



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 2392-I

Mitochondrial DNA Sequencing (Human HL-60 DNA)

This Standard Reference Material (SRM) is intended to provide quality control when performing the polymerase chain reaction (PCR) and sequencing of human mitochondrial DNA (mtDNA) for forensic identification, medical diagnosis, or mutation detection. It may also serve as a control when amplifying (PCR) and sequencing any DNA. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is certified for the sequences of the entire human mtDNA (16 569 base pairs) from a promyelocytic cell line (HL-60) prepared from the peripheral blood leukocytes from an individual with acute promyelocytic leukemia. A unit of SRM 2392-I consists of 65 μL of extracted DNA from cell culture line HL-60 at a nominal concentration of 1.4 $\text{ng}/\mu\text{L}$, which is contained in a vial packaged in a protective plastic box.

Certified Values: The certified sequence information of extracted human DNA from HL-60 is provided in Table 1. Also provided in Table 1 is the certified sequence information for two additional entire mtDNA templates, CHR and GM09947A, which are provided in SRM 2392. SRM 2392-I only contains the HL-60 template. Table 2 contains the sequences of 58 unique primer sets that were designed to amplify any portion or the entire human mtDNA [1].

Supplemental Information: The sequence information of an additional two DNA templates, (GM03798 [1] and GM10742A [2]), that were amplified and sequenced in their entirety multiple times at NIST are provided in references 1 or 2. Although the extracted DNA from GM03798 and GM10742A are not provided, the cell cultures can be obtained from NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ. A schematic of the differences from the Cambridge Reference Sequence [3] found in the mtDNA from all five templates is shown in Figure 1.

Expiration of Certification: The certification of this SRM is valid until **31 March 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. This certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The analytical determination, technical measurements and analysis of data for the certification of this SRM were performed by D.K. Hancock, K.L. Richie, K.A. Holland (on sabbatical from Gettysburg College, Gettysburg, PA), and B.C. Levin of the NIST DNA Technologies Group, Biotechnology Division.

The overall direction and coordination of the technical measurements leading to the certification was performed by B.C. Levin of the NIST DNA Technologies Group, Biotechnology Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

Vincent L. Vilker, Chief
Biotechnology Division

John Rumble, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: 13 June 2003

Support for the preparation and certification of this SRM was provided by the National Institute of Justice through the NIST Office of Law Enforcement Standards.

NOTICE AND WARNINGS TO USER

Warning: SRM 2392-I IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

Permissions: The research to use HL-60 DNA in SRM 2392-I was deemed exempt from the policy of Part 27 of Title 15 of the Code of Federal Regulations by the NIST Institutional Review Board and the Director of the Chemical Science and Technology Laboratory. This work fit into the exemption category described in 15 CFR 27.101(b)(4) which states: "Research, involving the collection or study of existing data, documents, pathological specimens, or diagnostic specimens, if, these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects."

ATCC also waived condition 3(c) in their Material Transfer Agreement which states that the "purchaser shall not sell, lend, distribute or otherwise transfer the material or replicates to any others" for the use of HL-60 in the NIST mitochondrial DNA SRM. They stated that, in their view, "as a government agency, NIST will not be providing this material as a commercial product despite the collection of fees for the SRM."

Storage: Store frozen at a temperature of -20°C . **DO NOT** use a self-defrosting freezer because of periodic cycling of temperatures may shorten the shelf life of this SRM.

INSTRUCTIONS FOR USE

It is recommended that once thawed, each SRM component should be used in its entirety. Repeated freezing and thawing is **NOT** recommended as this might shorten the shelf life of the SRM. If it is necessary to perform repeated analyses, thaw the SRM and divide the tube contents into aliquots that will be kept frozen until use. Thawing can be conducted at refrigerator temperatures, room temperature, or at 37°C . Once thawed, the sample should be processed without delay. DNA concentrations given are nominal values and are **NOT** intended for use as concentration standards.

SOURCE AND ANALYSIS¹

Source of Material: DNA from HL-60 was prepared by the Professional Services Department of the American Type Culture Collection (ATCC), Manassas, VA. This material was subsequently vialled at NIST into 65 μL portions (nominal DNA concentration of 1.4 ng/ μL) and labeled SRM 2392-I Component D (Components A, B, and C are available in SRM 2392).

NIST Analysis: PCR was used to amplify the HL-60 DNA in its entirety multiple times using all 58 primer sets. The PCR products were sequenced with an Applied Biosystems, Inc. 310 automated sequencer. The sequences of representative PCR products of the final HL-60 DNA included in SRM 2392-I were reanalyzed to ensure sequence accuracy.

Interlaboratory Analyses: An interlaboratory evaluation of the amplification, sequencing and data analysis of the HL-60 template was conducted by four laboratories, including NIST. These laboratories were: The Armed Forces DNA Identification Laboratory (AFDIL), Rockville, MD; Federal Bureau of Investigation Laboratory (FBI), Quantico, VA; and The Georgia Bureau of Investigation (GBI), Decatur, GA. The sequences obtained by all of the laboratories were identical. Description of the interlaboratory analysis of HL-60 is described in reference 2.

¹Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified Human mtDNA Sequence Differences from the Cambridge Reference Sequence (CRS) [3,4] Found in the Two Templates (CHR and GM09947A) in NIST SRM 2392 and One Template (HL-60) in NIST SRM 2392-I

Comparison with the Cambridge Reference Sequence (CRS)						
CRS						
# ^a	Base ^b 1981/ 1999	Template CHR ^d	Template 9947A ^d	Template HL-60 ^e	Amino acid change	Region
73	A	G	-	G		HV2
93	A	-	G	-		HV2
150	C	-	-	T		HV2
152	T	-	-	C		HV2
195	T	C	C	-		HV2
204	T	C	-	-		HV2
207	G	A	-	-		HV2
214	A	-	G	-		HV2
263*R	A	G	G	G		HV2
295	C	-	-	T		HV2
303-309	-	C (ins)	CC (ins)	-		HV2
311-315*R	-	C (ins)	C (ins)	C (ins)		HV2
489	T	-	-	C		HV2
709	G	A	-	-		12sRNA
750 *R	A	G	G	G		12sRNA
1438*R	A	G	G	G		12sRNA
1719	G	A	-	-		16sRNA
2706	A	G	-	G		16sRNA
3106-3107*E	CC/del	del C	del C	del C		16sRNA
3423*E	G/T	T	T	T	Silent	ND1
4135	T	-	C	-	Tyr → His	ND1
4216	T	-	-	C	Tyr → His	ND1 LHON
4769*R	A	G	G	G	Silent	ND2
4985*E	G/A	A	A	A	Silent	ND2
5186	A	G	-	-	Silent	ND2
5228	C	-	-	G	Silent	ND2
5633	C	-	-	T		tRNA Ala
6221	T	C	-	-	Silent	COI
6371	C	T	-	-	Silent	COI

Comparison with the Cambridge Reference Sequence (CRS)						
CRS						
# ^a	Base ^b 1981/ 1999	Template CHR ^d	Template 9947A ^d	Template HL-60 ^e	Amino acid change	Region
6791	A	G	-	-	Silent	COI
6849 ^h	A	G(0.3A) ^h	-	-	Thr → Ala ^h	COI
7028	C	T	-	T	Silent	COI
7476	C	-	-	T		tRNA Ser
7645	T	-	C	-	Silent	COII
7861	T	-	C	-	Silent	COII
8448	T	-	C	-	Met → Thr	ATPase 8
8503	T	C	-	-	Silent	ATPase 8
8860*R	A	G	G	G	Thr → Ala	ATPase 6
9315	T	-	C	-	Phe → Leu	COIII
9559*E	G/C	C	C	C	Arg → Pro	COIII
10172	G	-	-	A	Silent	ND3
10398	A	-	-	G	Thr → Ala	ND3
11251	A	-	-	G	Silent	ND4
11335*E	T/C	C	C	C	Silent	ND4
11719	G	A	-	A	Silent	ND4
11878	T	C	-	-	Silent	ND4
12071 ^{het}	T	-	-	C/T ^{het}	Phe→Leu ^{het}	ND4
12612	A	G	-	G	Silent	ND5
12705	C	T	-	-	Silent	ND5
13572	T	-	C	-	Silent	ND5
13702*E	G/C	C	C	C	Gly → Arg	ND5
13708	G	A	-	A	Ala → Thr	ND5 LHON
13759	G	-	A	-	Ala → Thr	ND5
13966	A	G	-	-	Thr → Ala	ND5
14199*E	G/T	T	T	T	Pro → Thr	ND6
14272*E	G/C	C	C	C	Phe → Leu	ND6
14365*E	G/C	C	C	C	Silent	ND6
14368*E	G/C	C	C	C	Phe → Leu	ND6
14470	T	C	-	-	Silent	ND6
14569	G	-	-	A	Silent	ND6
14766*E	T/C	T	C	T	Ile → Thr	ND6
15257	G	-	-	A	Asp → Asn	CYT B LHON

Comparison with the Cambridge Reference Sequence (CRS)						
CRS						
# ^a	Base ^b 1981/ 1999	Template CHR ^d	Template 9947A ^d	Template HL-60 ^e	Amino acid change	Region
15326*R	A	G	G	G	Thr → Ala	CYT B
15452	C	-	-	A	Leu → Ile	CYT B
15812	G	-	-	A	Val → Met	CYT B LHON
16069	C	-	-	T		HV1
16183	A	C	-	-		HV1
16184-93	-	C (ins)	-	-		HV1
16189	T	C	-	-		HV1
16193	C	-	-	T		HV1
16223	C	T	-	-		HV1
16278	C	T	-	T		HV1
16311	T	-	C	-		HV1
16362	T	-	-	C		HV1
16519	T	C	C	-		HV1

^a Numbers correspond to Cambridge Reference Sequence [3]

^b Base found in 1981[3] Base found in 1999 [4]

^d SRM 2392, reference 1

^e SRM 2392-I, reference 2

- Base pair same as in 1981 Cambridge Reference Sequence [3]

^h Possible heteroplasmic site. This heteroplasmy seen in the mtDNA from the first CHR cell culture line is not seen in the mtDNA from the second CHR cell culture line. The second CHR cell culture line agrees with the CRS at np 6849. It is DNA from the second CHR cell culture line that is supplied in NIST SRM 2392.

*R Rare polymorphisms in Cambridge Reference Sequence discovered by reanalysis of original placenta by Andrews et al., 1999 [4].

*E Error in Cambridge Reference Sequence discovered by reanalysis of original placenta by Andrews et al., 1999 [4].

del Deletion

ins Insertion

het Heteroplasmy found in HL-60 at np 12071

HV1 Non-coding region found from 16024 and 16569

HV2 Non-coding region found from 1 and 576

CHR DNA Sequence based on two amplifications and cycle sequencing procedures with DNA from the first cell culture line and at least one amplification and cycle sequencing procedure with DNA from the second cell culture line.

GM09947A DNA Sequence based on two amplifications and cycle sequencing procedures.

HL-60 DNA Sequence based on two amplifications and cycle sequencing procedures in both the forward and reverse directions for a total of 4 sequences.

ATPase 6 ATP synthase 6

ATPase 8 ATP synthase 8

CYTB Cytochrome B

COI Cytochrome C Oxidase I

COII Cytochrome C Oxidase II

COIII Cytochrome C Oxidase III

LHON Leber Hereditary Optic Neuropathy

ND1 NADH dehydrogenase 1

ND2 NADH dehydrogenase 2

ND3 NADH dehydrogenase 3

ND4 NADH dehydrogenase 4

ND5 NADH dehydrogenase 5

ND6 NADH dehydrogenase 6

Table 2. Reference Sequences for Primer Sets Used for PCR Amplification of Human mtDNA

Primer Set Number	Primer Sequence
1(HV2)	F15 CACCCTATTAACCACTCACG
	R484 TGAGATTAGTAGTATGGGAG
2	F361 ACAAAGAACCCTAACACCAGC
	R921 ACTTGGGTTAATCGTGTGACC
3	F756 CATCAAGCACGCAGCAATG
	R1425 AATCCACCTTCGACCCTTAAG
4	F873 GGTTGGTCAATTCGTGCCAG
	R1425 AATCCACCTTCGACCCTTAAG
5	F1234 CTCACCACCTCTTGCTCAGC
	R1769 GCCAGGTTTCAATTTCTATCG
6	F1587 TGCACCTGGACGAACCAGAG
	R2216 TGTTGAGCTTGAACGCTTTC
7	F1657 CTTGACCGCTCTGAGCTAAAC
	R2216 TGTTGAGCTTGAACGCTTTC
8	F1993 AAACCTACCGAGCCTGGTG
	R2216 TGTTGAGCTTGAACGCTTTC
9	F2105 GAGGAACAGCTCTTTGGACAC
	R2660 AGAGACAGCTGAACCCTCGTG
10	F2417 CACTGTCAACCCAACACAGG
	R3006 ATGTCCTGATCCAACATCGAG
11	F2834 CCAACCTCCGAGCAGTACATG
	R3557 AGAAGAGCGATGGTGAGAGC
12	F2972 ATAGGGTTTACGACCTCGATG
	R3557 AGAAGAGCGATGGTGAGAGC
13	F3234 AGATGGCAGAGCCCGGTAATC
	R3557 AGAAGAGCGATGGTGAGAGC
14	F3441 ACTACAACCCTTCGCTGACG
	R3940 TGAAGCCTGAGACTAGTTCGG
15	F3635 GCCTAGCCGTTTACTCAATCC
	R4162 TGAGTTGGTCGTAGCGGAATC
16	F3931 TCAGGCTTCAACATCGAATACG
	R4728 TTATGGTTCATTGTCCGGAGAG
17	F4183 TTTCTACCACTCACCTAGCATTAC
	R4728 TTATGGTTCATTGTCCGGAGAG
18	F4392 CCCATCCTAAAGTAAGGTCAGC
	R4983 GGTTTAATCCACCTCAACTGCC
19	F4447 TTGGTTATAACCCTTCCCCTAC
	R4982 GTTTAATCCACCTCAACTGCC
20	F4797 CCCTTTCACCTTCTGAGTCCCAG
	R5553 AGGGCTTTGAAGGCTCTTG
21	F4976 ATTAACCAGACCCAGCTACG
	R5553 AGGGCTTTGAAGGCTCTTG
22	F5318 CACCATCACCTCCTTAACC
	R5882 GCTGAGTGAAGCATTGGACTG
23	F5700 TAAGCACCTAATCAACTGGC
	R6262 GCCTCCACTATAGCAGATGCG
24	F5999 TCTAAGCCTCCTTATTCGAGC
	R6526 ATAGTGATGCCAGCAGCTAGG
25	F6242 CGCATCTGCTATAGTGGAGG
	R6526 ATAGTGATGCCAGCAGCTAGG
26	F6426 GCCATAACCAATACCAAACG
	R7030 TGGGCTACAACGTAGTACGTG
27	F6744 GGCTTCCTAGGGTTTATCGTG
	R7255 TTTCATGTGGTGTATGCATCG

Primer Set Number	Primer Sequence
28	F7075 GAGGCTTCATTCCTGATTTCC R7792 GGGCAGGATAGTTCAGACGG
29	F7215 CGACGTTACTCGGACTACCC R7792 GGGCAGGATAGTTCAGACGG
30	F7645 TATCACCTTTCATGATCACGC R8215 GACGATGGGCATGAAACTG
31	F7901 TGAACCTACGAGTACACCGACTAC R8311 AAGTTAGCTTTACAGTGGGCTCTAG
32	F8164 CGGTCAATGCTCTGAAATCTGTG R8669 CATTGTTGGGTGGTATTAGTCG
33	F8539 CTGTTCGCTTCATTCATTGCC R9059 GTGGCGCTCCAATTAGGTG
34	F8903 CCCACTTCTTACCACAAGGC R9403 GTGCTTTCTCGTGTACATCG
35	F9309 TTTCACTTCCACTCCATAACGC R9848 GAAAGTTGAGCCAATAATGACG
36	F9449 CGGGATAATCCTATTTATTACCTCAG R9995 AGAGTAAGACCCTCATCAATAGATGG
37	F9754 AGTCTCCCTCACCATTTCGG R10275 AAAGGAGGGCAATTTCTAGATC
38	F10127 ACTACCACAACCAACGGCTAC R10556 GGAGGATATGAGGTGTGAGCG
39	F10386 GGATTAGACTGAACCGAATTGG R11166 CATCGGGTGATGATAGCCAAG
40	F10704 GTCTCAATCTCCAACACATATGG R11267 TGTGTGAGTGAAATTAGTGCG
41	F11001 AACGCCACTTATCCAGTGAACC R11600 CTGTTTGTCTAGGCAGATGG
42	F11403 GACTCCCTAAAGCCCATGTCTG R11927 TTGATCAGGAGAACGTGGTTAC
43	F11760 ACGAACGCACTCACAGTCG R12189 AAGCCTCTGTTGTCAGATTAC
44	F11901 TGCTAGTAACCACGTTCTGGTG R12876 GATATCGCCGATACGGTTG
45	F12357 AACCACCCTAACCCCTGACTTCC R12876 GATATCGCCGATACGGTTG
46	F12601 TTCATCCCTGTAGCATTGTTCTG R13123 AGCGGATGAGTAAGAAGATTCC
47	F12793 TTGCTCATCAGTTGATGATACG R13343 TTGAAGAAGGCGTGGGTACAG
48	F13188 CACTCTGTTCCGAGCAGTATG R13611 TCGAGTGCTATAGGCGCTTGTC
49	F13518 CATCATCGAAACCGCAAAC R13935 TGTGATGCTAGGGTAGAATCCG
50	F13715 GAAGCCTATTTCGAGGATTC R14118 TGGGAAGAAGAAAGAGAGGAAG
51	F13899 TTTCTCCAACATACTCGGATTC R14388 TTAGCGATGGAGGTAGGATTGG (New Primer)
	R14388 TTAGCGATGGAGGTAGGATTCC (Old Primer)
52	F14189 ACAAACAATGGTCAACCAGTAAC R14926 TGAGGCGTCTGGTGAGTAGTGC
53	F14470 TCCAAAGACAACCATCATTCC R14996 CGTGAAGGTAGCGGATGATTC
54	F14909 TACTACCAGACGCCTCAACCG R15396 TTATCGGAATGGGAGGTGATTC
55	F15260 AGTCCCACCCTCACACGATTC R15774 ACTGGTTGTCCTCCGATTCAGG

Primer Set Number		Primer Sequence
56	F15574	CGCCTACACAATTCTCCGATC
	R16084	CGGTTGTTGATGGGTGAGTC
57 (HV1)	F15971	TAACTCCACCATTAGCACC
	R16451	GCGAGGAGAGTAGCACTCTTG
58	F16097	TACATTACTGCCAGCCACCATG
	R336	TTAAGTGCTGTGGCCAGAAG
-21M13	F	TGAAAACGACGGCCAGT

HV2: Hypervariable region 2

HV1: Hypervariable region 1

F: forward primer

R: reverse primer

These are the same primers used for SRM 2392 and reference 1 except the reverse primer of set 51 has been changed to: TTAGCGATGGAGGTAGGATTGG. The change (C to G) occurs at np 14368 and is in bold and underlined. Those using SRM 2392 should also use the new reverse primer 51.

REFERENCES

- [1] Levin, B.C.; Cheng, H.; Reeder, D.J.; *A Human Mitochondrial DNA Standard Reference Material for Quality Control in Forensic Identification, Medical Diagnosis, and Mutation Detection*; Genomics, Vol. 55, pp. 135-146 (1999).
- [2] Levin, B.C.; Holland, K.A.; Hancock, D.K.; Coble, M.; Parsons, T.J.; Kienker, L.J.; Williams, D.W.; Jones, M.P.; Richie, K.L.; *Comparison of the Complete mtDNA Genome Sequences of Human Cell Lines - HL-60 and GM10742A - from Individuals with Promyelocytic Leukemia and Leber Hereditary Optic Neuropathy, Respectively, and the Inclusion of HL-60 in the NIST Human Mitochondrial DNA Standard Reference Material - SRM 2392-I*; Mitochondrion, Vol. 2, pp. 386-399 (2003).
- [3] Anderson, S.; Bankier, A.T.; Barrell, B.G.; deBruijn, M.H.L.; Coulson, A.R.; Drouin, J.; Eperon, I.C.; Nierlich, D.P.; Roe, B.A.; Sanger, F.; Schreier, P.H.; Smith, A.J.H.; Staden, R.; Young, I.G.; *Sequence and Organization of the Human Mitochondrial Genome*; Nature, Vol. 290, pp. 457-465 (1981).
- [4] Andrews, R.M.; Kubacka, I.; Chinnery, P.F.; Lightowlers, R.N.; Turnbull, D.M.; Howell, N.; *Reanalysis and Revision of the Cambridge Reference Sequence for Human Mitochondrial DNA*; Nature Genetics, Vol. 23, p. 147 (1999).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet <http://www.nist.gov/srm>.

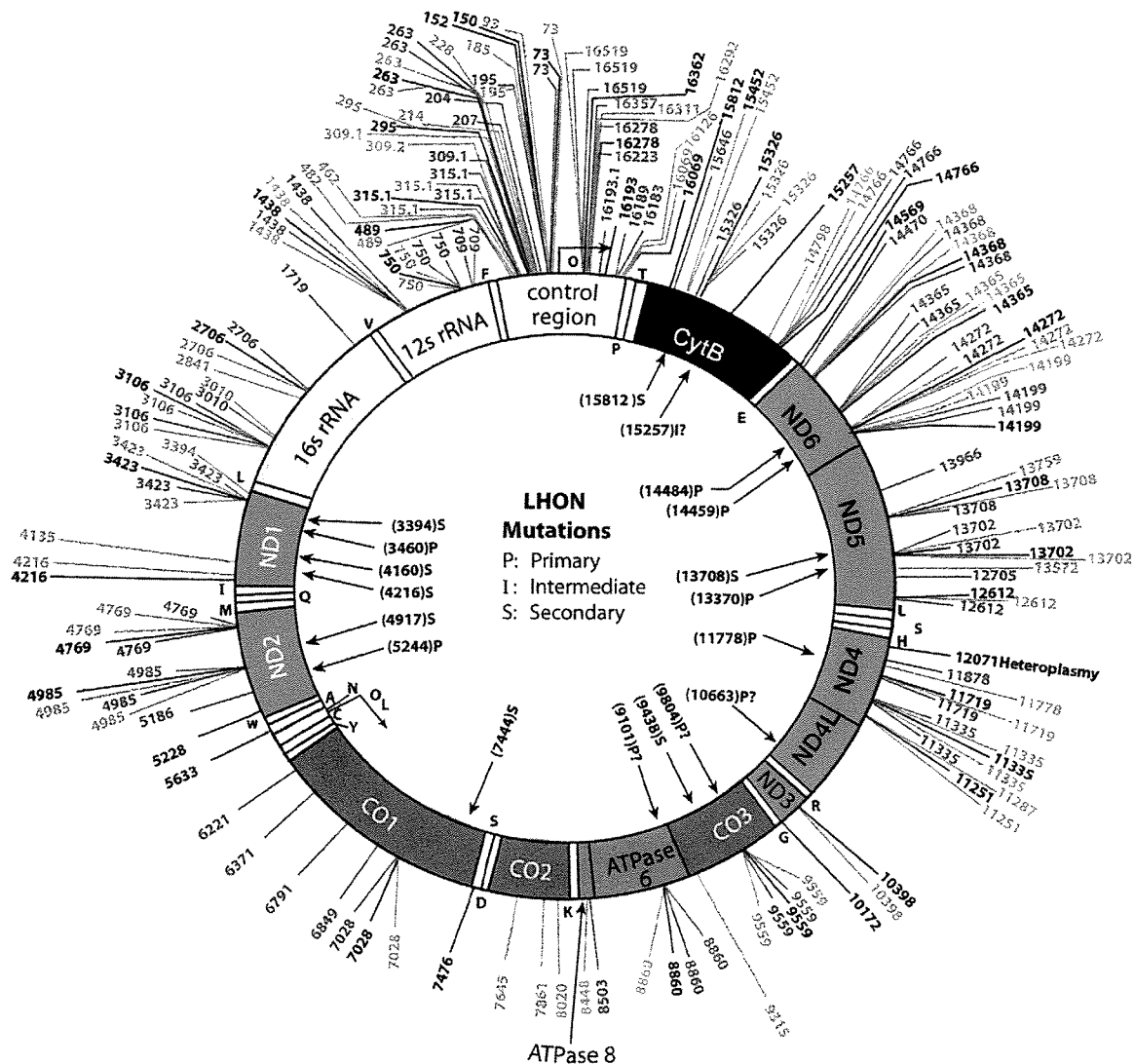


Figure 1. Schematic of human mtDNA showing its circular double-stranded DNA and all the differences from Cambridge Reference Sequence (1981) found in CHR (red), 9947A (green), HL-60 (black), GM03798 (blue), and GM10742A (purple) as numbers along the outside of the color-coded circle. Locations of the control region, rRNAs and genes coded by human mtDNA are shown. The locations of the 22 tRNAs are noted by white areas in the circle and designated by their single letter amino acid code. Since a number of mutations found in GM10742A and HL-60 and one change in CHR have been associated with primary, intermediate or secondary mutations linked to the disease Leber Hereditary Optic Neuropathy (LHON), the position of these mutations plus other LHON mutations are shown on the inside of the circle (Wallace et al., 1997). The question mark following the np of the LHON mutations indicates the assignment is not confirmed. One of the primary mutations that have been associated with LHON, G11778A, was found in GM10742A [2] but not found in the other DNA templates examined in this research. (Modified from Levin et al., 1999).