Colorectal cancer (CRC) is one of the most prevalent types of cancer, causing significant morbidity and mortality worldwide. CRC is curable if diagnosed at an early stage. Mutations in the oncogene KRAS play a critical role in early development of CRC. Detection of activated KRAS is of diagnostic and therapeutic importance. In this study, KRAS gene fragments containing mutations in codon 12 were amplified by multiplex PCR using a 5'-Cy5-labeled reverse primer in combination with 3'-mutation-specific forward primers that were linked with four unique nucleotide-sequence tags at the 5'-end. The Cy5-labeled reverse primer was extended under PCR amplification to the 5'-end of the mutation-specific forward primers and thus included the complimentary sequence of the tag. PCR products were hybridized to tag-probes immobilized on various substrates and detected by a scanner. Our results indicate that all mutations at codon 12 of KRAS derived from cancer cells and clinical samples could be unambiguously detected. KRAS mutations were accurately detected when the mutant DNA was present only in 10% of the starting mixed materials including wild-type genomic DNA, which was isolated from either cancer cells or spiked fecal samples. The immobilized tag-probes were stable under multiple thermal cycling treatments, allowing re-use of the tag-microarray and further optimization to solid PCR. Our results demonstrated that a novel oligonucleotide-tagged microarray system has been developed which would be suitable to be used for detection of KRAS mutations and clinical diagnosis of CRC.
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Keywords: ONCOLOGY, K-RAS MUTATIONS, METASTATIC COLORECTAL-CANCER, FRAGMENT LENGTH POLYMORPHISM, POINT MUTATIONS, GENE-MUTATIONS, ENRICHED PCR, DNA PROBES, GROWTH, SAMPLES, TUMORS, colon cancer, KRAS, mutation, microarray, hybridization, diagnostics, Primates Mammalia Vertebrata Chordata Animalia (Animals, Chordates, Humans, Mammals, Primates, Vertebrates) - Hominidae [86215] human common MCF7 cell line cell_line human breast cancer cells LNCaP cell line cell_line human prostate cancer cells PC3 cell line cell_line human prostate cancer cells LS174T cell line cell_line human colon cancer cells HT29 cell line cell_line human colon cancer cells A549 cell line cell_line human lung cancer cells A427 cell line cell_line human lung cancer cells CALU-1 cell line cell_line human lung cancer cells SW620 cell line cell_line human ovarian cancer cells Colon205 cell line cell_line human colon cancer cells SW480 cell line cell_line human ovarian cancer cells, human KRAS gene [Hominidae] mutation, codon 12, 3'-mutation-specific forward primers, 5'-Cy5-labeled reverse primer, DNA, 02508, Cytology - Human, 03502, Genetics - General, 03508, Genetics - Human, 10062, Biochemistry studies - Nucleic acids, purines and pyrimidines, 12504, Pathology - Diagnostic, 14004, Digestive system - Physiology and biochemistry, 14006, Digestive system - Pathology, 24001, Neoplasms - Diagnostic methods, 24004, Neoplasms - Pathology, clinical aspects and systemic effects, Gastroenterology, Methods and Techniques, Molecular Genetics, Oncology, colon cancer Colonic Neoplasms (MeSH), digestive system disease, neoplastic disease diagnosis, genetics, Biochemistry and Molecular Biophysics, Human Medicine, Medical Sciences, feces digestive system, genotyping laboratory techniques, genetic techniques, multiplex PCR laboratory techniques, genetic techniques, oligonucleotide-tagged microarray analysis laboratory techniques, genetic techniques, PCR assay laboratory techniques, genetic techniques
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