UNIVERSITÄT DES SAARLANDES

Precision diagnostics

miRMAs as non-invasive biomarkers

Saarbücken, 25.11.2013

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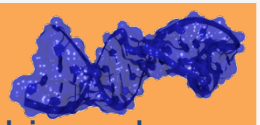
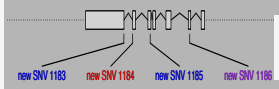
Chair for Clinical Bioinformatics
Saarland University
University Hospital

Non-Invasive Biomarkers

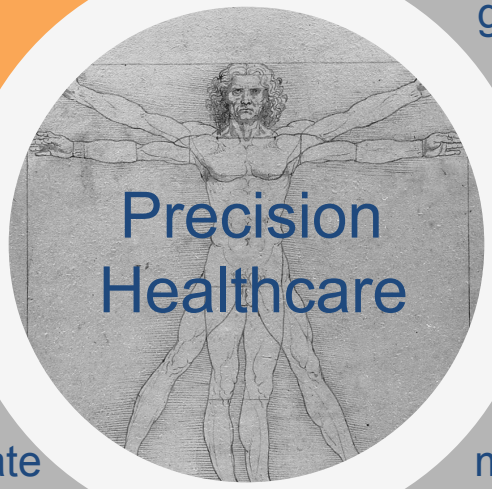


Detection of miRNA and protein biomarker patterns from human blood or serum samples using microarrays, NGS, qRT-PCR & mass spectrometry. Biostatistical evaluation & validation of the complex profiles.

Genetic Testing by NGS



Whole genome, exome or gene panel sequencing of DNA in order to detect genetic causes for human diseases. Understanding the effect of the respective genetic variants for different disease phenotypes.

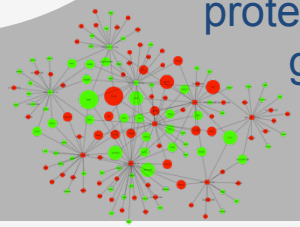


Bacterial Resistance

Understanding the genetic cause of bacterial resistance and correlate the bacterial resistance to classical culture based tests in order to derive the minimal inhibitory concentration and best therapy with anti bacterial agents.

Systems Biology

Model and understand how DNA, mRNA, microRNA, methylation and proteins mutually interact in order generate a holistic and multi-scale molecular representation of human pathologies.



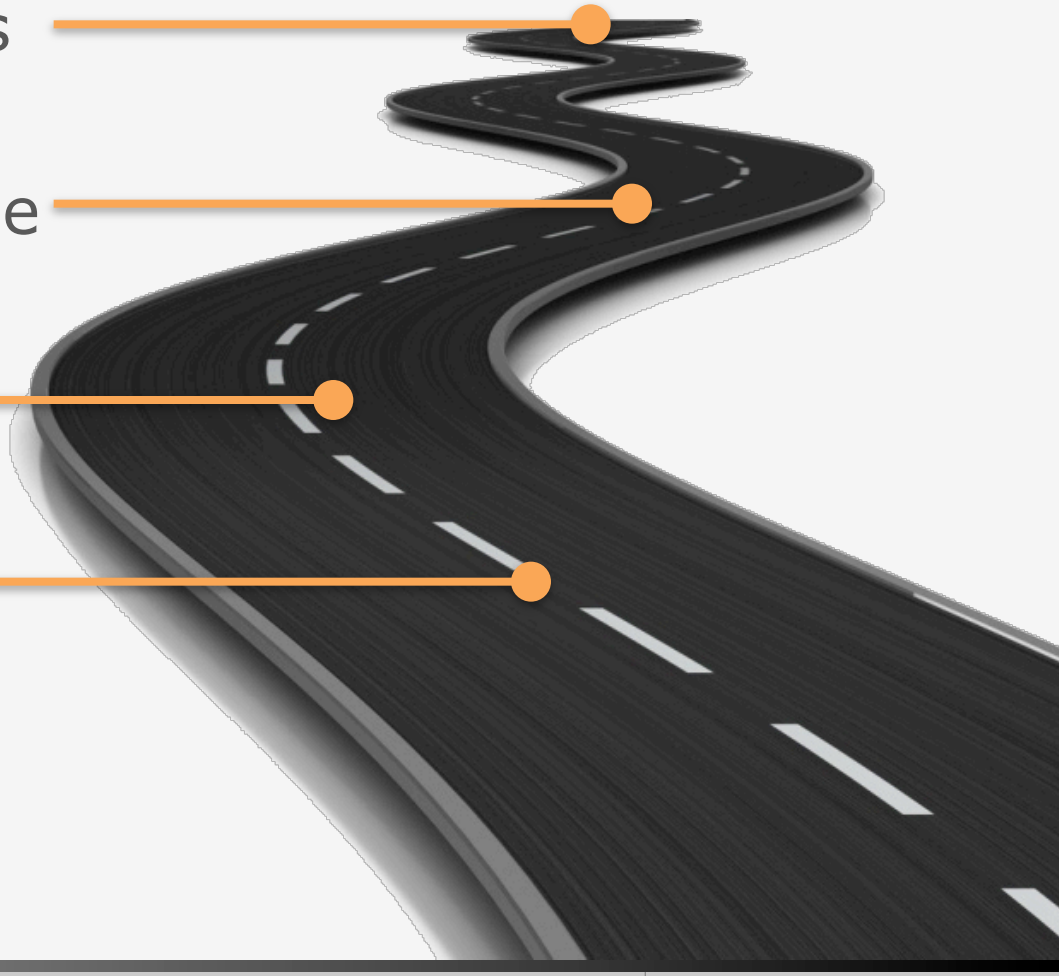
> Agenda

A brief intro to biomarkers

Steps in biomarker pipeline

Regulatory basics

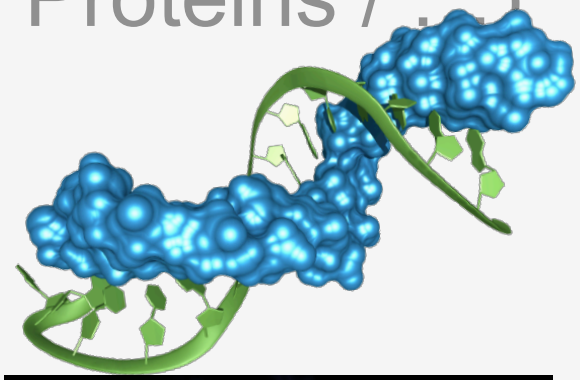
miRNAs as markers



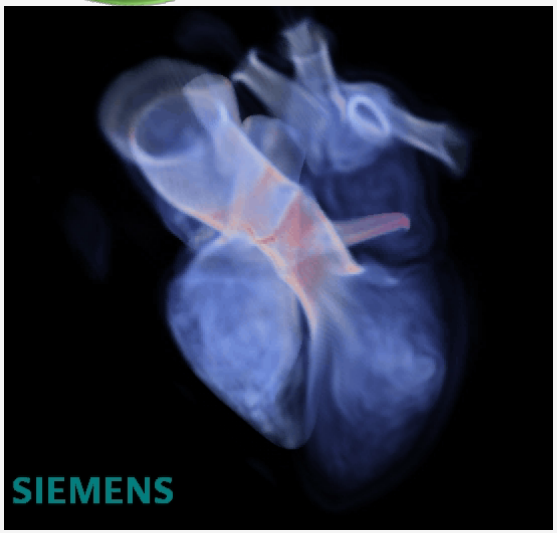
A biomarker is in general a substance used as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a intervention.

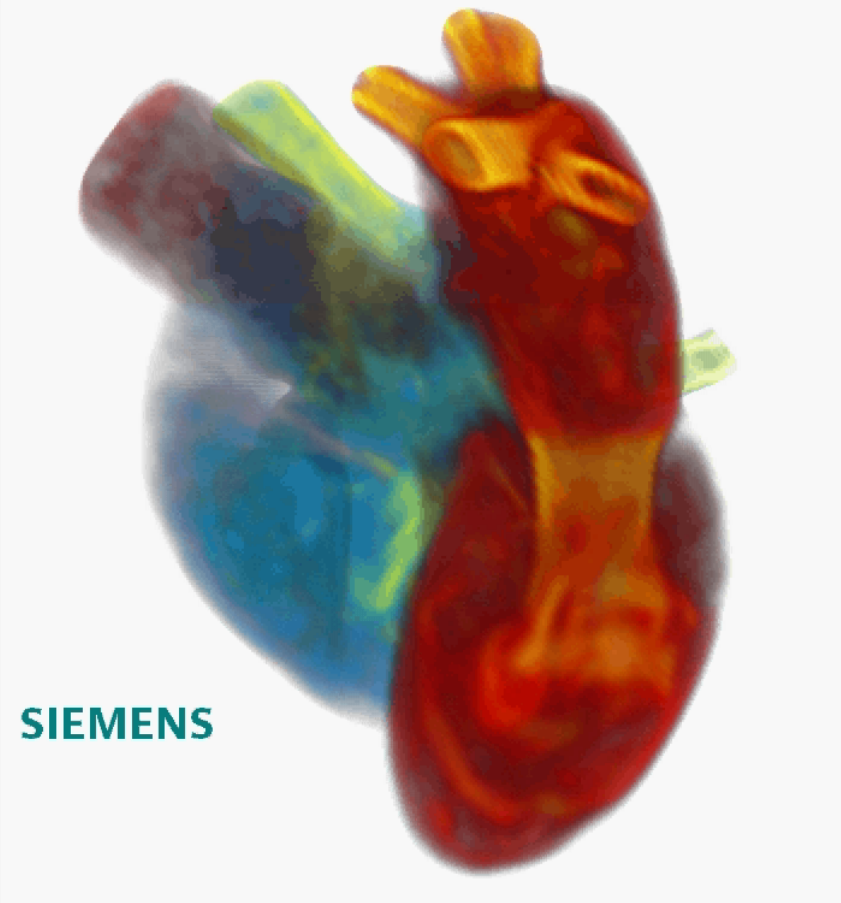
[wikipedia]

- Molecules (DNA / RNA / Proteins / …)
- Cells
- Organ function
- …

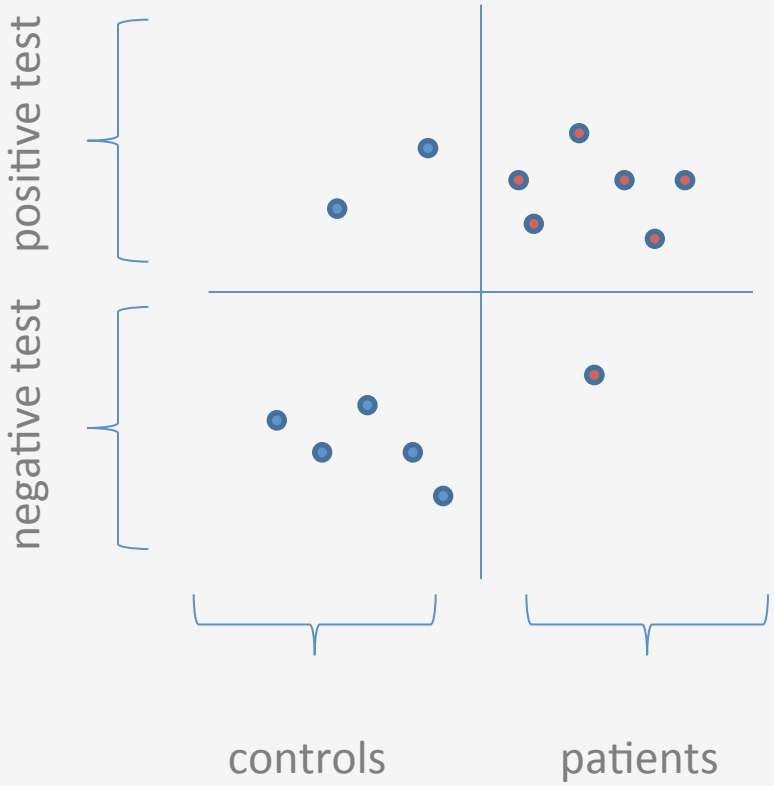


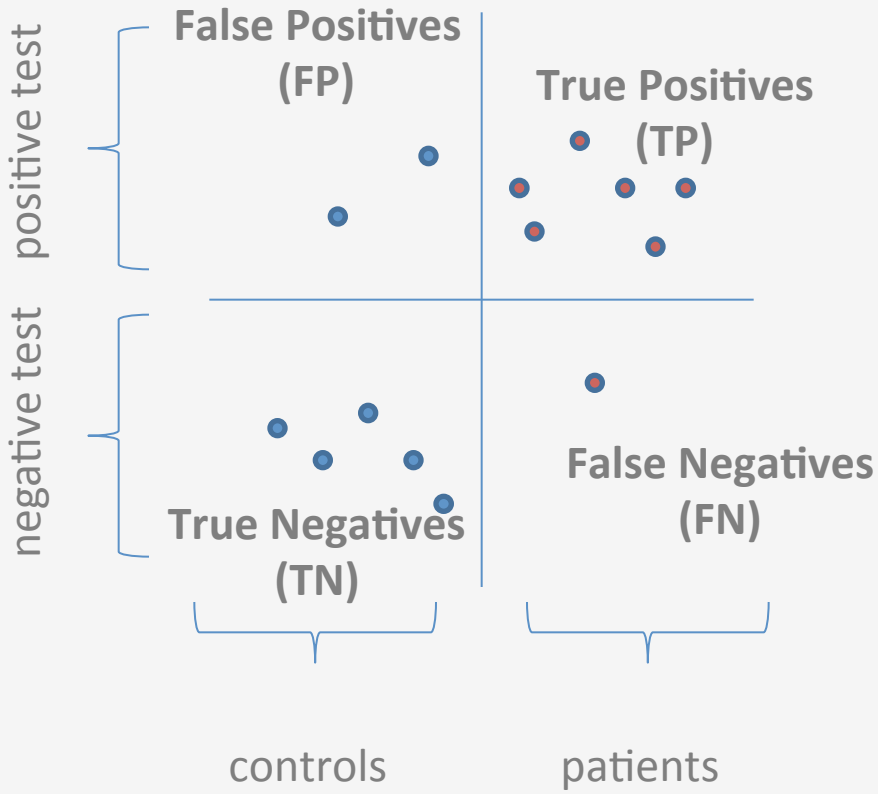
- In-vivo biomarkers




SIEMENS

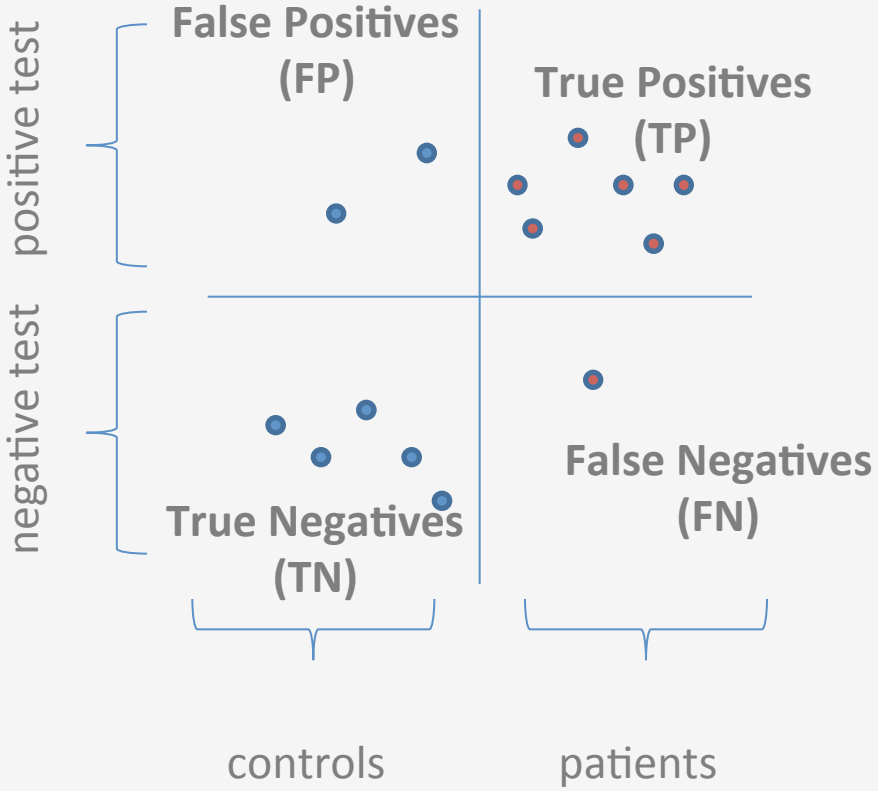
- Specificity
- Sensitivity
- Accuracy
- Positive Predictive Value
- Negative Predictive Value
- Positive Likelihood Ratio
- Negative Likelihood Ratio
- Odds ratio
- Area under the ROC curve
- Wilcoxon Mann-Whitney test
- T-tests


positive test
negative test
controls
patients


positive test
negative test
False Positives
(FP)
True Positives
(TP)
True Negatives
(TN)
False Negatives
(FN)
controls
patients

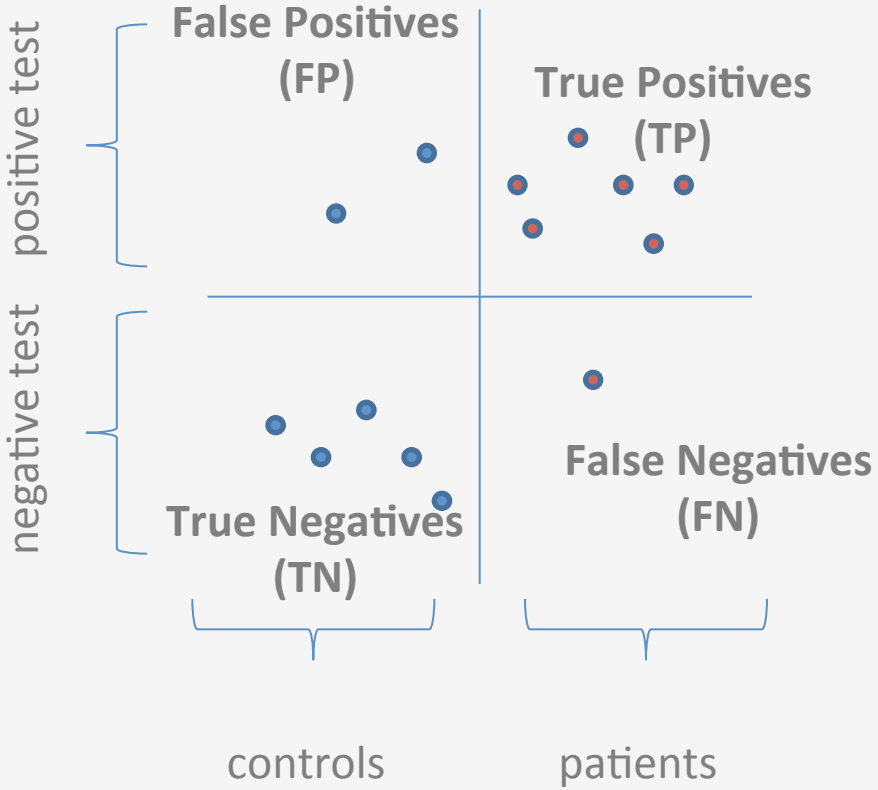
sens = TP / (TP + FN)

= 6 / (6 + 1)
= 85.7%



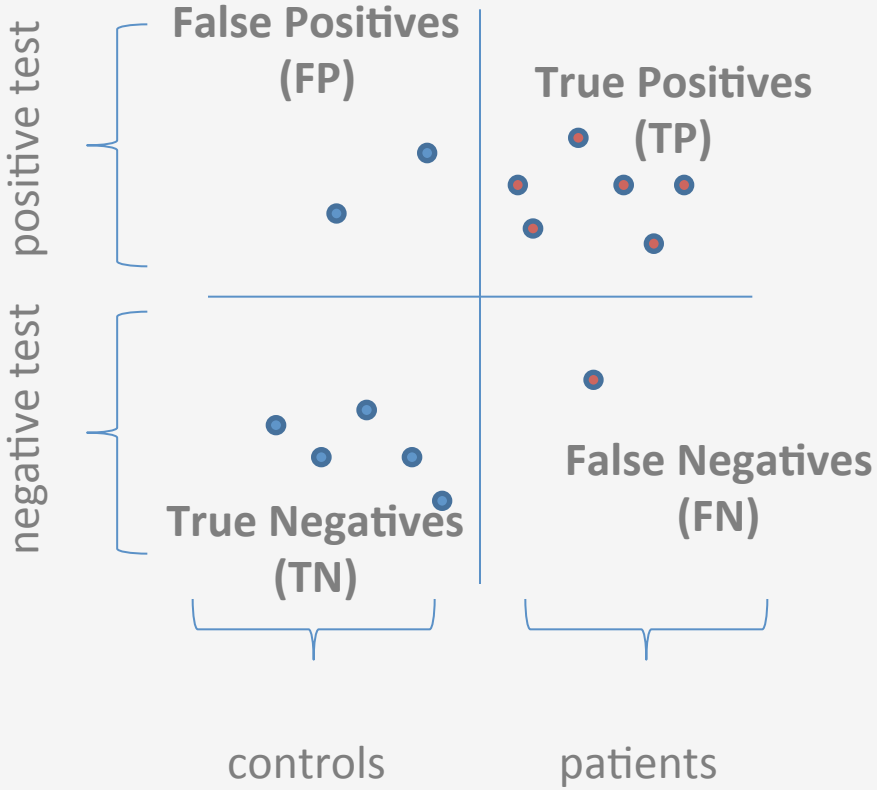
spec = TN / (TN + FP)

= 5 / (5 + 2)
= 71.4%



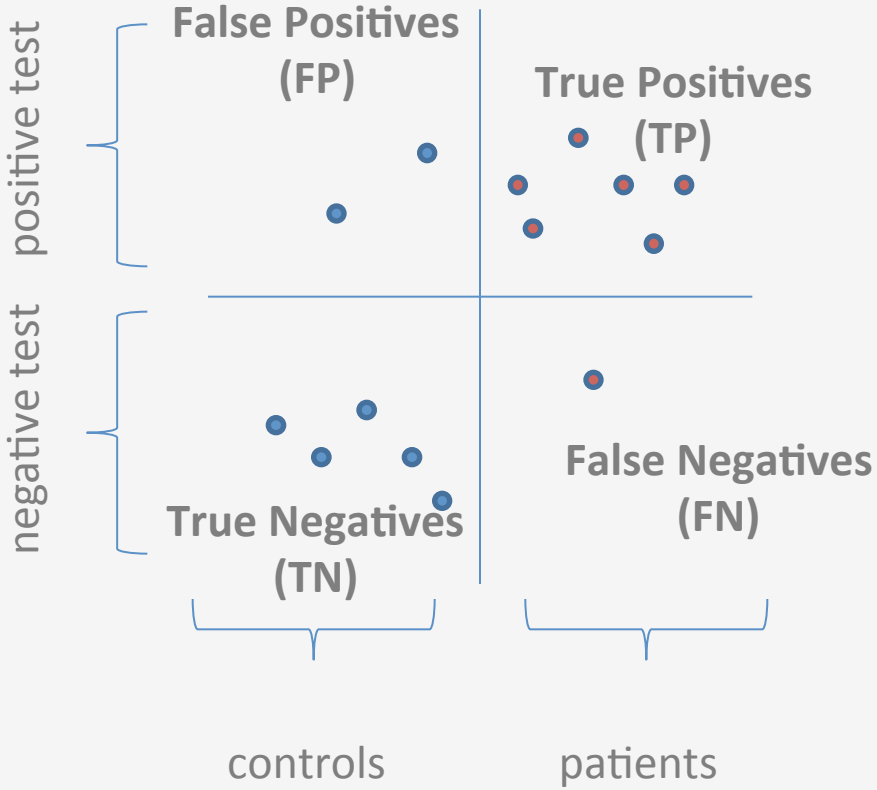
acc = (TP + TN)/ (TP + FN + FP + TN)

= (6 + 5) / (6 + 1 + 2 + 5)
= 78.6%



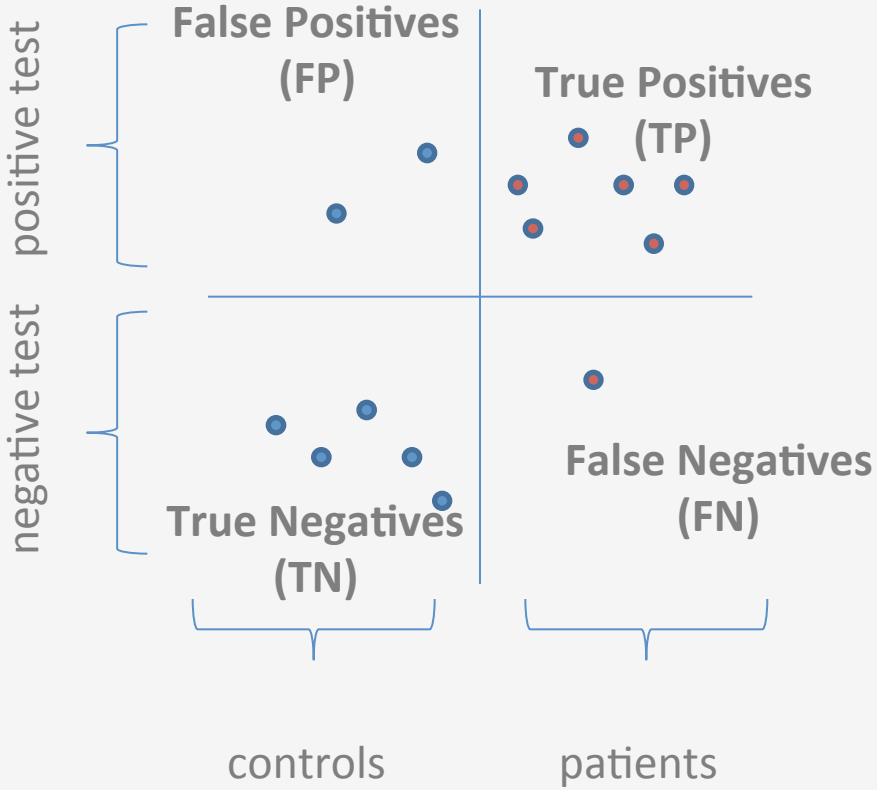
ppv = TP / (TP + FP)

= 6 / (6 + 2)
= 75%



npv = TN / (TN + FN)

= 5 / (5 + 1)
= 83.3%



in this case, the prevalence of the disease is equal in both classes, in reality, this must not hold and the prevalence has to be included in the consideration

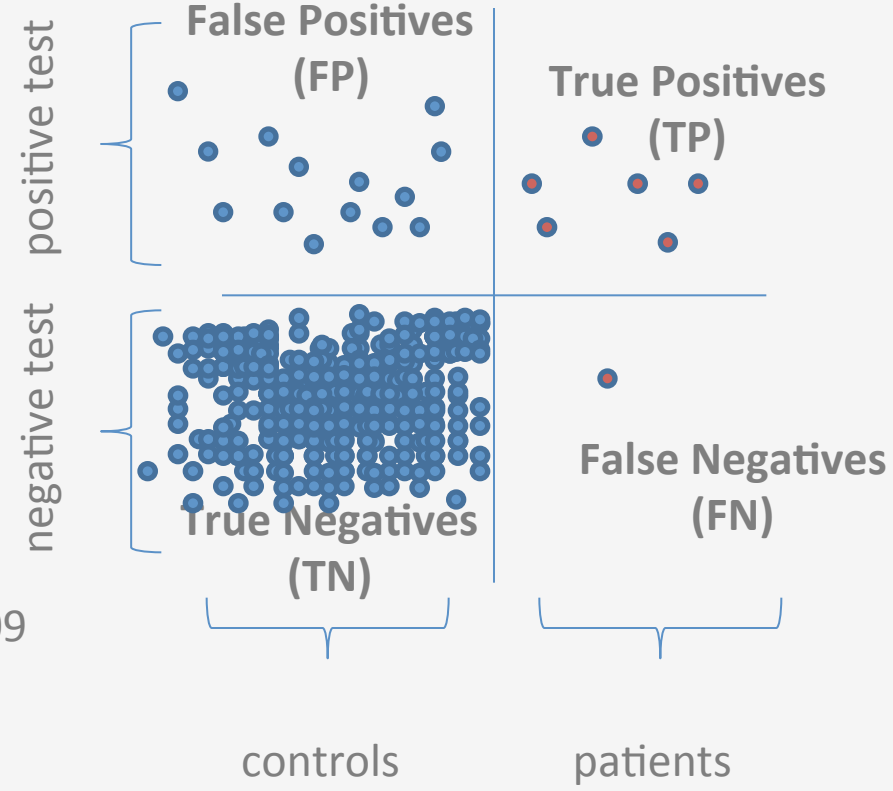
example: test with 80% specificity, 90% sensitivity and prevalence of 1%

ppv = TP / (TP + FP)

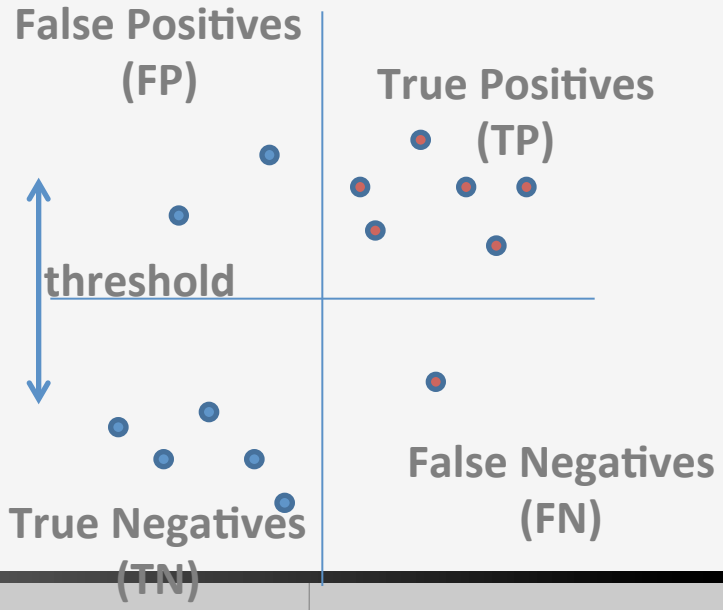
TP = sensitivity * prevalence = 0.9 * 0.01 = 0.009
FP = (1-specificity)*(1-prevalence)
= 0.2 * 0.99 = 0.198

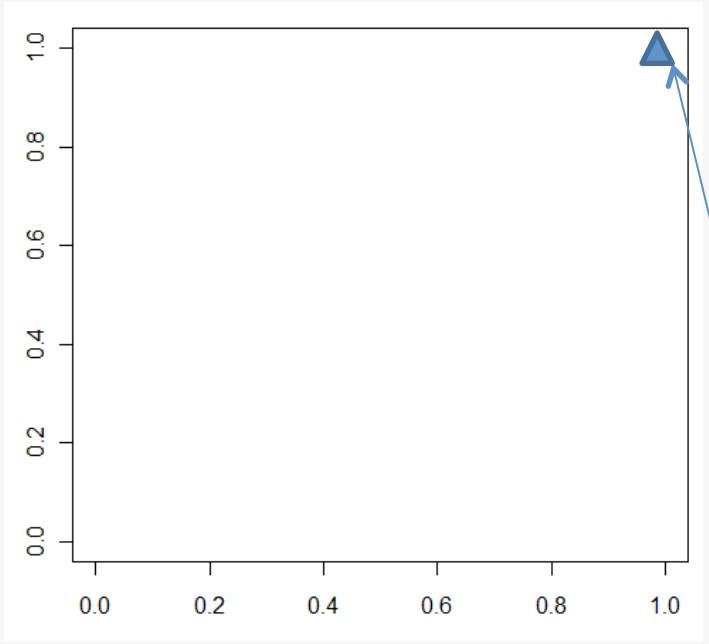
ppv = 0.009/(0.009+0.198) = 0.043

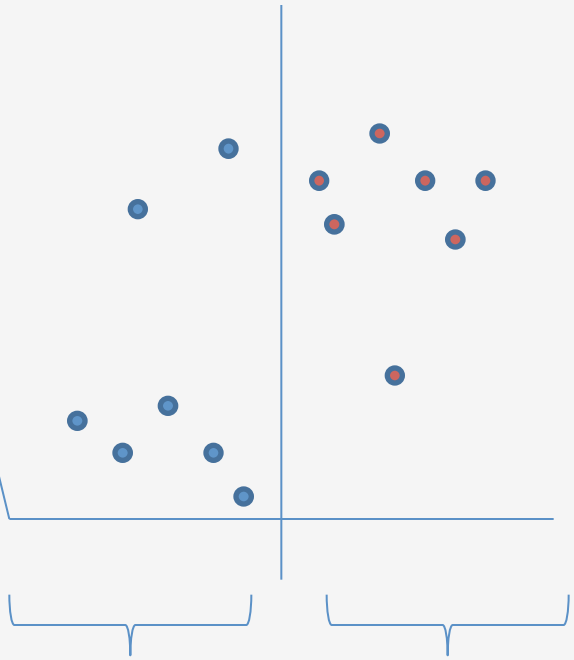
if the test is positive you have a chance of 4.3% to be diseased



- ROC (receiver operator characteristics) curve is a plot of sensitivity versus 1 – specificity
- Specificity and Sensitivity are computed depended on a threshold for a binary classifier
- ROC analysis helps to find the optimal classification threshold
- The closer the area under the curve (AUC) to 1 the better the classifier





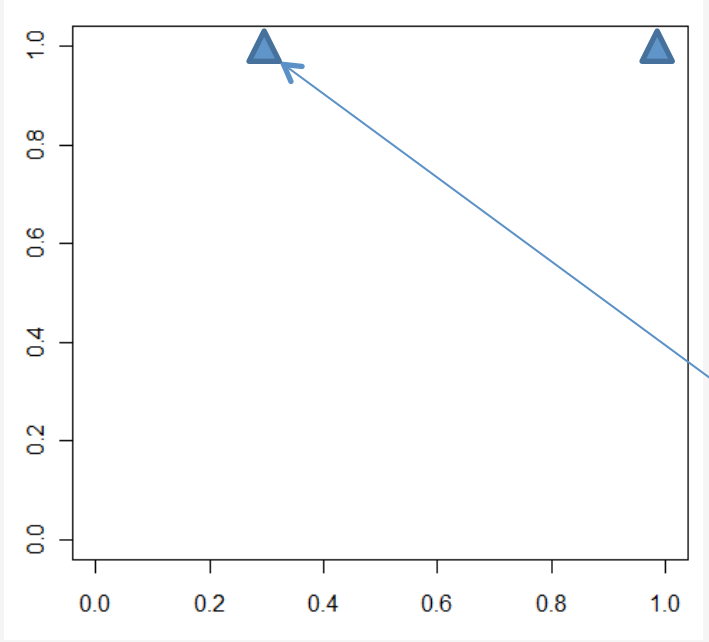


spec; sens

0; 1

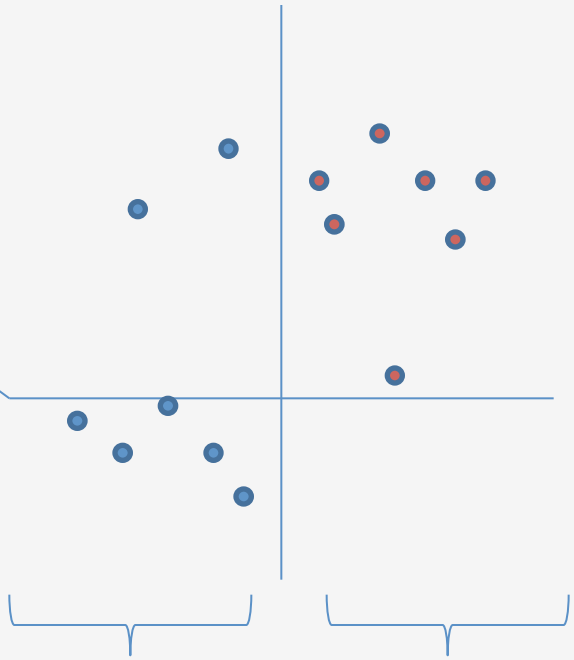
controls

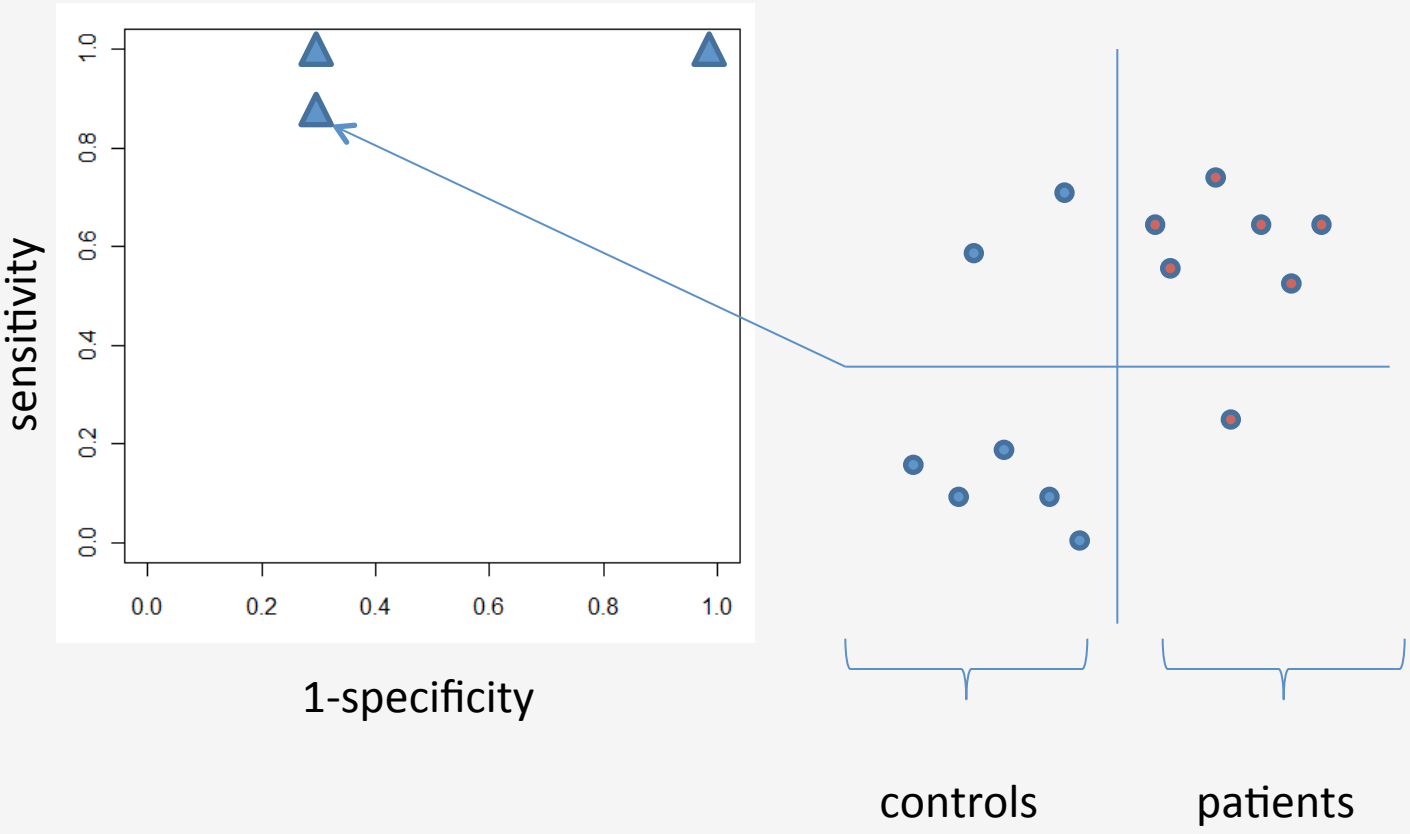
patients

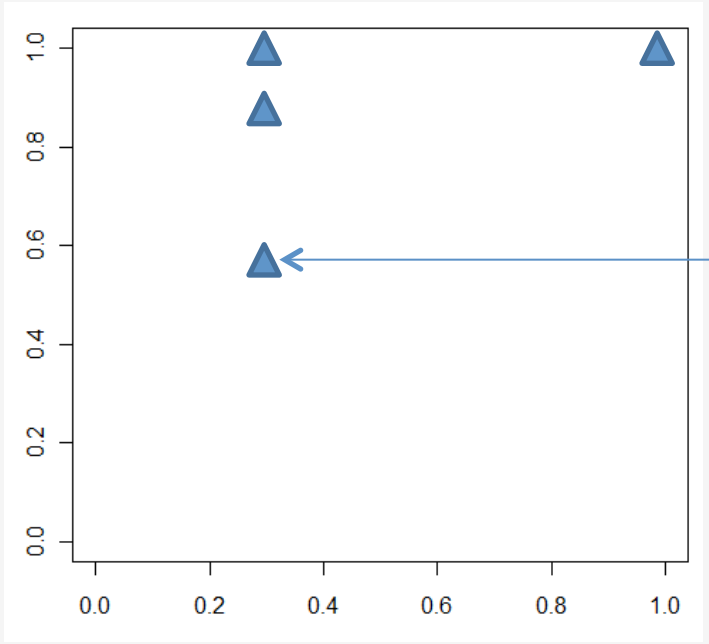


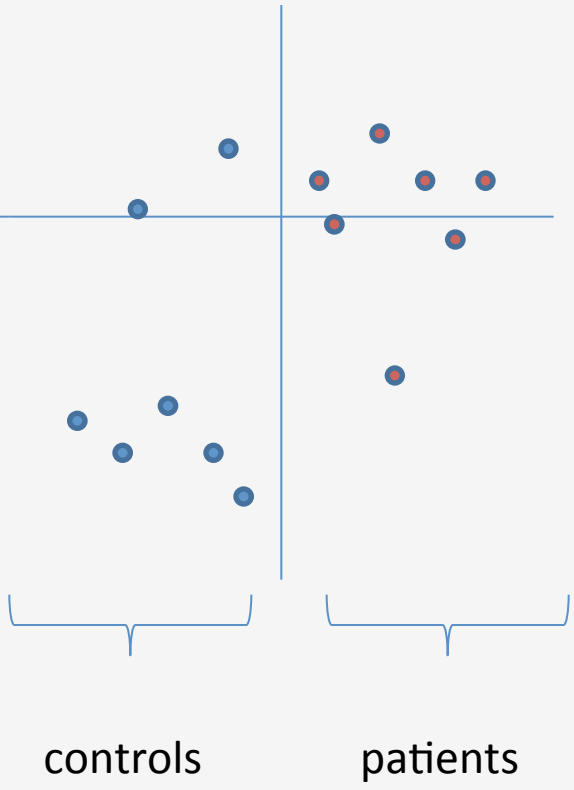
spec; sens

0.71; 1



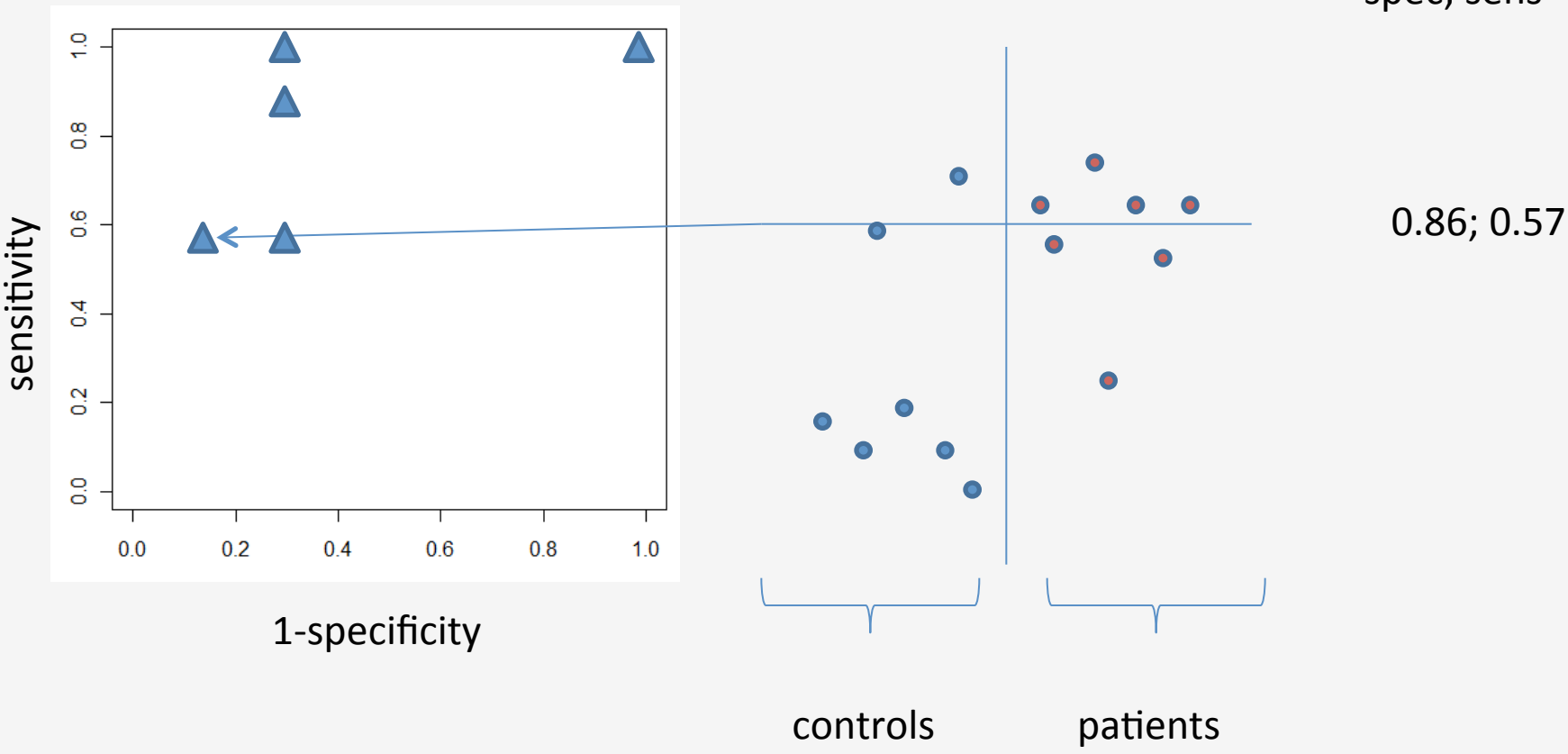

sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1-specificity
spec; sens
0.71; 0.86
controls
patients

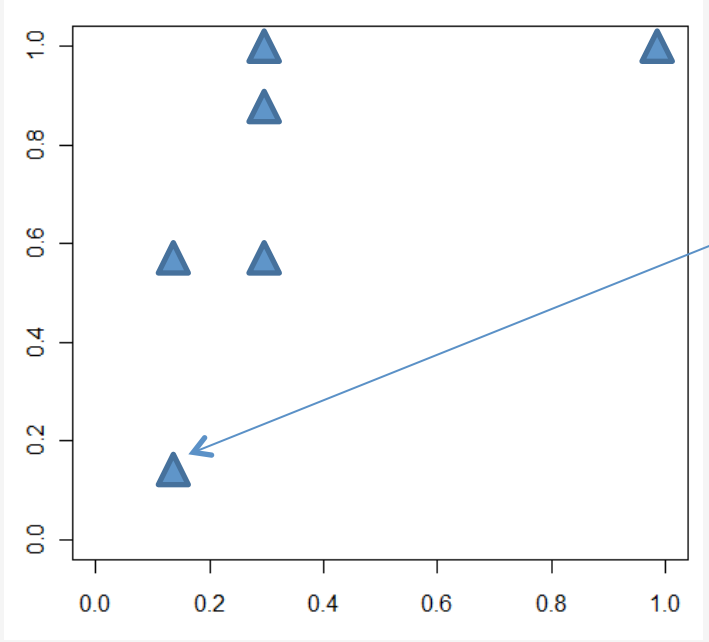


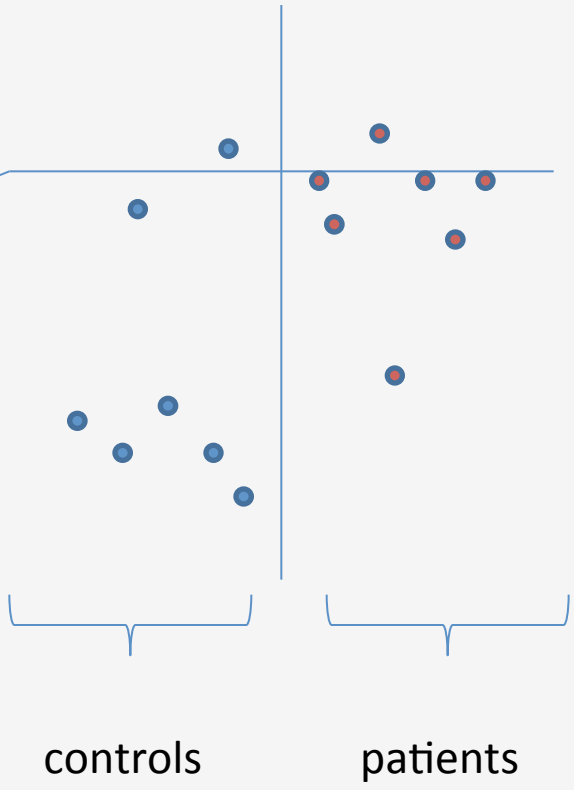


spec; sens

0.71; 0.57

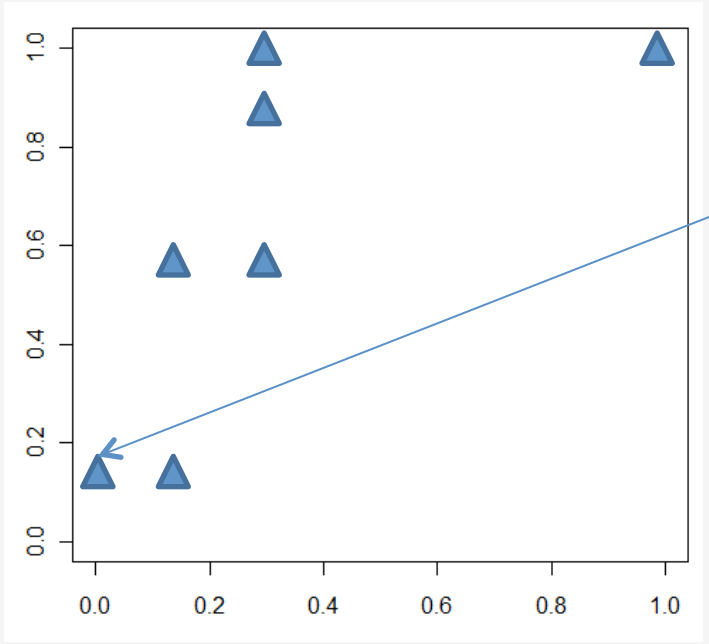

spec; sens
0.86; 0.57
sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1-specificity
controls
patients

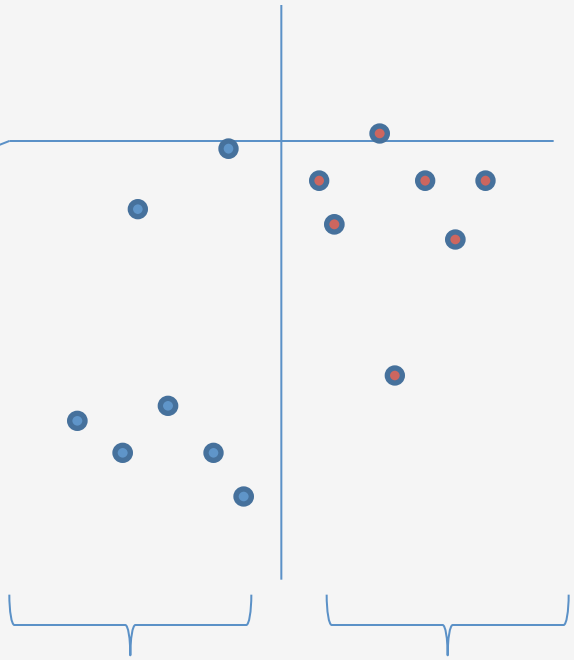




spec; sens

0.86; 0.14



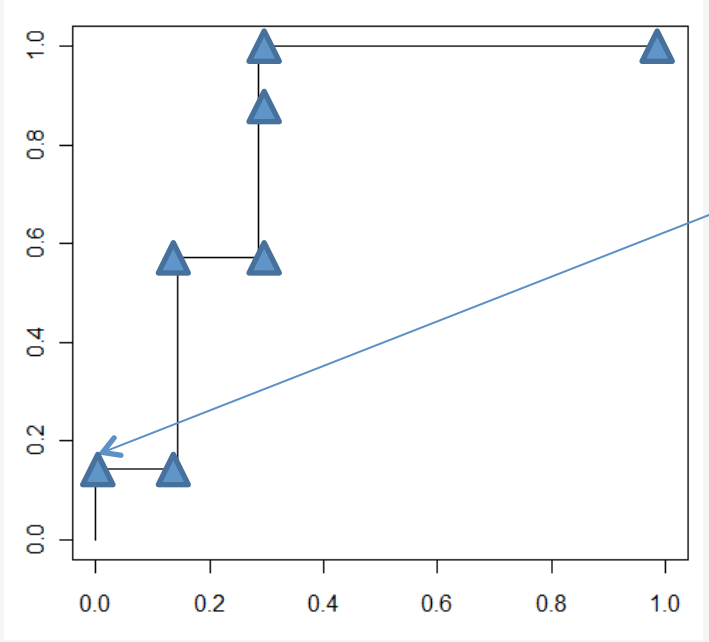


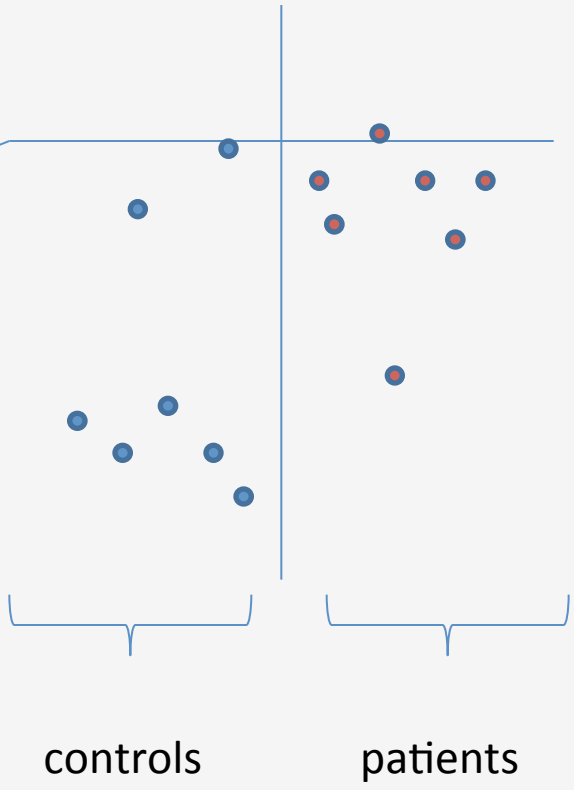
spec; sens

1; 0.14

controls

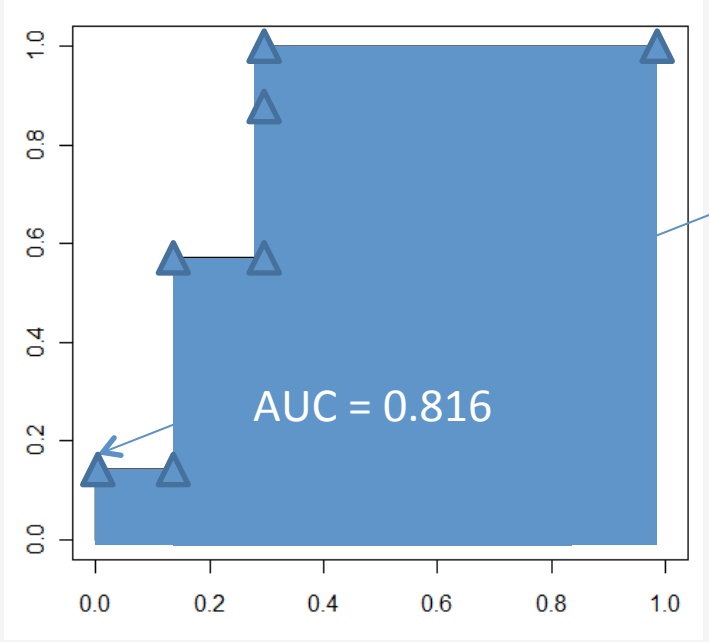
patients

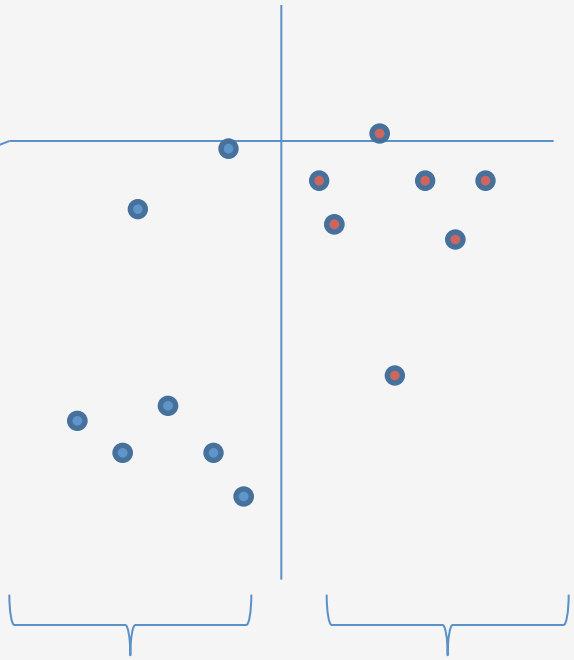




spec; sens

1; 0.14





spec; sens

1; 0.14

controls

patients

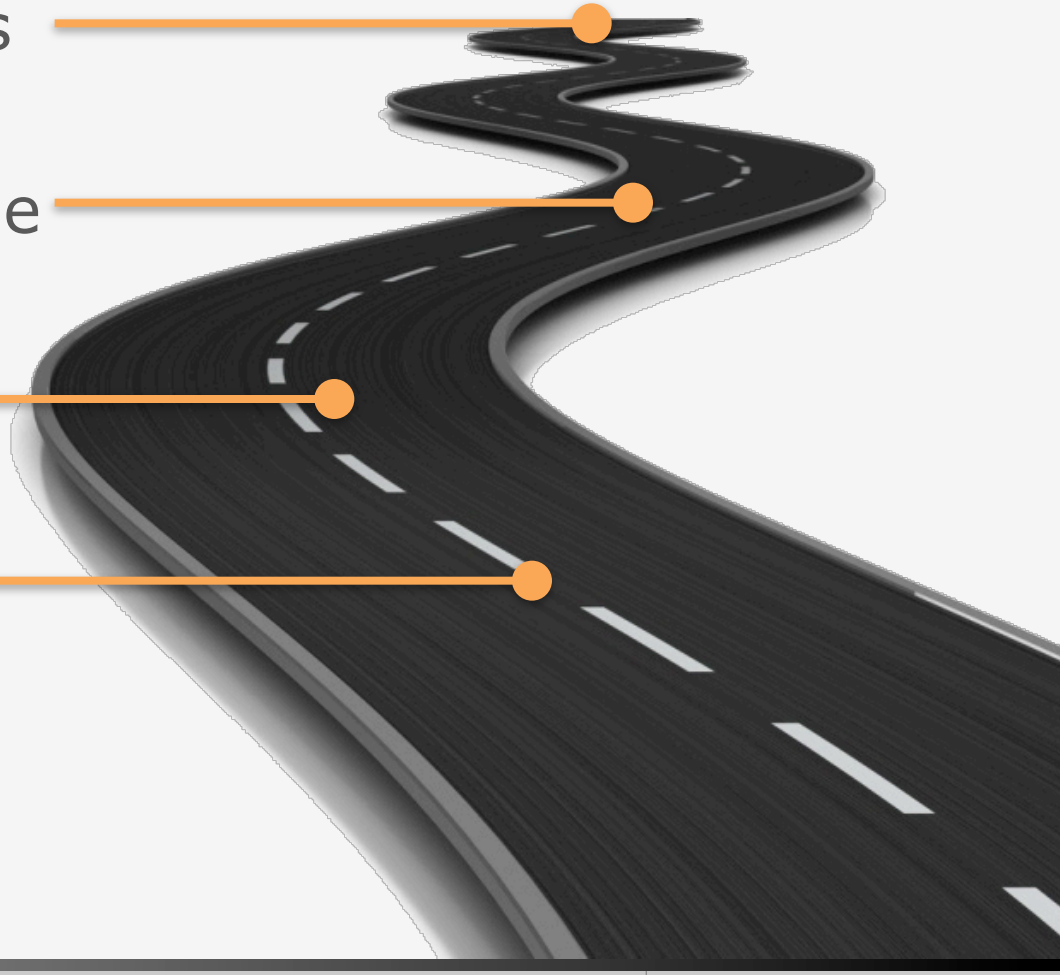
> Agenda

A brief intro to biomarkers

Steps in biomarker pipeline

Regulatory basics

miRNAs as markers



basic research

↓

Clinical Assay Formats

Discovery phases

Patent protection

Development phases

Regulatory & reimbursement issues

Assay production

Marketing

Competition

many hurdle to overcome

- Specifications
- IP issues
- Make immunogens (inject, wait, fuse, screen, repeat)
- Candidate antibodies

- Conjugation and reagent research (prototype assays)
- Select antibodies

- Reagent components
- Assay configuration
- Reagent manufacturing processes
- Begin stability studies
- Repeat until final design achieved

- Design verification testing
- Validation lots
- produced
- Submit 510K/ PMA

- Complete design validation
- Inventory build

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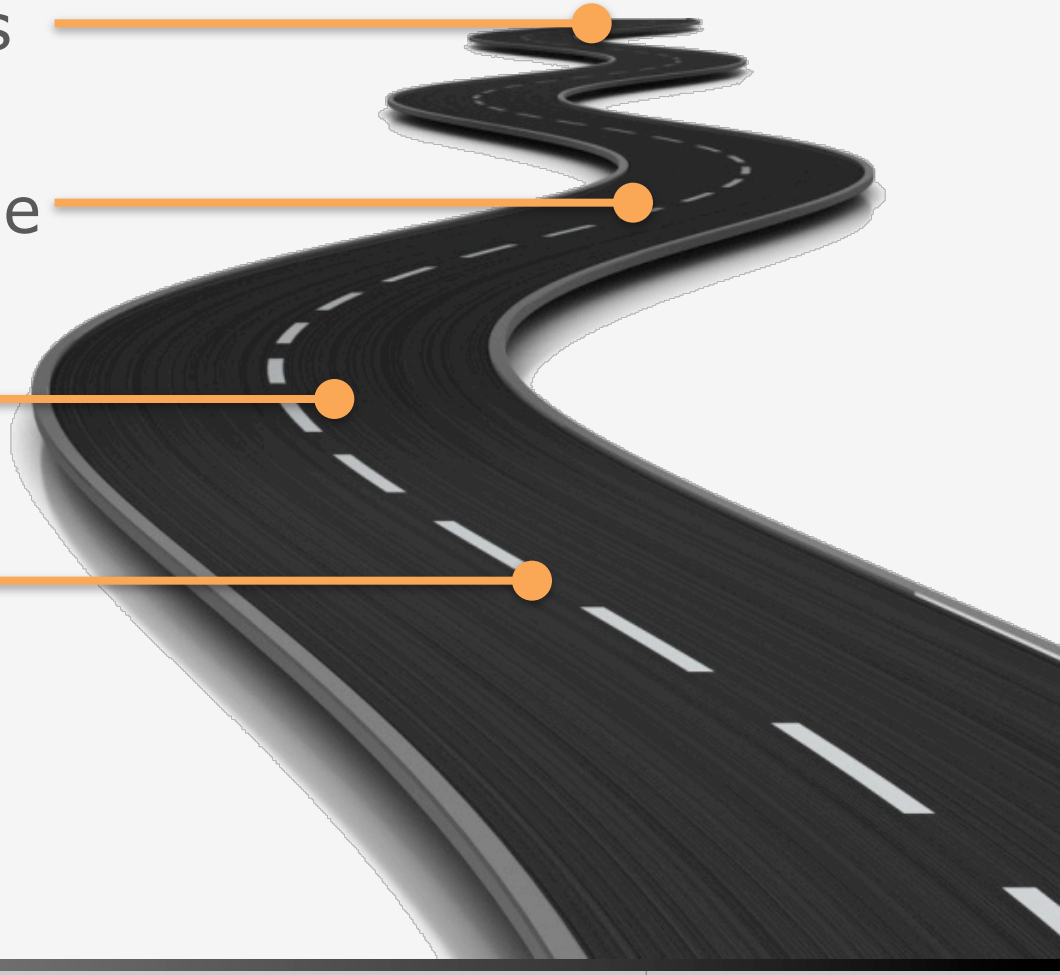

- In the US 3 classes of medical devices exist:
  - Class I: Devices that do not require premarket approval or clearance but must follow general controls.
  - Class II: Devices that are cleared using the 510(k) process, which uses a similar approved device in the market (predicate device) for comparison. The process is referred as Premarket Notification (PMN).
  - Class III: Devices are approved by the Premarket Approval (PMA) process, similar to new drug approvals. These include devices to sustain human life and are of substantial importance in preventing impairment of human health. (Section 515)

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/default.htm

> FDA: determining the class

Medical Specialty		Regulation Citation (21CFR)
73	Anesthesiology	Part 868
74	Cardiovascular	Part 870
75	Chemistry	Part 862
76	Dental	Part 872
77	Ear, Nose, and Throat	Part 874
78	Gastroenterology and Urology	Part 876
79	General and Plastic Surgery	Part 878
80	General Hospital	Part 880
81	Hematology	Part 864
82	Immunology	Part 866
83	Microbiology	Part 866
84	Neurology	Part 882
85	Obstetrical and Gynecological	Part 884
86	Ophthalmic	Part 886
87	Orthopedic	Part 888
88	Pathology	Part 864
89	Physical Medicine	Part 890
90	Radiology	Part 892
91	Toxicology	Part 862

T 866 -- IMMUNOLOGY AND MICROBIOLOGY DEVICES

part C--Microbiology Devices

c. 866.2300 Multipurpose culture medium.

)*Identification.* A multipurpose culture medium is a device that consists primarily of liquid or solid ological materials intended for medical purposes for the cultivation and identification of several pes of pathogenic microorganisms without the need of additional nutritional supplements. Test sults aid in the diagnosis of disease and also provide epidemiological information on diseases used by these microorganisms.

)*Classification.* Class I (general controls). The device is exempt from the premarket notification ocedures in subpart E of part 807 of this chapter subject to the limitations in 866.9.

Subpart C--Microbiology Devices

§ 866.2050 - Staphylococcal typing bacteriophage.
§ 866.2120 - Anaerobic chamber.
§ 866.2160 - Coagulase plasma.
§ 866.2170 - Automated colony counter.
§ 866.2180 - Manual colony counter.
§ 866.2300 - Multipurpose culture medium.
§ 866.2320 - Differential culture medium.
§ 866.2330 - Enriched culture medium.
§ 866.2350 - Microbiological assay culture medium.
§ 866.2360 - Selective culture medium.

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/ClassifyYourDevice/ucm051530.htm

> FDA: determining the class

Medical Specialty		Regulation Citation (21CFR)
73	Anesthesiology	Part 868
74	Cardiovascular	Part 870
75	Chemistry	Part 862
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82	Immunology	Part 866
83	Microbiology	Part 866
84	Neurology	Part 882
85	Obstetrical and Gynecological	Part 884
86	Ophthalmic	Part 886
87	Orthopedic	Part 8
88	Pathology	Part 8
89	Physical Medicine	Part 8
90	Radiology	Part 8
91	Toxicology	Part 8

PART 866 -- IMMUNOLOGY AND MICROBIOLOGY DEVICES

Subpart G--Tumor Associated Antigen immunological Test Systems

Sec. 866.6040 Gene expression profiling test system for breast cancer prognosis.

(a)*Identification* . A gene expression profiling test system for breast cancer prognosis is a device that measures the ribonucleic acid (RNA) expression level of multiple genes and combines this information to yield a signature (pattern or classifier or index) to aid in prognosis of previously diagnosed breast cancer.

(b)*Classification* . Class II (special controls). The special control is FDA's guidance document entitled "Class II Special Controls Guidance Document: Gene Expression Profiling Test System for Breast Cancer Prognosis." See 866.1(e) for the availability of this guidance document.

[72 FR 26291, May 9, 2007]

§ 866.5785 - Anti-Saccharomyces cerevisiae(S. cerevisiae) antibody (ASCA) test systems.
§ 866.5800 - Seminal fluid (sperm) immunological test system.
§ 866.5820 - Systemic lupus erythematosus immunological test system.
§ 866.5860 - Total spinal fluid immunological test system.
§ 866.5870 - Thyroid autoantibody immunological test system.
§ 866.5880 - Transferrin immunological test system.
§ 866.5890 - Inter-alphatrypsin inhibitor immunological test system.
§ 866.5900 - Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system.
§ 866.5910 - Quality control material for cystic fibrosis nucleic acid assays.

Subpart G--Tumor Associated Antigen immunological Test Systems

§ 866.6010 - Tumor-associated antigen immunological test system.
§ 866.6020 - Immunomagnetic circulating cancer cell selection and enumeration system.
§ 866.6030 - AFP-L3% immunological test system.
§ 866.6040 - Gene expression profiling test system for breast cancer prognosis.
§ 866.6050 - Ovarian adnexal mass assessment score test system.

> FDA: determining the class

Medical Specialty		Regulation Citation (21CFR)
73	Anesthesiology	Part 868
74	Cardiovascular	Part 870
75	Chemistry	Part 862
76	Dental	Part 872
77	Ear, Nose, and Throat	Part 874
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86	Ophthalmic	Part 886
87	Orthopedic	Part 888
88	Pathology	Part 864
89	Physical Medicine	Part 890
90	Radiology	Part 892
91	Toxicology	Part 862

PART 870 -- CARDIOVASCULAR DEVICES

Subpart D--Cardiovascular Prosthetic Devices

Sec. 870.3925 Replacement heart valve.

(a)*Identification.* A replacement heart valve is a device intended to perform the function of any of the heart's natural valves. This device includes valves constructed of prosthetic materials, biologic valves (e.g., porcine valves), or valves constructed of a combination of prosthetic and biologic materials.

(b)*Classification.* Class III (premarket approval).

§ 870.3690 - Pacemaker test magnet.
§ 870.3700 - Pacemaker programmers.
§ 870.3710 - Pacemaker repair or replacement material.
§ 870.3720 - Pacemaker electrode function tester.
§ 870.3730 - Pacemaker service tools.
§ 870.3800 - Annuloplasty ring.
§ 870.3850 - Carotid sinus nerve stimulator.
§ 870.3925 - Replacement heart valve.
§ 870.3935 - Prosthetic heart valve holder.
§ 870.3945 - Prosthetic heart valve sizer.

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/ClassifyYourDevice/ucm051530.htm

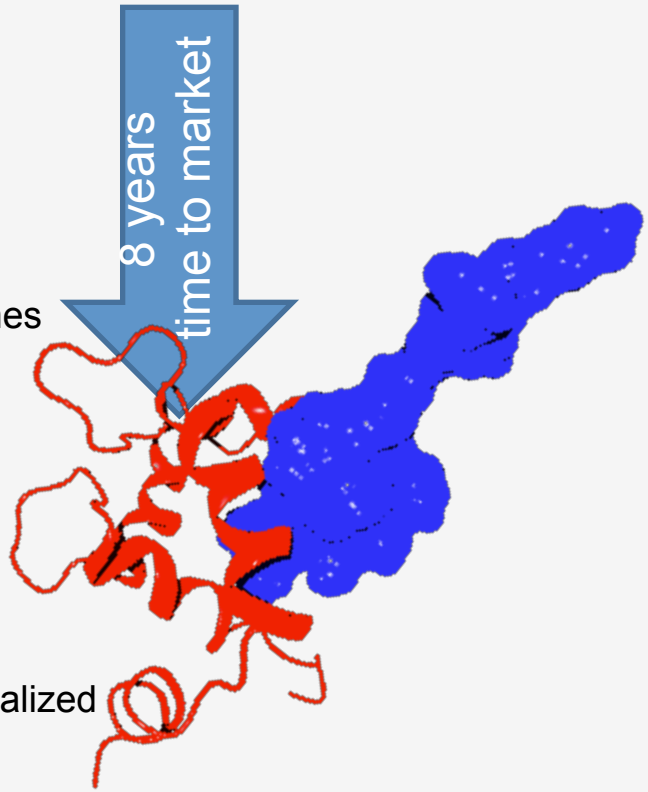
- IVD directive 98/79/EG (IVDD - *In vitro diagnostic directive*)
- Directive 90/385/EWG (e.g. implants)
- Medical Device Directive (MDD) 93/42/EWG
- All in-vitro diagnostics have to be CE-labeled

Troponin-I

- 1987 first polyclonal antibodies reported
- 1992 first monoclonal antibodies reported
- 1995 first automated commercial assay
- 1997/8 acceptance comparable to CKMB
- 2001 new gold standard test for AMI per ACC/ESC Guidelines

BNP / NT-proBNP

- 1995 Research activity reaches a high level
- 1997 Monoclonal antibodies available
- 2001 First commercial BNP assay available
- 2003 First commerical NT-proBNP (Bayer) assay commercialized
- 2005/6 ESC guideline; NACB/AHA guideline
- TBD Acceptance as a gold standard test for CHF



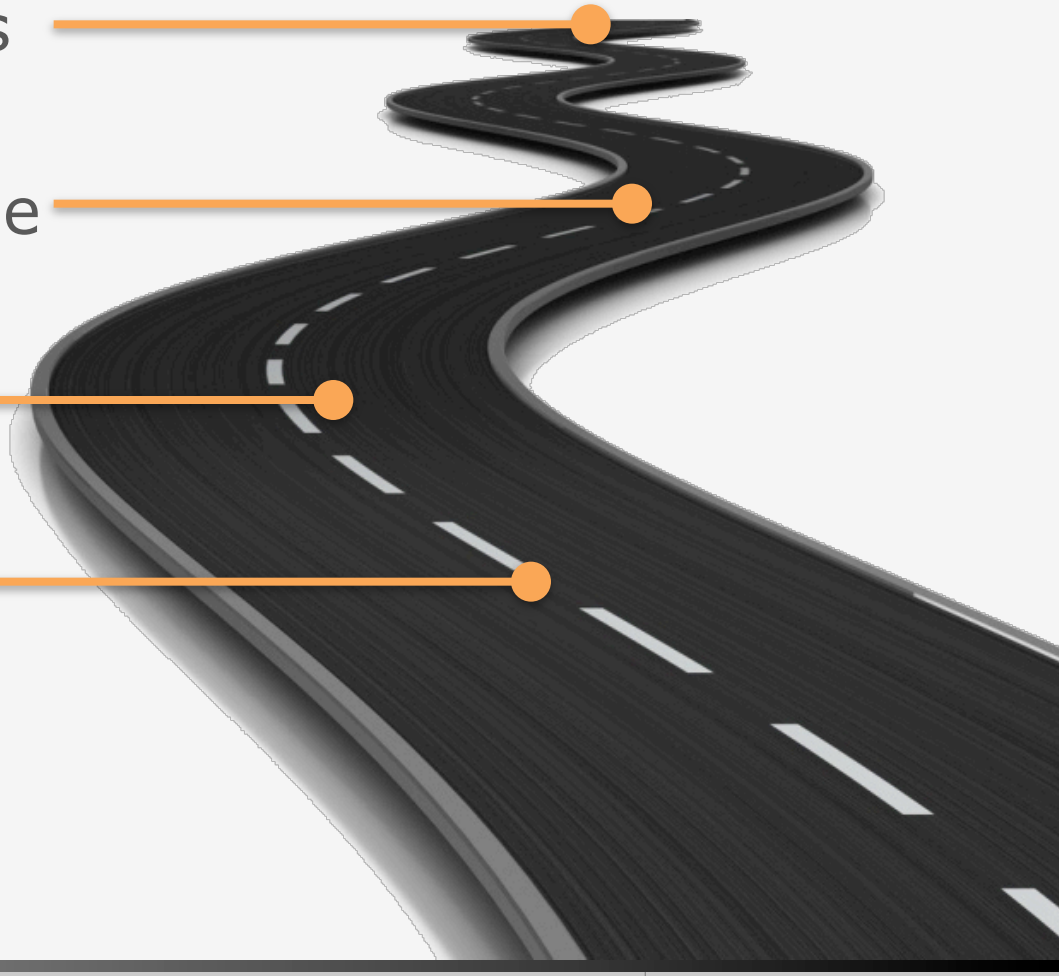
> Agenda

A brief intro to biomarkers

Steps in biomakrer pipeline

Regulatory basics

miRNAs as markers



Three paradigms:

Develop a minimal-invasive test (screening blood cells, serum, urine)

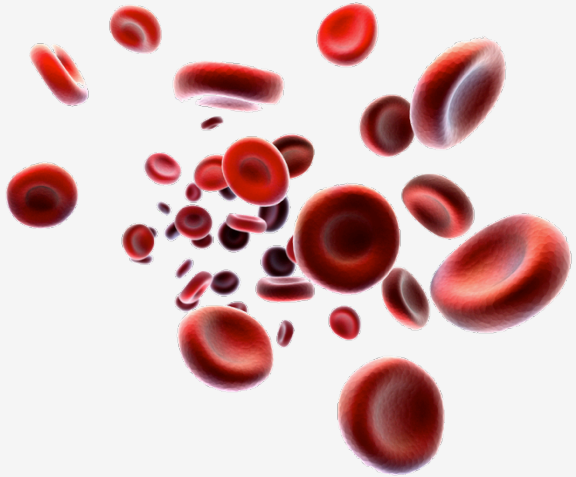
Combine the diagnostic power of many markers

Investigate different diseases to understand the specificity of the profiles

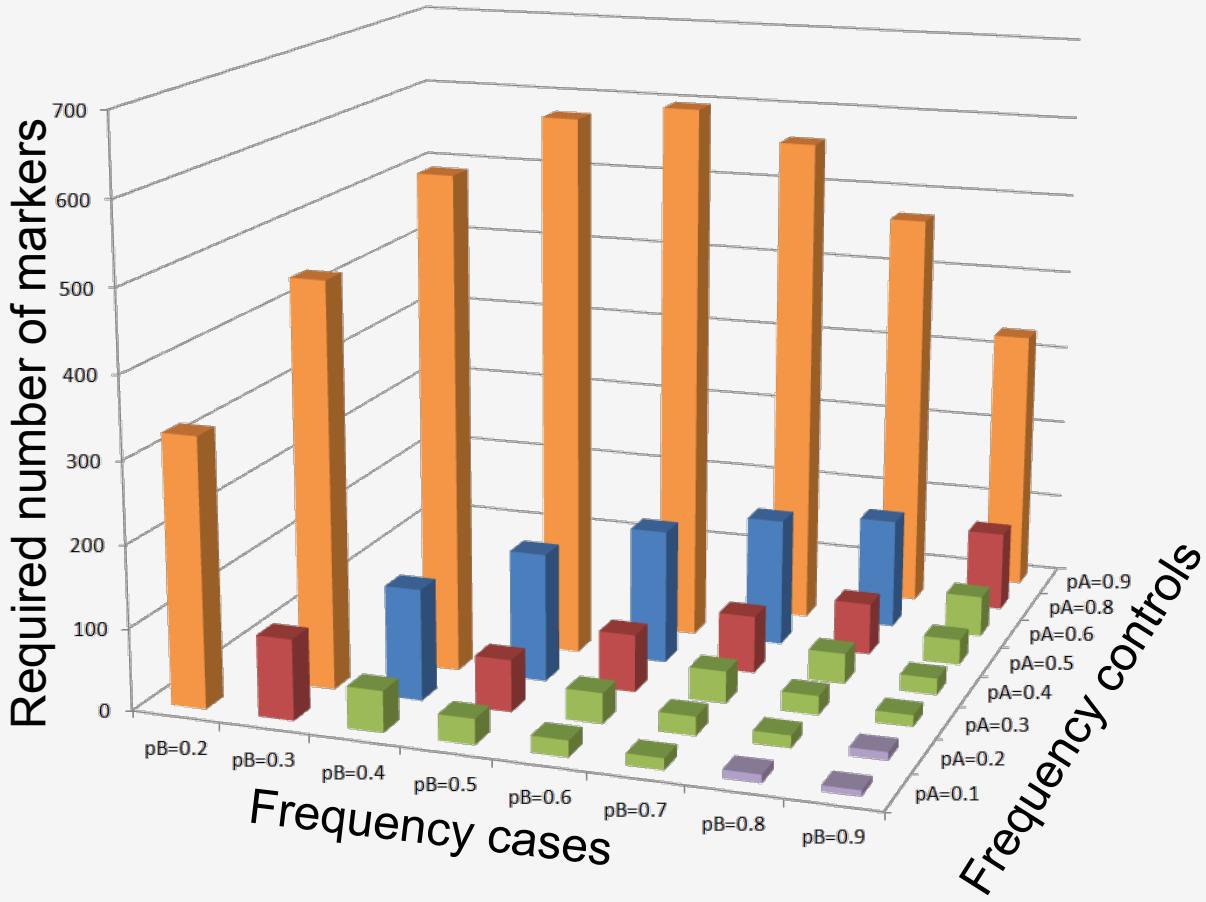
nature methods
www.nature.com/naturemethods
Techniques for life scientists and chemists

Toward the blood-borne miRNome of human diseases

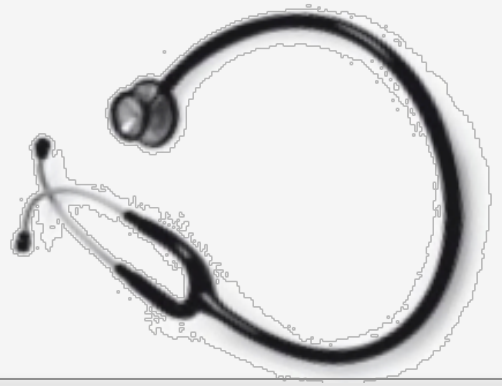
Andreas Keller[1,2,21], Petra Leidinger[2,21], Andrea Bauer[3], Abdou ElSharawy[4], Jan Haas[5], Christina Backes[2], Anke Wendschlag[6], Nathalia Giese[7], Christine Tjaden[7], Katja Ott[7], Jens Werner[7], Thilo Hackert[7], Klemens Ruprecht[8], Hanno Huwer[9], Junko Huebers[10], Gunnar Jacobs[4], Philip Rosenstiel[4], Henrik Dommisch[11], Arne Schaefer[4], Joachim Müller-Quernheim[12], Bernd Wullich[13], Bastian Keck[13], Norbert Graf[14], Joerg Reichrath[15], Britta Vogel[5], Almut Nebel[4], Sven U Jager[16], Peer Staehler[6], Ioannis Amarantos[6], Valesca Boisguerin[6], Cord Staehler[6], Markus Beier[6], Matthias Scheffler[6], Markus W Büchler[7], Joerg Wischhusen[17,18], Sebastian F M Haeusler[17], Johannes Dietl[17], Sylvia Hofmann[4], Hans- Peter Lenhof[19], Stefan Schreiber[4,20], Hugo A Katus[5], Wolfgang Rottbauer[5], Benjamin Meder[5], Joerg D Hoheisel[3], Andre Franke[4,21] & Eckart Meese[2,21]



The advantage of marker sets

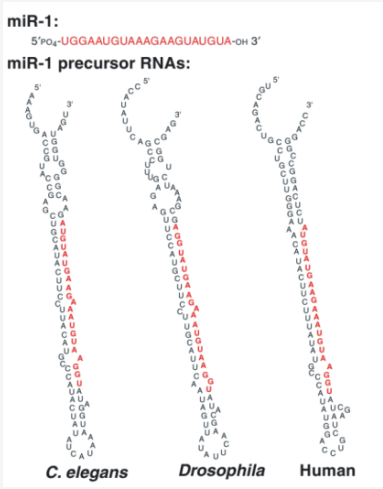


Required number of independent biomarkers to achieve specificity and sensitivity of 99%.



Structure

- Small non-coding RNAs (17-23 nucleotides length)
- Very stable and conserved across organisms
- Per ~120 base precursor molecule transcribed from the genome usually two mature forms are build and exported from nucleus, -3p and -5p

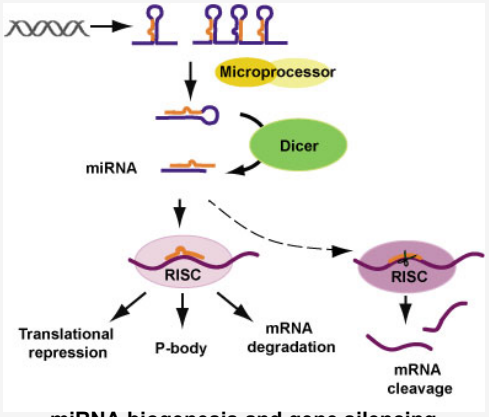


Biology

- > 20,000 miRNAs in >100 species are known
- For *h. sapiens* > 2,2000 are annotated
- These build 0.0002% of the human genome
- … while regulating > 70% of genes and majority of biochemical pathways

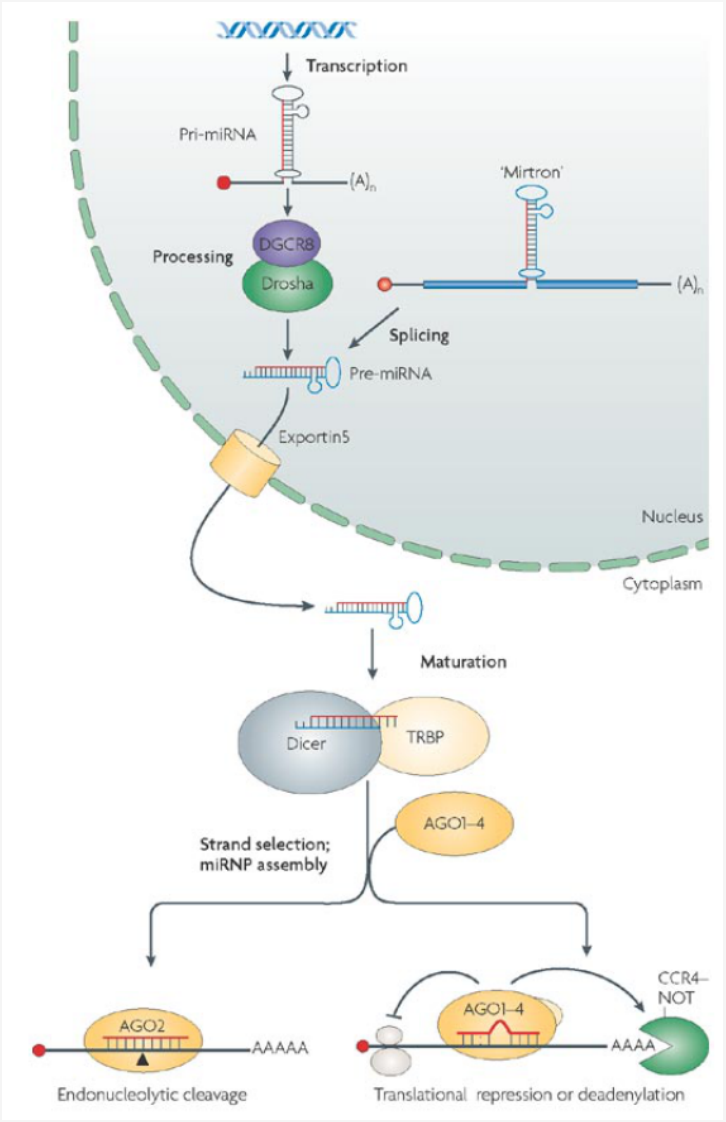
Potential

- Are expressed specifically in tissues and body fluids
- Have demonstrated a wide potential as diagnostic biomarkers



miRNA biogenesis and gene silencing

- Transcription of the pre-miRNAs is done via the RNA-Polymerase II
- The pre-miRNAs can contain multiple miRNA sequences in their hairpin structure
- The typical hairpin-structure is due to two symmetrical strands.
- Additional characteristics are a 5'-cap and a 3'-poly-A tail.
- The pre-miRNAs are exported from the nucleus to the cytoplasm via Exportin5
- DICER splices out the loop-structures of the pre-miRNA.
- Result is the mature miRNA

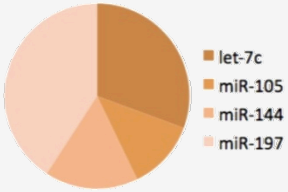


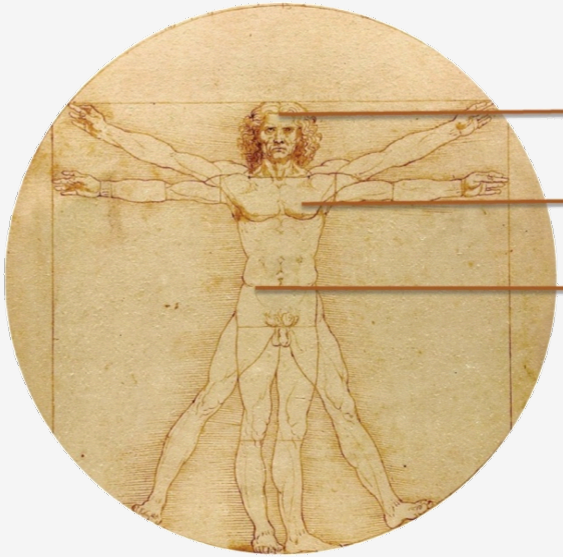
- miRNAs are often very similar to each other (see let-7 family as example)
- it is however, especially in the light of multiplex assays, important to distinguish between these miRNAs
- standard hybridization assays, e.g., may show a high degree of so-called „cross-hybridization“ introducing noise to the multiplex profiles

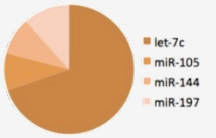
Multiple alignment

```
let-7b UGAGGUAGUAGGUUGUGUGGUU
let-7c UGAGGUAGUAGGUUGUAUGGUU
let-7d AGAGGUAGUAGGUUGCAUAGUU
let-7e UGAGGUAGGAGGUUGUAUAGUU
let-7a UGAGGUAGUAGGUUGUAUAGUU
let-7f UGAGGUAGUAGAUUGUAUAGUU
let-7g UGAGGUAGUAGUUUGUACAGUU
let-7i UGAGGUAGUAGUUUGUGCUGUU
        ******* ** ***    ***
```

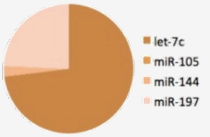
blood
(leukocyte)



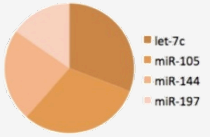




Brain

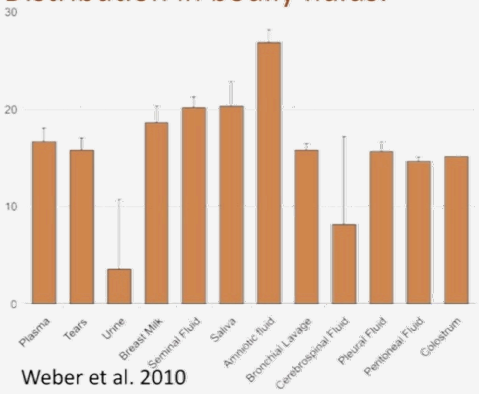


Heart



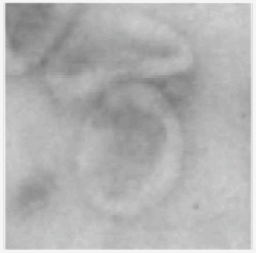
Liver

Distribution in bodily fluids:



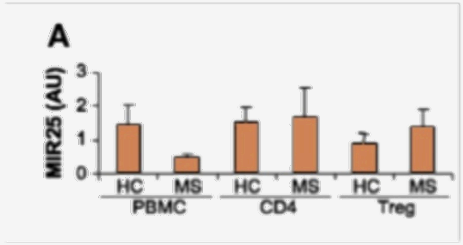
Weber et al. 2010

Communication via
Exosomes



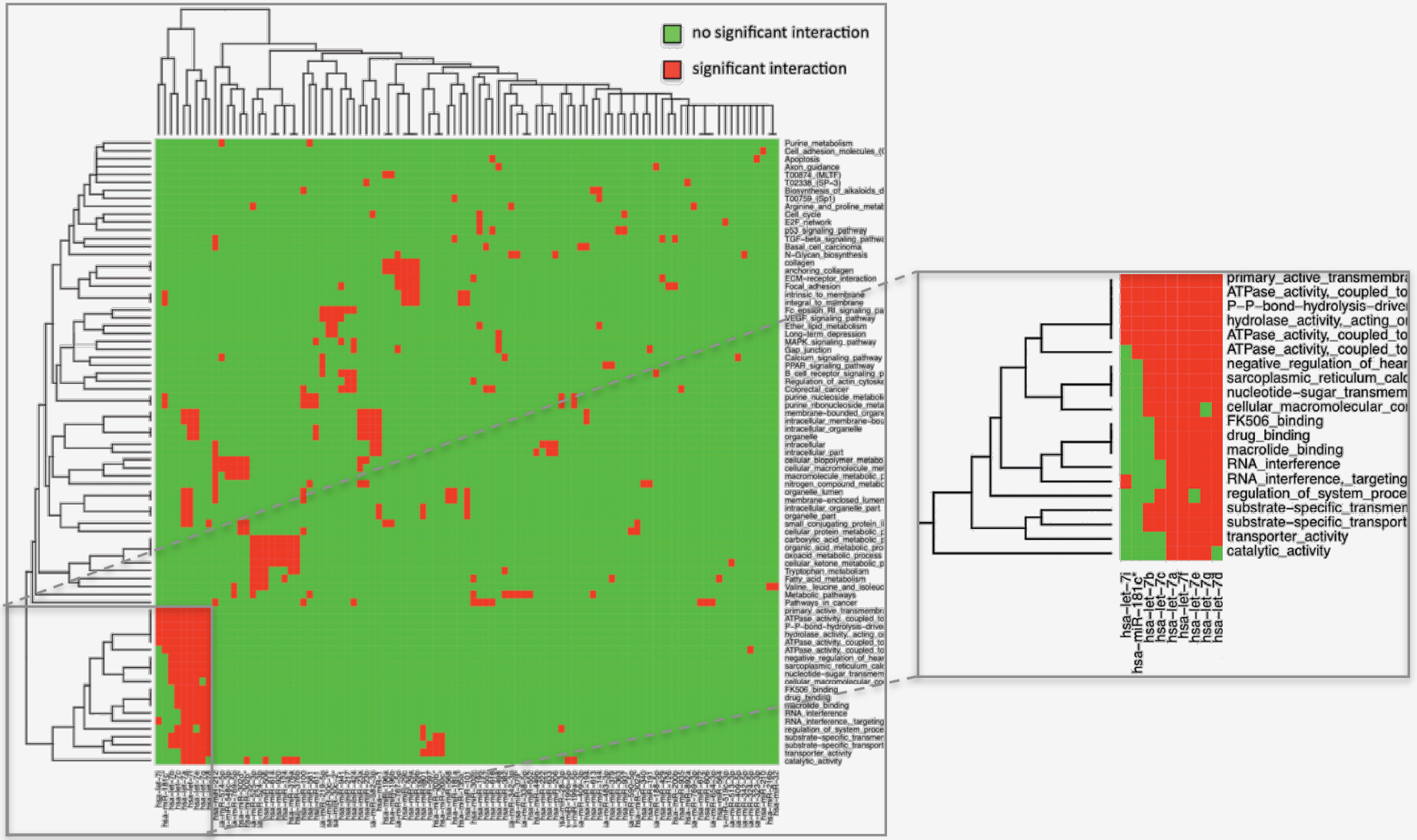
Zomer et al. 2010

PBMC versus T regulatory cells

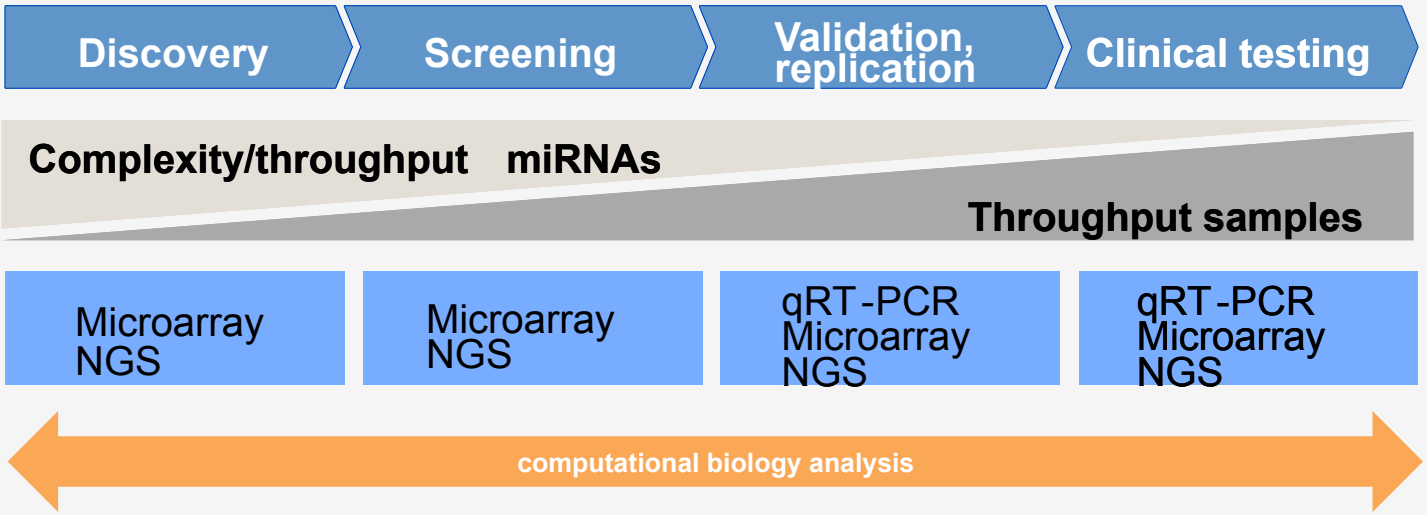


De Santiset al. 2010

> miRNAs regulate pathways



Technologies for measuring miRNAs



Data from blood cells and serum

- 2,000 microarray samples (10MB)	20.00 GB
- 1,000 Illumina HiSeq NGS samples (5GB)	5,000.00 GB
- 4,000 qRT-PCR samples (10KB)	0.05 GB

Cohort

Category	ICD10	# Samples
Normal		203
long-lived individuals (lli)		15
tumor of stomach	C16	13
colon cancer	C18	29
lung cancer	C24	73
pancreatic cancer ducatal	C25	45
Melanoma	C43	35
breast cancer	C50	48
ovarian cancer	C56	24
prostate cancer	C61	65
wilms tumor	C64	124
renal cancer	C65	20
Glioma	C71	20
Sarcoidosis	D86.0	45
multiple sclerosis	G35	23
acute myocardial infarction	I21.3	62
dilatative cardiomyopathie	I42	33
COPD	J40-47	47
Peridontitis	K05.4	18
crohn's disease	K50	62
Colitis	K51	46
Pancreatitis	K85	37
Psoriasis	L40	43
benign prostate hyperplasia	N40	35
pre-eclampsia	O14	16
Others		133

Key characteristics

1,314 samples screened using microarray technology

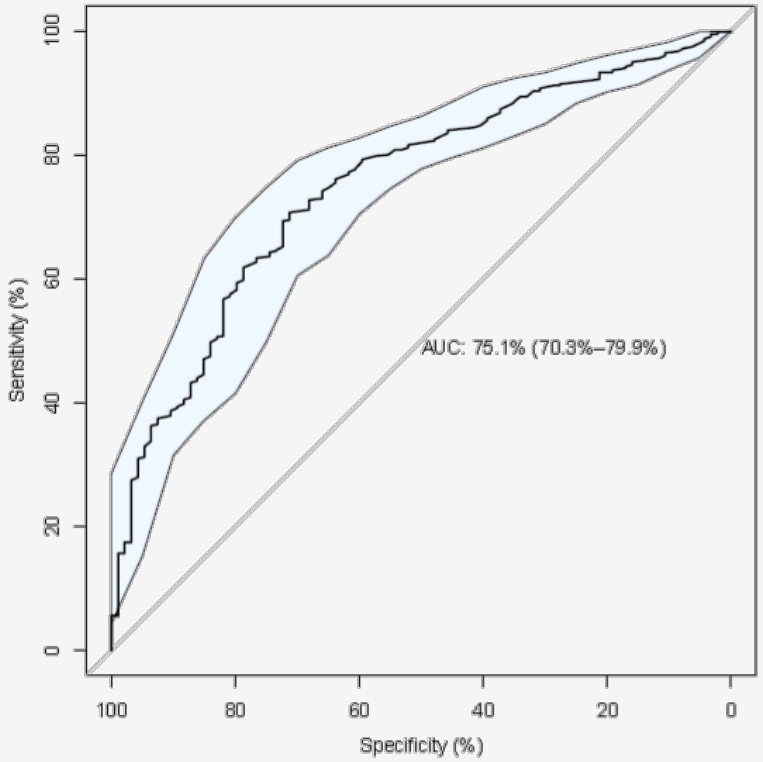
863 miRNAs (miRBase 16) screened

On average 10 replicates per miRNA measured

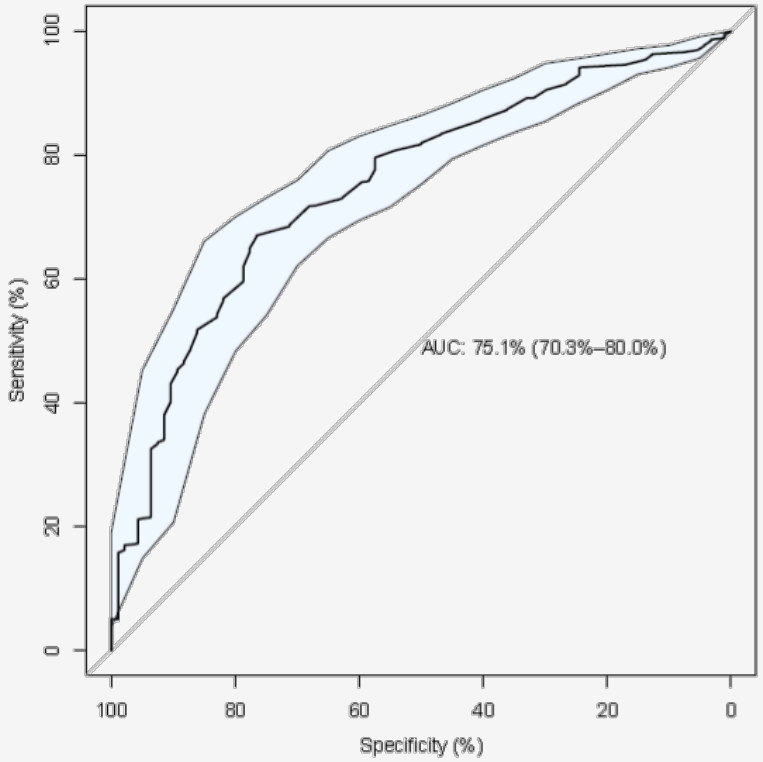
Corresponds to ≈12 million single measurements

Unsupervised and supervised analysis carried out.

Classification is carried out as 10-fold cross validation radial basis function SVM with stepwise forward subset selection together with non-parametric permutation tests

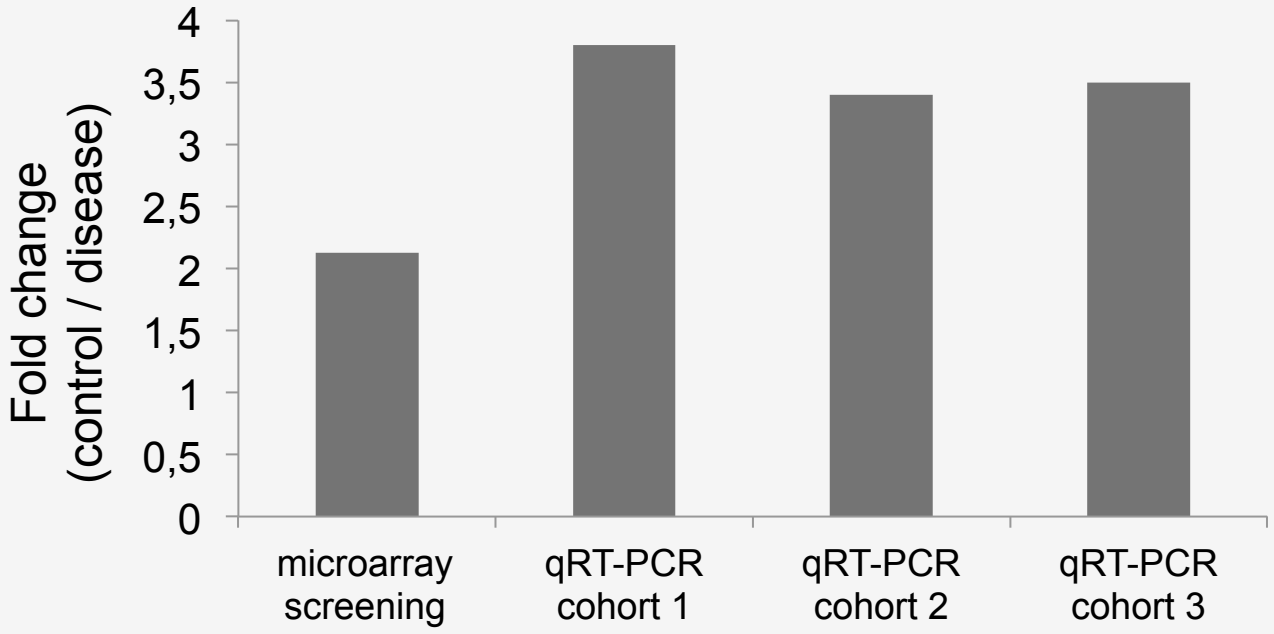


miR-144*, diseases vs. control

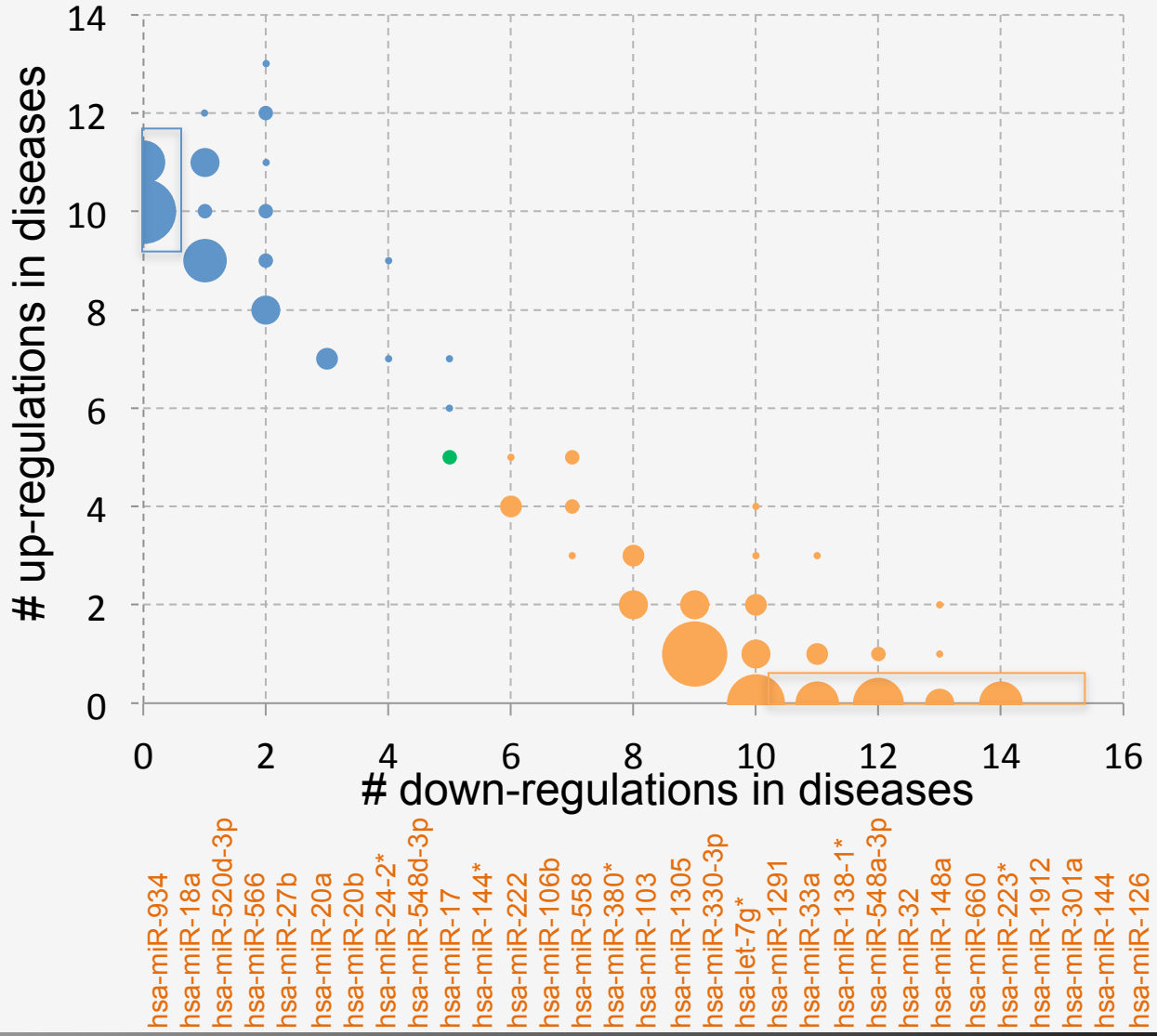


miR-144*, cancer vs. control

Constant down-regulation in all cohorts



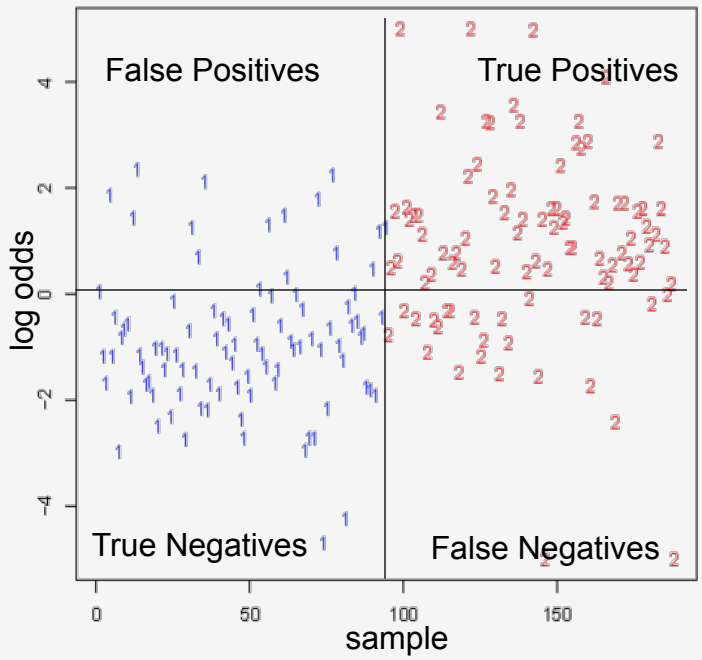
hsa-miR-1246
hsa-miR-223
hsa-let-7e*
hsa-miR-1303
hsa-miR-25*
hsa-miR-491-5p
hsa-miR-885-5p
hsa-miR-539
hsa-miR-194*
hsa-miR-658
hsa-miR-145
hsa-miR-130b*
hsa-miR-499-5p
hsa-miR-484
hsa-miR-126*



diseases vs. controls

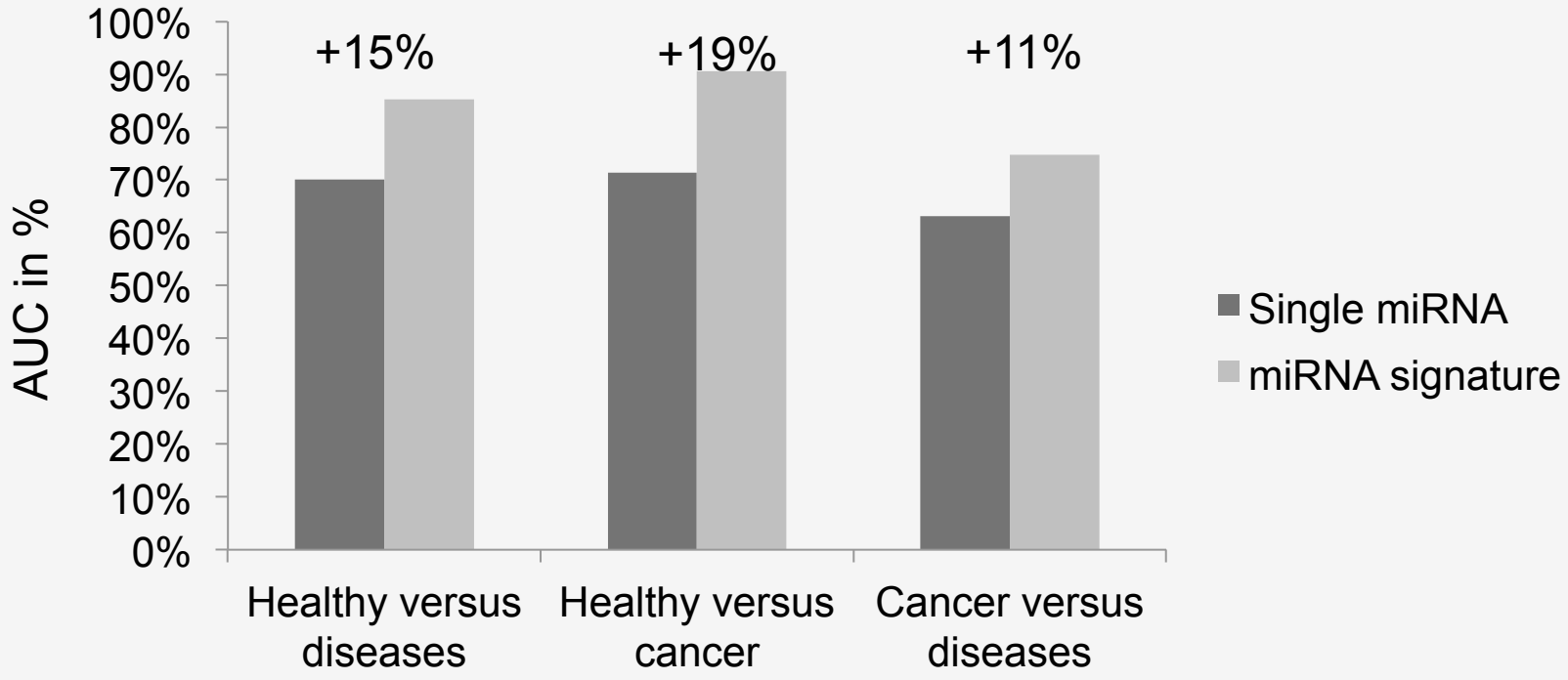
Accuracy	78%
Specificity	81%
Sensitivity	75%

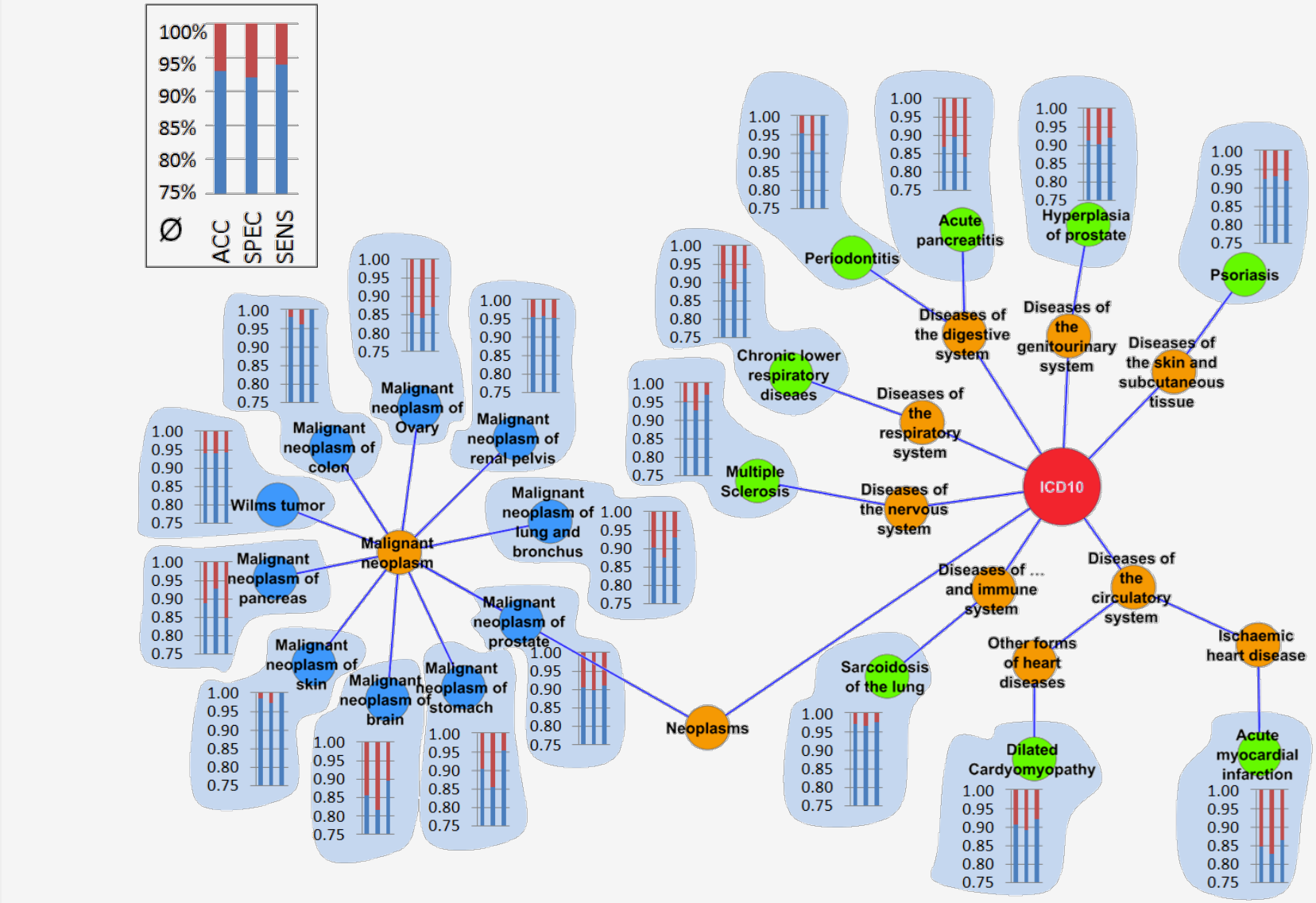
Cancer

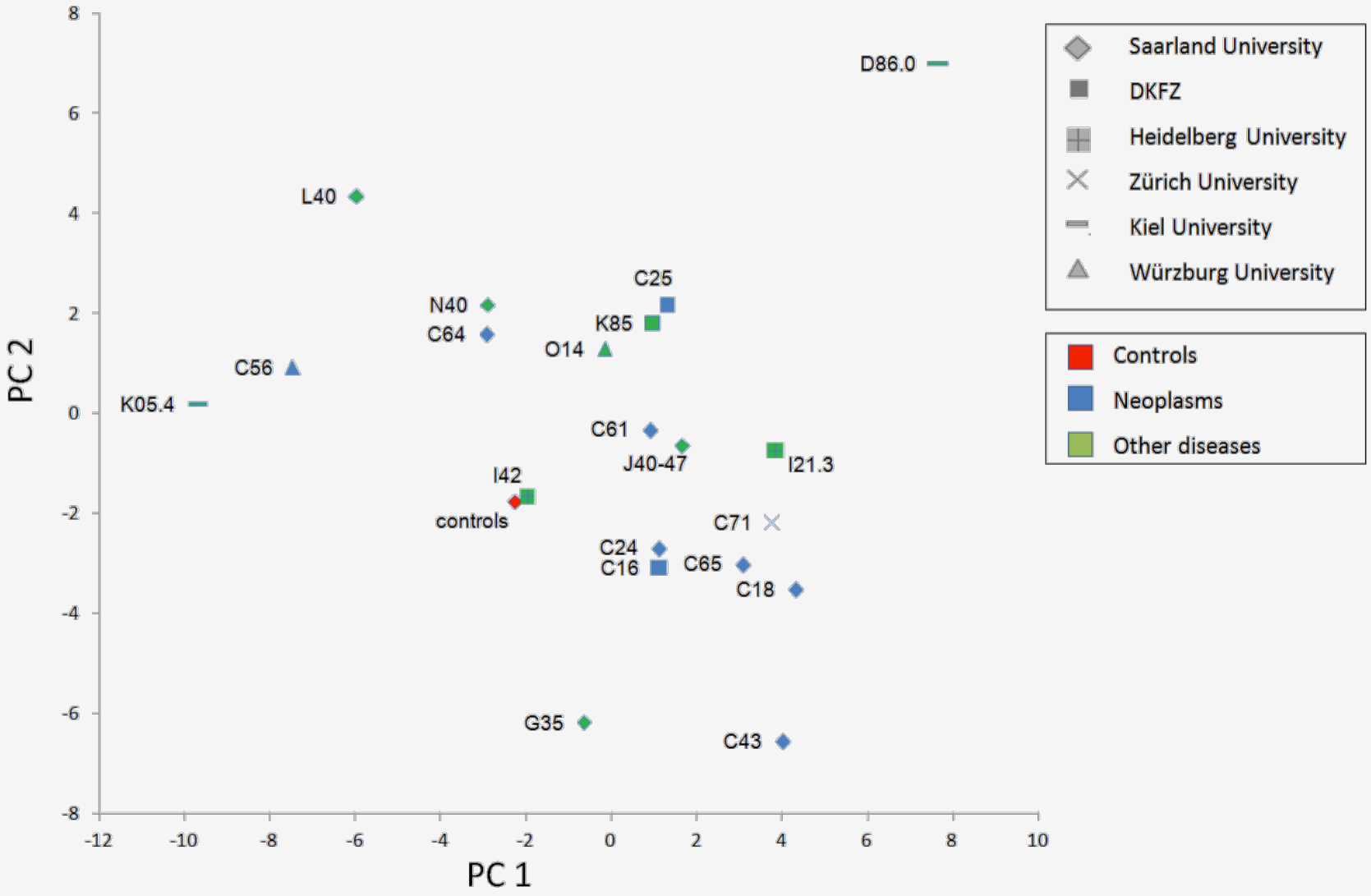


Accuracy	82%
Specificity	81%
Sensitivity	83%

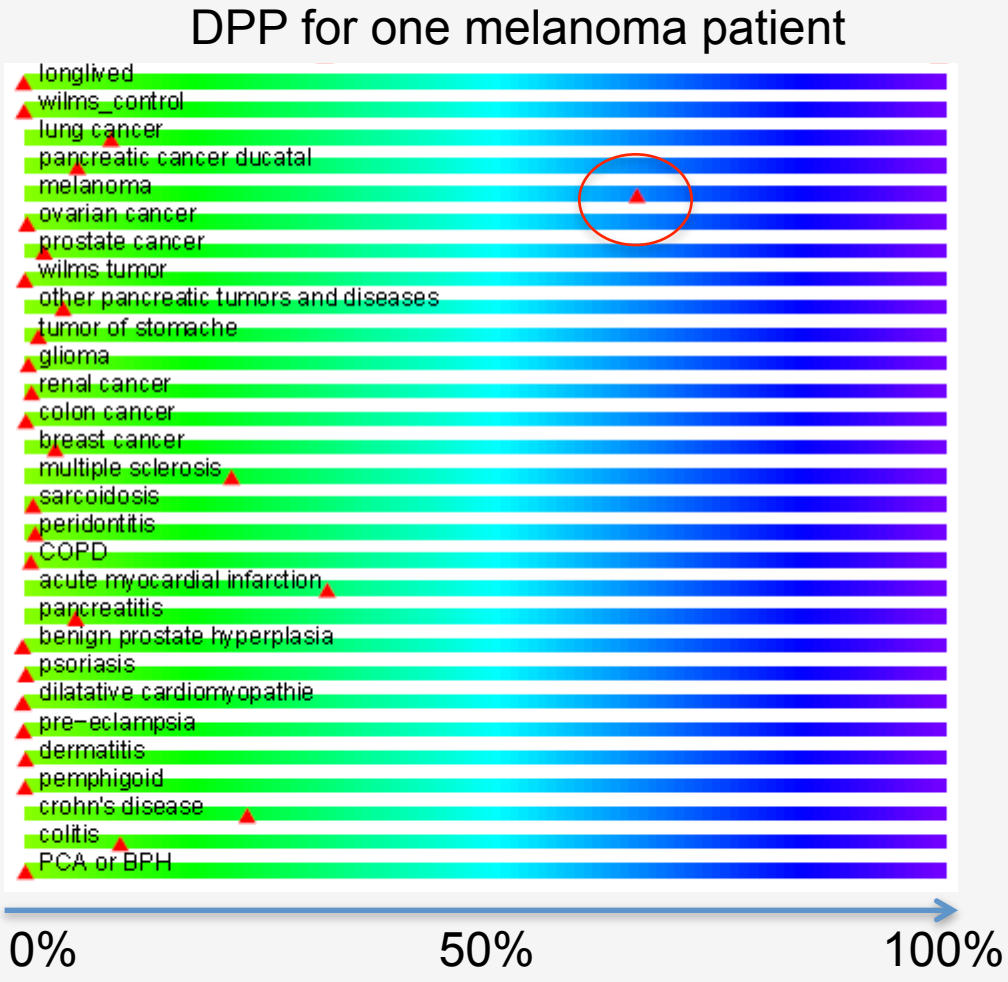
Improved AUC by at least 11%




100%
95%
90%
85%
80%
75%
Ø
ACC
SPEC
SENS
ICD10
Neoplasms
Malignant neoplasm
Malignant neoplasm of Ovary
Malignant neoplasm of colon
Malignant neoplasm of renal pelvis
Wilms tumor
Malignant neoplasm of lung and bronchus
Malignant neoplasm of pancreas
Malignant neoplasm of prostate
Malignant neoplasm of skin
Malignant neoplasm of brain
Malignant neoplasm of stomach
Diseases of the digestive system
Periodontitis
Acute pancreatitis
Diseases of the genitourinary system
Hyperplasia of prostate
Psoriasis
Diseases of the skin and subcutaneous tissue
Diseases of the respiratory system
Chronic lower respiratory diseaes
Diseases of the nervous system
Multiple Sclerosis
Diseases of ... and immune system
Sarcoidosis of the lung
Diseases of the circulatory system
Other forms of heart diseases
Dilated Cardyomyopathy
Ischaemic heart disease
Acute myocardial infarction


PC 2
PC 1
8
6
4
2
0
-2
-4
-6
-8
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
D86.0
L40
C25
N40
K85
C64
O14
C56
K05.4
C61
J40-47
I21.3
I42
controls
C71
C24
C16
C65
C18
G35
C43
Saarland University
DKFZ
Heidelberg University
Zürich University
Kiel University
Würzburg University
Controls
Neoplasms
Other diseases

- For each patient i and each disease j we compute the probability that the patient is positive for this disease as $p_{(i,j)}$ relying on the distance from the SVM hyperplane
- For each patient the disease with highest probability is selected OR
- Alternatively all diseases with probability above a selected threshold
- The hit rate of the "best" disease was 48%

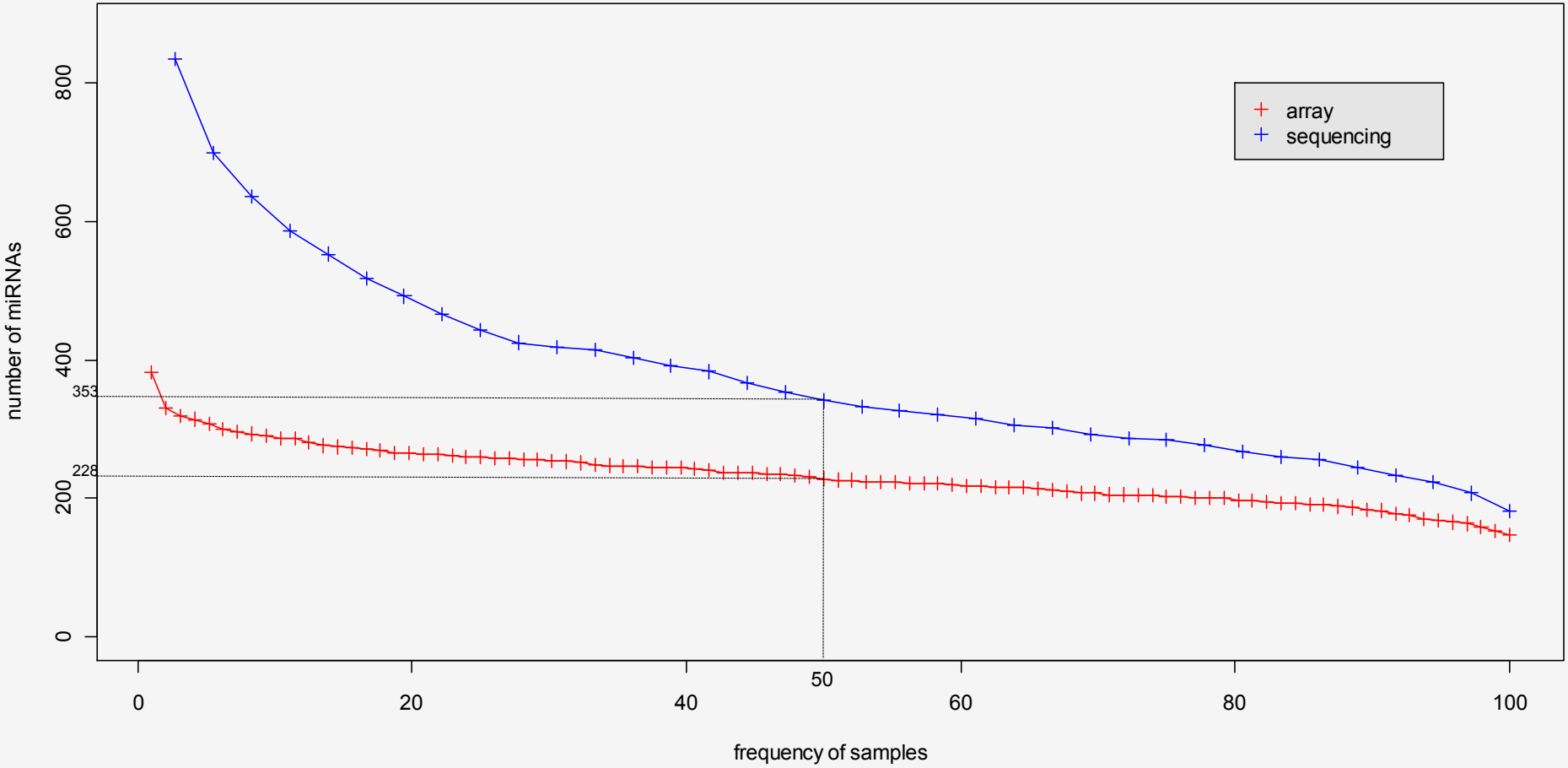


Keller et al, in preparation

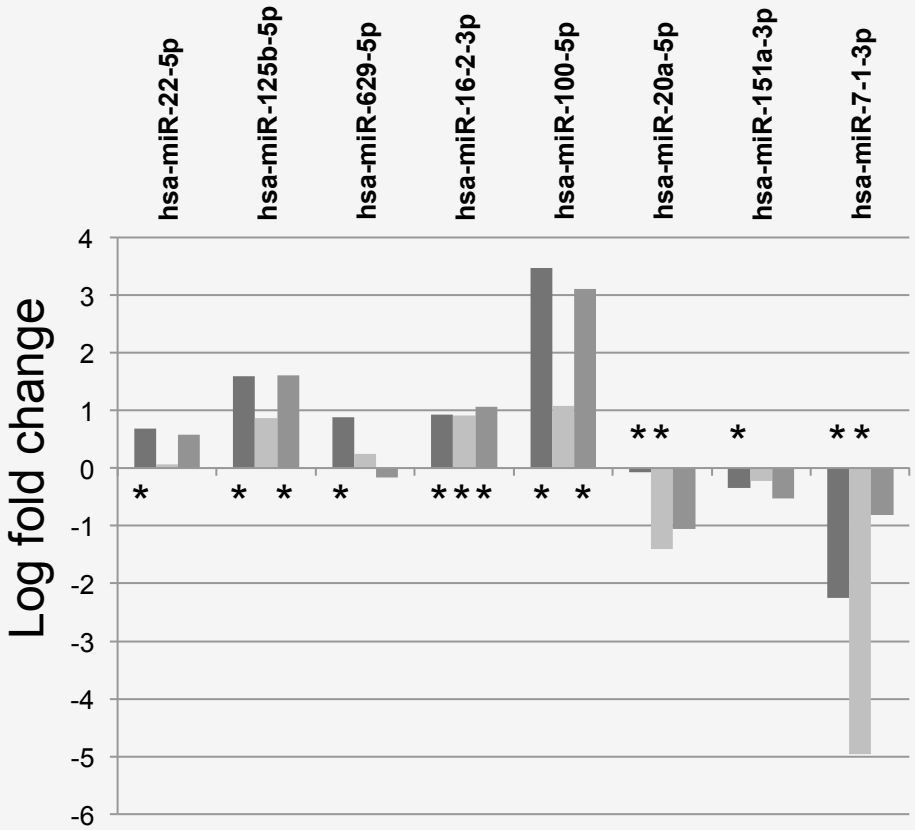
Five advantages of NGS

- NGS offers to screen for all known miRNAs at once
- NGS enables the detection of novel miRNAs
- NGS allows for detecting SNPs in miRNAs
- NGS enables ultra-high throughput screening
- NGS is more sensitive

➔ We screened about 1,000 patients using NGS


array
sequencing
number of miRNAs
0
200
400
600
800
353
228
0
20
40
50
60
80
100
frequency of samples

> 8-miRNA signature
> to differentiate between MS and control



Sample group	Number	Age (mean ± SD)	Sex (female/male)	MMSE (mean ± SD)	cohort size NGS	cohort size RT-qPCR
Alzheimer disease	106	72.7 (10.4)	53/53	18.9 (3.4)	48	94
healthy control	22	67.1 (7.5)	11/11	29.3 (1.2)	22	21
mild cognitive impairment	18	73.9 (6.2)	9/9	25.3 (1.4)	-	18
multiple sclerosis	16	32.3 (10.7)	12/4	not available	-	16
Parkinson disease	9	69.7 (9.0)	1/8	25.2 (4.2)	-	9
major depression	15	45.2 (9.1)	0/15	not available	-	15
bipolar disorder	15	41.9 (13.7)	14/1	29.5 (1.6)	-	15
schizophrenia	14	41.7 (7.9)	1/13	26.1 (4.3)	-	14

Genome Biology IMPACT FACTOR 10.3

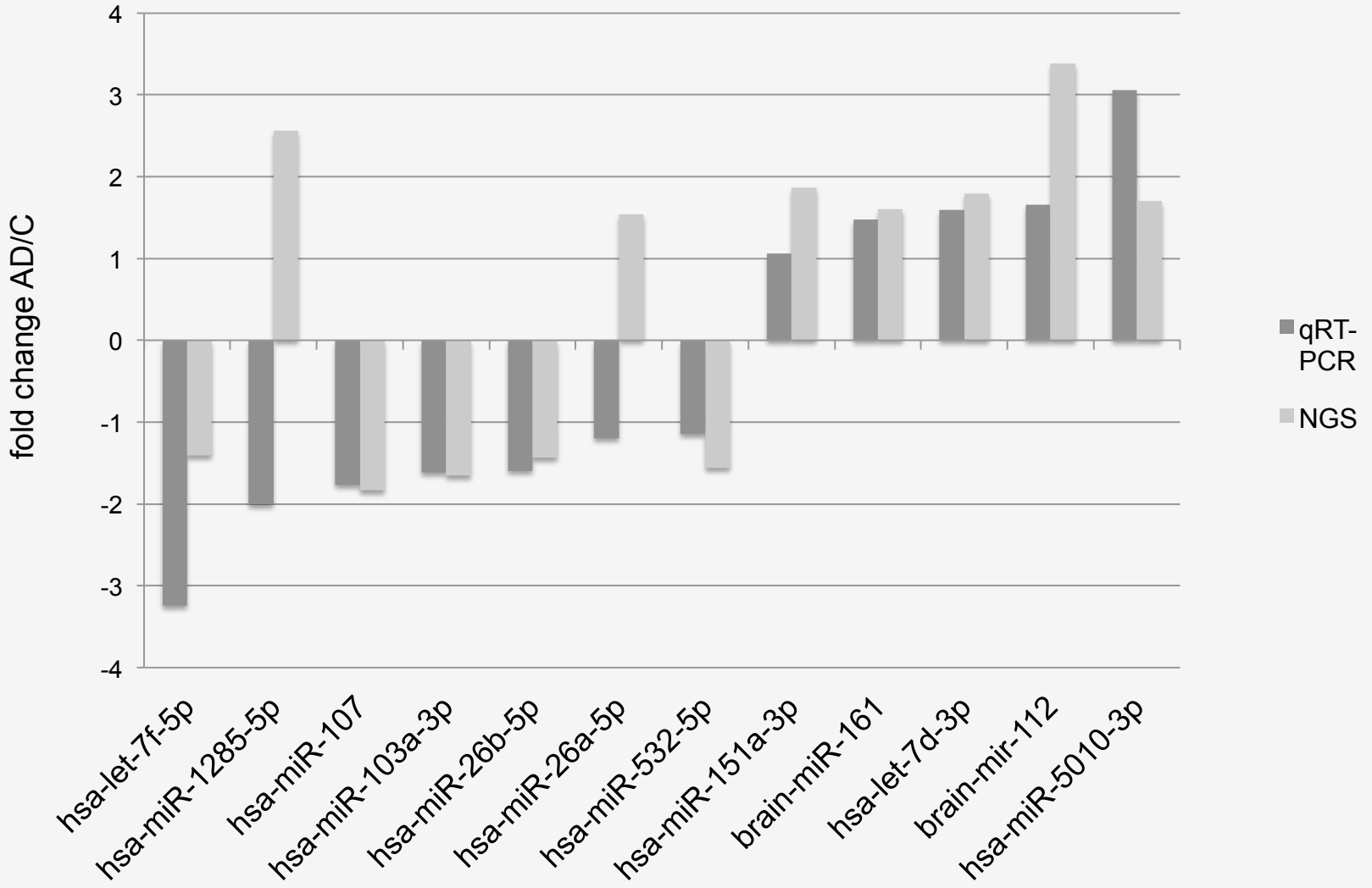
Research

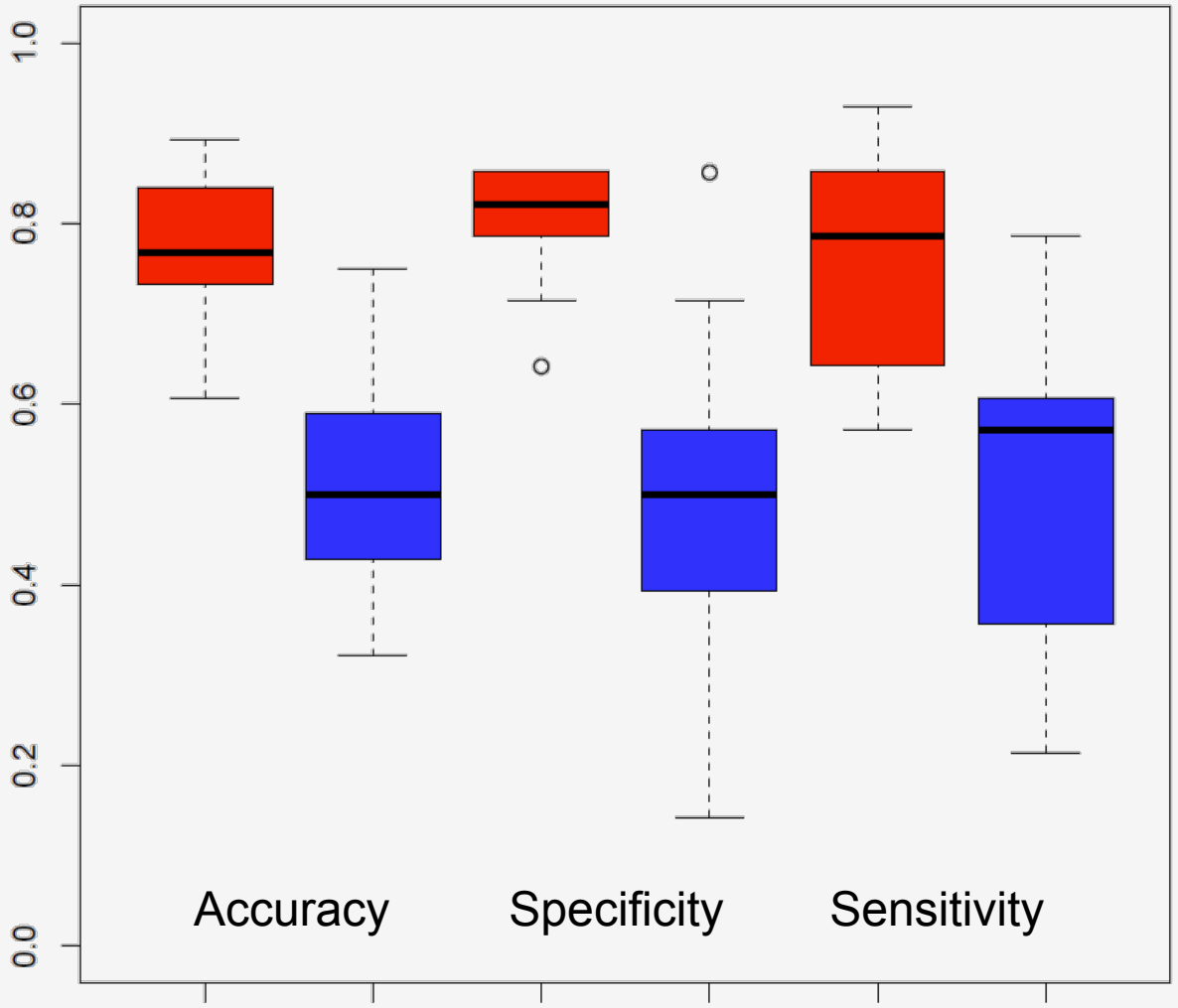
Highly accessed Open Access

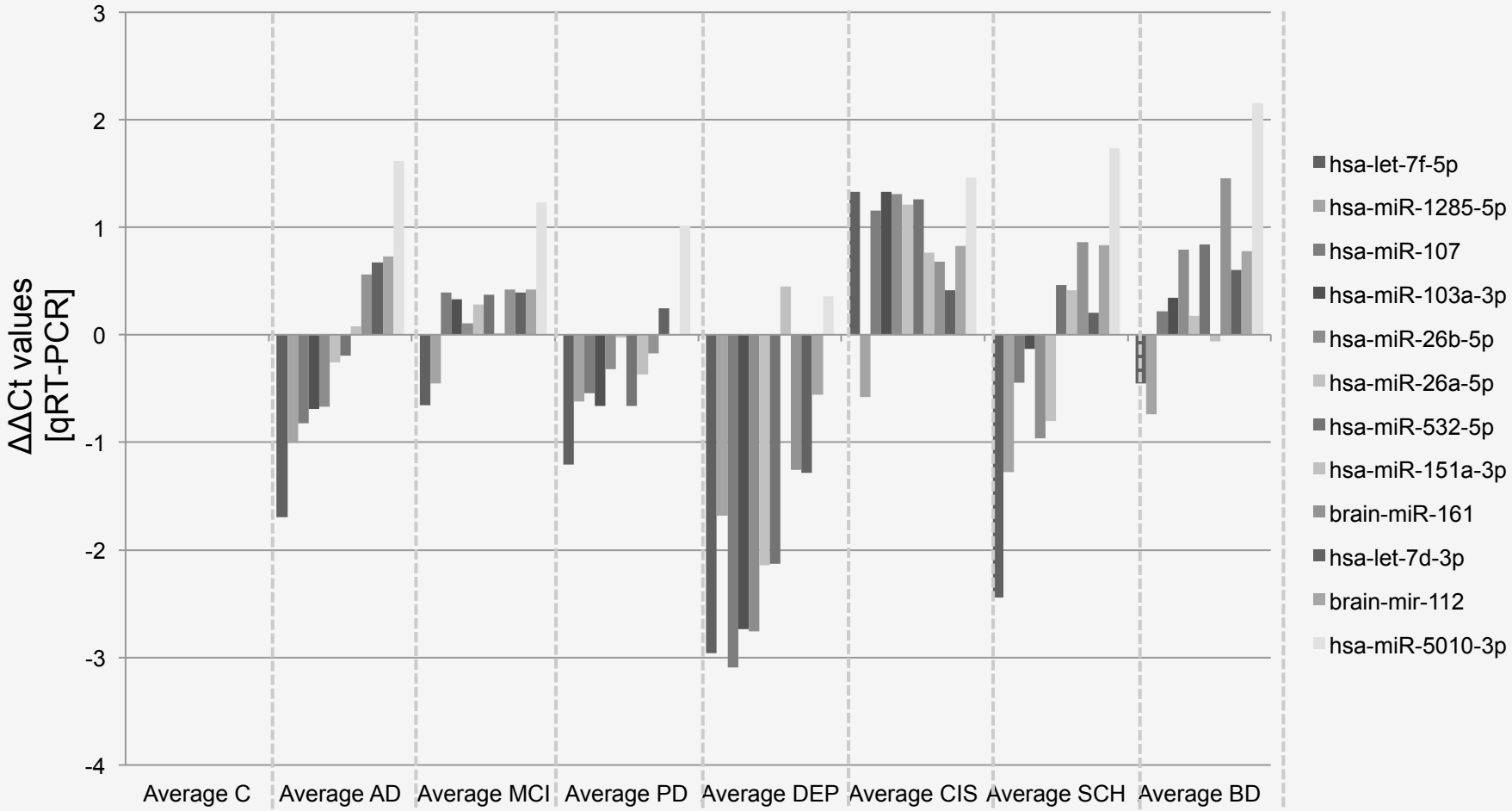
A blood based 12-miRNA signature of Alzheimer disease patients

Petra Leidinger, Christina Backes, Stephanie Deutscher, Katja Schmitt, Sabine C Muller, Karen Frese, Jan Haas, Klemens Ruprecht, Friedemann Paul, Cord Stahler, Christoph JG Lang, Benjamin Meder, Tamas Bartfai, Eckart Meese and Andreas Keller

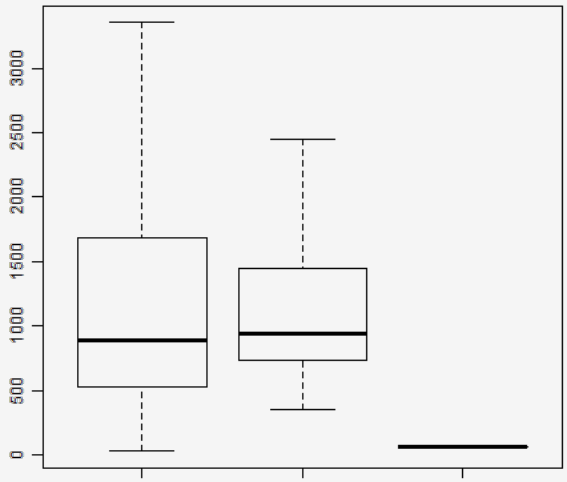
Genome Biology 2013, **14**:R78 doi:10.1186/gb-2013-14-7-r78


fold change AD/C
4
3
2
1
0
-1
-2
-3
-4
hsa-let-7f-5p
hsa-miR-1285-5p
hsa-miR-107
hsa-miR-103a-3p
hsa-miR-26b-5p
hsa-miR-26a-5p
hsa-miR-532-5p
hsa-miR-151a-3p
brain-miR-161
hsa-let-7d-3p
brain-mir-112
hsa-miR-5010-3p
qRT-PCR
NGS

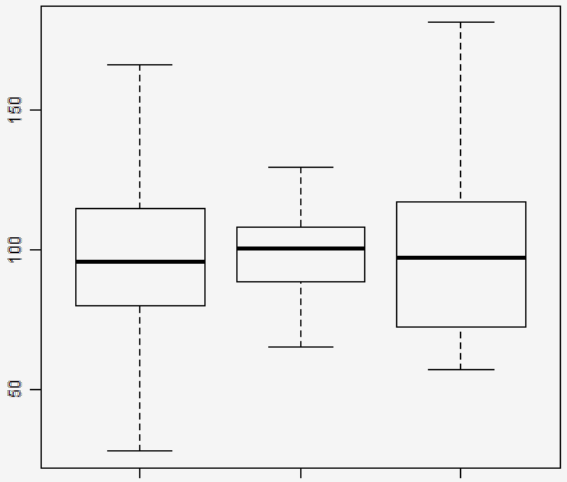

1.0
0.8
0.6
0.4
0.2
0.0
Accuracy
Specificity
Sensitivity


ΔΔCt values [qRT-PCR]
3
2
1
0
-1
-2
-3
-4
Average C
Average AD
Average MCI
Average PD
Average DEP
Average CIS
Average SCH
Average BD
hsa-let-7f-5p
hsa-miR-1285-5p
hsa-miR-107
hsa-miR-103a-3p
hsa-miR-26b-5p
hsa-miR-26a-5p
hsa-miR-532-5p
hsa-miR-151a-3p
brain-miR-161
hsa-let-7d-3p
brain-mir-112
hsa-miR-5010-3p

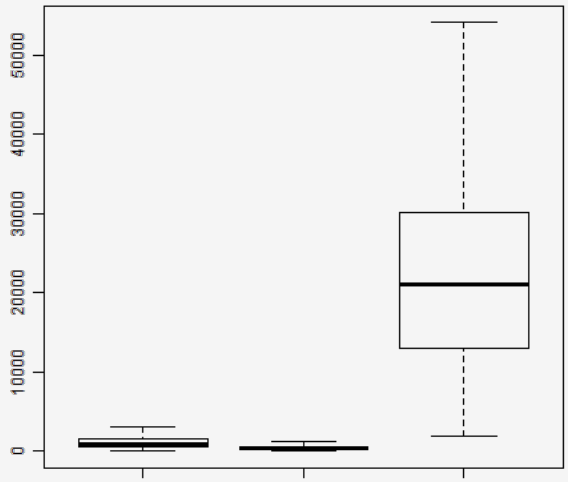
Technological bias



DOWN in NGS



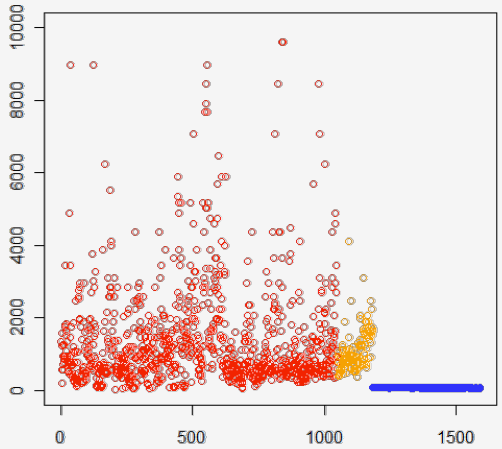
equal



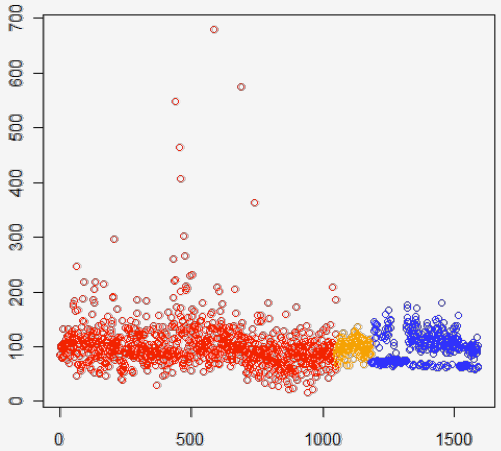
UP in NGS

The technological bias leads to results that can't be reproduced by qRT-PCR technically, causing avoidable cost and precious biological material.

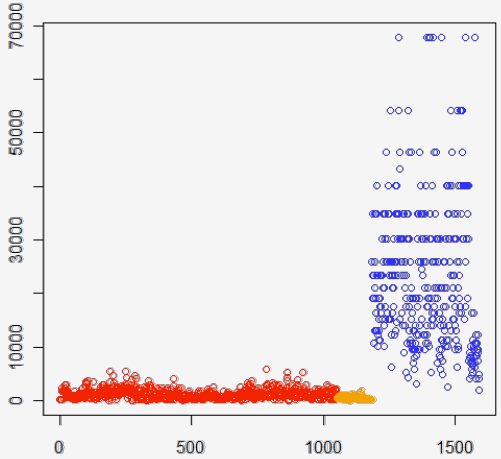
Technological bias



DOWN in NGS



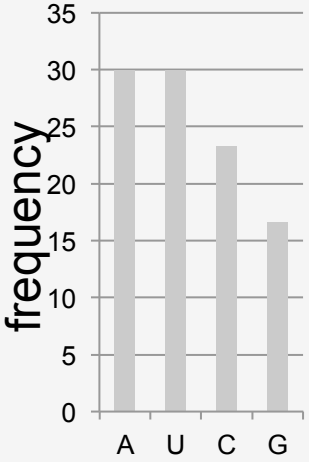
equal



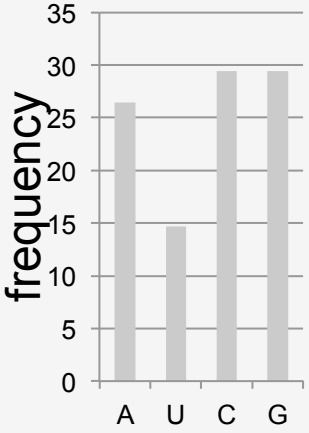
UP in NGS

The technological bias leads to results that can't be reproduced by qRT-PCR technically, causing avoidable cost and precious biological material.

up:
array / NGS



down:
array / NGS



Concept

For many miRNAs array, NGS and qRT-PCR data are available. Use them to train three statistical models.

For new studies use

a) NGS data & sequence OR
b) Array data & sequence OR
c) NGS & Array data & sequence OR
d) sequence

in order to predict whether effects come from technical bias or biological effects

Training & prediction

$$f(s,a) = x \qquad a \in R^n$$
$$f(s,b) = x \qquad b \in R^m$$
$$f(s,a,b) = x \qquad x \in \{0,1\}$$
$$f(s) = x \qquad s \in \{0,1\}^k$$

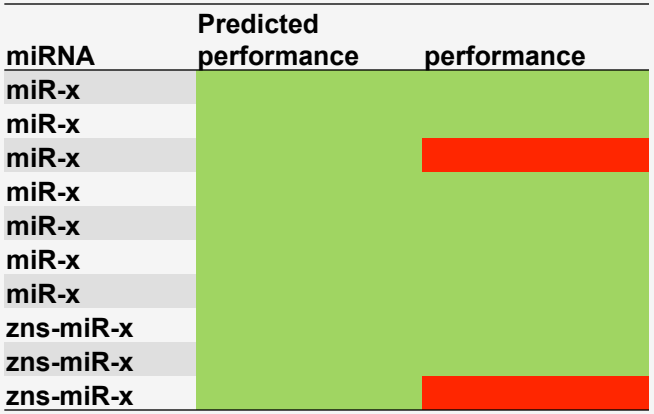
In Silicio prediction of PCR validation (isiPCR validation)

Result for case a)

Special problems in this case:

a) Very old samples of bad quality and severely affected sequencing quality
b) Only small amount of samples available

➔ No trial and error validation possible



Validation of 8 of 10 markers despite weak quality

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SIEMENS

> Selected references
> Focus: minimally-invasive miRNA biomarkers

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