

AmpliTaq Gold® DNA Polymerase and PCR for FFPE Tissue Studies

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 deparaffination 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.

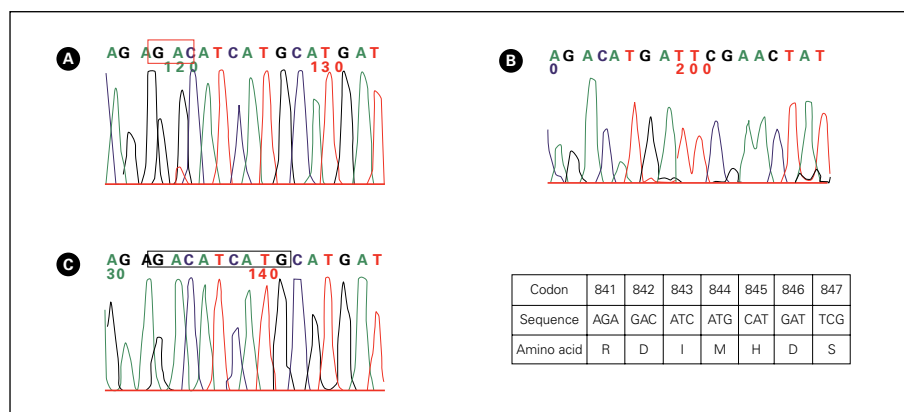


Figure 1. Mutational Analysis using AmpliTaq Gold® DNA Polymerase on Paraffin-Embedded Tissues. Differential expression of *KIT/PDGFR*A mutant isoforms in epithelioid and mixed GIST variants depends pre-dominantly on the tumor site. In panel 1A, the wild type amino acid at position 842 in exon 18 of *PDGFR*A is aspartic acid (D), specified by codon GAC. In the D842V missense mutation shown, the heterozygous DNA sequence is GAC/GTC, aspartic acid (D), and valine (V); hence, D842V. In panel 1B, the 3 codon deletion DIMH842-844 is compared to the wild type sequence in panel 1C. Wild type: AGAGACATCATGCATGAT; DIMH842-844: AGA-CATGATTCGAACAT.

Mutational Analysis using Paraffin-Embedded Tissues

Gastrointestinal stromal tumors (GISTs) form a distinctive group of mesenchymal neoplasms. Mutations in the *KIT* and *PDGFR*A 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 *PDGFR*A gene.

Amplicons are quickly sorted by their sizes using ethidium bromide-stained agarose gel electrophoresis. Column-purified amplicons can be analyzed for mutations by denaturing high pressure liquid chromatography (DHPLC) or by DNA sequencing on an Applied Biosystems DNA analyzer with the BigDye® Terminator Cycle Sequencing Kit.

Figure 1 summarizes the result of *PDGFR*A gene mutational analysis for three representative samples in a GIST study.

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.

Research Results

AmpliTaq Gold DNA Polymerase has been a valuable component in countless studies and has been cited extensively. Koppert et al.⁴ used AmpliTaq Gold DNA Polymerase for PCR-SSCP analysis and found the enzyme superior to other polymerases for detection of SNPT1942C 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.⁵ microdissected samples from FFPE tissues, amplified

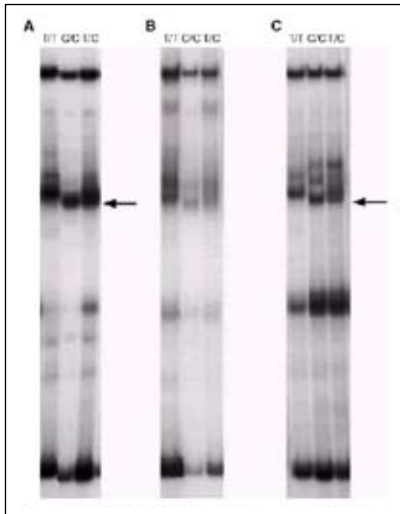


Figure 2. Frequent Loss of the *AXIN1* Locus but Absence of *AXIN1* Gene Mutations in Adenocarcinoms of the Gastroesophageal Junction with Nuclear β -catenin Expression. T1942C PCR-SSCP. Three DNA samples, 1942T/T (homozygous normal), 1942C/C (homozygous polymorphic), 1942T/C (heterozygous) were amplified with AmpliTaq Gold[®] Polymerase and AmpliTaq Gold[®] 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 Gold Buffer (A, C).

them with AmpliTaq Gold[®] DNA Polymerase, and sequenced them on an Applied Biosystems DNA Analyzer. The histopathology data reveal that a *BRAF* mutational status alone cannot unambiguously distinguish Spitz nevi from non-Spitz nevi melanocytic lesions.

Monitoring the Time Course of Induction of Expression

Rubinfeld et al.⁶ used real-time PCR to validate results from oligonucleotide microarray transcript expression profiling. AmpliTaq Gold DNA Polymerase detects mRNA transcripts coding for cell-surface proteins, which are induced by CPT-11 (a topoisomerase inhibitor) in xenograft tumors. In addition, qPCR is used to

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/tma.html
2. "What is Pathology Informatics?" www.netautopsy.org/whatpinf.htm
3. Josefsson, A, Livak, K, Gyllensten U (1999). Detection and quantitation of human papillomavirus by using the fluorescent 5' exonuclease assay. *J Clin. Microbiol.* 37(3):490-496.
4. Koppert, LB (2004) Frequent loss of the *AXIN1* locus but absence of *AXIN1* gene mutations in adenocarcinoms of the gastroesophageal junction with nuclear b-catenin expression. *Br. J. Cancer* 90:892-899.
5. Fullen, D (2006) BRAF and NRAS mutations in spitzoid melanocytic lesions. *Modern Pathol.* 1-9.
6. Rubinfeld, B (2006) Identification and immunotherapeutic targeting of antigens induced by chemotherapy. *Nat. Biotechnol.* 24(2):205-209. 4.

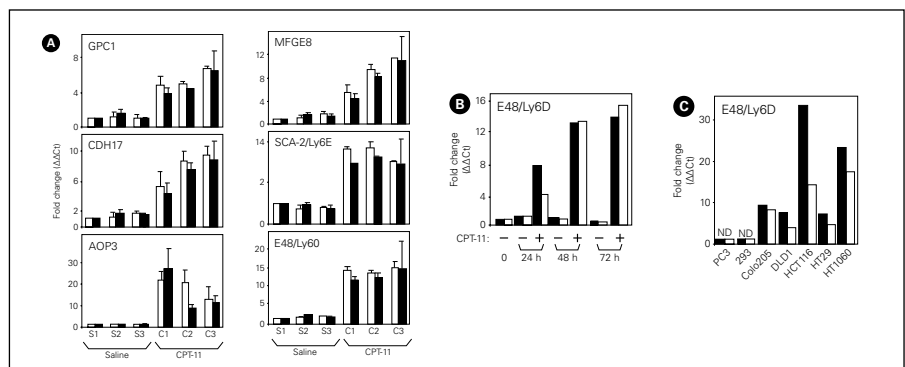


Figure 3. Identification and Immunotherapeutic Targeting of Antigens Induced by Chemotherapy. mRNA transcripts coding for cell-surface proteins induced by CPT-11. Scale bars, 150 μ m. (A) Real-time PCR analysis of RNA from xenograft tumors grown in mice administered CPT-11 (C1-3) or saline (S1-3). Relative 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 (C_t) were normalized to GAPDH (white bars) and action (black bars). (B) Time course of LY6D/E48 mRNA induction after addition of 10 μ M CPT-11 to Colo205 cells *in vitro*. Fold change is relative to vehicle control at time zero. (C) Induction of LY6D/E48 mRNA in cultured cell lines incubated with 10 μ M CPT-11 for 48 h. Fold change is relative to vehicle control. Transcript was not detected (ND) in PC3 and 293 cell lines.

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