Bioinformatics 사용법 (1) – Site List

2015. 8.

연세프로테옴연구원 정슬기

Sequence homology search	
http://blast.ncbi.nlm.nih.gov/Blast.cgi	서열 유사도 분석
http://www.ebi.ac.uk/Tools/msa/clustalo/	Multiple sequence alignment tool
http://www.ebi.ac.uk/interpro/	Amino acid sequence를 이용 domain/motif 검색
Sequence 분석	
http://web.expasy.org/compute_pi/	sequence의 pI와 mw를 계산해 주는 툴
http://web.expasy.org/peptide_mass/	tryptic peptide의 mw를 계산
http://web.expasy.org/peptide_cutter/	Peptide cleavage site 예측
PTM 예측	
http://www.cbs.dtu.dk/services/NetAcet/	N-acetyltransferase A 의 substrate site 예측
http://www.cbs.dtu.dk/services/NetCGlyc/	C-mannosylation site 예측
http://www.cbs.dtu.dk/services/NetNGlyc/	N-Glycosylation site 예측
http://www.cbs.dtu.dk/services/NetPhos/	Phosphorylation site 예측
Protein localization/topology 예측	
http://www.psort.org/	Subcellular localization 예측
http://www.cbs.dtu.dk/services/SignalP/	Signal peptide cleavage site 예측
http://www.cbs.dtu.dk/services/TMHMM-2.0/	Trans-membrane domain 예측
Functional annotation tool	
https://david.ncifcrf.gov/	Functional annotation tool
Useful sites	
http://www.expasy.org/proteomics	Proteomics 관련 tool list
http://www.oxfordjournals.org/our_journals/nar/webserver/c/	Nucleic Acid Research의 web-server issue에 나온 tool list

Bioinformatics 사용법 (2) – Fasta format

2015. 8.

연세프로테옴연구원 정슬기

FASTA format is a text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes. A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (">") symbol in the first column. It is recommended that all lines of text be shorter than 80 characters in length.

1. 많은 bioinformatics tool들이 fasta format의 서열을 input값으로 받음.
2. 많은 경우 "It is recommended that all lines of text be shorter than 80 characters in length." 이 제한은 지킬 필요 없음

Example

```
> title1

MANEVIKCKAAVAWEAGKPLSIEEIEVAPPKAHEVRIKIIATAVCHTDAYTLSGADPEGCFPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCG
ECKFCLNPKTNLCQKIRVTQGKGLMPDGTSRFTCKGKTILHYMGTSTFSEYTVVADISVAKIDPLAPLDKVCLLGCGISTGYGAAVNTAKLEPGSV
CAVFGLGGVGLAVIMGCKVAGASRIIGVDINKDKFARAKEFGATECINPQDFSKPIQEVLIEMTDGGVDYSFECIGNVKVMRAALEACHKGWG
VSVVVGVAASGEEIATRPFQLVTGRTWKGTAFGGWKSVESVPKLVSEYMSKKIKVDEFVTHNLSFDEINKAFELMHSGKSIRTVVKI

>title2

MFAEIQIQDKDRMGTAGKVIKCKAAVLWEQKQPFSIEEIEVAPPKTKEVRIKILATGICRTDDHVIKGTMVSKFPVIVGHEATGIVESIGEGVTTVKP
```

Additional information

https://en.wikipedia.org/wiki/FASTA_format

http://zhanglab.ccmb.med.umich.edu/FASTA/

Bioinformatics 사용법 (3) – BLAST

2015. 8.

연세프로테옴연구원 정슬기

BLAST for Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences

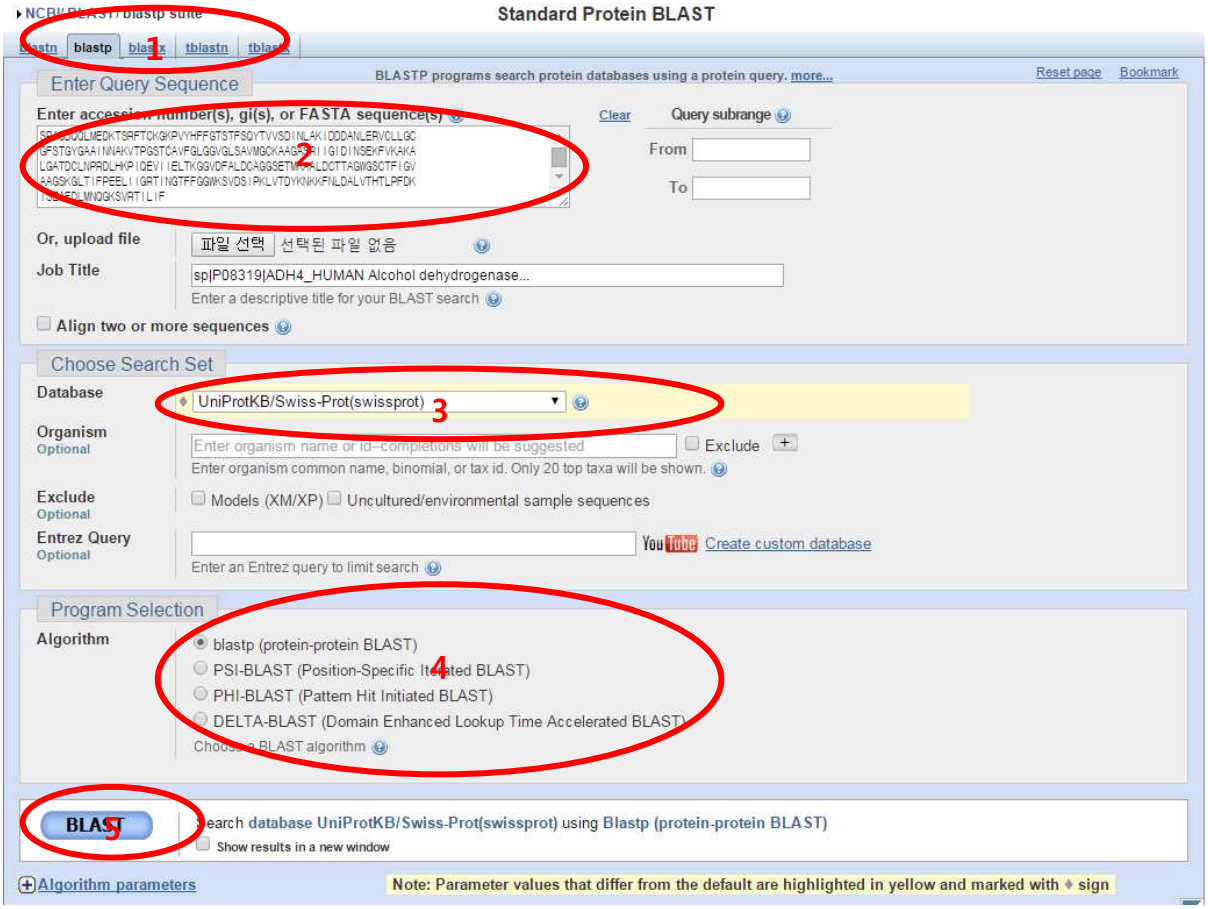
URL: http://blast.ncbi.nlm.nih.gov/Blast.cgi

Basic BLAST types:

1. blastn (nucleotide blast): Search a nucleotide database using a nucleotide query
2. blastp (protein blast): Search protein database using a protein query
3. blastx: Search protein database using a translated nucleotide query
4. tblastn: Search translated nucleotide database using a protein query
5. tblastx: Search translated nucleotide database using a translated nucleotide query

Example of Standard Protein BLAST

1. blast type 선택
2. query sequence를 입력: fasta format의 서열을 입력
3. database 선택
4. algorithm 선택
5. 실행



References

1. http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastDocs

2. Altschul, SF., Madden, TL., Schäffer, AA., Zhang, J., Zhang, Z. et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs., *Nucleic Acids Res.* **1997**, 25, 3389-402

Bioinformatics 사용법 (4) – ClustalO

2015. 8.

연세프로테옴연구원 정슬기

Clustal Omega is a multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences.

URL: http://www.ebi.ac.uk/Tools/msa/clustalo/

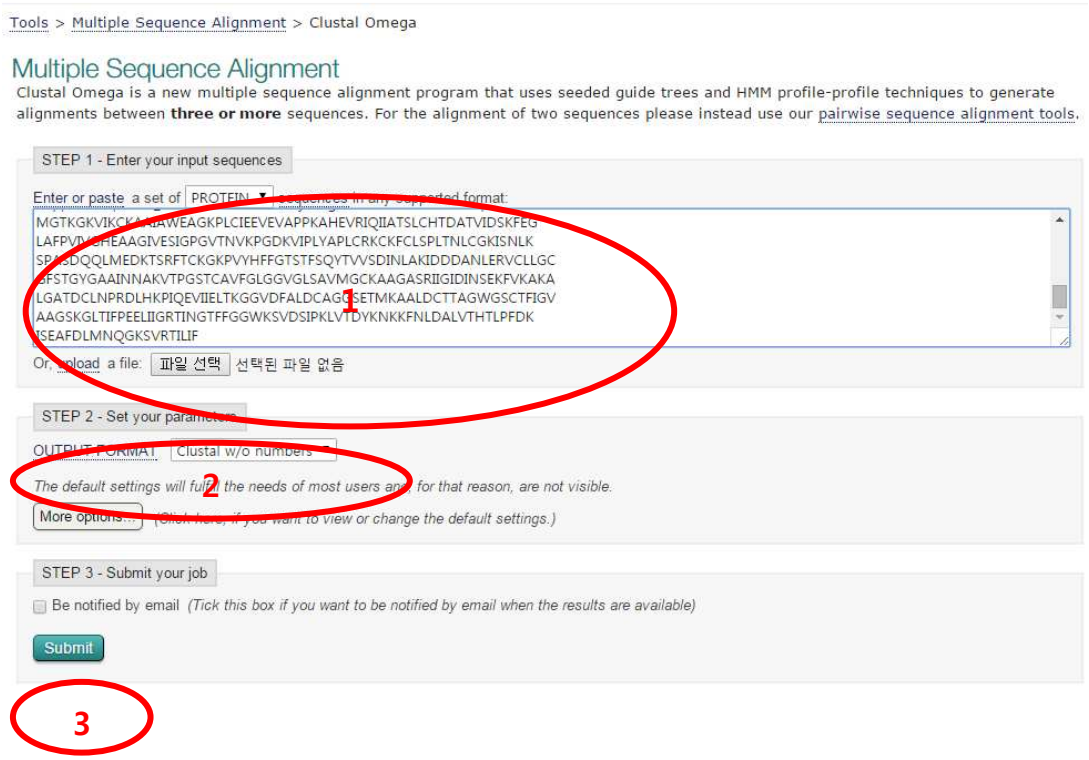
Example of ADH family alignment

1. 3개 이상의 sequence를 fasta format으로 입력 (e.g)

```
> ADHX_HUMAN

MANEVIKCKAAVAWEAGKPLSIEEIEVAPPKAHEVRIKIIATAVCHTDAYTLSGADPEGCFPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCG
ECKFCLNPKTNLCQKIRVTQGKGLMPDGTSRFTCKGKTILHYMGTSTFSEYTVVADISVAKIDPLAPLDKVCLLGCGISTGYGAAVNTAKLEPGSV
CAVFGLGGVGLAVIMGCKVAGASRIIGVDINKDKFARAKEFGATECINPQDFSKPIQEVLIEMTDGGVDYSFECIGNVKVMRAALEACHKGWG
VSVVVGVAASGEEIATRPFQLVTGRTWKGTAFGGWKSVESVPKLVSEYMSKKIKVDEFVTHNLSFDEINKAFELMHSGKSIRTVVKI

> ADH7_HUMAN

MFAEIQIQDKDRMGTAGKVIKCKAAVLWEQKQPFSIEEIEVAPPKTKEVRIKILATGICRTDDHVIKGTMVSKFPVIVGHEATGIVESIGEGVTTVKP
GDKVIPLFLPQCRECNACRNPDGNLCIRSDITGRGVLADGTTRFTCKGKPVHHFMNTSTFTEYTVVDESSVAKIDDAAPPEKVCLIGCGFSTGY
GAAVKTGKVKPGSTCVVFGLGGVGLSVIMGCKSAGASRIIGIDLNKDKFEKAMAVGATECISPKDSTKPISEVLSEMTGNNVGYTFEVIGHLETM
IDALASCHMNYGTSVVVGVPPSAKMLTYDPMLLFTGRTWKGCVFGGLKSRDDVPKLVTEFLAKKFDLDQLITHVLPFKKISEGFELLNSGQSIRT
VLTF

> ADH4_HUMAN

MGTKGKVIKCKAAIAWEAGKPLCIEEVEVAPPKAHEVRIQIIATSLCHTDATVIDSKFEGLAFPVIVGHEAAGIVESIGPGVTNVKPGDKVIPLYAPL
CRKCKFCLSPLTNLCGKISNLKSPASDQQLMEDKTSRFTCKGKPVYHFFGTSTFSQYTVVSDINLAKIDDDANLERVCLLGCGFSTGYGAAINNA
KVTPGSTCAVFGLGGVGLSAVMGCKAAGASRIIGIDINSEKFVKAKALGATDCLNPRDLHKPIQEVIIELTKGGVDFALDCAGGSETMKAALDCT
TAGWGSCTFIGVAAGSKGLTIFPEELIIGRTINGTFFGGWKSVDSIPKLVTDYKNKKFNLDALVTHTLPFDKISEAFDLMNQGKSVRTILIF
```

2. output format 선택 (optional)

3. 실행


Tools > Multiple Sequence Alignment > Clustal Omega
Multiple Sequence Alignment
Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences. For the alignment of two sequences please instead use our pairwise sequence alignment tools.
STEP 1 - Enter your input sequences
Enter or paste a set of PROTEIN sequences in any supported format:
MGTKGKVIKCKAAVLWEQKKPFSIEEIEVAPPKTKEVRIKILATGICR
LAFPVVSGHEAAGIVESIGPGVTNVKPGDKVIPLYAPLCRKCKFCLSPLTNLCGKISNLK
SPKSDQQLMEDKTSRFTCKGKPVYHFFGTSTFSQYTVVSDINLAKIDDDANLERVCLLGC
GFSTGYGAAINNAKVTPGSTCAVFGLGGVGLSAVMGCKAAGASRIIGIDINSEKFVKAKA
LGATDCLNPRDLHKPIQEVIIELTKGGVDFALDCAGSETMKAALDCTTAGWGSCTFIGV
AAGSKGLTIFPEELIIGRTINGTFFGGWKSVDSIPKLVTDYKNKKFNLDALVTHTLPFDK
ISEAFDLMNQGKSVRTILIF
1
Or upload a file: 파일 선택 선택된 파일 없음
STEP 2 - Set your parameters
OUTPUT FORMAT Clustal w/o numbers
The default settings will fulfil the needs of most users and for that reason, are not visible.
More options... (Click here, if you want to view or change the default settings.)
2
STEP 3 - Submit your job
Be notified by email (Tick this box if you want to be notified by email when the results are available)
Submit
3

결과


Tools > Multiple Sequence Alignment > Clustal Omega
Results for job clustalo-I20150729-094100-0598-68213748-oy
Alignments
Result Summary
Phylogenetic Tree
Submission Details
Download Alignment File
Hide Colors
Send to ClustalW2_Phylogeny
CLUSTAL O(1.2.1) multiple sequence alignment
sp|P40394|ADH7_HUMAN
sp|P11766|ADHX_HUMAN
sp|P08319|ADH4_HUMAN

References

1. http://www.ebi.ac.uk/Tools/msa/clustalo/help/

2. Sievers, F., Wilm, A., Dineen, D., Gibson, TJ., Karplus, K. et al., Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol.* **2011**, 7, 539

Bioinformatics 사용법 (5) – InterProScan

2015. 8.

연세프로테옴연구원 정슬기

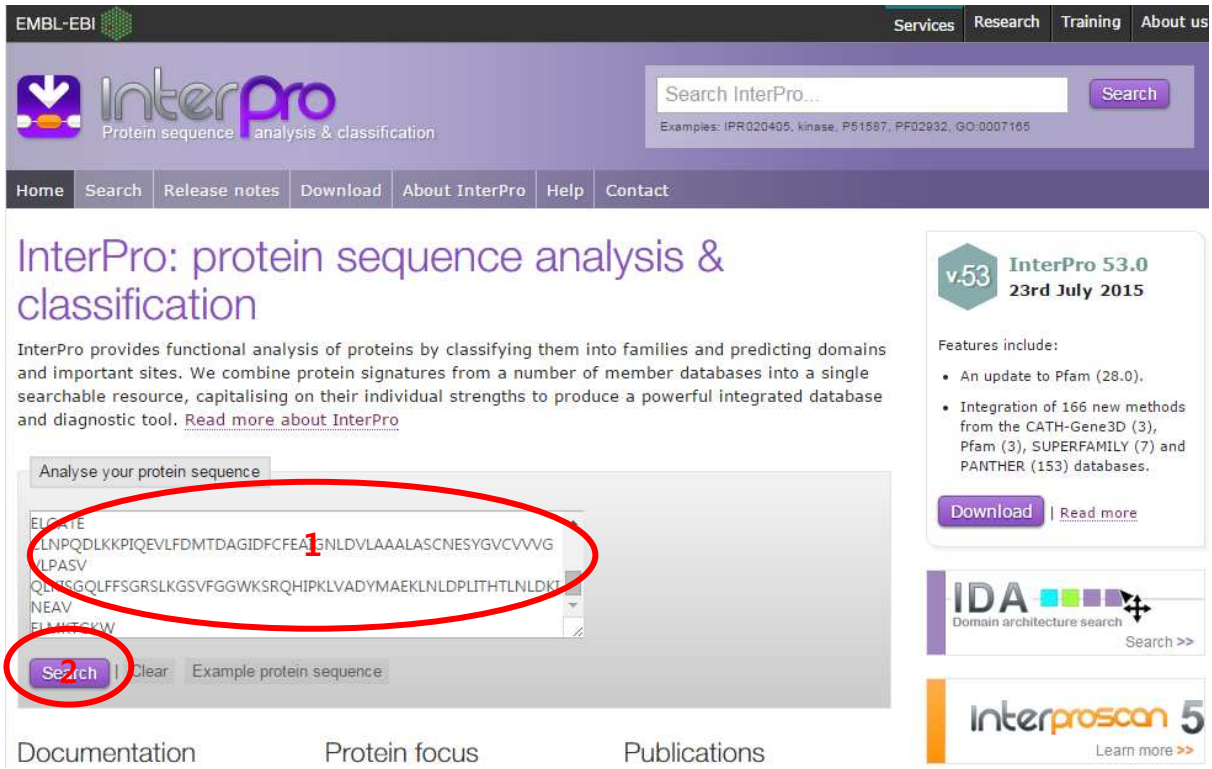
InterPro provides functional analysis of proteins by classifying them into families and predicting domains and important sites.

URL: http://www.ebi.ac.uk/interpro/

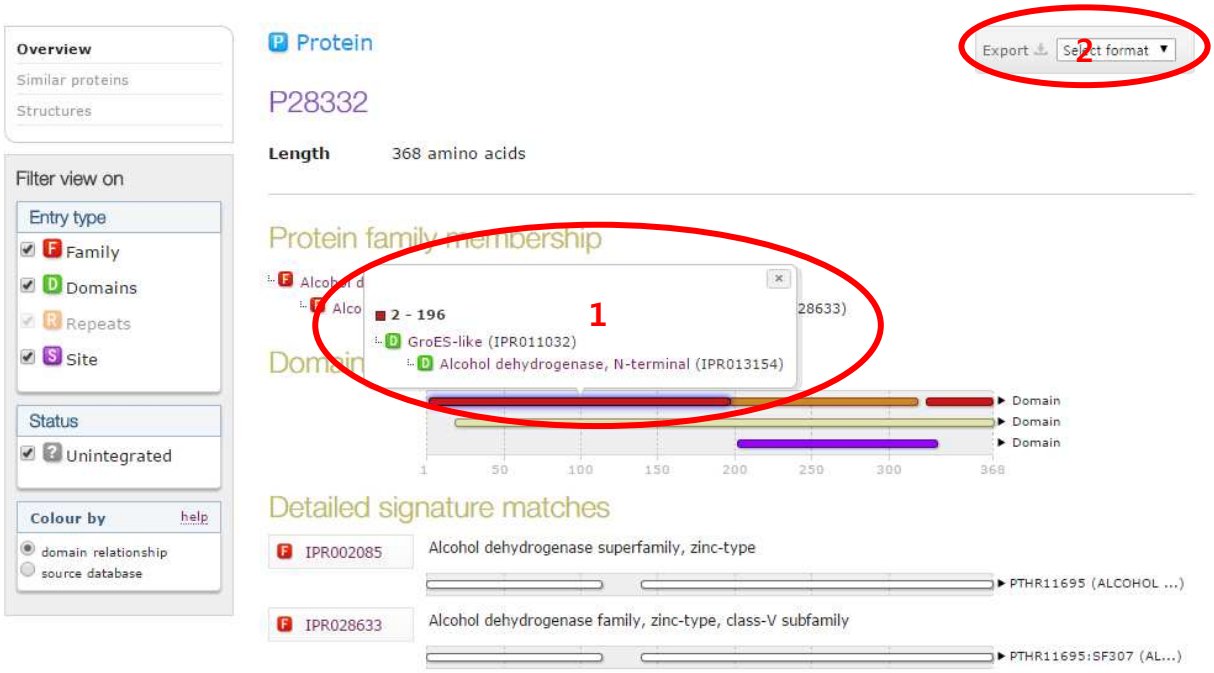
Example of ADH6 domain prediction

1. sequence를 fasta format으로 입력

2. 실행



결과



1. domain search 결과 위에 마우스 포인터를 올려두면 간략한 설명이 나옴 (클릭하면 InterPro database로 이동)

2. export 명령을 이용 원하는 format의 파일로 저장 가능 (tsv, xml, etc...)

References

1. http://www.ebi.ac.uk/interpro/training.html

2. Jones, P., Binns, D., Chang, HY., Fraser, M., Li, W. et al., InterProScan 5: genome-scale protein function classification. *Bioinformatics*. **2014**, 30, 1236-40

Bioinformatics 사용법 (6) – DAVID functional annotation tool

2015. 8.

연세프로테옴연구원 정슬기

The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (**DAVID**) provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes. For any given gene list, DAVID tools are able to: (1) Identify enriched biological themes, particularly GO terms; (2) Discover enriched functional-related gene groups; (3) Cluster redundant annotation terms; (4) Visualize genes on BioCarta & KEGG pathway maps; (5) Display related many-genes-to-many-terms on 2-D view; (6) Search for other functionally related genes not in the list; (7) List interacting proteins; (8) Explore gene names in batch; (9) Link gene-disease associations; (10) Highlight protein functional domains and motifs; (11) Redirect to related literatures; (12) Convert gene identifiers from one type to another; (13) And more

URL: https://david.ncifcrf.gov/

사용법

1. Click "start analysis" or "Functional Annotation"

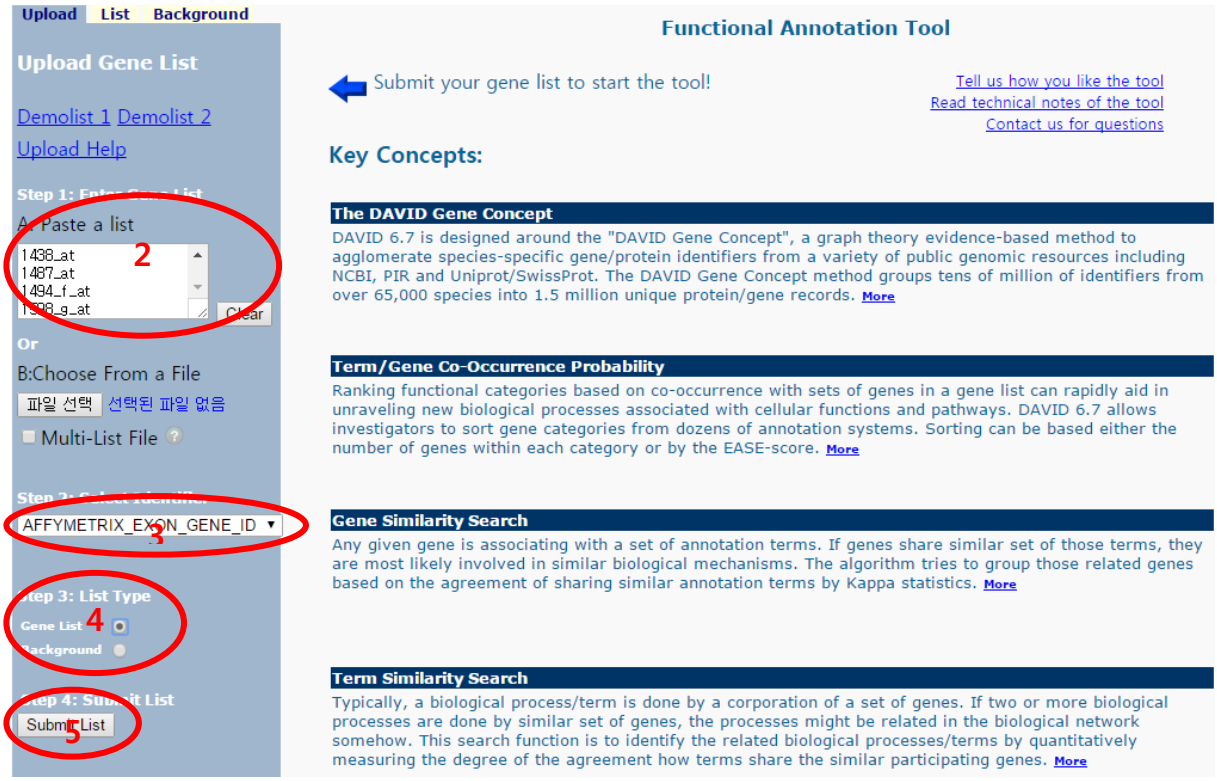
2. Submit gene/protein list

3. Select identifier

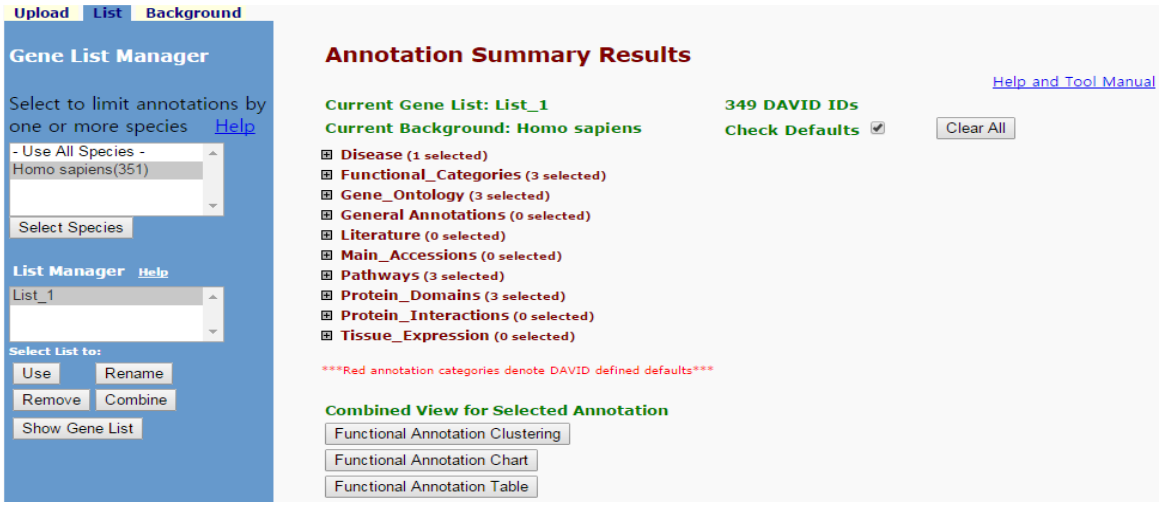
4. Select list type (Gene list)

5. 실행


DAVID Bioinformatics Resources 6.7
National Institute of Allergy and Infectious Diseases (NIAID), NIH
Home
Start Analysis
Shortcut to DAVID Tools
Technical Center
Downloads & APIs
Term of Service
Why DAVID?
About Us
1
Shortcut to DAVID Tools
Functional Annotation
Gene Functional Classification
Gene ID Conversion
Gene Name Batch Viewer
Recommending: A paper published in Nature Protocols describes step-by-step procedure to use DAVID!
Welcome to DAVID 6.7
2003 - 2015
Search
What's Important in DAVID?
Current (v 6.7) release note
New requirement to cite DAVID
IDs of Affy Exon and Gene arrays supported
Novel Classification Algorithms
Pre-built Affymetrix and Illumina backgrounds
User's customized gene background
Enhanced calculating speed
The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 is an update to the sixth version of our original web-accessible programs. DAVID now provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes. For any given gene list, DAVID tools are able to:
Identify enriched biological themes,
Statistics of DAVID

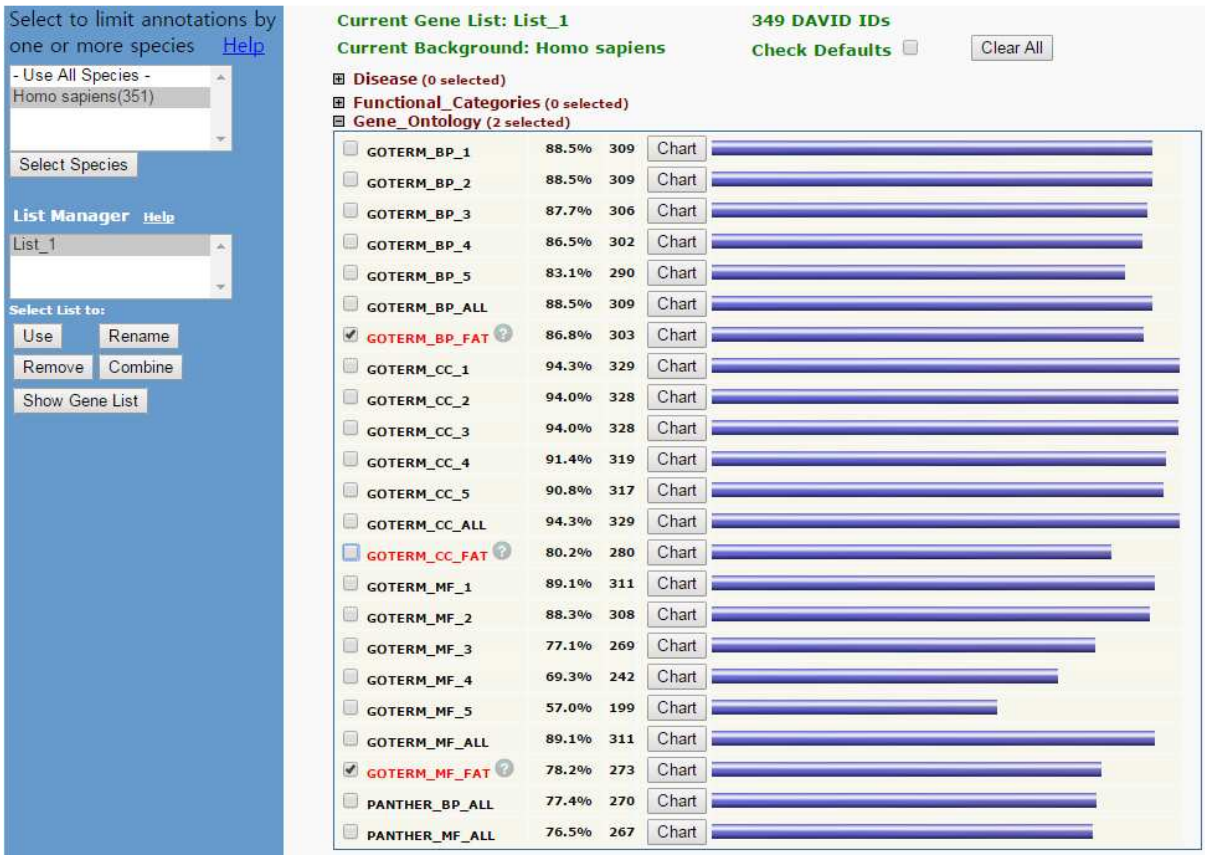

Upload
List
Background
Upload Gene List
Demolist 1
Demolist 2
Upload Help
A: Paste a list
1438_at
1487_at
1494_f_at
Clear
2
Or
B:Choose From a File
파일 선택
선택된 파일 없음
Multi-List File
AFFYMETRIX_EXON_GENE_ID
3
Step 3: List Type
Gene List
Background
4
Step 4: Submit List
Submit List
5
Functional Annotation Tool
Submit your gene list to start the tool!
Tell us how you like the tool
Read technical notes of the tool
Contact us for questions
Key Concepts:
The DAVID Gene Concept
DAVID 6.7 is designed around the "DAVID Gene Concept", a graph theory evidence-based method to agglomerate species-specific gene/protein identifiers from a variety of public genomic resources including NCBI, PIR and Uniprot/SwissProt. The DAVID Gene Concept method groups tens of million of identifiers from over 65,000 species into 1.5 million unique protein/gene records. More
Term/Gene Co-Occurrence Probability
Ranking functional categories based on co-occurrence with sets of genes in a gene list can rapidly aid in unraveling new biological processes associated with cellular functions and pathways. DAVID 6.7 allows investigators to sort gene categories from dozens of annotation systems. Sorting can be based either the number of genes within each category or by the EASE-score. More
Gene Similarity Search
Any given gene is associating with a set of annotation terms. If genes share similar set of those terms, they are most likely involved in similar biological mechanisms. The algorithm tries to group those related genes based on the agreement of sharing similar annotation terms by Kappa statistics. More
Term Similarity Search
Typically, a biological process/term is done by a corporation of a set of genes. If two or more biological processes are done by similar set of genes, the processes might be related in the biological network somehow. This search function is to identify the related biological processes/terms by quantitatively measuring the degree of the agreement how terms share the similar participating genes. More

결과



1. 확인하고 싶은 annotation 만 선택하여 분석 가능

2. e.g., gene ontology 의 Biological process 와 Molecular function 만 선택한 경우



3. annotation cluster 결과

88 Cluster(s) Download File

Annotation Cluster 1	Enrichment Score: 6.07				Count	P_Value	Benjamini
GOTERM_BP_FAT	RNA processing	RT			34	2.0E-7	3.9E-4
GOTERM_BP_FAT	mRNA processing	RT			25	2.3E-7	2.2E-4
GOTERM_BP_FAT	mRNA metabolic process	RT			27	2.4E-7	1.6E-4
GOTERM_BP_FAT	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	RT			16	1.7E-6	8.0E-4
GOTERM_BP_FAT	RNA splicing, via transesterification reactions	RT			16	1.7E-6	8.0E-4
GOTERM_BP_FAT	nuclear mRNA splicing, via spliceosome	RT			16	1.7E-6	8.0E-4
GOTERM_BP_FAT	RNA splicing	RT			21	6.0E-6	1.4E-3
Annotation Cluster 2	Enrichment Score: 4.46				Count	P_Value	Benjamini
GOTERM_BP_FAT	DNA metabolic process	RT			29	9.3E-6	2.0E-3
GOTERM_BP_FAT	cellular response to stress	RT			31	1.0E-5	2.0E-3
GOTERM_BP_FAT	DNA replication	RT			16	2.4E-5	4.1E-3
GOTERM_BP_FAT	response to DNA damage stimulus	RT			22	9.9E-5	1.6E-2
GOTERM_BP_FAT	DNA repair	RT			18	2.2E-4	2.8E-2
Annotation Cluster 3	Enrichment Score: 3.18				Count	P_Value	Benjamini
GOTERM_BP_FAT	in utero embryonic development	RT			14	1.6E-4	2.4E-2
GOTERM_BP_FAT	chordate embryonic development	RT			18	1.3E-3	8.9E-2
GOTERM_BP_FAT	embryonic development ending in birth or egg hatching	RT			18	1.4E-3	9.4E-2

4. Annotation chart 결과

216 chart records Download File

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_BP_FAT	RNA processing	RT		34	9.7	2.0E-7	3.9E-4
	GOTERM_BP_FAT	mRNA processing	RT		25	7.2	2.3E-7	2.2E-4
	GOTERM_BP_FAT	mRNA metabolic process	RT		27	7.7	2.4E-7	1.6E-4
	GOTERM_MF_FAT	RNA binding	RT		37	10.6	9.7E-7	5.3E-4
	GOTERM_BP_FAT	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	RT		16	4.6	1.7E-6	8.0E-4
	GOTERM_BP_FAT	nuclear mRNA splicing, via spliceosome	RT		16	4.6	1.7E-6	8.0E-4
	GOTERM_BP_FAT	RNA splicing, via transesterification reactions	RT		16	4.6	1.7E-6	8.0E-4
	GOTERM_BP_FAT	protein localization	RT		43	12.3	2.5E-6	9.5E-4
	GOTERM_BP_FAT	protein transport	RT		39	11.2	2.7E-6	8.6E-4
	GOTERM_BP_FAT	establishment of protein localization	RT		39	11.2	3.4E-6	9.2E-4
	GOTERM_BP_FAT	RNA splicing	RT		21	6.0	6.0E-6	1.4E-3
	GOTERM_BP_FAT	DNA metabolic process	RT		29	8.3	9.3E-6	2.0E-3
	GOTERM_BP_FAT	cellular response to stress	RT		31	8.9	1.0E-5	2.0E-3
	GOTERM_BP_FAT	DNA replication	RT		16	4.6	2.4E-5	4.1E-3
	GOTERM_MF_FAT	transcription factor binding	RT		26	7.4	7.7E-5	2.1E-2
	GOTERM_BP_FAT	response to DNA damage stimulus	RT		22	6.3	9.9E-5	1.6E-2
	GOTERM_BP_FAT	in utero embryonic development	RT		14	4.0	1.6E-4	2.4E-2
	GOTERM_MF_FAT	helicase activity	RT		12	3.4	1.7E-4	3.1E-2
	GOTERM_BP_FAT	negative regulation of macromolecule metabolic process	RT		33	9.5	2.2E-4	3.0E-2
	GOTERM_BP_FAT	DNA repair	RT		18	5.2	2.2E-4	2.8E-2
	GOTERM_MF_FAT	nucleotide binding	RT		71	20.3	2.6E-4	3.5E-2

5. Download file 기능을 이용하여 tap separated text file 로 down load 가능 (download file 을 click 하면 txt file 이 web-browser 에 바로 열림으로 마우스 오른쪽 버튼 click 하여 "다른이름으로 링크저장"을 사용하는 것이 편함)

References

1. https://david.ncifcrf.gov/helps/functional_annotation.html#summary

2. Huang, da W., Sherman, BT., Lempicki, RA., Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* **2009**, 4, 44-57

3. Dennis, G., Sherman, BT., Hosack, DA., Yang, J., Gao, W. et al., DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* **2003**, 4, P3