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Ultra-sensitive detection of BRAF V600E and G469A mutations by Ice COLD-PCR and BLOCker-Sequencing

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BRAF, a serine/threonine kinase, mediates the RAS/RAF/MAPK signal transduction pathway. Over 90% of BRAF mutations are V600E (c.1796T>A); this activates mitogenic cascades leading to tumorigenesis. This mutation is present in 70% melanoma, 100% hairy cell leukemia, 40% papillary thyroid cancer and, less frequently, other cancer types. V600E mutation status is valuable for determining clinical diagnosis, companion diagnostic tests, treatment guidance and outcome prediction. Ice COLD-PCR (Improved & Complete Enrichment CO-amplification at Lower Denaturation temperature) is technology that enriches mutated DNA sequences in an excess of wild-type DNA through selective amplification of the mutant DNA population using LNA-modified oligonucleotides (RS-oligo) complementary to wild-type sequence. After Ice COLD-PCR enrichment, V600E mutations could be detected at 0.05% as confirmed by standard Sanger sequencing. For the BRAF mutation G469A (c.1406G>C), limit of detection was 0.01%. We combined Ice COLD-PCR enrichment and dye terminator sequencing using a novel sequencing methodology, BLOCker-Sequencing (BLocking Oligonucleotide Cycle Sequencing). Wild-type strand sequencing is blocked by a BLOCker-oligo but mutant DNA is concurrently sequenced with one primer and amplified with a 5' phosphate primer . Lambda exonuclease selectively removed all products resulting from incorporation of the primer containing the 5' phosphate. BLOCker-Sequencing after standard PCR increased the limit of V600E detection from 20% for standard Sanger sequencing to 1%. This compares favorably with other commercially available non-sequencing based mutation detection systems. The results presented here will demonstrate enhanced limits of detection when BLOCKer-Sequencing is used following Ice COLD-PCR amplification.

Biography

Dr. Yanggu Shi completed his Ph.D. from New York University and postdoctoral studies from Columbia University College of Physicians and Surgeons. He is a Sr. Scientist at Transgenomic, Inc. Prior to this he was a Sr. Scientist at Human Genome Sciences, Inc.