



RNA-Seq 실험방법 및 최신 분석기술

Never Ending Service



BIO-Experiment & Analysis Service

Microarray

- ❖ Gene Expression
- ❖ miRNA
- ❖ CGH / CNV
- ❖ ChIP on chip
- ❖ Methylation
- ❖ Agilent/Affymetrix
- ❖ Exiqon

NGS

- ❖ RNA-Seq
- ❖ small RNA-Seq
- ❖ ChIP-Seq
- ❖ MBD-Seq
- ❖ Exome-Seq
- ❖ Target DNA-Seq
- ❖ Illumina/IonProton

qRT-PCR

- ❖ mRNA
- ❖ microRNA
- ❖ PCR Array
- ❖ Qiagen
- ❖ Genosensor
- ❖ Others

Antibody Array

- ❖ Protein Profiling
- ❖ Phosphorylation
- ❖ FullmoonBio
- ❖ RayBiotech
- ❖ Others

nature COMMUNICATIONS

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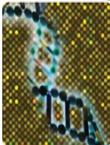
NATURE COMMUNICATIONS | ARTICLE

Metabolic engineering of *Corynebacterium glutamicum* for L-arginine production

Seok Hyun Park, Hyun Uk Kim, Tae Yong Kim, Jun Seok Park, Suok-Su Kim


Affiliations | Contributions | Corresponding author

Microarray



❖ Meaningful Data from Any Genome

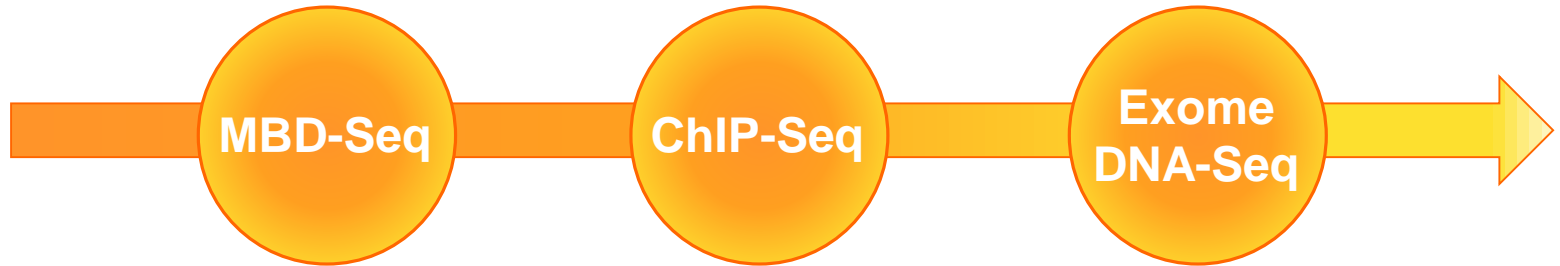
NGS



❖ Next Generation Sequencing



DNA

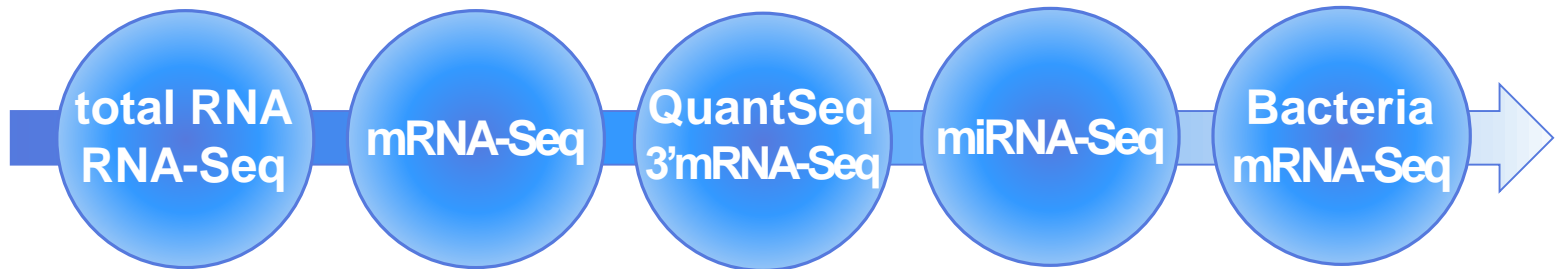


- DNA Methylation Profiling
- Epigenome 분석
- ~ 2 μ g gDNA

- DNA-Protein Interactions
- Histone modifications
- ~20 μ l IP-DNA

- Whole Exome Sequencing
- SNP, CNV, Mutation
- ~ 2 μ g gDNA

RNA



- mRNA-Seq 분석외 lncRNA 분석
- Whole Transcriptome
- ~ 2 μ g total RNA

- Gene Expression, Isoform, GO/Pathway
- Splicing, Gene Fusion
- ~ 2 μ g total RNA

- mRNA Expression Profiling
- Low Quantity & Quality RNA 샘플 실험가능
- ~ 2 μ g total RNA

- microRNA Expression Profiling
- Target Gene 분석외
- ~ 2 μ g total RNA

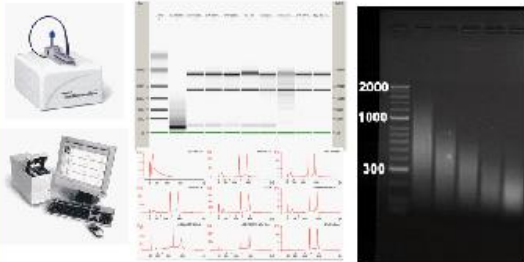
- Gene Expression Profiling
- GO/Pathway/Network
- ~ 2 μ g total RNA

RNA-Seq Process

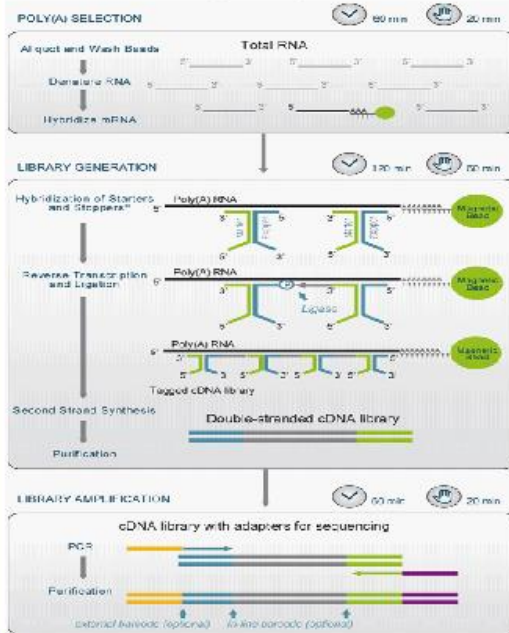


Sample Preparation

Sample Prep & QC

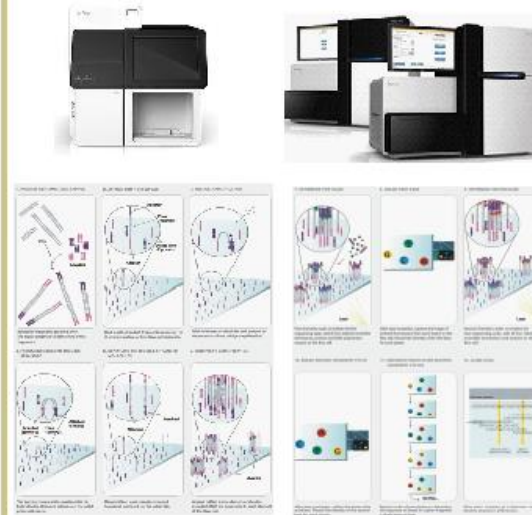


Library Prep & QC



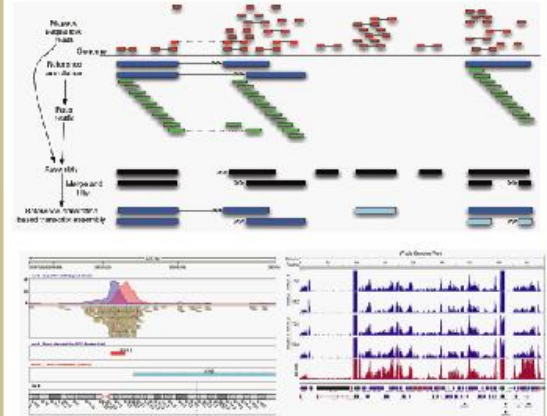
Sequencing

Cluster Generation & Sequencing

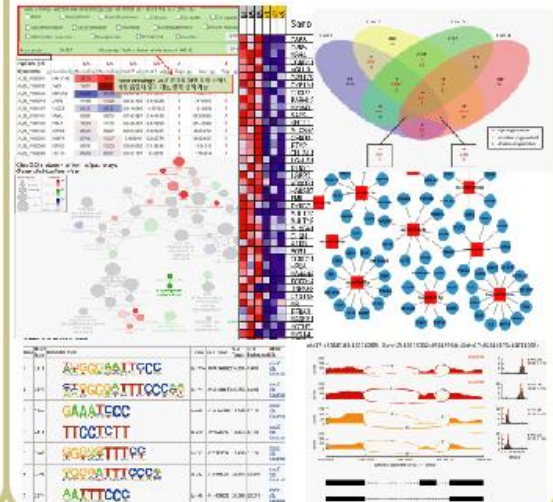


Data Analysis

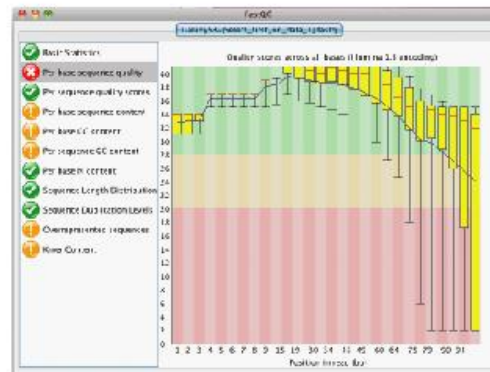
Genome Mapping & Analysis



Data Mining & Visualization

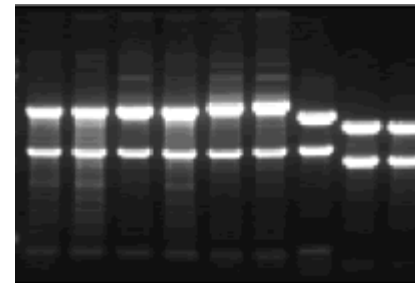


FastQC





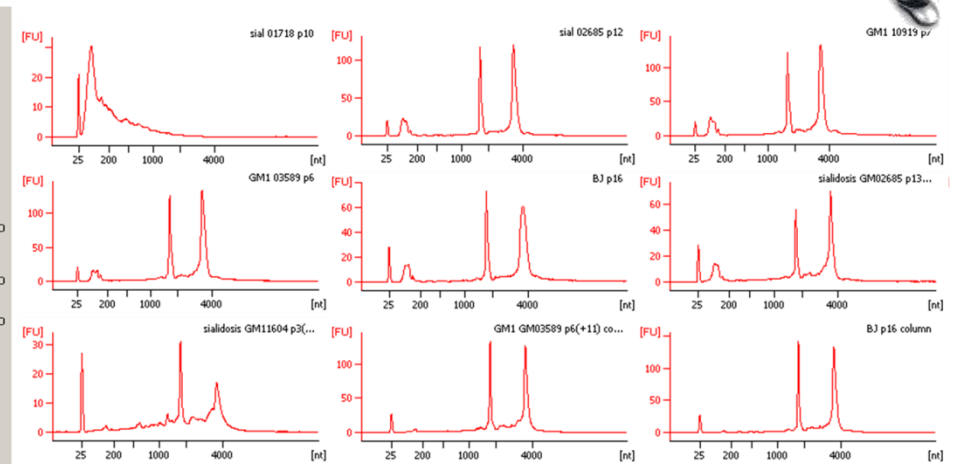
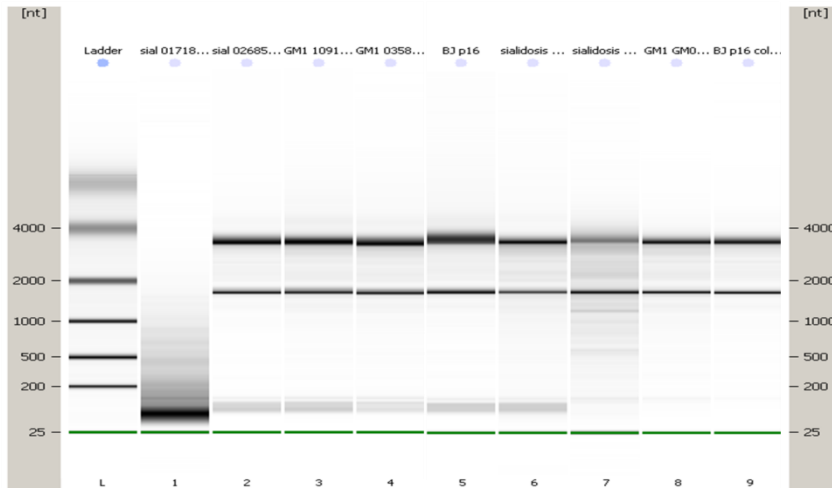
- TriZol 이용 시 예상되는 RNA양 (tissue 1mg, cultured cell 1*10⁶당)
 - Liver and spleen, 6-10 μ g
 - Kidney, 3-4 μ g
 - Skeletal muscles and brain, 1-1.5 μ g
 - Placenta, 1-4 μ g
 - Epithelial cells (1 \times 10⁶ cultured cells), 8-15 μ g
 - Fibroblasts, (1 \times 10⁶ cultured cells) 5-7 μ g
- RNA 분리
 - Total RNA 분리는 TriZol 시약을 표준 방법으로 권하고 상용화된 키트도 가능
 - 분리된 RNA는 Rnase free water나 DEPC DW에 녹여 냉동보관(-70도 이하)
- RNA QC
 - Spectrophotometer : OD260/OD280값
 - Denaturing agarose gel electrophoresis
 - RNA의 양(농도)와 깨짐정도 확인
- 500pg ~ 2 μ g total RNA for RNA-seq
- Sample to Data : RNA QC 후 3~4 weeks



RNA Quality Control



Sample	μg/μl	OD _{260/280}	OD _{260/230}	Total (μg)	Ratio(28s/18s)	RIN
Sample 1	0.1898	1.72	1.10	2.8475	0.0	2.3
Sample 2	0.1842	1.79	1.08	2.7629	1.9	9.8
Sample 3	0.6542	1.89	1.31	6.5419	1.8	9.5
Sample 4	0.3111	1.77	1.28	3.1108	1.8	9.6
Sample 5	0.2822	1.74	1.16	2.8223	1.8	9.8
Sample 6	0.2351	1.83	1.03	3.5264	2.1	8.9
Sample 7	0.0356	2.01	1.27	0.3560	0.6	7.7
Sample 8	0.1356	2.01	1.53	2.0340	1.7	9.2
Sample 9	0.0721	2.02	1.78	0.7210	1.8	9.8



● RIN(RNA Integrity Number) :

Agilent社에서 제공하는 software tool로 Eukaryote total RNA sample에 적용되며, electrophoretic trace에 의해 integrity가 결정됩니다. 1~10까지의 값을 가지며, RIN값에 대한 다양한 결과는 <http://www.chem.agilent.com/rin/rinSearch.aspx> 에서 찾아 보실 수 있습니다. 일반적인 허용 기준은 RIN > 8.0 이상입니다.

Poly(A) RNA Selection Kit

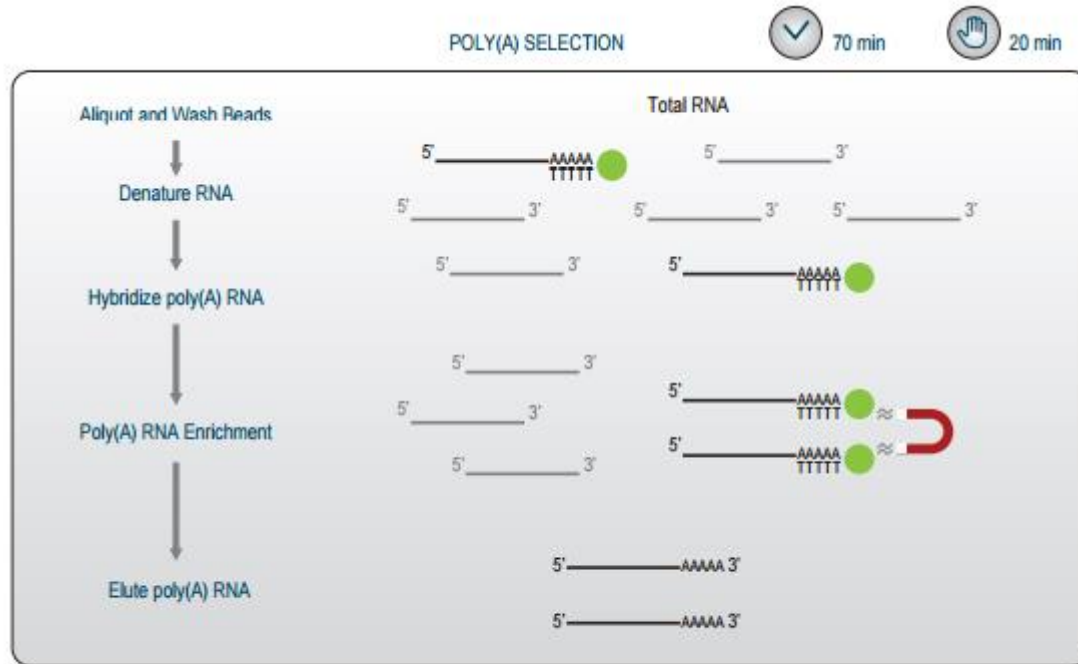
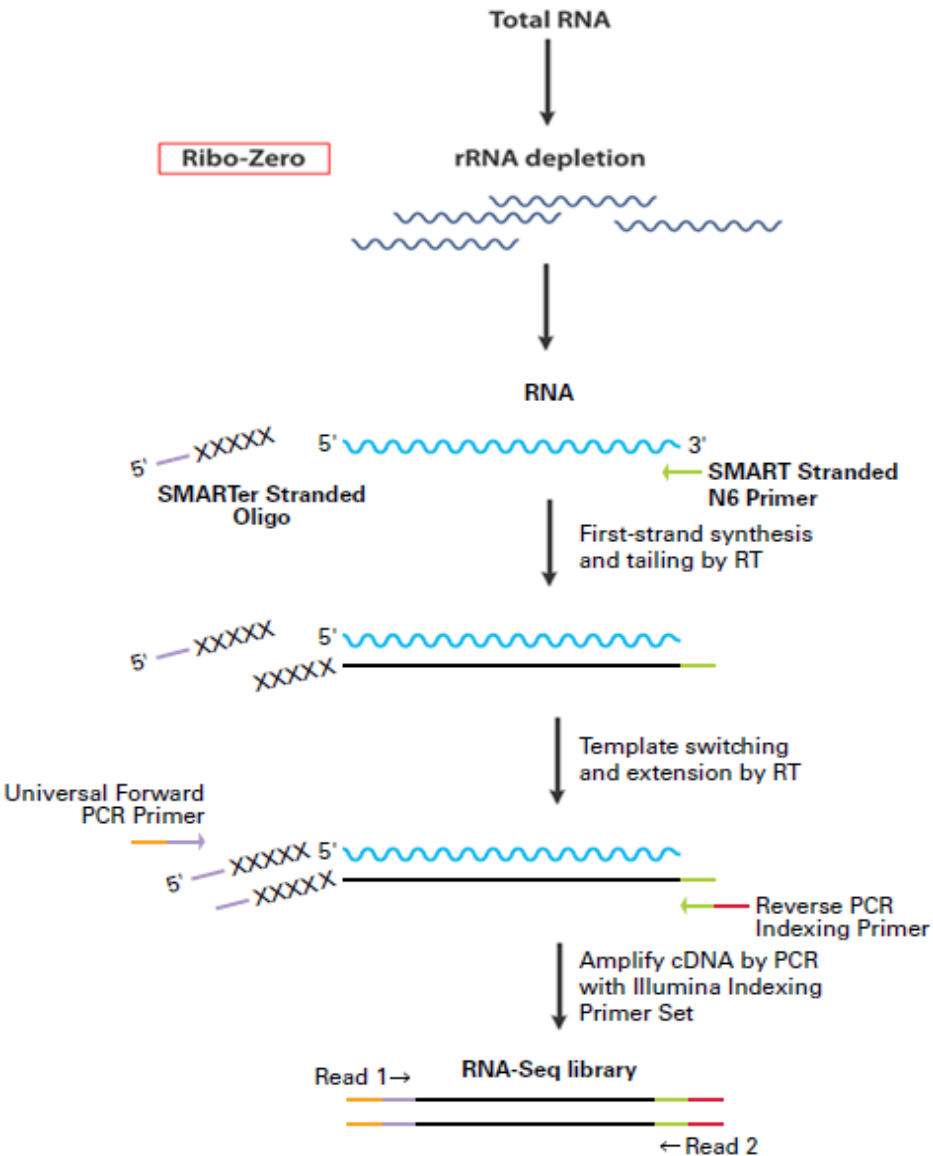


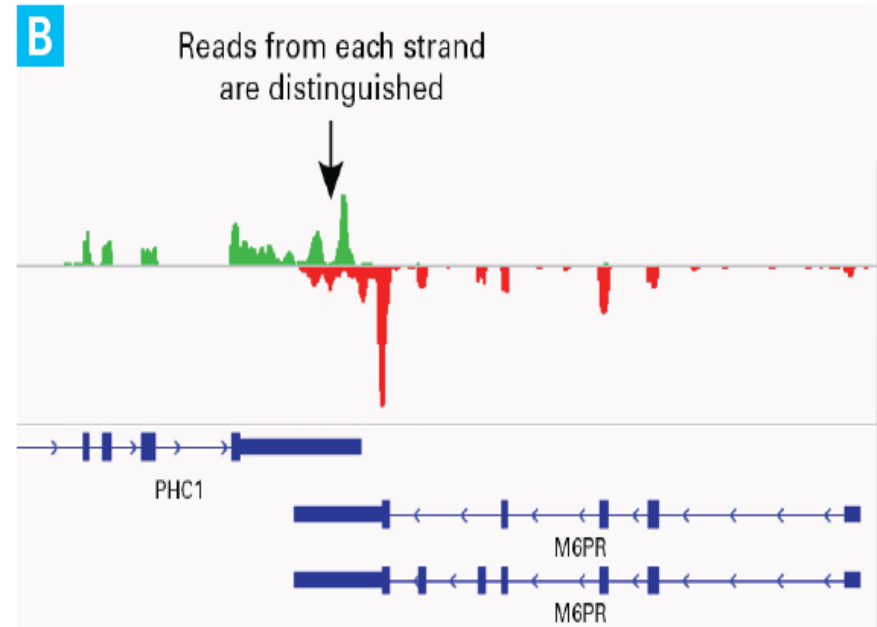
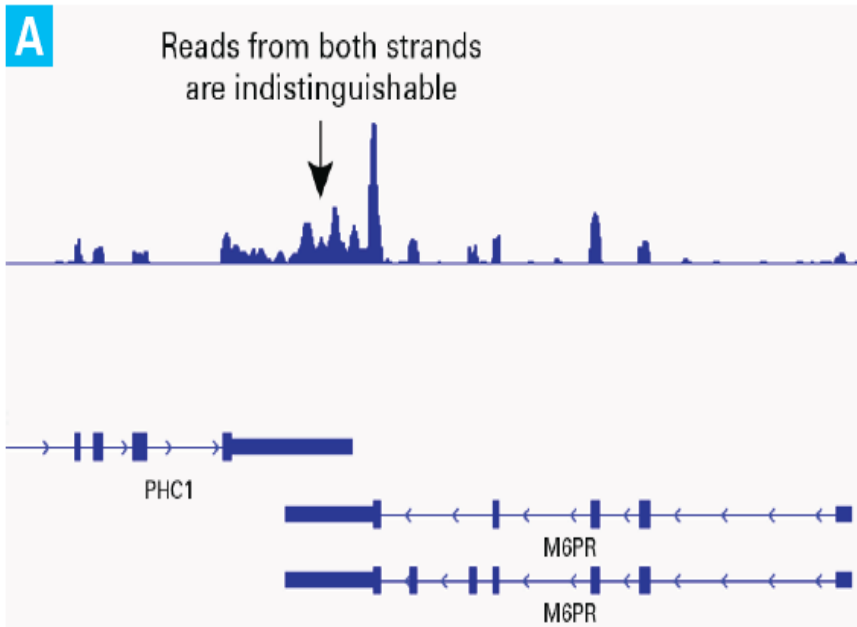
Figure 1 | Schematic overview of the Poly(A) RNA Selection.

- ✓ Highly specific poly(A) enrichment
- ✓ Magnetic bead-based purification
- ✓ Various downstream applications such as RNA-Seq

SMARTer® Stranded RNA-Seq



- **Superior performance** : Get uniform transcriptome coverage, high reproducibility, and high correlation across a broad input range (100 ng – 100 pg RNA)
- **99% accurate strand identification** : Determine every transcript's strand of origin
- **Simplified workflow** : Produce an Illumina®-ready library in less than 4 hours
- **Versatile kit** : Suitable for eukaryotic and prokaryotic samples, and for coding and noncoding RNA-Seq



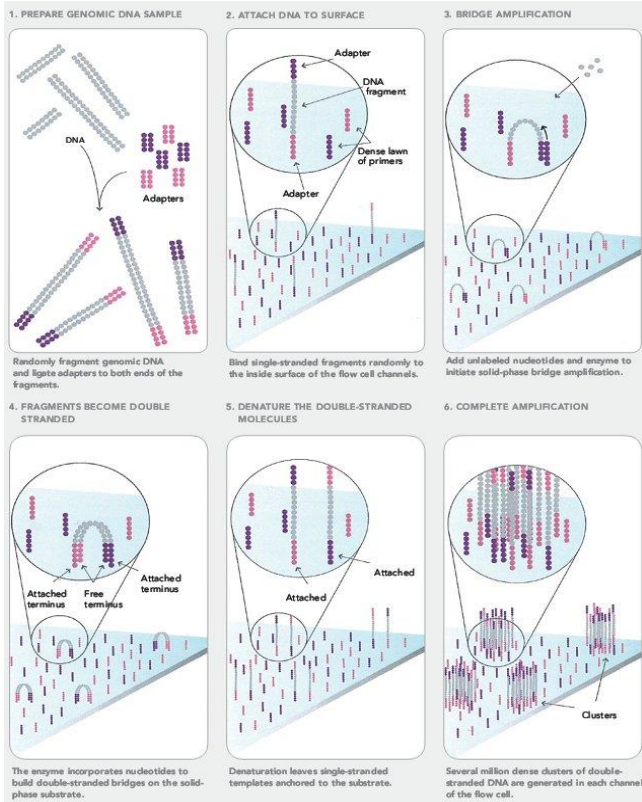
Capturing strand-specific information allows you to assign RNA-Seq reads to the correct strand of DNA.

Panel A. Non-stranded RNA-seq cannot distinguish the reads from RNA transcribed from different strands of the same genomic sequence. Panel B. The SMARTer Stranded RNA-Seq Kit produces libraries that can be assigned to a strand with $\geq 99\%$ accuracy. Short, overlapping sequences that originate from different DNA strands are distinguished from each other, enabling quantitative expression analysis and accurate genome annotation.

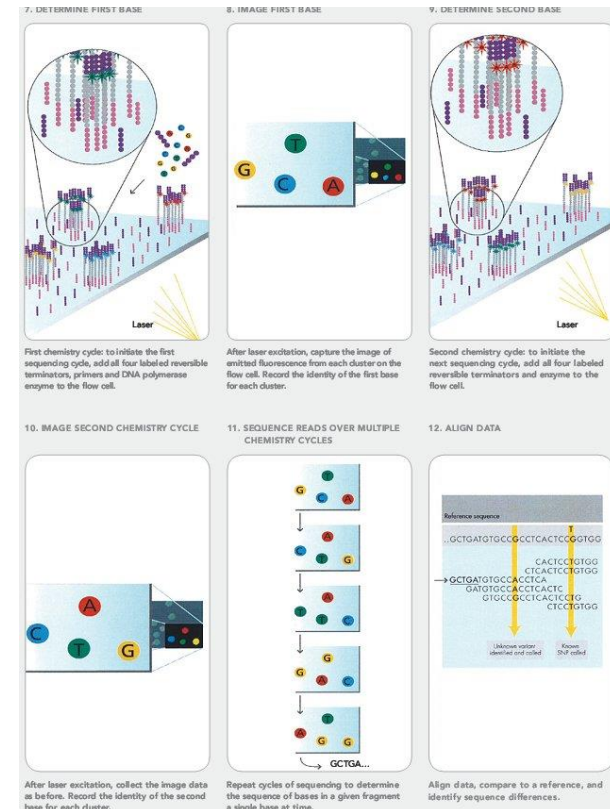
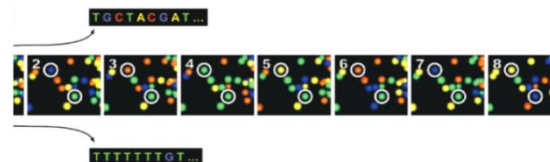
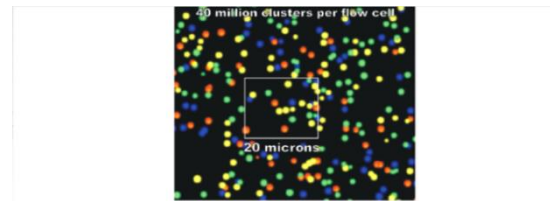
Sequencing



Run Format	Read Length	# of Reads	High-Quality Output (Gb)	
			/lane	/FlowCell
Single End	1 x 50bp	~150 million/lane	7.5 Gb	135-150 Gb
	1 x 100bp		15 Gb	270-300 Gb
Paired End	2 x 50bp	~300 million/lane	15 Gb	270-300 Gb
	2 x 100bp		30 Gb	540-600 Gb



Illumina HiSeq-2000/2500 Illumina NextSeq 500



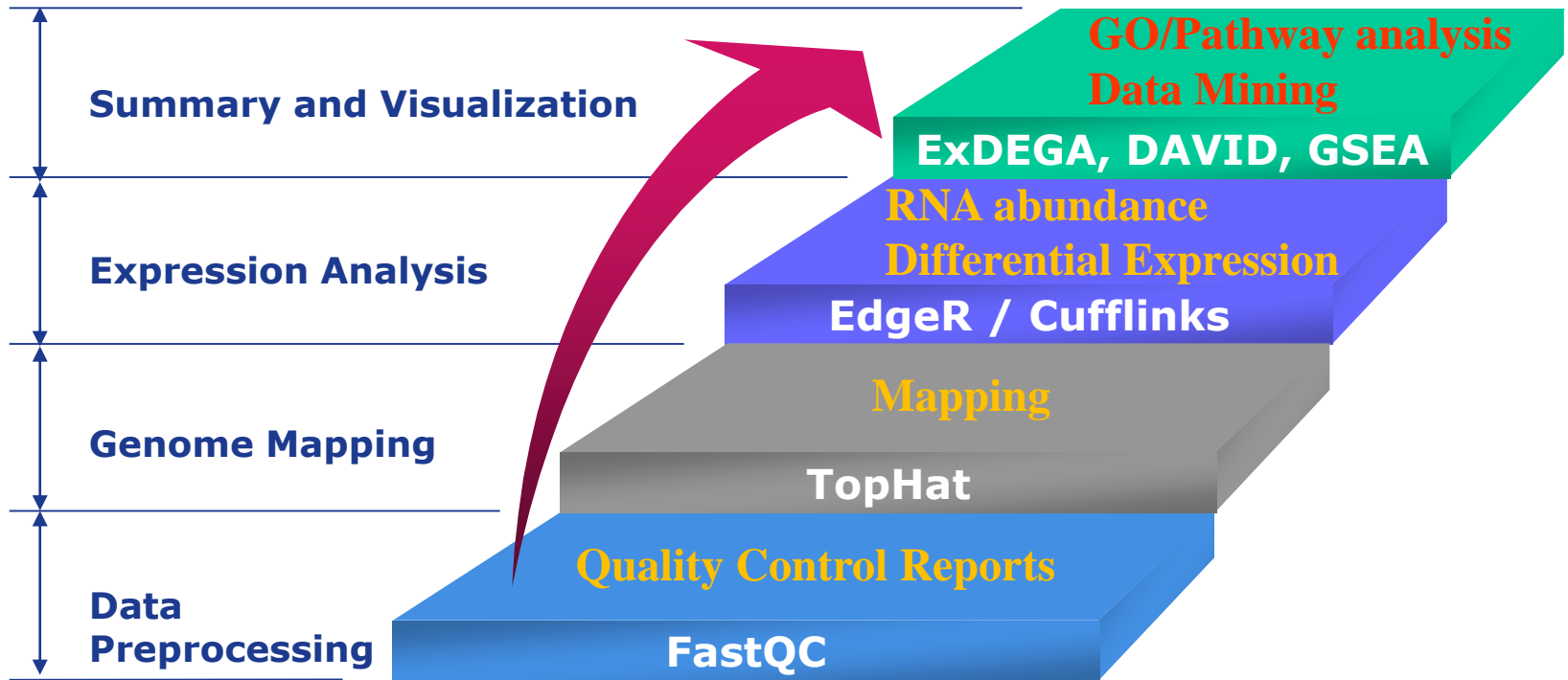
Work Flow of Data Analysis



NGS ↓



Discoveries



Other Data Analysis Tools for Data Processing, Mining, Visualization : MISO, Quick GO, ClueGO, CytoScape, DAVID, GSEA, UCSC Genome, IGV, IPA, Others

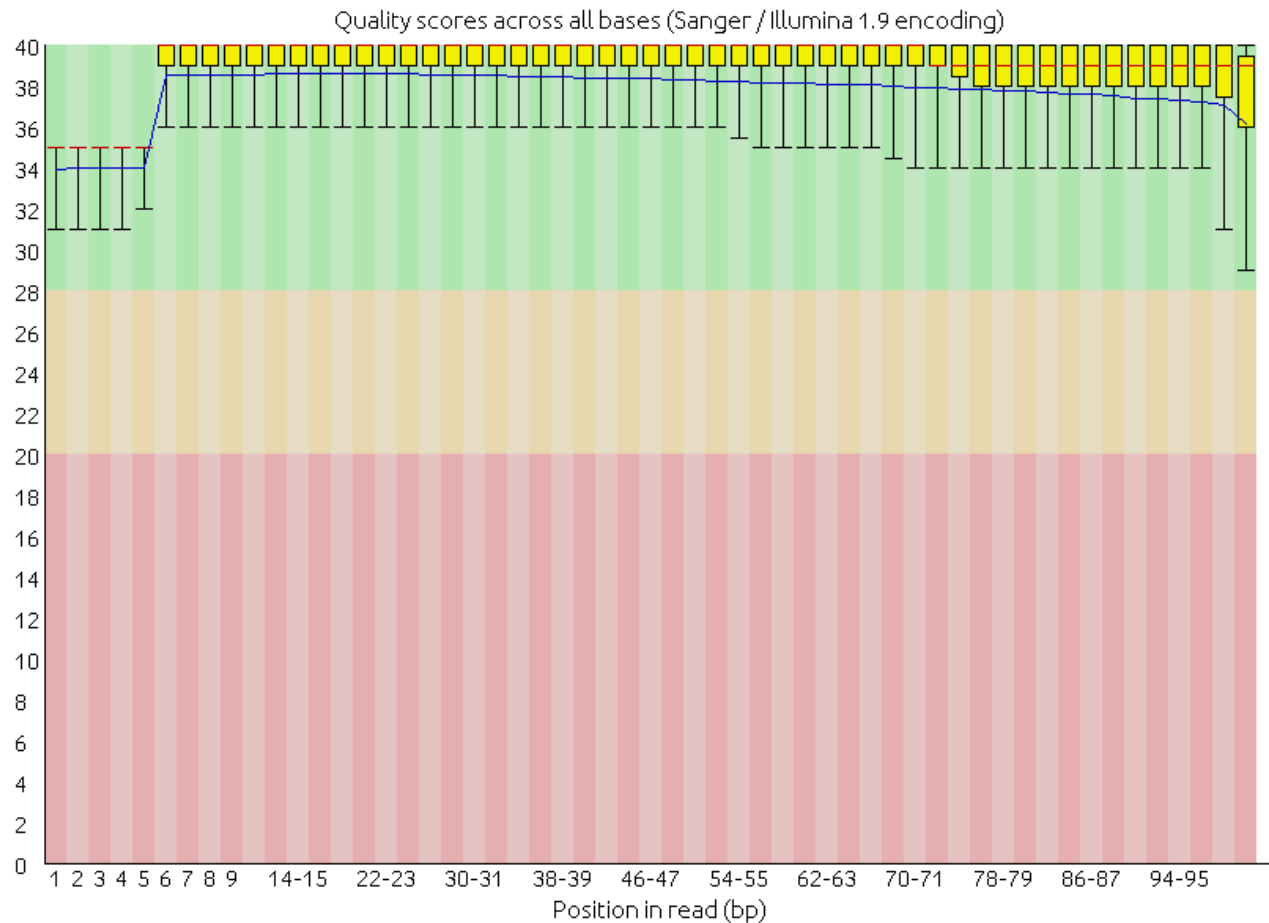


FastQ 파일 안에서 각 position의 base에 대한 quality 값의 영역을 보여 줌

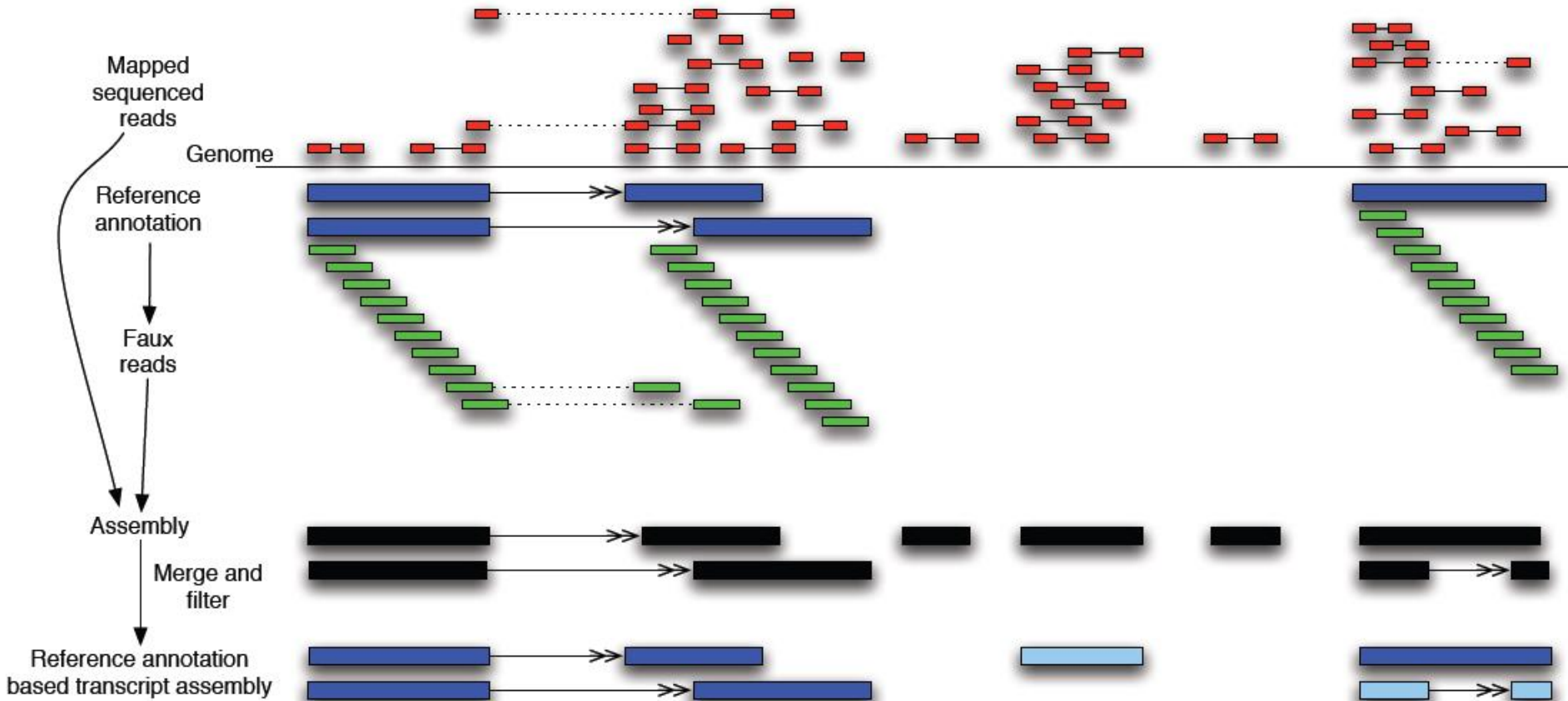
Summary

- ✔ [Basic Statistics](#)
- ✔ [Per base sequence quality](#)
- ✔ [Per tile sequence quality](#)
- ✔ [Per sequence quality scores](#)
- ✘ [Per base sequence content](#)
- ! [Per sequence GC content](#)
- ✔ [Per base N content](#)
- ✔ [Sequence Length Distribution](#)
- ✔ [Sequence Duplication Levels](#)
- ✔ [Overrepresented sequences](#)
- ✔ [Adapter Content](#)
- ✘ [Kmer Content](#)

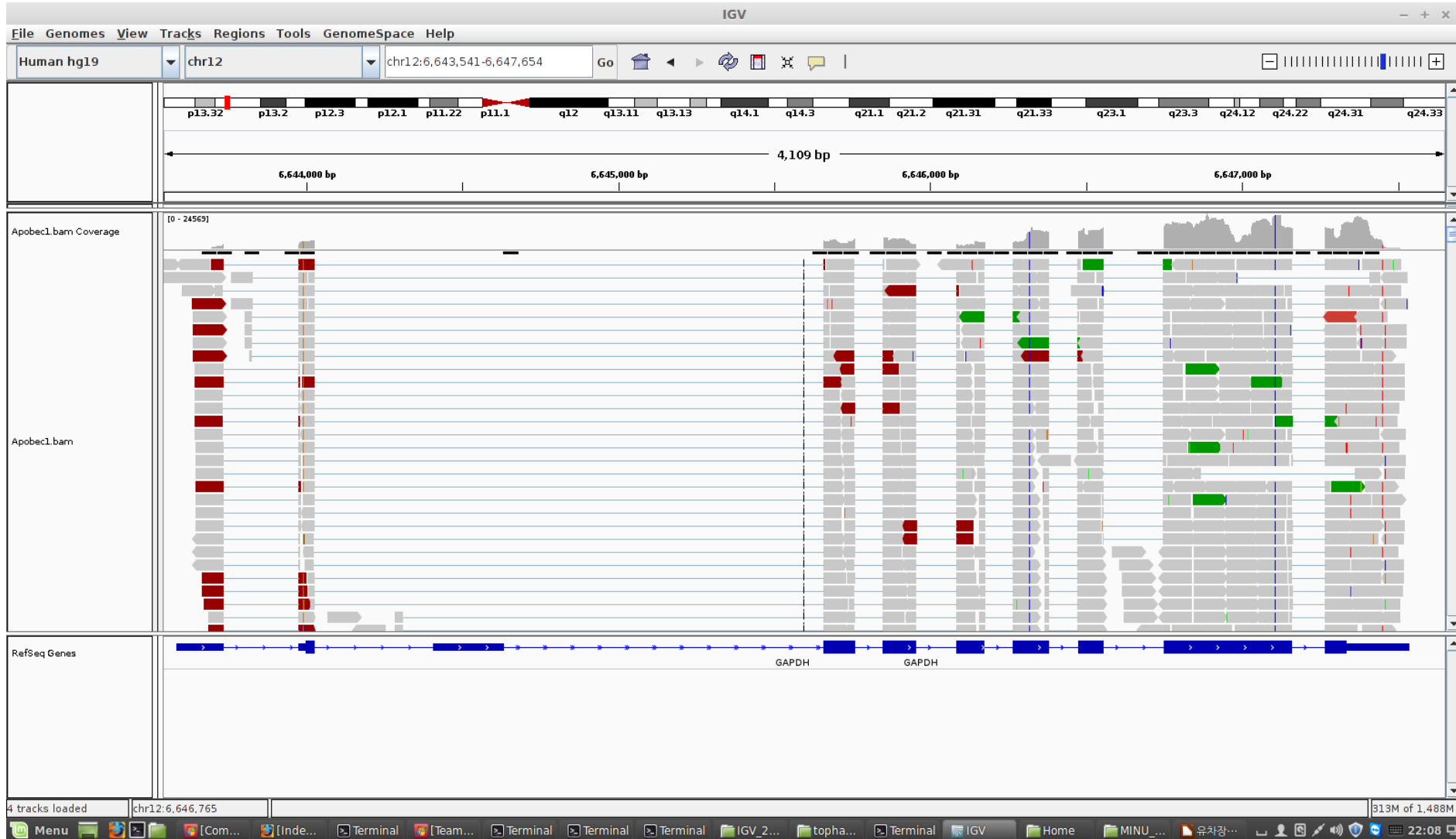
✔ Per base sequence quality



TopHat(Bowtie2), Bedtools, Cufflinks



Genome Browser - IGV



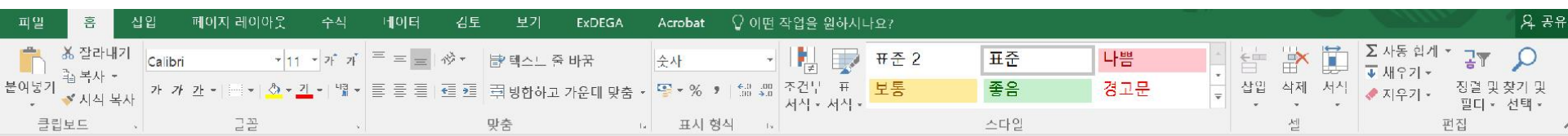


ExDEGA : Excel based Differentially Expressed Gene Analysis tool

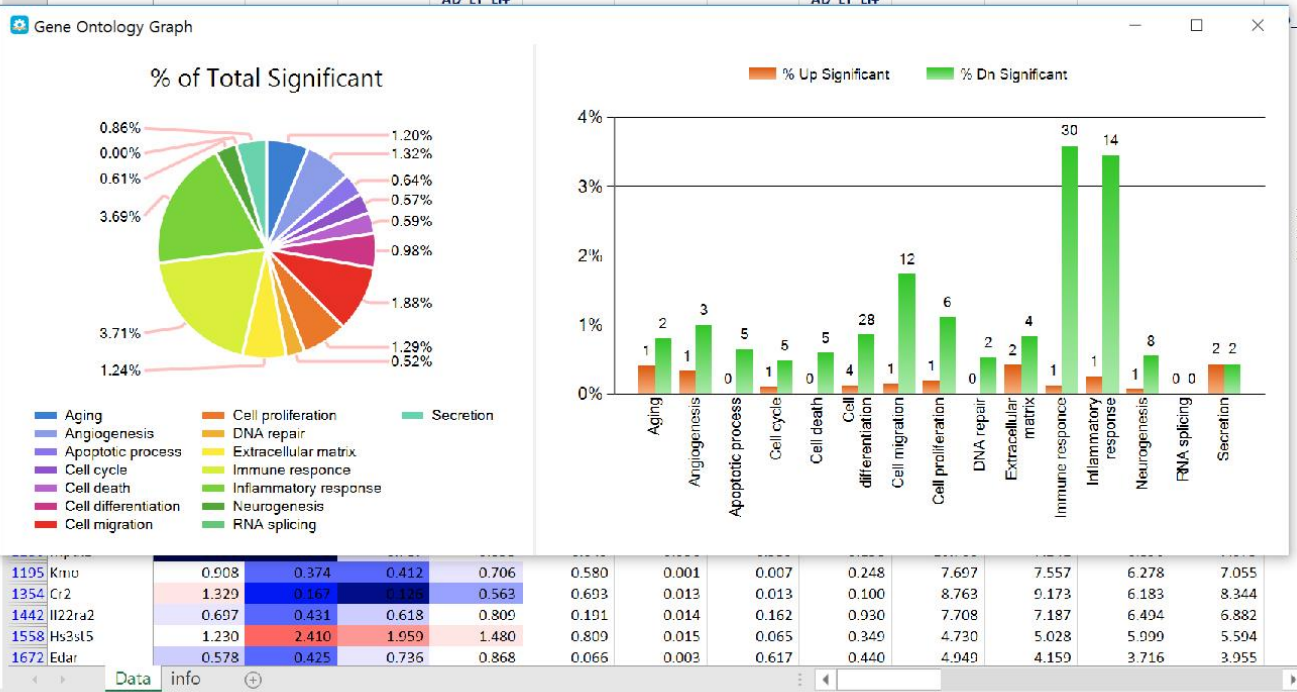
Microsoft Excel interface showing the ExDEGA tool. The spreadsheet displays a list of genes with columns for Gene Symbol, Fold change, p-value, and Average of Normalized RC (log2). The 'Filter: 200' is applied. The 'Gene Category' sidebar on the left lists various biological processes like Aging, Angiogenesis, Apoptotic process, etc. The 'DCG Analysis' sidebar on the right shows 'Significant Gene Selection' with a 'Fold change' filter set to 2 and a 'p-value' filter set to 0.05. The 'Sample Comparison / Filter' section has 'AD_FMT_LI / AD_LT_LI' checked.

Gene Symbol	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI+ / AD_FMT_LI+ / AD_TG_LI	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI+ / AD_FMT_LI+ / AD_TG_LI	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI	AD_TG_LI	AD_FMT_LI	AD_LT_LI+ / AD_FMT_LI	AD_LI
22 Mybl1	0.561	0.401	0.715	0.857	0.053	0.004	0.115	0.670	7.279	6.445	5.961	6.224				
41 Sico5a1	0.752	0.419	0.557	0.779	0.230	0.005	0.049	0.711	4.923	4.513	3.669	4.151				
253 Pms1	0.669	0.497	0.742	0.871	0.017	0.009	0.218	0.891	6.274	5.694	5.264	5.495				
288 Aux3	0.571	0.203	0.356	0.678	0.090	0.000	0.101	0.936	4.389	3.581	2.091	3.021				
326 Icus	0.654	0.472	0.722	0.861	0.222	0.201	0.989	7.292	6.679	6.209	6.463					
489 Ccl20	0.612	0.191	0.313	0.656	0.655	0.035	0.045	0.392	5.343	4.635	2.957	4.027				
499 A530032D15Rik	1.175	0.408	0.348	0.674	0.974	0.001	0.021	0.166	7.769	8.002	6.477	7.432				
501 C130026I21Rik	1.033	0.256	0.248	0.624	0.958	0.005	0.010	0.168	10.417	10.465	8.452	9.784				1
502 Sp110	1.000	0.378	0.378	0.689	0.854	0.037	0.017	0.172	12.297	12.297	10.893	11.759				1
503 Sp140	0.914	0.259	0.284	0.642	0.886	0.016	0.023	0.292	11.258	11.128	9.310	10.488				1
505 A630001G21Rik	1.266	0.471	0.372	0.686	0.478	0.016	0.008	0.057	7.612	7.952	6.527	7.408				
729 Trincm163	0.739	0.412	0.557	0.779	0.369	0.019	0.254	0.857	5.292	4.855	4.012	4.494				
742 Cxcr4	0.833	0.379	0.455	0.727	0.463	0.001	0.003	0.267	10.017	9.754	8.617	9.294				
752 Fcarnr	1.143	0.470	0.411	0.705	0.599	0.042	0.010	0.094	8.873	9.066	7.783	8.562				
754 Fcarnr	1.867	0.176		0.547	0.452	0.009	0.008	0.050	9.491	10.392	6.984	9.522				
890 Rgs13	0.638	0.396	0.620	0.810	0.253	0.044	0.320	1.000	6.491	5.843	5.153	5.539				
894 Brinp3	1.245	2.003	1.489	1.245	0.064	0.006	0.089	0.854	4.884	5.312	5.887	5.628				
1025 Sell	1.312	0.166	0.127	0.563	0.565	0.001	0.001	0.040	9.018	9.409	6.428	8.581				
1036 Xcl1	0.643	0.425	0.660	0.830	0.162	0.011	0.475	0.710	5.871	5.234	4.636	4.966				
1081 Sh2d1b1	0.841	0.418	0.497	0.748	0.369	0.016	0.081	0.488	5.672	5.422	4.412	5.004				
1089 Furla	1.216	0.270	0.222	0.611	0.884	0.001	0.007	0.112	10.526	10.808	8.636	10.097				
1163 BC094916	1.709	0.363	0.212	0.606	0.412	0.008	0.008	0.043	5.384	6.157	3.922	5.435				
1165 Pyhin1	1.150	0.350	0.302	0.651	0.445	0.029	0.003	0.050	7.123	7.236	5.609	6.717				
1186 Mpx1			0.787	0.893	0.049	0.036	0.939	0.198	10.700	7.242	6.896	7.079				
1195 Kmo	0.908	0.374	0.412	0.706	0.580	0.001	0.007	0.248	7.697	7.547	6.278	7.055				
1354 Cr2	1.329	0.167	0.126	0.563	0.693	0.013	0.013	0.100	8.763	9.173	6.183	8.344				
1442 Il2ra2	0.697	0.431	0.618	0.809	0.191	0.014	0.162	0.930	7.708	7.187	6.494	6.882				
1558 Hs3st5	1.230	2.410	1.959	1.480	0.809	0.015	0.065	0.349	4.720	5.028	5.999	5.594				
1672 Eclar	0.578	0.425	0.736	0.868	0.066	0.003	0.617	0.440	4.949	4.159	3.716	3.955				

- ❖ Add Another GO 기능을 통해 사용자가 원하는 GO를 자유롭게 구성할수 있습니다.
- ❖ Significant gene을 매우 빠르고 쉽게 데이터를 취할수 있고 그래프로 표현할수 있습니다.



Filter: 200	Fold change	p-value	Average of Normalized RC (log2)
AD	LT	LI+	



DEG Analysis

Significant Gene Selection

Fold change: 2

Average of Normalized RC (log2): 4

p-value: 0.05

Sample Comparison / Filter

- ADTG_LI / AD_LT_LI
- ADFMT_LI / AD_LT_LI
- ADFMT_LI / AD_TG_LI
- ADLT_LI+ AD_FMT_LI/AD_TG_LI

Gene Category Graph

순번: 24421개 중 200개의 레코드가 있습니다.

- ❖ Pie chart를 통해 Significant gene 대상으로 BioFunction 분포를 확인하고 영역을 클릭하면 해당 up/down 유전자가 필터링됨.
- ❖ Bar graph를 통해 BioFunction 별로 up/down의 분포를 확인하고 bar를 클릭하면 해당 유전자를 확인할 수 있음.



파일 홈 삽입 페이지 레이아웃 수식 데이터 검토 보기 ExDEGA Acrobat 이면 작업을 원하시나요?

Calibri 11 가 가 텍스트 주 바꿈

숫사 표준 2 표준 나뎠

사동 함께 새우기

Venn Diagram Analysis

A - AD_TG_LI / AD_LT_LI
 B - AD_FMT_LI / AD_LT_LI
 C - AD_FMT_LI / AD_TG_LI

0 up-regulated
 16 contra regulated
 0 down-regulated

Venn Diagram tool

Sample Comparison

ADTG_LI / AD_LT_LI
 ADFMT_LI / AD_LT_LI
 ADFMT_LI / AD_TG_LI
 ADLT_LI + AD_FMT_LI

Fold change: 1.5
 Average of Normalized RC (log2): 4
 p-value: 0.05

Diagram View

All
 Save Image

Gene Symbol	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI + AD_FMT_LI / AD_TG_LI
2761 Gabrg2	1.693	2.506	1.480	1.240
4366 Gm6682	1.838	1.518	0.826	0.913
4728 Arg2	1.620	1.772	1.094	1.047
6797 Pdlim2	1.511	1.581	1.046	1.023
8284 Ctdc58	1.581	1.531	0.968	0.984
9538 Ai661453	1.501	1.516	1.010	1.005
11529 Adamts12	1.710	2.147	1.256	1.128
11637 Lcn2	1.507	1.832	1.216	1.108
13673 Flhc1	1.583	1.807	1.141	1.071
15046 Abcb4	1.573	1.535	0.976	0.988
18860 Hif3a	1.585	1.618	1.021	1.010
19258 Lrn3	1.560	1.606	1.030	1.015
20336 Trnc1	1.767	2.518	1.425	1.213
20734 Mti483	1.625	1.838	1.131	1.065
21806 Osgin1	1.547	1.585	1.024	1.012
24366 Mid1	2.092	1.641	0.784	0.892
24424				
24425				
24426				
24427				
24428				
24429				
24430				
24431				
24432				
24433				
24434				
24435				
24436				

AD_TG_LI / AD_LT_LI

AD_FMT_LI / AD_LT_LI

AD_FMT_LI / AD_TG_LI

42
86

16
0
55

0
0
0

0
16
0

123
309

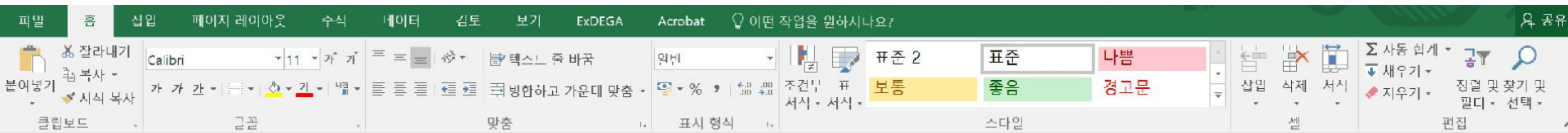
25
0
179

85
164

Data info

Volcano Plot

순번 24421개 중 16개의 레코드가 있습니다.



A11637 Lcn2

Gene Symbol	Fold change				p-value				Average of Normalized RC (log2)			
	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI+ / AD_FMT_LI / AD_TG_LI	AD_TG_LI / AD_LT_LI	AD_FMT_LI / AD_LT_LI	AD_FMT_LI / AD_TG_LI	AD_LT_LI+ / AD_FMT_LI / AD_TG_LI	AD_LT_LI	AD_TG_LI	AD_FMT_LI	AD_LT_LI+ / AD_FMT_LI
Lcn2	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Fhd1	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Abcb4	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Hif3a	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Lrfr3	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Tmc7	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Mir483	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6
Osgin1	~10.5	~11.5	~11.5	~11.5	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6	~0.6

Gene Graph Tool

DEG Analysis

Gene Category Graph

Analysis Graph

Venn Diagram

Volcano Plot

Selected Gene Graph (Gene Symbol)

Abcb4
Hif3a
Lrfr3
Tmc7
Mir483
Osgin1

Graph View

Gene Search

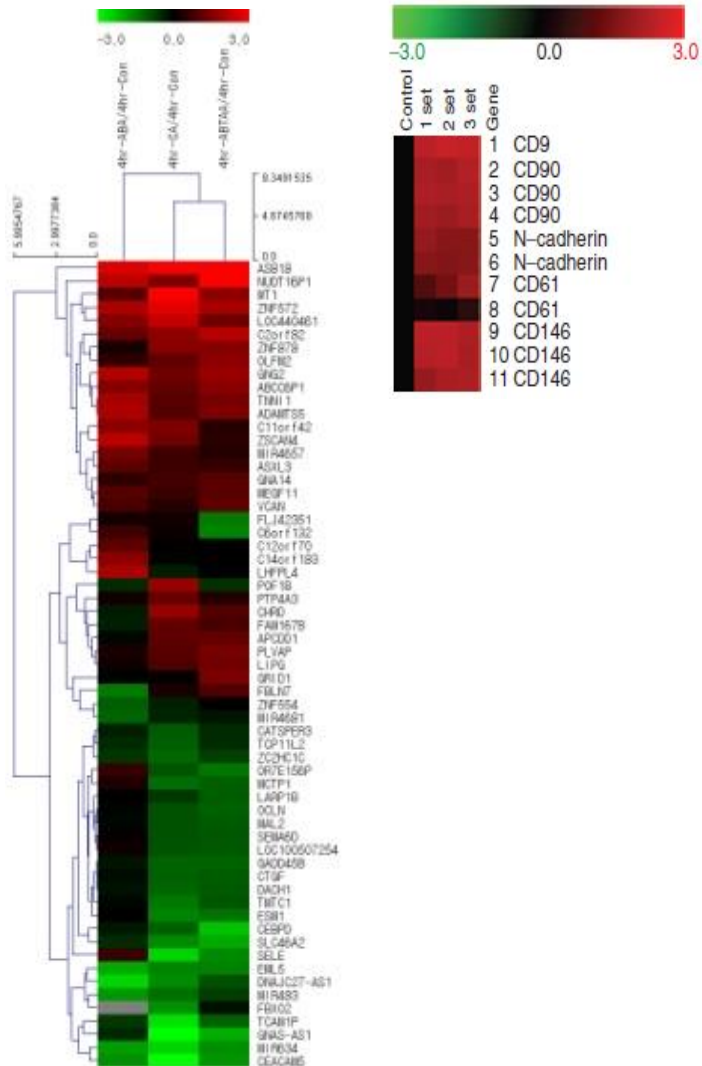
대상을 선택한 다음 <Enter> 키를 누르거나 (붙여넣기)를 선택합니다.

개수: 8 83%

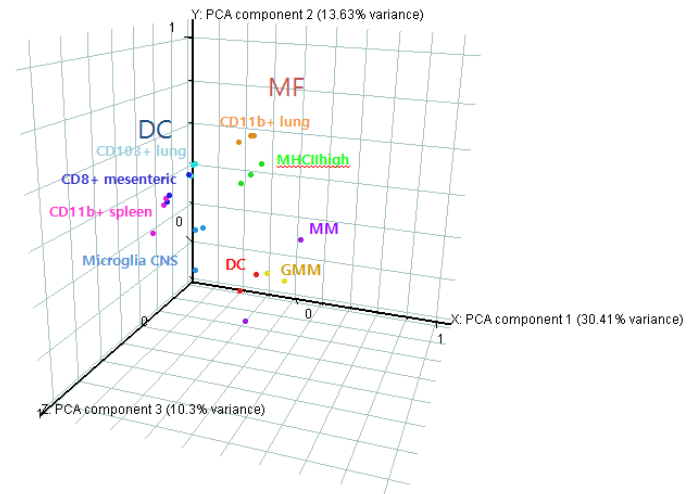
Clustering & Classification



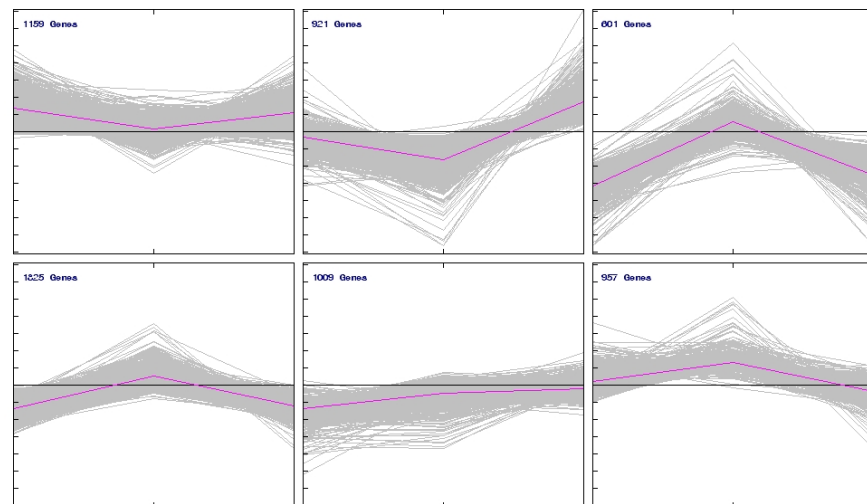
Hierarchical Clustering (HCL)



Principal Component Analysis (PCA)



K-means clustering (KMC)



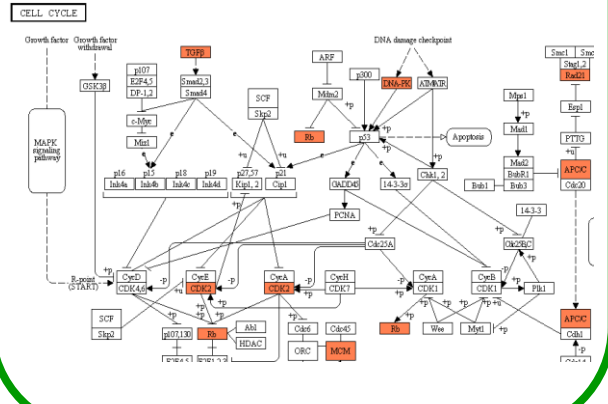
Additional Data Analysis



GO & Pathway

QuickGO

KEGG Mapper



Gene Set Enrichment

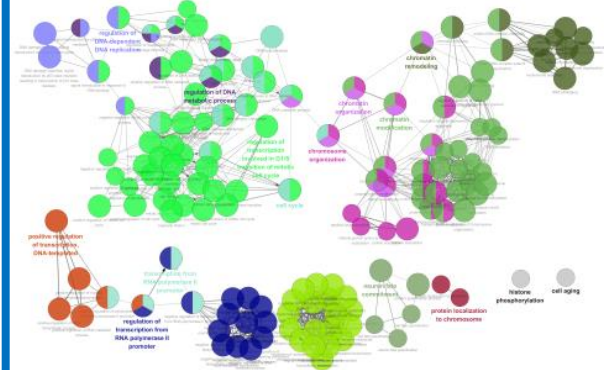
DAVID

GSEA

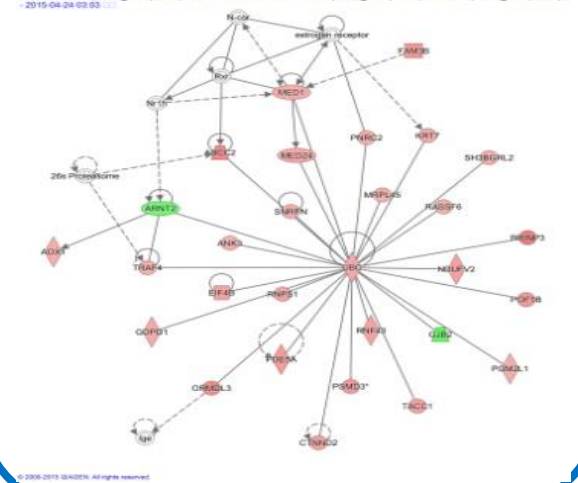
GENESET	ES	NES	NP	FDR	QVAL
gene_setsgmtPRACTOME_INSULIN_RECEPTOR_RECYCLING	0.7051	1.6903	0.0108	0.4703	0.93
gene_setsgmtPKEGG_ALLOGRAFT_REJECTION	0.6831	1.7429	0.0029	0.3306	0.713
gene_setsgmtPKEGG_TYPE_1_DIABETES_MELLITUS	0.6831	1.8278	0	0.1508	0.258
gene_setsgmtPRACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	0.6804	1.5191	0.0398	1	1
gene_setsgmtPKEGG_GRAFT_VERSUS_HOST_DISEASE	0.6777	1.7734	0.0016	0.2722	0.54
gene_setsgmtPRACTOME_PD1_SIGNALING	0.6696	1.5048	0.0478	1	1
gene_setsgmtPKEGG_ASTHMA	0.6609	1.6649	0.0076	0.4892	0.975
gene_setsgmtPRACTOME_TRANSFERRIN_ENDOCYTOSIS_AND_RECYCLING	0.6577	1.5666	0.0219	1	1
gene_setsgmtPRACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOAD	0.6528	1.5084	0.0538	1	1
gene_setsgmtPBIOCARTA_COMP_PATHWAY	0.6393	1.4677	0.0521	1	1
gene_setsgmtPKEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.635	1.8849	0	0.1041	0.091
gene_setsgmtPKEGG_AUTOIMMUNE_THYROID_DISEASE	0.6196	1.6902	0.0056	0.4034	0.93
gene_setsgmtPKEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	0.6157	1.4886	0.0279	1	1
gene_setsgmtPRACTOME_GENERATION_OF_SECOND_MESSENGER_MOLECULES	0.6102	1.4898	0.0464	1	1
gene_setsgmtPBIOCARTA_IL10_PATHWAY	0.6035	1.3206	0.1449	1	1
gene_setsgmtPKEGG_LEISHMANIA_INFECTION	0.5932	1.6972	0.0014	0.5178	0.914
gene_setsgmtPKEGG_OTHER_GLYCAN_DEGRADATION	0.5819	1.2691	0.1631	1	1
gene_setsgmtPRACTOME_RNA_POLL_TRANSCRIPTION_INITIATION	0.5771	1.3739	0.0972	1	1
gene_setsgmtPKEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	0.5703	1.553	0.02	1	1
gene_setsgmtPRACTOME_TIE2_SIGNALING	0.5694	1.2409	0.1968	1	1
gene_setsgmtPBIOCARTA_HSP27_PATHWAY	0.5639	1.2429	0.2194	1	1
gene_setsgmtPKEGG_BACH1_TRANSCRIPTION_FACTOR	0.5501	1.444	0.0448	1	1

Network Analysis

Cytoscape (ClueGO)



IPA (Option)



Gene Ontology & Pathway



Quick GO DB를 이용한 Gene Ontology 분석

KEGG DB를 이용한 Pathway 분석

EMBL-EBI

QuickGO
A fast browser for Gene Ontology terms

QuickGO
Help
Reference
FAQs
Video tutorials
Downloads
geneontology.org
UniProt-GOA project
Web Services

EBI > Databases > QuickGO

QuickGO

growth

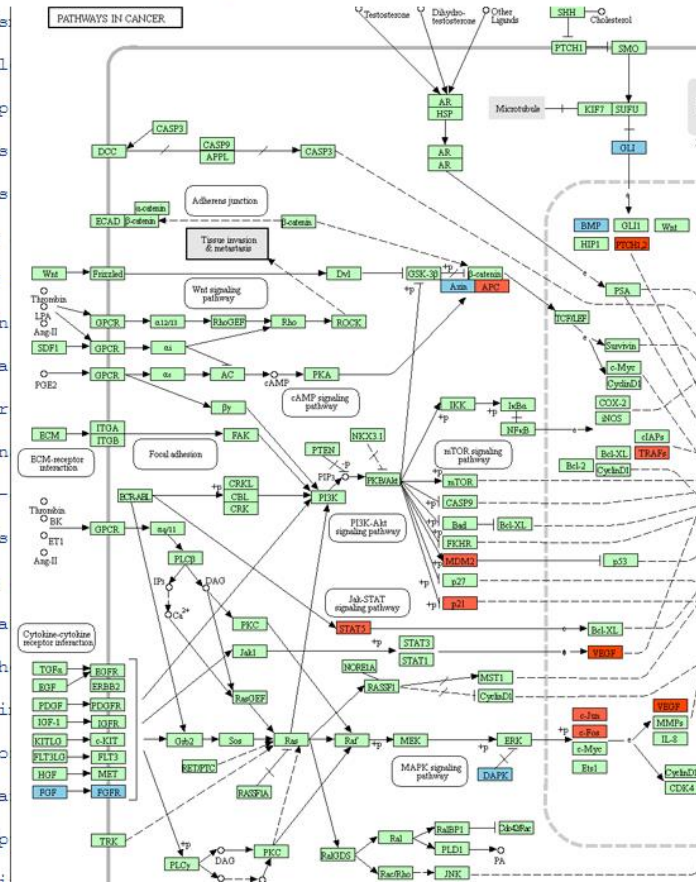
- GO:0009542 granum
- GO:0040007 growth
- GO:0043186 P granule
- GO:0042332 gravitaxis
- GO:0016049 cell growth
- GO:0030426 growth cone
- GO:0044840 gut granule
- GO:0060437 lung growth
- GO:0070088 PHA granule
- GO:0080112 seed growth
- GO:0098868 bone growth
- GO:0009630 gravitropism
- GO:0035265 organ growth
- GO:0042718 yolk granule
- GO:0043036 starch grain
- GO:0060419 heart growth
- GO:0001555 oocyte growth
- GO:0030448 hyphal growth
- GO:0098594 mucin granule
- GO:0033095 aleurone grain
- GO:0040007 growth
- GO:0016049 cell growth
- GO:0030426 growth cone
- GO:0060437 lung growth
- GO:0080112 seed growth
- GO:0098868 bone growth

Pathway Search Result

Sort by the pathway list

Show all objects

- hsa01100 Metabolic pathways - Homo sapiens (human) (28)
- hsa05200 Pathways in cancer - Homo sapiens (human) (19)
- hsa05034 Alcoholism
- hsa04110 Cell cycle
- hsa05165 Human papillomavirus infection
- hsa05206 MicroRNAs
- hsa04217 Necroptosis
- hsa04151 PI3K-Akt signaling pathway
- hsa05322 Systemic lupus erythematosus
- hsa05166 HTLV-I infection
- hsa04115 p53 signaling pathway
- hsa05203 Viral carcinogenesis
- hsa04010 MAPK signaling pathway
- hsa04060 Cytokine-cytokine receptor interaction
- hsa04210 Apoptosis
- hsa05167 Kaposi's sarcoma
- hsa04668 TNF signaling pathway
- hsa04510 Focal adhesion
- hsa05168 Herpes simplex infection
- hsa04810 Regulation of lipids
- hsa05224 Breast cancer
- hsa05202 Transcription
- hsa04090 Neurospora



Gene Set Enrichment Analysis



DAVID tool을 이용한 Functional Annotation 분석

Current Gene List: List_1
Current Background: Homo sapiens

94 DAVID IDs

Check Defaults [x] Clear All

DAVID Database for Annotation, Visualization and Integrated Discovery (Laboratory of Immunology - Windows Internet Explorer)

55 chart records

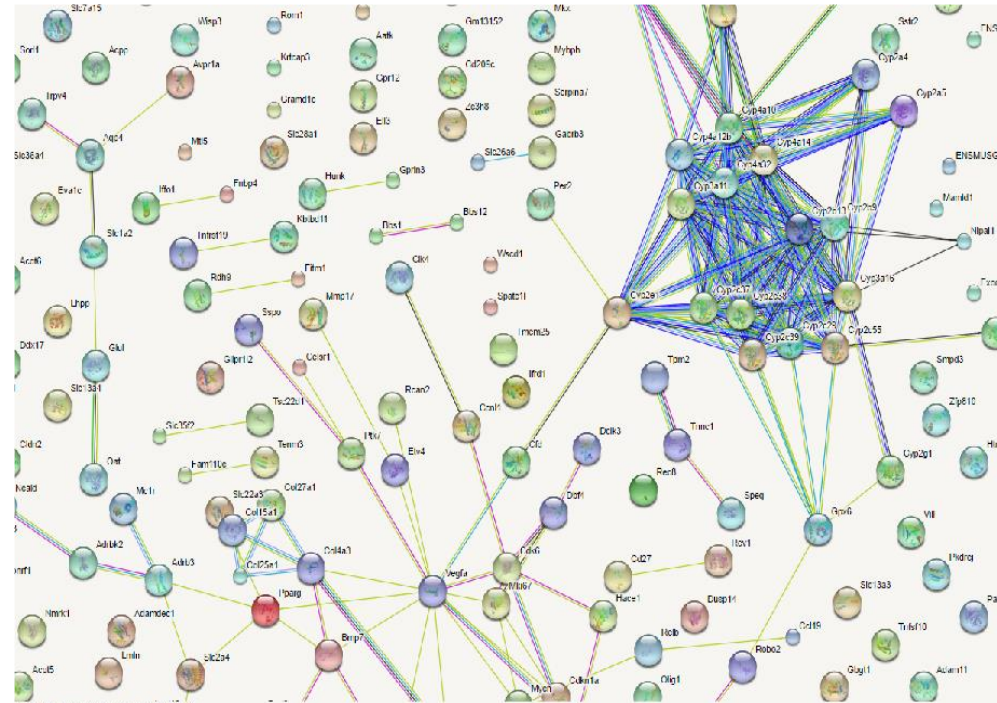
Label	Category	Term	Q-RT	Genes	Count
GOTERM_BP_FAT	cellular adhesion		RT	8	8
GOTERM_BP_FAT	cell-cell adhesion		RT	8	8
GOTERM_BP_FAT	cell adhesion		RT	11	11
GOTERM_BP_FAT	hematopoiesis		RT	11	11
GOTERM_BP_FAT	inflammatory response		RT	7	7
GOTERM_BP_FAT	integrin-mediated signaling pathway		RT	4	4
GOTERM_BP_FAT	cellular cation homeostasis		RT	6	6
GOTERM_BP_FAT	defense response		RT	9	9
GOTERM_BP_FAT	cell homeostasis		RT	6	6
GOTERM_BP_FAT	liver development		RT	4	4
GOTERM_BP_FAT	cellular metal ion homeostasis		RT	5	5
GOTERM_BP_FAT	metal ion homeostasis		RT	5	5
GOTERM_BP_FAT	uric acid metabolism		RT	4	4
GOTERM_BP_FAT	osteoblast development		RT	3	3

Gene Report

Current Gene List: List_1
Current Background: Homo sapiens
94 DAVID IDs
6 record(s)

OFFICIAL_GENE_SYMBOL	GENE_NAME	Q
TEK	TEK tyrosine kinase, endothelial	BQ
AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	BQ
ITGB1BP1	integrin beta 1 binding protein 1	BQ
ITGA8	integrin, alpha 8	BQ
LAMA5	laminin, alpha 5	BQ
RND2	rhodanese 2 (rhodanodase)	BQ

String-db tool을 이용한 gene set 분석



MSigDB기반 GSEA 분석

Gene Set Name [# Genes (K)]	Description	# Genes in overlap (k)	k/K	p-value	LODR q-value
GO:R01:GOAL:GO:0001634:CELLULAR DIFFERENTIATION [1492]	Any process that modulates the frequency, rate or extent of cell differentiation, the process in which relatively unspecialized cells acquire specialized structural and functional features.	40	0.125	1.78×10^{-12}	2.41×10^{-15}
GO:R01:GOAL:GO:0001634:CELLULAR DIFFERENTIATION [1672]	Any process that modulates the frequency, rate or extent of multicellular organismal development.	40	0.125	9.15×10^{-13}	2.01×10^{-14}
GO:REGULATION OF NERVOUS SYSTEM DEVELOPMENT [750]	Any process that modulates the frequency, rate or extent of nervous system development, the origin and formation of nervous tissue.	25	0.15	1.53×10^{-14}	2.26×10^{-11}
GO:REGULATION OF CELL DEVELOPMENT [836]	Any process that modulates the rate, frequency or extent of the progression of the cell over time, from its formation to the mature	26	0.15	2.2×10^{-14}	2.43×10^{-11}

Biological Process (GO)

pathway ID	pathway description	count in gene set	false discovery rate
GO:001676	long-chain fatty acid metabolic process	1	2.69e-06
GO:001973	epoxygenase P450 pathway	7	3.53e-06
GO:004505	regulation of cell differentiation	41	7.41e-05
GO:004238	carcinogen drug catabolic process	7	0.00116
GO:003793	regulation of developmental process	62	0.00316

Molecular Function (GO)

pathway ID	pathway description	count in gene set	false discovery rate
GO:0016712	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	1	2.3e-09
GO:007030	isomerase activity	10	1.14e-08
GO:004491	monooxygenase activity	12	1.59e-07
GO:0038792	arachidonic acid epoxidase activity	8	1.59e-07
GO:002057	hematidling	15	1.23e-06

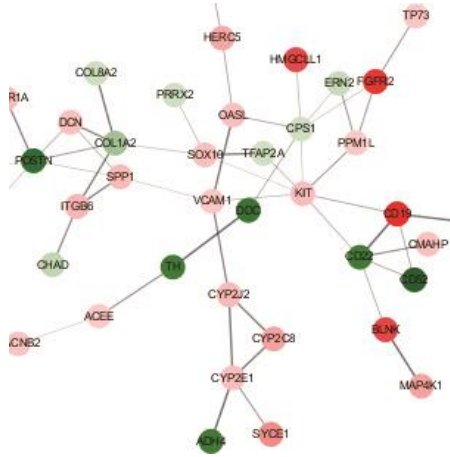
KEGG Pathways

pathway ID	pathway description	count in gene set	false discovery rate
00170	Pentose metabolism	15	9.07e-12
00330	Arachidonic acid metabolism	12	3.28e-07
00140	Steroid hormone biosynthesis	10	3.89e-06
00561	Lipidic acid metabolism	8	4.72e-06

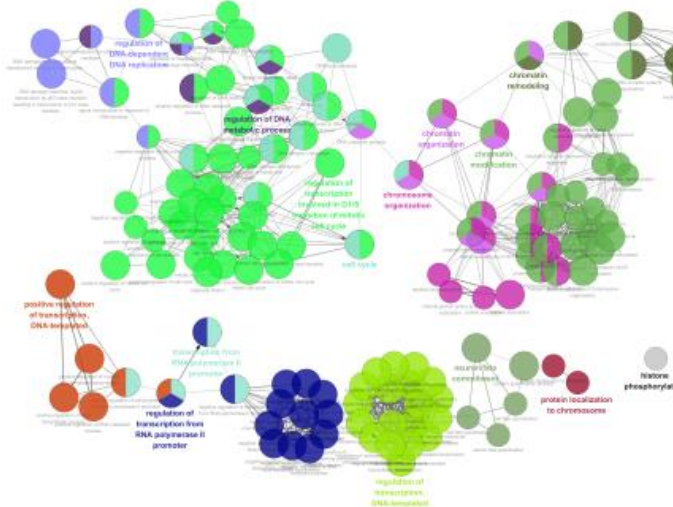
Network Analysis (Cytoscape / IPA)



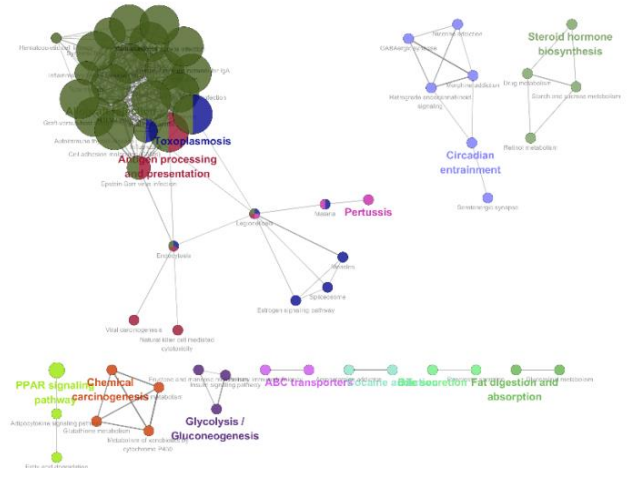
PPI(String)



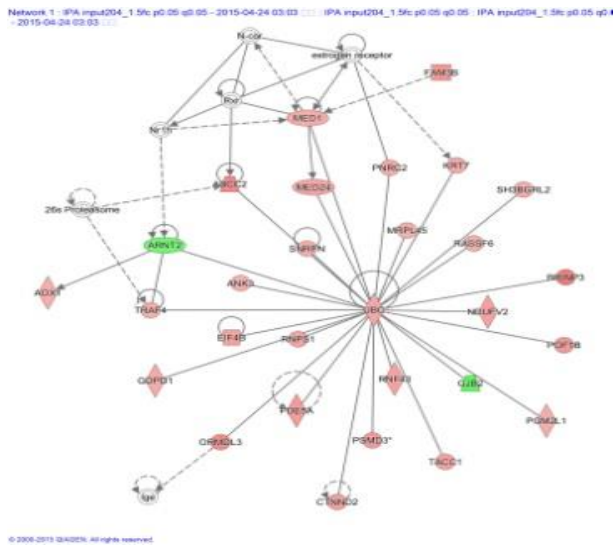
GO network (ClueGO)



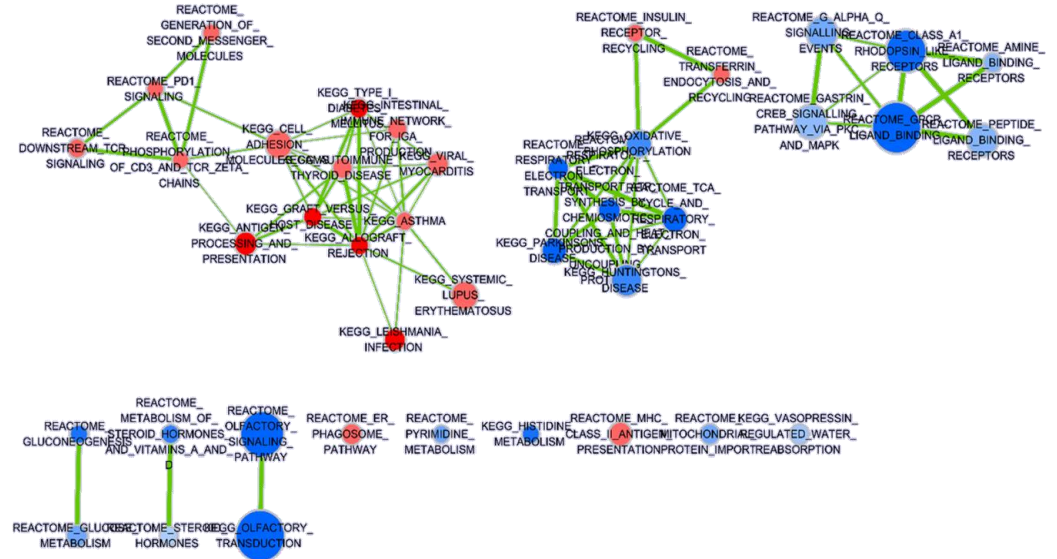
Pathway network (ClueGO)



IPA network (Optional)



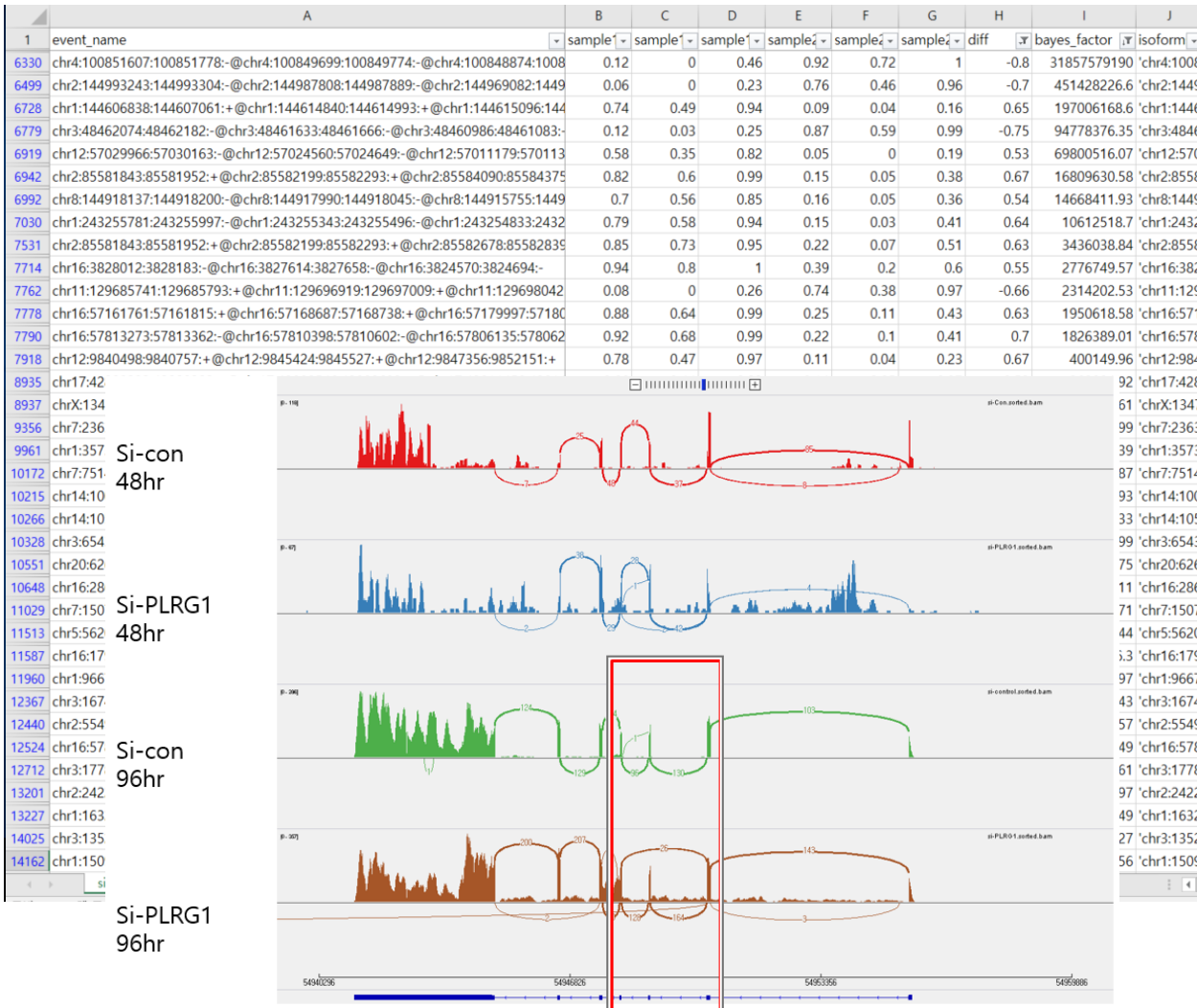
Gene set network (Enrichment map)



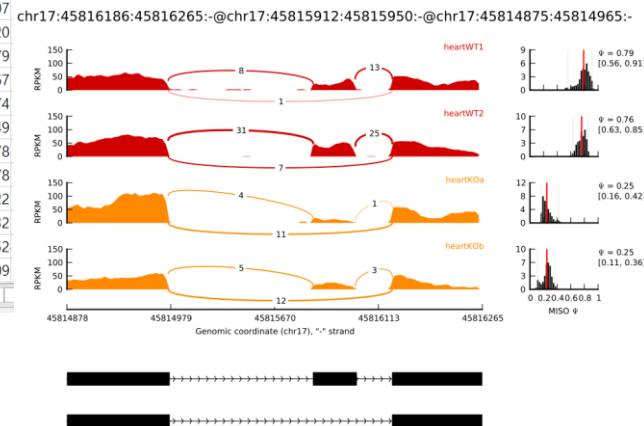
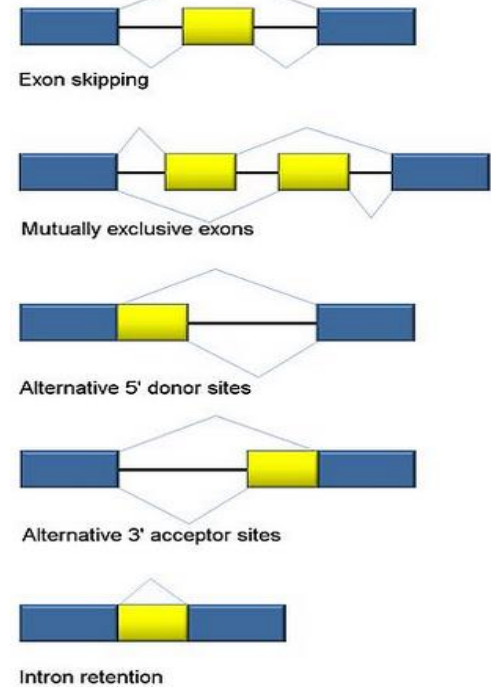
Alternative Splicing Events



MISO (Mixture of Isoforms) : Probabilistic analysis and design of RNA-Seq experiments for identifying isoform regulation



Splicing Patterns



Other Analysis Support



Gene search (GeneCards)

Relevant diseases search (FunDO)

GeneCards®: The Human Gene Database

GeneCards is a searchable, integrative database that provides comprehensive, user-friendly information on all annotated and predicted human genes. It automatically integrates gene-centric data from ~125 web sources, including genomic, transcriptomic, proteomic, genetic, clinical and functional information.

Explore a Gene

ALB

Jump to section for this gene:

Aliases Disorders Domains Drugs Expression Function Genomics Localization Orthologs Paralogs Pathways Products Proteins Publications Sources Summaries Transcripts Variants

GeneCards Database Statistics

Category	# of Genes	Example Genes
Total GeneCards genes	152,704	
Protein-coding	21,965	IGF1R, FGFR1, SOD1, EGFR, MAP2K1, AKT1, AKT2, ERBB2, FGFR2, FGFR3
RNA genes	107,049	PRNT, TCL6, DLEU1, HCP5, DSCR8, MEG3, H19, KIAA0087, NR2F, KIAA1875
Pseudogenes	16,329	GNRHR2, GUCY1B2, PRKY, HL4H, FMO6P, NSUN6P2, MMP23A, SXX0, TDH, LPAL2
Genetic loci	1,754	FAM201B, ST2, CYP3A, CYP2A, FRAXA, XM, FRAXE, AAVS1, AZF1, CMM
Gene Clusters	134	IGLV@, PCDHA@, RNS51@, IGKV@, HOXA@, HOXD@, HOXB@, IFN1@, SAA@, PCDHG@
Uncategorized	5,473	C20orf181, TRAV35, ZNF807, LOC441956, PPI3, IGHV3-9, IGHV1-9, IGHV1-8, IPRN, LOC100500557

The GeneCards human gene database index: 135ABCDEGHIJLKMNOPQRSTUUVWXYZ

FunDO Exploring Genes Using Functional Disease Ontology Annotations

The top 8 diseases and the genes in the list which map to them. The sizes of the disease nodes are proportional to the number of edges. The table below shows all diseases which map to at least 2 genes from the list. The p-value is calculated using Fisher's exact test. The table itself is interactive, to see by a particular column, click on the column heading.

DO Term	Number of Genes in List	% of Disease Gene List	Fold Enrichment	p-value	Bonferroni corrected p-value
1. Cancer	21	2.85	4.2205	3.951e-8	0.00000241
2. Gastrointestinal tumor	3	23.08	34.1352	0.00008312	0.06507
3. Oral Cancer	4	7.41	10.957	0.0004954	0.03022
4. Multiple myeloma	4	8.9	10.2013	0.0005507	0.03969
5. Primary hyperparathyroidism	2	28.57	42.2626	0.00039351	0.05704
6. Multiple endocrine neoplasia	2	22.22	32.8709	0.001589	0.09691
7. Wiskott-Aldrich syndrome	2	20	29.5838	0.001977	0.1206
8. Osteoporosis	3	7.5	11.0939	0.00251	0.1531

miRNA target gene analysis (miRWalk 2.0)

miRNA target gene network (CyTargetLinker)

miRWalk 2.0: a comprehensive atlas of predicted and validated miRNA-target interactions

Pathway information retrieval system

Step 1: Select a species, database and pathway

Select database: KEGG pathways, RCGO pathways, Wiki pathways, Pathway pathways

Select species: Human, Mouse, Rat

Select pathway: ABC transporters, Acute myeloid leukemia, Adherens junction, Adipocyteline signaling pathway, Alanine aspartate and glutamate metabolism, Alkoxisterone regulated sodium reabsorption, Allograft rejection, alpha Lipoic acid metabolism

Step 2: Result tables i.e. information on gene location, other identifiers and their associations

Gene information: Gene Table, Location, Synonym, mRNA Table, Homolog, External links

Functional association: Gene-miRNA interactions, Enriched miRNAs

Step 3: Putative miRNA list

Output fields: miRNA, Identifiers

Select start position of miRNA seed: Position 1

Input parameters: Promoter/2, 5' UTR, CDS, 3' UTR, Minimum seed length?, and/or p-value 0.05

Other databases: miRWalk, miRDB, miRMap, PITA, miRanda, miRMap, miRBase, miRMap, PICTAR2, RNAhybrid, RNA22, RNAhybrid, TargetScan

Step 4: Output

Query output: [] [SEARCH]

CyTargetLinker

Session Name: Session

File Edit View Select Layout Apps Tools Help

Control Panel: Networks, Mapper, Filters, CyTargetLinker

Select extended network: miRNAs

Overlap threshold: 1.00

miRNA	#	Color	Show/Hide
hs17-5p	17127	[Red]	[Show]
hs17-5p	0/1543	[Blue]	[Hide]
hs17-5p	0/2350	[Blue]	[Hide]

TargetScan v3.0.10.1 (version 4.2)

Table Panel: shared, name, miRNAs, cl type, cl nodes, biologic, ensmid, label, stream, ensid2, organism, degree

Data Visualization

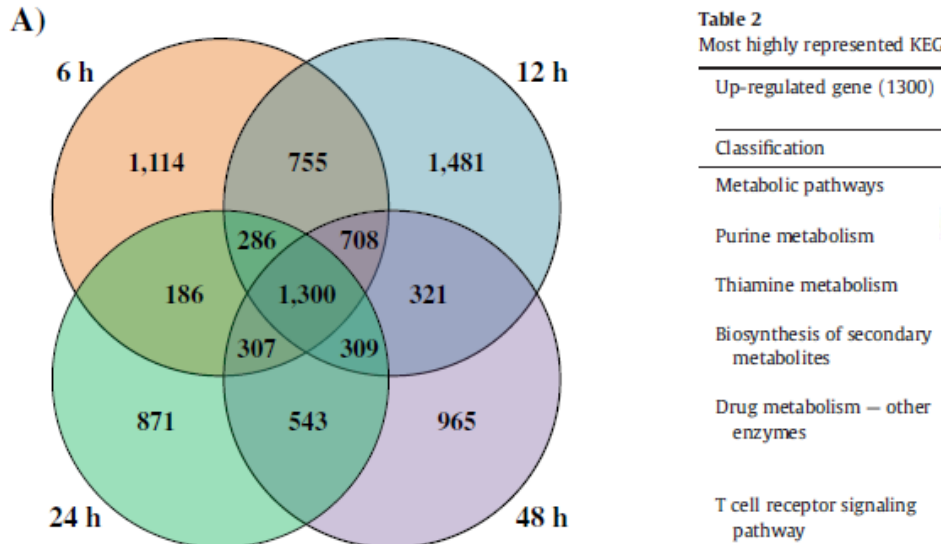


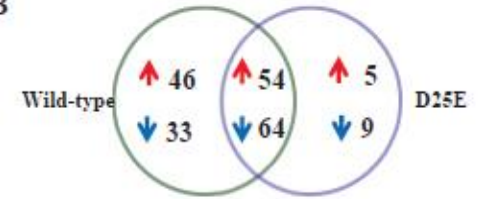
Table 2
Most highly represented KEGG classifications in up- and down-regulated transcripts.

Classification	Up-regulated gene (1300)		Down-regulated genes (588)		
	#	%	#	%	t2.4
Metabolic pathways					t2.1
Purine metabolism					t2.2
Thiamine metabolism					t2.3
Biosynthesis of secondary metabolites					
Drug metabolism – other enzymes					
T cell receptor signaling pathway					

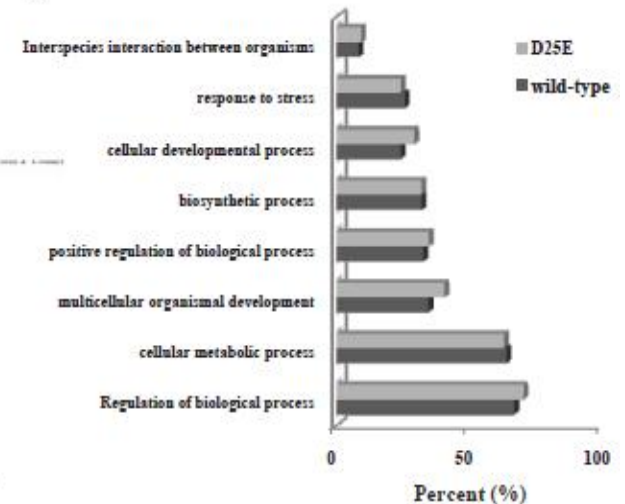
A



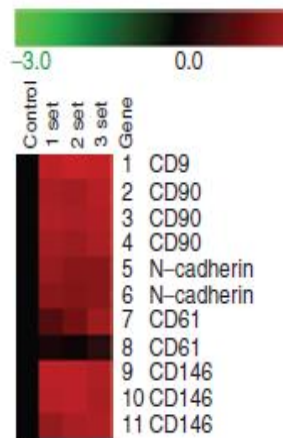
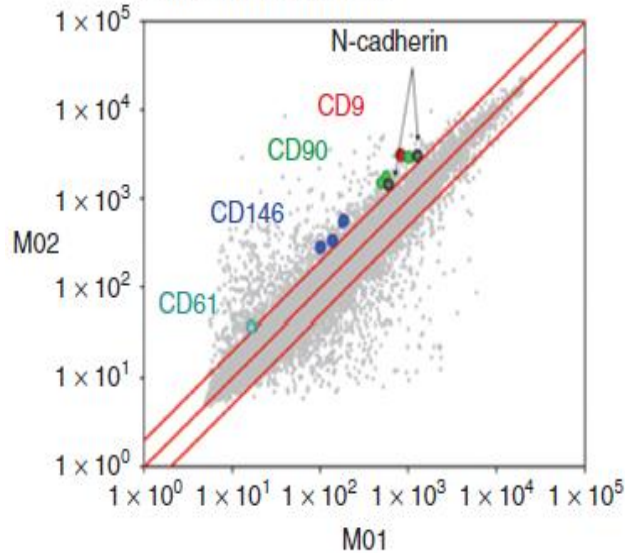
B



C



Scatter plot of whole gene expression profiles between M01 and M02



Paper Support



NCBI » GEO

Gene Expression Omnibus: a public functional genomics data repository supporting MIAME submissions. Array- and sequence-based data are accepted. Tools are provided to help download experiments and curated gene expression profiles. [More information »](#)

GEO navigation

DataSets

Gene profiles

QUERY

A	B
1	SERIES
2	title Murine ES Cells: Control vs. Triple-Fusion Transfected
3	summary Transcriptional profiling of mouse embryonic stem cells comp
4	overall design Two-condition experiment, ES vs. TF-ES cells. Biological repl
5	contributor Jane,Doe
6	contributor John,A,Smith
7	
8	SAMPLES
9	# The corresponding example matrix table is included in the next worksheet.
10	Sample name title
11	ES-rep1 Control Embryonic Stem Cell Replicate 1
12	ES-rep2 Control Embryonic Stem Cell Replicate 2
13	ES-rep3 Control Embryonic Stem Cell Replicate 3
14	ES-rep4 Control Embryonic Stem Cell Replicate 4
15	TF-ES-rep1 Triple-Fusion Transfected Embryonic Stem Cells Replicate 1
16	TF-ES-rep2 Triple-Fusion Transfected Embryonic Stem Cells Replicate 2
17	TF-ES-rep3 Triple-Fusion Transfected Embryonic Stem Cells Replicate 3
18	

Jang et al. *Virology Journal* 2011, 8:453
<http://www.virologyj.com/content/8/1/453>



American Heart Association
 Learn a

Long-Term Effects of Sildenafil in a Rat Model of Chronic Mitral Regurgitation: Benefits of Ventricular Remodeling and Exercise Capacity
 Kyung-Hee Kim, Yong-Jin Kim, Jung-Hun Ohn, Jimin Yang, Sang-Eun Lee, Sae-Won Lee, Hyung-Kwan Kim, Jeong-Wook Seo and Dae-Won Sohn

SHORT REPORT **Open Access**

Gene Expression Profiles are Altered in Human Papillomavirus-16 E6 D25E-Expressing Cell Lines

Mi Jang[†], Jee Eun Rhee[†], Dai-Ho Jang and Sung Soon Kim^{*}



Hepatology Research 2011; 41: 170-183

doi: 10.1111/j.1872-034X.2010.007

Original Article

Expression profiles of miRNAs in human embryonic stem cells during hepatocyte differentiation

Nury Kim,¹ Hyemin Kim,¹ Inkyung Jung,² Yeji Kim,¹ Dongsup Kim² and Yong-Mahn Han¹

N-cadherin Determines Individual Variations in the Therapeutic Efficacy of Human Umbilical Cord Blood-derived Mesenchymal Stem Cells in a Rat Model of Myocardial Infarction

Eun Ju Lee¹, Eue-Keun Choi^{1,2}, Soo Kyoung Kang¹, Gi-Hwan Kim¹, Ju Young Park¹, Hyun-Jae Kang^{1,2}, Sae-Won Lee¹, Keum-Hyun Kim¹, Jin Sook Kwon³, Ki Hong Lee⁴, Youngkeun Ahn⁴, Ho-Jae Lee¹, Hyun-Jai Cho^{1,2}, Soo Jin Choi⁵, Won Il Oh⁵, Young-Bae Park^{1,2} and Hyo-Soo Kim^{1,2,6}



For only Expression Profiling





Whole Transcriptome Library Library for Expression Profiling



Assembly, Align and RPKM



Many reads for 1 transcript are needed

Expression Profiling Library Library for Expression Profiling



Only 1 read for 1 transcript is needed
if we can sequence the same positions of all different transcripts.



- Normally 1/4 of sequence read is needed.
- Higher number of multiplexing is possible.
- Significantly less amount of computational resources are required.
- Still the question remains :
Which position of the transcripts to sequence?

3 types of QuantSeq



- QuantSeq 3` mRNA-Seq Library prep kit
- QuantSeq 5` mRNA-Seq Library prep kit
- QuantSeq-Flex Targeted mRNA-Seq Library prep kit

5`end with Cap

3`end with poly(A)



QuantSeq 5

QuantSeq-Flex

QuantSeq 3

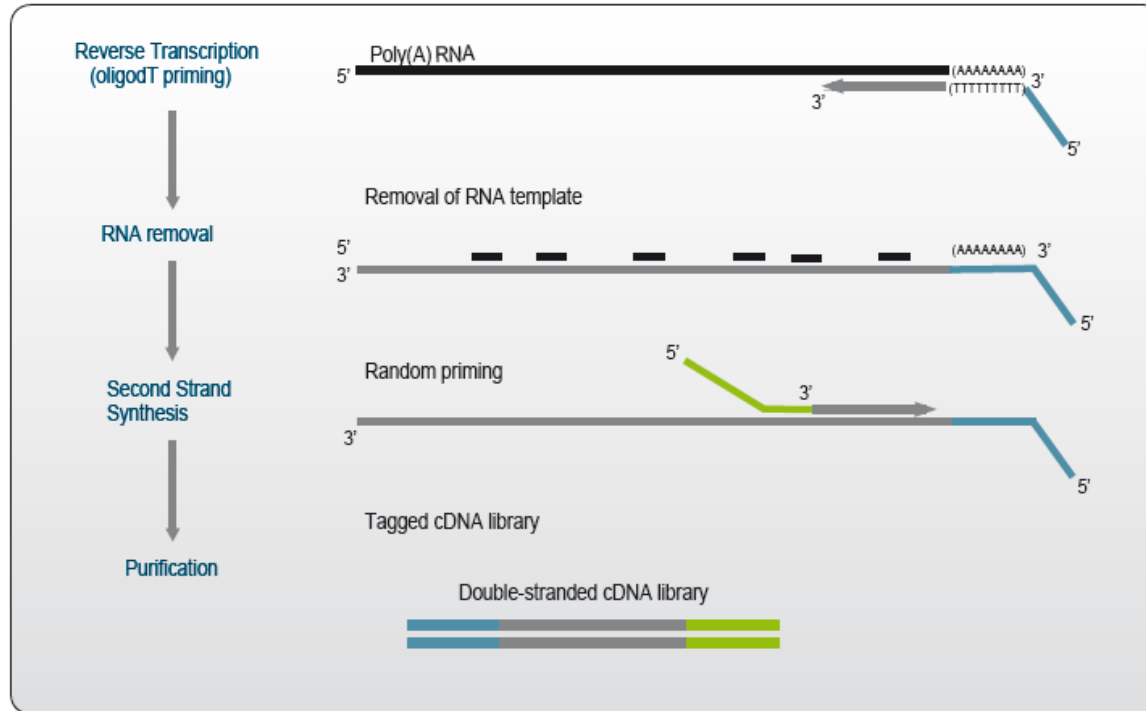
Library Generation (QuantSeq 3)



LIBRARY GENERATION

200 min

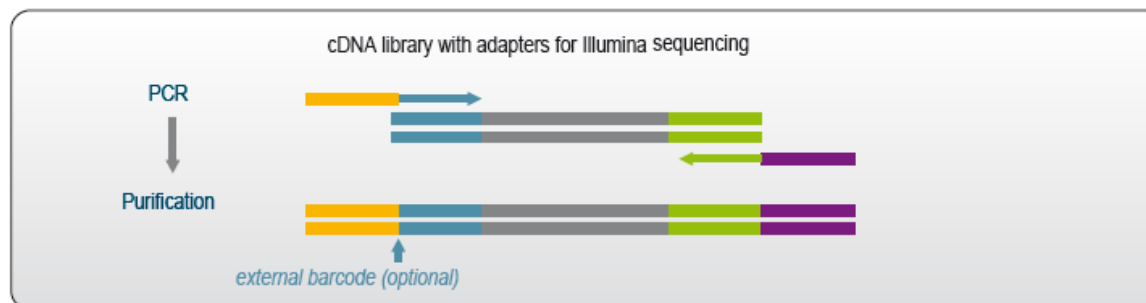
60 min



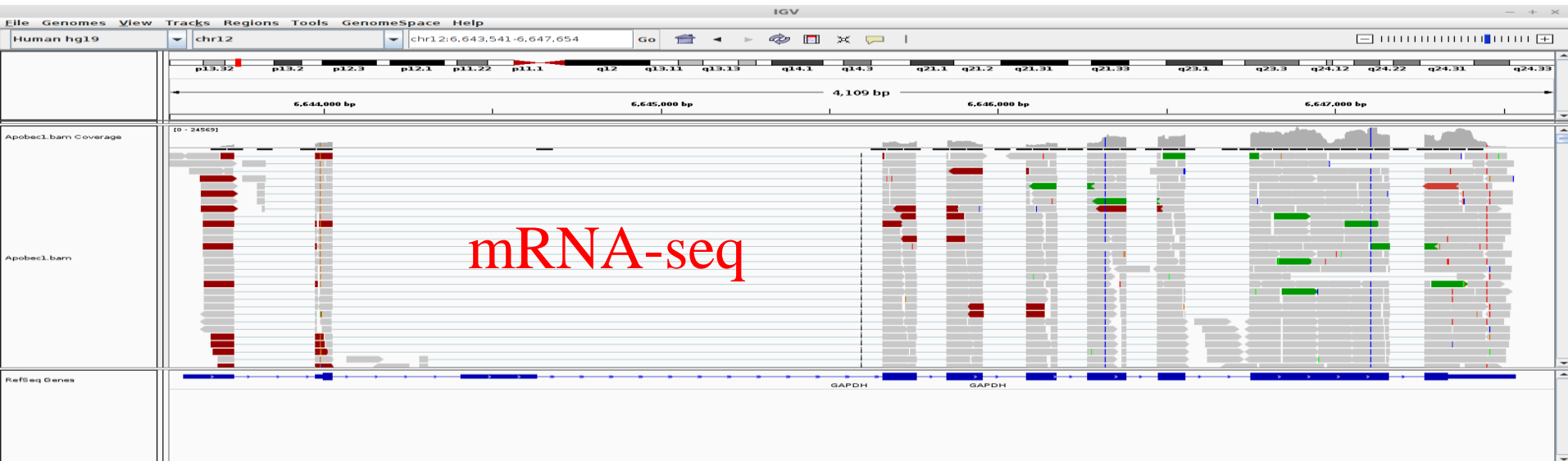
LIBRARY AMPLIFICATION

70 min

45 min



Data Analysis – IGV





- High number of multiplexing (up to 48 samples)
- No need for length normalization
- Easy protocol and automation-friendly
- Massive screening of diverse samples with high (around 6-order of magnitude) dynamic range for detection is possible.
- 75 or 50bp SR is enough for the data analysis.
- Significant reduction of cost
- Degraded sample could be done.
- Wide input range, from 100pg ~ 2ug of total RNA

Validation by qRT-PCR



DEGs selection in mRNA-seq

ExDEGA Acrobat

GO DEG Tools

11322 14.011556197299

1	A	B		C		D		E		F		G		H		I		J		K		L	
		Filter: 88	Fold change		p-value		Average of Normalized RC(log2)																
2	Gene Symbol	WO/WY	MO/MY	WO/WY	MO/MY	WY	WO	MY	MO	WY_03	WY_13	WY_42											
2948	CCL19	0.290	0.393	0.011678	0.517271	11.98813	10.20472	12.3461	10.99972	12.76157	11.60205	11.60796											
3189	CDH4	2.890	1.908	0.01187	0.270571	8.485512	10.01676	9.508852	10.4408	7.778705	9.205009	8.663686											
3473	CHAC1	0.221	49.926	0.041535	0.063741	7.782682	5.605333	4.108761	9.75049	7.63026	7.81653	7.72944											
3895	COL11A2	17.999	1.902	0.001213	0.026304	9.121619	13.29148	12.99901	13.92667	8.950766	9.164232	8.872125											
3938	COL8A1	2.688	2.663	0.03134	0.151371	7.001103	8.427835	7.564946	8.977964	6.706391	7.797833	6.92921											
4046	CPEB3	2.505	1.650	0.041193	0.225426	8.40998	9.734917	9.484561	10.20702	7.268705	9.489562	7.865645											
4126	CRHR1	3.797	1.142	0.007099	0.903696	8.898861	10.82365	9.884293	10.07614	8.883787	9.450195	8.065582											
4230	CSNK2B	11.106	1.235	1.21E-05	0.06741	14.52628	17.99954	17.45746	17.76243	14.30721	14.12078	14.77864											
4393	CUX2	0.099	0.512	0.029186	0.743966	7.826325	4.490452	8.010138	7.043935	6.009318	9.214438	7.497493											
4890	DHRS2	0.128	0.802	0.04212	0.901452	9.584293	6.623453	9.401526	9.083977	10.89492	8.797833	8.674855											
4899	DHRS9	0.258	0.883	0.01262	0.740589	11.37145	9.417269	10.83558	10.65631	11.80767	11.72672	11.14738											
5233	DPYSL5	4.326	2.369	0.004222	0.096878	5.130609	7.243529	6.23934	7.483616	4.871815	5.929623	4.826116											
5519	EHMT2	14.871	1.462	4.71E-07	0.075718	13.51128	17.40567	16.83218	17.38021	13.40203	13.62289	13.42465											
5714	EOMES	2.734	1.751	0.036716	0.394935	6.844435	8.295309	7.882011	8.69035	7.644404	7.039806	6.300048											
5969	FA2H	0.363	0.731	0.008504	0.91667	10.46977	9.006928	10.34628	9.894109	10.83837	10.14093	10.79062											
6558	FFAR2	2.363	1.379	0.022413	0.256899	6.330503	7.570992	6.73488	7.198711	6.930708	6.181162	5.685939											
6598	FGF9	2.629	2.726	0.015427	0.047819	5.352367	6.746685	4.803301	6.250095	4.488486	5.378608	5.280682											
6724	FLOT1	11.256	1.546	3.52E-04	0.041775	13.29133	16.78393	16.04394	16.67272	13.72085	12.2363	13.31321											

Gene Ontology Selection

All Genes

AND OR

- Aging
- Angiogenesis
- Apoptotic process
- Cell cycle
- Cell death
- Cell differentiation
- Cell migration
- Cell proliferation
- DNA repair

Add Another GO

Significant Gene S...

Fold: 2

Normalized RC (log2): 6

p-value: 0.05

Sample Comparisor

WO/WY

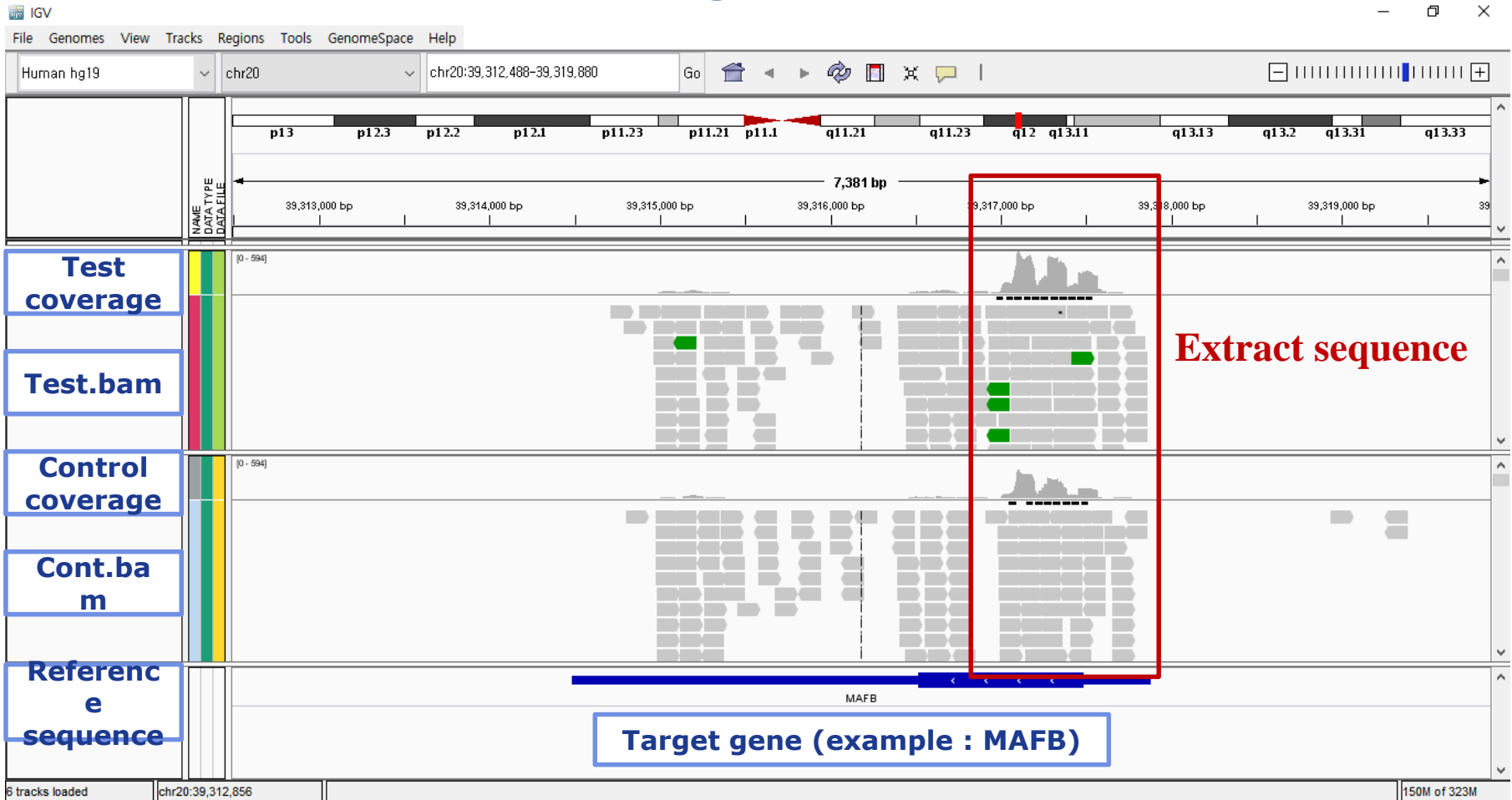
MO/MY

88개의 레코드가 있습니다.

Validation by qRT-PCR



Selection of Primer region



Validation by qRT-PCR

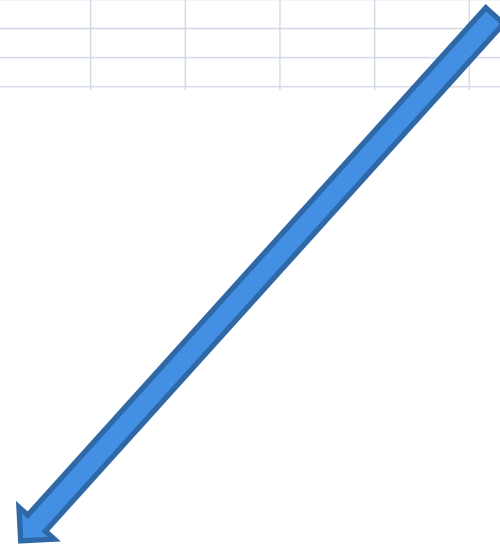


Primer design

Extracted sequence

MAFB	
FULL sequence	TCCTCTAAATAAACACCATTTCCATGGTTTTAATAAGCAAATAGGAAACCATTTAATAACCAAAAAACAGAAACCAGTCTGAATAAACTACAATAGACTAGGTTTTAATATTTTCATATC
Expected sequence	GCCGATGAGCGCTTCCACCGCGTCTCGGGCGTCAGGTTGAGCGCCTCGGGTTTCATCTGCTGGTAGTTGCTCGCCATCCAGTACAGATCCTCGAGGTGTGTCTTCTGTTCCGGT

TCTGTCTTCTCTTCTTCGCTCAGCCTCGCTCTCGCTGCCGGGACGCCAGAGTCCTCTCTTCTCCTGTTGCTCTTCTCTGGC
GCGGACTACTCTCCGGGAGCAAAGCAGCTGTCCGGCTTAACGCGCAAAGTTCAAAAAGCCAGGAGTCTCCAGATGGCCTTG
GTGACTTCTCGGGACTATGCTCGCCGCCCTCAGCTGGAGAGAAGTTACTCCGGGACCTCCACCCCTCTGCTCTCTCTCCG
GCTGTGCTCGAGTCTAGGAGGCGGCGCTGGCGTGCCTACTCTCGCTTAGCCAAGGTCCCCGCCCCGCCGCGCACCCGCCG
TCGCCCCGCGGCCGCGCGCCCTTCTCCTCTCGCTCAAGTCAAACAGGTCAAAGGCTGGGGCCCGGCCAGGACAGGGTCCG
GGGTAGCTGGGACTAGGACGTGGACAGGAGTCCGGGCGGGCAAGGGCGGGGCCAGGACCGGCCAGACTCA CA
GAAAGAACTCGGGAGAGGAGGGGCTGTCTGCTGGTGGAGCCCCTCCGTAAGCCGGAGTTGGCGAGTTTCTGCACTTGAC
CTTGTAGGCGTCTCTCTCGCGGCCAGCCGGGACACCTCTGCTTAAGCTGCTCACCTGCTGAATGAGCTGCGTCTCTCATTCT
CCAGGTGGTGTCTCTGCTGAGCGGTTTATACTGCAAGACTGGCGTAGCCCCGGTCTTTCAGGGTCCGCCGCTCTGCTTCAG
GCGGATCACCTGCTCTTGGTGAAGCCCCGAGGTGCGGTTCAAGCTCGCGCACGGACATGGACAGAGCTGGTCTCGGA GA
AGCGGTCTCCACGCTGCCGTTGCCGCCCGCCGCGTCCGCGAGGCCGTCGCGTCCGCCCCGGCCCGGGTGGTAGTGGG
CAGCTGTTGCCCGGGCTAGCGCGCTGACGCGCGCGCGACGCTTGGTGTGATGATGGTGTGCGGGTGAAGCGTGGCGG
CCCAGCTCGTCTGGGCCACGCGCGCCGGTACGCGTGGTGGCGGGTGAAGGGTGGTGGTGTGGTGGTGGTGAAGCGCC
GCGAAAGCTGTGAAAGCTTTCAGCGGCTGTGGCACTGGTGCAGCGCATGAGCGCTTCCACCGCGTCTCGGGCGTCAAGT
TGAGCGCCTCGGGGTTTCATCTGCTGGTAGTTGCTCGCCATCCAGTACAGATCTCGAGGTGTGTTCTGTTCCGGTGGGCTGAA
GCTGGGCGACGAGGGCACGGAGCTACACGGAGTGTGAGCGGTGGTGGAGGACCCGAGCCGGCTGGCTGACGGCGTGTGCA
GGGCTGCCCCGACGCTCCGCGGCCCAAGTGGCTCTTCTCACGTCGAACTTGAGCAGGTGAAAGTGTGACATACTCCAT
GGCCAGCGGGTGGTGGGAGCTCTGGCCCATATGCTCAGCTCCGCGGCCATCGCTGAAAGCGAGGCGACAGCCGCCGCTGCCG
CCGGGAAACTTTGCGCCGGCCGGAGCGCGCCGAGCAAGCGCGGGGGGGAAGAGCGGAGAAAGAGCTGGGGAGGCGGGG
AGCGAGGGCGACGCGGGCCGGGCGCCGCCCAAGCCTTTGCTGGGGACGCGGCGCGCGCCGGAGAGTCCCGAGGCTG
CCTGCACCGCCCAAGAGCTCTGGGCTGTGCTCCGCGCAGGACCGGGCGGGTAGAGTGGCGGGGTGGAGAGGCAAGCGG
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GCAGCCGCTCGAGCTGGCGGTGCAGCTGTGCTG

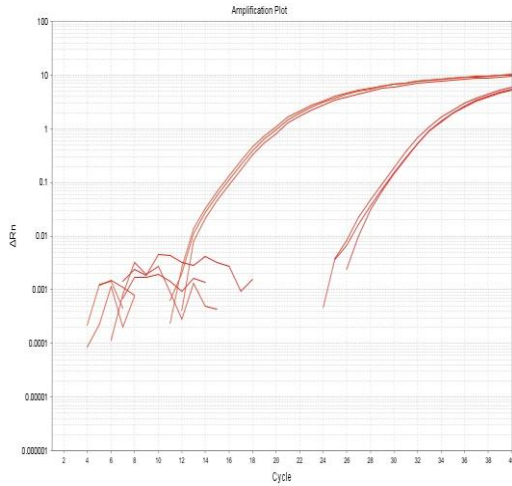


Validation by qRT-PCR

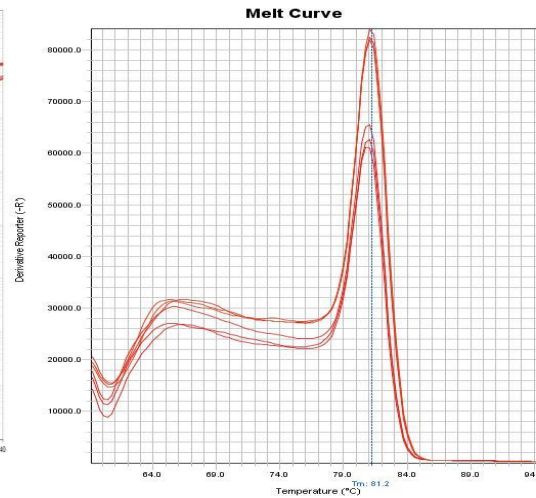


FABP6

Amplification Plot



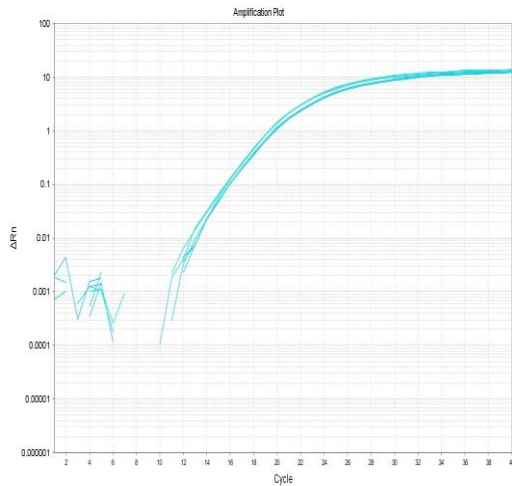
Melt Curve



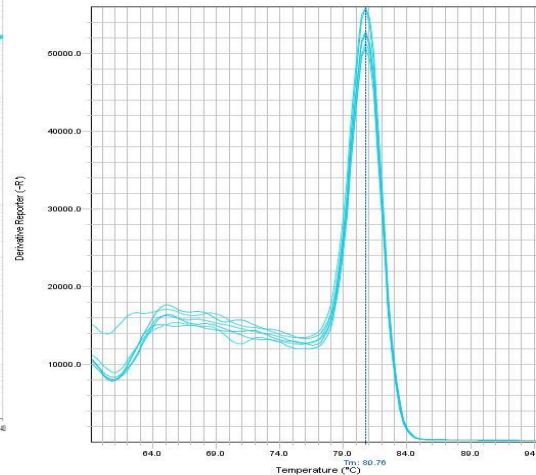
Fold Change

Gene	NGS	qRT-PCR
FABP6	275.71	10,742.00± 2296.91
TFF3	43.83	71.02±5.53
SPINK4	41.45	98.43±6.08
POU2AF1	24.63	1,457.37±8 4.54
RBP2	18.40	13.36±2.47
GZMH	13.45	21.92±1.53
CLCA1	12.93	283.53±52. 92
LGALS3	10.07	13.00±0.91
GZMB	9.11	0.01±0.00
LYZ	7.48	396.30±7.0 8

GAPDH



Melt Curve





<https://www.nature.com/sdata/policies/repositories>

Scientific Data >> Recommended Data Repositories

View data repositories

Biological sciences:

nucleic acid sequence; protein sequence; molecular & supramolecular structure; neuroscience; omics; taxonomy & species diversity; mathematical & modelling resources; cytometry & immunology; organism-focused resources

Health sciences

Chemistry & chemical biology

Earth and environmental sciences

Physics, astrophysics & astronomy

Social sciences

Generalist repositories

Other repositories

<https://www.ncbi.nlm.nih.gov/geo/>

Gene Expression Omnibus

GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles



Gene Set Enrichment Analysis

- <https://david.ncifcrf.gov/>
- <http://software.broadinstitute.org/gsea/msigdb/index.jsp>

Gene Search & ID Conversion

- <https://www.ncbi.nlm.nih.gov/gene>
- <https://david.ncifcrf.gov/>
- <http://www.uniprot.org/uploadlists/>
- <http://www.genecards.org/>

Gene Ontology & Pathway

- <https://www.ebi.ac.uk/QuickGO/>
- http://www.genome.jp/kegg/tool/map_pathway2.html
- <http://pantherdb.org/>

Genomic Data Analysis

- <https://www.bioconductor.org/>
- <http://apps.cytoscape.org/>
- mev.tm4.org/

Other web based DB & tools

- <http://bioinformatics.psb.ugent.be/webtools/Venn/>
- <http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>
- <http://www.mirbase.org/>

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