ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»

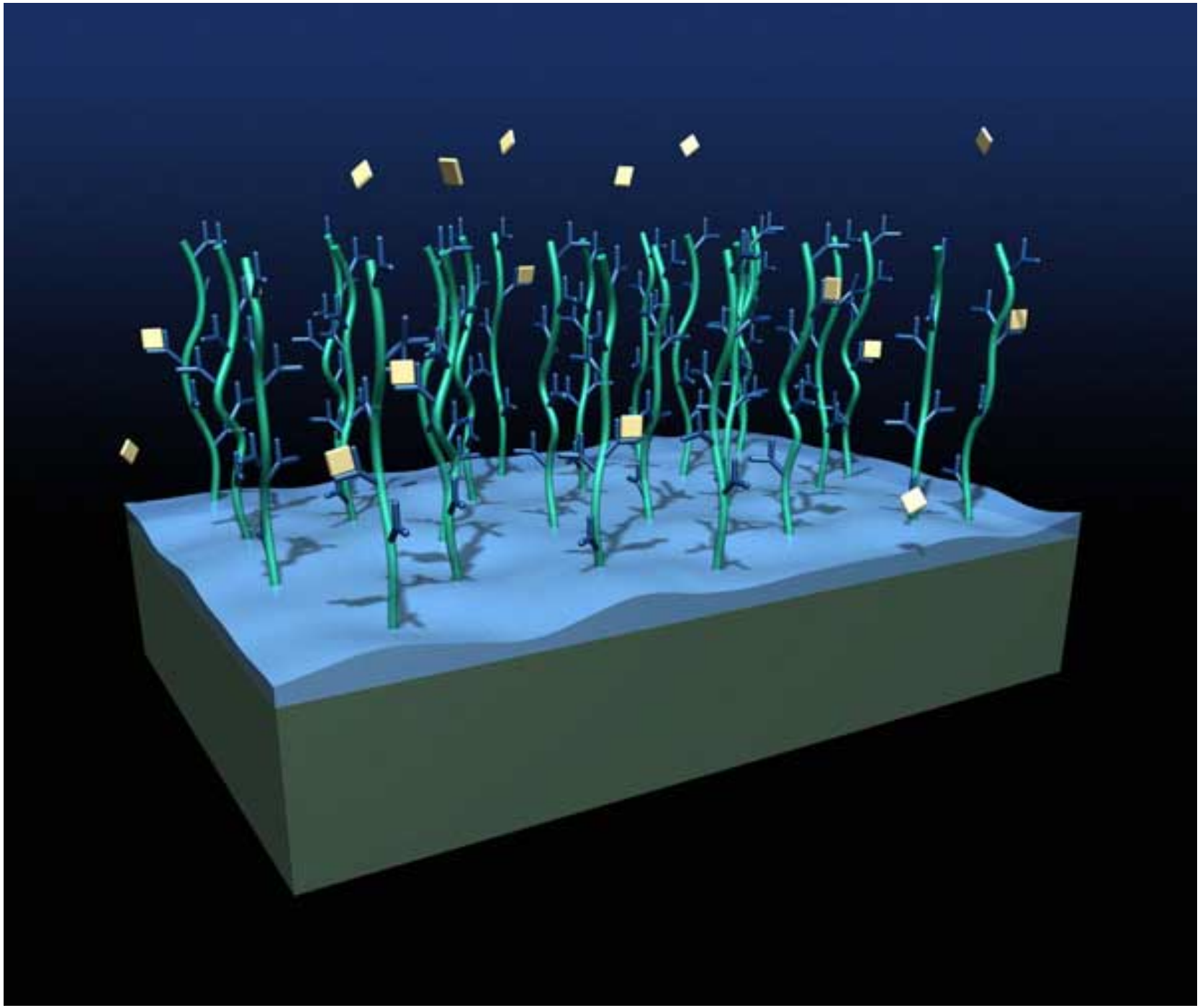
ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ
ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ

Τεχνικές μελέτης γονιδιακής έκφρασης:

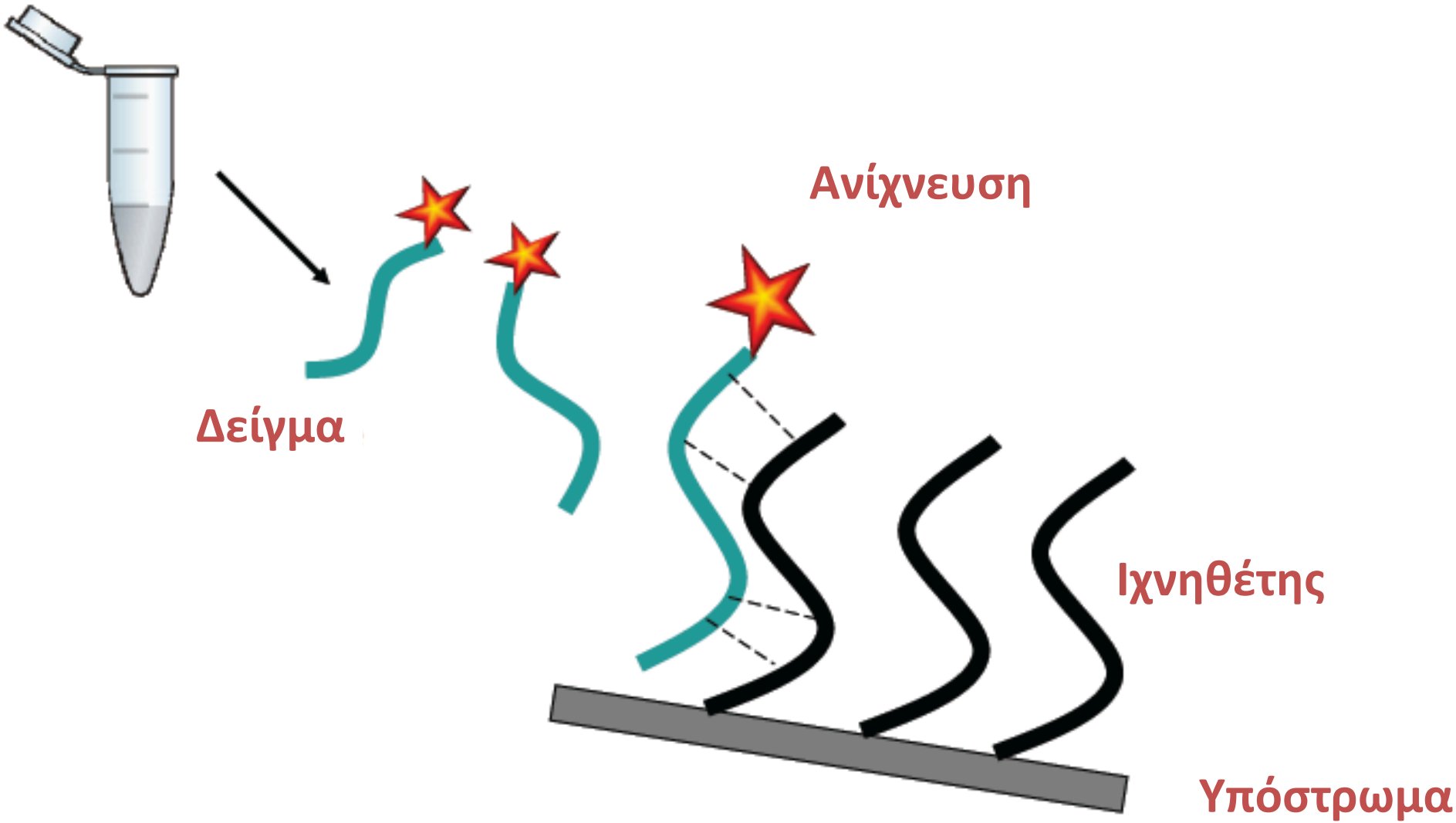
Μικροσυστοιχίες

Νικόλαος Μπαλατσός

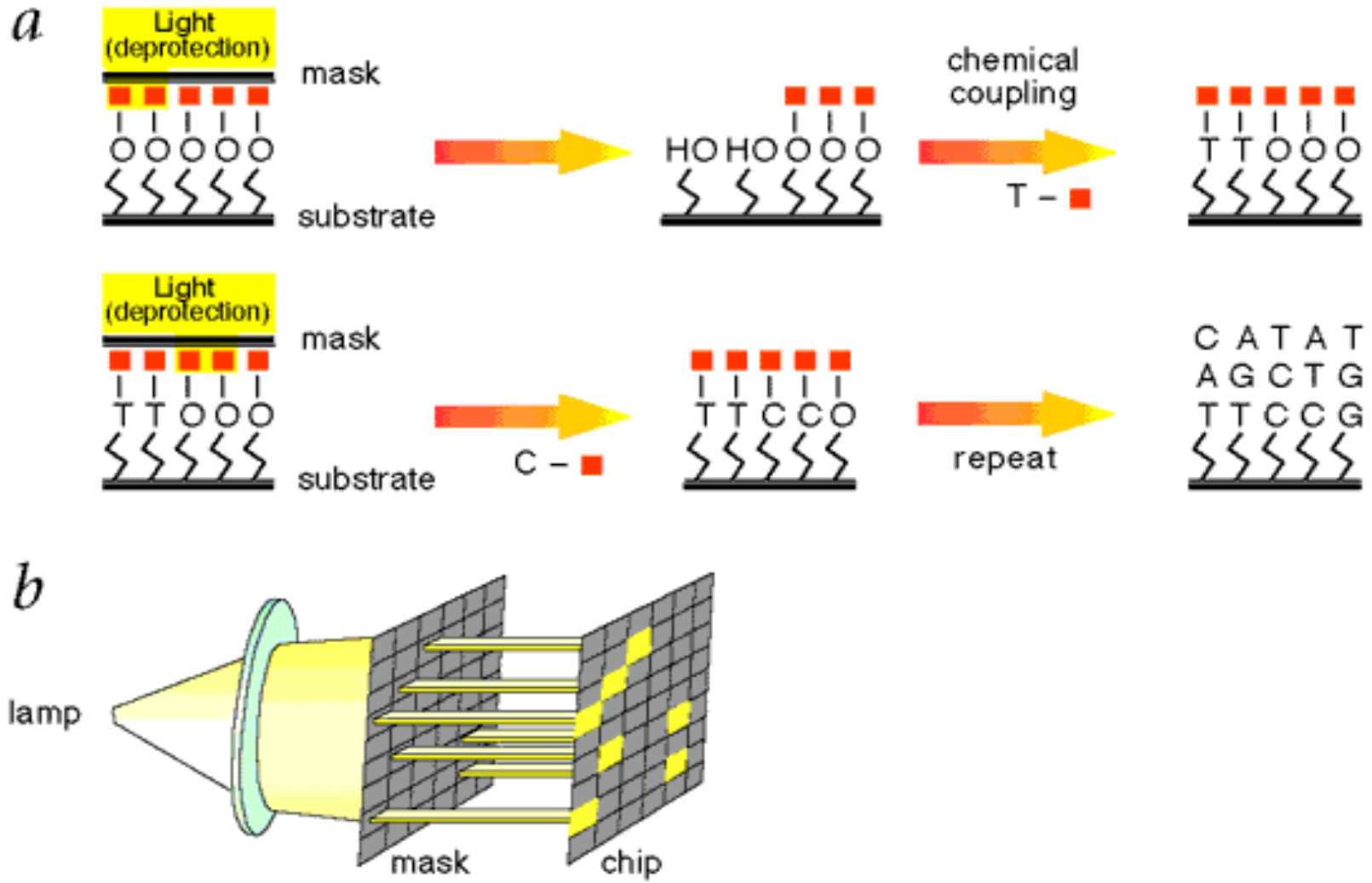


http://www.arrayit.com/Products/Microarray_Slides/Hydrogel_Slides/hydrogel_slides.html

Μικροσυστοιχίες

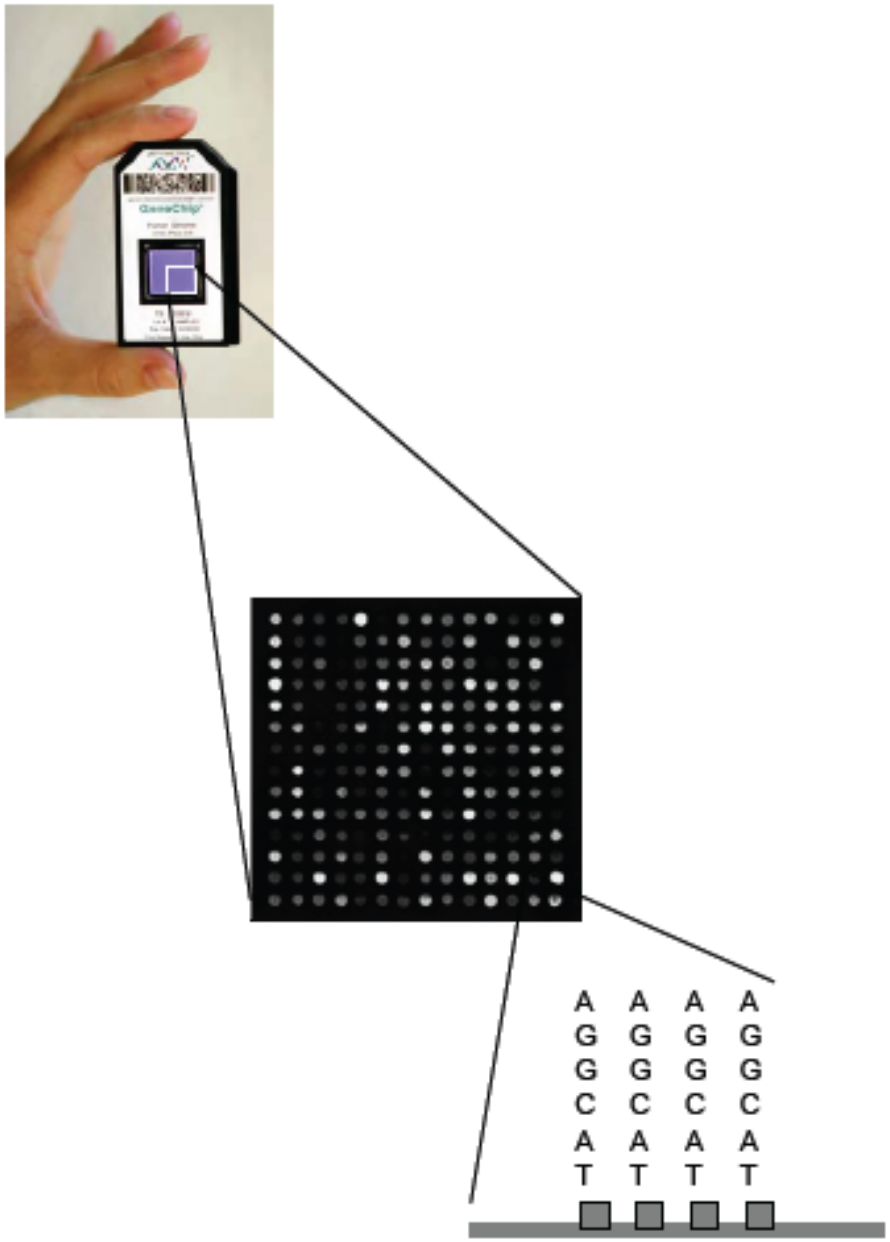


Μικροσυστοιχίες



Fodor et al.Science 1991;251:767-773)

Μικροσυστοιχίες



Μικροσυστοιχίες

Εφαρμογές μικροσυστοιχιών

- Μελέτη γονιδιακής έκφρασης
- Ανίχνευση πολυμορφισμών
  (Single nucleotide polymorphism, SNP)
- Εκφραση πρωτεϊνών
- Αλληλεπίδραση πρωτεϊνών

Παροχή μεγάλης παράλληλης πληροφορίας

Πλεονεκτήματα μικροσυστοιχιών

- Μικρός όγκος δείγματος (nL)
- Ελάχιστη σπατάλη αντιδραστηρίων
- Ταυτόχρονη ανάλυση πολλών γονιδίων/πρωτεϊνών
- Αυτοματοιποίηση
- Ποσοτικοποίηση

Ωφέλειες μικροσυστοιχιών

Κλινική φροντίδα

Διάγνωση

Πρόγνωση

Πρόβλεψη απόκρισης θεραπείας

Παρακολούθηση

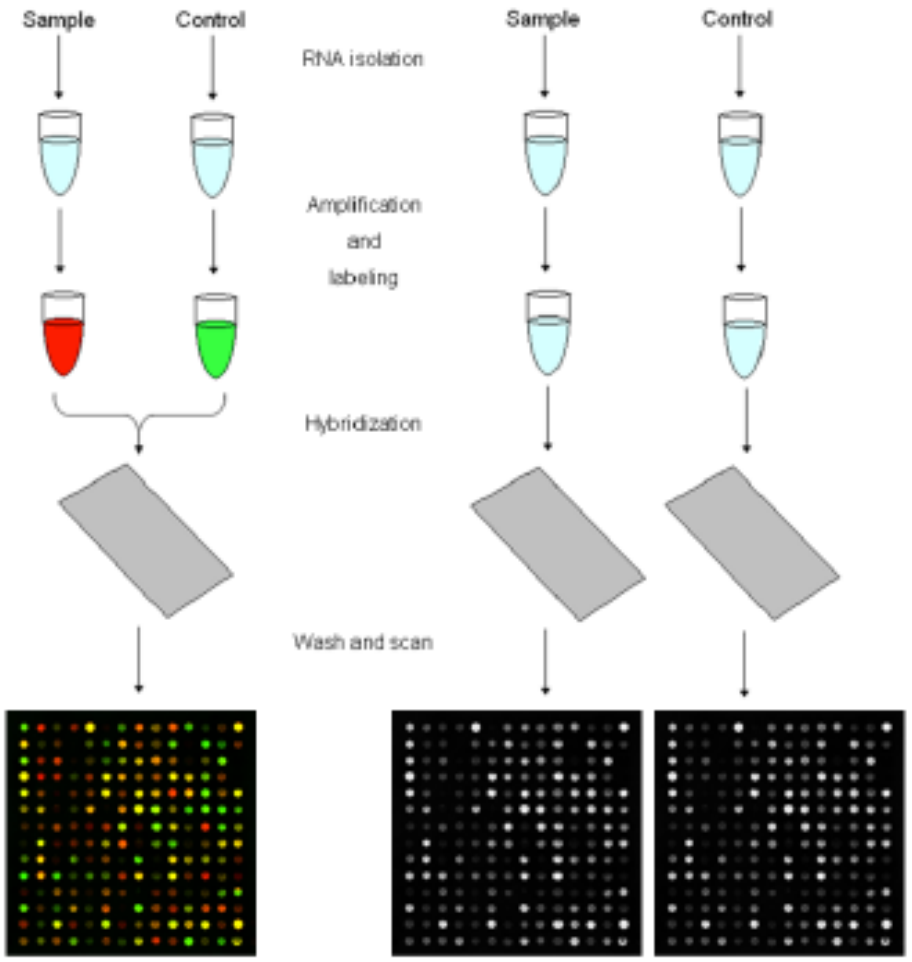
Ερευνα

Κατανόηση παθογένεσης ασθένειας

Περιορισμοί μικροσυστοιχιών

- Νέα (σχετικά) τεχνολογία
- Τεχνικά θέματα (θόρυβος, επαναληψιμότητα)
- Καλύτερος καθορισμός γονιδίων (πολλά ESTs)
- Ακριβή τεχνολογία

Μικροσυστοιχίες



Υπόθεση

Η φαινοτυπική διαφορετικότητα (diversity) του καρκίνου μπορεί να συνοδεύεται από ανάλογη διαφορετικότητα στη γονιδιακή έκφραση (μικροσυστοιχίες)

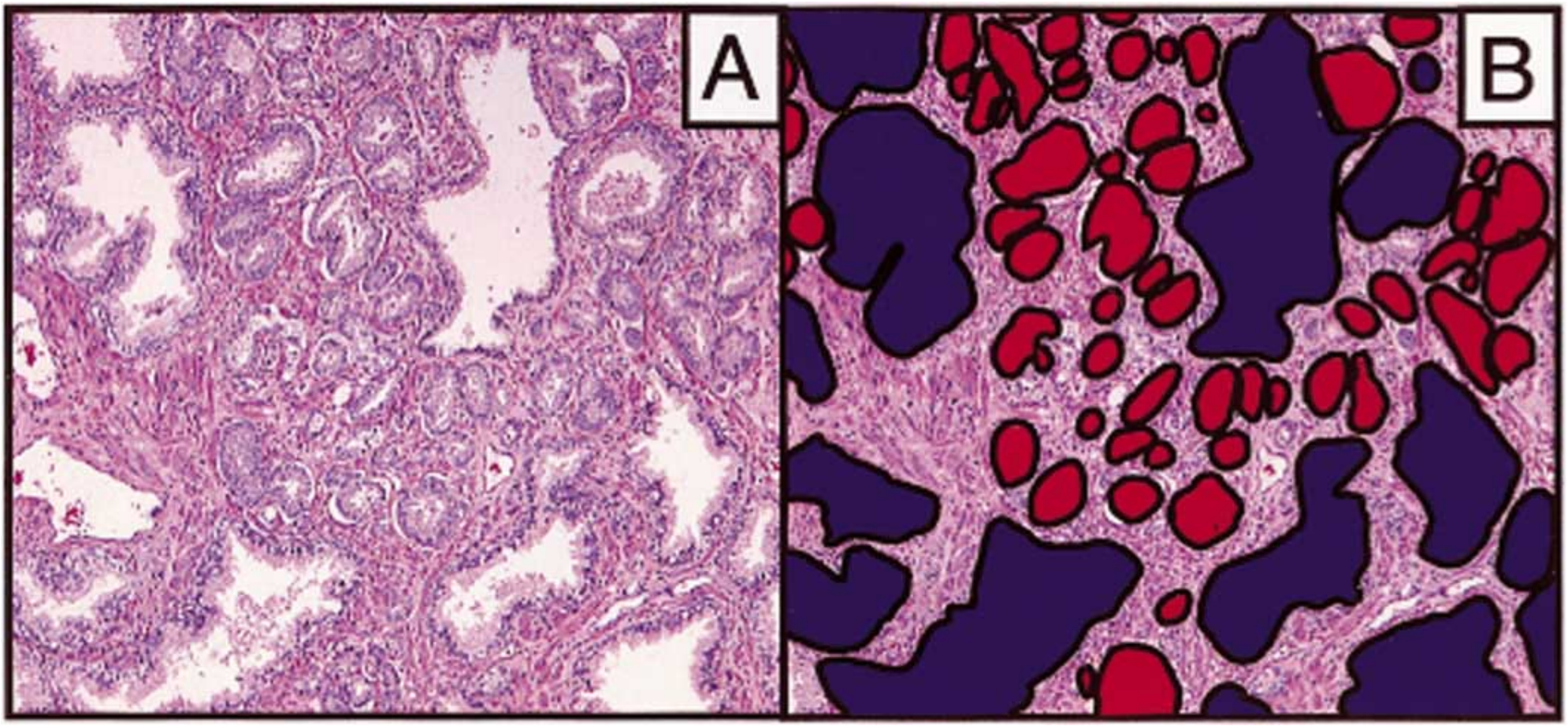
⇓

Μελέτη της γονιδιακής έκφρασης μπορεί να οδηγήσει σε καλύτερη ταξινόμηση του καρκίνου

⇓

Μοριακά πορτρέτα
Μοριακές υπογραφές

Tumor Heterogeneity (Prostate Cancer)



Tumor Cells, Red
Benign Glands,Blue

Rubin MA *J Pathol* 2001;195;80-86

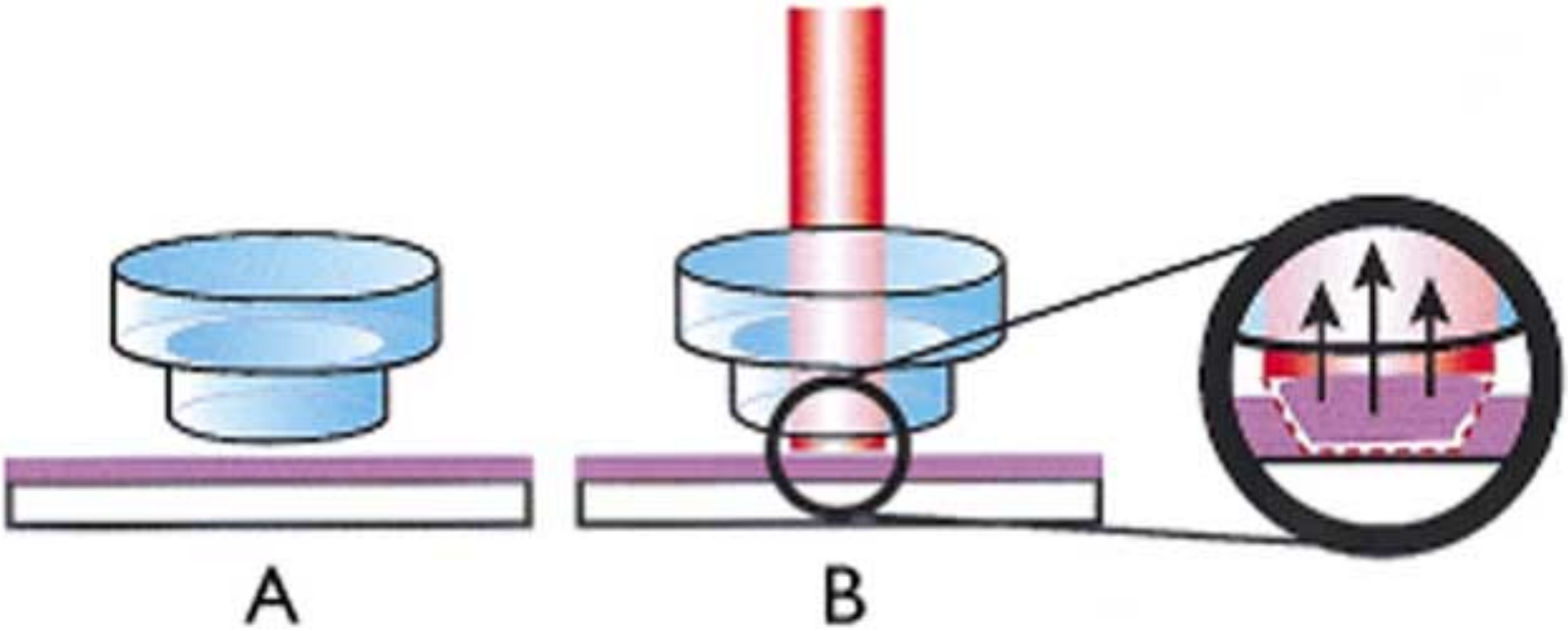
Laser Capture Microdissection, LCM

Χρήση μικροσκοπίας και ακτινοβολίας laser για απομονωση καθορισμένης ομάδας κυττάρων (π.χ. με παθολογικό φαινότυπο) από δείγματα ιστών σε παραφίνη.

↓

Επιτρέπει τη μελέτη σε καθορισμένο πληθυσμό κυττάρων (αποφυγή προσμίξεων υγιών – παθολογικών κυττάρων)

Laser Capture Microdissection



LCM uses a laser beam and a special thermoplastic polymer transfer cup (A). The cap is set on the surface of the tissue and a laser pulse is sent through the transparent cap, expanding the thermoplastic polymer. The selected cells are now adherent to the transfer cap and can be lifted off the tissue and placed directly onto an eppendorf tube for extraction (B).

Rubin MA, *J Pathol* 2001;195:80-86

Κατατομή έκφρασης ιστών

- Πλήθος βάσεων δεδομένων ως αποτέλεσμα χρήσης μικροσυστοιχιών
- Αναζητήσεις όπως:
  - ποια γονίδια εκφράζονται σε ποιούς ιστούς
    (ιστοειδική έκρφαση)
  - μοναδικά γονίδια που εκφράζονται σε ένα μόνο ιστό
  - ποσοτικές σχέσεις μεταξύ επιπέδων έκφρασης
  - έκφραση μεταξύ παθολογικού και μή παθολογικού ιστού

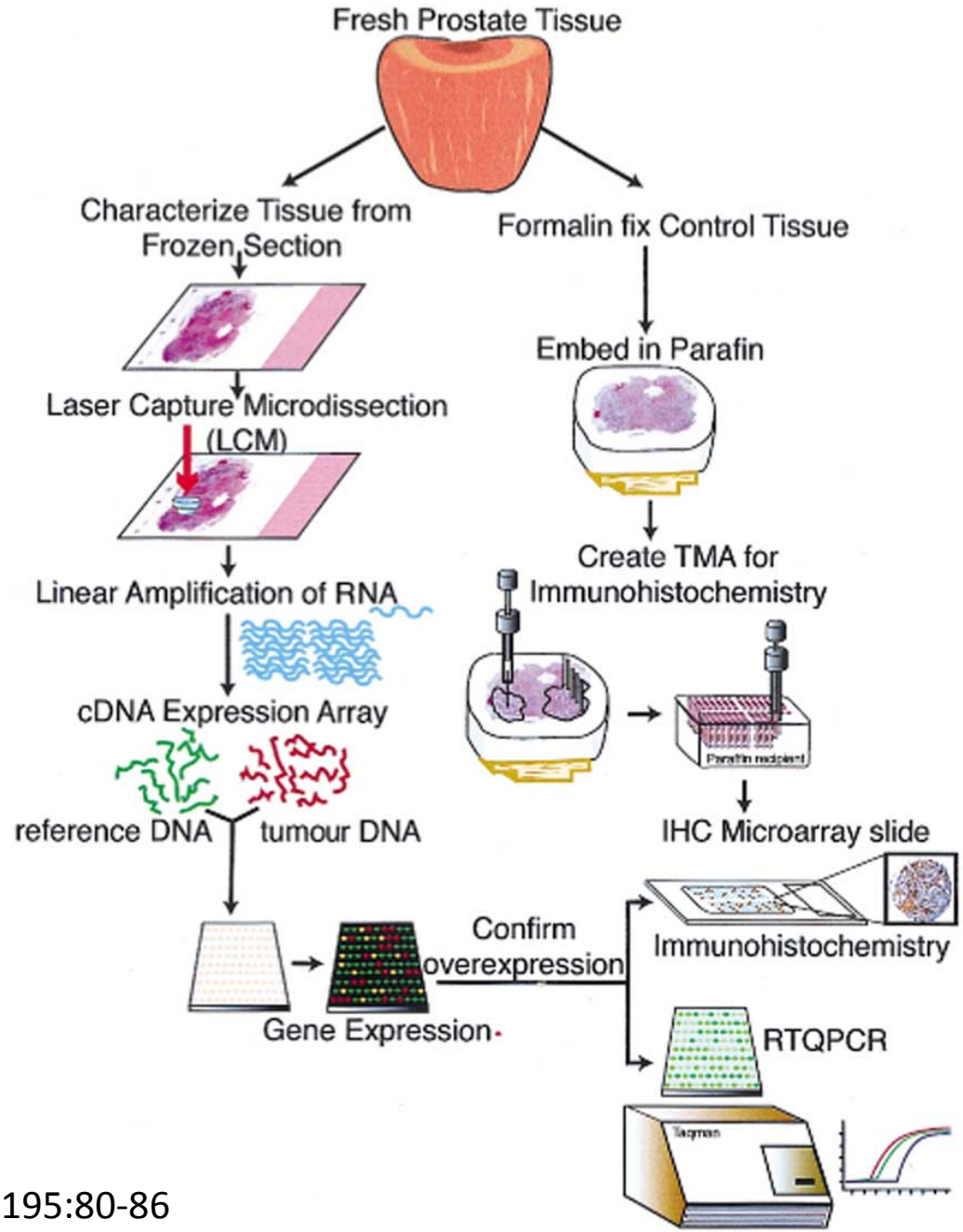
Περιορισμοί:

- Δεδομένα RNA. Οχι πρωτεϊνών
- Μεγάλη απόκλιση στα αποτελέματα

Μικροσυστοιχίες ιστών

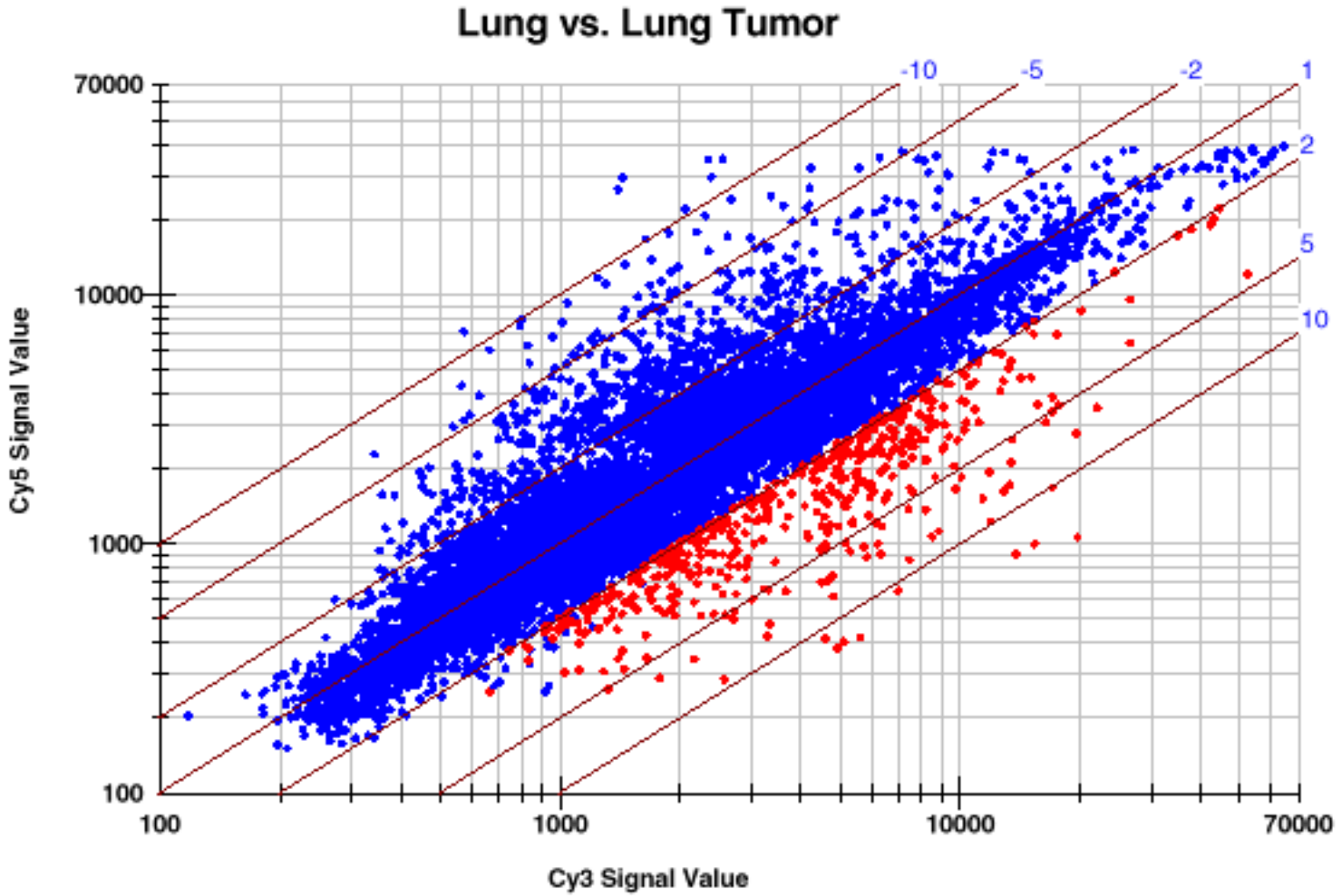
- Απότύπωση σε ένα πλακίδιο (slide) μικροποσοτήτων ιστού
- Συστοίχιση πολλών δειγμάτων σε ένα πλακίδιο (π.χ. 500)
- Ταυτόχρονη επεξεργασία (π.χ. Ανοσοιστοχημεία)
- Χρήση αποθηκευμένου ιστού (μπλόκ παραφίνης)

Μοριακή κατατομή (profiling) καρκίνου προστάτη



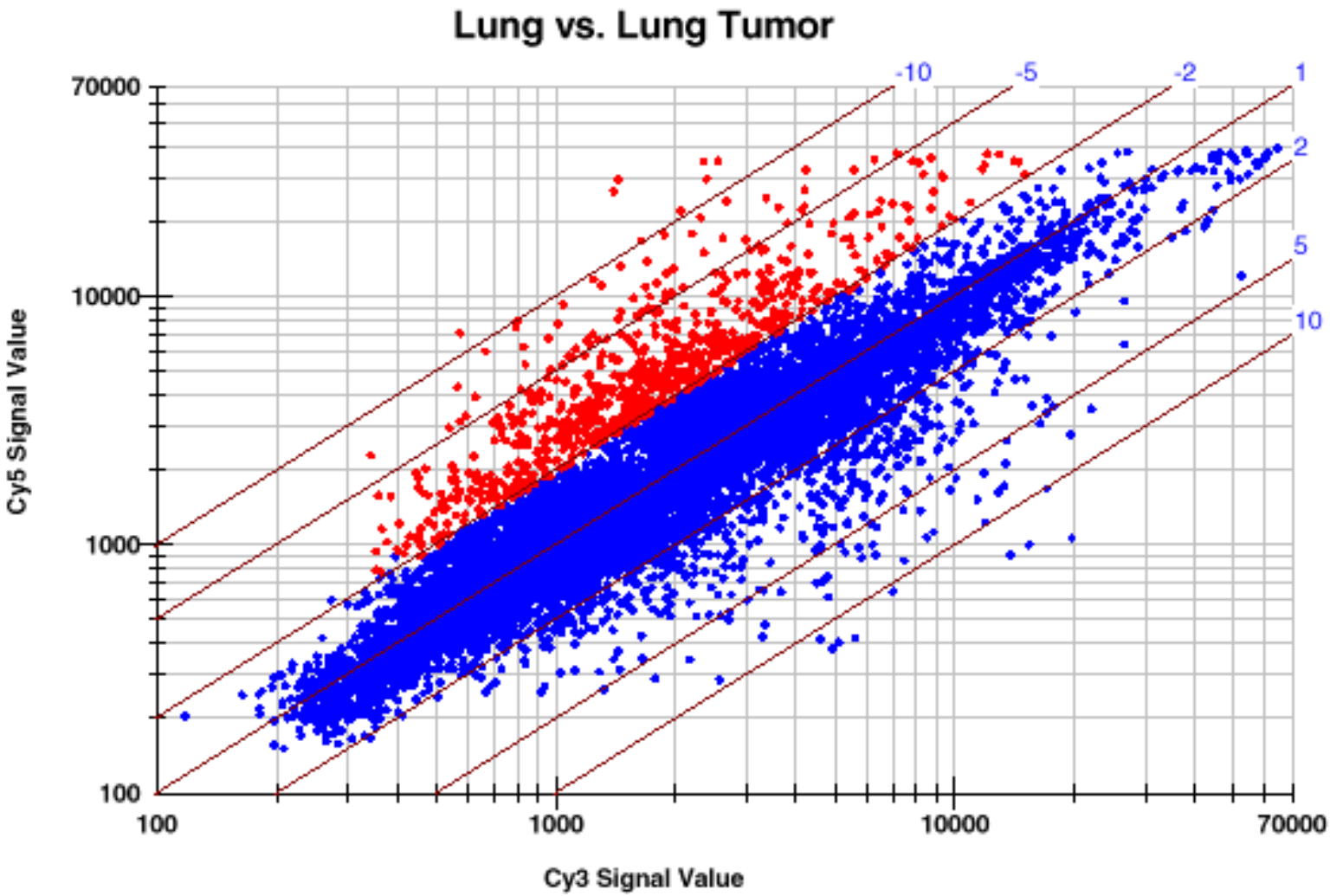
Rubin MA, *J Pathol* 2001;195:80-86

Lung Tumor: Up-Regulated



http://www.freepptdb.com/details-cancer-diagnostics-the-old-and-the-new-467023.html

Lung Tumor: Down-Regulated



http://www.freepptdb.com/details-cancer-diagnostics-the-old-and-the-new-467023.html

Διαγνωστική εφαρμογή μικροσυστοιχιών:

Case Study:

The Agendia **MammaPrint** Test

the first Genomic Test approved by the FDA – Feb. 2007

Close to the finish line - Approval of the Agendia 70 gene signature for breast cancer outcomes

FDA News

FOR IMMEDIATE RELEASE **Media Inquiries:**
P07-13 Karen Riley, 301-827-6242 February 6, 2007
Consumer Inquiries: 888-INFO-FDA

FDA Clears Breast Cancer Specific Molecular Prognostic Test

The U.S. Food and Drug Administration (FDA) today cleared for marketing a test that determines the likelihood of breast cancer returning within 5 to 10 years after a woman's initial cancer. It is the first cleared molecular test that profiles genetic activity.

Πως φτάσανε εκεί– 1) Ανακάλυψη

Gene expression profiling predicts clinical outcome of breast cancer

Laura J. van 't Veer*†, Hongyue Dai†‡, Marc J. van de Vijver*†, Yudong D. He‡, Augustinus A. M. Hart*, Mao Mao‡, Hans L. Peterse*, Karin van der Kooy*, Matthew J. Marton‡, Anke T. Witteveen*, George J. Schreiber‡, Ron M. Kerkhoven*, Chris Roberts‡, Peter S. Linsley‡, René Bernards* & Stephen H. Friend‡

** Divisions of Diagnostic Oncology, Radiotherapy and Molecular Carcinogenesis and Center for Biomedical Genetics, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands*
‡ Rosetta Inpharmatics, 12040 115th Avenue NE, Kirkland, Washington 98034, USA
† These authors contributed equally to this work

NATURE | VOL 415 | 31 JANUARY 2002 | www.nature.com

- Γονιδιακή έκφραση με μικροσυστοιχίες για ανακάλυψη υποψηφίων
- Συστοιχίες 25,000 γονιδίων
- 117 ασθενείς, 98 όγκοι
- Αρκετά χρόνια παρακολούθησης (follow-up data on progression)
- Ομαδοποίηση ~5000 ρυθμιζόμενων γονιδίων
- Συσχέτιση ομάδων γονιδίων με παρατηρούμενα κλινικά συμπεράσματα (καλή και μέτρια πρόγνωση)
- Η έκφραση 231 γονιδίων συνδέθηκε στατιστικά με την ασθένεια
- Μείωση σε έναν πυρήνα 70 γονιδίων (γονδιακή υπογρφή ή κατατομή -gene signature or profile) που επιτρέπει ακριβή κατάταξη ασθενών σε αυτούς με καλή ή μέτρια πρόγνωση.

Πως φτάσανε εκεί– 2) Πρώιμη αξιολόγηση

The New England
Journal of Medicine

VOLUME 347 DECEMBER 19, 2002 NUMBER 25

A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

Marc J. van de Vijver, M.D., Ph.D., Yudong D. He, Ph.D., Laura J. van 't Veer, Ph.D., Hongyue Dai, Ph.D., Augustinus A.M. Hart, M.Sc., Dorien W. Voskuil, Ph.D., George J. Schreiber, M.Sc., Johannes L. Peterse, M.D., Chris Roberts, Ph.D., Matthew J. Marton, Ph.D., Mark Parrish, Douwe Atsma, Anke Witteveen, Annuska Glas, Ph.D., Leonie Delahaye, Tony van der Velde, Harry Bartelink, M.D., Ph.D., Sjoerd Rodenhuis, M.D., Ph.D., Emiel T. Rutgers, M.D., Ph.D., Stephen H. Friend, M.D., Ph.D., and René Bernards, Ph.D.

- *Επέκταση της προηγούμενης εργασίας για να συμπεριλάβει 295 επιπλέον ασθενείς*
- *Ικανότητα πρόβλεψης πιθανότητας μακρινής μετάστασης σε 5 χρόνια*

Πως φτάσανε εκεί– 3) Μετάβαση από ερευνητικό εργαλείο σε διαγνωστικό τεστ

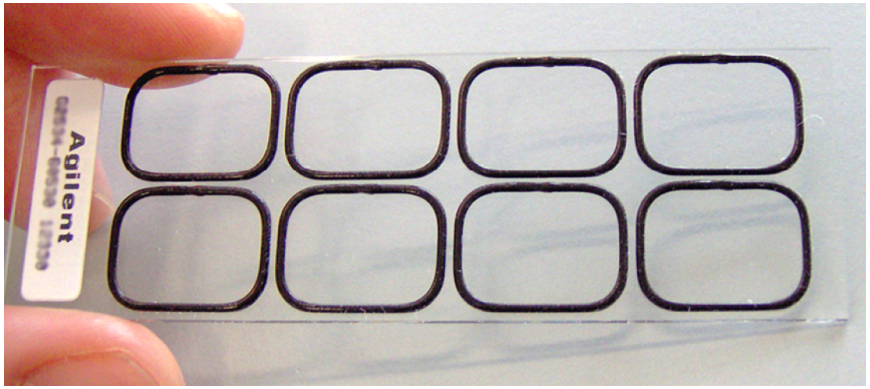
Converting a breast cancer microarray signature into a high-throughput diagnostic test

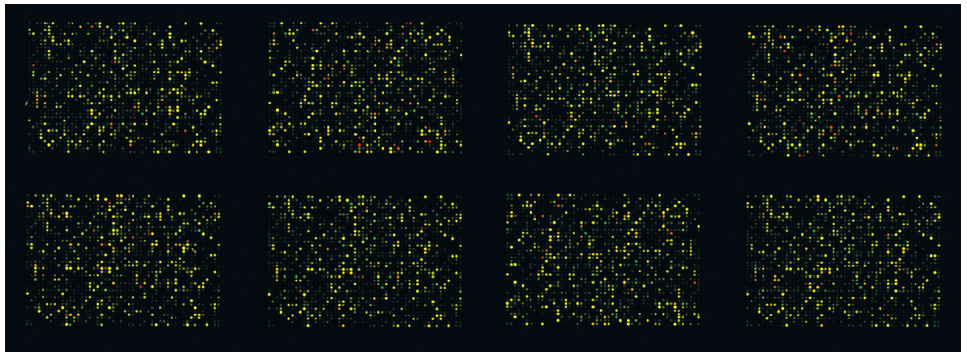
Annuska M Glas*[1], Arno Floore[1], Leonie JMJ Delahaye[1], Anke T Witteveen[1], Rob CF Pover[1], Niels Bakx[1], Jaana ST Lahti-Domenici[1], Tako J Bruinsma[1], Marc O Warmoes[1], René Bernards[1], Lodewyk FA Wessels[2] and Laura J Van 't Veer[1]

BMC Genomics 2006, **7**:278

- *Εστίαση στην εκτέλεση της δοκιμής*
- *Επιπλέον αξιολόγηση*

Πως φτάσανε εκεί– 3) Μετάβαση από εργαλείο σε τεστ





- Adopted new array format termed *MammaPrint*
- 1,900 vs. 25,000 array probes, each gene represented in triplicate
- Goal is to achieve higher throughput, better reproducibility
- Analyzed RNA from the original study, many replicates/ repeats (some samples analyzed 40X over four months)

Based on http://research.lunenfeld.ca/techTransfer/download_box/seminars/2008-01-31.pdf

Πως φτάσανε εκεί– 4) Ανεξάρτητη αξιολόγηση

Validation and Clinical Utility of a 70-Gene Prognostic Signature for Women With Node-Negative Breast Cancer

Marc Buyse, Sherene Loi, Laura van't Veer, Giuseppe Viale, Mauro Delorenzi, Annuska M. Glas, Mahasti Saghatchian d'Assignies, Jonas Bergh, Rosette Lidereau, Paul Ellis, Adrian Harris, Jan Bogaerts, Patrick Therasse, Arno Floore, Mohamed Amakrane, Fanny Piette, Emiel Rutgers, Christos Sotiriou, Fatima Cardoso, Martine J. Piccart

On behalf of the TRANSBIG Consortium

Journal of the National Cancer Institute, Vol. 98, No. 17, September 6, 2006

- External validation using optimized platform
- Analysis of samples from 307 patients from 5 European hospitals
- Concluded that the MammaPrint assay will provide more accurate information on recurrence risk as compared to conventional criteria and will improve the guidance for the requirement for adjuvant therapy for breast cancer patients

Based on http://research.lunenfeld.ca/techTransfer/download_box/seminars/2008-01-31.pdf

Συνοπτικά, πως φτάσαμε εκεί

- 2002 – ανακάλυψη 70 γονιδίων (γονιδιακή υπογραφή) (117 ασθενείς)
- 2002 – διπλασιασμός αποτελεσμάτων (σε άλλο δείγμα 295 ασθενών)
- 2006 – Εκτέλεση δοκιμής
- 2006 – Βελτίωση συστοιχιών (array format): επαναληψιμότητα, πίσων στο αρχικό δείγμα
- 2006 – Εξωτερική επιβεβαίωση (307 ασθενείς, 5 νοσοκομεία)
- 2007 – Εγκριση από FDA

Based on http://research.lunenfeld.ca/techTransfer/download_box/seminars/2008-01-31.pdf

Single Nucleotide Polymorphisms (SNP)

- DNA variation at one base pair level; found at a frequency of 1 SNP per 1,000 - 2,000 bases

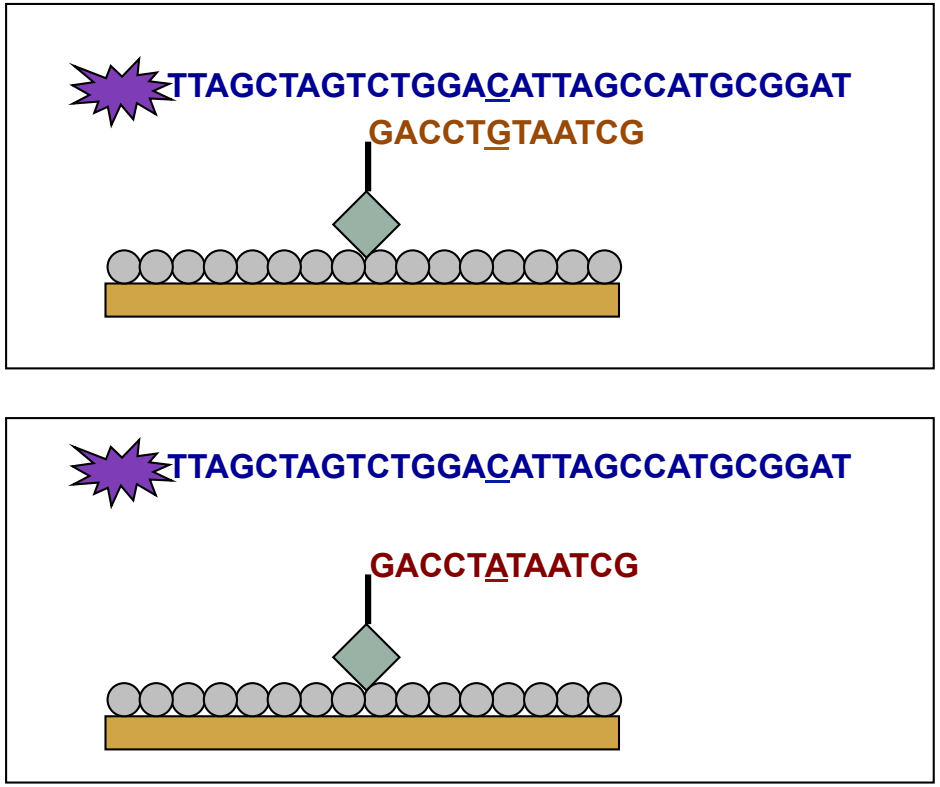
- Currently, a map of 1.42 x 10^6 SNPs have been described in humans (*Nature 2001; 409:928-933*) by the International SNP map working group)

- Identification: Mainly a by-product of human genome sequencing at a depth of x10 and overlapping clones

- 60,000 SNPs fall within exons; the rest are in introns

Genotyping: SNP Microarray

- Immobilized allele specific oligo probes
- Hybridize with labeled PCR product
- Assay multiple SNPs on a single array

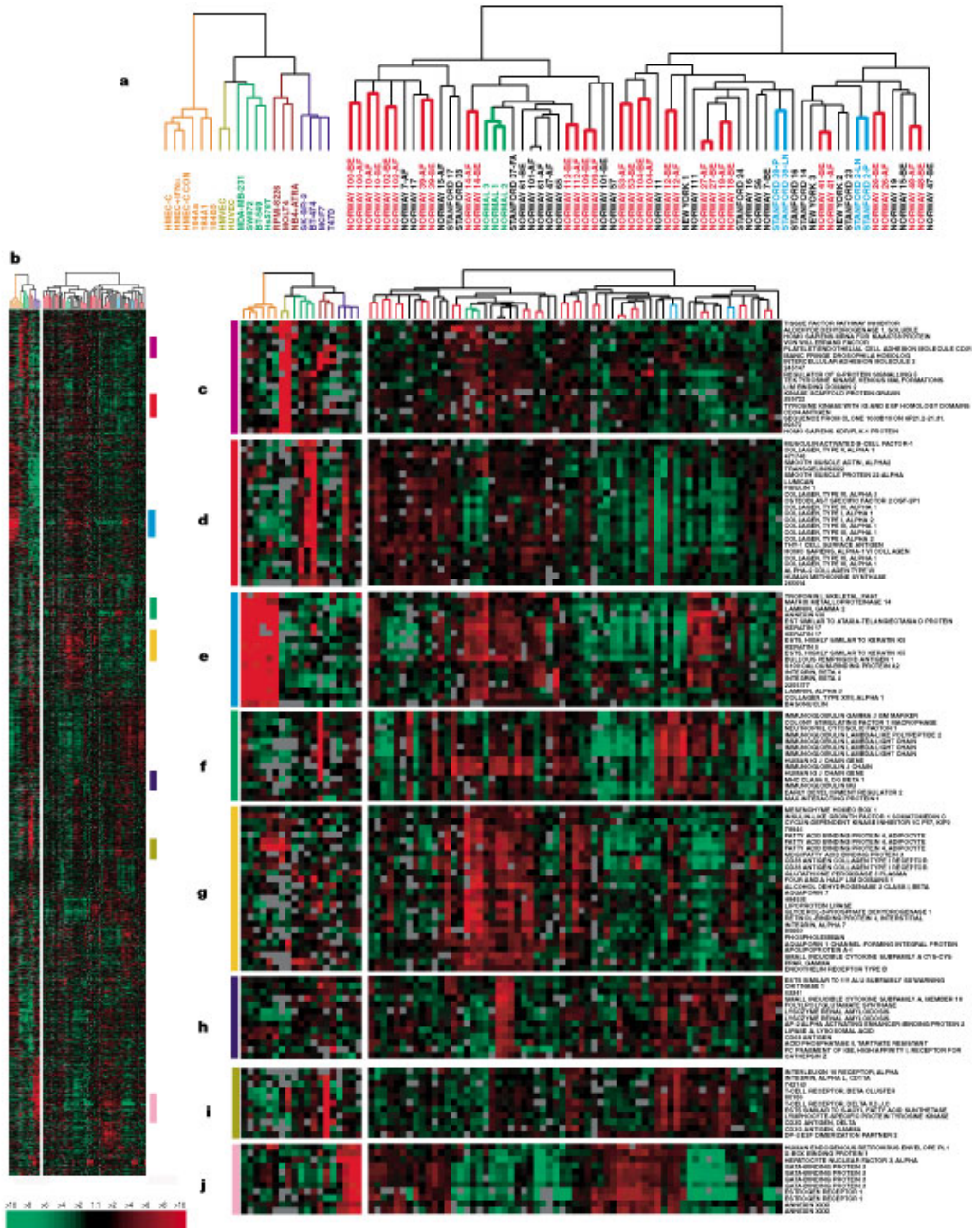


http://www.freepptdb.com/details-cancer-diagnostics-the-old-and-the-new-467023.html

Rationale For Improved Subclassification of Cancer by Microarray Analysis

- Classically classified tumors are clinically very heterogeneous – some respond very well to chemotherapy; some do not.

Molecular Portraits of Cancer



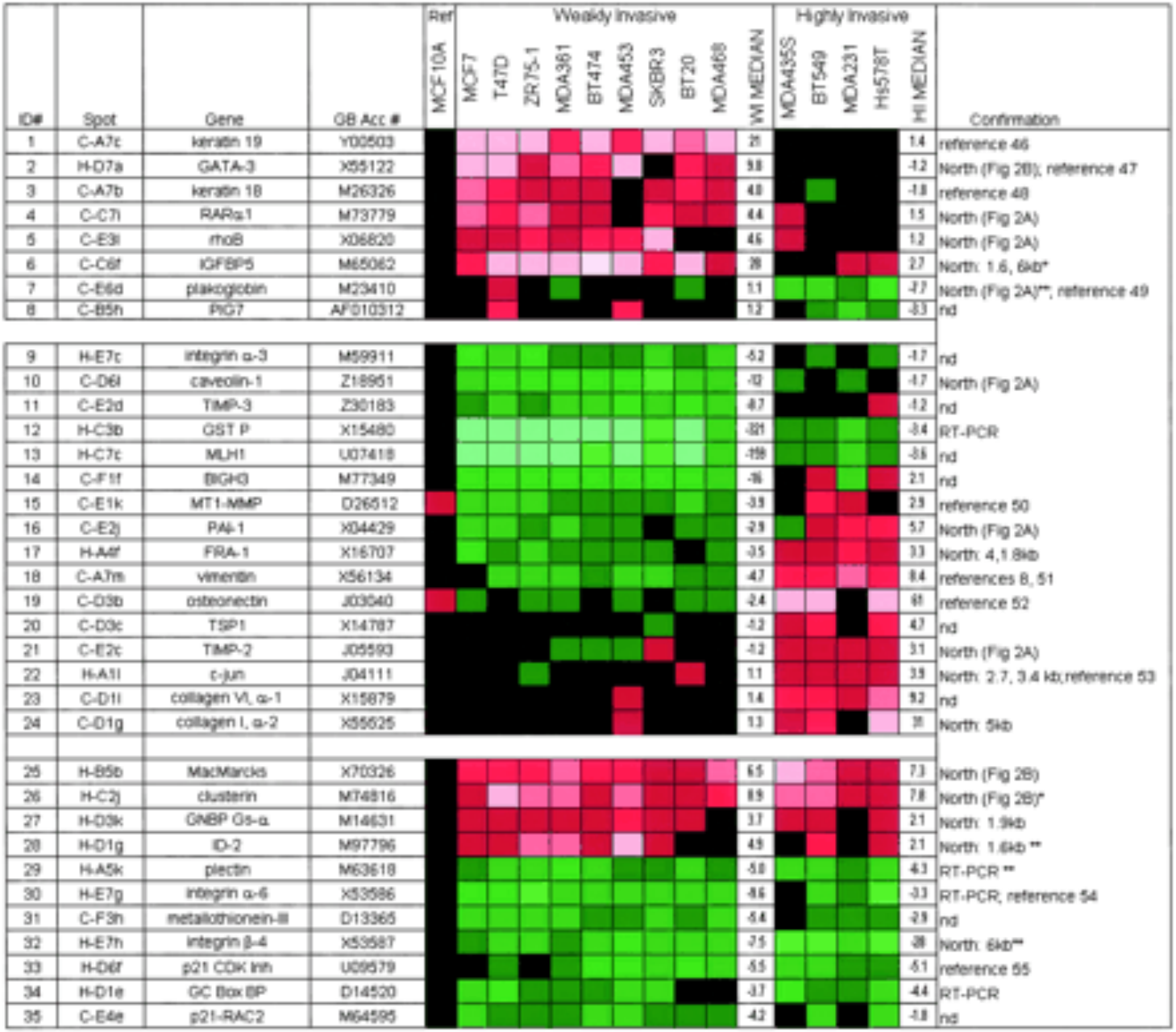
Breast Cancer
analysis of 1753 genes

Green: Gene underexpression
Black: Equal Expression
Red: Overexpression

Left Panel: Cell Lines
Right Panel: Breast Tumors

Perou et al Nature 2000;406:747-752

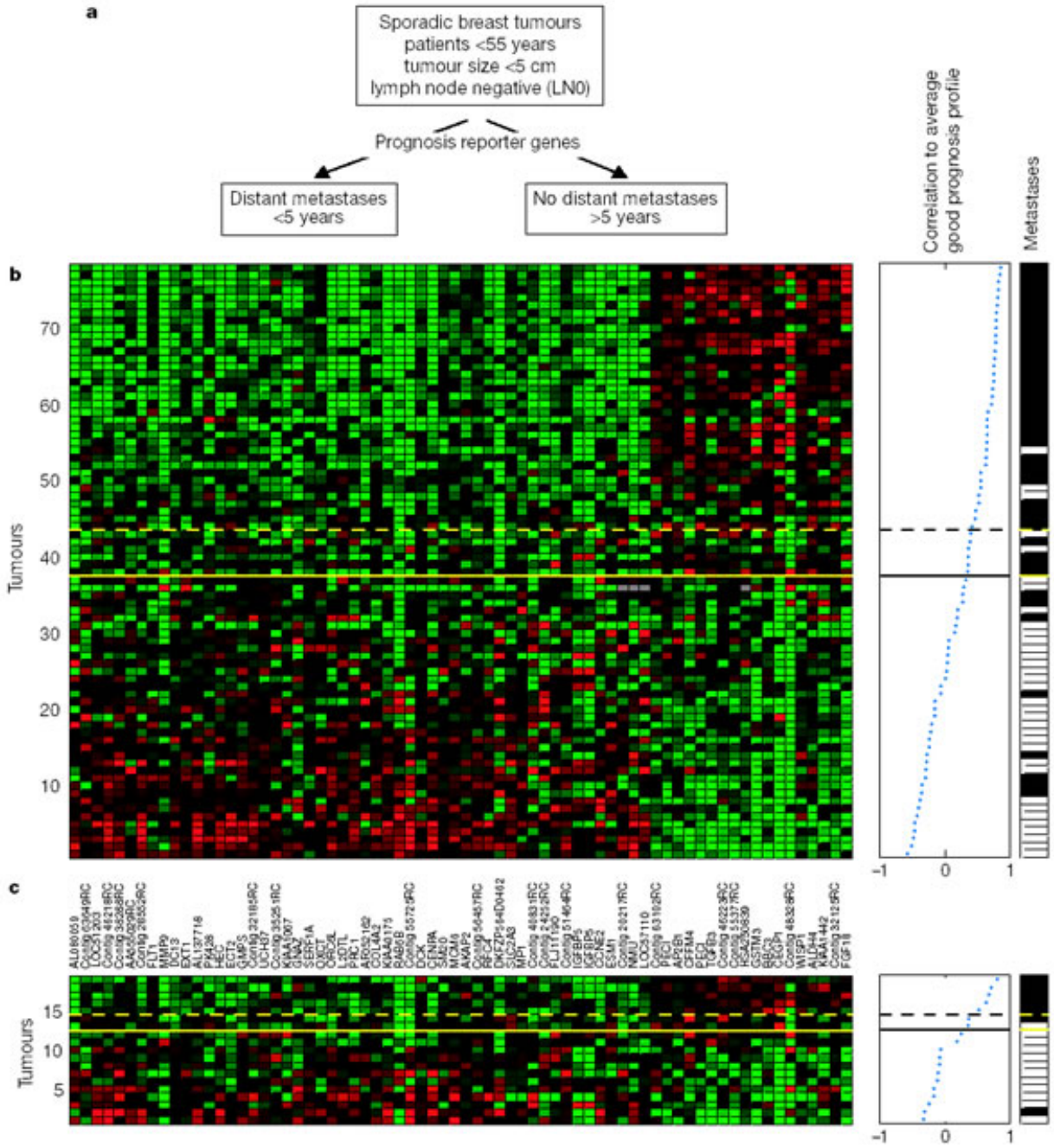
Aggressive vs Non-Aggressive Breast Cancer Cell Lines



Can accurately predict aggressiveness with a set of only 24 genes

Zajchowski et al *Cancer Res* 2001;61:5168-78

Prognostic Signature of Breast Cancer



Patients above line
No Distant Metastasis

Patients Below Line
Distant metastasis

Van't Veer et al.Nature 2002;415:530-536

Μελλοντικά (;)

Ασθενής

↓

Χειρουργείο/βιοψία

↓

Καρκινικός ιστός

↓

Ανάλυση μικροσυστοιχιών

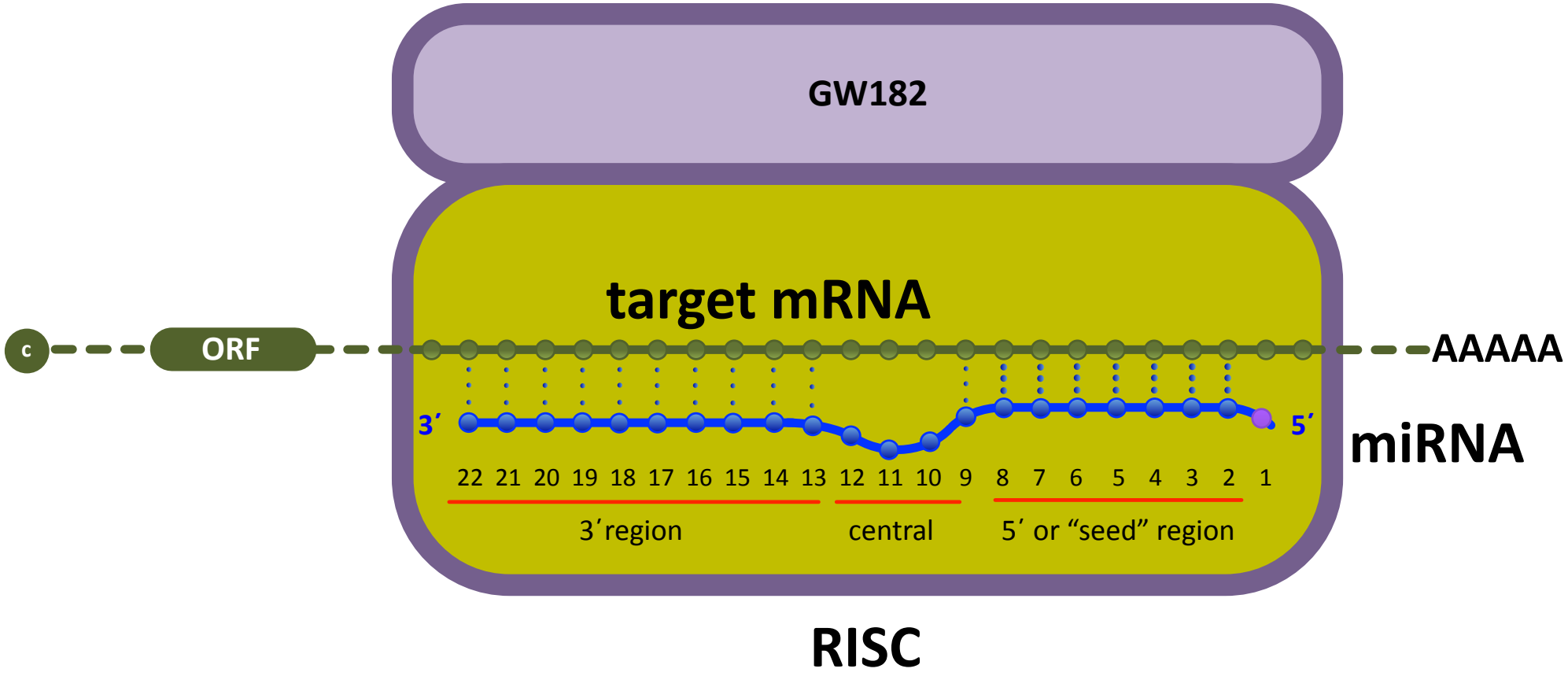
↓

Αποτύπωμα όγκου

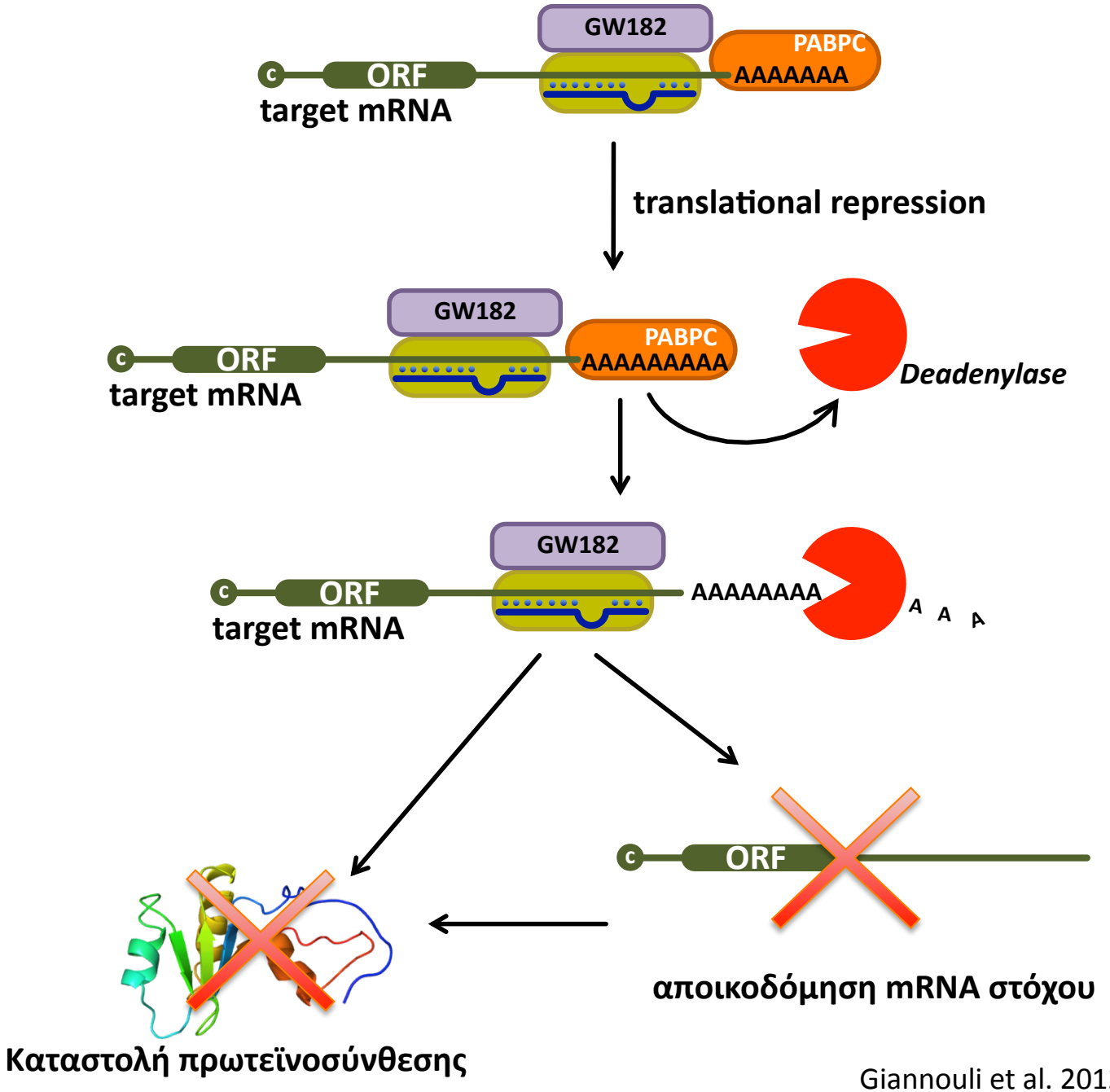
↓

Εξατομικευμένη θεραπευτική προσέγγιση

miRISC targets mRNA

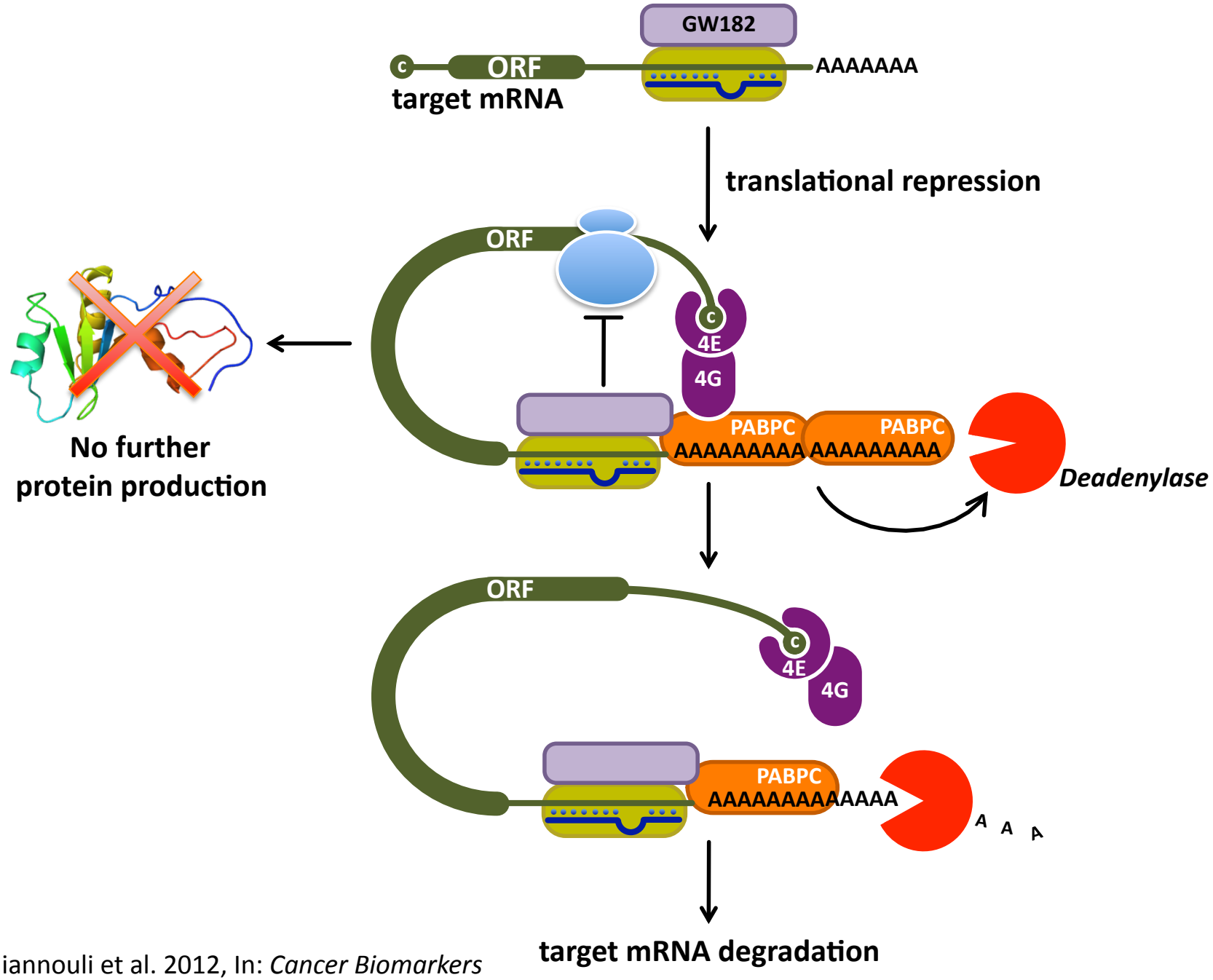


miRNA: Αποικοδόμηση mRNA, καταστολή μετάφρασης



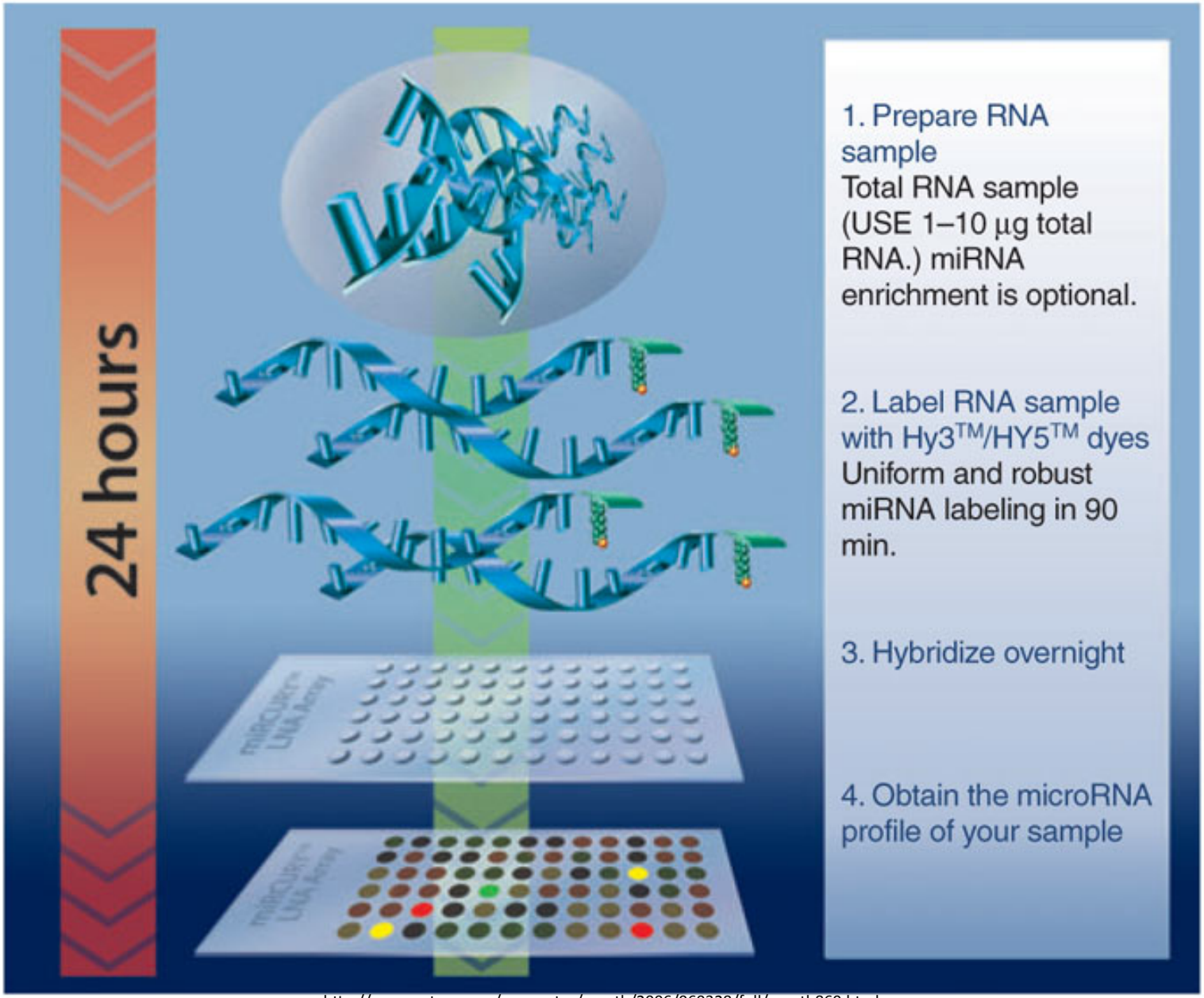
Giannouli et al. 2012, In: *Cancer Biomarkers*

miRNA - mediated mRNA degradation



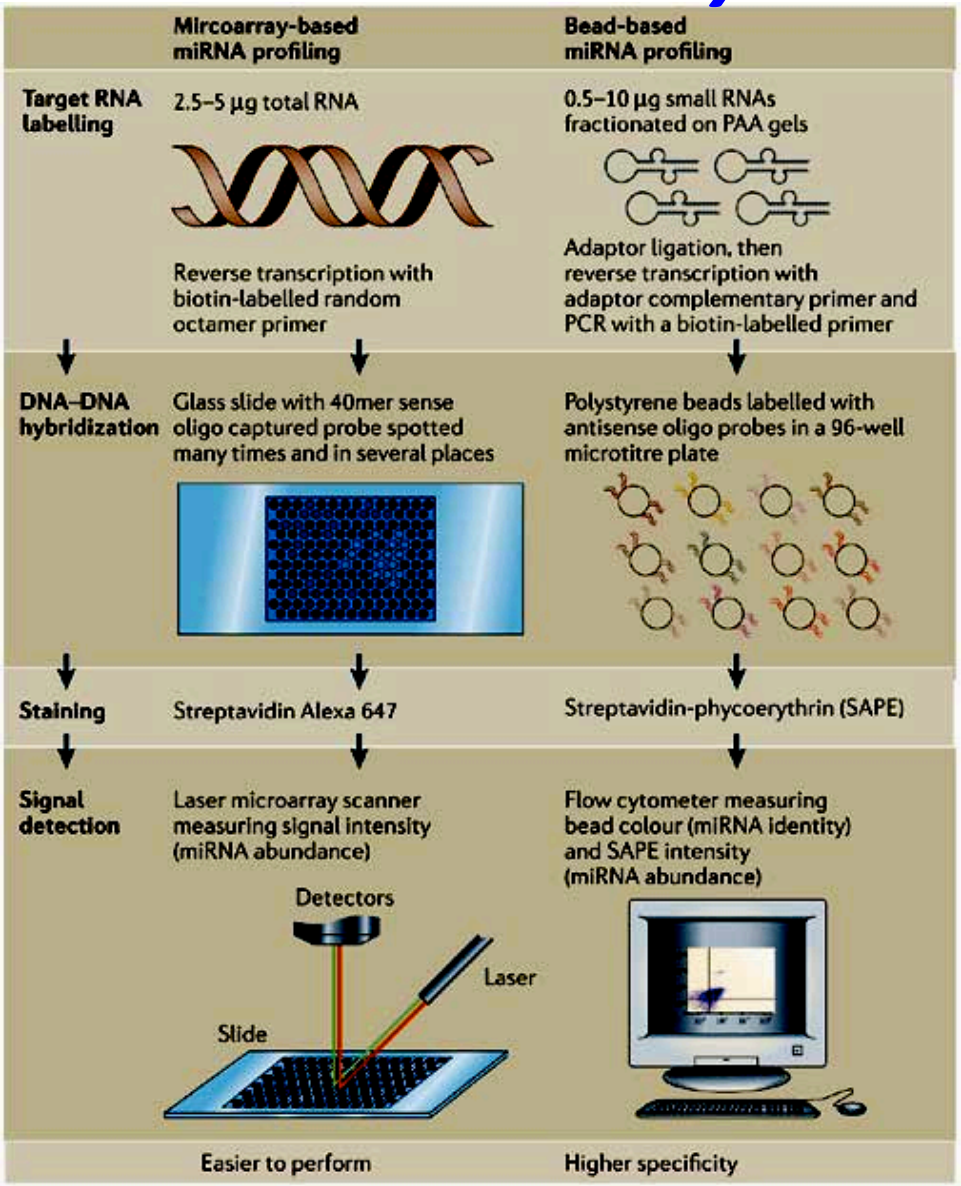
Giannouli et al. 2012, In: *Cancer Biomarkers*

Συστοιχίες microRNA
microRNA arrays



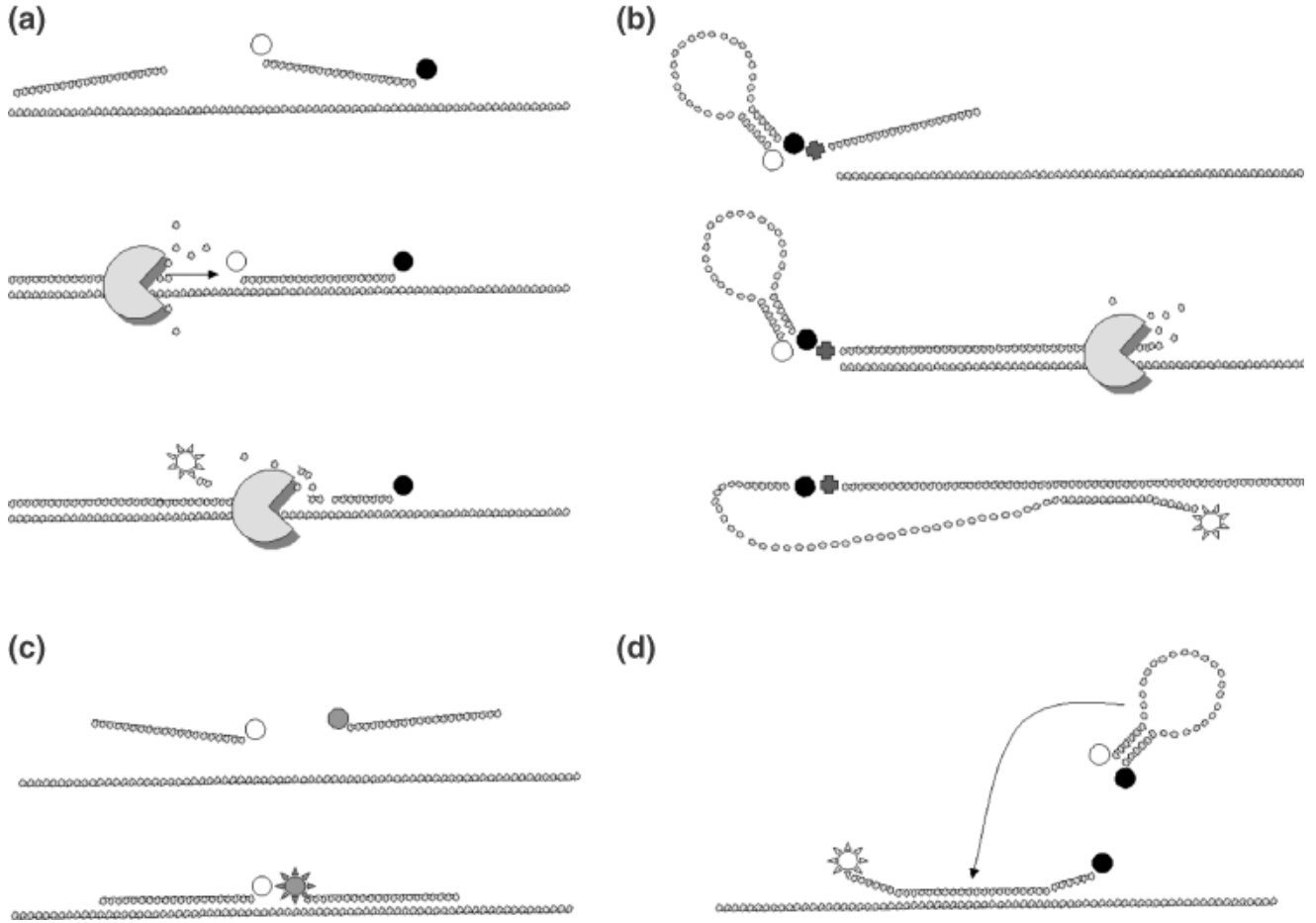
http://www.nature.com/app_notes/nmeth/2006/060328/full/nmeth869.html

Συστοιχίες microRNA
microRNA arrays



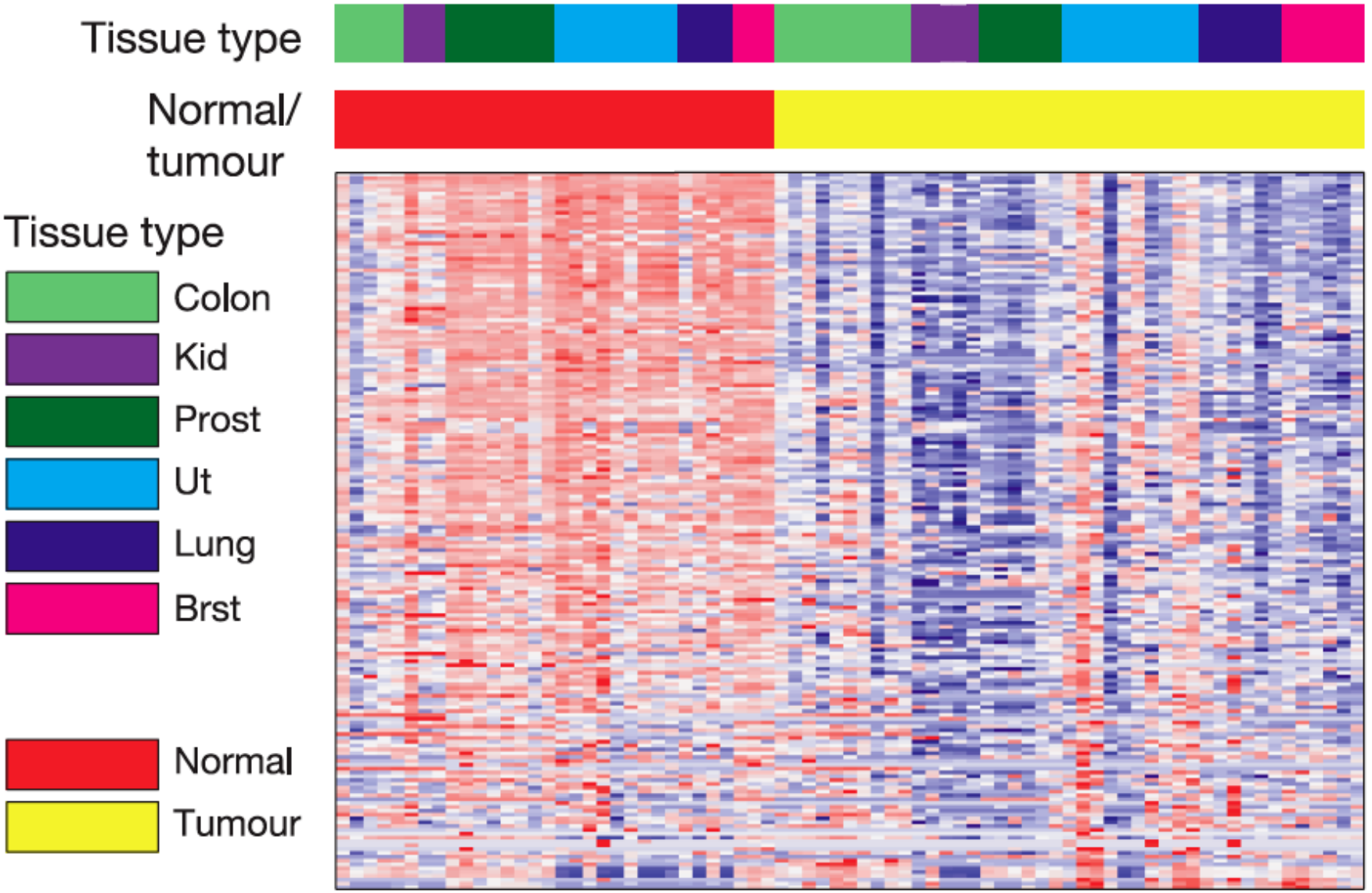
Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

RNA Diagnostics: Fluorogenic probe chemistries



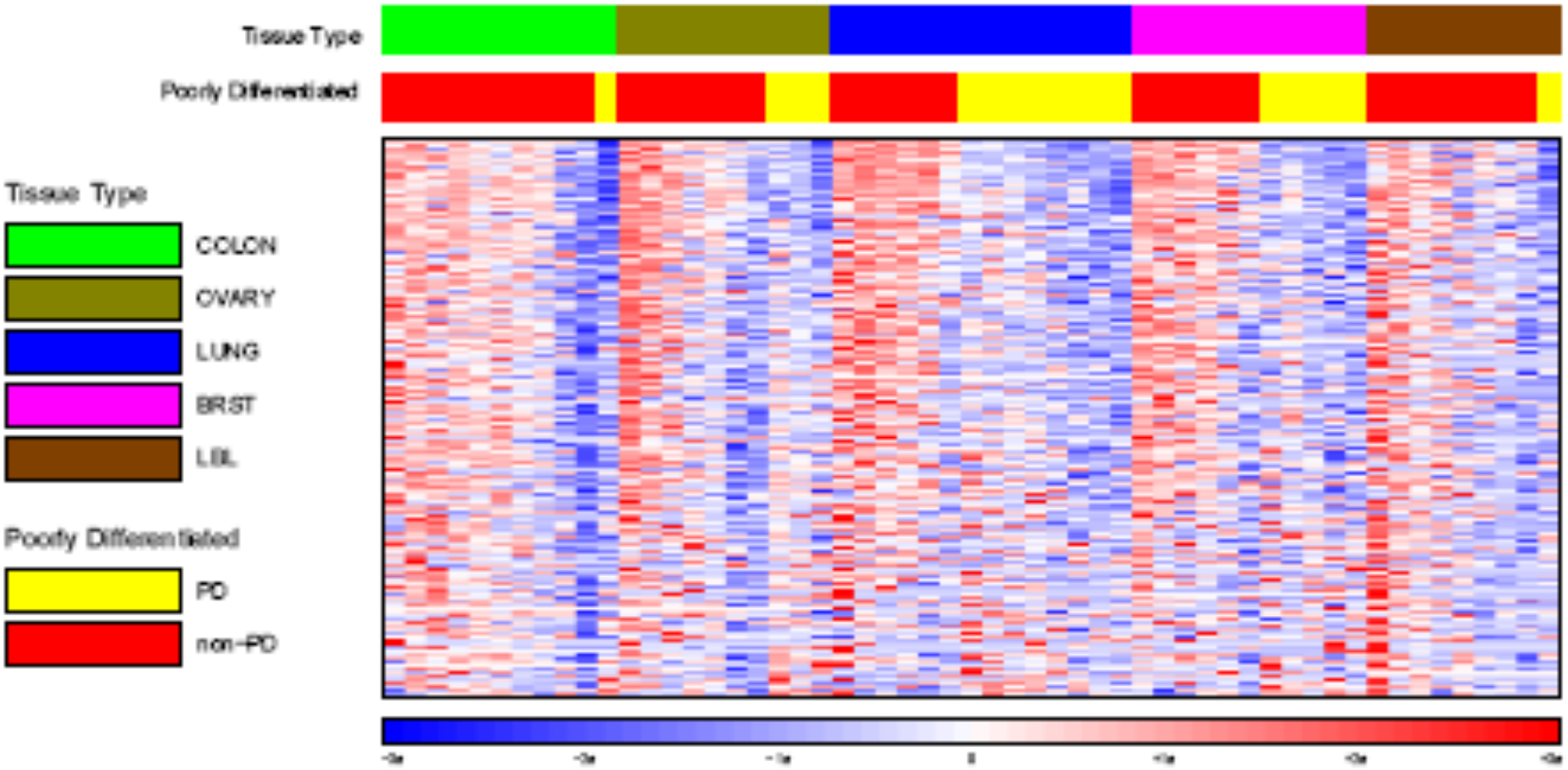
(a) 5′ nuclease probes. Downstream of one of the primers, additionally a dual-labeled hybridization probe anneals to the DNA target molecule. During primer extension the 5′ → 3′ exonuclease activity of Taq DNA polymerase hydrolyzes the probe, separating a fluorescent reporter dye (o) from an intramolecular quencher (•), giving rise to specific fluorescence emission. **(b)** Scorpion primers. After annealing, the primer section is extended by the DNA polymerase. After strand separation, the probe section of the Scorpion oligodeoxynucleotide hybridizes to a region downstream from the primer sequence during the annealing step of the PCR reaction. The hairpin structure of the Scorpion primer is blocked from extension (+) to ensure that the reporter dye (o) and quencher (•) are only separated by specific hybridization of the probe section to the target sequence. **(c)** Adjacent hybridization probes. Two linear fluorogenic oligoprobes hybridize next to each other to the target sequence during the annealing step of the PCR reaction. The acceptor dye (•) then emits fluorescence due to corresponding excitation by the excited donor dye () in close proximity. **(d)** Molecular beacons. Hybridization of the probe sequence to the target sequence during the annealing step separates the reporter dye (o) from the quencher (•).
http://onlinelibrary.wiley.com/doi/10.1002/wrna.46/full

miRNA expression profile classify human cancers



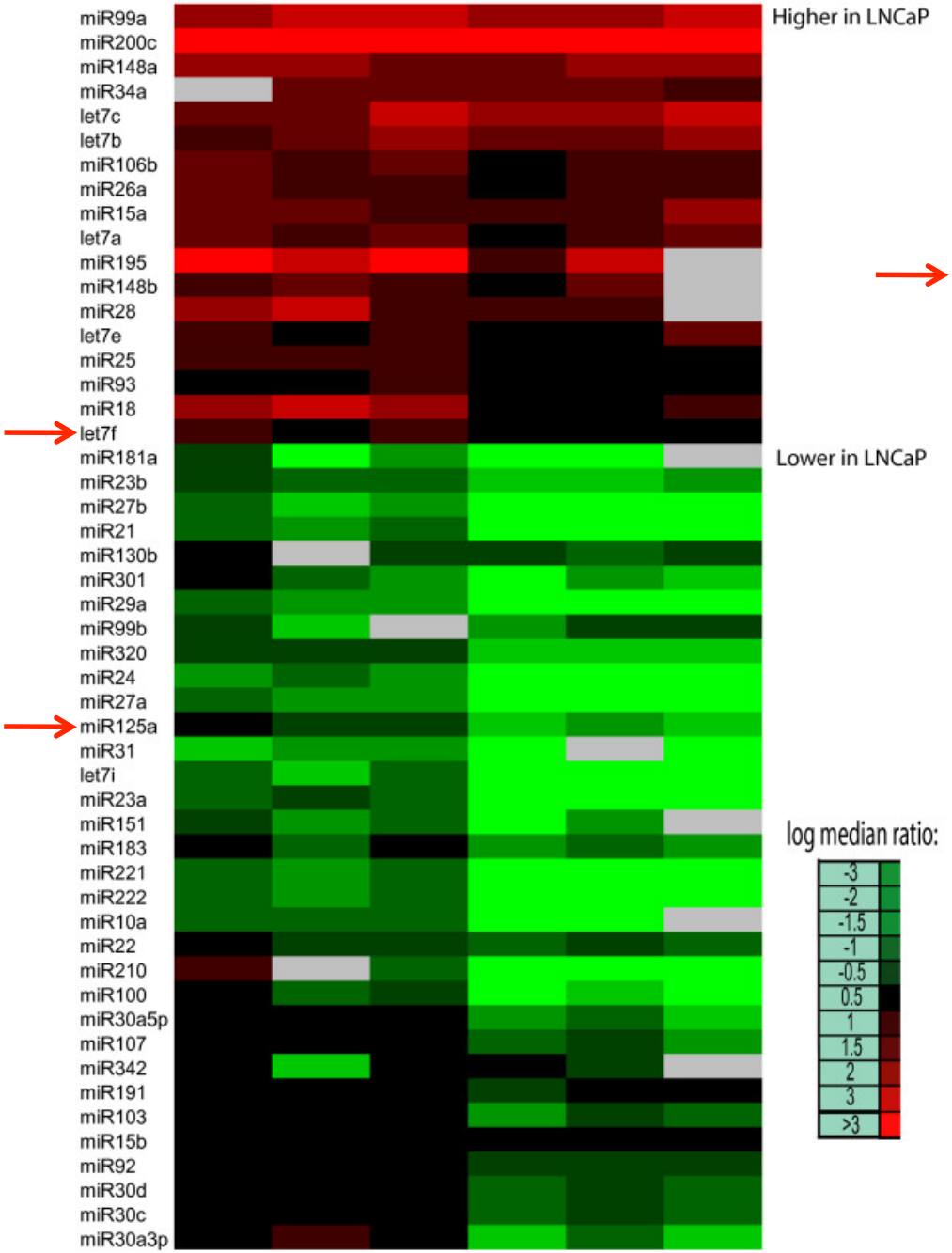
Lu J et al., Nature 435:834-838, 2005

Comparison of miRNA expression of poorly differentiated and more-differentiated tumors



LBL: Diffuse large-B cell lymphoma
PD: Poor Diagnosed

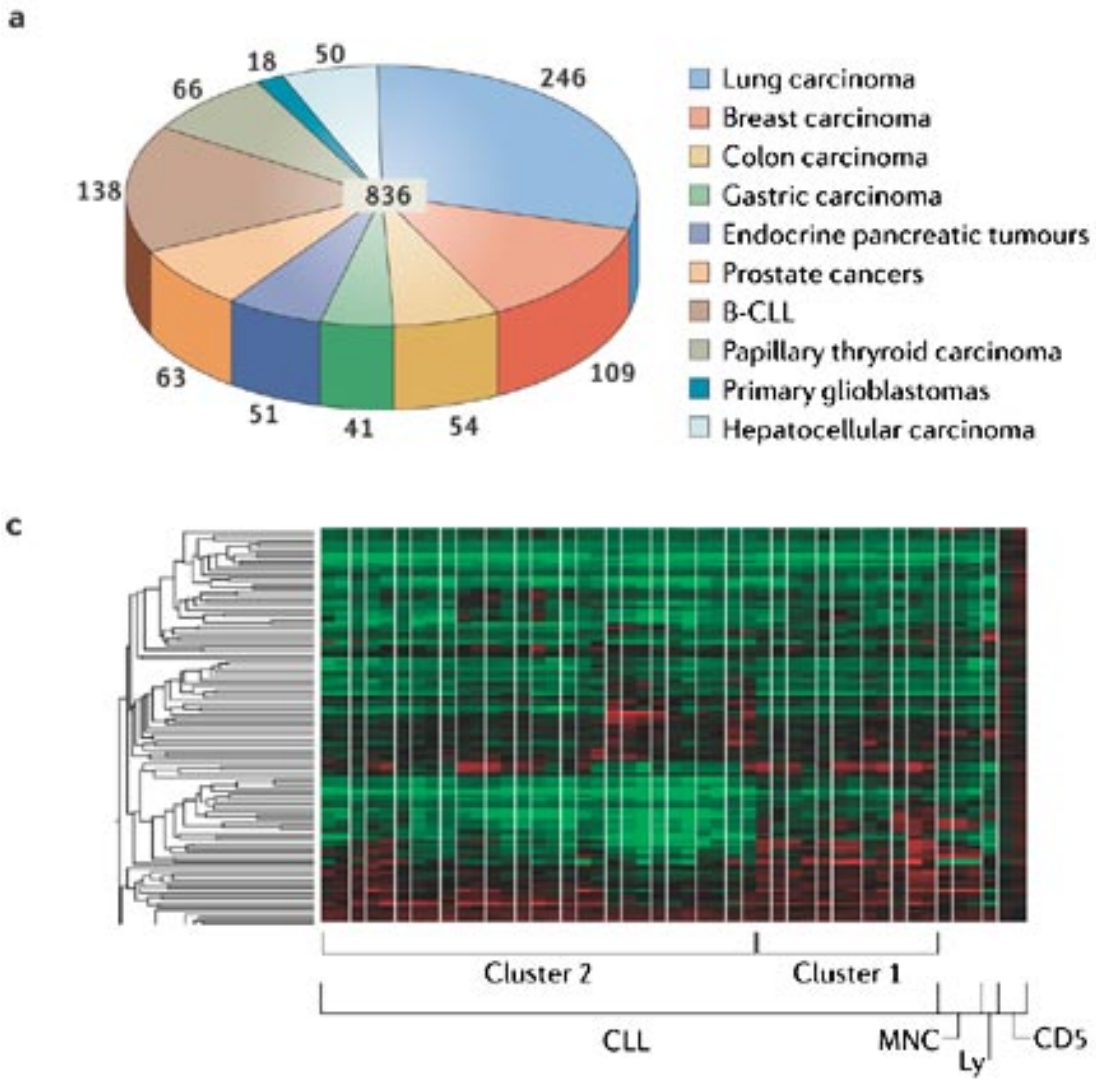
miRNAs and breast cancer – profiling data

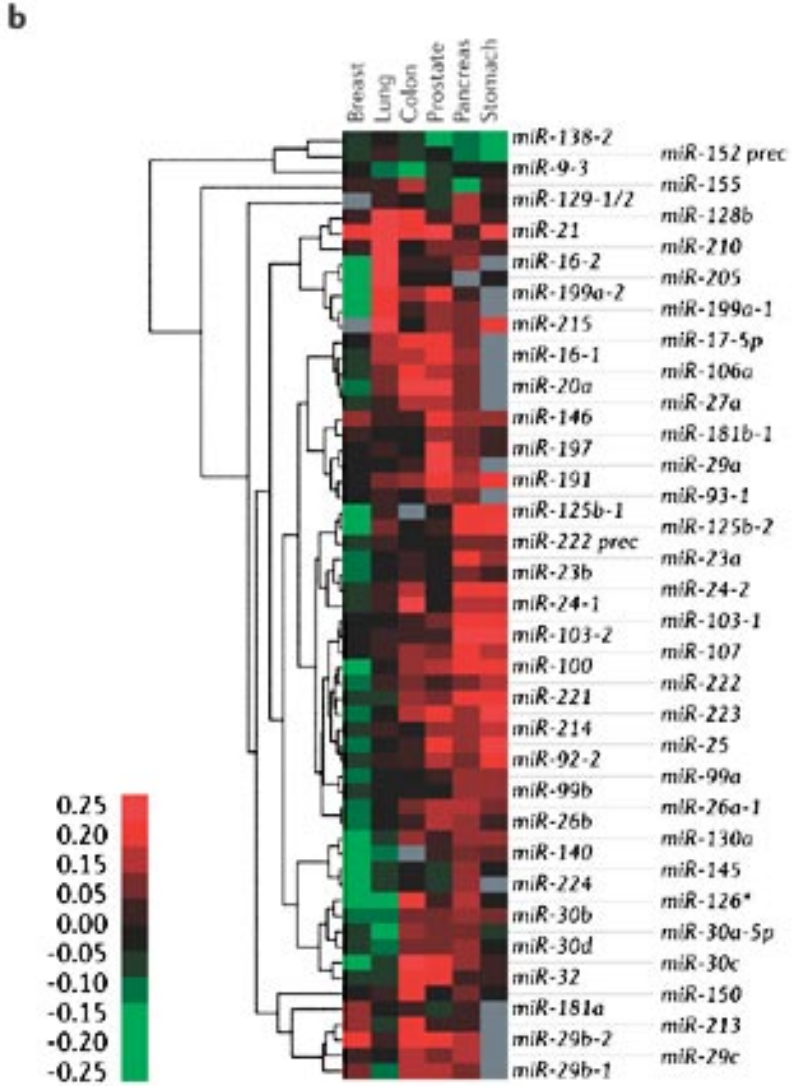


→ : HER2-acossiated
(*let7f, let7g, miR107, miR10b, miR125a, miR125b, miR126, miR154 and miR195)*

Mattie MD, Mol Cancer 2006

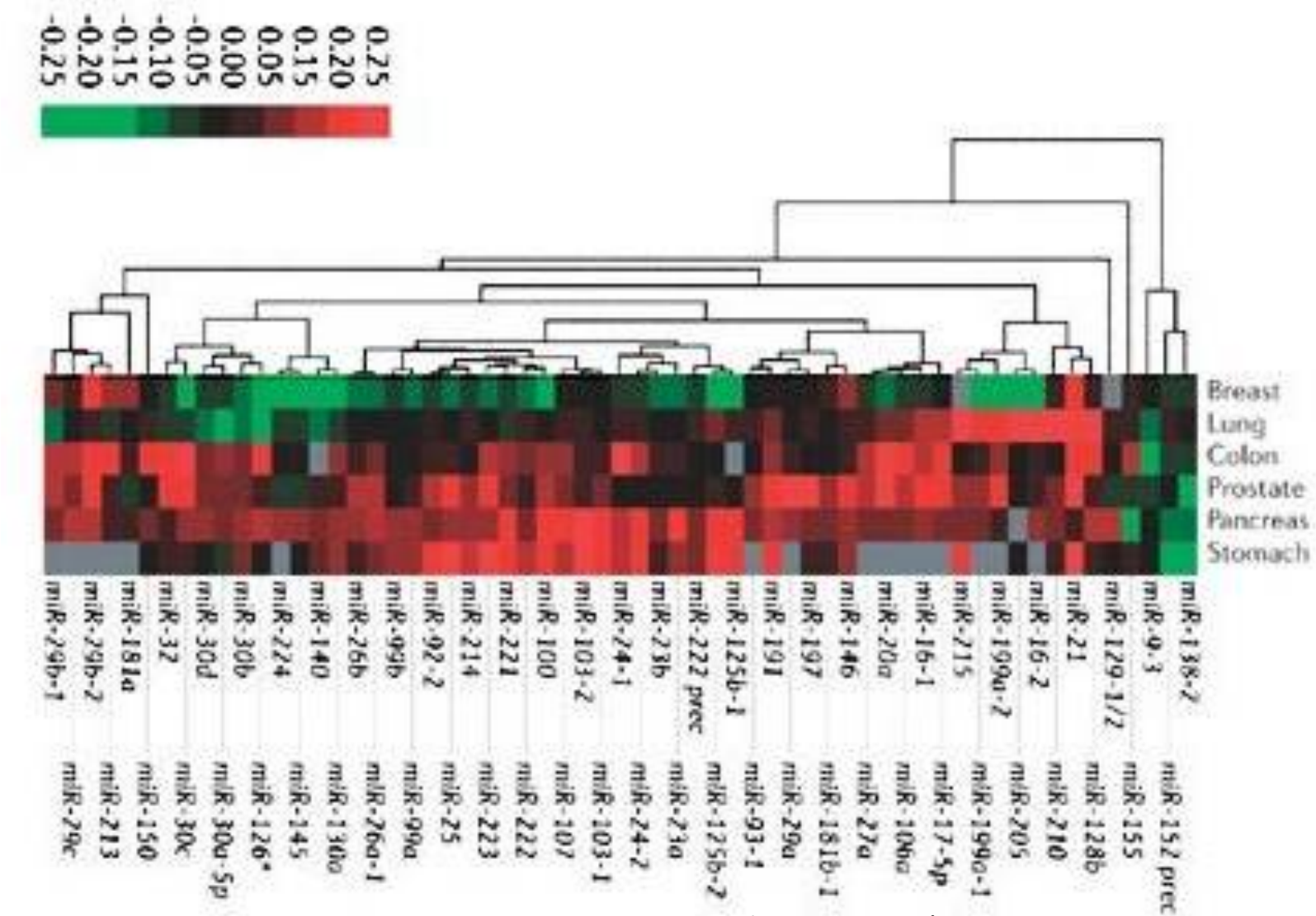
Examples of microRNA profiles in human solid and liquid cancers





Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

Examples of microRNA profiles in human solid and liquid cancers



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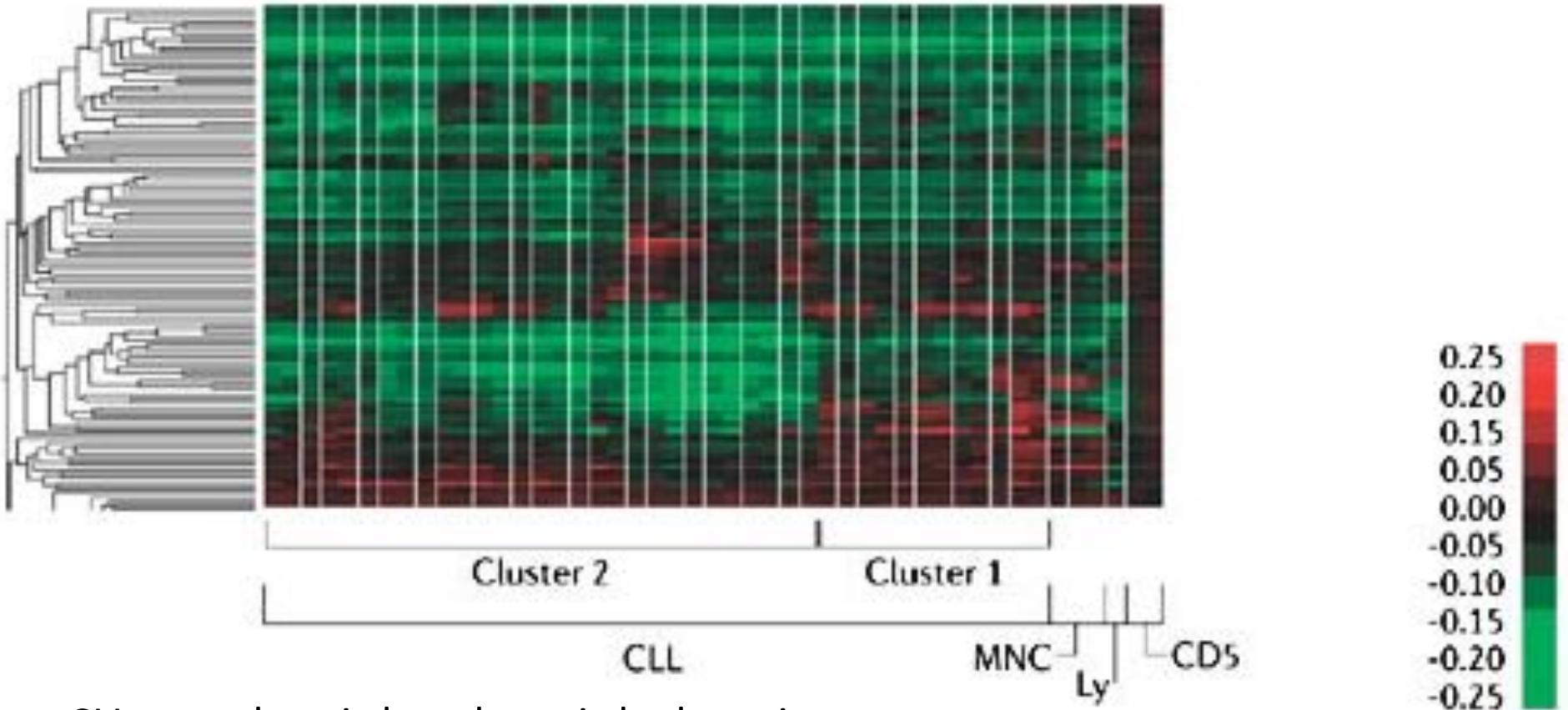
Examples of microRNA profiles in human solid and liquid cancers



CLL, chronic lymphocytic leukaemia;
MNC, mononuclear cells;
Ly, B lymphocytes;
CD5, a subset of B lymphocytes largely accepted to represent the equivalent of malignant cells in CLL

Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

miRNA expression profiles as diagnostic and prognostic markers of lung cancer

Expression of let-7 miRNA

Frequently reduced in human lung cancers
Reduced shorter postoperative survival.

***let-7 miRNA* overexpression in A549 lung adenocarcinoma cells**

inhibited lung cancer cell growth *in vitro.*

Takamizawa *et al. Cancer Res 2004*

miRNA expression profiles discriminate lung cancers from noncancerous lung tissues

Molecular signatures that differed in tumor histology
High *hsa miR-155 and low hsa-let-7a-2 precursor miRNA expression*
correlated with poor survival of lung adenocarcinomas

Yanaihara et al. *Cancer Cell* 2006

Κατατομή έκφρασης microRNA σε καρκίνους

Cancer type*	MiRNA profiling data	Significance	Refs
Chronic lymphocytic leukaemia	A unique signature of 13 genes associated with prognostic factors (ZAP70 and IgVH mutation status) and progression (time from diagnosis to therapy)	MiRNAs as diagnostic markers (the identification of two categories of patients)	49,35
Lung adenocarcinoma	Molecular signatures that differ with tumour histology; miRNA profiles correlated with survival (*miR-155* and *let-7*)	MiRNAs as prognostic and diagnostic markers	53
Breast carcinoma	MiRNA expression correlates with specific pathological features	MiRNAs as prognostic markers	50
Endocrine pancreatic tumours	A signature that distinguishes endocrine from acinar tumours; the overexpression of *miR-21* is strongly associated with both a high Ki67 proliferation index and the presence of liver metastases	MiRNAs as diagnostic and prognostic markers	54
Hepatocellular carcinoma	MiRNA expression correlated with differentiation	MiRNAs as prognostic markers	52
Papillary thyroid carcinoma	MiRNA upregulation (for example, *miR-221* and *miR-222*) in tumoral cells and normal cells adjacent to tumours, but not in normal thyroids without cancers	MiRNAs probably involved in cancer initiation	37 114
Glioblastoma	A specific signature compared with normal tissues	MiRNAs as diagnostic markers	51
Human cancers	MiRNA-expression profiles accurately classify cancers; an miRNA classifier classes poorly differentiated samples better than a messenger RNA classifier	MiRNAs as diagnostic markers	41
Human solid cancers	Common signature for distinct types of solid carcinomas	Specific miRNAs are involved in common molecular pathways	47

*Only data from microarray studies reporting results on human primary tumours were included in this table. IgV_H, immunoglobulin heavy-chain variable-region, MiRNA, microRNA. ZAP70, 70 kDa zeta-associated protein.

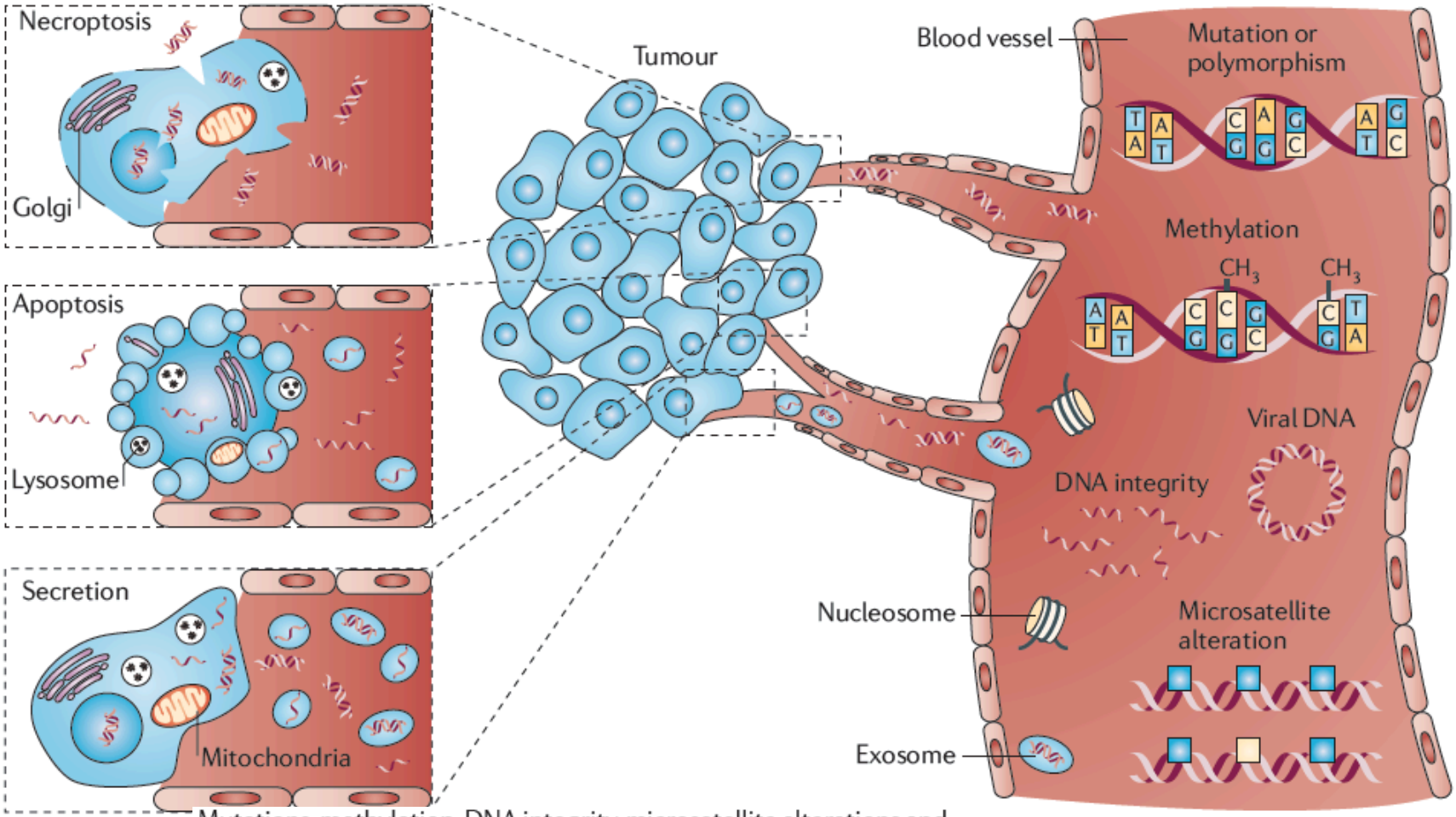
Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

OncomiRs

microRNAs	Tumorigenesis	Diagnosis	Prognosis
miR-9	Neuroblastoma		
miR-10b	Breast cancer		
miR-15, miR-15a	Leukemia, pituitary adenoma		
miR-16, miR-16-1	Leukemia, pituitary adenoma		
miR-17-5p, miR-17-92	Lung cancer, lymphoma		
miR-20a	Lymphoma, lung cancer		
miR-21	Breast cancer, cholangiocarcinoma, head & neck cancer, leukemia, cervical cancer		Pancreatic cancer
miR-29, miR-29b	Leukemia, cholangiocarcinoma		
miR-31	Colorectal cancer		
miR-34a	Pancreatic cancer		Neuroblastoma
miR-96	Colorectal cancer		
miR-98	Head & neck cancer		
miR-103	Pancreatic cancer		
miR-107	Leukemia, pancreatic cancer		
miR-125a, miR-125b	Neuroblastoma, breast cancer		
miR-128	Glioblastoma		
miR-133b	Colorectal cancer		
miR-135b	Colorectal cancer		
miR-143	Colon cancer, cervical cancer		
miR-145	Breast cancer, colorectal cancer		
miR-146	Thyroid carcinoma		
miR-155	Breast cancer, leukemia, pancreatic cancer		Lung cancer

Cho W, Mol Cancer 2007

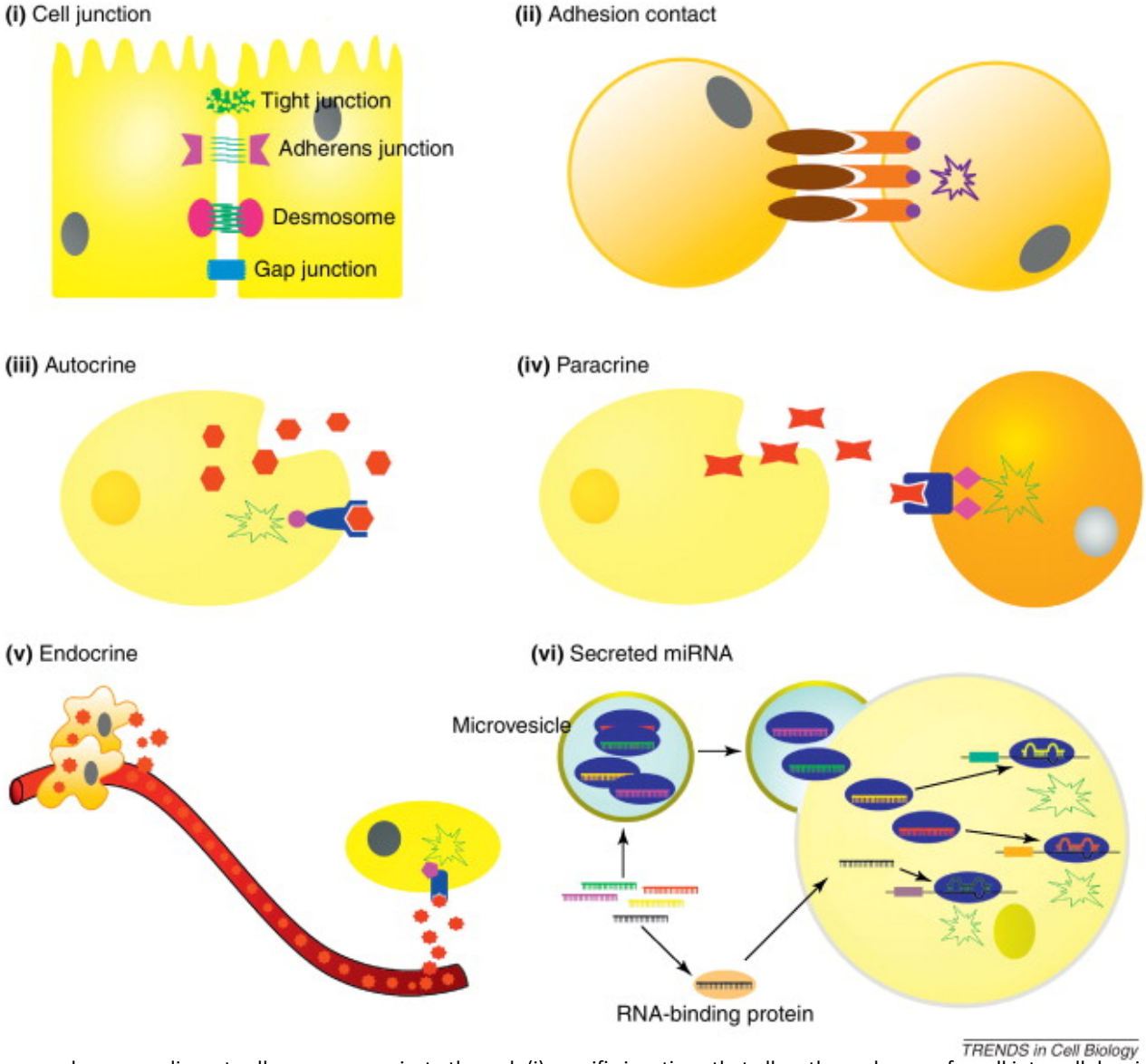
Ελεύθερα νουκλεϊκά οξέα στο αίμα (Cell-free nucleic acids)



Mutations, methylation, DNA integrity, microsatellite alterations and viral DNA can be detected in cell-free DNA (cfDNA) in blood. Tumour-related cfDNA, which circulates in the blood of cancer patients, is released by tumour cells in different forms and at different levels. DNA can be shed as both single-stranded and double-stranded DNA. The release of DNA from tumour cells can be through various cell physiological events such as apoptosis, necrosis and secretion. The physiology and rate of release is still not well understood; tumour burden and tumour cell proliferation rate may have a substantial role in these events. Individual tumour types can release more than one form of cfDNA.

Schwarzenbach et al. *Nature Reviews Cancer* 2011; 11: 426.

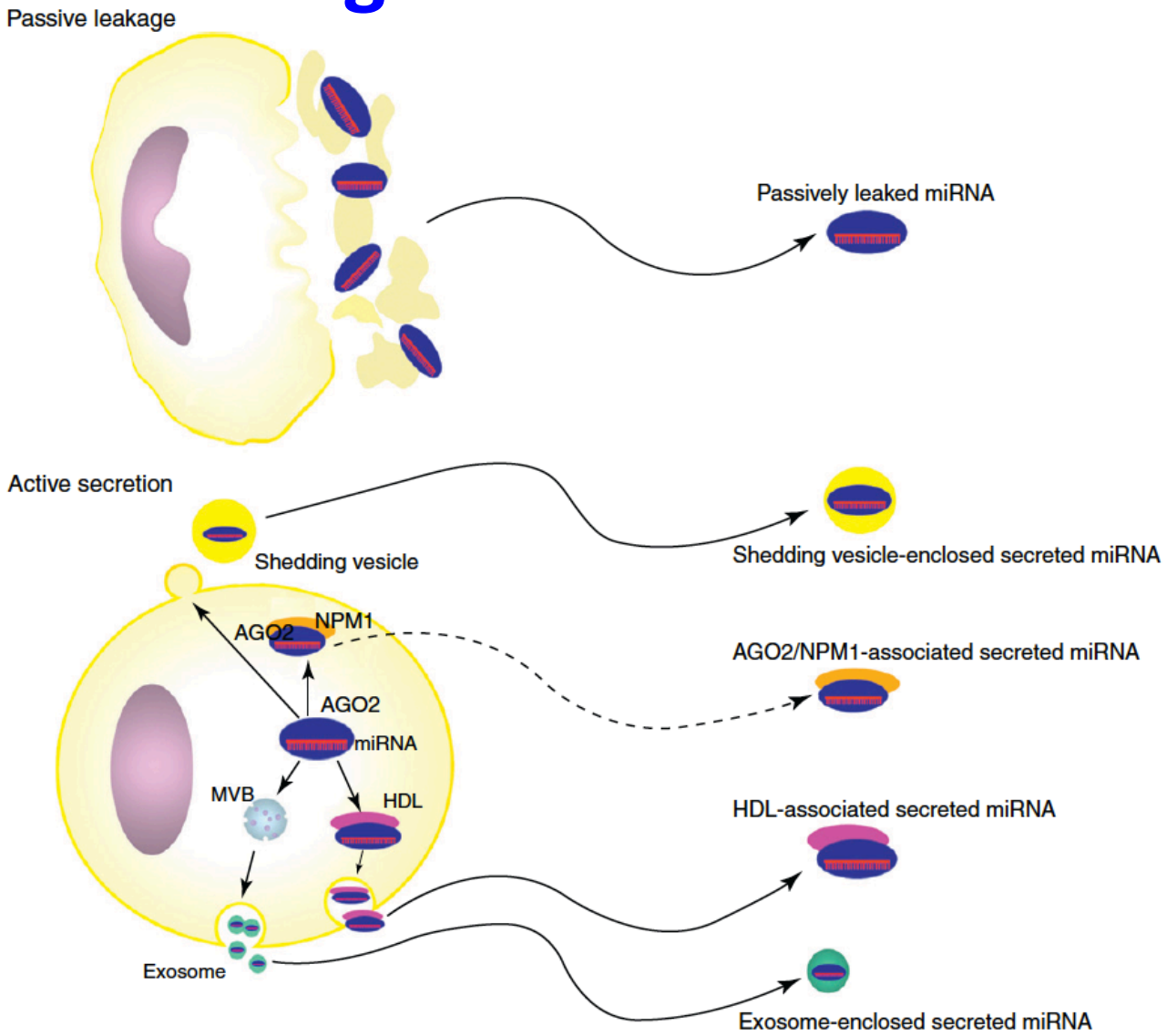
Secreted miRNA-mediated gene regulatory network as a novel form of intercellular communication



Cells can communicate by several means: adjacent cells can communicate through (i) specific junctions that allow the exchange of small intracellular signaling molecules or (ii) direct adhesion contacts between a membrane-bound signaling molecule on one cell and a receptor on the surface of another cell. Cells also can communicate via soluble messengers, such as hormones, cytokines and chemokines, which may act (iii) on the original cells (autocrine action) or (iv) on adjacent cells (paracrine action) or (v) travel long distances through intercellular nanotubes to affect target cells (endocrine action). In addition to these methods, (vi) secreted miRNA-mediated gene regulatory networks represent another type of intercellular communication in which a group of specific miRNAs can be transferred to target cells via microvesicles or RNA-binding proteins. These exogenous miRNAs can then activate myriad signaling events in the recipient cells by modulating expression of their target genes.

Chen et al., *Trends in Cell Biology*, 2012; 22: 125

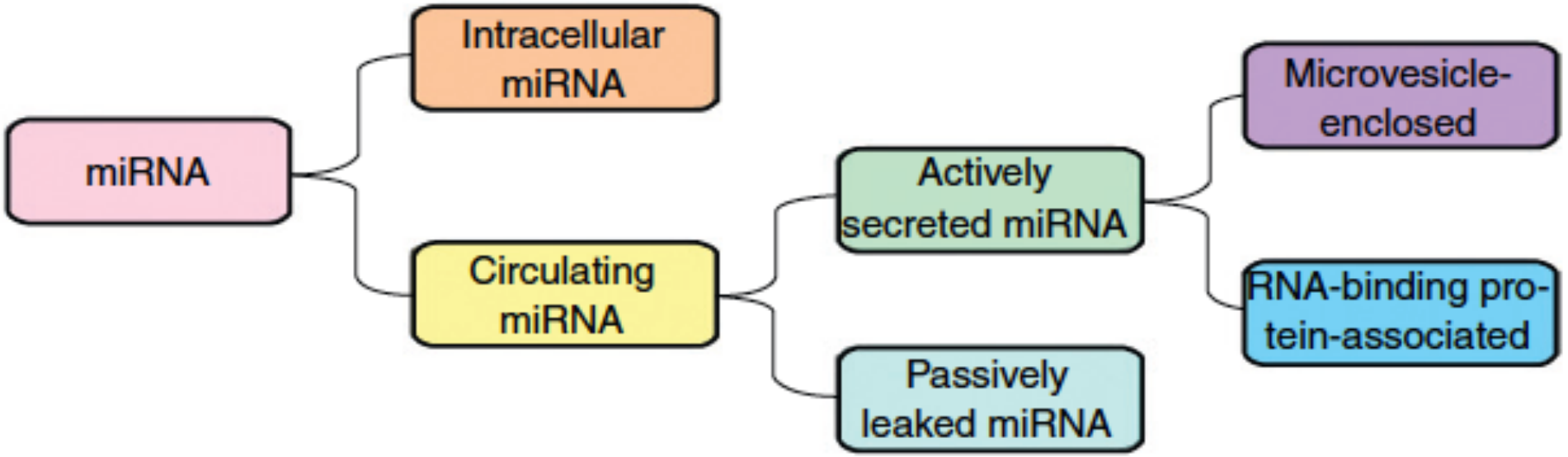
Circulating vs. secreted miRNAs



miRNAs can enter the circulation through three pathways: (i) passive leakage from broken cells; (ii) active secretion via microvesicles, including exosomes and shedding vesicles; and (iii) active secretion in conjunction with the RNA-binding protein high-density lipoprotein (HDL). Other RNA-binding proteins, including Argonaute2 (AGO2) and nucleophosmin 1 (NPM1), are found to bind circulating miRNAs; however, whether AGO2- or NPM1-bound miRNAs are actively released from cells and can be taken up by recipient cells is currently unclear. miRNA secretion via microvesicles and HDL is active and energy dependent, and this is the key characteristic that distinguishes secreted miRNAs from passively leaked miRNAs.

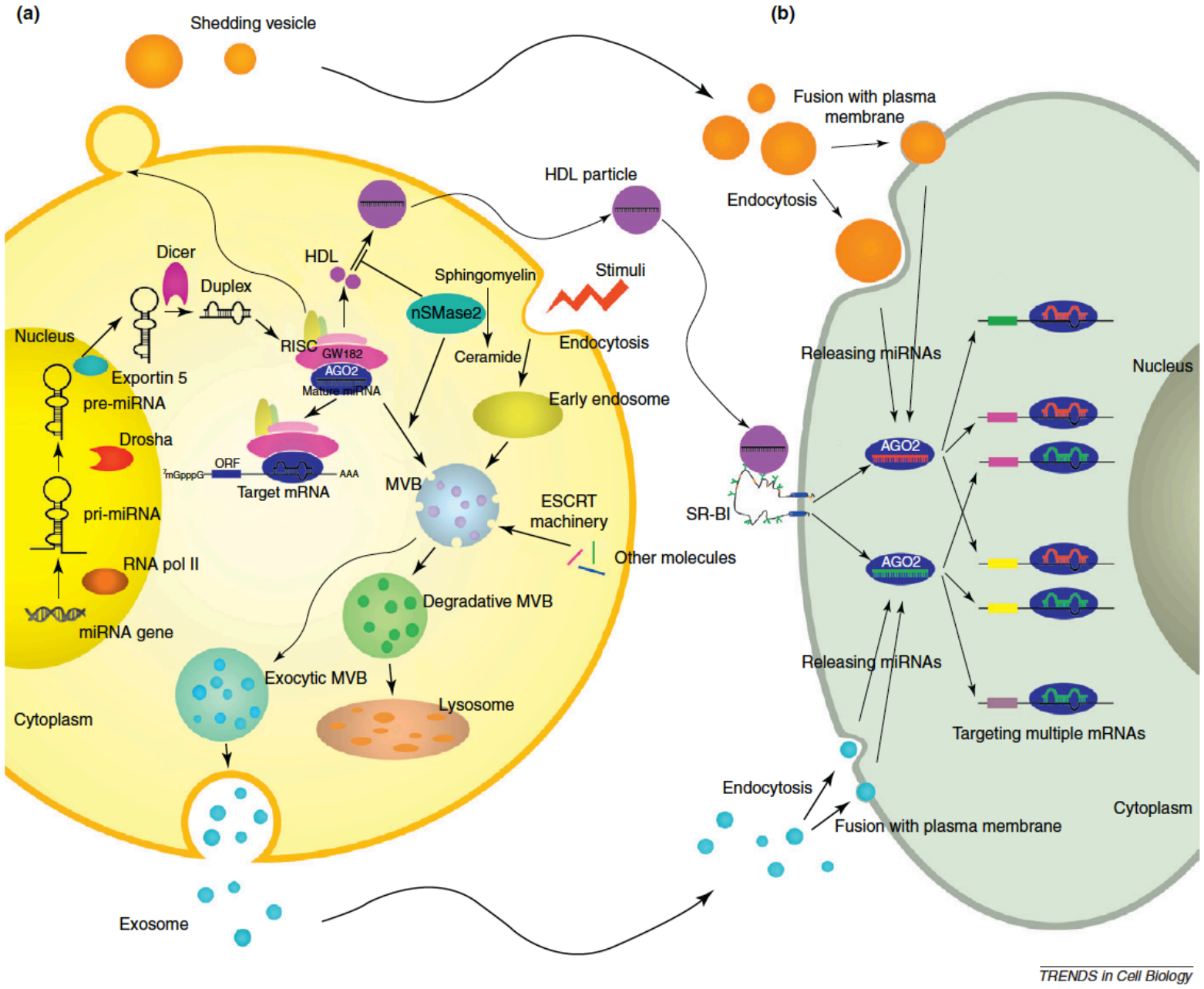
Chen et al., *Trends in Cell Biology*, 2012; 22: 125

Circulating vs. secreted miRNAs

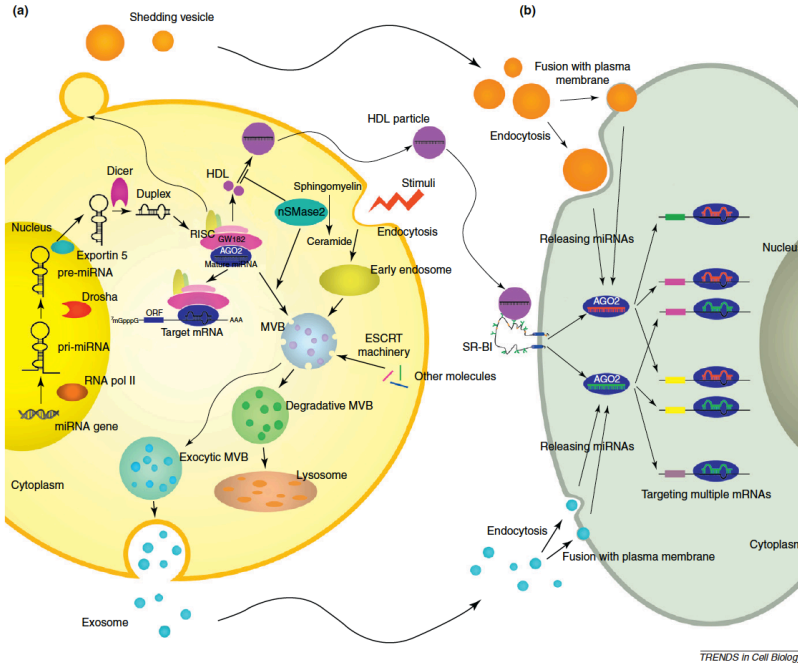


Chen et al., *Trends in Cell Biology*, 2012; 22: 125

Biogenesis and proposed model for secreted miRNAs



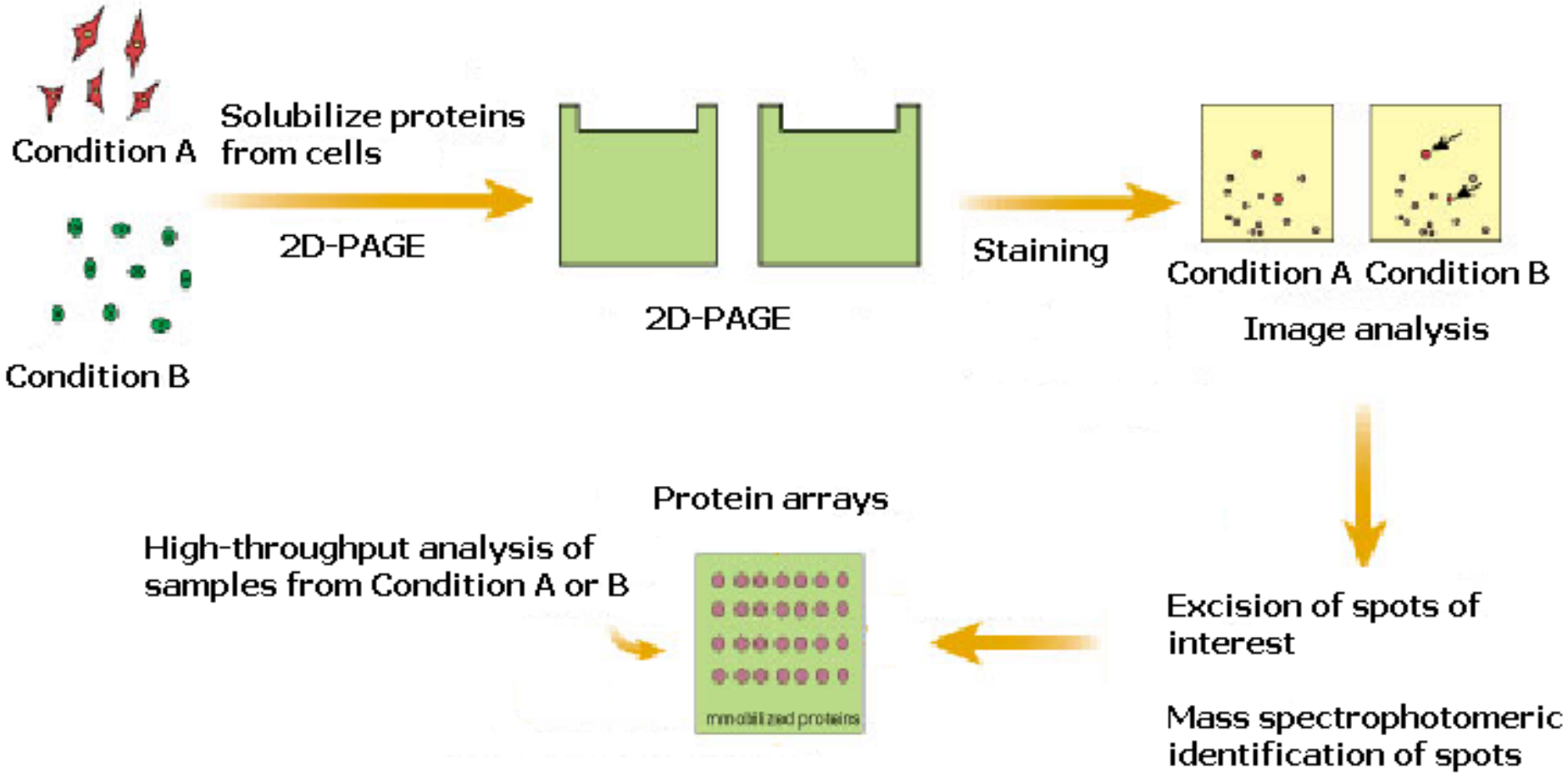
Chen et al., *Trends in Cell Biology*, 2012; 22: 125



Schematic description of the sorting and release of secreted miRNAs. After being transcribed in the nucleus, exported to the cytoplasm and processed into a mature form, miRNAs can bind to complementary sequences on target mRNAs to repress translation or trigger mRNA cleavage. They can also be packaged and transported to the extracellular environment via three different pathways. (i) The generation of exosomal miRNAs requires ceramide production on the cytosolic side by neutral sphingomyelinase 2 (nSMase2), and other molecules that are targeted to lysosomes depend on the endosomal sorting complex required for transport (ESCRT) machinery. Thus, a ceramide-dependent, ESCRT-independent pathway may control the incorporation of miRNAs into exosomes. Furthermore, exosomes may deliver cellular components of the RNA-induced silencing complex (RISC), such as GW182 and Argonaute2 (AGO2), to enhance the biological function of the secreted miRNAs. After fusion of multivesicular bodies (MVBs) with the plasma membrane, exosomal miRNAs are released into the circulation accompanying the release of exosomes. (ii) Shedding vesicles are formed by the process of blebbing or shedding from the plasma membrane. However, it is currently unknown how miRNAs are shed at the cell surface. (iii) miRNA inside the donor cell can be stably exported in conjunction with RNA- binding proteins, such as high-density lipoprotein (HDL). nSMase2 represses cellular export of miRNAs to HDL. (b) Schematic description of the uptake of secreted miRNAs in recipient cells. Exosomes and shedding vesicles can donate their miRNAs to recipient cells by the process of endocytosis, phagocytosis or direct fusion with the plasma membrane. HDL-associated miRNAs are taken up by recipient cells through binding to scavenger receptor class B type I (SR-BI) receptors present at the recipient cellular membrane. Because one miRNA can target numerous mRNAs and numerous miRNAs can target one mRNA simultaneously, secreted miRNAs may function in networks that form a complex system regulating myriad signaling events in the target cells.

Chen et al., *Trends in Cell Biology*, 2012; 22: 125

High- Throughput Proteomic Analysis By Mass Spectrometry

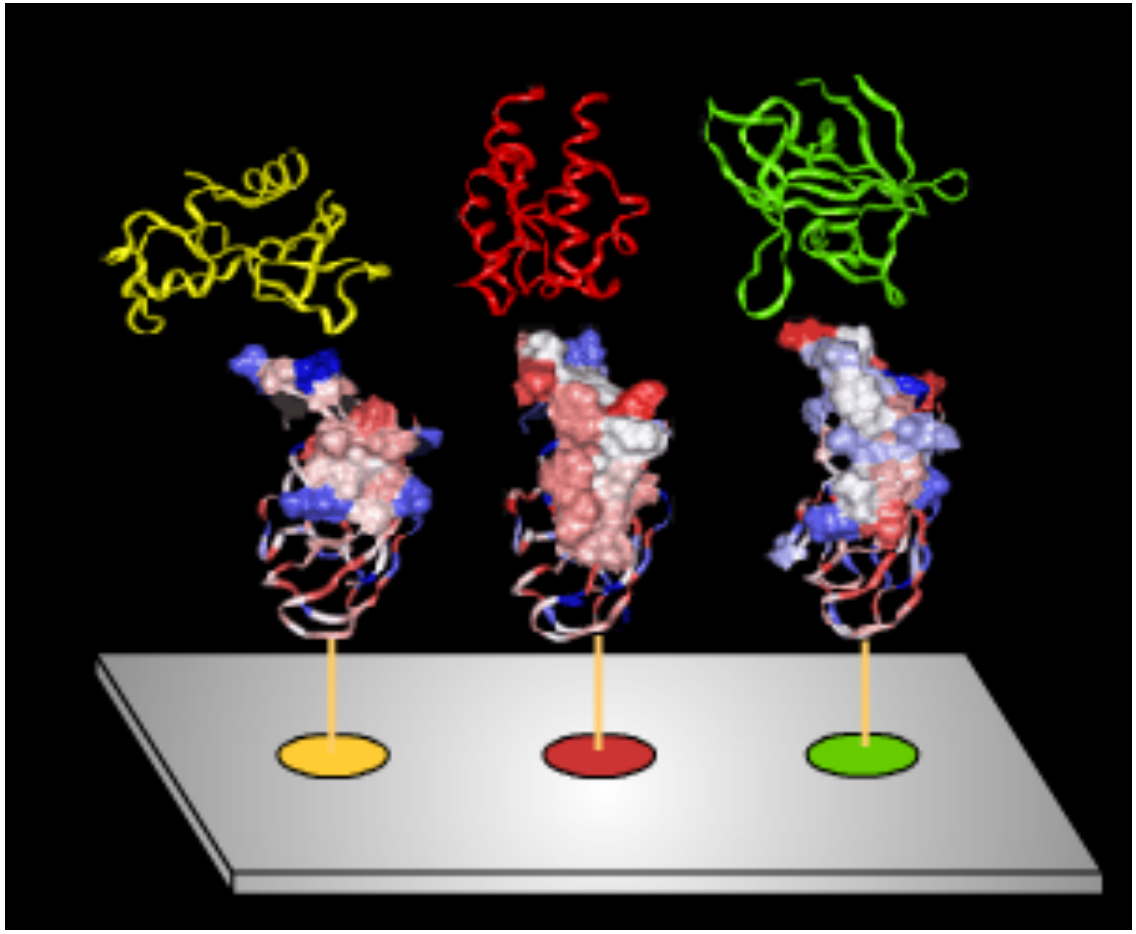


Haab et al Genome Biology 2000;1:1-22

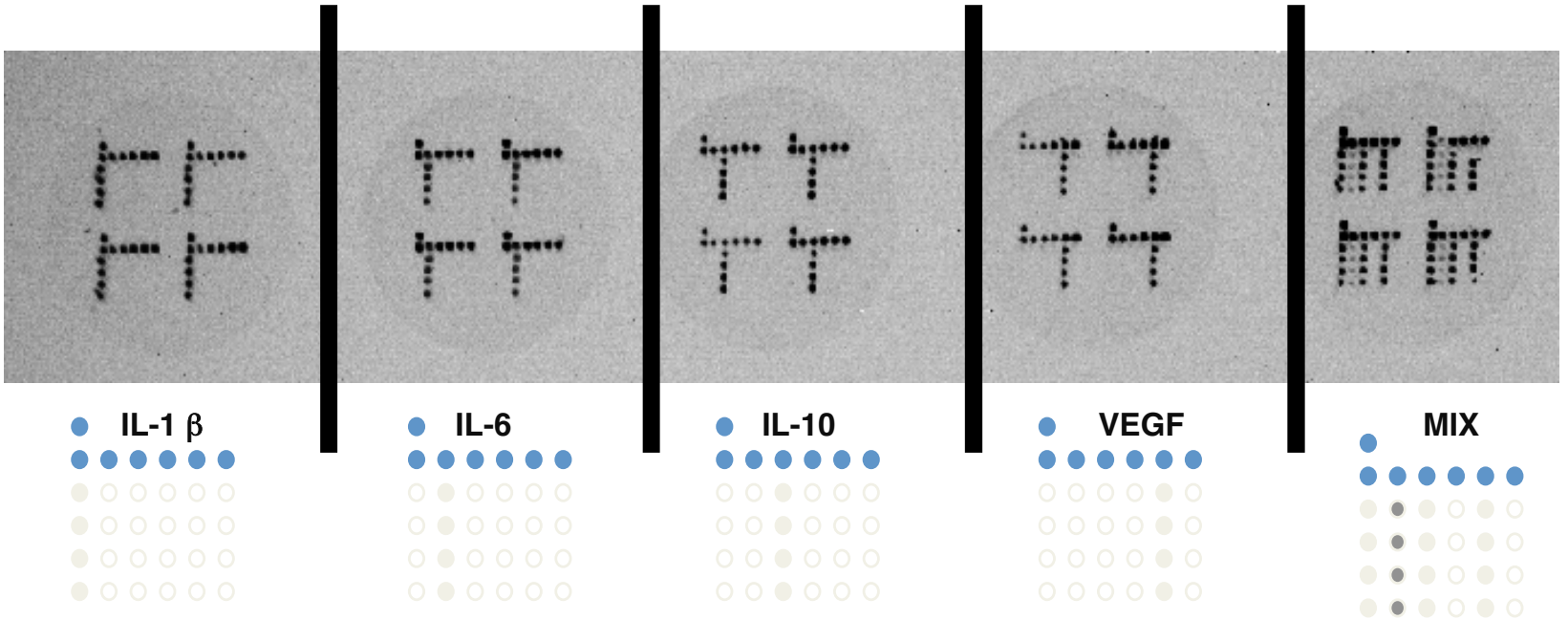
Μικροσυστοιχίες πρωτεϊνών

Σάρωση για:

- **μικρά μόρια στόχους**
- **μεταμεταφραστικές τροποποιήσεις**
- **Αλληλεπιδράσεις πρωτεϊνών**
- **Αλληλεπιδράσεις DNA-πρωτεϊνών**
- **Ενζυμικές δοκιμές**
- **Χαρτογράφηση επιτόπων**



Cytokine Specific Microarray ELISA



http://www.freepptdb.com/details-cancer-diagnostics-the-old-and-the-new-467023.html

Mass Spectrometry for Proteomic Pattern Generation

- Serum analysis by SELDI-TOF mass spectrometry after extraction of lower molecular weight proteins

- Data analyzed by a “pattern recognition” algorithm

Serum Fingerprint by Mass Spectrometry

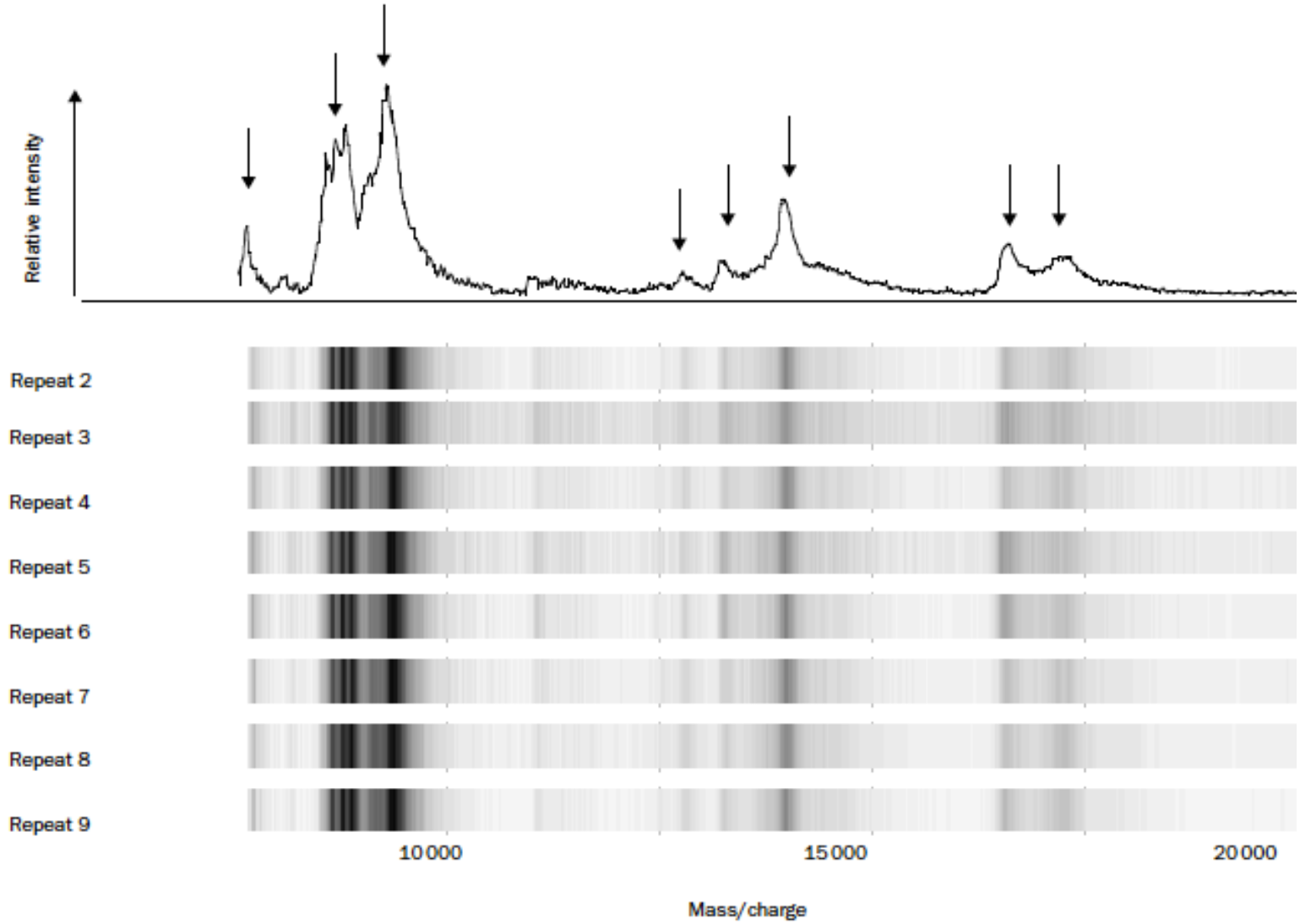


Figure 2: **Example of between-chip reproducibility of mass spectra**
Serum from an unaffected female control was individually applied to a single bait surface region on 100 separate C18 chips and analysed by SELDI-TOF. Nine randomly obtained spectra from the 100 used in the analysis are shown. The eight proteins with the highest consistent amplitudes (arrows), were used as a surrogate for reproducibility by calculation of the coefficient of variance of the normalised peak amplitudes for each of the eight.

Petricoin III EF, et al. *Lancet* 2002;359:572-577

Results

Classification by Proteomic Pattern

	Cancer	Unaffected	New Cluster
Unaffected Women			
No evidence of ovarian cysts	2/24	22/24	0/24
Benign ovarian cysts <2.5cm	1/19	18/19	0/19
Benign ovarian cysts >2.5cm	0/6	6/6	0/6
Benign gynecological inflammatory disorder	0/7	0/7	7/7
Women with Ovarian Cancer			
Stage I	18/18	0/18	0/18
Stage II, III, IV	32/32	0/32	0/32

Petricoin III EF, et al. *Lancet* 2002;359:572-577