Overview

In a variety of disease investigations, tissue samples are preserved for analysis by fixing them in 10% formalin and then embedding them in paraffin. Histopathological examinations involve thin sectioning of the paraffin block, followed by immunohistochemical staining by specific antibodies and light microscopy to assess morphology. For molecular studies, genomic DNA can be extracted from the paraffin sections by microdissection and analyzed by PCR and DNA sequencing techniques.

Because FFPE tissue preservation is universally popular, samples for retrospective molecular analyses are readily available for investigating almost every disease state in the past decade and a half. In situ PCR of FFPE samples has generated several thousand research publications.

DNA Extraction from FFPE Tissues

DNA extraction from FFPE tissues often involves a deparaffinization step with xylene, then successive ethanol washes followed by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The extracted DNA is often damaged, but AmpliTaq Gold DNA Polymerase produces multifold amplification. PCR efficiency can be negatively affected (by a factor of 100–1000, compared to high molecular weight DNA) by inhibitors such as those found in purified Pap-stained cervical cancer samples. The addition of an optimal amount of BSA restores PCR efficiency, and with an internal control such as β-actin, AmpliTaq Gold DNA Polymerase achieves reliable PCR results.

PCR and DNA sequencing reliably assess the mutational status of a wide variety of tissue samples. Because the technique is so highly developed and specific, statistically meaningful correlations can be made about the presence or absence of mutations in the samples. This data set helps to illuminate trends and relationships between gene defects.

Mutational Analysis using Paraffin-Embedded Tissues

Gastrointestinal stromal tumors (GISTs) form a distinctive group of mesenchymal neoplasms. Mutations in the KIT and PDGFRA genes have been associated with the pathogenesis of GISTS. When combined with AmpliTaq Gold DNA Polymerase and an Applied Biosystems thermal cycler, PCR can specifically amplify exons 12 and 18 of the PDGFRA gene.

Amplicons are quickly sorted by their gene. PDGRFA-specific amplifier exons 12 and 18 is compared to the wild type

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Research Results

AmpliTaq Gold DNA Polymerase has been a valuable component in countless studies and has been cited extensively. Koppert et al. used AmpliTaq Gold DNA Polymerase for PCR-SSCP analysis and found the enzyme superior to other polymerases for detection of SNP T1942C in the AXIN1 gene (Figure 2).

Molecular Methods Evaluate a Spectrum of Melanocytic Lesions

In another study involving BRAF and NRAS mutations in various stages of melanoma, Fullen et al. microdissected samples from FFPE tissues, amplified
monitor the time course of an antigen whose expression is induced by CPT-11, a chemotherapeutic agent in metastatic colorectal cancer (Figure 3).

Mutational analysis of FFPE tissues is easily achievable via PCR with AmpliTaq Gold DNA Polymerase and an Applied Biosystems thermal cycler, and via DNA sequencing with an Applied Biosystems DNA analyzer and BigDye® Terminator reagents. In a typical study, multiple exons in a variety of genes can be analyzed for mutations on dozens of tumor samples. Online databases (NCBI) often provide the sequence data required to design primers.

Conclusion

The ability to link quantitative molecular determinations, sequence analysis, and immunohistochemical data may be useful for determining the type or anatomical site of a tumor. This ability might also enable the unraveling of the carcinogenesis mechanism.

AmpliTaq Gold DNA Polymerase has been successful in a variety of studies involving FFPE tissue samples, in which inhibitors and DNA quality might otherwise cause difficulties. Because FFPE tissue sample repositories are widespread and comprehensive, small or very large retrospective molecular studies can be undertaken with confidence.

References

1. United States Military Cancer Institute, Tissue Microarrays. www.usmci.org/mta.html
2. “What is Pathology Informatics?” www.netautopsy.org/whatpinf.htm

Figure 2. Frequent Loss of the AXIN1 Locus but Absence of AXIN1 Gene Mutations in Adenocarcinomas of the Gastroesophageal Junction with Nuclear β-catenin Expression. T1942C PCR-SSCP. Three DNA samples, 1942T/T (homozygous normal), 1942C/C (homozygous polymorphic), 1942T/C (heterozygous) were amplified with AmpliTaq Gold® Polymerase and AmpliTaq Gold® DNA Polymerase detects and AmpliTaq Gold Buffer (A), Promega Taq DNA Polymerase and Promega buffer (B), Promega Taq DNA Polymerase and AmpliTaq Gold Buffer (C) and with AmpliTaq Gold Buffer and Promega buffer (no PCR products obtained). All samples were amplified and SSCP-electrophoresed in the same experiment. Arrows point to the polymorphic SSCP fragment. Note that the polymorphic fragments are clearly visible only after amplification in AmpliTaq GoldBuffer (A, C).

Figure 3. Identification and Immunotherapeutic Targeting of Antigens Induced by Chemotherapy. mRNA transcripts coding for cell-surface proteins induced by CPT-11. Scale bars, 150 µm. (A) Real-time PCR analysis of transcripts from xenograft tumors grown in mice administered CPT-11 (C1-3) or saline (S1-3). Relative amounts of the 6 transcripts were measured in 3 tumors from each group and plotted as fold change relative to S1, arbitrarily set to one. Cycle thresholds (ΔΔCt) were normalized to GAPDH (white bars) and used to design primers.

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