



Technology Transition Workshop | *Rixun Fang*

GenPlex™ HID Training Class I

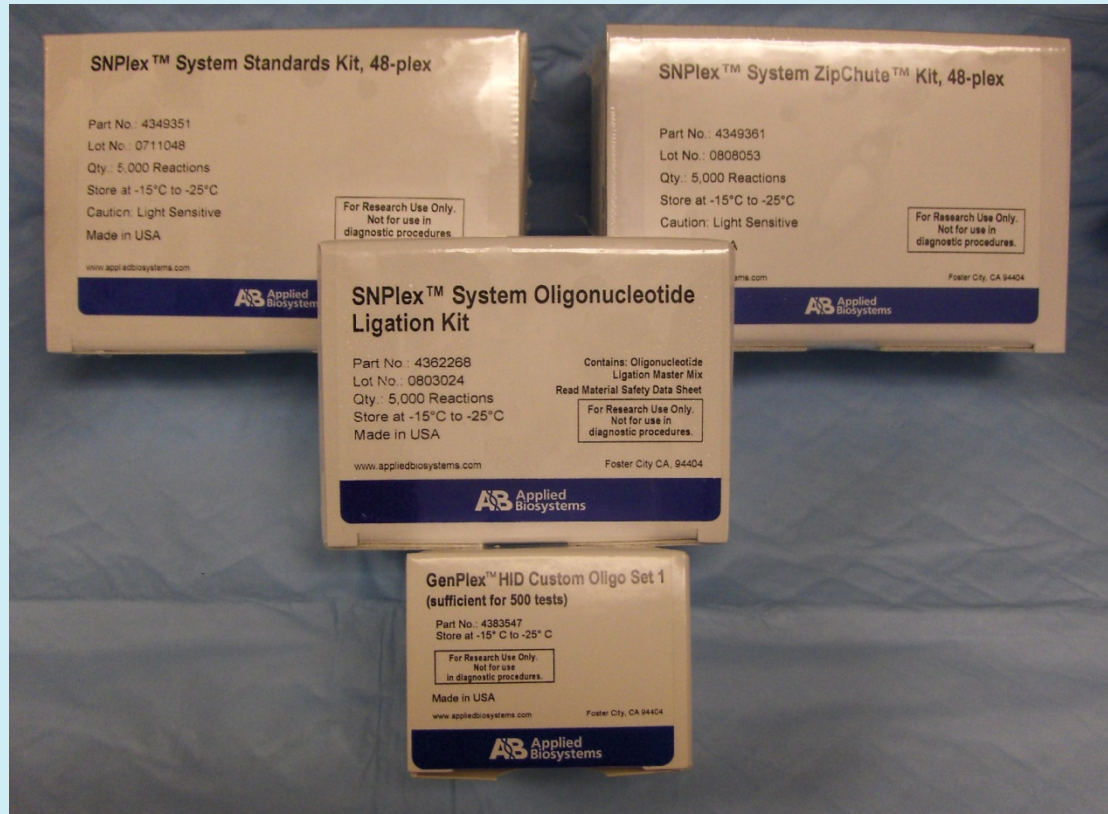
Outline of Presentation

- **Introduction**
- **GenPlex™ HID kit**
- **Experimental plan**
- **Class schedule**

Potential Forensic Applications

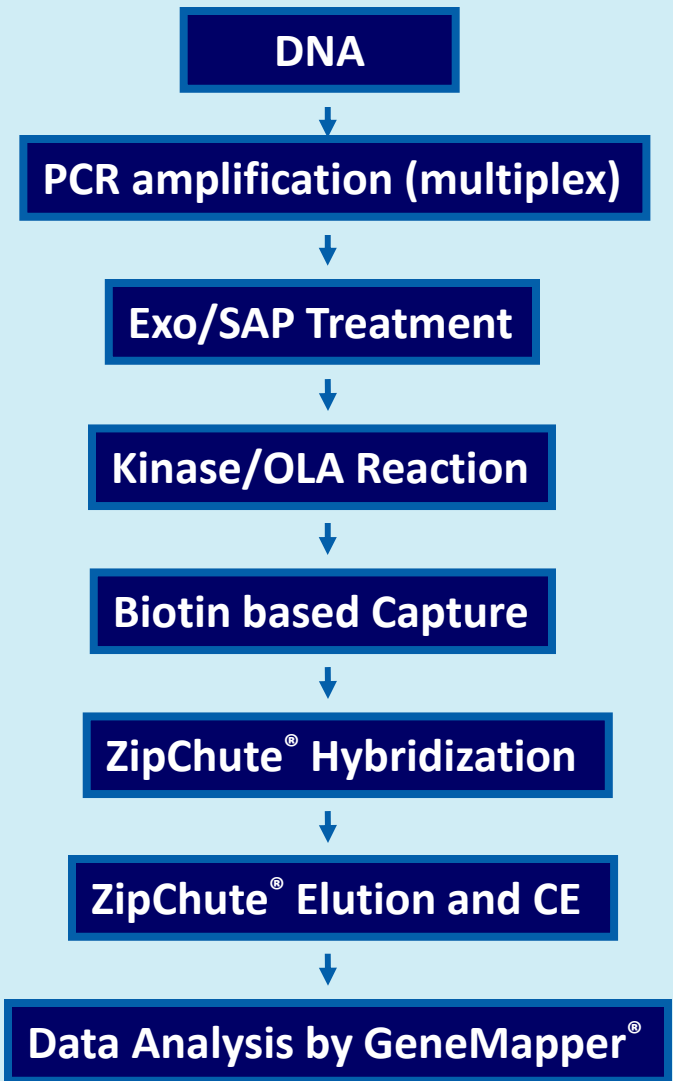
- **Identification**
 - **Missing persons**
 - **Mass disaster**
 - **Animal species identification**
 - **Microbial forensics**
- **Paternity and maternity testing**
- **Genealogy studies**
- **Trait ID and race prediction: eye, skin, or hair color**
- **SNPs identified by SNPforID consortium and Ken Kidd are leading SNP panels**

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

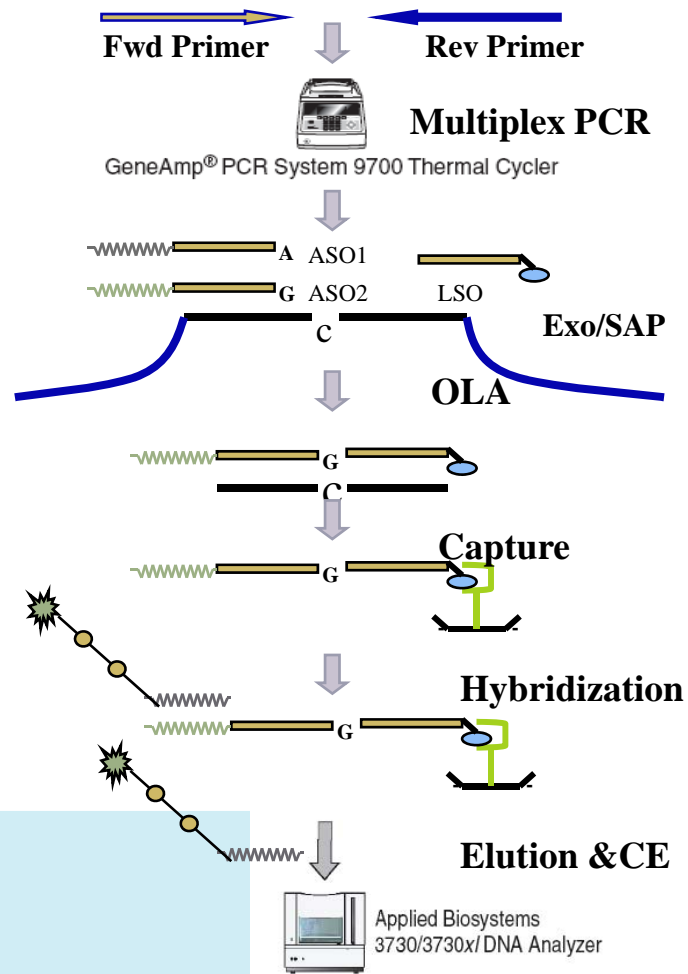


Major System Features

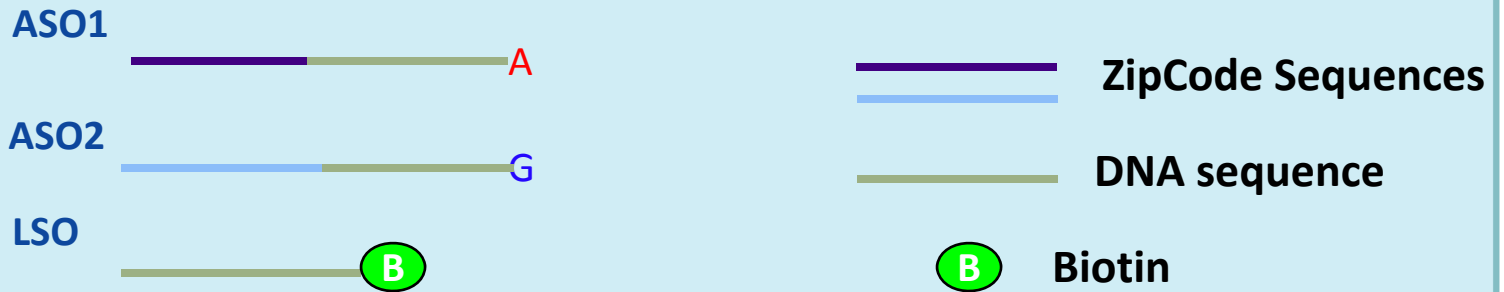
- **High sensitivity**
 - 100 pg gDNA
- **Highly specific**
 - Specificity at the PCR and OLA step
- **Short target size (<115 bp)**
- **Rapid time-to-result turn around time**
 - About 5 - 6 hours
- **Multiplex system (SNPforID core set of 48 SNP + gender determination marker)**
- **High probability of identity**
 - Average probability of identity approximately 1×10^{-19}
- **Use the current instruments and similar workflow for STR analysis**
 - CE instrument
 - Size standard, allelic ladder, etc.
- **Currently requires GeneMapper[®] 4.0 software**
- **Automatable**



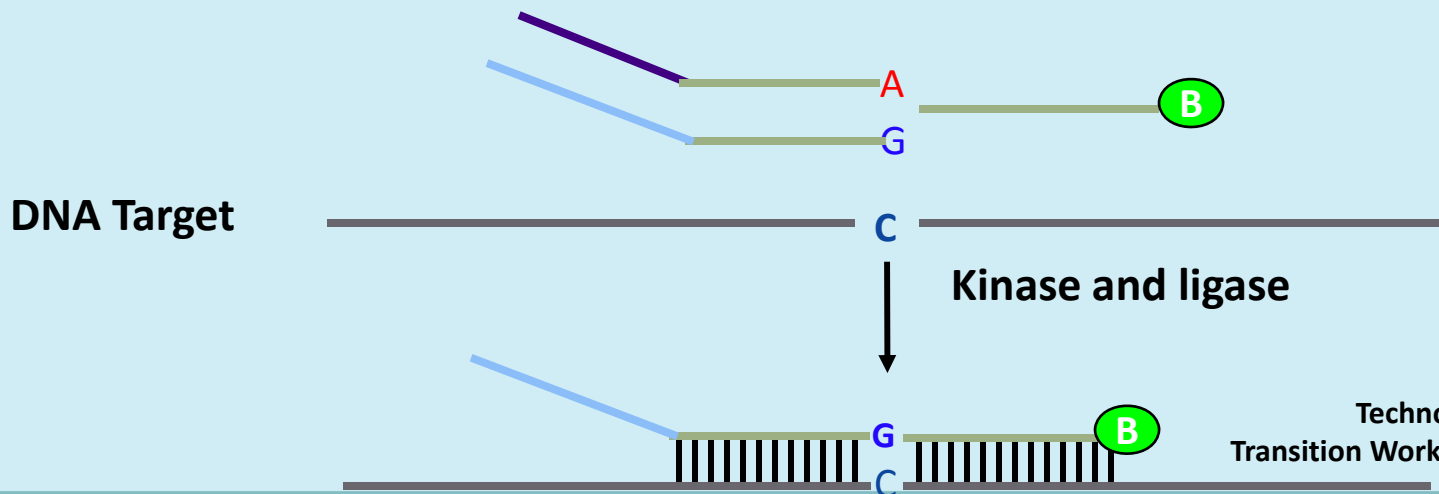
Chemistry Overview



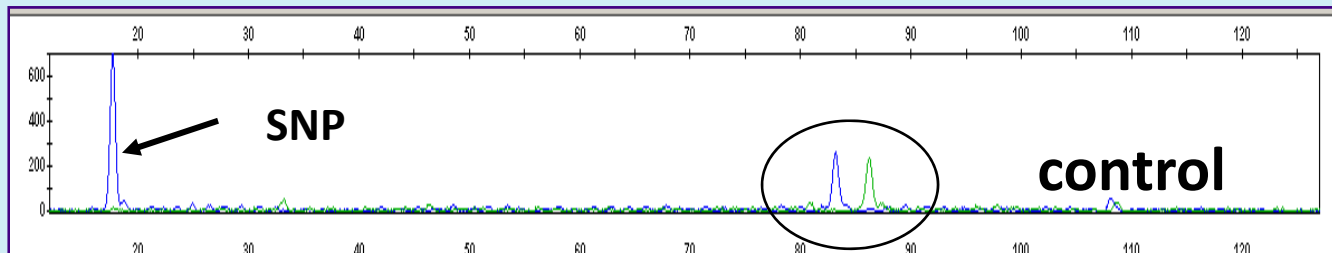
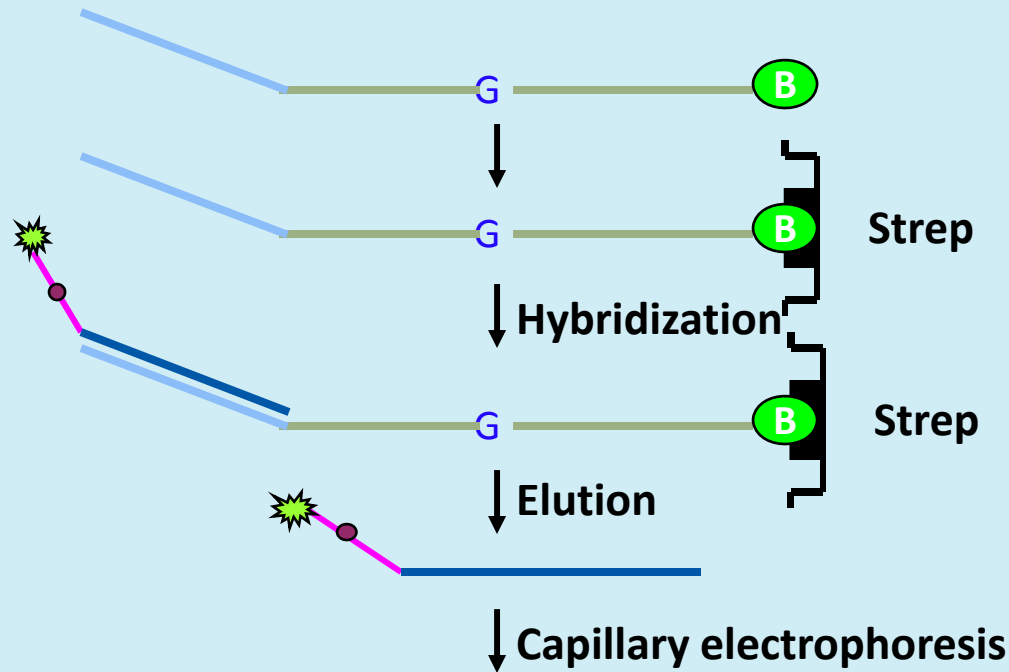
Bioinformatic Design of OLA Probes



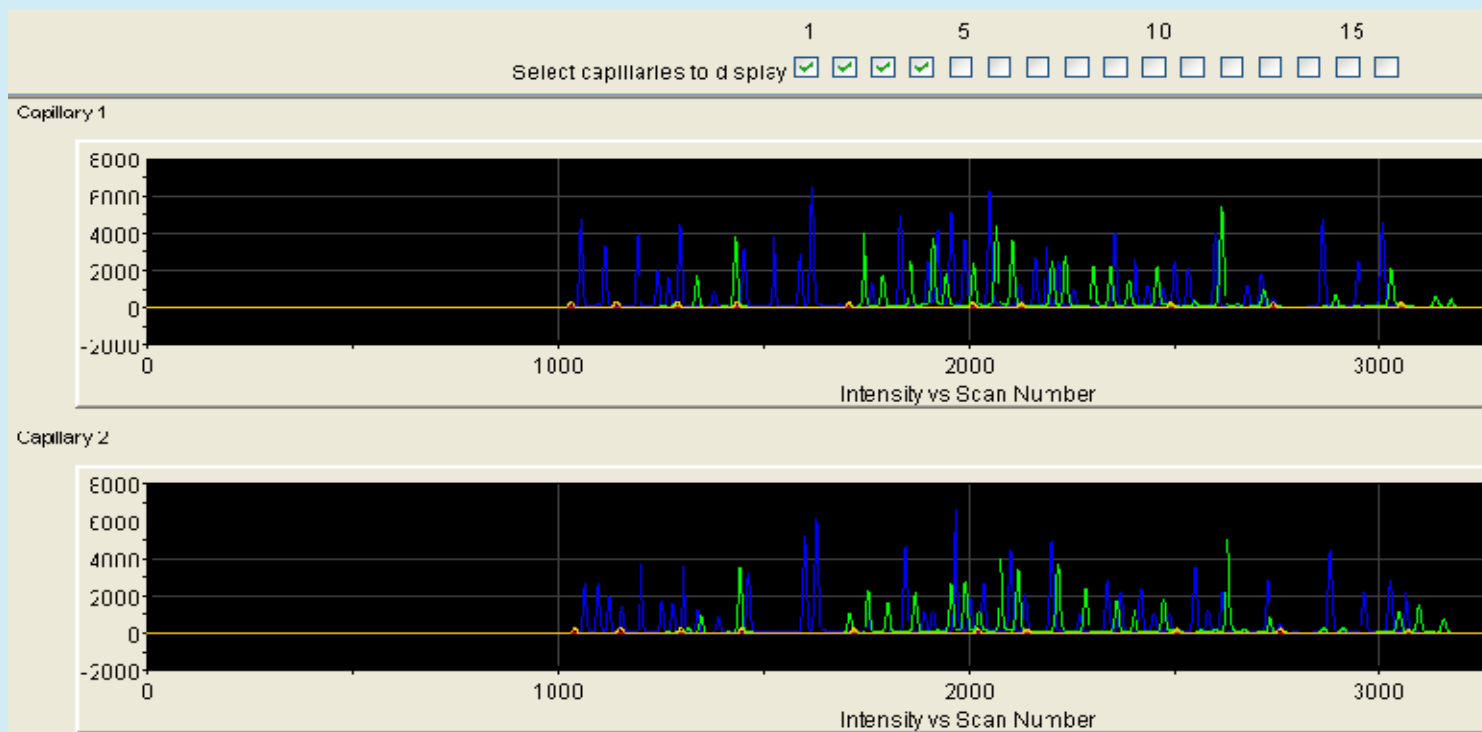
Allele-specific discrimination by OLA reaction



Multiple SNP Detection Using Capillary Electrophoresis



Automatic Separation of Allele-specific ZipChute[®] Using Capillary Electrophoresis

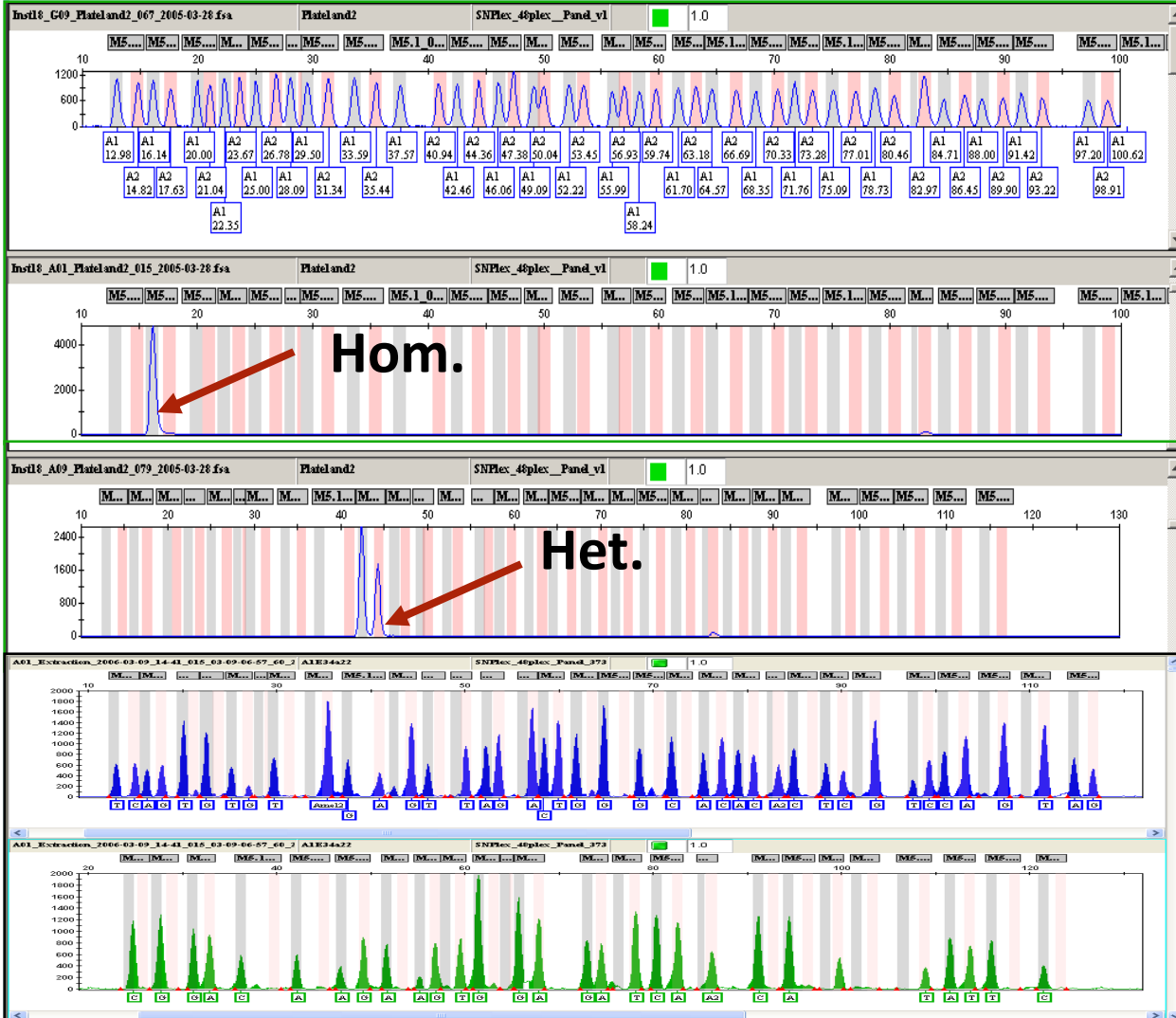


3130xl or 3730xl Genetic Analyzers

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SNP Genotyping Using GeneMapper® Software 4.0



Allelic ladder

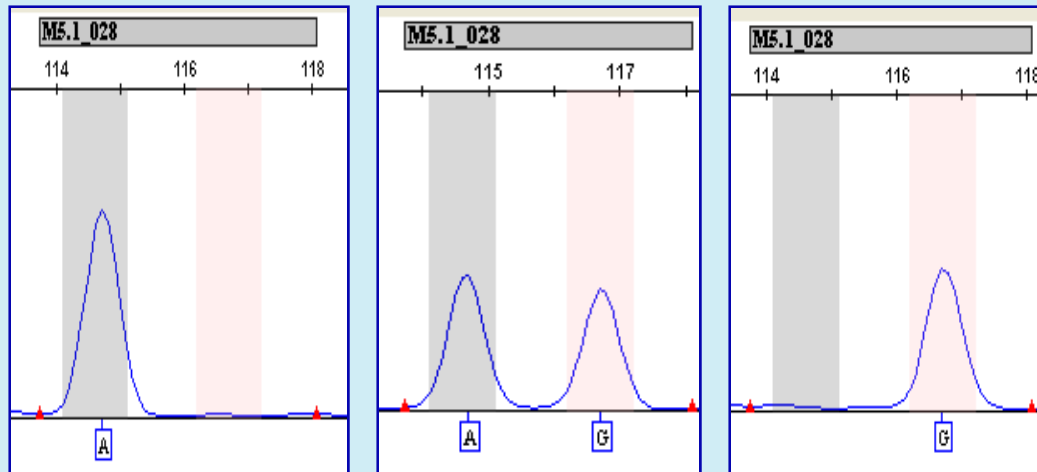
Singleplex

Multiplex
(49-plex)

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Automated Allele Calling – ZipChute[®] Probe Identification



e.g. Hom. 1
AA

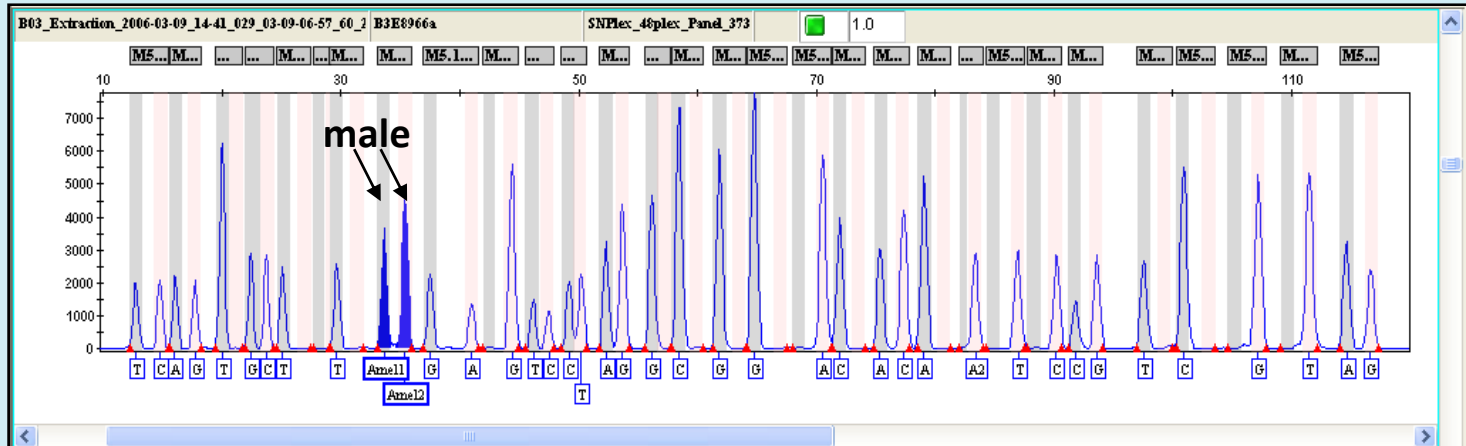
Het.
AG

Hom. 2
GG

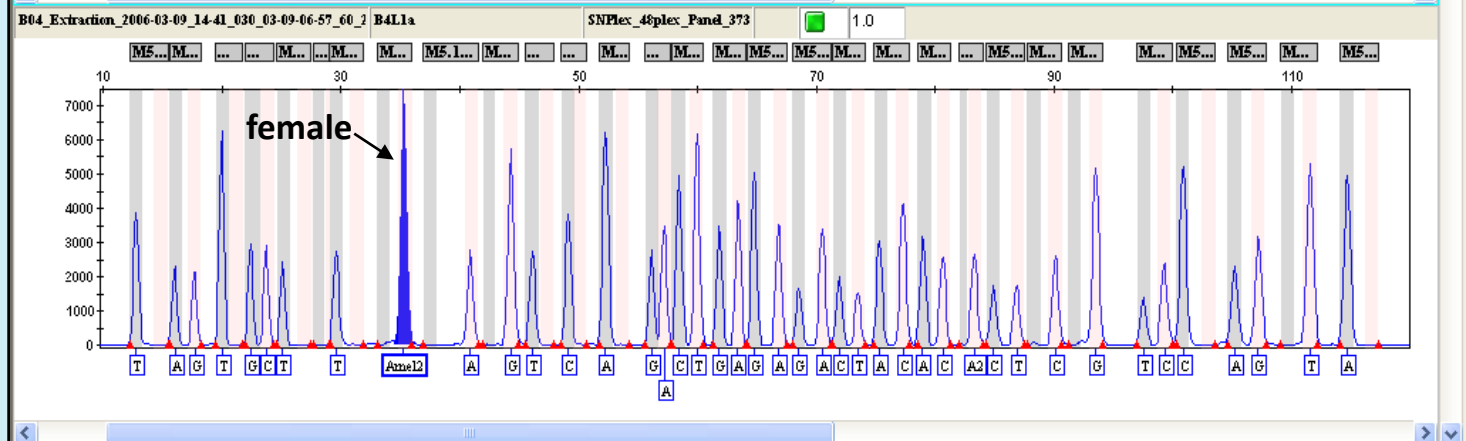
Marker = SNP
Marker = 2 bins
Bin = Allele

Gender Determination

DNA1



DNA2



System Components

- **Oligo mixes**
 - **Multiplex PCR primer mix**
 - **Oligo ligation assay oligonucleotide mix**
- **Materials and reagents**
- **Software**
 - **GeneMapper[®] Software 4.0**
- **Instruments**
 - **3130x/ or 3730x/ Genetic Analyzers**

Reagents

- **PCR**
 - **Multiplex PCR Master Mix (2x)**
- **PCR reaction clean-up**
 - **ExoSAP-IT[®]**
- **Oligo ligation reaction**
 - **100 mM dATP**
 - **SNPlex[™] OLA Master Mix**

Reagents (continued)

- **Binding OLA products to hybridization plate and biotinylated strand isolation**
 - **SNPlex™ Hybridization Wash Buffer (10X)**
 - **SNPlex™ Hybridization Binding Buffer**
 - **SNPlex™ Positive Hybridization Control**
- **ZipChute® hybridization**
 - **SNPlex™ ZipChute® Dilution Buffer**
 - **SNPlex™ ZipChute® Mix, 48-plex**
 - **SNPlex™ Denaturant**

Reagents (continued)

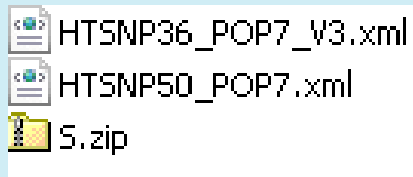
- **ZipChute[®] elution**
 - **SNPlex[™] Sample Loading Reagent**
- **Plate preparation for electrophoresis on a 3130x/
Genetic Analyzer**
 - **SNPlex[™] Size Standard, 48-plex**
 - **SNPlex[™] Allelic Ladder, 48-plex**
 - **SNPlex[™] Sample Loading Reagent**

Instruments and Other Supplies






- **GeneAmp[®] PCR System 9700 thermal cycler**
 - Multiplex PCR
 - Exo/SAP cleanup
 - OLA reaction
- **3130x/ or 3730 and 3730x/ Genetic Analyzer: capillary electrophoresis (CE) for ZipChute[®] separation**
- **96-well PCR plate: PCR, OLA, and CE setup**
- **96-well streptavidin coated plate: OLA product binding and ZipChute[®] hybridization**

Softwares and Other Patches Needed: Online or CD

- <http://www.appliedbiosystems.com/support/software/snplex/updates.cfm>
 - **SNPlex™ 3130x/ Data Collection v3.0**



- **SNPlex™ 3130x/ GeneMapper® v4.0**

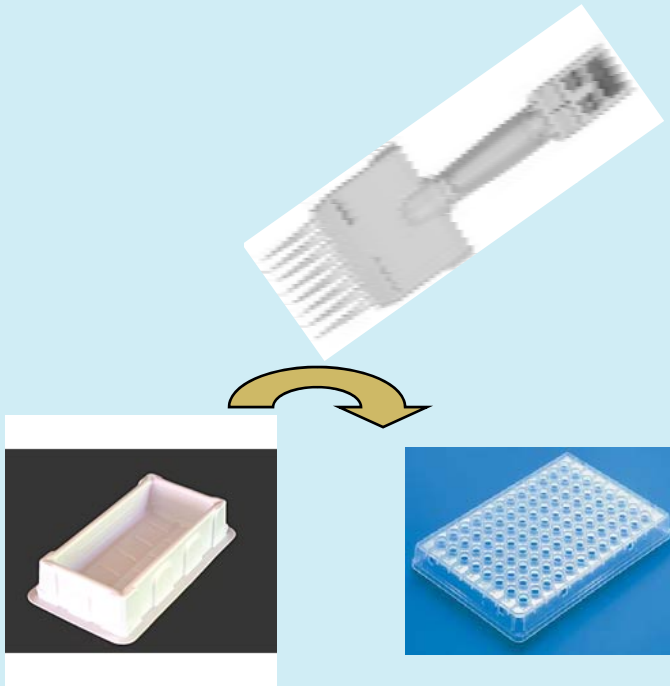
 3130_Global_QC_parameters.xml	XML Document
 SNPlex_48plex_3130_Bins.txt	Text Document
 SNPlex_48plex_3130_Panels.txt	Text Document
 SNPlex_48plex_v1.xml	XML Document
 SNPlex_Rules_3130.xml	XML Document

- **Assay information file (AIF): SNP Set**

3130xl Electrophoresis

- **Capillary:**
 - **New array: conditioned by SNPlex™ System Array Conditioning Kit**
 - **Old array (>50 runs): use it directly**
- **Polymer:**
 - **POP-7 polymer**
- **Spectral Calibration:**
 - **DS-40 Spectral Calibration Standard Kit (Dye Set S)**
- **Capillary electrophoresis run module:**
 - **HTSNP36_POP7_V3.xml**

Plate Washing Procedure



Dispose of buffer in sink

Centrifuge the plate upside down on paper towel

GenPlex™ High Throughput Solution

DNA preparation



Multiplex PCR



1.30 hr



OLA reaction



1.20 hr



MULTIMEK™ 96
AUTOMATED 96-CHANNEL PIPETTOR



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



CE



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Lab Demonstration

- 1. PCR setup and thermal cycling**
- 2. ExoSAP-IT[®] preparation and incubation**
- 3. Phosphorylation reaction setup and incubation**
- 4. OLA setup and thermal cycling**
- 5. Binding to Streptavidin-coated plate, setup and incubation**
- 6. ZipChute[®] Hybridization setup and incubation**
- 7. Wash and elution**
- 8. Capillary electrophoresis**

Questions?

Contact Information

Rixun Fang
Applied Biosystems™
Part of Life Technologies™
(650) 638-6672
Rixun.fang@lifetech.com

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