Microarray-based methods for monitoring rare transcripts, DNA methylation, and protein binding to promoters.

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Microarray-based technologies have allowed the acquisition of data on (1) rare transcripts, (2) DNA methylation, and (3) proteins bound to DNA. (1) PCR with "arbitrary" primers generates a low complexity representation of the mRNA population that can detect rare transcripts, including nascent transcripts, which cannot be detected by conventional microarray methods. The amplified products are identified by hybridization to an array of transcript sequences, including intron sequences. The method is called "RNA arbitrarily primed PCR-Array" (RAP-Array). The next two methods use an array of 10,771 human promoter regions, encompassing most known protein coding genes: (2) Monitoring DNA methylation and copy number. Genomic DNA is digested with a methylation-sensitive enzyme *Hpa*II, followed by linker ligation. After removal of repeats, ligated fragments are PCR amplified, labeled, and hybridized to the array. Only those parts of the genomic DNA that have unmethylated restriction sites within a few hundred base pairs generate PCR products detectable on an array. Thus, methylated regions are distinguished from unmethylated regions. The method is named "Methylation-sensitive restriction complexity reduction" (MRCR). Application of this method to monitor methylation differences in prostate cancer cell lines is described. (3) Finally, ChIP is a well known method of cross-linking chromatin and specific immunoprecipitation of a protein bound to DNA. In "ChIP-on-chip" the precipitated DNA is amplified and applied to the microarray in order to identify the profile of precipitated sequences. Application of this method to generate transcription factors binding profiles is described. The arrays described here are freely available to collaborators.