

EXPression ANalyzer and DisplayER

Tom Hait Aviv Steiner Igor Ulitsky Chaim Linhart Amos Tanay Seagull Shavit Rani Elkon Adi Maron-Katz Dorit Sagir Eyal David Roded Sharan Israel Steinfeld Yossi Shiloh Ron Shamir

Ron Shamir's Computational Genomics Group

Rani Elkon's Group

Schedule

- Data, preprocessing, grouping (14:15-14:30)
- Hands-on part I (14:30-14:40)
- Grouping analysis (14:40-14:55)
- Hands-on part II (14:55-15:10)
- Enrichment analysis (15:10-15:20)
- Hands-on part III (15:20-15:30)
- ChIP-seq and GSEA (15:30-15:45)
- Hands-on part IV (15:45-16:00)

EXPANDER – an integrative package for analysis of gene expression and NGS data

• Built-in support for 18 organisms:

human, mouse, rat, chicken, fly, zebrafish, C.elegans, yeast (s. cereviciae and s. pombe), arabidopsis, tomato, listeria, leishmania, E. coli (two strains), aspargillus, rice. And v.vinifera (grape)

- Demonstration on human CAL51 cell line experiments*:
 - RNA-Seq data, which contains expression profiles measured in several time points after IR-induction.
 - P53 ChIP-Seq data after 2 hours of IR-induction.

*Data from Rashi-Elkeles, Warnatz and Elkon et al, 2014, Science Signaling, DOI: 10.1126/scisignal.2005032

EXPANDER status

- 829 citations since 2003
- 63 citations since 2017
- 18,062 downloads since 2003
- 914 since 2017

What can it do?

Low level analysis

- Data adjustments (missing values, merging, divide by base, log)
- Normalization
- Probes & condition filtering
- High level analysis
 - Group detection (supervised clustering, differential expression, clustering, bi-clustering, network based grouping).
 - Ascribing biological meaning to patterns via enrichment analysis

Links to public annotation databases



EXPANDER – Data

Expression matrix (probe-row; condition-column)

- One-channel data (e.g., Affymetrix)
- Dual-channel data, in which data is log R/G (e.g. cDNA microarrays)
- '.cel' files
- RNA-Seq counts OR absolute/relative intensities data

ChIP-Seq data: in BED or GFF3 formats

- □ <u>ID conversion file</u>: maps probes to genes
- Gene groups data: defines gene groups
- Gene ranks data: defines gene ranking for GSEA
- □ Network information (e.g. PPI network) .sif format

First steps with the data – load, define, preprocess

- Load dialog box , "Data menu", "Preprocessing menu"
- Data definitions
 - Defining condition subsets
 - Data type & scale (log)
 - Define genes of interest

Data Adjustments

- Missing value estimation (KNN or arbitrary)
- Flooring
- Condition reordering
- Merging conditions
- Merging probes by gene IDs
- Assigning genes to ChIP-Seq peaks
- Divide by base
- Log data (base 2)

Data preprocessing

- Normalization = removal of systematic biases
 - Quantile = equalizes distributions
 - Lowess (locally weighted scatter plot smoothing) = a non linear regression to a base array
- Visualizations to inspect normalization:
 - box plots
 - Scatter plots (simple and M vs. A)

 $M = \log_2(A1/A2)$ A = 0.5*log₂(A1*A2)

Data preprocessing

Probe filtering

Focus downstream analysis on the set of "responding genes"

- Fold-Change
- Variation
- Statistical tests: T-test, SAM (Significance Analysis of Microarrays), edgeR and deseq2 (RNAseq count data)
- It is possible to define "VIP genes".
- □ <u>Standardization</u> : Mean=0, STD=1 (visualization)
- Condition filtering
- Order of operations

Hands-on (1-2)





Links to public annotation databases

Visualization utilities

Supervised Grouping



Differential expression:

- a) Under normality assumption: t-test, SAM b) No normality assumption (RNA Seq data): Wilcoxon rank sum test , Negative binomial (edgeR/DESeq2)
- Similarity group (correlation to a selected probe/gene)
- □ Rule based grouping (define a pattern)

Unsupervised grouping - cluster Analysis

Partition into distinct groups, each with a particular expression pattern

- co-expression \rightarrow co-function
- co-expression \rightarrow co-regulation

Partition the genes attempts to maximize:

- Homogeneity within clusters
- Separation between clusters

Cluster Analysis within Expander

- Implemented algorithms:
 - CLICK, K-means, SOM, Hierarchical
- Visualization:
 - Mean expression patterns
 - <u>Heat-maps</u>
 - <u>Chromosomal positions</u>
 - Network sub-graph (Cytoscape integration)
 - PCA
 - Clustered heat map

Biclustering

Clustering seeks global partition according to similarity across <u>ALL</u> conditions >> becomes too restrictive on large datasets.

- Relevant knowledge can be revealed by identifying genes with common pattern across a <u>subset</u> of the conditions
- Novel algorithmic approach is needed: *Biclustering*



Biclustering II

* Bicluster = subset of genes with similar behavior under a subset of conditions

Computationally challenging: has to consider many combinations

Biclustering methods in EXPANDER:

ISA (Iterative Signature Algorithm) - Ihmels et.al Nat Genet 2002

SAMBA = Statistical Algorithmic Method for Bicluster Analysis (A. Tanay, R. Sharan, R. Shamir *RECOMB 02*)

Drawbacks/limitations:

- Useful only for over 20 conditions
- Parameters
- How to asses the quality of Bi-clusters

Biclustering Visualization

				Probe ID YHL028W YOR273C Bicluster 11	Gene Symbol WSC4 TPO4	Gene ID
			YHLD228	00000000000000000000000000000000000000	WSC4 Expression	·
Bic num	Bic Score	# Conditions	YOR313	žč	SPS4 3.2	
1	230.475	7	37 YLR034 YGR143		SMF3 SKN1 1.6	
2	495.917	7	76YEL065	Ŵ	Siti no	
3	433.011	6	76 YIL1190 YIR142	Ŵ	PUT1 1 6	
4	248.93	7	38YÖR153	šivi 🛛 🗖	PDR5	
5	461.604	7	68 YPL058 YML120		NDI1 -3.2	↓
6	177.526	7	22 YMR145	jč	NDE1	
7	116.431	5	34 YPR16/		MEI16 MEP3	
8	374.292	9	27 YOL119	č –	MCH4	
9	331.779	10	46 YRL183 YPL250		ICY2	
10	320.373	6	55 YMR189	ΞΨ.	GCV2	
11	417.158	7	75 YKR039 YMR058		GAP1 FET3	
12	286.944	6	55 YOR317	Ŵ	FAA1	
13	144.321	12	13 YPL205 YNL111		CYB5	
14	201.665	10	23 ÝMĽ116	Ŵ	ÁTRÍ	
15	435.368	7	68 YDR380	W III	ARO9 ARO10	
16	680.887	8	81 YCL025	Ç,	AGP1	
17	148.6	10	12 YDR029		N/A	
18	200.601	14	19 YCLX09	Ŵ	N/A	
19	206.593	6	44 YOL150	č	N/A	
20	372.519	10	53 YOR315	ŚŴ	N/A	
21	575.098	5	107 107 107 107	Ŵ	N/A	
22	387.311	6	71 YML033	W NAC	N/A N/A	
23	213.658	4	56 YOL114	Č	N/A	
24	196.952	14	15 YDL180	W SAC	N/A N/A	
25	458.704	12	45 YHR029	ič 🛛	N/A	ě
26	522.565	12	42 YDL089	W	N/A	d5.
27	179.189	7	27			Y

Hands-on (3-4)





Visualization utilities

Functional enrichment analysis - Ascribing functional meaning to gene groups

- Gene Ontology (GO) annotations for all supported organisms
- <u>TANGO</u>: Apply statistical tests that seek overrepresented GO functional categories in the groups

Functional Enrichment - Visualization

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🗧 xpander5 - Default Session

File Data Preprocessing Grouping Group Analysis Visualizations Options Help

Diagrams Enrichment Table multicellular organismal development - GO:00 Set Enriched with #genes Raw p-value Corrected p-Val... Frequency in s. positive regulation of cellular process - GO:00 3.509E-12 0.0010 Cluster 1 immune response - GO:0006955 13 9.02 immune response - GO:0006955 response to external stimulus - GO:0009605 12 1.798E-10 0.0010 8.33 protein dimerization activity - GO:0046983 Cluster_1 0.0010 7.63 cellular developmental process - GO:0048869 Cluster_1 defense response - GO:0006952 11 1.825E-9 structural constituent of cytoskeleton - GO:000 Cluster 1 taxis - GO:0042330 6 1.67E-7 0.0020 4.16 defense response - GO:0006952 chemokine activity - GO:0008009 1.471E-6 0.0050 2.77 Cluster 1 4 nuclear part - GO:0044428 nuclear part - GO:0044428 15 7.072E-7 0.0040 12.19 Cluster 3 regulation of developmental process - GO:005 regulation of cellular process - GO:0050794 28 1.083E-6 0.0040 22.76 Cluster_3 nucleus - GO:0005634 Cluster 3 nucleus - GO:0005634 24 3.038E-6 0.0080 19.51 response to external stimulus - GO:0009605 regulation of cellular process - GO:0050794 40 1.472E-11 0.0010 34.18 Cluster 4 negative regulation of biological process - GO Cluster 4 regulation of progression through cell cycle - GO:0000074 13 1.879E-9 0.0010 11.11 chemokine activity - GO:0008009 multicellular organismal development - GO:0007275 7.478E-9 0.0010 Cluster_4 28 23.93 regulation of progression through cell cycle - C transcription factor activity - GO:0003700 Cluster 4 18 8.693E-9 0.0010 15.38 response to stress - GO:0006950 anatomical structure development - GO:0048856 26 1.032E-8 0.0010 22.22 Cluster 4 regulation of cellular process - GO:0050794 Cluster_4 apoptosis - GO:0006915 15 1.831E-8 0.0010 12.82 anatomical structure development - GO:00488 positive regulation of cellular process - GO:0048522 Cluster 4 16 2.187E-8 0.0010 13.67 taxis - GO:0042330 cytoplasmic part - GO:0044444 Cluster 4 cellular developmental process - GO:0048869 26 3.548E-8 0.0020 22.22 Cluster 4 negative regulation of biological process - GO:0048519 16 4.0E-7 0.0020 13.67 actin cytoskeleton - GO:0015629 myeloid cell differentiation - GO:0030099 Cluster_4 regulation of developmental process - GO:0050793 9 7.271E-7 0.0040 7.69 contractile fiber part - GO:0044449 14 8.002E-7 0.0040 11.96 Cluster 4 response to stress - GO:0006950 muscle contraction - GO:0006936 Cluster 4 nucleus - GO:0005634 28 8.377E-7 0.0040 23.93 cvtoskeletal part - GO:0044430 Cluster 4 protein dimerization activity - GO:0046983 8 1.309E-6 0.0040 6.83 transcription factor activity - GO:0003700 Cluster 4 myeloid cell differentiation - GO:0030099 6 1.804E-6 0.0080 5.12 apoptosis - GO:0006915 Cluster_5 muscle contraction - GO:0006936 11 6.271E-20 0.0010 26.19 Cluster 5 actin cytoskeleton - GO:0015629 10 1.116E-13 0.0010 23.8 Cluster_5 contractile fiber part - GO:0044449 7 6.862E-12 0.0010 16.66 Cluster 5 cytoskeletal part - GO:0044430 10 1.587E-9 0.0010 23.8 structural constituent of cytoskeleton - GO:0005200 6 2.653E-9 0.0010 14.28 Cluster_5 2.612E-8 0.0020 40.47 Cluster 5 cytoplasmic part - GO:0044444 17 Analysis Info: Analyzed Gene Groups: CLICK 1.1 Background Set Selection: all genes Can be saves as a Threshold p-Value: 0.01 Max Size of Class to consider: 3000 tabular .txt file Annotation sub-types: Process, Function Number of iterations: 1000 Number of enriched sets: 4

Data Sheet 1 CLICK 1.1 CLICK 1.1 GO Enrich.1 CLICK 1.1 GO Enrich.2

Currently working on: Data Sheet 1

Pathway analysis

- Searches for biological pathways that are overrepresented in gene groups
- KEGG: Kyoto Encyclopedia of Genes and Genomes (mainly metabolic),all 18 orgs
- WikiPathways various biological pathways(~20 species, 1765 pathways) – open resource
- Statistical hyper-geometric (HG) cumulative distribution score + multiple testing correction

C 🕺 🗅 www.genome.jp/kegg-bin/show_pathway?hsa04060+6347+6387+3625+3624+2919+3575+51330+3589+3554+3569

Cytokine-cytokine receptor interaction - Homo sapiens (human)

[Pathway menu | Organism menu | Pathway entry | Download KGML | Show description | User data mapping] Homo sapiens (human) ▼ Go 100% ▼





Visualization utilities

Inferring regulatory mechanisms from gene expression data

Assumption:

co-expression → transcriptional *co*-regulation → *common cis-regulatory promoter elements*

- Computational identification of *cis*-regulatory elements over-represention
- PRIMA PRomoter Integration in Microarray Analysis (Elkon, et. Al, Genome Research, 2003)
- **AMADEUS** novel motif enrichment analysis

PRIMA – general description

- Input:
 - Target set (e.g. co-expressed genes)
 - Background set (e.g. all genes on the chip)
- *Analysis:* Detects TFs with high target set prevalence
- TF binding site models TRANSFAC DB
- Default: From -1000 bp to 200 bp relative the TSS

Promoter Analysis - Visualization



		Select All	
	-1000 -950		
	-1000 -550		
388 (RHUB) 487 (ATE2)			
8553 (BHLHB2)			
3726 (JUNB)	GREAT TTA GRAAGA GA	M00807[EGR]	
8870 (IER3)			
1958 (EGRÍ)	CCT GCCCAGGCGGGCCCAGC <mark>CGCTCCTCCCCGCAC</mark> T CCCGGTT CGCT CT CACGGT CCCT GAGGT GGG	MO0716[ZF5]	
10221 (TRIB1)	GGCCCTGGCTCAGGAAGCTCTTTCTGCGAGTCACCGCGAAGGGGCGGCCCGGGAGCCTGGAGAAGCTA		
3164 (NR4A1)	T G G G G T G G C A T G C C A G C G A T G C A G G C G C G C G C G C G C G C G C		
7071 (KLF10) 0702 (SEPTAD2)		M01068(UE1H3BETA)	
2354 (EOSB)	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN		
9903 (KLHL21)	GGGGCT GGAAAACAGCACAAACCCAAGT GTT CT CT GCAAAGCAGCAGT CCT GGT GGT GAAAT AGGGGT	M00189[AP-2]	
3280 (HES1)	ACGCCGGCCGGCT GAT GT CAAACT GCAGCT CGGCT GGT GT AGCT CT T AAAG <mark>GGCCCGCGGGGCG</mark> CCGGG		
1969 (EPHA2)	T GCCACAGTT GCT CTT CCT GGACT GGT CAAAT GGT GATT GGCCAACAGGCAGCCGT GGGT GG	💌 🔄 MUU196[Sp1]	
2077 (ERF)	T CAGAT CT GCGCT GT GAT TT CCT GT TT CCCAGAGCCT CAGGCTT CAT CCTT CCCT CCC	M00243(Eqr.1)	
29970 (SCHIP1) 4002 (CM4077)	I TAAAAAATAAATITI GGACATGCTAAATATCI GAGAGGGGGTI TAGAAGTAGATTCTATATCCTTAG	M00245[Egi=1]	
4092 (SMAD7) 1944 (DUSP2)		M01104[MOVO-B]	
602 (BCL3)	GCAGCACCGGCCTCGGTCGCGCTGACTCTGGCCTGGTGTCCGTGTCTCTTGCTATCTCTCTTTCTCTC		
1959 (EGR2)	GGT GT GT GAAGAGGG <mark>CAGCGGGGGCGGGGAGT</mark> GT GT AGCGGAAGT GGAGCGANNNNNNNNNNNNNNNN	M00720[CAC-binding_protein]	
1960 (EGR3)	T CATT CTTT CCT CTT CACCACGAAAACCGCACAT CCT CGACCCCAT AT GGAT GG		
9021 (SOCS3)	GCCCCCTTCTCGGCCACCTTTCCAGCTCCGGAGACAGCCATTCCCGCAGATCCCTGGCGTGCCTATTC		
1746 (ULX2) dete /C ADD45D	CTATTAGCAATAATACCTTTAAGTTTATGTAGCTTCTTTGAAGCAACAAGGAAAACCCGTTTCAAT		
150094 (SNE11 K)	TARGET CRATCE AGAT TA ACT C C CAAAT GGT CT C C CGACT ACT T C C C C C C C C C C C C C C C		
3397 (ID1)	TAGCTAGACCAGTTTGTCGTCTCCATGGCGACCGCCGCGCGCG		
5292 (PIM1)	CCGGCCCTTTGACACACATCCCTTCCCAGAAATCAGGATTCGCTGGTGCTTTTGCATTTCTAAAATGG		
3400 (ID4)	CATTAATGGCCTAAATTAAGTTACAGGTATGAATTTTACATAAAACAGATTAATATTATATGTCATAA		
677 (ZFP36L1)	GGAACAAACCCTTGGTCGGCGGGGCCGGTAAACAACTCGGGAGCGAGC		
25976 (TIPARP) 4665 (NADO)	RACAAAGGCIIAGAAICAIAAIGICIAIGAIAAIACTICITAGCICCIAGCIIIACIGACIICCAI		
221749 (C6orf145)	TACACAGGAAACATAT GCGTTCATTTAGCAAGTGTATATAAAAAACATCATAGACAAAGCAAAAAAAA		
26039 (\$\$18L1)	CGCTCCCCAGCCACCCGGCTGCGTGAACAGCCCCCCAGCGAGCG		
23135 (JMJD3)	CCCT GGGGGCAGT GT CAT CAGCAGCCACAGAAGCTT GCGGAACATT GCAT CAT GGAGACT GGGGGCT A		
11007 (CCDC85B)	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN		
51339 (DACT1)	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTT GT AAACGCAGACT AAGT AAT AAT		
22520 (CLCE1)	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN		
80176 (SPSB1)	TATTCT GAGTTCAAACAAAGCTAAACCCAAACTGATTTTCAGCACCGTAAAAATGAGAGACCATCAAACTT		
56672 (C11orf17)	AGAGCT GCGCT GAGGGATTAT GAGAGACCCT ACAACTT CT CAGGCGCCCCCT GCCCCGGGGGCCAGGA		
54877 (ZCCHC2)	CNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN		
29950 (SERTAD1)			
5488D (BCOR)	CGCCCAGGACGTGCGCCCGBGTCGBGCGTGCGCAGCCAACTCGGCCCGAGCTNNNNNNNNNNNNN		
415116 (PPPTRIDB)	A NONNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN		
148479 (PHE13)	TACTTACCTTTTTCCCCCCACTTTCGAATATTAAAAATGTTCCCGTCAGACCGCCTTTTGGTCACCGCA		
51274 (KLF3)	CT GCGCAAAT GCCGCTTTTT AGCT CCAGCGGCAGGCCT GGCCCGT GGGAGTTTT GCCCCAGGGGT CT C		
114789 (SLC25A25)	ACAGGGGATAGGCCAGGCTTGCTCGAGGCCAAGCACTAGGCCTTTGGTAACCCCCCTCGCTACGCAAA		
64661 (AXUD1)			
91748 (C14orf43) 122964 (CBER2)	AALGALATIC GUT GUT CUT CUT CUT CUT CUT GAGAAAGT GAGGGALGCACCT GGTT CT CAGGT		
84848			
284023	T G G G G C C AT C A C G A T G T G T G G G T G T C C A G G C C T C C G G A A G G A A G G A T T C C C A G C A T C C T A A A G C A		



Amadeus

A Motif Algorithm for Detecting Enrichment in multiple Species

- Supports diverse motif discovery tasks:
 - 1. Finding over-represented motifs in one or more given sets of genes.
 - 2. Identifying motifs with **global spatial features** given **only** the genomic **sequences**.

• Possible Gene-sets:

- 1. Identified gene sets clusters vs. all genes promoters.
- 2. ChIP-Seq peaks sequences using Expander built-in FASTA sequences generation.

AMADEUS on ChIP-Seq peaks



Hands-on (4-7)





Gene Sets Enrichment analysis

- Goal: Determine whether an a priori defined set of genes shows concordance with a biological pattern (e.g. differences between two phenotypes)
- Gene set sources:
 - ✓ MSigDB (Broad molecular signature database)
 - ✓ KEGG
 - ✓ Wiki pathways
- Gene rank sources
 - ✓ Phenotype labels
 - ✓ Imported
 - ✓ Selected condition
- Significance estimated with permutations
- FDR correction for multiple comparisons



3,000 4.000 5.000 6,000 Rank in ordered dataset

0

ES | Hits 🛕 Ranking Metric

ChIP-Seq enrichment analysis

- Searches for over-representation of genes closest to ChIP-Seq data peaks
- Uses hyper geometric test
- Multiple testing correction (Bonferroni)
- Enrichment results visualization (same as other group analysis results)

ChIP-Seq visualization

- Peaks to genomic region distributions
- Closest gene to peak chromosome visualization
- Peaks enrichment in genomic regions
- Peaks annotation table including closest gene and genomic region (e.g., 5UTR, Exon etc)



Peaks Distribution



Peak ID	Chromosome P	. Gene ID	Gene Symbol	Transcript ID	Strand	Dist from TSS	Seq Type	Intensity
1	Chr1: 118625	388581	FAM132A	ucoo1adl.2	-	-4255	Upstream of the	0.0
2	Chr1: 183892	163688	CALML6	ucoo1aih.1	+	-7238	Upstream of the	0.0
3	chr1: 215902	6497	SKI	ucoo1aja.4	+	-937	Upstream of the	0.0
4	chr1: 359766	7161	TP73	uco10nzj.2	+	-9468	Upstream of the	0.0
5	chr1: 371273	57470	LRRC47	ucoo1akx.1	-	154	Exon	0.0
6	chr1: 613422	8514		ucoo1aly.2	+	28473	Intergenic	0.0
7	chr1: 647438	54626		ucoo1amx.3	-	5190	Intergenic	0.0
8	Chr1: 661881	80835	TAS1R1	ucoorant.3	+	3580	5UTR	0.0
9	chr1: 666245	9903	KLHL21	ucootanz.1	-	377	Exon	0.0
10	chr1: 832649	50651		ucoo1apb.3	+	-57792	Intergenic	0.0
11	chr1: 924144			ucoo9vmq.3	-	328	Exon	0.0
12	chr1: 104902	378708	APITD1	ucootare.3	+	214	5UTR	0.0
13	chr1: 108046	54897		UC009VMX.2	-	-50285	Intergenic	0.0
14	chr1: 116190	57540		ucoo1asi.1	+	58305	Intergenic	0.0
15	chr1: 119682	90231		ucoo1atk.3	-	17825	Intergenic	0.0
16	chr1: 122671	55187		ucoo1atv.3	+	-22727	Intergenic	0.0
17	chr1: 126783	9249	DHRS3	UC001AUC.3	-	-762	Upstream of the	0.0
18	chr1: 127006	343066	AADACL4	ucoo1auf.3	+	-3818	Upstream of the	0.0
19	chr1: 153729	23254		UC001aVS.4	+	-54575	Intergenic	0.0
20	chr1: 155407	114827		UC001aWa.1	+	-32915	Intergenic	0.0
21	Chr1: 161611	23013		ucoo1axk.1	+	-12989	Intergenic	0.0
22	chr1: 164782	1969	EPHA ₂	ucoo1aya.2	-	3743	Intron	0.0
Geno	60						· · · · · · · · · · · · · · · · · · ·	· · · · ·
	70						Intergenic	

Genome Regions

Integration between different technologies



- ChIP-Seq vs. GE analysis:
 - GSEA ChIP-Seq target genes as a single set
 - ChIP-Seq enrichment of GE's clusters
 - ChIP-Seq target genes distribution in GE
 - Expander enrichment tools (e.g., TANGO, PRIMA)
 select ChIP-Seq target genes as a single cluster

GSEA – ChIP-Seq vs. GE



File Data Preprocessing Supervised Grouping Unsupervised Grouping Enrichment Analysis ChIP-Seq Analysis Visualization Options Help



Rank distribution – ChIP-Seq vs. GE





Hands-on (8-10)

