Supplementary Data

Mitochondrial DNA deletions are associated with non-B DNA conformations

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Contents

Supplemental Figure S1. List of published studies from which deletions were retrieved.

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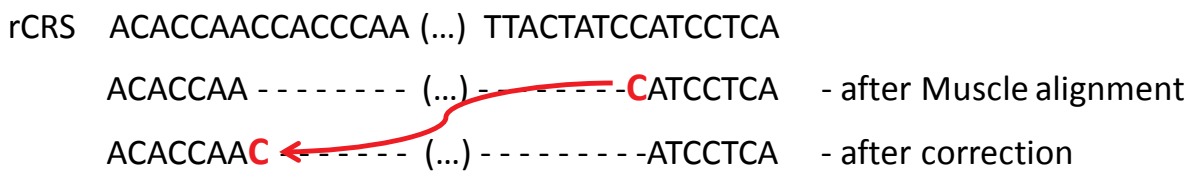
Supplemental Figure S2. Refinement of breakpoint locations on the revised Cambridge reference mtDNA sequence (rCRS). In cases where one or more bases could be equally aligned upstream or downstream of the deletion area, we corrected the limits of the deletion by retaining all of the possible matches at the 5' breakpoint.



Supplemental Figure S3. List of the 753 unique mtDNA deletions used in this study. Numbers according to the L-strand positions of the revised Cambridge reference mtDNA sequence (rCRS; NC_012920).

Breakpoints	
5’	3’
105	112
299	349
306	357
463	12080
471	5152
478	5160
488	8643
493	14242
504	5443
537	4431
544	13768
547	4443
585	4443
633	16262
708	13767
898	16116
1417	15219
1491	5206
1491	5323
1672	16071
1691	13925
1741	14502
1753	13101
1771	16072
1840	5451
1906	16072
1907	16079
1989	14366
2059	16071
2068	16074
2333	16075
2333	16078
2334	16073
2540	16047
2781	16071
2818	16078
3167	14154
3173	14161
3212	16071
3233	3237
3255	16070
3256	16072
3263	15123
3263	16070
3263	16071
3263	16072
3263	16073
3270	16034
3272	15068
3272	16034
3311	15741
3326	3591
3326	13923
3393	16072
3418	3432
3432	16071
3486	16068
3549	15440
3549	16072
3571	15540
3575	15968
3591	11519
3614	12987
3692	16068
3742	16074
3826	16071
3963	11432
4048	15949
4069	14306
4141	16073
4351	16071
4376	16078
4398	14822
4458	16074
4469	13923
4486	16075
4882	16068
5042	14552
5173	15607
5195	16071
5328	16076
5330	16077
5440	13030
5561	13405
5671	16071
5745	16073
5772	13411
5773	13411
5781	16069
5781	16071
5782	13483
5785	13923
5785	16072
5787	13920
5787	13924
5787	15576
5787	16035
5787	16071
5787	16072
5787	16073
5788	13239
5788	15448
5788	16069
5788	16071
5789	16075
5793	12767
5795	13920
5798	16072
5803	16072
5836	12662
5987	13537
6003	15441
6019	6025
6023	14424
6062	16070
6074	9179
6076	13799
6084	13808
6124	15893
6128	15434
6172	13057
6173	16078
6175	14162
6189	16072
6203	14258
6215	10976
6218	13447
6226	13447
6238	14103
6310	15611
6320	11272
6326	13989
6326	13990
6326	16071
6329	13994
6331	13994
6341	13994
6341	14005
6380	14096
6416	14182
6422	15208
6423	14344
6428	15270
6429	14101
6437	14077
6453	14288
6464	14874
6466	14135
6469	15586
6476	13777
6476	14146
6504	13805
6521	16071
6527	15055
6533	16075
6537	13838
6551	13852
6555	14345
6572	12304
6577	15154
6585	15144
6587	14285
6601	16284
6625	16074
6634	9925
6750	16072
6753	16075
6759	15865
6790	15918
6836	14380
6843	14388
6851	14816
6862	13061
6919	13564
6927	16072
6935	15447
6940	15451
6961	16071
7057	16071
7067	16047
7098	16072
7099	16071
7113	14693
7115	10546
7116	16073
7122	14000
7125	13050
7127	14393
7128	14006
7182	14566
7185	14120
7188	14335
7193	14587
7193	14596
7201	14809
7204	13903
7212	14261
7215	16072
7215	16073
7218	16071
7235	16072
7235	16074
7265	16035
7265	16071
7267	16077
7268	16077
7277	12300
7288	15900
7289	14943
7322	13464
7322	15239
7323	15056
7323	15435
7329	14531
7331	15946
7337	14931
7338	14598
7347	14245

7350	16071
7360	15276
7366	14060
7367	12063
7367	16072
7370	16072
7370	16073
7372	13239
7374	15957
7387	15930
7396	13118
7399	15383
7399	15405
7400	14290
7401	16068
7401	16074
7402	13677
7402	15128
7402	15372
7402	16068
7402	16071
7402	16072
7402	16073
7402	16076
7403	12392
7403	16075
7403	16078
7404	16080
7406	14560
7407	12396
7407	13620
7409	13688
7410	13688
7414	14568
7414	15609
7428	13415
7429	14895
7431	15093
7439	13477
7440	15882
7443	13468
7448	13451
7448	13475
7449	15167
7449	15926
7450	15378
7452	12263
7452	13723
7453	13487
7453	13644
7453	15435
7460	11847
7461	12977
7462	16071
7465	11426
7469	14285
7470	16074
7471	16073
7472	14814
7477	15998

7479	11440
7485	10997
7491	11004
7493	13985
7494	12763
7494	16073
7495	15439
7506	14433
7508	15939
7517	13096
7524	13395
7527	16065
7529	13474
7539	15237
7539	15913
7547	13475
7553	13546
7562	15381
7563	13724
7623	15036
7626	8196
7629	14813
7629	16072
7632	8206
7635	15310
7635	15440
7638	15916
7644	16071
7662	15429
7662	15552
7667	14359
7667	16071
7667	16072
7669	13810
7669	15437
7669	16072
7671	16071
7677	15013
7682	13722
7694	14053
7695	13107
7696	12365
7696	13987
7697	12364
7705	15945
7719	16053
7723	14108
7723	14110
7728	13806
7733	16073
7755	15236
7756	16072
7775	13532
7775	16072
7777	13794
7779	16071
7792	14181
7794	12561
7795	15287
7795	16073

7804	13795
7805	13844
7808	12388
7811	16071
7813	16077
7814	14805
7815	13581
7817	14536
7817	15382
7817	16071
7817	16072
7817	16074
7818	16075
7818	16077
7819	16070
7819	16077
7820	16074
7821	13760
7821	16072
7821	16073
7823	15389
7824	16077
7828	13385
7829	14135
7841	13905
7845	15761
7846	9749
7850	13474
7853	13887
7857	12283
7869	16069
7869	16076
7870	16076
7885	15698
7889	15435
7899	15642
7904	16285
7912	9560
7914	16070
7915	16276
7919	16072
7920	16078
7921	16072
7921	16080
7921	16283
7922	16075
7923	11423
7923	16075
7931	15761
7933	15604
7945	16073
7945	16074
7949	14315
7958	15927
7959	13787
7961	13374
7962	16020
7964	12432
7967	11312
7975	15496

7978	16072
7982	15504
7983	15504
7989	15435
7990	16068
7990	16078
7991	16070
7991	16073
7992	15730
7992	16072
7995	15213
7997	15568
7998	12300
8019	15519
8030	16070
8030	16075
8030	16078
8031	14564
8031	16070
8031	16074
8031	16077
8032	12300
8032	16069
8032	16071
8032	16072
8032	16073
8032	16075
8033	14337
8033	16071
8033	16078
8034	11423
8034	11434
8034	15882
8034	16071
8034	16076
8036	13095
8049	14115
8053	15444
8055	13095
8058	12215
8076	15911
8083	16071
8093	16071
8095	12393
8113	14849
8134	13573
8137	14771
8143	15271
8151	15783
8156	16037
8170	16333
8175	16073
8176	11614
8198	13433
8208	16070
8211	15340
8213	16070
8220	13998
8222	13476
8231	16072

8231	16280
8232	15542
8232	16071
8233	13383
8233	13967
8233	16067
8239	13407
8240	13341
8244	16381
8250	13410
8253	16066
8254	16070
8256	15261
8257	16162
8259	13359
8261	15652
8262	10844
8263	13531
8264	16071
8268	14906
8269	13447
8271	13410
8278	13770
8280	8290
8281	16371
8287	16072
8289	13791
8291	16276
8292	16034
8294	16074
8295	16073
8299	13153
8299	16071
8304	15055
8305	14619
8308	13382
8310	16078
8312	13667
8316	15802
8327	13358
8344	15633
8372	12085
8373	16067
8380	13338
8385	15918
8387	12821
8387	15515
8387	16067
8387	16071
8387	16078
8394	16070
8395	16071
8395	16073
8398	16072
8398	16073
8408	14118
8412	15664
8417	15127
8418	14127
8420	11498

8421	12399
8421	13564
8426	12894
8428	15482
8437	14068
8439	16075
8441	14505
8461	15603
8464	13138
8467	13444
8468	13446
8470	13447
8475	14381
8476	14812
8477	13590
8479	13594
8482	13460
8483	13448
8484	13448
8491	13448
8503	13425
8518	15422
8527	13718
8536	15439
8536	15642
8547	15727
8553	15585
8555	13980
8561	13985
8562	13759
8563	14596
8563	15435
8564	16072
8569	15879
8571	13237
8572	13997
8572	15826
8576	12984
8576	13962
8576	16075
8577	15731
8580	15239
8580	15539
8583	15958
8587	15738
8588	13730
8601	15731
8612	13575
8612	16072
8617	13877
8623	15662
8623	15663
8624	13886
8624	15662
8630	16067
8631	13513
8631	13580
8635	16074
8637	16073
8637	16084

8637	16236
8640	13378
8640	15369
8645	15656
8648	16085
8651	16071
8662	14817
8663	16075
8664	15363
8697	15938
8707	13723
8711	13550
8712	15990
8723	9215
8728	13699
8775	13991
8777	14006
8810	15638
8813	13304
8815	15846
8817	15610
8823	15855
8825	15658
8830	14897
8838	14906
8893	15503
8904	14903
8905	14110
8933	13768
8989	13997
8992	16072
8992	16074
8993	13563
8996	16070
9009	16354
9016	14576
9021	13833
9070	13476
9079	15312
9084	15438
9087	13155
9137	13808
9143	13333
9144	13816
9180	14281
9191	12909
9191	15322
9194	15323
9238	15576
9246	13058
9253	14425
9258	13627
9258	14429
9268	16068
9281	13810
9320	14273
9345	13148
9345	13851
9351	13154
9357	13865

9417	13684
9436	15913
9440	14083
9444	13964
9484	13724
9486	9502
9490	16072
9497	13735
9514	13052
9515	13055
9532	14135
9537	15519
9538	15537
9538	15658
9574	10166
9574	12972
9574	13951
9576	13942
9587	13953
9591	13052
9603	14141
9609	14147
9622	15812
9625	15435
9659	11536
9665	15183
9698	13735
9729	15338
9744	14439
9773	13756
9799	15557
9816	15744
9845	16065
9860	15026
9923	16073
9924	15194
9966	15801
9995	15897
9998	15895
10004	15360
10051	15077
10063	14598
10071	14622
10080	14959
10104	11868
10154	15945
10169	14435
10186	13759
10191	13753
10198	13761
10207	13766
10367	12829
10370	15570
10418	15570
10497	14760
10559	14981
10587	15913
10598	13206
10625	13059
10668	14859

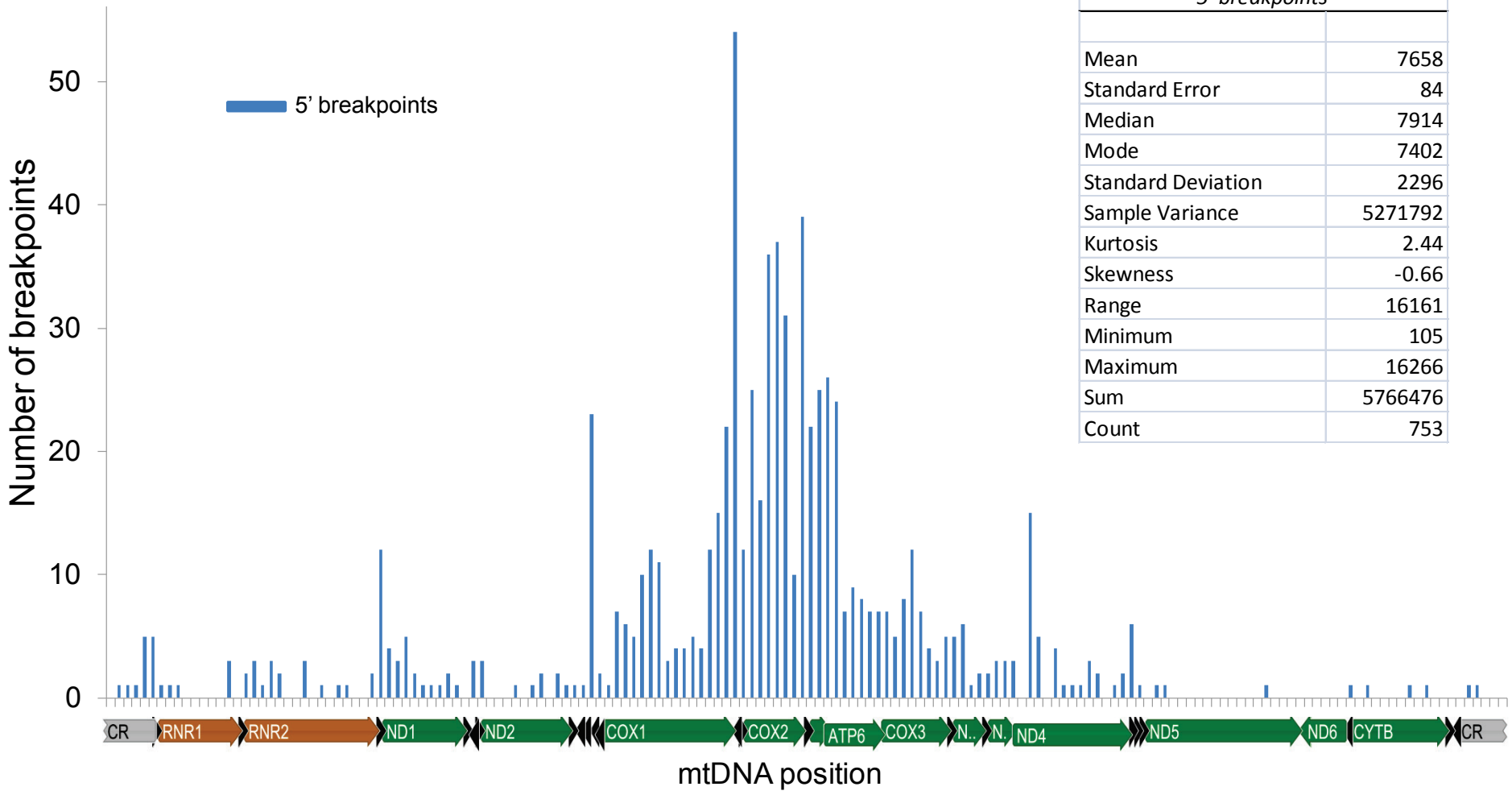
10676	14868
10746	14125
10749	14129
10758	14138
10925	14152
10935	13773
10939	15530
10940	15546
10943	15363
10943	15372
10946	13781
10951	15372
10952	13990
10952	15373

10953	14133
10953	15837
10961	15846
10969	14119
10984	15543
11035	15159
11035	15183
11036	15028
11036	15441
11065	15860
11232	13980
11237	13984
11240	15036
11258	14369

11368	15786
11479	16071
11514	13810
11648	13811
11668	15916
11680	14258
11731	14450
11739	15572
11986	13792
12021	16075
12084	15854
12103	14414
12104	14413
12112	14422

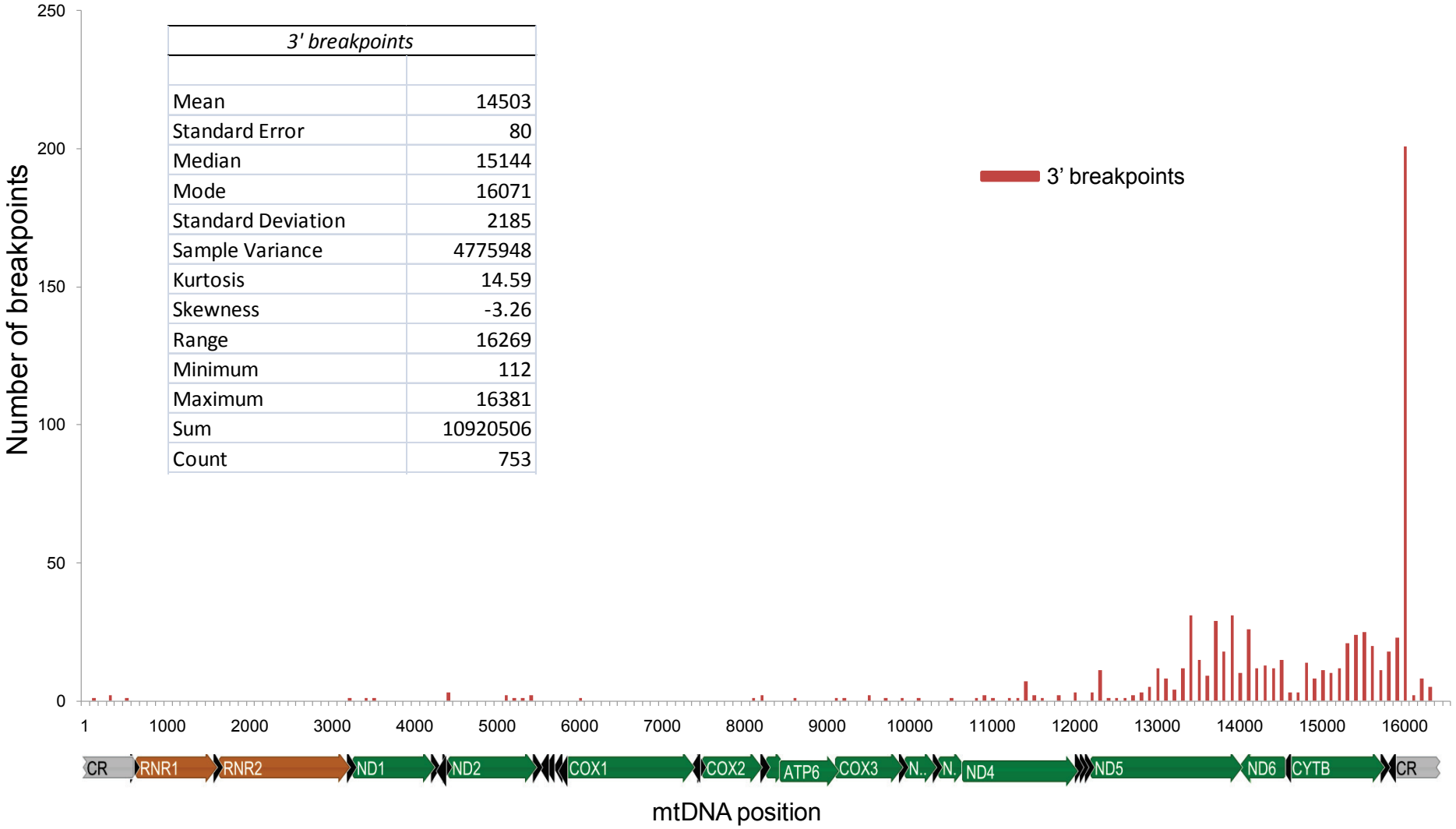
12113	14421
12113	14422
12122	15844
12203	15355
12439	15390
12544	15525
13761	14252
14786	14791
14927	14943
15497	15522
15643	15665
16164	16319
16266	576

Supplemental Figure S4. Distribution of 5’ deletion breakpoints in the human mtDNA. The values were grouped in 100-nt windows. The locations of the mitochondrial genes are shown below the x-axis.



5' breakpoints	
Mean	7658
Standard Error	84
Median	7914
Mode	7402
Standard Deviation	2296
Sample Variance	5271792
Kurtosis	2.44
Skewness	-0.66
Range	16161
Minimum	105
Maximum	16266
Sum	5766476
Count	753

Supplemental Figure S5. Distribution of 3’ deletion breakpoints in the human mtDNA. The values were grouped in 100-nt windows. The locations of the mitochondrial genes are shown below the x-axis.



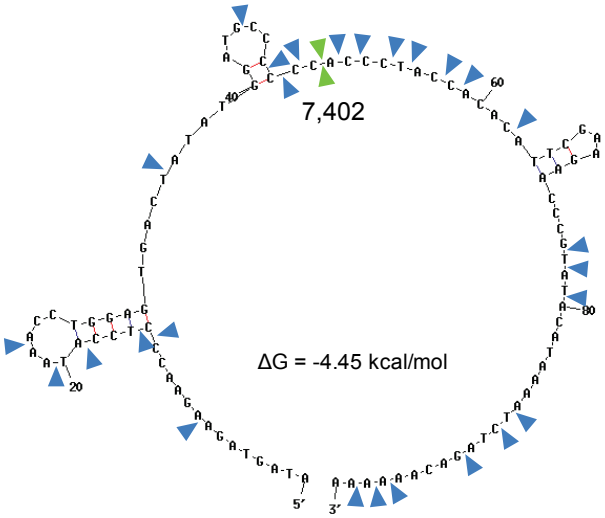
Supplemental Figure S6. Distribution of 3’ breakpoints around the 16,071 hotspot.

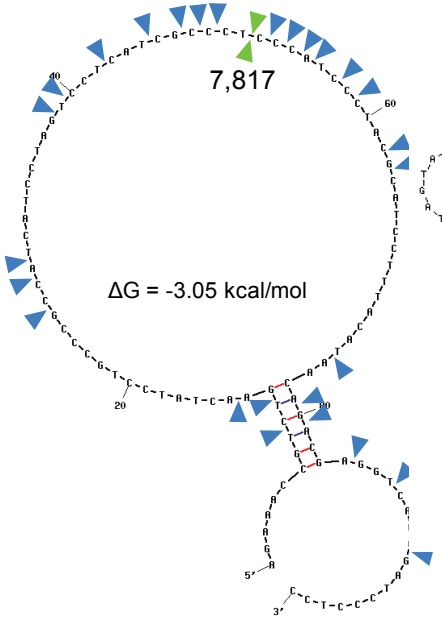
mtDNA position	Number of 3' breakpoints	
	n	%
16065	2	0.27
16066	1	0.13
16067	4	0.53
16068	7	0.93
16069	4	0.53
16070	13	1.73
16071	41	5.44
16072	38	5.05
16073	23	3.05
16074	14	1.86
16075	15	1.99
16076	5	0.66
16077	8	1.06
16078	11	1.46
16079	1	0.13
16080	2	0.27
Total	189	25.09

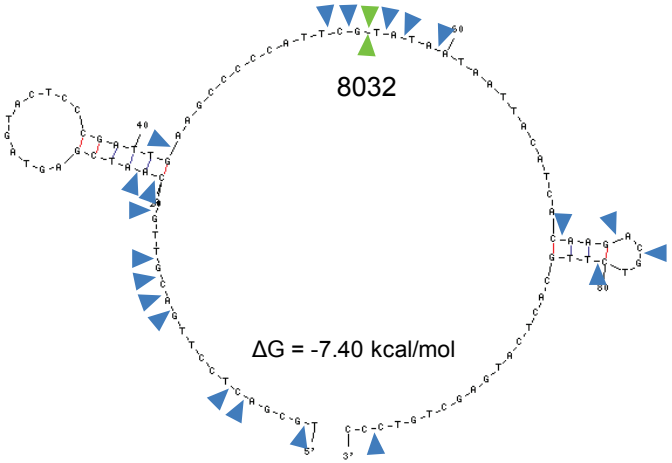
Supplemental Figure S7. List of the most frequent breakpoints detected in real and simulated mtDNA deletions. In cases where adjacent sites had several breakpoints, only the most frequent one in that cluster is represented. The dataset of 20,000 random deletions was generated using in-house python scripts.

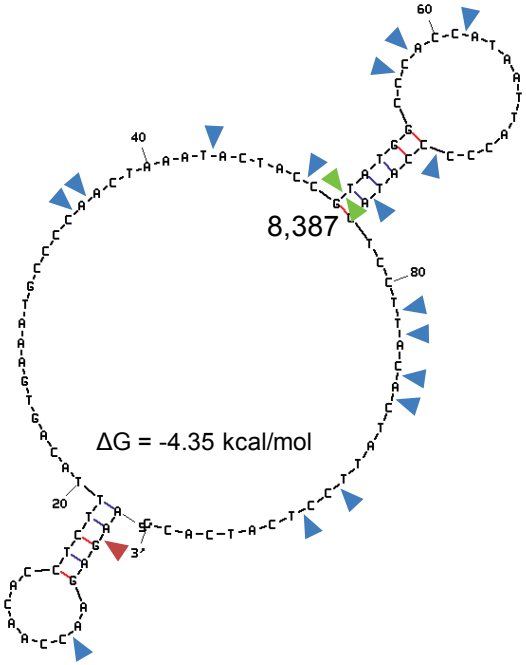
mtDNA position	Type	*n*	Frequency
753 real deletions			
16,071	3′	41	$5.4X10^{-2}$
7,402	5′	8	$1.1X10^{-2}$
5,787	5′	7	$9.3X10^{-3}$
15,435	3′	6	$8.0X10^{-3}$
8,032	5′	6	$8.0X10^{-3}$
3,263	5′	5	$6.6X10^{-3}$
7,817	5′	5	$6.6X10^{-3}$
8,387	5′	5	$6.6X10^{-3}$
13,447	3′	4	$5.3X10^{-3}$
12,300	3′	3	$4.0X10^{-3}$
20,000 random deletions			
13,556	5′	8	$4.0X10^{-4}$
14,291	5′	8	$4.0X10^{-4}$
4,613	3′	7	$3.5X10^{-4}$
9,529	3′	7	$3.5X10^{-4}$
13,312	3′	7	$3.5X10^{-4}$
4,255	5′	7	$3.5X10^{-4}$
4,443	5′	7	$3.5X10^{-4}$
6,785	5′	7	$3.5X10^{-4}$
8,198	5′	7	$3.5X10^{-4}$
9,682	5′	7	$3.5X10^{-4}$
16,156	5′	7	$3.5X10^{-4}$

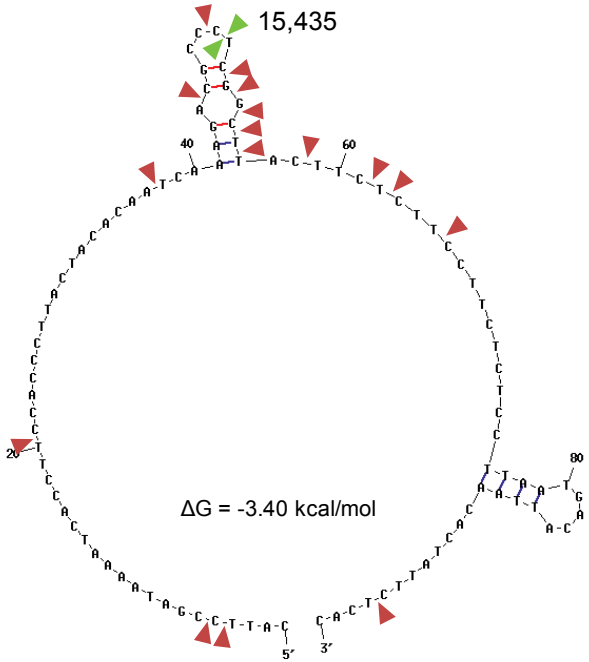
Supplemental Figure S8. Secondary structures around the most frequent 5’ and 3’ breakpoint sites of the human mtDNA L-strand. The 100-nt windows around five of the ten most frequent breakpoint sites (mtDNA positions 7,402, 7,817, 8,032, 8,387, 15,435, indicated by green arrows) were used for folding predictions with the UNAFold software package. The breakpoints were used as window midpoints. The blue and red arrows indicate less frequent 5’ and 3’ breakpoints, respectively. The secondary structures were obtained in the mfold-util software v4.6.


7,402
ΔG = -4.45 kcal/mol

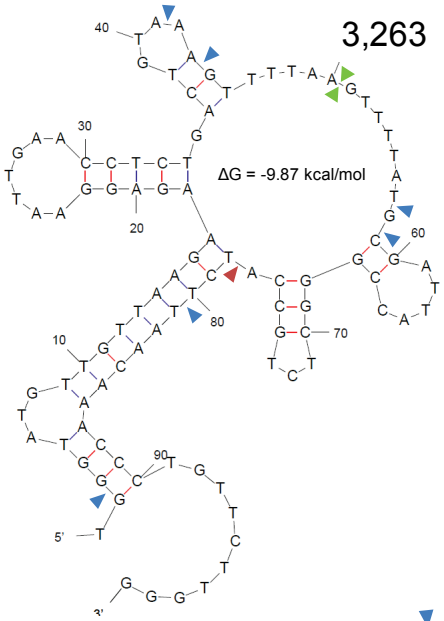

7,817
ΔG = -3.05 kcal/mol

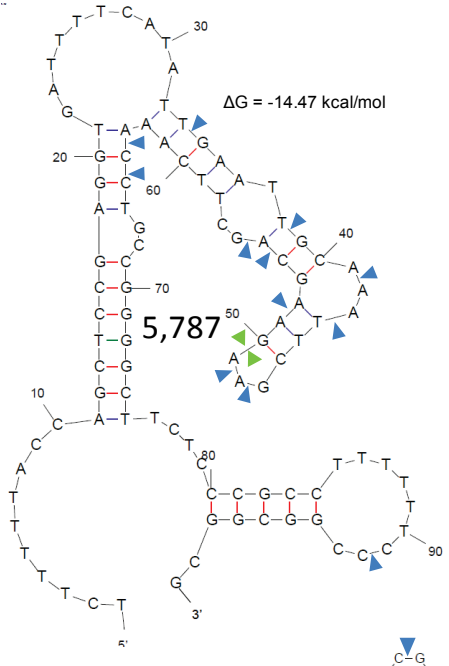

8032
ΔG = -7.40 kcal/mol

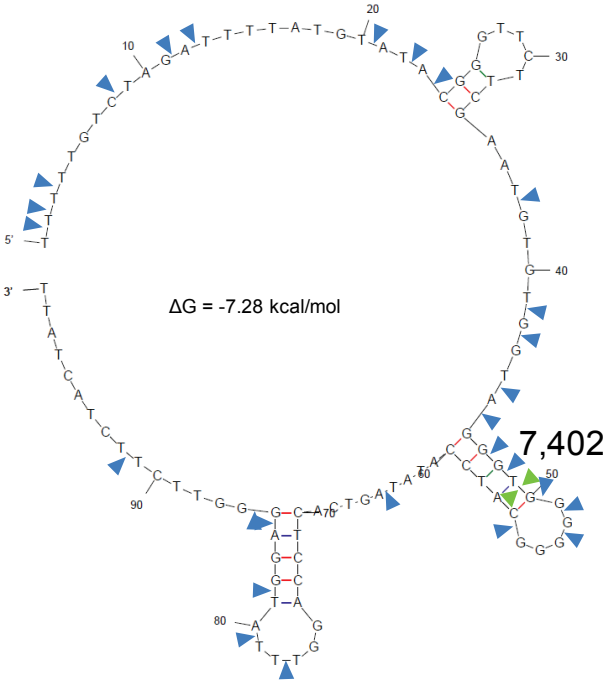

8,387
ΔG = -4.35 kcal/mol

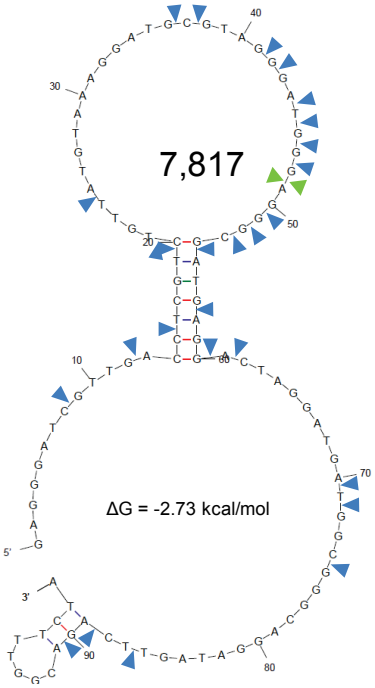

15,435
ΔG = -3.40 kcal/mol

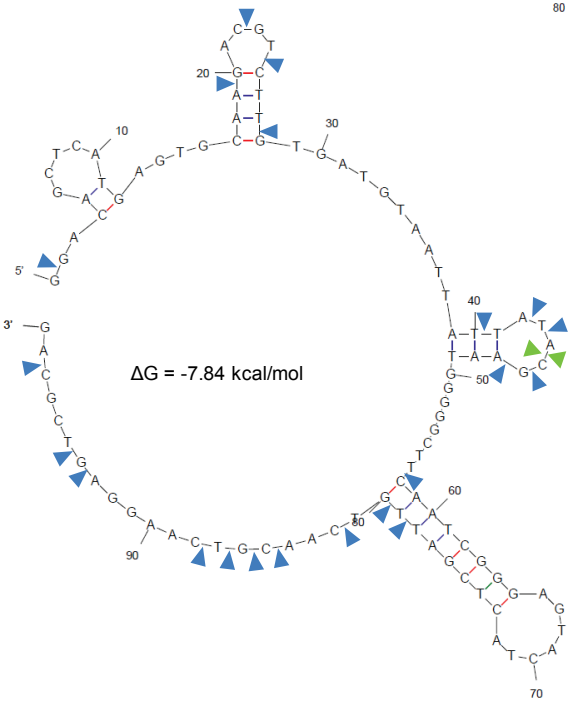
Supplemental Figure S9. Secondary structures around the most frequent 5' and 3' breakpoint sites of the human mtDNA H-strand. The 100-nt windows around the ten most frequent breakpoint sites (mtDNA positions 3,263, 5,787, 7,402, 7,817, 8,032, 8,387, 12,300, 13,447, 15,435 and 16,071 indicated by green arrows) were used for folding predictions with the UNAFold software package. The breakpoints were used as window midpoints. The blue and red arrows indicate less frequent 5' and 3' breakpoints, respectively. Highlighted in grey is the 13-bp direct repeat at the 3' breakpoint of the 'common deletion'. The secondary structures were obtained in the mfold-util software v4.6.

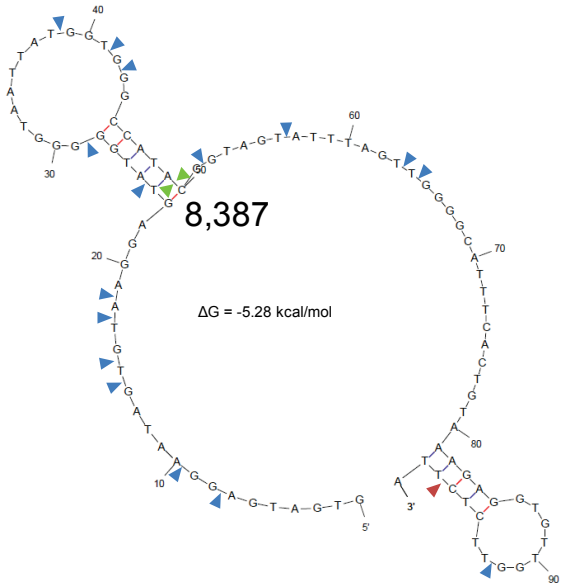

3,263
ΔG = -9.87 kcal/mol

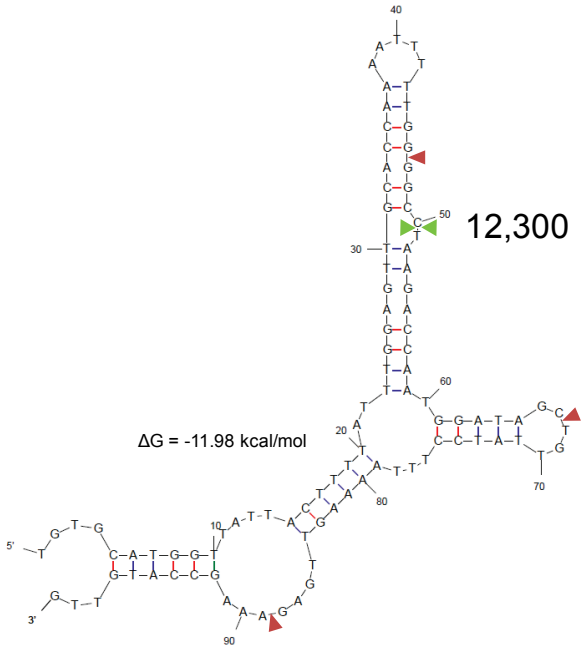

ΔG = -14.47 kcal/mol
5,787

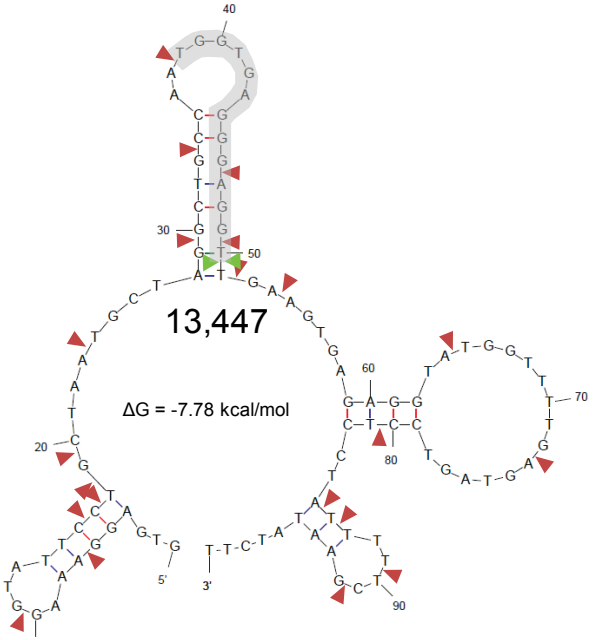

ΔG = -7.28 kcal/mol
7,402

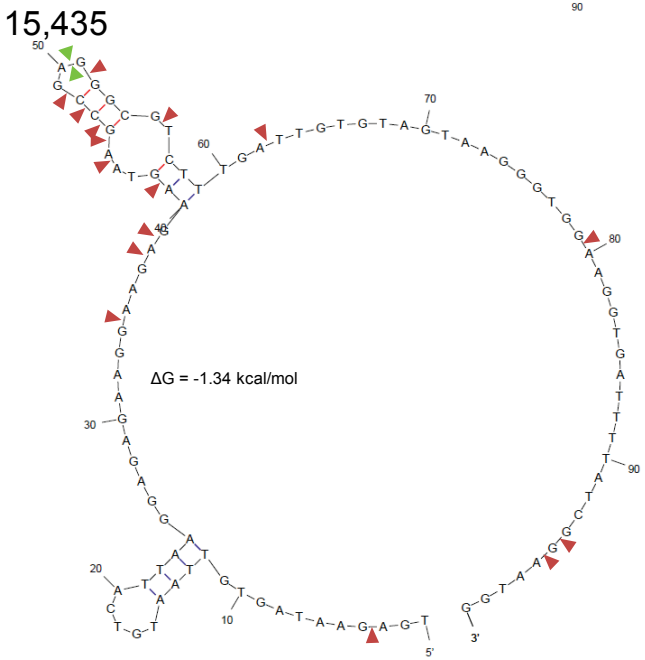

7,817
ΔG = -2.73 kcal/mol

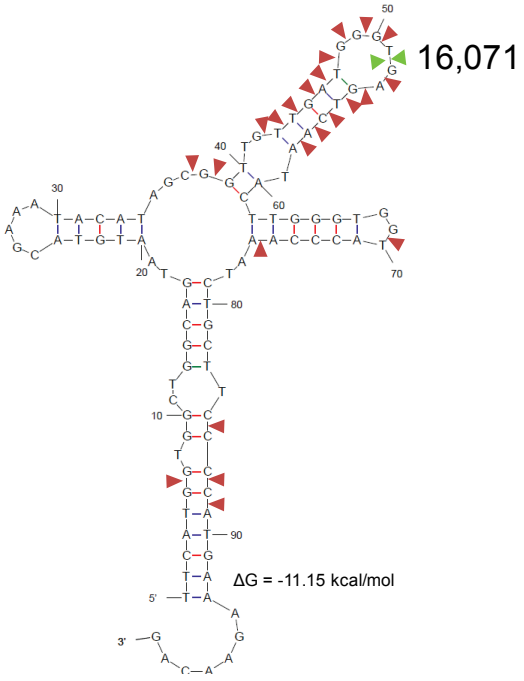

ΔG = -7.84 kcal/mol
8,032


8,387
ΔG = -5.28 kcal/mol

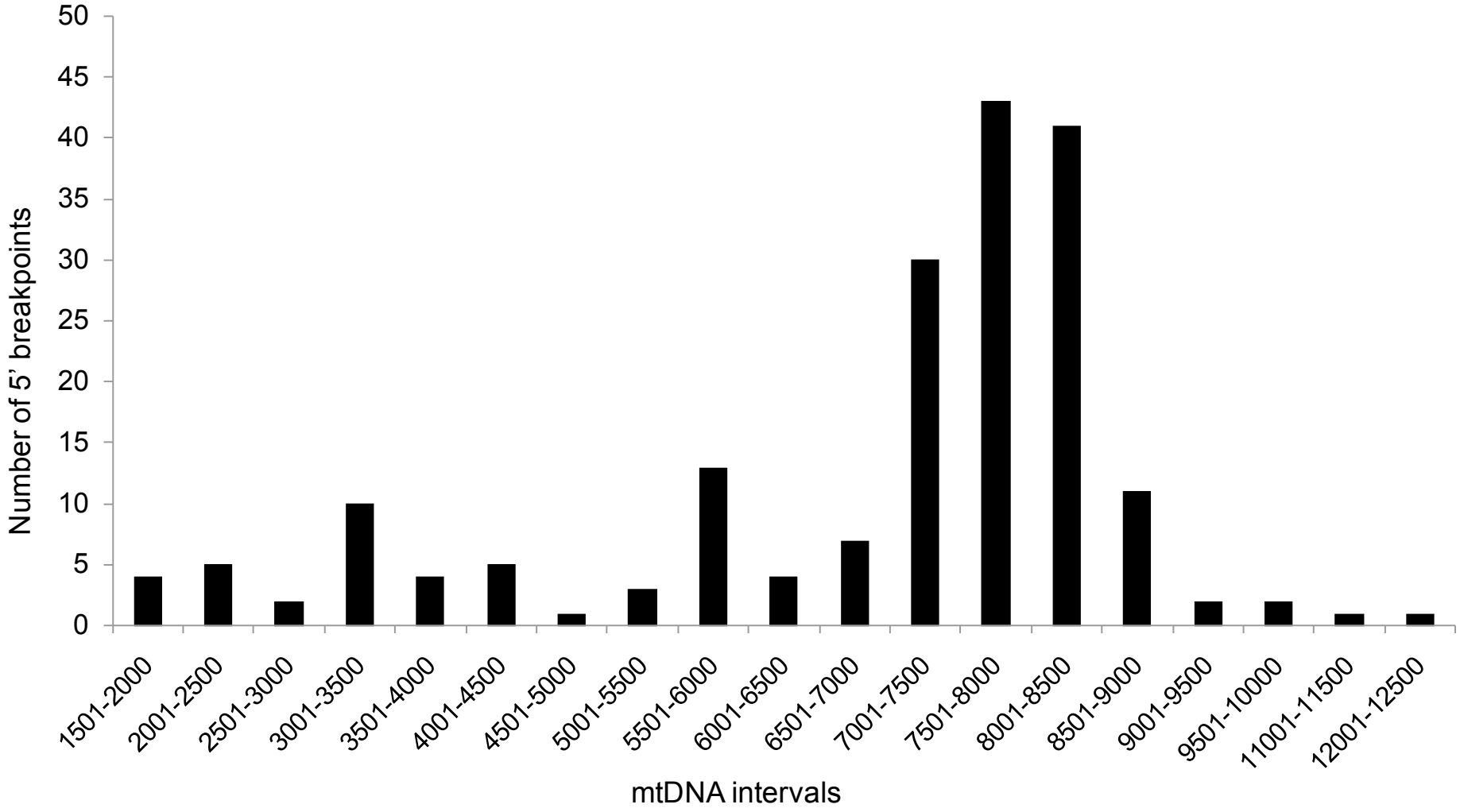

12,300
ΔG = -11.98 kcal/mol


13,447
ΔG = -7.78 kcal/mol

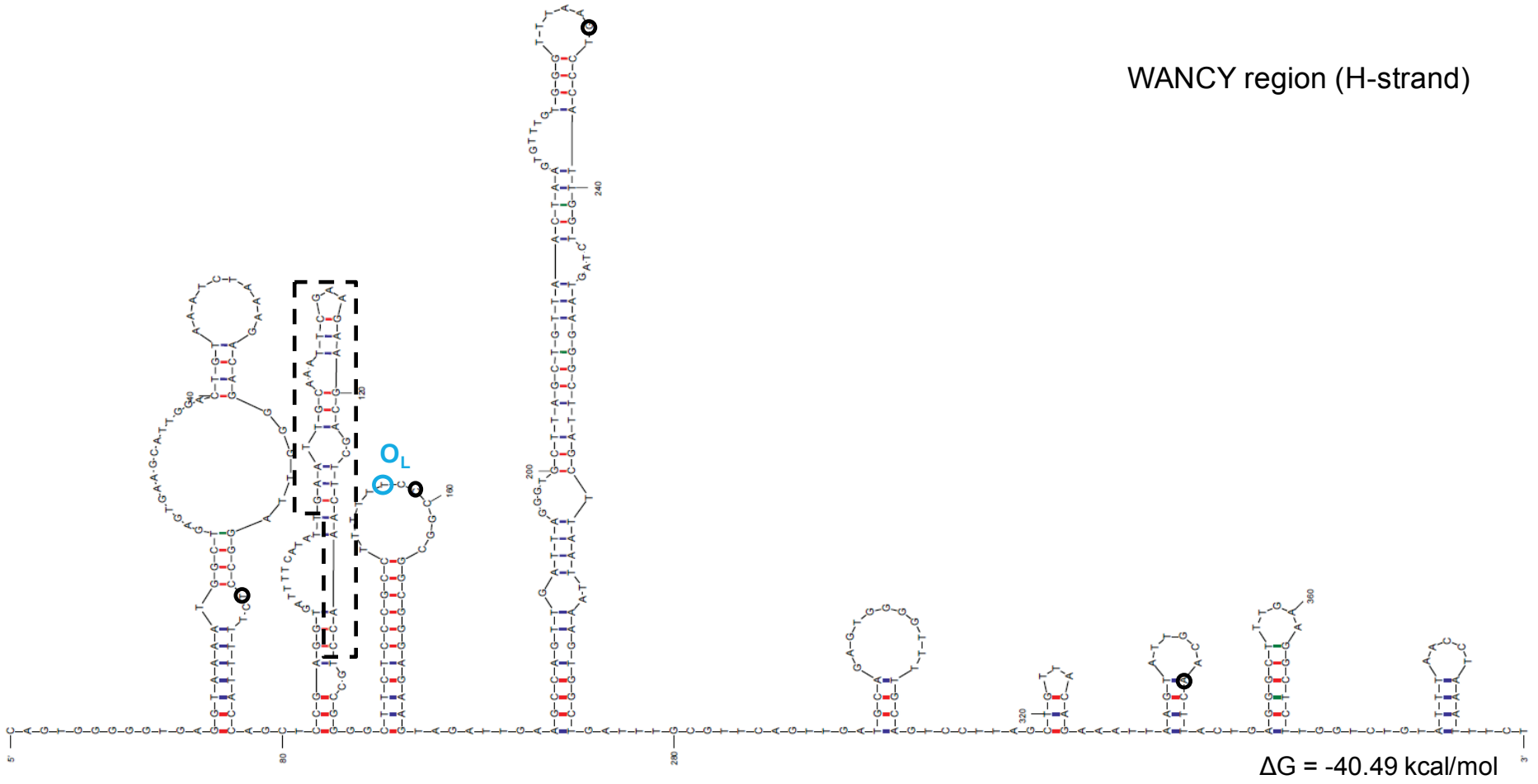

15,435
ΔG = -1.34 kcal/mol


16,071
ΔG = -11.15 kcal/mol

Supplemental Figure S10. Global distribution of 5' breakpoints from deletions with the 3' breakpoint at the 16,071 hotspot. The graph displays the distribution of 5' breakpoints (binned in 500-nt intervals along the mtDNA sequence) from deletions with a 3' breakpoint in the 16,075-16,080 mtDNA region.



Supplemental Figure S11. The WANCY cluster of tRNAs is a hotspot for mtDNA breakage. The 27 5’ deletion breakpoints (black circles and dashed line) identified in the WANCY region are located in hairpin elements predicted for the H-strand. The blue circle depicts the origin of L-strand replication (O_L). The secondary structure was obtained in the mfold-util software v4.6.



Supplemental Figure S12. Distribution of deletion breakpoints according to the coding features of the mitochondrial genome.

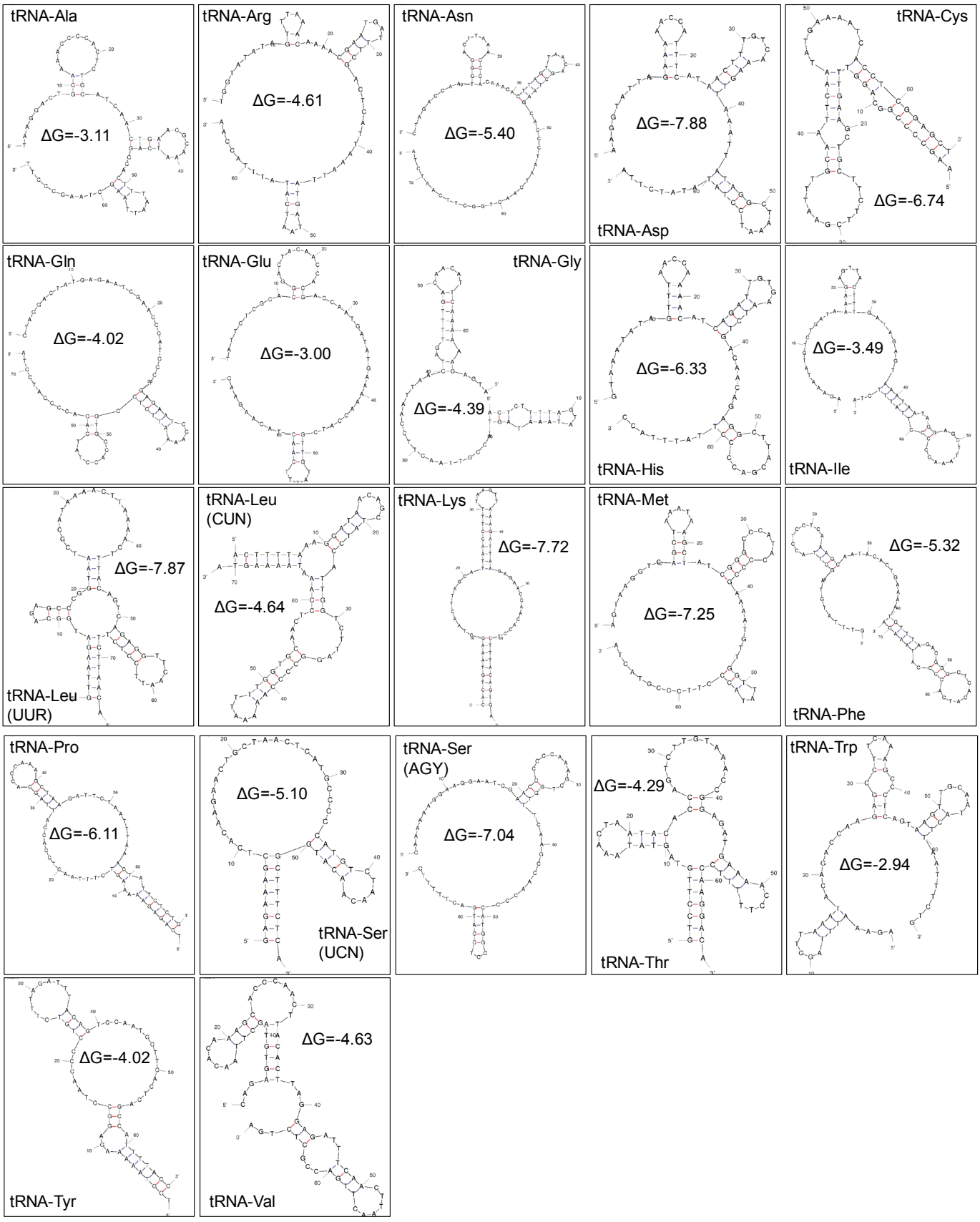
mtDNA region	mtDNA position (first - last)		Length (bases)	Number of breakpoints			% of total breakpoints
				5'	3’	Total	
tRNA-Phe	577	647	71	2	0	2	0.13
RNR1	648	1601	954	5	0	5	0.33
tRNA-Val	1602	1670	69	0	0	0	0.00
RNR2	1671	3229	1559	20	0	20	1.33
tRNA-Leu(UUA/G)	3230	3304	75	11	1	12	0.80
ND1	3307	4262	956	20	2	22	1.46
tRNA-Ile	4263	4331	69	0	0	0	0.00
tRNA-Gln	4329	4400	72	3	0	3	0.20
tRNA-Met	4402	4469	68	2	3	5	0.33
ND2	4470	5511	1042	8	6	14	0.93
tRNA-Trp	5512	5579	68	1	0	1	0.07
tRNA-Ala	5587	5655	69	0	0	0	0.00
tRNA-Asn	5657	5729	73	1	0	1	0.07
tRNA-Cys	5761	5826	66	23	0	23	1.53
tRNA-Tyr	5826	5891	66	1	0	1	0.07
COX1	5904	7445	1542	149	1	150	9.96
tRNA-Ser(UCN)	7446	7514	69	28	0	28	1.86
tRNA-Asp	7518	7585	68	9	0	9	0.60
COX2	7586	8269	684	182	2	184	12.22
tRNA-Lys	8295	8364	70	11	0	11	0.73
ATP8	8366	8572	207	56	0	56	3.72
ATP6	8527	9207	681	71	2	73	4.85
COX3	9207	9990	784	49	5	54	3.59
tRNA-Gly	9991	10058	68	4	0	4	0.27
ND3	10059	10404	346	12	1	13	0.86
tRNA-Arg	10405	10469	65	1	0	1	0.07
ND4L	10470	10766	297	10	1	11	0.73
ND4	10760	12137	1378	41	21	62	4.12
tRNA-His	12138	12206	69	1	0	1	0.07
tRNA-Ser(AGY)	12207	12265	59	0	2	2	0.13
tRNA-Leu(CUN)	12266	12336	71	0	5	5	0.33
ND5	12337	14148	1812	3	219	222	14.74
ND6	14149	14673	525	0	60	60	3.98
tRNA-Glu	14674	14742	69	0	1	1	0.07
CYTB	14747	15887	1141	4	174	178	11.82
tRNA-Thr	15888	15953	66	0	21	21	1.39
tRNA-Pro	15956	16023	68	0	0	0	0.00
Control region (CR)	16024	576	1122	14	219	233	15.47
Non-coding (other than CR)			89	11	7	18	1.20

Breakpoints located in overlapping genes were only considered once

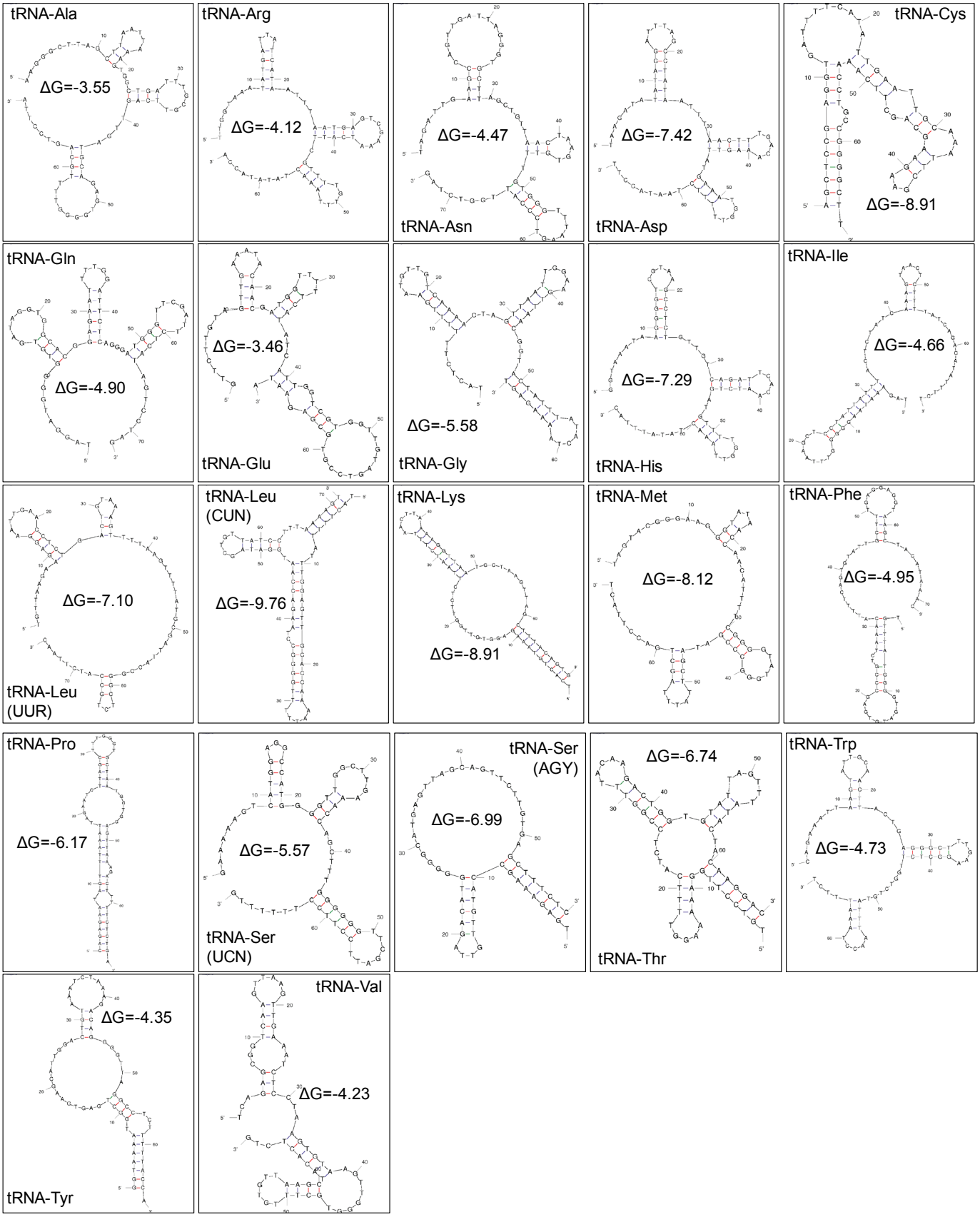
Supplemental Figure S13. Number of mtDNA deletion breakpoints per base. The breakpoints were grouped according to the coding features of the mitochondrial genome.

mtDNA region	Breakpoints/base		
	5’	3’	Total
tRNA-Phe	0.028	0.000	0.028
RNR1	0.005	0.000	0.005
tRNA-Val	0.000	0.000	0.000
RNR2	0.013	0.000	0.013
tRNA-Leu(UUA/G)	0.147	0.013	0.160
ND1	0.021	0.002	0.023
tRNA-Ile	0.000	0.000	0.000
tRNA-Gln	0.042	0.000	0.042
tRNA-Met	0.029	0.044	0.074
ND2	0.008	0.006	0.013
tRNA-Trp	0.015	0.000	0.015
tRNA-Ala	0.000	0.000	0.000
tRNA-Asn	0.014	0.000	0.014
tRNA-Cys	0.348	0.000	0.348
tRNA-Tyr	0.015	0.000	0.015
COX1	0.097	0.001	0.097
tRNA-Ser(UCN)	0.406	0.000	0.406
tRNA-Asp	0.132	0.000	0.132
COX2	0.266	0.003	0.269
tRNA-Lys	0.157	0.000	0.157
ATP8	0.271	0.000	0.271
ATP6	0.104	0.003	0.107
COX3	0.063	0.006	0.069
tRNA-Gly	0.059	0.000	0.059
ND3	0.035	0.003	0.038
tRNA-Arg	0.015	0.000	0.015
ND4L	0.034	0.003	0.037
ND4	0.030	0.015	0.045
tRNA-His	0.014	0.000	0.014
tRNA-Ser(AGY)	0.000	0.034	0.034
tRNA-Leu(CUN)	0.000	0.070	0.070
ND5	0.002	0.121	0.123
ND6	0.000	0.114	0.114
tRNA-Glu	0.000	0.014	0.014
CYTB	0.004	0.152	0.156
tRNA-Thr	0.000	0.318	0.318
tRNA-Pro	0.000	0.000	0.000
Control region (CR)	0.012	0.195	0.208
Non-coding (other than CR)	0.124	0.079	0.202

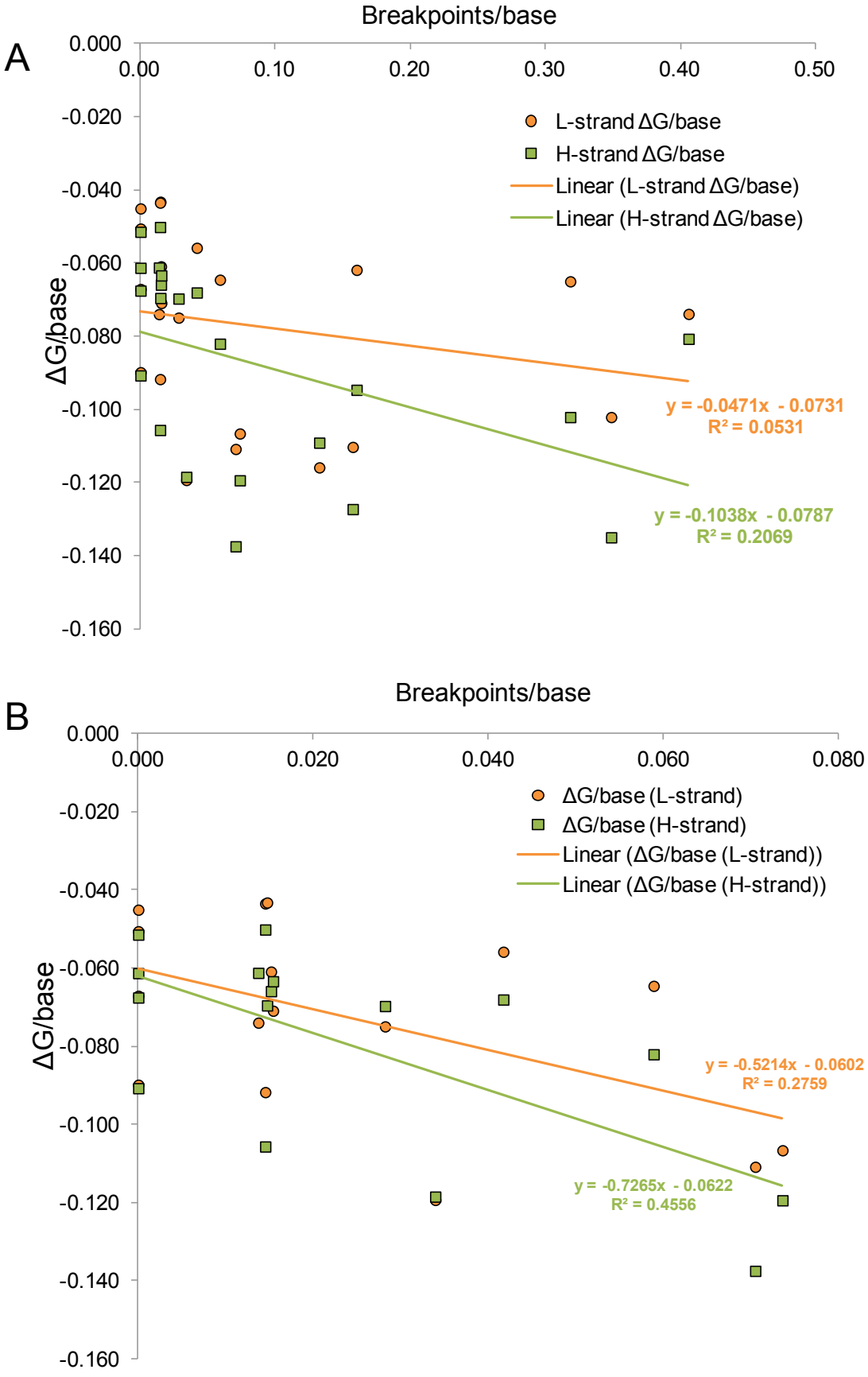
Supplemental Figure S14. Secondary structures of mitochondrial tRNA genes (L-strand). The secondary structures were obtained in the mfold-util software v4.6. ΔG values in kcal/mol.



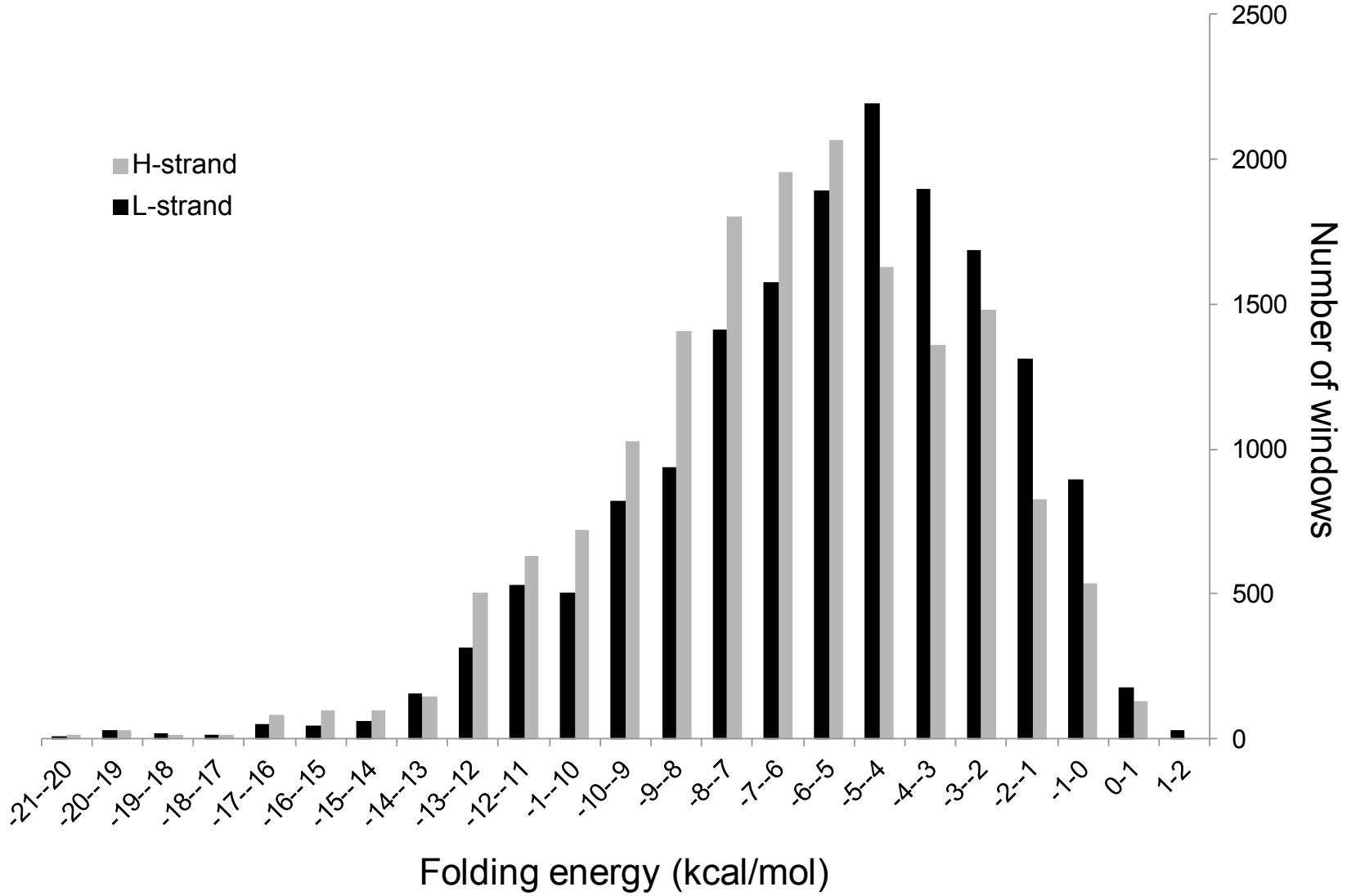
Supplemental Figure S15. Secondary structures of mitochondrial tRNA genes (H-strand). The secondary structures were obtained in the mfold-util software v4.6. ΔG values in kcal/mol.



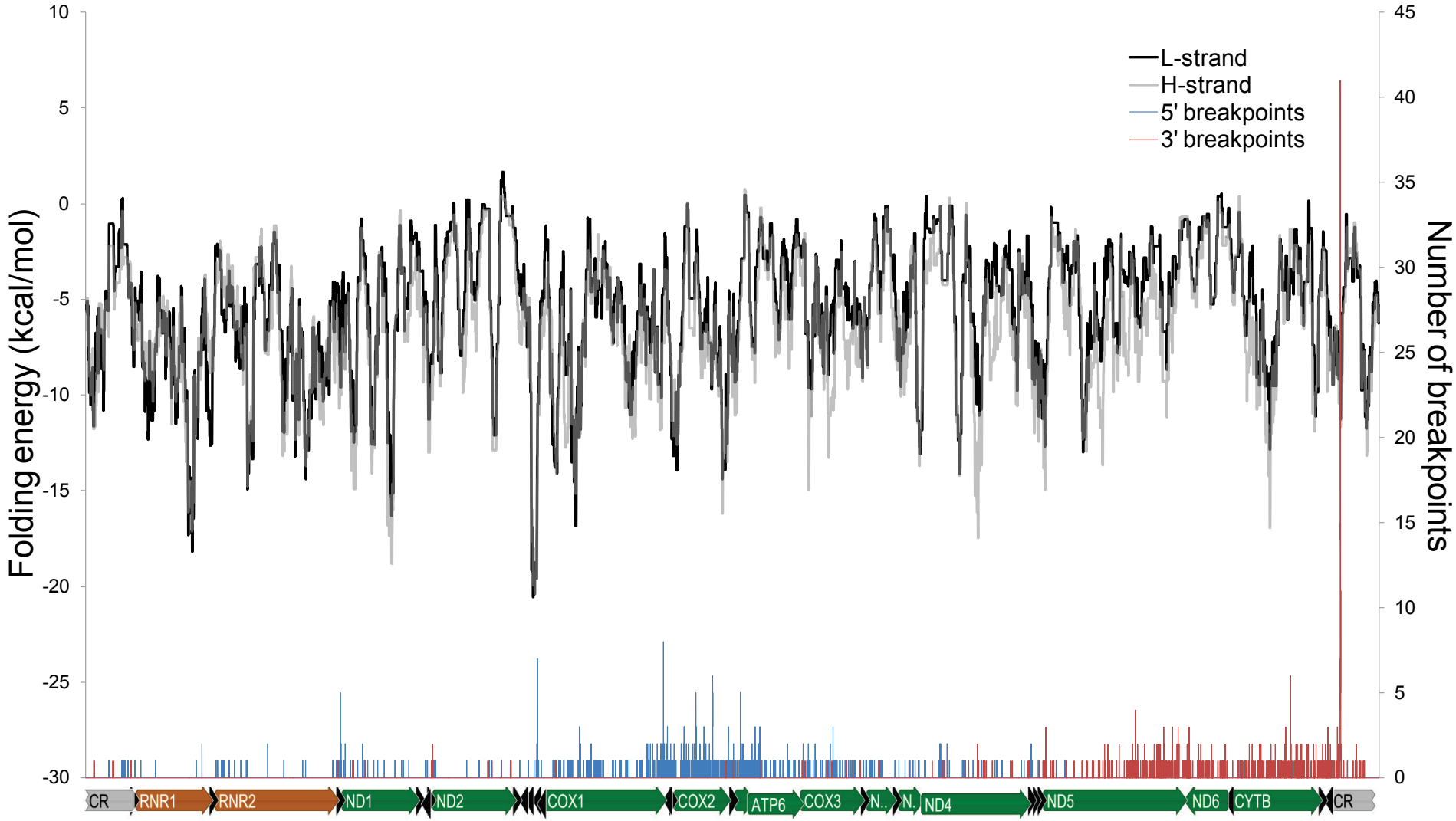
Supplemental Figure S16. Relationship between the folding free energy and the number of breakpoints in tRNA genes. (A) All tRNA gene sequences. (B) Only tRNA genes with less than 0.1 breakpoints per base. The linear trendlines are indicated for L- and H-strand tRNA gene sequences.



Supplemental Figure S17. The distribution of folding energies predicted for all of the 100-nt windows of the mitochondrial genome. The distribution of folding energies in 16,569 windows (length of 100 nt, overlapped by 1 nt) is shown for the L-strand (black bars) and H-strand (grey bars).



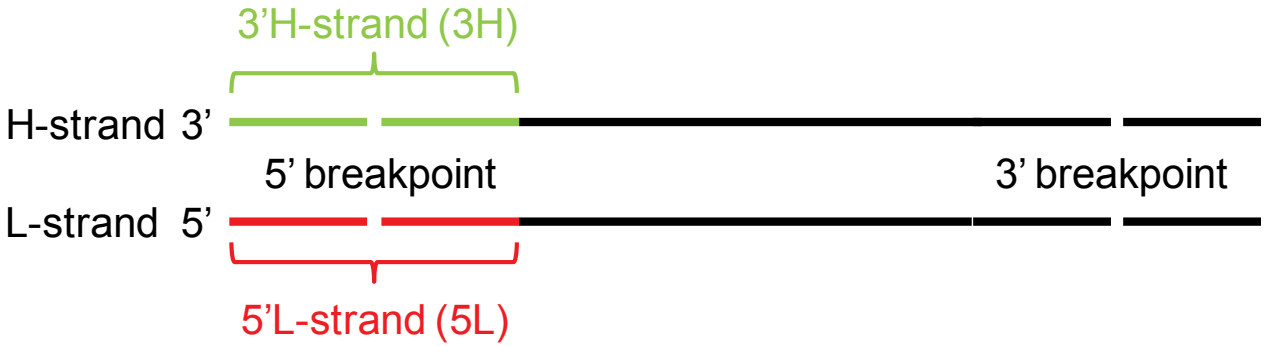
Supplemental Figure S18. Deletion hotspots and the mtDNA regions with high folding potentials. The profile of folding energy across the mitochondrial genome (sliding window of 100-nt with 1-nt overlap) is shown for the L-strand (black line) and H-strand (grey line). The distribution of 5’ (blue bars) and 3’ (red bars) deletion breakpoints is also indicated. The locations of the mitochondrial genes are shown below the x-axis.



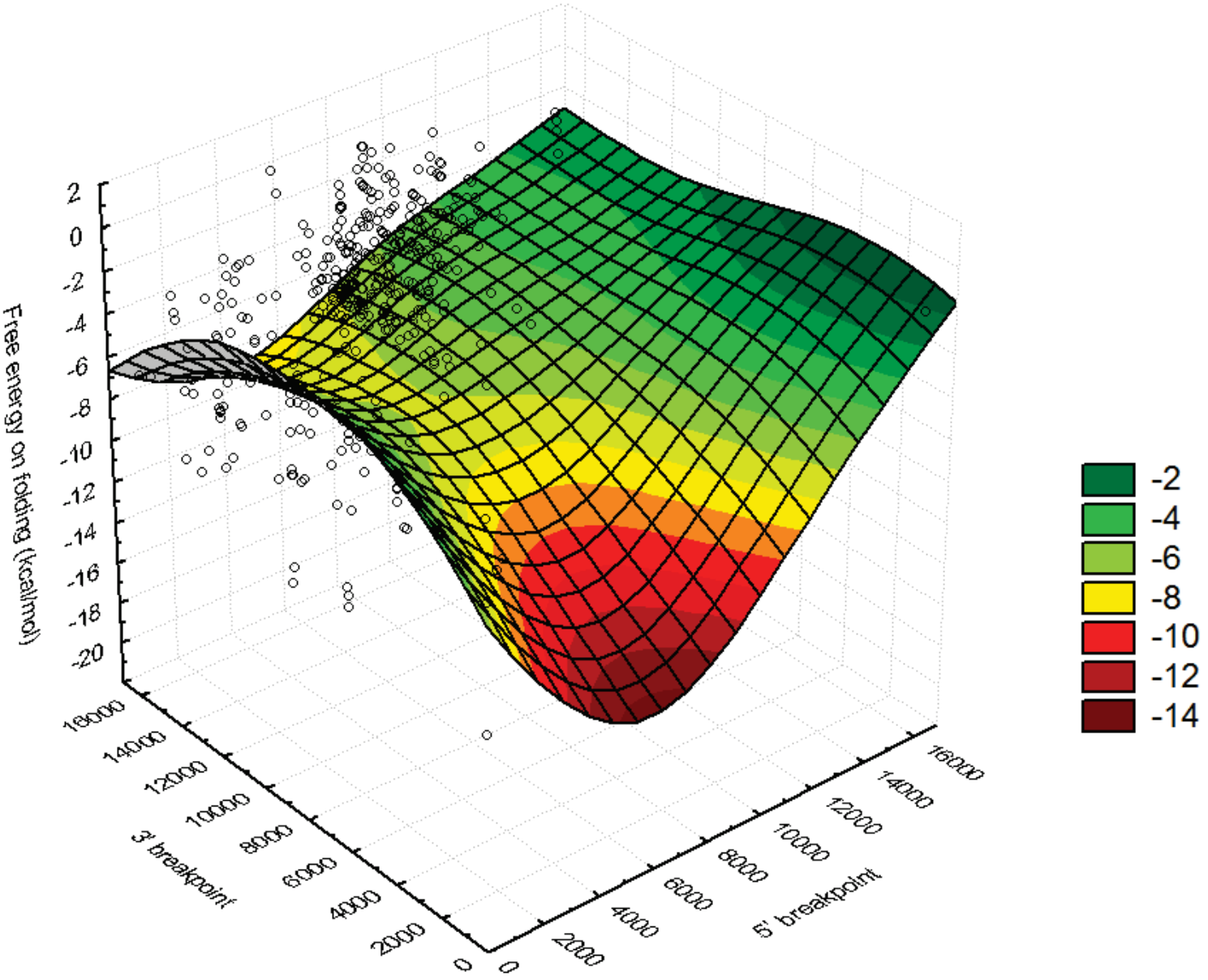
Supplemental Figure S19. Descriptive statistics of folding energies in deletion breakpoints areas. The energy of folding (kcal/mol) was estimated for the four 100-nt areas (in both H- and L-strands) around 5' and 3' breakpoints (breakpoints as midpoints) from 753 reported mtDNA deletions.

Breakpoint area	Mean	Median	Mode	Minimum	Maximum	Range
5L	-5.66	-5.38	-4.450	-19.580	1.27	20.85
3L	-5.93	-5.04	-11.27	-13.900	1.23	15.13
3H	-6.58	-6.73	-7.280	-19.900	0.76	20.66
5H	-7.01	-6.87	-11.65	-17.460	0.38	17.84

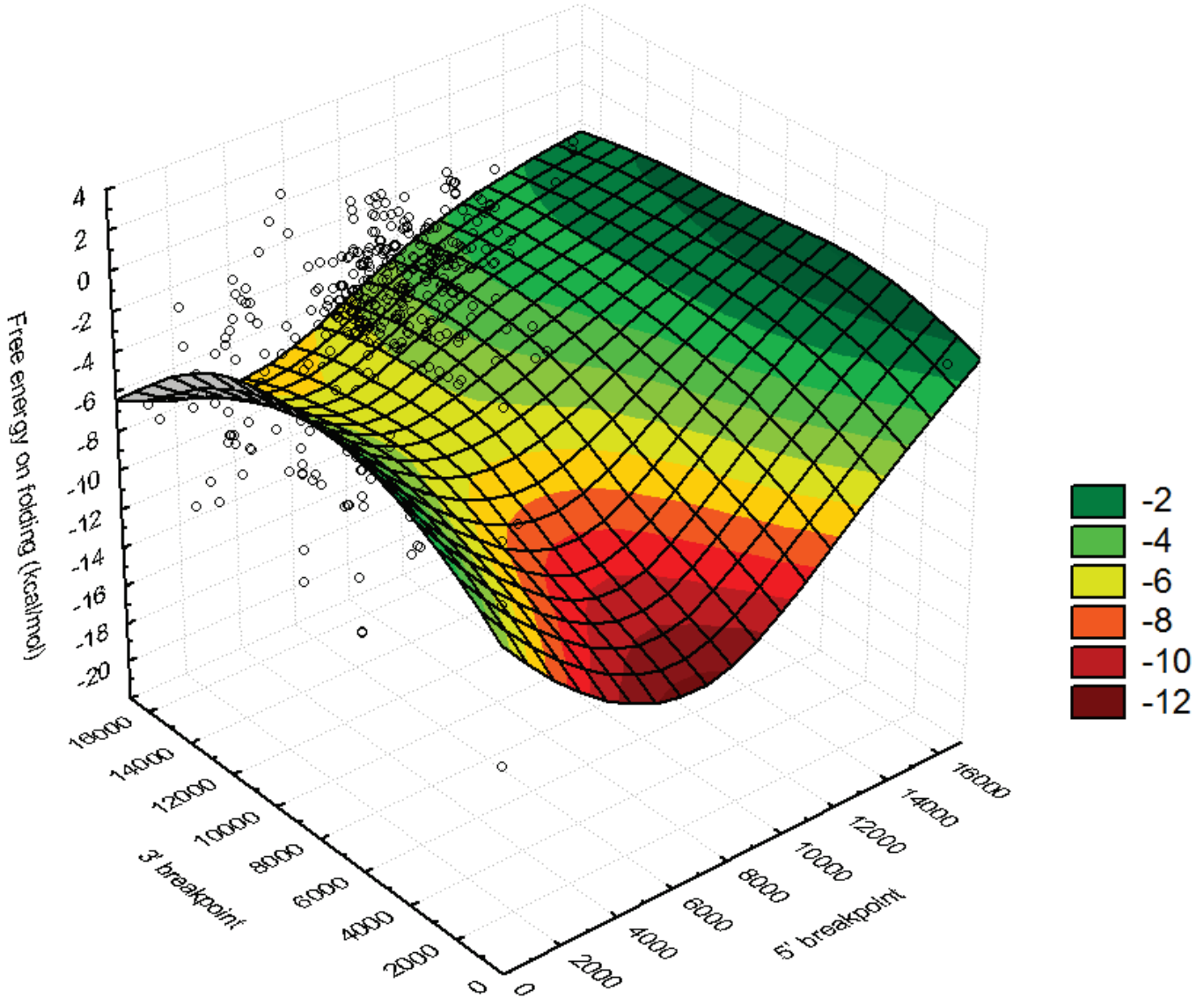
Supplemental Figure S20. Landscape of the folding potentials in the 5' breakpoint areas of mtDNA deletions. The three-dimensional plots display the locations of breakpoints (X and Y axes) according to the free energy of folding of the breakpoint area (z-axis). A surface is fitted to the data according to the distance-weighted least-squares smoothing procedure. A gradient from red (high folding potential) to green (low folding potential) is used in both graphs.



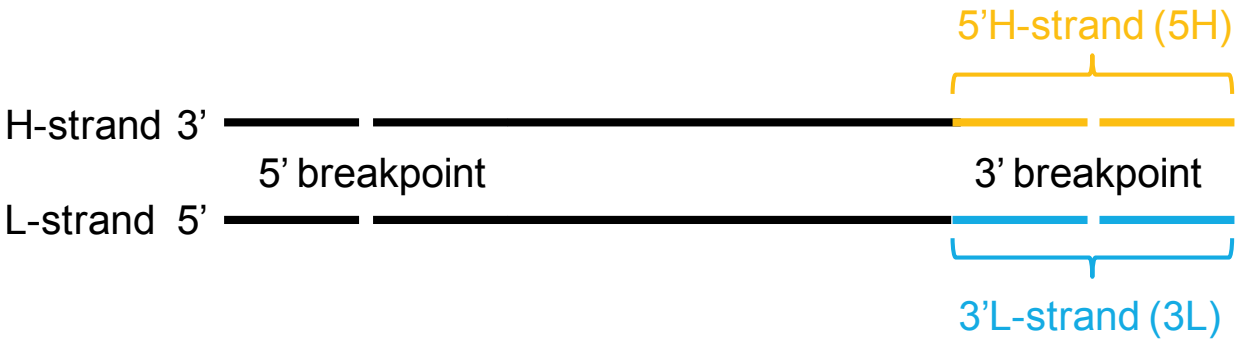
3H


Free energy on folding (kcal/mol)
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
3' breakpoint
16000
14000
12000
10000
8000
6000
4000
2000
0
5' breakpoint
0
2000
4000
6000
8000
10000
12000
14000
16000
-2
-4
-6
-8
-10
-12
-14

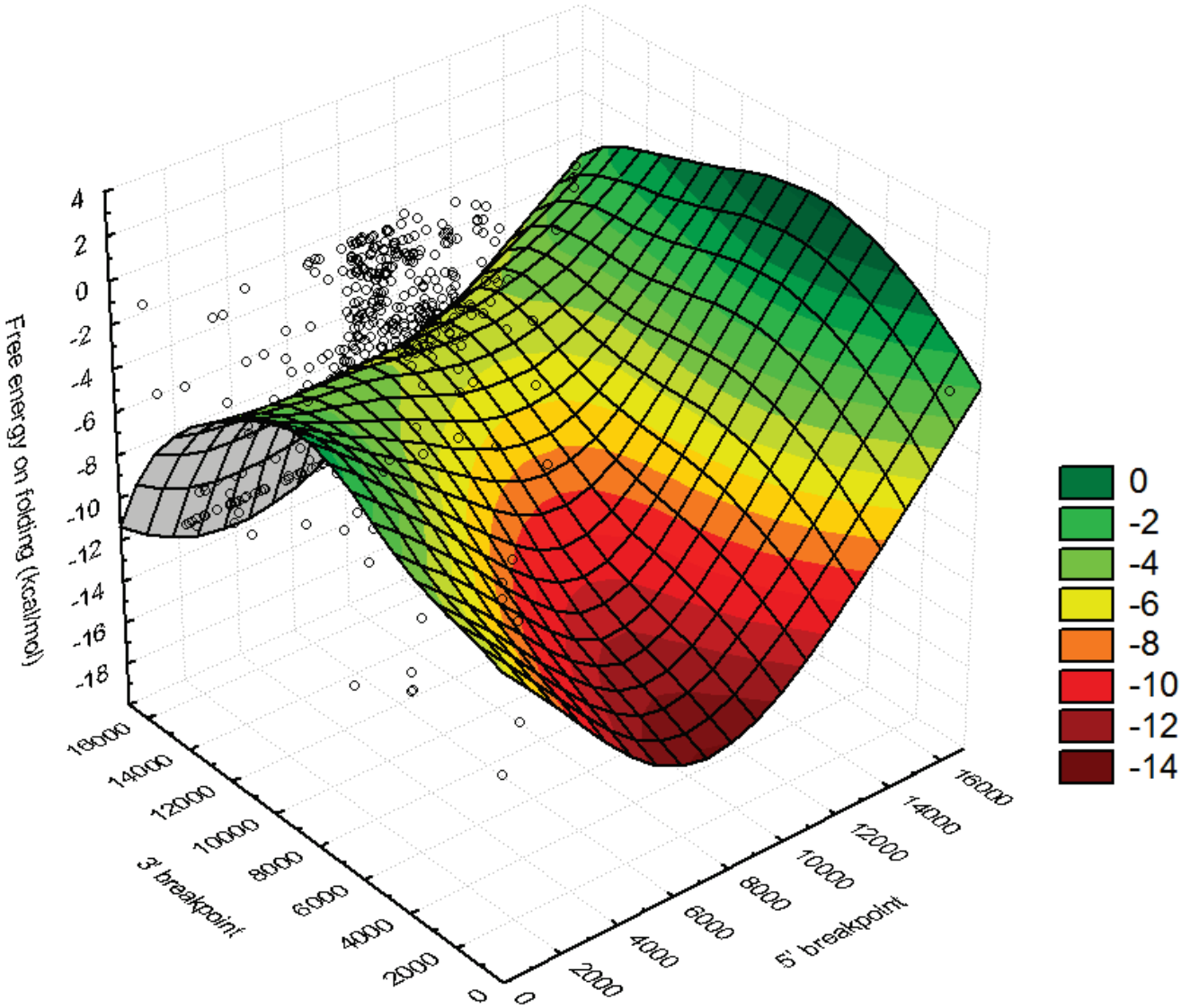
5L


Free energy on folding (kcal/mol)
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
3' breakpoint
16000
14000
12000
10000
8000
6000
4000
2000
0
5' breakpoint
-2
-4
-6
-8
-10
-12

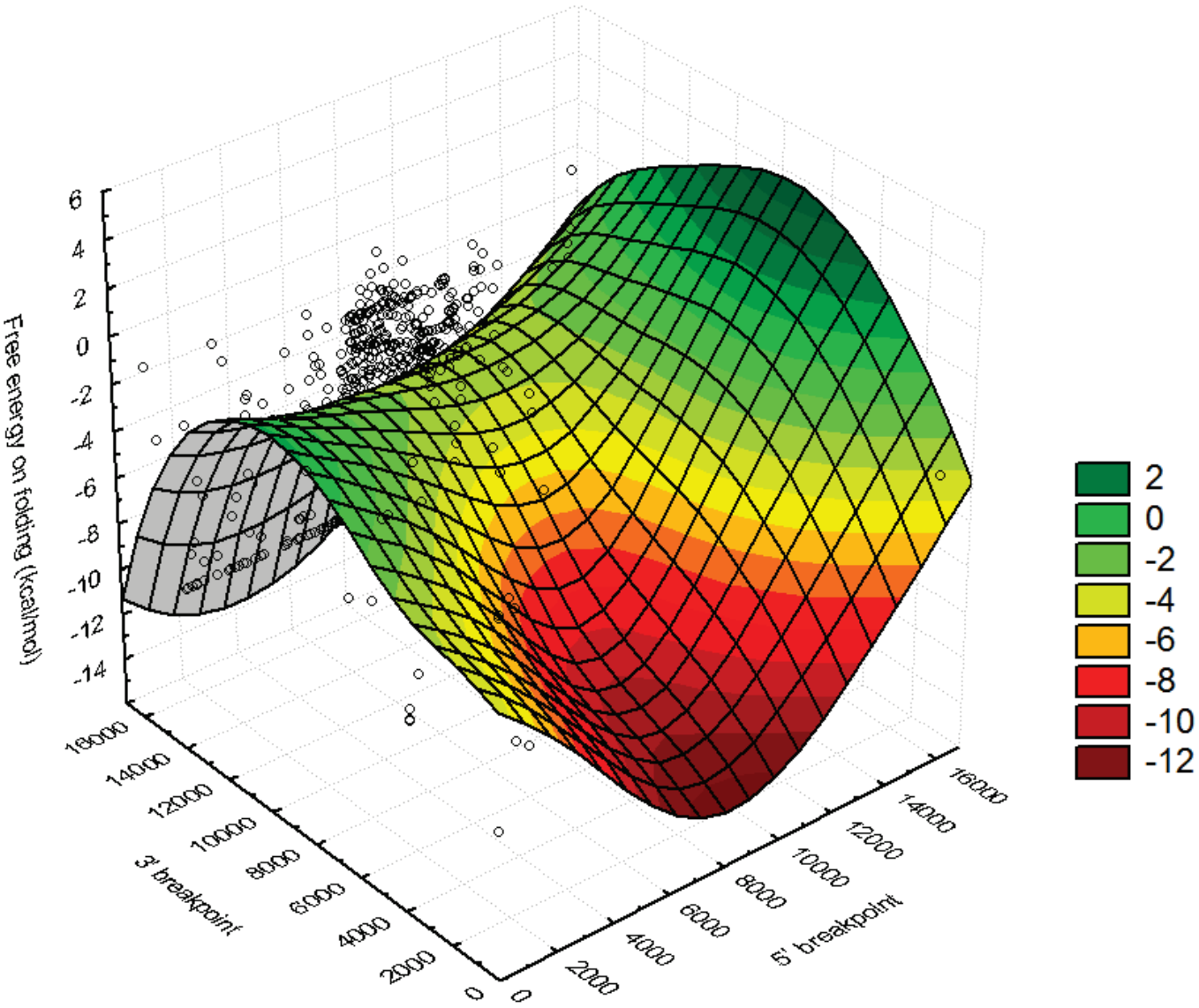
Supplemental Figure S21. Landscape of the folding potentials in the 3' breakpoint area of mtDNA deletions. The three-dimensional plots display the locations of breakpoints (X and Y axes) according to the free energy of folding of the breakpoint area (z-axis). A surface is fitted to the data according to the distance-weighted least-squares smoothing procedure. A gradient from red (high folding potential) to green (low folding potential) is used in both graphs.



5H


Free energy on folding (kcal/mol)
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
3' breakpoint
16000
14000
12000
10000
8000
6000
4000
2000
0
5' breakpoint
0
2000
4000
6000
8000
10000
12000
14000
16000
0
-2
-4
-6
-8
-10
-12
-14

3L


Free energy on folding (kcal/mol)
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
3' breakpoint
16000
14000
12000
10000
8000
6000
4000
2000
0
5' breakpoint
2
0
-2
-4
-6
-8
-10
-12

Supplemental Figure S22. Comparison between the two main breakpoint hotspots and their flanking regions. The table displays the genomic location, number of breakpoints and mean ΔG values for the two mtDNA segments where 5' and 3' breakpoints are more frequent and their upstream and downstream flanking segments with the same length.

	Upstream region	**Breakpoint hotspot**	**Downstream region**	**Upstream region**	**Breakpoint hotspot**	**Downstream region**
mtDNA region (length)	6,601 - 7,400 (800 nt)	7,401 - 8,200 (800 nt)	8,201 - 9,000 (800 nt)	6,601 - 7,400 (800 nt)	7,401 - 8,200 (800 nt)	8,201 - 9,000 (800 nt)
Gene or noncoding feature	*COX1* (partial)	*COX1* (partial) / *tRNA-Ser* / *tRNA-Asp* / *COX2* (partial)	*COX2* (partial) / *tRNA-Lys* / *ATP8* / *ATP6* (partial)	*COX1* (partial)	*COX1* (partial) / *tRNA-Ser* / *tRNA-Asp* / *COX2* (partial)	*COX2* (partial) / *tRNA-Lys* / *ATP8* / *ATP6* (partial)
Parameter	Number of 5' breakpoints (per base)			Mean ΔG (kcal/mol)		
Value	70 (0.09)	220 (0.28)	160 (0.20)	-6.18	-6.76	-4.26
***p*-value**		9.29×10^{-12}	1.36×10^{-2}		4.21×10^{-5}	$< 1.00 \times 10^{-17}$
mtDNA region (length)	15,901 – 16,000 (100 nt)	16,001 - 16,100 (100 nt)	16,101-16,200 (100 nt)	15,901 – 16,000 (100 nt)	16,001 - 16,100 (100 nt)	16,101-16,200 (100 nt)
Gene or noncoding feature	*tRNA-Thr* (partial) / *tRNA-Pro* (partial)	*tRNA-Pro* (partial) / control region (partial)	control region (partial)	*tRNA-Thr* (partial) / *tRNA-Pro* (partial)	*tRNA-Pro* (partial) / control region (partial)	control region (partial)
Parameter	Number of 3' breakpoints (per base)			Mean ΔG (kcal/mol)		
Value	22 (0.22)	201 (2.01)	2 (0.02)	-6.97	-8.32	-3.40
***p*-value**		7.05×10^{-3}	2.78×10^{-3}		1.85×10^{-11}	$< 1.00 \times 10^{-17}$