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# Quality Management Practices @ ICRISAT Genomics Lab

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# Contents



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Background

Measures being taken at ICRISAT- AGL

Use of LIMS

Summary

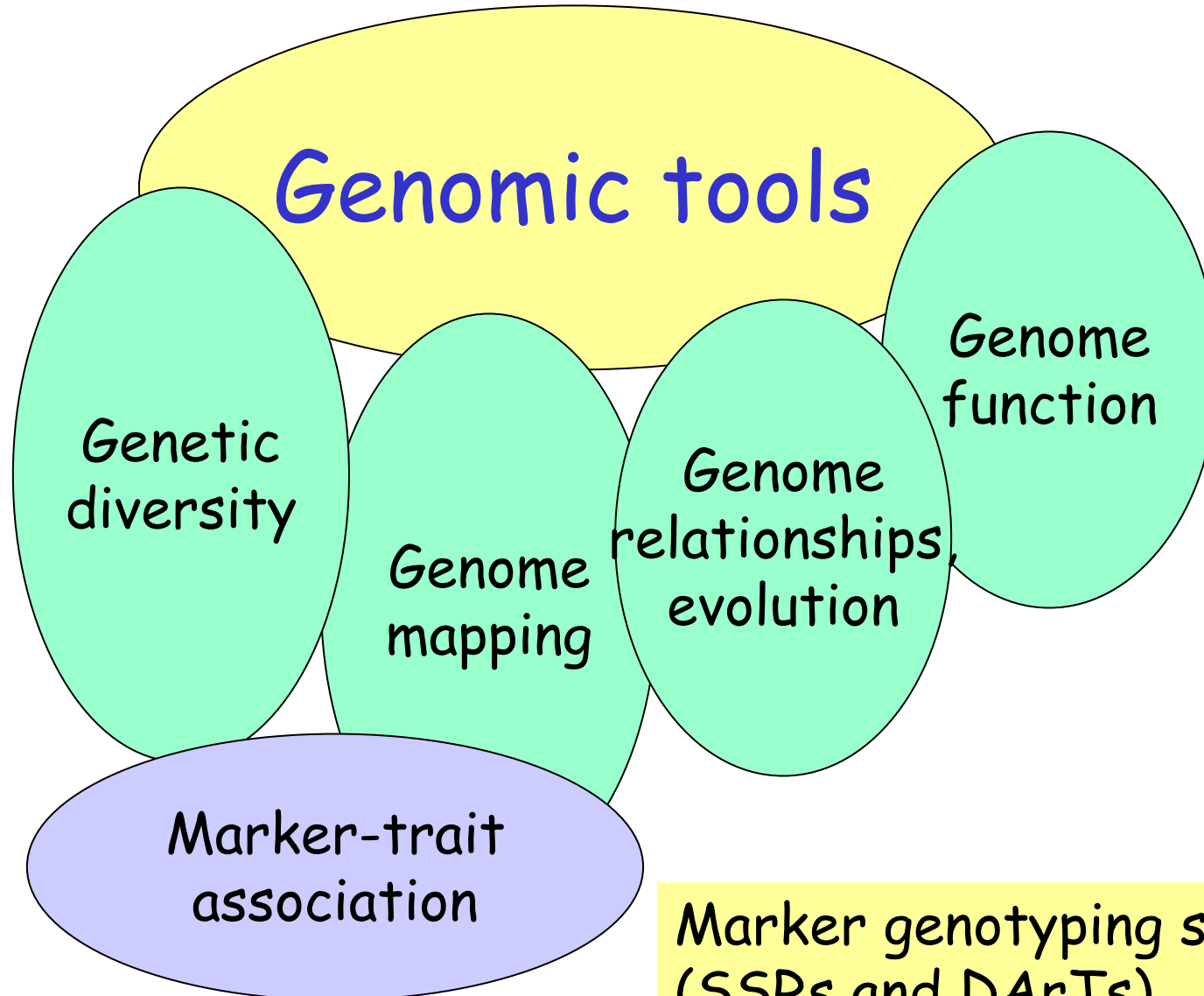


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## Background...

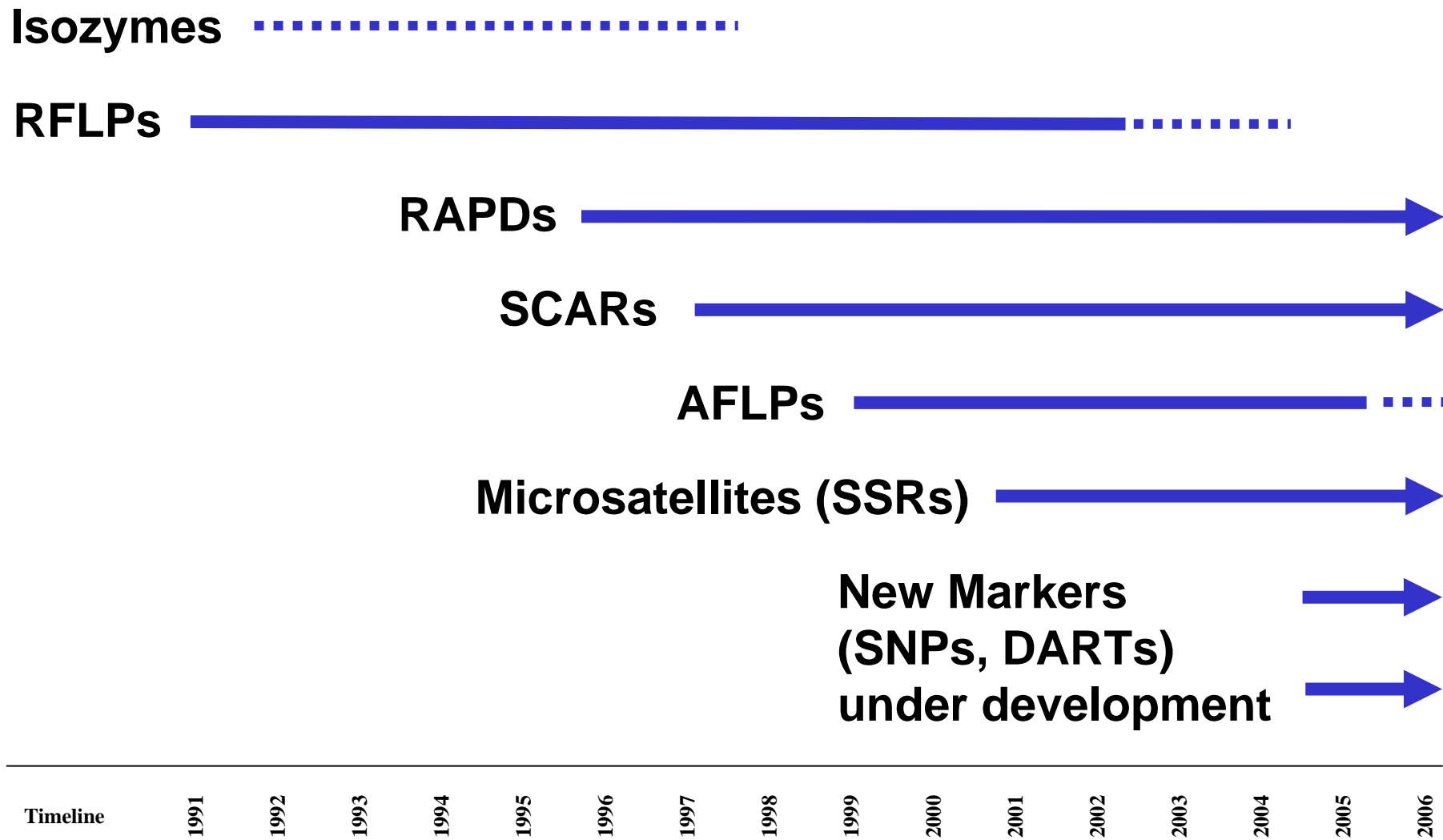


# Genomics research @ ICRISAT





# Genetic marker evolution





# Availability and use of markers

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## Availability

- Large number of molecular markers
- High throughput genotyping assays

Plant breeding- transitional phase

- high throughput genotyping

Genebank

- molecular characterization of large germplasm collection



# Genotyping facilities



LIMS

DNA extraction



DNA normalization and PCR-setup



384-well PCR



ABI3700  
24- & 96-capillary  
genetic analyzers





# Why should we care?



More data- more possibility of error?

How to minimize error in high throughput genotyping assays?

Prevention is better than cure!!



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**Measures being taken at ICRISAT- AGL...**



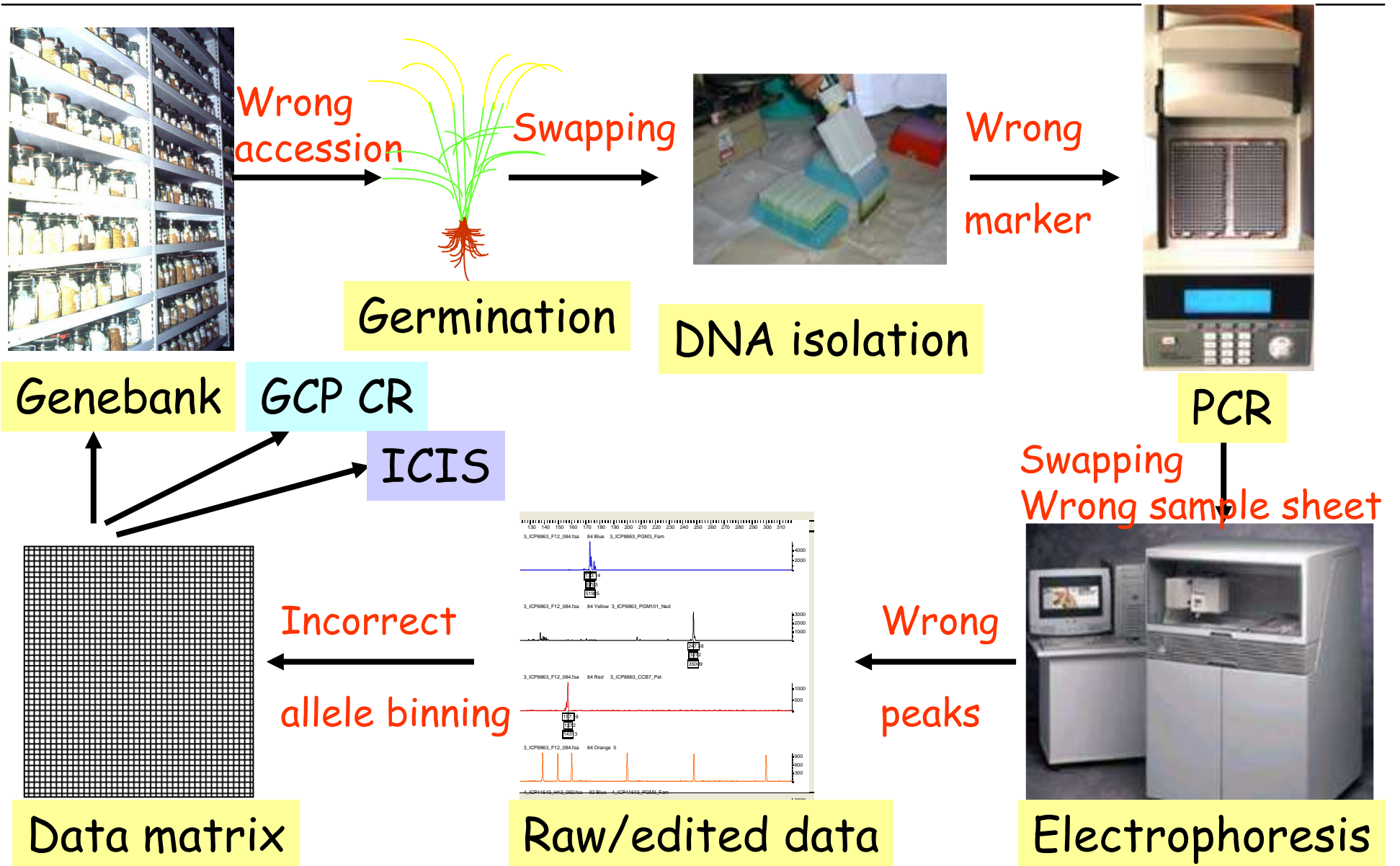
# What we have been doing?



	# Acc.	# SSRs	Collob.
Chickpea	4000	50	ICARDA
Groundnut	1000	20	EMBRAPA
Pigeonpea	1000	20	-
Sor	<b><math>11,500 \times 30 \times 20 = 6,900,000</math> datapoints Management and quality standards!</b>		
Pearl millet	1000	20	-
Finger millet	1000	20	-
Foxtail millet	500	20	-



# Genebank- Laboratory





# Precautionary measures



Genebank and lab technician together at the time of sowing and DNA isolation

DNA isolated from single plant/ accession

4 control genotypes in each 96 well plate

Control genotypes in fixed wells

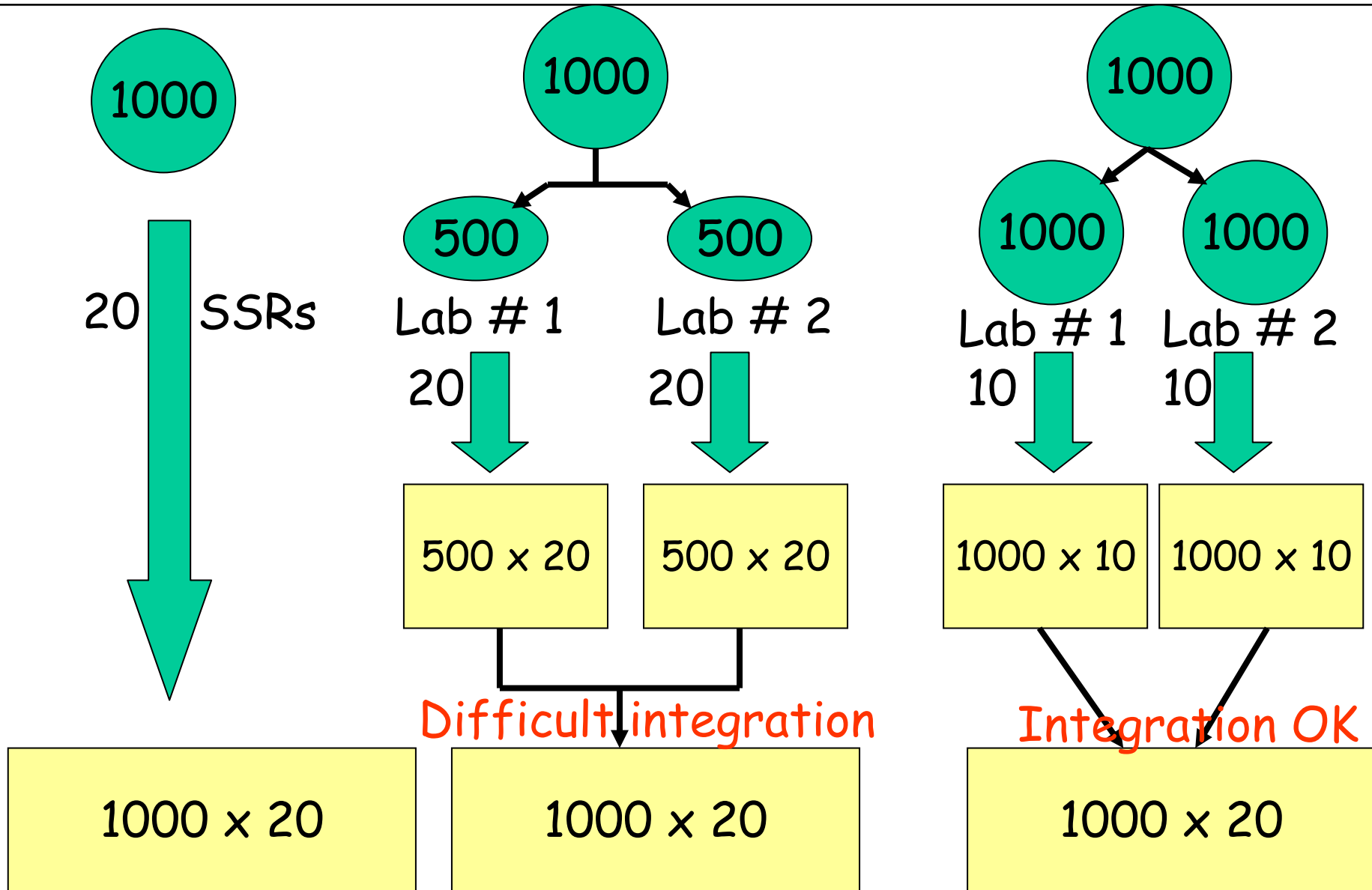
Use of LIMS, developed in-house

Peak determination by one technician

Allele calling/binning using Allelobin



# Division of work





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Use of LIMS...



# LIMS @ ICRISAT-AGL



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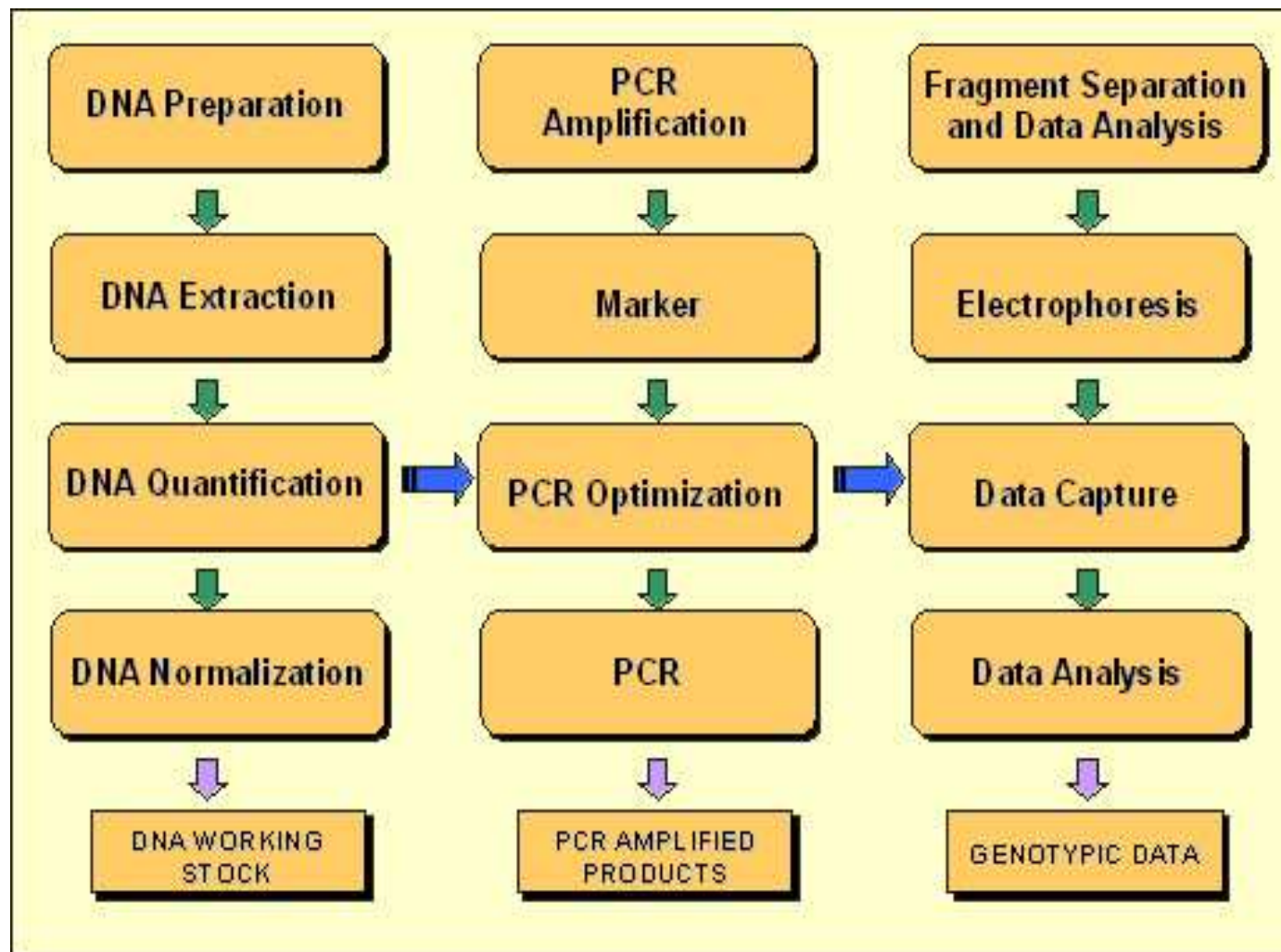
LIMS needs to keep track of the sample DNA from its source to the genotyping data/file

## Features:

- (1) modular: possibility to skip/add certain modules that would aid in data management
- (2) besides workflow manager, it provides visibility quality checks, centralization of data
- (3) ability to track data and communicate quality information- improves methods and work practices



# The genotyping workflow





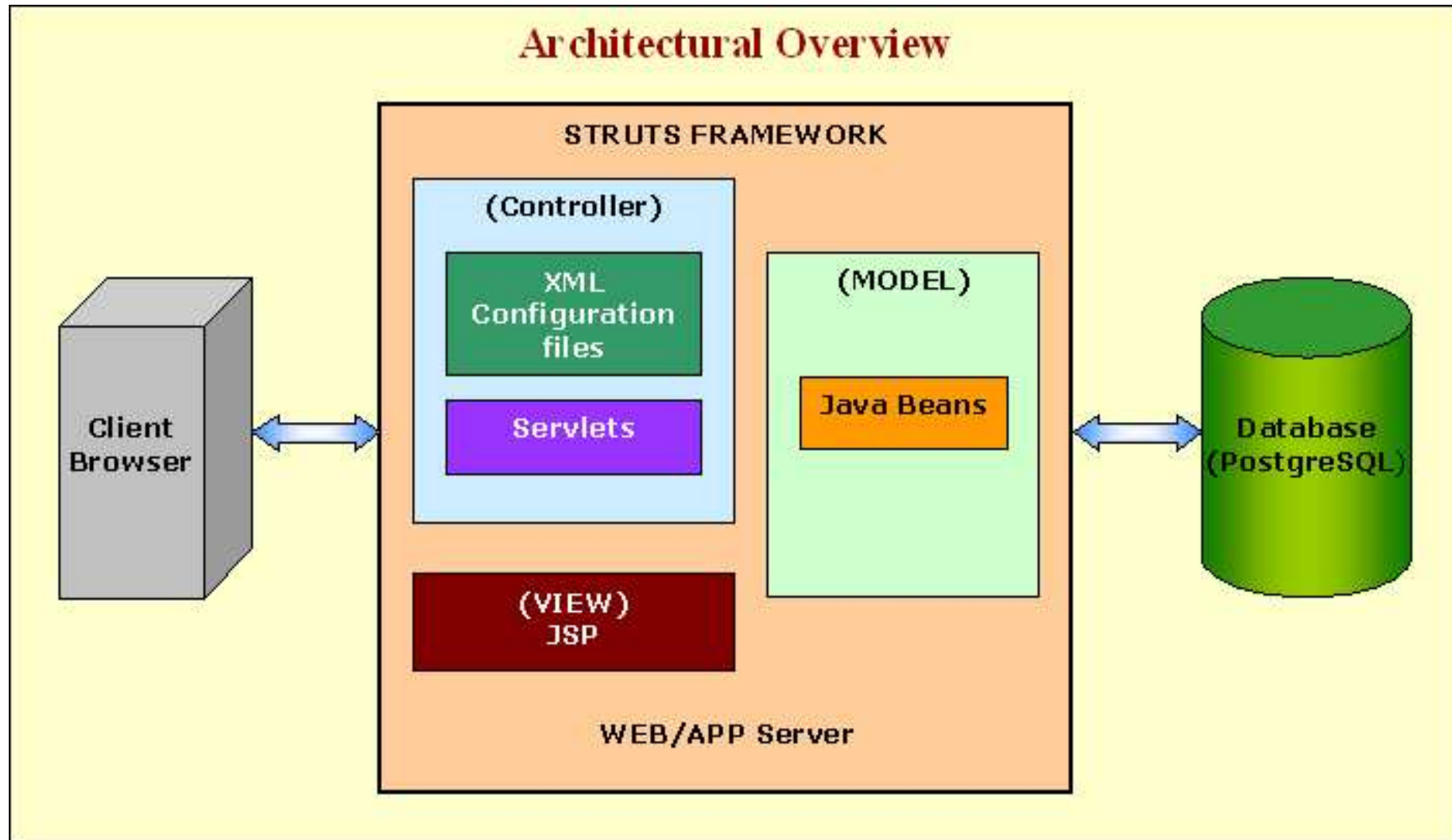
# Implementation of LIMS



- Implemented with open source, freely available
- Researcher accesses through the browser
- GUI and middleware implemented using Java Struts Framework technologies
- Two versions: (i) uses MS-SQL 2000 server  
(ii) another uses the Postgres SQL sever. Also has been tested with SQL server 2005 Express ed
- The Tomcat apache webserver used to provide GUI interconnections with the database



# System Architecture





# Modules of LIMS



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Four main modules:

- (a) Experimental design
- (b) Sample tracking
- (c) Generation of reports
- (d) Storing of data on markers/protocols



# Module I: Experimental Design

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- (a) Enters name for project, study, experiment and name/number of accessions
- (b) Data on genotype identities/names must be uploaded. Genotype identifier is used to create DNA extraction plate.
- (c) Germplasm passport data or characterization data may also be uploaded into the system at this stage



# Module II: Sample Tracking



- (a) Sample DNA localization in microtitre plate, DNA quantification, normalization, PCR setting up info held in database for each marker.
- (b) Assignment of multiple sets of markers based on allele size data for dye colours. Merging genotyper files across experiments for allele size binning.
- (c) Treatment of failed samples, e.g. can be replaced with data from repeat assays. Elimination of duplicate data by alerting the user to existence of 2 records for the same genotypes and seeks user intervention to decide which one to eliminate.
- (d) Automated allele binning



## Module III: Generation reports



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- (a) Data submitted can be viewed/printed as report
  - (b) Report may be downloaded as tab delimited file
  - (c) Generally used for printing DNA and PCR plate design, DNA quantification results, details of PCR markers, programmes and reagent calculation



# Module IV: Data storage



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- (a) Information on DNA extraction, quantification and PCR protocols entered through the "Protocol and Markers" module, archived in this module
  - (b) Simple form allows user to enter details on protocols, marker types, primer information (sequences,  $T_m$ , product size, mapping position, ref)



# Where to find?



## BMC Bioinformatics



Software

Open Access

### Laboratory Information Management Software for genotyping workflows: applications in high throughput crop genotyping

B Jayashree\*<sup>1</sup>, Praveen T Reddy<sup>1</sup>, Y Leeladevi<sup>1</sup>, Jonathan H Crouch<sup>3</sup>, V Mahalakshmi<sup>4</sup>, Hutokshi K Buhariwalla<sup>5</sup>, KE Eshwar<sup>2</sup>, Emma Mace<sup>6</sup>, Rolf Folksterma<sup>7</sup>, S Senthilvel<sup>2</sup>, Rajeev K Varshney<sup>2</sup>, K Seetha<sup>2</sup>, R Rajalakshmi<sup>1</sup>, VP Prasanth<sup>1</sup>, Subhash Chandra<sup>1</sup>, L Swarupa<sup>1</sup>, P SriKalyani<sup>1</sup> and David A Hoisington<sup>2</sup>

Address: <sup>1</sup>Bioinformatics and Biometrics Unit, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India, <sup>2</sup>Applied Genomics Laboratory, ICRISAT, Patancheru 502 324, Andhra Pradesh, India, <sup>3</sup>International Wheat and Maize Improvement Centre (CIMMYT), Apdo. Postal 6-641, 06600 México, D.F., México, <sup>4</sup>3-6-502, Satguru Apts, Himayat Nagar, Hyderabad 500029, Andhra Pradesh, India, <sup>5</sup>c/o International Wheat and Maize Improvement Centre (CIMMYT), Apdo. Postal 6-641, 06600 México, D.F., México, <sup>6</sup>Hermitage Research Station, Queensland Department of Primary Industries and Fisheries, Yangan Rd, via Warwick Q 4370 Australia and <sup>7</sup>De Ruiters Seeds Benelux, P.O. Box 1050, 2660 BB Bergschenhoek, The Netherlands

The genotyping LIMS project page:

Downloading: <http://www.icrisat.org/gt-bt/lims/lims.asp>

Demo: <http://test1.icrisat.org/lims/>



# Functional highlights of LIMS



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Ensures quality control in a medium scale genotyping

Data entry and retrieval functions are simple with a built-in error checking facility

Implements functions for automated allele binning, eliminating the errors, introduced by incorrect allele sizing

Ensures that all samples have been treated identically and all data entered/edited have date/time

Application with the database and tutorial available



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Summary...



# Summary



- ❖ Data generated and analyzed for chickpea (4000 x 50), sorghum (3000 x 50) and submitted to GCP Central Registry
- ❖ Data generated and being analyzed for groundnut (1000 x 20), pigeonpea (1000 x 20)
- ❖ Generation of data in progress for finger millet and foxtail millet
- ❖ Validation of subset of generated data planned under "Data Validation" project of GCP



# Take-home message



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- (1) Planned workflow essential
  - (2) Use of LIMS improves efficiency
  - (3) Combination of automation and human skills
  - (4) Error can be minimized
  - (5) Estimation of error rate



# Team involved



## Applied Genomics:

R K Varshney  
D A Hoisington  
C T Hash

## Bioinformatics/Statistics:

B Jayashree  
S Chandra  
V Prasanth

## Genetic Resources:

H D Upadhyaya  
S L Dwevidi  
R Bhattacharjee

### Financial support:



Generation  
Challenge  
Programme





Thank you !!!