



Certificate of Analysis

Standard Reference Material[®] 2392

Mitochondrial DNA Sequencing (Human)

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 identifications, medical diagnosis, or mutation detection. This SRM can also be used for quality assurance when assigning values to in-house control materials.

This SRM is composed of well-characterized extracted human deoxyribonucleic acid (DNA) from cell culture lines known as CHR and GM09947A as well as cloned DNA from the HV1 region of CHR. A unit of SRM 2392 is composed of three frozen components packaged in one box. See the section in this certificate entitled *Description of Components* for a complete listing.

Certified Values: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST. This SRM is certified for the sequences of the entire human mtDNA (16 569 base pairs) from two lymphoblastoid cell culture lines (CHR and GM09947A) plus the cloned HV1 region of CHR containing a C-stretch. Table 1 contains sequence differences from the Revised Cambridge Reference Sequence (rCRS) for the CHR component [1]. Table 2 contains the sequence differences from the rCRS for component GM09947A. Table 3 contains the sequence differences from the rCRS for the cloned HV1 region of component CHR.

Note: Information in the updated certificate has been tabulated as differences from the Revised Cambridge Reference Sequence [1]. The original SRM 2392 certificate listed differences from the original Cambridge Reference Sequence [2]. Nomenclature is in agreement with current forensic guidelines [3,4].

Expiration of Certification: The certification of **SRM 2392** is valid, within the measurement uncertainties specified, until **31 July 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage, Handling, and Use”). The 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 or register online) will facilitate notification.

Overall direction and coordination of the technical activities leading to the recertification were under the chairmanship of P.M. Vallone of the NIST Biomolecular Measurement Division.

Overall direction and coordination of the technical measurements leading to the original certification were performed by B.C. Levin and D.J. Reeder, formerly of NIST. The analytical determination and technical measurements for the certification of this SRM were performed by B.C. Levin, H. Cheng, L.A. Tully, M.P. Jones and D.J. Reeder, formerly of the NIST DNA Technologies Group, Biotechnology Division. Additional sequencing experiments for the recertification of this SRM were performed by P.M. Vallone, S. Maragh and M.C. Kline of the NIST Biomolecular Measurement Division along with assistance from the Armed Forces DNA Identification Laboratory (Rockville, MD).

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Gaithersburg, MD 20899
Certificate Issue Date: 10 August 2018
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

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

INSTRUCTIONS FOR STORAGE, HANDLING, AND USE

Storage: Store frozen at a temperature of $-20\text{ }^{\circ}\text{C}$. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM.

Warning: SRM 2392 IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE THE SRM 2392 COMPONENTS AS BIOSAFETY LEVEL 1 MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [5]. SRM 2392 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

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 $37\text{ }^{\circ}\text{C}$. Once thawed, the sample should be processed without delay.

SOURCE AND ANALYSIS⁽¹⁾

Source of Material: CHR DNA, both extracted and cloned, was prepared in the NIST Applied Genetics Group, Biomolecular Measurement Division. DNA for GM09947A was prepared by Life Technologies, Inc., Gaithersburg, MD.

NIST Analysis: Components were sequenced using a modified strategy based on the work of Levin et al. [6]. Fluorescently-labeled sequencing fragments were separated on an Applied Biosystems 3130 Genetic Analyzer capillary electrophoresis platform.

Description of Components: Three components are included in each unit; all components must be stored at $-20\text{ }^{\circ}\text{C}$.

#1 Extracted DNA from cell culture line CHR (tube contains $60\text{ }\mu\text{L}$ of DNA at a concentration of approximately $1\text{ ng}/\mu\text{L}$).

#2 Extracted DNA from cell culture line GM09947A (tube contains $60\text{ }\mu\text{L}$ of DNA at a concentration of approximately $1\text{ ng}/\mu\text{L}$).

#3 Cloned DNA from the CHR HV1 region containing the C-stretch (tube contains $10\text{ }\mu\text{L}$ of DNA at a concentration of approximately $100\text{ ng}/\mu\text{L}$).

Note: DNA concentrations given are nominal values; the components of this SRM are not intended for use as concentration standards.

⁽¹⁾Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify 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 Revised Cambridge Reference Sequence for SRM 2932 Component CHR.

Site	rCRS	CHR	Comment
64	C	Y = CT	C/T heteroplasmy
73	A	G	
195	T	C	
204	T	C	
207	G	A	
263	A	G	
309.1		C	insertion
315.1		C	insertion
709	G	A	
750	A	G	
1438	A	G	
1719	G	A	
2706	A	G	
3107	C		deletion
4769	A	G	
4929	C	T	
5186	A	G	
6221	T	C	
6371	C	T	
6791	A	G	
7028	C	T	
8503	T	C	
8860	A	G	
11719	G	A	
11878	T	C	
12612	A	G	
12705	C	T	
13708	C	A	
13966	A	G	
14470	T	C	
14766	C	T	
15326	A	G	
16183	A	C	
16189	T	C	
16193.1		C	insertion
16223	C	T	
16278	C	T	
16519	T	C	

Table 2. Certified Human mtDNA Sequence Differences from the Revised Cambridge Reference Sequence for SRM 2392 Component GM09947A

Site	rCRS	GM09947A	Comments
93	A	G	
195	T	C	
214	A	G	
263	A	G	
309.1		C	insertion
309.2		C	insertion
315.1		C	insertion
750	A	G	
1438	A	G	
3107	C		deletion
4135	T	C	
4769	A	G	
7645	T	C	
7861	T	C	
8448	T	C	
8860	A	G	
9315	T	C	
13572	T	C	
13759	G	A	
15326	A	G	
16311	T	C	
16519	T	C	

Table 3. Certified Human mtDNA Sequence Differences from the Revised Cambridge Reference Sequence for SRM 2392 Component CHR Clone

Site	rCRS	CHR clone	Comments
16183	A	C	
16189	T	C	
16193.1		C	insertion
16223	C	T	
16278	C	T	
16519	T	C	

REFERENCES

- [1] 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*; Nat. Genet., Vol. 23, p. 147 (1999).
- [2] 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).
- [3] Wilson, M.R.; Allard, M.W.; Monson, K.; Miller, K.W.; Budowle, B.; *Recommendations for Consistent Treatment of Length Variants in the Human Mitochondrial DNA Control Region*; Forensic Sci Int., Vol. 129, pp. 35–42 (2002).
- [4] Wilson, M.R.; Allard, M.W.; Monson, K.L; Miller, K.W.P.; Budowle, B.; *Further Discussion of the Consistent Treatment of Length Variants in the Human Mitochondrial DNA Control Region*; Forensic Science Communications, Vol. 4, Number 4 (2002);
<https://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/oct2002/wilson.htm>
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- [5] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition; Richardson, J.; Barkley, W.E.; Richmond, J.Y.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control (CDC) and Prevention and National Institutes of Health (NIH); US Government Printing Office: Washington, DC, (2007); available at <https://www.cdc.gov/biosafety/publications/bmbl5/> (accessed Aug 2018).
- [6] Levin, B.C.; Cheng, H.; Reeder, D.J.; *Human Mitochondrial DNA Standard Reference Material for Quality Control in Forensic Identification, Medical Diagnosis, and Mutation Detection*; Genomics, Vol. 55, pp. 135–146 (1999).

<p>Certificate Revision History: 10 August 2018 (Change of certification period; editorial changes); 15 May 2013 (Extension of the certification period; editorial changes); 12 January 2009 (Revised to adopt the Revised Cambridge Reference Sequence (rCRS), include differences from rCRS found for site 4929, include heteroplasmy site 64, and report an extension of the expiration date); 17 June 2003 (This revision reports an extension in the expiration date and replacement of reverse primer 51); 29 December 1999 (Original certificate date).</p>

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: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <https://www.nist.gov/srm>.