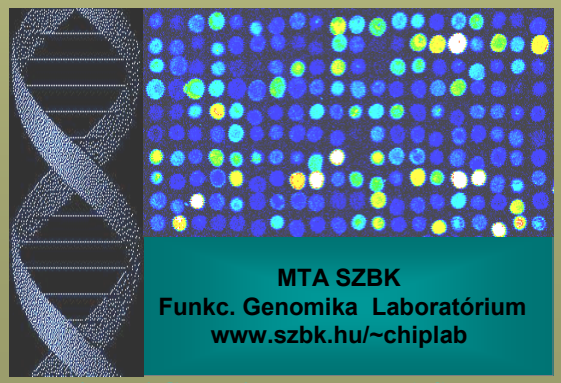
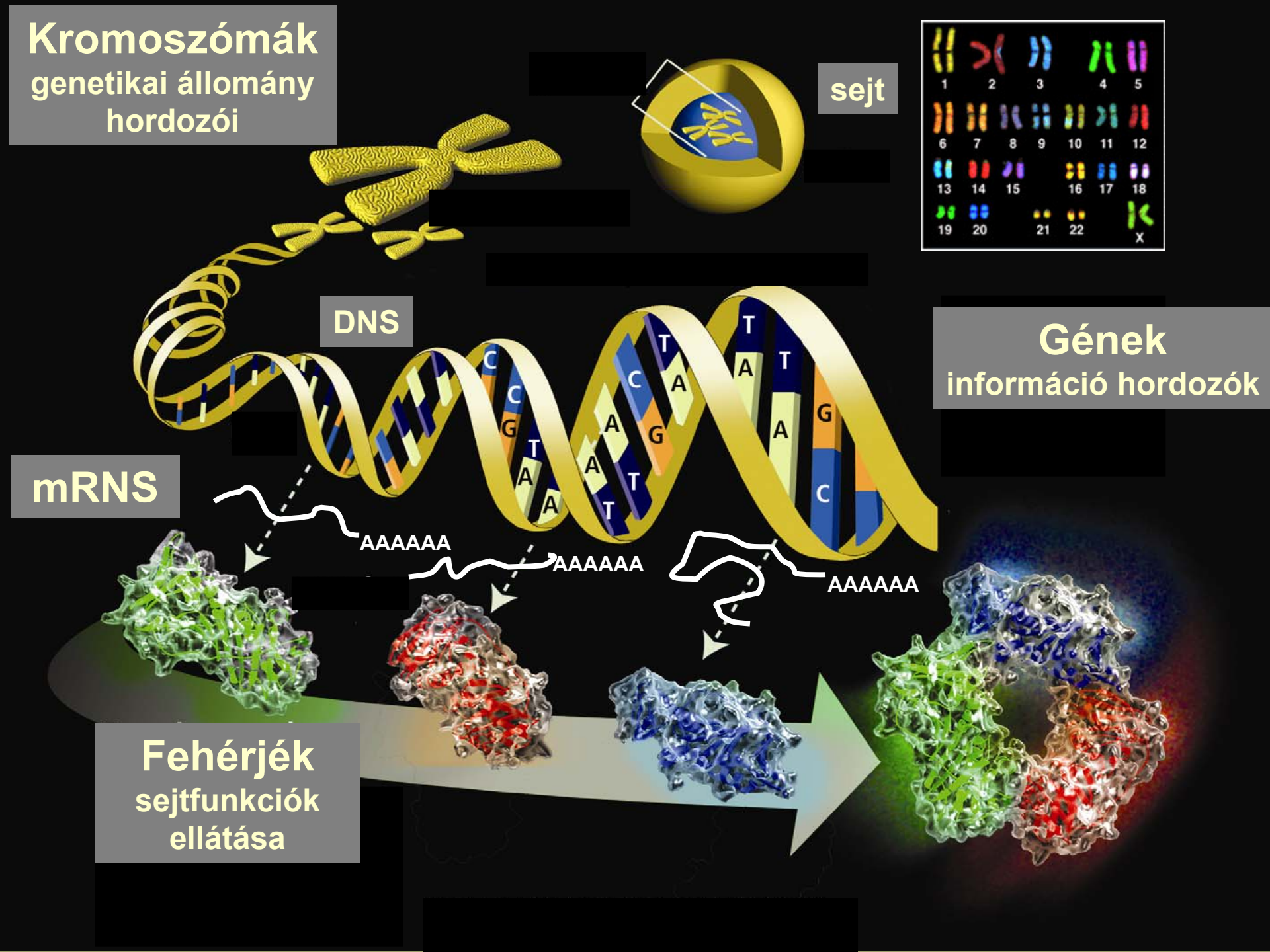
Microarray-technológia és funkcionális genomika

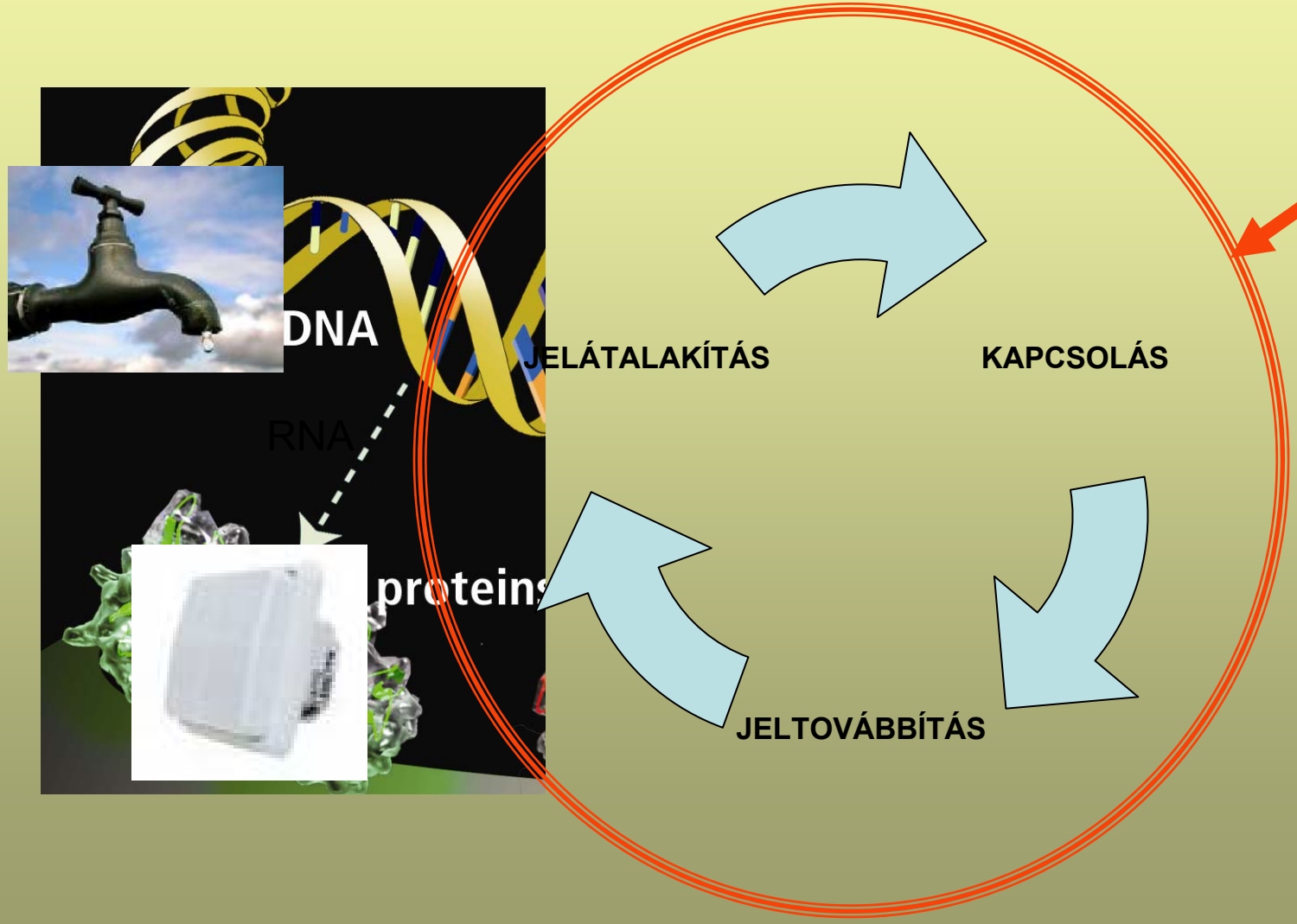
MTA SZBK, Genetika Intézet
Funkcionális Genomika Laboratórium

Semmelweis Egyetem, Genetika Intézet
Budapest
2011.12.08.




Kromoszómák
genetikai állomány hordozói
sejt
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X
DNS
Gének
információ hordozók
mRNS
AAAAAA
AAAAAA
AAAAAA
Fehérjék
sejtfunkciók ellátása

Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



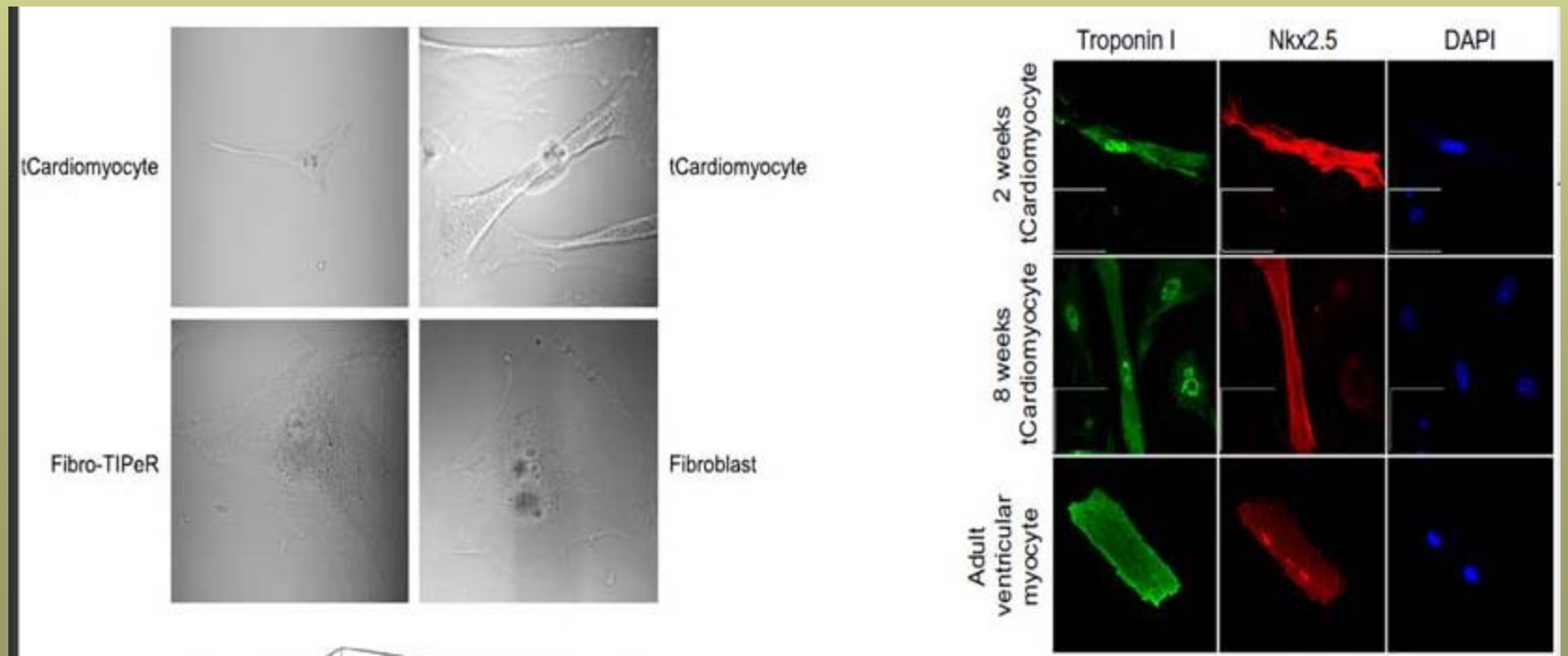
Kulcsszavak: kapcsolók

A fenotípus meghatározója a transzkriptom vagy a genom?

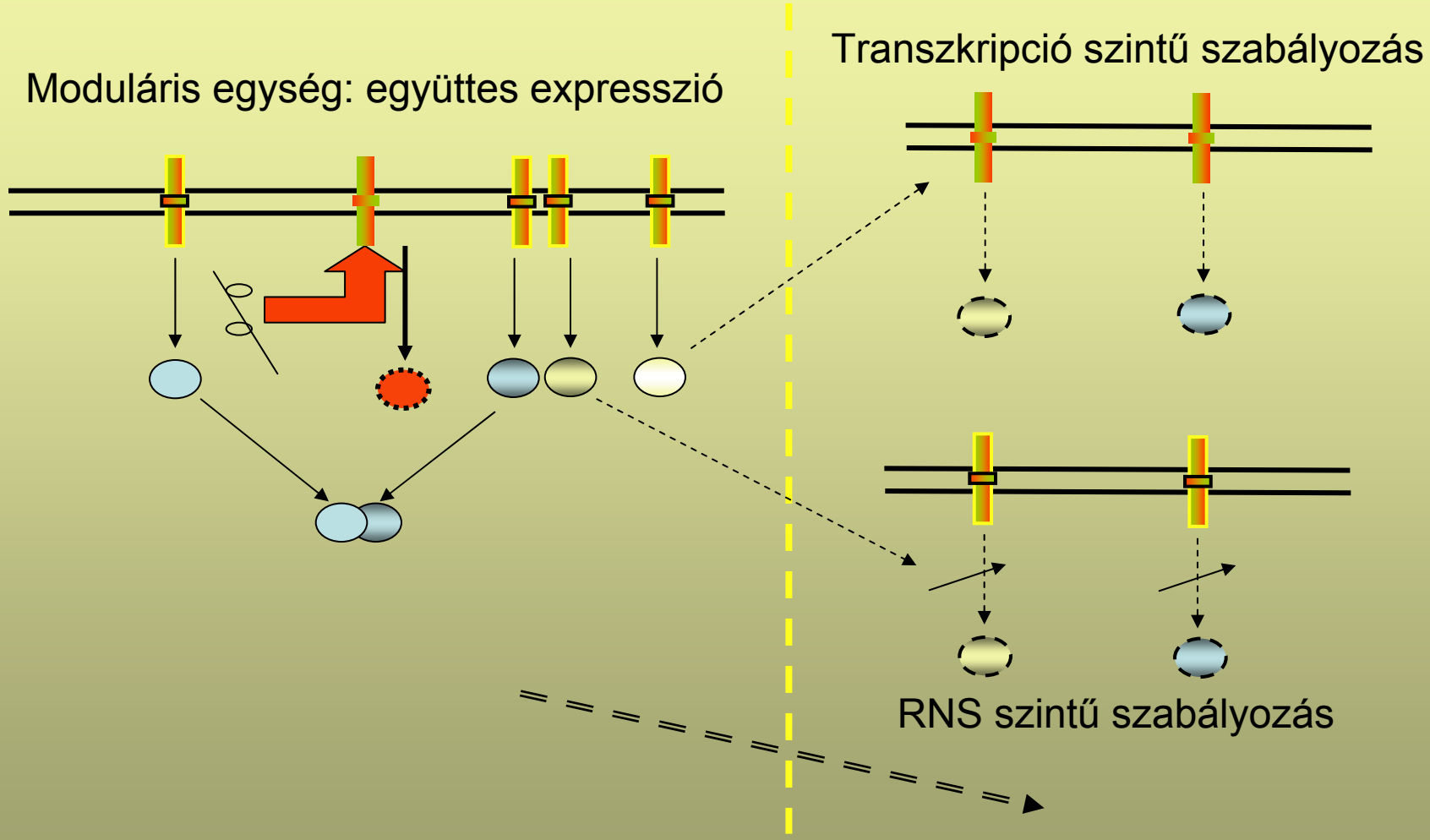
Gurdon JB, Elsdale TR, Fischberg M (1958) Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature* 182(4627):64–65.

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.

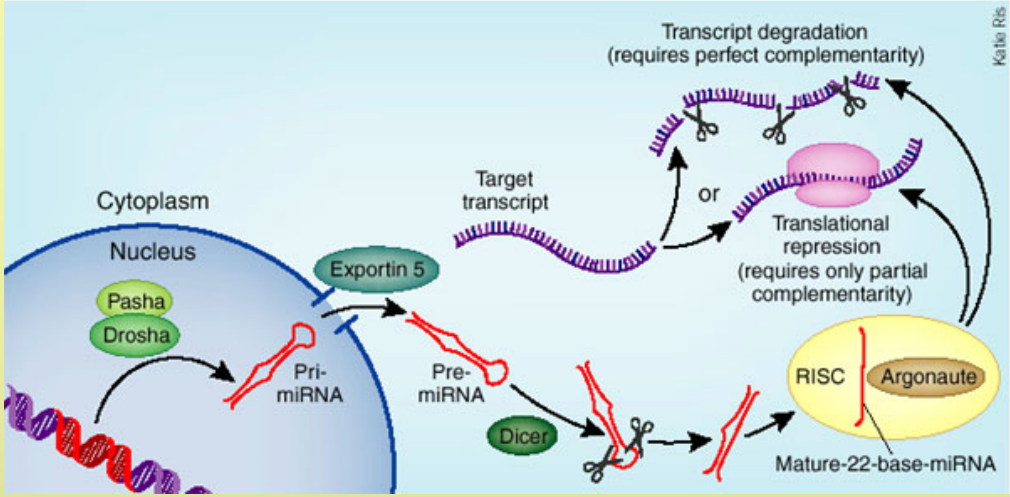


Molekuláris kapcsolók és hálózatok: a rendszerbiológia elemei



Kulcsszavak: kapcsolók és modulok

miRNS analízis

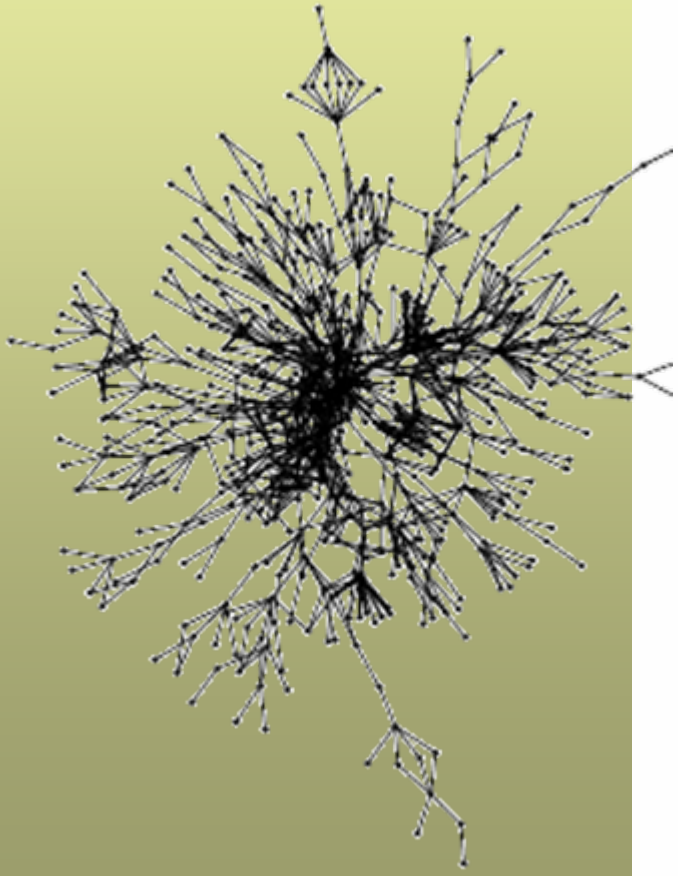


- miRNS: kis 19-25 nukleotid hosszú egyszálú RNS molekulák, amelyek a génexpresszió negatív szabályozásában játszanak szerepet
- a sejtmagban keletkeznek hosszú prekurzor RNSként: *pri-miR*

- emberben számuk meghaladja az ezret -> minden miRNS több száz mRNS 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



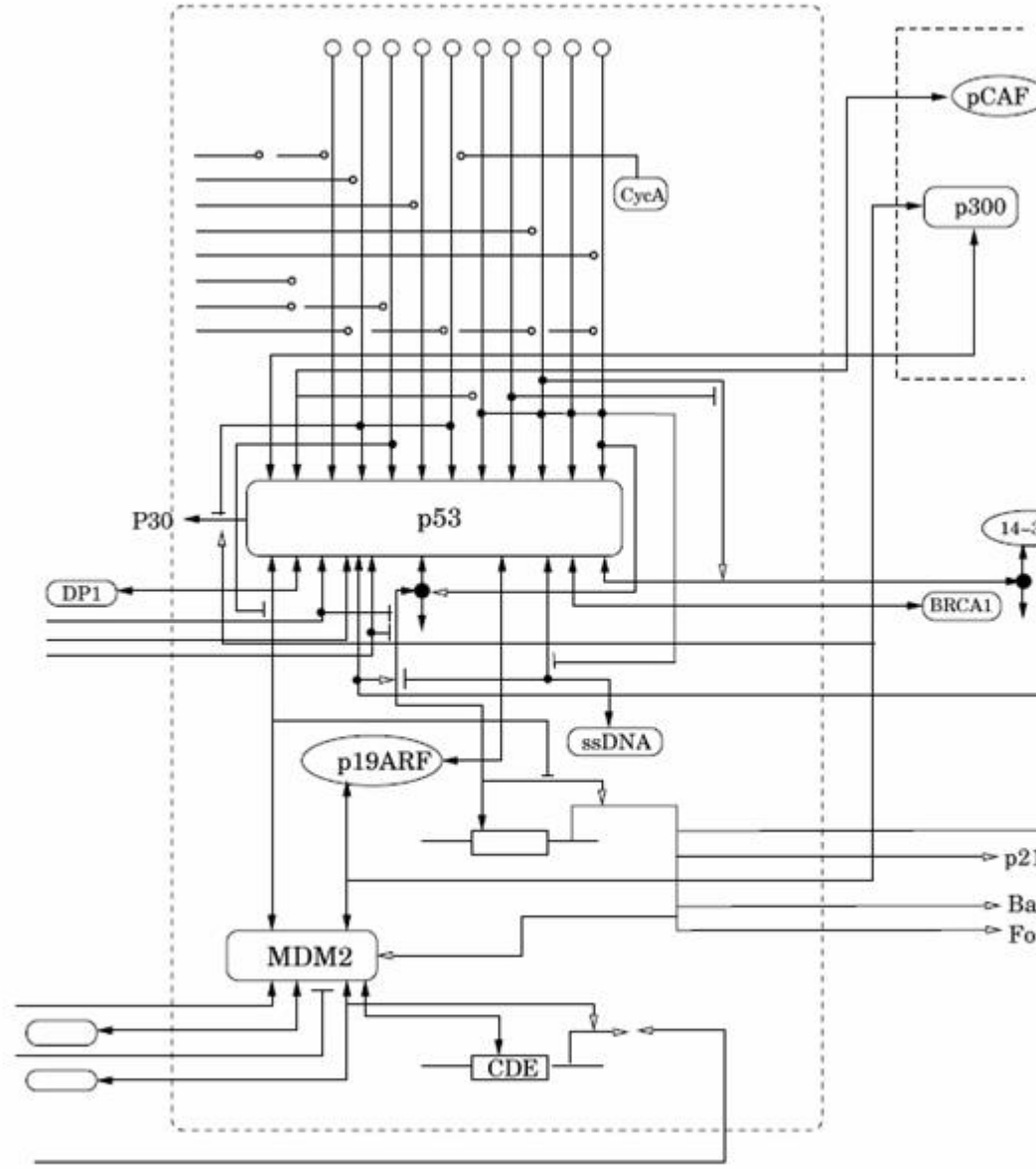


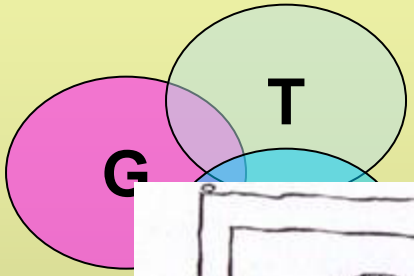
Figure 1.2: Schematic architecture of the p53 network. The p53 node integrates information from very different parts of the system. Only part of the cell circuitry is shown here. For a

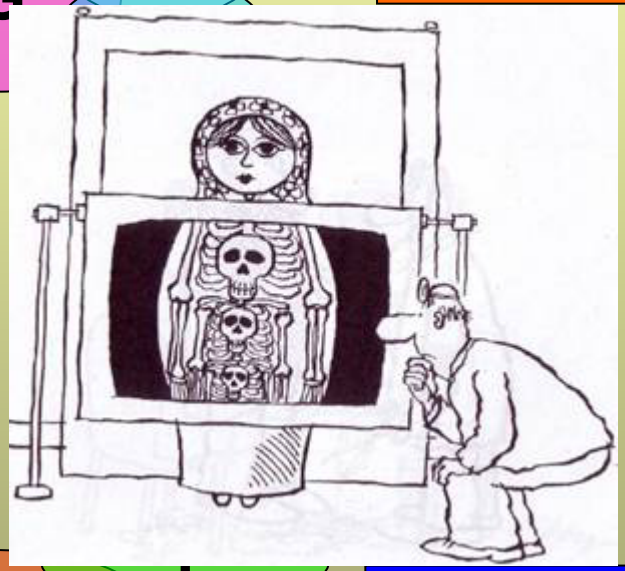
Funkcionális genomika lényege

Környezeti hatások, gyógyszerek, fertőzések

szövet, szerv szint

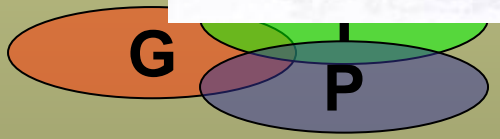
daganat képződés, gyulladás, különböző betegségek





Minőségi és mennyiségi változások

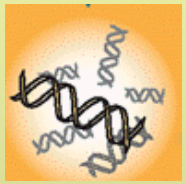
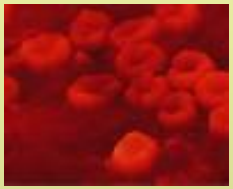
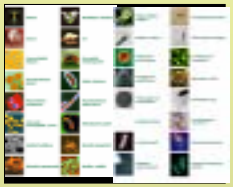
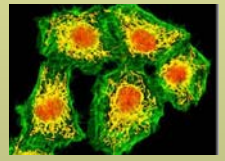
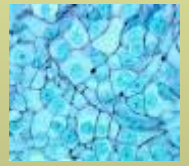
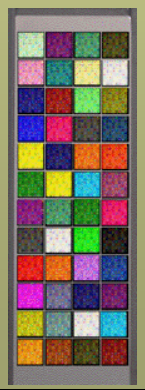
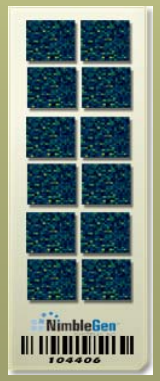
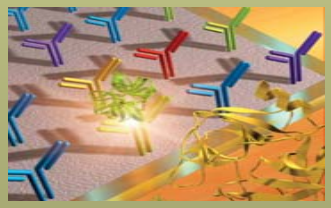
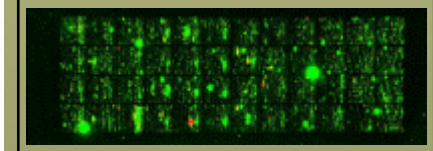
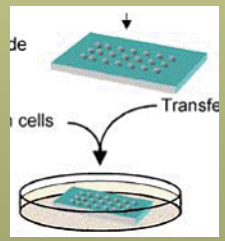
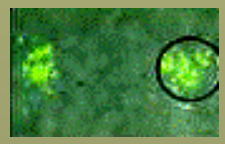
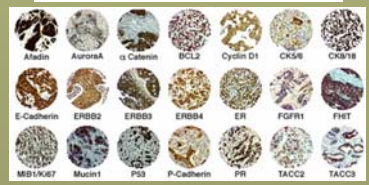
funkciók felderítése



sejtosztódás, differenciáció, sejthalál

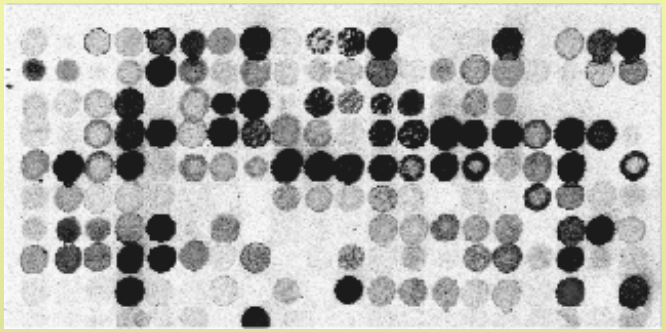
sejt szint

Microarray technológiák a funkcionális genomikában

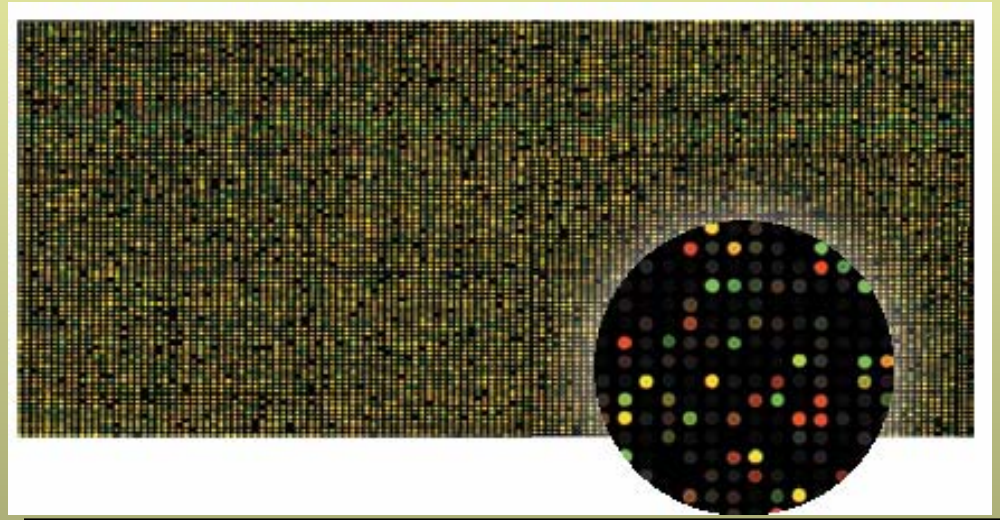
Minta						
	Fragmentek	Szubcelluláris	Sejtek	Szervek	Organizmusok	Közösségek
Cél-molekula						
	DNS, RNS	Fehérjék	Sejtek	Szövetek		
Microarray	   	 	 	 		
	Genomika	Proteomika	Citomika			

A csipek hordozó szerinti osztályozása

macroarray

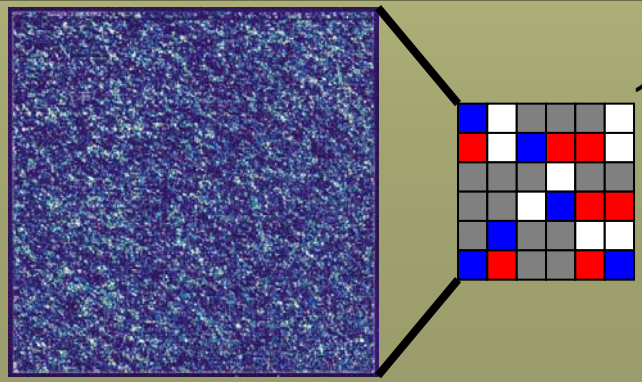


•nylon membránon néhány 100 génspecifikus minta (DNS darab)
•radioaktív jelölés
•kis minta sűrűség
(100-1000 pont/cm^2)



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

chip



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

Egy csipkísérlet általános lépései

cDNS klón és/vagy DNS szekvencia

nyomtatás

Makroarray

Mikroarray

Chip

biológiai minta szövetből, sejtkultúrából

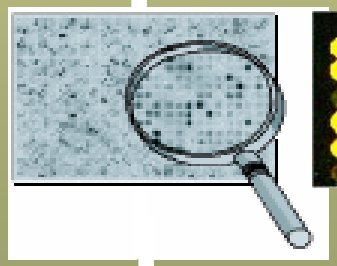
RNS, DNS, fehérje

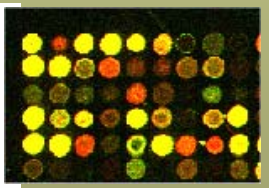
Hibridizálás, fehérje-ellenanyag kölcsönhatás

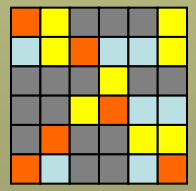
néhány 100, vagy több 1.000, 100.000 gén, fehérje

X

jelölt minta



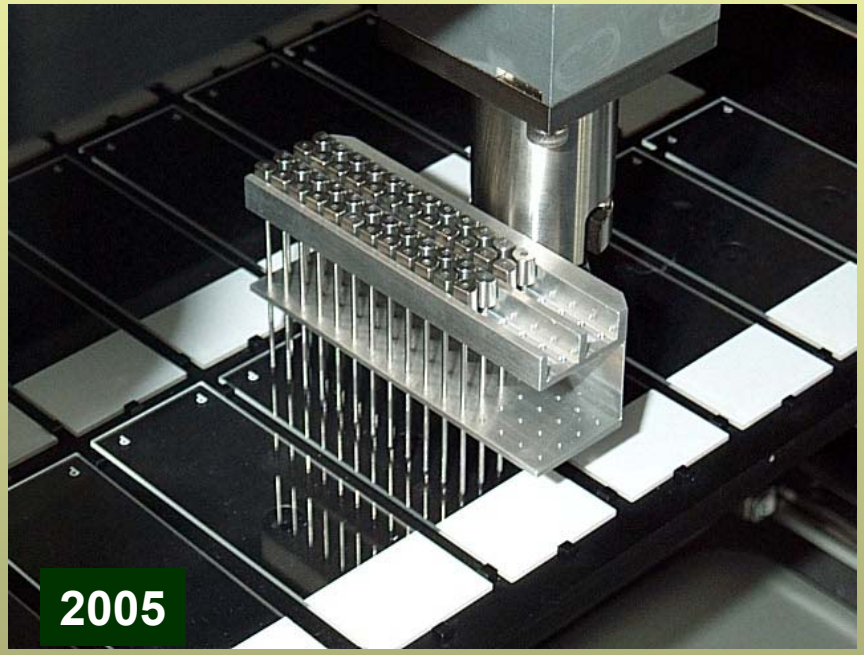


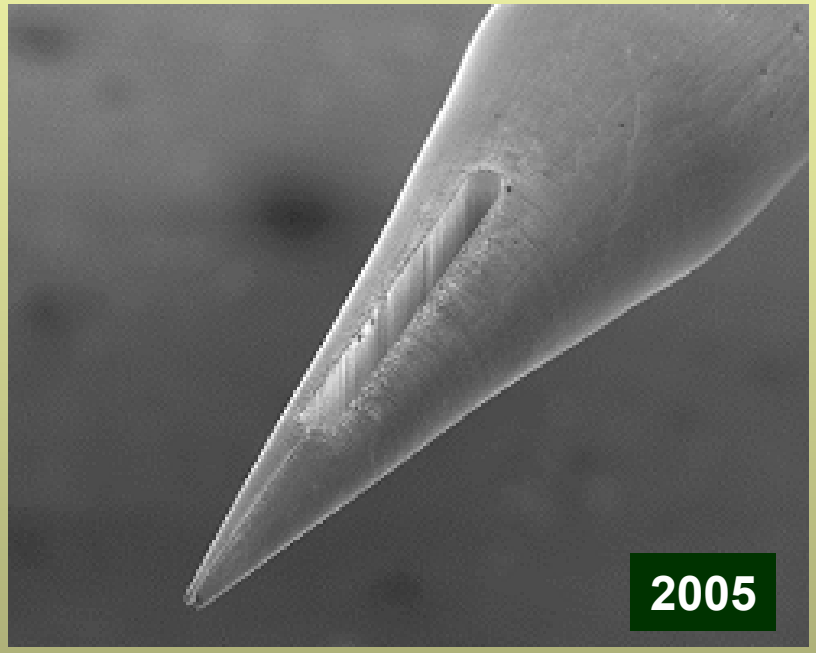


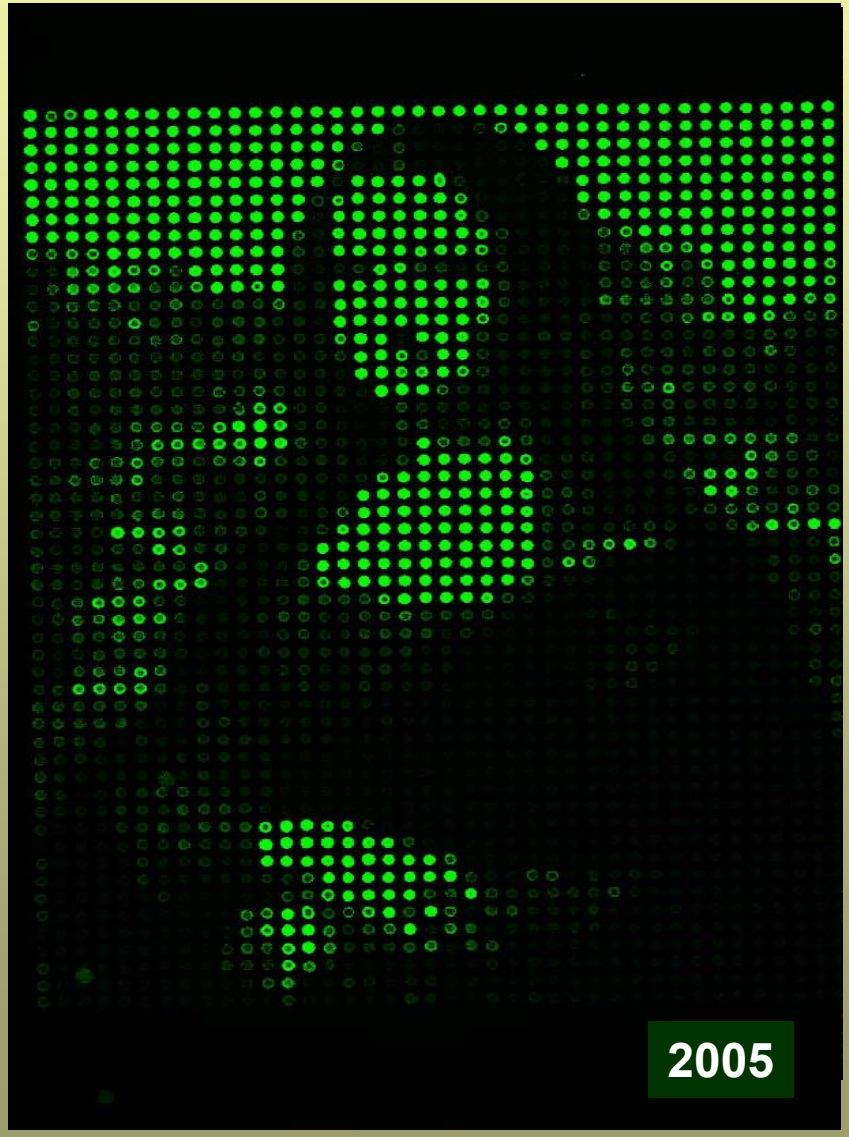
adatfeldolgozás, bioinformatika, génműködésre vonatkozó információk értékelése


MicroGrid
MicroGrid
BioRobotics
2005

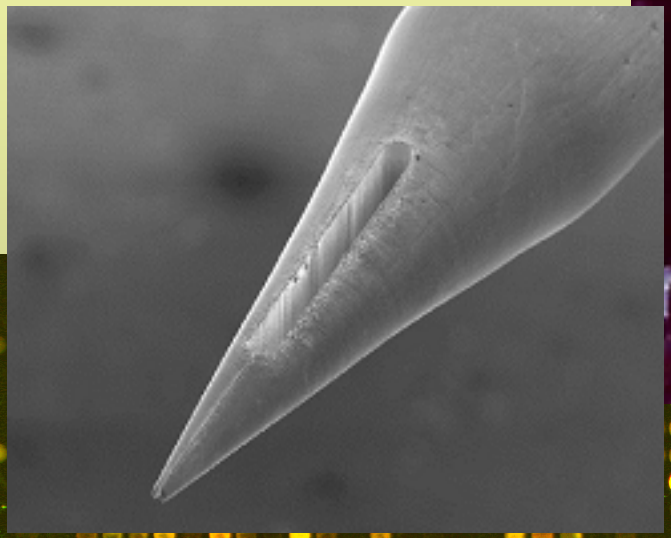

2004

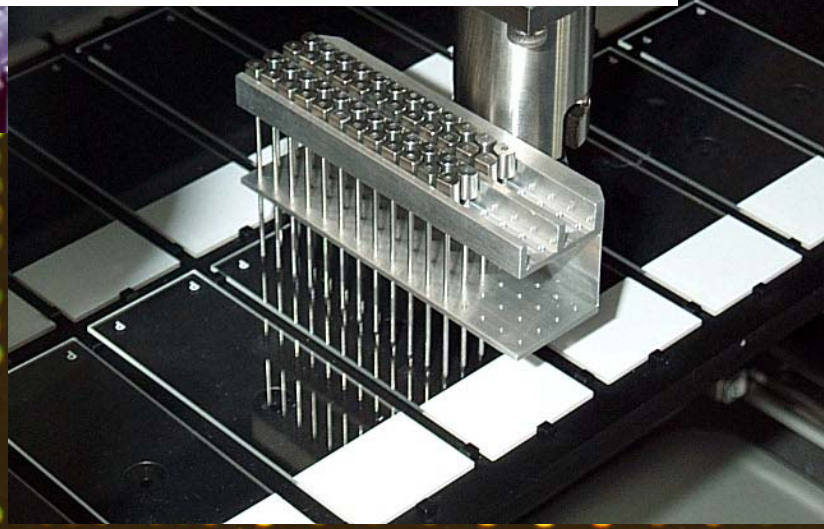

2005

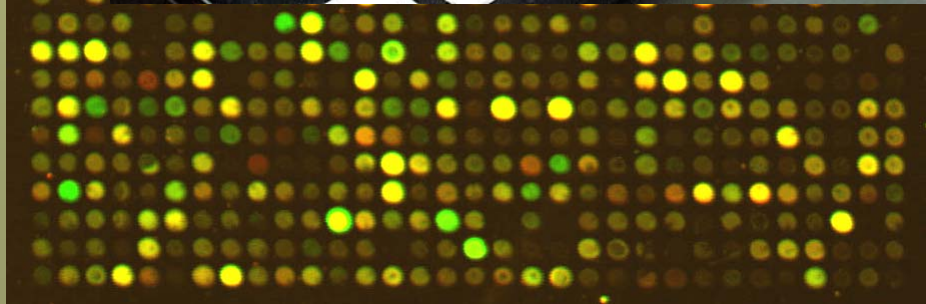

2005


2005

DNS-chipek/DNS-microarrayek készítése

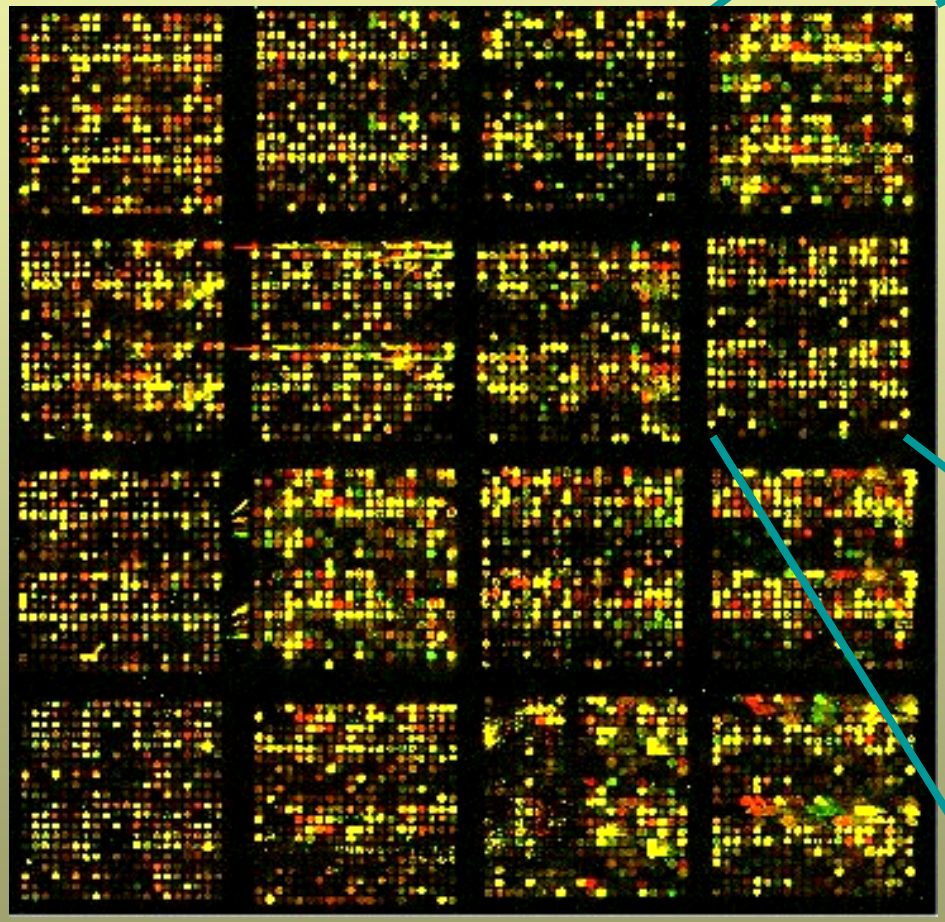


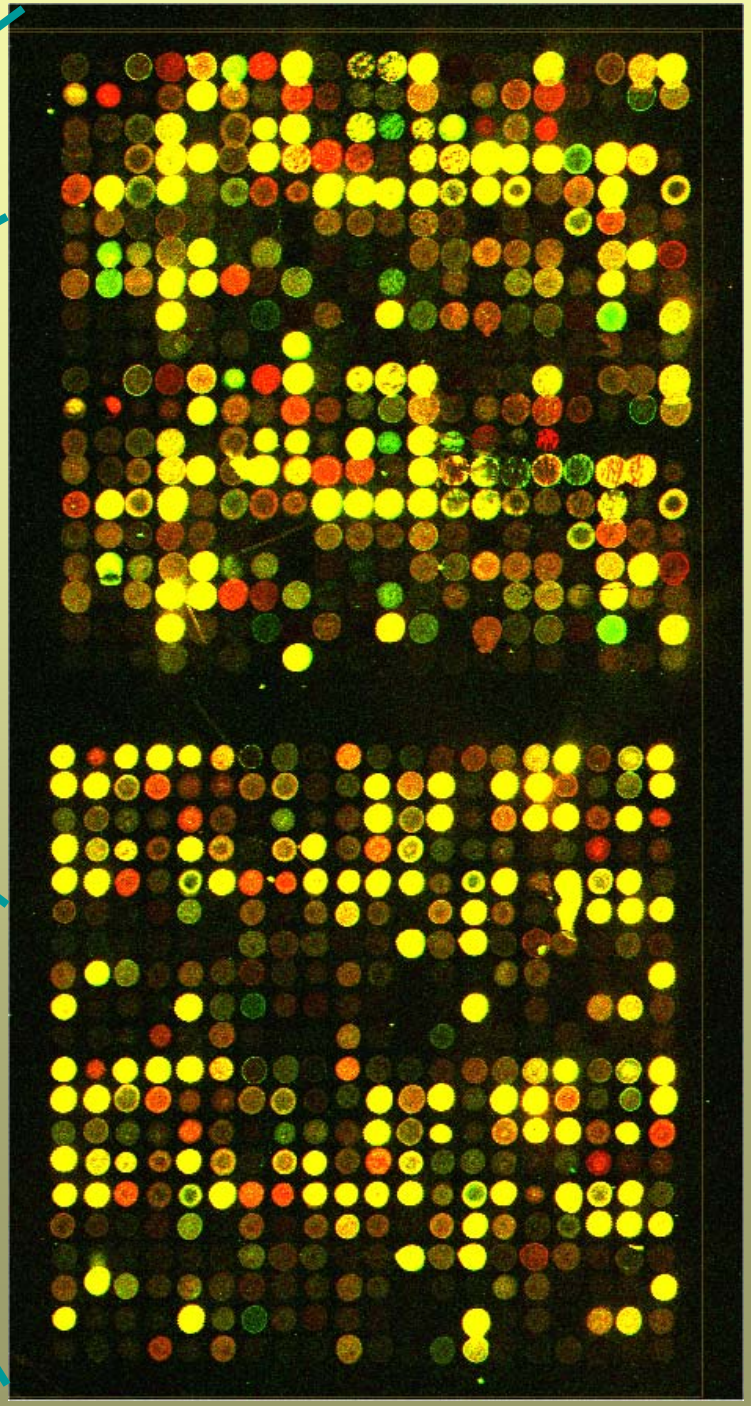




Laboratory of Functional Genomics.BRC, Szeged

Human-I
Mouse-I
Rat-I
DNS-chip

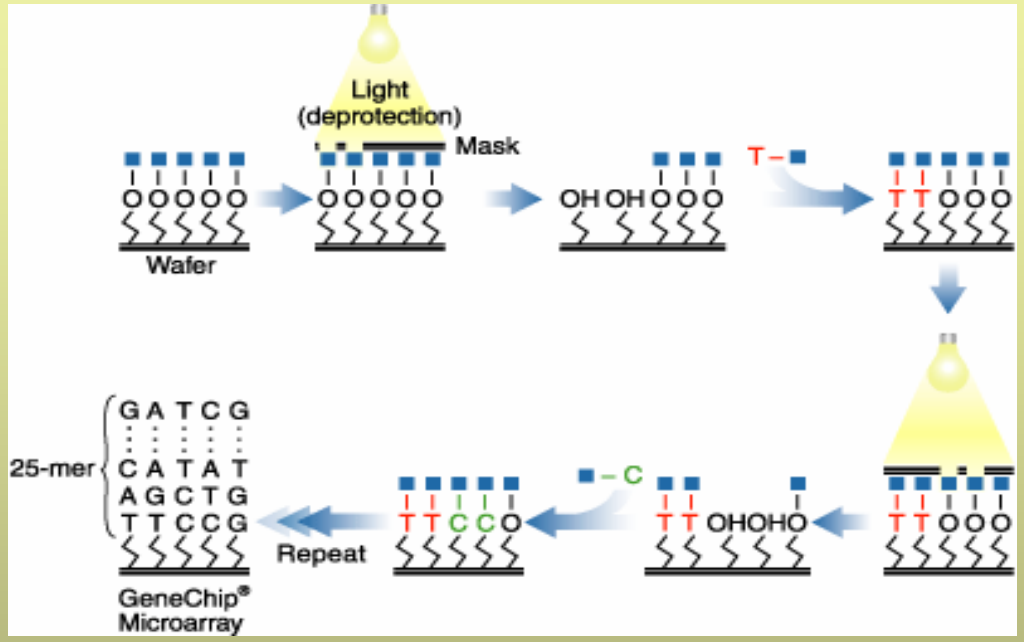


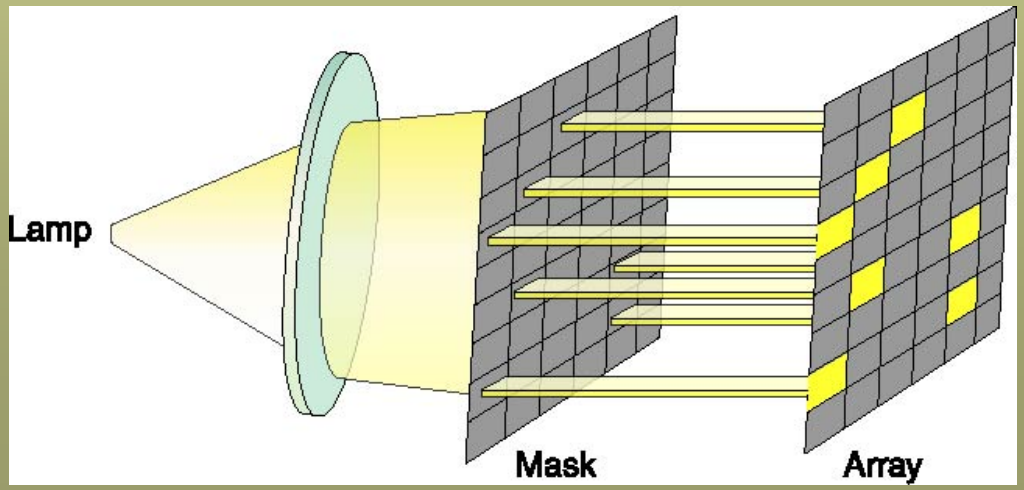


Oligonukleotid alapú chip készítése in situ szintézis

Affymetrix

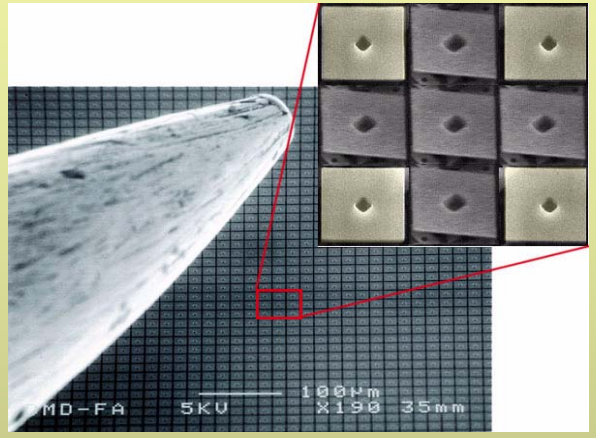
CHIPKÉSZÍTÉS





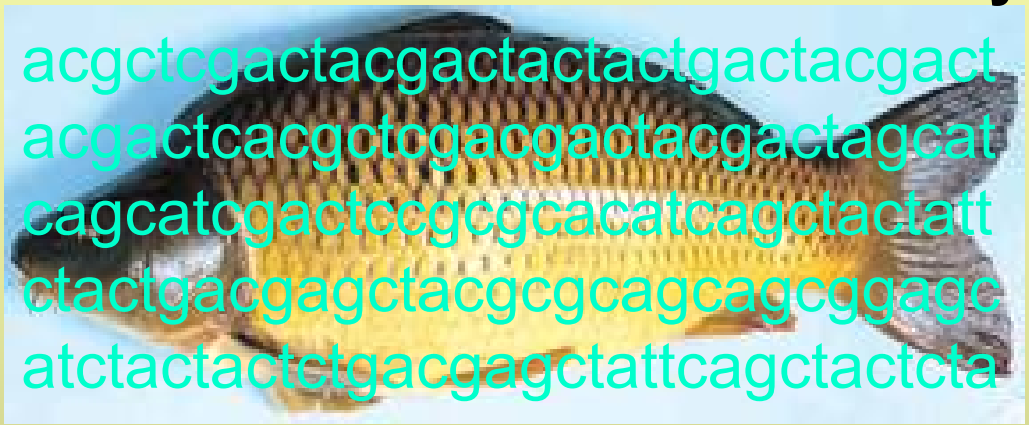
Alternatív technológiák oligonukleotid DNS-chipek gyártására

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

homology search Blast

sequence comparisons

EST contig construction

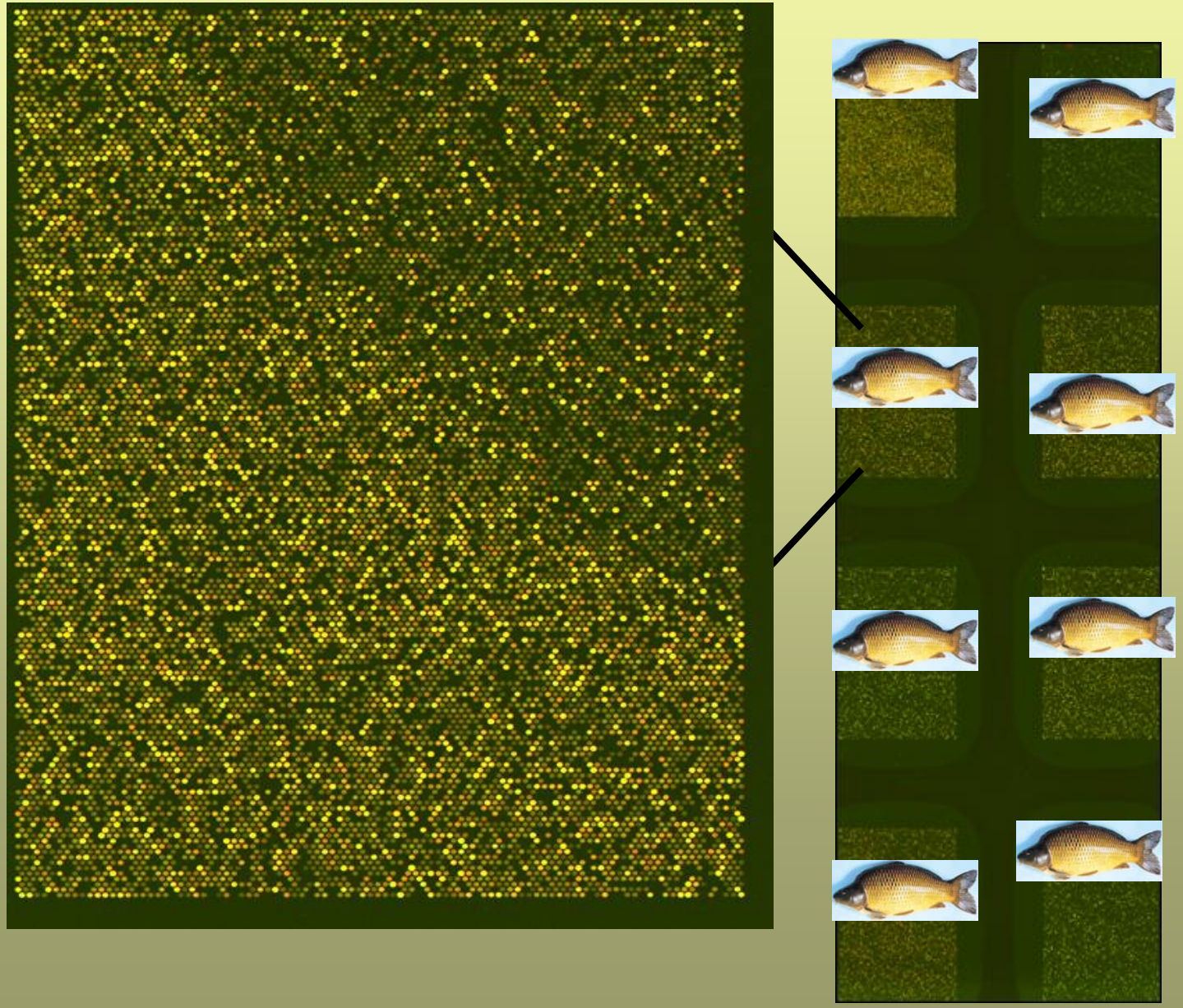
Probe Design

15.000 gene-specific oligonucleotide sequences

DNA-microarray construction

15.000 cured ESTs, functional groups, gene families, annotation

Ponty DNS-microarray formátum



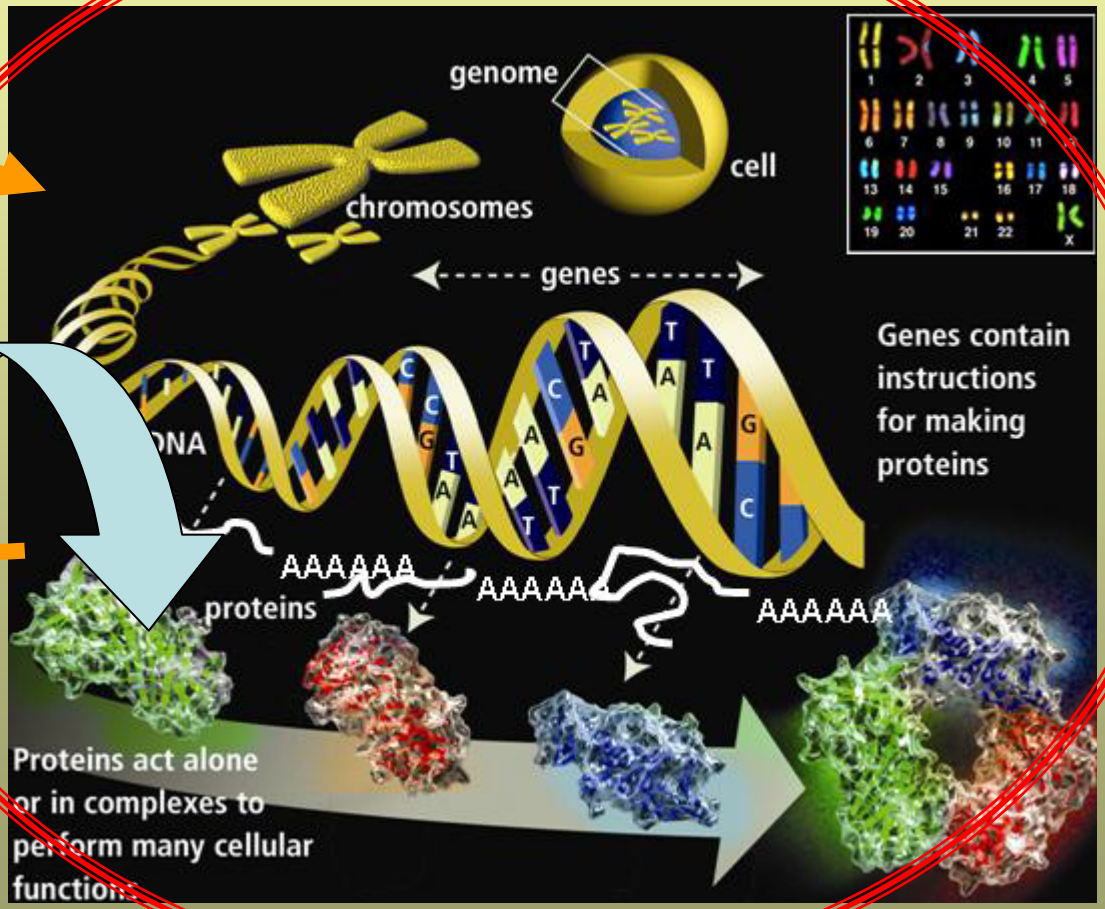
Analysis of
8 carps in
1 microarray
experiment
15.000 gene
analysis
each

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

hipoxia stressz

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

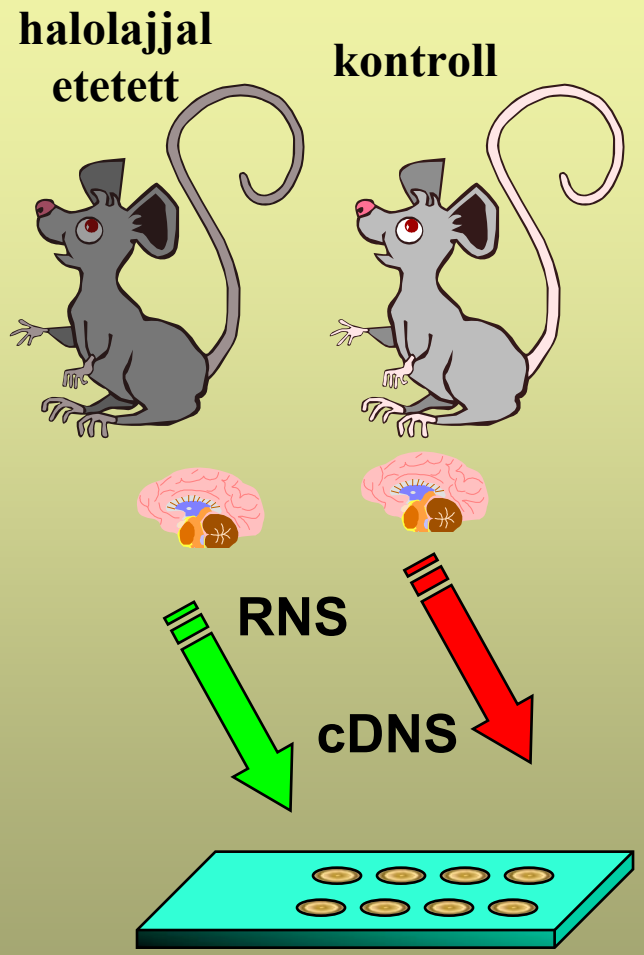
A táplálék kölcsönhatása a génekkel, génállománnyal



Rövidtávú és Hosszútávú hatások sejtszintű és Fiziológiás változások

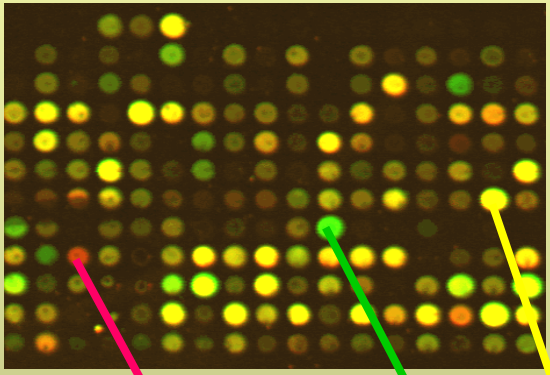
Kitajka et al. (2002) PNAS 99, 2619;
Barcelo-Coblijn, G (2003) *PNAS 100, 11321.*
Kitajka (2004) PNAS 101, 10931
Puskás et al. (2003) *PNAS* 100, 1580-1585.
Puskás et al. (2004) Biochimie 86, 817.
Jayasooriya et al (2005) *PNAS* **102**, 7133.
Puskas et al. (2006) *Curr Pharm Biotechnol*. 7, 525
Puskas 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

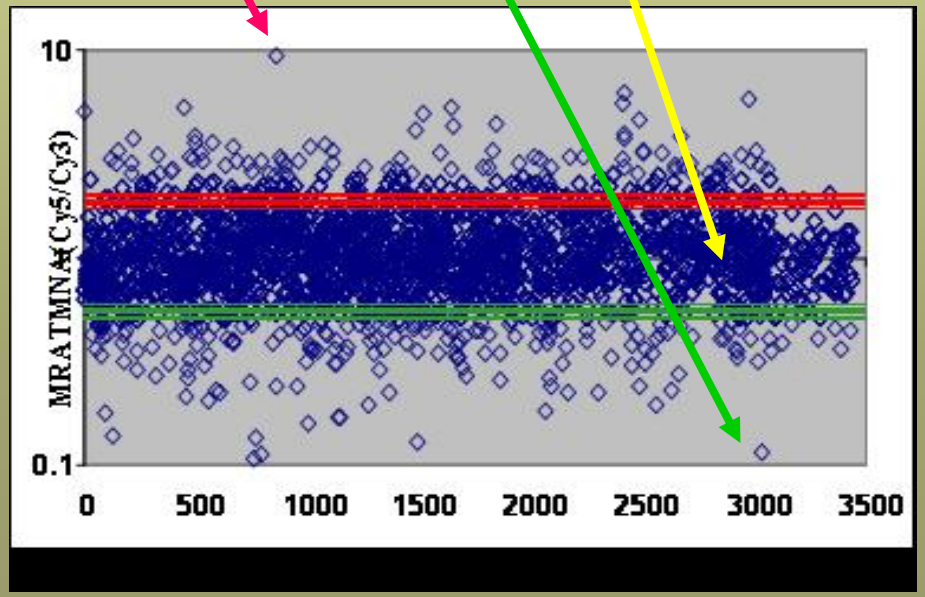


patkány cDNS-chip
3200 génspecifikus minta
6400 mintapont

Génexpressziós változások



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



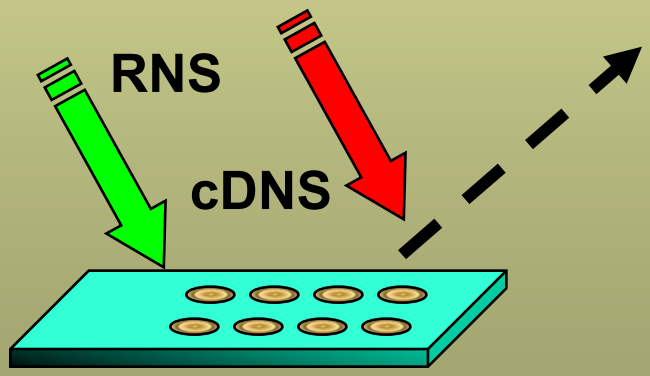
Pozitív hatás a mentális funkciókra, Tanulás, memória

Antidepresszánsok hatásának vizsgálata DNS- chip technológiával patkány agyban

kontroll

kezelt
haloperidol,
riszperidon

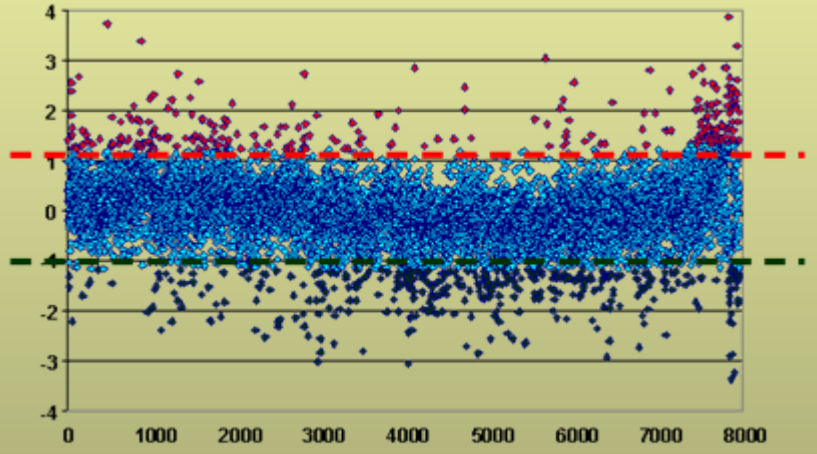
temporális agykéreg



patkány cDNS-chip
3200 génspecifikus minta
6400 mintapont

LEHETŐSÉGEK

Hatásmechanizmus, kinetika
(akut és krónikus kezelések) terápiás-toxikus dózis
potenciális gyógyszerek, gyógyszercélpontok felderítése



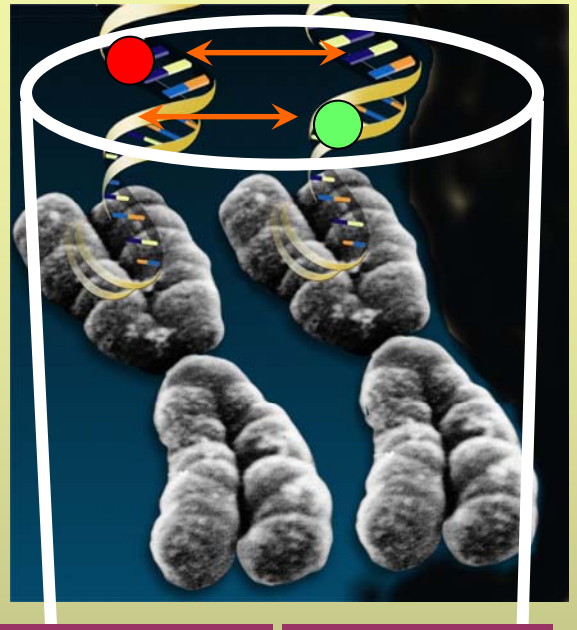
Génexpressziós változások

Jelátviteli folyamatok (Erk-2 kinase, ryk-related, Janus prot. Tyk 1)
Szerkezeti fehérjék (tubulin, fibronectin)
Fehérje metabolizmus (proteasomal subunits, ubiquitin proteins)
Sejt túlélés, apoptózis (midkine, stress-inducible protein)
Szinaptikus plaszticitás, növekedési faktorok, Ca-háztartás
(follistatin-like protein, basic fibroblast growth factor, pleiotrophin)

Feher LZ, Kalman J, Puskas LG et al. (2005) *Neurochem. Int*. **47,** 271.

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 chipek
•egy nukleotid eltérés azonosítása

Jelölt DNS

hibridizáció

mosás

1. SNP 3 pozíció

2. SNP 2 pozíció

A G T C

A G T C

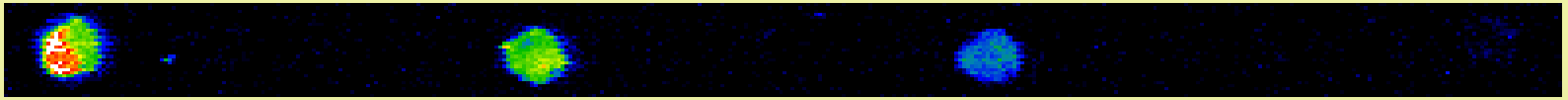
DNS

detektálás

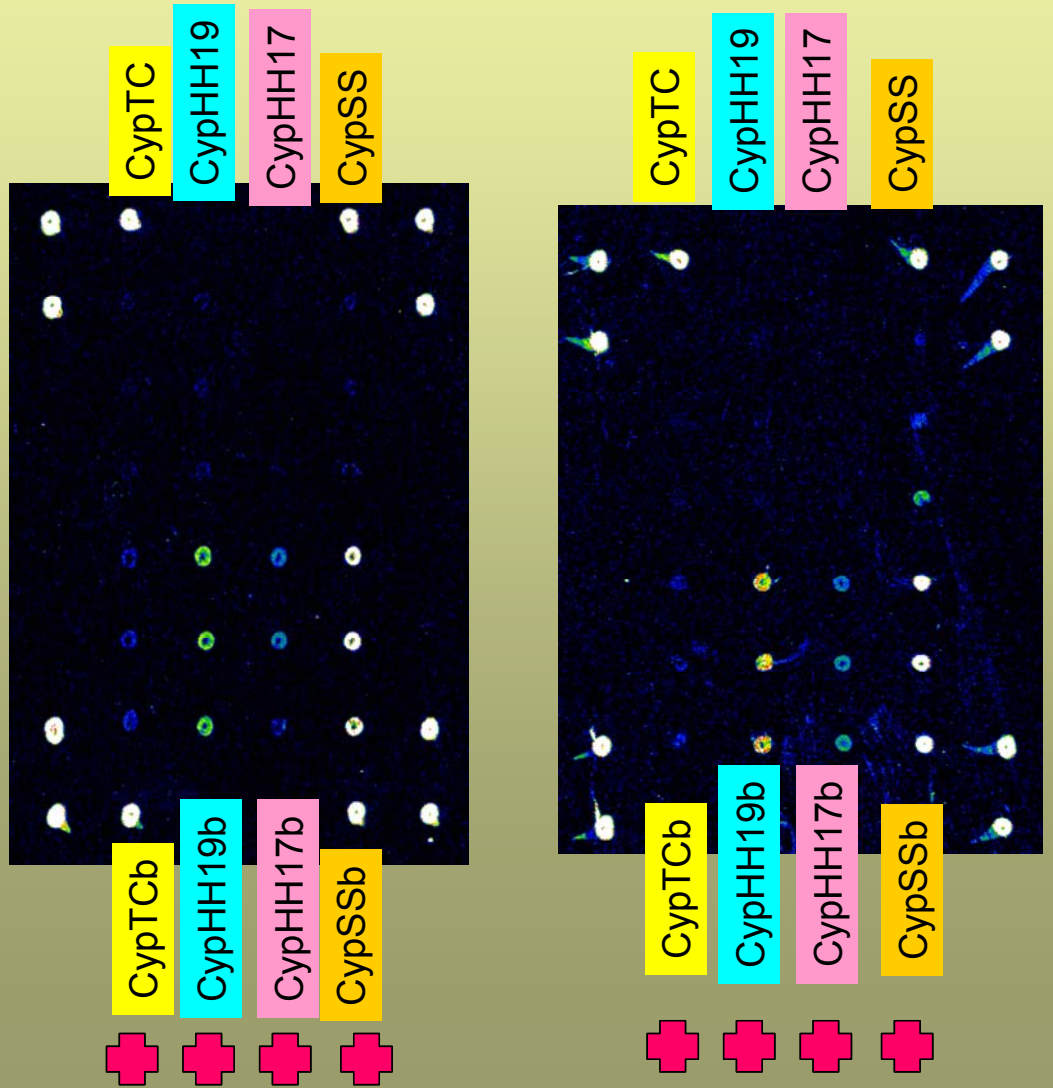
Adat analízis
1. SNP: 3. pozíció A-C
2. SNP: 2. pozíció C-T

DNA-chip Lab.BRC, Szeged

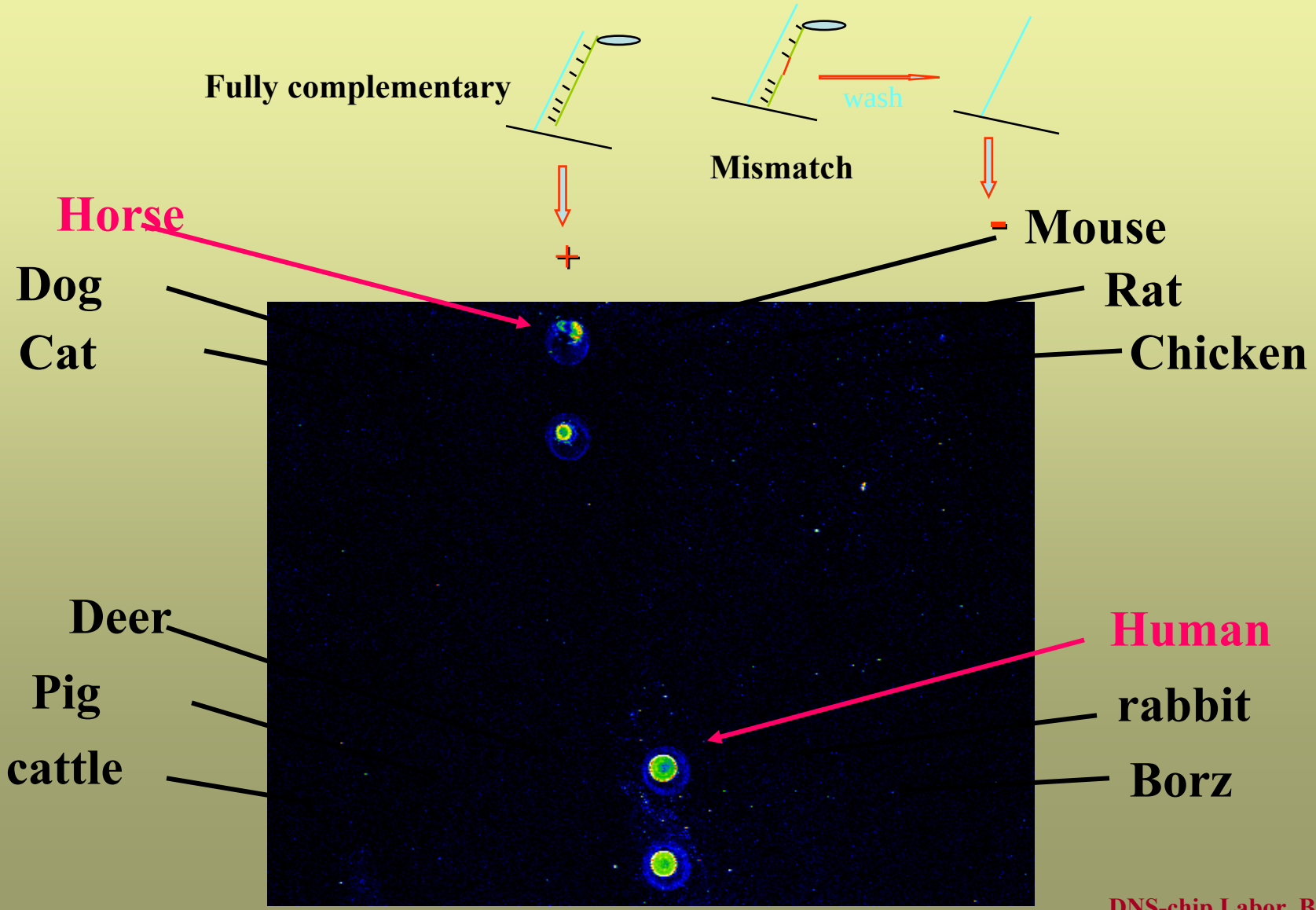
Mutációk detektálásának specificitása



Komplementer 1 mutáció 2 mutáció 3 mutáció



Fajmeghatározás mitokondriális cytB polimorfizmus alapján

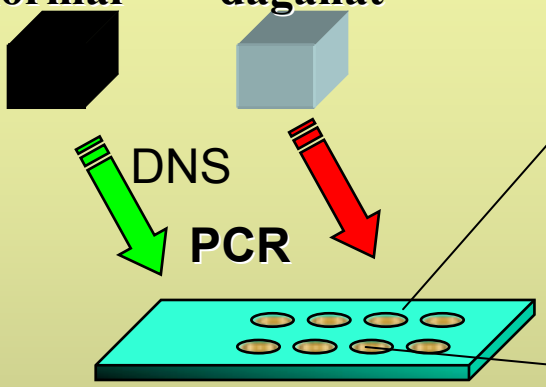


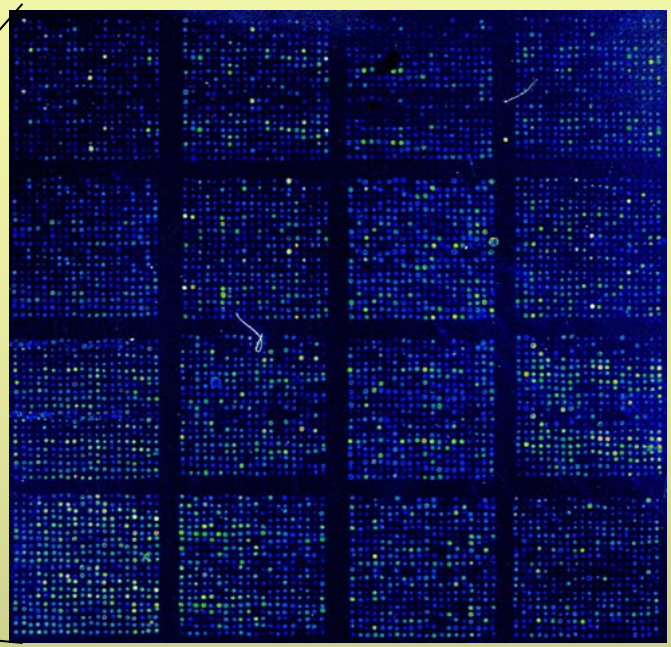
DNS-chip Labor, BRC.

Genomszintű változások,
kromoszóma rendellenességek,
amplifikációk, deléciók detektálása

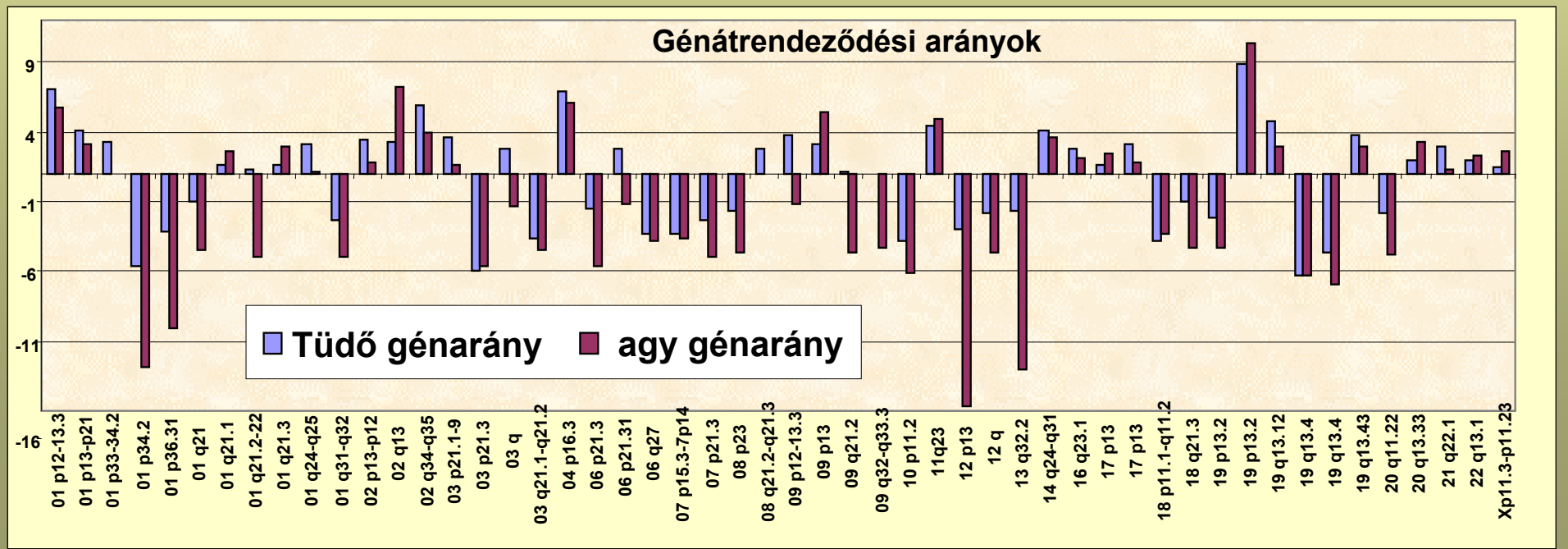
Paraffinba ágyazott minták

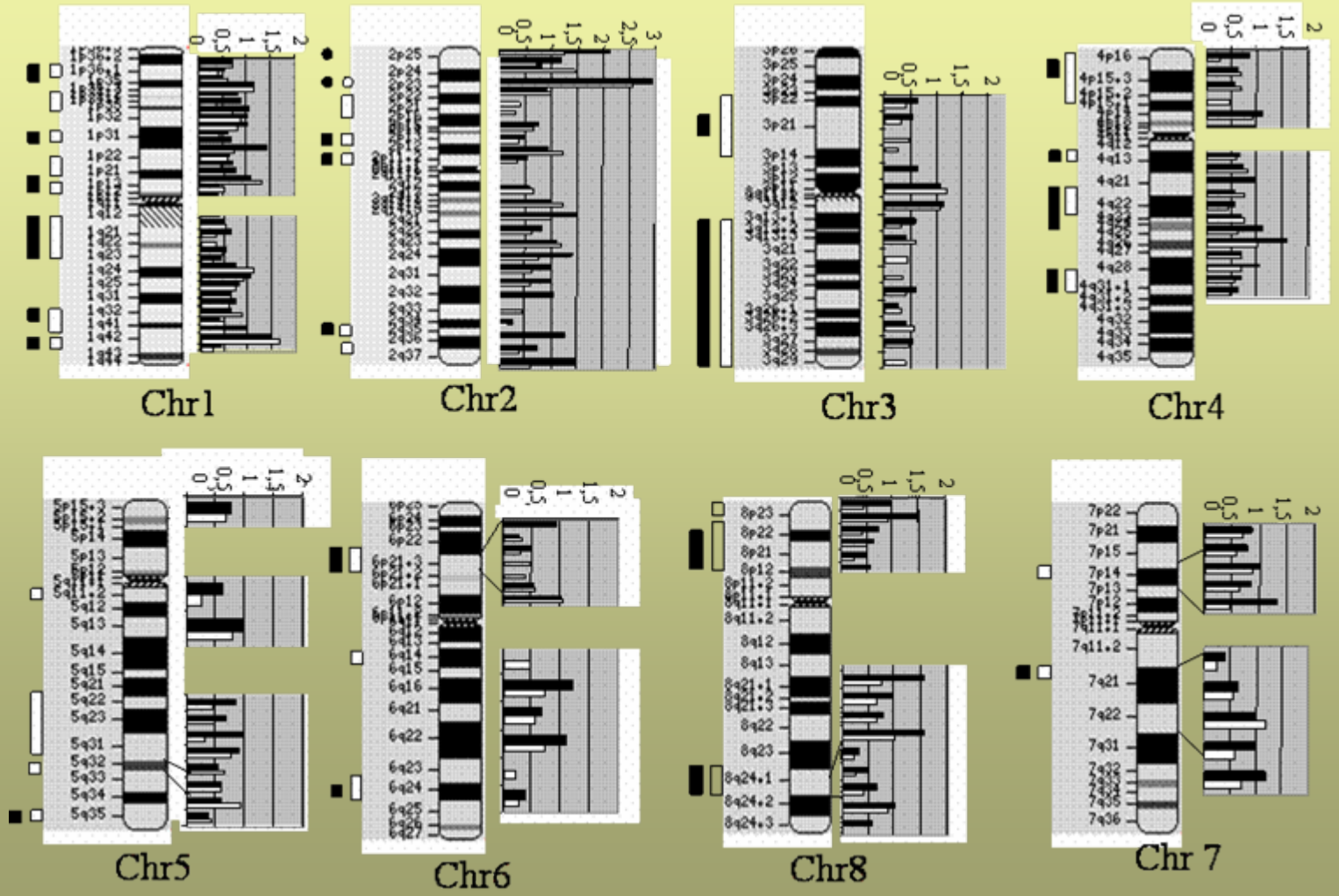
normál **daganat**



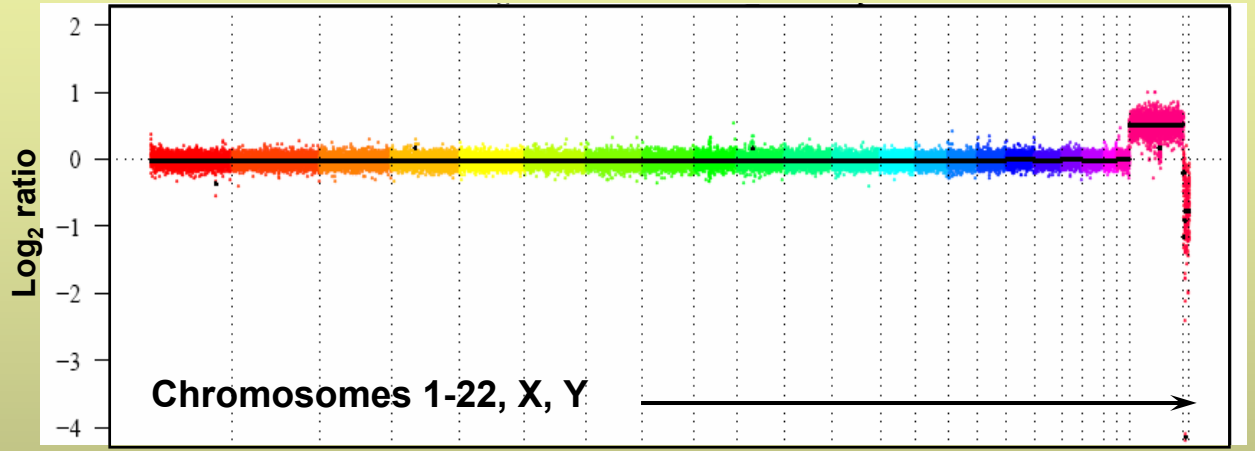


Metasztázisok, tumorok, multiplex tumorok jellemzése, igazolása

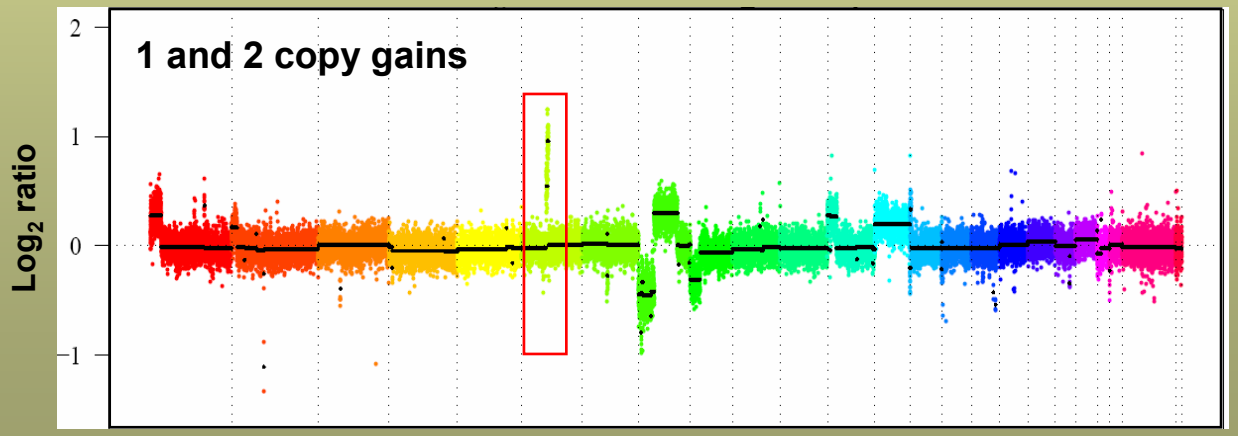



Chr1
Chr2
Chr3
Chr4
Chr5
Chr6
Chr8
Chr 7

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

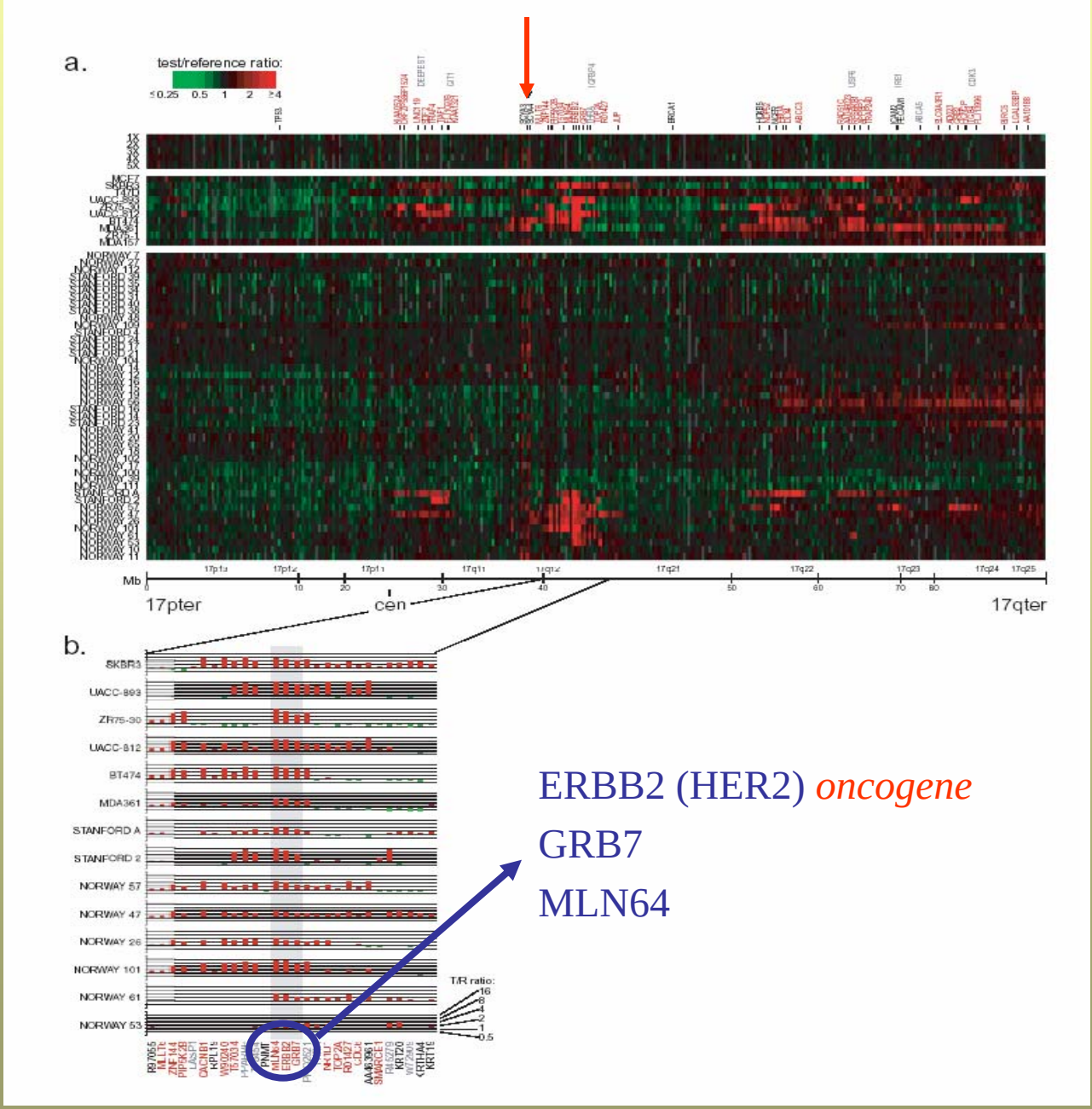


Nő vs. férfi gDNS



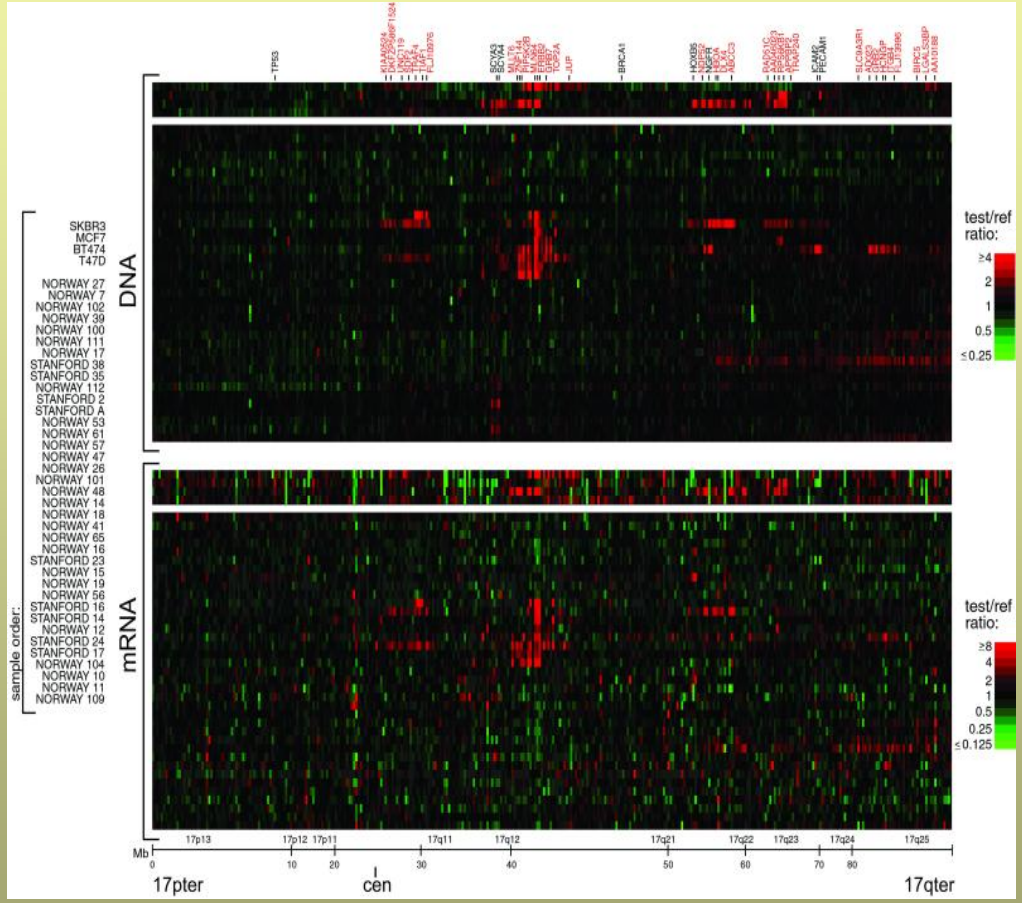
Pajzsmirigy tumor

Variation in copy number mapping to chr 17

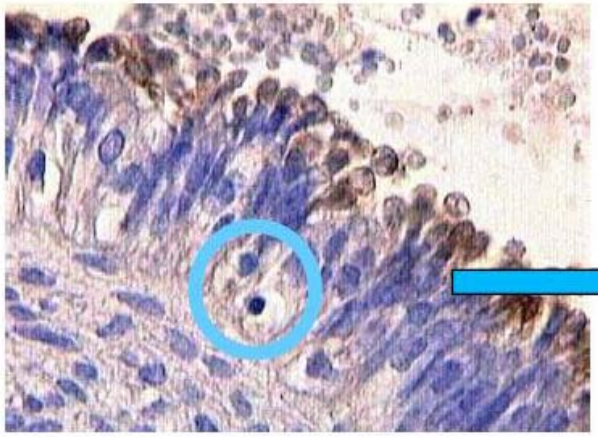


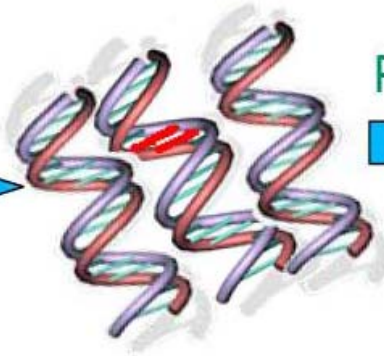
Párhuzamos mRNS szint vizsgálat

- 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 GGG TAC
AGC TCC TGG TAC
AGC TCC AGG TAC

KRAS mutaton correlates with resistance to e.g.Erbitux

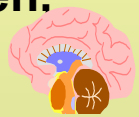
Drug resistance

Újgenerációs szekvenálási stratégiák

Átfogó fehérjekifejeződés és fehérjemódosítások vizsgálata fehérjecsip technikával

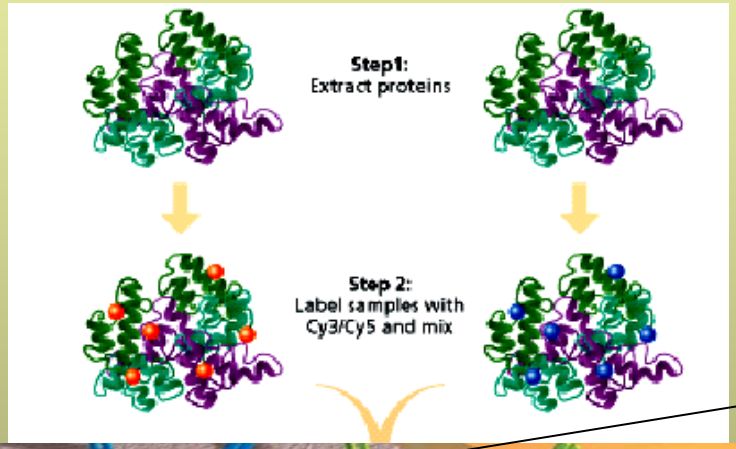
Fehérje-chipek - eltérően kifejeződő vagy módosított fehérjék azonosítására

Kezeletlen, kontroll

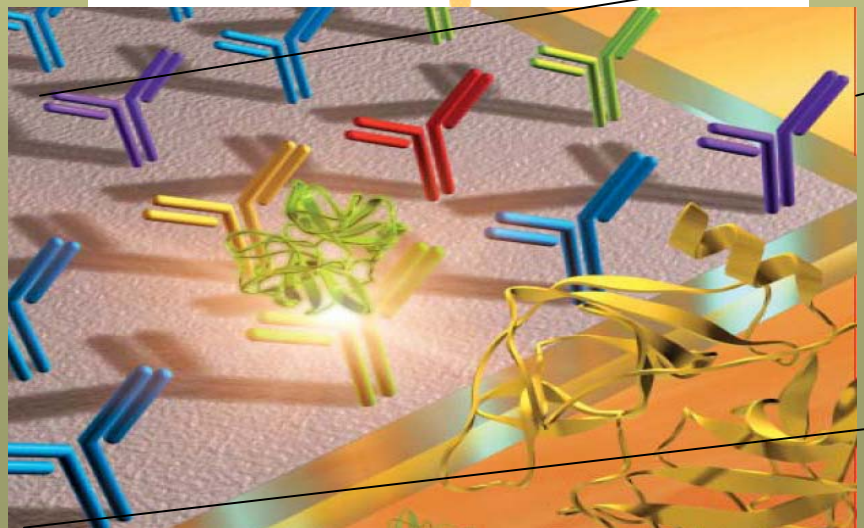


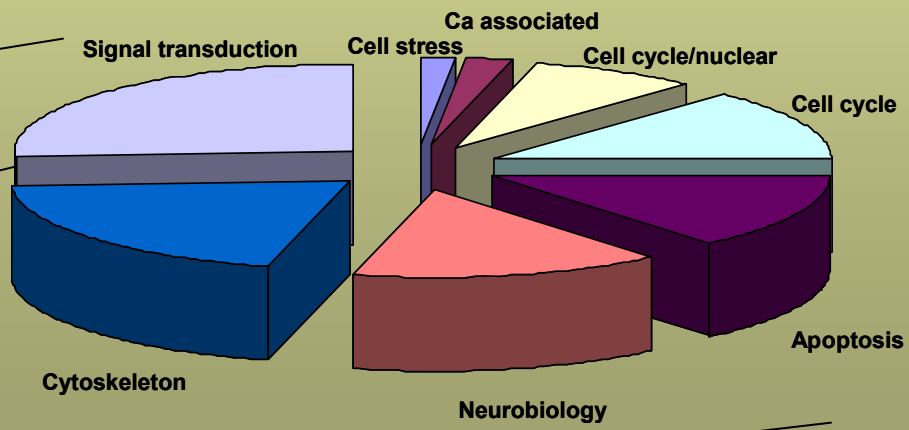
Kezelt

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

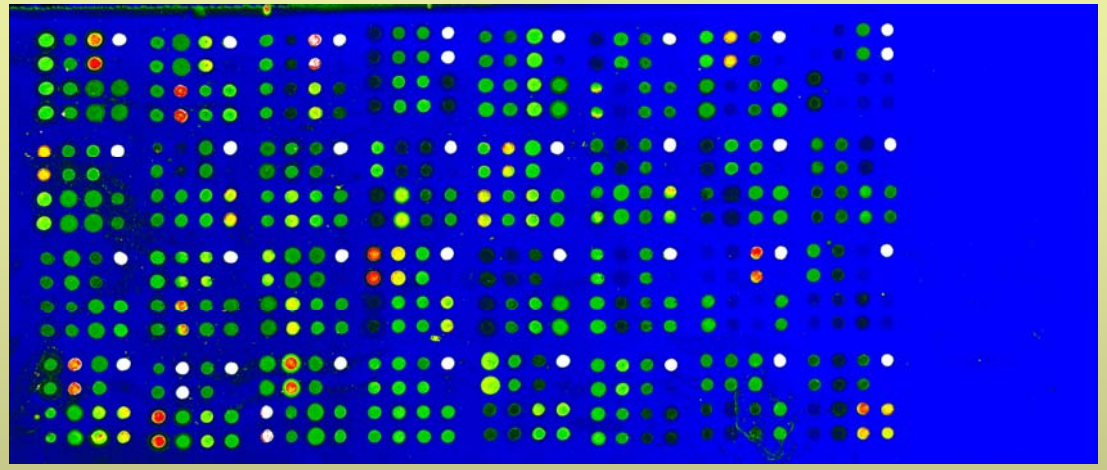


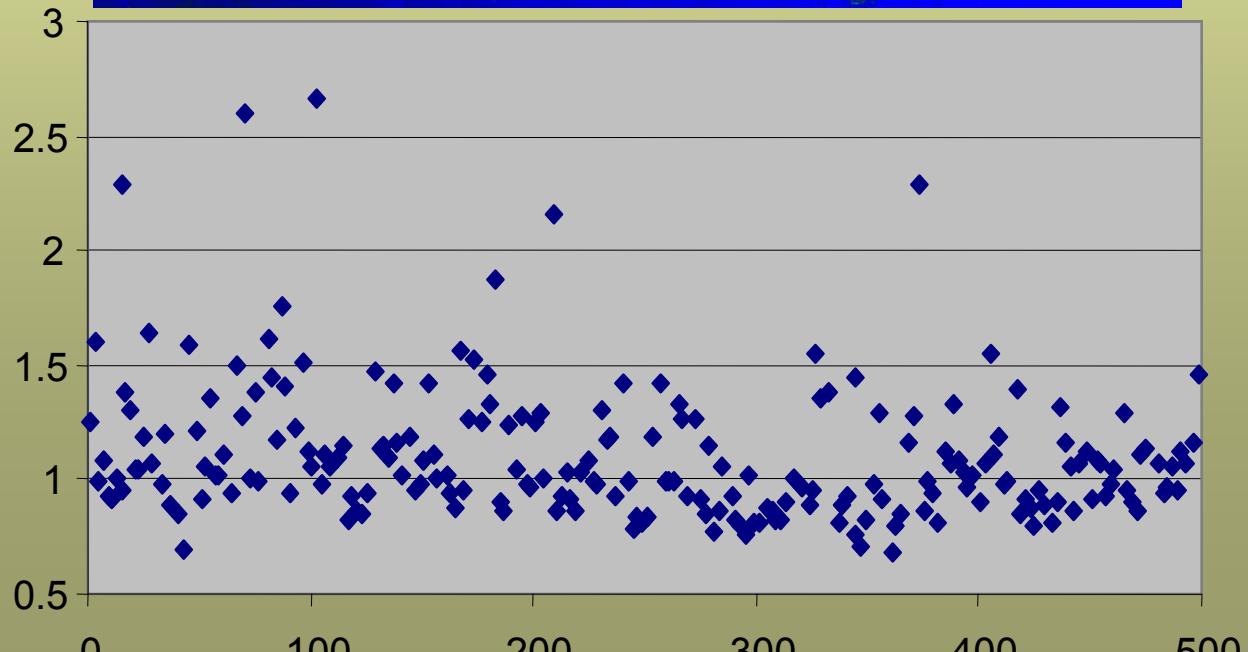
Antitestek, amelyek a jelátviteli és egyéb folyamatokban résztvevő fehérjéket ismeri fel





Tüdőtumor fehérjecsipes vizsgálata





- cdk5
- Caspase 7
- chk1
- HSP90
- Adaptin
- cdc26
- CyclinE

- Synaptotagmin
- Caspase11
- Nicastrin

Specificitás és érzékenység meghatározása

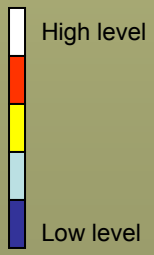
Caspase 9

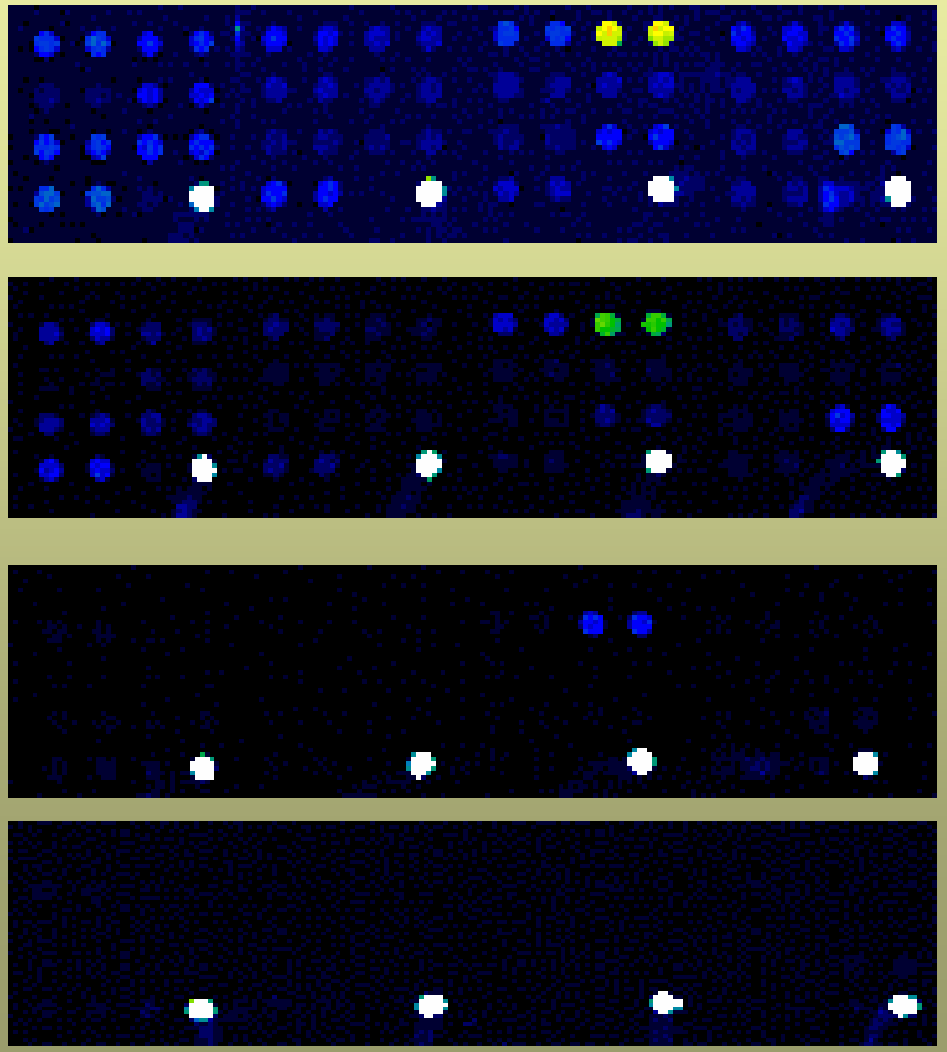
100ng/ml

30ng/ml

3ng/ml

buffer



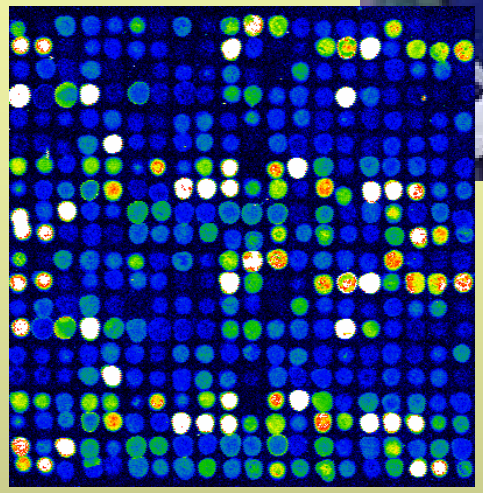


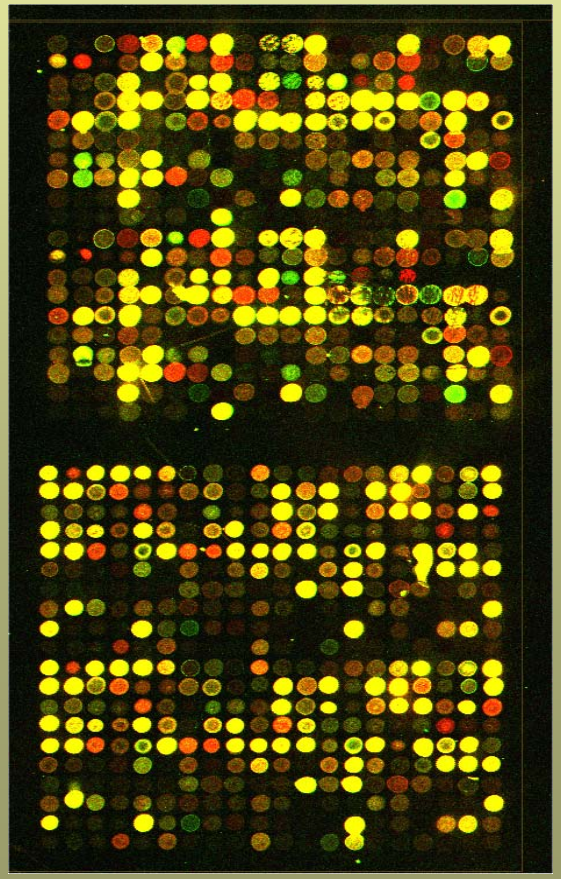
Chip kísérletek adatanalízise
elemzések,
bioinformatikai megoldások

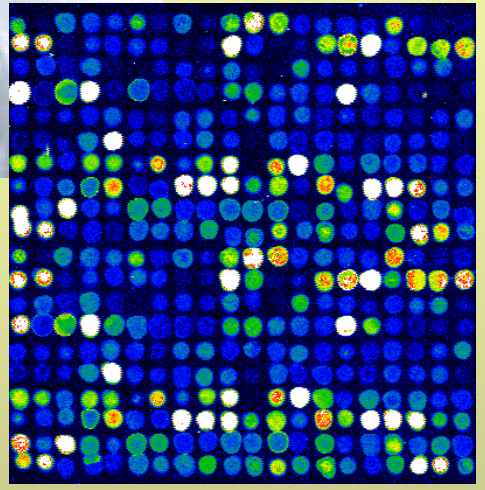
Lézer szkenner
Cy5

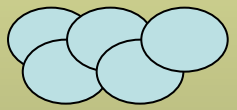
Képanalízis

Lézer szkenner
Cy3





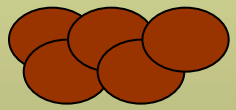




beteg, gyógyszerrel kezelt, különböző környezeti hatásoknak kitett sejt vagy szövet

Cy5 dCTP

jelölt cDNS

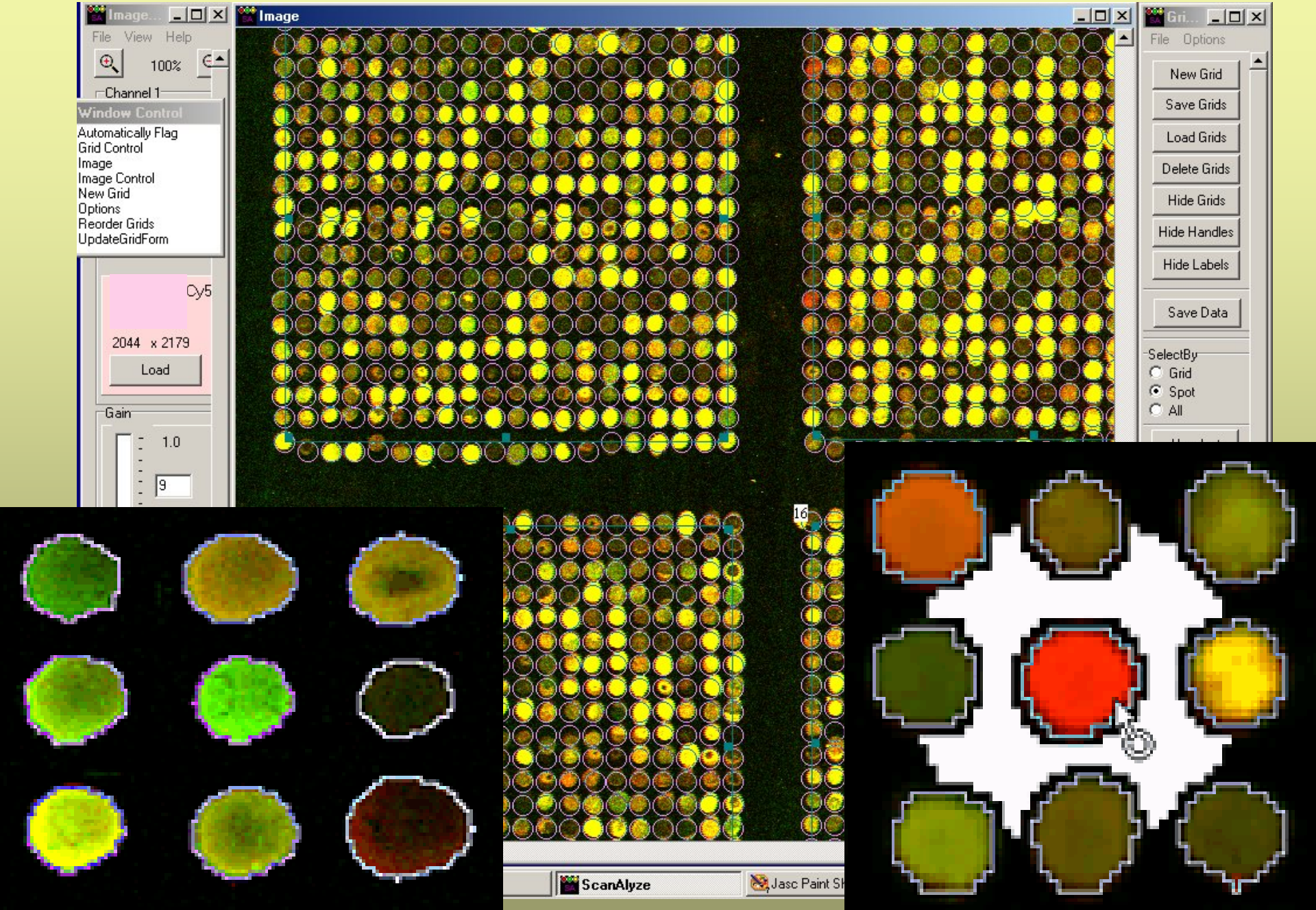


kontroll minta (egészséges, kezeletlen) sejt vagy szövet

Cy3 dCTP

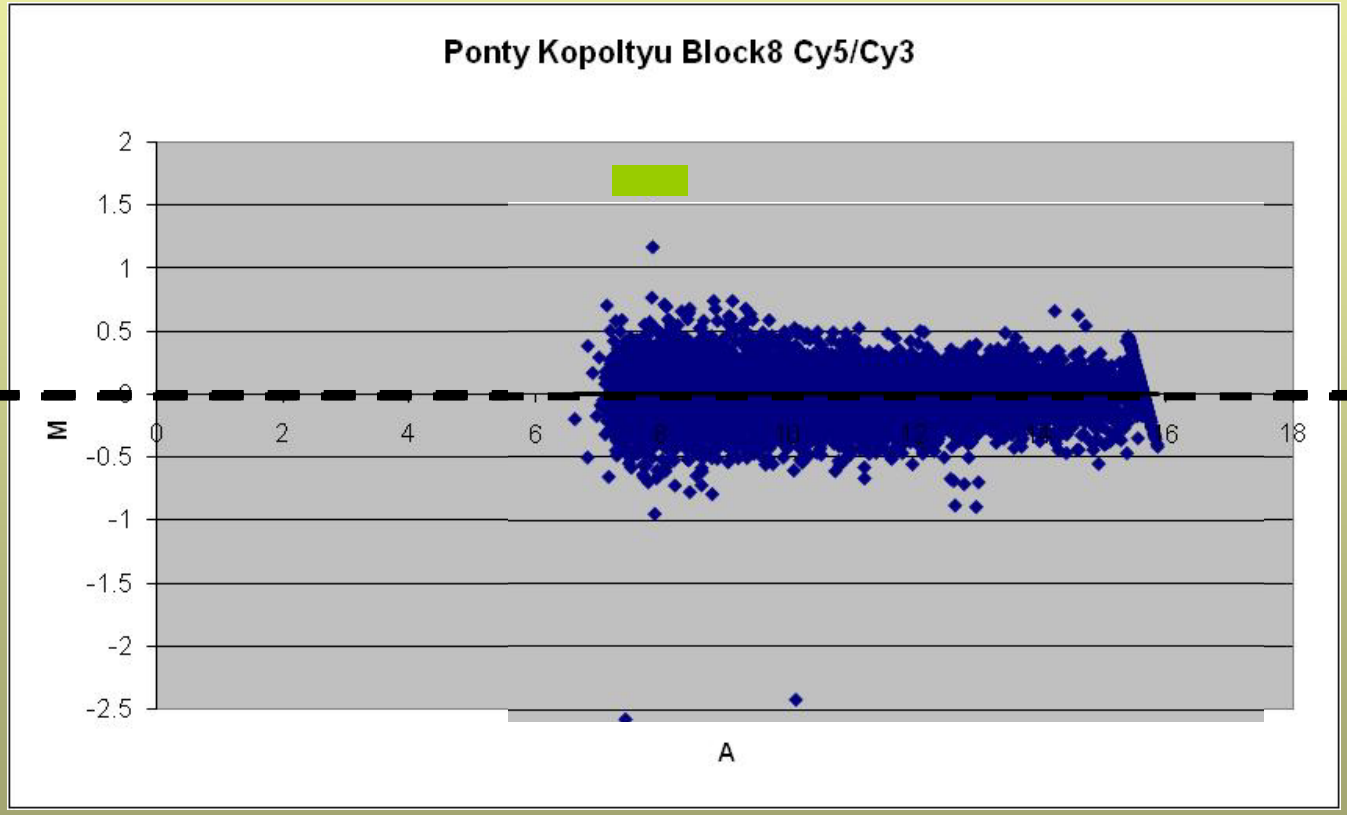
jelölt cDNS

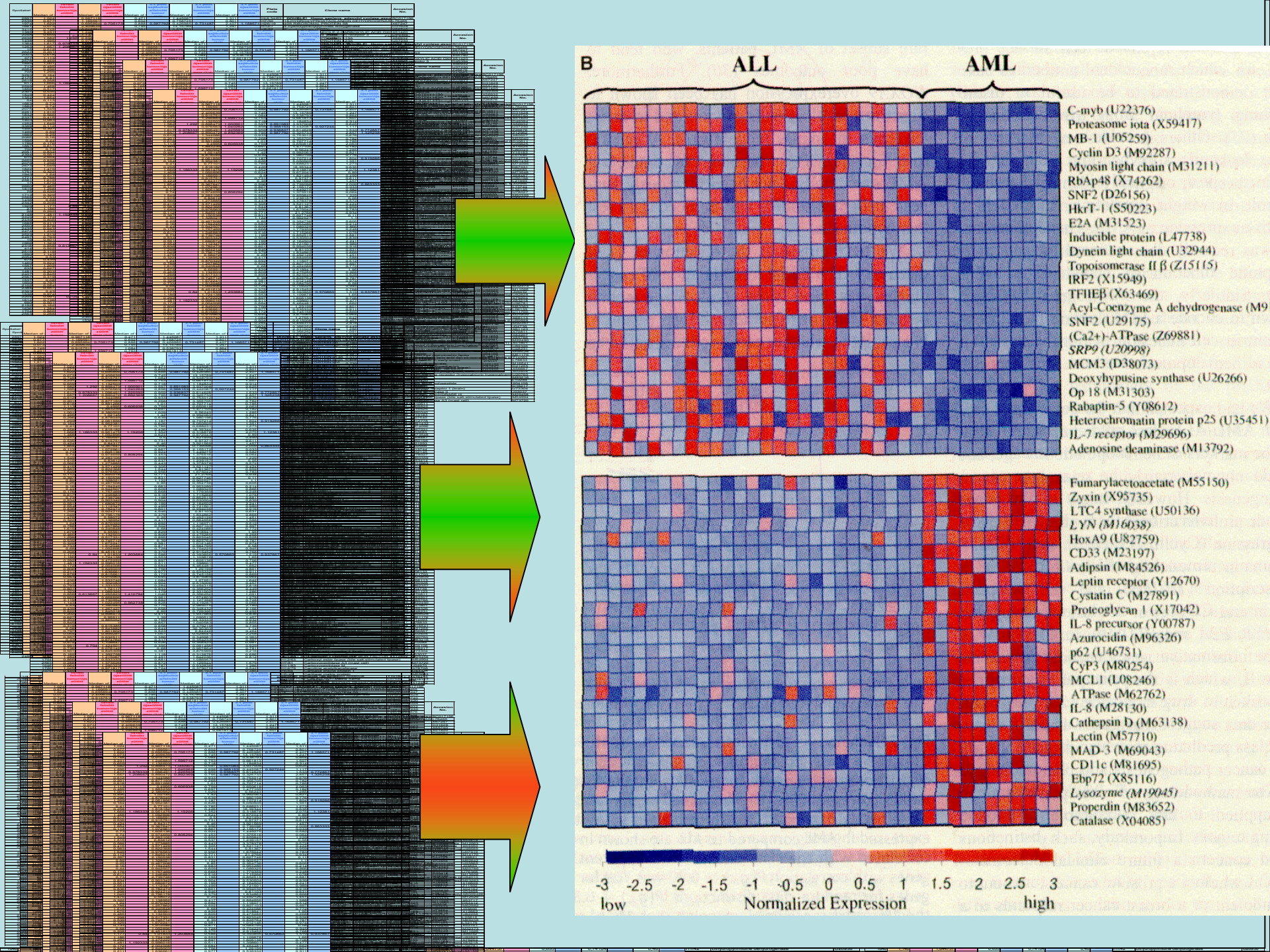
DNS-chipek képanalízise



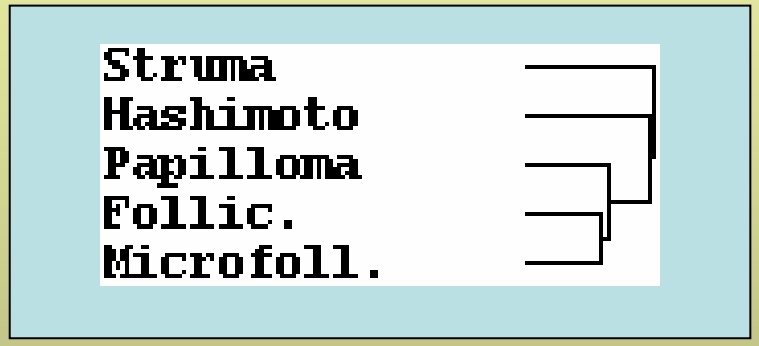
1. Informatikai feladat: NORMALIZÁCIÓ

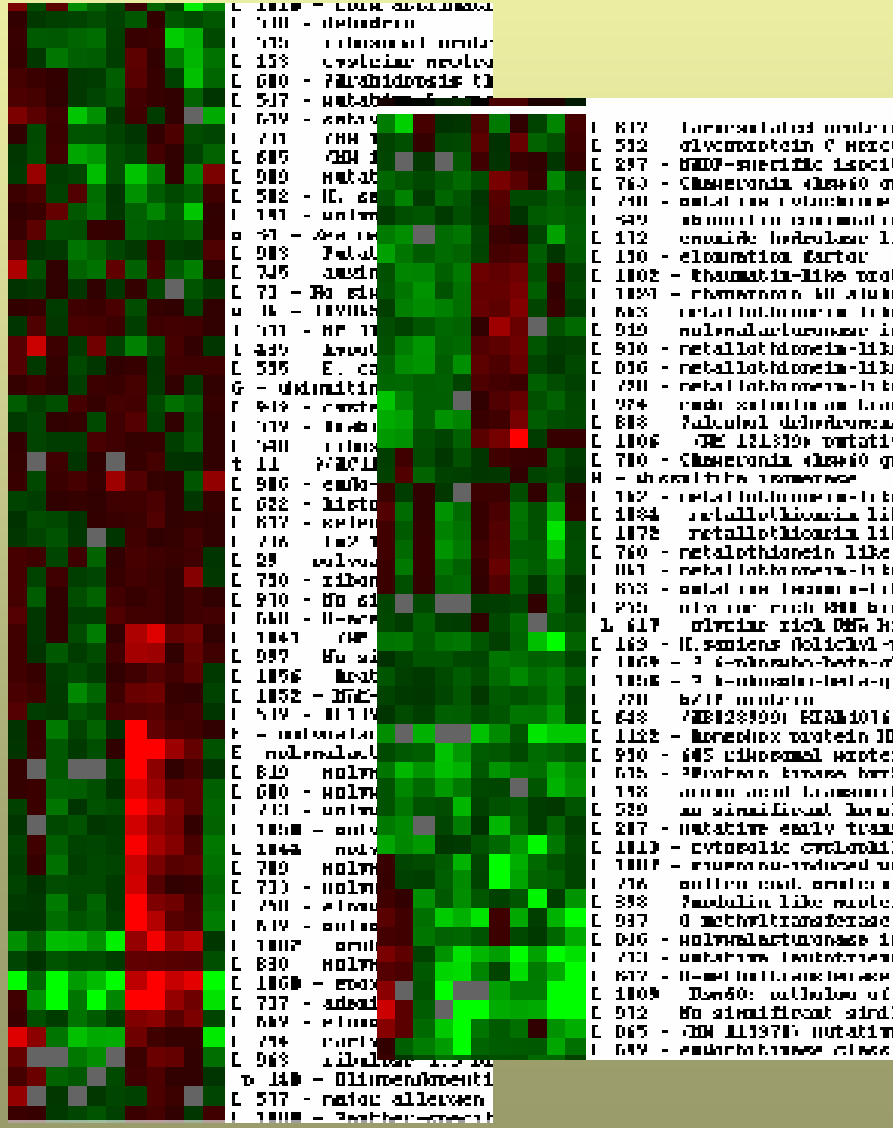
Globális és Lowess normalizációk




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 β (Z15115)
IRF2 (X15949)
TFIIEβ (X63469)
Acyl-Coenzyme A dehydrogenase (M9
SNF2 (U29175)
(Ca2+)-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)
-3 -2.5 -2 -1.5 -1 -0.5 0 0.5 1 1.5 2 2.5 3
low
Normalized Expression
high

Különböző pajzsmirigy eredetű betegségek hierarhikus klaszteranalítise





Hierarchical cluster analysis with Omniviz

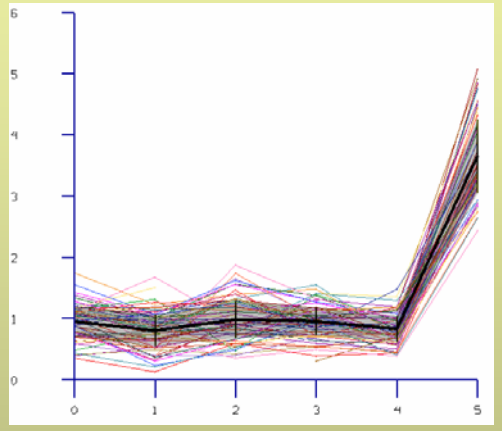
Betegség-specifikus klaszterek meghatározása

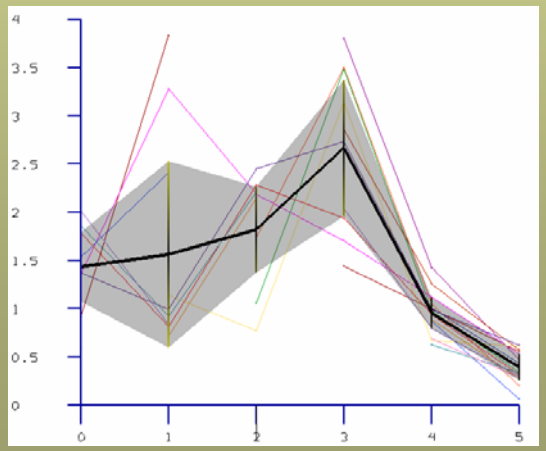
Legközelebbi kapcsolat: microfollicular & follicular carc., papilloma ehhez az alcsoporthoz hasonlít.

Struma and Hashimoto különböznek egymástól és más betegség csoportoktól.

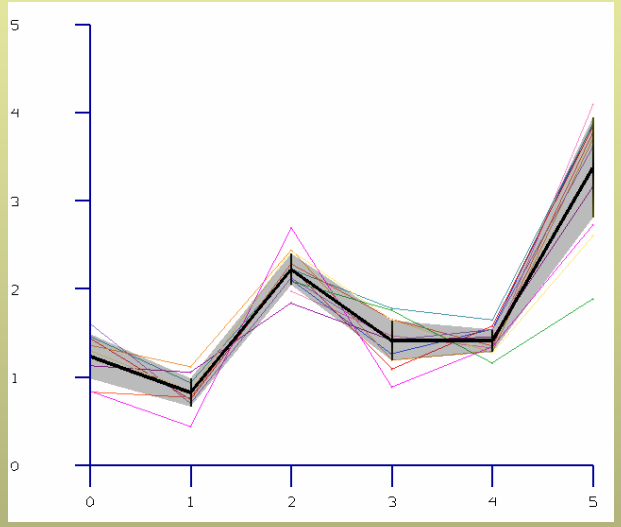
Betegségspecifikus génmarkerek azonosítása

Struma

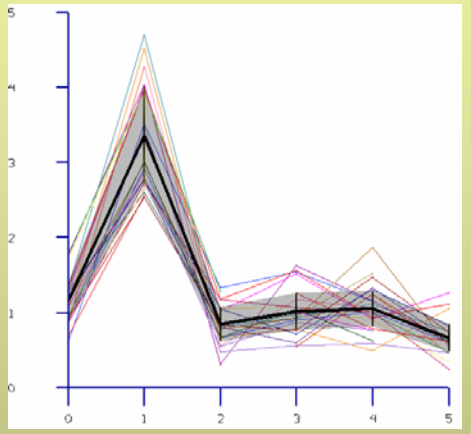


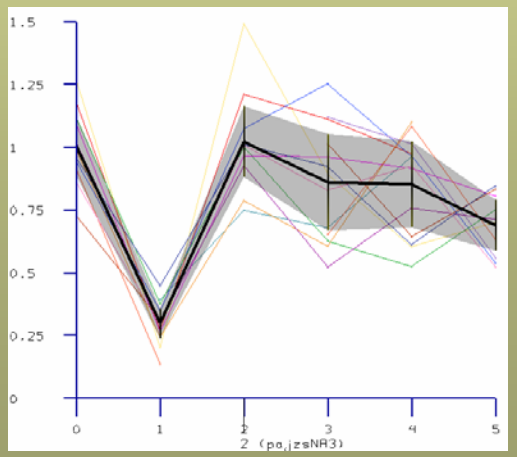


Microfollicular + Struma

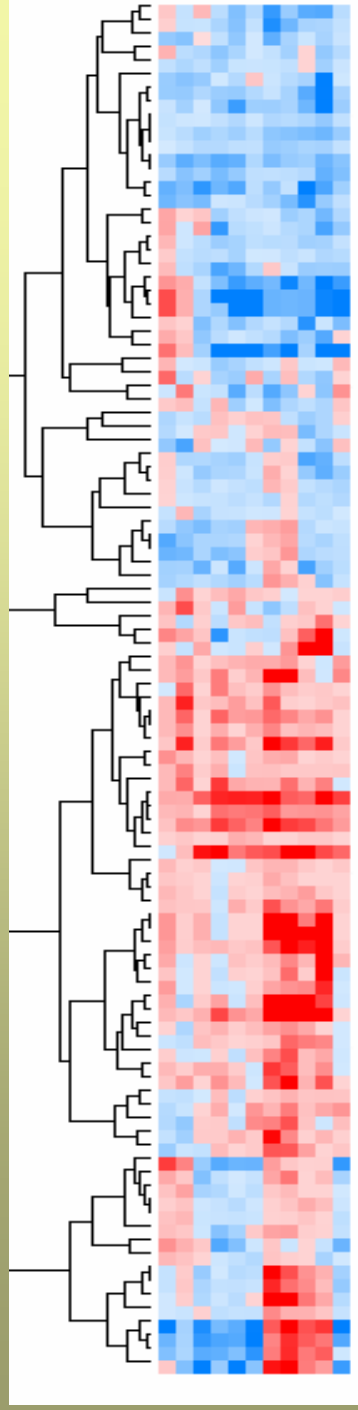


Hashimoto

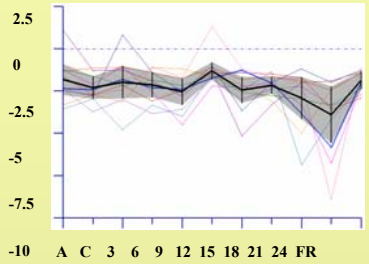




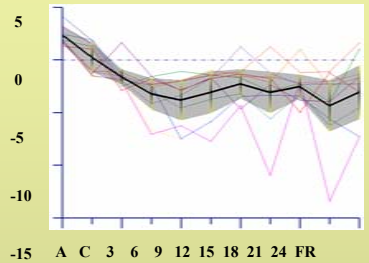
Körte érésének genomikai vizsgálata



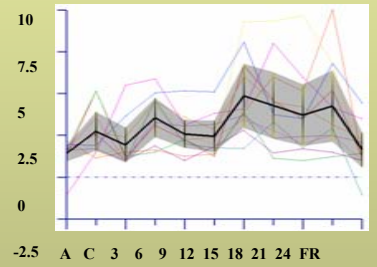
translationally controlled tumor prot.	AJ504884
pollen coat protein	AJ504885/AJ504757
early transcription factor small subu.	
glutathione reductase	AJ504702
glycine-rich RNA-binding prot.	
chloroplast protein-translocon-like	AJ504706
rod photoreceptor transducin a1	AJ504940
unknown protein	
bZIP protein	AJ504990
putative protein	AJ504931
6-phospho-beta-glucosidase	AJ504735/AJ504737
Protein kinase byr2	AJ504886/AJ504901
amino acid transporter-like prot.	AJ504942
EST	
cytosolic cyclophilin	AJ504887
fructose 1,6-bisphosphate aldolase	AJ504736/AJ504871
sim. to light-induced protein	AJ504762
serine/threonine prot. phosphatase	AJ504761/AJ504881
ribosomal protein YL16	AJ504882
60S ribosomal protein	AJ504870
putative leukotriene-A4 hydrolase	
EST	
unknown protein	AJ504768
nodulin-like protein	AJ504701
MAP kinase-like protein	AJ504742/AJ504883
putative protein	AJ504968
Putative serine/threonine kinase	AJ504880
auxin-repressed protein	AJ504879
putative dehydrin	
EST	
cysteine protease	AJ504704
chaperonin 60 alpha subunit	AJ504860
farnesylated protein	AJ504705
ribosomal prot. L44 isoform	AJ504862
cysteine protease inhibitor	AJ504859
putative protein	AJ504938
dehydrin	AJ504866
disulfite isomerase	
metallothionein-like protein	AJ504878
endo-xyloglucan transferase	AJ504876
alcohol dehydrogenase	AJ504903
adenosine triphosphatase	AJ504936
Chaperonin (hsp60 gene)	AJ504961
putative aminopeptidase	
hypothetical protein	
major allergen Mal d 1	AJ504707
thaumatin-like protein	AJ504926/AJ504927
glucan endo-beta-1,3-glucosidase	
hypothetical protein	AJ504782/AJ504984
beta-galactosidase 3'-fragment	AJ504849
hypothetical protein	AJ504779
Pbs 23kDa PSII	AJ504992
abscisic stress ripening protein	AJ504868
EST	
hypothetical protein	
Ca2+-transporting ATPase	AJ504695
lipoxigenase	
EST	
beta-D-galactosidase 3'	
O-methyltransferase	AJ504856
beta galactosidase	
hsp80	
sulfate transporter protein	AJ504708
polyphenol oxidase	AJ504916
hsp70	
ACC oxidase	AJ504864
methionine sulfoxide reductase	AJ504694
EST	
thaumatin-like protein	AJ504925
thaumatin-like protein 5'	AJ504928
endochitinase III	AJ504854
endo-chitinase class II	AJ504853
proteinase inhibitor se60-like prot.	AJ504693
soluble NSF attachment protein	AJ504709
poly(1,4-a-D-galacturonide) hydrol.	AJ504907/AJ504855
galactokinase like protein	AJ504965
pyroline-5-carboxylate synth.	AJ504700
putative RING zinc finger protein	AJ504869
abscisic stress ripening protein	
oxidoreductase	AJ504904
major allergen Pyrc1	AJ504858
b-alanine-pyruvate aminotransfer.	AJ504947
abscisic stress ripening-like prot.	AJ504852
capsanthin/capsorubin synthase	AJ504699
expansin	
early light-induced protein	AJ504872
putative protein	AJ504769



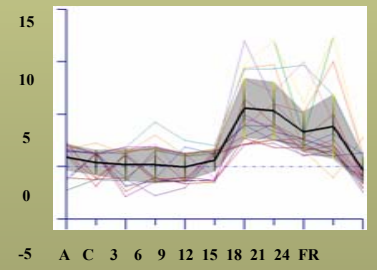
Nincs változás



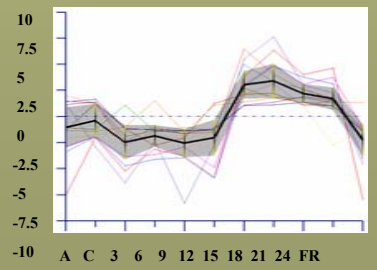
Gyümölcs növekedés



Érés és növekedés



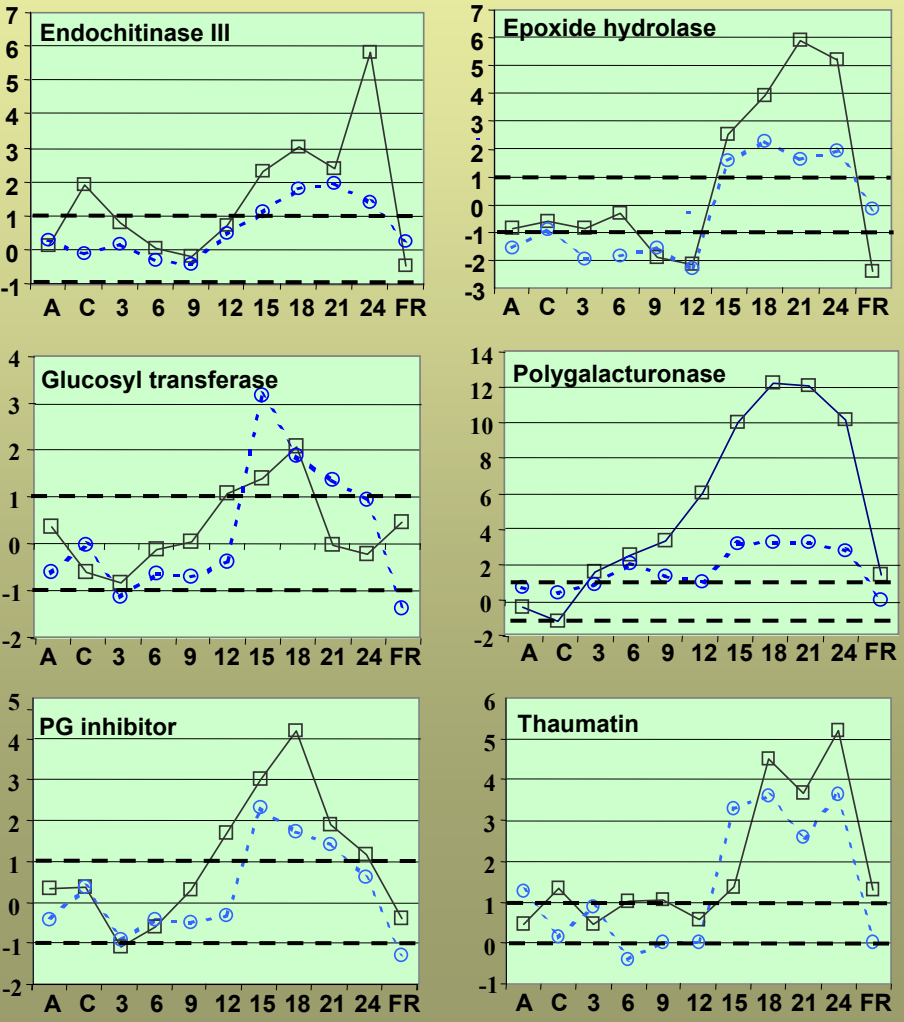
Éréssel összefüggő



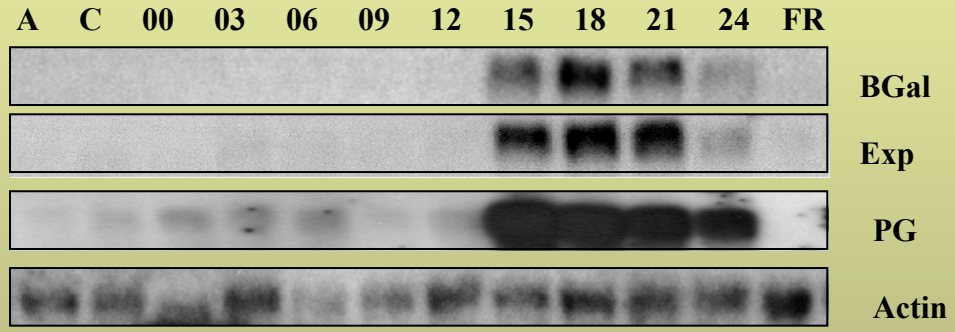
Éréssel összefüggő

Confirmation of gene expression data obtained from DNA-microarrays by different techniques

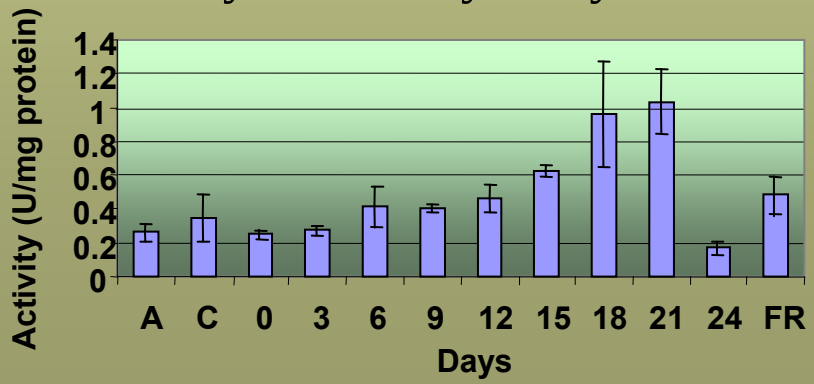
Real-time quantitative PCR



Northern-blot analysis

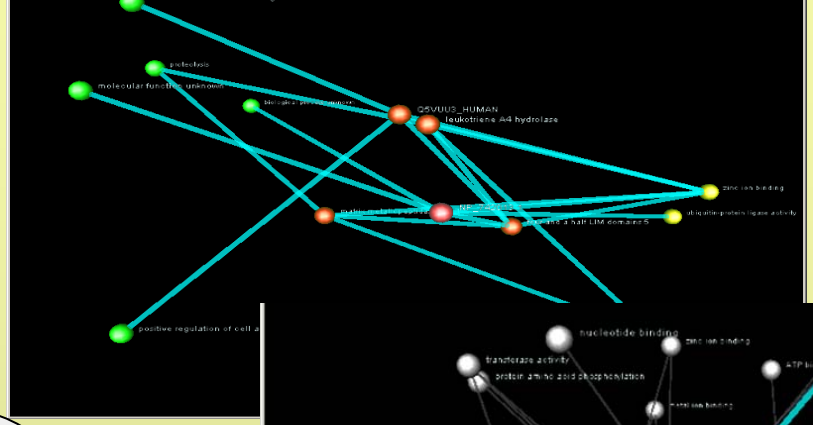


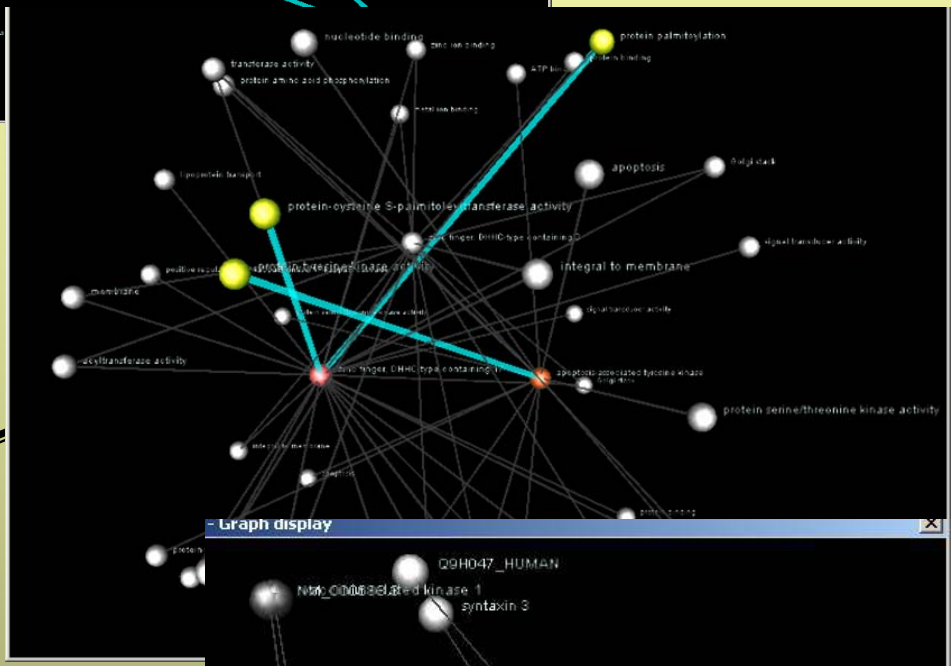
Enzyme activity assay

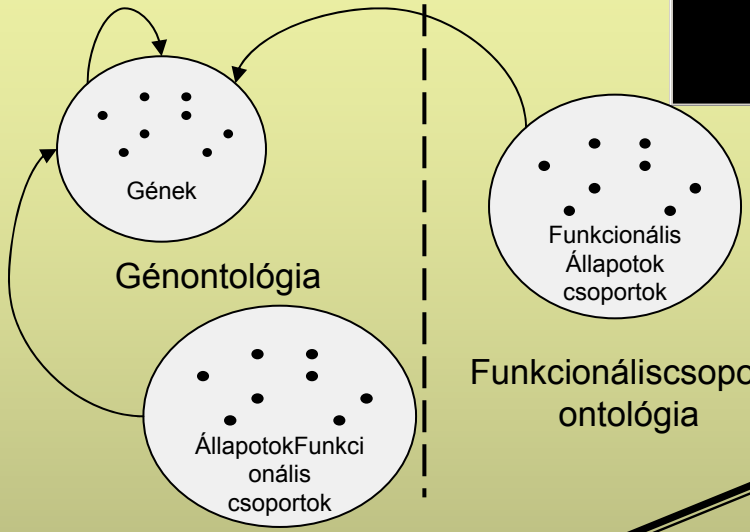


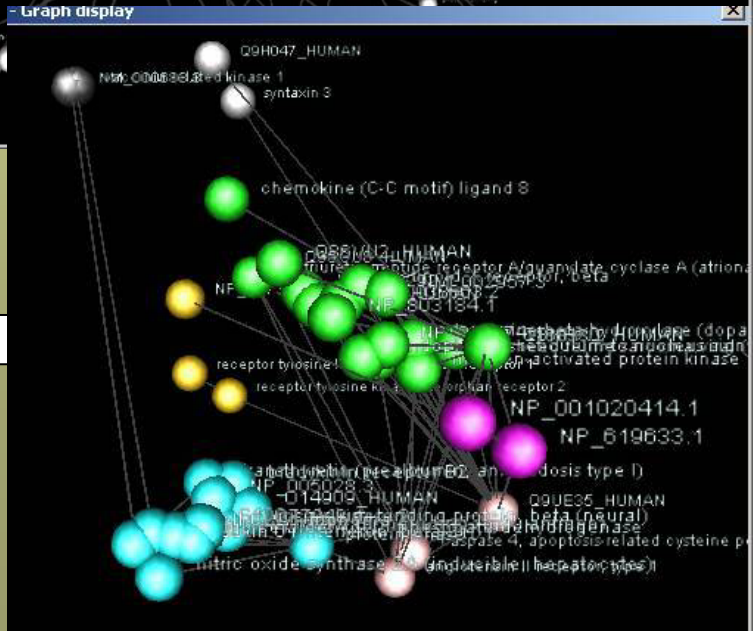
DNA-chip Laboratory, BRC, Szeged

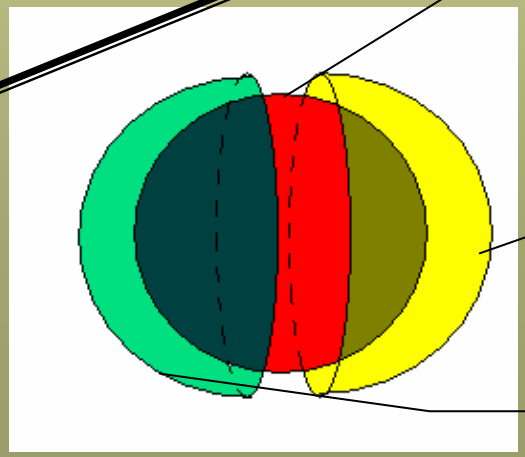
Adatbáziskutatás, adatbányászás











Ontológiák

Állapotok

Genomikai kutatások a Gyógyszerkutatásban, -fejlesztésben

Felfedezés (Discovery)

Optimalizáció (Optimization)

Hatásosság vizsgálat (POC, Preclinical)

ADME-Tox (Preclinical)

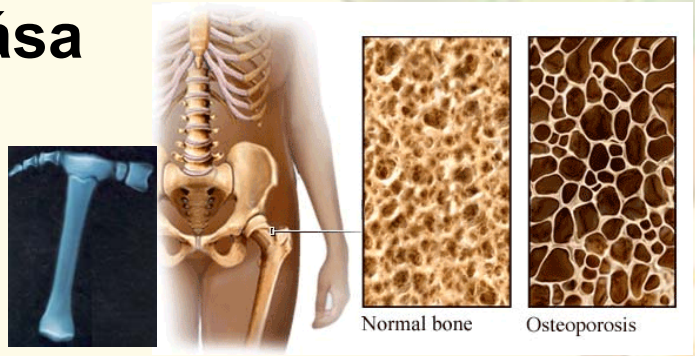
Klinikai vizsgálat (Clinical trials)

Felfedezés (Discovery)

1. <u>Target alapú szűrések</u>

1a: új target azonosítása

cathepsin K
– osteoclast formation



1b: off-target effektusok kimutatása

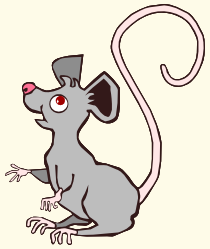
1c: target validálás (siRNS, expr. mintázat)

Felfedezés (Discovery)

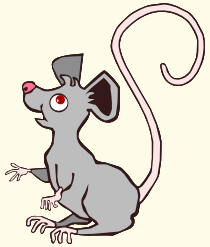
1. Target alapú szűrések

1a: új target azonosítása

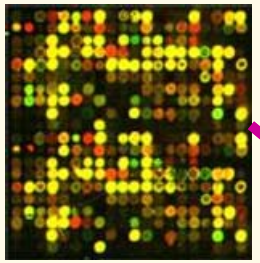
OVE

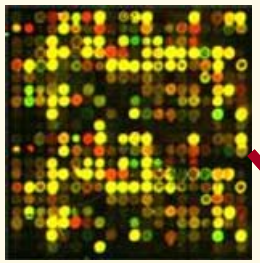


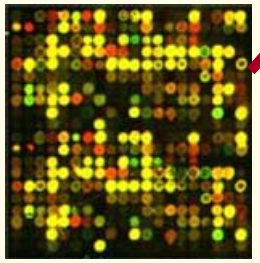
OVE + Estrogen



Control







Potenciális gyógyszer-célpontok

Expressziós mintázat: MARKER

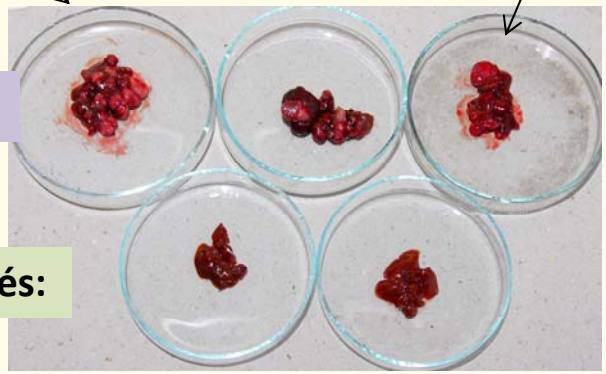
Génexpressziós változások Ac915-tel kezelt KO-egér májkarcinómában

Kezeletlen kontroll

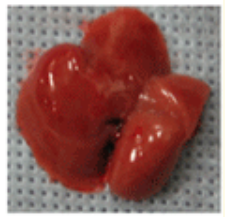
Tumorigenesis: DEN, 15 naposan egyszeri 50 ul-es kezelés, 4 hónap normál táp

Kezeletlen

Ac915 kezelés:

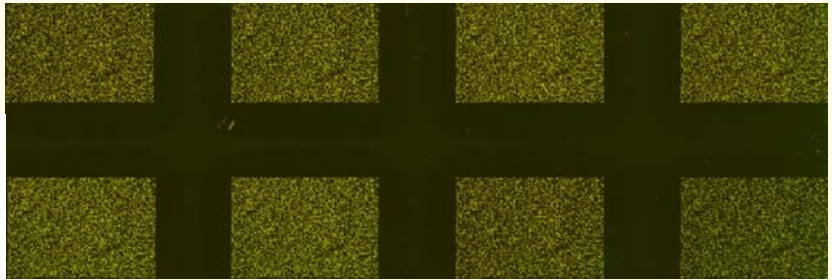


Tumor izolálás



Egészséges szövet izolálás

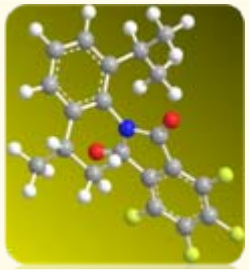
Egészséges szövet izolálás



AVIDIN BIOTECHNOLOGY

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
- Szingnifikancia analízis, p-value

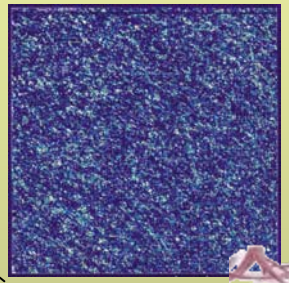
AVIDIN BIOTECHNOLOGY

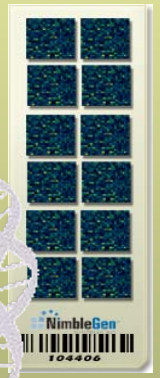
Új módszerek
fejlesztések,
lehetőségek

AVIDIN BIOTECHNOLOGY

Genomikai kutatások eszközei

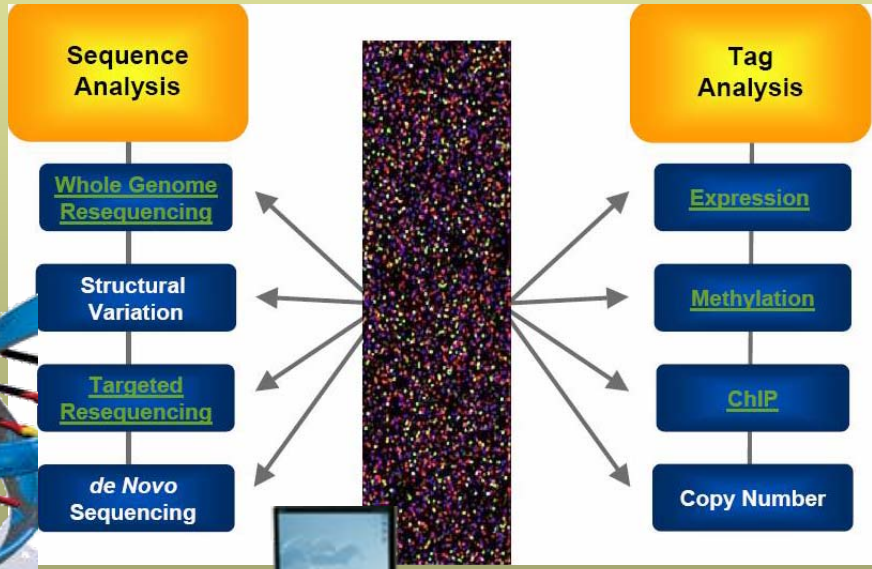
DNS-chipek,
microarrayek





valós-idejű PCR

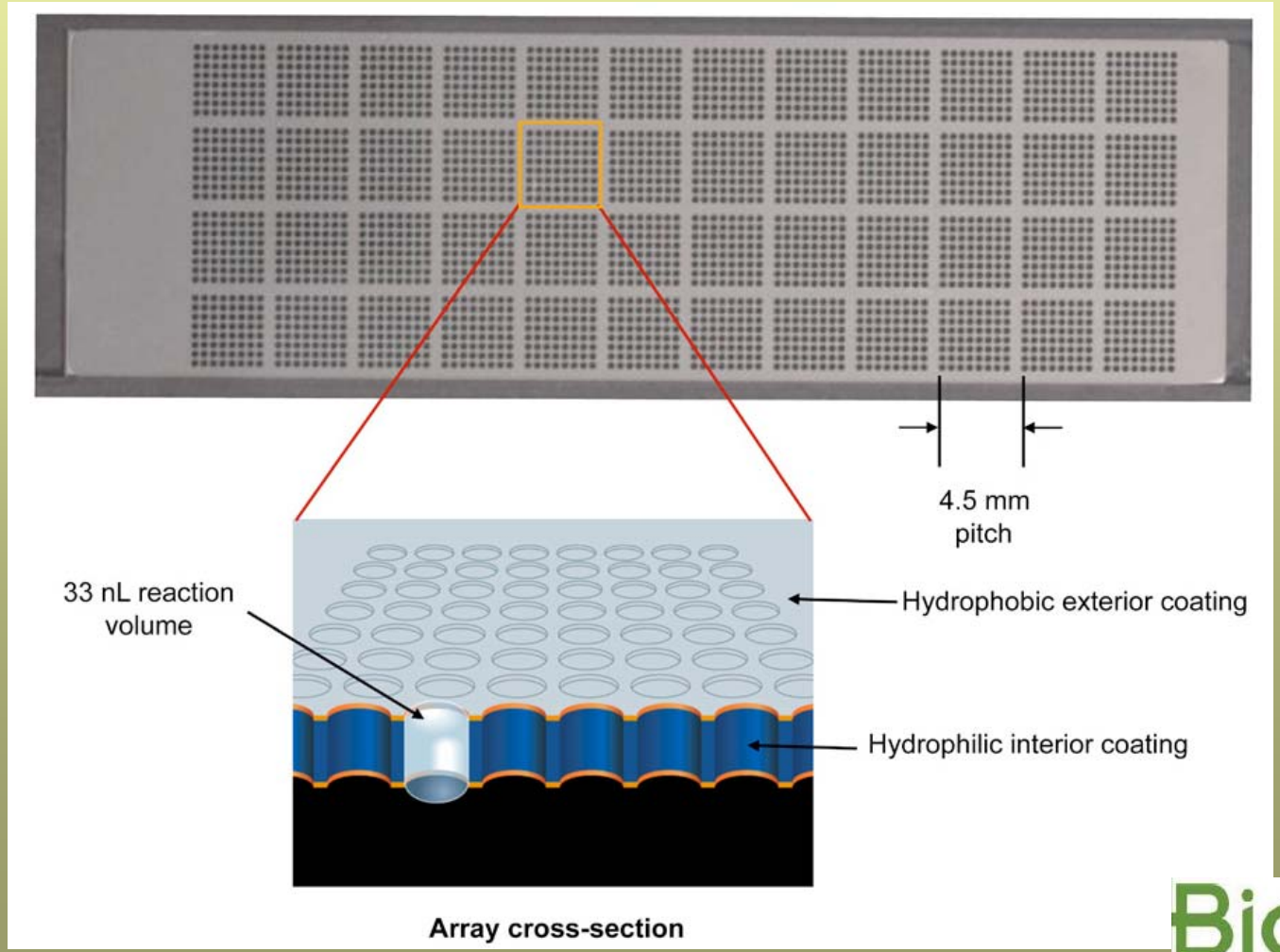
Új generációs szekvenátorok



SOLID/454

AVIDIN BIOTECHNOLOGY

Nanokapilláris valós-idejű PCR

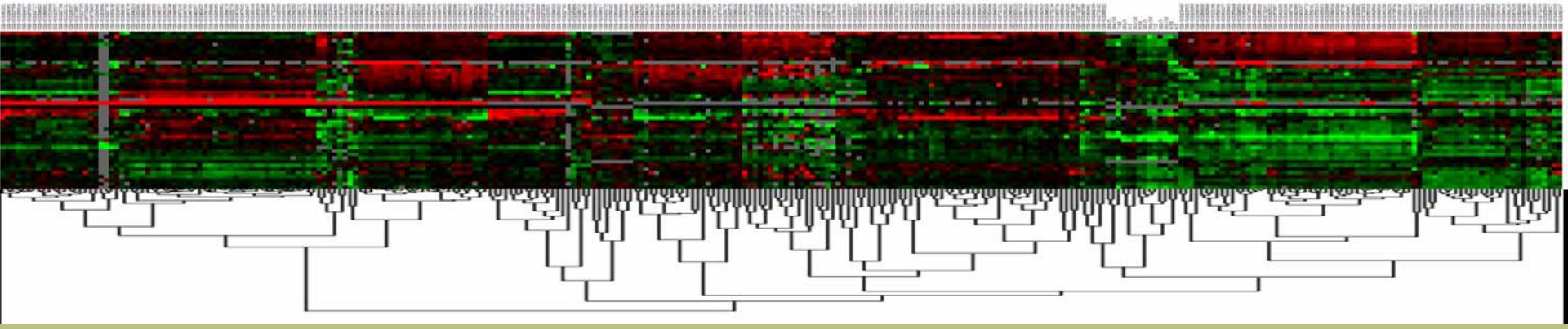


3.072 reakciótér
48x64 almátrix

BioTrove

AVIDIN BIOTECHNOLOGY

480 vizsgált, citotoxikus anyag szűrése toxikológiai panelen



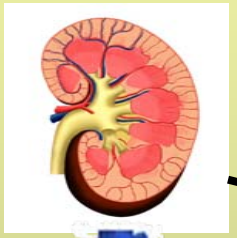
A normalizált génexpressziós értekeket hierarchikus klaszterezési eljárásnak vetettük alá.
A referencia-minták közös klaszterben mutatkoztak, amely hasonló hatásmechanizmusra utal

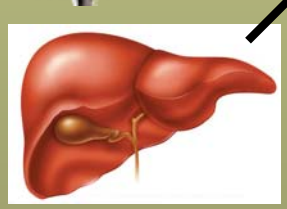
480 minta (52 gén/minta) 10 lemezen:
1 koncentrációban (EC10) 1 időpontban (24 h) máj sejteken
Összesen 24.960 QRT-PCR

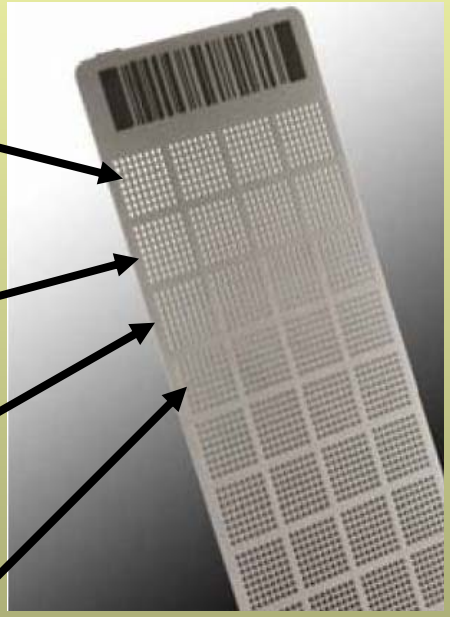
Vass L, et al. (2009) Toxicogenomics screening of small molecules using high-density, nanocapillary real-time PCR. Int J Mol Med.23:65-74.

Kooperáció: MTA SZBK, Comgenex zRT., Targetex Kft.

Protocol for toxicogenomics screening





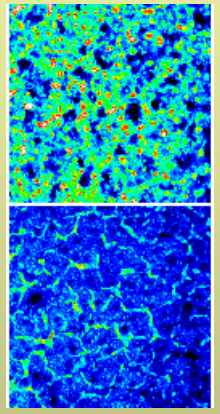


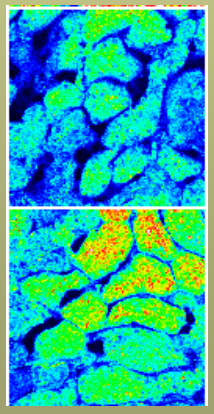
REFERENCE:
Heart and liver toxicity of doxorubicine, sulfasalazine; liver and kidney toxicity of aniline, rotenone, ID9637; safety of Ac929 and Q50 was confirmed with the Avidin's Toxicogenomics screening platform

3+3 x4 samples
2 compounds/plate

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	-1.69	-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				-0.88				-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	-1.11		-1.46	-1.92	-1.22		-1.22	-2.87	-1.70		-1.17	-0.07	-0.40		-1.04	-0.28	-1.26		-1.58	-0.49	-0.91		-0.92	0.04	-0.09
	-3.77		-5.82		-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
			0.34				-0.14				-0.82				2.32				nd				-0.34				-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	-0.95	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		-0.26
	-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	-0.52	-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	-1.66	-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
8	12	24	20	6	11	18	28	4	17	21	31	8	15	3	15	2	13	2	17	3	9	1	10	1	8	1	9
B	L	H	K	B	L	H	K	B	L	H	K	B	L	H	K	B	L	H	K	B	L	H	K	B	L	H	K
ID9637				Doxorubicin				Sulfasalzine				Rotenone				Aniline				Ac-915				Q-50			

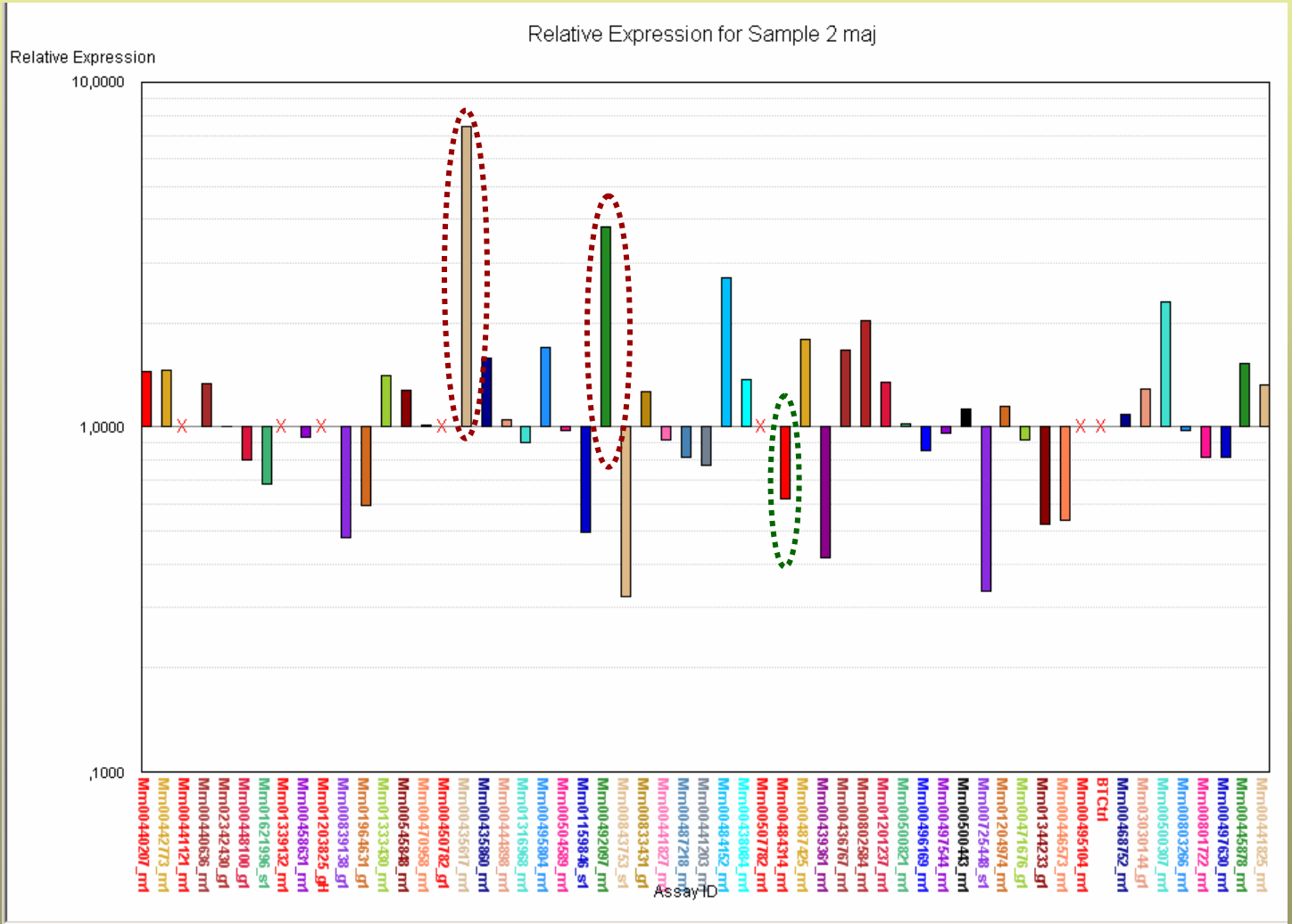


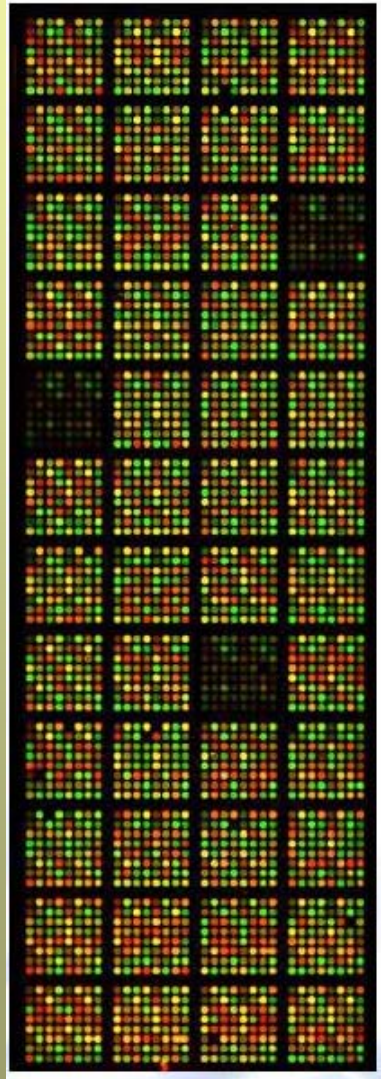


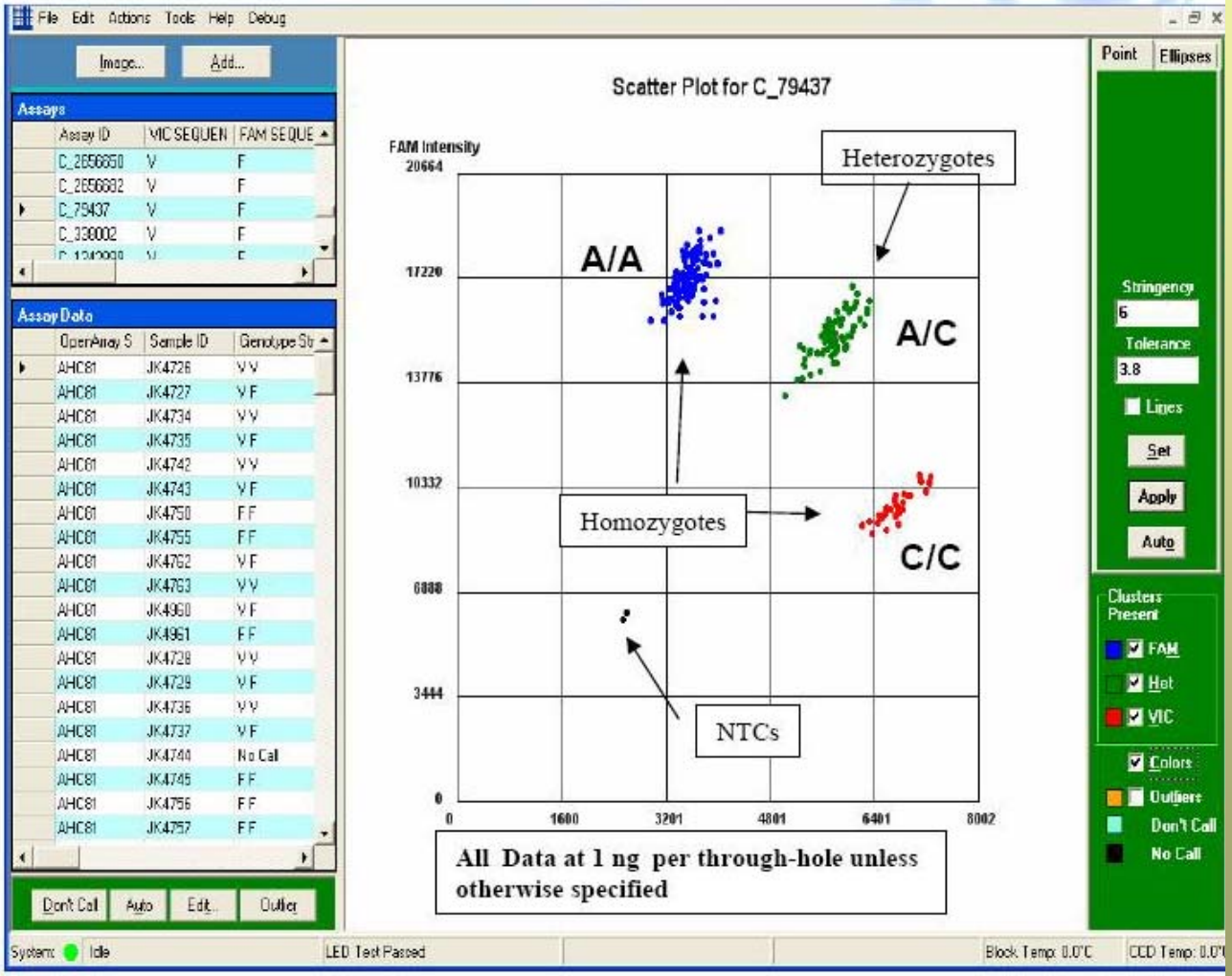
Liver

Nimesulide 50 mg/kg

Control gene: Mm02342430_g1 PPIA, cyclophilin A





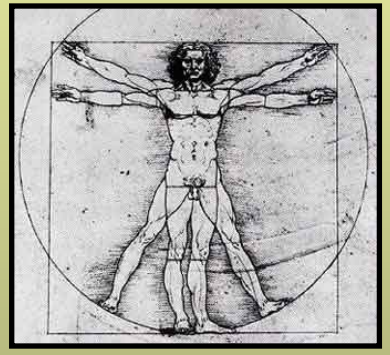


Duplex Taqman endpoint assay

>99.5% Accuracy >95% Call rate

SNP genotyping applications

Human



- Population Genetics
- Disease Associations
- PGx
- Genetic Identification

Salmon

- Population Genetics
- Migratory Patterns
- Breeding

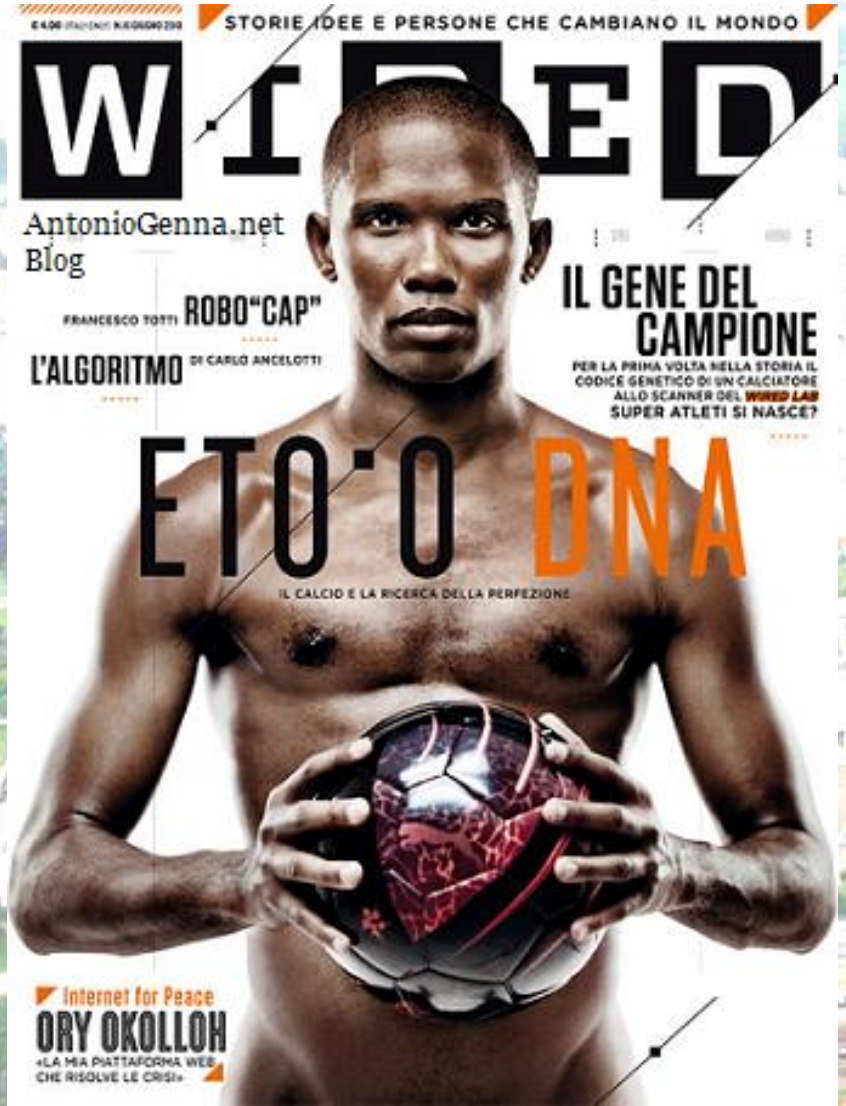
Corn

- Marker Assisted Breeding
- Transgenics QC/ Stewardship

Mouse

- Marker Assisted Breeding
- Transgenics
- Trait mapping
- Human disease models

Clinical assessment incorporating a personal genome

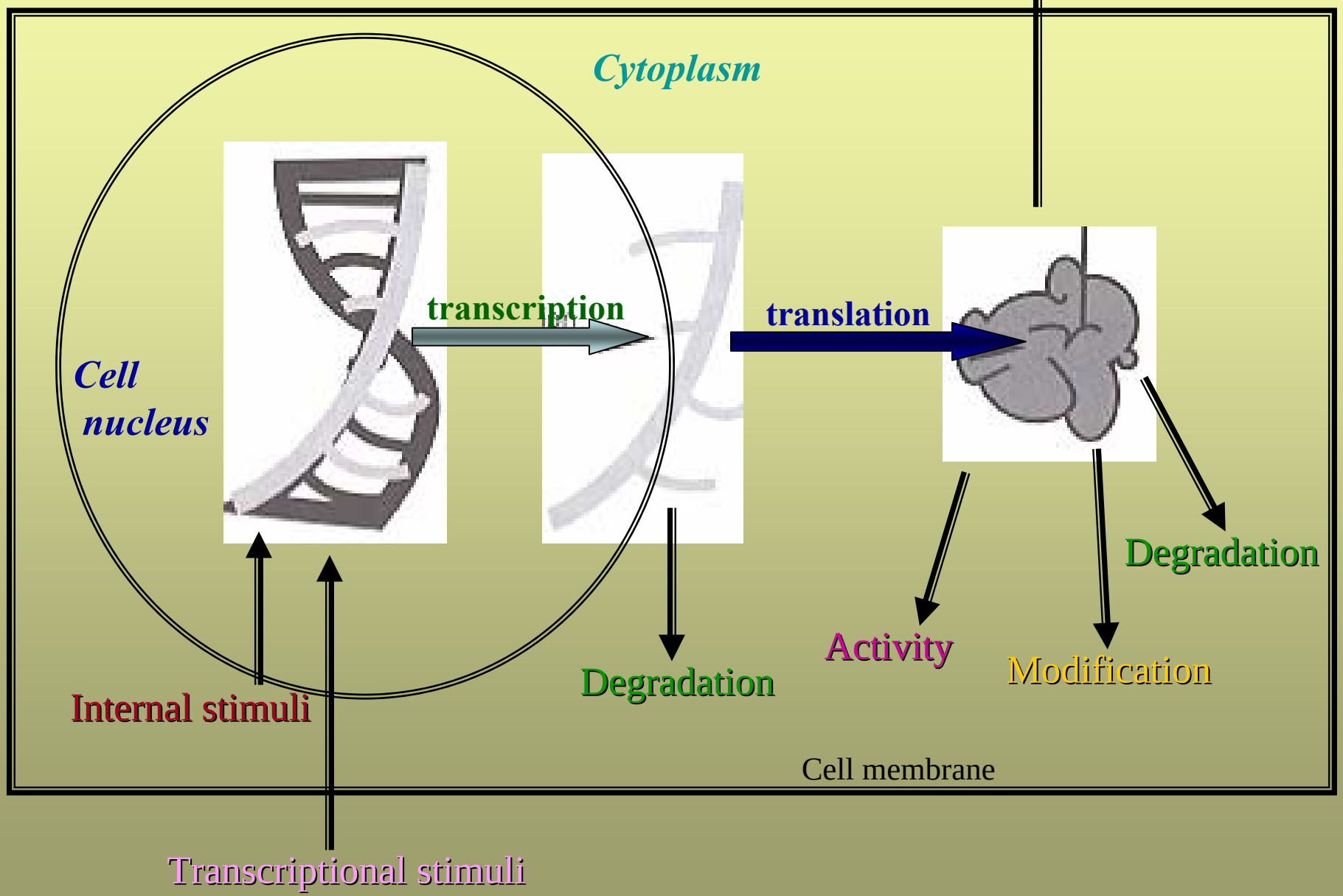


***ACTN3* p.R577X**

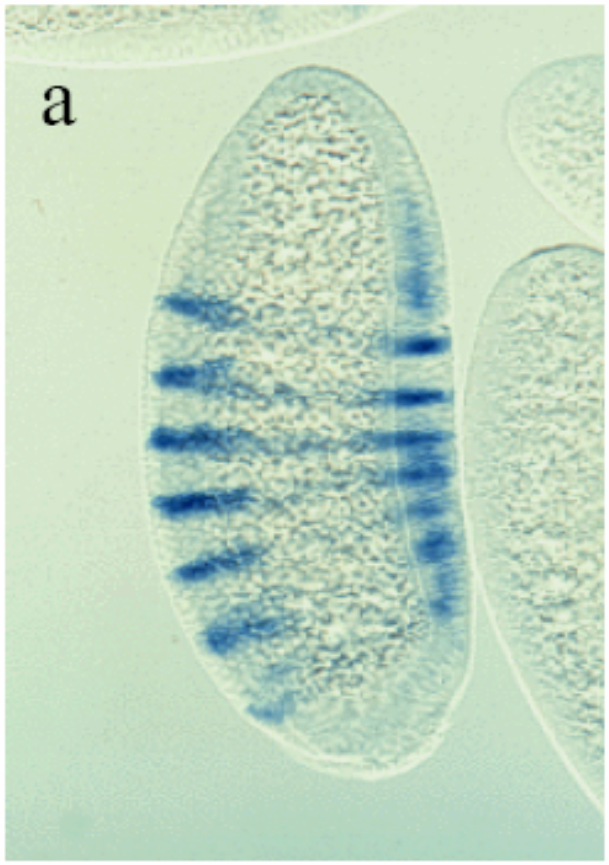
Samule Eto'o: p.R577R

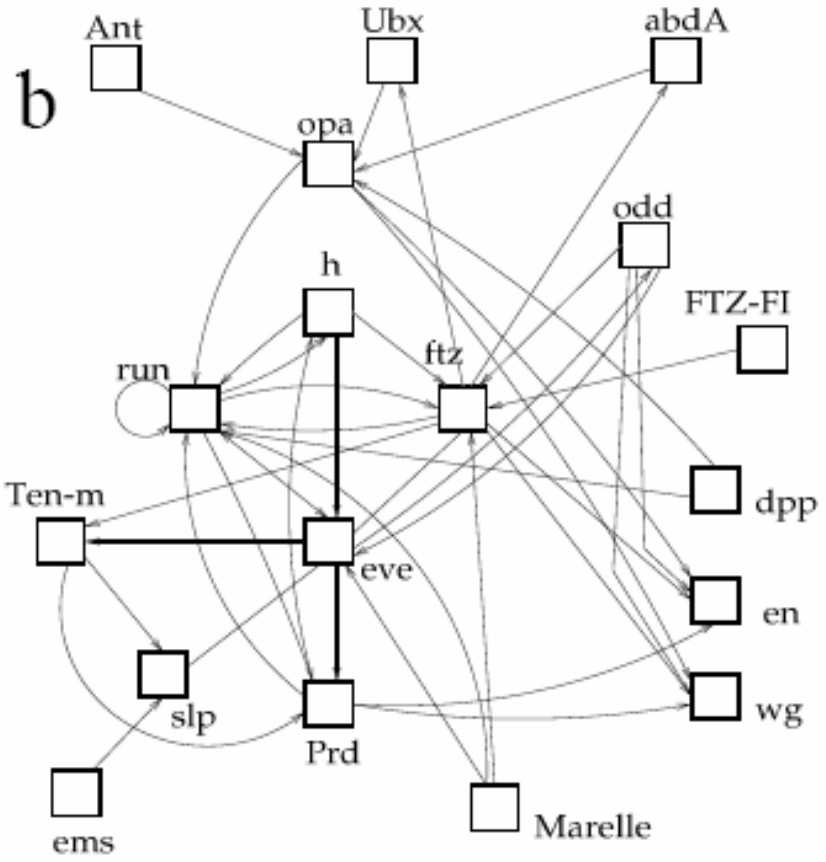
Korlátok, megoldások

Flow of genetic information



Sejthálózatok és génhálózatok, kapcsolatok és jelek





Neuronok csoportosítása

Osztályozási szempontok lehetnek:

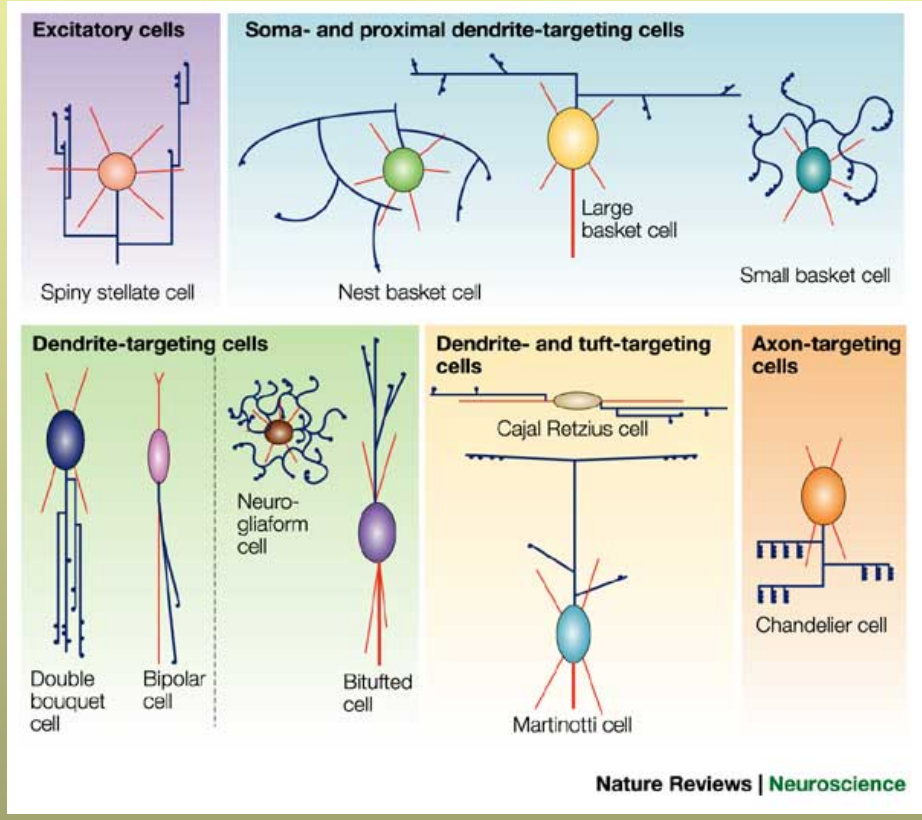
- a morfológia
- neurotranszmitterek
- néhány fehérje marker
- elektrofiziológiai jellemzők

↓

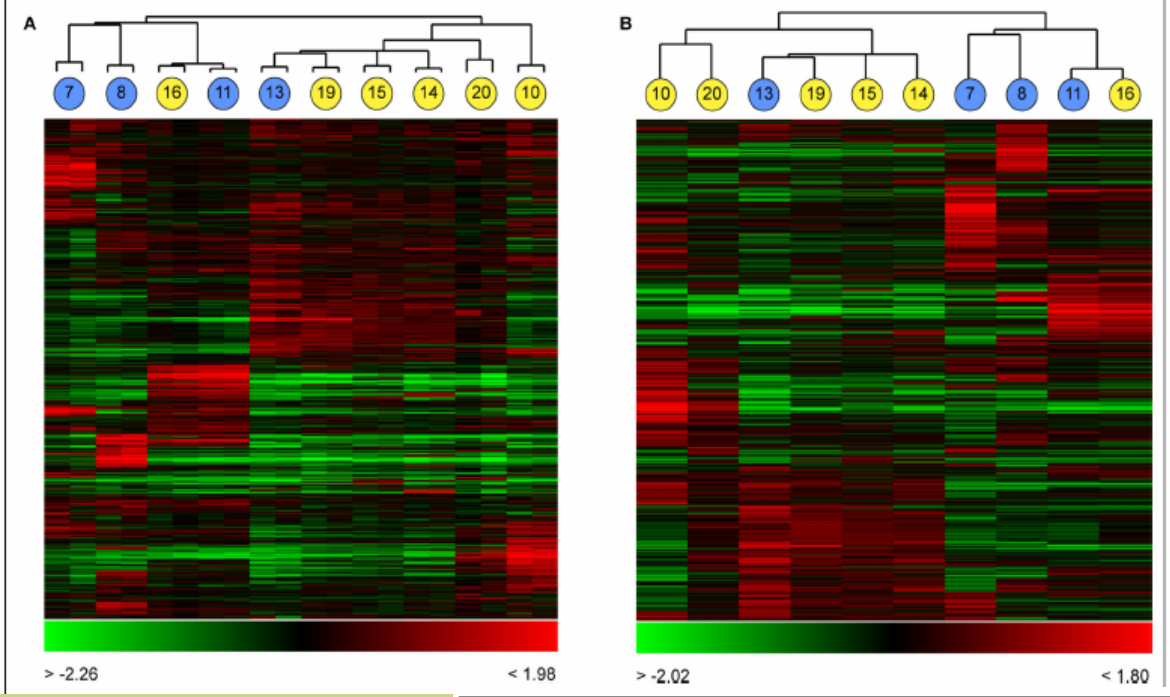
- single cell RT-PCR

↓

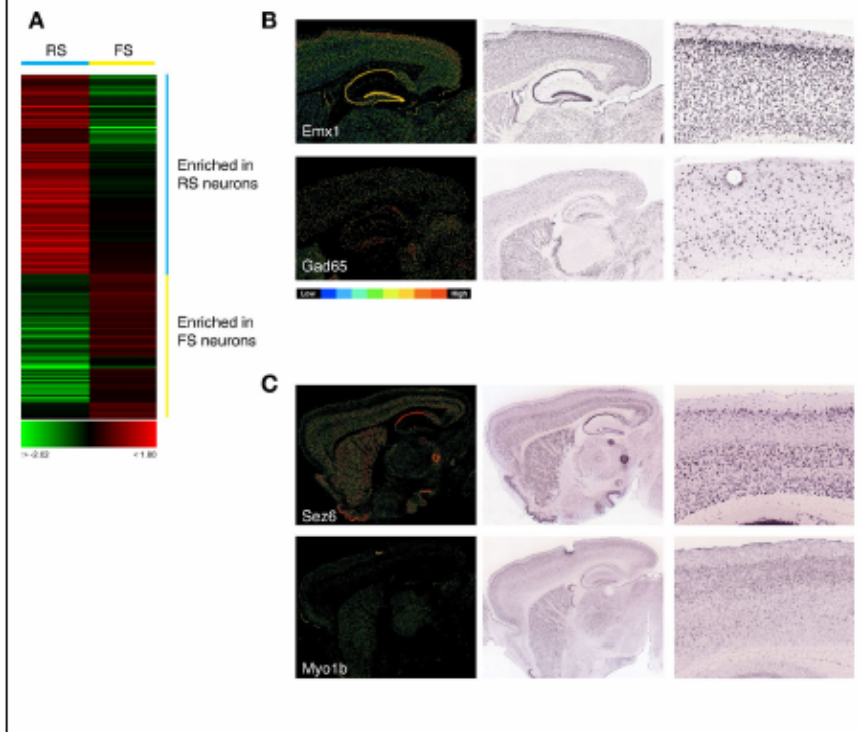
- globális génexpresszió analízis



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, *13 April 2010*

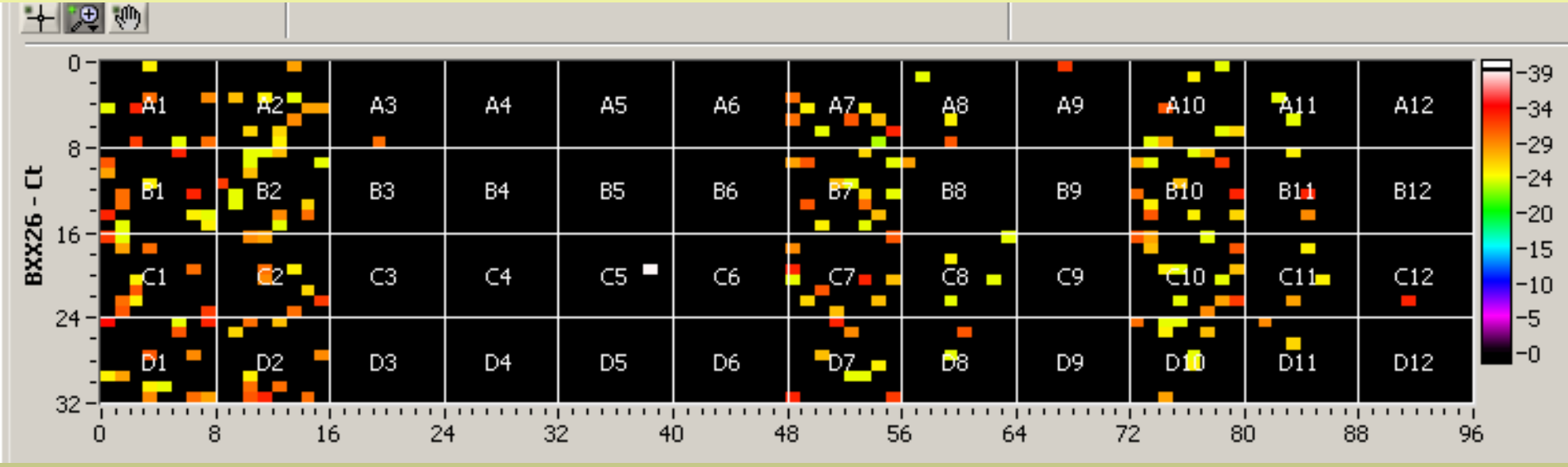


18 különböző neuron klaszterezése
4 posztnatális stádiumból



Nanokapilláris QRT-PCR digitális expresszióvizsgálatra

Digitális génexpresszió analízis egy sejtből



1. S18 ngf - 43
2. S18 p – 41
3. S18 negative - 1
7. X1 ngf – 40
8. X1 p – 10
9. X1 negative - 1
10. X2 ngf – 40
11. X2 p -10
12. X2 negative - 1

Hagyományos és chip adatok egy adott klinikai kórképre