Advancing forensic RNA profiling

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Chapter 7

Extended specificity studies of mRNA assays used to infer human organ tissues and body fluids

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Abstract

Messenger RNA (mRNA) profiling is a technique increasingly applied for the forensic identification of body fluids and skin. More recently, an mRNA-based organ typing assay was developed which allows for the inference of brain, lung, liver, skeletal muscle, heart, kidney, and skin tissue. When applying this organ typing system in forensic casework for the presence of animal, rather than human, tissue is an alternative scenario to be proposed, for instance that bullets carry cell material from a hunting event. Even though mRNA profiling systems are commonly in silico designed to be primate specific, physical testing against other animal species is generally limited. In this study, human specificity of the organ tissue inferring system was assessed against organ tissue RNAs of various animals. Results confirm human specificity of the system, especially when utilizing interpretation rules considering multiple markers per cell type. Besides, we cross-tested our organ and body fluid mRNA assays against the target types covered by the other assay. Marker expression in the nontarget organ tissues and body fluids was observed to a limited extent, which emphasizes the importance of involving the case-specific context of the forensic samples in deciding which mRNA profiling assay to use and when for interpreting results.

Introduction

The use of messenger RNA (mRNA) profiling for the identification of body fluids and skin has been increasingly investigated in the past decades [1-11]. More recently, an mRNA assay was developed which allows for the inference of seven organ tissue types [12-14]. Previously, enzyme-linked immunosorbent assays or histological methods were used to infer the type of cell [15-18]. These methods are, however, often not of sufficient sensitivity to assess the limited amount of cells present on forensic evidentiary traces [19]. The organ typing mRNA assay described in [12], in contrast, allows analysis of low level and degraded samples and can be used to determine multiple tissue types simultaneously [13]. As DNA is simultaneously coextracted alongside RNA, DNA profiles can be generated from the same evidentiary stain to identify the donor of a trace. In forensic settings, this organ typing mRNA assay (referred to as the Organtyper) can be used to aid in the identification of organ tissue remnants present on, for example, evidentiary items of a violent crime and therewith provide information on the course of events. An alternative scenario to be proposed in case of a violent crime is the presence of animal rather than human cells, for instance as the result of a hunting event, a car collision with an animal, or even from food preparation or spilling. Specificity to human tissues can, therefore, be very informative when RNA profiling is applied in forensic context.

In 1977, the DNA genome of bacteriophage PhiX174 [20] was fully sequenced and since then, thousands of organisms (including over 130 mammalian species) have followed [21], enabling comparative genome analyses to in silico assess the potential formation of unintended amplification products from nontarget genes and/or organisms. The amount and the positioning of mismatches at oligonucleotide binding sites are the key in amplification efficiency [22-26]. However, experimental confirmation of human specificity of RNA profiling multiplexes adds to addressing hypotheses in a forensic context, and we subjected organ typing RNA assays to a selection of animal and nontarget organ tissue RNAs. Additionally, we cross-tested our organ and body fluid mRNA assays against the target types covered by the other assay; an experiment inspired by a forensic case involving facial violence (head, nose, and throat area) that led to a request to subject the evidentiary samplings from knives to both organ and body fluid typing. The rationale was that (besides blood and skin) nasal mucosa, saliva, skeletal muscle, and brain could be present. This request made it necessary to cross-test the Organtyper and Cell-typer on body fluids and organs, including organs not targeted by the multiplex.

Materials and methods

Sample collection

A set of 19 animal target organ tissues was used to assess human specificity of organ-typing markers in nonhuman samples. This sample set included commercially available total RNAs (Zyagen) of whole brain for cow, chicken, dog, guinea pig, pig, and rabbit; cerebellum for cat and sheep; lung, liver, skeletal muscle, heart, and kidney for cow and dog; and universal RNA (encompassing brain, heart, kidney, liver, lung, skeletal muscle, pancreas, spleen, stomach, intestine, colon, testis, ovary, and uterus) for chicken. Besides, organ specimen RNAs as described in [12] and the FirstChoice® Human Total RNA Survey Panel (Applied Biosystems) were used; the latter containing pools of total RNA for 20 different human tissues including six of the organs targeted by the Organtyper multiplex [12]. Skin was added to this organ specimen set [12]. Lastly, six human body fluids (*i.e.*, blood, saliva, nasal mucosa, vaginal mucosa, menstrual secretion, and semen) as described in [1] were included (different donors were used throughout the experiments). All necessary approvals from ethical examination commissions at the medical institutions where organs were collected and informed consent of the voluntary donors contributing body fluids were obtained.

RNA profiling

Reverse transcription of the commercial RNAs (animal origin or Human Total RNA Survey Panel) was performed as described in [1]. As the RNA concentrations of these samples were provided, defined RNA inputs to an end concentration of 20 pg/µL were used during reverse transcription. A cDNA input representing 10 pg RNA was used in duplicate PCR amplifications for each animal sample (which is twice the amount of human RNA used to optimize the Organtyper system [12], as we aimed to challenge the multiplex for the animal specimens). A third amplification carried not only animal cDNA (~10 pg RNA), but also human kidney cDNA (~5 pg RNA) that served as a positive amplification control. In all these amplifications, the kidney markers responded. Amplification of the Human Total RNA Survey Panel samples used a cDNA input representing 5 pg RNA.

PCR amplification was performed using Brain-plex, Organtyper [12], and Cell-typer [3]. The Brain-plex is a small multiplex combining four markers specifically aiming for the identification of central nervous system tissue. These include astrocyte (astrocytes constitute nearly half of the human brain [27]) markers GFAP, S100B, ACSBG1, and oligodendrocyte (oligodendrocytes constitute the majority of myelinating cells in the brain [28]) marker OPALIN together with housekeeping marker 18S-rRNA. Brain-plex primers were designed using Ensembl and NCBI primer blast [21, 29], labeled

with a 6-FAM label to obtain the highest sensitivity (Brain-plex primer sequences are provided in Supplementary Table 1).

Minor adjustments were made to the Organtyper [12] and Cell-typer [3] multiplexes. In the Organtyper multiplex brain marker RTN1 was replaced by the more sensitive markers GFAP and OPALIN, which also extended the targeted central nervous system cell types. The primers for heart marker MYBPC3, housekeeping marker 18S-rRNA, skin marker LOR, and blood marker HBB were adjusted to increase annealing temperature and reduce primer–dimer formations. Also, the primer concentrations of lung marker SFTPB and kidney marker UMOD were reduced to improve signal balance. In the Cell-typer multiplex [3], the primers for 11 markers were adjusted to enable an increased annealing temperature and optimize marker spacing. Primer details for these updated Organtyper and Cell-typer multiplexes can be found in Supplementary Table 2 and Supplementary Table 3, respectively.

PCR amplifications involved the PCR protocol as described in [1] with the annealing temperature increased to 64°C. PCR products were purified [1] prior to detection using a 3130XL Genetic Analyzer (Life Technologies). Amplification products were analyzed using POP-4 (Life Technologies) separation matrix and 3 kV, 10 s injection settings. Profile analysis was performed using Genemapper ID-X version 1.1.1 (Life Technologies) with a detection threshold of 150 relative fluorescence units.

RNA data interpretation was performed according to the "$x=n/2$" guidelines as described in [30] based on multiple PCR replicates (three) per sample. This method compares the number of observed (x) to the number of theoretically possible peaks (n) in all replicates. A cell type is scored "observed" when at least half of the possible peaks are observed ($x \geq n/2$), denoted "sporadically observed" when less than half of the possible peaks are observed ($0 < x < n/2$), meaning that no reliable statement is possible. A cell type is scored "not observed" when no peaks are detectedt ($x=0$).

Appropriate controls (*i.e.*, positive controls including samples of known origins, and/or no template controls) were included during each step of the protocol.

In silico assessment of human specificity

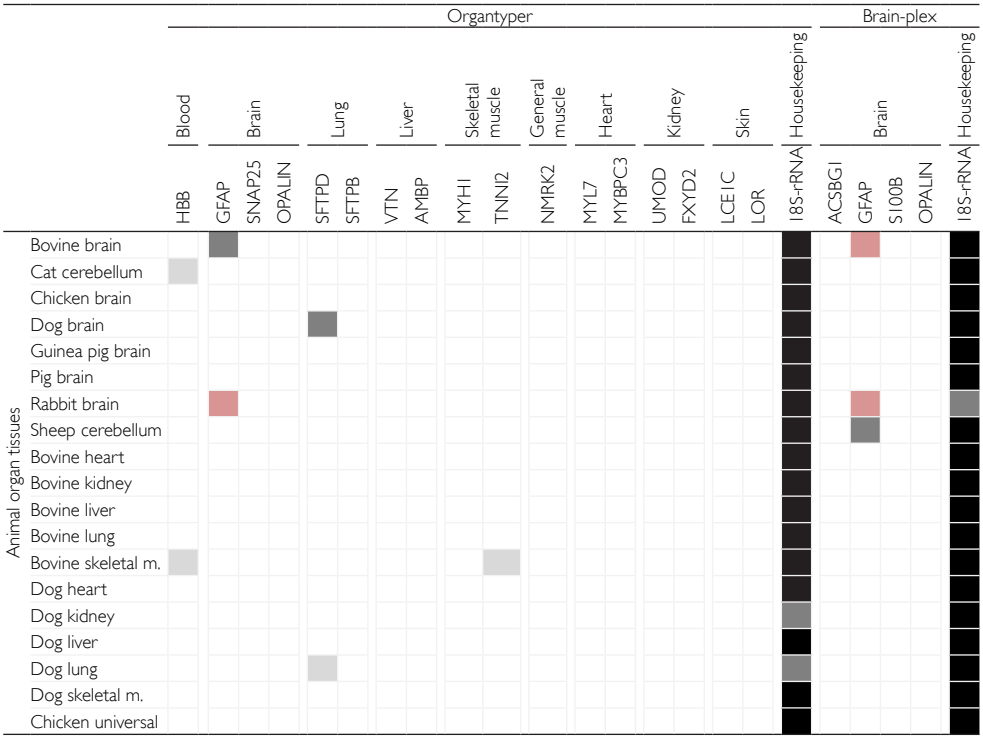
Primer sequences of the Brain-plex, Organtyper, and Cell-typer multiplex (Supplementary Tables 1–3) were subjected to in silico BLAST analyses [21] using primer specificity stringency settings in which a maximum of six mismatches were allowed on any target. RNA sequences for ribosomal RNA marker 18S-rRNA were obtained from the SILVA rRNA database project [31]. A summary of the possible products on animal templates considering cow, cat, chicken, dog, guinea pig, pig, rabbit, and sheep using these databases is shown in Supplementary Table 4.

Results and discussion

Animal organ tissues

Specificity of organ-typing markers (*i.e.*, markers residing in the Organtyper and Brain-plex) for human tissues was tested by using various animal RNAs. Animals were selected for their presence in an average household and included pets (cat, dog, guinea pig, and rabbit) and foods (cow, chicken, pig, and sheep). Results are presented in Table 1. Only few false positive signals (*i.e.*, cross-specific amplifications) were obtained, mainly for astrocyte marker GFAP (specifically with cow, rabbit, and sheep brain RNA, see Table 1). As a positive scoring for an organ tissue requires the detection of at least 50% of all markers for that tissue (section "RNA profiling", "RNA data interpretation"), this cross-reactivity will not result in a false "observed" scoring.

Table 1 Results of specificity tests for the markers residing in the Organtyper and Brain-plex (that are designed to be human specific) using animal organ tissue samples. Different colored fillings indicate the number of times a marker is detected out of three replicate profiles: white when no signals are detected, light grey when detected once, and dark grey when detected twice. Markers detected in all of the three replicates are colored black or red depending on whether marker signals were expected or not (18S-rRNA is not a human-specific marker so signals are to occur).



Housekeeping marker 18S-rRNA was detected in most animal amplifications and is, thus, not a human specific housekeeping marker, which is consistent with the results described in [32]. The main function of this housekeeping marker in these end-point RT-PCR assays is to confirm successful RNA analysis by indicating the presence of RNA, and the absence of factors inhibiting cDNA synthesis or PCR, which is especially informative when none of the other markers give a signal [12]. Few signals were observed for blood marker HBB, lung marker SFTPD, and skeletal muscle marker TNNI2, but these signals were sporadic. Overall, the detection of these marker signals can be explained by the positioning and number of mismatches in the primers when aligned to the animal templates (see Supplementary Table 4 for detailed BLAST results). For example in silico BLAST analyses for astrocyte marker GFAP to cow, rabbit, or sheep show one or two mismatches per primer that reside at least four nucleotides upstream of the 3' end of the primer (Supplementary Table 4A). Species that show more (3' end) mismatches (cat and guinea pig, Supplementary Table 4A) did not result in false positive GFAP signals (Table 1). The Brain-plex results more GFAP false positive responses than the Organtyper (Table 1), which is consistent with the higher sensitivity of a smaller multiplex.

No nonhuman body fluids (other than blood residing in organs) were analyzed as sample collection of vaginal mucosa, nasal mucosa, skin, and menstrual secretion was too complex or impossible and no full picture would be obtained.

Human tissues and case example

Specificity of the Organtyper and Cell-typer multiplex was cross-tested on nontarget human body fluids and organ tissues, including organs not targeted by the multiplex. For the Organtyper, similar tests have previously been performed and described in [12] and these findings were confirmed (Table 2A). Most of the detected Organtyper signals were sporadic marker amplifications seen in only one of the replicates. Skeletal muscle markers were however detected in esophagus, thymus, thyroid, and trachea (all residing in the upper chest/ throat area). Even though the biological function of the skeletal muscle markers in these tissues is unknown, expression of these markers in thyroid tissue has been reported in BioGPS [33]. Additionally, one of the lung markers is detected in thyroid tissue, and both skin markers in vaginal mucosa samples. These results are consistent with findings previously described in [2].

Brain-plex markers GFAP and OPALIN did not result in any unexpected signals when analyzing nontarget organ tissues or body fluids (Table 2). Reproducible ACSBG1 signals are, however, detected in trachea tissue. Additionally, S100B signals are reproducibly detected in adipose, colon, esophagus, small intestine and trachea tissue. S100B expression was reported predominantly in astrocytes [34]. Various studies, however, describe the detection of S100B in non-nervous tissues,

Table 2 Results of specificity tests for the markers residing in the Organtyper, Brain-plex, and Cell-typer multiplex (that are designed to be target-specific) using human organ tissue (A); and human body fluid (B) samples. Different colored fillings indicate the number of times a marker is detected out of three replicate profiles: white when no signals are detected, light grey when detected once, and dark grey when detected twice. Markers detected in all of the three replicates are colored green or red depending on whether markers are detected on target organs, or a result of cross-reacting markers, respectively. Dark red colorings indicate samples for which an "observed" scoring would occur using the "x = n/2" rule, light red when samples would be scored "sporadically observed" which is generally regarded not reliable.

such as adipocytes [35]. Additionally, deregulated expression of S100 family genes in human diseases, especially in cancer studies has been described [36]. Notwithstanding the evident role of S100B in astrocyte functioning, all functions of S100B are not fully clear [37], and explanations regarding the detection of this marker in these nontarget tissues cannot readily be given based on gene expression databases such as BioGPS or GTEx Portal [33, 38]. These findings support the choice of supplementing the original Organtyper multiplex with GFAP and OPALIN (section "RNA profiling"), for which no signals were detected in non-target tissues.

When analyzing the same set of human organ tissues and body fluids with the Cell-typer multiplex, marker signals for vaginal mucosa marker MYOZ1 were additionally detected in skeletal muscle samples. Even though the exact function of MYOZ1 in vaginal mucosa is unknown [7], various studies do describe that the protein encoded by the MYOZ1 (Myozenin-1) gene is predominantly expressed in skeletal muscle [39, 40] and this is also evident in the BioGPS and GTEx Portal gene expression databases [33, 38]. Menstrual secretion marker MMP7 is observed in kidney tissue and seminal fluid marker SEMG1 is detected in bladder tissue, which is consistent with results described in [3, 32, 41]. The detection of menstrual secretion marker MMP11 in placenta, seminal fluid marker SEMG1 in prostate, and spermatozoa marker PRM1 in testes tissue are implied by the association of these tissue types with the body fluids targeted by these markers and have previously been reported [42-44]. The markers indicative for the presence of nasal mucosa (*i.e.* STATH and BPIFA1) are also detected in trachea tissue, and thus appear expressed in multiple tissues (BPIFA1 in nasal, oropharyngeal, and lung epithelia [3, 45], and STATH in salivary glands and trachea [33]). The detection of vaginal mucosa marker MUC4 in trachea tissue, the cross-reactivity of skin markers in various body fluids, and expression of vaginal mucosa markers and white blood cell marker CD93 in nasal mucosa samples has previously been reported (Table 2) [2, 3, 46]. Although skin marker LCE1C is common in the Organtyper and Cell-typer, results for this marker on human body fluid samples were not identical (Table 2B). This is likely cause by the use of different body fluid donors, and emphasized the variability that may be seen between donors especially for cross-reactive signals.

When considering the false positive responses not per marker but for all markers indicating a body fluid or organ jointly by the use of the "$x=n/2$" interpretation guideline, trachea seems prone to false positive scorings while especially skeletal muscle markers may provoke false positive results (Table 2). In the context of the above-described case in which Organtyper and Cell-typer were requested for facial injuries, we discuss the following points of attention: (1) the MYOZ1 signals in the Cell-typer multiplex should be regarded both in context with the two vaginal mucosa markers and the two skeletal muscle markers in the Organtyper multiplex; in case skeletal muscle markers MYH1 and TNNI2 respond and vaginal mucosa markers CYP2B7P1 and MUC4 not, MYOZ1 signals may derive in the presence of skeletal muscle and MYOZ1 is best excluded from

the inference for vaginal mucosa; (2) when trachea may be injured, the presence of nasal mucosa and skeletal muscle is unsure; and (3) when multiple organs in the upper chest/ facial area are violated, one should be extremely careful to infer presence of skeletal muscle and nasal and vaginal mucosa.

Concluding remarks

In this study, specificity analysis of a set of mRNA markers used for the inference of body fluids and organ tissues was performed to gain insight and awareness regarding the possibilities and limitations of forensic mRNA profiling. Results stress the importance of using multiple markers per cell type, as single markers may be detected in nontarget tissues and the use of increased number of markers per cell type will allow inference with more certainty. Additionally, awareness of unintended marker detection in nontarget tissues is crucial when applying mRNA profiling in casework, as this may lead to incorrect associations. Results of this study emphasize the importance of furthering the knowledge and understanding of mRNA markers when applied to forensic casework.

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Supplementary material

Supplementary Table 1 Primer sequences for the markers residing in the Brain-plex.

Marker name	Tissue	[primer] µM	Forward primer (5'-3') Reverse primer (5'-3')	Size (bp)	Dye
GFAP	Astrocyte	0.04	TGGAGAGGAAGATTGAGTCGCTGG CGAACCTCCTCCTCGTGGATCTTC	70	6FAM™
OPALIN	Oligodendrocyte	0.8	GCCATGGAGGAAAGTGACAGACC CTCATGTGTGGGTGATCTCCTAGG	93	6FAM™
ACSBG1	Astrocyte	0.2	CTACACTTCCGGCACCACTGG GTCCACGTGATATTGTCTTGACTCAG	66	6FAM™
S100B	Astrocyte	0.3	GCAGCAAGGAGACCAGGAAGG AACGTCGATGAGGGCCACCATG	81	6FAM™
18S-rRNA	Housekeeping	0.013	GACTCAACACGGGAAACCTCACC CTCCACCAACTAAGAACGGCCATG	110	PET™

Supplementary Table 2 Primer sequences for the markers residing in the Organtyper multiplex.

Gene	Tissue	[Primer] µM	Forward primer (5'-3') Reverse primer (5'-3')	Size (bp)	Dye
SNAP25	Brain	0.02	TCGGCGGCTCCACCACAGTT TTGGCTCTGGACCTGGGCTTCTC	78	6FAM™
GFAP	Brain	0.04	TGGAGAGGAAGATTGAGTCGCTGG CGAACCTCCTCCTCGTGGATCTTC	70	6FAM™
OPALIN	Brain	0.8	GCCATGGAGGAAAGTGACAGACC CTCATGTGTGGGTGATCTCCTAGG	93	6FAM™
STFTB	Lung	0.04	CCATGATTCCCAAGGGTGCGCTA CGCCACCAGAGGTACCACGC	68	NED™
SFTPD	Lung	0.10	CCTGCCTGGTCGCGATGGAC CCAGGCATCCCTGCTTGCCC	99	6FAM™
AMBP	Liver	0.03	TTGGCTGACCGAGGTGAATGTGTCC[a] ACCAGTTGCCCACCCCCTGAT	119	VIC®
VTN	Liver	0.05	GTGCAAGCCCCAAGTGACTCG CATAGACCGTGTACTCATCCTCCG	65	VIC®
TNNI2	Skeletal muscle	0.13	ATGTCTGAAGTGCAGGAGCTCTGC GTCGTACTTCTCCTCTTCAGCCGC	72	PET™
MYH1	Skeletal muscle	0.8	TTCGCATCTCTACGCCAGGGTCCTTA[a] AGGAAAGGAGCAGCCTCCCCAAA	104	NED™
MYL7	Heart	0.08	AAGCTCAATGGGACAGACCCCG CACCACCCCTTTGCCGCTGG	78	VIC®
MYBPC3	Heart	0.4	AGGCAGAGAAGGCAGAGCCCAT AGCTTGACCCTTTGGGACTTGGG	129	VIC®
NMRK2	General muscle	0.4	GGAGGAGAAGTACCCGCAACTACA ACACCGTTGGCCTCCATCTCCT	109	6FAM™
UMOD	Kidney	0.06	ATCACACGGAAAGGTGTCCAGGC TTTTTTGGAGCACAGGGCTTTCCGC[a]	145	6FAM™
FXYD2	Kidney	0.06	CTCCATCCAGGCCCCAGGCA CTCTCCAAAAAGCAGAGACAGCAGG CGGTCTCATAGTCATAGTAGAACGGG	143	PET™
LCE1C	Skin	0.2	TGTGACCCCGCTCCTGAATCCG CTTGGGAGGGCACTTGGGGGTG	99	NED™
LOR	Skin	0.6	TGCTTTGGGCTCTCCTTCCTTCTC GGTCTTCACGCAGTCCACTGG	88	PET™
18S-rRNA	Housekeeping	0.013	GACTCAACACGGGAAACCTCACC CTCCACCAACTAAGAACGGCCATG	110	PET™
HBB	Blood	0.1	GCACGTGGATCCTGAGAACTTCAG ATGGGCCAGCACACAGACCAG	61	6FAM™

[a] Underlined nucleotides are 5' tails added to improve multiplex spacing.

Supplementary Table 3 Primer sequences for the markers residing in the Cell-typer multiplex.

Gene	Tissue	[Primer] µM	Forward primer (5'-3') Reverse primer (5'-3')	Size (bp)	Dye
HBB[a]	Blood	0.035	GCACGTGGATCCTGAGAACTTCAG ATGGGCCAGCACACAGACCAG	61	6FAM™
CD93[a]	Blood	0.075	GCTCTGGGGCTACTGGTCTATC TCCCAGGTGTCGGACTGTACTG	151	NED™
ALAS2	Blood	0.05	TTCTGCACCAGAAGGACTCAGCC[b] TAAATCTCGCACCCTGGCAGGATC	103	6FAM™
HTN3[a]	Saliva	0.2	CTTCACTTCAGCTTCACTGACTTCTG CTTTGCATGTGAATCAGCTCCAGTC	132	VIC®
STATH[a]	Saliva/ nasal mucosa	0.3	TTCATCTTGGCTCTCATGGTTTCCATG GCCATACCCATAACCGAATCTTCCA	93	6FAM™
BPIFA1	Nasal mucosa	0.15	CAAGTGAATACGCCCCTGGTCG GAATGGGTGCAGTCACCAAGGAC	131	PET™
SEMG1	Seminal fluid	0.6	GGAAGATGACAGTGATCGT CAACTGACACCTTGATATTGG	121	6FAM™
PRM1[a]	Spermatozoa	0.05	GAGAGCCATGAGGTGCTGCC AGGCAGGAGTTTGGTGGATGTGC	90	NED™
KLK3	Seminal fluid	0.05	GACGTGGATTGGTGCTGCACC CTTCTCGCACTCCCAGCCTC	64	PET™
MUC4[a]	Vaginal mucosa	0.8	CTGCTACAATCAAGGCCACTGCTAC AAGGGAAGTTCTAGGTTGACAGTTGG	141	6FAM™
CYP2B7P1[a]	Vaginal mucosa	0.05	CCTCATGTCGCAGAGAGAGTCTAC CCCATGGGGAGAAGGTCAGCA	146	VIC®
MYOZ1[a]	Vaginal mucosa	0.8	CGTGTTCTCCGGTCACAGCAG TGGATTCAGCCGGCTGCTCG	88	VIC®
MMP7	Menstrual secretion	0.4	GAACAGGCTCAGGACTATCTC TTAACATTCCAGTTATAGGTAGGCC[b]	127	VIC®
MMP10	Menstrual secretion	0.07	GCATCTTGCATTCCTTGTGCTGTTG GGTATTGCTGGGCAAGATCCTTGTT	107	VIC®
MMP11	Menstrual secretion	0.15	CAACCGACAGAAGAGGTTCG GAACCGAAGGATCCTGTAGG	76	NED™
CDSN[a]	Skin	0.05	TCCTCCTGCCAGGGACCTTG GATACGCGTGGGGTCCTTACAG	71	VIC®
LCE1C	Skin	0.03	TGTGACCCCGCTCCTGAATCCG CTTGGGAGGGCACTTGGGGGTG	99	NED™
18S-rRNA[a]	Housekeeping	0.013	GACTCAACACGGGAAACCTCACC CTCCACCAACTAAGAACGGCCATG	110	PET™
ACTB[a]	Housekeeping	0.045	CAGAGCCTCGCCTTTGCCGAT CGCGGCGATATCATCATCCATGGT	75	PET™

[a] Primer concentrations lowered compared to [3].
[b] Underlined nucleotides are 5' tails added to improve multiplex spacing.

Supplementary Table 4 BLAST results for the primers included in the Organtyper and Brain-plex (A), and the Cell-typer multiplex (B) when considering various animals as target. Shown are the locations at which mismatches occur, numbered in 5'-3' direction. To infer the distance from the 3' end of the primer, the primer length is indicated as well. Housekeeping marker 18S-rRNA resulted in full primer-template matches for all animals considered (not shown). Markers and animals without comments did not result in hits when considering the primer BLAST specificity stringency settings described in section "In silico assessment of human specificity". Colour coding for Organtyper and Brain-plex (A) correspond to results presented in Table 1, which indicates the number of times a marker is detected out of three replicate profiles: white when no signals are detected, light grey when detected once and dark grey when detected twice. Markers detected in all of the three replicates are coloured red. Indicated in the dotted square are samples with possible non-specific product formation, which were not assessed in this study as RNA samples for these organs were not included.

	Tissue	Gene	Primer length (bp)	Bos taurus Bovine	Canis lupus familiaris Dog	Gallus gallus Chicken	Felis catus Cat	Cavia porcellus Guinea pig	Sus scrofa Pig	Oryctolagus cuniculus Rabbit	Ovis aries Sheep
A	Blood	HBB	F 24			10T>C 13T>C*	13T>C				
			R 21			9G>C 13A>G 15A>T*	1A>G 16G>C				
	Brain	GFAP	F 24	20G>T			17G>A 20G>T	3G>A 8G>A 11G>A 20G>T		20G>C	20G>T
			R 24	3A>C 15G>A			1C>T 3A>C 15G>A	1C>T 3A>C 15G>A		3A>C 20T>C	3A>C 15G>A
		ACSBG1	F 21	7T>G 10C>G 19T>C	4C>T 7T>C		7T>C 10C>G 19T>C		7T>A 10C>G	7T>C 10C>G	
			R 26	18T>C	6C>T 18T>C		12A>G 18T>C		6C>T	12A>G 21A>G	
	Lung	SFTPD	F 20						13C>T 19A>T		
			R 20						12T>C 16T>C		
Organtyper	Skeletal muscle	TNNI2	F 24		6T>C		24C>T		6T>G 9A>G 12G>C	4T>G 6T>C 9A>G 21C>G	
			R 24	4G>A 16T>C 19A>G	4G>A 16T>C 19A>G 22C>T		4G>A 16T>C 19A>G	16T>C 19A>G	16T>C 19A>C	4G>A 16T>C 19A>G	4G>A 16T>C 19A>G
	General muscle	NMRK2	F 24	13C>A 14C>G 17C>A 19A>G 24A>G		8A>G 14C>A 19A>G					13C>G 14C>G 17C>A 19A>G 24A>G
			R 22	22T>C		3A>C 7T>C 10G>T					22T>C
	Heart	MYL7	F 22		15A>G 21C>G				9T>C		
			R 20		7C>A 10T>C				7C>G 10T>C		7C>G 16G>A
		MYBPC3	F 22							3G>T 13T>C 15G>A	
			R 23								
	Kidney	FXYD2	F 25	1C>G 7A>C 8A>C 18A>G 23A>G					7A>C 8A>C 9A>G 10A>C 18A>G		
			R 26	8A>G 23C>T					2G>T 14A>G 20G>A 23C>T		
B	Blood	HBB	F 24			10T>C 13T>C*	13T>C				
			R 21			9G>C 13A>G 15A>T*	1A>G 16G>C				
		CD93	F 22				6G>T 9G>A 12A>T			15G>T 21T>C	
			R 22				5A>T 8T>C 11C>T			8T>C 11C>G 20C>T	
		ALAS2	F 21				9G>A 12G>A 16T>G	12G>A 15C>T	1C>T 15C>T	15C>T	
			R 24	9G>A 20G>A			7T>G 8C>T	6C>T 9C>A	9G>A	12C>A	
	Nasal mucosa	BPIFA1	F 22				1C>A 3A>T			1C>A 2A>G 9T>C	
			R 23				3A>G 6G>A 21G>A			2A>G 3A>G 20G>T	
Cell-typer	Vaginal mucosa	MYOZ1	F 21		3T>A			2G>A 3T>G			3T>G 10C>T 11G>A
			R 20		1T>C 2G>A 9G>A 20G>A			1T>A 2G>T 4A>G 20G>A			6T>G 7C>G 20G>A
	Menstrual secretion	MMP7	F 21						14A>G		
			R 25						4A>G 20T>C 25C>G	6A>C 20T>C	
		MMP10	F 25							1G>C 9C>T 10A>C 11T>C 23T>C	
			R 25							12G>A 17A>T 21T>A	
		MMP11	F 20	14A>C						14A>C	
			R 20	7A>G						7A>C	7A>G
	Skin	CDSN	F 20	10C>A					10C>A	14G>A 19T>G	10C>A
			R 22	1G>A 7C>T 19A>G				4A>T 19A>G	1G>A 19A>G	4A>C 7C>T	7C>T 19A>G

*The product length of amplification using HBB markers to Gallus gallus template deviates from the expected product length (130 vs 61 bp).

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