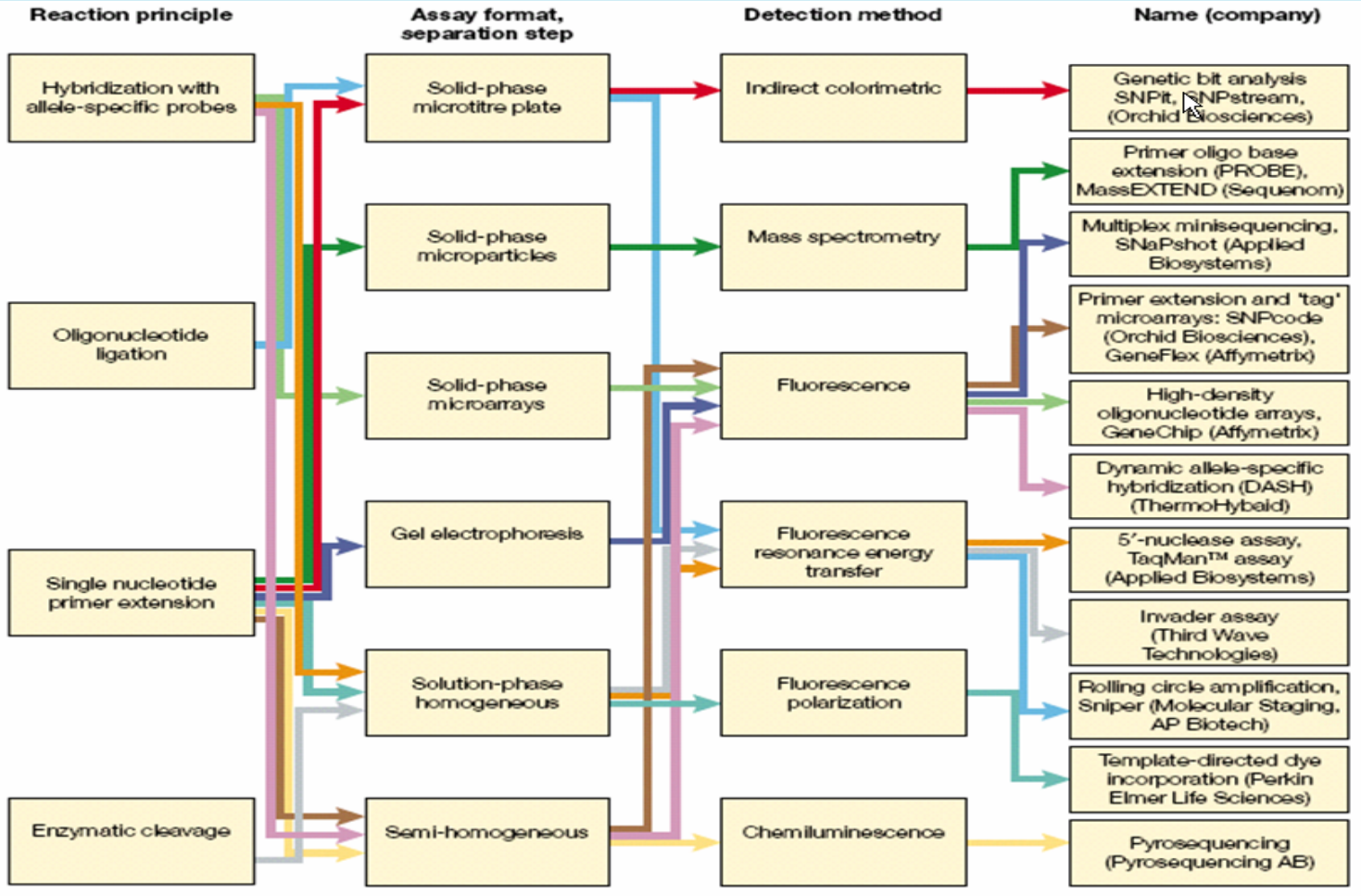
Technology Transition Workshop| *Manohar Furtado, Ph.D.*

GenPlex™: A CE-based SNP Detection Platform

Summary of SNP Detection Methods



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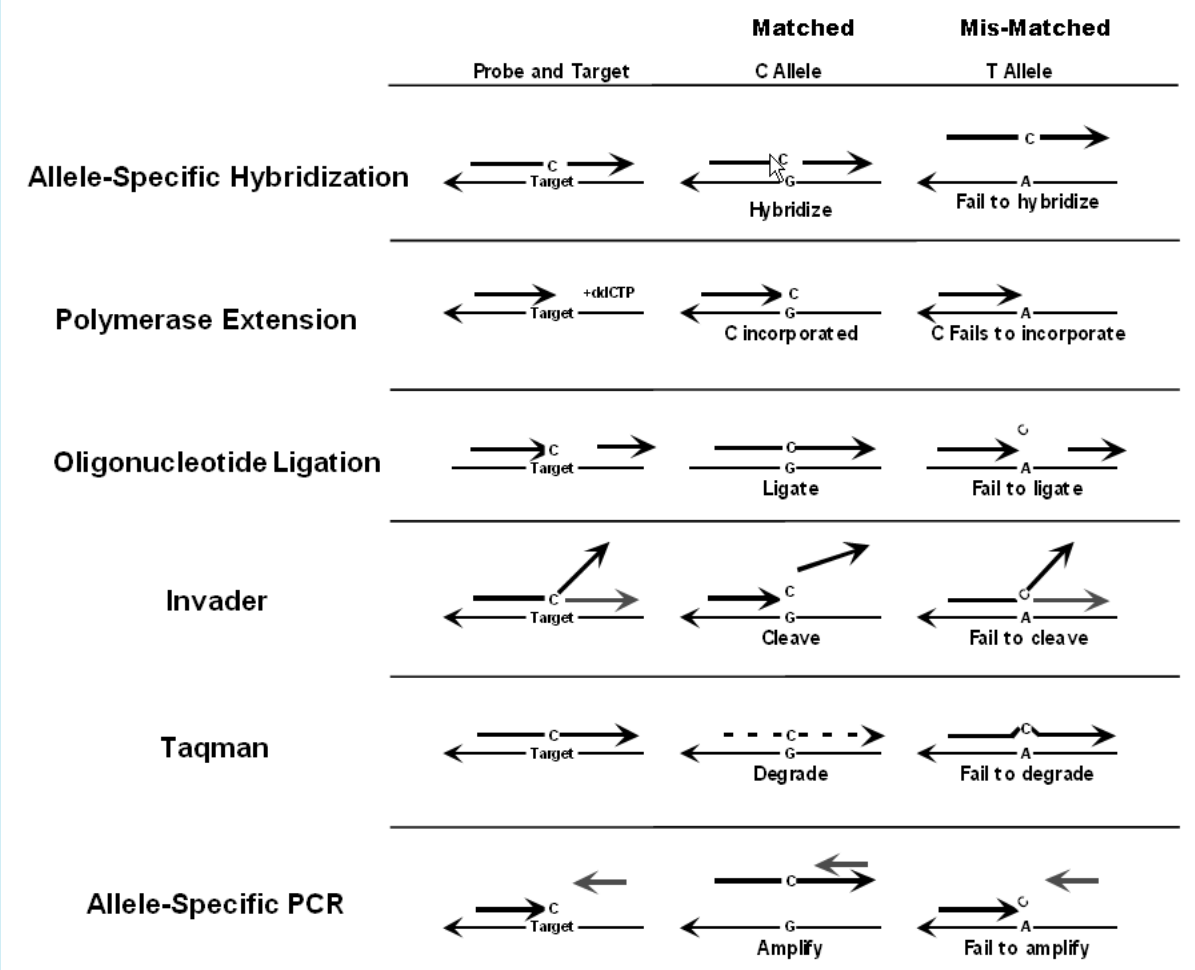
SNP / Mutation Detection



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Sample Types Work-Flow

Sample Types

- Swabs
- Blood
- Serum
- Semen
- Saliva
- Tissue
- Feces
- Urine
- Nasal wash
- Soil
- Food matrices
- Meat
- Milk
- Rinses
- Vegetables
- Beverage
- Creams
- Vaccines
- Injectables
- Water
- Air

Sample Pre-processing

- Sample Collection Devices
- Sample Clean-up
- Sample Conc. Devices

Nucleic Acid Isolation Station

- 6100
- GenePod
- Enigma
- IQuum, HandyLab®
- Network Bio, Microchip
- Robotics, KF, Tecan
- Irlekan, IGene

Amplification Detection Systems

Real-time Systems
7500, m2000, Aztec
Bax® Q7, Biotrove,
Fluidigm®,HandyLab®,
Enigma, IQuum

Thermal Cyclers
Fragment Analysis
Sequencing

CE Systems
31XX
Network Biosystems
Micro Chip/Mathies

Luminex®

E-TaqMan®
Mass Spec

Software Integration: Sample ID & tracking, LIMS, Data Analysis & Reporting, Archiving

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Mutation Detection Assay Formats Customer Needs

- **Low sample input (0.5 – 1 ng range), sensitivity**
- **Heterozygote detection / balance**
- **Accuracy, specificity, reproducibility**
- **Inhibitor tolerance**
- **Degraded samples / small amplicons**
- **Contamination controls**
- **Work-flow (ease of use)**
- **Detection software**
- **Assay cost**
  - **Ease of manufacturing**

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Mutation Detection Assay Formats

- **Single-plex PCR and sequencing (dye-labeled terminator) CE**
  - **MicroSeq® Fungal ID kits (330-500 bp)**
  - **ViroSeq® Genotyping System (FDA cleared) (1200 bp)**
    - **Detailed analysis of heterozygote / mixture detection capability for over 70 mutations**
    - **Detailed analysis of inhibitor effects**
  - **VariantSeQr™ (Cancer-related genes; > 65,000 amplicons)**
  - **MitoSeQr™ (Mito-sequencing)**

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Mutation Detection Assay Formats

- **Multiplex-PCR and SBE**
  - **SNaPShot® (dye-labeled terminator) CE**
  - **PinPoint (ddNTPs / no dye) MALDI**
- **Fundamental issue with dye-labeled terminators are incorporation efficiencies of polymerases on hetero / mixture detection capabilities**
  - **Mutation K103N**
    - **Detected at 5 to 10% mutant with a > 95% accuracy**
  - **Mutation D67N**
    - **Detected at 60% mutant with a < 90% accuracy**

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Mutation Detection Assay Formats

- **Multiplex-PCR and OLA on CE / Luminex™ / Qdot® Nanocrystals / Microarrays**
  - **Cystic fibrosis kit (FDA Cleared)**
  - **GenPlex® format for human ID etc.**
- **Multiplex-OLA and PCR**
  - **SNPLex® (variable SNP sets for disease association) CE**
  - **Universal TaqMan® (real-time allelic discrimination)**

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Mutation Detection Assay Formats

- **Allelic discrimination (multiplexed on arrays)**
  - **TaqMan® Arrays (48 to 384 single-plexes)**
  - **Open array formats (Mustang, Biotrove, Fluidigm®)**
- **Whole genome sequencing**
  - **The ultimate solution: Alec Jeffreys SOLiD™ System**

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Other Formats

- Real-time PCR (Taqman® and SYBR Green)
  - Gene expression profiling
  - Pathogen detection
  - Biothreat applications
- Allelic discrimination
  - Genotyping on TaqMan®
  - HCV genotyping (sequencing versus OLA versus TaqMan®)
- Allele-specific PCR formats
- Endonuclease cleavage
- Microarray-based formats
- LAMP
- FRET probes and melt curves
- Invader assay
- Scorpion probes (ARMS)

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Addressing Sample Requirements

- 1 ng of human DNA = 300 copies
- Sequencing formats
  - Need about 300 to 900 target genome copies
  - HIV 1000 copies (larger amplicon)
- Multiplex PCR formats (SBE, PCR – OLA formats) 40- to 50-plex
  - Short amplicons multiplexed: 150 to 300 target genome copies
  - < 1 ng for a 50-plex

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Addressing Sample Requirements

- **Methods requiring much higher sample input**
  - **SNPLex® (OLA-PCR) 30 to 40 ng / 48-plex reaction**
  - **Universal detector (OLA-TaqMan®) 3 ng / SNP or about 150 ng for a 50-plex**
  - **Allelic discrimination formats (high sample input)**
    - **TaqMan® 600 to 900 copies / SNP: 100 ng for a 50-plex**
    - **Open arrays (300 copies / SNP): 50 ng for a 50-plex**

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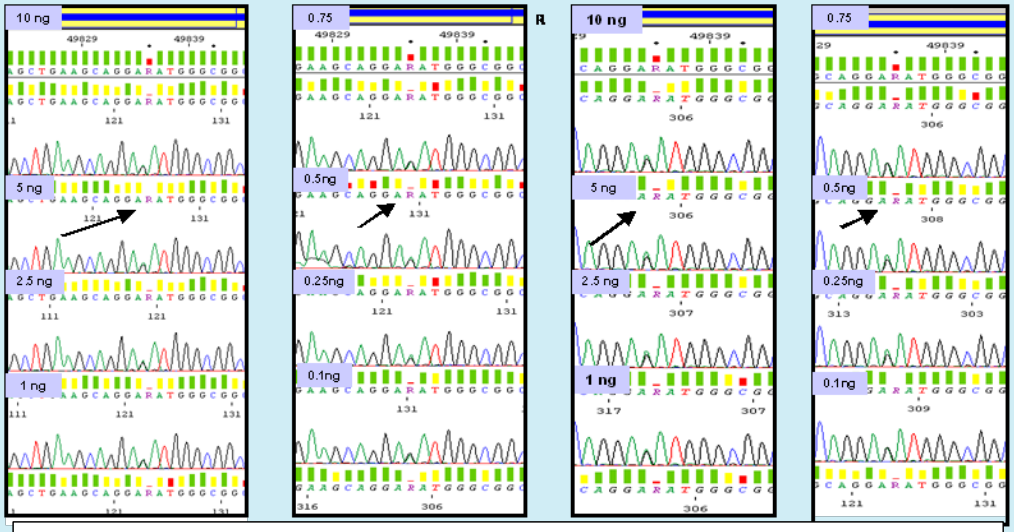
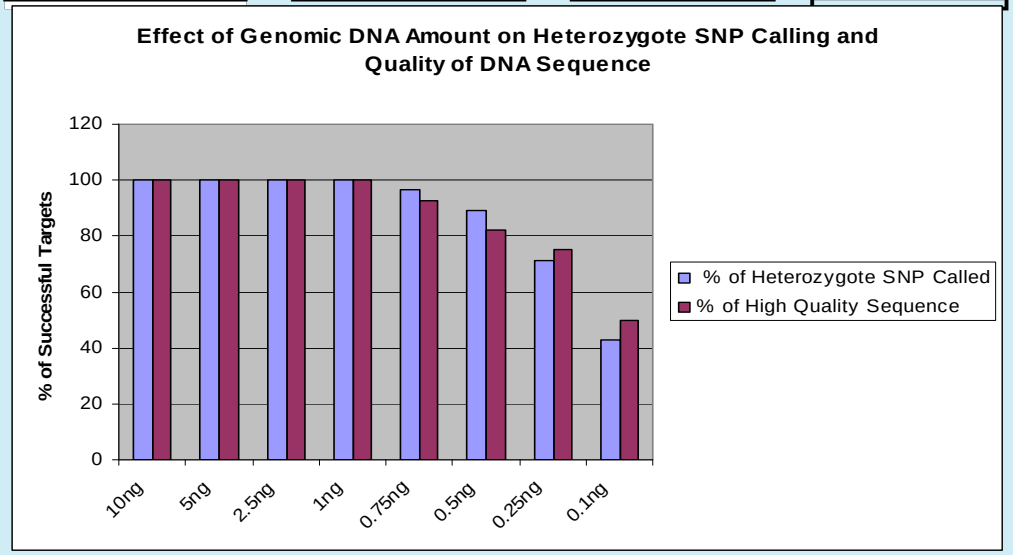
SeqScape® Analysis for PCR Template Titration Study





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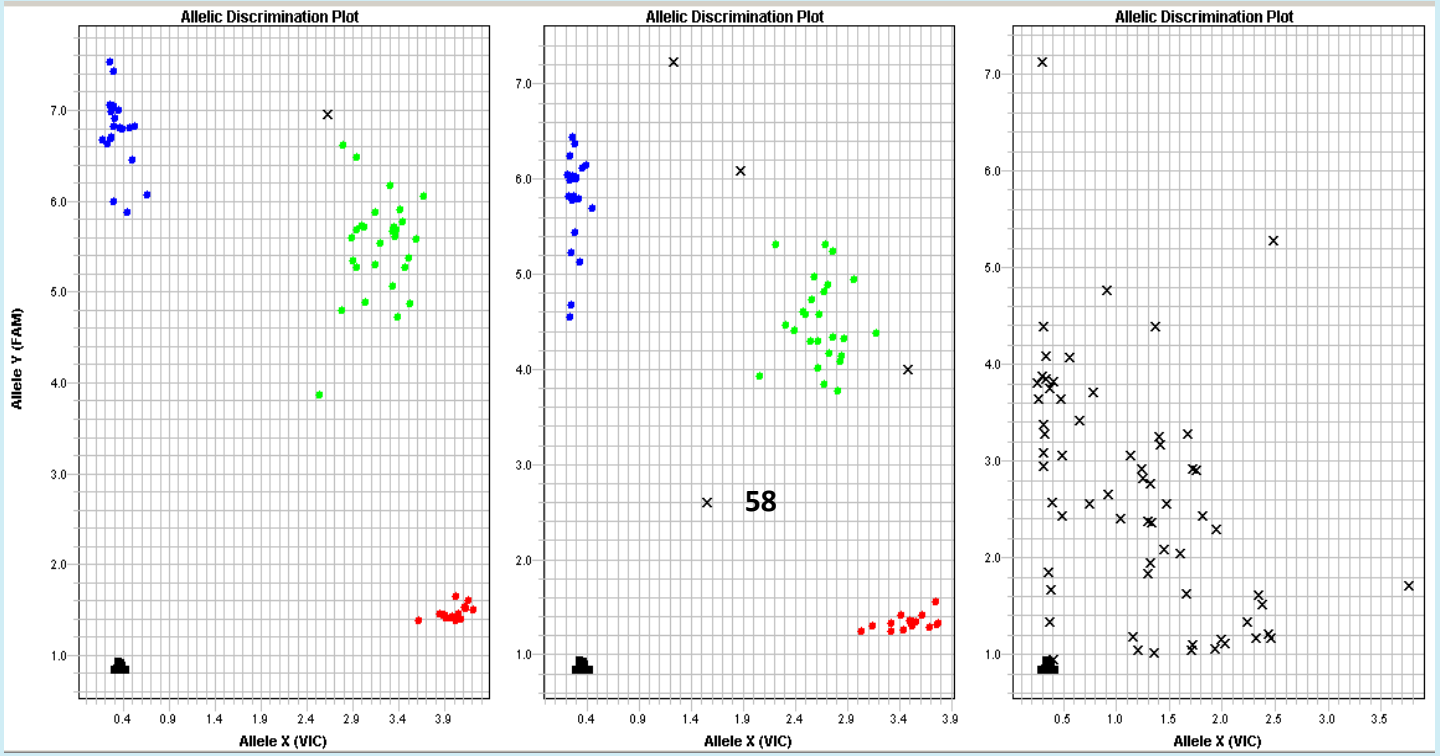
DNA Titration: Universal Detector

DNA NA17202

3 ng / SNP **0.3 ng / SNP** **0.03 ng / SNP**



Total: 144 ng **14.4 ng** **1.44 ng**

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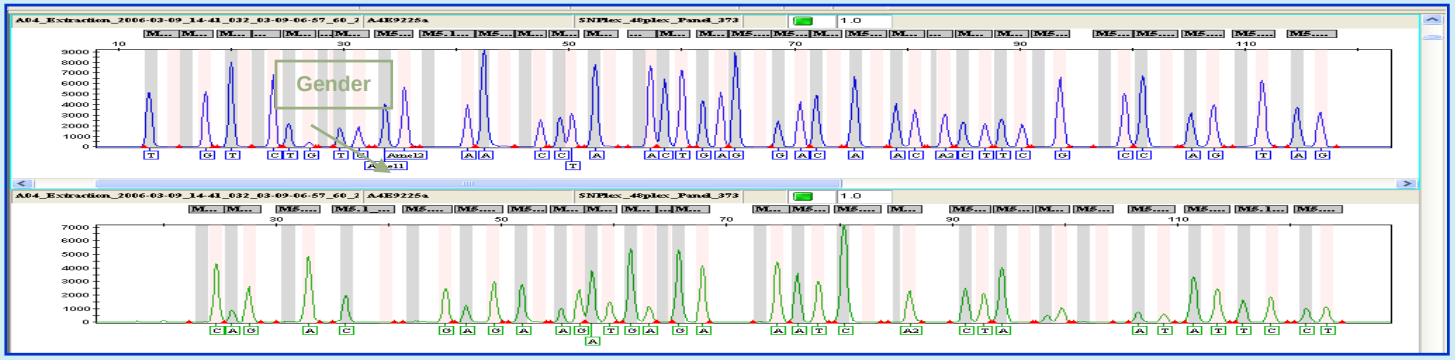
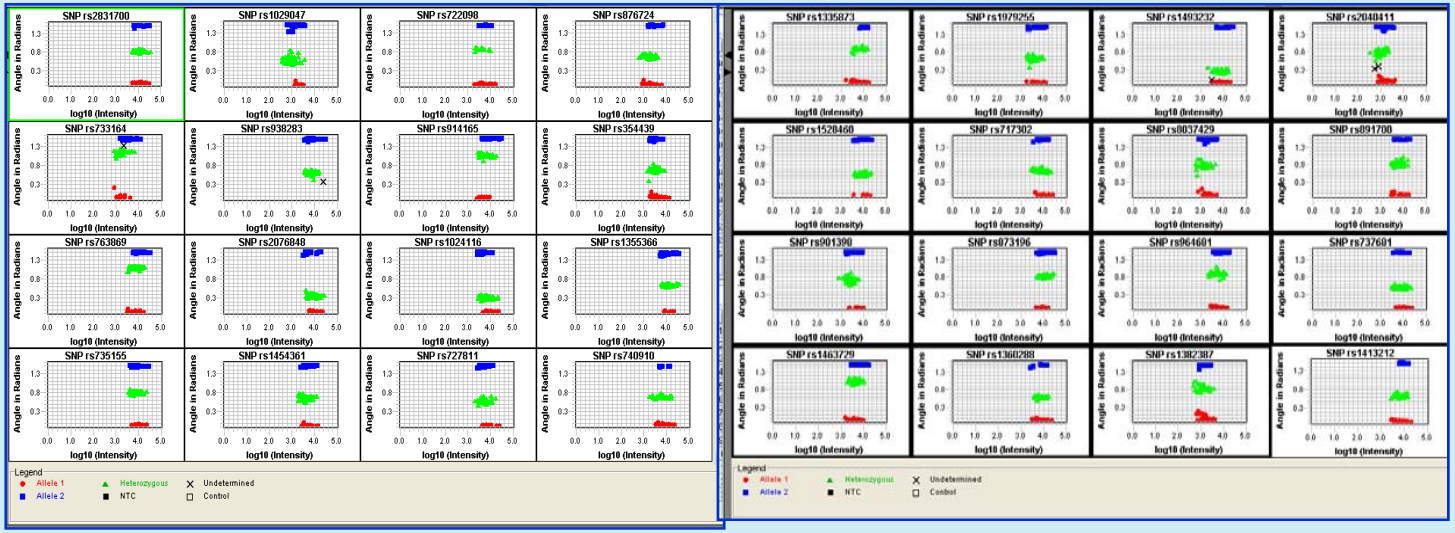
Protocol Development for 49-Plex Panel: A Sensitive Assay at 1 ng Input





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Pre-amplification

Tested for TaqMan® assays:

ITN	1000-plex
Body fluid ID	28-plex
Sequencing	24-plex
Identifiler	17-plex

heterozygote balance

Split sample for 10 assays

2 copies / assay

Limited sample: about 20 copies

10 cycle pre-amplification

Split sample for 1000 assays

20 copies / assay

As many as 100 to 1000 targets

Sample about 20,000 copies

TaqMan® detection limit of 5 to 10 copies / assay

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Carry-over Contamination in PCR

- **Exponential amplification followed by exponential amplification**
  - **Nested PCR**
  - **Pre-amplification by PCR followed by TaqMan®**
  - **Pre-amplification by PCR followed by PCR-OLA or OLA PCR**

needs containment

- **Exponential amplification followed non-exponential amplifications / other detection formats**
  - **PCR amplification followed by exo-SAP then cycle sequencing**
  - **PCR amplification followed by exo-SAP the OLA**
  - **PCR amplification followed by single–based extensions**
- **Detection instrument dynamic range**
  - **CE about 2 logs**
  - **TaqMan® about 6 logs**
  - **Luminex® about 4 logs**

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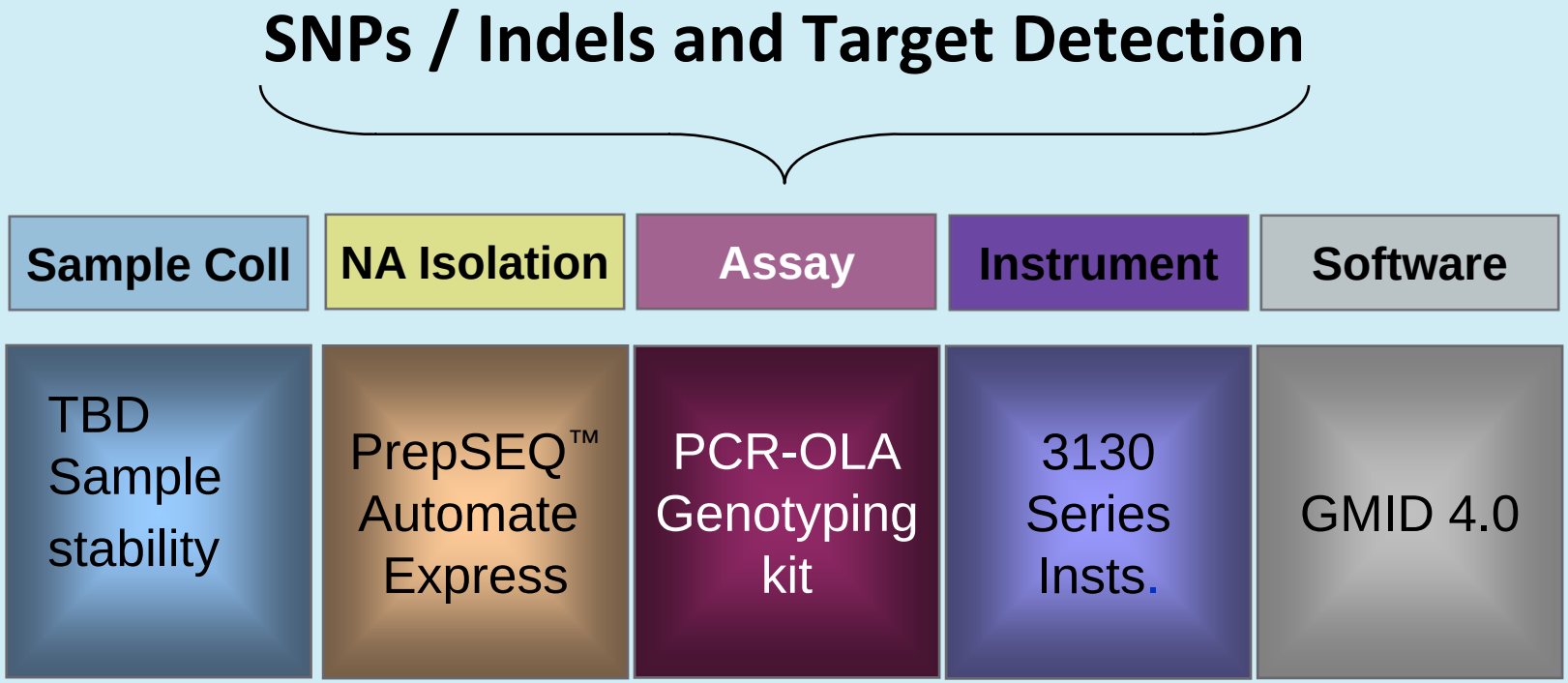
Complete Solution: Sample to Answer



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Sample Preparation Systems

- **Direct lysis in PCR compatible buffers**
- **Column-based**
  - **Rapid spin column**
  - **LySep column**
- **PrepSEQ™ & PrepFiler™ Kits**
  - **Manual**
  - **KingFisher®**
  - **Tecan**
  - **Automate Express**

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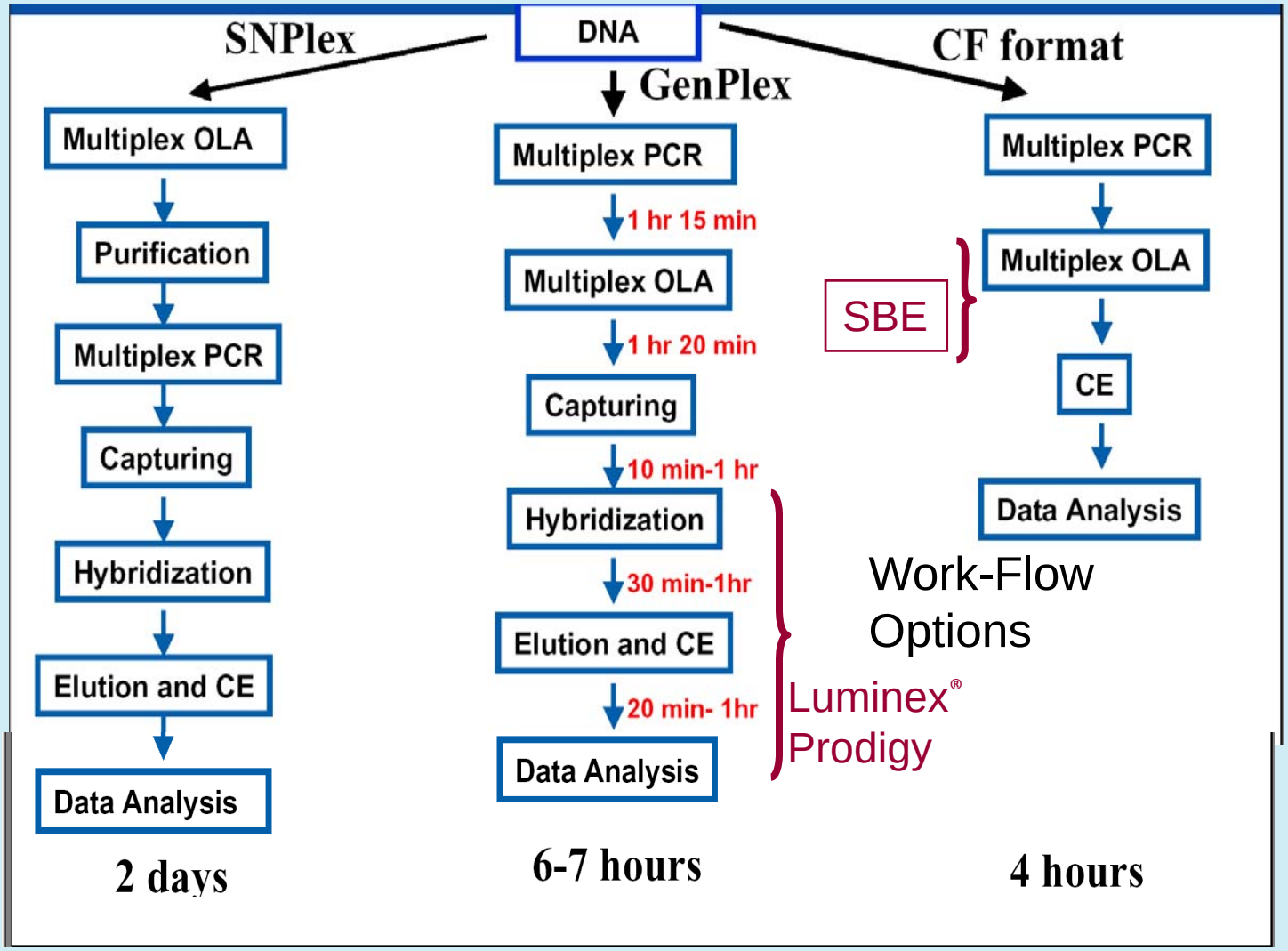

2007

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Chemistry Overview – The GenPlex™ Genotyping System

DNA

↓

PCR amplification (multiplex)

↓

Kinase / OLA reaction

↓

Biotin based capture

↓

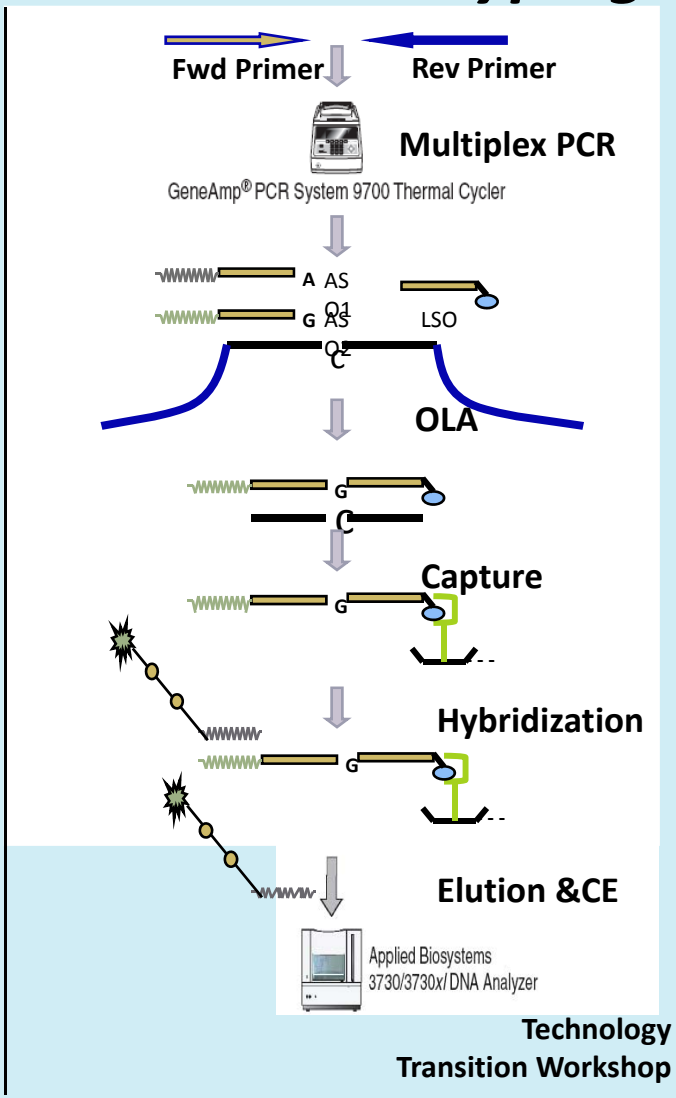
ZipChute® hybridization **4 - 5 hours**

↓

ZipChute® elution and CE

↓

Data analysis by GeneMapper®



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Pros and Cons

- **Pros**
  - **High sensitivity**
  - **Rapid time-to-result turn around time, < 7 hours**
  - **OLA reaction targets PCR products at high concentration; not gDNA; less complexity**
  - **Very useful for fixed / known sets of SNPs / mutations (eg. CF kit)**
  - **Highly specific; specificity at the PCR and OLA steps**
  - **Flexible; multiple applications possible**
- **Cons**
  - **< 1 ng gDNA**
  - **Multiplexed PCR needs some development**
  - **Not as useful for screening variable sets of SNPs needed for disease association studies**

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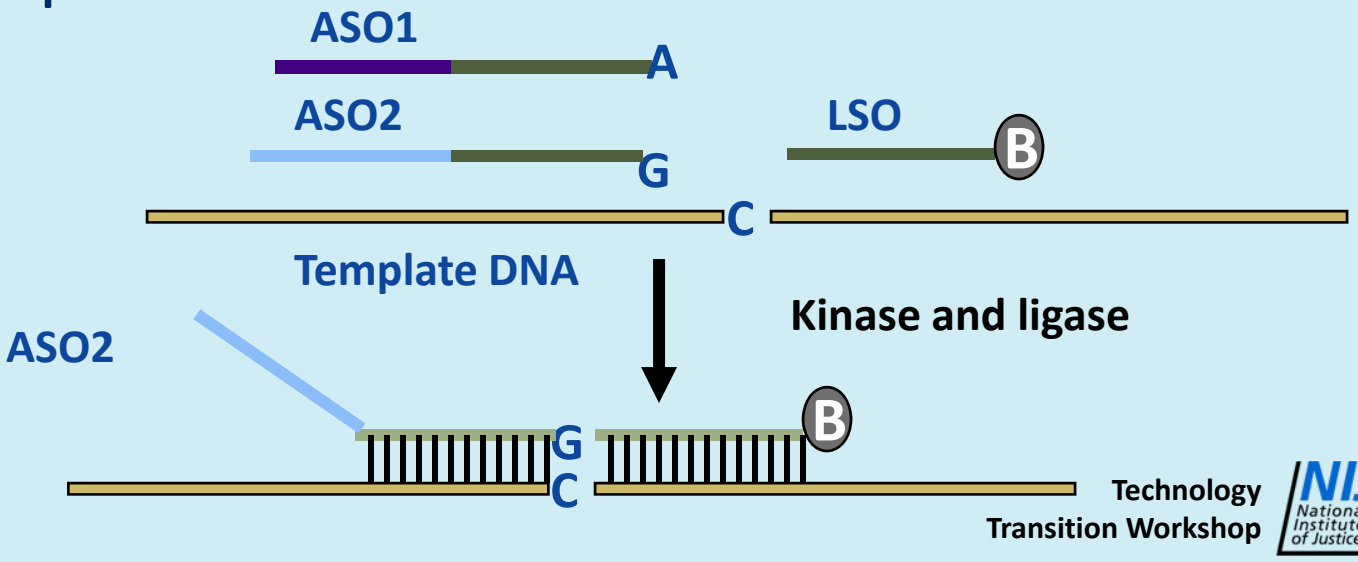
Three Unique OLA Probes per SNP Assay

OLA Principle



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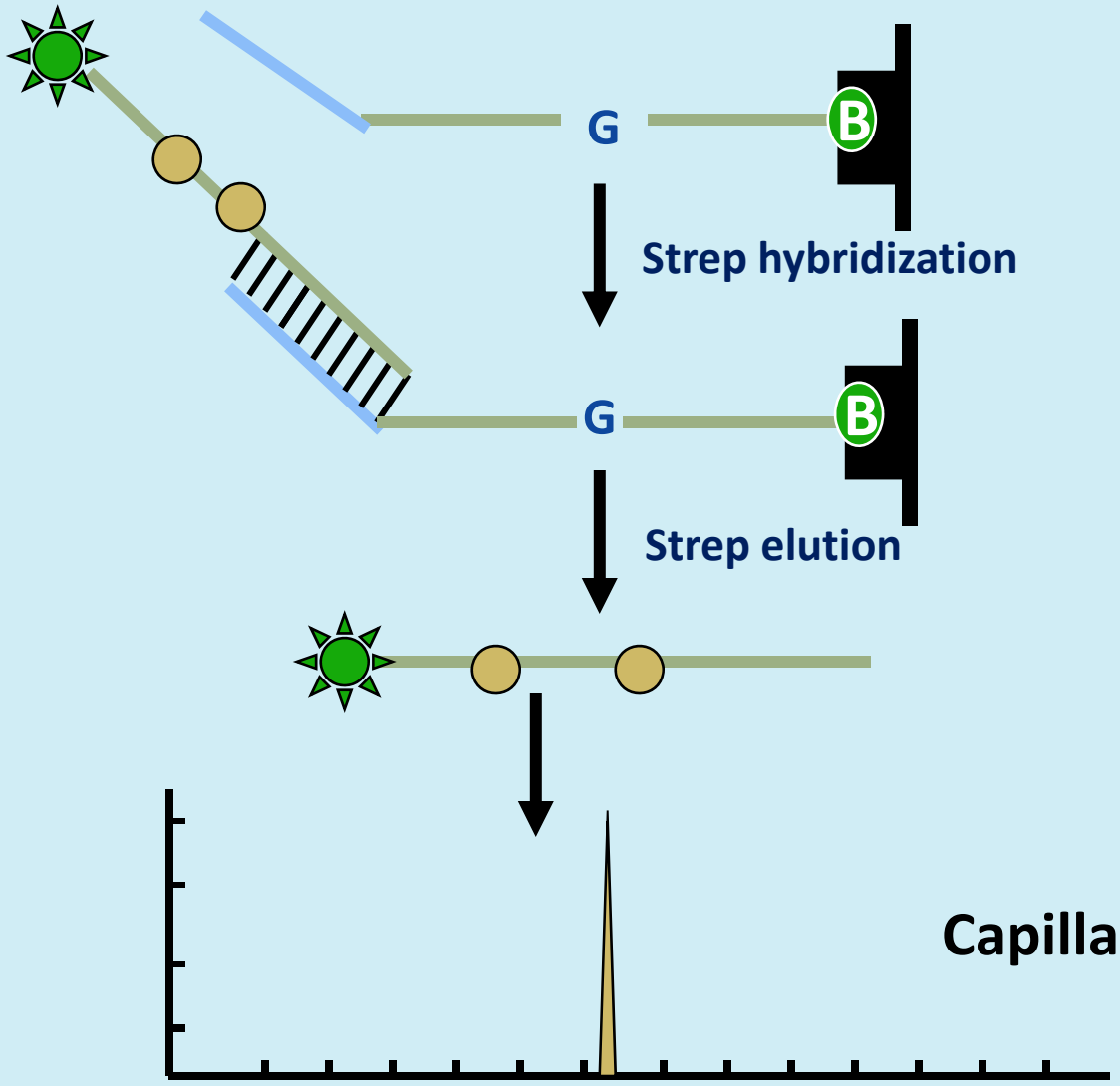

Capillary Electrophoresis

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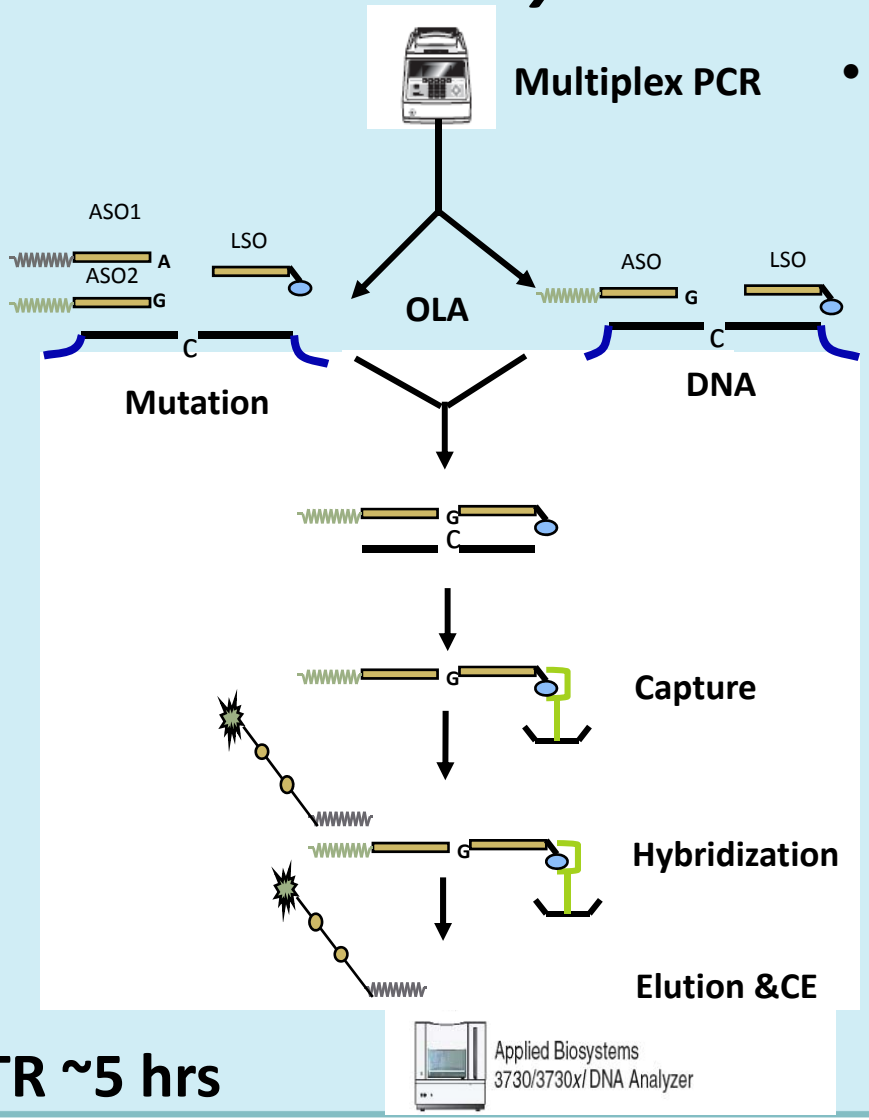
GenPlex™ Chemistry and Workflow: Current Format



- System features
  - High sensitivity
    - 100 pg gDNA
  - Highly specific
    - Specificity at the PCR and OLA steps
  - Rapid time-to-result turn around time
  - Automated
    - About 5 hours
  - Multiple applications possible
    - 48 SNPs
    - 96 DNA targets
    - 48 targets and 24 SNPs
    - Any combination

TTR ~5 hrs

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GenPlex™ Chemistry and Workflow: Other Formats

Eliminate the capture and wash steps

Multiplex PCR

↓

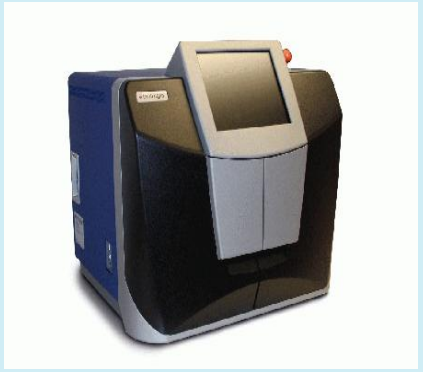
OLA

↓

Run on CE

Existing CF format

Run on Prodigy™ microarrays



Run on Luminex® bead-based

About 4 hrs

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GenPlex™ High Throughput Solution

DNA preparation

Multiplex PCR

1.30 hr

OLA reaction

1.20 hr

- **Binding** 10 min
- **Wash**
- **Hybridization** 30 min
- **Wash**

MULTIMEK™ 96 AUTOMATED 96-CHANNEL PIPETTOR

CE

Biomek-3000 and GenPlex SNP Genotyping in Forensic Genetics

Michael Stangegaard, Carmen Tomas, Anders J. Hansen, Rune Frank-Hansen, Claus Børsting, and Niels Morling*
University of Copenhagen, Copenhagen, Denmark

Stangegaard, M. et al., *JALA* (2008) 13(5) 297-303

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SNPforID Consortium

Electrophoresis 2006, *27*, 1713–1724

Juan J. Sanchez[1]
Chris Phillips[2]
Claus Børsting[1]
Kinga Balogh[3]
Magdalena Bogus[3]
Manuel Fondevila[2]
Cheryl D. Harrison[4]
Esther Musgrave-Brown[4]
Antonio Salas[2]
Denise Syndercombe-Court[4]
Peter M. Schneider[3]
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Niels Morling[1]

[1]Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark
[2]Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain
[3]Institute of Legal Medicine, Johannes Gutenberg University

Research Article

A multiplex assay with 52 single nucleotide polymorphisms for human identification

A total of 52 SNPs reported to be polymorphic in European, Asian and African populations were selected. Of these, 42 were from the distal regions of each autosome (except chromosome 19). Nearly all selected SNPs were located at least 100 kb distant from known genes and commonly used STRs. We established a highly sensitive and reproducible SNP-typing method with amplification of all 52 DNA fragments in one PCR reaction followed by detection of the SNPs with two single base extension reactions analysed using CE. The amplicons ranged from 59 to 115 bp in length. Complete SNP profiles were obtained from 500 pg DNA. The 52 loci were efficiently amplified from degraded samples where previously only partial STR profiles had been obtained. A total of 700 individuals from Denmark, Greenland, Somalia, Turkey, China, Germany, Taiwan, Thailand and Japan were typed, and the allele frequencies estimated. All 52 SNPs were polymorphic in the three major population groups. The mean match probability was at least 5.0×10^{-19} in the populations studied. Typical paternity indices ranged from 336 000 in Asians to 549 000 in Europeans. Details of

Sanchez, J. et al., *Electrophoresis* (2006) **27** 1713-1724

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Initial Strategy

Select p-arm and
q-arm SNPs on
each autosome

↓

Pilot multiplex designs
with 22 - 25plex SNaPshot™

Re-validate SNP allele
frequencies

↓

Review and modify the first 50
(on the basis of PCR performance and frequency)

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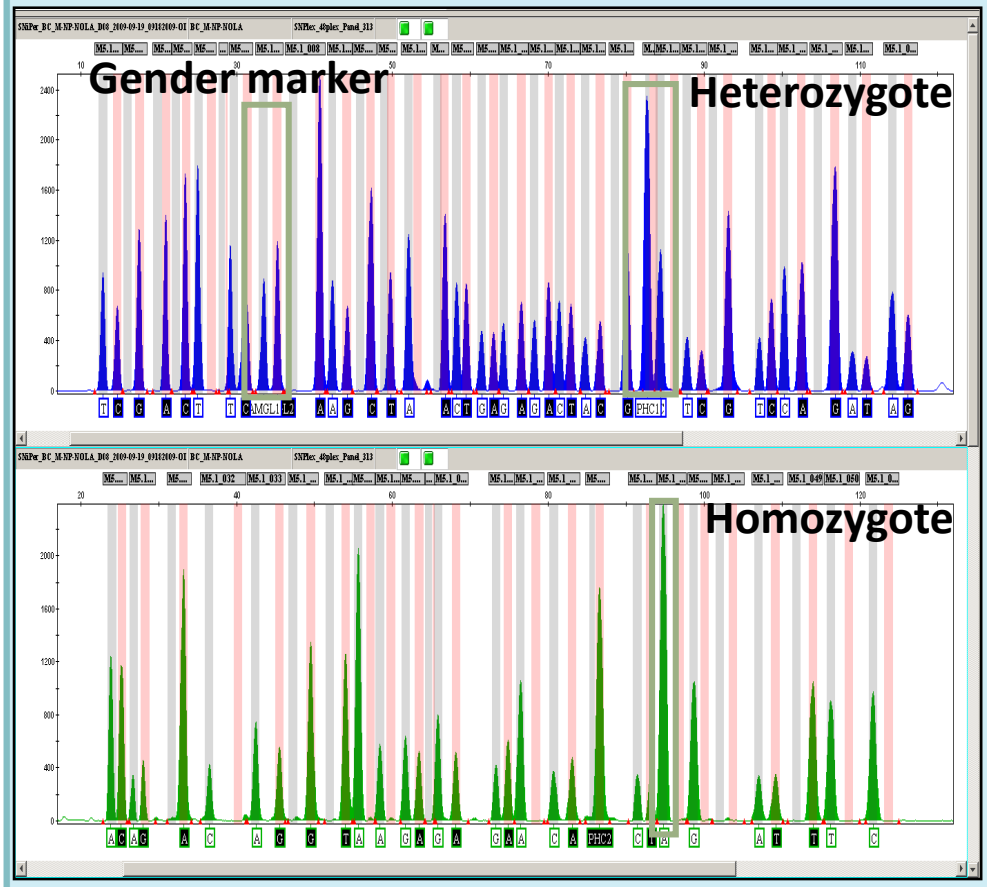
Custom GenPlex™ Kit for SNP and Indel Genotyping: 49-Plex



Custom Kit

Working prototype in the forensic area: SNPforID panel

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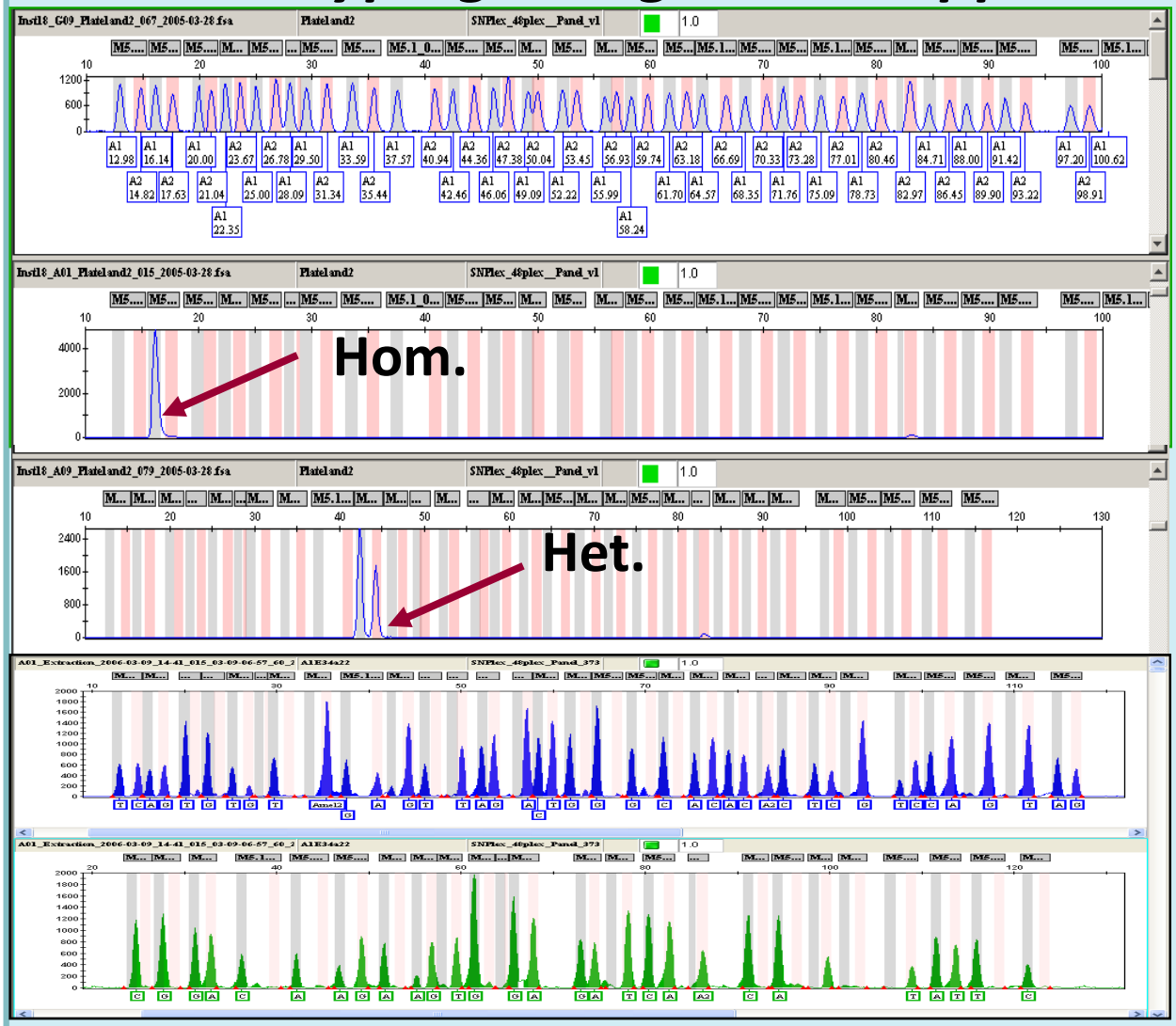
SNP Genotyping Using GeneMapper® Software 4.0



Allelic ladder

Singleplex

Multiplex (49-plex)

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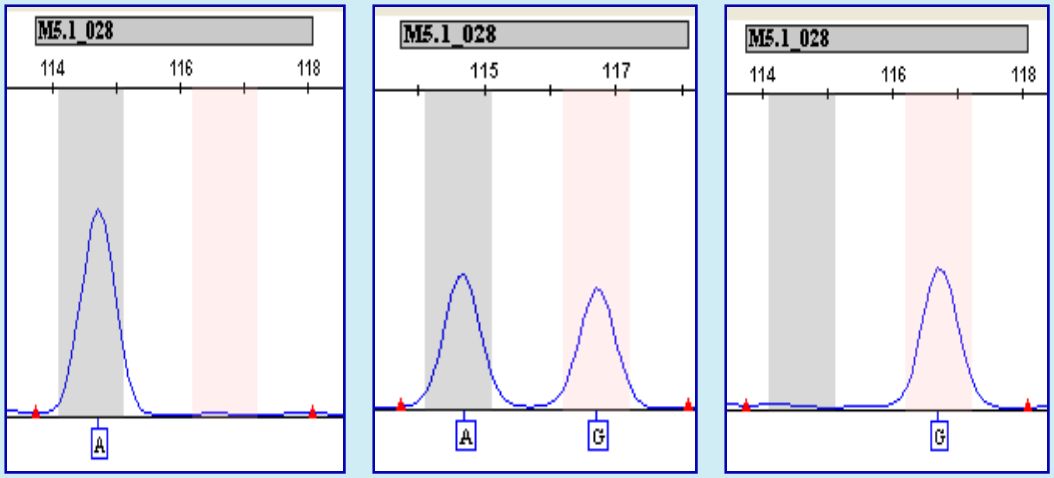
Automated Allele Calling – ZipChute® Probe Identification



Marker = SNP
Marker = 2 bins
Bin = Allele

	Hom. 1	Het.	Hom. 2
e.g.	AA	AG	GG

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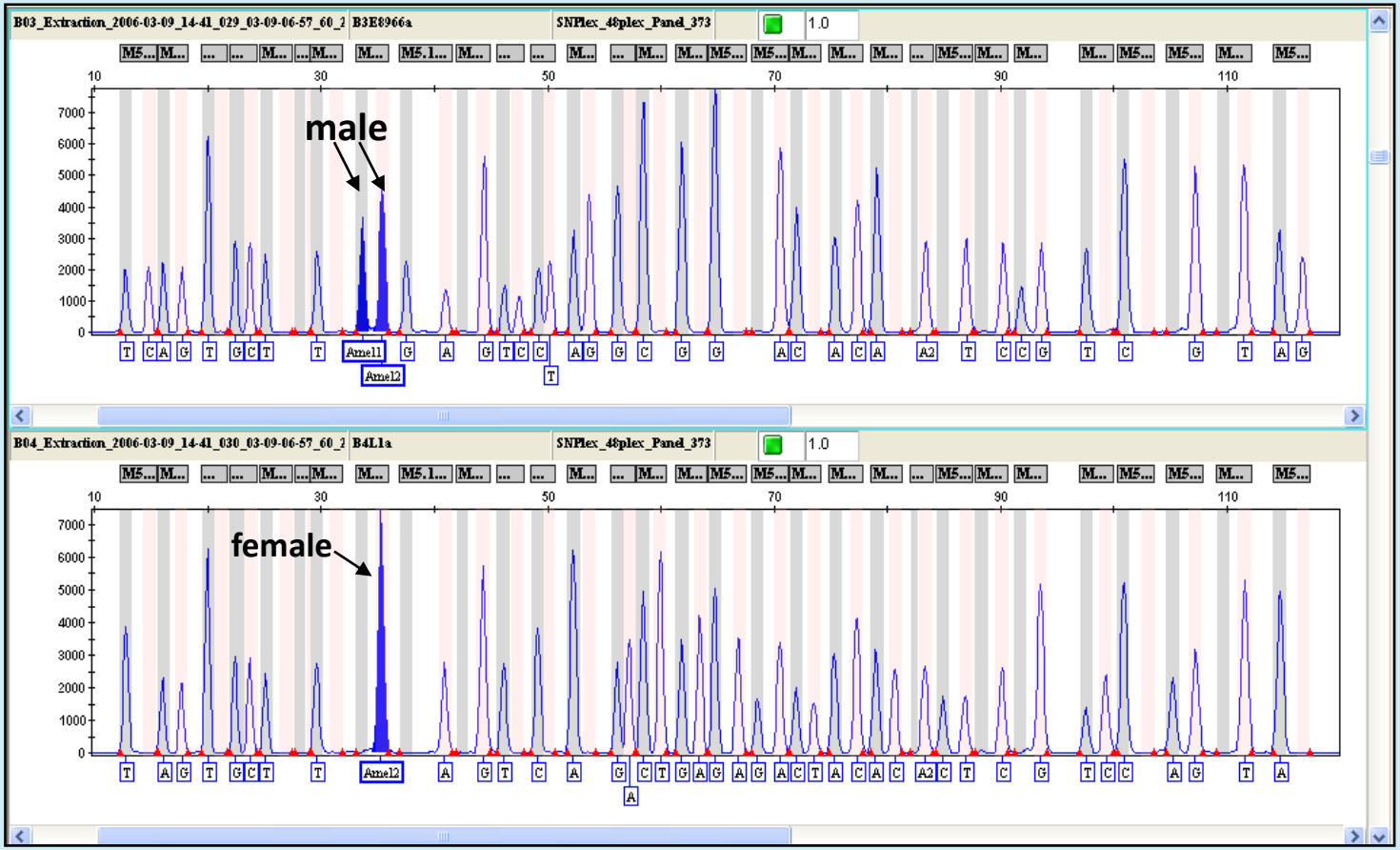
Gender Determination

DNA1

DNA2



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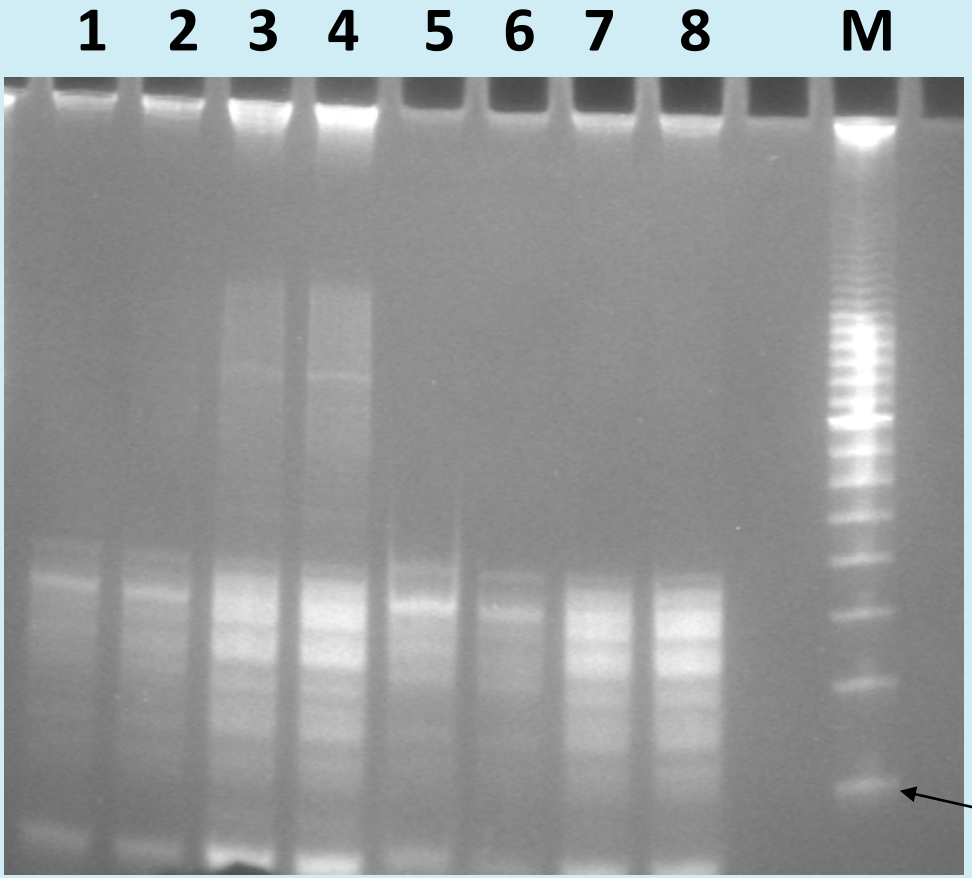
Polyacrylamide Gel QC for Multiplex PCR Products Targets 48 Multiplex Targets



DNA:
1, 3, 5, 7 = 14529
2, 4, 6, 8 = 09947A

Primers:
1, 2, 5, 6 = SNPforID
3, 4, 7, 8 = 1.2 µM

Annealing temperature:
1, 2, 3, 4 = 57° C
5, 6, 7, 8 = 60° C

Low molecular weight PCR products were observed

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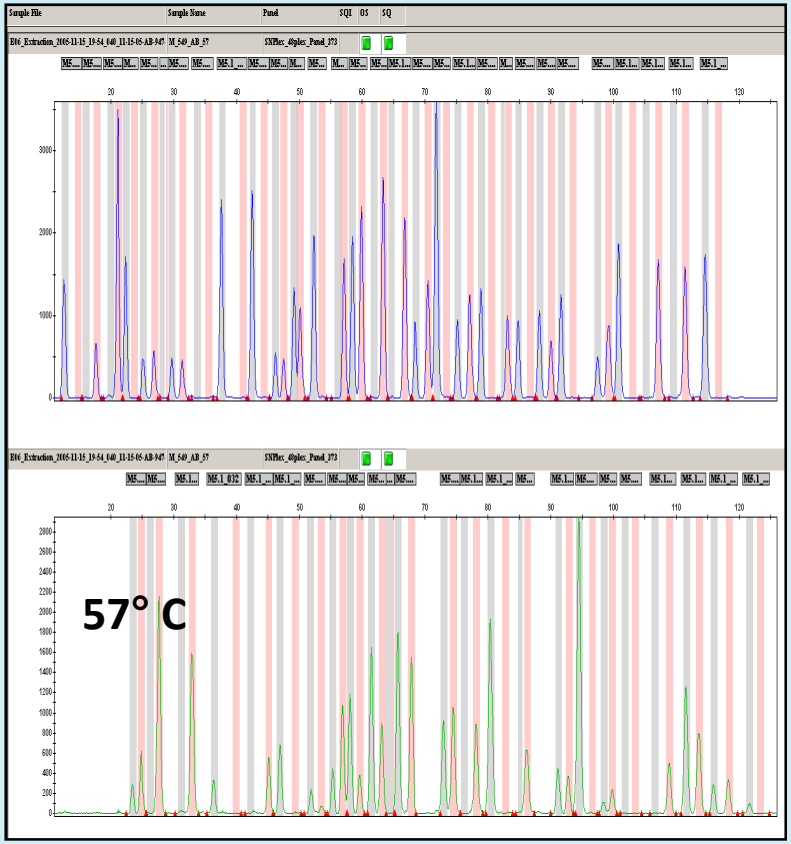
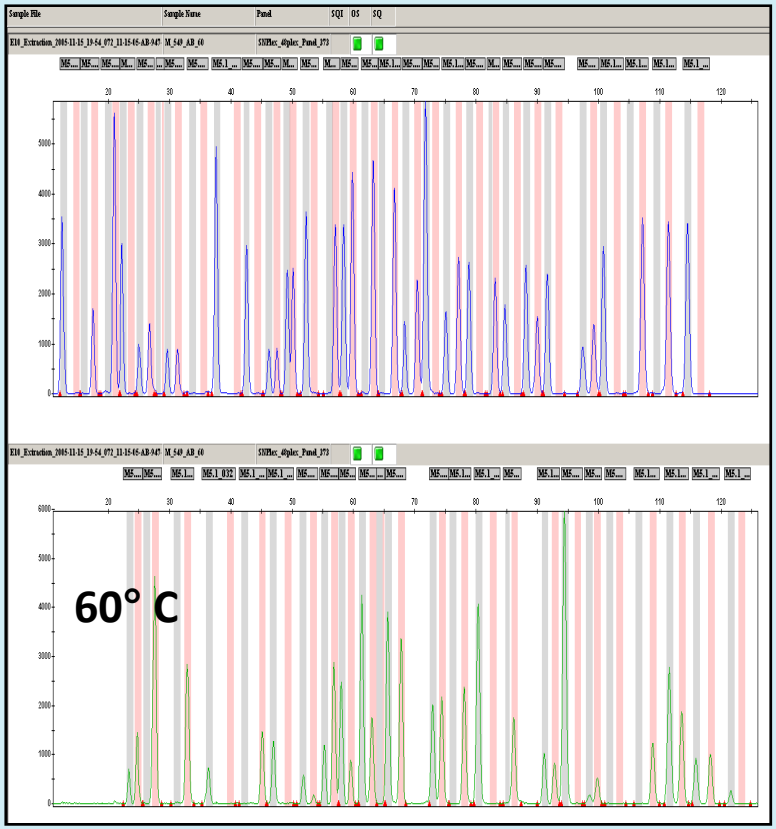
Annealing Temperature Testing: 57° C and 60° C





DNA: NA14529

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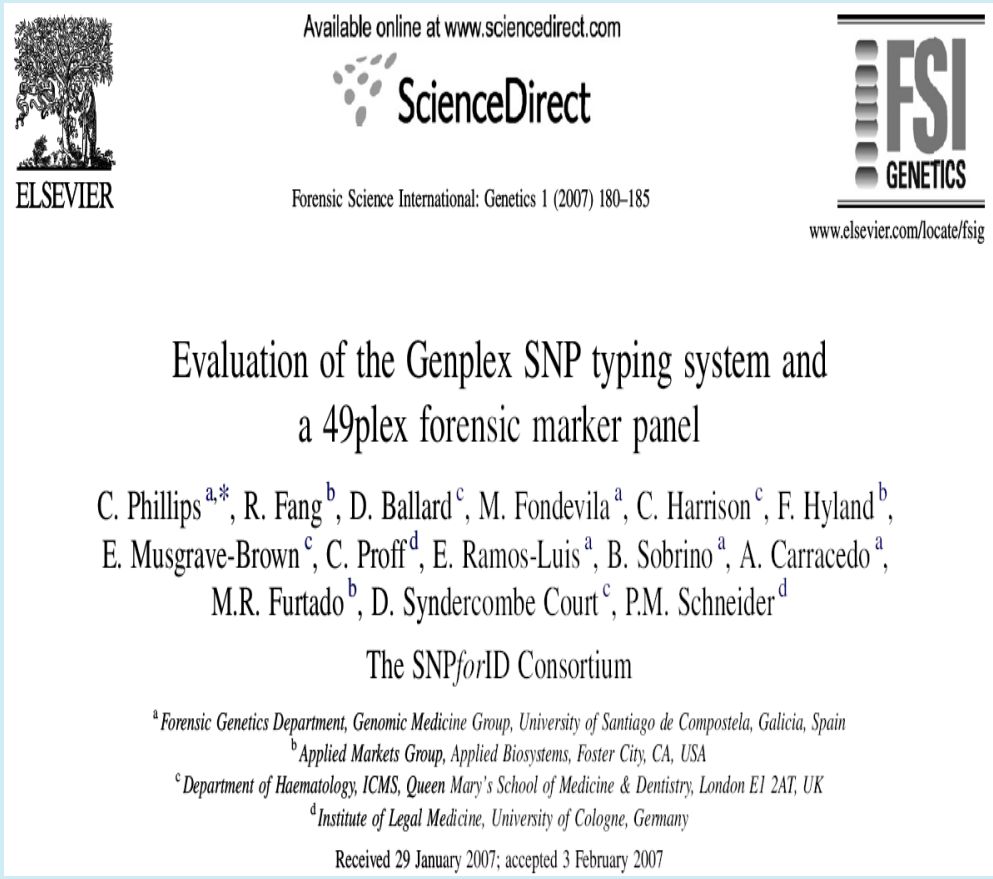
Accuracy Evaluation Studies Across Multiple Platforms



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ScienceDirect

Forensic Science International: Genetics 1 (2007) 180–185

FSI GENETICS

www.elsevier.com/locate/fsig

Evaluation of the Genplex SNP typing system and a 49plex forensic marker panel

C. Phillips[a,*], R. Fang[b], D. Ballard[c], M. Fondevila[a], C. Harrison[c], F. Hyland[b], E. Musgrave-Brown[c], C. Proff[d], E. Ramos-Luis[a], B. Sobrino[a], A. Carracedo[a], M.R. Furtado[b], D. Syndercombe Court[c], P.M. Schneider[d]

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[d] *Institute of Legal Medicine, University of Cologne, Germany*

Received 29 January 2007; accepted 3 February 2007

100% Concordance:

- **TaqMan®**
- **SNaPshot™**
- **Sequenom® iPLEX™**

Phillips, C. et al., *For Sci International Genetics* (2007) **1(2)** 180-185

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High Accuracy and Sensitivity

Forensic Science International: Genetics 3 (2008) 1–6

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journal homepage: www.elsevier.com/locate/fsig

Typing of 48 autosomal SNPs and amelogenin with GenPlex SNP genotyping system in forensic genetics

Carmen Tomas *, Michael Stangegaard, Claus Børsting, Anders Johannes Hansen, Niels Morling

The SNP*for*ID Consortium

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ABSTRACT

GenPlex (Applied Biosystems) is a new SNP genotyping system based on an initial PCR amplification followed by an oligo ligation assay (OLA). The OLA consists of the hybridization of allele and locus specific oligonucleotides (ASOs and LSOs) to PCR products and posterior ligation of ASOs and LSOs. The ligation products are immobilized to microtitre plates and reporter oligonucleotides (ZipChute® probes) are hybridized to the ligation products. ZipChute® probes are subsequently eluted and detected using capillary electrophoresis. Applied Biosystems developed the GenPlex SNP genotyping system with amelogenin and 48 of the 52 SNPs used in the 52 SNP-plex assay developed by the SNP*for*ID consortium. The system requires equipment that is usually found in forensic genetic laboratories. The use of a robot for performance of the pipetting steps is highly recommendable.

A total of 286 individuals from Denmark, Somalia and Greenland were investigated with GenPlex using a Biomek® 3000 (Beckman Coulter) robot. The results were compared to results obtained with an ISO 17025 accredited SNP typing assay based on single base extension (SBE). With the GenPlex SNP genotyping system, full SNP profiles were obtained in 97.6% of the investigations. Perfect concordance was obtained in duplicate investigations and the SNP genotypes obtained with the GenPlex system were concordant with those of the accredited SBE based SNP typing system except for one result in rs901398 in one of 286 individuals most likely due to a mutation 6 bp downstream of the SNP. Reproducible SNP genotypes were obtained from as little as 250 pg of DNA.

Sensitivity: 250 pg
Error Rate: 1/13728
(caused by mutation)

Tomas, C. et al., *For Sci International Genetics* (2008) **3(1)** 1-6

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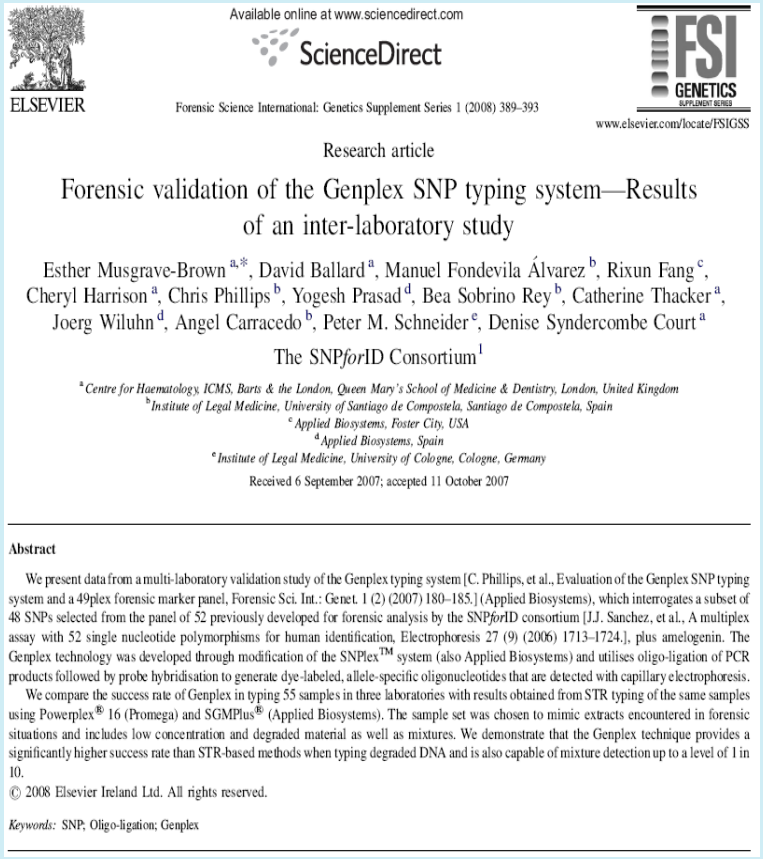
Mixture and Degraded DNA Sample SNP Genotyping



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Forensic Science International: Genetics Supplement Series 1 (2008) 389–393

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Research article

Forensic validation of the Genplex SNP typing system—Results of an inter-laboratory study

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The SNP*for*ID Consortium [1]

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Abstract

We present data from a multi-laboratory validation study of the Genplex typing system [C. Phillips, et al., Evaluation of the Genplex SNP typing system and a 49plex forensic marker panel, Forensic Sci. Int.: Genet. 1 (2) (2007) 180–185.] (Applied Biosystems), which interrogates a subset of 48 SNPs selected from the panel of 52 previously developed for forensic analysis by the SNP*for*ID consortium [J.J. Sanchez, et al., A multiplex assay with 52 single nucleotide polymorphisms for human identification, Electrophoresis 27 (9) (2006) 1713–1724.], plus amelogenin. The Genplex technology was developed through modification of the SNPlex™ system (also Applied Biosystems) and utilises oligo-ligation of PCR products followed by probe hybridisation to generate dye-labeled, allele-specific oligonucleotides that are detected with capillary electrophoresis.

We compare the success rate of Genplex in typing 55 samples in three laboratories with results obtained from STR typing of the same samples using Powerplex® 16 (Promega) and SGMPlus® (Applied Biosystems). The sample set was chosen to mimic extracts encountered in forensic situations and includes low concentration and degraded material as well as mixtures. We demonstrate that the Genplex technique provides a significantly higher success rate than STR-based methods when typing degraded DNA and is also capable of mixture detection up to a level of 1 in 10.

Keywords: SNP; Oligo-ligation; Genplex

Demonstrate:

- **Higher success rate than STR-based methods when typing degraded DNA**
- **Mixture detection up to a level of 1 in 10**

Musgrave-Brown, E. et al., *For Sci International Genetics Suppl. Series 1* (2008) 389-393

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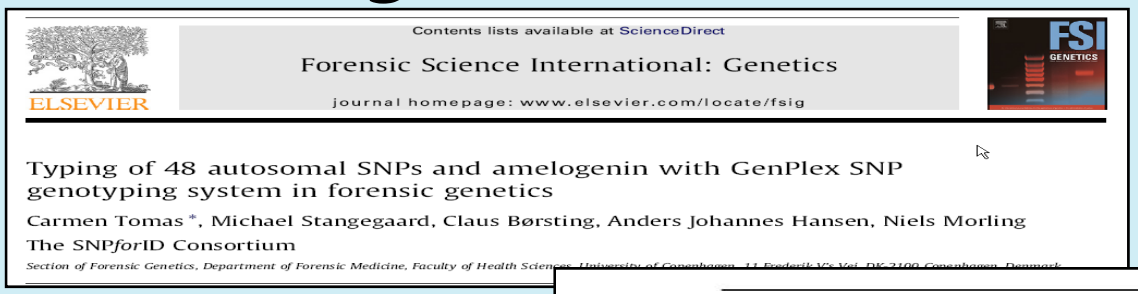
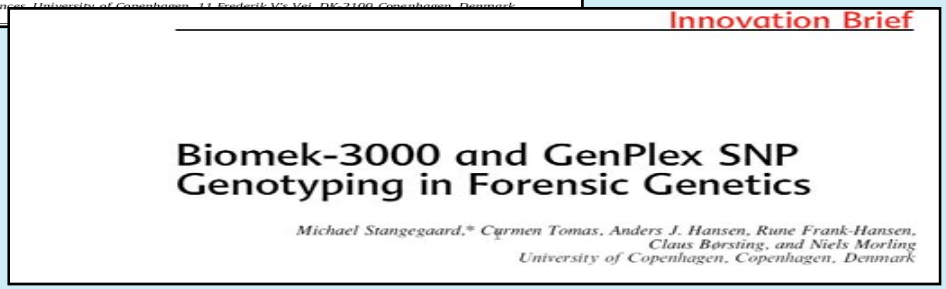
Automating GenPlex™





Tomas, C. et al., *For Sci International Genetics* (2008) **3(1)** 1-6
Stangegaard, M. et al., *JALA* (2008) **13(5)** 297-303

- **286 individuals typed**
- **Biomek 3000 robot used for automation**
- **Sensitivity at 250 pg**
- **No carry-over contamination**
- **High Accuracy: only 1/14014 allele discrepancy due to a mutation 6 bp downstream of the SNP**

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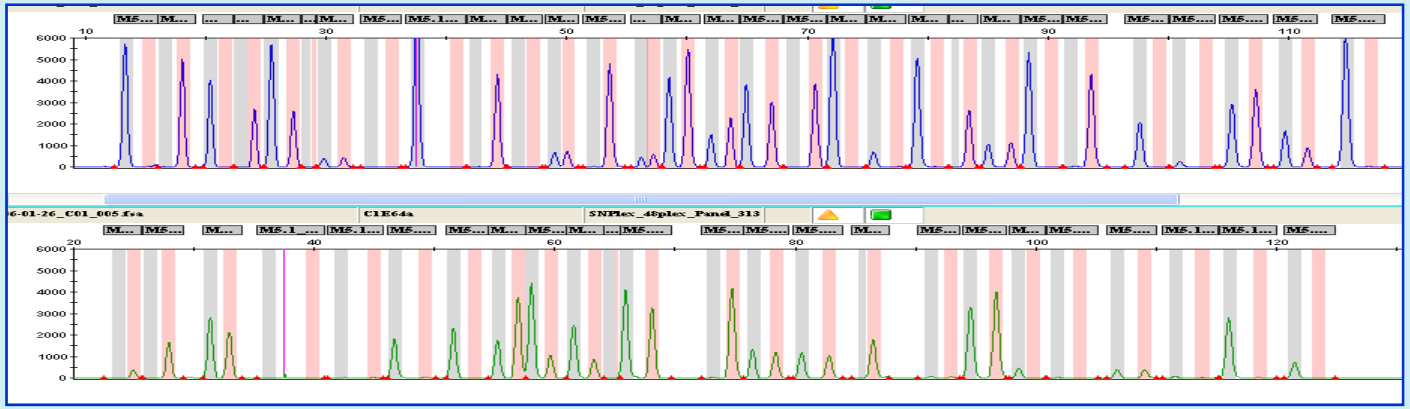
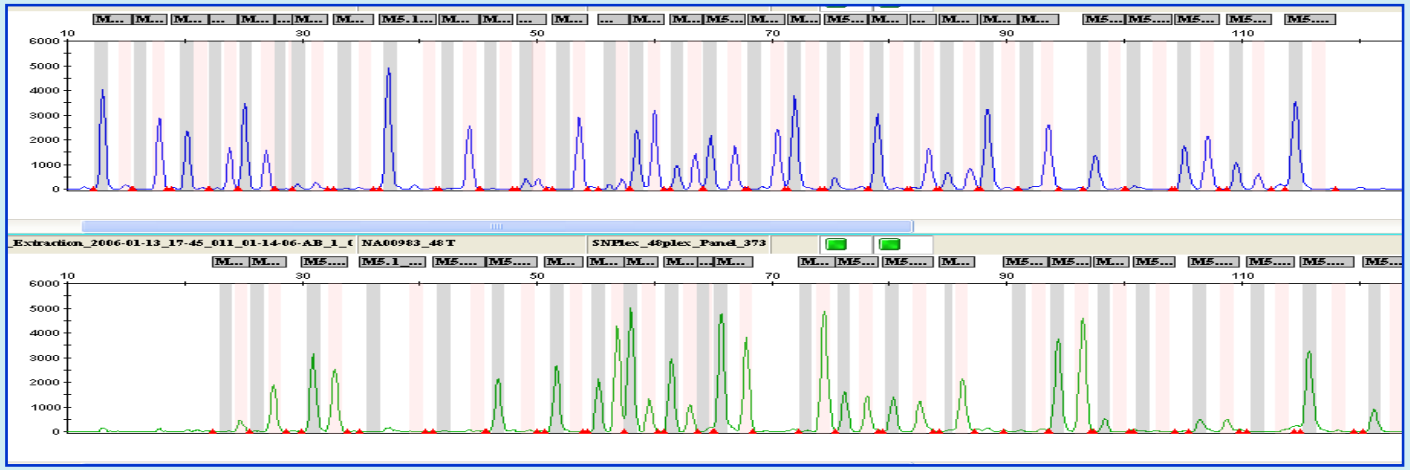
Profiles of 3130xl and 3730xl Platforms

3130*xl*



3730*xl*



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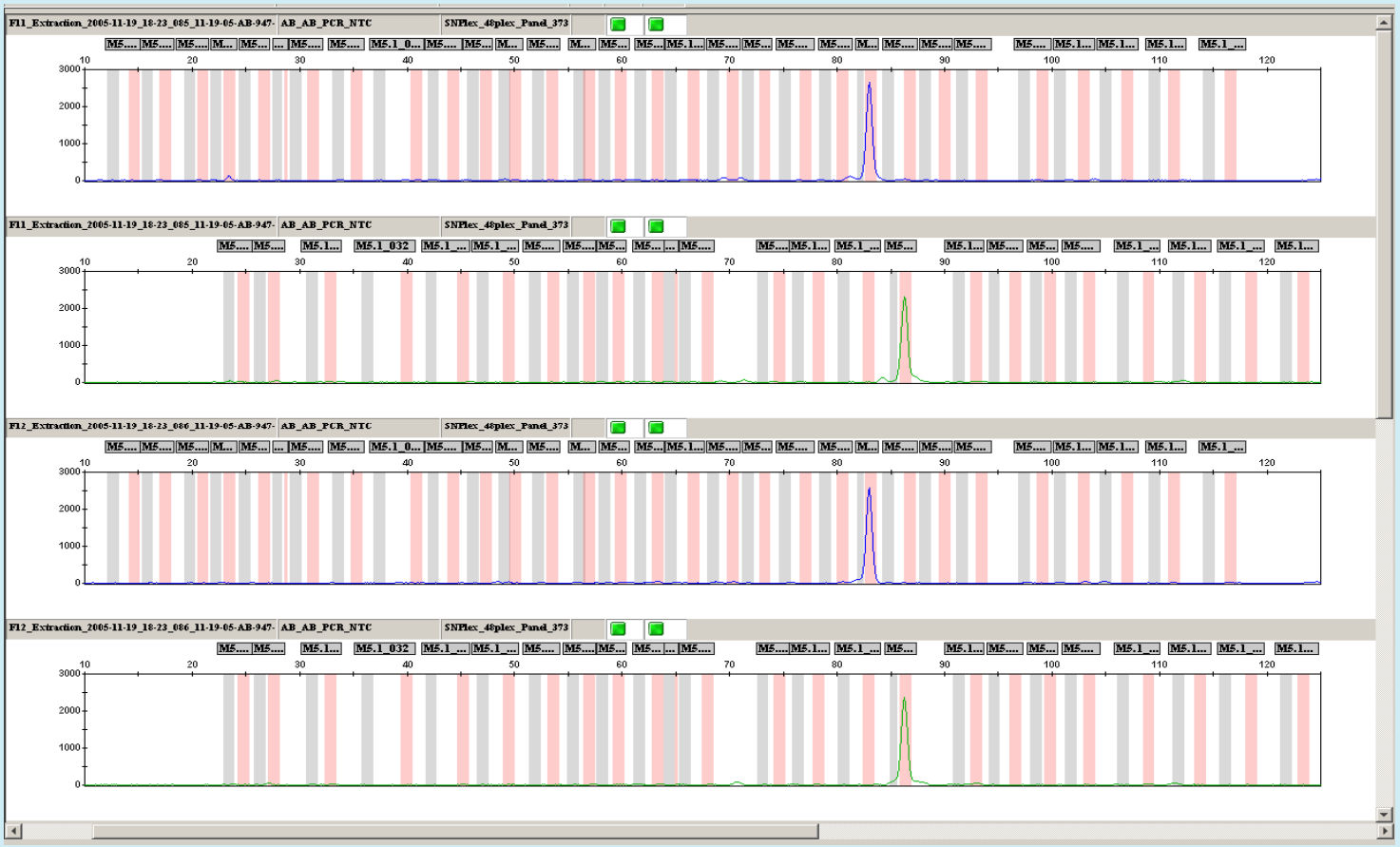
NTCs Were Clean

AB

SNP forID



PHC

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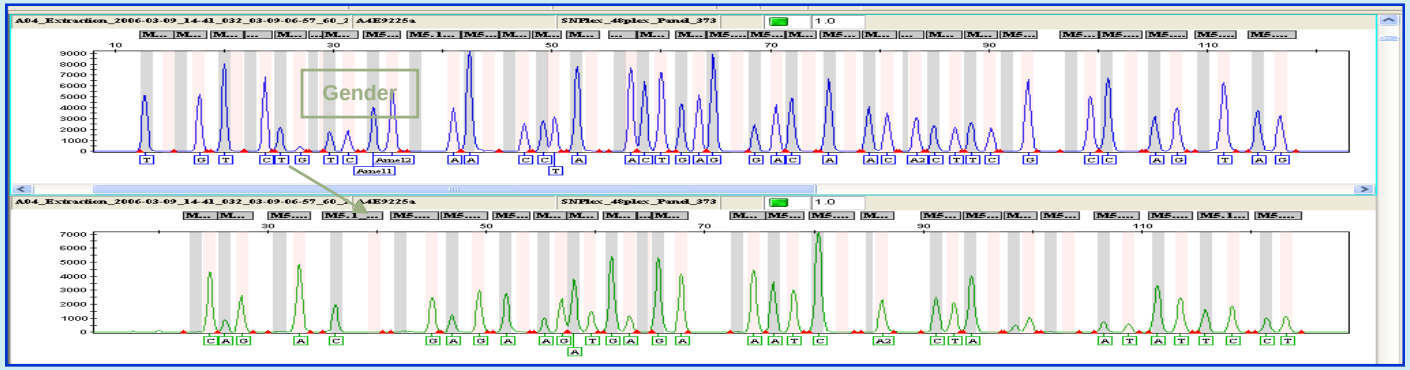
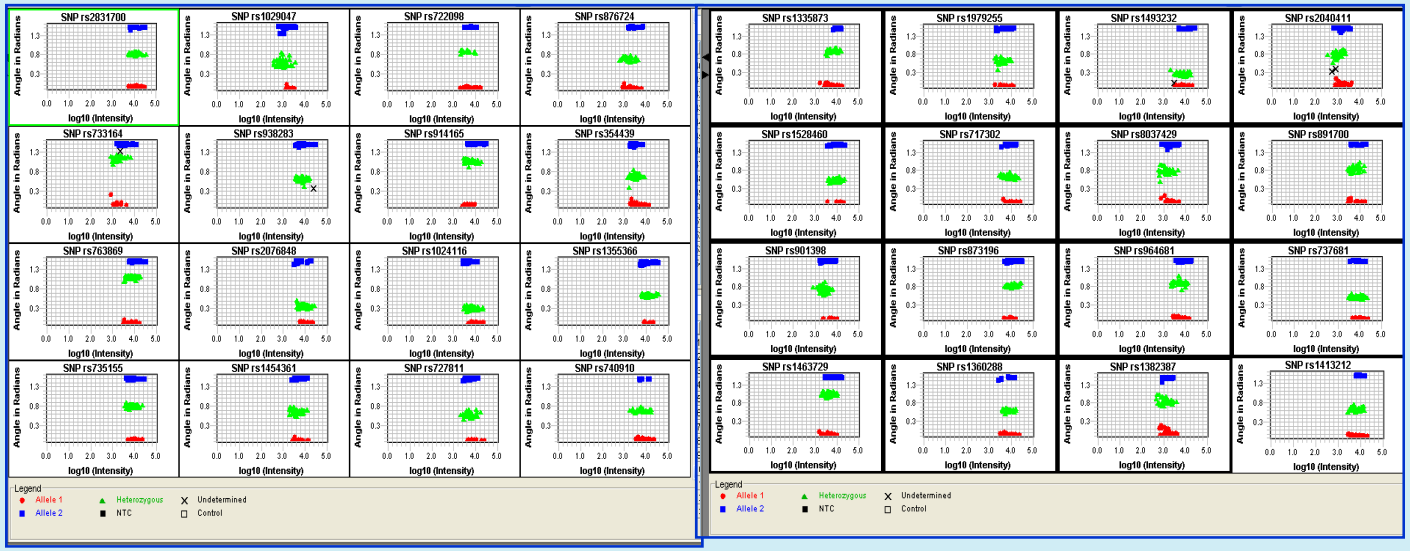
Protocol Development for 49-Plex Panel





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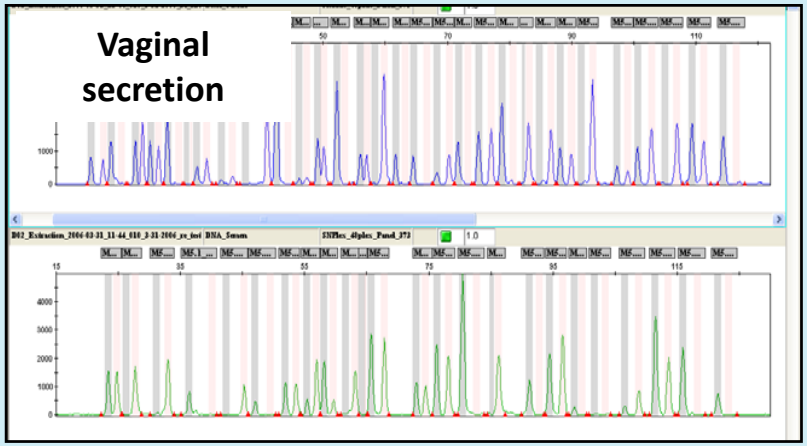
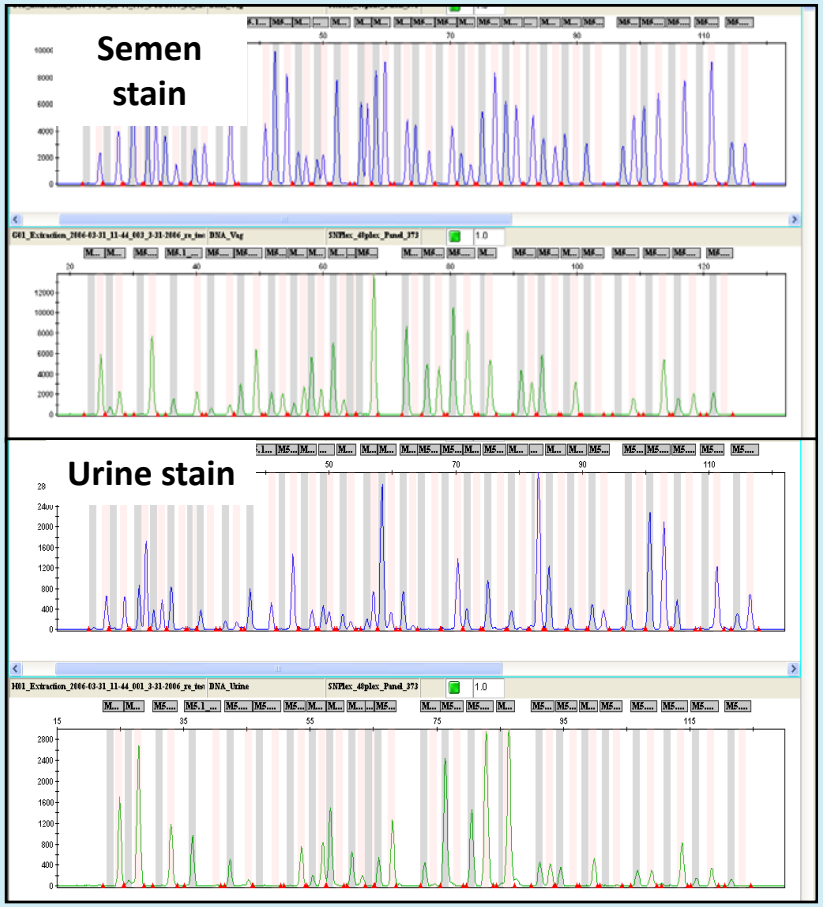
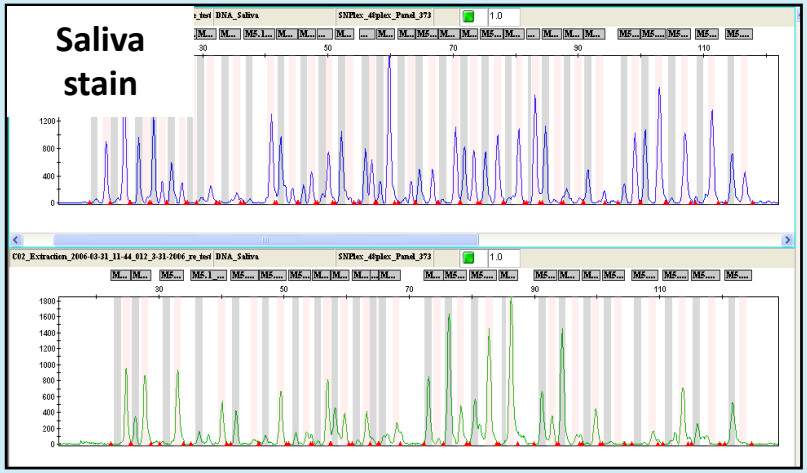
Forensic Samples







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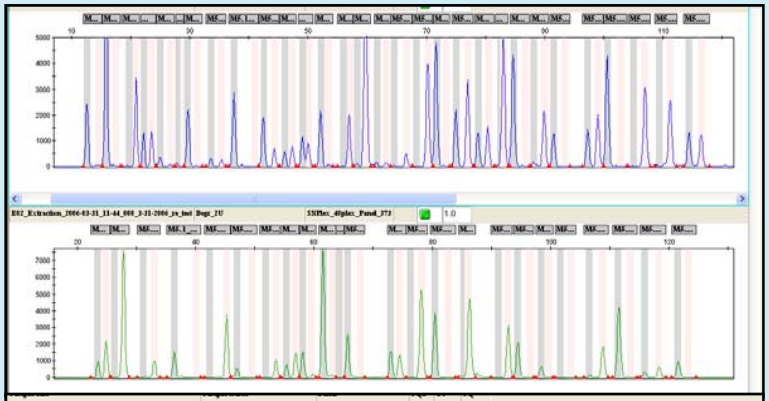
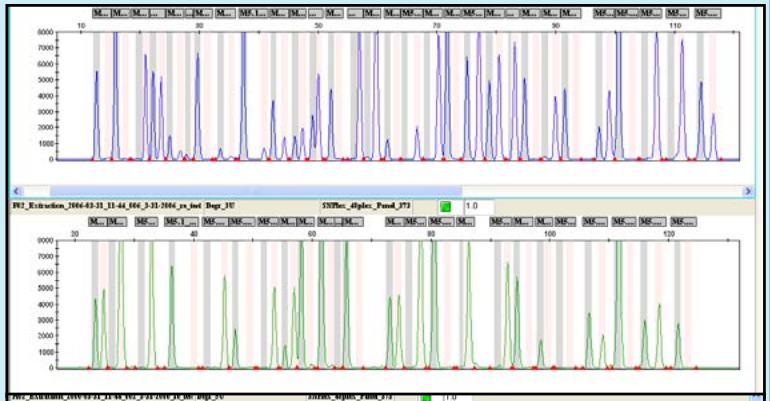
Degraded DNA Testing

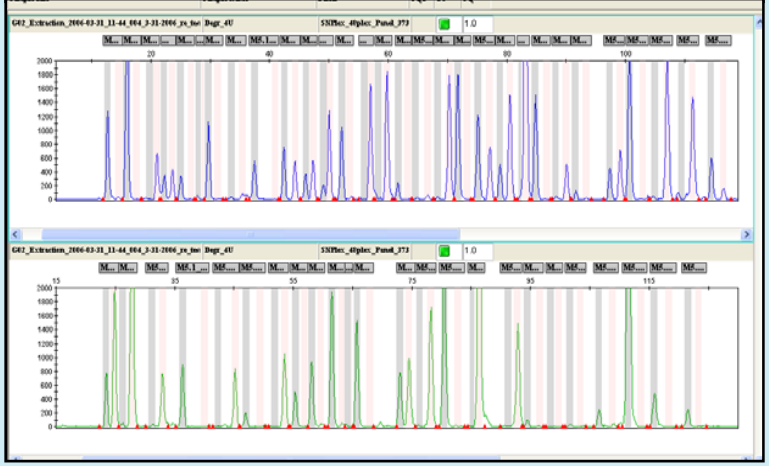
2U



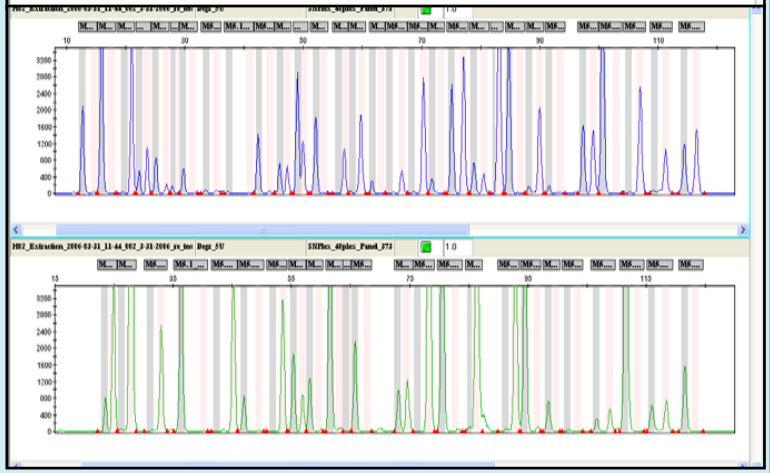
3U



4U



5U



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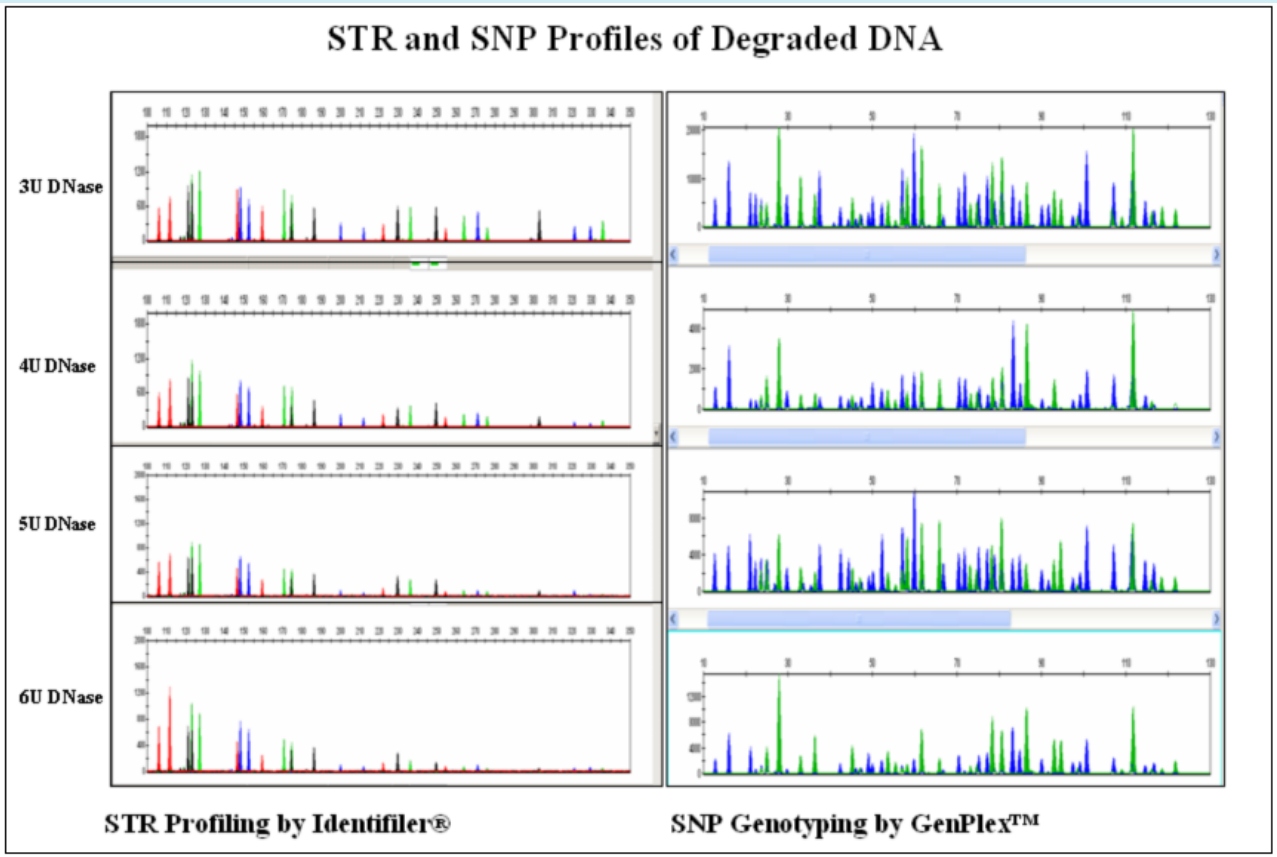
Degraded DNA: STR versus SNP Comparison



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Identifiler® and SNPs: Empirical Data Genotypes on CEPH families

- **Three 'CEPH' families, European origin**
- **3-generation families with genotyped parents and grandparents, many children**
- **Genotyped for Identifiler® and 49 SNPs**
  - **Power of discrimination**
  - **Paternity exclusion**

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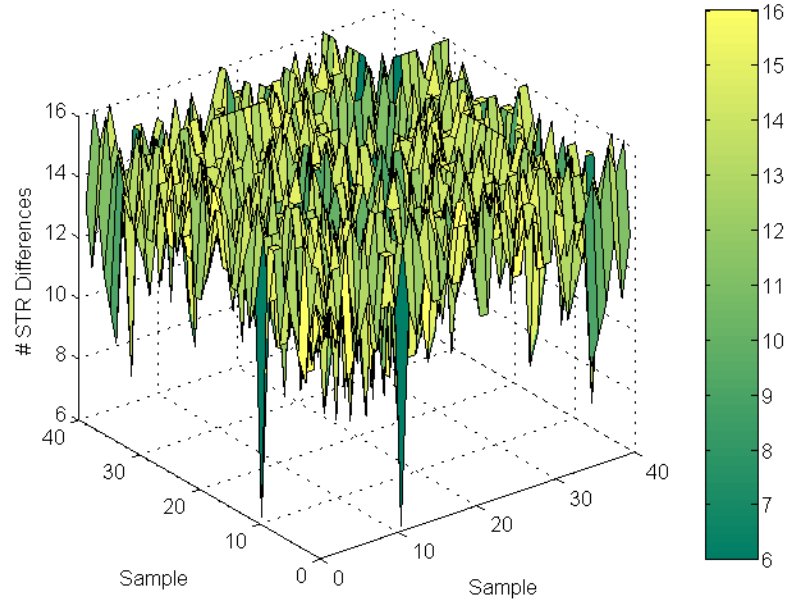
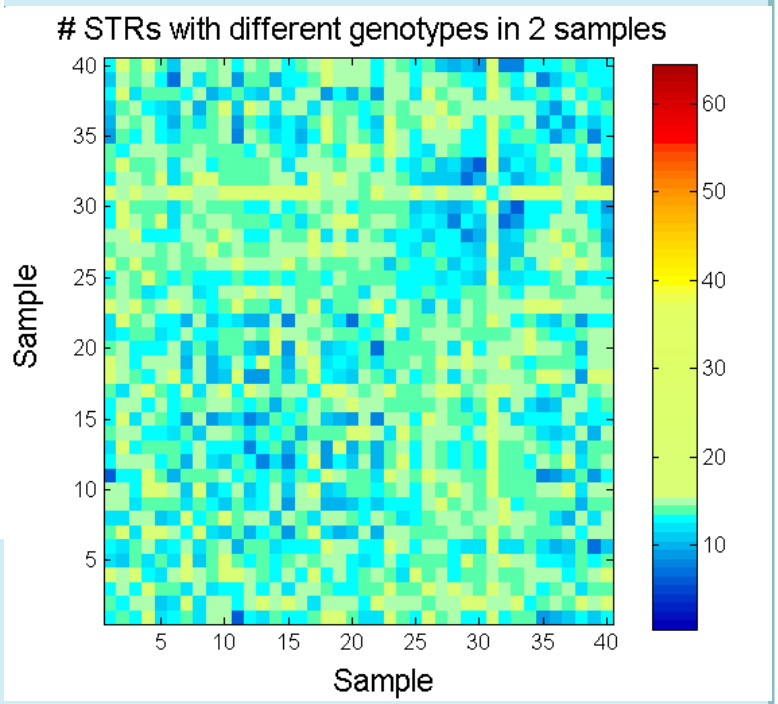
STRs: Number of Loci Different Between Samples

Overall mean # loci different = 13.8





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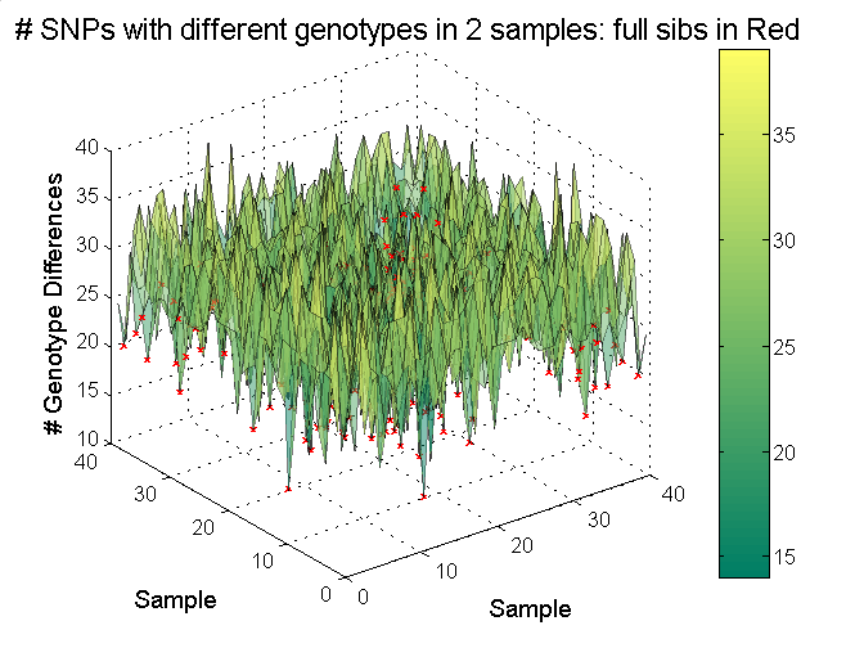
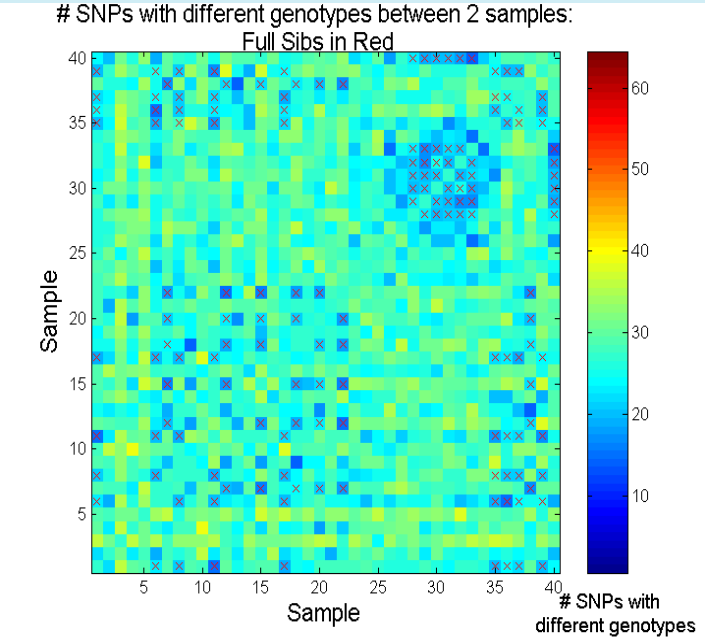
SNPs: Number of Genotype Differences Between Samples

Overall mean # loci different about 27





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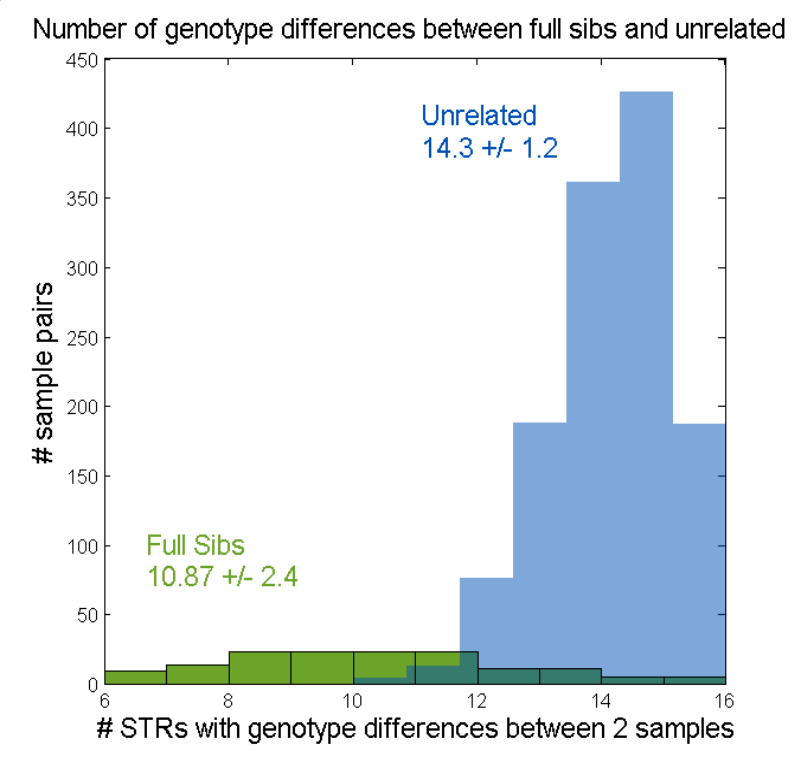
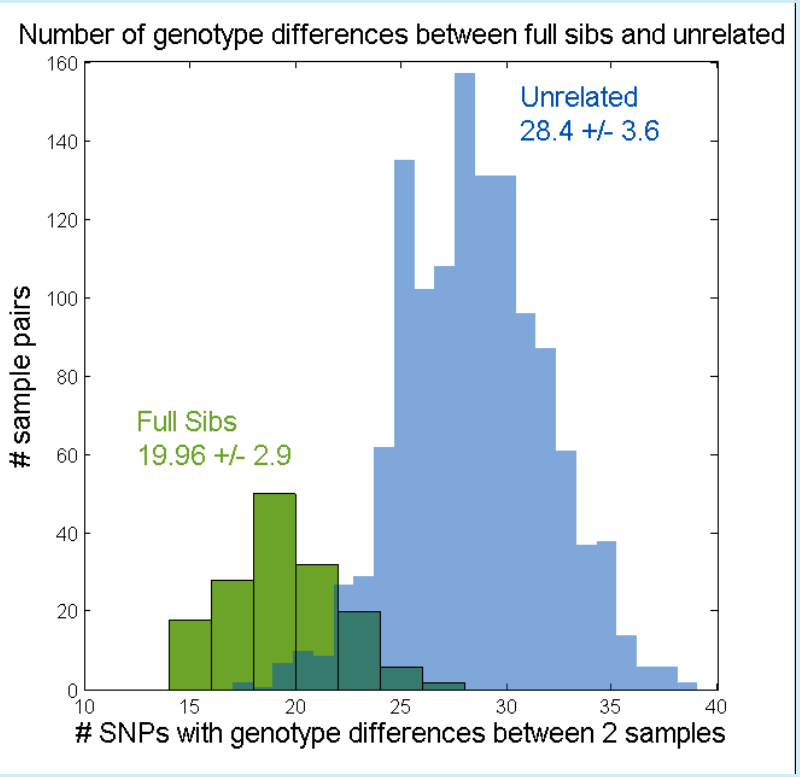
Number of Genotype Differences Between Related and Unrelated Samples





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Identifiler® and SNPforID Panels: Average Probability of Paternity Exclusion

	Identifiler®	49 SNPs	30 SNPs	20 SNPs
CEPH 20 samples	**$8.986 * 10^{-7}$**	**$2.08 * 10^{-5}$**	**$8.22 * 10^{-4}$**	**$1.36 * 10^{-2}$**

Paternity exclusion analysis: CERVUS 2.0

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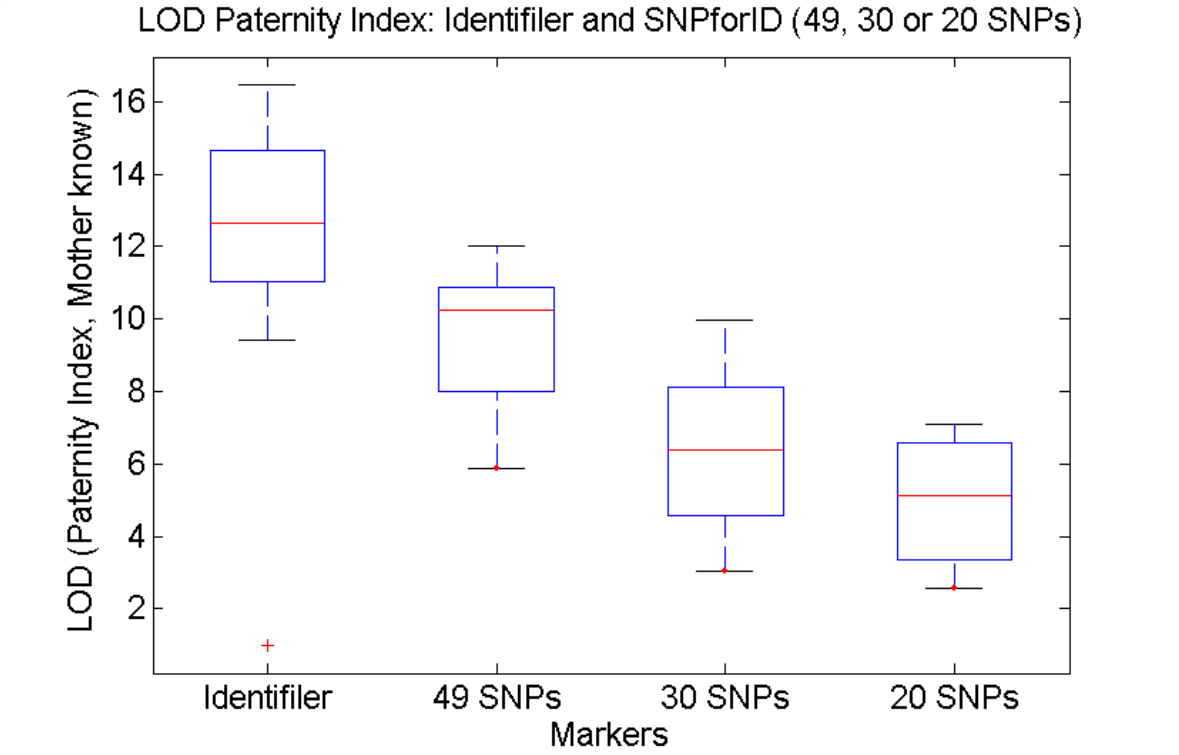
Identifiler® and SNPs

- **Identifiler® has higher LOD score for paternity detection in 20 CEPH children**



LOD score analysis: CERVUS 2.0

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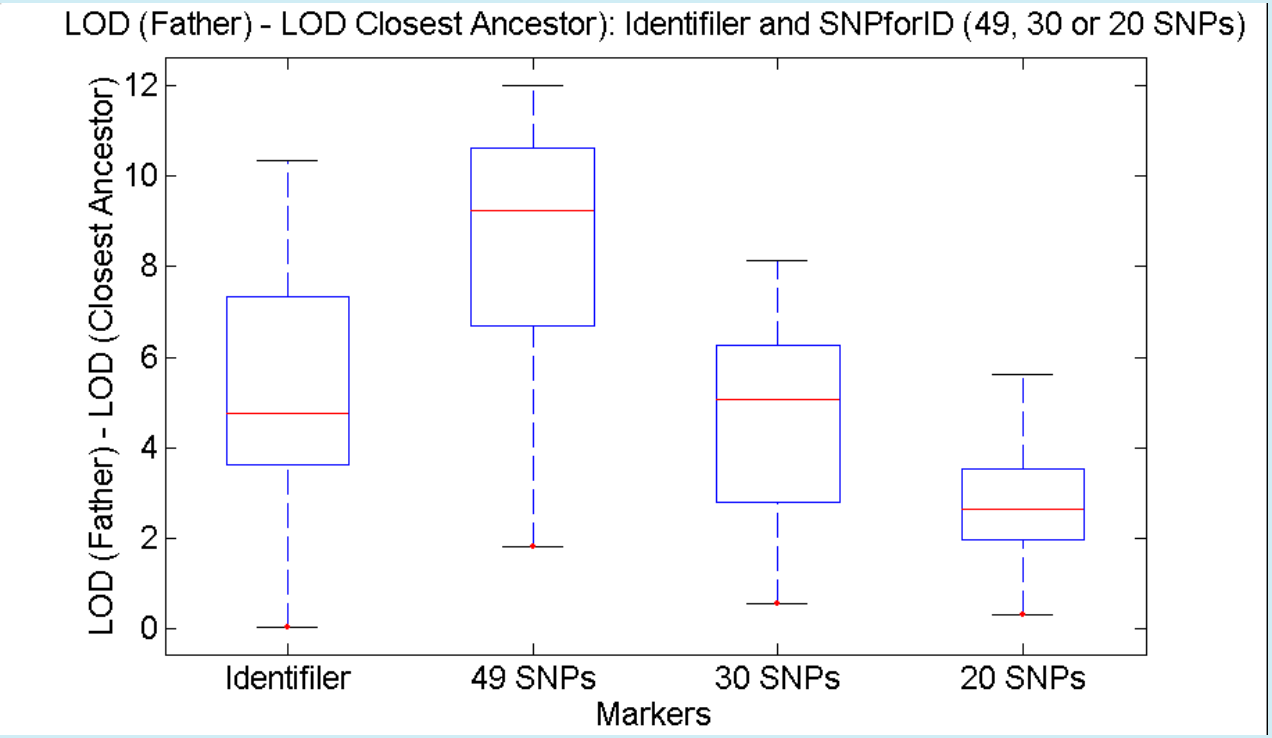
Identifiler® and SNPs

- **SNPs can better distinguish between close relatives for paternity identification**



LOD score analysis: CERVUS 2.0

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Conclusions

- A 49-plex single tube reaction was designed
- A gender-specific marker was included
- Able to reliably detect all SNPs on the 3130 and 3730
- Comparison with TaqMan® allelic discrimination assays indicated a concordance rate of 100%
- The method was used to successfully genotype 41 samples from three CEPH families
- The method worked with with several sample types (blood, buccal swabs, semen, etc.)
- Testing with DNA degraded using sonication and DNase I treatment indicated that the method works well with degraded DNA

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Conclusions: Identifiler® and SNPforID SNP Panel

- **Overall informativeness is comparable**
- **SNPforID panel is better for identity**
  - **About 45 SNPs are comparable to Identifiler®**
- **Identifiler® is better for paternity exclusion**
  - **But SNPs give greater distinction between closely related candidates**
- **SNPs give larger absolute number of loci different**

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SNP Panels From Ken Kidd

G Model
FSIGSS-442; No of Pages 2
ARTICLE IN PRESS

Forensic Science International: Genetics Supplement Series xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Research article

Multiplexed SNP detection panels for human identification

Rixun Fang [a], Andrew J. Pakstis [b], Fiona Hyland [a], David Wang [a], Jaiprakash Shewale [a], Judith R. Kidd [b], Kenneth K. Kidd [b], Manohar R. Furtado [a,*]

[a] *Applied Markets, Applied Biosystems/Life Technologies, 850 Lincoln Centre Dr, m/s 402, Foster City, CA 94404, United States*
[b] *Genetics Dept. Yale University School of Medicine, New Haven, CT 06520, United States*

Fang, R. et al., *For Sci International Genetics Suppl. Series* (2009) doi:10.1016/j.fsigss.2009.08.161

Features

- **A panel of 92 SNPs was assembled for human identification after testing more than 500 potential candidate SNPs from the list available since January 2009 at the site (http://info.med.yale.edu/genetics/kkidd/contents.html)**
- **SNPs were selected based on testing samples from 44 populations across the globe using TaqMan® allelic discrimination formats in a collaboration**
- **High average heterozygosity (> 0.4)**
- **Low global Fst values (< 0.06) as a set of highly discriminative SNPs suitable for human identification in all geographic populations**

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Features

- **The SNP list includes 45 essentially unlinked SNPs distributed across the human autosomes**
  - **These SNPS are an excellent panel for individual identification providing high match probabilities in the range of values comparable to those for the 13 CODIS STR markers**
- **The unlinked status of these 45 SNPs also make them useful for resolving close biological relationships**
- **Additional SNPs on the list can be used to provide a higher power of discrimination, if required**
- **No meaningful departures from Hardy-Weinberg ratios were seen for any SNP in the populations tested**

Features

- **Separating out six SNPs exhibiting some linkage disequilibrium (LD) left 86 SNPs in the panel with no significant pair-wise LD**
- **Two panels of 49 and 41 SNPs were multiplexed for initial testing**
- **Coding SNPs and SNPs with known functional or phenotypic manifestations were excluded**
- **To test the performance characteristics of the two panels of SNPs and to compare their utility to STR analysis for human identification and paternity testing, 41 individuals from three different CEPH families spanning three generations were genotyped**

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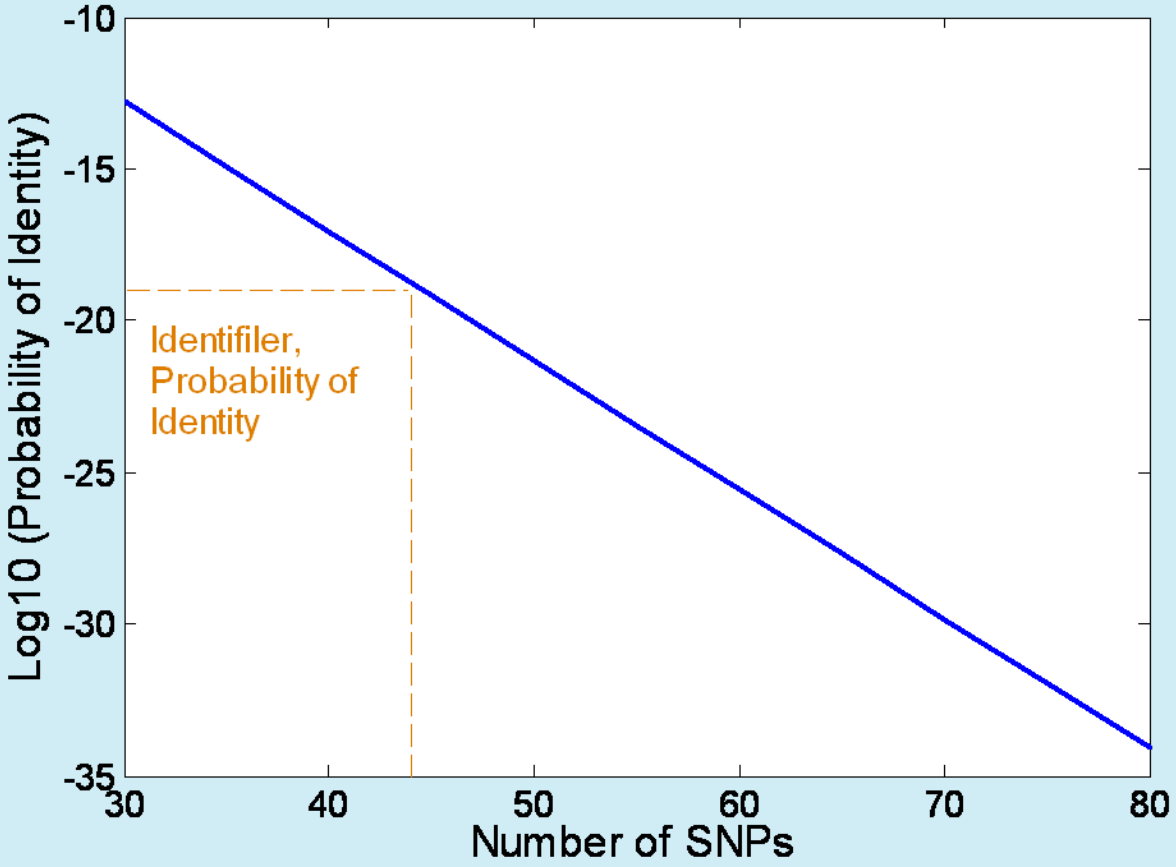
Informativeness: SNPs versus STRs

- **Probability of Identity: 42 SNPs = 15 STRs**



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Data Analysis

- **41 samples**
- **80 markers gave good results**
- **Mean # genotype differences between any pair of samples = 44.76**
- **Standard deviation of # genotype differences between any pair of samples = 6.7**
- **Minimum # genotype differences between pair of samples (many full-sibs) = 18**
- **Maximum # genotype differences between any pair of samples = 64**

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Identity

# of SNPs	Log_{10} Probability of Identity
30	-12.7774
35	-14.9069
40	-17.0367
45	-19.1661
50	-21.2955
55	-23.4252
60	-25.5550
65	-27.6845
70	-29.8138
75	-31.9433
80	-34.0728

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Leverage CE Platform Flexibility for Fixed Sets of SNPs / Mutations

SNPforID 49-plex

Custom kit on market. DtI for a new product

KenKidd SNPs ID 3 pools of 45

AIM markers 38-plex

Y-SNPs 42-plex

Mito SNPs 2-panels

Clock gene mutation set

50-plex ZipChutes®

MRSA typing panel

Bipolar mutation set

Multiplex design pipeline

Coccoidiosis typing panels

Ras mutation set (12)

EGFR mutation set (28)

Microbial panel with detection of over 36 targets, 4 drug resistance mutations 4 virulence factors in one tube

50 zip chutes with 2-dyes, expandable to 100 with additional dyes

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Platform Flexibility: Manufacturing

SNPfor-ID 49-plex

KenKidd SNPs ID 3 pools of 45

AIM markers 38-plex

Y-SNPs 42-plex

Mito SNPs 2-panels

Clock gene set

100 Luminex® beads / 500 Prodigy spots

MRSA panel

Bipolar set

Multiplex design pipeline

Coccoidiosis 2-panels

Ras mutation set (12)

EGFR mutation set (28)

Microbial panel with detection of over 36 targets, 4 drug resistance mutations 4 virulence factors in one tube

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Population Indicative Panel Study: Chris Phillips – Feasibility Study

- **SNP targets:**
  - **32 SNPs**
  - **30 bi-allelic**
  - **2 tri-allelic**
- **Tri-allelic OLA oligo design**
  - **Two bins were used for each locus**

SNP ID	SNP_NAME	GENOTYPE	ASO1	ASO2
rs5030240	NBS-1	A/C/G	M5.1_017_1_A40037=G	M5.1_017_2_A40118=C
			M5.1_005_1_A40033=G	M5.1_005_2_A30007=A
rs4540055	NBS-9	A/C/T	M5.1_032_1_A50055=T	M5.1_032_2_A50056=G
			M5.1_013_1_A40034=T	M5.1_013_2_A40027=A

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Creating the Final Combination of SNPs

- **Select best loci for between-population F_{st}**
- **Optimize the chromosome spread**

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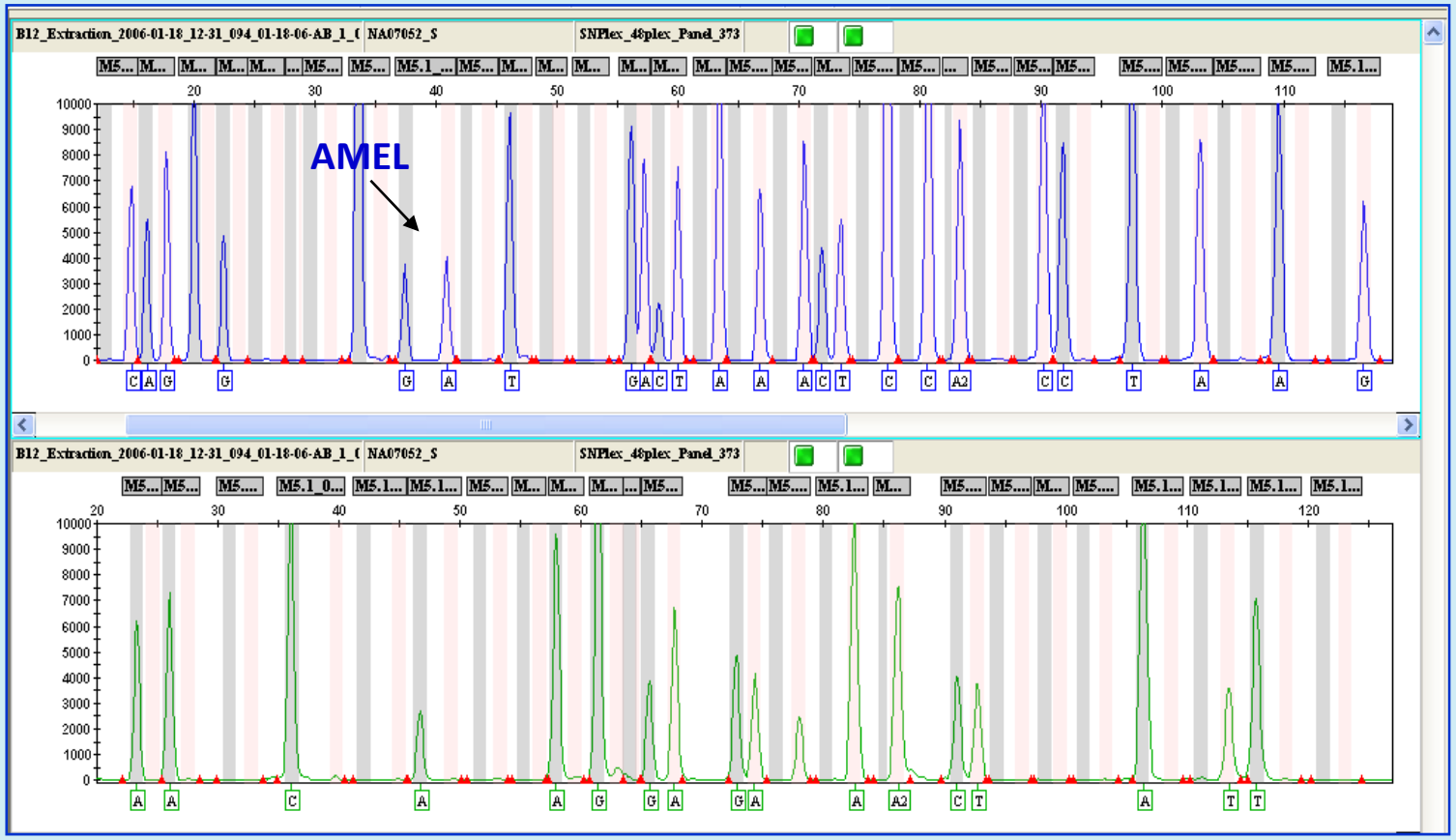
32 SNP Panel Study For a Male's DNA



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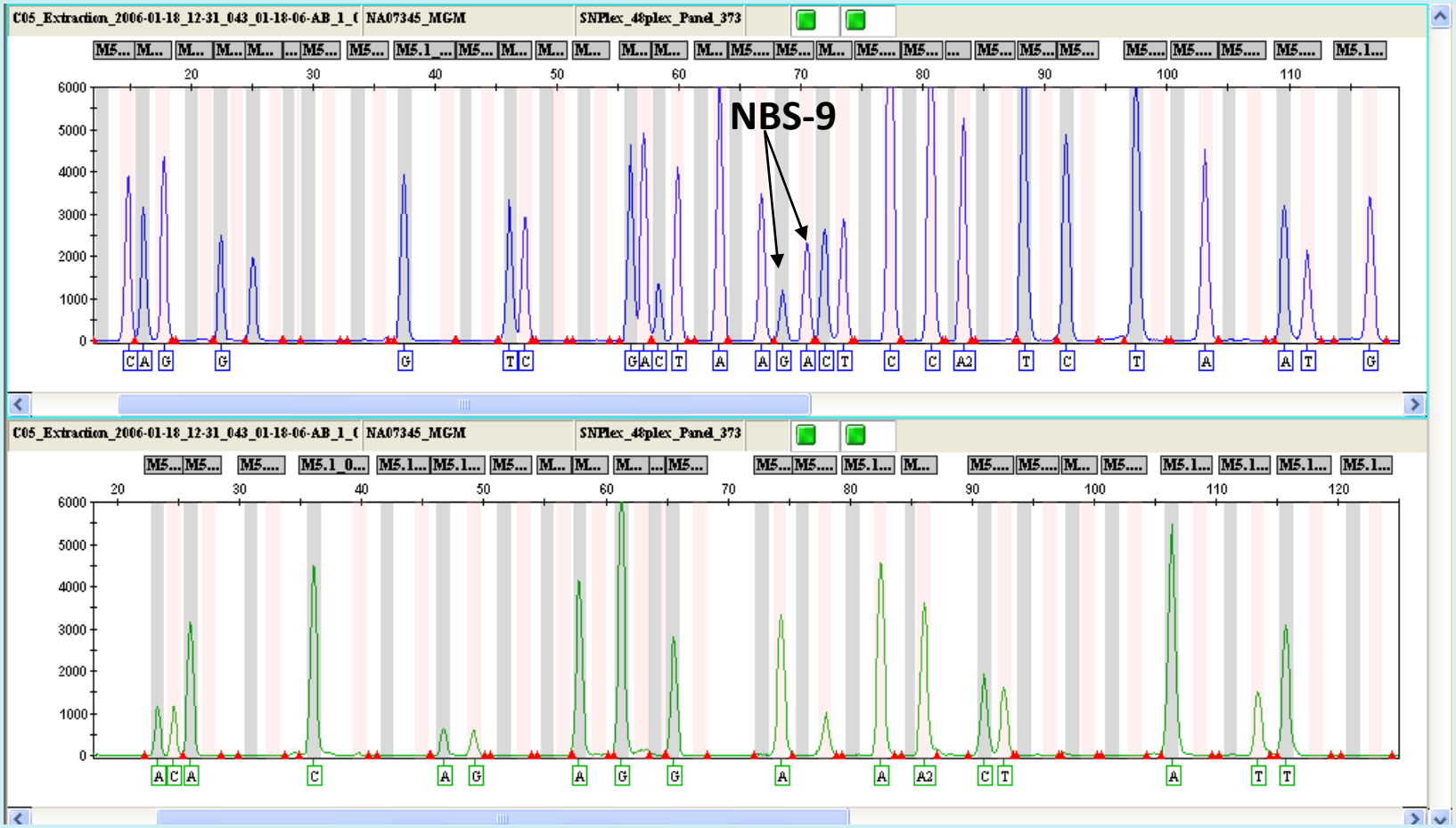
32 SNP Analysis For a Female's DNA



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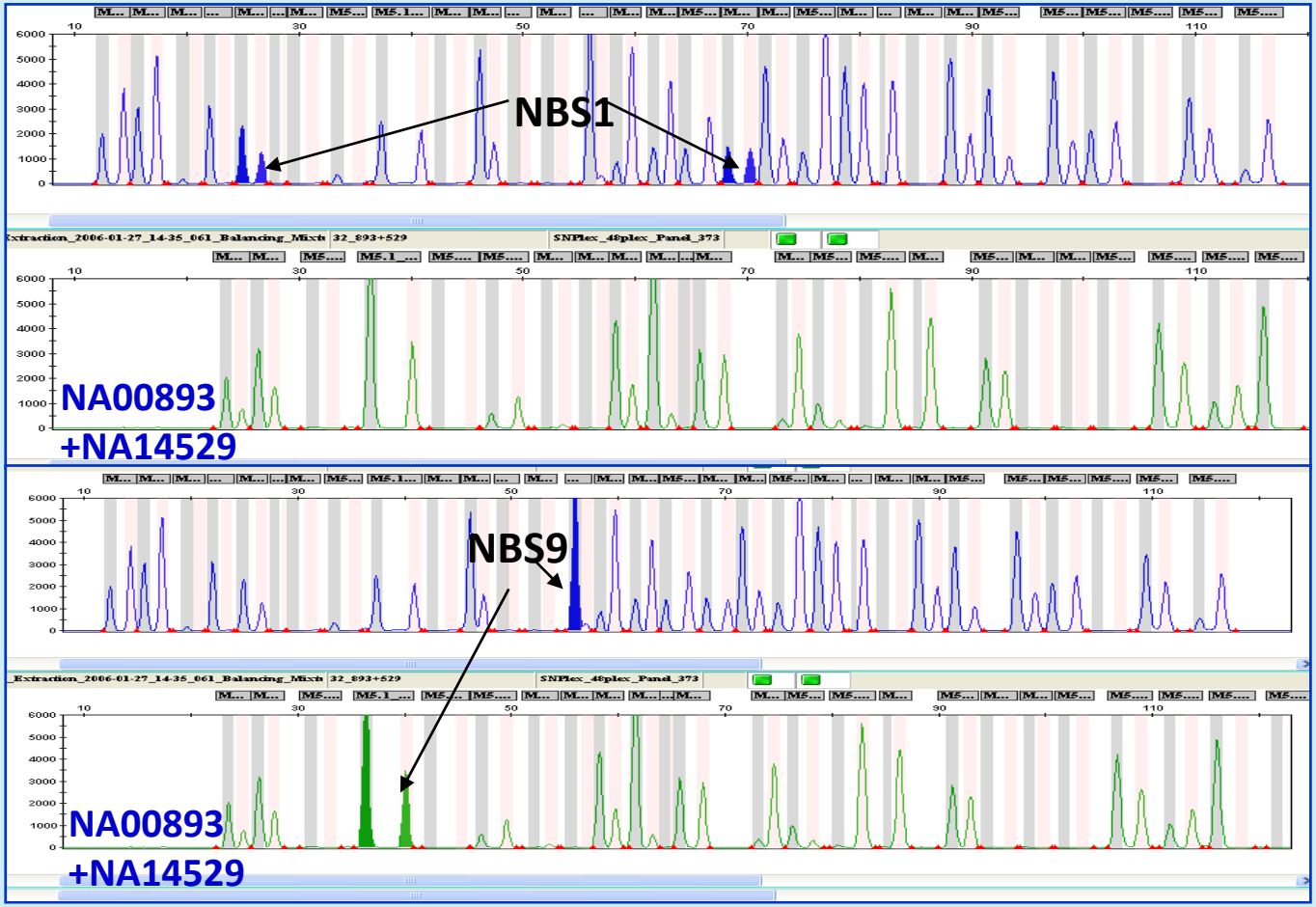
Tri-allelic Markers for DNA Mixture Study



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Y-SNP Study: Potential Collaboration with Genographic Project

- **Genographic Project:**
  - **A five-year effort to understand the human journey**
  - **Launched by The National Geographic Society, IBM, geneticist Spencer Wells, and the Waitt Family Foundation have launched the Genographic Project**
  - **M humanity's genetic journey through the ages**
- **131 targets were selected to cover all Y haplogroups**
  - **SNPs were selected based on information from Peter Underhill, YCC, and publications**
  - **Multiplex PCR primers and OLA oligos designed**

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Y-SNPs

- **Tree**

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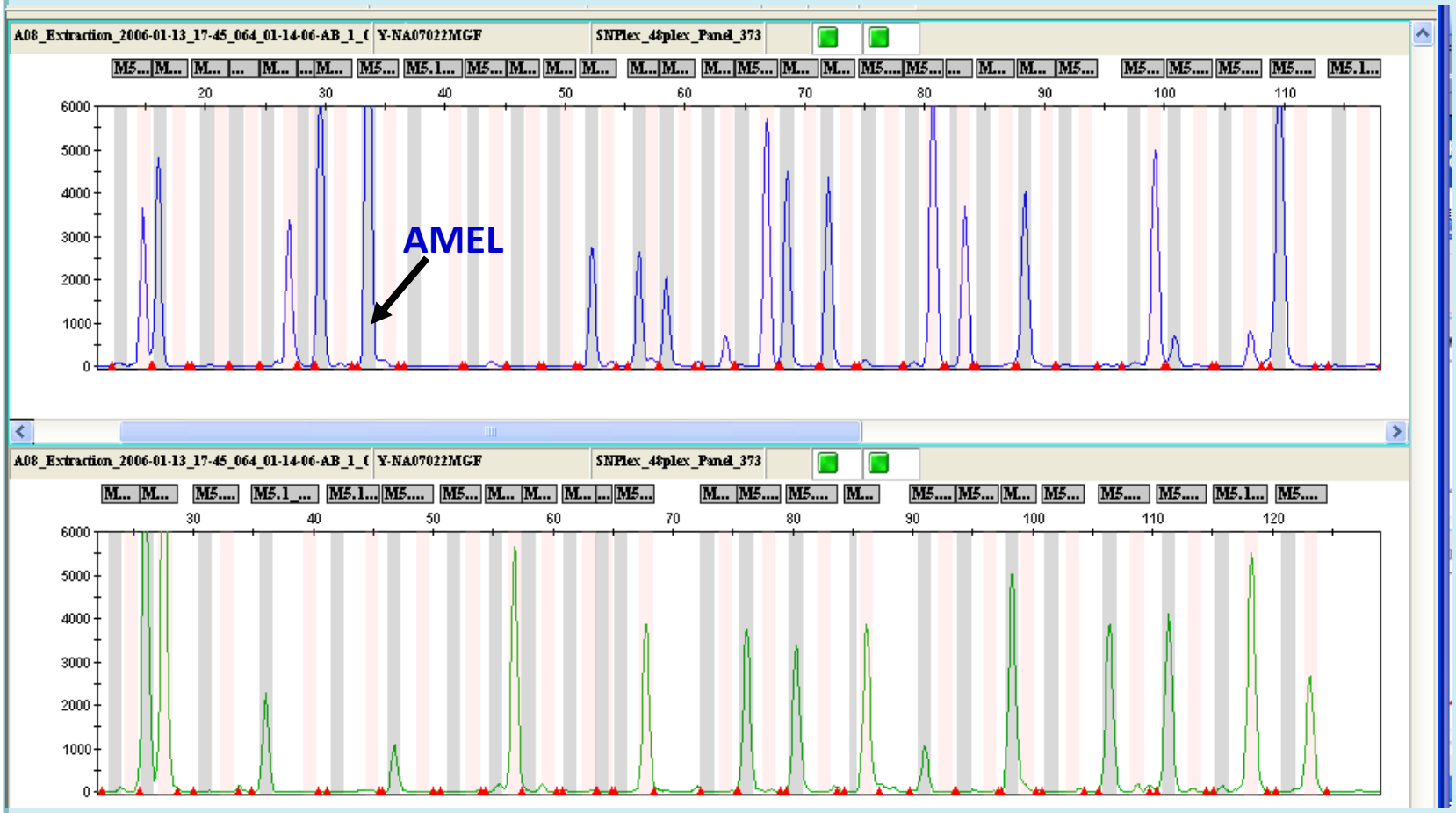
Y-SNPs – 29 Loci



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Multiplexed SNP Detection System for Mitochondrial DNA

- Rixun Fang PhD*
- Pius Brzoska PhD*
- Peter Ma PhD*
- Peter Vallone PhD**
- Manohar Furtado PhD*
  - *Applied Markets and Molecular and Cell Biology Division, Applied Biosystems, Foster City CA 94404
  - ** NIST Building 227, Room B24, 100 Bureau Drive Gaithersburg, MD 20899-8312 USA
- AAFS 2007

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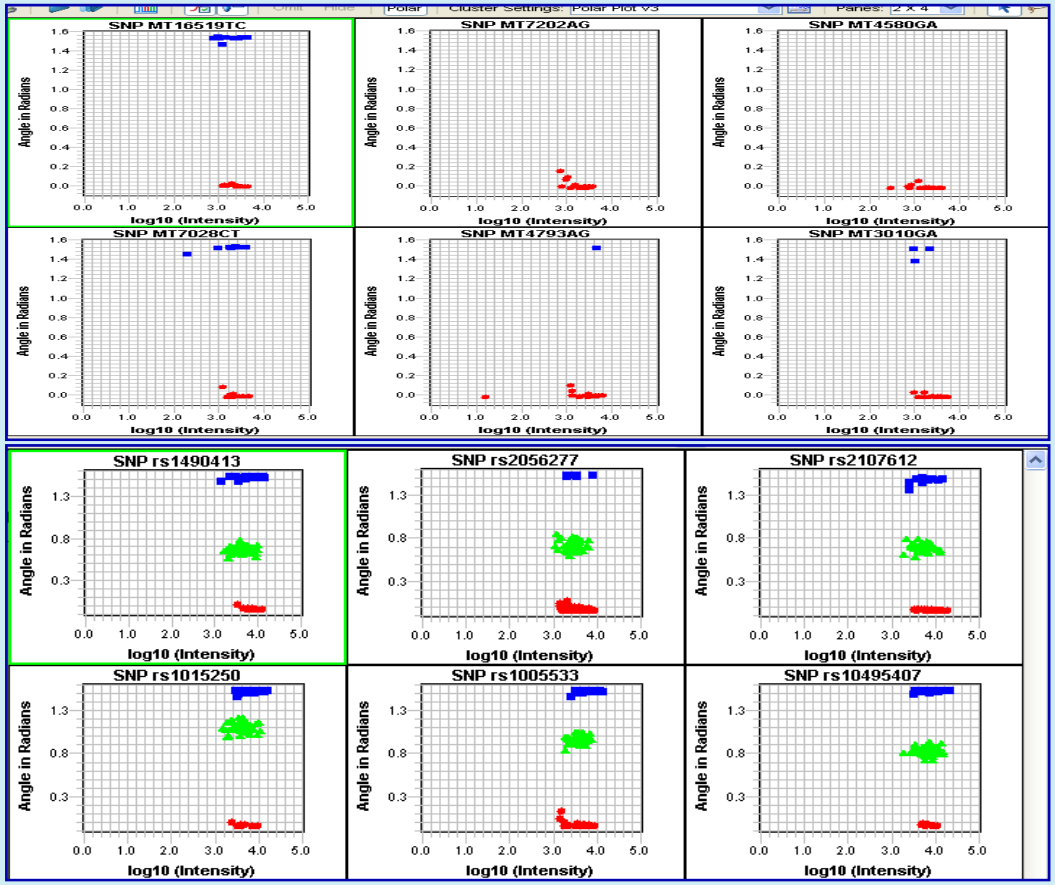
GeneMapper® Software Cluster Plots for SNP Genotyping

MtDNA

Nuclear DNA



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Caucasian Multiplex Panels for Distinguishing the Most Common HV1 / HV2 Types (Vallone et al., Coble et al.)

A	B	C	D	E	F	G	H
477 T/C	477	72	482	4808	64	3826	64
3010 G/A	3010	513	5198	5147	4745	3834	4688
4580 G/A	3915	4580	6260	9380	10211	4688	11377
4793 A/G	5004	5250	9548	9899	10394	6293	12795
5004 T/C	6776	11719	9635	11914	10685	7891	13293
7028 C/T	8592	12438	11485	15067	11377	11533	14305
7202 A/G	10394	12810	11914	16519	14470	12007	16519
10211 C/T	10754	14770	15355	14560	12795		
12858 C/T	11864	15833	15884	16390	15043		
14470 T/A	15340	15884	16368	14869	16390		
16519 T/C	16519	16519	16519				
H:1	H:2,H:3,H:6	V:1,H:5	J:1,J:2,K:2,K:3	J:4,T:2,T:3,H:4	V:1,H:1,H:2,H:3	J:1,J:3,T:1	K:1

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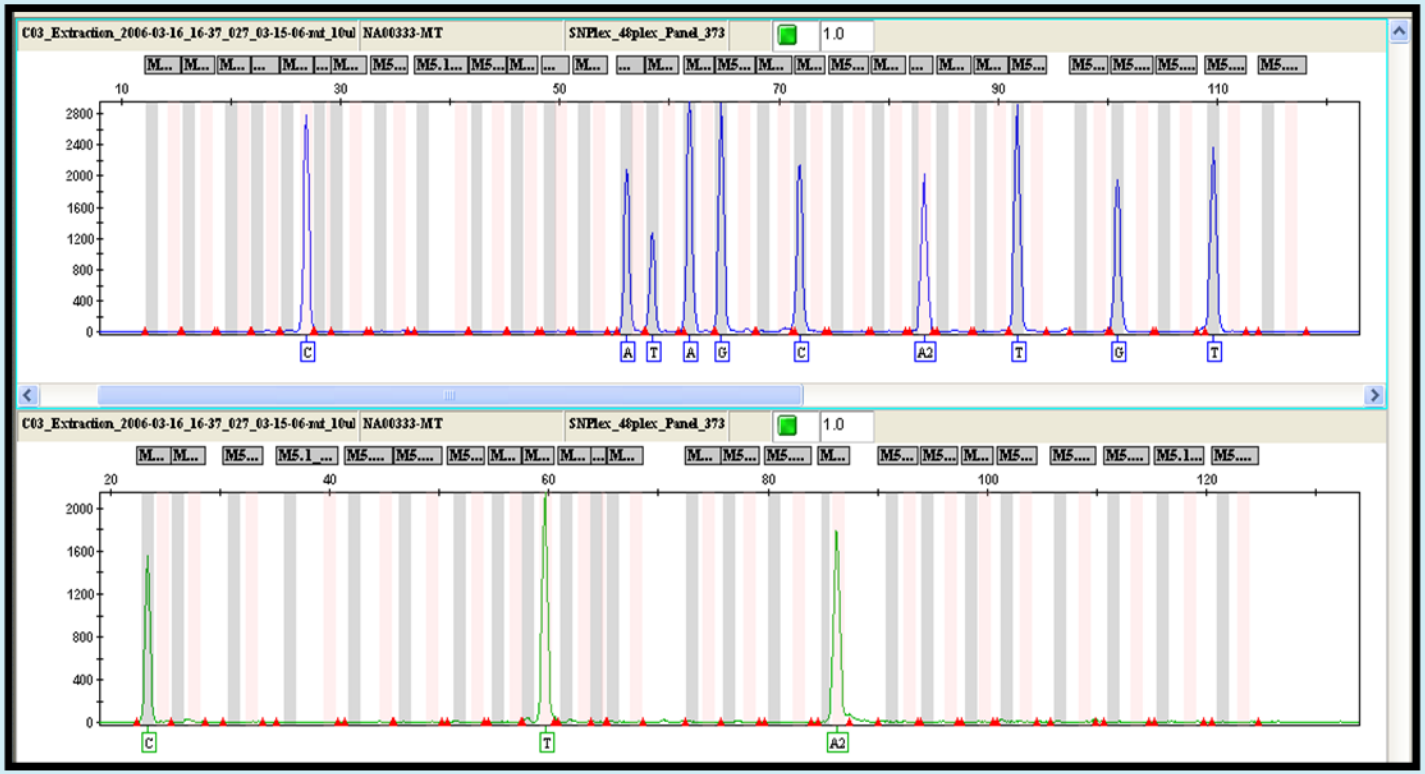
SNP Detection for Panel A Coding SNPs



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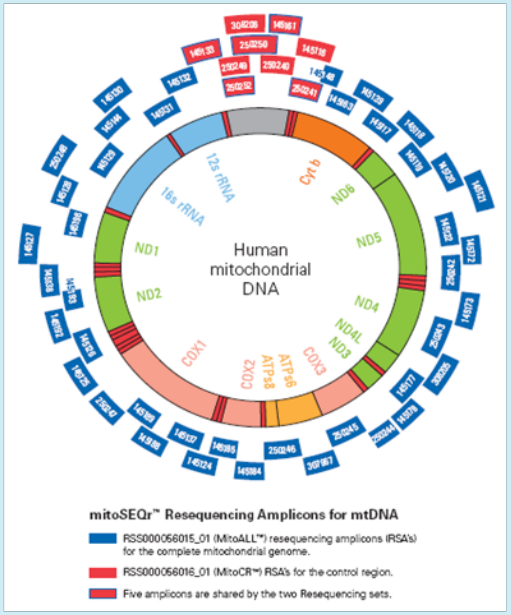
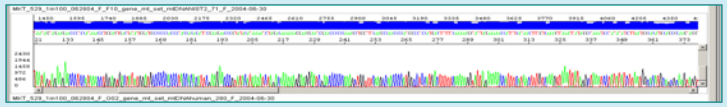
Mitochondrial Genotype Verification by mitoSEQr™ Sequencing System

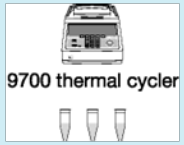




Human Genomic DNA

↓

PCR Reaction



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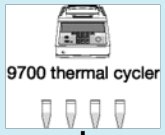
Sequencing Reaction



↓

Electrophoresis

3730/3730*xl* system

↓

Data Analysis

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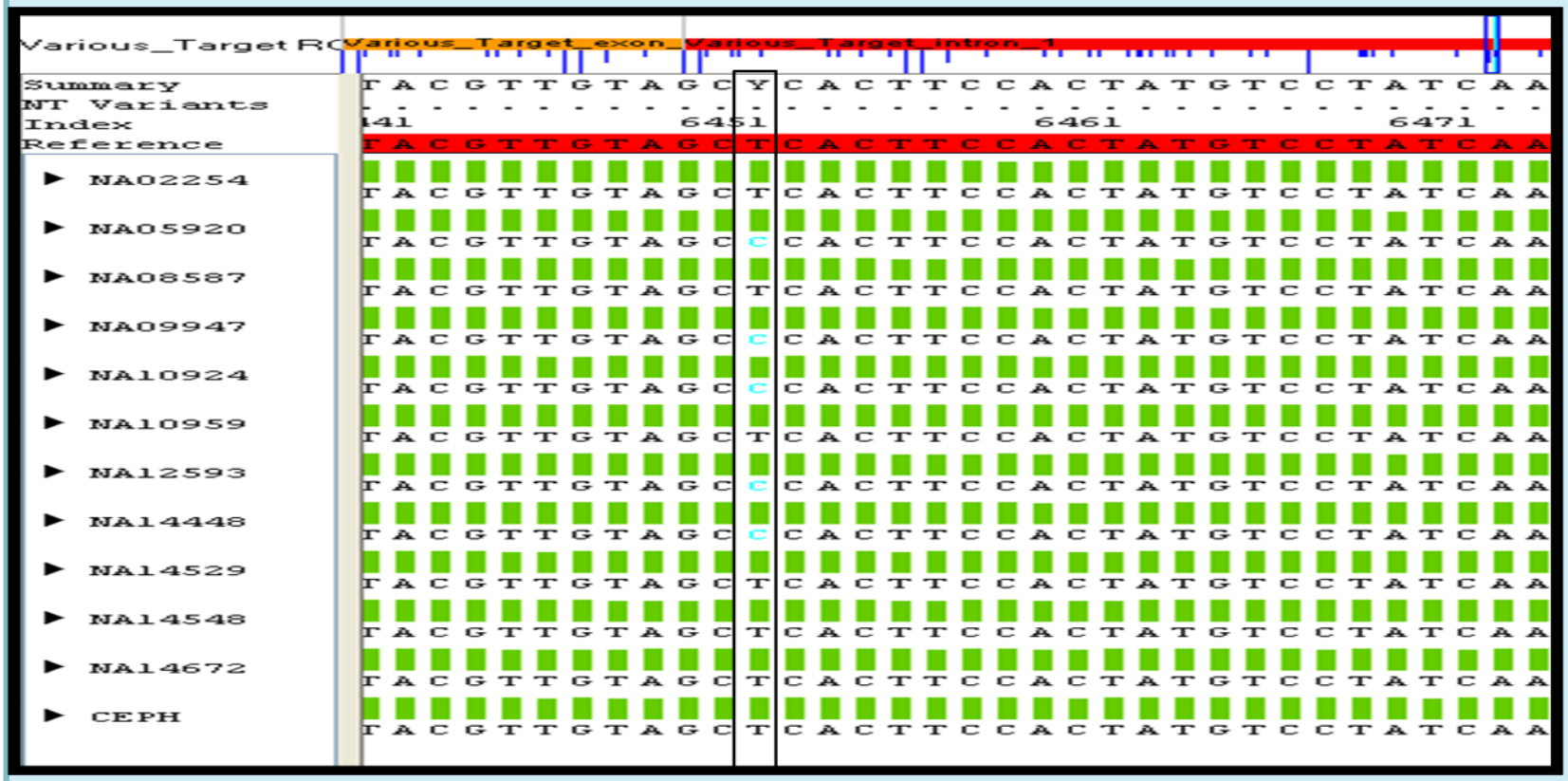
Mitochondrial SNP Detection by mitoSEQr™ System



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Correlation Between GenPlex™ and Sequencing Methods: 100%

	DNA	7202AG	5004TC	4793AG	3010GA	10211CT	14470TA	4580GA	477TC	12858CT	7028CT	16519TC
1	NA00131	A	T	A	A	C	T	G	T	C	C	C
2	NA00333	A	T	A	G	C	T	G	T	C	T	C
3	NA00546	A	T	A	G	C	T	G	T	C	C	T
4	NA00607	A	T	A	G	C	T	G	T	C	T	C
5	NA00893	A	T	A	G	C	T	G	T	C	C	T
6	NA00946	A	T	A	G	C	T	G	T	C	T	C
7	NA01805	A	T	A	A	C	T	G	T	C	C	C
8	NA01814	A	T	A	A	C	T	G	T	C	T	T
9	NA01953	A	T	A	G	C	T	G	T	C	C	T
10	NA01954	A	T	A	G	C	T	G	T	C	T	T
11	NA01990	A	T	G	G	C	T	G	T	C	C	C
12	NA02254	A	T	A	G	C	T	G	T	C	T	C
13	NA05920	A	T	A	G	C	T	G	T	C	C	T
14	NA08587	A	T	A	G	C	T	G	T	C	T	T
15	NA09947	A	T	A	G	C	T	G	T	C	C	C
16	NA10924	A	T	A	G	C	T	G	T	C	C	T
17	NA14448	A	T	A	G	C	T	G	T	C	C	T
18	NA14529	A	T	A	G	C	T	G	T	C	T	T
19	NA14548	A	T	A	G	C	T	G	T	C	T	C
20	NA14672	A	T	A	G	C	T	G	T	C	T	C

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Correlation with SNaPshot® Study by NIST

- **44 U.S. Caucasian samples sharing a common HVI / HVII type**
- **100% correlation**

NIST calls		3010	4793	10211	5004	7028	7202	16519	12858	4580	477	14470
mt1	GT38069	G	G	C	T	C	A	C	C	G	T	T
mt10	MT97163	G	G	C	T	C	A	C	C	G	T	T
mt11	TT50697	G	A	C	T	C	A	C	C	G	T	T
mt12	TT50715	A	A	C	T	C	A	C	C	G	T	T
mt13	TT50720	G	A	C	T	C	A	C	C	G	T	T
mt14	TT51702	G	A	C	T	C	A	C	C	G	T	T
mt15	TT51703	G	A	C	T	T	A	C	C	A	T	T
mt16	UA16894	A	A	C	T	C	A	C	C	G	C	T
mt17	UT57291	G	A	C	T	T	A	T	C	A	T	T
mt18	UT57293	A	A	C	T	C	A	C	C	G	T	T
mt19	UT57301	G	G	C	T	C	A	C	C	G	T	T
mt2	GT38075	G	G	C	T	C	A	C	C	G	T	T
mt20	UT57310	G	A	C	T	C	A	C	C	G	T	T
mt21	UT58314	G	A	C	T	C	A	C	C	G	T	A
mt22	UT58333	G	A	C	C	C	A	T	C	G	T	T
mt23	UT58334	A	A	C	T	C	A	C	C	G	T	T
mt24	UT58335	G	A	C	T	C	A	C	C	G	T	T
mt25	WT51345	A	A	C	T	C	A	C	C	G	T	T
mt26	WT51359	A	A	C	T	C	A	C	C	G	T	T
mt27	WT51378	G	A	C	T	C	A	C	C	G	T	T
mt28	WT52457	A	A	C	T	C	A	C	C	G	T	T
mt29	WT52472	A	A	C	T	C	A	C	C	G	T	T
mt3	GT38078	G	A	C	T	C	A	C	C	G	T	T
mt30	WT52474	A	A	C	T	C	A	C	C	G	T	T
mt31	MT94861	G	A	C	T	C	A	C	C	G	T	T
mt32	GT38094	G	A	C	T	C	A	C	C	G	T	T
mt33	JT51178	G	A	C	T	C	A	C	C	G	T	T
mt34	MT94846	G	A	C	T	C	A	T	C	G	T	T
mt35	MT94847	G	A	C	T	C	A	C	C	G	T	T
mt36	MT94850	A	A	C	T	C	A	C	C	G	T	T
mt37	MT94892	G	A	C	T	C	A	T	C	G	T	T
mt38	MT97152	G	A	C	T	C	A	C	C	G	T	T
mt39	MT97180	A	A	C	T	C	A	C	C	G	T	T
mt4	GT38081	G	A	C	T	T	A	T	C	A	T	T
mt40	TT51669	A	A	C	T	C	A	C	T	G	C	T
mt41	GT38091	G	A	C	T	T	A	T	C	A	T	T
mt42	MT94812	G	A	C	T	T	A	T	C	A	T	T
mt43	OT07753	G	A	C	T	T	A	C	C	A	T	T
mt44	PT86478	A	A	C	T	C	A	C	C	G	T	T
mt5	GT38086	A	A	C	T	C	A	C	C	G	C	T
mt6	GT38100	A	A	C	T	C	A	C	C	G	T	T
mt7	GT38106	G	A	C	T	C	A	T	C	G	T	T
mt8	GT38108	A	A	C	T	C	A	C	C	G	T	T
mt9	MT94842	A	A	C	T	C	A	C	C	G	T	T

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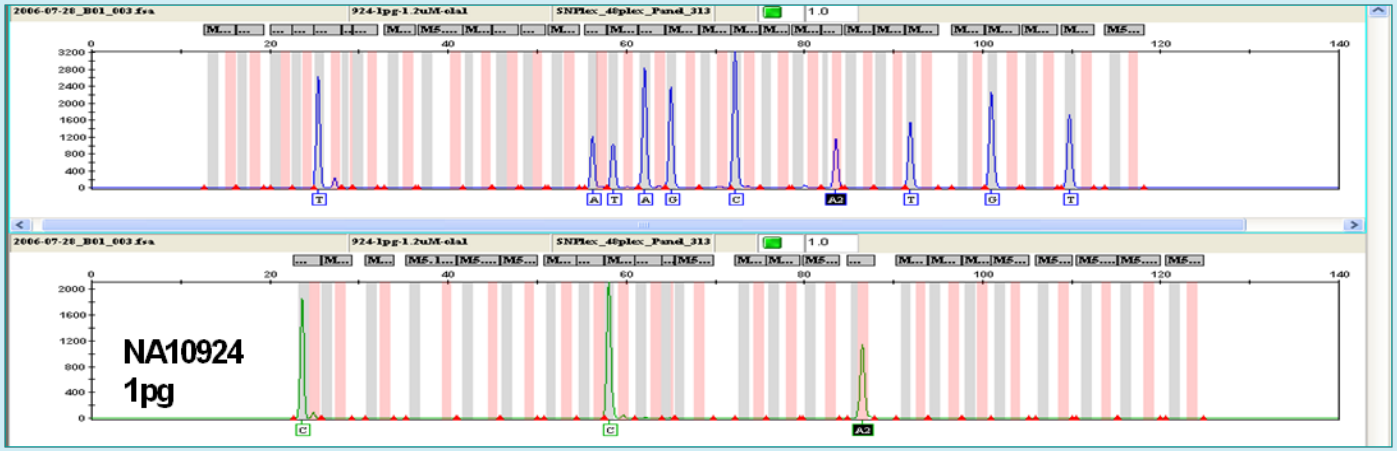
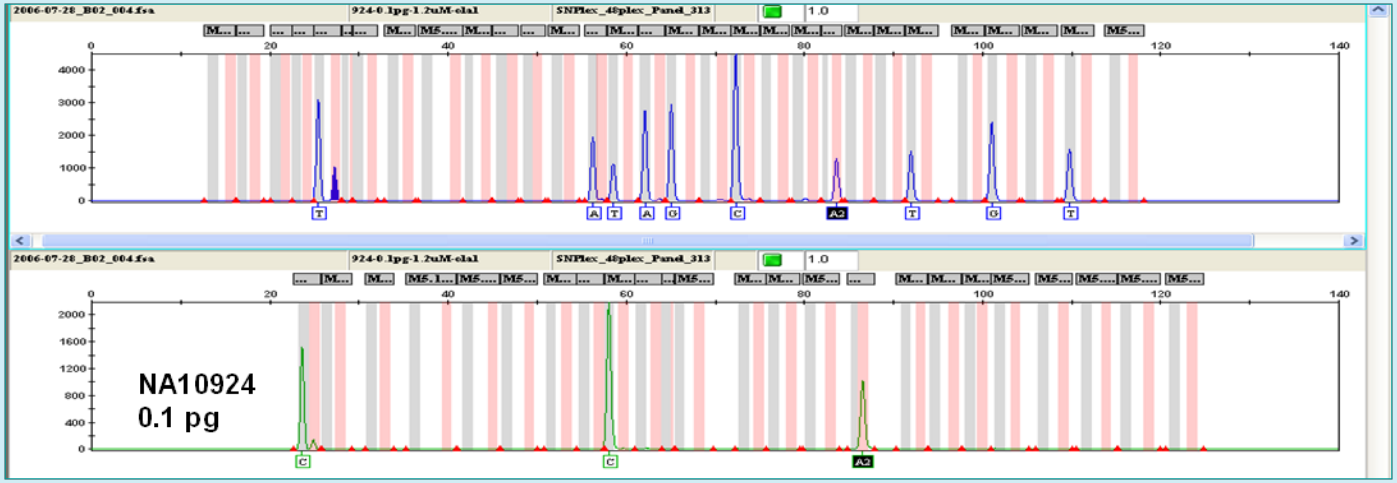
Sensitivity of 11-Plex SNP Detection System

1 pg gDNA



0.1 pg gDNA



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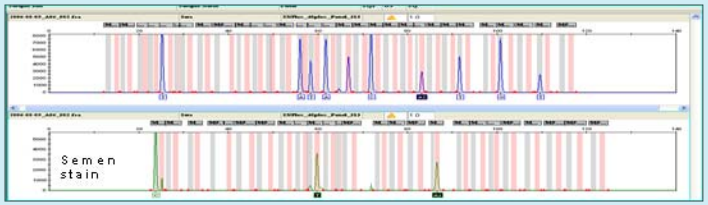
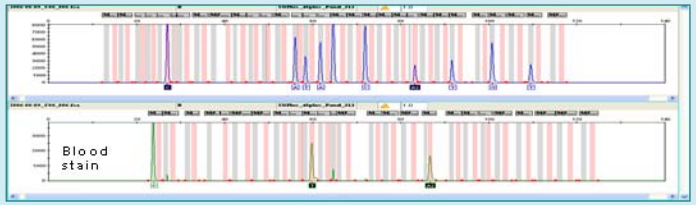
System Validation Using Forensic Samples

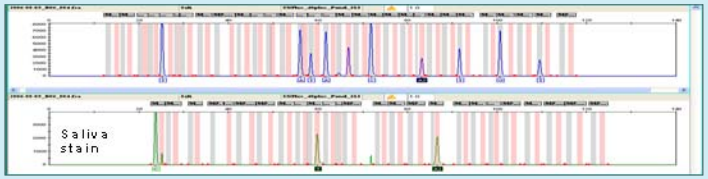
Semen stain

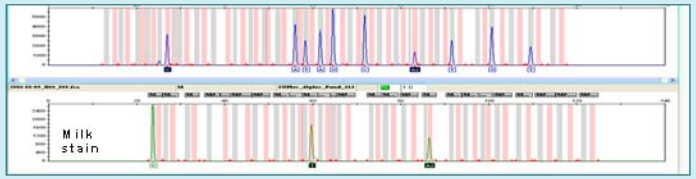




Blood-stain

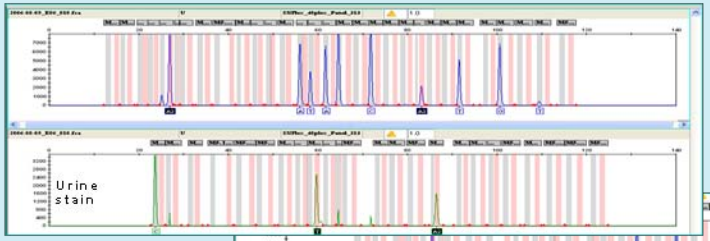
Saliva stain

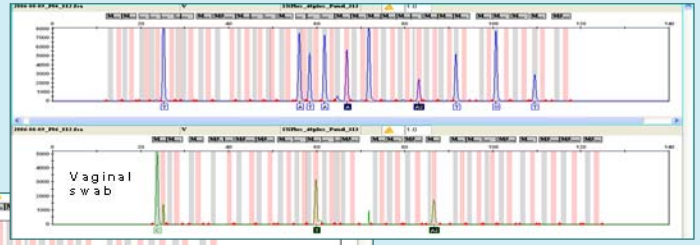




Milk stain

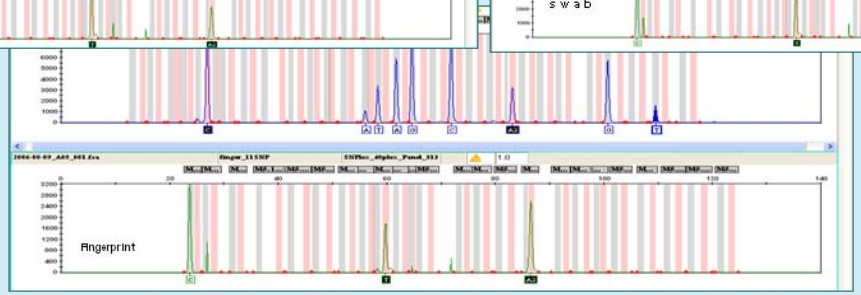
Urine stain





Vaginal swab

Fingerprint



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Potential to Genotype 58 SNPs

A	B	C	D	E	F	G	H
477 T/C	477	72	482	4808	64	3826	64
3010 G/A	3010	513	5198	5147	4745	3834	4688
4580 G/A	3915	4580	6260	9380	10211	4688	11377
4793 A/G	5004	5250	9548	9899	10394	6293	12795
5004 T/C	6776	11719	9635	11914	10685	7891	13293
7028 C/T	8592	12438	11485	15067	11377	11533	14305
7202 A/G	10394	12810	11914	16519	14470	12007	16519
10211 C/T	10754	14770	15355	14560	12795		
12858 C/T	11864	15833	15884	16390	15043		
14470 T/A	15340	15884	16368	14869	16390		
16519 T/C	16519	16519	16519				
H:1	H:2,H:3,H:6	V:1,H:5	J:1,J:2,K:2,K:3	J:4,T:2,T:3,H:4	V:1,H:1,H:2,H:3	J:1,J:3,T:1	K:1

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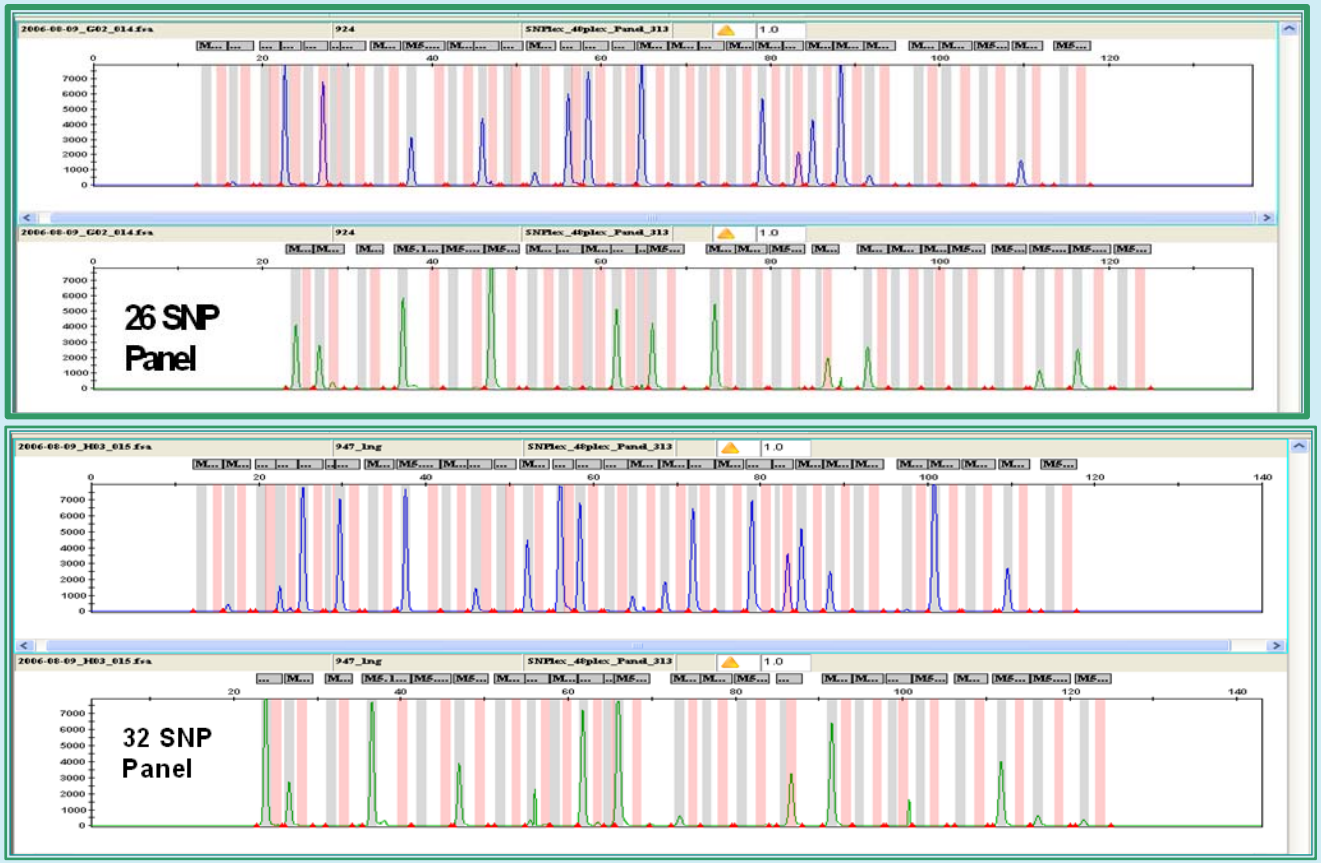
Potential of Scaling up to 58 SNPs Using Two Multiplex Designs



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Conclusions

- **An easy and robust method has been developed for mitochondrial SNP genotyping**
- **The results from this approach are completely concordant with sequencing and SNaPshot®**
- **This method fulfills the following requirements for forensic studies:**
  1. **Low consumption of gDNA samples (picogram of genomic DNA)**
  2. **High throughput (up to 48 SNPs / reaction)**
  3. **High accuracy**
  4. **Fast and ease of use (< 7 hours)**
- **This method can be used for varieties of forensic samples**

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References

- Peter M. Vallone, Rebecca S. Just, Michael D. Coble, John M. Butler, Thomas J. Parsons. “A multiplex allele-specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome” Int J Legal Med (2004) 118(3) 147–157
- Coble, M.D., Just, R.S., O'Callaghan, J.E., Letmanyi, I.H., Peterson, C.T., Irwin, J.A., Parsons, T.J. “Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians” Int J Legal Med (2004) 118(3) 137–146

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Clinical Applications: Pathogen and Drug Resistance Detection

- **Application for detection of multiple pathogens, drug resistant mutations and virulence factors**
- **Equal or better than existing formats**

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SIMULTANEOUS DETECTION OF 17 RESPIRATORY VIRUSES AND SUBTYPES WITH THE LUMINEX DIAGNOSTICS® RESPIRATORY VIRUS PANEL KIT

Christine Robinson, Kristin Murray, Kristi Lookner, and Qi Wei
The Children's Hospital, Denver CO

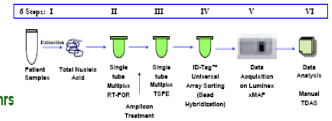
Respiratory Viral Panel

NAVY MEDICINE
World Class Care...Anytime, Anywhere

Virus	Type/Subtype	Gene Target
Influenza A		Matrix gene
	H1	Hemagluttinin gene
	H3	Hemagluttinin gene
	H5	Hemagluttinin gene
Influenza B		Matrix gene
Respiratory Syncytial Virus	Type A	Fusion gene
	Type B	Fusion gene
Coronavirus	229E	Nucleocapsid gene
	OC43	Nucleocapsid gene
	SARS	Nucleocapsid gene
	NL63	Nucleocapsid gene
	NHKU1	Nucleocapsid gene
Parainfluenza virus	Parainfluenza 1	HN gene
	Parainfluenza 2	HN gene
	Parainfluenza 3	HN gene
	Parainfluenza 4	HN gene
Metapneumovirus		Polymerase (L) gene
Enterovirus/ Rhinovirus		5'NCR
Adenovirus		Hexon gene

U.S. Naval Medical Research Unit 2

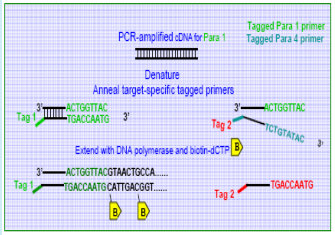
ID Tag™ RVP Overview



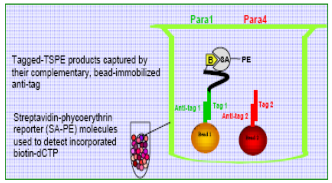
II. Setup & Multiplex PCR (3 hrs

			Comment	Results
Viruses	Influenza A	H1, H3	H5 not scored	3 (4)
	Influenza B			1
	RSV	A, B		2
	Coronaviruses	NL63, HKU1, 229E, OC43	SARS available outside the US	4 (5)
	Parainfluenza	1-4		4
	HMPV		Dutch sequence	1
	Entero/Rhino		No differentiation	1
	Adenovirus			1
Controls	MS-2 bacteriophage		Internal (extraction) control	1
	Lambda DNA		Run control	1
Total Analytes 19 (21)				

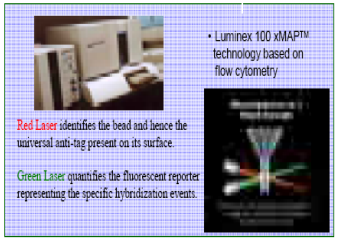
III: Amplicon Treatment & Multiplex TSPE (2.5 hrs)



IV: Bead Hybridization + Reporter Addition (1 hr)



V & VI: Detection and Analysis (30 minutes)



Total time about 6 to 7 hours

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Proof of Concept: GenPlex™ Assay Design for Multiple Pathogen Targets

(41 Targets and Six Mutations)

	Design	Assay Targets	Targets Tested
1	1	Human metapneumovirus	Human metapneumovirus
2	1	Influenza A virus (A/chicken/Chaiyi/2004(H5N1))	Influenza A virus (A/chicken/Chaiyi/2004(H5N1))
3	1	Bovine adenovirus A	Bovine adenovirus A
4	1	SARS coronavirus Frankfurt 1	SARS coronavirus Frankfurt 1
5	1	Influenza B virus	Influenza B virus
6	1	Human parainfluenza virus 1	Human parainfluenza virus 1
7	1	Human parainfluenza virus 2	Human parainfluenza virus 2
8	1	Human parainfluenza virus 3	Human parainfluenza virus 3
9	1	Influenza A virus (A/HongKong/2308(H3N2))	Influenza A virus (A/HongKong/2308(H3N2))
10	1	SARS coronavirus Frankfurt 1	SARS coronavirus Frankfurt 1
11	1	Human adenovirus type 4	Human adenovirus type 4
12	1	Influenza A virus (A/X31(H3N2))	Influenza A virus (A/X31(H3N2))
13	1	Human respiratory syncytial virus	Human respiratory syncytial virus
14	1	Influenza A virus (A/Kanagawa/1/2009(H1N1))	Influenza A virus (A/Kanagawa/1/2009(H1N1))
15	2	Influenza A virus (A/Nagasaki/1/2008(H3N2))	Influenza A virus (A/Nagasaki/1/2008(H3N2))
16	2	Bacillus anthracis	Bacillus anthracis
17	2	Bacillus sp CDC1B	
18	2	Francisella tularensis subsp tularensis	
19	2	Francisella tularensis	Francisella tularensis
20	2	Human parainfluenza virus 3	Human parainfluenza virus 3
21	2	Human papillomavirus type 16	Human papillomavirus type 16
22	2	Human T-lymphotropic virus 1	Human T-lymphotropic virus 1
23	2	Human T-lymphotropic virus 2	Human T-lymphotropic virus 2

	Design	Assay Targets	Targets Tested
24	2	EBV	EBV
25	2	Human herpesvirus 4	Human herpesvirus 4
26	2	Human herpesvirus 5	Human herpesvirus 5
27	2	BK polyomavirus	BK polyomavirus
28	2	JC polyomavirus	JC polyomavirus
29	2	Simian virus 40	
30	2	Human herpesvirus 7	Human herpesvirus 7
31	2	Human T-lymphotropic virus 2	
32	2	HIV-AII	
33	2	Hepatitis B virus	Hepatitis B virus
34	2	Hepatitis B virus	Hepatitis B virus
35	2	Hepatitis C virus	Hepatitis C virus
36	2	Human immunodeficiency virus 1	Human immunodeficiency virus 1
37	2	Human adenovirus 6	Human adenovirus 6
38	2	Human adenovirus 6	Human adenovirus 6
39	2	Human immunodeficiency virus 2	
40	2	Human immunodeficiency virus 2	
41	2	OW_UL97_C592G	OW_UL97_C592G
42	2	OW_UL97_C592G	OW_UL97_C592G
43	2	OW_UL97_del981-2	OW_UL97_del981-2
44	2	OW_UL97_del981-2	OW_UL97_del981-2
45	2	OW_UL97_M460V	OW_UL97_M460V
46	2	OW_UL97_M460V	OW_UL97_M460V
47	2	Human gender assay	Male, Female

All in one tube

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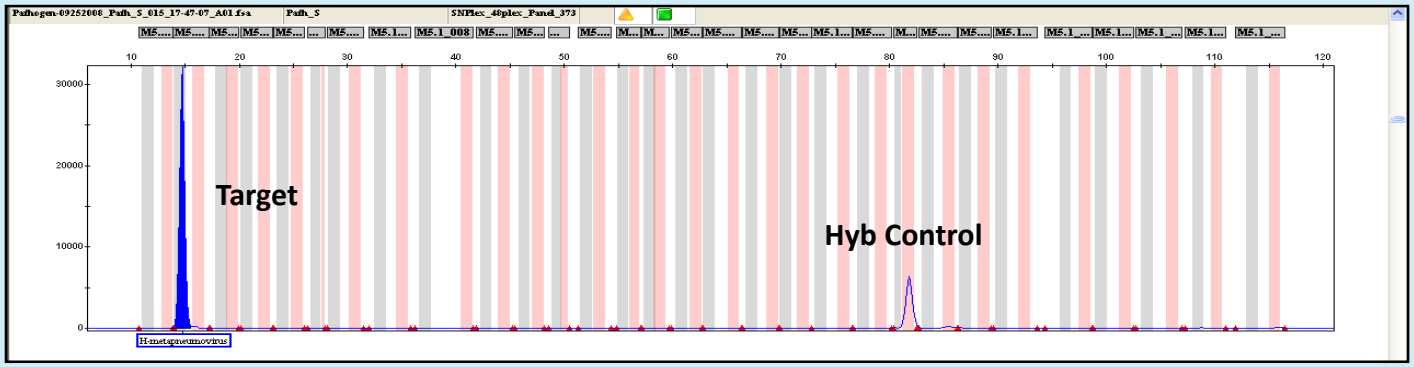
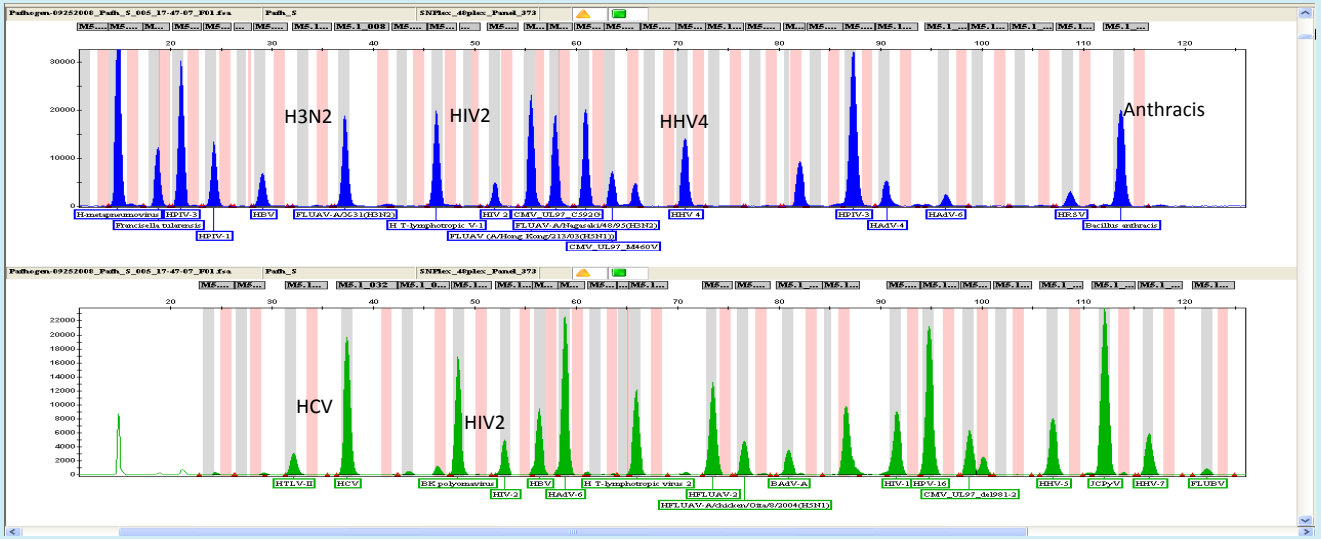
Multiplexing Study for Pathogen Detection

Singleplex Assay



Multiplex Assay



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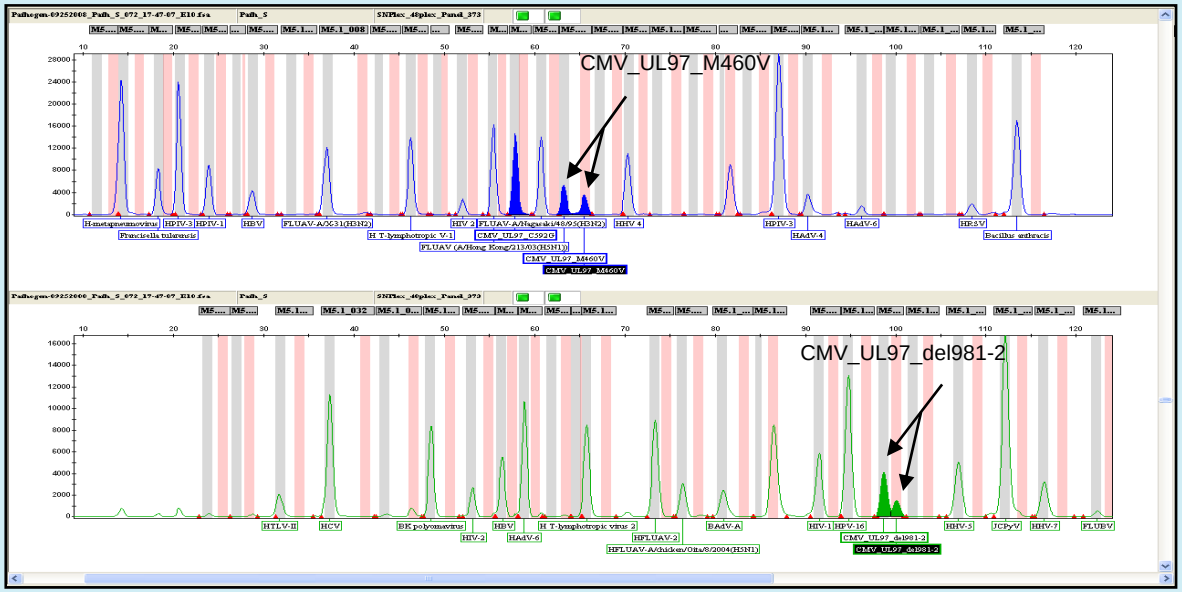
Cytomegalovirus Drug Resistance Mutations

- **Mutations tested: UL97, M460V, C592G, A594V, L595S and pol del981-2**

CMV_UL97_C592G	C[T/G]G
CMV_UL97_M460V	C[A/G]T
CMV_UL97_A594V	G[C/T]G
CMV_UL97_L595S	T[T/C]G
CMV_UL97_del981-2	C[ATCGAC/-]C

- **OLA assays: two alleles for mutation**



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Companion Diagnostics

- **Application for detection of mutation in EGFR and KRAS pathway**

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EGFR Pathway Mutations Influencing Cancer Therapy

- **Mutations in EGFR and Ras confer drug resistance**

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Current Technologies Used for EGFR Mutation Detection In Lung Cancer

Table 1. Methods for detecting *EGFR* mutations in lung cancer specimens

Technique	Reference	Sensitivity (% mutant DNA)	Mutations identified	Comprehensive detection of deletions and insertions?
Direct sequencing	Multiple	25	Known and new	Yes
PCR-SSCP	(10)	10	Known and new	Yes
TaqMan PCR	(11, 12)	10	Known only	No
Loop-hybrid mobility shift assay	(13)	7.5	Known only	Yes
Cycleave PCR	(14)	5	Known only	Yes
PCR-RFLP and length analysis	(15)	5	Known only	Yes
MALDI-TOF MS–based genotyping	(16)	5	Known only	No
PNA-LNA PCR clamp	(17)	1	Known only	No
Scorpions ARMS	(18)	1	Known only	No
dHPLC	(19–21)	1	Known and new	Yes
Single-molecule sequencing	(22)	0.2	Known and new	Yes
Mutant-enriched PCR	(23)	0.2	Known only	No
SMAP	(8)	0.1	Known only	No

Abbreviations: SSCP, single-strand conformation polymorphism; PNA-LNA: peptide nucleic acid–locked nucleic acid; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ARMS, amplified refractory mutation system; dHPLC, denaturing high performance liquid chromatography.

Clin Cancer Res 2007;13(17) September 1, 2007

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DxS Developed EGF29 and K-RAS Mutation Test Kits

- **DxS's EGF29 and K-RAS Mutation Test Kits detect key mutations from EGFR and K-RAS that correlate with responsiveness to tyrosine kinase inhibitors, such as gefitinib and erlotinib, with some mutations having a sensitizing effect and others being linked to resistance**
- **Total of (7 + 29) = 36 tubes for assay**
- **2 to 3 ng DNA input / tube**
- **Total about 70 ng**

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GenPlex™ Assay to Detect 34 Mutations In One Reaction

UID	Gene	Exon	SNP/Indel Mutation
EGFR_A750_E758del	EGFR	exon19	A[AGCAACATCTCCGAAAGCCAACAAGGA/-]A
EGFR_A767_S768_TLAins	EGFR	exon20	A[-/CGCTGGCCA]G
EGFR_E746_A748del	EGFR	exon19	G[GAATTAAGA/-]G
EGFR_E746_A750del	EGFR	exon19	A[GAATTAAGAGAAGCAACA/-]T
EGFR_E746_E749del	EGFR	exon19	G[GAATTAAGAGAA/-]G
EGFR_E746_T751del	EGFR	exon19	G[GAATTAAGAGAAGCAACA/-]T
EGFR_E746del	EGFR	exon19	A[AGG/-]A
EGFR_G719A	EGFR	exon18	G[G/C]C
EGFR_G719C	EGFR	exon18	G[G/T]G
EGFR_G719S	EGFR	exon18	G[G/A]G
EGFR_K745_E749del	EGFR	exon19	C[AAGGAATTAAGAGAA/-]
EGFR_K745_L747del	EGFR	exon19	C[AAGGAATTA/-]A
EGFR_L747_A750del	EGFR	exon19	A[TTAAGAGAAGCA/-]A
EGFR_L747_E749del	EGFR	exon19	A[TTAAGAGAA/-]G
EGFR_L747_K754del	EGFR	exon19	A[TTAAGAGAAGCAACATCTCCGAAA/-]G
EGFR_L747_P753del	EGFR	exon19	A[TTAAGAGAAGCAACATCTCCG/-]A
EGFR_L747_S752del	EGFR	exon19	A[TTAAGAGAAGCAACATCT/-]C
EGFR_L747_T751del	EGFR	exon19	A[TTAAGAGAAGCAACA/-]T
EGFR_L858R	EGFR	exon21	C[T/G]G
EGFR_L861Q	EGFR	exon21	C[T/A]G
EGFR_M766_A767_Alins	EGFR	exon20	G[-/GCCATA]G
EGFR_N756_L760del	EGFR	exon19	C[CAACAAGGAAATCCT/-]C
EGFR_P753_I759del	EGFR	exon19	T[CCGAAAGCCAACAAGGAAATC/-]C
EGFR_R748_P753del	EGFR	exon19	T[TAAGAGAAGCAACATCTC/-]C
EGFR_S752_I759del	EGFR	exon19	T[ATCTCCGAAAGCCAACAAGGAAAT/-]C
EGFR_S768I	EGFR	exon20	A[G/T]C
EGFR_T751_E758del	EGFR	exon19	C[AACATCTCCGAAAGCCAACAAGGA/-]A
EGFR_T790M	EGFR	exon20	A[C/T]G
EGFR_V769_D770_ASVins	EGFR	exon20	G[-/GCCAGCGTG]G
KRAS_Ala12Val	K-RAS	exon2	G[C/T]T
KRAS_Arg12Cys	K-RAS	exon2	T[C/T]G
KRAS_Gly12Asp	K-RAS	exon2	G[G/A]T
KRAS_Gly12Ser	K-RAS	exon2	T[G/A]G
KRAS_Gly13Asp	K-RAS	exon2	G[G/A]C

DNA Input = 0.5 ng

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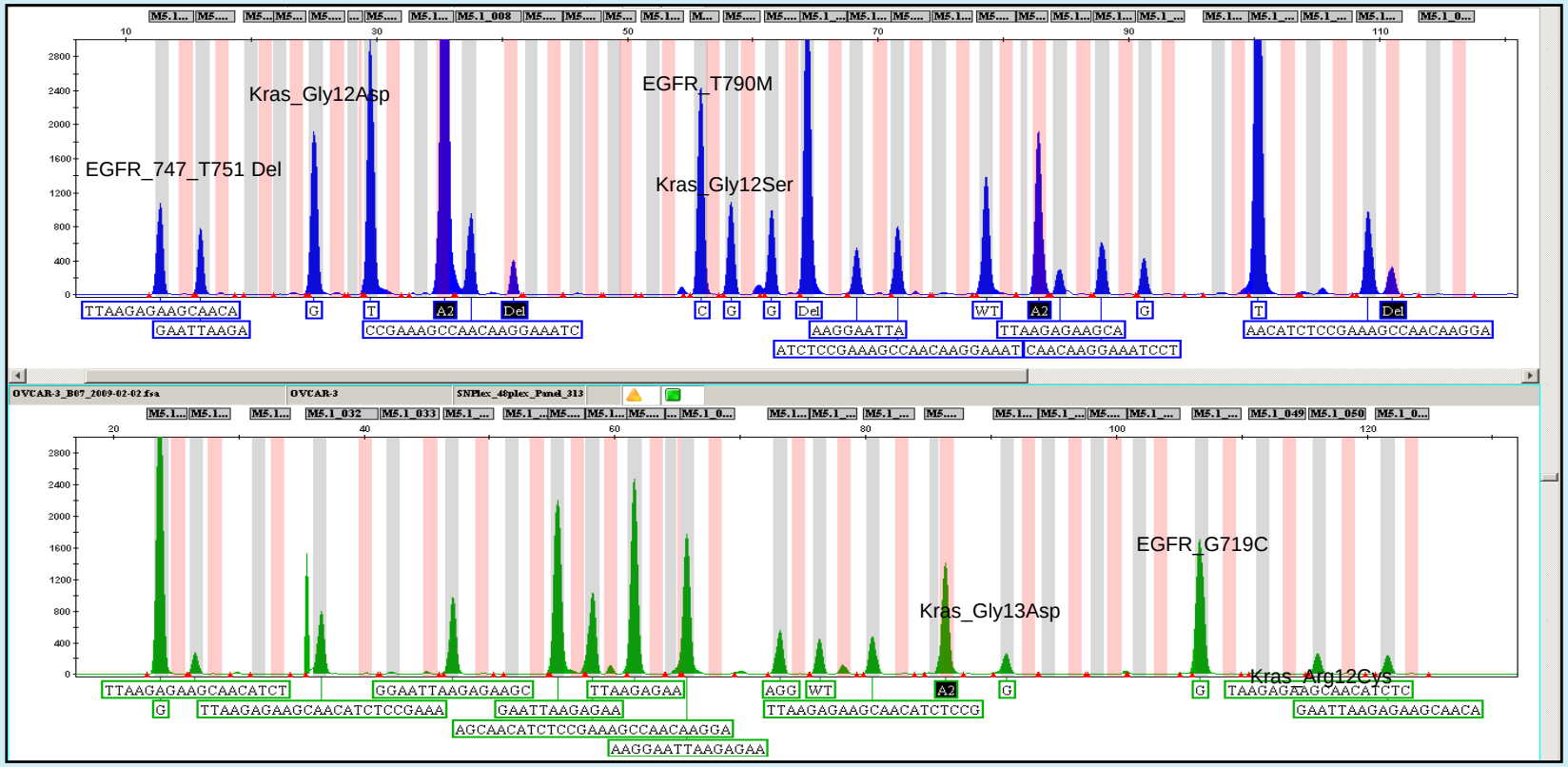
Cancer Cell Line DNA SNP and INDEL Detection Using GenPlex® Approach (NCI-60 Panel)



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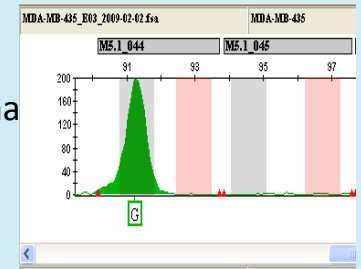
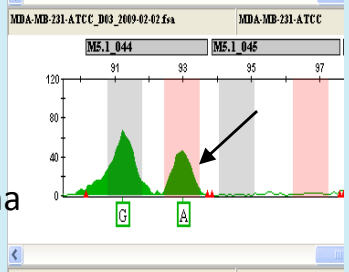
KRas Gly13Asp

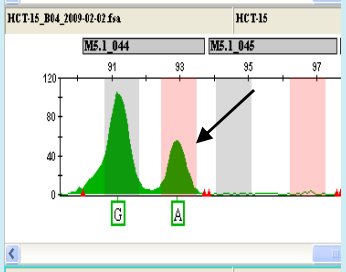
MDA-MB-435: M14 melanoma cells



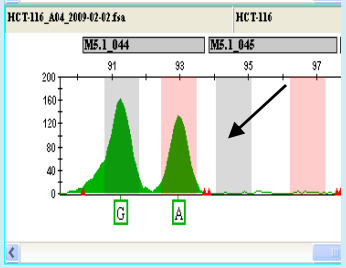
MDA-MB-231: Breast adenocarcinoma



HCT-15: colon carcinoma



HCT-116 cells:colorectal carcinoma

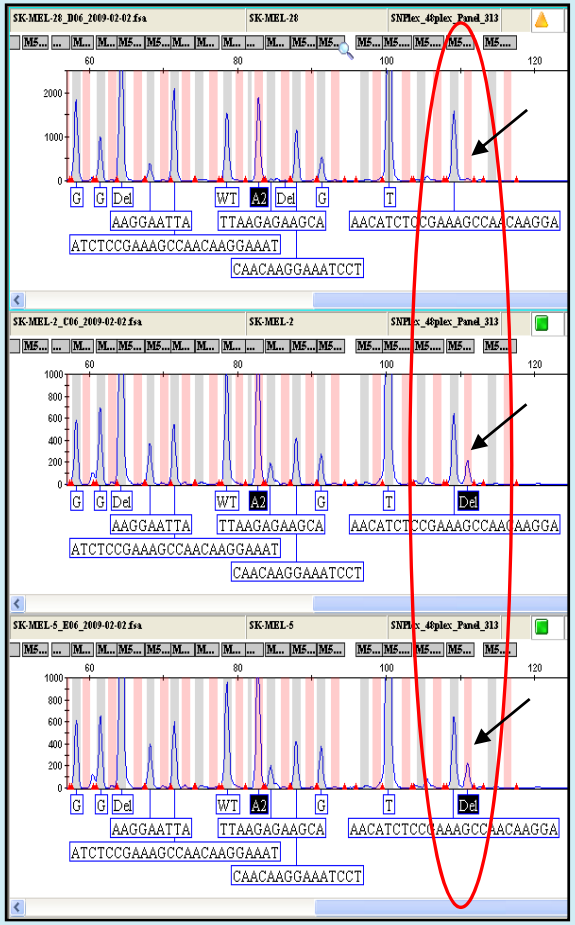


EGFR T751-E758 del

SK-MEL 28: Human melanoma cell

SK-MEL 2: Human melanoma cell

SK-MEL 5: Human melanoma cell



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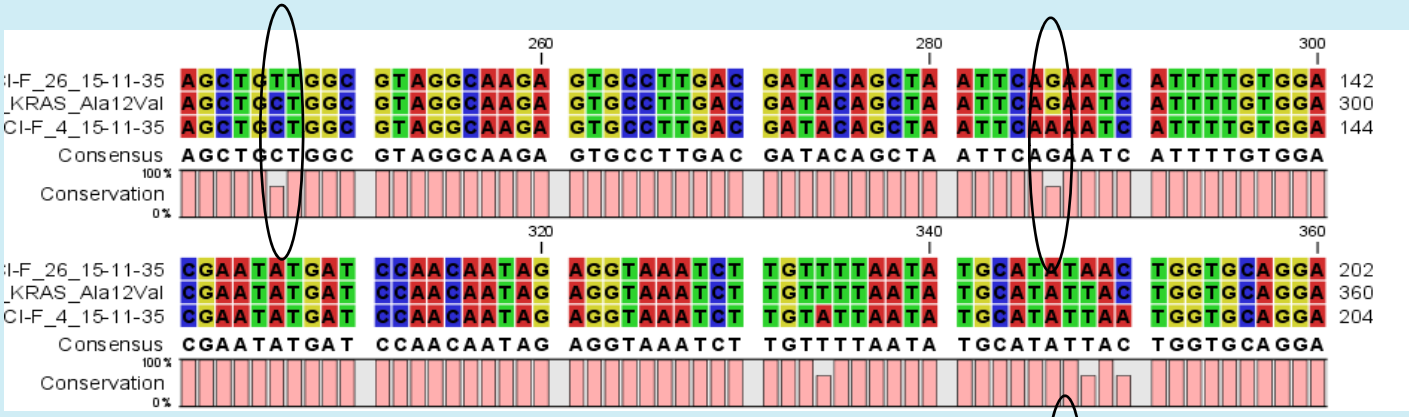
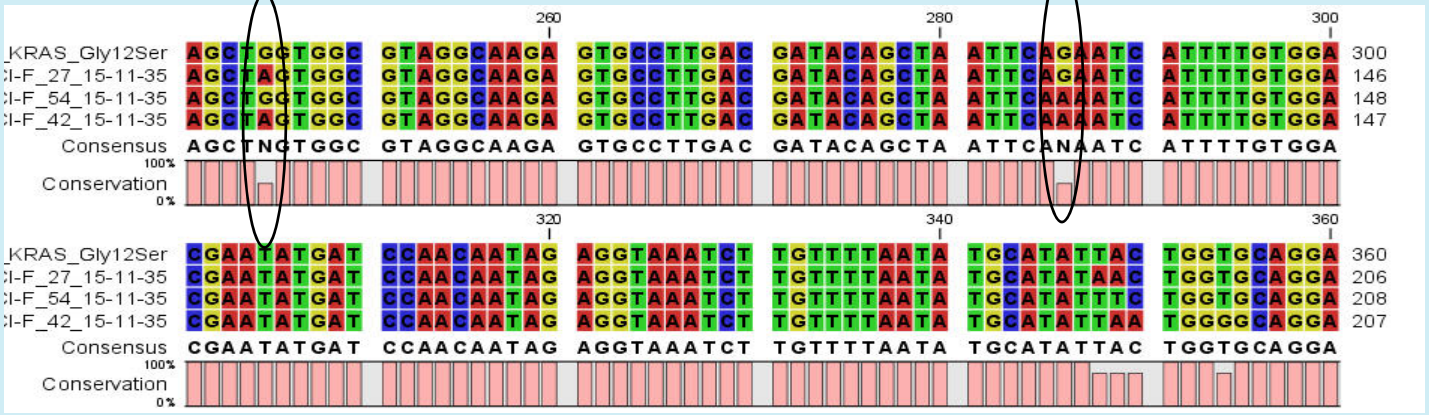
100% Concordance of Mutation Detection Between DNA Sequencing and GenPlex™ Platform

KRas _Ala12Val



KRas_ Gly12Ser



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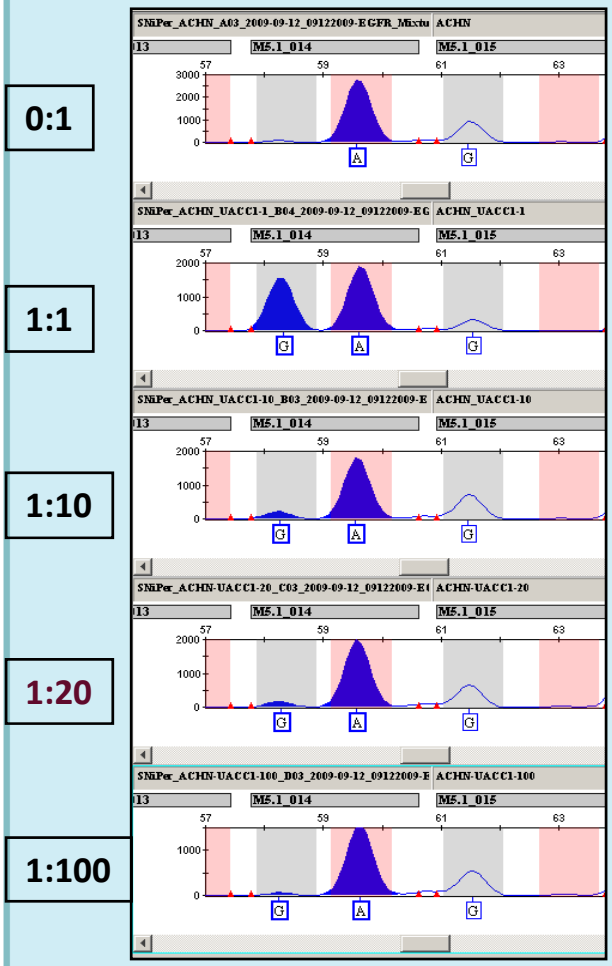
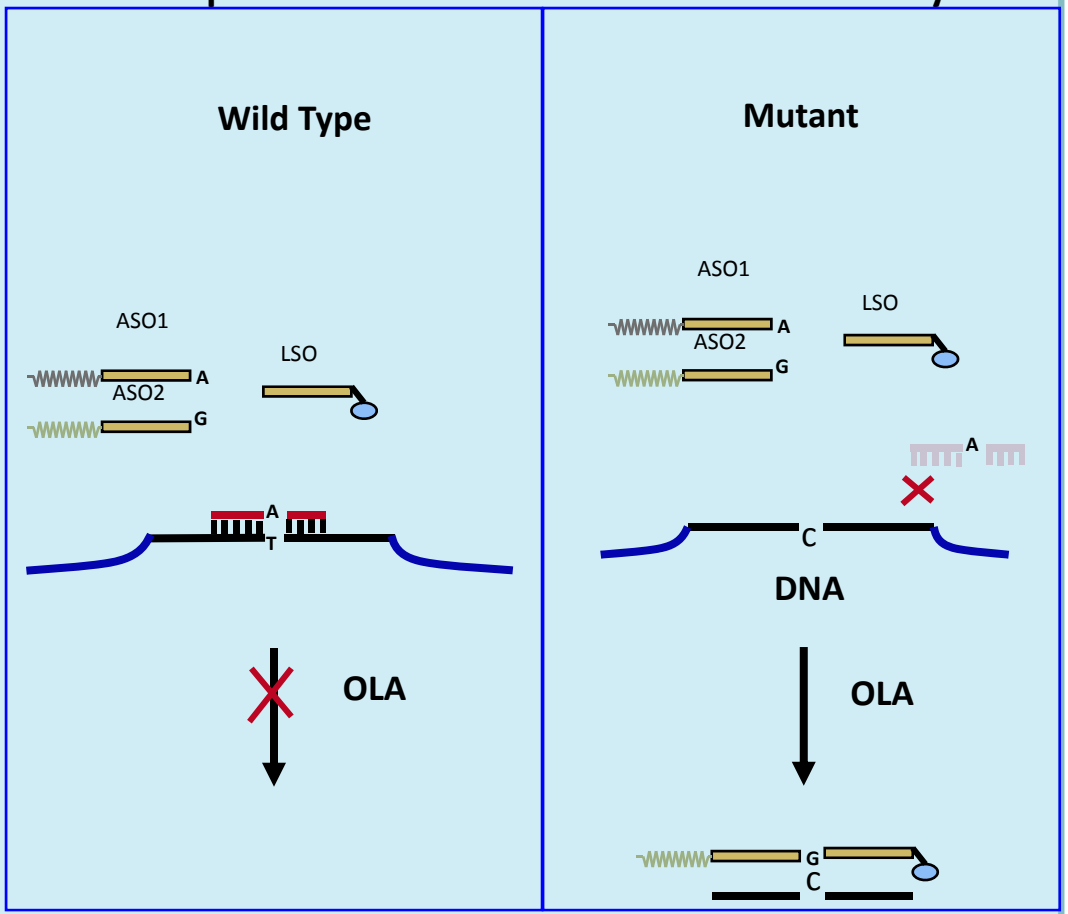
Minor Component Detection Sensitivity

Cell line DNA: ACHN/UACC1



Competitive OLA format to increase sensitivity



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Conclusions

- The GenPlex™ system provides a solution for multiple mutation and nucleic acid detection:
  - 98 nucleic acid targets
  - 49 SNP targets
  - Any combination of both per reaction
- GenPlex™ system has high sensitivity: suitable for low amount genomic DNA (0.1 ng to 1 ng of NA)
- High specificity is ensured at two levels; both at the PCR and OLA reactions
  - > 99.5% accuracy reported in customer studies
- The system is amenable to automation
- Streamlined protocol with time to result time of about 4 to 5 hours
- Highly Integrated GenPlex™ System will be an excellent platform for molecular diagnostic applications

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Questions?

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