



Addition of an adipose marker to the organ tissue identification mRNA assay

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ABSTRACT

At the Netherlands Forensic Institute, mRNA profiling for the purpose of organ tissue identification is frequently requested in casework. The previous version of the assay (denoted Organtyper) lacked markers for identifying adipose tissue, while this was occasionally questioned in casework. Here, three potential adipose markers are examined before incorporating one into the final Organtyper assay. The added value of the adipose marker becomes clear from looking at casework results.

1. Introduction

At the NFI, mRNA profiling is frequently used in casework for the identification of body fluids and organ tissues [1,2]. Organ tissue identification is performed using the Organtyper assay [1] and is often requested in violent cases, for example when knives or bullets are involved or when spatters of tissue-like structures are found on e.g. clothing of a suspect. Occasionally, these tissue-like substances have an adipose-like appearance but the previously published version of the Organtyper assay [1] only contained mRNA makers for brain, lung, liver, skeletal muscle, heart and kidney tissue and lacked markers for adipose. In order to identify adipose tissue, three potential adipose markers (SCD, ADIPOQ and TRARG1) were examined. After assessing the specificity of the markers on a range of organ tissues and the performance on different adipose donors, one marker (TRARG1) was added to the Organtyper assay that is currently used in casework.

2. Material and methods

Genes (SCD, ADIPOQ and TRARG1) were selected using literature and gene expression database BioGPS [3,4]. Primers were designed using Ensembl and NCBI Primer Blast [5,6]. DNA/RNA co-extraction, DNase treatment, reverse transcription, PCR and CE were performed according to standardized protocols [1].

Adipose-specificity of the three genes was assessed using the FirstChoice® Human Total RNA Survey Panel (Applied Biosystems), which are pools of total RNA for 20 different human tissues including adipose and the six organs targeted by the Organtyper assay [1]. For each tissue,

an input of 10 pg RNA was used during PCR, which is the input for which the Organtyper assay was developed and optimized [1].

Performance of the marker on its target tissue was assessed by testing adipose tissue of different donors. The adipose samples were collected from the upper arm of ten deceased donors who consented to scientific use of their body.

3. Results and discussion

Primer concentrations were optimized using adipose tissue RNA extract of five donors. The optimized primer concentrations were 0.05, 0.1 and 0.1 μ M for ADIPOQ, SCD and TRARG1, respectively (results not shown).

All three primer sets were included in the Organtyper assay [1] using their optimized primer concentrations. Specificity of the three markers to adipose tissue was assessed on the 20 organ tissues of the FirstChoice RNA Survey, including adipose tissue. An overview of the results of these specificity tests is shown in Table 1. All three potential adipose markers were detected in adipose tissue. Non-specific signals were detected for markers SCD and ADIPOQ in respectively 12 and 3 out of the 19 tested non-adipose tissues. SCD and ADIPOQ were therefore omitted from further testing. Specificity of TRARG1 was confirmed as it was only detected in adipose tissue and not in any of the other tissues. False positive signals for other markers in the Organtyper assay (lung marker SFTPB and skeletal muscle markers MYH1 and TNNI2) are in line with previous results [7].

The Organtyper assay was supplemented with only TRARG1 and performance of this marker on its target tissue was assessed by testing

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Table 1

Specificity results for the adipose markers in the Organtyper assay. Signals in target tissues are presented in green. Signals in non-target tissues are shown in grey. General muscle marker NMRK2 [1] results are not included; this marker has been omitted from the Organtyper assay due to limited value in case work.

	Blood	CNS			Lung		Liver		Skeletal muscle		Heart		Kidney		Adipose			House-keeping	
	HBB	GFAP	SNAP25	OPALIN	SFTPD	SFTPB	AMBP	VTN	MYH1	TNNI2	MYL7	MYBPC3	UMOD	FXD2	SCD	ADIPOQ	TRARG1	18S-rRNA	
Brain	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Lung	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Liver	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Skeletal muscle	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Heart	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Kidney	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Adipose	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Bladder	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Colon	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Cervix	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Esophagus	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Small intestine	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Prostate	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Placenta	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Thyroid	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Ovary	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Trachea	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Thymus	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Testes	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Spleen	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

Table 2

Primer information for all primers in the final Organtyper assay. Underlined nucleotides are 5' tails added to improve multiplex spacing, CNS = central nervous system. FXD2 has two forward primers to ensure amplification of different transcript variants. Primers from [1] are unmodified and are only included for completeness. The fluorescent dye is added to the forward primer unless stated otherwise.

Gene	[primer] (µM)	Tissue	Forward primer (5' – 3') Reverse primer (5' – 3')	Size (bp)	Dye	Ref.
SNAP25	0.02	CNS	F: TCGGCGGCTCCACCACAGTT R: TTGGCTCTGGACCTGGGCTTCTC	78	6FAM™	[1]
OPALIN	0.8	CNS	F: GCCATGGAGAAAGTGACAGACC R: CTCATGTGTGGGTGATCTCTAGG	93	6FAM™	[1]
GFAP	0.04	CNS	F: TGGAGAGGAAGATTGAGTCGCTGG R: CGAACCTCCTCCTCGTGGATCTTC	70	6FAM™	[1]
SFTPB	0.	Lung	F: CCATGATTC ^u CCAAAGGGTGCCTA R: CGCCACCAGAGGTACCACGC	68	NED™	[1]
SFTPD	0.10	Lung	F: CCTGCCTGGTCCGGATGGAC R: CCAGGCATCCTGCTTGCCC	99	6FAM™	[1]
AMBP	0.03	Liver	F: TTGGCTGACCGAGGTGAATGTGTC R: ACCAGTTGCCACCCCTGAT	119	VIC™	[1]
VTN	0.05	Liver	F: GTGCAAGCCCAAGTACTCG R: CATAGACCGTGTACTCATCCTCG	65	VIC™	[1]
TNNI2	0.13	Skeletal muscle	F: ATGTCTGAAGTGACAGAGCTCTGC R: GTCGTACTTCTCTTTCAGCCGC	72	PET™	[1]
MYH1	0.8	Skeletal muscle	F: TTCGATCTCTACGCCAGGGTCTTA R: AGGAAAGGAGCAGCCTCCCAAA	104	NED™	[1]
MYL7	0.08	Heart	F: AAGCTCAATGGGACAGACCCCG R: CACCACCCCTTTGCCGCTGG	82	VIC™	[1]
MYBPC3	0.4	Heart	F: AGGCAGAGAAGGCAGAGCCCAT R: AGCTTGACCCTTTGGGACTTGGG	129	VIC™	[1]
UMOD	0.06	Kidney	F: ATCACACGAAAGGTGCCAGGC R: TTTTGGAGCACAGGGCTTTCCGC	145	6FAM™	[1]
FXD2	0.06	Kidney	F1: CTCTCCAAAAGCAGAGACAGCAGG F2: CTCCATCCAGGCCAGGCA R: CGGTCTCATAGTCATAGTAGAACGGG	137 143	PET™ (rv)	[1]
TRARG1	0.1	Adipose	F: TGGTGGCCGTGACCCGCAA R: GAGCTCTCCGATGGCCAG	95	PET™	This paper
18S-rRNA	0.013	Housekeeping	F: GACTCAACAGGGAAACCTCACC R: CTCACCAACTAAGAAGGCCATG	110	PET™	[1]

adipose tissues of different donors. TRARG1 was detected in all ten of the adipose tissues, showing that it can be used to detect adipose tissues in a range of different donors (results not shown). An overview of the final Organtyper assay, including TRARG1 is presented in Table 2.

4. Conclusion

After assessing the specificity of the markers on a range of organ tissues and the performance on different adipose donors, TRARG1 was added to the Organtyper assay. The inclusion of this adipose marker is of added value especially in violent crimes, as adipose cells on violent

objects may indicate perforation.

The updated Organtyper assay has been applied in 27 cases on 78 forensic samples and adipose was detected in 13 of these samples. Five of these were knives, one was a tool, the remaining seven were either clothing or bed sheets.

Conflict of interest

None.

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