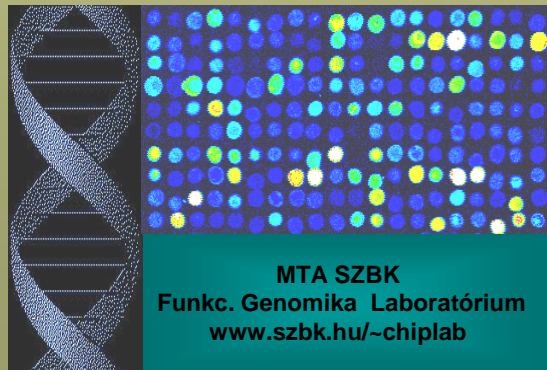


Funkcionális genomikai módszerek: technológia és alkalmazás

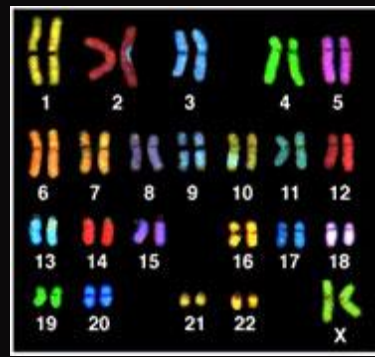
MTA SZBK, Genetika Intézet
Funkcionális Genomika Laboratórium

Genetikai Tanszék, ELTE TTK, Budapest
2019.11.25.



**Kromoszómák
genetikai állomány
hordozói**

sejt



DNS

**Gének
információ hordozók**

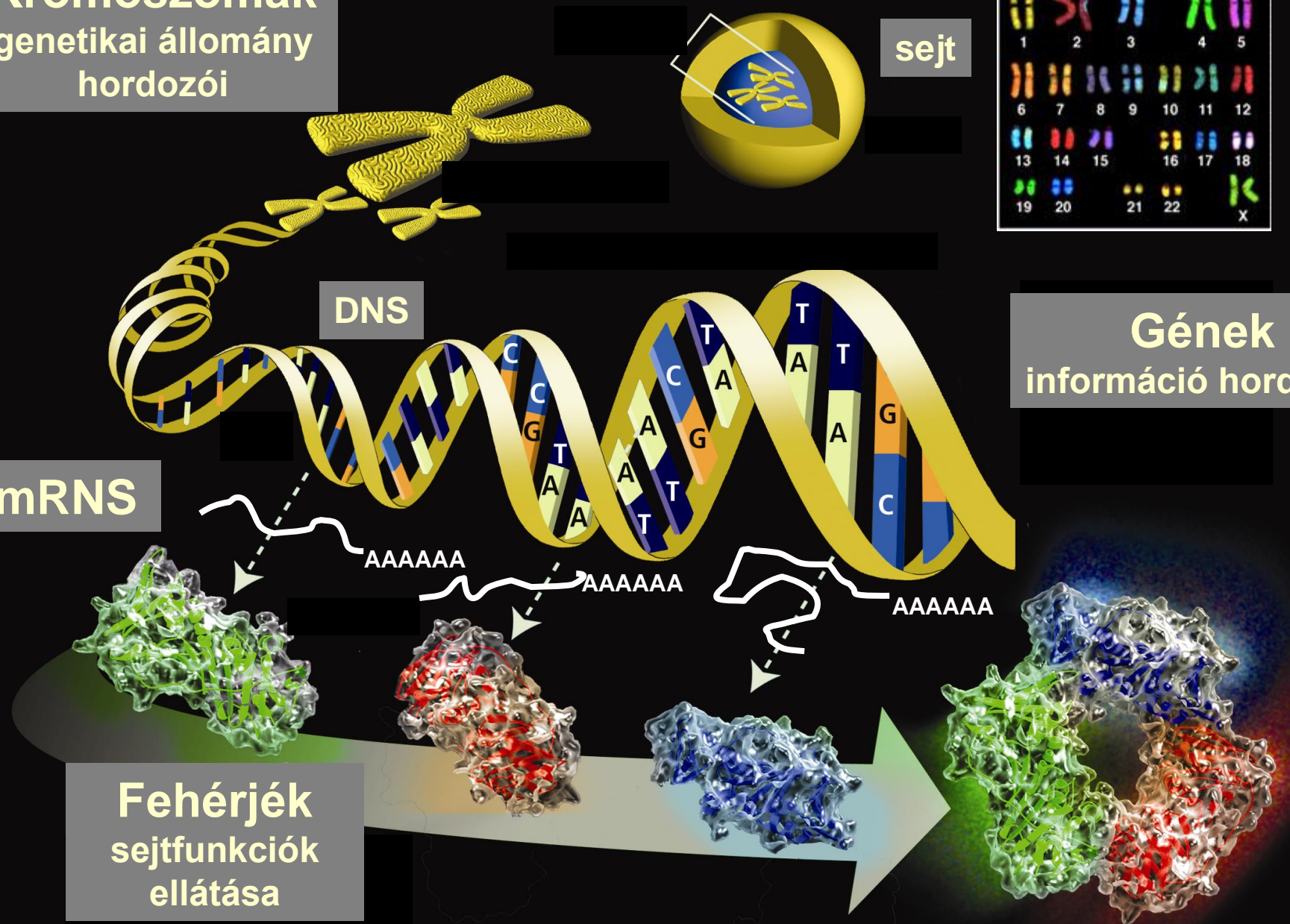
mRNS

AAAAAA

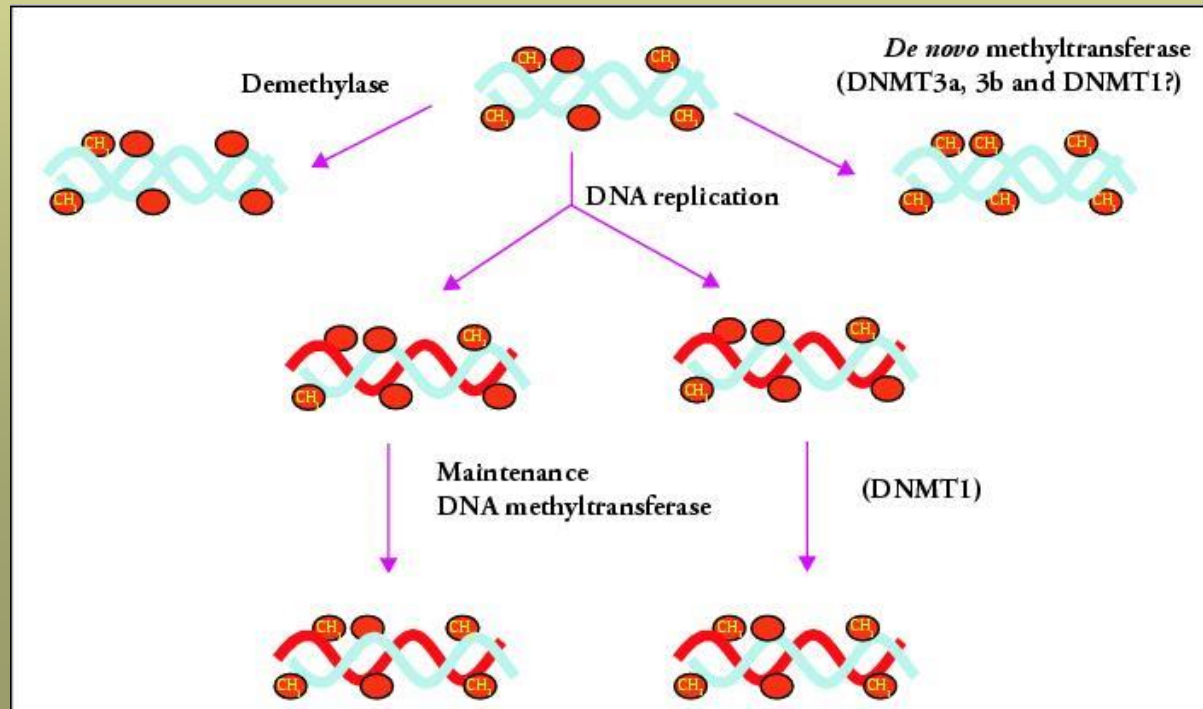
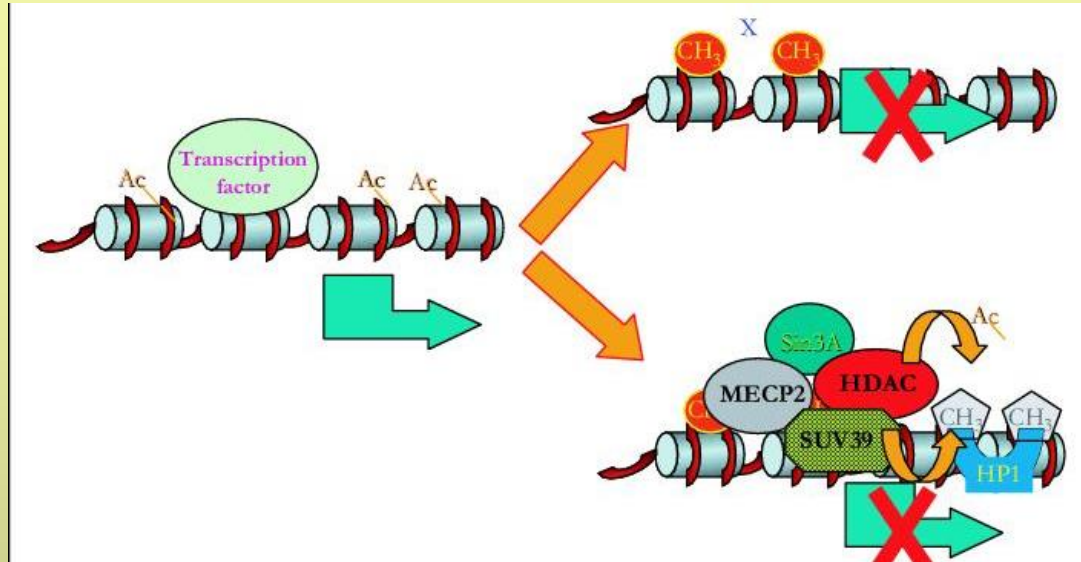
AAAAAA

AAAAAA

**Fehérjék
sejtfunkciók
ellátása**

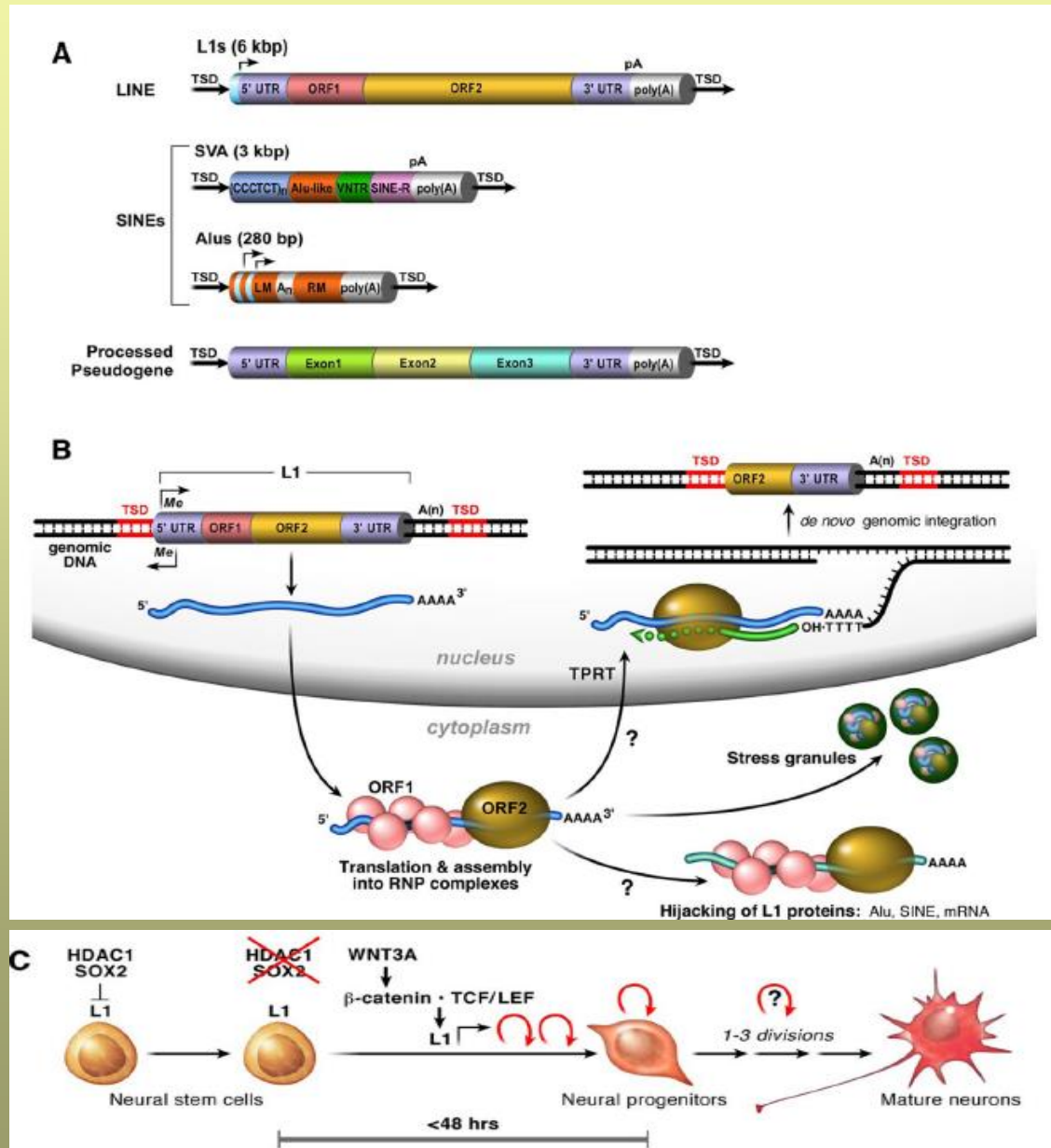


DNS metiláció és „hiszton-kód” – epigenetikai változások

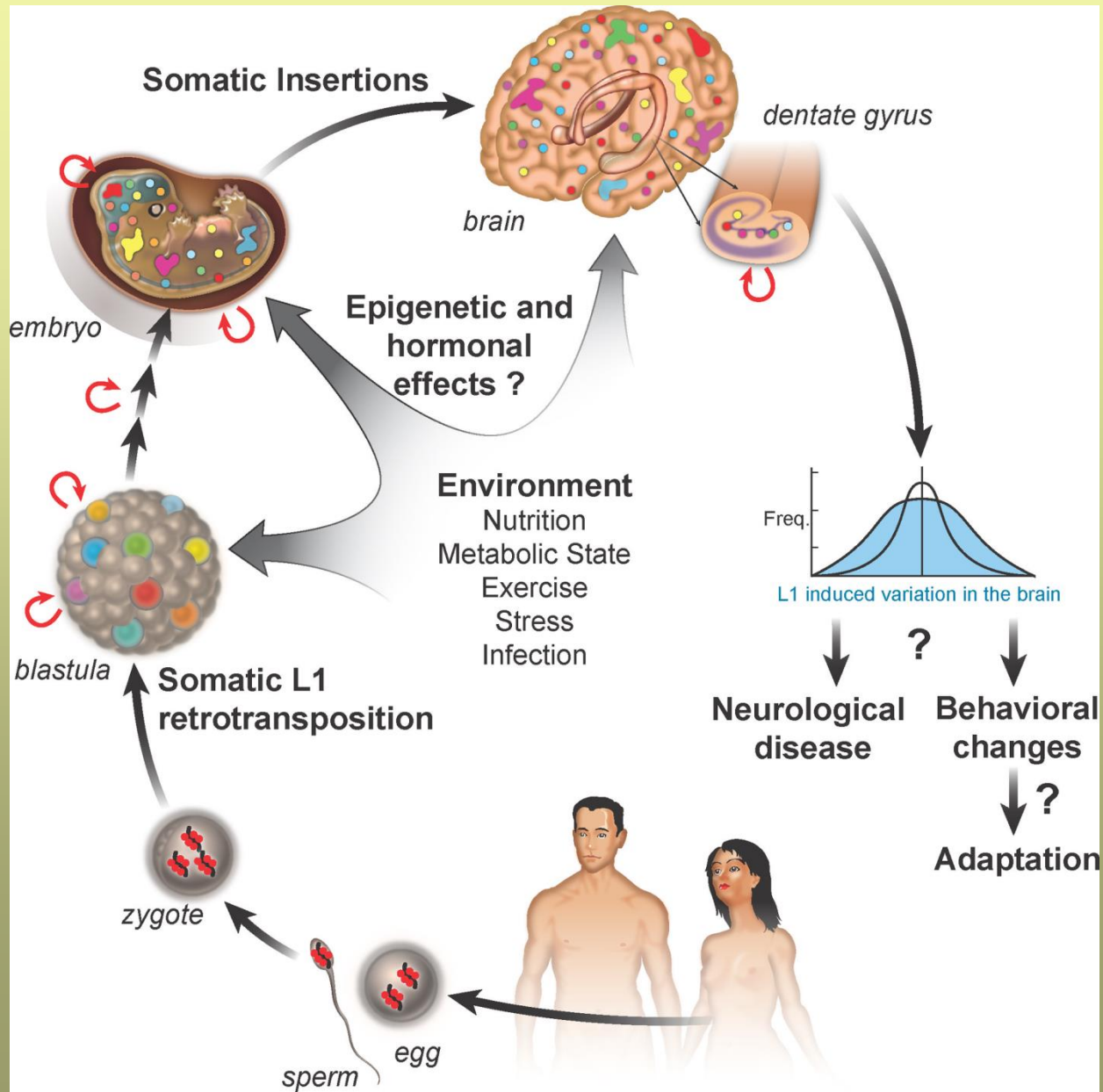


Repetitív szekvenciák – ártalmatlan „szemét”?

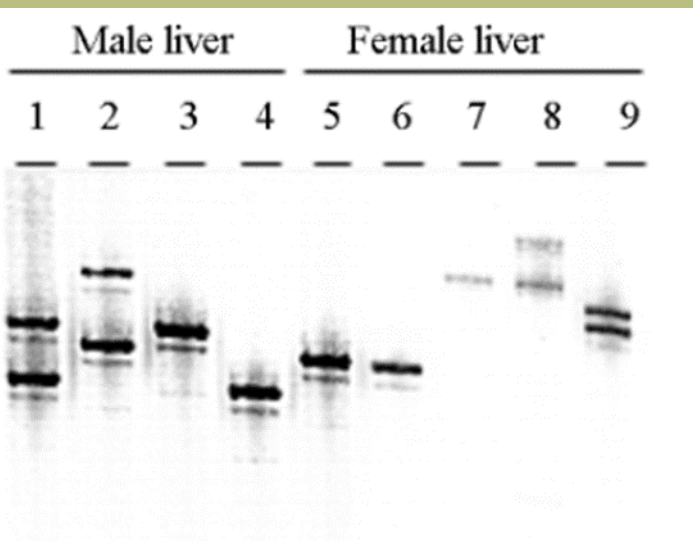
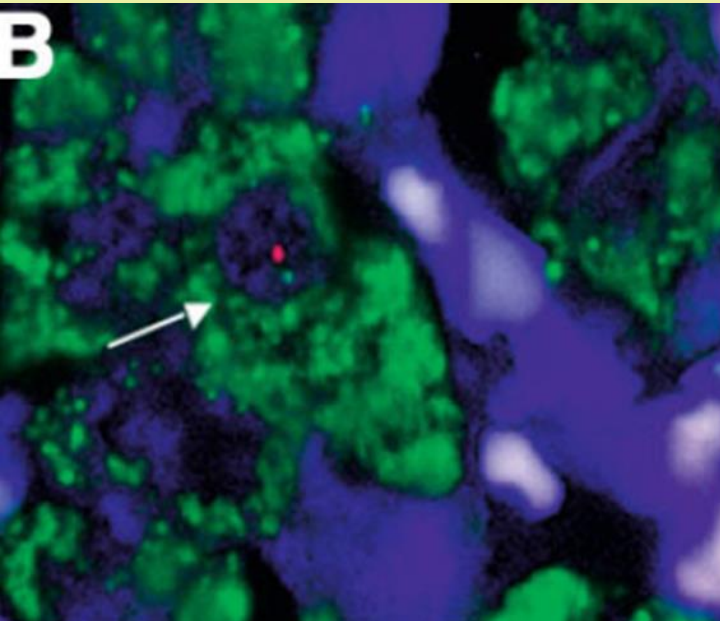
LINE
SINE



Repetitív szekvenciák – neuronális diverzitás oka?



Mikrokimerizmus – további sejt-diverzitás/heterogenitás



[Microchimeric fetal cells cluster at sites of tissue injury in lung decades after pregnancy.](#)

1. O'Donoghue K, Sultan HA, Al-Allaf FA, Anderson JR, Wyatt-Ashmead J, Fisk NM.

Reprod Biomed Online. 2008 Mar;16(3):382-90.

PMID: 18339261

[Similar articles](#)

[Chimerism and microchimerism of the human heart: evidence for cardiac regeneration.](#)

2. Bayes-Genis A, Roura S, Prat-Vidal C, Farré J, Soler-Botija C, de Luna AB, Cinca J.

Nat Clin Pract Cardiovasc Med. 2007 Feb;4 Suppl 1:S40-5.

PMID: 17230214

[Similar articles](#)

[Male cell microchimerism in normal and diseased female livers from fetal life to adulthood.](#)

3. Guettier C, Sebah M, Buard J, Feneux D, Ortin-Serrano M, Gigou M, Tricottet V, Reynès M, Samuel D, Féray C.

Hepatology. 2005 Jul;42(1):35-43.

PMID: 15962317

[Similar articles](#)

[Fetal microchimerism is common in normal and diseased vulvar skin.](#)

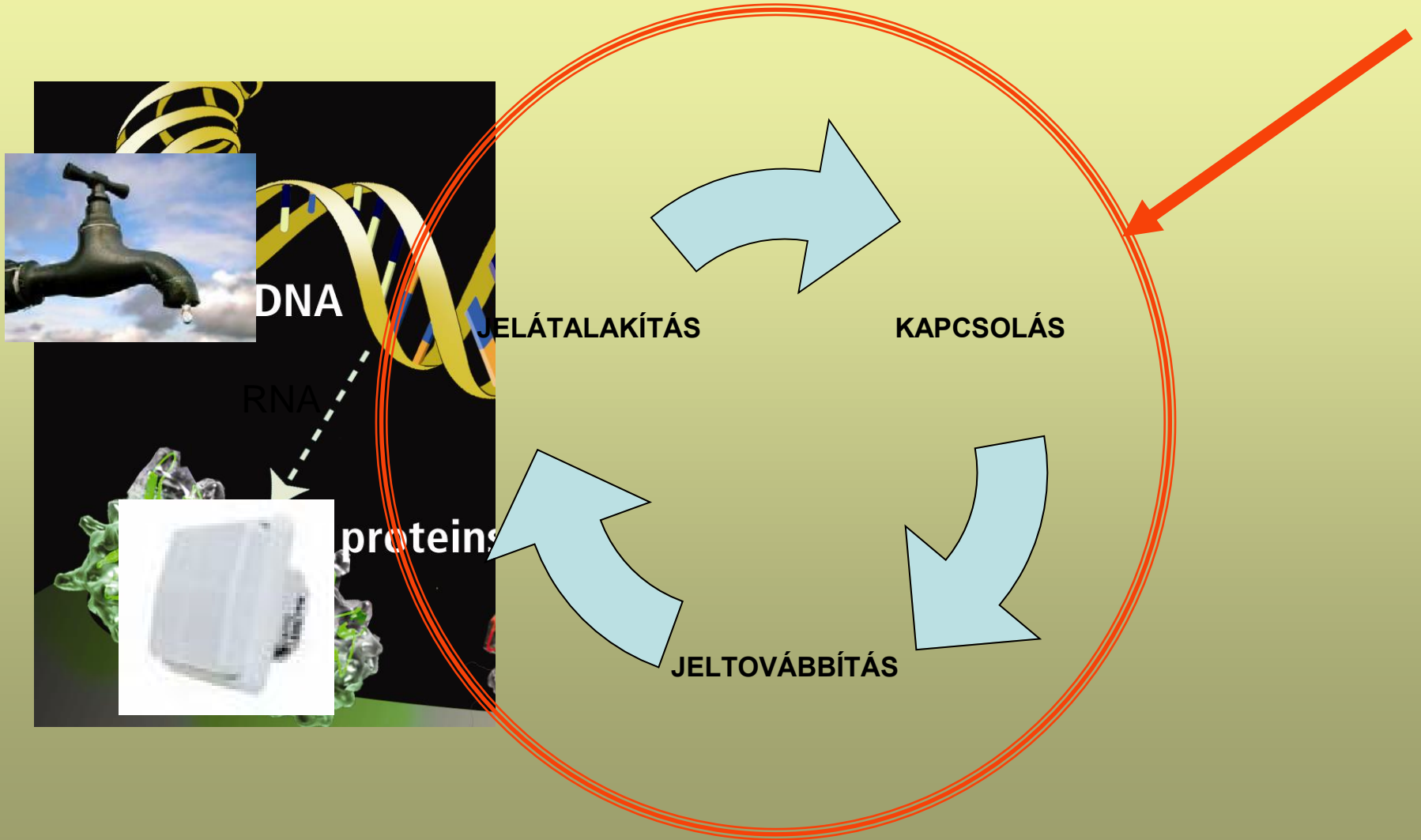
4. Regauer S, Schwaiger P, Liegl B, Klintschar M.

J Invest Dermatol. 2004 Apr;122(4):1059-60. No abstract available.

PMID: 15102100 **Free Article**

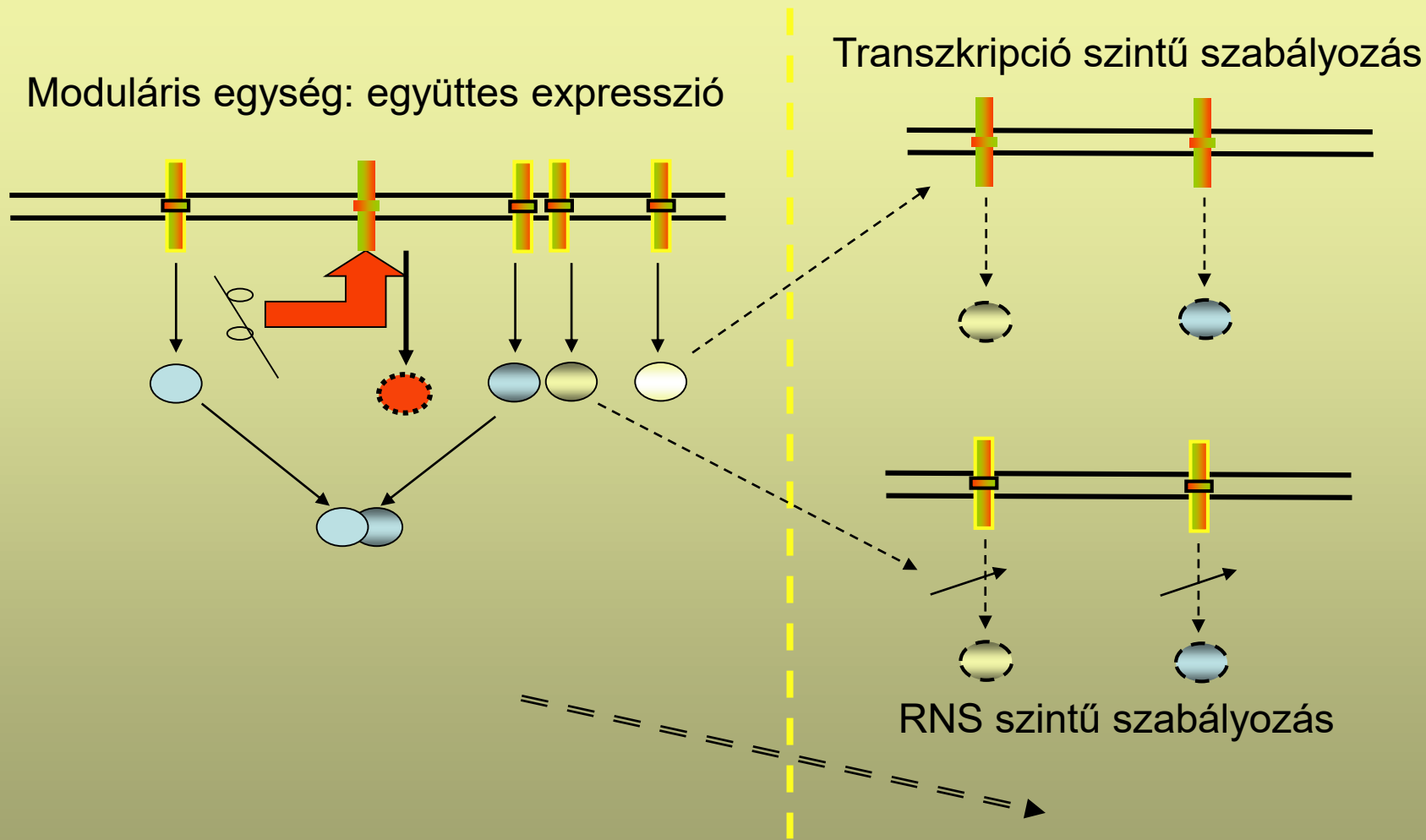
[Similar articles](#)

Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



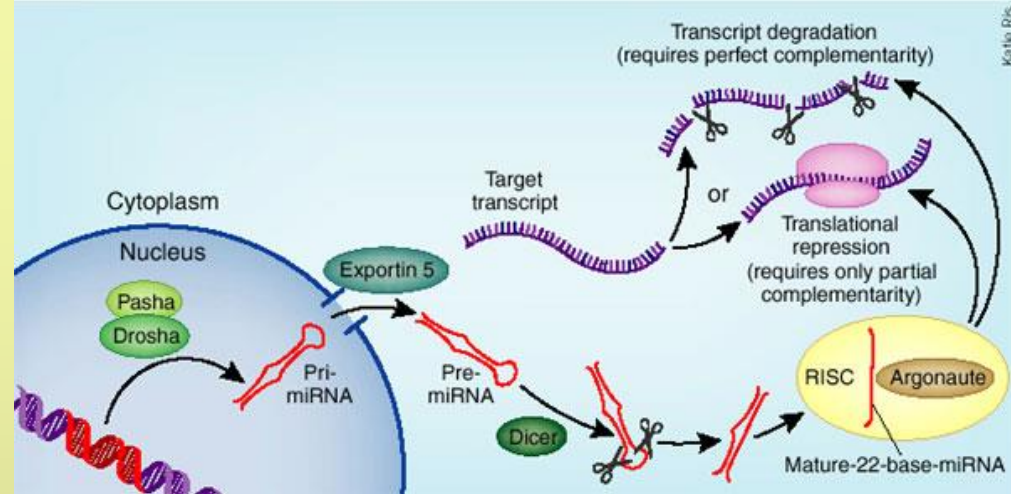
Kulcsszavak: kapcsolók

Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



Kulcsszavak: kapcsolók és modulok

miRNS analízis



- miRNS: kis 19-25 nukleotid hosszú egyszálú RNS molekulák, amelyek a génexpresszió negatív szabályozásában játszanak szerepet
- a sejtmagban keletkeznek hosszú prekurzor RNSként: *pri-miR*
- emberben számuk meghaladja az ezret -> minden miRNS több száz mRNA transzkripcióját szabályozza
- méretük és a nagyfokú szekvencia konzerváció (1-2 nukleotid eltérés) miatt detektálásuk nehéz
- megváltozott miRNS expresszió számos betegség kulcsa lehet -> target: rák, szív és érrendszeri megbetegedések

Génhálózatok, skála-független tulajdonság, redundancia és érzékenység

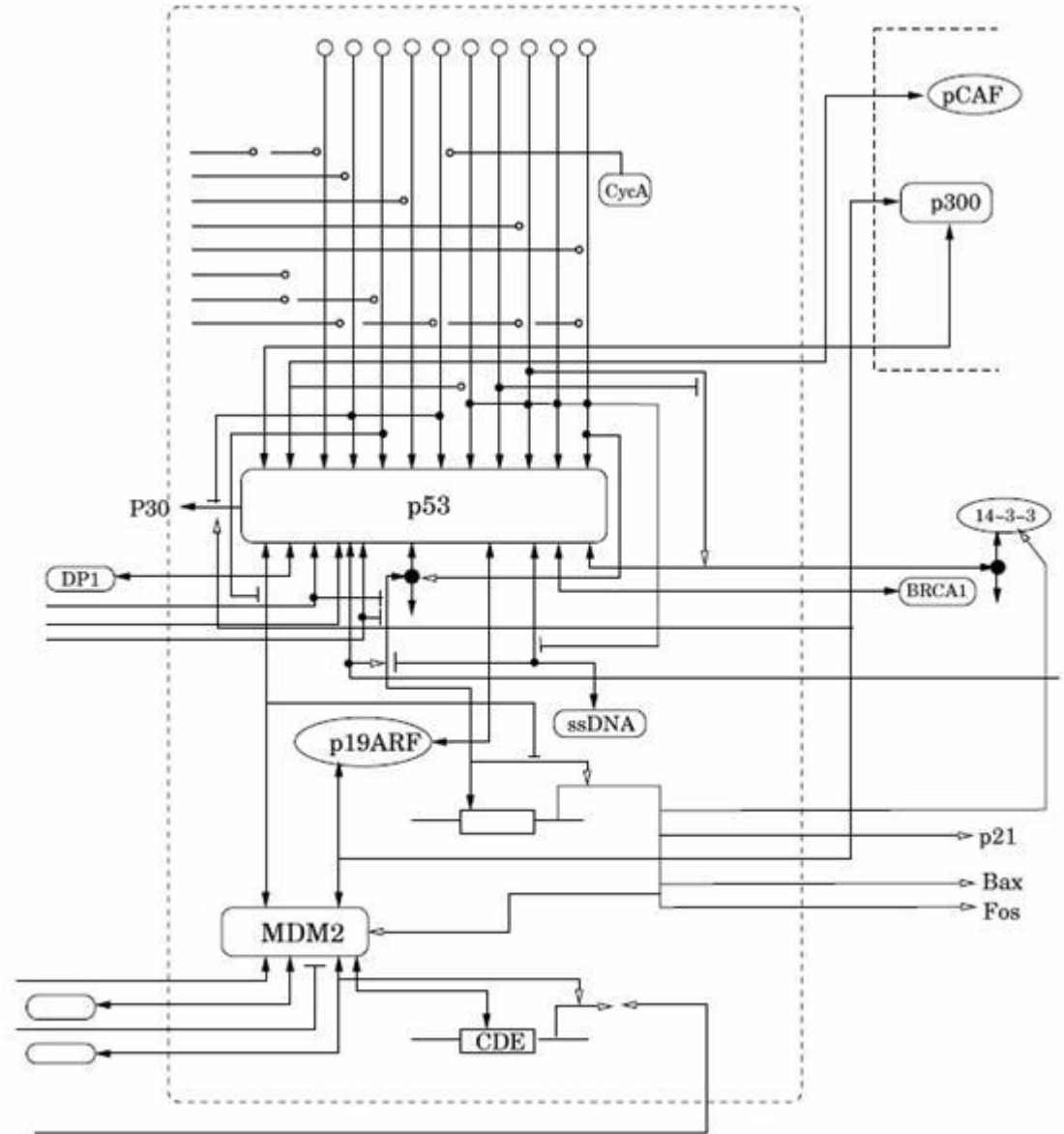
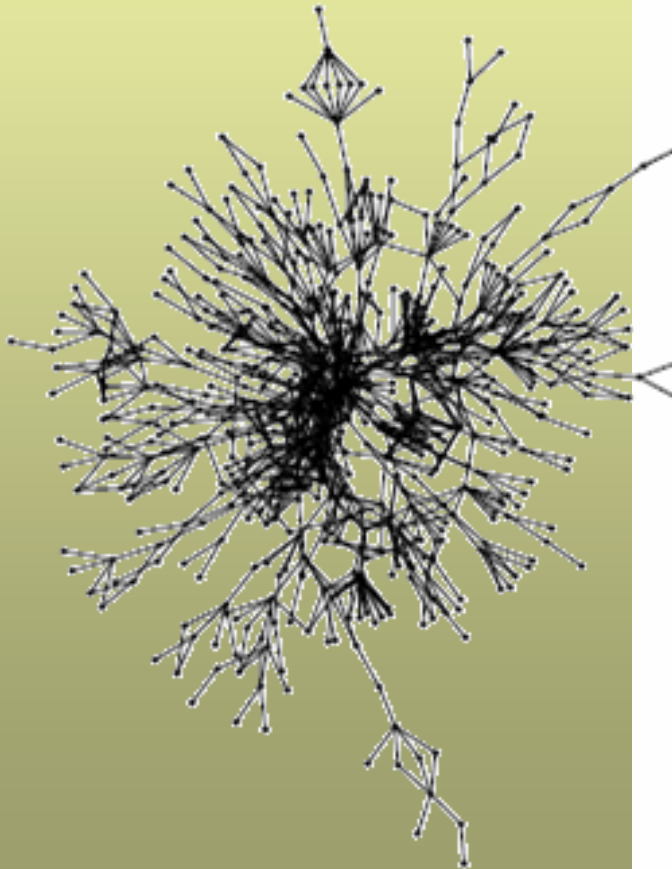


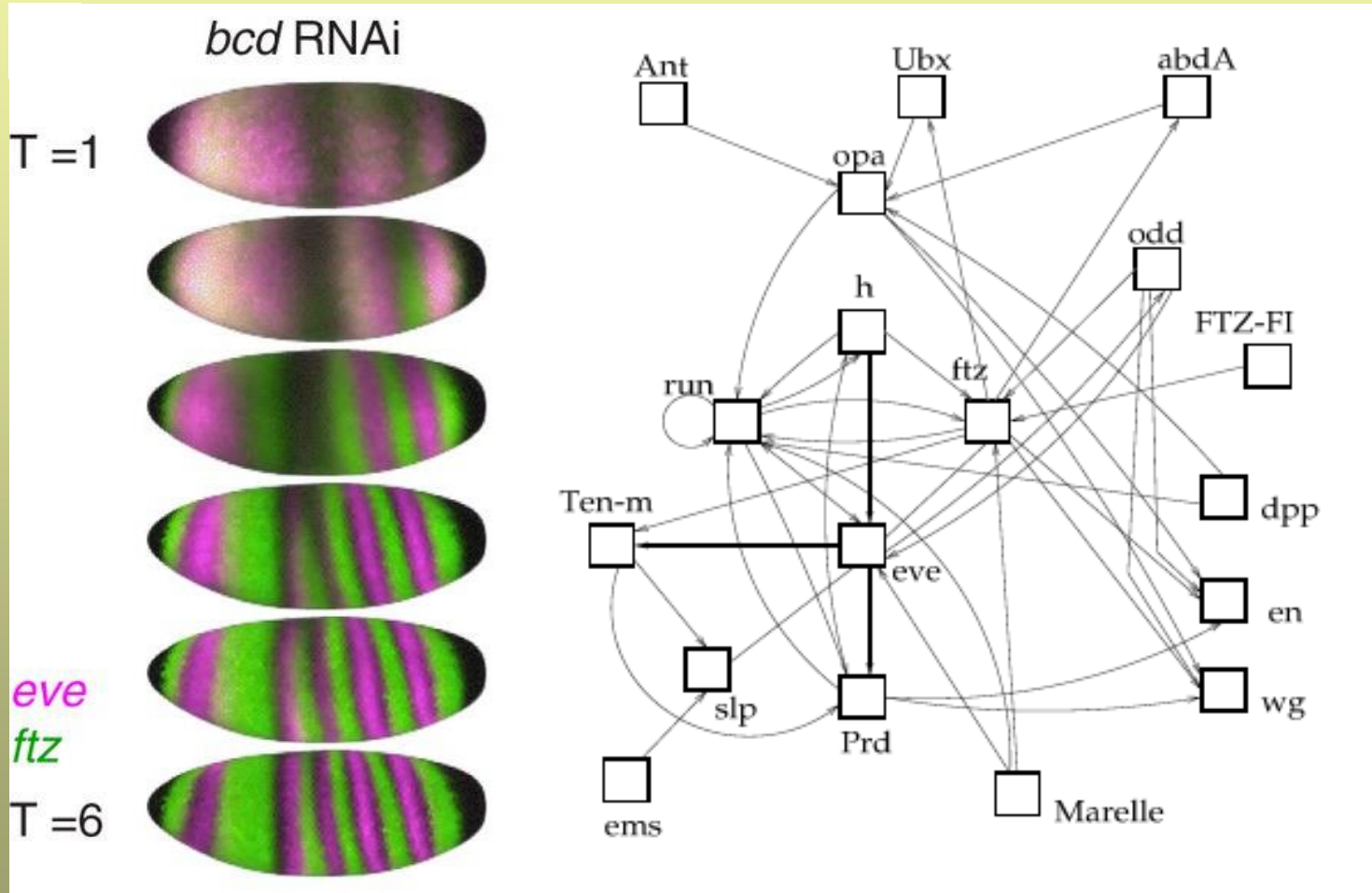
Figure 1.2: Schematic architecture of the p53 network. The p53 node integrates information from very different parts of the system. Only part of the cell circuitry is shown here. For a

Chicago nemzetközi célpontok, légvonal hálózat



Sejthálózatok és génhálózatok, kapcsolatok és jelek

Génaktivitás térbeli kifejeződése



Ftz and eve are involved in the establishment of the segmentation pattern of the Drosophila embryo

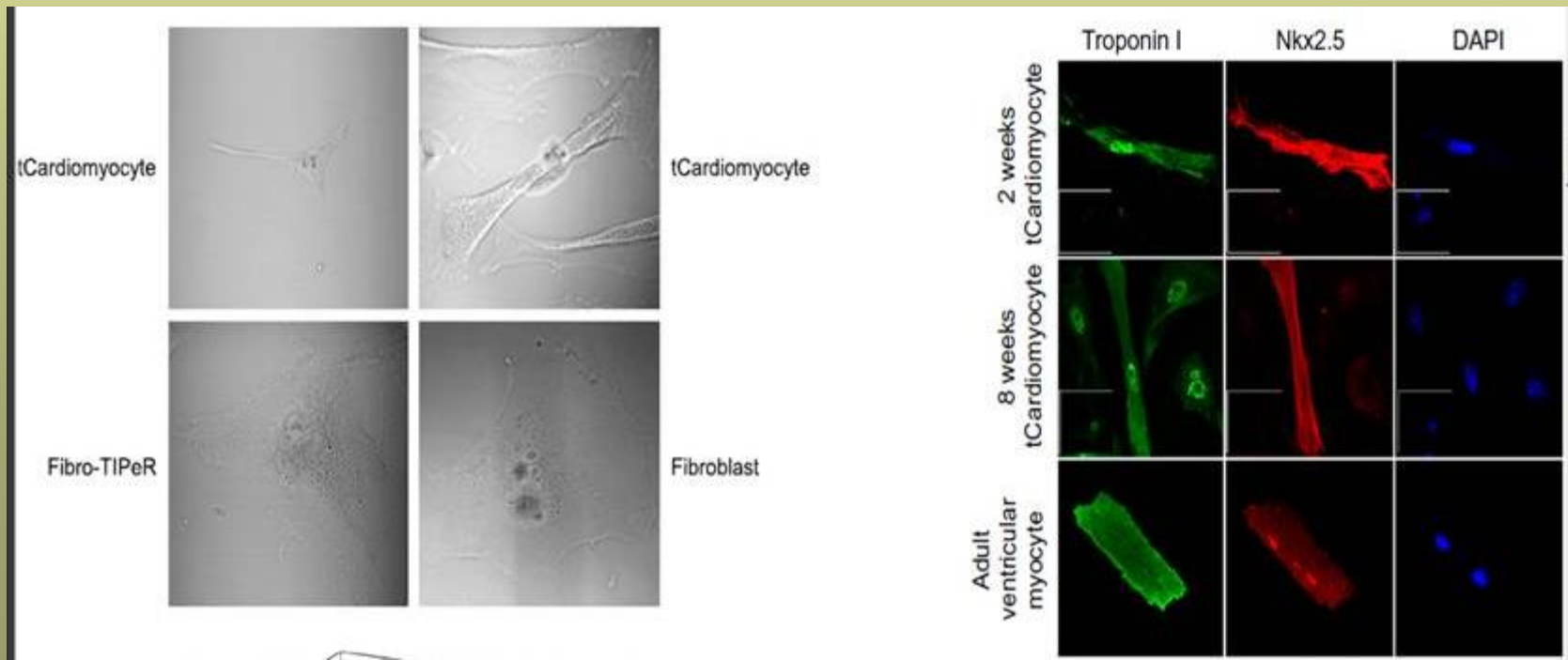
A fenotípus meghatározója a transzkriptom vagy a genom?

Gurdon JB, Elsdale TR, Fischberg M (1958) Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature* 182(4627):64–65.

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–676.

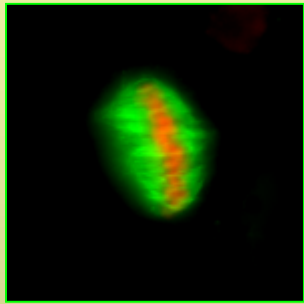
Sul JY, et al. (2009) Transcriptome transfer produces a predictable cellular phenotype. *Proc Natl Acad Sci USA* 106:7624–7629.

Kim TK, et al. (2011) Transcriptome transfer provides a model for understanding the phenotype of cardiomyocytes. *Proc Natl Acad Sci U S A*. 108(29):11918-23.

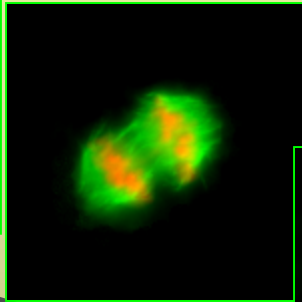


Sejtciklus-egyres fázisai

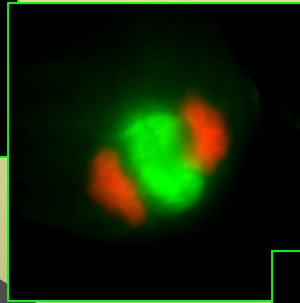
Ciklikus fehérje-módosítások, komplexek és degradációk



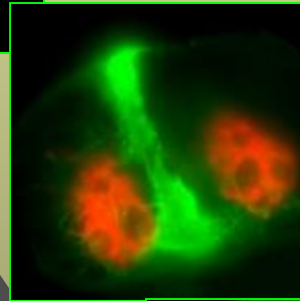
metaphase



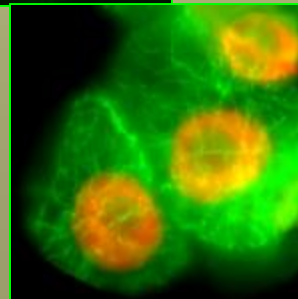
anaphase



early telophase

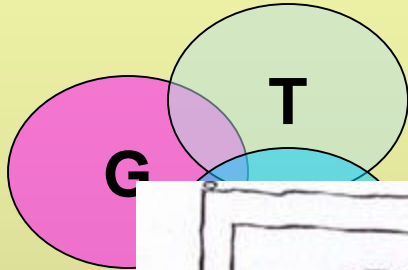
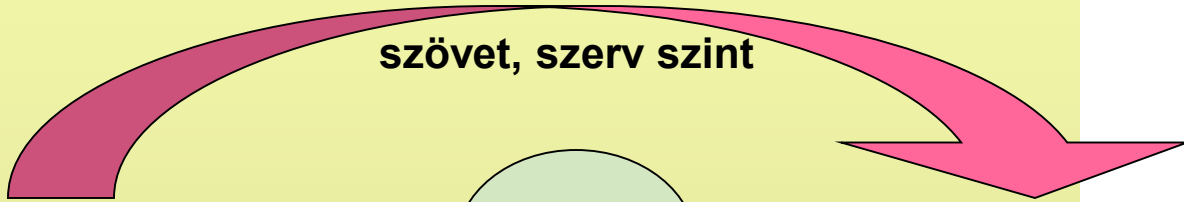


late telophase



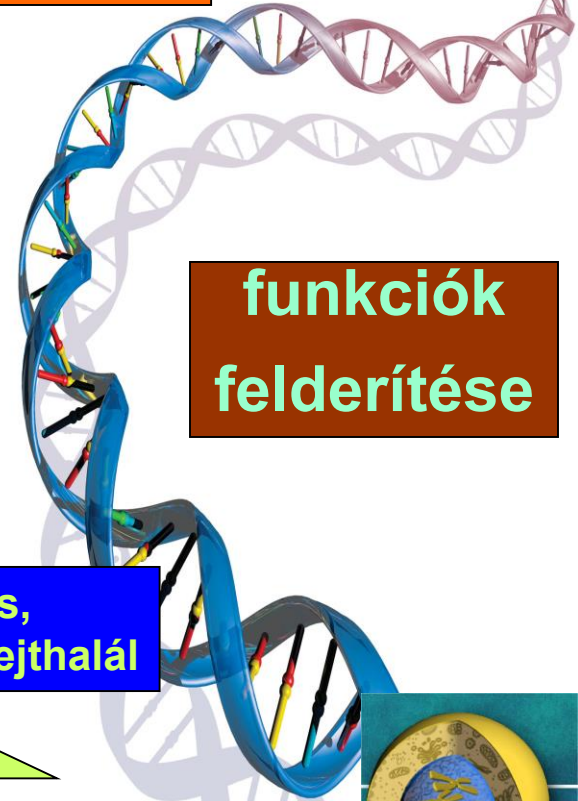
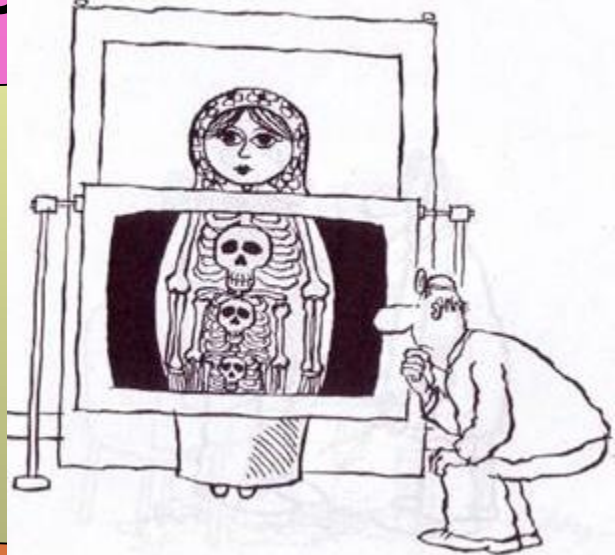
G1 phase

Funkcionális genomika lényege



daganat képződés, gyulladás, különböző betegségek

Minőségi és mennyiségi változások



funkciók felderítése

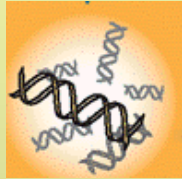


sejtosztódás, differenciáció, sejthalál



Microarray technológiák a funkcionális genomikában

Minta



Fragmentek



Szubcelluláris



Sejtek



Szervek



Organizmusok



Közösségek

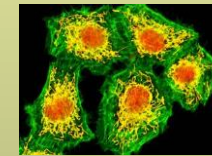
Cél-
molekula



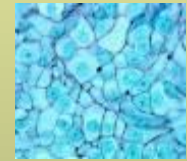
DNS, RNS



Fehérjék



Sejtek

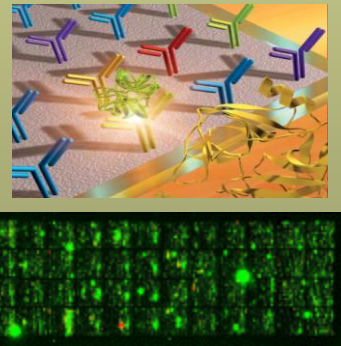


Szövetek

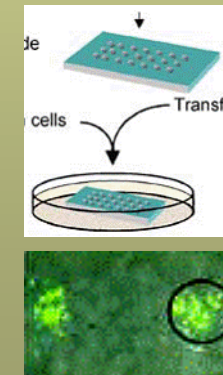
Microarray



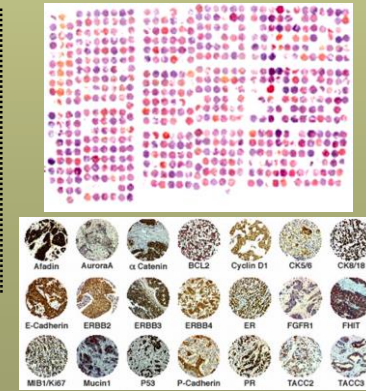
Genomika



Proteomika

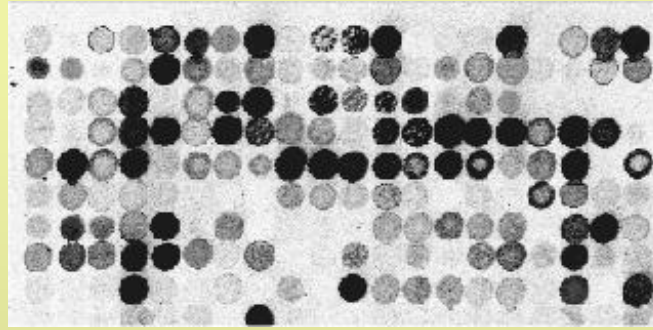


Citomika

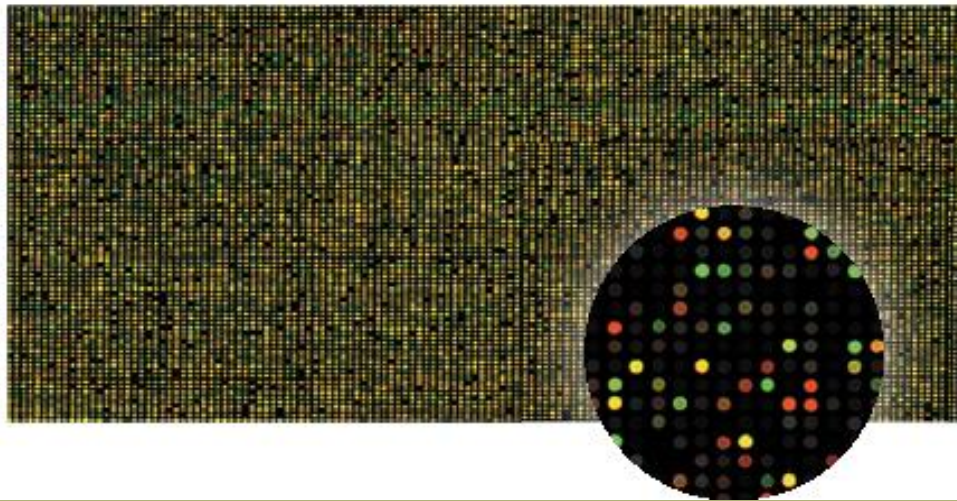


A csipek hordozó szerinti osztályozása

macroarray

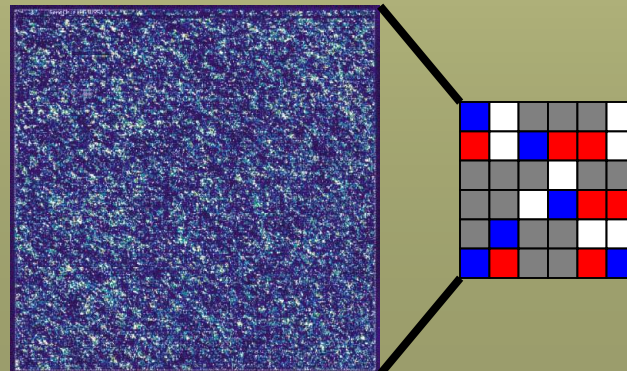


- nylon membránon néhány 100 génspecifikus minta (DNS darab)
- radioaktív jelölés
- kis minta sűrűség (100-1000 pont/cm²)



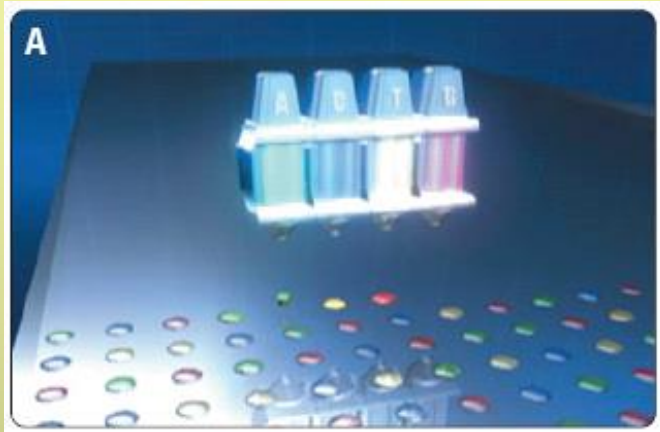
- üveglemezen több 10.000-100.000 génspecifikus minta (DNS darab)
- fluoreszcens jelölés
- közepes mintasűrűség (5000 pont/cm²)

chip

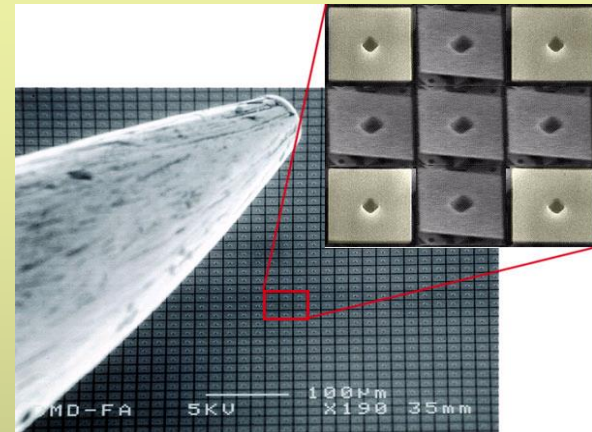


- üveglemezen több 100.000-1.000.000 génspecifikus minta (DNS darab)
- fluoreszcens jelölés
- nagy minta sűrűség (10.000 pont/cm²)

Alternatív technológiák oligonukleotid DNS-chipek gyártására



inkjet-printed microarrays
(e.g. Agilent)



maskless array synthesizer
(e.g. Nimblegen)

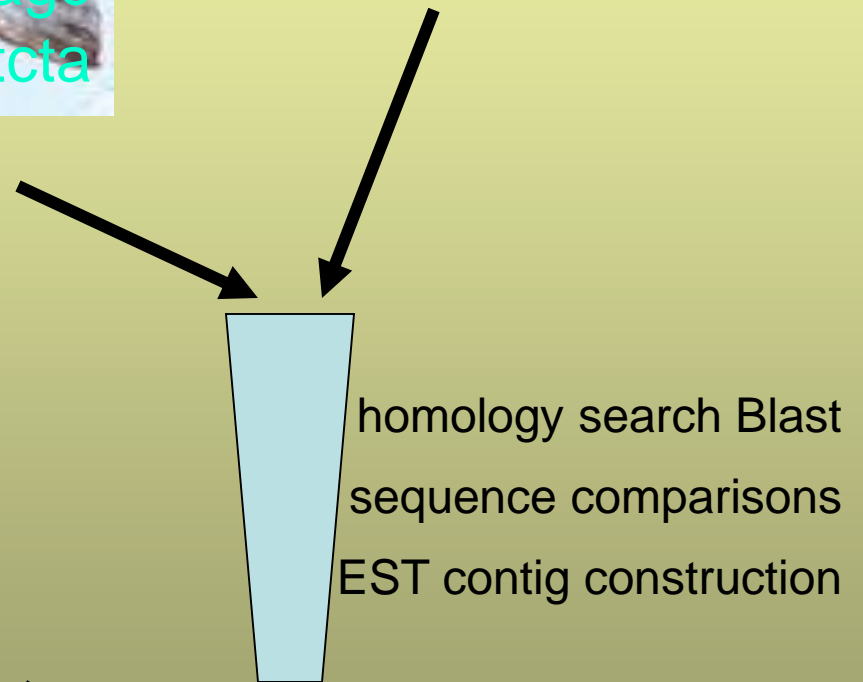


CARP Gene database & bioinformatics tools for microarray selection



CarpBase: over 15.000 expressed
sequence tags (ESTs)

NCBI: over 10.000 uncurated sequences



Probe Design

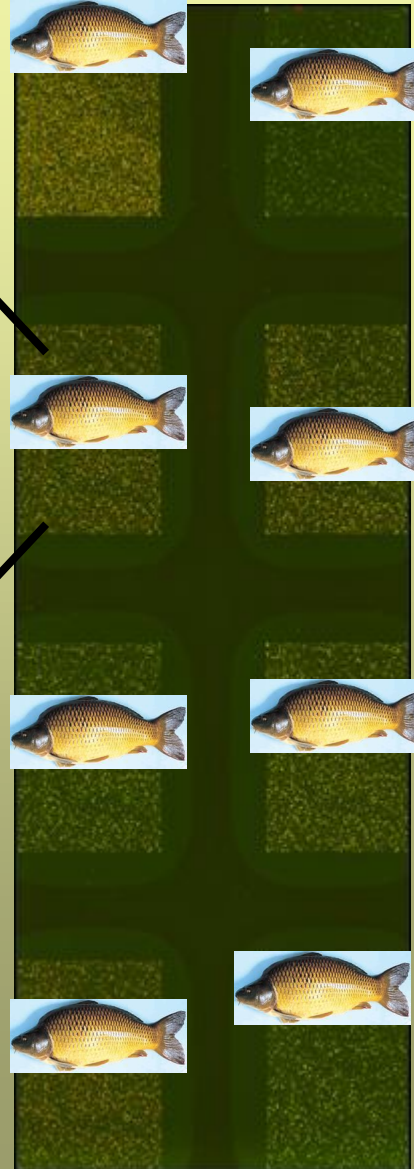
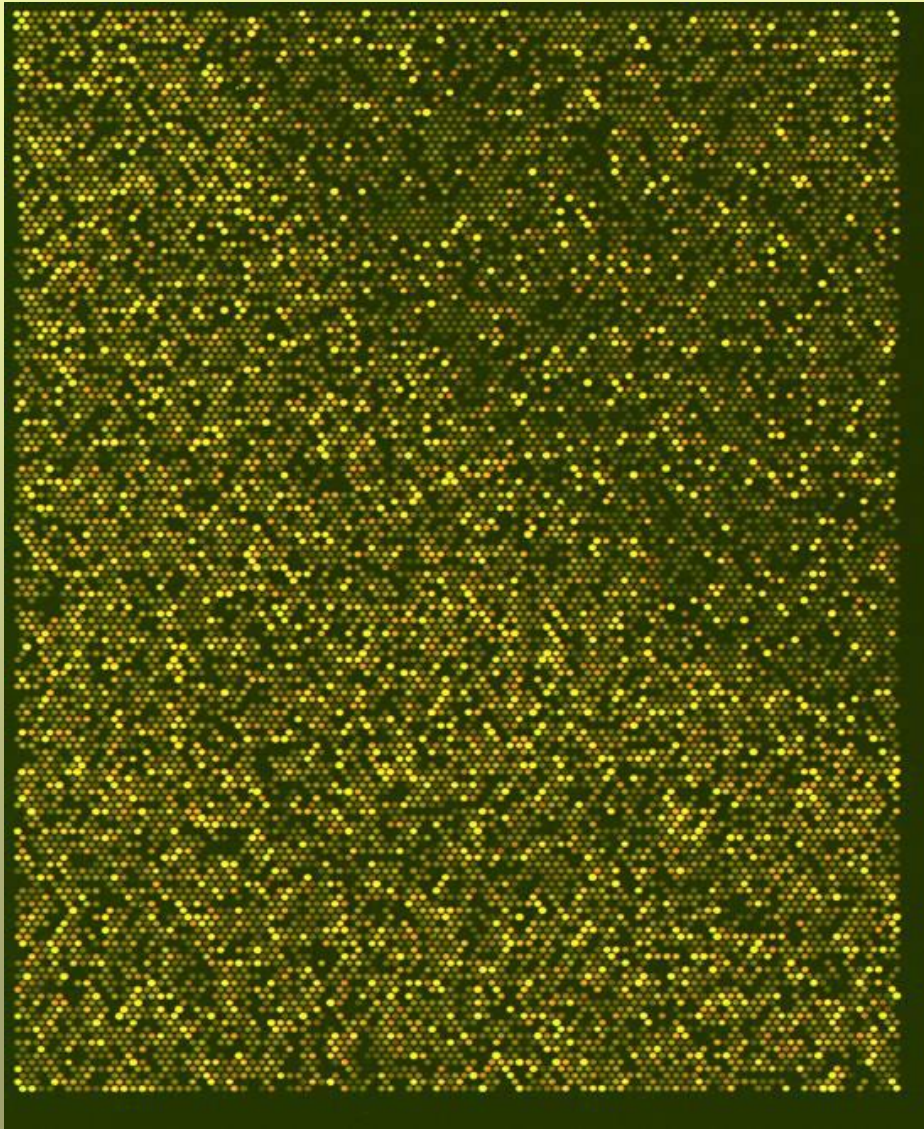
15.000 gene-specific
oligonucleotide sequences



DNA-microarray construction

15.000 cured ESTs,
functional groups,
gene families,
annotation

Ponty DNS-microarray formátum



**Analysis of
8 carps in
1 microarray
experiment
15.000 gene
analysis
each**

Különböző stressznek kitett pontyok (vér, máj, kopolytú) génextpressziós vizsgálata

hipoxia stressz

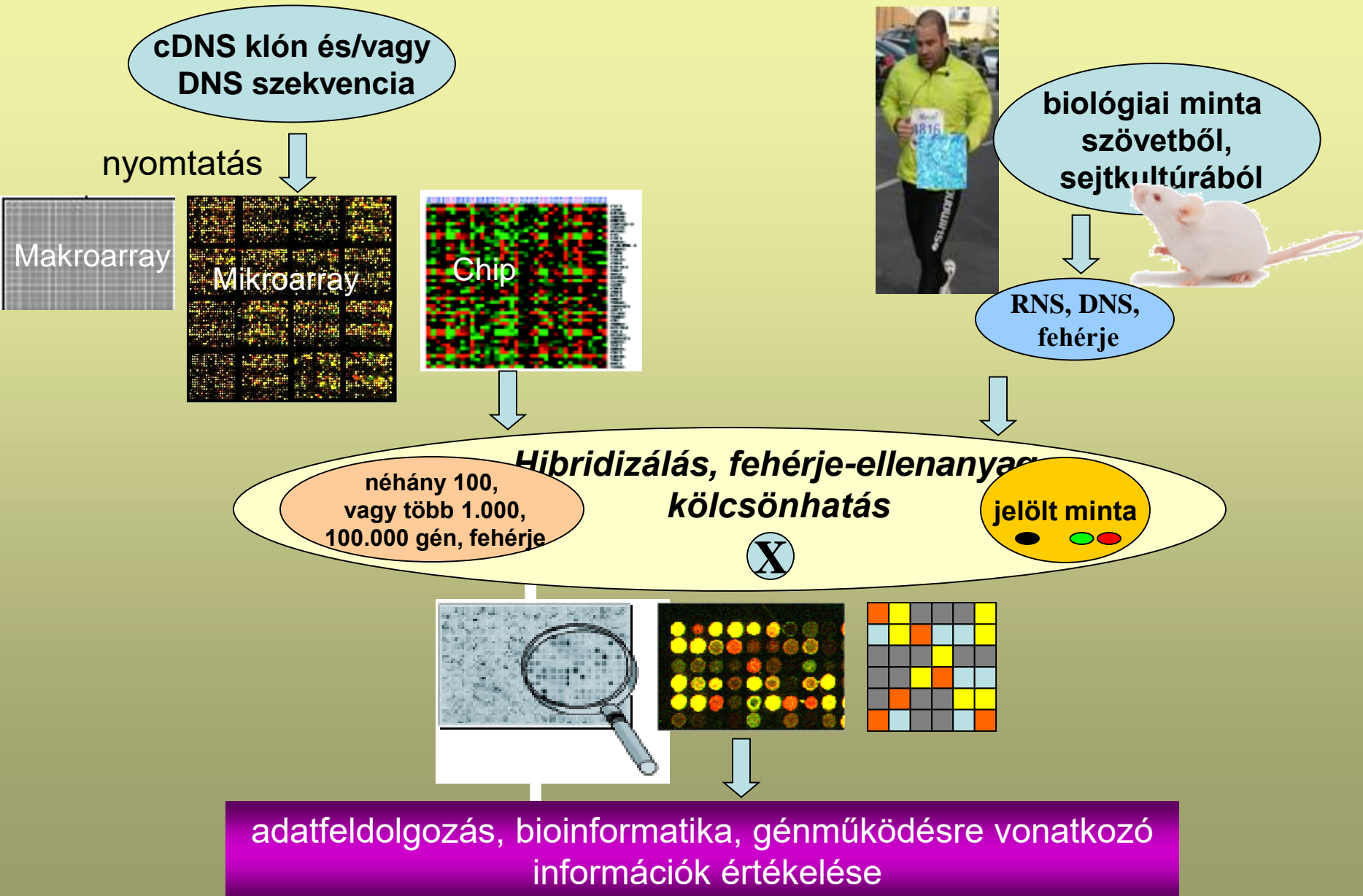
Szelénium hiány

Zsúfoltsági teszt

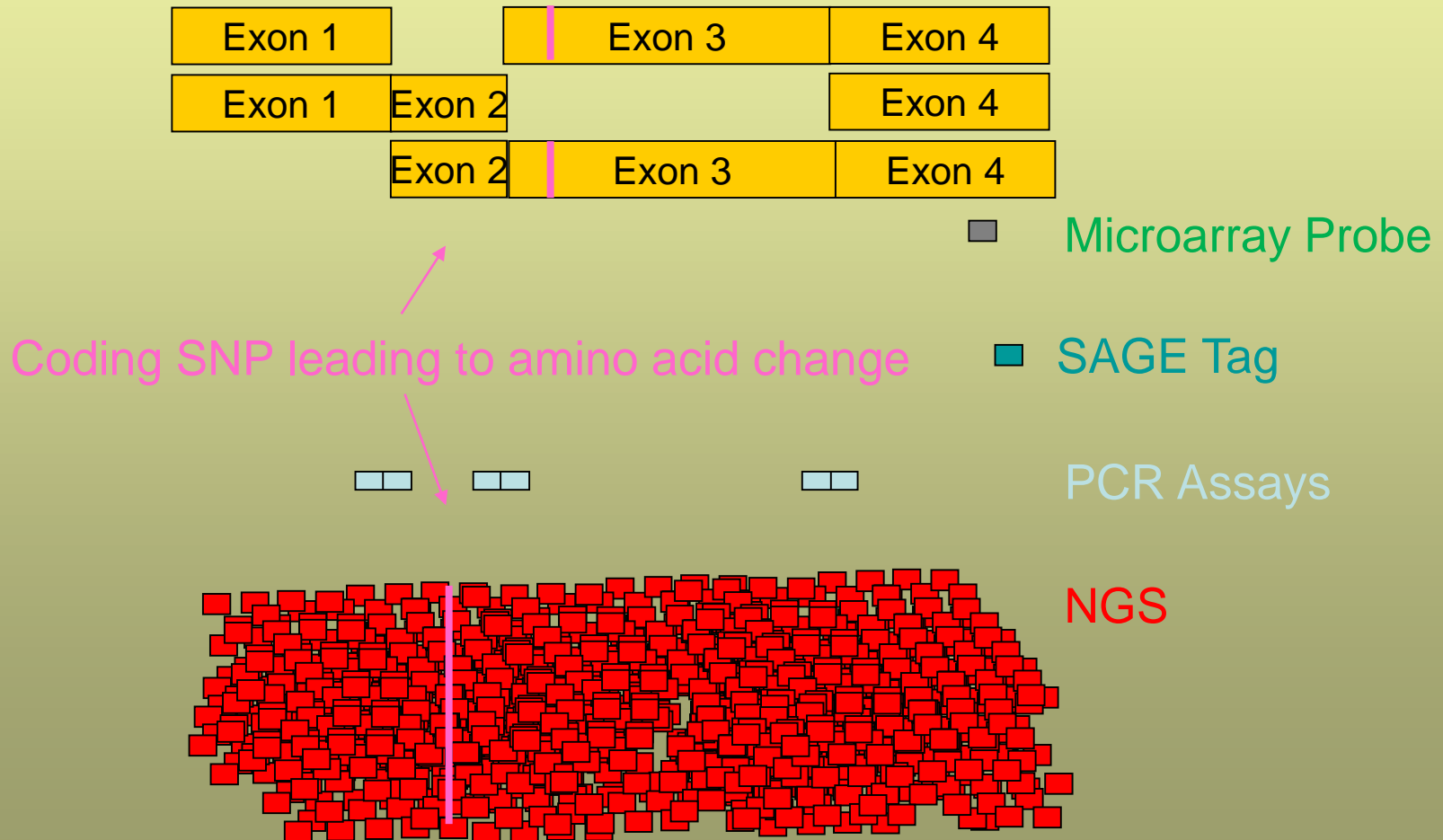


Sample collection (gill, blood, liver)
RNA preservation
transportation
RNA preparation
cDNA conversion
(banking for future studies)
DNA-microarray
HTS-QRT-PCR

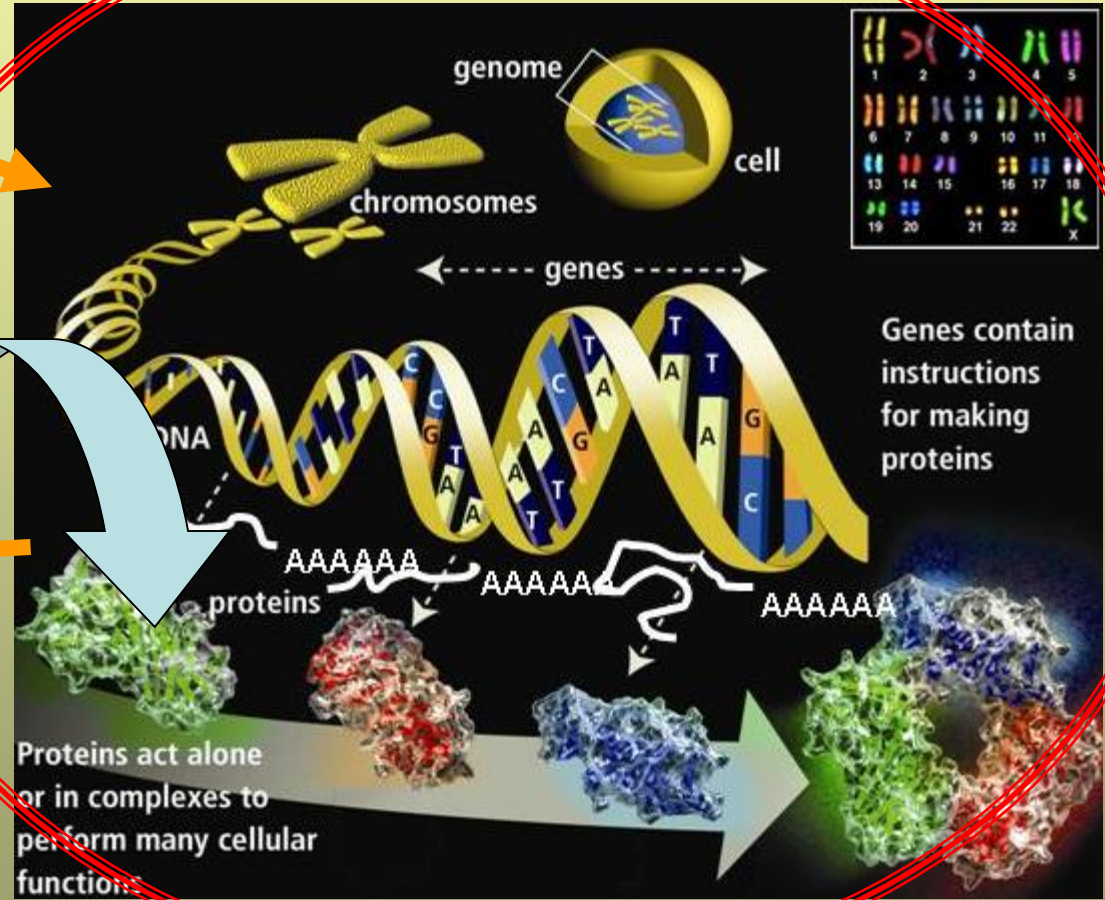
Egy csipkísérlet általános lépései



Microarray vs. SAGE vs. TaqMan vs. NGS



A táplálék kölcsönhatása a génekkel, génállománnyal



Rövidtávú és Hosszútávú hatások sejt szintű és Fiziológiás változások

Kitajka et al. (2002) PNAS 99, 2619;
Barcelo-Coblijn, G (2003) PNAS 100, 11321.
Kitajka (2004) PNAS 101, 10931
Puskás et al. (2003) PNAS 100, 1580-1585.
Puskás et al. (2004) Biochimie 86, 817.
Jayasooriya et al (2005) PNAS 102, 7133.
Puskas et al. (2006) Curr Pharm Biotechnol. 7, 525
Puskas LG, Kitajka K. (2006) Nutr Health. 18, 227
Ménesi D et al. (2009) Prostaglandins Leukot Essent Fatty Acids
DasUN, Puskas LG (2010) Lipids Health Disease

N-3 politelítetlen zsírsavakat tartalmazó diéta hatásának vizsgálata cDNS-chip technológiával

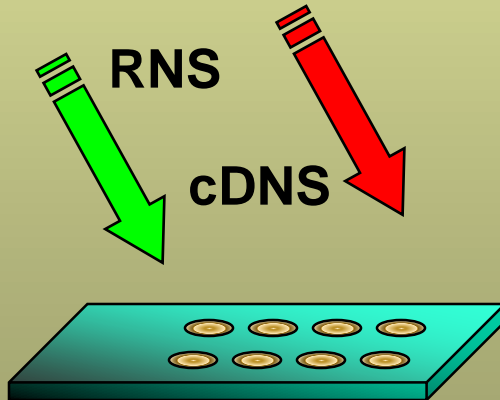
halolajjal
etetett

kontroll



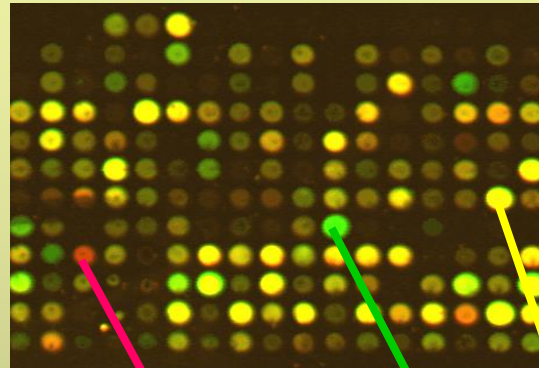
RNS

cDNS

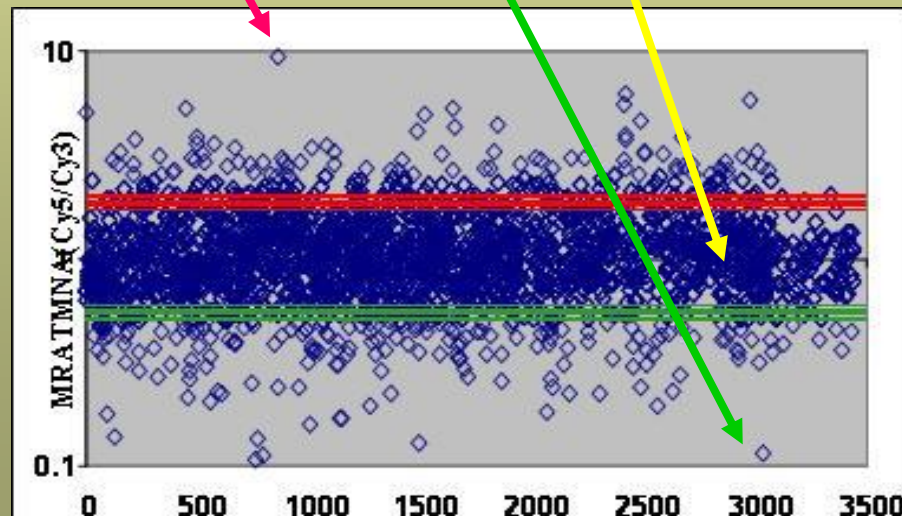


patkány cDNS-chip
3200 génspecifikus minta
6400 mintapont

Génexpressziós változások



szinaptikus plaszticitás
citoszkeleton
jelátviteli folyamatok
energia metabolizmus
membrán asszociáció
ioncsatorna képzés
transzkripció faktorok

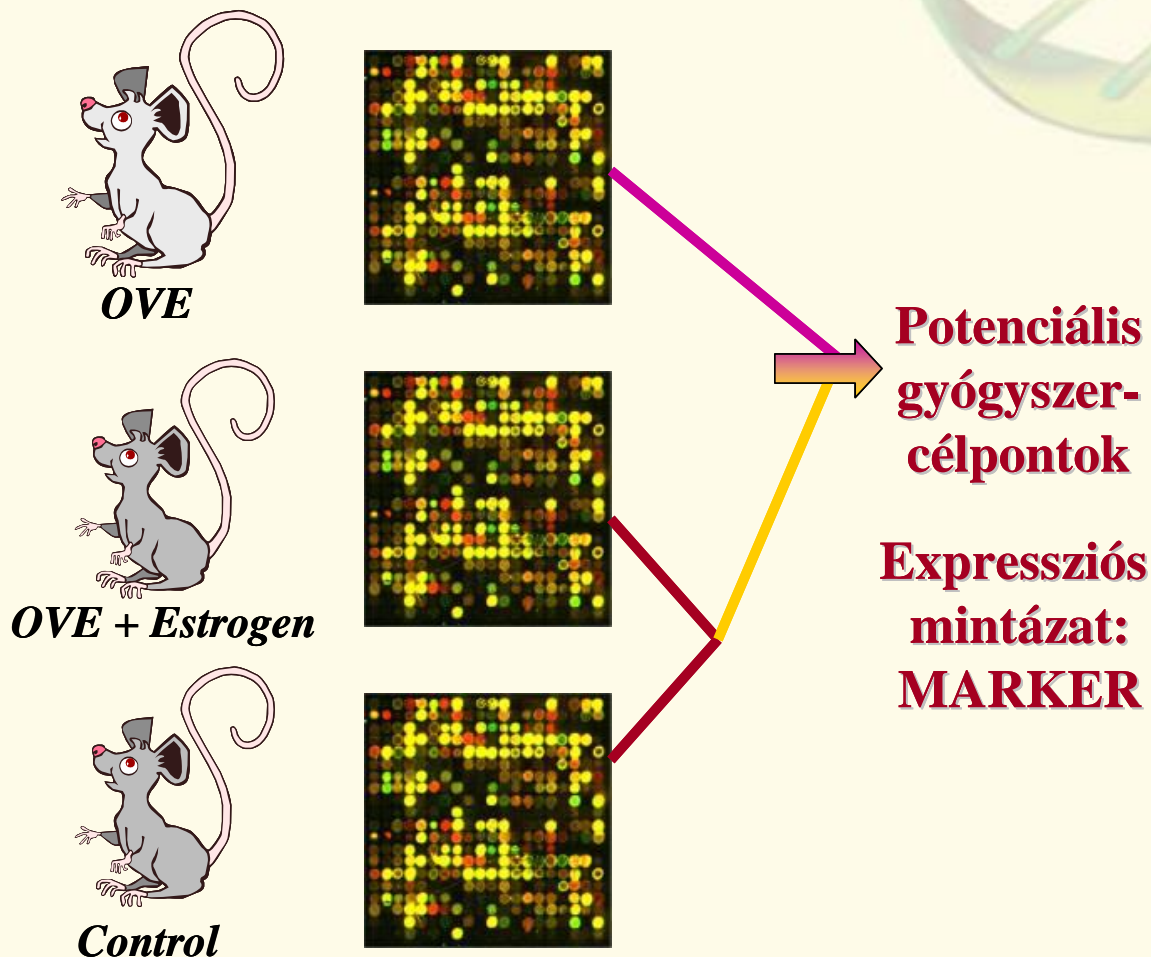


Pozitív hatás a mentális funkciókra, Tanulás, memória

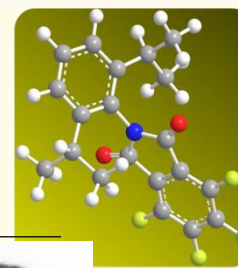
Felfedezés (Discovery)

1. Target alapú szűrések

1a: új target azonosítása



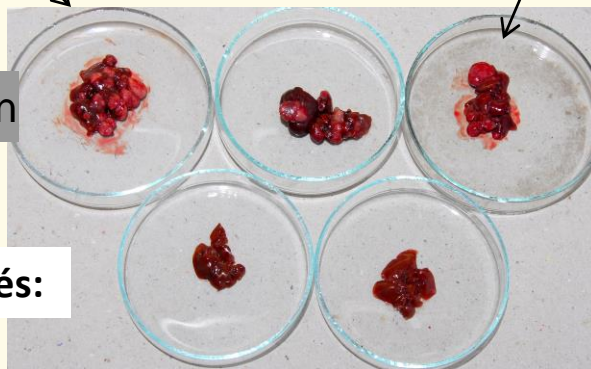
Génexpressziós változások Ac915-tel kezelt KO-egér májkarcinómában



Tumorigenesis: DEN, 15 naponan egyszeri 50 ul-es kezelés, 4 hónap normál táp

Kezeletlen kontroll

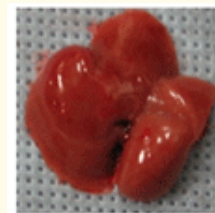
Kezeletlen



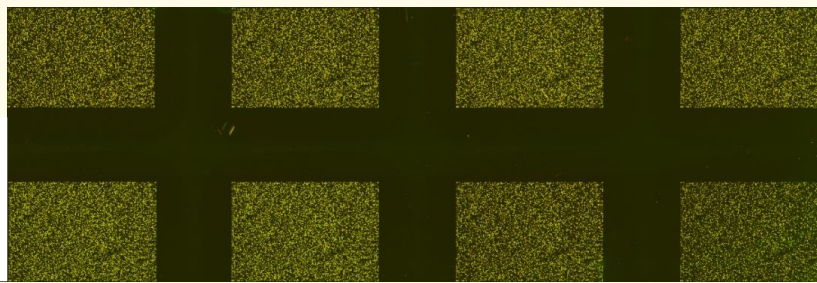
Ac915 kezelés:

Tumor izolálás

Egészséges szövet izolálás

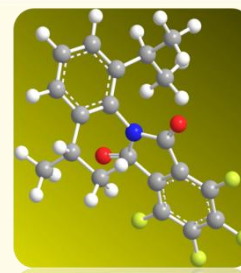


Egészséges szövet izolálás



Funkcionális elemzés

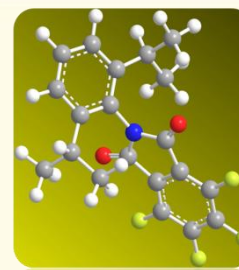
Ac-915 tumor / induced tumor



- DAVID bioinformatics
- WEB alapú rendszer
- Input: gén lista
- Funkcionális “csoportok” (GO terms, pathways, tissue expression pattern etc.)
- Géncsoportosítás azonos funkció szerint
- Szignifikancia analízis, p-value

Represszált gének.

Ac-915 tumor / induced tumor



Term	Count	%	PValue	Genes
:0007010~cytoskeleton organization and biogenesis	28	7.31%	1.17E-05	Myh11, Arhgef11, Myo7a, Epb4.1l2, Dlg1, Arpc2, Tagln, Trpm7, Tubd1, Snap23, Rdx, Ndel1, Birc5, Actg2, Krt1, Ppp4c, Pfn2, Tbce, Wasl, Lcp1, Tubb4, Cenpj, Vill, Tuba1a, Fmn1, Capn3, Dnaic1, Rhoq,
:0015630~microtubule cytoskeleton	16	4.18%	0.00664	Cetn3, Dynlt3, Tubb4 , Cenpj, Nek2, Akap9 , Cenpf, Tubd1 , Tuba1a , Cdc2a, Ndel1, Birc5, Brca1, Npm1, Dnaic1, Ppp4c,
:0007049~cell cycle	29	7.57%	9.93E-04	Gadd45a , Rras , Dlg1, Ncapd2, Cdca3, Anxa1, Cdc2a, Birc5, Brca1, Psm13, Npm1, Tgfa, Rbm7, Jun , Pcnp, Btg3, Cetn3, Tsc2, Mns1, Ccna2 , Maff, Nek2, Cdkn2c, Cenpf, Ddit3 (DNA-damage inducible transcript 3) , Txnip, Ccnb1 , Ube2c, Pttg1,
:0000278~mitotic cell cycle	14	3.66%	0.00237	Btg3 , Cetn3, Dlg1, Ccna2, Nek2, Ncapd2, Cenpf, Cdca3, Cdc2a, Birc5, Ccnb1, Ube2c, Tgfa, Pttg1,
:0051301~cell division	11	2.87%	0.02743	Arhgef11, Cetn3 , Birc5 , Ccnb1, Ccna2, Nek2, Ube2c, Ncapd2, Cdca3 (cell division cycle associated 3) , Pttg1, Cdc2a ,
:0000902~cell morphogenesis	21	5.48%	0.00541	Arhgef11, Ing2, Tsc2, Wasl, Tubb4, Myo7a, Egfr , Epb4.1l2, Dlg1, Igfbp4 , Ctnna1, Rdx, Ndel1, Alcam, Brms1l, Igfbp7 , Rhoq, Cdc42se1, Tbce, Btg1, Pak2,
:0031410~cytoplasmic vesicle	16	4.18%	0.00568	Pla2g4a, Snapin , Myo7a, Egfr , Snap23, Tff3, Pdpk1, Rab7 , Sec23ip , Spg21, Tlr1, Nostrin, Sec24b, Pip5k3, Ica1, Sec23a ,
:0007088~regulation of mitosis	5	1.31%	0.01555	Birc5 , Dlg1 , Tgfa , Cenpf , Cdc2a ,
:0048193~Golgi vesicle transport	6	1.57%	0.03495	Rab6 , Stx6 (syntaxin 6) , Sec24b , Lman1, Sec23a, Snap23,
:0015630~microtubule cytoskeleton	16	4.18%	0.00664	Cetn3, Dynlt3, Tubb4, Cenpj, Nek2, Akap9, Cenpf, Tubd1, Tuba1a, Cdc2a, Ndel1, Birc5, Brca1 , Npm1, Dnaic1, Ppp4c,

The **ArrayExpress Archive** is a database of functional genomics experiments including gene expression where you can query and download data collected to **MIAME** and **MINSEQE** standards. **Gene Expression Atlas** contains a subset of curated and re-annotated Archive data which can be queried for individual gene expression under different biological conditions across experiments.

Experiments Archive

33537 experiments, 969491 assays



Experiment, citation, sample and factor annotations

[Browse experiments](#) | [platform designs](#) | [protocols](#) | [files](#)

Query

Gene Expression Atlas

3558 experiments, 99484 assays, 20806 conditions

Genes

up/down in

Conditions

Any species

Query

[Gene Expression Atlas Home](#)

Experiment, citation, sample and factor annotations [clear]

ArrayExpress data only

[Advanced query syntax](#)

[Submitter/reviewer login](#)

[ArrayExpress Browser Help](#)

Filter on [reset]

Oryza sativa

All arrays

All assays by molecule by All technologies

Display options [reset]

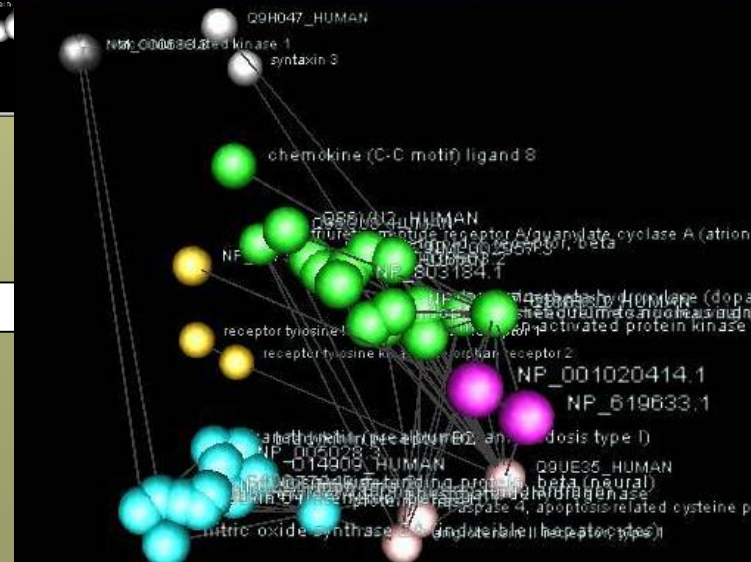
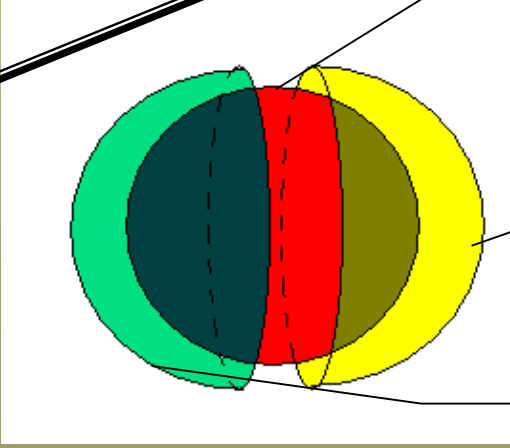
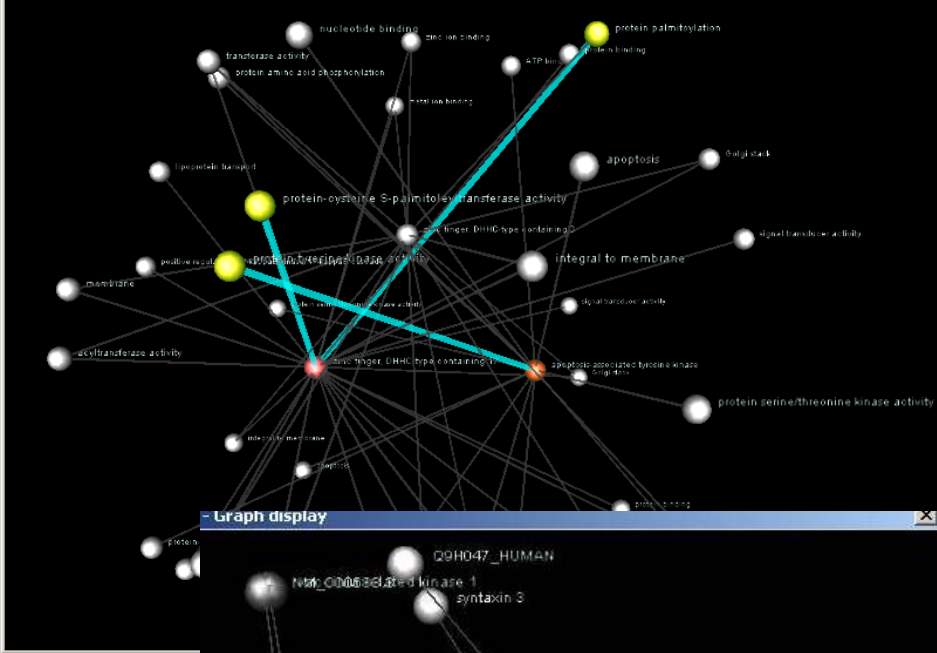
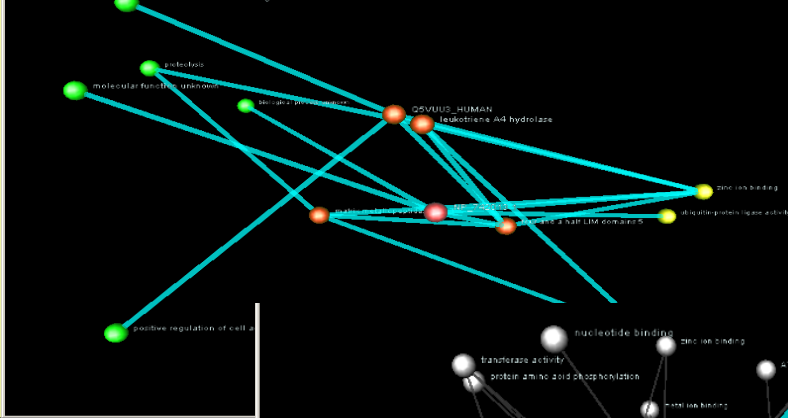
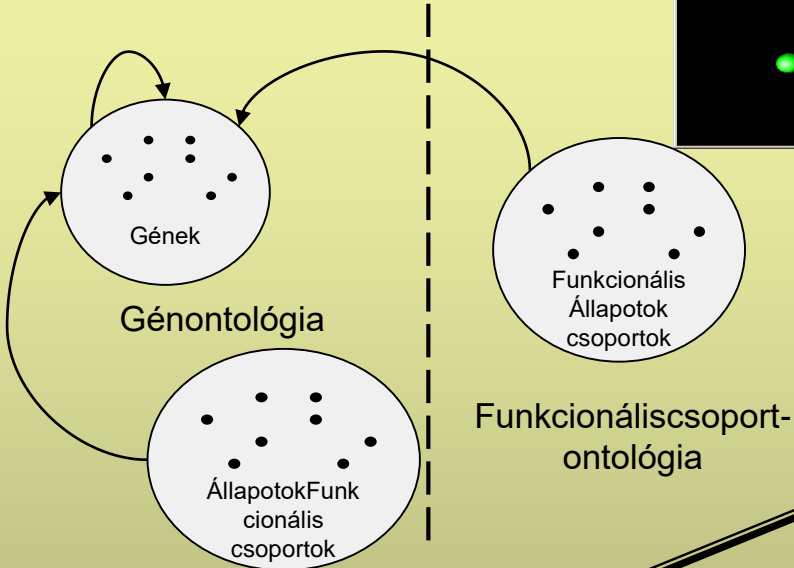
25 experiments per page

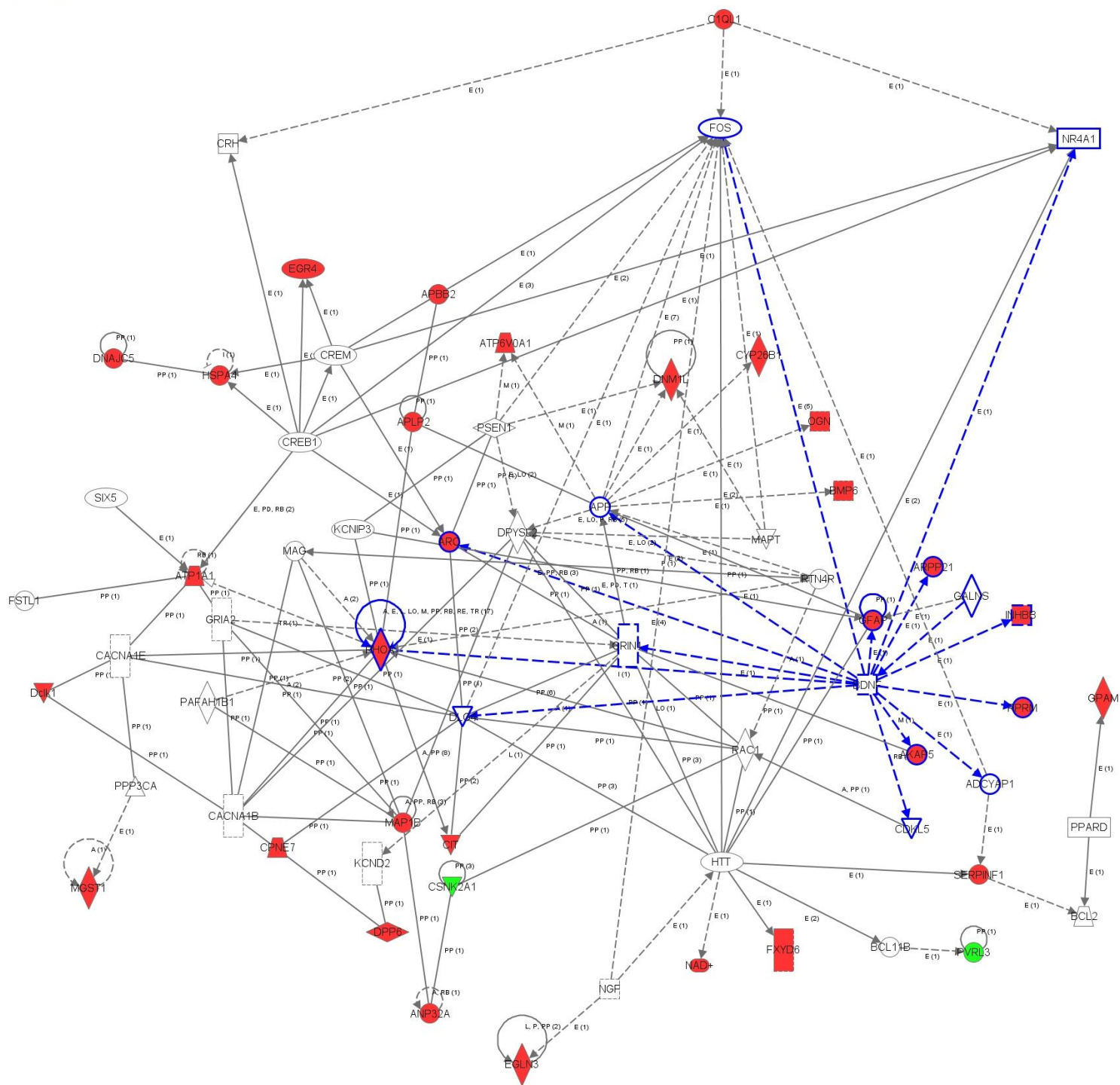
Detailed view

Query

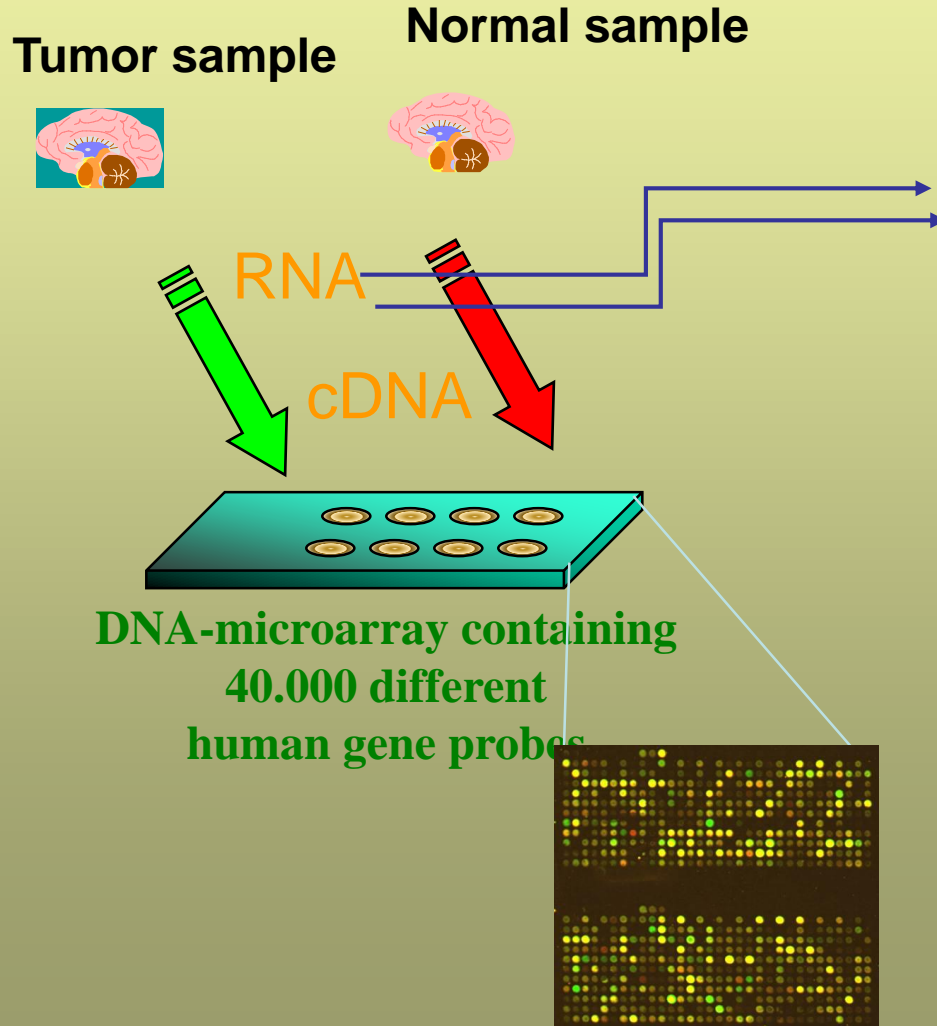
Accession	Title	Assays	Species	Date	Processed	Raw	Atlas
E-GEOD-11014	A diverse set of microRNAs and microRNA-like small RNAs in developing rice grains	6	Oryza sativa	2008-08-20			-
E-GEOD-22564	A global analysis of QTLs for expression variations in rice shoot at early seedling stage	226	Oryza sativa Indica Group	2011-06-24			-
E-GEOD-28308	A single functional blast resistance gene Pi54 activates complex defense mechanism in Rice	8	Oryza sativa Japonica Gro	2011-12-08			-
E-GEOD-13161	A transcriptome atlas of rice cell types uncovers cellular, functional and developmental hierarchies	168	Oryza sativa	2010-05-19			-
E-GEOD-14729	A transcriptomic analysis of super-hybrid rice LYP9 and its parental lines	96	Oryza sativa	2009-04-22			-
E-GEOD-33409	Abscission layer (AL) at the base of flower in rice using laser micro-dissection: NIL(qSH1) AL vs. Nipponbare AL, NIL(qSH1) AL vs.	11	Oryza sativa	2012-03-31			-
E-GEOD-21772	affy_meloidogyne_rice2-Oryza sativa genes expressed in the early compatible interaction with root-knot nematode	6	Oryza sativa	2011-09-24			-
E-GEOD-34192	affy_riz_2011_7-: Transcriptomic analysis of rice leaves challenged with virulent and avirulent Xoo strains	18	Oryza sativa	2012-10-15			-
E-GEOD-19844	affy_xoo_rice-Transcriptomics-based identification of Xoo strain BAI3 Talc targets in rice	9	Oryza sativa	2010-12-01			-
E-GEOD-40964	Agilent 44K Rice Oligo DNA Microarray RAP-DB (G2519F#15241); genome-wide transcriptional analysis of Aluminum-responsive g	16	Oryza sativa	2012-09-19			-
E-GEOD-14462	An Expression Alteration of Small RNA Profiling in Autotriploids Derived from Rice Twin-Seedlings	2	Oryza sativa	2009-07-14			-
E-GEOD-27726	Analysis of anther transcriptomes to identify genes contributing to meiosis and male gametophyte development in rice	12	Oryza sativa Indica Group	2011-06-10			-
E-GEOD-16796	Analysis of PHOTOPERIOD SENSITIVITY5 Sheds Light on the Role of Phytochromes in Photoperiodic Flowering in Rice	4	Oryza sativa	2010-05-16			-
E-GEOD-5986	Analysis of Rice microRNAs Expression under Drought Stress	24	Oryza sativa	2010-06-11			-
E-GEOD-8670	Analysis of the Interaction Transcriptome During Biotrophic Invasion of Rice by the Blast Fungus, Magnaporthe oryzae	24	Magnaporthe oryzae , Oryz	2009-04-08			-
E-GEOD-7567	BTH-responsive rice genes	8	Oryza sativa	2010-06-05			-
E-GEOD-17169	bulked RILs with high and low grain number per panicle derived from 2 cultivars at panicle primordia stage	4	Oryza sativa Indica Group	2009-12-31			-
E-GEOD-13152	Characterization of unique small RNA populations from rice grain	4	Oryza sativa	2008-11-24			-
E-GEOD-35502	Characterizing the role of rice NRAMP5 in Manganese, Iron and Cadmium Transport	20	Oryza sativa Japonica Gro	2012-02-03			-
E-GEOD-8767	Chilling stress transcriptional regulatory networks of japonica rice	44	Oryza sativa	2009-12-30			-

Adatbáziskutatás, adatbányászás

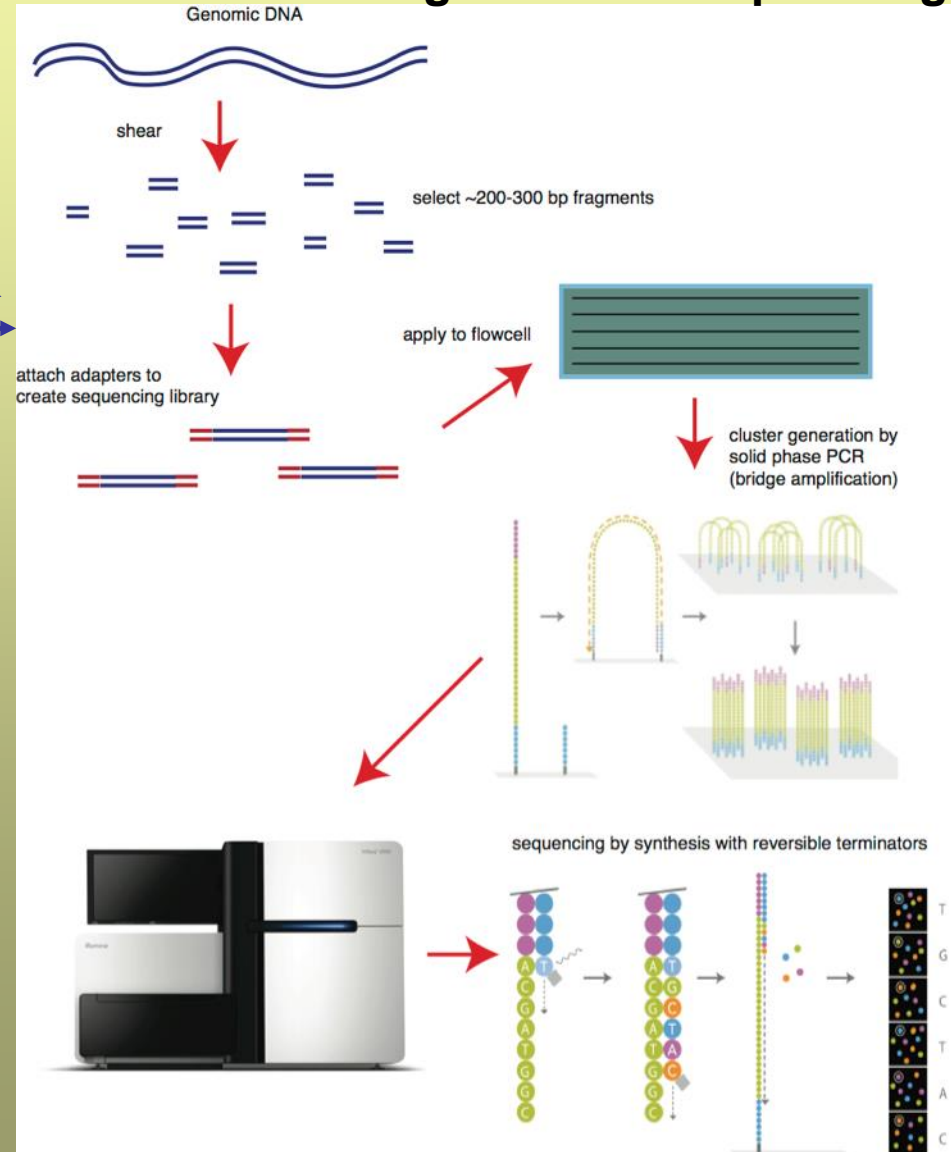




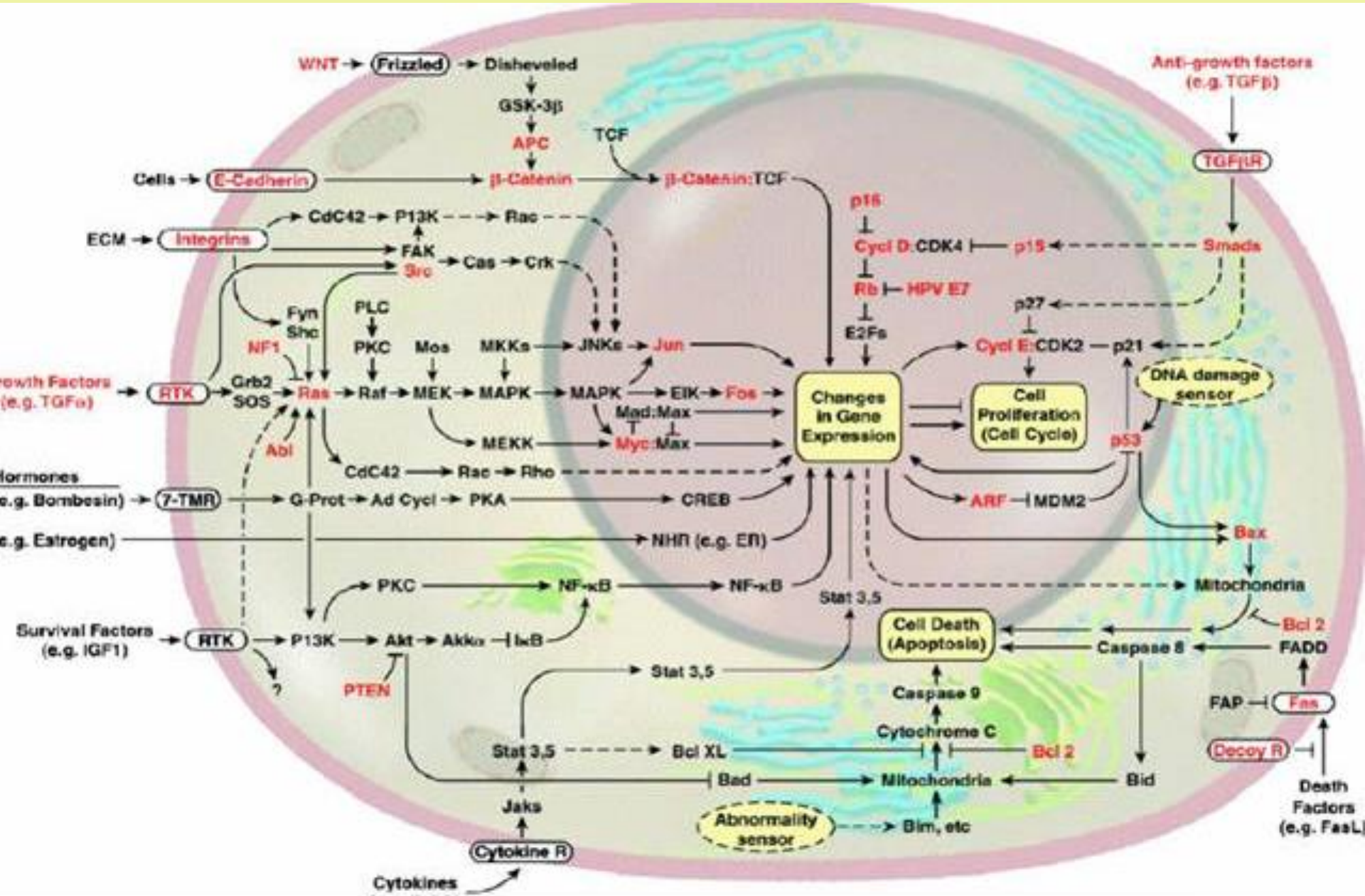
Globális genomikai szűrési módszerek alkalmazása tumorbiológiai kutatásokban

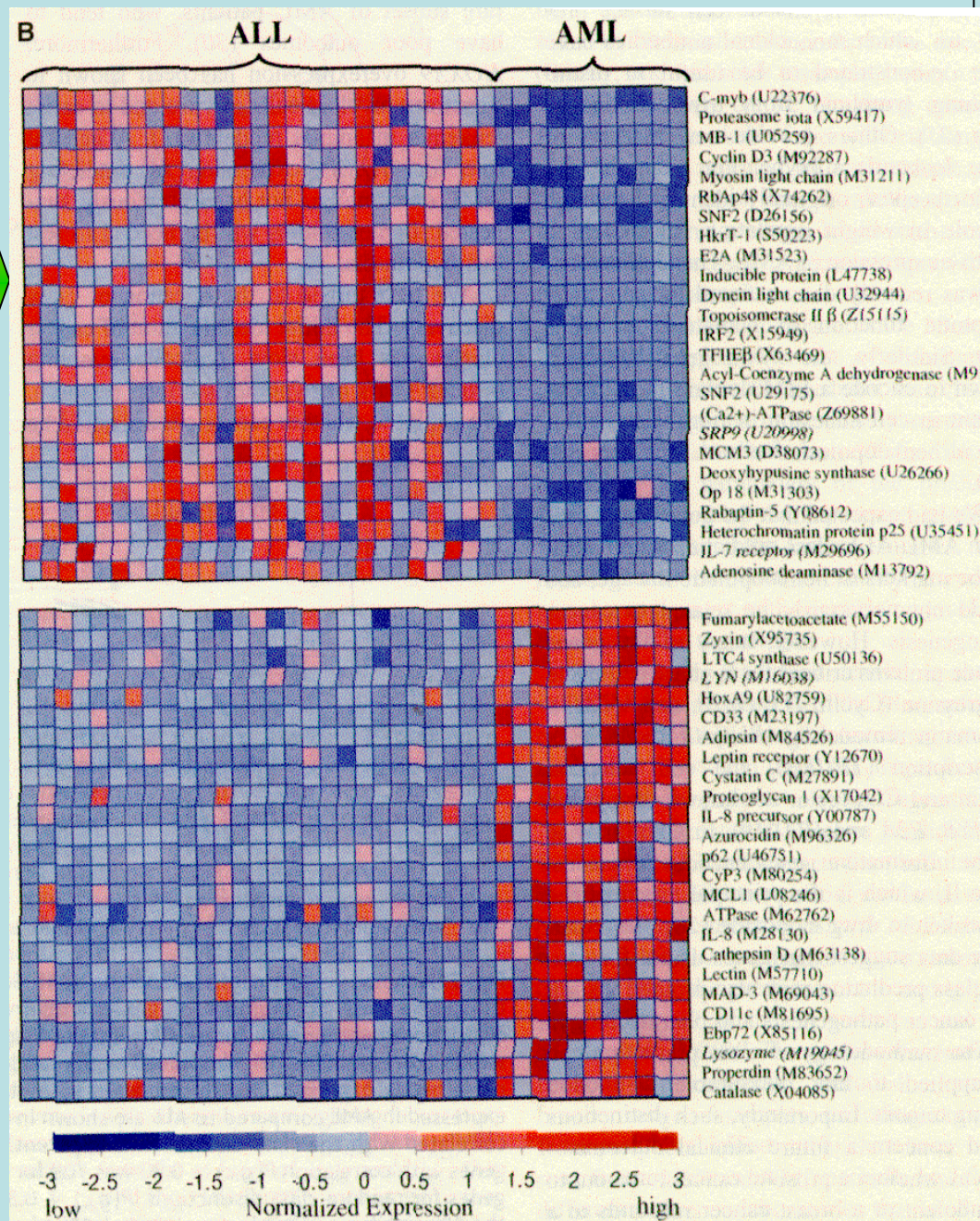
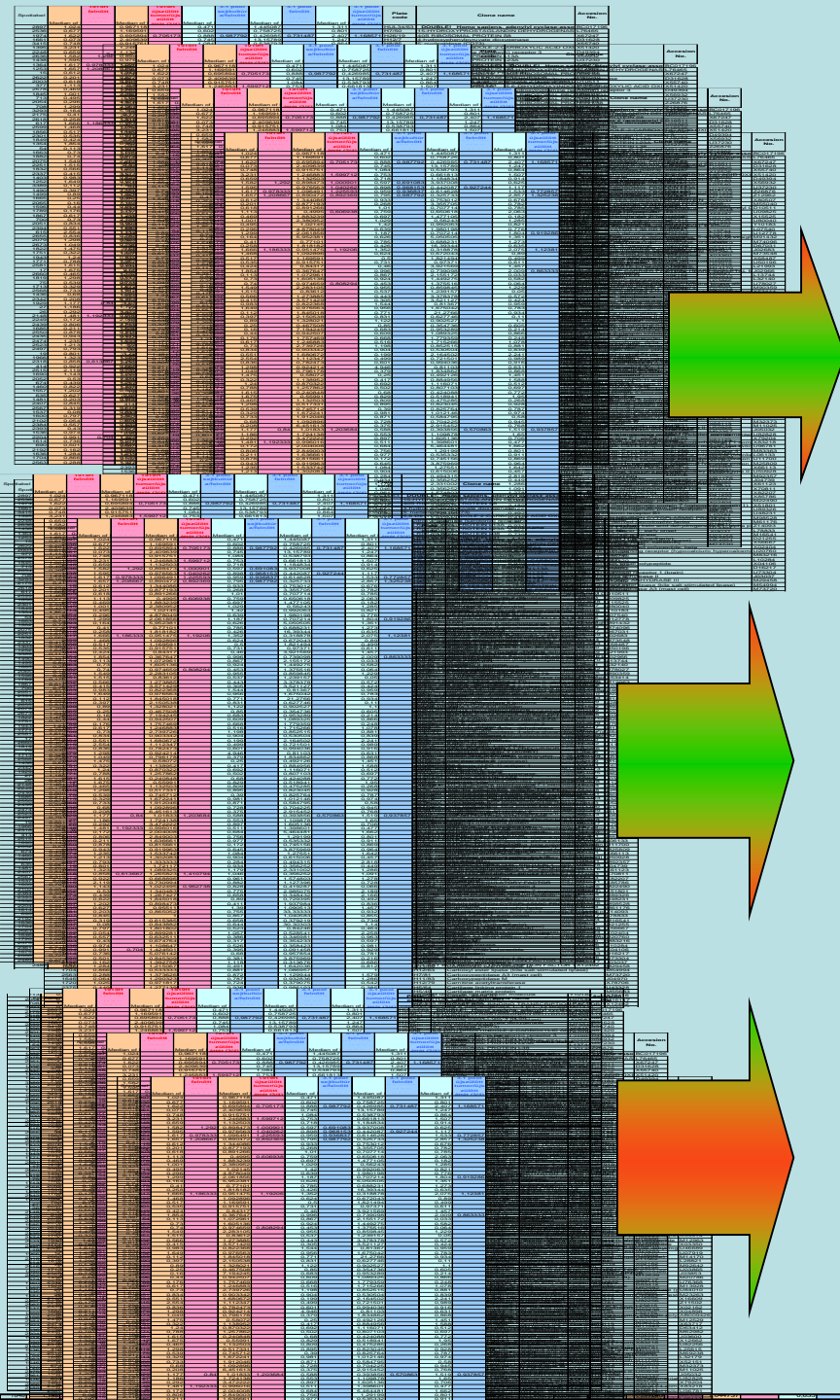


Illumina Next generation sequencing

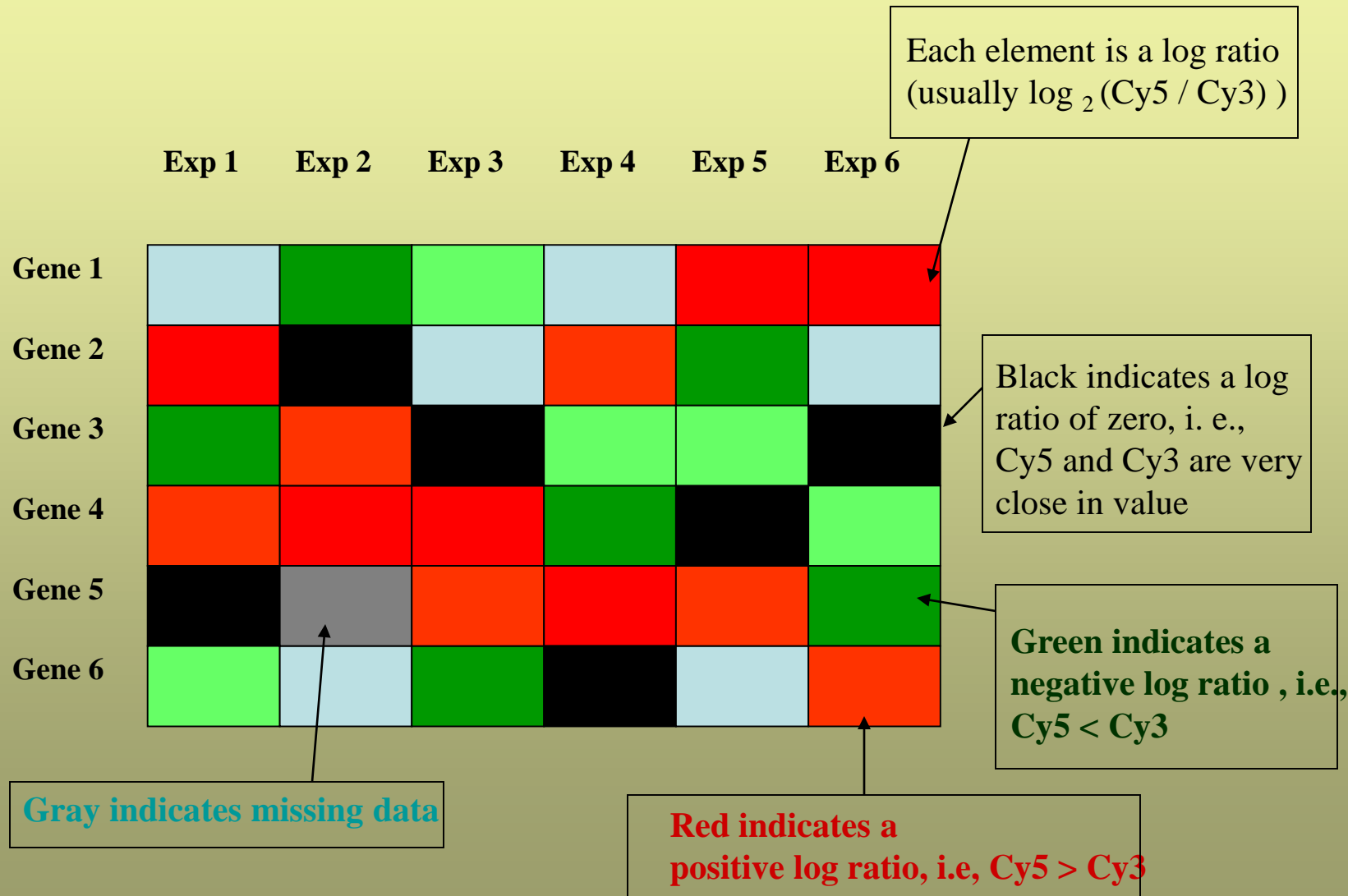


Complex regulatory networks, disregulation of signalling pathways result in gene expression differences (8000!)



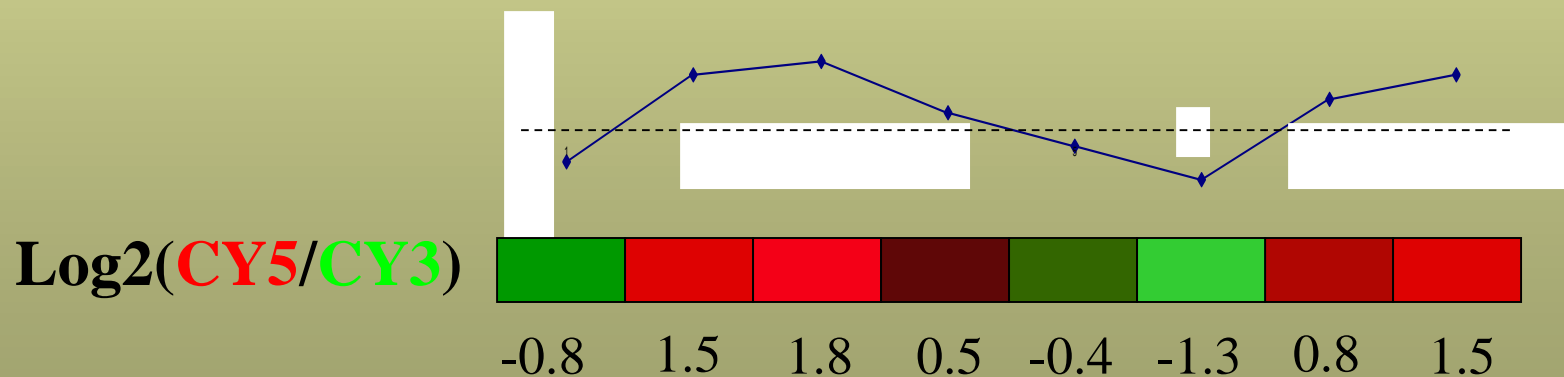


The Expression Matrix is a representation of data from multiple microarray experiments.

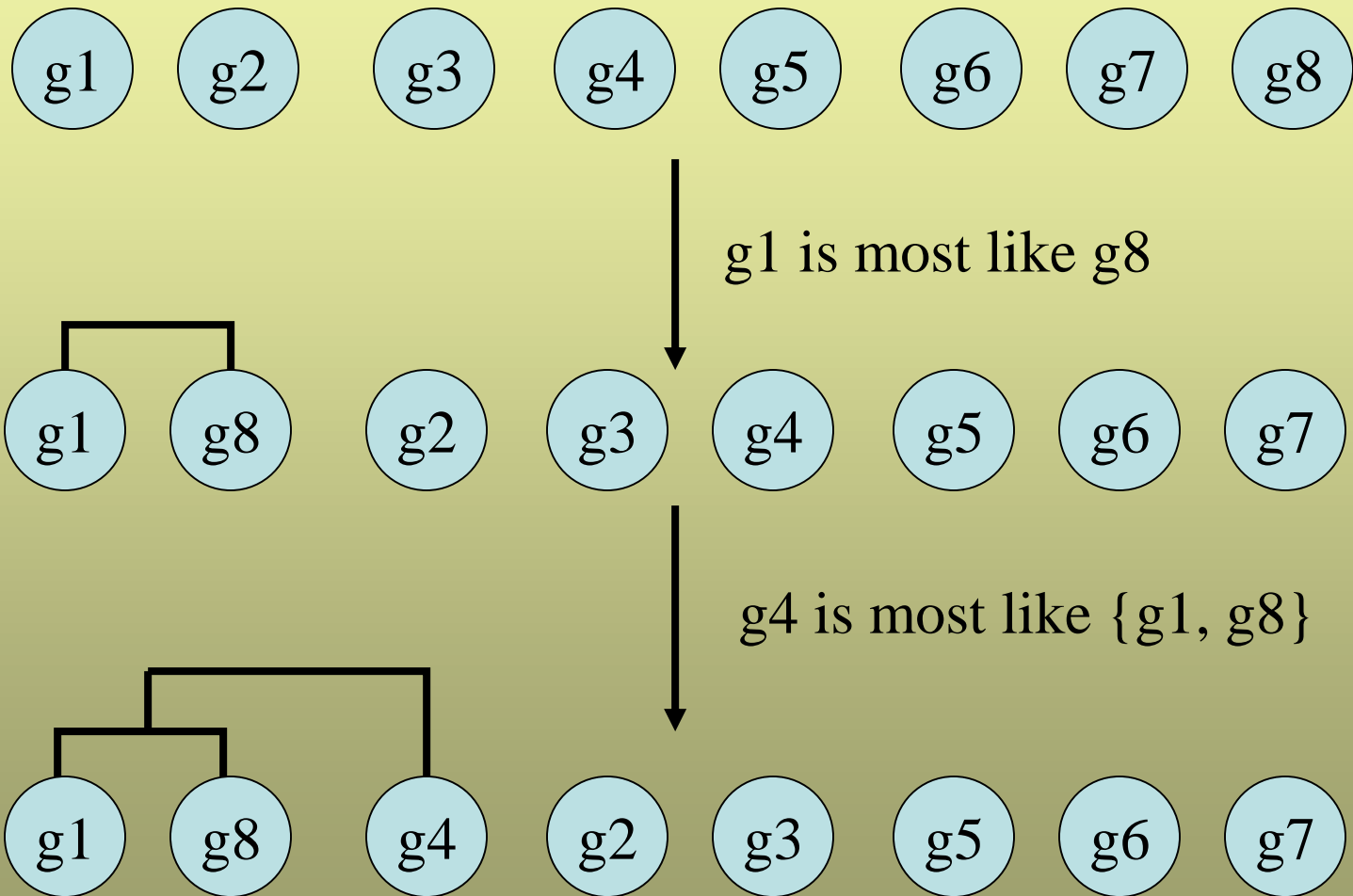


Expressziós Vektorok

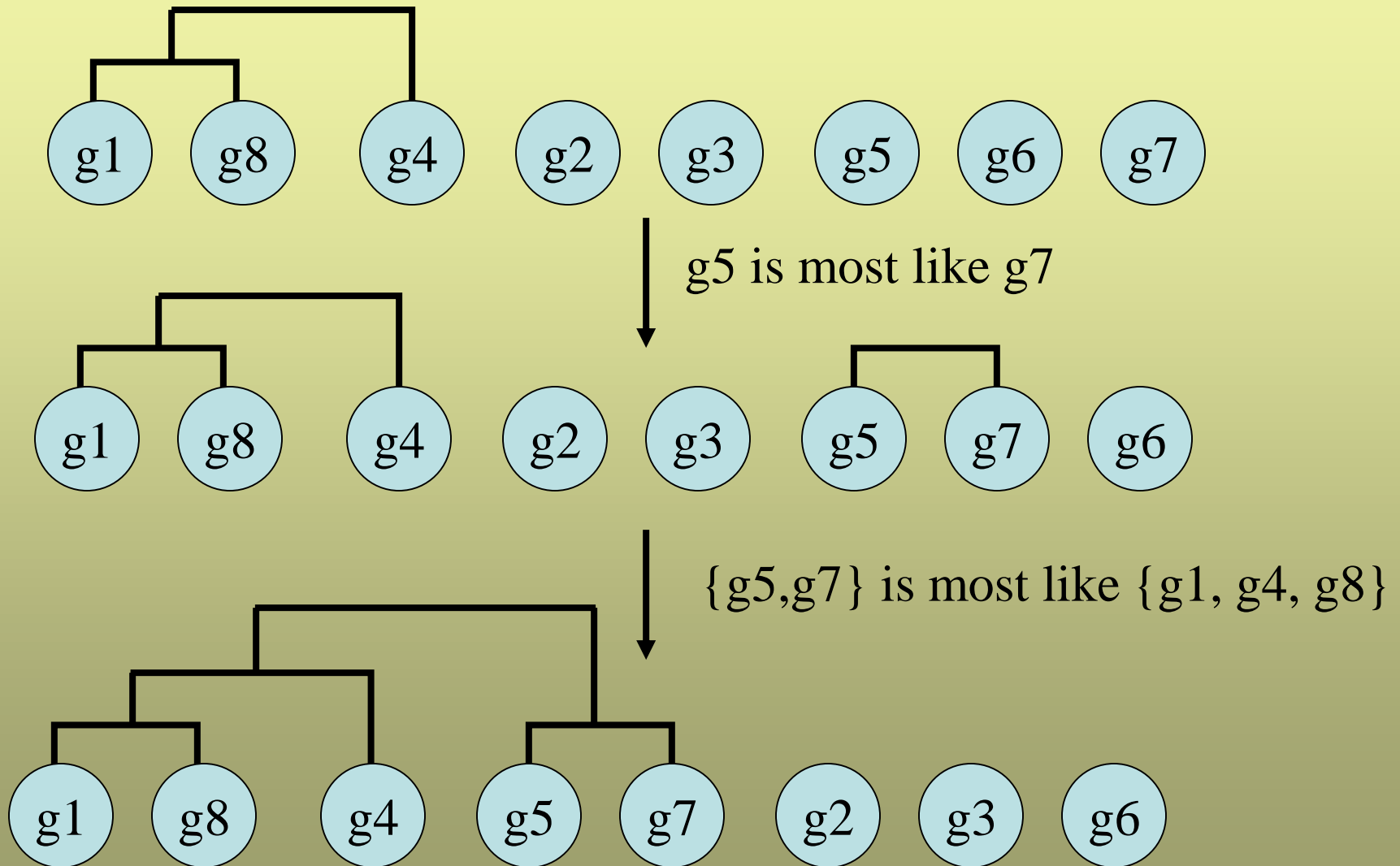
egy gén kifejeződését jeleníti meg több kísérleti körülmény vagy minta típus során



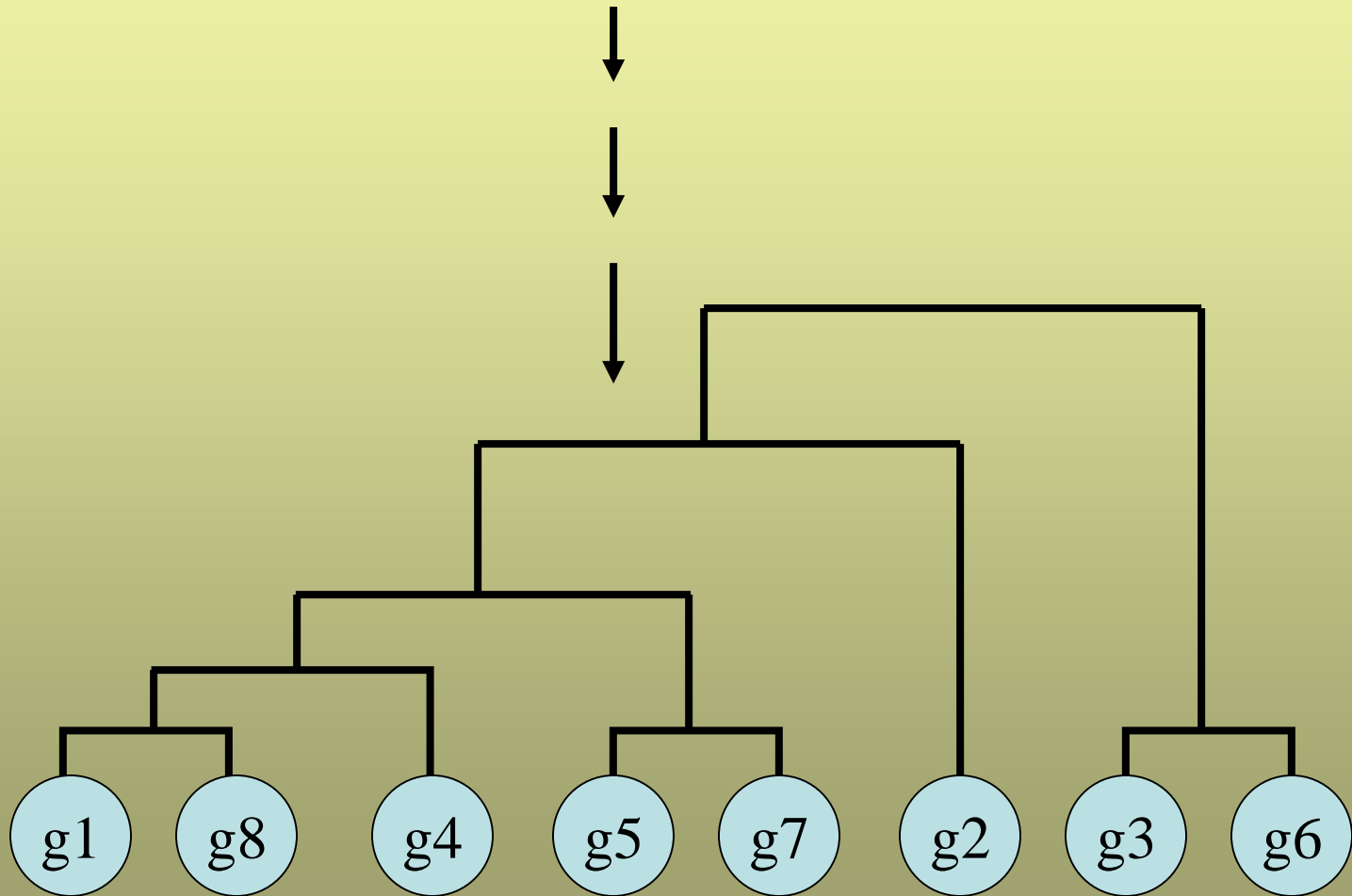
Hierarchikus klaszterezés



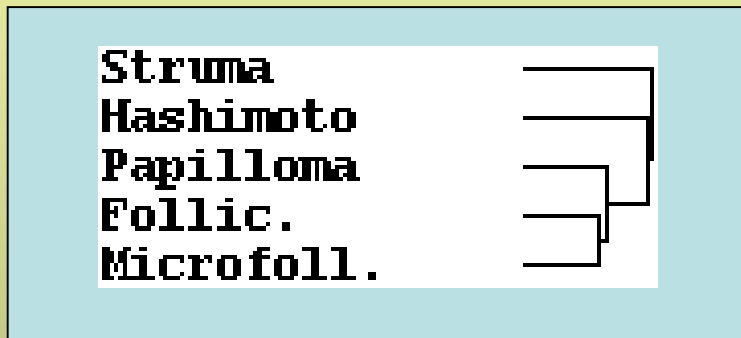
Hierarchikus klaszterezés



Hierarchikus fa



Különböző pajzsmirigy eredetű betegségek hierarhikus klaszteranalítise

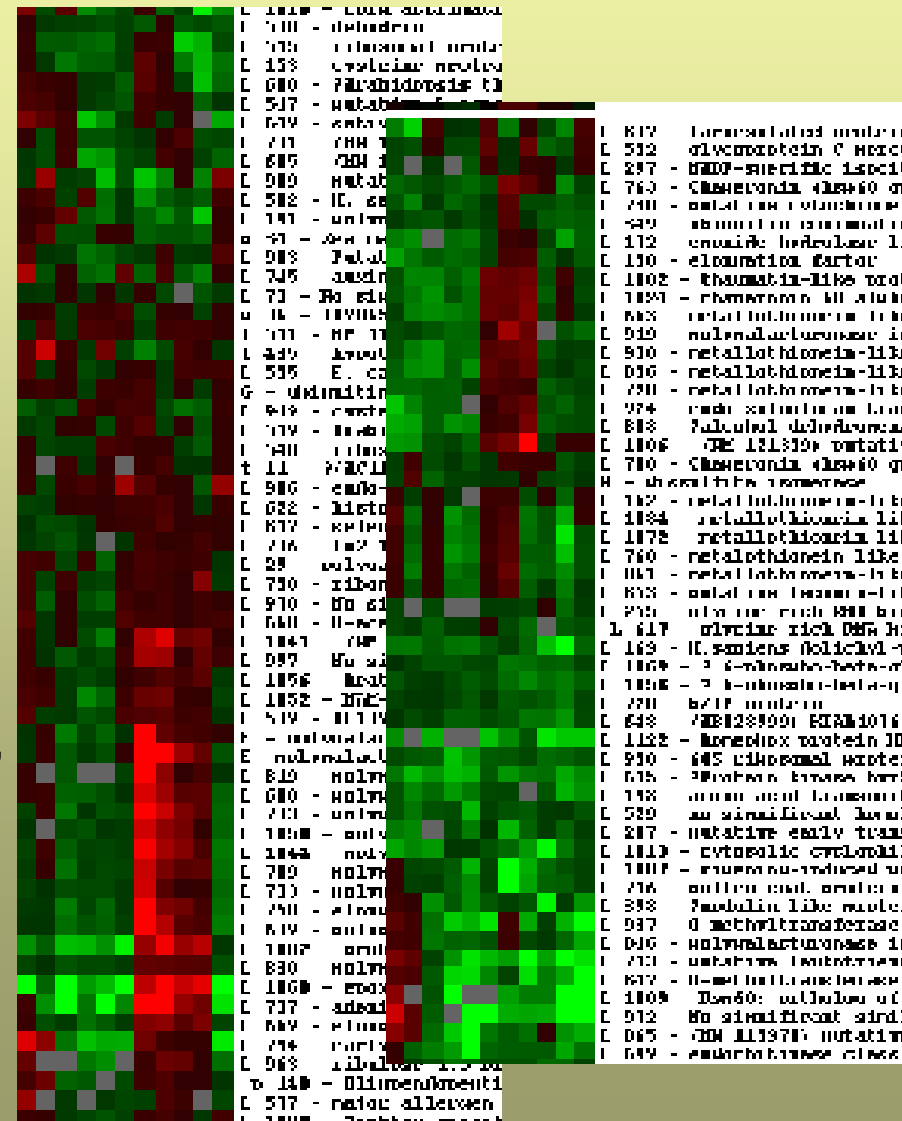


Hierarchical cluster analysis with Omniviz

Betegség-specifikus klaszterek meghatározása

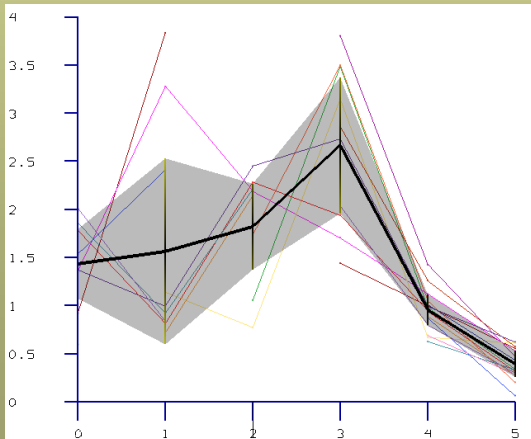
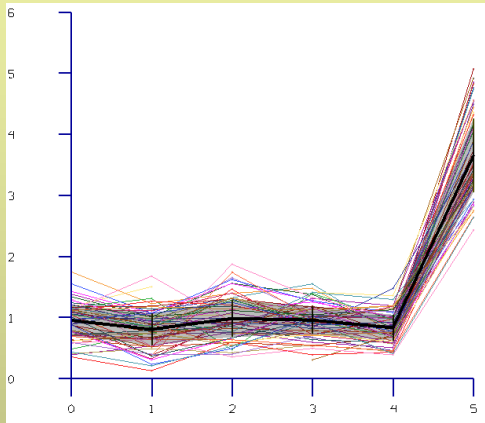
Legközelebbi kapcsolat: microfollicular & follicular carc., papilloma ehhez az alcsoporthoz hasonlít.

Struma and Hashimoto különböznek egymástól és más betegség csoportoktól.

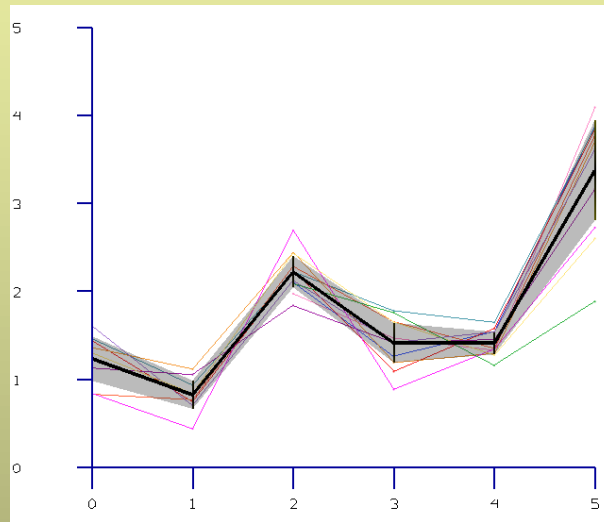


Betegségsspecifikus génmarkerek azonosítása

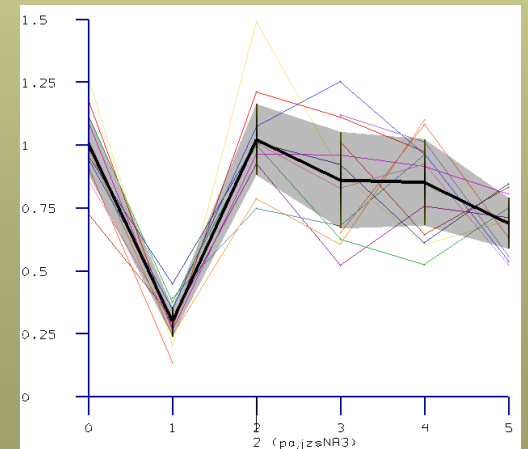
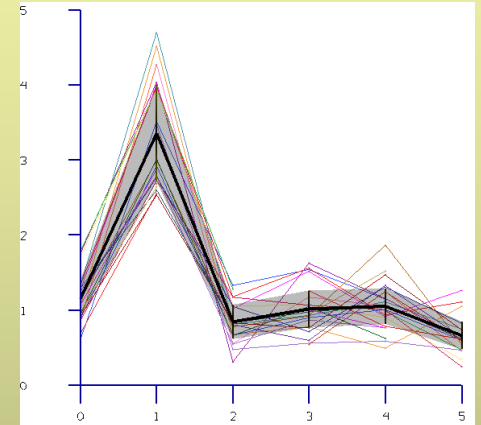
Struma



Microfollicular + Struma

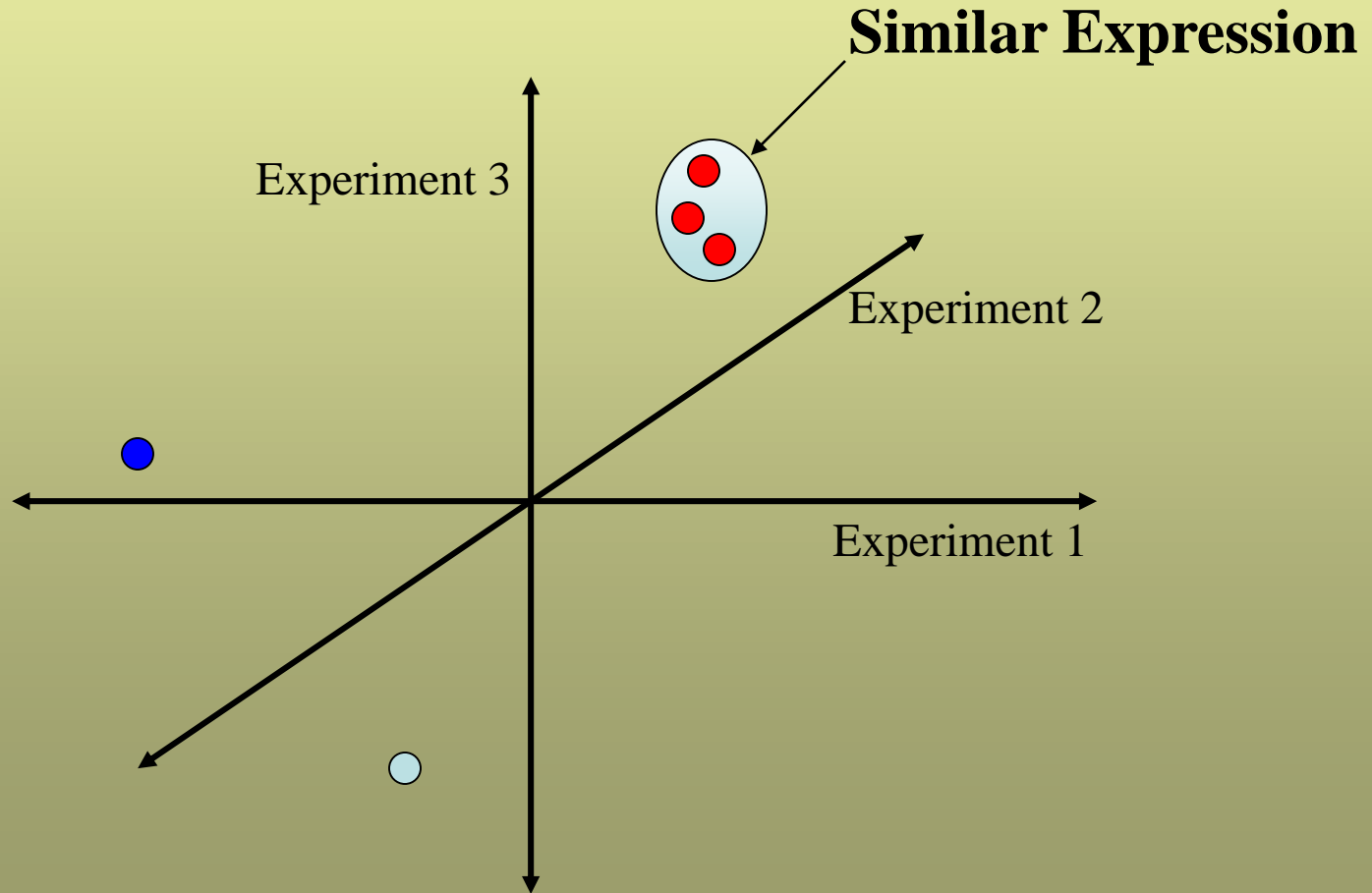
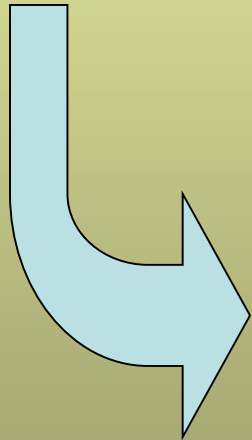


Hashimoto

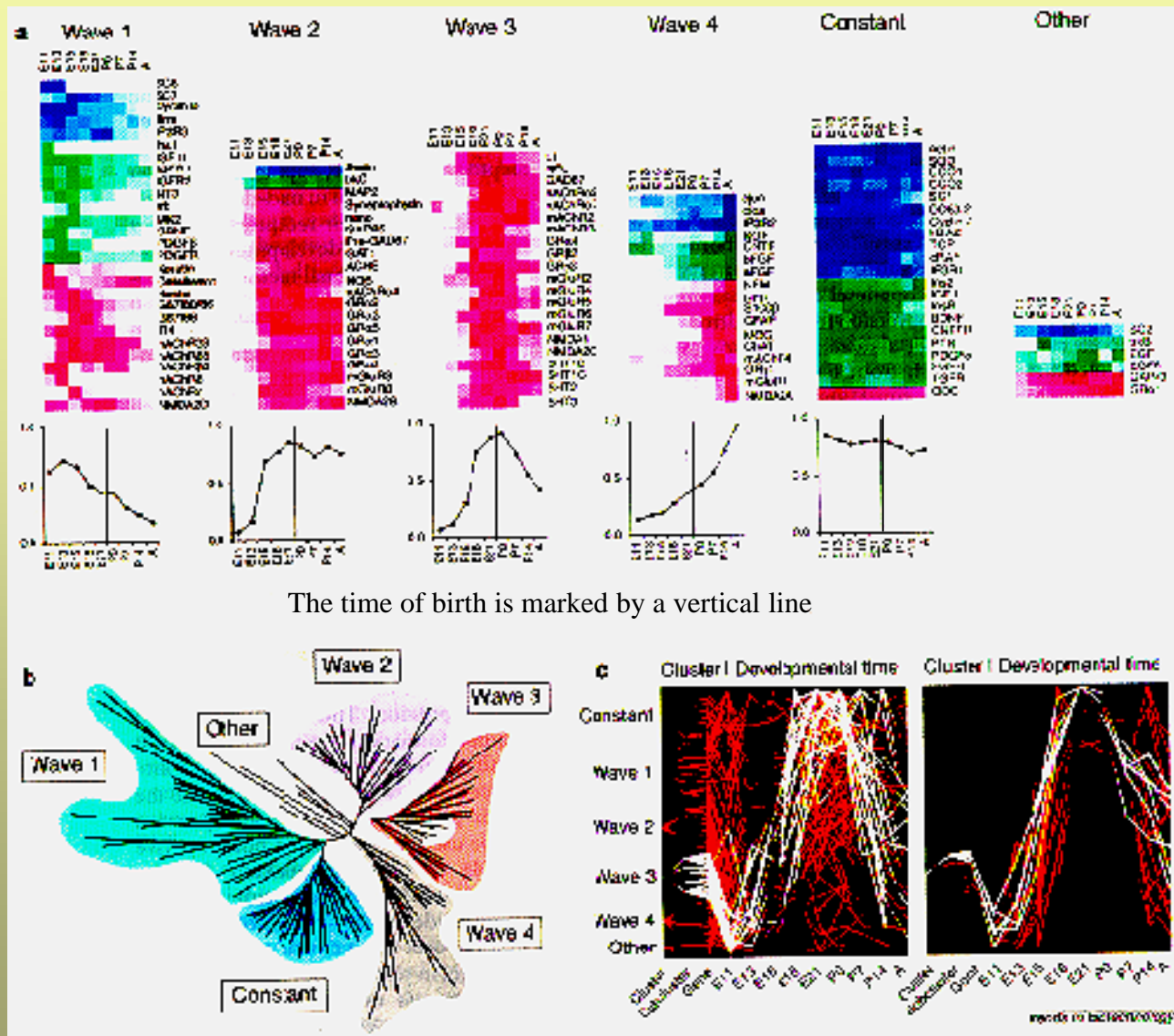


Expression Vectors As Points in 'Expression Space'

	Exp 1	Exp 2	Exp 3
G1	-0.8	-0.3	-0.7
G2	-0.4	-0.8	-0.7
G3	-0.6	-0.8	-0.4
G4	0.9	1.2	1.3
G5	1.3	0.9	-0.6



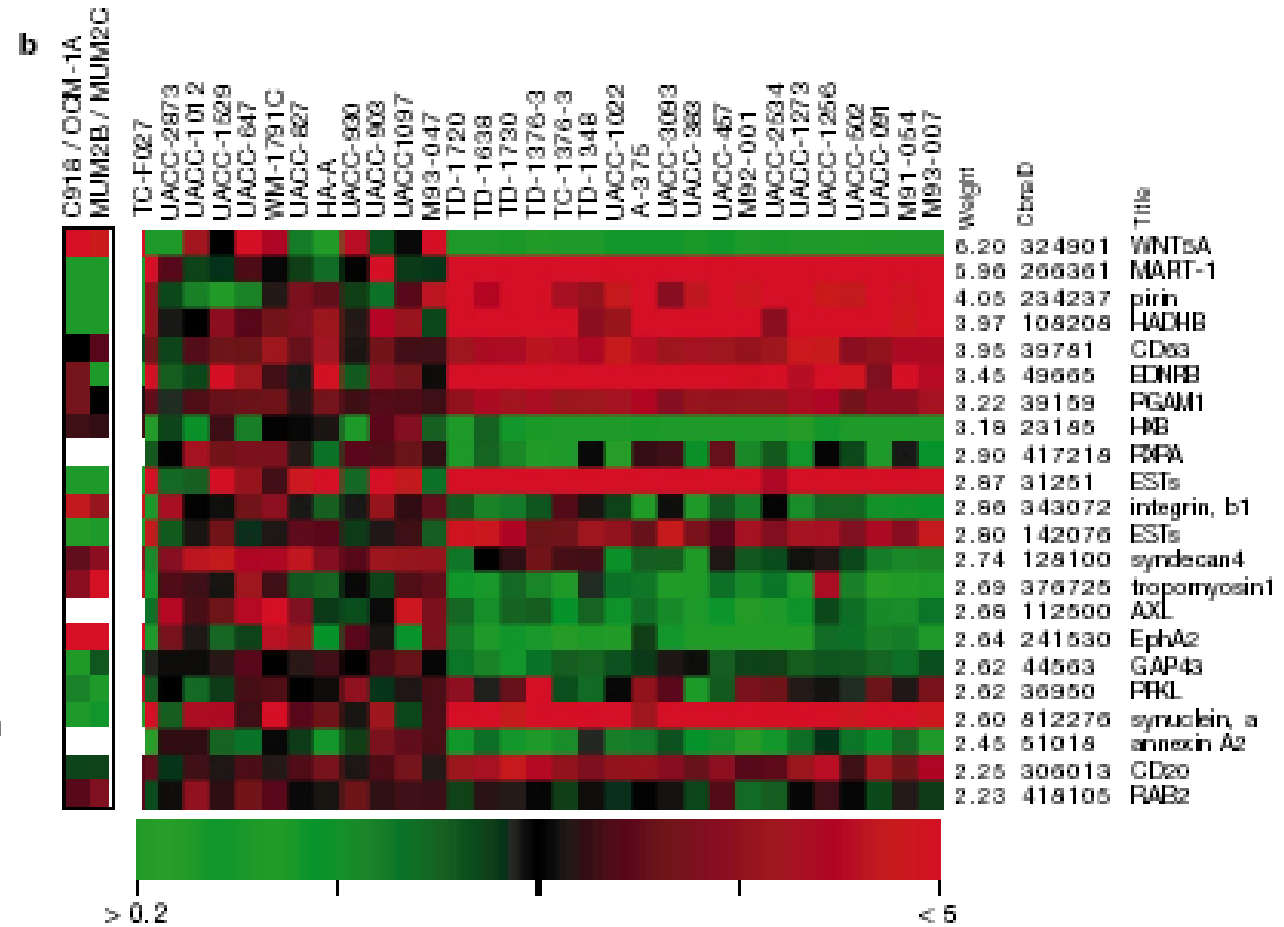
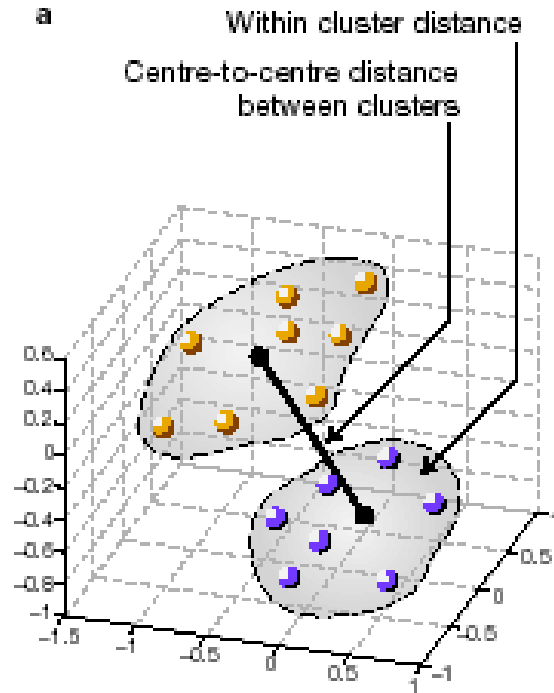
Génexpressziós klaszterek patkány fejlődése során



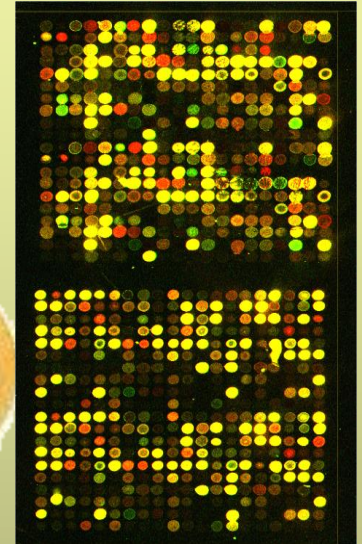
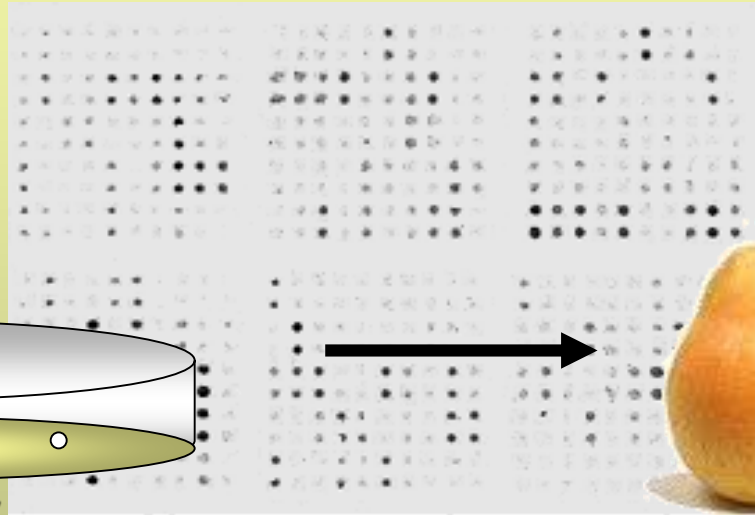
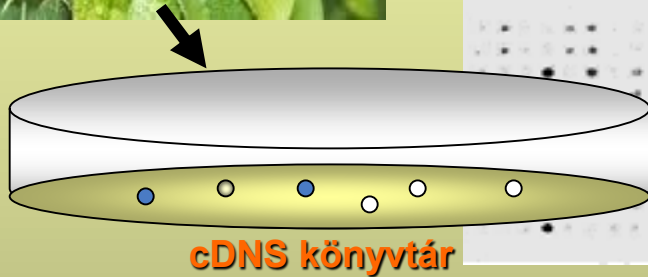
The time of birth is marked by a vertical line

- Normalized gene-expression trajectories are shown grouped by waves that are determined by clustering. The graphs below show the average normalized expression pattern or wave over the nine time points for all of the genes in each cluster.
- Tree of all gene-expression pattern. c. Plots of all normalized time series, highlighted wave 3.

Melanoma



Körte érésével kapcsolatos gének azonosítása

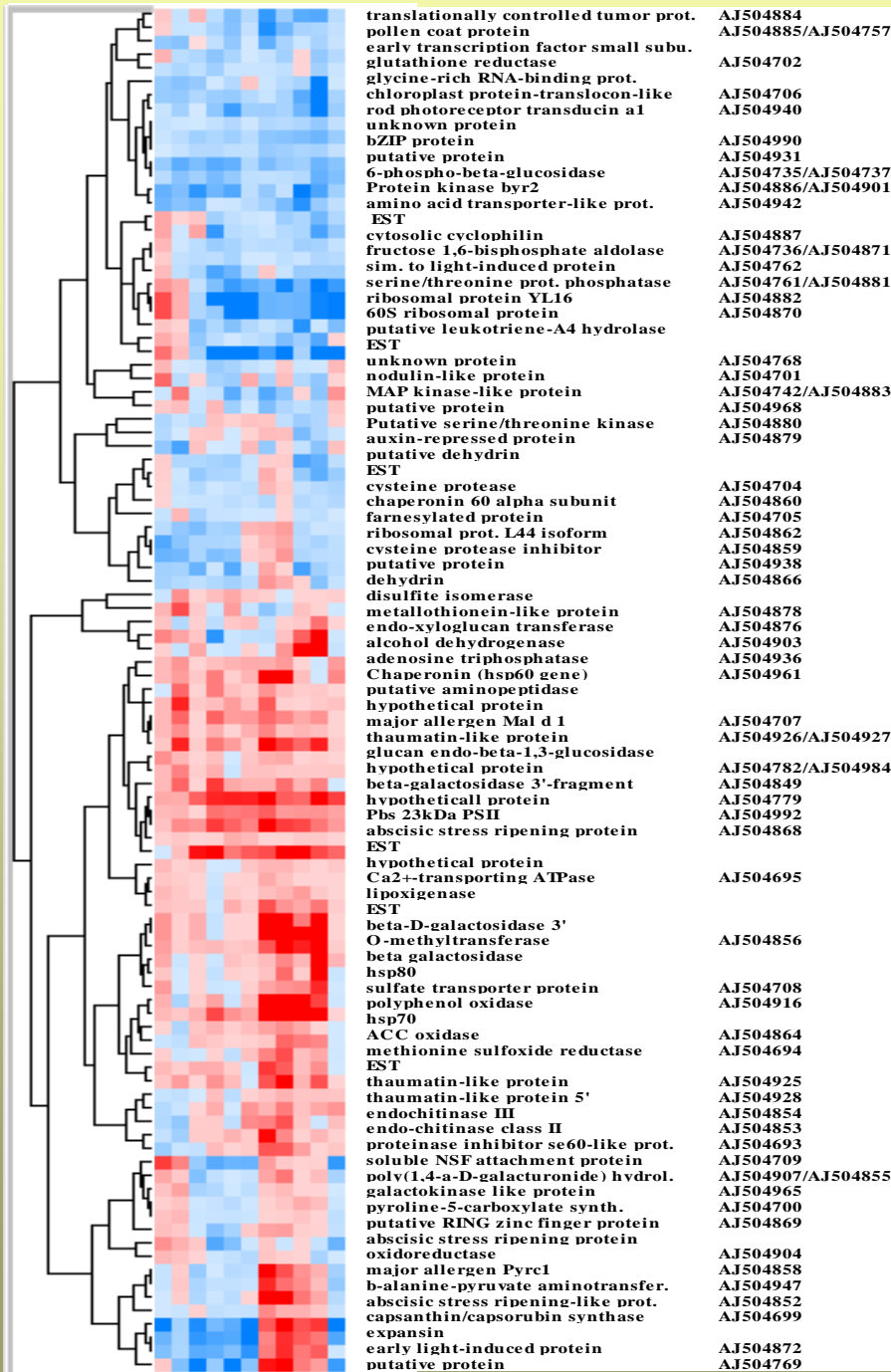


↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
A C 3 6 9 12 15 18 21 24 FR

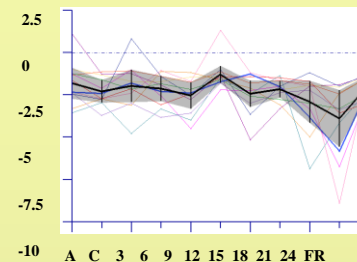
A: növekvő kis termés

FR: összeaszott, éretlen termés

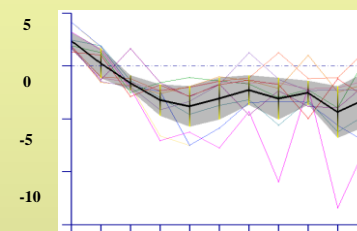
C: nem növekvő, éretlen
termés



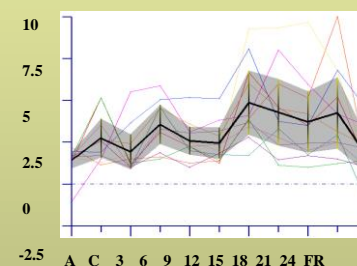
Nem érés specifikus



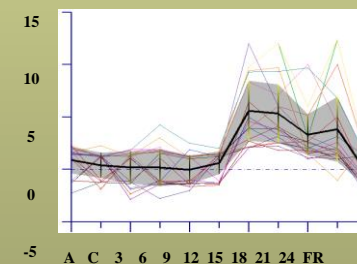
Gyümölcs növekedésre jellemző gének



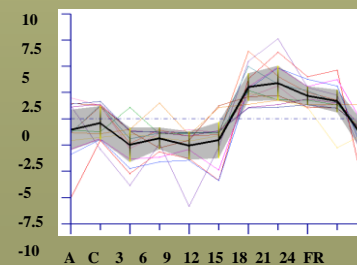
Gyümölcs növekedésre és érésre jellemző gének



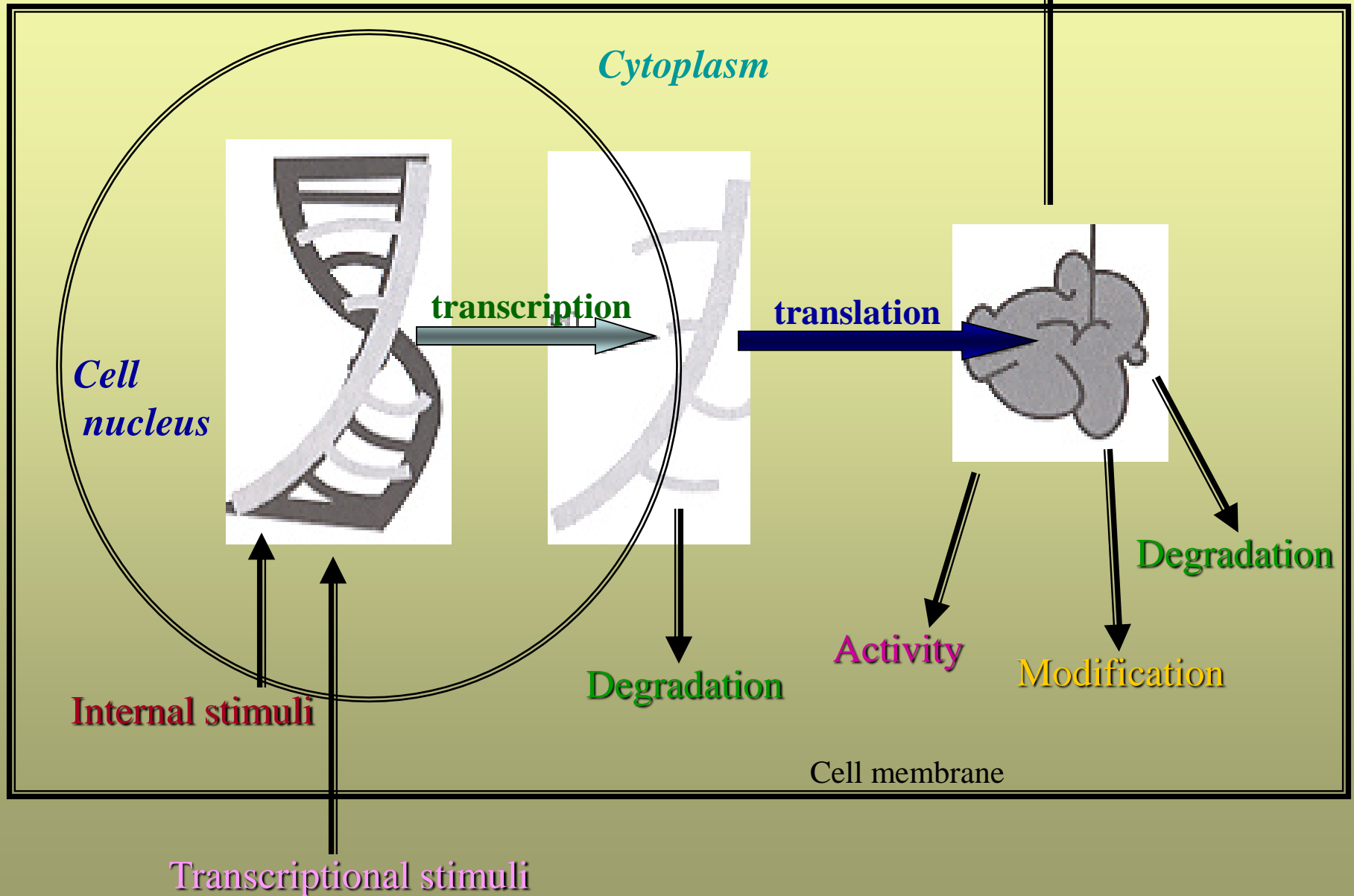
Gyümölcs érésre jellemző gének



Gyümölcs érésre jellemző gének



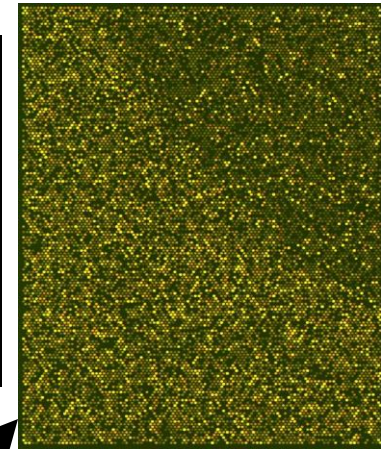
Flow of genetic information



Minták heterogenitása, heterogén minták genomikai elemzése

1. Tumorasszociált miofibroblasztok célpontjai?

Term	Count	%	PValue	Genes
hsa05210:Colorectal cancer	10	1.5244	0.00829	TCF7,PIK3CB,MSH2,JUN,PIK3CD,LEF1,BIRC5,AKT3,DVL1,TGFB2
hsa05215:Prostate cancer	16	0.9715	0.02993	FGFR1,AR,MAP2K1,NFKBIA,FOXO1,RAF1,LEF1,NFKB1,TCF7L2,TCF7L1,EP300,GS
h_cd40Pathway:CD40L Signaling Pathway	5	0.4378	0.04075	IKBKG,NFKB1,CD40,TNFAIP3,IKBKB
h_ceramidePathway:Ceramide Signaling Pathway	6	0.5254	0.0474	TNFRSF1A,BAX,MAPK3,SMPD1,NFKB1,FADD
h_compPathway:Complement Pathway	8	0.4857	0.00433	C7,MASP1,C3,CFB,C4B,C1R,C1S,CFD
h_nfkbPathway:NF-kB Signaling Pathway	7	0.613	0.01591	TNFRSF1A,IL1R1,IKBKG,NFKB1,FADD,TNFAIP3,IKBKB
hsa04340:Hedgehog signaling pathway	15	0.9107	1.00E-03	BMP4,BMP2,STK36,CSNK1A1L,GAS1,GLI2,GLI3,PRKX,GLI1,WNT2,SMO,GSK3B,C
hsa04960:Aldosterone-regulated sodium reabsorption	6	0.9146	0.02751	PDPK1,ATP1B3,PIK3CB,PIK3CD,IGF1,ATP1A1

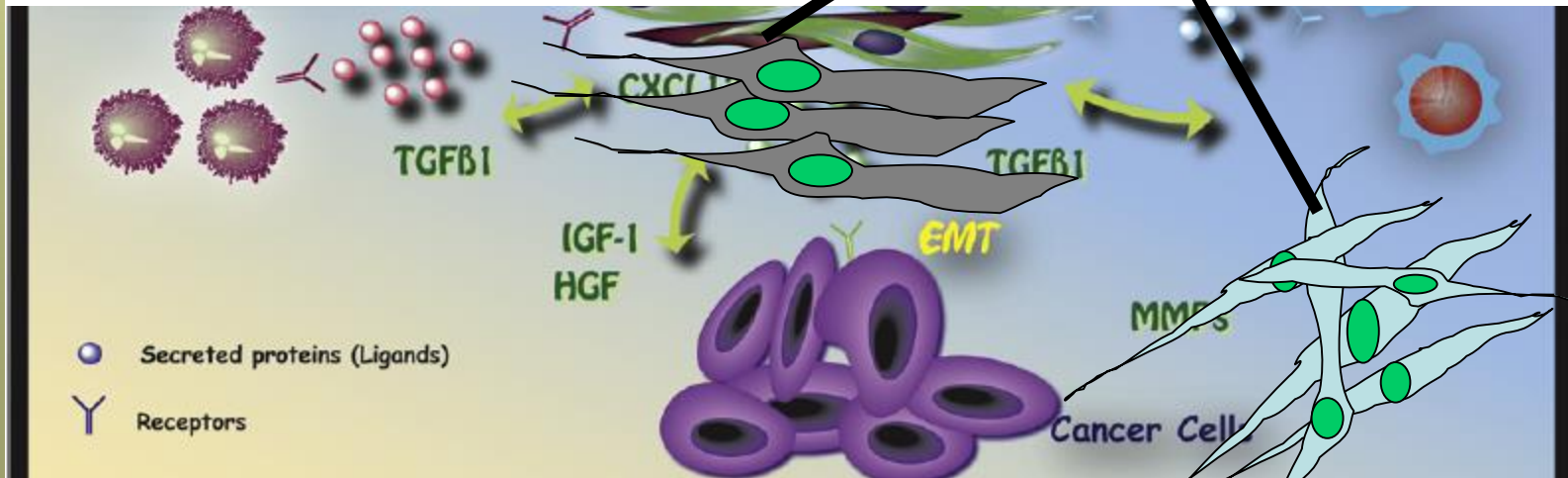


DNA-microarray

QRT-PCR

RNA

RNA



Péter Hegyi (Med. Univ. Szeged, 1st Intest.)

Imre Boros (BRC HAS, Univ Szeged)

Ágnes Zvara, Nóra Faragó (BRC HAS)

Tumorössejtek szerepe

CANCER GENOMICS & PROTEOMICS 6: 19-30 (2009)

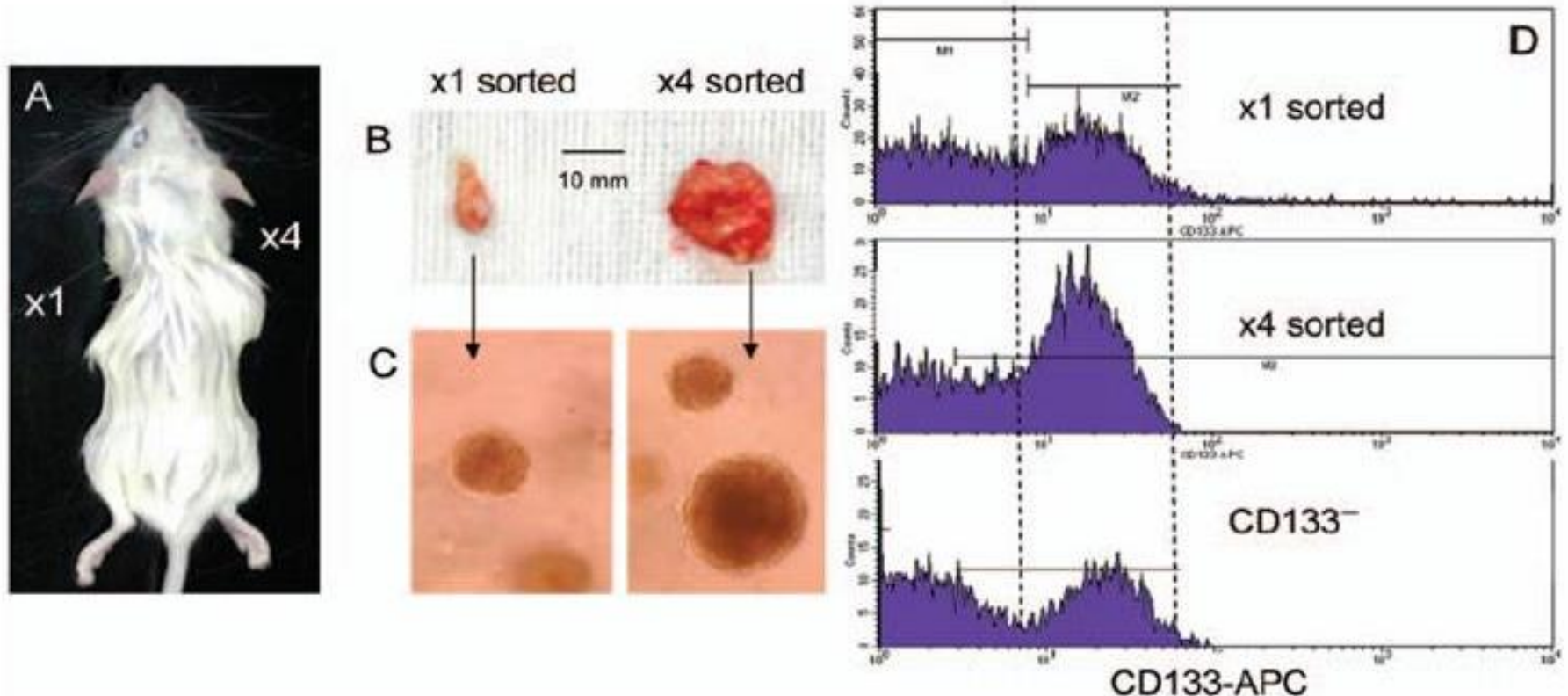
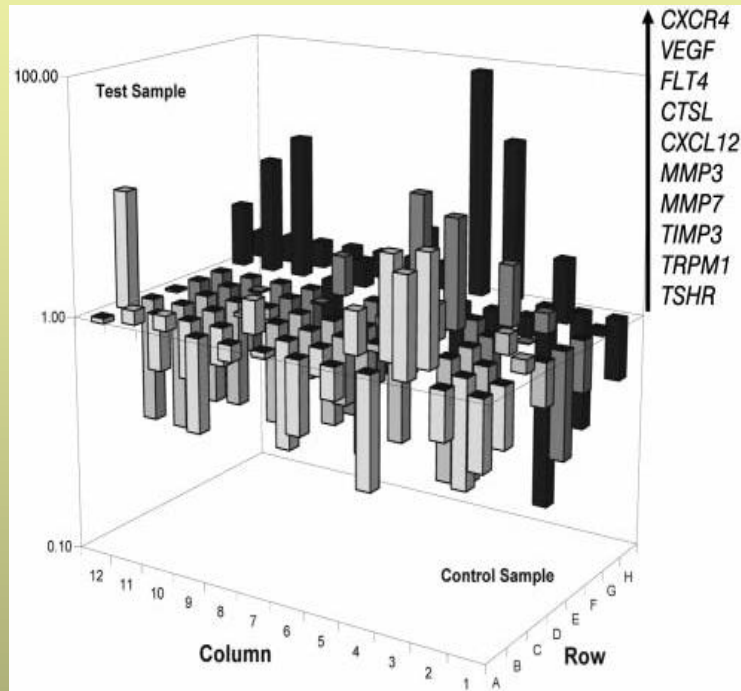


Figure 3. Increased in vivo tumorigenicity of repeatedly MACS-CD133⁺-sorted HCT116 cells. To minimize possible host differences, the same number (1.5×10^3) of one- and four-times sorted cells was injected into opposite flanks of NOD/SCID mice. Six out of six mice uniformly developed relatively small tumors after injection of one-time immunomagnetically sorted cells (A; left injection site, x1 CD133⁺), whereas repeatedly sorted cells induced significantly larger tumors in six of six out mice (A; right injection site; x4 CD133⁺). Removed tumors initiated by transplantation of repeatedly sorted CD133⁺ cells also displayed significantly higher levels of vascularization (B). Dissociated mouse tumor xenografts induced by repeatedly sorted CD133^{high} cells also possessed a higher ability to form floating spheres (C) and contained many more cells with high levels of CD133 (D).

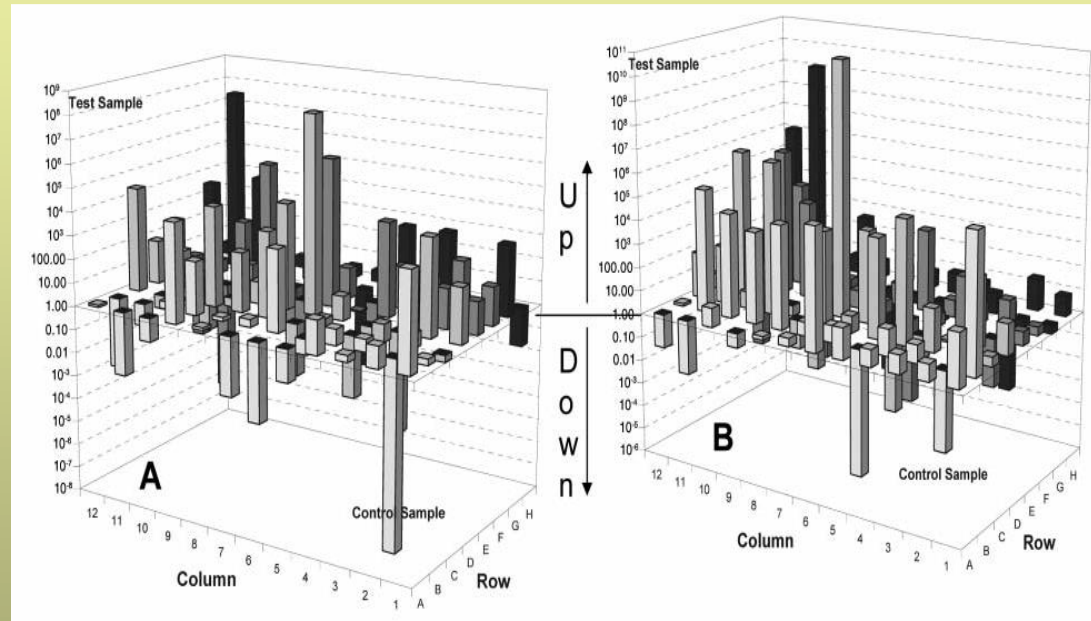
Tumorössejtek géneexpressziós vizsgálata (QRT-PCR array)



Metasztázisban szereplő gének

CD133+

CD133-



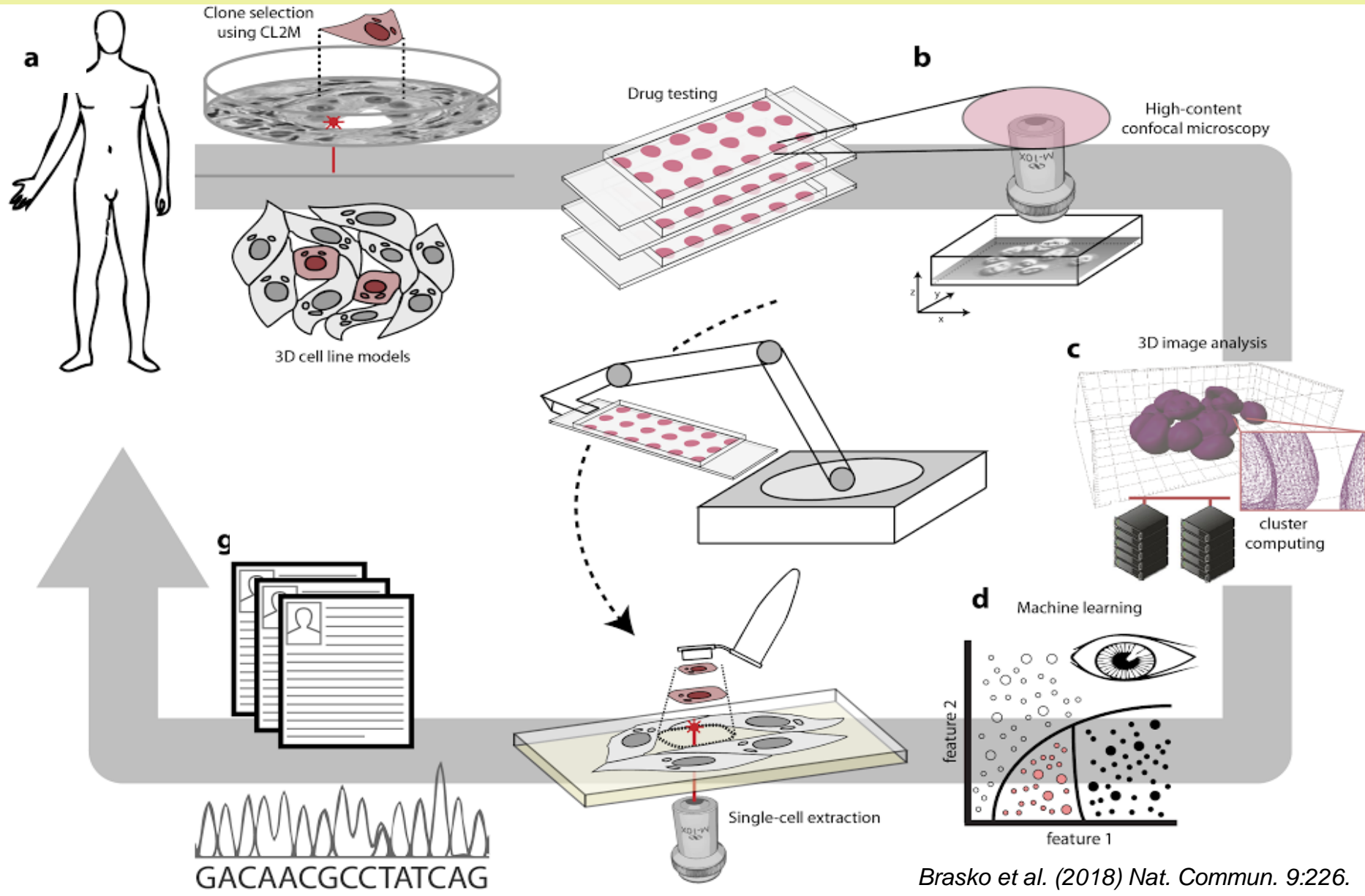
Össejt marker gének (ABCG2, NOTCH1/2, WNT1, FGF3,...)

Az agy sejtjeinek heterogenitása

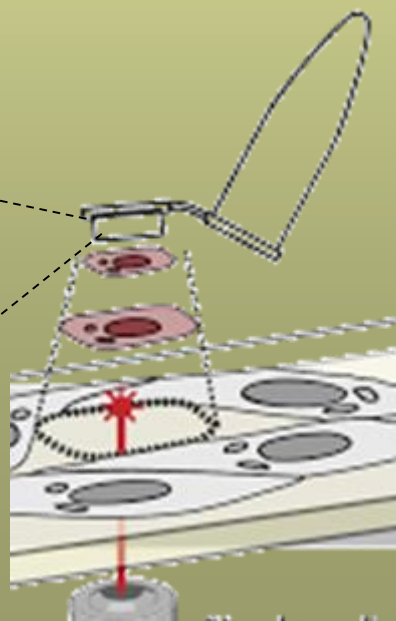
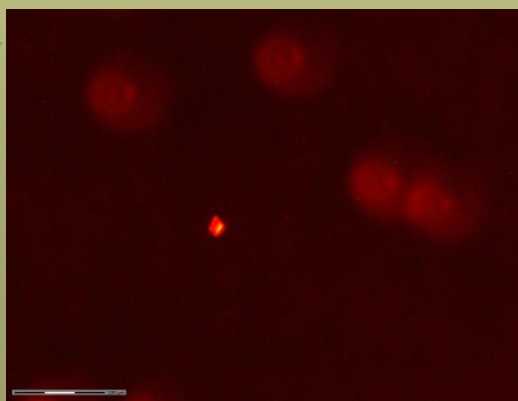
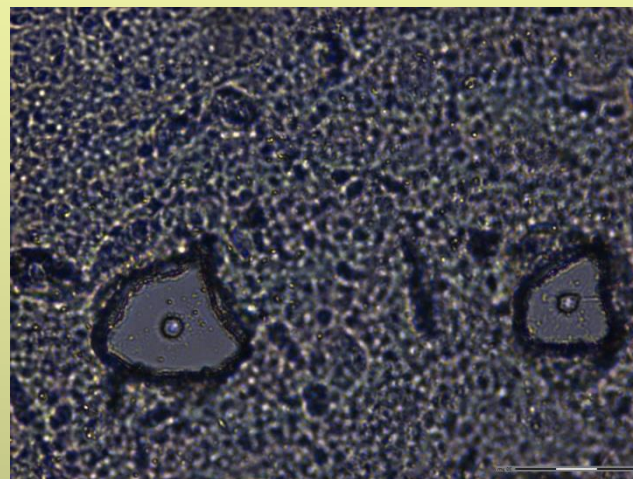
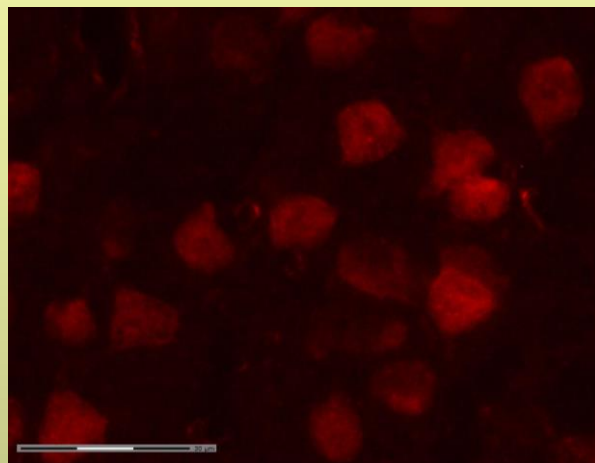
rengeteg különböző sejtípus
nagyfokú heterogenitás
megértés, adatfeldolgozás
értelem, memória
neuronális hálózatok
patológias állapotokban
különböző sejtípusok szerepe



Sejtek, sejtcsoportok gyűjtése lézerdisszekciós módszerrel



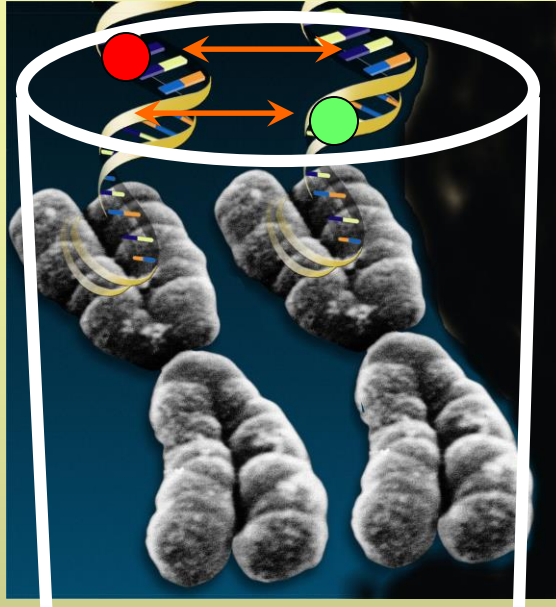
Különböző neuron típusok gyűjtése fixált, jelölt mintákból



Tamás Gábor
Nagy István
kollaboráció

Genomi átrendeződések és változások vizsgálata csiptechnikával

Pontmutációk (SNP) detektálása



CCATGG
Vad típus

CCCTGG
Mutáns

1. SNP

GCTACC
Vad típus

GTTACC
Mutáns

2. SNP

- Oligonukleotid alapú chipek
- egy nukleotid eltérés azonosítása

Jelölt DNS



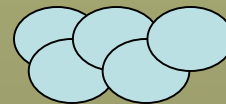
hibridizáció



mosás

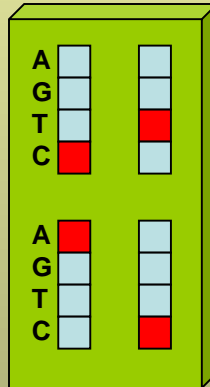


DNS



1. SNP
3 pozíció

2. SNP
2 pozíció



detektálás



Adat analízis

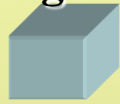
- 1. SNP: 3. pozíció A-C
- 2. SNP: 2. pozíció C-T

Genomszintű változások,
kromoszóma rendellenességek,
amplifikációk, deléciók detektálása

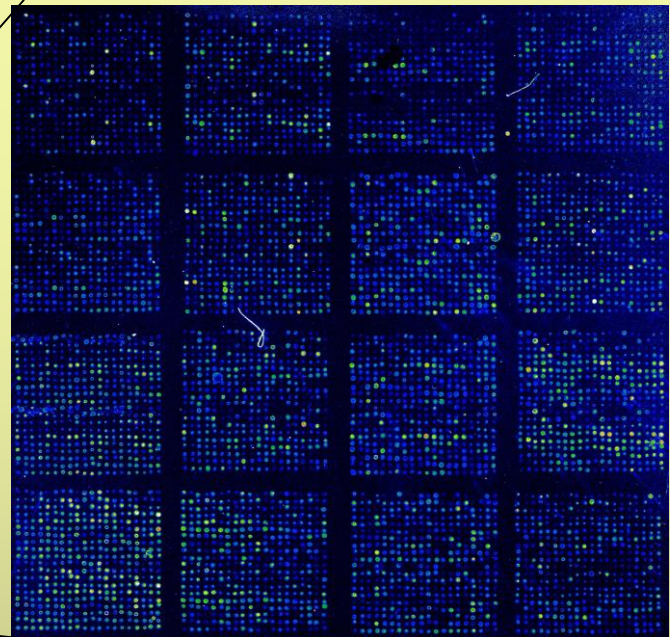
Paraffinba ágyazott minták

normál

daganat

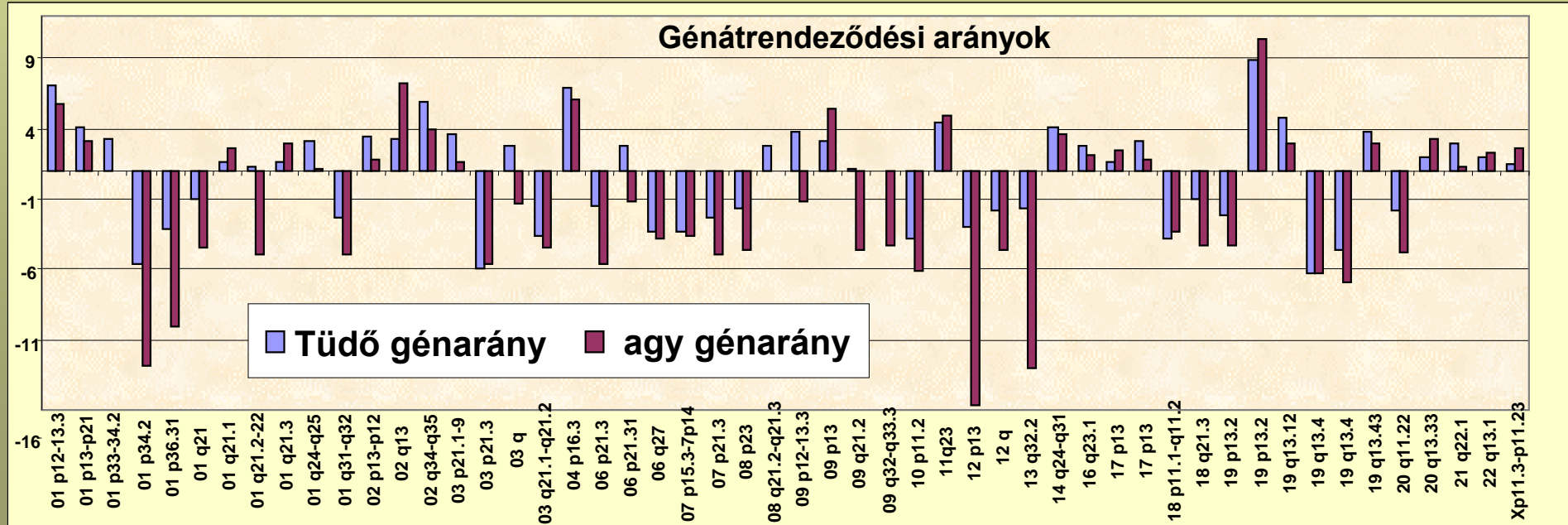


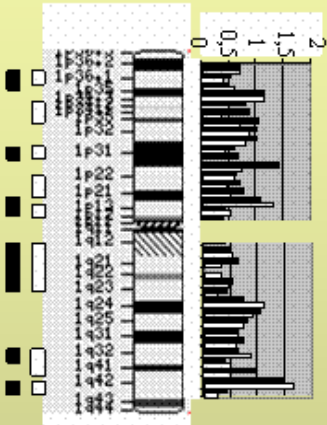
PCR



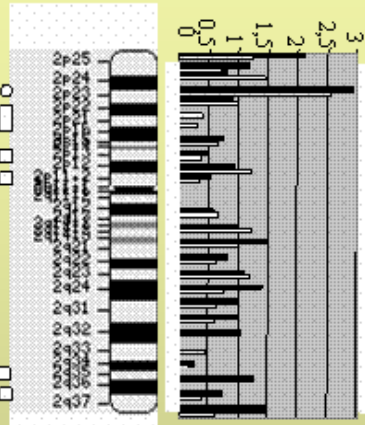
Metasztázisok, tumorok, multiplex tumorok jellemzése, igazolása

Génátrendeződési arányok

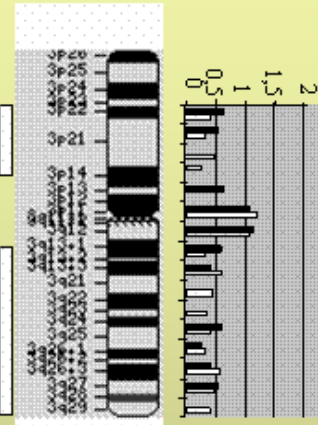




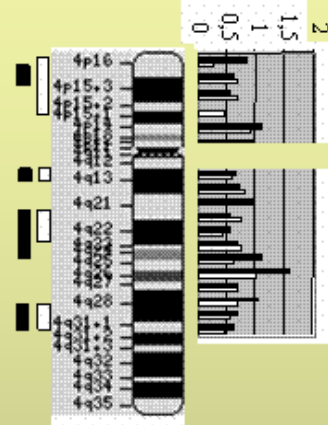
Chr1



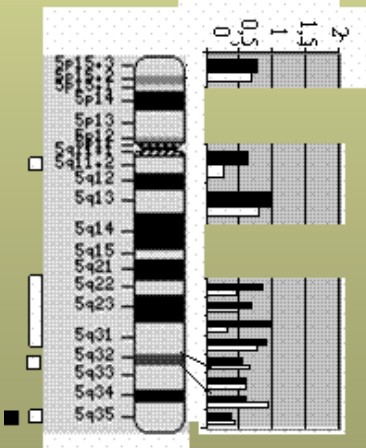
Chr2



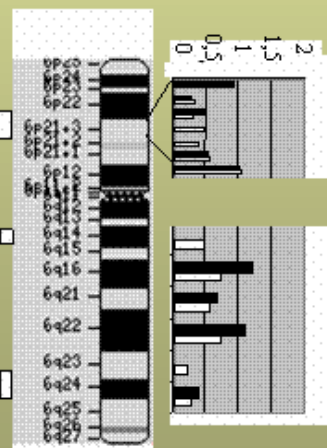
Chr3



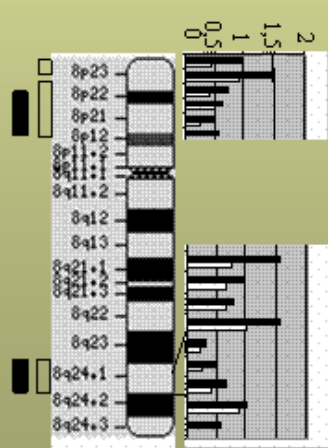
Chr4



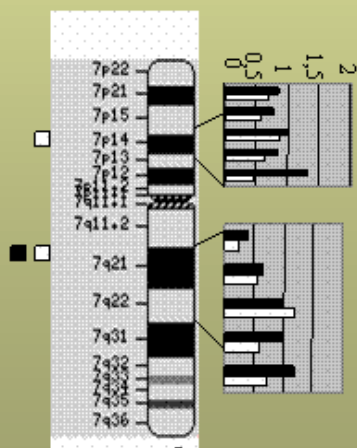
Chr5



Chr6

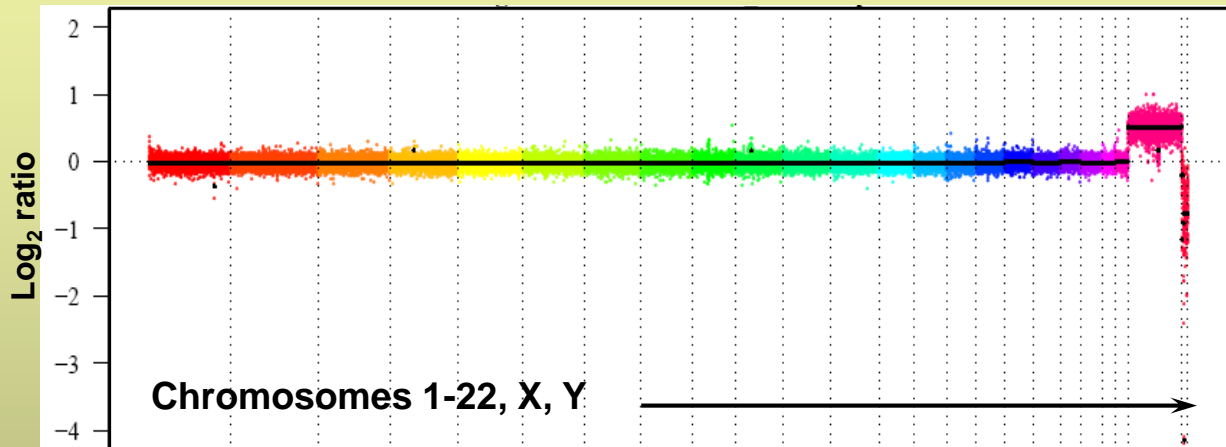


Chr8

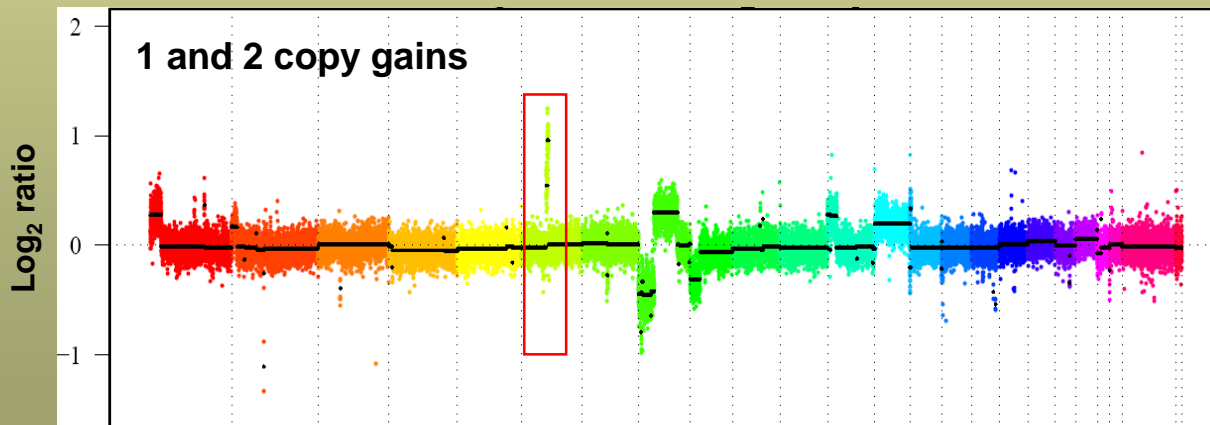


Chr 7

CGH nagyfelbontású (385K) teljes genom array-vel

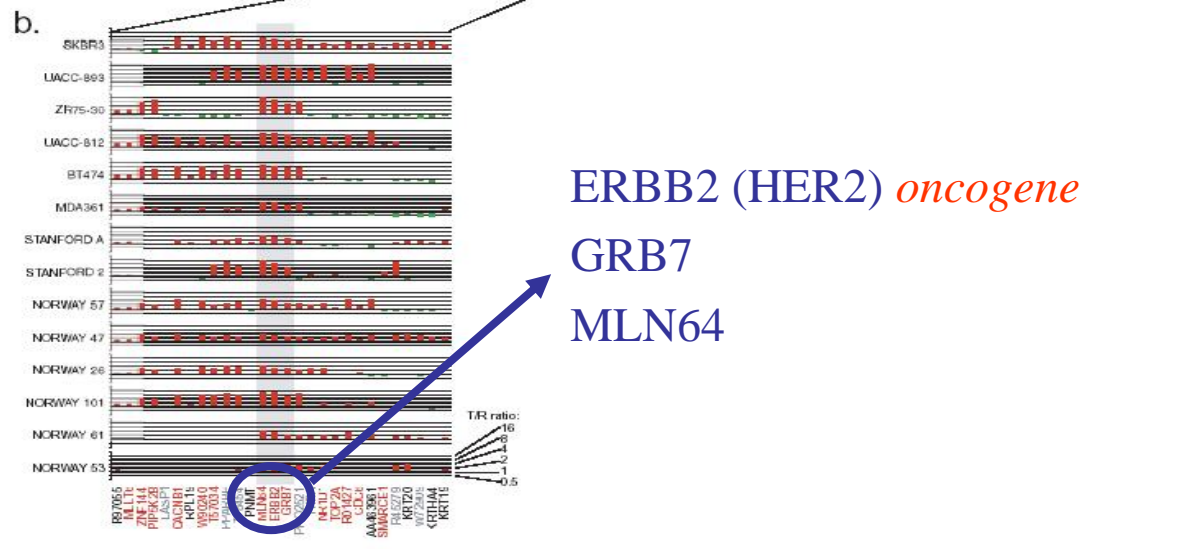
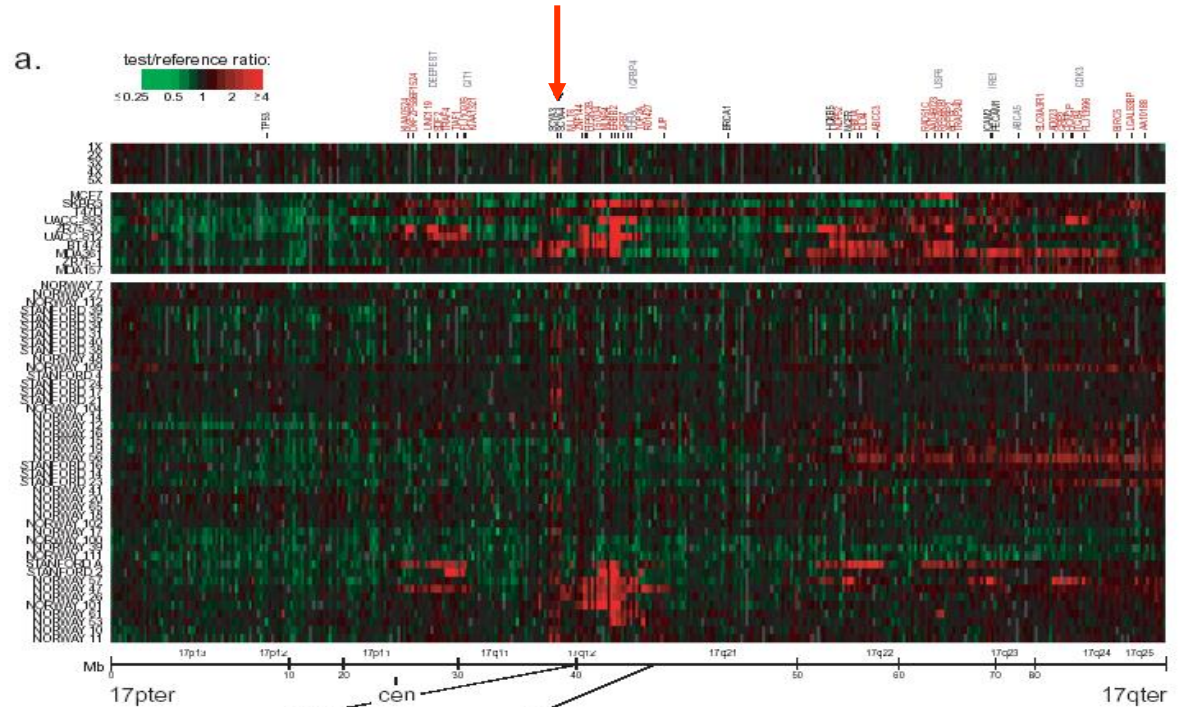


Nő vs. férfi gDNS



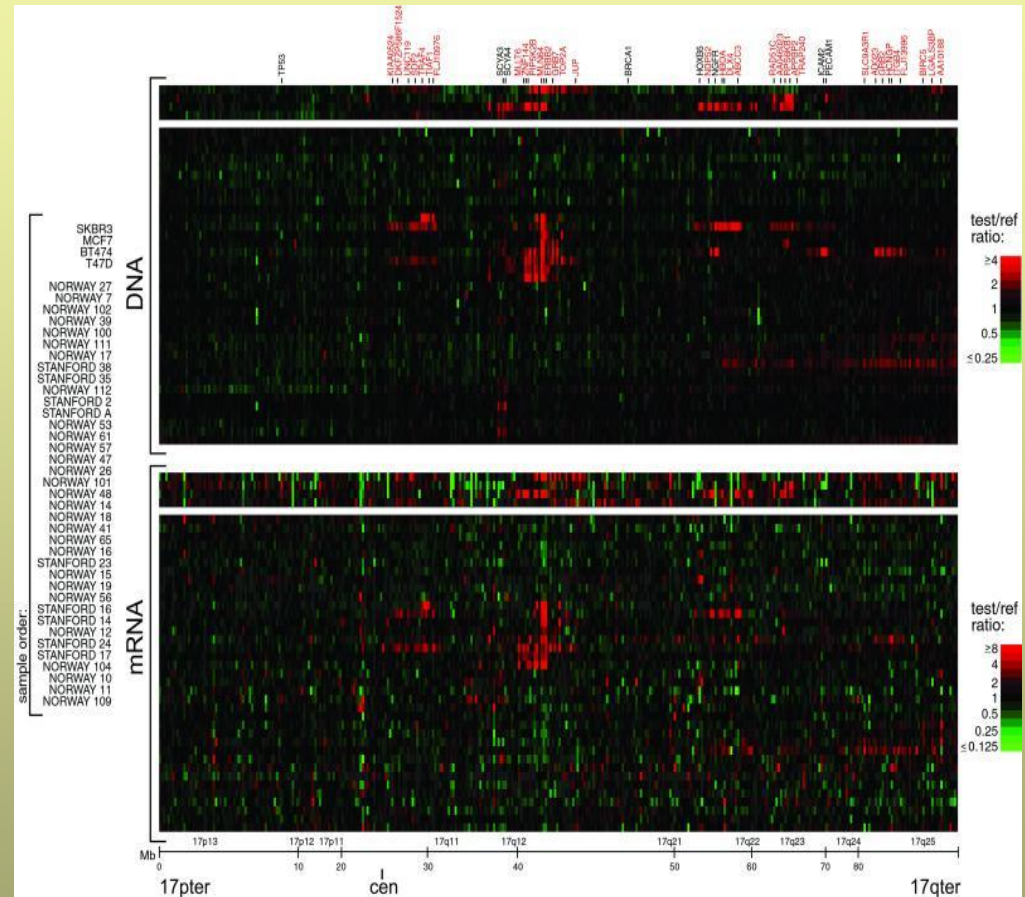
Pajzsmirigy tumor

Variation in copy number mapping to chr 17

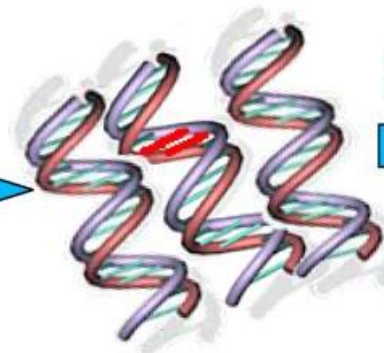
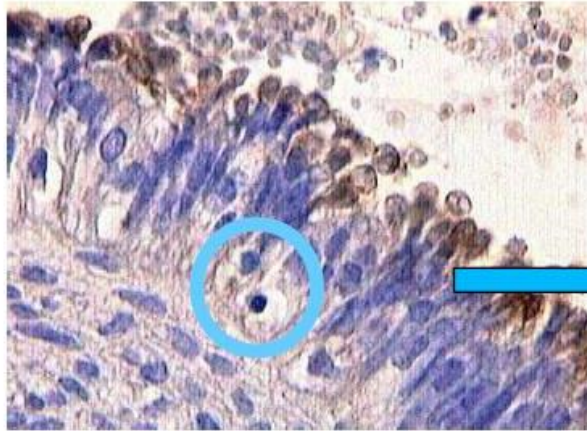


Párhuzamos mRNS szint vizsgálat

- 117 high level DNA amplifications (91 different genes)
- 62% (54 genes) found associated with at least moderately elevated mRNA
 - 12/54 genes are oncogenes or candidates
- 42% (36 genes) found associated with highly elevated mRNA.



Egyedi genomi eltérések detektálása?



PMES



AGC TCC CGG TAC
AGC TCC **G**GG TAC
AGC TCC TGG TAC
AGC TCC **A**GG TAC

Drug resistance

KRAS mutaton correlates with resistance to e.g. Erbitux

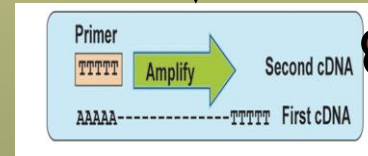
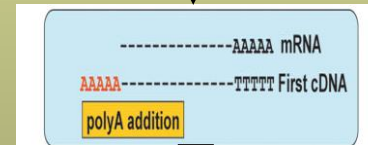
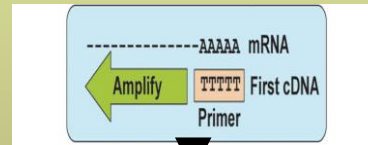
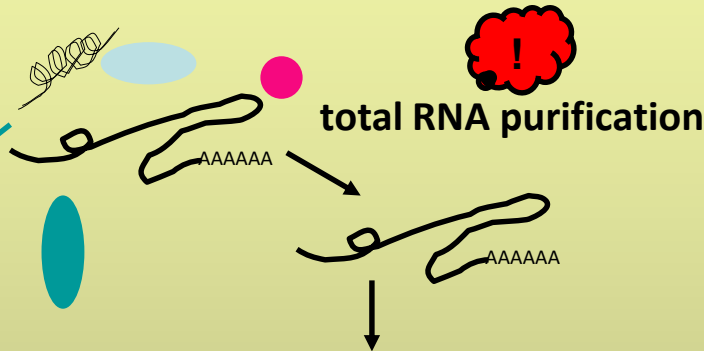
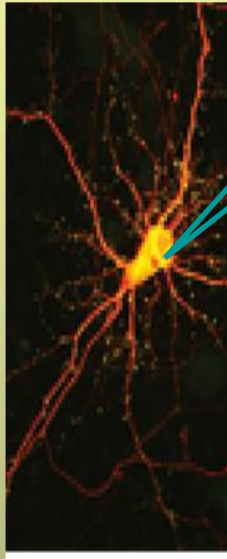
Újgenerációs szekvenálási stratégiák

Loss of heterogeneity in the entire genome verification of haploidy by next generation sequencing

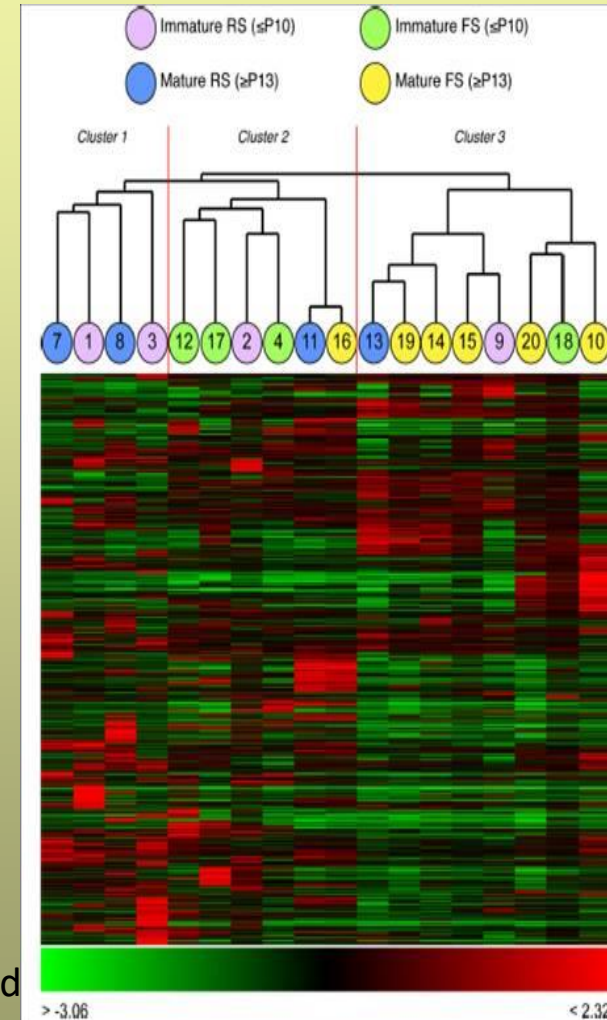
Reference Position	Variation Type	Length	Reference	Variants	Allele Variations	Frequencies	Counts	Coverage	Overlapping Annotations
220	SNP	1	A	1	G	100	259	259	rRNA: 12S ribosomal RNA, Gene: Rnr1
247	SNP	1	T	2	A/T	98.2/1.8	382/7	389	rRNA: 12S ribosomal RNA, Gene: Rnr1
348	SNP	1	T	1	C	100	169	169	rRNA: 12S ribosomal RNA, Gene: Rnr1
467	SNP	1	G	1	A	100	30	30	rRNA: 12S ribosomal RNA, Gene: Rnr1
482	SNP	1	C	1	T	100	201	201	rRNA: 12S ribosomal RNA, Gene: Rnr1
518	SNP	1	C	1	T	98.3	118	120	rRNA: 12S ribosomal RNA, Gene: Rnr1
569	SNP	1	T	1	C	100	171	171	rRNA: 12S ribosomal RNA, Gene: Rnr1
572	SNP	1	A	1	C	100	225	225	rRNA: 12S ribosomal RNA, Gene: Rnr1
646	SNP	1	T	1	C	98.4	183	186	rRNA: 12S ribosomal RNA, Gene: Rnr1
654	SNP	1	T	1	C	100	41	41	rRNA: 12S ribosomal RNA, Gene: Rnr1
815	SNP	1	A	1	T	100	73	73	rRNA: 12S ribosomal RNA, Gene: Rnr1
829	SNP	1	C	1	T	99.7	348	349	rRNA: 12S ribosomal RNA, Gene: Rnr1
900	SNP	1	C	1	T	99.7	385	386	rRNA: 12S ribosomal RNA, Gene: Rnr1
980	SNP	1	T	1	C	97.8	177	181	rRNA: 12S ribosomal RNA, Gene: Rnr1
1400	SNP	1	T	1	C	99.3	267	269	rRNA: 16S ribosomal RNA, Gene: Rnr2
1403	SNP	1	T	1	C	99.6	282	283	rRNA: 16S ribosomal RNA, Gene: Rnr2
1425	SNP	1	T	1	A	96.6	84	87	rRNA: 16S ribosomal RNA, Gene: Rnr2
1447	SNP	1	G	1	A	100	136	136	rRNA: 16S ribosomal RNA, Gene: Rnr2
1453	SNP	1	A	1	T	97.3	73	75	rRNA: 16S ribosomal RNA, Gene: Rnr2
1868	SNP	1	C	1	T	100	74	74	rRNA: 16S ribosomal RNA, Gene: Rnr2
1942	SNP	1	C	1	A	100	151	151	rRNA: 16S ribosomal RNA, Gene: Rnr2
1954	SNP	1	A	1	C	98.6	139	141	rRNA: 16S ribosomal RNA, Gene: Rnr2
1994	SNP	1	A	1	C	98.8	85	86	rRNA: 16S ribosomal RNA, Gene: Rnr2
2078	SNP	1	G	1	A	99.8	412	413	rRNA: 16S ribosomal RNA, Gene: Rnr2
2391	SNP	1	T	1	C	100	169	169	rRNA: 16S ribosomal RNA, Gene: Rnr2
2461	SNP	1	T	1	A	99.8	958	960	rRNA: 16S ribosomal RNA, Gene: Rnr2
2514	SNP	1	C	2	T/C	98.8/1.2	726/9	735	rRNA: 16S ribosomal RNA, Gene: Rnr2
2542	SNP	1	A	1	G	99.4	694	698	rRNA: 16S ribosomal RNA, Gene: Rnr2
2564	SNP	1	C	1	T	100	289	289	rRNA: 16S ribosomal RNA, Gene: Rnr2
3737	SNP	1	A	2	A/T	92.5/7.5	86/7	93	Gene: Trni, tRNA: Trni
3850	SNP	1	C	1	T	100	126	126	Gene: Trnm, tRNA: Trnm
7698	SNP	1	C	1	T	100	70	70	Gene: Trnk, tRNA: Trnk
8613	SNP	1	C	1	A	100	336	336	Gene: COX3, CDS: COX3
8622	SNP	1	C	1	T	100	378	378	Gene: COX3, CDS: COX3

Egyedi sejtek genomi és digitális expresszióvizsgálata

Elektrofiziológiailag különböző sejtek teljes genom analízise



mRNA was amplified by a global polyadenylated PCR-based method



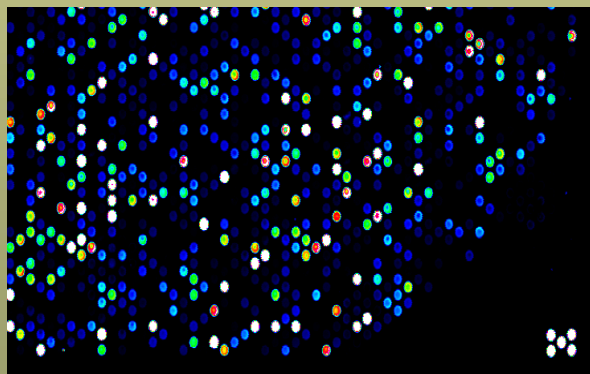
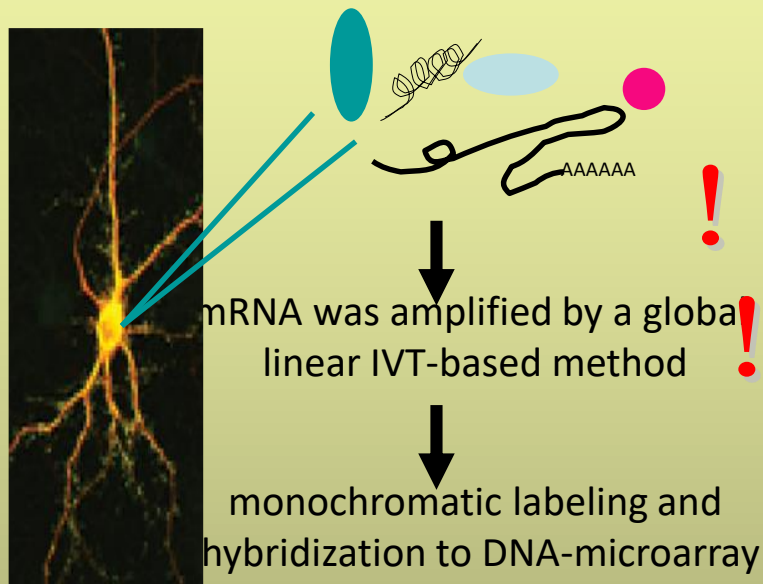
Grouping and classifying electrophysiologically defined classes of neocortical neurons by single cell, whole-genome expression profiling

Tatiana Subkhankulova, Kojiro Yano, Hugh P. C.

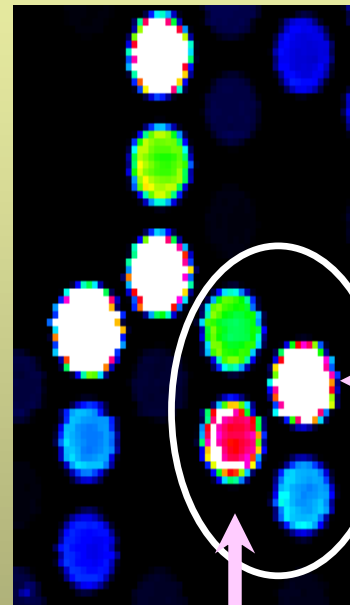
Robinson and Frederick J. Livesey

MOLECULAR NEUROSCIENCE, 2010

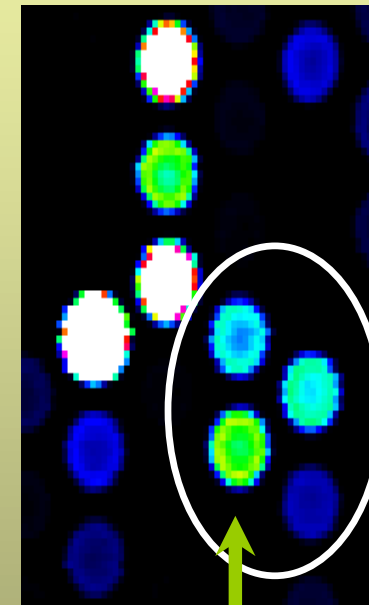
Elektrofiziológiailag különböző sejtek teljes genom analízise



Type I interneuron



Type II interneuron



Gábor Tamás, Ágnes Katalin Kocsis, Szeged University
Nóra Faragó, Ágnes Zvara, HAS BRC, Szeged, Hungary

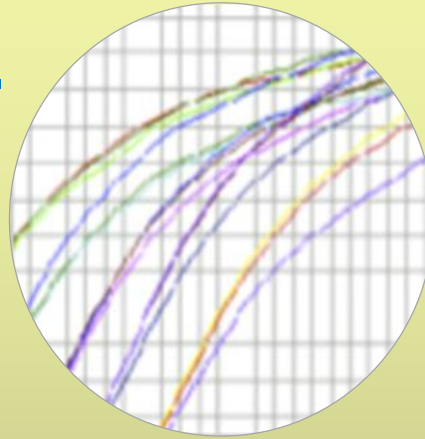
Digitális PCR – A PCR harmadik generációja

1.



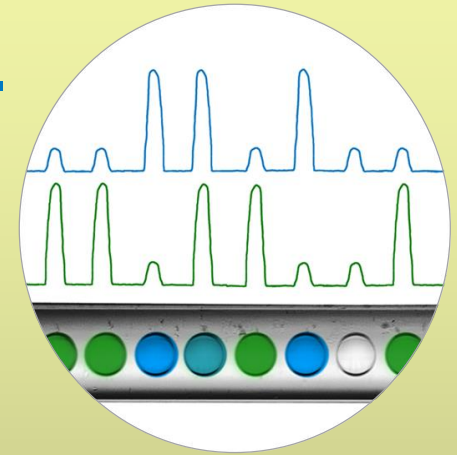
PCR
Kvalitatív

2.



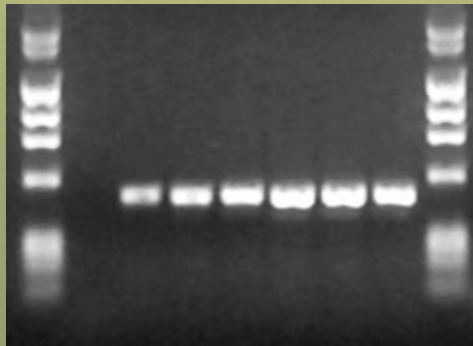
Real-time PCR
Relatív kvantitálás

3.

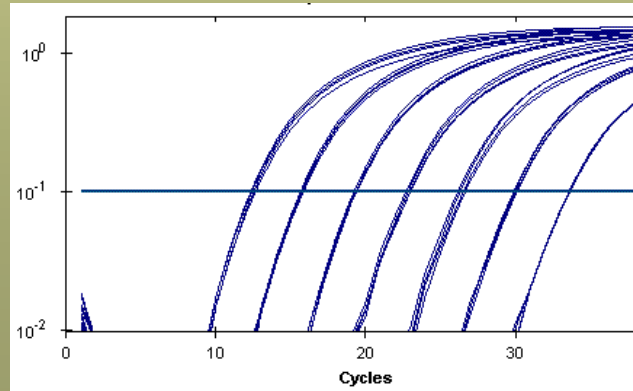


Digitális PCR
Abszolút kvantitálás

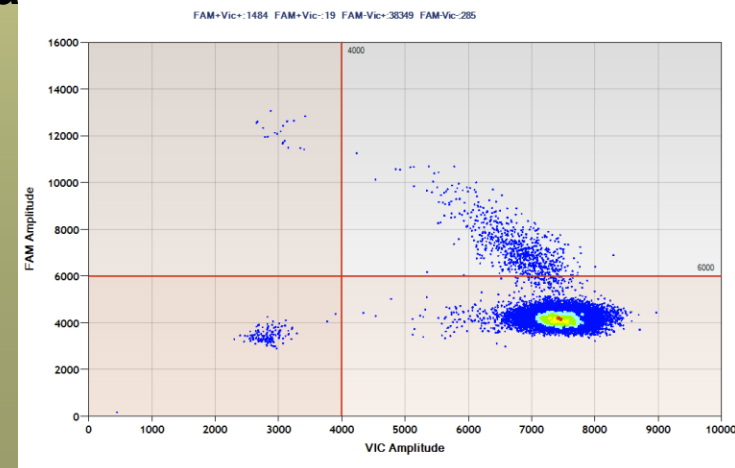
Végpont analízis



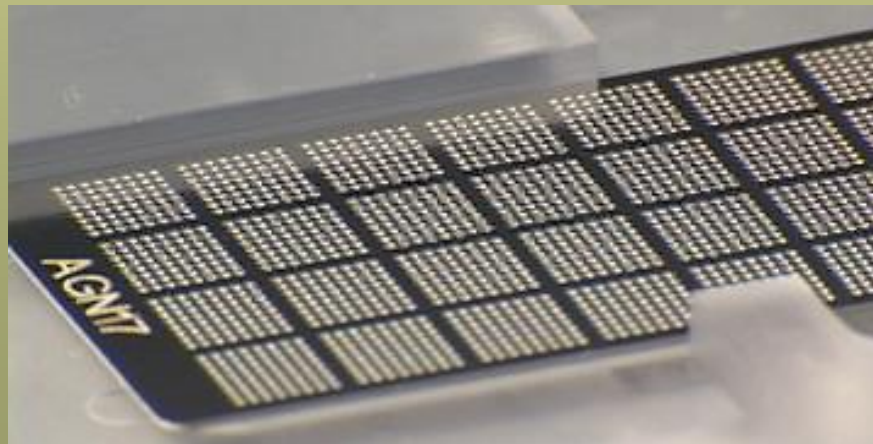
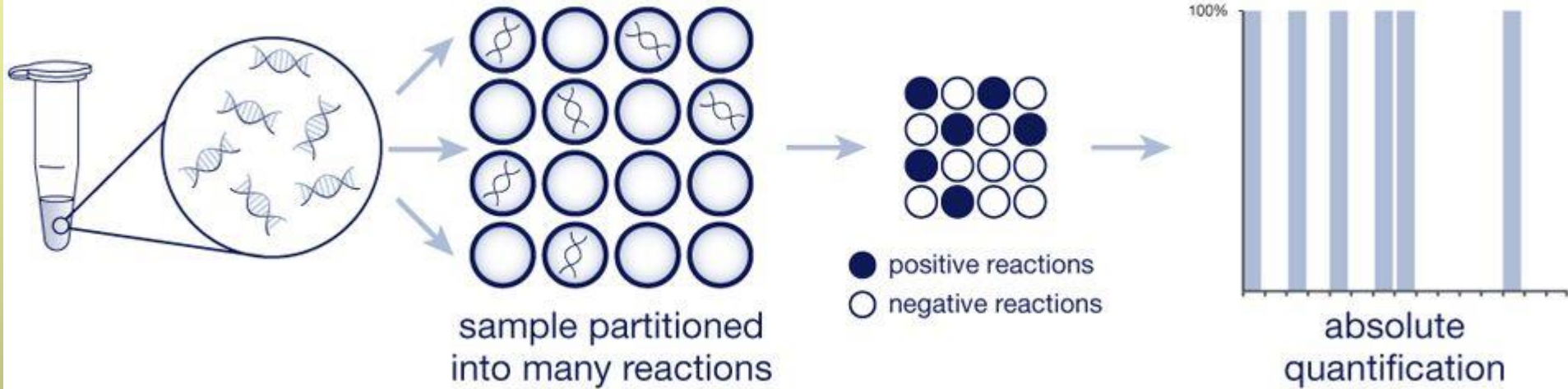
Fluoreszcencia detektálása



Egyedi reakciók elemzése

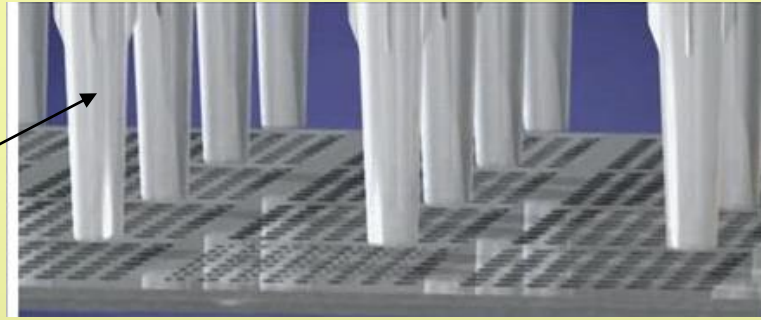


Digitális PCR nanokapilláris HTS QRT-PCR technikával

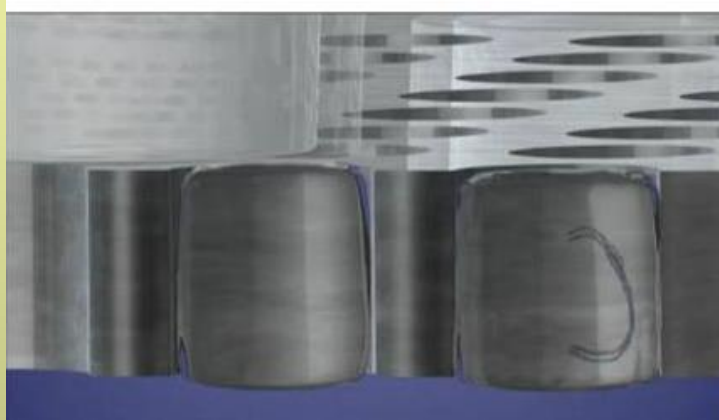




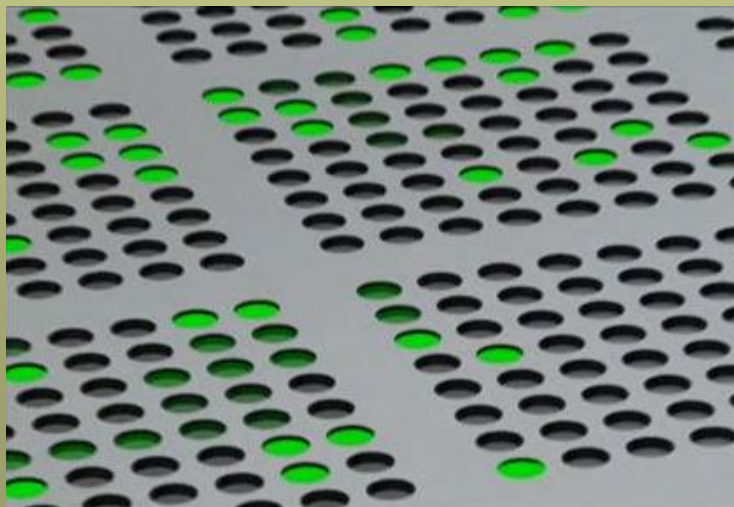
minta (1 sejt)
TaqMan próba
QRT-PCR reagens



feltöltés

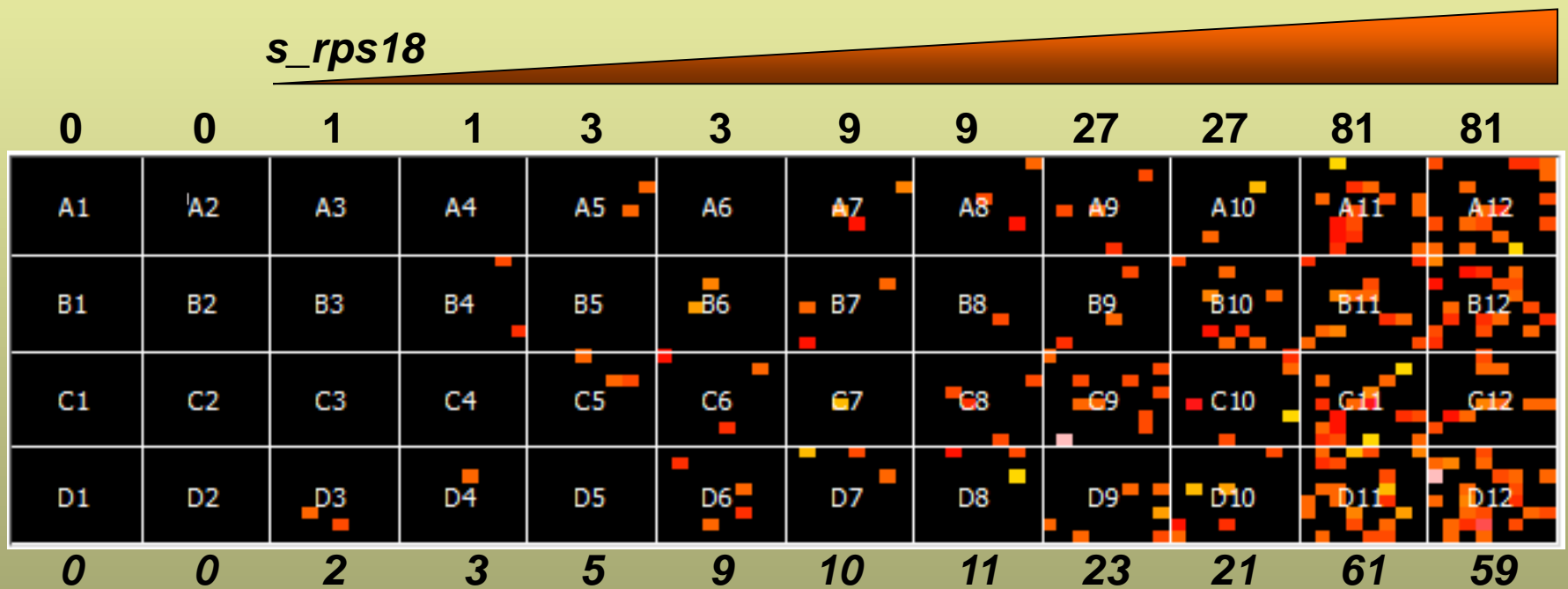


PCR



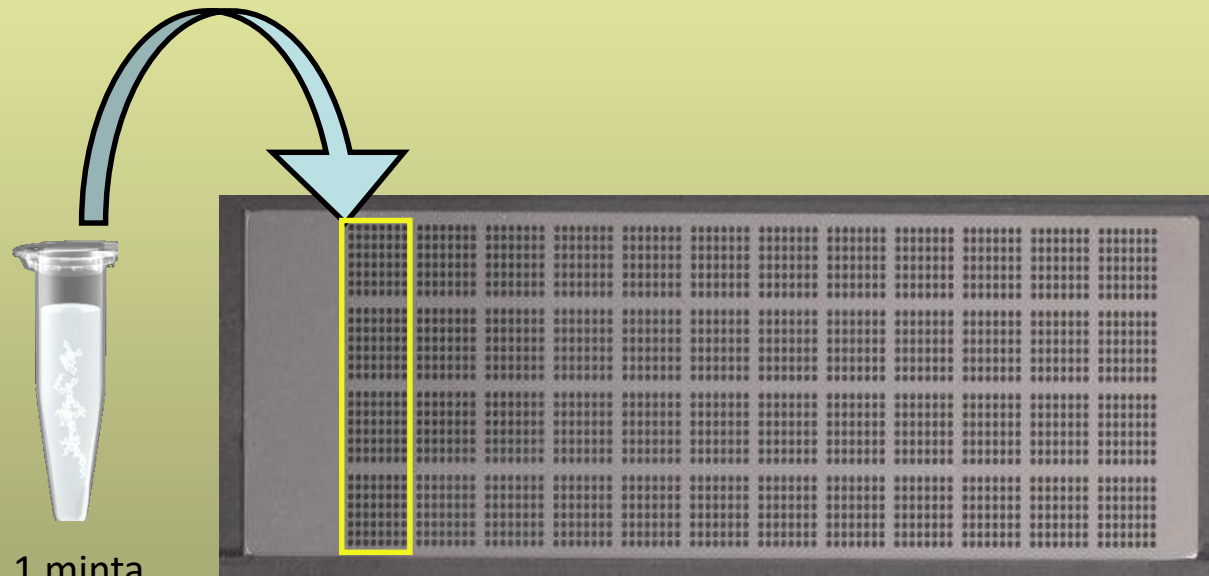
PCR vége

Spike-in DNS kópiaszámának meghatározása dQRT-PCR technikával



Egyedi neuronok génexpressziós elemzése digitális QRT-PCR módszerrel

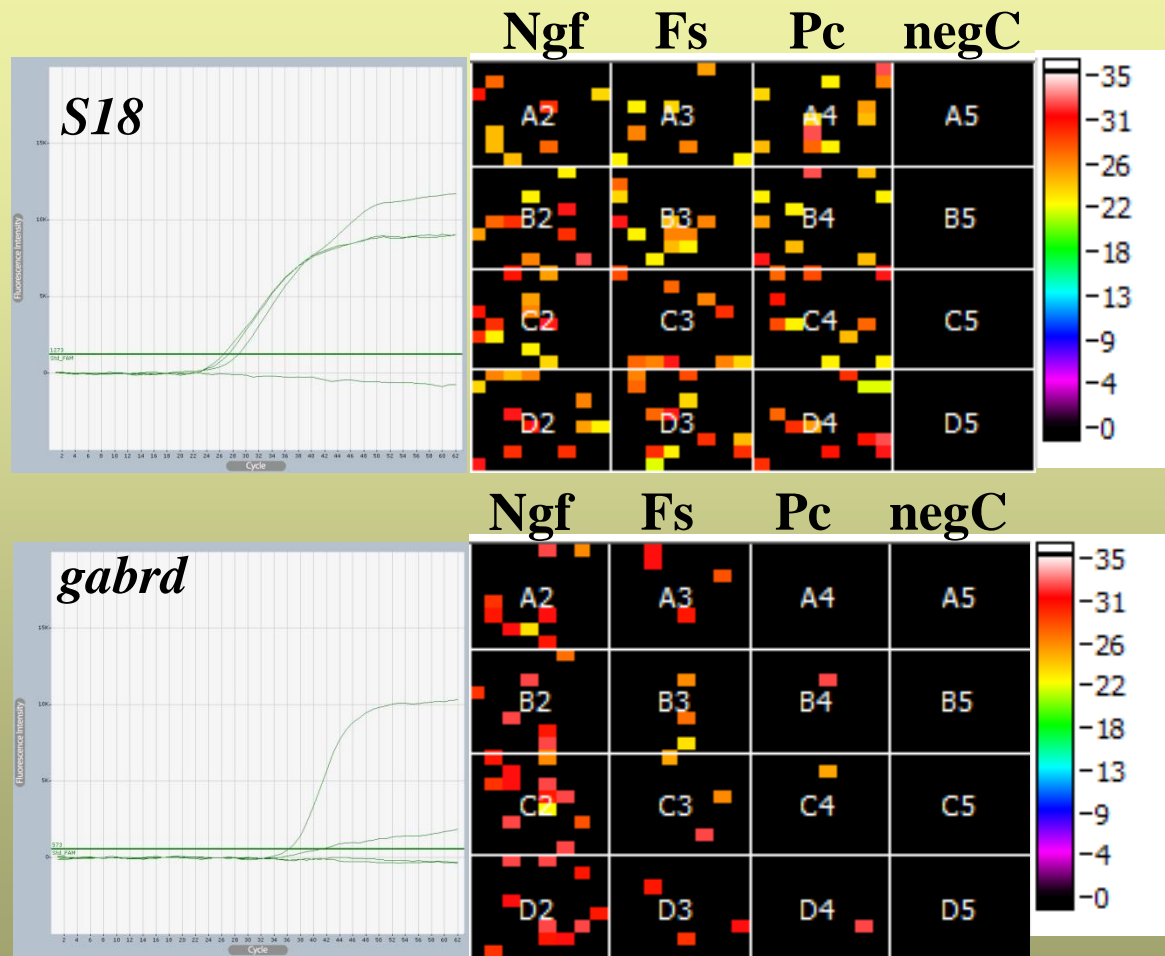
Kísérlet tervezése



1 minta

$4 \times 64 = 256$ egyedi PCR

Egyedi neuronok génexpressziós elemzése digitális QRT-PCR módszerrel



Ngf: Neurogliaform sejt

Fs: Fast spiking sejt

Pc: Piramis sejt

negC: RT- negatív kontroll

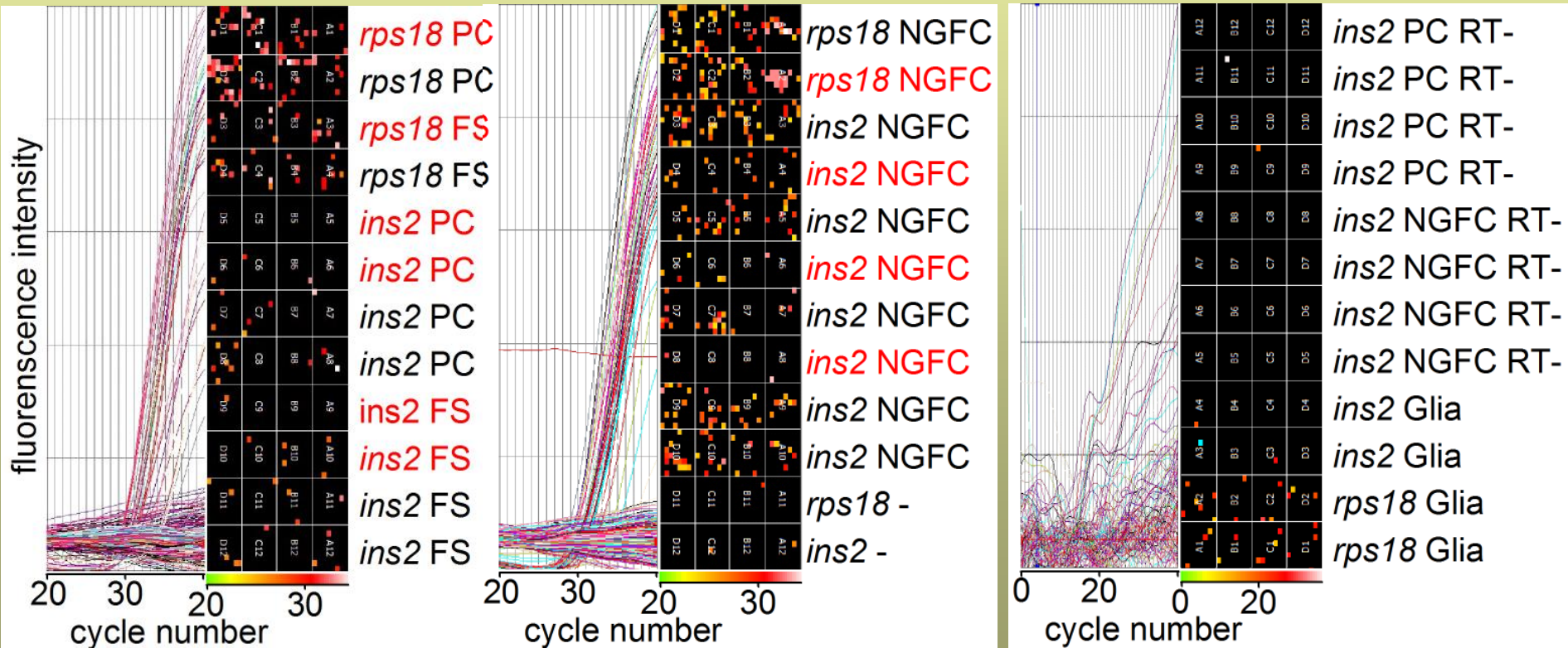
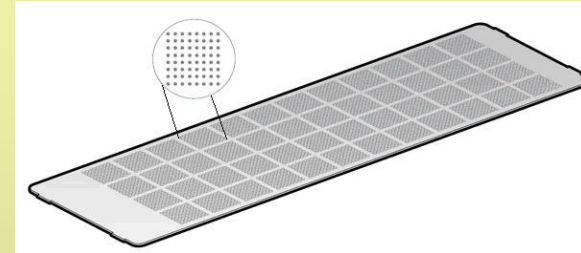
S18 háztartási gén hasonló kifejeződése minden neuron típusban

Neuron-típus specifikus expresszió: GABA receptor

Inzulin mRNS kópiaszám meghatározása egyedi sejtekben

Single cell digital PCR

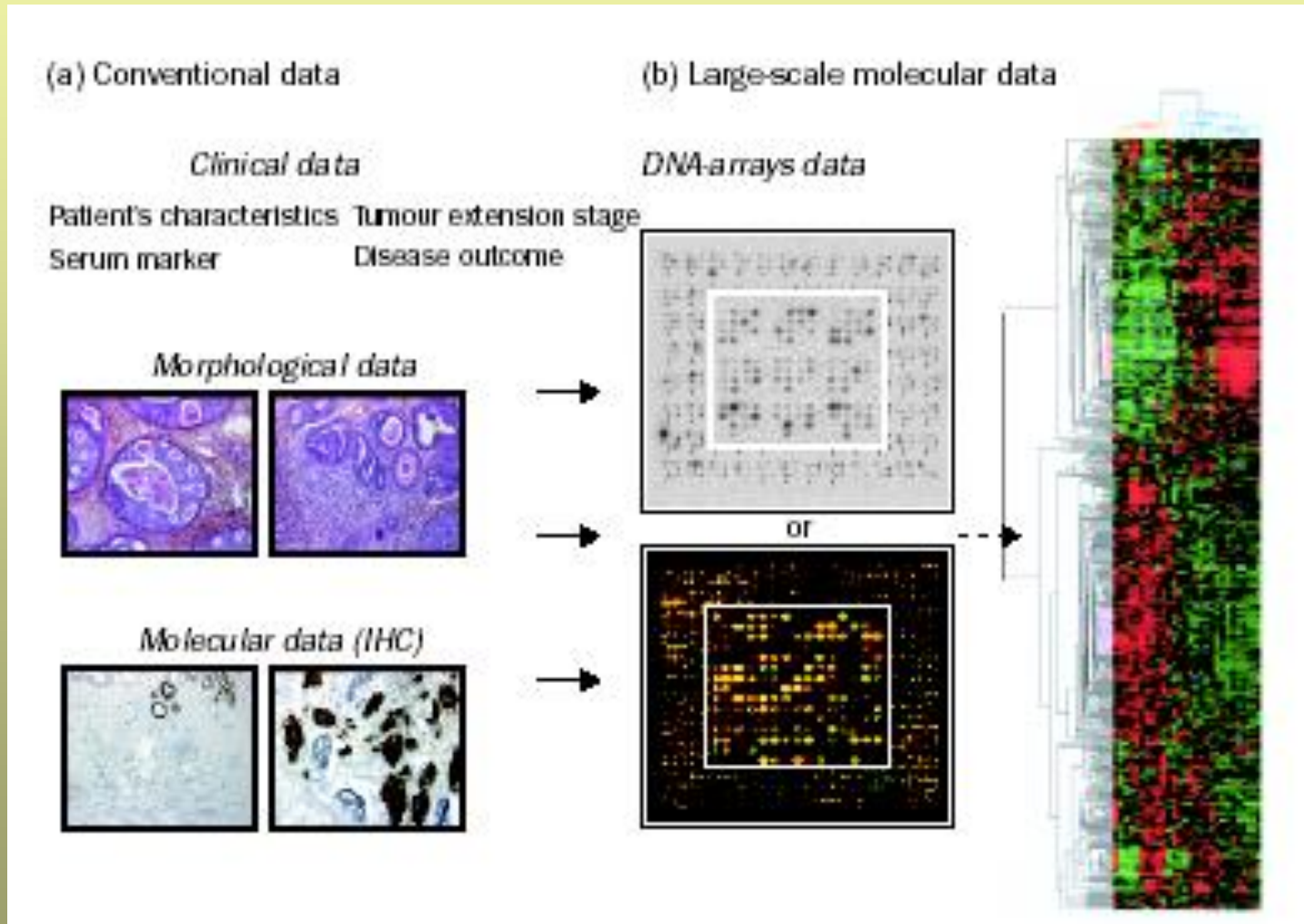
hypoglycemia / normoglycemia



rps18: small ribosomal subunit protein known to be involved in binding the aminoacyl-tRNA complex

Molnár et al. (2014) J. Neuroscience, 34:1133

Hagyományos és chip adatok egy adott klinikai kórképre



Köszönöm a figyelmet!

