

# **DEVELOPMENT OF A STANDARD REFERENCE MATERIAL FOR FRAGILE X SYNDROME (Update)**

**Kristy L. Richie**

**National Institute of Standards and Technology**

**Developing QC Materials for Genetic Testing Meeting  
Centers for Disease Control and Prevention  
Los Angeles, CA - November 9<sup>th</sup> , 2004**

# OBJECTIVE

- **Prepare an SRM for the accurate determination of the number of triplet repeats in the fragile X gene in normal and pre-mutation individuals and their relatives.**
  - **Conduct an interlaboratory evaluation to assess and correct problems experienced by other laboratories.**
  - **Provide a measure of quality control and quality assurance to clinical diagnostic laboratories.**

# Trinucleotide Repeat Diseases

Disease	Repeat	Repeat Length	Gene Product
<b>Fragile X Syndrome</b>	<b>CGG</b>	<b>N: 5 - 44</b> <b>P: 55 - 200</b> <b>D: 200 +</b>	<b>FMR - 1 protein</b>
<b>X-linked spinal &amp; bulbar muscular atrophy</b>	<b>CAG</b>	<b>N: 11 - 34</b> <b>D: 200 +</b>	<b>Androgen Receptor</b>
<b>Myotonic dystrophy</b>	<b>CTG</b>	<b>N: 5 - 30</b> <b>P: 42 - 180</b> <b>D: 200 +</b>	<b>Myotonin protein K</b>
<b>Huntington's Disease</b>	<b>CAG</b>	<b>N: 11 - 34</b> <b>D: 37 - 121</b>	<b>Huntingtin</b>
<b>Spinocerebellar ataxia type 1</b>	<b>CAG</b>	<b>N: 19 - 36</b> <b>D: 43 - 81</b>	<b>Ataxin - 1</b>
<b>FRAXE mental retardation</b>	<b>GCC</b>	<b>N: 6 - 25</b> <b>D: 200 +</b>	<b>?</b>
<b>Dentatorubral-pallidoluysian atrophy</b>	<b>CAG</b>	<b>N: 7 - 23</b> <b>D: 49 - 75</b>	<b>?</b>

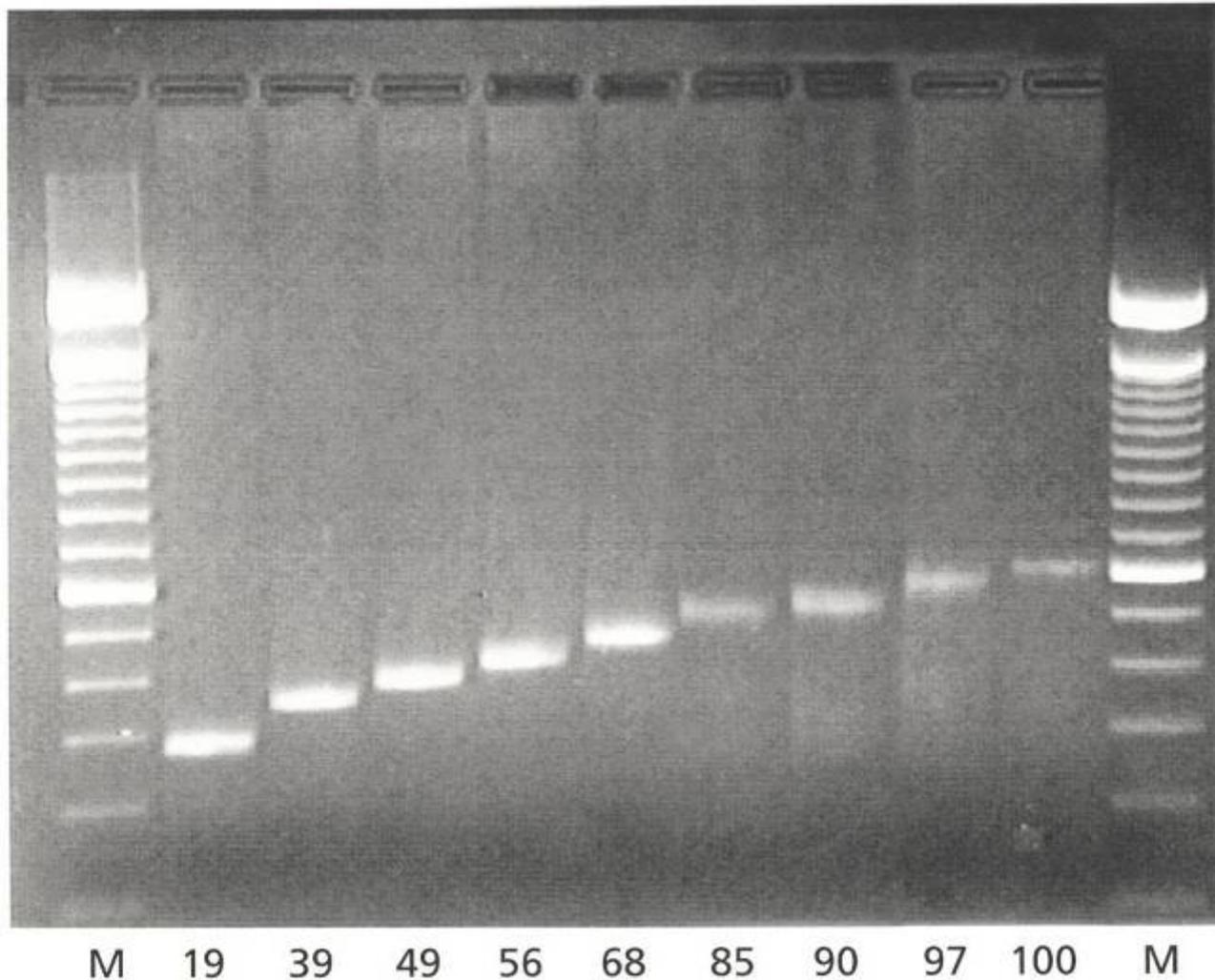
# EXPERIMENTAL DESIGN

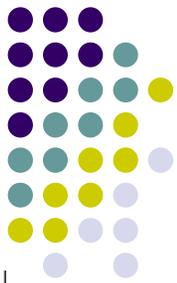
- DNA from fragile X cell lines or patient samples was obtained.
- Samples contained normal and pre-mutation fragile X alleles, with CGG repeat sizes ranging from 20 to 118.
- Amplification using a modified PCR technique for GC-rich templates. The forward and reverse primers were:  
5' GCT CAG CTC CGT TTC GGT TTC ACT TCC GGT 3'  
5' AG CCC CGC ACT TCC ACC ACC AGC TCC TCC A 3'.  
The forward flanking region: 121 base pairs.  
The reverse flanking region: 100 base pairs.
- PCR products analyzed on 2% agarose gels.
- Sequencing performed using 310 Genetic Analyzer.

# NIST Fragile X SRM 2399

Will Contain Nine

Fragile X Triplet Repeat Sizes





# 118 bp CGG Repeat

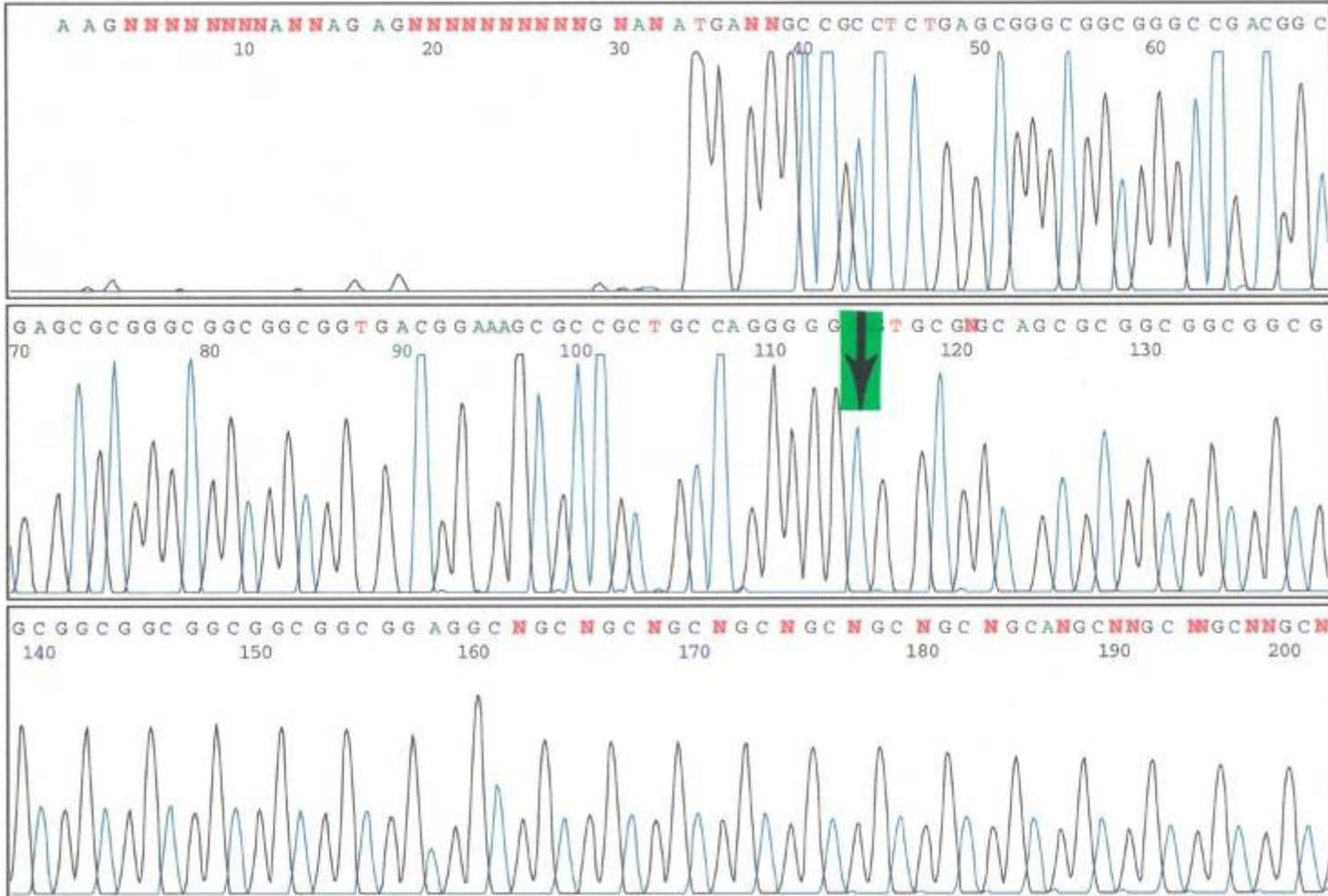


Model 310  
Version 3.0  
ABI-CE1  
Version 3.2

FX-D100SampleFX-D100  
FX-D100  
Lane 4

Signal G:1338 A:108 T:141 C:259  
DT POP6(BD Set-Any Primer)  
dRhod-BigDye-4-14-03  
Points 774 to 10240 Base 1: 774

Page 1 of 5  
Wed, Apr 30, 2003 11:13  
Tue, Apr 29, 2003 13:51  
Spacing: 13.30(13.30)





# Reproducibility of the Numbers of Fragile X Repeats

Experiment Number	SRM Components								
	A	B	C	D	E	F	G	H	I
1	20	30	41	51	60	73	93	96	119
2	20	30	41	51	60	73	93	97	118
3	20	30	41	51	60	73	94	97	118
4	20	30	41	ND	60	ND	ND	ND	120

ND: Not determined

## **Certified Number of Triplet Repeats in Fragile X SRM 2399**

<b>Component Designation</b>	<b>Number of Certified CGG Repeats</b>	<b>Gender of Donor</b>
<b>A</b>	<b>20</b>	<b>Female</b>
<b>B</b>	<b>30</b>	<b>Male</b>
<b>C</b>	<b>41</b>	<b>Female</b>
<b>D</b>	<b>51</b>	<b>Male</b>
<b>E</b>	<b>60</b>	<b>Male</b>
<b>F</b>	<b>73</b>	<b>Male</b>
<b>G</b>	<b>93</b>	<b>Male</b>
<b>H</b>	<b>96</b>	<b>Male</b>
<b>I</b>	<b>118</b>	<b>Male</b>

STANDARD REFERENCE MATERIAL®

**2399**

**Fragile X Triplet  
Repeat Standard**

Store at -20°C

[www.nist.gov/srm](http://www.nist.gov/srm)

**NIST**

**National Institute of Standards and Technology**  
Technology Administration, U.S. Department of Commerce



# LIST OF PARTICIPANTS IN ILE

- ARUP Laboratories, Salt Lake City, UT
- Baylor College of Medicine – Houston, TX
- Genzyme Genetics – Westborough, MA
- LabCorp – Research Triangle Park, NC
- NGRL – Manchester, U.K.
- NIST – Gaithersburg, MD
- OHSU – Portland, OR
- Specialty Laboratories – Santa Monica, CA
- University of Leuven – Leuven, Belgium

# INTERLABORATORY EVALUATION RESULTS

Lab#	Number of Triplet Repeats								
<b>NIST</b>	20	30	41	51	60	73	93	96	118
<b>1</b>	20	30	40	51	59	72	87	97	118
<b>2</b>	19	30	NC	NC	NC	NC	NC	NC	NC
<b>3</b>	19	NC	NC	NC	57	NC	NC	NC	NC
<b>4</b>	20	30	41	51	60	73	88	93	120
<b>5</b>	18	30	42	50	58	70	85	90	110
<b>6</b>	18	29	41	54	61	73	NC	NC	NC
<b>7-a</b>	20	30	42	51	60	73	89/94	99	124
<b>7-b</b>	21	30	42	51	60	74	91/96	NC	126
<b>7-c</b>	20	30	41	51	60	74	92/96	NC	128
<b>8</b>	16.7	26.7	37.7	47.7	56	68.3	82/87	91	114.3

# Techniques Used in the ILE

Lab #	Techniques
1	Alpha-labeled $^{32}\text{P}$ dCTP PCR
2	PCR/GeneScan
3	Denaturing PAGE/PCR/Gene Scan
4	PCR/Denaturing PAGE/end-labeled $(\text{CGG})_5$ probe
5	Alpha-labeled $^{32}\text{P}$ dCTP PCR
6	PCR/GeneScan
7	PCR/GeneScan
8	PCR/Fluorescent CE/GeneScan

# SUMMARY

- DNA containing various numbers of triplet repeats ranging from 20 to 118 have been identified and amplified.
- Confirmation of these sizes by sequencing using the 310 genetic analyzer.
- Interlaboratory Evaluation complete.
- Certified values have been obtained.
- SRM 2399 to be available later this year.

# ACKNOWLEDGEMENTS

**Dr. Barbara C. Levin - NIST**

**Dr. Diane K. Hancock - NIST**

**Dr. Jean Amos – Specialty Labs**

**Coriell Cell Repositories**

**Measurement Services Division - NIST**

# NIST SRM

## ■ NIST Standard Reference Material® (SRM®)

- ◆ A CRM issued by NIST. A SRM is a well-characterized material produced in quantity to improve measurement science. It is certified for specific chemical or physical properties, and is issued by NIST with a certificate that reports the results of the characterization and indicates the intended use of the material.

## ■ The three main purposes of an SRM:

1. to help develop accurate analytical methods
2. to calibrate measurement systems
3. to ensure the long-term adequacy and integrity of measurement quality assurance programs

# DEFINITIONS

- Reference Material (RM) – Material with homogeneous and well established property values to be used to calibrate an apparatus, assess a measurement method, or assign values to materials.
- Certified Reference Material (CRM) – RM plus certificate that certifies its property values by a procedure which also establishes its traceability.

# Why Fragile X?

- Fragile X Syndrome is the leading heritable cause of mental retardation, affecting ~ 1 in 1200 males.
- The number of Fragile X tests conducted for carrier screening, pre-natal screening and diagnostic testing is second only to cystic fibrosis testing.
- The number of CGG repeat elements present in an individual determines disease status.
  - >200 copies of the repeat leads to mental retardation in virtually all males.
  - Recent research indicates late-onset neurological symptoms in male carriers of pre-mutation alleles.