

GENOMELAB™
FROM TISSUES TO TARGETS.

Identify



Isolate



Prepare



Analyze



Evaluate



Validate



*The pathway to Genetic
Discovery begins here*

Genome Lab™ SNPstream

Design, Automation and Analysis
of SNP Assays

Jim Thorn
Prague May 2005

Introduction

- SNPstream in Genome Lab
- SNPstream system overview
- SNPstream technology
 - Assay design to genotype export
- Application of SNPstream technology
 - SNPstream in forensics
 - SNPstream in clinical research
- Conclusion

Genome Lab™ Platform Workflow Map

Identify

Identify phenotype normal vs. disease



- FC 500
- Vi-Cell™

Isolate

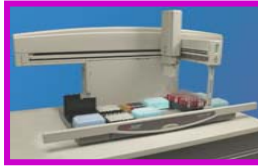
Isolate and purify nucleic acids



- Altra™
- Biomek®
- HPLC
- Biomek®
- Avanti®
- Optima™
- Allegra®

Prepare

Prepare the nucleic acids for specific detection



- DU® 800
- Biomek®
- Avanti®
- Optima™
- Allegra®
- DTX 880

Analyze

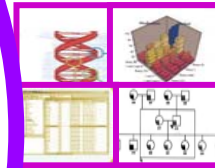
Analyze the genetic elements



- CEQ Family
- MDQ
- SNPstream

Evaluate

Evaluate genetic material as potential markers



- Software Informatics

Diagnose

Application of markers as diagnostic test



SNPstream Genotyping System

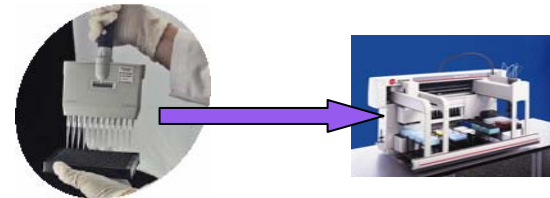
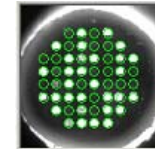
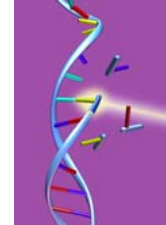
Complete Genotyping System

- Assay Design
- Automation
- Reagents
- Hardware
- Software
- Support



SNPstream System Design

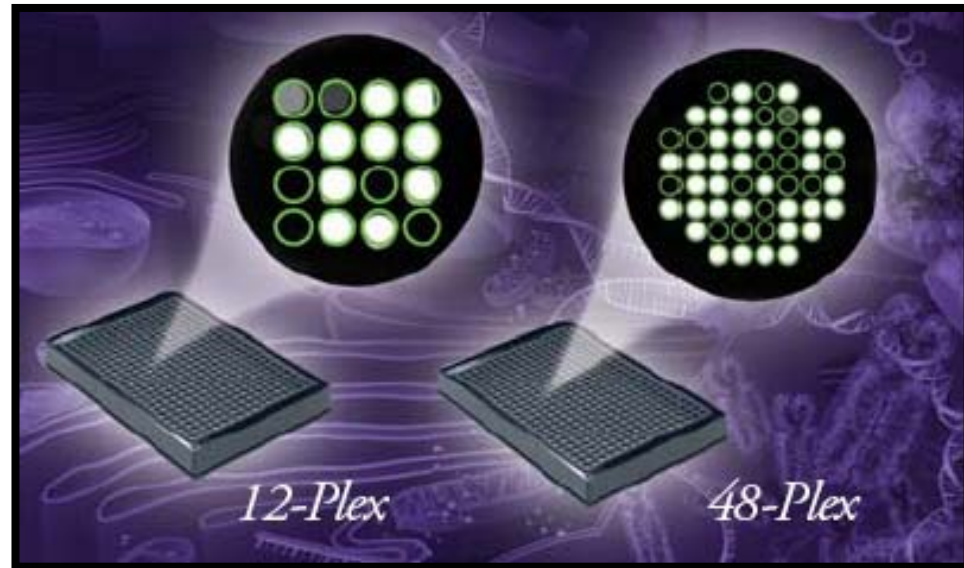
- High fidelity and accuracy
 - Single base extension technology
 - >99% accuracy of calling
- Sensitive
 - Less than 2ng gDNA per 12 or 48 plex assay
 - <0.04ng per SNP
- Scalable throughput
 - 4600 to 3,200,000 SNPs per day
 - Manual to full automation
- Simple
 - Automatic bar code reading
 - Sample tracking



SNPstream

Technology

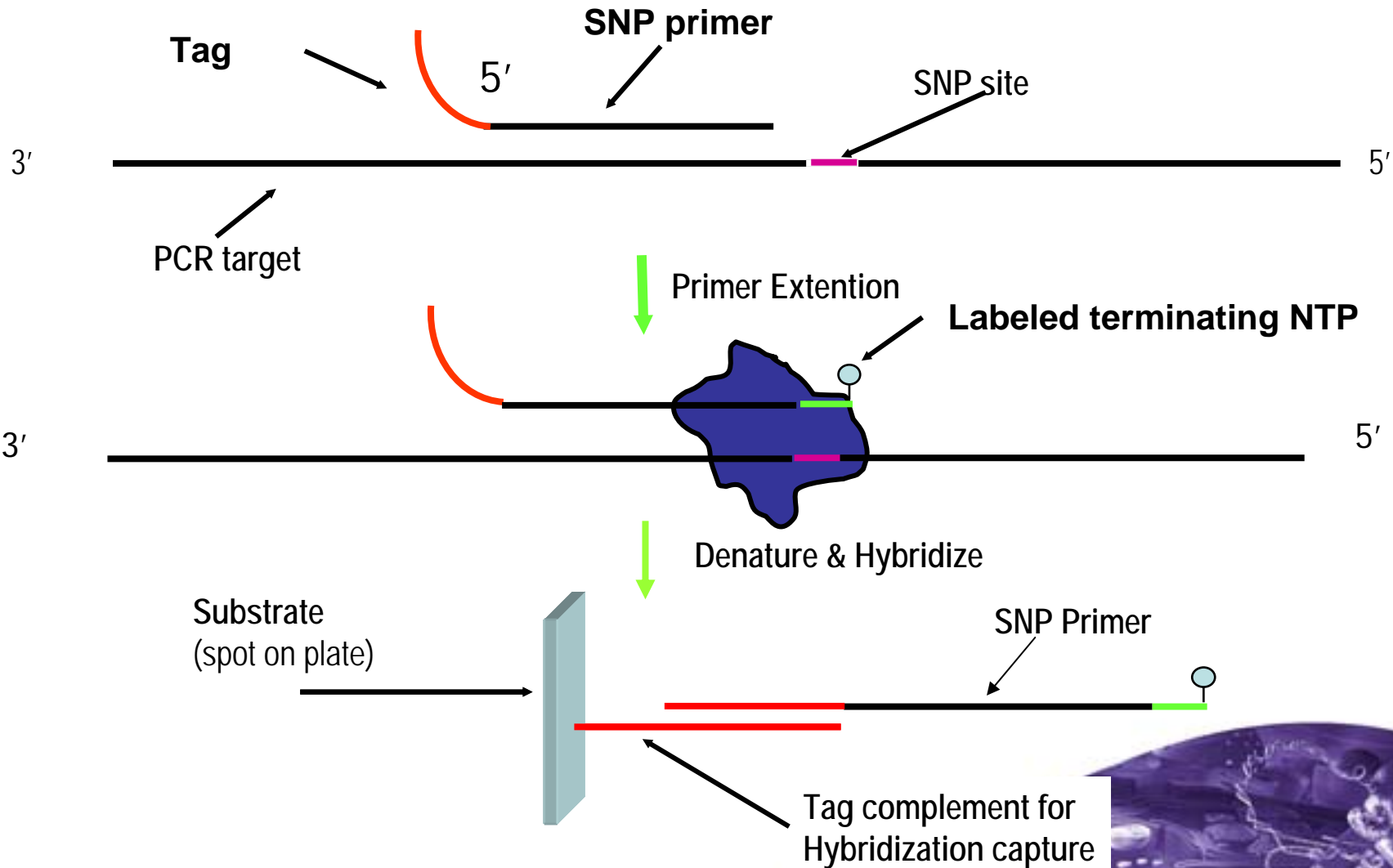
SNPware Tag Array Plates



- 384 well format
- Each well contains 16 or 52 'spots'
 - 12 or 48 tags per well, 4 control spots
- Each 'spot' = unique oligonucleotide of known sequence (a Tag)
- Each Tag on plate complementary to one extension primer Tag
- Size/shape compatible with standard microplate robotics
 - Flexibility for multiple SNP panels per plate

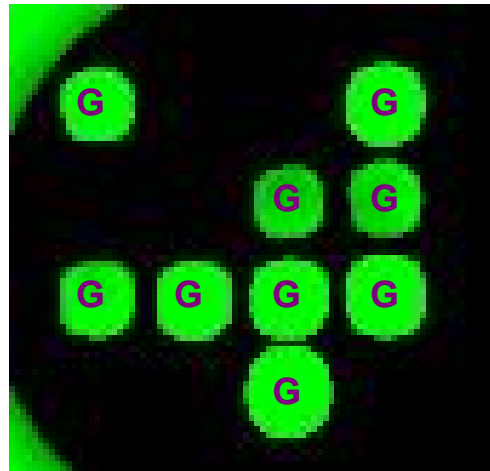
Single Base Primer Extension

The Genotyping Gold Standard

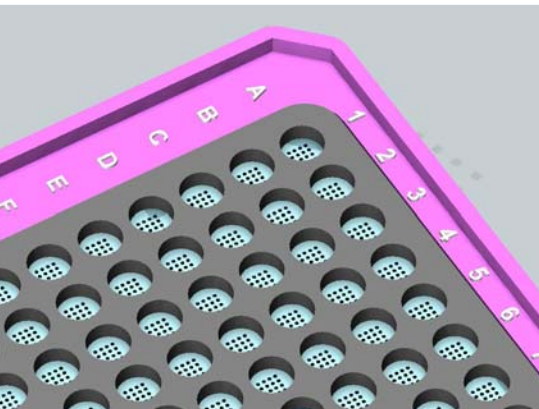
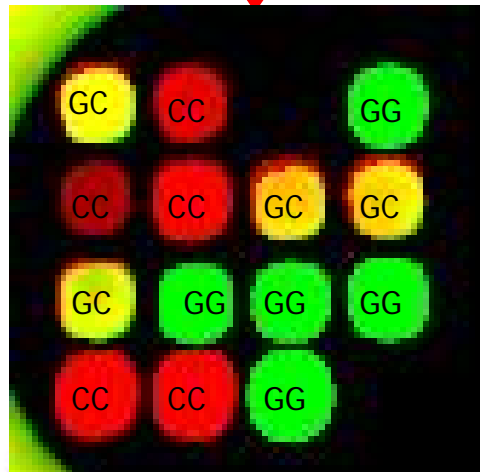
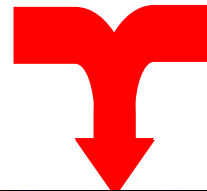
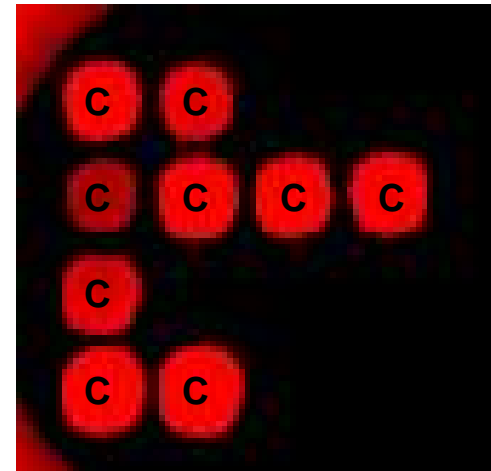


SNPstream- Detection

Imaging Channel 1
(Allele X)



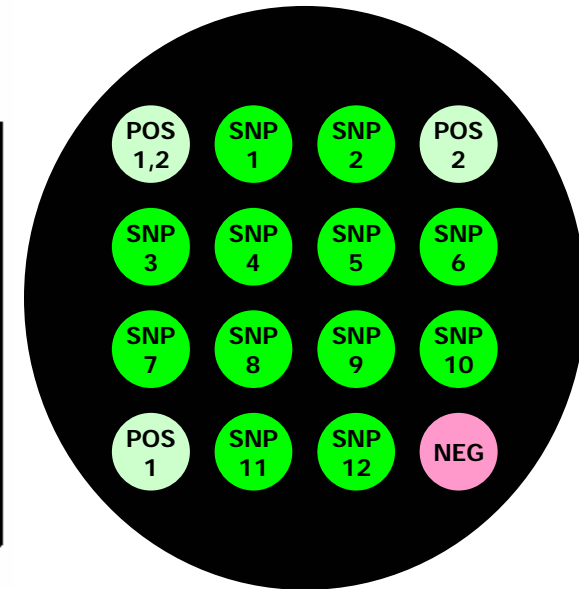
Imaging Channel 2
(Allele Y)



48-plex

12-plex

		52_1	52-XX	52-YY		52_2	
	52_3	52_4	52_5	52_6	52_7	52_8	
52_9	52_10	52_11	52_12	52_13	52_14	52_15	52_16
52-XY	52_17	52_18	52_19	52_20	52_21	52_22	52_23
52-Neg	52_24	52_25	52_26	52_27	52_28	52_29	52_30
52_31	52_32	52_33	52_34	52_35	52_36	52_37	52_38
	52_39	52_40	52_41	52_42	52_43	52_44	
		52_45	52_46	52_47	52_48		





Experimental Design

SNP Mining, Primer and Assay Design

SNP Mining

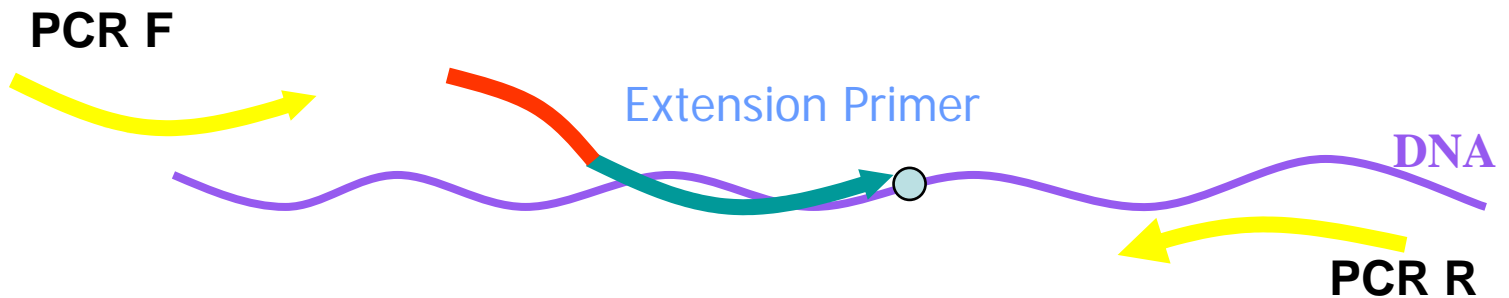
- Publications.
- In-house research: sequencing, mutation analysis.
- Public databases:
 - OMIM- online Medelian Inheritance in Man
 - SNP databases: dbSNP, HGMD, JSNP
 - Licensed SNP databases: Celera



Current SNP Validation Status

Database	Total SNPs	Validated	Comments
dbSNP	4,145,633	512,247	Allele Frequency has improved Quality
The SNP Consortium	1,255,326	57,000	42 African,Caucasian and Chinese
Celera	4,902,233	3,488,791 2,443,761	Celera+repassed sequence dbSNP revalidated
SNP on Demand	137,923	137,923	45 Africans and caucasian and all genes are centric
RealSNP	226,754	129,494	Database with Validation mainly dbSNP loci

SNPstream Primers



- Per SNP:
 - Two unmodified PCR primers
 - Approx 20bp each
 - SNP extension primer with 5' tag array tail
 - Approx 40 bp
- Per Panel:
 - 24/96 PCR primers
 - 12/48 Extension primers.



utoprimer.com

A Beckman Coulter, Inc. Web Tool

- Web-based design tool
 - Fast, secure, easy to use
 - Designs PCR and Extension primers
 - Assigns tags to extension Primers
 - Looks for inter and intra strand interactions
 - Groups sequences into panels
 - Generates output file
-
- THE ONLY PRIMER DESIGN TOOL THAT CAN DESIGN AND ASSEMBLE MULTIPLEX PANELS (with 80-90% assay success on SNPstream)





Submitted Sequences

Autoprimer.com File Confirm Page - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Search Favorites History Print

Address http://www.autoprimer.com/primer_app/confirm.asp Go Links

  **utoprimer.com**
A Beckman Coulter, Inc. Web Tool

home support design

File Confirm Page

Step 1: - Product to Design Primers

Step 2: Select file to process and submit. For help in creating a file, click here.
To "cut and paste" sequences, click here.

Step 3: Confirm Data:

```
SNP111_CT      AACTGAATTGCAGAGAGCTTAGATGACTTGAAGAGACTTGACAGCTGAGCAGGGAG,
SNP102_RC_GA  GCCTTGAGAGCTTCTAACATCTCCTATAACAATTGGTAACAGAAGACAGTTACTGGAAC
SNP162_CT      CTTTTAGAAGGTCACAGGTCACAGGTCAGCATGTGTCTGCCTCTGGCAGATTTATCCTTACA
SNP123_CT      TCCCAGGCTCCCCCTCCCTTTCTGTGGTTCCGTTTCTTGTGCACACAGTAGCTGCTG
SNP138_CT      AGTTATGAATAAGGGAAATTGGTTAATAACAACCTTAGCTTGTGCCCCCTCCCCCACTCG
SNP10_CT TCCCAGGCTCCCCCTCCCTTTCTGTGGTTCCGTTTCTTGTGCACACAGTAGCTGCTGTTTACTGA
SNP149_CT      TCAAGAGCCATCAGGCAGCGCGTGGAGGGCCCTACCTAGTTCCTCCTCCTGGGCTT(
SNP141_CT      CTCTTCTCTTTTCTCTCTTTTCTCCTCCTCTGCTATTTTATTTTATTCTTTGG(
SNP113_RC_GA  TAAAGGAACACTTTAAGTTTACTGCTCTTGTCTAGTGCAATCAGACAGCACCAGG
SNP152_RC_TC  AGGGACTCCTCCAGGACTTACTGGCACCCCTTTGTGAACAAGGGCCATGCCTTACCC
SNP117_CT      CTCTTGGTGTCTTTCATTGTTACAGGACTTGTGCTCTGAAGTCTGATGAACAACATACAC
SNP129_CT      GGCAGATAATATTTCTCCCTGAACCACTCATCCATATCTGCTCTATTAGATAATTTG
SNP139_CT      CAAACTCAAGTCAAAAAGAAATAGCTTTGTTGGGAGGGGAAGCAGAGTCAATTACATA
```

Step 4: - Number of Primer Designs Desired (type ALL for All Available)

Done Internet

Primer Designs

Autoprimer.com Results - Microsoft Internet Explorer

Address: http://www.autoprimer.com/primer_app/process.asp

SNP Name- Probe Name	SNP Type	Primer Type-Mod.	Amplicon Length	Sequence
Panel # 1				
SNP10_CT-U11	CT	PCR PCR SNPU	136	TTTCTTGTGCACACAGTAGCTG TAACTTCTGAGCCTCAGCTCC AGAGCGAGTGACGCATACTACTCCTCCATTTTTATTTAATCCTCA Possible SNP-IT Cross Hybridization...
SNP123_CT-U12	CT	PCR PCR SNPU	136	TTTCTTGTGCACACAGTAGCTG TAACTTCTGAGCCTCAGCTCC CGACTGTAGGTGCGTAACTCCTCCATTTTTATTTAATCCTCA Possible SNP-IT Cross Hybridization...
SNP152_RC_TC-U10	CT	PCR PCR SNPU	151	ACTTACTGGCACCCCTTTGTG TCAGTGCTATTTCTCTGAGCTAGG AGATAGAGTCGATGCCAGCTTGGAGGATTCTCTTTGTAACC
SNP149_CT-U9	CT	PCR PCR SNPU	89	CTTGCCCTGCTGAGGACT CTGTGAGGACTGGTGAGA GACCTGGGTGTCGATACCTATATTTCTTGAATTCTGCCTACCTA
SNP111_CT-U7	CT	PCR PCR SNPU	112	TCCTGTGCACAAAGGAAGC TTTCCAATAGGCTAGCAGA AGGGTCTCTACGCTGACGATACATGTAAGTTACTCCACCCCTGCC Possible SNP-IT Cross Hybridization... Possible SNP-IT Primer vs Amplicon Cross Hybridization...
SNP129_CT-U8	CT	PCR PCR SNPU	133	ATATCTGCTCTATTCAGATATTTGTGC AGATGTTTGGGATAGCTTTTTAAT GTGATTCTGTACGTGTCGCCCCACAGATCCTCAAACCAAGCCC Possible SNP-IT Primer vs Amplicon Cross Hybridization...
SNP141_CT-U5	CT	PCR PCR SNPU	97	CCCAGGGCCATTAATAATG TTTAGCTTTTTCCAAGGTCGT GCGGTAGGTTCCCGACATATTTAGAATGAGATTGGTTAAGA
SNP117_CT-U4	CT	PCR PCR SNPU	90	TTTCTGGGGAAATATGCC ATAAAATAAAGAGGGGAAGAACCT AGCGATCTGCGAGACCGTATGTTGAGTGTGAGAGCAGGGACTTT
SNP139_CT-U6	CT	PCR PCR SNPU	151	TCACATTACATATCAAGAAGAAATATAACTC TACTTGTACTATCTCCATCCAGAA GGCTATGATTGCAATGCTTTGAGGGAGAGGCATTGAGAAAGTA
SNP138_CT-U2	CT	PCR PCR SNPU	90	CCTTGGTTGTCTGGAGCTC TTCCCTCCCAACAAAGC GGATGGCGTTCCTGCTCTATTAGTTGAGATGCAGTTGCAGAAGGTA
SNP162_CT-U3	CT	PCR PCR SNPU	110	AATATTTTCATGCCAGGGAAGTA AACAAAGTACATTTAGGTAATTATCACCAT CGTGCCGCTCGTGATAGAATTCACACTAGGAAATTCAGGG
SNP102_RC_GA-U1	CT	PCR	152	TCCTATACAATTGGTAACAGAAGACA



Assay Design

- No optimising of reaction conditions needed
 - Autoprimer design of primers
 - Thermodynamic properties match standard conditions
- Standard PCR conditions
 - 35 cycles of:
 - 94°C, 30 seconds
 - 55°C, 30 seconds
 - 72°C, 1 minute
- Standard primer extension conditions
 - 45 cycles of:
 - 94°C, 20 seconds
 - 40°C, 11 seconds



Data Analysis

Quality Control and Genotype Export

Quality Control (Manual or Automatic)

Run GetGenos

Run on DB | Run on File

Process all pending plates
 Reprocess selected plates

Reprocess plates with #: 2580603

Retrieve Plate List

Plate: 2580603

Reprocess a plate

All segments
 Segment: 1

Panel/Snp Info

All spots/snps
 Select a spot/SNP

Please select which spot to run the GetGenos:

	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X

SNP: Seg1 : 2129772_CG

Run GetGenos | Stop Running | Close

GetGenos Message box

07/09/2004,8:50:53 AM, successfully updated plate:2580603 segment:1 Spot: (4,7) and Insert 384 data
 07/09/2004,8:50:59 AM, successfully updated plate:2580603 segment:1 Spot: (5,7) and Insert 384 data
 07/09/2004,8:51:05 AM, successfully updated plate:2580603 segment:1 Spot: (6,7) and Insert 384 data
 07/09/2004,8:51:10 AM, successfully updated plate:2580603 segment:1 Spot: (7,7) and Insert 384 data
 07/09/2004,8:51:15 AM, successfully updated plate:2580603 segment:1 Spot: (3,8) and Insert 384 data
 07/09/2004,8:51:20 AM, successfully updated plate:2580603 segment:1 Spot: (4,8) and Insert 384 data

GetGenos

File Run View Settings Utility Help snp/snpstream48/V2 DB

Current spot:(5,8) SNP: 2129772_CG at Segment 1 of plate: 2580603
 Total: 384 XX: 191 XY: 56 YY: 128 NEG: 8
 Org Fail: 0 Geno Fail: 1
 Call Rate: 375/376=99.73 HW score: 179.9
 View all Data Points

Insert well:h14 for Spot: (5,8) at segment: 1 of plate:2580603

QC Review

New Reviewed

Select Category: SNP Plate Number

Select by SNP Plate Number: plates within 5 days

Select QC Plate from list: 2580603

Select QC Segment from list

GetGenos Message box

07/09/2004,8:51:15 AM, successfully updated plate:2580603 segment:1 Spot: (3,8) and Insert 384 data
 07/09/2004,8:51:20 AM, successfully updated plate:2580603 segment:1 Spot: (4,8) and Insert 384 data
 07/09/2004,8:51:26 AM, successfully updated plate:2580603 segment:1 Spot: (5,8) and Insert 384 data
 07/09/2004,8:51:32 AM, successfully updated plate:2580603 segment:1 Spot: (6,8) and Insert 384 data
 GetGenos of plate: 2580603 completed.
 Manually stopped running!

GetGenos

File Run View Settings Utility Help snp/snpstream48/V2 DB

QC Review

New Reviewed

Select Category: SNP Plate Number

Select by SNP Plate Number: plates within 5 days

Select QC Plate from list

Constant Settings for Plate Type 48plex

Cluster Parameters | **Automatic QC** | Output

Perform Automatic QC on SNPs as assays are processed
 Ignore Grid Shifted wells up to 2 % of wells in segment

Use	Description	Value
<input type="checkbox"/>	Fail if Hardy Weinberg value is >	60
<input type="checkbox"/>	No decision if Hardy Weinberg value is >	68
<input checked="" type="checkbox"/>	Fail if Call Rate % <	29
<input checked="" type="checkbox"/>	No decision if Call Rate % <	28
<input checked="" type="checkbox"/>	Fail if > nn% of spots too low energy	30
<input checked="" type="checkbox"/>	No decision if > nn% of spots too low energy	20
<input checked="" type="checkbox"/>	Fail if YY lower relative energy >	0.14
<input checked="" type="checkbox"/>	No decision if YY lower relative energy >	0.07

Reset to Default Settings

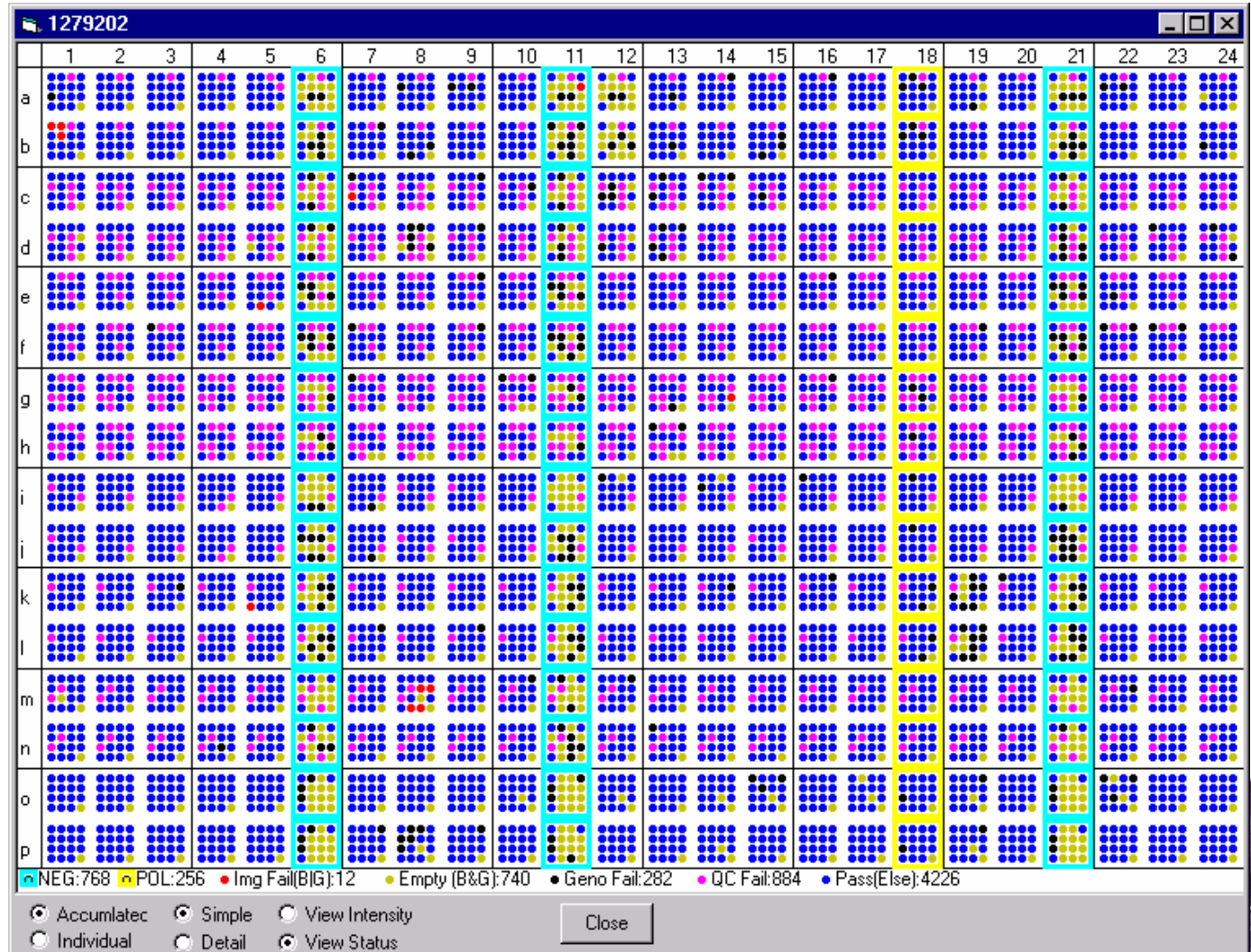
View Summary Report after finish each batch run

OK | Cancel

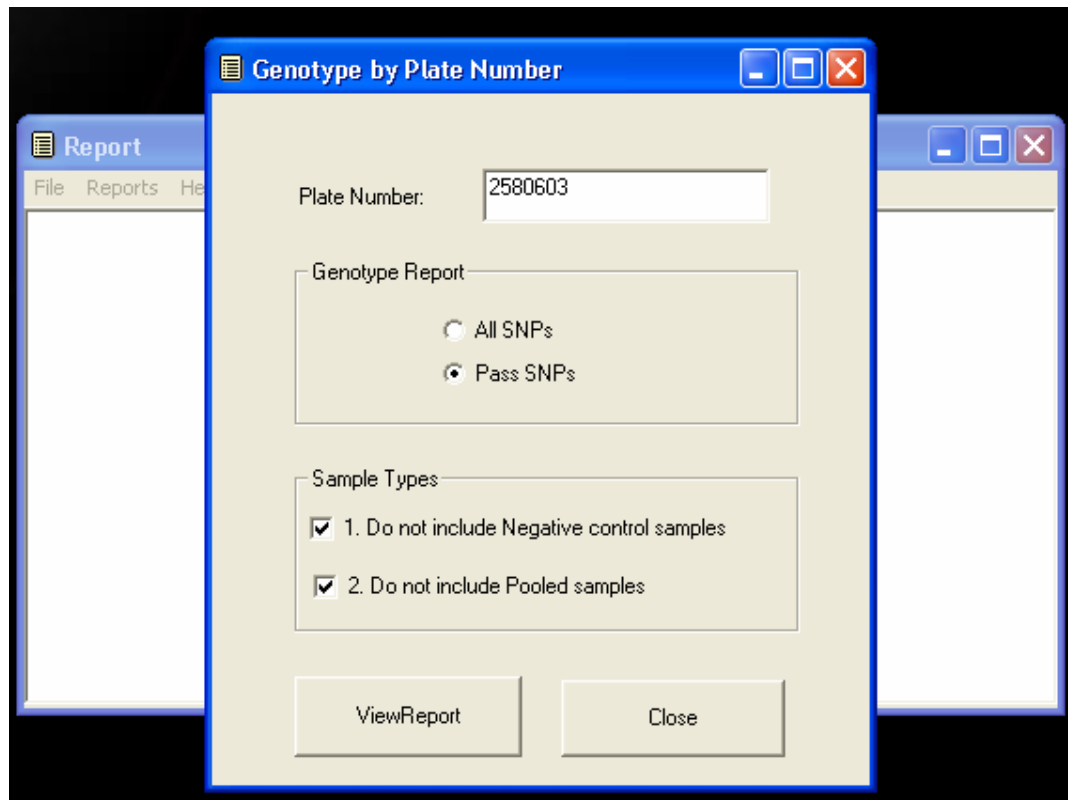
Check here to enable Auto QC



Whole Plate View



Genotype Summary Report

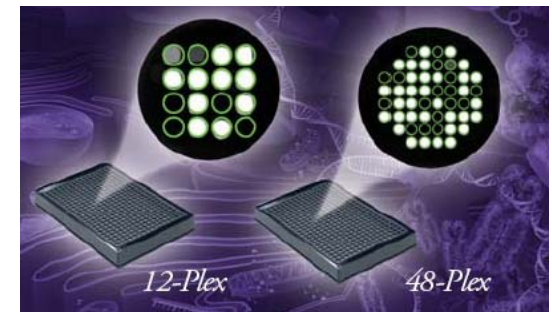


Genotype Report								
Plate Number: 12580603	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6	SNP 7	SNP 8
Sample ID	1329034_CG	586528_CG	786376_CG	1259700_CG	852753_CG	209611_CG	881482_CG	763949_CG
PD1	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CC	CC	GG	CC	CG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC
	CG	CC	GG	CC	GG	CC	CC	CC



SNPstream Genotyping System Performance

- Scalable throughput
 - 4,608 to 800,000 genotypes in 24 hours (12 plex)
 - 18,432 to 3,200,000 genotypes in 48 hours (48 plex)
- Multiplex level
 - 12 and 48 plex PCR and Primer extension
- Conversion rate
 - 80 - 90% with quality input sequences
- Accuracy
 - >99%
- Call Rate
 - >95%



SNPstream Application

Forensic Science



SNPstream in Forensic Science

- Forensic investigation challenges
 - Low amounts of sample available
 - DNA may be too degraded for STR analysis
 - STR gives no information about subject's physical appearance

 - Less than 2ng gDNA per multiplex assay
 - <0.17ng per SNP in 12-plex
 - <0.04ng per SNP in 48-plex
 - Autoprimer designs short amplicons
 - Typically 90 – 150bp
 - SNPs may be used that predict characteristics
 - hair and eye colour
 - Racial group

American Forensic investigation of World Trade Center attack



- American Forensic investigation of World Trade Center attack to identify victims
 - Some DNA badly degraded
- SNPstream technology
 - Small amplicons
 - Low quantity of gDNA
- Results obtained on samples not typed by microsatellite technologies



DNA Print Genomics Inc

- Louisiana serial killer case
 - Eyewitness accounts = white male
- DNAPrint developed DNAWitness test
 - SNPstream instrument + Proprietary software
 - Unknown offender's DNA tested
 - 85% sub-Saharan African and 15% Native American
 - After 2 months a man whose genetic heritage is consistent with the test results provided by DNAPrint, was linked to the crimes via STR (human identity) testing and arrested



SNP Stream in Clinical research

180 disease significant SNPs
(15 panels)



Recent Publications

Clinical Research

Functional variants of OCTN cation transporter genes are associated with Crohn disease. Peltekova VD et al 2004. Nature Genetics. 36: 471- 475

Polymorphisms in the Tyrosine Kinase2 and Interferon Regulatory Factor 5 Gene are Associated with Systemic Lupus Erthematosus. S. Sigurdsson et al 2005. Am. J. Hum. Genet. 76: 528-537.



Recent Publications

Blood Transfusion

High-throughput multiplex single-nucleotide polymorphism analysis for red cell and platelet antigen genotypes. G.A. Denomme and M. Van Oene 2005. Transfusion. 45: 5, 660.

- Transfusion recipients develop immunity to donor antigens
- Require antigen negative blood
- Current screening by antisera is costly
- Tested screening by genotype using SNPstream
- Potential to reduce screening cost up to 20 times with SNPstream



Conclusion

- True multiplexing from primer design to genotypes
- Fully automated from reaction to data analysis
- Lower costs
 - Both PCR and assay costs
- Saves time
 - Multiplexing increases throughput
- Saves DNA
 - Only 2ng of gDNA required per reaction

GENOMELAB™

FROM TISSUES TO TARGETS.

Identify



Isolate



Prepare



Analyze



Evaluate



Validate



Questions?

