Update on high throughput marker facility and genotyping services at IRRI

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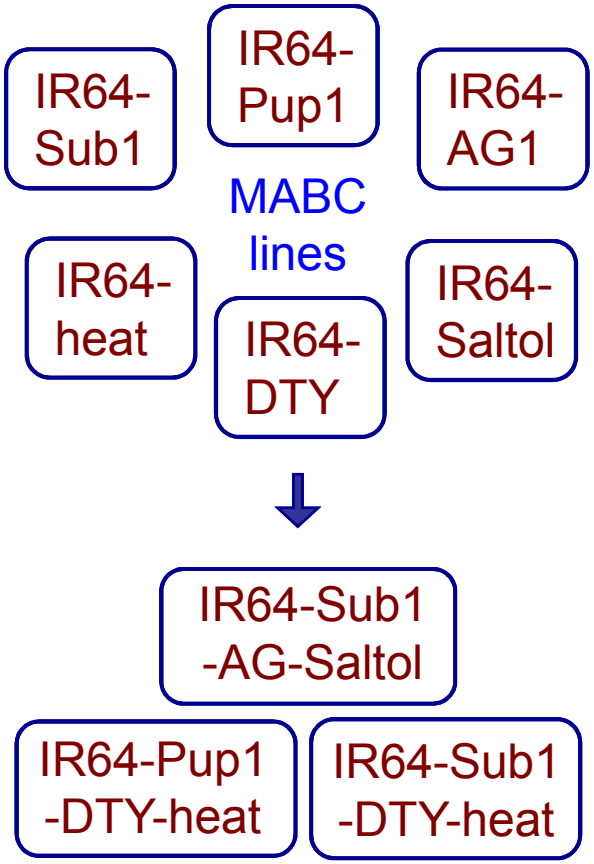
IRRI

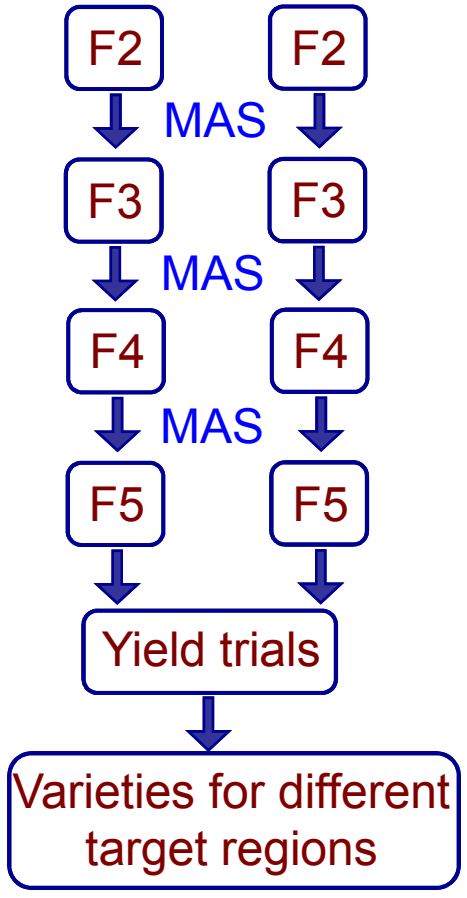
Integrating markers into breeding programs

MABC and QTL pyramiding



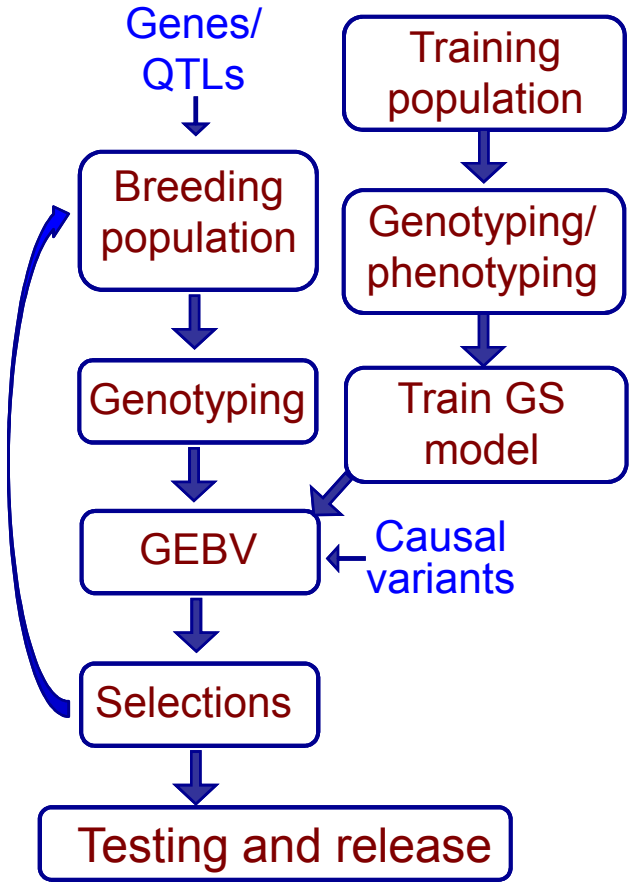
QTL pyramid lines

MAS in the pedigree breeding programs



New released varieties

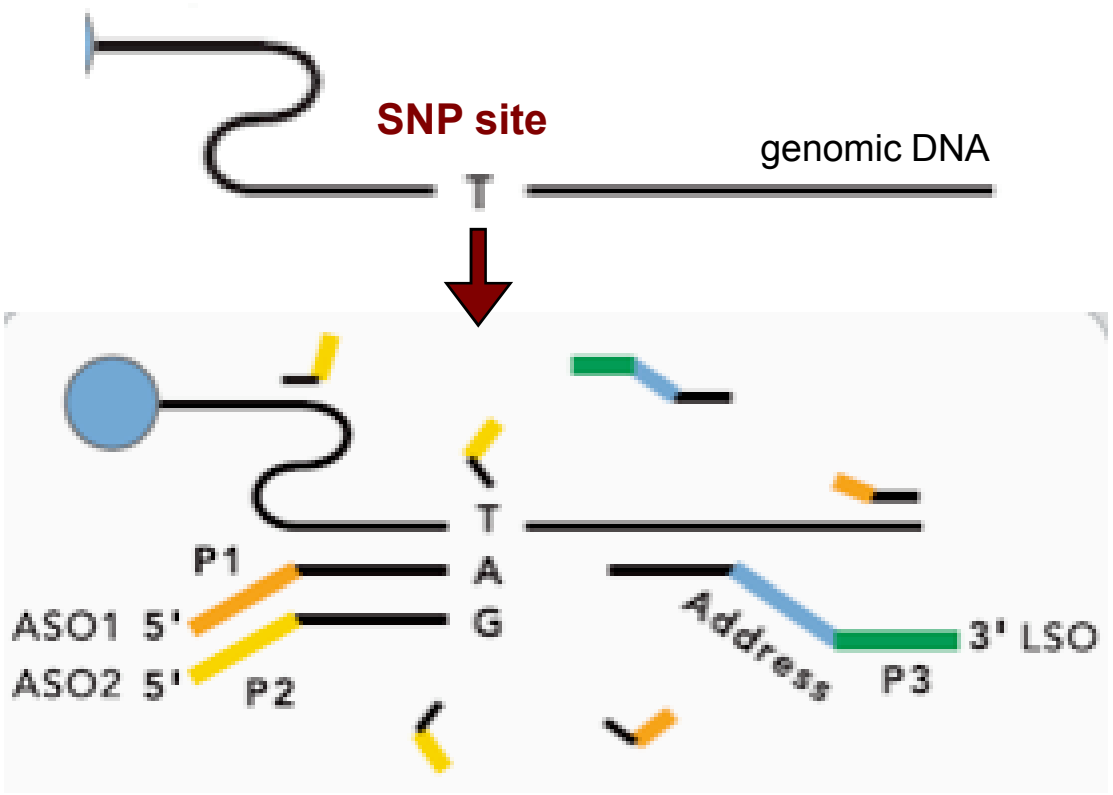
Genomic selection



New released varieties

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

SNP marker platforms

Fludigm
24 and 96 SNPs

Fine-mapping, trait-based SNPs for breeding, MABC

BeadXpress
384 SNPs

Diversity analysis, fingerprinting, MABC, QTL mapping

GBS
3k-20k SNPs

High resolution mapping, genomic selection, association studies

SNP chips
6k-50k SNPs

New initiative under the Global Rice Science Partnership (GRiSP)

- Product 2.1.3 High-throughput SNP genotyping platform for breeding applications:
  - **Facilities for high-throughput SNP genotyping** set up at IRRI and CIAT with access to GRiSP partners
  - **SNP fingerprinting platform** in place to support activities for variety identification, quality assurance and characterizing breeding lines and released varieties
  - **Trait-based SNP markers** for key traits validated, optimized, and deployed in breeding programs

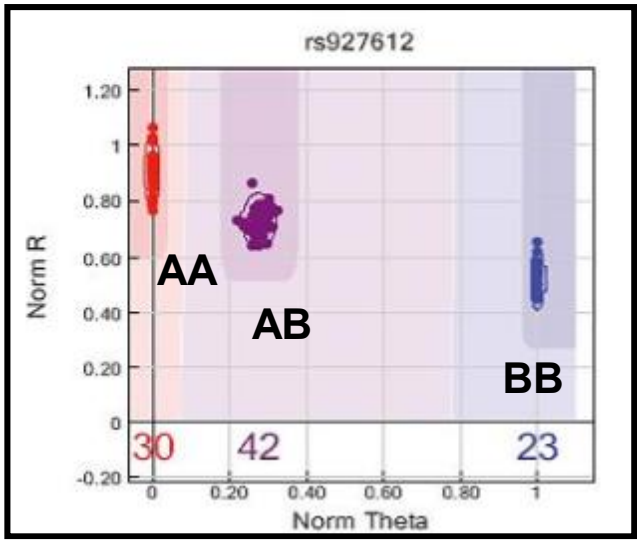
IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

AfricaRice

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

384-SNP marker genotyping at IRRI

- Illumina BeadXpress Reader at IRRI
- 96 samples x 384 SNP markers per run



Over 16,000 rice samples (6 million data points) run at IRRI in past 3 years

Breeding applications:

- Diversity analysis
- DNA fingerprinting
- QTL mapping
- Marker-assisted selection

Thomson et al. 2012 (Mol. Breed. 29:875)

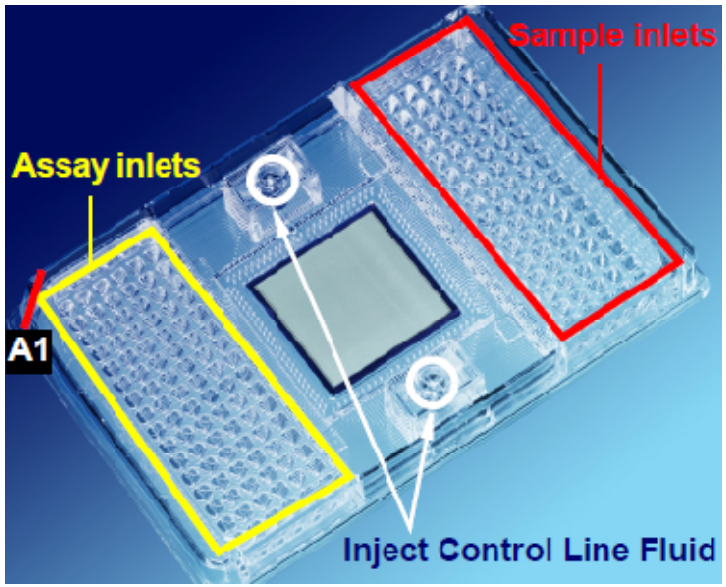
Seven custom VeraCode OPA sets

Rice SNP set	Illumina OPA ID	No. of SNPs	Description
RiceOPA1.0	VC0011438-OPA	96	Quality control
RiceOPA2.1	GS0011861-OPA[a]	384	indica/indica
RiceOPA3.1	GS0011862-OPA[b]	384	indica/japonica
RiceOPA4.0	VC0013043-OPA	384	japonica/japonica
RiceOPA5.0	GS0011972-OPA	384	indica/ *O. rufipogon*
RiceOPA6.0	VC0011530-OPA	384	japonica/ *O. rufipogon*
RiceOPA7.0	VC0011440-OPA	384	indica/japonica

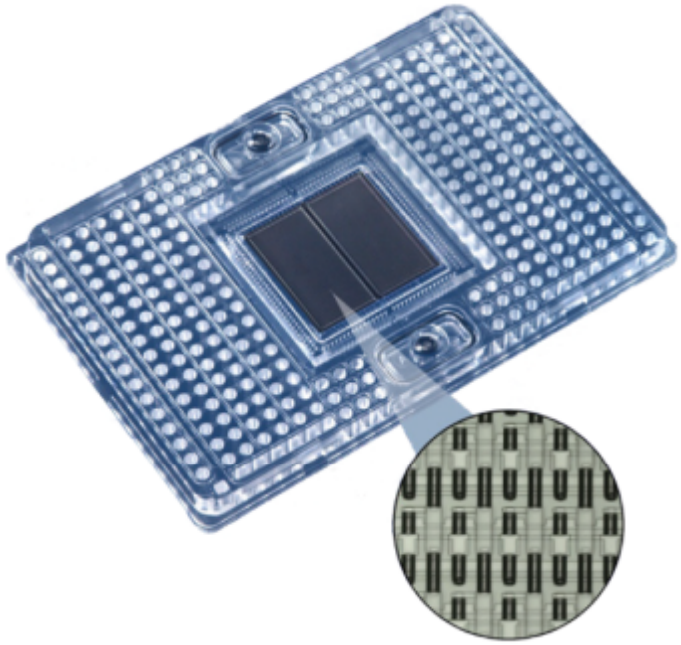
Thomson et al. 2012 (Molecular Breeding 29:875–886)

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)



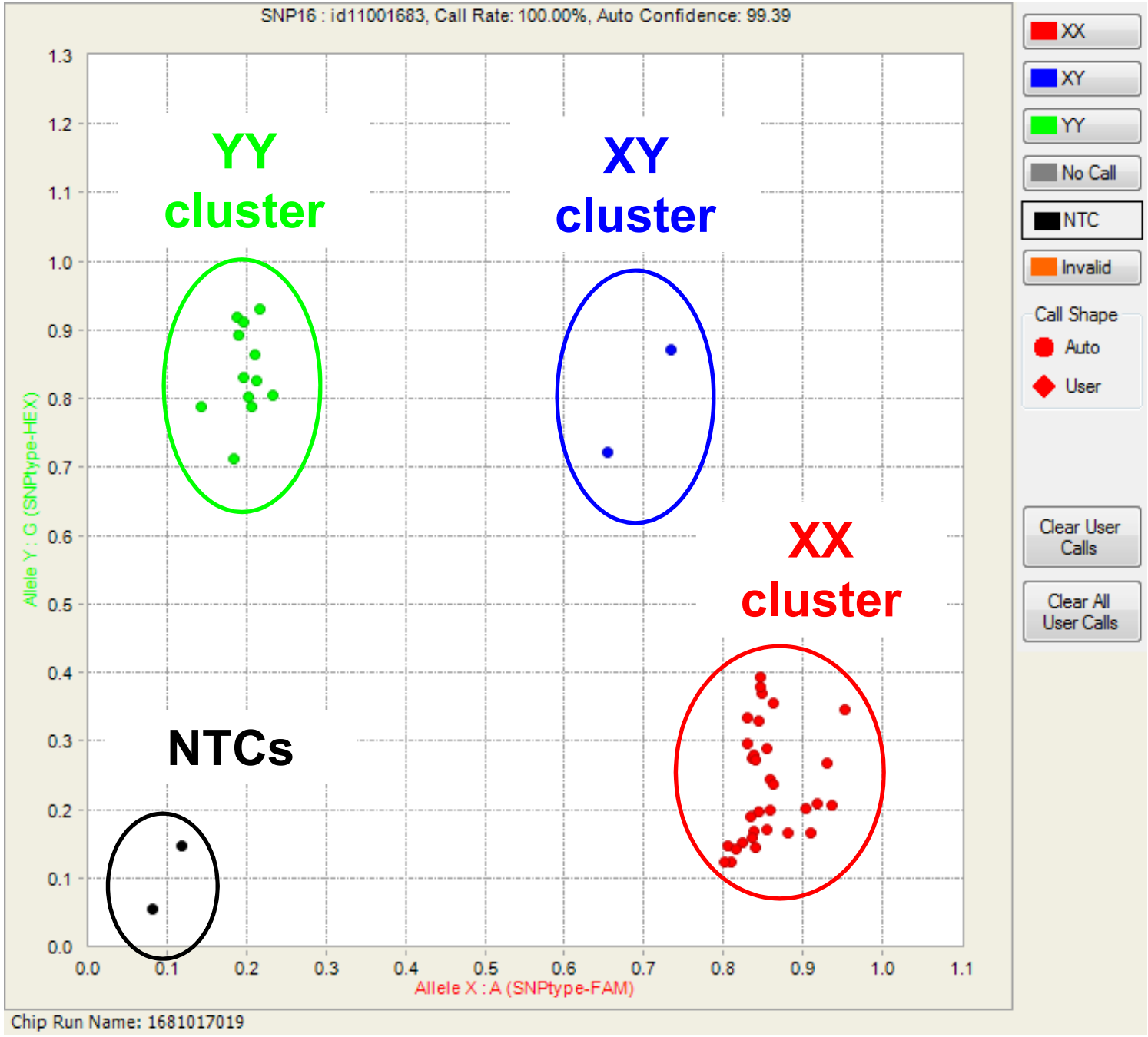
24 SNPs x 192 samples
(4,608 reactions)

- Different sets of custom SNPs can be used for each chip run

SNP genotyping services now offered at IRRI starting Mar. 2013

Scatter-plot view for a single SNP

Well defined clusters

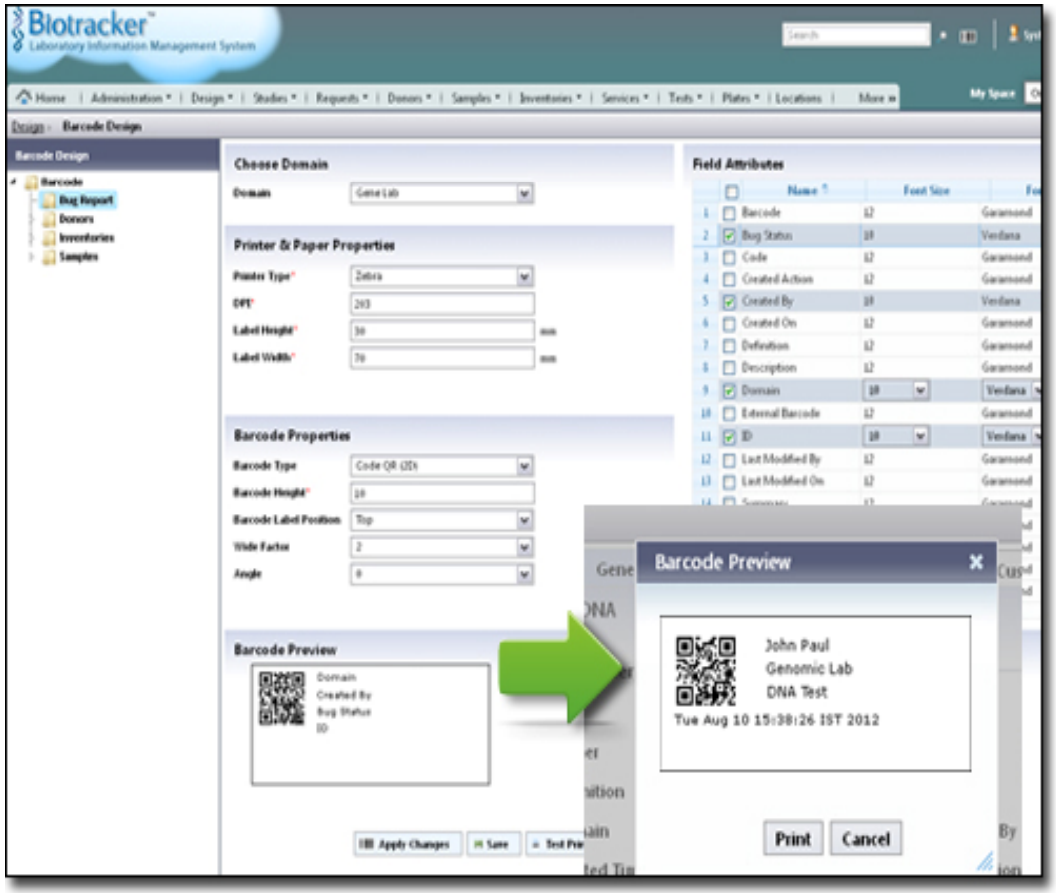


96-capillary Fragment Analyzer

- Functional indels, SSRs, NGS library QC

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)



Genotyping Services Lab at IRRI

External for-profit customers (private sector)

Service	Costs - per set	Samples per set	Costs - per sample	Costs - per SNP	Fixed or custom	Shared or exclusive
BXP-384 SNPs	$1,608	24	$67.00	$0.17	Fixed	Shared
Fluidigm-96 SNPs	$2,065	94	$21.97	$0.23	Fixed	Shared
Fluidigm-96 SNPs	$2,362	94	$25.13	$0.26	Custom	Shared
Fluidigm-96 SNPs	$2,488	94	$26.47	$0.28	Custom	Exclusive
Fluidigm-24 SNPs	$1,400	188	$7.45	$0.31	Fixed	Shared
Fluidigm-24 SNPs	$1,502	188	$7.99	$0.33	Custom	Shared
Fluidigm-24 SNPs	$1,574	188	$8.37	$0.35	Custom	Exclusive
DNA extraction	$348	94	$3.70		Fixed	Shared

Services now offered for DNA extraction (from lyophilized leaf tissue), Fluidigm 24 and 96 SNP sets (fixed and custom), and BeadXpress 384-SNP sets (fixed)

GSL price list (public sector partners)

External non-profit (Universities, NARES, other CG)

Service	Costs - per set	Samples per set	Costs - per sample	Costs - per SNP	Fixed or custom	Shared or exclusive
BXP-384 SNPs	$1,296	24	$54.00	$0.14	Fixed	Shared
Fluidigm-96 SNPs	$1,666	94	$17.72	$0.19	Fixed	Shared
Fluidigm-96 SNPs	$1,906	94	$20.28	$0.21	Custom	Shared
Fluidigm-96 SNPs	$2,009	94	$21.37	$0.22	Custom	Exclusive
Fluidigm-24 SNPs	$1,128	188	$6.00	$0.25	Fixed	Shared
Fluidigm-24 SNPs	$1,213	188	$6.45	$0.27	Custom	Shared
Fluidigm-24 SNPs	$1,270	188	$6.76	$0.28	Custom	Exclusive
DNA extraction	$282	94	$3.00		Fixed	Shared

Training and capacity building for molecular breeding in rice

Molecular Breeding Course

- **Molecular Breeding course**

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

September 2-13, 2013

- **SNP Data Analysis Course**

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

April 22-27, 2013

SNP Data Analysis

SNP data analysis workflow

BeadXpress → ALCHEMY

Fluidigm EP1 → SNP GENOTYPING ANALYSIS

IRRI Galaxy Bioinformatics Workbench

Web-based tools for user-friendly analysis

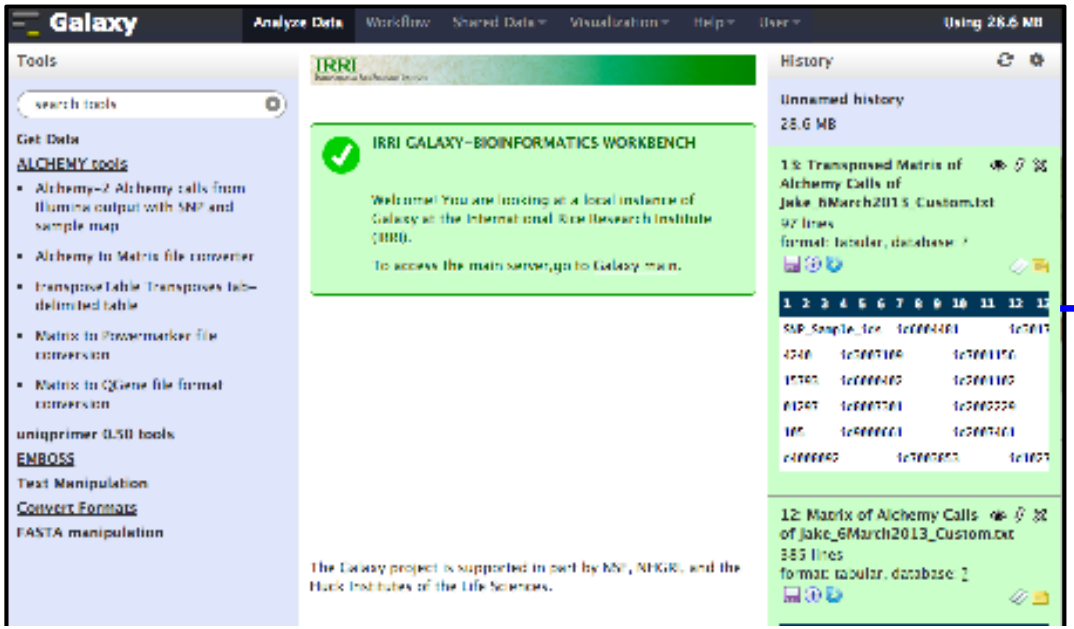
(R. Mauleon, V. Juanillas, IRRI)

POWERMARKER → Diversity analysis

TASSEL → Association Analysis

FLAPJACK → Graphical Genotypes

QGENE → QTL Mapping



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Thank You!