Large-scale gene expression analysis

Qualitative and quantitative information on all transcribed sequences ("Transcriptome")

- >Changes during developmental processes and cell differentiation
- >Cell-type and tissue specific gene expression profiles
- >Genes induced and repressed in response to drugs or environmental hits
- ≻Genes regulated in response to cellular communication (signal transduction)
- Genes regulated in response to hormones
- >Individual specificity of gene expression
- >Changes during neoplastic transformation or other disease

How to measure the activity of all genes (genome-wide) in cells/tissues (mRNA).

Sequencing methods

(EST)

SAGE (LongSAGE, CAGE)

direct re-sequencing (deep sequencing)

Sequence approach

DNA microarrays, oligonucleotide microarrays.

Spotted arrays

In situ synthesized oligo arrays

Bead-arrays®

Hybridization approach

### Spotted

cDNA microarrays DNA microarrays	10,000 - 40,000 probes / cm <sup>2</sup> are spotted on glass slides using an automated microarrayer.
	Probes are cDNA or PCR products representing known genes or simply EST
	Chemically synthesized long oligonucleotides (50 – 70 nt) representing cds of known genes/EST

DNA chips: there are currently several types available:

## 2. In situ synthesized

Oligonucleotide arrays (Affychip®) Affymetrix	Up to 450,000 20-25nt long oligonucleotides / cm <sup>2</sup> are sythesized directly on the chip surface, using a photolitographic technique. Each gene is represented by a "probeset" of 12-13 probes.		
Long oligonucleotide arrays	Up to 250,000 different 30-60 nt long oligonucleotides / cm <sup>2</sup> are sythesized directly on the chip surface, using an ink-jet technique.		
Bead Arrays®	Oligonucleotide probes (30-50 nt) are synthesized on		
Illumina	beads, with a identification address. Beads are randomly arrayed on surfaces and position of each oligonucleotide determined using addresses.		

#### Spotted (pinspotted) DNA microarrays

A 1 - 40,000 probes / cm<sup>2</sup> are spotted on glass slides using an automated microarrayer.

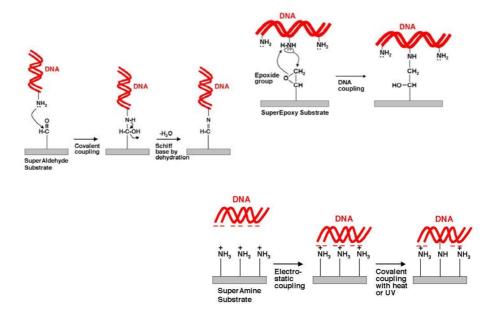
Probes are cDNA fragments or PCR products representing known genes or simply EST

Probe size: 200-1,000 nt

B Same as above, but with:

Oligonucleotide probes (30 - 70 nt in lenght)

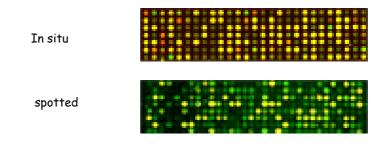
Single-strand



Up to 250,000 oligonucleotides /  $cm^2$  are sythesized directly on the chip surface, using an ink-jet technique.

These 60 nt long oligonucleotides represent sequences of known genes or EST

**Chemical synthesis of oligonucleotides** 



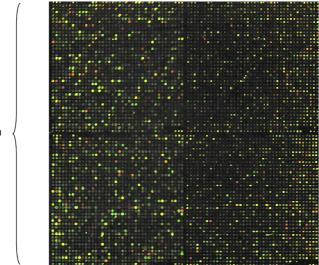
Spotted arrays, or ink-jet oligo arrays are commonly used for **relative** measurements, i.e. to compare gene expression between two biological samples.

RNA from sample and from reference are labeled by introducing two different fluorochromes.

This allows co-hybridization of the two samples to the same chip, providing direct comparison by two-color analysis

The most common fluorochromes are the cyanines Cy3 (red) and Cy5 (green)

## How a spotted microarrays hybridized with two-colors probes looks like



1 cm

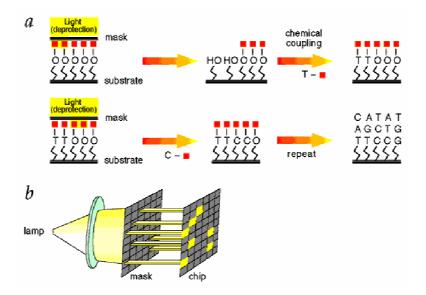
#### Short Oligonucleotide arrays (Affymetrix)

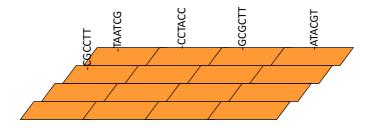
Up to millions of oligonucleotides / cm<sup>2</sup> are synthesized directly on the chip surface, using a photolithographic technique.

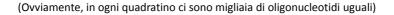
These 20-25 nt long oligonucleotides represent sequences of known genes or EST

**Chemical synthesis of oligonucleotides** 

Affymetrix technology allows direct synthesis of oligonucleotides in microzones on the glass slide by photolithograpy





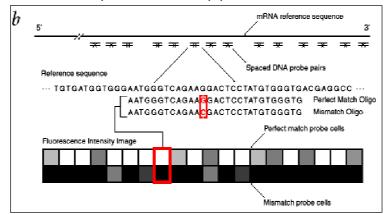


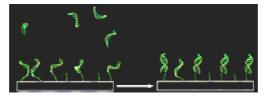
One limitation: the lenght of oligonucleotides (20-25 nt)

This can give problems of aspecific hybridization due to: -Similar sequences can be present in different mRNAs -Hybridization is done at the same temperature that is average of the optimal temperature for individual probe-target pairs

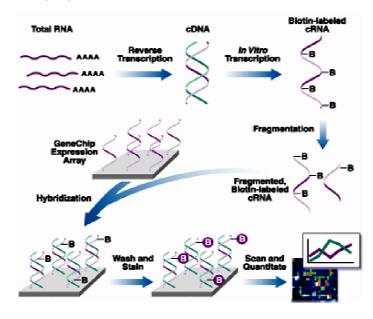
Affymetrix gives one solution to these problems:

#### Affymetrix GeneChip probe set



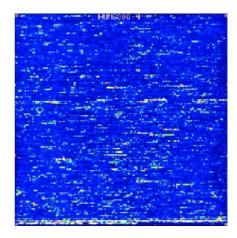


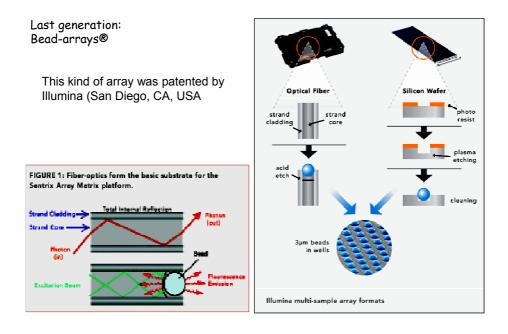
## How an Affychip is used



## How an Affychip result looks like

An oligonucleotide array (Affychip®) hybridized to biotin-labelled cRNA and revealed with fluorochromeconjugated avidin





illumina.



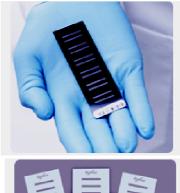
Each array cluster contains about 50,000 3-micron beads, or features, assembled in dense geometries.



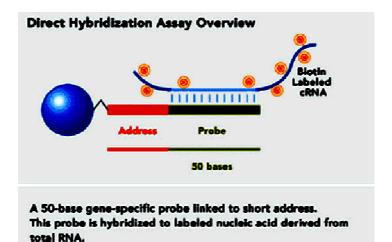
2 Over 1500 probes, or bead types, at >30x average feature redundancy, are represented in each array cluster.



3 Labeled sample targets hybridize to capture probes immobilized on the beads.







Reading of microarrays is performed with laser scanners, which allow a quantitation of fluorescence in different channels



Scanners produce a **table of values** that are intensities at each spot. If using double colors, relative fluorescence intensities in two channels are read.

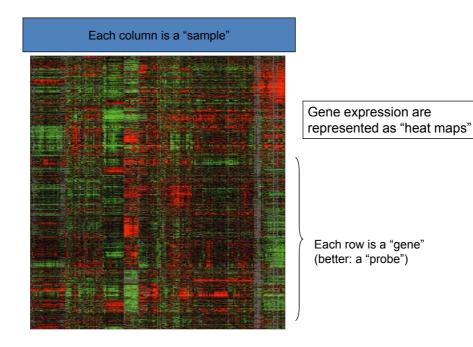
First important difference to know is therefore:

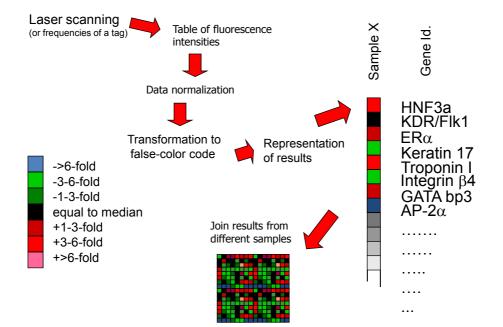
Absolute versus relative measurement

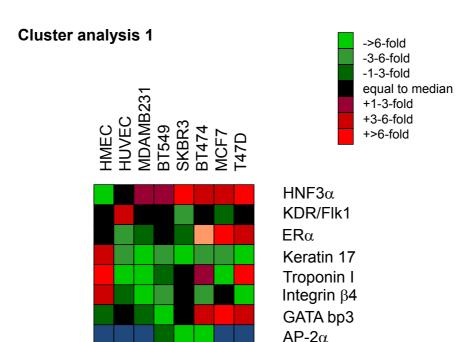
Using hybridization reaction as an absolute measurement of RNA requires that the amount of probe on each spot be uniform and reproducible.

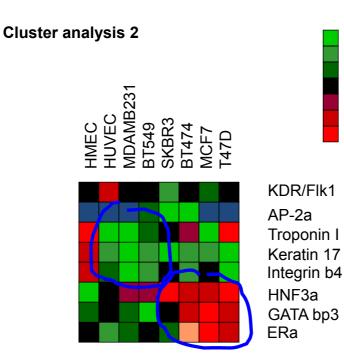
This is a requirement fullfilled by Affymetrix arrays and by the latest generation of in situ synthesized long-oligo arrays and bead arrays.

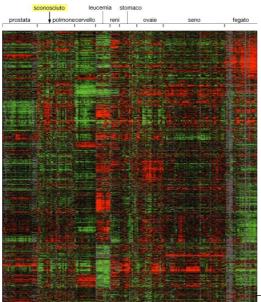
In all the other cases, the amount of probe / spot is variable and unassessable, so that **relative** measurements are necessary.











Gene expression are represented as "heat maps"

->6-fold -3-6-fold -1-3-fold

+1-3-fold

+3-6-fold +>6-fold

equal to median

Different expression profiles in human cells of different tissues: 1800 genes probes

# The Transcriptional Program of Sporulation in Budding Yeast

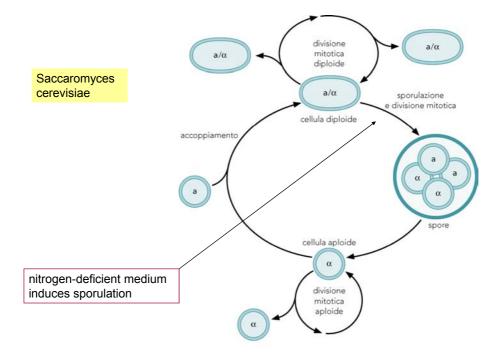
S. Chu,\* J. DeRisi,\* M. Eisen, J. Mulholland, D. Botstein, P. O. Brown,† I. Herskowitz†

Diploid cells of budding yeast produce haploid cells through the developmental program of sporulation, which consists of meiosis and spore morphogenesis. DNA microarrays containing nearly every yeast gene were used to assay changes in gene expression during sporulation. At least seven distinct temporal patterns of induction were observed. The transcription factor Ndt80 appeared to be important for induction of a large group of genes at the end of meiotic prophase. Consensus sequences known or proposed to be responsible for temporal regulation could be identified solely from analysis of sequences of coordinately expressed genes. The temporal expression pattern provided clues to potential functions of hundreds of previously uncharacterized genes, some of which have vertebrate homologs that may function during gametogenesis.

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This paper is included as a resource in the Moodle site. It makes part of the course and students should read and study it



How to measure the activity of all genes (genome-wide) in cells/tissues (mRNA).

Sequencing methods	
(EST)	
SAGE (LongSAGE, CAGE)	
direct re-sequencing (deep sequencing)	Sequence approach
DNA microarrays, oligonucleotide microarrays.	
Spotted arrays	
In situ synthesized oligo arrays	
Bead-arrays®	Hybridization approach

#### EST = expressed sequence tags

- 1. mRNA extraction from cells or tissues
- 2. cDNA synthesis (oligo-dT or random-primed)
- 3. cloning into plasmid vectors
- 4. sequencing from vector primers (200-300nt)
- 5. Estimate expression from frequency

[Current EST Databases contain millions of EST]

automated DNA sequencing

how to create a cDNA library

on line sequence databases, BLAST





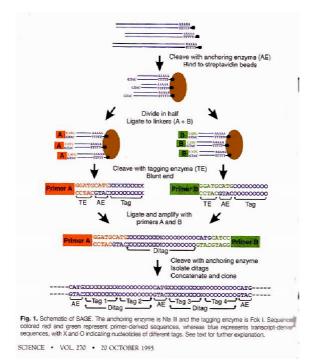
#### SCIENCE • VOL. 273 • 20 OCTOBER 1995

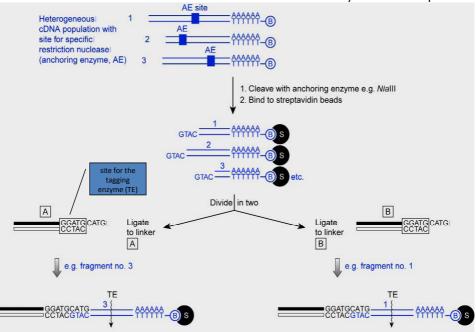
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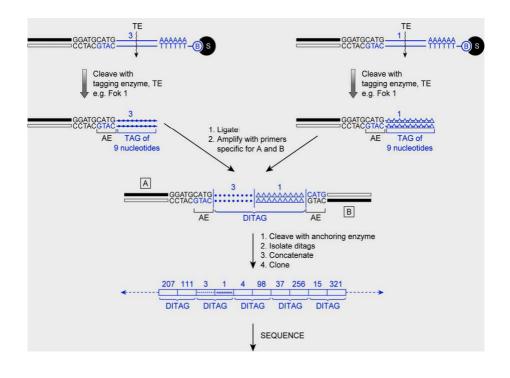
#### Serial Analysis of Gene Expression

Victor E. Velculescu, Lin Zhang, Bert Vogelstein, Kenneth W. Kinzler\*

The characteristics of an organism are determined by the genes expressed within it. A method was developed, called serial analysis of gene expression (SAGE), that allows the quantitative and simultaneous analysis of a large number of transcripts. To demonstrate this strategy, short diagnostic sequence tags were isolated from pancreas, concatenated, and cloned. Manual sequencing of 1000 tags revealed a gene expression pattern characteristic of pancreatic function. New pancreatic transcripts corresponding to novel tags were identified. SAGE should provide a broadly applicable means for the quantitative cataloging and comparison of expressed genes in a variety of normal, developmental, and disease states.







#### SAGE= Serial Analysis of Gene Expression

SAGE analysis produces a table of frequencies for each tag

Frequencies are proportional to representation of that tag in the sample, i.e. to the level of expression of that specific mRNA Table 1, Pancrestic SAGE tags. Tag indicates the 9-to sequence identifying each tag, adjacent to the 4-bp anchormy Na II sig. n and Percent indicate the number of times the tag was identified and its frequency, respectively. Gene indicates the description and accesson number of the GenBark release 87 entry found to exactly match the indicates tag when the SAGE software group was used, with the topware sectors. When multiple entries were identified because of duplicated entries (7, only one entry is table. For drymolyperingen and hypelingen 1, other genes (adencine tiphosphitase and myscin alkal light chain, respectively) were identified that ware predicted to contain the same tags, but subscience and the contained analysis identified that ware predicted to contain the same tags, but users quark individual and contains entry for a transcript that contained at least one copy of the Alu corresteve sequence (16).

GAAGACAAA         Chymolyppingen (M24400)         37           CAGGGTGAA         Pencrestic trysin (M2460)         31           GCGTAACAA         Pencrestic trysin (M22612)         31           GCGTAACAA         Pencrestic trysin (M2285)         16           CCAAGAAGAT         Proceed by M32859         16           CCAAGAAGAT         Proceed by M32859         16           CCAAGAAGAT         Proceed by M32859         17           CCAAGAAGAT         Proceed by M32859         16           CCCAAGAAGAT         Proceed by M32859         17           CCAAGAAGAC         No match (see Table 2 P2)         9           GTGAACCC         No match         8           COCCIGTGT         No match         8           GGTGACCTC         No match         5           COCTGGAACC         No match	Тад	Gene	n	Percen
GMACACAAA         Chymrolynpiancym (M24400)         37           TCAGGGTGA         Percreatic tryson (M24512)         31           GCGTAACAA         Percreatic tryson (M22512)         31           TCATTGOCC         Percreatic tryson (M3285)         16           TCATGOCC         Percreatic tryson (M3285)         16           TCOTCAAAA         No match (see Table 2, P1)         14           TCOCAAACC         No match (see Table 2, P2)         9           GTGAACCC         No match (see Table 2, P2)         9           GTGAACCT         No match (see Table 2, P2)         9           GTGAACCT         No match (see Table 2, P2)         9           GTGAACCT         No match (see Table 2, P2)         9           GTGAACCTG         No match (see Table 2, P2)         9           GTGAACCTGA         No match (see Table 2, P2)	BAGCACACO	Procarboxypeoticiase A1 (X87 318)	64	7.6
TO/GGOTGA         Hercreest: typesh 1 (M22512)         31           GOTOTA/CA         Elestesh B/ M (H92512)         31           GOTOTA/CA         Elestesh B/ M (H92512)         20           GTOTA/CA         Elestesh B/ M (H92612)         20           GTOTA/CA         Elestesh B/ M (H92628)         16           CODA/GAGOT         Procent boxypepticitize B (M5028)         16           CODA/GAGOT         Procent boxypepticitize B (M5027)         14           ADOCTINGGT         Bie saft stimulated (gase (JS4457)         12           GTOTOA/A         No match (see Table 2 P2)         9           GTGMACOC         No match         8           AGOTA/CA         Secretary typesin inibitor (M11040)         6           GTGMACOCA         No match         5           AGOCTA/CA         No match         5           AGOCTA/CA         No match         5           AGOCTA/CA         No match         5           AGOCTA/CA         No match         5           AGOCA/CA         No match	TCTGTGTG	Pancreatic trypsinogen 2 (M27602)		5.5
SCSTRACCA         Electron 11 (M19862)         20           SCSTRACCA         Electron 11 (M39285)         20           TCATTGUCC         Parceradio Ipace (M39285)         16           TCATCAAAA         No match (see Table, 2, P1)         14           TCCCAAACC         No match (see Table, 2, P2)         12           GTGMACCC         No match (see Table, 2, P2)         9           GTGAACCC         No match         5           GCCCTGACA         No match         5           GCCCTGACA         No match         5           GCCCTGACA         No match         5           GCCCTGACA         No match (see Table, 2, P3)         5           GCCCGACA         No m	BAACACAAA	Chymotrypsinogen (M24400)	37	4.4
GTG1G1GCT         Processe E_(00306)         16           CCACAGAGT         Procerboxyper(idse E M45(027)         16           CCACAGAGT         Procerboxyper(idse E M45(027)         14           CCCCAGAGT         Procerboxyper(idse E M45(027)         14           CCCCAGAGT         No match (see Table 2, P1)         14           ACCOTTGGT         Bite saft stimulated (pase (054457)         12           GTG1GCOCT         No match (see Table 2 P2)         9           GTGAACCC         21 Alw orthold (see Table 2 P2)         9           GTGAACCC         No match         8           AGOTACCA         Scentropy trysin infibior (M11940)         6           GTGAACACC         No match         5           AGOTACA         Scentropy trysin infibior (M11940)         5           GTGAACACC         No match         5           AGOTACA         No match         5           AGOTACA         No match         5           AGOTACA         No match         5           AGOTACA         No match	CAGGGTGA	Pancreatic trypsin 1 (M22612)	31	3.7
TCATTG2CC         Parcinato Egoso (M30285)         16           CCRA0AGA         Processo (M30285)         16           CCRA0AGA         No match (see Table, 2, P1)         14           TCOTTGAAA         No match (see Table, 2, P1)         14           ADCOTTGCT         Bits stimulate (gase (M5457)         12           GTGTGACCC         No match (see Table, 2, P1)         14           TCCAAGAC         No match (see Table, 2, P2)         9           GTGAACCC         No match         5           GCCGTGAAC         No match         5           GCGGAGCO         No match (see Table, 2, P3)         5           AGCCGTGCA         No match (see Table, 2, P3)         5           AGCCGAGA         No match (see Table, 2, P3)         5           AGCCAGCO         No match (see Table, 2, P3)         5           AGCCAGCAC         No match (see Table, 2, P3)         5	ACGTGACCA	Elastase IIIB (M19692)		2.4
CZRACAGAGT         Procerboxyperitides B (MSIC67)         14           CZRACAGAGT         Procerboxyperitides B (MSIC67)         14           AGCCTIGGT         Bile saft stimulatot (gasse (054457)         12           GIGTGCOCT         No match (see Table 2 P2)         9           GIGTACACC         21 Alw orthos         8           GGTGACCC         No match (see Table 2 P2)         9           GIGTACACC         No match (see Table 2 P3)         6           GIGTACACCA         Secretary tryasin inhibitor (M11940)         6           GIGTACACCA         No match         5           GIGTACACCA         No match         5           GIGTACACCA         No match         5           AGCOCACCA         No match         5           AGCACACA         No match         5           AGCACACA         No match         5           AGCACACA         No match         5           AGCACACA         No match         6           GIGTACACC         No match         5	aTGTGTGCT	Protease E (D00306)		1.9
TCOTCAAAA         No match (see Table, 2, P1)         14           AGCOTTIGGT         Bile stimulated (pase QIS4457)         12           GTGTOGOT         No match         11           TCOCAGACC         No match         11           TCOCAGACC         No match         11           TCOCAGACC         No match         11           GTGAACCC         No match         11           TCOCAGACC         No match         11           TCOCAGACC         No match         11           TCOCAGACC         No match         11           TCOCCTT         No match         11           TCOCCTGTA         No match         11           TCOCCTGTA         No match         11           TCOCCTGTA         No match         11           TCOCCTGTA         No match         12           GTGAACTCA         No match         5           GACGTTGGA         No match         5           AGCCAGTOC         No match         14           TTCATAGA         No match         14           GTGAACTCA         No match         14           TTCATAGA         No match         14           TTCATAGA         No match         14 <td>CATTGGCC</td> <td>Pancreatic lipase (M93285)</td> <td>16</td> <td>1.9</td>	CATTGGCC	Pancreatic lipase (M93285)	16	1.9
AGCONTIGGT         Bile saft stimulator (pase (254457))         12           GIGTGOCOCT         No match (see Table 2 P2)         9           GIGTACACC         No match (see Table 2 P3)         6           GIGTACACCA         Secretary tryain initibior (M11940)         6           GIGTACACA         Secretary tryain (see Table 2 P3)         5           GIGTACAC         No match         5           GIGTACACCA         No match         5           AGCOCACAC         No match         5           AGCACACA         No match         5           AGCACACA         No match         5           AGCACACA         No match (see Table 2 P3)         5           AGCACACA         No match (see Table 2 P4)         5           TICITICIGG         No match (see Table 2 P4)         4           GITACACACA         No match (see Table 2 P4)         4           GITACACACA         <				1.7
GTGT000CT         No match         11           GTGT000CT         No match         11           TOC0AGACC         No match         11           GTGAACCC         No match         11           GTGAACCC         No match         11           GTGAACCC         No match         8           GGTGACTCT         No match         8           GGTGACTC         No match         8           COCCTGTACA         Secretary trypsin inhibitor (M11940)         6           GCCGTGAACC         No match         5           GTGACOCG         No match         5           GACGTTGGA         No match         5           AGCCCTACA         No match         5           AGCAGTTCC         Elengation factor 2 (211622)         5           AGCAGTTCC         Isonath (sec Table 2, P3)         5           AGTGAACAC         No match (sec Table 2, P3)         5           AGTGAACCC         No match (sec Table 2, P3)         5           AGTGAACAC         No match (sec Table 2, P3)         5           TTCATACAC         No match (sec Table 2, P3)         4           GTGAACCC         No match         4           GTGAACCC         No match         4 </td <td>AAAAA</td> <td>No match (see Table, 2, P1)</td> <td></td> <td>1.7</td>	AAAAA	No match (see Table, 2, P1)		1.7
TOCGAGACC         No match (see Table 2 P2)         9           OTGAAACC         21 Aw orbits         8           GGTGAACCC         No match (see Table 2 P2)         9           GGTGAACCC         No match (see Table 2 P2)         8           ARGOTACC         No match (see Table 2 P2)         8           ARGOTACC         No match (see Table 2 P2)         6           ARGOTACC         No match (see Table 2 P3)         5           OCTGTGAATC         Mo match (see Table 2 P1)         5           AGCACACA         No match (see Table 2 P3)         5           TICITICISCS         No match (see Table 2 P4)         4           GTAPACCC         NF-e80 P6 (490), As entry (\$04541)         4           GTAPACCC         TWF researcht (No 5049), As entry (\$04541)         4           GTGGACACA         No match (see Table 2 P3)         4           GCCATCACC         No match (see Table 2 P3)         4 <td>GOCTTEGT</td> <td>Bile salt stimulated lipase (X54457)</td> <td></td> <td>1.4</td>	GOCTTEGT	Bile salt stimulated lipase (X54457)		1.4
GTGAACC     21 Ale ordis     8       GGTGACTC     No match     8       AAGGTAACCA     Secretary tryasin inhibitor (M1 1940)     6       TOCOCTIGTC     No match     5       GTGACCCC     No match     5       COTGTAACCA     Secretary tryasin inhibitor (M1 1940)     6       COCCTGTATC     No match     5       COTGTATACCA     No match     5       AGCACROC     Mo Instance     221 1052)       SAGCACROCA     No match (sec Table 2, P3)     5       AGCACROCA     No match (sec Table 2, P3)     5       AGCACROCA     No match (sec Table 2, P3)     5       ATTGAACA     No match (sec Table 2, P3)     5       ATTGAACAC     No match (sec Table 2, P4)     4       TTCATACAC     No match (sec Table 2, P4)     4       GTGACACC     No match (sec Table 2, P4)     4	atg tgogót	No match		1.3
GartaActCit         No match         8           AxeGotTACA         Secretary tryosin initibilar (M11940)         6           TOCCOURTG         No match         5           GITAGCACO         No match         5           GOTGTAATC         Meintak         5           GOTGTAATC         Meintak         5           ACADITIGGA         No match         5           ACCOUNT         Meintak         5           AGCACOTCC         Romatch         5           AGCACOCA         No match         6           AGCACOCA         No match         6           AGCACOCA         No match         6           GTAACOCC         NF-eB (26140), Ab entry (504541)         4           GTAACOCC         NF-eB (26140), Ab entry (504541)         4           GATAACOCC         No match         6           GATAACOCC         No match         6           GATAACOCC         No match         4           GOTGGAAG         Parcenetior much (05	GOGAGAOC	No match (see Table 2 P2)		1.1
AGGCTANCA         Secretary typesin inhibitor (M11940)         6           GTGACACC         No match         5           GTGACACC         No match         5           GTGACACC         No match         5           COCTGTART         MB 1159, M22065, 11 ALL antrias         5           CACGTTGCA         No match         5           AGCACGTCC         MB 1159, M22065, 12 ALL antrias         5           AGCACGTCA         No match         5           AGCACGTCC         Elengation factor 2 (211692)         5           AGCACGTCC         No match (sec Table 2, P3)         5           ATTGMAGA         No match (sec Table 2, P4)         5           TTCATACAC         No match (sec Table 2, P4)         4           GTGGCAGC         NF-#4 (261496), AU entry (S04541)         4           GTGGCAGC         NF-#4 (261496), AU entry (S01446)         4           GTGGCAGC         NF match         4           GTGGCAGC         No match         4           GCTGGGAAC	3TGAAAOOO	21 Alu entries		1.0
TOCCOURTIC     No match     5       GTGACCAOG     No match     5       COTOTTAATC     M61199, M2066, 11 ALi antriaa     5       CADGTTGCAA     No match     5       AGCACTOCAC     No match     5       AGCACTOCAC     No match     5       AGCACTOCCC     Elengthon factor 2 (211692)     5       AGCACACCA     No match     6       AGTGGAGA     No match     5       AGCACACCA     No match     6       AGTGGAGA     No match     6       TATGAACAC     No match     4       GTGACACCOC     NF-e80(64)(A5 entry (50454))     4       GTGACACCA     No match     4       GTGACACCA     No match     4       GCTGGGAG     Parcreater much (105569)     4       GCCATOGTC     Mitochondrial CytD oxidase (X15759)     4       SAGE     tags occurring:     Greater than three times     360				1.0
GTGACOCC         No metch         5           CCTGTGAR         No match         5           CACGTTGGA         No match         5           AGCOCTACA         No match         5           AGCACTICGA         No match         5           AGCACTICA         No match         5           AGCACTICA         No match         5           AGCACTICA         No match         2(211602)           SACCTACA         No match (see Table 2, P3)         5           ANTTGANGA         No match (see Table 2, P4)         5           TTCATACAC         No match (see Table 2, P4)         4           GTGACACCC         NF-#4 (241402)         4           GTGACACC         No match (see Table 2, P4)         4           G	MGGTAACA	Secretory trypsin inhibitor (M1 1949)		0.7
ÓZTGTÄÄTC         M9 1199, M20966, 11 ALL entrikas         5           ÓZTGTÄÄTC         M9 1199, M20966, 11 ALL entrikas         5           ÓZDGTÄÄTC         No match         5           ÁGEXCITACA         No match         5           ÁTGTÁAACA         No match         4           ÓTGCAQGC         NF-leiß P61490, Ab entry (504541)         4           ÓTGAACACO         No match         4           ÓTGAACACO         No match         4           ÓCTGGGAGA         Paromatch (105562)         4           ÓCCATOGTC         Mochandral CyrtO addase (X15759)         4           ÓXGE tágs occurring:         Greater than three times         360	COOCTIGTG	No match		0.6
CAUCITICICA         No match         5           CAUCITICICA         No match         5           AGCOCIACA         No match         5           AGCOCIACA         No match         22116021         5           AGCARGICA         No match (see Table 2, P3)         5         5           ACTGARAGA         No match (see Table 2, P4)         5         5           TICATACAC         No match (see Table 2, P4)         4         4           TICATACAC         No match         4         4           GTAGARACCC         NF-match (see Table 2, P4)         4           GTAGARACCC         No match         4         4           GTAGARACCC         No match         4         4           QCT06QGAAC         Parcreate much         105582)         4           XGE tags occurring:         Greater than three times         360	3TGACCACG			0.6
ÁGCÓC TÁCA         No metch         5           AGCACOTOC         Biorgation factor 2 (211692)         5           AGCACAGCA         No metch (sea Table 2, P3)         5           AGTGAAGA         No match (sea Table 2, P4)         5           TICTGTGGG         No match (sea Table 2, P4)         5           TICTGTGGG         No match         4           GTGACACAC         No match         4           GTGACACC         NF-e8 (P6149), Ab entry (504541)         4           GTAMACOC         TW respector II (M55994), Ab entry (501448)         4           GCTGGGAAG         Parmeteris much (M55994), Ab entry (501448)         4           GCTGGGAGA         Parmeteris much (M55994), Ab entry (501448)         4           GCTGGGAGAG         Parmeteris much (M55994)         4           Machaetas cocurring:         Greater than three times         380	CTGTAATC	M91159, M29366, 11 Alu entries		0.6
AGGACOTICC         Eargigtion factor 2 (21 16)(2)         5           AGGACOTICC         Eargigtion factor 2 (21 16)(2)         5           AGGACOGCA         No match (sea Table 2, Pd)         5           THCTGTGAG         No match (sea Table 2, Pd)         5           THCTGTGAG         No match (sea Table 2, Pd)         5           THCTGTGAG         No match (sea Table 2, Pd)         4           GTGCACAGC         No match         4           GTGCACAGC         No match         4           GTGCACAGC         The motor in (MS5994), Au entry (S04541)         4           GACACACA         No match         4           COCTGGGAG         Parcreater much (MS5994), Au entry (X01448), 4         4           GCCCATCOGTC         Microchondrial CyrtD oxidase (X15759)         4           SAGE tags occurring:         Greater than three times         380	SADGTTGGA	No match		0.6
ACICAGOGA         No metath isse Table 2, P(3)         5           ACICAGOGA         No match isse Table 2, P(4)         5           TICTGTGGG         No match         4           TICATACARC         No match         4           GTOCACACC         No match         4           GTOCACACC         No match         4           GTOCACACC         NF-e8 061469), Au entry (\$04541)         4           GTAAACCC         TW meteritic (\$05594), Au entry (\$01448)         4           GACACACA         No match         4           COCGAGGAG         Parceptor II (\$05594), Au entry (\$01448)         4           COCGTOGGAG         Parceptor II (\$05594), Au entry (\$01448)         4           GOCATOGTC         Monchard CyrD oxidase (\$15759)         4           SAGE tags occurring:         Greater than three times         360	AGCCCTACA	No match		0.6
AATTGANGA         No match leas Table 2, P4)         5           TITCATAGAG         No match leas Table 2, P4)         4           TITCATAGAG         No match leas Table 2, P4)         4           GTGGCAGC         No match least 1, Ab entry (\$94541)         4           GTGACAGC         TWP respirate 11 (M55994), Ab entry (\$94541)         4           GAACAGACA         No match least 1, M55994), Ab entry (\$94541)         4           GAACAGACA         No match least 1, M55994), Ab entry (\$94541)         4           GCTGGGAAG         Parcenter much (J05582)         4           GCCATOGTC         Mitochondrial CyrtC oxidase (X15759)         4           SAGE tags occurring:         Greater than three times         380	ACCACCTCC	Elongation factor 2 (21 1692)	5	0.6
TTCTGTGGG     No match     4       TTCATACAC     No match     4       GTGCACAGC     No match     4       GTGCACAGC     NF-#8 (261469), Abj entry (504541)     4       GTGACACC     TW receptor III (M55994), Abj entry (501448)     4       GAACACAC     No match     4       GCCGGGACA     Parcestor III (M55994), Abj entry (501448)     4       GCTGGGACAC     No match     4       GCCGTGGGAC     Parcestor much (J05682)     4       SAGE tags occurring:     Greater than three times     380	CGCAGGGA	No match (see Table 2, P3)	5	0.6
TTCATACAG         No instituin         4           GTGCACAGC         NF-w8 (x61499), Au entry (\$94541)         4           GTGCACAGC         TWF receptor II (M55994), Au entry (\$94541)         4           GAACACAC         TWF receptor II (M55994), Au entry (\$94541)         4           GAACACACA         No match         4           CCCTGGGAAG         Parcreate mucini (J05582)         4           CCCGTGGCAG         Mitochondrial CyrtD oxidiase (X15759)         4           SAGE tags occurring:         Greater than three times         380	WITTGAAGA	No match (see Table 2, P4)		0.6
GTGGCAGCC         NF-wE (261496), Abs entry (504541)         4           GTGACAGCC         TNF receptor II (N55994), Abs entry (501448)         4           GMACAGACA         No match         4           GCGGGAAG         Par compation much (N55892)         4           GCGGGGAGC         Mit conclusion (N55892)         4           GCGGGGGAC         Mit conclusion (N55892)         4           SAGE tags occurring:         Greater than three times         360	TOTGTGGG	No match		0.5
CTAGAACCC TTNE reseptor II (N55994), Au anny (X01446) 4     GAACACACA No match     GAACACACA No match     GAACACACA A No match     GCCATGGCAAG Parcreate mucin (J05562) 4     CCCATGCTC Mitochondrial CyrtC oxidase (X15759) 4     SAGE tags occurring: Greater than three times 380	TCATAGAG			Q.5
GMACACACA COCATOGACA COCATOGACA COCATOGACA SAGE tags occurring: Greater than three times 380	STGGCAGGC	NF-xB (X61499), Alu entry (\$94541)		0.5
OCTOGGANG         Parcreatic mucin (J05582)         4           OCCATOGTC         Mitochondrial CyrtD oxidiae (X15759)         4           SAGE tags occurring:         Greater than three times         380	STAAAACCC	TNF receptor II (M55994), Alu entry (X01448)	4	0.5
COCATOSTC Whoch and is Cyto Datase (X15759) 4 SAGE tags occurring: Greater than three times 380	BAACACACA	No match		0.5
SAGE tags occurring: Greater than three times 380	CTGGGAAG	Pancreatic mucin (J05582)		0.5
	COCATOGTC	Mitochondrial CytC oxidase (X15759)	4	0.5
	SAGE tags occurring:	Greater than three times	380	45.2
		Three times (15 × 3 =)	45	5.4
Two times (32 × 2 =) 64				7.6
One time 351			351	41.8
Total SAGE tags 840				100.0

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Students are expected to explore and use resources that are allocated in the Moodle website.

Some interactive problems and exercise are also present: I strongly invite students to perform these activities.