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Optimization of gene expression microarray protocol for formalin-fixed paraffin-embedded tissues

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ABSTRACT

Formalin-fixed paraffin-embedded (FFPE) tissue is a widely available clinical specimen for retrospective studies. The possibility of long-term clinical follow-up of FFPE samples makes them a valuable source to evaluate links between molecular and clinical information. Working with FFPE samples in the molecular research area, especially using high-throughput molecular techniques such as microarray gene expression profiling, has come into prominence. Because of the harmful effects of formalin fixation process such as degradation of nucleic acids, cross-linking with proteins, and chemical modifications on DNA and RNA, there are some limitations in gene expression profiling studies using FFPE samples. To date many studies have been conducted to evaluate gene expression profiling using microarrays (Thomas et al., Thomas et al. (2013) [1]; Scicchitano et al., Scicchitano et al. (2006) [2]; Frank et al., Frank et al. (2007) [3]; Fedorowicz et al., Fedorowicz et al. (2009) [4]). However, there is still no generally accepted, efficient and standardized procedure for microarray analysis of FFPE samples. This paper describes the microarray data presented in our recently accepted to be published article showing a standard protocol from deparaffinization of FFPE tissue sections and RNA extraction to microarray gene expression analysis. Here we represent our data in detail, deposited in the gene expression omnibus (GEO) database with the accession number GSE73883. Four combinations of two different cRNA/cDNA preparation and labeling protocols with two different array platforms (Affymetrix Human Genome U133 Plus 2.0 and U133_X3P) were evaluated to determine which combination gives the best percentage of present call. The study presents a dataset for comparative analysis which has a potential in terms of providing a robust protocol for gene expression profiling with FFPE tissue samples.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/colon FFPE tissue
Sex	Female
Sequencer or array type	Affymetrix Human Genome U133 Plus 2.0, Affymetrix U133_X3P Array
Data format	Raw microarray data (.CEL files) and analyzed data (.CHP files)
Experimental factors	HGU133 Plus 2.0 + 3′ IVT kit, X3P Array + 3′ IVT kit, HGU133 Plus 2.0 + Nugen Ovation FFPE WTA System Kit, X3P Array + Nugen Ovation FFPE WTA System Kit and tumor vs. normal for X3P Array + Nugen Ovation FFPE WTA System Kit
Experimental features	Four 8-μm-thick colon paraffin-embedded tissue sections were used for RNA extractions. Four different hybridization combinations were performed on four labeled samples using two different kits (Affymetrix 3′ IVT kit and the NuGEN Ovation FFPE WTA system) and two different arrays (Human Genome U133 Plus 2.0 and U133_X3P arrays). Three of these four samples have matched control samples. Gene expression data analysis was also performed for these three matched samples. Totally, this dataset includes 19 arrays.
Consent	Informed consent was obtained from all patients.
Sample source location	Ankara University, Faculty of Medicine, Department of Pathology, Ankara, Turkey

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73883

2. Experimental design, materials and methods

2.1. Experimental design

A comparison study was conducted assessing two different microarray chips and amplification kits for performance comparison. All steps are represented in Fig. 1.

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RNA was extracted from four sporadic colorectal cancer FFPE samples using Qiagen RNeasy extraction kit with modified deparaffinization step. The yield and quality of RNA samples were evaluated using a Nanodrop Spectrophotometer and a Agilent 2100 Bioanalyzer. We assessed microarray performance by comparing four combinations of two different cDNA preparation & labeling protocols and microarray chips. Finally, gene expression data analysis was performed for three matched samples using U133 X3 + NuGEN Ovation FFPE WTA System combination.

2.2. FFPE tissue materials

FFPE tissue samples were obtained from four colon adenocarcinoma patients. Local ethical approval was obtained for using the human material (Research Ethics Committee of Medical Faculty of Ankara University, Ankara Ref: 153-4854). Four different 6–10 year old FFPE blocks were used in this study (08/40 T(TT01), 08/95 T(TT08), 09/137 T(TT24) and 10/12 T(TT31) were collected in 2007, 2005, 2009, and 2006 respectively). Three of these four samples have matched control samples (08/40 T(NT01), 08/95 T(NT08) and 10/12 T(NT31)).

2.3. Characteristics of patients

Four tumor samples and three matched controls of these four samples were used for microarray analysis. All cases were sporadic colorectal cancers from female patients within the age of 27 to 48 years. The histotype of the tumors was conventional adenocarcinoma. The patients had stage III disease and 75% of the patients have left colon sided. All clinico-pathological parameters of the patients are given in Table 1.

2.4. Preparation of FFPE tissue samples and RNA extraction

All steps were performed under RNase-free conditions. Four of 8 μm-thick sections were cut from FFPE blocks, on a microtome with a disposable blade. Two of the sections were placed on per microscope slide. The tumor area containing ~90% tumor cells was used for macrodissection.

Four sections were used for RNA extraction. Total RNA was isolated using Qiagen RNeasy FFPE kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions except deparaffinization step. The deparaffinization step was optimized by comparing our laboratory deparaffinization protocol with Qiagen RNeasy FFPE kit's deparaffinization step. These comparisons and their results were given in detail in our manuscript accepted to be published in Pathology Research and Practice [5]. RNA samples extracted from FFPE tissues were analyzed in terms of RNA concentration and purity using NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The quality of RNA samples was assessed with RNA 6000 Nano Assay on Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). RNA degradation degree assessment was also checked with PCR which was performed by amplifying seven different amplicon sets that produced fragments ranging from 101 to 246 bp (B2M-101 bp, HPRT-113 bp, VEGFA-168 bp, MAPK14-198 bp, TCF19-201 bp, TGFB1-240 bp, NCAPG-246 bp).

2.5. In vitro transcription, amplification, labeling and hybridization for gene expression analysis

The RNA samples extracted from FFPE samples were amplified and labeled using the 3′ IVT kit (Affymetrix, Santa Clara, CA) and the Ovation FFPE WTA System (NuGEN San Carlos, CA, USA). Human Genome U133 Plus 2.0 and U133_X3P arrays were used for hybridization. Four combinations were used to determine which pairwise combination gives the best result in terms of percentage of present call.

Four combinations depicted in Table 2 were applied on the four tumor samples. One of these four combinations was applied on the matched normal samples of three of the four samples. In the combinations where 3′ IVT kit (Affymetrix, USA) is used manufacturer's protocol

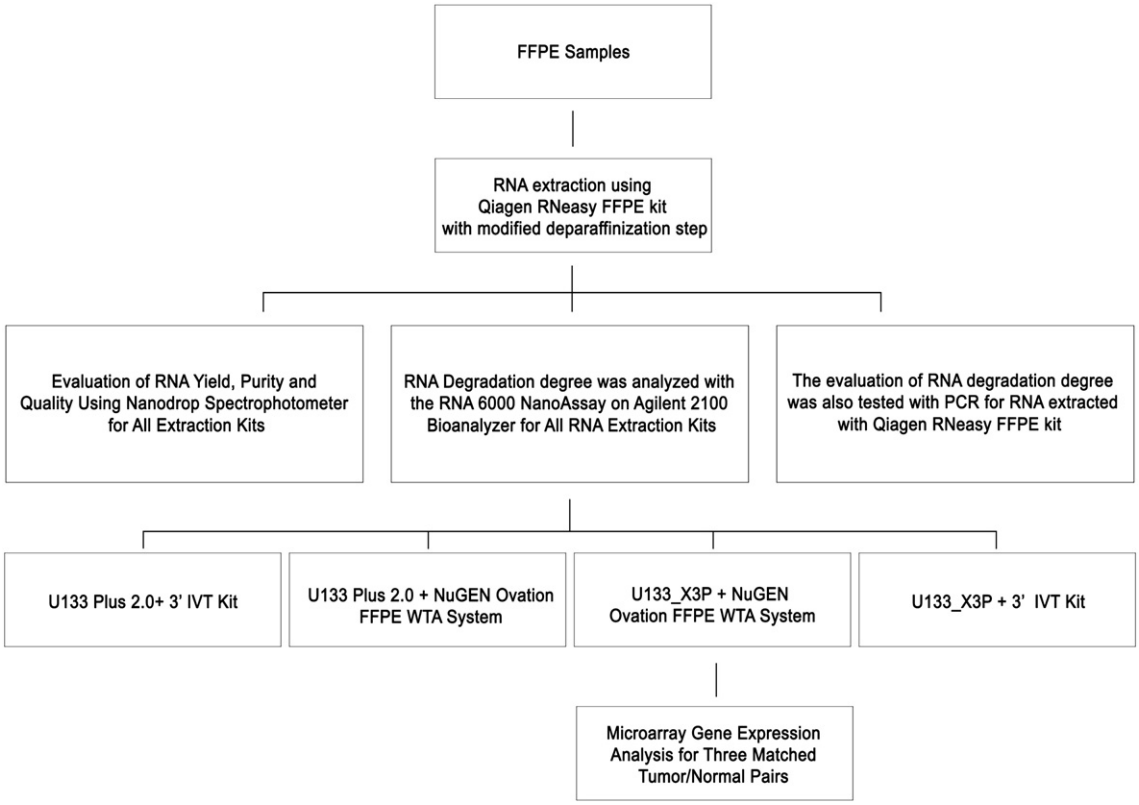

Fig. 1. A schematic representation of entire experimental workflow.