Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2013

Supplementary Information

Materials and methods

Primers were designed to amplify 24 human mRNA targets using the LAMP Designer program v 1.02 (Premier Biosoft). Primers and the targets NCBI accession numbers/versions are shown in Supplementary Table 1.

RNA was purified from whole blood using the QIAamp RNA Blood Mini Kit according to the manufacturer's instructions. In brief, 1.5 mL of whole blood was processed resulting in 100 μL RNA eluate. Concentration and purity of the RNA eluate was determined using the Nanodrop 2000 (Thermo Scientific). The RNA eluate was allocated and stored at -80 °C until further processing.

The two-stage RT-isoPCR assay was performed as follows. A first-stage one-step reverse transcription PCR was performed using the AffinityScript One-Step RT-PCR kit (Agilent Technologies) according to the manufacturer's instruction. In brief, RT-isoPCR reactions were performed as singleplex reactions amplifying a single target or as multiplex reactions amplifying all 24 targets. First-stage reaction volumes (50 μL) contained: FIP/BIP primer set(s) in concentrations of 0.4 μM, 50% Hercules II RT-PCR 2x Master Mix, 1% AffinityScript RT/Rnase block and 1.5 μL RNA eluate. Using a thermocycler (2720 Thermal Cycler from Applied Biosystems), the RT-PCR reagent mixture was subjected to an initial incubation at 45°C for 5 min to facilitate reverse transcription, this was followed by a 1 min hotstart at 95°C and 8 thermocycles of 20 s at 95°C, 20 s at 60°C and 20s at 72°C. Final elongation was performed for 5 min at 72°C. First-stage RT-PCR products were stored at -20°C if not processed directly in second-stage reactions.

Subsamples of the first-stage product were used as template in 24 individual isothermal amplification/detection reactions each with a single target. Second-stage reaction mixture (10 μL) consisted of: 6 μL of Isothermal Mastermix (Optigene), 1 μL first-stage RT-PCR amplified product and primers in concentrations of 1.6 μM FIP, 1.6 μM BIP, 0.8 μM LF and 0.8 μM LB. Reactions were performed at 63°C for at least 45 min using the Genie II device (Optigene). The device measured the development in fluorescent signal in real-time. The time of detection (ToD) was determined as the time point at which the maximum slope in fluorescent increase was recorded.

A melting temperature analysis was performed by the Genie II device, following each individual isothermal reaction.

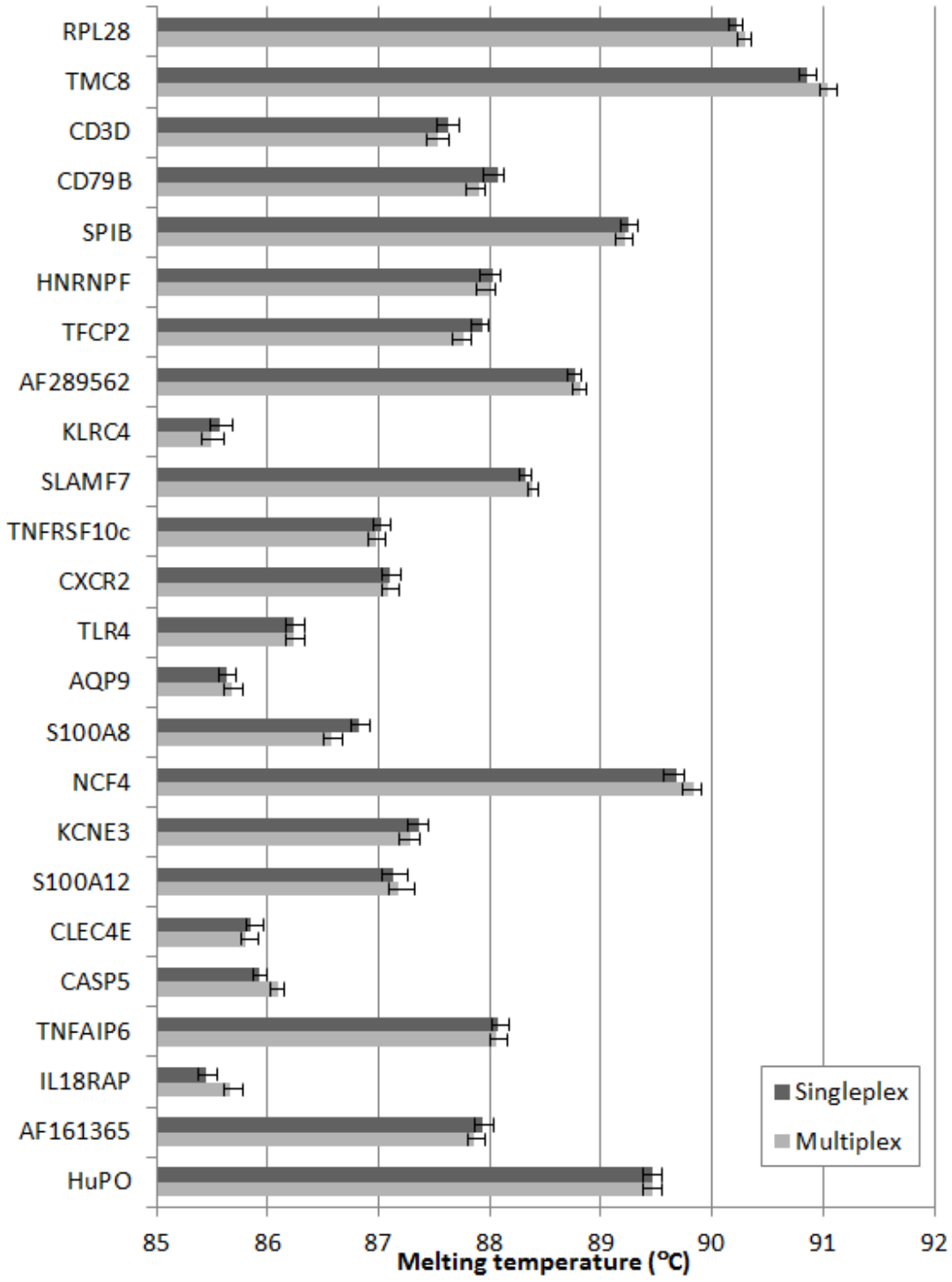
No template controls (NTC) were performed by substituting RNA eluate in the first-stage reaction and first-stage product in the second-stage reaction with water.

This journal is © The Royal Society of Chemistry 2013

Supplementary Table 1: Primer design shown for 24 targets. The NCBI accession/version number is shown for the mRNA target sequence. All primer sequences are shown as 5' to 3'.

#	Target	NCBI accession/version	FIP	BIP	LF	LB
1	RPL28	NM_001136134.1	CCTACGCTCACTCACCATGCGCA GGTGTCTCAGTGTTC	GCCCAAAGTTGTGCAGAGCTGA ACAGAGGATGCCTCA	ACTGGACTAAGAGCTGGAGA	CCACAGCAGCAGAGACTC
2	TMC8	NM_152468.4	GTGACCAGGAAGGCGAAGGGC TGTACAAGCTGAGTATCTT	GGTTCTCAGGCCGGTTCTGCAC GATGTCCAGCACATT	CACGGTGAGGAGGAAGTTG	CTGGAACGGGAGGAGTTC
3	CD3D	NM_000732.4	TCCTCCAAGGTGGCTGTACTCTC TGTTGAGGAATGACCAG	AACTGGGCTCGGAACAAGTGAC AGTTGGTAATGGCTGC	GCATCATCTCGATCTCGGAG	CCTGAGACTGGTGGCTTC
4	CD79B	NM_000626.2	CCAGCAGCAGGAAGATAGGCC ACGCTGAAGGATGGTATC	GGCTGGCATGGAGGAAGATCA GCGTCACTATGTCCTCATA	AGCAGCGTCTGGATCATG	TGGACATTGACCAGACAGC
5	SPIB	NM_003121.4	TACAGGTGTGAGCCATTGCCCA CTGTAGCACTTGGTGG	AGCACTTTGGGAGGCGAGCCA GGCTGGTCTTGAATG	TGATGGCCTGCTGTTAATGT	GGGAATGGCTTGAACCCA
6	HNRNPF	NM_001098208.1	GCTCTGCACTGTGAACTCACTA GAGACCTCAGCTACTGTC	GCCACTGTGTCCACATGAGGTG AGAGGAGAGAAGAAGTTGTA	GCCGTATCTGTGGTCATACAT	GCGACCGAGAACGACATT
7	TFCP2	NM_005653.4	AGCAAGCTACTTAGCCACTGTG ACACTATCTGAAGTAGGTGCTA	AGCTGGCAGGCAGTGATGAGC ACTGGTGGTATGAGA	TTTCCTCATCTTCGGTTGGG	CTGAAATCCAAACCAGGCAATC
8	AF289562	AF289562	AACTGCTGCTCTGCTGTCCCTGT GGATGGTTGTCAGTC	CTCCAGGATCGGCCATGTTCAA TGCAGCTTGAGGTTTCA	TCTCATCATCTGACTCACTCCT	ACCGCCTCTACCTGTCTC
9	KLRC4	NM_001199805.1	CTTCCCACTGCCAAGATCCATTC TGGTGAAGTCATATCATTGGA	GGCTCCATTCTCTCACCCAACAA GCTCGAGGCATAGAGT	GTTGGAATGTGTACTAGTCCCA	ATTGAAATGCAGAAGGGAGACT
10	SLAMF7	NM_021181.3	GGAGTGCAGTGGTGCCATGGC ATGAGAATCGCTTGA	ACACCTGTGCTAGGTCAGTCTA GATGGTGAGCTCTGTGT	CTCAGCTCACTGCAACCT	CGTAAGATGAACATCCCTACCA
11	TNFRSF10c	NM_003841.3	CACTTCCGGCACATCTCTGGACC ATGACCAGAGACACA	TAGCAGGTGCCCTAGTGGGCCA AATTCTTCAACACACTGG	TGCCTTCTTTACACTGACACA	AGTCAGTAATTGTACGTCCTGG
12	CXCR2	NM_001557.3	ATGAGTAGACGGTCCTTCGGAA ATTCATATGTCTCAGCATCTGG	GTTAGCCCAGCCTGCTATGAGG ATCCGTAACAGCATCCG	CAGGAGCAAGGACAGACC	GACATGGGCAACAATACAGC
13	TLR4	NM_138554.4	GTTCTCCTGGCAGTGAGAAGGT TGATGGACCTCTGAATCTCT	ACGTGTGAAGGTATTCAAGGCA ATGTGGTCAAGGAGCATTG	CCAGCCATCTGTGTCTCC	CATTGCTGTTTCCTGTTGGG
14	AQP9	NM_020980.3	ACAATGGCTCACAGATTCCTGG AAGCCACAGCCTCTAATTG	TCATCTGGCTGTGAAAGTGAGG CTTCTCTGAGGACTCCTGT	TCTGGTAAGAGTCTCTGACTGT	ACCACAACAGGTAGGTATTGG
15	S100A8	NM_002964.4	TCCAGCTCGGTCAACATGATGC CTGCATGTCTCTTGTCAG	ACGTCTACCACAAGTACTCCCTC AATTTCTTCAGGTCATCCCT	CACCAGGTCTTCTGAAAGACA	GGGAATTTCCATGCCGTCTA
16	NCF4	NM_000631.4	AAGCATGGAACTGGCGGTAGTC GTCATCGAGGTGAAGA	GCCAGACAGCAAGAGCAGTCTC CTGTTTCACACCCAC	GCGGTAGATGAGGTACTTGG	CACACTCCCAGCCAAAGT
17	KCNE3	NM_005472.4	TCAGTCAGTTTCAGGAGTCCCT ATGTGTCCAGAGACATCCT	AGCAGTCTGAGCTTCTACCGAG TTCCATTGGTAGTCTCCATAG	CACACTAAGGCTCCTCCAC	CCCACCTCAATCCCTGTTG
18	S100A12	NM_005621.1	AGCGCAATGGCTACCAGGGCCT GGATGCTAATCAAGAT	CCCATTACCACACCCACAAAGAT CATTGAGGACATTGCTGG	ATTCTTGAAAGTCGACCTGTTC	TAGGTAGCTCTCTGAAGGCTT
19	CLEC4E	NM_014358.2	TTCCTGCTCCTCCTGTGAGTAAG AACTGCTCAGCCATG	GGACTGTCAGACCAGGTTGTCC TCAGAGACTTTGTCAAAGGT	GATAACCACCAGGTGAGCC	GAGGGTCAGTGGCAATGG
20	CASP5	NM_004347.3	CCTTCTCCTCGTGGATCTTGCCA TCCTTGGCACTCATCTC	AACACCACATAACGTGTCCTGG TTCTGGAAGCATGTGATGAG	TGCCTCCAGGTTCTCAGA	GGCTCCATCTTCATTACGGAA
21	TNFAIP6	NM_007115.3	CCTTAGCTTCTGCGTAGGTGAG ATATGGCTTGAACGAGCAG	TTGAAGGCGGCCATCTCGCAGC AGCACAGACATGAA	CCAGACCGTGCTTCTCTG	ACTTACAAGCAGCTAGAGGC
22	IL18RAP	NM_003853.2	CCAAGCGTCTGATCCTTGCTTCT ACAGGCACTGGATTGA	TTCCAAGTGAGGCCACTTCATCC ATCAGGAAATAGGCTCAGG	CCGGTACAGCAGCACTATT	TCTGAGTGAAGAACACTTGGC
23	AF161365	AF161365.1	ACCTCGGCCAATGAACGGACAT CTCCAACCTCACAGA	ATAGAGTGGGAGGTGGGAGCT CTTCCTCTAGCACAGACC	GTACTGATCACTTCCTGTCCTC	CCTCGTGCTGCCTTCATT
24	HuPO	NM_053275.3	CGCATCATGGTGTTCTTGCCAA GCAGATGCAGCAGATC	CTCTGGAGAAACTGCTGCCTCT CAGTGAGGTCCTCCTTG	ATCAGCACCACAGCCTTC	GGAATGTGGGCTTTGTGTTC

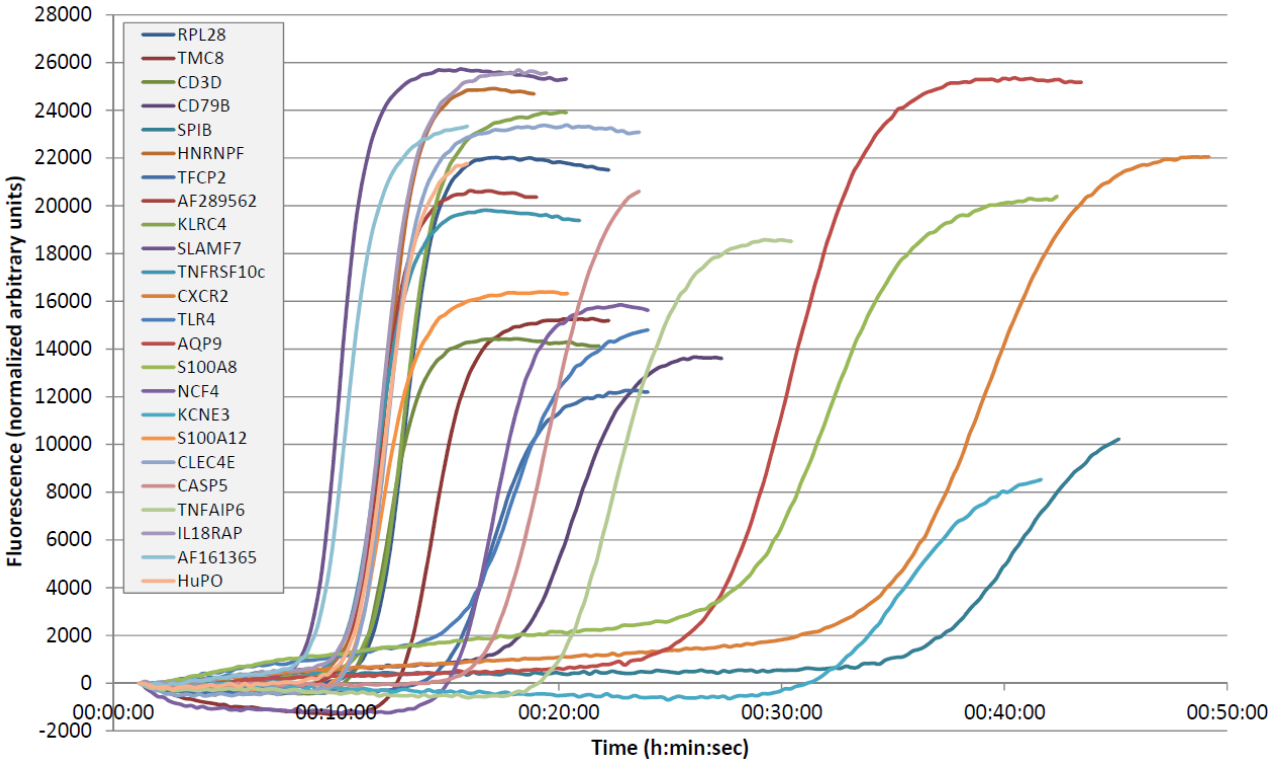
This journal is © The Royal Society of Chemistry 2013



Supplementary Figure 1:

Melting temperature for singleplex versus multiplex detection.

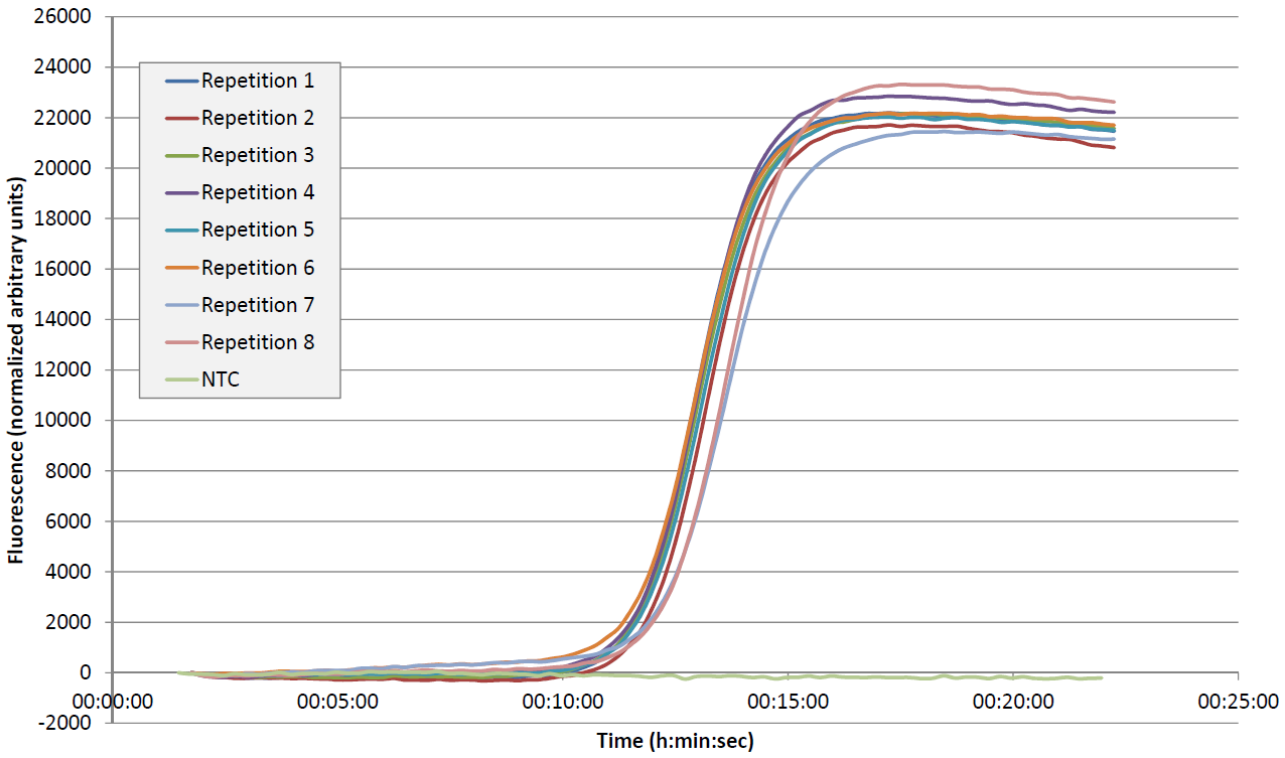
This journal is © The Royal Society of Chemistry 2013



Supplementary Figure 2:

Fluorescent development in isothermal amplification for detection of all 24 mRNA targets.
RNA was purified from whole blood from which the RT-isoPCR method was performed. A first-stage multiplex PCR with all 24 primer sets was performed, the product of which was used as template in individual isothermal amplification reactions targeting each of the 24 mRNAs. For simplicity and ease of viewing, the fluorescent developments after the plateaus are reached are not shown. In general, the fluorescence level decreased after the plateaus were reached.

This journal is © The Royal Society of Chemistry 2013



Supplementary Figure 3:

Fluorescence development in isothermal amplification for detection of RPL28.
RNA was purified from whole blood from which the RT-isoPCR method was performed for 8 repetitions. Each RT-isoPCR repetition was performed using a first-stage multiplex PCR with all 24 primer sets. The products were then detected in isothermal amplifications with RPL28 specific primer sets. The time of detection is determined as the time point at which the highest slope (fluorescence vs time) is observed. No template controls were included as negative controls.