

SUPPLEMENTARY MATERIAL FOR:

5'-Tailed sequencing primers improve sequencing quality of PCR products

Jonas Binladen, M. Thomas P. Gilbert, Paula F. Campos, and Eske Willerslev

University of Copenhagen, Copenhagen, Denmark

BioTechniques 42:174-176 (February 2007)

Supplementary Table S1. Sample Details

Sample Type and No.	C14 Age	Location
Musk Ox (IEM 199-007)	Late Pleistocene	Taymir Peninsula
Musk Ox (IEM 202-0860)	Late Pleistocene	New Siberian Islands
Musk Ox (GIN 367/117)	Late Pleistocene	Aldan
Woolly Rhino (5-18438)	Pleistocene	Not Applicable
Woolly Rhino (9-10693)	Pleistocene	Nizhnyi Novgorod District, Irset River

Primer Details

The primers were designed to the desired length by adding a by adding a neutral 40-bp sequence (5'-AACT GACTAAACTAGGTGCCACGTC GTGAAAGTCTGACAA-3') and a poly(C) tail to the 5' end of the PCR primer (1,2). The neutral sequence is a randomly generated sequence not matching any sequence when a simple Basic Local Alignment Search Tool (BLAST) search is done in GenBank®. Primers for woolly rhino (WR15500F and WR15600R) were designed for this study, the musk ox dloop L1 and H1 primers are from MacPhee et al (3).

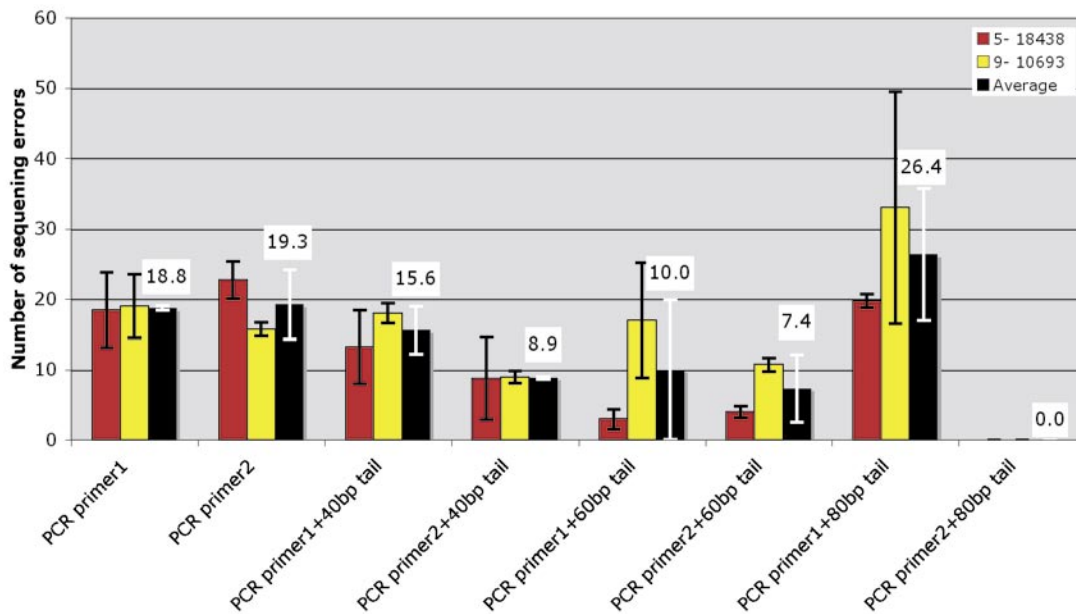
Benchmarks

Supplementary Table S2. Primer Details

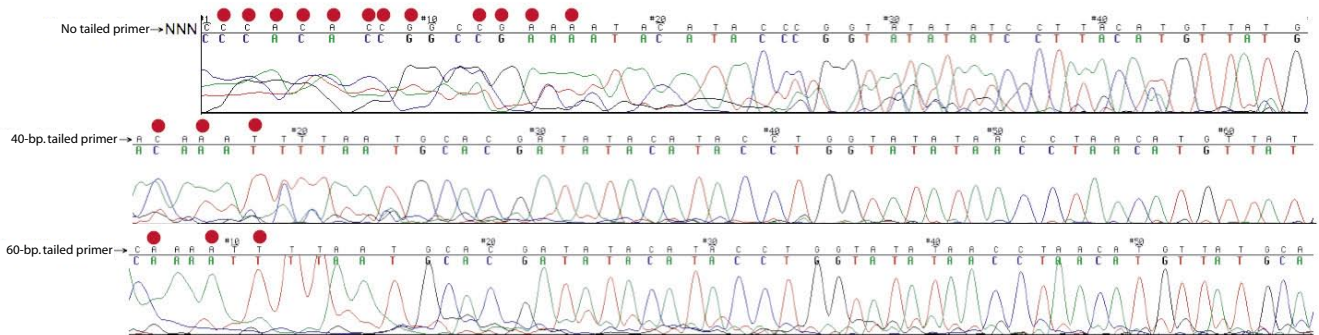
Name	Full Sequence ^a	Performance ^b
Wr15500F	5'-CACTCCCTTCTTAAACCA(C/G)AAG-3'	1
Wr15600R	5'-CATGCTTATATGCATGGGGC-3'	1
Wr15500F + 40-bp tail	5'-AACTGACTAAACTAGGTGCCACGTCGTGAAAGTCTGACAA CACTCCCTTCTTAAACC A(C/G)AAG-3'	1.2
Wr15600R + 40-bp tail	5'-AACTGACTAAACTAGGTGCCACGTCGTGAAAGTCTGACAA CATGCTTATATGCAT GGGGC-3'	2.2
Wr15500F + 60-bp tail	5'-CCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT GACAA CACTCCCTTCTTAAACCA(C/G)AAG-3'	1.9
Wr15600R + 60-bp tail	5'-CCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT GACAA CATGCTTATATGCATGGGGC-3'	2.6
Wr15500F + 80-bp tail	5'-CCCCCCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT GCCACGTCGTGAAAGTCTGACAA CACTCCCTTCTTAAACCA(C/G)AAG-3'	0.7
Wr15600R + 80-bp tail	5'-CCCCCCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT TAGGTGCCACGTCGTGAAAGTCTGACAA CATGCTTATATGCATGGGGC-3'	N.A.
dloop L1	5'-AAAGGATCCTAAACTACTCCCTGAATT-3'	1
dloop H1	5'-AAAGGATCCATCATGCGTTGTTGCGT-3'	1
dloop L1 + 40-bp tail	5'-AACTGACTAAACTAGGTGCCACGTCGTGAAAGTCTGACAAA AAGGATCCTAAAC TACTCCCTGAATT-3'	2.4
dloop H1 + 40-bp tail	5'-AACTGACTAAACTAGGTGCCACGTCGTGAAAGTCTGACAAA AAGGATCCATCAT GCGTTGTTGCGT-3'	4.6
dloop L1 + 60-bp tail	5'-CCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT GACAAA AAGGATCCTAAACTACTCCCTGAATT-3'	2.4
dloop H1 + 60-bp tail	5'-CCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT GACAAA AAGGATCCATCATGCGTTGTTGCGT-3'	2.6
dloop L1 + 80-bp tail	5'-CCCCCCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT CACGTCGTGAAAGTCTGACAAA AAGGATCCTAAACTACTCCCTGAATT-3'	1.9
dloop H1 + 80-bp tail	5'-CCCCCCCCCCCCCCCCCCCCCAACTGACTAAACTAGGTGCCACGTCGTGAAAGTCT CACGTCGTGAAAGTCTGACAAA AAGGATCCATCATGCGTTGTTGCGT-3'	0.5

^aThe specific part of the tailed primers is written in bold.
^bPerformance is calculated by dividing the average sequencing errors of nontailed PCR primer with the average sequencing errors of the tailed sequencing primer. N.A., not available due to very divergent sequence produced, that could not be aligned in Sequencher™ 3.1.1 using default settings.

Woolly Rhino Data



Supplementary Figure S1. Number of sequencing errors in a 113-bp region of the D-loop of two woolly rhino (*Coelodonta antiquitatis*) samples (5-18438 and 9-10693). Black columns and numbers are the average number of sequencing errors for each sequencing primer for the two samples with 3–4 sequence runs for each sample. Error bars indicate standard deviation. One primer (PCR primer2 + 80-bp tail) performed poorly and did not produce a sequences that could be aligned in Sequencher 3.1.1, thus no data is available for this primer.



Supplementary Figure S2. Examples of electropherograms showing the beginning of sequencing runs with nontailed primer and 40- and 60-bp tailed primers, respectively. The electropherograms are shown from the first position after the primer, except for the nontailed primer electropherogram in which the three first nucleotides were not detected (indicated by NNN). Red circles show where Sequencher 3.1.1 misreads the electropherograms compared to the known sequence (only for the first 20 nucleotides). The examples are from woolly rhino sample 5-18438 using WR15600R primers.

Positions and Types of Sequencing Errors

	1	11	21	31	41	51	61	71	81	91	101	111
IEM 202-0860 CONSENSUS	GTGGAGTA-G	GA-AAATTA-G	T-GTTATATT	ATTGAAA-GT	TTT-GGT-AG	ATTTGATACT	GGGAAGGC-T	CT-TAATGTT	TTTAGTGGA	TA-TT-AAATA	GA-A-G-GTG	TTA-GTGGT
IEM 202-0860 H1-no tail	.C.TGCG...	.G.T.....G	GGG..GG.A.A.A.A.A.A.A.A.
IEM 202-0860 H2-no tail	A.....A...	.G.....A.A.A.A.A.A.A.A.A.A.A.
IEM 202-0860 H3-no tailAT..	.G...A..A.A.A.A.A.A.A.A.A.A.
IEM 202-0860 H1-40bp tail	AG.....
IEM 202-0860 H2-40bp tail	.AA.....
IEM 202-0860 H3-40bp tail	.A.....
IEM 202-0860 H1-60bp tail	T.CTT.GG.T	TC.CCC.G.TC..CT..
IEM 202-0860 H2-60bp tail	.CTA..G..AAA..A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.A.
IEM 202-0860 H3-60bp tail	TCA.T..C..AGA.C..i..	..CTTC:.
IEM 202-0860 H1-80bp tail	..AT...G.T..
IEM 202-0860 H2-80bp tail	T..T:.....	..TT...T.
IEM 202-0860 H3-80bp taili.G.AT.T
IEM 202-0860 CONSENSUS	GTGGAGTA-G	GA-AAATTA-G	T-GTTATATT	ATTGAAA-GT	TTT-GGT-AG	ATTTGATACT	GGGAAGGC-T	CT-TAATGTT	TTTAGTGGA	TA-TT-AAATA	GA-A-G-GTG	TTA-GTGGT
IEM 202-0860 L1-no tail:.T.T.	C..CCCC.
IEM 202-0860 L2-no tailT:T.	C.CTC
IEM 202-0860 L3-no tail
IEM 202-0860 L1-40bp tail
IEM 202-0860 L2-40bp tail
IEM 202-0860 L3-40bp tail	.A.....
IEM 202-0860 L1-60bp tail
IEM 202-0860 L2-60bp tail
IEM 202-0860 L3-60bp tail
IEM 202-0860 L3-80bp tailG..G.T..A.

Supplementary Figure S3. Alignment showing the types and positions of the sequencing errors for each sequencing run. Primer sequences have been removed. The number following each sequence is the total number of sequencing errors. ":"s are insertions added when comparing each sequence with consensus sequence in Sequencher version 3.1.1, these are counted as sequencing errors. "-"s are used to align all sequences together and are not counted as sequencing errors.

	1	11	21	31	41	51	61	71	81	91	101	111	121
GIN 367/117 CONSENSUS	GTGGA-GT-A	-GGAA-A-TT	A-CT-GTTAT	ATTATTGAA-	AGTTTT-GGT	AGATTTC-AT	AC-TGGG-AA	GGC-TCT--T	AAATGTTTTT-	A-GTGG-TA-	TA-TT-A-AT	AGAAGG-TGT	TA GTGGT
GIN 367/117H1-no tail	GGG	55
GIN 367/117H2-no tail	.G.T...G.	.TGG...A.	11
GIN 367/117H3-no tail	..TG...G	.AT.G...:	G:	20
GIN 367/117H1-40bp tail	TG.....	C.....	C.....	04
GIN 367/117H2-40bp tail	TG.....	04
GIN 367/117H3-40bp tail	TA.....	TT.A.....	C.C.....	11
GIN 367/117H1-60bp tail	A.....	02
GIN 367/117H2-60bp tail	.G.A.....	A.....	T.T.....	07
GIN 367/117H3-60bp tail	A.....	03
GIN 367/117H1-80bp tail	AA.....	79
GIN 367/117H2-80bp tail	T.A.....	A.....	T.T.....	14
GIN 367/117H3-80bp tail	T.A.....	A.....	C.....	74
GIN 367/117 CONSENSUS	GTGGA-GT-A	-GGAA-A-TT	A-CT-GTTAT	ATTATTGAA-	AGTTTT-GGT	AGATTTC-AT	AC-TGGG-AA	GGC-TCT--T	AAATGTTTTT-	A-GTGG-TA-	TA-TT-A-AT	AGAAGG-TGT	TA GTGGT
GIN 367/117L1-no tail	21
GIN 367/117L2-no tail	12
GIN 367/117L3-no tail	14
GIN 367/117L1-40bp tail	06
GIN 367/117L2-40bp tail	08
GIN 367/117L3-40bp tail	01
GIN 367/117L1-60bp tail	21
GIN 367/117L2-60bp tail	17
GIN 367/117L3-60bp tail	12
GIN 367/117L1-80bp tail	13
GIN 367/117L2-80bp tail	.G.G...G	11
GIN 367/117L3-80bp tail	.G.G...G	12

Supplementary Figure S4. Alignment showing the types and positions of the sequencing errors for each sequencing run. Primer sequences have been removed. The number following each sequence is the total number of sequencing errors. “.”s are insertions added when comparing each sequence with consensus sequence in Sequencher version 3.1.1, these are counted as sequencing errors “.”s are used to align all sequences together and are not counted as sequencing errors.

1	11	21	31	41	51	61	71	81	91	101	111
IEM 199-007	CONSENSUS	ATGGA-G-T- A-GG-AAATT AG-TGTTATA TTATTGAAAG TTTTGGTAGA TTTGAT-ACT GGAAGGCT- C-T-TAATCT TTTTAGTGGT APATTAATAG AA-GG-TGTT A-G-TGCT 00									
IEM 199-007H1-no tail	.G.....GC .T..GT..::	..GG..i.....A..A
IEM 199-007H2-no tail	GA.....G. G.AT.GG.AGGG..i.....A..
IEM 199-007H3-no tail	.G.....G. C..A.T.GCC .TA.....AGG:..A..:T:GGGGGGG
IEM 199-007H1-40bp tail	GG.....
IEM 199-007H2-40bp tail	GT.....
IEM 199-007H3-40bp tail	GA.....
IEM 199-007H1-60bp tail	T...G.....G.....
IEM 199-007H2-60bp tail	T...G.....G.....T...T.....
IEM 199-007H3-60bp tail	T...G.....A.....
IEM 199-007H1-80bp tail	.G..A..G.....A.....C.....G.....A.....G..A..A..A.....C..G.....
IEM 199-007H2-80bp tail	T...G.....G.....T.....T.....T.....A.....G.....
IEM 199-007H3-80bp tail	CCCCC.C.C. G.....CCCCA C.C.CC.A. AC.....T.....A.....
IEM 199-007	CONSENSUS	ATGGA-G-T- A-GG-AAATT AG-TGTTATA TTATTGAAAG TTTTGGTAGA TTTGAT-ACT GGAAGGCT- C-T-TAATCT TTTTAGTGGT APATTAATAG AA-GG-TGTT A-G-TGCT 00									
IEM 199-007L1-no tailGG.....G.....T.C 08
IEM 199-007L2-no tailG.....C...AC.C.....GAG...G.CC T.A.A.GG 17	
IEM 199-007L3-no tailT.....T.....C.C.CC.....G...G.....	TT..G.: 15
IEM 199-007L1-40bp tailG...G.....	..C.:C 04
IEM 199-007L2-40bp tailA.....	..C 02
IEM 199-007L3-40bp tailA 01
IEM 199-007L1-60bp tailT..... 01
IEM 199-007L3-60bp tailT..... 02
IEM 199-007L1-80bp tailA..... 02
IEM 199-007L2-80bp tailG.....A...G... ..A.G.G. 06
IEM 199-007L3-80bp tailT.T.....A.....T.....TC 07

Supplementary Figure S5. Alignment showing the types and positions of the sequencing errors for each sequencing run. Primer sequences have been removed. The number following each sequence is the total number of sequencing errors. “.”s are insertions added when comparing each sequence with consensus sequence in Sequencher version 3.1.1, these are counted as sequencing errors. “..”s are used to align all sequences together and are not counted as sequencing errors.

	1	11	21	31	41	51	61	71	81	91	101	111	121
5-18438 CONSENSUS	AAACAATTTA	A-TG-CACG-	A-TATACATA	CCTGGTATAT	AACTTAACAT	GTTATGCACG	ATAFACA-TA	AGGGGTGAC	AGATATTATT	GATPAC-TG--	AGG-T-GT-C	A-CA-TGGT-	GGGATGT
5-18438-15500F1-no tail	..G.:	..A.	..A.	..A.	..A.	..G.	..G.	..G.	..G.	..T.	..T.	..C.	..:
5-18438-15500F2-no tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F3-no tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F4-no tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F1-40bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F2-40bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F3-40bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F4-40bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F1-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F2-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F1-60bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F2-60bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F3-60bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F4-60bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F1-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F2-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F3-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438-15500F4-80bp tail	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:	..:
5-18438 CONSENSUS	AAACAATTTA	A-TG-CACG-	A-TATACATA	CCTGGTATAT	AACTTAACAT	GTTATGCACG	ATAFACA-TA	AGGGGTGAC	AGATATTATT	GATPAC-TG--	AGG-T-GT-C	A-CA-TGGT-	GGGATGT
5-18438-15600R1-no tail	...GTCC.C	G.G.	...C.A.A.	...C.A.G.	...G.T.C	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R2-no tail	...GC.T	G.AA.	...G	...CA.A	...AT.	...TG.	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R3-no tail	...:	...A.	...GGG.	...AA.	...A.	...TC.	...A.G.	...:	...:	...:	...:	...:	...:
5-18438-15600R4-no tail	...CCACAC	C.G.	...CGA.	...A.	...:	...C.	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R1-40bp tail	C.TATC.	A.	...C.	...G.	...A	...T.	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R2-40bp tail	.C.T.T.	...A.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R3-40bp tail	G.G.G.T.A.	...A.A.	...:	...T.	...T.	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R4-40bp tail	.C.A.T.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R1-60bp tail	...ATTC.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R2-60bp tail	...ATTC.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R3-60bp tail	C..ATTC.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:
5-18438-15600R4-60bp tail	C..A.T.	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:	...:

Supplementary Figure S6. Alignment showing the types and positions of the sequencing errors for each sequencing run. Primer sequences have been removed. The number following each sequence is the total number of sequencing errors. ":"s are insertions added when comparing each sequence with consensus sequence in Sequencher version 3.1.1, these are counted as sequencing errors. "."s are used to align all sequences together and are not counted as sequencing errors.

1	11	21	31	41	51	61	71	81	91	101	111	121	
9-10693_CONSENSUS	AAACAATTTA	-AT-GCACGA	-TATACATAC	CTGGTATATA	ATCTAGCATG	TTATGTA-CG	A-CGTACATA	A-GCGGGTGA	CAGATATTAC	-TGATAC-TG	AG-GT-G-T	C-AC-ATGG-	-TGAGATGT
9-10693_15500F1-no tail
9-10693_15500F2-no tail
9-10693_15500F3-no tail
9-10693_15500F4-no tail
9-10693_15500F1-40bp tail
9-10693_15500F2-40bp tail
9-10693_15500F3-40bp tail
9-10693_15500F4-40bp tail
9-10693_15500F1-60bp tail
9-10693_15500F2-60bp tail
9-10693_15500F3-60bp tail
9-10693_15500F4-60bp tail
9-10693_15500F2-80bp tail
9-10693_CONSENSUS	AAACAATTTA	-AT-GCACGA	-TATACATAC	CTGGTATATA	ATCTAGCATG	TTATGTA-CG	A-CGTACATA	A-GCGGGTGA	CAGATATTAC	-TGATAC-TG	AG-GT-G-T	C-AC-ATGG-	-TGAGATGT
9-10693_15600R1-no tail	TT.TG.GCA.	.T...T.GG
9-10693_15600R2-no tail	TT.GG..T	G.C.T.T.T.	.GC.G:
9-10693_15600R3-no tail	.CCTTGG.A.	.G...TT.TC	.TGG.....
9-10693_15600R4-no tail	CCT.GG.A:	.G...AG.TC	.TGG.....
9-10693_15600R1-40bp tail	CG.AGG...AGG
9-10693_15600R2-40bp tail	CG.AGG..AG.GG
9-10693_15600R3-40bp tail	CGTTGG...AGG
9-10693_15600R4-40bp tail	TCGTTG...AT..T..GG
9-10693_15600R1-60bp tail	G..GGT.A.G.G	.AG.....
9-10693_15600R2-60bp tail	CG.AGG...T	.TG...G.G	.G.....
9-10693_15600R3-60bp tail	G..GTT...TG.G	.AG.....
9-10693_15600R4-60bp tail	CG.AGG...A	.T...G.A.G	G.G.....

Supplementary Figure S7. Alignment showing the types and positions of the sequencing errors for each sequencing run. Primer sequences have been removed. The number following each sequence is the total number of sequencing errors. "."s are insertions added when comparing each sequence with consensus sequence in Sequencher version 3.1.1, these are counted as sequencing errors. "-"s are used to align all sequences together and are not counted as sequencing errors.

REFERENCES

1. **Lindblad-Toh, K., E. Winchester, M.J. Daly, D.G. Wang, J.N. Hirschhorn, J.P. Lavolette, K. Ardlie, D.E. Reich, et al.** 2000. Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. *Nat Genet.* 24:381-386.
2. **Sanchez, J.J., C. Borsting, C. Hallenberg, A. Buchard, A. Hernandez, and N. Morling.** 2003. Multiplex PCR and minisequencing of SNPs—a model with 35 Y chromosome SNPs. *Forensic Sci Int.* 137:74-84.
3. **MacPhee, R.D.E., A.N. Tikhonov, D. Mol, and A.D. Greenwood.** 2005. Late quaternary range collapse and loss of genetic diversity in muskox (*Ovibos*). *BMC Evolutionary Biology* 5:49