

Comparison of Four SNP Genotyping Technologies:

- Taqman Allelic Discrimination
- Pyrosequencing
- Sequenom MassARRAY MALDI-TOF
- ABI SNPLex

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Director SNP Genotyping Resource

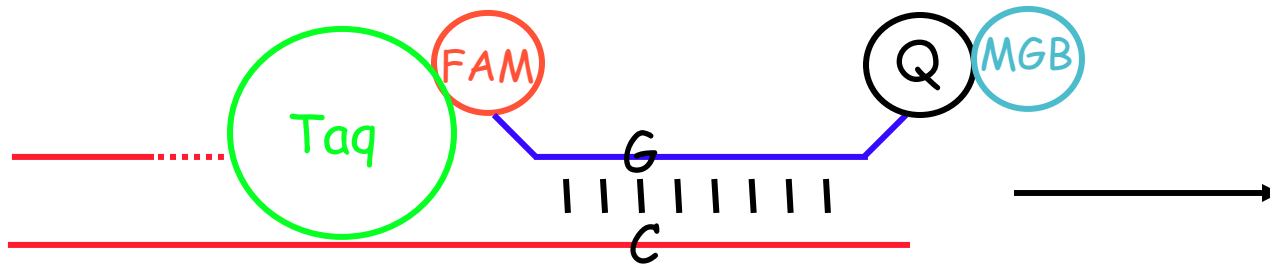
W.M. Keck Foundation Biotechnology Resource
Laboratory at Yale University
(<http://keck.med.yale.edu/>)



Choosing a Technology

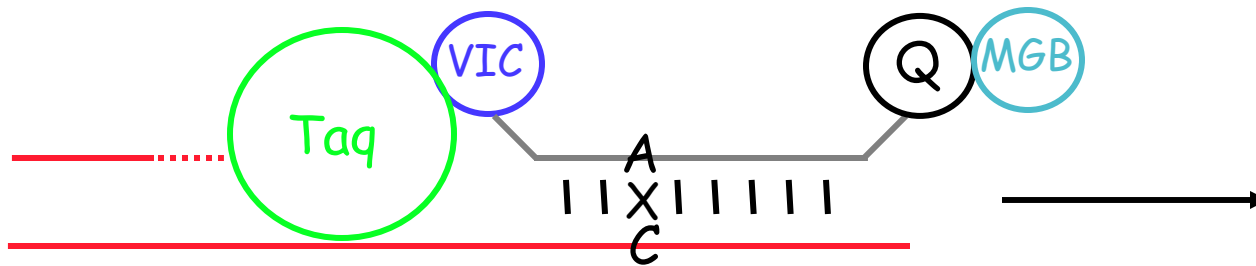
- Total # Genotypes
- Project Size = # SNPs X # genomic DNAs

Taqman Allelic Discrimination



Taq Nuclease
Digestion of
Probe

Fam Fluorescence



Strand
Displacement

Probe Intact

Vic Fluorescence
Quenched

(C Homozygote Shown)

Taqman Allelic Discrimination

Procedure:

Mix genomic DNA with real time PCR reagents

Thermocycle

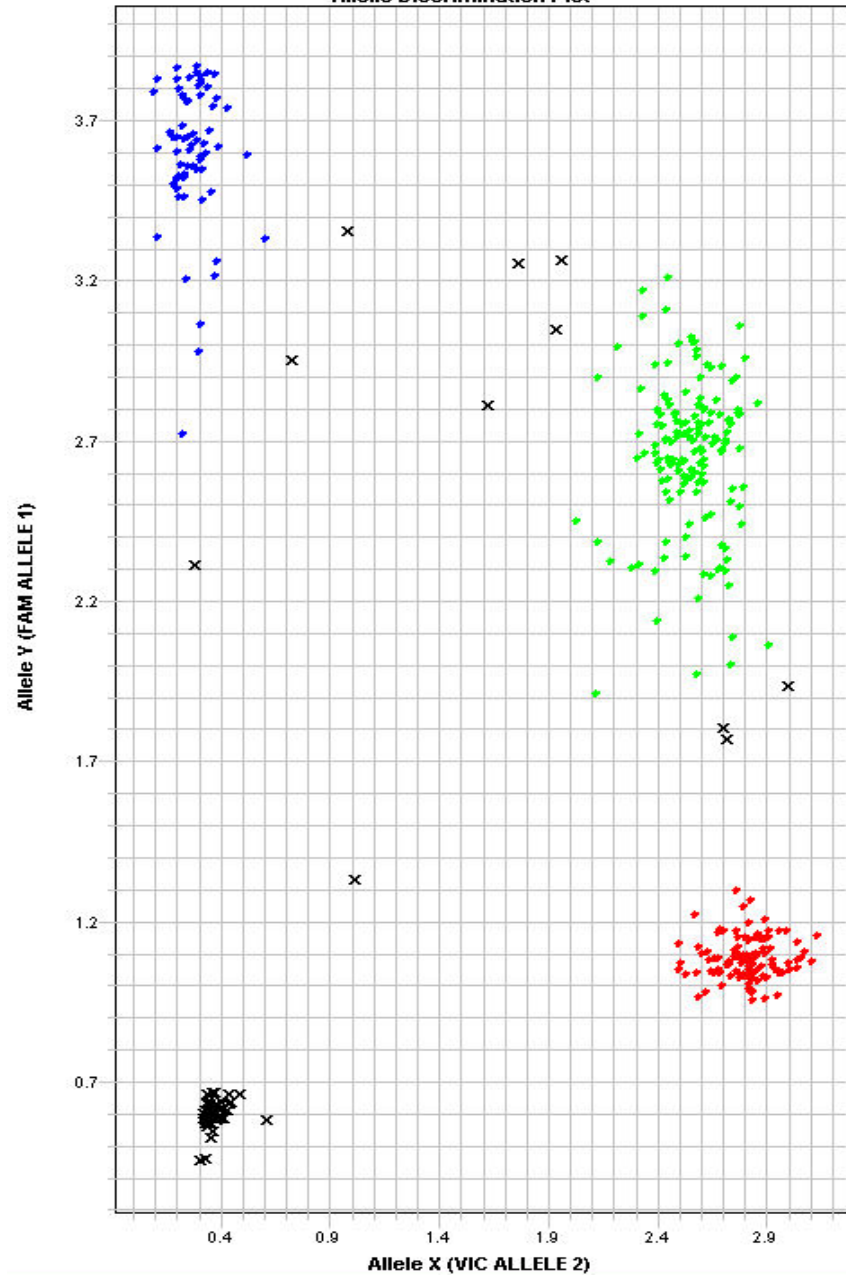
Read Fam & Vic fluorescence in 7900HT

Marker: C_7461306_10

Call: Undeter...



Allelic Discrimination Plot



Legend

- x Undetermined
- Allele X
- Both
- Allele Y
- NTC

Taqman Genotyping Project

556 human genomic DNAs (WGA)

1.2 Mb region of chromosome 6

103 Assays on Demand

95/103 assays produced high quality genotype data

7 SNPs submitted to the Assay by Design service

6/7 developed into effective SNP assays.

Jeff Gruen

Haiying Meng

Matthew Held

Taqman Allelic Discrimination

Project Size:

1 SNP X 96 genomic DNAs

48 SNPs X 96 genomic DNAs

48 SNPs X 384 genomic DNAs

Scales linearly in terms of labor and cost

About \$0.50-0.70 per genotype

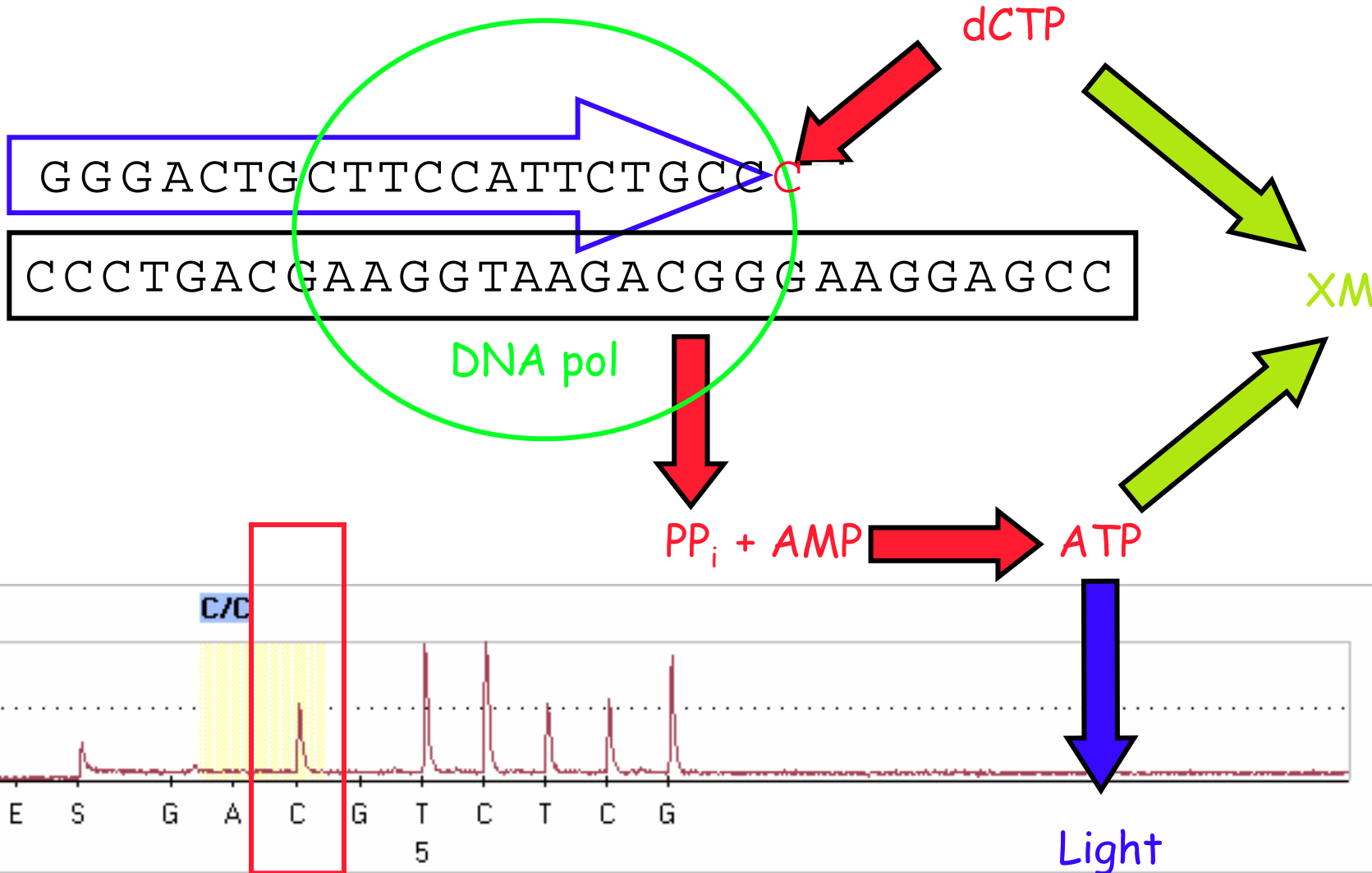
Advantages:

Simple & Robust Chemistry

Very Simple Procedure

Capital Equipment Cost: \$100,000-\$200,000

Pyrosequencing



Pyrosequencing

Procedure:

Mix genomic DNA with PCR reagents & thermocycle

Purify biotinylated PCR products using Streptavidin-Sepharose

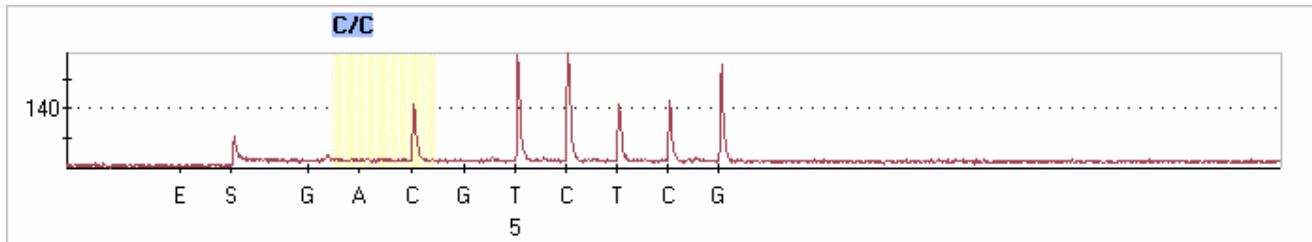
Anneal extension primer to single stranded biotinylated PCR product

Pyrosequencing reaction in PSQ96MA

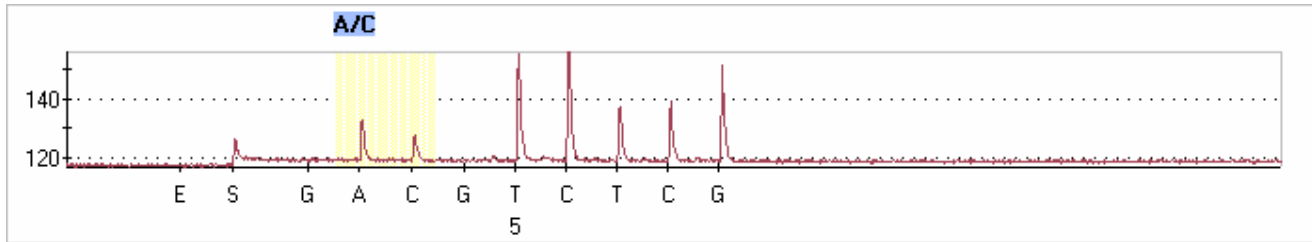
34 ADD1 SNP Pyrosequencing Assay

GGGACTGCTTCCATTCTGCC[C/A]TTCCTCGG

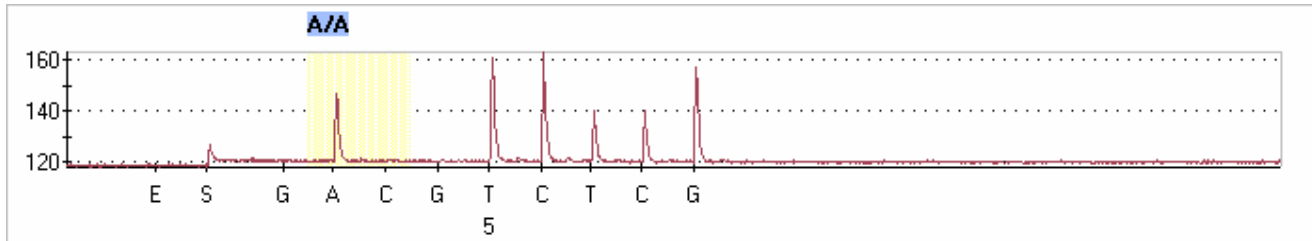
C/C



A/C

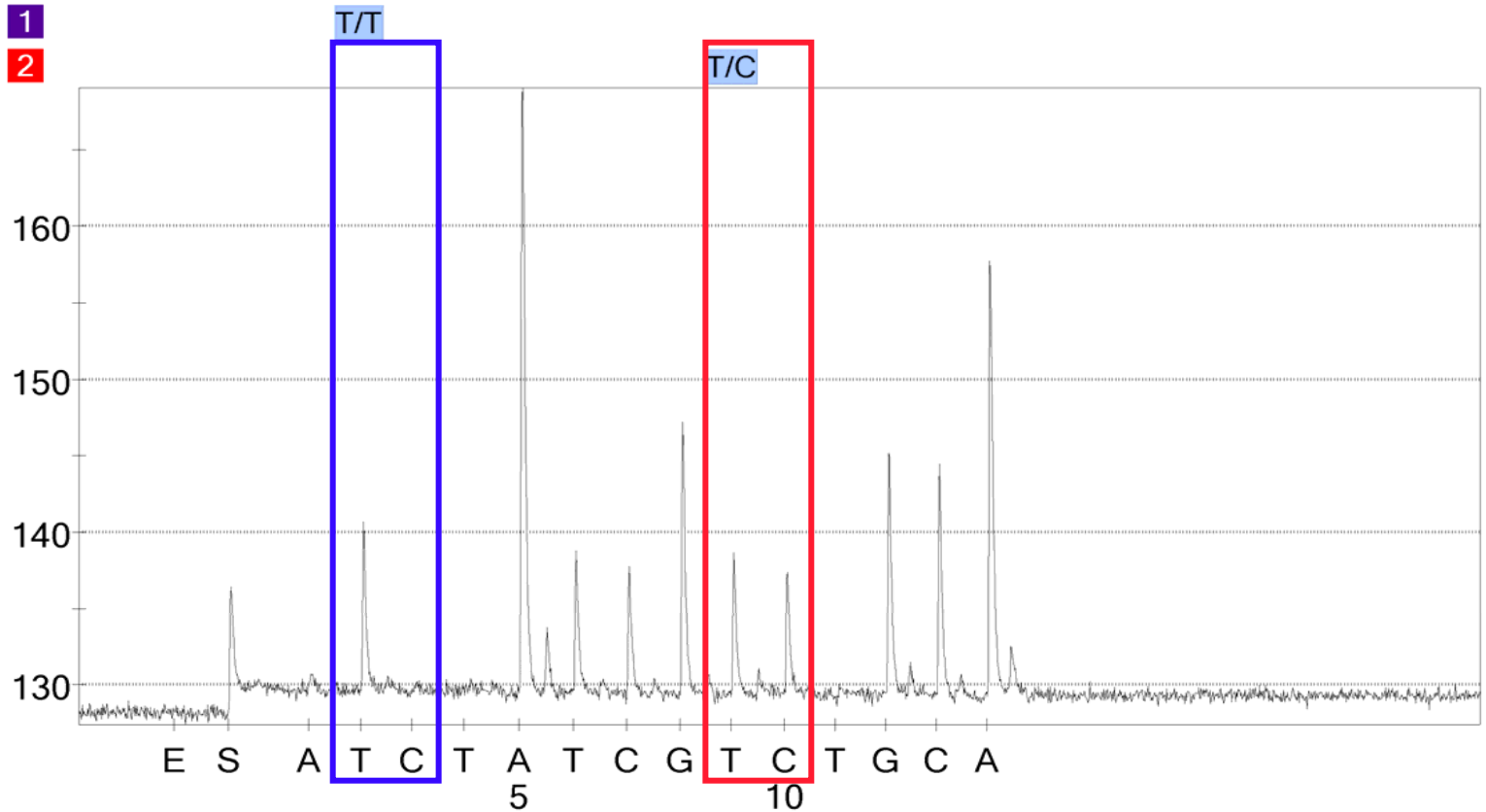
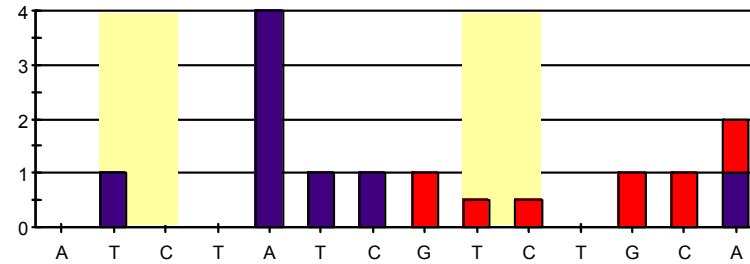


A/A



1) rs708580: [T/C]AAAATCATT

2) rs552075: G[T/C]GCATTGAC



Pyrosequencing

Project Size:

1 SNP X 96 genomic DNAs

48 SNPs X 96 genomic DNAs

48 SNPs X 384 genomic DNAs

About \$1.60 per genotype (uniplex) and \$0.80
per genotype (2-plex)

Advantages:

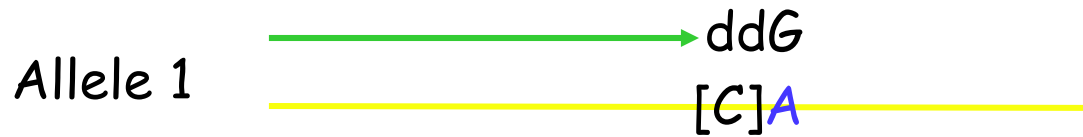
Simple & Robust Chemistry

Simple Procedure

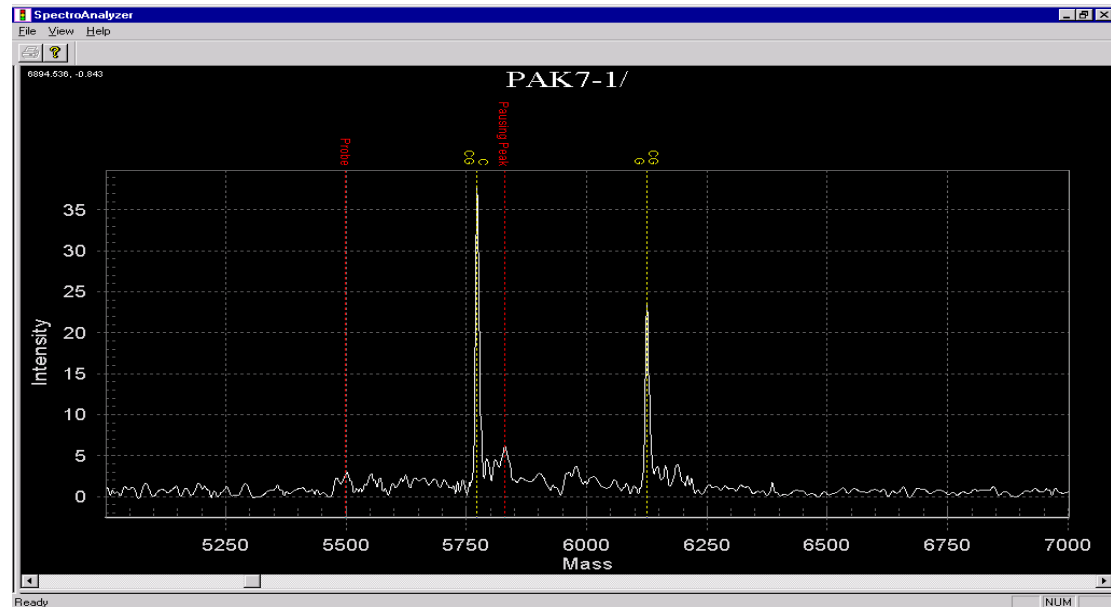
Quantitative allele signal

Capital Equipment Cost: \$100,000-\$200,000

Sequenom MassARRAY



(heterozygote shown)



Sequenom MassARRAY

5-Step Process:

- 1) PCR amplification
- 2) SAP treatment
- 3) Extension
- 4) Ion Exchange Clean-up
- 5) MALDI-TOF m/z Measurement

Sequenom MassARRAY

- High Throughput Automated MALDI-TOF
- 384 position Silicon Target Chip
- Automated Real-Time Genotype Calling
- Three major instrument components:
 - Multimek pipetting robot
 - Custom Pin Tool
 - Bruker Autoflex MALDI-TOF Mass Spectrometer

Sequenom Genotyping Performance

Researchers design assays and perform PCR

38 markers X 4000 genomic DNAs

38 markers were distributed among a total of ten 3-plex and 4-plex assays

average multiplex about 3.8

> 140,000 high quality genotypes

Sequenom MassARRAY

Project Size:

48 SNPs X 384 genomic DNAs

48 SNPs X 3840 genomic DNAs

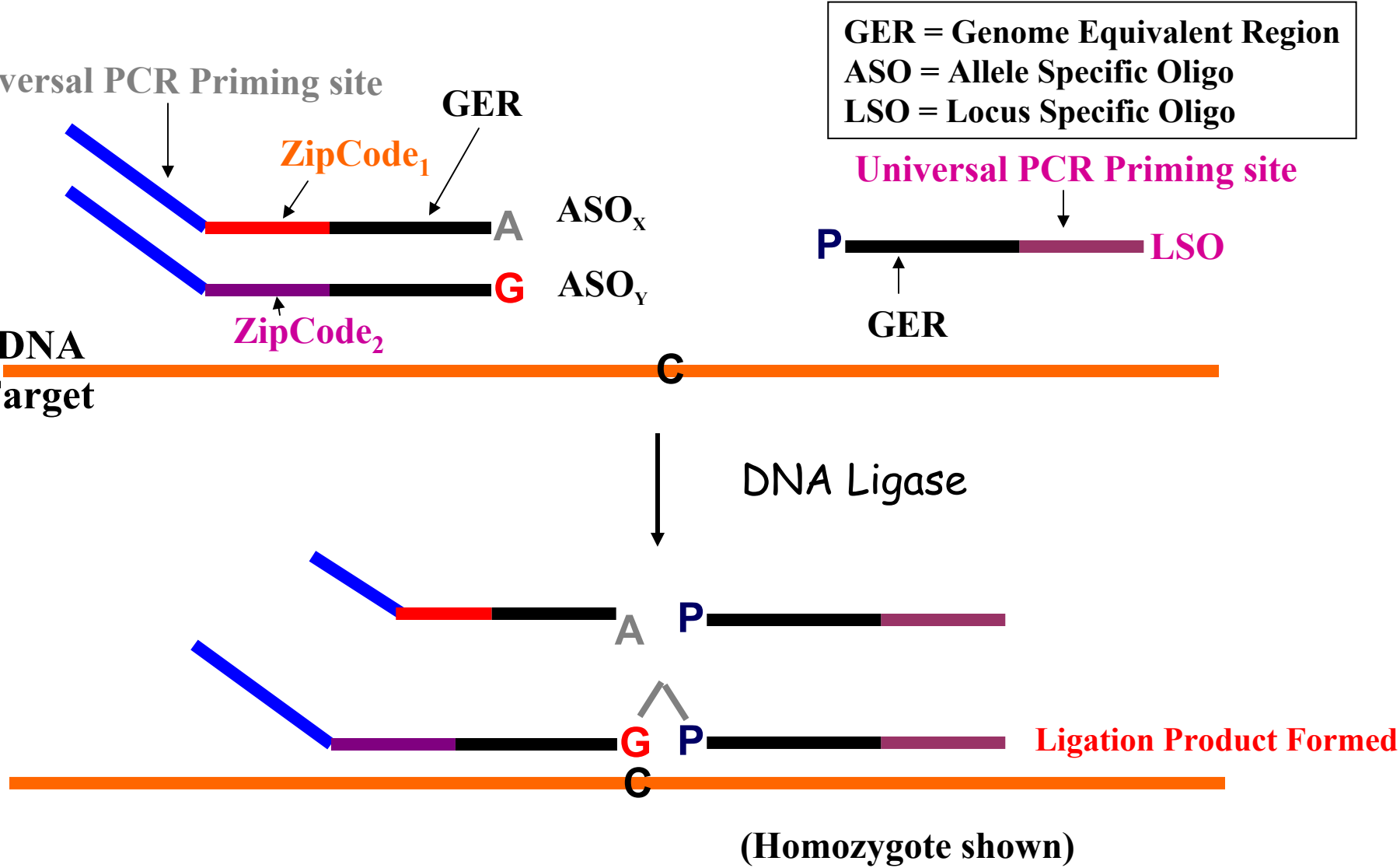
About \$0.50-0.60 per genotype (4-plex)

Advantages:

Efficient when processing thousands of DNAs

Capital Equipment Cost: \$500,000

SNIPlex OLA Assay



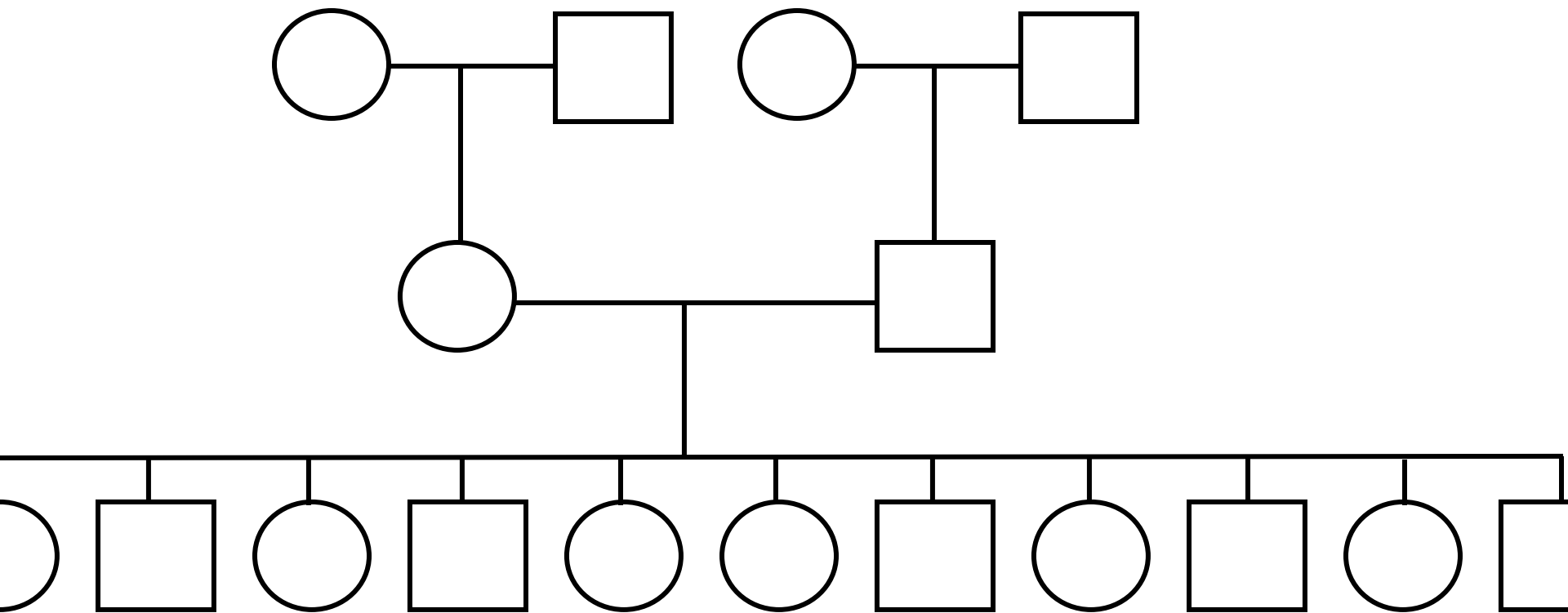
SNPlex

9-Step Procedure:

- 1) Kinase oligonucleotide probes
- 2) OLA reaction
- 3) Exonuclease digestion
- 4) PCR amplification
- 5) Binding of Biotinylated PCR products to Streptavidin plates
- 6) Hybridize Zipchutes
- 7) Wash & Elute Zipchutes
- 8) Capillary electrophoresis on ABI3730
- 9) Assign genotypes with Genemapper

SNPlex Trial Experiment

17 genomic DNAs in Coriell CEPH family 1331



SNPlex Trial Experiment

17 genomic DNAs in Coreill CEPH family 1331 X 4 wells per DNA

1 control DNA ABI X 4 wells

72 total wells genomic DNA

22 wells no template control

ABI probe set A (48-plex)

Total genotypes attempted = 3456

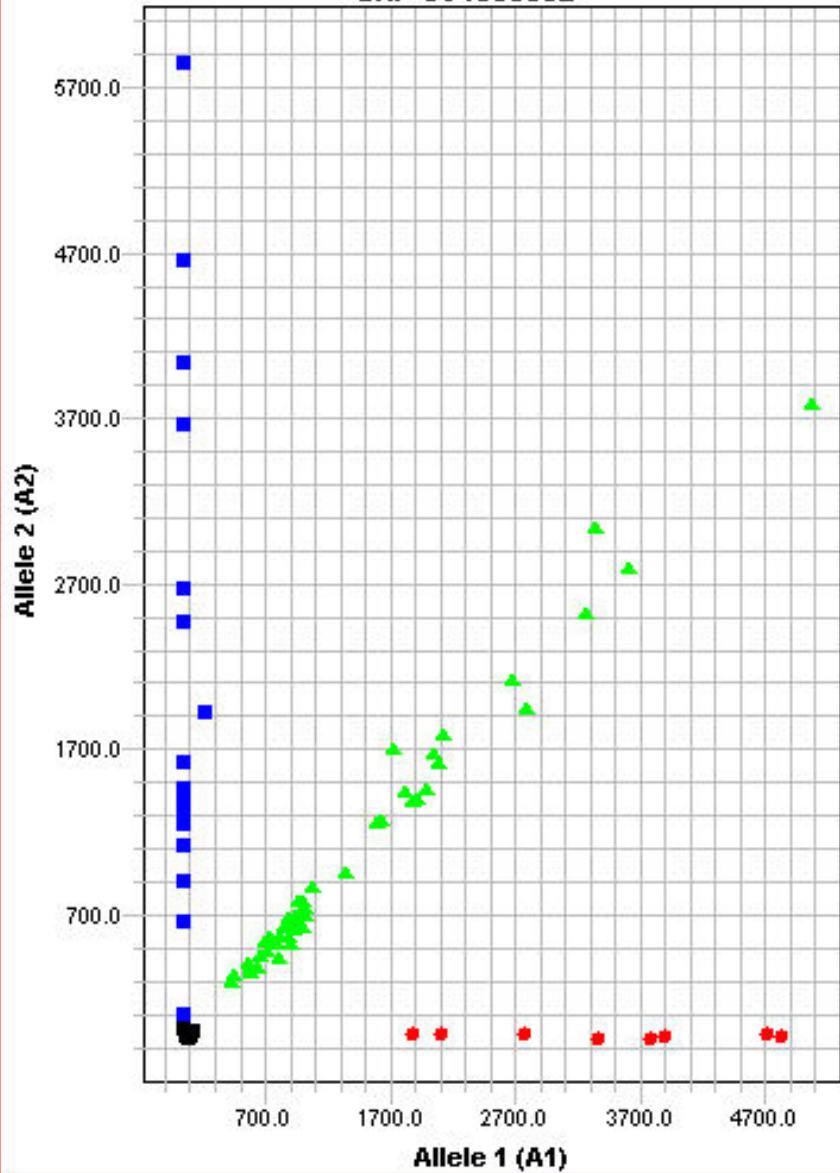
Genemapper called genotypes for 47 of the 48 markers = 3384 total

% Mis Call = $1/3384 = 0.03\%$

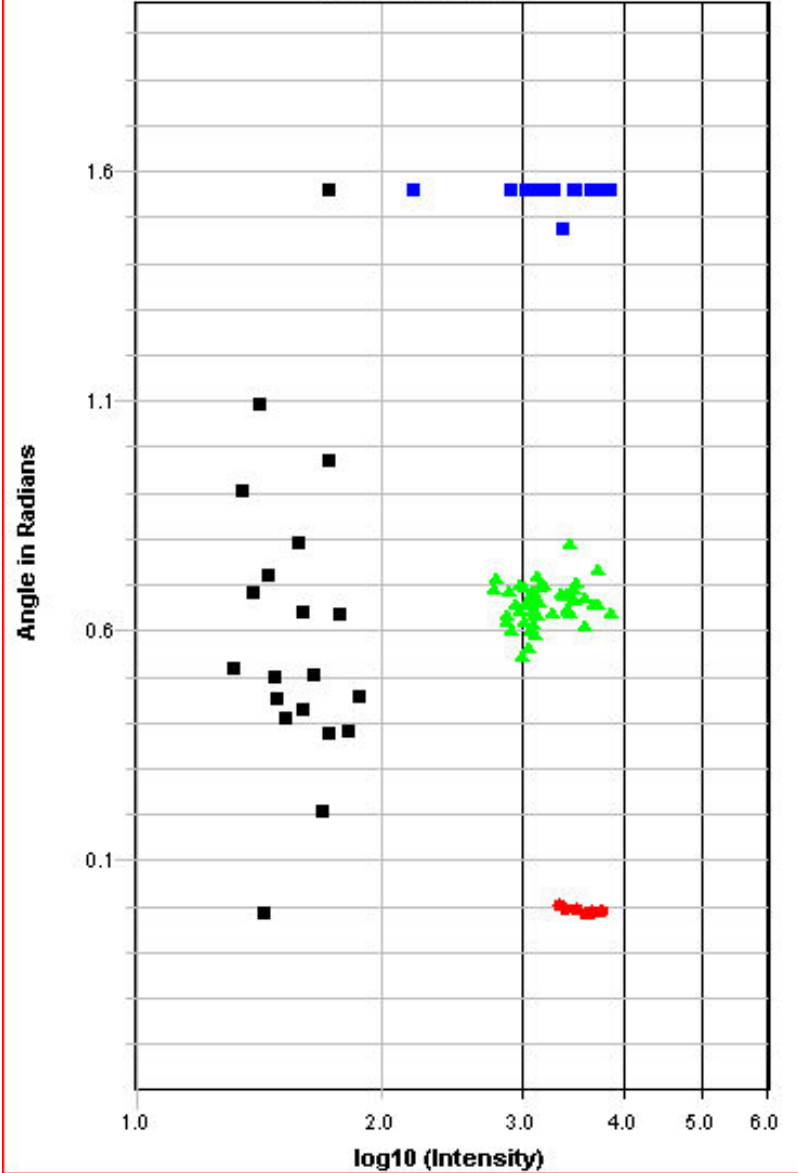
% No Call = $9/3384 = 0.26\%$

For CEPH 1331 samples, Mendelian inheritance of 47 markers

SNP CV1688032



SNP CV1688032



SNPlex

Project Size:

48 SNPs X 384 genomic DNAs

48 SNPs X 3840 genomic DNAs

About \$0.25-0.07 per genotype (48-plex)

Advantage:

Efficient when processing thousands of DNAs

Capital Equipment Cost: \$500,000

Comparison of SNP Genotyping Technologies

	Genotypes per day	Plex	Project Size	Pro	Con
SNPlex	>17,856	24-48	>100,000	Very High Throughput	Complicated Procedure
Beckman Coulter iSeq 100	>3840	1-6	>100,000	Very High Throughput	Printing of Spectrochip
Roche 454 GS FLX	960	1-3	96-100,000	Sequence Info Robust	3-plex Design is Difficult
Applied Biosystems Taqman	3840	1	> 384	Simple Robust	Uniplex Only