



Real-time PCR assays for the detection of tissue and body fluid specific mRNAs

R. Fang^a, C.F. Manohar^b, C. Shulse^a, M. Brevnov^a, A. Wong^a,
O.V. Petrauskene^a, P. Brzoska^a, M.R. Furtado^{a,*}

^a *Applied Biosystems, Foster City, CA, United States*

^b *Lawrence Livermore National Laboratories, Livermore, CA, United States*

Abstract. Identification of tissue parts and body fluids is frequently required in crime scene investigations. We analysed expression data from public databases and internal studies to identify 110 highly expressed mRNAs as potential tissue specific markers. Further, we identified specific assays targeting mRNA in body fluids like saliva, semen, vaginal secretions and blood. The capability to pre-amplify small amounts of RNA enabling testing for the presence of multiple mRNA species was demonstrated. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

In forensic casework traditional methods for tissue identification are labour intensive, expensive and often not confirmatory [1,2]. Messenger RNA profiling has great promise in identifying tissue and body fluids as demonstrated by the recognition of specific markers for saliva, semen and menstrual blood [3,4]. We scrutinized expression data from 37 tissues [5] using arrays representing 26,000 genes and in-house data from 17 tissues generated using the Applied Biosystems Expression Arrays representing 29,098 genes to select 480 highly expressed tissue specific genes. Next, we screened the GeneCard[®] expression database that contains expression data from microarray systems, Northern analysis and serial analysis of gene expression (SAGE) to define a sub-set of 110 genes

* Corresponding author.

E-mail address: furtadmr@appliedbiosystems.com (M.R. Furtado).

Table 1
Six of the 10 tissue specific genes with high levels of expression

Gene name	Tissue specificity	Source
SEMG1, SEMG2	Seminal vesicle, Ductus deferens	Public
KLK3	Prostate	Public
TGM4	Prostate	AB
MCSP	Prostate	AB
PRB4	Trachea, Salivary gland	AB

useful in discriminating between tissue types. These markers were screened using tissue and body fluid RNA to define their utility in identification.

2. Materials and methods

Total tissue RNA was purchased from commercial sources (Clontech, BioChain and Invitrogen). Total nucleic acid was isolated from semen stains, saliva, blood stains and vaginal swabs using a modification of the ABI Prism™ TransPrep system. RNA was converted to cDNA using the High Capacity cDNA Archive kit (Applied Biosystems) using random primers. We used Applied Biosystems Taqman® Gene Expression [6] assays (www.allgenes.com) specific for these targets to screen RNA from tissues and body fluids and identify specific mRNA markers.

3. Results and discussion

We selected 44 genes from public datasets and 66 from Applied Biosystems (AB) Expression Array system. Thirty of the 66 genes in the second set are proprietary to the Celera Discovery System™. This 110-gene set was tested against RNA from a 48-tissue panel. We used the HeatMap Builder (Stanford University) to view expression profiles across tissues and select genes with tissue specific expression. We identified 10 genes that had >100–1000 fold expression levels in certain tissue systems compared to the rest of the tissues (Table 1) and 40 with smaller differences. Two novel genes that could serve as markers for semen stains were SEMG1 (GeneID 6406) and SEMG2 (Gene ID 6407) that encode the semenogelin proteins involved in formation of a gel matrix that encases ejaculated spermatozoa (Fig. 1).

The mRNAs identified as potential candidates for identification of tissues systems were tested against corresponding body fluid stains (Serological Inc.). The results are shown in

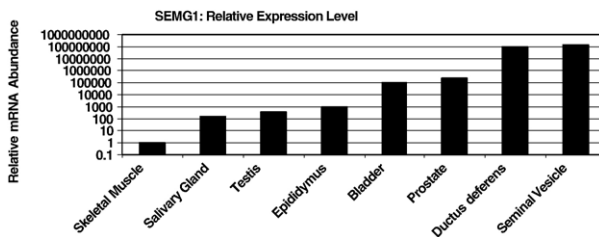


Fig. 1. Tissue expression profile for SEMG1 indicates the highest levels of expression in the seminal vesicle and the Ductus deferens and a 1000 fold lower level in the prostate and bladder. The lowest level was observed in the skeletal muscle, which was set at 1 to determine the relative fold difference in expression between tissues.

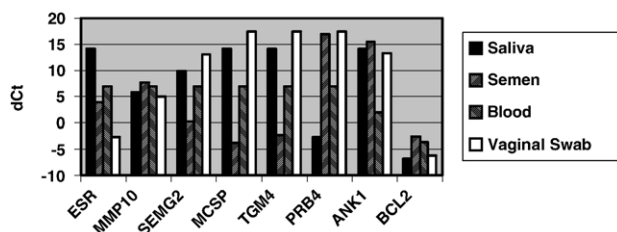


Fig. 2. The expression levels of 8 genes were tested with RNA from saliva, semen, blood and vaginal swabs. Expression levels shown reflect normalization to GAPDH. Lower dCt values equate to higher expression.

Fig. 2. The results presented indicate that: (i) The ESR1 gene (estrogen receptor 1) is a potentially good marker for discriminating vaginal secretions from other fluids with a >100-fold higher expression while MMP10 did not exhibit such differences; (ii) SEMG2/SEMG1, MCSP and TGM 4 clearly exhibit high expression levels in semen compared to other body fluids; (iii) PRB4 and Ankyrin 1 would serve as markers for saliva and blood, respectively. Further, we did dilution experiments to lower the amount of input RNA to that obtained from <1 cell equivalent. Routine testing with 40 cycles did not detect the mRNAs. Pre-amplification (10–15 cycles) of the RNA samples with a primer mix targeting multiple genes provided sufficient template for detection of multiple genes (data not shown). The protocol is useful when dealing with extremely low amounts of material requiring testing against multiple targets in that increasing the target copy number allows splitting the sample into multiple assays.

4. Conclusions

We identified a set of 110 genes that exhibited tissue specific expression. Following testing with RNA from 48 tissues we were able to identify 10 genes with high expression levels in specific tissues (Table 1). Testing of 8 of these markers with body fluids identified ESR1, SEMG1/SEMG2, MCSP, TGM4, PRB4 and ANK1 as a set of potential markers. Additional studies with larger sample sets are required to validate these findings. We have also shown that pre-amplification is useful when sample is limiting.

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