

# **The kinship of two 12<sup>th</sup> Dynasty mummies revealed by ancient DNA sequencing**

Konstantina Drosou, Campbell Price and Terence A. Brown

## **SUPPLEMENTARY INFORMATION**

**Supplementary text.** Authentication of Y chromosome SNPs.

**Supplementary Table 1.** Y chromosome SNPs.

**Supplementary Fig. 1.** Median joining network showing the relationship of the mtDNA haplotypes of Nakht-Ankh (NAII) and Khnum-Nakht (KNIII) to modern members of the M haplogroup.

**Supplementary Fig. 2.** Fragmentation and misincorporation patterns for the sequence read datasets.

## Supplementary text

### Authentication of Y chromosome SNPs

We assessed the authenticity of Y-chromosome SNPs by the following methods. First, comparisons were made between the shotgun and enriched datasets and between the two enrichment rounds for NAII. We discarded variants that exhibited zero coverage in the second enrichment round and also excluded variants that showed higher coverage in the first capture round (e.g. positions 8,461,171, 16,280,355; Supplementary Table 1), as this pattern is not consistent with the target enrichment principle. Second, variants present in parts of the Y chromosome that were not covered by the capture baits (e.g. positions 8,496,955, 8,606,268; Supplementary Table 1) were treated as contaminants. This meant that the majority of the variants detected in the repeated regions of the NRY (Francalacci et al., 2015), which were not included in the capture design, were discarded. Following the same rationale, we treated variants present in the heterochromatic region as contaminants. Third, considering the multitude of Y-chromosome structural variants (Skaletsky et al., 2003), we discarded SNPs that fell within repeated regions (e.g. positions 17,319,993, 17,319,997, 18,366,183 and 18,366,196; Supplementary Table 1) and segmental duplicated regions (e.g. positions 9,105,173 and 9,397,228; Supplementary Table 1). Fourth, potential SNPs were tested against the UCSC database and their phylogenetic values assessed using the ISOGG browser. The recovered variants were absent from the databases apart from the ones found in the heterochromatic region. As the database has been built using modern DNA variants, we conclude that the latter SNPs are most likely a product of contamination, whereas the former are more likely to be authentic ancient SNPs. Finally, because of the greater misincorporation frequency close to the ends of aDNA fragments (Briggs et

al., 2007), potential SNPs that were located within 3-4 bp of the end of a read (e.g. positions 8,450,681, 16,332,091; Supplementary Table 1), and were not confirmed from the central region of a second read, were discarded.

## References

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## Supplementary Table 1

Y chromosome SNPs. SNPs coloured pink did not pass one or more of the quality filters and were discarded.

Position	Nakht-Ankh				Khnum-Nakht		RefSNP ID	Present in capture design?	Region
	SNP	1 <sup>st</sup> capture coverage	2 <sup>nd</sup> capture coverage	Shotgun coverage	SNP	Double capture coverage			
2,715,515	C→T	4	4	–	–	–	–	✓	X-degenerate
2,715,521	C→T	4	4	–	–	–	–	✓	X-degenerate
2,722,774	–	–	–	–	G→A	3	–	x	X-degenerate
2,734,431	G→A	3	4	–	–	–	–	✓	X-degenerate
3,713,584	G→A	3	5	–	–	–	–	✓	X-transposed
3,713,756	C→T	3	7	–	–	–	–	✓	X-transposed
3,713,809	G→A	3	7	–	–	–	–	✓	X-transposed
6,963,148	C→T	3	6	–	–	–	–	✓	X-degenerate
6,973,749	C→T	3	5	–	–	–	–	✓	X-degenerate
6,973,751	C→T	3	5	–	–	–	–	✓	X-degenerate
6,973,810	G→A	3	4	–	–	–	–	✓	X-degenerate
8,234,080	G→A	1	5	–	–	–	–	✓	ampliconic
8,234,092	G→A	1	5	–	–	–	–	✓	ampliconic
8,450,681	C→T	3	8	–	–	–	–	✓	ampliconic
8,450,740	G→A	3	6	–	G	3	–	✓	ampliconic
8,450,741	G	2	6	–	G→A	3	–	✓	ampliconic
8,455,255	G→A	1	3	–	G	2	–	✓	ampliconic
8,455,257	G→A	1	3	–	G	2	–	✓	ampliconic
8,461,171	C→T	3	2	–	–	–	–	✓	ampliconic
8,476,675	G→A	1	4	–	–	–	–	✓	ampliconic
8,477,307	C→T	8	17	–	–	–	–	✓	ampliconic
8,477,413	G→A	8	17	–	–	–	–	✓	ampliconic
8,478,647	–	–	–	–	G→A	3	–	✓	ampliconic
8,496,955	C→T	3	6	–	–	–	–	x	ampliconic
8,496,967	C→T	3	6	–	–	–	–	x	ampliconic
8,497,017	C→T	3	6	–	–	–	–	x	ampliconic
8,497,018	C→T	3	6	–	–	–	–	x	ampliconic
8,526,417	G→A	3	7	–	–	–	–	✓	ampliconic
8,536,676	G→A	3	4	–	–	–	–	✓	ampliconic
8,536,693	G→A	3	4	–	–	–	–	✓	ampliconic
8,540,634	G→A	1	3	–	–	–	–	x	ampliconic
8,540,906	–	–	–	–	C→T	3	–	x	ampliconic
8,548,074	G→A	5	3	–	–	–	–	✓	ampliconic
8,570,618	G→A	16	32	–	–	–	–	x	ampliconic
8,572,472	C→T	3	4	–	–	–	–	x	ampliconic
8,576,977	G→A	2	4	–	–	–	–	x	ampliconic
8,606,268	C→T	3	5	–	–	–	–	x	ampliconic
8,636,343	G→A	1	4	–	–	–	–	✓	ampliconic
8,636,347	G→A	1	4	–	–	–	–	✓	ampliconic
8,636,356	G→A	1	4	–	–	–	–	✓	ampliconic
8,656,504	–	–	–	–	C→T	11	–	✓	ampliconic
8,656,564	–	–	–	–	G→A	9	–	✓	ampliconic
9,105,173	C→T	2	4	–	–	–	–	x	ampliconic
9,397,228	G→A	4	4	–	–	–	–	x	ampliconic
13,137,85	–	–	–	5	–	–	rs75824801	x	heterochromatic
7									
13,667,35	G→A	3	0	4	–	–	rs75168767	x	heterochromatic
3									
13,667,35	A→G	3	0	4	–	–	rs76121765	x	heterochromatic
8									

13,667,43	G→A	3	0	2	–	–	rs113971352	x	heterochromatic
6									
13,667,44	A→G	3	0	2	–	–	rs112140261	x	heterochromatic
1									
13,667,44	A→G	3	0	1	–	–	rs202118775	x	heterochromatic
9									
13,682,94	A→G	5	0	1	–	–	rs62602632	x	heterochromatic
0									
13,682,96	G→A	5	0	1	–	–	rs62602634	x	heterochromatic
3									
13,694,98	T→A	4	0	4	–	–	rs62603802	x	heterochromatic
3									
13,801,08	T→A	4	1	4	–	–	rs62602333	x	heterochromatic
2									
13,801,08	G→T	4	1	4	–	–	rs62602334	x	heterochromatic
4									
13,802,26	G→A	6	2	4	–	–	rs62602337	x	heterochromatic
5									
13,802,28	A→G	14	4	8	–	–	rs62602338	x	heterochromatic
7									
13,802,30	G→A	13	3	8	–	–	rs62602339	x	heterochromatic
2									
13,851,65	A→T	3	0	3	–	–	rs62617861	x	heterochromatic
5									
13,851,66	A→T	4	0	3	–	–	rs62617862	x	heterochromatic
8									
15,045,86	G→A	5	13	–	–	–	–	✓	X-degenerate
1									
15,054,14	C→T	2	3	–	–	–	–	✓	X-degenerate
8									
15,054,19	G→A	2	3	–	–	–	–	✓	X-degenerate
8									
15,085,73	–	–	–	–	C→T	3	–	✓	X-degenerate
3									
15,321,37	G→A	4	0	–	–	–	–	x	X-degenerate
2									
15,447,68	–	–	–	–	G→A	5	–	x	X-degenerate
6									
15,447,69	–	–	–	–	G→A	5	–	x	X-degenerate
5									
15,447,69	–	–	–	–	G→A	5	–	x	X-degenerate
8									
15,978,44	C→T	3	4	–	–	–	–	✓	X-degenerate
9									
16,205,00	C→T	3	8	–	–	–	–	✓	X-degenerate
8									
16,268,80	G→A	7	11	–	–	–	–	✓	X-degenerate
5									
16,268,80	G→A	7	11	–	–	–	–	✓	X-degenerate
7									
16,268,86	G→A	4	8	–	–	–	–	✓	X-degenerate
3									
16,268,86	G→A	4	8	–	–	–	–	✓	X-degenerate

7											
16,280,35	C→T	5	4	-	-	-	-	✓	X-degenerate		
5											
16,280,35	C→T	5	4	-	-	-	-	✓	X-degenerate		
9											
16,331,50	G→A	3	5	-	-	-	-	✓	X-degenerate		
8											
16,332,09	G→A	6	5	-	-	-	-	✓	X-degenerate		
1											
16,344,14	C→T	1	3	-	-	-	-	✓	X-degenerate		
2											
16,344,15	C→T	1	3	-	-	-	-	✓	X-degenerate		
1											
16,344,15	C→T	1	3	-	-	-	-	✓	X-degenerate		
4											
16,419,511	G→A	2	3	-	-	-	-	✓	X-degenerate		
16,560,26	-	-	-	-	G→A	4	-	✓	X-degenerate		
9											
16,560,27	-	-	-	-	G→A	4	-	✓	X-degenerate		
2											
16,561,10	-	-	-	-	G→A	4	-	✓	X-degenerate		
6											
16,751,95	G→A	5	4	-	-	-	-	✓	X-degenerate		
7											
16,751,96	G→A	5	4	-	-	-	-	✓	X-degenerate		
0											
16,911,323	C→T	3	10	-	-	-	-	✓	X-degenerate		
16,912,03	C→A	3	4	-	-	-	-	✓	X-degenerate		
2											
16,912,05	G→A	3	4	-	-	-	-	✓	X-degenerate		
4											
16,955,21	G→A	1	3	-	-	-	-	✓	X-degenerate		
4											
17,113,120	G→A	15	50	-	-	-	-	✓	X-degenerate		
17,113,383	-	-	-	-	C→T	3	-	✓	X-degenerate		
17,148,58	G→A	6	7	-	-	-	-	✓	X-degenerate		
6											
17,173,39	-	-	-	-	G→A	3	rs376481998	✓	X-degenerate		
1											
17,184,00	G→A	4	1	-	-	-	-	✓	X-degenerate		
4											
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6											
17,184,99	G→A	1	5	-	-	-	-	✓	X-degenerate		
8											
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3											
17,319,99	G→A	4	0	-	-	-	-	x	X-degenerate		
7											
17,397,67	C→T	2	4	-	-	-	-	x	X-degenerate		

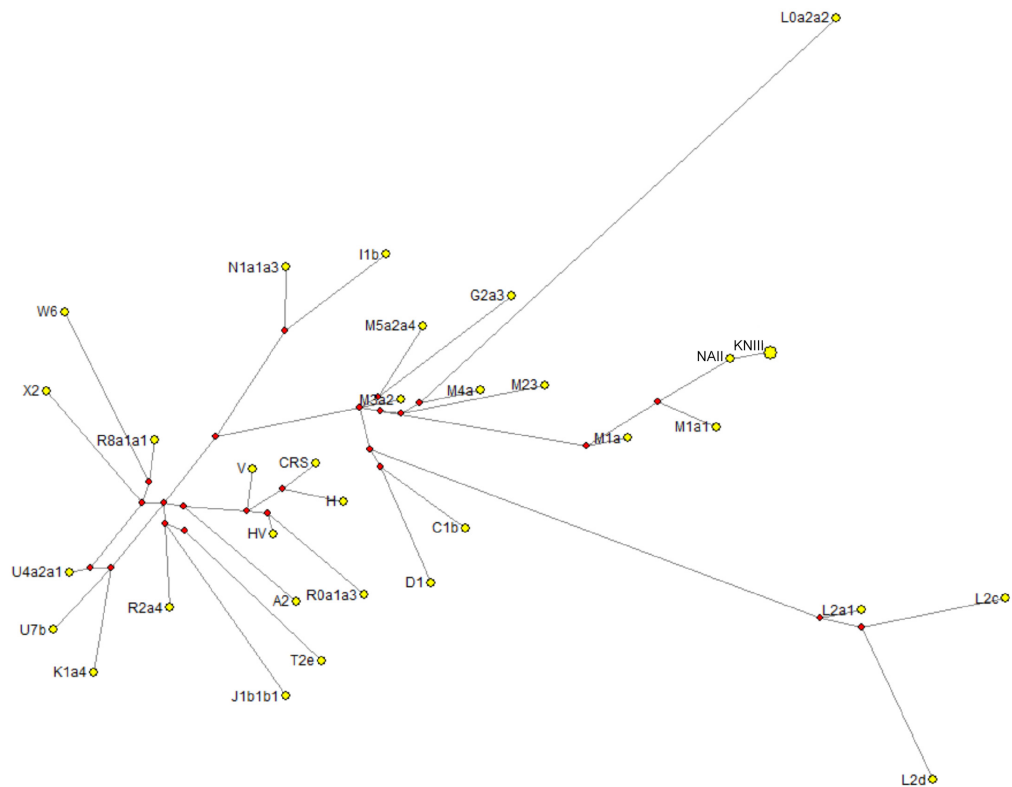
7											
17,614,12	C→T	4	5	-	-	-	-	✓	X-degenerate		
5											
17,614,16	G→A	2	4	-	-	-	-	✓	X-degenerate		
3											
17,761,35	C→T	3	6	-	-	-	-	✓	X-degenerate		
7											
17,907,58	G→A	3	9	-	-	-	-	✓	X-degenerate		
3											
18,084,25	G→A	3	5	-	-	-	-	✓	X-degenerate		
3											
18,203,37	G→A	1	4	-	-	-	-	✓	X-degenerate		
4											
18,366,18	G→A	3	1	-	-	-	-	x	ampliconic		
3											
18,366,19	G→A	3	1	-	-	-	-	x	ampliconic		
6											
18,551,56	-	-	-	-	G→A	3	-	✓	X-degenerate		
1											
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0											
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3											
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3											
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1											
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1											
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5											
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8											
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0											
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2											
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5											
18,882,50	C→T	2	11	-	-	-	-	✓	X-degenerate		
1											
18,885,46	C→T	2	5	-	-	-	-	✓	X-degenerate		
4											
18,910,22	C→T	2	7	-	-	-	-	✓	X-degenerate		
6											
19,383,65	G→A	3	5	-	-	-	-	✓	X-degenerate		
6											
19,437,311	-	-	-	-	G→A	4	-	x	X-degenerate		
19,442,86	-	-	-	-	G→A	4	-	✓	X-degenerate		
3											
19,468,91	G→A	2	4	-	-	-	-	✓	X-degenerate		
8											
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3											

20,836,56	C→T	2	6	-	-	-	-	✓	ampliconic
2									
20,836,59	G→A	2	6	-	-	-	-	✓	ampliconic
7									
21,570,00	G→A	4	-	-	-	-	-	x	X-degenerate
5									
21,717,54	C→T	1	4	-	-	-	-	✓	X-degenerate
8									
21,717,54	C→T	1	4	-	-	-	-	✓	X-degenerate
9									
21,717,55	C→	1	3	-	-	-	-	✓	X-degenerate
7									
21,724,112	G→	3	4	1	-	-	-	✓	X-degenerate
×									
21,754,33	G→A	5	9	-	-	-	-	✓	X-degenerate
0									
21,755,29	C	1	1	-	C→T	3	-	✓	X-degenerate
1									
21,755,31	G→A	1	1	-	G→A	3	-	✓	X-degenerate
4									
21,755,31	G	1	1	-	G→A	3	-	✓	X-degenerate
5									
21,759,59	G→A	1	6	-	-	-	-	✓	X-degenerate
9									
21,759,60	G→A	1	6	-	-	-	-	✓	X-degenerate
5									
21,765,77	C→T	4	6	-	-	-	-	✓	X-degenerate
8									
21,825,86	G→A	7	22	-	-	-	-	✓	X-degenerate
0									
21,825,86	G→A	6	16	-	-	-	-	✓	X-degenerate
2									
21,841,16	G→A	2	6	-	-	-	-	✓	X-degenerate
7									
21,872,46	G→T	4	5	-	-	-	-	✓	X-degenerate
0									
21,872,46	C→T	11	13	-	-	-	-	✓	X-degenerate
8									
21,878,62	G→A	4	5	-	-	-	-	✓	X-degenerate
2									
21,878,62	G→A	5	6	-	-	-	-	✓	X-degenerate
6									
21,878,62	G→A	5	6	-	-	-	-	✓	X-degenerate
8									
21,878,65	G→A	5	6	-	-	-	-	✓	X-degenerate
8									
21,897,88	C→T	3	2	-	-	-	-	✓	X-degenerate
2									
21,904,17	C→T	1	3	-	-	-	-	✓	X-degenerate
4									
21,904,18	C→T	2	4	-	-	-	-	✓	X-degenerate
0									
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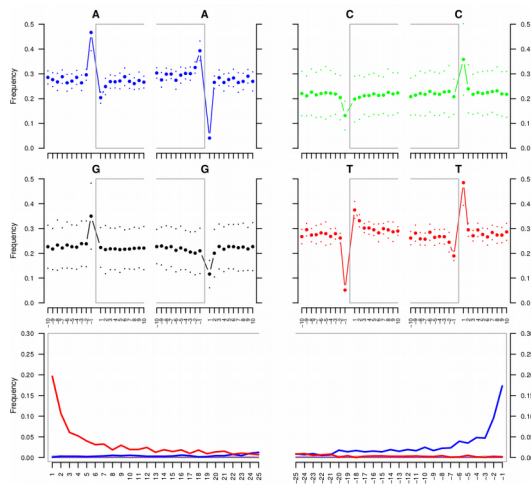
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6	21,931,19	G→A	2	6	-	-	-	-	✓	X-degenerate
9	22,625,38	-	-	-	-	C→T	5	-	✓	X-degenerate
7	22,625,39	-	-	-	-	C→T	5	-	✓	X-degenerate
2	22,625,72	G→A	3	3	-	-	-	-	✓	X-degenerate
7	22,677,32	G→A	1	4	-	-	-	-	✓	X-degenerate
1	22,677,44	G→A	3	1	-	-	-	-	✓	X-degenerate
2	22,678,02	C→T	1	3	-	-	-	-	✓	X-degenerate
9	22,894,80	-	-	-	-	C <sub>o</sub> T	3	-	✓	X-degenerate
8	22,927,43	G→A	3	7	-	-	-	-	✓	X-degenerate
7	22,928,41	G→A	3	3	-	-	-	-	✓	X-degenerate
8	22,931,98	G→A	2	8	-	-	-	-	x	X-degenerate
3	22,939,71	-	-	-	-	C→T	4	-	✓	X-degenerate
9	22,940,80	C→T	3	3	-	-	-	-	✓	X-degenerate
9										

**Supplementary Fig. 1.** Median joining network showing the relationship of the mtDNA haplotypes of Nakht-Ankh (NAII) and Khnum-Nakht (KNIII) to modern members of the M haplogroup. Empty nodes are shown in red. Edge lengths are proportional to the degree of difference between pairs of nodes.

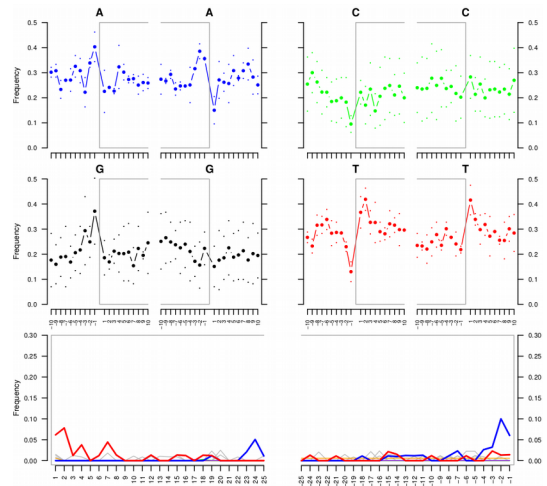


**Supplementary Fig. 2.** Fragmentation and misincorporation patterns for the sequence read datasets. **a**, NAII, mtDNA enrichment; **b**, KNI, mtDNA enrichment; **c**, KNII, mtDNA enrichment; **d**, KNIII, mtDNA enrichment; **e**, NAII, shotgun (mapped to the mitochondrial rCRS); **f**, KNII, shotgun (mapped to the mitochondrial rCRS); **g**, NAII, first NRY enrichment; **h**, NAII, double NRY enrichment; **i**, KNIII, double NRY enrichment; **j**, NAII shotgun (mapped to the Y chromosome); **k**, KNII shotgun (mapped to the Y chromosome). Data generated with MapDamage v.0.3.3 (Ginolhac et al., 2011). In each analysis, the upper four panels show the frequency of each of the four nucleotides at positions  $-10$  to  $+10$  relative to the 5' and 3' ends of the sequence reads, and the lower two panels show the C $\rightarrow$ T (red) and G $\rightarrow$ A (blue) misincorporation patterns within the initial 25 and final 25 nucleotides of each read. The patterns are consistent with the fragmentation and misincorporation models typical for aDNA (Ginolhac et al., 2011), although the mtDNA enriched (**b**) and shotgun datasets (**f**, **k**) for KNII are so damaged that no distinct patterns are observed. The NRY plots have more noise but discernible patterns of aDNA damage can be seen.

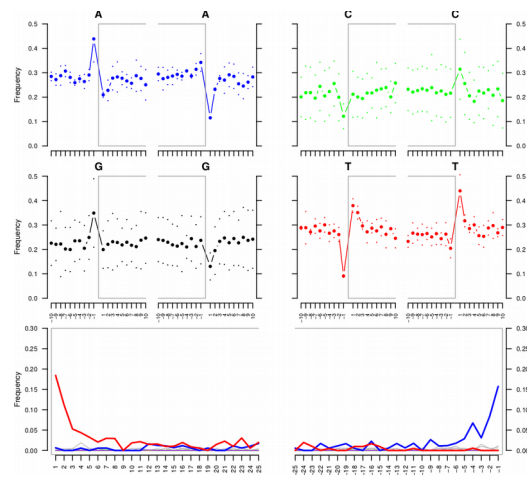
**a. NAII, mtDNA enrichment**



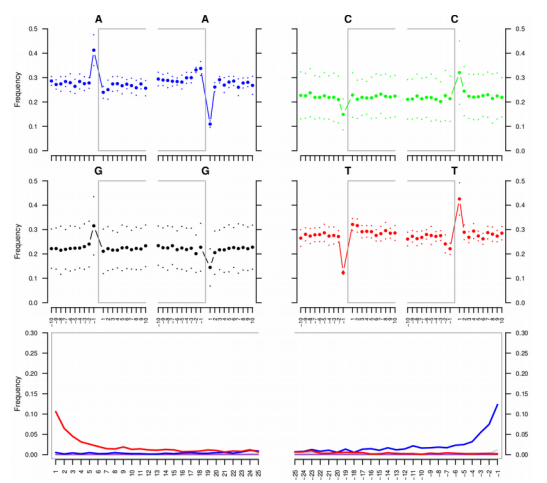
**b. KNI, mtDNA enrichment**



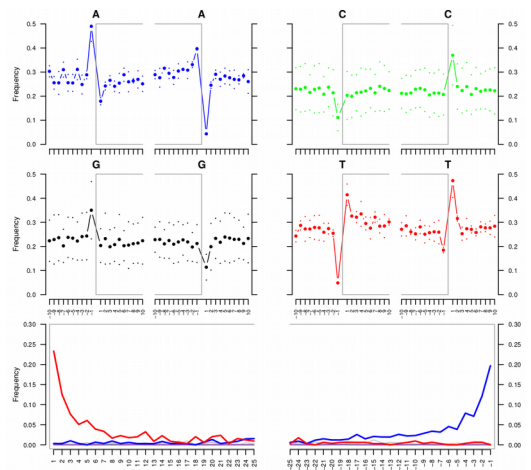
**c. KNII, mtDNA enrichment**



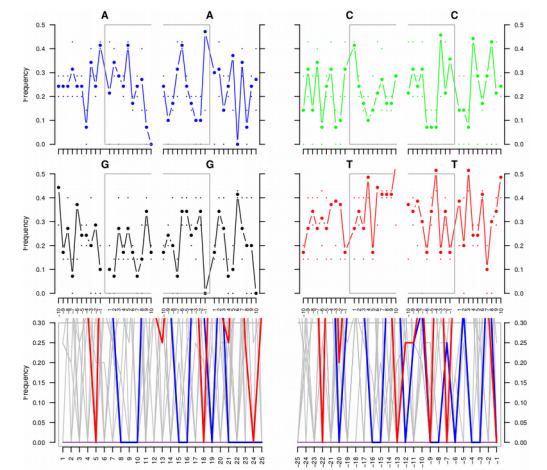
**d. KNIII, mtDNA enrichment**



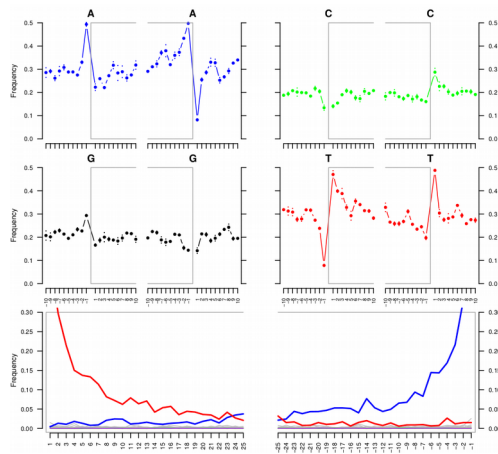
**e. NAII, shotgun (mapped to mt rCRS)**



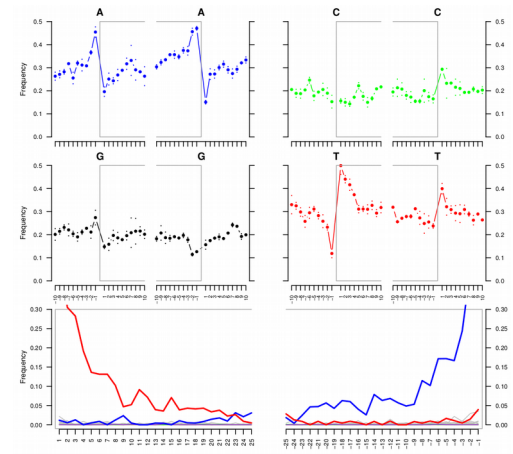
**f. KNII, shotgun (mapped to mt rCRS)**



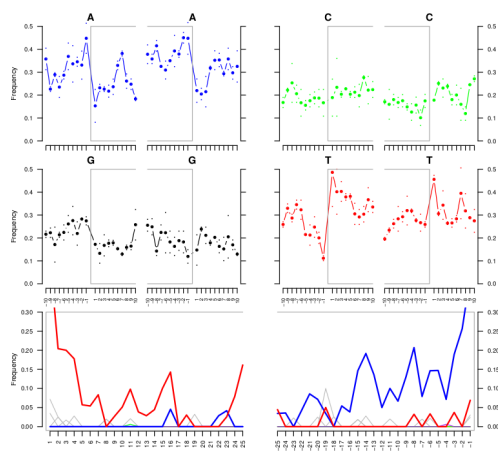
**g. NAI1, first NRY enrichment**



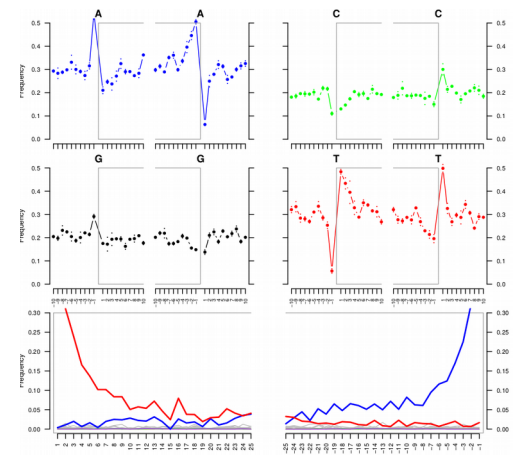
**h. NAI1, double NRY enrichment**



**i. KN111, double NRY enrichment**



**j. NAI1, shotgun (mapped to Y)**



**k. KN111, shotgun (mapped to Y)**

