NCBI resources III: GEO and expression data analysis

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Fall 2015

Homework assignment 2

- Given the publication http://www.ncbi.nlm.nih.gov/pubmed/19723656, find GEO datasets that are associated with the paper.

- Choose the first data series and perform a GEO2R analysis

- Find the top two differentially expressed genes and search their gene symbol at Gene database and explain what they are

- Write a report (in word or ppt) to include all the operations and screen shots

Due on 9/25 (send by email or bring printed hard copy to class)

Office hour:
Tue, Thu and Fri 2-4pm, MO325A
Or email: yyin@niu.edu

Gene Expression Omnibus (GEO)

http://www.ncbi.nlm.nih.gov/geo/

GEO is an international public repository that archives and freely distributes microarray, next-generation sequencing, and other forms of high-throughput functional genomics data submitted by the research community.

The three main goals of GEO are to:

Provide a robust, versatile database in which to efficiently store high-throughput functional genomic data

Offer simple submission procedures and formats that support complete and well-annotated data deposits from the research community

Provide user-friendly mechanisms that allow users to query, locate, review and download studies and gene expression profiles of interest (Query and analysis)

Gene expression

- Genome analysis tells us what genes are present, but before we can determine the organism's phenotype, we need to know how those genes are expressed: under what conditions, in what tissues, how much gene product is made, etc.
  - Also, understanding and curing diseases is tied to the analysis of what genes are expressed in disease states.
- There is some progress in deciphering gene control signals, but right now it is more profitable to do experiments and use bioinformatics tools to analyze their results. Later, with some luck, we will be able to make better predictions about gene expression just from sequence data.

Gene Expression and Regulation

Genes are differentially expressed in...

- Different cell types (e.g. muscle cells, fibroblasts)
- Environmental conditions (e.g. heat shock, nutrient deprivation)
- Developmental phases (e.g. embryonic day 10)
- Cell-cycle stages (e.g. G1 phase)
- Disease states (e.g. tumor cells, virus-infected cells)

Gene expression is primarily regulated at the level of transcription

Hence, the number of mRNA copies in a cell for a particular gene is a good indicator of that gene's expression (number of proteins)

Dynamic range of mRNA levels:

- Highly expressed genes can have up to 9400 mRNA copies per cell
- Poorly expressed genes can have < 1 mRNA copies per cell

What Gene Expression Can Do?

- Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product.

- The most fundamental process through which the genotype gives rise to the phenotype.

- For a specific cell at a specific time, only a subset of the genes coded in the genome are expressed.

- Measure of mRNA expression level can
  - Provide a good indicator of corresponding protein expression level
  - Provide insight on the mechanisms of transcriptional and post-transcriptional regulation
  - Shed light on disease mechanisms and treatments

- Transcriptome: The complete set of messenger RNA (mRNA) and noncoding RNA (ncRNA) transcripts produced by a particular cell, cell type, or organism.

High-throughput transcriptome profiling

- Hybridization based approaches: incubating fluorescently labeled cDNA with microarrays. Hybridization signal is measured.
  - Hybridization: the process of establishing a sequence-specific interaction between two strands of nucleic acids through complementary hydrogen bonding between nucleobases.
  - Printed arrays (cDNA microarray)
  - Synthesized arrays (High density oligo arrays)

- Sequencing based approaches: directly determine the cDNA/mRNA sequence. Count is measured.
  - Sanger sequencing-based approaches
    - EST sequencing
    - Serial Analysis of Gene Expression (SAGE)
  - High-throughput sequencing-based approach
    - RNA-Seq

Concepts

Gene expression profiling: the simultaneous assessment of the expression level of a large number of genes, often an entire genome, to obtain a global snapshot of the complete mRNA component of the cell at a given time; helps to distinguish between different cell types, different disease states, and different time points during development

Expressed sequence tag (EST): a single-pass sequencing read from the 3′ or 5′ end of a cDNA clone. In contrast, full-length cDNA (FLcDNA) sequencing involves generation and assembly of sequencing reads spanning the full length of cDNA clones

Microarray: a method for high-throughput gene expression profiling involving hybridization of mRNA to an array of complementary DNA probes corresponding to genes of interest. Hybridization intensity to a particular probe is related to the expression level of the corresponding transcript. The microarray method has dominated expression profiling research for the past decade

More concepts

Resequencing: sequencing of the genome or transcriptome of an individual of a species for which a reference genome sequence is available. In contrast, sequencing and assembly of the reference genome itself is termed de novo sequencing. Resequencing is commonly conducted to gauge sequence diversity within the species

Serial Analysis of Gene Expression (SAGE): the first sequencing-based method for high-throughput gene expression profiling. SAGE involves the generation of short sequence tags from 3′ ends of mRNA transcripts. The tags are then concatenated, sequenced, and counted providing estimates of transcript abundance

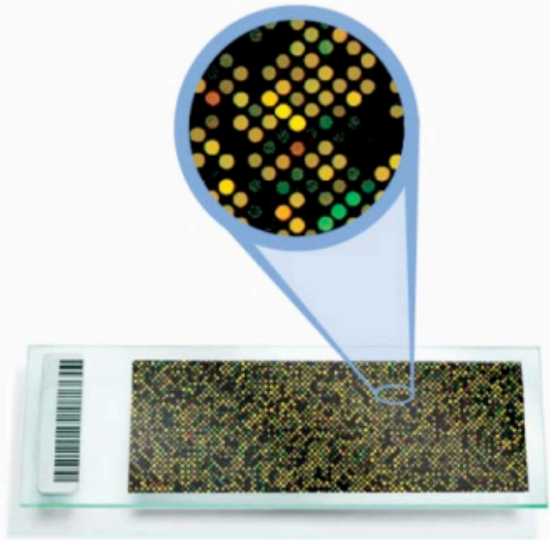
Sequencing depth: the total number of sequencing reads generated from a sequencing library. The higher the sequencing depth the higher the chance of detecting rare transcripts and sequence variants present in the cell

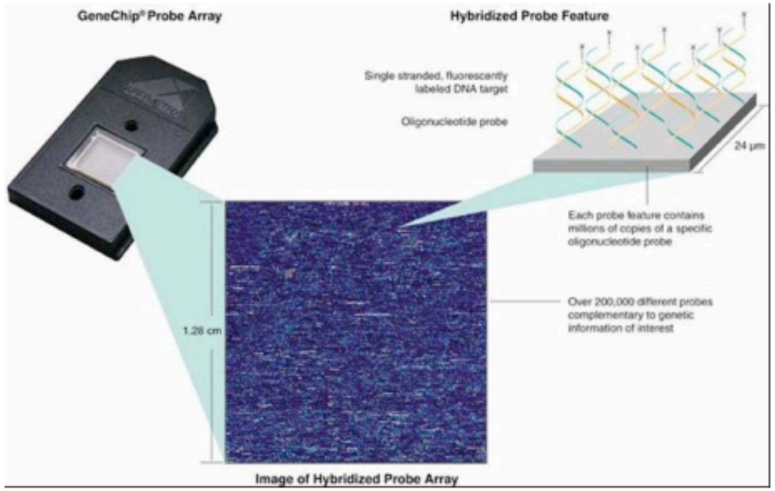
DNA microarrays

DNA microarray: a solid support (glass slide, silicon chip, etc) on which DNA of known sequence is deposited in a regular grid-like array.

- **Spotted or printed arrays (aka cDNA array)**: DNA feature physically transferred from a plate or reservoir to a solid support, typically a chemically modified glass microscope slide. (Agilent, GE, ABI)

- **Synthesized arrays (aka oligonucleotide array)**: DNA features chemically synthesized in-situ on the substrate. (Affymetrix, NimbleGen, Combimatrix)

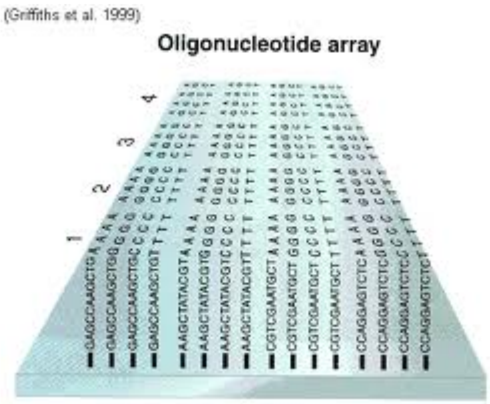


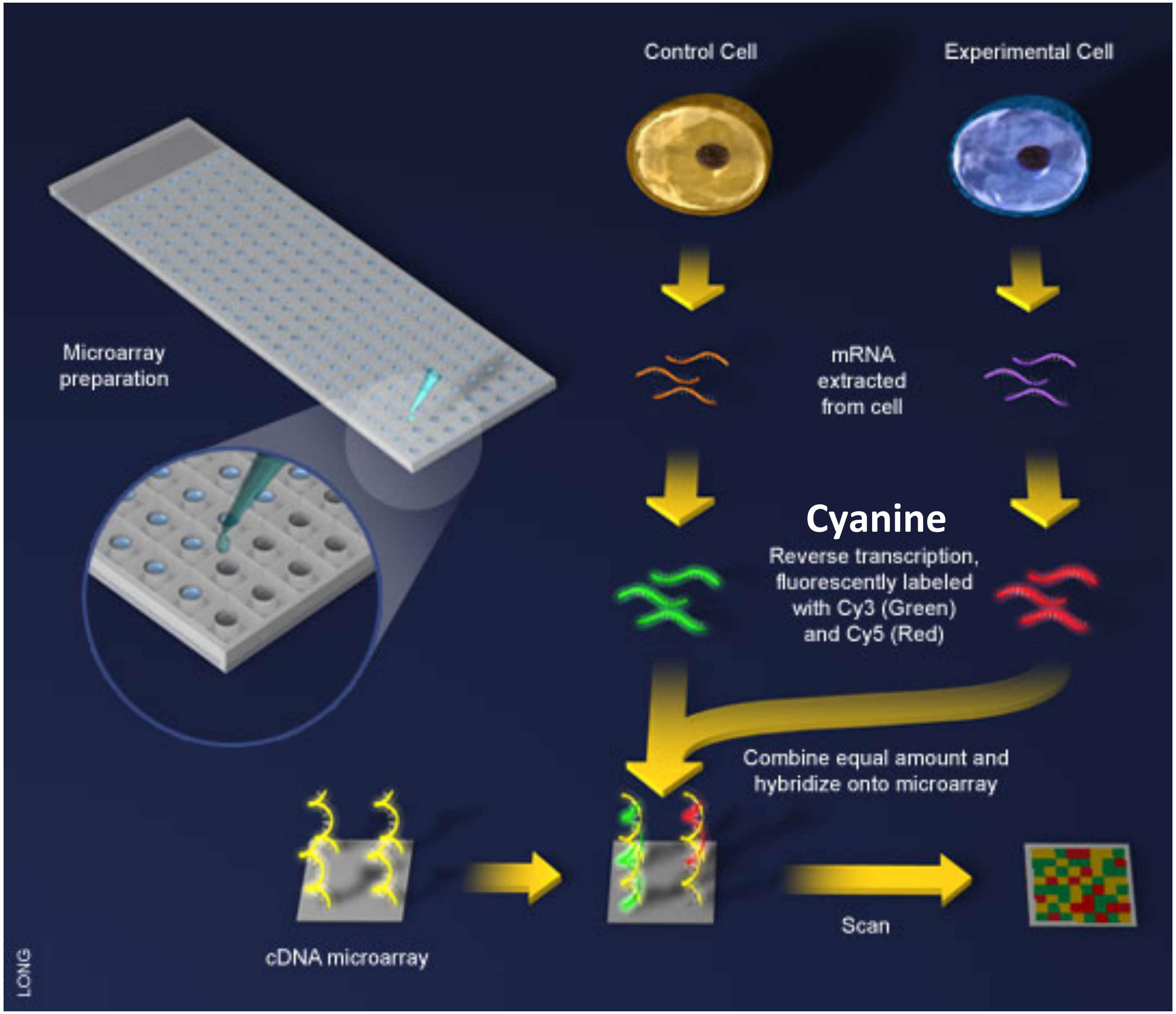


A simplified protocol for DNA microarray experiment

1. Prepare or purchase DNA microarray
2. Isolate mRNA from cell cultures or tissue samples
3. Reverse transcribe mRNA into cDNA
4. Label cDNA by incorporating fluorescently-labeled nucleotides
5. Hybridize labeled cDNA to DNA microarray
6. Wash and scan microarray in scanner
7. Analyze data

Basic intro to microarray



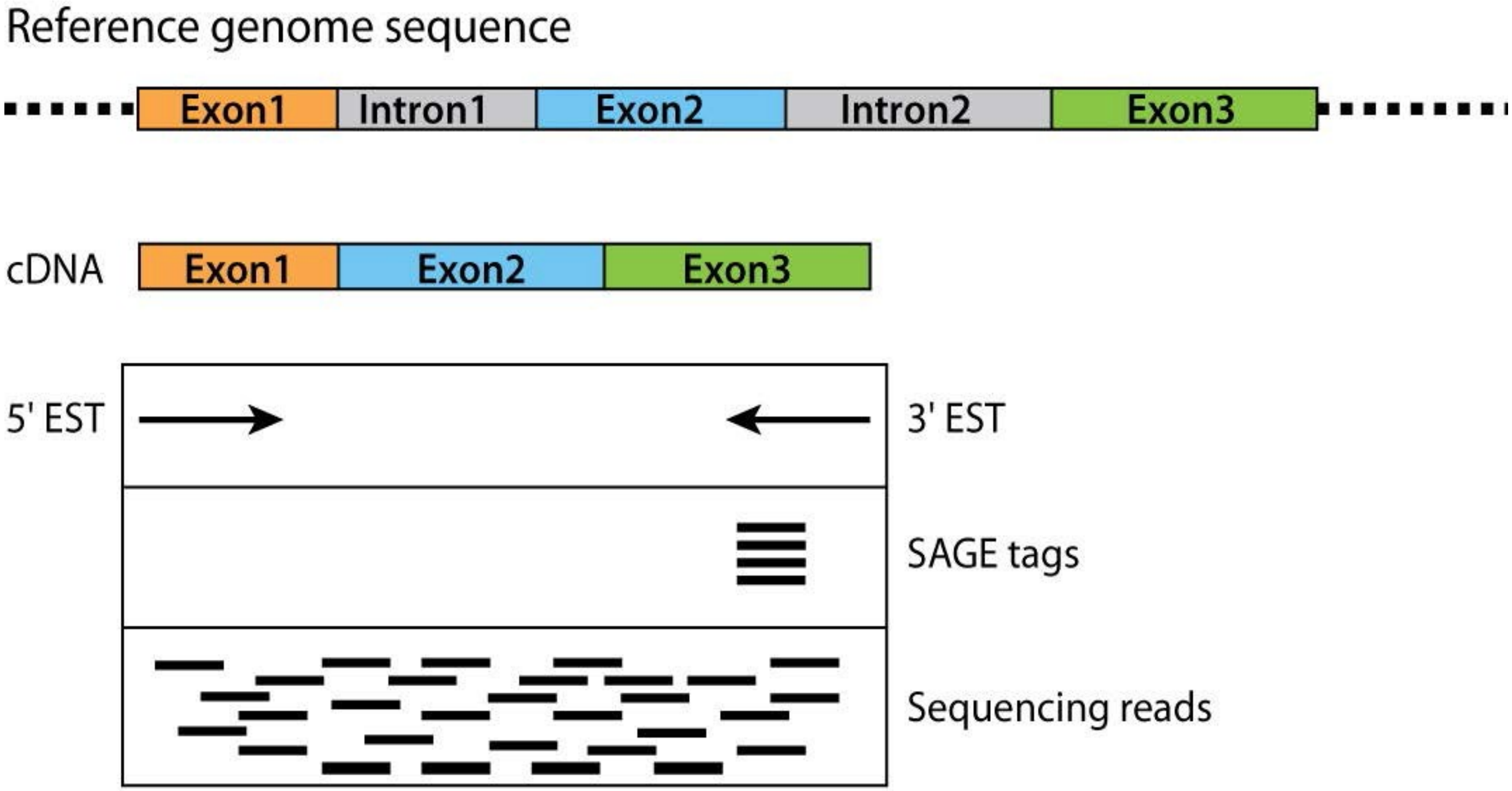


People are moving from microarray to high throughput sequencing

Technology	**Tiling microarray**	**EST sequencing**	**RNA-Seq**
Technology specifications			
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to 100 bp	Single base	Single base
Throughput	High	Low	High
Reliance on genomic sequence	Yes	No	In some cases
Background noise	High	Low	Low
Practical issues			
Required amount of RNA	High	High	Low
Cost for mapping transcriptomes of large genomes	High	High	Relatively low
Application			
Dynamic range to quantify gene expression level	Up to a few hundred-fold	Not practical	>,8000-fold
Simultaneously map transcribed regions and gene expression	Yes	Limited for gene expression	Yes
Ability to distinguish different isoforms	Limited	Yes	Yes
Ability to distinguish allelic expression	Limited	Yes	Yes

Wang et al. Nature Rev Genet, 10:57, 2009

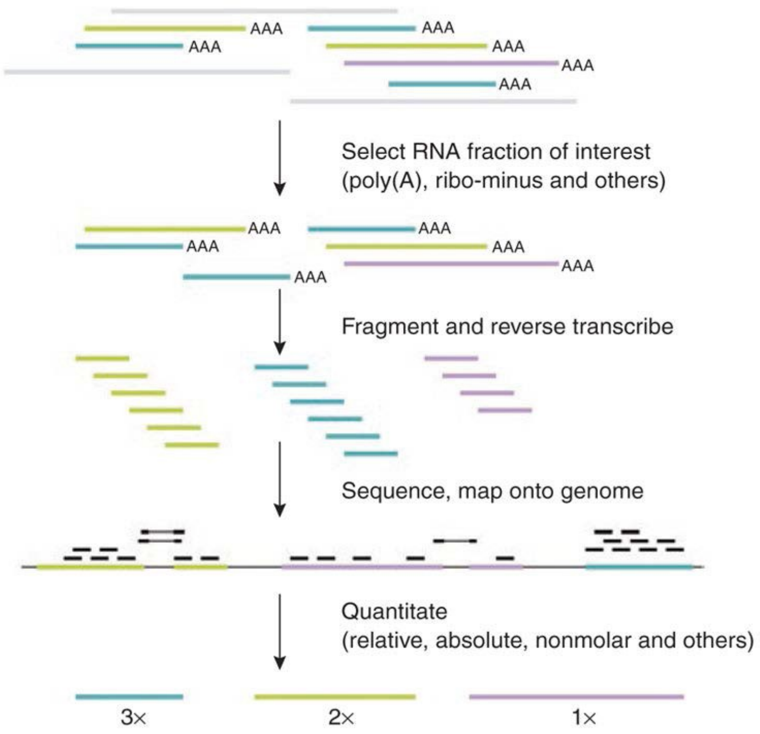
Sequences targeted by different sequencing-based approaches



Morozova et al. Annu Rev Genom Human Genet, 2009

RNA-Seq

- RNA-Seq: the use of next generation sequencing technologies to sequence cDNA in order to get information about a sample's RNA content.
- A RNA fraction of interest is selected, fragmented and reverse transcribed.
- Resulting cDNA are sequenced using one of the next generation sequencing technologies to obtain ten to a hundred million reads.
- The reads are mapped back onto the genome and analyzed to calculate expression levels.

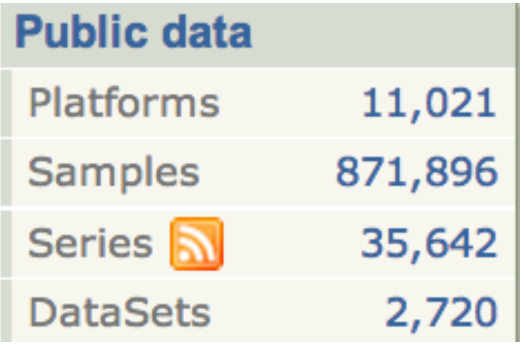


Pepke et al. Nature Methods 6:S22 - S32, 2009

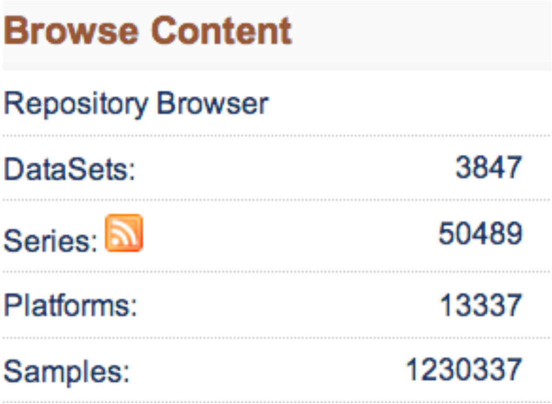
What data does GEO have?

http://www.ncbi.nlm.nih.gov/geo/

- Submitter supplied: Platform, Sample, Series
- NCBI curated: DataSets and Profiles
- Tools: GEO BLAST and GEO2R



Public data	
Platforms	11,021
Samples	871,896
Series	35,642
DataSets	2,720

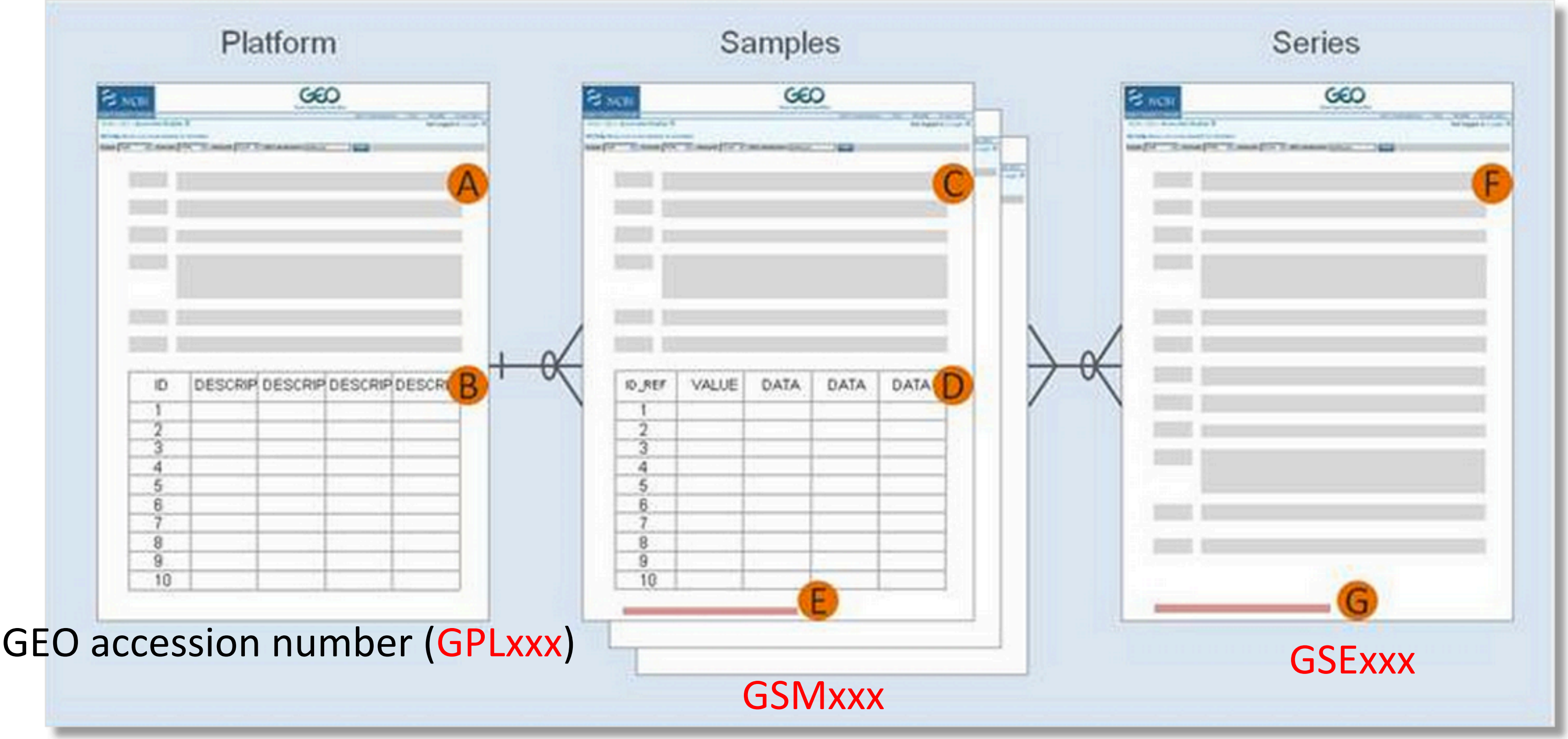


Browse Content

Repository Browser	
DataSets:	3847
Series:	50489
Platforms:	13337
Samples:	1230337

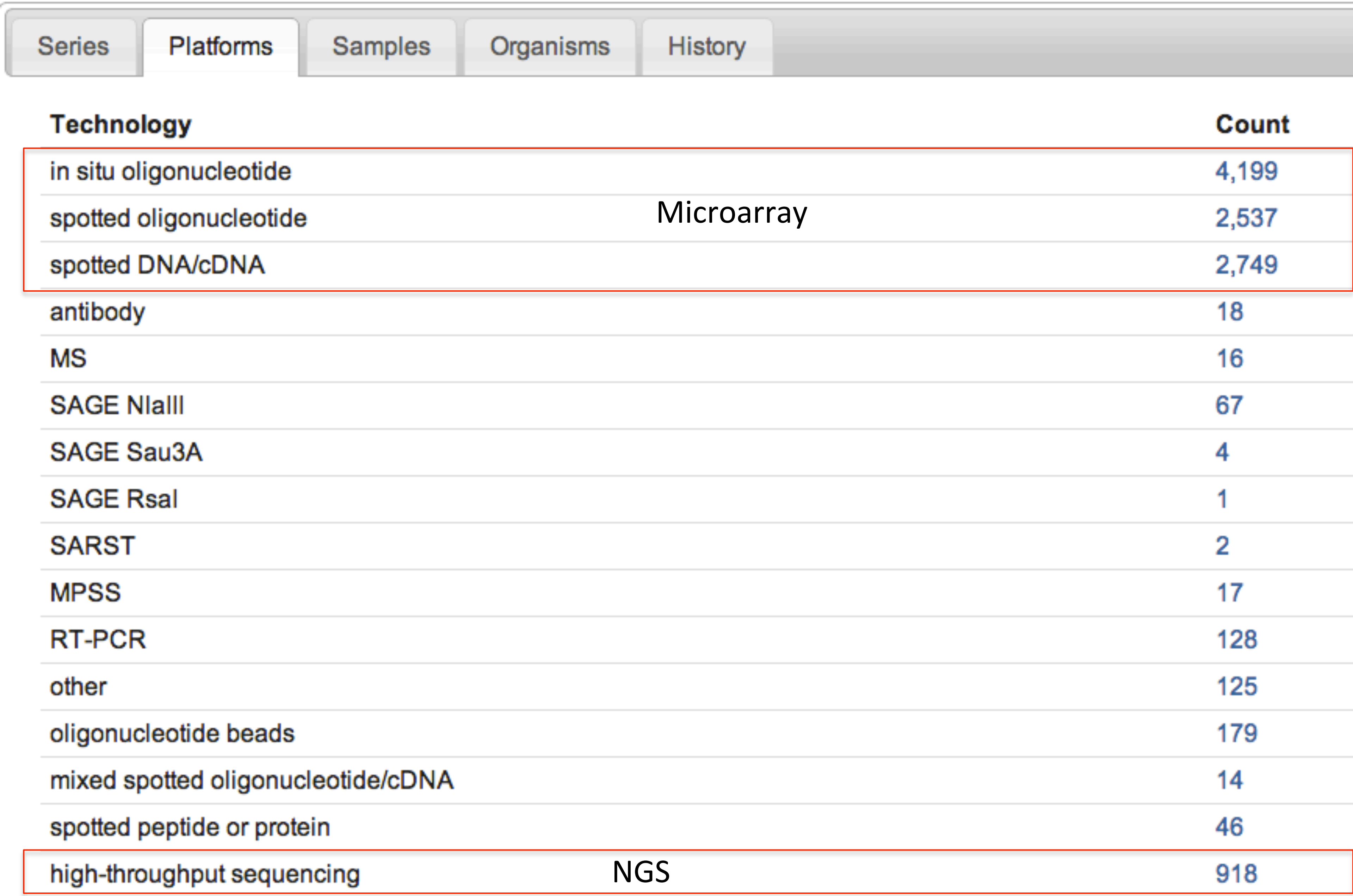
Omics data:

Genomics
Transcriptomics
Epigenomics
Proteomics
…



For almost all array data submissions, you will be asked to provide the following information:

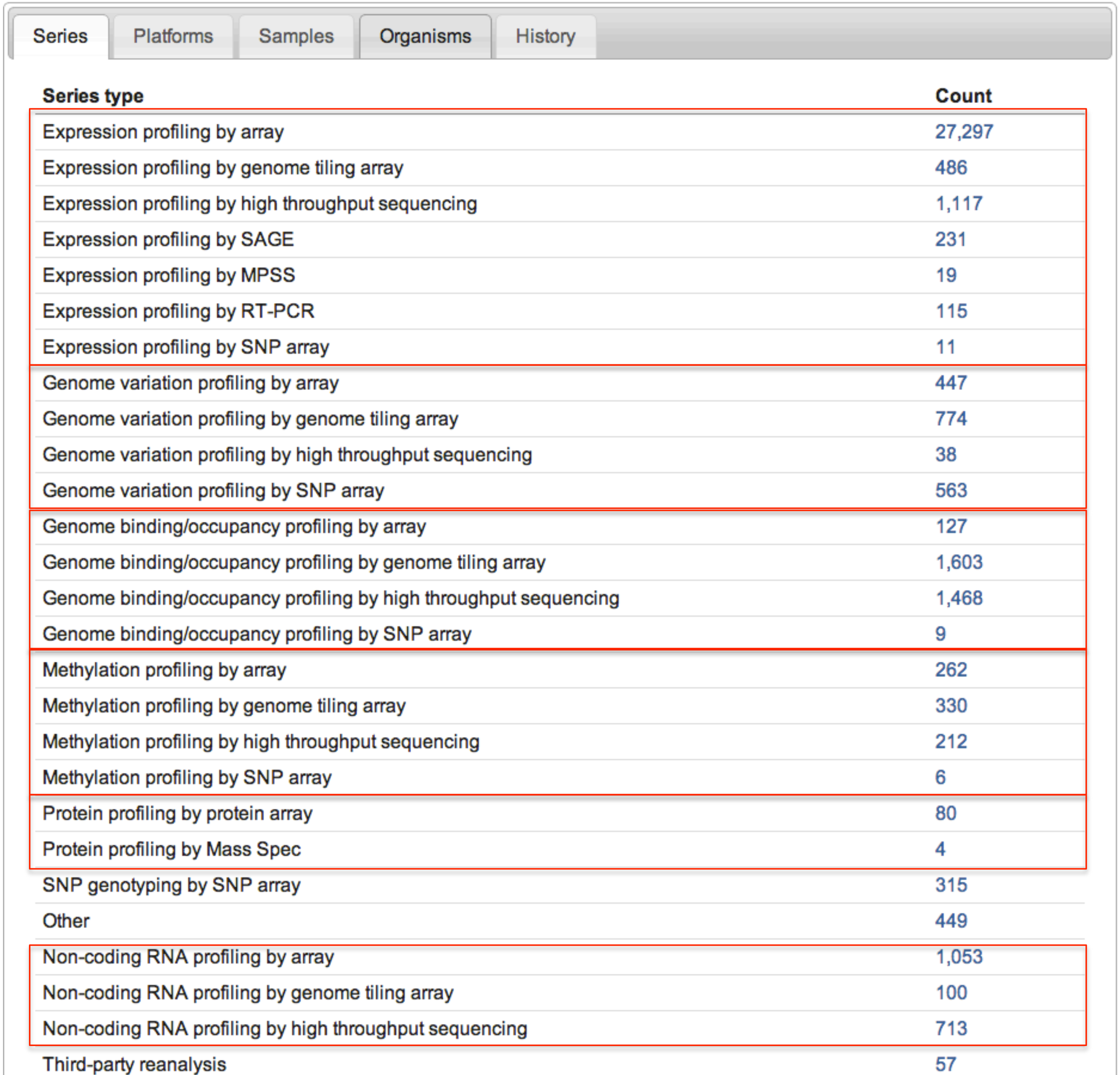
A	Text description of the array or sequencer
B	Text tab-delimited table of the array template
C	Text description of the biological sample and protocols to which it was subjected
D	Text tab-delimited table of processed hybridization result or sequence counts
E	Raw data file, or processed sequence data file
F	Text description of the overall experiment
G	Tar archive of raw data files, or processed sequence data files



Technology	Count
in situ oligonucleotide	4,199
spotted oligonucleotide	2,537
spotted DNA/cDNA	2,749
antibody	18
MS	16
SAGE NlaIII	67
SAGE Sau3A	4
SAGE RsaI	1
SARST	2
MPSS	17
RT-PCR	128
other	125
oligonucleotide beads	179
mixed spotted oligonucleotide/cDNA	14
spotted peptide or protein	46
high-throughput sequencing	918

Microarray

NGS



Series type	Count
Expression profiling by array	27,297
Expression profiling by genome tiling array	486
Expression profiling by high throughput sequencing	1,117
Expression profiling by SAGE	231
Expression profiling by MPSS	19
Expression profiling by RT-PCR	115
Expression profiling by SNP array	11
Genome variation profiling by array	447
Genome variation profiling by genome tiling array	774
Genome variation profiling by high throughput sequencing	38
Genome variation profiling by SNP array	563
Genome binding/occupancy profiling by array	127
Genome binding/occupancy profiling by genome tiling array	1,603
Genome binding/occupancy profiling by high throughput sequencing	1,468
Genome binding/occupancy profiling by SNP array	9
Methylation profiling by array	262
Methylation profiling by genome tiling array	330
Methylation profiling by high throughput sequencing	212
Methylation profiling by SNP array	6
Protein profiling by protein array	80
Protein profiling by Mass Spec	4
SNP genotyping by SNP array	315
Other	449
Non-coding RNA profiling by array	1,053
Non-coding RNA profiling by genome tiling array	100
Non-coding RNA profiling by high throughput sequencing	713
Third-party reanalysis	57

Expression

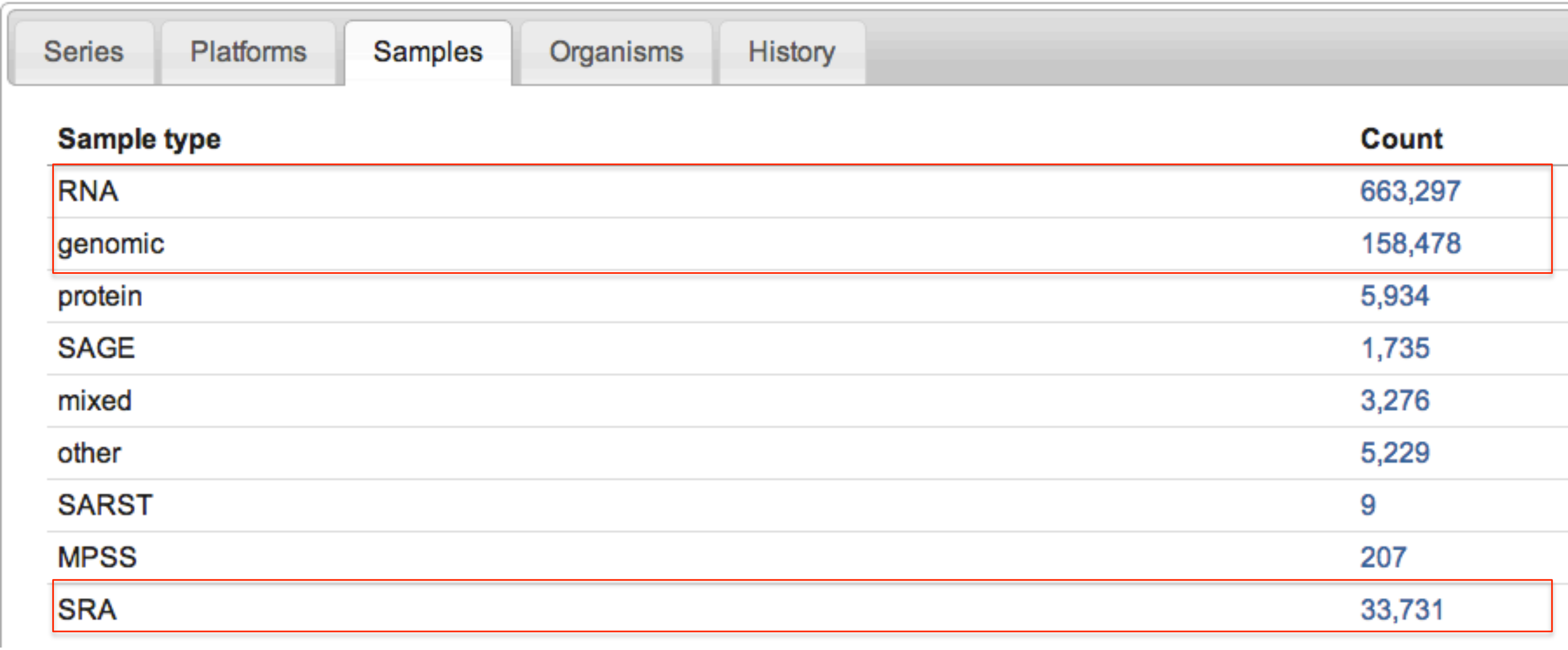
Genome variation

DNA-binding

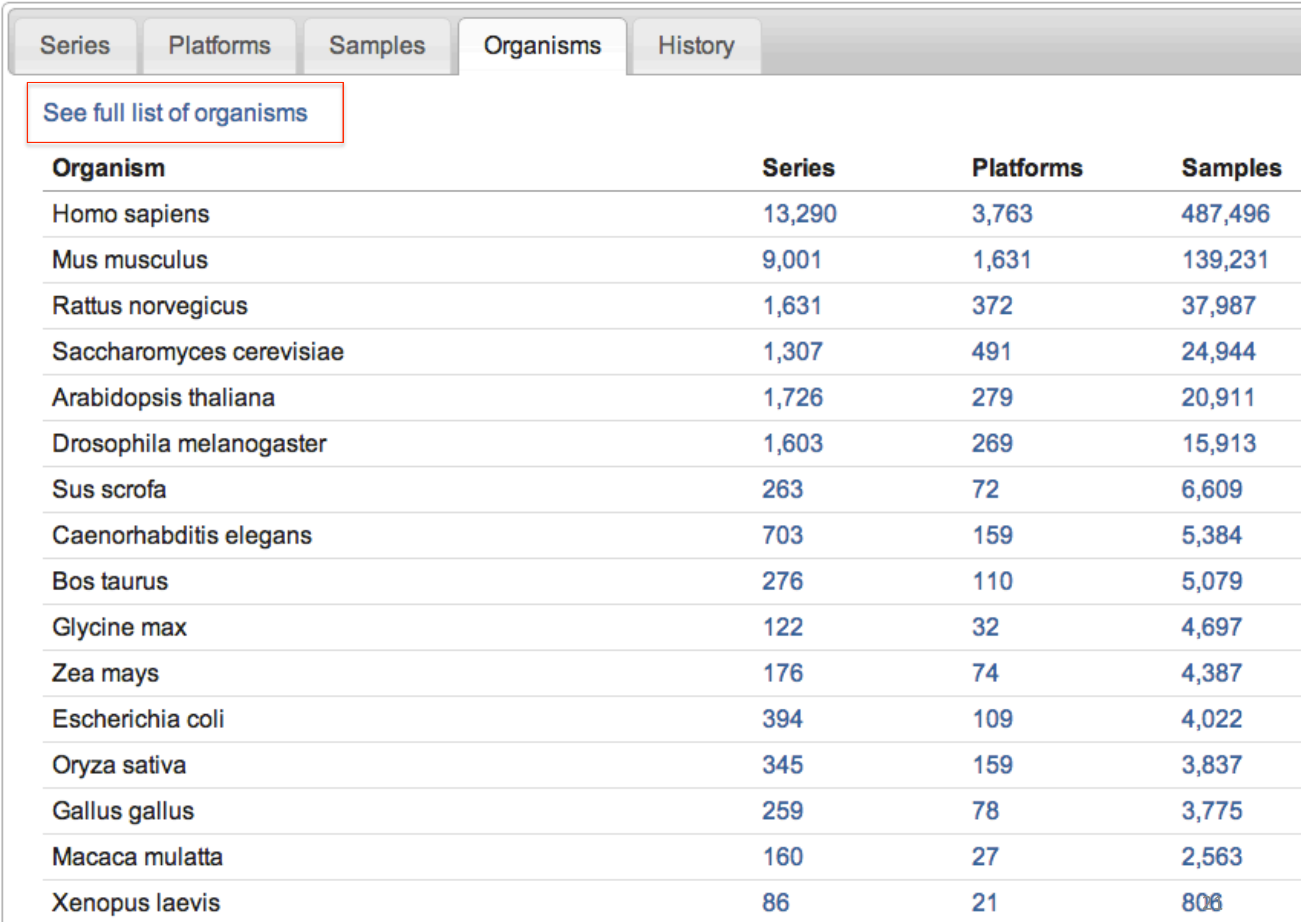
Methylation/
Epigenomics

Protein array

ncRNAs



Sample type	Count
RNA	663,297
genomic	158,478
protein	5,934
SAGE	1,735
mixed	3,276
other	5,229
SARST	9
MPSS	207
SRA	33,731

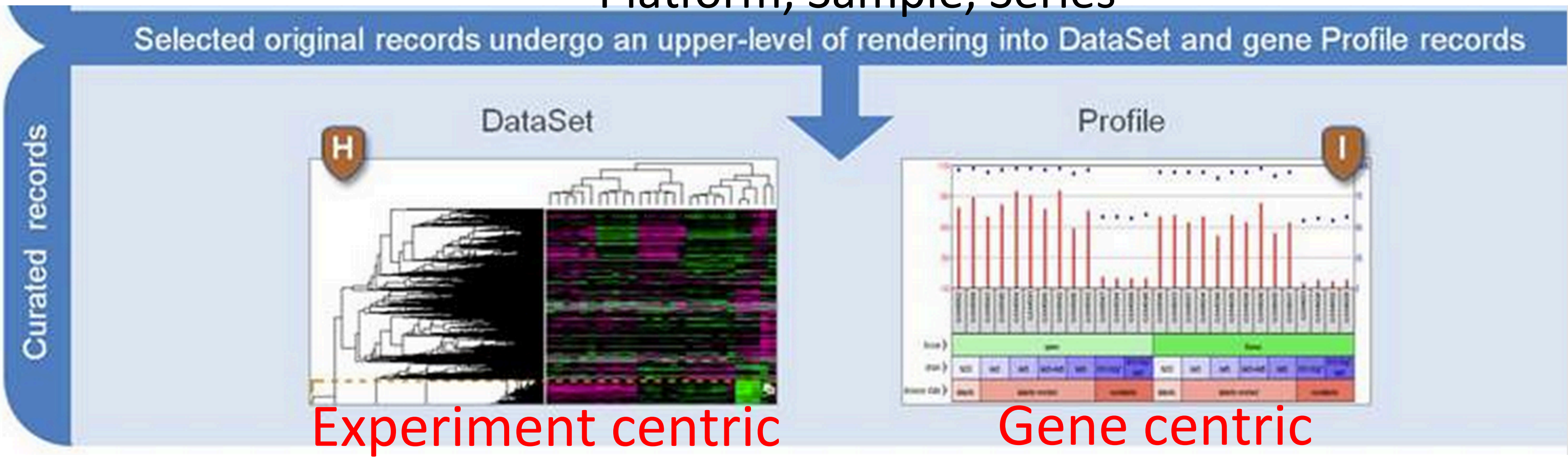


See full list of organisms

Organism	Series	Platforms	Samples
Homo sapiens	13,290	3,763	487,496
Mus musculus	9,001	1,631	139,231
Rattus norvegicus	1,631	372	37,987
Saccharomyces cerevisiae	1,307	491	24,944
Arabidopsis thaliana	1,726	279	20,911
Drosophila melanogaster	1,603	269	15,913
Sus scrofa	263	72	6,609
Caenorhabditis elegans	703	159	5,384
Bos taurus	276	110	5,079
Glycine max	122	32	4,697
Zea mays	176	74	4,387
Escherichia coli	394	109	4,022
Oryza sativa	345	159	3,837
Gallus gallus	259	78	3,775
Macaca mulatta	160	27	2,563
Xenopus laevis	86	21	806

http://www.ncbi.nlm.nih.gov/geo/info/overview.html

Platform, Sample, Series



Experiment centric

Gene centric

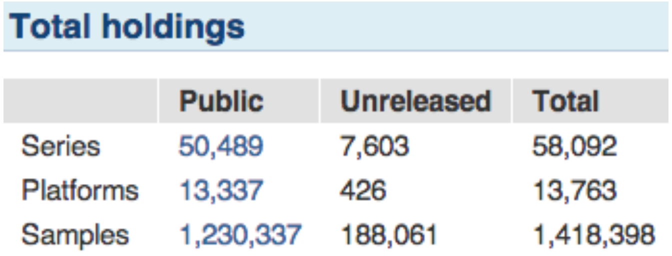
Data of a GEO Series are reassembled by GEO staff into GEO Dataset records (GDSxxx).

A DataSet represents a curated collection of biologically and statistically comparable GEO Samples and forms the basis of GEO's suite of data display and analysis tools.

Not all submitted data are suitable for DataSet assembly, so not all Series have corresponding DataSet record(s).

Profiles are derived from DataSets

A Profile consists of the expression measurements for an individual gene across all Samples in a DataSet.



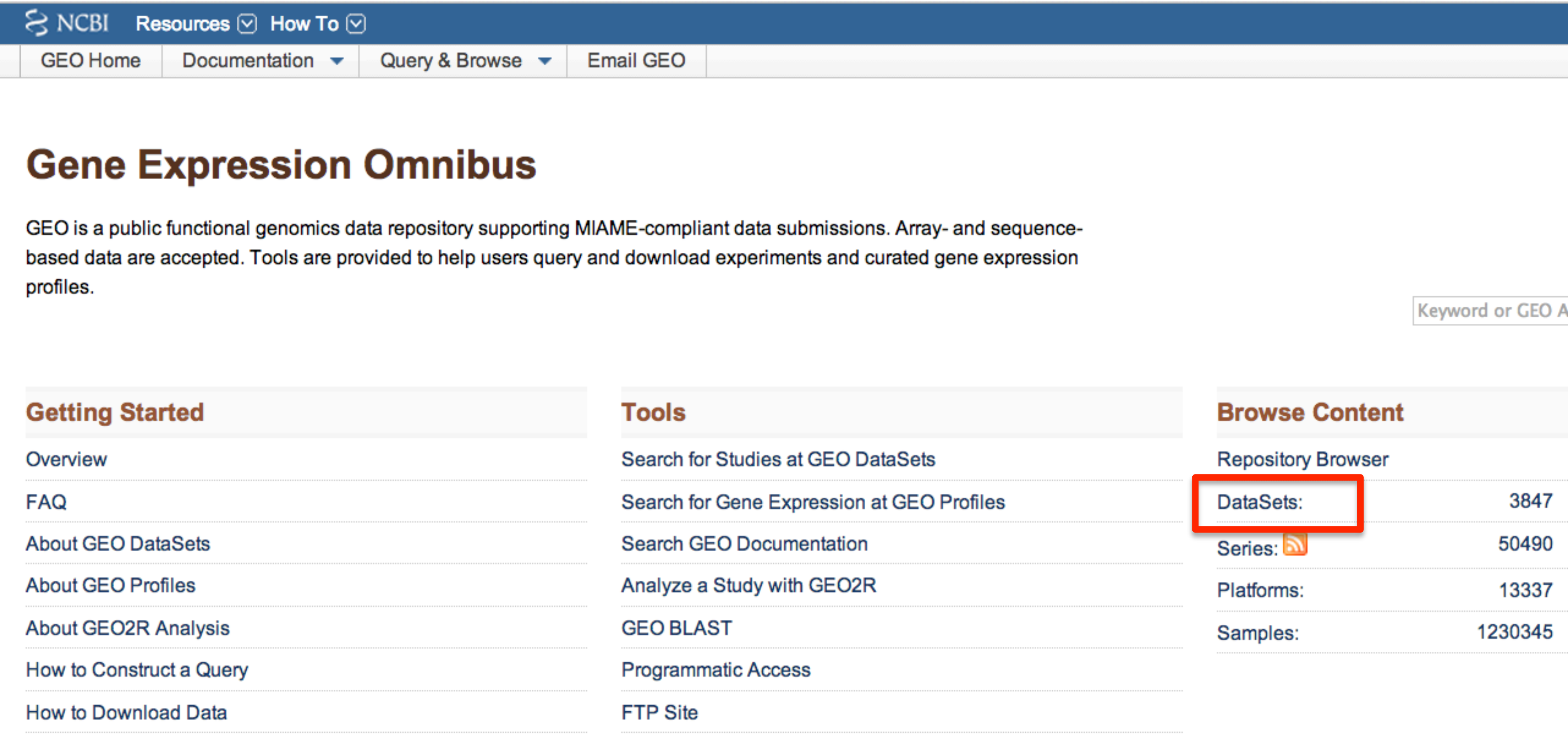
Total holdings

	Public	Unreleased	Total
Series	50,489	7,603	58,092
Platforms	13,337	426	13,763
Samples	1,230,337	188,061	1,418,398

Hands on exercise 1

GEO browse and query

http://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.

Getting Started

- Overview
- FAQ
- About GEO DataSets
- About GEO Profiles
- About GEO2R Analysis
- How to Construct a Query
- How to Download Data

Tools

- Search for Studies at GEO DataSets
- Search for Gene Expression at GEO Profiles
- Search GEO Documentation
- Analyze a Study with GEO2R
- GEO BLAST
- Programmatic Access
- FTP Site

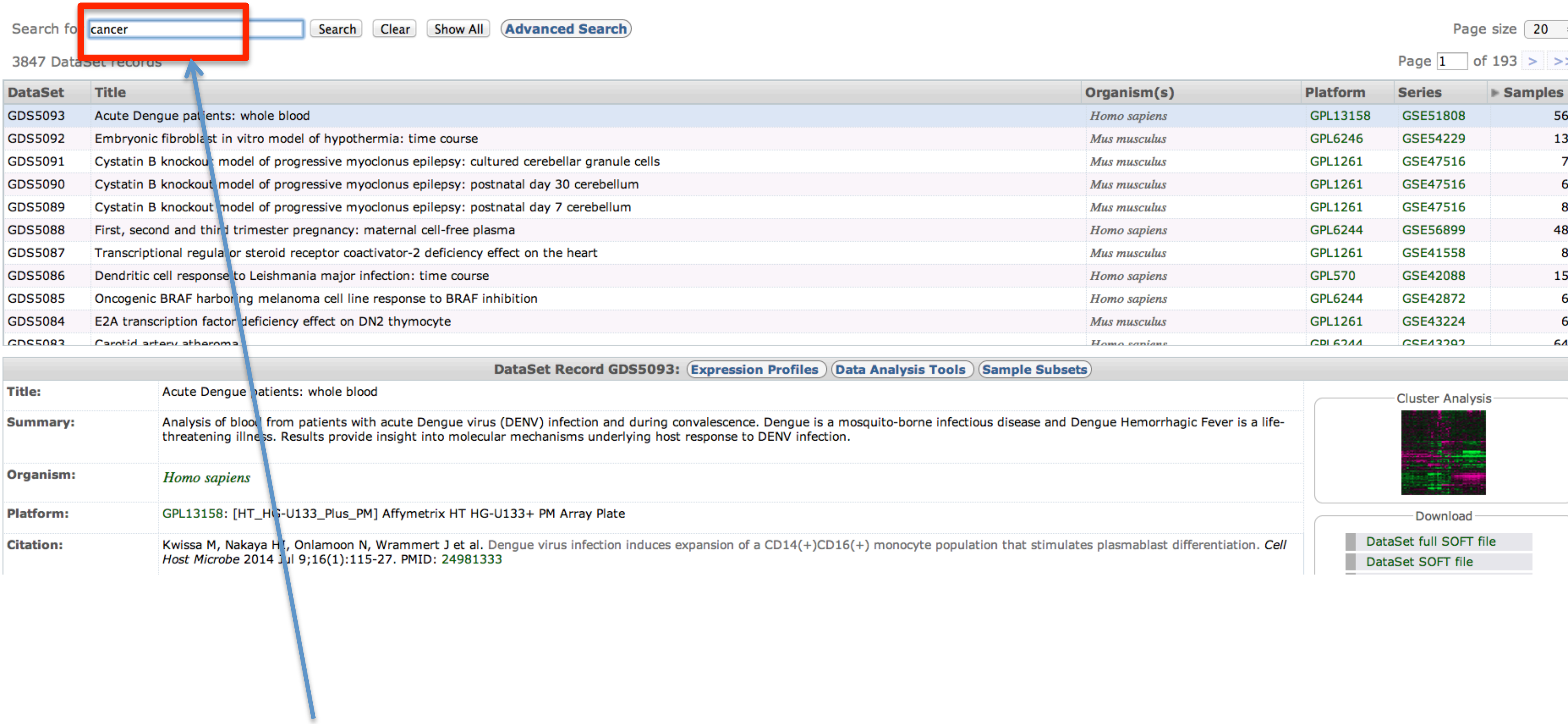
Browse Content

Repository Browser

DataSets:	3847
Series:	50490
Platforms:	13337
Samples:	1230345

Try:
cancer
colon cancer
arabidopsis

These are only DataSets



3847 DataSet records

DataSet	Title	Organism(s)	Platform	Series	Samples
GDS5093	Acute Dengue patients: whole blood	*Homo sapiens*	GPL13158	GSE51808	56
GDS5092	Embryonic fibroblast in vitro model of hypothermia: time course	*Mus musculus*	GPL6246	GSE54229	13
GDS5091	Cystatin B knockout model of progressive myoclonus epilepsy: cultured cerebellar granule cells	*Mus musculus*	GPL1261	GSE47516	7
GDS5090	Cystatin B knockout model of progressive myoclonus epilepsy: postnatal day 30 cerebellum	*Mus musculus*	GPL1261	GSE47516	6
GDS5089	Cystatin B knockout model of progressive myoclonus epilepsy: postnatal day 7 cerebellum	*Mus musculus*	GPL1261	GSE47516	8
GDS5088	First, second and third trimester pregnancy: maternal cell-free plasma	*Homo sapiens*	GPL6244	GSE56899	48
GDS5087	Transcriptional regulator steroid receptor coactivator-2 deficiency effect on the heart	*Mus musculus*	GPL1261	GSE41558	8
GDS5086	Dendritic cell response to Leishmania major infection: time course	*Homo sapiens*	GPL570	GSE42088	15
GDS5085	Oncogenic BRAF harboring melanoma cell line response to BRAF inhibition	*Homo sapiens*	GPL6244	GSE42872	6
GDS5084	E2A transcription factor deficiency effect on DN2 thymocyte	*Mus musculus*	GPL1261	GSE43224	6
GDS5083	Carotid artery atheroma	*Homo sapiens*	GPL6244	GSE43292	64

DataSet Record GDS5093:

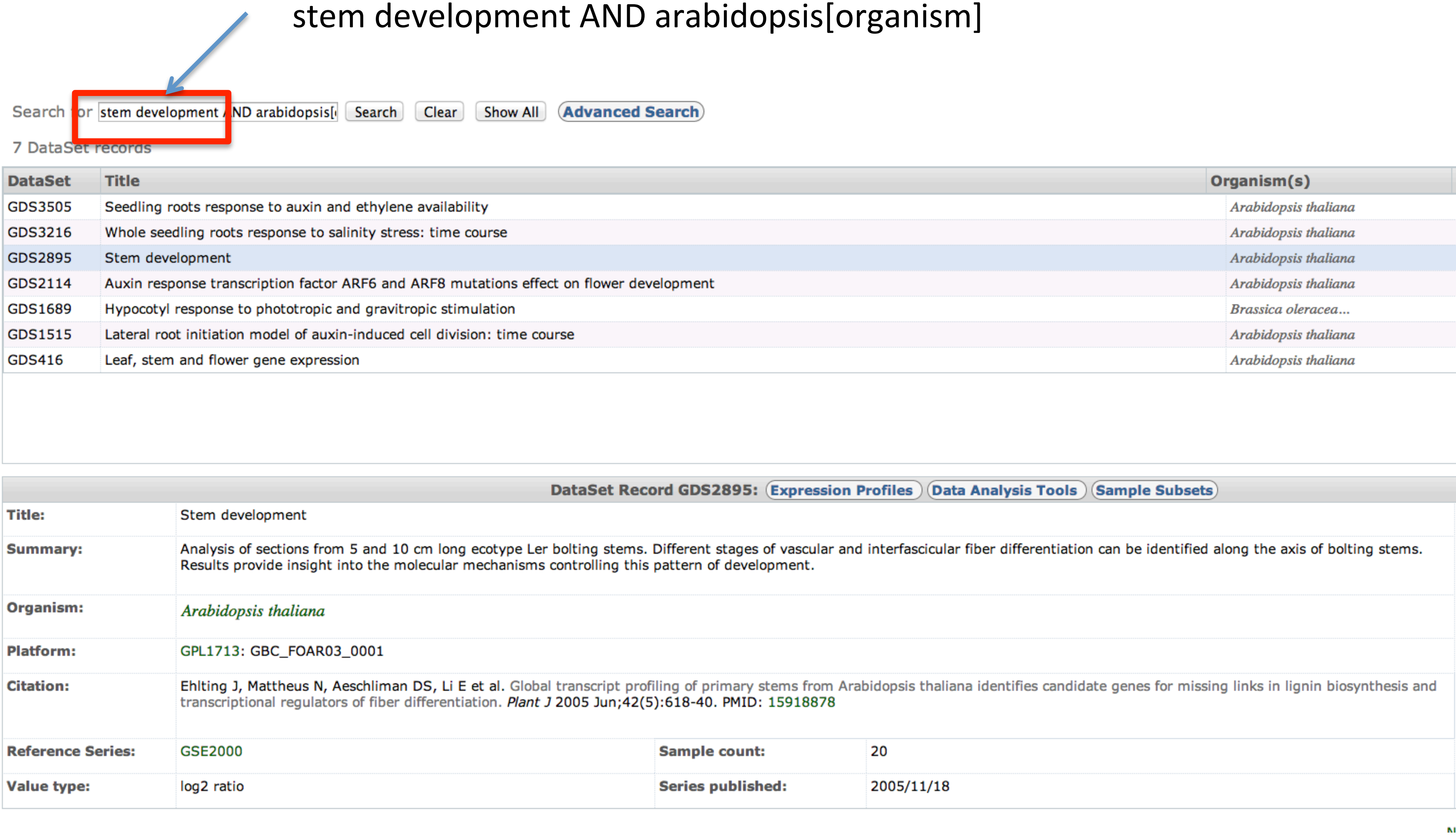
Title:	Acute Dengue patients: whole blood
Summary:	Analysis of blood from patients with acute Dengue virus (DENV) infection and during convalescence. Dengue is a mosquito-borne infectious disease and Dengue Hemorrhagic Fever is a life-threatening illness. Results provide insight into molecular mechanisms underlying host response to DENV infection.
Organism:	*Homo sapiens*
Platform:	GPL13158: [HT_HG-U133_Plus_PM] Affymetrix HT HG-U133+ PM Array Plate
Citation:	Kwissa M, Nakaya HI, Onlamoon N, Wrammert J et al. Dengue virus infection induces expansion of a CD14(+)CD16(+) monocyte population that stimulates plasmablast differentiation. *Cell Host Microbe* 2014 Jul 9;16(1):115-27. PMID: 24981333

Type the keyword in the search box and click search

Construct queries to narrow down the results

term [field] OPERATOR term [field]

stem development AND arabidopsis[organism]



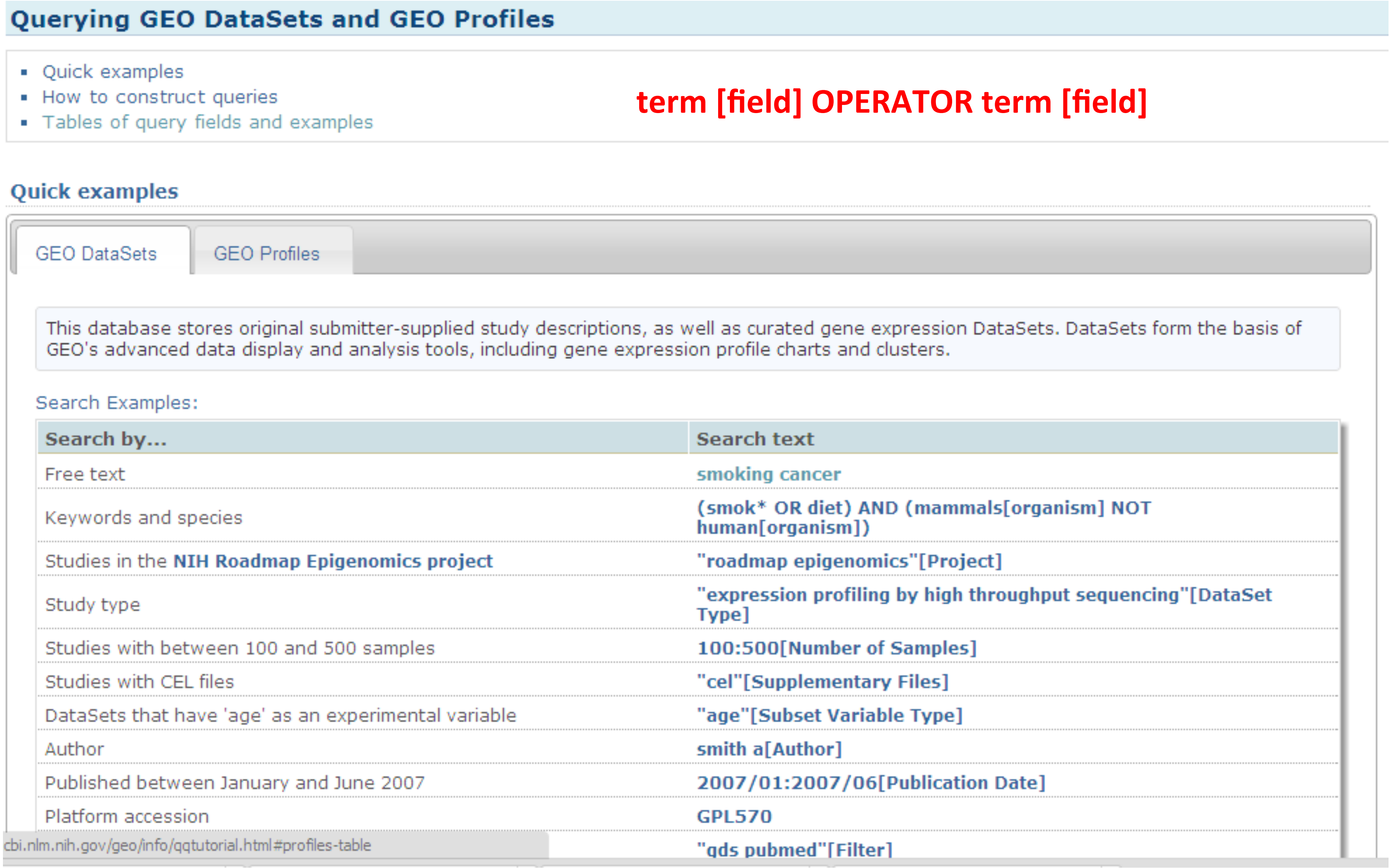
7 DataSet records

DataSet	Title	Organism(s)
GDS3505	Seedling roots response to auxin and ethylene availability	*Arabidopsis thaliana*
GDS3216	Whole seedling roots response to salinity stress: time course	*Arabidopsis thaliana*
GDS2895	Stem development	*Arabidopsis thaliana*
GDS2114	Auxin response transcription factor ARF6 and ARF8 mutations effect on flower development	*Arabidopsis thaliana*
GDS1689	Hypocotyl response to phototropic and gravitropic stimulation	*Brassica oleracea...*
GDS1515	Lateral root initiation model of auxin-induced cell division: time course	*Arabidopsis thaliana*
GDS416	Leaf, stem and flower gene expression	*Arabidopsis thaliana*

DataSet Record GDS2895:

Title:	Stem development		
Summary:	Analysis of sections from 5 and 10 cm long ecotype Ler bolting stems. Different stages of vascular and interfascicular fiber differentiation can be identified along the axis of bolting stems. Results provide insight into the molecular mechanisms controlling this pattern of development.		
Organism:	*Arabidopsis thaliana*		
Platform:	GPL1713: GBC_FOAR03_0001		
Citation:	Ehlting J, Mattheus N, Aeschliman DS, Li E et al. Global transcript profiling of primary stems from Arabidopsis thaliana identifies candidate genes for missing links in lignin biosynthesis and transcriptional regulators of fiber differentiation. *Plant J* 2005 Jun;42(5):618-40. PMID: 15918878		
Reference Series:	GSE2000	**Sample count:**	20
Value type:	log2 ratio	**Series published:**	2005/11/18

http://www.ncbi.nlm.nih.gov/geo/info/qqtutorial.html



Querying GEO DataSets and GEO Profiles

- Quick examples
- How to construct queries
- Tables of query fields and examples

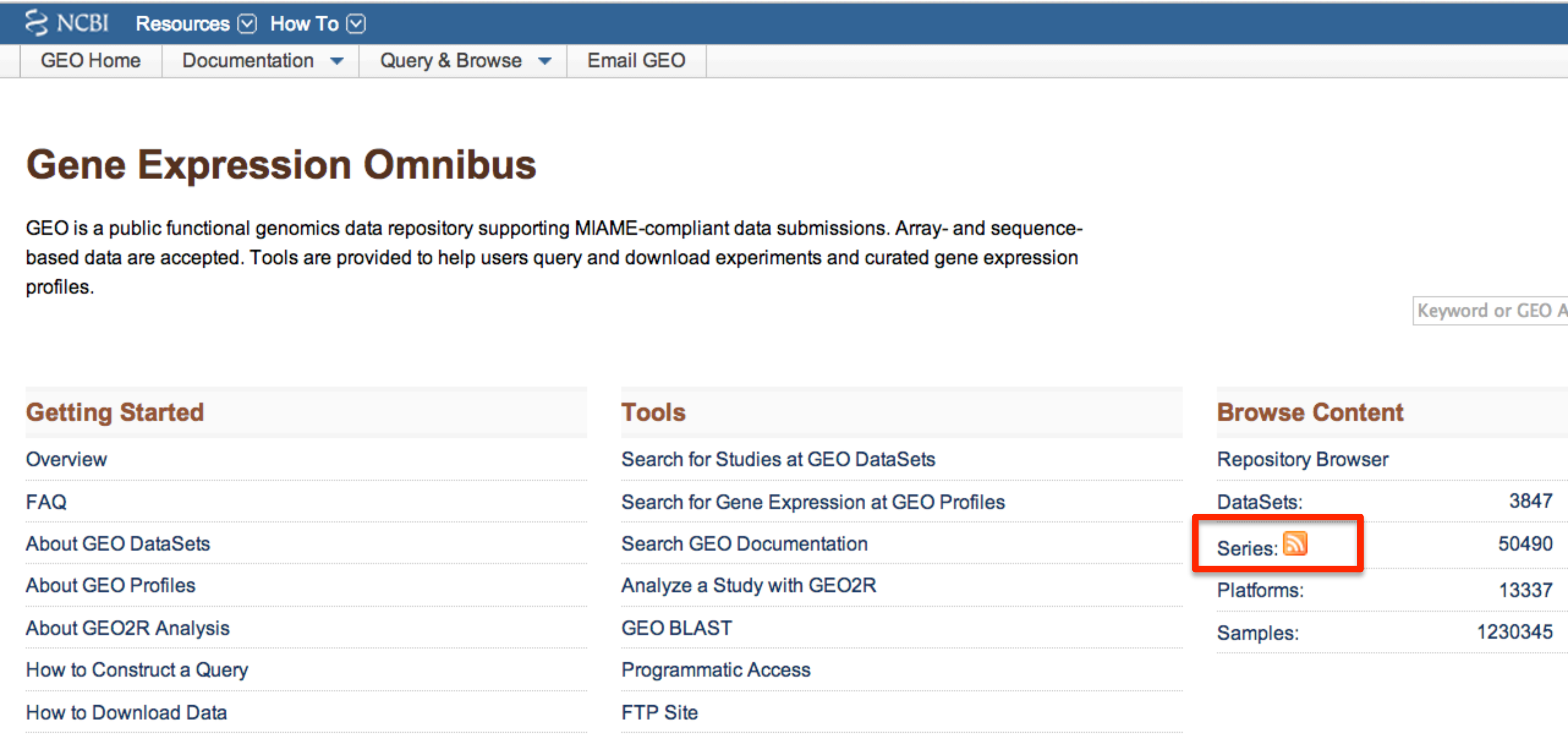
term [field] OPERATOR term [field]

Quick examples

This database stores original submitter-supplied study descriptions, as well as curated gene expression DataSets. DataSets form the basis of GEO's advanced data display and analysis tools, including gene expression profile charts and clusters.

Search Examples:

Search by...	Search text
Free text	smoking cancer
Keywords and species	**(smok* OR diet) AND (mammals[organism] NOT human[organism])**
Studies in the **NIH Roadmap Epigenomics project**	**"roadmap epigenomics"[Project]**
Study type	**"expression profiling by high throughput sequencing"[DataSet Type]**
Studies with between 100 and 500 samples	**100:500[Number of Samples]**
Studies with CEL files	**"cel"[Supplementary Files]**
DataSets that have 'age' as an experimental variable	**"age"[Subset Variable Type]**
Author	**smith a[Author]**
Published between January and June 2007	**2007/01:2007/06[Publication Date]**
Platform accession	**GPL570**
	"gds pubmed"[Filter]



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Repository Browser

DataSets:	3847
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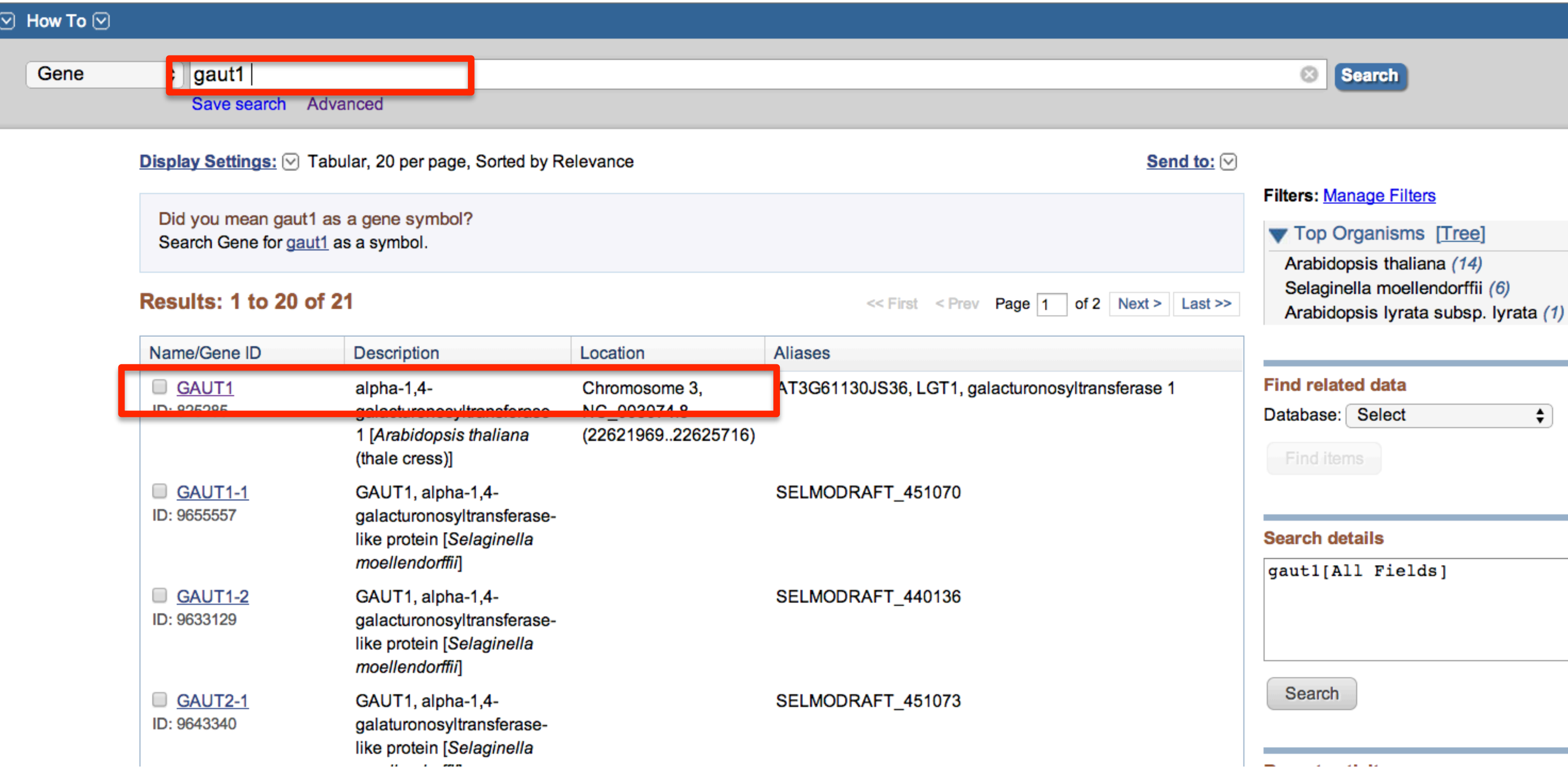
Hands on exercise 2

GEO gene profiles

Search for a gene: GAUT1



gaut1

Display Settings: Tabular, 20 per page, Sorted by Relevance

Did you mean gaut1 as a gene symbol?
Search Gene for gaut1 as a symbol.

Results: 1 to 20 of 21

<< First < Prev Page 1 of 2 Next > Last >>

Name/Gene ID	Description	Location	Aliases
☐ GAUT1 ID: 825285	alpha-1,4-galacturonosyltransferase 1 [*Arabidopsis thaliana* (thale cress)]	Chromosome 3, NC_003074.8 (22621969..22625716)	AT3G61130JS36, LGT1, galacturonosyltransferase 1
☐ GAUT1-1 ID: 9655557	GAUT1, alpha-1,4-galacturonosyltransferase-like protein [*Selaginella moellendorffii*]		SELMODRAFT_451070
☐ GAUT1-2 ID: 9633129	GAUT1, alpha-1,4-galacturonosyltransferase-like protein [*Selaginella moellendorffii*]		SELMODRAFT_440136
☐ GAUT2-1 ID: 9643340	GAUT1, alpha-1,4-galaturonosyltransferase-like protein [*Selaginella*		SELMODRAFT_451073

Filters: Manage Filters

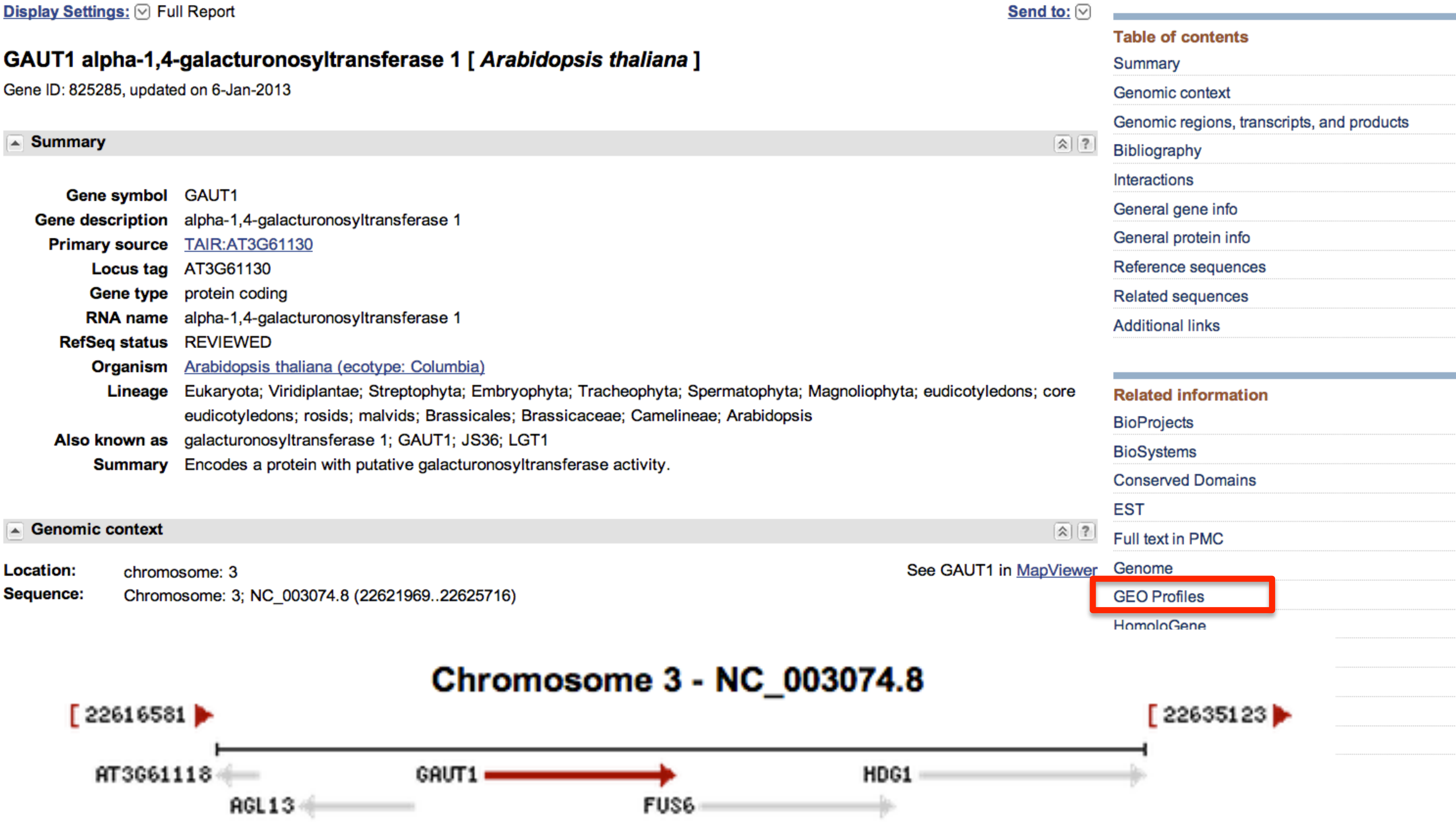
Top Organisms [Tree]
- Arabidopsis thaliana *(14)*
- Selaginella moellendorffii *(6)*
- Arabidopsis lyrata subsp. lyrata *(1)*

Find related data

Database: Select

Search details

```
gaut1[All Fields]
```



Display Settings: Full Report

Send to:

GAUT1 alpha-1,4-galacturonosyltransferase 1 [*Arabidopsis thaliana*]

Gene ID: 825285, updated on 6-Jan-2013

Summary

Gene symbol	GAUT1
Gene description	alpha-1,4-galacturonosyltransferase 1
Primary source	TAIR:AT3G61130
Locus tag	AT3G61130
Gene type	protein coding
RNA name	alpha-1,4-galacturonosyltransferase 1
RefSeq status	REVIEWED
Organism	Arabidopsis thaliana (ecotype: Columbia)
Lineage	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; malvids; Brassicales; Brassicaceae; Camelineae; Arabidopsis
Also known as	galacturonosyltransferase 1; GAUT1; JS36; LGT1
Summary	Encodes a protein with putative galacturonosyltransferase activity.

Genomic context

Location: chromosome: 3

Sequence: Chromosome: 3; NC_003074.8 (22621969..22625716)

See GAUT1 in MapViewer

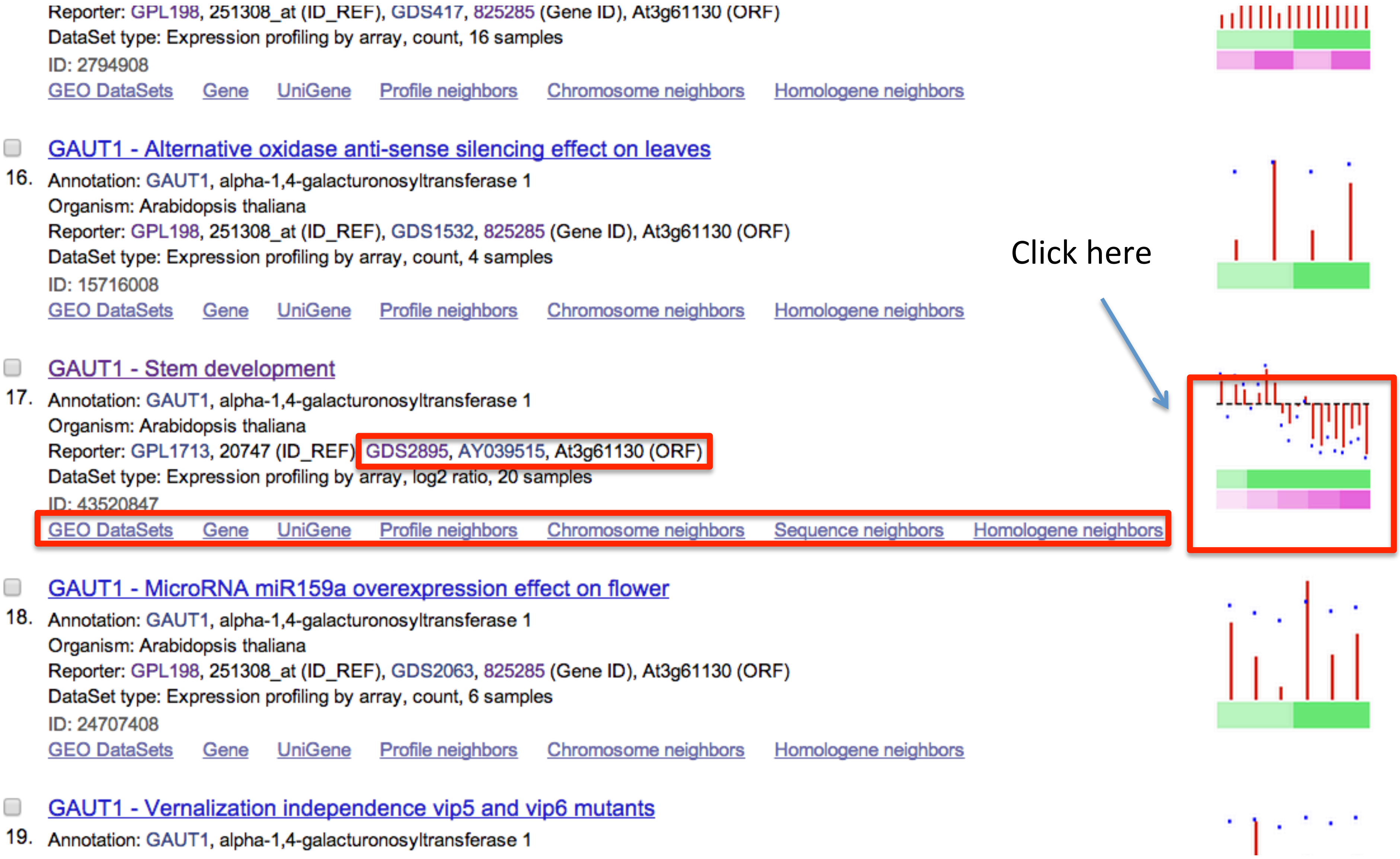
Table of contents

- Summary
- Genomic context
- Genomic regions, transcripts, and products
- Bibliography
- Interactions
- General gene info
- General protein info
- Reference sequences
- Related sequences
- Additional links

Related information

- BioProjects
- BioSystems
- Conserved Domains
- EST
- Full text in PMC
- Genome
- GEO Profiles
- HomoloGene

Scroll down to find record 17



Reporter: GPL198, 251308_at (ID_REF), GDS417, 825285 (Gene ID), At3g61130 (ORF)
DataSet type: Expression profiling by array, count, 16 samples
ID: 2794908
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Homologene neighbors

☐ GAUT1 - Alternative oxidase anti-sense silencing effect on leaves
16. Annotation: GAUT1, alpha-1,4-galacturonosyltransferase 1
Organism: Arabidopsis thaliana
Reporter: GPL198, 251308_at (ID_REF), GDS1532, 825285 (Gene ID), At3g61130 (ORF)
DataSet type: Expression profiling by array, count, 4 samples
ID: 15716008
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Homologene neighbors

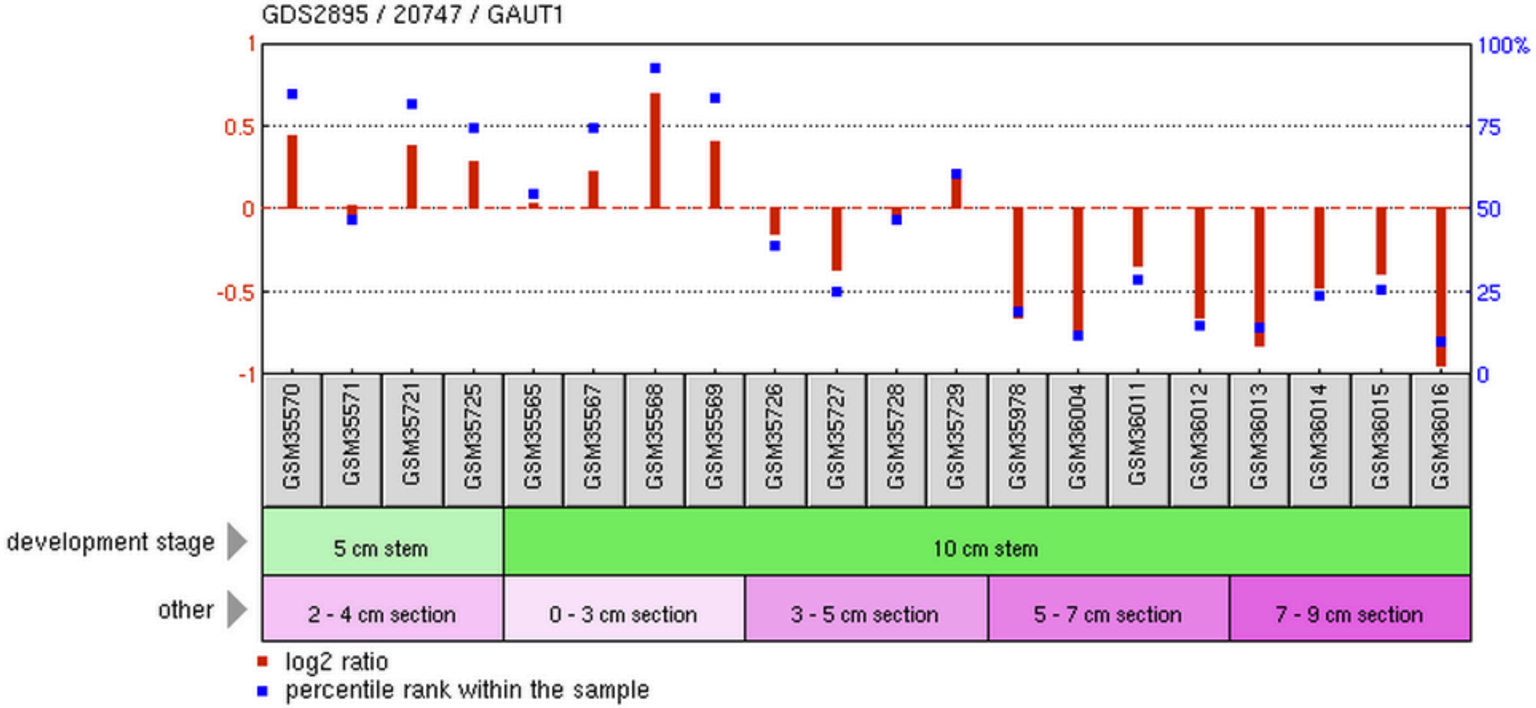
☐ GAUT1 - Stem development
17. Annotation: GAUT1, alpha-1,4-galacturonosyltransferase 1
Organism: Arabidopsis thaliana
Reporter: GPL1713, 20747 (ID_REF), GDS2895, AY039515, At3g61130 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43520847
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors Homologene neighbors

☐ GAUT1 - MicroRNA miR159a overexpression effect on flower
18. Annotation: GAUT1, alpha-1,4-galacturonosyltransferase 1
Organism: Arabidopsis thaliana
Reporter: GPL198, 251308_at (ID_REF), GDS2063, 825285 (Gene ID), At3g61130 (ORF)
DataSet type: Expression profiling by array, count, 6 samples
ID: 24707408
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Homologene neighbors

☐ GAUT1 - Vernalization independence vip5 and vip6 mutants
19. Annotation: GAUT1, alpha-1,4-galacturonosyltransferase 1

Click here

Profile GDS2895 / 20747 / GAUT1
Title Stem development
Organism Arabidopsis thaliana



Graph caption help

Sample	Value	Rank
GSM35570	0.456367	85
GSM35571	-0.00232472	47
GSM35721	0.388293	82
GSM35725	0.297085	75
GSM35565	0.0420401	55
GSM35567	0.23765	75
GSM35568	0.7	93
GSM35569	0.421382	84

Go back to result page

Profile neighbors: what are the co-expressed genes sharing similar expression profiles?

Summary, 20 per page, Sorted by Default order

Results: 1 to 20 of 200

☐ GAUT1 - Stem development

1. Annotation: GAUT1, GAUT1 (GALACTURONOSYLTRANSFERASE 1); polygalacturonate 4-alpha-galacturonosyltransferase/ transferase, transferring glycosyl groups
Organism: Arabidopsis thaliana
Reporter: GPL1713, 20747 (ID_REF), GDS2895, AY039515, At3g61130 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43520847
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors Homologene neighbors

☐ Stem development

2. Annotation: Arabidopsis thaliana chromosome 1 BAC F3C3 genomic sequence, complete sequence
Organism: Arabidopsis thaliana
Reporter: GPL1713, 28681 (ID_REF), GDS2895, AC084165, At1g32080 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43528781
GEO DataSets Profile neighbors Sequence neighbors

☐ Stem development

3. Annotation: Arabidopsis thaliana DNA chromosome 4, contig fragment No. 43
Organism: Arabidopsis thaliana
Reporter: GPL1713, 24698 (ID_REF), GDS2895, AL161543, At4g16180 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43524798
GEO DataSets Profile neighbors Sequence neighbors

☐ Stem development

4. Annotation: Arabidopsis thaliana genomic DNA, chromosome 5, TAC clone:K21H1
Organism: Arabidopsis thaliana
Reporter: GPL1713, 19369 (ID_REF), GDS2895, AB020742, At5g67180 (ORF)

atabase: Select

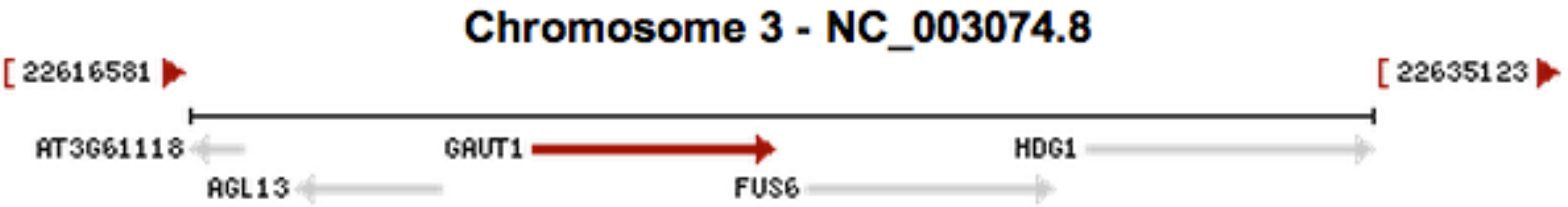
Profile neighbors for GEO Profiles (Sel
43520847) (200) GEC

GEO Profiles for Gene (Select 825285)
GEC

gaut1 (21)

GAUT1 [Arabidopsis thaliana]

Gene Links for GEO Profiles (Select



Chromosome neighbors: are neighboring genes co-expressed?

Results: 20

☐ 1. AT3G60880 - Stem development
Annotation: AT3G60880, dihydrodipicolinate synthase 1 (DHDPS1) (DHDPS) (DHPS1)
Organism: Arabidopsis thaliana
Reporter: GPL1713, 24307 (ID_REF), GDS2895, X72971, At3g60880 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43524407
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors Homologene neighbors

☐ 2. AtPP2-A13 - Stem development
Annotation: AtPP2-A13, AtPP2-A13 (Arabidopsis thaliana phloem protein 2-A13); carbohydrate binding
Organism: Arabidopsis thaliana
Reporter: GPL1713, 22586 (ID_REF), GDS2895, AY034967, At3g61060 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43522686
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors Homologene neighbors

☐ 3. FUS6 - Stem development
Annotation: FUS6, FUS6 (FUSCA 6)
Organism: Arabidopsis thaliana
Reporter: GPL1713, 21904 (ID_REF), GDS2895, AF360295, At3g61140 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43522004
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors Homologene neighbors

☐ 4. SYP73 - Stem development
Annotation: SYP73, SYP73 (SYNTAXIN OF PLANTS 73); protein transporter
Organism: Arabidopsis thaliana
Reporter: GPL1713, 21301 (ID_REF), GDS2895, AF355759, At3g61450 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43521401
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors

☐ GAUT1 - Stem development

Profile data

Profile pathways

Find related data
Database: Select

Recent activity

Chromosome neighbors for GEO Profiles (Select 43520847) (20) GEO P
Similar studies for GEO DataSets (Selec 200013043) (20) GEO Da
(poplar stem) AND "Populus trichocarpa" [porgn] (59) GEO Da
poplar stem (319) GEO Da
arabidopsis stem (467) GEO Da
See m

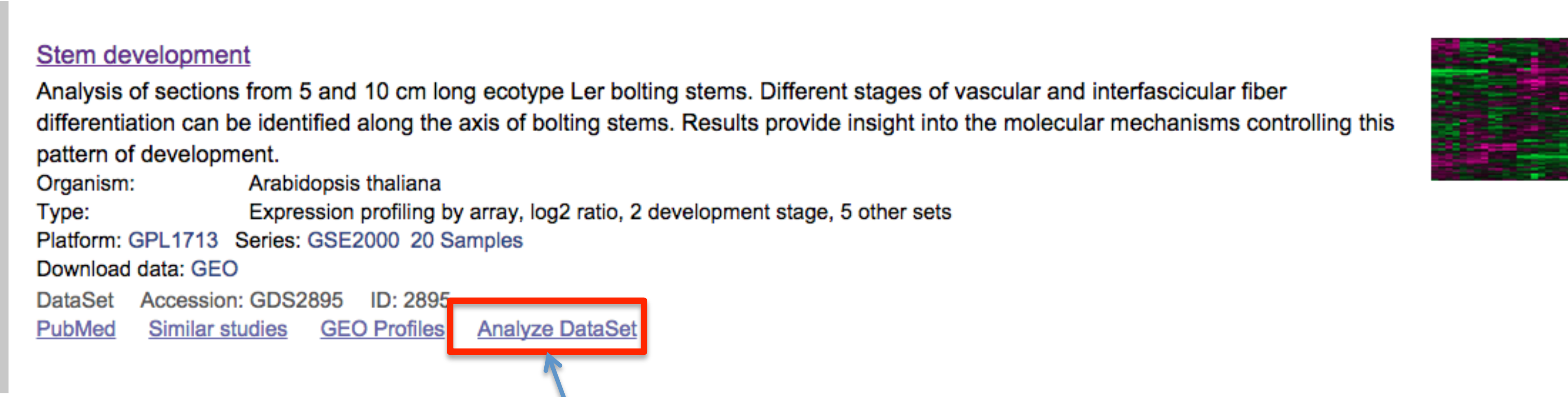
Hands on exercise 3

GEO DataSets analysis tool

GAUT1 - Stem development

Annotation: GAUT1, alpha-1,4-galacturonosyltransferase 1
Organism: Arabidopsis thaliana
Reporter: GPL1713, 20747 (ID_REF), GDS2895, AY039515, At3g61130 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43520847

Click



Stem development

Analysis of sections from 5 and 10 cm long ecotype Ler bolting stems. Different stages of vascular and interfascicular fiber differentiation can be identified along the axis of bolting stems. Results provide insight into the molecular mechanisms controlling this pattern of development.

Organism: Arabidopsis thaliana

Type: Expression profiling by array, log2 ratio, 2 development stage, 5 other sets

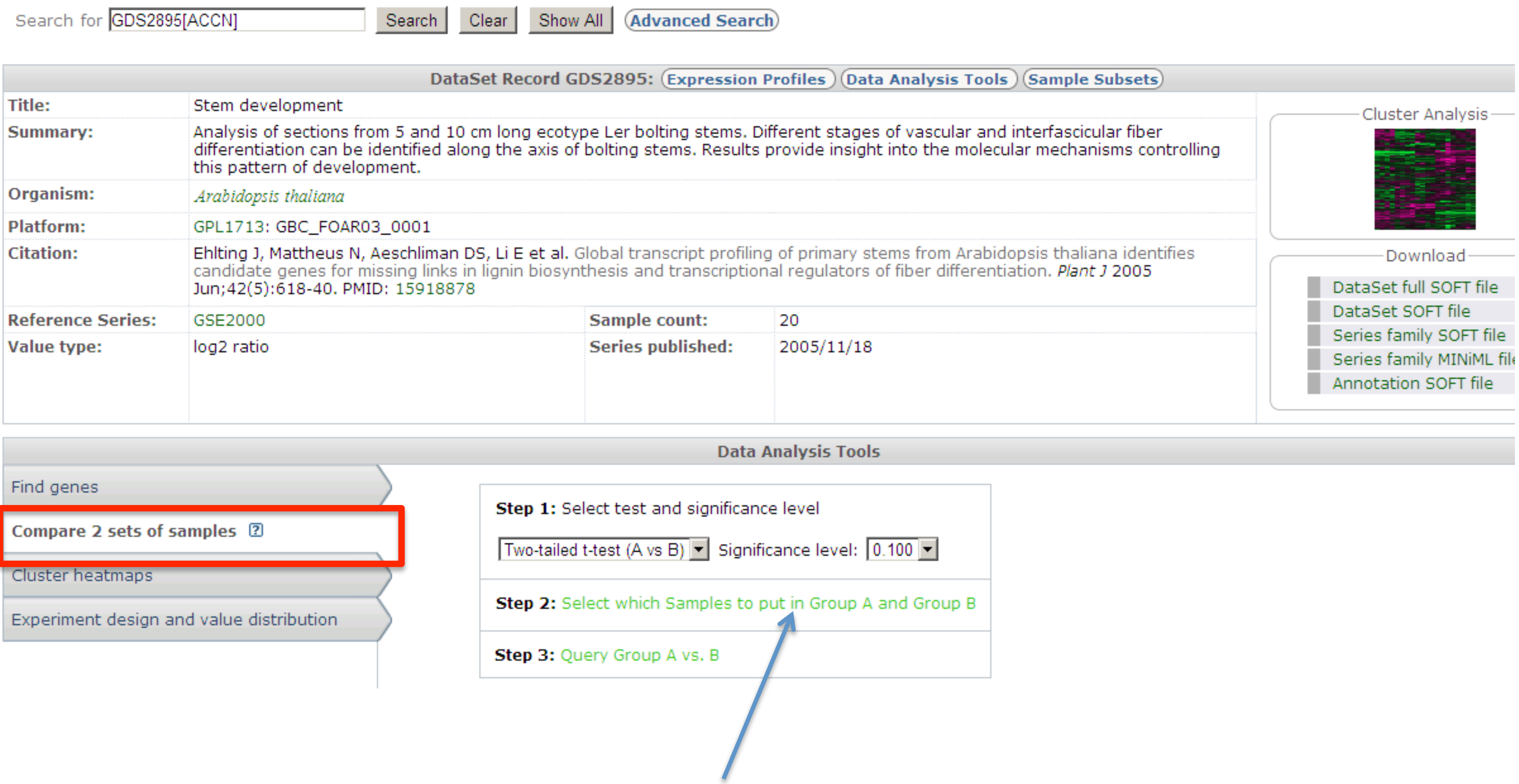
Platform: GPL1713 Series: GSE2000 20 Samples

Download data: GEO

DataSet Accession: GDS2895 ID: 2895

Click

We want to use this DataSet to identify differentially expressed genes in stem development
How: define two groups of samples and run two sample t test



Search for GDS2895[ACCN]

DataSet Record GDS2895:

Title:	Stem development		
Summary:	Analysis of sections from 5 and 10 cm long ecotype Ler bolting stems. Different stages of vascular and interfascicular fiber differentiation can be identified along the axis of bolting stems. Results provide insight into the molecular mechanisms controlling this pattern of development.		
Organism:	*Arabidopsis thaliana*		
Platform:	GPL1713: GBC_FOAR03_0001		
Citation:	Ehlting J, Mattheus N, Aeschliman DS, Li E et al. Global transcript profiling of primary stems from Arabidopsis thaliana identifies candidate genes for missing links in lignin biosynthesis and transcriptional regulators of fiber differentiation. *Plant J* 2005 Jun;42(5):618-40. PMID: 15918878		
Reference Series:	GSE2000	**Sample count:**	20
Value type:	log2 ratio	**Series published:**	2005/11/18

Data Analysis Tools

Step 1: Select test and significance level

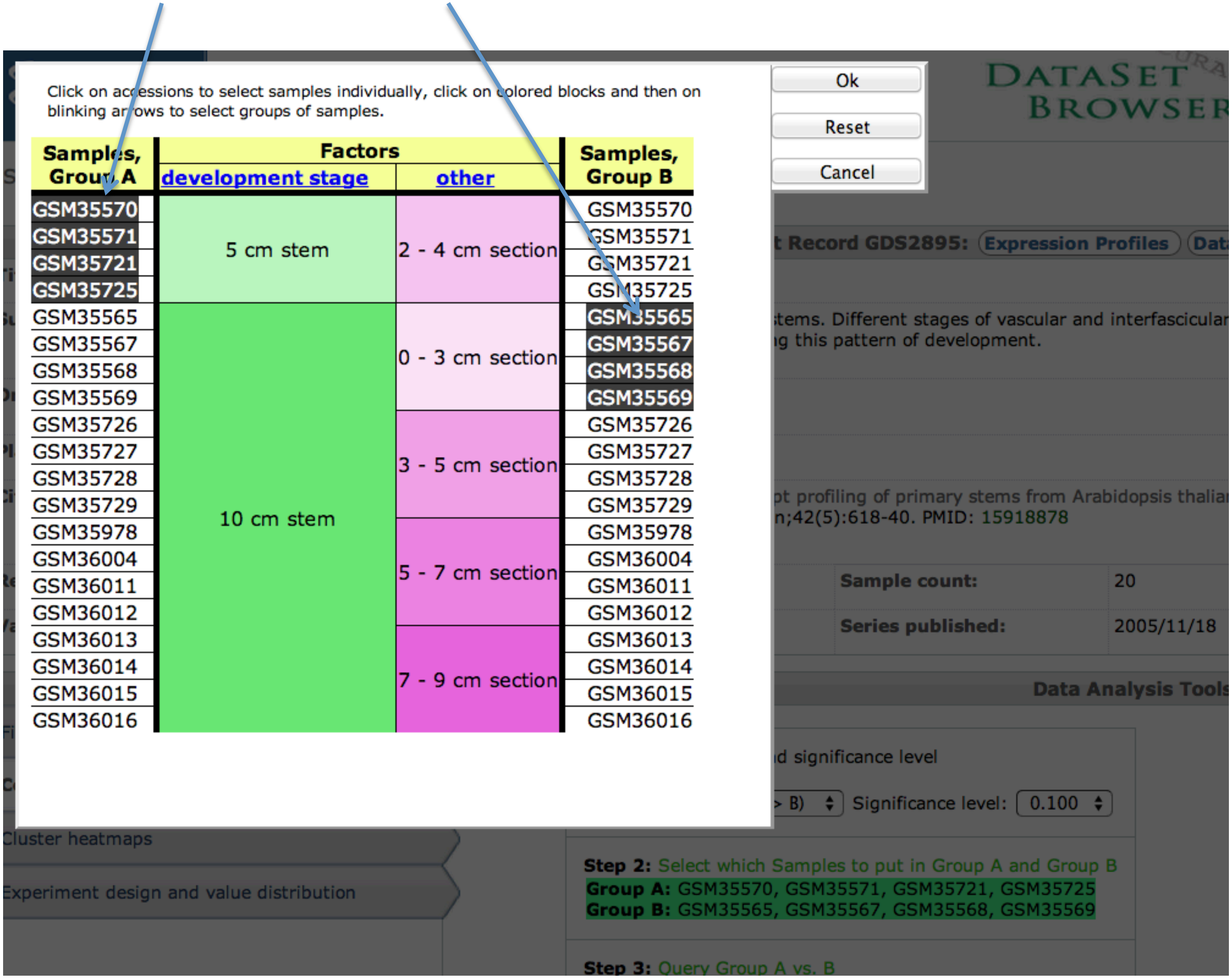
Two-tailed t-test (A vs B) Significance level: 0.100

Step 2: Select which Samples to put in Group A and Group B

Step 3: Query Group A vs. B

Click on step 2 to define two groups of samples

Click samples to select



Click on accessions to select samples individually, click on colored blocks and then on blinking arrows to select groups of samples.

Samples, Group A	Factors		Samples, Group B
	development stage	other	
GSM35570	5 cm stem	2 - 4 cm section	GSM35570
GSM35571			GSM35571
GSM35721			GSM35721
GSM35725			GSM35725
GSM35565	10 cm stem	0 - 3 cm section	GSM35565
GSM35567			GSM35567
GSM35568			GSM35568
GSM35569			GSM35569
GSM35726		3 - 5 cm section	GSM35726
GSM35727			GSM35727
GSM35728			GSM35728
GSM35729			GSM35729
GSM35978		5 - 7 cm section	GSM35978
GSM36004			GSM36004
GSM36011			GSM36011
GSM36012			GSM36012
GSM36013		7 - 9 cm section	GSM36013
GSM36014			GSM36014
GSM36015			GSM36015
GSM36016			GSM36016

t Record GDS2895:

tems. Different stages of vascular and interfascicular
g this pattern of development.

pt profiling of primary stems from Arabidopsis thalia
n;42(5):618-40. PMID: 15918878

Sample count: 20

Series published: 2005/11/18

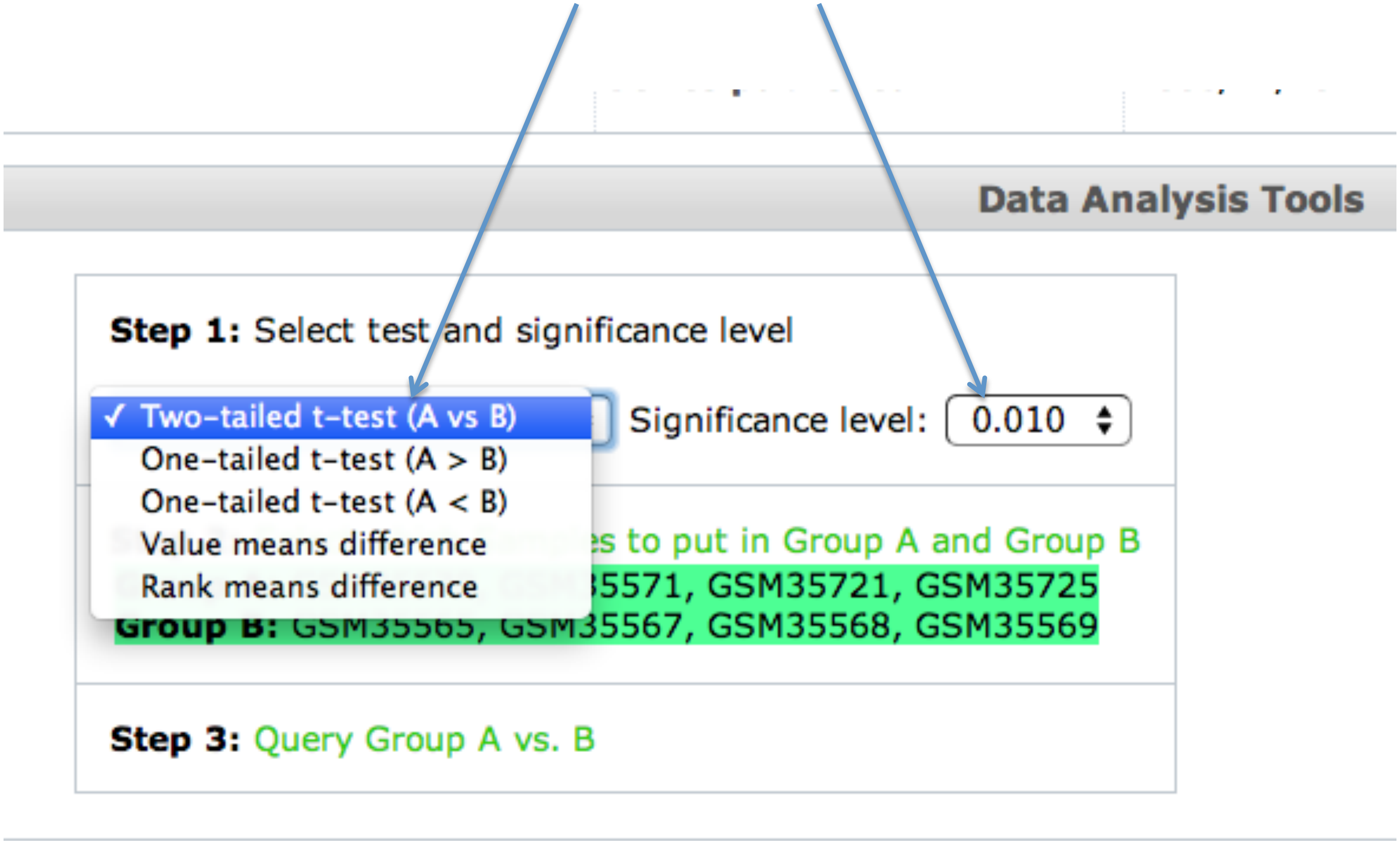
d significance level

> B) Significance level: 0.100

Step 2: Select which Samples to put in Group A and Group B
Group A: GSM35570, GSM35571, GSM35721, GSM35725
Group B: GSM35565, GSM35567, GSM35568, GSM35569

Step 3: Query Group A vs. B

Step 1: you can choose different statistical methods for analysis



Step 1: Select test and significance level

Significance level: 0.010

es to put in Group A and Group B

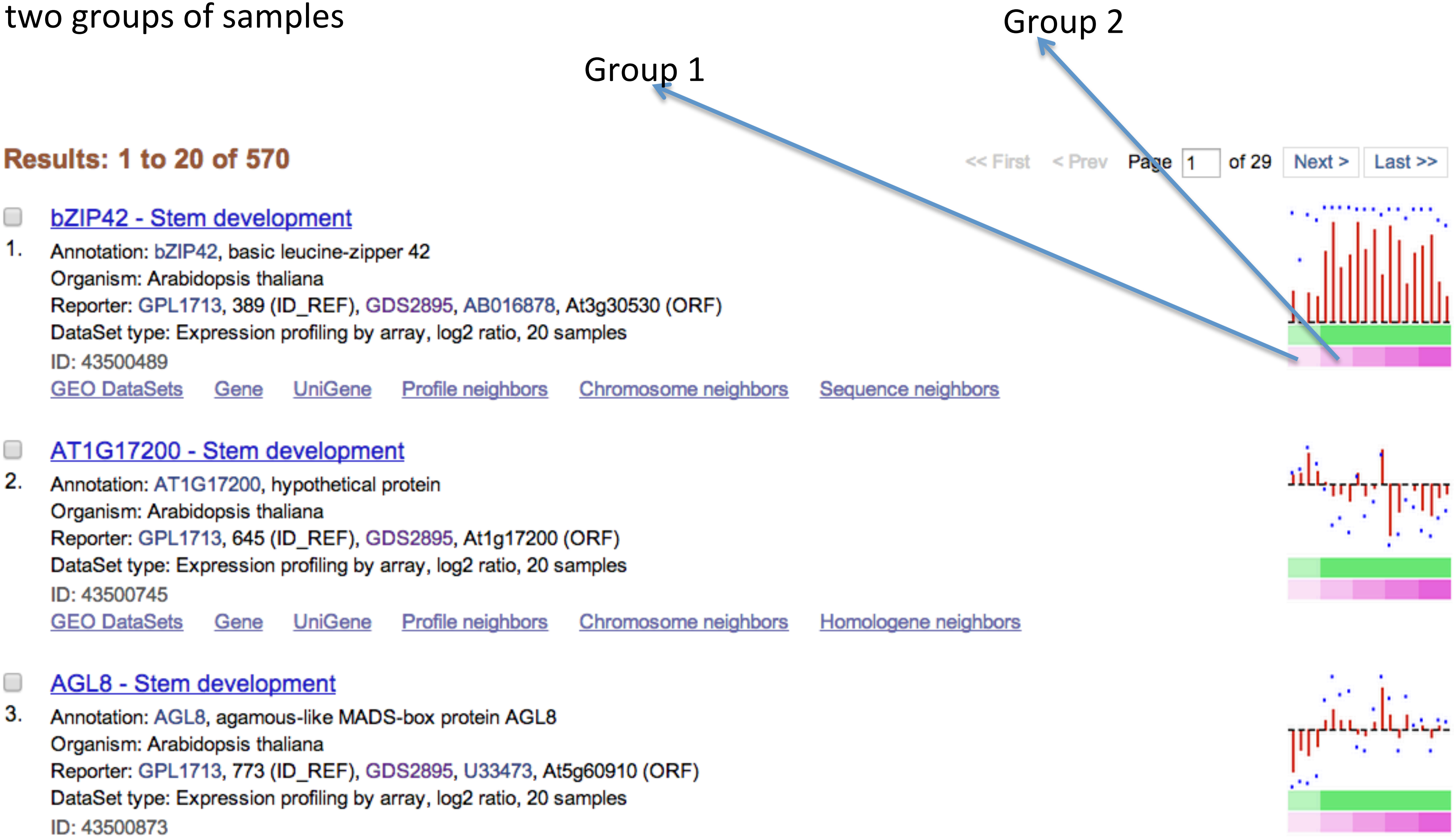
35571, GSM35721, GSM35725

Group B: GSM35565, GSM35567, GSM35568, GSM35569

Step 3: Query Group A vs. B

Step 3 to perform analysis

Result page is a list of genes with significantly different expression between two groups of samples



Results: 1 to 20 of 570

☐ bZIP42 - Stem development

1. Annotation: bZIP42, basic leucine-zipper 42
Organism: Arabidopsis thaliana
Reporter: GPL1713, 389 (ID_REF), GDS2895, AB016878, At3g30530 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43500489
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Sequence neighbors

☐ AT1G17200 - Stem development

2. Annotation: AT1G17200, hypothetical protein
Organism: Arabidopsis thaliana
Reporter: GPL1713, 645 (ID_REF), GDS2895, At1g17200 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43500745
GEO DataSets Gene UniGene Profile neighbors Chromosome neighbors Homologene neighbors

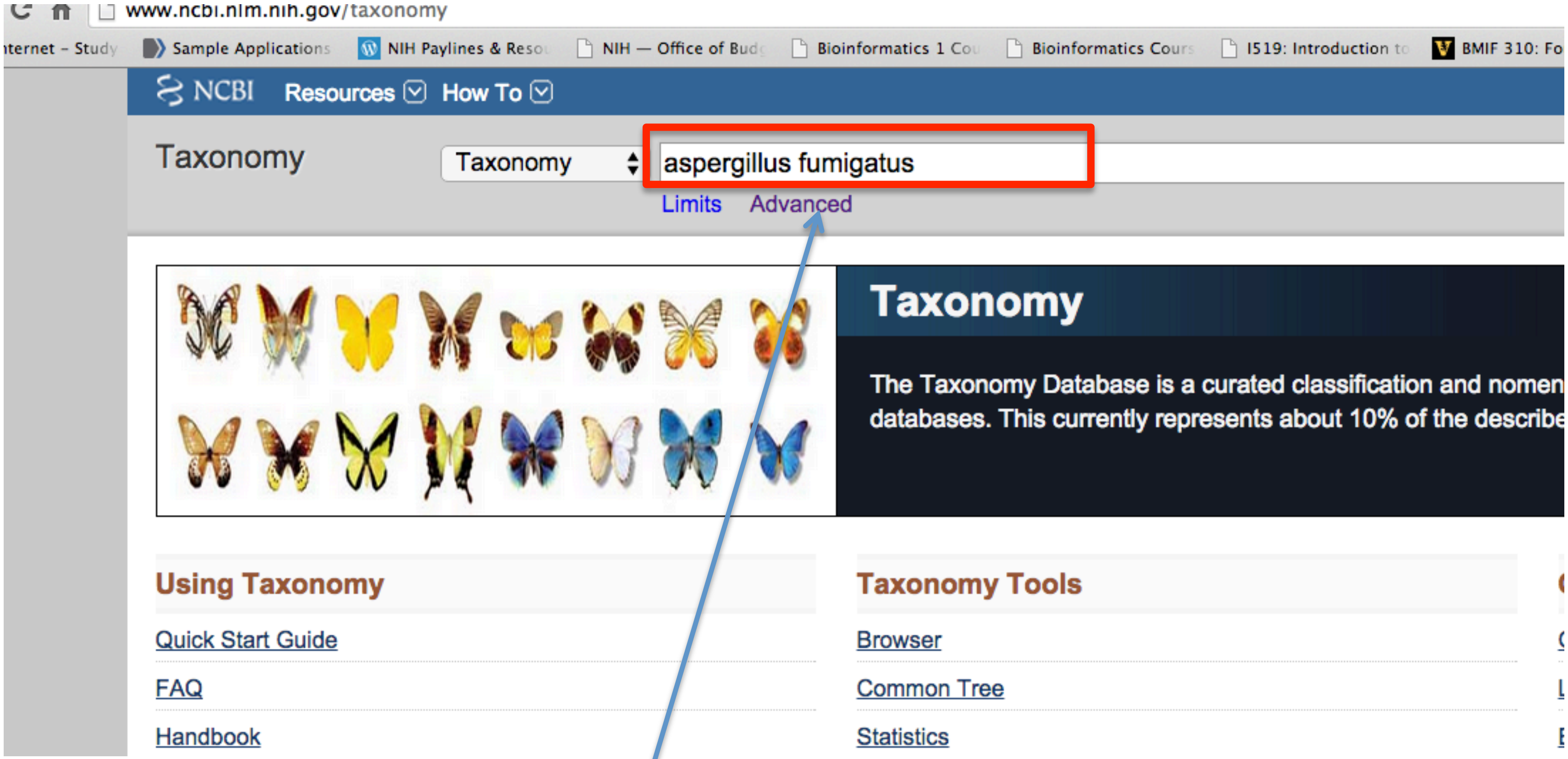
☐ AGL8 - Stem development

3. Annotation: AGL8, agamous-like MADS-box protein AGL8
Organism: Arabidopsis thaliana
Reporter: GPL1713, 773 (ID_REF), GDS2895, U33473, At5g60910 (ORF)
DataSet type: Expression profiling by array, log2 ratio, 20 samples
ID: 43500873

"Analyze DataSet" is for GEO DataSets
"GEO2R" is for GEO Series

GEO2R: differentially expressed genes

http://www.youtube.com/watch?v=EUPmGWS8ik0



www.ncbi.nlm.nih.gov/taxonomy

Taxonomy: aspergillus fumigatus

Taxonomy

The Taxonomy Database is a curated classification and nomen
databases. This currently represents about 10% of the describe

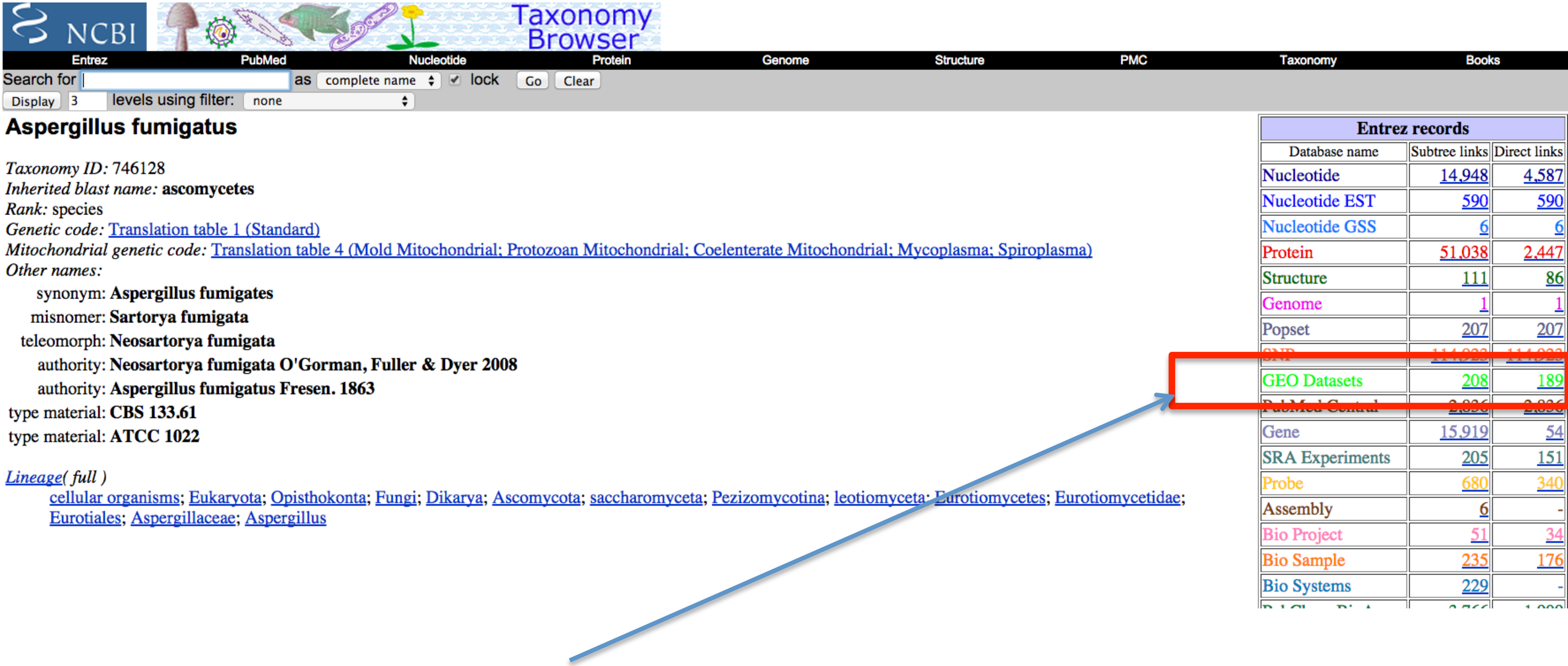
Using Taxonomy

- Quick Start Guide
- FAQ
- Handbook

Taxonomy Tools

- Browser
- Common Tree
- Statistics

Go to taxonomy page, and put the species name you want to search



Aspergillus fumigatus

Taxonomy ID: 746128
Inherited blast name: **ascomycetes**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 4 (Mold Mitochondrial; Protozoan Mitochondrial; Coelenterate Mitochondrial; Mycoplasma; Spiroplasma)
Other names:
synonym: **Aspergillus fumigates**
misnomer: **Sartorya fumigata**
teleomorph: **Neosartorya fumigata**
authority: **Neosartorya fumigata O'Gorman, Fuller & Dyer 2008**
authority: **Aspergillus fumigatus Fresen. 1863**
type material: **CBS 133.61**
type material: **ATCC 1022**

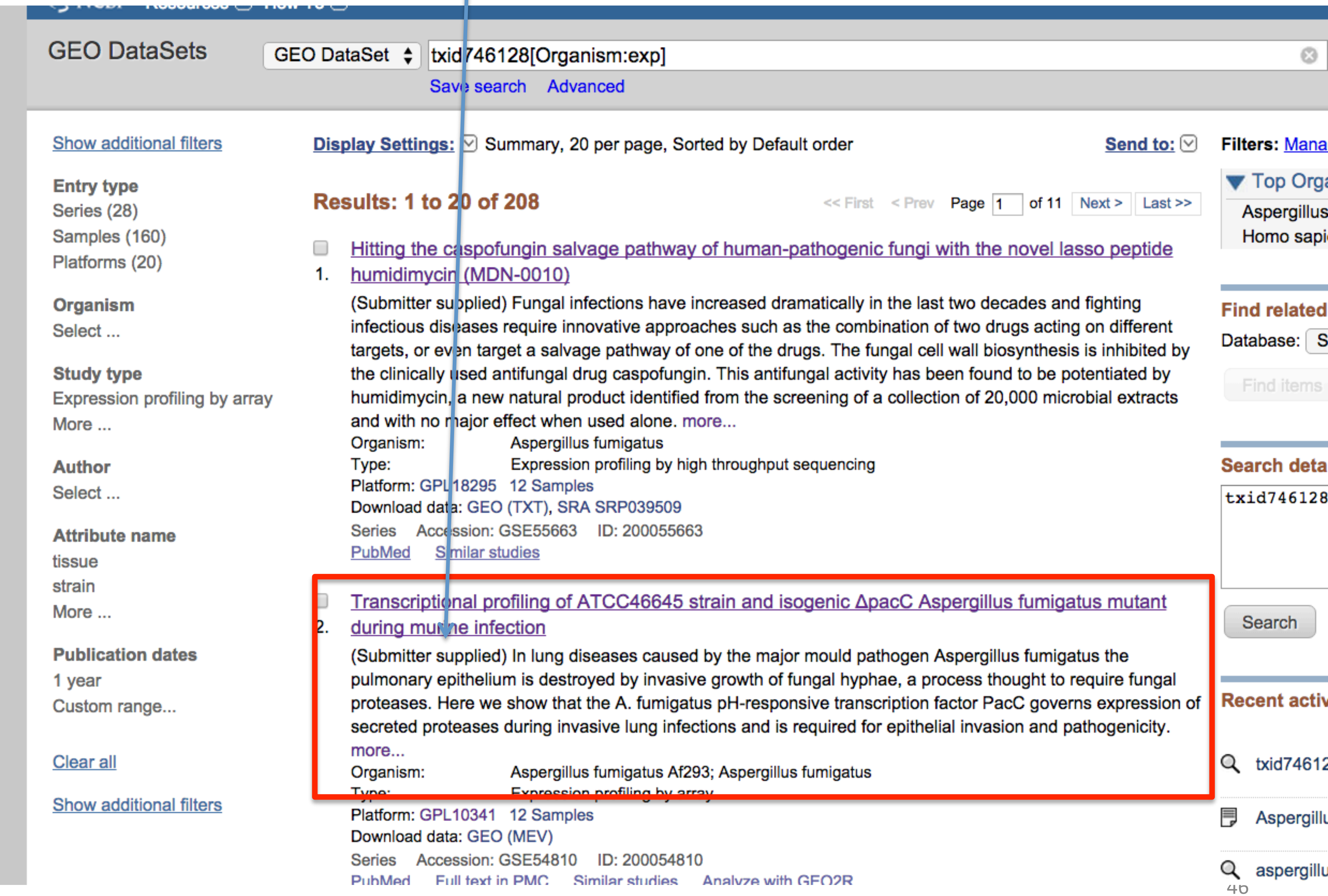
Lineage(full)
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae; Aspergillus

Entrez records

Database name	Subtree links	Direct links
Nucleotide	14,948	4,587
Nucleotide EST	590	590
Nucleotide GSS	6	6
Protein	51,038	2,447
Structure	111	86
Genome	1	1
Popset	207	207
SNP	114,923	114,923
GEO Datasets	208	189
PubMed Central	2,836	2,836
Gene	15,919	54
SRA Experiments	205	151
Probe	680	340
Assembly	6	-
Bio Project	51	34
Bio Sample	235	176
Bio Systems	229	-

Go to GEO datasets

Let’s try the second one



Summary, 20 per page, Sorted by Default order

Entry type
Series (28)
Samples (160)
Platforms (20)

Organism
Select ...

Study type
Expression profiling by array
More ...

Author
Select ...

Attribute name
tissue
strain
More ...

Publication dates
1 year
Custom range...

Clear all

Show additional filters

Results: 1 to 20 of 208

☐ 1. Hitting the caspofungin salvage pathway of human-pathogenic fungi with the novel lasso peptide humidimycin (MDN-0010)

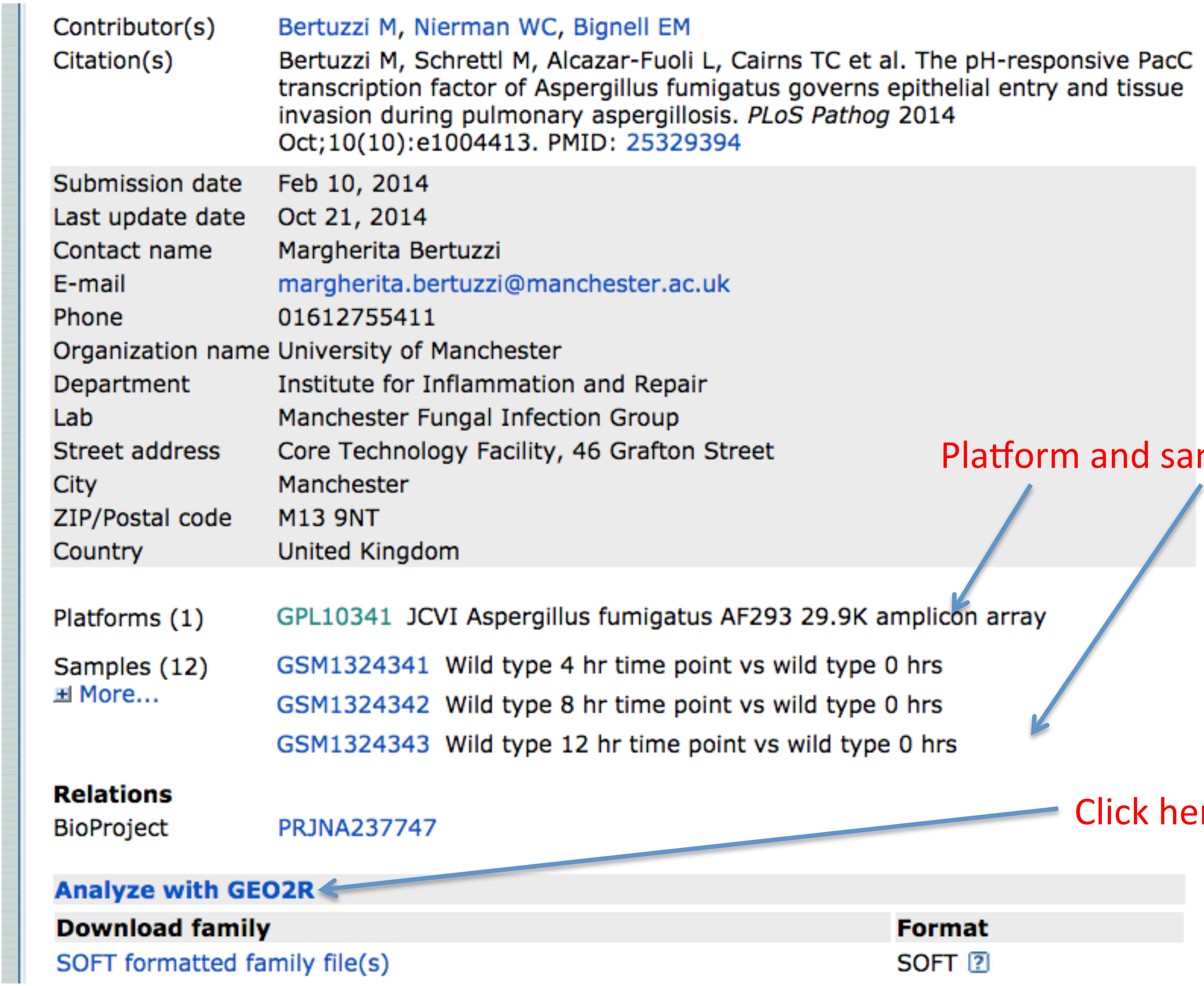
(Submitter supplied) Fungal infections have increased dramatically in the last two decades and fighting infectious diseases require innovative approaches such as the combination of two drugs acting on different targets, or even target a salvage pathway of one of the drugs. The fungal cell wall biosynthesis is inhibited by the clinically used antifungal drug caspofungin. This antifungal activity has been found to be potentiated by humidimycin, a new natural product identified from the screening of a collection of 20,000 microbial extracts and with no major effect when used alone. more...

Organism: Aspergillus fumigatus
Type: Expression profiling by high throughput sequencing
Platform: GPL18295 12 Samples
Download data: GEO (TXT), SRA SRP039509
Series Accession: GSE55663 ID: 200055663
PubMed Similar studies

☐ 2. Transcriptional profiling of ATCC46645 strain and isogenic ΔpacC Aspergillus fumigatus mutant during murine infection

(Submitter supplied) In lung diseases caused by the major mould pathogen Aspergillus fumigatus the pulmonary epithelium is destroyed by invasive growth of fungal hyphae, a process thought to require fungal proteases. Here we show that the A. fumigatus pH-responsive transcription factor PacC governs expression of secreted proteases during invasive lung infections and is required for epithelial invasion and pathogenicity. more...

Organism: Aspergillus fumigatus Af293; Aspergillus fumigatus
Type: Expression profiling by array
Platform: GPL10341 12 Samples
Download data: GEO (MEV)
Series Accession: GSE54810 ID: 200054810
PubMed Full text in PMC Similar studies Analyze with GEO2R



Contributor(s)	Bertuzzi M, Nierman WC, Bignell EM
Citation(s)	Bertuzzi M, Schrettl M, Alcazar-Fuoli L, Cairns TC et al. The pH-responsive PacC transcription factor of Aspergillus fumigatus governs epithelial entry and tissue invasion during pulmonary aspergillosis. *PLoS Pathog* 2014 Oct;10(10):e1004413. PMID: 25329394
Submission date	Feb 10, 2014
Last update date	Oct 21, 2014
Contact name	Margherita Bertuzzi
E-mail	margherita.bertuzzi@manchester.ac.uk
Phone	01612755411
Organization name	University of Manchester
Department	Institute for Inflammation and Repair
Lab	Manchester Fungal Infection Group
Street address	Core Technology Facility, 46 Grafton Street
City	Manchester
ZIP/Postal code	M13 9NT
Country	United Kingdom
Platforms (1)	GPL10341 JCVI Aspergillus fumigatus AF293 29.9K amplicon array
Samples (12) ⊞ More...	GSM1324341 Wild type 4 hr time point vs wild type 0 hrs GSM1324342 Wild type 8 hr time point vs wild type 0 hrs GSM1324343 Wild type 12 hr time point vs wild type 0 hrs

Relations

BioProject	PRJNA237747

Analyze with GEO2R

Download family	**Format**
SOFT formatted family file(s)	SOFT ?

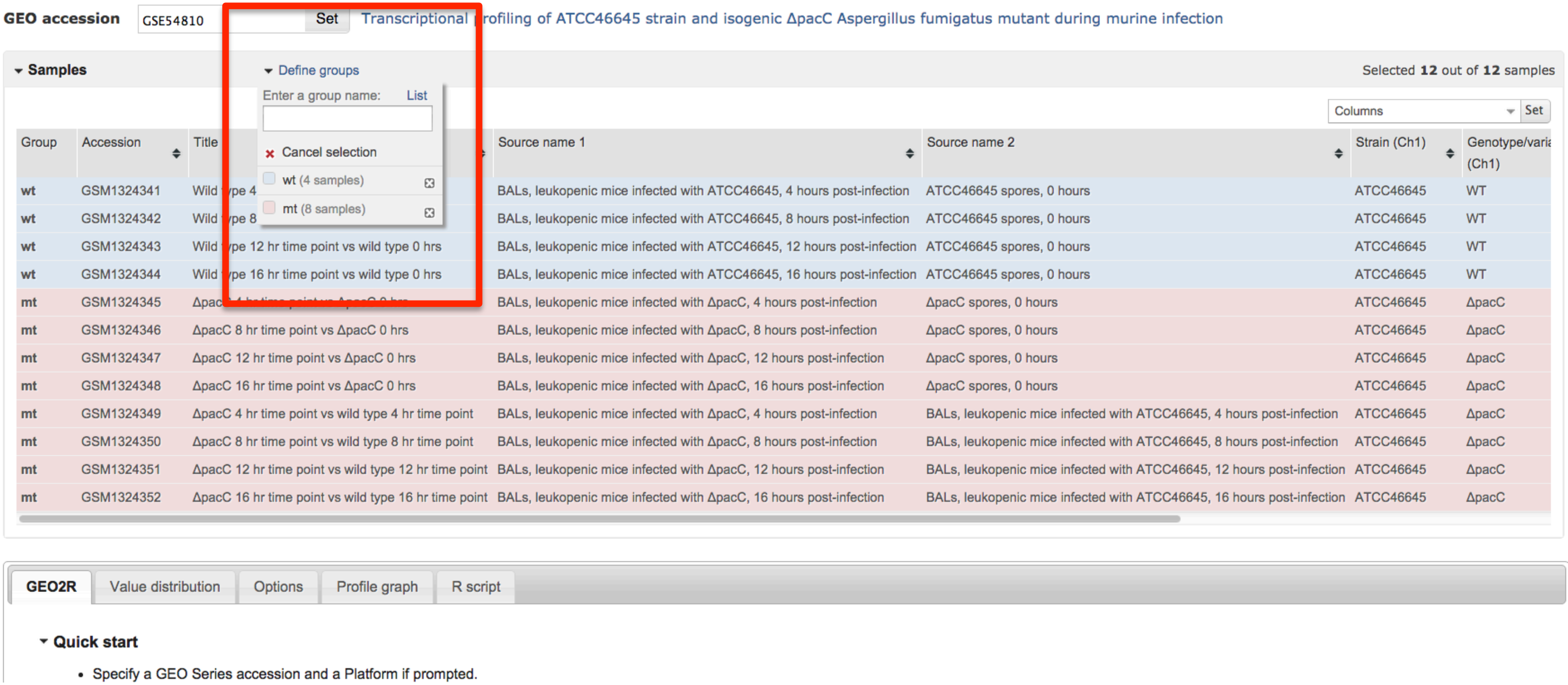
Platform and sample data

Click here

Click on Define groups and type in group names

Select samples from the table and click on the defined group to assign to the group

Click on Top 250 in the bottom of the page to run the job



GEO accession GSE54810 Set Transcriptional profiling of ATCC46645 strain and isogenic ΔpacC Aspergillus fumigatus mutant during murine infection

Samples

Define groups

Enter a group name: List

✖ Cancel selection

☐ wt (4 samples)

☐ mt (8 samples)

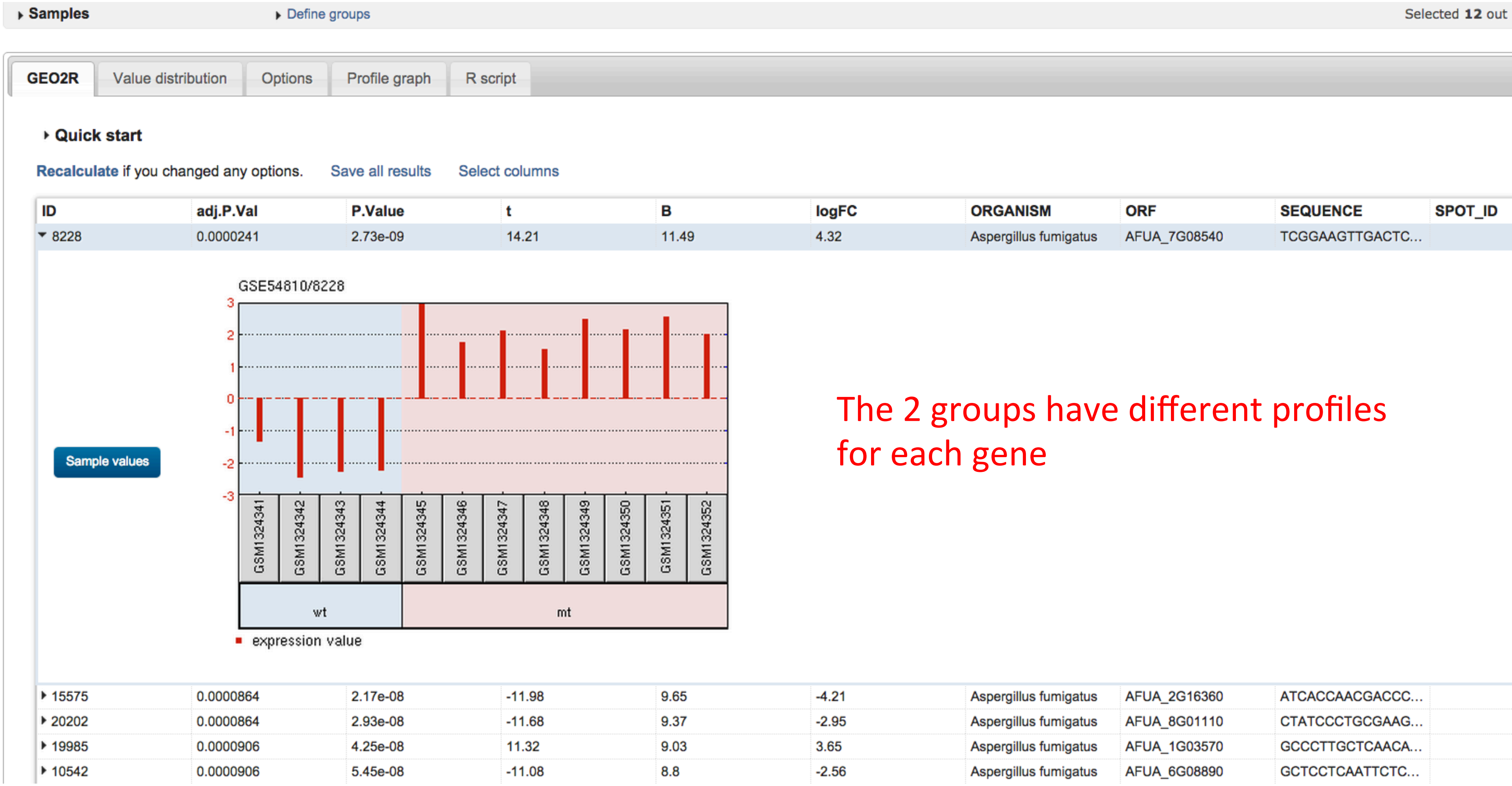
Selected **12** out of **12** samples

Group	Accession	Title	Source name 1	Source name 2	Strain (Ch1)	Genotype/varia (Ch1)
wt	GSM1324341	Wild type 4	BALs, leukopenic mice infected with ATCC46645, 4 hours post-infection	ATCC46645 spores, 0 hours	ATCC46645	WT
wt	GSM1324342	Wild type 8	BALs, leukopenic mice infected with ATCC46645, 8 hours post-infection	ATCC46645 spores, 0 hours	ATCC46645	WT
wt	GSM1324343	Wild type 12 hr time point vs wild type 0 hrs	BALs, leukopenic mice infected with ATCC46645, 12 hours post-infection	ATCC46645 spores, 0 hours	ATCC46645	WT
wt	GSM1324344	Wild type 16 hr time point vs wild type 0 hrs	BALs, leukopenic mice infected with ATCC46645, 16 hours post-infection	ATCC46645 spores, 0 hours	ATCC46645	WT
mt	GSM1324345	ΔpacC 4 hr time point vs ΔpacC 0 hrs	BALs, leukopenic mice infected with ΔpacC, 4 hours post-infection	ΔpacC spores, 0 hours	ATCC46645	ΔpacC
mt	GSM1324346	ΔpacC 8 hr time point vs ΔpacC 0 hrs	BALs, leukopenic mice infected with ΔpacC, 8 hours post-infection	ΔpacC spores, 0 hours	ATCC46645	ΔpacC
mt	GSM1324347	ΔpacC 12 hr time point vs ΔpacC 0 hrs	BALs, leukopenic mice infected with ΔpacC, 12 hours post-infection	ΔpacC spores, 0 hours	ATCC46645	ΔpacC
mt	GSM1324348	ΔpacC 16 hr time point vs ΔpacC 0 hrs	BALs, leukopenic mice infected with ΔpacC, 16 hours post-infection	ΔpacC spores, 0 hours	ATCC46645	ΔpacC
mt	GSM1324349	ΔpacC 4 hr time point vs wild type 4 hr time point	BALs, leukopenic mice infected with ΔpacC, 4 hours post-infection	BALs, leukopenic mice infected with ATCC46645, 4 hours post-infection	ATCC46645	ΔpacC
mt	GSM1324350	ΔpacC 8 hr time point vs wild type 8 hr time point	BALs, leukopenic mice infected with ΔpacC, 8 hours post-infection	BALs, leukopenic mice infected with ATCC46645, 8 hours post-infection	ATCC46645	ΔpacC
mt	GSM1324351	ΔpacC 12 hr time point vs wild type 12 hr time point	BALs, leukopenic mice infected with ΔpacC, 12 hours post-infection	BALs, leukopenic mice infected with ATCC46645, 12 hours post-infection	ATCC46645	ΔpacC
mt	GSM1324352	ΔpacC 16 hr time point vs wild type 16 hr time point	BALs, leukopenic mice infected with ΔpacC, 16 hours post-infection	BALs, leukopenic mice infected with ATCC46645, 16 hours post-infection	ATCC46645	ΔpacC

Quick start

- Specify a GEO Series accession and a Platform if prompted.

The result page, click on the ID will give the graph



Recalculate if you changed any options. Save all results Select columns

ID	adj.P.Val	P.Value	t	B	logFC	ORGANISM	ORF	SEQUENCE	SPOT_ID
8228	0.0000241	2.73e-09	14.21	11.49	4.32	Aspergillus fumigatus	AFUA_7G08540	TCGGAAGTTGACTC...	
15575	0.0000864	2.17e-08	-11.98	9.65	-4.21	Aspergillus fumigatus	AFUA_2G16360	ATCACCAACGACCC...	
20202	0.0000864	2.93e-08	-11.68	9.37	-2.95	Aspergillus fumigatus	AFUA_8G01110	CTATCCCTGCGAAG...	
19985	0.0000906	4.25e-08	11.32	9.03	3.65	Aspergillus fumigatus	AFUA_1G03570	GCCCTTGCTCAACA...	
10542	0.0000906	5.45e-08	-11.08	8.8	-2.56	Aspergillus fumigatus	AFUA_6G08890	GCTCCTCAATTCTC...	

The 2 groups have different profiles for each gene

You may check the detailed info of the gene in GPL table

ftp

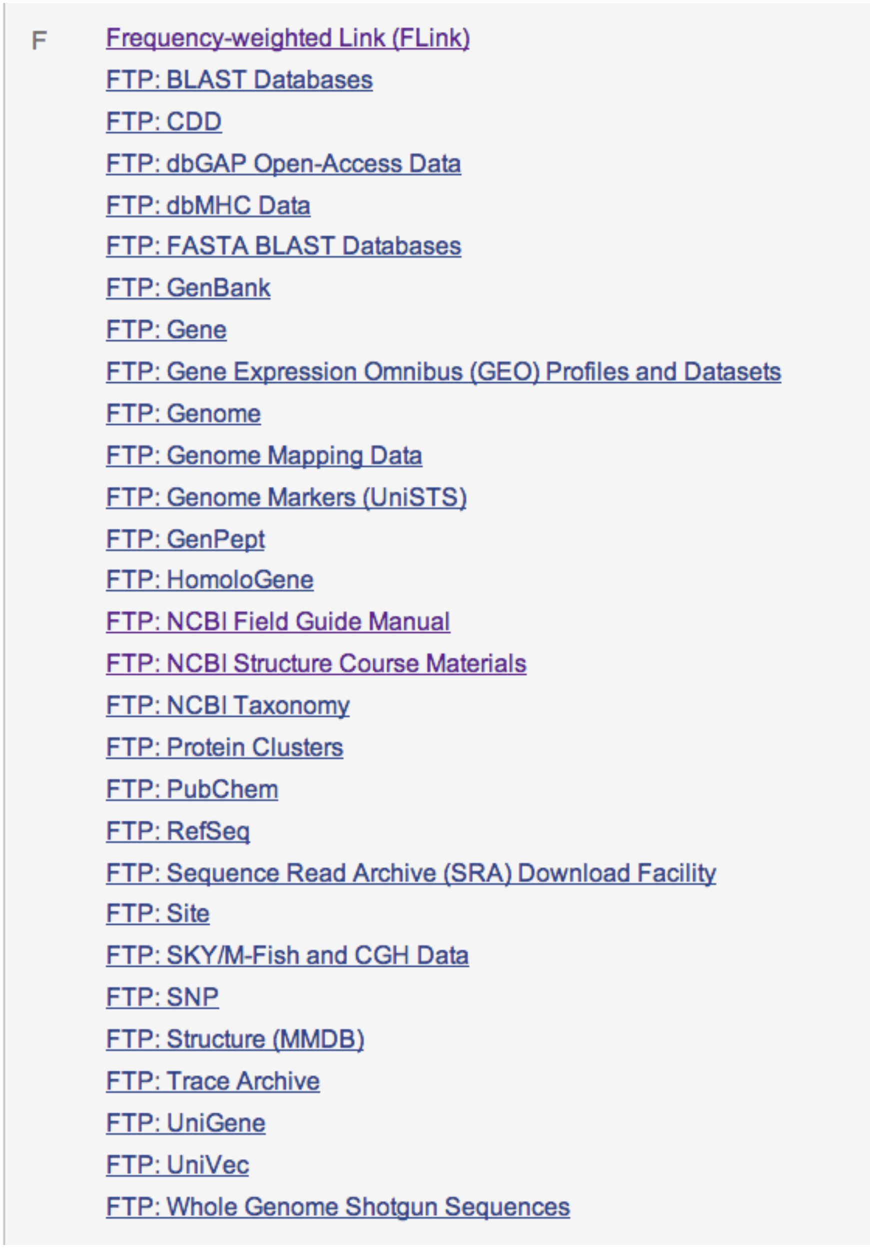
FTP stands for File Transfer Protocol.
HTTP stands for Hyper Text Transfer Protocol.

When ftp appears in a URL it means that the user is connecting to a file server and not a Web server and that some form of file transfer is going to take place.

When http appears in a URL it means that the user is connecting to a Web server and not a file server. The files are transferred but not downloaded, therefore not copied into the memory of the receiving device.

http://wiki.answers.com/Q/What_is_the_difference_between_FTP_and_HTTP

ftp server of NCBI



F

Frequency-weighted Link (FLink)
FTP: BLAST Databases
FTP: CDD
FTP: dbGAP Open-Access Data
FTP: dbMHC Data
FTP: FASTA BLAST Databases
FTP: GenBank
FTP: Gene
FTP: Gene Expression Omnibus (GEO) Profiles and Datasets
FTP: Genome
FTP: Genome Mapping Data
FTP: Genome Markers (UniSTS)
FTP: GenPept
FTP: HomoloGene
FTP: NCBI Field Guide Manual
FTP: NCBI Structure Course Materials
FTP: NCBI Taxonomy
FTP: Protein Clusters
FTP: PubChem
FTP: RefSeq
FTP: Sequence Read Archive (SRA) Download Facility
FTP: Site
FTP: SKY/M-Fish and CGH Data
FTP: SNP
FTP: Structure (MMDB)
FTP: Trace Archive
FTP: UniGene
FTP: UniVec
FTP: Whole Genome Shotgun Sequences

ftp resources

- Refseq genomes, proteins, mRNAs
- Microbial genomes
- Plant genomes
- Fungal genomes
- Blast database folder
- Sra reads
- Geo datasets

Next lecture: `EBI resources I`