

Proof-of-Principle Evidence for a Clinically-Compatible, Single-Tube Method for Detection of Loss of Heterozygosity in Human Neoplasias Based on Linear-After-The-Exponential (LATE) PCR.

J. Aquiles Sanchez, Ph.D., Dpt. of Biology, Brandeis University, Waltham, MA

Authors

J. Aquiles Sanchez¹, Jesse J. Salk², Jessica D. Abramowitz¹, Kaiane Habeshian¹, David Steffin¹, John E. Rice¹, Carissa A. Sanchez², Thomas G. Paulson², Brian J. Reid², and Lawrence J. Wangh¹

¹Dpt. of Biology, Brandeis University, Waltham, MA 02454 and ²Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Abstract

LOH involving tumor suppressor genes occur in human cancers and LOH in premalignant cells potentially provides diagnostic biomarkers of future cancer risk. However, conventional laboratory methods for detection of LOH are too complex and labor-intensive for routine clinical use. We have devised a clinically-compatible strategy for convenient detection of LOH based on LATE PCR. LATE-PCR LOH assays are remarkably easy-to-use, sensitive, and robust. The assays integrate genomic DNA preparation, PCR amplification, and endpoint LOH detection in a single closed-tube format, require minimal operator involvement, detect LOH even when present in at least 10% of the cells tested, accept sample sizes down to single cells, use a single-detection probe for maximal multiplex capacity, and endpoint results are independent of the extent of amplification past the initial point of product detection or the amount of starting genomic DNA. LATE-PCR LOH assays are used to first identify heterozygous SNPs in the vicinity of chromosomal DNA regions of interest in the normal genomes of a patient. These informative SNPs are then tested in premalignant genomes from the same individual to determine if these SNPs are still heterozygous or if they have lost either of their alleles due to LOH. Each LATE-PCR assay efficiently generates large amounts of single-stranded DNA products that remain available for hybridization at multiple temperatures to a single mismatch-tolerant fluorescent probe. Detection of LOH involves determining the ratio of fluorescent intensities from the mismatch-tolerant probe hybridized to LATE-PCR products at three defined temperatures at the end of the amplification reaction. Such fluorescent ratio identifies the SNP allele configuration and reveals with 99.7% accuracy whether the sample has undergone LOH or not. LATE-PCR LOH assays are demonstrated here for detection of 9p21 LOH and 17p13 LOH, two of three key biomarkers of cancer progression in Barrett's esophagus (BE), a premalignant precursor to esophageal adenocarcinoma. LATE-PCR identifies the same LOH events in BE cell lines and in patient BE biopsies as those previously identified in the same samples by conventional microsatellite analysis and Pyrosequencing, the current gold standards for detection of LOH. We anticipate that clinical assays based on LATE-PCR endpoint assays will be reliable, rapid, and relatively inexpensive compared to current methods of LOH detection and will facilitate diagnostic of pre-malignant conditions such as Barrett's esophagus, oral premalignant lesions, and other neoplastic conditions where LOH is a cancer risk biomarker.