#3720 **Rapid sequencing of the p53 gene with the MicroGene Blaster automated DNA sequencer.** Bharaj, B., Angelopoulou, K., and Diamandis, E.P. *Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada.*

p53 is the most commonly mutated gene in human cancers. It regulates cellular growth after DNA damage by arresting growth at the G1 phase of the cell cycle and permitting apoptosis in cells that fail to repair. Approximately 92% of the p53 mutations are localized in the domains encoding exons 5 through 8. Sequencing of codons 175 to 282 alone has resulted in the detection of nearly 85% of the mutations. Sixteen breast cancer tumors, with previously identified mutations in the p53 gene were analyzed with a newly developed automated sequencing technique. Exons 5 through 8 were amplified using PCR and the reactions were subsequently subjected to cycle sequencing, using Cy5.5-labelled primers. These products were resolved on the MicroGene Blaster automated DNA sequencer (Visible Genetics Inc., Ontario, Canada), which can sequence 300 bases in 30 minutes. Of these 16 tumors, two had mutations in exon 5 (codon 163 Tyr to Cys and codon 132 Lys to Glu),two in exon 6 (codon 220 Tyr to Cys), three in exon 7 (codon 245 Gly to Ser; codon 248 Arg to Tyr and codon 249 Arg to Thr), and three in exon 8 (codon 278 Pro to Ser, codon 280 Arg to Thr and codon 283 Arg to Cys). Two tumors had polymorphisms in exon 6 (codon 213). In all cases we identified the same mutations by the MicroGene Blaster and the ALF Express sequencer (Pharmacia Biotech., Uppsala, Sweden). These data demonstrate that the MicroGene Blaster can sequence very rapidly as compared to the conventional automated sequencers, without compromising accuracy and resolution.

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