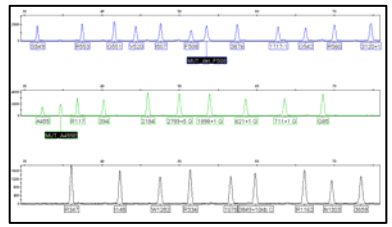
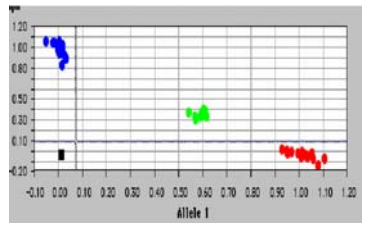
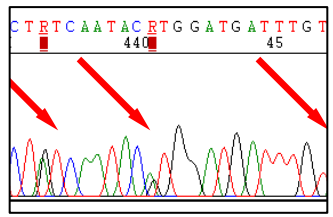
ABI Genotyping total solution

SNP Genotyping Technologies Available from AB

SNP ANALYSIS	PLATFORM	
Chromosome & Genome-Wide LD Studies	• SNPlex™ on electrophoretic genetic analyzer • High throughput	
Candidate Gene / Regional LD Studies	• TaqMan® on Sequence Detection System • Cost-effective, easy to use, validated assays	
Dense Mutational Spectra	• Resequencing Primers on electrophoretic genetic analyzer • Dual use: mutation discovery & assay	

ABI Genotyping platform

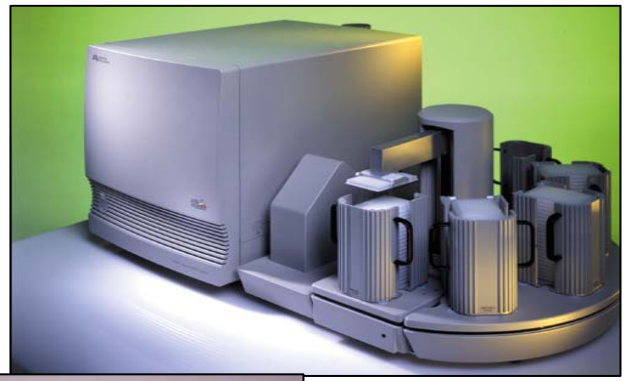
- 5’ Nuclease Assay =TaqMan Probe mismatch
  - Allele Discrimination
  - 7900HT, 7700, 7000

- Sequencing =actually determine/compare sequences
  - 3730, 3100, 310
  - VariantSEQr resequencing primer
  - Sequencing reagent:
  - SeqScape v2.1

- Multiplexed OLA/PCR Assay Extension = SNPlex assay
  - 3730, (3100)
  - GeneMapper v 3.5

ABI Tool Box of Technologies...

Sequence Detection Systems



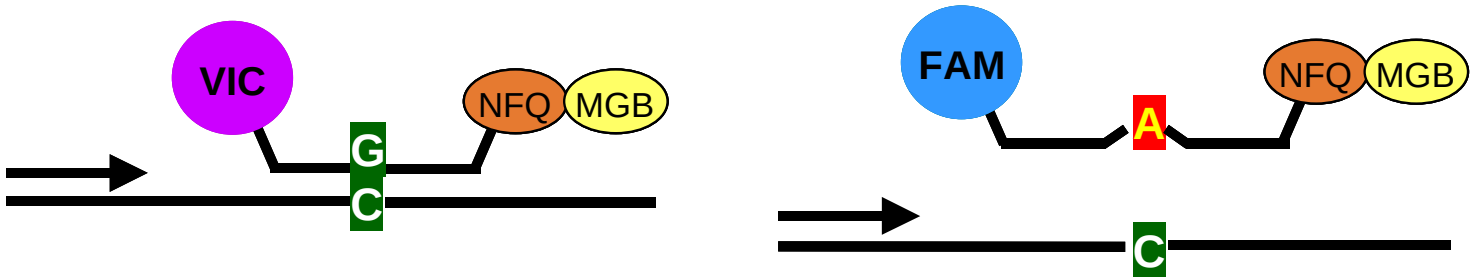
5' Nuclease Assay

Electrophoresis Platforms

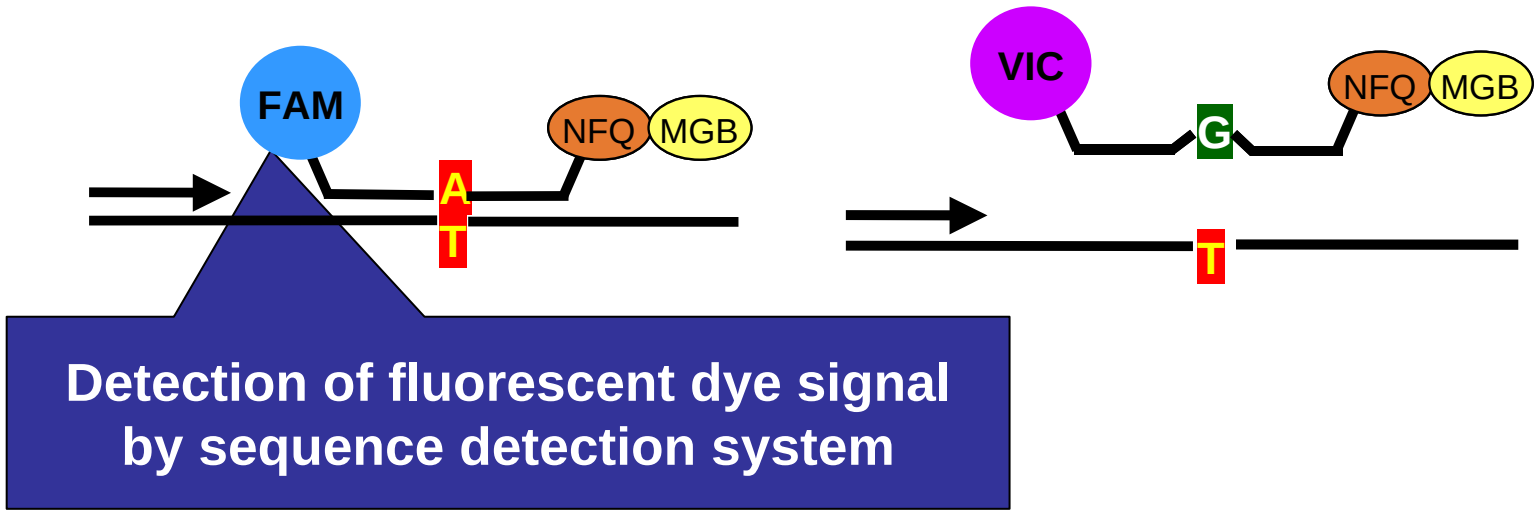
Resequencing SNPlex Assay

TaqMan 5’ Nuclease Assay

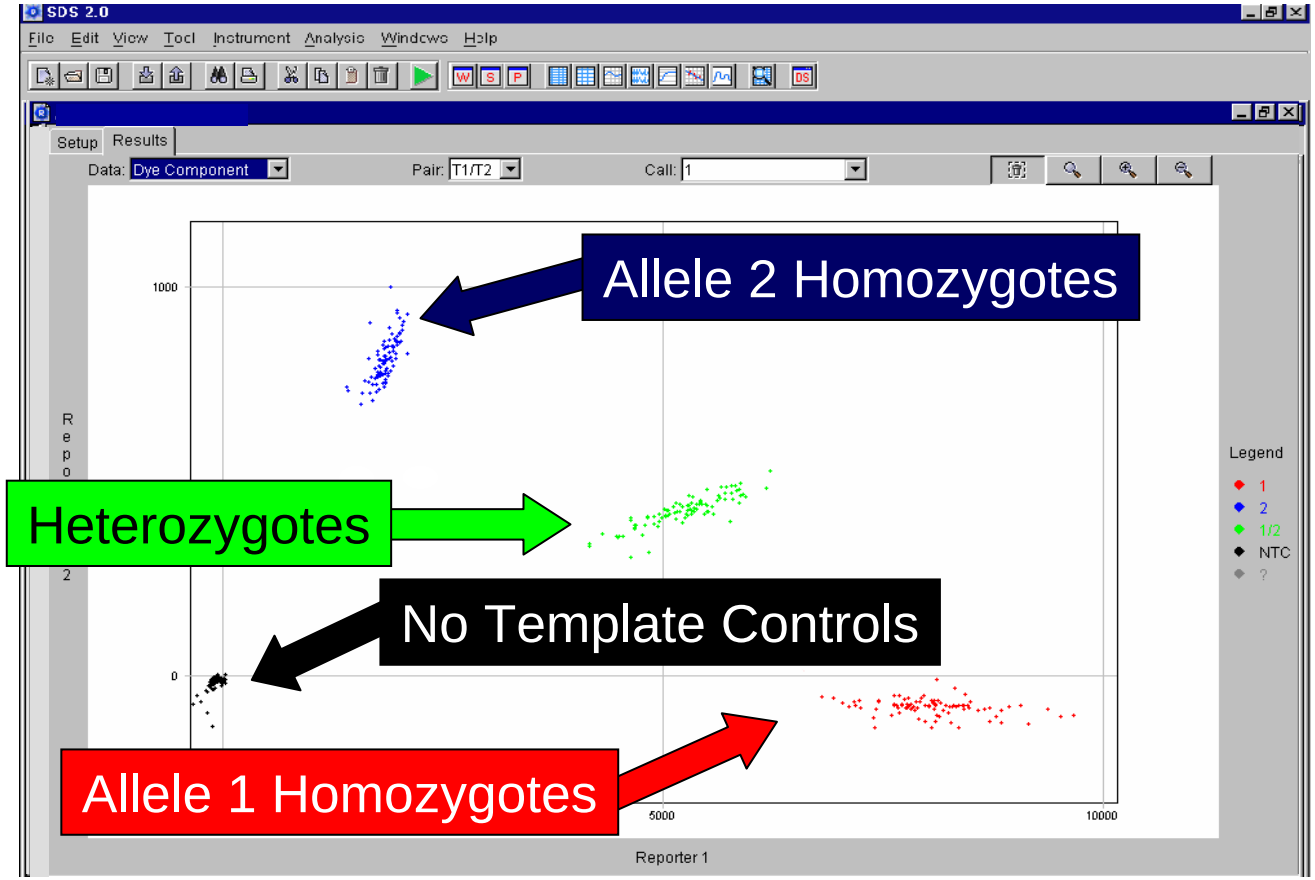
For Allele 1 - only VIC™ dye signal is generated



For Allele 2 - only FAM™ dye signal is generated



Allelic Discrimination Cluster Data View



TaqMan® Assay→ Simple Workflow

One step, single tube assay

Setup

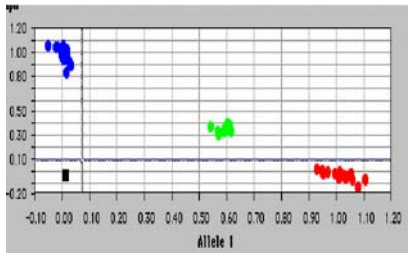
Pipette: Master Mix + Assay mix + DNA Sample & Seal plate

Cycle

Read

ABI PRISM® 7900HT SDS
with integrated robotics

Analyze



Three Solutions for SNP design

- Ready made assays
- 網路選購
- Updated Human Genome information

- 代客設計與測試 for SNPs
- 85% 成功率

- Design primer or probe by software

Applied Biosystems

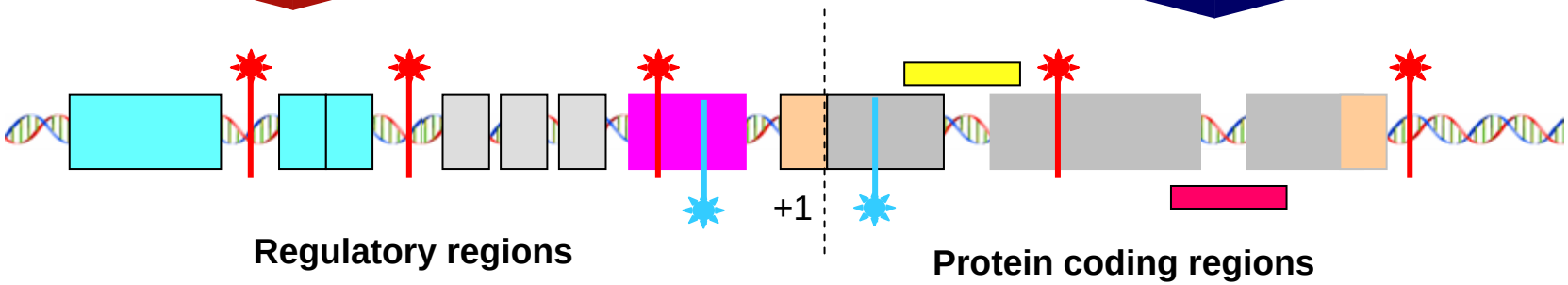
針對人類3萬個基因,推出20萬個SNP-AoD基因分析試劑組

SNP Assays

Assays-on-Demand products

Gene Expression

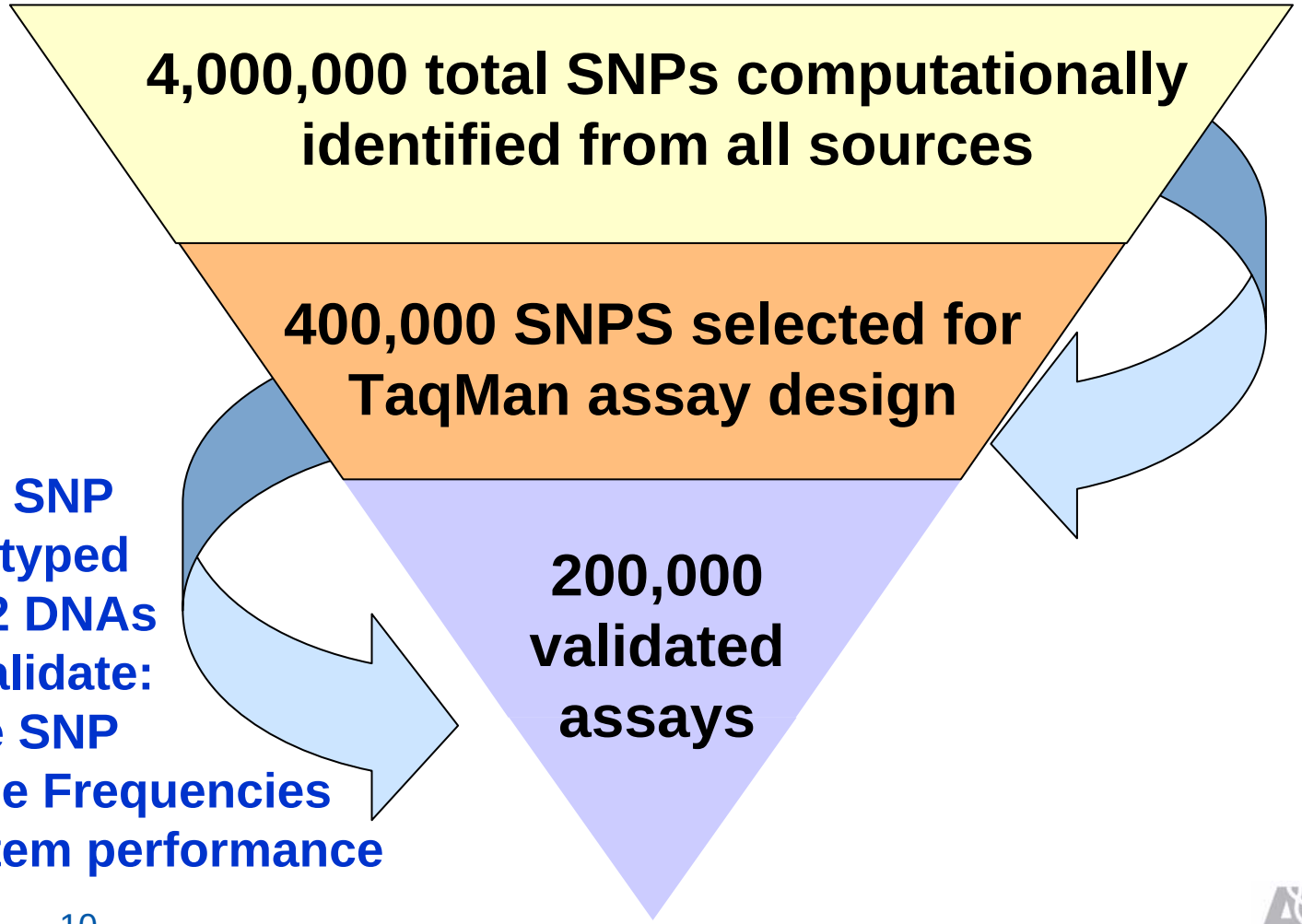
Assays-on-Demand products



SNP **Gene Expression**

Assays-by-Design℠ Service

AB SNP Validation Overview



SNP AoD Selection Goals

- A genome-wide SNP marker set useful for LD mapping
  - 200,000 5’ Nuclease SNP Assays
  - SNPs in gene-regions / gene centric approach
  - Minor allele frequency >10% in one population
  - Coverage of 1 SNP per 10 Kb of gene length

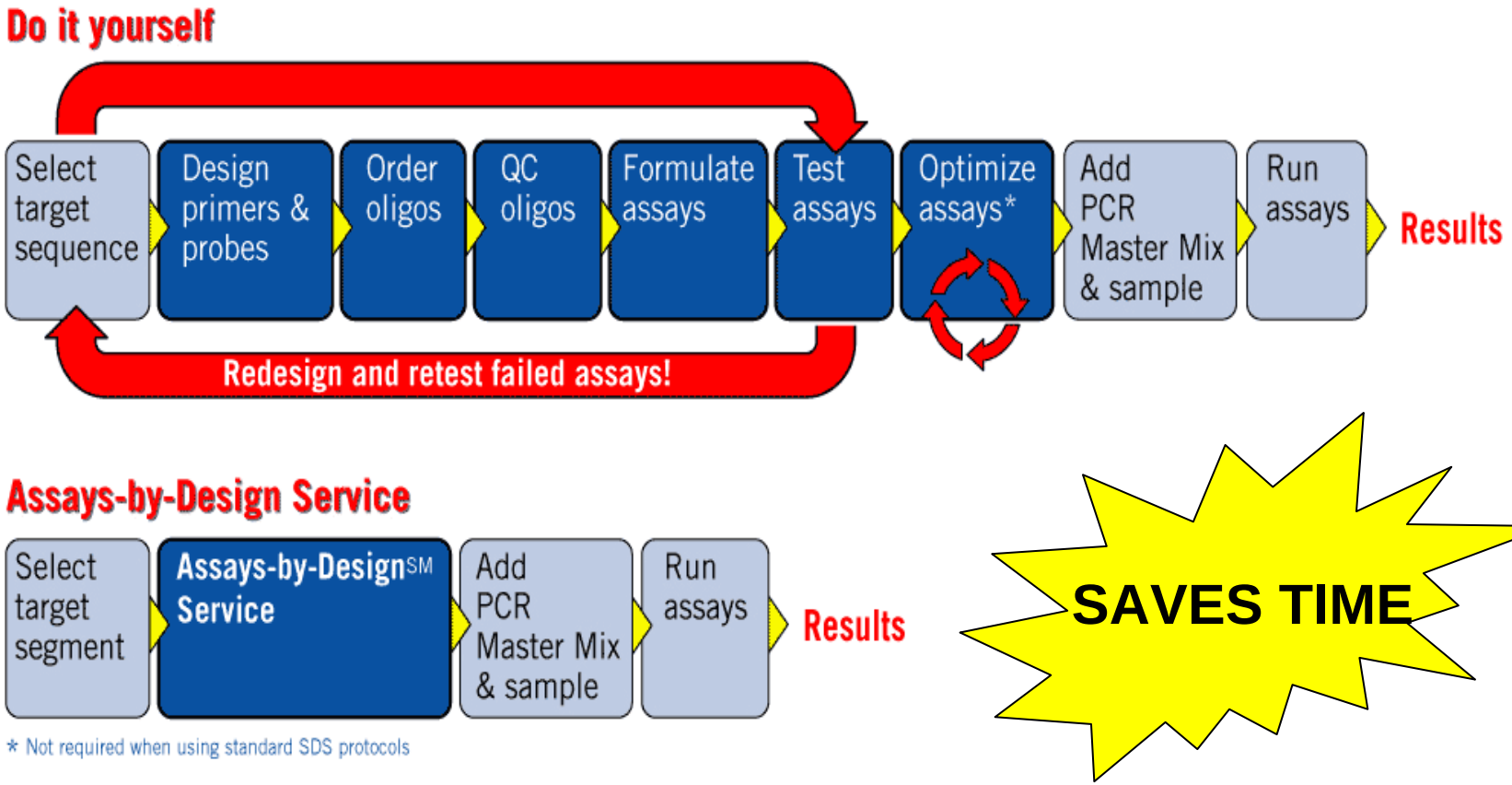
AbD Position as

- Custom Assay
  - Unlike off the shelf you get what you want
- Convenience
  - Unlike oligos in many tubes
  - All in one tube
  - Assay is designed and optimized (standard thermal cycling conditions)
- High Quality Control
  - Mass spec
  - HPLC purified probe
  - Functional tested??

•85% 成功率

Do-It-Yourself vs. The Assays-by-Design℠ Service

Streamlining Your Workflow

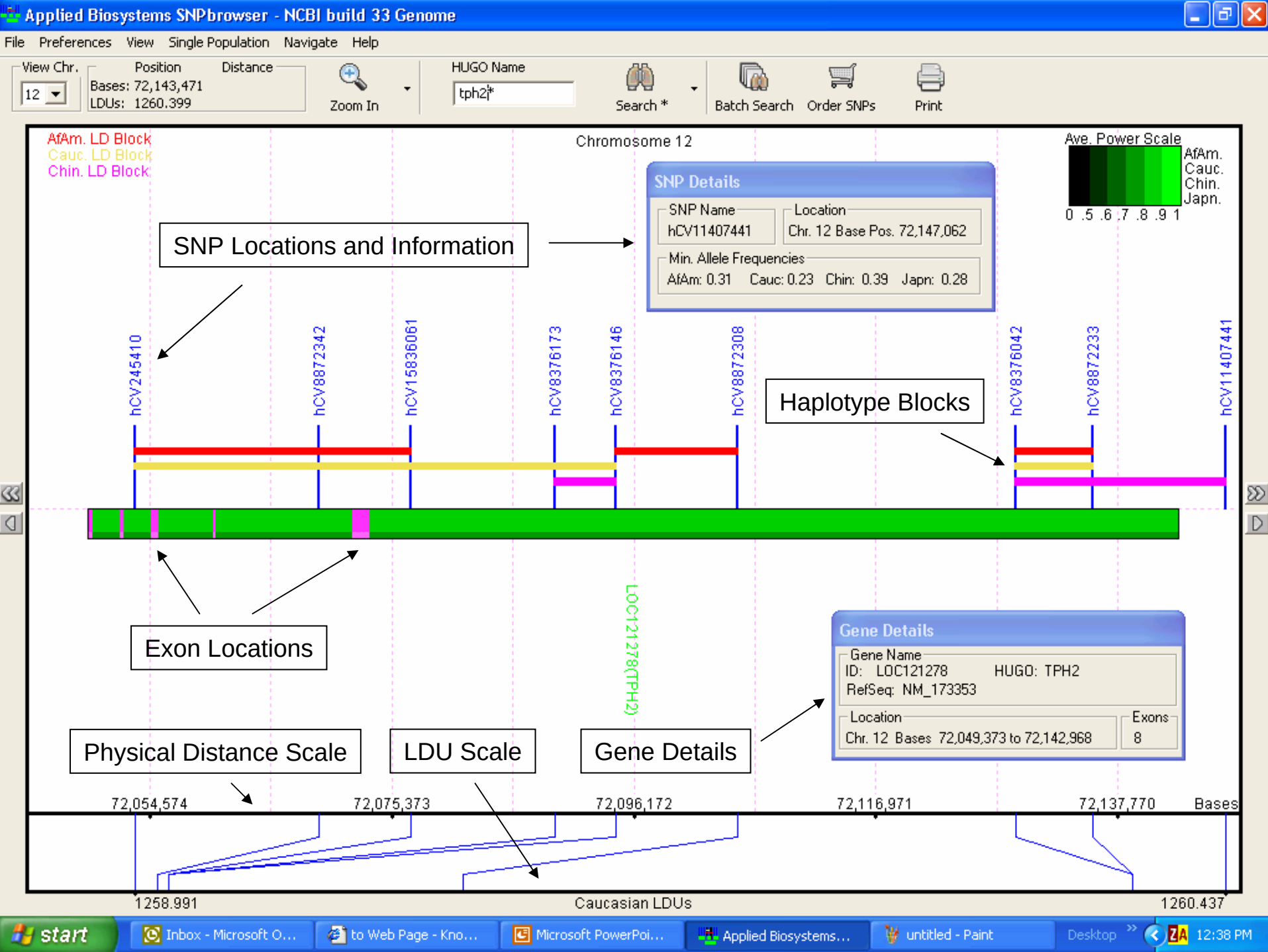


Using SNPbrowser™ to Help Design Genetic Association Studies

Selecting SNPs for Association Studies

Factors to be considered when selecting SNPs:

- minor allele frequency
- distance between adjacent SNPs
- location with respect to exons or regulatory elements
- non-coding or coding
- local LD architecture


Applied Biosystems SNPbrowser - NCBI build 33 Genome
File Preferences View Single Population Navigate Help
View Chr. 12
Position
Bases: 72,143,471
LDUs: 1260.399
Distance
Zoom In
HUGO Name
tph2*
Search *
Batch Search
Order SNPs
Print
AfAm. LD Block
Cauc. LD Block
Chin. LD Block
Chromosome 12
Ave. Power Scale
AfAm.
Cauc.
Chin.
Japn.
0 .5 .6 .7 .8 .9 1
SNP Details
SNP Name
hCV11407441
Location
Chr. 12 Base Pos. 72,147,062
Min. Allele Frequencies
AfAm: 0.31 Cauc: 0.23 Chin: 0.39 Japn: 0.28
SNP Locations and Information
hCV245410
hCV8872342
hCV15836061
hCV8376173
hCV8376146
hCV8872308
hCV8376042
hCV8872233
hCV11407441
Haplotype Blocks
Exon Locations
LOC121278(TPH2)
Gene Details
Gene Name
ID: LOC121278 HUGO: TPH2
RefSeq: NM_173353
Location
Chr. 12 Bases 72,049,373 to 72,142,968
Exons
8
Physical Distance Scale
LDU Scale
Gene Details
72,054,574
72,075,373
72,096,172
72,116,971
72,137,770
Bases
1258.991
Caucasian LDUs
1260.437
start
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to Web Page - Kno...
Microsoft PowerPoi...
Applied Biosystems...
untitled - Paint
Desktop
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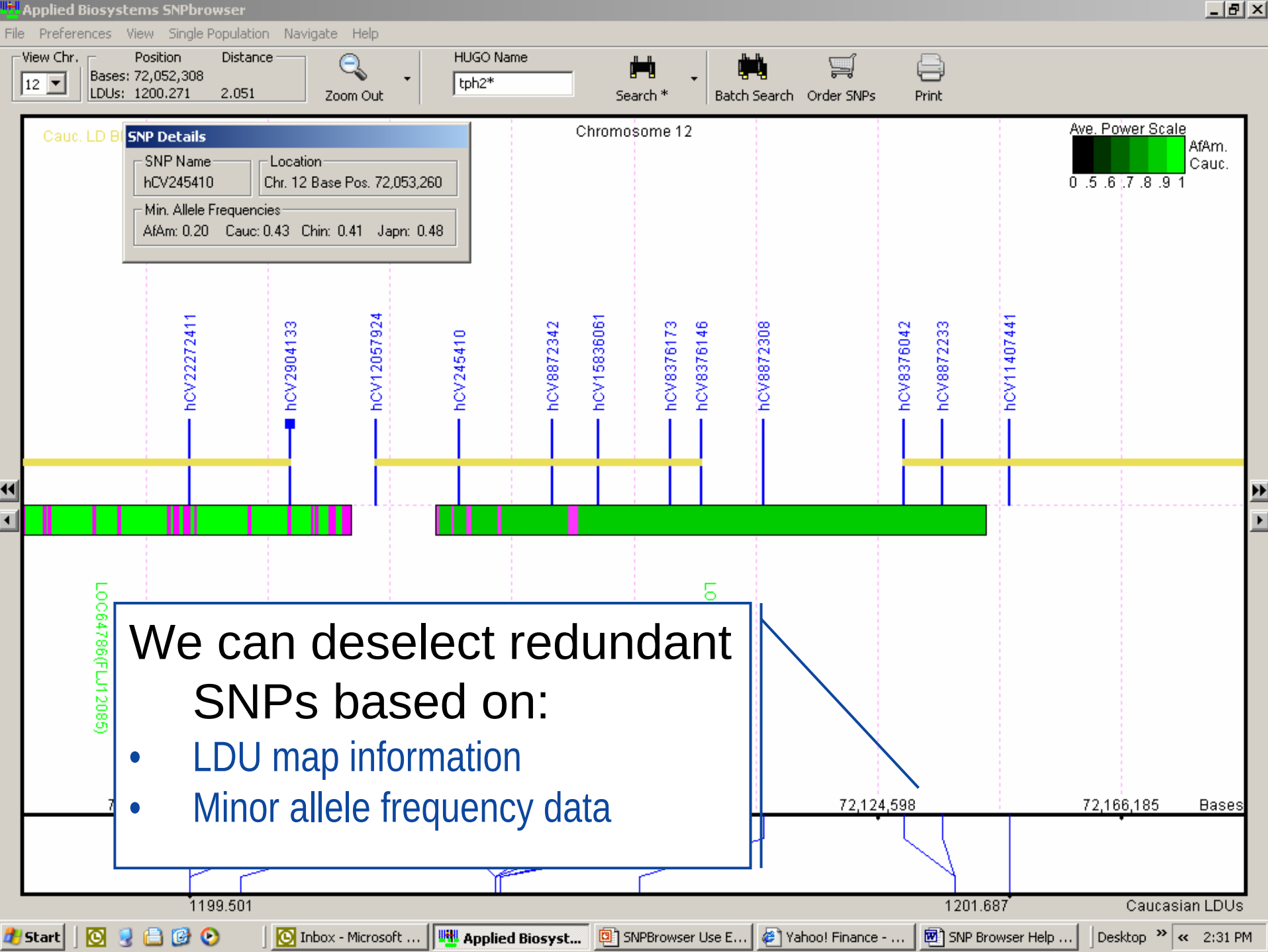
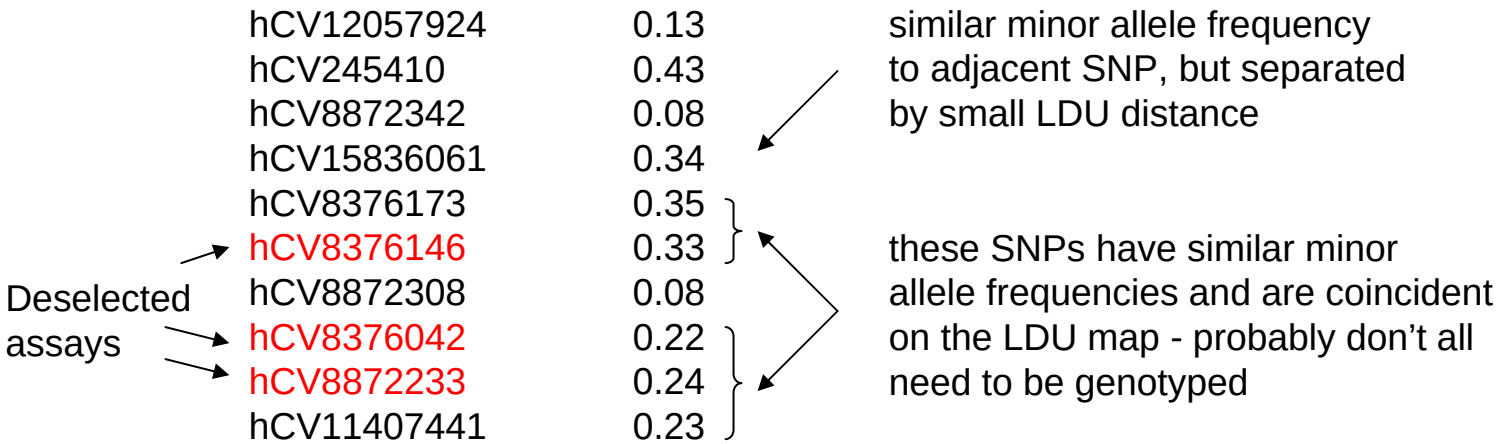

Applied Biosystems SNPbrowser
File Preferences View Single Population Navigate Help
View Chr. 12
Position
Bases: 72,052,308
LDUs: 1200.271
Distance
2.051
Zoom Out
HUGO Name
tph2*
Search *
Batch Search
Order SNPs
Print
SNP Details
SNP Name
hCV245410
Location
Chr. 12 Base Pos. 72,053,260
Min. Allele Frequencies
AfAm: 0.20 Cauc: 0.43 Chin: 0.41 Japn: 0.48
Chromosome 12
Ave. Power Scale
AfAm.
Cauc.
0 .5 .6 .7 .8 .9 1
hCV22272411
hCV2904133
hCV12057924
hCV245410
hCV8872342
hCV15836061
hCV8376173
hCV8376146
hCV8872308
hCV8376042
hCV8872233
hCV11407441
LOC647986(FLJ12085)
72,124,598
72,166,185
Bases
1199.501
1201.687
Caucasian LDUs
We can deselect redundant SNPs based on:
• LDU map information
• Minor allele frequency data
Start
Inbox - Microsoft ...
Applied Biosyst...
SNPBrowser Use E...
Yahoo! Finance - ...
SNP Browser Help ...
Desktop
2:31 PM

Using SNPbrowser to Help Design Genetic Association Studies

Which SNPs Should be Chosen for Genotyping?

For reasons of efficiency, we may not want to type all 10 of these SNPs (at least in the first round of genotyping).

Using allele frequency information and the LDU scale we can deselect those SNPs that might not give much additional information.



ABI Genotyping platform

- 5’ Nuclease Assay =TaqMan Probe mismatch
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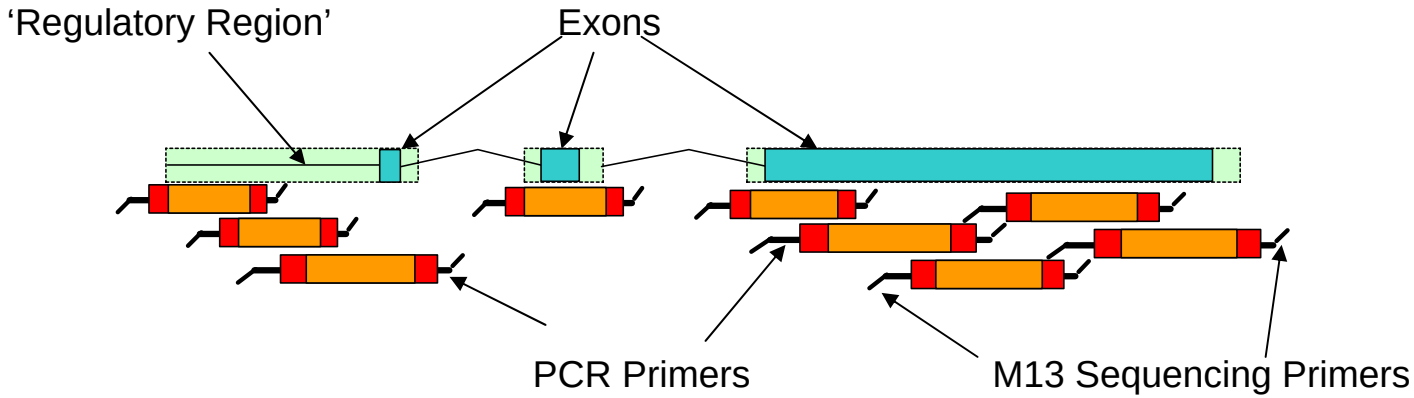
  - VariantSEQr resequencing primer
  - Sequencing reagent:
  - SeqScape v2.1

- Multiplexed OLA/PCR Assay Extension = SNPlex assay
  - 3730, (3100)
  - GeneMapper v 3.5

When is sequencing the best technology for detecting variants?

- Discovering new mutations
- Detection of insertions/deletions as well as novel SNPs
- Dense mutational spectrum
- Evaluating known variants

VariantSEQr™ Resequencing System
Complete Gene Primer Sets



- **Includes all Genbank RefSeqs (transcripts) for each gene → 'supertranscript'**
- **Primers for all exons (coding and non-coding)**
- **'Regulatory region' = 1kb 5' of exon 1 start**
- **Splice junctions**
- **Primers tailed with M13 universal F and R sequencing primers**
- **Annotation of regions where coverage is < 100%**

An Application Product That Is Applied Biosystems Optimized

AmpliTaq Gold® DNA Polymerase

- ✓ ***Resequencing Primers:***
  - ❖ *Ready-To-Pipet Into PCR Reaction*
  - ❖ *Enough Liquid Volume For 500 DNA Samples*
- ✓ ***Protocol:***
  - ❖ *PCR Thermal Cycling Profile*
  - ❖ *PCR Cleanup*
  - ❖ *Sequencing Thermal Cycling Profile*
  - ❖ *Sequencing Cleanup*
- ✓ ***Optimized For Use On:***
  - ❖ *Applied Biosystems 9700 thermal cycler*
  - ❖ *AmpliTaq Gold® DNA Polymerase*
  - ❖ *BigDye® Terminator v3.1*
  - ❖ *Applied Biosystems 3730 & 3730xl & 3100 systems*
  - ❖ *Applied Biosystems SeqScape® Software 2.1*

VariantSEQr™ System Summary

- Validated PCR primers to provide consistent and specific amplification
- Primers pre-mixed, diluted and aliquoted for easy use
- Streamlined and optimized protocols for efficient workflow
- Automated and highly accurate data analysis

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Complete Solution for High Throughput SNP Genotyping

Locus Specific Assays

Fixed Content

Human Linkage Mapping Set

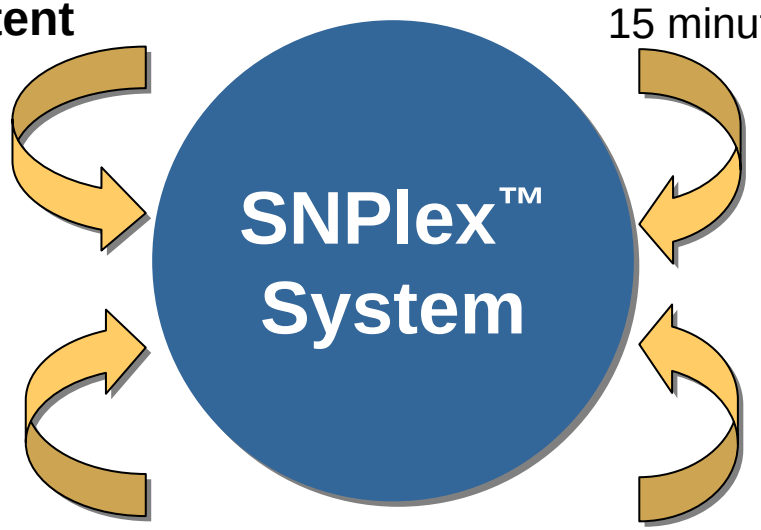
Custom Content

Instrumentation

Applied Biosystems 3730/3730xl

POP-7™, 36 cm array

15 minutes per run



Universal Reagents

OLA/PCR modules

Reporter Probes

Multi-color fluorescence

Mobility Modifiers

Data Analysis

Data Collection Software v2.0

GeneMapper™ Software v 3.5

Assay Products

- **SNPlex™ System Custom: (Flexible,made-to-order)**:
  - Customer specified/selected
  - AB designs probes & multiplex groups, synthesizes oligos, pools probes, ships to customer

- **SNPlex™ System Fixed (off-the-shelf)**:
  - Human Linkage Mapping Set ~ 3500 markers
  - Additional Human and Mouse Sets planned

Basic Assay Steps Involved in SNPlex™ System

Ligation
Generation of genotype (GT) specific products through multiplex oligonucleotide ligation reaction (OLA)

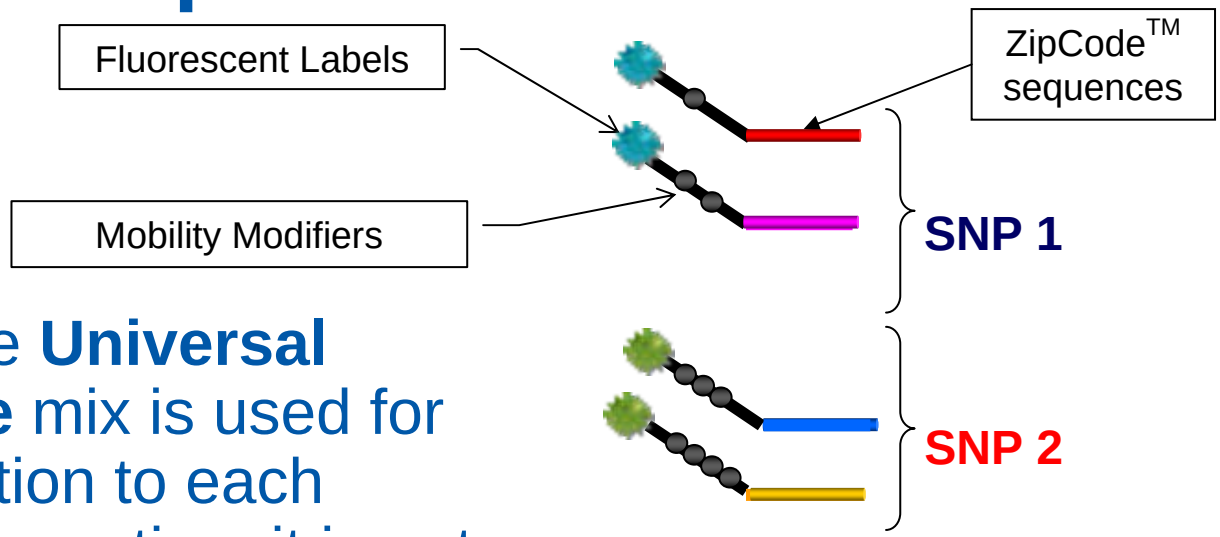
↓

Amplification
Multiplex PCR with universal primers

↓

Detection
Hybridization of universal ZipChute™ probes to amplicons and identification of eluted ZipChutes by CE

Universal ZipChute™ Probes



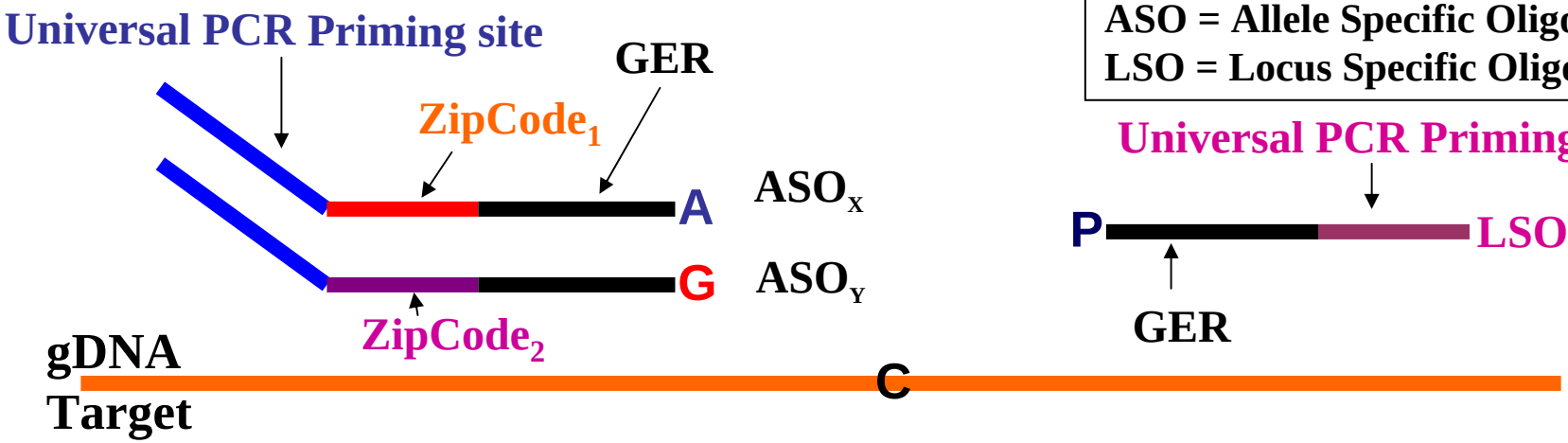
- The same **Universal ZipChute** mix is used for hybridization to each multiplex reaction, it is not SNP specific
- ZipChute probes that specifically hybridize to genotype specific amplicons are eluted and analyzed by capillary electrophoresis
- ZipChute identification is used for genotype determination

Whole Product View

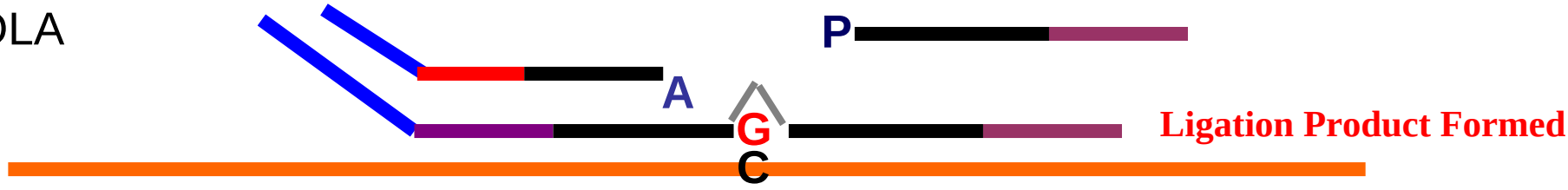
- **OLA/PCR Reagent Kits (Universal)**
  1. Phosphorylation Module
  2. Ligation Module
  3. Amplification Module
  4. Purification Module
  5. Hybridization Module
  6. Controls
  7. Standards Module
- **SNP specific probes (Assays)**
- **GeneMapper™ 3.5, Data Collection 2.0, BioTrekker™ 1.0 (optional)**
- **Electrophoresis instruments & consumables**

SNPlex™ System OLA/PCR Assay

GER = Genome Equivalent Region
ASO = Allele Specific Oligo
LSO = Locus Specific Oligo



1. OLA

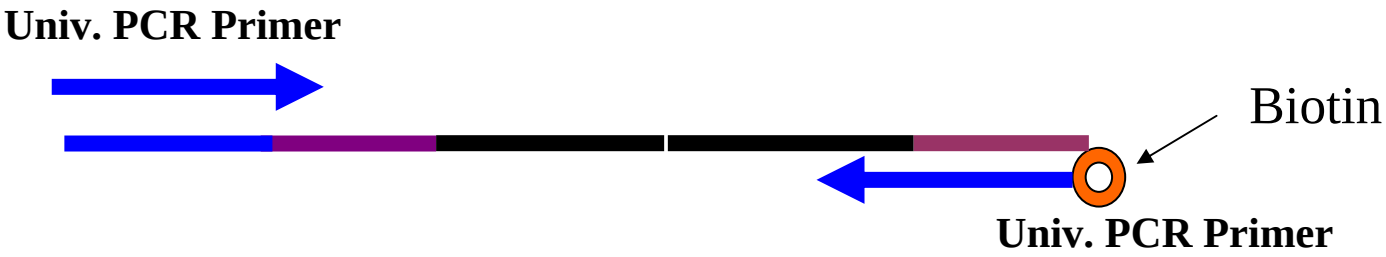


(Homozygote shown in this case)

2. Clean-up

PCR & ZipChute™ Probe Hybridization

3. Multiplexed Universal PCR

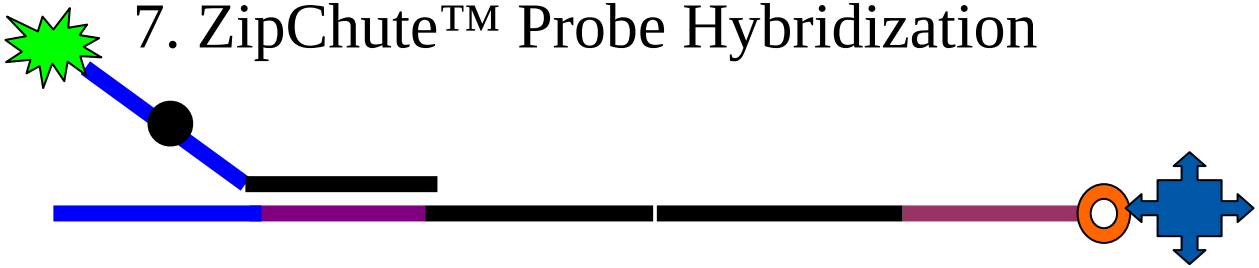


4. Capture double stranded DNA

5. Denature double stranded DNA

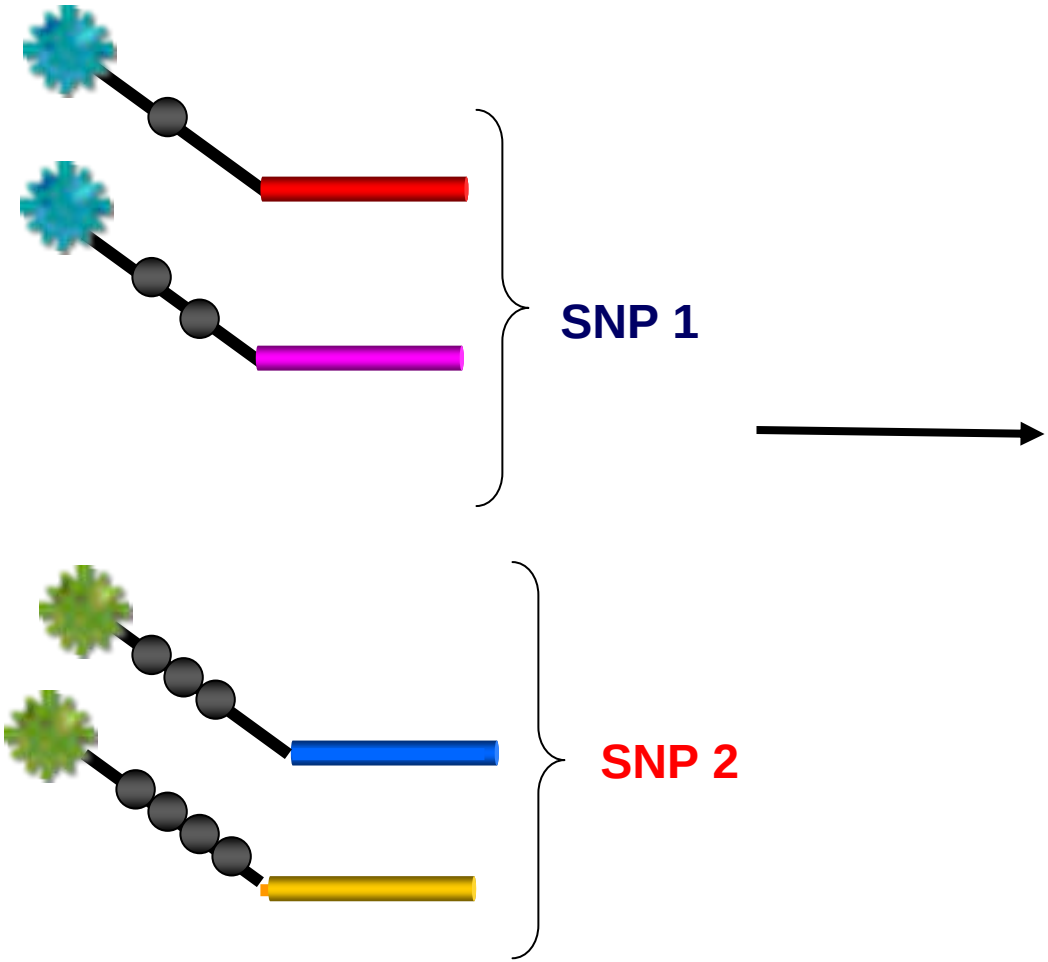
6. Wash away one strand

7. ZipChute™ Probe Hybridization



Detection

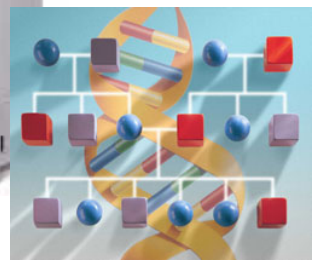
8. Wash and release ZipChute™ Probes



9. Load on CE instrument

2004: Experimental Design

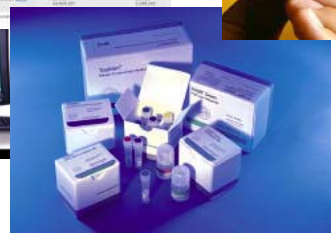
Applied Biosystems
3730xl DNA Analyzer



SNPlex™ Genotyping
System

CELERA DISCOVERY SYSTEM™
The essential tool for the life science researcher

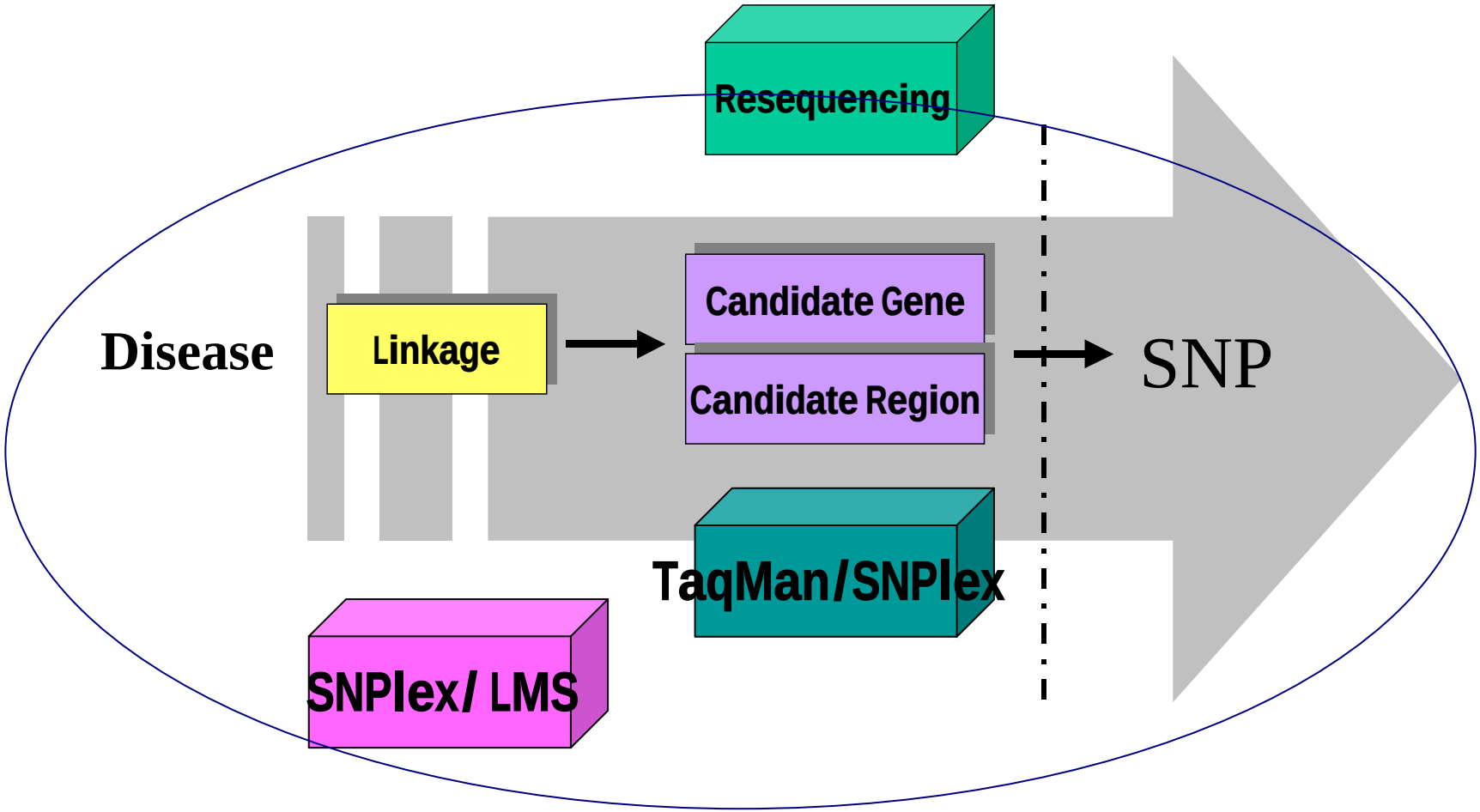
ABI PRISM® 7900HT
Sequence Detection System



TaqMan® SNP Genotyping Assays
(Formerly Assays-on-Demand™
products)

Applied Biosystems

Disease Gene Hunting:



AB offer total solution

以計畫的需求來決定適用方案:
唯有ABI能提供SNP Total Solutions

	# SNP Markers	# Samples	Recommended Platform
Whole Genome	1000's-500K	1000's	SNPlex
Linkage Mapping	1000's	100's-1000	SNPlex
Candidate Gene/Region	10-1000's	500-1000's	TaqMan/SNPlex
ADME/Tox	100's	10-1000's	TaqMan

SNPlex vs TaqMan

	SNPlex	TaqMan
實驗流程	Ligation Probe Hybridization Detection	Hybridization Detection
Throughput (genotypes per day)	437,760 - 1,300,000	27,648 - 255,000
Running cost (per SNP)	0.02-0.08	0.4-0.5

Why customers will not switch from Taqman to SNPlex

- Don’t have CE instr. or budget
- Don’t have robotics or budget
- Have projects with critical SNPs (i.e. can’t substitute)
- Have projects with small numbers of SNPs (< 50)
- Working exclusively with non-Human species
  - SNPlex can support this, but optimized for Human

Why Customers Might Switch from Taqman to SNPlex…

- Attractiveness of price/GT
  - This can be the wrong reason based upon approach/study needs
- Already has CE platform
- Wants to do sequencing, re-sequencing, linkage or other fragment analysis applications
- Existing expertise/hardware for automation
- Project size is large
  - Dictates need for high throughput capability

Two Technology Choices

Choose TaqMan

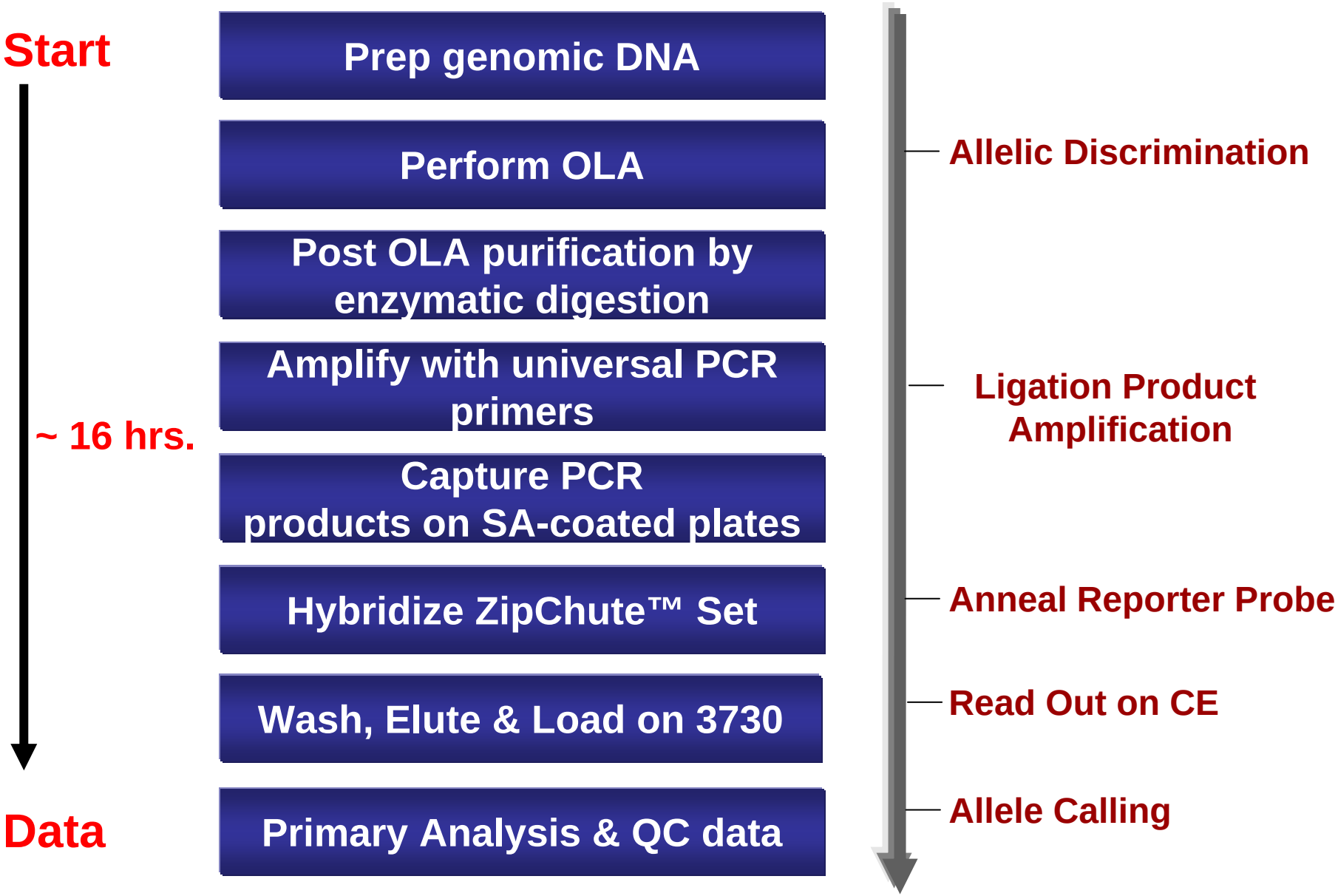
1. To take advantage of singleplex technology for “mix & match” of individual SNPs
2. When you are limited to a small number of SNPs to genotype
3. For simplified one-step workflow and convenience of validated assays
4. Additionally require gene expression capability in your laboratory

Choose SNPlex

1. To take advantage of multiplex technology to lower overall project cost
2. When you have desired “set” of 48 or more SNPs to genotype
3. Use robotics/automation in your laboratory
4. Additionally require sequencing capability in your laboratory

Complete SNP genotyping product portfolio:
Candidate gene/candidate region studies
Whole chromosome/genome studies

SNPlex™ Assay Workflow Overview



5’ Nuclease based solution is simplest to implement

- Easiest to implement
  - Assays by Design^{SM} service
  - Ready to use Assays on Demand^{TM}
  - Universal master mix
  - Minimum of pipetting steps
  - no transfer or additions post PCR.
- Easiest to transfer between collaborators
  - PCR infrastructure + 7900,7700,or 7000
  - No multiplexing required

Assays are portable from study to study, lab to lab

AB SNPS- 100% Ready To Use

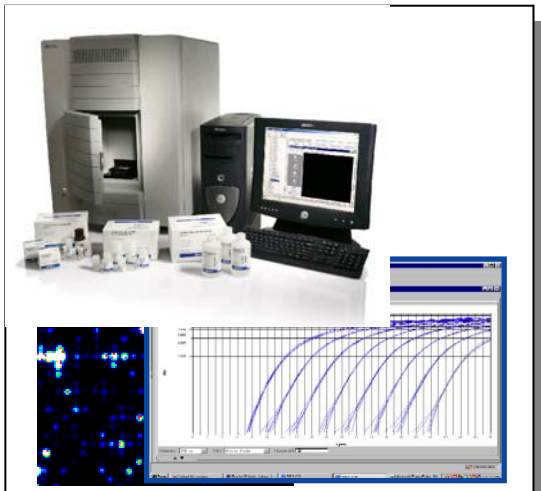
- 200,000
  - fully-validated
  - true SNPs
  - Real allele frequency data
  - Hardy-Weinberg Equilibrium
  - Gene coverage plus
  - Ready-to-use assays

Choose it and use it!

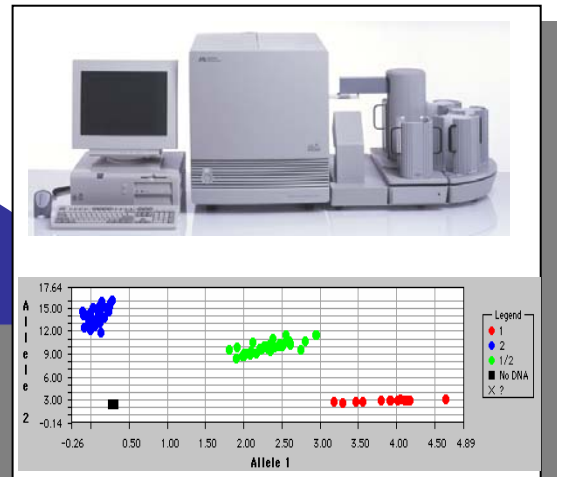
The Strategy

- Shift customer focus from per genotype costs to project costs.
- AB providing enabling resources that are redefining project time and cost

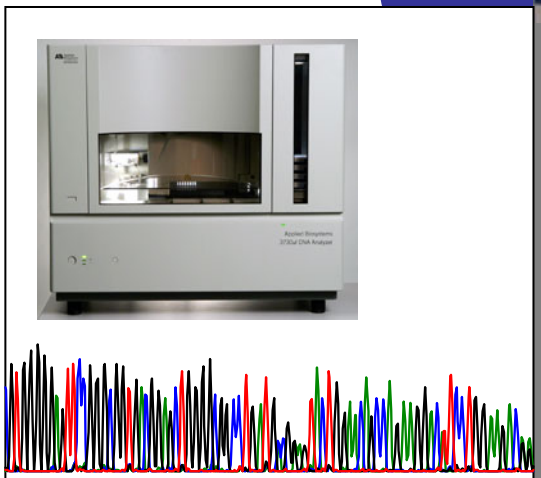
ABI 基因體研究相關的全貌



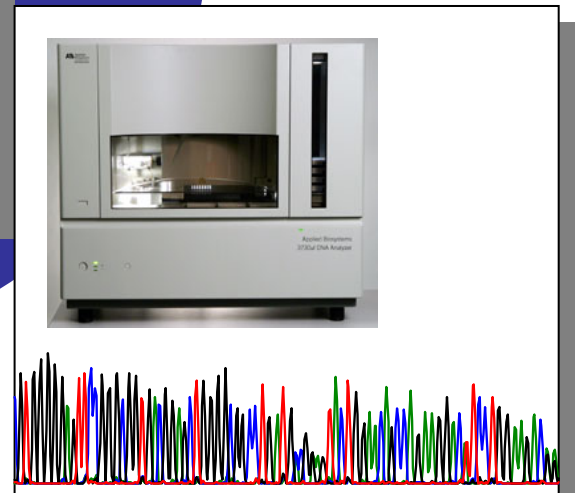
GENE EXPRESSION



SNP GENOTYPING



SEQUENCING



RESEQUENCING