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APPLICATION OF SSR AND SNP IN MAIZE VARIETY IDENTIFICATION AND DATABASE CONSTRUCTION

Document prepared by an expert from China

Application of SSR and SNP in maize variety identification and database construction

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SSR

- expressed co-dominantly
- generally easy to record
- robust
- repeatable in different laboratories
- have automated analyses with high throughput

Select SSR for the present maize variety identification and database construction.

- The following four aspects were optimized and standardized , which would be helpful for effective use of SSR to construct high-throughput DNA fingerprint database for variety identification.
  1. DNA and reagent
  2. detection platform
  3. core primers
  4. standard

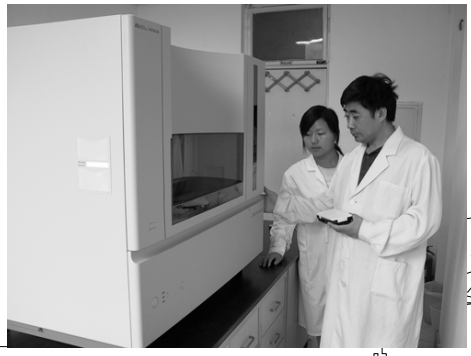
1. To guarantee quality of DNA and reagent

- DNA: OD260/OD280 value is 1.8-2.0
- Primer: be fluorescent-labeled or not, which color should be selected
- Taq and PCR reaction reagent: designated suppliers and specifications
- PCR reaction: be amplified with uniform procedure and system

2. Selection of detection platform

- Select the five-color fluorescence capillary detection system after comparing the following four different systems:
  - Agarose gel electrophoresis
  - Non-denaturing polyacrylamide gel electrophoresis
  - Denaturing polyacrylamide gel electrophoresis combined with silver staining
  - Capillary electrophoresis combined with multi-color fluorescence detection

ABI3730xl DNA analyzer

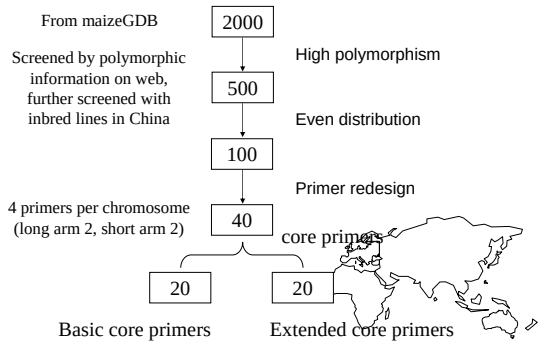


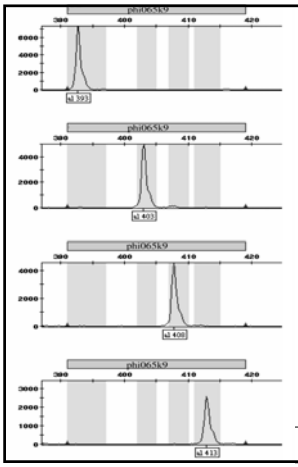
3. Determination of core primers

What are core primers?

primers with high polymorphism, high stability, good repetition, etc., were preferably selected in preliminary research.

Development steps of core primers





A single locus:

(1) Product quality was good under standard assay conditions, i.e. no non-specific fragments, acceptable peak morphology and low stutter.

(2) Alleles must be differentiated easily therefore intervals between adjacent alleles were required.

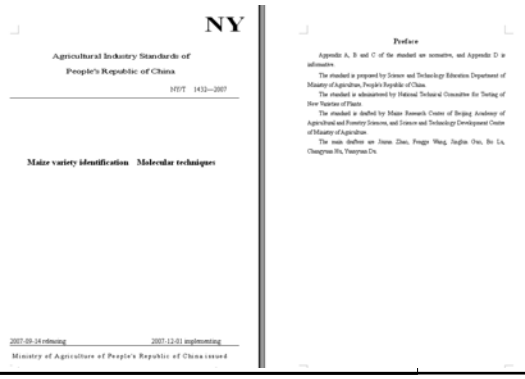
(3) Primers must exhibit useful discrimination power (DP) values. It was inefficient to construct a database using low DP primers.

The combination patterns of the 20 primers of Ⅰ group (two 10-plex sets) and the 20 primers of Ⅱ group (two 10-plex sets)

Set	Locus name	Chr BIN	Allele range	Fluorescence labeled	Set	Locus name	Chr BIN	Allele range	Fluorescence labeled
Ⅰ-1	umc1432r6	10.02	211-259	VIC	Ⅱ-1	umc1429y7	5.03	125-145	VIC
Ⅰ-1	umc2105k3	3.00	250-350	VIC	Ⅱ-1	umc1999y3	4.09	167-200	VIC
Ⅰ-1	phi072k4	4.01	405-432	VIC	Ⅱ-1	umc1459y3	3.07	234-265	VIC
Ⅰ-1	umc1545y2	7.00	150-249	NED	Ⅱ-1	umc114"y4	1.07	149-172	NED
Ⅰ-1	bnlg1702k1	6.05	260-347	NED	Ⅱ-1	phi299952y2	6.07	200-254	NED
Ⅰ-1	phi065k9	9.03	391-415	NED	Ⅱ-1	umc2163w3	10.04	250-352	NED
Ⅰ-1	bnlg2406k1	8.06	220-239	PET	Ⅱ-1	phi2333"6y1	8.09	150-222	PET
Ⅰ-1	bnlg439w1	1.03	319-369	PET	Ⅱ-1	bnlg490y4	4.04	245-331	PET
Ⅰ-1	bnlg2305k4	5.07	240-312	FAM	Ⅱ-1	umc2054w2	9.01	184-214	FAM
Ⅰ-1	bnlg1940k7	2.08	324-388	FAM	Ⅱ-1	phi96109y1	2.00	231-257	FAM
Ⅰ-2	umc1125r3	7.04	149-175	VIC	Ⅱ-2	umc1956k4	7.03	153-176	VIC
Ⅰ-2	umc1335r5	1.06	233-257	VIC	Ⅱ-2	umc2160k3	7.01	195-244	VIC
Ⅰ-2	phi053k2	3.05	333-363	VIC	Ⅱ-2	phi041y6	10.00	296-334	VIC
Ⅰ-2	bnlg1618k8	6.00	154-216	NED	Ⅱ-2	bnlg2235y5	8.02	174-198	NED
Ⅰ-2	umc2007y4	2.04	233-300	NED	Ⅱ-2	umc0536k9	2.07	216-238	NED
Ⅰ-2	bnlg2191k4	4.06	362-421	NED	Ⅱ-2	bnlg249k2	6.01	259-313	NED
Ⅰ-2	umc1504k12	10.05	163-196	PET	Ⅱ-2	bnlg1529k3	2.09	156-204	PET
Ⅰ-2	umc1"05w1	5.03	254-349	PET	Ⅱ-2	umc1231k4	9.05	239-283	PET
Ⅰ-2	phi050k15	8.08	202-238	FAM	Ⅱ-2	bnlg16"1y17	1.10	173-255	FAM
Ⅰ-2	umc1492y13	9.04	270-290	FAM	Ⅱ-2	umc2115k3	5.02	265-295	FAM

The combination pattern was 3+3+2+2. Each set of primers were divided into four groups based on the four labeling types (VIC, NED, PET, FAM). Markers with the same label and allele size ranges separated by more than 10bp were considered a group.

4. The standard for SSR in maize variety identification



NY

Agricultural Industry Standards of People's Republic of China

NY/T 1432—2007

Maize variety identification Molecular techniques

Preface

2007-09-14 released 2007-12-01 implemented

Ministry of Agriculture of People's Republic of China issued

Extensive application area of SSR marker

Main aspects:

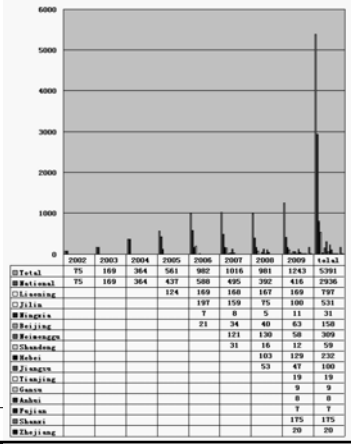
- ✓ Varieties protection
- ✓ Supervision of varieties in regional trial
- ✓ Varietal purity identification
- ✓ Varietal authenticity identification

1. Database construction of varieties applied for plant varieties protection

- Up to Dec. 31, 2009, 2371 maize varieties had been applied for protection, of which 1194 were authorized.
- Now the database of 1300 applied varieties have been finished.

2. Supervision in maize regional trial

- Up to Dec. 31, 2009, the database of 5391 maize varieties in regional trial had been constructed, including national and 13 major corn provinces'.



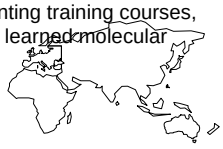
	2002	2003	2004	2005	2006	2007	2008	2009	total
Total	75	169	364	561	982	1016	981	1243	5391
National	75	169	364	437	588	495	392	416	2936
Liaoning				124	169	168	167	169	797
Jilin					197	159	75	100	531
Ningxia					7	8	5	11	31
Beijing					21	34	40	63	158
Heinongye						121	130	58	309
Shandong						31	16	12	59
Hebei							103	129	232
Jingsyn							53	47	100
Tianjing								19	19
Guzn								9	9
Anhui								8	8
Fujian								7	7
Shanxi								175	175
Zhejing								20	20

Supervision content

- Distinction identification
- Uniformity identification
- Change of hybrid composition during different years
- Construction of DNA fingerprint database
- Tracking and monitoring varieties which have passed the trial

3. Application in varietal purity identification

- Provide technical service of varietal purity identification: detect more than 500 samples annually for research academies, seed production and managerial departments to avoid great losses.
- Published <DNA fingerprint of maize hybrids>, including 192 maize hybrids' purity fingerprint by SSR.
- Hold several national DNA fingerprinting training courses, in which hundreds of technicians have learned molecular techniques.



4. Application in authenticity identification, judicial identification of infringing cases

- Since the variety protection was carried out in 1999, cases on infringing variety rights increased.
- DNA fingerprint becomes an important method in these cases.
- Has rich experience in varietal authenticity identification using SSR.

SNP

Compare to SSR, SNP

- occurs at a much higher genomic density
- higher throughput
- has lower genotyping error rate
- easier data collection

Technological upgrading from SSR to SNP is necessary.

Explore 2 detecting systems:

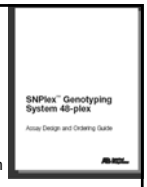
- SNPlex™ Genotyping System (ABI):

  48-plex

- MaizeSNP50 Beadchip (illumina):

  features more than 55,000 evenly spaced SNPs across the entire maize genome.

SNPlex™ Genotyping System



7600 SNP loci released on public database, e.g. *Panzea*

⇩ PIC value＞0.45, Chromosomal locations are known

424 Candidate SNP loci

⇩ Even distribution; Highest PIC value; Satisfy the requirements of assay design

48 selected SNP loci

⇩

SNPlex™ Genotyping System

1. Phosphorylation Module
2. Ligation Module
3. Amplification Module
4. Purification Module
5. Hybridization Module
6. Controls
7. Standards Module

⇩

Data collection and analysis

Materials:

- 112 Chinese inbred lines representing diverse pedigrees and geographic origins
- 38 main hybrids

Advantages:

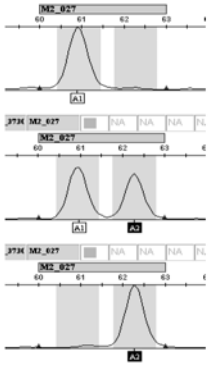
- ➢High throughput
- ➢Bi-allelic and easy of data collection

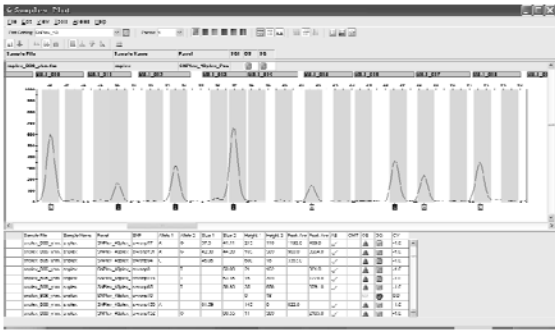
In this SNPset,

Number of total SNPs: 48

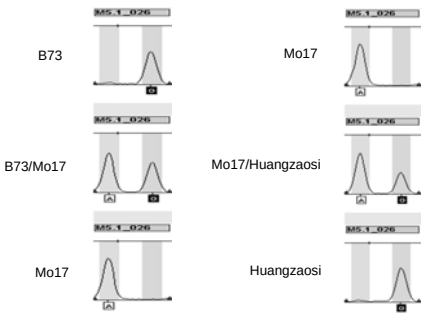
Number of passed SNPs: 42

Assay pass rate: 87.5%

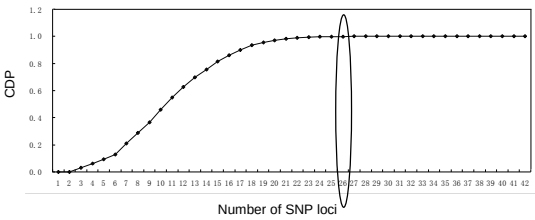




Genotyping result of 7 SNP loci for Huangzaosi maize line



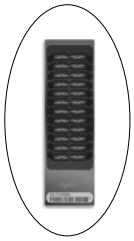
Genotyping result of the locus M026



Cumulative discrimination power (CDP) value of 42 SNP loci

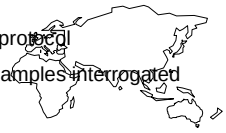
CDP value of 26 loci reaches 0.9991. While more SNPs may be used to discriminate close-related or essential derivation varieties, the 42 SNPs panel presents enough power for identity control in 150 Chinese main inbred lines and hybrids.

MaizeSNP50 Beadchip



Highlights:

- ❖ >99% average call rates and 99.99% reproducibility
- ❖ Genome-wide coverage using over 55,000 evenly spaced markers
- ❖ PCR-free protocol
- ❖ Up to 24 samples interrogated in parallel



Materials: 96

- main hybrids and their parents
- three sets of near-isogenic lines

Now data are being analyzed.

Hope:

- Screen and obtain 500-1000 core SNP loci for maize database construction and variety identification instead of SSR.

Thanks for your attention!

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