

**Working Group on Biochemical and Molecular Techniques  
and DNA-Profiling in Particular**

**BMT/18/6 Rev.**

**Eighteenth Session  
Hangzhou, China, October 16 to 18, 2019**

**Original:** English  
**Date:** November 14, 2019

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**ADVANCES IN THE CONSTRUCTION AND APPLICATION OF DNA FINGERPRINT DATABASE IN MAIZE**

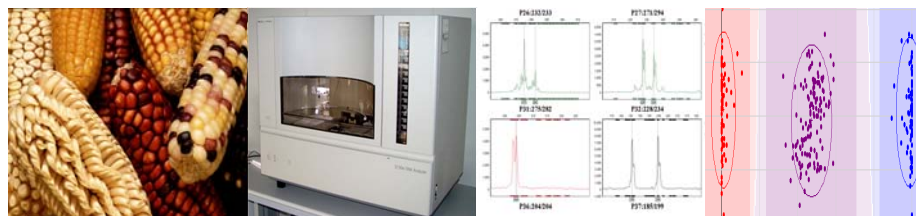
*Document prepared by an expert from China*

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The annex to this document contains a copy of a presentation on “Use of SSR and SNP markers in Maize variety identification”, made at the eighteenth session of the BMT.

[Annex follows]

## Use of SSR and SNP markers in maize variety identification



Maize Research Center, Beijing Academy of  
Agriculture and Forestry Sciences, China

BMT/18, Hangzhou, China

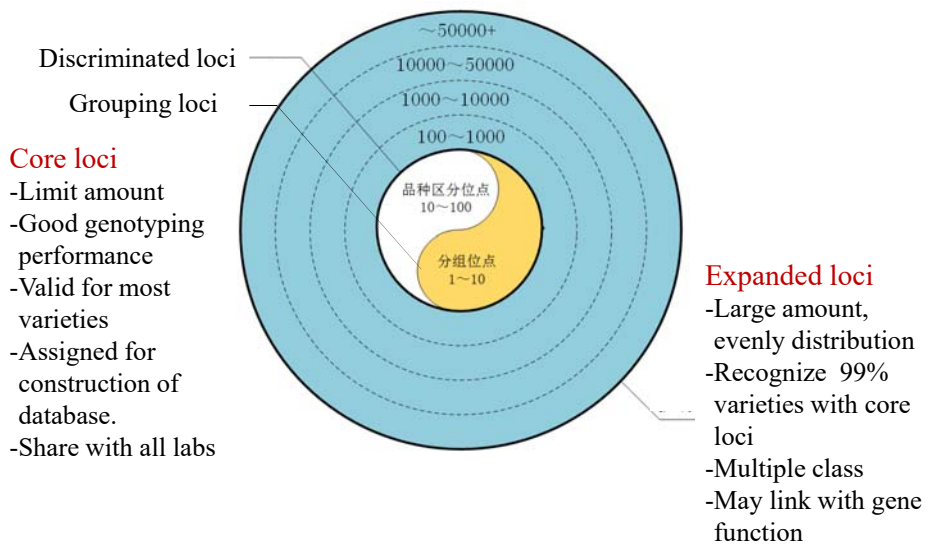
Oct 16 - 18, 2019



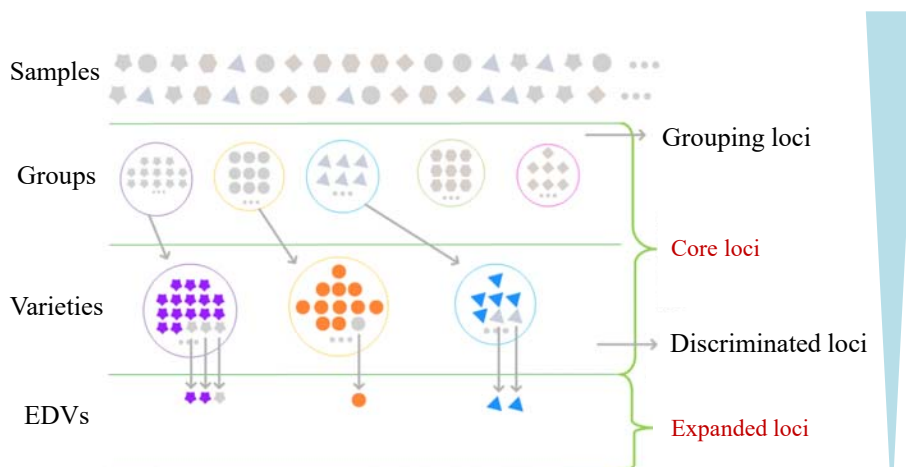
## Questions?

- **Why use SSR and SNP markers in maize variety identification?**
- **Why use the method of the combination of core loci in maize variety identification?**

### 1.1 The method of the combination of core loci - accuracy, cost, efficiency and compatible

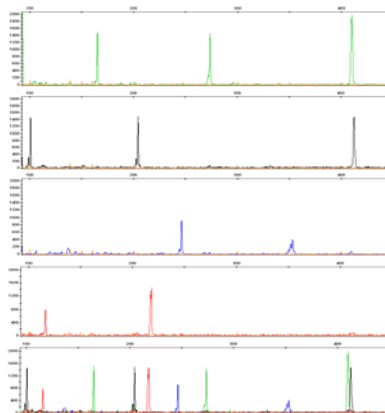
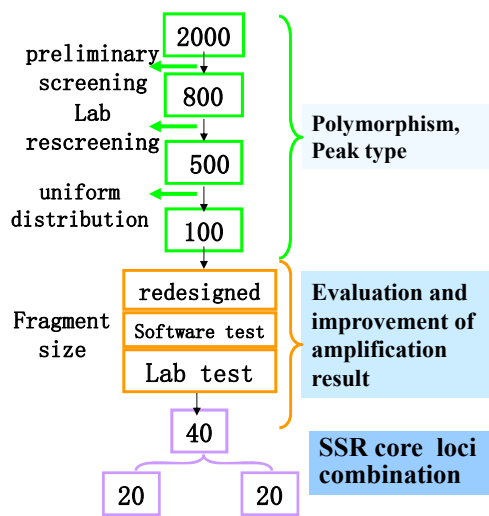


### The process of method of the combination of core loci



## 1.2 Development and selection of SSR core loci

selected process



5 colors - 10 plex fluorescence electrophoresis  
increasing efficiency by 10 times



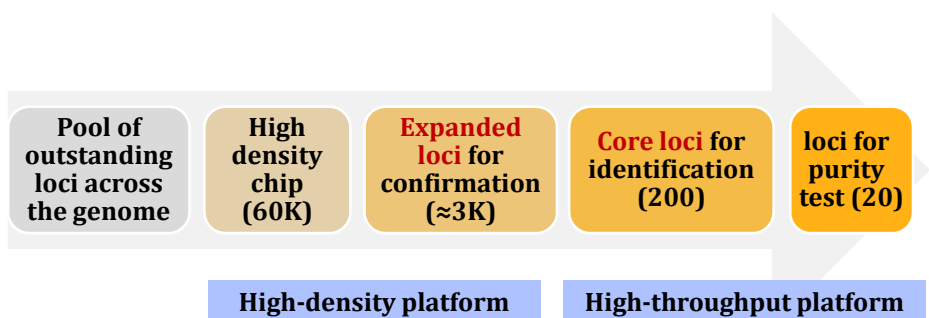
Maize variety identification molecular techniques



General guideline for identification of plant varieties by DNA fingerprinting

Drafted two agricultural industry standards in China

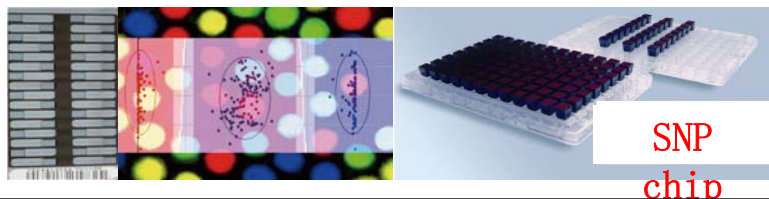
### 1.3 Determination of expanded and core SNP loci



### 1.4 The development of the novel maize SNP chip Maize6H-60K

**Independent property rights and multi purpose:** Maize6H-60K is developed by the Maize Research Center, Beijing Academy of Agriculture and Forestry Sciences with independent IP right. It is the first maize SNP chip for maize variety identification, IP confirmation and molecular breeding in China.

➤ **Outstanding loci with multiple validation:** based on the deep sequencing of 400 Chinese and/or foreign inbred lines with broad representation, outstanding loci were revealed. Along with the previous 6 maize chips, the Maize6H-60K was finalized after multiple rounds of validation.



### Features of maize SNP chip Maize6H-60K

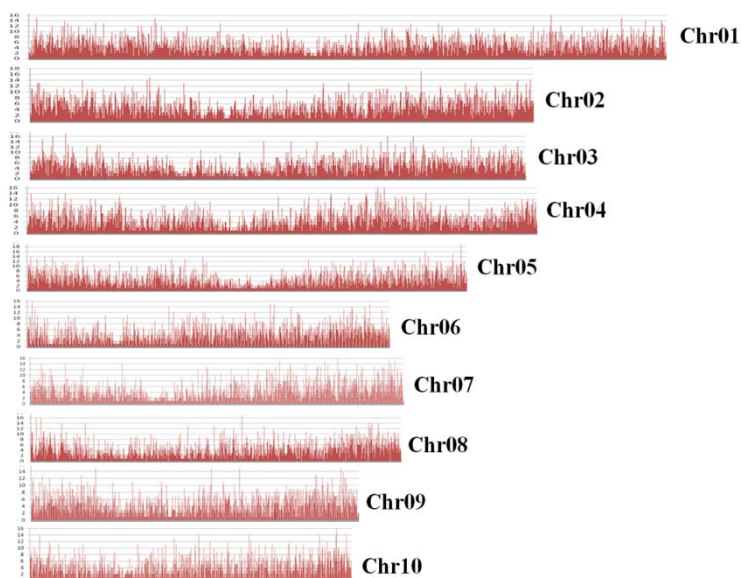
- High Density: **more than 60,000** loci, evenly distributed on the genome;
- High Quality: **over 90%** are effective loci, and the genotyping data can be analyzed automatically ;
- High Discrimination Power: Loci **MAF  $\geq 0.2$** ;
- High Compatibility : compatible with both **Affymetrix** and **Illumina** chip platform;
- High Flexibility: both of **384** and **96** formats are available;
- High Cost Efficiency: the **best cost performance** among chips with mid- to high-density.



384 chip

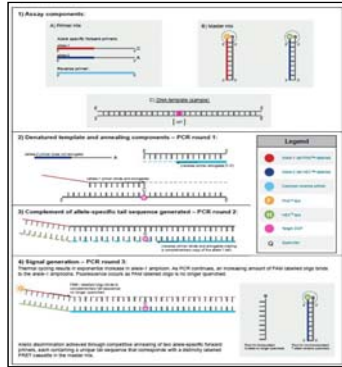


Mini-96 chip



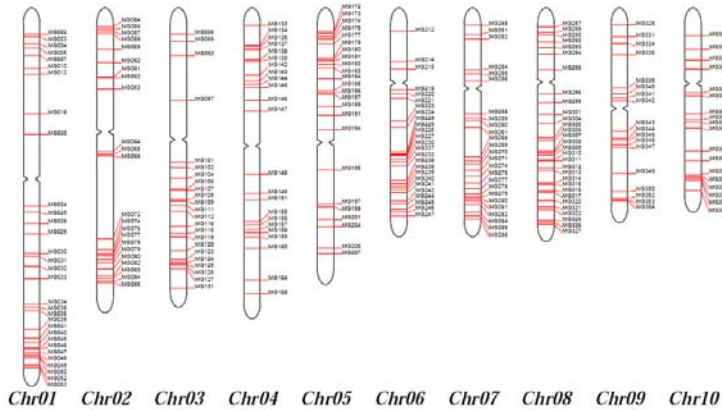
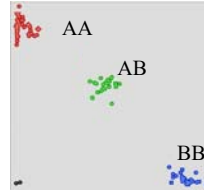
The distribution of loci in Maize6H-60K on the maize genome based on 100kb window.  
X-axis indicates the window number, and y-axis represents the number of loci.

### 1.5 Features of core SNP loci for maize variety identification



- High polymorphism
- Desired genotype
- High conservative
- Highly stable
- Mendelian heredity
- Compatible with platforms (KASP, chip, NGS...)

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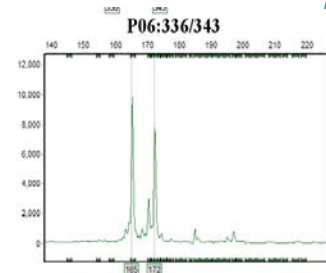
Distribution of SNP Core Loci for Maize Variety Identification on Chromosomes

## 2 Maize standard DNA fingerprint database

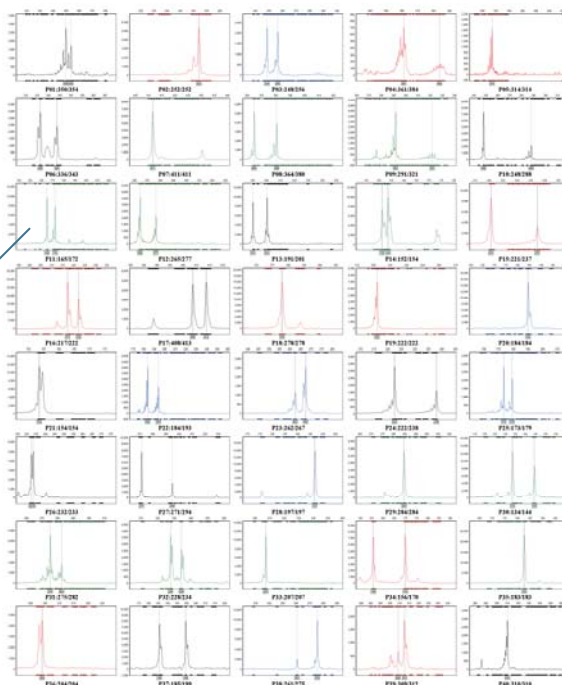
	SSR-DNA fingerprint database	SNP-DNA fingerprint database
Samples	> 30,000 samples (VCU, PVP, Registered varieties, inbred lines)	> 10,000 samples (Registered, PVP varieties, representative lines)
Loci	40 SSR Core loci	200 SNP Core loci
Platform	CE	KASP & Chip
Data	Allele name-number (standardized by core loci Panel)	Deoxynucleotide code-ATCG (standardized by SNP management system)
Reference samples	2 homozygous DH lines	2 homozygous DH lines
Function	Support for authenticity identification of DUS, PVP, random inspection, VCU testing, EDV; purity testing; paternity testing; clustering...	

### SSR-DNA fingerprint management system display

- Graphical format
- Digital format



Standard DNA fingerprint for hybrid Jingke968



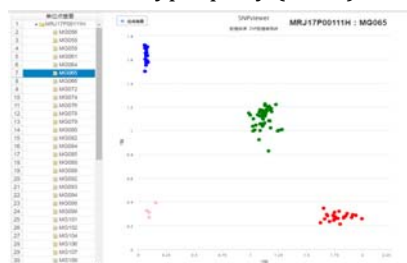


## Demonstration of the SNP-DNA fingerprint management system

Sample query

序号	样品编号	样品名称	样品来源	样品类型	负责人	所属	创建时间	操作
1	8002	CP024	81001022	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
2	8003	德豆13	81001075	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
3	8007	德豆25	81000429	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
4	8008	德豆4号	81000373	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
5	8009	德豆4号	81001676	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
6	80010	德豆1号	81000419	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
7	80011	德豆2号	81000310	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
8	80012	长绿7号	81000314	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
9	80013	长绿7号	81000310	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除
10	80014	长绿5号	81000741	芸豆种质资源样品	张彦坤	芸豆	2018-08-09	删除

Genotype query (KASP)



Fingerprint query

序号	样品编号	名称	来源	负责人	所属
1	8100001	MQ001A	芸豆	张彦坤	芸豆
2	8100002	MQ002A	芸豆	张彦坤	芸豆
3	8100003	MQ003A	芸豆	张彦坤	芸豆
4	8100004	MQ004A	芸豆	张彦坤	芸豆
5	8100005	MQ005A	芸豆	张彦坤	芸豆
6	8100006	MQ006A	芸豆	张彦坤	芸豆
7	8100007	MQ007A	芸豆	张彦坤	芸豆
8	8100008	MQ008A	芸豆	张彦坤	芸豆
9	8100009	MQ009A	芸豆	张彦坤	芸豆

Comparison outcomes

序号	样品编号	名称	来源	负责人	所属
7	8100007	MQ007A	芸豆	张彦坤	芸豆
24	8100024	MQ024A	芸豆	张彦坤	芸豆
25	8100025	MQ025A	芸豆	张彦坤	芸豆
215	8100215	MQ021A	芸豆	张彦坤	芸豆

**Automatically genotyping calling and comparison**

**Q: What are the advantages of SSR and SNP markers in variety identification?**

**SSR - accuracy confirming**

- High polymorphism
- Strong power of distinguish varieties
- Mid-throughput

**complementary**

**SNP - fast preliminary screening and identifying EDV**

- High-density method to distinguish EDV
- High-throughput platform
- Automatically genotyping calling

### 3 A case of integration of SSR and SNP markers in maize variety identification

#### First stage - fast preliminary screening (SNP)

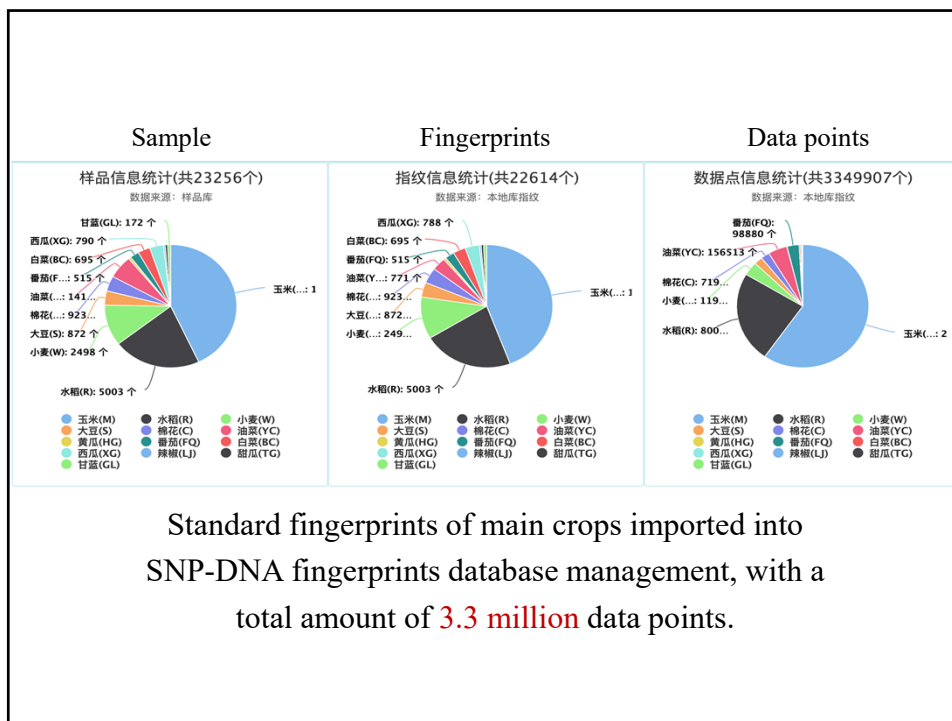
- 1047 samples 200 loci 2 repeats (418,800 data point)
- Construction database 1d
- Genotyping calling <1d
- Comparing with standard SNP-DNA database ~10min

#### Second stage – accuracy confirming (SSR)

- 138 suspectable samples 40 loci (5520 data point)
- Construction database 0.5d
- Genotyping calling 1h
- Comparing with standard SSR-DNA database 1h
- Finally 90 samples were found out
- About 1000 samples reduction in the confirming part
- About 65% suspectable samples were verified.
- Time cost largely reduced.

### 4 Support Funds: Research and application of molecular fingerprint detection technology for main crop (National key R & D plan) 2017.7-2020.12

- Maize, rice, wheat, soybean, cotton, pepper, watermelon, cucumber, Chinese cabbage, tomato, oil seed rape
- Goals:
  - Development of the combination of **core loci** and **extension loci** of each crop
  - Establish **fingerprint detection technology system** based on SNP and draft **national or industrial standards**
  - Develop a **DNA fingerprint data management system** compatible with multi crop, multi marker and multi platform, **build more than 50000 varieties of standard DNA fingerprints**
  - Research on the **common technology of DNA molecular identification** of main crops
  - Used in the administrative management, market monitoring and technical services such as the test, approval, registration, power confirmation and law enforcement of crop varieties.



## 4 Looking forward in the future

Authorities	DUS	Electrophoresis
Purity	Protein	KASP
GMO	SSR	Hydrolysis Probe
Specific trait	SNP	High-density chip
Gene editing	InDels	MS
...	...	NGS
		...

**accuracy, cost, efficiency and compatible**

....ATGAC.... ACACGCCA.... TCGGGGTC.... GTCGACCG.... TCGT....  
....GTGAC.... ACACGCCA.... TCGAGGTC.... GTCACCG.... TCGC....  
....GTGAC.... ACATGCCA.... TCGGGGTC.... GTCACCG.... TCGT....  
....GTGAC.... ACACGCCA.... TCGGGGTC.... GTCGACCG.... TCGT....

Thanks for your  
attention!



[End of Annex and of document]