molecular biology

Whole Gene Amplification from Archived Formalin-fixed, Paraffin-embedded Tissues

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Application Notes

- **High yield from limited template** – Amplify nanogram amounts of genomic DNA into microgram yields in less than three hours
- **Preserve precious samples** – Only ten nanograms of starting material required
- **Amplify DNA from any source** – Suitable with numerous sources of DNA including FFPE, buccal swabs, cell culture, plants and bacteria
- **Unlimited genetic analysis** – Compatible with many downstream applications such as TaqMan® and BeadArray® assays

Introduction

Archived formalin-fixed and paraffin-embedded (FFPE) tissue collections represent invaluable resources for studying pathogenesis of cancer and a variety of other diseases. The possibility of retrospective analysis of pathogenic as well as normal specimen and correlation of the molecular finding to disease and drug development is a critical issue. The accessibility of nucleotides from FFPE tissue is limited, because of extensive cross-linking of all tissue components. The DNA fragmentation depends on the tissue type and the fixation condition.

With the development of whole genome amplification (WGA) techniques, large quantities of DNA can be generated from limited starting quantities. Procedures have been used such as primer extension pre-amplification (PEP) with random 15-mer primers to amplify genomic DNA from single cells or low available amounts of DNA.[1] Other strategies like linker adaptor PCR,[2] tagged PCR,[3] and degenerate oligonucleotide primed PCR (DOP)[4] were designed, improving the yield, but the coverage of the genome in the amplification products was not optimal. Applying multiple displacement amplification (MDA)[5] with random primers and ϕ29 DNA polymerase ensured accurate whole genome amplification from small amounts of clinical samples, but typically has not been successful with FFPE tissue. We evaluated the ability of the GenomePlex™ WGA Kit developed by Rubicon Genomics, Inc. (Ann Arbor, MI) to amplify fixed tissue. GenomePlex WGA is based on random fragmentation of genomic DNA and amplification by PCR using ligated adapters to create the OmniPlex™ Library.[6] The resulting library can be amplified more than thousand-fold.

DNA extraction and whole genome amplification

One or two sections from FFPE prostate samples (10-15 years old) were cut 5 µm thick and genomic DNA was extracted following the protocol shown in Figure 1. Quantification of the DNA was determined using the PicoGreen™ assay (Molecular Probes, Eugene, OR). Samples were normalized to 20 ng DNA in 10 µl TE (10 mM Tris, 1 mM EDTA at pH 8.0). For complete and accurate whole genome amplification, the GenomePlex Kit was applied and an OmniPlex library created according to the supplied protocol. Quantitative real-time PCR amplifications were performed on an iCycler™ thermocycler (Bio-Rad, Hercules, CA) with SYBR®Green dye (Cambrex BioScience Rockland, Rockland, ME) detection. To each sample well 0.75 µl of SYBR Green (1:1000 dilution in TE) and 0.75 µl of Fluorescein Calibration Dye (1:1000 dilution in TE) (Bio-Rad, Hercules, CA) were added and amplified using a two step program: initial denaturation at 95 °C for three minutes, followed by 16 cycles with denaturation at 94 °C for 15 seconds and primer annealing/extension at 65 °C for two minutes. Amplification products were purified using a commercially available kit and quantified by spectrophotometer. The average yield was 4 µg with an average amplification of 200-fold amplification (data not shown).

The real-time PCR amplification cycle plot was displayed with the background subtracted and variable baselines. In order to interpret the curves and make them more comparable, the individual readings for each sample well were displayed, copied and transferred to a spreadsheet program. From each data point on a particular sample curve the lowest relative fluorescent units (RFU) value was subtracted and the fluorescence intensity was normalized to the maximum of the sample curve (Figure 2). Samples qualified for downstream applications when their threshold values (C_T) were within four cycles of the control DNA, unless the amplification continued not too far into saturation. The amplified tissue samples were normalized to 4 ng/µl for testing.

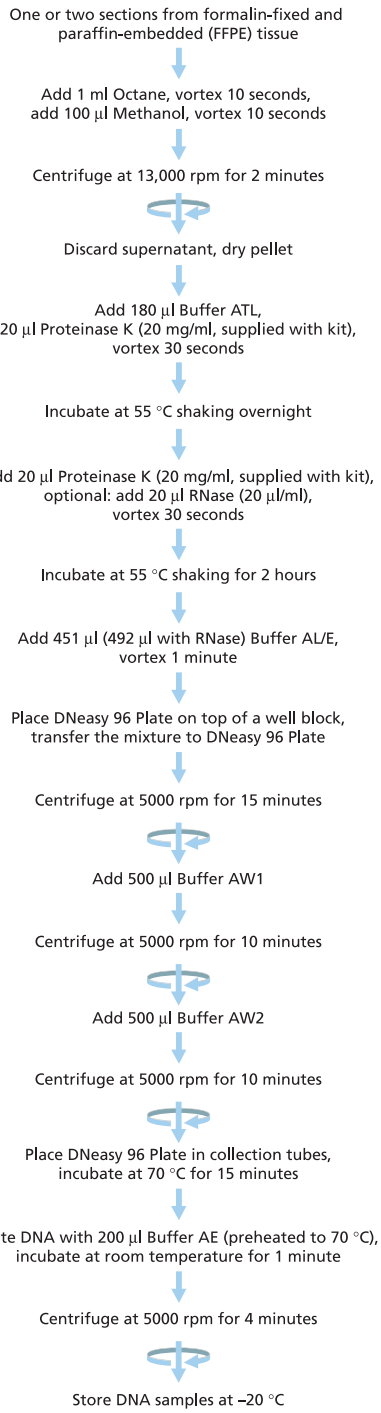

Figure 1. DNA extraction from formalin-fixed and paraffin-embedded tissue. *Modified procedure using commercially available kit for high-throughput DNA modification.*

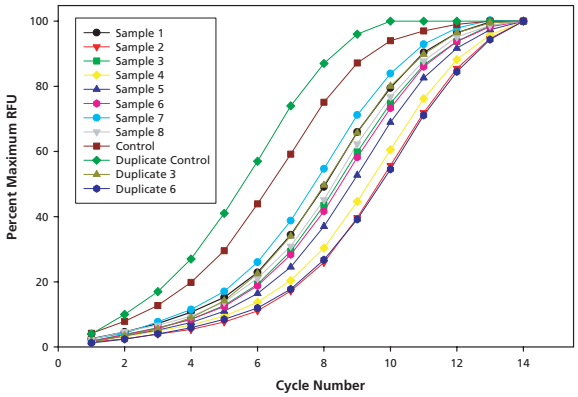

Figure 2. Amplification plots for different real-time PCR assays. *After each PCR cycle fluorescent intensities were normalized to the maximum intensity. *Samples are duplicates.*

Summary

The GenomePlex WGA Kit developed by Rubicon Genomics and offered exclusively by Sigma-Aldrich is ideal for isolation of human DNA from tissue samples like blood or mouthwash, fresh frozen and formalin-fixed and paraffin-embedded. It is a robust method for generating limited DNA into microgram quantity within less than three hours. We are currently evaluating the success of the amplified products for genotyping and other molecular assays.

References

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Ordering Information

Product	Description	Unit
WGA-1	GenomePlex WGA Kit	50 rxn