Electronic Supplementary Material to

A novel amplification strategy for genotyping with liquid chromatography-electrospray ionization mass spectrometry

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Table S1. Different APEX-2 multiplex sets generated for ICEMS analysis.

CALL RATE (%)

100	95	86	90	74	70

AMPLICON LENGTH

5-plex	10-plex	15-plex	20-plex	25-plex	30-plex
79	79	79	79	79	79
89	89	89	89	89	89
100	100	100	100	100	100
110	110	110	110	110	110
126	126	126	126	126	126
	84	84	84	84	84
	94	94	94	94	94
	104	104	104	104	104
	107	107	107	107	107
	115	115	115	115	115
		82	82	82	82
		92	92	92	92
		97	97	97	97
		112	112	112	112
		121	121	121	121
			86	86	86
			88	88	88
			95	95	95
			102	102	102
			122	122	122
				80	80
				99	99
				108	108
				113	113
				119	119
					83
					87
					98
					103
					123

Material and Methods S1. Confirmatory sequencing experiments.

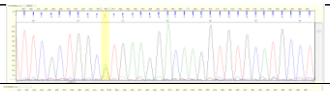
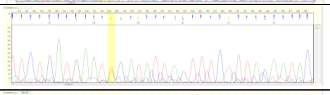
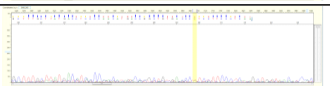
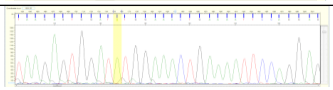
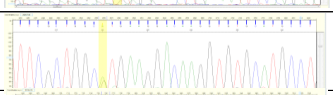
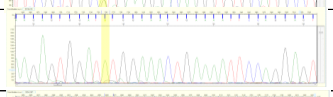
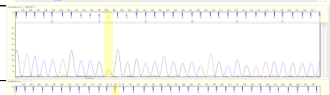
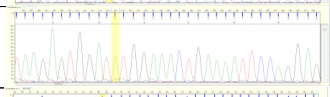
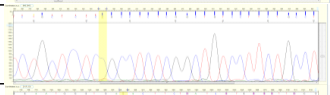
The 23 candidate regions were sequenced in 19 individuals (6.7 % of all study subjects). The studied regions were amplified by PCR in a 15 µl volume containing 1× Reaction Buffer B (Naxo, Tartu, Estonia), 0.25 mM of each dNTP (Fermentas), 2.5 mM $MgCl_2$, 1.25 U Hot Fire Polymerase (Solis Biodyne, Tartu, Estonia), 1.2 µM of each primer (Metabion) and 33–56 ng of template DNA. For this purpose, the same primers were used as for the multiplex PCR protocol. After the first round of both strand sequencing, six primer pairs were re-designed (Table S2). Cycling was performed with a GeneAmp PCR System 2700 thermocycler (Life Technologies) under the following conditions: 95˚C/15 min (initial denaturation), 10 cycles of 95˚C/30 sec, 64˚C/30 sec, 62˚C/30 sec, 60˚C/30 sec and 72˚C/20 sec, 27 cycles of 95˚C/30 sec, 55˚C/30 sec and 72˚C/20 sec 64˚C, and 72˚C/1 min.

PCR product purification was carried out with Exonuclease I and Shrimp Alkaline Phosphatase treatment. Ten units of ExoI and 1 U SAP (both Fermentas) were added to 5 µl of PCR product and incubated at 37˚C/20 min and at 80˚C/15 min. The BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) was applied in a 10 µl reaction volume containing 1 µl of purified PCR product, 0.7 µl BigDyeTerminator Ready Reaction Premix, 2 µl of BigDye Terminator Sequencing Buffer and 0.9 µM sequencing primer (universal primer 1 and 2). Cycling was carried out by a GeneAmp PCR System 2700 thermocycler under following conditions: 95˚C/20 sec, 30 cycles of 50˚C/15 sec, and 60˚C/1 min. The extension products were purified according to dextran/ethanol precipitation protocol. The purified pellet was dissolved in 10 µl of formamide, followed by electrophoresis on the ABI Prism 3730 DNA Analyzer (Life Technologies).

Table S2. Sequences of the newly designed sequencing primers.

SNP	Fw	Rev
rs4680	GATCCAAGTTCCCCTCTCT	GGGCCTGGTGATAGTGG
rs2306283	CTGTAAGAGTCAAATGTTTTTCC	GGGAAATTGACAGAAAGTACTCT
rs1805008	CACTCACCCATGTACTGCTTCA	AGGATGGTGAGGGTGACAGC
rs9479757	TTGATCGATACATTGCAGTC	ATTTGCCATGTAGTCAGCC
rs11568563	ACAAGGGGCCATGGTCATA	TTTTATGGAAGGCCAACTGTG
rs7439366	TTGCCTACATTTTTGCCTAC	TCCCATCTTTCTTTCAGTGT

Table S3. Comparison of the genotypes determined by ICEMS and those assessed in the first and second round of Sanger sequencing.

		ICEMS results		Sanger sequencing		
Sample	SNP	Multiplex	Singleplex	1st round	2nd round	2nd round read display
Ko54	rs4680	**G/A**	**G/A**	**A/A**	**G/A**	
Ko40	rs2306283	**C/T**	**C/T**	**C/C**	**C/T**	
Ko82	rs9479757	**G/A**	**G/A**	**A/A**	**G/A**	
Ko41	rs7439366	**T/T**	**T/T**	**C/T**	**T/T**	
Met76	rs4680	**G/A**	**G/A**	**A/A**	**G/A**	
Met14	rs7439366	**T/T**	**T/T**	**C/T**	**T/T**	
Bup62	rs1805008	**C/T**	**C/T**	**C/C**	**C/T**	
Bup01	rs7439366	**T/T**	**T/T**	**C/T**	**T/T**	
Met57	rs948854	**A/G**	**G/G**	**G/G**	**G/G**	
Ko40	rs11568563	**A/A**	**A/C**	**C/C**	**C/C**