National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2399

Fragile X Human DNA Triplet Repeat Standard

This Standard Reference Material (SRM) is intended to provide quality control by serving as a positive control to clinical laboratories that test human samples for Fragile X and who need to determine the number of CGG trinucleotide repeats present in samples. This SRM is composed of human deoxyribonucleic acid (DNA) from fragile X cell lines or patient samples that have been amplified using polymerase chain reaction (PCR) techniques. This SRM consists of a single box containing nine vials, designated A through I. Each vial contains 20 μL of a frozen PCR product with a different number of CGG repeats suspended in a buffer (10 mM Tris-Cl pH 8.5).

The American College of Medical Genetics Guidelines requires a positive control for all genetic testing [1]. In addition to medical diagnoses, the ability to detect the correct number of triplet repeats will help in genetic counseling and genetic research in the area of triplet repeats. SRM 2399 will also help to ensure the accuracy and comparability of results from different laboratories.

Certified Values: Table 1 contains the certified values for the number of trinucleotide repeats in eight of the nine samples. Table 2 summarizes the triplet repeat data obtained from experiments conducted at NIST. Table 3 contains the reference sequences of the forward and reverse primers used by NIST to amplify and sequence the CGG repeat area and 222 flanking base pairs [2].

Expiration of Certification: The certification of this SRM is valid until **31 August 2012**, 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. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification were performed by K.L. Richie and B.C. Levin of the NIST Biochemical Science Division.

The analytical determination, technical measurements and analysis of data for the certification of this SRM were performed by K.L. Richie and B.C. Levin of the NIST Biochemical Science Division.

Reevaluation performed by K.L. Richie, J.P. Jakupciak, and M.C. Kline of the NIST Biochemical Science Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

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Certificate Issue Date: 19 June 2007
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
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Permissions: The research needed to prepare SRM 2399 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 exempts: "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." Permission to use Fragile X samples from Coriell Institute for Medical Research (Camden, NJ) [1] for SRM 2399 was provided by J.C. Beck, Ph.D., Director, Coriell Institute for Medical Research.

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

INSTRUCTIONS FOR USE

Minimize repeated freezing and thawing of these materials, as this might shorten the shelf-life of the SRM. If it is necessary to perform many repeated analyses, the SRM may be thawed and the vial contents aliquoted into sterile vials that can be kept frozen until use. Thawing can be conducted at refrigerator temperatures or room temperature. After thawing, briefly centrifuge the sample tube to spin down any condensate present in the tube cap and gently mix to obtain a homogenous solution. The vial manufacturer warns against over-tightening the vial caps.

Note: As these are PCR products, you will need to dilute these samples at least 1 x 10^5 before proceeding with any technique that requires additional PCR amplification. Please note that as components "G", "H", and "I" are less concentrated than components "A" thru "F", you may need to adjust your dilutions accordingly.

SOURCE AND ANALYSIS

Source of Material: DNA was obtained from Coriell Institute for Medical Research (Camden, NJ) and Quest Diagnostics, Inc. (Van Nuys, CA). The PCR products were prepared by the NIST DNA Measurements Group, Biochemical Science Division.

NIST Analysis: Modified PCR conditions, using the primers shown in Table 3, were developed at NIST [2] in order to successfully amplify the extended CGG regions in each triplet repeat sample. To obtain the certified number of triplet repeats in each sample, the PCR products were purified using the Qiaquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and then cycle-sequenced using fluorescent dye-labeled terminators and analyzed on an Applied Biosystems, Inc. 310 Genetic Analyzer. Multiple PCR and sequencing reactions for each of the nine samples were performed. Table 2 shows the results obtained from the multiple sequencing experiments.

[1]Certain commercial equipment, instruments, materials, or companies are identified in this paper to specify the experimental procedure. Such identification does not imply recommendation or endorsement by NIST, nor does it imply that the materials or equipment identified are the best available for this purpose.

Table 1. Certified Number of Trinucleotide Repeats in Fragile X Samples

Component Designation	Number of Certified CGG Repeats	Gender of Donor
A	20 [(a)]	Female
B	30	Male
C	41 [(a)]	Female
D	51	Male
E	60	Male
F	73	Male
G	__ [(b)]	Male
H	96	Male
I	118	Male

[(a)] Due to the two X chromosomes in female DNA, each chromosome may have different numbers of triplet repeats. In the DNA from these two samples, there was a certifiable number of repeats in addition to a full expansion, i.e., triplet repeats greater than 200. We are only certifying the smaller number of repeats.

[(b)] "G" – genotyping analysis confirms the presence of two distinct CGG fragment sizes, 88/89 and 93. Due to the complexity of this sample, the number of CGG repeats are not certified, but are provided as information values only.

Note: These values have all been confirmed through DNA sequencing. Sequencing data were obtained with a BigDye Terminator Cycle Sequencing Kit, version 1.0 (Applied Biosystems, Foster City, CA). For each component, the certified value is the next smallest integer of the average of the numbers of triplet repeats for that component as listed in Table 2, which also shows the variability of the actual count from experiment to experiment.

Table 2. Reproducibility of the Numbers of Fragile X Triplet Repeats Determined by Multiple Sequencing Reactions at NIST

Experiment Number	Number of Triplet Repeats (component designation)								
	A	B	C	D	E	F	G(*)	H	I
1	20	30	41	51	60	73	88/89	96	119
2	20	30	41	51	60	73	88/89	97	118
3	20	30	41	51	60	73	88/89	97	118
4	20	30	41	ND	60	ND	ND	ND	120

ND: Not determined

(*) Provided as information value only.

Table 3. Primers Used to Amplify the Region of Interest [3]

Primer	Primer Sequence
Forward 286 (a)	5' GCT CAG CTC CGT TTC GGT TTC ACT TCC GGT 3'
Reverse 555 (b)	5' AGC CCC GCA CTT CCA CCA CCA GCT CCT CCA 3'

(a) Position 286 in the sequence of the 1 kb PstI fragment containing the CGG repeats.
(b) Position 555 in the sequence of the 1 kb PstI fragment containing the CGG repeats.

REFERENCES

[1] *Standards and Guidelines for Clinical Genetics Laboratories*; American College of Medical Genetics: Bethesda, MD (2004); available at http://www.acmg.net/Pages/ACMG_Activities/stds-2002/stdsmenu-n-htm.

[2] O'Connell, C.D.; Atha, D.H.; Jakupciak, J.P.; Amos, J.A.; Richie, K.L.; *Standardization of PCR Amplification for Fragile X Trinucleotide Repeat Measurements*; Clinical Genetics, Vol. 61, pp. 13–20 (2002).

[3] Fu, Y.H.; Kuhl, D.P.A.; Pizzuti, A.; Pieretti, M.; Sutliffe, J.S.; Richards, S.; Verkerk, A.J.; Holden, J.J.; Fenwick, R.G., Jr.; Warren, S.T.; Oostra, B.A.; Nelson, D.L.; Caskey, C.T.; *Variation of the CGG Repeat at the Fragile X Site Results in Genetic Instability*: *Resolution of the Sherman Paradox*; Cell, Vol. 67, pp. 1047–1058 (1991).

Certificate Revision History: 19 June 2007 (Change component G Trinucleotide Repeats to Information value and expiration date); 22 December 2004 (Original certificate date).

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, email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.