

Marker Lab Service for HRDC members

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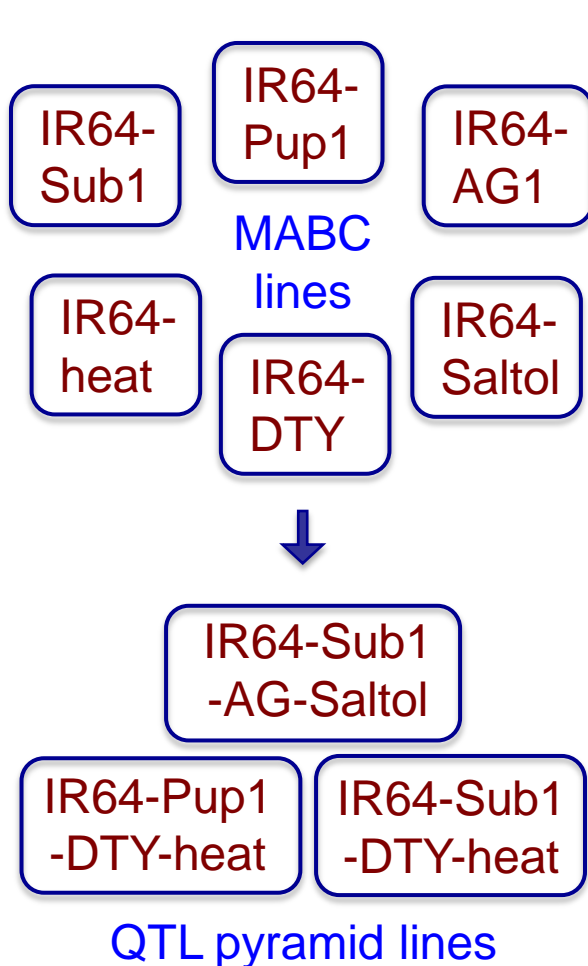
2014 HRDC Annual Meeting, IRRI

19 March 2014

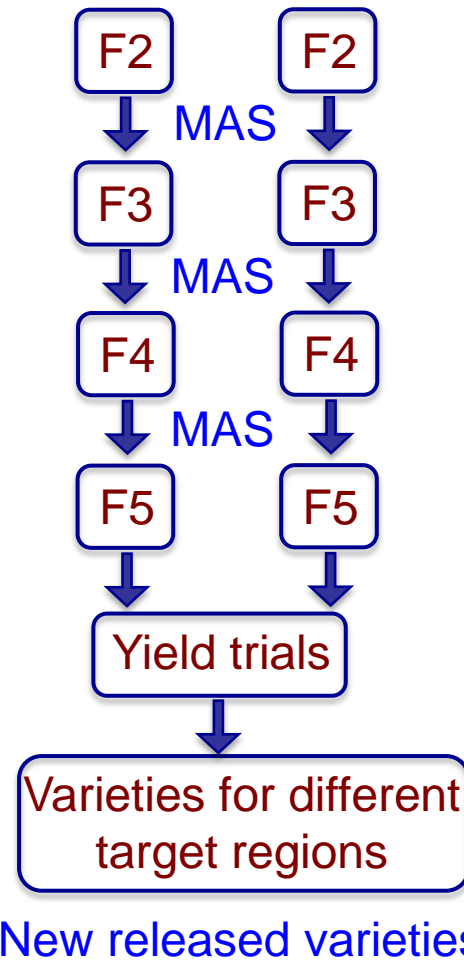


Integrating markers into breeding programs

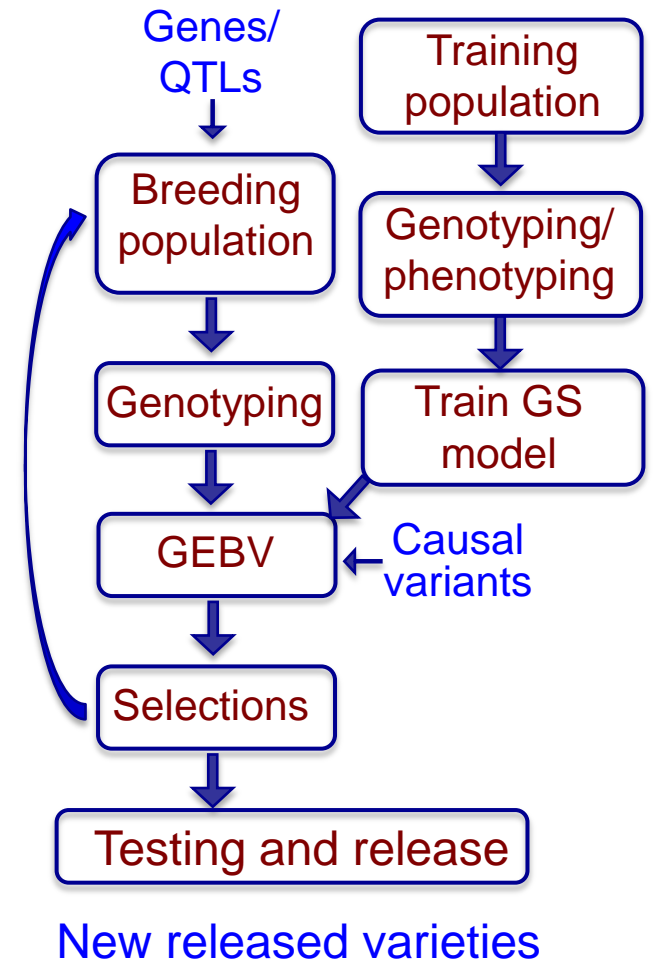
MABC and QTL pyramiding



MAS in the pedigree breeding programs



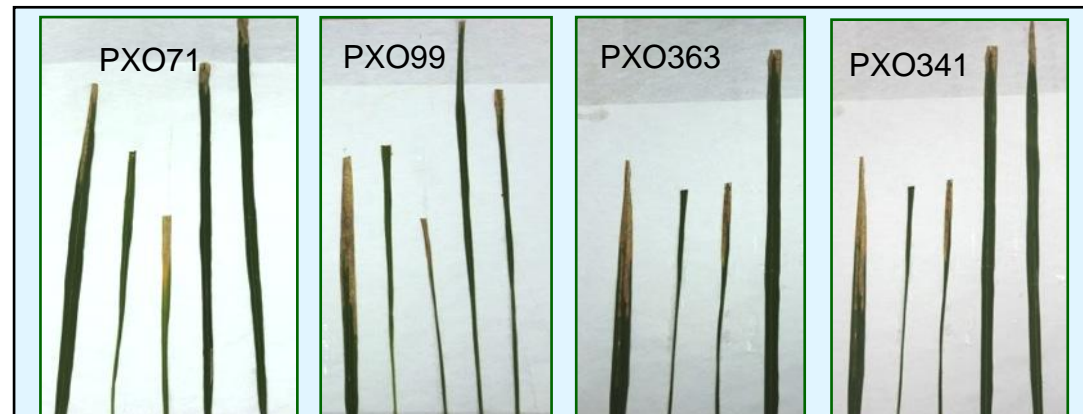
Genomic selection



Using major QTLs/genes for breeding



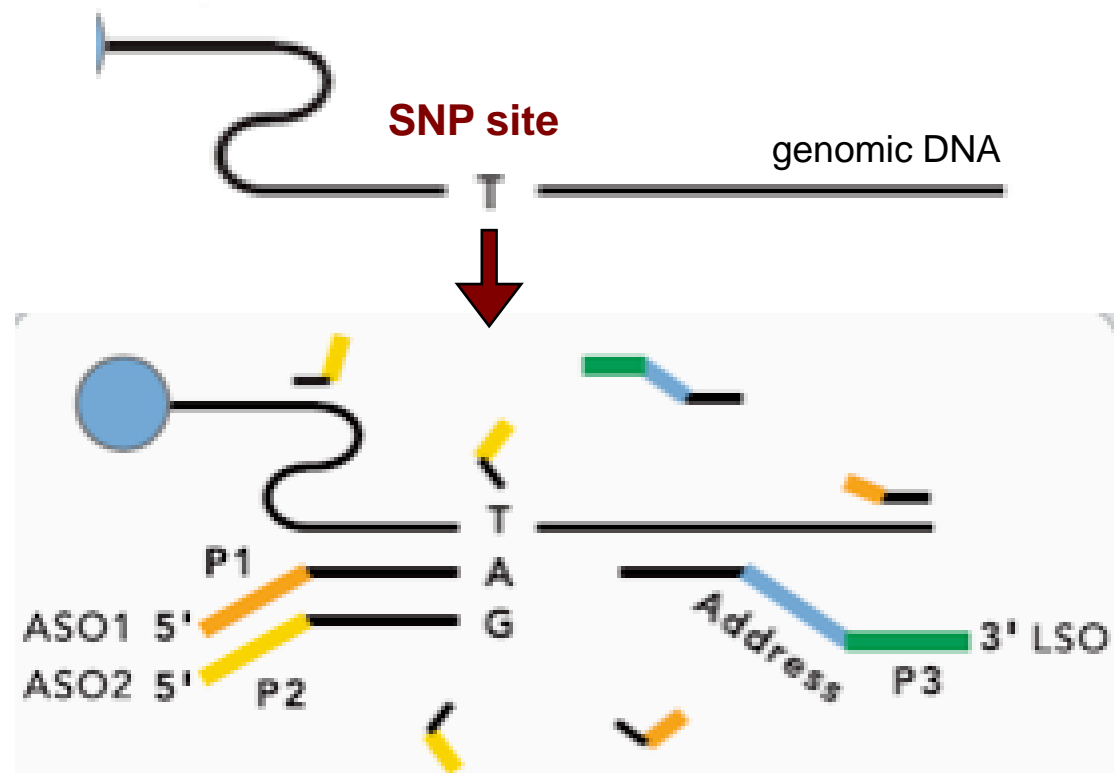
- QTLs and major genes for stress tolerance and disease resistance are known
- Flanking SSR/STS markers are being replaced with SNP markers for more efficient selection



Xa genes for bacterial leaf blight

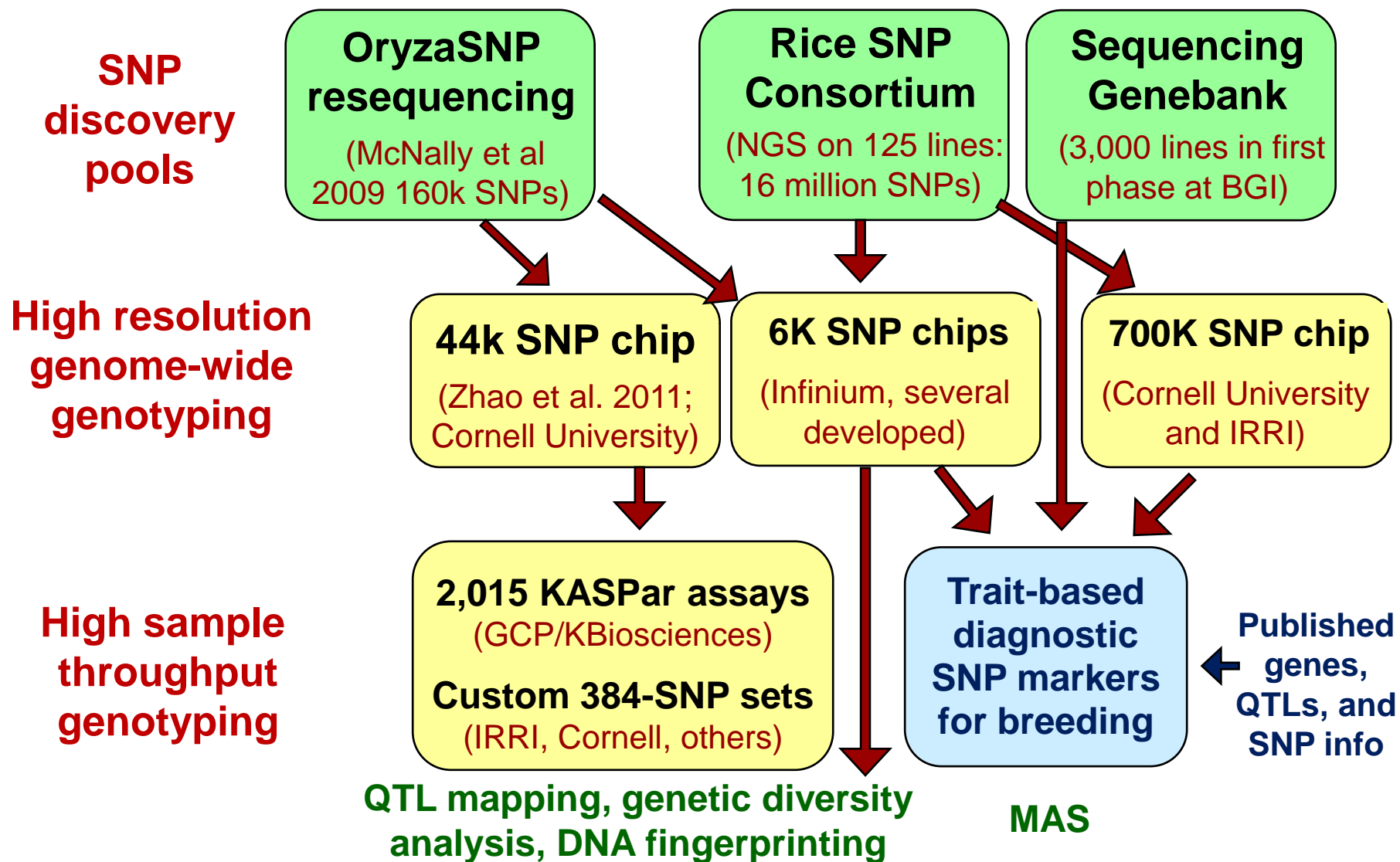
Advantages of SNP markers

- Millions of SNP loci across the genome
- Most SNP markers are bi-allelic
- SNP data can be easily merged in a database
- Rapid high-throughput SNP genotyping systems are available
- SNP haplotypes can track specific alleles



Single nucleotide polymorphism (SNP) marker

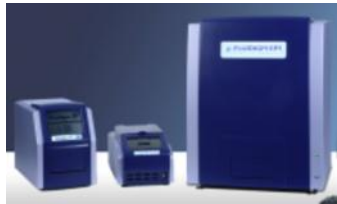
Resources for SNP development in rice



SNP genotyping platforms for breeding applications



Fragment Analyzer
or KASPar/TaqMan
SSRs/indels or SNPs



Fludigm
24 and 96 SNPs



BeadXpress
384 SNPs



Infinium
6K SNP chip
5,000 SNPs



GBS
10k-30k SNPs

Trait-based MAS,
Fine-mapping,
Rapid QC scans,
Background MABC,
Diversity analysis,
QTL mapping,
SNP fingerprinting,
High-resolution
mapping,
Genomic selection

Workflow for the IRRI Genotyping Services Lab

Tissue Preparation:

Leaf sampling in 96 well format



DNA extraction:

Automated magnetic bead system



QC check:

Check DNA quality and concentration



SNP genotyping:

Custom Fluidigm and Infinium sets of 24 – 4,800 SNPs



Data storage & analysis:

SNP database/tools

Automated DNA extraction



Tissue harvesting in 96 well format

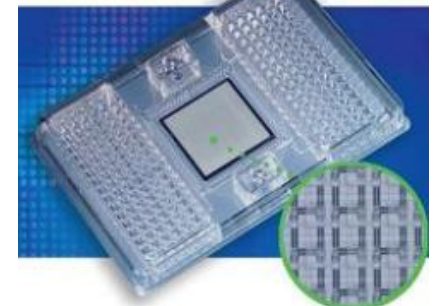


DNA quality control



Infinium 6K chip

Fluidigm
24 or 96 SNPs



Automated leaf sampling with PlantTrak

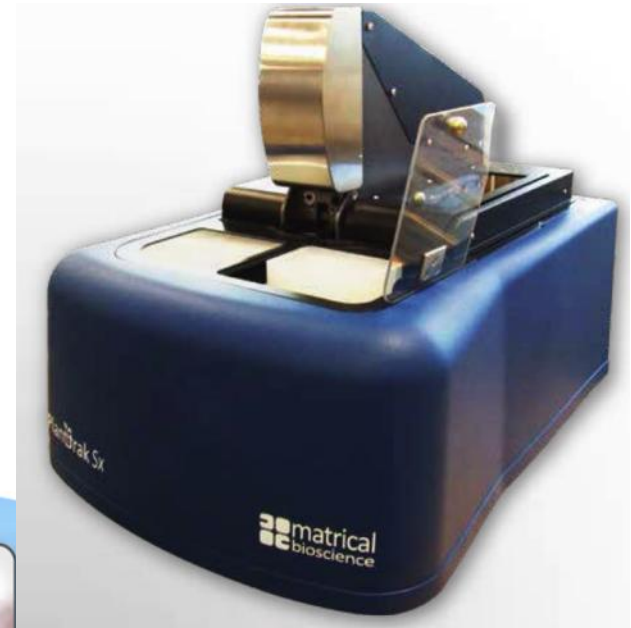


Handheld sampling and barcode device

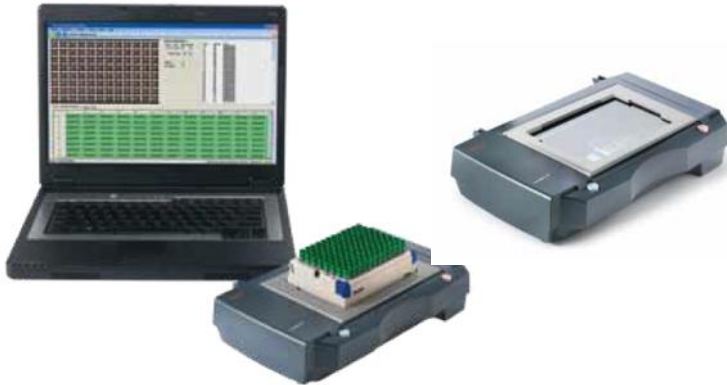
- Lightweight, easy to use
- Barcoding system automatically correlates genetic sample with plant location
- Increase the work efficiency while decreasing human error



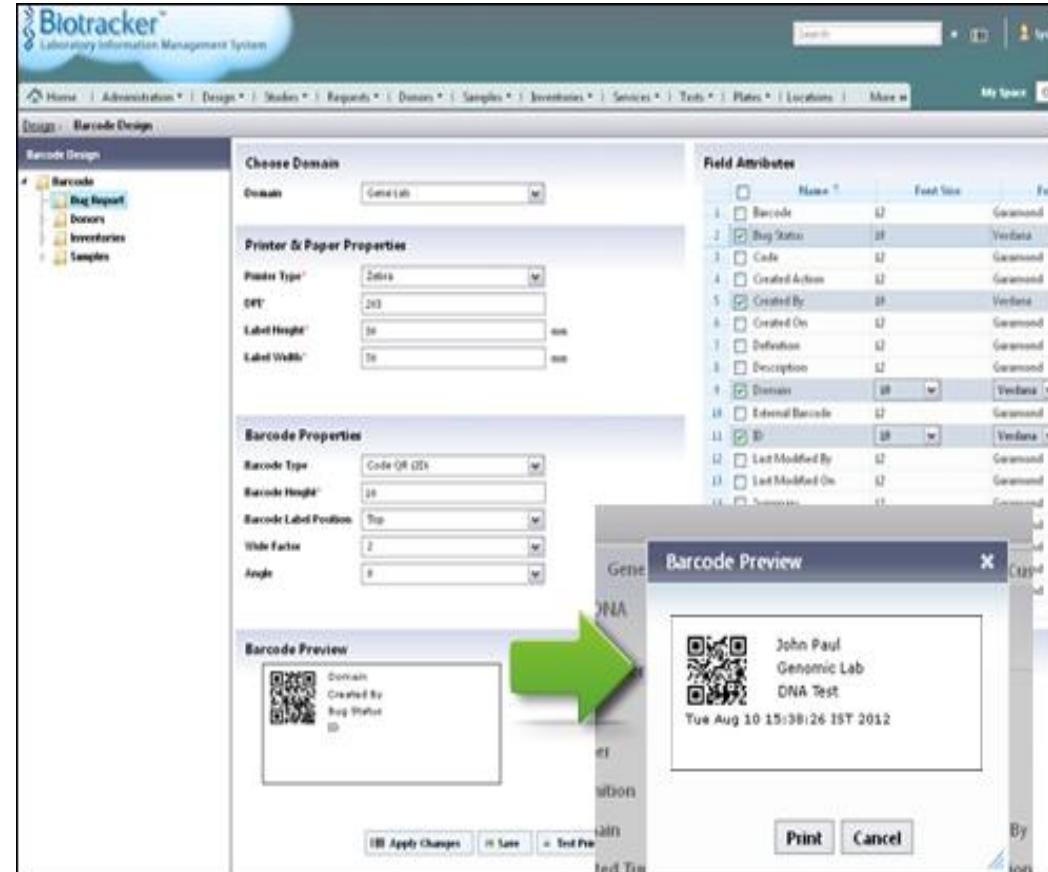
Magazines hold 96 leaf samples
Benchtop unloader automatically
transfers to 96 well plates



LIMS and barcoding for lab data management

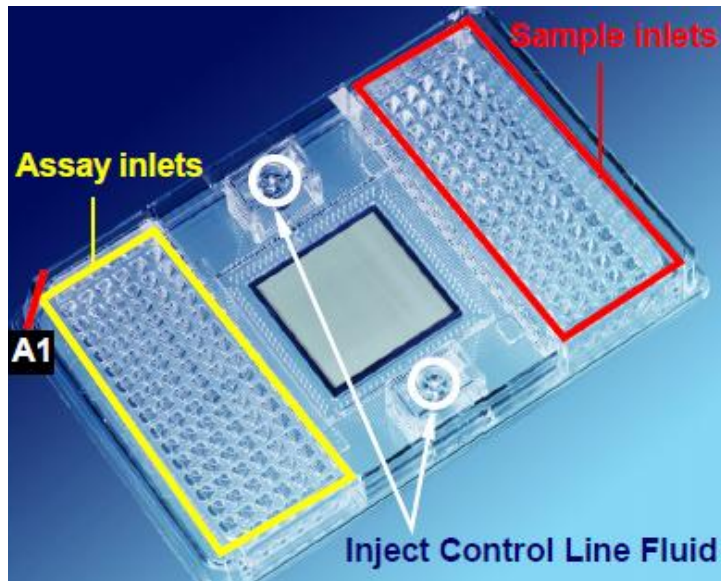


- Barcoding for plots/individual plants, tissue and DNA samples, SNP assays
- Integrating with web-based Biotracker 5.0 LIMS (Ocimum Biosolutions)

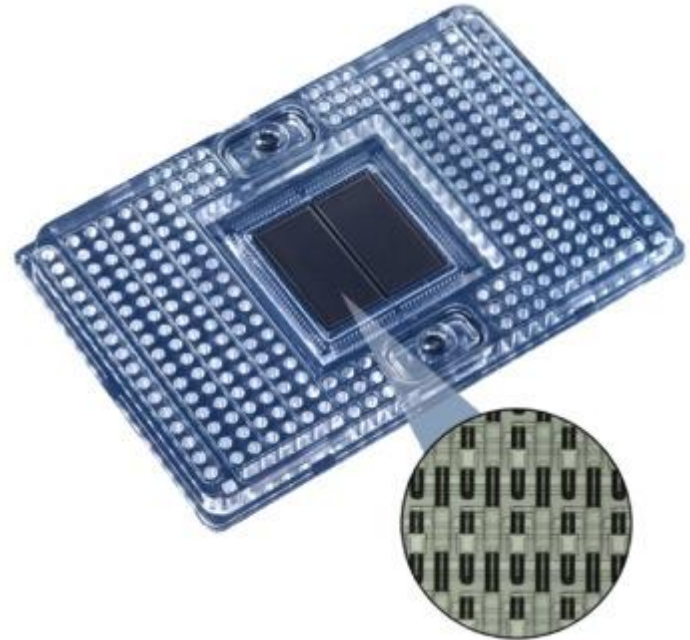


Fluidigm EP1 system for high sample throughput applications

- **Fluidigm Dynamic Arrays** provide nano-liter size reactions for rapid genotyping at a low cost per sample



96 SNPs x 96 samples
(9,216 reactions)



24 SNPs x 192 samples
(4,608 reactions)

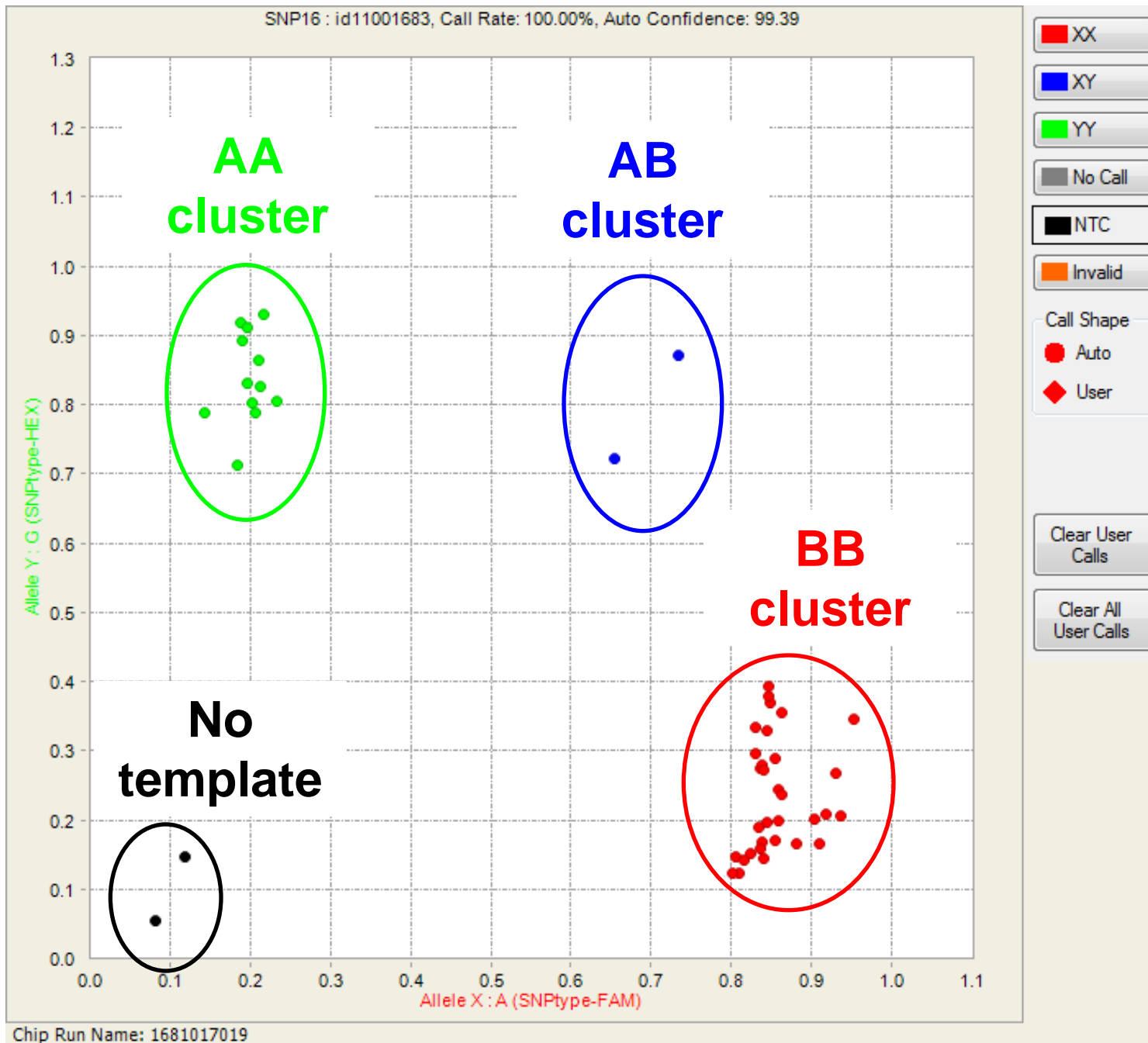
- Different sets of custom SNPs can be used for each chip run
- Inventory of assays being increased for common use

Using Fluidigm® SNPtype™ Assays in rice

SNP16 : id11001683, Call Rate: 100.00%, Auto Confidence: 99.39

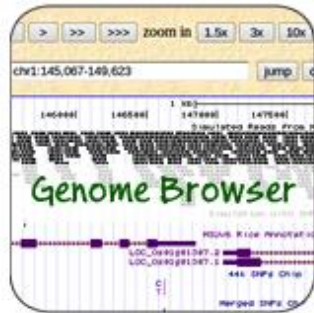
Scatter-plot view for a single SNP

Well defined clusters for the 3 genotype classes



44K SNP chip data online

Rice Diversity



The Rice Diversity Project is a collaborative effort to explore the genetic basis of variation in rice and its wild ancestors. The project evaluates genotypes and phenotypes in a diverse set of rice accessions and uses association mapping to link genotype and phenotype. Our genotyping platforms are based on thousands of SNP markers. Our phenotyping efforts use controlled vocabularies to systematically describe a broad array of plant characteristics.

News & Views

- Publication of 44K-SNP GWAS paper in Nature Communications and data set
- Publication of new paper describing seven new 384-SNP assays in Molecular Breeding and data set
- Rice: Research to Production course application for 2012 now available

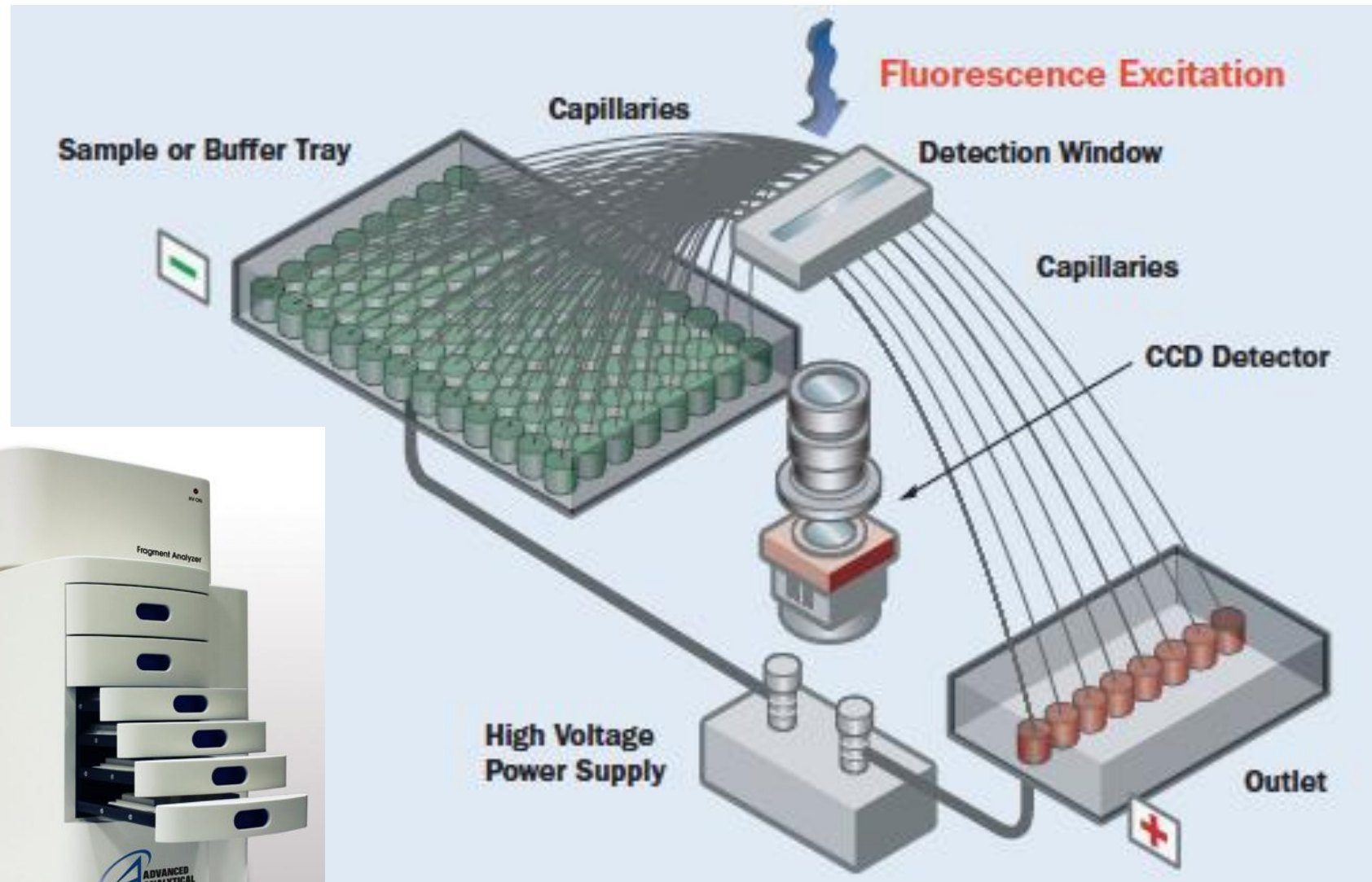
Zhao et al. 2011 (Nat. Comm.)



This project is funded by the National Science Foundation (PGRP #1026555).

www.ricediversity.org

Genotyping indel and SSR markers



- **96-capillary Fragment Analyzer**

Infinium 6K SNP chip from Cornell University

Custom 6K Infinium chip:

- Designed at Cornell University (M. Wright and Susan McCouch) from 120 genome data + legacy BXP SNPs
- Independent design from RICE6K chip being used in China (Yu et al. 2013)

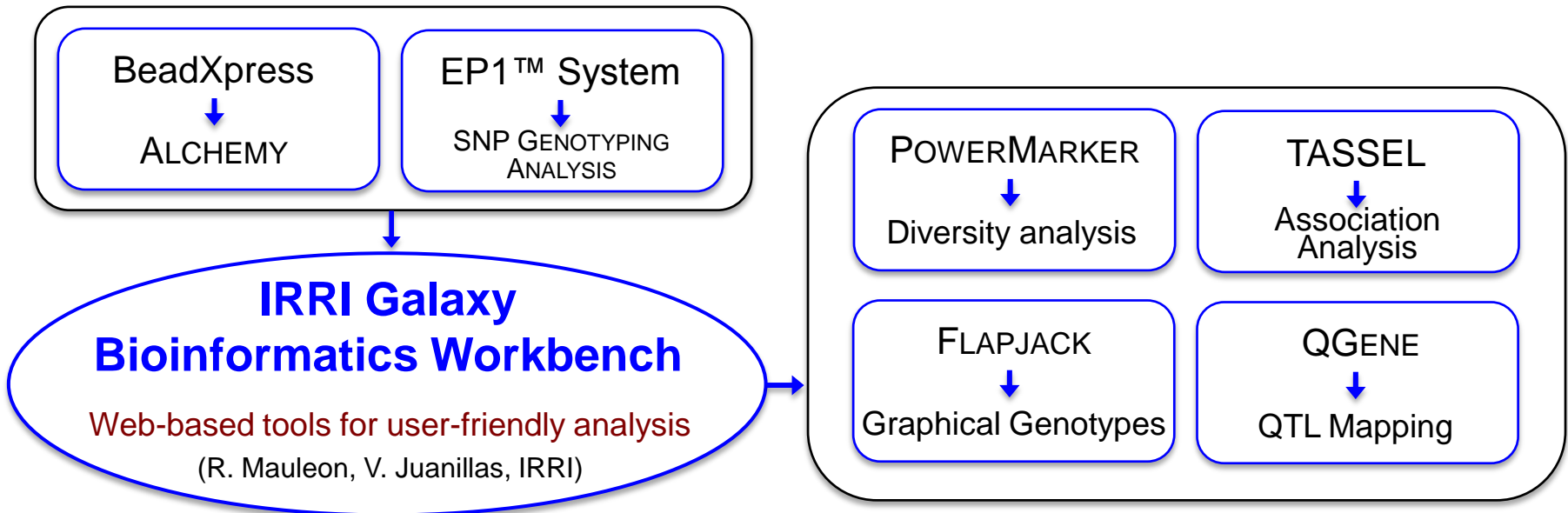
Preliminary data:

- 5,274 SNP loci on chip (6,000 beads)
- 4,736 SNP loci with >85% call rate
- Example polymorphism rates:
 - Indica/japonica ~2,250 SNPs
 - Indica/aus ~ 1,350 SNPs
 - Indica/indica ~ 1,000 SNPs



1 x 24 sample format,
can process 4 chips at a time

SNP data analysis workflow



Galaxy Analyze Data Workflow Shared Data Visualization Help User Using 28.6 MB

Tools

search tools

Get Data

ALCHEMY tools

- Alchemy-2 Alchemy calls from Illumina output with SNP and sample map
- Alchemy to Matrix file converter
- transposeTable Transposes tab-delimited table
- Matrix to Powermarker file conversion
- Matrix to QGene file format conversion

uniqprimer 0.50 tools

EMBOSS

Text Manipulation

Convert Formats

FASTA manipulation

IRRI GALAXY-BIOINFORMATICS WORKBENCH

Welcome! You are looking at a local instance of Galaxy at the International Rice Research Institute (IRRI).
To access the main server, go to [Galaxy main](#).

History

Unnamed history
28.6 MB

13: Transposed Matrix of Alchemy Calls of Jake_6March2013_Custom.txt
97 lines
format: tabular, database: ?

	1	2	3	4	5	6	7	8	9	10	11	12	13
SNP_Sample_ids	id6004481												id3817
4248	id3007189												id7001156
15793	id6000402												id2001102
81297	id8007301												id2002229
105	id9000661												id2007461
d4008092	id7003853												id1027

12: Matrix of Alchemy Calls of Jake_6March2013_Custom.txt
385 lines
format: tabular, database: ?

The Galaxy project is supported in part by [NSF](#), [NHGRI](#), and the [Huck Institutes of the Life Sciences](#).

Nita_1319snps_464acc.flapjack - Flapjack - 1.12.05.25

New Project Open Project Import Data

Data Sets

1319_464acc_Flapjack 4

Trait Data

Default View

Chromosome: 1 464 lines, 383 markers, length: 45,027,304

Overview

Line: Marker: Genotype:

Zoom:

Flapjack Tip: Hold % while clicking and dragging lines or markers to move them to new positions

8C, 11T, 70.91MB

Training and capacity building at IRRI for molecular breeding in rice



Molecular Breeding Course

- **Molecular Breeding course**

Case studies in using markers to accelerate breeding efforts, including SNP genotyping.

September 2014

- **SNP Data Analysis Course**

SNPs for genetic diversity analysis, QTL mapping, graphical genotyping, association mapping and MAS.

May 2014



SNP Data Analysis

www.training.irri.org

<http://gsl.irri.org>



Genotyping Services
Laboratory



Genotyping services available for HRDC members for 2014

- DNA extraction
- Fragment Analyzer
 - Indel and SSR markers
- Fluidigm SNP genotyping
 - 288 SNP polymorphism survey
 - 24 and 96 SNPs (flexible sets)
- Infinium rice 6K SNP chip:
 - Approx. 4,800 SNPs (fixed set)

Product Description
DNA Extraction
BeadXpress 384 plex
Fluidigm 96.96 Dynamic Array
Fluidigm 192.24 Dynamic Array
Fluidigm Polymorphism Survey
Fragment Analyzer
Fragment Analyzer (without PCR)
Infinium SNP chip

<http://gsl.irri.org>

Note: The BeadXpress 384-SNP sets have been phased out and are not available for 2014 genotyping



Genotyping prices for 2014

Prices for Non Profit Clients

Product Description	Number of samples per Unit Description	Price per Unit Description	Price per sample
DNA Extraction	94-96	345	3.63
BeadXpress 384 plex	24	1400	58.33
Fluidigm 96.96 Dynamic Array	94	1935	20.59
Fluidigm 192.24 Dynamic Array	188	1400	7.45
Fluidigm Polymorphism Survey	1	60	60.00
Fragment Analyzer	94	190	2.00
Fragment Analyzer (without PCR)	94	170	1.80
Infinium SNP chip	24	1935	80.62

Note: prices subject to final approval and may change without notice;
There are additional surcharges to test and optimize custom Fluidigm SNP sets

<http://gsl.irri.org>



Genotyping prices for 2014

Prices for Commercial Clients

Product Description	Number of samples per Unit Description	Price per sample	Price per Unit Description
DNA Extraction	94-96	4.53	430
BeadXpress 384 plex	24	72.29	1735
Fluidigm 96.96 Dynamic Array	94	25.53	2400
Fluidigm 192.24 Dynamic Array	188	9.20	1730
Fluidigm Polymorphism Survey	1	75.00	75
Fragment Analyzer	94	2.50	235
Fragment Analyzer (without PCR)	94	2.23	210
Infinium SNP chip	24	100.00	2400

Note: prices subject to final approval and may change without notice;
There are additional surcharges to test and optimize custom Fluidigm SNP sets

<http://gsl.irri.org>



Acknowledgments

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Genotyping Services Lab (GSL) team at IRRI

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- Socorro Carandang
- Annalhea Jarana
- Grace Cariño

Marker Validation:

- Maria S. Dwiyantri
- C. Jade Dilla-Ermita
- Erwin Tandayu
- Crisostomo Dizon

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- Geraldine Malitic
- Geisha Sanchez
- Venice Juanillas

IRRI Scientists:

J.H. Chin,
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E. Redona,
C. Vera Cruz,
K. McNally,
E. Nissila

GSL team

