

Appendices

Appendix A: Molecular markers

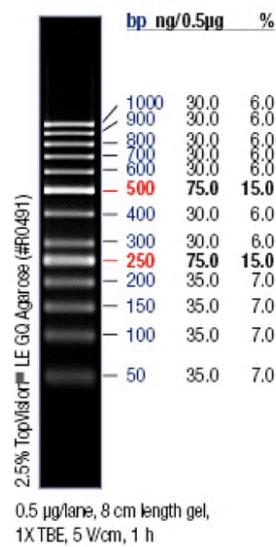


Figure A1: DNA ladder. GeneRuler 50 bp DNA Ladder (Thermo Scientific, Fermentas, Lithuania).

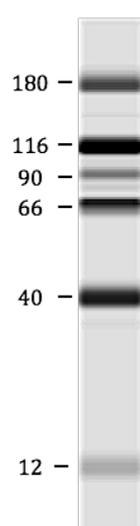


Figure A2: Protein marker. Biotinylated Ladder 1 (Protein Simple, California, USA)

Appendix B: Calibration curves.

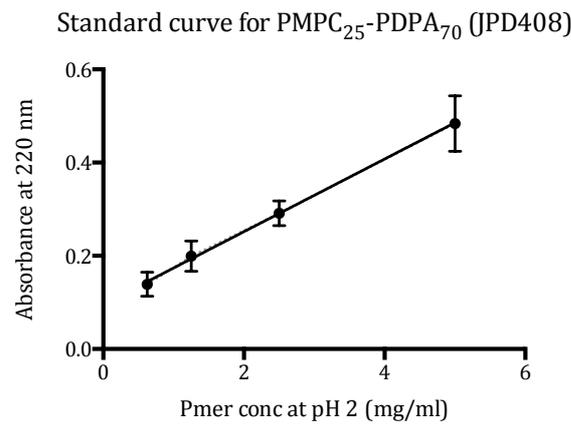


Figure B1: Standard curve of polymer concentration. PMPC₂₅-PDPA₇₀ was dissolved in PBS buffer at pH 2.0. Polymer concentration was measured at the absorbance of 220 nm by UV-Vis spectrophotometer.

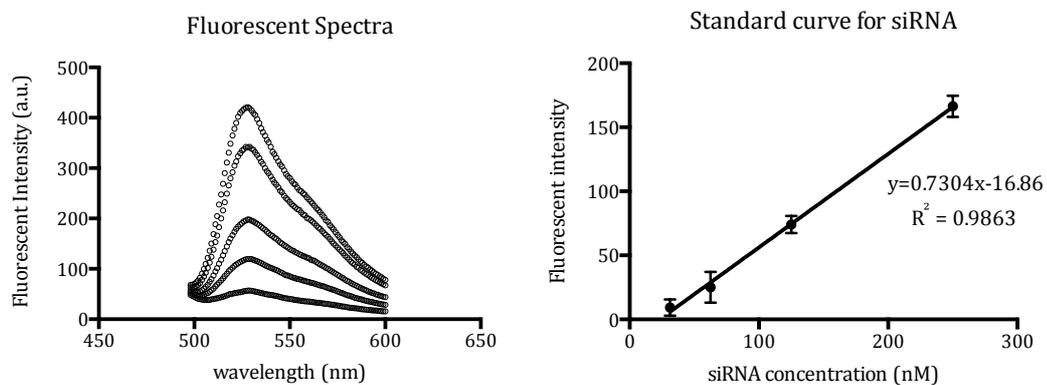


Figure B2: Standard curve of siRNA concentration. siRNA concentration was determined using the Quant-it™ Picogreen® dsDNA assay (Invitrogen), Fluorescent signal from siRNA incorporated dye was determined using Spectrofluorometry (at excitation wavelength of 480 nm and emission wavelength of 528 nm).

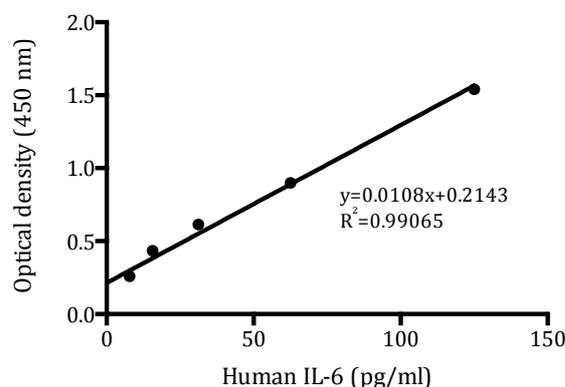


Figure B3: Calibration curve for Human Interleukin-6 (IL-6). Various standards of Human IL-6 over the range of 0 to 500 pg/ml were prepared, performed ELISA and measured at absorbance of 450 nm by microplate reader and plotted as the calibration curve.

Appendix C: MATLAB script for image analysis

```

clear all
[p n]=uigetfile('*.','Choose DAPI');
dapi=imread([n p]);
[p n]=uigetfile('*.','Choose RHD');
rhd=imread([n p]);
[r c]=size(dapi);
culo=1;
th=100;
while culo==1
    nuc=255.*ones(r,c);
    f=find(dapi<th);
    nuc(f)=0;
    nucer=imerode(nuc,strel('disk',2));
    nuccdil=imdilate(nuc,strel('disk',2));
    subplot(2,2,1)
    imshow(dapi)
    title('original dapi')
    subplot(2,2,2)
    imshow(nucer)
    title('mask for nucleus')
    subplot(2,2,3)
    imshow(nuccdil)
    title('area escluded from cytoplasm')
    culo=input('do it again? yes=1; no=0 ');
    drawnow
    pause(0.1)
    if culo==1
        th=input('new threshold? ');
    end
end

close all

culo=1;
th=100;
while culo==1
    cit=255.*ones(r,c);
    f=find(rhd<th);
    cit(f)=0;
    cit=imerode(cit,strel('disk',4));
    f=find(nuccdil==255);
    cit(f)=0;
    def=rhd;
    def(:,:,2)=nucer;
    def(:,:,3)=cit;
    subplot(2,2,1)
    imshow(rhd)
    title('original rhd')
    subplot(2,2,2)
    imshow(cit)
    title('citoplasm mask')
    subplot(2,2,3)
    imshow(def)
    title('All masks')
    culo=input('do it again? yes=1; no=0 ');
    drawnow
    pause(0.1)
    if culo==1
        th=input('new threshold? ');
    end
end

close all

culo=1;
th=3;
while culo==1
    cited=edge(cit);
    nuccdil2=imdilate(nuccdil,strel('disk',12));
    f=find(nuccdil2==255);
    cited(f)=0;
    cited2=imdilate(cited,strel('disk',th));
    imshow(cited2)
    culo=input('do it again? yes=1, no=0 ');
    drawnow
    pause(0.1)
    if culo==1
        th=input('new strel size: ');
    end
end
end

```

```
citdil=imdilate(cit,strel('disk',th));  
  
nucdil2=nucdil;  
fer=find(nucer==255);  
nucdil(fer)=0;  
  
f=find(nucer==255);  
nucval=rhd(f);  
f=find(cit==255);  
citval=rhd(f);  
f=find(nucdil==255);  
nucring=rhd(f);  
f=find(cited2==1);  
citring=rhd(f);  
  
nuc_avg=mean(nucval)  
cit_avg=mean(citval)  
nucring_avg=mean(nucring)  
citring_avg=mean(citring)  
  
display('the variables in the workspace nucval and citval contains all the intensity values,  
pixels by pixels')
```


1381 aagctcttgg agggcgagga ggagaggcta cgctgtccc ccagccctac ctgcgagcgc
 1441 agccgtggcc gtgcttcctc tcactcatcc cagacacagg gtgggggag cgtcaccaaa
 1501 aagcgcaaac tggagtccac tgagagccgc agcagcttct cacagcacgc acgcactagc
 1561 gggcgcgtag ccgtggagga ggtggatgag gagggaagt ttgtccggt gcgcaacaag
 1621 tccaatgagg accagtccat gggcaattgg cagatcaagc gccagaatgg agatgatccc
 1681 ttgctgactt accggttccc accaaagtcc accctgaagg ctgggcaggt ggtgacgatc
 1741 tgggctgcag gagctggggc caccacagc cccctaccg acctgggtgtg gaaggcacag
 1801 aacacctggg gctgcgggaa cagcctgcgt acggctctca tcaactccac tggggaagaa
 1861 gtggccatgc gcaagctggt gcgctcagt actgtggttg aggacgacga ggatgaggat
 1921 ggagatgacc tgctccatca ccaccacgtg agtggtagcc gccgctgagg ccgagcctgc
 1981 actggggcca ccagccagg cctgggggca gcctctcccc agcctccccg tgccaaaaat
 2041 cttttcatta aagaatgttt tggaacttta aaaaaaa

>>> FWD primer

<<<< RV primer

_____ Silencer Select Validated siRNA from ambion (P/N:4390826)

===== anti-Lamin A/C siRNA, sequence²

² Elbashir, S.M., et al., Analysis of gene function in somatic mammalian cells using small interfering RNAs. *Methods*, 2002. 26(2): p. 199-213.

Gene Match for siRNA scrambled sequence

Database: Homo sapiens build 37.3 genome database (reference assembly
GRCh37.p5 [GCF_000001405.17] and alternate assemblies HuRef
[GCF_000002125.1] and CRA_TCAGchr7v2 [GCF_000002135.2])
4,900 sequences; 5,937,867,303 total letters

Query=
Length=19

Sequences producing significant alignments:	Score (Bits)	E Value
ref NT_010498.15	Homo sapiens chromosome 16 genomic contig, ... 30.2	9.4
ref NT_006576.16	Homo sapiens chromosome 5 genomic contig, G... 30.2	9.4
ref NW_001838329.1	Homo sapiens chromosome 16 genomic contig... 30.2	9.4
ref NT_167205.1	Homo sapiens chromosome Y genomic contig, GR...28.2	37
ref NT_079573.4	Homo sapiens chromosome X genomic contig, GR...28.2	37
ref NT_167197.1	Homo sapiens chromosome X genomic contig, GR...28.2	37
ref NT_011515.12	Homo sapiens chromosome 21 genomic contig, ... 28.2	37
ref NT_010966.14	Homo sapiens chromosome 18 genomic contig, ... 28.2	37
ref NT_010194.17	Homo sapiens chromosome 15 genomic contig, ... 28.2	37
ref NT_026437.12	Homo sapiens chromosome 14 genomic contig, ... 28.2	37
ref NT_024524.14	Homo sapiens chromosome 13 genomic contig, ... 28.2	37
ref NT_029419.12	Homo sapiens chromosome 12 genomic contig, ... 28.2	37
ref NT_008413.18	Homo sapiens chromosome 9 genomic contig, G... 28.2	37
ref NT_008183.19	Homo sapiens chromosome 8 genomic contig, G... 28.2	37
ref NT_033968.6	Homo sapiens chromosome 7 genomic contig, GR...28.2	37
ref NT_007592.15	Homo sapiens chromosome 6 genomic contig, G... 28.2	37
ref NT_016354.19	Homo sapiens chromosome 4 genomic contig, G... 28.2	37
ref NT_016297.16	Homo sapiens chromosome 4 genomic contig, G... 28.2	37
ref NT_005612.16	Homo sapiens chromosome 3 genomic contig, G... 28.2	37
ref NT_032977.9	Homo sapiens chromosome 1 genomic contig, GR.. 28.2	37
ref NW_001842361.2	Homo sapiens chromosome X genomic contig,... 28.2	37
ref NW_001842360.1	Homo sapiens chromosome X genomic contig,... 28.2	37
ref NW_001842356.1	Homo sapiens chromosome X genomic contig,... 28.2	37
ref NW_001838716.1	Homo sapiens chromosome 21 genomic contig... 28.2	37
ref NW_001838467.2	Homo sapiens chromosome 18 genomic contig... 28.2	37
ref NW_001838289.1	Homo sapiens chromosome 16 genomic contig... 28.2	37
ref NW_001838219.1	Homo sapiens chromosome 15 genomic contig... 28.2	37
ref NW_001838113.2	Homo sapiens chromosome 14 genomic contig... 28.2	37
ref NW_001838073.2	Homo sapiens chromosome 13 genomic contig... 28.2	37
ref NW_001838061.2	Homo sapiens chromosome 12 genomic contig... 28.2	37
ref NW_001839149.2	Homo sapiens chromosome 9 genomic contig,... 28.2	37
ref NW_001839132.1	Homo sapiens chromosome 8 genomic contig,... 28.2	37
ref NW_001839007.1	Homo sapiens chromosome 7 genomic contig,... 28.2	37
ref NW_001838973.1	Homo sapiens chromosome 6 genomic contig,... 28.2	37
ref NW_001838921.1	Homo sapiens chromosome 4 genomic contig,... 28.2	37
ref NW_001838915.1	Homo sapiens chromosome 4 genomic contig,... 28.2	37
ref NW_001838901.1	Homo sapiens chromosome 4 genomic contig,... 28.2	37
ref NW_001838884.2	Homo sapiens chromosome 3 genomic contig,... 28.2	37
ref NW_001838590.2	Homo sapiens chromosome 1 genomic contig,... 28.2	37
ref NT_079592.2	Homo sapiens chromosome 7 genomic contig, al.. 28.2	37

Appendix E: Gene name**Table E1: List of genes on PCR array: PAHS-016**

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
1	Hs.12341	NM_001111	ADAR	Adenosine deaminase, RNA-specific	1.01	0.64	0.88	0.95	0.02
2	Hs.530291	NM_001157	ANXA11	Annexin A11	1.21	0.77	1.21	1.16	0.58
3	Hs.470233	NM_177985	ARL5A	ADP-ribosylation factor-like 5A	1.03	1.19	1.15	1.78	1.02
4	Hs.25362	NM_178815	ARL5B	ADP-ribosylation factor-like 5B	0.92	0.77	1.05	1.38	0.74
5	Hs.9754	NM_012068	ATF5	Activating transcription factor 5	0.85	0.72	1.02	1.30	0.72
6	Hs.523309	NM_004281	BAG3	BCL2-associated athanogene 3	0.90	0.76	1.27	1.49	0.88
7	Hs.118110	NM_004335	BST2	Bone marrow stromal cell antigen 2	1.13	0.81	1.23	1.28	0.79
8	Hs.2490	NM_033292	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	0.94	1.61	1.31	1.04	4.93
9	Hs.74034	NM_001753	CAV1	Caveolin 1, caveolae protein, 22kDa	1.03	1.16	1.12	2.82	1.26
10	Hs.460988	NM_001755	CBFB	Core-binding factor, beta subunit	0.99	0.76	1.01	1.24	0.76
11	Hs.501497	NM_001252	CD70	CD70 molecule	1.04	0.59	1.26	2.49	0.57
12	Hs.238990	NM_004064	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	1.03	1.4	1.07	1.23	1.07
13	Hs.273621	NM_033133	CNP	2',3'-cyclic nucleotide 3' phosphodiesterase	0.99	0.59	1.01	1.28	0.55
14	Hs.368921	NM_001856	COL16A1	Collagen, type XVI, alpha 1	1.12	1.34	1.68	1.55	1.52

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
15	Hs.632586	NM_001565	CXCL10	Chemokine (C-X-C motif) ligand 10	0.62	1.04	26.16	5.70	17.54
16	Hs.82890	NM_001344	DAD1	Defender against cell death 1	1.02	1.74	1.54	1.75	3.01
17	Hs.169611	NM_019887	DIABLO	Diablo, IAP-binding mitochondrial protein	1.18	0.75	0.92	1.08	0.43
18	Hs.77768	NM_006736	DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2	1.14	0.77	1.54	1.42	0.69
19	Hs.131431	NM_002759	EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2	1.02	0.99	1.09	1.44	0.85
20	Hs.62661	NM_002053	GBP1	Guanylate binding protein 1, interferon-inducible	1.00	1.34	1.93	1.40	1.25
21	Hs.386567	NM_004120	GBP2	Guanylate binding protein 2, interferon-inducible	1.18	1.38	1.41	1.44	0.91
22	Hs.86724	NM_000161	GCH1	GTP cyclohydrolase 1	0.93	1.18	6.71	1.55	0.7
23	Hs.181244	NM_002116	HLA-A	Major histocompatibility complex, class I, A	1.00	0.68	1.43	1.26	0.59
24	Hs.654404	NM_005514	HLA-B	Major histocompatibility complex, class I, B	1.06	0.59	1.15	1.03	0.51
25	Hs.449621	NM_002117	HLA-C	Major histocompatibility complex, class I, C	0.30	1.19	20.28	4.39	0.04
26	Hs.631991	NM_002119	HLA-DOA	Major histocompatibility complex, class II, DO alpha	1.54	1.65	44.63	1.43	0.66
27	Hs.387679	NM_002122	HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1	0.52	7.73	28.01	7.59	2.09
28	Hs.650174	NM_005516	HLA-E	Major histocompatibility complex, class I, E	0.99	0.81	0.96	0.69	0.51
29	Hs.519972	NM_018950	HLA-F	Major histocompatibility complex, class I, F	1.02	1.14	3.12	4.88	1.34
30	Hs.512152	NM_002127	HLA-G	Major histocompatibility complex, class I, G	0.45	24.67	1.84	270.59	6.84

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
31	Hs.514289	NM_002145	HOXB2	Homeobox B2	0.98	1.52	1.66	1.61	0.68
32	Hs.690634	NM_005527	HSPA1L	Heat shock 70kDa protein 1-like	0.80	1.15	7.1	1.53	0.74
33	Hs.380250	NM_005531	IFI16	Interferon, gamma-inducible protein 16	1.16	0.97	2.46	2.04	2.82
34	Hs.532634	NM_005532	IFI27	Interferon, alpha-inducible protein 27	1.01	1.17	1.92	2.16	3.35
35	Hs.14623	NM_006332	IFI30	Interferon, gamma-inducible protein 30	1.01	0.76	0.62	1.10	0.76
36	Hs.730125	NM_002038	IFI6	Interferon, alpha-inducible protein 6	1.10	1.5	1.26	2.21	9.82
37	Hs.163173	NM_022168	IFIH1	Interferon induced with helicase C domain 1	1.15	3.18	0.92	2.27	10.72
38	Hs.20315	NM_001548	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	0.90	1.17	0.97	2.18	2.79
39	Hs.714337	NM_001549	IFIT3	Interferon-induced protein with tetratricopeptide repeats 3	1.04	0.9	0.59	2.18	3.03
40	Hs.458414	NM_003641	IFITM1	Interferon induced transmembrane protein 1 (9-27)	1.10	0.72	0.76	1.01	0.65
41	Hs.709321	NM_006435	IFITM2	Interferon induced transmembrane protein 2 (1-8D)	1.16	1.08	1.12	1.31	1.51
42	Hs.37026	NM_024013	IFNA1	Interferon, alpha 1	6.64	1.48	1.39	2.35	1.04
43	Hs.211575	NM_000605	IFNA2	Interferon, alpha 2	0.40	0.03	0.95	0.10	3.02
44	Hs.1510	NM_021068	IFNA4	Interferon, alpha 4	N/A	N/A	N/A	N/A	N/A
45	Hs.529400	NM_000629	IFNAR1	Interferon (alpha, beta and omega) receptor 1	0.77	1.88	0.73	1.24	1.41

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
46	Hs.708195	NM_000874	IFNAR2	Interferon (alpha, beta and omega) receptor 2	1.12	1.05	0.93	1.75	0.9
47	Hs.93177	NM_002176	IFNB1	Interferon, beta 1, fibroblast	2.63	1.16	17.76	12.01	15.55
48	Hs.436061	NM_002198	IRF1	Interferon regulatory factor 1	1.16	0.7	0.44	0.72	0.61
49	Hs.654566	NM_002199	IRF2	Interferon regulatory factor 2	1.98	0.85	0.58	1.19	0.93
50	Hs.75254	NM_001571	IRF3	Interferon regulatory factor 3	1.04	0.81	2.5	1.47	0.8
51	Hs.521181	NM_001098629	IRF5	Interferon regulatory factor 5	1.39	2.77	80.68	472.34	5.08
52	Hs.166120	NM_001572	IRF7	Interferon regulatory factor 7	0.73	0.61	43.97	51.36	2.86
53	Hs.1706	NM_006084	IRF9	Interferon regulatory factor 9	1.09	1.35	6.51	12.96	1.84
54	Hs.458485	NM_005101	ISG15	ISG15 ubiquitin-like modifier	1.14	0.86	1.25	2.41	1.25
55	Hs.459265	NM_002201	ISG20	Interferon stimulated exonuclease gene 20kDa	1.05	0.99	6.2	7.24	1.8
56	Hs.75285	NM_002216	ITIH2	Inter-alpha (globulin) inhibitor H2	1.28	2.2	2.73	6.07	2.62
57	Hs.80395	NM_002371	MAL	Mal, T-cell differentiation protein	0.28	0.12	385.5	5.75	15.07
58	Hs.132966	NM_000245	MET	Met proto-oncogene (hepatocyte growth factor receptor)	1.18	0.97	0.75	2.12	0.89
59	Hs.626579	NM_020310	MNT	MAX binding protein	1.06	2.46	1.83	1.76	6.7
60	Hs.517307	NM_002462	MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	0.85	0.69	4.12	1.47	1.38

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
61	Hs.926	NM_002463	MX2	Myxovirus (influenza virus) resistance 2 (mouse)	0.51	0.34	1.32	5.80	5.53
62	Hs.82116	NM_002468	MYD88	Myeloid differentiation primary response gene (88)	0.97	0.82	2.14	1.17	0.65
63	Hs.54483	NM_004688	NMI	N-myc (and STAT) interactor	1.04	1.91	0.88	1.77	4.47
64	Hs.443837	NM_006310	NPEPPS	Aminopeptidase puromycin sensitive	1.04	0.8	0.77	0.79	0.7
65	Hs.453951	NM_013957	NRG1	Neuregulin 1	1.28	1.79	1.91	3.18	3.99
66	Hs.524760	NM_002534	OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	0.93	0.65	0.75	1.11	0.67
67	Hs.414332	NM_002535	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	0.81	1.34	1.42	2.47	2.26
68	Hs.526464	NM_033238	PML	Promyelocytic leukemia	0.96	0.71	1.61	1.53	0.51
69	Hs.496255	NM_002744	PRKCZ	Protein kinase C, zeta	0.93	0.64	1.82	0.87	0.66
70	Hs.570274	NM_003690	PRKRA	Protein kinase, interferon-inducible double stranded RNA dependent activator	0.98	2.09	7.86	1.42	4.75
71	Hs.434081	NM_002818	PSME2	Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	1.00	1.06	0.92	1.22	1.22
72	Hs.350966	NM_004219	PTTG1	Pituitary tumor-transforming 1	1.18	3.12	1.09	1.90	12.41
73	Hs.508021	NM_018191	RCBTB1	Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1	0.98	0.93	0.69	1.21	0.89
74	Hs.473341	NM_022136	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	1.79	1.17	0.78	3.61	12.95

No.	UniGene	RefSeq	Symbol	Description	Fold change (2 hrs)		Fold change (20 hrs)		
					Psomes	Pneg	Psomes	Pneg	Lipofec
75	Hs.349094	NM_002351	SH2D1A	SH2 domain containing 1A	N/A	N/A	N/A	N/A	N/A
76	Hs.521482	NM_003028	SHB	Src homology 2 domain containing adaptor protein B	1.08	0.98	1.5	2.16	0.81
77	Hs.489201	NM_006304	SHFM1	Split hand/foot malformation (ectrodactyly) type 1	1.06	1.91	1.38	1.68	3.72
78	Hs.502338	NM_004171	SLC1A2	Solute carrier family 1 (glial high affinity glutamate transporter), member 2	1.35	1.81	3.24	1.07	6.51
79	Hs.642990	NM_007315	STAT1	Signal transducer and activator of transcription 1, 91kDa	1.12	0.92	0.75	0.47	0.75
80	Hs.530595	NM_005419	STAT2	Signal transducer and activator of transcription 2, 113kDa	0.96	0.76	1.25	1.06	0.72
81	Hs.352018	NM_000593	TAP1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0.96	0.63	0.73	0.99	0.68
82	Hs.478275	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	0.95	1.46	2.05	1.66	2.43
83	Hs.510528	NM_003300	TRAF3	TNF receptor-associated factor 3	1.09	1.13	0.6	2.10	1.56
84	Hs.73793	NM_003376	VEGFA	Vascular endothelial growth factor A	0.78	0.99	0.64	1.32	0.78
85	Hs.534255	NM_004048	B2M	Beta-2-microglobulin	-	-	-	-	-
86	Hs.412707	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1	-	-	-	-	-
87	Hs.728776	NM_012423	RPL13A	Ribosomal protein L13a	-	-	-	-	-
88	Hs.592355	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	-	-	-	-	-
89	Hs.520640	NM_001101	ACTB	Actin, beta	-	-	-	-	-

Appendix F: Western blot

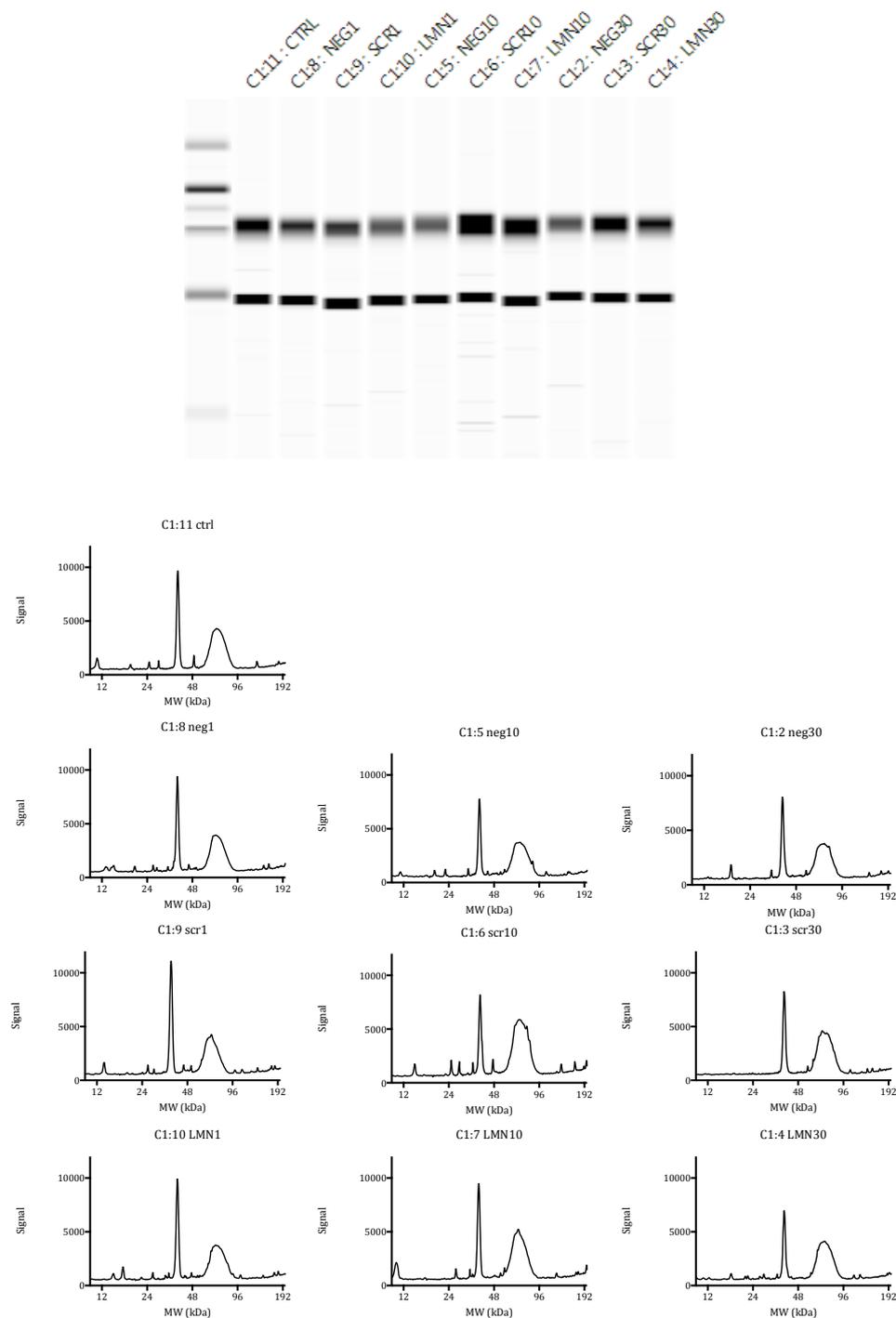


Figure F1: Electropherogram of protein samples on the Compass software. The proteins obtained in samples were identified according to their molecular weight by comparing with the protein marker. The area under the peak was used to analysed the relative protein expression. The calculation was performed by comparing with similar proteins in the untreated control sample.