applied biosystems

# NGM Detect™ PCR Amplification Kit

## USER GUIDE

**Catalog Number** A31832

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**Revision** D



ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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**Revision history:** Pub. No. 100044085

| Revision | Date | Description |
|---|---|---|
| D | 2 January 2019 | • We redesigned primers at the IQC Large and TH01 markers to correct high background seen in samples with a high soil burden, then performed subsequent validation work. Accordingly, we made the following changes to this user guide:<br>– Chapter 1—Updated "About the primers", Figure 1, and Figure 2.<br>– Chapter 2—Updated Figure 3.<br>– Chapter 6—Updated text and figures throughout.<br>• Chaper 4—Updated analysis file references to v3. |
| C | 13 March 2017 | • Added information for artifact (p 85).<br>• Removed IQC from precision and accuracy graphs (update three figures) and tables; noted that they were not included in the study (p 60–66).<br>• Updated wording on how stutter filters are calculated; updated minus stutter values; added plus stutter values (pp 75, 81–82).<br>• Corrected typo—change 401 to 410 (p 88).<br>• Updated SWGDAM references from 2012 to 2016. |
| B | 28 December 2016 | • Added Chapter 6, "Experiments and results".<br>• Added information about direct amplification to Chapter 2. |
| A | 21 September 2016 | New document |

# Contents

# 1 Product information



**IMPORTANT!** Before using this product, read and understand the information in the "Safety" appendix in this document.

## Product description

### Kit overview

The Applied Biosystems™ NGM Detect™ PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of specific loci in human genomic DNA.

The kit amplifies:

- 16 autosomal STR loci:
  - The 12 loci in the extended European Standard Set (ESS): FGA, TH01, vWA, D3S1358, D8S1179, D18S51, D21S11, D12S391, D1S1656, D2S441, D10S1248, and D22S1045
  - 3 additional loci that are not in the ESS, but that are present in the AmpFℓSTR™ SGM Plus™ PCR Amplification Kit: D16S539, D2S1338, and D19S433
  - SE33, a highly polymorphic locus
- Two internal quality control markers (IQCS and IQCL)
- 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel)
- Amelogenin (sex determining marker)

### Internal quality control system for PCR

The NGM Detect™ kit is an Applied Biosystems™ STR kit that includes an internal quality control (IQC) system for PCR. The IQC system has two synthetic targets, one low molecular weight and one high molecular weight, that are amplified with the sample. The behavior of the IQC target peaks can be used to evaluate the success of the PCR reaction and give an indication of sample quality.

### Validated DNA input amounts and PCR cycles

The kit is validated for use with 500 pg DNA (15-µL input volume) for 30 cycles.

## About the primers

The NGM Detect™ kit primers are manufactured using the same synthesis and purification improvements as the primers in the GlobalFiler™ kit, Identifiler™ Plus kit, and NGM SElect™ kit. These improvements enhance the assay signal-to-noise ratio and simplify the interpretation of results.

The primers that are used in the NGM Detect™ kit are unique when compared with primers for corresponding STR loci in the GlobalFiler™, Identifiler™ Plus, and NGM SElect™ kits except for one D12 primer and one TH01 primer that are shared with the GlobalFiler™ and NGM SElect™ kits.

### Changes to the IQC Large and TH01 primers

A small number of laboratories reported high background with the original formulation of the NGM Detect™ kit when samples with a high soil burden were run. After internal investigations, we determined that cross-reactivity of the IQC Large and TH01 primers with soil-associated microbial DNA caused the background artifact peaks. Accordingly, we redesigned primers for both markers. We did not make any other changes to the NGM Detect™ kit formulation, protocols, or workflow.

The updated formulation of the NGM Detect™ kit was re-validated with internal and external testing. Other than correcting the soil-associated background issue, the performance of the updated formulation assay is fully comparable to that of the original kit. Unless otherwise indicated, the data in Chapter 6, "Experiments and results" are from the re-validation study.

See the *Technical Note: Updated NGM Detect™ PCR Amplification Kit: Validation and Comparative Study* for studies directly related to soil specificity and a direct comparison of the NGM Detect™ kit original formulation to the updated formulation.

## Dyes used in the kit

| Dye | Color | Label |
|---|---|---|
| 6-FAM™ | Blue | Samples, allelic ladders, and controls |
| VIC™ | Green | |
| TED™ | Yellow | |
| TAZ™ | Red | |
| SID™ | Purple | |
| LIZ™ | Orange | GeneScan™–600 LIZ™ Size Standard v2.0 |

## Loci amplified by the kit

**Table 1** NGM Detect™ kit loci and alleles

| Locus designation | Chromosome location | Alleles included in Allelic Ladder | Dye label | DNA Control 007 |
|---|---|---|---|---|
| IQCS | N/A | 1, 2 | 6-FAM™ | 2 |
| D2S1338 | 2q35 | 11–28 | | 20,23 |
| SE33 | 6q14 | 4.2, 6.3, 8, 9, 11–20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2,33.2, 34.2, 35, 35.2, 36–39, 42 | | 17, 25.2 |
| IQCL | N/A | 1, 2 | | 2 |
| D16S539 | 16q24.1 | 5, 8–15 | VIC™ | 9,10 |
| D18S51 | 18q21.33 | 7, 9, 10, 10.2, 11–13, 13.2, 14, 14.2, 15–27 | | 12, 15 |
| TH01 | 11p15.5 | 4–9, 9.3, 10–13, 13.3 | | 7, 9.3 |
| D12S391 | 12p13.2 | 14–19, 19.3, 20–27 | | 18, 19 |
| D3S1358 | 3p21.31 | 9–20 | TED™ | 15, 16 |
| FGA | 4q28 | 13–30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2 | | 24, 26 |
| Y indel | Yq11.221 | 1, 2 | TAZ™ | 2 |
| Amelogenin | X p22.1–22.3, Y: p11.2 | X, Y | | X, Y |
| vWA | 12p13.31 | 11–24 | | 14, 16 |
| D21S11 | 21q11.2–q21 | 24, 24.2, 25–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38 | | 28, 31 |
| D1S1656 | 1q42.2 | 9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3 | | 13, 16 |
| D2S441 | 2p14 | 8–11, 11.3, 12–17 | | 14, 15 |
| D8S1179 | 8q24.13 | 5–19 | SID™ | 12, 13 |
| D19S433 | 19q12 | 6–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2 | | 14, 15 |
| D22S1045 | 22q12.3 | 7–20 | | 11, 16 |
| D10S1248 | 10q26.3 | 8–19 | | 12, 15 |

## Standards and controls that are required

For the NGM Detect™ kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **DNA Control 007**—A positive control for evaluating the efficiency of the amplification step and STR genotyping using the NGM Detect™ Allelic Ladder. DNA Control 007 is present in the kit. See "DNA Control 007" on page 13.
- **GeneScan™–600 LIZ™ Size Standard v2.0**—Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for PCR products. Order the GeneScan™–600 LIZ™ Size Standard v2.0 (Cat. No. 4408399) separately.
- **NGM Detect™ Allelic Ladder**—Developed for accurate characterization of the alleles amplified by the kit. The Allelic Ladder is present in the kit and allows automatic genotyping of most of the reported alleles for the loci in the kit. See "Allelic ladder profile" on page 11.

## Allelic ladder profile

The allelic ladder profile appears on the next page.

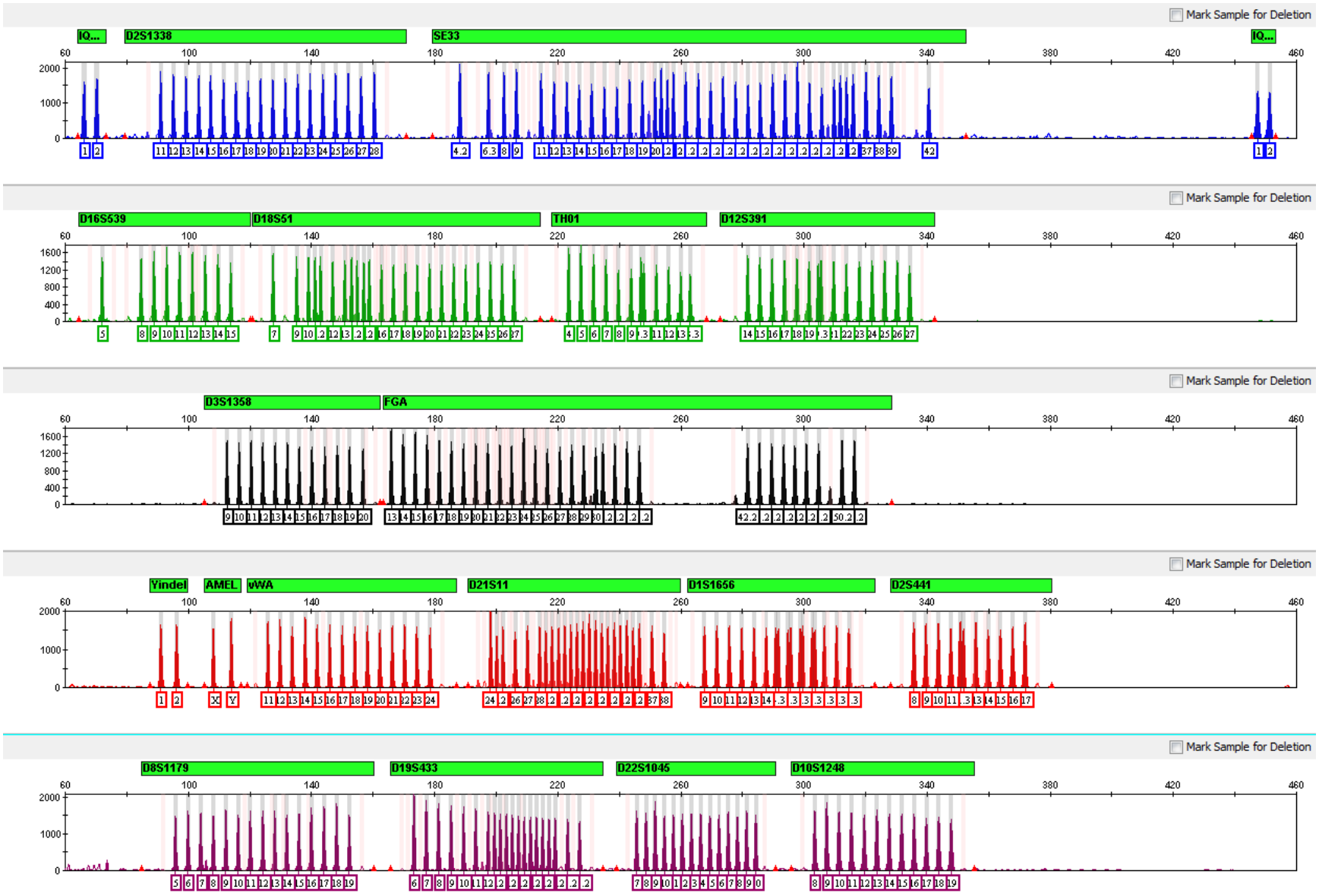



**Figure 1** GeneMapper™ *ID-X* Software plot of the NGM Detect™ Allelic Ladder

## DNA Control 007

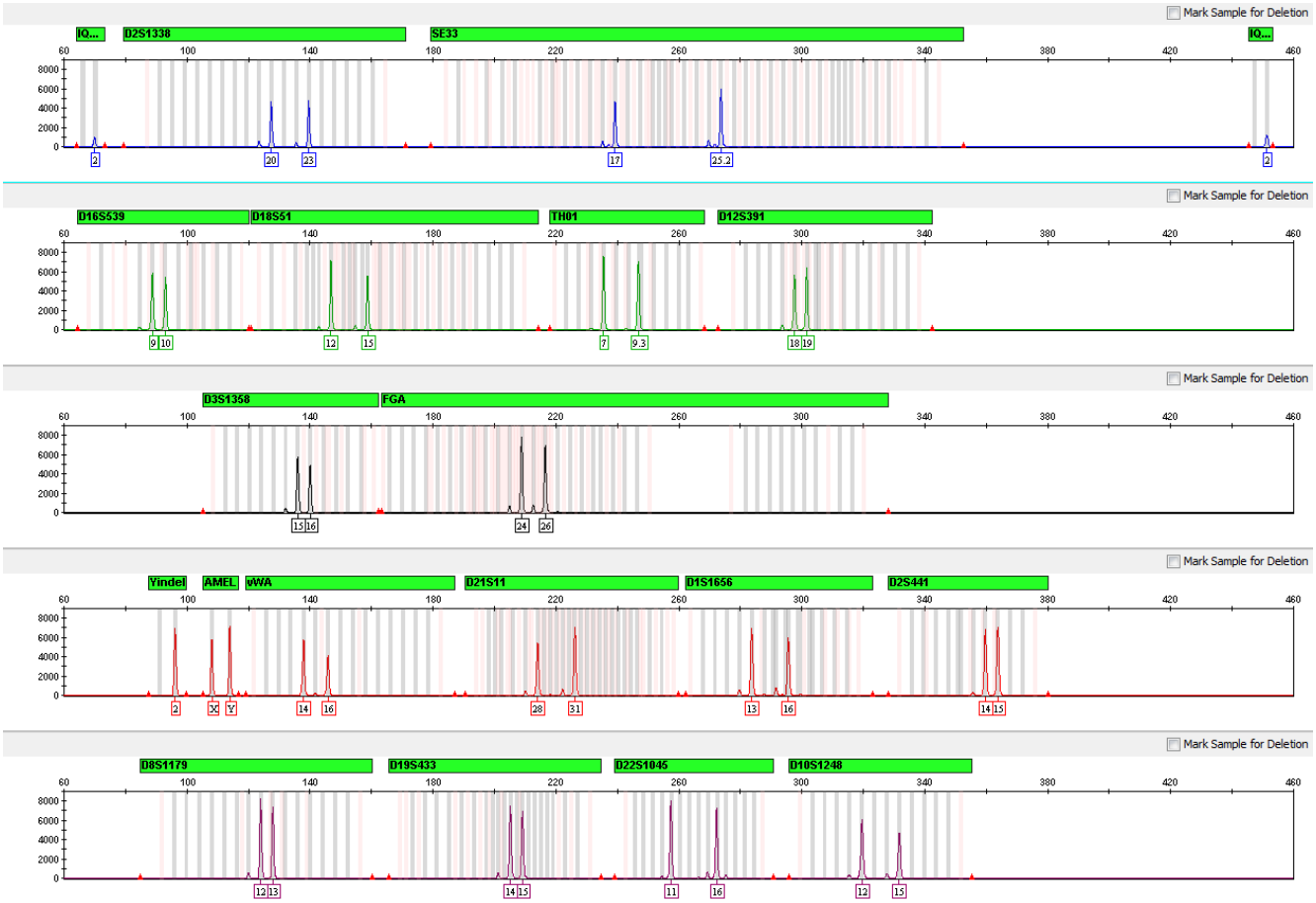



**Figure 2** DNA Control 007 (500 pg) amplified with the NGM Detect™ kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 8,000 RFU).

# Contents and storage

The NGM Detect™ kit (Cat. No. A31832) contains sufficient quantities of the following reagents to perform 200 amplifications with a 25 µL total reaction volume.

**IMPORTANT!** The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.

**IMPORTANT!** Do not refreeze kit components after thawing.

**Table 2** NGM Detect™ PCR Amplification Kit (Cat. No. A31832; 200 reactions)

| Contents | Description | Amount | Storage |
|---|---|---|---|
| NGM Detect™ Master Mix | Contains enzyme, salts, dNTPs, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt. | 2 × 0.75 mL | –25°C to –15°C on receipt.<br>2°C to 8°C after first use, for up to 6 months or up to the expiration date stated on the kit (whichever comes first). |
| NGM Detect™ Primer Set | Contains forward and reverse primers to amplify DNA targets. | 2 × 0.25 mL | –25°C to –15°C on receipt.<br>2°C to 8°C after first use, for up to 6 months or up to the expiration date stated on the kit (whichever comes first).<br>Store protected from light. |
| NGM Detect™ Allelic Ladder | Contains amplified alleles.<br>See "Allelic ladder profile" on page 11 for information. | 1 × 0.05 mL | –25°C to –15°C on receipt.<br>2°C to 8°C after first use, up to the expiration date stated on the kit.<br>Store protected from light. |
| DNA Control 007 | Contains 0.1 ng/µL human male genomic DNA from cell line in 0.05% sodium azide and buffer[1]<br>See "DNA Control 007" on page 13 for information. | 1 × 0.3 mL | –25°C to –15°C on receipt.<br>2°C to 8°C after first use, up to the expiration date stated on the kit. |

[1] DNA Control 007 is included at a concentration that is appropriate for use as an amplification control (that is, to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). It is not designed for use as a DNA quantification control. If you quantify aliquots of DNA Control 007, the concentration may differ from the labeled concentration.

# Required materials not supplied

See Appendix B, "Materials required but not supplied".

# Instruments and software compatibility

| Instrument type | Validated models |
|---|---|
| Thermal cyclers | • ProFlex™ 96-well PCR System (Cat. No. 4484075)<br>• ProFlex™ 2 × 96-well PCR System (Cat. No. 4484076)<br>• ProFlex™ 3 × 32-Well PCR System (Cat. No. 4484073)<br>• Veriti™ 96-Well Thermal Cycler (Cat. No. 4479071)<br>• GeneAmp™ PCR System 9700, 96-Well Silver (Cat. No. N8050001)<br>• GeneAmp™ PCR System 9700, 96-Well Gold-Plated (Cat. No. 4314878)<br><br>**IMPORTANT!** The NGM Detect™ kit is NOT validated for use with:<br>• ProFlex™ 2 × Flat PCR System (Cat. No. 4484078)<br>• ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077)<br>• Veriti™ 96-Well *Fast* Thermal Cycler (Cat. No. 4375305)<br>• GeneAmp™ PCR System 9700 with the aluminium 96-well block (Cat. No. 4314879) |
| Genetic analyzers[1] | • 3500/3500xL Genetic Analyzer with any of the following:<br>– 3500 Data Collection Software v1 (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)<br>– 3500 Data Collection v2 Software (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)<br>– 3500 Data Collection v3 Software (Windows™ 7 operating system)<br>• 3130/3130*xl* Genetic Analyzer with:<br>– Data Collection Software v4 (Windows™ 7 operating system)<br>– 3130/3730 Data Collection v4 6-Dye Module v1 |
| Analysis software | GeneMapper™ *ID-X* Software v1.5.2 or later[2]<br><br>Windows™ 7 operating system |

[1] We conducted validation studies using the 3130*xl*, 3500, and 3500xL configurations.

[2] GeneMapper™ *ID-X* Software v1.2 to v1.5 can be used to analyze NGM Detect™ PCR Amplification Kit data. However, some genotype quality assessment features of the NGM Detect™ kit are not included in earlier versions of the software. Refer to Chapter 4, "Analyze data with GeneMapper™ ID-X Software" for more details.

# Workflow

**Extract DNA**, see:

www.thermofisher.com/hid-sampleprep

▼

**Quantify DNA**

"DNA quantification" on page 17

▼

**Perform PCR**

"Prepare the amplification kit reactions" on page 19

▼

"Perform PCR" on page 21

▼

**Perform electrophoresis**

"Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)" on page 24 or
"Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)" on page 28

▼

"Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)" on page 27

▼

**Analyze data**

"Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 35

▼

"Create an analysis method" on page 40

▼

"Create a size standard definition file if needed" on page 48

▼

"Analyze and edit sample files with GeneMapper ID-X Software" on page 50

▼

"Examine or edit a project" on page 51

▼

Chapter 5, "Assess the PCR reaction with the Internal Quality Control System"

# 2 Perform PCR



## DNA quantification

### Importance of quantification before STR analysis

DNA quantification can be used to determine:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with short tandem repeat (STR) analysis.
- The amount of sample to use in STR analysis applications.
- The relative quantities of human male and female DNA in a sample (using the Quantifiler™ Trio DNA Quantification Kit). This guides selection of the applicable STR chemistry.
- The DNA quality, with respect to the inhibition level and the DNA degradation level. This metric is useful for determining the likelihood of recovery of STR loci with larger amplicon sizes.
- If the sample contains highly degraded DNA. Such samples may require an alternative approach to STR analysis by capillary electrophoresis. Precision ID NGS System and Panels are optimized for degraded samples. The Precision ID Identity Panel provides discrimination of individuals similar to STR genotype match probabilities. Also, the Precision ID Ancestry Panel infers biogeographical ancestry for investigative leads.
- If PCR inhibitors are present in a sample. Such that samples may require additional purification before proceeding to STR analysis.

### Effect of DNA quantity on results

If too much DNA is added to the PCR reaction, the increased amount of PCR product that is generated can result in:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the capillary electrophoresis instrument ("off-scale" data).

Off-scale data are a problem because:

- Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause a corresponding stutter peak to appear higher in relative intensity, therefore increasing the calculated percent stutter.
- Multicomponent analysis of off-scale data are not accurate. This inaccuracy results in poor spectral separation ("pull-up").

- False signals of inhibition by the IQC system, although none is present.
- A reduction in the IQCL peak height.
- Incomplete +A nucleotide addition.

To address these problems, rerun the amplification reaction using less DNA.

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR is extremely low. Unbalanced amplification of the alleles may occur because of stochastic fluctuation.

## Methods of quantifying DNA

For information on recent innovations in quantification chemistry, go to **thermofisher.com**.

| Kit and user guide | Detects | How it works |
|---|---|---|
| Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911)<br>For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354) | • Total human DNA (two targets—one small amplicon and one larger amplicon)<br>• Degraded DNA | • Uses 5′ nuclease assays with multiple-copy target loci, for improved detection sensitivity:[1]<br>– The human-specific target loci are multiple copy, and dispersed on various autosomal chromosomes.<br>– The primary quantification targets have relatively short amplicons (75 to 80 bases), to improve the detection of degraded DNA samples.<br>• Uses features that maximize consistency of quantification:<br>– Genomic targets have conserved primer- and probe-binding sites.<br>– Minimal copy number variation between different individuals and population groups.<br>• Contains a Large Autosomal target with a longer amplicon (>200 bases) to help determine if a DNA sample is degraded.<br>• Contains an Internal PCR control (IPC) 5′ nuclease assay which amplifies an integrated synthetic DNA sequence. The performance of this assay can be used to assess whether real-time PCR of the sample has been impacted by inhibition. |
| Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910)<br>For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354) | • Total human DNA (two targets—one small amplicon and one larger amplicon)<br>• Human male DNA<br>• Degraded DNA | |

[1] The detection sensitivity of the Quantifiler™ HP Kit and the Quantifiler™ Trio Kit is improved over the Quantifiler™ Duo Kit.

# Before you begin

## Prepare low-TE buffer

For optimal results, we recommend using low-TE buffer for sample preparation. Prepare it as described in this procedure or buy it from Teknova (Cat. No. T0223).

1. Mix together:
   - 10 mL of 1 M Tris-HCl, pH 8.0
   - 0.2 mL of 0.5 M EDTA, pH 8.0
   - 990 mL glass-distilled or deionized water

   **Note:** Adjust the volumes accordingly for specific needs.
2. Aliquot, then autoclave the solutions.
3. Store the aliquots at room temperature.

## Thaw reagents (before first use of the kit)

Thaw the Master Mix and Primer Set.

**IMPORTANT!** The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.

**IMPORTANT!** Thawing is required only during first use of the kit. After first use, reagents are stored at 2°C to 8°C and do not require subsequent thawing. Do not refreeze the reagents.

# Prepare the amplification kit reactions

**IMPORTANT!** The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.

**IMPORTANT!** Use adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Caps may not provide a consistent seal across all plate wells.

1. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes, remove droplets from the caps by centrifuging the tubes briefly.
2. Pipette the required volumes of components into an appropriately sized clear (non-colored) polypropylene tube:

| Reaction component | Volume per reaction |
|---|---|
| Master Mix | 7.5 µL |
| Primer Set | 2.5 µL |

**Note:** Include volume for additional reactions to provide excess volume for the loss that occurs during reagent transfers.

3. Vortex the reaction mixture for 3 seconds, then centrifuge briefly.

4. Dispense 10 µL of reaction mixture into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.

5. Adjust the sample input amount and volume as needed:

   **Note:** The optimum DNA input is 500 pg.

   - If total sample input amount is >500 pg of DNA, dilute with low-TE buffer to achieve a 500 pg input in a 15-µL volume.
   - If total sample input volume is <15 µL, bring to volume with low-TE buffer to achieve a 15-µL input volume.

6. Prepare samples as shown in the following table, then add them to the appropriate well or tube (final reaction volume is 25 µL).

| Sample | Add |
|---|---|
| Negative control | 15 µL of low-TE buffer |
| Test sample | 15 µL of DNA[1] |
| Positive control | Combine, then add to the reaction well or tube:<br>• 5 µL DNA Control 007 (0.1 ng/µL)<br>• 10 µL of low-TE buffer |

[1] Prepared in step 5.

7. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film.

8. Vortex the plate or tubes at medium speed for 3 seconds.

9. Centrifuge the tubes or plate at 3,000 rpm for approximately 30 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

10. Amplify the samples.

**IMPORTANT!** See "Instruments and software compatibility" on page 15 for a list of validated thermal cyclers.

# Perform PCR

**IMPORTANT!** This kit is validated for use with the thermal cyclers listed in "Instruments and software compatibility" on page 15.

1. Program the thermal cycling conditions.

   **IMPORTANT!** If you are using the:

   - ProFlex™ PCR System, select **9700 Simulation Mode**.
   - GeneAmp™ PCR System 9700, select the Max ramping mode.
   - Veriti™ Thermal Cycler, set up the method using the **Convert a Method** tool and select **9700 Max Mode**.

   *Do not* use 9600 emulation mode.

| Initial incubation step | Cycle (30 cycles) | | | Final extension | Final hold |
|---|---|---|---|---|---|
| | Denature | Anneal | Extend | | |
| HOLD | CYCLE | | | HOLD | HOLD |
| 95°C, 1 minute | 96°C, 5 seconds | 59°C, 21 seconds | 65°C, 29 seconds | 60°C, 5 minutes | 4°C[1] |

[1] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate into the thermal cycler, close the heated cover, then start the run.

   **IMPORTANT!** If you are using a GeneAmp™ PCR System 9700 *and* adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti™ Thermal Cycler and the ProFlex™ PCR System do not require a compression pad.

3. When the run is complete, store the amplified DNA.

| If you are storing the DNA... | Then place at... |
|---|---|
| <2 weeks | 2°C to 8°C |
| >2 weeks | –25°C to –15°C |

   **IMPORTANT!** Protect the amplified DNA from light.

# Direct amplification

FTA™ cards and non-chemically treated bloodstain cards are useful for the collection, storage, and processing of biological samples. A small punch disc of the card containing the sample can be placed directly into an amplification tube or 96-well plate, washed, then amplified, without transferring the disc.

Our studies indicate that a 1.2-mm bloodstained disc contains approximately 5 ng–20 ng of DNA. Because of the high quantity of DNA, a lower cycle number is required to produce on-scale data. In our testing, an appropriate cycle number for this high quantity DNA was 26 cycles. We recommend that each laboratory determine the optimum cycle number which is based on internal validation studies.

**Note:** This kit is not fully validated for use with direct amplification. Perform your own validation for this purpose or use the NGM SElect™ Express kit for direct amplification of database samples.

In the example that is shown in Figure 3, a 1.2-mm disc of a bloodstained FTA™ card was purified using one wash with FTA™ Purification Reagent and one wash with 1X low TE buffer, followed by a short drying step. The sample was then amplified directly in the well of a standard 96-well amplification MicroAmp™ plate for 26 cycles.

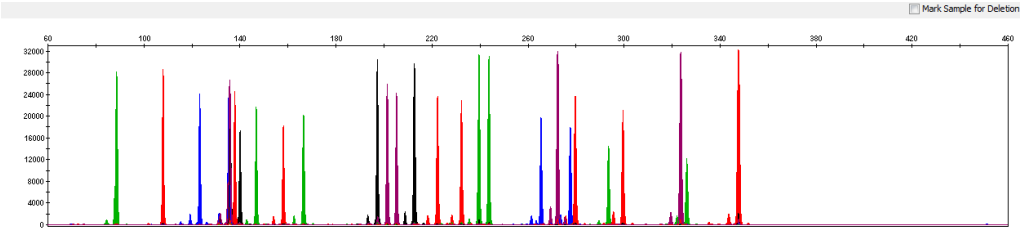



**Figure 3** Combined dyes electropherogram of a 1.2-mm FTA™ bloodstain disc amplified for 26 cycles with the NGM Detect™ kit on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 33,000 RFU).

# 3 Perform electrophoresis



## Allelic ladder requirements for electrophoresis

To accurately genotype samples, you must run an allelic ladder with the samples.

| Instrument | Number of allelic ladders to run | One injection equals | Number of samples per allelic ladder(s) |
|---|---|---|---|
| 3500 | 1 per 3 injections | 8 samples | 23 samples + 1 allelic ladder |
| 3500*xl* | 1 per injection | 24 samples | 23 samples + 1 allelic ladder |
| 3130 | 1 per 4 injections | 4 samples | 15 samples + 1 allelic ladder |
| 3130*xl* | 1 per injection | 16 samples | 15 samples + 1 allelic ladder |

**IMPORTANT!** Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs. Follow the guidelines in the preceding table, which should account for normal variation in run speed. Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

It is critical to genotype using an allelic ladder run under the same conditions as the samples. Size values obtained for the same sample can differ between instrument platforms, because of different polymer matrices and electrophoretic conditions.

# Materials required for electrophoresis

Appendix B, "Materials required but not supplied" lists the required materials that are not supplied with this kit.

**IMPORTANT!** The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.

# Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)

## Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products that are generated by this kit. For details on the procedures, see the documents that are listed in "Documentation and support" on page 136.

**Note:** We conducted validation studies for the kit using the 3130*xl*, 3500, or 3500xL configurations.

| Genetic Analyzer | Operating System | Data Collection Software | Additional software | Instrument protocols, run modules, and conditions |
|---|---|---|---|---|
| 3500<br>3500xL | Windows™ Vista | 3500 Data Collection Software v1 | HID Updater 3500 DC v2 (Cat. No. 4480670) | Set up the following conditions:<br>• Run module: HID36_POP4 (HID36_POP4xl for 3500xL)<br>• Injection conditions[1]: 1.2 kV/11 sec (20 sec for 3500xL)<br>• Run conditions: 13 kV/1500 sec (13 kV/1500 sec for 3500xL)<br>• Dye Set J6-T |
| 3500<br>3500xL | Windows™ 7 | 3500 Data Collection Software v2 | HID Updater 3500 DC v2 (Cat. No. 4480670) | Same as 3500 Data Collection Software v1 listed above |
| 3500<br>3500xL | Windows™ 7 | 3500 Data Collection Software v3 | None | Same as 3500 Data Collection Software v1 listed above |

[1] Our studies indicate that the injection conditions that are documented generate profiles from 0.5 ng of input DNA with heterozygous peak height averages between 4,000–10,000 RFU (3500 or 3500xL) with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary, therefore changes to injection parameters may need to be explored and validated to deliver the best results on your specific system. Large deviations from the recommended injection parameters could affect the performance of the size standard and/or allelic ladder, therefore validation is recommended.

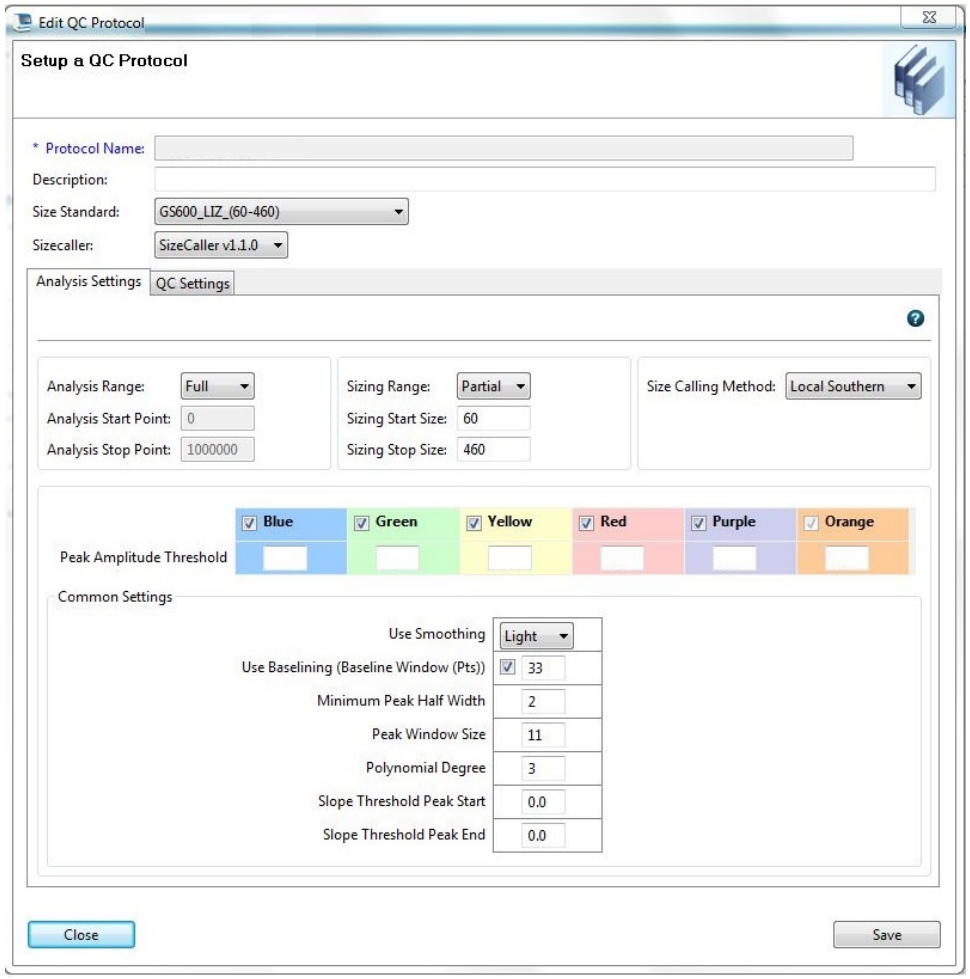
## Create a 3500 QC protocol

The NGM Detect™ kit has been validated with data that was analyzed using the Local Southern method (60–460 base pairs).

1. In the **Library** tab, open the **QC Protocol** window.
2. Create a new QC protocol :
   a. Name the new QC protocol according to your laboratory naming convention.
   b. Set the following parameters:

| Parameter | Setting |
|---|---|
| Size Standard | GS600_LIZ_(60-460) |
| Size Range<br>Sizing Start Size<br>Sizing Stop Size | Partial<br>60 bp<br>460 bp |
| Size Calling Method | Local Southern Method |
| After checking the "Use Baselining" box: Baseline Window Pts. | 33 |
| Peak Window Size | 11 |

c. Click **Save**.




3. Add the QC protocol to the HID assay.

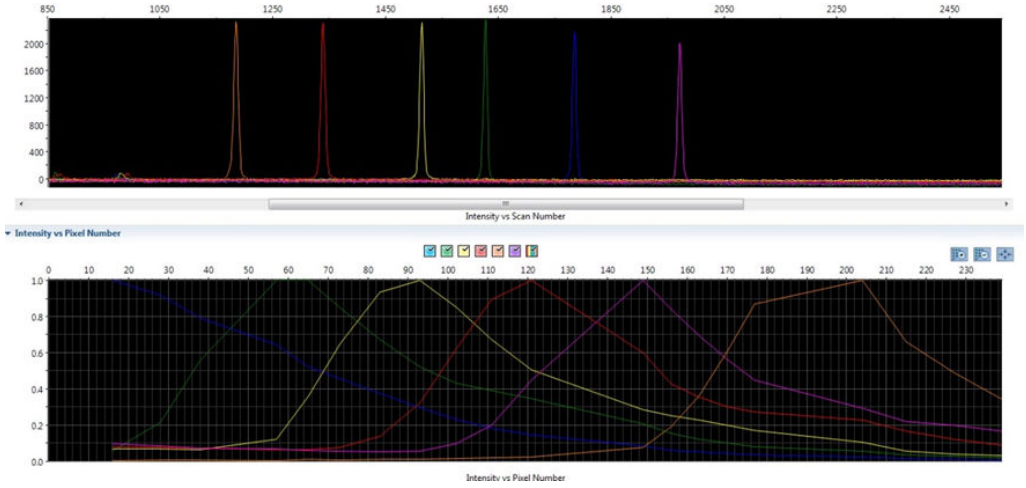
## Perform spectral calibration

Perform a spectral calibration using the DS-37 Matrix Standard Kit (Dye set J6-T, 6-dye) (J6-T Dye Set) (Cat. No. A31234).

**Note:** Since J6-T is a new dye set, create a new dye set in the Data Collection Software before running a spectral calibration. For instructions on creating a new dye set, see the "Create a New Dye Set" section of the *3500/3500xL Genetic Analyzer with 3500 Series*

*Data Collection Software v2 User Guide* (Cat. No. 4476988). Use the J6 template to set up the J6-T dye set.

The following figure is an example of a passing 6-dye spectral calibration.




# Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

This procedure applies to the 3500 Series and 3130 Series instruments.

Prepare the samples for electrophoresis immediately before loading.

1. Pipet the required volumes of components into an appropriately sized polypropylene tube:

| Reagent | Volume per reaction |
|---|---|
| GeneScan™–600 LIZ™ Size Standard v2.0 | 0.4 µL |
| Hi-Di™ Formamide | 9.6 µL |

**Note:** Include volume for additional samples to provide excess volume for the loss that occurs during reagent transfers.

**IMPORTANT!** The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Vortex the tube, then briefly centrifuge.

3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide/size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   **Note:** For blank wells, add 10 µL of Hi-Di™ Formamide.

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
5. Heat the reaction plate in a thermal cycler at 95°C for 3 minutes.
6. Immediately place the plate on ice for 3 minutes.
7. Place the sample tray on the autosampler, then start the electrophoresis run.

# Set up the 3130/3130*xl* instruments for electrophoresis (before first use of the kit)

## Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 136.

| Genetic Analyzer | Operating System | Data Collection Software | Additional software | Run modules and conditions |
|---|---|---|---|---|
| 3130[1] | Windows™7 | Data Collection Software v4 | 3130/3730 DC v4 6-Dye Module v1 | Set up the following conditions:<br>• HIDFragmentAnalysis36_POP4_1<br>• Injection conditions[2]: 3 kV/4 sec<br>• Run conditions: 14 kV/1600 sec<br>• Dye Set J6-T |
| 3130*xl* | | | | Set up the following conditions:<br>• HIDFragmentAnalysis36_POP4_1<br>• Injection conditions[2]: 3 kV/5 sec<br>• Run conditions: 14 kV/1600 sec<br>• Dye Set J6-T |

[1] We conducted validation studies using the 3130*xl*, 3500, and 3500xL configurations.

[2] Our studies indicate that the injection conditions that are documented generate profiles from 0.5 ng of input DNA with heterozygous peak height averages between 2,000– 4,000 RFU (3130 or 3130*xl*) with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary, therefore changes to injection parameters may need to be explored and validated to deliver the best results on your specific system. Large deviations from the recommended injection parameters could affect the performance of the size standard and/or allelic ladder, therefore validation is recommended.

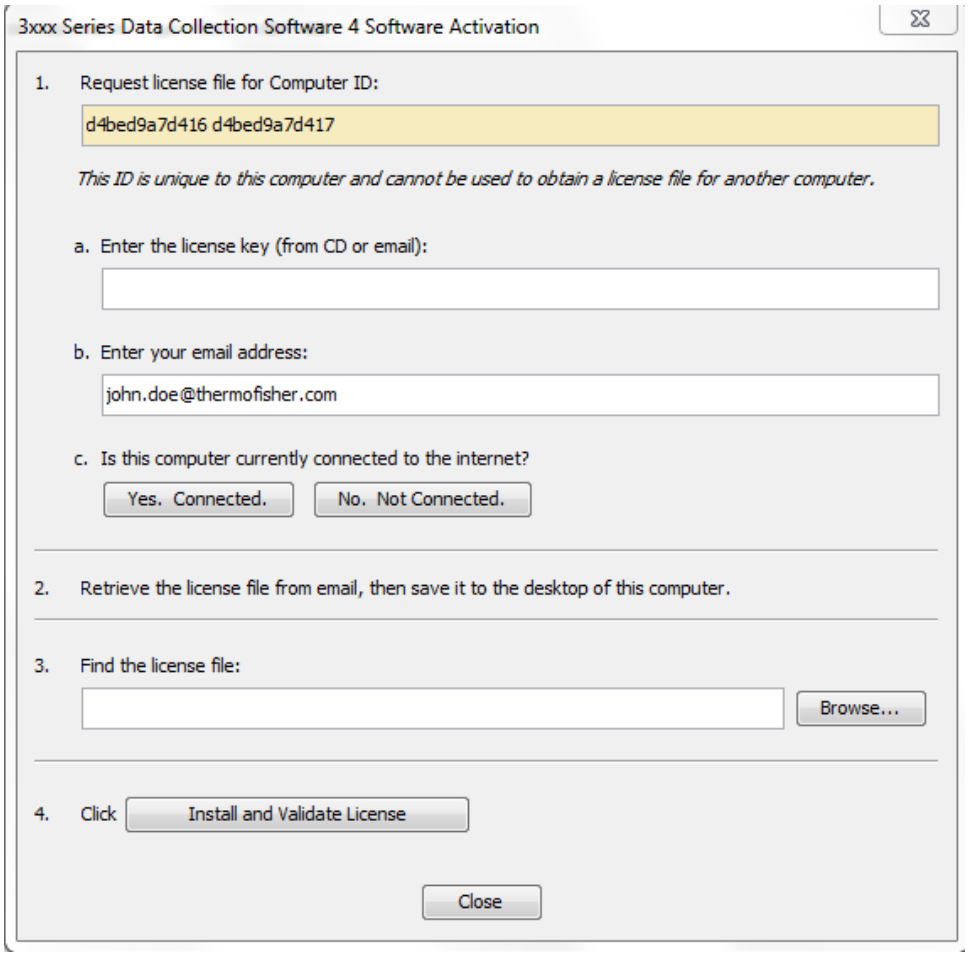
## Obtain and activate 6-dye license

1. Confirm that you are running Data Collection Software v4 (**Help ▸ About**).
2. Obtain a 3130 DC v4 6-Dye Module v1 License key. Contact your local Human Identification representative for information.

3. Ensure that all network cards in the computer are enabled.

   **IMPORTANT!** You can run the 3130 Series Data Collection Software v4 using only the network cards that are enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

4. Select **Tools** ▸ **License Manager** to display the **Software Activation** dialog box.




5. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen. The license file will be emailed to you.

6. Obtain the software license file from your email.

7. Make a copy of the software license file and keep it in a safe location.

8. Copy the software license file to the desktop of the Data Collection Software v4 computer.

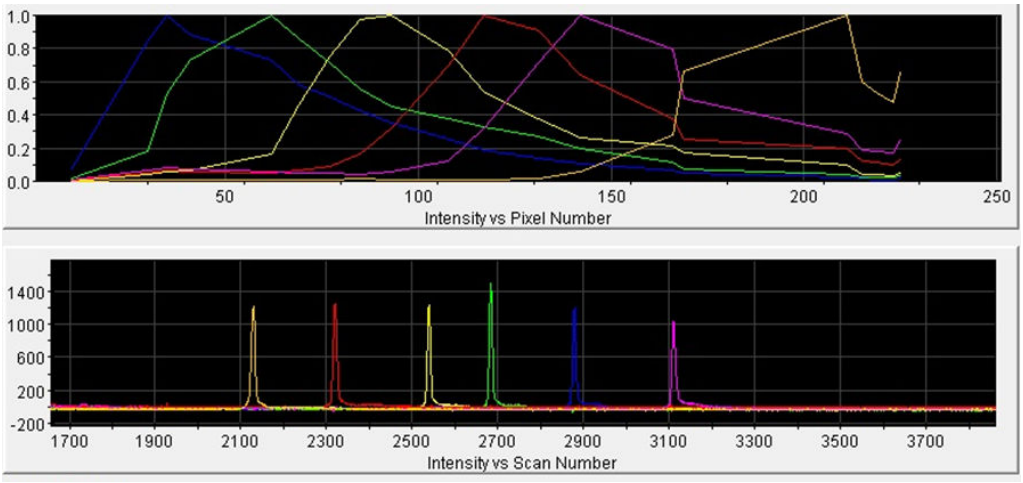
9. If the Software Activation dialog box has closed, select **Tools** ▸ **License Manager**.

10. Click **Browse**, then navigate to the software license file saved on your computer.
11. Click **Install and Validate License**.
    A message is displayed when the license is installed and validated.
12. Click **Close**.

### Perform spectral calibration

Perform a spectral calibration using the DS-37 Matrix Standard Kit (Dye set J6-T, 6-dye) (J6-T Dye Set) (Cat. No. A31234).

The following figure is an example of a passing 6-dye spectral calibration.




## Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

This procedure applies to the 3500 Series and 3130 Series instruments.

Prepare the samples for electrophoresis immediately before loading.

1. Pipet the required volumes of components into an appropriately sized polypropylene tube:

| Reagent | Volume per reaction |
|---|---|
| GeneScan™ –600 LIZ™ Size Standard v2.0 | 0.4 µL |
| Hi-Di™ Formamide | 9.6 µL |

**Note:** Include volume for additional samples to provide excess volume for the loss that occurs during reagent transfers.

**IMPORTANT!** The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Vortex the tube, then briefly centrifuge.

3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide/size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   **Note:** For blank wells, add 10 µL of Hi-Di™ Formamide.

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.

5. Heat the reaction plate in a thermal cycler at 95°C for 3 minutes.

6. Immediately place the plate on ice for 3 minutes.

7. Place the sample tray on the autosampler, then start the electrophoresis run.

# 4 Analyze data with GeneMapper™ *ID-X* Software



## Overview of GeneMapper ID-X Software

GeneMapper™ *ID-X* Software is an automated genotyping software application for forensic casework, databasing, and paternity data analysis.

GeneMapper™ *ID-X* Software v1.5.2 or later analyzes 4-dye, 5-dye, and 6-dye data and is required to correctly analyze data that is generated using the NGM Detect™ kit. After electrophoresis, the data collection software stores information for each sample in a .fsa or .hid file. The GeneMapper™ *ID-X* Software v1.5.2 or later allows you to analyze and interpret the data from the .fsa or .hid files.

Prior versions of GeneMapper™ *ID-X* Software (v1.2 and above) are capable of analyzing data that is generated using the NGM Detect™ kit. However, some data quality assessment tools are not fully functional on versions prior to v1.5.2. Details on the data assessment functionality added to specific versions of GeneMapper™ *ID-X* Software are found in the release notes for each version. To summarize:

- In versions prior to v1.4, samples show a red flag for the CGQ if the Y-indel peak is missing, for example, with a female DNA sample. This occurs because the absence of the Y-indel peak triggers the Allele Number Rule.
- In versions prior to v1.5.2, when the sample type is set as "Negative Control" the software will assign red CGQ flags. This occurs even if the negative control sample is valid, that is, it contains no peaks above the PAT in the STR, AMEL, and Y-indel marker ranges. The red flags appear because the presence of the IQC peaks triggers the Allele Number Rule for this sample type (no peaks are expected in a valid negative control sample).

# GeneMapper™ *ID-X* Software analysis of the NGM Detect™ kit

GeneMapper™ *ID-X* Software allows you to designate four sample types:

- Sample
- Positive Control
- Allelic Ladder
- Negative Control

GeneMapper™ *ID-X* Software has logic for Negative Control sample type assignments, whereby the software generates red flags on the Genotype Quality (GQ) indicators when peaks are present above the Peak Amplitude Threshold (PAT) designated in the analysis method.

The NGM Detect™ PCR Amplification Kit includes the internal quality control (IQC) system for PCR. In this system, two synthetic targets in the primer mix are amplified with the sample under test. The IQC system allows you to determine whether PCR has been successful in the absence of peaks from the sample.

In versions prior to v1.5.2, when the sample type is set as "Negative Control" the software will assign red CGQ flags. This occurs even if the negative control sample is valid, that is, it contains no peaks above the PAT in the STR, AMEL, and Y-indel marker ranges. The red flags appear because the presence of the IQC peaks triggers the Allele Number Rule for this sample type (no peaks are expected in a valid negative control sample).

Thermo Fisher Scientific has developed the NGM Detect™ kit using version 1.5.2 of the GeneMapper™ *ID-X* Software. This version includes updated logic for the Negative Control sample type. It allows the IQC marker GQ to flag green in the presence of the IQC peaks.

A software patch that updates GeneMapper™ *ID-X* Software v1.5 to v1.5.2 is available at **thermofisher.com/us/en/home/technical-resources/software-downloads/genemapper-id-x-software.html**.

# Allelic ladder requirements for data analysis

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies before you use multiple allelic ladder samples in an analysis.

  For multiple allelic ladder samples, the GeneMapper™ *ID-X* Software calculates allelic bin offsets by using an average of all allelic ladders that use the same panel in a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run. When the software imports multiple run folders into a project, only the ladders in their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "**Allelic Ladder**" in the **Sample Type** column in a project. Analysis will fail if the **Allelic Ladder Sample Type** is not specified.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples, to ensure proper allele calling.
- Alleles that are not in the allelic ladders do exist. Off-ladder (OL) alleles can contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the bin window of any known allelic ladder allele or virtual bin.

  **Note:** If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory protocol.

# File names and versions used in this section

The file names and version numbers of panel, bin, and stutter files that are shown in this section may differ from the file names that you see when you download or import files.

If you need help to determine the correct files to use, contact your local Human Identification representative, or go to **thermofisher.com/support**.

# Set up the GeneMapper™ *ID-X* Software for analysis (before first use of the kit)

## Workflow: Set up GeneMapper™ *ID-X* Software

Before you use GeneMapper™ *ID-X* Software to analyze data for the first time, you must do the following:

"Check panel, bin, and stutter file versions on your computer" on page 35

▼

"(If needed) Download newer versions of panel, bin, and stutter files" on page 35

▼

"Import panels, bins, and marker stutter" on page 36

▼

"(Optional) Define custom table or plot settings" on page 39

## Check panel, bin, and stutter file versions on your computer

1. Start the GeneMapper™ *ID-X* Software , then log in with the appropriate user name and password.
2. Select **Tools ▸ Panel Manager**.
3. Check the version of files that are currently available in the **Panel Manager**:
   a. Select **Panel Manager** in the navigation pane.
   b. Expand the **Panel Manager folder** and any subfolders to identify the analysis file version that is already installed for your kit choice.
4. Check the version of files available for import into the **Panel Manager**:
   a. Select **Panel Manager**, then select **File ▸ Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to, then open the **Panels** folder, then check the version of panel, bin, and stutter files installed.
5. Check for newer versions of the files as described in the next procedure.

## *(If needed)* Download newer versions of panel, bin, and stutter files

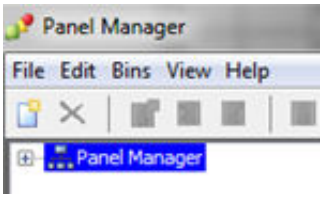
1. Go to **thermofisher.com/us/en/home/technical-resources/software-downloads/genemapper-id-x-software.html**.
2. If the file versions listed are newer than the versions on your computer, download the file **NGM Detect Analysis Files**.

   **Note:** When downloading new versions of analysis files, see the associated **Read Me** file for details of changes between software file versions. Perform the appropriate internal validation studies before using new file versions for analysis.
3. Unzip the file.

## Import panels, bins, and marker stutter

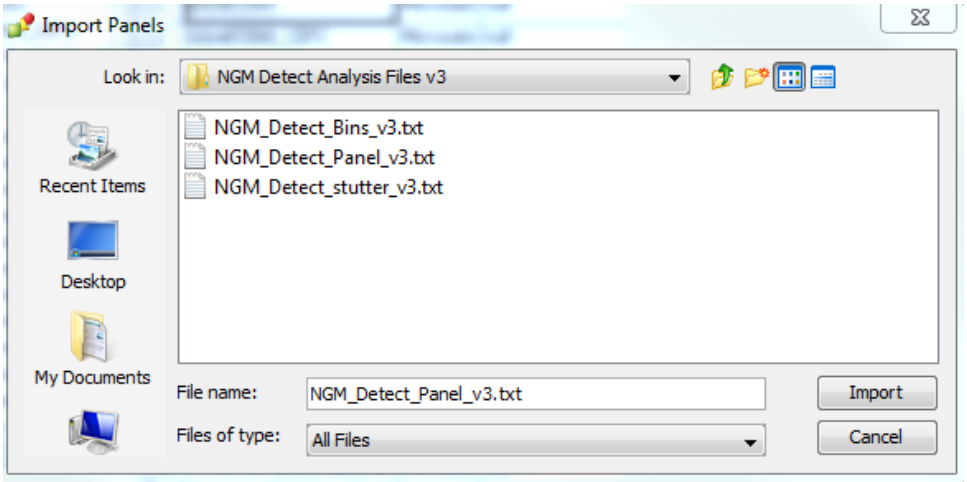
To import the latest panel, bin set, and marker stutter from the website into the GeneMapper™ *ID-X* Software database:

1. Start the GeneMapper™ *ID-X* Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.

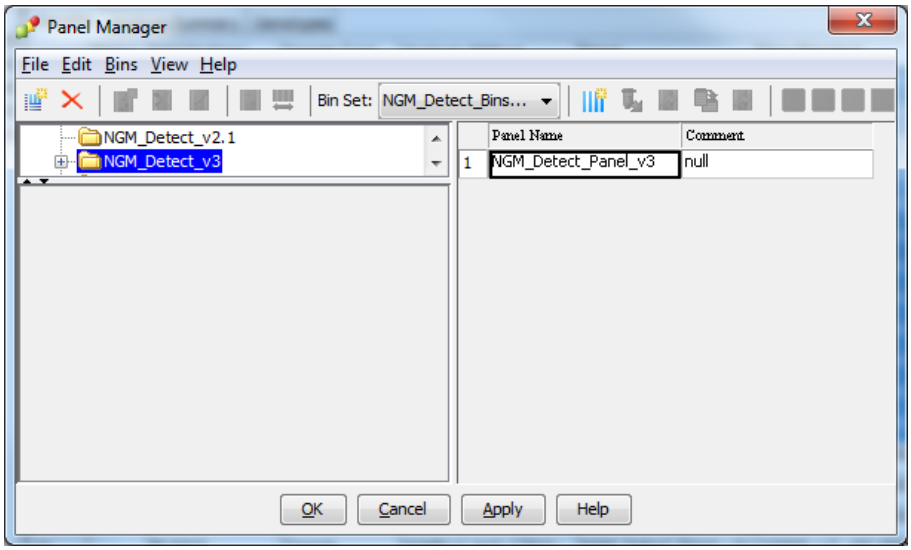



3. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to, then open the **NGM Detect Analysis Files** folder that you unzipped in the previous procedure.
4. Select **NGM_Detect_Panels.txt**, then click **Import**.

   **Note:** Importing this file creates a new folder in the navigation pane of the **Panel Manager**, **NGM_Detect_Panel**. This folder contains the panel and associated markers.



5. Import the bins file:
   a. Select the **NGM_Detect_Panel** folder in the navigation pane.

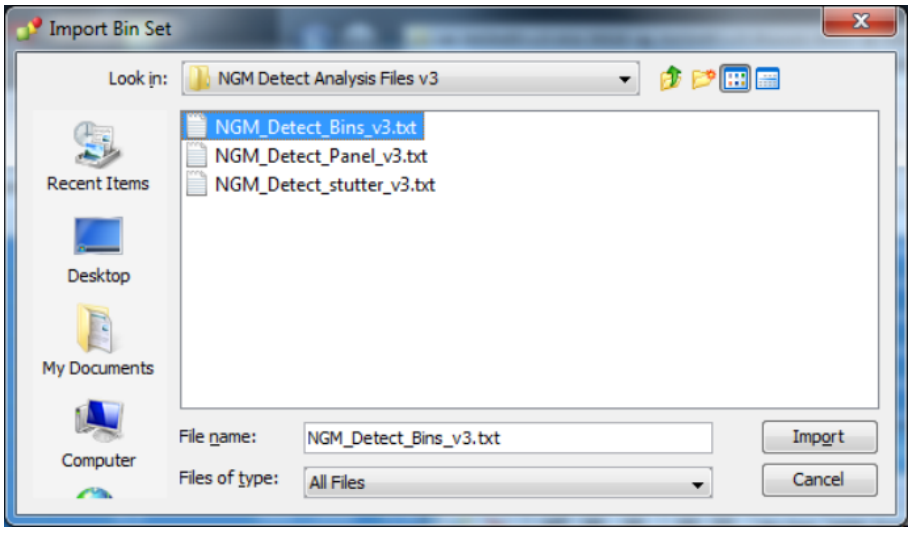



   b. Select **File** ▸ **Import Bin Set** to open the **Import Bin Set** dialog box.

   c. Navigate to, then open the **NGM Detect Analysis Files** folder.

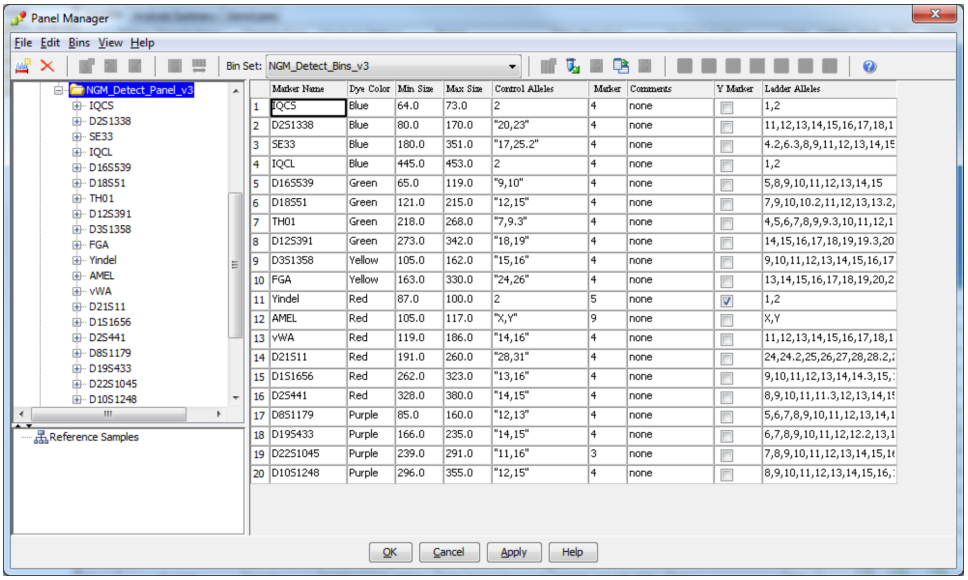
   d. Select **NGM_Detect_Bins.txt**, then click **Import**.

   **Note:** Importing this file associates the bin set with the panels in the **NGM_Detect_Panel** folder.



6. *(Optional)* View the imported panels and bins in the navigation pane: Double-click the **NGM_Detect_Panel** folder.

   The panel information is displayed in the right pane and the markers are displayed below it.




7. Import the stutter file:

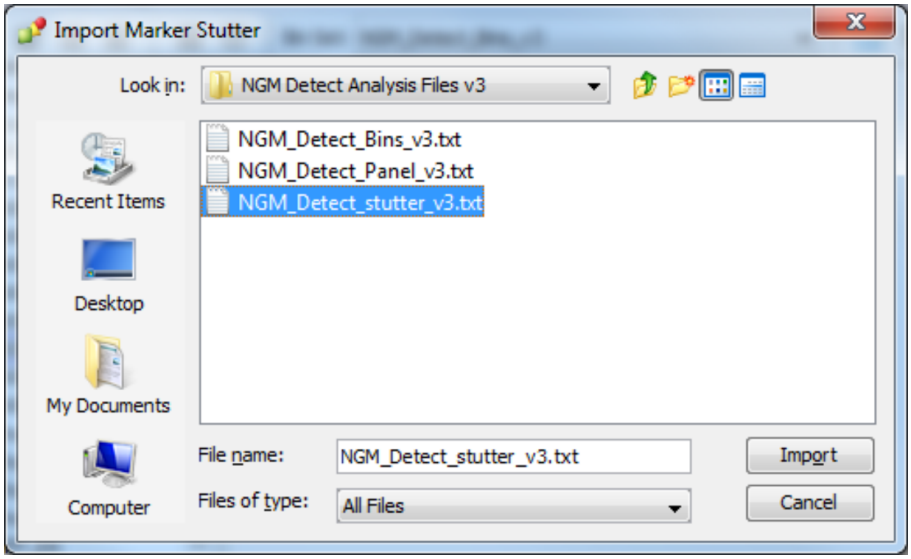
   a. Select the **NGM_Detect_Panel** folder in the navigation panel.

   b. Select **File ▸ Import Marker Stutter** to open the **Import Marker Stutter** dialog box.

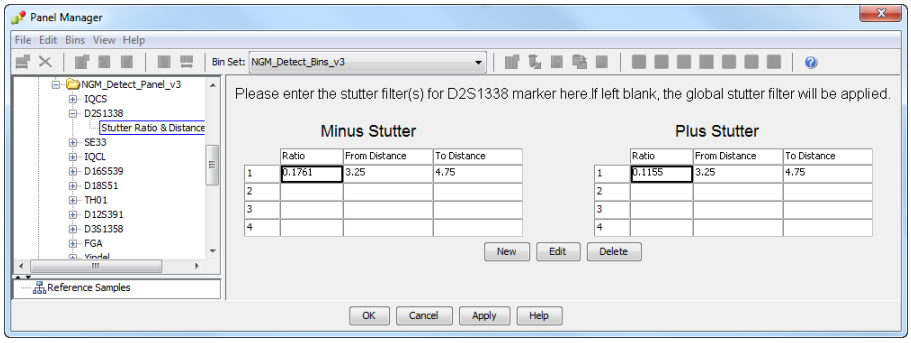
   c. Navigate to, then open the **NGM Detect Analysis Files** folder.

d. Select **NGM_Detect_Stutter.txt**, then click **Import**.

**Note:** Importing this file associates the marker stutter ratio with the bin set in the **NGM_Detect_Panel** folder and overwrites any existing stutter ratios associated with the panels and bins in that folder.




8. View the imported marker stutters in the navigation pane:
   a. Double-click the **NGM_Detect_Panel** folder to display the folder.
   b. Double-click the folder to display its list of markers below it.
   c. Double-click a marker to display the **Stutter Ratio & Distance** view for the marker in the right pane.




9. Click **Apply**, then click **OK** to add the panel, bin set, and marker stutter to the GeneMapper™ *ID-X* Software database.

**IMPORTANT!** If you close the **Panel Manager** without clicking **Apply**, the panels, bin sets, and marker stutter are not imported into the GeneMapper™ *ID-X* Software database.

## *(Optional)* Define custom table or plot settings

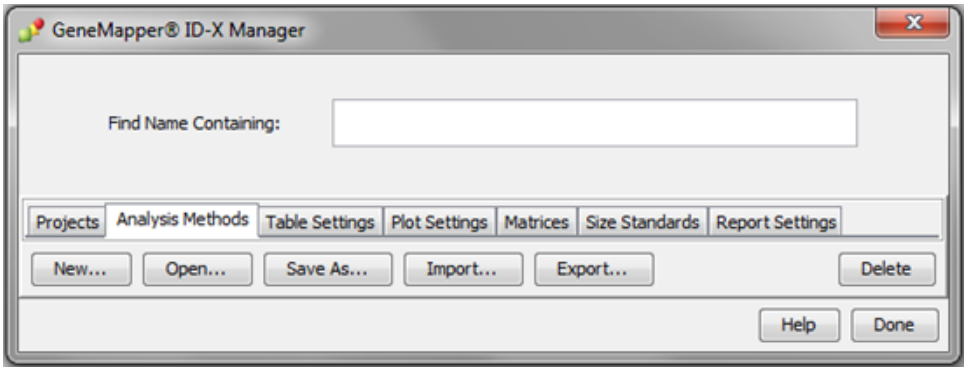
Default views for table and plot settings are provided with the software. For information on defining custom views, see *GeneMapper™ ID-X Software Getting Started Guide— Basic Features*.

# Create an analysis method

## Create an analysis method

**IMPORTANT!** Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method that is created in GeneMapper™ *ID-X* Software version 1.2 is not compatible with analysis methods that are created in earlier versions of software, or with GeneMapper™ Software v3.2.1.

1. Select **Tools** ▸ **GeneMapper® ID-X Manager** to open the **GeneMapper ID-X Manager**.




2. Click the **Analysis Methods** tab, then click **New** to open the **Analysis Method Editor** with the **General** tab selected.

3. Enter the settings shown in the figures on the following pages.

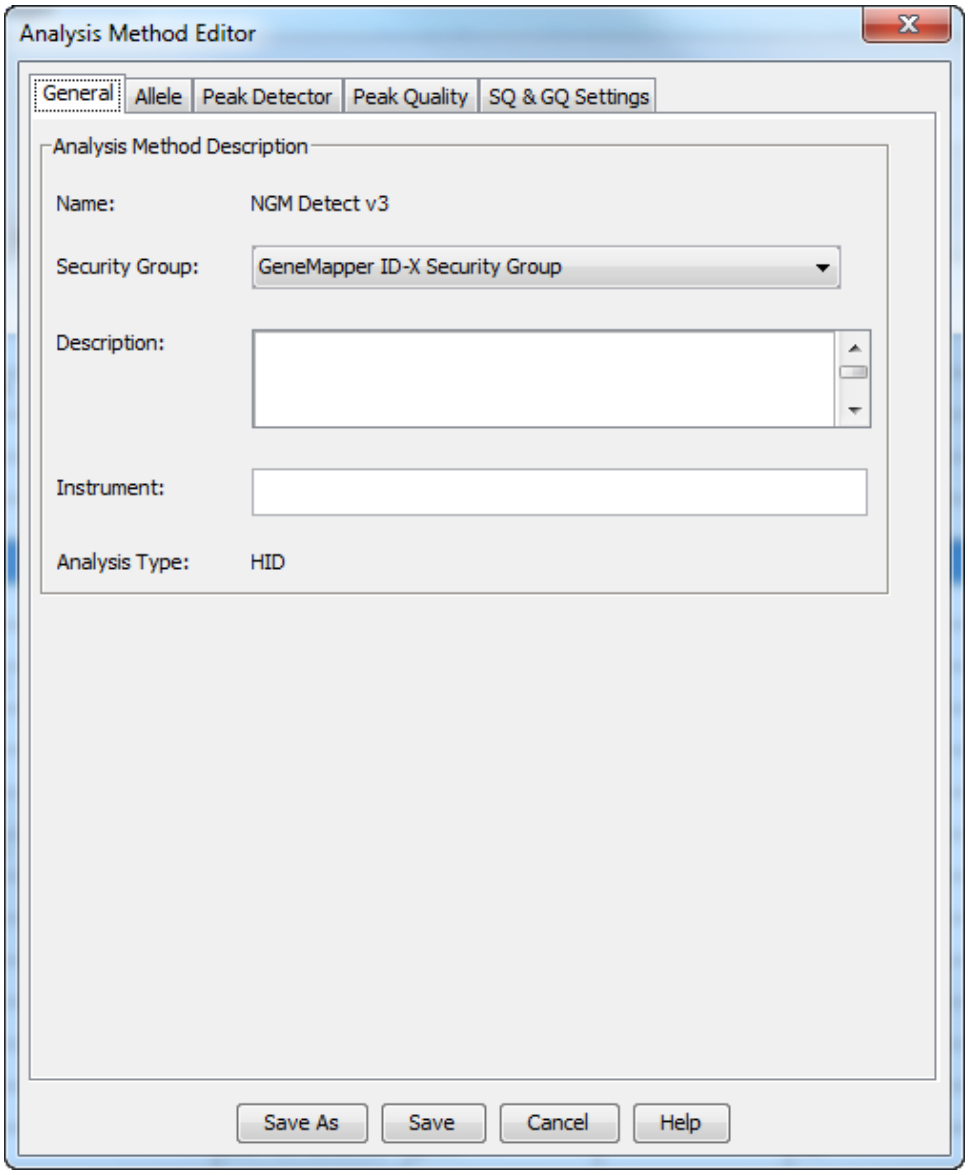
   **Note:** The **Analysis Method Editor** closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

4. After you enter the settings on all tabs, click **Save**.

## Enter Analysis Method settings

### Enter General tab settings

1. Enter a **Name** and select the **Security Group** appropriate for your software configuration.




2. *(Optional)* Enter a **Description** and **Instrument**.

## Enter Allele tab settings

**IMPORTANT!** Perform appropriate internal validation studies to determine the appropriate settings to use.

1. Select the **NGM_Detect_Bins_v3** bin set.

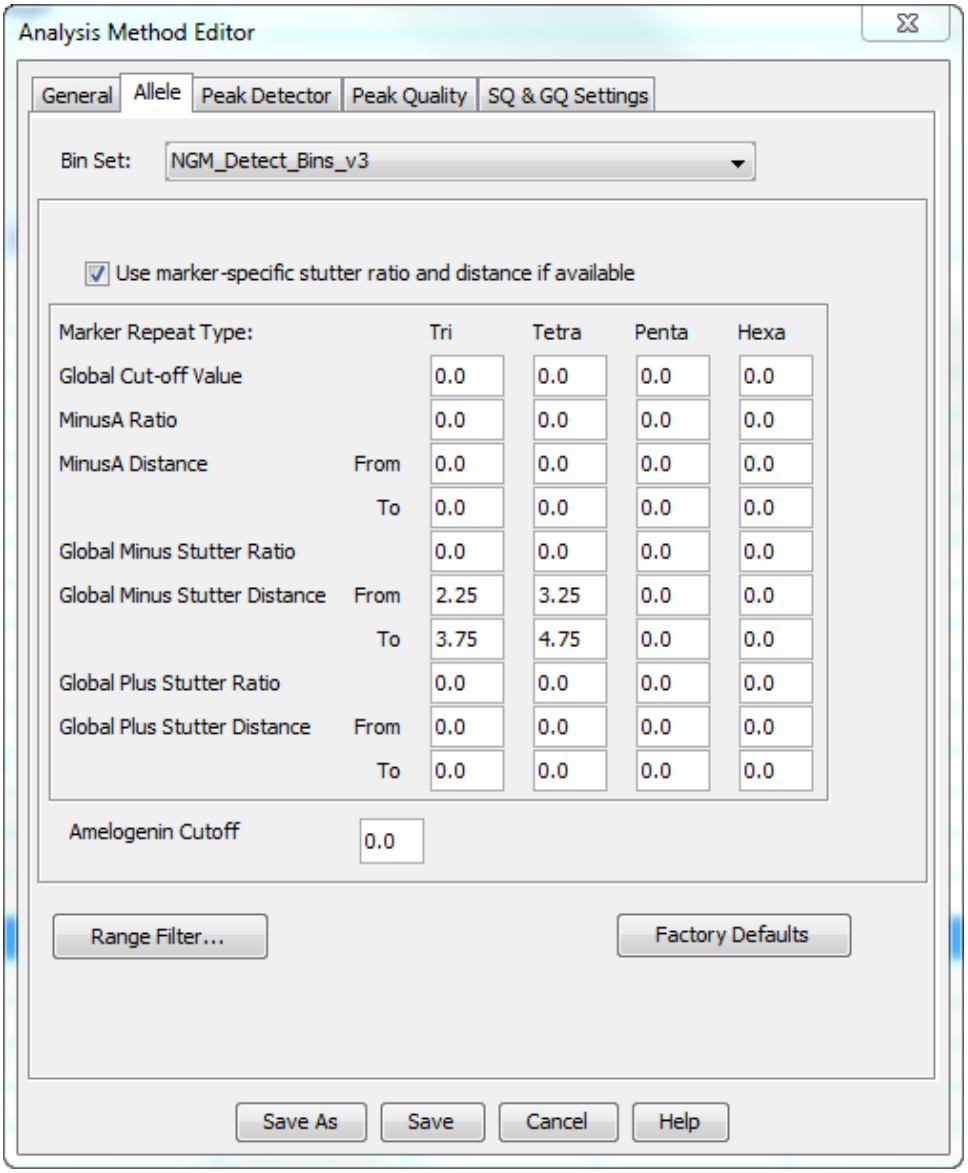



**Figure 4** Settings used in developmental validation of the kit

2. *(Optional)* To apply the stutter ratios contained in the **NGM_Detect_Stutter.txt**, select the **Use marker-specific stutter ratio and distance if available** checkbox (selected by default).

3. If using GeneMapper™ *ID-X* Software v1.0.1 or later, enter values for the 4 **Marker Repeat Types**.

4. Enter the appropriate filter settings.

### Enter Peak Detector tab settings

Enter the appropriate values:

| Field | Values to enter or select | Additional information |
|---|---|---|
| **Ranges** | Enter the values shown in Figure 5.<br>**Note:** The read region for the NGM Detect™ kit is 64 to 458 bp. | — |
| **Peak Detection** | Enter the appropriate settings.<br>**IMPORTANT!** Perform appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of data. | The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper™ *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks. |

| Field | Values to enter or select | Additional information |
|---|---|---|
| **Smoothing and Baseline** | Enter the values shown in Figure 5, or adjust as needed dependent on the polymer you are using. | — |
| **Size Calling Method** | Select **Local Southern Method**, or another method if validated by your internal validation. | — |
| **Normalization** | *(Optional)* Select the **Normalization** checkbox. | A **Normalization** checkbox is available on this tab in GeneMapper™ *ID-X* Software for use in conjunction with data run on the 3500 Series Genetic Analyzers. |

**Note:** The read region for the NGM Detect™ kit is 64 to 458 bp.

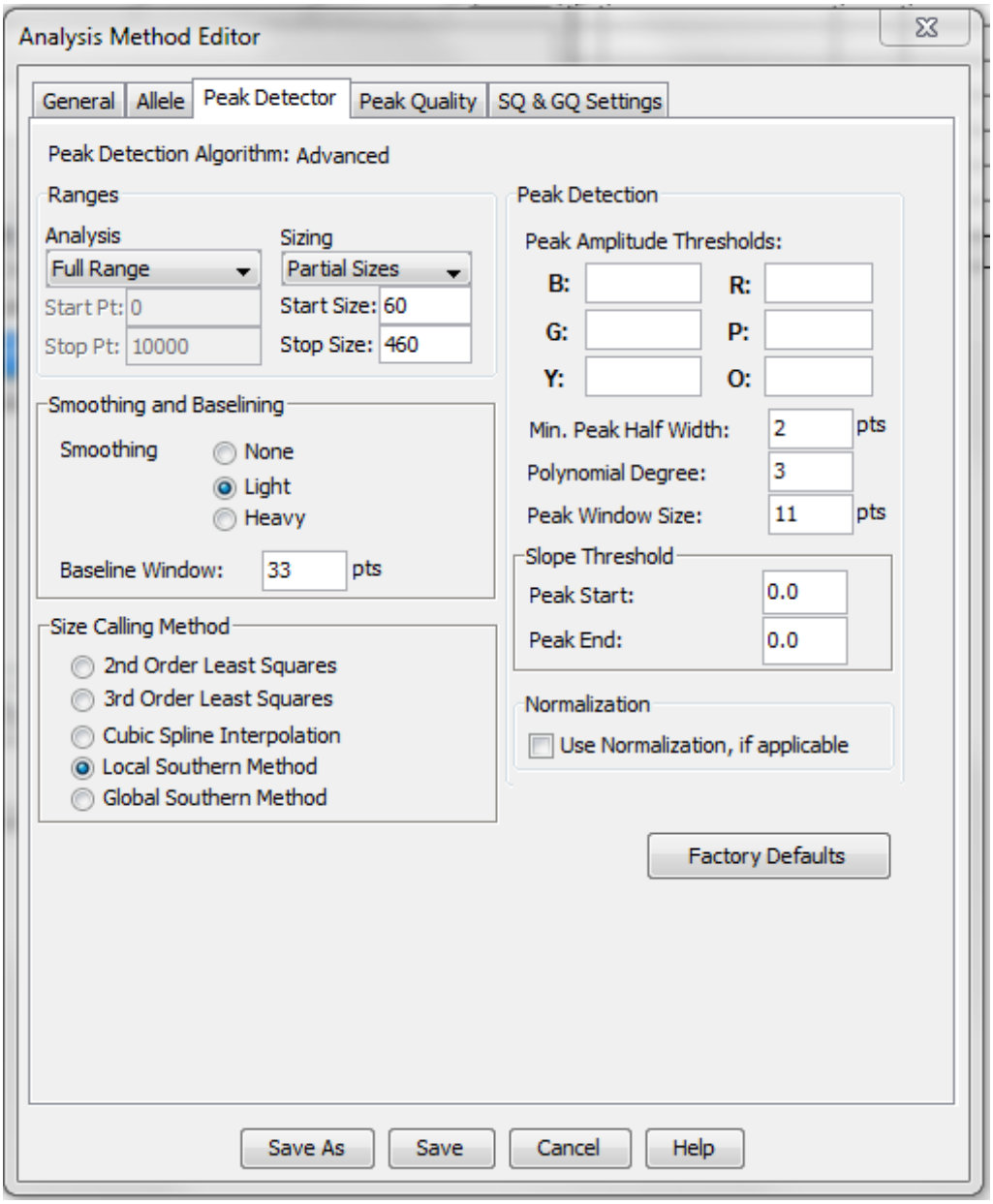
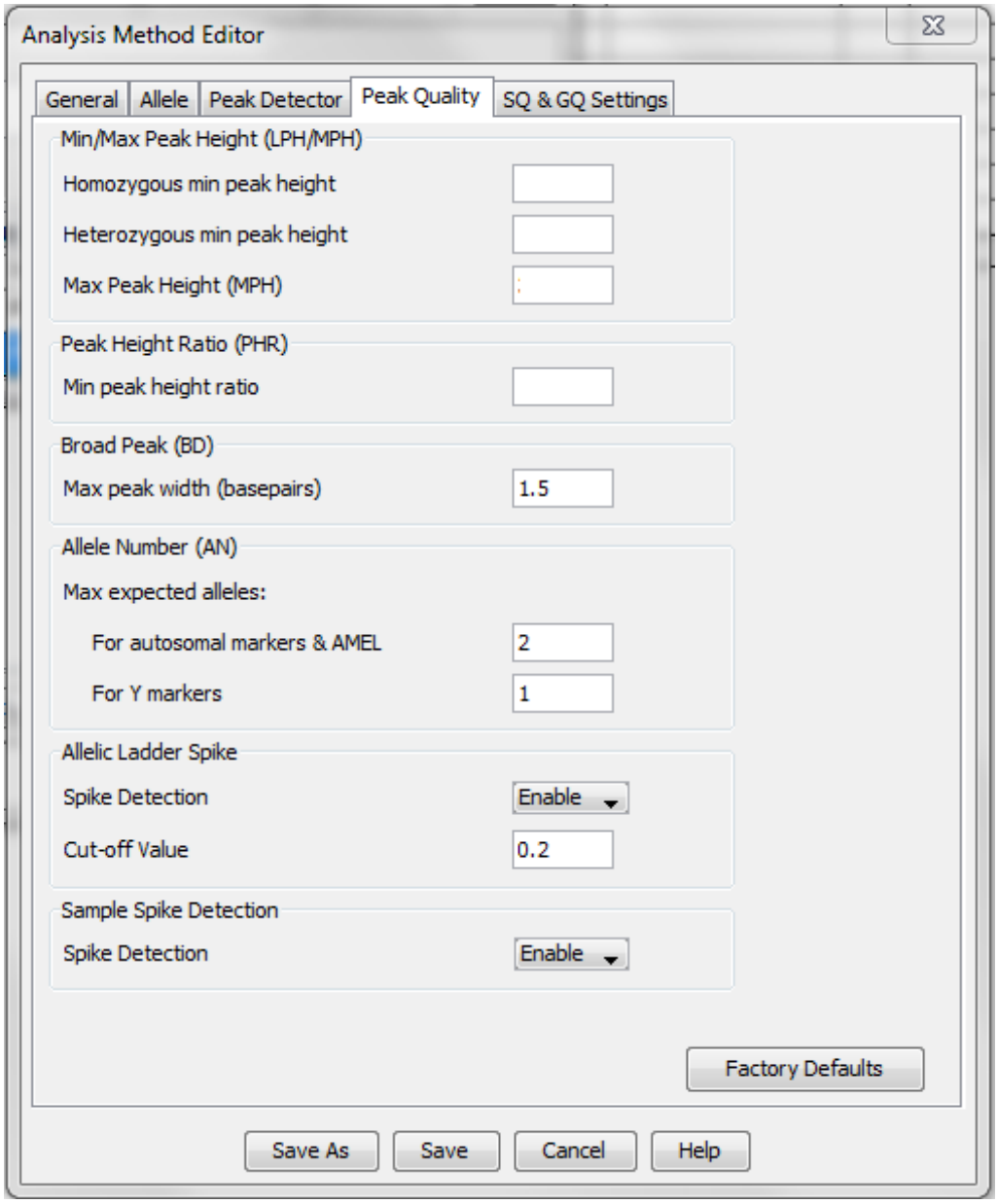



**Figure 5** Settings used in developmental validation of the kit

### Enter Peak Quality tab settings

**IMPORTANT!** Perform the appropriate internal validation studies to determine the heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold, and the minimum peak height ratio threshold for interpretation of data.

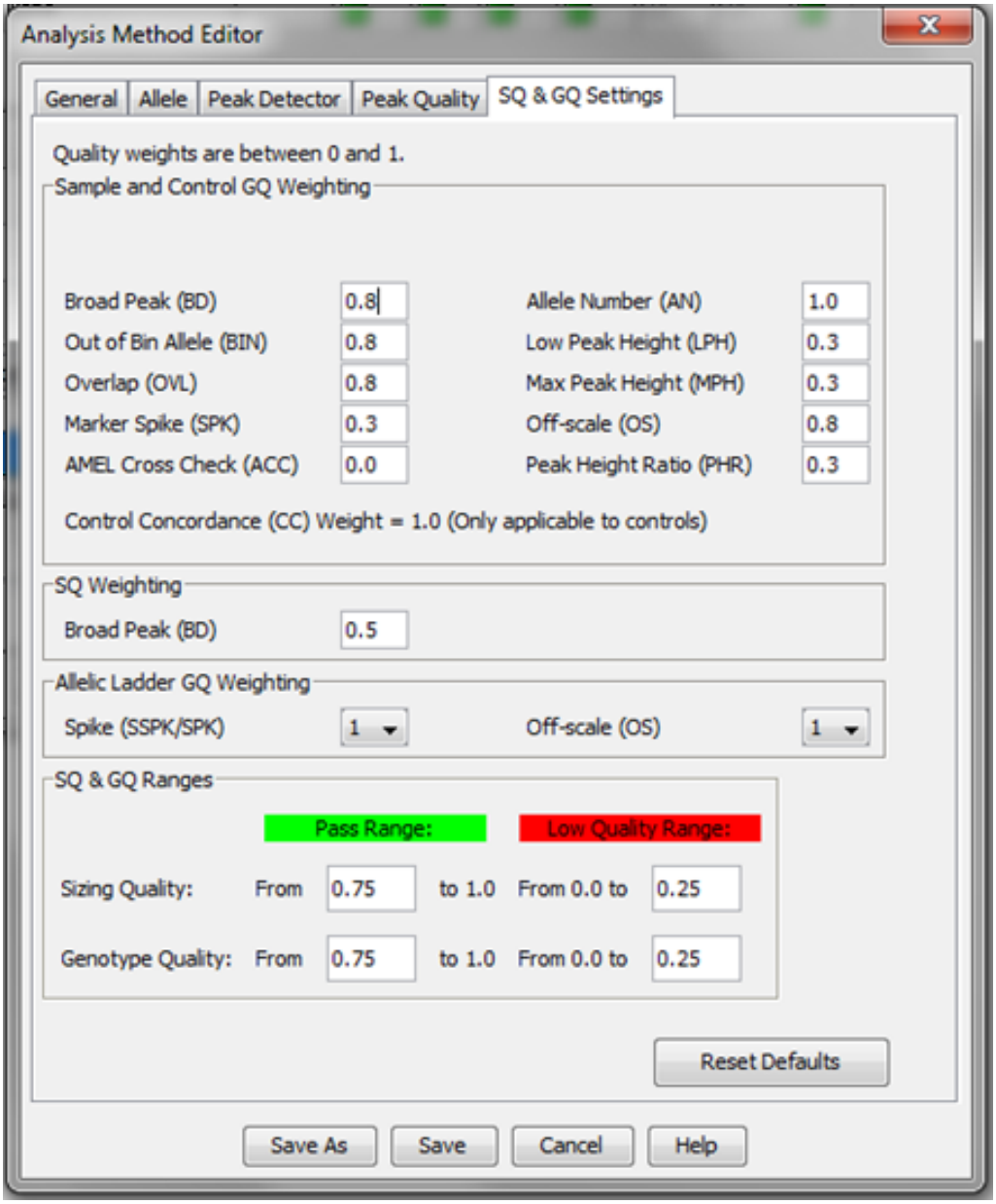
Enter the following values:



### Enter SQ and GQ tab settings

**IMPORTANT!** The values that are shown are the software defaults and are the values we used during developmental validation. Perform appropriate internal validation studies to determine the appropriate values to use.

Enter the following values:




**Note:** Set the **ACC GQ Weighting** according to the values you determine during internal validation studies of the **ACC PQV**. For example, set the **ACC GQ Weighting** to 0.3 or higher to flag samples in which the Amelogenin result is anything other than X, X or X, Y, or does not agree with the results for the Y indel marker.

# Create a size standard definition file if needed

If you cannot use the default settings that are provided, create a new size standard definition file.

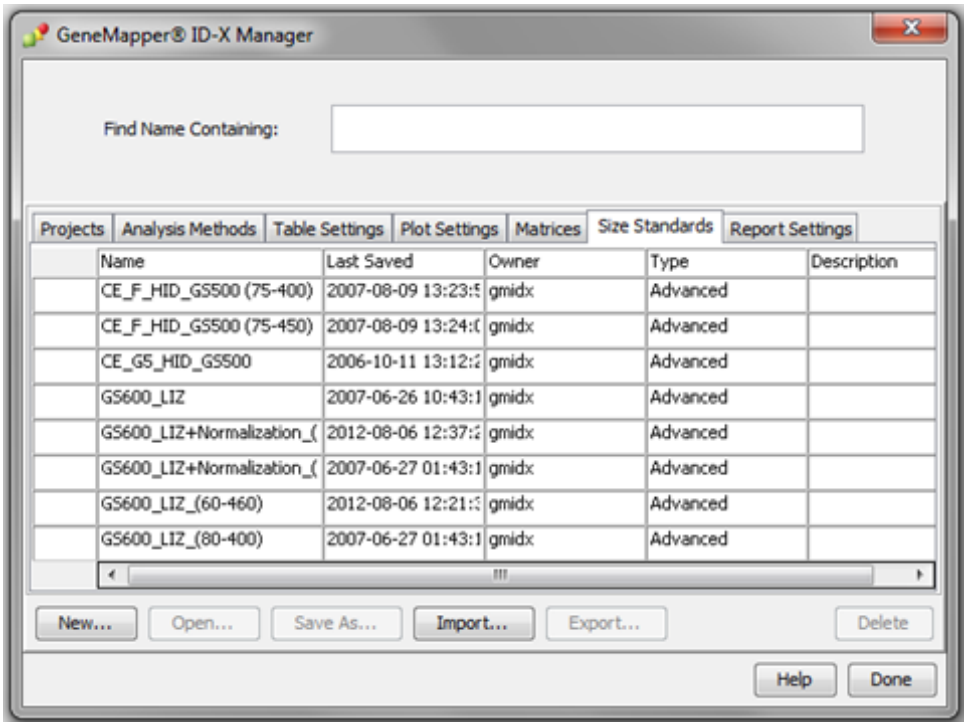
## About the GS600_LIZ_ (60–460) size standard definition file

The GS600_LIZ_(60–460) size standard definition that is provided with GeneMapper™ *ID-X* Software and used with the Local Southern size calling method contains the following peaks: 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460.

This size standard definition has been validated for use with this kit on the genetic analyzers listed in "Instruments and software compatibility" on page 15. If you need to create your own size standard definition, see "Create a size standard definition file" on page 48.

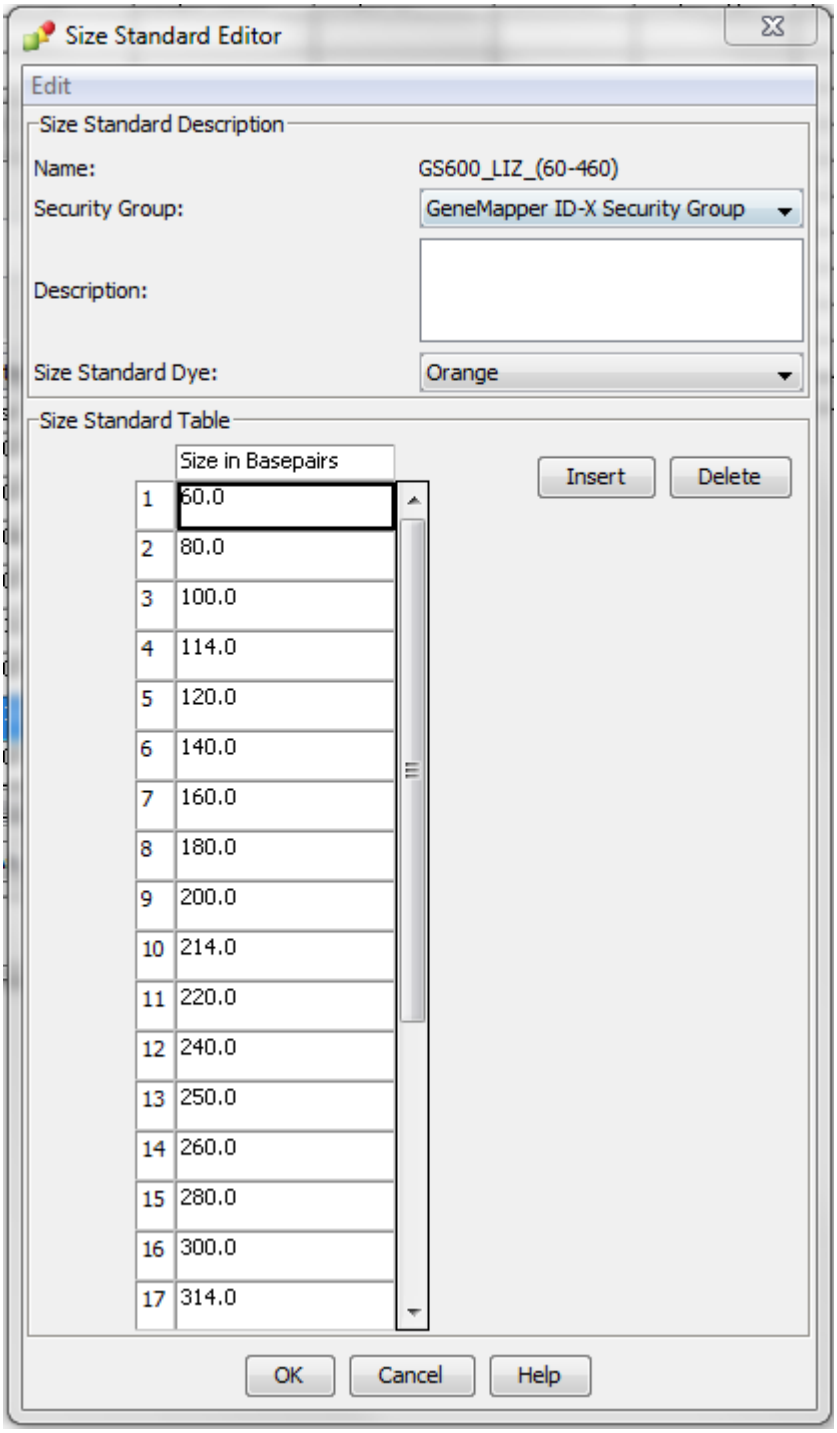
## Create a size standard definition file

1. Select **Tools** ▸ **GeneMapper ID-X Manager** to open the **GeneMapper ID-X Manager**.
2. Click the **Size Standards** tab, then click **New**.




3. Specify settings in the **Size Standard Editor**:
   a. Enter a name as shown in the following figure or enter a new name.
   b. In the **Security Group** field, select the **Security Group** appropriate for your software configuration.
   c. In the **Size Standard Dye** field, select **Orange**.

d. In the **Size Standard Table**, enter the peak sizes that correspond to your size standard.



# Analyze and edit sample files with GeneMapper *ID-X* Software

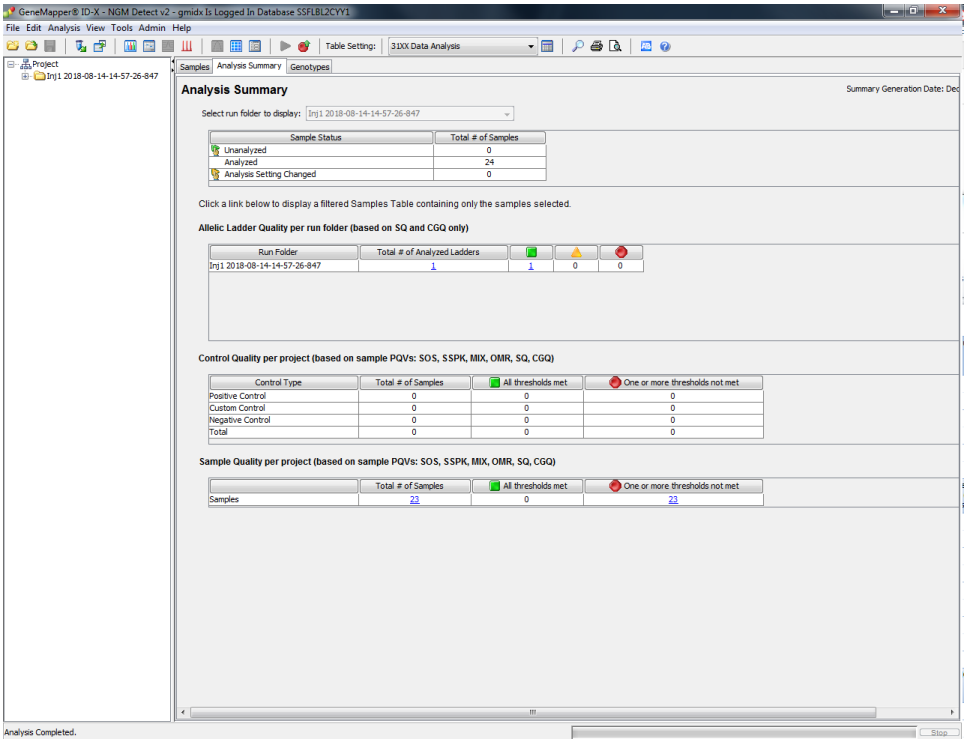
1. In the **Project** window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory that contains the sample files.

2. Apply analysis settings to the samples in the project.

| Parameter | Settings |
|---|---|
| **Sample Type** | Select the sample type. |
| **Analysis Method** | Select **NGM Detect Analysis Method** (or the name of the analysis method you created). |
| **Panel** | Select **NGM_Detect_Panel**. |
| **Size Standard** | Use a size range of 60–460 bp for **Local Southern** size calling method [1] |

[1] The NGM Detect™ kit was originally validated with the GeneScan™–600 LIZ™ Size Standard v2.0. If you use a different size standard, perform the appropriate internal validation studies to support the use of this size standard with the NGM Detect™ kit.

3. Click **Analyze**, enter a name for the project (in the **Save Project** dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The **Analysis Summary** tab is displayed, and the **Genotypes** tab is available when the analysis is complete.



## Examine or edit a project

Display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data.

## For more information on using the GeneMapper™ *ID-X* Software

See "Related documentation" on page 136 for a list of available documents.

# 5 Assess the PCR reaction with the Internal Quality Control System



## Overview of the Internal Quality Control System

The Internal Quality Control System (IQC) is a tool that helps you to assess the PCR reaction and, in conjunction with the STR marker data properties, infer whether a sample is showing signs of degradation or inhibition. The primers for the two IQC markers, IQCS and IQCL, amplify synthetic DNA targets included in the primer mix. IQCS is a low molecular weight amplicon, 70 nt in length. Whereas, IQCL is a high molecular weight amplicon, 456 nt in length.

**Note:** The allele designation for both IQCS and IQCL will always be 2 (as shown in Figure 2) for all samples. Two alleles for each of the IQC markers are present in the NGM Detect™ Allelic Ladder (designated 1 and 2, see Figure 1). This is because the software dictates that in order for a ladder to be valid, it must have more than one allele per locus.

The IQC system enables you to:

- Confirm the success or failure of the PCR reaction, by looking for the presence or absence of the IQCS and IQCL primer peaks on the electropherogram.
- Determine if PCR inhibitors are present in the PCR reaction, or if PCR reaction conditions are not optimal, by comparing the relative peak heights of IQCS and IQCL.

## Evaluate the PCR reaction

To evaluate the PCR performance of the samples, review the relative peak heights of the IQCS and IQCL. Under ideal PCR conditions, the peak height of IQCL is approximately the same or slightly higher than IQCS (termed "balanced" in Table 3). Under sub optimal PCR conditions (for example inhibition), the height of the IQCL is substantially reduced relative to the ICQS. Note that when high inputs of DNA are amplified (greater than 2 ng) some suppression of the IQC peaks may also be seen.

See the following table for outcome scenarios.

**Table 3** IQC peak interpretation

| Sample DNA profile | IQC peaks | Interpretation | Example |
|---|---|---|---|
| Balanced | Balanced | PCR performance is optimal, no sample degradation or inhibition | See Figure 6 |
| Ski-slope | IQCL peak height significantly decreased or not present | Inhibition | See Figure 7 and Figure 8 |
| Ski-slope | Balanced | Degraded sample DNA | See Figure 9 |
| No peaks | Balanced | No DNA or very little sample DNA | See Figure 10 |
| No peaks | No peaks | PCR failure | — |

## Balanced profile

In this example of a balanced profile, the IQC peaks and the DNA profile peaks are balanced. This indicates that PCR has occurred optimally.

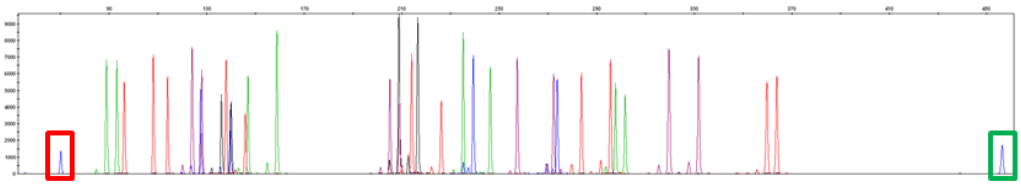


**Figure 6** Combined dyes electropherogram showing IQCS and IQCL peaks with 0.5 ng DNA (scaled to 9,000 RFU). The IQCS peak is highlighted by the red box and the IQCL peak is highlighted by the green box.

## Ski slope profile with decreased IQCL peak height

Figure 7 shows a significantly lower IQCL peak height relative to the IQCS peak height. This indicates that the PCR reaction has been compromised by inhibition. If high levels of PCR inhibition occur, lower IQCL peaks exhibiting some shouldering due to incomplete +A nucleotide addition may be observed. Figure 8 shows the complete absence of an IQCL peak, indicating a high level of inhibition.

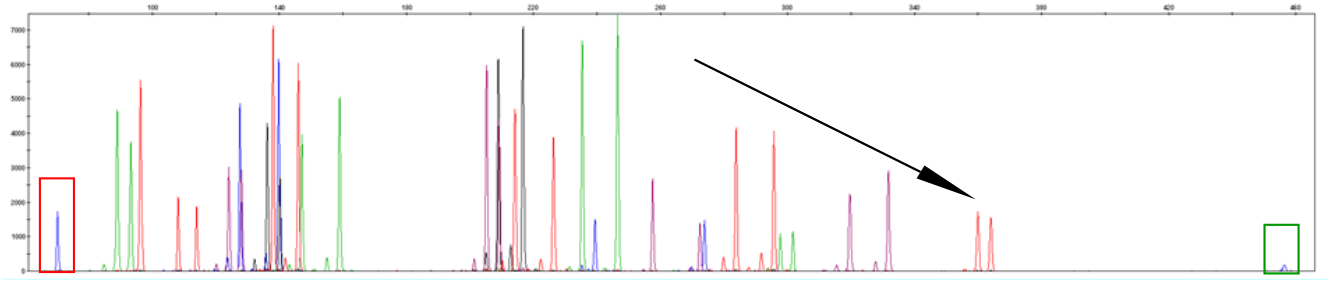


**Figure 7** Combined dyes electropherogram for the NGM Detect™ kit in the presence of 140 ng/µL humic acid. The IQCS peak is highlighted by the red box and the IQCL peak is highlighted by the green box. The arrow indicates the ski slope peak pattern observed in the DNA profile.

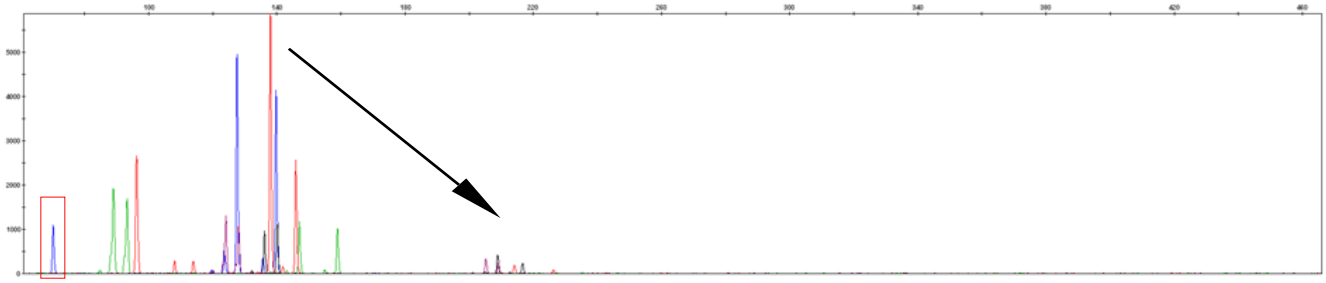


**Figure 8** Combined dyes electropherogram for the NGM Detect™ kit in the presence of 250 ng/µL humic acid. The IQCS peak is highlighted by the red box and the IQCL peak is absent. The arrow indicates the ski slope peak pattern observed in the DNA profile.

## Ski slope profile with balanced IQC peaks

The presence of both the IQCS and IQCL with balanced relative peak heights indicates that PCR has occurred optimally.

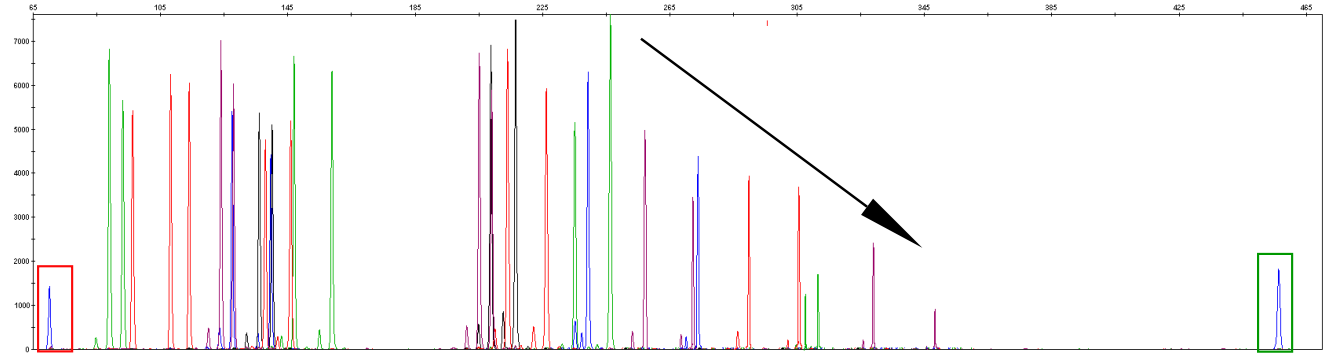


**Figure 9** Combined dyes electropherogram from degraded DNA. The IQCS peak is highlighted by the red box and the IQCL peak is highlighted by the green box. The ski slope nature of the DNA profile is highlighted by the arrow.

## No sample peaks with balanced IQC peaks

Although there are no DNA profile peaks in the following figure, the presence of both the IQCS and IQCL peaks with balanced relative peak heights indicates that PCR has occurred optimally.

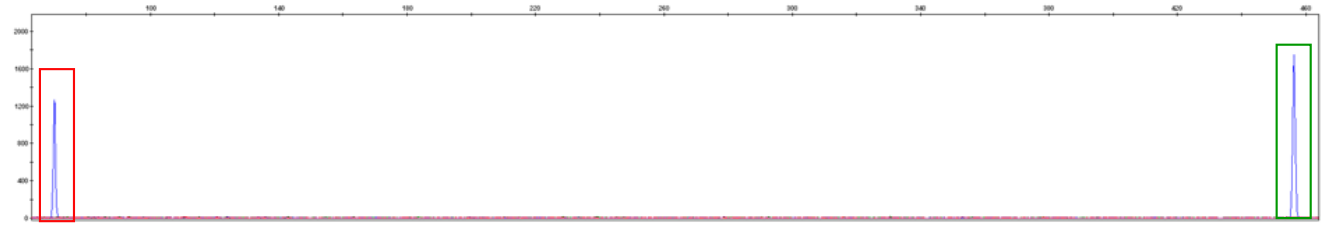


**Figure 10** Combined dyes electropherogram showing IQCS and IQCL peaks with 0 ng DNA (scaled to 2,000 RFU). The IQCS peak is highlighted by the red box and the IQCL peak is highlighted by the green box.

# 6 Experiments and results



## Importance of validation

Validation of a DNA typing procedure for human identification applications is an evaluation of the efficiency, reliability, and performance characteristics of the procedure. By challenging the procedure with samples that are commonly encountered in forensic and parentage laboratories, the validation process uncovers attributes and limitations that are critical for sound data interpretation (Sparkes, Kimpton, Watson, 1996; Sparkes, Kimpton, Gilbard, 1996; Wallin, 1998).

## Experiment conditions

We conducted developmental validation experiments according to the updated and revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, December 2016). Based on these guidelines, we conducted experiments that comply with guidelines 2.0 and 3.0 and its associated subsections. This DNA methodology is not novel. (Moretti *et al.*, 2001; Frank *et al.*, 2001; Wallin *et al., 2002*; and Holt *et al.*, 2000).

We used conditions that produced optimum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use.

# Laboratory requirements for internal validation

Each laboratory using this kit must perform internal validation studies. Performance of this kit is supported when used according to the following developmentally validated parameters. Modifications to the protocol should be accompanied by appropriate validation studies performed by the laboratory.

# Developmental validation

Except where noted, all developmental validation studies were performed using the GeneAmp™ PCR System 9700 96-Well thermal cycler or the ProFlex™ PCR System according to the protocol described in the Perform PCR chapter.

Unless otherwise indicated, the data in this chapter are from the re-validation of the NGM Detect™ kit after redesigning the IQCL and TH01 markers. The performance of the updated kit formulation assay is fully comparable to that of the original kit. See the *Technical Note: Updated NGM Detect™ PCR Amplification Kit: Validation and Comparative Study* for studies directly related to soil specificity and a direct comparison of the NGM Detect™ kit original formulation to the updated formulation.

**SWGDAM guideline 2.2.1**

"Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic, database, known or casework reference samples." (SWGDAM, December 2016)

**SWGDAM guideline 3.9.2**

"The reaction conditions needed to provide the required degree of specificity and robustness should be determined. These include, but are not limited to, thermal cycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents." (SWGDAM, December 2016)

**SWGDAM guideline 3.9.6**

"Criteria for detection of amplified product should be determined based on the platform and/or method. " (SWGDAM, December 2016)

## PCR components

We examined the concentration of each component of the kit. The concentration of each component was in the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, 0.5 ng of DNA Control 007 was amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Figure 11). The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

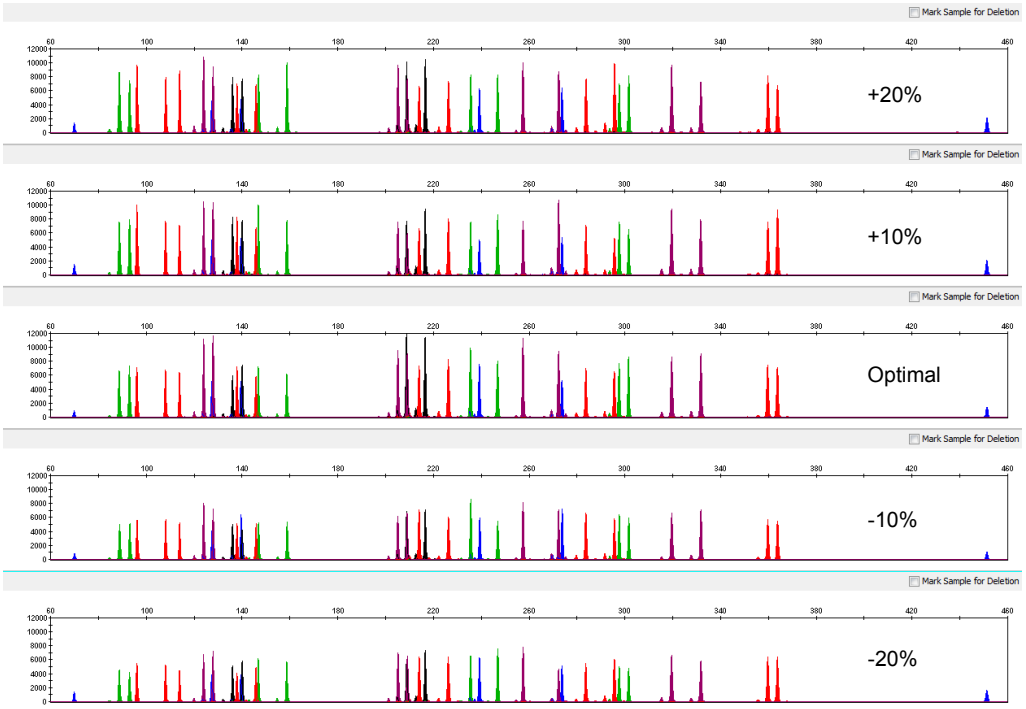



**Figure 11** DNA Control 007 (0.5 ng) amplified with the NGM Detect™ kit in the presence of varying concentrations of magnesium chloride and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 12,000 RFU).

## Thermal cycling temperatures

Thermal cycling parameters were optimized using a Design of Experiments (DOE) approach that seeks to identify the combination of temperatures and hold times that produce the best assay performance. Optimal assay performance was determined through evaluation of several factors, including; evaluation of assay sensitivity, peak-height balance, and resistance to PCR inhibitors.

For example, annealing/extension temperatures of 57, 58, 59, 60, and 61°C were tested using a GeneAmp™ PCR System 9700 (Figure 12). The PCR products were analyzed using a 3500xL Genetic Analyzer.

Of the tested annealing temperatures, 57°C to 61°C produced robust profiles. The optimal combination of specificity, sensitivity, and resistance to PCR inhibition was observed at 59°C. Thermal cycler temperature is critical to assay performance; therefore routine, regularly scheduled thermal cycler calibration is recommended.

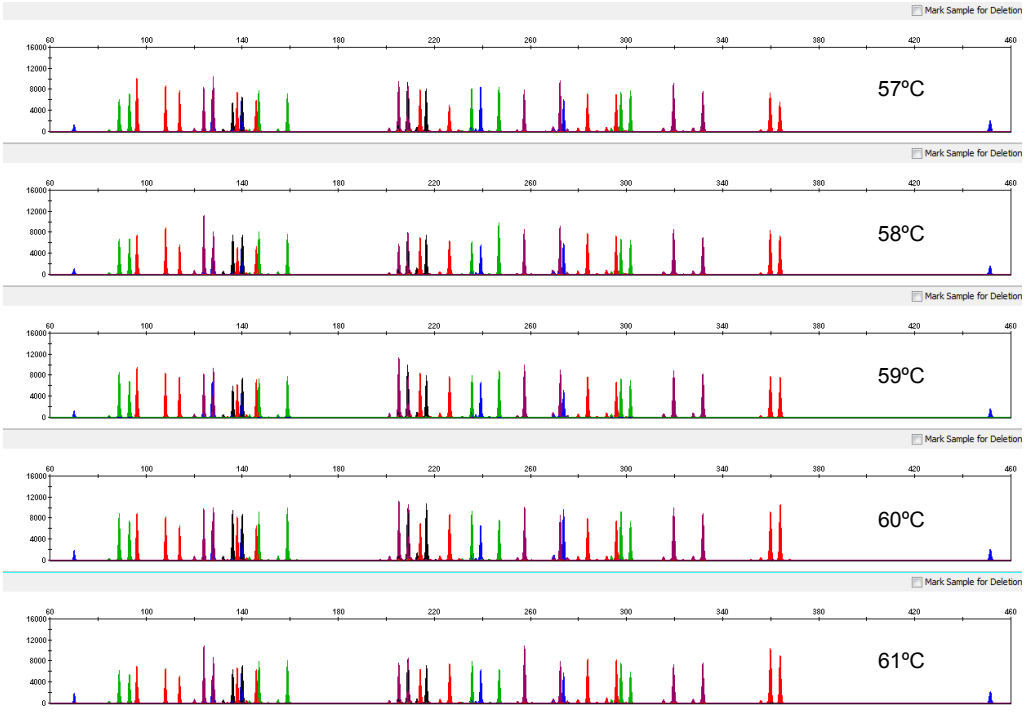



**Figure 12** Electropherograms obtained from amplification of 0.5 ng of DNA Control 007 at annealing temperatures of 57, 58, 59, 60, and 61°C, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 16,000 RFU).

## PCR cycle number

Reactions were amplified for 28, 29, 30, 31, and 32 cycles on the GeneAmp™ PCR System 9700 using 0.5 ng of DNA Control 007. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated for all numbers of thermal cycles (28–32) and off-scale data were collected for several allele peaks at 32 cycles (Figure 13).

We recommend using 30 cycles to optimize signal peak height and minimize artifact or other undesirable peaks.

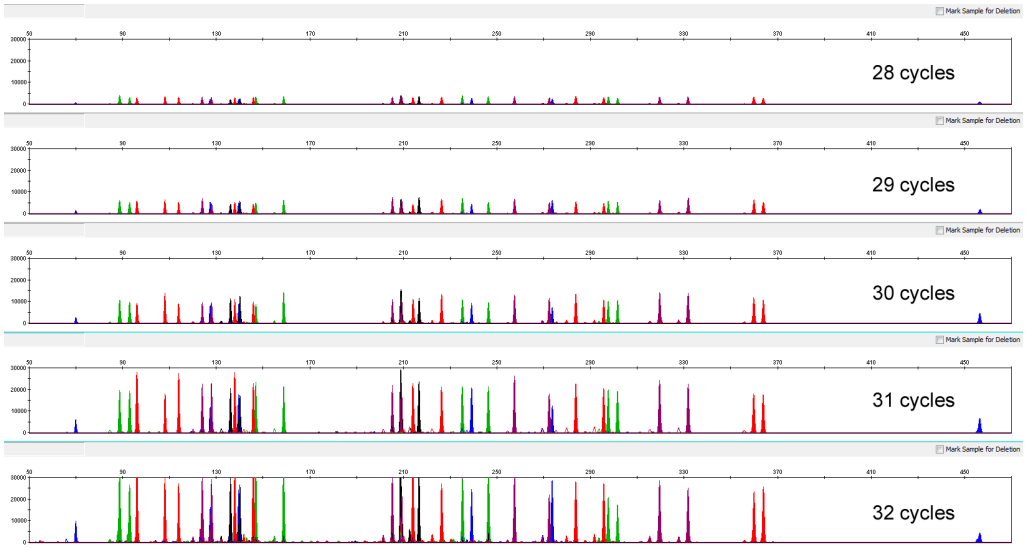



**Figure 13** Representative NGM Detect™ kit profiles obtained from amplification of 0.5 ng of DNA Control 007 using 28, 29, 30, 31, and 32 cycles, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 30,000 RFU).

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

## CE injection time

The effect of injection times on heterozygous peak heights observed for NGM Detect™ assays was studied on three capillary electrophoresis instruments; the 3500 (8-capillary), 3500xL (24-capillary), and 3130*xl* (16-capillary). As shown in Figure 14 through Figure 16, increasing or decreasing injection times affected profile peak heights in an approximately linear manner. All genomic DNA samples were amplified for 30 cycles.

Our developmental validation studies indicate that the injection conditions that are recorded in Chapter 3 generate profiles from 0.5 ng of input DNA with heterozygous peak height averages between 4,000–10,000 RFU (3500 or 3500xL) or 2,000–4,000 RFU (3130 or 3130*xl*). This is with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary, therefore laboratories are encouraged to optimize injection times. Optimized injection times provide the most efficacious level of assay sensitivity, minimize the

occurrence of off-scale peaks or undesirable artifacts, and do not adversely impact the performance of the size standard and allelic ladder.

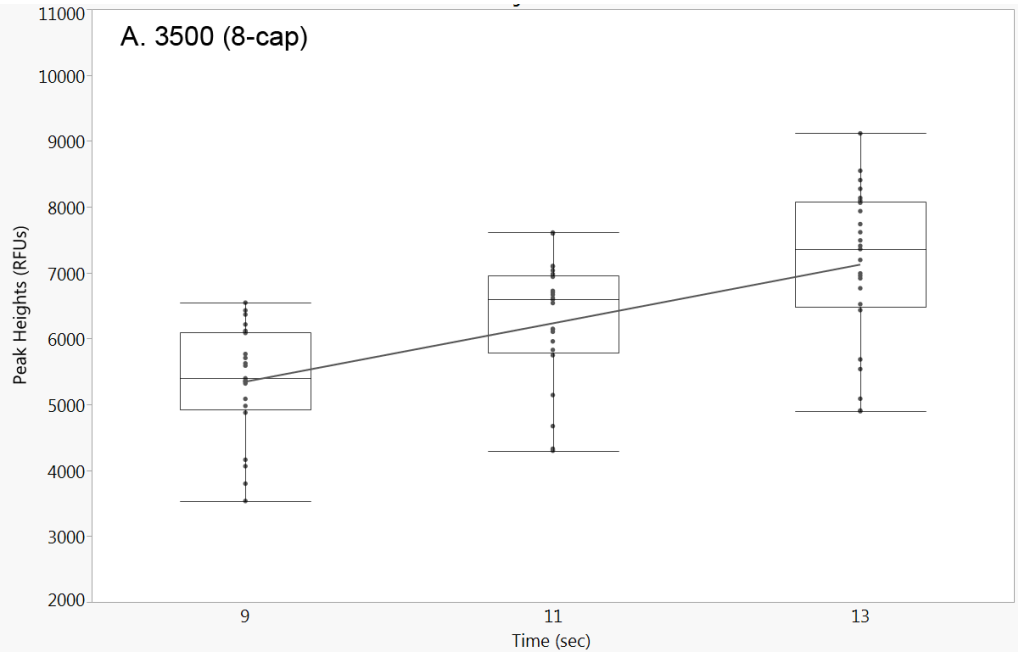



**Figure 14** Box plots show the effect of varying injection times (X-axis) on peak heterozygous heights (Y-axis) observed for NGM Detect™ assays with 0.5 ng of human male 007 genomic DNA input on a 3500 Genetic Analyzer.

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

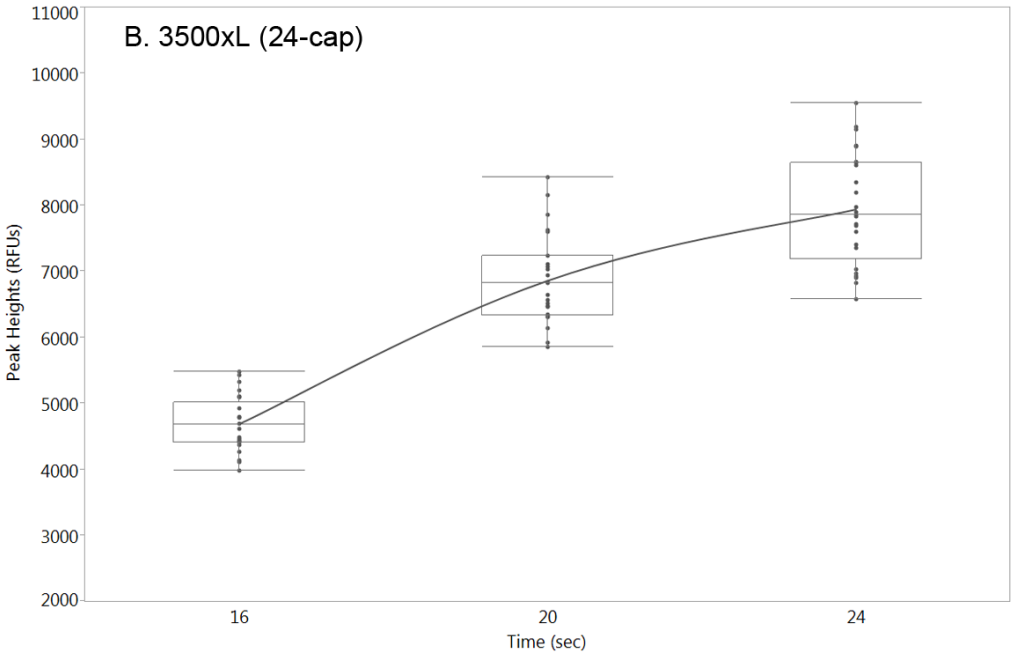



**Figure 15** Box plots show the effect of varying injection times (X-axis) on heterozygous peak heights (Y-axis) observed for NGM Detect™ assays with 0.5 ng of human male 007 genomic DNA input on a 3500xL Genetic Analyzer.

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

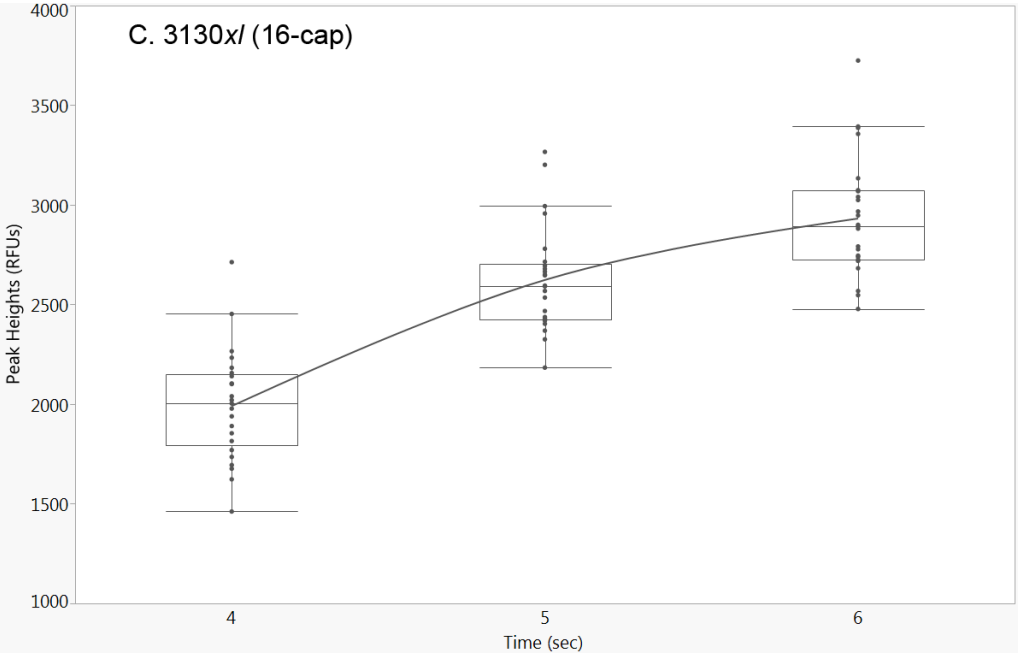



**Figure 16** Box plots show the effect of varying injection times (X-axis) on heterozygous peak heights (Y-axis) observed for NGM Detect™ assays with 0.5 ng of human male 007 genomic DNA input on a 3130*xl* Genetic Analyzer.

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

# Accuracy, precision, and reproducibility

## SWGDAM guideline 3.5

"Precision and accuracy of the assay should be demonstrated: Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. Precision depends only on the distribution of random errors and does not relate to the true value or specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value." (SWGDAM, December 2016)

## Accuracy observation

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al*., 2000; Wallin *et al*., 2002). However, accuracy and reproducibility of profiles have been determined from various sample types.

Figure 17, Figure 18, and Figure 19 show the size differences that were observed between sample alleles and allelic ladder alleles on the Applied Biosystems™ 3130 *xl*, 3500, and 3500xL Genetic Analyzers with POP-4™ Polymer. The X-axis in the following figures represents the nominal nucleotide sizes for the NGM Detect™ Allelic Ladder. The dashed lines parallel to the X-axis represent the ±0.25-nt windows. The y-axis represents the deviation of each sample allele size from the corresponding Allelic Ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the Allelic Ladder, irrespective of the capillary electrophoresis platforms.

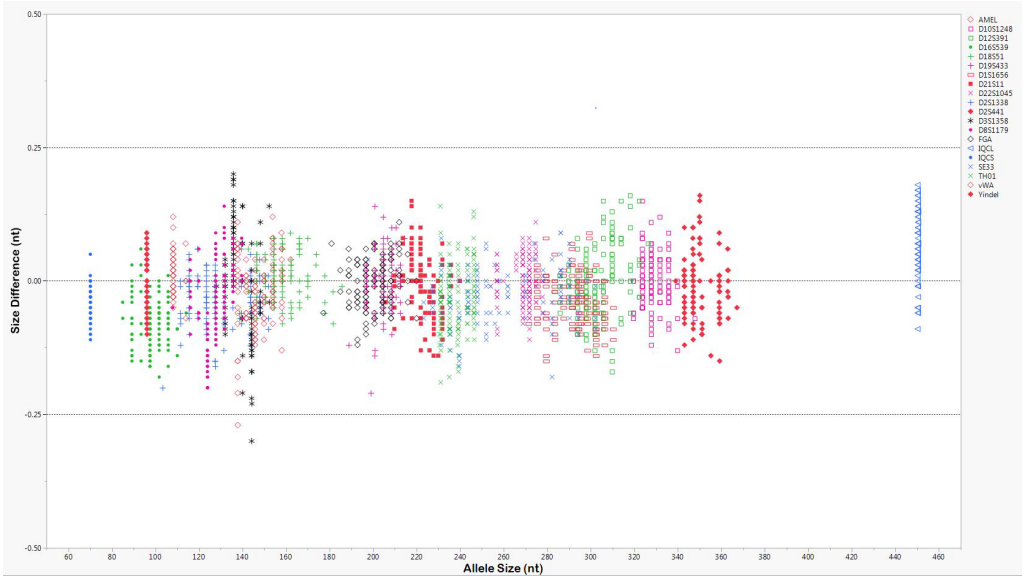



**Figure 17** Allele size vs. Allelic Ladder sizing for 81 samples analyzed on a 3130 *xl* Genetic Analyzer. Size and ladder sizing for the NGM Detect™ kit were calculated using the GeneScan™–600 LIZ™ Size Standard v2.0.

**Note:** Except for the TH01 marker data, the data in this figure are from the original formulation of the NGM Detect™ kit.

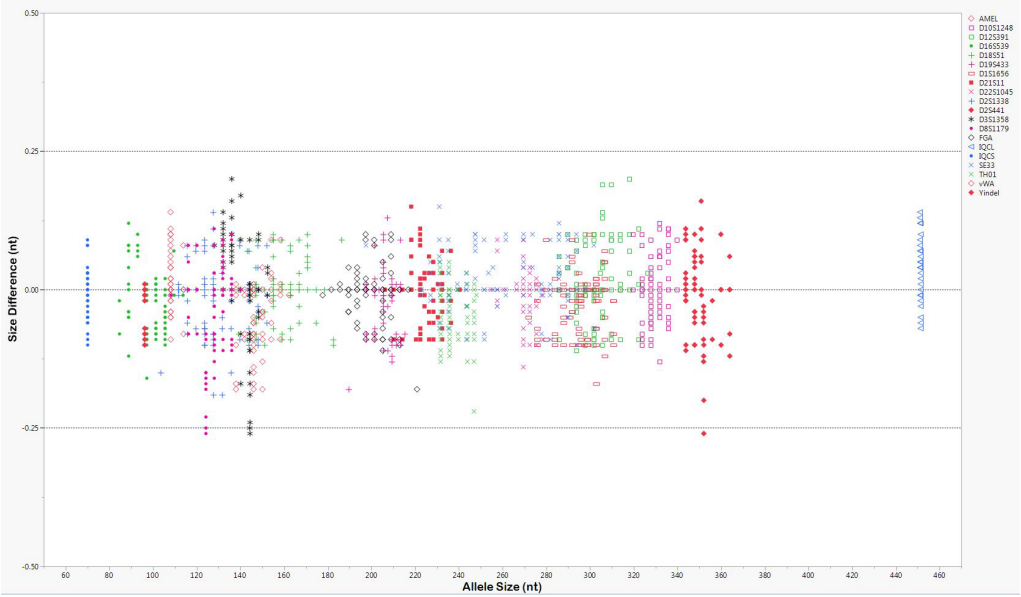



**Figure 18** Allele size vs. Allelic Ladder sizing for 81 samples analyzed on a 3500 Genetic Analyzer. Size and ladder sizing for the NGM Detect™ kit were calculated using the GeneScan™ –600 LIZ™ Size Standard v2.0.

**Note:** Except for the TH01 marker data, the data in this figure are from the original formulation of the NGM Detect™ kit.


**Figure 19** Allele size vs. Allelic Ladder sizing for 81 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the NGM Detect™ kit were calculated using the GeneScan™ –600 LIZ™ Size Standard v2.0.

**Note:** Except for the TH01 marker data, the data in this figure are from the original formulation of the NGM Detect™ kit.

## Precision and size window description

Sizing precision enables the determination of accurate and reliable genotypes. The recommended method for genotyping is to use a ±0.5-nt "window" around the size obtained for each allele in the allelic ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be either:

- An "off-ladder" allele, that is, an allele of a size that is not represented in the allelic ladder.
- An allele that does correspond to an allele in the allelic ladder, but whose size is just outside a window because of measurement error.

The measurement error inherent in any sizing method can be defined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections on a capillary instrument.

## Precision and size window observation

Table 4 lists typical precision results obtained from multiple runs of the NGM Detect™ Allelic Ladder using the GeneScan™–600 LIZ™ Size Standard v2.0. The results were obtained within a set of injections on a single capillary array. The number of repeated injections for each genetic analyzer platform is shown in the following table:

| CE platform | Capillaries | # Injections | Sizing method |
|---|---|---|---|
| 3130*xl* | 16/injection | 5 | Local Southern, 60–460 bp |
| 3500 | 8/injection | 12 | Local Southern, 60–460 bp |
| 3500xL | 24/injection | 4 | Local Southern, 60–460 bp |

The mean sizes and the standard deviation for the allele sizing were calculated for all the alleles in each run (Table 4). The mean range and the standard deviation range show the lowest and highest values obtained across multiple runs.

Sample alleles can occasionally size outside of the ±0.5-nt window for a respective Allelic Ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems with the smallest standard deviations in sizing. The figures in "Accuracy observation" on page 63 illustrate the tight clustering of allele sizes obtained on the Applied Biosystems™ genetic analyzers, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off–ladder allele versus measurement error of a sample allele that corresponds to an allele in the Allelic Ladder. Repeat analysis, when necessary, provides an added level of confidence in the final allele assignment.

GeneMapper™ *ID-X* Software automatically flags sample alleles that do not size within the prescribed window around an allelic ladder allele by labeling the allele as OL (off-ladder).

Maximum sizing precision is obtained within the same set of capillary injections. Cross–platform sizing differences occur due to several factors including type and concentration of polymer, run temperature, and electrophoresis conditions. Variations

in sizing can also occur between runs on the same instrument and between runs on different instruments of the same platform type because of these factors.

**IMPORTANT!** To minimize the variation in sizing between runs and to ensure accurate genotyping, follow the guidelines in "Allelic ladder requirements for data analysis" on page 34 and use allelic ladders obtained from the same run as samples to analyze the samples.

For more information on precision and genotyping, see (Lazaruk *et al.*, 1998; Mansfield *et al.*, 1998).

**Note:** The IQCS and IQCL markers were omitted from this study because they are not used for genotyping.

**Table 4** Precision results of multiple runs of the NGM Detect™ Allelic Ladder

**Note:** Except for the TH01 marker data, the data in this table are from the original formulation of the NGM Detect™ kit.

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| **D2S1338** | | | | | | |
| 11 | 90.76–90.87 | 0.023–0.034 | 91.05–91.13 | 0.003–0.040 | 90.96–91.03 | 0.028–0.035 |
| 12 | 94.90–95.00 | 0.021–0.031 | 95.17–95.24 | 0.021–0.041 | 95.10–95.16 | 0.024–0.034 |
| 13 | 99.01–99.13 | 0.023–0.035 | 99.31–99.38 | 0.001–0.041 | 99.22–99.28 | 0.027–0.034 |
| 14 | 103.16–103.28 | 0.020–0.036 | 103.45–103.52 | 0.028–0.044 | 103.38–103.44 | 0.031–0.038 |
| 15 | 107.09–107.19 | 0.025–0.037 | 107.31–107.38 | 0.015–0.058 | 107.23–107.28 | 0.028–0.037 |
| 16 | 111.29–111.38 | 0.028–0.033 | 111.55–111.60 | 0.005–0.043 | 111.45–111.50 | 0.025–0.041 |
| 17 | 115.34–115.43 | 0.022–0.035 | 115.56–115.62 | 0.001–0.032 | 115.49–115.52 | 0.011–0.031 |
| 18 | 119.29–119.36 | 0.012–0.033 | 119.52–119.57 | 0.024–0.042 | 119.43–119.47 | 0.015–0.040 |
| 19 | 123.27–123.36 | 0.020–0.034 | 123.50–123.56 | 0.004–0.041 | 123.42–123.47 | 0.034–0.036 |
| 20 | 127.26–127.35 | 0.026–0.041 | 127.51–127.57 | 0.019–0.054 | 127.41–127.46 | 0.028–0.042 |
| 21 | 131.28–131.39 | 0.024–0.039 | 131.53–131.62 | 0.017–0.051 | 131.46–131.51 | 0.029–0.039 |
| 22 | 135.34–135.44 | 0.023–0.038 | 135.56–135.63 | 0.011–0.046 | 135.50–135.53 | 0.027–0.035 |
| 23 | 139.39–139.49 | 0.020–0.040 | 139.62–139.69 | 0.001–0.048 | 139.54–139.59 | 0.002–0.033 |
| 24 | 143.51–143.61 | 0.020–0.040 | 143.77–143.82 | 0.008–0.043 | 143.68–143.74 | 0.026–0.042 |
| 25 | 147.63–147.74 | 0.017–0.031 | 147.89–147.94 | 0.016–0.048 | 147.79–147.85 | 0.024–0.043 |
| 26 | 151.76–151.85 | 0.023–0.029 | 152.02–152.06 | 0.018–0.041 | 151.92–151.97 | 0.030–0.036 |
| 27 | 155.91–156.01 | 0.018–0.031 | 156.15–156.20 | 0.028–0.043 | 156.07–156.11 | 0.026–0.040 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| 28 | 160.45–160.49 | 0.008–0.033 | 160.51–160.57 | 0.007–0.042 | 160.45–160.47 | 0.037–0.040 |
| **SE33** | | | | | | |
| 4.2 | 188.38–188.44 | 0.024–0.037 | 188.22–188.26 | 0.018–0.044 | 188.17–188.18 | 0.034–0.036 |
| 6.3 | 197.70–197.76 | 0.022–0.038 | 197.54–197.6 | 0.005–0.045 | 197.49–197.50 | 0.027–0.038 |
| 8 | 202.76–202.80 | 0.022–0.032 | 202.58–202.63 | 0.011–0.043 | 202.53–202.55 | 0.022–0.033 |
| 9 | 206.78–206.85 | 0.023–0.040 | 206.60–206.65 | 0.017–0.044 | 206.56–206.56 | 0.030–0.036 |
| 11 | 214.87–214.92 | 0.031–0.036 | 214.65–214.69 | 0.035–0.051 | 214.59–214.62 | 0.029–0.038 |
| 12 | 219.05–219.12 | 0.021–0.037 | 218.83–218.88 | 0.033–0.053 | 218.78–218.80 | 0.035–0.042 |
| 13 | 223.16–223.20 | 0.032–0.047 | 222.92–222.97 | 0.021–0.050 | 222.88–222.90 | 0.033–0.043 |
| 14 | 227.22–227.29 | 0.032–0.046 | 227.01–227.06 | 0.011–0.054 | 226.96–226.98 | 0.033–0.049 |
| 15 | 231.26–231.34 | 0.028–0.047 | 231.06–231.09 | 0.018–0.070 | 231.01–231.03 | 0.037–0.045 |
| 16 | 235.40–235.46 | 0.024–0.041 | 235.16–235.22 | 0.013–0.053 | 235.14–235.14 | 0.039–0.044 |
| 17 | 239.50–239.56 | 0.029–0.045 | 239.27–239.32 | 0.031–0.048 | 239.23–239.24 | 0.039–0.045 |
| 18 | 243.65–243.71 | 0.020–0.031 | 243.38–243.43 | 0.004–0.044 | 243.35–243.36 | 0.027–0.042 |
| 19 | 247.78–247.84 | 0.030–0.037 | 247.47–247.54 | 0.009–0.049 | 247.47–247.48 | 0.026–0.035 |
| 20 | 251.90–251.95 | 0.026–0.036 | 251.58–251.65 | 0.009–0.049 | 251.57–251.59 | 0.033–0.037 |
| 20.2 | 253.86–253.92 | 0.021–0.041 | 253.56–253.63 | 0.025–0.045 | 253.55–253.56 | 0.028–0.036 |
| 21 | 255.86–255.93 | 0.028–0.038 | 255.57–255.63 | 0.015–0.048 | 255.55–255.57 | 0.028–0.040 |
| 21.2 | 257.83–257.90 | 0.026–0.042 | 257.55–257.60 | 0.031–0.043 | 257.52–257.54 | 0.021–0.030 |
| 22.2 | 261.81–261.88 | 0.019–0.043 | 261.53–261.58 | 0.005–0.052 | 261.51–261.53 | 0.030–0.039 |
| 23.2 | 265.91–265.98 | 0.025–0.047 | 265.62–265.68 | 0.027–0.055 | 265.60–265.62 | 0.030–0.036 |
| 24.2 | 269.97–270.02 | 0.026–0.041 | 269.66–269.72 | 0.025–0.055 | 269.64–269.66 | 0.035–0.041 |
| 25.2 | 273.95–274.00 | 0.025–0.036 | 273.65–273.69 | 0.034–0.053 | 273.62–273.64 | 0.031–0.045 |
| 26.2 | 278.10–278.16 | 0.029–0.043 | 277.8–277.84 | 0.007–0.058 | 277.78–277.80 | 0.033–0.038 |
| 27.2 | 282.16–282.22 | 0.027–0.047 | 281.84–281.89 | 0.005–0.058 | 281.81–281.84 | 0.026–0.050 |
| 28.2 | 286.14–286.21 | 0.022–0.045 | 285.83–285.86 | 0.023–0.054 | 285.80–285.82 | 0.027–0.038 |
| 29.2 | 290.15–290.22 | 0.028–0.046 | 289.85–289.89 | 0.016–0.052 | 289.82–289.83 | 0.031–0.036 |
| 30.2 | 294.20–294.24 | 0.028–0.039 | 293.87–293.92 | 0.029–0.066 | 293.85–293.85 | 0.037–0.045 |
| 31.2 | 298.16–298.24 | 0.028–0.045 | 297.84–297.92 | 0.001–0.050 | 297.82–297.83 | 0.032–0.043 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** |
| 32.2 | 302.17–302.23 | 0.027–0.041 | 301.83–301.90 | 0.004–0.051 | 301.81–301.83 | 0.042–0.045 |
| 33.2 | 306.14–306.20 | 0.025–0.050 | 305.81–305.86 | 0.007–0.043 | 305.76–305.80 | 0.030–0.033 |
| 34.2 | 310.16–310.22 | 0.033–0.045 | 309.81–309.88 | 0.007–0.048 | 309.78–309.80 | 0.034–0.043 |
| 35 | 312.20–312.27 | 0.025–0.050 | 311.84–311.89 | 0.005–0.047 | 311.82–311.83 | 0.034–0.036 |
| 35.2 | 314.24–314.30 | 0.027–0.041 | 313.87–313.94 | 0.001–0.050 | 313.83–313.87 | 0.037–0.048 |
| 36 | 316.37–316.43 | 0.024–0.042 | 316–316.04 | 0.010–0.038 | 315.97–315.99 | 0.024–0.048 |
| 37 | 320.60–320.66 | 0.032–0.048 | 320.21–320.27 | 0.035–0.053 | 320.19–320.23 | 0.037–0.048 |
| 38 | 324.76–324.83 | 0.035–0.054 | 324.35–324.41 | 0.009–0.064 | 324.35–324.38 | 0.042–0.048 |
| 39 | 328.85–328.92 | 0.027–0.047 | 328.45–328.51 | 0.029–0.055 | 328.45–328.48 | 0.029–0.039 |
| 42 | 341.00–341.06 | 0.031–0.048 | 340.58–340.65 | 0.001–0.064 | 340.57–340.59 | 0.037–0.044 |
| **D16S539** | | | | | | |
| 5 | 72.32–72.38 | 0.032–0.041 | 72.00–72.05 | 0.009–0.051 | 71.93–71.98 | 0.024–0.031 |
| 8 | 84.95–84.99 | 0.024–0.040 | 84.64–84.70 | 0.008–0.044 | 84.58–84.61 | 0.027–0.040 |
| 9 | 89.15–89.18 | 0.020–0.034 | 88.84–88.9 | 0.004–0.045 | 88.78–88.82 | 0.027–0.041 |
| 10 | 93.30–93.33 | 0.022–0.036 | 92.99–93.05 | 0.004–0.040 | 92.94–92.97 | 0.028–0.040 |
| 11 | 97.45–97.49 | 0.020–0.039 | 97.15–97.22 | 0.007–0.045 | 97.11–97.14 | 0.025–0.035 |
| 12 | 101.63–101.67 | 0.026–0.033 | 101.33–101.39 | 0.030–0.046 | 101.28–101.33 | 0.034–0.039 |
| 13 | 105.83–105.86 | 0.021–0.043 | 105.53–105.59 | 0.009–0.047 | 105.47–105.52 | 0.038–0.039 |
| 14 | 109.97–110.01 | 0.027–0.042 | 109.66–109.74 | 0.032–0.048 | 109.63–109.67 | 0.030–0.043 |
| 15 | 114.08–114.11 | 0.024–0.036 | 113.77–113.85 | 0.028–0.051 | 113.72–113.78 | 0.027–0.038 |
| **D18S51** | | | | | | |
| 7 | 127.11–127.18 | 0.030–0.038 | 127.52–127.58 | 0.014–0.046 | 127.44–127.47 | 0.028–0.042 |
| 9 | 134.69–134.79 | 0.022–0.047 | 135.21–135.27 | 0.010–0.051 | 135.12–135.17 | 0.028–0.045 |
| 10 | 138.52–138.63 | 0.025–0.051 | 139.07–139.15 | 0.001–0.041 | 139.00–139.06 | 0.037–0.042 |
| 10.2 | 140.66–140.77 | 0.028–0.054 | 141.21–141.30 | 0.001–0.042 | 141.15–141.21 | 0.035–0.043 |
| 11 | 142.41–142.53 | 0.023–0.047 | 143.00–143.10 | 0.008–0.052 | 142.93–142.99 | 0.020–0.038 |
| 12 | 146.33–146.45 | 0.023–0.050 | 146.95–147.03 | 0.020–0.044 | 146.88–146.95 | 0.029–0.038 |
| 13 | 150.27–150.39 | 0.027–0.042 | 150.90–151.01 | 0.024–0.045 | 150.83–150.91 | 0.031–0.038 |
| 13.2 | 152.41–152.53 | 0.027–0.050 | 153.05–153.16 | 0.013–0.046 | 153.00–153.07 | 0.033–0.044 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| 14 | 154.21–154.33 | 0.030–0.048 | 154.86–154.96 | 0.024–0.046 | 154.80–154.87 | 0.032–0.036 |
| 14.2 | 156.35–156.48 | 0.021–0.040 | 157.02–157.14 | 0.004–0.047 | 156.96–157.03 | 0.035–0.043 |
| 15 | 158.15–158.27 | 0.032–0.051 | 158.83–158.94 | 0.001–0.043 | 158.76–158.83 | 0.040–0.045 |
| 16 | 162.05–162.18 | 0.027–0.049 | 162.76–162.86 | 0.004–0.043 | 162.69–162.77 | 0.030–0.037 |
| 17 | 165.92–166.07 | 0.027–0.054 | 166.64–166.77 | 0.003–0.039 | 166.58–166.66 | 0.029–0.046 |
| 18 | 169.80–169.93 | 0.034–0.049 | 170.54–170.66 | 0.016–0.044 | 170.49–170.57 | 0.030–0.039 |
| 19 | 173.66–173.81 | 0.031–0.049 | 174.44–174.55 | 0.004–0.044 | 174.39–174.47 | 0.029–0.045 |
| 20 | 177.54–177.69 | 0.035–0.051 | 178.34–178.48 | 0.003–0.047 | 178.28–178.36 | 0.032–0.040 |
| 21 | 181.47–181.63 | 0.039–0.049 | 182.30–182.45 | 0.006–0.044 | 182.26–182.35 | 0.035–0.044 |
| 22 | 185.33–185.52 | 0.035–0.047 | 186.21–186.37 | 0.013–0.042 | 186.17–186.26 | 0.033–0.046 |
| 23 | 189.25–189.44 | 0.030–0.067 | 190.17–190.32 | 0.022–0.046 | 190.12–190.22 | 0.032–0.042 |
| 24 | 193.19–193.37 | 0.032–0.054 | 194.13–194.26 | 0.021–0.050 | 194.07–194.17 | 0.029–0.055 |
| 25 | 197.14–197.32 | 0.030–0.064 | 198.11–198.25 | 0.005–0.048 | 198.05–198.15 | 0.033–0.047 |
| 26 | 200.98–201.15 | 0.038–0.065 | 201.92–202.07 | 0.024–0.048 | 201.88–201.98 | 0.034–0.043 |
| 27 | 204.81–204.98 | 0.035–0.067 | 205.76–205.91 | 0.020–0.040 | 205.72–205.83 | 0.038–0.046 |
| **TH01** | | | | | | |
| 4 | 222.50–222.65 | 0.035–0.073 | 223.26–223.39 | 0.004–0.049 | 223.19–223.31 | 0.035–0.041 |
| 5 | 226.48–226.64 | 0.029–0.076 | 227.24–227.39 | 0.011–0.060 | 227.19–227.32 | 0.031–0.040 |
| 6 | 230.47–230.65 | 0.034–0.057 | 231.25–231.39 | 0.029–0.049 | 231.19–231.31 | 0.027–0.044 |
| 7 | 234.46–234.65 | 0.028–0.059 | 235.27–235.39 | 0.008–0.048 | 235.18–235.31 | 0.033–0.041 |
| 8 | 238.47–238.65 | 0.033–0.068 | 239.25–239.40 | 0.005–0.050 | 239.19–239.32 | 0.029–0.043 |
| 9 | 242.55–242.71 | 0.028–0.065 | 243.34–243.47 | 0.027–0.053 | 243.26–243.38 | 0.025–0.040 |
| 9.3 | 245.61–245.78 | 0.035–0.058 | 246.38–246.51 | 0.005–0.049 | 246.31–246.43 | 0.034–0.047 |
| 10 | 246.63–246.81 | 0.033–0.074 | 247.40–247.54 | 0.024–0.050 | 247.33–247.45 | 0.031–0.041 |
| 11 | 250.70–250.86 | 0.036–0.066 | 251.43–251.54 | 0.003–0.046 | 251.36–251.47 | 0.034–0.043 |
| 12 | 254.62–254.79 | 0.031–0.069 | 255.36–255.48 | 0.016–0.046 | 255.29–255.40 | 0.030–0.044 |
| 13 | 258.53–258.71 | 0.036–0.063 | 259.28–259.39 | 0.003–0.045 | 259.19–259.31 | 0.033–0.040 |
| 13.3 | 261.46–261.65 | 0.042–0.065 | 262.21–262.34 | 0.005–0.038 | 262.15–262.27 | 0.027–0.042 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| **D12S391** | | | | | | |
| 14 | 281.70–281.76 | 0.022–0.037 | 281.71–281.75 | 0.039–0.054 | 281.68–281.70 | 0.033–0.041 |
| 15 | 285.72–285.76 | 0.026–0.033 | 285.73–285.75 | 0.010–0.048 | 285.69–285.71 | 0.030–0.043 |
| 16 | 289.68–289.72 | 0.023–0.040 | 289.68–289.71 | 0.001–0.049 | 289.65–289.67 | 0.030–0.038 |
| 17 | 293.71–293.77 | 0.018–0.037 | 293.71–293.76 | 0.001–0.046 | 293.68–293.69 | 0.027–0.041 |
| 18 | 297.70–297.75 | 0.026–0.040 | 297.7–297.74 | 0.001–0.050 | 297.67–297.69 | 0.036–0.040 |
| 19 | 301.69–301.74 | 0.020–0.042 | 301.70–301.75 | 0.024–0.048 | 301.65–301.67 | 0.032–0.040 |
| 19.3 | 304.64–304.68 | 0.021–0.033 | 304.62–304.67 | 0.011–0.047 | 304.58–304.61 | 0.031–0.037 |
| 20 | 305.62–305.66 | 0.025–0.037 | 305.61–305.65 | 0.007–0.048 | 305.56–305.59 | 0.030–0.039 |
| 21 | 309.65–309.70 | 0.022–0.043 | 309.65–309.71 | 0.012–0.054 | 309.60–309.64 | 0.036–0.044 |
| 22 | 313.76–313.81 | 0.028–0.041 | 313.77–313.79 | 0.031–0.050 | 313.71–313.74 | 0.034–0.040 |
| 23 | 318.00–318.04 | 0.022–0.037 | 317.97–318.00 | 0.032–0.051 | 317.92–317.96 | 0.026–0.039 |
| 24 | 322.19–322.22 | 0.026–0.046 | 322.13–322.18 | 0.004–0.056 | 322.11–322.13 | 0.016–0.047 |
| 25 | 326.3–326.33 | 0.022–0.041 | 326.24–326.28 | 0.011–0.070 | 326.21–326.23 | 0.034–0.052 |
| 26 | 330.39–330.42 | 0.027–0.033 | 330.34–330.38 | 0.029–0.067 | 330.31–330.34 | 0.037–0.048 |
| 27 | 334.42–334.46 | 0.025–0.039 | 334.38–334.40 | 0.016–0.060 | 334.34–334.38 | 0.027–0.042 |
| **D3S1358** | | | | | | |
| 9 | 112.46–112.49 | 0.019–0.047 | 112.54–112.59 | 0.038–0.046 | 112.50–112.53 | 0.017–0.035 |
| 10 | 116.39–116.42 | 0.021–0.027 | 116.47–116.51 | 0.001–0.036 | 116.43–116.45 | 0.024–0.032 |
| 11 | 120.26–120.29 | 0.018–0.035 | 120.33–120.37 | 0.028–0.044 | 120.29–120.30 | 0.022–0.037 |
| 12 | 124.05–124.08 | 0.023–0.034 | 124.11–124.15 | 0.007–0.049 | 124.08–124.09 | 0.023–0.044 |
| 13 | 128.11–128.15 | 0.024–0.038 | 128.17–128.22 | 0.025–0.049 | 128.14–128.15 | 0.031–0.037 |
| 14 | 132.04–132.09 | 0.016–0.035 | 132.12–132.16 | 0.026–0.050 | 132.09–132.10 | 0.029–0.038 |
| 15 | 135.93–135.96 | 0.024–0.034 | 136.01–136.04 | 0.006–0.037 | 135.98–135.98 | 0.030–0.035 |
| 16 | 140.07–140.11 | 0.017–0.035 | 140.16–140.20 | 0.001–0.046 | 140.13–140.13 | 0.039–0.043 |
| 17 | 144.27–144.29 | 0.020–0.036 | 144.33–144.37 | 0.034–0.052 | 144.31–144.32 | 0.027–0.037 |
| 18 | 148.33–148.36 | 0.017–0.031 | 148.4–148.44 | 0.029–0.049 | 148.38–148.38 | 0.028–0.033 |
| 19 | 152.35–152.37 | 0.019–0.030 | 152.40–152.45 | 0.027–0.045 | 152.38–152.39 | 0.032–0.039 |
| 20 | 156.66–156.70 | 0.023–0.037 | 156.70–156.76 | 0.008–0.038 | 156.69–156.71 | 0.020–0.037 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| **FGA** | | | | | | |
| 13 | 165.50–165.58 | 0.029–0.039 | 165.95–166.04 | 0.015–0.047 | 165.93–165.97 | 0.023–0.040 |
| 14 | 169.35–169.45 | 0.030–0.036 | 169.84–169.91 | 0.003–0.043 | 169.81–169.86 | 0.030–0.035 |
| 15 | 173.21–173.31 | 0.031–0.039 | 173.72–173.83 | 0.009–0.043 | 173.70–173.74 | 0.028–0.038 |
| 16 | 177.06–177.17 | 0.032–0.041 | 177.59–177.71 | 0.004–0.047 | 177.58–177.63 | 0.031–0.041 |
| 17 | 180.94–181.05 | 0.026–0.042 | 181.51–181.61 | 0.005–0.047 | 181.48–181.55 | 0.027–0.047 |
| 18 | 184.85–184.97 | 0.032–0.045 | 185.45–185.56 | 0.013–0.051 | 185.43–185.50 | 0.030–0.039 |
| 19 | 188.76–188.89 | 0.024–0.040 | 189.38–189.51 | 0.021–0.039 | 189.38–189.45 | 0.031–0.042 |
| 20 | 192.67–192.80 | 0.032–0.042 | 193.34–193.44 | 0.010–0.049 | 193.31–193.39 | 0.028–0.049 |
| 21 | 196.56–196.70 | 0.030–0.050 | 197.23–197.38 | 0.004–0.046 | 197.24–197.32 | 0.035–0.043 |
| 22 | 200.46–200.59 | 0.034–0.054 | 201.15–201.27 | 0.027–0.054 | 201.13–201.22 | 0.026–0.043 |
| 23 | 204.28–204.39 | 0.033–0.049 | 204.97–205.11 | 0.020–0.044 | 204.96–205.05 | 0.031–0.050 |
| 24 | 208.09–208.22 | 0.037–0.048 | 208.83–208.93 | 0.011–0.056 | 208.81–208.89 | 0.035–0.048 |
| 25 | 211.94–212.05 | 0.027–0.048 | 212.67–212.82 | 0.004–0.048 | 212.67–212.74 | 0.039–0.050 |
| 26 | 215.83–215.95 | 0.029–0.061 | 216.58–216.74 | 0.023–0.041 | 216.59–216.69 | 0.037–0.043 |
| 27 | 219.83–219.97 | 0.034–0.059 | 220.62–220.79 | 0.001–0.050 | 220.64–220.73 | 0.036–0.050 |
| 28 | 223.71–223.86 | 0.035–0.058 | 224.55–224.68 | 0.011–0.053 | 224.54–224.64 | 0.037–0.040 |
| 29 | 227.55–227.70 | 0.034–0.063 | 228.41–228.58 | 0.016–0.061 | 228.43–228.53 | 0.030–0.056 |
| 30 | 231.34–231.50 | 0.034–0.056 | 232.23–232.40 | 0.015–0.057 | 232.25–232.36 | 0.035–0.056 |
| 30.2 | 233.71–233.86 | 0.030–0.054 | 234.60–234.76 | 0.014–0.050 | 234.62–234.73 | 0.039–0.052 |
| 31.2 | 237.59–237.74 | 0.029–0.062 | 238.50–238.68 | 0.003–0.050 | 238.52–238.64 | 0.040–0.051 |
| 32.2 | 241.50–241.66 | 0.038–0.053 | 242.46–242.62 | 0.034–0.050 | 242.47–242.59 | 0.038–0.047 |
| 33.2 | 245.48–245.64 | 0.035–0.049 | 246.43–246.61 | 0.022–0.053 | 246.47–246.56 | 0.041–0.052 |
| 42.2 | 280.49–280.68 | 0.034–0.067 | 281.59–281.80 | 0.001–0.048 | 281.61–281.75 | 0.038–0.052 |
| 43.2 | 284.32–284.52 | 0.041–0.073 | 285.46–285.67 | 0.016–0.047 | 285.47–285.62 | 0.032–0.052 |
| 44.2 | 288.33–288.51 | 0.041–0.070 | 289.46–289.66 | 0.016–0.053 | 289.48–289.62 | 0.033–0.055 |
| 45.2 | 292.23–292.41 | 0.040–0.070 | 293.35–293.57 | 0.001–0.055 | 293.38–293.52 | 0.033–0.048 |
| 46.2 | 295.73–295.93 | 0.037–0.065 | 296.95–297.17 | 0.004–0.051 | 296.98–297.12 | 0.038–0.059 |
| 47.2 | 299.50–299.7 | 0.037–0.073 | 300.72–300.95 | 0.007–0.059 | 300.76–300.90 | 0.036–0.065 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** |
| 48.2 | 303.40–303.60 | 0.034–0.075 | 304.64–304.87 | 0.018–0.053 | 304.67–304.82 | 0.040–0.055 |
| 50.2 | 311.14–311.37 | 0.048–0.081 | 312.47–312.69 | 0.007–0.063 | 312.46–312.64 | 0.043–0.057 |
| 51.2 | 314.87–315.12 | 0.052–0.083 | 316.30–316.56 | 0.021–0.066 | 316.34–316.51 | 0.039–0.055 |
| **Yindel** | | | | | | |
| 1 | 90.97–91.00 | 0.021–0.031 | 91.04–91.10 | 0.028–0.044 | 90.97–90.98 | 0.030–0.033 |
| 2 | 96.09–96.12 | 0.022–0.033 | 96.18–96.22 | 0.001–0.035 | 96.10–96.11 | 0.022–0.035 |
| **AMEL** | | | | | | |
| X | 108.18–108.22 | 0.024–0.039 | 108.08–108.16 | 0.007–0.046 | 108.05–108.09 | 0.030–0.035 |
| Y | 113.96–114.01 | 0.001–0.035 | 113.94–113.99 | 0.028–0.042 | 113.91–113.94 | 0.025–0.035 |
| **vWA** | | | | | | |
| 11 | 125.73–125.77 | 0.017–0.040 | 125.79–125.85 | 0.015–0.046 | 125.74–125.78 | 0.024–0.038 |
| 12 | 129.63–129.66 | 0.021–0.036 | 129.72–129.76 | 0.026–0.046 | 129.67–129.69 | 0.026–0.036 |
| 13 | 133.62–133.65 | 0.023–0.039 | 133.74–133.75 | 0.017–0.050 | 133.67–133.70 | 0.031–0.036 |
| 14 | 137.82–137.86 | 0.021–0.035 | 137.96–137.99 | 0.004–0.045 | 137.92–137.93 | 0.025–0.040 |
| 15 | 141.72–141.75 | 0.024–0.036 | 141.87–141.90 | 0.005–0.045 | 141.81–141.83 | 0.025–0.030 |
| 16 | 145.81–145.83 | 0.022–0.035 | 145.96–146.01 | 0.026–0.043 | 145.92–145.93 | 0.027–0.036 |
| 17 | 149.88–149.92 | 0.024–0.035 | 150.05–150.10 | 0.028–0.042 | 150.02–150.03 | 0.034–0.040 |
| 18 | 153.94–153.97 | 0.027–0.035 | 154.12–154.16 | 0.021–0.046 | 154.07–154.08 | 0.028–0.040 |
| 19 | 158.03–158.07 | 0.023–0.040 | 158.23–158.27 | 0.004–0.046 | 158.18–158.20 | 0.031–0.038 |
| 20 | 162.08–162.13 | 0.022–0.048 | 162.29–162.32 | 0.024–0.045 | 162.24–162.27 | 0.023–0.037 |
| 21 | 166.07–166.11 | 0.024–0.033 | 166.26–166.33 | 0.026–0.045 | 166.24–166.25 | 0.022–0.038 |
| 22 | 170.08–170.11 | 0.016–0.036 | 170.29–170.34 | 0.020–0.041 | 170.25–170.27 | 0.032–0.035 |
| 23 | 173.97–174.03 | 0.020–0.032 | 174.20–174.26 | 0.010–0.047 | 174.17–174.18 | 0.031–0.039 |
| 24 | 178.37–178.42 | 0.029–0.037 | 178.59–178.64 | 0.003–0.046 | 178.56–178.57 | 0.029–0.038 |
| **D21S11** | | | | | | |
| 24 | 197.75–197.85 | 0.024–0.039 | 198.20–198.26 | 0.032–0.046 | 198.16–198.20 | 0.034–0.045 |
| 24.2 | 199.77–199.87 | 0.033–0.040 | 200.23–200.30 | 0.001–0.046 | 200.19–200.24 | 0.023–0.042 |
| 25 | 201.76–201.85 | 0.023–0.045 | 202.20–202.25 | 0.031–0.043 | 202.16–202.2 | 0.027–0.035 |
| 26 | 205.74–205.81 | 0.020–0.046 | 206.16–206.22 | 0.024–0.050 | 206.12–206.16 | 0.031–0.044 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| 27 | 209.74–209.81 | 0.025–0.043 | 210.16–210.23 | 0.031–0.047 | 210.12–210.17 | 0.037–0.046 |
| 28 | 213.69–213.75 | 0.025–0.037 | 214.11–214.16 | 0.031–0.048 | 214.08–214.11 | 0.025–0.042 |
| 28.2 | 215.69–215.75 | 0.029–0.044 | 216.11–216.19 | 0.025–0.046 | 216.09–216.13 | 0.028–0.040 |
| 29 | 217.69–217.77 | 0.024–0.032 | 218.13–218.22 | 0.025–0.045 | 218.13–218.16 | 0.029–0.042 |
| 29.2 | 219.81–219.88 | 0.027–0.045 | 220.23–220.31 | 0.001–0.049 | 220.22–220.26 | 0.030–0.041 |
| 30 | 221.77–221.86 | 0.024–0.037 | 222.23–222.3 | 0.005–0.043 | 222.21–222.25 | 0.032–0.047 |
| 30.2 | 223.77–223.84 | 0.027–0.045 | 224.20–224.29 | 0.008–0.056 | 224.20–224.24 | 0.034–0.045 |
| 31 | 225.78–225.86 | 0.027–0.049 | 226.26–226.32 | 0.021–0.059 | 226.24–226.28 | 0.038–0.043 |
| 31.2 | 227.76–227.85 | 0.030–0.041 | 228.23–228.32 | 0.011–0.054 | 228.21–228.28 | 0.037–0.039 |
| 32 | 229.78–229.86 | 0.033–0.047 | 230.28–230.35 | 0.022–0.056 | 230.26–230.31 | 0.038–0.043 |
| 32.2 | 231.76–231.86 | 0.030–0.049 | 232.27–232.34 | 0.019–0.055 | 232.25–232.29 | 0.027–0.042 |
| 33 | 233.79–233.89 | 0.027–0.043 | 234.28–234.38 | 0.009–0.048 | 234.28–234.33 | 0.036–0.038 |
| 33.2 | 235.74–235.83 | 0.031–0.043 | 236.27–236.33 | 0.011–0.046 | 236.23–236.29 | 0.037–0.040 |
| 34 | 237.87–237.98 | 0.023–0.040 | 238.38–238.47 | 0.007–0.048 | 238.38–238.42 | 0.017–0.043 |
| 34.2 | 239.80–239.89 | 0.036–0.041 | 240.33–240.40 | 0.001–0.048 | 240.30–240.37 | 0.033–0.044 |
| 35 | 241.92–242.02 | 0.029–0.044 | 242.46–242.54 | 0.030–0.052 | 242.43–242.49 | 0.036–0.041 |
| 35.2 | 243.92–244.00 | 0.029–0.038 | 244.43–244.51 | 0.024–0.053 | 244.41–244.47 | 0.028–0.041 |
| 36 | 245.94–246.05 | 0.025–0.037 | 246.46–246.55 | 0.004–0.048 | 246.46–246.49 | 0.033–0.040 |
| 37 | 250.09–250.18 | 0.032–0.039 | 250.59–250.67 | 0.001–0.046 | 250.58–250.63 | 0.028–0.040 |
| 38 | 253.95–254.05 | 0.024–0.044 | 254.46–254.53 | 0.018–0.048 | 254.45–254.51 | 0.028–0.043 |
| **D1S1656** | | | | | | |
| 9 | 267.24–267.33 | 0.029–0.037 | 267.69–267.75 | 0.019–0.049 | 267.64–267.68 | 0.035–0.040 |
| 10 | 271.29–271.37 | 0.027–0.034 | 271.71–271.79 | 0.021–0.047 | 271.67–271.72 | 0.035–0.037 |
| 11 | 275.31–275.40 | 0.026–0.041 | 275.76–275.82 | 0.010–0.048 | 275.70–275.76 | 0.031–0.040 |
| 12 | 279.35–279.42 | 0.021–0.039 | 279.76–279.83 | 0.001–0.049 | 279.71–279.76 | 0.035–0.046 |
| 13 | 283.38–283.45 | 0.017–0.043 | 283.80–283.86 | 0.001–0.051 | 283.73–283.79 | 0.031–0.039 |
| 14 | 287.35–287.40 | 0.022–0.035 | 287.75–287.82 | 0.005–0.037 | 287.70–287.74 | 0.030–0.041 |
| 14.3 | 290.42–290.48 | 0.027–0.040 | 290.80–290.86 | 0.013–0.055 | 290.75–290.81 | 0.038–0.047 |
| 15 | 291.31–291.37 | 0.018–0.037 | 291.72–291.79 | 0.005–0.050 | 291.67–291.72 | 0.031–0.040 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** | **Mean** | **Standard deviation** |
| 15.3 | 294.41–294.45 | 0.025–0.037 | 294.80–294.85 | 0.010–0.050 | 294.74–294.78 | 0.035–0.047 |
| 16 | 295.31–295.38 | 0.020–0.038 | 295.75–295.80 | 0.008–0.048 | 295.66–295.72 | 0.033–0.039 |
| 16.3 | 298.41–298.45 | 0.029–0.036 | 298.78–298.84 | 0.031–0.049 | 298.71–298.76 | 0.038–0.045 |
| 17 | 299.24–299.29 | 0.022–0.043 | 299.64–299.72 | 0.001–0.052 | 299.56–299.61 | 0.018–0.044 |
| 17.3 | 302.34–302.39 | 0.028–0.040 | 302.71–302.77 | 0.023–0.052 | 302.64–302.69 | 0.023–0.042 |
| 18 | 303.19–303.24 | 0.021–0.037 | 303.62–303.65 | 0.007–0.047 | 303.53–303.58 | 0.030–0.036 |
| 18.3 | 306.24–306.32 | 0.024–0.040 | 306.66–306.70 | 0.018–0.048 | 306.57–306.64 | 0.026–0.048 |
| 19.3 | 310.25–310.32 | 0.026–0.044 | 310.65–310.70 | 0.007–0.057 | 310.57–310.63 | 0.029–0.036 |
| 20.3 | 314.22–314.30 | 0.008–0.041 | 314.65–314.71 | 0.001–0.048 | 314.56–314.62 | 0.018–0.045 |
| **D2S441** | | | | | | |
| 8 | 334.96–335.09 | 0.033–0.047 | 335.78–335.91 | 0.031–0.050 | 335.70–335.84 | 0.027–0.045 |
| 9 | 338.84–338.99 | 0.035–0.049 | 339.68–339.78 | 0.031–0.053 | 339.57–339.71 | 0.018–0.053 |
| 10 | 342.87–343.01 | 0.029–0.049 | 343.71–343.87 | 0.032–0.054 | 343.64–343.78 | 0.035–0.047 |
| 11 | 346.91–347.05 | 0.035–0.046 | 347.80–347.91 | 0.016–0.056 | 347.68–347.82 | 0.030–0.049 |
| 11.3 | 349.92–350.06 | 0.032–0.061 | 350.83–350.96 | 0.016–0.049 | 350.72–350.87 | 0.026–0.054 |
| 12 | 351.00–351.15 | 0.026–0.054 | 351.91–352.03 | 0.021–0.055 | 351.80–351.94 | 0.035–0.053 |
| 13 | 354.97–355.12 | 0.032–0.051 | 355.88–356.01 | 0.010–0.050 | 355.78–355.94 | 0.027–0.047 |
| 14 | 358.95–359.10 | 0.036–0.056 | 359.88–360 | 0.001–0.053 | 359.78–359.90 | 0.037–0.048 |
| 15 | 362.94–363.09 | 0.040–0.058 | 363.86–363.99 | 0.010–0.051 | 363.77–363.92 | 0.040–0.044 |
| 16 | 366.93–367.07 | 0.037–0.049 | 367.83–367.97 | 0.024–0.051 | 367.77–367.91 | 0.033–0.039 |
| 17 | 370.94–371.08 | 0.030–0.051 | 371.86–372.01 | 0.035–0.049 | 371.79–371.94 | 0.032–0.043 |
| **D8S1179** | | | | | | |
| 5 | 95.58–95.63 | 0.025–0.030 | 95.79–95.84 | 0.027–0.045 | 95.73–95.76 | 0.025–0.035 |
| 6 | 99.66–99.71 | 0.016–0.032 | 99.88–99.92 | 0.001–0.042 | 99.83–99.84 | 0.016–0.034 |
| 7 | 103.81–103.86 | 0.023–0.033 | 104.04–104.09 | 0.010–0.047 | 103.98–104.01 | 0.028–0.036 |
| 8 | 107.91–107.96 | 0.023–0.037 | 108.15–108.19 | 0.007–0.044 | 108.09–108.10 | 0.033–0.042 |
| 9 | 111.97–112.02 | 0.017–0.034 | 112.21–112.26 | 0.007–0.045 | 112.17–112.18 | 0.025–0.035 |
| 10 | 115.96–116.00 | 0.017–0.033 | 116.19–116.24 | 0.001–0.039 | 116.15–116.16 | 0.019–0.026 |
| 11 | 119.87–119.91 | 0.024–0.031 | 120.12–120.16 | 0.001–0.051 | 120.07–120.08 | 0.014–0.024 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| 12 | 123.91–123.96 | 0.027–0.033 | 124.17–124.22 | 0.007–0.052 | 124.13–124.15 | 0.026–0.040 |
| 13 | 127.72–127.79 | 0.022–0.038 | 128.01–128.06 | 0.021–0.043 | 127.96–127.98 | 0.030–0.041 |
| 14 | 131.64–131.70 | 0.021–0.038 | 131.94–132.00 | 0.019–0.050 | 131.91–131.93 | 0.031–0.041 |
| 15 | 135.62–135.69 | 0.024–0.040 | 135.95–136.00 | 0.033–0.046 | 135.90–135.93 | 0.023–0.036 |
| 16 | 139.62–139.69 | 0.031–0.037 | 139.95–140.01 | 0.001–0.041 | 139.92–139.94 | 0.023–0.038 |
| 17 | 143.71–143.78 | 0.018–0.043 | 144.08–144.14 | 0.010–0.044 | 144.04–144.08 | 0.029–0.040 |
| 18 | 147.81–147.88 | 0.028–0.039 | 148.19–148.24 | 0.020–0.042 | 148.14–148.18 | 0.033–0.039 |
| 19 | 151.93–152.01 | 0.024–0.038 | 152.31–152.37 | 0.018–0.048 | 152.27–152.30 | 0.031–0.042 |
| **D19S433** | | | | | | |
| 6 | 172.80–172.89 | 0.025–0.039 | 173.29–173.39 | 0.013–0.043 | 173.28–173.33 | 0.028–0.034 |
| 7 | 176.77–176.85 | 0.030–0.038 | 177.25–177.35 | 0.003–0.047 | 177.25–177.30 | 0.035–0.042 |
| 8 | 180.72–180.82 | 0.028–0.042 | 181.25–181.35 | 0.004–0.047 | 181.25–181.30 | 0.023–0.040 |
| 9 | 184.79–184.92 | 0.019–0.043 | 185.37–185.47 | 0.013–0.045 | 185.37–185.44 | 0.031–0.040 |
| 10 | 188.80–188.92 | 0.028–0.043 | 189.42–189.51 | 0.021–0.042 | 189.40–189.47 | 0.035–0.042 |
| 11 | 192.70–192.81 | 0.023–0.047 | 193.33–193.42 | 0.010–0.053 | 193.31–193.39 | 0.028–0.045 |
| 12 | 196.67–196.79 | 0.033–0.044 | 197.32–197.44 | 0.007–0.045 | 197.32–197.40 | 0.029–0.046 |
| 12.2 | 198.81–198.94 | 0.025–0.046 | 199.44–199.56 | 0.001–0.046 | 199.46–199.53 | 0.038–0.048 |
| 13 | 200.64–200.75 | 0.035–0.043 | 201.28–201.40 | 0.005–0.043 | 201.28–201.36 | 0.028–0.048 |
| 13.2 | 202.61–202.71 | 0.030–0.045 | 203.26–203.36 | 0.010–0.045 | 203.26–203.33 | 0.027–0.043 |
| 14 | 204.53–204.64 | 0.033–0.045 | 205.19–205.29 | 0.016–0.052 | 205.19–205.26 | 0.037–0.040 |
| 14.2 | 206.51–206.62 | 0.023–0.047 | 207.17–207.27 | 0.019–0.052 | 207.16–207.23 | 0.029–0.043 |
| 15 | 208.44–208.54 | 0.031–0.048 | 209.11–209.21 | 0.008–0.050 | 209.10–209.18 | 0.028–0.046 |
| 15.2 | 210.41–210.50 | 0.029–0.050 | 211.08–211.19 | 0.009–0.050 | 211.09–211.16 | 0.020–0.044 |
| 16 | 212.36–212.45 | 0.027–0.051 | 213.03–213.13 | 0.003–0.048 | 213.03–213.11 | 0.037–0.039 |
| 16.2 | 214.34–214.43 | 0.032–0.047 | 215.03–215.14 | 0.031–0.048 | 215.03–215.11 | 0.032–0.045 |
| 17 | 216.42–216.52 | 0.033–0.048 | 217.12–217.25 | 0.023–0.046 | 217.15–217.23 | 0.029–0.044 |
| 17.2 | 218.34–218.44 | 0.027–0.049 | 219.05–219.19 | 0.003–0.062 | 219.07–219.15 | 0.035–0.048 |
| 18.2 | 222.38–222.48 | 0.031–0.044 | 223.12–223.25 | 0.017–0.054 | 223.14–223.22 | 0.036–0.055 |
| 19.2 | 226.31–226.42 | 0.036–0.057 | 227.06–227.22 | 0.015–0.061 | 227.11–227.19 | 0.034–0.052 |

| Allele | 3130*xl* | | 3500 | | 3500xL | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| **D22S1045** | | | | | | |
| 7 | 245.00–245.10 | 0.024–0.044 | 245.61–245.70 | 0.003–0.051 | 245.59–245.66 | 0.031–0.041 |
| 8 | 248.05–248.14 | 0.026–0.041 | 248.65–248.74 | 0.007–0.053 | 248.63–248.69 | 0.039–0.045 |
| 9 | 251.04–251.15 | 0.025–0.042 | 251.63–251.71 | 0.035–0.046 | 251.61–251.67 | 0.022–0.034 |
| 10 | 253.99–254.08 | 0.021–0.035 | 254.56–254.66 | 0.014–0.044 | 254.55–254.61 | 0.032–0.040 |
| 11 | 256.91–257.00 | 0.031–0.047 | 257.50–257.58 | 0.001–0.043 | 257.47–257.53 | 0.030–0.035 |
| 12 | 259.81–259.90 | 0.034–0.046 | 260.43–260.52 | 0.031–0.050 | 260.40–260.47 | 0.039–0.044 |
| 13 | 262.79–262.89 | 0.031–0.057 | 263.39–263.51 | 0.026–0.046 | 263.38–263.45 | 0.031–0.039 |
| 14 | 265.76–265.87 | 0.031–0.059 | 266.41–266.49 | 0.013–0.040 | 266.36–266.44 | 0.030–0.049 |
| 15 | 268.74–268.85 | 0.032–0.044 | 269.36–269.47 | 0.020–0.059 | 269.35–269.42 | 0.027–0.034 |
| 16 | 271.74–271.85 | 0.029–0.040 | 272.36–272.46 | 0.030–0.046 | 272.33–272.41 | 0.032–0.037 |
| 17 | 274.73–274.83 | 0.028–0.048 | 275.35–275.45 | 0.012–0.056 | 275.33–275.40 | 0.033–0.042 |
| 18 | 277.73–277.83 | 0.026–0.043 | 278.36–278.47 | 0.030–0.050 | 278.33–278.41 | 0.034–0.043 |
| 19 | 280.71–280.81 | 0.015–0.049 | 281.34–281.45 | 0.029–0.049 | 281.31–281.39 | 0.033–0.042 |
| 20 | 283.70–283.78 | 0.027–0.037 | 284.32–284.42 | 0.001–0.048 | 284.28–284.37 | 0.026–0.048 |
| **D10S1248** | | | | | | |
| 8 | 303.34–303.37 | 0.021–0.039 | 303.46–303.49 | 0.012–0.049 | 303.42–303.45 | 0.031–0.040 |
| 9 | 307.25–307.29 | 0.021–0.036 | 307.39–307.43 | 0.010–0.049 | 307.33–307.37 | 0.031–0.042 |
| 10 | 311.24–311.27 | 0.027–0.039 | 311.37–311.40 | 0.005–0.046 | 311.30–311.35 | 0.030–0.045 |
| 11 | 315.30–315.33 | 0.024–0.042 | 315.42–315.46 | 0.024–0.050 | 315.38–315.41 | 0.034–0.048 |
| 12 | 319.51–319.53 | 0.022–0.040 | 319.60–319.64 | 0.035–0.053 | 319.58–319.6 | 0.019–0.044 |
| 13 | 323.62–323.63 | 0.021–0.042 | 323.67–323.72 | 0.007–0.064 | 323.65–323.69 | 0.030–0.045 |
| 14 | 327.67–327.68 | 0.028–0.039 | 327.73–327.76 | 0.016–0.053 | 327.70–327.73 | 0.033–0.042 |
| 15 | 331.70–331.71 | 0.024–0.037 | 331.74–331.80 | 0.023–0.048 | 331.72–331.76 | 0.030–0.043 |
| 16 | 335.70–335.72 | 0.022–0.039 | 335.76–335.79 | 0.012–0.049 | 335.74–335.76 | 0.030–0.046 |
| 17 | 339.68–339.69 | 0.019–0.032 | 339.73–339.78 | 0.041–0.053 | 339.71–339.73 | 0.031–0.038 |
| 18 | 343.71–343.74 | 0.023–0.038 | 343.76–343.8 | 0.011–0.048 | 343.74–343.77 | 0.030–0.036 |
| 19 | 347.76–347.78 | 0.012–0.035 | 347.80–347.85 | 0.014–0.049 | 347.78–347.81 | 0.032–0.038 |

# Extra peaks in the electropherogram

## Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram. Causes for the appearance of extra peaks include stutter products, incomplete 3′ A nucleotide addition (at the n-1 position), dye artifacts, and mixed DNA samples (see DNA Advisory Board (DAB) Standard 8.1.2.2).

## Extra peaks: Stutter

### Stutter definition

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller than the target STR allele product (minus stutter), or less frequently, one repeat larger (plus stutter) (Butler, 2005; Mulero *et al*., 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the minus stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al*., 1996). Although plus-stutter is normally much less significant than minus-stutter in STR loci with tetranucleotide repeats, the incidence of plus-stutter may be more significant in trinucleotide repeat-containing loci.

Contact HID Support for more information on plus stutter.

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak.

### Stutter observations

Plus-stutter was regularly observed and was more significant in trinucleotide repeat-containing loci (Figure 25 on page 83).

Peak heights were measured for amplified samples at the loci that are used in the kit. All data were generated on the 3500xL Genetic Analyzer. Some conclusions from these measurements and observations are:

- For each locus, the stutter percentage generally increases with allele length.
- Each allele within a locus displays a relatively consistent average stutter percentage.
- Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered.
- The measurement of stutter percentage for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.
- Stutter can be elevated when minus stutter and plus stutter overlap. This is typically observed when a given allele flanks another allele that is 2 repeat units away (as seen with the FGA locus in control 007 DNA).
- The magnitude and/or variability of stutter may increase with low DNA input amounts.

Figure 20 through Figure 24 show the stutter observed in the population study that are one repeat unit away from the alleles recorded. All data were generated on the 3500xL Genetic Analyzer.

The stutter filter settings that are derived from this data are listed in "Stutter percentage filter settings provided with GeneMapper™ ID-X Software" on page 83.

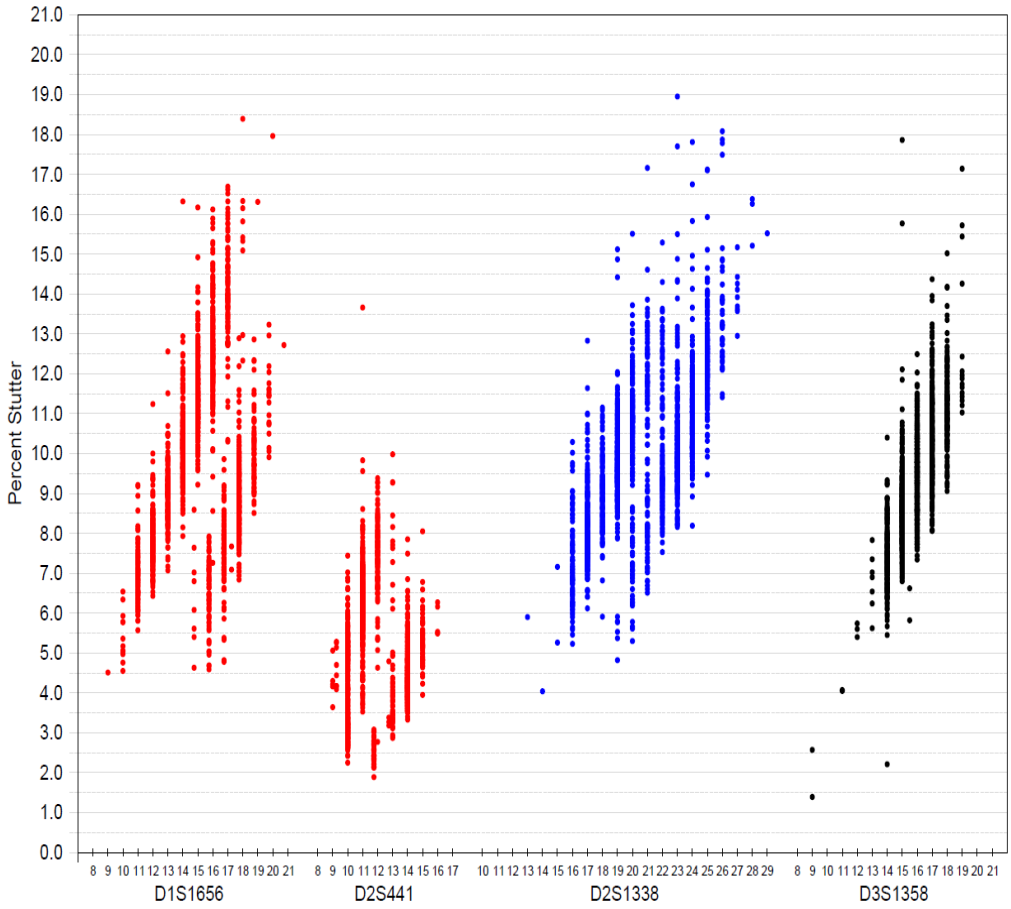



**Figure 20** Stutter percentages for D1S1656, D2S441, D2S1338, and D3S1358 loci (Blue=FAM™ dye, black=TED™ dye, red=TAZ™ dye)

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

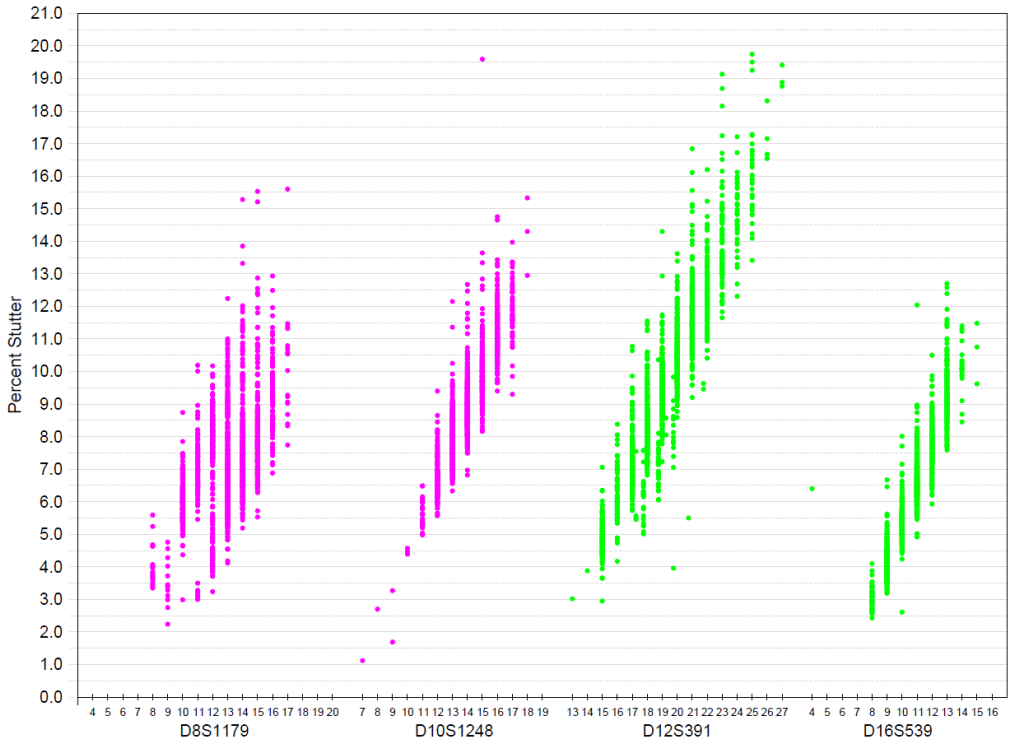



**Figure 21** Stutter percentages for D8S1179, D10S1248, D12S391, and D16S539 (Green=VIC™ dye and purple=SID™ dye)

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

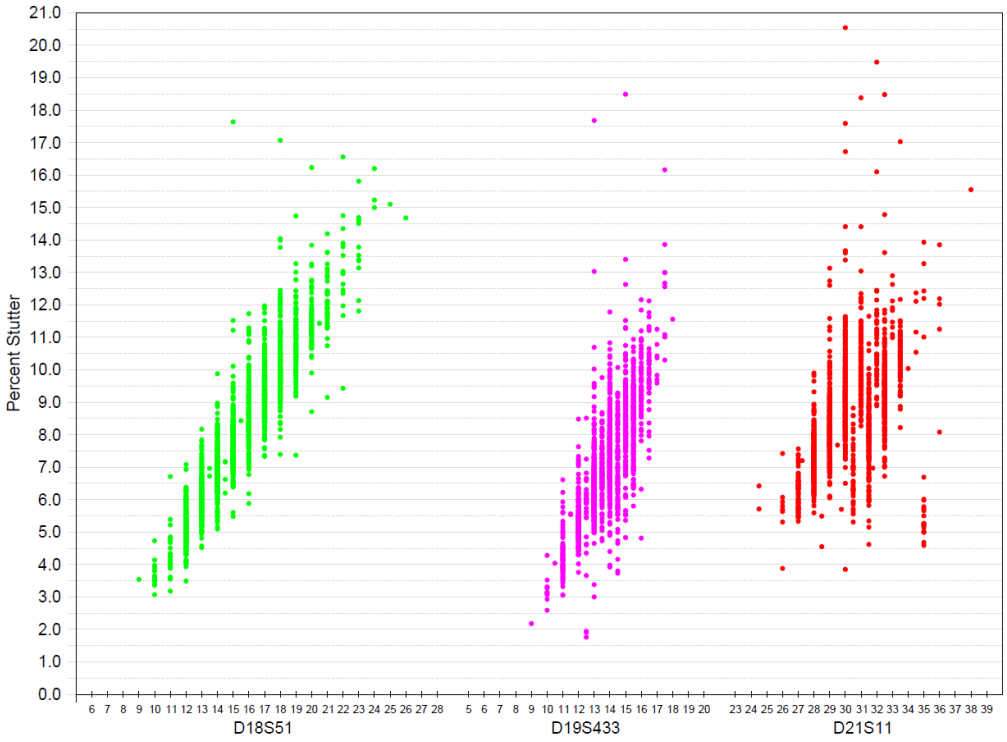



**Figure 22** Stutter percentages for D18S51, D19S433, and D21S11 loci (Green=VIC™ dye, red=TAZ™ dye, purple=SID™ dye)

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

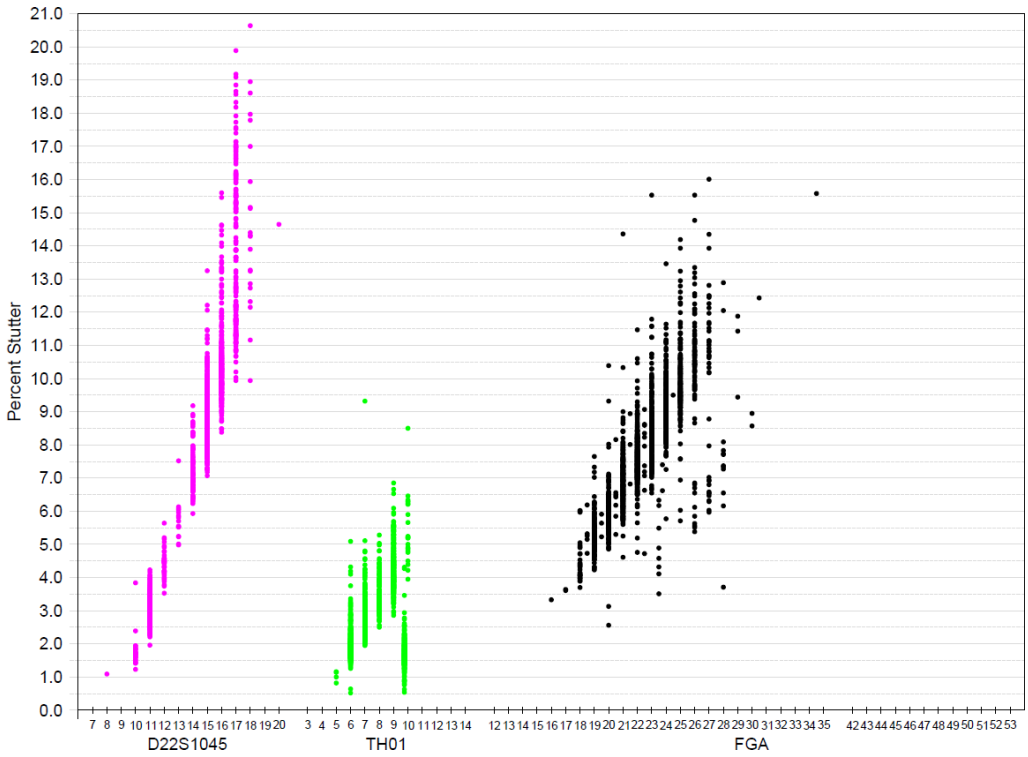



**Figure 23** Stutter percentages for D22S1045, TH01, and FGA loci (Purple=SID™ dye, Green=VIC™ dye, black=TED™ dye)

**Note:** Except for the TH01 marker data, the data in this figure are from the original formulation of the NGM Detect™ kit.

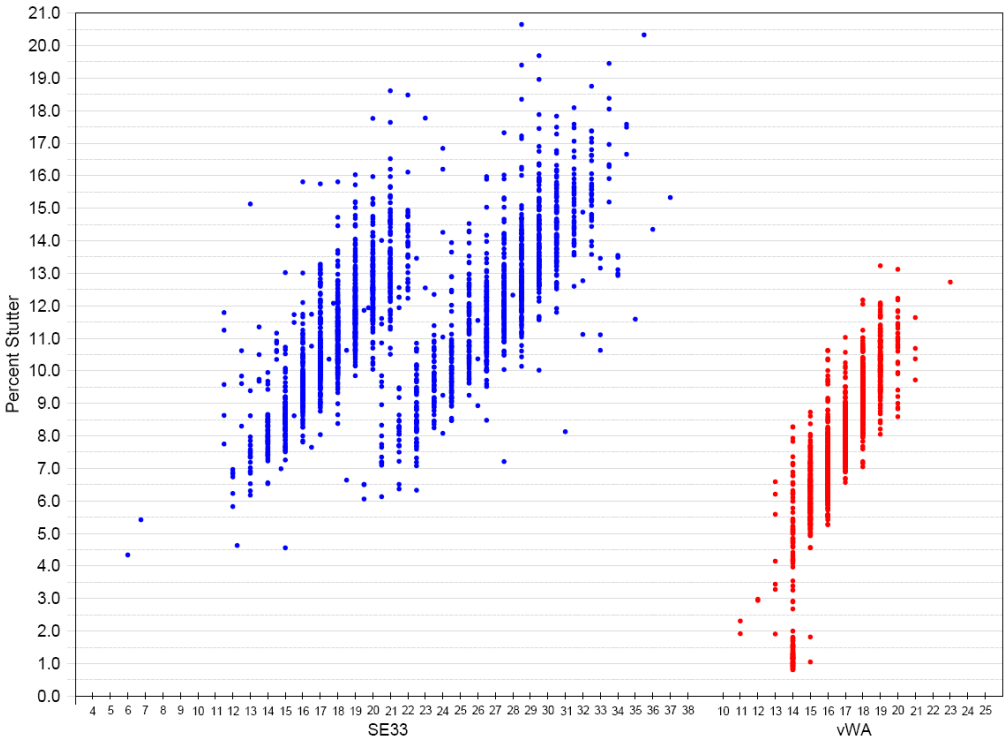



**Figure 24** Stutter percentages for SE33 and vWA loci (Blue=FAM™ dye, red=TAZ™ dye)

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

### Non-standard stutter peaks at the D22S1045 and SE33 loci

The D22S1045 locus in the NGM Detect™ kit is a trinucleotide repeat locus, and shows an elevated level of plus stutter (Figure 25). Other loci, such as THO1 and D2S1338, may also exhibit relatively elevated plus stutter.

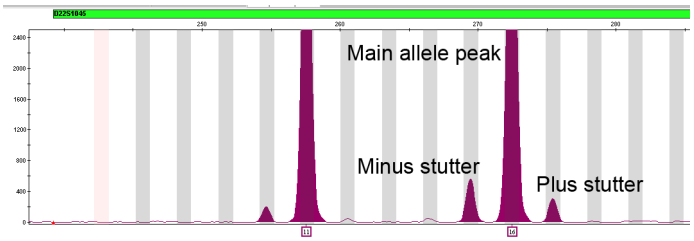



**Figure 25** NGM Detect™ kit electropherogram showing minus and plus stutter associated with the D22S1045 STR locus. Data produced on a 3500xL Genetic Analyzer.

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

STR loci such as D1S1656 and SE33 (Figure 26) contain more complex nucleotide sequences including regions of dinucleotide repeats which can yield additional stutter peaks. If these stutter peaks exceed the peak amplitude threshold (typically 175 RFU), they may be detected as additional alleles in the profile. The stutter file that is provided with the GeneMapper™ *ID-X* Software for analysis of NGM Detect™ kit data contains a minus 2–nt stutter filter for SE33 and D1S1656, as well as filters for commonly observed plus stutter, to prevent these peaks from being called in normal profiles.

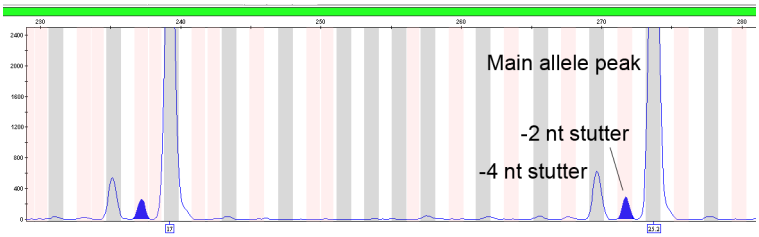



**Figure 26** Example of a –2-nt reproducible stutter artifact at the SE33 locus. Data produced on a 3500xL Genetic Analyzer.

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

### Stutter percentage filter settings provided with GeneMapper™ *ID-X* Software

The settings in Table 5 and Table 6 were derived using the data that is shown in "Stutter observations" on page 77. The proportion of the stutter product relative to the main allele (stutter percent) is measured by dividing the height of the stutter peak by the height of the main allele peak.

Analysis showed that observed stutter data points were not normally distributed. As such, at each locus a best-fit, non-parametric statistical model was applied to the data and a threshold filter level that emulated historical stutter filter levels (approximately 99.7%) was derived.

**IMPORTANT!** The values that are shown in the table are the values that were determined during developmental validation studies using specific data sets. To determine the appropriate values to use for your applications, always perform internal validation studies.

**Table 5** Minus stutter percentage filter settings provided with the GeneMapper™ *ID-X* Software

**Note:** The data in this table are from the original validation study of the NGM Detect™ kit. Based on the validation study with the updated kit formulation, no changes to the stutter filter values were required.

| Locus [1] | % Stutter |
|---|---|
| D2S1338 | 17.61 |
| SE33 | 18.63 |
| SE33 (–2 nt) | 6.41 |
| D16S539 | 11.97 |
| D18S51 | 16.15 |
| TH01 | 7.66 |
| D12S391 | 18.99 |
| D3S1358 | 15.42 |
| FGA | 14.70 |
| vWA | 12.51 |
| D21S11 | 16.88 |
| D1S1656 | 16.68 |
| D1S1656 (–2 nt) | 3.90 |
| D2S441 | 9.55 |
| D8S1179 | 13.80 |
| D19S433 | 13.57 |
| D22S1045 | 19.02 |
| D10S1248 | 14.32 |

[1] These percentages are used as stutter filters in NGM_Detect_Stutter.txt

**Table 6** Plus stutter percentage filter settings provided with the GeneMapper™ *ID-X* Software

**Note:** The data in this table are from the original validation study of the NGM Detect™ kit. Based on the validation study with the updated kit formulation, no changes to the stutter filter values were required.

| Locus [1] | % Stutter |
|---|---|
| D2S1338 | 11.55 |
| SE33 | 9.80 |
| D16S539 | 4.68 |
| D18S51 | 8.19 |
| TH01 | 6.14 |
| D12S391 | 7.76 |
| D3S1358 | 4.36 |
| FGA | 9.16 |
| vWA | 7.59 |
| D21S11 | 9.14 |
| D1S1656 | 5.47 |
| D2S441 | 4.92 |
| D8S1179 | 5.39 |
| D19S433 | 6.91 |
| D22S1045 | 8.22 |
| D10S1248 | 2.60 |

[1] These percentages are used as stutter filters in NGM_Detect_Stutter.txt

## Extra peaks: Addition of 3' A nucleotide

### 3′ A nucleotide addition definition

Many DNA polymerases can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*, 1996). This nontemplate addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

### 3′ A observations

The efficiency of +A addition is related to the particular sequence of the DNA at the 3′ end of the PCR product.

The NGM Detect™ kit includes two main design features that promote maximum +A addition:

- The primer sequences have been optimized to encourage +A addition.
- The PCR chemistry allows complete +A addition with a short final incubation at 60°C for 5 minutes.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Figure 27 shows examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended time can result in double +A addition, in which two nontemplate adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks.

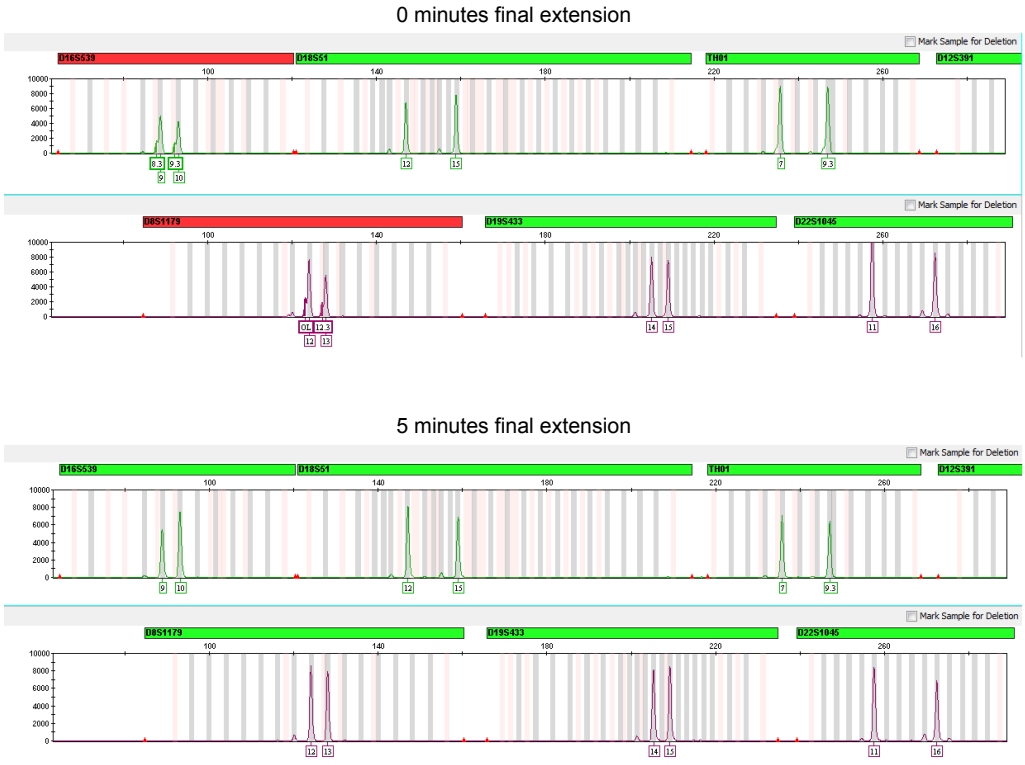



**Figure 27** Omitting the final extension step results in shoulders on main allele peaks due to incomplete +A nucleotide addition. Examples shown are the smaller amplicons of VIC™ and SID™ dye channel data from a 3500xL Genetic Analyzer using the NGM Detect™ kit.

If the amount of input DNA is greater than recommended concentration, "shouldering" of allele peaks can be observed. Amplification of excess input DNA can also result in off-scale data and lowered IQCL peak heights. In this situation, the IQCL may also exhibit some shouldering due to incomplete +A nucleotide addition.

## Extra peaks: Artifacts

### Artifact definition

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible. Anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise).

### Dye artifact observation

Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the NGM Detect™ kit. Internal population studies show that kit electropherograms are free of reproducible dye artifacts in the kit read region of 64–458 nt. Two exceptions are as follows:

- A low level 113–117 nt dye artifact in the VIC™ dye channel that has been detected below commonly used analytical thresholds.
- A low level ~66 nt dye artifact in the TED™ dye channel. This artifact was approximately 40–80 RFU in our studies. The peak height observed may vary depending on the sensitivity of individual CE instruments.

Figure 28 shows the low baseline-level fluorescence that is observed in a typical negative control PCR. However, it is important to consider noise and other amplification-related artifacts when interpreting data.

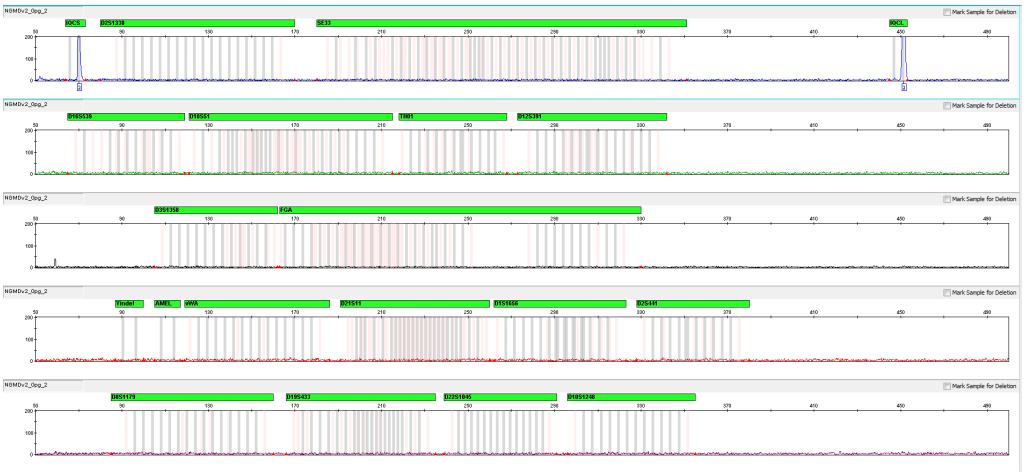


**Figure 28** Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0 to 200 RFU).

Some small PCR artifacts were occasionally observed with the human male 007 DNA provided as the control DNA in the NGM Detect™ kit. The most prominent and consistent artifacts were:

- A 170-bp artifact in the SID™ channel
- Two artifacts consistent with the size of a –2 and –3 repeat unit stutter for allele 14 of D2S441

In all cases, the peak heights of the artifacts were typically 1% or less than peak heights of the nearest true allele. If higher amounts of DNA (that is, 1 ng or more) are amplified, or if a lower PAT is used, then the peak heights could theoretically exceed the analytical threshold. The peak height of the artifacts appeared to be proportional to the amount of input 007 DNA in the PCR.

# Characterization of loci

## SWGDAM guideline 3.1

"The basic characteristics of a genetic marker should be determined and documented." (SWGDAM, December 2016)

## Loci in this kit

This section describes basic characteristics of the 16 autosomal STR loci, Y indel locus, and sex-determining marker (Amelogenin), that are amplified with the NGM Detect™ kit. Most of these loci have been extensively characterized by other laboratories.

## Nature of polymorphisms

The primers for the Amelogenin locus flank a 6-nucleotide deletion in intron 1 of the X homolog. Amplification generates 108-nt and 114-nt products from the X and Y chromosomes, respectively. The primers for the Y indel flank a region in the q arm of the Y chromosome (Yq11.221). Depending on the haplotype of the sample, the amplification generates either a 91-nt or a 96-nt product. (Sizes are the actual nucleotide size according to sequencing results, including 3′ A nucleotide addition, and size may not correspond exactly to allele mobility observed on capillary electrophoresis platforms.) Except for D22S1045, a trinucleotide STR, the remaining loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus are caused by differences in the number of repeat units.

We have sequenced all the alleles in the NGM Detect™ kit Allelic Ladder, including microvariants. In addition, other groups in the scientific community have sequenced alleles at some of these loci (Nakahori *et al*., 1991; Puers *et al*., 1993; Möller *et al*., 1994; Barber *et al*., 1995; Möller and Brinkmann, 1995; Barber *et al*., 1996; Barber and Parkin, 1996; Brinkmann *et al*., 1998; Momhinweg *et al*., 1998; Watson *et al*., 1998). Among the various sources of sequence data on the loci, there is consensus on the repeat patterns and structure of the STRs.

## Inheritance

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich *et al*., 1992).

## Mapping

The NGM Detect™ kit loci have been mapped, and the chromosomal locations have been published (Nakahori *et al*., 1991; Edwards *et al*., 1992; Kimpton *et al*., 1992; Mills *et al*., 1992; Sharma and Litt, 1992; Li *et al*., 1993; Straub *et al*., 1993; Barber and Parkin, 1996; and Lareu, *et al*., 1996).

## Genetic linkage

Two sets of STR loci in the NGM Detect™ kit are located on the same chromosomes. vWA and D12S391 are located approximately 6.3 million bp apart on the p arm of chromosome 12; D2S1338 and D2S441 are located approximately 150 million bp apart on opposite arms of chromosome 2. Linkage disequilibrium analysis was conducted on the genotype results from 1,034 individuals of three ethnic groups (350 African American, 349 Caucasian, and 335 Hispanic). STR locus genotype results from the population study were analyzed using the Linkage Disequilibrium module of GenePop software version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). See Table 7 for results.

The relatively high probability values indicate that there is no statistically significant linkage disequilibrium found between the pairs of loci that are located on the same chromosome.

An independent analysis of data from the same collection of population samples (Budowle, *et al*., 2010) also concluded that the 15 STR loci that are shared between the NGM™ and NGM SElect™ kits were independent at the population level (note that the SE33 locus was not part of this analysis). Therefore, to calculate the rarity of a profile for comparison to single-source and mixture samples, the frequencies of all loci including vWA and D12S391 could be multiplied. However, the analysis of the CEPH pedigree families demonstrated a degree of linkage between vWA and D12S391 that does not support the assumption of independence for kinship analysis.

**Table 7** GenePop software LD Result (p-value for pairwise analysis of loci)

| Locus | Chromosome map position[1] | Chromosome Nuclear Coordinates[1] (million bp) | African-American (n = 350) | Caucasian (n = 350) | Hispanic (n = 293) |
|---|---|---|---|---|---|
| vWA | 12p13.31 | 5.9 | 0.86 | 0.29 | 0.27 |
| D12S391 | 12p13.2 | 12.2 | | | |
| D2S441 | 2p14 | 68 | 0.11 | 0.32 | 0.19 |
| D2S1338 | 2q35 | 218 | | | |

[1] STR locus mapping data was obtained from the NCBI Map Viewer **http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9606** or the UCSC Genome Browser (**http://genome.ucsc.edu/**). GenePop LD analysis probability results (p values) greater than 0.05 were considered to indicate that linkage disequilibrium between the loci within the population tested was not statistically significant.

# Species specificity

## SWGDAM Guideline 3.2

"The ability to detect genetic information from non-targeted species (e.g., detection of microbial DNA in a human assay) should be determined. The detection of genetic information from non-targeted species does not necessarily invalidate the use of the assay, but may help define the limits of the assay." (SWGDAM, December 2016)

## Nonhuman studies

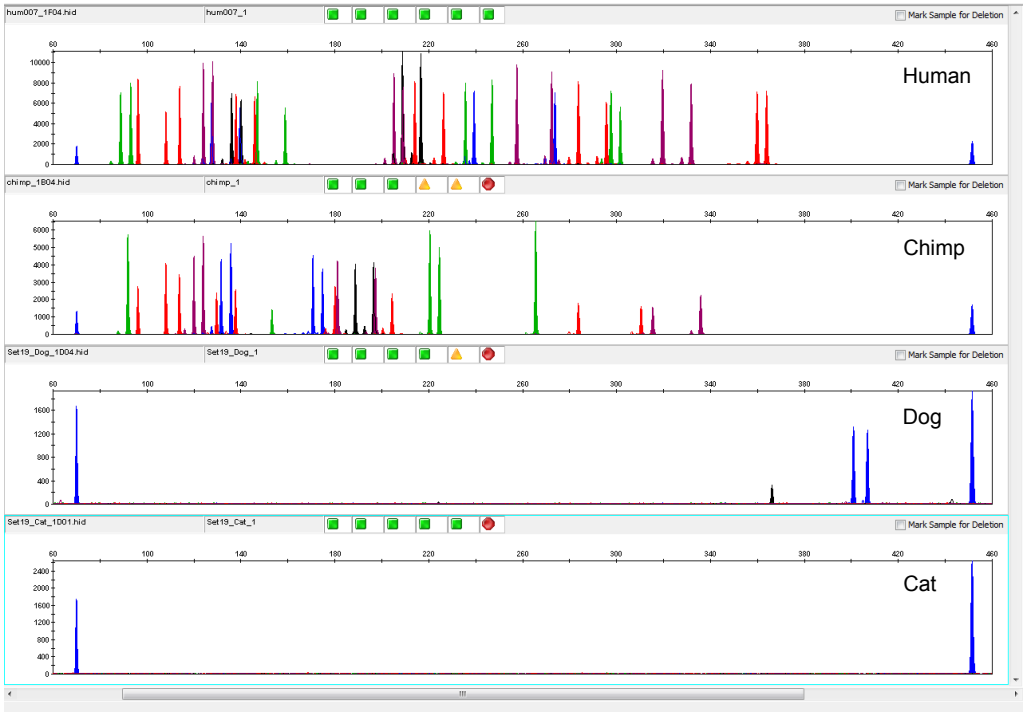
The NGM Detect™ kit provides the required specificity for detecting human alleles. Species specificity testing was performed to ensure that there is no cross-reactivity with nonhuman DNA that may be present in forensic casework samples.

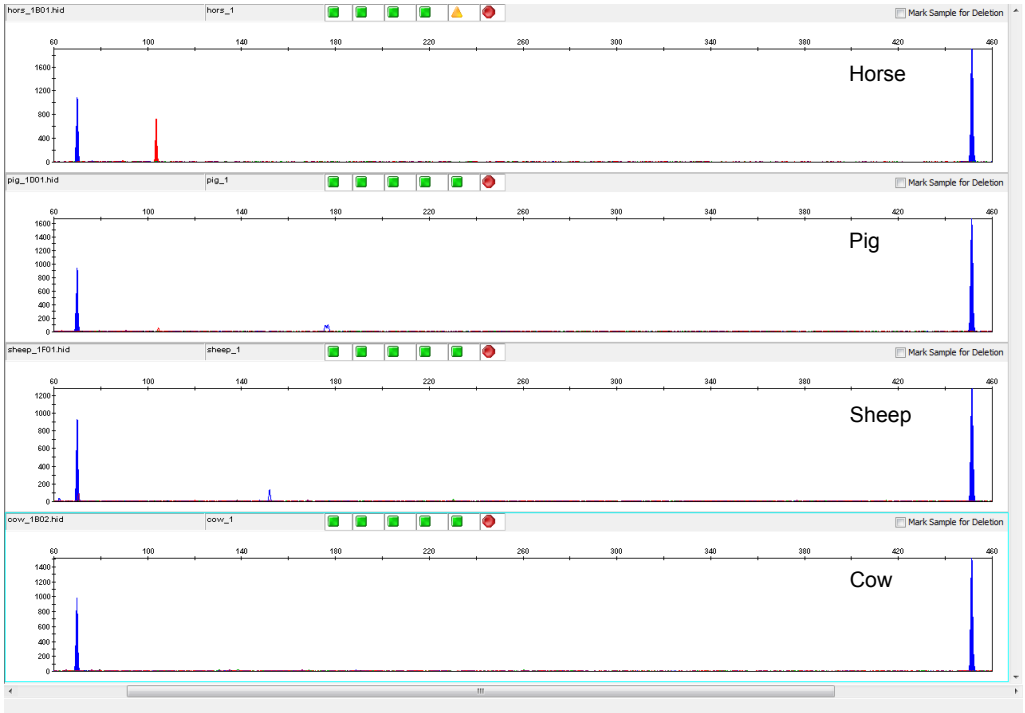
The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for the NGM Detect™ kit kit:

- Primates: gorilla, chimpanzee, orangutan, and cynomolgous (macaque) (0.5 ng each)
- Non-primates: mouse, dog, sheep, pig, rabbit, cat, horse, hamster, rat, chicken, and cow (5.0 ng each)
- Microorganisms: *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Lactobacillus rhamnosus* (pooled genomic DNAs, with approximately 50,000 copies of DNA from each species, per reaction)

Results were evaluated for the presence of any amplified peaks that would indicate cross reactivity of the NGM Detect™ kit with any of these non-human species.

Figure 29 shows select electropherogram results from the species specificity tests. The chimpanzee, gorilla (data not shown), orangutan (data not shown), and macaque (data not shown) DNA samples produced partial profiles in the 70–400 nucleotide region.



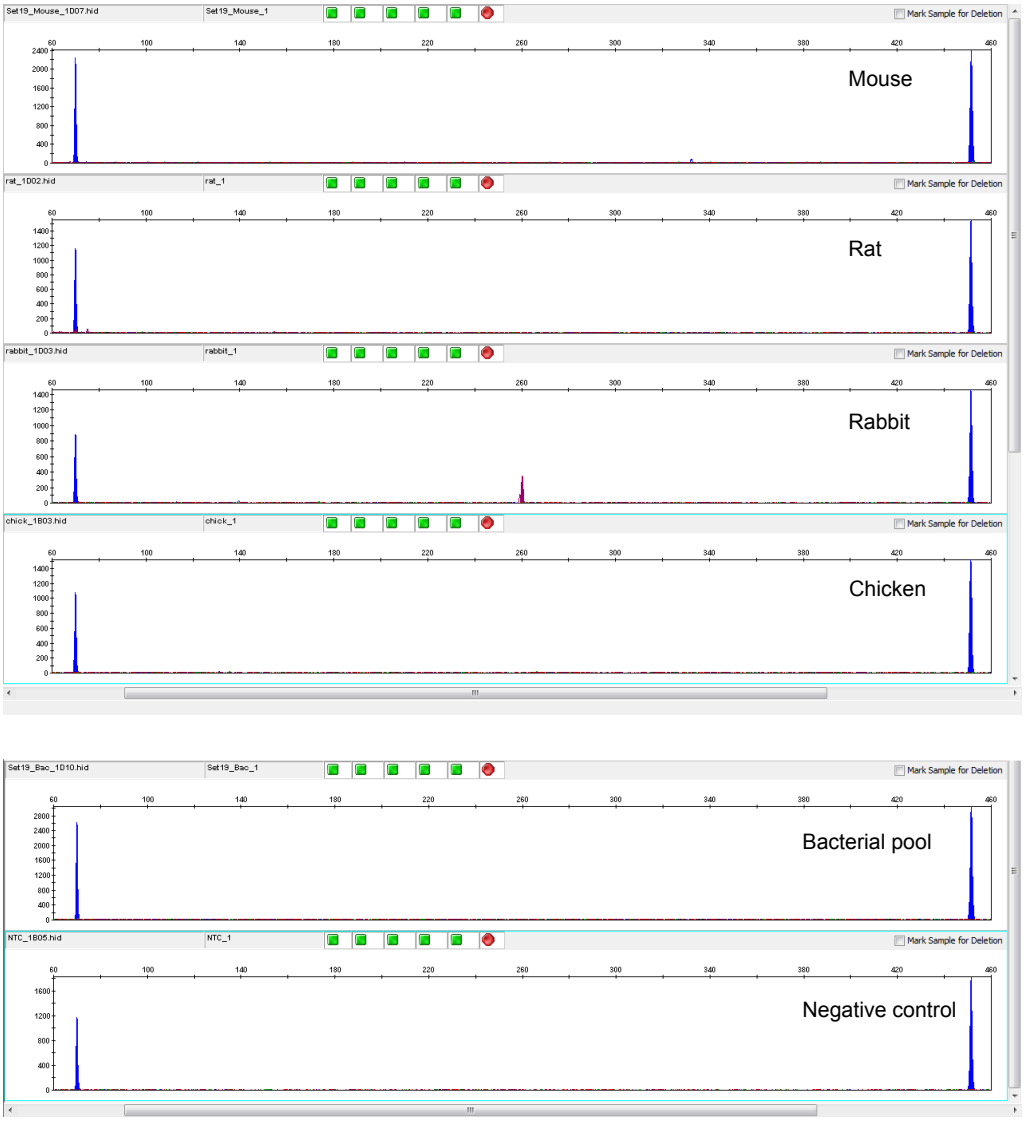



**Figure 29** Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer.

Table 8 shows the most significant cross-reactive peaks that were observed among non-human, non-primate, genomic DNAs (that is, peaks over a 175 RFU Peak Amplitude Threshold on the 3500xL Genetic Analyzer). Peaks were observed for dog, horse, and rabbit. Most peaks did not fall into human STR locus bins or marker ranges, and would therefore not be confused with human STR alleles. This data shows that the likelihood of obtaining an allelic profile consistent with that from a human sample, from non-primates or microorganisms, is low.

**Table 8** Observed cross-reactive peaks for non-human, non-primate animals.

| Species | Dye channel | Size | Peak height |
|---|---|---|---|
| Dog | B | 401 bp | 1,294 RFU |
| | | 407 bp | 1,046 RFU |
| | Y | 366 bp | 289 RFU |
| Horse | R | 104 bp | 915 RFU |
| Rabbit | P | 260 bp | 389 RFU |

# Sensitivity

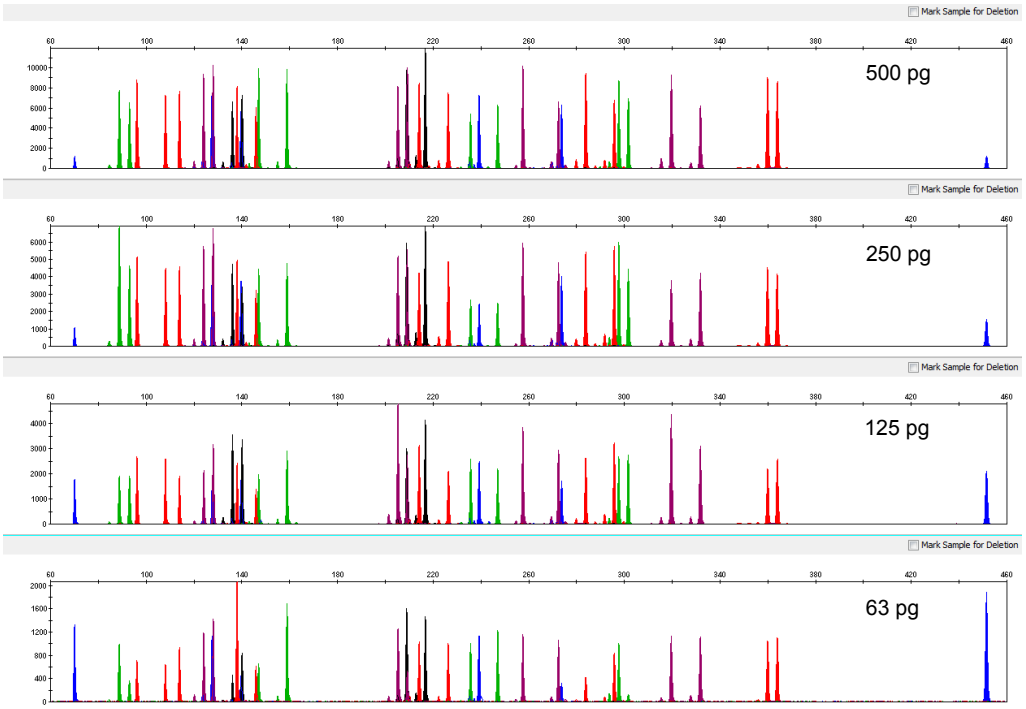
## SWGDAM guideline 3.3

"The ability to obtain reliable results from a range of DNA quantities, to include the upper and lower limits of the assay, should be evaluated." (SWGDAM, December 2016)

## Sensitivity observation

The recommended amount of input DNA for the NGM Detect™ kit is 0.5 ng for 30 cycles of amplification based on real-time PCR quantification, such as with the Quantifiler™ Trio DNA Quantification Kit or the Quantifiler™ HP DNA Quantification Kit. To determine the optimum input DNA, perform studies according to the quantification kit that you use.

If the sample contains degraded or inhibited DNA, amplification of a higher amount of DNA may be beneficial. In Figure 30, DNA Control 007 was serially diluted from 0.5–0.008 ng. Full profiles (35 PCR products) were consistently obtained at 0.125 ng, but occasional partial profiles resulted at lower concentrations.



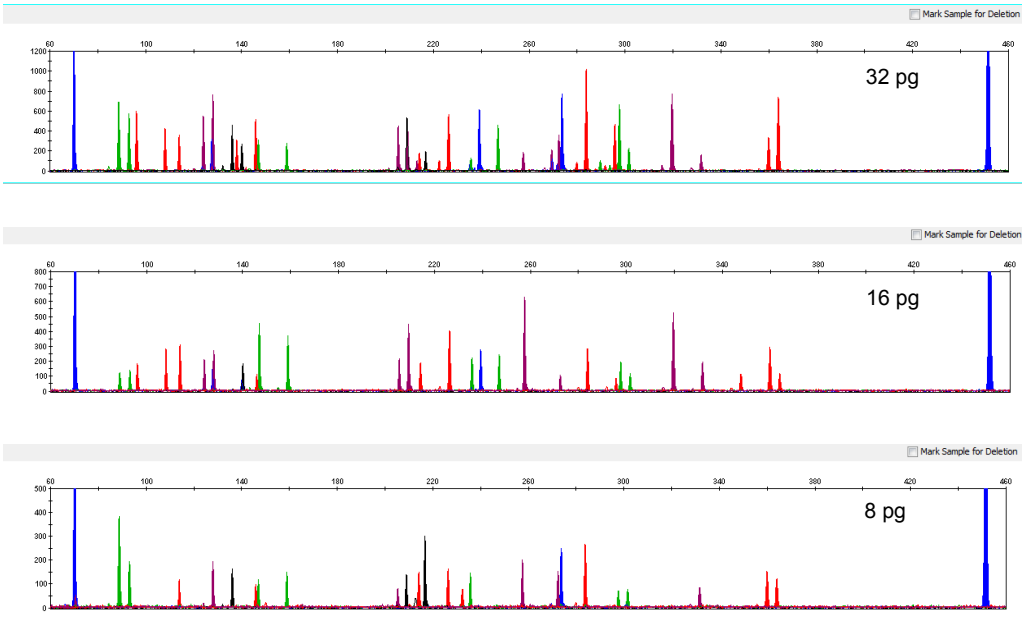



**Figure 30** Electropherograms for amplifications using 500, 250, 125, 63, 32, 16, and 8 pg of DNA Control 007. Electrophoresis was performed on a 3500xL Genetic Analyzer. Note that as the DNA input is serially diluted by 2-fold, the Y-axis scale is also adjusted, as needed, to accommodate lower peak heights.

# Stability

## SWGDAM guideline 3.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults should be evaluated. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated to determine the effects of such factors." (SWGDAM, December 2016)

## Degraded DNA

As the average size of degraded DNA approaches the size of the target sequence, the amount of PCR product that is generated is reduced, because of the reduced number of intact templates in the size range required for amplification. Degraded DNA was prepared to examine the potential for differential amplification of loci. Approximately 10 µg of high molecular weight DNA was sonicated and subjected to digestion with 0.25 U/µL of DNase I for various lengths of time (from ~5–10 minutes) to produce a range (low, medium, and high) of degradation levels (Bender *et al.*, 2004). The DNA was examined by agarose gel analysis to determine the average size of the DNA fragments at each time point. Amplification of 0.5 ng of degraded DNA using the NGM Detect™ kit was performed. As the DNA became progressively degraded, the loci failed to amplify robustly in order of decreasing size. Preferential amplification was not observed.

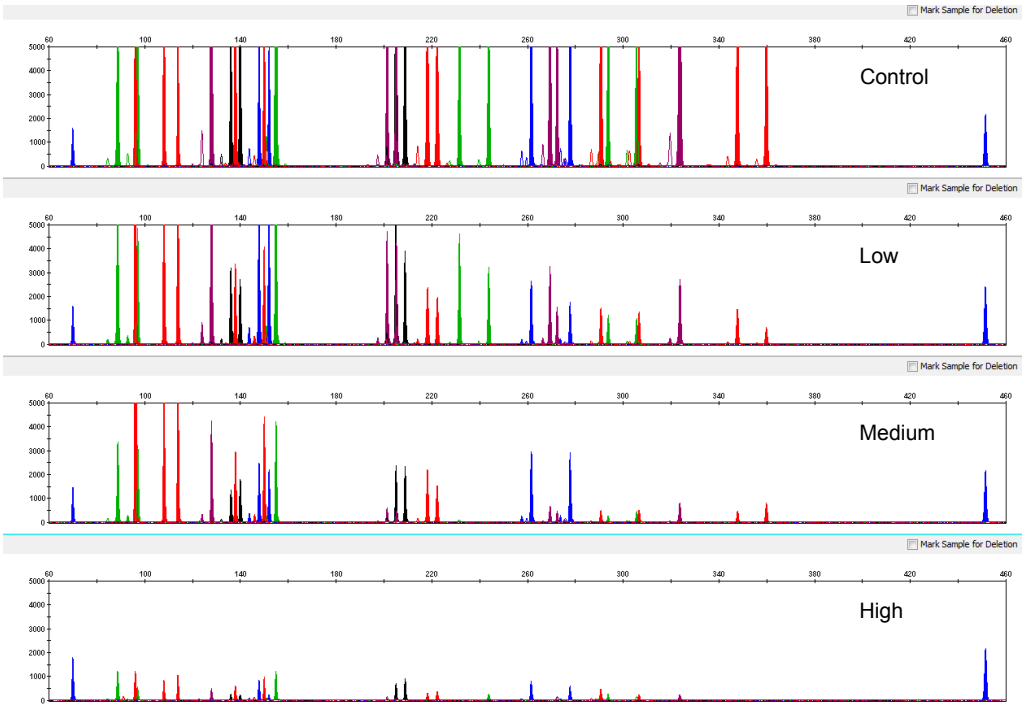



**Figure 31** Amplification of a single donor DNA sample sonicated and incubated with 0.25 U/µL of DNase I for various lengths of time (from ~5–10 minutes).

## Effect of inhibitors: hematin

Heme compounds have been identified as PCR inhibitors in DNA samples that are extracted from bloodstains (DeFranchis *et al*., 1988; Alkane *et al*., 1994). It is believed that the inhibitor is co-extracted and co-purified with the DNA and then interferes with PCR by inhibiting polymerase activity. To examine the effects of hematin on the performance of the NGM Detect™ kit, 0.5 ng of DNA Control 007 was amplified in the presence of increasing concentrations of hematin for 30 cycles of amplification (Figure 32). The final concentrations of hematin that is used in the reaction were 0, 450, 575, and 700 µM (Table 9).

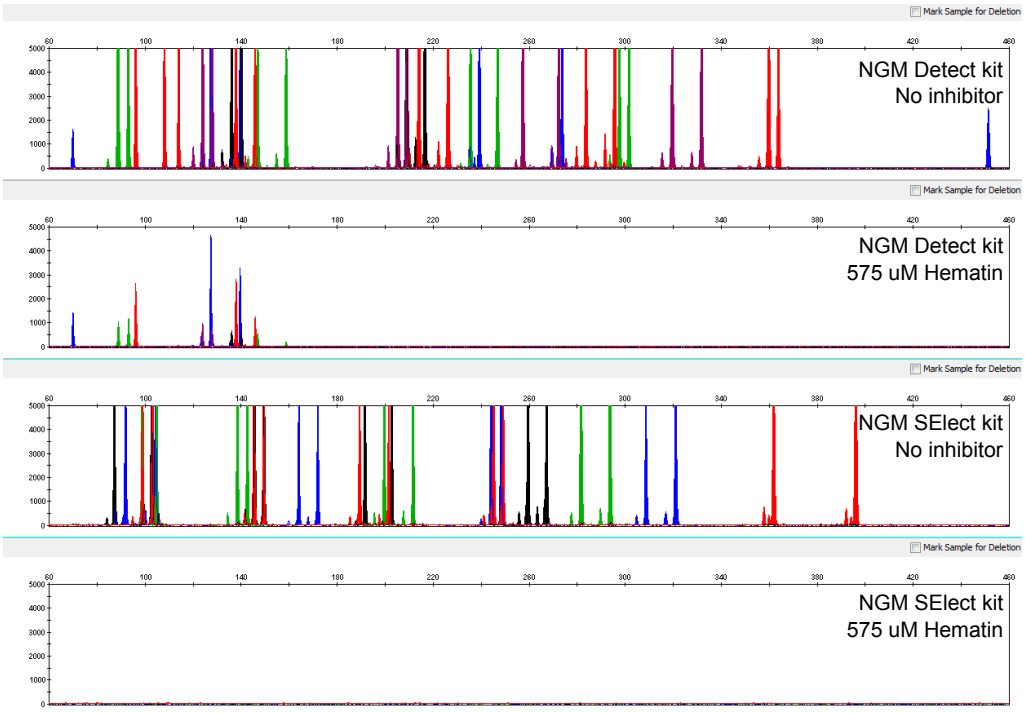



**Figure 32** Electropherograms for the NGM Detect™ kit and the NGM SElect™ kit show the improved performance of the NGM Detect™ kit in the presence of hematin compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the NGM Detect™ kit and the NGM SElect™ kit for 30 cycles of amplification.

**Table 9** NGM Detect™ kit performance in simulated hematin inhibition (N = 5).

| Hematin concentration | Average number of alleles detected[1] | Minimum, maximum |
|---|---|---|
| 0 µM | 35 | 35, 35 |
| 450 µM | 35 | 35, 35 |
| 575 µM | 12.4 | 12, 13 |
| 700 µM | 0 | 0, 0 |

[1] Only those peaks ≥175 RFU were counted. A complete profile with DNA Control 007 yields 35 peaks using the NGM Detect™ kit.

## Effect of inhibitors: humic acid

Traces of humic acid may inhibit the PCR amplification of DNA evidence that is collected from soil. Amplification of 0.5 ng of DNA Control 007 in the presence of increasing amounts of humic acid was performed using the NGM Detect™ kit for 30 cycles of amplification (Figure 33). The final concentrations of humic acid tested in the reactions were 0, 140, 250 and 400 ng/µL (Table 10).

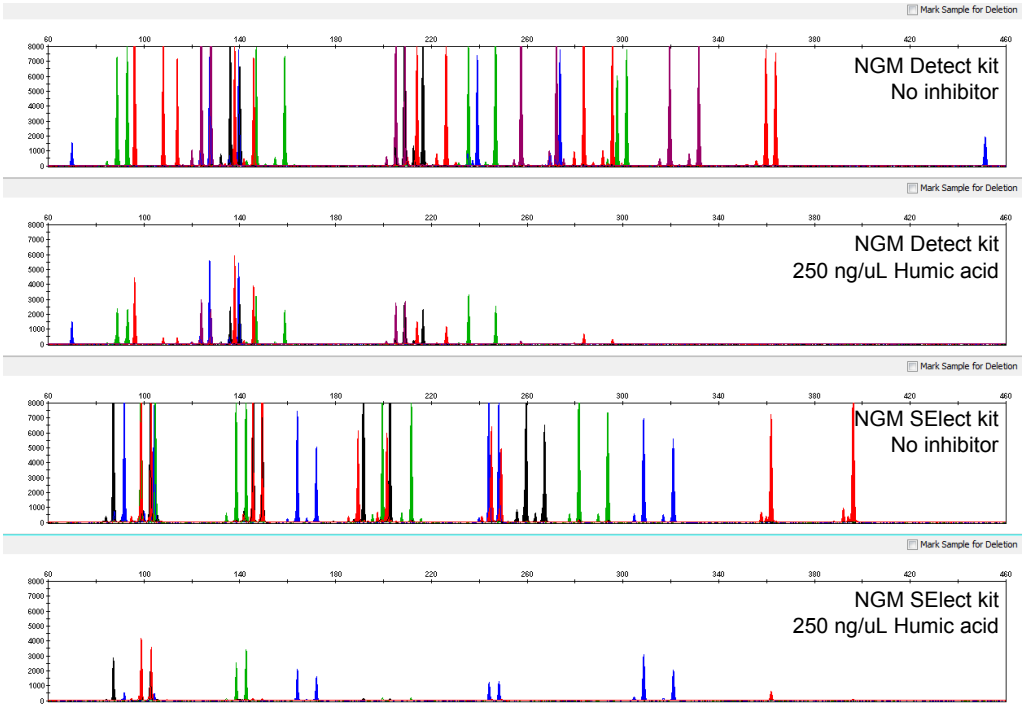



**Figure 33** Electropherograms for the NGM Detect™ kit and the NGM SElect™ kit show the improved performance of the NGM Detect™ kit in the presence of humic acid compared with previous AmpFℓSTR™ kits. The same set of inhibited samples was analyzed with the NGM Detect™ kit and the NGM SElect™ kit for 30 cycles of amplification.

**Table 10** NGM Detect™ kit performance in simulated humic inhibition (N = 5).

| Humic acid concentration | Number of alleles detected[1] | Minimum, maximum |
|---|---|---|
| 0 ng/µL | 35 | 35, 35 |
| 140 ng/µL | 35 | 35, 35 |
| 250 ng/µL | 26 | 26, 26 |
| 400 ng/µL | 1 | 1, 1 |

[1] Only those peaks ≥175 RFU were counted. A complete profile with DNA Control 007 yields 35 peaks using the NGM Detect™ kit.

# Mixture studies

## SWGDAM guideline 3.8

"The ability to obtain reliable results from mixed source samples should be determined." (SWGDAM, December 2016)

## Mixture study overview

Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting the results. Perform studies to determine a minimum peak height threshold to avoid typing when stochastic effects are likely to interfere with accurate interpretation of mixtures.

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an important category of forensic casework.

It is essential to ensure that the DNA typing system is able to detect DNA mixtures. Typically, mixed samples can be distinguished from single-source samples by:

- The presence of more than two alleles at one or more loci
- The presence of a peak at a stutter position that is significantly greater in percentage than typically observed in a single-source sample
- Significantly imbalanced alleles for a heterozygous genotype

The peak height ratio is defined as the height of the lower peak (in RFU) divided by the height of the higher peak (in RFU), expressed as a percentage.

If an unusually low peak height ratio is observed for one locus, and there are no other indications that the sample is a mixture, reamplify and reanalyze the sample to determine if the imbalance is reproducible. Possible causes of imbalance at a locus are:

- Degraded DNA
- Presence of inhibitors
- Extremely low amounts of input DNA
- A mutation in one of the primer binding sites
- Presence of an allele containing a rare sequence that does not amplify as efficiently as the other allele

## Mixture study observation

Median, minimum, and maximum peak height ratios observed for alleles in the NGM Detect™ kit loci in unmixed human population database samples (N=60) are shown in Figure 34. The donor samples that were used are listed in "Population samples used in these studies" on page 102.

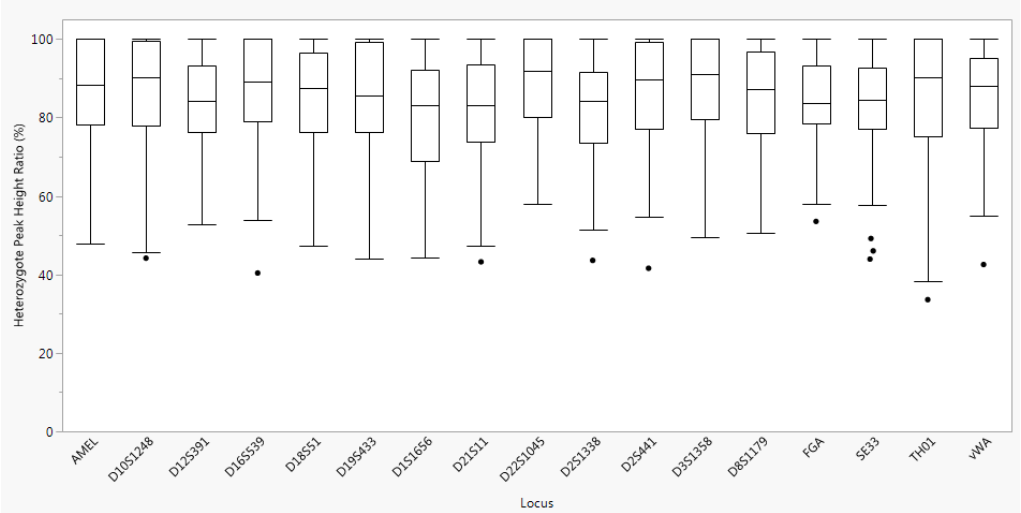



**Figure 34** Heterozygote ratios for 0.5 ng of input DNA. The distribution of intra-locus peak height ratio is expressed as a percentage, by locus (low peak height allele divided by high peak height allele). Boxes show the middle 50% or interquartile range (IQR). Box halves below and above median show the second and third quartile, respectively. "Whiskers" indicate 1.5 IQR from the upper and lower margins of the IQR. Black dots are outlier data points more than 1.5 IQR from the median (N=60).

## Resolution of genotypes in mixed samples

A sample containing DNA from two sources can comprise (at a single locus) any of the seven genotype combinations (see below).

- Heterozygote + heterozygote, no overlapping alleles (four peaks)
- Heterozygote + heterozygote, one overlapping allele (three peaks)
- Heterozygote + heterozygote, two overlapping alleles (two peaks)
- Heterozygote + homozygote, no overlapping alleles (three peaks)
- Heterozygote + homozygote, overlapping allele (two peaks)
- Homozygote + homozygote, no overlapping alleles (two peaks)
- Homozygote + homozygote, overlapping allele (one peak)

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether or not it is possible to resolve the genotypes of the major and minor components at a single locus.

The ability to obtain and compare quantitative values for the different allele peak heights on Applied Biosystems™ instruments provides additional valuable data to aid in resolving mixed genotypes.

Ultimately, the likelihood that any sample is a mixture must be determined by the analyst in the context of each particular case, including the information provided from known reference samples.

**Note:** Peak detection is a complex process that involves the STR chemistry, capillary electrophoresis conditions, and the data analysis software. Contact HID Support for a Technical Note with additional information on detecting peaks in electropherograms.

## Limit of detection of the minor component

Mixtures of two DNA samples were examined at various ratios (0:1, 1:1, 1:3, 1:7, 1:15, 1:30, 30:1, 15:1, 7:1, 3:1 and 1:0 M:F ratios). The total amount of genomic input DNA mixed at each ratio was 0.5 ng. The samples were amplified in a GeneAmp™ PCR System 9700, then electrophoresed and detected using a 3500xL Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 35. The male 007 and female 9947a were mixed according to the ratios indicated. The minor component allele calls at non-overlapping loci are highlighted. Detection of full profiles for the minor contributor was possible at ratios of 7:1 (0.438 ng of 007 and 0.063 ng of 9947a) and 1:7 (0.063 ng of 007 and 0.438 ng of 9947a, with 2/3 replicates giving full profiles of the 007 minor contributor). 15:1, 30:1, 1:30, and 1:15 ratios resulted in partial profiles for the minor component. The genotypes of these samples are shown in Table 11.

Table 12 shows mixture sample compositions and STR allele counts from NGM Detect™ assays.

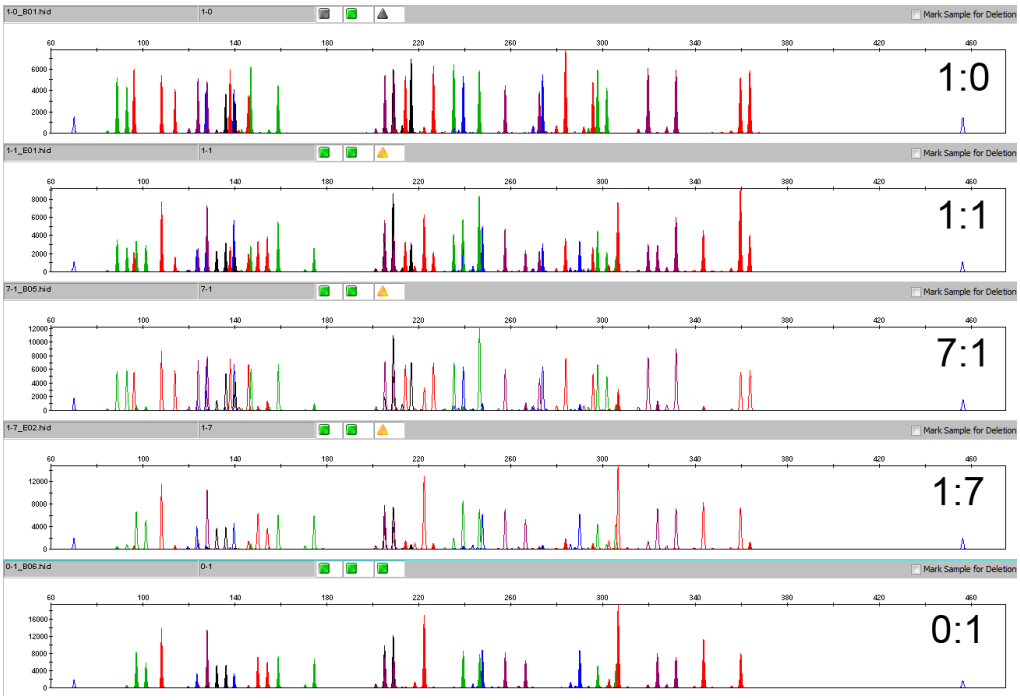



**Figure 35** Amplification of DNA mixtures at various ratios. Panels show electropherograms for 1:0 (male 007 DNA only), 1:1 mixture, 7:1 mixture, 1:7 mixture, and 0:1 (female 9947a DNA only).

**Note:** The data in this figure are from the original formulation of the NGM Detect™ kit.

**Table 11** Genotypes of mixed DNA samples

**Note:** The data in this table are from the original validation study of the NGM Detect™ kit.

| Locus | Male 007 alleles | | Female 9947a alleles | |
|---|---|---|---|---|
| IQCS | — | — | — | — |
| D2S1338 | 20 | 23 | 19 | 23 |
| SE33 | 17 | 25.2 | 19 | 29.2 |
| IQCL | — | — | — | — |
| D16S539 | 9 | 10 | 11 | 12 |
| D18S51 | 12 | 15 | 15 | 19 |
| TH01 | 7 | 9.3 | 8 | 9.3 |
| D12S391 | 18 | 19 | 18 | 20 |
| D3S1358 | 15 | 16 | 14 | 15 |
| FGA | 24 | 26 | 23 | 24 |
| Y indel | 2 | — | — | — |
| Amelogenin | X | Y | X | — |
| vWA | 14 | 16 | 17 | 18 |
| D21S11 | 28 | 31 | 30 | — |
| D1S1656 | 13 | 16 | 18.3 | — |
| D2S441 | 14 | 15 | 10 | 14 |
| D8S1179 | 12 | 13 | 13 | — |
| D19S433 | 14 | 15 | 14 | 15 |
| D22S1045 | 11 | 16 | 11 | 14 |
| D10S1248 | 12 | 15 | 13 | 15 |

**Table 12** Mixture sample compositions and STR allele counts from NGM Detect™ assays

**Note:** The data in this table are from the original validation study of the NGM Detect™ kit.

| Ratio (M:F) | ng per reaction | | | Number of unique alleles in non-stutter positions per contributor[1] | |
|---|---|---|---|---|---|
| | Male 007 | Female 9947a | Total DNA | Male 007 | Female 9947a |
| 1:0 | 0.500 | 0.00 | 0.5 | 16.00 | 0.00 |
| 1:1 | 0.250 | 0.250 | 0.5 | 16.00 | 13.00 |
| 1:3 | 0.125 | 0.375 | 0.5 | 16.00 | 13.00 |
| 1:7 | 0.063 | 0.438 | 0.5 | 15.67 | 13.00 |

| Ratio (M:F) | ng per reaction | | | Number of unique alleles in non-stutter positions per contributor[1] | |
|---|---|---|---|---|---|
| | Male 007 | Female 9947a | Total DNA | Male 007 | Female 9947a |
| 1:15 | 0.031 | 0.469 | 0.5 | 12.33 | 13.00 |
| 1:30 | 0.016 | 0.484 | 0.5 | 8.00 | 13.00 |
| 30:1 | 0.484 | 0.016 | 0.5 | 16.00 | 8.67 |
| 15:1 | 0.469 | 0.031 | 0.5 | 16.00 | 11.67 |
| 7:1 | 0.438 | 0.063 | 0.5 | 16.00 | 13.00 |
| 3:1 | 0.375 | 0.125 | 0.5 | 16.00 | 13.00 |
| 0:1 | 0.000 | 0.500 | 0.5 | 0.00 | 13.00 |

[1] Mixture sample compositions and STR allele counts from NGM Detect™ assays. Average number of unique, distinct alleles (that is, alleles neither present in the other contributor genotype nor located in a stutter position) obtained in 3 replicate reactions per mixture sample. Full profiles of unique alleles in 007 and 9947a DNAs were 16 and 13, respectively.

# Population data

## SWGDAM guideline 3.7

"The distribution of genetic markers in populations should be determined in relevant population groups." (SWGDAM, December 2016)

## Population data overview

To interpret the significance of a match between genetically typed samples, you must know the population distribution of alleles at each locus in question. If the genotype of the relevant evidence sample is:

- Different from the genotype of the reference sample for a suspect, then the suspect is *excluded* as the donor of the biological evidence that was tested. An exclusion is independent of the frequency of the two genotypes in the population.
- The same as the genotype of the reference sample for a suspect, then the suspect is *included* as a possible source of the evidence sample.

The probability that another, unrelated individual would also match the evidence sample is estimated by the frequency of that genotype in the relevant populations.

## Loci in the kit

The NGM Detect™ kit contains loci for which extensive population data are available. For additional information on the loci shared between many of the AmpFℓSTR™ kits, see the population data and additional studies section of the *AmpFℓSTR™ NGM SElect™ PCR Amplification Kit Kit User Guide* (Pub. No. 4458841) and the *AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit User Guide* (Pub. No. 4440211).

## Population samples used in these studies

**Note:** The data in this section are from the original validation study of the NGM Detect™ kit.

The NGM Detect™ kit has high genotypic concordance (>99.5%) to the NGM SElect™ kit and by extension to the GlobalFiler™ kit. (All STR markers in the NGM SElect™ kit are included in the GlobalFiler™ kit with both the GlobalFiler™ and NGM SElect™ kits sharing the same primers for common loci.) Therefore, the existing population data from the GlobalFiler™ kit was used, as applicable, to generate the population data that is provided in this section. Whole blood samples, provided by the Interstate Blood Bank (Memphis, Tennessee) and Boca Biolistics (Coconut Creek, Florida), were collected in the United States (with no geographical preference) from randomly selected individuals of known ethnicities. Ethnicities of sample donors were:

- African-American—330 samples
- Asian—153 samples
- Caucasian—343 samples
- Hispanic—368 samples

DNA was extracted using a 6100 Nucleic Acid Prep Station.

In addition to the alleles that we observed and recorded in our databases, other alleles have been published or reported to us by other laboratories (see the STRBase at **www.cstl.nist.gov/div831/strbase**).

## Concordance studies

In the validation study of the original NGM Detect™ kit formulation, a concordance population study with 1,165 in-house samples identified two samples that were discordant because of allele dropout or severe allele imbalance in the NGM Detect™ assay. The samples were characterized and degenerate oligos were added to the SE33 (2 oligos) and D8S1179 (1 oligo) markers in the final stages of development. The resulting concordance between the NGM SElect™ kit and the NGM Detect™ kit was greater than 99.5%.

In the validation study of the updated NGM Detect™ kit formulation, we used the new primer set with 1,092 individuals from the same donor population. We observed 100% concordance between the new primer set and the original primer set at the TH01 locus.

## Probability of Identity definition

The $P_I$ value is the probability that two individuals selected at random will have an identical genotype (Sensabaugh, 1982).

## Probability of identity

Table 13 shows the Autosomal STR allele frequencies at NGM Detect™ kit loci by population group.

Table 14 shows the Y-specific allele frequencies by population group for NGM Detect™ kit Y indel locus. The Y-specific allele frequencies were not included in the probability of identity calculation.

Table 15 shows the Probability of identity ($P_I$) values of the NGM Detect™ kit loci individually and combined.

**Table 13** Autosomal allele frequencies by population group for NGM Detect™ kit STR loci. (*=Alleles not detected or not detected in significant quantities)

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| **D2S1338** | | | | |
| 10 | * | * | * | * |
| 11 | * | * | * | * |
| 12 | * | * | * | * |
| 13 | 0.15* | * | 0.15* | * |
| 14 | * | * | 0.15* | * |
| 15 | 0.30* | * | 0.15* | * |
| 16 | 5.3 | 1.63 | 4.08 | 3.8 |
| 17 | 10 | 14.05 | 18.37 | 17.8 |
| 18 | 4.85 | 13.07 | 8.31 | 6.52 |
| 19 | 16.21 | 16.67 | 14.14 | 17.53 |
| 20 | 10.45 | 8.82 | 15.74 | 13.86 |
| 21 | 11.97 | 2.94 | 2.92 | 3.67 |
| 22 | 12.42 | 5.88 | 1.75 | 6.52 |
| 23 | 9.24 | 18.3 | 10.06 | 14.27 |
| 24 | 8.79 | 11.11 | 10.2 | 8.83 |
| 25 | 6.97 | 5.88 | 12.1 | 5.43 |
| 26 | 2.58 | * | 1.6 | 1.49 |
| 27 | 0.76 | 0.33* | 0.29* | 0.14* |
| 28 | * | 0.98* | * | 0.14* |
| 29 | * | 0.33* | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| **SE33** | | | | |
| 3.2 | * | * | * | * |
| 4.2 | * | * | * | * |
| 5 | * | * | * | * |
| 6 | * | * | * | * |
| 6.3 | * | * | 0.15* | * |
| 7 | * | * | * | * |
| 8 | * | * | * | * |
| 8.2 | * | * | * | * |
| 9 | * | * | * | * |
| 9.2 | * | * | * | * |
| 10 | * | * | * | * |
| 10.2 | * | * | * | * |
| 11 | * | * | * | * |
| 11.2 | 0.76 | * | * | 0.14* |
| 12 | 0.15* | 0.33* | 0.44* | 0.14* |
| 12.1 | * | * | 0.15* | * |
| 12.2 | 0.30* | * | 0.15* | 0.14* |
| 13 | 1.36 | * | 0.87 | 1.22 |
| 13.2 | 0.45* | * | * | 0.14* |
| 14 | 3.33 | * | 3.64 | 1.77 |
| 14.2 | 0.15* | * | * | 0.82 |
| 14.3 | * | * | 0.15* | * |
| 15 | 4.24 | 1.31* | 3.64 | 4.89 |
| 15.2 | 0.15* | * | 0.15* | 0.14* |
| 15.3 | * | * | * | * |
| 16 | 6.97 | 3.59 | 5.39 | 5.57 |
| 16.2 | 0.30* | * | * | 0.27* |
| 16.3 | * | * | * | 0.14* |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 17 | 7.73 | 5.23 | 6.56 | 8.7 |
| 17.2 | 0.15* | * | * | * |
| 17.3 | * | * | 0.15* | * |
| 18 | 10.76 | 4.9 | 7.87 | 10.05 |
| 18.2 | 0.15* | * | * | 0.27* |
| 19 | 15 | 9.48 | 8.31 | 8.15 |
| 19.2 | 0.30* | * | 0.29* | * |
| 19.3 | * | * | 0.29* | * |
| 20 | 9.55 | 6.86 | 5.25 | 4.48 |
| 20.2 | 0.91 | 0.33* | 0.87 | 0.82 |
| 21 | 5.76 | 6.21 | 2.04 | 3.12 |
| 21.1 | * | * | * | * |
| 21.2 | 0.91 | 1.63 | 1.17 | 1.09 |
| 22 | 1.97 | 2.61 | 0.58* | 1.09 |
| 22.2 | 1.36 | 2.29 | 3.35 | 2.31 |
| 23 | 0.30* | * | * | * |
| 23.2 | 0.61* | 2.61 | 2.62 | 2.85 |
| 23.3 | * | * | * | 0.14* |
| 24 | 0.30* | 0.33* | 0.15* | 0.14* |
| 24.2 | 1.67 | 6.54 | 4.52 | 2.31 |
| 25 | * | * | * | * |
| 25.2 | 2.42 | 7.19 | 3.79 | 3.12 |
| 26 | 0.15* | * | * | 0.27* |
| 26.2 | 5.61 | 7.52 | 4.52 | 6.39 |
| 27 | * | * | * | * |
| 27.2 | 5.91 | 3.59 | 6.85 | 7.07 |
| 28 | * | * | * | * |
| 28.2 | 3.94 | 7.84 | 7.73 | 6.25 |
| 29 | * | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 29.2 | 2.58 | 8.5 | 7.87 | 5.84 |
| 30 | * | * | * | * |
| 30.2 | 1.21 | 7.52 | 4.66 | 3.8 |
| 31 | * | * | * | 0.14* |
| 31.2 | 1.06 | 1.63 | 2.77 | 2.31 |
| 32 | * | * | 0.44* | * |
| 32.2 | 0.76 | 1.31* | 1.6 | 2.04 |
| 33 | * | * | 0.29* | 0.41* |
| 33.2 | 0.45* | 0.33* | 0.15* | 0.54* |
| 34 | * | * | 0.29* | 0.41* |
| 34.2 | 0.15* | * | * | 0.27* |
| 35 | * | * | 0.15* | * |
| 35.2 | * | 0.33* | * | * |
| 36 | * | * | 0.15* | * |
| 36.2 | * | * | * | * |
| 37 | * | * | * | 0.14* |
| 37.2 | * | * | * | * |
| 38 | * | * | * | * |
| 38.2 | * | * | * | * |
| 39 | * | * | * | * |
| 39.2 | * | * | * | * |
| 40 | * | * | * | * |
| 41 | * | * | * | * |
| 42 | * | * | * | * |
| 43 | * | * | * | * |
| **D16S539** | | | | |
| 4 | * | * | * | * |
| 5 | * | * | * | * |
| 6 | * | * | * | 0.14* |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 7 | * | * | * | * |
| 8 | 3.33 | * | 1.46 | 2.04 |
| 8.3 | * | * | * | * |
| 9 | 21.67 | 31.05 | 12.68 | 10.19 |
| 9.3 | * | * | * | * |
| 10 | 11.52 | 14.05 | 4.08 | 15.76 |
| 11 | 30 | 20.59 | 32.22 | 31.79 |
| 11.3 | * | * | * | * |
| 12 | 19.09 | 21.57 | 30.9 | 24.18 |
| 12.1 | * | * | * | * |
| 12.2 | * | * | * | * |
| 13 | 13.03 | 11.44 | 16.76 | 14.4 |
| 13.3 | * | * | * | * |
| 14 | 1.36 | 1.31* | 1.75 | 1.22 |
| 15 | * | * | 0.15* | 0.27* |
| 16 | * | * | * | * |
| **D18S51** | | | | |
| 6 | * | * | * | * |
| 7 | * | * | * | * |
| 8 | | | | |
| 9 | * | * | * | 0.14* |
| 9.2 | * | * | * | * |
| 10 | 0.15* | * | 1.17 | 0.68 |
| 10.2 | 0.15* | * | * | * |
| 11 | 0.45* | 1.31* | 0.87 | 1.22 |
| 11.2 | * | * | * | * |
| 12 | 6.21 | 5.56 | 15.01 | 10.46 |
| 12.2 | * | * | * | * |
| 12.3 | * | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 13 | 3.94 | 17.32 | 11.95 | 11.41 |
| 13.1 | * | * | * | * |
| 13.2 | 0.30* | * | * | * |
| 13.3 | * | * | * | * |
| 14 | 5.91 | 22.88 | 17.64 | 16.3 |
| 14.2 | 0.45* | * | * | 0.14* |
| 15 | 16.52 | 16.99 | 15.31 | 12.23 |
| 15.2 | * | * | * | 0.14* |
| 15.3 | * | * | * | * |
| 16 | 18.18 | 12.42 | 11.95 | 12.91 |
| 16.1 | * | * | * | * |
| 16.2 | * | * | * | * |
| 17 | 16.36 | 6.54 | 10.79 | 17.39 |
| 17.2 | * | * | * | * |
| 17.3 | * | * | * | * |
| 18 | 14.09 | 4.9 | 8.31 | 7.74 |
| 18.1 | * | * | * | * |
| 18.2 | * | * | * | * |
| 19 | 9.7 | 5.23 | 4.08 | 3.53 |
| 19.2 | * | * | * | * |
| 20 | 4.7 | 1.96 | 1.31 | 1.9 |
| 20.2 | 0.15* | * | * | * |
| 21 | 1.82 | 1.96 | 1.02 | 2.17 |
| 21.2 | * | * | * | * |
| 22 | 0.61* | 0.98* | 0.29* | 0.68 |
| 22.2 | * | * | * | * |
| 23 | 0.30* | 0.98* | 0.29* | 0.54* |
| 23.2 | * | * | * | * |
| 24 | * | 0.65* | * | 0.27* |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 25 | * | * | * | 0.14* |
| 26 | * | 0.33* | * | * |
| 27 | * | * | * | * |
| 28 | * | * | * | * |
| **TH01** | | | | |
| 3 | * | * | * | * |
| 4 | * | * | * | * |
| 5 | 0.45* | * | 0.15* | * |
| 5.3 | * | * | * | * |
| 6 | 15.45 | 13.07 | 21.72 | 27.17 |
| 6.3 | * | * | * | * |
| 7 | 37.42 | 26.14 | 17.64 | 32.74 |
| 7.3 | * | * | * | * |
| 8 | 20.61 | 3.59 | 11.37 | 8.7 |
| 8.3 | * | * | * | * |
| 9 | 16.06 | 51.63 | 17.06 | 12.77 |
| 9.3 | 8.33 | 4.25 | 31.2 | 17.12 |
| 10 | 1.52 | 1.31* | 0.87 | 1.49 |
| 10.3 | * | * | * | * |
| 11 | * | * | * | * |
| 12 | * | * | * | * |
| 13 | * | * | * | * |
| 13.3 | * | * | * | * |
| 14.3 | * | * | * | * |
| **D12S391** | | | | |
| 13 | * | * | * | 0.14* |
| 14 | * | * | * | 0.14* |
| 15 | 7.58 | 2.61 | 4.37 | 4.08 |
| 16 | 5.15 | 0.98* | 3.35 | 5.03 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 17 | 16.52 | 8.17 | 10.35 | 7.34 |
| 17.1 | 0.45* | * | * | 0.27* |
| 17.3 | 0.61* | * | 1.9 | 1.22 |
| 18 | 24.55 | 28.43 | 16.18 | 19.7 |
| 18.1 | * | * | * | * |
| 18.3 | 1.21 | * | 2.19 | 2.17 |
| 19 | 13.94 | 23.86 | 12.54 | 18.75 |
| 19.1 | 0.61* | * | * | * |
| 19.3 | 0.30* | * | 0.58* | 1.22 |
| 20 | 11.52 | 16.99 | 9.77 | 17.12 |
| 20.1 | * | * | * | * |
| 20.3 | * | * | 0.15* | * |
| 21 | 7.27 | 10.78 | 13.56 | 8.7 |
| 21.3 | 0.15* | * | 0.15* | * |
| 22 | 5 | 3.59 | 10.79 | 6.79 |
| 23 | 3.64 | 3.27 | 8.16 | 3.67 |
| 24 | 0.61* | 0.98* | 3.64 | 1.9 |
| 24.3 | * | * | * | * |
| 25 | 0.61* | 0.33* | 1.9 | 1.36 |
| 26 | * | * | 0.29* | 0.27* |
| 27 | * | * | 0.15* | 0.14* |
| **D3S1358** | | | | |
| 8 | * | * | * | * |
| 9 | 0.30* | * | * | 0.14* |
| 10 | * | * | * | * |
| 11 | * | * | 0.29* | * |
| 12 | 0.15* | 0.33* | * | 0.14* |
| 13 | 0.61* | * | 0.15* | 0.41* |
| 14 | 9.09 | 2.61 | 15.16 | 9.1 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 15 | 28.18 | 49.02 | 27.26 | 34.65 |
| 15.2 | 0.30* | * | * | * |
| 16 | 32.42 | 21.9 | 24.34 | 26.9 |
| 16.2 | * | * | * | * |
| 17 | 22.27 | 19.61 | 19.68 | 17.93 |
| 17.1 | * | * | * | * |
| 17.2 | * | * | * | * |
| 18 | 6.06 | 6.54 | 11.66 | 9.92 |
| 18.2 | * | * | * | * |
| 19 | 0.61* | * | 1.46 | 0.82 |
| 20 | * | * | * | * |
| 20.1 | * | * | * | * |
| 21 | * | * | * | * |
| **FGA** | | | | |
| 12.2 | * | * | * | * |
| 13 | * | * | * | * |
| 14 | * | * | * | * |
| 15 | * | * | * | * |
| 16 | * | 0.33* | 0.15* | * |
| 16.1 | 0.30* | * | * | * |
| 16.2 | * | * | * | * |
| 17 | * | 0.33* | 0.15* | * |
| 17.2 | * | * | * | * |
| 18 | 0.91 | 3.27 | 1.02 | 0.68 |
| 18.2 | 0.61* | * | * | * |
| 19 | 6.97 | 4.25 | 5.69 | 7.61 |
| 19.2 | 0.45* | * | * | * |
| 19.3 | * | * | * | * |
| 20 | 6.82 | 3.92 | 14.87 | 8.7 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 20.1 | * | * | * | * |
| 20.2 | 0.30* | * | 0.44* | 0.27* |
| 20.3 | * | * | * | * |
| 21 | 11.67 | 13.07 | 18.22 | 13.45 |
| 21.2 | 0.15* | 0.33* | 0.29* | * |
| 21.3 | * | * | * | * |
| 22 | 17.27 | 14.38 | 19.24 | 14.4 |
| 22.1 | * | * | * | * |
| 22.2 | 0.15* | * | 0.87 | 0.54* |
| 22.3 | * | * | * | * |
| 23 | 17.27 | 27.12 | 14.87 | 12.91 |
| 23.1 | * | * | * | * |
| 23.2 | * | 0.65* | 0.44* | 0.41* |
| 23.3 | 0.30* | * | * | * |
| 24 | 18.94 | 18.3 | 14.43 | 15.62 |
| 24.1 | * | * | * | * |
| 24.2 | * | 0.33* | * | * |
| 24.3 | * | * | * | * |
| 25 | 9.55 | 9.8 | 6.71 | 13.72 |
| 25.1 | * | * | * | * |
| 25.2 | * | * | * | * |
| 25.3 | * | * | * | * |
| 26 | 4.09 | 3.27 | 1.9 | 7.07 |
| 26.1 | * | * | * | * |
| 26.2 | * | * | * | * |
| 27 | 2.58 | 0.65* | 0.58* | 3.12 |
| 27.2 | * | * | * | * |
| 28 | 1.21 | * | 0.15* | 0.95 |
| 28.2 | * | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 29 | * | * | * | 0.41* |
| 29.2 | * | * | * | * |
| 30 | 0.15* | * | * | 0.14* |
| 30.2 | 0.15* | * | * | * |
| 31 | * | * | * | * |
| 31.2 | * | * | * | * |
| 32 | * | * | * | * |
| 32.2 | * | * | * | * |
| 33.2 | * | * | * | * |
| 34.2 | 0.15* | * | * | * |
| 41.2 | * | * | * | * |
| 42.2 | * | * | * | * |
| 43.2 | * | * | * | * |
| 44.2 | * | * | * | * |
| 45.2 | * | * | * | * |
| 46.2 | * | * | * | * |
| 47.2 | * | * | * | * |
| 48.2 | * | * | * | * |
| 49.2 | * | * | * | * |
| 50.2 | * | * | * | * |
| 51.2 | * | * | * | * |
| 52.2 | * | * | * | * |
| **vWA** | | | | |
| 10 | * | * | * | * |
| 11 | 0.45* | * | * | 0.14* |
| 12 | * | * | * | 0.27* |
| 13 | 0.91 | * | 0.15* | 0.14* |
| 14 | 7.27 | 23.53 | 8.75 | 6.52 |
| 15 | 20.91 | 1.63 | 12.24 | 9.78 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 15.2 | * | * | * | * |
| 16 | 27.58 | 15.36 | 22.3 | 30.57 |
| 17 | 19.85 | 29.74 | 27.41 | 27.17 |
| 18 | 13.79 | 19.61 | 17.78 | 18.07 |
| 18.2 | * | * | * | * |
| 19 | 6.52 | 9.15 | 10.06 | 6.39 |
| 20 | 1.97 | 0.98* | 1.31 | 0.82 |
| 21 | 0.61* | * | * | * |
| 22 | * | * | * | * |
| 23 | 0.15* | * | * | * |
| 24 | * | * | * | * |
| 25 | * | * | * | * |
| **D21S11** | | | | |
| 23 | * | * | * | * |
| 23.2 | * | * | * | * |
| 24 | * | * | * | * |
| 24.2 | * | * | * | 0.27* |
| 24.3 | * | * | * | * |
| 25 | * | * | * | * |
| 25.2 | * | * | * | * |
| 25.3 | * | * | * | * |
| 26 | 0.30* | * | 0.58* | 0.41* |
| 26.2 | * | * | * | * |
| 27 | 5.91 | * | 2.62 | 1.49 |
| 27.1 | * | * | * | * |
| 27.2 | * | * | * | * |
| 28 | 25.15 | 4.9 | 16.76 | 11.41 |
| 28.1 | * | * | * | * |
| 28.2 | * | 0.65* | * | 0.14* |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 28.3 | * | * | * | * |
| 29 | 15.61 | 26.8 | 23.76 | 21.06 |
| 29.1 | * | * | * | * |
| 29.2 | * | * | 0.15* | * |
| 29.3 | 0.15* | * | 0.15* | * |
| 30 | 20.76 | 30.72 | 23.18 | 27.17 |
| 30.1 | * | * | * | * |
| 30.2 | 1.67 | 0.65* | 2.77 | 1.77 |
| 30.3 | * | * | * | * |
| 31 | 8.79 | 9.48 | 6.85 | 5.16 |
| 31.1 | * | * | * | * |
| 31.2 | 4.55 | 3.92 | 8.89 | 11.14 |
| 31.3 | * | 0.33* | * | * |
| 32 | 1.36 | 2.61 | 2.33 | 1.36 |
| 32.1 | * | * | * | * |
| 32.2 | 7.12 | 14.38 | 9.62 | 12.5 |
| 32.3 | * | * | * | * |
| 33 | 0.91 | 0.98* | * | 0.14* |
| 33.1 | * | * | * | * |
| 33.2 | 3.18 | 4.58 | 1.9 | 5.3 |
| 33.3 | * | * | * | * |
| 34 | 0.15* | * | * | * |
| 34.1 | * | * | * | * |
| 34.2 | * | * | 0.44* | 0.14* |
| 35 | 3.64 | * | * | 0.27* |
| 35.1 | * | * | * | * |
| 35.2 | * | * | * | * |
| 36 | 0.76 | * | * | 0.14* |
| 36.1 | * | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 36.2 | * | * | * | * |
| 37 | * | * | * | * |
| 37.2 | * | * | * | * |
| 38 | * | * | * | 0.14* |
| 38.2 | * | * | * | * |
| 39 | * | * | * | * |
| **D1S1656** | | | | |
| 8 | * | * | * | * |
| 9 | 0.15* | * | * | 0.14* |
| 10 | 1.36 | * | 0.29* | 0.41* |
| 11 | 5.3 | 3.59 | 6.27 | 3.94 |
| 12 | 8.48 | 4.25 | 15.74 | 9.38 |
| 13 | 11.06 | 13.73 | 7 | 7.07 |
| 13.3 | * | * | * | * |
| 14 | 25 | 6.21 | 6.27 | 11.28 |
| 14.3 | 0.91 | * | 0.29* | 0.27* |
| 15 | 16.97 | 20.26 | 15.31 | 15.49 |
| 15.3 | 1.82 | * | 8.75 | 2.99 |
| 16 | 10 | 31.05 | 9.33 | 15.08 |
| 16.3 | 7.27 | 0.65* | 4.96 | 5.16 |
| 17 | 2.73 | 14.05 | 4.96 | 6.79 |
| 17.1 | * | * | 0.29* | * |
| 17.3 | 5.76 | 3.59 | 12.68 | 15.76 |
| 18 | 0.45* | 0.33* | 0.29* | 0.82 |
| 18.3 | 1.82 | 1.63 | 5.98 | 4.48 |
| 19 | 0.15* | * | * | * |
| 19.3 | 0.61* | 0.33* | 1.6 | 0.68 |
| 20 | * | 0.33* | * | * |
| 20.3 | 0.15* | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 21 | * | * | * | * |
| 21.3 | * | * | * | * |
| **D2S441** | | | | |
| 7 | * | * | * | * |
| 8 | 0.15* | * | * | * |
| 9 | * | * | 0.58* | 0.14* |
| 9.1 | * | 2.94 | * | * |
| 10 | 9.09 | 20.59 | 19.83 | 30.3 |
| 11 | 35.61 | 36.27 | 33.09 | 31.93 |
| 11.3 | 2.88 | 2.61 | 5.1 | 4.62 |
| 12 | 20.45 | 20.92 | 4.08 | 3.8 |
| 12.3 | 0.15* | * | 0.29* | 0.41* |
| 13 | 3.48 | 6.21 | 3.35 | 1.9 |
| 13.3 | * | * | * | * |
| 14 | 26.21 | 9.8 | 28.86 | 23.1 |
| 14.3 | * | * | * | * |
| 15 | 1.97 | 0.65* | 4.37 | 3.4 |
| 16 | * | * | 0.44* | 0.41* |
| 17 | * | * | * | * |
| 18 | * | * | * | * |
| **D8S1179** | | | | |
| 4 | * | * | * | * |
| 5 | * | * | * | * |
| 6 | * | * | * | * |
| 7 | * | * | * | * |
| 8 | 0.30* | * | 2.04 | 0.68 |
| 9 | 0.30* | * | 1.31 | 0.27* |
| 10 | 3.33 | 9.8 | 10.5 | 9.51 |
| 10.2 | * | * | * | * |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 11 | 5.61 | 9.48 | 6.71 | 5.03 |
| 12 | 11.36 | 13.4 | 15.16 | 12.5 |
| 12.3 | * | * | * | * |
| 13 | 18.18 | 24.18 | 33.24 | 33.15 |
| 13.3 | * | * | * | * |
| 14 | 35.91 | 15.69 | 18.8 | 23.23 |
| 15 | 18.03 | 22.22 | 9.04 | 11.41 |
| 15.3 | * | * | * | * |
| 16 | 5.91 | 4.25 | 2.77 | 3.53 |
| 17 | 1.06 | 0.98* | 0.44* | 0.68 |
| 18 | * | * | * | * |
| 19 | * | * | * | * |
| 20 | * | * | * | * |
| **D19S433** | | | | |
| 5 | * | * | * | * |
| 5.2 | * | * | * | * |
| 6 | * | * | * | * |
| 6.2 | * | * | * | * |
| 7 | * | * | * | * |
| 8 | * | * | * | * |
| 9 | 0.30* | 0.33* | * | * |
| 9.2 | * | * | * | * |
| 10 | 1.21 | * | 0.15* | 0.41* |
| 10.2 | 0.15* | * | * | * |
| 11 | 9.85 | * | * | 1.63 |
| 11.2 | 0.30* | * | * | 0.27* |
| 12 | 10.45 | 4.58 | 7.29 | 8.42 |
| 12.1 | * | * | 0.15* | * |
| 12.2 | 3.94 | 0.33* | 0.15* | 1.49 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 13 | 27.88 | 28.1 | 27.26 | 18.48 |
| 13.1 | * | * | * | * |
| 13.2 | 5.3 | 2.61 | 1.6 | 6.93 |
| 14 | 18.94 | 23.2 | 35.13 | 30.71 |
| 14.2 | 5.3 | 9.48 | 2.04 | 4.62 |
| 14.3 | * | * | * | * |
| 15 | 6.67 | 7.52 | 16.18 | 13.04 |
| 15.2 | 4.39 | 20.26 | 3.5 | 6.79 |
| 16 | 1.52 | 0.33* | 5.69 | 4.08 |
| 16.2 | 3.18 | 2.61 | 0.29* | 2.17 |
| 17 | * | * | 0.29* | 0.54* |
| 17.2 | 0.61* | 0.65* | 0.15* | 0.41* |
| 18 | * | * | 0.15* | * |
| 18.2 | * | * | * | * |
| 19 | * | * | * | * |
| 19.2 | * | * | * | * |
| 20.2 | * | * | * | * |
| **D22S1045** | | | | |
| 6 | * | * | * | * |
| 7 | * | * | * | * |
| 8 | 0.61* | * | * | * |
| 9 | * | * | * | * |
| 10 | 4.09 | * | 0.44* | 0.68 |
| 11 | 14.7 | 15.36 | 13.85 | 7.61 |
| 12 | 6.21 | 0.33* | 0.58* | 0.95 |
| 13 | 0.30* | 0.33* | 1.02 | 1.09 |
| 14 | 7.88 | 0.33* | 3.35 | 2.04 |
| 15 | 23.33 | 33.66 | 36.3 | 43.48 |
| 16 | 20.3 | 23.86 | 36.3 | 34.65 |

| Allele | African American (n=330) | Asian (n=153) | U.S. Caucasian (n=343) | U.S. Hispanic (n=368) |
|---|---|---|---|---|
| 17 | 20.45 | 24.18 | 7.58 | 8.42 |
| 18 | 2.12 | 1.96 | 0.58* | 0.95 |
| 19 | * | * | * | * |
| 20 | * | * | * | 0.14* |
| 21 | * | * | * | * |
| **D10S1248** | | | | |
| 7 | 0.15* | * | * | * |
| 8 | * | * | * | 0.14* |
| 9 | 0.15* | * | * | 0.14* |
| 10 | * | 0.33* | * | 0.14* |
| 11 | 3.64 | * | 0.58* | 0.27* |
| 12 | 14.09 | 10.78 | 3.5 | 4.48 |
| 13 | 22.88 | 36.93 | 29.45 | 25.95 |
| 14 | 27.88 | 22.55 | 29.74 | 36.14 |
| 15 | 18.48 | 22.55 | 19.39 | 22.69 |
| 16 | 10.15 | 5.23 | 13.41 | 7.74 |
| 17 | 2.27 | 1.63 | 3.64 | 2.31 |
| 18 | 0.30* | * | 0.29* | * |
| 19 | * | * | * | * |
| 20 | * | * | * | * |

**Table 14** Y-specific frequencies by population group for the NGM Detect™ kit Y indel locus. (*=Alleles not detected or not detected in significant quantities)

| Allele | African American (n = 246) | Asian (n = 65) | U.S. Caucasian (n = 233) | U.S. Hispanic (n = 182) |
|---|---|---|---|---|
| **Y indel** | | | | |
| 1 | 1.22 | 67.69 | * | 0.55* |
| 2 | 98.78 | 32.31 | 100 | 99.45 |

**Table 15** Probability of identity ($P_I$) values for the NGM Detect™ kit STR loci

| Locus | African American (n = 330) | Asian (n = 153) | U.S. Caucasian (n = 343) | U.S. Hispanic (n = 368) |
|---|---|---|---|---|
| D10S1248 | 0.0693 | 0.1045 | 0.0943 | 0.1131 |
| D12S391 | 0.0377 | 0.0664 | 0.0231 | 0.0318 |
| D16S539 | 0.0727 | 0.0915 | 0.1043 | 0.0809 |
| D18S51 | 0.0322 | 0.0402 | 0.0311 | 0.0281 |
| D19S433 | 0.0388 | 0.0663 | 0.0862 | 0.0484 |
| D1S1656 | 0.034 | 0.0564 | 0.0223 | 0.0247 |
| D21S11 | 0.0453 | 0.0671 | 0.052 | 0.0487 |
| D22S1045 | 0.0559 | 0.1073 | 0.1309 | 0.1604 |
| D2S1338 | 0.0225 | 0.0337 | 0.0316 | 0.0316 |
| D2S441 | 0.103 | 0.0961 | 0.0976 | 0.1079 |
| D3S1358 | 0.0984 | 0.1689 | 0.0749 | 0.0949 |
| D8S1179 | 0.0762 | 0.0527 | 0.0631 | 0.0661 |
| FGA | 0.0322 | 0.0555 | 0.0384 | 0.0282 |
| SE33 | 0.0118 | 0.0139 | 0.0085 | 0.0081 |
| TH01 | 0.0949 | 0.175 | 0.0801 | 0.0902 |
| vWA | 0.0622 | 0.084 | 0.065 | 0.0926 |
| Combined | $7.96 \times 10^{-22}$ | $2.25 \times 10^{-19}$ | $2.29 \times 10^{-21}$ | $3.12 \times 10^{-21}$ |

## Probability of paternity exclusion observation

Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), Match Probability (MP), and p-value of each locus was calculated using a software program developed by Ge (Li *et al*., 2013) and shown in the following table.

Departures from Hardy-Weinberg Equilibrium (HWE) expectations of each locus were derived using Arlequin (Excoffier *et al*., 2010). After Bonferroni correction (Weir, 1990), (p-value = 0.05/21 = 0.0024), no departures from HWE were observed at any locus.

**Table 16** Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), Match probability (MP), and p-value of STR loci

| Marker | African American | | | | Asian | | | | U.S. Caucasian | | | | U.S. Hispanic | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ho | He | MP | p-value | Ho | He | MP | p-value | Ho | He | MP | p-value | Ho | He | MP | p-value |
| Y-indel | - | - | 0.976 | - | - | - | 0.556 | - | - | - | 1 | - | - | - | 1 | - |
| D3S1358 | 0.772 | 0.762 | 0.094 | 0.902 | 0.686 | 0.682 | 0.151 | 0.012 | 0.753 | 0.786 | 0.079 | 0.698 | 0.698 | 0.758 | 0.098 | 0.291 |
| vWA | 0.752 | 0.798 | 0.068 | 0.116 | 0.829 | 0.784 | 0.08 | 0.309 | 0.841 | 0.807 | 0.064 | 0.621 | 0.842 | 0.782 | 0.08 | 0.463 |
| D16S539 | 0.772 | 0.797 | 0.071 | 0.026 | 0.8 | 0.764 | 0.092 | 0.457 | 0.801 | 0.749 | 0.103 | 0.047 | 0.77 | 0.774 | 0.084 | 0.835 |
| D8S1179 | 0.782 | 0.793 | 0.068 | 0.662 | 0.814 | 0.828 | 0.052 | 0.795 | 0.793 | 0.797 | 0.065 | 0.122 | 0.755 | 0.802 | 0.064 | 0.518 |
| D21S11 | 0.861 | 0.849 | 0.039 | 0.553 | 0.8 | 0.791 | 0.069 | 0.667 | 0.873 | 0.837 | 0.046 | 0.385 | 0.827 | 0.839 | 0.044 | 0.315 |
| D18S51 | 0.931 | 0.868 | 0.031 | 0.324 | 0.829 | 0.853 | 0.038 | 0.572 | 0.873 | 0.872 | 0.03 | 0.962 | 0.849 | 0.87 | 0.031 | 0.945 |
| D2S441 | 0.772 | 0.756 | 0.099 | 0.421 | 0.714 | 0.746 | 0.101 | 0.238 | 0.757 | 0.766 | 0.09 | 0.077 | 0.791 | 0.763 | 0.094 | 0.611 |
| D19S433 | 0.812 | 0.825 | 0.051 | 0.663 | 0.714 | 0.802 | 0.064 | 0.13 | 0.785 | 0.774 | 0.083 | 0.859 | 0.82 | 0.834 | 0.046 | 0.446 |
| TH01 | 0.762 | 0.747 | 0.102 | 0.418 | 0.614 | 0.656 | 0.171 | 0.381 | 0.753 | 0.783 | 0.081 | 0.326 | 0.77 | 0.767 | 0.091 | 0.845 |
| FGA | 0.782 | 0.866 | 0.033 | 0.082 | 0.9 | 0.841 | 0.044 | 0.24 | 0.829 | 0.857 | 0.037 | 0.337 | 0.842 | 0.882 | 0.025 | 0.127 |
| D22S1045 | 0.842 | 0.822 | 0.055 | 0.062 | 0.743 | 0.742 | 0.112 | 0.966 | 0.705 | 0.714 | 0.131 | 0.026 | 0.698 | 0.672 | 0.162 | 0.064 |
| SE33 | 0.96 | 0.929 | 0.009 | 0.776 | 0.943 | 0.936 | 0.008 | 0.526 | 0.968 | 0.947 | 0.005 | 0.532 | 0.921 | 0.941 | 0.007 | 0.597 |
| D10S1248 | 0.792 | 0.789 | 0.075 | 0.823 | 0.757 | 0.764 | 0.091 | 0.928 | 0.785 | 0.769 | 0.09 | 0.63 | 0.691 | 0.724 | 0.124 | 0.336 |
| D1S1656 | 0.921 | 0.863 | 0.033 | 0.351 | 0.757 | 0.818 | 0.056 | 0.043 | 0.912 | 0.899 | 0.019 | 0.55 | 0.871 | 0.896 | 0.02 | 0.048 |
| D12S391 | 0.861 | 0.864 | 0.032 | 0.19 | 0.771 | 0.808 | 0.063 | 0.65 | 0.904 | 0.896 | 0.02 | 0.45 | 0.842 | 0.874 | 0.028 | 0.071 |
| D2S1338 | 0.911 | 0.894 | 0.02 | 0.763 | 0.871 | 0.872 | 0.03 | 0.356 | 0.88 | 0.878 | 0.027 | 0.23 | 0.906 | 0.877 | 0.027 | 0.929 |

The following table shows the Probability of paternity exclusion (PE) values of the NGM Detect™ kit STR loci individually and combined.

The PE value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing using the NGM Detect™ kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

**Table 17** Probability of paternity exclusion values for the NGM Detect™ kit STR loci

| Locus | African American (n = 330) | Asian (n = 153) | Caucasian (n = 343) | Hispanic (n = 368) |
|---|---|---|---|---|
| D10S1248 | 0.6623 | 0.5353 | 0.5649 | 0.4644 |
| D12S391 | 0.7401 | 0.631 | 0.8032 | 0.6588 |
| D16S539 | 0.5548 | 0.6063 | 0.5915 | 0.5623 |
| D18S51 | 0.7892 | 0.656 | 0.7557 | 0.7121 |
| D19S433 | 0.6332 | 0.5238 | 0.5135 | 0.6431 |
| D1S1656 | 0.7462 | 0.5703 | 0.8032 | 0.7338 |
| D21S11 | 0.728 | 0.6063 | 0.7264 | 0.7013 |
| D22S1045 | 0.7038 | 0.4795 | 0.4507 | 0.397 |
| D2S1338 | 0.814 | 0.7463 | 0.7498 | 0.7392 |
| D2S441 | 0.5228 | 0.5353 | 0.4986 | 0.5051 |
| D3S1358 | 0.4918 | 0.3976 | 0.5338 | 0.4689 |
| D8S1179 | 0.599 | 0.6063 | 0.6187 | 0.5381 |
| FGA | 0.728 | 0.8397 | 0.6632 | 0.7175 |
| SE33 | 0.8639 | 0.88 | 0.9231 | 0.8781 |
| TH01 | 0.5124 | 0.3424 | 0.5036 | 0.5381 |
| vWA | 0.6103 | 0.6186 | 0.6576 | 0.6276 |
| PEi | $7.8605 \times 10^{-9}$ | $1.3136 \times 10^{-7}$ | $1.2071 \times 10^{-8}$ | $7.3910 \times 10^{-8}$ |
| Combined | 0.9999999921 | 0.9999998686 | 0.9999999879 | 0.9999999261 |

A

# Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| Faint or no signal from both the DNA Control 007 and the DNA test samples at all loci, including the IQC markers | The incorrect volume of Master Mix or Primer Set was used. | Use the correct volume of Master Mix or Primer Set. |
| | The DNA Polymerase was not activated. | Repeat the amplification with an initial hold at 95°C for 1 minute. |
| | The Master Mix was not vortexed thoroughly before aliquoting. | Vortex the Master Mix thoroughly. |
| | The Primer Set was exposed to too much light. | Replace the Primer Set and store it protected from light. |
| | Evaporation. | Ensure that the plate is properly sealed with film and that a compression pad was used with the GeneAmp™ PCR System 9700. (A compression pad should not be used with other validated thermal cyclers.) |
| | The thermal cycler malfunctioned. | See the thermal cycler user manual and check the instrument calibration. |
| | Incorrect thermal cycler conditions were used. | Use correct thermal cycler conditions. |
| | A MicroAmp™ base was used with a tray/retainer set and tubes in GeneAmp™ PCR System 9700. | Remove the MicroAmp™ base. |
| | The tubes or plate were not seated tightly in the thermal cycler during amplification. | Push the tubes or plate firmly into the block after first cycle. |
| | The wrong PCR reaction tubes or plate were used. | Use MicroAmp™ Reaction Tubes with Caps or the MicroAmp™ Optical 96-well Reaction Plate for the GeneAmp™ PCR System 9700 or Veriti™ Thermal Cycler. |
| | Insufficient PCR product was electrokinetically injected. | Use correct genetic analyzer settings. |
| | Degraded formamide was used. | Check the storage of formamide. Do not thaw and refreeze multiple times. Try Hi-Di™ Formamide. |
| Positive signal from DNA Control 007 but partial or no signal from DNA test samples when IQC peaks are present and balanced | The quantity of test DNA sample is below the assay sensitivity. | Quantify DNA and (when possible) add 500 pg of DNA. For low concentration samples, add up to 15 µL of the DNA sample to the reaction mix. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Positive signal from DNA Control 007 but partial or no signal from DNA test samples when IQC peaks are present and balanced | The test sample DNA is severely degraded. | Use the Quantifiler™ HP DNA Quantification Kit or the Quantifiler™ Trio DNA Quantification Kit to evaluate sample quality during the quantification step. If DNA is degraded, reamplify with an increased amount of DNA or consider using the Precision ID GlobalFiler™ NGS STR Panel. |
| | The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration). | Redilute DNA using low-TE buffer (with 0.1 mM EDTA). |
| Positive signal from DNA Control 007 but partial or no signal from DNA test samples when IQC peaks are present and unbalanced | The test sample contains a high concentration of PCR inhibitor (for example, heme compounds, certain dyes). | Quantify the DNA, then use the minimum necessary volume of test sample DNA. |
| | | Wash the sample in a Centricon™-100 centrifugal filter unit. |
| | The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration). | Redilute DNA using low-TE buffer (with 0.1 mM EDTA). |
| Positive signal from DNA Control 007 and elevated signal from DNA test samples when IQC peaks are present and unbalanced | The quantity of the test sample DNA is in excess of recommended input amount, which can cause loss of balance in IQC peaks. | Quantify DNA, then use 500 pg. |
| More than two alleles present at a locus | Exogenous DNA is present in the sample. | Use appropriate techniques to avoid introducing foreign DNA during laboratory handling. |
| | Stutter product (–1 repeat unit position) was amplified. | Ensure that stutter filters are applied. |
| | Stutter filters apply to sample input amounts equal or greater than 250 pg. Samples well below the recommended input amount may exhibit stutters values exceeding the filters due to the stochastic effects of the PCR. | Increase the sample input above 250 pg, if possible. (Note, the optimum sample input is 500 pg.) |
| | The test sample contained mixed DNA. | If a mixed profile is not expected, check that laboratory protocols relating to cleanliness are followed. |
| | Incomplete 3´ A base addition (n–1 nt position) occured. | Include the final extension step of 60°C for 5 minutes in the PCR. |
| | | Remove amplified plate from storage (thaw if necessary) and place on thermal cycler at 60°C for 15 minutes. |
| | | Check the quantity of the original sample DNA to ensure input is less than 750 pg per reaction. Adjust input as necessary during re-amplification. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| More than two alleles present at a locus | | If the total amount of DNA in the reaction exceeds 1 ng, adjust the final extension time to 15 minutes to minimize incomplete 3′ A base addition. |
| | The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels. | Check that you are using the recommended number of PCR cycles. Repeat PCR amplification using reduced input DNA amount, or interpret the off-scale data according to your laboratory procedure. |
| | | Check that you are using the recommended injection conditions on the instrument. |
| | Poor spectral separation occurred. | Perform a spectral calibration. |
| | | Confirm that Filter Set J6-T modules are installed and used for analysis. |
| | Too much DNA was present in the reaction. | Use the recommended amount of template DNA: 500 pg for 30 PCR cycles. |
| | The double-stranded DNA was not completely denatured. | Use the recommended amount of Hi-Di™ Formamide and heat the sample plate at 95°C for 3 minutes. |
| Poor peak height balance | Incorrect thermal cycler conditions were used. | Use correct thermal cycler conditions. |
| Some but not all loci visible on electropherogram of DNA Test Samples | The PCR reaction volume you used is lower than the volume required for the amplification. | Use the correct PCR reaction volume: 25 µL |
| STR profiles contain many off-scale alleles | DNA quantification was not performed or not accurate. | Ensure that DNA quantification is accurate. |

# B Materials required but not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

## STR kit required materials

| Item | Source |
|---|---|
| NGM Detect™ PCR Amplification Kit, 200 reactions | A31832 |

## Sample preparation required materials

| Item | Source |
|---|---|
| GeneScan™ 600 LIZ™ Size Standard v2.0, 2 × 200 µL<br><br>**IMPORTANT!** Do not use GeneScan™ 350 ROX™, GeneScan™ 500 ROX™, or GeneScan™ 500 LIZ™ Size Standards with this kit. | 4408399 |
| Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) | Teknova T0223<br><br>or see "Prepare low-TE buffer" on page 19. |
| Hi-Di™ Formamide, 25-mL | 4311320 |

## Thermal cycler required materials

### ProFlex™ PCR System

| Item | Source |
|---|---|
| ProFlex™ 96-well PCR System | 4484075 |
| ProFlex™ 2 × 96-well PCR System | 4484076 |
| ProFlex™ 3 × 32-Well PCR System | 4484073 |

### Veriti™ Thermal Cycler

| Item | Source |
|---|---|
| Veriti™ 96-Well Thermal Cycler | 4479071 |
| (*Optional*) Tabletop centrifuge with 96-Well Plate Adapters | MLS |

### GeneAmp™ PCR System 9700

| Item | Source |
|---|---|
| GeneAmp™ PCR System 9700, 96-Well Silver | N8050001 |
| GeneAmp™ PCR System 9700, 96-Well Gold-Plated | 4314878 |
| Silver 96-Well Sample Block | N8050251 |
| Gold-Plated 96-Well Block | 4314443 |

## Genetic analyzer required materials

### 3500 Series Genetic Analyzer

| Item | Source |
|---|---|
| 3500 Series Data Collection Software 3.1 Upgrade (RUO) | A26287[1] |
| 3500 Series Data Collection Software 3.1 (RUO) | 4475183[1] |
| HID Updater 3500 Data Collection Software v2 | 4480670 |
| Anode buffer container (ABC) | 4393927 |
| Cathode buffer container (CBC) | 4408256 |
| POP-4™ Polymer (960 samples) for 3500/3500xL Genetic Analyzers | 4393710 |
| POP-4™ Polymer (384 samples) for 3500/3500xL Genetic Analyzers | 4393715 |
| DS-37 Matrix Standard Kit (Dye Set J6-T) | A31234 |
| Conditioning reagent | 4393718 |
| 8-Capillary array, 36 cm for 3500 Genetic Analyzers | 4404683 |
| 24-Capillary array, 36 cm for 3500xL Genetic Analyzers | 4404687 |
| 96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers | 4410228 |
| 8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers | 4410231 |
| 8-Strip Septa for 3500/3500xL Genetic Analyzers | 4410701 |

| Item | Source |
|---|---|
| 96-Well Septa for 3500/3500xL Genetic Analyzers | 4412614 |
| Septa Cathode Buffer Container, 3500 series | 4410715 |

[1] Contact your Thermo Fisher Scientific HID representative.

### 3130 Series Genetic Analyzer required materials

| Item | Source |
|---|---|
| 3130 Data Collection Software v4 | 4475105 |
| 3130*xl* Data Collection Software-v4 | 4475126 |
| 3130/3730 Data Collection Software-v4 6-Dye Module v1 | 4478404 |
| 96-Well Plate Septa | 4315933 |
| Reservoir Septa | 4315932 |
| 3130/3130*xl* Genetic Analyzer 16-Capillary Array, 36 cm | 4315931 |
| POP-4™ Polymer for the 3130/3130*xl* Genetic Analyzer | 4352755 |
| Running Buffer, 10× | 402824 |
| DS-37 Matrix Standard Kit (Dye Set J6-T) | A31234 |
| MicroAmp™ Optical 96-Well Reaction Plate | N8010560 |

## Analysis software required materials

### GeneMapper™ *ID-X* Software v1.5.2 patch

In addition to the GeneMapper™ *ID-X* Software listed in the following table, a v.1.5.2 patch is required. The patch enables full functionality of the data quality assessment tools when using the NGM Detect™ PCR Amplification Kit.

The patch is available for free download at **thermofisher.com/us/en/home/technical-resources/software-downloads/genemapper-id-x-software.html**.

### GeneMapper™ *ID-X* Software

| Item | Source |
|---|---|
| GeneMapper™ *ID-X* Software v1.5 Full Installation | A27884 |
| GeneMapper™ *ID-X* Software v1.5 Client Installation | A27886 |
| GeneMapper™ *ID-X* Software v1.4 Full Installation | 4479707 |
| GeneMapper™ *ID-X* Software v1.4 Client Installation | 4479711 |

# Miscellaneous required materials

## Plates and tubes

| Item | Source |
|---|---|
| MicroAmp™ 96-Well Tray | N8010541 |
| MicroAmp™ Reaction Tube with Cap, 0.2 mL | N8010540 |
| MicroAmp™ 8-Tube Strip, 0.2 mL | N8010580 |
| MicroAmp™ Optical 8-Cap Strips | 4323032 |
| MicroAmp™ 96-Well Tray/Retainer Set | 403081 |
| MicroAmp™ 96-Well Base | N8010531 |
| MicroAmp™ Clear Adhesive Film | 4306311 |
| MicroAmp™ Optical Adhesive Film | 4311971 |
| MicroAmp™ Optical 96-Well Reaction Plate | N8010560 |

## Laboratory supplies

| Item | Source |
|---|---|
| **Various procedures** | |
| Aerosol resistant pipette tips | MLS[1] |
| Microcentrifuge tubes | MLS |
| Pipettors | MLS |
| Tape, labeling | MLS |
| Tube, 50-mL Falcon™ | MLS |
| Tube decapper, autoclavable | MLS |
| Deionized water, PCR grade | MLS |
| Vortex | MLS |

[1] Major laboratory supplier

C

# PCR work areas



## Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using this kit for:

- Forensic DNA testing, see "Forensic Laboratories: Handbook for Facility Planning, Design, Construction, and Moving", National Institute of Justice, 1998
- Parentage DNA testing, see the "Guidance for Standards for Parentage Relationship Testing Laboratories", American Association of Blood Banks, 7th edition, 2004

The sensitivity of this kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified (Kwok and Higuchi, 1989).

Process samples carefully to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

**Note:** We do not intend these references for laboratory design to constitute all precautions and care necessary for using PCR technology.

## PCR setup work area materials

**IMPORTANT!** Do not remove these items from the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate nuclease-free tube (for master mix preparation)
- Microcentrifuge tube rack
- Pipette tips, sterile, disposable hydrophobic filter-plugged
- Pipettors

- Tube decapper, autoclavable
- Vortex

## Amplified DNA work area

**IMPORTANT!** Place the thermal cyclers in the Amplified DNA Work Area.

Use only the validated thermal cyclers listed in "Instruments and software compatibility" on page 15.

D

# Safety

**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

# Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

# Biological hazard safety

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  **www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**

# Documentation and support

## Related documentation

| Document title | Pub. No. |
|---|---|
| **STR kits** | |
| *NGM Detect™ PCR Amplification Kit – PCR Amplification and CE Quick Reference* | 100044088 |
| *NGM Detect™ PCR Amplification Kit – PCR Setup Quick Reference* | 100044087 |
| *Technical Note: Updated NGM Detect™ PCR Amplification Kit: Validation and Comparative Study*<br>**Note:** Go to **thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/GSD/Technical-Notes/ngm-detect-results-tech-note.pdf** to open the Technical Note. | — |
| **Quantification kits** | |
| *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* | 4485355 |
| **Thermal cyclers** | |
| *ProFlex™ PCR System User Guide* | MAN0007697 |
| *Veriti™ Thermal Cycler User Guide* | 4375799 |
| *GeneAmp™ PCR System 9700 Base Module User Manual* | 4303481 |
| **3500 Series Genetic Analyzer** | |
| *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v1 User Guide* | 4401661 |
| *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide* | 4476988 |
| *HID Updater 3500 Data Collection Software v2.0 User Bulletin* | NA |
| *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide* | 100031809 |
| *3500 Series Data Collection Software v3 User Bulletin: New Features and HID Validation Summary* | MAN0010812 |
| *3500 Series Data Collection Software v3.1 User Bulletin: New Features and HID Validation Summary* | MAN0014110 |
| **3130*xl* Series Genetic Analyzer** | |
| *3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* | 4352716 |
| *3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin* | 4363787 |
| *3130/3130xl Genetic Analyzers Getting Started Guide* | 4352715 |

| Document title | Pub. No. |
|---|---|
| *3130/3130xl Genetic Analyzers Quick Reference Card* | 4362825 |
| *3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide* | 4359472 |
| **GeneMapper™ *ID-X* Software all versions** | |
| *GeneMapper™ ID-X Software Bin Overlap User Bulletin* | 100029546 |
| **GeneMapper™ *ID-X* Software v1.0** | |
| *GeneMapper™ ID-X Software v1.0 Getting Started Guide— Basic Features* | 4375574 |
| *GeneMapper™ ID-X Software v1.0 Quick Reference— Basic Features* | 4375670 |
| *GeneMapper™ ID-X Software v1.0 Installation Guide* | 4476603 |
| *GeneMapper™ ID-X Software v1.0 Administrator Guide* | 4376327 |
| *GeneMapper™ ID-X Software v1.0 Reference Guide* | 4375671 |
| **GeneMapper™ *ID-X* Software v1.1** | |
| *GeneMapper™ ID-X Software v1.1 Getting Started Guide— Mixture Analysis Tool* | 4396773 |
| **GeneMapper™ *ID-X* Software v1.2** | |
| *GeneMapper™ ID-X Software v1.2 Verification Experiments and Installation Procedures User Bulletin* | 4462639 |
| *GeneMapper™ ID-X Software v1.2 Quick Reference— Mixture Analysis Tool* | 4426482 |
| *GeneMapper™ ID-X Software v1.2 Reference Guide* | 4426481 |
| **GeneMapper™ *ID-X* Software v1.3** | |
| *GeneMapper™ ID-X Software v1.3 Verification Experiments and Installation Procedures User Bulletin* | 4470483 |
| **GeneMapper™ *ID-X* Software v1.4** | |
| *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* | 4477684 |
| **GeneMapper™ *ID-X* Software v1.5** | |
| *GeneMapper™ ID-X Software v1.5 New Features and Verification User Bulletin* | 100031708 |
| *GeneMapper™ ID-X Software v1.5 Getting Started Guide— Basic Features* | 100031701 |
| *GeneMapper™ ID-X Software v1.5 Quick Reference— Basic Features* | 100031702 |
| *GeneMapper™ ID-X Software v1.5 Getting Started Guide— Mixture Analysis Tool* | 100031704 |
| *GeneMapper™ ID-X Software v1.5 Quick Reference— Mixture Analysis Tool* | 100031705 |
| *GeneMapper™ ID-X Software v1.5 Installation Guide* | 100031706 |
| *GeneMapper™ ID-X Software v1.5 Administrator Guide* | 100031703 |
| *GeneMapper™ ID-X Software v1.5 Reference Guide* | 100031707 |

# Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

# Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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