



Supplementary

Primer design for extremely damaged DNA specimens of Asian rhinoceros species

Yada Katanyuphan, Wunrada Surat*

Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

Supplementary Table

Table S1 Profiles of complete D-loop sequences of Asian rhinoceros samples used in this study

Number	Species	Accession number	Location	Length of D-loop (bp)	Position of D-loop in mtDNA	Reference
1	<i>Dicerorhinus sumatrensis</i>	FJ905816	Cincinnati Zoo, USA	1014	15453–16466	Willerslev et al., 2009
2		KY117545	Sumatra, Indonesia	1015	15454–16468	Salleh et al., 2017
3		MF066629	Cincinnati Zoo, USA	1013	15453–16465	Steiner et al., 2018
4		MF066630	Sabah–Borneo	1013	15453–16465	Steiner et al., 2018
5		MF066631	Sabah–Borneo	1013	15453–16465	Steiner et al., 2018
6		MF066632	Sabah–Borneo	1013	15453–16465	Steiner et al., 2018
7		MF066633	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
8		MF066634	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
9		MF066635	Malaysia	1013	15453–16465	Steiner et al., 2018
10		MF066636	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
11		MF066637	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
12		MF066638	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
13		MF066639	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
14		MF066640	Sumatra–Indonesia	1013	15453–16465	Steiner et al., 2018
15		MF066641	Malay Peninsula	1013	15453–16465	Steiner et al., 2018
16		MF066642	Malay Peninsula	1013	15453–16465	Steiner et al., 2018
17		MF066643	Malay Peninsula	1013	15453–16465	Steiner et al., 2018
18		MK909153	Malaysia	1089	15455–16543	Margaryan et al., 2020

* Corresponding author.

E-mail address: fsciwrds@ku.ac.th (W. Surat)

Table S1 Continued

Number	Species	Accession number	Location	Length of D-loop (bp)	Position of D-loop in mtDNA	Reference
19	<i>Rhinoceros unicornis</i>	NC_001779	–	1376	15454–16829	Xu et al., 1996
20		MZ736708	India	1375	15454–16828	Ghosh et al., 2022
21		MZ736707	India	1375	15454–16828	Ghosh et al., 2022
22		MZ736706	India	1375	15454–16828	Ghosh et al., 2022
23		MZ736705	India	1375	15454–16828	Ghosh et al., 2022
24		MZ736703	India	1375	15454–16828	Ghosh et al., 2022
25		MZ736702	India	1375	15454–16828	Ghosh et al., 2022
26		MZ736701	India	1375	15454–16828	Ghosh et al., 2022
27		MZ736700	India	1375	15454–16828	Ghosh et al., 2022
28		MZ736699	India	1375	15454–16828	Ghosh et al., 2022
29		MZ736698	India	1375	15454–16828	Ghosh et al., 2022
30		MZ736697	India	1375	15454–16828	Ghosh et al., 2022
31		MZ736696	India	1375	15454–16828	Ghosh et al., 2022
32		MZ736695	India	1375	15454–16828	Ghosh et al., 2022
33		MZ736694	India	1375	15454–16828	Ghosh et al., 2022
34		MZ736693	India	1375	15454–16828	Ghosh et al., 2022
35	<i>Rhinoceros sondaicus</i>	FJ905815	–	968	15450–16417	Willerslev et al., 2009
36		KY117574	Java, Indonesia	962	15446–16407	Salleh et al., 2017
37		MK909142	Culcutta	953	15448–16400	Margaryan et al., 2020
38		MK909146	Java, Indonesia	967	15447–16413	Margaryan et al., 2020
39		MK909148	Java, Indonesia	970	15448–16417	Margaryan et al., 2022

Accession number in Genbank

References

- Ghosh, T., Kumar, S., Sharma, K., Kakati, P., Sharma, A., Mondol, S. 2022. Consideration of genetic variation and evolutionary history in future conservation of Indian one-horned rhinoceros (*Rhinoceros unicornis*). BMC Ecol. Evol. 22: 92. doi.org/10.1186/s12862-022-02045-2
- Margaryan, A., Sinding, M.S., Liu, S., et al. 2020. Recent mitochondrial lineage extinction in the critically endangered Javan rhinoceros. Zool. J. Linn. Soc. 190: 372–383. doi.org/10.1093/zoolinnean/zlaa004
- Salleh, F.M., Ramos-Madriral, J., Peñaloza, F., et al. 2017. An expanded mammal mitogenome dataset from Southeast Asia. GigaScience 6: gix053. doi.org/10.1093/gigascience/gix053
- Steiner, C.C., Houck, M.L., Ryder, O.A. 2018. Genetic variation of complete mitochondrial genome sequences of the Sumatran rhinoceros (*Dicerorhinus sumatrensis*). Conserv. Genet. 19: 397–408. doi.org/10.1007/s10592-017-1011-1
- Willerslev, E., Gilbert, M.T.P., Binladen, J., et al. 2009. Analysis of complete mitochondrial genomes from extinct and extant rhinoceroses reveals lack of phylogenetic resolution. BMC Evol. Biol. 9: 95. doi.org/10.1186/1471-2148-9-
- Xu, X., Janke, A., Arnason, U. 1996. The complete mitochondrial DNA sequence of the Greater Indian rhinoceros, *Rhinoceros unicornis*, and the phylogenetic relationship among Carnivora, Perissodactyla, and Artiodactyla (+ Cetacea). Mol. Biol. Evol. 13: 1167–1173. doi.org/10.1093/oxfordjournals.molbev.a025681

Table S2 Polymorphic sites within sequences produced by Primer I in three Asian rhino species (Sumatran, Indian and Javan)

Species/ Haplotype/ Number of samples	Variable site																																													
	17	19	20	21	23	26	27	29	32	36	38	43	44	45	46	47	48	56	57	62	63	64	65	67	68	74	77	78	79	84	88	90	91	92	94	98	99	100	101	103	105	106	117			
S/H1/3	A	A	A	C	A	A	A	T	A	A	A	T	G	A	T	T	T	G	A	A	G	A	T	C	T	C	T	A	A	C	A	C	A	T	A	A	A	A	A	G	T	A	G	T	T	
S/H2/2	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	
S/H3/3	.	G	G	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	
S/H4/1	.	G	G	.	G	.	.	.	G	G	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	
S/H5/3	.	.	G	.	G	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	
S/H6/4	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
S/H7/1	.	G	G	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	
S/H8/1	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I/H1/6	T	T	.	T	.	G	.	C	.	G	.	A	G	G	.	C	T	G	T	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	.	G	C	G	-	.	.	.	.	.	
I/H2/4	T	C	.	T	.	G	.	C	.	G	.	A	G	G	.	C	T	G	T	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	T	G	-	.	.	.	.	.	
I/H3/3	T	T	.	T	.	G	.	C	.	G	.	A	G	G	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	C	G	-	.	.	.	.	.	.	
I/H4/4	T	T	.	T	.	G	.	C	.	G	.	A	G	G	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	C	G	-	.	.	.	.	.	C	
J/H1/4	T	T	.	T	.	.	G	A	.	G	C	A	G	G	.	.	G	T	.	A	.	T	C	.	G	T	.	.	G	T	.	G	T	T	.	G	T	.	G	T	.	-	A	C	.	
J/H2/1	T	T	.	T	.	.	G	A	.	G	.	A	G	G	.	.	G	T	.	A	.	T	C	.	G	T	.	.	G	T	.	G	T	T	.	G	T	.	G	T	.	-	A	C	.	

Dot (.) = identical sequence to reference sequence in first line; S = Sumatran rhinoceros (*Dicerorhinus sumatrensis*); I = Indian or Greater one-horned rhinoceros (*Rhinoceros unicornis*); J = Javan rhinoceros (*R. sondaicus*)

Table S3 Polymorphic sites within sequences produced by Primer II in three Asian rhino species (Sumatran, Indian and Javan)

Species/ Haplotype/ Number of samples	Variable site																																									
	1	2	10	11	16	17	18	19	21	22	28	31	32	33	38	42	44	45	46	48	52	53	54	55	57	59	60	71	78	83	84	85	86	88	89	93	98	109				
S/H1/8	T	T	G	A	A	G	A	G	A	T	C	T	A	A	C	A	C	A	T	A	A	A	G	T	A	G	T	A	T	T	T	A	T	A	T	A	A	A	C			
S/H2/2	C	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	G	.	.			
S/H3/3	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.			
S/H4/1	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.			
S/H5/3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	G	.	.			
S/H6/1	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	G	.	.			
I/H1/6	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	.	G	C	G	-	.	.	.	.	.	A	C	A	G	C	.	.	.	T			
I/H2/4	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	T	G	-	.	.	.	.	.	.	A	C	A	G	C	.	.	.	T			
I/H3/3	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	C	G	-	.	.	.	.	.	.	A	C	A	G	C	.	.	G	T			
I/H4/4	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	C	G	-	.	.	.	.	C	.	A	C	A	G	C	.	.	.	T			
I/H1/5	.	.	.	G	.	A	.	T	C	.	G	T	.	.	G	T	T	.	G	T	.	G	T	.	-	A	C	.	.	A	C	A	T	.	.	G	.	.	.			

Dot (.) = identical sequence to reference sequence in first line; S = Sumatran rhinoceros (*Dicerorhinus sumatrensis*); I = Indian or Greater one-horned rhinoceros (*Rhinoceros unicornis*); J = Javan rhinoceros (*R. sondaicus*)

Table S3 Polymorphic sites within sequences produced by Primer II in three Asian rhino species (Sumatran, Indian and Javan)

Species/ Haplotype/ Number of samples	Variable site																																													
	1	2	10	11	16	17	18	19	21	22	28	31	32	33	38	42	44	45	46	48	52	53	54	55	57	59	60	71	78	83	84	85	86	88	89	93	98	109								
S/H1/8	T	T	G	A	A	G	G	A	T	C	T	A	A	C	A	C	A	T	A	A	A	A	G	T	A	G	T	T	T	T	T	A	T	A	T	A	A	A	C							
S/H2/2	C	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	C	.	.	.	G	.	.							
S/H3/3	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.							
S/H4/1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.							
S/H5/3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	C	.	C	.	.	.	G	.	.								
S/H6/1	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	G	.	.								
I/H1/6	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	.	G	C	G	-	.	.	.	.	.	A	C	A	G	C	.	.	T								
I/H2/4	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	T	G	-	.	.	.	.	.	A	C	A	G	C	.	.	T								
I/H3/3	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	C	G	-	.	.	.	.	.	A	C	A	G	C	.	.	G	T							
I/H4/4	.	C	A	G	.	A	.	C	.	T	C	T	G	T	.	.	T	C	G	C	G	G	C	G	-	.	.	C	.	A	C	A	G	C	.	.	T									
J/H1/5	.	.	.	G	.	A	.	T	C	.	G	T	.	.	G	T	T	.	G	T	.	G	T	-	A	C	.	.	A	T	.	.	.	.	G	.	.	.								

Dot (.) = identical sequence to reference sequence in first line; S = Sumatran rhinoceros (*Dicerorhinus sumatrensis*); I = Indian or Greater one-horned rhinoceros (*Rhinoceros unicornis*); J = Javan rhinoceros (*R. sondaicus*)

Table S4 Polymorphic sites within sequences produced by Primer III in three Asian rhino species (Sumatran, Indian and Javan)

Species/ Haplotype/ Number of samples	Variable site																			
	2	13	30	33	39	42	48	55	56	59	60	61	62	64	75	101	102	106	107	113
S/H1/3	A	C	T	A	T	A	T	T	-	A	T	G	-	G	C	T	C	T	C	C
S/H2/2	.	.	.	.	.	.	.	C	-	.	.	.	.	.	.	.	.	.	T	.
S/H3/4	.	.	.	C	.	.	.	.	-	.	.	.	.	.	.	.	.	.	T	.
S/H4/1	.	.	.	C	.	C	.	.	-	.	.	.	.	.	.	.	.	.	T	.
S/H5/6	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	T	.
S/H6/2	.	.	.	.	G	.	.	.	-	.	.	.	.	.	.	.	.	.	T	.
I/H1/7	.	T	C	T	.	G	.	.	A	C	C	.	T	C	G	C	T	C	.	.
I/H2/7	.	T	C	T	.	.	.	.	A	C	C	.	T	C	G	C	T	C	.	.
I/H3/3	G	T	C	T	.	.	.	.	A	C	C	.	T	C	G	C	T	C	.	.
J/H1/4	.	.	.	T	.	.	.	.	A	C	C	A	C	C	G	C	T	C	T	T
J/H2/1	.	.	C	T	.	.	.	.	A	C	C	A	C	C	G	C	T	C	T	T

Dot (.) = identical sequence to reference sequence in first line; S = Sumatran rhinoceros (*Dicerorhinus sumatrensis*); I = Indian or Greater one-horned rhinoceros (*Rhinoceros unicornis*); J = Javan rhinoceros (*R. sondaicus*).