

# DNA-mikromreže

DNA-mikroraster = DNA-mikromreža

makroraster: točke sond so  $>300 \mu\text{m}$ ;

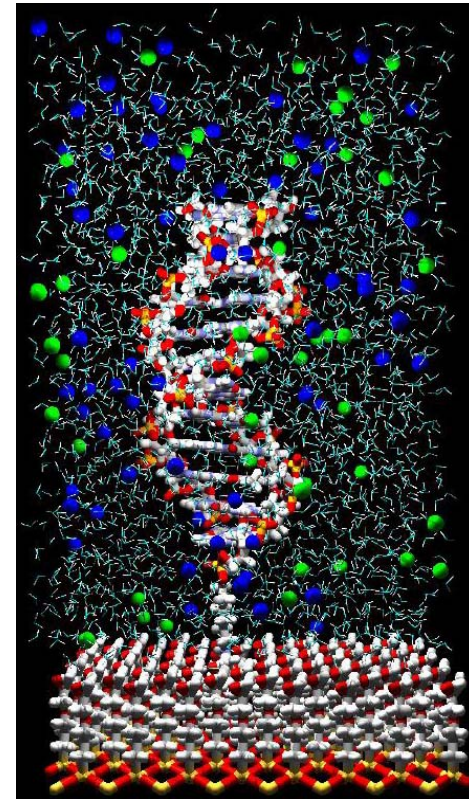
mikroraster:  $<200 \mu\text{m}$

za delo z mikromrežami so potrebne posebne avtomatizirane naprave

Primer: DNA-sonda je vezana na čip  $\rightarrow$  iščemo po vzorcu cDNA (fluorescenčno označena) iz preiskovanega vira, če vsebuje komplementarno zaporedje.

Podobno lahko vežemo peptide (npr. substrate) in z njimi iščemo tarčne proteine (npr. encime)  $\rightarrow$  proteinske mikromreže.

Pri DNA-mikromrežah je pri klasični izvedbi na stekleno podlago vezana sonda cDNA (0,5-5 kb), pri alternativni pa je oligonukleotid (20 - 80-mer) vezan direktno na čip.



# DNA-mikromreže /2

Z mikromrežami lahko preiskujemo bistveno večje število vzorcev kot pri klasičnih analizah. Pomembna je uporaba za primerjavo izražanja genov v različnih celicah: npr. normalne in obolele celice, tkivno-specifično izražanje, odziv celic na okolje (zdravila),...

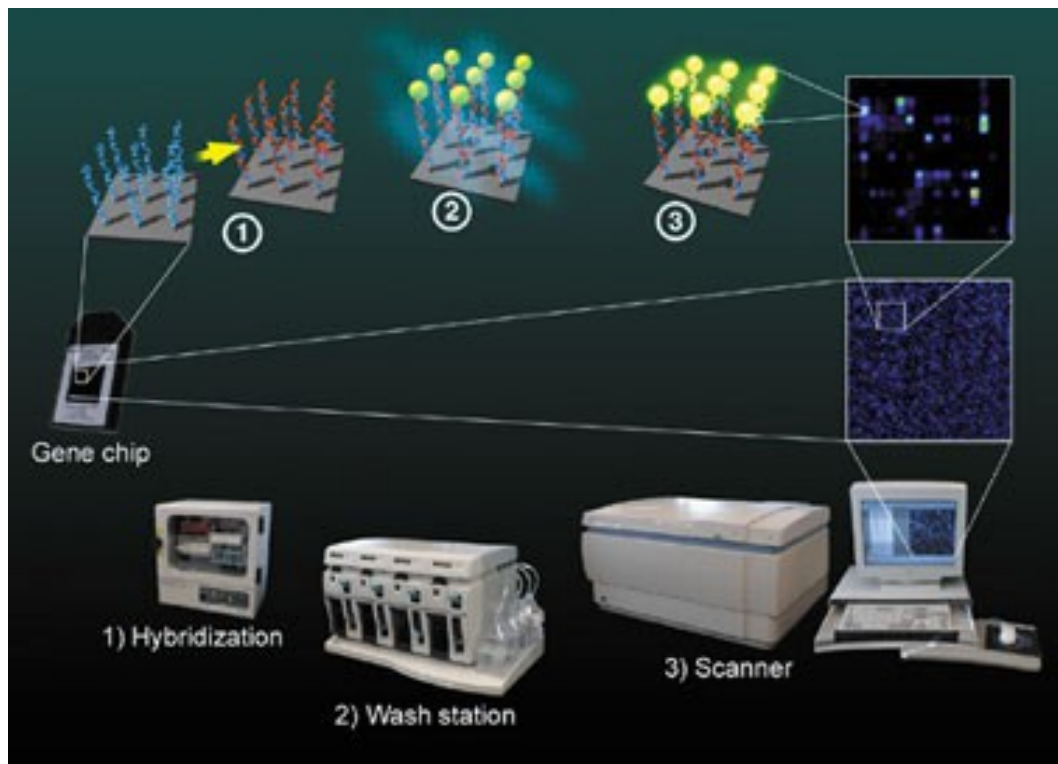
## Faze v pripravi eksperimenta

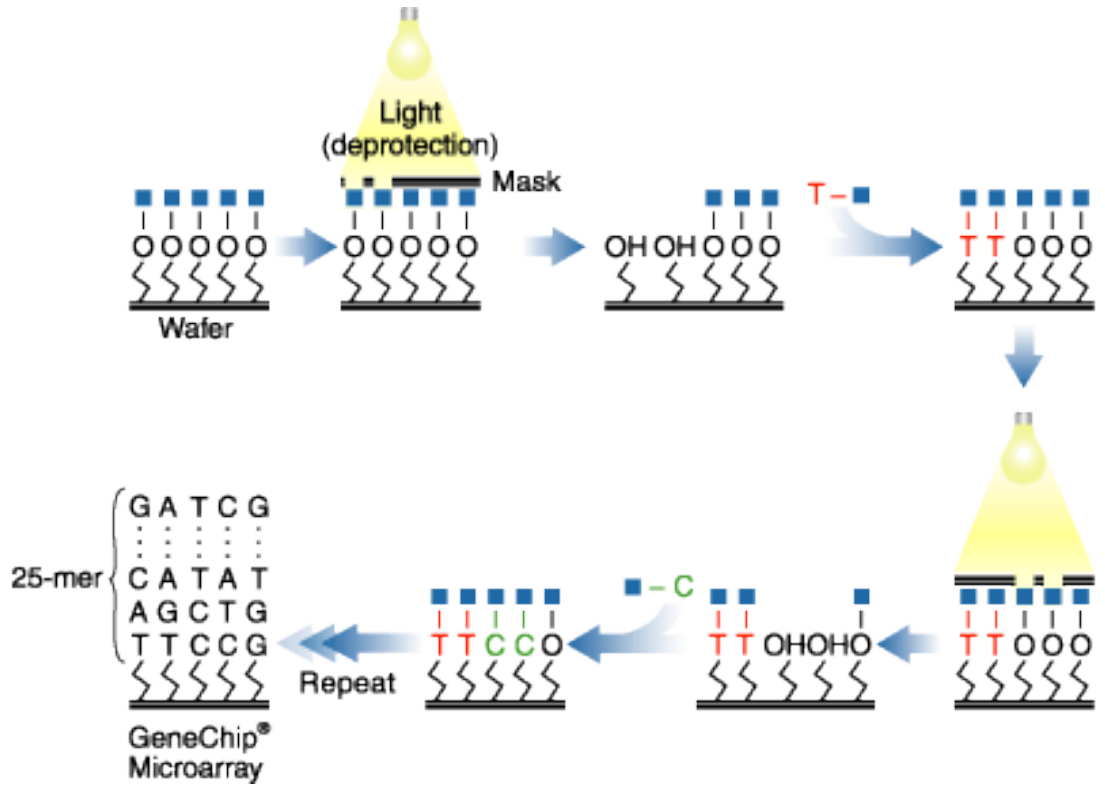
(primerjalna hibridizacija):

- izbor vira mRNA  
(populacije celic, ki jih preučujemo)
- izolacija mRNA in pretvorba v cDNA
- fluorescenčno označevanje cDNA
- hibridizacija z DNA-mikromrežo
- skeniranje hibridiziranega rastra
- interpretacija slike

## Priprava mikromreže:

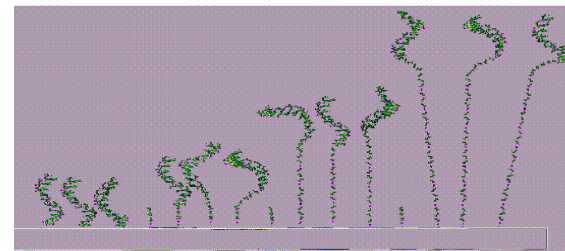
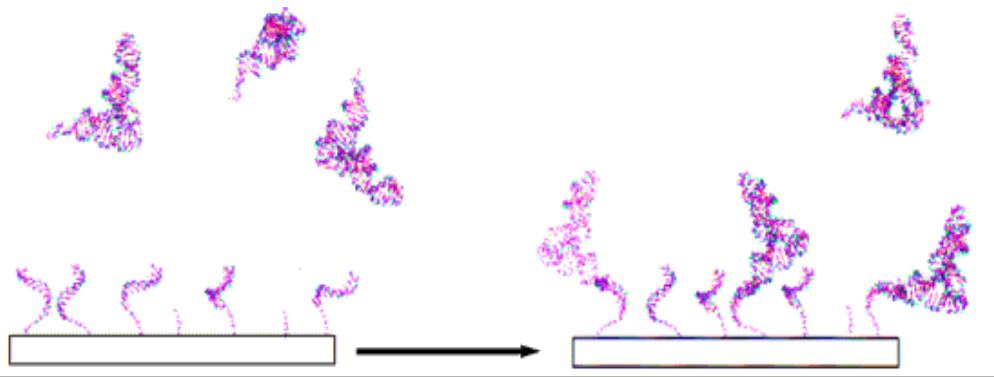
- izbor, izolacija/sinteza sond
- nanos sond na čip  
(ali komercialni čipi)





# DNA-mikromreže /3

- Nosilec za nanos sond je iz stekla ali plastike.
- Oligonukleotidne sonde sintetiziramo *in situ* (na steklu).
- Daljše sonde lahko pripravimo s pomočjo PCR.
- Sonde se na steklo vežejo preko oligoetilen-glikola ali poli-L-lizina.
- Sonde so vedno ssDNA.
- Na eni ploščici je lahko do ~2 milijona točk.



- nespecifične interakcije (50 % formamid, 0,5 % SDS, 6xSSC,...)  
8-24 h pri 42-65 °C





# GeneChip® Human Gene 1.0 ST Array

[Printer-Friendly](#)  [Email](#) [Overview](#)[Comprehensive Coverage](#)[Performance](#)[Literature](#)[How to Order](#)[Tools & Data](#)

The GeneChip® Human Gene 1.0 ST Array is the latest product in the family of Affymetrix expression arrays offering whole-transcript coverage. Each of the 28,869 genes is represented on the array by approximately 26 probes spread across the full length of the gene, providing a more complete and more accurate picture of gene expression than 3' based expression array designs. The small format of the array makes it a cost-effective expression profiling solution for new microarray users.

The Gene 1.0 ST Array is part of a complete solution for gene expression analysis that includes Whole Transcript (WT) Sense Target Labeling and Control Reagents, fluidics and scanning instrumentation and basic analysis software. The array contains the most up-to-date content of well-annotated genes, and data is analyzed using a simple workflow.

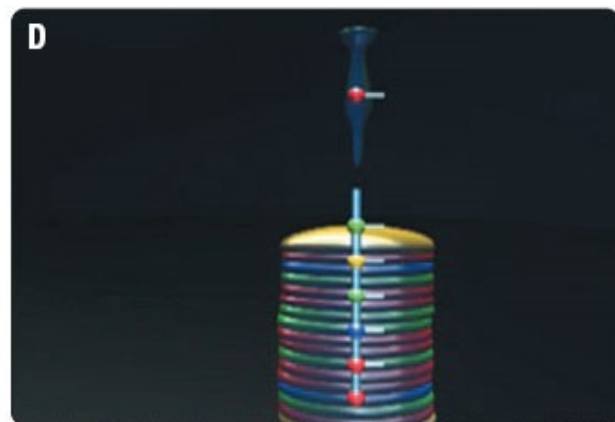
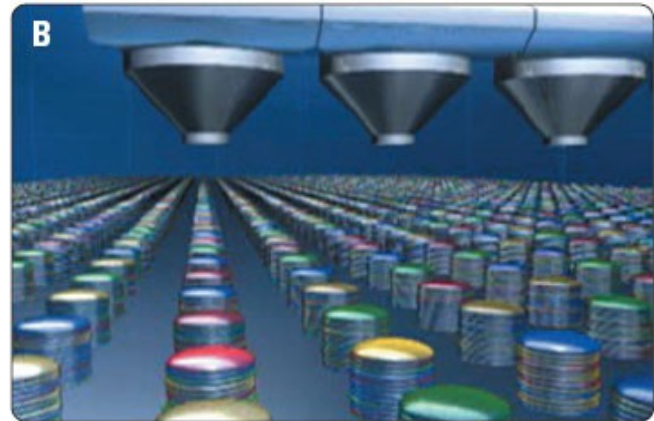
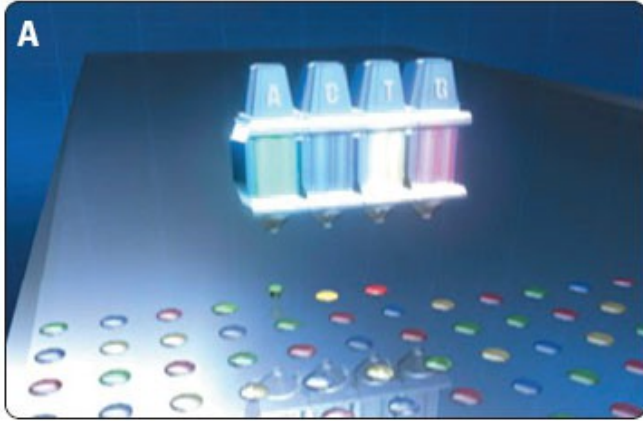
The Gene 1.0 ST Array uses a subset of probes from the [Human Exon 1.0 ST Array](#) and covers only well-annotated content. Like the Exon 1.0 ST Array, "gene-level" analysis of multiple probes on different exons is summarized into an expression value representing all transcripts from the same gene.

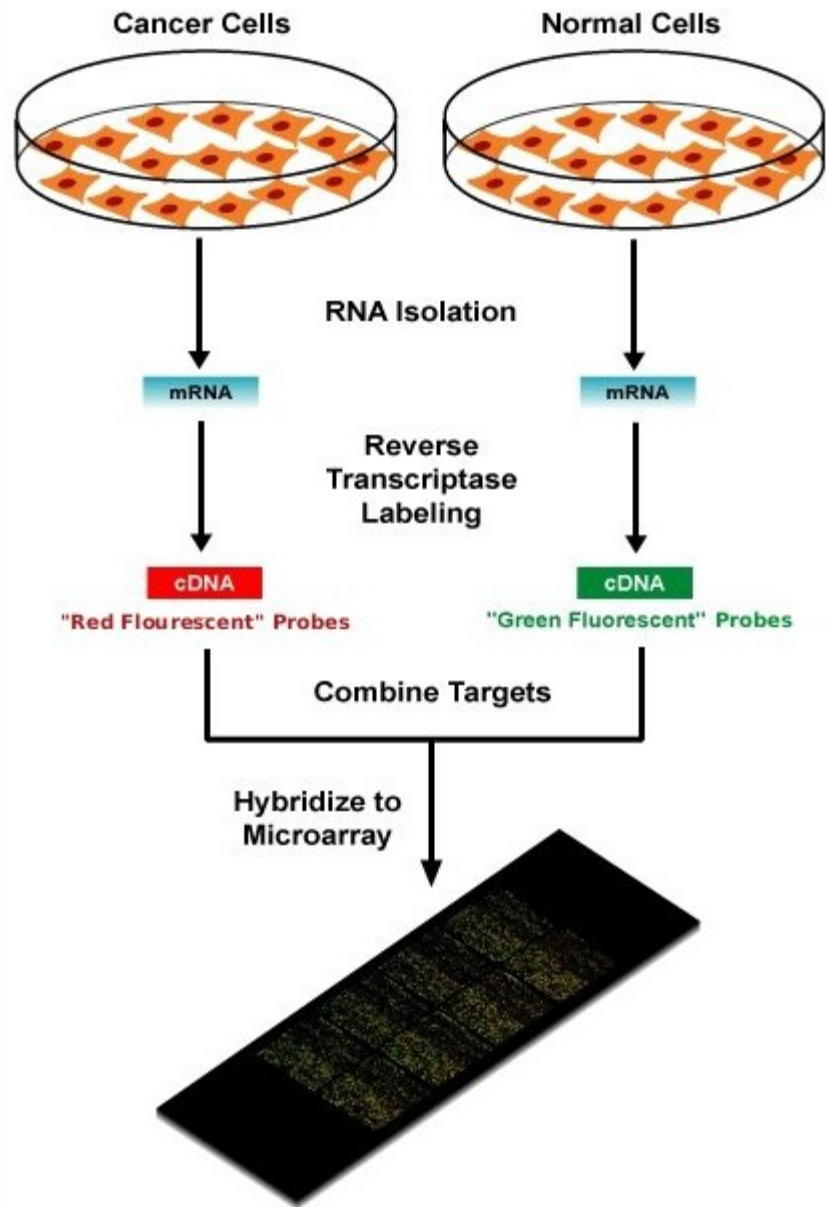
Array Format	169
Feature Size	5 µm
Total Number of Distinct Probes	764,885
Oligonucleotide Probe Length	25-mer probes
Required Orientation of Labeled Targets to be Hybridized to the Array	Sense
Gene-level Probe Sets with Ensembl Support	28,132
Gene-level Probe Sets with Putative Full-length Transcript Support (GenBank and RefSeq)	19,734

## More Information

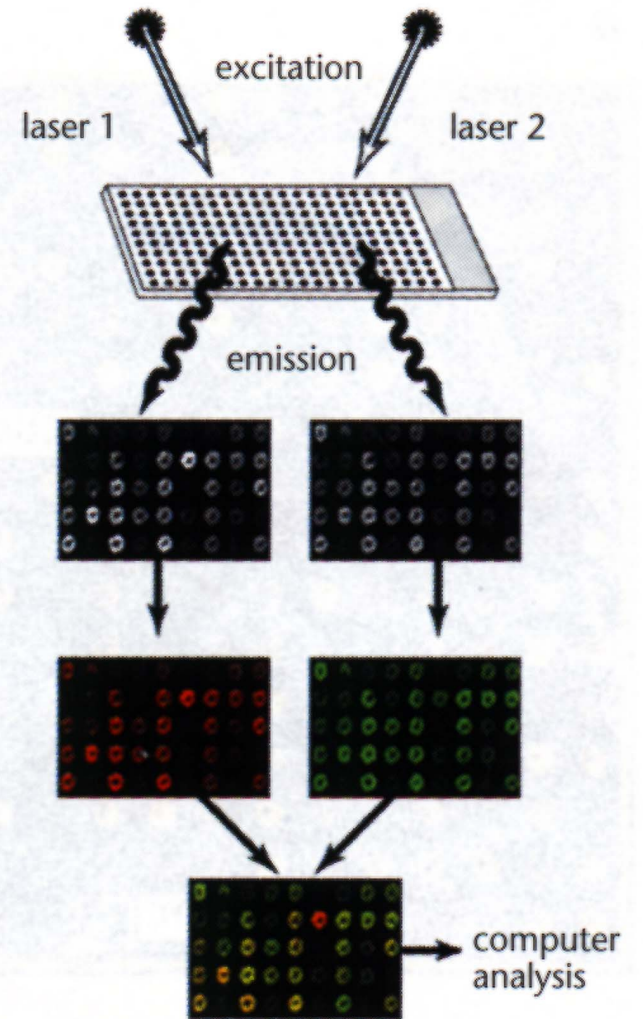
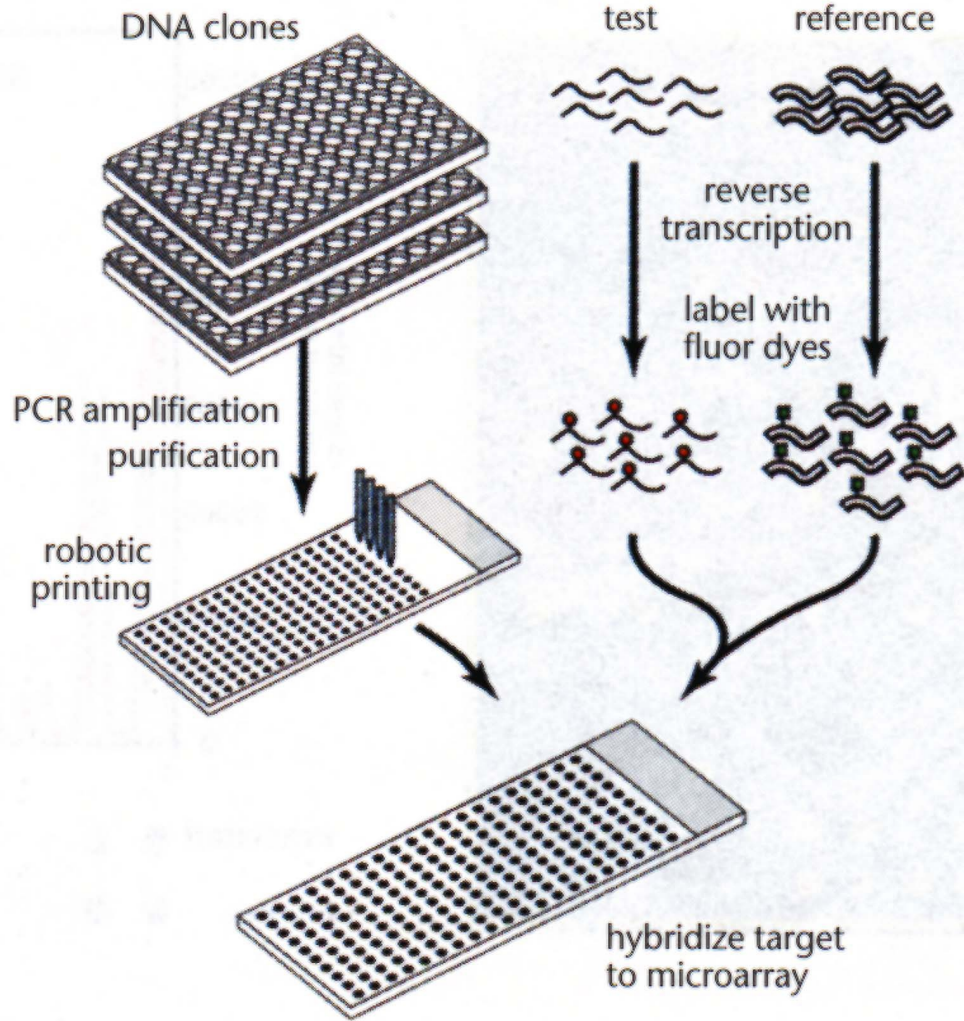
[Details about the complete system for expression analysis with the Gene 1.0 ST Array](#)

**SurePrint** (Agilent) – tehnologija tiskanja oligonukleotidov na steklenem nosilcu. Dolžine oligonukleotidov, pripravljenih *in situ*, so standardno 60 nt. Tiskalnik brizga  $pI$  količine reagentov na točno določena mesta na podlagi, sinteza pa poteka po fosforamiditni metodi.



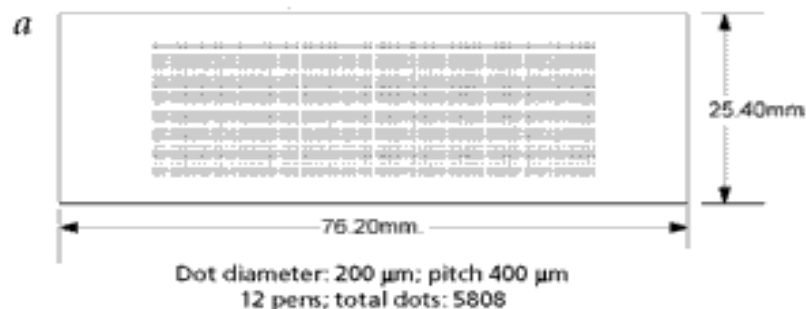






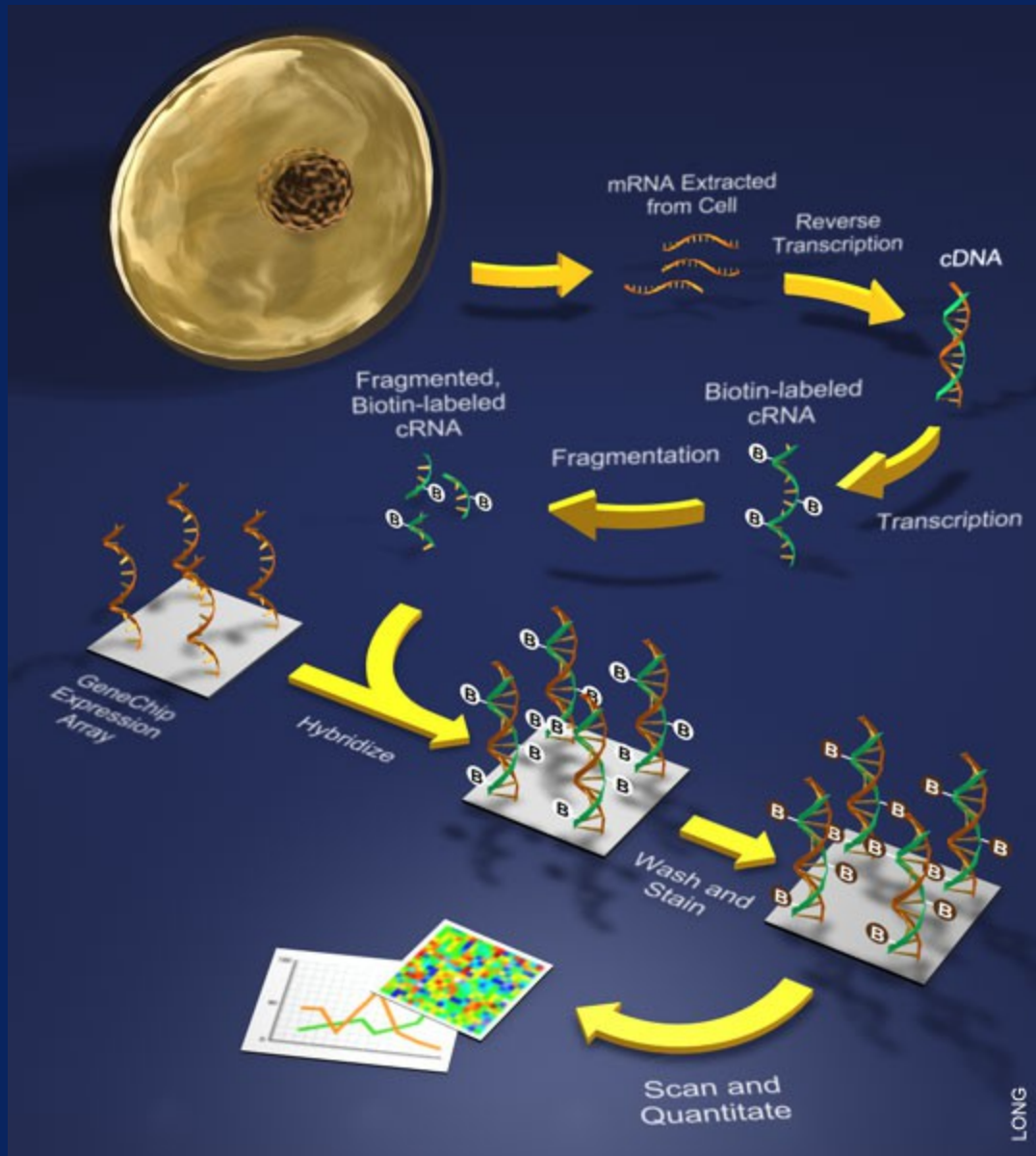
# DNA-mikromreže /4

- cDNA za imobilizacijo lahko dobimo iz zbir klonov
- za fiksiranje na steklo rabimo nekaj *nI* sonde (0,1-0,5 µg/ml)
- steklo je obdelano s poli-Lys ali aminosilani
- DNA kovalentno vežemo na steklo z UV (254 nm)
- cDNA pretvorimo v ss obliko s segrevanjem ali z NaOH
- vzorčno cDNA označimo s fluorescenčnima označevalcema Cye3-dUTP in/ali Cye5-dUTP (zelen / rdeč) v stopnji reverzne transkripcije (pričakovana vgradnja 2 Cye/100 b)
- za vsako ploščico (~800 mm<sup>2</sup>) z vzorci rabimo 2-5 µg mRNA

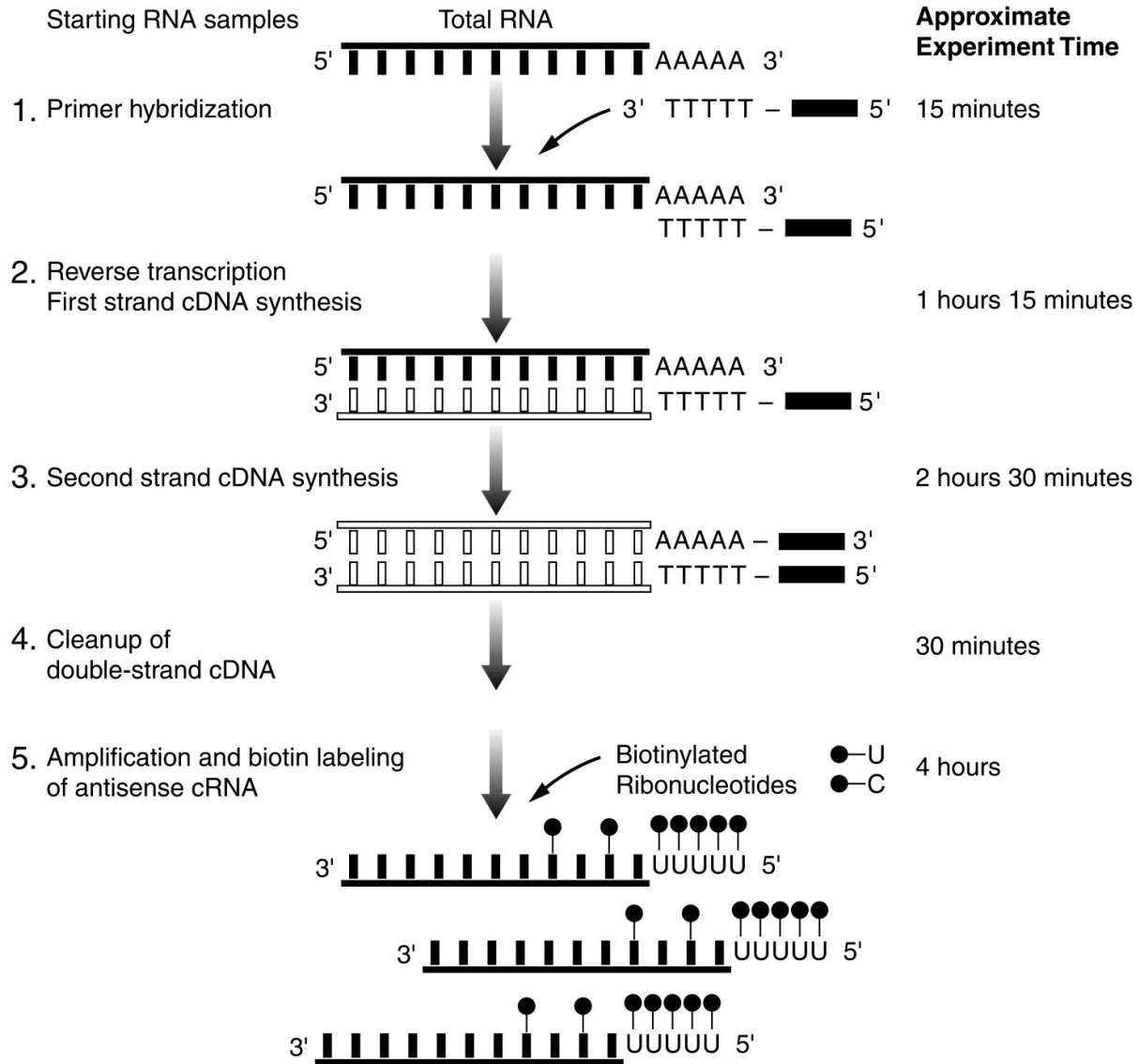


← tehnologija leta ~2000

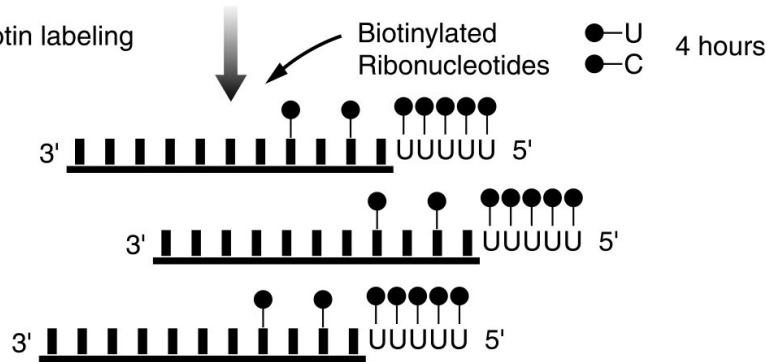
- detektiramo s pomočjo prirejenega laserskega vrstičnega konfokalnega mikroskopa; laserja sta zeleni (532 nm) in rdeči (633 nm)
- zaradi visoke ponovljivosti rastrskega nanašanja (roboti) lahko analiziramo rezultate s superimpozicijo
- programska oprema omogoča preračunavanja, primerjave, itd.



## Eukaryotic Target Labeling for GeneChip® Probe Arrays



5. Amplification and biotin labeling of antisense cRNA



6. Cleanup of biotinylated cRNA

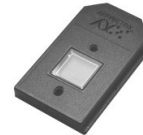
30 minutes

7. Fragmentation

45 minutes

8. Hybridization

16 hours



9. Washing/Staining

Streptavidin-phycoerythrin  
Biotinylated anti-streptavidin antibody

75 minutes

10. Scanning

< 10 minutes



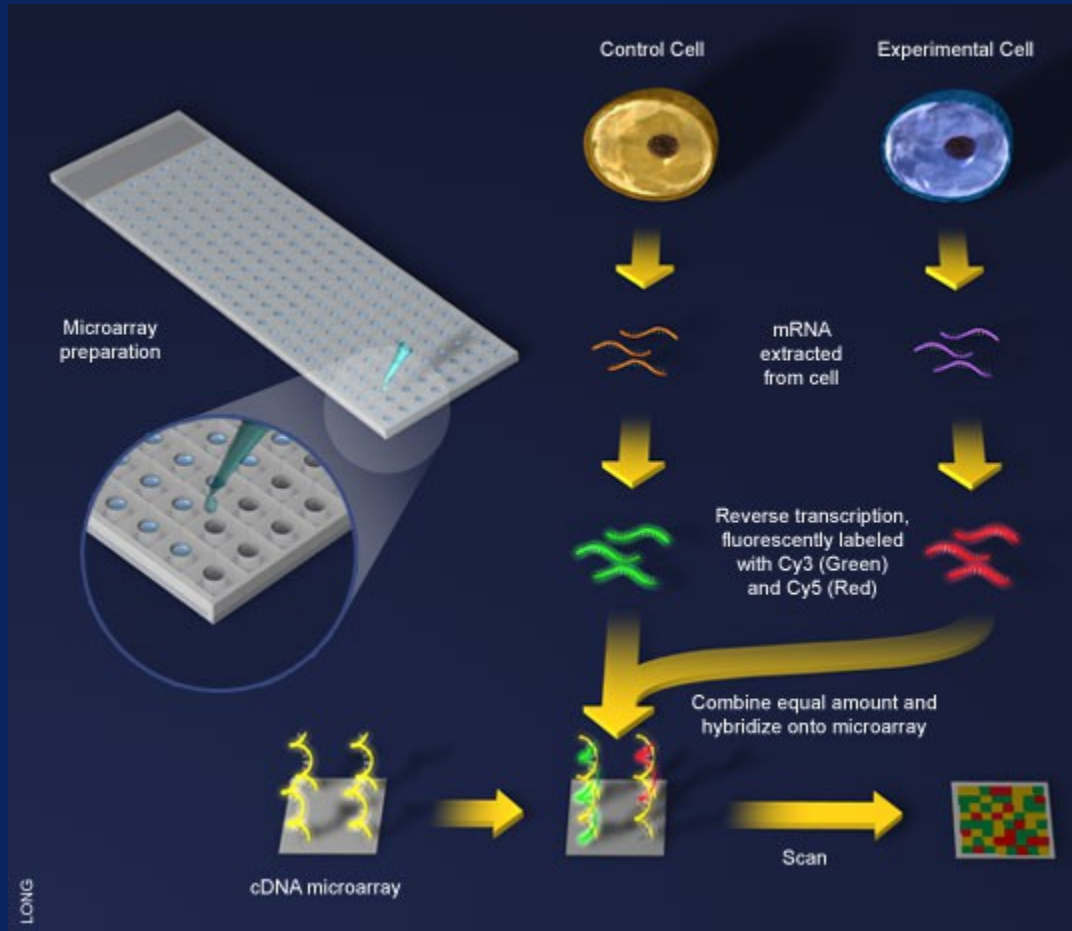
Legend:

TTTTTT RNA

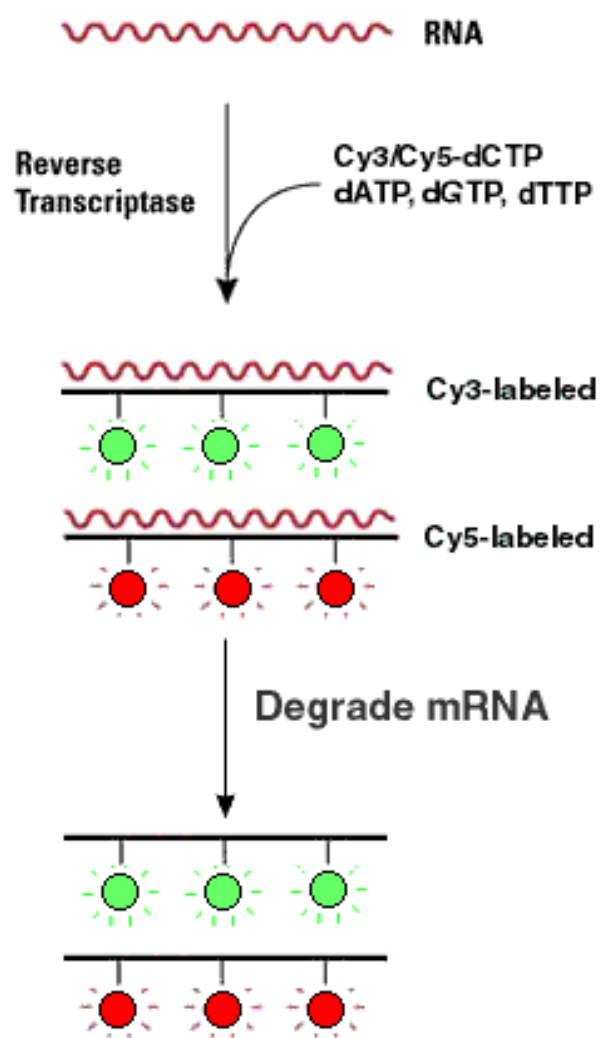
TTTTTT DNA

TTTTTT T7 Primer

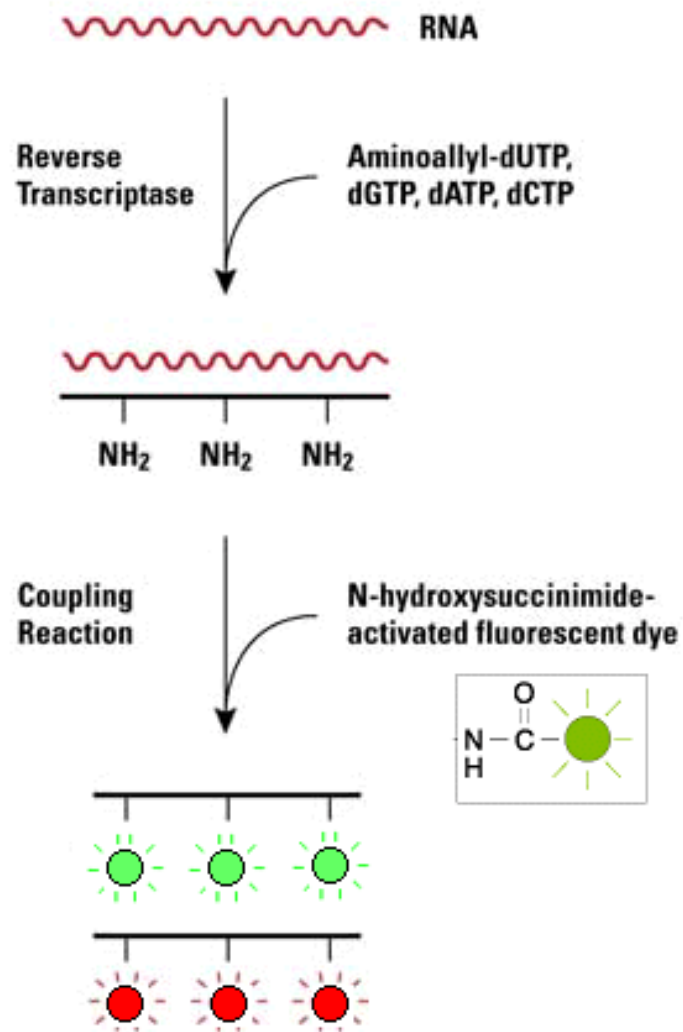
●— Biotin



## A Direct Labeling

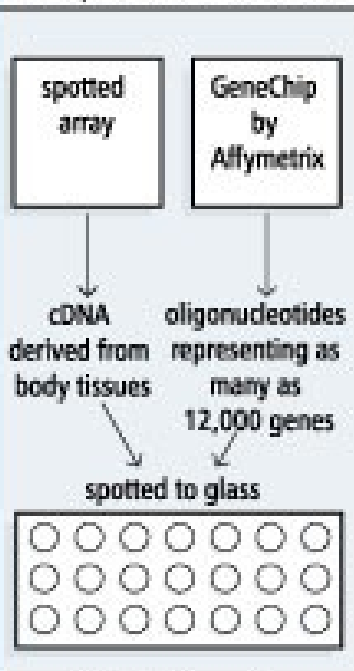


## B aa-dUTP Indirect Labeling

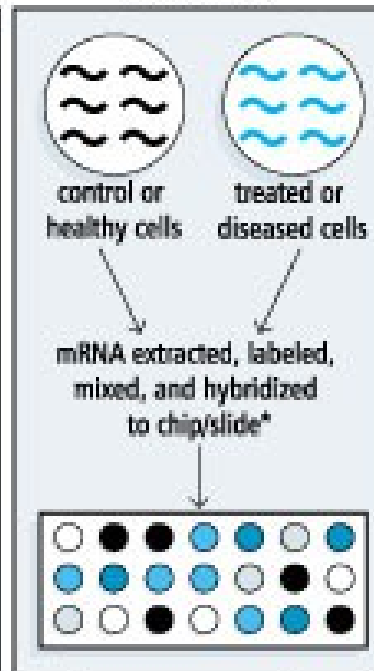


# MICROARRAY TECHNOLOGY

## CHIP/SLIDE PREPARATION

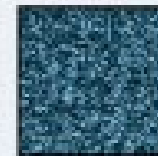


## EXPERIMENT

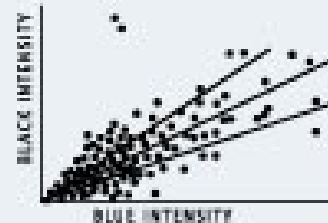


## ANALYSIS

scan chip/slide with laser; the resulting image is a complex pattern



algorithms turn data into graphical representations



**\*Note:** Affymetrix experiments use two chips. Software analyzes pattern differences between the two.

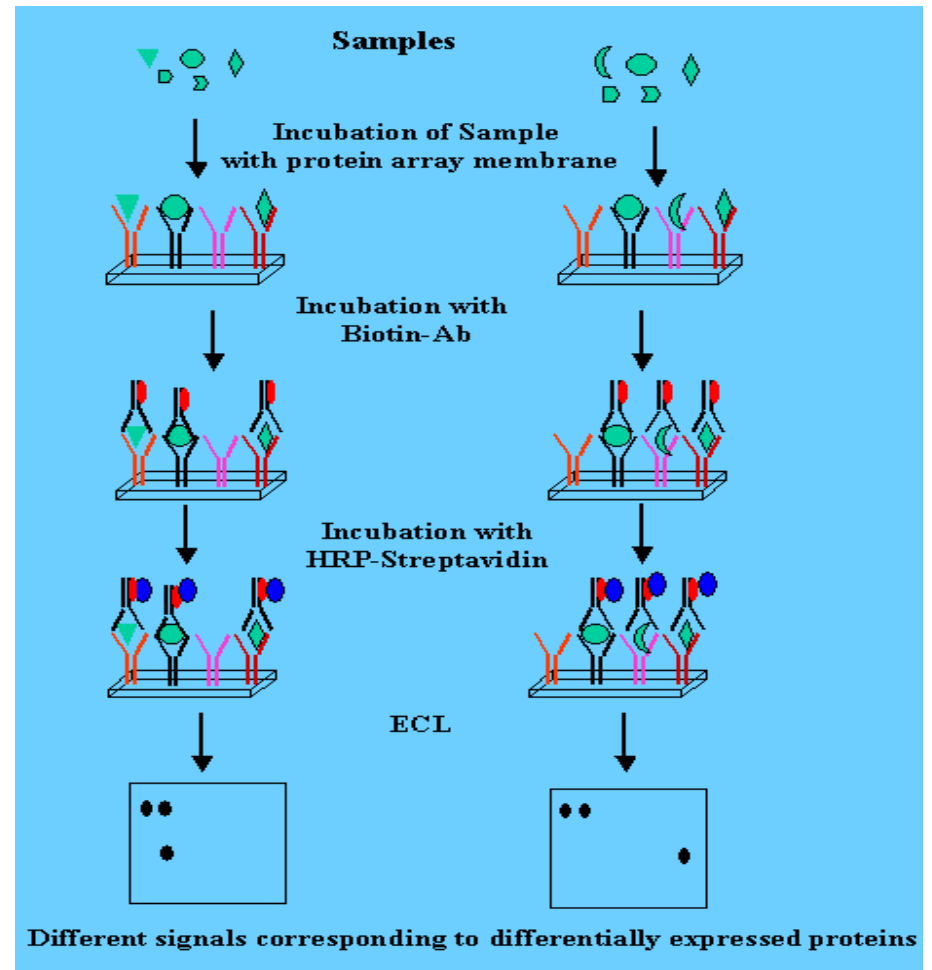
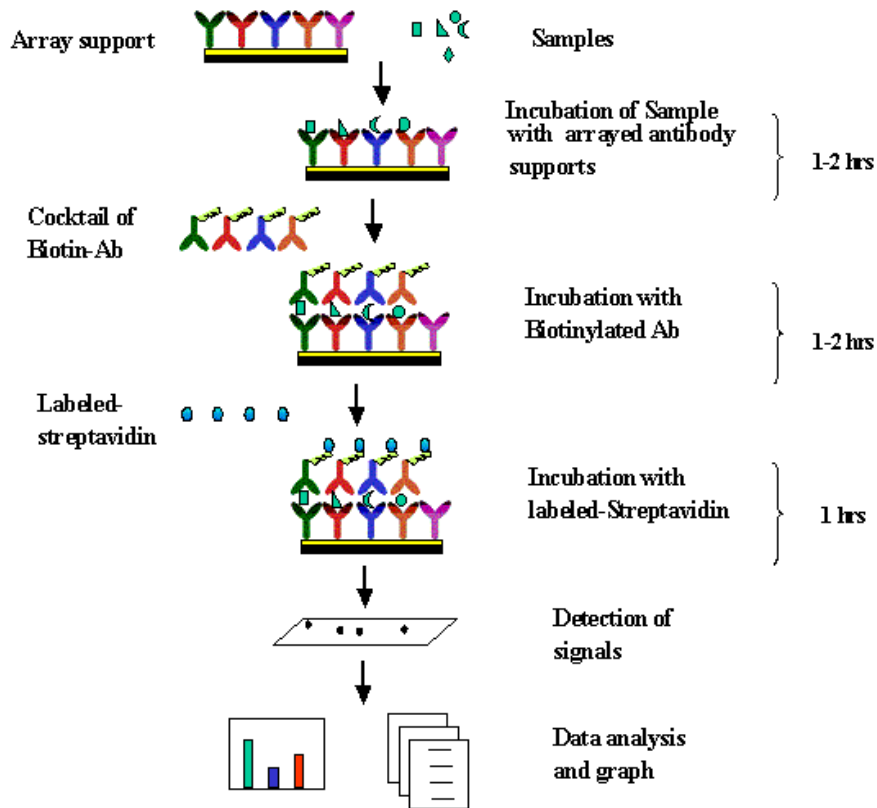
### **Result:**

Gene expression is represented as a function of the ratio of the intensities.

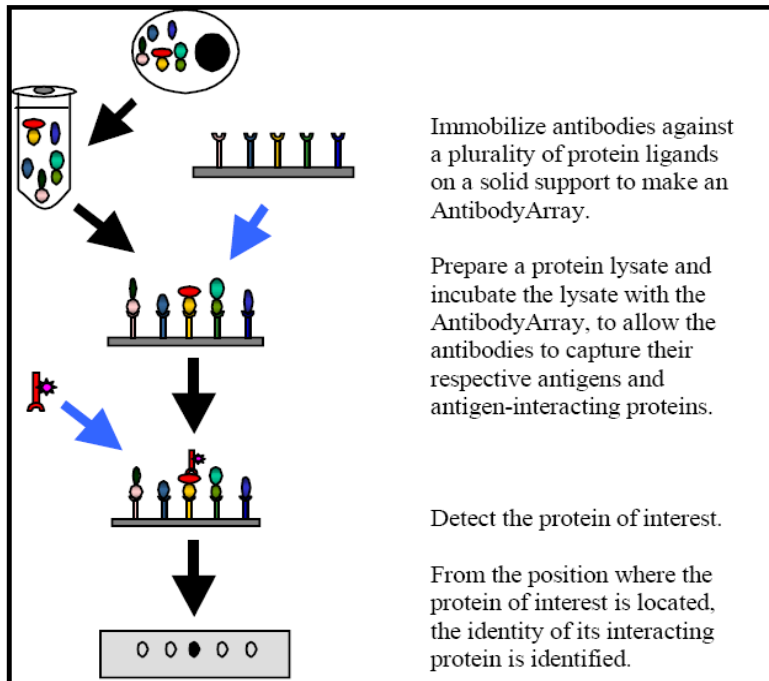
- a. ↑ black – ↑ expression in untreated.
- b. ↑ blue – ↑ expression in treated.
- c. blue/black – genes equally expressed in both types of cells.



# Proteinske mikromreže

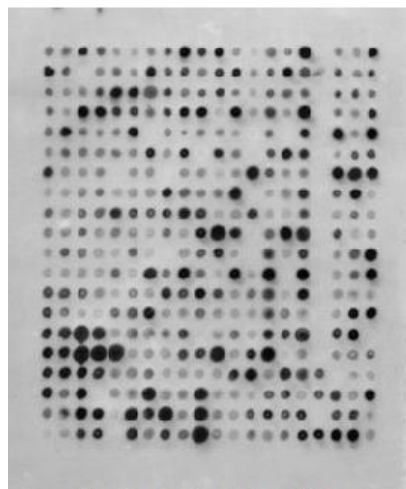


# Proteinske mikromreže: mreže s protitelesi

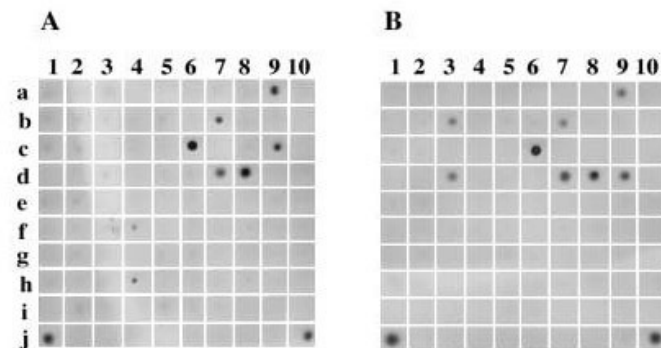


Mreže vsebujejo protitelesa proti proteinom, za katere vemo, da sodelujejo v določenem celičnem / fiziološkem procesu (npr. apoptoza, prenos signalov ipd.). Z njihovo pomočjo lahko identificiramo tudi interakcije med proteini – pogoj je, da imamo neimobilizirana protitelesa proti proteinu, ki nas zanima in za katerega predpostavljamo, da interagira s proteinom, proti kateremu so protitelesa vključena v mrežo.

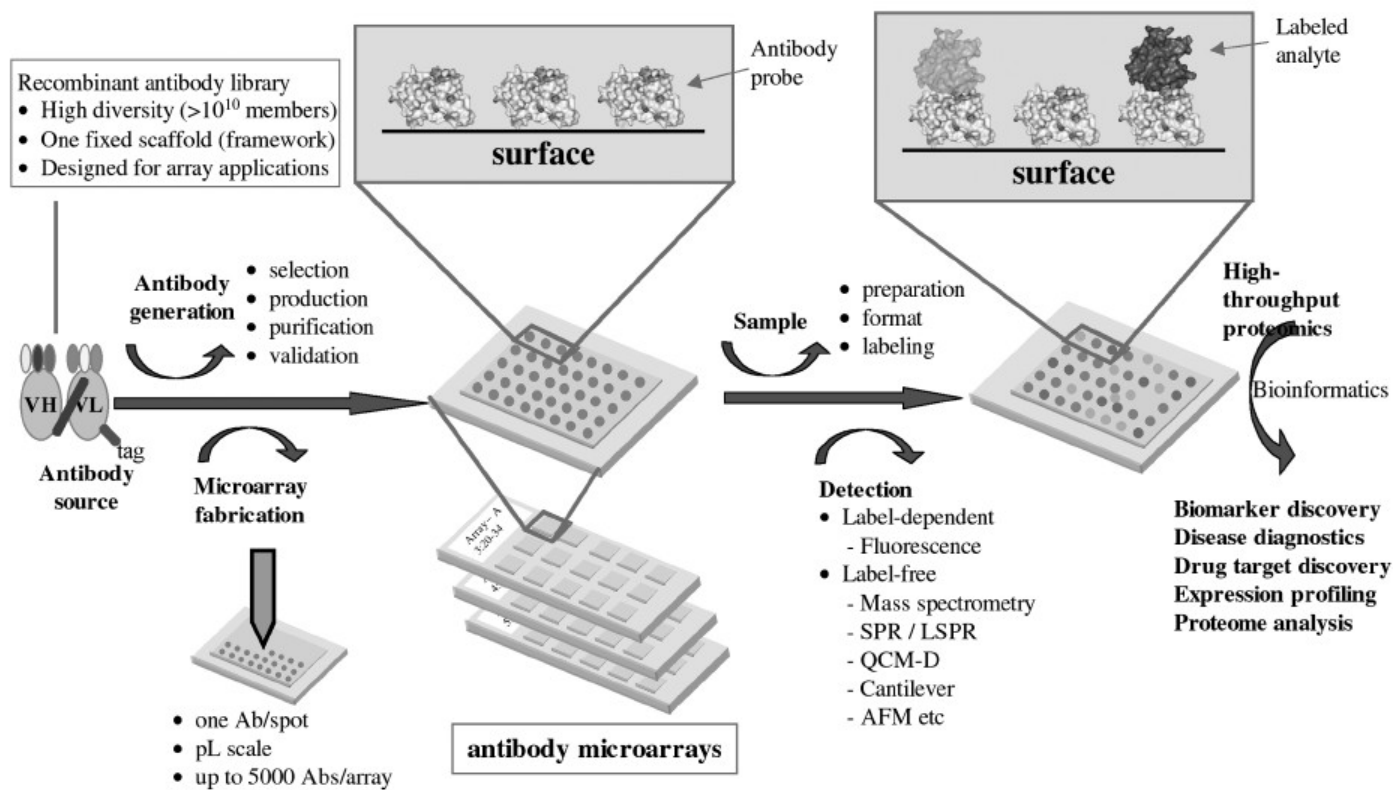
Preučujemo lahko tudi ekspresijske profile v določenih tkivih in posttranslacijske modifikacije.



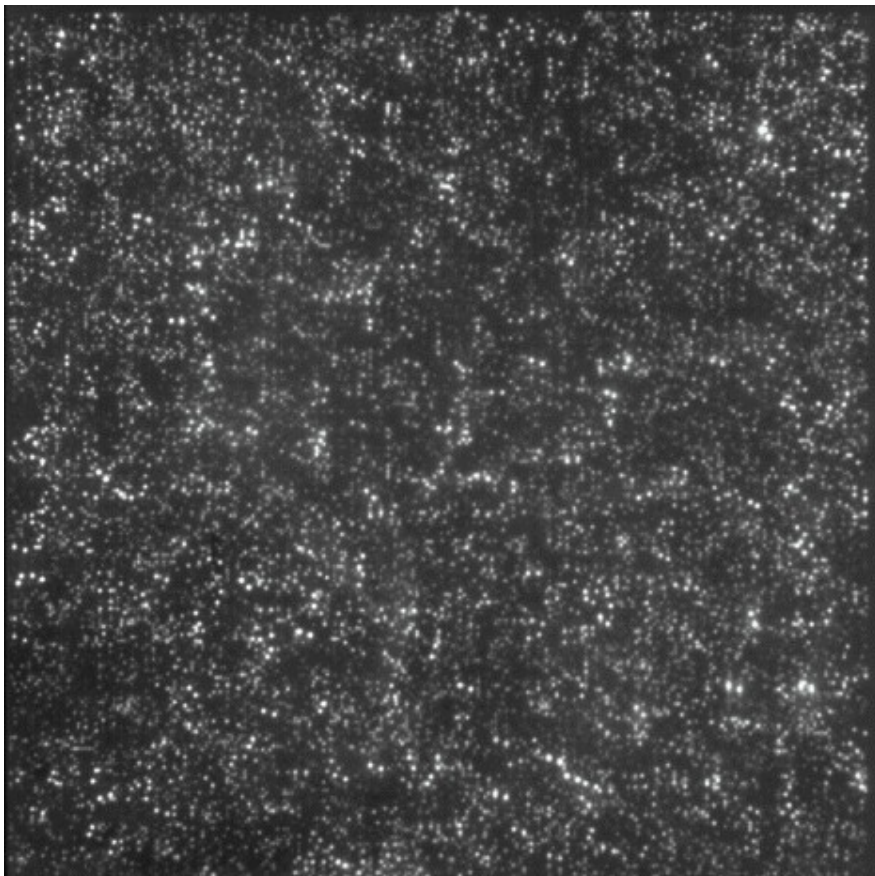
Tyrosine phosphorylation profiling with Signal Transduction Antibody Array™



Two identical antibody arrays each containing 100 antibodies were used to screen potential Stat1 interacting proteins. One array was incubated with the whole cell lysates from HeLa cells ( $2 \times 10^7$ ) received no treatment (A); while the other array was incubated with lysates from cells treated with recombinant human TNF-alpha (10ng/ml) for 30 min (B). In this example, Stat1 proteins were found to interact with several proteins, including TNFR1 (7d), TNFR2 (8d), FADD (3b), TRADD (9d). The interactions between Stat1 and FADD and between Stat1 and TRADD are regulated by signaling initiated by TNF treatment (compare A and B).



# Proteinske ekspresijske mreže

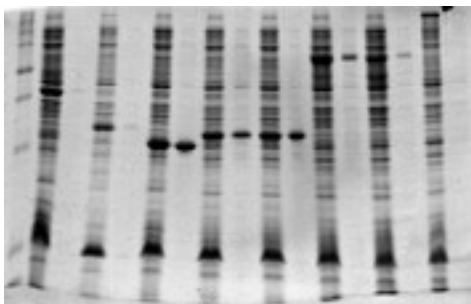


Iz tkiva izolirajo mRNA → cDNA; vstavijo v ekspresijski vektor in izrazijo v *E. coli*.  
Rekomb. proteine izolirajo (His6) in jih nanesejo kot mrežo na PVDF-membrano (22 cm x 22 cm) v duplikatu:  
~27 600 proteinov (x2) na 1 membrani.

S tovrstnimi mrežami lahko npr. določamo specifičnost protiteles, prisotnost točno določenih protiteles v serumu ipd.

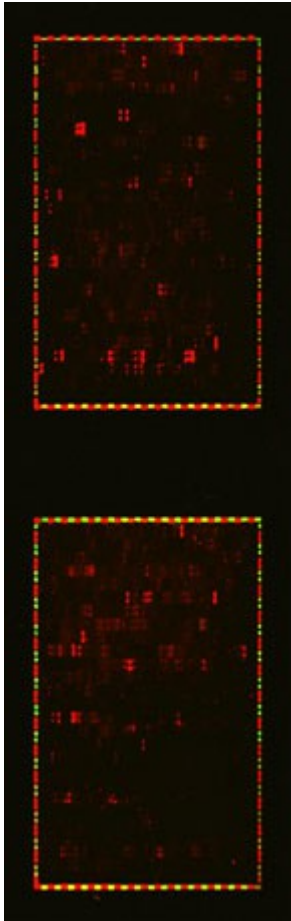
Library	Proteins
Human Brain	38.016
Human Lung	24.576
Human Colon	18.432
Human Testis	26.880
Human Uniclone Set (Protagen)	15.744
Human T-Lymphocytes	16.128
Human T-Lymphocytes (PHA induced)	17.280

<http://www.rzpd.de/products/proteinarrays/>



<http://www.proteinstrukturfabrik.de/hex1>

# Peptidne mikromreže



Peptidni mikromreži po analizi tumorskega oz. kontrolnega seruma.

Mikromrežno so razporejeni peptidi, iščemo pa specifična protitelesa v vzorcu.

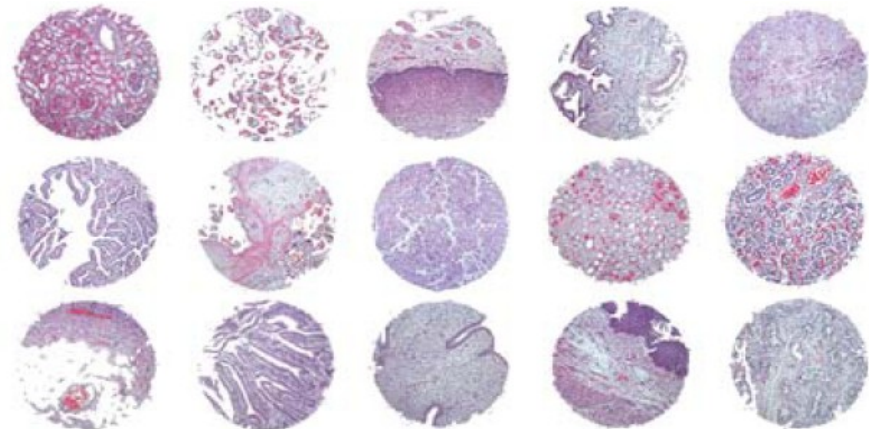
Npr.: 6 tumorskih antigenov prestavljenih s prekrivajočimi se peptidi – po 2000 peptidov nanesenih v duplikatu → inkubiramo z 10  $\mu$ l tumorskega oz. kontrolnega seruma.

Lise obarvajo s sekundarnimi protitelesi (anti-človeška Ab).

# Tkivne (mikro)mreže



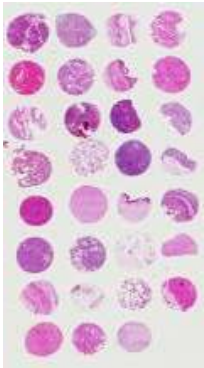
Tkivne mikromreže predstavljajo urejene vzorce tkivnih rezin s premerom 0,6 mm do 2 mm, ki omogočajo imunohistokemijske analize in analize hibridizacije *in situ* (FISH). Na vsaki plošči je več deset do več sto vzorcev. Vsi so (pato)fiziološko in histološko okarakterizirani in običajno neobarvani. Lahko so v parafinu in fiksirani s formalinom ali pa samo z zamrzovanjem.



### Technical Information

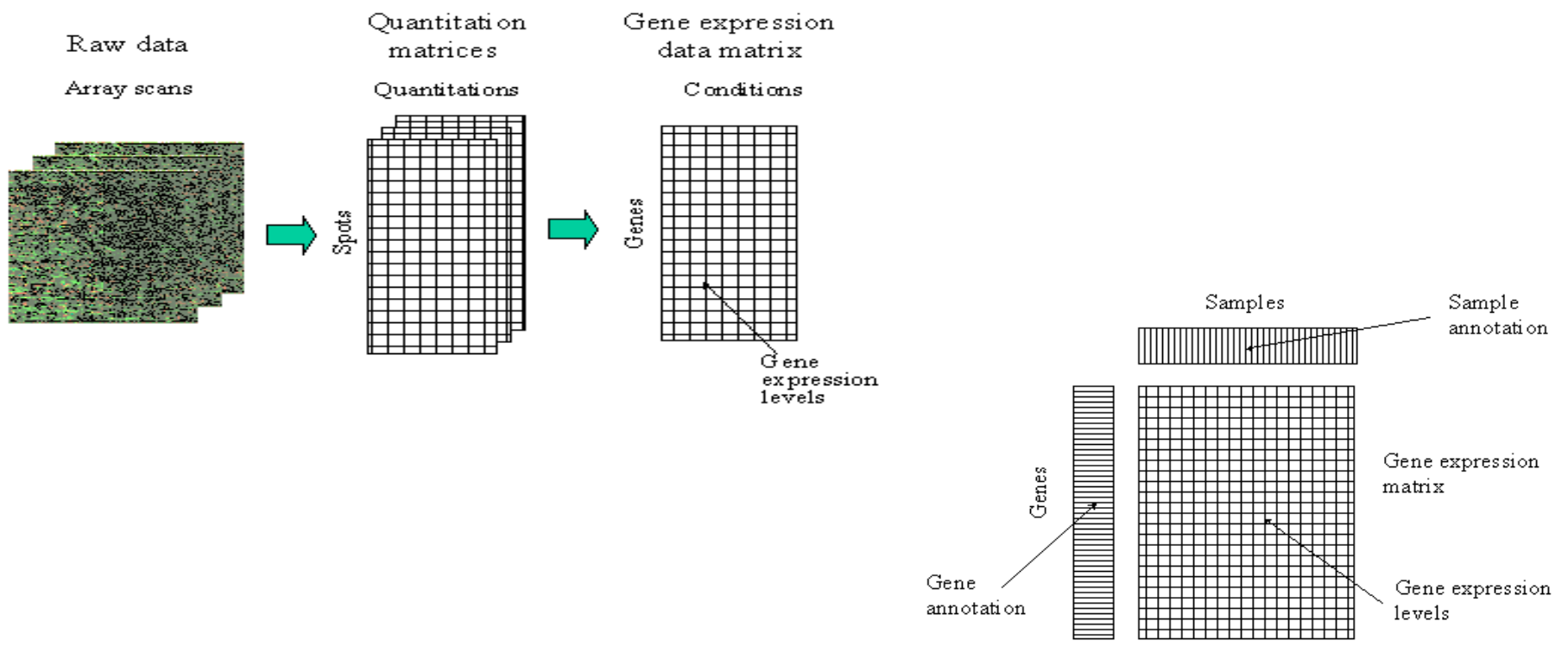
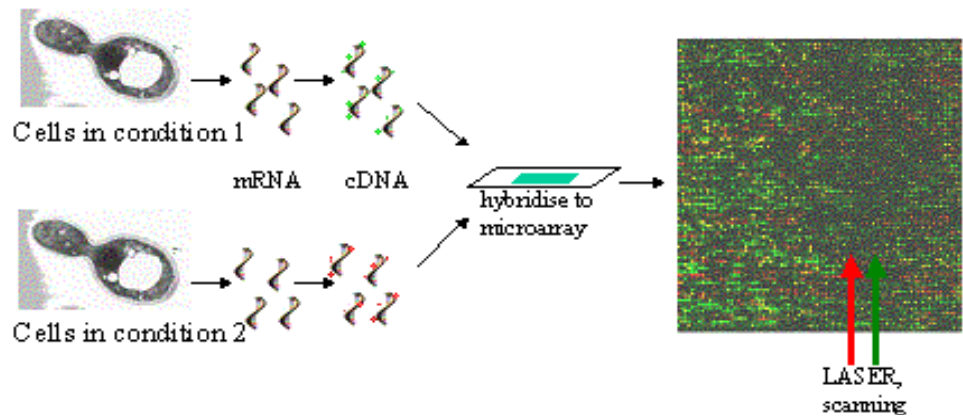
- Spot diameter: 2mm
- Fixation in 4 % paraformaldehyde in PBS
- Paraffin embedded
- Tissue type validated by immunohistochemistry /classified according to synovitis score by Krenn (Surgical Pathology Update 2001)

## Tissue Microarray Synovitis, human



	a	b	c	d	e
1	●	●		●	●
2	●	●		●	●
3	●	●		●	●
4	●	●	●	●	
5	●	●		●	●
6	●	●		●	●
7	●	●		●	●

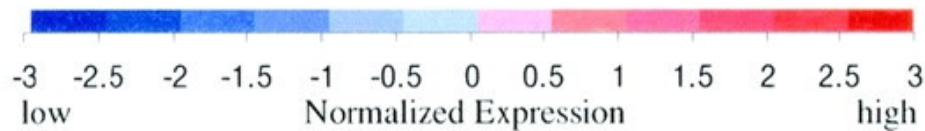
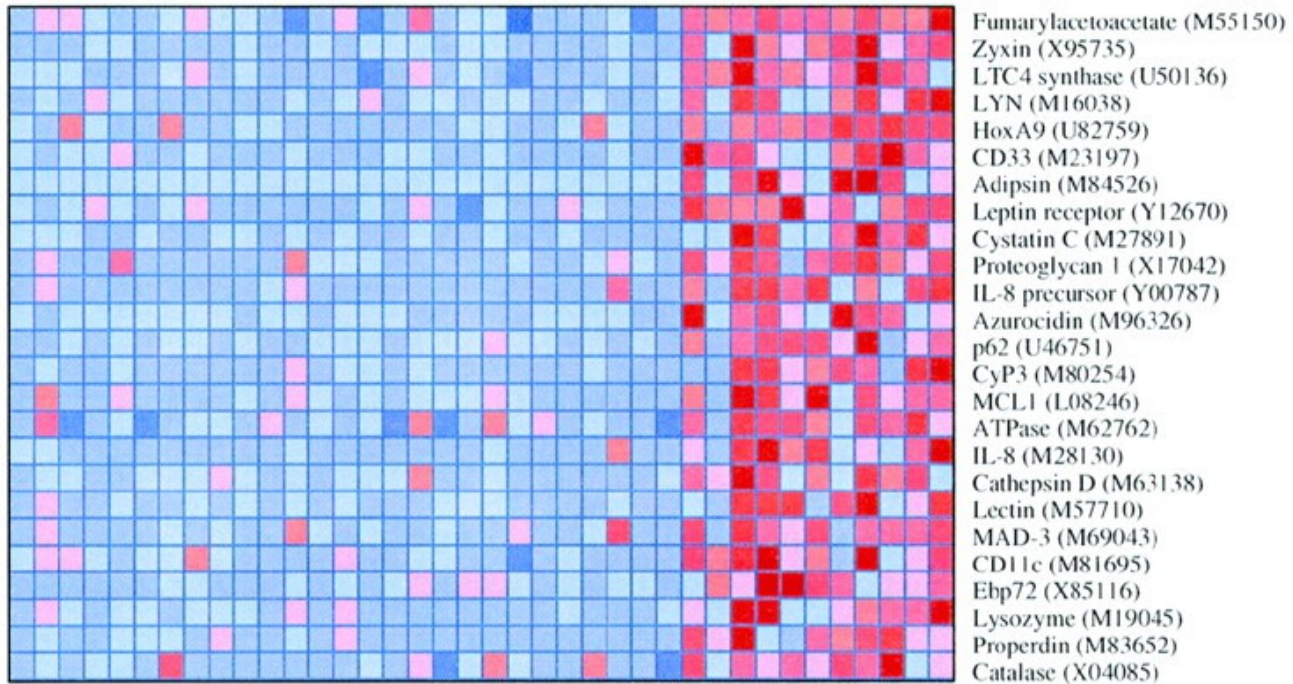
Position	localisation	Diagnosis	Sex	Age
1 a	knee	RA	f	76
1 b	knee	RA	f	76
1 d	left knee	RA	f	62
1 e	left knee	RA	f	62
2 a	knee	RA	f	57
2 b	knee	RA	f	57
2 d	knee	RA	m	19
2 e	knee	RA	m	19
3 a	knee	RA	f	61
3 b	knee	RA	f	61
3 d	hip	PSA	m	28
3 e	hip	PSA	m	28
4 a	knee	normal tissue	m	24
4 b	knee	normal tissue	m	24
4 c	knee	normal tissue	m	37
4 d	knee	normal tissue	m	37
5 a	knee	OA	m	66
5 b	knee	OA	m	66
5 d	hip	OA	f	66
5 e	hip	OA	f	66
6 a	hip	OA	m	54
6 b	hip	OA	m	54
6 d	knee	OA	f	65
6 e	knee	OA	f	65
7 a	knee	OA	f	75
7 b	knee	OA	f	75
7 d	knee	OA	m	82
7 e	knee	OA	m	82





fenotip 1

fenotip 2



ArrayExpress is a public repository for **transcriptomics data**, which is aimed at storing **MIAME-** and **MINSEQE-** compliant data in accordance with **MGED** recommendations. The ArrayExpress Warehouse stores gene-indexed **expression profiles** from a curated subset of experiments in the repository.

» [More Info](#)

## Experiments

6849 experiments, 206300 assays available



Experiment, citation, sample and factor annotations

- » [Browse experiments](#)
- » [Advanced query interface](#)

Query

» [Submitter/reviewer login](#)

## Expression Profiles

740 experiments, 169983 genes available



Genes

Experiment and sample annotations

Any species



Query

» [Advanced query interface](#)

## News



- **27 Aug 2008 - Sorting by Experiment Technology Type**  
Quickly identify [ChIP-Chip](#) or [RNAi](#) experiments using the new drop down filter.
- **27 Aug 2008 - Links to variably expressed genes in the Atlas from the browse interface**  
E.g. query [E-GEOD-4262](#) and identify genes where expression varies with the experimental factors.

## Links

- [ArrayExpress Atlas Beta](#) *new!*
- [ArrayExpress User Survey](#)
- [Help](#) | [FAQ](#) | [Tutorials](#) | [Citing](#)
- [Submit data](#) to ArrayExpress
- [Programmatic Access](#)
- [FTP server](#) for public data
- [Software Downloads](#) and [Statistics](#)
- [Quality metrics](#) for microarrays
- [ArrayExpress Scientific Advisory Board](#)

## ArrayExpress (EMBL)

<http://www.ebi.ac.uk/microarray-as/ae/>

Zbirka podatkov o izražanju genov, dobljenih z mikromrežami.