G80 Analysis of Gene Expression Patterns to Identify Tissue and Body Fluid Specific mRNA Species Using Real Time PCR Assays

Rixun Fang, PhD, Christine Shulse, BS, Pius Brzoska, PhD, and Manohar R. Furtado, PhD, Applied Biosystems, 850 Lincoln Center Dr., Foster City, CA 94404; and Chitra F. Manohar, PhD, Lawrence Livermore National Laboratory, 7000 East Ave, Livermore, CA 94550*

After attending this presentation, attendees will learn that it is possible to test for specific mRNAs that serve as markers for tissue and body fluid identification. Attendees will be informed about pre-amplification protocols that can be employed to simultaneously amplify and detect multiple targets when the amount of material available is limiting.

This presentation will impact the forensic community and/or humanity by teaching the forensic community that gene expression profiles and specific mRNA can be used to identify a large number of body parts and fluids. Attendees will understand that this can be done with very small amounts of material and therefore useful in forensic investigations when sample available is limiting.

This presentation proposes that by screening microarray and SAGE based tissue expression data in multiple databases, both public and internal, it is possible to identify candidate mRNA species that would show tissue specific expression. Additionally, this would select highly expressed messages for use in forensic applications.

In this presentation, and from the screening of human tissue and body fluids, it is possible to define specific markers that can be used for identification. Identification of tissue parts and body fluids is frequently required in crime scene investigations. Conventional methods are often laborintensive, not confirmatory and employ a diverse range of methodologies. Several forensic laboratories have pioneered the selection of specific protein or mRNA markers for identification of tissues and body fluids. Some laboratories have designed and tested real-time PCR based assays that target the detection of mRNAs encoded by > 20,000 genes in identified in the human genome. The presented methods employ proprietary methods to design assays specific to a target transcript avoiding amplification of related gene transcripts. It also allowed for the development of methods for pre-amplification of hundreds of targets present in a single sample preserving relative quantification information. These methods will be useful when dealing with heterogeneous mixtures.

In this study, the performance of assays targeting saliva specific markers was analyzed such as Statherin, Histatin Ge3, PRB1, PRB2, PRB3, menstrual blood markers like metalloproteinases, and semen specific markers like protamines. These were targets selected based on literature reports. A total of 480 genes from the analysis of microarray data from specific tissues were selected. TData was used to further limit this to a set of 130 genes. Next, RNA from 48 tissues was selected and converted to cDNA by reverse transcription reaction using random primers and commercially available kits. Initially, cDNA was tested using six endogenous controls (GAPDH, GUSB, 18S RNA and ACTB, HPRT and B2M) for normalization purposes. Next, assays targeting transcripts from these genes were used to analyze gene expression relative to endogenous controls across 48 tissues. HeatMap views of gene expression patterns were constructed to identify tissue specific patterns. Based on these expression profiles researchers identified ~20 genes, not reported in the literature as specific markers that are highly expressed in single tissues. Additionally, the authors identified some genes that were expressed in a few (2 to 3) tissues and could still serve as specific markers when used in specific combinations. This research also shows that pre-amplification protocols can be used to detect mRNA when the sample is limiting. Detection of saliva specific markers was demonstrated following pre-amplification.

This study demonstrates that, in many instances, single markers can be defined as specific for a given tissue. These include SEMG1, SEMG2, KLK3, and PRB4/HGNC that have not been reported in the literature as tissue specific markers. In some instances, using a combination of targets will provide identification. This research has shown that if the sample is limiting, pre-amplification of a large set of mRNAs in a single reaction is useful for identification.

Tissue Identification, Gene Expression, Real Time PCR

 ** Presenting Author*