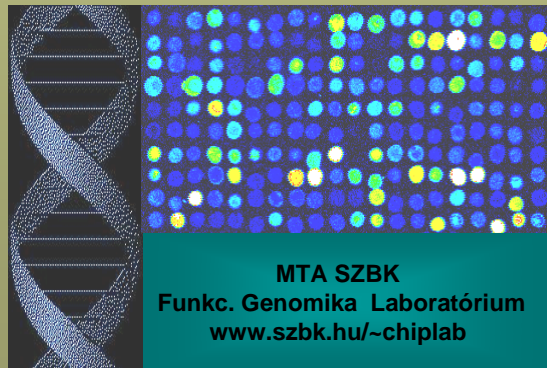


# Microarray-technológia és funkcionális genomika

MTA SZBK, Genetika Intézet  
Funkcionális Genomika Laboratórium

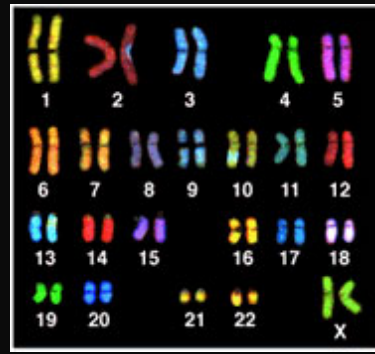
Semmelweis Egyetem, Genetika Intézet  
Budapest  
2017.12.01.



**Kromoszómák**  
genetikai állomány  
hordozói



sejt



DNS

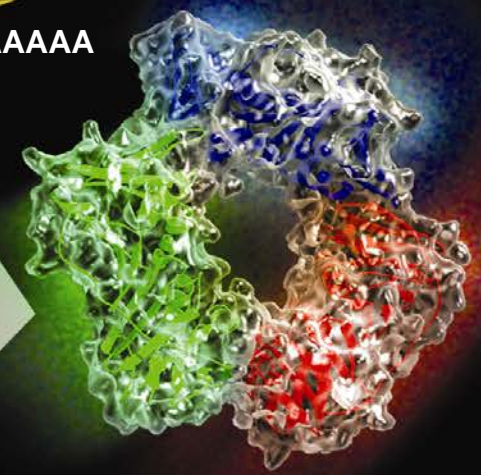
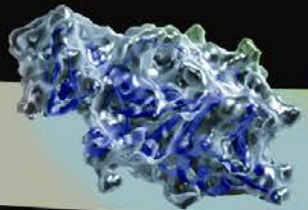
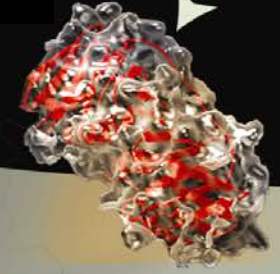
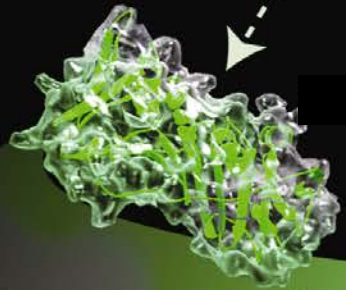
**Gének**  
információ hordozók

mRNS

AAAAAA

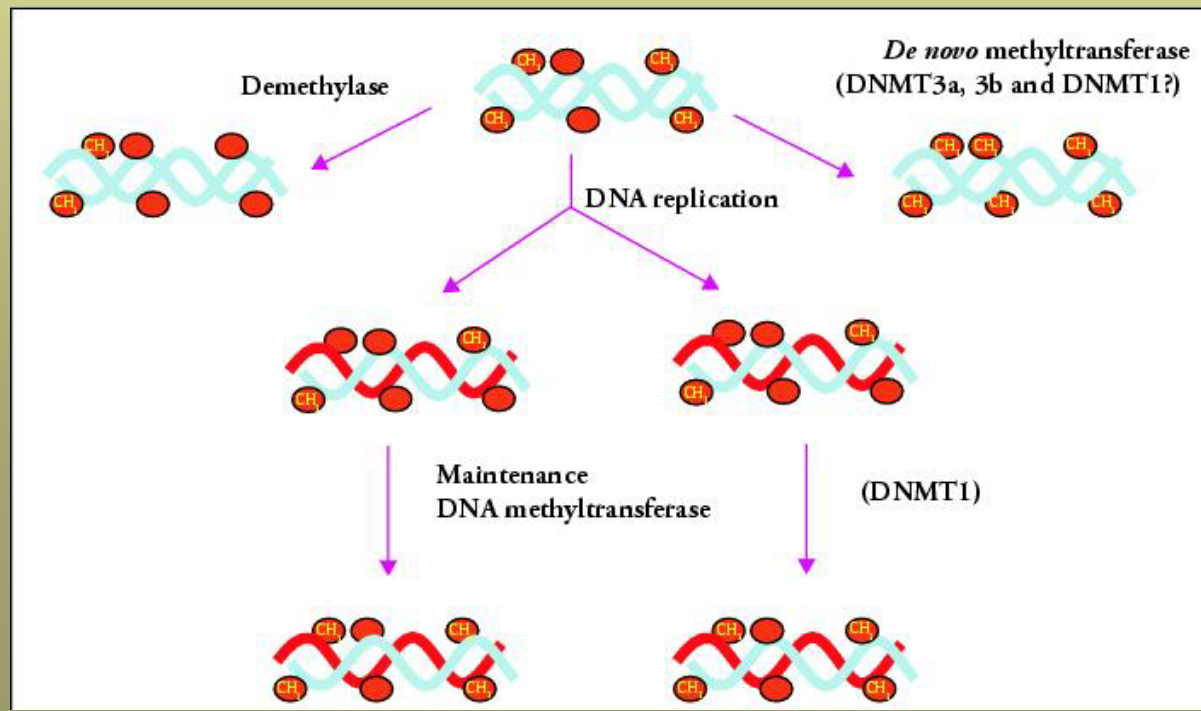
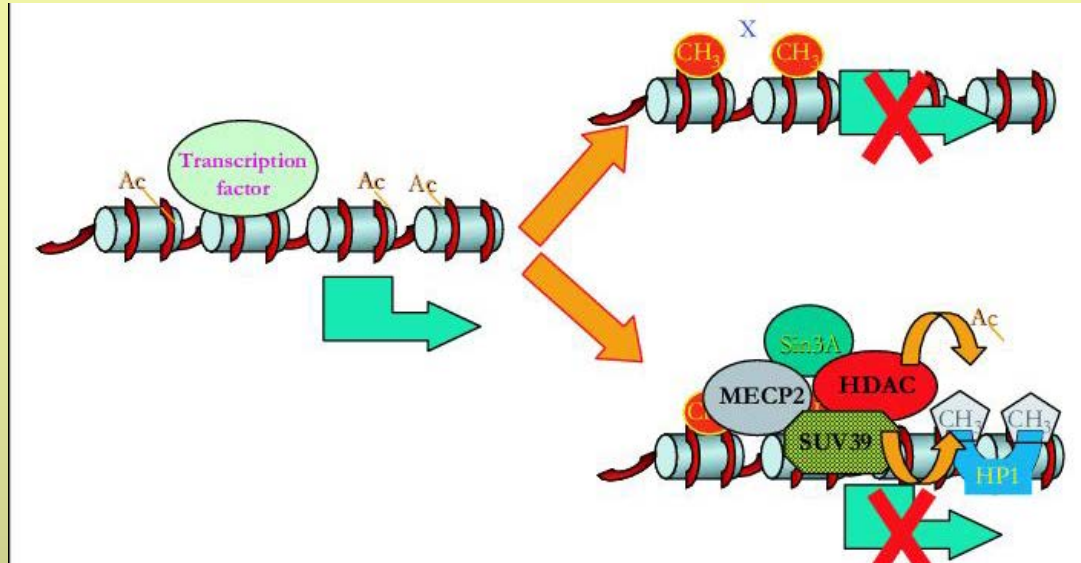
AAAAAA

AAAAAA



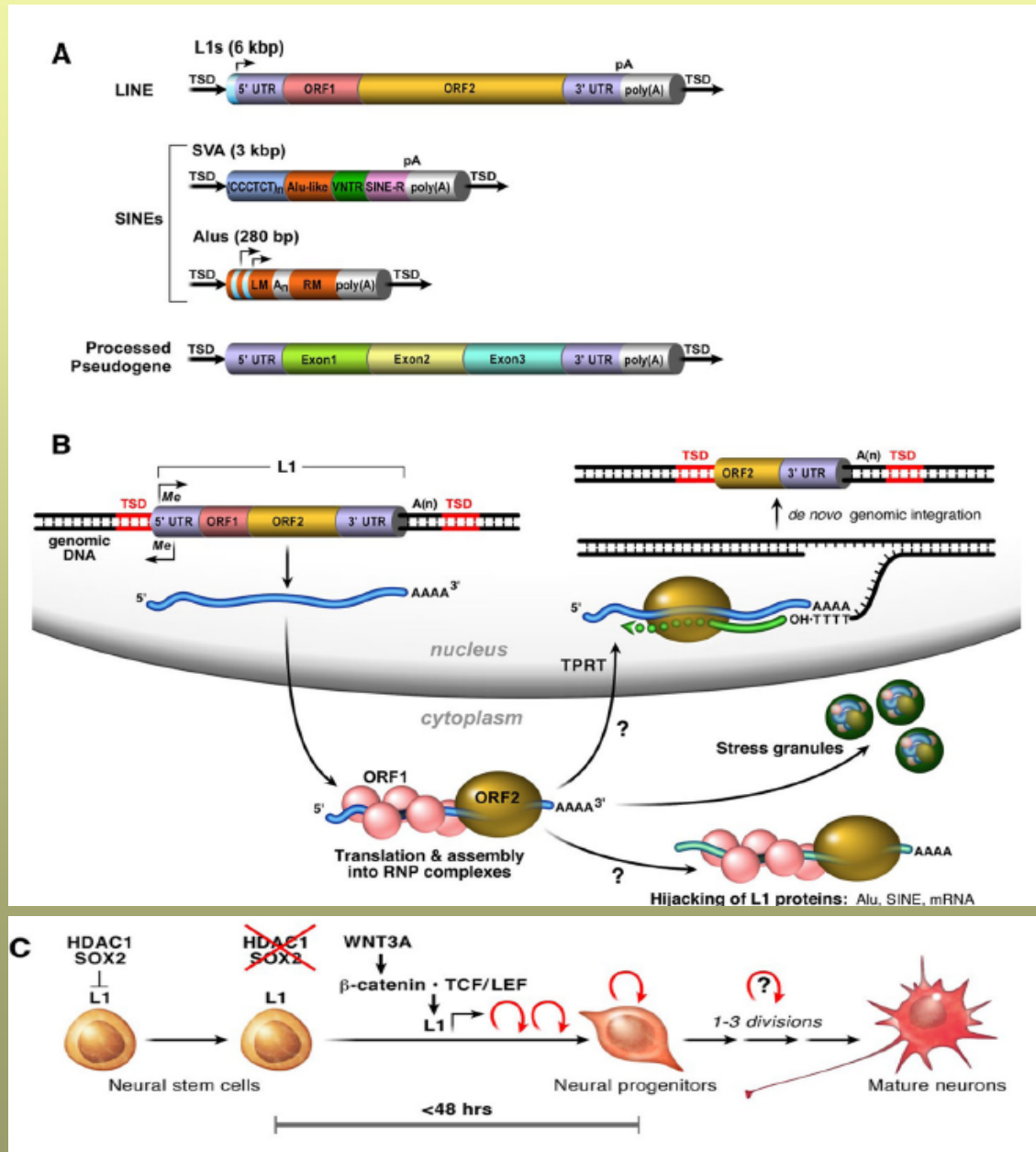
**Fehérjék**  
sejtfunkciók  
ellátása

# DNS metiláció és „hiszton-kód” – epigenetikai változások



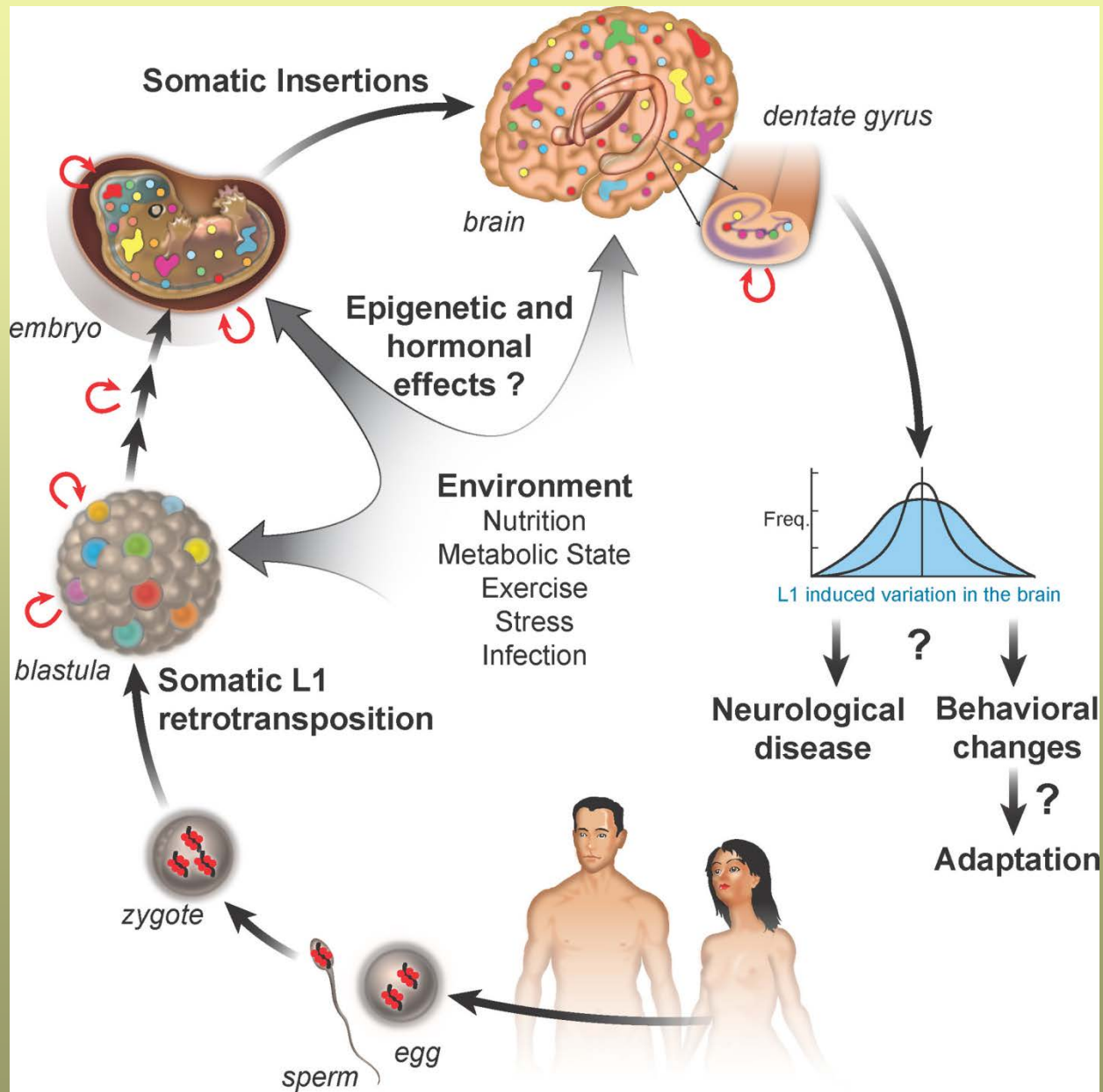
# Repetitív szekvenciák – értelmetlenül „szemét”?

LINE  
SINE

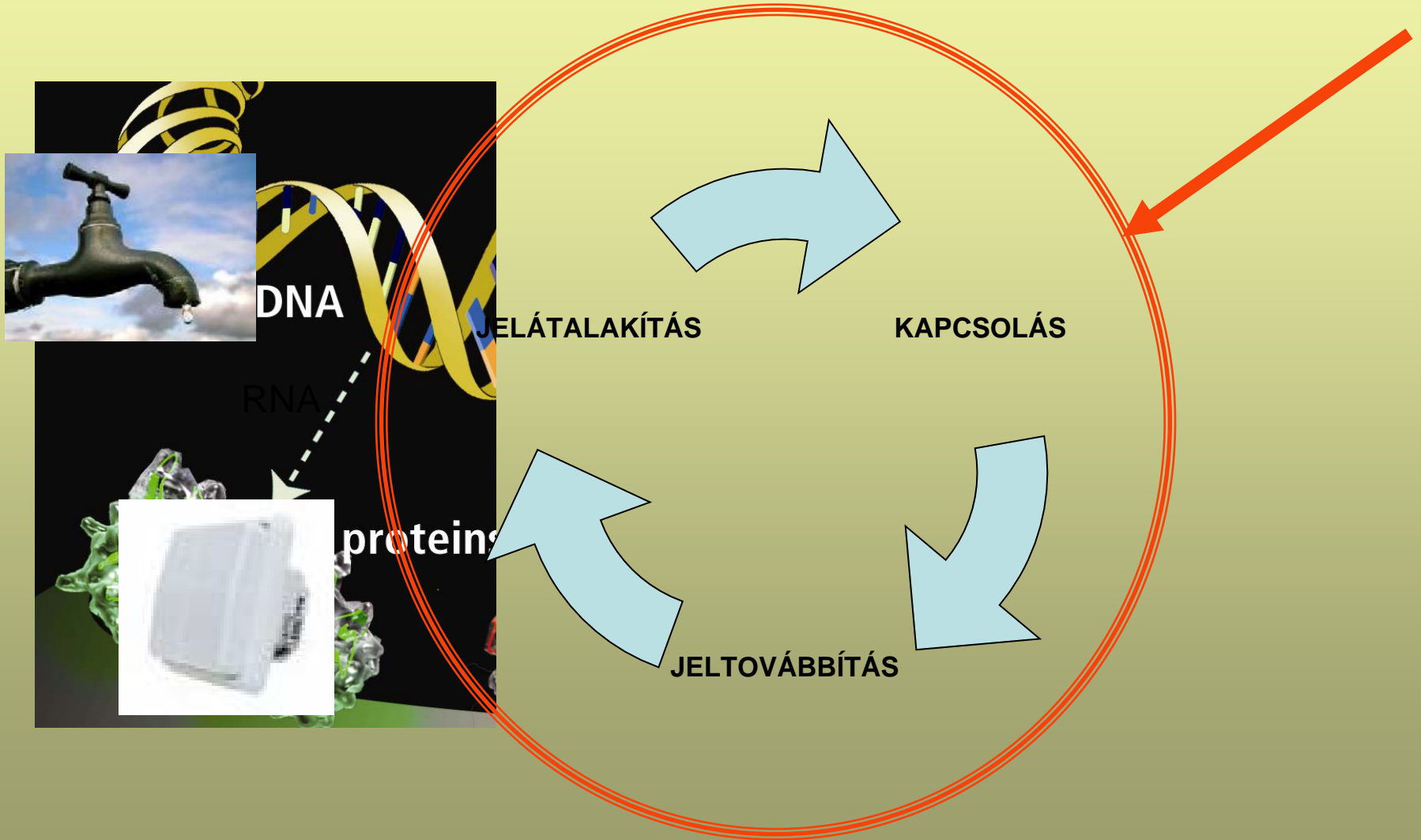




# Repetitív szekvenciák – neuronális diverzitás oka?

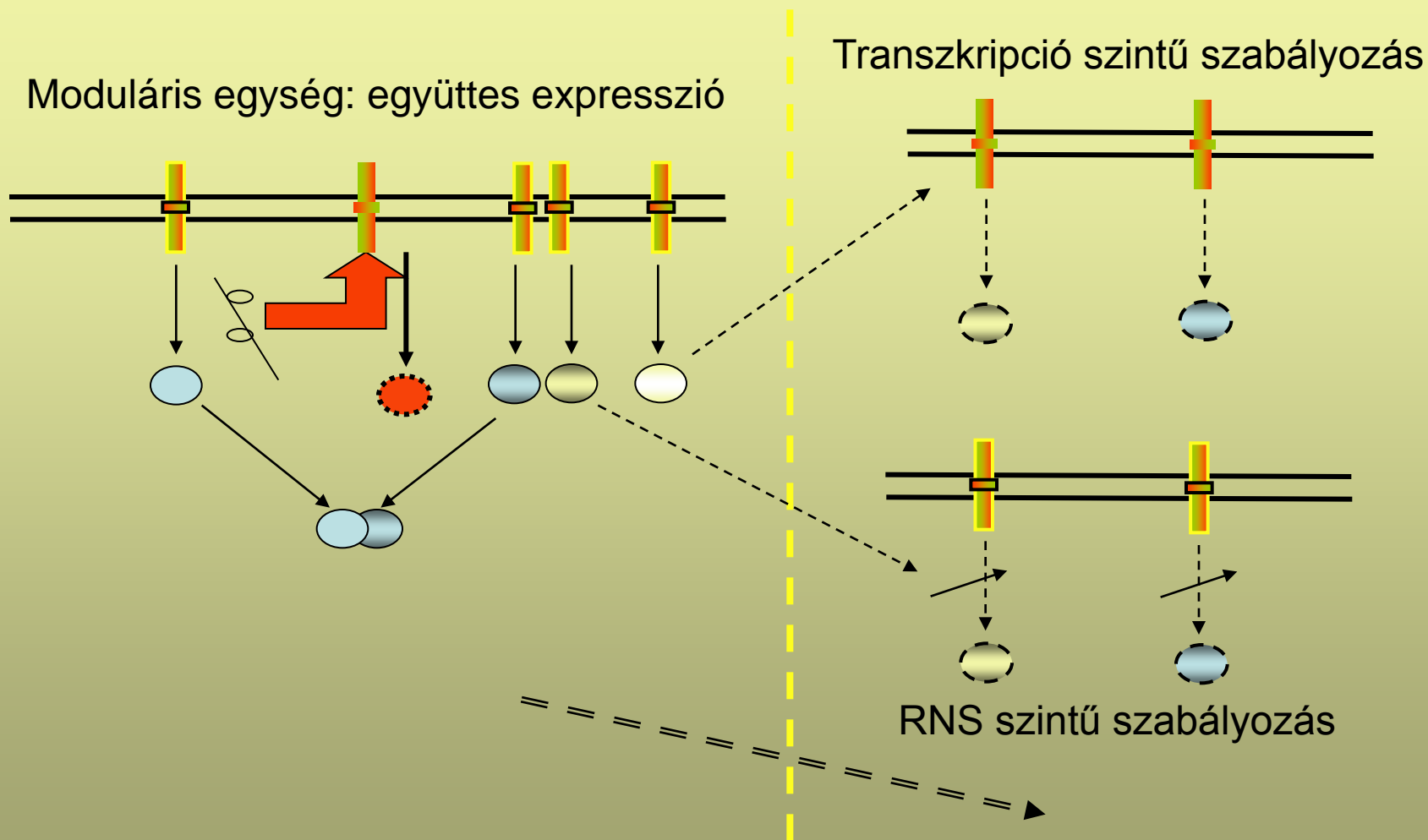


# Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



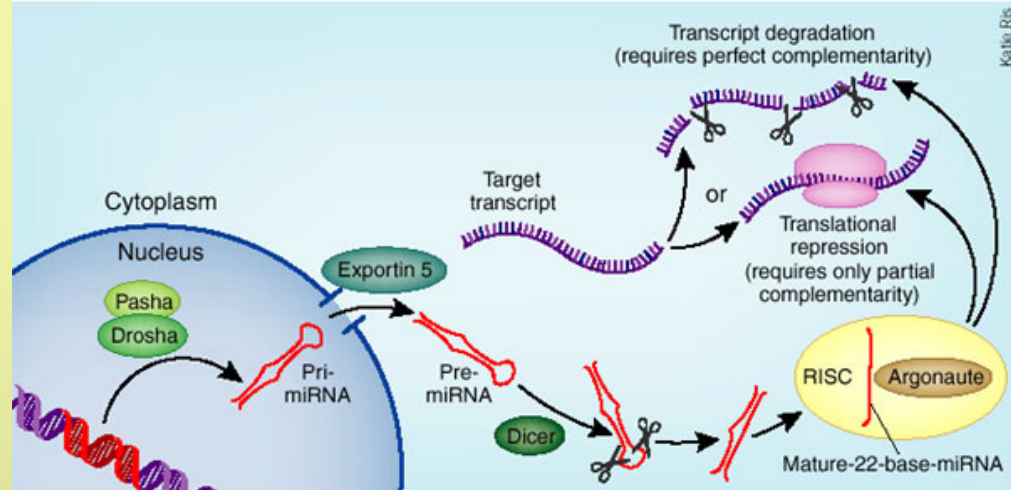
Kulcsszavak: kapcsolók

# Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



Kulcsszavak: kapcsolók és modulok

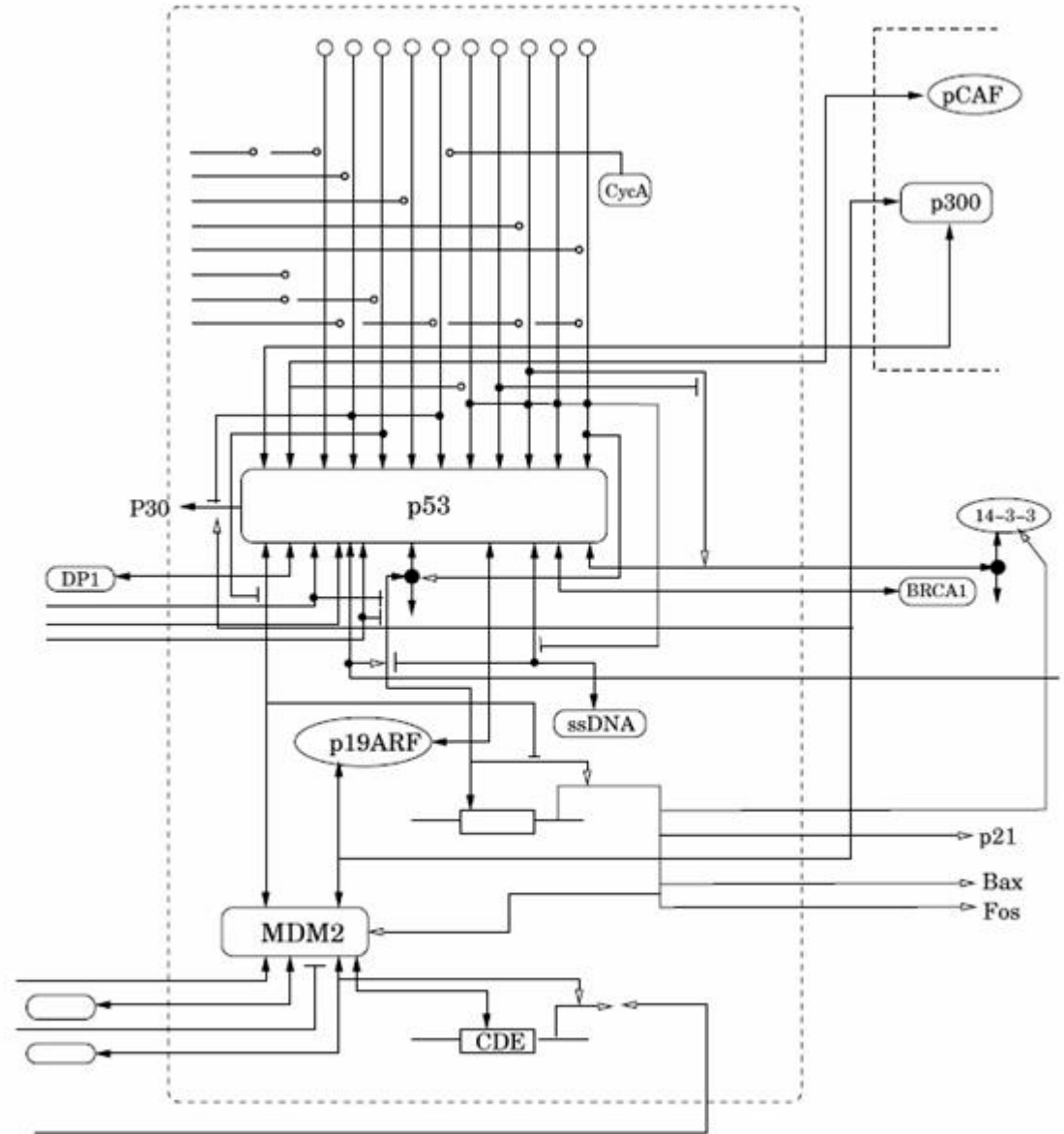
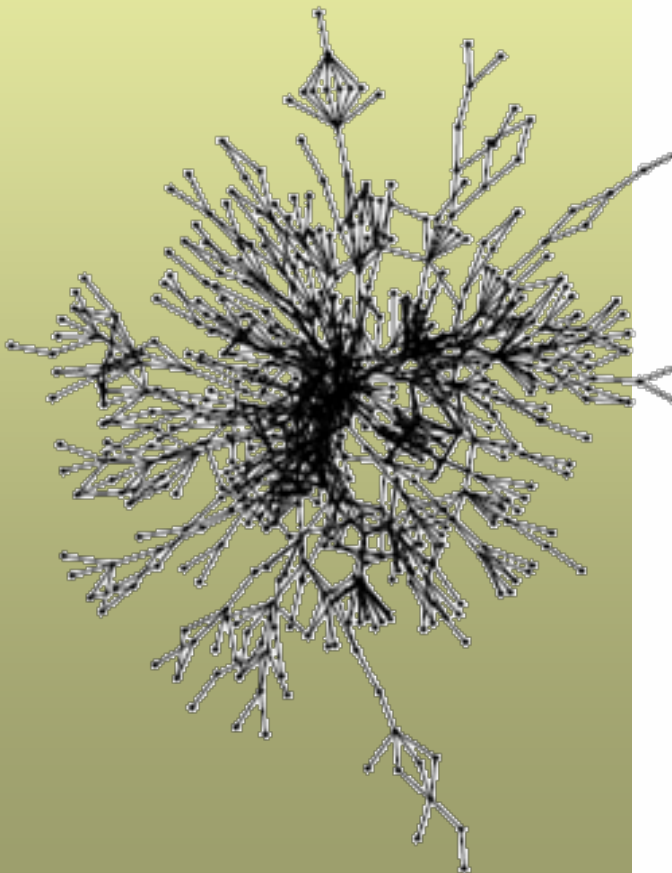
# miRNS analízis



- miRNS: kis 19-25 nukleotid hosszú egyszálú RNS molekulák, amelyek a génexpresszió negatív szabályozásában játszanak szerepet
- a sejtmagban keletkeznek hosszú prekursor 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

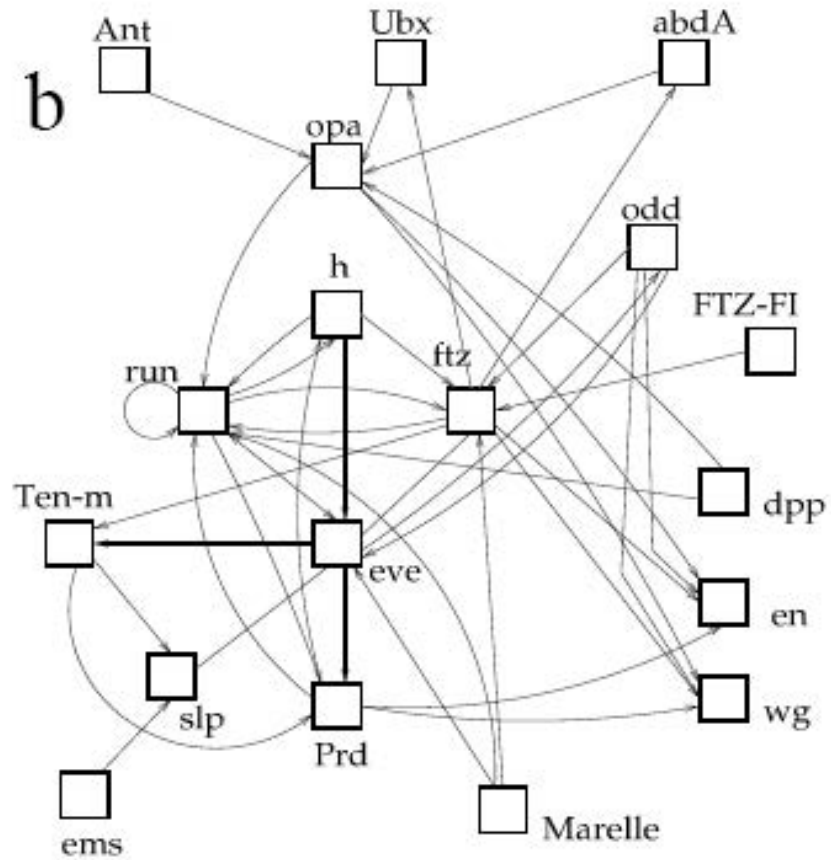


# Génhálózatok, skála-független tulajdonság, redundancia és érzékenység



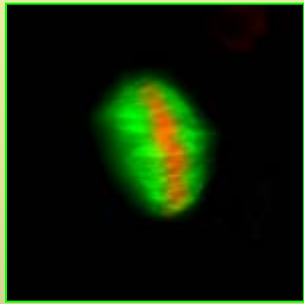
**Figure 1.2:** Schematic architecture of the p53 network. The p53 node integrates information from very different parts of the system. Only part of the cell circuitry is shown here. For a

# Sejthálózatok és génhálózatok, kapcsolatok és jelek

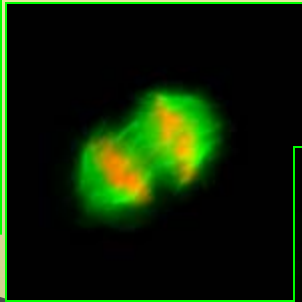


# Sejtciklus-egyres fázisai

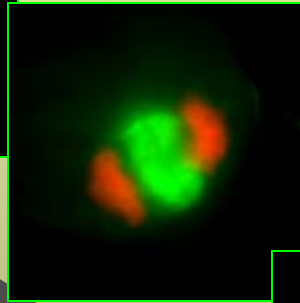
## Ciklikus fehérje-módosítások, komplexek és degradációk



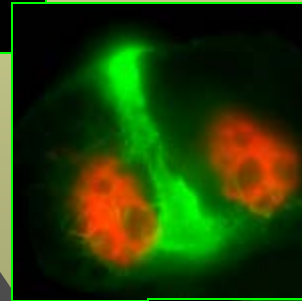
metaphase



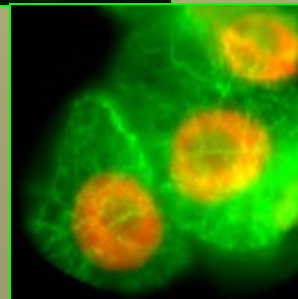
anaphase



early telophase



late telophase



G1 phase

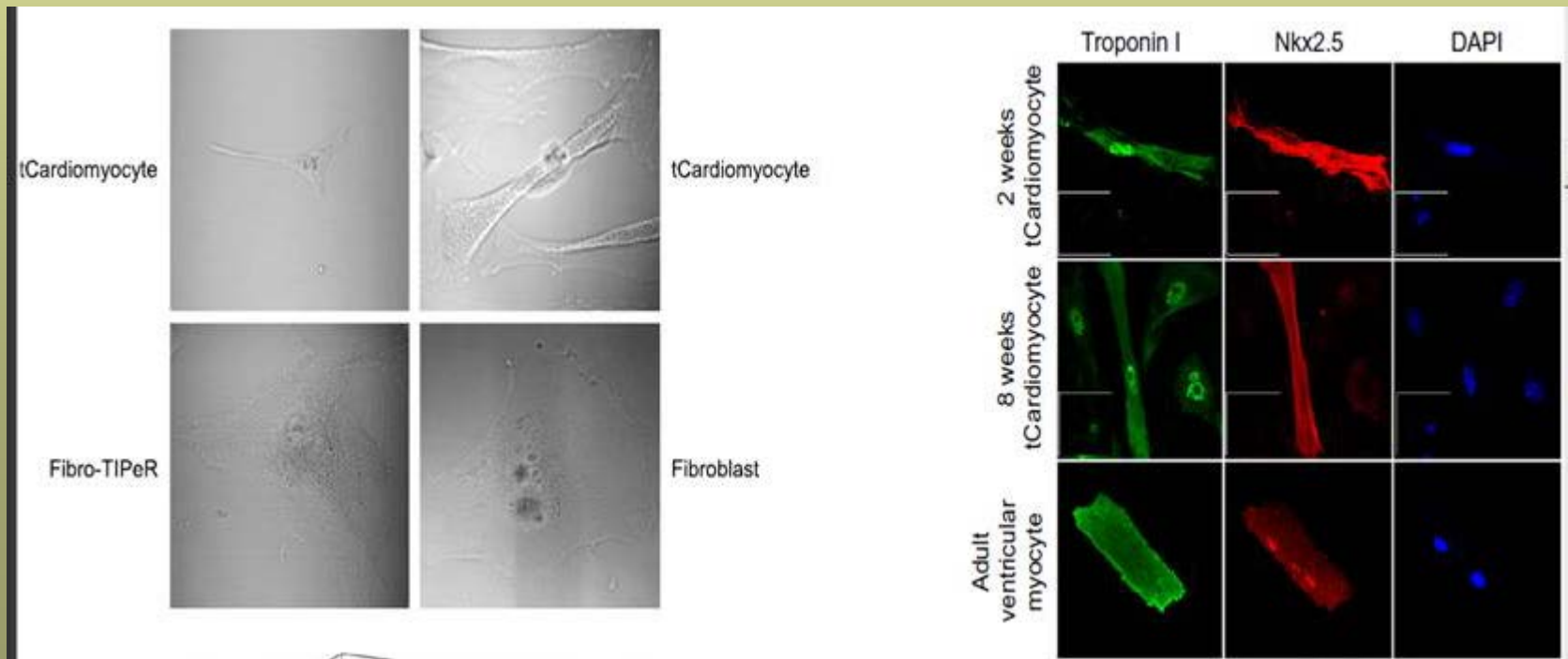
# A fenotípus meghatározója a transzkriptom vagy a genom?

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.

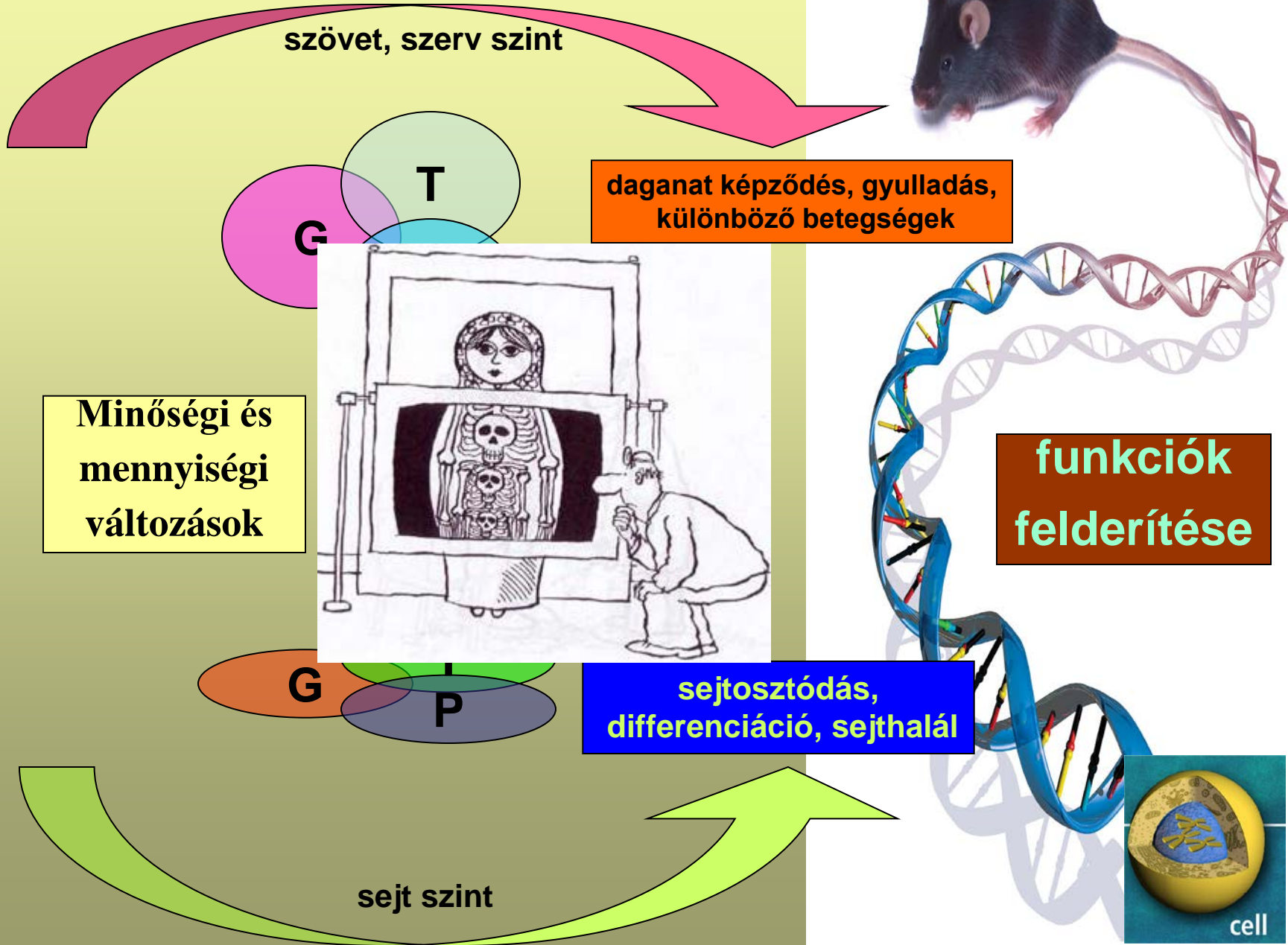
Kim TK, et al. (2011) Transcriptome transfer provides a model for understanding the phenotype of cardiomyocytes. *Proc Natl Acad Sci U S A*. 108(29):11918-23.



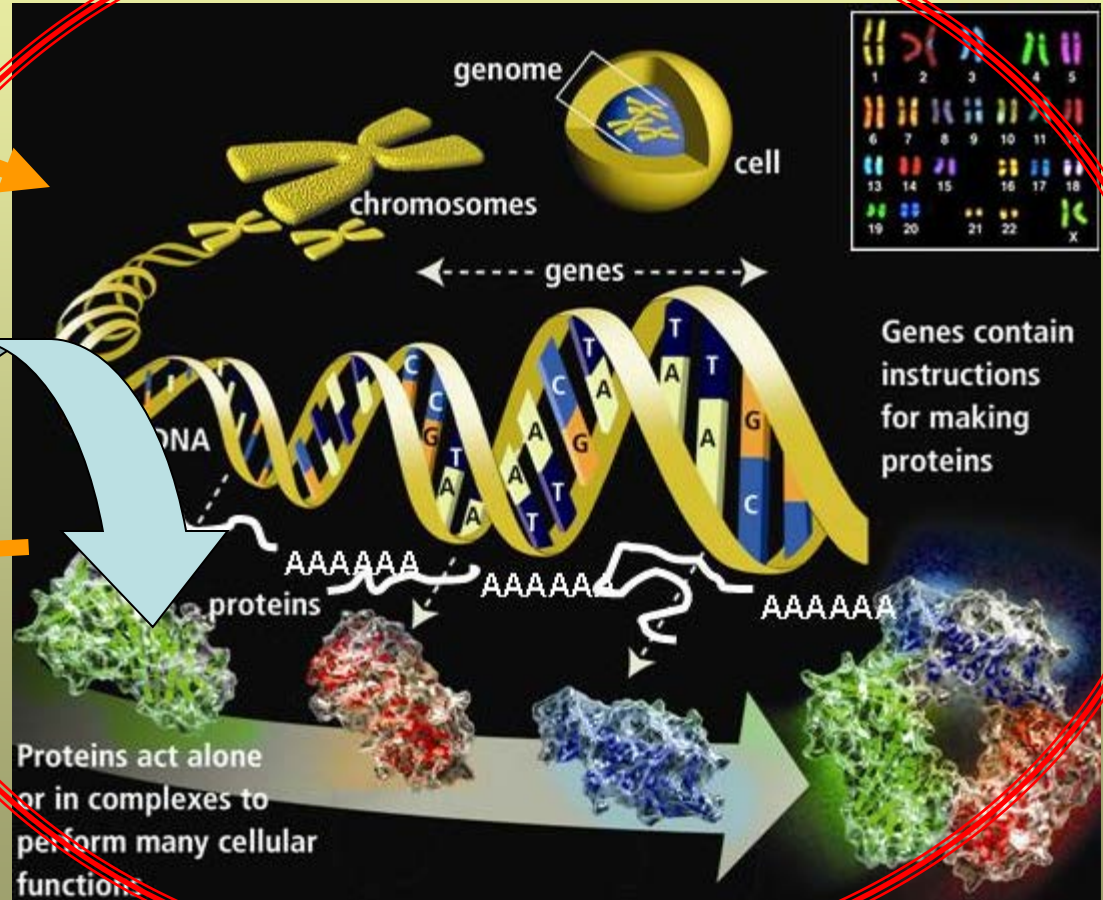


Környezeti hatások, gyógyszerek, fertőzések

# Funkcionális genomika lényege



# A táplálék kölcsönhatása a génekkel, génállománnyal



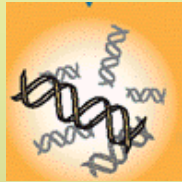
Rövidtávú és Hosszútávú hatások sejt szintű és Fiziológias változások

*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*



# Microarray technológiák a funkcionális genomikában

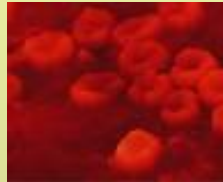
Minta



Fragmentek



Szubcelluláris



Sejtek



Szervek



Organizmusok



Közösségek

Cél- molekula



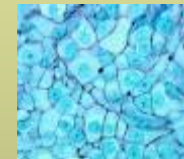
DNS, RNS



Fehérjék



Sejtek

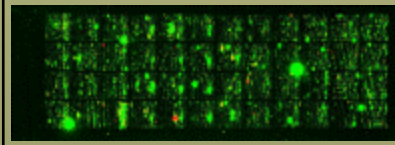
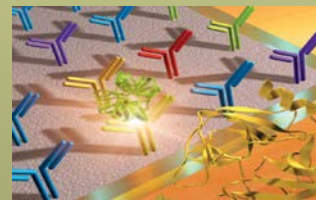


Szövetek

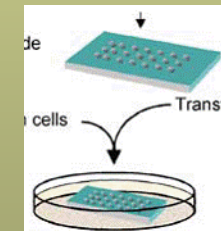
Microarray



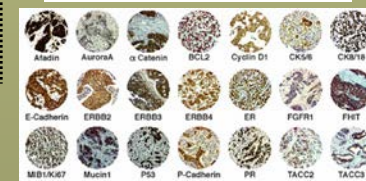
Genomika



Proteomika

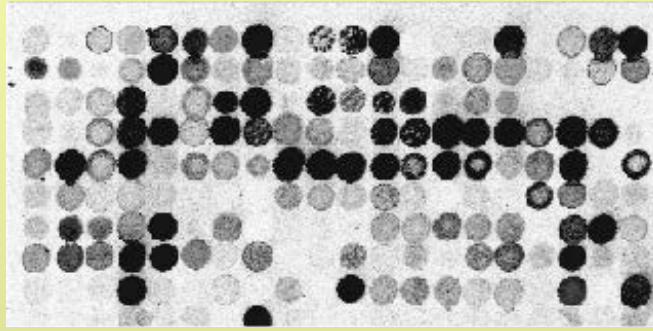


Citomika

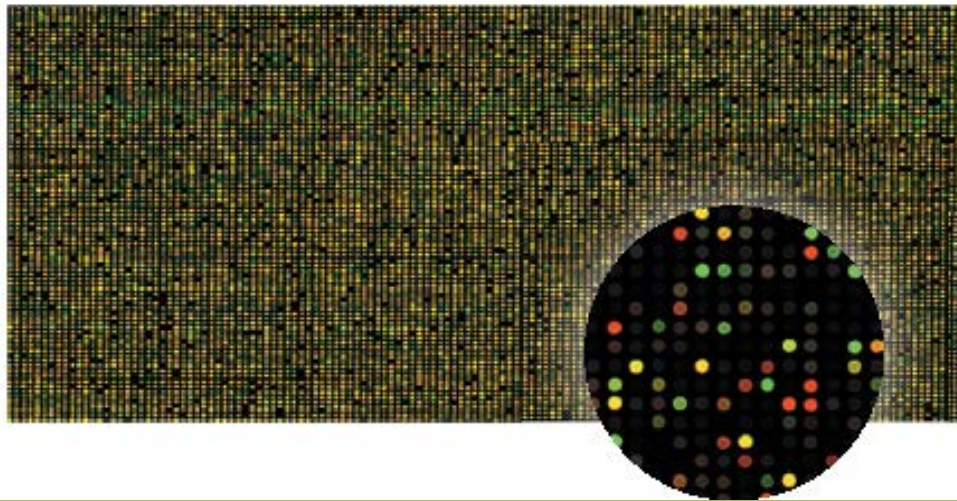


# A csipek hordozó szerinti osztályozása

macroarray

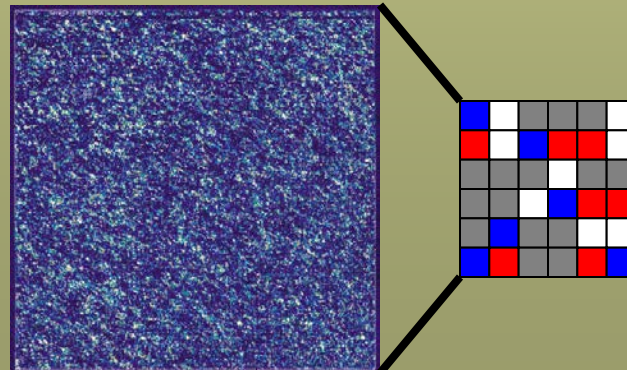


- nylon membránon néhány 100 génspecifikus minta (DNS darab)
  - radioaktív jelölés
  - kis minta sűrűség (100-1000 pont/cm<sup>2</sup>)



- üveglemezen több 10.000-100.000 génspecifikus minta (DNS darab)
  - fluoreszcens jelölés
  - közepes mintasűrűség (5000 pont/cm<sup>2</sup>)

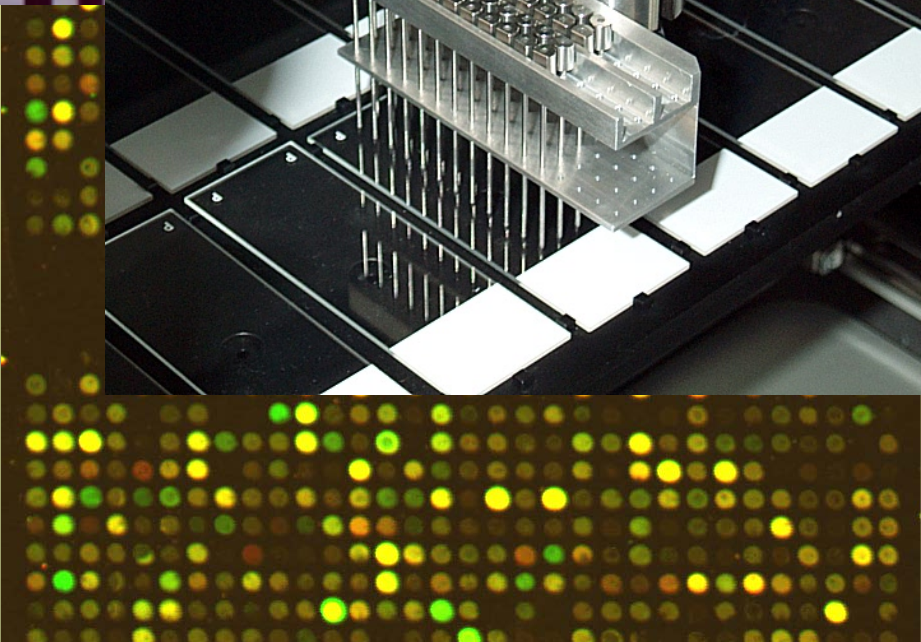
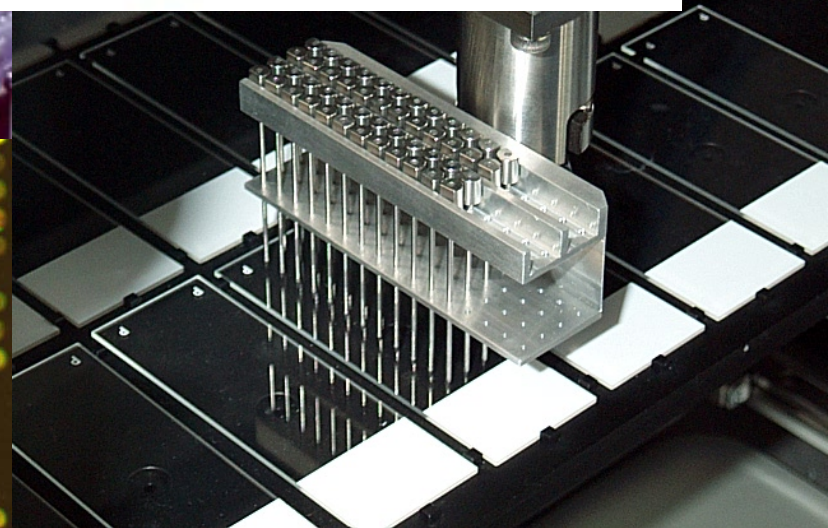
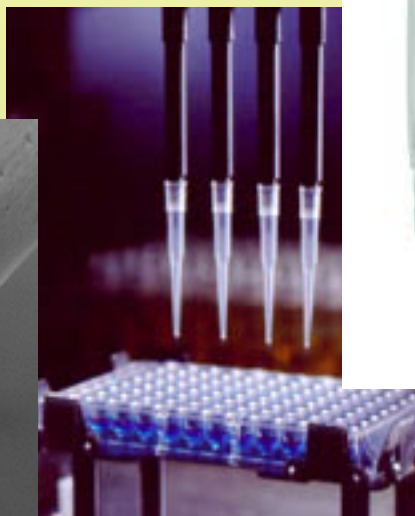
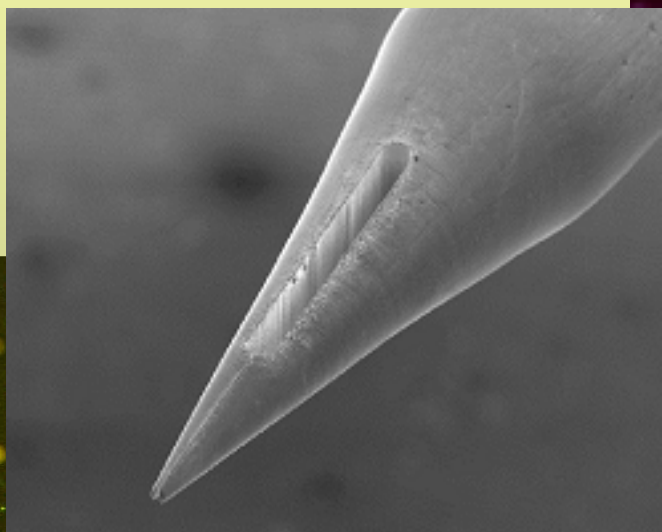
chip



- üveglemezen több 100.000-1.000.000 génspecifikus minta (DNS darab)
  - fluoreszcens jelölés
  - nagy minta sűrűség (10.000pont/cm<sup>2</sup>)

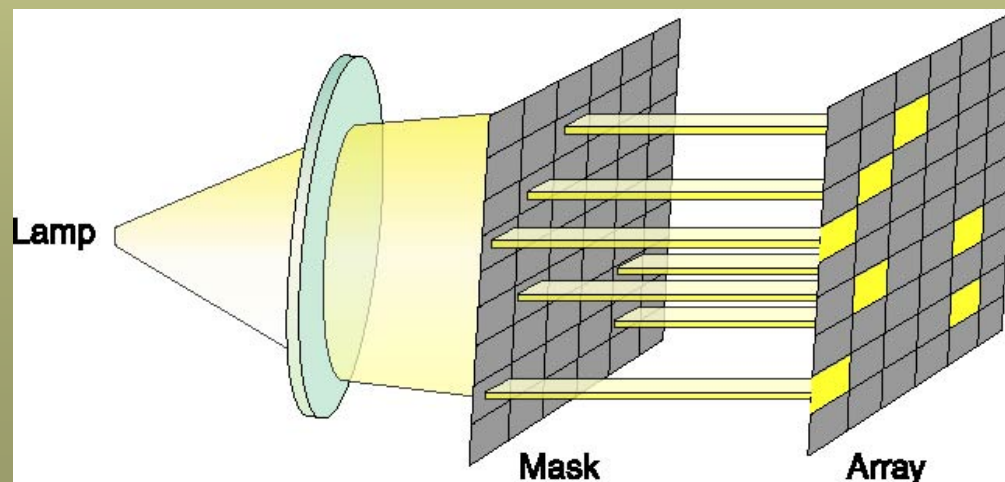
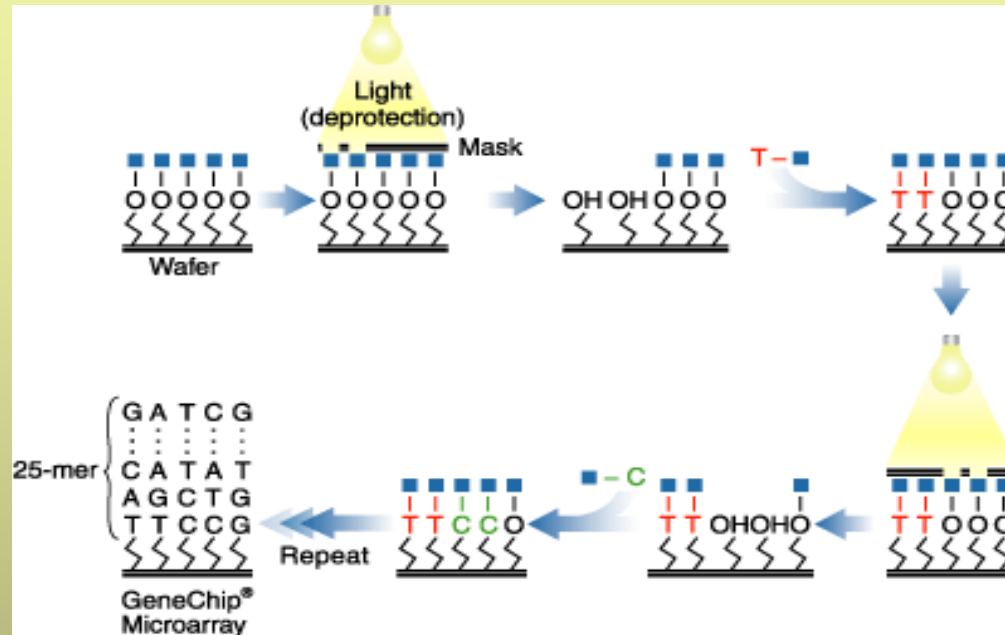


# DNS-chipek/DNS-microarrayek készítése



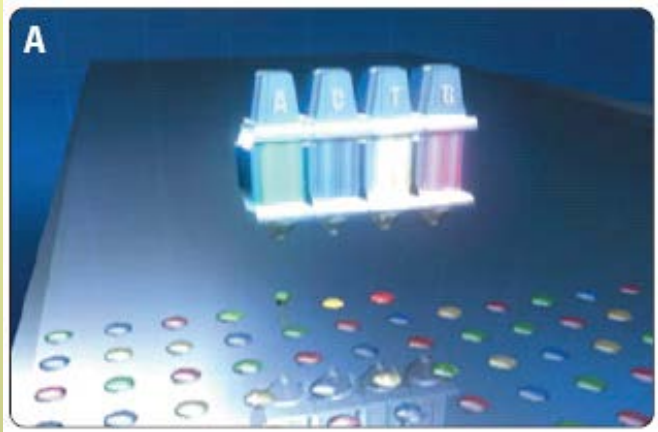
# Oligonukleotid alapú chip készítése in situ szintézis

Affymetrix

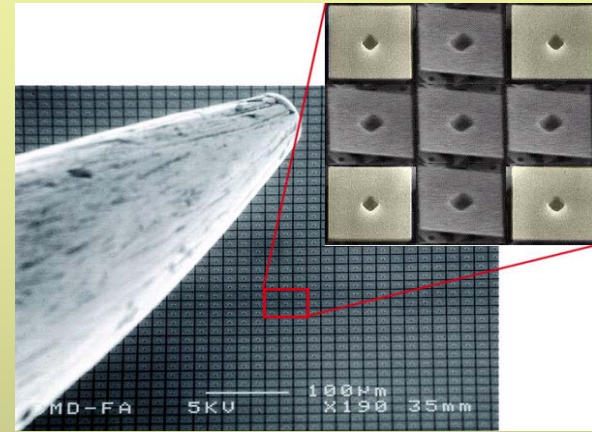


CHIPKÉSZÍTÉS

# Alternatív technológiák oligonukleotid DNS-chipek gyártására



inkjet-printed microarrays  
(e.g. Agilent)

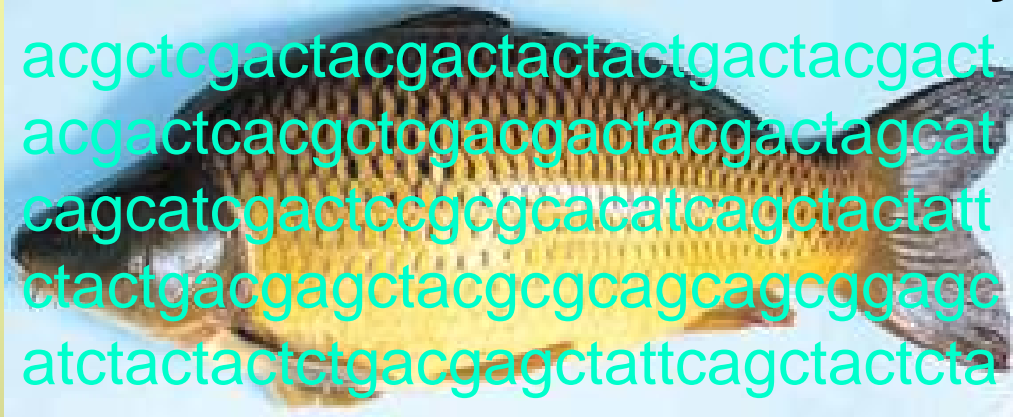


maskless array synthesizer  
(e.g. Nimblegen)



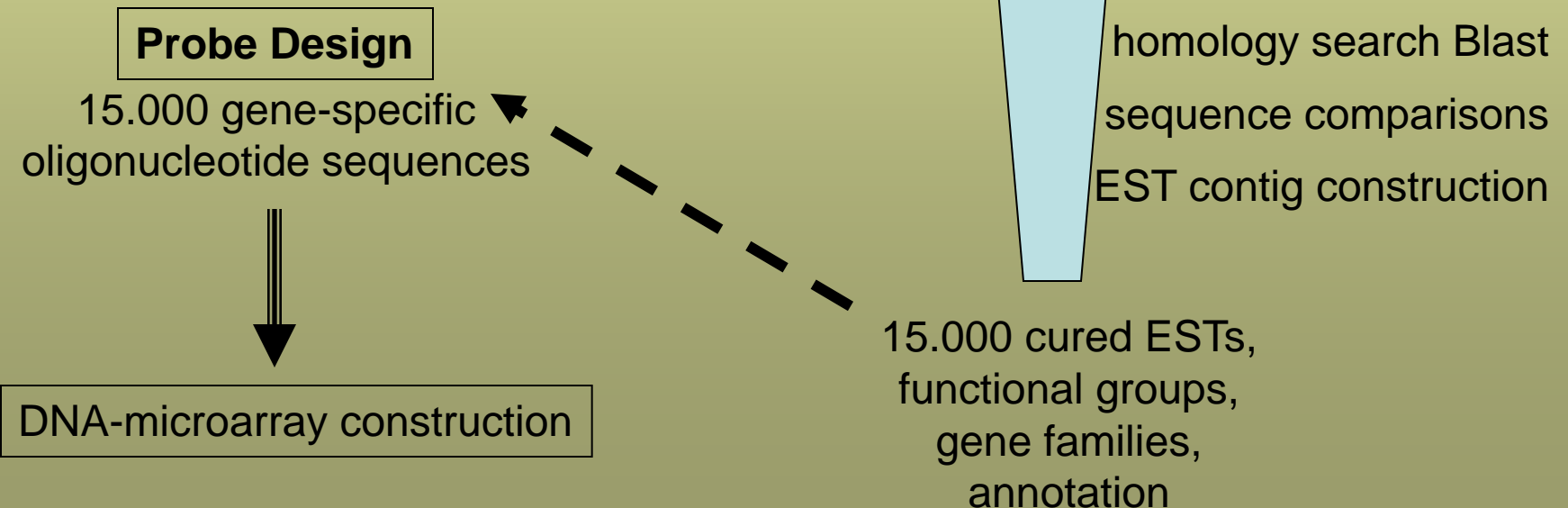


# CARP Gene database & bioinformatics tools for microarray selection



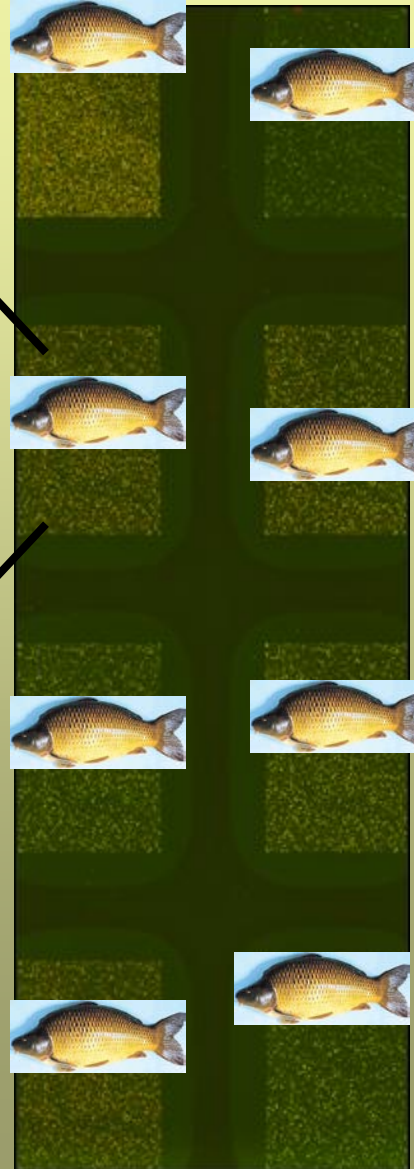
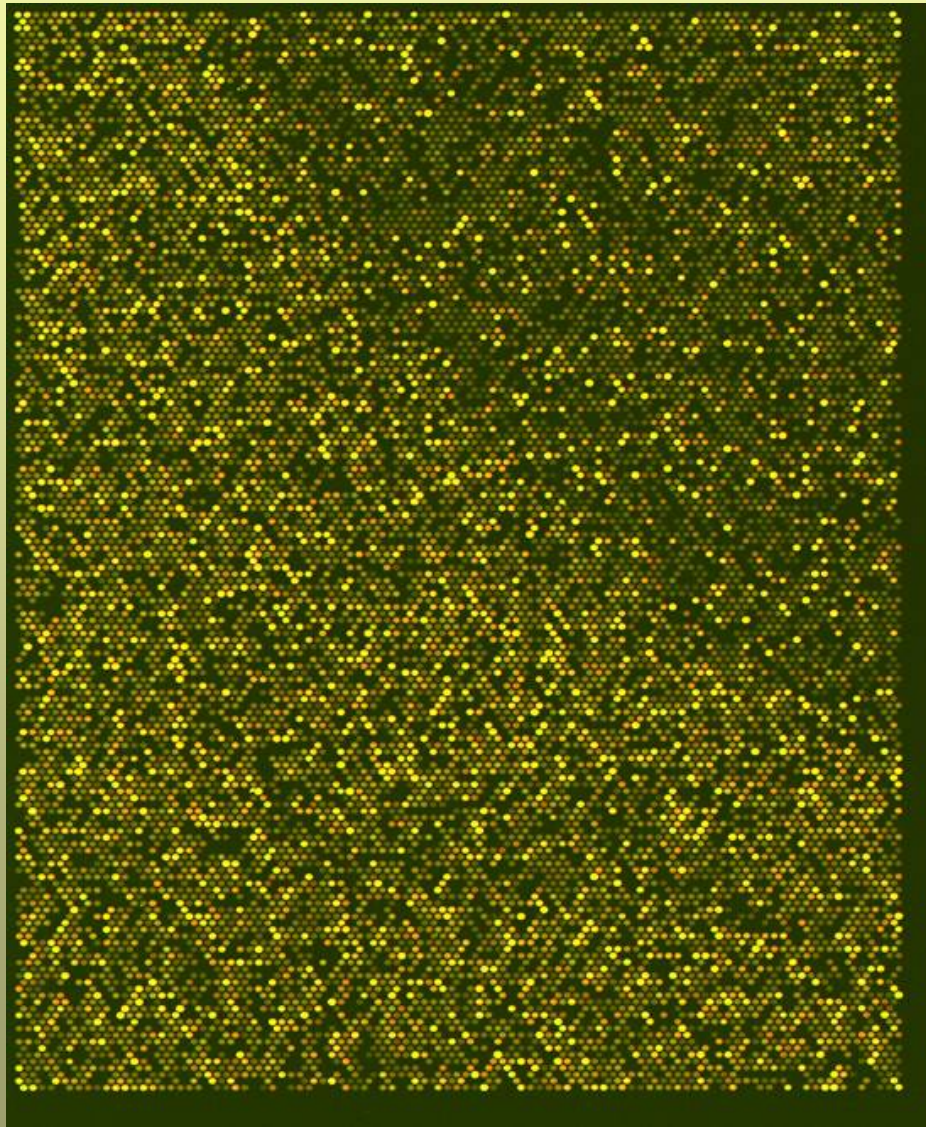
CarpBase: over 15.000 expressed  
sequence tags (ESTs)

NCBI: over 10.000 uncurated sequences





# 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

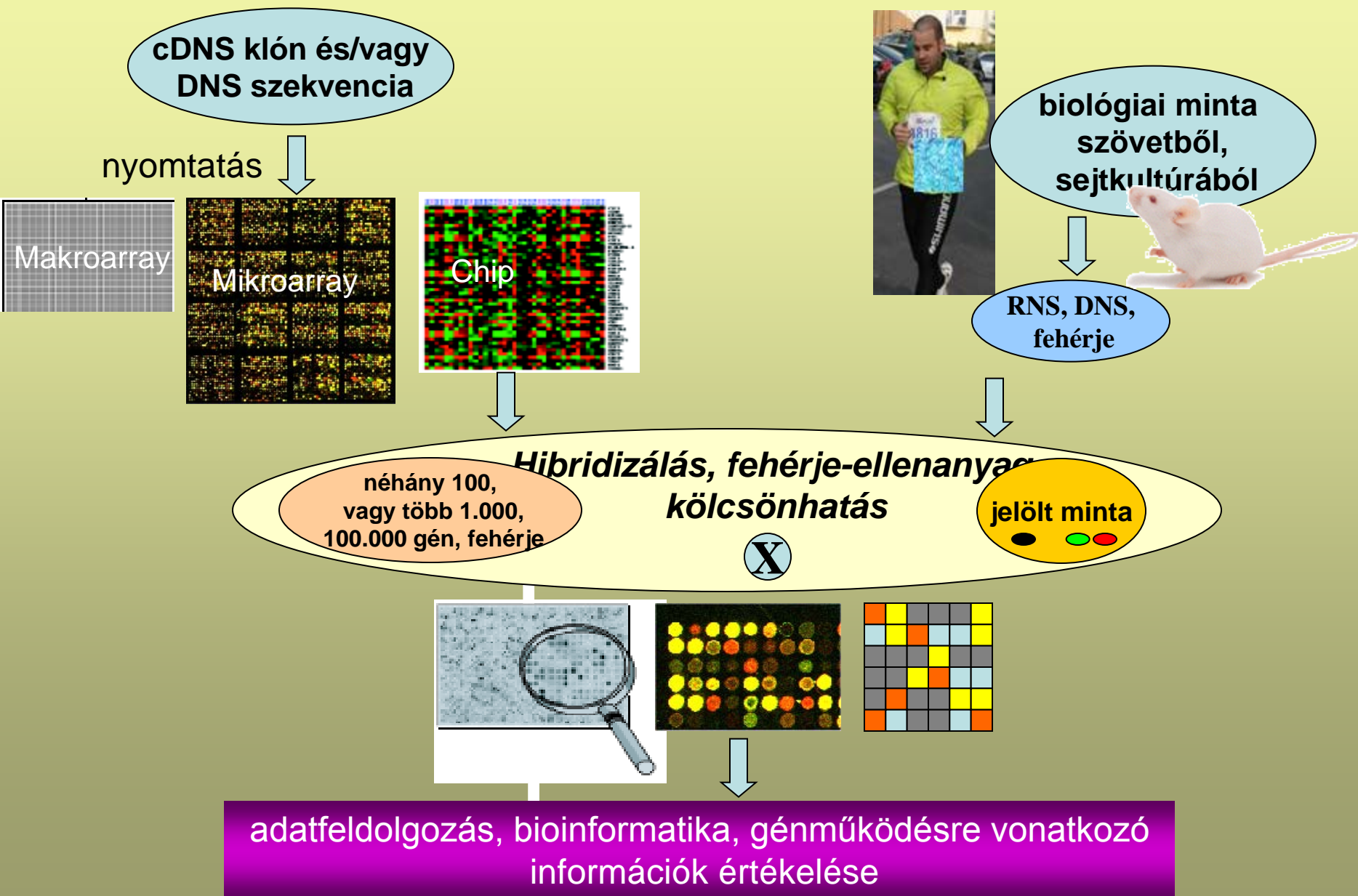
Zsúfoltsági teszt



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



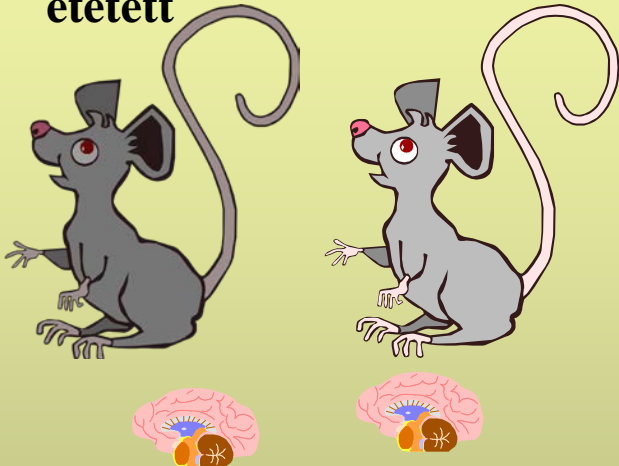
# Egy csipkísérlet általános lépései



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

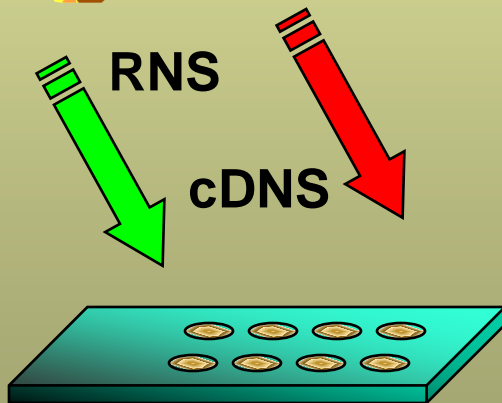
halolajjal  
etetett

kontroll



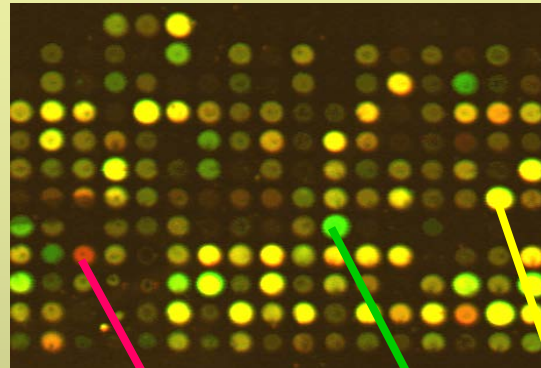
RNS

cDNS

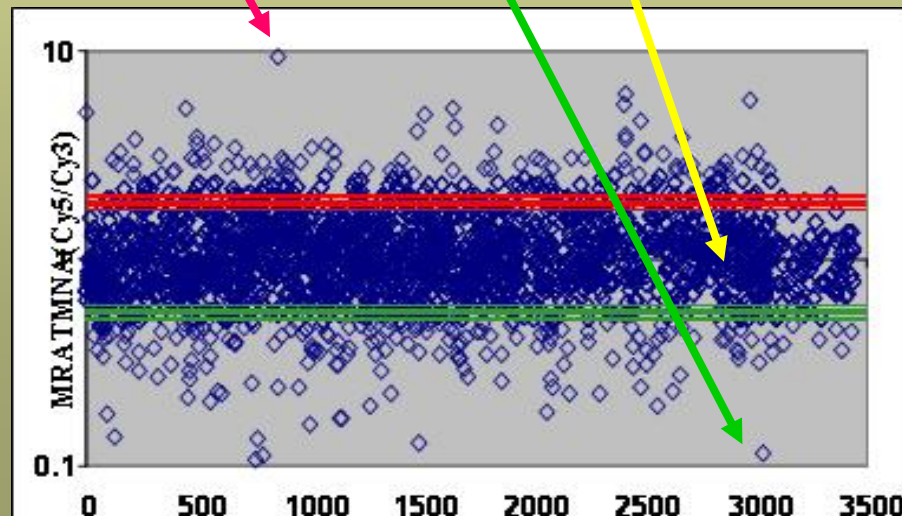


patkány cDNS-chip  
3200 génspecifikus minta  
6400 mintapont

Génexpressziós változások



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

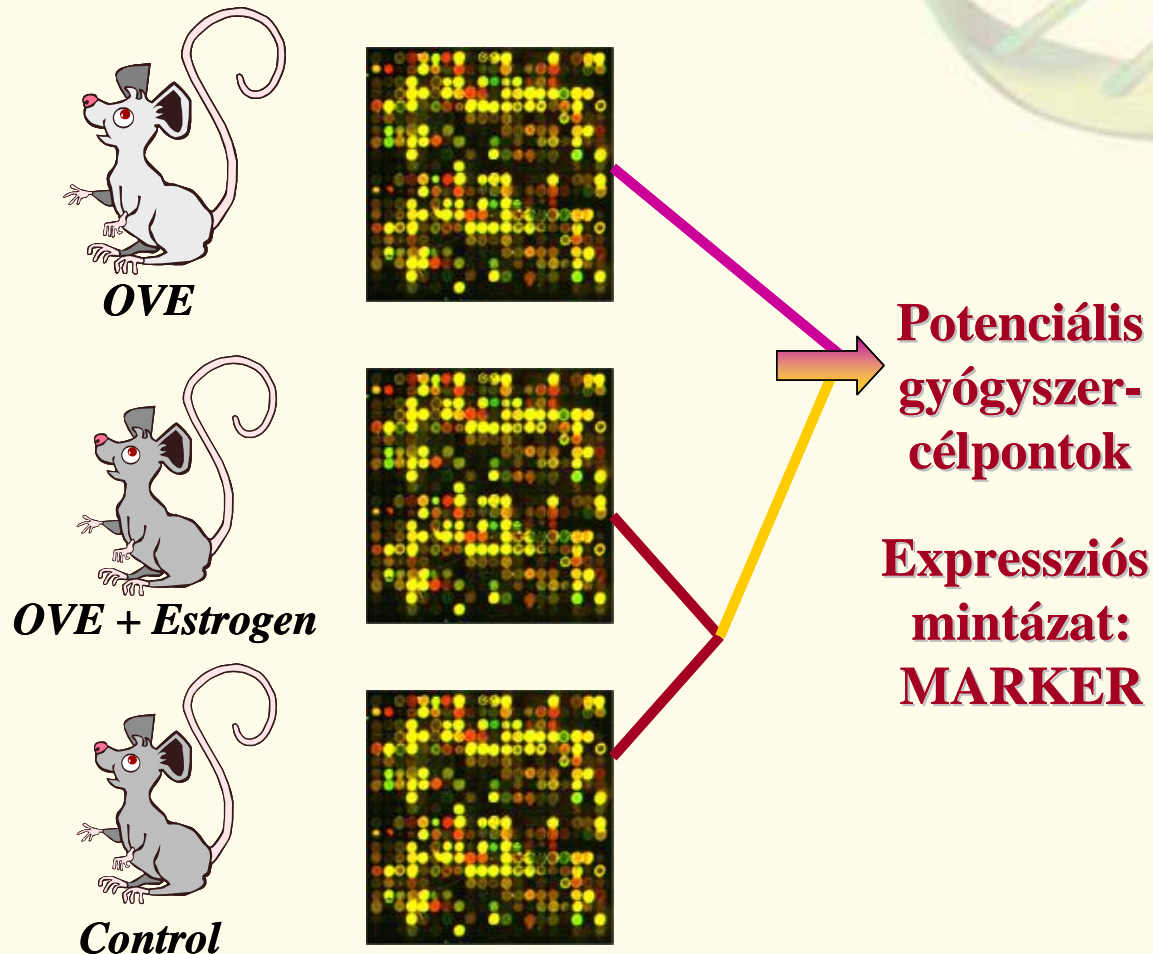


Pozitív hatás a mentális funkciókra, Tanulás, memória

# Felfedezés (Discovery)

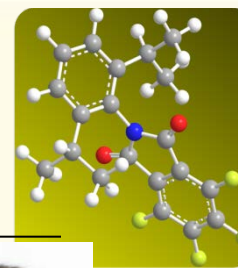
## 1. Target alapú szűrések

### 1a: új target azonosítása





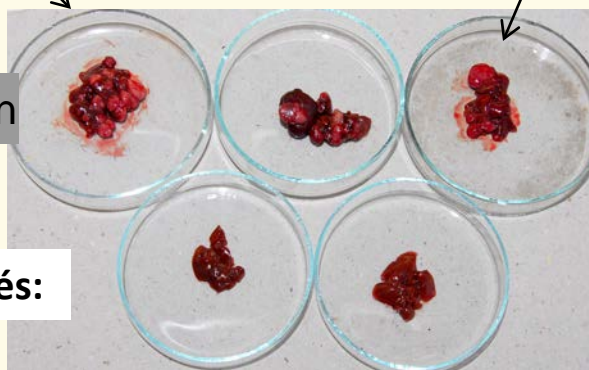
# Génexpressziós változások Ac915-tel kezelt KO-egér májkarcinómában



Kezeletlen kontroll

Tumorigenesis: DEN, 15 napos  
egyszeri 50  $\mu$ l-es kezelés, 4 hónap  
normál táp

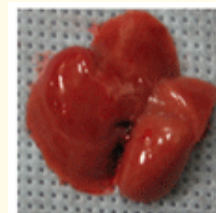
Kezeletlen



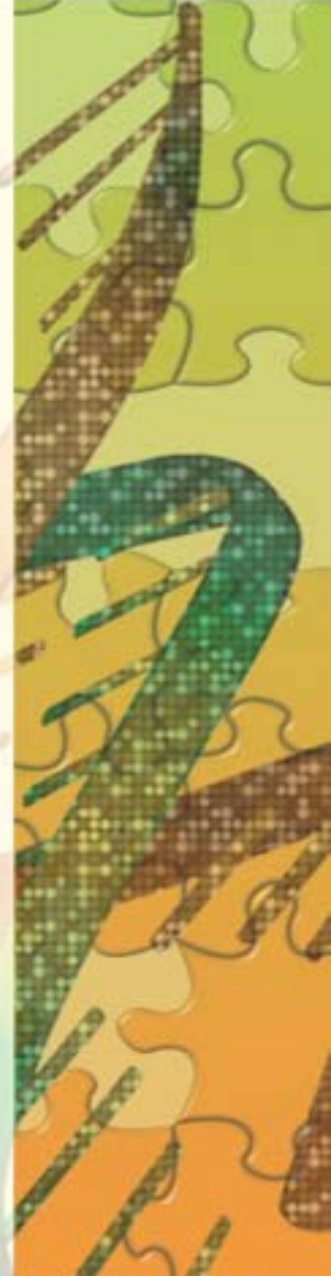
Ac915 kezelés:

Tumor  
izolálás

Egészséges  
szövet izolálás

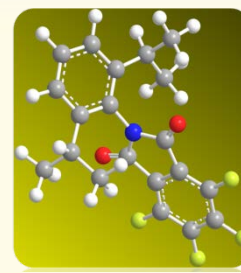


Egészséges  
szövet izolálás



# Funkcionális elemzés

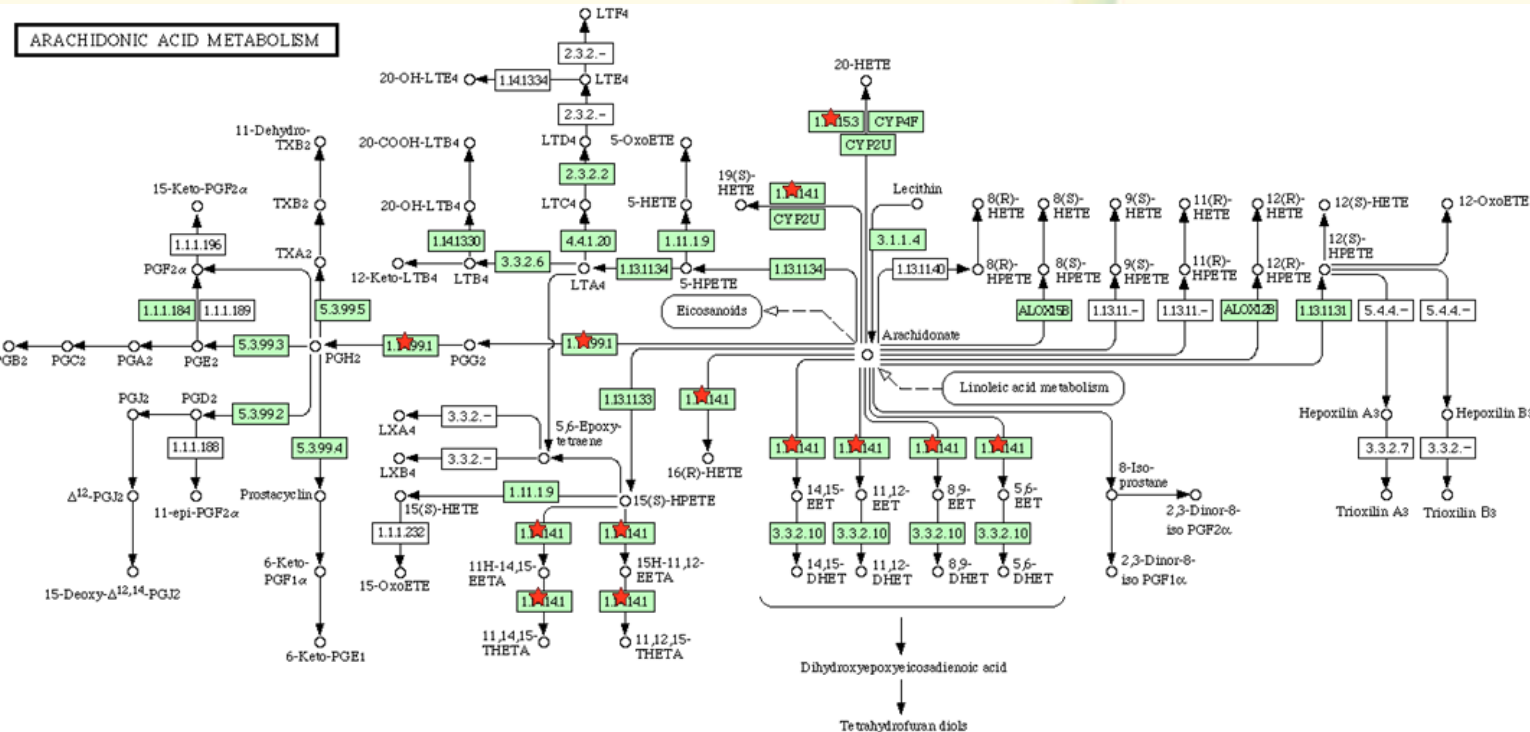
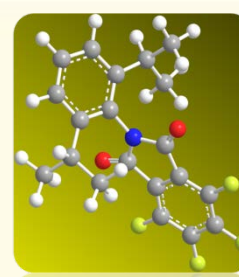
*Ac-915 tumor / induced tumor*



- 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

# Overexpresszált gének.

Ac-915 tumor / induced tumor



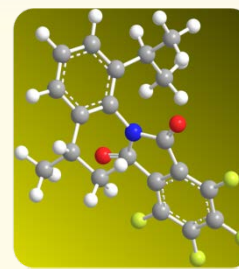
Cyp2c54  
Cyp2j9  
Cyp2e1  
Cyp2c55  
Cyp2c37  
Ptgs1  
Cyp4a12b

[cytochrome p450](#)  
[cytochrome p450, family 2, subfamily j, polypeptide 9](#)  
[cytochrome p450, family 2, subfamily e, polypeptide 1](#)  
[cytochrome p450, family 2, subfamily c, polypeptide 55](#)  
[cytochrome p450, family 2. subfamily c, polypeptide 37](#)  
[prostaglandin-endoperoxide synthase 1](#)  
[cdna sequence bc060945](#)



# Represszált gének.

Ac-915 tumor / induced tumor



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

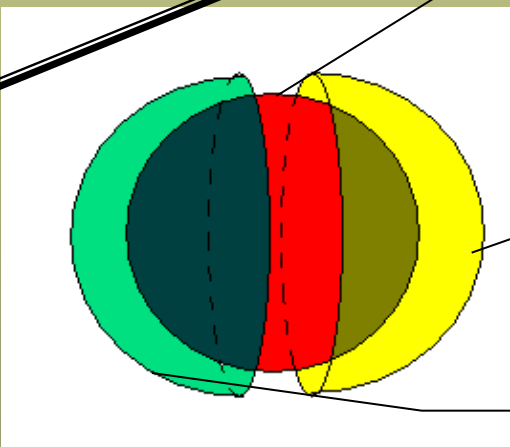
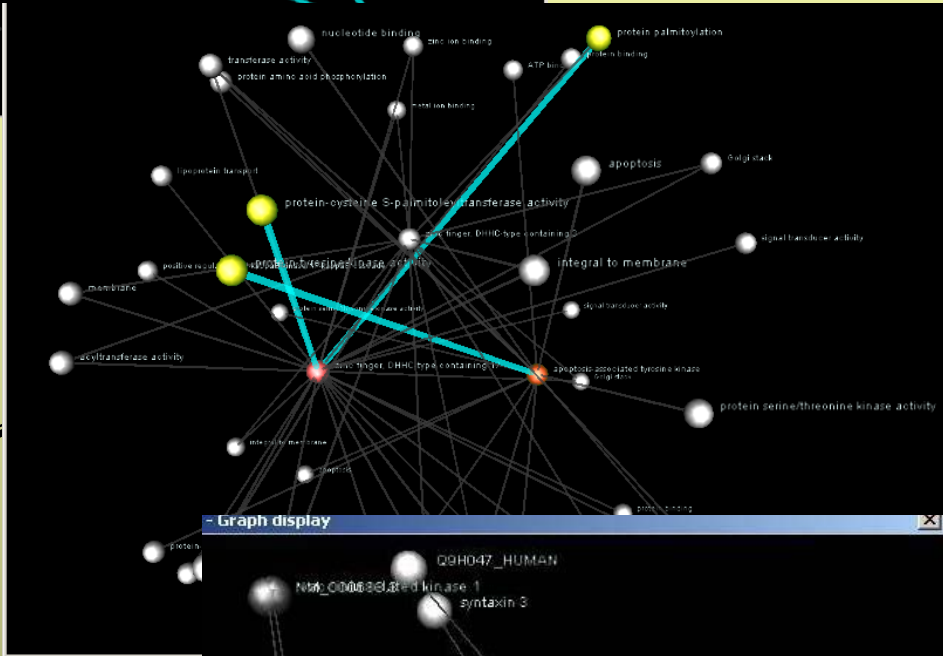
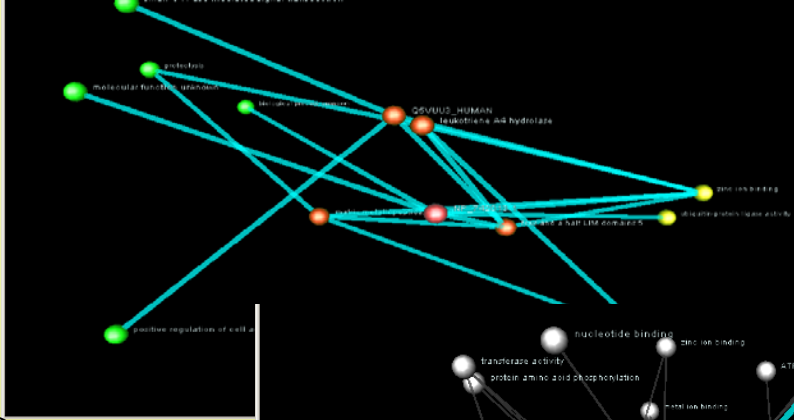
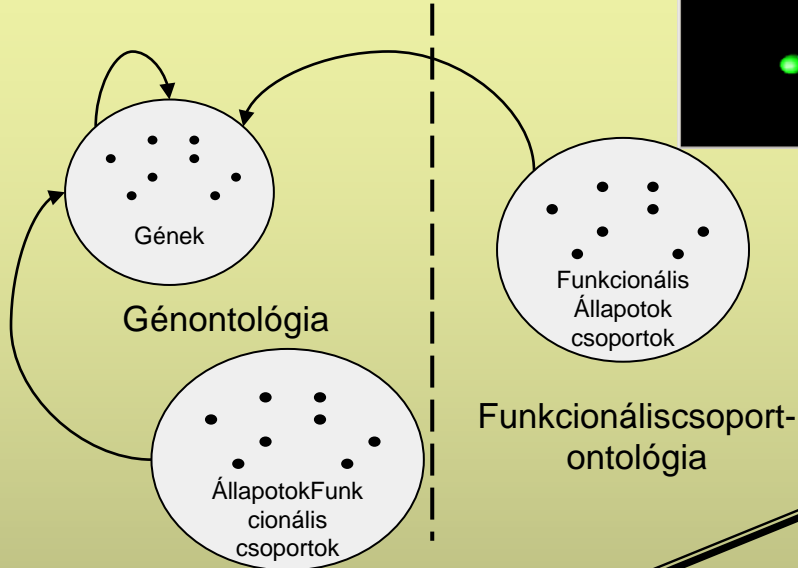
Detailed view

Query

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

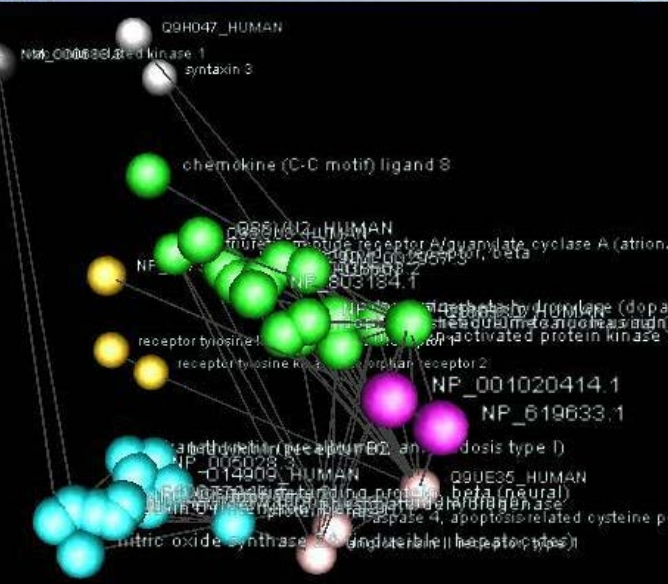


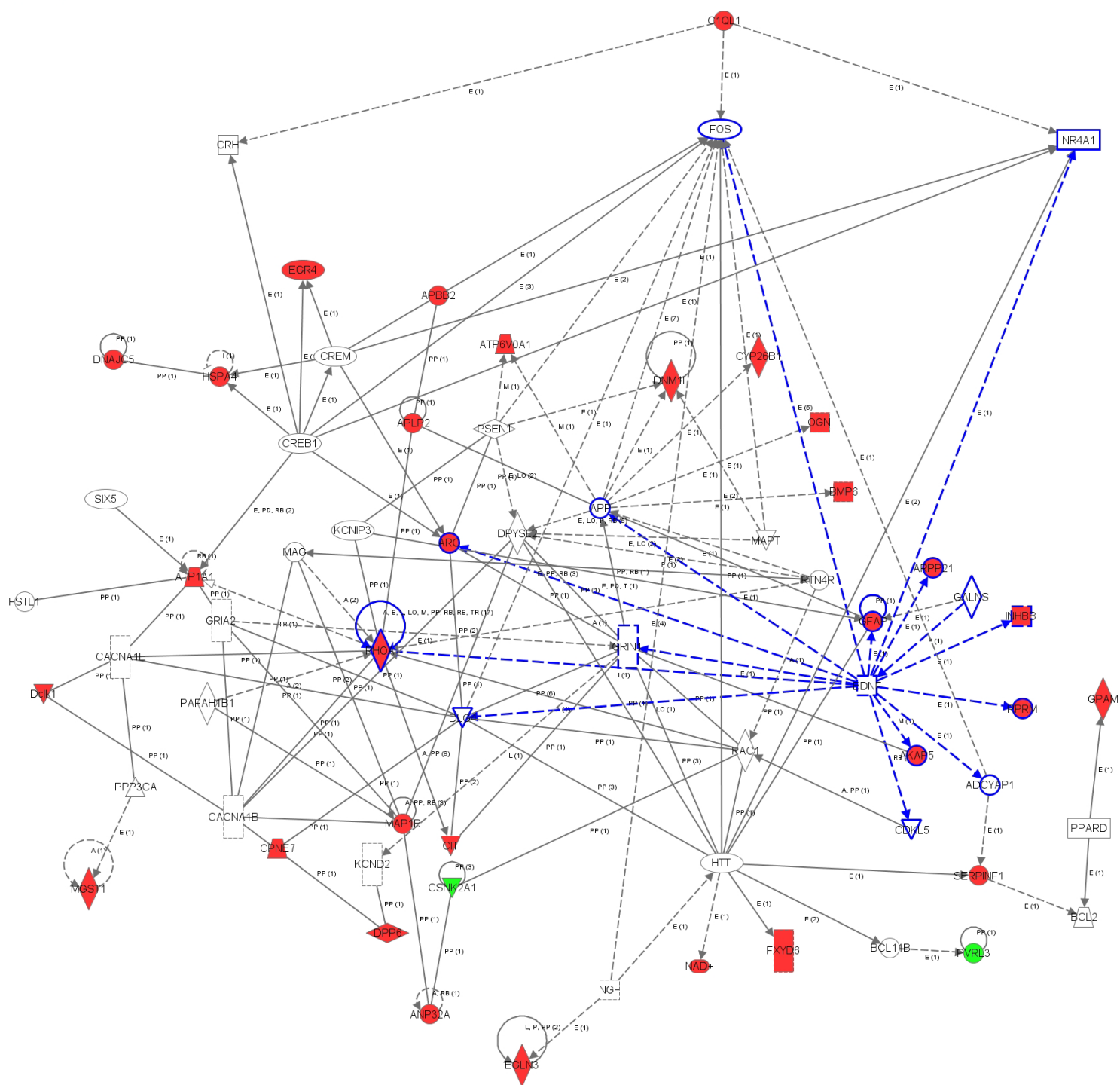
# Adatbáziskutatás, adatbányászás



Ontológiák

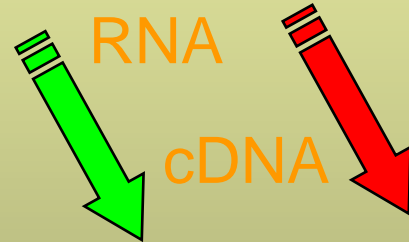
Állapotok



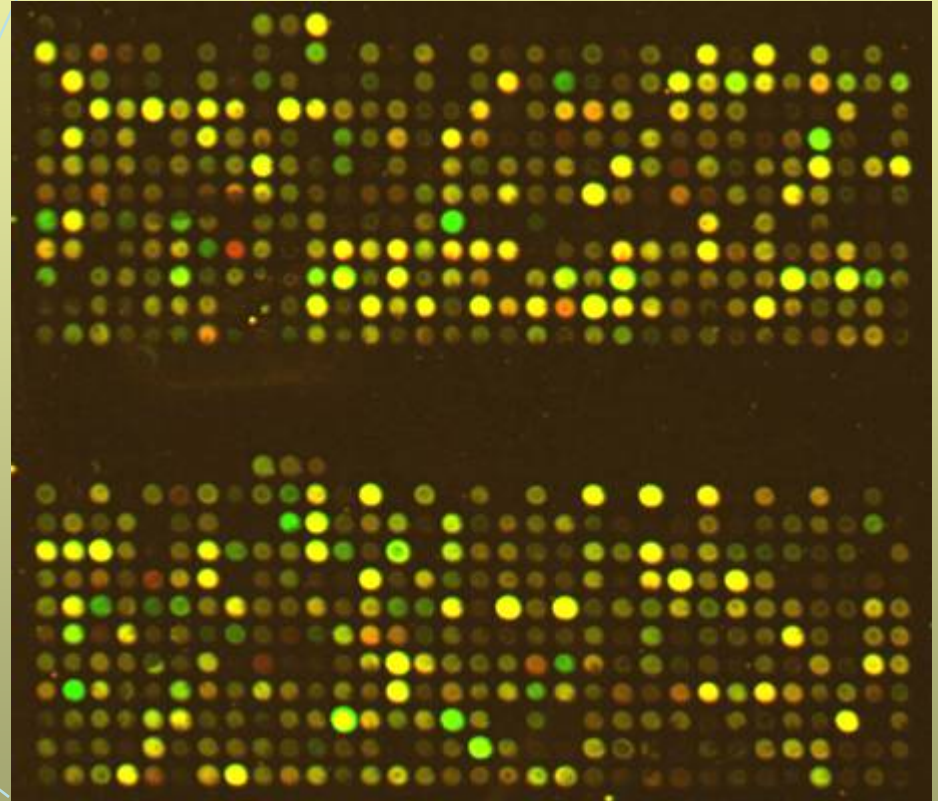


# Application of DNA-microarrays in cancer genomics: Global gene expression analysis

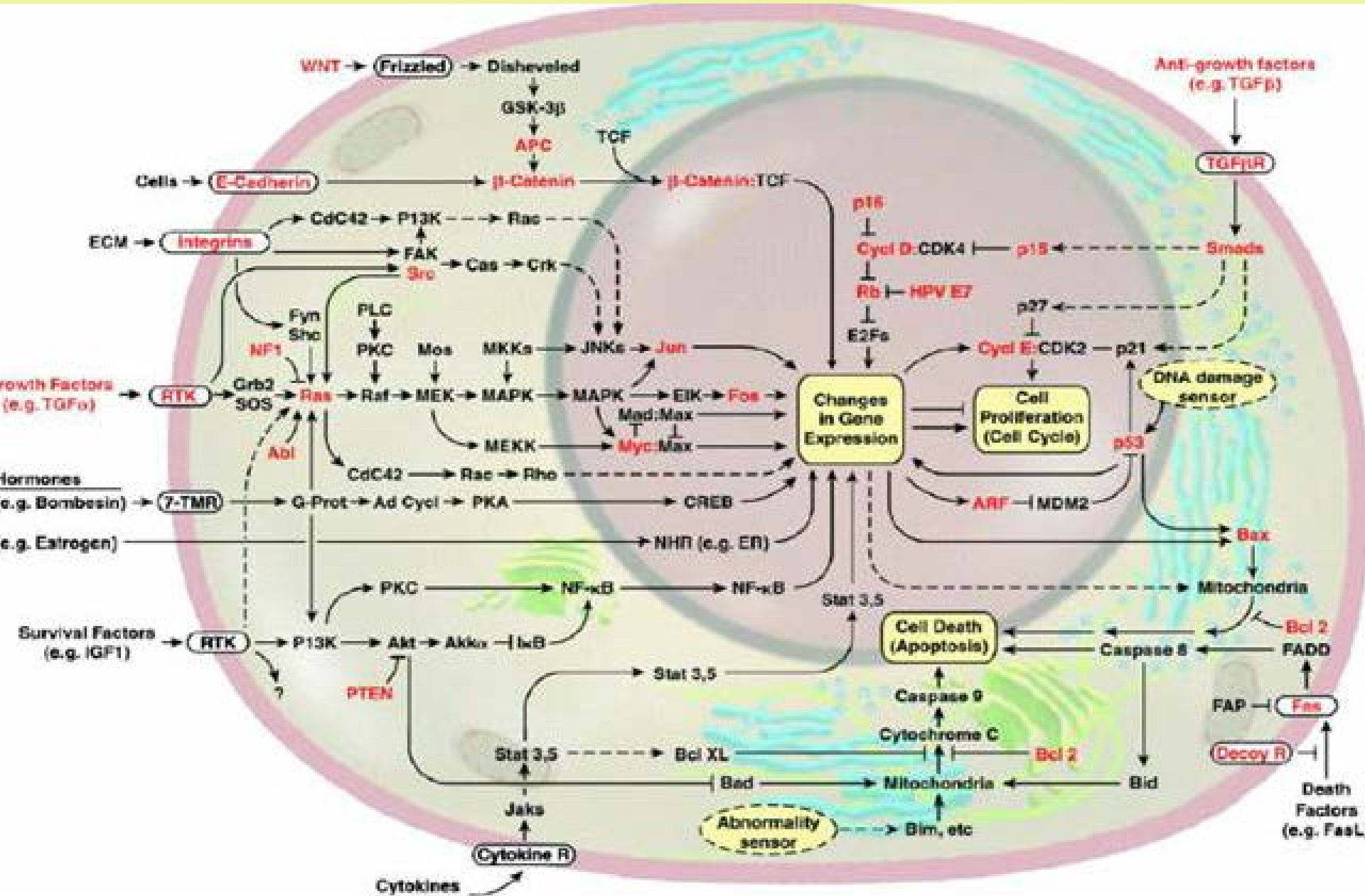
Tumor sample      Normal sample



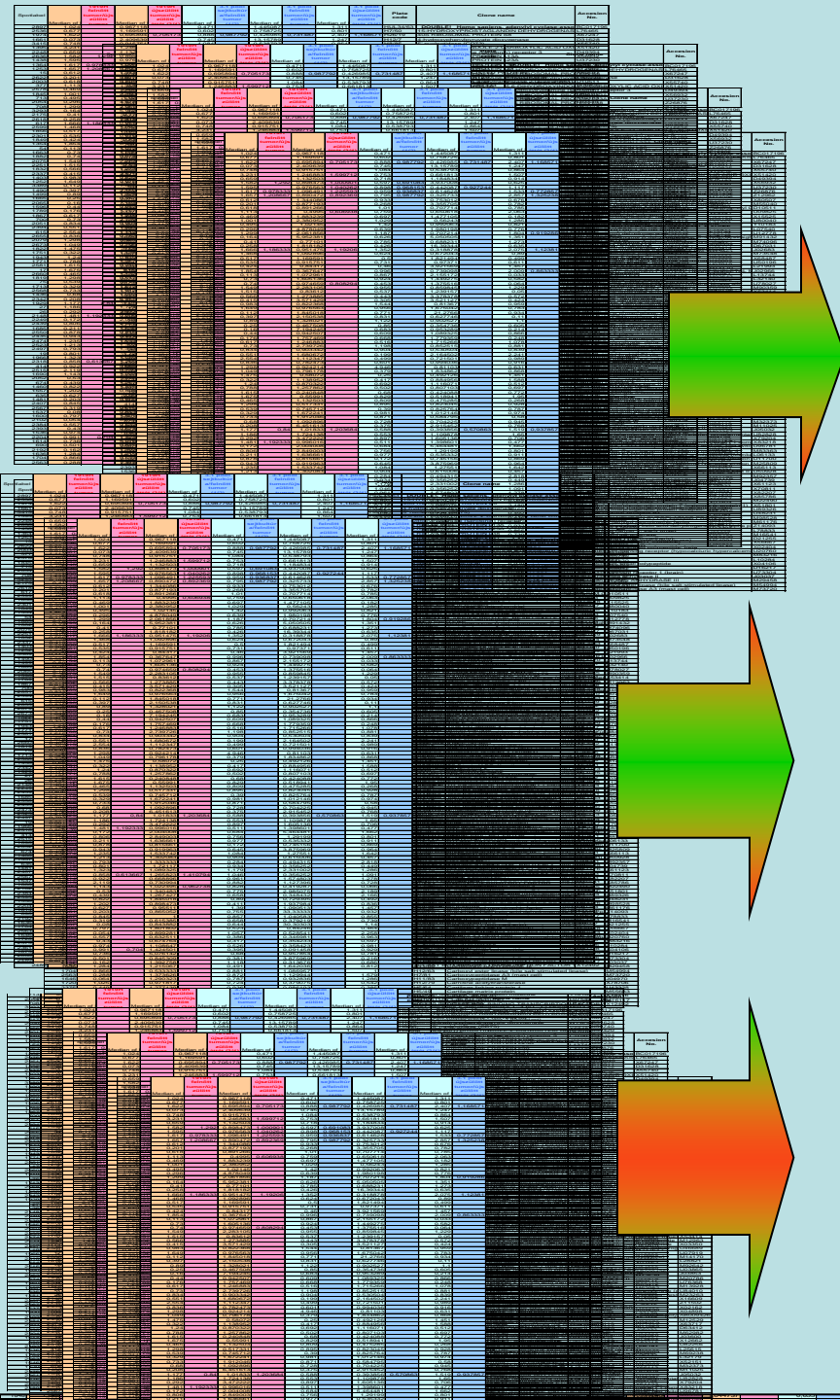
DNA-microarray containing  
40.000 different  
human gene probes



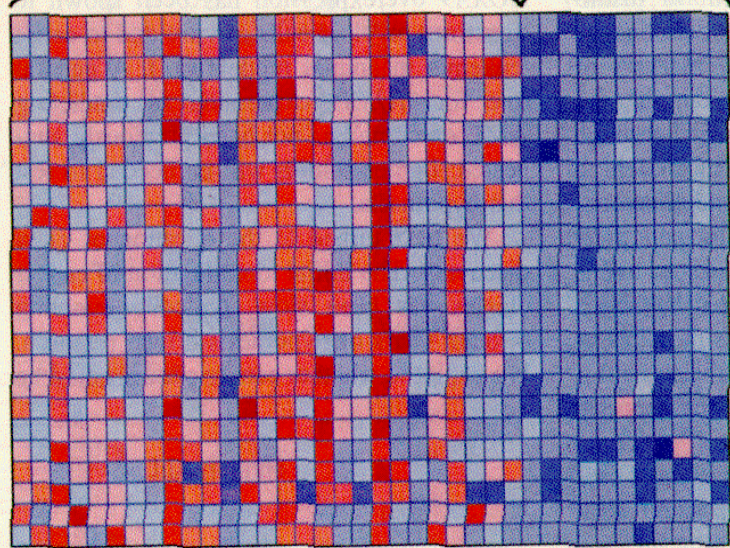
# Complex regulatory networks, disregulation of signalling pathways result in gene expression differences (8000!)



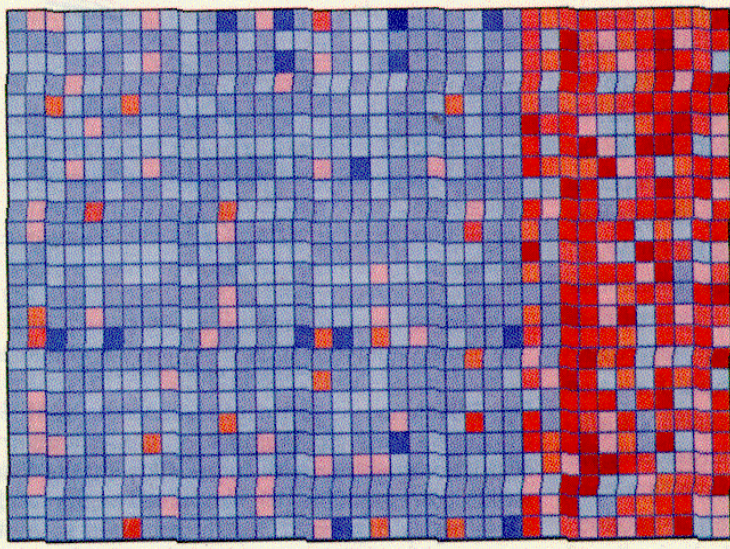




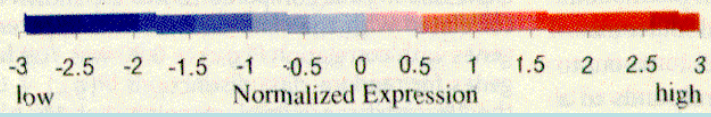
**B** ALL AML



- C-myb (U22376)
- Proteasome iota (X59417)
- MB-1 (U05259)
- Cyclin D3 (M92287)
- Myosin light chain (M31211)
- RbAp48 (X74262)
- SNF2 (D26156)
- HkrT-1 (S50223)
- E2A (M31523)
- Inducible protein (L47738)
- Dynein light chain (U32944)
- Topoisomerase II  $\beta$  (Z15115)
- IRF2 (X15949)
- TFHIE $\beta$  (X63469)
- Acyl-Coenzyme A dehydrogenase (M91001)
- SNF2 (U29175)
- (Ca<sup>2+</sup>)-ATPase (Z69881)
- SRP9 (U20998)
- MCM3 (D38073)
- Deoxyhypusine synthase (U26266)
- Op 18 (M31303)
- Rabaptin-5 (Y08612)
- Heterochromatin protein p25 (U35451)
- IL-7 receptor (M29696)
- Adenosine deaminase (M13792)

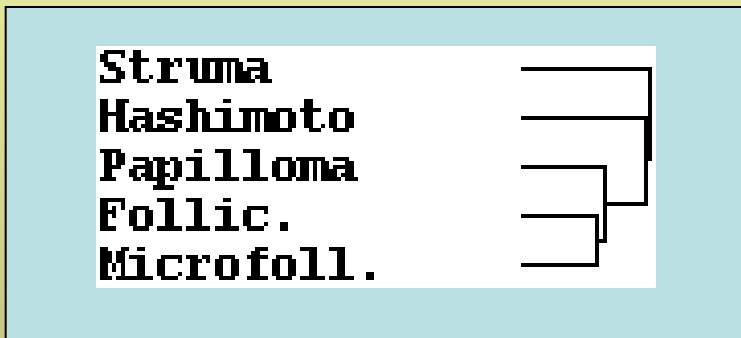


- Fumarylacetoacetate (M55150)
- Zyxin (X95735)
- LTC4 synthase (U50136)
- LYN (M16038)
- HoxA9 (U82759)
- CD33 (M23197)
- Adipsin (M84526)
- Leptin receptor (Y12670)
- Cystatin C (M27891)
- Proteoglycan 1 (X17042)
- IL-8 precursor (Y00787)
- Azurocidin (M96326)
- p62 (U46751)
- CyP3 (M80254)
- MCL1 (L08246)
- ATPase (M62762)
- IL-8 (M28130)
- Cathepsin D (M63138)
- Lectin (M57710)
- MAD-3 (M69043)
- CD11c (M81695)
- Ebp72 (X85116)
- Lysozyme (M19045)
- Properdin (M83652)
- Catalase (X04085)





# Különböző pajzsmirigy eredetű betegségek hierarhikus klaszteranalítise

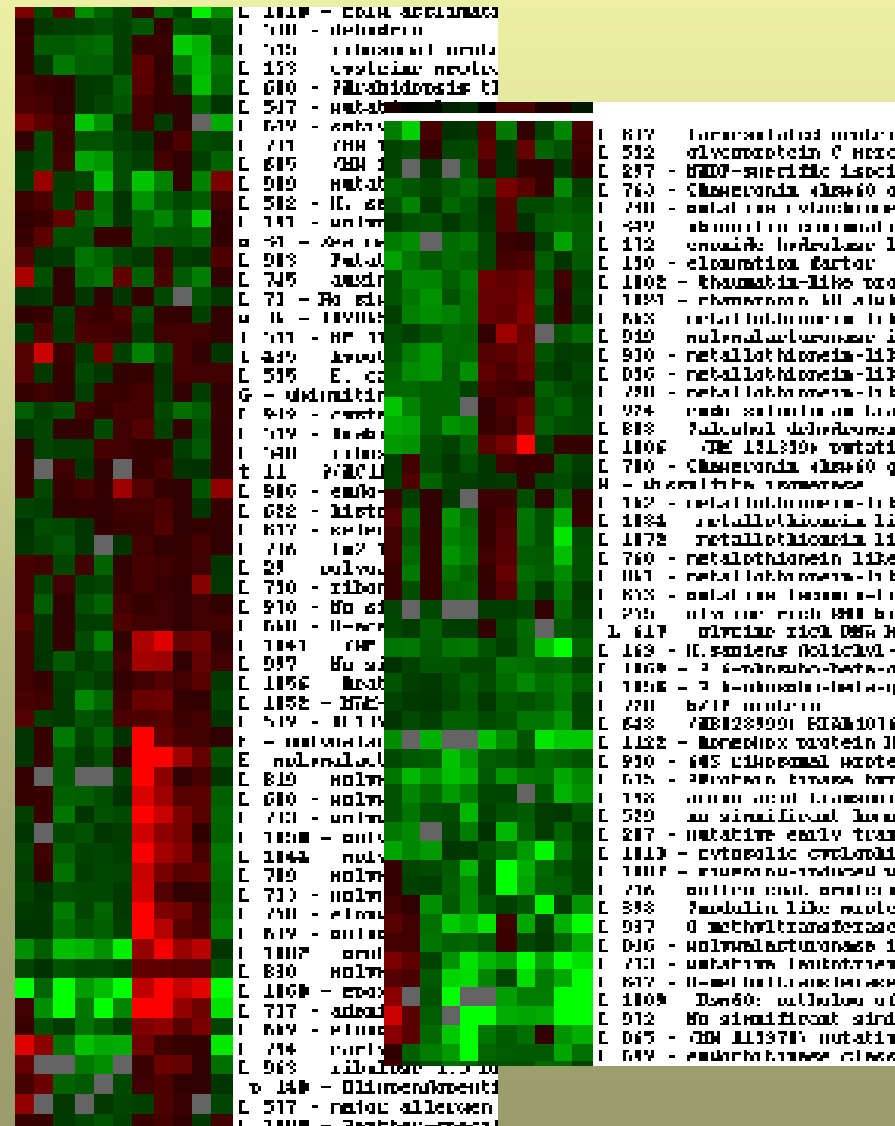


Hierarchical cluster analysis with Omniviz

Betegség-specifikus klaszterek meghatározása

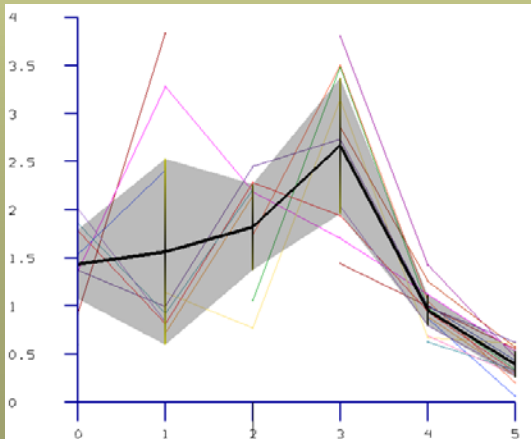
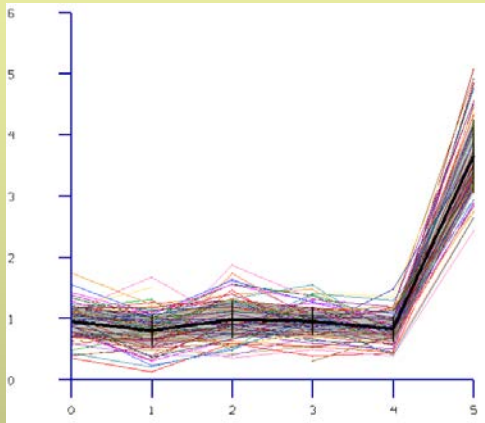
Legközelebbi kapcsolat: microfollicular & follicular carc., papilloma ehhez az alcsoporthoz hasonlít.

Struma and Hashimoto különböznek egymástól és más betegség csoportoktól.

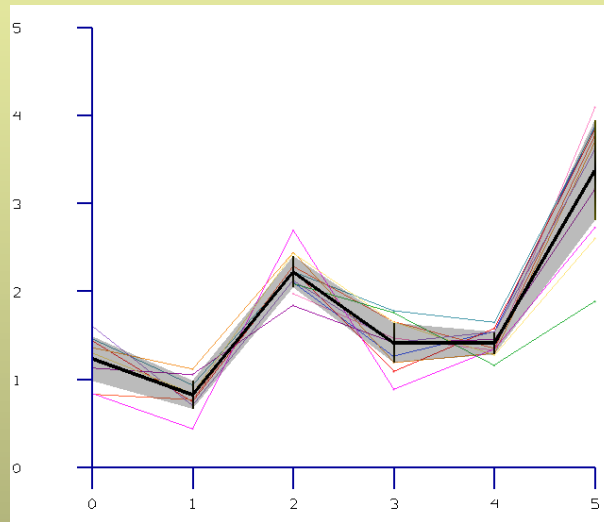


# Betegségspecifikus génmarkerek azonosítása

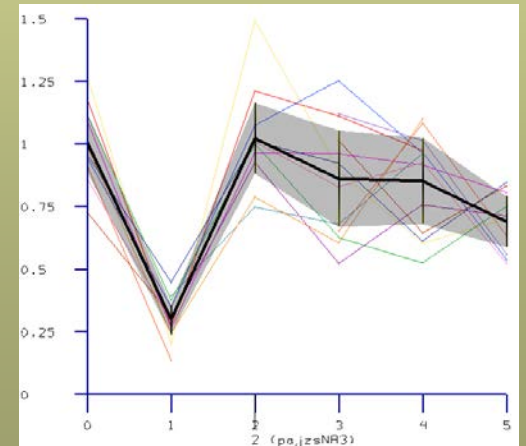
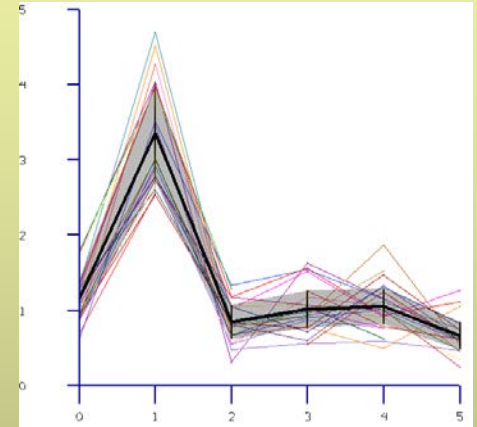
## Struma



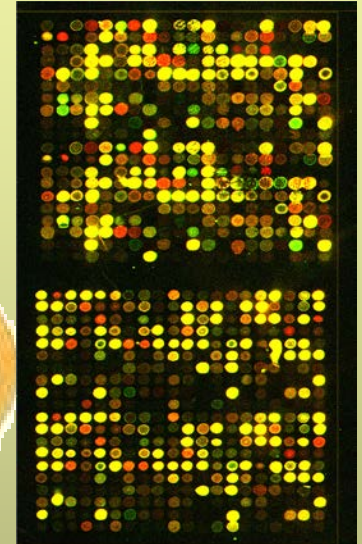
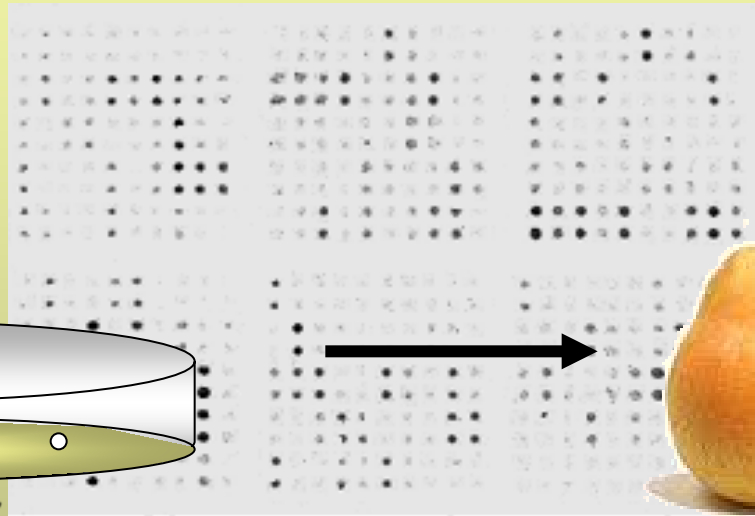
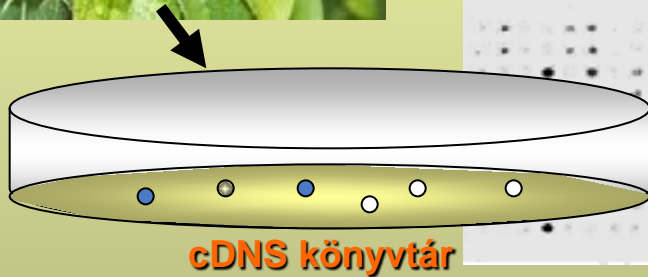
## Microfollicular + Struma



## Hashimoto



# Körte érésével kapcsolatos gének azonosítása



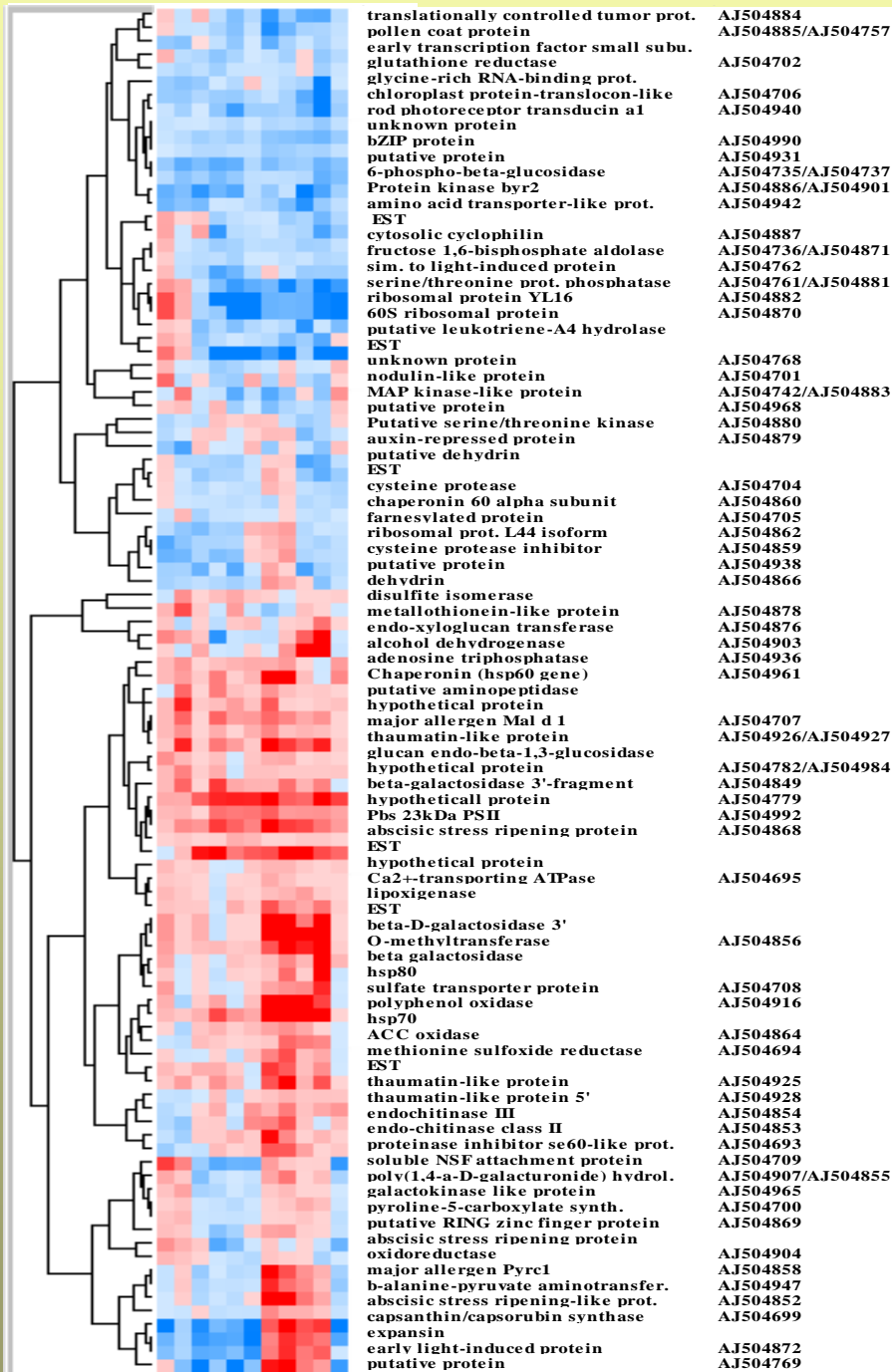
↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓  
**A C 3 6 9 12 15 18 21 24 FR**

A: növekvő kis termés

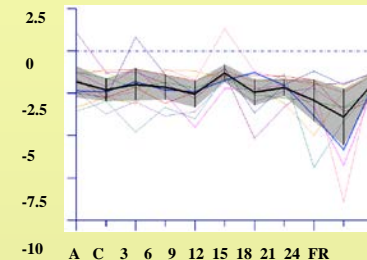
FR: összeaszott, éretlen termés

C: nem növekvő, éretlen  
termés

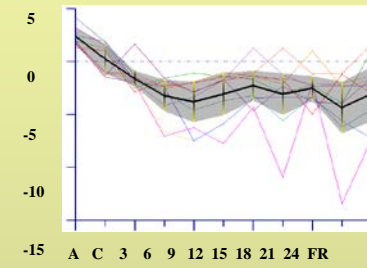




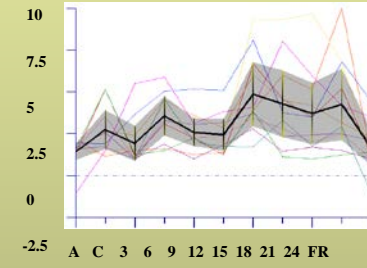
Nem érés specifikus



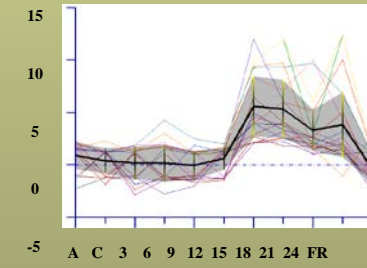
Gyümölcs növekedésre jellemző gének



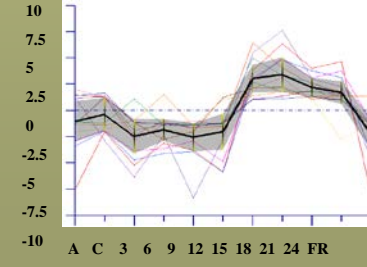
Gyümölcs növekedésre és érésre jellemző gének



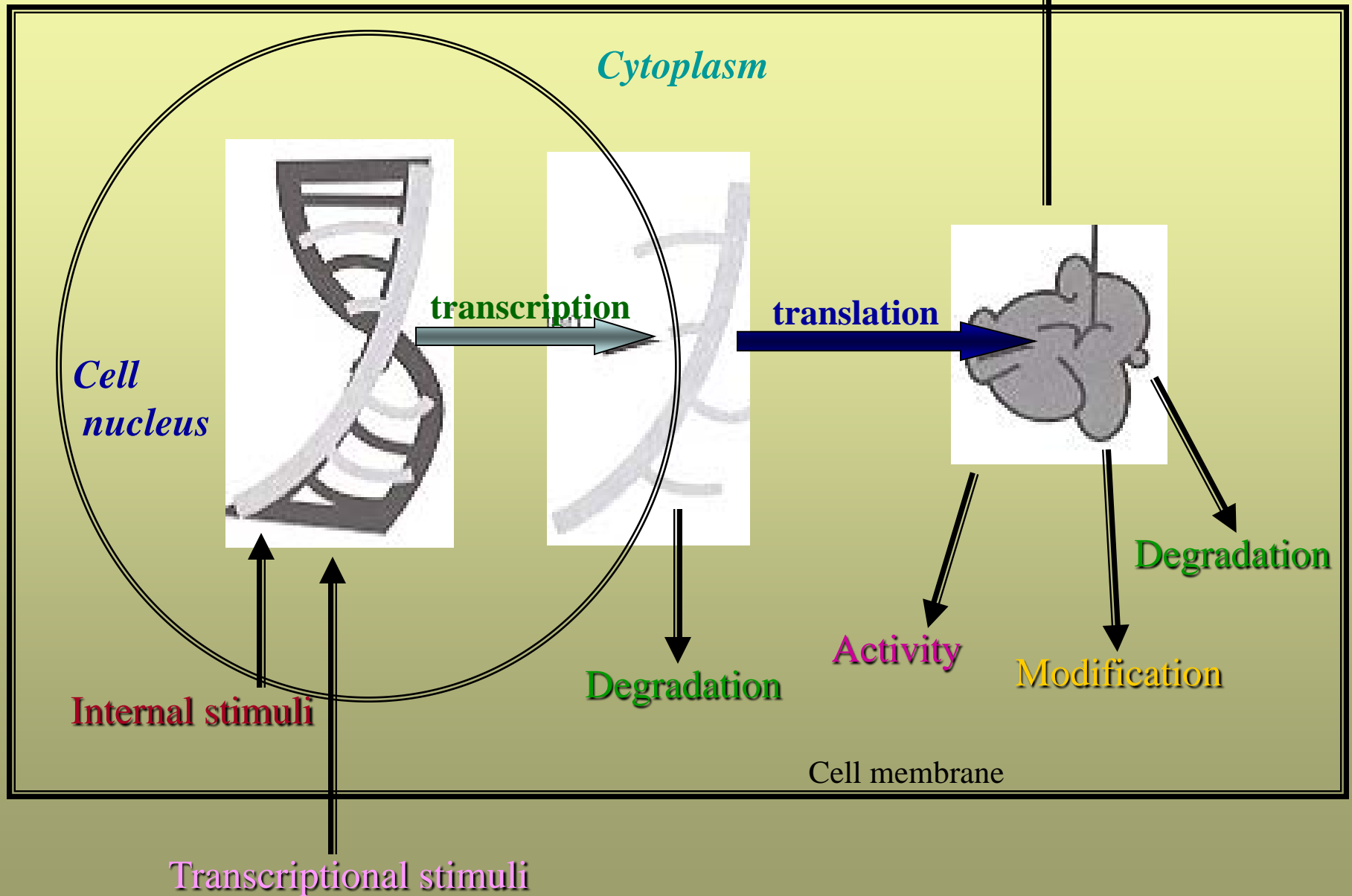
Gyümölcs érésre jellemző gének



Gyümölcs érésre jellemző gének

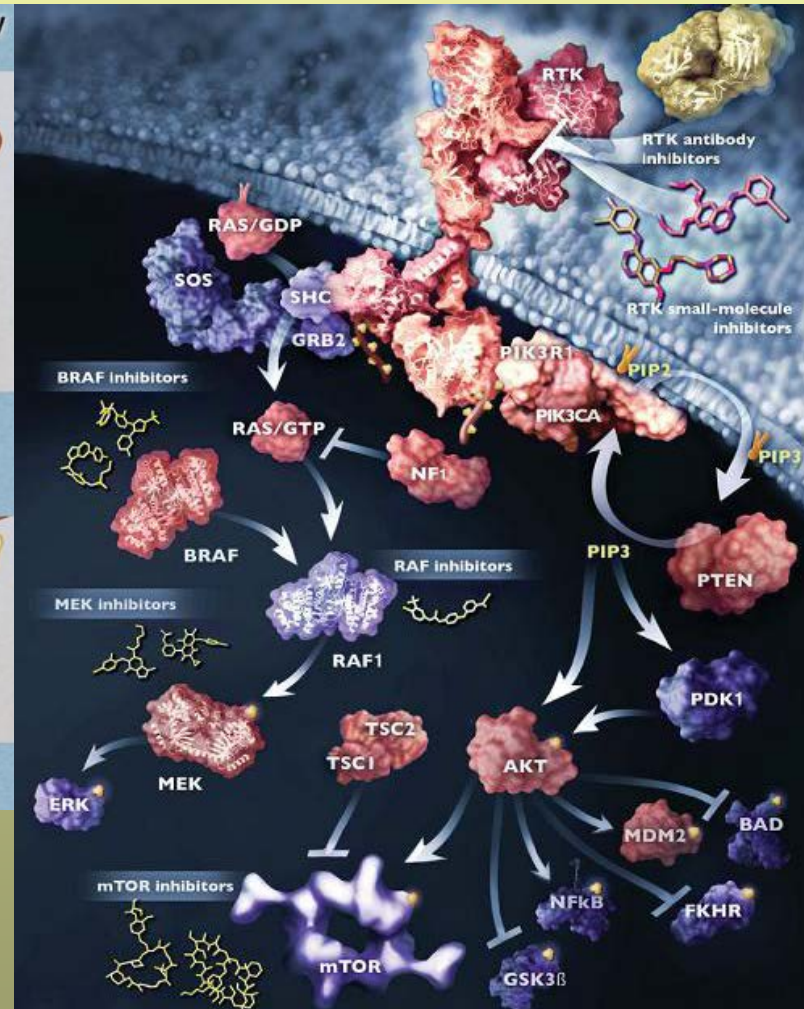
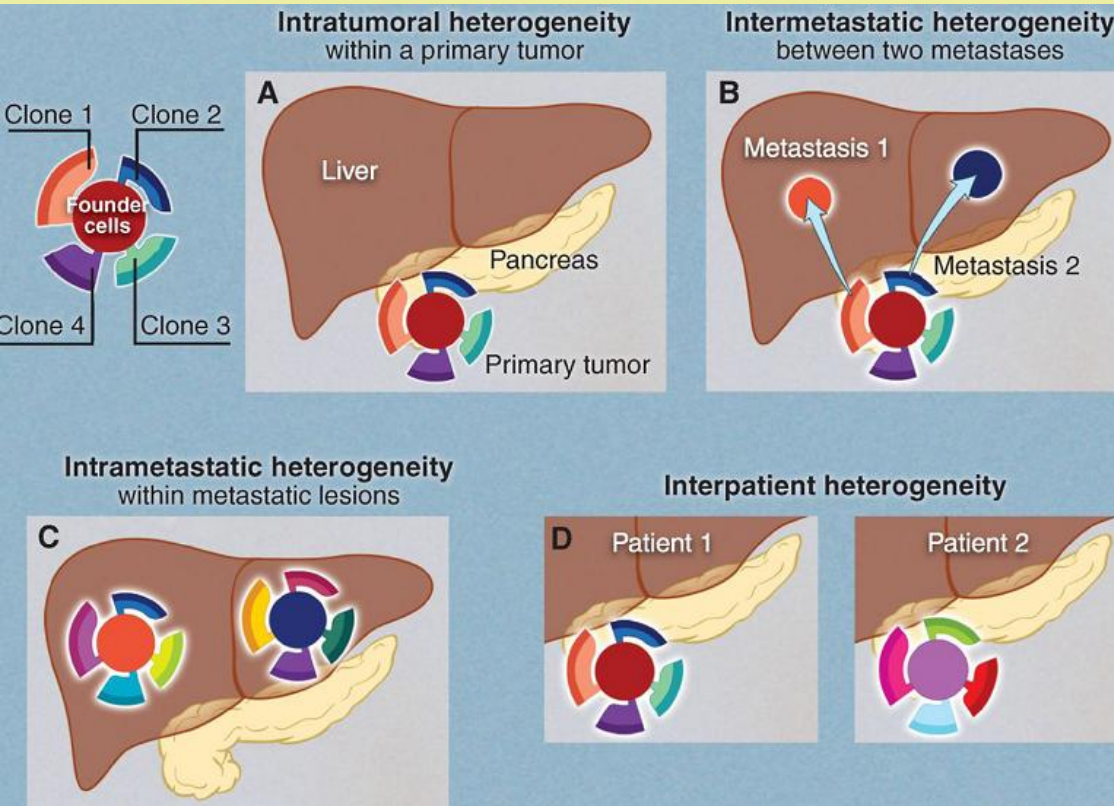


# Flow of genetic information



# **Minták heterogenitása, heterogén minták genomikai elemzése**

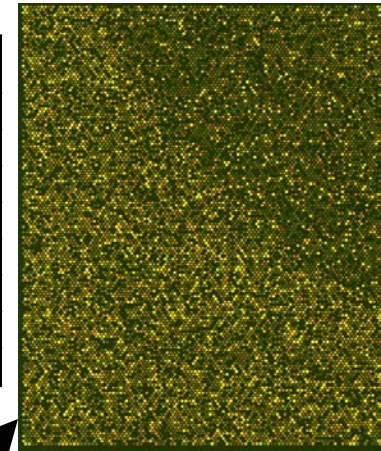
# A rák heterogenitása és egyedisége





# 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

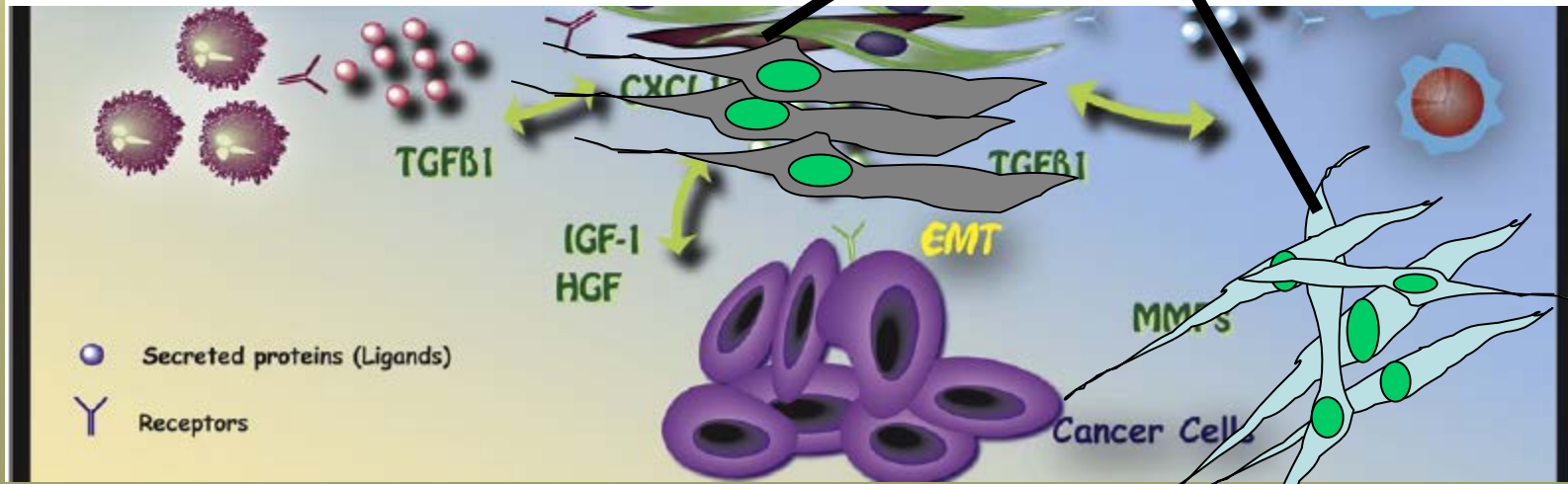


DNA-microarray

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)

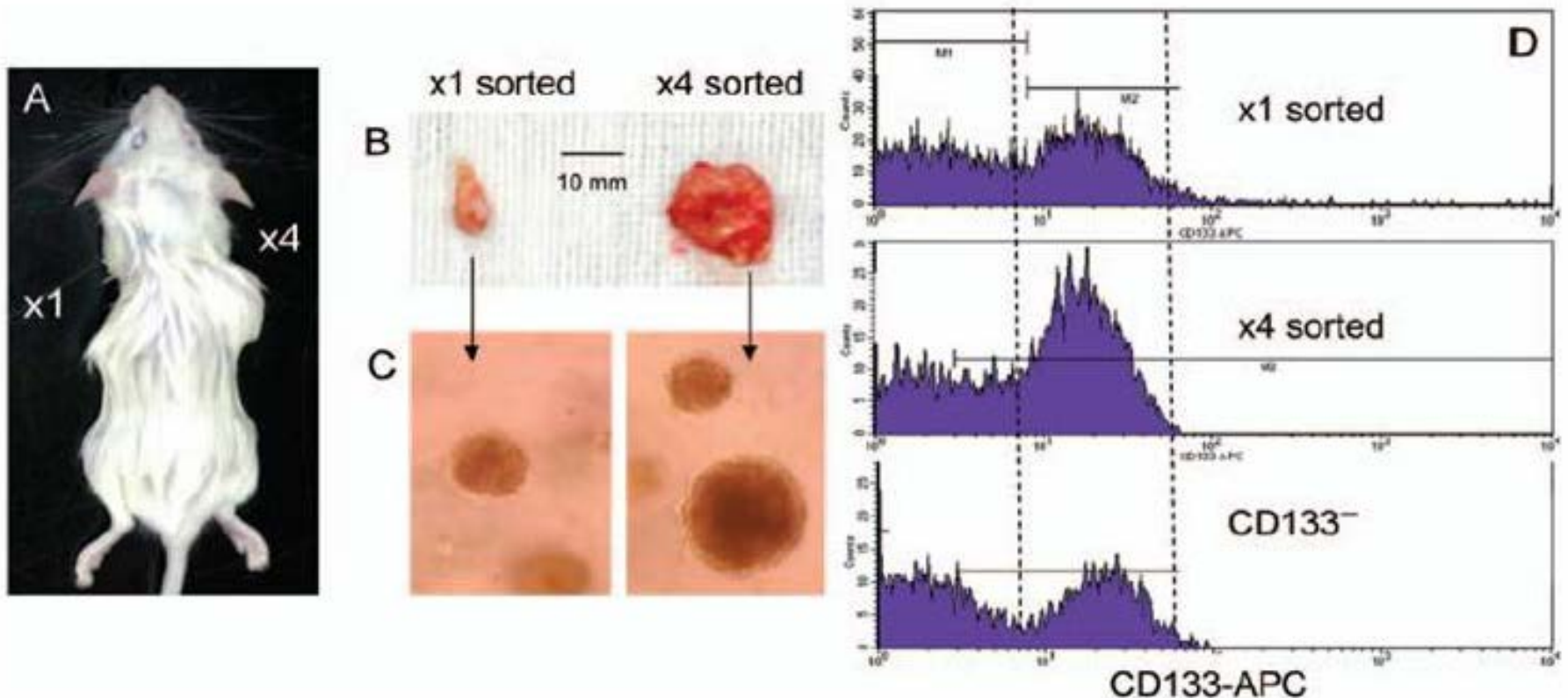
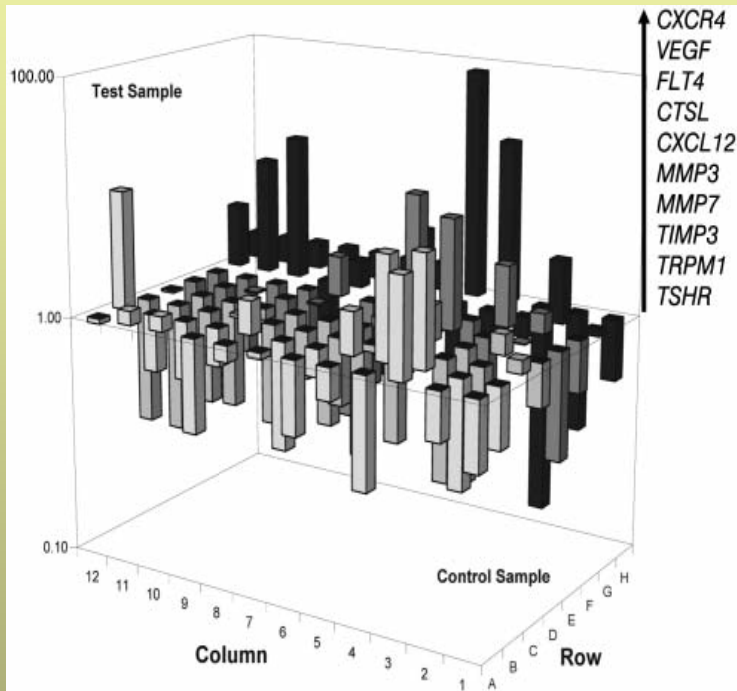


Figure 3. Increased in vivo tumorigenicity of repeatedly MACS-CD133<sup>+</sup>-sorted HCT116 cells. To minimize possible host differences, the same number ( $1.5 \times 10^3$ ) of one- and four-times sorted cells was injected into opposite flanks of NOD/SCID mice. Six out of six mice uniformly developed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

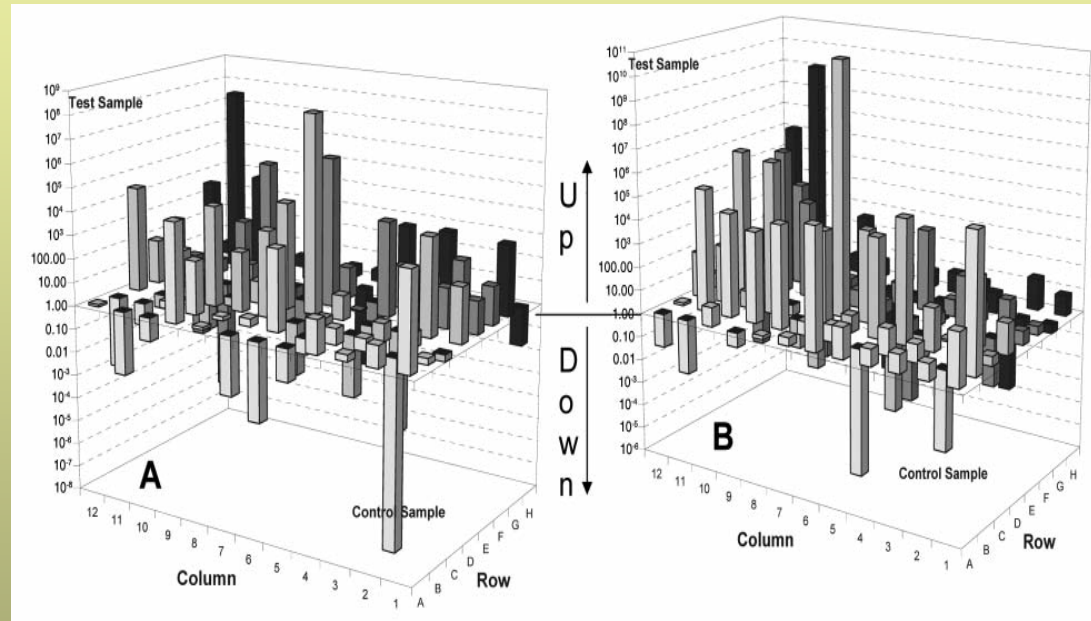
# Tumorössejtek géneexpressziós vizsgálata (QRT-PCR array)



Metasztázisban szereplő gének

**CD133+**

**CD133-**



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

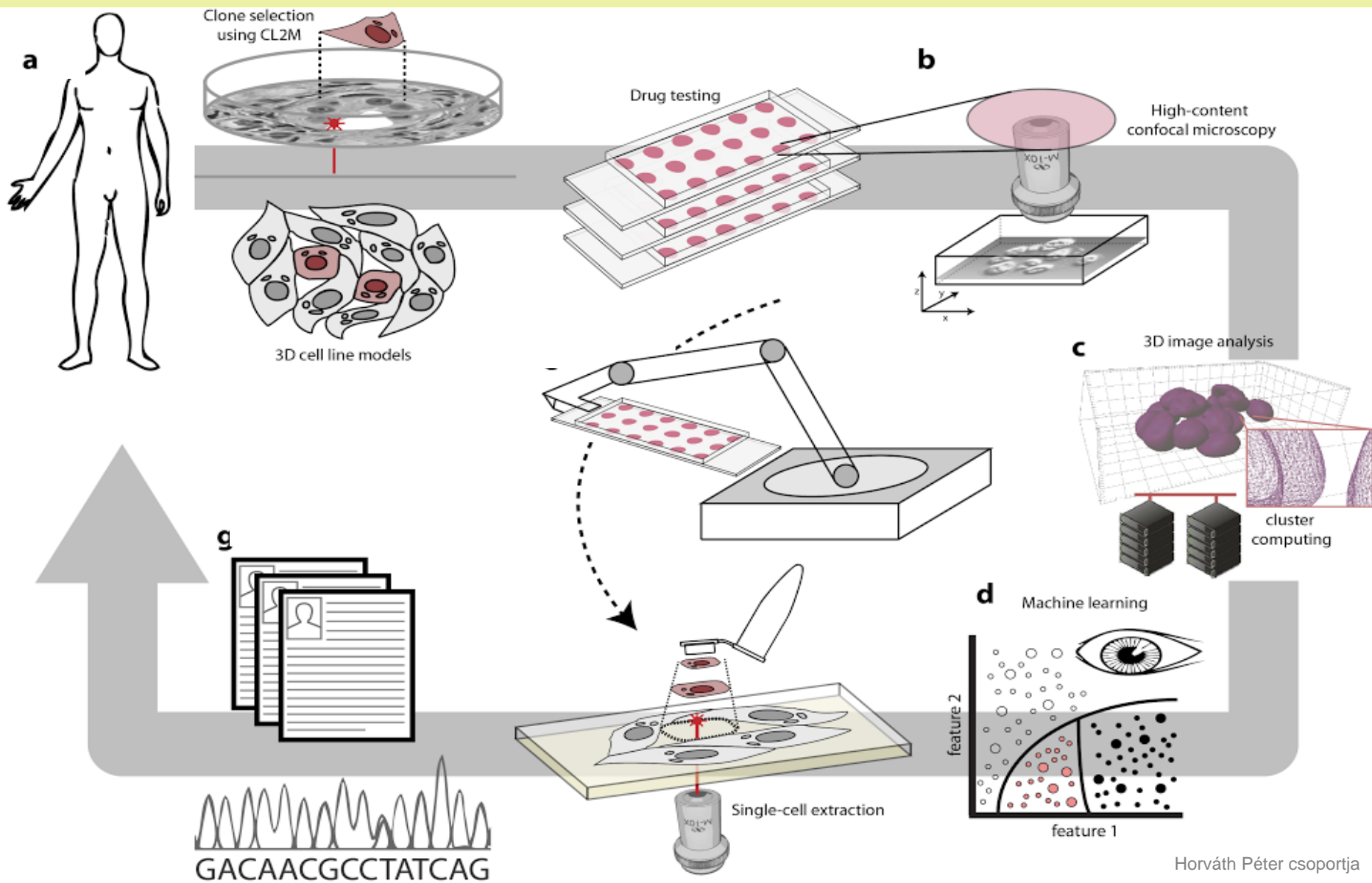


# Az agy sejtjeinek heterogenitása

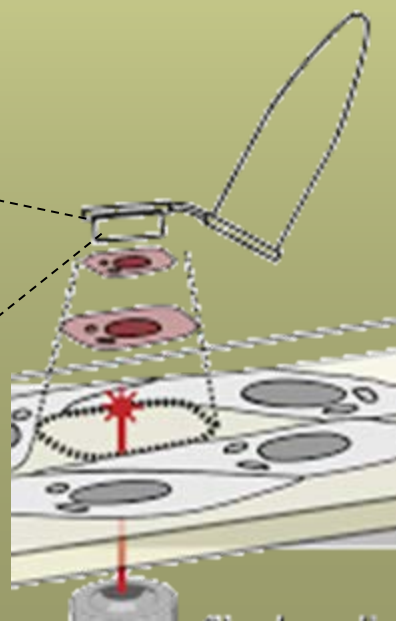
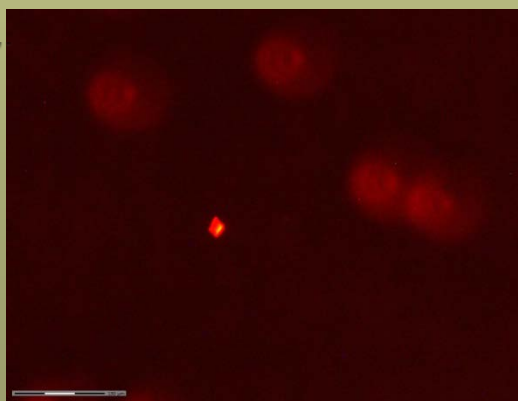
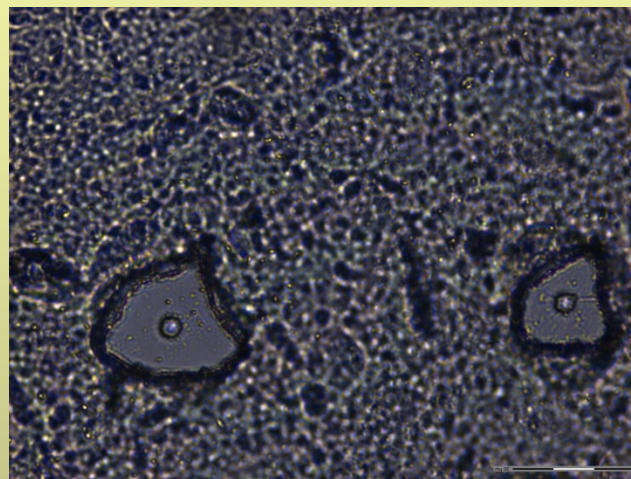
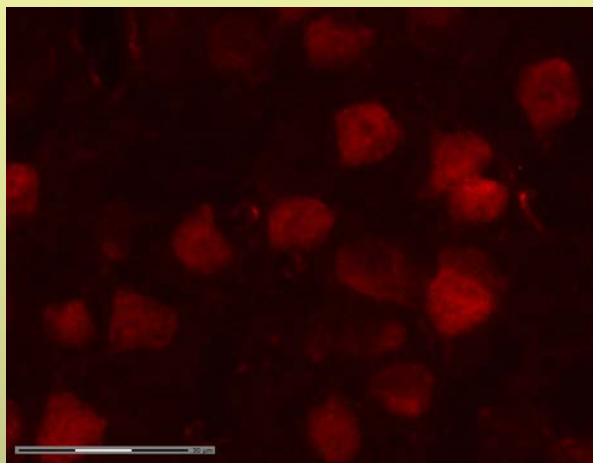
rengeteg különböző sejtípus  
nagyfokú heterogenitás  
megértés, adatfeldolgozás  
értelem, memória  
neuronális hálózatok  
patológias állapotokban  
különböző sejtípusok szerepe



# Sejtek, sejtcsoportok gyűjtése lézerdisszekciós módszerrel



# Különböző neuron típusok gyűjtése fixált, jelölt mintákból

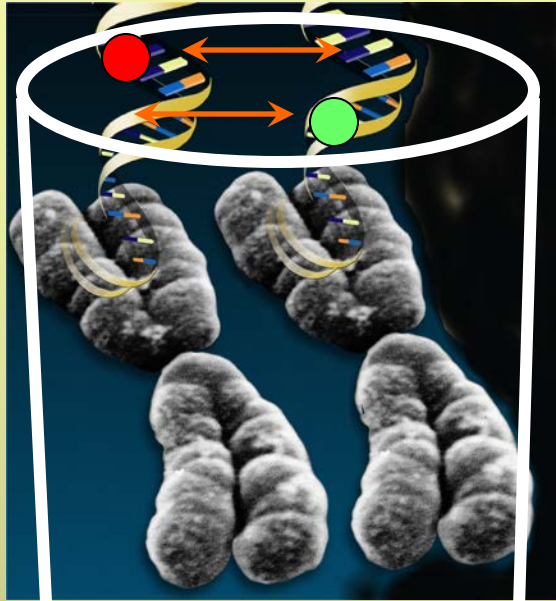


Tamás Gábor  
Nagy István  
Horváth Péter  
kollaboráció



# Genomi átrendeződések és változások vizsgálata csiptechnikával

# Pontmutációk (SNP) detektálása



CCATGG  
Vad típus

GCTACC  
Vad típus

CCCTGG  
Mutáns

GTTACC  
Mutáns

1. SNP

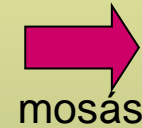
2. SNP

- Oligonukleotid alapú chip
- egy nukleotid eltérés azonosítása

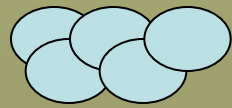
Jelölt DNS



hibridizáció



DNS



1. SNP  
3 pozíció

2. SNP  
2 pozíció

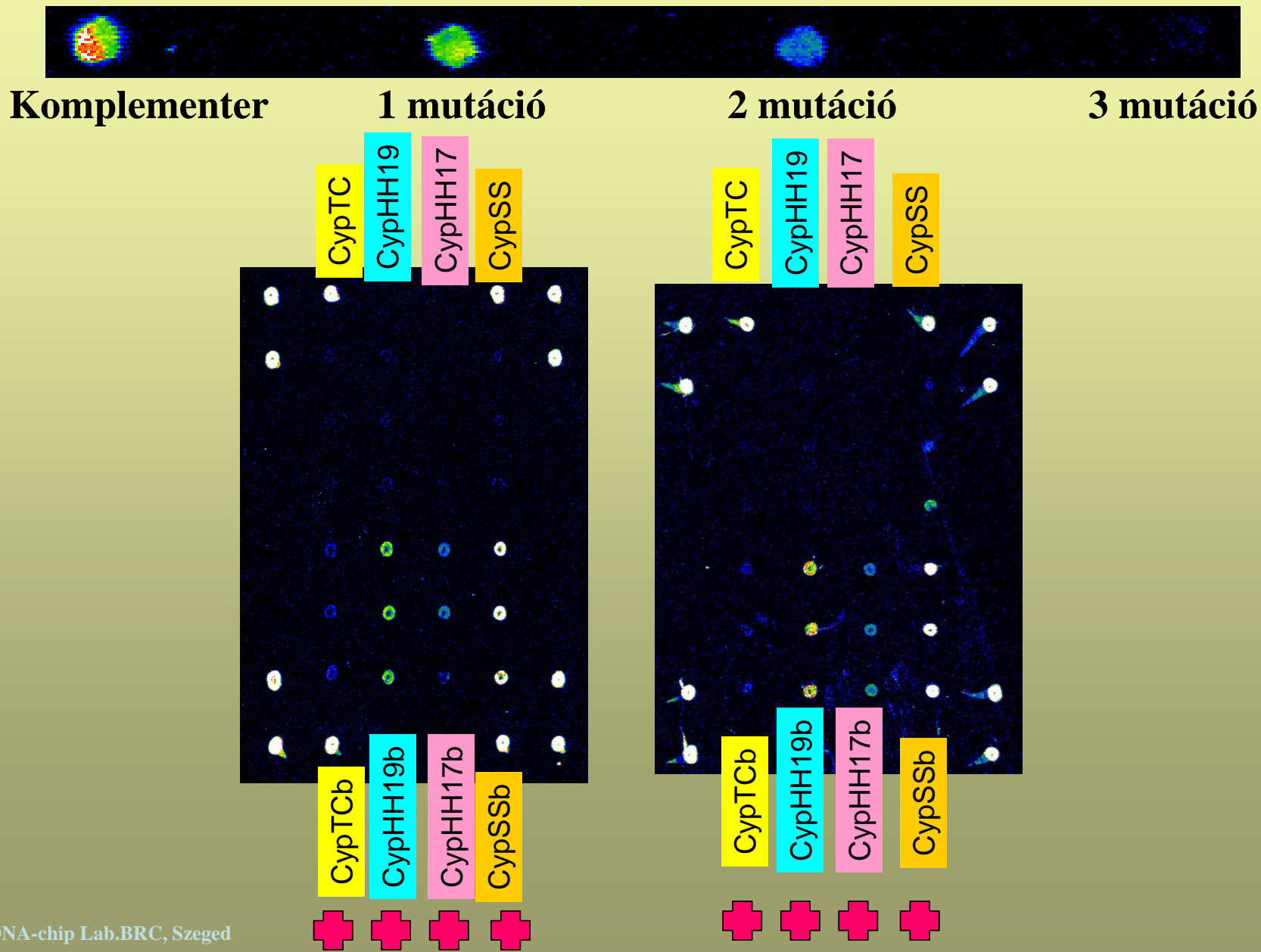
A		
G		
T		
C		
A		
G		
T		
C		

detektálás



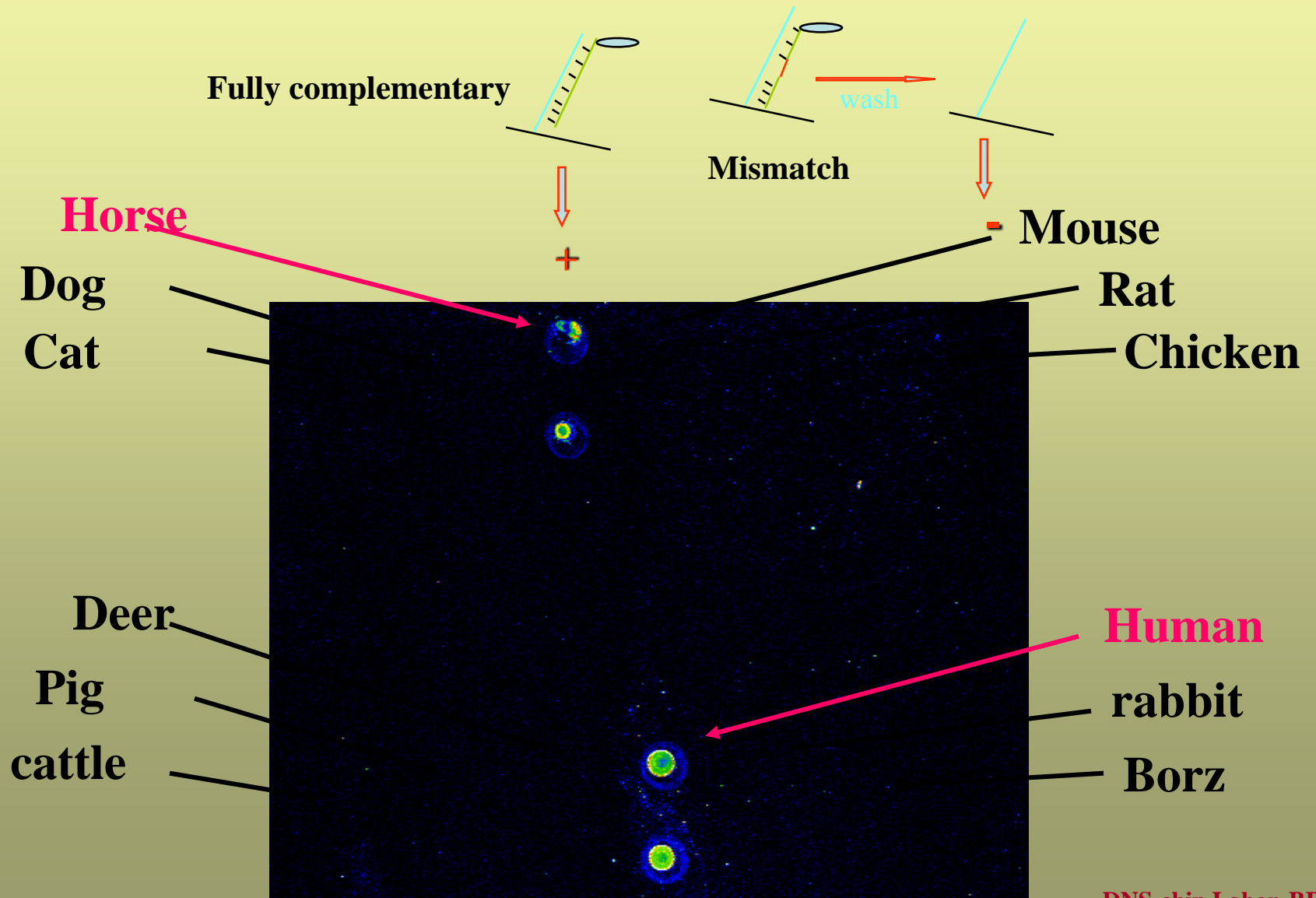
**Adat analízis**  
 1. SNP: 3. pozíció A-C  
 2. SNP: 2. pozíció C-T

# Mutációk detektálásának specificitása





# Fajmeghatározás mitokondriális cytB polimorfizmus alapján



Genomszintű változások,  
kromoszóma rendellenességek,  
amplifikációk, deléciók detektálása

Paraffinba ágyazott minták

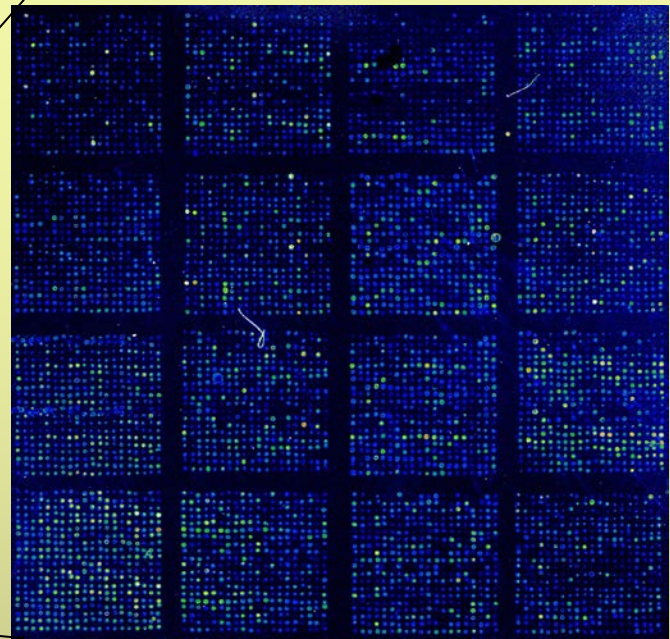
normál

daganat



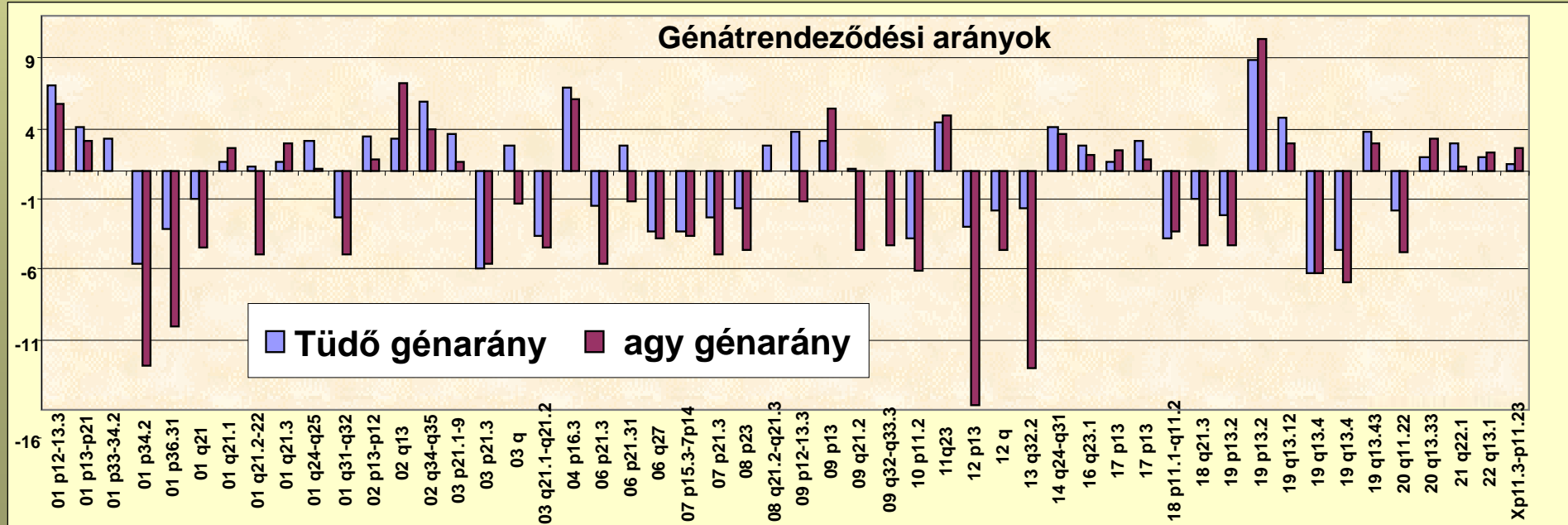
DNS

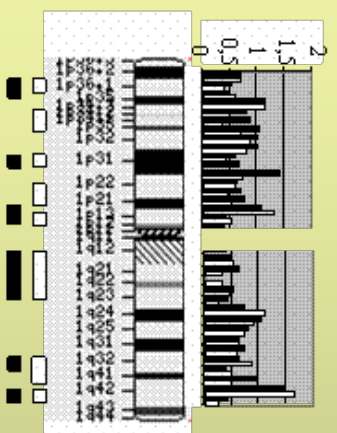
PCR



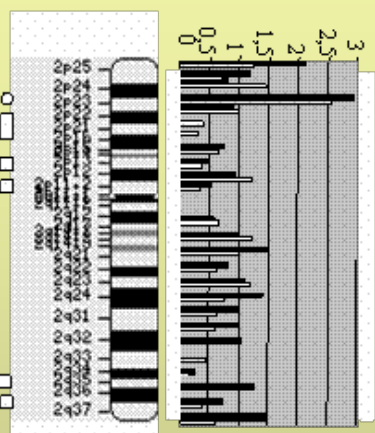
Metasztázisok, tumorok, multiplex tumorok jellemzése, igazolása

Génátrendeződési arányok

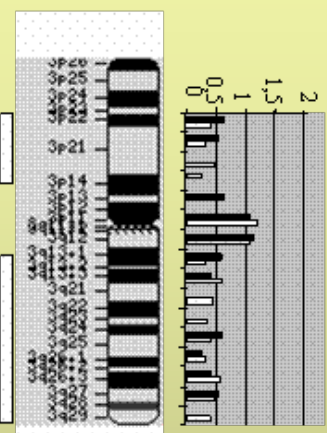




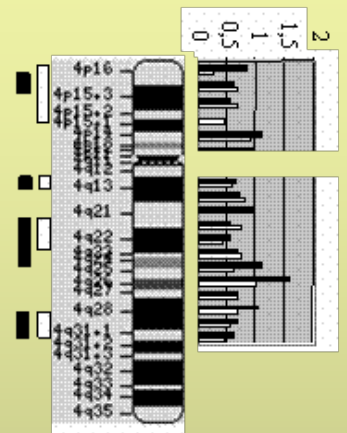
Chr1



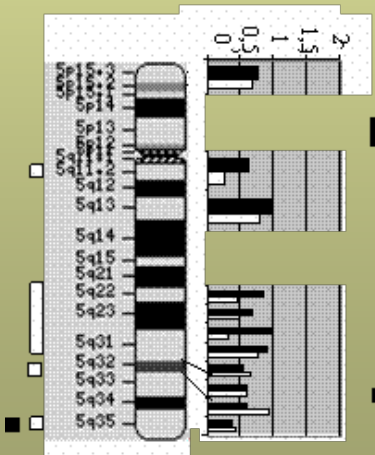
Chr2



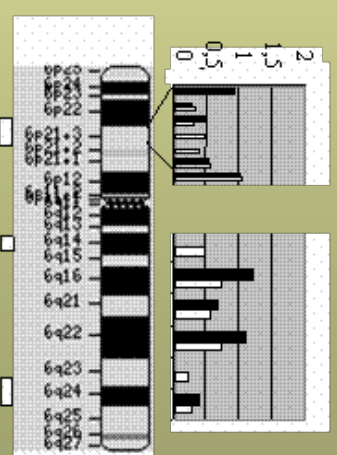
Chr3



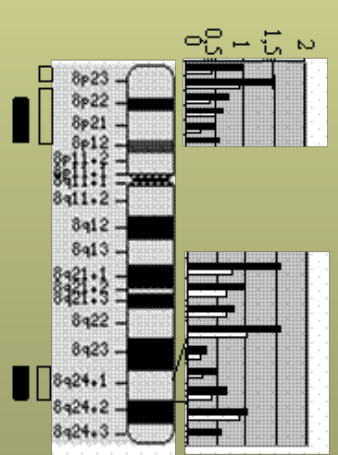
Chr4



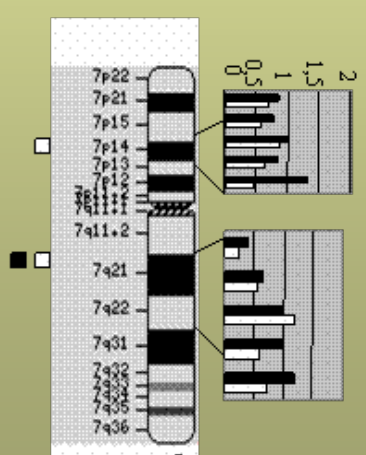
Chr5



Chr6



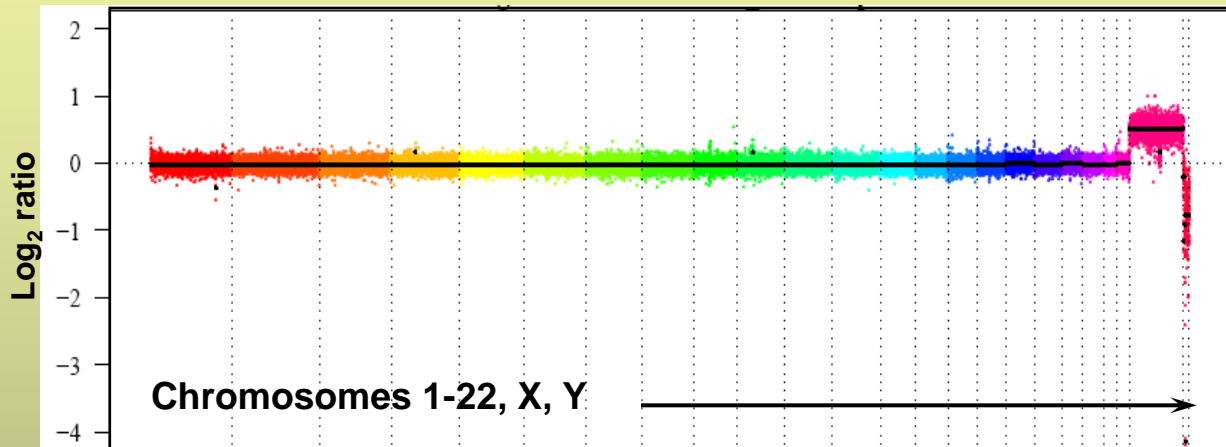
Chr8



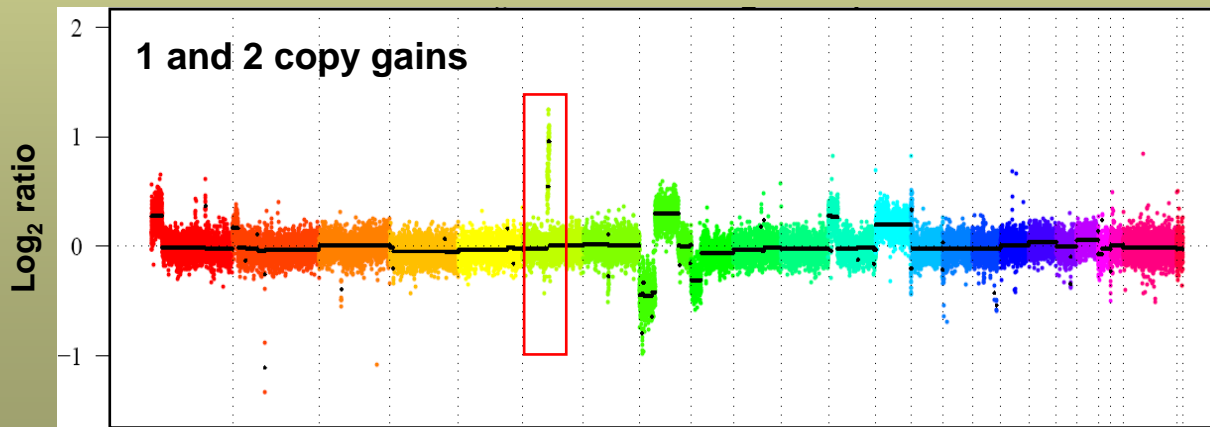
Chr 7



# CGH nagyfelbontású (385K) teljes genom array-vel

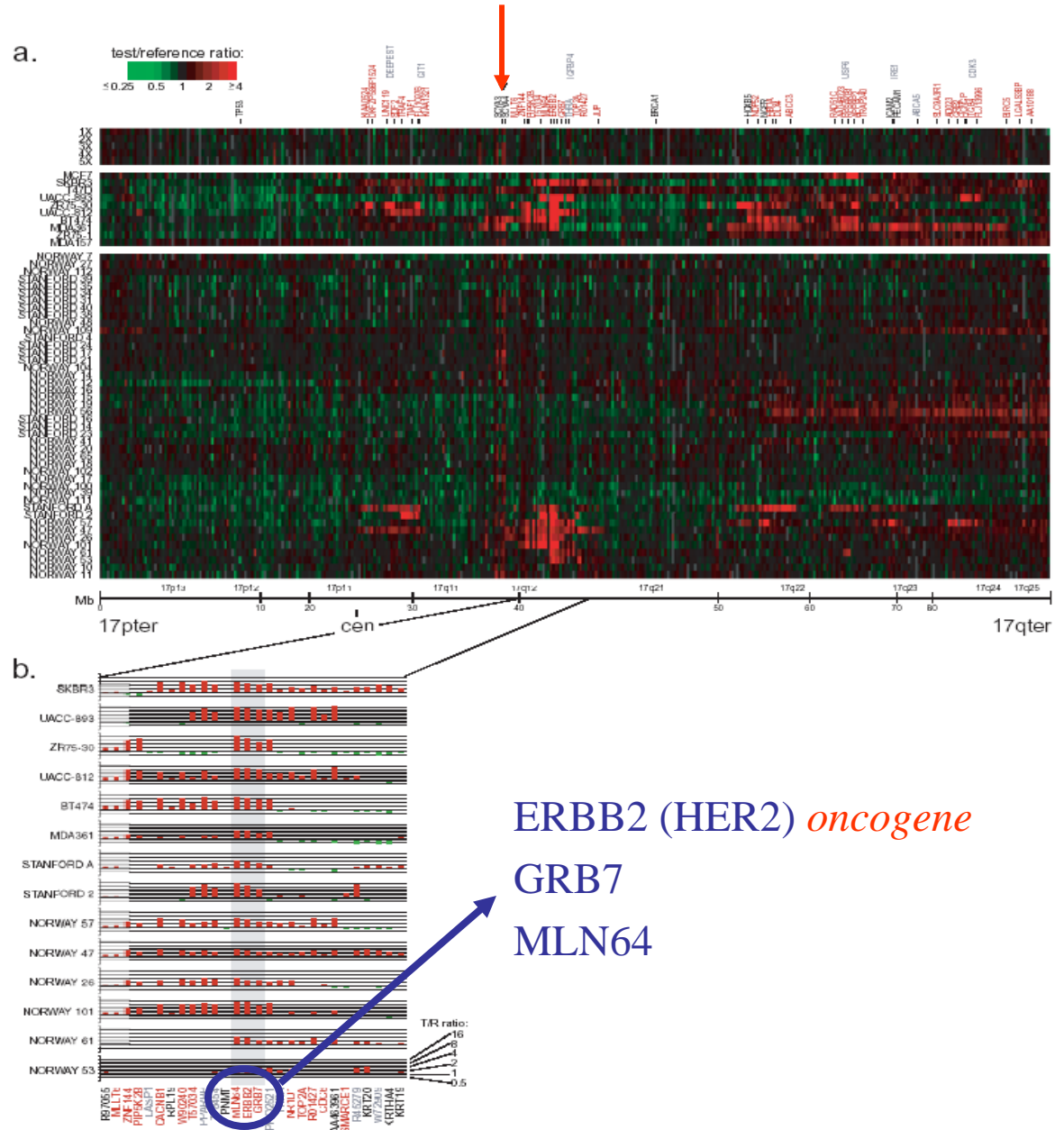


Nő vs. férfi gDNS



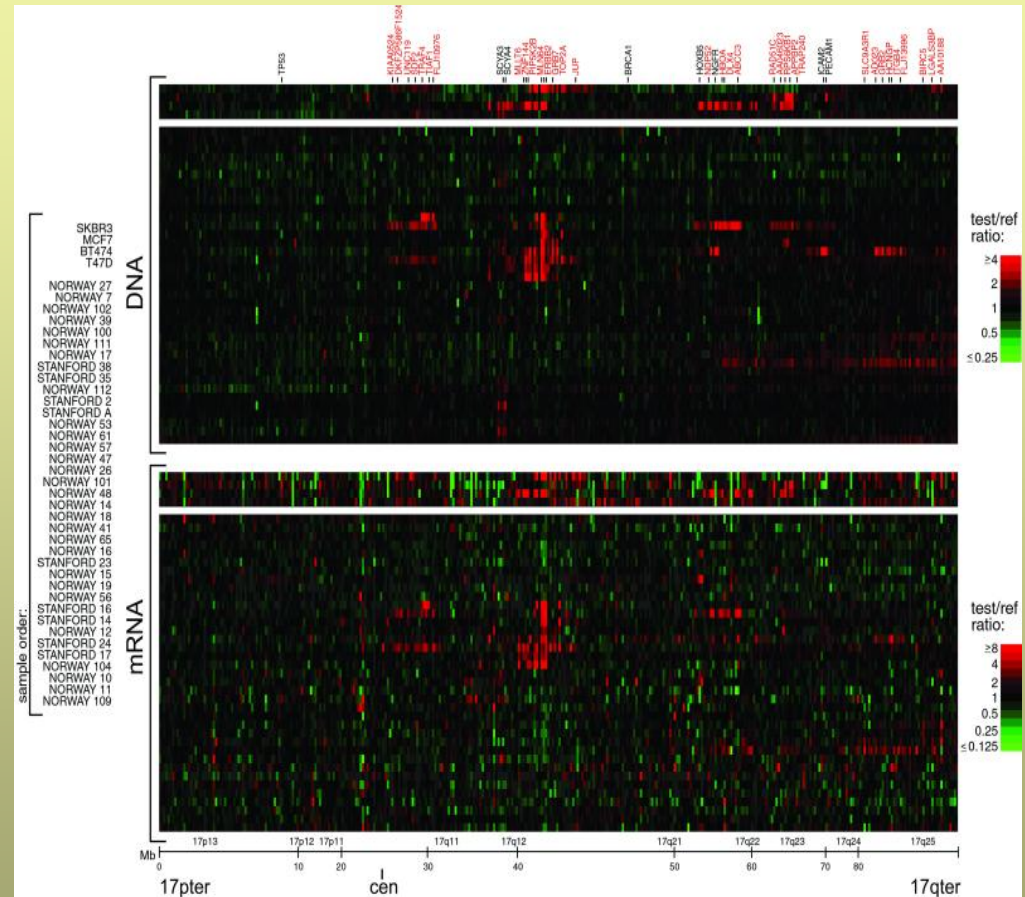
Pajzsmirigy tumor

# Variation in copy number mapping to chr 17



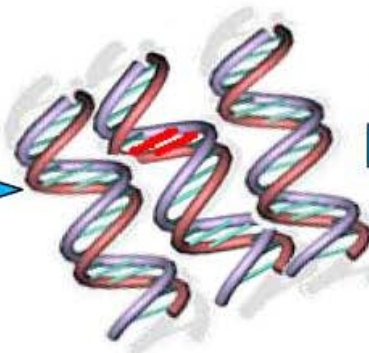
# Párhuzamos mRNS szint vizsgálat

- 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



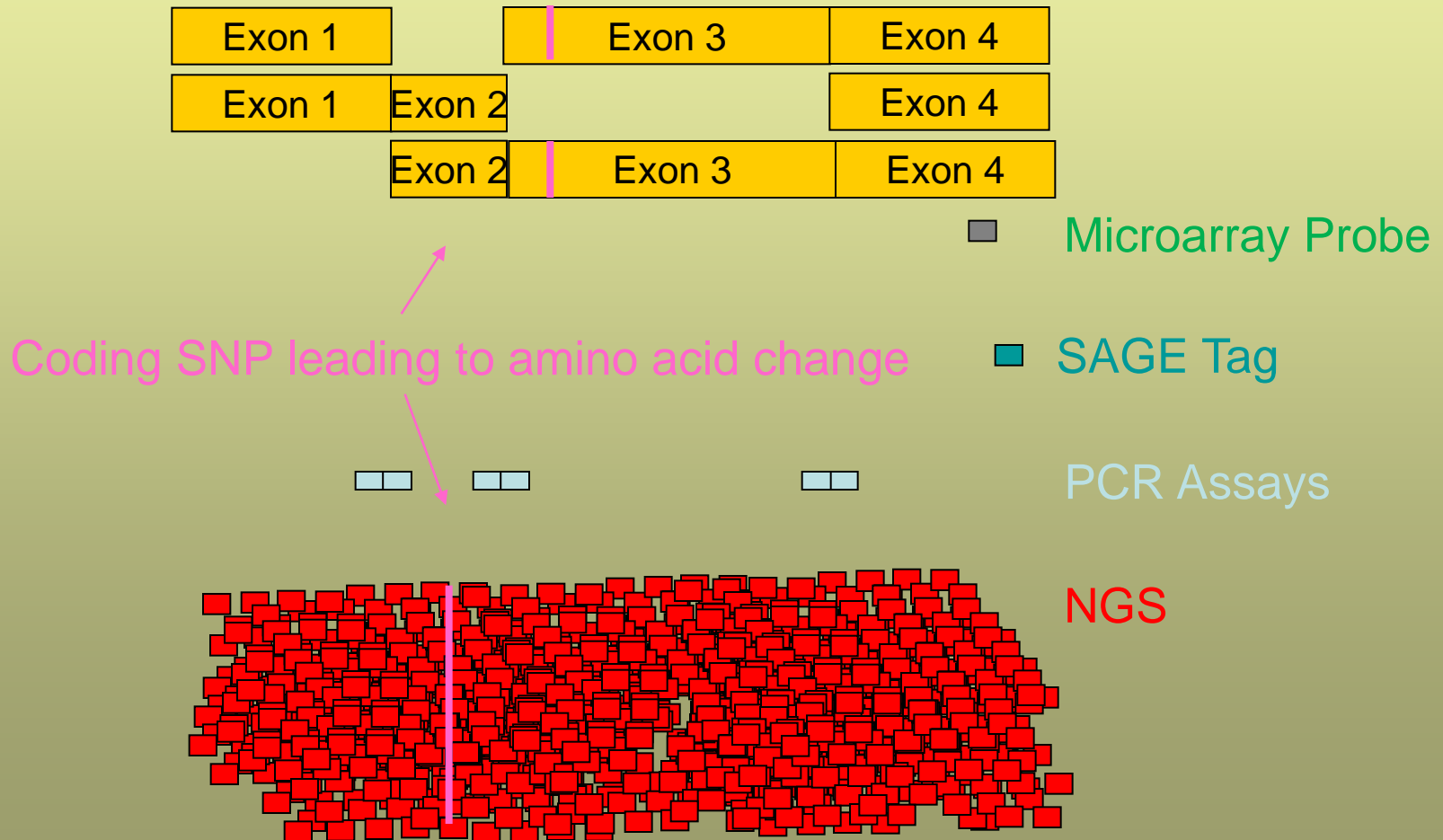
AGC TCC CGG TAC  
AGC TCC **G**GG TAC  
AGC TCC TGG TAC  
AGC TCC **A**GG TAC

Drug resistance

KRAS mutaton correlates with resistance to e.g. Erbitux

Újgenerációs szekvenálási stratégiák

# Microarray vs. SAGE vs. TaqMan vs. NGS



# Átfogó fehérjekifejeződés és fehérjemódosítások vizsgálata fehérjecsip technikával



# Fehérje-chipek - eltérően kifejeződő vagy módosított fehérjék azonosítására



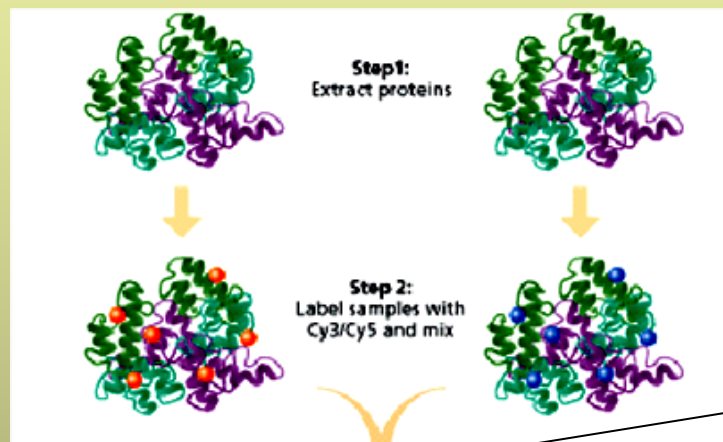
Kezeletlen.  
kontroll



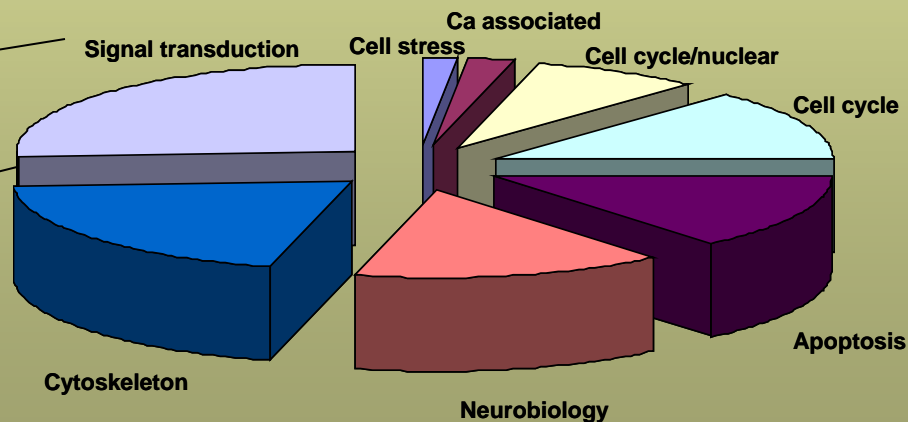
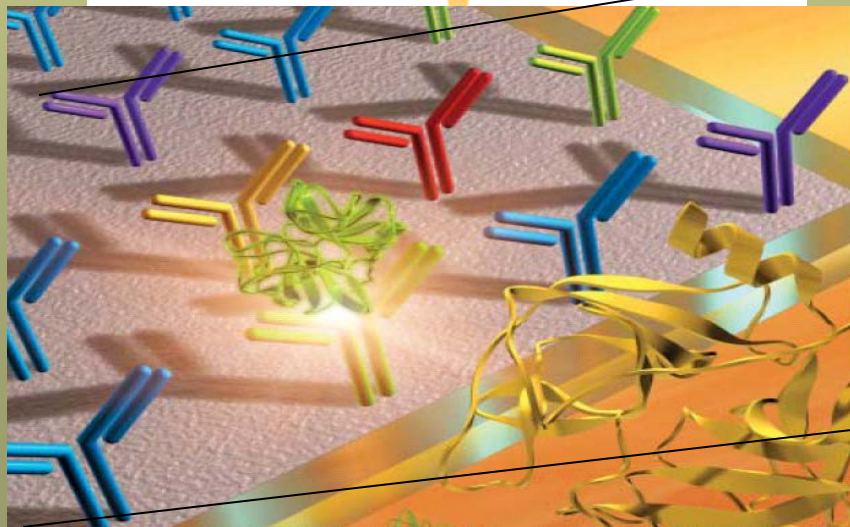
Kezelt



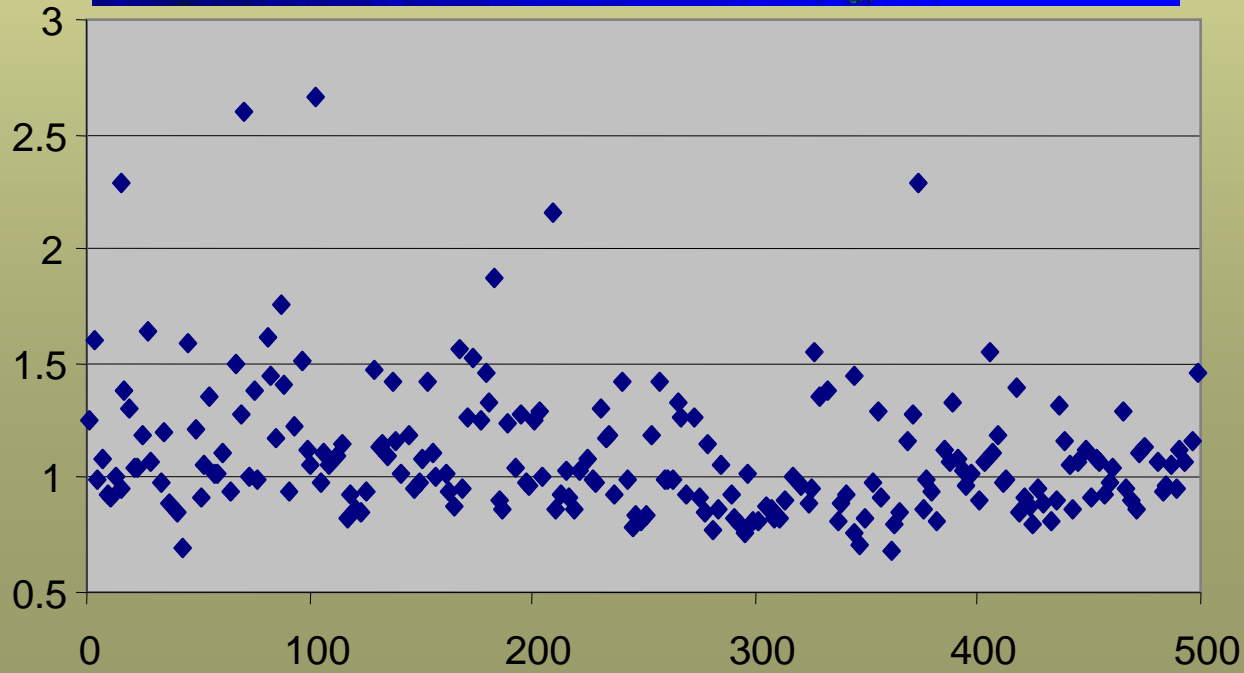
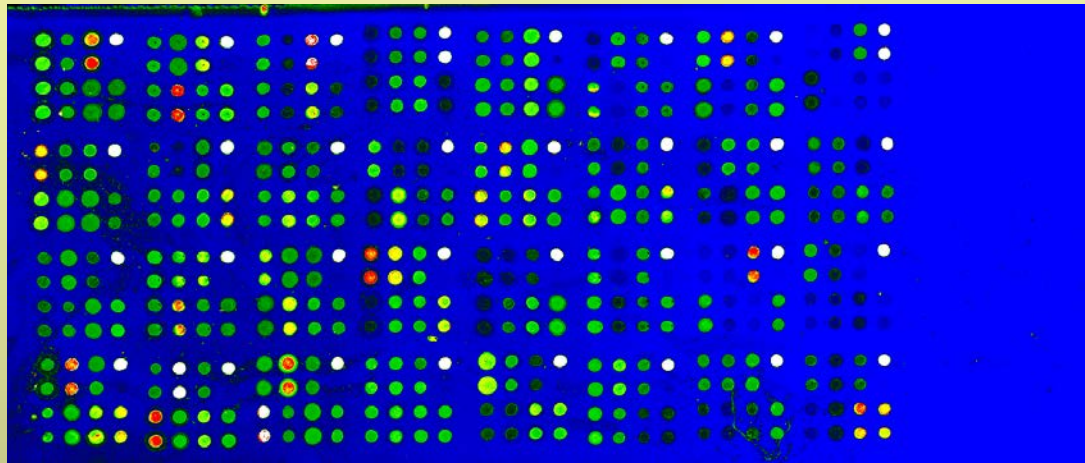
„bár ígéretes technológia,  
mégsem annyira megalapozott,  
mint a DNS-csip módszer”



Antitestek, amelyek  
a jelátviteli és egyéb  
folyamatokban  
résztevő fehérjéket ismeri fel



# Tüdőtumor fehérjecsipes vizsgálata



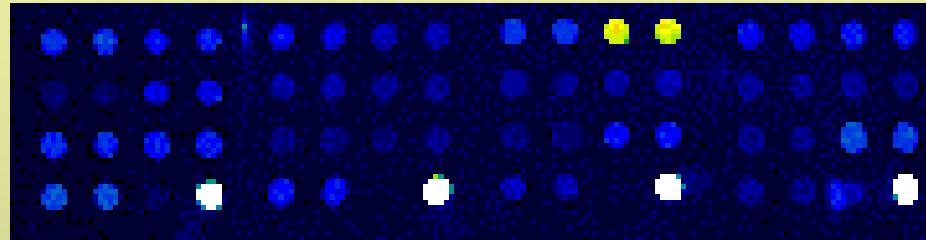
cdk5
Caspase 7
chk1
HSP90
Adaptin
cdc26
CyclinE

Synaptotagmin
Caspase11
Nicastrin

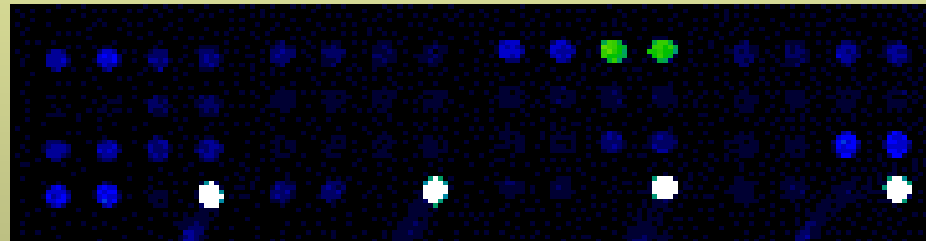
# Specificitás és érzékenység meghatározása

## Caspase 9

100ng/ml



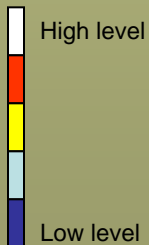
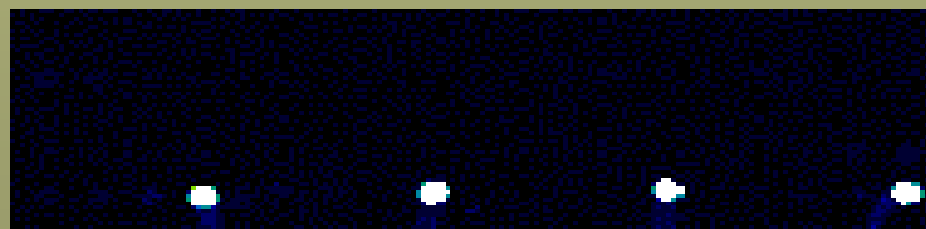
30ng/ml



3ng/ml



buffer

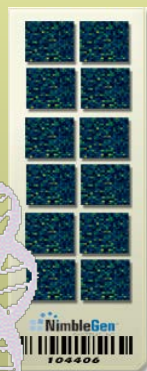
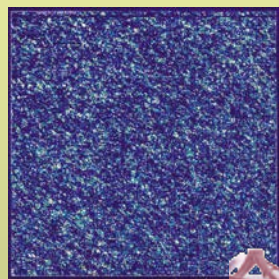




**Új módszerek  
fejlesztések,  
lehetőségek**

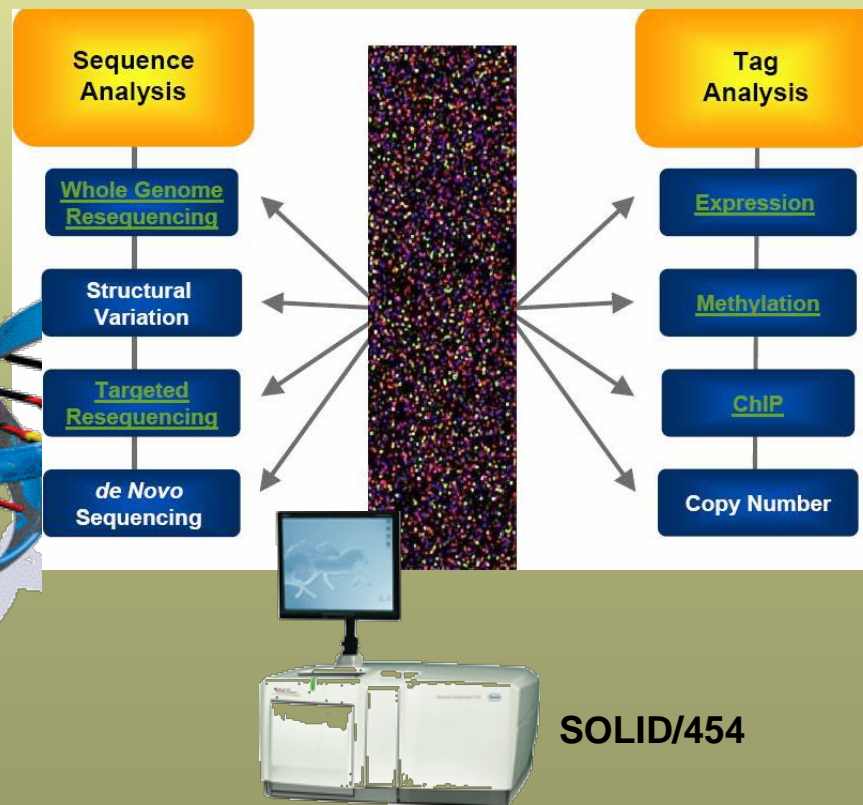
# Genomikai kutatások eszközei

DNS-chipek,  
microarrayek



valós-idejű PCR

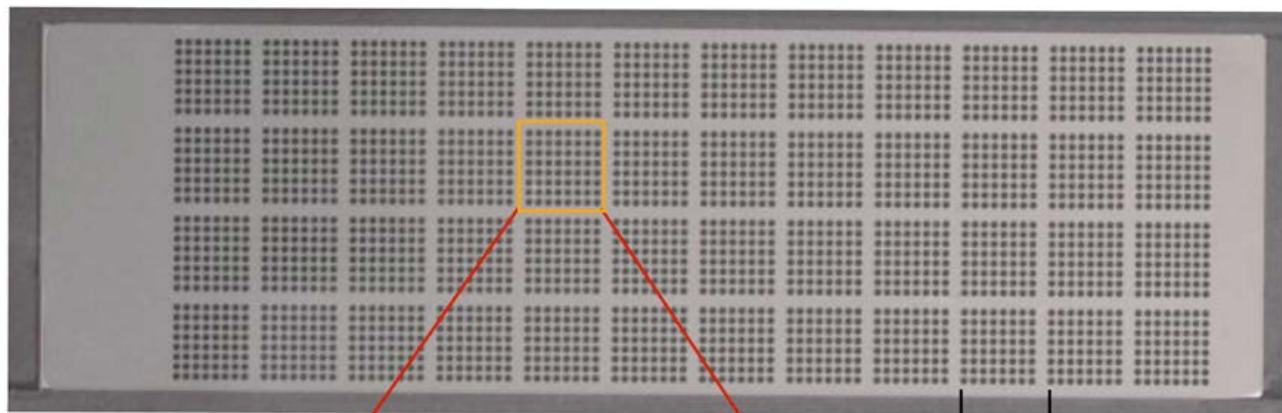
Új generációs szekvenátorok



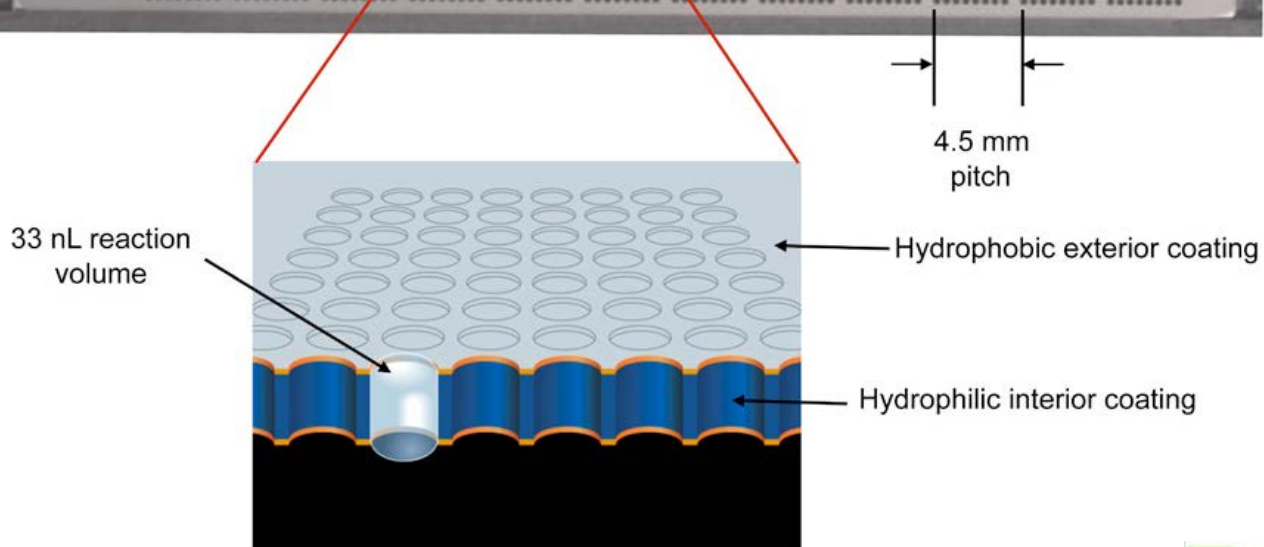


**AVIDIN**  
BIOTECHNOLOGY

# Nanokapilláris valós-idejű PCR



3.072 reakciótér  
48x64 almátrix



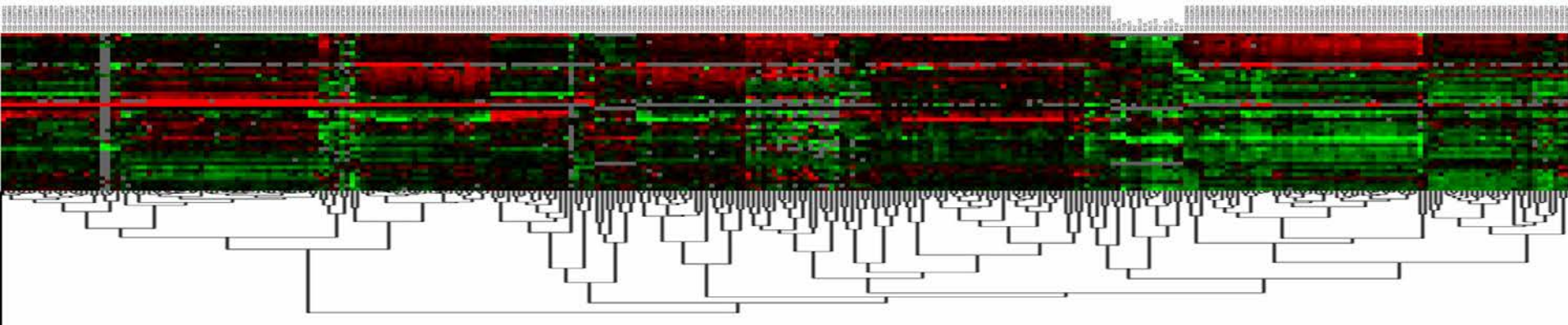
Array cross-section





**AVIDIN**  
BIOTECHNOLOGY

## 480 vizsgált, citotoxikus anyag szűrése toxikológiai panelen

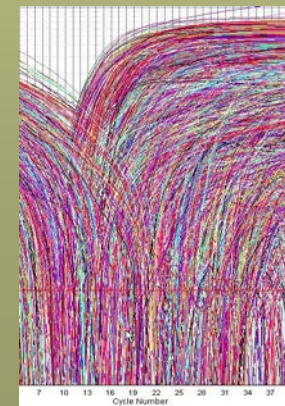
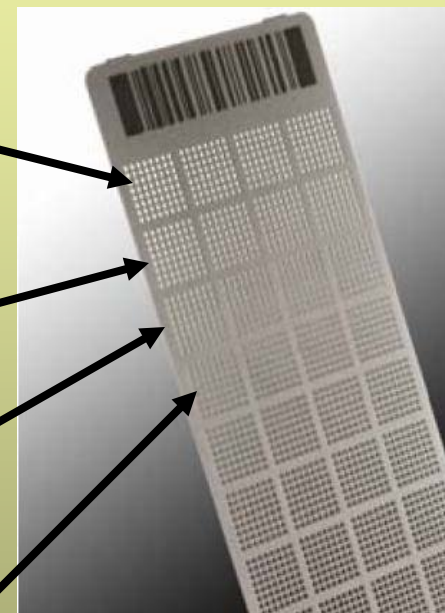
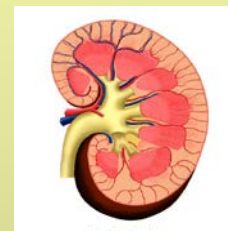


A normalizált génexpressziós értékeket hierarchikus klaszterezési eljárásnak vetettük alá. A referencia-minták közös klaszterben mutatkoztak, amely hasonló hatásmechanizmusra utal.

480 minta (52 gén/minta) 10 lemezen:  
1 koncentrációban (EC10) 1 időpontban (24 h) máj sejteken  
Összesen 24.960 QRT-PCR



## Protocol for toxicogenomics screening



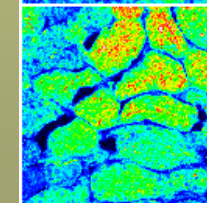
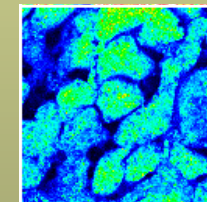
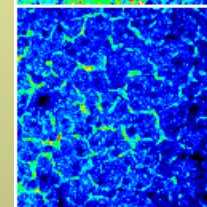
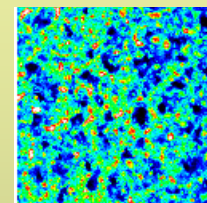
3+3 x4 samples  
2 compounds/plate

### REFERENCE:

Heart and liver toxicity of doxorubicine, sulfasalazine;  
liver and kidney toxicity of aniline, rotenone, ID9637;  
safety of Ac929 and Q50 was confirmed with the  
Avidin's Toxicogenomics screening platform

# Toxicogenomics screening with reference compounds

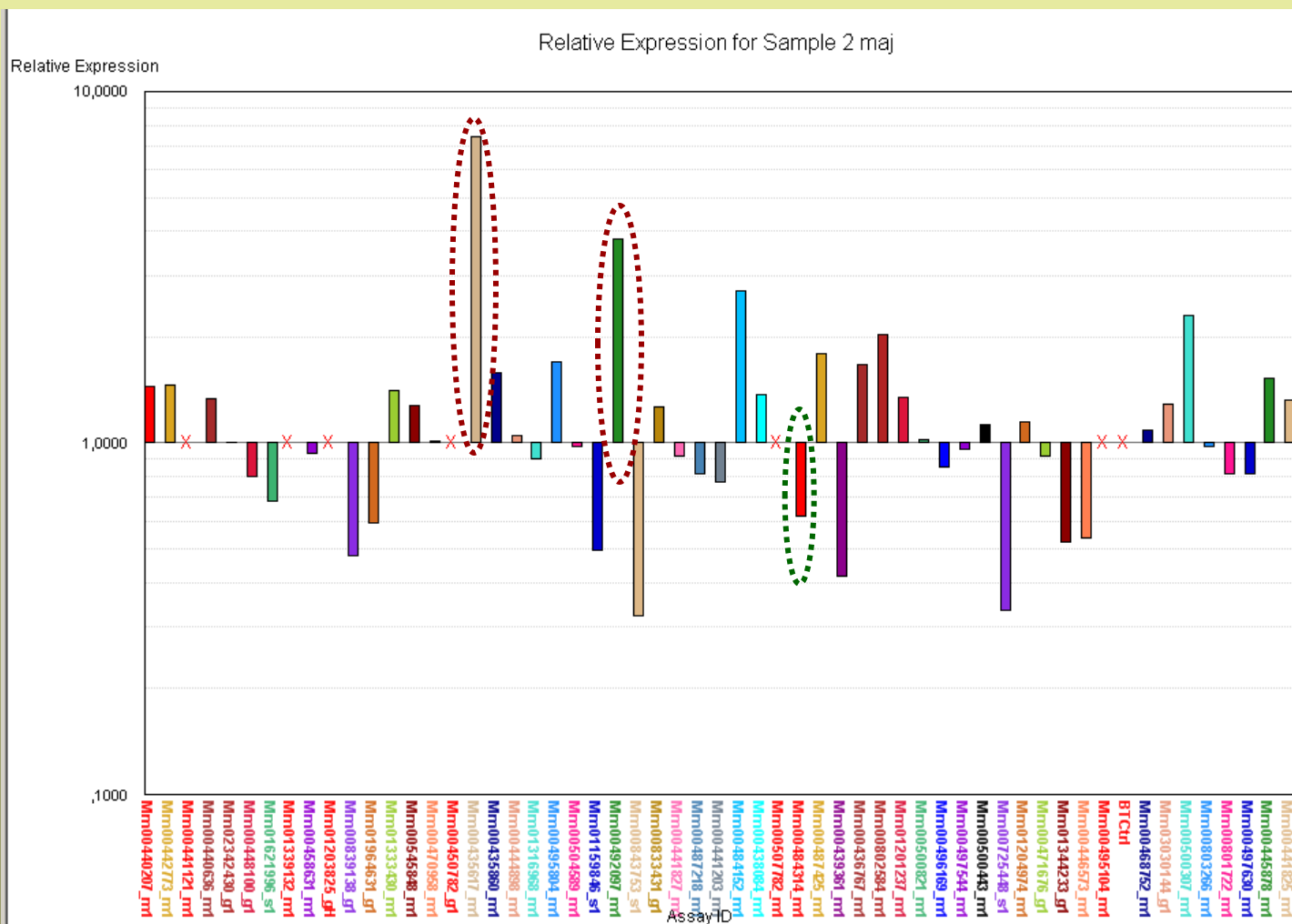
	0.41		-0.41			-0.32	-2.30		0.00		-2.42			0.28	2.12		-0.04	-0.24		0.11		-0.02		-0.07		-0.61				
-0.85	-2.24	-1.63	-1.72	-1.00	-0.01	-0.76	-2.30	0.09	-0.45	-3.00	-2.55			-0.31	0.37	0.12	-1.40	-0.74	-0.47	0.60	-1.89	-0.43	-0.58	0.45	-0.99	-0.87	-0.59	0.28	-0.55	
-0.31	-1.25	-4.54	-1.63	-0.81	-1.64	-1.69	-3.41	0.55	-0.91	-2.01	-2.65			-0.21	-0.77	-0.96	0.15	-0.32	-0.61	0.11	-1.74	-0.64	-0.03	0.20	-1.37	-0.12	-1.11	-0.92	-2.25	
-0.32	-0.16	-0.65	-2.75	-0.87	-0.55	0.11	-2.36	-0.51	-0.50	-1.85	-1.59			-1.23	-0.60	0.52	0.42	-0.44	-0.15	0.34	-0.07	-0.42	-0.60	-0.09	-1.18	-0.76	-0.47	0.37	-1.64	
0.02	-1.19	-4.02	-1.25	-0.75	-1.14	-1.34	-2.03	0.09	-1.23	-2.12	-2.57			-0.17	-1.50	0.15	-1.62	-0.08	-1.18	0.03	-1.34	-0.18	-0.91	0.35	-0.62	-0.22	-0.71	0.18	-0.90	
			-0.73				-2.48				-2.83						-0.63								-0.29				-0.99	
-0.03	0.62	-3.24	-0.70	0.03	-0.70	-1.04	-1.69	0.72	-1.06	-2.32	-2.73			0.19	-0.99	0.13	-0.83	-0.18	-1.36	-0.36	-1.09	-0.46	-0.70	-0.39	-0.65	0.33	-0.92	0.48	-0.23	
		-3.59	-1.97			-1.46	-1.92			-1.22	-2.87	-1.70				-1.17	-0.07	-0.40		-1.04	-0.28		-1.58	-0.49	-0.91		-0.92	0.04	-0.09	
		-3.77				-1.68	-1.91			-1.00		-2.32				-1.76		-3.10		-0.65		0.59		-1.91		-0.87		-1.48	-0.30	
-1.35	-0.24	-3.34	-2.06	-0.57	-0.80	-0.87	-2.49	0.00	-1.19	-3.09	-2.86			-0.82	-1.77	-1.50	-1.90	-0.05	-1.49	0.10	-2.16	-0.39	-0.99	-0.26	-1.14	0.01	-0.96	0.65	-1.01	
-0.49	-2.14	-1.62	-2.70	-0.86	-0.74	-1.08	-2.47	-0.23	-1.18	-2.25	-2.39			-1.12	-3.19	-0.38	nd	-0.53	-1.61	0.54	-1.95	-0.66	-1.42	0.41	-1.41	-0.31	-0.26	0.21	-0.76	
	-0.20	-0.53			0.59	0.10			0.51	nd					0.00	0.37			0.05	1.13			1.12	1.12		1.01	2.16			
-0.86	-1.06	-4.68	-1.32	-1.06	-1.16	-1.65	-2.52	-0.40	-1.26	-2.83	-2.56			-0.70	-1.35	-1.00	-1.22	-0.30	-1.27	-0.54	-1.36	-0.46	-0.94	-0.70	-0.58	-0.91	-1.08	-0.43	-0.74	
-0.89	0.08	-4.34	-2.25	-0.72	-0.58	-1.07	-2.62	-0.39	-1.13	-2.27	-2.40			-0.92	-1.35	-0.90	-1.30	-0.59	-1.19	0.35	-0.94	-0.20	-0.70	0.00	-0.73	-0.71	-0.97	0.00	-0.96	
-3.04			0.01	-1.36			-1.70	-0.85			-1.75			-0.71			0.79	0.40			1.48	-0.80			-0.87	0.12			-0.16	
-1.70			-0.72	-0.61			0.75	0.48			-1.01			-0.10			nd	0.50			-1.08	-0.46			-1.14	-0.18			0.28	
-0.24	-0.04	-3.35	-1.76	-0.85	-1.35	0.33	-2.82	-0.43	-1.15	-0.31	-2.18			-0.91	-2.51	-0.44	0.36	-0.38	-2.07	0.20	-2.12	-0.33	-1.59	0.17	-1.09	-0.64	-0.79	0.16	-1.73	
-0.53	-0.40	-4.01	-1.47	-0.51	-0.75	-1.51	-2.54	0.08	-0.89	-2.56	-2.91			-0.28	-1.08	-0.61	-1.67	-0.09	-0.54	-0.06	-1.31	-0.17	-0.89	0.27	-0.53	-0.46	-0.65	0.26	-0.88	
							-0.14				-0.82						2.32				nd									-0.67
-0.85		-2.57	-1.75	0.06		-1.87	-1.73	0.16		-1.77	-2.22			-0.15		0.06	-0.63	-0.25		-0.20	-0.30	-0.03		0.39	-0.09	-0.22		0.12	0.16	
-0.12	0.13	-3.61	-0.10	0.24	-0.50	-1.44	-1.58	-0.05	-0.72	-2.26	-2.40			-0.28	-0.98	-0.28	-0.67	-0.18	-0.72	-0.14	-0.04	0.26	0.18	-0.28	-0.02	-0.16	-0.13	-0.67	-0.03	
-0.82	-0.20	-2.05	-0.90	0.56	0.06	-2.29	-2.23	-0.12	-0.30	nd	-2.49			-0.25	-0.04	-0.34	0.62	-0.17	-0.55	0.37	0.68	0.47	-0.60	-0.16	-0.18	-0.20	-0.41	-0.15	-0.95	
-1.95	0.90	-2.39	0.55	-0.71	0.93	0.05	-0.88	0.14	1.55	0.19	-2.23			1.15	0.96	0.24	2.42	0.50	1.01	0.30	0.34	-0.17	0.49	0.70	0.09	0.40	0.15	0.12	-0.73	
-0.16	-0.51	nd	-0.79	-0.10	0.02	-0.37	-0.52	0.84	0.46	-2.13	-1.74			0.60	0.71	0.41	nd	0.11	0.76	1.28	-0.54	0.07	0.70	0.62	0.36	0.70	1.10	0.86	1.46	
-2.44			-3.29	-2.53			-1.78	-1.97			-0.57			-3.46			1.69	-1.62			-1.43	-2.38			-1.83	-1.75			-1.53	
-0.13		-1.68	-0.24	-0.43		-0.37	-1.13	0.22		-0.90	-0.98			-0.34		-0.10	4.77	-0.39		0.42	-0.27	-0.26		0.49	-0.62	-0.23		0.75	-1.06	
-1.92	2.39	-2.36	-0.45	0.18	0.52	-2.13	-0.29	0.46	0.37	-3.22	-2.28			1.25	1.42	-0.45	nd	0.77	0.55	0.02	-0.77	-0.06	0.62	0.55	0.27	0.70	0.47	-0.84	0.85	
-0.12	-1.16	-3.44	0.15	0.41	-0.74	-1.26	-1.26	0.48	-1.26	0.65	-0.64			-0.07	-1.15	-0.77	nd	-0.21	-0.99	-0.34	-0.30	0.07	-1.21	-0.08	-0.25	-0.10	-0.42	-0.47	-0.75	
		nd				-0.27				-0.10					0.17					0.22				-0.43				-1.00		
-0.44	-1.44	-3.48	-2.89	-0.69	-0.96	-1.27	-2.03	0.48	-0.95	-2.63	-2.59			0.16	-1.27	-0.99	-1.80	-0.10	-0.59	0.62	-1.08	-0.45	-0.52	0.68	-1.21	0.10	-0.70	0.62	-1.17	
0.99	-0.14		-1.44	0.18	-1.01		-0.82	1.06	-1.02		-0.57			-0.10	-1.34		-0.05	-0.02	-1.06		-0.93	0.59	-0.79	-0.03	0.50	-0.71	-0.71	-0.26		
		-7.17				-2.82				-1.14					-0.78				0.79				-4.19				-1.75			
	-0.19					-0.96				-1.06					-0.82				-0.85				-0.70				-0.93			
		-3.38				-0.16					0.02					-0.49					-0.31			0.13				0.61		
-1.46	-0.65		-1.79	-1.53	-0.36		-0.91	-1.37	-1.24		-1.76			-0.98	-0.14		nd	-1.88	-1.11		nd	0.80	-1.14		0.35	-0.82	-0.35		-0.88	
-0.40	-0.40	-3.06	-0.86	-0.20	-0.90	-1.19	-2.17	0.37	-1.14	-2.16	-2.68			0.10	-0.48	0.45	-0.71	-0.08	-0.59	0.33	-1.61	0.00	-0.56	-0.11	-0.21	-0.12	-0.27	0.45	-0.48	
-3.19	-1.54		-4.83	-1.15	-1.81		-2.56	-0.07	-0.67		-2.53			-0.23	0.89		-2.21	-0.38	-0.53		-1.18	-1.76	-1.24		-1.43	-0.08	-0.39		-0.64	
-0.24	0.54	nd	-0.86	-0.51	-0.35	-0.97	-1.73	-0.44	-0.56	-2.72	-2.11			-1.23	-0.23	0.09	-0.54	-0.28	-0.56	-0.09	-0.67	-0.21	0.05	-0.21	0.14	-0.52	-0.65	0.20	0.32	
-0.39	-0.87	-5.10	-1.91	0.39	-1.29	-1.37	-3.52	0.09	-1.62	-3.18	-2.53			-1.19	-2.06	-1.55	-1.28	0.26	-1.55	-0.67	-2.17	0.07	0.76	-0.81	-1.19	0.05	-1.27	-0.13	-1.82	
-0.17	0.25				-1.58	-0.50			-1.14	-0.15				-1.25	-1.32			-0.68	-1.14			-1.07	-0.98			-0.56	-0.66			
0.43	0.05	-3.52	-1.05	0.35	-0.68	-1.85	-1.46	0.93	-0.10	-1.98	-1.19			-0.12	-0.52	-0.27	0.90	0.64	-0.47	0.51	-0.62	0.39	-0.05	-0.05	-0.09	0.42	-0.08	0.41	-0.45	
-0.33	0.71	-1.22	0.20	0.13	-1.51	-1.38	-0.84	0.63	-1.62	-2.37	-1.85			-0.25	-0.01	-0.19	-0.04	0.14	-0.85	-0.23	-0.33	-0.09	-0.82	-0.11	0.33	0.50	-0.95	0.09	0.54	
<b>8</b>	<b>12</b>	<b>24</b>	<b>20</b>	<b>6</b>	<b>11</b>	<b>18</b>	<b>28</b>	<b>4</b>	<b>17</b>	<b>21</b>	<b>31</b>	<b>8</b>	<b>15</b>	<b>3</b>	<b>15</b>	<b>2</b>	<b>13</b>	<b>2</b>	<b>17</b>	<b>3</b>	<b>9</b>	<b>1</b>	<b>10</b>	<b>1</b>	<b>8</b>	<b>1</b>	<b>9</b>			
<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>	<b>B</b>	<b>L</b>	<b>H</b>	<b>K</b>			
<b>ID9637</b>				<b>Doxorubicin</b>				<b>Sulfasalazine</b>				<b>Rotenone</b>				<b>Aniline</b>				<b>Ac-915</b>				<b>Q-50</b>						



# Liver

Nimesulide 50 mg/kg

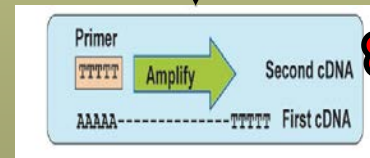
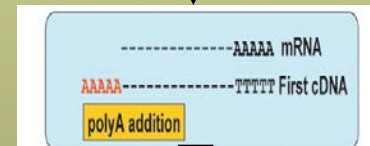
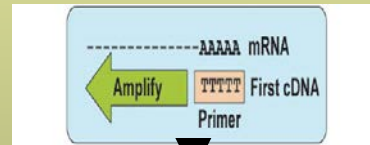
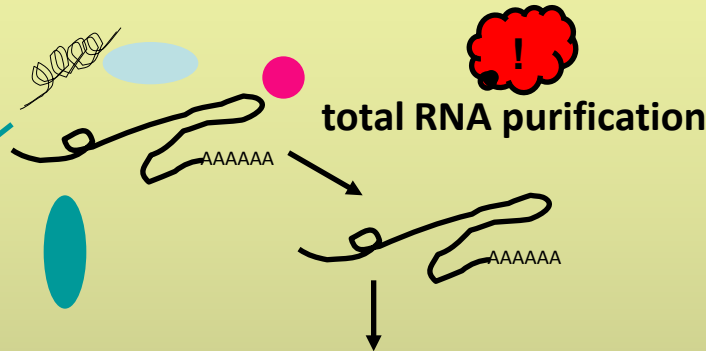
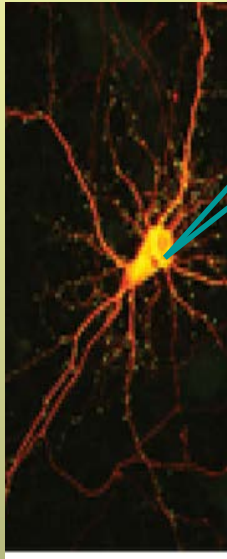
Control gene: Mm02342430\_g1 PPIA, cyclophilin A



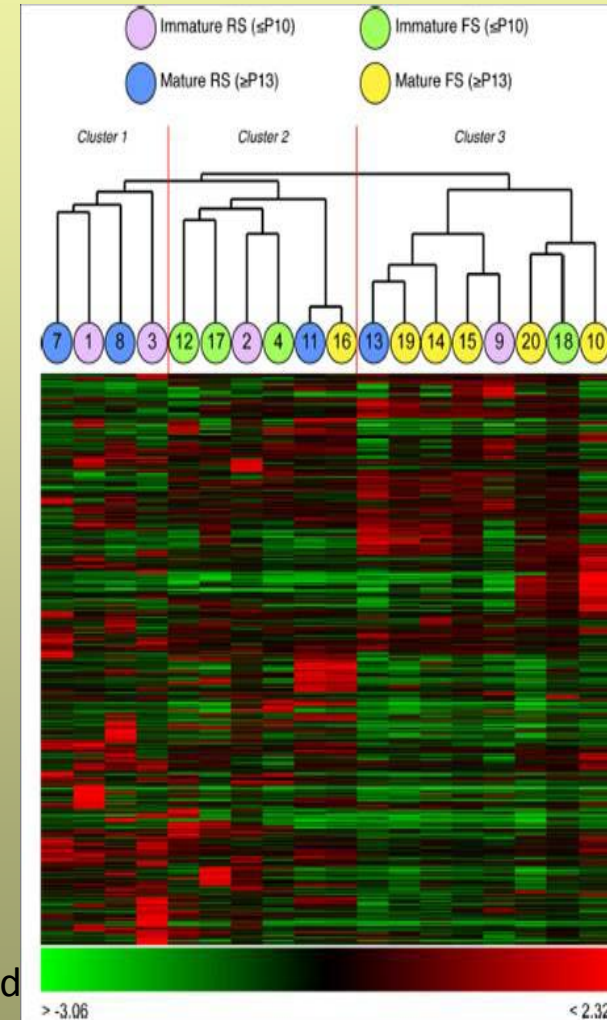
# **Egyedi sejtek genomi és digitális expresszióvizsgálata**



# Elektrofiziológiailag különböző sejtek teljes genom analízise



mRNA was amplified by a global polyadenylated PCR-based method



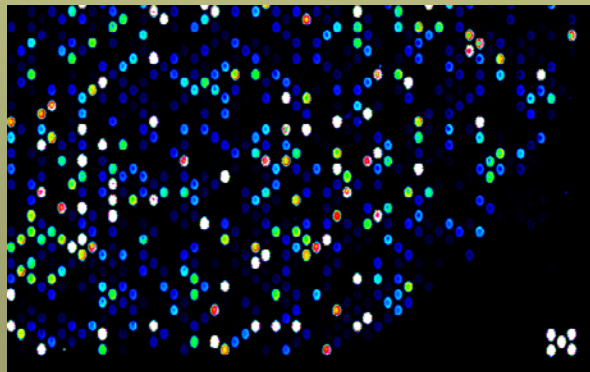
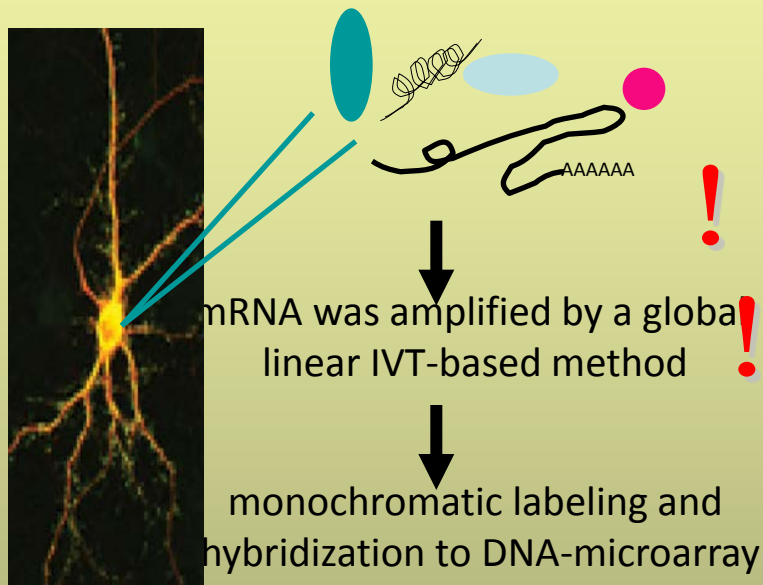
Grouping and classifying electrophysiologically defined classes of neocortical neurons by single cell, whole-genome expression profiling

Tatiana Subkhankulova, Kojiro Yano, Hugh P. C.

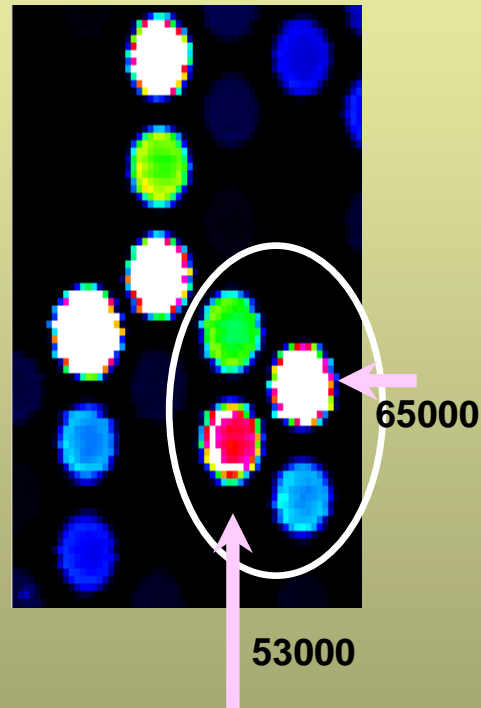
Robinson and Frederick J. Livesey

MOLECULAR NEUROSCIENCE, 2010

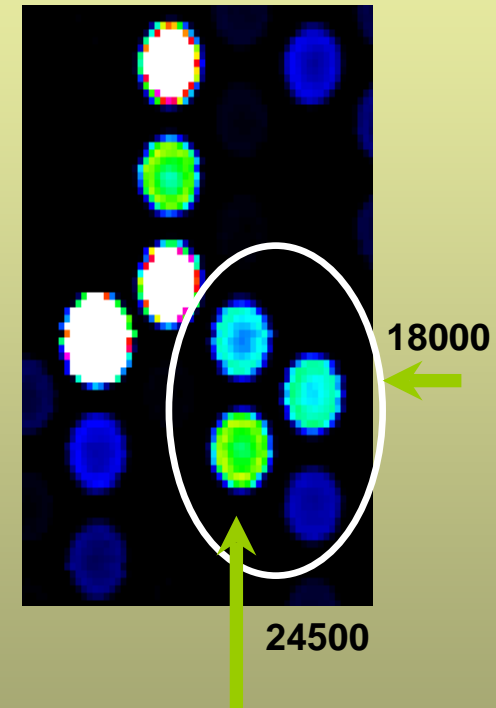
# Elektrofiziológiailag különböző sejtek teljes genom analízise



Type I interneuron



Type II interneuron



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

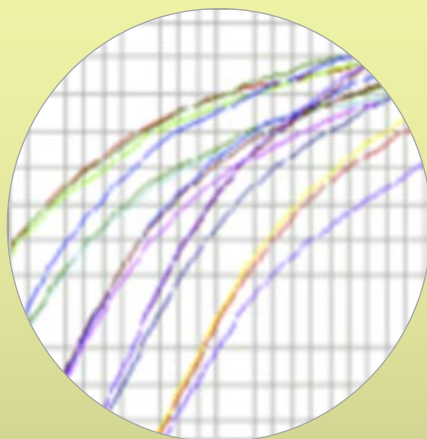
# Digitális PCR – A PCR harmadik generációja

1.



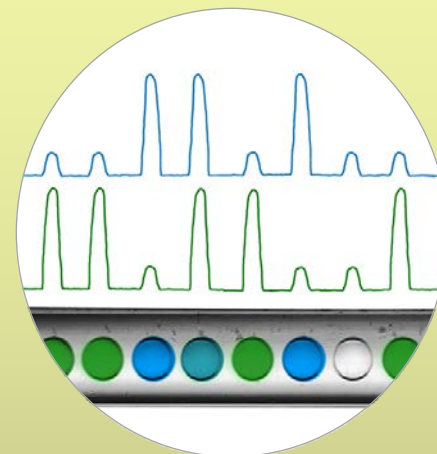
**PCR**  
Kvalitatív

2.



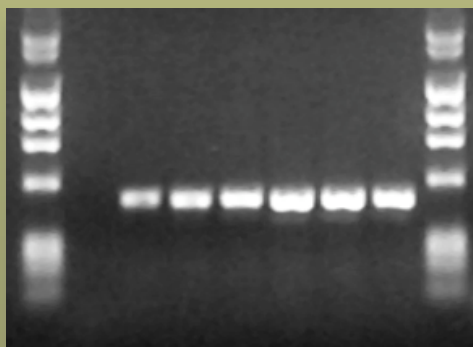
**Real-time PCR**  
Relatív kvantitálás

3.

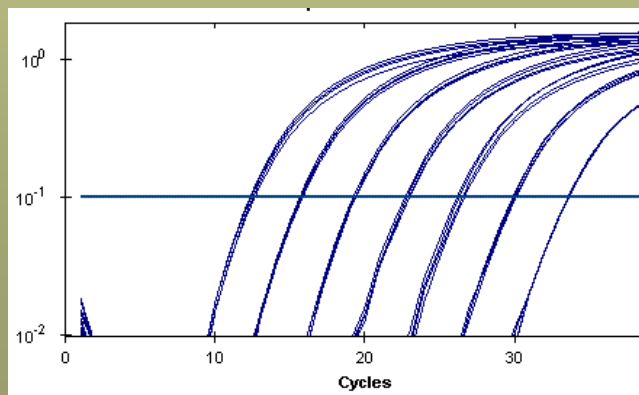


**Digitális PCR**  
Abszolút kvantitálás

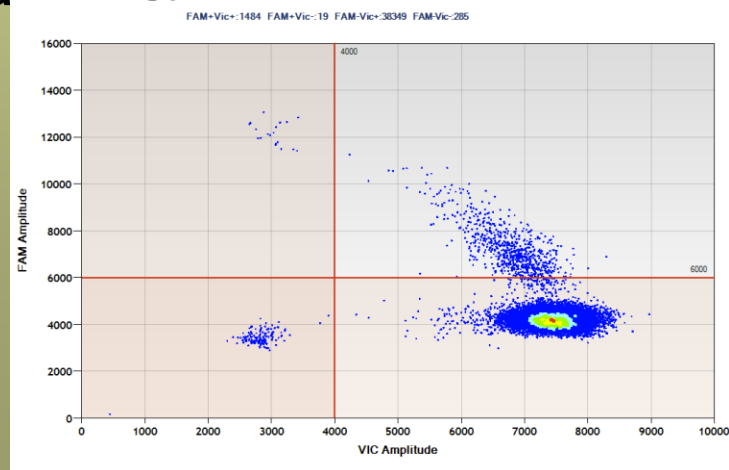
**Végpont analízis**



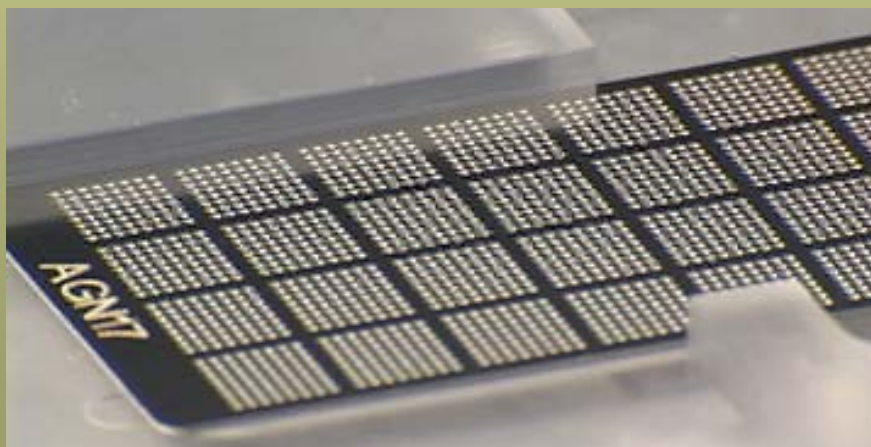
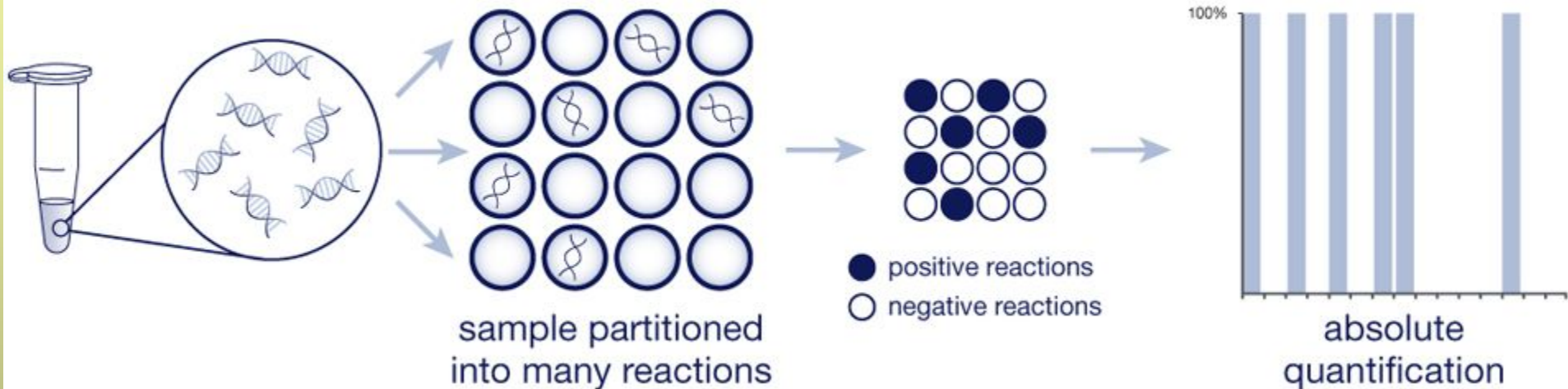
**Fluoreszcencia detektálása**



**Egyedi reakciók elemzése**



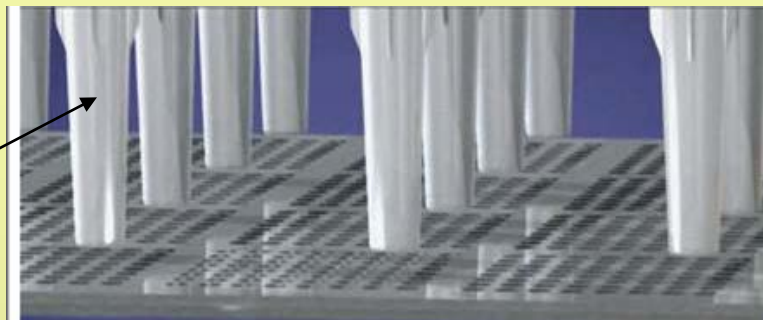
# Digitális PCR nanokapilláris HTS QRT-PCR technikával



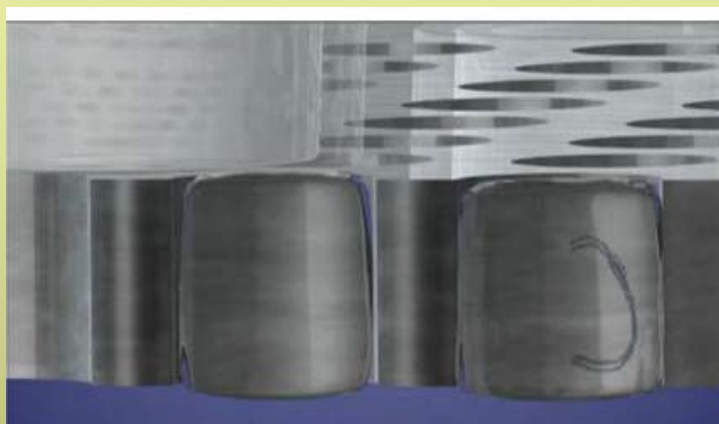




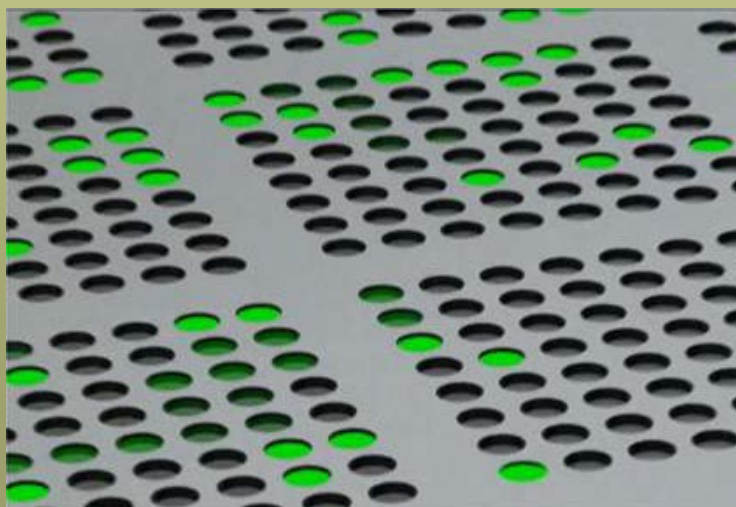
minta (1 sejt)  
TaqMan próba  
QRT-PCR reagens



**feltöltés**

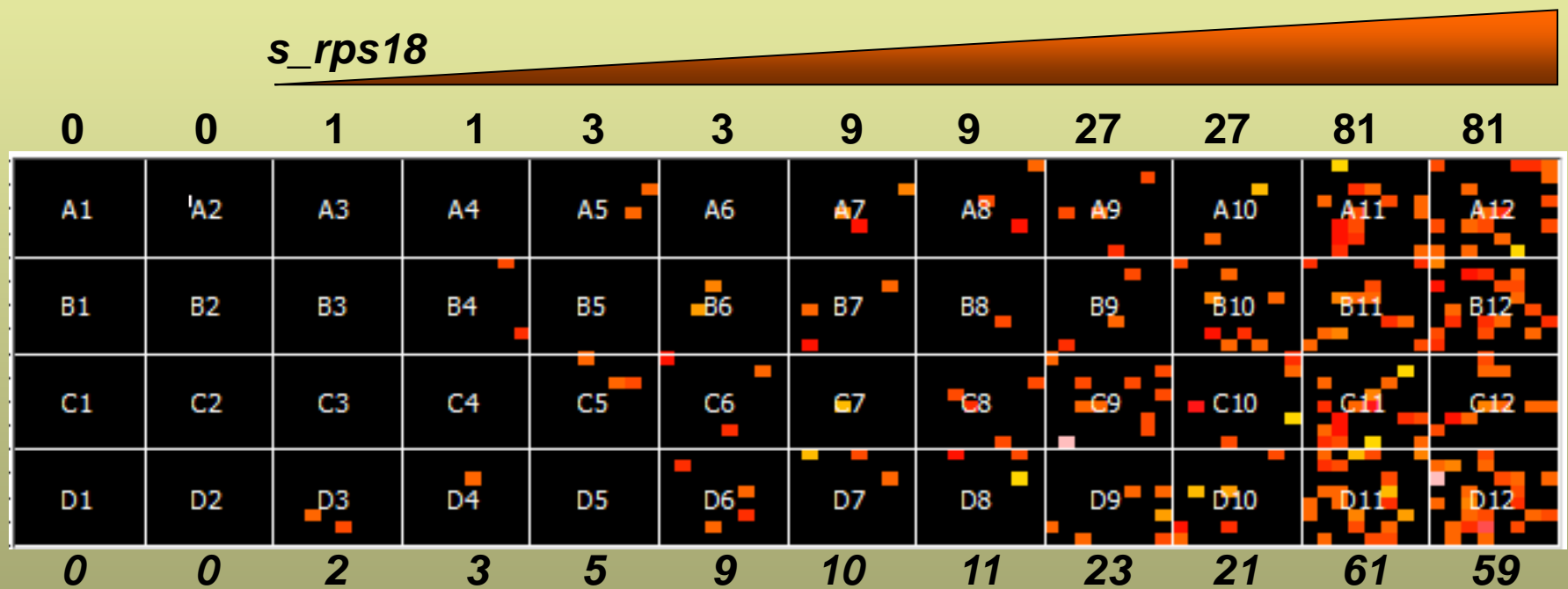


**PCR**



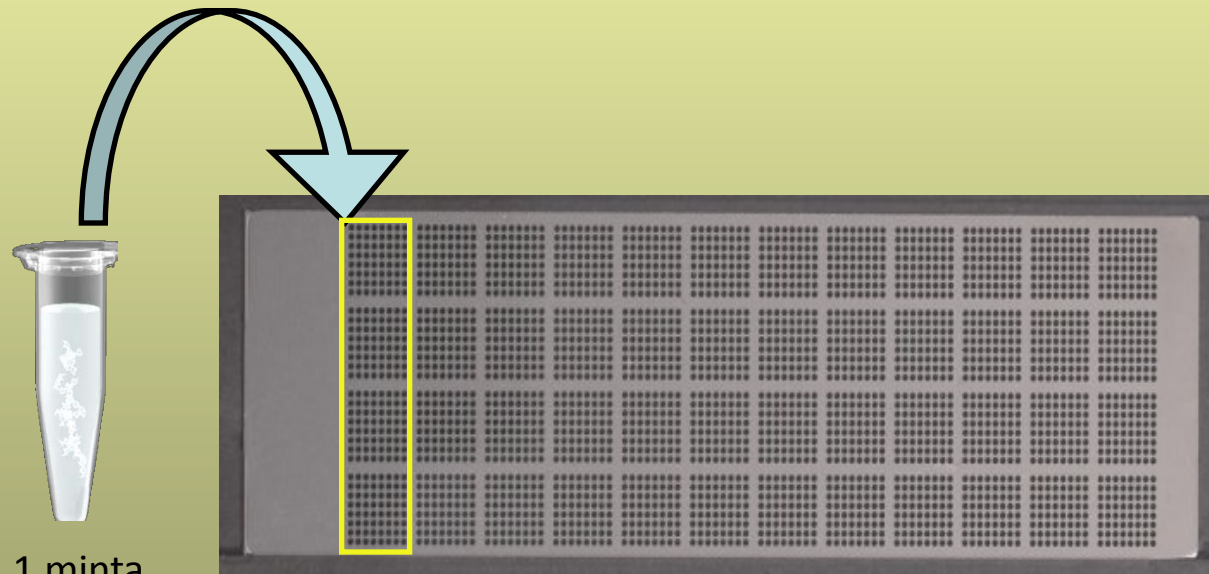
**PCR vége**

# Spike-in DNS kópiaszámának meghatározása dQRT-PCR technikával

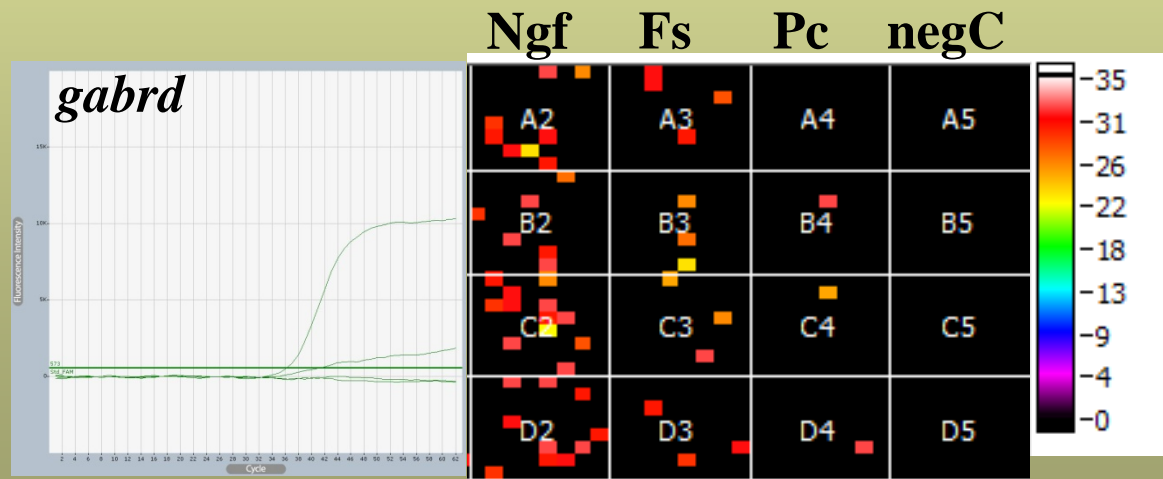
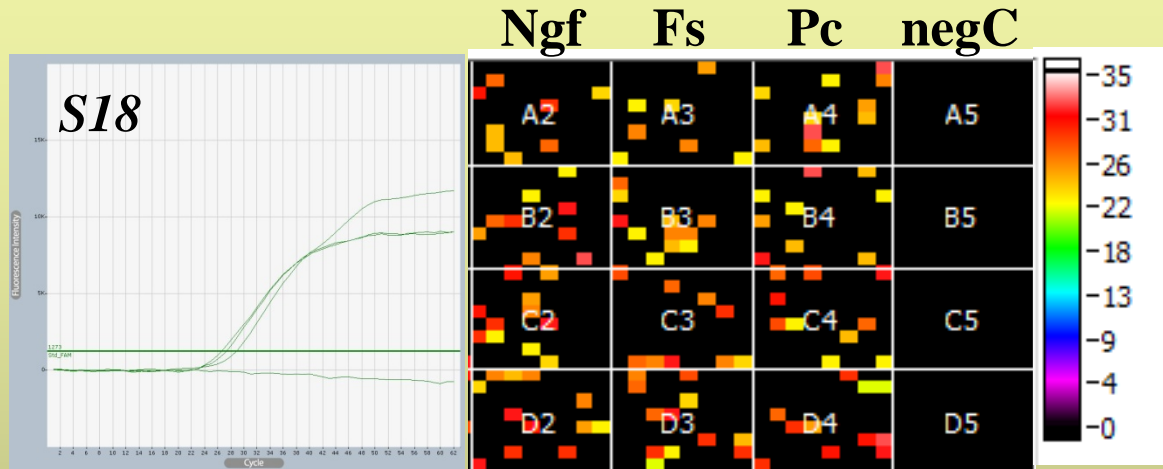


# Egyedi neuronok génexpressziós elemzése digitális QRT-PCR módszerrel

Kísérlet tervezése



# Egyedi neuronok génexpressziós elemzése digitális QRT-PCR módszerrel



Ngf: Neurogliaform sejt  
Fs: Fast spiking sejt  
Pc: Piramis sejt  
negC: RT- negatív kontroll

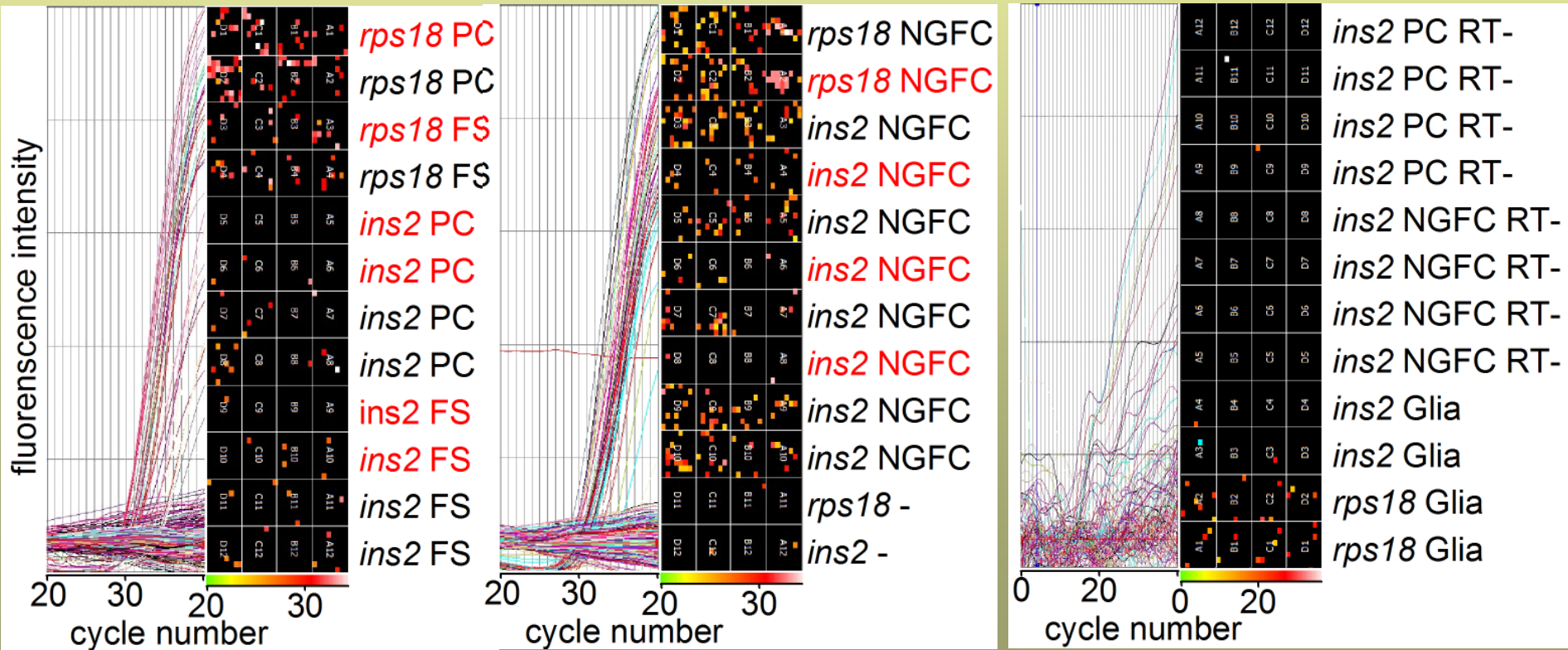
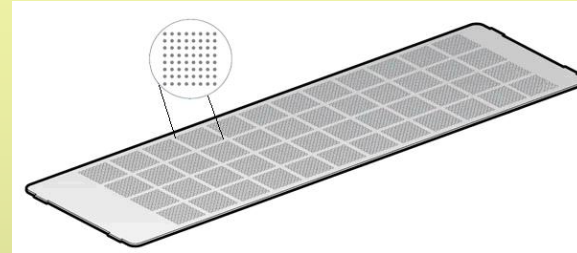
S18 háztartási gén hasonló kifejeződése minden neuron típusban  
Neuron-típus specifikus expresszió: GABA receptor



# Inzulin mRNS kópiaszám meghatározása egyedi sejtekben

## Single cell digital PCR

hypoglycemia / normoglycemia



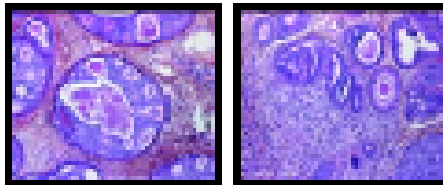
# Hagyományos és chip adatok egy adott klinikai kórképre

(a) Conventional data

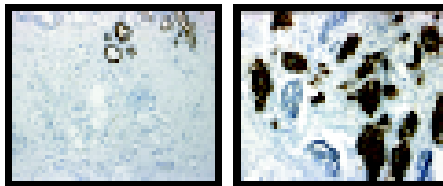
*Clinical data*

Patient's characteristics    Tumour extension stage  
Serum marker                      Disease outcome

*Morphological data*

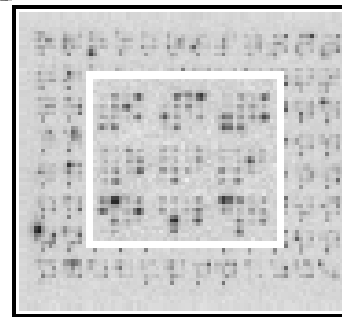


*Molecular data (IHC)*

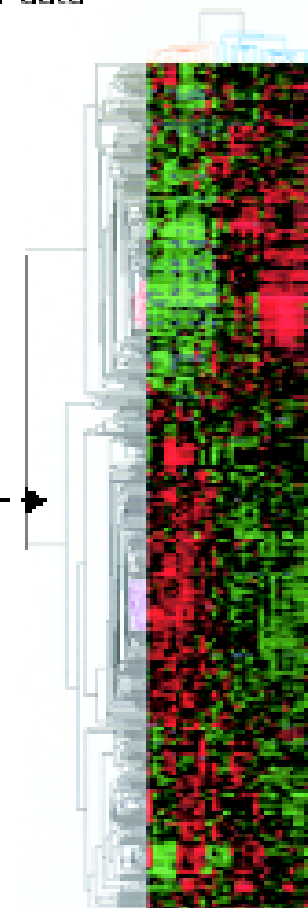
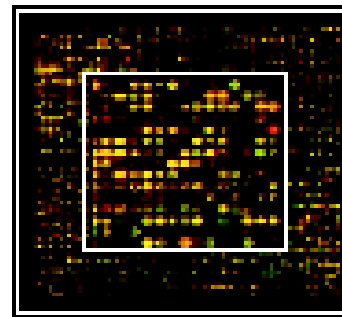


(b) Large-scale molecular data

*DNA-arrays data*



or



Köszönöm a figyelmet!

