

MICROSPHERE ASSAYS FOR Y-CHROMOSOME SNPs

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The human genome contains vast amounts of sequence variation. Current DNA typing methods capture a tiny fraction of that variation. We have explored methods of exploiting the remaining diversity of human DNA, while reducing assay costs and time. Liquid Arrays of microspheres, developed by the Luminex Corporation, allow hybridization of up to 100 allele-specific oligonucleotides in a single tube. In this study, the application of this technology to screening Y-chromosome SNPs was investigated.

Liquid arrays of allele-specific oligonucleotides were coupled to fluorescently tagged latex microspheres. Each oligonucleotide was associated with a particular "color" fluorescent microsphere. DNA samples were amplified by PCR with primers labeled with a fluorescent reporter dye. The labeled amplification products were hybridized for 30 min to the virtual array of oligonucleotides on microspheres, and then analyzed directly, without washing, in a Luminex 100™ flow reader. The Luminex 100™ measures the median fluorescent intensity of reporter dye associated with each color classification of microsphere, and hence with each allele-specific oligonucleotide.

A panel of 10 Y-chromosome SNPs plus the amelogenin locus were used to successfully genotype 17 male individuals. The total assay could be completed in 1 hour after PCR was completed. The recent availability of a large number of additional Y-chromosome SNPs will allow expansion of the panel up to the capacity of the reader.