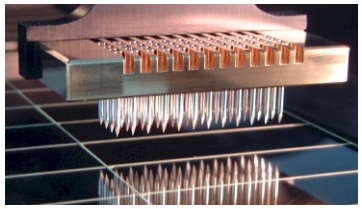
Microarrays – novel molecular tools in medical diagnostics taking advantage of specific biomolecule interactions



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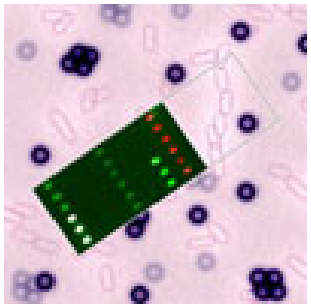
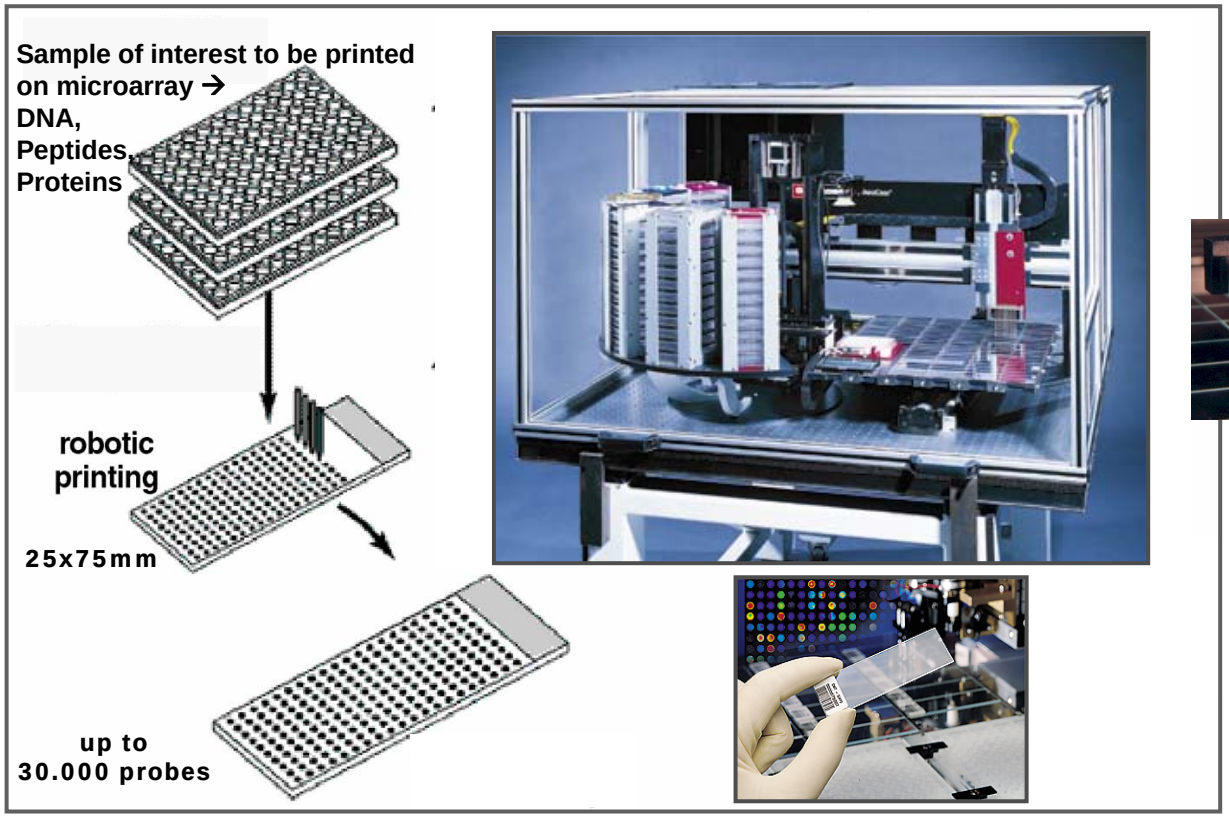
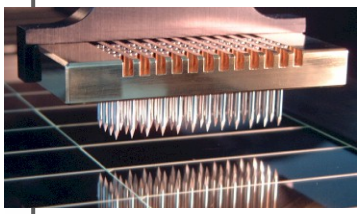


OUTLINE

- Introduction in microarrays (the principle behind)
- Overview of possible microarray applications
- Examples of ARC-sr in-house microarray developments

Microarrays

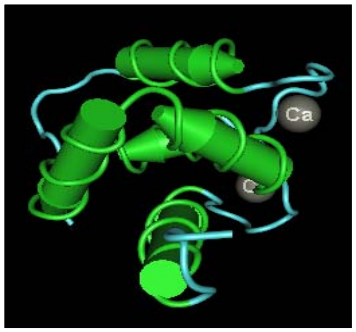


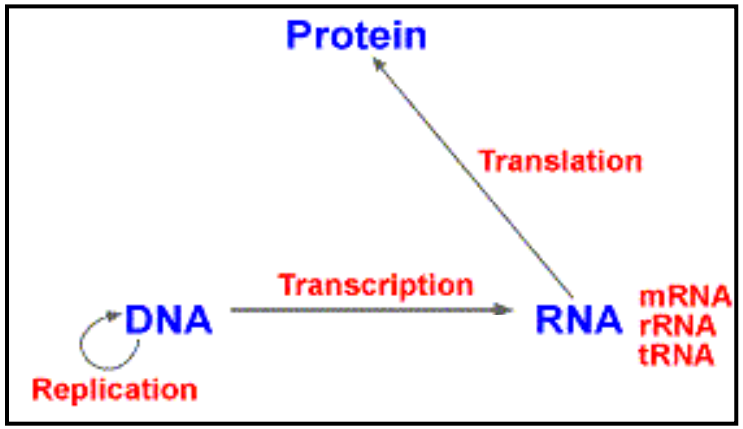


Microarrays - great tools to study

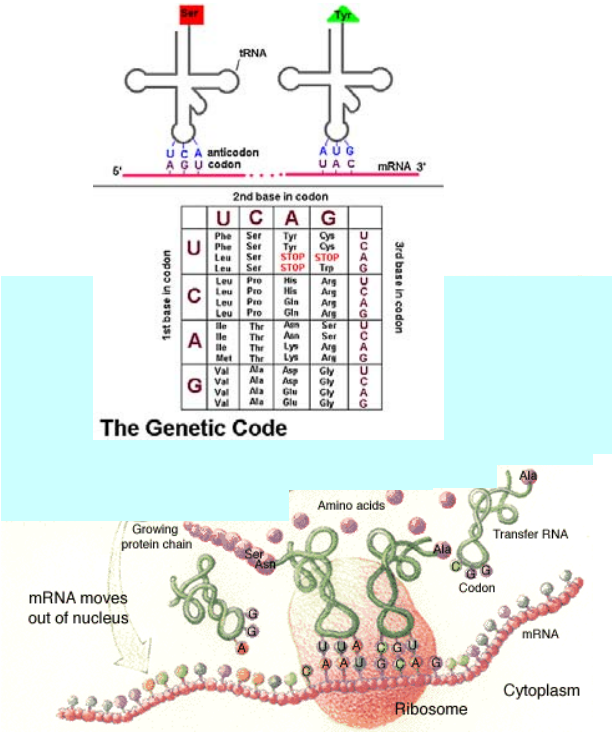
the magic triangle of LIFE in a highly multiplexed manner

Deoxyribo**N**ucleic **A**cid



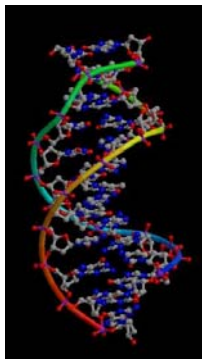


Ribo**N**ucleic **A**cid



To make the story of microarrays short + simple

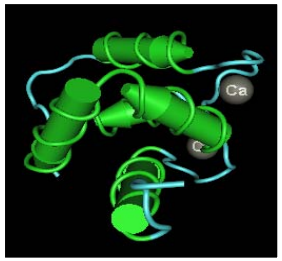
It‘s all about specific interaction of biomolecules made visible by fluorescence labeling of the target molecule, which binds to the respective probe on the microarray



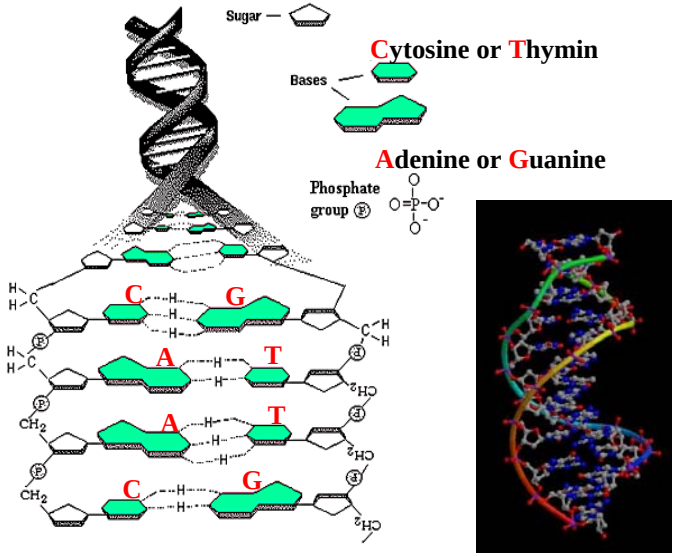
Probes immobilized on the microarray can be

DNA

Protein



DNA Microarrays
Base-pairing principle

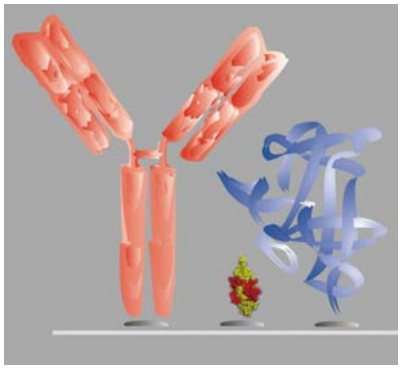


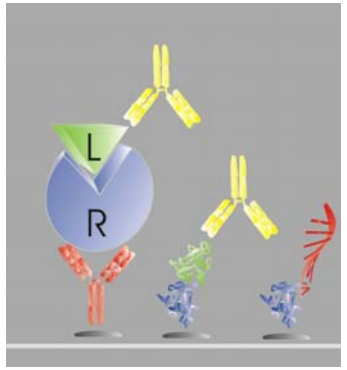
Gene Detection (DNA-DNA)

Gene Expression (DNA - mRNA / cDNA)

DNA Methylation (DNA-DNA)

Protein Microarrays
Specific protein interaction
(Ab-Ag, Receptor-Ligand)

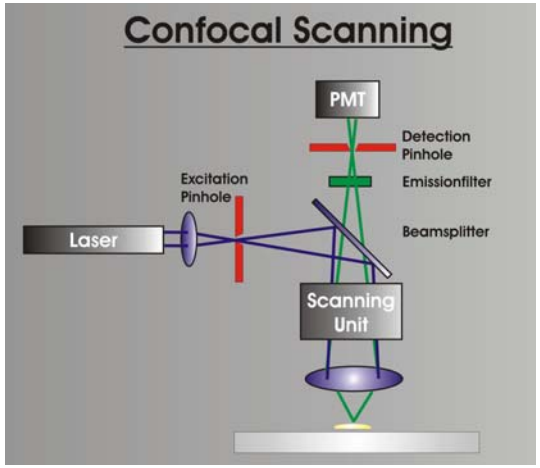




Protein abundance studies (Antibody – Protein)

Specific Interaction (Receptor-Ligand Protein-Protein, Protein-DNA)

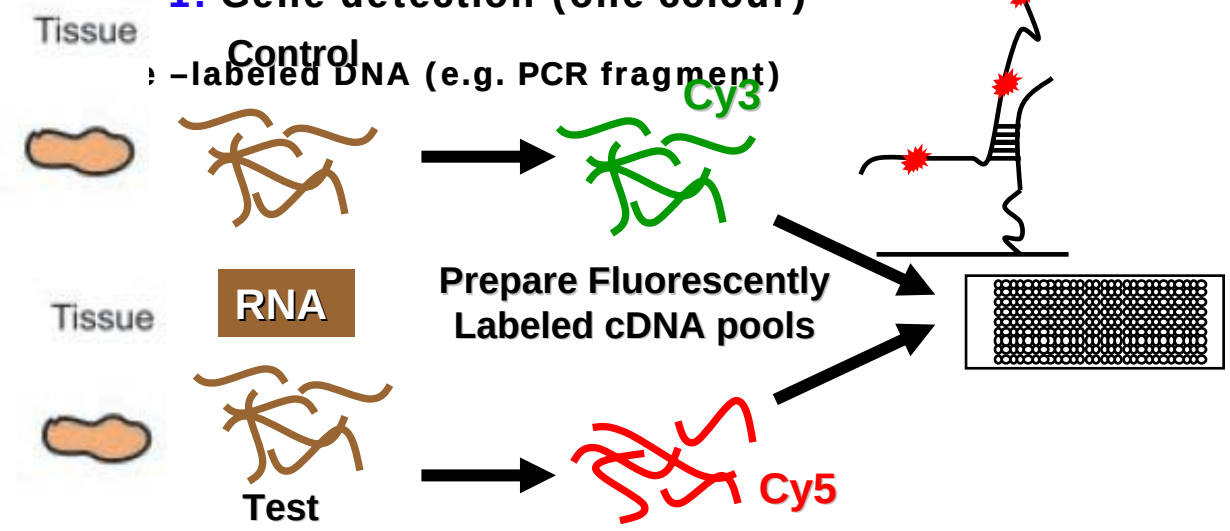
How to detect probe-target interaction on microarrays ?



„Use Case“ 2: Gene expression (two colour → ratio detection)

„Use Case“ 1: Gene detection (one colour)

→ D… –labeled DNA (e.g. PCR fragment)



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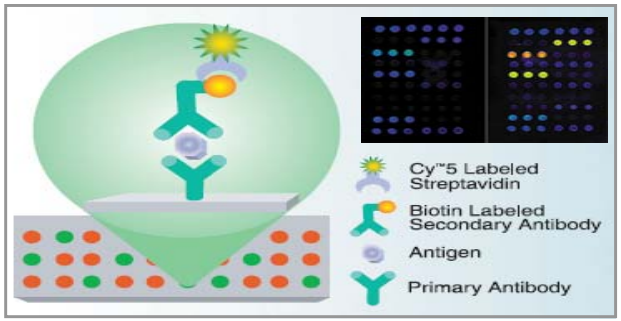
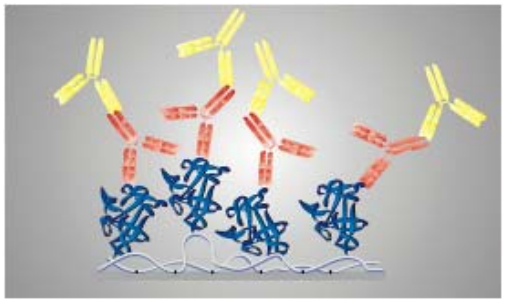
How to detect target-probe interaction on microarrays ?

„Use Case“ 3: Sandwich ELISA on Chip
→ specific antibody – antigen to be analysed detected by a biotinylated ag-specific sec. ab

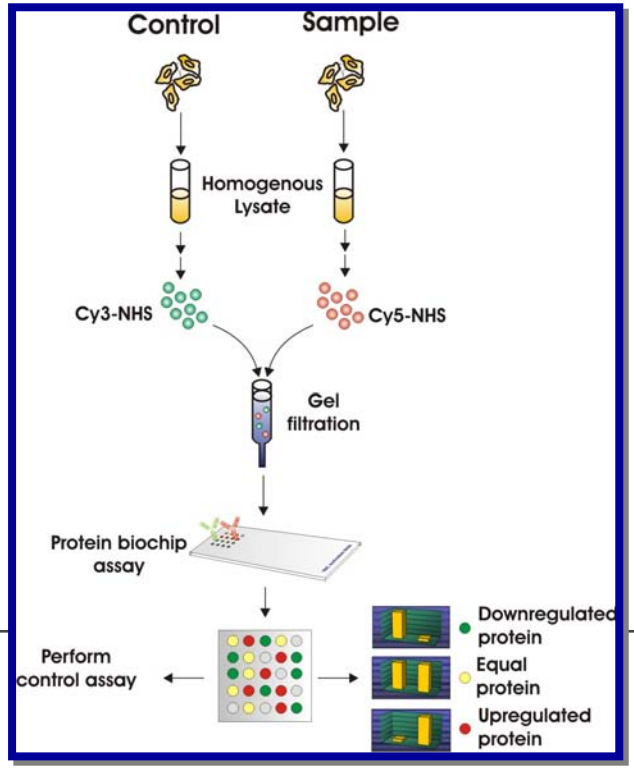


Courtesy: Schleicher&Schuell

„Use Case“ 4: Analysis of specific antibodies
→ antigen-specific antibody – labeled secondary antibody



„Use Case“ 5: Protein abundance Chips
→ specific antibodies – proteins to be analysed (ref.- and sample protein lysate labeled red+green)



Sreekumar et al., *Cancer Research*, 2001

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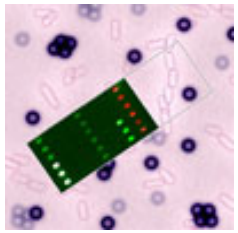
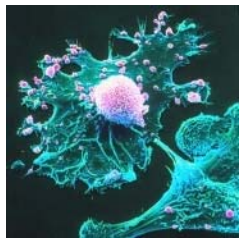
Microarray application fields ARC-sr

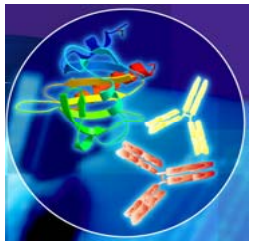
- Infectious disease diagnostics



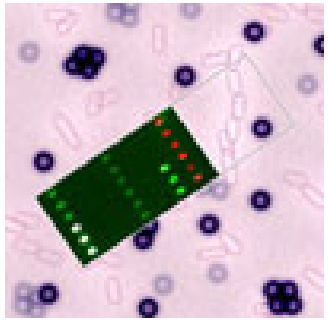
- Tumor diagnostics



- Allergy diagnostics

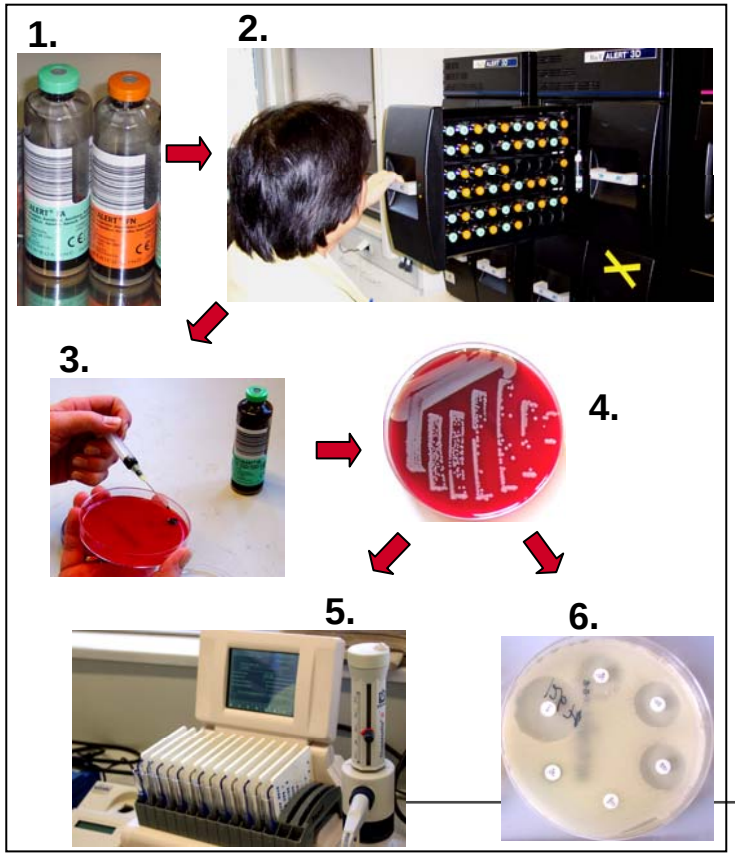


- Infectious disease diagnostics



Rationale for developing DNA microarrays for diagnosis of blood-stream infections

Conventional testing



0 h
4 h
8 h
12 h
16 h
20 h
24 h
28 h
32 h

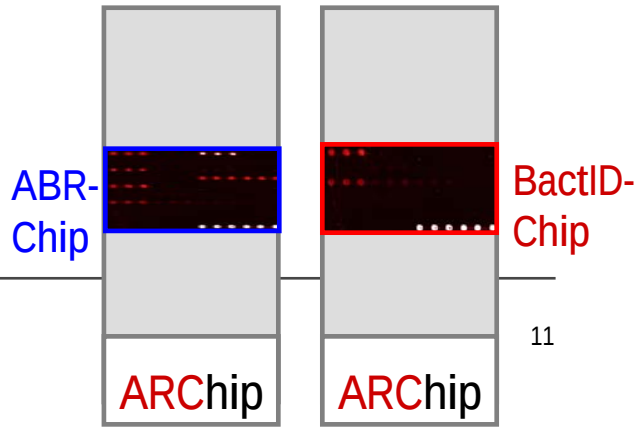
DNA Chips

0 h
4 h
8 h
12 h
16 h
20 h
24 h
28 h
32 h

DNA isolation direct from blood (ev. short enrichment)

Amplification of pathogen DNA (incl. fluorescence labeling) + detection on Chip

DIAGNOSIS
pathogen + AB-resistance



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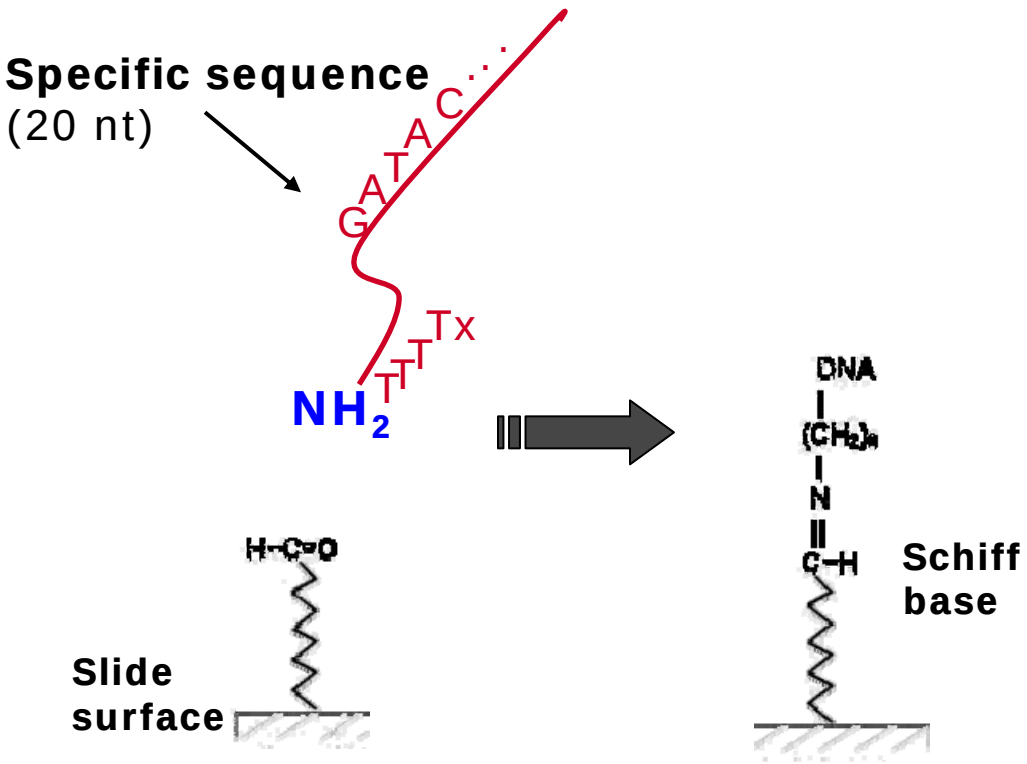
Antibiotic Resistance Chip (ABR-Chip)



Phenotypic resistance	Gene	Probe (position)
ß-Lactam-antibiotics, Oxacillin	**mecA = PBP2a = PBP2'**	ID 1
Kanamycin	**aphA-3**	ID 2
Methicillin (Oxacillin)	**mecR**	ID 3
Trimethoprim	**dhfrA**	ID 4
Streptomycin	**aadA**	ID 5
Vancomycin	**vanB**	ID 6
Erythromycin	**ermC**	ID 7
Penicillin, Aminopenicillin	**blaZ**	ID 8
Chloramphenicol	**cat**	ID 9
Tetracyclin	**tetC**	ID 10
Fosfomycin	**fosB**	ID 11
Gentamycin	**aacA-aphD**	ID 12

Probe Design
Tm +/- 2°C

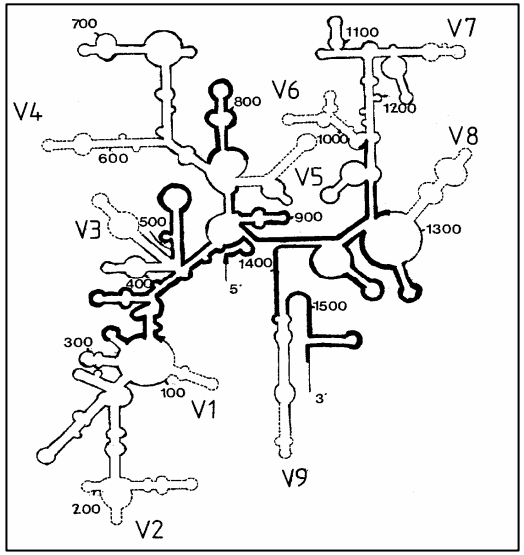
Staphylococcus epidermidis

BactID-Chip

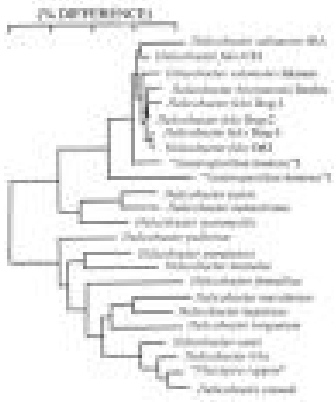
- Stapyhlococcus epidermidis
- Staphylococcus aureus
- Enterococcus faecalis
- Enterococcus faecium
- E. coli

= BactID prototype

16S rRNA approach



Probe design



PROBE	clk1361	clk469	clk639	klep203hp	klep216	klep61	ecl449	ecl471	ecl461	ecl633	ecl643	ecl652	ecl90hp
Le	22	22	22	18	21	20	26	21	28	21	21	22	1
Tm	61	60	60	59	60	61	60	61	59	60	60	60	6
GC%	55	55	55	67	57	65	46	57	39	57	57	55	7
CitKose2	0,0	0,0	0,0	1,2	0,8		1,2	0,3		3,8	2,4	2,0	2,
CitKoser	0,0	0,0	0,0	1,2	0,8		1,2	0,3		3,8	2,4	2,0	2,
CitFarme	0,7	3,4	0,8	1,2	1,3		4,3	2,3		0,6	2,4	2,0	2,
EteGroup	0,7	3,9	0,8	1,2	1,3		2,7	3,5		0,6	2,4	2,0	1,
CitAmalo	0,7	3,4	0,8	1,2	1,3			2,3		0,6	2,4	2,0	2,
CitRoden	0,0	2,7	0,8	1,2	2,5		2,4	2,3		0,6	2,4	2,1	2,
CitSedla	0,7	2,3	0,8	1,2	2,5		2,4	2,6		0,6	2,4	2,0	2,
KlePne17	1,5		2,4	0,0	0,0	0,0				0,6	1,2	0,8	1,
KlePne41	1,5		2,4	0,0	0,0	0,0				0,6	1,2	0,8	1,
KlePne26	1,5	2,9	2,4	0,0	0,0	0,0		1,8		0,6	1,2	0,8	1,
KlePne27	1,5		2,4	0,0	0,0	0,0				0,6	1,2	0,8	1,
KleSpec3	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KleSpec4	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KleSing2		2,8	2,4	0,0	0,0			2,0		0,6	1,2	0,8	
NtxBact2	0,8	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KleSpec5	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KlePne42	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KlePne43	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KlePne39	1,5	2,9	2,4	0,0	0,0	0,0		1,8		0,6	1,2	0,8	1,
SerLiqu2	1,5	2,9	2,4	0,0	0,0	0,0		1,8		0,6	1,2	0,8	1,
KlePne28	1,5	2,8	2,4	0,0	0,0	0,0		2,0		0,6	1,2	0,8	1,
KleSpeci	1,5		2,4		0,0	1,2				0,6	1,2	0,8	1,
KlePne25	1,5	3,3	2,4	0,0	0,0	0,0		2,4		0,6	1,2	0,8	1,

Recently extended

includes now
22 bacterial species +
4 Candida species
relevant for blood-stream infections

ARB software, CalcOligo

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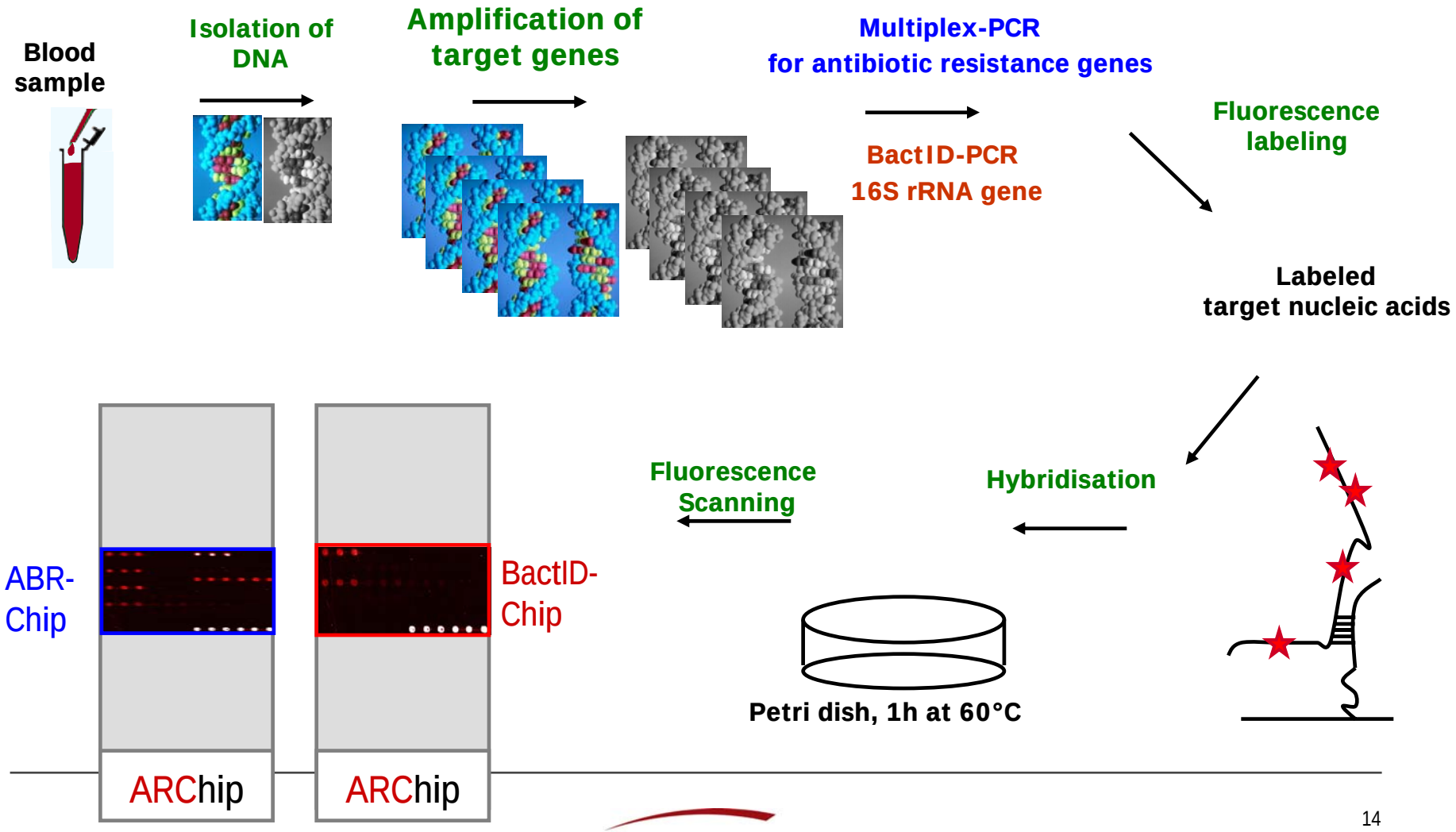
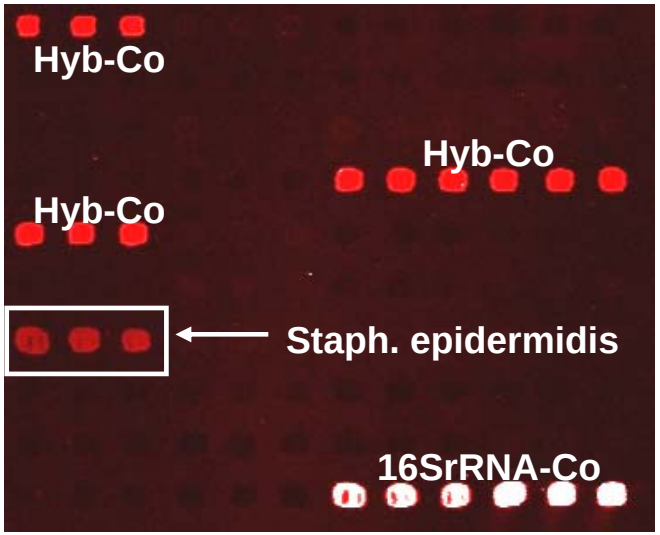
Procedure ABR / BactID-Chip

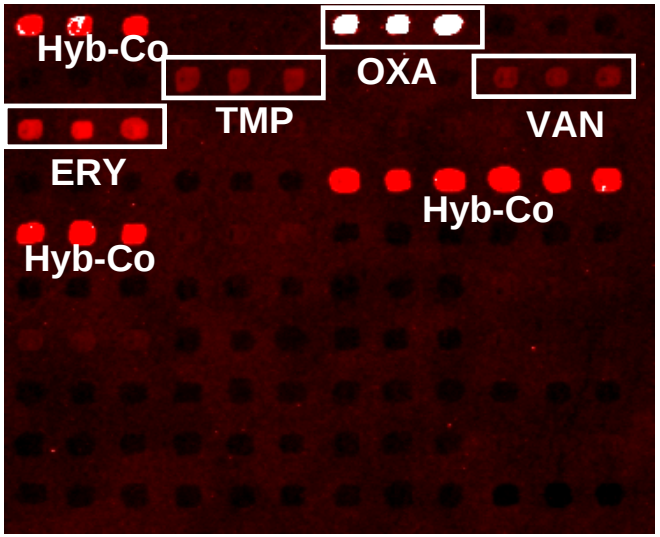


Typical Scanner Image ABR / BactID-Prototype-Chip

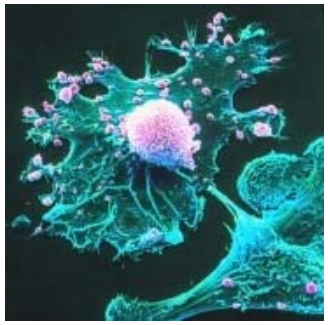
Bact-ID



ABR

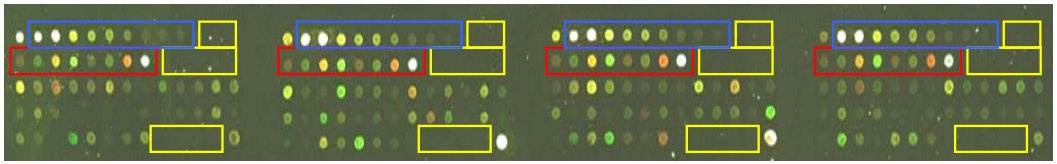


- Tumor diagnostics



Tumor Diagnostics: Breast Cancer Chip

Use gene signatures for better cancer diagnosis / prognosis

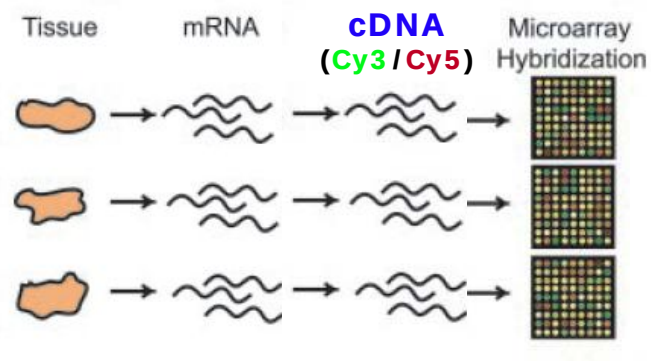


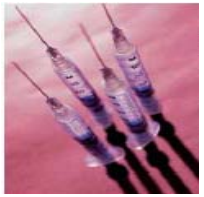
Surface: ARC Epoxy
Spotter: Omnigrid Arrayer (GeneMachines)

Oligo length: 65mers

Calibration Controls
Ratio Controls
Buffer

- **Prototype chip established**
  → 91 breast cancer relevant genes,
  (incl. calibration-, neg. – and pos. controls)
- **Working protocol optimized**
- **Reliable RNA Amplification method established**





Human growth hormone targeted Microarray:

Development of a targeted DNA microarray to identify specific changes in blood cell gene expression related to the administration of human growth hormone
→ DOPING CONTROL

First project phase:

Feasibility study

- **in vitro** - studies in hgh-treated cell lines representing defined types of blood cells → monocytes (THP-1), T-lymphocytes (H9) und B-lymphocytes (RA-1)

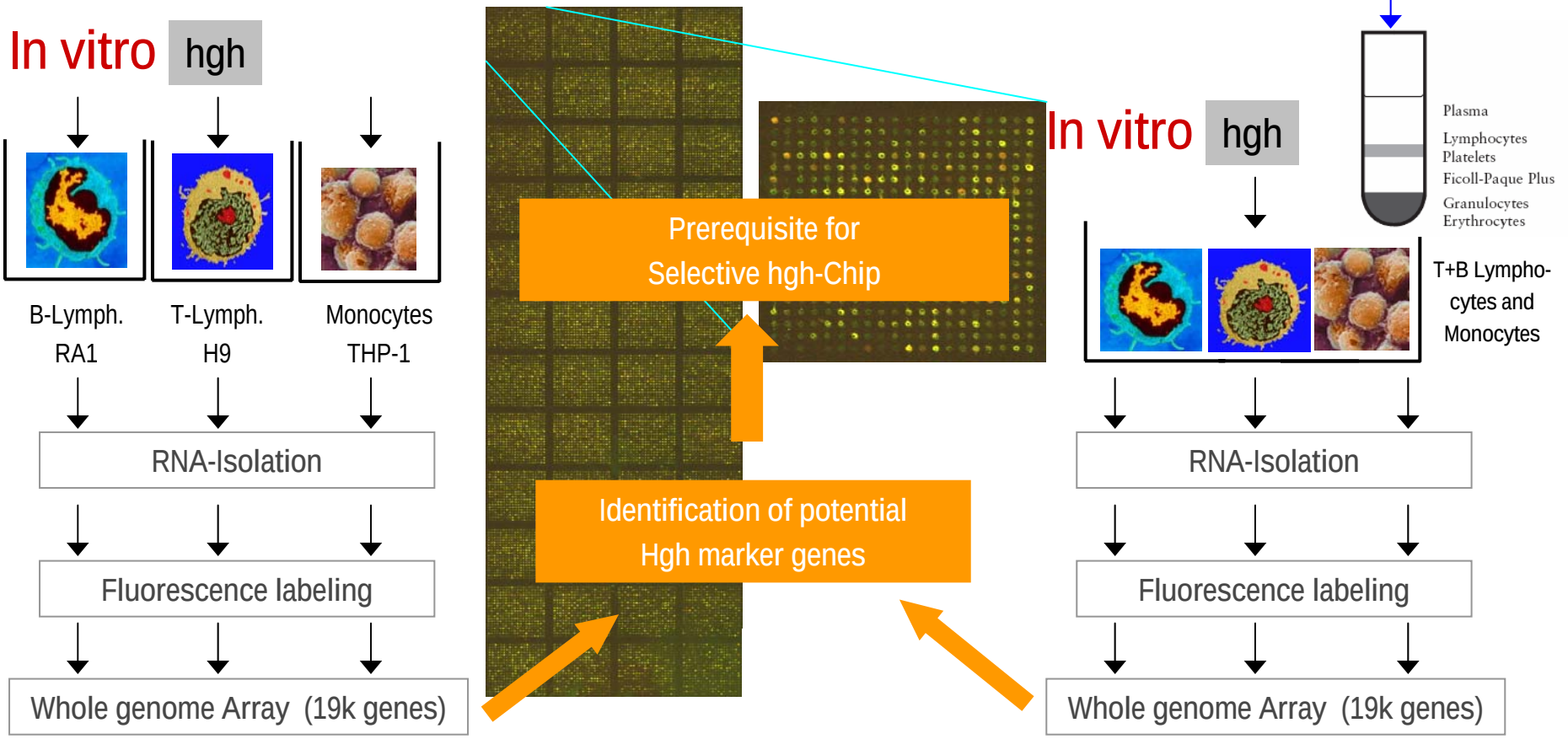
- **in vitro** - studies in peripheral blood mononuclear cells (PBMCs) from untreated individuals stimulated with growth hormone

Approach so far

Cell lines
in cell culture

1. ←

→ 2. **Lymphocytes/Monocytes**
Obtained from blood donors by gradient centrifugation (Ficoll)



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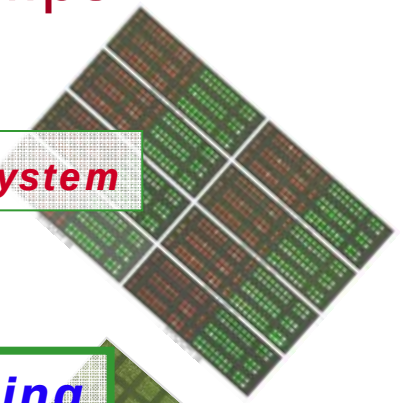
Tumor Diagnostics : DNA Methylation chips



„proof-of-concept“ via CpG methylation test system

methods optimisation

enrichment strategies

Whole-Genome Methylation Screening

30 tumor vs normal DNAs
12k CpG Island Microarray

microarray-optimization

Candidate Tumor-Markers Chip

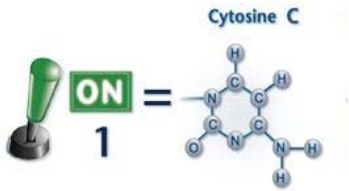
200 patient samples
CpG OligoArray

Final Tumor-Markers

clinical validation

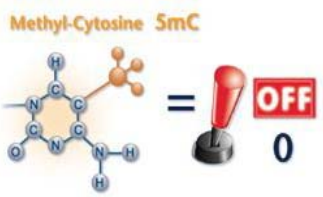
Diagnostic Test

Genome wide hypomethylation



Activation of Protooncogenes → CANCER

Focal hypermethylation



Deactivation of tumor suppressor genes → CANCER

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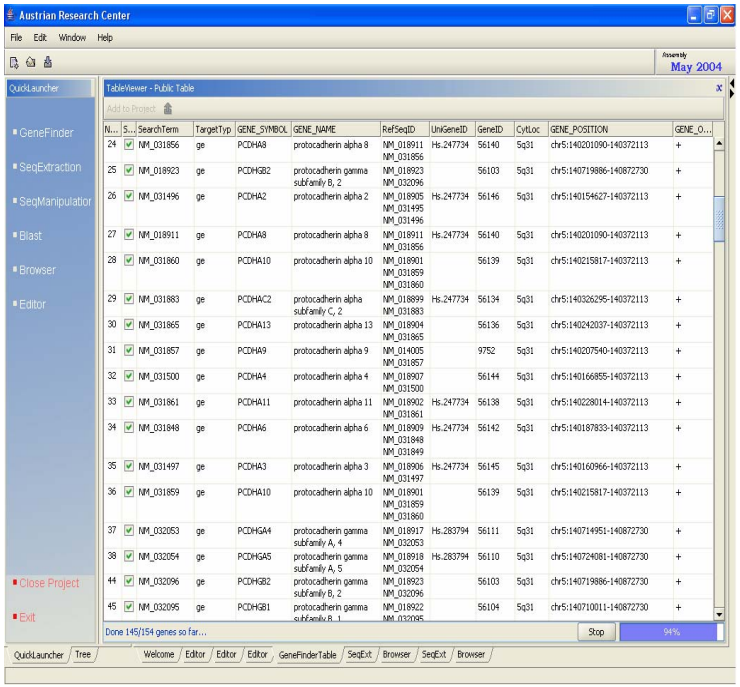
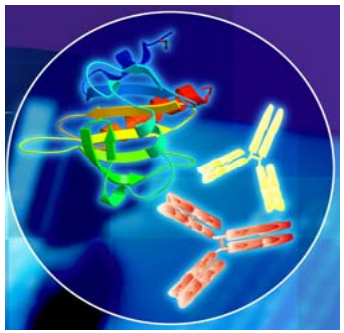
Bioinformatics:
Software for high-throughput probe design

ARC Gene Filter

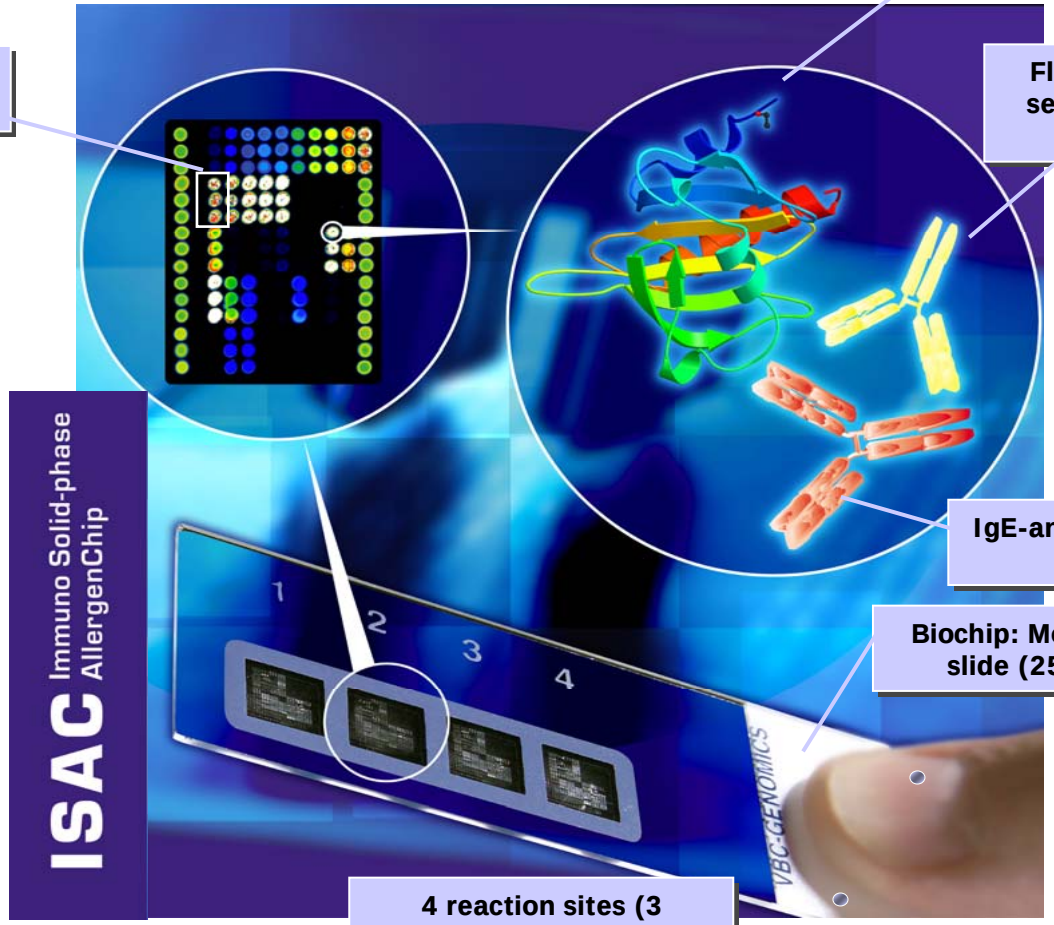
- Modules „Gene Finder“, „Sequence Extractor“, „MethAnalyzer“
- Target sequence selection and high-throughput probe design
- Applications: DNA-Methylation -, SNP – and gene expressions chips



- Allergy diagnostics



Allergy Diagnostics : Protein chips



Allergen molecule

Allergen-triplet after fluorescence-reaction

Fluorescence-labelled secondary anti-human IgE antibody

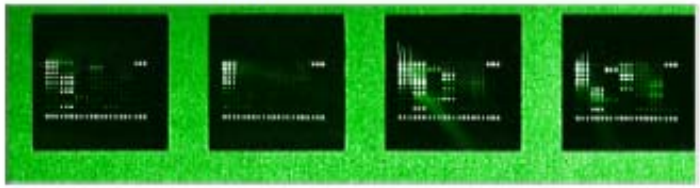
IgE-antibdy (in patient's-serum)

Biochip: Modified glass slide (25x75 mm)

4 reaction sites (3 samples + 1 control)

Tumordiagnostic-Chips: Protein-Pilot-Chip

ISAC CRD48a



ProteoBind (VBC

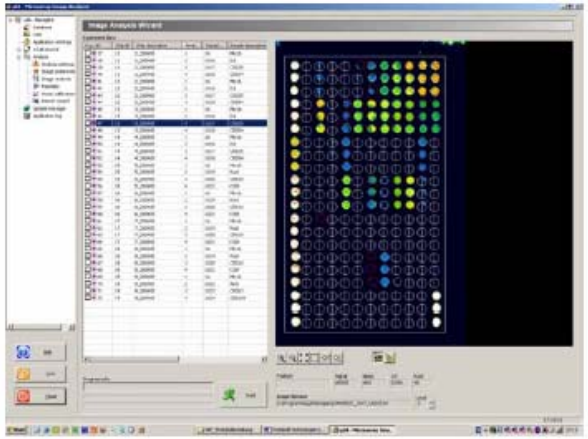
ISAC CRD48a

ARChip Epoxy

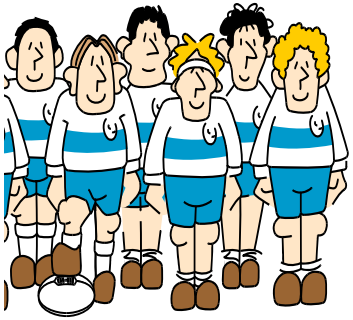
Immuno Solid-Phase Allergen Chip (ISAC)

component resolved diagnosis of allergies

- Panel of 48 recombinant allergenes tested (CRD48a)
- Surface comparison: ARChip Epoxy vs. VBC ProteoBind
- Chip spotting:
  Pin-tool Arrayer (ARC-sr) vs. Ring&Pin Arrayer (VBC)
- ISAC-software evaluation



The MOLECULAR DIAGNOSTICS Team



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THANK YOU