



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 μ L of extracted DNA from cell culture line HL-60 at a nominal concentration of 1.4 ng/ μ L, which is contained in a vial packaged in a protective plastic box.

Certified Sequence Information: 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 (CRS) [3] found in the mtDNA from all five templates is shown in Figure A1 of the Appendix.

Expiration of Certification: The certification of **SRM 2392-I** is valid, within the measurement uncertainty specified, until **31 March 2018**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise 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. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification was performed by B.C. Levin of the NIST Applied Genetics Group, Biomolecular Measurement Division. 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.

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.

Support aspects involved in the preparation of this SRM were coordinated through the NIST Office of Reference Materials.

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WARNING TO USERS

Warning: SRM 2392-I is a human source material. Since there is no consensus on the infectious status of extracted DNA, handle SRM 2392-I components as Biosafety Level 1 Material, capable of transmitting infectious disease [3]. SRM 2391-I components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

NOTICE TO USERS

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 fits into the exemption category described in 15 CFR 27.101(b)(4) which states as follows. “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.”

The Professional Services Department of the American Type Culture Collection (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”.

INSTRUCTIONS FOR STORAGE AND USE

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.

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 ANALYSIS⁽¹⁾

Source of Material: DNA from HL-60 was prepared by the 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.

⁽¹⁾Certain commercial equipment, instruments, or materials are identified in this report 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 ^(c)	Template 9947A ^(c)	Template HL-60 ^(d)	Amino Acid Change	Region
73	A	G	- ^(e)	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
6791	A	G	-	-	Silent	COI
6849 ^(het1)	A	G(0.3A) ^(het1)	-	-	Thr → Ala ^(het1)	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

Comparison with the Cambridge Reference Sequence (CRS)						
CRS						
# ^(a)	Base ^(b) 1981/1999	Template CHR ^(c)	Template 9947A ^(c)	Template HL-60 ^(d)	Amino Acid Change	Region
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 ^(het2)	T	-	-	C/T ^(het2)	Phe→Leu ^(het2)	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
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 CRS [3].

^(b) Base found in 1981 [3]/Base found in 1999 [4].

^(c) The certified sequence information for two additional entire mtDNA templates, CHR and GM09947A, which are provided in SRM 2392 and detailed in reference 4.

^(d) Reference 2.

^(e) “-” Base pair same as in 1981 CRS [3].

^(het1) 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.

^(het2) Heteroplasmy found in HL-60 at np 12071.

Definitions and Acronyms for Table 1

*R:	Rare polymorphisms in Cambridge Reference Sequence discovered by reanalysis of original placenta [4]
*E	Error in Cambridge Reference Sequence discovered by reanalysis of original placenta [4].
del	Deletion
ins	Insertion
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	CACCC	TATTA	ACCAC	TCACG	
	R484	TGAGA	TTAGT	AGTAT	GGGAG	
2	F361	ACAAA	GAACC	CTAAC	ACCAG	C
	R921	ACTTG	GGTTA	ATCGT	GTGAC	C
3	F756	CATCA	AGCAC	GCAGC	AATG	
	R1425	AATCC	ACCTT	CGACC	CTTAA	G
4	F873	GGTTG	GTCAA	TTTCG	TGCCA	G
	R1425	AATCC	ACCTT	CGACC	CTTAA	G
5	F1234	CTCAC	CACCT	CTTGC	TCAGC	
	R1769	GCCAG	GTTTC	AATTT	CTATC	G
6	F1587	TGCAC	TTGGA	CGAAC	CAGAG	
	R2216	TGTTG	AGCTT	GAACG	CTTTC	
7	F1657	CTTGA	CCGCT	CTGAG	CTAAA	C
	R2216	TGTTG	AGCTT	GAACG	CTTTC	
8	F1993	AAACC	TACCG	AGCCT	GGTG	
	R2216	TGTTG	AGCTT	GAACG	CTTTC	
9	F2105	GAGGA	ACAGC	TCTTT	GGACA	C
	R2660	AGAGA	CAGCT	GAACC	CTCGT	G
10	F2417	CACTG	TCAAC	CCAAC	ACAGG	
	R3006	ATGTC	CTGAT	CCAAC	ATCGA	G
11	F2834	CCCAA	CCTCC	GAGCA	GTACA	TG
	R3557	AGAAG	AGCGA	TGGTG	AGAGC	
12	F2972	ATAGG	GTTTA	CGACC	TCGAT	G
	R3557	AGAAG	AGCGA	TGGTG	AGAGC	
13	F3234	AGATG	GCAGA	GCCCG	GTAAT	C
	R3557	AGAAG	AGCGA	TGGTG	AGAGC	
14	F3441	ACTAC	AACCC	TTCGC	TGACG	
	R3940	TGAAG	CCTGA	GACTA	GTTTCG	G
15	F3635	GCCTA	GCCGT	TTACT	CAATC	C
	R4162	TGAGT	TGGTC	GTAGC	GGAAT	C
16	F3931	TCAGG	CTTCA	ACATC	GAATA	CG
	R4728	TTATG	GTTCA	TTGTC	CGGAG	AG
17	F4183	TTTCT	ACCAC	TCACC	CTAGC	ATTAC
	R4728	TTATG	GTTCA	TTGTC	CGGAG	AG
18	F4392	CCCAT	CCTAA	AGTAA	GGTCA	GC
	R4983	GGTTT	AATCC	ACCTC	AACTG	CC
19	F4447	TTGGT	TATAC	CCTTC	CCGTA	C
	R4982	GTTTA	ATCCA	CCTCA	ACTGC	C
20	F4797	CCCTT	TCACT	TCTGA	GTCCC	AG
	R5553	AGGGC	TTTGA	AGGCT	CTTG	
21	F4976	ATTAA	ACCAG	ACCCA	GCTAC	G
	R5553	AGGGC	TTTGA	AGGCT	CTTG	
22	F5318	CACCA	TCACC	CTCCT	TAACC	
	R5882	GCTGA	GTGAA	GCATT	GGACT	G
23	F5700	TAAGC	ACCCT	AATCA	ACTGG	C
	R6262	GCCTC	CACTA	TAGCA	GATGC	G
24	F5999	TCTAA	GCCTC	CTTAT	TCGAG	C
	R6526	ATAGT	GATGC	CAGCA	GCTAG	G
25	F6242	CGCAT	CTGCT	ATAGT	GGAGG	
	R6526	ATAGT	GATGC	CAGCA	GCTAG	G
26	F6426	GCCAT	AACCC	AATAC	CAAAC	G
	R7030	TGGGC	TACAA	CGTAG	TACGT	G

Primer Set Number	Primer Sequence						
27	F6744	GGCTT	CCTAG	GGTTT	ATCGT	G	
	R7255	TTTCA	TGTGG	TGTAT	GCATC	G	
28	F7075	GAGGC	TTCAT	TCACT	GATTT	CC	
	R7792	GGGCA	GGATA	GTTCA	GACGG		
29	F7215	CGACG	TFACT	CGGAC	TACCC		
	R7792	GGGCA	GGATA	GTTCA	GACGG		
30	F7645	TATCA	CCTTT	CATGA	TCACG	C	
	R8215	GACGA	TGGGC	ATGAA	ACTG		
31	F7901	TGAAC	CTACG	AGTAC	ACCGA	CTAC	
	R8311	AAGTT	AGCTT	TACAG	TGGGC	TCTAG	
32	F8164	CGGTC	AATGC	TCTGA	AATCT	GTG	
	R8669	CATTG	TTGGG	TGGTG	ATTAG	TCG	
33	F8539	CTGTT	CGCTT	CATTC	ATTGC	C	
	R9059	GTGGC	GCTTC	CAATT	AGGTG		
34	F8903	CCCAC	TTCTT	ACCAC	AAGGC		
	R9403	GTGCT	TTCTC	GTGTT	ACATC	G	
35	F9309	TTTCA	CTTCC	ACTCC	ATAAC	GC	
	R9848	GAAAG	TTGAG	CCAAT	AATGA	CG	
36	F9449	CGGGA	TAATC	CTATT	TATTA	CCTCA	G
	R9995	AGAGT	AAGAC	CCTCA	TCAAT	AGATG	G
37	F9754	AGTCT	CCCTT	CACCA	TTTCC	G	
	R10275	AAAGG	AGGGC	AATTT	CTAGA	TC	
38	F10127	ACTAC	CACAA	CTCAA	CGGCT	AC	
	R10556	GGAGG	ATATG	AGGTG	TGAGC	G	
39	F10386	GGATT	AGACT	GAACC	GAATT	GG	
	R11166	CATCG	GGTGA	TGATA	GCCAA	G	
40	F10704	GTCTC	AATCT	CCAAC	ACATA	TGG	
	R11267	TGTTG	TGAGT	GTAAA	TTAGT	GCG	
41	F11001	AACGC	CACTT	ATCCA	GTGAA	CC	
	R11600	CTGTT	TGTCT	TAGGC	AGATG	G	
42	F11403	GACTC	CCTAA	AGCCC	ATGTC	G	
	R11927	TTGAT	CAGGA	GAACG	TGGTT	AC	
43	F11760	ACGAA	CGCAC	TCACA	GTCG		
	R12189	AAGCC	TCTGT	TGTCA	GATTC	AC	
44	F11901	TGCTA	GTAAC	CACGT	TCTGG	TG	
	R12876	GATAT	CGCCG	ATACG	GTTG		
45	F12357	AACCA	CCCTA	ACCCT	GACTT	CC	
	R12876	GATAT	CGCCG	ATACG	GTTG		
46	F12601	TTCAT	CCCTG	TAGCA	TTGTT	CG	
	R13123	AGCGG	ATGAG	TAAGA	AGATT	CC	
47	F12793	TTGCT	CATCA	GTTGA	TGATA	CG	
	R13343	TTGAA	GAAGG	CGTGG	GTACA	G	
48	F13188	CACTC	TGTTT	GCAGC	AGTAT	G	
	R13611	TCGAG	TGCTA	TAGGC	GCTTG	TC	
49	F13518	CATCA	TCGAA	ACCGC	AAAC		
	R13935	TGTGA	TGCTA	GGGTA	GAATC	CG	
50	F13715	GAAGC	CTATT	CGCAG	GATTT	C	
	R14118	TGGGA	AGAAG	AAAGA	GAGGA	AG	
51	F13899	TTTCT	CCAAC	ATACT	CGGAT	TC	
	R14388	TTAGC	GATGG	AGGTA	GGATT	GG (New Primer) ^(a)	
	R14388	TTAGC	GATGG	AGGTA	GGATT	CG (Old Primer)	
52	F14189	ACAAA	CAATG	GTCAA	CCAGT	AAC	
	R14926	TGAGG	CGTCT	GGTGA	GTAGT	GC	
53	F14470	TCCAA	AGACA	ACCAT	CATTC	C	
	R14996	CGTGA	AGGTA	GCGGA	TGATT	C	

Primer Set Number	Primer Sequence	
54	F14909	TACTC ACCAG ACGCC TCAAC CG
	R15396	TTATC GGAAT GGGAG GTGAT TC
55	F15260	AGTCC CACCC TCACA CGATT C
	R15774	ACTGG TTGTC CTCCG ATTCA GG
56	F15574	CGCCT ACACA ATTCT CCGAT C
	R16084	CGGTT GTTGA TGGGT GAGTC
57 (HV1)	F15971	TTAAC TCCAC CATTG GCACC
	R16451	GCGAG GAGAG TAGCA CTCTT G
58	F16097	TACAT TACTG CCAGC CACCA TG
	R336	TTAAG TGCTG TGGCC AGAAG
-21M13	F	TGTAA AACGA CGGCC AGT

^(a) These are the same primers used for SRM 2392 and reference 1 except the reverse primer of set 51 has been changed to: TTAGC GATGG AGGTA GGATT GG. The change (C to G) occurs at np 14 368 and is in bold and underlined.

Acronyms for Table 2

HV2: Hypervariable region 2

HV1: Hypervariable region 1

F: forward primer

R: reverse primer

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).
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<p>Certificate Revision History: 31 October 2012 (Certification expiration period extended; editorial changes); 07 December 2007 (Update of expiration date and editorial changes); 13 June 2003 (Original certificate date).</p>
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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.

