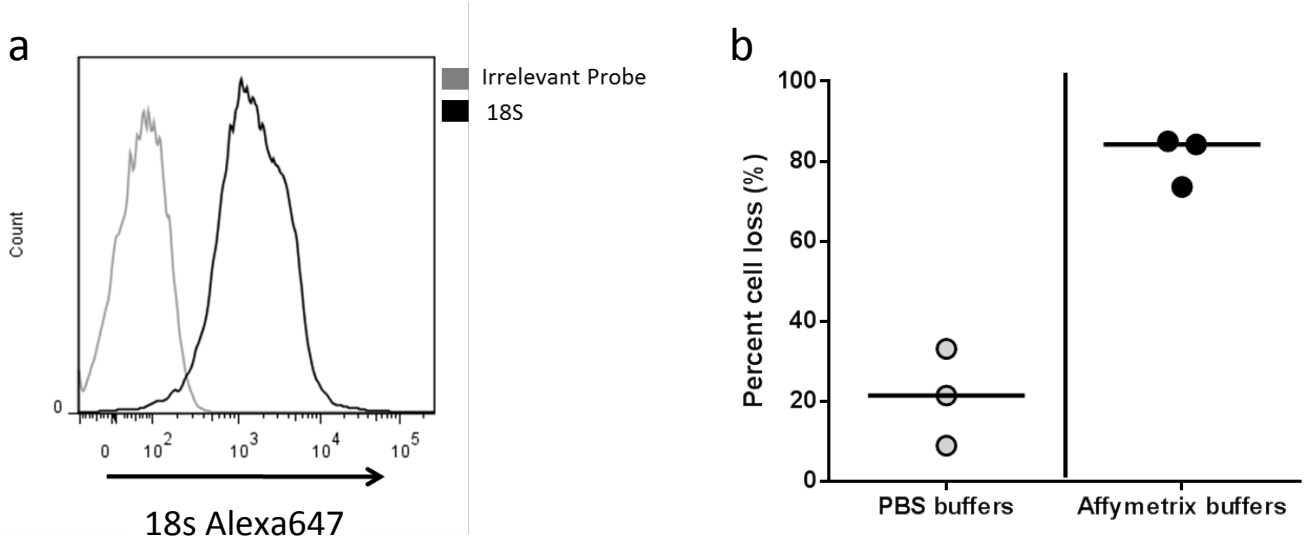
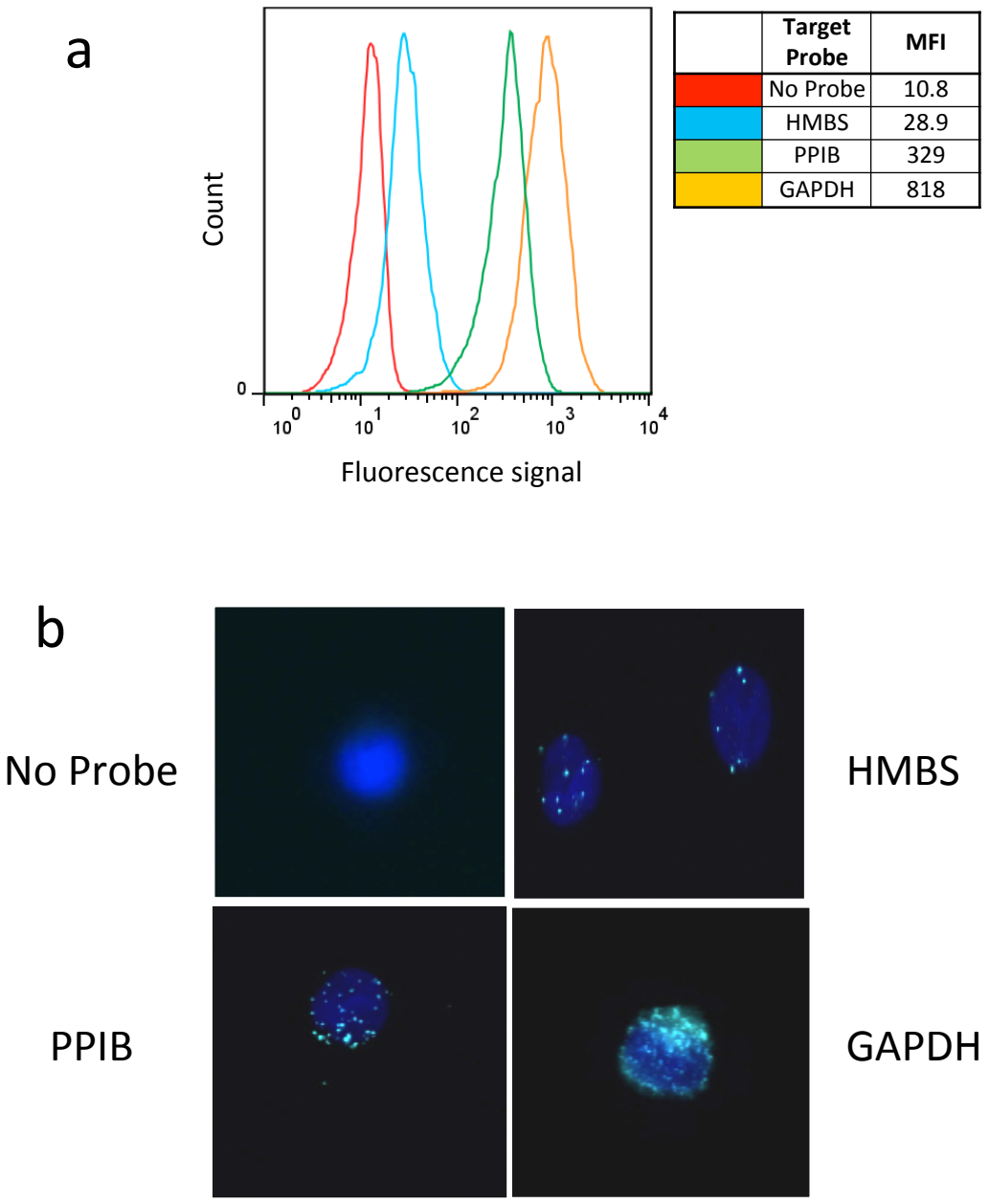
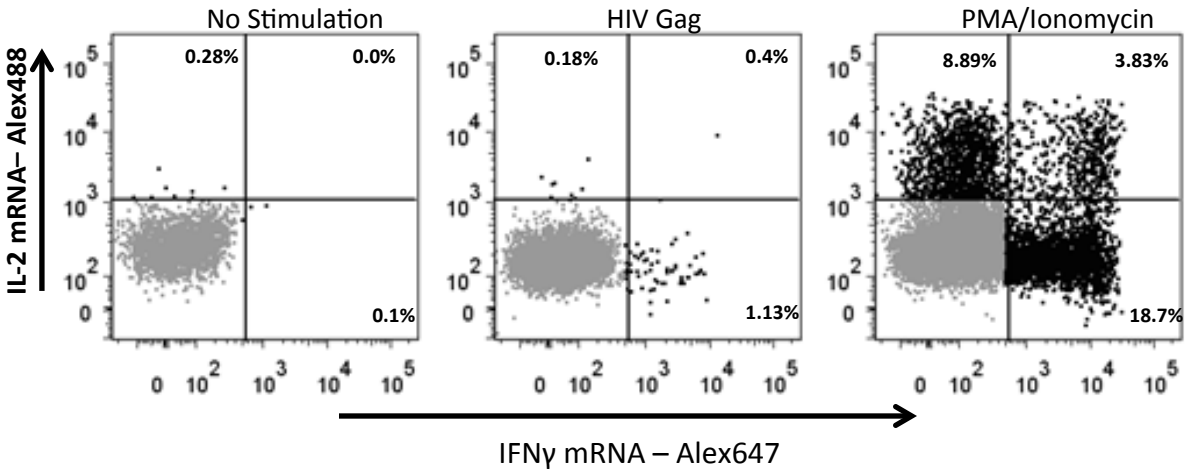


Supplementary Figure 1. Adaptation of the QuantiGene ViewRNA for cells in suspension and detection with flow cytometry. (a) PBMCs from healthy control individuals were stained either with probes for 18s or and irrelevant probe, DapB, according to the QuantiGene ViewRNA protocol for microscopy. Instead of performing the assay on slides we performed the assay on cells in suspension in 3ml facs tubes and the cells we analyzed on the LSR Fortessa**. (b)** Comparison of cell loss after using either PBS, according to a standard surface stain flow protocol, or the flow-FISH buffers.

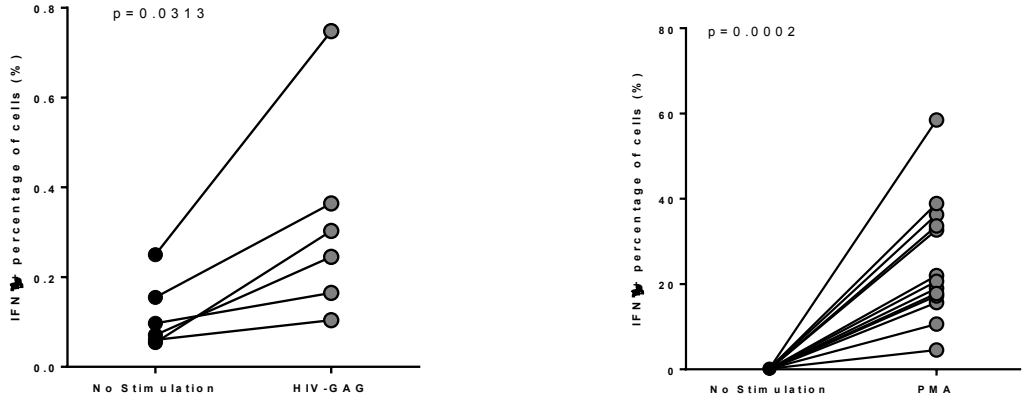


Supplementary Figure 2. Sensitivity of Flow-FISH assay.
Detection of three housekeeping genes with variable expression with flow cytometry and confocal microscopy. U937 cells were stained using the Flow-FISH assay with probes for HMBS (low expression), PPIB (medium expression) and GABDH (high expression). Samples were split in two and half were analyzed with flow cytometry (a) and the other half was analyzed with confocal microscopy (b).

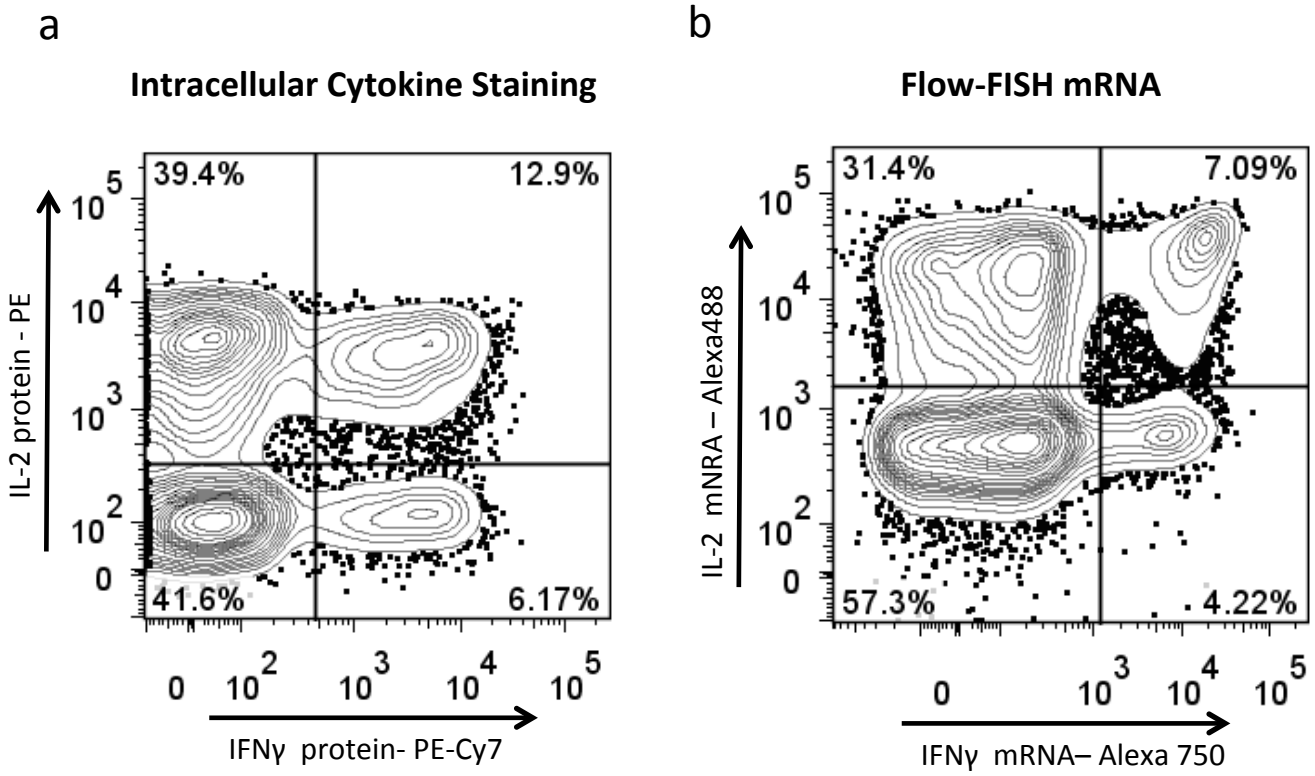
a



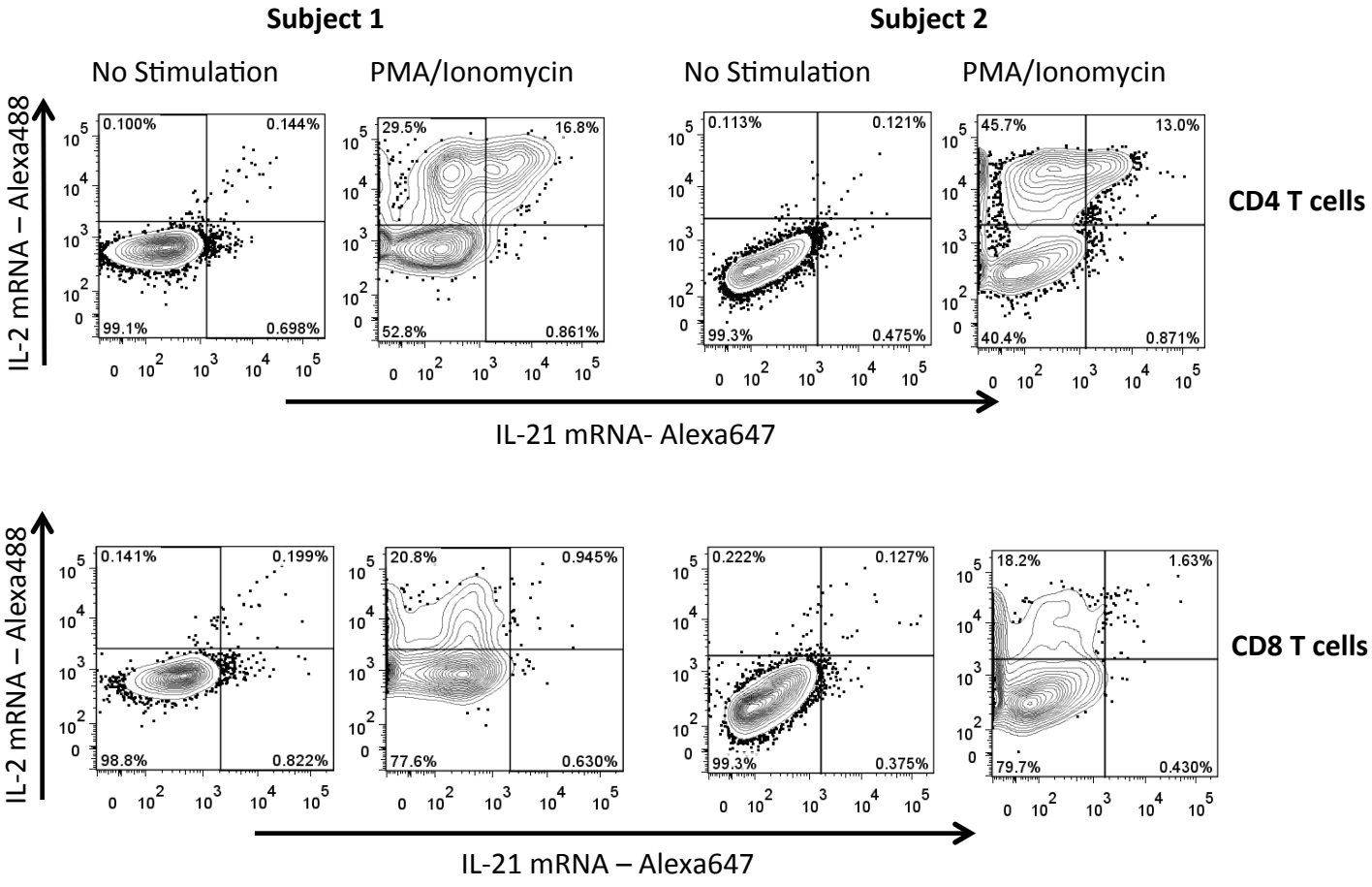
b



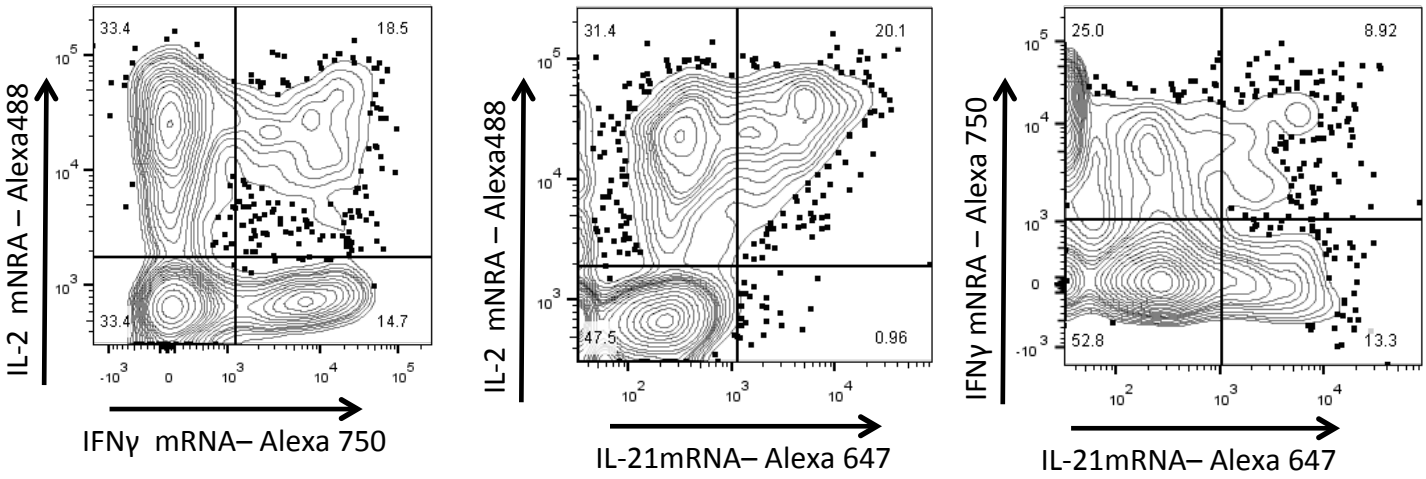
Supplementary Figure 3. Detection of IL-2 and IFN-γ mRNA in antigen-specific CD8 T cells. IL-2 and IFN-γ mRNA detection in CD8 T cells from PBMCs of an HIV-infected individual stimulated with an HIV-Gag peptide pool, a CMV lysate or PMA/Ionomycin for 12 hours (one representative experiment out of five). (**b**) Collective data of IFN-γ mRNA detection after HIV-Gag peptide pool, a CMV lysate or PMA/Ionomycin stimulation. Statistical analysis was performed using a Wilcoxon matched-pairs test.



Supplementary Figure 4. Comparison of cytokine detection with intracellular cytokine staining protocol or flow-FISH mRNA assay. PBMCs from healthy control individuals were stimulated overnight with PMA/ Inomycin. A) intracellular cytokine staining form IL-2 and IFNγ protein and B) Flow-FISH assay for detection of IL-2 and IFNγ mRNA.



Supplementary Figure 5. Detection of IL-21 mRNA in CD4 T cells but not CD8 T cells. Lymphocytes isolated by a spleen sample of two subjects suffering from idiopathic thrombocytopenic purpura. Cells were stimulated overnight either following an ICS protocol or following the new flow-RNA protocol.



Supplementary Figure 6. Simultaneous detection of 3 different mRNA cytokines using flow-RNA fish. IL2, IFNγ and IL-21 mRNA detection on CD4 T cells from a spleen sample of a subject suffering from idiopathic thrombocytopenic purpura. Cells were stimulated overnight with PMA/ Ionomycin.

Supplementary Table 1. Sequence of probe sets used in this study.

TARGET GENE (ACCESSION NUMBER)	PROBE BINDING REGION	SEQUENCE
Human IL-2 (NM_021803)	2-24bp	ccttggtctcgttttcacttcag
	25-52bp	tctcataagtaccaacagtagagctaga
	53-72bp	tccatgttgccaggactgga
	73-96bp	accatcagacagatgacaatcctc
	97-118bp	gaccagtgtccccaagaagatg
	119-139bp	accttgggagcttgatttgtg
	140-162bp	attctaatcatgtggcgatcttg
	163-189bp	tgatcaacaatatctataagttgacgc
	190-214bp	caagtcattcacataatttttcagc
	215-234bp	gctggcagaaattcagggac
	235-260bp	cacagtttgtctctacatcttctgga
	261-282bp	aagcaggaaaaagctgaccact
	283-304bp	tgactttagttgggccttctga
	305-328bp	cctttcattgtttcctgtatttgc
	329-356bp	tcagctttttaattgatacattgattat
	357-378bp	tttgtggaaggtggtttcctct
	379-400bp	gtgtttctgtcttctccctgca
	401-423bp	tcacatgaagggcatgttagtct
	424-446bp	tgggtggtttttttctcataagaa
	447-473bp	gtgatttgaatctttctaggaattctt
	474-497bp	gctgatgaatcatcttttggagaa
	498-521bp	ttccgtgtgttctagaggacagat
	522-547bp	caagttagatcctcaggaatcttcac
Human IL-21 (NM_000586)	2-28bp	tagtgattaaagagagtgatagggaac
	29-52bp	ggcaggagttgaggttactgtgag
	53-76bp	caggagttgcatcctgtacattgt
	77-99bp	gcaagacttagtgcaatgcaaga
	100-124bp	agtaggtgcactgtttgtgacaagt
	125-150bp	agctgtgttttctttgtagaacttga
	151-173bp	ccagcagtaaatgctccagttgt
	174-199bp	aattccattcaaaatcatctgtaaat
	200-225bp	gtgagtttgggattcttgtaattatt
	226-251bp	tgtaaaacttaaatgtgagcatcctg
	252-269bp	ctgtggccttcttgggca
	270-297bp	tcttctagacactgaagatgtttcagtt
	298-320bp	cttcctccagaggtttgagttct
	321-344bp	ttttgctttgagctaaatttagca
	345-367bp	gtccctgggtcttaagtgaaagt
	368-395bp	gaactattacgttgatattgctgattaa
	396-419bp	ttgtttcagatccctttagttcca
	420-444bp	tcatcagcatattcacacatgaatg
	445-469bp	cagaaattctacaatggttgctgtc
	470-494bp	tttgacaaaaggtaatccatctgtt
	495-519bp	tatcaagtcagtgttgagatgatgc
	520-544bp	atgttttaagtgggaagcacttaat

	545-572bp	aaatatttaaataaatagaaggcctgat
	573-601bp	ccatacattcaacaataaatataaaattt
	632-662bp	tcataaaagatccatatttatagttttaaga
	663-684bp	cccctagggcttacaaaaagaa
	685-712bp	gggataaataagtgaaaccattttagag
Human IFN-gamma (NM_000619)	26-53bp	ttaactgatctttctcttctaatagctg
	54-75bp	aagctgatcaggtccaaaggac
	76-104bp	aagaagttgaaatcagtagttcttgtatc
	105-126bp	cgtttccgagagaattaagcca
	127-155bp	aaagccaagatataacttgtatatttcat
	156-175bp	ccaaaacgatgcagagctga
	176-197bp	tggcagtaacagccaagagaac
	198-221bp	tctgcttcttttacatatgggtcc
	222-248bp	cctgcattaaaatatttcttaaggttt
	249-272bp	ccattatccgctacatctgaatga
	273-297bp	cttcaaaatgcctaagaaaagagtt
	298-321bp	tctgtcactctcctctttccaatt
	322-345bp	gacaatttggctctgcattatttt
	346-372bp	gtttttaaaaagtttgaagtaaaagga
	373-395bp	tggatgctctggtcatctttaaa
	396-418bp	ccttgatggtctccacactcttt
	419-444bp	attgaaaaacttgacattcatgtctt
	445-467bp	tcatctcgtttctttttgttgct
	468-492bp	cgaataattagtcagcttttcgaag
	493-516bp	gcgttggacattcaagtcagttac
	517-540bp	ttggatgagttcatgtattgcttt
	541-560bp	ggcgacagttcagccatcac
	561-581bp	cgcttccctgttttagctgct
	582-605bp	cgaaacagcatctgactccttttt
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	647-678bp	ttaataaatagatttagatttaaaattcaaat
	768-805bp	atataggaattataaataatacatatattaatagatat
	806-830bp	aaggattaagtgagacagtcacagg
	831-855bp	gccttgcctaattagtcagaaaaca
	856-882bp	cctgagataaagccttgtaatcacata
	883-901bp	gttggctgcctagttggcc
	902-923bp	caacccatgggatcttgcttag
	924-951bp	cattgtatcatcaagtgaaataaacaca
	952-981bp	ctggatagtatcacttcacttataagtgtt
	982-1004bp	gcatattttcaaaccggcagtaa
	1005-1025bp	aagcactggctcagattgcag
	1026-1049bp	caagttctgtctgacatgccatta
	1050-1071bp	catcagggtcacctgacacatt
	1072-1096bp	gaaatctcctgagatgctatgtttt
	1097-1117bp	atatttggaagcaccaggcat
	1118-1141bp	tgggtacagtcacagttgtcaaca
	1142-1168bp	attttaacaaatgagttactttccatt

Human IDO (NM_002164)	116-135bp	gttttccatagcgtgtgcca
	136-160bp	ggtactctttactgattgtccagga
	161-184bp	caaagcccacttcttcatcaatat
	185-205bp	tttcctgtggatttggcagag
	206-232bp	tccagtcattataaaaatcaggtagat
	233-255bp	aggcagatgtttagcaatgaaca
	256-278bp	agctggccagactctatgagatc
	279-304bp	tgtttaacttctcaactctttctcga
	305-327bp	tgtgagatgatcaatgctgagca
	328-347bp	aggcgctgtgacttgtggtc
	348-369bp	gcatcccagaactagacgtgca
	370-391bp	cccacacatatgccatggtgat
	392-412bp	ggacatctccatgacctttgc
	413-437bp	gcaatatttcttggcaagaccttac
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	461-483bp	aataggaggcagttccagtttct
	484-507bp	caagacacagtctgcataaaccaa
	508-530bp	ggatccttttttcttccagtttgc
	531-555bp	gttctcataagtcaggggcttatta
	556-578bp	cgaaatgagaacaaaacgtccat
	579-602bp	aatcctttactgcagtctccatca
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	851-873bp	ccagaacccttcatacaccagac
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	1060-1081bp	ttgaaaggacaaactcacggac
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	1120-1141bp	tcagggagaccagagctttcac
	213-232bp	ggtacccacgcgaatcactc
	233-250bp	agcaagctggctcttgcg
	251-271bp	cacactgtccgtctgtatgcg
	272-292bp	cgaggctttcaatgttgccac

Human HMBS (NM_000190)	293-312bp	tcaaactgcaggccagggta
	313-335bp	ctgtggtggacatagcaatgatt
	336-358bp	tgcagtatcaagaatcttgtccc
	359-383bp	ggcttttctctccaatcttagagag
	384-406bp	atgttcaagctccttggtaaaca
	407-428bp	ccacttcattcttctccagggc
	429-451bp	cttcaaggagtgaacaaccaggt
	452-471bp	ggaagcacagtgggcaggtc
	472-490bp	tccgatggtgaagccagga
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	508-529bp	gacaacagcatcatgagggttt
	530-550bp	cccaacaaattttgggtgaaa
	551-571bp	tggcagggtttctagggtctt
	572-593bp	tggttcccaccacactcttctc
	594-612bp	gctgctcttcgcagggagc
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	767-786bp	tcctcagggtgcaggatctg
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	1331-1351bp	agtctcccggggtaatcactc

	1352-1371bp	aggcttgaaccctgcagttc
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Human PPIB (NM_000942)	97-113bp	cggtggctagggagccg
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	915-932bp	ggagtgggtccgctccac
	63-80bp	gctggcgacgcaaaagaa
	81-100bp	tgtctgagcgatgtggctcg
	101-120bp	gaccttcaccttccccatgg
	121-140bp	cgaccaaatccgttgactcc

Human GAPDH (NM_002046)	141-158bp	gtgaccaggcgcccaata
	159-179bp	ccagagttaaaagcagccctg
	180-203bp	ttgatggcaacaatatccacttta
	204-224bp	ttgaggtcaatgaaggggtca
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	853-876bp	gatgtcatcatatttggcaggttt
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	974-994bp	cagcgtcaaaggtggaggagt
	995-1011bp	gagggcaatgccagccc
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	1057-1076bp	accaccctgttgctgtagcc
	1117-1133bp	ctggggctggtggtcca
	1134-1157bp	ctctctcttcctcttgtgctcttg
	1158-1177bp	gactccccagcagtgagggt
hsa-miR-21 (MI0000077)	8-18bp	ctgataagcta
	19-29bp	tcaacatcagt
hsa-miR-155 (MI0000681)	4-15bp	gattagcattaa
	16-26bp	acccctatcac