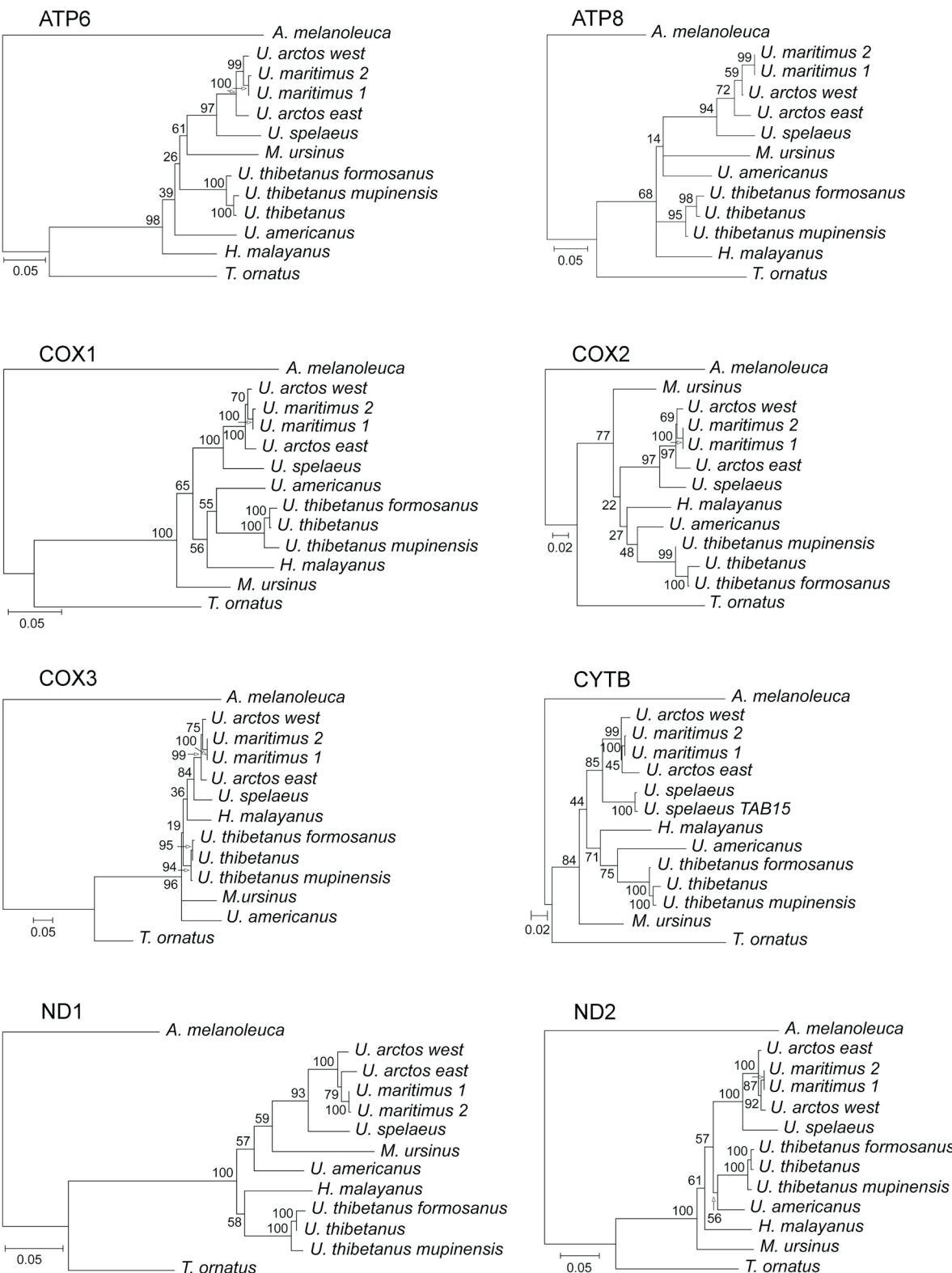
**Radiocarbon Dating by Accelerator Mass Spectrometry (AMS).** The samples underwent standard chemical pretreatment for removal of contaminants (3), and collagen was extracted using the method of Longin (4). The collagen was combusted into CO<sub>2</sub>. CO<sub>2</sub> was cryogenically trapped using an automatic device (5), transformed into graphite, and analyzed for <sup>14</sup>C by the Groningen AMS facility (6). The <sup>14</sup>C activities in the graphite were measured, relative to a standard radioactivity, corrected for isotopic fractionation using the stable isotope ratio <sup>13</sup>C/<sup>12</sup>C to  $\delta^{13}\text{C} = -25\text{ ‰}$ , and calculated using the conventional half-life (7). They are reported in years before present (B.P.). The errors quoted are all 1-sigma. For samples relatively old on the <sup>14</sup>C time scale, the exponential decay law of radioactivity conveys asymmetric errors for the reported ages (8).

1. Poinar HN, Höss M, Bada JL, Pääbo S (1996) Amino acid racemization and the preservation of ancient DNA. *Science* 272:864–866.
2. Nimura N, Kinoshita T (1986) o-phthalaldehyde-N-acetyl-L-cysteine as a chiral derivatization reagent for liquid chromatographic optical resolution of amino acid enantiomers and its application to conventional amino acid analysis. *J Chromatogr* 352:169–177.
3. Mook WG, Streurman HJ (1983) Physical and chemical aspects of radiocarbon dating. *PACT* 8:31–55.
4. Longin, R (1971) *Nature* 230:241–242.
5. Aerts-Bijma AT, van der Plicht J, Meijer HAJ (2001) Automatic AMS sample combustion and CO<sub>2</sub> collection. *Radiocarbon* 43:293–298.
6. van der Plicht J, Wijma S, Aerts AT, Pertuisot MH, Meijer HAJ (2000) Status report: The Groningen AMS facility. *Nuclear Instr Methods Phys Res B* 172:58–65.
7. Mook WG, van der Plicht J (1999) Reporting <sup>14</sup>C activities and concentrations. *Radiocarbon* 41:227–239.
8. Olsson IU (1989) The <sup>14</sup>C method – its possibilities and some pitfalls. *PACT* 24:161–177.

PNAS For Figs. S1 and S2, please see page 14 of the Supporting Information.



**Fig. S3.** Phylogeny based on the amino acid sequence of all 13 proteins encoded by the mitochondrial genome. Tree construction was performed using the giant panda (*Ailuropoda melanoleuca*) as an outgroup. The topology is supported by MrBayes, PhyML, and neighbor joining analysis; the corresponding support values for each node are indicated in black, blue, and green characters, respectively. The scale bar depicts the genetic distance relevant for MrBayes analysis. The *Ursus spelaeus* and *Ursus arctos* western lineage (west) sequences are from this study. GenBank accession numbers for the other sequences are indicated in Fig. 4.



**Fig. S4.** Phylogeny based on individual mitochondrial coding genes. The nucleotide sequence of each protein coding gene was used to infer phylogenetic relationships by PhyML analysis, with the giant panda (*Ailuropoda melanoleuca*) serving as an outgroup. Bootstrap values for each node and the scale for the genetic distance are indicated. The *Ursus spealeus* and *Ursus arctos* western lineage (west) sequences are from this study. GenBank accession numbers for the other sequences are indicated in Fig. 4.

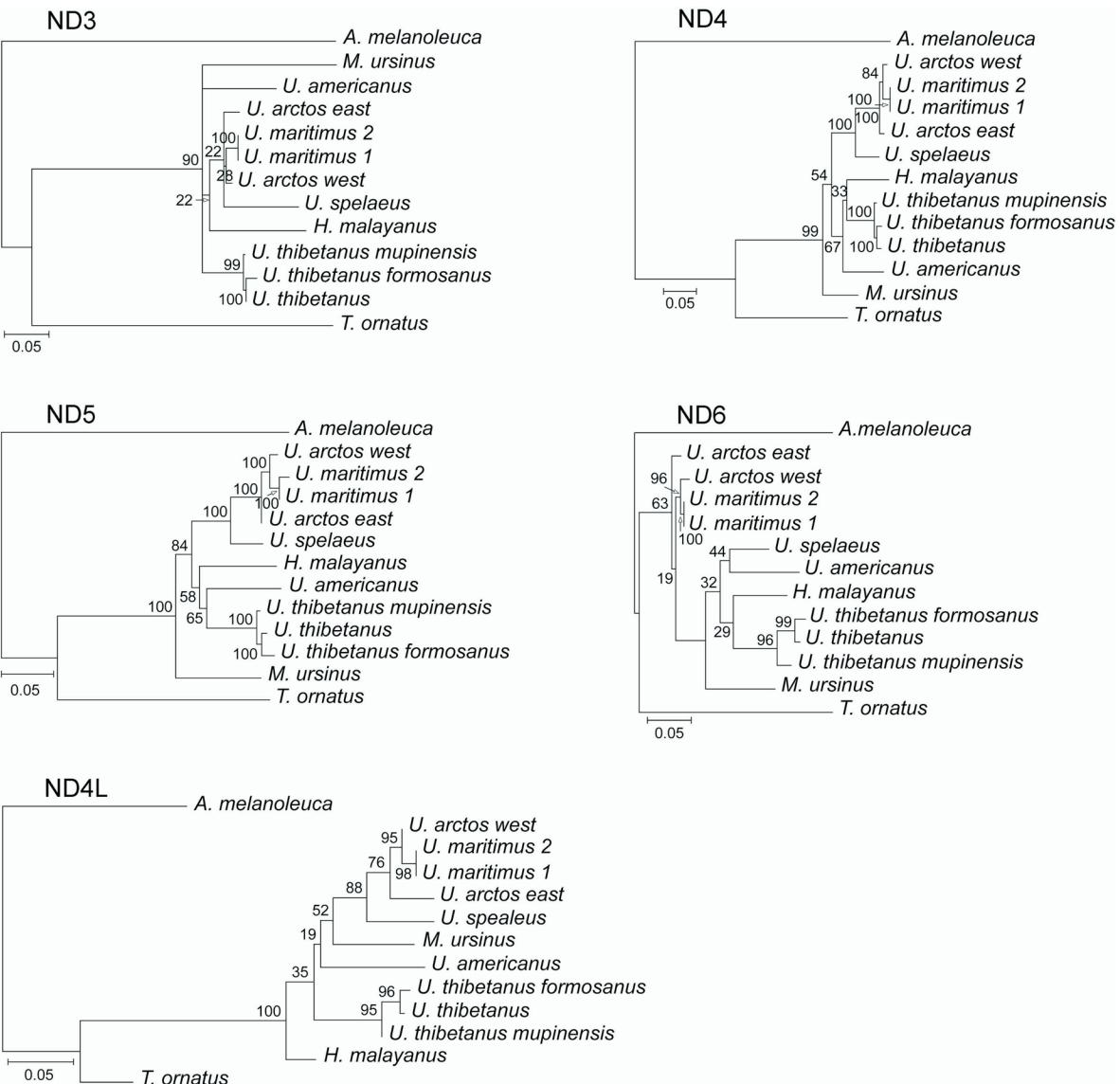
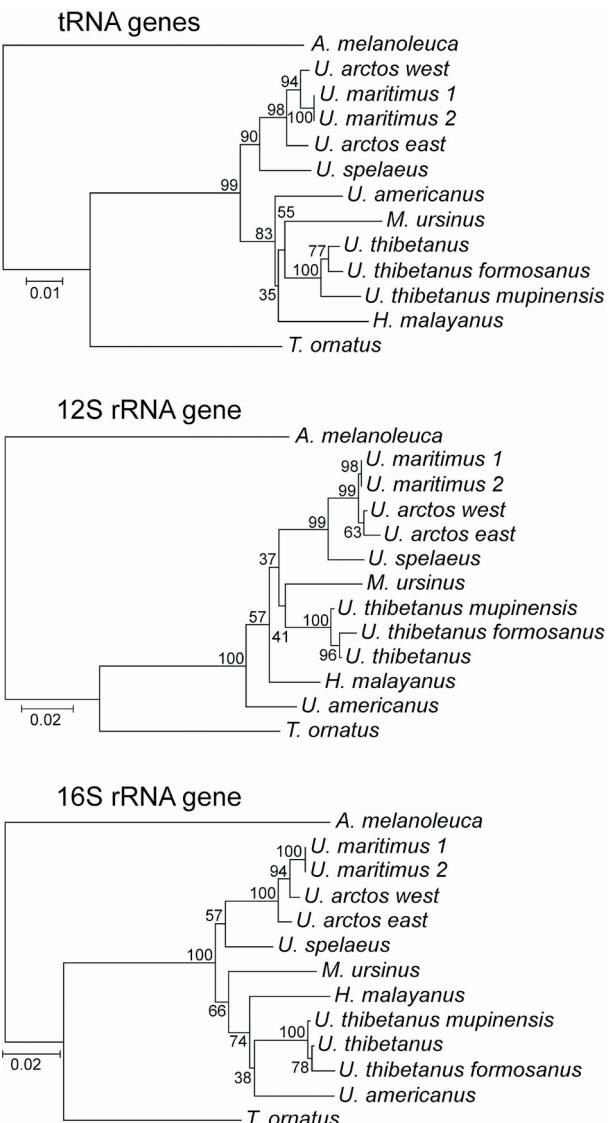
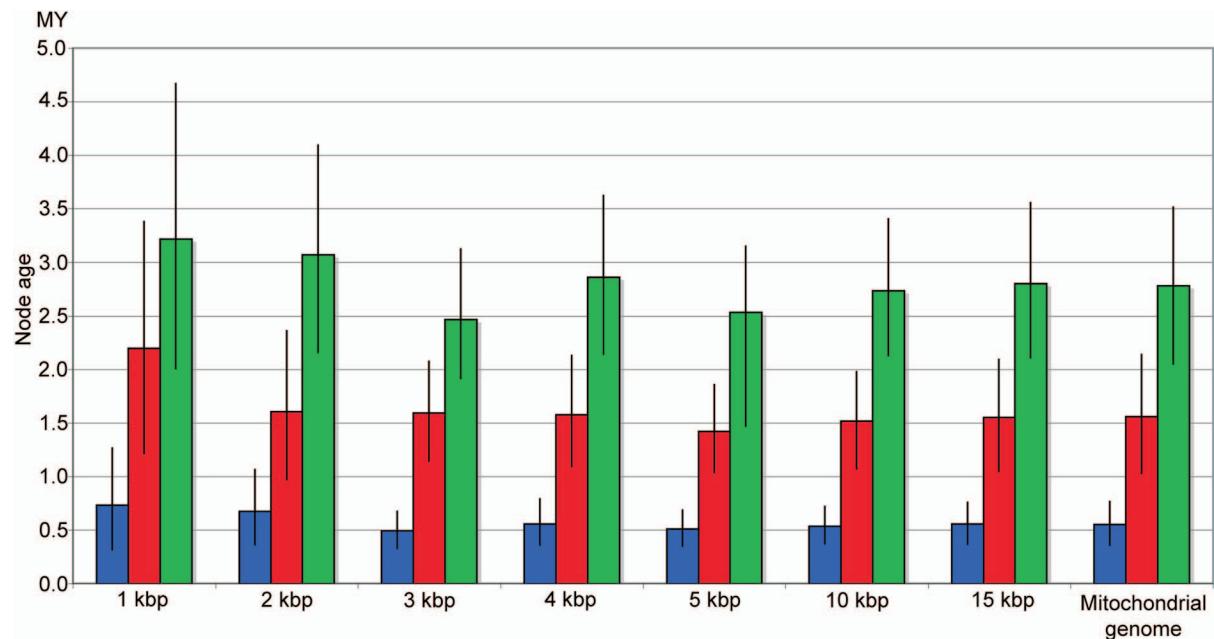


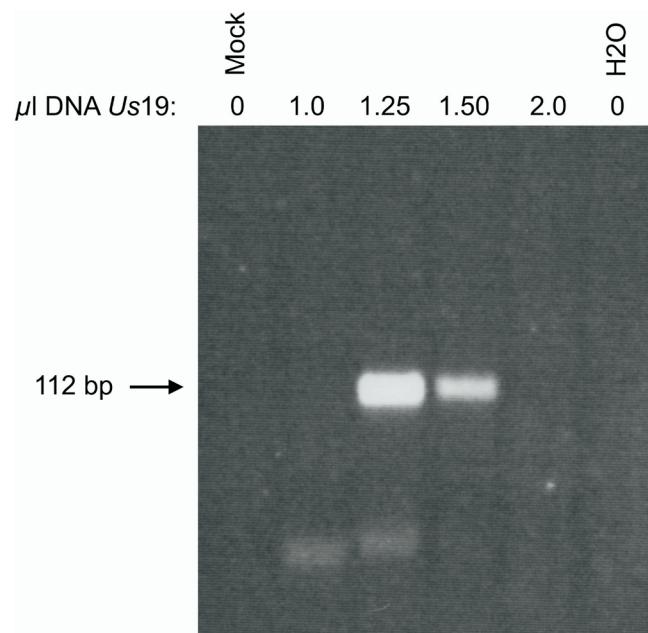
Fig. S4 (continued).



**Fig. S5.** Phylogeny based on mitochondrial tRNA and rRNA genes. PhyML analysis was performed on tRNA, 12S rRNA, and 16S rRNA genes, using the giant panda (*Ailuropoda melanoleuca*) as an outgroup. The tRNA genes sequences were concatenated as a single string of data to perform the analysis. Bootstrap values for each node and the scale for the genetic distance are indicated. The *Ursus spelaeus* and *Ursus arctos* western lineage (west) sequences are from this study. GenBank accession numbers for the other sequences are indicated in Fig. 4.



**Fig. S6.** Comparison of the divergence dates obtained for dataset of various lengths. Only node 3 (blue), 6 (red) and 7 (green), as defined in Fig. 5, are shown. Black bars represent posterior densities (95% credibility interval) of nodal age estimates.



**Fig. S7.** Amplification of a fragment of the cave bear 16S ribosomal RNA gene. Variable amounts of the Chauvet-Pont d'Arc *Us19* DNA extract were amplified using primer pair 46, yielding a 112-bp DNA fragment. The total reaction volume was electrophoresed through an acrylamide gel stained with SYBR Green I. Negative controls included reactions carried out on a mock extract (Mock) or in the absence of any extract ( $\text{H}_2\text{O}$ ). Sequencing analysis of the cloned PCR products confirmed that they correspond to cave bear DNA.

**Table S1.** Oligonucleotide primers used for PCR amplification of cave bear mitochondrial genome fragments

Primer pair	Primer position	Forward primer	Reverse primer	Amplicon, bp
1	83F 206R	AAAGGTCTCGTCGAGTCA	GCGTCGTTCGTCGATT	142
2	83F 207R	AAAGGTCTCGTCGAGTCAA	TGCGTCGTTCGTCGATTT	143
3	170F 267R	TCAATGGTCGAGGACATATAGTA	TGCGTACACGTGCGTACAC	106–256
4	201F 267R	GCCACTAAATCGAACGAACA	TGCGTACACGTGCGTACAC	205–265
5	546F 592R	CGTGTACGCACGTGTACG	GGGGTTTGATTAAGCTAAGTT	148–228
6	546F 644R	CGTGTACGCACGTGTACG	GGCAAGACAACCATAAATGTG	119–239
7	546F 657R	CGTGTACGCACGTGTACG	TTTGGGTTGGCAAGAC	119
8	572F 705R	CGCACCGCTTTTAGATA	GTGGGATTTGGATATGATAGC	155
9	599F 730R	CTTAATCAAACCCCCCTTAC	TATTTGGCTGGAAATTTAGCTT	156
10	658F 829R	TCTTGCCAAACCCCCAAAAA	GATAATAACGGAAAAATCAATAGGA	196
11	758F 890R	ACTGTAATCATAGGCATGAGACTTT	GTTGGATTTGGGATTTGTA	152
12	862F 1021R	GGCCACGAGCTTACTAGAT	CGGAGACTTGCATGTGAAT	179
13	862F 1024R	GGCCACGAGCTTACTAGAT	GGGGAGACTTGCATGTGT	180
14	997F 1163R	GCCTTCCATTAGCTACTAACAA	CTTTCGTTCATGGCTTAATTTT	189
15	1060F 1163R	TTGGATCTTAAAGCGATTGA	CTTTCGTTCATGGCTTAATTTT	126
16	1143F 1273R	CCACGGGATACAGCAGTG	TTTTCTTCACACGCTTACG	151
17	1238F 1358R	GGTTATACGATTGACCCGAGTT	GAGAGCATTAAAGTCACTTCGTAGC	146
18	1317F 1467R	TTTATCCAGCTGAAAAAGCTAC	GCTAGTAGTTCTGGCGAATAA	173
19	1422F 1599R	CCCACTATGCTTAGCCTTAA	TTGCTGAAGATGGCGGTATA	197
20	1429F 1576R	ATGCTTAGCCTTAAACATAAAT	AGACTGAATTAGCAAGAGGTGG	169
21	1554F 1663R	CGATAAACCCCGATAGACC	ATGGGTTACACCTTGACCTAACTT	133
22	1637F 1743R	AGTAAGCACAATCATCTCGCATA	AATCCACCTTATGTTTATGTTCAT	132
23	1699F 1799R	AATGGGCTACATTTCTATTCAAG	CATGCTTATTGCTTATTCA	121
24	1767F 1899R	TTAGTAGTAAATCAAGAATAGAGAG	ATGCTTACCTGTTACGACTT	153
25	1768F 1903R	TTAGTAGTAAATCAAGAATAGAGAGC	CAGTATGCTTACCTGTTACG	156
26	1877F 2001R	CAAAATAAACGCAAGAGGGAGC	CTAGCTCTGGGTTCAAAGTGG	145
27	1882F 2033R	AAAACGCAAGAGGGAGACAAG	AATTGGTTATGGTAGTTGGTTAGT	177
28	1971F 2094R	GCTTACACCCAGAGGATTCA	AGCTCGATTAAAAGAATTCTATCT	149
29	2009F 2168R	AACCCAGAGCTAGCCAGATAA	AGGAGGGTAATCTTGTGTT	181
30	2009F 2168R	AACCCAGAGCTAGCCAGATAA	AGGTAGAAGGGATAATCTTGTGTT	185
31	2022F 2166R	CCCAGATAACAACTACCAAAC	GAAGGGATAATCTTGTGTTA	167
32	2134F 2275R	GCAAGGGAATGATGAAAGATTAC	GAGTTTACCCCAGTGGATTGT	162
33	2182F 2300R	TCCCTTCTACCTTTGCTATAAT	TCTTCTACTATTTGCGACAT	140
34	2250F 2398R	CCGAAACCAGACGAGCTACC	AATTTTAAGTTTGTAGGCAAGTT	173
35	2271F 2360R	ATGAAACAATCCACTGGGTA	CTATTCTGGCAACCAGCTA	109
36	2365F 2521R	GGTTGCCAGAAATAGAATTT	TTAATTGATGGCTGCTCTAGG	178
37	2467F 2635R	GAATCAAGGATACAGCCTTACT	TTACTCATATTAGCATTGTTGCTT	194
38	2470F 2627R	TCAAGGATACAACCTTACTTAGAGAG	TTAGCATTGTTGCTTATATTTAA	182
39	2600F 2721R	CCTAATATAATACTGGGCTAATCT	TGTTGGATTATTCTACGTTGTTG	145
40	2693F 2856R	ACAGCAACGGATAACCACTGA	GGGAATGCTGGAGGTGATG	182
41	2818F 2973R	GAACTCGGCAAACACAAAC	CGTGTGCCCTTACACAA	174
42	2835F 2964R	ACCCCGCTGTTACCA	GTGGCCTTACACAAAGTCCTTATT	154
43	2935F 3061R	GCAAAAGGTAGCATAATCATTGTTCT	GCTCCATAGGGCTCTCGTCTTA	150
44	3037F 3210R	CGTGAAGAGGGCGGGAAATA	GTGAGCATTTGACTGGTACGTCTA	198
45	3038F 3155R	GTAAAGAGGGCGGGAAATAAAAT	TCGCCCAACCTAAATTG	135
46	3129F 3215R	AACAAACATATTCTTCCATGAGC	AAGTAAGTGAGCATTTGACTGGTAC	112
47	3183F 3330R	CAAATAACCTCCGAGTGTATTA	ATCCACATCGAGGTGCTAA	167
48	3303F 3420R	TTTAAGAGTCCATATGACAATAGGG	CTGGATTGCTCCGGTCTGA	139
49	3392F 3542R	TTCAACGATTAAGTCCTACGTGAT	TGGGGGTAGACTTACTAGGTTAG	174
50	3515F 3609R	CGCCTTAAGACCAATAGATG	AAAGGTTAAGTTTATGCAATCAC	119
51	3575F 3692R	AAGAGACAGGGCCTGTTAG	CGAGGAGAAATGGGTACGACTA	138
52	3576F 3736R	AGAGACAGGGCCTGTTAG	AGCCAGTACTTTGTT	179
53	3586F 3746R	CTTGTAGGGTGGCAGAG	ACGAAGTTGATATAGCCAGTA	183
54	3606F 3735R	CCGGTGTGATGCATAAAACTT	ATAGCCAGTACTTTGTT	151
55	3711F 3840R	CGCCGTAGCCTCTTAAACA	AGTGGGCACAGGGTCT	147
56	3712F 3840R	GCCGTAGCCTCTTAAACA	AGTGGGCACAGGGTCT	146
57	3735F 3822R	AGAACGAAAAGTACTGGGCTATA	GGGTTCTTGGTAAAGTTTATA	112
58	3807F 3981R	ACCTATCGCAGATGCTACAA	TACAGCTAAGCTTGTATGCTAAT	199
59	3815F 3974R	CAGATGCTATAAAACTTTTACCA	TAAGCTTGTATGCTAATATAAAC	185
60	3926F 4051R	TTCTCTACCAATGCCATA	GCTCGTAGAGCTCAATTAG	145
61	3950F 4136R	TTGTCACATAAACCTGGAGTA	GTAGAAAGTGTGAATGAGCCAT	208
62	4025F 4175R	GATGGGCTTCAAACCTAAAC	GGGAAAATTAGTCAGAGGTGCT	172
63	4104F 4272R	AGCCATTATTCTCTATCAGTCCTAC	AACCTGAGACTAATTGACTCTCCT	194
64	4114F 4244R	CTCCTATCAGTCCTACTATGAATG	CAAAAGGGCTCGGTTAG	148

Primer pair	Primer position	Forward primer	Reverse primer	Amplicon, bp
65	4218F 4397R	ATGATTATCTCCACTCTAGCAGAA	GGGGCTGTGAAATGCTC	196
66	4364F 4492R	TAATAATGCCCTCACGACTATCC	TCGGAATCGGGGGTATGA	146
67	4459F 4593R	CTCTAACAGCTTCTTCCTATGA	AGGAGGAATGCTGCTGTGATA	156
68	4541F 4695R	ATTCCTACCCCTCACACTG	TTAAGATTAGGTTGATTCTATAA	179
69	4668F 4812R	GAGGTTAAAATCTTATTCTAG	GTACGGGAAGGGATGAA	162
70	4791F 4910R	CCCATACCCCAGAAATGTT	TTCAAAGCCAATTCAAGATTATGAG	143
71	4886F 5007R	ATCGTTCTATTCACTCCCCTACTG	CGGTGGCTTGTTGAGGAAAT	142
72	4977F 5123R	ATCCACGGGCATAGAAG	TGCTAGGGCAATGGTGAT	164
73	5102F 5254R	AATCCAATCGCATCAACTGT	TGGATGGTGAATTGATATAGG	175
74	5228F 5317R	TGACAAAAAAATCGCACCTCTAT	GTCCTCTCAGCCTCTACTAAT	112
75	5228F 5337R	TGACAAAAAAATCGCACCTCTAT	TCGGAGTTGAGTTGGTTAGTC	132
76	5313F 5467R	CCGTATTAGTAGGAGGCTGAG	ACATGAATAGTATAAATGCTCTAGA	180
77	5345F 5455R	CAAACCTCAACTCGAAAAATC	AAATGTCCTAAAGTTATTATGATG	135
78	5439F 5575R	TCAACCTACAATCTACATCATAATA	GAGGCCGCTAGTGTAGT	155
79	5541F 5691R	TACCACTAATTGCCTCACTAATC	CGCATGTAGAAATAAGGTTAATA	175
80	5541F 5699R	TACCACTAATTGCCTCACTAATC	TAGGCGCATGTAGAAATAAG	179
81	5666F 5813R	CCCATATTCTAGCCATCACAGC	GTATGGTTGATGTCAGATTAGAGG	172
82	5720F 5881R	GCATACGCTACAGCACTAACAT	GCCCTGGTCTATTCTAGCTA	183
83	5796F 5892R	AAATAATCTCCCTACCCCTCTAA	CTTGAGGCCCTGGTC	114
84	5824F 5980R	AGCATCAACCATACTTCTTCACTAA	AGCGGTTGTTTGCCTCA	175
85	5949F 6085R	TAAGGACTGCAAGAGCATATCTCA	GCGGCGGGAGAAGTAGATT	155
86	6059F 6204R	GCTAAAACCCCCAAACAC	TGAAATCTAAGAATAGAGGTCAAGT	171
87	6059F 6224R	GCTAAAACCCCCAAACAC	GGCTGATAAAAGTATTAGACTGTA	191
88	6179F 6318R	CACCAAGAGCTGGCAAA	GGCTCATGCACCGAACAGA	158
89	6237F 6420R	GCTTTTATCAGCCATTCTACCT	ATGGGCACTACGATTACATTG	205
90	6296F 6398R	AAGACATTGGCACCTTACCT	ATCTGATCATCCCCAACAA	121
91	6394F 6529R	GCCCTGTTGGGGATGAT	ATTATTGAGGGAACGCTATGTC	159
92	6498F 6630R	CTGATTAGTGCCTTAATGATCG	GGGGTAGACAGTCATCCA	152
93	6592F 6766R	CTTCTGGCCTCTTATGGTA	TTGCAGGGGTTCTAGTT	193
94	6610F 6722R	GTAGAAGCAGGTGCAGGAA	AATAGCTCCAGAACATAGAGA	135
95	6649F 6762R	CCTCTAGCGGGTAATCTG	GGGGGCTCATGTTAATA	131
96	6732F 6883R	TCTGGGAGCTATTAATTCTACCA	GTGTTAAGGTTGATCTGTAAGTAG	177
97	6857F 6995R	CAGTCTTAGCAGCTGGATTACTAT	CCGAACCCAGGAAGGAGTA	157
98	6857F 7007R	CAGTCTTAGCAGCTGGATTACTAT	TGTGAGAGATCATTGCAACC	171
99	6969F 7124R	CTTCGGACATCTGAGGTTACA	ACCTACGGTAAACATGTGGT	178
100	6969F 7145R	CTTCGGACATCTGAGGTTACA	AAGCTCGTGTCAAGCTATAC	200
101	7095F 7254R	CATTGGATTCTTAGGATTATCG	TCATAGCGGGAGATCATT	179
102	7234F 7387R	ACTCTGACGGAGGGAATA	TCCCATTGATAACACATAGTGAA	177
103	7360F 7508R	CATGACACGTACTATGTGGTAGC	GAATGTCATGTTAACATGAA	174
104	7468F 7616R	ACACTTAACAACACATGAGCAAAAT	TGAAATGAATGAGCTACAGAA	174
105	7552F 7657R	GGCCTGTCAGGAATACCTC	TCCCAATCATGAAAATTATCAG	128
106	7624F 7775R	ATAGGCTATTATTCCTACTAACAG	TTAGTGTGACGTAGGCAGGTTCT	175
107	7624F 7775R	ATAGGCTATTATTCCTACTAACAG	TATAACGTAGGCGGGTTCT	171
108	7751F 7887R	GCCCTCCTCATATCATACTATT	AACCTGACAAAGTTATGTA	162
109	7832F 7991R	TGGTTCAAGCCAATATCATAAC	CATGAAATGCGAGAAGTCTCTAT	184
110	7965F 8101R	GCCTCAAGACGCAACTCTC	CTTCTTGCGTCCATTGTACTT	159
111	8071F 8211R	AACTATACTAACACCAAAATTAA	TTTACAGTTAGTGAAGGGATTATTGA	165
112	8107F 8211R	AATGGACGCAAGAACAGTAGAG	TTTACAGTTAGTGAAGGGATTATTGA	129
113	8175F 8323R	TCCCATCACTACGGATCTCTAT	CGAACATTCTCAGGTTAGT	169
114	8191F 8334R	CCTCTACATGATGGATGAAGCTAA	TCTACTCTAACAGCCAGTTCTC	168
115	8296F 8393R	TGACTCTTATATGATTCGACACAA	TCTTCTGACGAGATCAGCATAC	120
116	8298F 8394R	ACTCTTATATGATTCGACACAA	ATCTCTGACGAGATCAGCATAC	119
117	8298F 8452R	ACTCTTATATGATTCGACACAA	CCTGGGATTGATCAGTTTT	175
118	8363F 8513R	CGAGTAGTGTGCCATAGAA	TGAGCATTGGCCGTAATATAG	171
119	8492F 8641R	CTTATAGCCATGCGACACAG	AACTAAAAGGTTAACGCTATGTTA	174
120	8601F 8763R	AAGAATGATCCGCTTAATACT	TTTGAGACTTTAACGCTATGAGA	188
121	8611F 8757R	CGCCTCAACTATAGAACATTA	GACTTTAACGCTATGAGAATAAA	173
122	8687F 8842R	CTCTTAATGAAGATGCCACAACTA	TCATTTTCTCTCAAGGCACAA	178
123	8731F 8876R	TTACAATTCTATCCATAGCTTAACA	TGGGGTGATAAAAGAGGTAA	166
124	8847F 8994R	CCTTGAGAAGAAAAATGAACGAAA	AGCGGAACATAGTCAGTGTGAA	169
125	8953F 9080R	TCCTCTCCAGTCGATTGA	TTGAGCCAATAAATAGGATTAGTGA	152
126	9053F 9202R	GGACAAACCTGGGCACTAATA	GGTCTTGATCGGAATCCAGTA	171
127	9125F 9276R	CCGCACTATTACACCTAC	TGTTTCGATAATTACGAGCATTG	174
128	9180F 9337R	CCTTGCGAGGTACAGTC	GCCTGCAGTAATATTAGCGGTTAGT	182
129	9232F 9335R	CCTGGCTACTTCTACCTCAA	GCAGTAATATTAGCGGTTAGTC	126

Primer pair	Primer position	Forward primer	Reverse primer	Amplicon, bp
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132	9379F 9492R	AATTGGGGAGCTACCTTG	CTTACTAGCAGGGTAAAGACGTAGG	138
133	9379F 9533R	AATTGGGGAGCTACCTTG	ATGTGTTGGTAGTCATTAAGTGT	180
134	9468F 9591R	AGTCGCTGTAGCCCTATT	GCTGAGAGGGCTCTGTTAGT	144
135	9468F 9595R	AGTCGCTGTAGCCCTATT	GGCTGAAAGGGCTCTGT	145
136	9572F 9721R	TCAATCCAAGCCCATGAC	CCTTGAATGTAATCTCTCGAATAAT	175
137	9573F 9703R	CAATCCAAGCCCATGACC	GTCCC GCCACCATTGATA	148
138	9573F 9717R	CAATCCAAGCCCATGACC	GAAATGTAATCTCTCGAATAATGTCC	170
139	9685F 9839R	ACCAATATACTTACCATGTATCAATG	GCTAGGCTTGAGTGTATAAAAAG	176
140	9720F 9845R	CATTATTGAGAGAGTACCTTCAG	GTAGGTGCTAGGCTTGAGTGT	147
141	9815F 9943R	TCTTCTTGCGAGGATTCTTCTG	CCGGATGCAAGAAGTACTGA	148
142	9889F 10019R	CCGCCACAGGTATTACTC	GAGATAGTAATGAATAGGGCCTGAA	155
143	9910F 10040R	CTGAATCGCTAGAAGTGC	AGTGTGAAGTATAACGCCAGAGAGA	155
144	10000F 10108R	GGAAGCCGCAAGCACATA	AAAGTTGAGCCATAGACTCCATC	131
145	10082F 10228R	ACTATGAGGCATCCTTCACTATT	CAAAGCCGAAATGGTACT	165
146	10165F 10263R	ATCATTGGCTCAACTTTCTCAC	GTCATACGACATCTACGAAGTGTCAA	124
147	10170F 10313R	TGGCTCAACTTTCTCACAGT	TAAAGAAGCAGGACCCATC	164
148	10242F 10396R	CTTGAAAGCAGCAGCATGAT	GGTGAATAGTGTAGGATTAAGTTG	179
149	10248F 10377R	AGCAGCAGCATGATATTGACACTTC	AAAGTTGATTGCTTCTCTGGACTG	154
150	10374F 10528R	GCGCAGTCCAGAAAGAAGCAA	GAAAAAAGGGAGGGCTGCGCAT	175
151	10494F 10645R	CAAGCCCCATGAATGCGGGTTT	ACGCTATGGTAGTATCGTATT	174
152	10605F 10755R	TACTCTCCCTCTCCCTGAGCA	AGTCGAATCACTTGTGTTGGTT	173
153	10725F 10871R	TAGAAATGGGCGCAATATGATA	GGATATTAGATGGGATCGATAAA	169
154	10834F 10937R	TTCTTAGCATTCAATTGTATCCCTAAT	ATTGTTAAAACTGTTACTGTAGCA	129
155	10907F 11041R	TAGAGGGTATAGTGTATCCTTATT	CCGTAAGTGTGGAGGACTATTACTAG	160
156	10907F 11046R	TAGAGGGTATAGTGTATCCTTATT	GTTCCGTAAAGTGTGGAGGACTATT	163
157	11017F 11092R	GAGGCAGCACTAGGACTGT	AAGATGATTTTAGCATTGTAAAGAG	100
158	11017F 11092R	GAAGCAGCACTAGGACTGT	AAGATGATTTTAGCATTGTAAAGAG	100
159	11065F 11196R	GGAACGTACTATGTACAAACCTC	TACGTCAAACGTGATGAGACTAATAAG	157
160	11156F 11290R	GCCCAATATAATTGGATCAATA	CATGTTGTAGGGCTAATAGAG	156
161	11156F 11294R	GCCCAATATAATTGGATCAATA	GTAGTCATGTTGTAGGGCTAAT	161
162	11157F 11290R	CCCAATATAATTGGATCAATACA	CATGTTGTAGGGCTAATAGAG	155
163	11263F 11394R	TATTCTTACCGACTCCCTATCG	TCAGGAGTAATTGTAGGAGGATTAG	156
164	11355F 11516R	AAGGAAACTCTGACCCGAAAG	CCCGCTTGTAGTCGTTCTGTT	181
165	11481F 11594R	CCCACTCTGGTATTATCACTCG	AGCCTAGATTATTTGGGTGT	136
166	11569R 11693R	CTTGCCTTACTAATTGCACTAC	TTTACCATAAATGCCATTACAC	149
167	11657F 11818R	GCCAAACTCTGATCCAGTATCT	TGTAATTGATCATGCCGTAGC	184
168	11665F 11818R	CCTGATCCAGTATCTTCTATGACT	ATTCGTATCATGCCGTAGC	172
169	11793F 11918R	GCTGCCGTACTCTAAAACTA	TTGGCGTAGGAGATAGAA	144
170	11878F 11993R	CCTTCATAATACTCTGCTATGG	GGGGTTGGATTAATACTGCTACA	139
171	11970F 12101R	GTCAGTCACATGCCCTAGT	CGGCTATGGATTGTTCA	149
172	12075F 12211R	CTATTCTGTCTGCAAACCTCAA	TTCTCGATAAGGTTGATGGTAG	159
173	12181F 12305R	TAGCAAGTCTCACTAACCTAGCAC	GTAGAGGGAGTAGAGGGCAGTA	146
174	12274F 12358R	CCATCACTTAATAGGAACCAACAT	GGTTAAATACTTTGACGTGGTCTG	109
175	12318F 12438R	TCCCTCTACATACTAATCACCACACA	GAATTATTTGGGGTTAAGGGATAG	145
176	12392F 12538R	ACGAGAAAACGCCCTGATAG	CTTGCCTACTTTTCGGTAAATAA	170
177	12392F 12542R	ACGAGAAAACGCCCTGATAG	GTTCTTGGCTACTTTTCGGTAA	173
178	125111F 12622R	AATCTAATAATAAAAGCCCAAGTC	GGTCCCTAAGACCAATGGATTACT	135
179	12590F 12754R	AATCATGGCTTTCAACTTTA	TGTGGGTAGGATTATTTGTG	187
180	12705F 12874R	AACTTTGCTCATATTAACGATACC	TTAGGGTTGAATAGTTATTCAATG	194
181	12830F 12962R	TGATATTCTTACTCAGGGCAAGA	GAAAATCCATGATTGATCATGTGA	157
182	12942F 13095R	CATGCCAGTAGCCCTGTT	TCCTTCAGCCGATGAAT	172
183	12946F 13082R	CCAGTAGCCCTATTGTCACA	CAGCCGATGAATAGTGTAAAGAT	161
184	13066F 13192R	CTAGTGCACGCCAAATAATCT	TCTCCGATGCGATTGTATAG	146
185	13154F 13277R	GACGAATAGACGCCAACACAG	GGTTTCATGTTCAATTGCAAAGA	147
186	13169F 13351R	ATACAGCCGCTACAAG	TGGATGAAGGCCGAATTG	200
187	13249F 13351R	AATACAAATGCTAGAGACTTCCAACA	TGGGTGAAGGCCGAATTG	120
188	13326F 13482R	CCTAGCAGCCACTGGAAAAT	GGCTGTTGGATAGTCTGTT	178
189	13330F 13482R	GCAGCCACTGGAAAATCA	GGCTGTTGGATAGTCTGTT	174
190	13441F 13627R	GGGGTATTCTTCAATCGATT	TGCTAGGTGAGGCTGATTAATTCC	210
191	13459F 13618R	CGATTTACCCCTGATAGAAC	GGCTGGTTAATTCCAATTGTTAC	182
192	13594F 13760R	TCAAGCCAGTAGGACTAATAATCG	GGTAAGTGGCAGGGCTTGAATA	189
193	13598F 13760R	GCCAGCTAGGACTAATAATTGTAACA	GGTAAGTGGCAGGGCTTGAATA	185
194	13732F 13832R	GAACAAGACATCGAAAAATAG	TTAGGTCTTAGAGTGAATCCTGTTA	127

Primer pair	Primer position	Forward primer	Reverse primer	Amplicon, bp
195	13803F 13927R	AGCTCTAGCACTCACAGGCATAC	GAGTACTGTAGGCAGCGGTTAT	146
196	13891F 14001R	GCCTGAGCCCTACTAACAACTCT	AGGAGAGGGTTATTCTCGTTGATA	134
197	13892F 14002R	CCTGAGCCCTACTAACAACT	AGAGGGTTATTCTCGTTGAT	130
198	13892F 14058R	CCTGAGCCCTACTAACAACT	TTAGGTATCCTGCAAATAA	186
199	13966F 14077R	CTTCTAGGACAACCTCGCTTA	TAGTGGGTGAATATTGGAAGAT	136
200	14049F 14201R	TCTAGGAAGTATTCGAGATAC	TGTGGGTAGTCAAGTTGAGAGTT	177
201	14163F 14300R	AGGTTTATCCTGGCATAGAA	CCATTTTGCGTTATTGATA	158
202	14274F 14396R	TCACCGCCTCTGCCAATAATG	GTCCTTCTGGTTAGAGACTGTGATTG	149
203	14336F 14479R	ACGCAATGACTAGAAAATGACT	GGAAGTCACCTGTGAAATTAAG	166
204	14369F 14552R	CTATCTCTATTCAAATAAAATCT	GGCTTATACAGTTATGGAACTTGA	208
205	14471F 14565R	TCTTGTACTTAATTCACGAGTGA	TAGGGATTGCGGCTTATACAG	116
206	14517F 14610R	CCAATAAGAAGAGATACCCAGTG	TAATTATGATAACGGGGGATTC	115
207	14585F 14725R	TATGGCTCCTCACTAAAAAAC	AGCTGTATTAGGTGATTATCG	163
208	14693F 14844R	ACAGGCAATCAATAGCTCAGACAAT	TGGGGGGTCTTTTAGGTTAAT	175
209	14693F 14846R	ACAGGCAATCAATAGCTCAGACAAT	TTGGGGGTCTTTTAGGTTAAT	176
210	14812F 14953R	CAAGTATCCACCCAAATAAACTA	ACTATCTTGTGATGGGTTTAG	166
211	14886F 14998R	CACCAACACCCCCACTA	GACTCCATGTGAGAATAATGATAATGT	139
212	14886F 15039R	CACCAACACCCCCACTA	AAGTACAACGGTATTTTATGTC	178
213	14888F 14998R	CCCAACACCCCCACTAAT	GACTCCATGTGAGAATAATGATAATGT	137
214	14949F 15081R	AACCCCTACAAAACCCATCACAA	GTTTTTCCGATGTTGGTCAT	152
215	15039F 15135R	GACATGAAAAATCACCGTTGACTT	TGATGGTGTGGGAGGTCAT	117
216	15081F 15224R	ATGACCAACATCGAAGAAC	TAGTGTATGGCTAGAACACAGG	164
217	15159F 15297R	ATCTCAGCATGATGAAACTTTG	GGATAACTCAACCGTAGTGAAC	160
218	15159F 15369R	ATCTCAGCATGATGAAACTTTG	AGTACAGGCCCCGTCCTACGTG	232
219	15268F 15369R	TTTCATCAATCACCATAATTGC	AGTACAGGCCCCGTCCTACGTG	123
220	15331F 15448R	CAAACGGAGCCTCCATATTCT	CTATGAATGCGGTGGCTATGA	138
221	15417F 15525R	TGAAACATGGCATTATTCTC	ATAGGGATGGCCGATAGTAG	129
222	15475F 15626R	TCCTACCTGAGGCCAATGTC	GATGATGAACGGGAGAATAAAG	173
223	15592F 15736R	ATAAGGCAACTCTAACAGATTCT	CTTAATTGTATAGTATGGGTGAAATG	170
224	15701F 15843R	CCCCCTTGGAAATCCCCTG	GGGTTTGCBBBBBGTATAGTT	162
225	15808F 15926R	TCCTATTCTCGCTGACTTA	TCCTCTAGTTGTTAGGGATAAT	143
226	15812F 15904R	ATTCTCGCTGACTTA	CGTAGGATAGCGTAGGCAAATA	114
227	15881F 16036R	CATCAAACCCGAGTGGTACTT	TCAGAAATAGGCATTGGCTTAGAG	178
228	15918F 16039R	ATCCTACGATCTATCCCTAACAAAC	AGTCAGAAATAGGCATTGGCTTA	143
229	16003F 16174R	ACACATCCAAACAAACGAGGAATGA	CAATAATTCCAGCGATGGGTATGA	195
230	16036F 16154R	CTCTAACCCAATGCCTATTCTG	ATGAGTACTAGGGGGATTGTAAG	142
231	16148F 16288R	TCTCTACTTACAATCCCCCTAGT	GGAGCTGTTGCTTCTCCTTGAGT	164
232	16180F 16332R	CCATCGCTGGATTATTGAA	CCAGGAAATAGTTAACGTAGGACAT	177
233	16305F 16477R	CAGCTCCACTATCAGCACCAAGC	GGGCACGCCATTAAATGCACG	192
234	16337F 16477R	CTACTTAAACTATTCCCTGGTACATAC	GGGCACGCCATTAAATGCACG	160
235	16409F 16529R	TGTACTGTACCATCATAGTATGTCC	TTTGAGATGAACTCTCATGAAAGA	147
236	16488F 16584R	GGCGTCCCCATGCATAT	CCCGGACTAAGTGAATACAT	117
237	16492F 16592R	TGCCCCATGCATATAGGC	TAAGCTCCCGACTAAGTG	119
238	16492F 16650R	TGCCCCATGCATATAGGC	CCGGAGCGAGAAGAGGTACAC	179
239	16546F 16679R	CATCTAAAGTTATTCAGTGTAA	AACATAGAAACCCCCCACATT	153
240	16597 F 16679 R	AGTCCGGGAGCTTAATCAC	AACATAGAAACCCCCCACATT	102
241	16597F 16721R	AGTCCGGGAGCTTAATCAC	CATGGCCCTGAGGTAAGA	142
242	16679F 42R	AATGTGGGGTTCTATG	AGTCCCCCTAAAGATACCAAA	191
243	16696F 29R	GTTGAAACTATACCTGGCATCTG	CCCCCTAAAAATAAAAGATACCAAA	167
244	16696F 36R	GTTGAAACTATACCTGGCATCTG	TCCCCCCCCTAAAAATAAAAG	171
245	16802F 125R	TCAGCCCCATGATCACACATAA	CCCGACCCGTGAAAGATA	151

The position of each forward (F) and reverse (R) primer is numbered according to the *Us18* mitochondrial genome sequence.

**Table S2. Oligonucleotide primers used for PCR amplification of brown bear mitochondrial genome fragments**

Primer pair	Primer position	Forward primer	Reverse primer	Amplicon, bp
1	170F 600R	TCAATGGTCGAGGACATATAAGTA	GGCAAGACAACCATAATGTG	451
2	555F 686R	CTTAATCAAACCCCCCTTACC	TATTGGCTGGGAATTAGCTTAT	156
3	614F 785R	TCTTGCCAAACCCCAAAAAA	GATAATAACGGAAAAATCAATAGGA	196
4	555F 1117R	CTTAATCAAACCCCCCTTACC	CTTCGTTCATGGCTTAATT	585
5	614F 1311R	TCTTGCCAAACCCCAAAAAA	GAGAGCATTAAAGTCACTTCGTAGC	723
6	951F 1117R	GCCTCCCATTAGCTACTAACAA	CTTCGTTCATGGCTTAATT	189
7	951F 1420R	GCCTCCCATTAGCTACTAACAA	GCTAGTAGTTCTGGCGAATAA	492
8	1270F 1420R	TTAATCCAGCTAAAAAGCTATC	GCTAGTAGTTCTGGCGAATAA	173
9	1270F 1553R	TTAATCCAGCTAAAAAGCTATC	TTGCTGAAGATGGCGGTATA	303
10	1270F 1617R	TTAATCCAGCTAAAAAGCTATC	ATGGGTTACACCTTGACCTAATT	371
11	1270F 2122R	TTAATCCAGCTAAAAAGCTATC	AGGAGGGTAATCTTGCTGTT	874
12	1963F 2475R	AACCCAGAGCTAGCCAGATAA	TTAATTGATGGCTGCTCTAGG	534
13	2421F 2927R	GAATCAAGGATACGCCCTACTT	CGTGTGGCCTTCATACAA	525
14	2772F 3284R	GAACCTGGCAAACACAAAC	ATCCAACATCGAGGTCGTA	532
15	2883F 3164R	GACCGTCAAAGGTAGCATAA	GTGAGCATTGACTGGTACGTCTA	306
16	3133F 3599R	AGTACAAAATAACCTCCGAGGTGAT	CATAGTGTAGGGAGAGGATTGAA	491
17	3257F 3794R	TTAAGAGTCCATATGACAATAGGG	AGTGGCGCAAGGGTTCT	555
18	3761F 4226R	ACCTATCGCAGATGCTACAAA	AACCTGAGACTAATTCTGACTCTCT	491
19	4202F 4864R	CCGAGCCCCTTTGACCTA	TCAAAGCCAATTCAAGATTAGAG	686
20	4745F 5271R	CCCATACCCCGAAAAATGTT	GTCCTCTCAGCCTCTACTAAT	549
21	5182F 5835R	TGACAAAAAAATCGCACCTAT	GCCCTGGCTATTTAGCTTA	674
22	5750F 6373R	AAATAATCTTCTACCCCTCTAA	ATGGGCAGTTACGATTACATTG	645
23	6346F 6947R	GGCTCTGGGGGATGAT	CCGAACCCAGGAAGGATTAG	621
24	6922F 7508R	CTTCGGACATCCTGAGGTTACA	GCCGAGGTATTCTGACAG	605
25	7463F 7839R	TTCATGGGGTTAATATGAC	CCCTGACAAAGTTATGTAATT	401
26	7785F 8405R	TGGTTCAAGCCAATATCATAAC	CCTGGGATTGATCAGTTTT	641
27	8316F 8947R	CGAGTAGTGTGCCATAGAAA	AGCCGAACTAGTCAGTTGAA	653
28	8684F 9155R	TTACAATTCTATCCATAGCTCAAC	GGTCTGTATCGGAATCCAGTA	493
29	9130F 9550R	TTCCCTTATGGCAGGTACA	GAGGGCTGAGAGGGCTCT	439
30	9529F 10068R	CCCAAGCCCATTGACCACTA	TATGAAAAAAAGTTGAGCCGTAGA	562
31	10035F 10708R	ACTATGAGGCATCCTTCACTATT	AGTCGAAATCACTGTTTTGTT	696
32	10678F 10922R	TAGAAATGGGCGGAATATGATA	GATAGGAGCTATATTAGCCAGTGT	268
33	10785F 11547R	TTTCTTAGCCTTCATTGTATCC	AGCCTAGATTATTTGGGTGT	785
34	11487F 12148R	GGGCCTTACTTCTGTTTTA	TGGTAGGAGGGAGTGATAGATT	683
35	12110F 12575R	CACTATGGCAGCTTGTGAC	GGTCCCTAAGACCAATGGATTACT	489
36	12464F 12707R	AATCTAATAATAAAAGGCCAAGTC	TGTGGGTTAGGATTATTTGTG	266
37	12464F 12827R	AATCTAATAATAAAAGGCCAAGTC	TTAGGGTTGAATAGTTATTCAATG	388
38	12464F 12932R	AATCTAATAATAAAAGGCCAAGTC	GTGTGTATGTATCAAATGGAAAAC	494
39	12464F 13145R	AATCTAATAATAAAAGGCCAAGTC	TCTCCGATGCGATTGTAG	701
40	12543F 13304R	AATCATGGTTTTCAACTTTA	TGGATGAAGGCCAATTG	779
41	12658F 12827R	AACTTGTCTATTAACGATACC	TTAGGGTTGAATAGTTATTCAATG	194
42	13279F 13571R	CCTAGCAGCCACTGGAAAAT	GGCTGGTTAATTCAAATTGTTAC	315
43	13279F 14004R	CCTAGCAGCCACTGGAAAAT	GGTATCCTCGGAAATACTCC	749
44	13811F 14349R	ATTATCGAAACCGCCAACACG	GTCCTTCTGGTTAGAGACTGTGATTG	565
45	14227F 14797R	TCACCGCCTCTGCCAATAATG	TGGGGGTCTTTAGGTTAAT	594
46	14662F 15112R	TCAGATAATAGACCCACGATAAATG	CAAAGTTCATGTCGAGAT	472
47	14992F 15478R	GACATGAAAAATCACCGTTACTT	ATAGGGAATGGCCGATAG	507
48	15333F 15836R	GGGCCTGTACTATGGCTCATAC	AGAAAGTACCACTCGGGTTGA	525
49	15654F 16241R	CCCTCTGGAATCCATCTGAC	GGAGCTGTTGCTTCTCCTGAGT	611
50	16133F 16527R	CCATCGCTGGAATTATTGAA	CCCGGACTAAGTGAATACAT	415
51	16258F 16593R	CAGCTCACTATCAGCACCAAAGC	CCGGAGCGAGAAGAGGTACAC	356
52	16435F 207R	TGCCCCATGCATATAAGC	TGCGTCGTTCGTTGATT	544

The position of each forward (F) and reverse (R) primer is numbered according to the Guzet *Ursus arctos* mitochondrial genome sequence.

**Table S3. Summary of statistics obtained for the various datasets used in this study**

Dataset	PhyML	MrBayes
Whole-genome (16,542 nt)		
In(Likelihood)	-57567	-57568
$\alpha$ parameter	0.20	1.60
Proportion of invariant	0	0.57
transition vs transversion	22.5	22.7
Protein Coding genes (amino acid) (3 797 aa)		
In(Likelihood)	-16851	-16872
$\alpha$ parameter	0.59	0.23
Proportion of invariant	0.39	
Protein coding genes (nucleotide) (11,385 nt)		
In(Likelihood)	-42107	-42110
$\alpha$ parameter	0.23	1.21
Proportion of invariant	0	0.29
Transition vs. transversion	28.3	28.0
tRNA (1,525 nt)		
In(Likelihood)	-3942	
$\alpha$ parameter	0.27	
Proportion of invariant	0.31	
Transition vs. transversion	20.3	
16S (1,590 nt)		
In(Likelihood)	-4325	
$\alpha$ parameter	0.26	
Proportion of invariant	0.32	
Transition vs. transversion	14.2	
12S (987 nt)		
In(Likelihood)	-2718	
$\alpha$ parameter	0.16	
Proportion of invariant	0	
Transition vs. transversion	14.3	
ATP6 gene (678 nt)		
In(Likelihood)	-2759	
$\alpha$ parameter	4.51	
Proportion of invariant	0.48	
Transition vs. transversion	24.9	
ATP8 (201 nt)		
In(Likelihood)	-851	
$\alpha$ parameter	0.99	
Proportion of invariant	0.23	
Transition vs. transversion	13.8	
Cox1 (1,542 nt)		
In(Likelihood)	-5471	
$\alpha$ parameter	0.67	
Proportion of invariant	0.44	
Transition vs. transversion	23.9	
Cox2 (681 nt)		
In(Likelihood)	-2406	
$\alpha$ parameter	14.2	
Proportion of invariant	0.62	
Transition vs. transversion	12.8	
Cox3 (783 nt)		
In(Likelihood)	-2789	
$\alpha$ parameter	6.00	
Proportion of invariant	0.64	
Transition vs. transversion	36.1	
Cytb (1,140 nt)		
In(Likelihood)	-4104	
$\alpha$ parameter	0.25	
Proportion of invariant	0	
Transition vs. transversion	16.1	
ND1 (954 nt)		
In(Likelihood)	-3357	
$\alpha$ parameter	0.27	
Proportion of invariant	0.08	
Transition vs. transversion	11.0	

Dataset	PhyML	MrBayes
ND2 (1,041 nt)		
In(Likelihood)	-3521	
$\alpha$ parameter	0.31	
Proportion of invariant	0.14	
Transition vs. transversion	26.2	
ND3 (345 nt)		
In(Likelihood)	-1324	
$\alpha$ shape parameter	0.54	
Proportion of invariant	0.33	
Transition vs. transversion	23.9	
ND4 (1,377 nt)		
In(Likelihood)	-5064	
$\alpha$ parameter	2.98	
Proportion of invariant	0.56	
Transition vs. transversion	34.5	
ND4L (294 nt)		
In(Likelihood)	-1150	
$\alpha$ parameter	0.44	
Proportion of invariant	0.19	
Transition vs. transversion	4.48	
ND5 (1,827 nt)		
In(Likelihood)	-7024	
$\alpha$ parameter	0.27	
Proportion of invariant	0	
Transition vs. transversion	22.8	
ND6 (525 nt)		
In(Likelihood)	-1980	
$\alpha$ parameter	3.62	
Proportion of invariant	0.52	
Transition vs transversion	2.04	

The table displays statistical results obtained from analyses with PhyML and MrBayes. Gene by gene analyses were only conducted with PhyML. The size of each dataset in nucleotides (nt) or amino acids (aa) is indicated.

## Other Supporting Information Files

[Fig. S1.](#)  
[Fig. S2.](#)