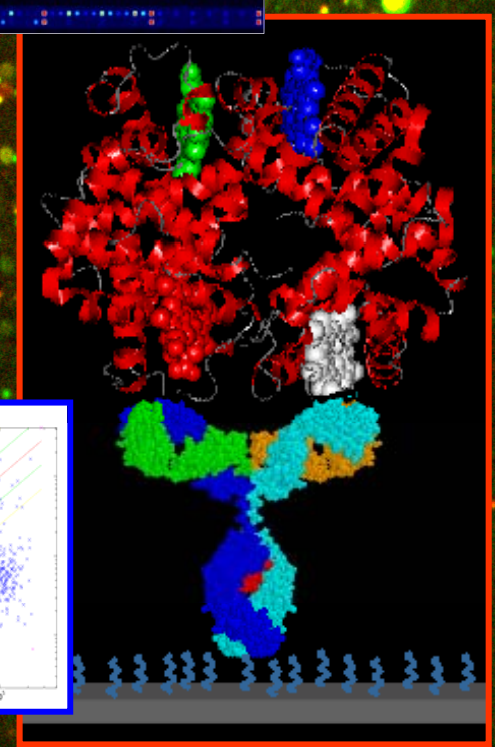
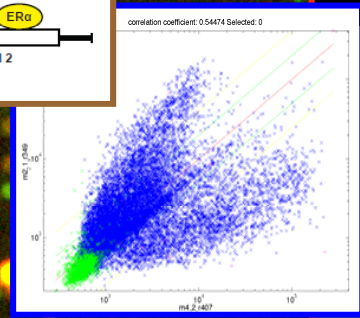
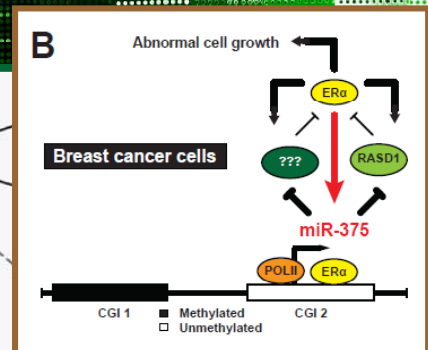
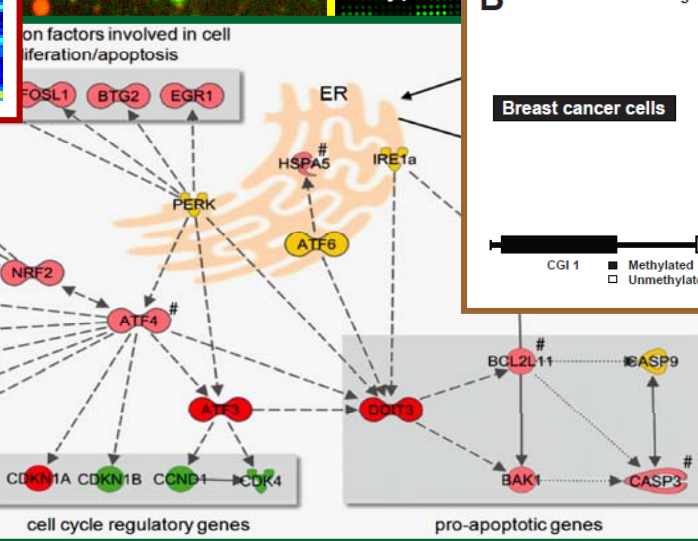
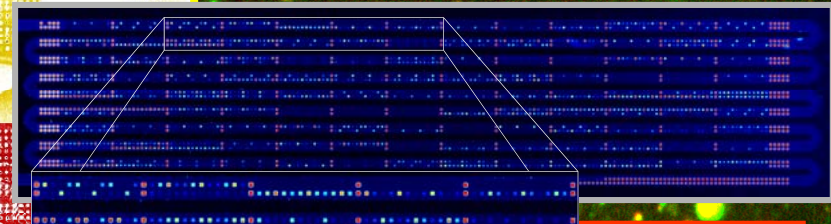
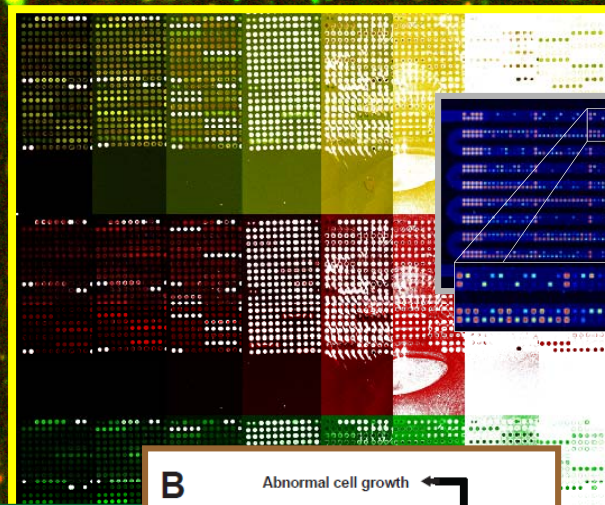
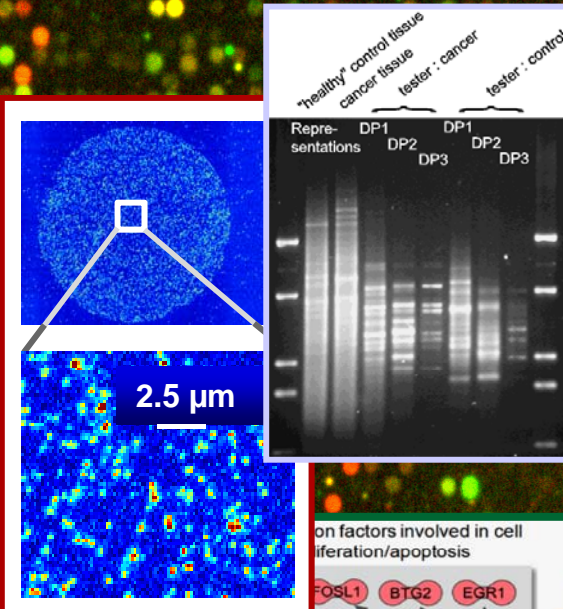
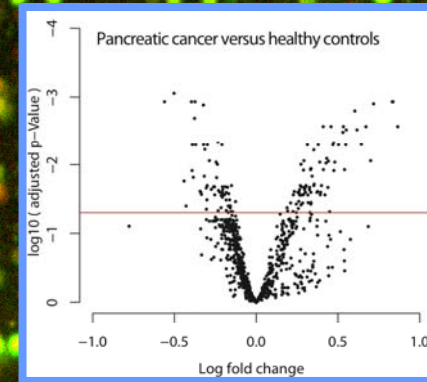


DKFZ | FUNCTIONAL GENOME ANALYSIS





dkfz.



Nationales Centrum für
Tumor Erkrankungen

dkfz.

Genom Aktivitäten

dkfz.



Grundlegende Ebenen funktioneller Analysen

DNA → **RNA** → **Protein** ➔➔

Methylation

Transcription

Expression

Structure

Splicing

Localisation

Organisation

Interaction

Modification

SNPs

RNAi

Combination

Copy number

Structure

Composition

Enzymatic activity

Degradation

Analyse des Pankreaskrebs

Material von 1200 Patienten

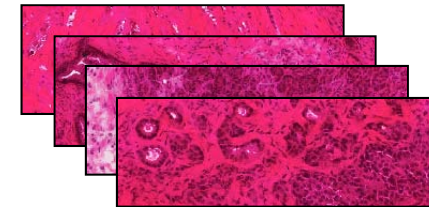
Tumore T1-T4, zystische Tumore, Pancreatitis und gesundes Gewebe.

Gefrorene Tumorgewebe

20 µm Schnitte



Histochemie



Mischen

Trennung in drei homogene Fraktionen; getrennte Isolation

<p>> 1000 DNA-Proben</p> <ul style="list-style-type: none">• Epigenetische Analyse• Mutationsanalyse• SNP-Typisierung	<p>> 550 RNA Proben</p> <ul style="list-style-type: none">• DNA-Microarrays> 550 mRNA Analysen> 250 miR Analysen	<p>> 700 Protein Proben</p> <ul style="list-style-type: none">• Antikörper-Microarrays• Protein-Microarrays
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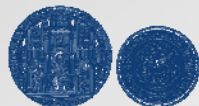
160 genomische Sequenzen



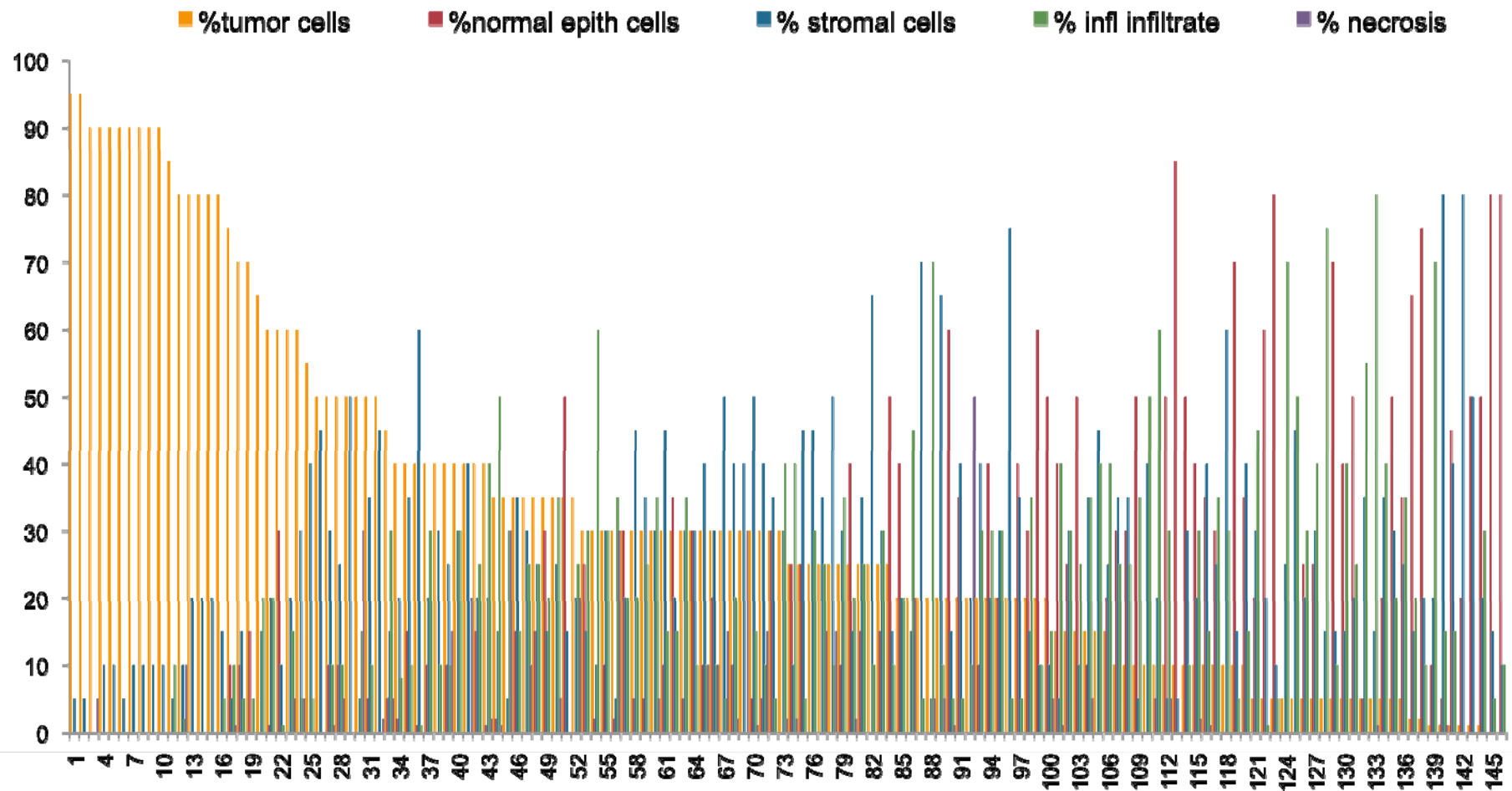
> 350 Blut- & Urinproben



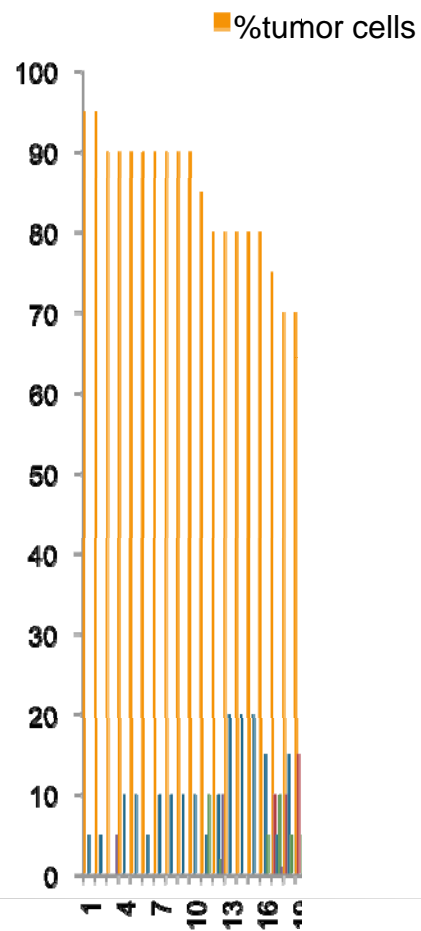
Queen Mary
University of London



Profilierung des Pankreas Adenokarzinoms



Profilierung des Pankreas Adenokarzinoms

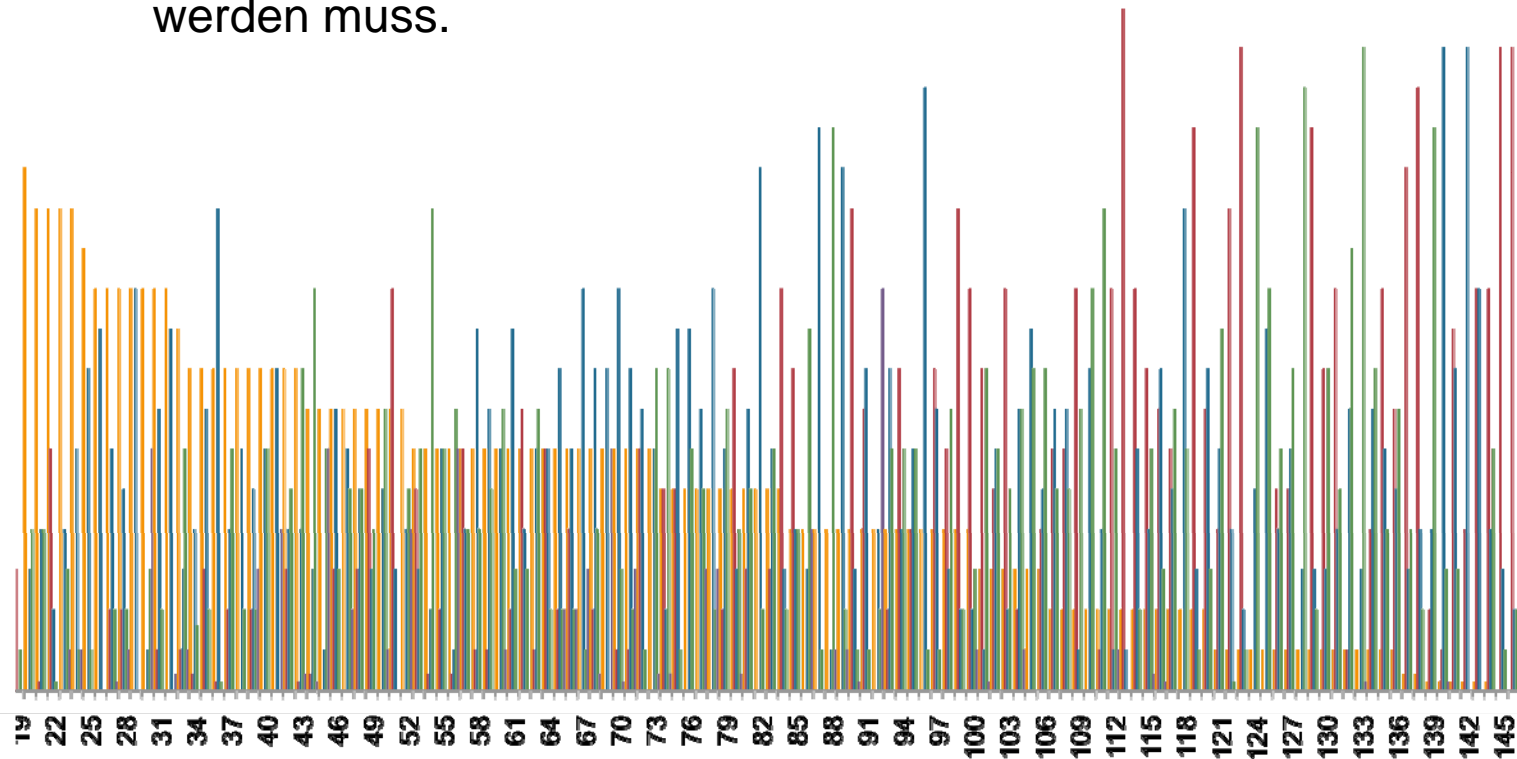


Material, das üblicherweise zur Biomarker Definition genutzt wird.

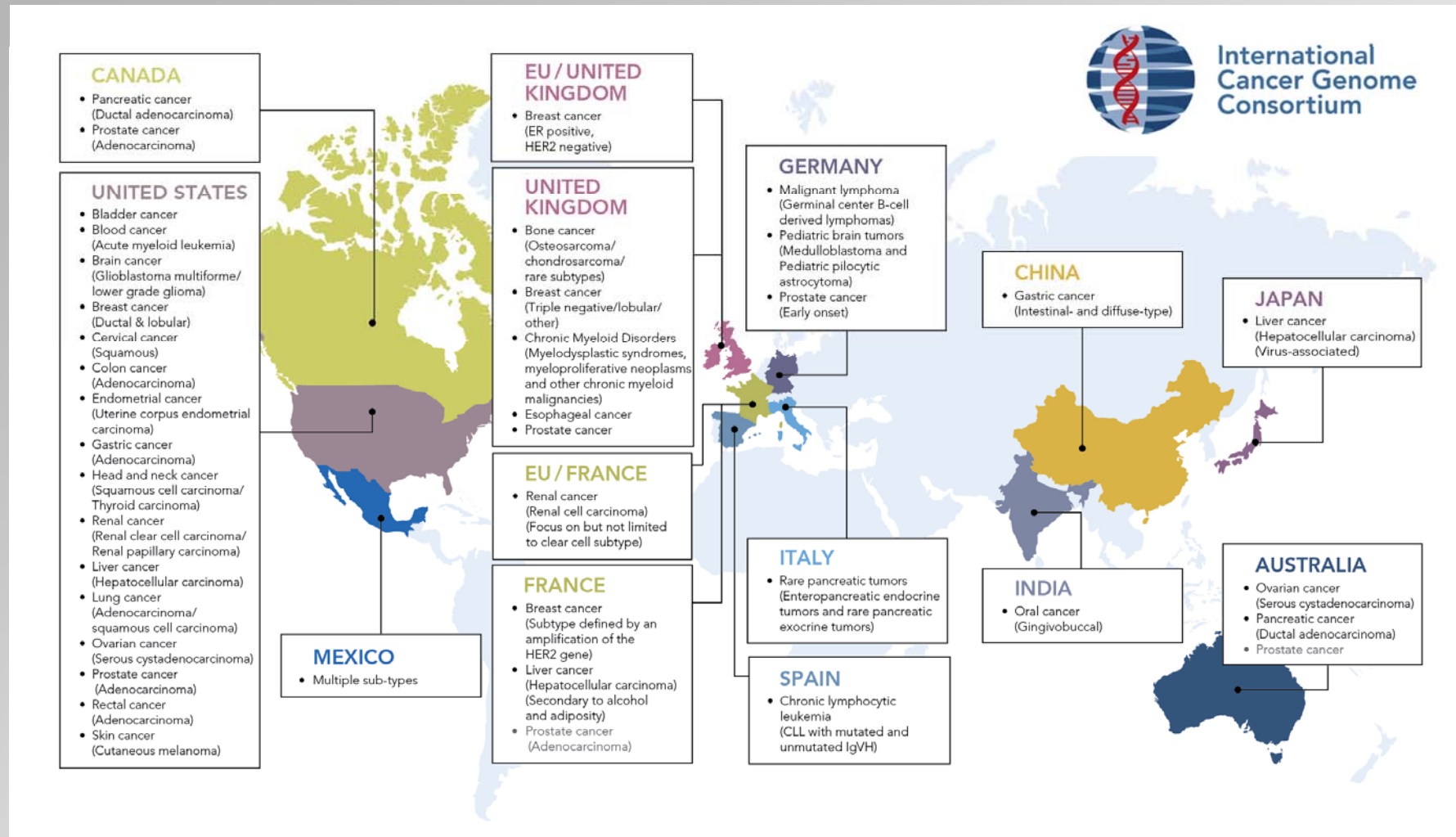
Profilierung des Pankreas Adenokarzinoms

■ %tumor cells ■ %normal epith cells ■ % stromal cells ■ % infl infiltrate ■ % necrosis

Material, das analysiert
werden muss.

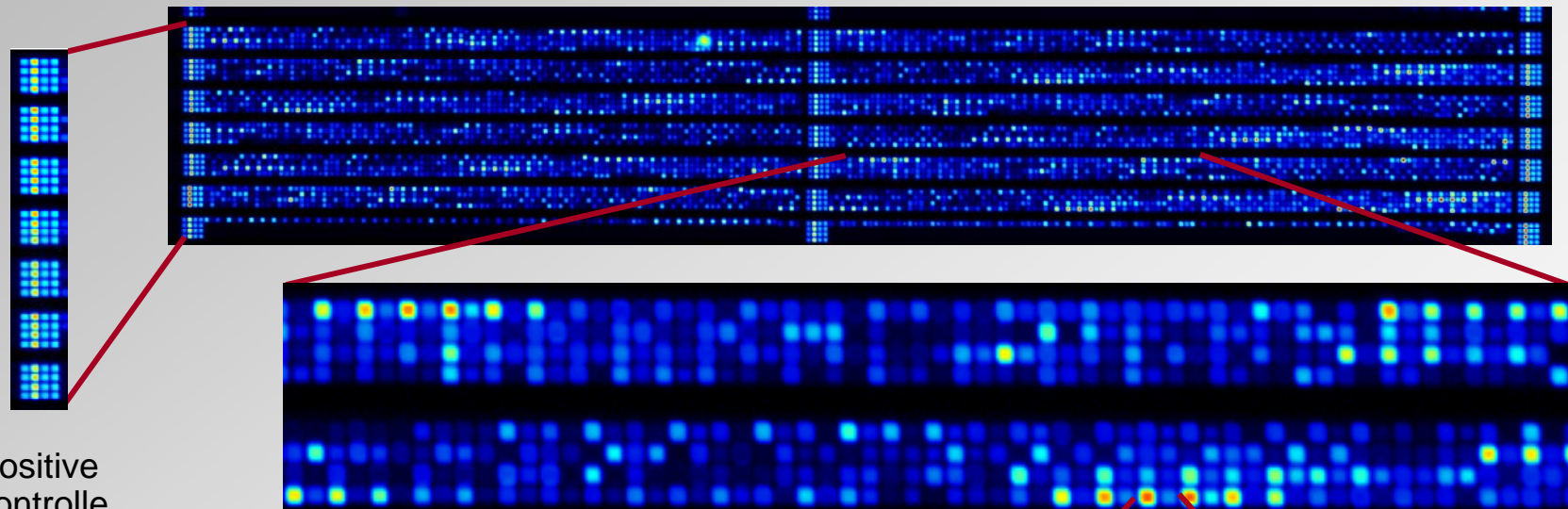
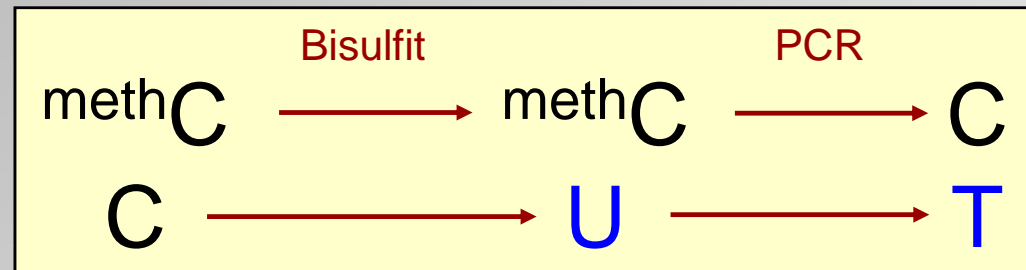
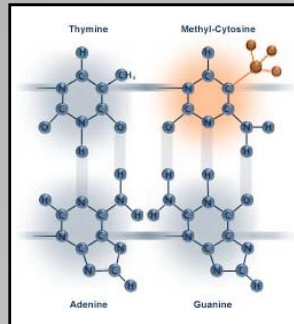


Patienten Sequenzierung



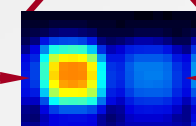
Epigenetische Messungen

Epigenetics



positive Kontrolle

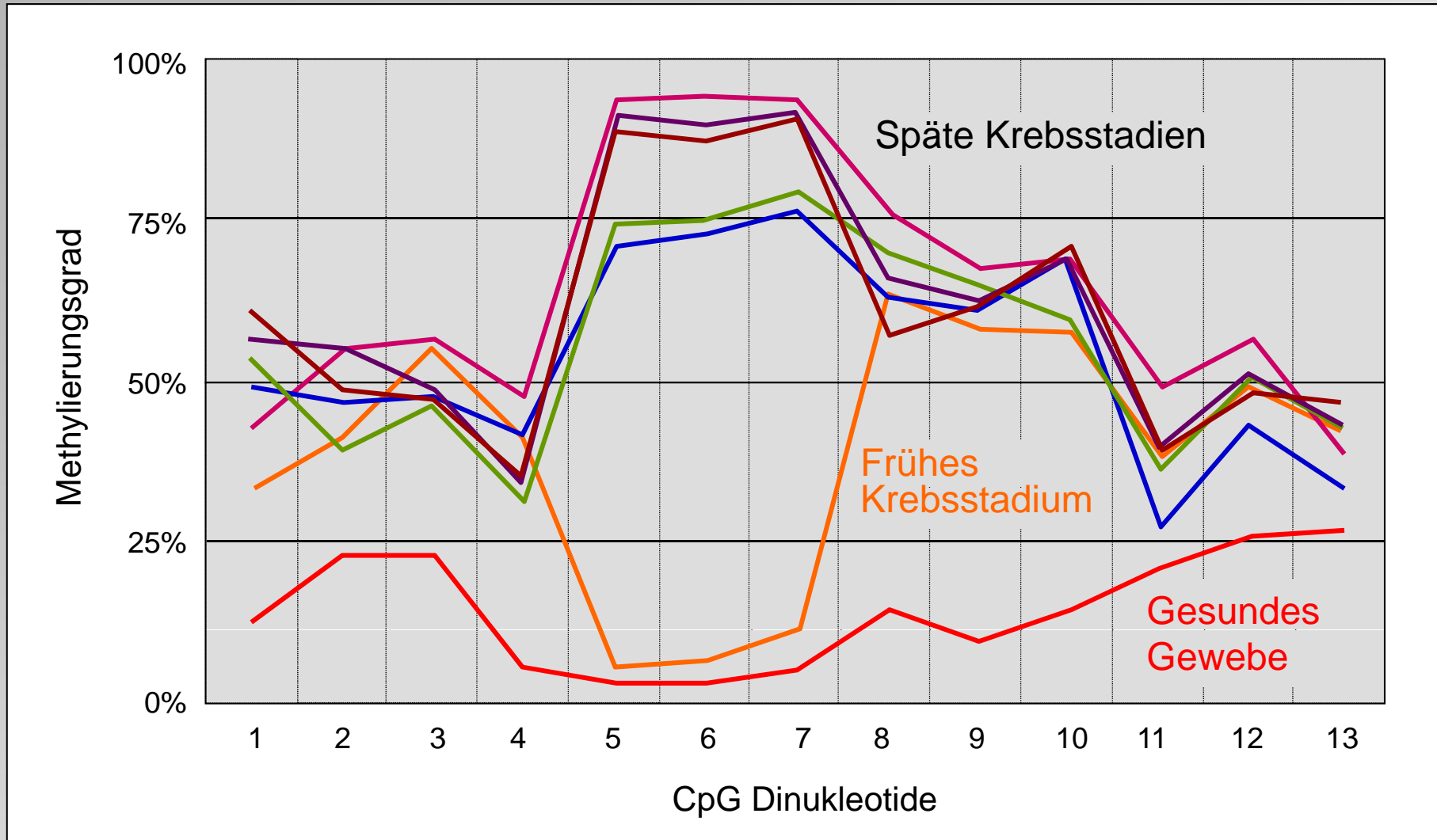
methyliert



unmethyliert

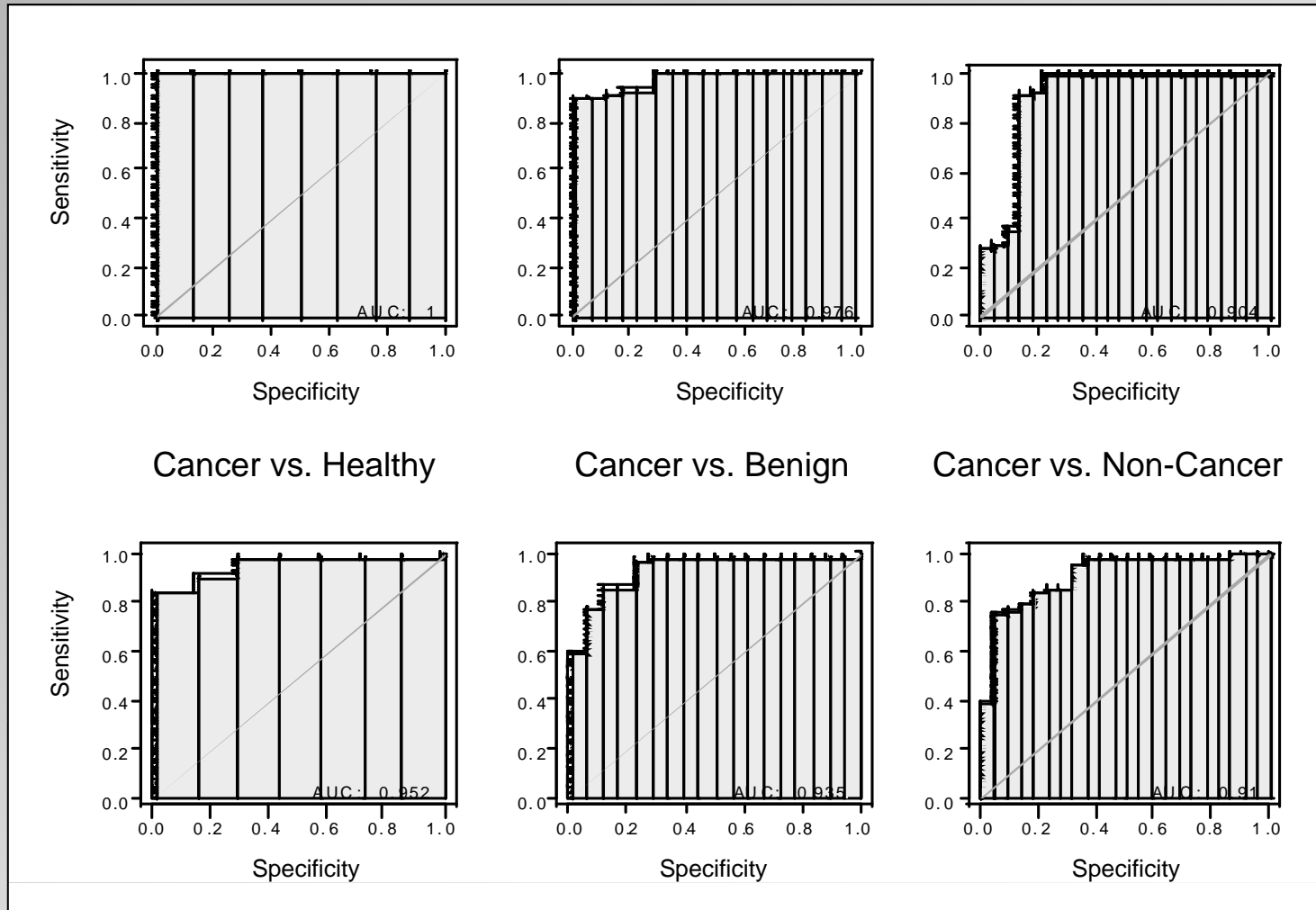
Pullat *et al.* (2007) *BMC Genomics* **8**, 282.
 Schanné *et al.* (2008) *Anal. Bioanal. Chem.* **391**, 1661-1669.
 Pullat *et al.* (2008) *New Biotechnol.* **25**, 131-141.

Epigenetische Diagnose



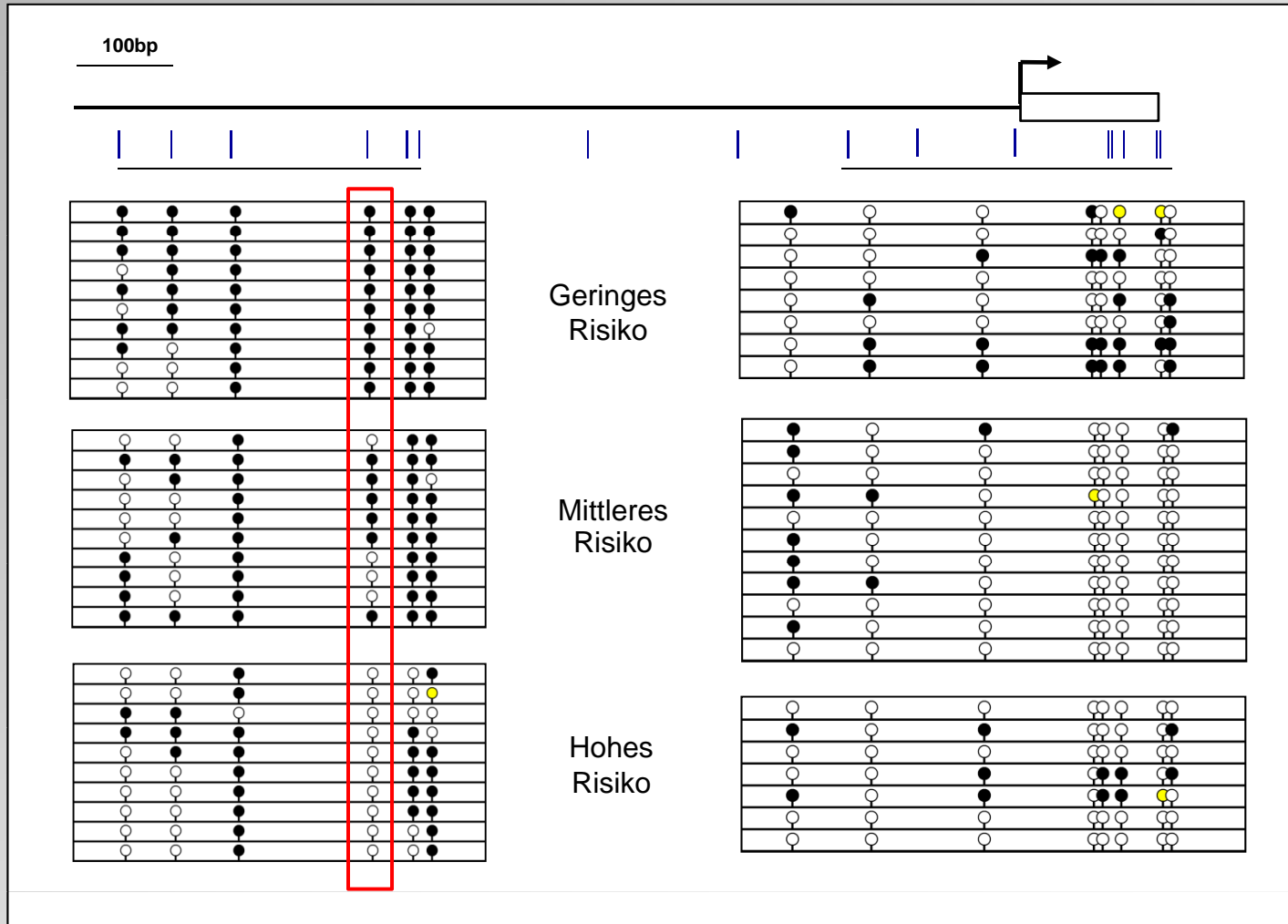
Signatur Qualität

Epigenetics



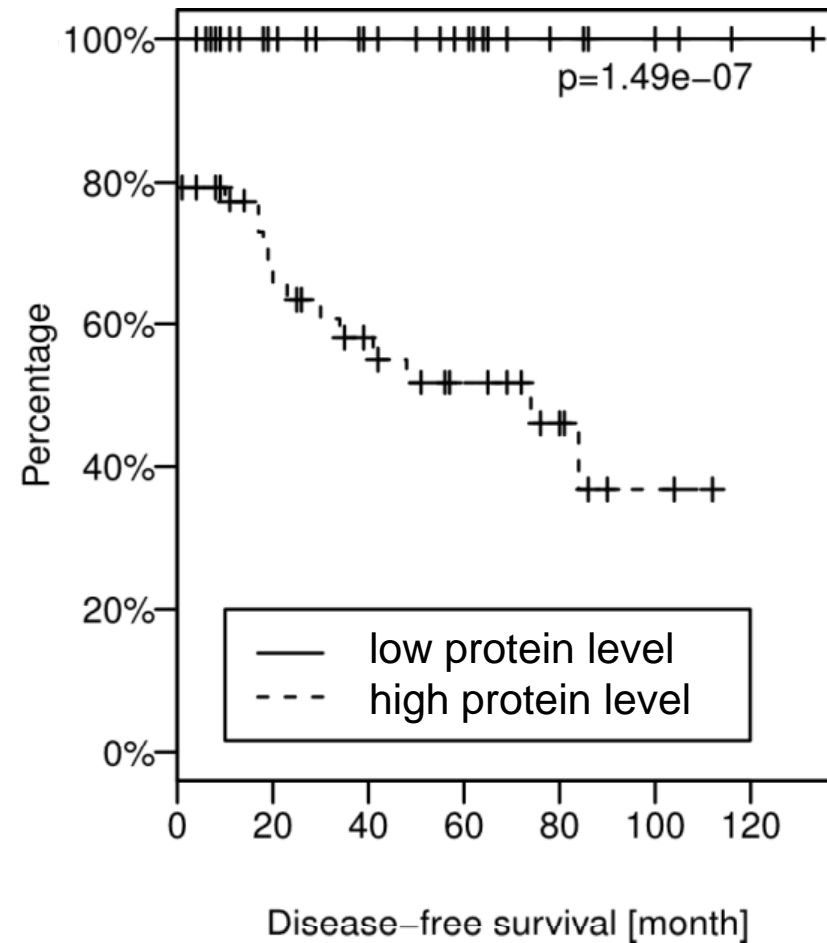
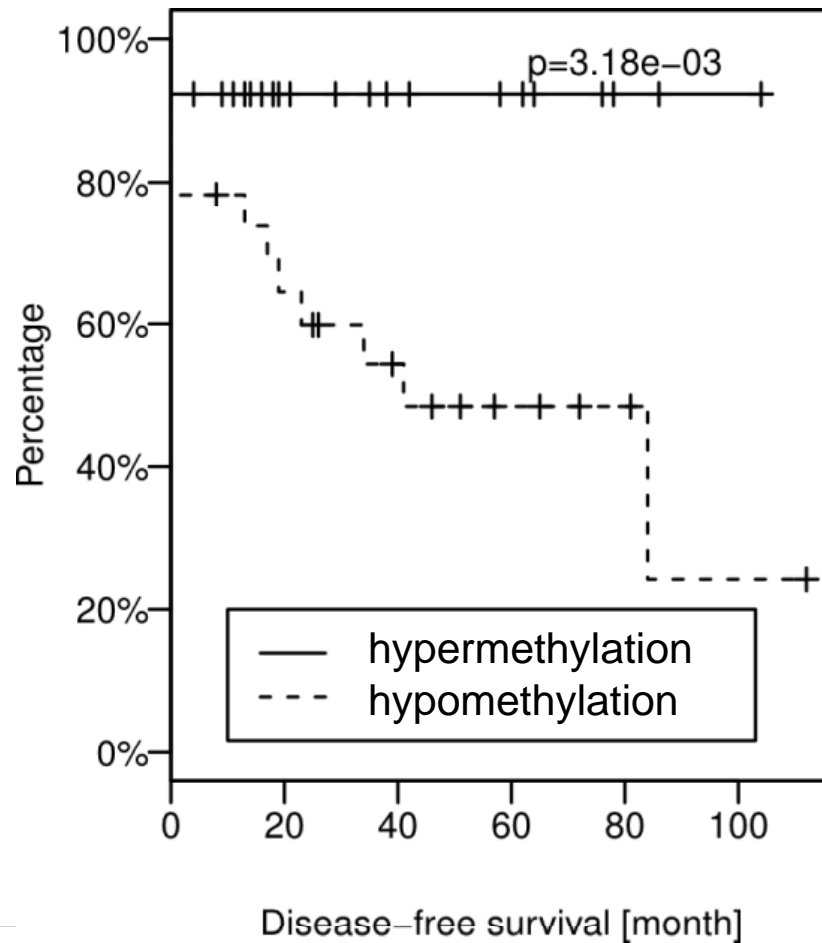
Definition prognostischer Methylierungsmarker

Epigenetics

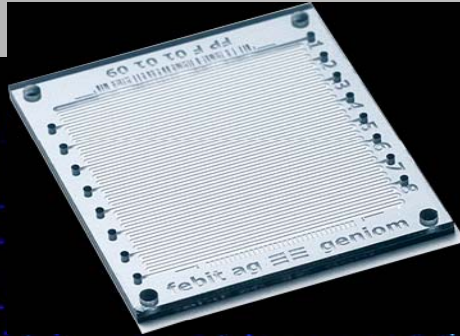


Definition prognostischer Methylierungsmarker

Epigenetics



miR

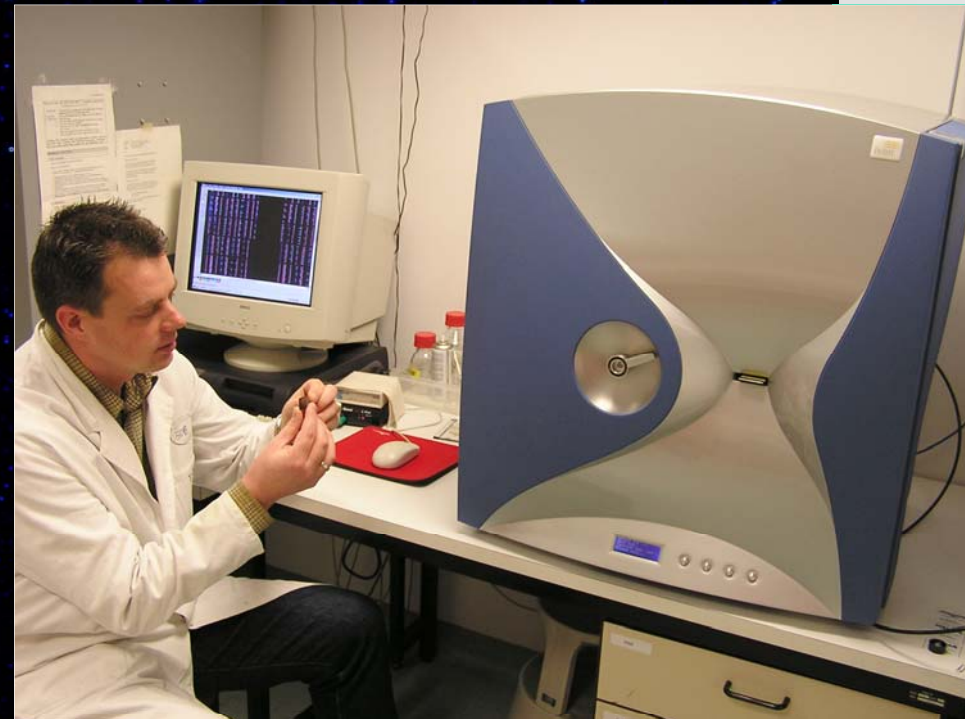


94 blood samples
45 cancer samples
38 chronic pancreatitis
11 normal donors

185 tissue samples
137 cancer samples
22 chronic pancreatitis
26 normal donors

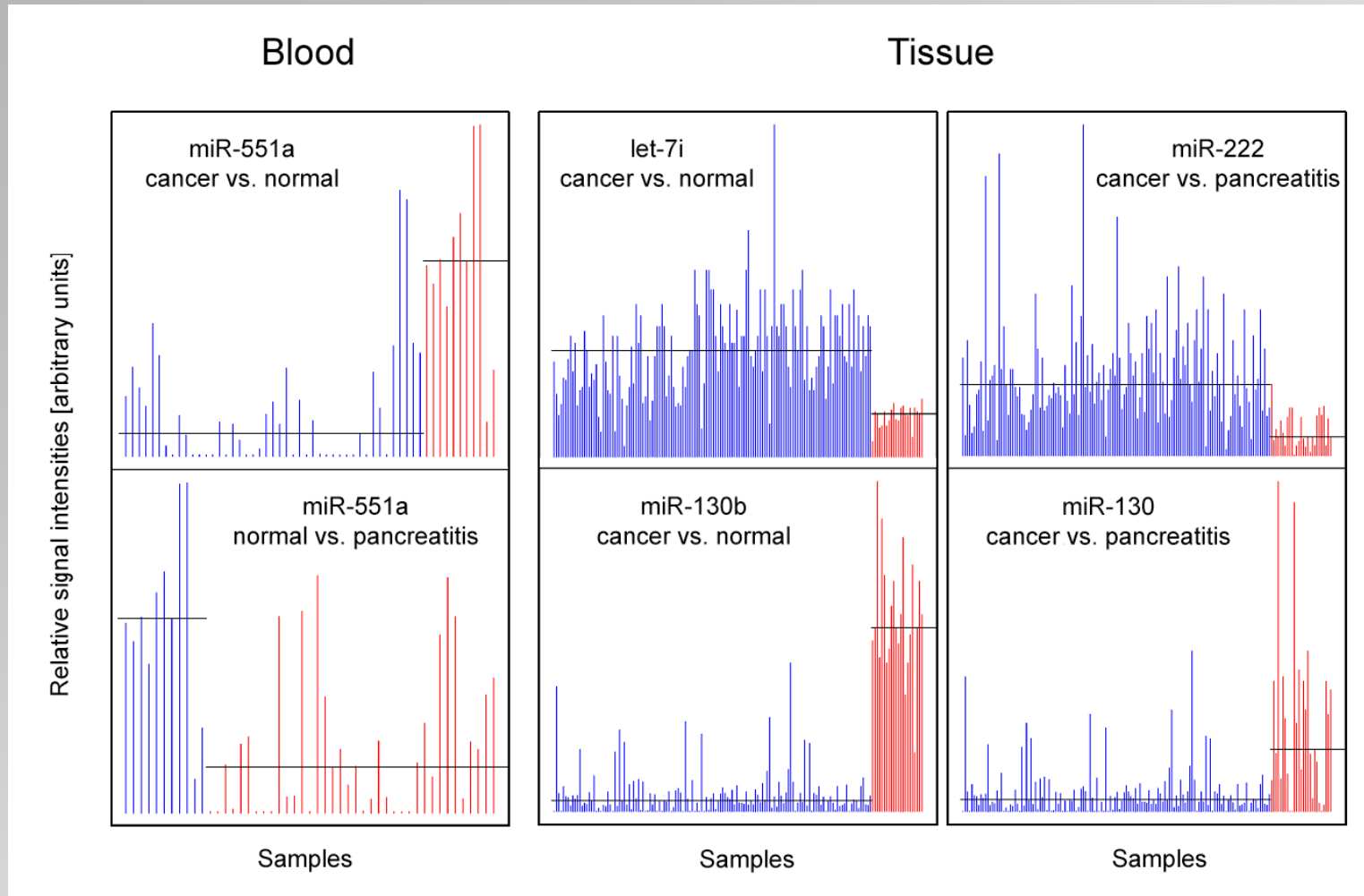
Chip design
featuring 863 miRs
common to miRBase 12.0 to 14.0

RT-PCR confirmation
960 reactions



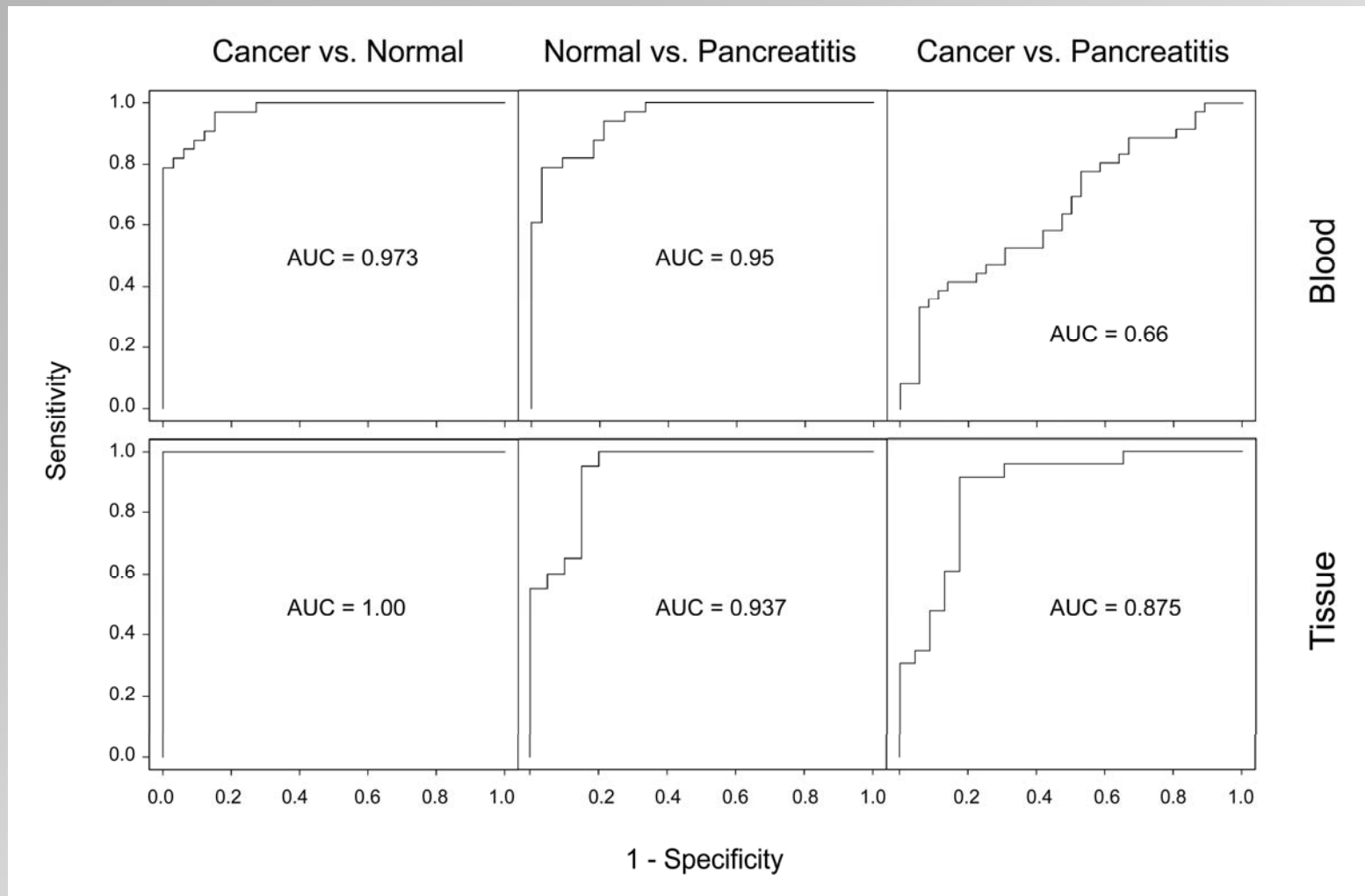
Individuelle miR Profile

miR



MicroRNA Diagnose im Blut

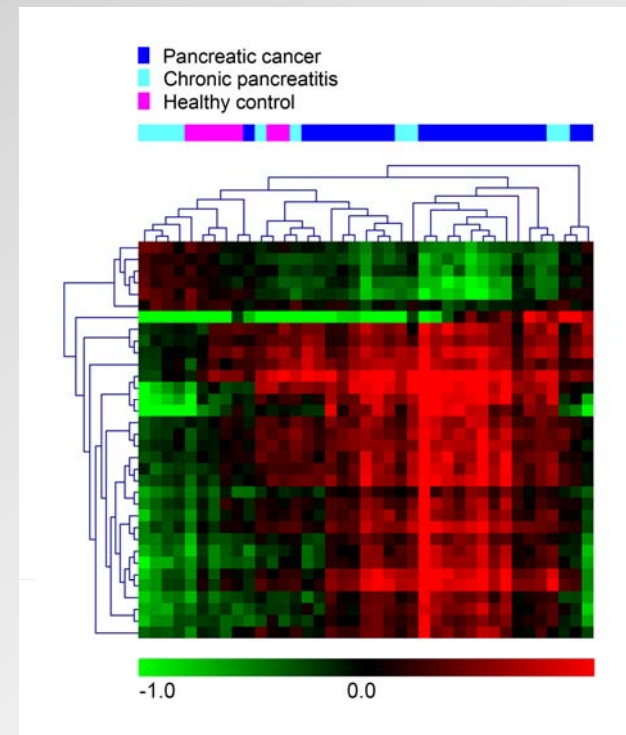
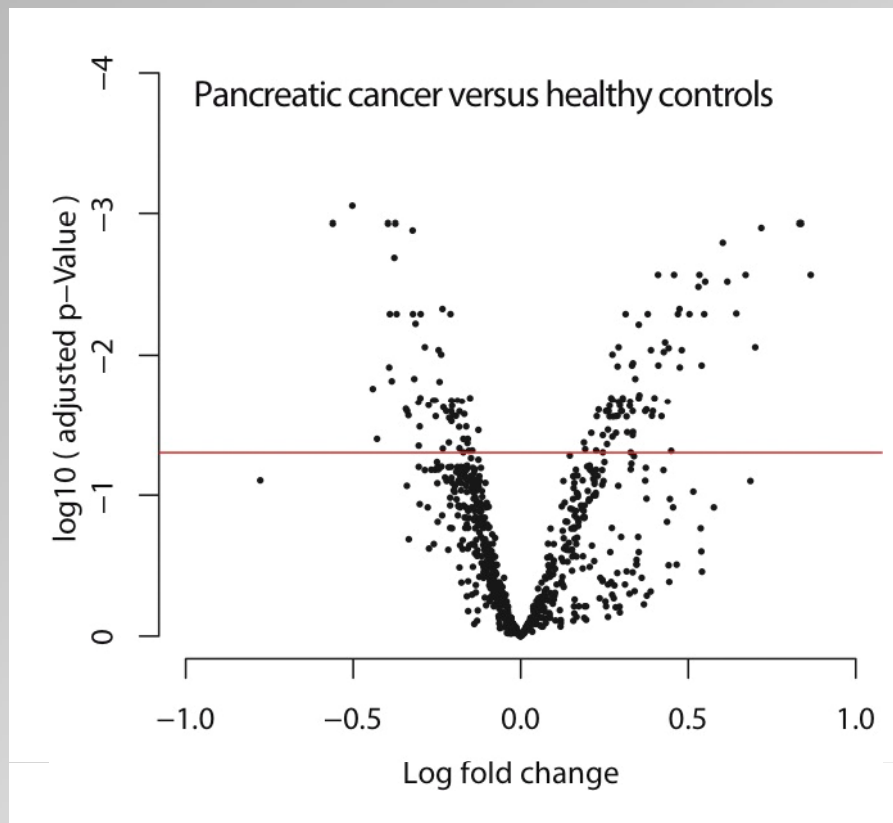
miR



Nicht-invasive Diagnose: Plasma

Antibody Microarrays

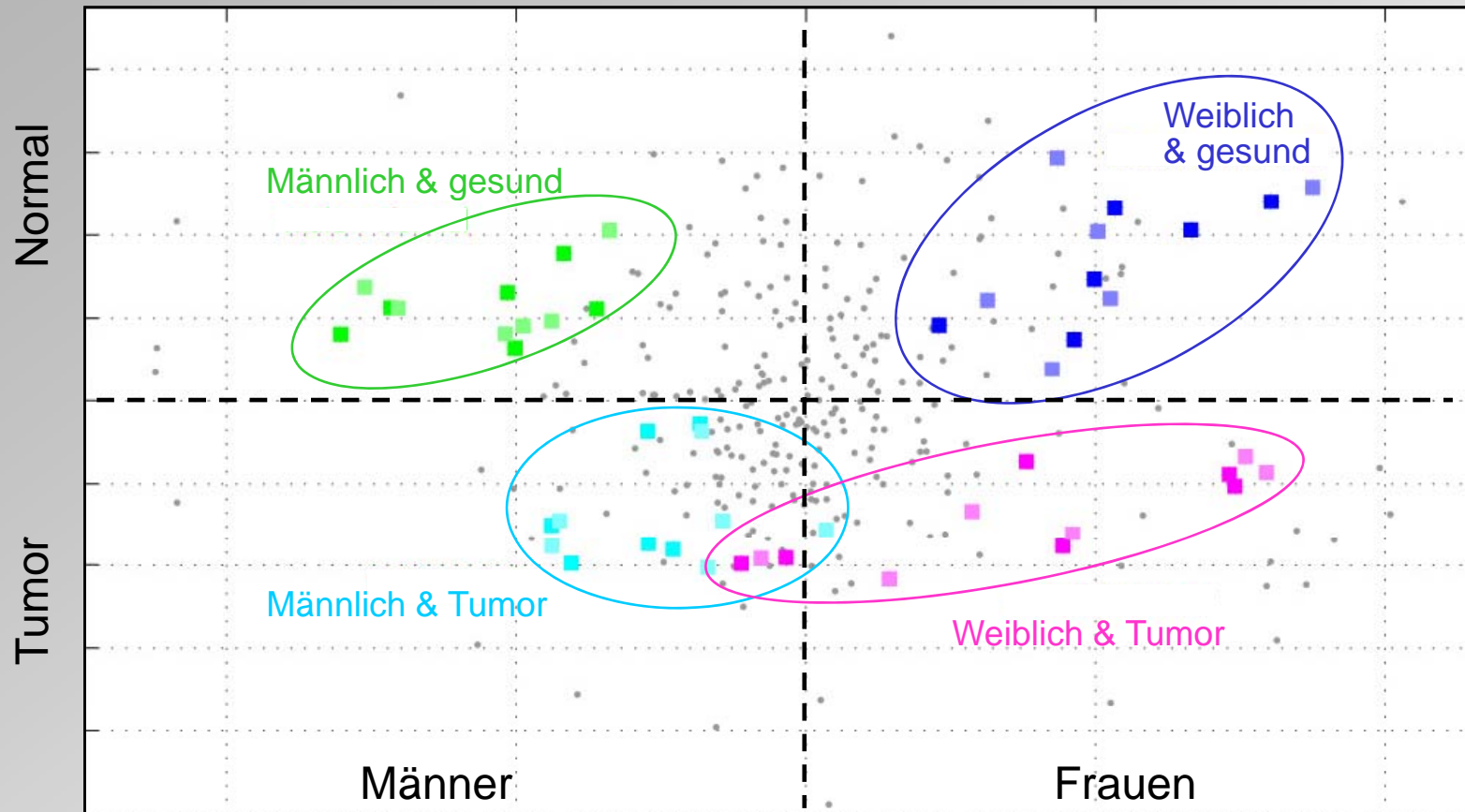
Differenzierung PDAC vs. Gesund & Pancreatitis: Specificity 76%
Sensitivity 77%



Cluster Analyse
PAM-definierter Markerproteine

PDAC Diagnose im Urin

Antibody Microarrays



MicroRNA Diagnose in Blut

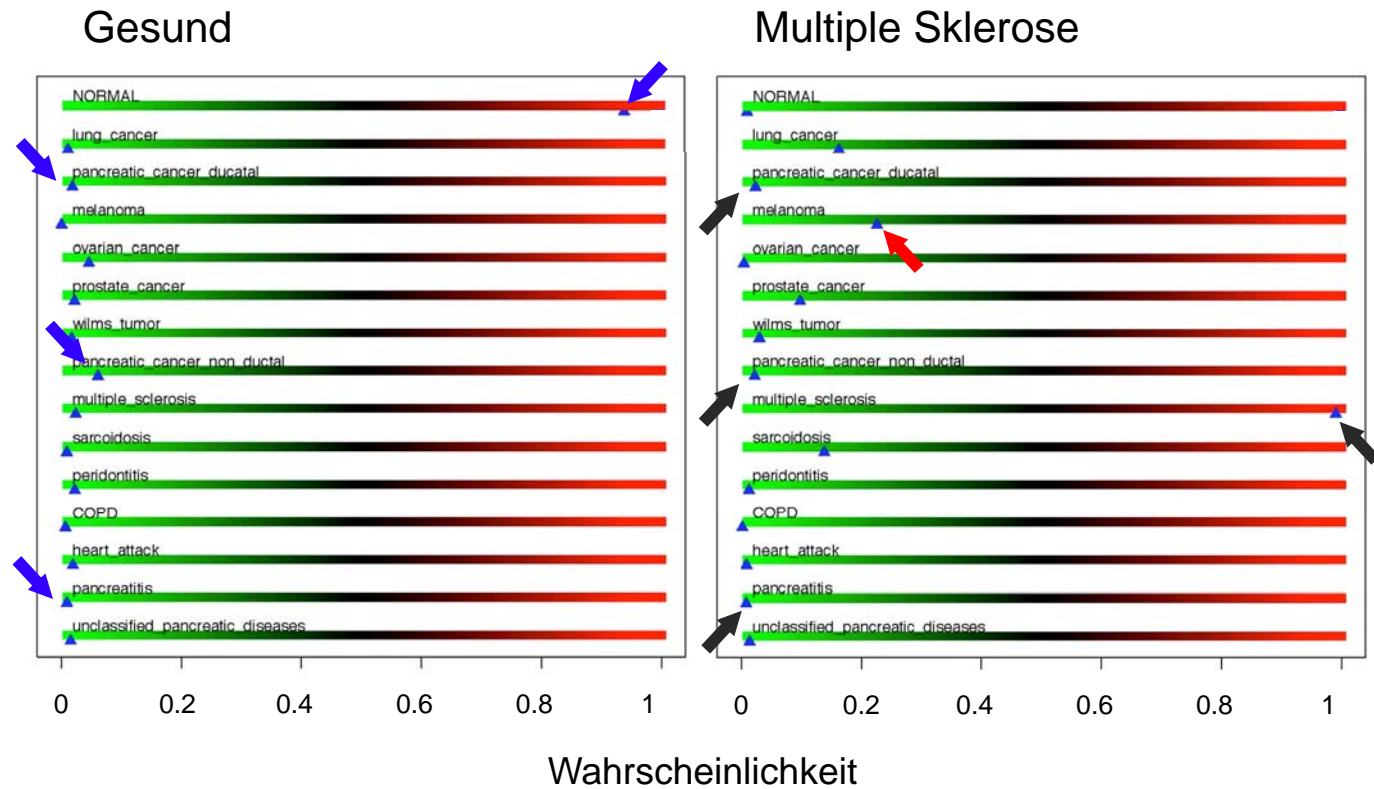
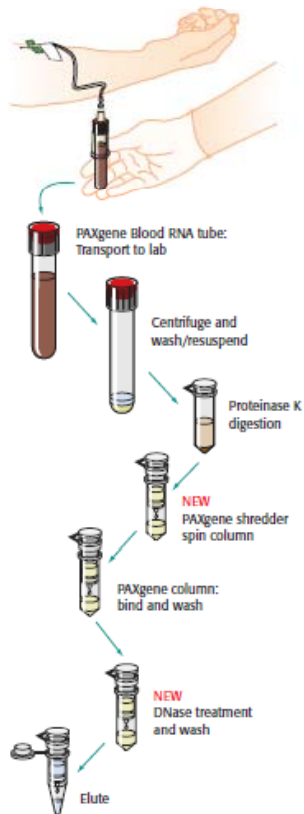
miR

normal	94
longlived	15
wilms_control	52
parkinson_control	7
lung cancer	73
pancreatic cancer ducatal	45
melanoma	35
ovarian cancer	24
prostate cancer	65
wilms tumor	124
other pancreatic tumors and diseases	48
tumor of stomache	13
glioma	20
renal cancer	20
colon cancer	29
multiple sclerosis	23
sarcoidosis	45
peridontitis	18
COPD	47
acute myocardial infarction	20
pancreatitis	37
benign prostate hyperplasia	35
psoriasis	17
rheumatoide arthritis	65
dilatative kardiomyopathie	34
parkinson	22
PCA or BPH	24
Adenocarcinoma or pankreatitis	3
acute myocardial infarction kinetics	41

Keller *et al.* (2011)
Nature Meth. **8**, 841-843.

MicroRNA Diagnose in Blut

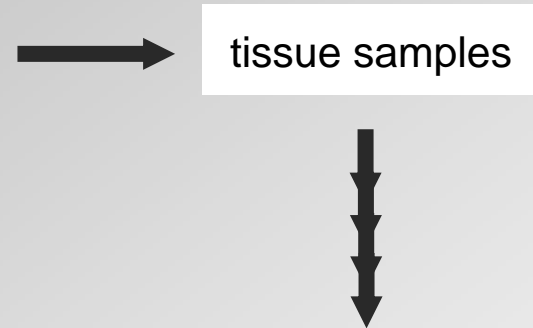
miR



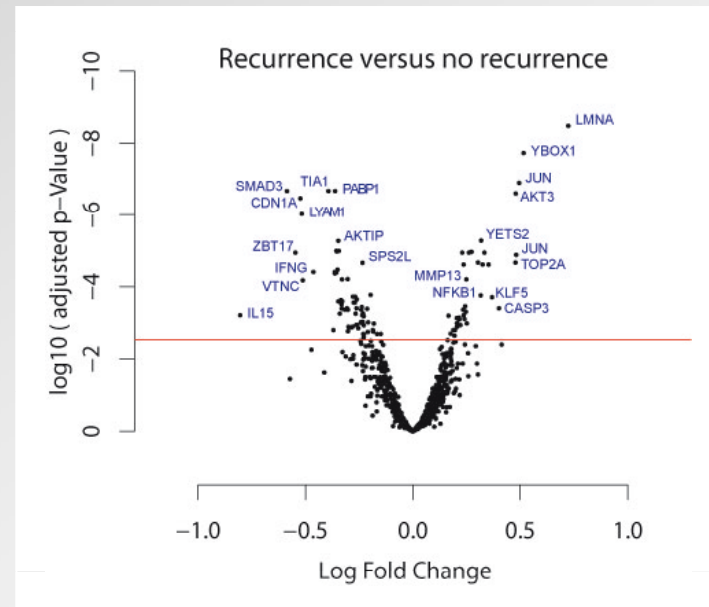
Vorhersage Wiederauftreten Blasenkrebs

Antibody Microarrays

- Fourth & ninth most common type of cancer in men & women
- 70% to 80% are low-stage, low-grade nonmuscle-invasive
- **Standard treatment: transurethral resection of bladder tumour**
- 50% to 70% of the tumours will recur
- 10% to 30% will progress to become muscle-invasive
- Recommended preventive procedure:
surveillance cystoscopy and urine cytology
=> surveillance cost: >100,000 US\$ per patient



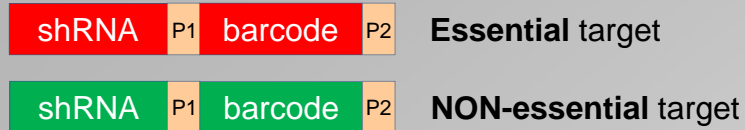
Uniprot entryname	Uniprot accession	HGNC symbol	Log-fold change	Adjusted p-value
LMNA_HUMAN	P02545	LMNA	0.72	3.3e-09
YBOX1_HUMAN	P67809	YBX1	0.52	1.9e-08
JUN_HUMAN	P05412	JUN	0.50	1.3e-07
AKT3_HUMAN	Q9Y243	AKT3	0.48	2.5e-07
SMAD3_HUMAN	P84022	SMAD3	-0.59	2.1e-07
IYAM1_HUMAN	P14151	SFI1	-0.57	9.6e-07
PABP1_HUMAN	P11940	PABPC1	-0.36	2.1e-07
TIA1_HUMAN	P31483	TIA1	-0.39	2.1e-07
CASP3_HUMAN	P42574	CASP3	0.40	4.0e-04
CDN1A_HUMAN	P36936	CDKN1A	-0.52	3.4e-07
CASP9_HUMAN	P55211	CASP9	0.30	1.4e-02
YEATS2_HUMAN	Q9JULM3	YEATS2	0.32	5.3e-06
PO2F2_HUMAN	P09086	POU2F2	-0.35	2.6e-04
IQP2A_HUMAN	P11388	IQP2A	0.48	2.2e-05
RPSA_HUMAN	P08865	RPSA	-0.35	1.1e-05
NFAC4_HUMAN	Q14934	NFATC4	0.35	2.5e-05
ZBT17_HUMAN	Q13105	ZBTB17	-0.55	1.1e-05
AKTIP_HUMAN	Q9J18T0	AKTIP	-0.35	5.4e-06
HSP7C_HUMAN	P11142	HSPA8	-0.34	1.0e-05
LIFR_HUMAN	P42702	LIFR	0.30	2.2e-05



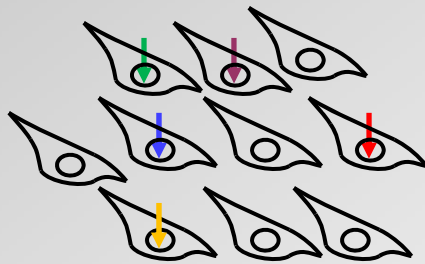
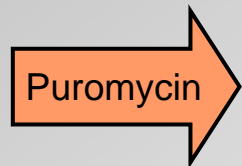
Classifier for the prediction of recurrence
with a sensitivity of 88% and a specificity of 100%

Simultaneous shRNA Screening

Böttcher et al. (2010) *Curr. Genomics* 11, 162-167



pooled infection:
one virus per cell



↓ Gene knockdown

