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SAM 2.0 Chip

A 14,080 element microarray chip (SAM2.0 chip) was generated at Iowa State University's Center for Plant Genomics. Of the 14,080 elements printed on the UltraGAPs slide (Corning, Inc.), 12,585 cDNA clones from the Stanford UnigeneI , UniGeneII_library947, UniGeneII_library949, UniGeneIII_952, UniGeneIV_3524, UniGeneV_946 (http://www.genome.arizona.edu/orders/), 793 cDNA clones from the ISUM3, ISUM4, ISUM5, ISUM6 and ISUM7 libraries (http://schnablelab.plantgenomics.iastate.edu/research/genomics/htp_est/) prepared at Iowa State University as part of a National Science Foundation Plant Genome Project. Also 47 lab interested genes and 192 human gene spots are included on the chip. The full-length inserts from the Stanford UnigeneI , UniGeneII_library947, UniGeneII_library949, UniGeneIII_952, UniGeneIV_3524, UniGeneV_946 were PCR amplified from denatured O/N E. coli culture, then purified and quantified (Nakazono et al. 2003 *Plant Cell*, 583-596). Most PCR negative (no band or smears) wells were removed, and a few were spotted onto the chips with labeling badPCR in the GeneID files. The 3' ends of cDNA inserts from mapped 793 cDNA clones and 47 lab interested genes were PCR amplified using gene-specific primers in 100 µl PCR reactions containing 1X PCR buffer (200 mM Tris-HCl, pH 8.4 and 500 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, and Taq polymerase. The PCR program cycle was: 94°C for 3 min followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec, 72°C for 2 min 30 sec, and a final elongation step of 2 min. PCR products were purified using Millipore 96-well multiscreen filter plates (LSKC09601) as recommended by the manufacturer and eluted in 50 µl of water. Five microliters of each purified PCR product were electrophoresed on a 1% agarose gel to examine quantity quality. The remaining 45 µl of purified PCR product were dried to completion in a concentrator/evaporator (Labconco, Kansas City, MO) and resuspended in 50% with a final DNA concentration 300 to 500 ng/µl. The PCR amplified cDNA inserts were printed on UltraGAPS, which is uniform covalently bound of pure gamma amino propyl silane coating (Corning, NY, Cat. No. 40015). The whole array consists of 48 subgrids printed in 18- x 54-mm array area. PixSys 5500 arrayer (Cartesian Technologies, Irvine, CA) equipped with ChipMaker3 pins (TeleChem, Santa Clara, CA) is used for printing. Slides were UV cross-linking at 300 mJ with a Stratalinker (Stratagene) for immobilization, and stored in desiccator at ambient temperature.