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mRNA MPS tissue identification assay to aid in the investigation of traumatic injuries

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ABSTRACT

Molecular analysis of the RNA transcriptome from a putative tissue fragment should permit assignment to a specific organ since each tissue will exhibit a unique pattern of gene expression. Determination of the organ source of tissues from crime scenes may aid in shooting and stabbing investigations. We have developed a new prototype massively parallel sequencing (MPS) mRNA profiling assay for organ tissue identification, designed to definitively identify 13 organ/tissue types using a targeted panel of 48 mRNA biomarkers. The identifiable organs and tissues include brain, spinal cord, lung, trachea, liver, skeletal muscle, heart, kidney, adipose, intestine, stomach, skin and spleen. The biomarkers were chosen after iterative specificity testing of numerous candidate genes in various tissue types. The assay is very specific with little cross reactivity with non-targeted tissue, and can detect RNA mixtures from different tissues, including two- to five-tissue admixtures. The sensitivity of the assay was evaluated as well as assay reproducibility between library preparations and sequencing runs. We also demonstrate the ability of the assay to successfully identify the tissue source of origin in cadaver samples, tissue samples with varying post mortem intervals (PMI) and mock and *bona fide* casework samples. We are using the data to train a multivariate statistical model that predicts the tissue type based on the mRNA profile. By considering co-expression of markers the model can recognize distinct expression patterns in each tissue.

1. Introduction

Criminal cases can sometimes involve significant trauma to the human body in which internal organ tissue may be transferred from the injured party to another individual, item or location [1]. The definitive identification of the source of the tissue can provide valuable probative information. The identification of tissue normally requires the expertise of a pathologist and/or histologist. The limited material present in some cases may make this type of typical identification difficult or even impossible. We previously evaluated the use of MPS technology to develop an internal organ tissue identification assay [2]. After successful development of a prototype assay, we have continued to develop the assay including the inclusion of additional biomarkers for trachea as well as new biomarkers for additional tissue types including spinal cord, skin and spleen. Here, we present performance evaluations for the updated MPS tissue identification assay including an evaluation of specificity, admixed tissue samples as well as cadaver and *bona fide* casework tissue samples.

2. Materials and methods

2.1. Tissue samples

Total RNA samples were purchased from commercial sources. Cadaver and casework tissue samples were collected by the Zurich Institute of Forensic Medicine and the Zurich Forensic Science Institute.

2.2. RNA extraction, DNase and quantitation

Cadaver and casework tissue samples were homogenized with a Beadruptor homogenizer using ceramic beads. Cadaver tissue samples were extracted using the Maxwell 48 Robot tissue protocol. Casework

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tissue samples were extracted with an RNA organic extraction as previously described [2].

2.3. TruSeq® targeted RNA library preparation, quantification and MiSeq sequencing

MPS libraries were prepared using the TruSeq® Targeted RNA kit (January 2016 protocol version, Illumina Inc., San Diego, CA, USA) as previously described [2] with the exception of a modified custom oligonucleotide pool (TOP). Pooled RNA libraries were quantitated using the 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA) and High Sensitivity D1000 Screen tape according to the manufacturer's protocol. Pooled libraries were diluted to 4 nM and denatured according to the manufacturer's protocol. A 600 µl 6 pM sample was used for sequencing with the MiSeq v3 150 cycle reagent cartridge using a v3 flow cell. The sequencing runs consisted of 51 single-end sequencing cycles.

2.4. Data analysis

After sequencing, local sequencing software on the MiSeq analyzed the data (base calling, demultiplexing and alignment to the provided manifest file using a banded Smith Waterman algorithm) resulting in a target hits file that displays total reads per amplicon per sample. A minimum sample total read count (MTR) of 5000 was used as an individual sample threshold, and samples below the MTR were excluded from analysis. In addition, a minimum biomarker read count (MBR) count of 500 was used as an individual biomarker threshold, with any count below this threshold removed. A third threshold was then used in which individual biomarker read count values that were less than 0.5% of the total reads for the sample were also removed.

3. Results and discussion

3.1. Candidate selection

The current 48-plex assay consists of biomarkers for the following tissues: brain (N = 4), spinal cord (N = 3), lung (N = 3), trachea (N = 3), liver (N = 5), skeletal muscle (N = 4), heart muscle (N = 1), kidney (N = 4), adipose (N = 3), intestine (N = 3), stomach (N = 3), skin (N = 3) and spleen (N = 4).

3.2. Specificity

The percent composition of total reads from each sample attributable to each class (e.g. tissue) of biomarkers was evaluated for numerous target and non-target tissue samples. Overall, 84–100% of the total reads for each sample was attributable to the target biomarker class. This demonstrates a high degree of specificity of the developed assay.

3.3. Admixed tissue samples

Two- to five-tissue admixtures were prepared using commercially purchased total RNA tissue samples. For the two-tissue admixtures (N = 5, lung-heart), 25 ng of each tissue was used in varying rations of each (20–5 ng, 15–10 ng, 12.5–12.5 ng, 10–15 ng, 5–20 ng). For the three-tissue admixtures (N = 7), 17 ng of each tissue was used. For the four-tissue admixtures (N = 4), 12.5 ng of each tissue was used. For the five-tissue admixtures (N = 3), 10 ng of each tissue was used. For the two-tissue admixtures, lung and heart were detected in each of the mixtures and the percent composition decreased or increased accordingly depending on the ratio of the inputs. For the three-tissue admixtures, all three tissues were identified in 4 of the 7 samples. In the remaining 3 samples, only 2 of the 3 tissues were identified. For the four-tissue admixtures, all four tissues were identified in 2 of the 4 samples. In the remaining 2 samples either 2 or 3 of the four tissues were identified. For the five-tissue mixtures, all five tissues were not identified in any of the samples. 3 and 4 tissues out of the 5 were identified in two and one of the samples, respectively. Overall, the ability to detect multiple tissues in admixed samples was demonstrated. Some tissues when found in lower than optimal input amounts fail to be detected. No false positive tissue inferences were obtained.

3.4. Cadaver tissue samples

Various tissues (brain, spinal cord, lung, liver, skeletal muscle, heart, kidney, intestine, stomach and adipose) were obtained from three different corpse samples. The percent composition of total reads attributable to biomarkers for the target tissue ranged from 93 to 100% for most samples. One heart sample indicated 20% composition attributable to liver biomarkers which could indicate contamination of genuine liver tissue in this sample. One intestine sample indicated 59% of total reads attributable to intestine biomarkers and 41% to skeletal muscle biomarkers. It is likely that associated genuine skeletal muscle was collected along with the intestines in this sample.

3.5. Bona fide casework samples

Casework samples from two suicide cases were available for testing: 1) material recovered from train tracks and 2) material recovered from a hooded jacket. For the train track sample, skeletal muscle was identified which would be expected as part of the material recovered after a person is hit by a train. For the hooded jacket sample, brain was identified which is consistent with a case in which the cause of death was a self-inflicted gunshot wound to the head. The results of the cadaver and *bona fide* casework samples demonstrate the usefulness of such an assay in the identification of the tissue source of origin of samples in traumatic injury investigations.

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Declaration of Competing Interest

None.

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