THE USE OF THE BEADXPRESS IN DISEASE ASSOCIATION STUDIES

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The Spanish National Genotyping Centre (**CeGen**, **www.cegen.org**), was created in 2004 as an initiative of ***Genoma España***. It provides support to scientists in their genotyping projects in every aspect of research, from **experiment design, DNA extraction, state-of-the-art genotyping, data interpretation, through to statistical analysis**.

CEGEN structure:

Coordination Node (UPF) **(cegencoordination@upf.edu)**
Santiago de Compostela Node (USC)
Madrid Node (CNIO)
Barcelona Node (CRG)

SNP GENOTYPING METHODS

Ideal platform for SNP genotyping:

- high-throughput capacity
- affordable price
- little input material
- automated
- accurate
- high success rate

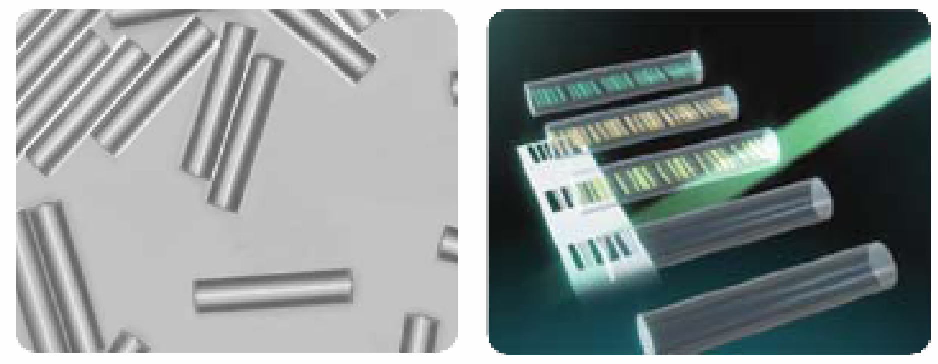
1-10 SNPs	10-384 SNPs	384-1536 SNPs	100.000- 2 M SNPs
TaqMan	**BeadXpress**	**BeadArray**	**BeadArray**
Pyrosequencing	**SNPlex**	Parallel	**Affymetrix**
DASH	**MassArray**		
	SNPstream		
	LabMap		
	Luminex		
	Fluidigm		

Technologies available at CEGEN genotyping nodes

BEADXPRESS AND VERACODE TECHNOLOGY

BeadXpress reader, with a dual-color laser detection system

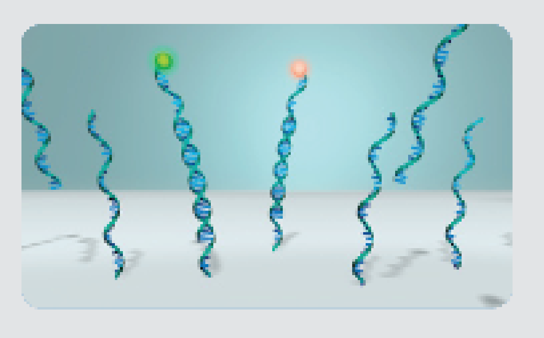
Veracode beads are glass cylindrical microbeads (28 um), with unique holographic codes that diffract the laser beam into multiple components (optical signature for each bead)



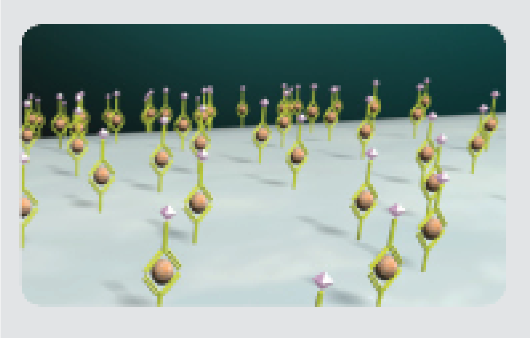
Veracode microbeads are introduced into groove plates and read by a CCD camera within the BeadXpress reader

BEADXPRESS AND VERACODE TECHNOLOGY

Beads with oligonucleotides



Beads with antibodies



VARIOUS SOLUTION-BASED BIOASSAYS:

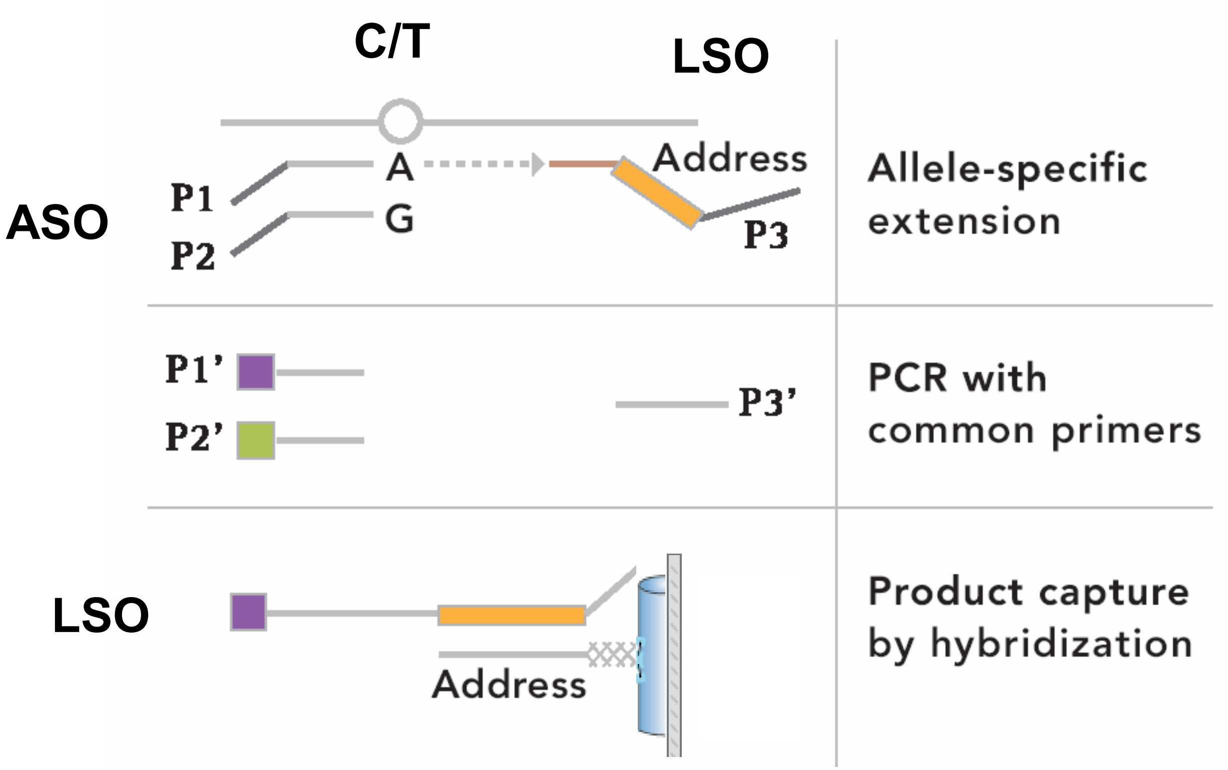
Genotyping
Gene Expression
Protein-based

VARIOUS MULTIPLEXING LEVELS:

48-, 96-, 144-, 192 and 384-plex for SNP genotyping if using GoldenGate chemistry

1- to 144-plex if using Universal Capture Beads (pooling different tubes of uniquely coded beads)

GOLDENGATE ASSAY

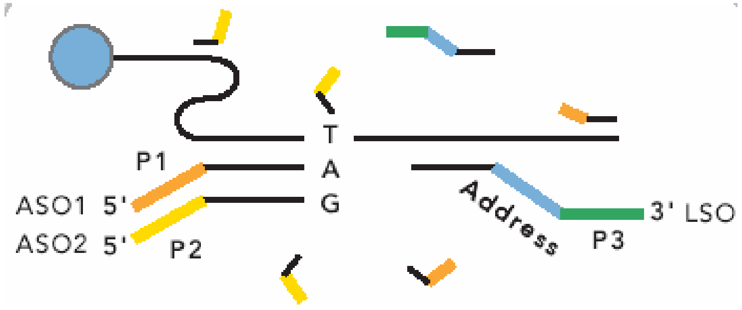


Allele-specific extension

PCR with common primers

Product capture by hybridization

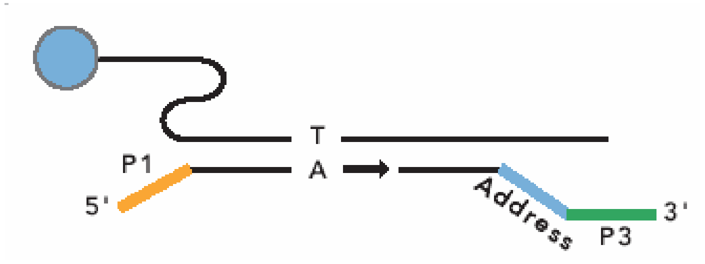
GOLDENGATE ASSAY



DNA activation by adding biotin

The complementary oligos hybridize to the genomic DNA sample

Washes



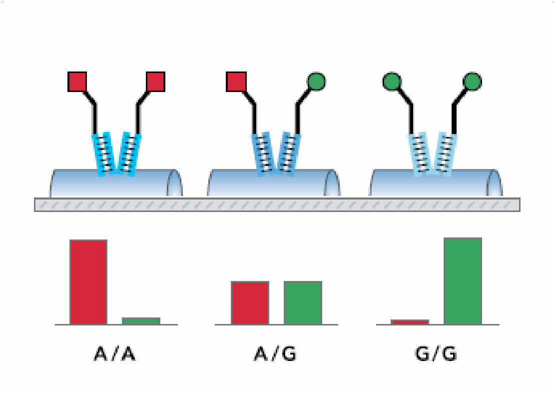
Extend, ligate and clean-up

Universal PCR

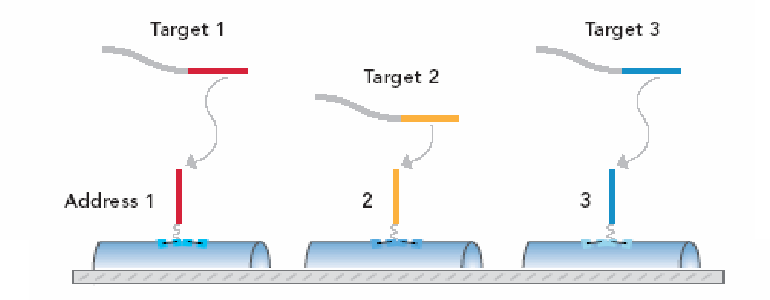
Hybridization occurs prior to amplification and thus no amplification bias is introduced

GOLDENGATE ASSAY

Elute dye-labelled and biotinylated strand



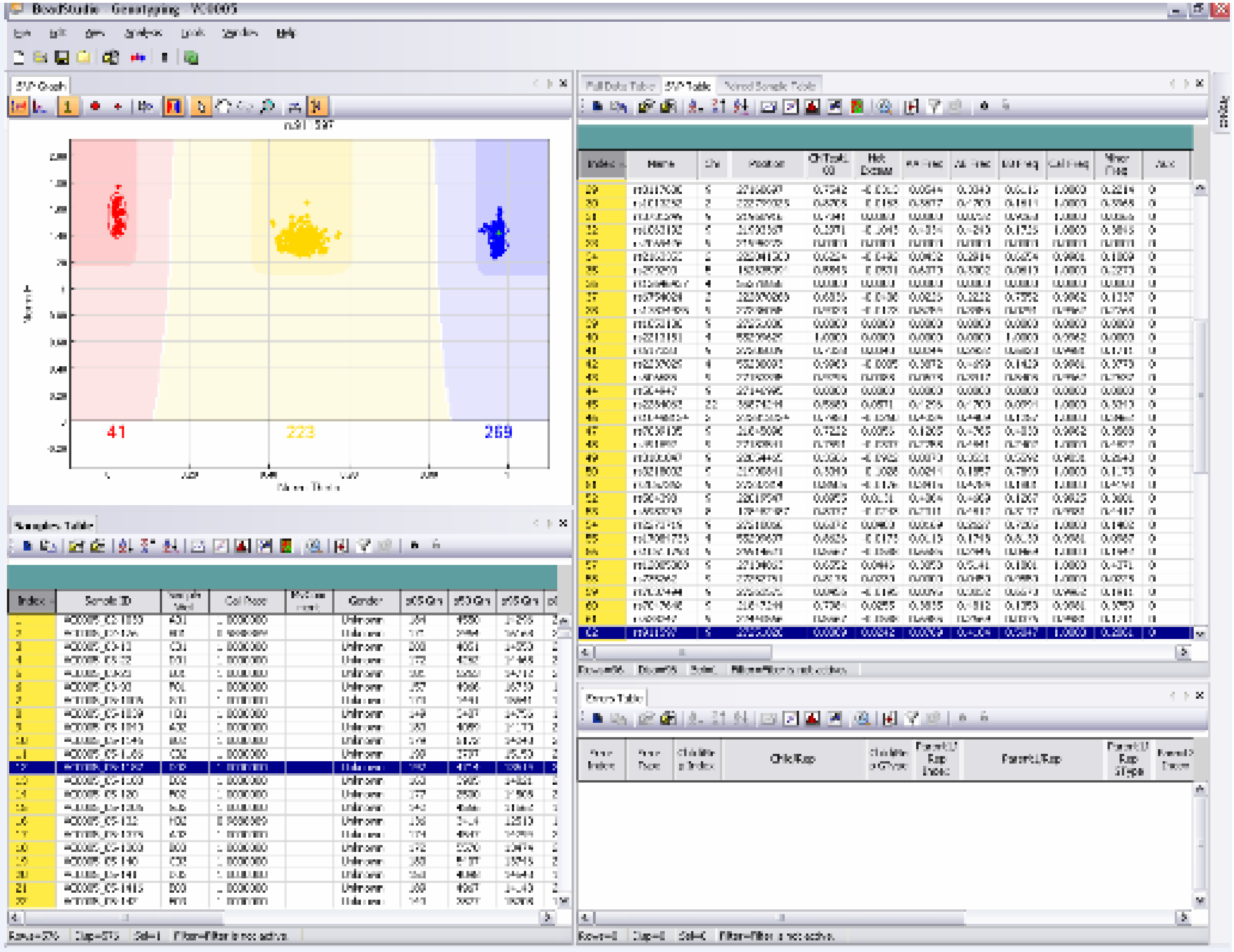
Hybridize to the VeraCode microbeads through the unique address sequence
Analyze fluorescent signal with the scanner



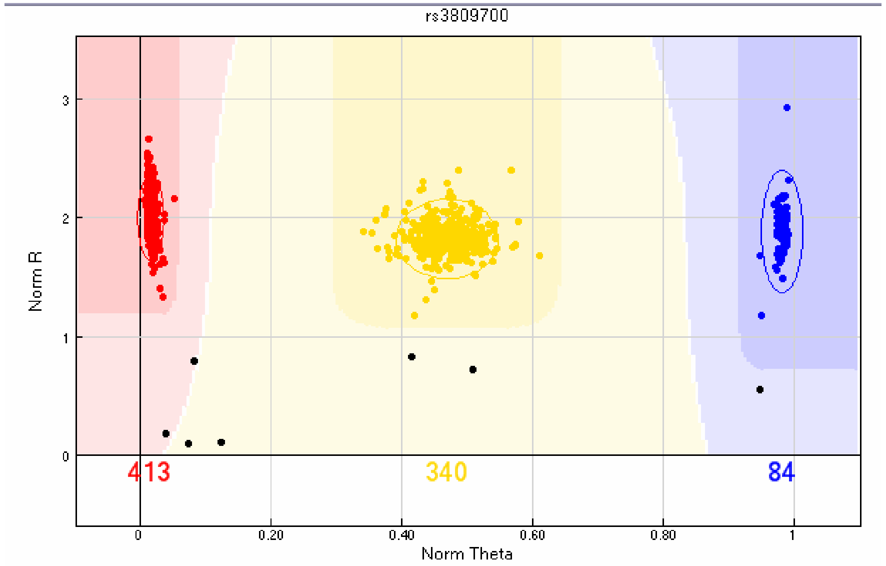
384 different beads, each with a unique address sequence and a unique holographic code

GOLDENGATE ASSAY

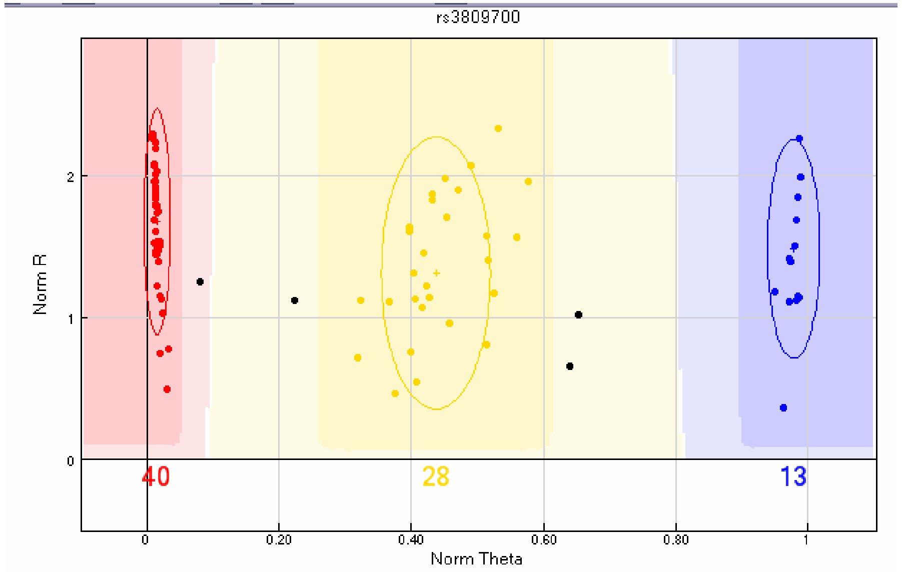
Verascan: to load assays and perform scans
BeadStudio: to analyze data and report



GOLDENGATE ASSAY



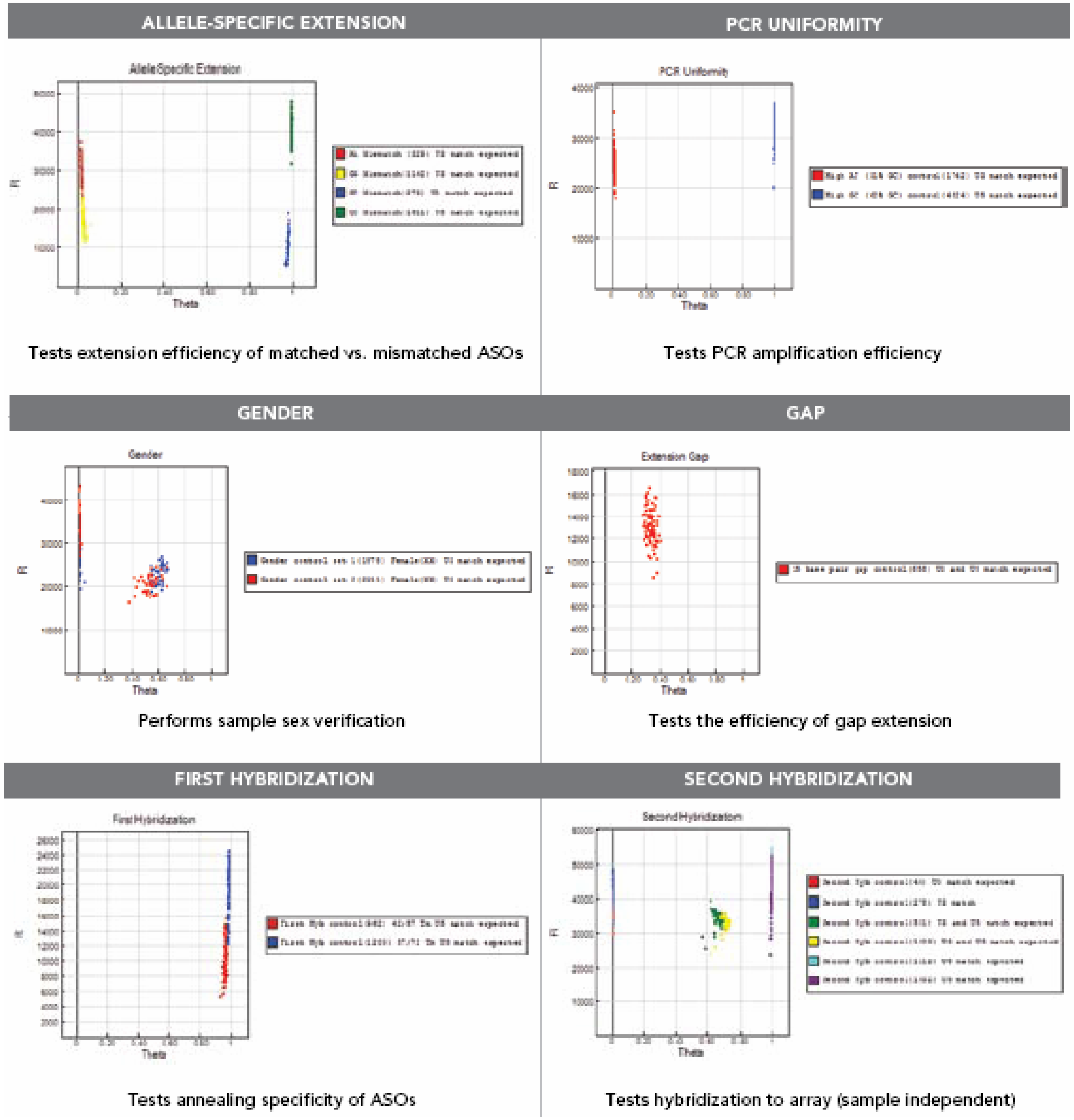
Genomic DNA
(250 ng)



GenomiPhi amplified DNA
(800 ng)

Less compact clusters

GOLDENGATE ASSAY



ALLELE-SPECIFIC EXTENSION

Tests extension efficiency of matched vs. mismatched ASOs

PCR UNIFORMITY

Tests PCR amplification efficiency

GENDER

Performs sample sex verification

GAP

Tests the efficiency of gap extension

FIRST HYBRIDIZATION

Tests annealing specificity of ASOs

SECOND HYBRIDIZATION

Tests hybridization to array (sample independent)

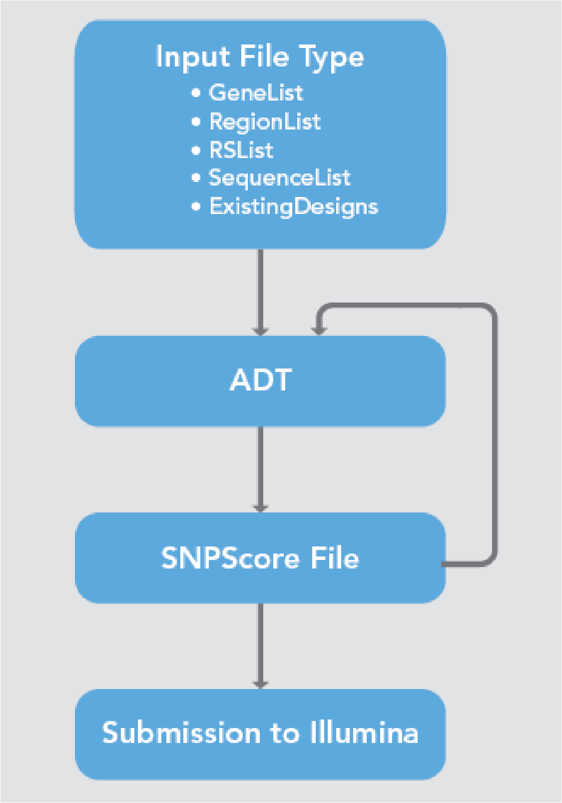
48 assay control to troubleshoot errors

PCR contamination controls (UDG treatment)

Most controls only valid for human samples

BEADXPRESS AND VERACODE TECHNOLOGY

Assay Design Tool (ADT): to create custom SNP panels
Assays scored using sequence information and a database of previous assays
SNP Score: expected success for designing a given assay, 0-1.1 (if GoldenGate validated)



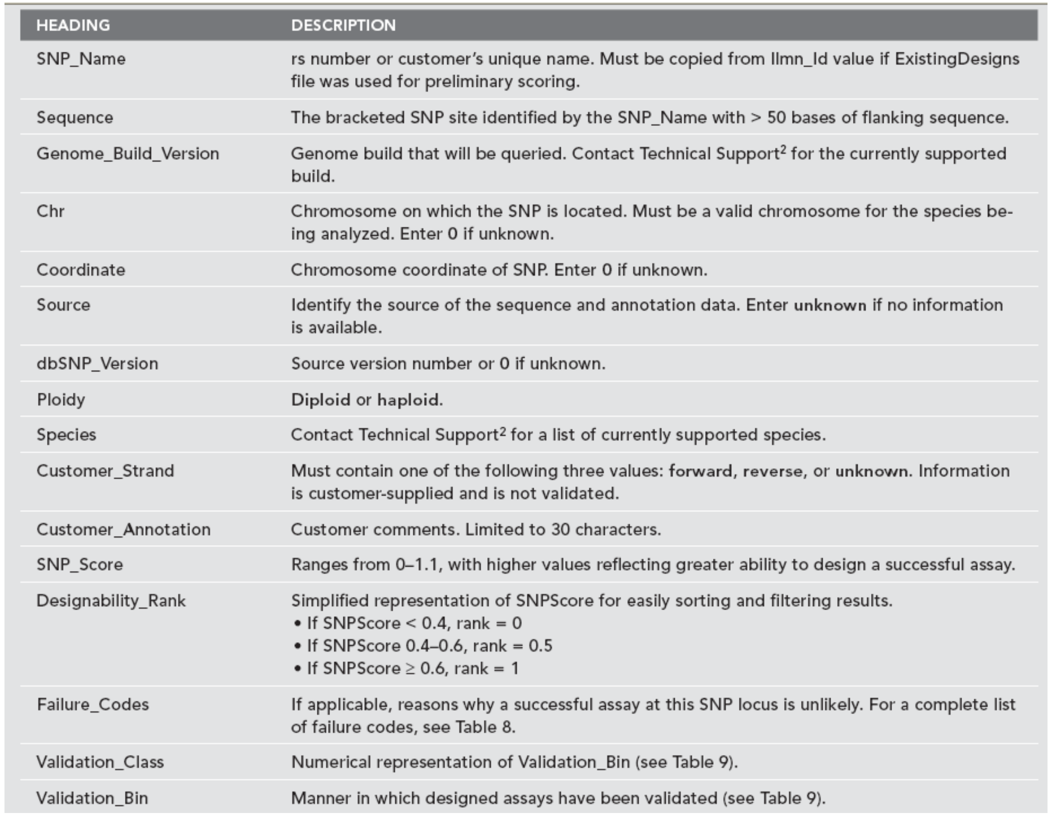
Input File Type

- GeneList
- RegionList
- RSList
- SequenceList
- ExistingDesigns

ADT

SNPScore File

Submission to Illumina



HEADING	DESCRIPTION
SNP_Name	rs number or customer's unique name. Must be copied from Illmn_Id value if ExistingDesigns file was used for preliminary scoring.
Sequence	The bracketed SNP site identified by the SNP_Name with > 50 bases of flanking sequence.
Genome_Build_Version	Genome build that will be queried. Contact Technical Support[2] for the currently supported build.
Chr	Chromosome on which the SNP is located. Must be a valid chromosome for the species being analyzed. Enter 0 if unknown.
Coordinate	Chromosome coordinate of SNP. Enter 0 if unknown.
Source	Identify the source of the sequence and annotation data. Enter **unknown** if no information is available.
dbSNP_Version	Source version number or 0 if unknown.
Ploidy	**Diploid** or **haploid**.
Species	Contact Technical Support[2] for a list of currently supported species.
Customer_Strand	Must contain one of the following three values: **forward**, **reverse**, or **unknown**. Information is customer-supplied and is not validated.
Customer_Annotation	Customer comments. Limited to 30 characters.
SNP_Score	Ranges from 0–1.1, with higher values reflecting greater ability to design a successful assay.
Designability_Rank	Simplified representation of SNPScore for easily sorting and filtering results. • If SNPScore < 0.4, rank = 0 • If SNPScore 0.4–0.6, rank = 0.5 • If SNPScore ≥ 0.6, rank = 1
Failure_Codes	If applicable, reasons why a successful assay at this SNP locus is unlikely. For a complete list of failure codes, see Table 8.
Validation_Class	Numerical representation of Validation_Bin (see Table 9).
Validation_Bin	Manner in which designed assays have been validated (see Table 9).

VeraCode Assay designer: to create custom SNP panels if using Universal Capture Beads (design multiplex assays incorporating capture bead sequences)

SAMPLES QC

>250 ng DNA per OPA (800 ng if GenomiPhi amplified)
Storage buffer (TE: 10 mM Tris, pH7.5; 1 mM EDTA)

Picogreen assay to measure the DNA concentration of samples to be genotyped:
-Measures only dsDNA (no single-stranded DNA, oligonucleotides, RNA, and/or proteins)
-Samples diluted to <1 ng/ul (final concentration in the assay)

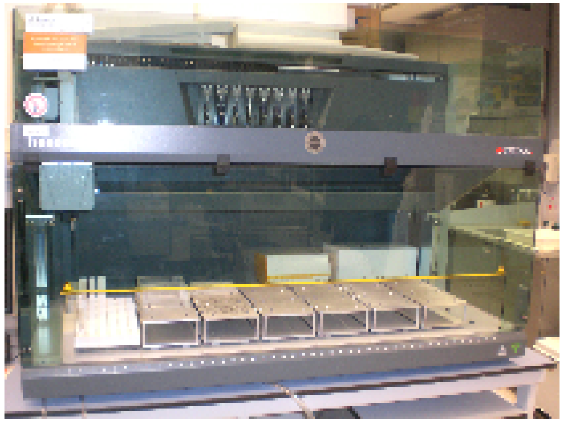
TECAN ROBOT

GEMINI XPS fluorometer

AUTOMATED PROTOCOL

BeadXpress installed on October 2008
Automated protocol

PRE PCR ROBOT



POST PCR ROBOT

BEADXPRESS

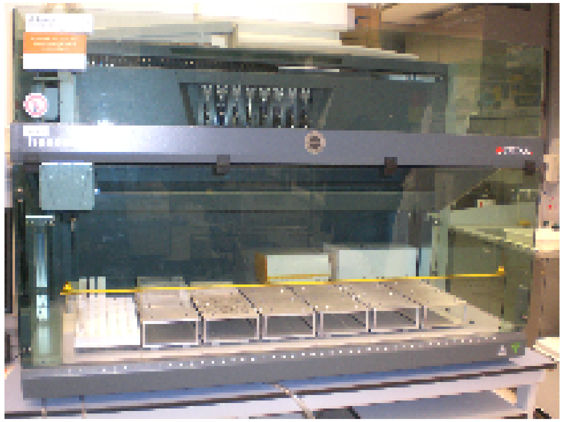
AUTOMATED PROTOCOL

	ROBOT	OTHER	DAY
Make activated DNA	40 min/ plate	40 min incubations	1
Add DNA to oligonucleotides and hybridize	20 min / plate	>2 h incubation	1
Extension and ligation of oligos	45 min / plate	15 min incubation	1
Universal PCR	50 min/ plate	2 h 45 min PCR	1
Bind PCR products and elute dye-labeled strand	15 min/ plate	1 h incubation	2
Hybridize to Veracode BeadPlate	45 min/ plate	3 h incubation	2
Wash Veracode BeadPlate	20 min/ plate		2
Scan the Veracode BeadPlate	1 h 10 min / plate[1] 3 h 30 min / plate[2]		2-3

[1] If 96plex
[2] If 384plex

AUTOMATED PROTOCOL

1 PRE PCR ROBOT



1 POST PCR ROBOT

1 BEADXPRESS

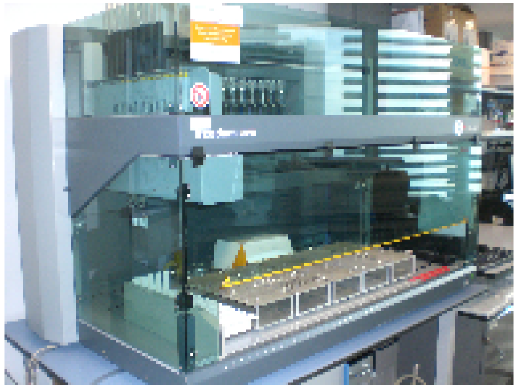
15 plates (1440 samples) per week, 2 technicians

	Monday	Tuesday	Wednesday	Thursday	Friday
PrePCR	3 plates	3 plates	3 plates	3 plates	3 plates
PostPCR	4 plates	4 plates	4 plates	3 plates	
Scanning		4 plates	4 plates	4 plates	3 plates

AUTOMATED PROTOCOL

2 PRE PCR ROBOTS

POST PCR ROBOT



BEADXPRESS

20 plates per week (1920 samples), 2 technicians

	Monday	Tuesday	Wednesday	Thursday	Friday
PrePCR	5 plates	5 plates	5 plates		5 plates
PostPCR	5 plates	5 plates	5 plates	5 plates	
Scanning		5 plates	5 plates	5 plates	5 plates

Protocol will be reduced in time soon (one day)

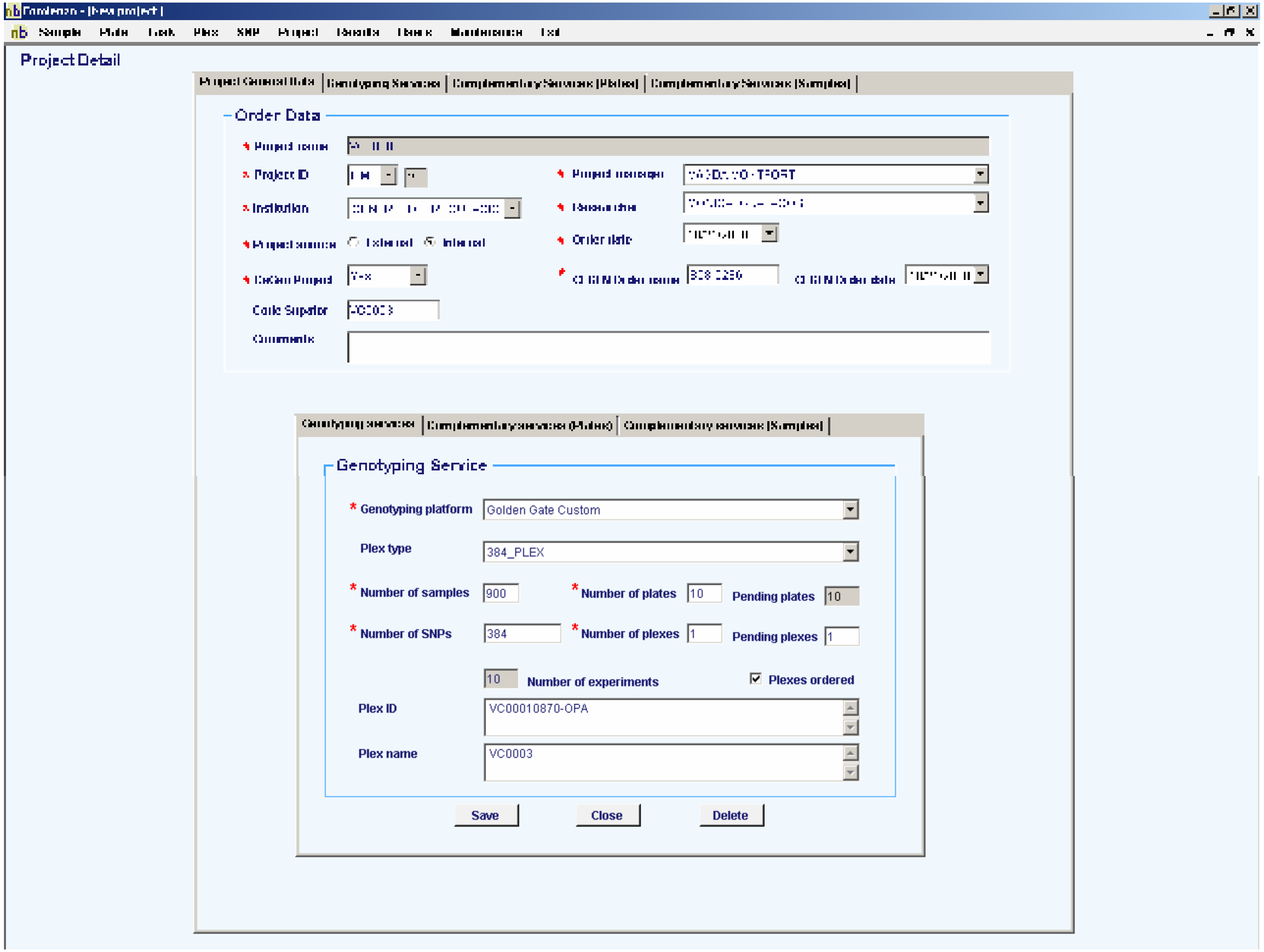
BEADXPRESS AND VERACODE TECHNOLOGY

>100 plates processed, 10,000 samples

Project Name	Nº SNPs	Samples	Plates	Failed SNP	SNP succes rate	Call rate
VC0001	96	900	10	9	90.6%	99.8%
VC0002	96	2,300	25	1	99.0%	99.3%
VC0003	96	1,564	17	5	94.8%	99.8%
VC0004	144	361	4	7	95.1%	95.8%
VC0005	96	2,753	30	3	96.9%	99.9%
VC0006	192	913	10	14	92.7%	98.9%
VC0007	144	453	5	4	97.2%	99.7%
VC0008	192	4,040	44	ongoing	ongoing	ongoing

LIMS

Laboratory Management Information System (LIMS): Oracle database, Linux server



Project name [illegible]

Project ID [illegible]

Project manager [illegible]

Institution [illegible]

Researcher [illegible]

Project source ○ External ● Internal

Order date [illegible]

CeGen Project [illegible]

CEGEN Order name [illegible]

CEGEN Order date [illegible]

Comments

* Genotyping platform: Golden Gate Custom

Plex type: 384_PLEX

* Number of samples: 900

* Number of plates: 10

Pending plates: 10

* Number of SNPs: 384

* Number of plexes: 1

Pending plexes: 1

10 Number of experiments

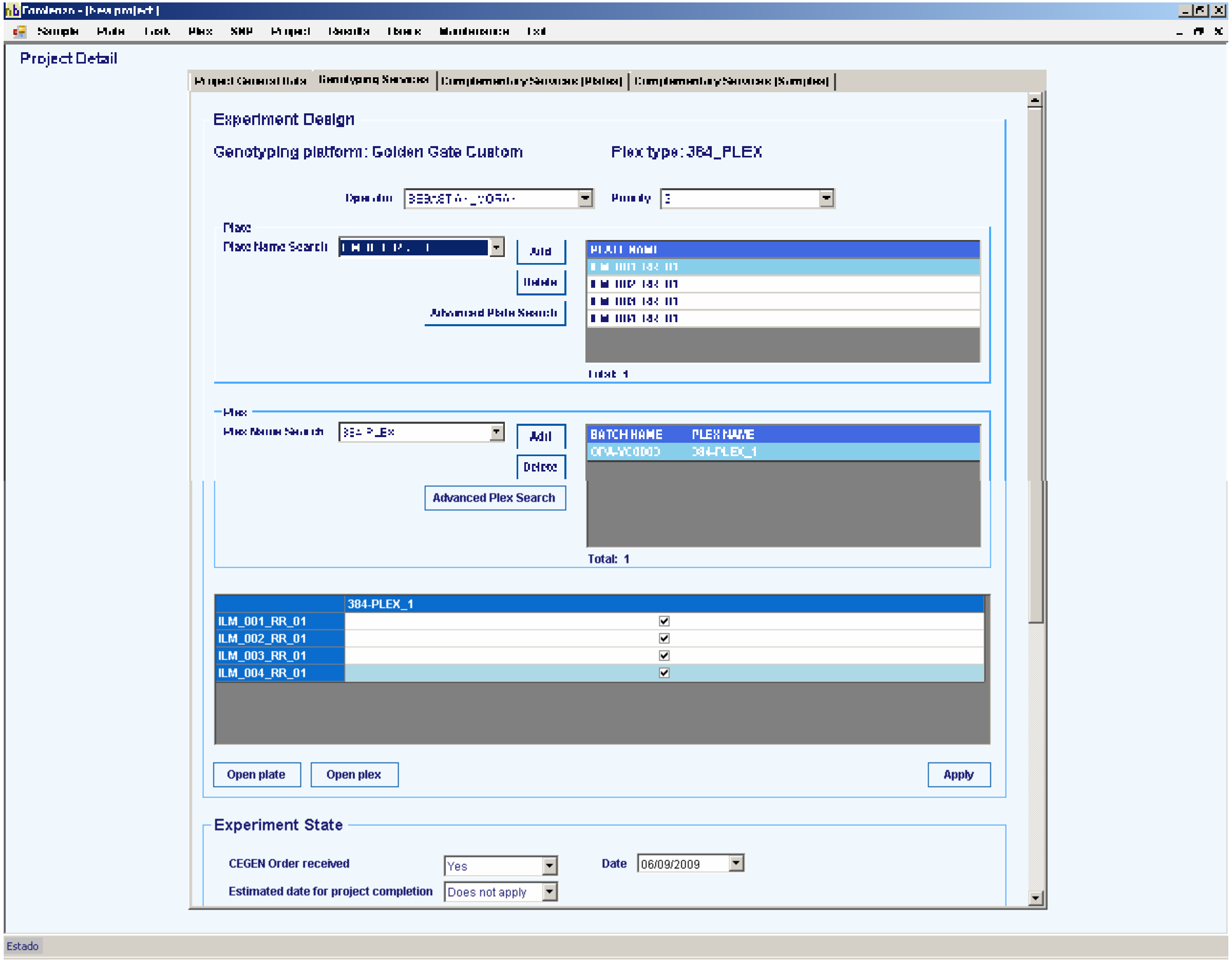
☑ Plexes ordered

Plex ID: VC00010870-OPA

Plex name: VC0003

LIMS

Experiment design: define plates and OPAs



Project Detail

Experiment Design

Genotyping platform: Golden Gate Custom

Plex type: 384_PLEX

Total: 1

	384-PLEX_1
ILM_001_RR_01	☑
ILM_002_RR_01	☑
ILM_003_RR_01	☑
ILM_004_RR_01	☑

Experiment State

CEGEN Order received: Yes

Date: 06/09/2009

Estimated date for project completion: Does not apply

LIMS

Plate and samples: ID (barcode) and user name
DNA Concetntration, Plate rearrangement, Plate export

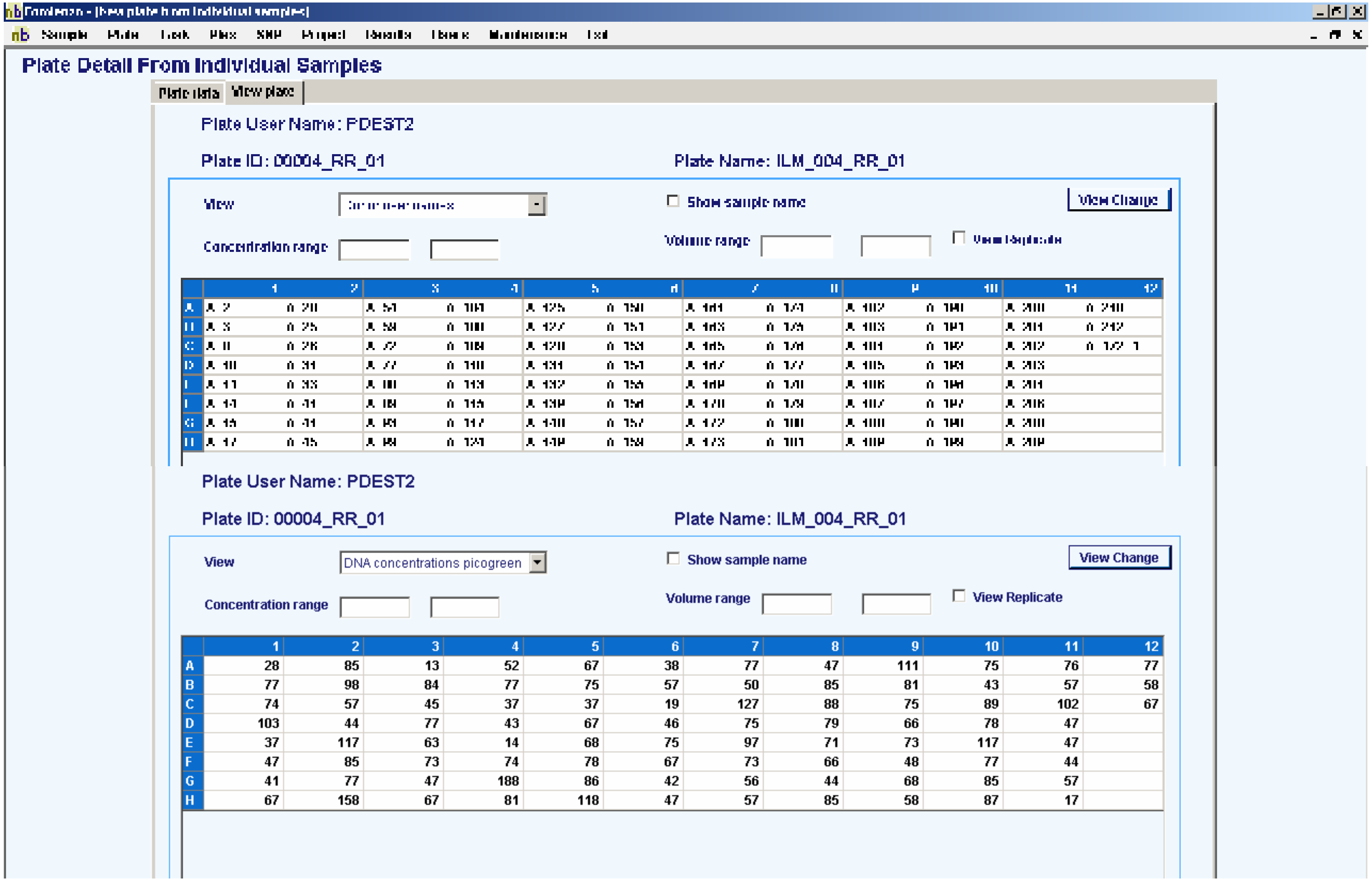


Plate User Name: PDEST2

Plate ID: 00004_RR_01

Plate Name: ILM_004_RR_01

Plate User Name: PDEST2

Plate ID: 00004_RR_01

Plate Name: ILM_004_RR_01

View: DNA concentrations picogreen

☐ Show sample name

Concentration range:

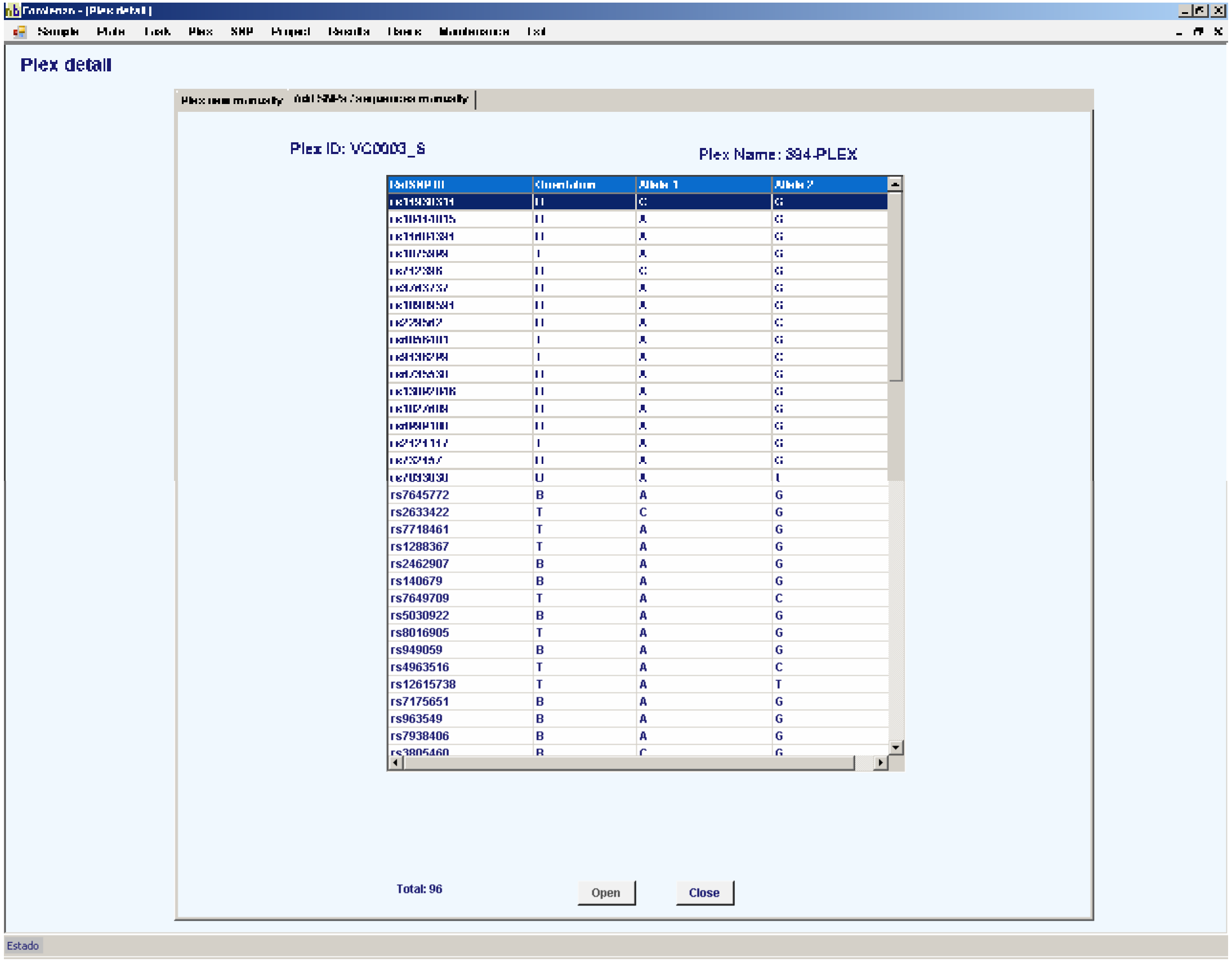
Volume range:

☐ View Replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	28	85	13	52	67	38	77	47	111	75	76	77
B	77	98	84	77	75	57	50	85	81	43	57	58
C	74	57	45	37	37	19	127	88	75	89	102	67
D	103	44	77	43	67	46	75	79	66	78	47	
E	37	117	63	14	68	75	97	71	73	117	47	
F	47	85	73	74	78	67	73	66	48	77	44	
G	41	77	47	188	86	42	56	44	68	85	57	
H	67	158	67	81	118	47	57	85	58	87	17	

LIMS

OPA: list of SNPs with strand orientation



Plex detail

Plex ID: VC0003_S

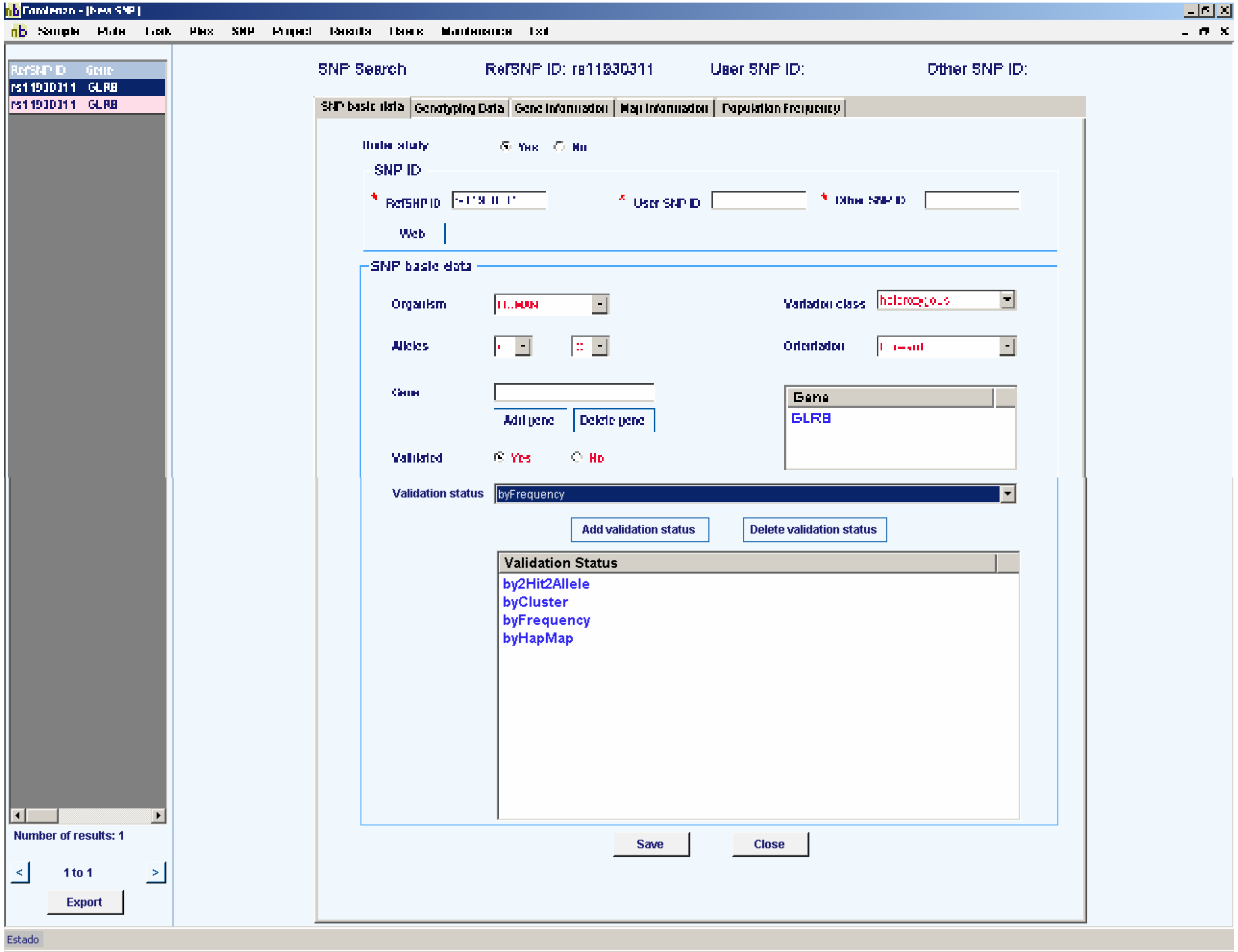
Plex Name: 384-PLEX

dbSNP ID	Orientation	Allele 1	Allele 2
[illegible]	B	C	G
[illegible]	B	A	G
[illegible]	B	A	G
[illegible]	T	A	G
[illegible]	B	C	G
[illegible]	B	A	G
[illegible]	B	A	G
[illegible]	B	A	C
[illegible]	T	A	G
[illegible]	T	A	C
[illegible]	B	A	G
[illegible]	B	A	G
[illegible]	B	A	G
[illegible]	B	A	G
[illegible]	T	A	G
[illegible]	B	A	G
[illegible]	B	A	T
rs7645772	B	A	G
rs2633422	T	C	G
rs7718461	T	A	G
rs1288367	T	A	G
rs2462907	B	A	G
rs140679	B	A	G
rs7649709	T	A	C
rs5030922	B	A	G
rs8016905	T	A	G
rs949059	B	A	G
rs4963516	T	A	C
rs12615738	T	A	T
rs7175651	B	A	G
rs963549	B	A	G
rs7938406	B	A	G
rs3805460	B	C	G

Total: 96

LIMS

SNP information from dbSNP



RefSNP ID	Gene
rs11930311	GLRB
rs11930311	GLRB

Number of results: 1

SNP Search RefSNP ID: rs11930311 User SNP ID: Other SNP ID:

SNP ID

RefSNP ID

User SNP ID

Other SNP ID

Web

SNP basic data

Organism

Variation class

Alleles

Orientation

Gene

Gene
GLRB

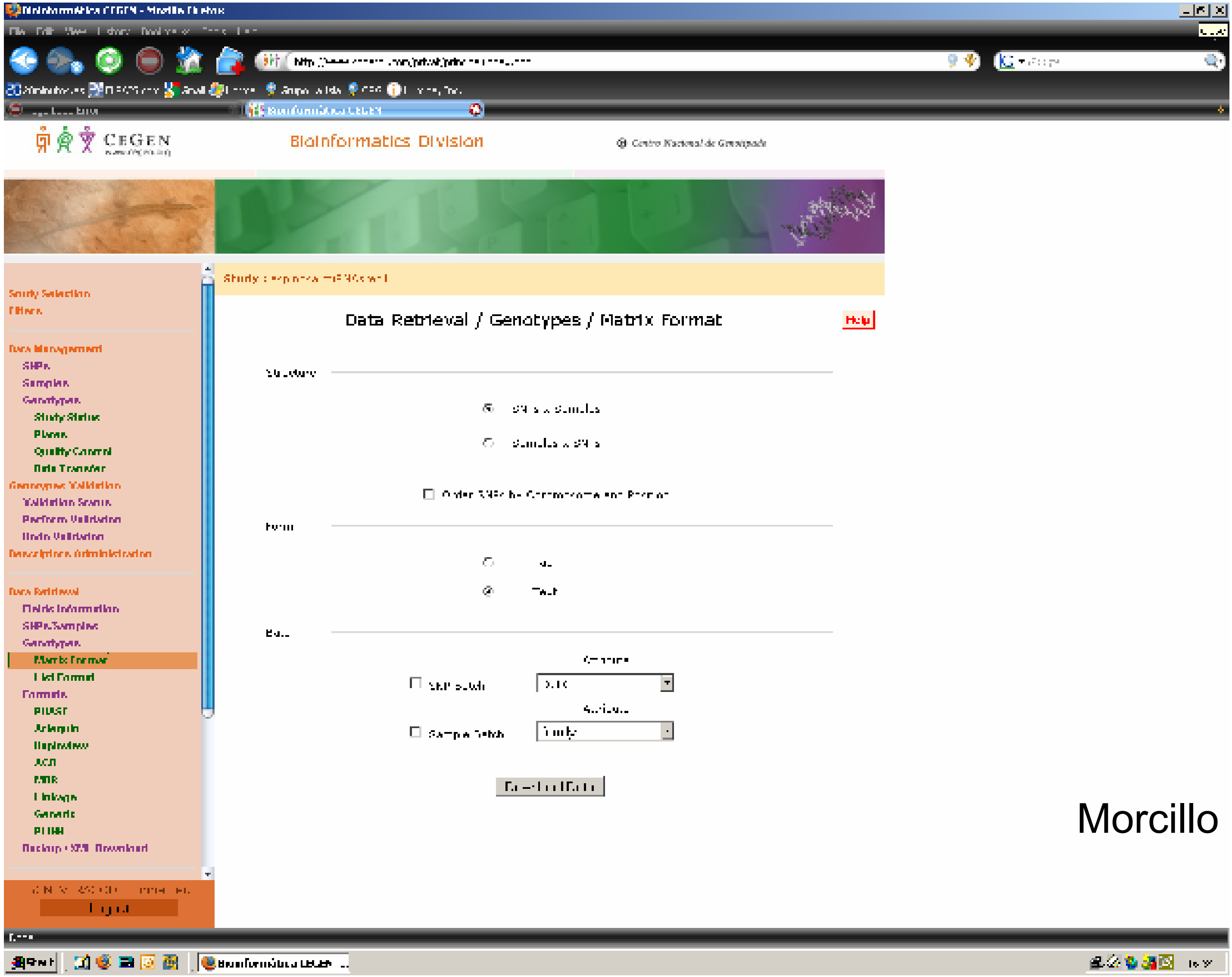
Validated ● Yes ○ No

Validation status byFrequency

Validation Status
by2Hit2Allele
byCluster
byFrequency
byHapMap

SNPator

SNPator: web-based application to deliver results to users



Morcillo et al 2008

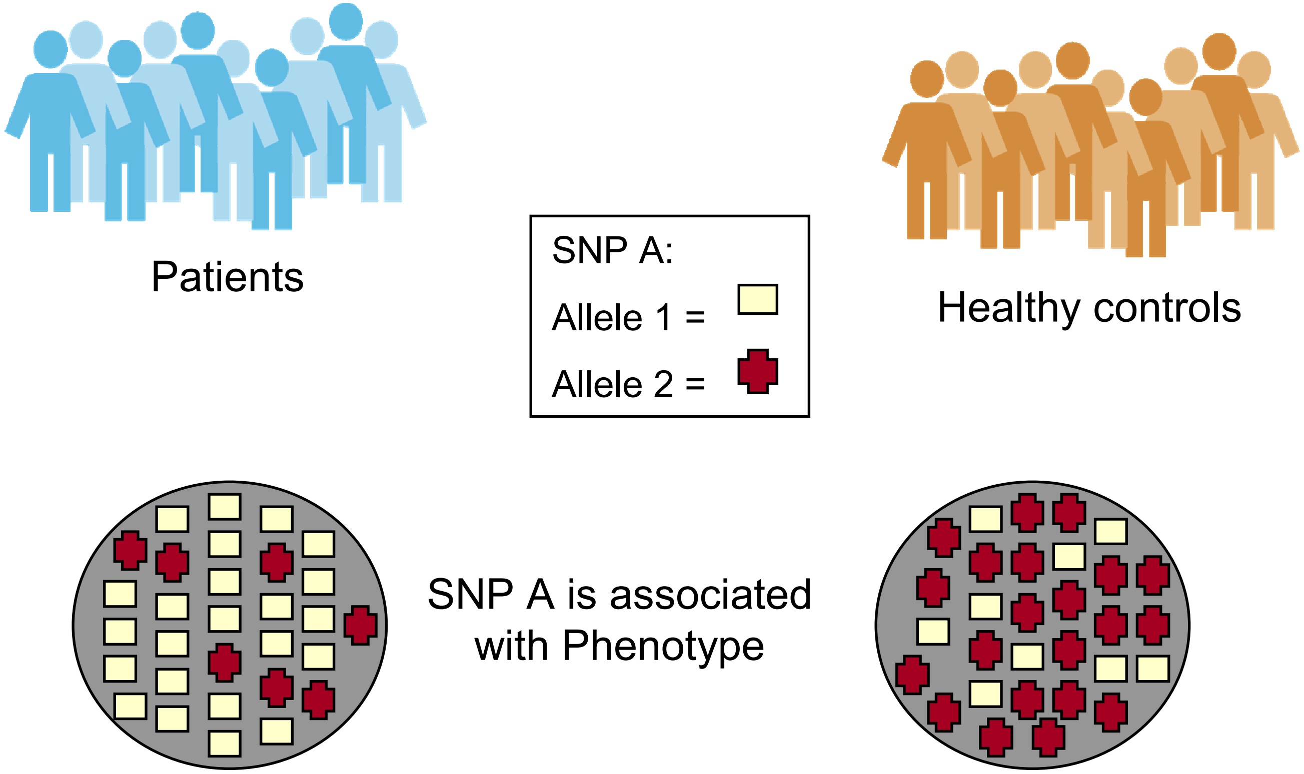
	rs10068039	rs10252922	rs10413657	rs10512391	rs1057558	rs1057559	rs10753142	rs10911111	rs11090910	rs11090911	rs11249664
A_10	CT	AA	GG	AA	CT	CC	TT	AA	TT	CC	CT
A_104	CT	GG	AG	AG	TT	CC	AT	AA	TT	CC	TT
A_108	CT	AG	GG	AG	CT	CC	AT	AG	TT	CC	CT
A_109	CC	AA	GG	AG	TT	CC	AA	AA	CT	CC	TT
A_11	CT	GG	GG	AG	TT	CC	AT	AG	CT	CC	CT
A_110	TT	GG	AG	AA	TT	CC	AA	AA	TT	CC	CC
A_113	CC	AG	AA	AA	CT	CC	AT	AG	CT	CC	TT
A_115	CT	AG	GG	AA	TT	CC	AA	AA	TT	CC	TT
A_117	TT	AG	AG	AG	TT	CC	AT	AG	TT	CC	CC
A_124	CT	GG	GG	AG	TT	CC	AT	AG	TT	CC	CT
A_125	CT	AA	GG	GG	CT	CC	AT	AA	CC	CC	TT
A_127	CT	AA	GG	AG	TT	CC	AA	AG	CT	CC	CT
A_128	CT	AG	AG	AA	TT	CC	AT	AA	TT	CC	CT
A_131	CT	AA	GG	AA	CT	CC	TT	AG	CT	CC	CT
A_132	CT	GG	GG	AG	TT	CC	TT	AG	TT	CC	CT
A_139	CT	AA	GG	AG	TT	CC	AT	AG	CC	CC	CT
A_14	CC	AG	GG	AG	CT	CC	TT	AA	TT	CC	TT
A_148	TT	AA	AG	AA	CC	CC	AA	AA	CT	CC	CT
A_149	TT	AG	GG	AA	TT	CC	TT	AA	TT	CC	CC
A_15	CT	GG	GG	AG	TT	CC	AT	AA	TT	CC	CT

SNPator

```
Plates     : PDEST1,PDEST2,PDEST3,PDEST4,PDEST5,PDEST6,PDEST7
Study      : VC0003
User       : Mbayés
Date       : 2008-09-16 12:03:04
Technology : Veracode
Source     : PDEST Genotypes.txt


Final Report
---------------------------------------------------------------------

Genotypes ....
---------------------------------------------------------
Total genotypes tested          : 57408
Unique genotypes                : 54870 (95.5%)
Empty genotypes                 : 1794 (3.1%)
Concordant repetitions          : 558 (100%)
Non concordant repetitions      : 0 (0%)


SNPs ....
---------------------------------------------------------
SNPs with more than two alleles  : 0 (0.00%)
Total SNPs                       : 96

Samples ....
---------------------------------------------------------
Total samples                    : 598
```

BEADXPRESS GENOTYPING APPLICATIONS: ASSOCIATION STUDIES

ASSOCIATION STUDIES: to identify DNA variants that predispose individuals to common multifactorial disorders or that contribute to differential drug response among individuals.



Patients

Healthy controls

SNP A:

Allele 1 = 🟨

Allele 2 = ✚

SNP A is associated
with Phenotype

BEADXPRESS GENOTYPING APPLICATIONS

ASSOCIATION STUDIES

Candidate gene studies (10-20 genes in a 384_plex)

Fine mapping of Genome Wide Association Studies (GWAS) or linkage studies

Independent replication of associations: follow-up studies to eiminate false positives, to extend the findings to diverse populations (ethnicity, environmental exposures)

OTHERS

Tracing evolutionary history of different populations

Diagnostics

Forensics

Selection of traits of interest for agricultural, cattle farming or aquiculture applications

ADHD (Attention-deficit/hyperactivity disorder)

SYMPTOMS

Inattention

Hyperactivity

Impulsiveness

PREVALENCE

6-8% children

3-5% adults

More prevalent in males

SUBTYPES

Inattentive (20-30%)

Hyperactive-impulsive (<15%)

Combined (50-75%)

COMPLEX DISORDER

Heretability is 76% from twin studies

2-8 fold Increased risk for ADHD in first degree relatives of patients with ADHD

ADHD: CANDIDATE GENES AND SNPs

Serotoninergic system

5HT1A, 5HT1B, 5HT1D, 5HT1E, 5HT1F, 5HT2A, 5HT2B, 5HT2C, 5HT3A, 5HT3B, 5HT4, 5HT5A, 5HT6, 5HT7,SLC6A4, TPH1, DDC, MAOA, MAOB

Neurotrophins and related genes

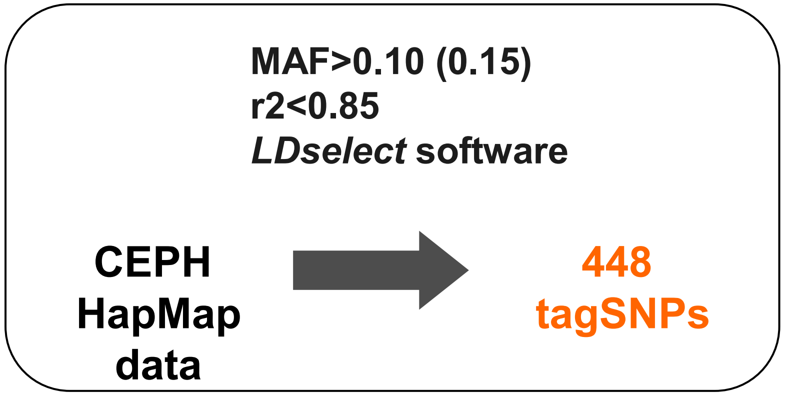
NGF, BDNF, NTF3, NTF4/5, CNTF, NTRK1, NTRK2, NTRK3, NGFR, CNTFR

Differentially expressed genes between left and right human embryonic cortex

BAIAP2, DAPPER1, ID2, LMO4, NEUROD6, ATP2B3

Dopaminergic system

DRD1, DRD2, DRD3, DRD4, DRD5, TH, COMT, DBH, SLC6A3



MAF>0.10 (0.15)
r2<0.85
***LDselect* software**

CEPH HapMap data

448 tagSNPs

ADHD: A CASE-CONTROL APPROACH

600 ADHD cases (**DSMIV**, 78% males, 22% females)

380 children (average age at assessment: **9.18 years** +/-2.56)
- combined ADHD (73%)
- inattentive ADHD (22%)
- hyperactive-impulsive ADHD (5%)

220 adults (average age at assessment: **29.86 years** +/-11.86)
- combined ADHD (67%)
- inattentive ADHD (29.3%)
- hyperactive-impulsive ADHD (3.7%)

700 controls

Caucasoid unrelated adult subjects
w/o DSM-IV ADHD symptoms
Matched for sex the clinical group
Average age: **42.38 years** +/-13.6

ADHD: SIGNIFICANT RESULTS AFTER 10% FDR CORRECTION

	Adults			Children		
Haplotypes	**Cases (N=216)**	**Controls (N=546)**	**Haplotype-specific P-value; OR (CI)**	**Cases (N=330)**	**Controls (N=546)**	**Haplotype-specific P-value; OR (CI)**
DDC						
C A A T	17.3%	8.8%	0.00053; **2.17** (1.42-3.33)	15.5%	8.8%	0.0017; **1.90** (1.27-2.84)
CNTFR						
C G C	42.3%	34.6%	0.0077; **1.38** (1.10-1.74)	42.7%	34.6%	9.1e-04; **1.40** (1.15-1.72)

Common susceptibility factors being involved in both adulthood and childhood ADHD

First genetic evidence that supports the diagnostic continuity of ADHD throughout lifespan

Ribasés et al Biol Psychiatry (2008)
Ribasés et al Mol Psychiatry (2009)

ADHD: SIGNIFICANT RESULTS AFTER 10% FDR CORRECTION

	Adults			Children		
Haplotypes	**Cases (N=216)**	**Controls (N=546)**	**Haplotype-specific P-value; OR (CI)**	**Cases (N=330)**	**Controls (N=546)**	**Haplotype-specific P-value; OR (CI)**
MAOB						
C G C	28.2%	17.1%	0.0029; **1.90** (1.28-2.82)			
BAIAP2						
A G C C T	16%	8.1%	1.7e-04; **2.13** (1.43-3.17)			
NEUROD6						
C T	13%	8.3%	0.011 ; **1.66** (1.16-2.39)			

MAOB, BAIAP2 and NEUROD6 genes may be involved in the persistence of the disorder through lifespan

BAIAP2 and NEUROD6 specifically associated with performance deficits in neuropsychological tasks (Conners Continuous Performance Test)

Ribasés et al Mol Psychiatry (2009)
Ribasés et al Biol Psychiatry (in press)

ADHD: SIGNIFICANT RESULTS AFTER 10% FDR CORRECTION

	Adult combined ADHD			Child combined ADHD		
Haplotypes	**Cases (N=216)**	**Controls (N=546)**	**Haplotype-specific P-value; OR (CI)**	**Cases (N=546)**	**Controls (N=422)**	**Haplotype-specific P-value; OR (CI)**
5HT2A						
A T T				25.1%	34.3%	0.0032 ; **1.56** (1.16-2.08)&
G C C	35.3%	25.1%	0.0036; **1.63** (1.17-2.25)	33.4%	25.1%	0.0084; **1.49** (1.12-1.97)
G C T	20.2%	34.2%	2.39e-05; **2.05** (1.42-2.96)&			

Association between a haplotype in the 5HT2A gene ONLY with the combined ADHD subtype, both in adults and children, suggesting the existence of some differential genetic components in different ADHD subtypes

Ribasés et al Mol Psychiatry (2009)

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