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Addressing the Unique Requirements of Human Identification Applications

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Human Identification differs from research in many ways

- **High public profile**
- **Routine, repetitive testing**
- **Substantial consequences of poor performance**
- **Validation key product requirement**
- **Regulatory considerations**
  - e.g. SWGDAM, ENSFI, NDIS
- **Aversion to stream of product updates/revisions**
- **Need for consistency between laboratories**
  - e.g. 15-20M database records globally

Challenges facing forensic laboratories

- Increasing sample volume
- Difficult and compromised samples
- Increasing database size
- Shortage of trained analysts
- Increasing quality standards and standardization
- Workflow bottlenecks
  - System validation
  - Sample preparation
  - Data analysis and report writing

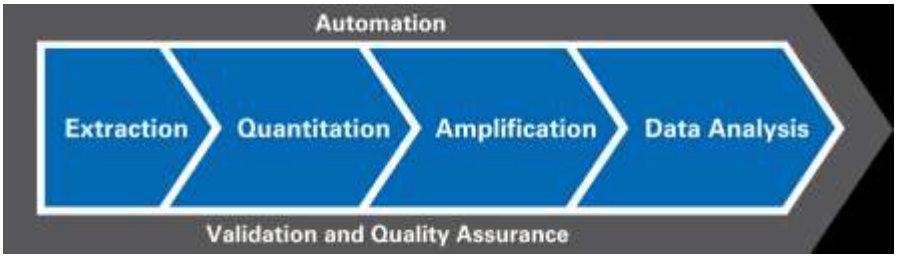
Expanding along the workflow

Sample Collection	DNA Extraction	Quantitation	Amplification	Detection	Data Analysis
					
	PrepFiler™ Forensic DNA Extraction kit	**Quantifiler® Human** **Quantifiler® Y** **7500 RT-PCR System** **HID RT-PCR Software**	 **9700 Thermal Cycler**	**310 System** **3130 System** **3130xl System**	**GeneMapper® *ID* v3.2.1 Software** **Genemapper® 3.3 software (Chinese)**

Ease of use
Ease of interpretation
Higher throughput
Streamlined workflow
Difficult samples

Automate Express™	Quantifiler® Duo	Next generation STR kits	3500 Genetic Analyser	GeneMapper® *ID-X* expert system software
				

The Forensic DNA Analysis Workflow



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The Forensic DNA Analysis Workflow

Short Tandem Repeat Assay Requirements

STR assays require:

- Small amplicon size <400 bp
- Specific amplification
- Minimal stutter by-products
- Maximal non-template-dependent adenylation (+A)
- Good peak height balance between loci
- Minimal artifacts

What are the challenges?

- Multiple primers in one reaction
  - Primer dimer
  - Specificity
    - Human genome and other non-human genomes interactions
    - Buffer components and thermal cycling conditions
- Peak morphology
  - +A addition, asymmetry, secondary structure
- Dye artifacts
- Limited “real estate”

Multiplex PCR Issues

Restrictions and Complications

- Fragment size for Capillary Electrophoresis
  - Allele size ranges from different loci in the same dye cannot overlap
- Primer Location
  - STRs are located in highly polymorphic and repetitive regions of the genome making primer specificity an issue
- Cascade effect
  - Change one primer and the entire reaction can be affected

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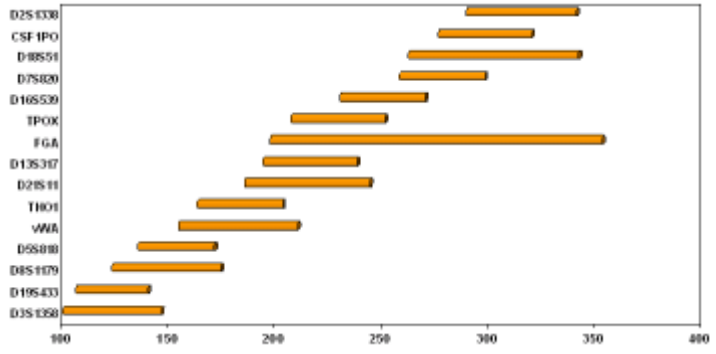
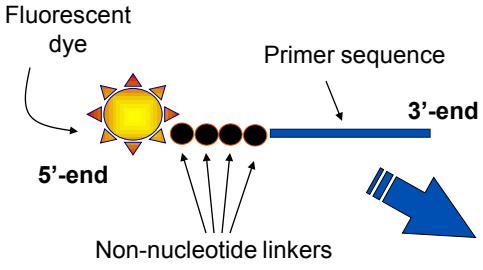
Overlapping amplicon sizes of Identifiler® Kit Loci



Challenging to space all loci in 100-400 bp region

Application of Non-Nucleotide Linkers to Shift Mobility

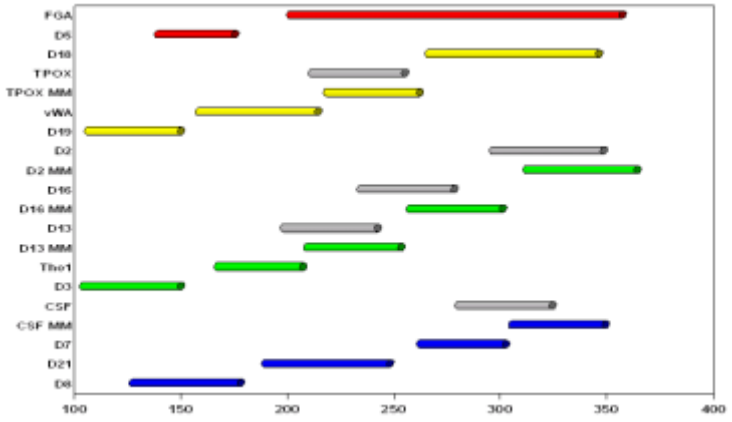


- **PCR amplification generates a labeled PCR product**
- ***For each linker unit added, there is an apparent migration shift of ~2.5 bp***

Non-nucleotide linkers are synthesized into the primer between the fluorescent dye and 5'-end of the primer sequence. During PCR amplification, the dye and linker are incorporated into the amplicon. With the added non-nucleotide linker, the mobility of the generated STR allele will be shifted to a larger apparent size during electrophoresis.

Figure 5.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Positioning of 15 STR Loci with Non-Nucleotide Linkers – Identifiler Kit



Next generation STR kit development

Recognizing the diverse demands of casework and databasing

Databasing Samples (Reference Samples)

- Predictable
- Standardized collection
- High yield, high quality DNA
- Single source
- Fresh

Casework Samples (Challenged Samples)

- Unpredictable
- Highly variable substrates
- Low yield, low quality DNA
- Mixed source
- Aged

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A New Generation of Application-Specific AmpFℓSTR® Kits

Identifiler® Kit

High Throughput Chemistry for Database Applications

AmpFℓSTR® Identifiler® ***Direct*** PCR Amplification Kit

High Performance Chemistry for Casework Applications

AmpFℓSTR® Identifiler® ***Plus*** PCR Amplification Kit

Key Requirements* of a Forensic Casework Analysis Kit

Ranking	Requirement
1	Amplify all loci in the presence of PCR inhibitors
2	Obtain NDIS uploadable profiles from degraded samples
3	Detect low levels of male DNA in mixed Male-Female samples
4	Obtain maximum number of alleles for the minor contributor in a mixture
5	Amplify enough highly discriminatory loci to minimize shared alleles in mixtures
6	Obtain NDIS uploadable profile from less than 100 pg
7	Obtain profiles with minimal stutter or artifact peaks

Casework samples often suffer from multiple challenges, all of which a chemistry designed specifically for casework analysis must be able to address

*Feedback from 125 Customer Interviews in North America

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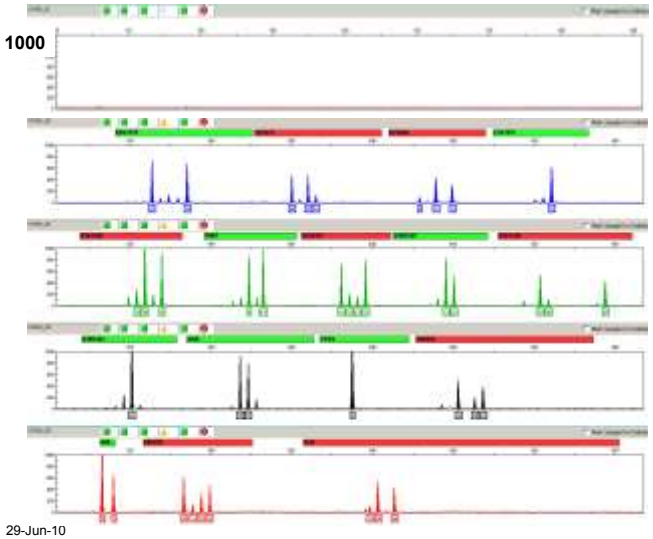
The AmpFℓSTR® Identifiler® Plus PCR Amplification Kit

AmpFℓSTR® Identifiler® Plus Kit

- Improved resistance to PCR inhibitors
  - Improved buffer formulation helps overcome significant levels of inhibition
- Enhanced sensitivity
  - Improved buffer formulation enables increased amplification efficiency maximizing signal, even for the minor contributor in mixed samples
  - Chemistry validated at two different PCR cycle numbers for standard and lower input amplifications
- Reduced occurrence of PCR artifacts
  - Improved primer manufacturing processes produces a significantly cleaner baseline to facilitate interpretation

www.appliedbiosystems.com/idplus

Leather Glove Swab: 1ng Input DNA, 28 Cycles

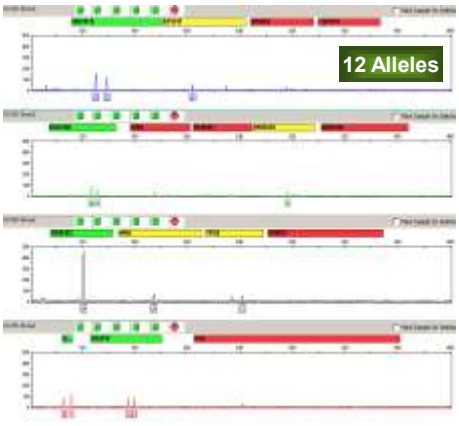


Identifiler® Kit No Profile

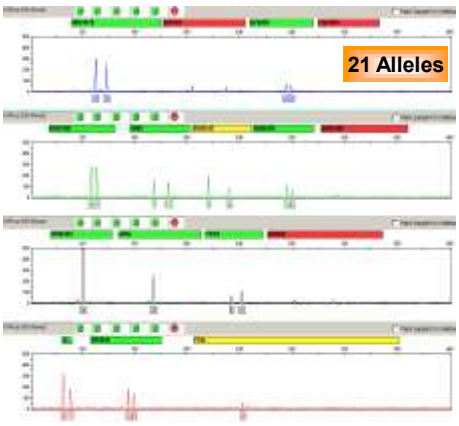
Identifiler® Plus Kit Mixed Profile

Missing Person Bone Sample: 173pg Input DNA, 29 Cycles

Identifiler® Kit



Identifiler® Plus Kit



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Quality in Manufacturing

Final Design and Formulations

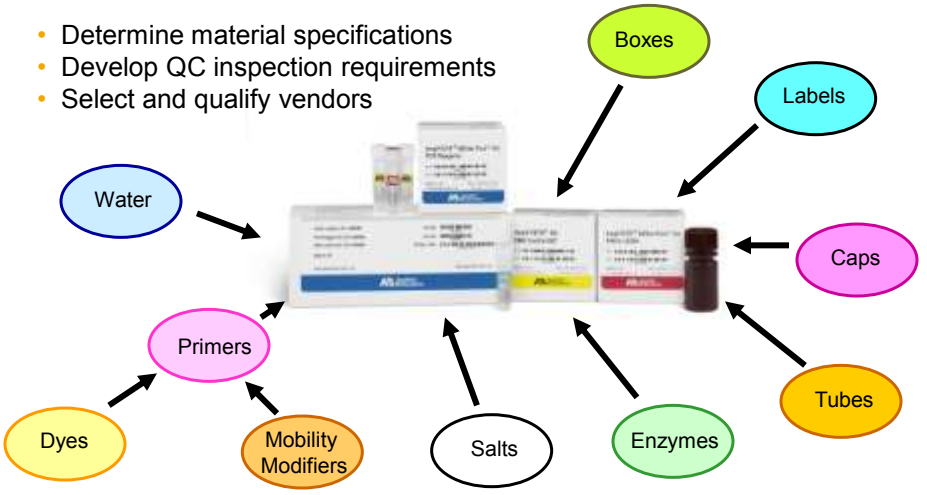
- Raw material specifications
- Formulations
  - Experiments to optimize buffer and PCR conditions
  - Guard band studies to determine allowed variances
  - Specification and QC method development to promote quality and lot to lot consistency
  - Scale-up to manufacturing size batches
- Allelic ladder development
- Confirmation through customer test sites

Product Transfer

- Select manufacturing location
  - Capacity – scale, throughput
  - Capability – facility setup, instrumentation, skill sets
- Ensure raw material supply
- Develop manufacturing and product documentation
- Determine product storage conditions and shelf life
- Build infrastructure
- Train manufacturing personnel
- Transfer support to Product Assurance Group

Raw Material Supply

- Determine material specifications
- Develop QC inspection requirements
- Select and qualify vendors



Product Stability

- Accelerated and real-time stability studies for all components

→ Product storage conditions
→ Shelf life determination

Quality Initiatives in Forensic DNA Analysis

- Joint ENFSI/SWGDAM/ BSAG position statement, September 2009
- Discussion of manufacturer contamination of disposable plastic-ware and other reagents
- Importance of appropriate QA/QC procedures in manufacturing facilities and individual DNA analysis laboratories

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AB HID Kits are manufactured in Warrington, UK

HID Kit Manufacturing Highlights

- Tens of thousands of AmpFℓSTR® and Quantifiler® forensic kits manufactured and sold in 2009
- Oligonucleotides are manufactured both in Pleasanton,CA and Warrington, UK
- Invested over $3.0 million on new automated tube fill and packaging machine and facility strictly for manufacture of Allelic Ladder
- General kit manufacturing separated from Allelic Ladder manufacturing by several miles

Duplicated Manufacturing sites required for all processes

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Oligonucleotide Production

- Over 11,000 sq ft of Manufacturing space in two locations
- 30-40 Manufacturing & QC Scientists devoted to HID oligonucleotide synthesis
- Over 20 Oligonucleotide synthesizers
- Over 35 Preparative & Analytical HPLC
- Stringent Process & Quality Control for all oligonucleotides

Automation of Manufacturing Lines

- Fully automated tube filling & capping
- No operator intervention required during a filling run

Minimal Interaction of Staff & Manufacturing Lines

- Clean suite entry is strictly controlled and recorded
- Entry permitted only to those staff members trained in gowning and clean room operations
- One-way flow procedure strictly enforced throughout the site

Adequate Protection of Products During Staff Interaction

- Extensive gowning precautions in operation at each site
  - Open product
    - Full body suit (hooded & booted), double gloves, face mask
  - Closed product
    - Lab coat, gloves, hair net, face mask

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Use of Class 10,000 Clean Rooms

- Dedicated suite of separate ISO 7/Class 10,000 clean rooms for formulation and tubing
- Fully automated tube filling machine enclosed in its own ISO 5/class 100 hepa-filtered enclosure
- Line clearance after manufacture of each lot
- Validated cleaning process
- Clean rooms tested for human DNA, microbial & fungal contamination to MHRA orange guide limit
- Validated, external contamination monitoring

Allelic Ladder Manufacturing

- Production site separated by several miles from general kit production facility
- Advanced Technology Air Filtration system
- Cross contamination avoided by work areas maintained under negative pressure.
- Production & packaging separated to avoid contamination
- Documented and validated cleaning and maintenance procedures

Instrumentation Custom designed and built solely for allelic ladder production.
This is the only machine of it's kind in the world designed to tube fill and packaging allelic ladder without contamination

Quality in Manufacturing: Tiered QC Testing

- AmpF*ℓ*STR® Kits undergo up to 7 levels of PCR-based quality control testing, from the individual components through to the completed kit
  - e.g. Singleplex primer, small batch primer set, large batch primer set, DNA controls, reaction mix

Post Production QC Analysis

- Each finished lot must pass a 3 tier batch review process prior to release
- Each finished lot undergoes a full functional test
  - Performance measured against detailed specifications & previous lots

On-Market Product Support

- Validation of new products and changes to existing products
  - Performed by validation specialists in Foster City
  - Ensures that products still function as designed
- Technical Troubleshooting
  - Experienced field support teams work with customers directly to resolve technical issues
  - Escalation to in-house Forensic Scientists, Product Assurance and Manufacturing teams to resolve quality issues quickly

Summary

- Challenges to the development of large multiplex STR assays with small amplicon size may be addressed using non-nucleotide linkers and 5-dye fluorescent technology
- Advances in buffer optimization have enabled us to address the most challenging forensic sample types
- Quality control processes in manufacturing ensure delivery of high quality products
- We continue to invest in facilities and staff to further improve the integrity of our manufacturing operation and the quality of our products

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AmpFISTR® Kits:

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Complete Integration Across the Forensic DNA Workflow

- Ease of Use
- Ease of Interpretation
- Higher Throughput
- Streamlined Workflow & Implementation
- Success with Difficult Samples

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Thank You!