AmpF™ STR® NGM™ PCR Amplification Kit - Overview
The most advanced STR kits optimized for analysis of forensic casework and database samples in Europe

Data Quality Worth Sharing!
The AmpFLSTR® NGM™ Kit: Performance Enhanced!

- **Greater power of discrimination**
  - Amplifies the 12 loci in the European Standard Set plus D2S1338, D19S433, D16S539 & Amelogenin

- **Exceptional profile quality**
  - Implementation of the latest improvements in primer synthesis and purification procedures to produce an artifact-free baseline

- **Increased sensitivity**
  - Improved buffer formulation increases amplification efficiency and maximises signal, even for the minor contributor in mixed samples
  - Ability to amplify and analyze challenging samples without increasing cycle number or changing reaction conditions
  - Option of a 30 cycle protocol for greater flexibility

- **Maximum success with compromised samples**
  - Improved buffer formulation overcomes significant levels of inhibition and supports enhanced profile and locus balance, even when inhibitors are present
  - Concentration of loci in the low molecular weight region of the profile increases the power of discrimination in partial profiles
  - Works in conjunction with the MiniFiler™ Kit to recover the maximum number of ESS loci when amplifying highly degraded samples
The AmpFLeSTR® NGM™ Kit: Quality Maintained!

- **Maximized concordance with previously typed samples**
  - Maintains all STR primer sequences for loci common to the SGM Plus®, Identifiler®, & SEfiler Plus™ kits
  - Contains the additional primer for D8S1179 included in all AmpFLeSTR® kits since the Identifiler® kit

- **Optimal detection**
  - Uses the same highly optimized dye set as the Identifiler® & SEfiler Plus™ Kits

- **Familiar workflow**
  - Uses the same reaction volume and instrument workflow as the Identifiler® & SEfiler Plus™ Kits
  - Requirement for only a single kit & a single amplification protocol for both casework and database samples

- **Optimal locus spacing**
  - Use of proprietary and proven mobility modifier technology to maximize locus spacing
NGM™ Kit Configuration: Building Blocks for Success

**Components**
- Master mix
- Primer set
- Control DNA 007
- Allelic ladder

**Reaction Conditions**
- 25µL Reaction Volume
- 1 ng Input DNA
- 29/30 Cycle Amplification
- Reduced Cycling Time

**Size Standard**
- GeneScan™ 500 LIZ® or GeneScan™ 600 LIZ® v2.0

**Instruments**
- 9700 or Veriti™ PCR Systems, 310, 3100, 3130 & 3500 Series Analyzers

**Dye Set**
- G5 Dye Set, 5 Dye Chemistry
AmpF\textregistered STR\textsuperscript{TM} NGM\textsuperscript{TM} PCR Amplification Kit

Locus Configuration

Use of mobility modifiers permits optimal spacing of loci
AmpFISTR® NGM™ PCR Amplification Kit
Allelic Ladder
AmpF™STR® NGM™ PCR Amplification Kit 1ng Control DNA 007
# AmpFLESTR® Kit Configurations

## Comparison of Pi Values

<table>
<thead>
<tr>
<th>Kit Configuration</th>
<th>Hispanic</th>
<th>African American</th>
<th>US Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGM Plus® Kit</td>
<td>Not Available</td>
<td>7.9 x 10^{-14}</td>
<td>2.99 x 10^{-13}</td>
</tr>
<tr>
<td>SEfiler™ Plus Kit</td>
<td>Not Available</td>
<td>6.47 x 10^{-15}</td>
<td>7.46 x 10^{-14}</td>
</tr>
<tr>
<td>Identifiler® Kit</td>
<td>7.65 x 10^{-18}</td>
<td>1.31 x 10^{-18}</td>
<td>5.01 x 10^{-18}</td>
</tr>
<tr>
<td>MiniFiler™ Kit</td>
<td>1.05 x 10^{-10}</td>
<td>6.52 x 10^{-11}</td>
<td>8.21 x 10^{-11}</td>
</tr>
<tr>
<td>Identifiler® Kit &lt; 200bp (+TPOX)</td>
<td>7.32x 10^{-08}</td>
<td>2.01x 10^{-08}</td>
<td>6.10x 10^{-08}</td>
</tr>
<tr>
<td>NGM™ Kit</td>
<td>1.60 x 10^{-19}</td>
<td>4.61 x 10^{-20}</td>
<td>2.21 x 10^{-19}</td>
</tr>
<tr>
<td>NGM™ Kit &lt; 200 bp (+D21S11)</td>
<td>3.31^{-12}</td>
<td>8.75 x 10^{-13}</td>
<td>2.64 x 10^{-12}</td>
</tr>
</tbody>
</table>
### AmpF\(\ell\)STR® NGM™ PCR Amplification Kit Population Study Genotype Concordance

<table>
<thead>
<tr>
<th>AmpF(\ell)STR® Kit Comparison</th>
<th>Number of Individuals</th>
<th>Number of Loci</th>
<th>Number of Alleles</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGM™ Kit v Profiler Plus® Kit</td>
<td>1231</td>
<td>6</td>
<td>14772</td>
<td>100%</td>
</tr>
<tr>
<td>NGM™ Kit v SGM Plus® Kit</td>
<td>1231</td>
<td>11</td>
<td>27082</td>
<td>100%</td>
</tr>
<tr>
<td>NGM™ Kit v Identifiler® Kit</td>
<td>1231</td>
<td>11</td>
<td>27082</td>
<td>100%</td>
</tr>
<tr>
<td>NGM™ Kit v NGM SElect™ Kit</td>
<td>1231</td>
<td>16</td>
<td>39392</td>
<td>100%</td>
</tr>
</tbody>
</table>

The NGM™ Kit contains an additional reverse primer at the D8S1179 locus to accommodate a rare mutation identified in Chamorro and Filipino populations from Guam. This primer was developed for inclusion in the Identifiler® kit and is included as standard in all subsequent AmpF\(\ell\)STR® kits containing D8S1179. This primer is not found in the SGM Plus® kit.
AmpFℓSTR® Kit Primer Strategy

- AmpFℓSTR® Kit primer sequences and primer complements are maintained wherever possible to enable maximum concordance between data generated with different AmpFℓSTR® kits
  - Consistent genotypes between historical and contemporary data sets
  - Consistent genotypes between database and the majority of casework samples
  - Simplified analysis, interpretation and reporting of genotype results

- Changes are made to AmpFℓSTR® Kit primer sequences or primer complements only when the result is of significant benefit to the analysis of forensic samples
  - Reduction of amplicon size in the MiniFiler™ kit for the analysis of degraded samples
    > Only those markers demonstrated to perform poorly in degraded samples are re-engineered to minimise the number of alternate primer sequences in use in the laboratory
  - Improvement of genotyping accuracy for known mutations by the inclusion of degenerate primers
    > Potential for a homozygote to heterozygote change at the affected locus only
Promoting Performance and Concordance
NGM™ Kit Primer Information: D8S1179

- Rare mutation identified during development of the Identifiler® Kit
- Introduced into all subsequent AmpFISTR® Kits to address this mutation
- Kits containing the primer will yield heterozygote genotypes for samples containing the mutation

**Cause:** A single G-A transition in the primer binding region for the standard AmpFISTR® reverse D8S1179 primer
Promoting Performance and Concordance
NGM™ Kit Primer Information: Amelogenin

- Amelogenin primer sequences redesigned from previous AmpFLESTR® Kits to reduce cross species reactivity significantly
Promoting Performance and Concordance

NGM™ Kit Primer Information: Amelogenin, D2S441, D22S1045

- Kit updated in 2011 to include three SNP-specific primers to address mutations at the Amelogenin, D2S441 and D22S1045 loci

- Amelogenin mutation identified as a result of the redesign of the primers to improve cross-species reactivity, D2S441 and D22S1045 mutations reported by NIST

- Mutation Frequency
  - Amelogenin: Frequency ~0.9% of Caucasian genomes
  - D2S441: Frequency ~5.1% of Korean genomes
  - D22S1045: Frequency ~1.4% of African American genomes
# AmpFLESTR® Kit Primer Set Comparison

<table>
<thead>
<tr>
<th>Locus</th>
<th>Identifiler® Kit</th>
<th>SEfiler Plus™ Kit</th>
<th>NGM™ Kit</th>
<th>NGM SElect™ Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogenin</td>
<td>SGM Plus® Kit Primers</td>
<td></td>
<td>New F/R Primers to Improve Specificity (includes 1 x SNP-Specific Primer)</td>
<td></td>
</tr>
<tr>
<td>vWA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D16S539</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S1338</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3S1358</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D21S11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D18S51</td>
<td>SGM Plus® Kit Primers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D19S433</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S1179</td>
<td>SGM Plus® Kit Primers + 1 x SNP-Specific Primer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE33</td>
<td>N/A</td>
<td>Published F/R Primers</td>
<td></td>
<td>1 x SEfiler Plus™ Primer + 1 x New Primer</td>
</tr>
<tr>
<td>D10S1248</td>
<td></td>
<td></td>
<td></td>
<td>New Loci: Identical Primers</td>
</tr>
<tr>
<td>D1S1656</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D21S391</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D22S1045</td>
<td></td>
<td></td>
<td></td>
<td>New Loci: Identical Primers (includes 1 x SNP-Specific Primer per Locus)</td>
</tr>
<tr>
<td>D2S441</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Developmental Validation of the AmpF&STR® NGM™ PCR Amplification Kit

- Based on SWGDAM guidelines for manufacturers

- Studies included (among others)
  - Sensitivity
  - Reproducibility
    > Heterozygote Peak Height Ratio, Intra-color Balance
  - PCR inhibition
  - DNA degradation
  - Selection of Guard Band
    > Effect of Cycle Number, Annealing Temperature etc.
  - Reproducible Artifacts
  - Mixtures and Species Specificity
  - Instrument concordance (310 NT, 3100 and 3130 Systems)
    > Validated for use with the 3500 Series Genetic Analyzers during instrument validation
Greater Sensitivity

1 ng
500 pg
250 pg
125 pg
62.5 pg
32.25 pg
Increased Allele Recovery at Lower Input DNA Levels
Peak Height Ratio Maintained at Decreasing Input DNA Concentrations

Amplification Kit

Heterozygote Peak Height Ratio

1 ng, 500 pg, 250 pg, 125 pg
## Improved Performance in the Presence of Inhibitors

<table>
<thead>
<tr>
<th>Loci</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Inhibitor</td>
<td></td>
</tr>
<tr>
<td>Humic Acid 40 ng/µL</td>
<td></td>
</tr>
<tr>
<td>Hematin 100 µM</td>
<td></td>
</tr>
<tr>
<td>Hematin 200 µM</td>
<td></td>
</tr>
</tbody>
</table>

### SEfiler Plus™ Kit

<table>
<thead>
<tr>
<th>Loci</th>
<th>No Inhibition</th>
<th>40 ng/µL Humic Acid</th>
<th>80 ng/µL Humic Acid</th>
<th>100 µM Hematin</th>
<th>200 µM Hematin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

### Identifiler® Kit

<table>
<thead>
<tr>
<th>Loci</th>
<th>No Inhibition</th>
<th>40 ng/µL Humic Acid</th>
<th>80 ng/µL Humic Acid</th>
<th>100 µM Hematin</th>
<th>200 µM Hematin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

### NGM™ Kit

<table>
<thead>
<tr>
<th>Loci</th>
<th>No Inhibition</th>
<th>40 ng/µL Humic Acid</th>
<th>80 ng/µL Humic Acid</th>
<th>100 µM Hematin</th>
<th>200 µM Hematin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

Green = Full Profile, Red = Allelic Dropout

9/7/2011 | © Life Technologies™
Improved Performance on Degraded Samples

The longer loci which fail due to extreme degradation may be recovered using the MiniFiler™ Kit.
The Importance of Mobility Modifiers: Amplicon Mobility v Amplicon Size for the NGM™ Kit

Amplicons can be engineered as short as possible while increasing the run size on the CE platform to ensure optimal locus spacing.
The Importance of Mobility Modifiers:
Amplicon Mobility v Amplicon Size for the MiniFiler™ Kit

Amplicon Mobility

Amplicon Size

Together, the NGM™ & MiniFiler™ Kits provide an unparalleled solution for the amplification of highly degraded samples.
A Powerful Solution for Degraded Samples: The NGM™ & MiniFiler™ Kits

Physical amplicon size ranges when using the NGM™/MiniFiler™ Kit Combination
Maximum Profile Quality: Baseline Comparisons Between the NGM™, SGM Plus® & Identifiler® Kits
Maximum Profile Quality: Mixture Interpretation

Major Component: 775 pg; Minor Component 125 pg

Full profile obtained for the minor contributor
Performance on Casework Samples

All Data Courtesy of Forensic Laboratories
Bone sample amplified using the Identifiler® Kit

Extraction: Qiagen QIAamp DNA MiniKit; DNA Input Concentration: Not Detected;
Cycle Number = 28

Data Courtesy of RIS Carabinieri Roma, Italy
Bone sample amplified using the NGM™ Kit

Extraction: Qiagen QIAamp DNA MiniKit; DNA Input Concentration: Not Detected; Cycle Number = 29

Data Courtesy of RIS Carabinieri Roma, Italy
Swab from a cap headband amplified using the SGM Plus® Kit

Extraction: Chelex; DNA Input Concentration: 0.02 ng; Cycle Number = 28

Data Courtesy of National Bureau of Investigation, Forensic Laboratory, Finland
Swab from a cap headband amplified using the NGM™ Kit

Extraction: Chelex; DNA Input Concentration: 0.02 ng; Cycle Number = 29

Data Courtesy of National Bureau of Investigation, Forensic Laboratory, Finland
Swab from a window handle amplified using the SGM Plus® Kit

Extraction: Organic; DNA Input Concentration: Not Detected; Cycle Number = 28

Data Courtesy of Eesti Kohtuekspertiisi Instituut, Tallinn, Estonia
Swab from a window handle amplified using the NGM™ Kit

Extraction: Organic; DNA Input Concentration: Not Detected; Cycle Number = 29

Data Courtesy of Eesti Kohtuekspertiisi Instituut, Tallinn, Estonia
Swab from a car dashboard amplified using the SEfiler Plus™ Kit

Extraction: Qiagen QIAamp DNA Mini kit; DNA Input Concentration: Not Known; Cycle Number = 30

Data Courtesy of a German State Police Laboratory
Swab from a car dashboard amplified using the NGM™ Kit

Extraction: Qiagen QIAamp DNA Mini kit; DNA Input Concentration: Not Known; Cycle Number = 29

Data Courtesy of a German State Police Laboratory
Sample from a fired bullet head amplified with the Identifiler® Kit

Extraction: Nucleospin Columns; DNA Input Concentration: 0.11 ng; Cycle Number = 28

Data Courtesy of IGNA, France
Sample from a fired bullet head amplified with the NGM™ Kit

Extraction: Nucleospin Columns; DNA Input Concentration: 0.11 ng; Cycle Number = 29

Data Courtesy of IGNA, France
AmpFISTR® NGM™ PCR Amplification Kit
Impact on Laboratory Workflow

- **More information per sample**
  - Amplifies the 12 loci in the European Standard Set plus D2S1338, D19S433 and D16S539
  - Facilitates international comparability of profiles

- **Maximum reliability of results**
  - Proven Applied Biosystems product and data quality
  - ISO 13485 manufacturing, rigorous quality control testing
  - Validated according to DAB and SWGDAM guidelines
  - Optimized as part of an instrument-reagent-software system
  - Professional training and support

- **Reduced labor and decreased time to result**
  - Single amplification protocol for both casework and database samples
  - Increased first-pass success rate for difficult casework samples
  - Reduced time needed for data analysis due to production of highest quality profiles
  - Maximised concordance with data generated with previous AmpFISTR® kits
Thank You

© 2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners

For Research, Forensic or Paternity Use Only. Not intended for any animal or human therapeutic or diagnostic use