

Supplemental Digital Contents

Methods

RNA extraction and sequencing – Sequencing center #1

We used an approach to construct RNA-sequencing libraries that yields an increased proportion of 5' end sequences. These steps are adapted from published methodologies^{1,2} and notably do not include normalization or fragmentation.

We performed two-rounds of selecting RNAs possessing poly-A tails by annealing them to poly-T oligos bound to beads (Invitrogen, Life Technologies, Grand Island, NY) so as to remove ribosomal RNA. After the RNAs were annealed to the poly-T-beads, unbound material was washed. The polyA species were eluted by melting the annealed structures at high temperature. After 2 rounds of polyA selection, the ribosomal RNA was ~5% of total RNA. Messenger RNAs (mRNAs) were reverse transcribed using random hexamers (Invitrogen). Double-stranded DNA (dsDNA) synthesis was performed using Pol I and RNase H. End-repair was performed using enzymes such as polymerases and exonucleases to obtain dsDNA fragments with no overhangs. Adenosine addition at 3' ends of both DNA strands was performed with subsequent ligation of appropriate next-generation sequencing adapters. The

Adenosine overhangs at 3' ends to prevent self-ligation of dsDNA fragments during adapter ligation.

Size selection was performed by fractionating the DNA library in an agarose gel to excise a size of about ~150-400bp. Size selection allows for appropriate size molecules for next-generation sequencing and enriches fragments that resulted from annealing of random hexamers near the 5' ends of transcripts.

Uniform amplification of the library with polymerase chain reaction was performed. To achieve uniform amplification of all fragments, the reaction was stopped at the stage of exponential amplification. A library constructed and sequenced with this approach contains directional information owing to the polarity of the complementary DNA (cDNA) molecule.

RNA extraction and sequencing - Sequencing center #2

Beads with oligodeoxythymidine (oligo[dT]) were used to isolate poly(A) mRNA. mRNA fragmentation was performed using divalent cations under elevated temperatures. Taking these short fragments as templates, random hexamer primer were used to synthesize the first-strand cDNA. The second-strand cDNA was synthesized using buffer, dNTPs, RNase H and DNA polymerase I. Short fragments were purified with QiaQuick p extraction kit (Qiagen, Hilden, Germany) and resolved with elution buffer for end reparation and poly(A) addition. Subsequently, the short fragments were connected with sequencing adaptors. For polymerase chain reaction amplification, we selected suitable fragments, as templates, with respect to the result of agarose gel

electrophoresis.

mRNA samples from RNA-sequencing were prepared using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA). Template molecules were used for cluster generation and sequencing on the Illumina HiSeq 2000 (Illumina, San Diego, CA). All samples were sequenced using 91 base-pairs paired-end sequencing (table 1).

References:

1. Wang Z, Gerstein M, Snyder M: RNA-Seq: A revolutionary tool for transcriptomics. *Nat Rev Genet* 2009; 10:57-63
2. Christodoulou DC, Gorham JM, Herman DS, Seidman JG: Construction of normalized RNA-seq libraries for next-generation sequencing using the crab duplex-specific nuclease. *Curr Protoc Mol Biol* 2011; Chapter 4:Unit4.12

Table 1. Description of Samples at the Two Sequencing Centers

	Total # samples	# samples single- end	Read length (avg bp)	# samples paired- end	Read length (avg bp)	# of alignments (millions)
Center 1	29	12	60	17	50	29 (20-48)
Center 2	16			16	91	63 (61-64)

Avg = average with range; bp = base pairs.

of alignments: reads are only considered if they have at least one alignment to a chromosome. Shown are median number of alignments in millions with interquartile range.

Table 2. GO Annotation

Down-regulated										
GO Identifier	Gene symbol	GO Term	Ontology	#Hits in group	Group size	#Hits expected	Fold enrichment	p-value	FDR	
GO:0005576	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, CCL14, CCL18, CCL21, CD14, CFD, CFH, CILP, CLU, CSTB, DCN, EFEMP1, F13A1, FBLN1, FBLN2, FOLR2, FTL, HP, IFI30, IGFBP4, ITLN1, KRT19, MGP, PLA2G2A, PLTP, PODN, PRELP, RARRES2, RNASE1, S100A4, SEPP1, SERPING1, SLPI, SPP1, WISP2	extracellular region	Cellular component	41	1,435	8	4.9	5.73E-21	7.16E-17	
GO:0006956	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CFD, CFH, CLU, F13A1, SERPING1	complement activation	Biological process	13	65	1	9.0	1.36E-17	8.52E-14	
GO:0072376	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CFD, CFH, CLU, F13A1, SERPING1	protein activation cascade	Biological process	13	74	1	9.0	8.37E-17	3.49E-13	
GO:0002252	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CD14, CFD, CFH, CLU, CYBA, F13A1, FCER1G, HCST, SERPING1, SLPI, SPP1	immune effector process	Biological process	20	467	3	5.9	3.39E-13	1.06E-09	
GO:0006959	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CFD, CFH, CLU, CYBA, F13A1, PLA2G2A, SERPING1	humoral immune response	Biological process	16	286	2	6.6	1.89E-12	4.72E-09	
GO:0002253	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CD52, CFD, CFH, CLU, F13A1, SERPING1	activation of immune response	Biological process	14	206	2	5.8	3.78E-12	7.89E-09	

GO:0048584	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CCL21, CD14, CD52, CFD, CFH, CLU, DCN, F13A1, MGP, PLTP, RARRES2, S100A4, SERPING1, SLPI, SPP1	positive regulation of response to stimulus	Biological process	24	835	5	4.5	5.45E-12	9.73E-09
GO:0006952	AIF1, C1QB, C1S, C3, C6, C7, CCL18, CCL21, CD14, CD52, CD68, CFD, CFH, CYBA, DCN, F13A1, FCER1G, HCST, HP, IFI30, LGMN, PLA2G2A, PRELP, RARRES2, SERPING1, SLPI, SPP1, TYROBP	defense response	Biological process	28	1,322	8	3.4	8.73E-11	1.21E-07
GO:0050778	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CD52, CFD, CFH, CLU, F13A1, SERPING1	positive regulation of immune response	Biological process	14	260	2	5.8	8.64E-11	1.35E-07
GO:0002684	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CD52, CFD, CFH, CLU, F13A1, SERPING1, SLPI	positive regulation of immune system process	Biological process	16	392	3	4.7	2.12E-10	2.65E-07
GO:0044421	C1QA, C1QB, C1QC, C1S, C6, CCL14, CCL18, CCL21, CFH, CLU, DCN, EFEMP1, FBLN1, FBLN2, ITLN1, KRT19, MGP, PLA2G2A, PODN, PRELP, RARRES2, SERPING1, SPP1	extracellular region part	Cellular component	23	933	5	4.3	3.54E-10	4.02E-07
GO:0006957	C3, C6, C7, CFD, CFH, SERPING1	complement activation, alternative pathway	Biological process	6	19	1	4.3	4.79E-10	4.61E-07
GO:0006958	C1QA, C1R, C1S, C6, C7, SERPING1	complement activation, classical pathway	Biological process	6	19	1	4.3	4.79E-10	4.61E-07
GO:0050776	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CD52, CFD, CFH, CLU, F13A1, RARRES2, SERPING1	regulation of immune response	Biological process	16	413	3	4.7	4.55E-10	4.74E-07

GO:0002682	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CD14, CD52, CFD, CFH, CLU, F13A1, IFI30, PTGIS, RARRES2, SERPING1, SLPI, TYROBP	regulation of immune system process	Biological process	21	798	5	3.9	7.56E-10	4.97E-07
GO:0005602	C1QA, C1QB, C1QC, C1S	complement component C1 complex	Cellular component	4	4	1	3.0	7.37E-10	5.12E-07
GO:0006955	C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL18, CCL21, CD14, CD52, CFD, CFH, CLU, CTSC, CYBA, F13A1, FCER1G, HCST, HP, IFI30, PLA2G2A, RARRES2, SERPING1, SPP1	immune response	Biological process	26	1,247	7	3.5	7.10E-10	5.22E-07
GO:0009611	AIF1, C1QB, C1QC, C1S, C3, C6, C7, CCL18, CCL21, CD14, CD52, CD68, CFH, DCN, EFEMP1, F13A1, FBLN1, FCER1G, HP, LYVE1, PLA2G2A, RARRES2, SERPING1, SLPI, SPP1	response to wounding	Biological process	25	1,150	7	3.4	7.01E-10	5.48E-07
GO:0002455	C1QA, C1R, C1S, C6, C7, SERPING1	humoral immune response mediated by circulating im	Biological process	6	20	1	4.3	6.82E-10	5.68E-07
GO:0002376	AIF1, C1QA, C1QB, C1QC, C1R, C1S, C3, C6, C7, CCL14, CCL18, CCL21, CD14, CD52, CFD, CFH, CLU, CTSC, CYBA, F13A1, FCER1G, HCST, HP, IFI30, LAPTM5, LGMN, PLA2G2A, PTGIS, RARRES2, SERPING1, SLPI, SPP1, TYROBP	immune system process	Biological process	33	2,074	11	2.9	1.93E-09	1.20E-06

GO:0016064	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, SERPING1	immunoglobulin mediated immune response	Biological process	8	71	1	5.7	3.65E-09	2.18E-06
GO:0051704	AKR1C1, C3, C6, C7, CCL18, CCL21, CD14, CFH, CYBA, EIF1AY, F13A1, FTL, HP, IFI30, IGFBP4, ITLN1, KRT18, LRP1, PLA2G2A, PLTP, PODN, PRELP, PTGIS, RARRES2, SLPI, SPP1, TYROBP	multi-organism process	Biological process	27	1,471	8	3.2	4.94E-09	2.81E-06
GO:0030246	C3, CCL21, CD14, CFH, CTSK, ITLN1, LYVE1, PLA2G2A, PLTP, PRELP, SEPP1, SERPING1, SLPI	carbohydrate binding	Molecular function	13	298	2	5.4	5.58E-09	3.03E-06
GO:0019724	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, SERPING1	B cell mediated immunity	Biological process	8	81	1	5.7	1.06E-08	5.51E-06
GO:0006954	AIF1, C1QB, C1S, C3, C7, CCL18, CCL21, CD14, CD52, CD68, CFH, DCN, F13A1, FCER1G, HP, PLA2G2A, RARRES2, SLPI, SPP1	inflammatory response	Biological process	19	754	4	4.3	1.14E-08	5.70E-06
GO:0001848	C1R, C1S, C3, C6, CFH, LRP1	complement binding	Molecular function	6	38	1	4.3	4.50E-08	2.16E-05
GO:0006950	AIF1, AKR1C1, C1QB, C1QC, C1S, C3, C6, C7, CCL18, CCL21, CD14, CD52, CD68, CFD, CFH, CLU, CYBA, CYBRD1, DCN, EFEMP1, F13A1, FBLN1, FCER1G, FTL, HCST, HP, IFI30, KRT18, LGMN, LRP1, LYVE1, MGST1, PLA2G2A, PRELP, RARRES2, SEPP1, SERPING1, SLPI, SPP1, TYROBP	response to stress	Biological process	40	3,312	18	2.2	5.36E-08	2.48E-05

GO:0009617	C3, C6, C7, CCL18, CCL21, CD14, CFH, F13A1, IFI30, ITLN1, PLA2G2A, PLTP, PRELP, RARRES2, SLPI	response to bacterium	Biological process	15	507	3	4.4	6.23E-08	2.78E-05
GO:0005539	CD14, CFH, CTSK, LYVE1, PLA2G2A, PLTP, PRELP, SEPP1, SERPING1, SLPI	glycosaminoglycan binding	Molecular function	10	191	2	4.2	6.66E-08	2.87E-05
GO:0001871	CD14, CFH, CTSK, LYVE1, PLA2G2A, PLTP, PRELP, SEPP1, SERPING1, SLPI	pattern binding	Molecular function	10	199	2	4.2	9.80E-08	4.08E-05
GO:0030247	CD14, CFH, CTSK, LYVE1, PLA2G2A, PLTP, PRELP, SEPP1, SERPING1, SLPI	polysaccharide binding	Molecular function	10	199	2	4.2	9.80E-08	4.08E-05
GO:0051707	C3, C6, C7, CCL18, CCL21, CD14, CFH, CYBA, F13A1, FTL, HP, IFI30, ITLN1, KRT18, PLA2G2A, PLTP, PODN, PRELP, RARRES2, SLPI, SPP1, TYROBP	response to other organism	Biological process	22	1,165	7	3.0	1.17E-07	4.57E-05
GO:0005615	C1QA, C1QB, C1QC, C1S, C6, CCL14, CCL18, CCL21, CFH, CLU, FBLN1, ITLN1, KRT19, PLA2G2A, PODN, RARRES2, SERPING1	extracellular space	Cellular component	17	709	4	3.9	1.55E-07	5.87E-05
GO:0009607	C3, C6, C7, CCL18, CCL21, CD14, CFH, CYBA, F13A1, FTL, HP, IFI30, ITLN1, KRT18, PLA2G2A, PLTP, PODN, PRELP, RARRES2, SLPI, SPP1, TYROBP	response to biotic stimulus	Biological process	22	1,231	7	3.0	3.05E-07	1.12E-04
GO:0002460	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, SERPING1, SPP1	adaptive immune response based on somatic recomb	Biological process	9	172	1	6.3	3.16E-07	1.13E-04
GO:0002250	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, SERPING1, SPP1	adaptive immune response	Biological process	9	179	1	6.3	4.43E-07	1.54E-04

GO:0000323	ACP2, CD14, CD68, CSTB, CTSC, CTSK, IFI30, LAPTM5, LGMN	lytic vacuole	Cellular component	9	187	1	6.3	6.40E-07	2.16E-04
GO:0005764	ACP2, CD14, CD68, CSTB, CTSC, CTSK, IFI30, LAPTM5, LGMN	lysosome	Cellular component	9	187	1	6.3	6.40E-07	2.16E-04
GO:0016485	C1S, C7, CFH, CORIN, CTSC, DCN, LGMN, LRP1, S100A4	protein processing	Biological process	9	200	2	3.8	1.12E-06	3.60E-04
GO:0051604	C1S, C7, CFH, CORIN, CTSC, DCN, LGMN, LRP1, S100A4	protein maturation	Biological process	9	211	2	3.8	1.75E-06	5.34E-04
GO:0065008	AKR1C1, C1QC, C3, CCL18, CCL21, CD52, CFH, CLU, CORIN, CSTB, CYBA, CYBRD1, DCN, EFEMP1, F13A1, FBLN1, FBLN2, FITM2, FTL, HCST, ITLN1, LRP1, PLA2G2A, PLIN4, PLTP, RARRES2, RPS29, S100A4, SERPING1, SLC40A1, SLC41A1, SLPI, SPP1	regulation of biological quality	Biological process	33	2,742	15	2.2	1.73E-06	5.40E-04
GO:0002449	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, HCST, SERPING1	lymphocyte mediated immunity	Biological process	9	224	2	3.8	2.86E-06	8.53E-04
GO:0051605	C1S, C7, CFH, CORIN, DCN, S100A4	protein maturation by peptide bond cleavage	Biological process	6	77	1	4.3	3.28E-06	9.52E-04
GO:0005773	ACP2, CD14, CD68, CSTB, CTSC, CTSK, IFI30, LAPTM5, LGMN	vacuole	Cellular component	9	233	2	3.8	3.95E-06	1.12E-03
GO:0008201	CFH, PLA2G2A, PLTP, PRELP, SEPP1, SERPING1, SLPI	heparin binding	Molecular function	7	125	1	5.0	4.44E-06	1.23E-03
GO:0005579	C6, C7, CLU	membrane attack complex	Cellular component	3	7	1	2.3	4.96E-06	1.35E-03
GO:0001503	CTSK, IGFBP4, LGMN, MGP, PRELP, PTGIS, SPP1, WISP2	ossification	Biological process	8	189	2	3.4	7.19E-06	1.91E-03

GO:0008228	C3, C7, CFH	opsonization	Biological process	3	8	1	2.3	7.91E-06	2.06E-03
GO:0002443	C1QA, C1R, C1S, C6, C7, CCL18, FCER1G, HCST, SERPING1	leukocyte mediated immunity	Biological process	9	262	2	3.8	1.02E-05	2.55E-03
GO:0001846	C1R, C1S, CD14, CFH	opsonin binding	Molecular function	4	26	1	3.0	1.01E-05	2.57E-03
GO:0070328	FITM2, PLIN4, PLTP	triglyceride homeostasis	Biological process	3	9	1	2.3	1.18E-05	2.90E-03
GO:0006909	C3, C7, CD14, CFH, CYBA, F13A1, HP	phagocytosis	Biological process	7	147	1	5.0	1.29E-05	3.10E-03
GO:0008009	CCL14, CCL18, CCL21, RARRES2, SPP1	chemokine activity	Molecular function	5	63	1	3.7	2.06E-05	4.86E-03
GO:0031639	C7, DCN, S100A4	plasminogen activation	Biological process	3	11	1	2.3	2.30E-05	5.33E-03
GO:0045087	C3, C6, C7, CCL18, CFD, CFH, HCST, HP, IFI30, SERPING1	innate immune response	Biological process	10	375	2	4.2	2.89E-05	6.58E-03

Up-regulated

GO Identifier	Gene symbol	GO Term	Ontology	#Hits in group	Group size	#Hits expected	Fold enrichment	p-value	FDR
GO:0043085	C8orf4, CCL5, CDH5, DUSP6, FLT1, GADD45B, GADD45G, GNG11, IGFBP3, IL32, JUN, NPPA, POSTN, PSIP1, RBM25, RGS5, RHOB, RRAS, S100A8, S100A9, S1PR2, SLC9A3R2	positive regulation of catalytic activity	Biological process	22	1,299	7	3.0	1.59E-07	6.63E-04
GO:0065009	ANXA3, C8orf4, CCL5, CDH5, CYR61, DUSP1, DUSP6, FLT1, GADD45B, GADD45G, GADD45GIP1, GNG11, HBB, IGFBP3, IL32, JUN, MAP1B, NPPA, POSTN, PSIP1, RBM25, RGS5, RHOB, RRAS, S100A8, S100A9, S1PR2, SLC9A3R2,	regulation of molecular function	Biological process	31	2,428	12	2.5	1.40E-07	8.74E-04

TAF10, TAGLN, ZFP36

GO:0050790	ANXA3, C8orf4, CCL5, CDH5, CYR61, DUSP1, DUSP6, FLT1, GADD45B, GADD45G, GADD45GIP1, GNG11, IGFBP3, IL32, JUN, MAP1B, NPPA, POSTN, PSIP1, RBM25, RGS5, RHOB, RRAS, S100A8, S100A9, S1PR2, SLC9A3R2	regulation of catalytic activity	Biological process	27	1,968	10	2.6	3.09E-07	9.66E-04
GO:0044093	ANXA3, C8orf4, CCL5, CDH5, CYR61, DUSP6, FLT1, GADD45B, GADD45G, GNG11, IGFBP3, IL32, JUN, NPPA, POSTN, PSIP1, RBM25, RGS5, RHOB, RRAS, S100A8, S100A9, S1PR2, SLC9A3R2	positive regulation of molecular function	Biological process	24	1,505	8	2.9	1.14E-07	1.42E-03

Each row of the table presents a matched Gene Ontology (GO) term. The columns contain (from left to right) the GO identifier and a link to its description, Gene symbols, the GO term description, Ontology in general (Biological Process, Molecular Function or Cellular Component), the number of hits from the input set to the ontology group, the size of the group in the database, the number of randomly expected hits, the fold enrichment for the group between baseline and postischemia, the *P*-value of the observation, and the false-discovery rate (FDR).

Table 3. Differential Expression GO Annotation

GO Identifier	GO Term	Ontology	Group size	Down-regulation		Up-regulation	
				#Hits in group down	p-value down	#Hits in group up	p-value up
GO:0006956	complement activation	Biological process	41	11	7.69E-17	0	1
GO:0005576	extracellular region	Cellular component	2,057	40	3.01E-15	18	0.01
GO:0006958	complement activation, classical pathway	Biological process	28	9	8.69E-15	0	1
GO:0006954	inflammatory response	Biological process	442	20	4.74E-14	6	0.02
GO:0051605	protein maturation by peptide bond cleavage	Biological process	102	12	1.14E-13	0	1
GO:0044421	extracellular region part	Cellular component	1,075	28	1.84E-13	13	1.99E-03
GO:0006959	humoral immune response	Biological process	93	11	1.20E-12	2	0.08
GO:0002526	acute inflammatory response	Biological process	126	12	1.49E-12	2	0.13
GO:0016064	immunoglobulin mediated immune response	Biological process	88	10	2.02E-11	0	1
GO:0001944	vasculature development	Biological process	441	0	1	15	3.03E-09

Table 4: Gene Set Enrichment Analysis SwissProt

keyword ID	keyword	category	Group size	Down-regulation		Up-regulation	
				#Hits in group down	<i>p</i> -value down	#Hits in group up	<i>p</i> -value up
KW0179	Complement alternate pathway	Biological process	12	4	2.94E-07	0	1
KW0180	Complement pathway	Biological process	27	10	5.13E-17	0	1
KW0325	Glycoprotein	PTM	4,264	46	2.03E-08	23	0.35
KW0391	Immune response	Biological process	194	13	1.61E-11	1	0.62
KW0399	Innate immunity	Biological process	64	12	3.46E-16	0	1
KW0768	Sushi	Domain	56	6	3.75E-07	0	1
KW0838	Vasoactive	Molecular function	11	0	1	3	1.87E-05
KW1015	Disulfide bond	PTM	2,882	43	2.33E-12	18	0.17

PTM = posttranslational modifications.

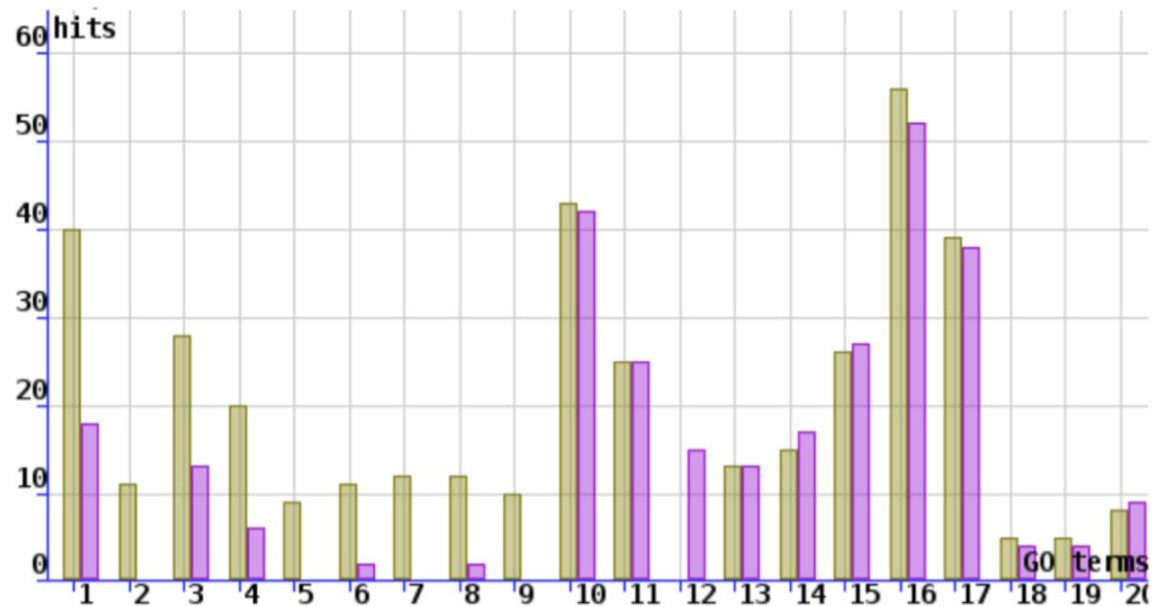


Fig.1. Shown is a graphical representation of the functional analysis comparing up- and down-regulated Gene Ontology (GO) terms. The X-axis shows the term, the Y-axis the number of hits in each term. Purple bars: down-regulated genes, Beige bars: up-regulated genes.

Terms:

1. Cellular component:extracellular region
2. Biological process:complement activation
3. Cellular component:extracellular region part
4. Biological process:inflammatory response
5. Biological process:complement activation, classical pathway
6. Biological process:humoral immune response
7. Biological process:protein maturation by peptide bond cleavage
8. Biological process:acute inflammatory response
9. Biological process:immunoglobulin mediated immune response
10. Biological process:response to stimulus

- 11. Biological process:response to chemical stimulus
- 12. Biological process:vasculature development
- 13. Biological process:response to external stimulus
- 14. Biological process:response to organic substance
- 15. Biological process:positive regulation of biological process
- 16. Biological process:biological regulation
- 17. Biological process:multicellular organismal process
- 18. Biological process:aging
- 19. Molecular function:polysaccharide binding
- 20. Biological process:response to biotic stimulus

Terms highlighted in red and green correspond to significantly different GO categories between upregulated (green) and downregulated (red) and numeric values are shown in table 3.

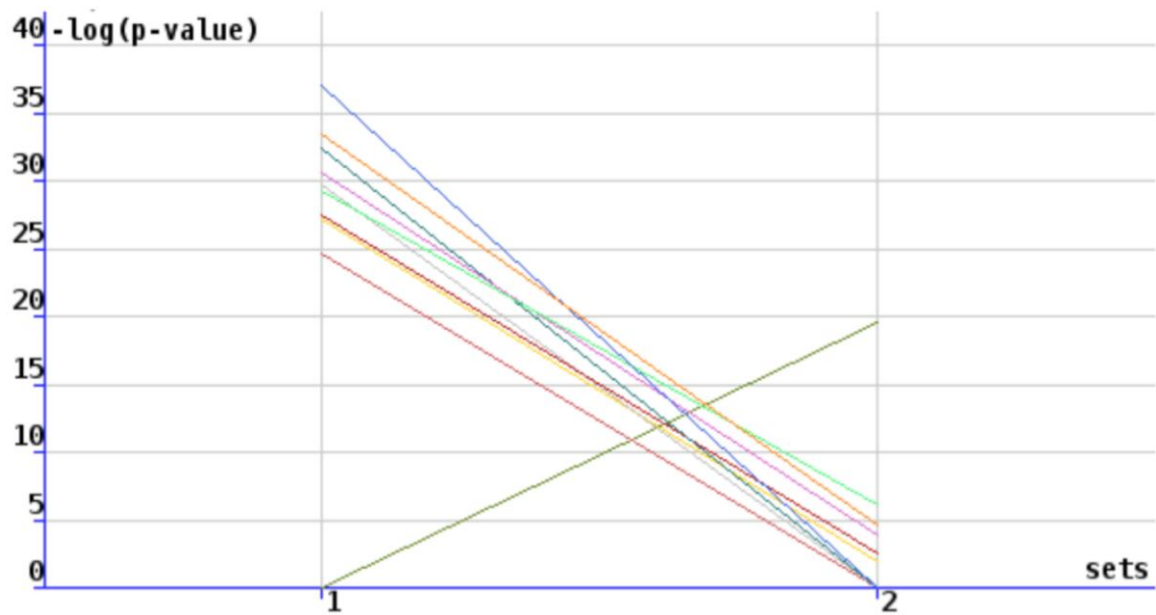


Fig. 2. Shown is a graphical representation of the $-\log_{10} p$ -values for the up- and down-regulated gene sets corresponding to table 3. Each colored line represents a Gene Ontology (GO) term #1: Down-regulated genes, #2: Up-regulated genes

Terms:

- Cellular component:extracellular region
- Biological process:complement activation
- Cellular component:extracellular region part
- Biological process:inflammatory response
- Biological process:complement activation, classical pathway
- Biological process:humoral immune response
- Biological process:protein maturation by peptide bond cleavage
- Biological process:acute inflammatory response
- Biological process:immunoglobulin mediated immune response
- Biological process:vasculature development