PUBLICATION NOTE

Illuminate Meiotic Crossover Events with myTags® Fluorescent Probes for Haplotyping Analysis

INTRODUCTION

The generation and study of genetic diversity are critical for both plant and animal breeding. This diversity is propagated by the exchange of DNA between homologous parental chromosomes during meiotic crossover. DNA-FISH technology has undergone many recent advances in applications of chromosomal studies in plants (1). Recently, a team of researchers from Michigan State University, Fujian Agriculture and Forestry University, Iowa State University, and University of Missouri combined efforts with Arbor Biosciences to develop a technique to track the meiotic crossover events in maize (2). The myTags® fluorescent probes generated for haplotype-specific analysis were able to effectively identify parental and recombinant regions of metaphase chromosomes in hybrids and F_2 progeny of Maize B73 and Mo70 hybrid. This technique facilitates the rapid, high-resolution study of genome inheritance patterns essential for plant breeding research.

DESIGNING, SELECTING AND VALIDATING HAPLOTYPE SPECIFIC PROBES

The authors needed to generate oligo-FISH probes that can be used to differentially paint the chromosome 10 from maize inbreds B73 and Mo17. The selection of the probes is based on presence-absence variation (PAV), single nucleotide polymorphisms (SNPs), and/or insertions and deletions (indels) in chromosome 10 sequences derived from the two inbreds. They obtained the following probe sets:

- 6,251 sequences unique to B73 and 5,506 unique to Mo17 (PAV probe sets)
- 4,353 sequences with 5 or more differences (≥5 SNPs probe set)
- 3,894 sequences with 3 and 4 differences (3-4 SNPs probe set)
- 6,506 sequences with 2 differences (2 SNPs probe set)
- 19,885 sequences with a single difference (1 SNP probe set)

The SNPs probes were synthesized as pairs by Arbor Biosciences, with one probe specific to the B73 sequence and the other to the Mo17 sequence. DNA-FISH was performed with these 5 different SNPs probe sets (Figure 1). While single-SNP probes produce a high-cross hybridization signal, they can still discriminate between B73 and Mo17 in competitive co-hybridization (Figure 1e). The combined pools of probes with PAV, ≥5 SNPs, and 3-4 SNPs produced the best contrast of haplotype-specific FISH signals with minimal cross hybridization. These two probe sets were designated hapB (haplotype B73, red) and hapM (haplotype Mo17, green) and were used in all haplotyping FISH experiments.

POTENTIAL OF HAPLOTYPE-SPECIFIC CHROMOSOME PAINTING

- **PAV and multi-SNPs probes give better contrast, but probes with only one SNP can still discriminate between homologous chromosomes**
- **Meiotic crossovers between homologous chromosomes can be easily visualized**
- **Chromosomal breakpoints from historical or multiple crossovers can be mapped**
- **Specific chromosomes derived from a single genotype can be tracked**
- **Extent of somatic recombination could potentially be examined**
- **True homologous chromosome pairing could potentially be distinguished from pairing of homeologous chromosomes with minor structural variation in polyploid species**

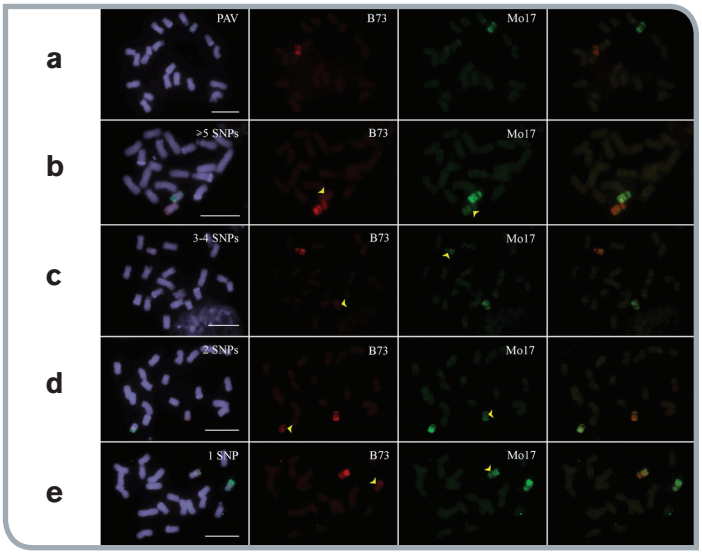

Figure 1. Development of maize chromosome 10 oligo-FISH probes specific to inbreds B73 or Mo17. *Probes specific to the B73 haplotype were detected in red color and probes specific to the Mo17 haplotype were detected in green color.* **a.** *Two oligo-FISH probes based on presence-absence variation (PAV).* **b.** *Two oligo-FISH probes based on 5 or more single nucleotide polymorphisms (SNPs).* **c.** *Two oligo-FISH probes based on 3 or 4 SNPs.* **d.** *Two oligo-FISH probes based on 2 SNPs.* **e.** *Two oligo-FISH probes based on 1 SNP. Images in first column: Complete metaphase cells hybridized with the two FISH probes; Images in the second column: digitally separated red FISH signals derived from the B73-specific probes; Images in the third column: digitally separated green FISH signals derived from the Mo17-specific probes; Images in the fourth column: merged FISH signals derived from both B73 and Mo17. Bar = 10 µm*

IDENTIFYING B73 AND MO17 CHROMOSOME 10 CROSSOVER IN HYBRIDS

The production of F2 plants was generated by pollinating sibling B73 X Mo17 hybrid plants. DNA-FISH hybridization was performed on somatic metaphase chromosomes from 58 F2 plants (BM1-BM58) for a total of 116 copies of chromosome 10 (Figure 2). The chromosomes can be cataloged as 8 different types based on the positions of chromosomal exchanges (Figure 2c). In 50 (43%) of the 116 chromosomes at least one unambiguous B73-Mo17 chromosomal exchange was identified, including 6 chromosomes with an exchange on both arms. Three or more crossovers per chromosome were never identified in this analysis.

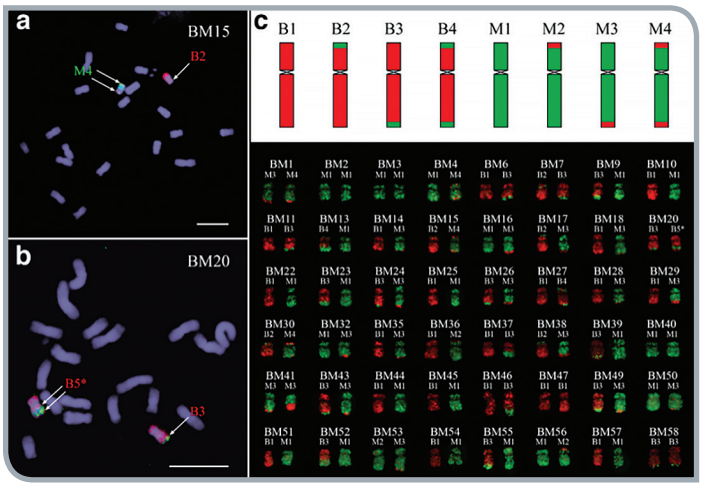

Figure 2. Crossovers between B73 and Mo17 chromosome 10 revealed by oligo-FISH mapping using probes hapB and hapM. **a.** *Oligo-FISH mapping of B73 × Mo17 F2 plant BM15. Probes hapB and hapM are shown in red and green, respectively. The single arrow identifies a single chromosomal exchange position (EP) on the B2-classified chromosome. Double arrows point to the two chromosomal EPs on the M4-classified chromosome. Bar = 10 µm.* **b.** *Oligo-FISH mapping of B73 × Mo17 F2 plant BM20. The single arrow points a single EP on the B3-classified chromosome. Double arrows point to the two EPs of double crossovers (COs) on the B5*-classified chromosome.* **c.** *Upper panel: diagrams of the 8 types of parental or recombinant chromosomes identified in F2 plants. B indicates B73; M indicates Mo17. Lower panel, representative images of the two copies of chromosomes 10 in 48 F2 plants. One copy of chromosome 10 in BM20 is marked as B5*, which is the only chromosome that does not belong to any of the eight types listed in the upper panel. Bar = 10 µm*

CONCLUSION

The authors have demonstrated the application of haplotype-specific DNA-FISH probes to clearly visualize meiotic crossovers between homologous chromosomes derived from different maize inbreds (2). There are multiple traditional methods to map crossovers, but these techniques present a number of limitations. By using synthetic, haplotype-specific DNA-FISH probes, the researchers were able to generate detailed maps of the chromosomal breakpoints resulting from historical crossovers, including breakpoints from multiple crossovers on recombinant inbred lines from intermated populations. This technique is a powerful addition to the DNA-FISH toolkit, and can be applied to a wide variety of agriculturally-relevant genomic research applications.

REFERENCES

1. Jiang, J., *et al.* (2019) Fluorescence in situ Hybridization in Plants: Recent Developments and Future Applications. *Chromosome Res.*

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3. Mikel, M. A. & Dudley, J. W., (2006) Evolution of North American Dent Corn From Public to Proprietary Germplasm. *Crop Sci.*

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