

**National DNA Index System (NDIS)
DNA Data Acceptance Standards**

Operational Procedures

FBI Laboratory

Created 11 January 2000

Revised 4 May 2005

Revision History

Date	Author	Comments
11 November 1996	Barry Brown	Revised per TWGDAM - Originally entitled "NDIS Standards for Acceptance of DNA Data"
12 June 1998	Barry Brown	Revised per TWGDAM
4 January 1999	Barry Brown	Revised per SWGDAM
12 July 1999	Barry Brown	Reviewed at SWGDAM
11 January 2000	Barry Brown	Revised per SWGDAM
29 April 2002	Dawn Herkenham	Revised document to be incorporated into NDIS Procedures Manual.
30 April 2002	Dawn Herkenham	Revised to include an Appendix describing the criteria used in reviewing requests to add new or modify PCR kits accepted at NDIS.
2 May 2002	Dawn Herkenham	Revised to include new PCR kits approved for use at NDIS.
9 May 2002	Dawn Herkenham	Incorporated revisions from May 9, 2002 NDIS Board Meeting.
30 July 2002	Dawn Herkenham	Incorporate suggestions from the SWGDAM CODIS Subcommittee for Appendix A, as approved and revised by the NDIS Board.
23 September 2002	Dawn Herkenham	Incorporated revisions (mtDNA provisions) discussed at the September 18-19, 2002 NDIS Procedures Board Meeting.
3 October 2002	Dawn Herkenham	Revised the listing of approved PCR kits to include the PowerPlex® 16 BIO kit approved by the FBI.
15 March 2003	Dawn Herkenham	Add Pentas D and E to list of approved PCR STR loci, correct table references and update FSSU address.
31 March 2003	Dawn Herkenham	Incorporate "4 by 4" rule for mixtures, correct chromosomal locations for STR loci, add Appendix B containing Guidelines for approval of Expert Systems and remove references to FSSU as approved at the March 2003 NDIS Procedures Board meeting.
13 May 2003	Dawn Herkenham	Incorporate revisions to Appendix B relating to developmental validation performed by the sponsoring laboratory and requiring maintenance of dual review for six months- changes approved at May, 2003 NDIS Board meeting.
22 October 2003	Dawn Herkenham	Delete RFLP sections and incorporate clarification to data acceptance standards for forensic profiles in Forensic Index.
19 May 2004	Dawn Herkenham	Incorporated the Appendix B approved by the NDIS Procedures Board in May, 2004 via e-mail vote.

Date	Author	Comments
1 July 2004	Dawn Herkenham	To clarify the '4 by 4' rule in section 6.4 at page 3 and to make a conforming change to footnote 2 on page 4 relating to the minimum number of loci required for Unidentified Human (Remains) at NDIS.
6 January 2005	Dawn Herkenham	To incorporate recommendations made by the SWGDAM Expert System Committee relating to monitoring Expert Systems approved for use at NDIS; new section 8 of Procedure.
13 January 2005	Dawn Herkenham	Incorporate revisions approved to Section 8 and footnote by the NDIS Procedures Board on January 12, 2005.
11 February 2005	Dawn Herkenham	Incorporate recommendations from the SWGDAM Ad Hoc Working Group on Contractor Technical Review into a new section 8 and renumbering of the former section 8 as section 9.
15 February 2005	Dawn Herkenham	To delete the waiver provisions applicable to DNA profiles developed prior to 1989 since RFLP DNA data is no longer maintained or searched at NDIS (Section 4.1 and 4.2). And to include the required PCR loci for juvenile, indicted person, arrestee, suspect and missing person DNA profiles contributed to NDIS in Table 3 of Section 6.6.
25 February 2005	Dawn Herkenham	To clarify that the new Section 8 was recommended by the SWGDAM Ad Hoc Working Group on Contractor Review by adding footnotes 3 and 4. Incorporated clarifications to Section 6.5 to address comments by Robert Jones relating to inclusion of arrestees and suspects at NDIS; arrestee and suspect specimens would only be permitted to be searched, <i>and not included in NDIS</i> , under the one-time search provision contained in the Justice for All Act of 2004.
7 March 2005	Dawn Herkenham	Incorporate discussions at the March 3, 2005 NDIS Procedures Board Meeting relating to Sections 8 and 9.
4 April 2005	Dawn Herkenham	Approved changes circulated in March 2005. Incorporated technical changes from NDIS Board members. Please see the following Sections: 7.3.1 7.3.2
4 May 2005	Dawn Herkenham	Incorporated revisions approved by the NDIS Procedures Board at their May 4, 2005 meeting. Additionally, new provision added to Section 9.0 to expressly state that contractor review of contractor generated casework data is not permitted.

1.0 Purpose

The purpose of this document is to define the data acceptance standards for DNA profiles to be accepted at NDIS. This document is written for CODIS Administrators.

2.0 Process for Changing the Document

Revisions to this document are controlled via a defined process, as described in NDIS procedure *Changing NDIS Procedures and Policies*.

3.0 Background

To ensure the reliability, accuracy and compatibility of DNA data uploaded to NDIS, the Federal Bureau of Investigation Laboratory developed data acceptance standards governing the various types of DNA technologies that are used in forensic DNA laboratories. Previous versions of this document entitled “*NDIS Standards for Acceptance of DNA Data*,” covered DNA data generated by Restriction Fragment Length Polymorphism (RFLP) and for Polymerase Chain Reaction (PCR) based methods.

NDIS shall accept a DNA profile after it is determined to be compliant with the DNA Data Acceptance Standards in effect at the time the DNA profile was generated or compliant with the standards that are in place at the time the DNA profile is offered.

4.0 Quality Assurance Standards

All DNA profiles offered to NDIS by NDIS participating laboratories shall be produced in accordance with the FBI Director’s Quality Assurance Standards, as required by the DNA Identification Act of 1994, as amended [42 U.S.C. §14132(b)]. The Quality Assurance Standards for Forensic DNA Testing Laboratories were approved by the Director of the FBI and became effective October 1, 1998. The Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories, also approved by the Director of the FBI, became effective April 1, 1999. These Quality Assurance Standards supersede the quality assurance guidelines adopted by the Technical Working Group on DNA Analysis Methods or TWGDAM, entitled “Guidelines for a Quality Assurance Program for DNA Analysis¹” (known as the TWGDAM Guidelines).

¹Guidelines for a Quality Assurance Program for DNA RFLP Analysis, Crime Laboratory Digest, April-July 1989, Vol. 16 (2), pp. 40-59; Guidelines for a Quality Assurance Program for DNA Analysis, Crime Laboratory Digest, April 1991, Vol 18 (2), pp. 44-75; and Guidelines for a Quality Assurance Program for DNA Analysis, Crime Laboratory Digest, April 1995, Vol. 22 (2), pp. 21-43.

5.0 RESERVED [Previously contained standards for RFLP data]

6.0 Standards for PCR DNA Data

6.1 Protocols for PCR

PCR DNA Controls, allelic ladders and primer sets that were validated together shall be used together.

6.1.1. The laboratory shall demonstrate that it continues to use a protocol that produces NDIS compatible DNA results by its application of a positive PCR DNA Control that has been appropriately validated.

6.1.2. All DNA profiles offered to NDIS must be associated with an accurate result for PCR DNA Controls.

6.1.3. Only DNA profiles derived from analysis of NDIS Accepted PCR Kits (Table 4) shall be accepted at NDIS.

6.2 Changes to the PCR Protocols

6.2.1. Any significant changes made to a protocol must be demonstrated to be non-detrimental to the PCR results, as indicated by appropriate PCR DNA Control results.

6.2.2. The use of a protocol that does not achieve the correct results for the PCR DNA Controls shall be discontinued.6.2.3. At the request of NDIS, a laboratory shall demonstrate the reliability of data generated by the proposed protocol.

6.3 Allelic Ladders

6.3.1. The allelic ladders used must be from the list of NDIS Accepted PCR Kits (Table 4).

6.3.2. The allelic ladders used for each locus must give NDIS compatible results, as demonstrated by the PCR DNA Controls.

6.3.3. At each locus, the allelic ladder should have the commonly occurring alleles of the repeat element.

6.3.4. An NDIS Accepted Allelic ladder must be associated with each sample set.

6.4 Interpretation of DNA Profiles

6.4.1. DNA profiles submitted to NDIS shall be interpretable (interpretable - any DNA

data that could be used to make an exclusion).

- 6.4.2. A laboratory submitting a DNA profile to the Forensic Index at NDIS that is derived from forensic evidence, shall only offer those alleles that are attributed to the putative perpetrator(s). Alleles derived from forensic profiles that are unambiguously attributed to a victim or individuals other than the perpetrator(s), such as, but not limited to a husband or boyfriend, shall not be offered to NDIS.
- 6.4.3. The DNA results from any locus in which an ambiguity exists in the assignment of one or more alleles to the putative perpetrator(s) may be offered to NDIS. The mere observation of alleles that may be attributed to individuals other than the putative perpetrator, does not in itself, preclude offering DNA profiles to NDIS at that locus.
- 6.4.4. Forensic mixture DNA profiles submitted to NDIS shall have up to 4 alleles at a maximum of 4 core loci, any of the remaining 9 core loci shall have no more than 2 alleles at each locus.

6.5 Acceptable PCR Loci

The searching of PCR profiles in NDIS derived from offender (convicted offender, juvenile, indicted person, arrestee, suspect), forensic samples, missing person, relatives of missing person, and unidentified human remains require conclusive results from a minimum number of required PCR loci. DNA profiles, which fail to include the minimum number of loci, shall not be autosearched at NDIS. Additional loci listed in Table 3 as acceptable shall be accepted for DNA profiles containing the required minimum number of PCR loci.

Table 3 constitutes all PCR loci from which results shall be accepted by NDIS. The absence of any particular locus from this table does not suggest the unsuitability of the locus for forensic application. The addition of new PCR loci shall be accepted by NDIS, upon approval by the NDIS Custodian.

Applications for the addition of new loci to the Acceptable PCR Loci may be submitted to the NDIS Custodian by a NDIS Participating Laboratory. The addition of new loci may be made by the NDIS Custodian only after data presented to NDIS demonstrates that the new loci have been appropriately validated including forensic and population studies, and provide NDIS comparable results. Please refer to Appendix A for the criteria used in determining and reviewing a request to add a new or modified PCR kit which can be used as a guide in preparing applications to add new loci.

Table 3 - PCR Loci Accepted at NDIS					
Locus	Chromosome	Offender ¹	Forensic ²	Missing Person and	Relatives of

	Location			Unidentified Human (Remains) ²	Missing Person ¹
CSF1PO	5q33.3-34	Required	Required	Required	Required
FGA	4q28	Required	Required	Required	Required
TH01	11p15-15.5	Required	Required	Required	Required
TPOX	2p23-2pter	Required	Required	Required	Required
VWA	12p12-pter	Required	Required	Required	Required
D3S1358	3p	Required	Required	Required	Required
D5S818	5q21-q31	Required	Required	Required	Required
D7S820	7q	Required	Required	Required	Required
D8S1179	8	Required	Required	Required	Required
D13S317	13q22-q31	Required	Required	Required	Required
D16S539	16q22-24	Required	Required	Required	Required
D18S51	18q21.3	Required	Required	Required	Required
D21S11	21	Required	Required	Required	Required
Amelogenin	X:p22.1-22.3 Y:p11.2	Accepted	Accepted	Accepted	Accepted
Penta D	21q	Accepted	Accepted	Accepted	Accepted
Penta E	15q	Accepted	Accepted	Accepted	Accepted
Completeness		13	10	8	13

Any of these PCR loci so indicated shall be accepted at NDIS.

¹The number required to be a complete profile for the Offender category (includes Convicted Offender, Juvenile, Indicted Person, Arrestee, Suspect) and Relatives of Missing Person is the required 13.

²An analysis of all 13 required loci must be attempted for Forensic, Missing Person and Unidentified Human (Remains). The minimum number of PCR loci required for search purposes is 10 for Forensic and 8 for Missing Person and Unidentified Human (Remains).

6.6 Acceptable PCR Kits

6.6.1. The following table (Table 4) provides the PCR Kits accepted by NDIS.

6.6.2. The absence of a PCR Kit from Table 4 does not suggest the unsuitability of that particular PCR Kit for forensic application.

6.6.3. The addition of a PCR Kit to Table 4 (NDIS Acceptable PCR Kits) or modification of an existing PCR Kit, shall be made only after data are presented that demonstrates that the new PCR Kit generates NDIS compatible results, or the modification is justified. Criteria used in determining and reviewing a request to add a new or modified PCR kit are described in Appendix A. Applications for the approval of a new or modified PCR kit may be submitted by a criminal justice agency. Acceptance of PCR Kits for use at NDIS is determined by the FBI.

Table 4 - NDIS Acceptable PCR Kits	
Manufacturer	Kit Name
Applied Biosystems	AmpF/STR® Profiler Plus™ (Part Number 4303326)
Applied Biosystems	AmpF/STR® COfiler™ (Part Number 4305246)
Applied Biosystems	AmpF/STR® Profiler Plus™ and AmpF/STR® COfiler™ (Part Number 4305979)
Applied Biosystems	AmpF/STR® Profiler Plus™ ID (Part Number 4330284)
Applied Biosystems	AmpF/STR® Profiler Plus™ ID and AmpF/STR® COfiler™ (Part Number 4330621)
Applied Biosystems	AmpF/STR® Identifier™ (Part Number 4322288)
Promega	PowerPlex® 1.1 (Catalog numbers DC6091/6090)
Promega	PowerPlex® 1.2 (Catalog numbers DC 6101/6100)
Promega	PowerPlex® 2.1 (Catalog numbers DC 6471/6470)
Promega	PowerPlex® 16 (Catalog numbers DC 6531/6530)
Promega	PowerPlex® 16 BIO (Catalog numbers DC 6541/6540)
Promega Monoplex*	Monoplex D5S818 (Catalog number DC6161)
Promega Monoplex*	Monoplex D7S820 (Catalog number DC6141)
Promega Monoplex*	Monoplex D13S317 (Catalog number DC6151)
Promega Monoplex*	Monoplex D16S539 (Catalog number DC6131)
Promega Monoplex*	Monoplex TH01 (Catalog number DC5081)
Promega Monoplex*	Monoplex TPOX (Catalog number DC5111)
Promega Monoplex*	Monoplex CSF1PO (Catalog number DC5091)
Promega Monoplex*	Monoplex vWA (Catalog number DC5141)

* Monoplexes are all fluorescence-labeled and have same chemistry as when in multiplex kits.

6.7 Format for Offering PCR Profiles to NDIS

6.7.1. The DNA result from each locus will be in the form p,q for heterozygotes and mixtures (in ascending order) and p,p for homozygotes.

6.7.2. Alleles below or above the allelic ladder are entered as < (lowest allele) or > (highest allele), respectively.

6.7.3. Alleles will be entered according to their relative base pair size even if they are between designated points on the allelic ladder.

7.0 Standards for MtDNA Data

7.1 Protocols for Mitochondrial DNA (mtDNA) Sequencing

7.1.1. Controls are required to assess analytical procedures and monitor the level of contamination. Each laboratory shall establish evaluation criteria for controls, including but not limited to, reagent blank control, negative control and positive control. Each of these controls shall be processed through sequencing along with the sample.

7.1.2. Nucleotide sequence obtained from population database samples shall include a minimum of hypervariable region I ("HV1"; positions 16024-16365) and hypervariable region II ("HV2"; positions 73-340). Nucleotide sequence from known (K) samples should include HV1 and HV2 positions. No minimum length requirements are set for nucleotide sequence data obtained from questioned (Q) samples. Both strands of the amplified product shall be sequenced to reduce ambiguities in sequence determination, provided, however, that following a homopolymeric stretch, a readable sequence may not always be obtained from both strands.

7.2 Contamination Controls

7.2.1. Laboratories shall establish a method to define and quantify contamination and determine the maximum threshold for contamination.

7.2.2. A positive control is a sample of known mtDNA sequence used to monitor the success of the analysis. The positive control shall be processed starting at amplification. For inclusion of mtDNA data in the National DNA Index System or NDIS, the HL60 cell line shall be required as the positive control.

7.2.3. Reagent blanks and negative controls shall be used to monitor levels of contamination. Reagent blanks monitor contamination from extraction to final sequence analysis. Negative controls monitor contamination from amplification to final sequence analysis. Both reagent blanks and negative controls are processed along with the sample.

7.2.3.1. If the reagent blank and/or negative control of a particular amplicon yields a sequence that is the same as that of the sample, the results from the amplicon shall be rejected. The analysis shall be repeated, starting with the extraction of the sample.

7.2.3.2. If contamination in the reagent blank is present above the threshold established by the laboratory, then the sample cannot be used for interpretative purposes and re-extraction or re-amplification is required.

7.2.3.3. If contamination in the negative control is present above the threshold established by the laboratory, then the extract may be re-used. However, the sample cannot be used for interpretative purposes and re-amplification is required.

7.3 Interpretation of Results

The laboratory shall establish criteria to assign nucleotide base calls to appropriate peaks or bands and to determine whether the results are of sufficient quality for interpretation purposes. The overall quality of the electropherogram data must be assessed. The results must be examined to determine if they meet the laboratory's analytical and interpretation threshold(s) established through internal validation studies. If the overall quality of the electropherogram is not suitable for analysis, the data should be rejected and the sample should be re-extracted, re-amplified and/or re-sequenced.

7.3.1. A consensus sequence obtained from the sample will be compared to the Revised Cambridge Reference Sequence (rCRS) described by Andrews (Andrews, et al. 1999). Differences between the reference sequence (rCRS) and the sample sequence shall be noted as polymorphisms. The nucleotide position and the DNA base difference from the reference shall be noted (e.g. 16089 C).

7.3.2. DNA base call designation shall be based on the nomenclature system set forth by the International Union of Pure and Applied Chemistry (I.U.P.A.C.). At confirmed positions of ambiguity the following I.U.P.A.C. codes shall be used:

G/T = K	A/C = M
A/G = R	A/G/T = D
G/C = S	A/C/T = H
A/T = W	A/C/G = V
C/T = Y	C/T/G = B
A/C/G/T = N	

7.3.2.1. Insertions are described by noting the site immediately three prime (3') to the insertion with respect to the light strand of the rCRS followed by a point and a "1" for the first inserted base and sequential numbering for each inserted base afterwards. With homopolymeric regions, the insertion will be placed at the highest numbered end of the homopolymeric region with respect to rCRS. Insertions should not alter subsequent numbering of the sequence. Variants from the rCRS shall be coded in accordance with the guidelines proposed by Wilson *et al.* 2002a and Wilson *et al.* 2002b.

7.3.2.2. The only approved exception to the I.U.P.A.C. nomenclature is the use of a '-' for deletions.

7.3.3. All relevant sequence traces shall be imported into a software program for analysis and alignment. The heavy strand sequences shall be reverse-complemented so that the bases are aligned in the light strand orientation. Strands shall be compared and bases designated.

7.3.4. Each laboratory shall define heteroplasmy within the operational limits of the

system used for sequencing. Heteroplasmy is defined as more than one mtDNA type present in an individual that can be detected at an operational level. When the samples being analyzed differ by a single nucleotide, additional samples shall be run in order to attempt to resolve the interpretative issue.

7.3.4.1. Heteroplasmy can be observed as point heteroplasmy where two DNA bases are observed at the same nucleotide position.

7.3.4.2. Heteroplasmy can also be seen as length heteroplasmy, which typically is observed as a variation in the number of bases in a homopolymeric stretch of bases (i.e., C-stretch).

7.3.5. Each laboratory shall develop their own interpretation guidelines for HVII length variants. Long stretches of the same nucleotide are referred to as homopolymeric tracts. In HVI the homopolymeric C-stretch region typically starts at nucleotide position 16184, in HVII the homopolymeric tract is found between nucleotide positions 303 to 315. Homopolymeric tracts can differ in length within the same individual. In most cases, no attempt will be made to determine the exact number of bases in an HVI C-stretch. However, laboratories shall develop their own guidelines for interpretation. A common length variant can usually be determined in the HVII homopolymeric tract. A length variant alone must not be used to support an interpretation of exclusion (Stewart *et al.* 2001).²

² References for the MtDNA Standards are as follows:

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., et al. Sequence and organization of the human mitochondrial genomes. *Nature* 1981; 290:457-465.

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* 1999; 23: 147.

Stewart, J.E.B., et al. Length variation in HV2 of the human mitochondrial DNA control region. *Journal of Forensic Sciences* 2001; 46:862-870. Wilson, M.R., Allard, M.W., Monson, K., Miller, K.W.P., and Budowle, B. Recommendations for consistent treatment of length variants in the human mitochondrial DNA control region. *Forensic Science International* Vol. 129/1: 35-42.

Wilson, M.R., Allard, M.W., Monson, K., Miller, K.W.P., and Budowle, B. Further discussion of the consistent treatment of length variants in the human mitochondrial DNA control region. *Forensic Science Communications* 2002, Vol. 4, No. 4.

8.0 Standards for an NDIS Participating Laboratory's Use of a Subcontractor for the Analysis of Offender DNA Samples³

Any laboratory using a subcontractor for the analysis of offender DNA samples shall ensure that they and the subcontractor comply with the FBI Director's Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories, including most particularly that all DNA data and results provided by the subcontractor receive an appropriate technical review before being entered into CODIS. The DNA profiles being entered into CODIS shall be considered the property and responsibility of the laboratory.

The laboratory shall require that the subcontractor certify its compliance with the FBI Director's Quality Assurance Standards.

8.1 Procedures for the Review of the DNA Data Generated by the Subcontractor

8.1.1 The laboratory shall establish and use appropriate technical review and quality assurance procedures to verify the integrity of the data received from the subcontractor including, but not limited to, the following:

- (a) Random reanalysis of samples;
- (b) Visual inspection and evaluation of one hundred percent (100%) of the data and results generated by the subcontractor;
- (c) Inclusion of QC samples; and
- (d) Onsite visits.

The combination of samples included in the random reanalysis and QC samples shall be equal to a minimum of five percent (5%) of the total number of samples provided to the subcontractor.

The laboratory may accept documentation of an onsite visit of the subcontractor conducted by an NDIS Participating Laboratory having similar analyses/contract criteria in satisfaction of subsection (d).

³ Section 8 is the recommendation of the Ad Hoc Working Group on Contractor Review within the Scientific Working Group on DNA Analysis Methods (SWGDM).

9.0 Interim Procedure for the Temporary Use of Contract Personnel to Perform the Technical Review of Offender DNA Data Generated by a Subcontractor⁴

Until such time as Expert Systems have been approved for use at NDIS and laboratories have had an opportunity to validate and implement such systems into their operations, NDIS Participating Laboratories may use, as a temporary measure, contract personnel to perform the technical review of their offender DNA data in accordance with the requirements in subsection 9.1 and upload that DNA data to NDIS.

The use of contract personnel to perform the technical review of casework DNA data generated by a subcontractor that will be uploaded to NDIS is not permitted.

The NDIS Procedures Board will establish a termination date for the temporary use of contract personnel and will provide NDIS Participating Laboratories notice of the termination date at least one year in advance of such termination date.

9.1 Requirements for NDIS Laboratories Using Contract Personnel to Review Offender DNA Data Generated by a Subcontractor

9.1.1. The laboratory's review procedures may include the use of contract personnel to perform the visual inspection and evaluation of offender DNA data and results by the subcontractor if the contract personnel satisfy the following requirements:

- (a) Is currently or was previously a qualified DNA analyst on the platform (for example, capillary electrophoresis, real time gel-based detection, or end product gel-based detection) used to generate the DNA data by the subcontractor laboratory;
- (b) Is currently a CODIS User with logon access;
- (c) Is currently an employee of a laboratory participating in NDIS;
- (d) Is current in semi-annual external proficiency testing on the platform used to generate the DNA data by the subcontractor laboratory; and
- (e) Has no direct or indirect financial interest in the subcontractor laboratory.

9.1.2. The laboratory shall develop, use and document a training program and

⁴ Section 9 is the recommendation of the Ad Hoc Working Group on Contractor Review within the Scientific Working Group on DNA Analysis Methods (SWGAM). The work product of the Ad Hoc group was provided to the CODIS Committee within SWGDAM at the January 2005 Meeting and minor additional recommendations were incorporated before submission to the NDIS Procedures Board.

the successful completion of a qualifying test for all technical review personnel.

9.1.3 In exigent circumstances and subject to the approval of the NDIS Custodian, an SDIS laboratory may temporarily store and search offender DNA data and results provided by the subcontractor at SDIS prior to the completion of an appropriate technical review. These DNA results may temporarily remain at SDIS for one year; provided, however, that the SDIS laboratory may request an extension of no more than one additional year for such DNA results to remain at SDIS subject to the approval of the NDIS Custodian upon demonstration that sufficient progress has been achieved in conducting an appropriate technical review of the subcontractor's DNA data and results temporarily stored at SDIS. **The DNA data and results that have been temporarily stored at SDIS and have not been subject to an appropriate technical review shall not be uploaded to or searched at NDIS.**

9.1.3.1 Any candidate match resulting from such offender DNA results shall be confirmed and an appropriate technical review performed prior to notification and reporting of the match.

10.0 Standards for Expert Systems for the Review of Offender DNA Data

10.1 Expert Systems for the Review of Offender DNA Data Approved for Use at NDIS

A laboratory's review procedures may include the use of an NDIS approved Expert System to assist in performing the visual inspection and evaluation of convicted offender DNA results/data by the subcontractor⁵.

10.2 Quality Assurance Documentation Required for an NDIS Approved Expert System for the Review of Offender DNA Data

Once an Expert System is approved for use in the review of offender DNA data and implemented in an NDIS Participating Laboratory, the laboratory shall monitor the use of the Expert System in accordance with the recertification requirements of Section 10.3 below. This quality control procedure or recertification has been designed to test for unexpected drift in the performance of the Expert System software due to unintended changes in rules and thresholds, hardware changes, new operating systems, etc... This quality control procedure is separate and distinct from the internal validation procedures required when intentional changes and/or modifications are made to the settings and/or

⁵ This provision was included in the recommendation of the Ad Hoc Working Group on Contractor Review within the Scientific Working Group on DNA Analysis Methods (SWGAM).

new versions of the Expert System are introduced.

10.3 Recertification of an NDIS Approved Expert System for the Review of Offender DNA Data

- 10.3.1 To verify the integrity of the Expert System, a re-certification shall be performed by the laboratory using a defined data set of no less than 200 unique samples. A “sample” shall mean a genotype resulting from the analysis of one DNA specimen from one person. This quality control challenge data set may consist of sample data used in the original NDIS validation to calibrate the system or other data with the variety of challenging results necessary to ensure the continued integrity and accuracy of the DNA typing results generated by the NDIS-approved expert system (see Appendix B: Table I). In order to be re-certified for continued use, the Expert System shall demonstrate complete concordance of the defined data set with the known DNA typing results.
- 10.3.2 This quality control challenge data set shall be run through the NDIS approved Expert System on a quarterly basis (regular intervals of three months). The intent of such an interval is to ensure that at least four (4) such challenges, spaced approximately three months apart, are made to the system each calendar year.
- 10.3.3 Documentation of the concordance results shall be available for review during the quality assurance audit process.

Appendix A

*Guidelines for Submitting Requests
for Approval of New PCR Loci
or New or Modified PCR Kits*

A.1 Background.

To ensure the compatibility of DNA profiles submitted by federal, state and local forensic laboratories and ensure the integrity and accuracy of the DNA data submitted for inclusion in the National DNA Index System, the Federal Bureau of Investigation (FBI) has, and will continue to specify, CODIS core loci and required controls for submission of DNA data to NDIS.

The FBI, in consultation and collaboration with the forensic science community, has determined core loci for PCR DNA data for inclusion in NDIS (also known as “CODIS core loci”). The use of these same loci by the laboratories participating in NDIS should ensure compatibility of the DNA data for database searching purposes and the exchange of information. For the PCR data, the FBI has accepted specific PCR kits for inclusion at NDIS.

The FBI encourages the forensic community to continue in these collaborations and when appropriate, submit requests for the approval of new loci and/or new PCR kits.

A.2 Request for Approval of New PCR Loci and/or New or Modified PCR Kits.

Requests to add a new PCR kit or locus or a modified PCR kit must be submitted by a sponsoring forensic DNA laboratory participating in the National DNA Index System (NDIS) or a federal, state or local laboratory using CODIS but not yet participating in NDIS. Requests must be in writing, accompanied by the appropriate documentation and forwarded to the NDIS Custodian at the following address:

NDIS Custodian
FBI Laboratory
Room 1120
2501 Investigation Parkway
FBI Academy Complex
Quantico, VA 22135

The FBI will provide a written acknowledgement of each request received (*see* Figure 1).

A.3 Criteria Used in Reviewing Requests.

Requests submitted to the NDIS Custodian for the approval of new loci or a new/modified PCR kit will be reviewed and evaluated by a panel of FBI personnel. The Panel will consider the following criteria to the extent appropriate in determining whether to approve a new locus or a new PCR kit for a specific application:

1. Concordant Studies (including performance and validation studies for different platforms)
2. Mixed Samples
3. Non-Probative Samples
4. Population Studies
5. Precision Studies
6. Proficiency/Qualifying Samples
7. Reproducibility
8. Sensitivity Assays (to determine the best template for successful amplification)
9. Articles, if any, submitted for publication relating to the internal validation studies
10. And such other information as may be needed by the FBI in order to make a determination on the compatibility and suitability of the loci or kit for use at NDIS

Sponsoring agencies submitting a request should address the criteria referenced above, including supporting documentation to facilitate the review and evaluation. The documentation relating to the studies referenced above should include the number of samples or cases analyzed and a brief summary of the study and results. Additionally, if one or more other federal, state or local laboratory has conducted similar internal validation studies and wishes to join in the request, please include that laboratory's request with the supporting documentation or the laboratory or laboratories involved may submit their request(s) separately. Any documentation submitted in support of a request will be returned to the sponsoring laboratory upon determination of the request.

The FBI reserves the right to request additional information and documentation, as necessary, in order to conduct a thorough review and evaluation.

A.4 Response to Request and Notification of Changes, if applicable.

Once a determination is made by the FBI Panel, the submitting laboratory will be contacted by the NDIS Custodian with the response to the request. In the event that a new PCR locus or PCR kit is approved for use at NDIS, the NDIS Procedure entitled "*DNA Data Acceptance Standards*" will be revised to reflect the addition and NDIS and CODIS participating laboratories will be notified through the CJIS WAN of the new addition.

Letter will be on FBI Letterhead

(Date)

(Name & Address of NDIS Participating Laboratory)

Dear Mr./Ms.:

This will acknowledge receipt of your request to add a new locus or PCR kit for use at the National DNA Index System (NDIS).

A panel will be convened to review your request and you will be notified of any decision or need for additional information in a timely manner.

If you have any questions concerning the processing of your request, please contact [NDIS Custodian] at (703) 632-8315.

Sincerely,

[Signature]
NDIS Custodian
FBI Laboratory

Figure 1. Sample Letter Acknowledging Receipt of Request to Add a New Locus or PCR Kit

APPENDIX B
***Guidelines for Submitting Requests
for Approval of an Expert System
for Review of Offender Samples***

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Appendix B

Guidelines for Submitting Requests for Approval of an Expert System for Review of Offender Samples⁶

B.1 Background

To ensure the integrity and accuracy of the nuclear DNA data submitted by federal, state and local forensic laboratories for inclusion in the National DNA Index System, the Federal Bureau of Investigation (FBI) has, and will continue to specify, required controls for submission of DNA data to NDIS.

Pursuant to recommendations made by the SWGDAM Subcommittee on Expert Systems, the NDIS Board approves the following guidelines for submitting requests for approval of the use of an Expert System for the interpretation of DNA samples for laboratories performing DNA analyses on known samples collected in a controlled manner.

The purpose of an Expert System is to increase laboratory efficiency and/or effectiveness by automatically interpreting nuclear DNA allele/profile data. Data that has been analyzed by an Expert System can be classified into one of the following categories:

Accept: Profiles and/or alleles identified by the Expert System that do not contain any anomalies or problems that would prevent them from being entered into CODIS without further review.

Edit: Profiles and/or alleles identified by the Expert System that contain one or more anomalies that require manual review before the data can be entered into CODIS.

Reject: Profiles and/or alleles identified by the Expert System that have one or more problems that prevent them from being entered into CODIS. Typically, data is considered “reject” and not “edit” when the number of problems exceeds some user-defined threshold. That is, the data is so bad there is no point in having a manual review.

It is permissible for Expert Systems to not make a distinction between “Edit” and “Reject”; that is, data can be classified as either “Accept” or “Edit/Reject”. Figure 1 depicts all of the possibilities.

⁶These guidelines also apply to the use of Expert Systems on “known” samples collected in a controlled manner (e.g., court ordered blood draw, buccal swab, etc.) that are analyzed in the course of forensic casework or mass fatality identifications.

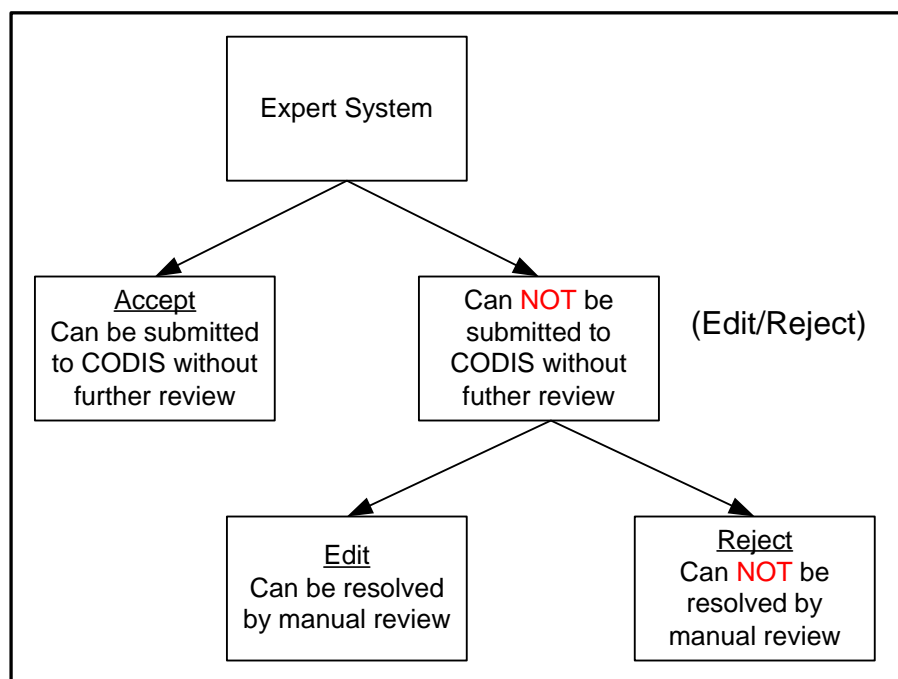


Figure 1. Results of Interpretation by Expert System

An Expert System is defined as follows:

An Expert System is a software program or set of software programs that meets all of the following criteria:

1. *A laboratory intends to use the system to replace one or both of the manual review processes defined in Section 12.1 of Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories;⁷*
2. *The system performs all of the following functions without human interpretation⁸ and with minimal possibilities for human error⁹:*
 - *Identifies peaks / bands*
 - *Assigns alleles*

⁷ None of these requirements are relevant if a laboratory does not intend to use the system to replace manual review.

⁸ As soon as a human is inserted into one of these steps, all downstream steps have potential for human error. For example, if human interpretation occurs while Identifying Peaks, then that step, and all subsequent steps, require manual review as specified in Section 12.1 of Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories.

⁹ Opening a file using a software program is NOT considered a significant "possibility for human error". However, for example, opening Genotyper in non locked mode is considered "opportunity for human error" because alleles can inadvertently be clicked on or off.

- Ensures data meet laboratory-defined quality checks including, at a minimum, validating positive and negative controls and ladders; ensure alleles from overlapping loci are concordant (when applicable); ensuring acceptable RFU values, and ensuring acceptable peak height/area ratios.
 - Describes the rationale behind decisions (e.g., Why a peak wasn't considered an allele; etc.)¹⁰;
3. The system does not make incorrect allele calls in those cases where the results are classified as "Accept" (i.e., the data can be entered directly into CODIS without manual review).¹¹

B.2 Request for Approval of an Expert System for Review of Offender Samples

Requests for approval of an Expert System for the review of offender samples shall be submitted by a sponsoring forensic DNA laboratory participating in the National DNA Index System (NDIS).¹² Requests shall be in writing, accompanied by the appropriate documentation and forwarded to the NDIS Custodian at the following address:

NDIS Custodian
FBI Laboratory
Room 1120
2501 Investigation Parkway
FBI Academy Complex
Quantico, VA 22135

The FBI will provide a written acknowledgement of each request received (*see* Figure 2).

B.3 Criteria Used in Reviewing Request for Approval of an Expert System

NDIS review and approval of an Expert System will be specific only for the version of the

¹⁰ It is important that each decision made by the Expert System is electronically documented.

¹¹ The primary concern of NDIS is that profiles (or alleles) documented as "Accept" are always correct. Therefore, an Expert System that cannot interpret stutter (or any other data interpretation challenge) ***MAY STILL BE*** approved by NDIS, provided that profiles with stutter are never classified as "Accept". This is subtle but important! An Expert System does not have to automatically call alleles for all of the types of challenges -- it just has to recognize those situations when it is incapable of making an interpretation. And, obviously, Expert Systems capable of interpreting more challenges are more useful to laboratories.

¹² An NDIS participating laboratory shall sponsor the approval of Expert Systems used by private laboratories (e.g., vendors that provide DNA analyses services to states under contract). In these cases it is expected that the Expert System is either: 1) proprietary to the vendor; or 2) a commercial product that is only being used by the vendor.

software submitted. The NDIS Custodian grants approval for the use of the Expert Systems on a specific combination of instrument platforms and kits.

After a particular version of an Expert System has been approved, laboratories wanting to use that system¹³ (adopting laboratories) shall follow the procedures described in Section B.5, *Implementation of an Approved Expert System for Use in Producing NDIS Acceptable Data*.

Requests submitted to the NDIS Custodian for the approval of an Expert System will be reviewed and evaluated by a panel established by the FBI. The Panel will consider the following criteria to the extent appropriate in determining whether to approve such an Expert System:

1. The sponsoring laboratory shall perform and complete the appropriate components of a developmental validation in accordance with Section 8 of the *Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories*.
 - a. Validation shall include studies to demonstrate accuracy, precision, and reproducibility.
 - b. The sponsoring laboratory shall provide a copy of their complete validation documents with the request for data review and evaluation. These documents shall demonstrate the Expert System, using established rules and thresholds, will provide review data equivalent to the samples routinely analyzed by the sponsoring laboratory.
 1. At least 200 unique samples shall be analyzed for the calibration of the software and presented for review. For purposes of this Appendix, a “sample” shall mean a genotype resulting from the analysis of one DNA specimen from one person.
 2. The initial 200 unique calibration samples shall be re-run and evaluated with each trial.
 3. The software review procedure shall be documented.
2. A concordance study of a minimum of 1,000 unique samples shall be conducted to demonstrate that the system performs as well as, or better than, the current system used by the sponsoring laboratory^{14, 15}. The 200 unique calibration samples described in paragraph 1.b. above shall not be included in the samples required for the concordance study. This means that a total of 1,200 unique samples shall

¹³ Expert Systems may only be used in conjunction with the kits and instruments for which approval was granted.

¹⁴ It is expected that the laboratory conducting the concordance study provides all of the samples (the test set) used in the study. However, in the case of private DNA laboratories (vendors), the test set may consist of samples provided by several states, provided the analytical methods and interpretation guidelines used by the vendor will be the same for all states included in the study.

¹⁵ The 1,000 samples represent the qualifying test for the Expert System.

be included in the data provided for approval.

- a. Using data from the concordance study, the sponsoring laboratory shall complete the table shown in Table 1 to demonstrate that the Expert System does not incorrectly document alleles as “Accept”. The sponsoring laboratory shall subject the Expert System to all of the challenges listed in Table 1, and provide appropriate references to the supporting documentation. The manual review of the data shall precede the electronic review.
3. And such other information as may be needed by the FBI in order to make a determination on the use of an Expert System in the review of offender samples.
4. Criteria 1 through 3 shall apply to all combinations of kits and instrument platforms (e.g., ABI 3100 class, ABI 310 class, etc.) that will be used in conjunction with the Expert System.

Sponsoring laboratories submitting a request shall address the criteria referenced above, including supporting documentation to facilitate the review and evaluation. Any documentation submitted in support of a request will be returned to the sponsoring laboratory upon determination of the request.

The FBI reserves the right to request additional information and documentation, as necessary, in order to conduct a thorough review and evaluation.

B.4 Response to Request and Notification of Changes, if applicable

Once a determination is made by the FBI Panel, the sponsoring laboratory will be contacted by the NDIS Custodian with the response to the request. In the event that an Expert System is approved for the review of offender samples, the NDIS Procedure entitled “*DNA Data Acceptance Standards*” will be revised to reflect the addition and NDIS and CODIS participating laboratories will be notified through the CJIS WAN of the new addition.

The following information will be included in the notification:

Manufacturer	Expert System and Version(s)	Instrument Platform(s)	Kit(s)

B.5 Implementation of an Approved Expert System for use in Producing NDIS Acceptable Data

Laboratories adopting an NDIS approved Expert System shall complete an internal validation process to demonstrate that the Expert System performs as expected within the individual

laboratory.¹⁶ This internal validation shall include, but may not necessarily be limited to, the following:

1. Establishing rules and thresholds for the laboratory in a study consisting of at least 200 unique samples. (These samples are for the purpose of calibrating the Expert System software.)
2. A one time assessment of the concordance of allele designations between the Expert System and the validated system currently in place in the laboratory for ten percent (10%) of the number of unique samples annually produced by the laboratory or 500 unique samples, whichever is less.¹⁷ Samples included in this concordance test shall be representative of samples analyzed in the laboratory (including representation of the various substrates);
 - a. Evaluation of non-concordant data to determine if the Expert System performs, at least as well as, the current validated allele calling procedure in the laboratory.
3. The Expert System shall be included in the laboratory's routine, external proficiency testing program.
4. Criteria B.5.1 through B.5.3 apply to all combinations of kits and instrument platforms (e.g., ABI 3100 class, ABI 310 class, etc.) that the laboratory will use in conjunction with the Expert System.

The internal validation shall be documented and available for review during the quality audit process. The internal validation shall be executed whenever that laboratory adjusts any of its interpretation thresholds, changes any parameters in the Expert System software, or makes any changes to its collection or analyses protocols that could affect the efficacy of the Expert System.

B.6 Approval of New Versions of Expert Systems

It is expected that Expert System vendors will periodically provide new versions of their software. Before using any new version in production, the sponsoring laboratory is required to

¹⁶ A laboratory that has performed the developmental validation described in Section B.3 is not required to perform the internal validation described in this section.

¹⁷ This represents the qualifying test for the Expert System.

validate the new software by re-performing the concordance study described in Section B.3.¹⁸ Although the vendor may claim that a new version contain only cosmetic changes (e.g., screen layouts, reports, etc.), the laboratory is still required to perform the concordance validation study in its entirety. After completing the concordance study, the sponsoring laboratory shall submit a notification letter describing the results to the NDIS Custodian. The FBI reserves the right to request the supporting validation data.

¹⁸ In those instances when the vendor is adding one or more features for the benefit of a non-sponsoring laboratory (that is, a laboratory that falls under Section B.5 instead of Section B.3), that laboratory shall either: 1) follow Section B.3; or 2) find a sponsoring laboratory to validate the new version of the Expert System.

Letter will be on FBI Letterhead

(Date)

(Name & Address of NDIS Participating Laboratory)

Dear Mr./Ms.:

This will acknowledge receipt of your request for approval of an Expert System for review of offender samples.

A panel will be convened to review your request and you will be notified of any decision or need for additional information in a timely manner.

If you have any questions concerning the processing of your request, please contact me at (703) 632-8315.

Sincerely,

[Signature]
NDIS Custodian
FBI Laboratory

Figure 2. Sample Letter Acknowledging Receipt of Request for Approval of an Expert System.

**Instructions for Completion of Table 1:
“Expert System Performance on Challenged Samples”**

1. **Name of Submitting Agency:** Please list the laboratory that encompasses the purview of this submission.
2. ***Point of Contact:*** The name, phone number and e-mail of the individual(s) that is (are) responsible for correspondence related to this submission.
3. ***Total Number of Calibration Samples Tested (Appendix B.5.1):*** This number shall represent at a minimum 200 unique samples. For purposes of this Appendix, a “sample” shall mean a genotype resulting from the analysis of one DNA specimen from one person.
4. ***Total Number of Concordance Samples Analyzed to test Expert System Rules (Appendix B.3.2).***
 - a. This number shall represent at a minimum 1000 unique samples, and shall not include any of the samples reported in line 3. This means that a total of 1,200 unique samples shall be included in the data provided for approval.
 - b. ***Number samples "Accept" by ES:*** Out of the number of samples evaluated by the ES (4a), how many samples met the criteria to be entered into CODIS without human review
 - c. ***Number of samples with 100% concordance between the ES "Accept" and the manual review (B.3, 2 study):*** Out of the number of samples evaluated by the ES (4a), how many samples showed 100% agreement with the original manual allele calls.
 - d. ***Number discordant samples where manual review was incorrect:*** Upon comparison with the manual reviewed data and the ES data (4a), how many samples had been incorrectly called by the manual review.

TABLE 1

A. This table represents the summary of analysis of selected challenged samples that have been previously confirmed. Although one sample may contain more than one challenge, each sample may count for only one example of a challenge. For example, one sample may contain stutter and spikes, but it cannot count for both stutter and spikes.

B. The following are working definitions of the information requested in the columns labeled:

- **# Times Challenged:** The laboratory shall document five or more instances of each specified challenge (where appropriate) for the NDIS Board to

review.

- # Times Challenge Classified as “Accept”: The number of times the Expert System classified the challenge as “Accept”. That is, the Expert System evaluated the profile and determined that it could be entered into CODIS without human review.
- # Times Challenge Classified as “Edit/Reject”: The number of times the Expert System classified the challenge as “Edit/Reject”. That is, the Expert System determines that it cannot evaluate the profile without human assistance.

Note: *# Times Challenge Classified as “Accept” + # Times Challenge Classified as “Edit/Reject” = # Times Challenged*

- #Times Challenge Sample “Accept” was Incorrect: The number of times the Expert System incorrectly classified a profile as “Accept”. This means the Expert System incorrectly determined that it was acceptable for upload to CODIS.
- List Location of Data (notebook, file etc) in Submission Documents where this data may be found: The laboratory shall identify where the data listed in Table 1 are located. This may be accomplished via an Appendix (e.g., notebook number and tab, color coded tabs etc.).

5. Stutter: Definition of stutter shall be as defined in the submitting laboratory’s internal Standard Operating Procedure.

a) % Range for Loci: Define range of stutter (across all loci, lowest threshold to highest threshold). Example: CSF1P0 = 8%; TH01 = 16%, therefore the range = 8% to 16%

b) > Threshold Stutter %: Number of Challenges for which the stutter percentage observed exceeds the user defined threshold at a given locus. (“5 by 5” means at least five challenges at a minimum of five different loci.)

6. Locus Peak Amplitude Imbalance: Calculated as either peak-height or peak-area imbalance within a locus.

7. Artifacts: Definition of the following artifacts shall be as defined in the submitting laboratory’s internal Standard Operating Procedure.

a) Pullup / Bleedthrough

b) Shoulders (+A and –A)

c) Spikes

8. Peaks

- a) Tri-allelic pattern: Challenge involving the observation of three alleles at a given locus
- b) Mixture: Challenge involving the observation of three or more peaks at two or more loci. (If necessary, the laboratory may prepare samples containing DNA from two or more individuals).
- c) Contamination: Challenge involving the observation of signal in the negative control and/or reagent blank. (Contamination may be simulated by renaming a sample that exhibits peaks as a negative control.)

9. Off Ladder Alleles:

- a) Microvariant Allele: Challenge involving the observation of an off ladder allele within the allelic ladder due to the presence of an allele that is one, two or three nucleotides shorter than the canonical allele size thus causing the amplified allele to migrate below that standard allele in the AL.
- b) Above/Below Allelic Ladder: Challenge involving the observation of an allele that migrates above or below an allelic ladder for a specific locus.

10. Missing Loci: Challenge involving the observation of a partial profile. (Missing Loci can be simulated by diluting a sample or using degraded material)

TABLE 1: EXPERT SYSTEM PERFORMANCE ON CHALLENGED SAMPLES

1	Name Of Submitting Laboratory:						
2	Name(s) for Point of Contact:						
		2a. Phone#:			2b. e-mail:		
3	<---Total Number of Calibration Samples Tested (Appendix B.3, 1)						
4	a)	<---Total Number of Concordance Samples to test ES Rules (Appendix B.3, 2)					
	b)	<---Number samples "Accept" by ES					
	c)	<---Number of samples with 100% concordance between the ES "Accept" and the manual review(B.3, 2 study)					
	d)	<---Number discordant samples where manual review was incorrect					
	Challenge	Minimum # Events that MUST be observed	# of Challenges	# Times Challenge Classified as "Accept"	# Times Challenge Classified as "Edit/Reject"	# Times Challenge Classified as "Accept" was Incorrect	List Location of Data (notebook, file etc) in Submission Documents where this data may be found
5	Stutter a) % Range for loci: b) >Threshold Stutter %						
		5 by 5					
6	Locus Peak Amplitude Imbalance	5					
7	Artifacts a) Pullup / Bleedthrough b) Shoulders (+A and -A) c) Spikes	5					
		5					
		5					
8	Peaks a) Tri-allelic patterns b) Mixture c) Contamination	5					
		5					
		5					
9	Off Ladder Alleles a) Microvariant Allele b) Above/Below Allelic Ladder						
		5					
10	Missing Loci	5					

I certify that this study was performed under the same rules as the 200 unique calibration samples described in Section B.3.1.