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CHINESE MAIZE STANDARD DNA FINGERPRINT DATABASE ARE BEING CONSTRUCTED

Document prepared by experts from China

Chinese maize standard DNA fingerprint database are being constructed

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Character a maize DNA fingerprint database should have

- 1) Representativeness
- 2) Openness
- 3) With simple, reliable and stable operating procedure
- 4) Standardization
- 5) integration with morphological database
- 6) With quick and convenient database managerial system
- 7) Extensive application areas

Development in China

1. Varieties selected into database

1000 varieties: including main inbreds, released hybrid varieties, varieties with plant varieties right, varieties in national or provincial regional trial.

Pattern database include 500 representative varieties; extended database include other 500 varieties and should open to unceasingly increased varieties.

2. optimization of SSR procedure from DNA extraction, PCR amplification and electrophoresis detecting.

Developed quick DNA extraction method: 96 samples extracted in only 10 mins.

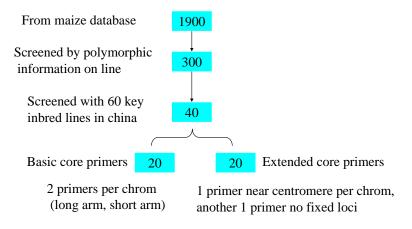
electrophoresis detecting by ABI3730xl DNA analyzer with five-dye fluorescent (10 multiplex PCR, 96-well capillary electrophoresis): average 30 mins per plate ($96 \times 10=960$ samples).

3.determination of a set of core SSR primers

What is Core primer? primers with high polymorphism, high stability, good repetition, et al, preferably selected in preliminary research.

Evaluation criterion of core primer used in maize DNA fingerprint database: (1) accordance with mendelian inheritance; (2) have been localized at fixed chromosome; (3) no or low linkage degree between primers; (4) easy amplification; (5) accurate and easy band tying; (6) sufficiently low mutation rate; (7) coincidence among different tissues of the same individual; (8) high polymorphism; (9) specific among different species; (10) potential of compound amplification; (11) known Allele frequency distribution in maize.

How to screen core SSR primers in maize



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Improve efficiency by multiplex-fluorescent PCR and making a set of maize purity and authenticity identification kit: two 10-multiplex PCR with core primers in maize in our research.

4. Make a standard for DNA fingerprint detection in maize

The national standard of distinction identification in maize are going to be enacted. Including: optimized SSR procedure; 20 pairs of basic core primers and 10 pairs of extended core primers; criteria for whether a variety is distinct from others

Application

1. Application in maize DUS testing

A DNA fingerprint database of about 500 maize varieties has been preliminarily constructed with 20 basic core primers, which will be used for screening most similar variety of a candidate variety in DUS testing.

2. Supervision in national regional trial in maize

Already four years since 2002, have detected nearly 500 accessions. Include uniformity identification, whether hybrid composition changed during different years, distinction identification, tracting and monitoring variety which have passed the trial.

3. Application in Judicial identification of infringing case

Since the variety protection were carried out in 1999, cases on infringing variety right increased greatly. morphological identification couldn't fit for the quick and accurate judicial detection, DNA fingerprint become an important method in these cases. Maize research center of Beijing agricultural & forestry academy of science has rich experience in DNA fingerprint identification, and has taken 91 maize infringing cases entrusted by court by now. These DNA fingerprint was as the important basis for judgement.

4. Application in maize purity identification

published <DNA fingerprint of maize hybrids> in 2004, including 192 maize hybrid' purity fingerprint by SSR techniques. Hold several training classes nationally. Provide service of purity identification more than 4000 samples by now.