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Constitution of microarray platforms for the analysis of the maize mRNA and small RNA transcriptome

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The completion of maize B73 inbred line genome sequencing project (Schnable et al., Science 326: 1112-1115, 2009; http://www.maizesequence.org) makes possible an implementation of genome-wide approaches to investigate several aspects of the maize genome activity. Using the database from the maize sequencing project we have developed custom microarray-based platforms to be employed as tools to analyze maize mRNA and small RNA (sRNA) transcriptome. Both arrays are based on the Combimatrix technology (http://www.customarrayinc.com). This microarray platform contains 35-40 mers oligos synthesized *in situ* using a semiconductor-based electrochemical-synthesis process, employs single channel Cy5 hybridization, and allows for array stripping and reusing.

The maize mRNA array was prepared using the Combimatrix CustomArray 90K (90.000 oligos). The filtered set of cDNA sequences (ZmB73_4a.53 version) released on March 2010 from the B73 Maize Genome Sequencing Consortium and the OligoArray 2.1 free software (Rouillard et al., Nucl Acid Res 31: 3057-3062, 2003; http://berry.engin.umich.edu/oligoarray2_1) were used to design oligo probes. A specificity check for oligo cross-hybridization against all other genes and for unique genome position was applied. The result was a maize mRNA array containing 45.000 probes (2 replicate for each selected target), representing 51.109 transcripts and 30.190 protein-encoding genes, which are the 96% and 93%, respectively, of the filtered cDNAs set and related genes identified by the Maize Genome Consortium (Schnable et al., Science 326: 1112- 1115, 2009). Information from the Maize Genome Consortium initiative also allowed to associate many of the mRNA probes with a Gene Ontology (GO) and Interpro annotation.

The maize sRNA array was prepared using the Combimatrix CustomArray 4X2K (4 arrays for each slide, and each array containing 2.240 oligos). For each sRNA probe were enclosed two mismatch controls designed to maximally destabilize hybridization, thus allowing to detect specificity of hybridization by comparing match to mismatch signal. Three replicates for both match and mismatch probes were included; therefore, each sRNA is represented by nine probes. Accordingly, the 4X2K sRNA array represents 204 sRNA maize sequences. The tRNA and U6 sequences were also included as positive controls, while anti-sense probes representing abundant mRNAs were the negative controls for degraded mRNA. The sRNA sequences were selected to represent mainly microRNAs (miRNAs). The probes selection criteria were as follows: i) sRNAs encoded by different loci, but with identical sequence or with a single nucleotide

polymorphism in the 5'- or 3'-end, were represented by only one probe, due to impossibility to distinguish differences by array hybridization; ii) all maize miRNA sequences from miRBase database release 14.0 (www.mirbase.org) were included; iii) all monocotyledonous miRNAs, distinct from maize miRNAs, with at least one predicted putative target into the maize genome (see below) were included; and iv) 78 sRNAs derived from high-throughput maize sRNA sequencing (Wang et al., Plant Cell 21:1053-1069, 2009), distinct to miRBase maize miRNAs and exhibiting a predicted ability to form short hairpin structure (shRNA), were selected as putative new miRNAs. Specifically, both monocotyledonous miRNAs and maize shRNAs were chosen by applying bioinformatics analysis to identify their putative targets according the method employed by Zhang et al. (PLoS Genetics 5: e716, 2009) and using the B73 genome sequence as a database. This method allows us to enclose in the array the shRNAs that have as putative targets the exon or intron sequence of both a low (less than five) and a high (from five to approximately one hundred) number of genes. In some cases these shRNAs target MITE-like repeats located within the same gene. The shRNAs may, therefore, represent new maize miRNAs or new type of sRNAs, exhibiting a mix of miRNA and short interfering sRNA (siRNAs) features, thus representing possible ancestor of miRNAs.

Both maize mRNA and miRNA custom arrays described above were employed as a tool to analyze transcriptome change in response to abiotic stresses (Altana et al. b, this issue of MGC Newsletter) and are available for scientific community for investigation of gene regulation.