Service Opportunities provided by the DKFZ Genomics & Proteomics Core Facility

19.11.2008, ZMBH

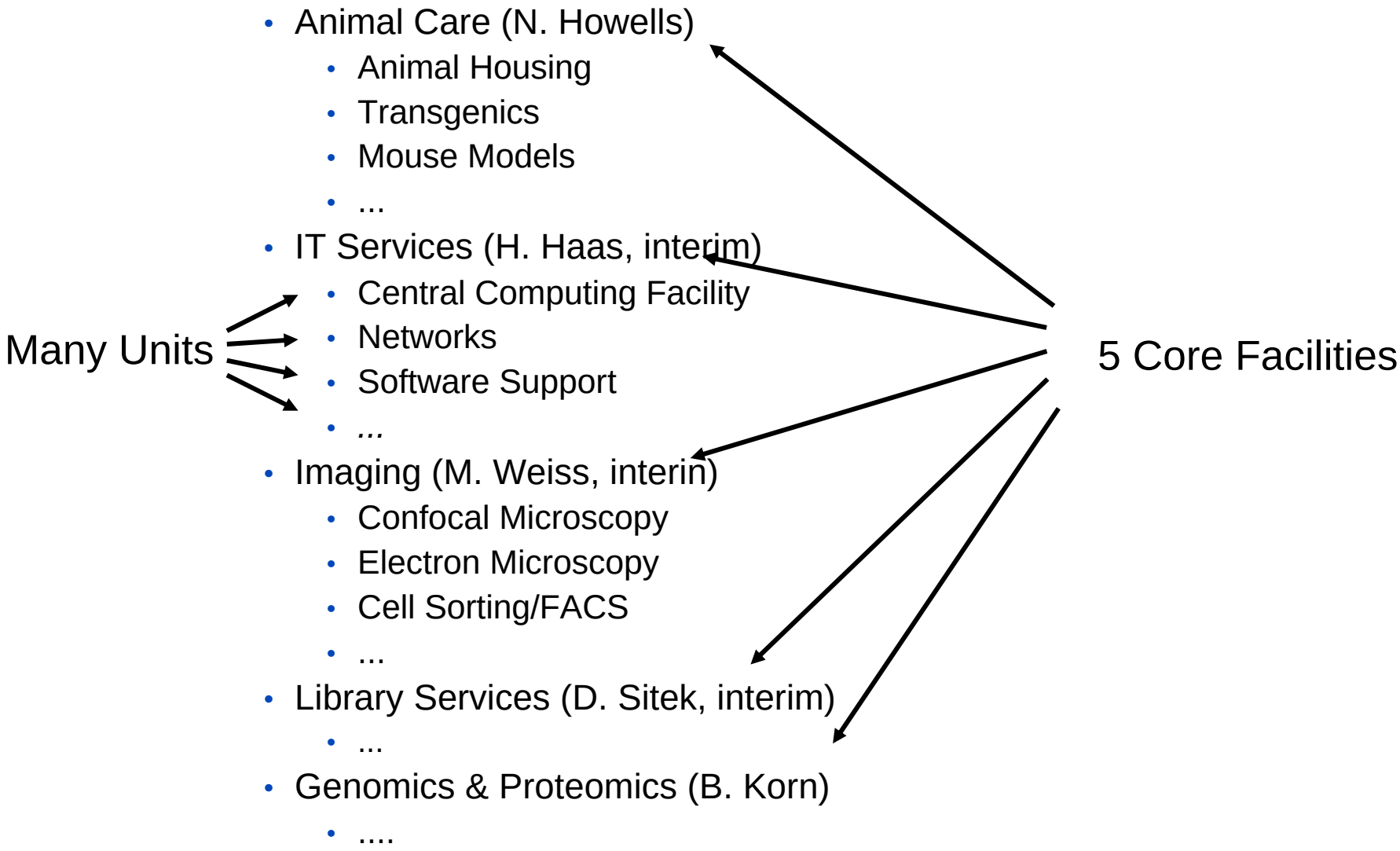
dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Objectives

- State-of-the-art Services and Technologies
- Service at highest Quality
- Ensure short Turn-around Time
- Equal Access
- Dedicated Personel and Responsibility for Service Duties
- Education
- Discriminate between Service and Research
- Capacity Utilization / Investment Cost / Efficiency
- Fee for Service
- Annual User Review

- Scientific Infrastructure (W1xx)
- CF Committee (WiRa)

Structure of Core Facilites



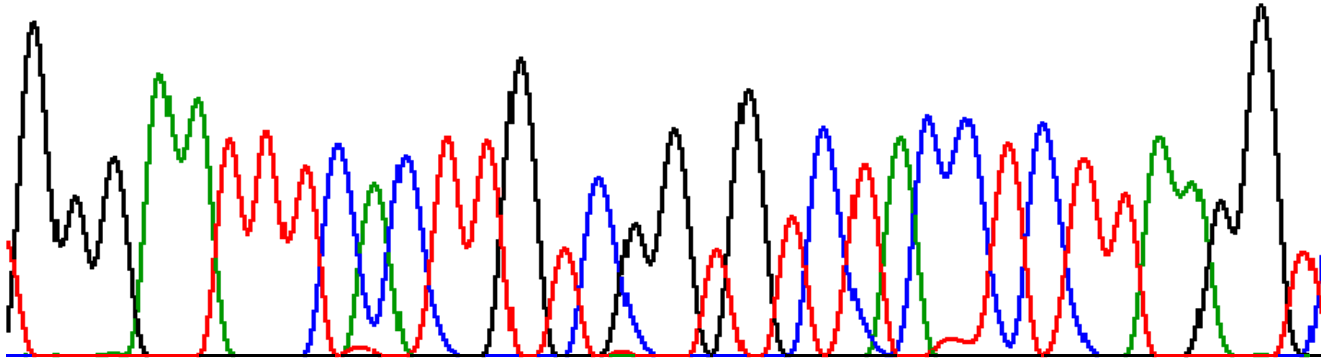
Ways to provide service

- Full service
  - Order of user, return of product/information (e.g. peptide, expression profile...)
- Supervised access
  - Central investment and expertise provided, but user is key for work (e.g. 2D gels, Real-time PCR)
- Trans-center service
  - One center provides established servcie for another/multiple other centers (e.g. chemical screening service by Joe Lewis (EMBL/DKFZ)
- Public-private service
  - Service provided together with private company (e.g. sample preparation in-house, external service, analysis in-house (Rahmenvertrag Protagen))
- Outsourcing

Genomics & Proteomics Core Facility (GPCF)

- **Sequencing**
- **High Throughput Sequencing**
- Genotyping
- Methylation Analysis
- **Expression Profiling**
- **miRNA Profiling**
- **Peptide Synthesis**
- Protein Analysis
- **Protein Interaction Screening**
- Molecular Structure Analysis (**NMR**, non-protein MS, Modeling)
- **Vector and Clone Repository**
- **Contamination Control**
- HUSAR
- Assisted Access
- **wetlab wiki (ww@DKFZ)**

DNA-Sequencing



Sanger Sequencing

Contact

- Andreas Hunziker (-42 4669, a.hunziker@dkfz.de)
- INF580, room B1.306.

We offer

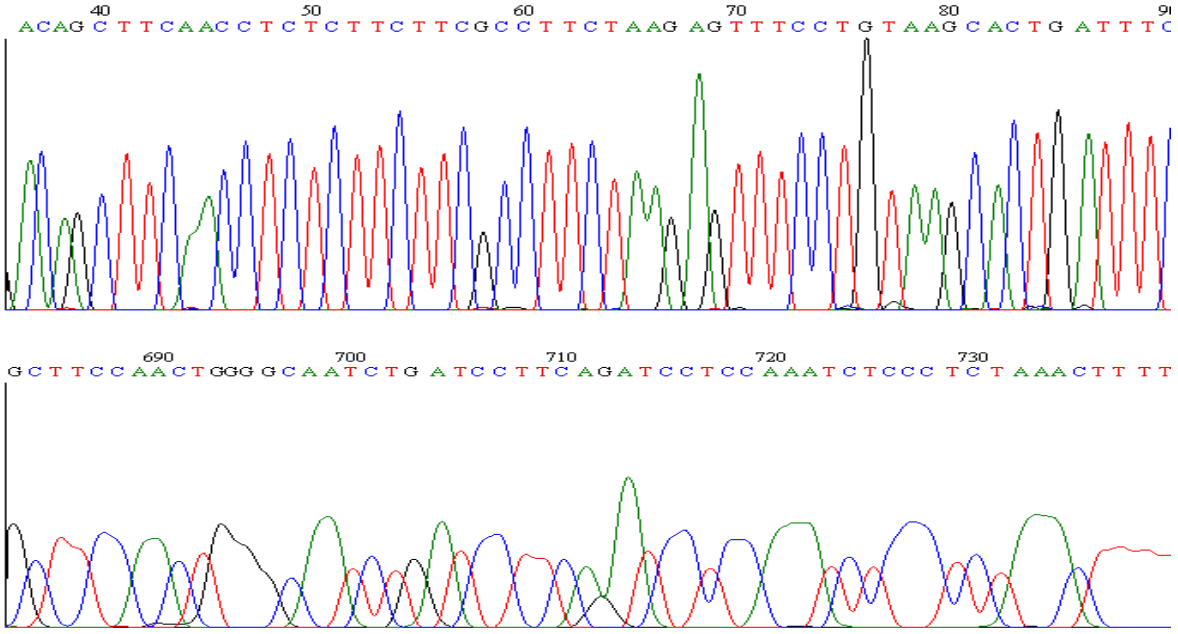
- DNA-sequencing of purified DNA
  - Plasmid (~300 ng at >40 ng/µl)
  - PCR product (~10 ng/100 bp)
- >100 vector primers on stock

Turn-around Time

- 1-3 working days
- You may be able to get the fastest turnaround time by bringing the samples before 9:30 AM.

Read-length

Our sequencing reaction read-lengths are normally between **600 and 800 bases** and starts **1 to 20 bases after the primerbinding site**.



What you get

- Normally we send the sequence as a text file by Email. When desired, you can also get the chromatogram.

Cost of Service

- The basic cost of DNA sequencing for processing one template with one primer is
- € 5 for raw data,
- € 10 for an edited sequence and
- € 5 for a new designed primer

Next Generation Sequencing

Stephan Wolf

Genomics & Proteomics Core Facilities, DKFZ

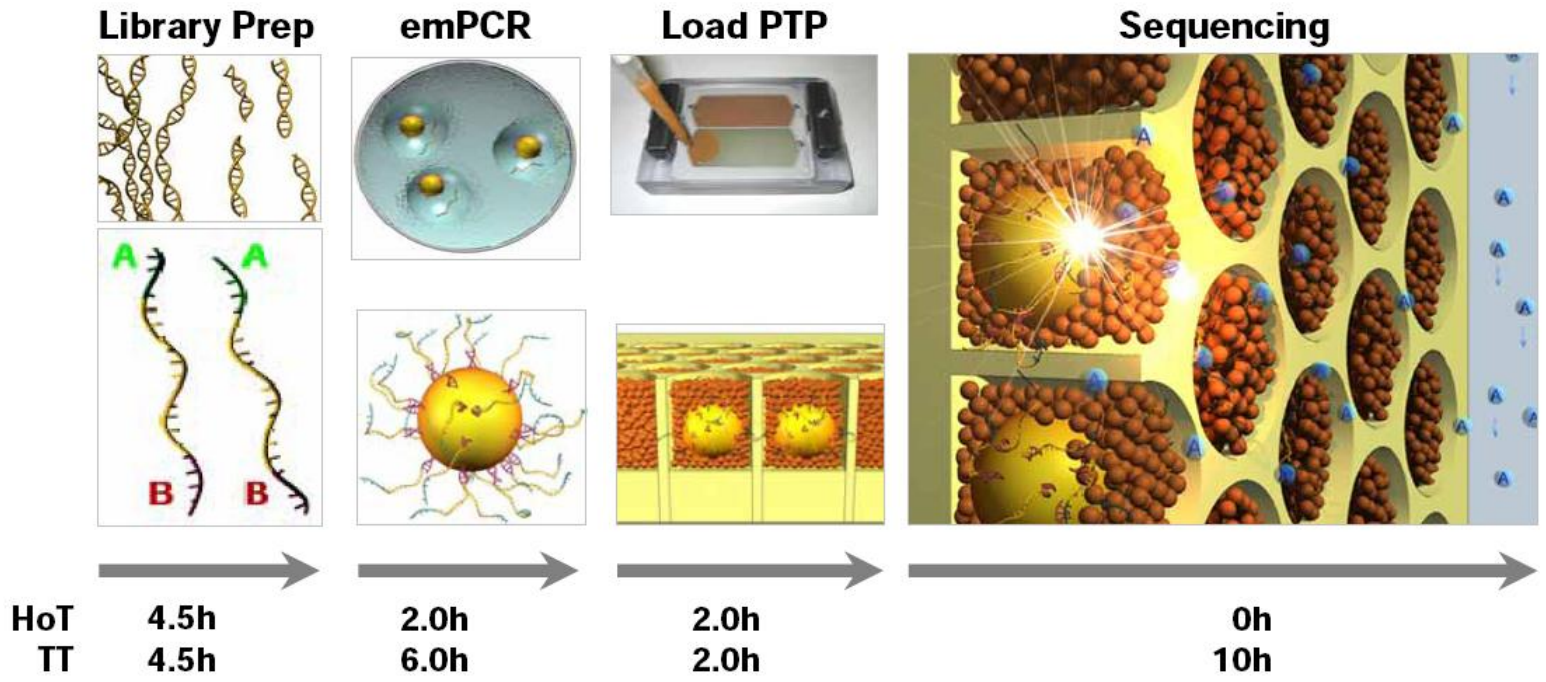
GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Next Generation Sequencing: Overview

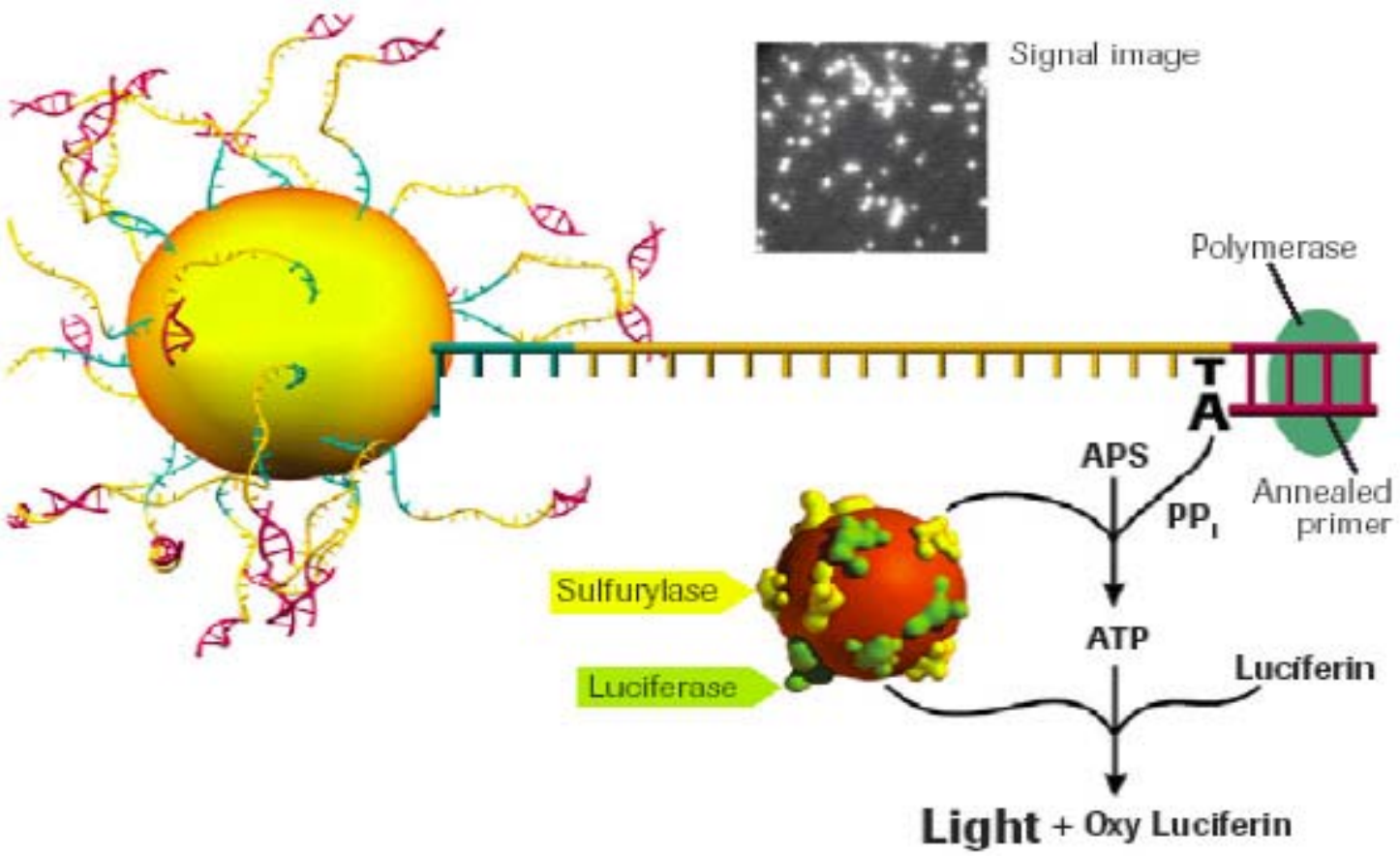
- Contact
  - Stephan Wolf, -42 3409
- Offer
  - Highly parallel DNA sequencing service based on the 454/Roche technology (“next generation sequencing”)
  - Assistance in experimental design
  - Sample preparation starting from customer prepared DNA (typically 3-5 µg of DNA)
  - Sequence analysis
  - Basic bioinformatics support
- Outlook
  - Tag System in early 2009 planned (Solexa, Solid)
  - DNA-Chip based preselection of relevant DNA regions from DNA libraries

Next Generation Sequencing: Workflow

- FLX/Titanium Workflow



Next Generation Sequencing: Workflow



See http://www.dkfz.de/gpcf for details, application and references

Next Generation Sequencing: Performance

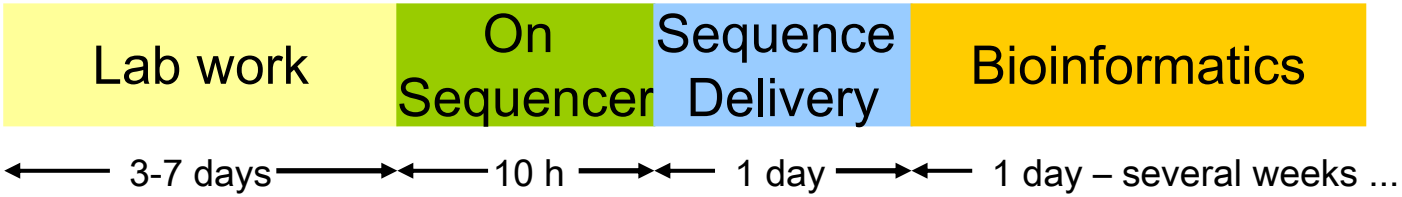
	Standard-series	Titanium-series
Run Time	<8 hours	<10 hours
Read Length	250-300 bases	350-450 bases
HQ Reads per Run	~400,000	~1,000,000
Single-Read Accuracy	>99.5% over 250 bp	>99% over 400 bp
Sample Input	Typically 3-5 µg of DNA (can be as little as 100 ng)	
Data	SFF trace data, FASTA, Consed	

Next Generation Sequencing: Set Up

- 75x75 PicoTiterPlate
- 2,4,8,16 lanes – 100, 67, 52, 46 Mbp
- 25x75 PicoTiterPlate
- 1,4 lanes – 12, 20 Mbp
- Long reads (250bp) versus short reads (100bp)
- 12 GS Multiplex Identifiers (MIDs) to pool up to 12 different samples per lane
- ...

Next Generation Sequencing: Summary

- Personell
  - Stephan Wolf
  - Anne Arens (Bioinformatics)
  - André Leischwitz (Technical assistance)
- Turn-around Time
  - ~ 2-3 weeks per single run



- Quality
  - Quality control at all levels
- Cost
  - ~ Euro 3500 – 12000

Expression Profiling

Bernhard Korn

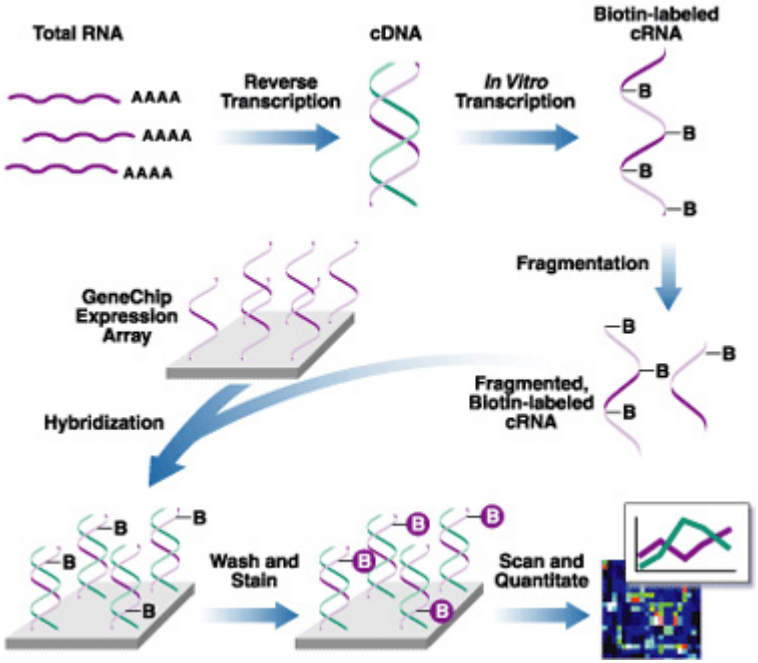
Genomics & Proteomics Core Facilities, DKFZ

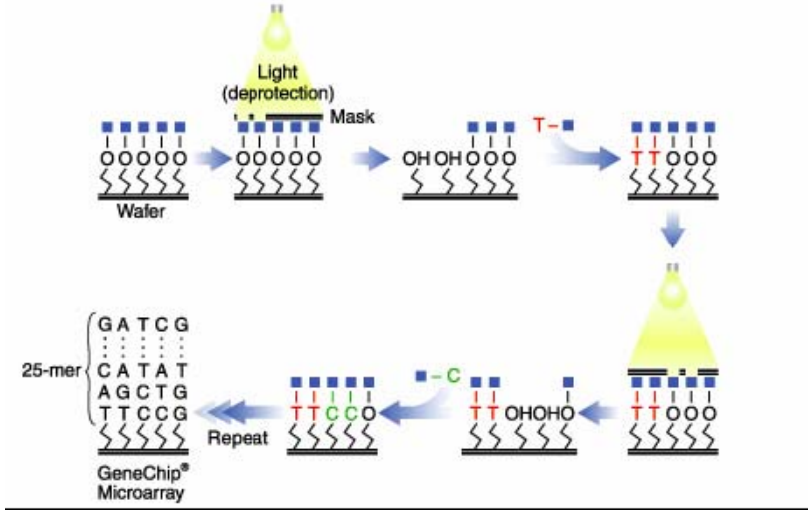
dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

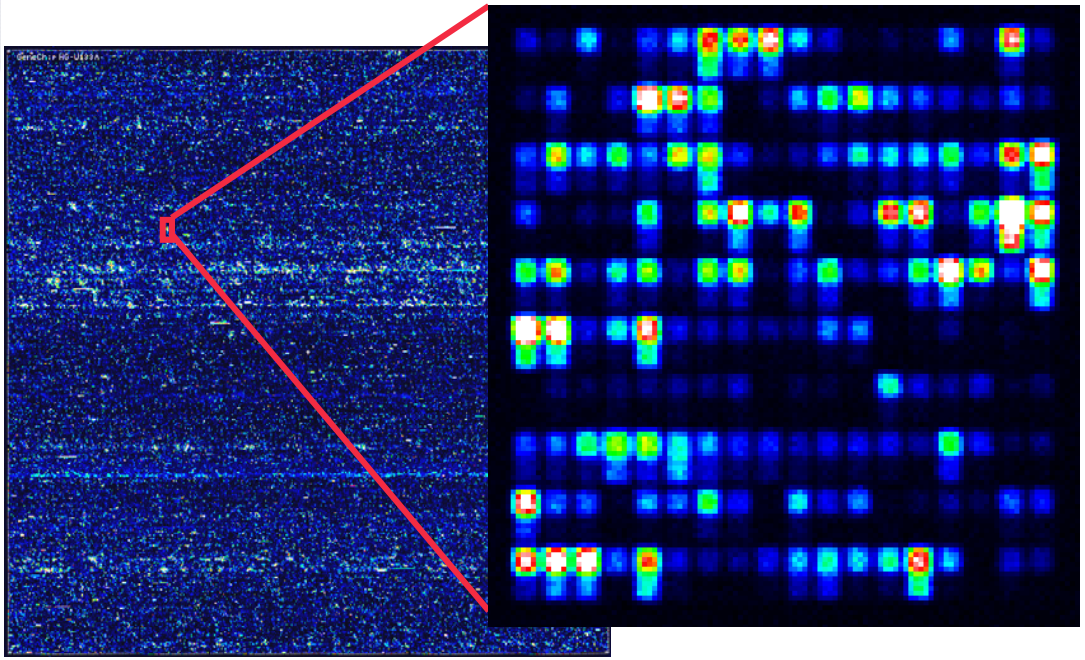
Overview: Expression Profiling

- Contact
  - B. Korn (b.korn@dkfz.de, -42 4700)
- Offer
  - Whole Genome profiling
    - Illumina (human, mouse, rat; ~750 ng total RNA required)
    - Affymetrix (wide species portfolio, exon chips; 5 µg total RNA required)
    - Small comparison projects (list of regulated genes)
    - Large scale studies (complex analysis, gene signatures)
  - Support in experimental set-up, basic data analysis
  - Courses on expression profiling and analysis, central software support
- Personal
  - Sabine Henze
  - Oliver Heil

Affymetrix

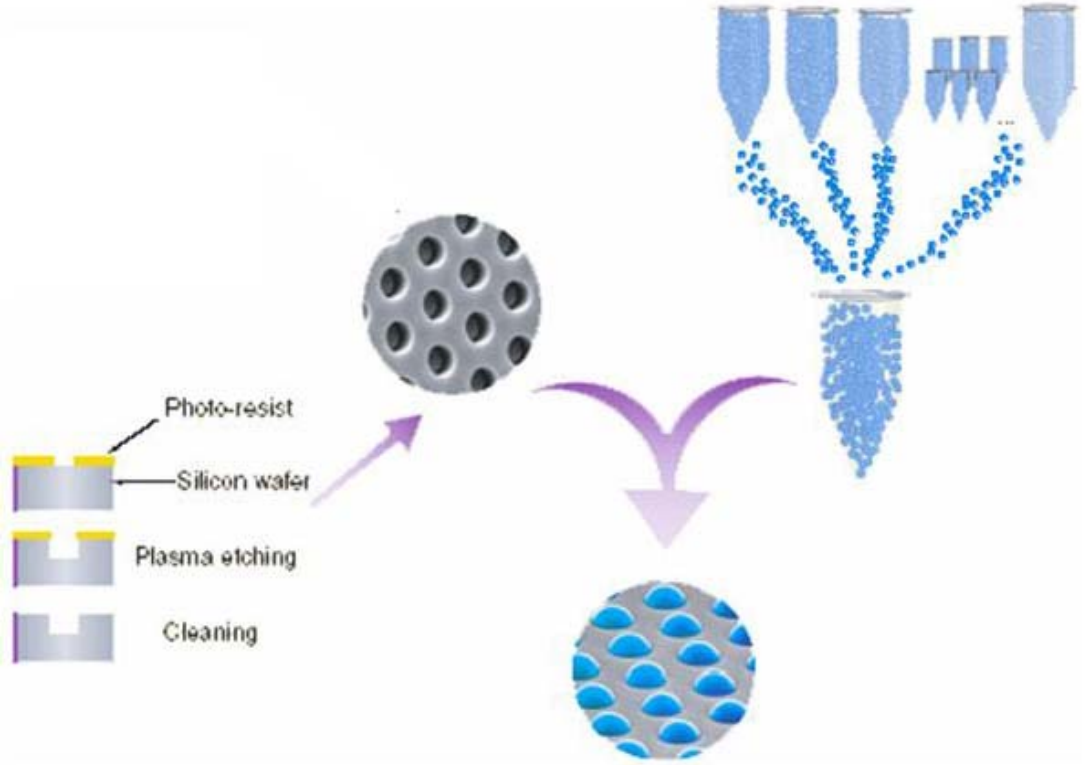




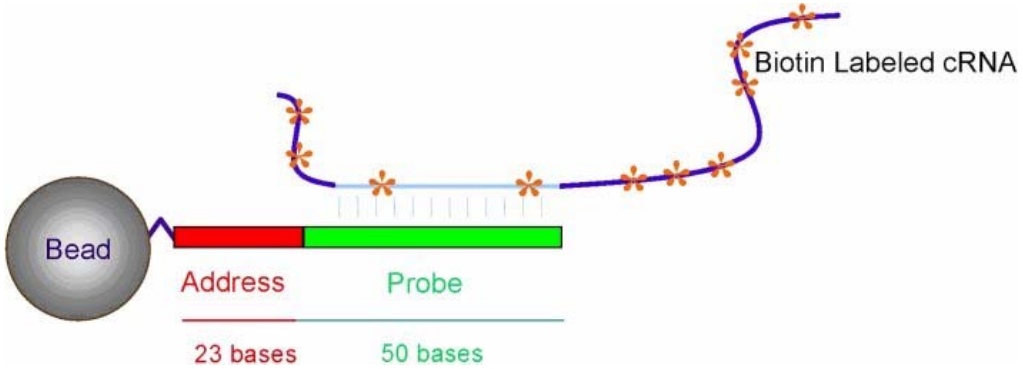


- 25mers
- 11-20 / gene
- 46.000 clusters
- PM / MM

Illumina



- 50mers
- 1 / transcript
- av. >30 replicates
- 23.00 - 48.000 clusters
- 800 – 7200 ctrls
- 1,7 mio beads (statistics)



BeadChip® and GeneChip® Systems

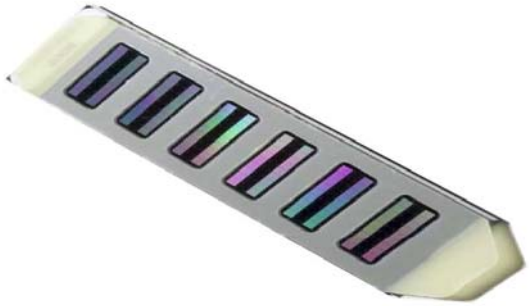
Chip

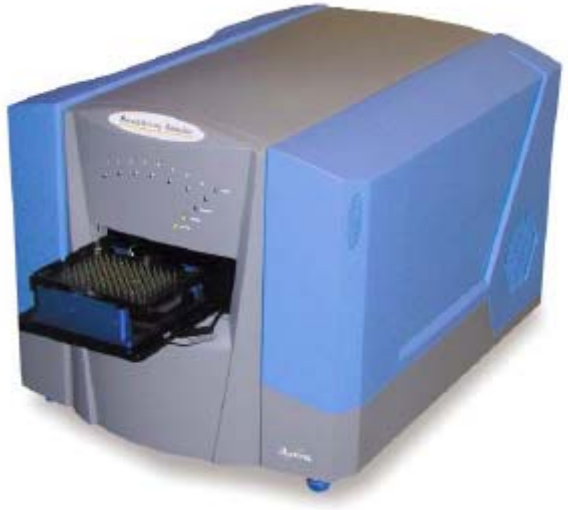
Fluidics Station

Scanner

Affymetrix

Software
Data Analysis





Illumina

Summary: Expression Profiling

- Turn-around Time
  - 2-3 weeks
- Quality
  - Quality control at all levels (total RNA, labeling, fragmentation, hybridization, scanning, analysis)
- Data Return
  - QC Data, Raw Expression Data
  - Interpreted Data (MS Office compatible)
  - Personal Meeting
- Cost
  - Starting at ~200€ / whole genome profile
- Outlook
  - Genome-wide profiling on formalin-fixed tissue
  - DKFZ expression analysis tool ‘Chipster’

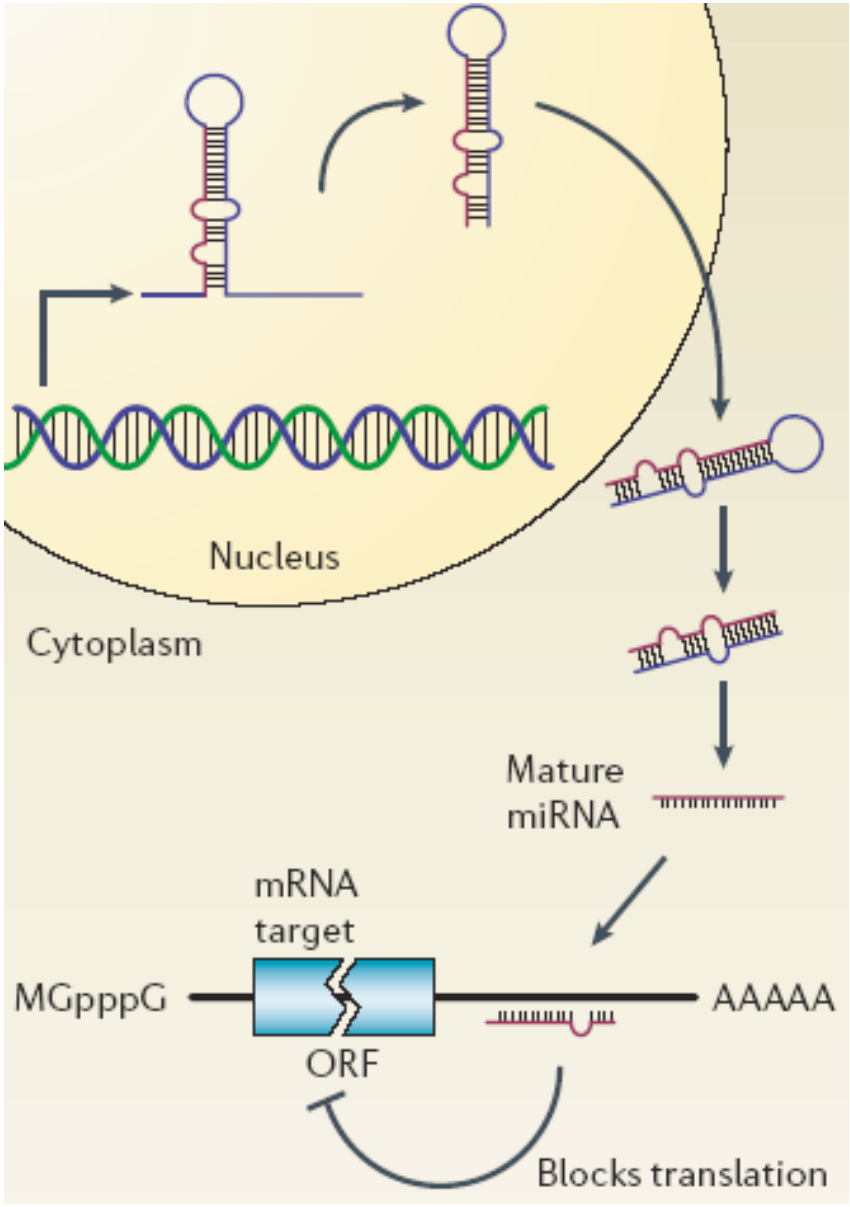
miRNA Overview

- A class of small non-coding RNAs
- Conserved through evolution
- Critical role in development
- Some miRNA species are relevant in variuos types of cancer
- Found in plants, yeast and animals
- Regulate expression of ~30% of all genes
- Deregulation implicated in disease

Genome-wide miRNA Profiling

- ~1150 human mature miRNAs, or >500 mature mouse miRNAs
- Microarray-based
- av. 30-fold redundancy
- Input: 0.5-1 µg of total RNA
- Single color assay

- Procedure:
  - Input QC (conc., quality)
  - Sample handling, hybridization, scanning
  - QC analysis
  - Basic expression analysis



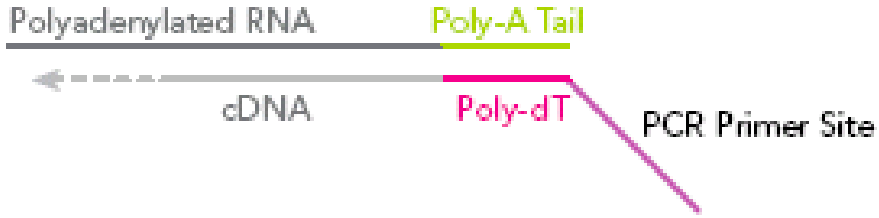
Esquela-Kerscher & Slack, 2006, Nat Rev. Cancer 6, 259

Assay Work Flow: miRNA Profiling

QC:
PAP ctrls

Polyadenylation
(Poly-A-Polymerase)

QC:
mRNA vs.
miRNA ctrls



1st strand cDNA Synthesis
(biotinylated dT-univPrimer)

QC:
internal MM,
neg. ctrls

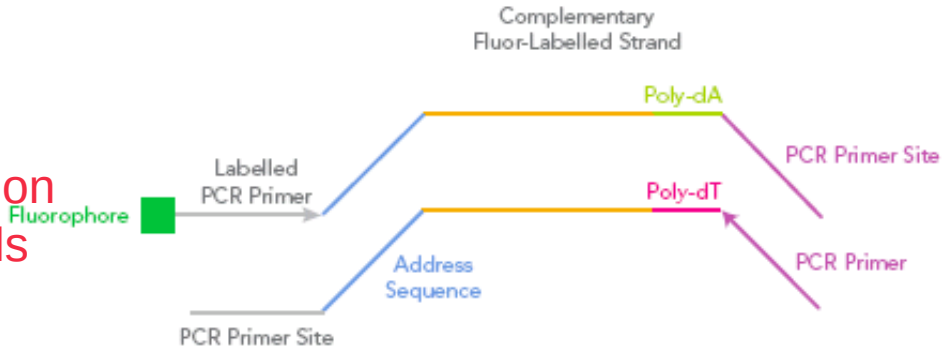
Hybridization of MSOs to immobilized 1st strand cDNA
(introducing address code and 2nd PCR site)

Assay Work Flow: miRNA Profiling

QC:
extension
MM ctrls

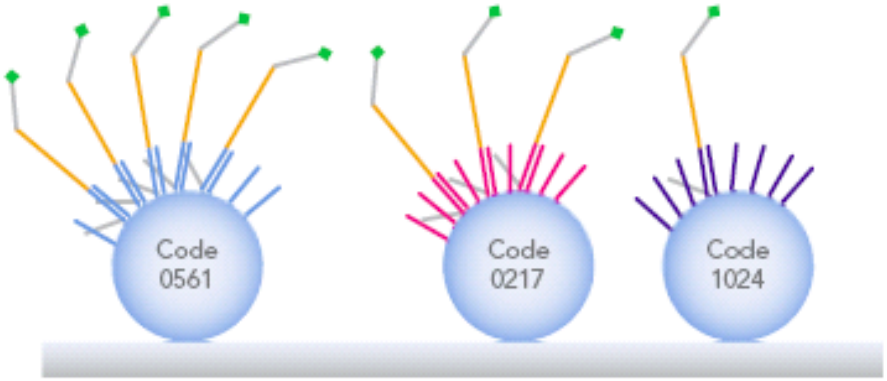
miRNA-specific 2nd strand Synthesis

QC:
contamination
detect. ctrls



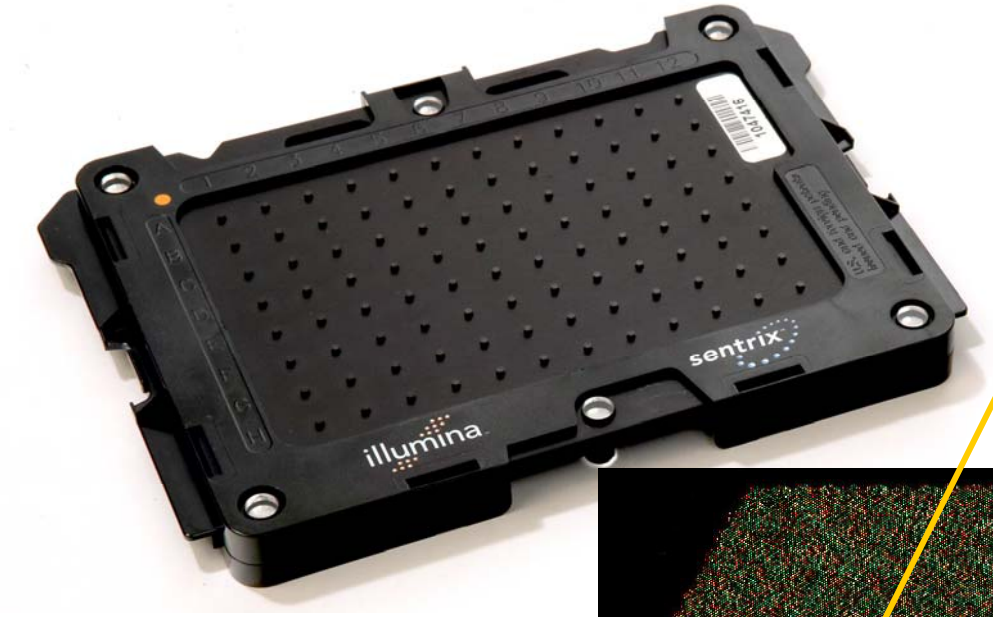
Universal PCR
(introducing fluorophores)

QC:
array hyb.
ctrls



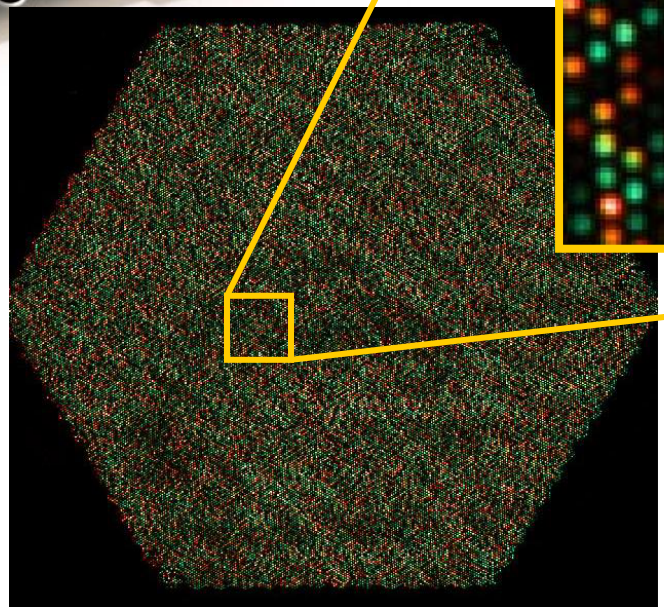
Hybridization to Array
(correlation of address code, miRNA species and intensity)

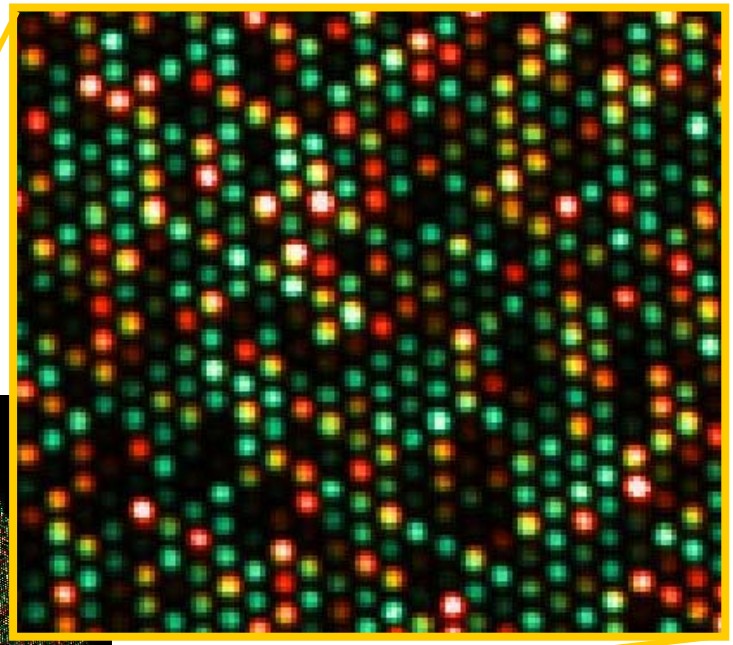
Microarray Format



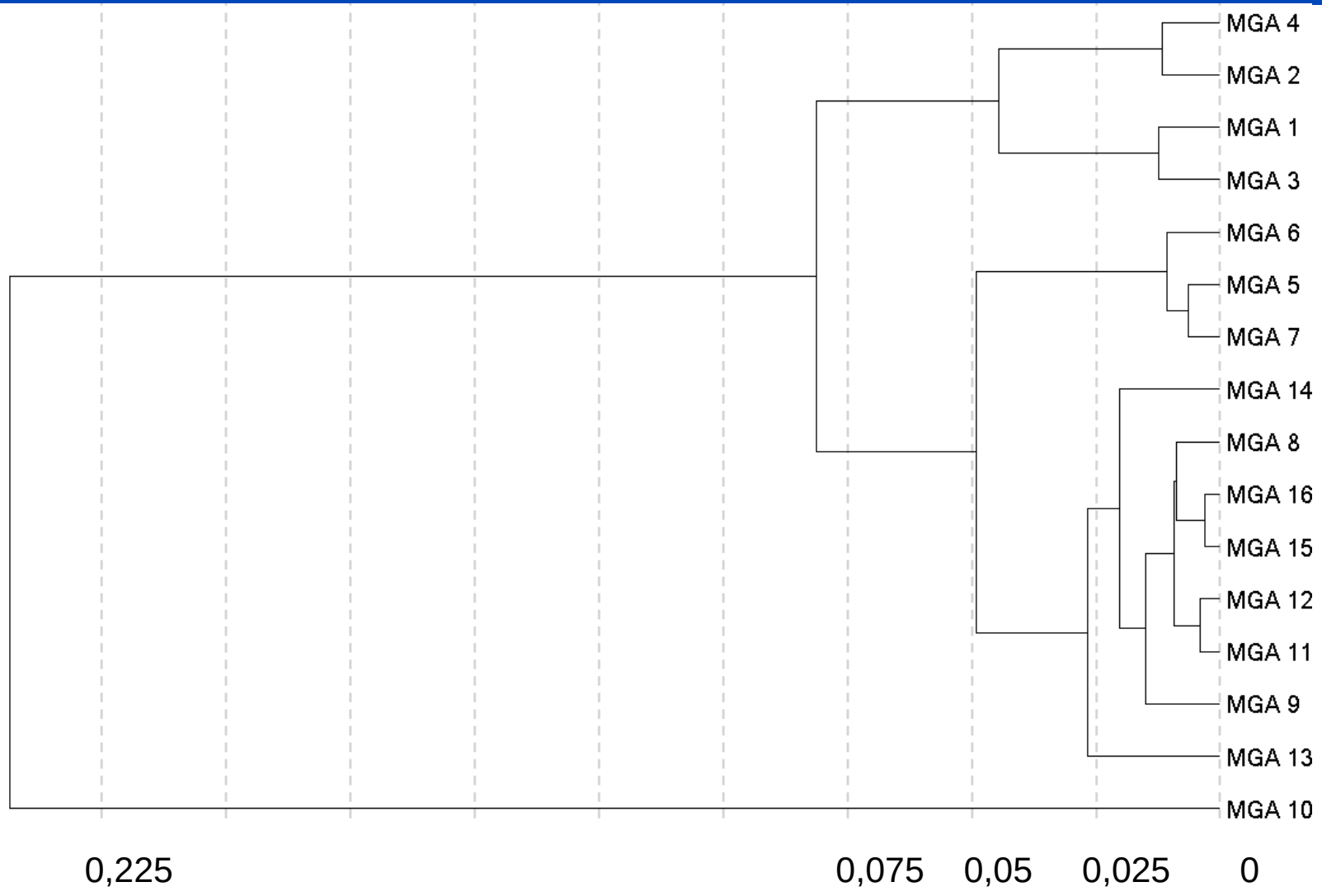
Sentrix® Array Matrix

96 samples in 1 experiment





Sample Correlations (quantile normalized data); Pearson correlation (1-r)



Technical replicates are below 0.01

Tamoxifen treatment leads to modulated miRNA expression (in cell lines and patients)

Summary: Expression Profiling

- Turn-around Time
  - 4-5 weeks
- Quality
  - Quality control at all levels (total RNA, labeling, hybridization, scanning, analysis)
- Data Return
  - QC Data, Raw Expression Data
  - Interpreted Data (MS Office compatible)
  - Personal Meeting
- Cost
  - 155€ / sample (min. 12 samples!)
- Outlook
  - Combined analysis of mRNA and miRNA profiling data
- Personal
  - Sabine Henze
  - Oliver Heil

Genotyping

Bernhard Korn

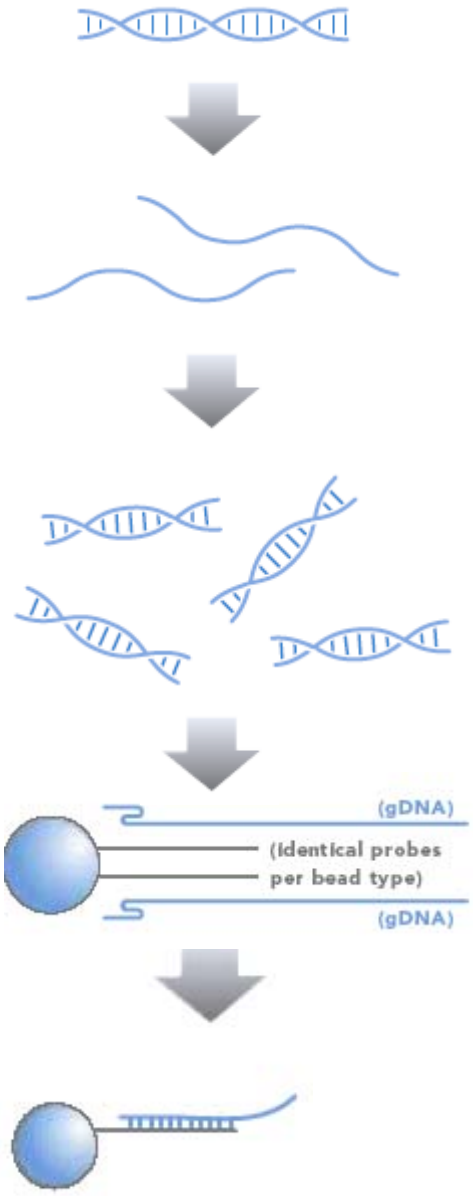
Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview: Genotyping

- Contact
  - B. Korn (b.korn@dkfz.de, -4700)
- Offer
  - Whole genome analysis, using Infinium Technology (370k, 550k, 610k, 1m SNPs)
    - Genotyping
    - Copy number variations
    - Linkage
    - LOH
  - Customized SNP set screening, using GoldenGate Technology
    - <1536 markers
    - >384 samples
  - Basic support for data analysis

GPCF: Infinium Technology I



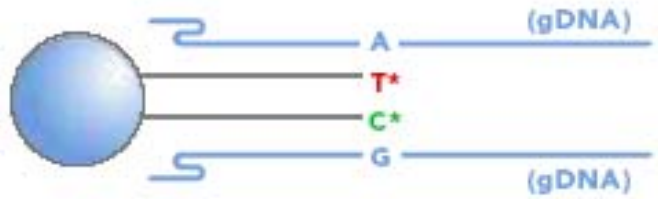
Genomic DNA amplification (WGA)
750 ng

Fragmented DNA
>10 µg

Hybridize DNA to beads on chip

Select at high stringency

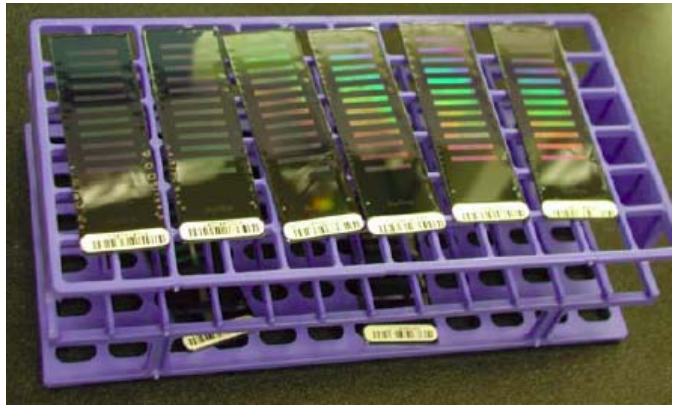
GPCF: Infinium Technology II

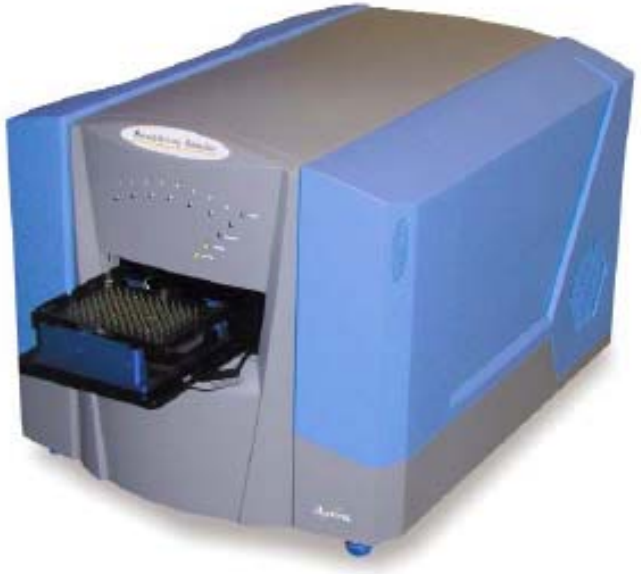


*Stain in red channel
*Stain in green channel

Single base extension, depending on allele

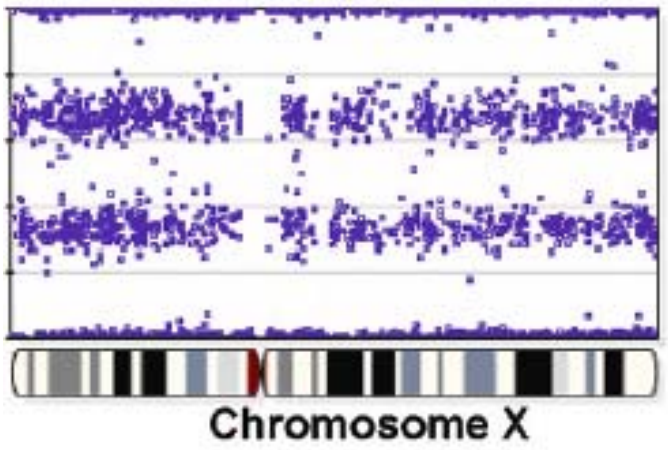
av. >20 replicates



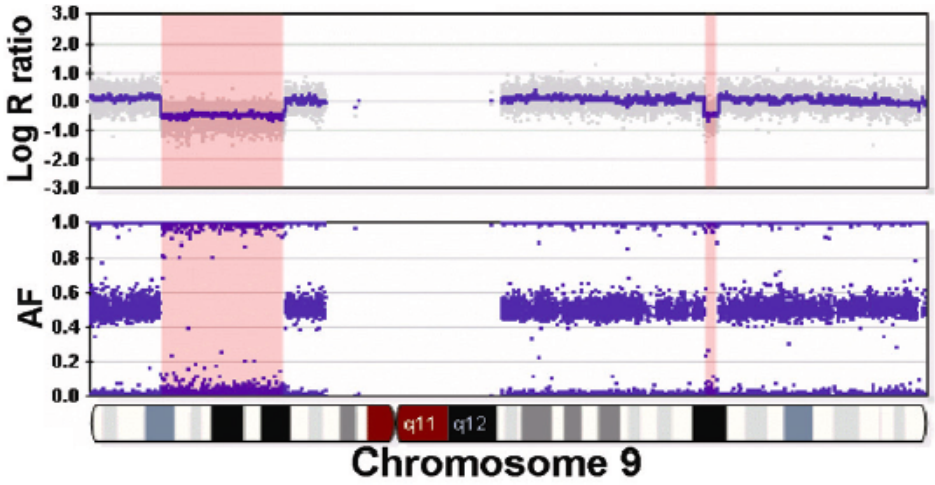


Beadchip: scanning is 15-45 min per genome

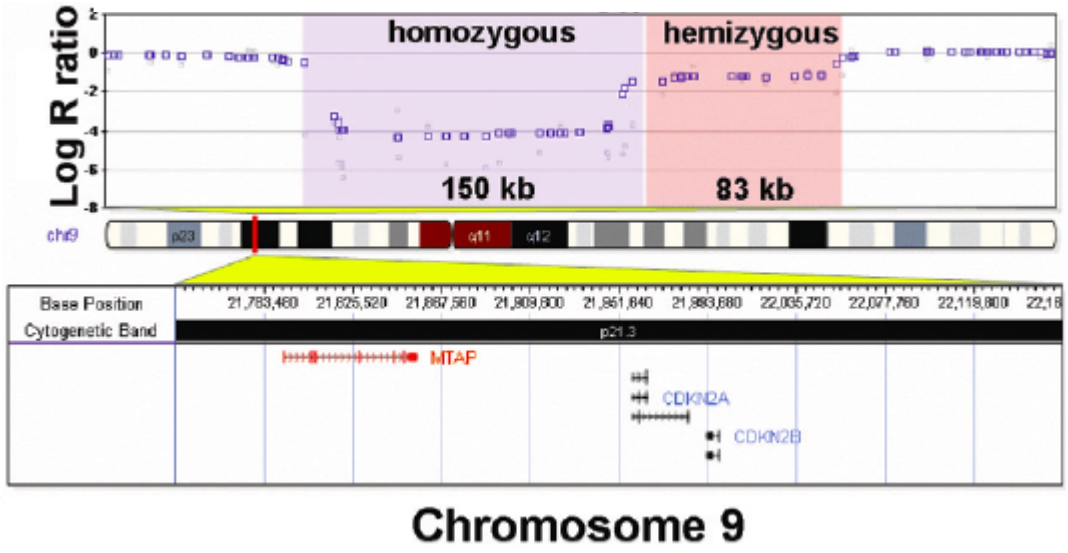
GPCF: Infinium Analysis II



3X female



HL-60
Hemizygous Del

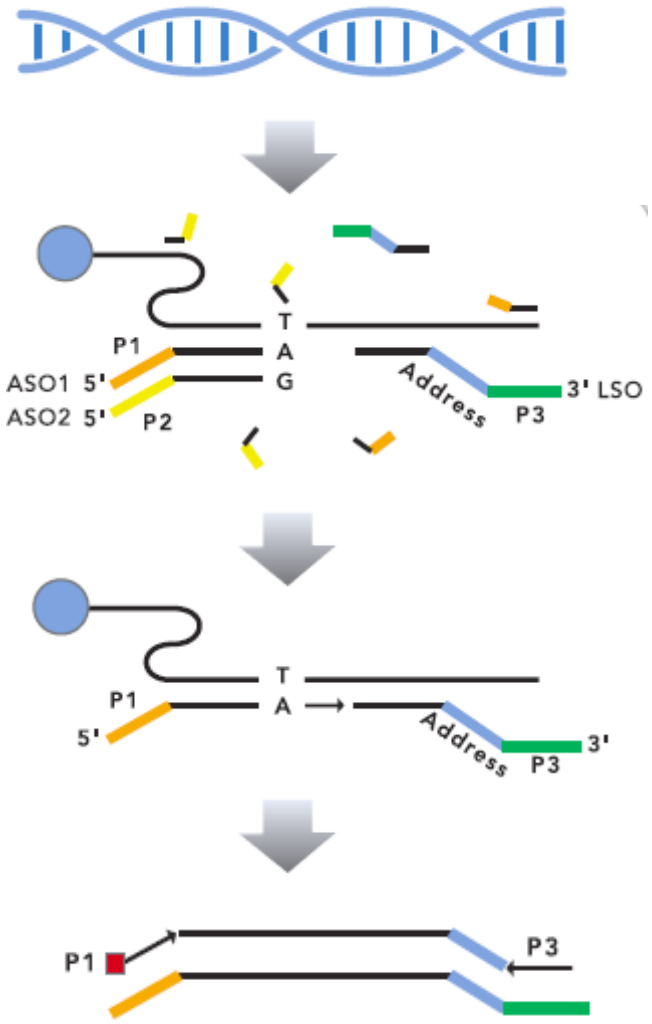


MCF-7
Homozygous and
Hemizygous Del

GPCF: GoldenGate Assays

- Customized genome analysis (96-1536 marker/SNPs)
  - Genotyping
  - *Expression*
  - *Methylation*
  - High throughput (multiples of 96 patients)
- Quality
  - Input DNA QC and conc. (picogreen)
  - Labeling, hybridization and scanning controls
  - Call rates >97-98 % (depending on selected SNP set)
  - Basic analysis (on-board tools)
- Status
  - Pilot projects since 05/07
  - First large scale screen (4.000 patients)
- Outlook
  - Up to 12.000 samples per year
  - Validation of established marker sets and signatures

GPCF: GoldenGate Technology



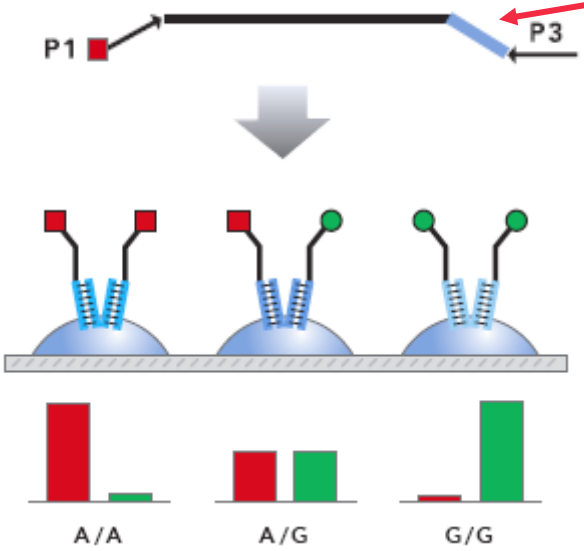
Genomic DNA (>250 ng)

Activate and add primers
(3 oligos per SNP)

Extension and ligation

Universal PCR

GPCF: GoldenGate Technology



address code

Hybridize address code to Beadarray

SAM Beadarray (96 samples)

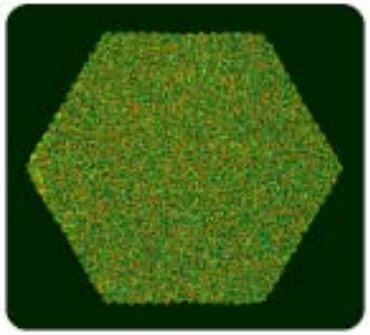


Image of pin
(1 sample, 1536 SNPs, av. 30 replications)

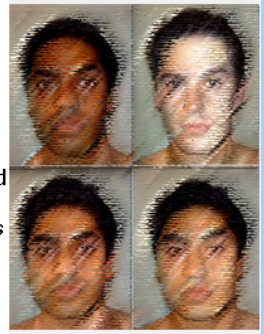
Summary: Genotyping

- Personal
  - Matthias Schick
  - Roger Fischer
  - (Oliver Heil)
- Turn-around Time
  - 2-3 Weeks (Infinium)
  - 1 to >4 Months (GoldenGate)
- Quality
  - Input 500-750 ng genomics DNA (70 ng/µl, Picogreen)
  - Quality control at all levels (WGA, labeling, hybridization, scanning, analysis)
  - Call Rates: Infinium (>99%), GoldenGate (>97%)
- Data Return
  - Raw Data
  - Interpreted Data (MS Office compatible)
  - Personal Meeting
- Cost
  - Starting at 290 € per sample (genome-wide)
  - 11 € per sample GoldenGate processing

Genotyping Webpage

Genomics & Proteomics Core Facilities
Expression Profiling
Genotyping
└ Genome-wide GT
└ FAQs - Genotyping
└ Glossary
Sequencing
454/GS20 Sequencing
Protein Analysis
Peptide Synthesis
Yeast Two-Hybrid
Oligonucleotide Synthesis
Vector & Clone Repository
Secure Login
Username:
korn
Password:
******
Login

Introduction to Genotyping



Genotyping provides a measurement of the genetic variation between members of a species. Single nucleotide polymorphisms (**SNP**) are the most common type of genetic variation. A SNP is a single base pair mutation at a specific locus, usually consisting of two alleles (where the rare allele frequency is >1%; random definition). SNPs are often found to be the etiology of many human diseases and are becoming of particular interest in pharmacogenetics (**Evans and McLeod** 2003, **Weinshilboum** 2003). Because SNPs are evolutionary conserved, they have been proposed as a markers for use in quantitative trait loci (**QTL**) analysis and in association studies. Previously these studies were done using microsatellites. The use of SNPs is being extended in the **HapMap project**, which is attempting to provide the minimal set of SNPs needed to genotype the human genome.

At the DKFZ Genotyping Core Facility, we provide access to full service of state-of-the-art genotyping of customized SNP sets, focused SNP sets, and genome-wide screens covering up to 650.000 SNPs on BeadChips (Illumina). Beside standard genotyping, we support loss-of heterocygosity (LOH), and copy number variation (CNV) studies.
For the analysis of genetic variation and function, we make use of the Illumina Beadchip technology.

Genome-wide SNP Analysis

We provide service, using Illuminas Infinium BeadChips that carry a fixed-content of SNPs for whole genome genotyping screens. This offers virtually unconstrained locus selection for high-value content coupled with high resolution. **More details** on genome-wide genotyping...

Applications include

- Whole-genome association studies
- Fine-mapping studies
- Whole genome LOH / copy number variation analysis
- Disease-associated copy number variation analysis

Focused and Customized SNP Analysis

Focused SNP panels can be used for cost-effective linkage analysis in human and mouse, or for genome-wide characterization of **non-synonymous SNPs** (nsSNPs).

Contact

Dr. Bernhard Korn
German Cancer Research Center
Im Neuenheimer Feld 515
69120 Heidelberg
Germany

Fon: +49 6221 42 4700
Email: **contact form**

Genome-wide Methylation Analysis

Bernhard Korn

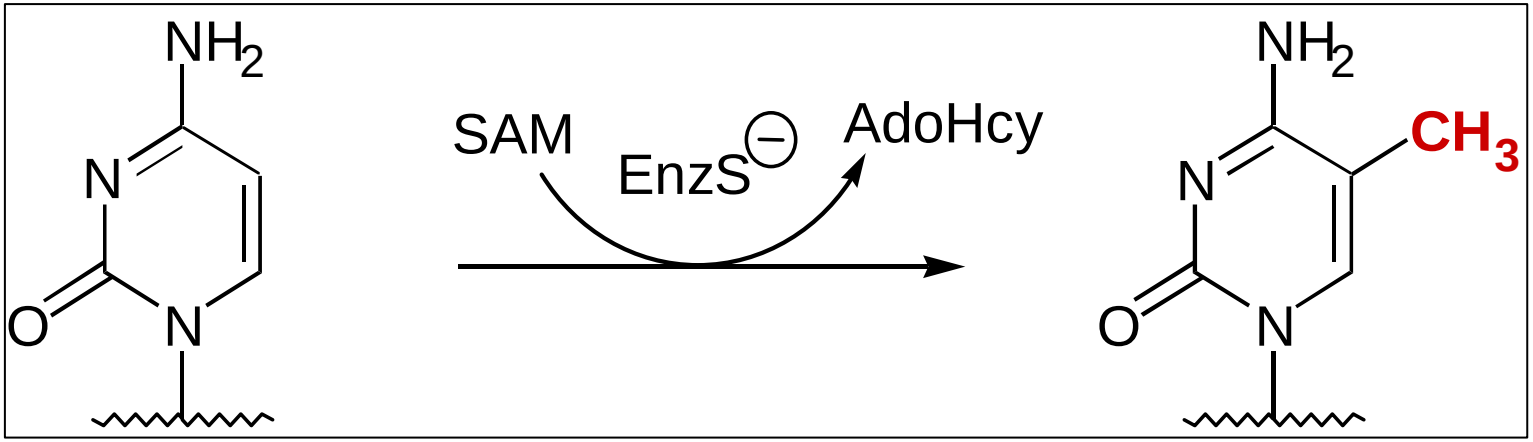
Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview: Methylation Analysis

- Contact
  - B. Korn (b.korn@dkfz.de, -4700)
- Offer
  - Bisulfite treatment of genomics DNA
  - Genome-wide analysis of >27.000 CpGs, representing more than **13.000 promoter**
  - QC at all levels
  - Basic support for data analysis

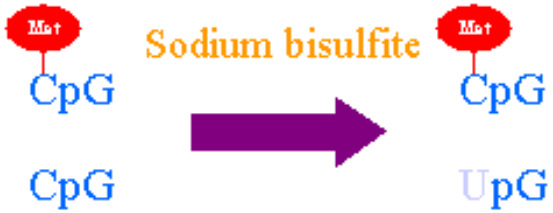
DNA Methylation



- Methyl group introduced in the 5’ position of cytosine
- In mammals, occurs in CpG dinucleotides
- Catalyzed by DNA methyltransferases (DNMT)

Infinium HumanMethylation27 Beadchip

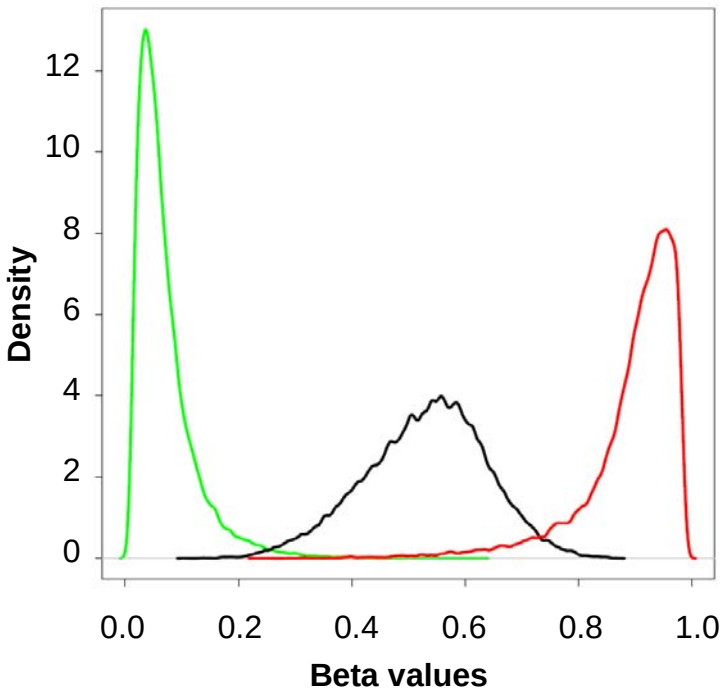
- 27.578 CpG sites (incl. 254 miRNA CpGs)
- Representing >13.000 promoters / genes
- 1-3 CpGs / promoter
- 12 arrays / BeadChip



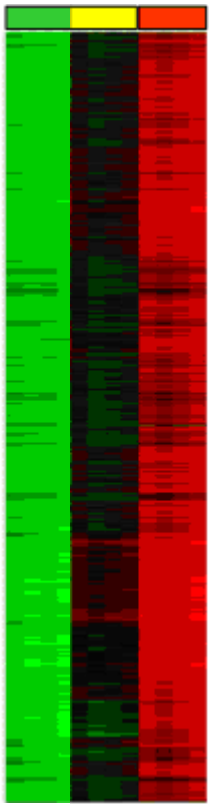
- 1 µg genomic DNA
  - ~500 ng bisulfite treated DNA
  - Infinium assay

- Two-color fluorescent scanning
- Methylation measure: ß-value (0-1)

Do ß-Values reflect Methylation Levels?



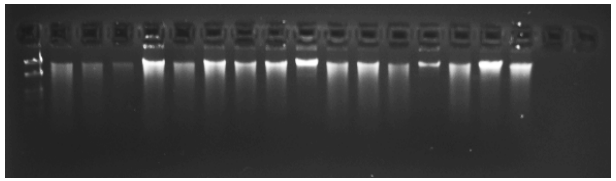
Density plot
mean ß: 0.070, 0.530, 0.894

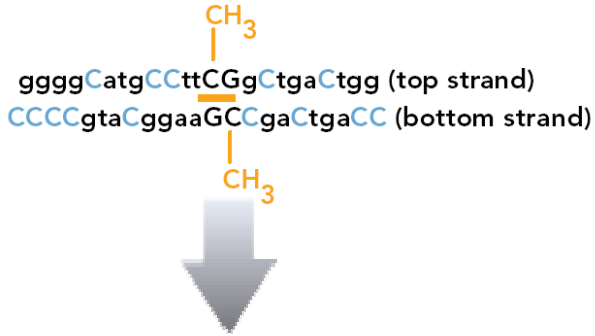


Cluster analysis of 26,492 CpGs

→ Assays are capable to discriminate methylation status
→ Quantitation is possible

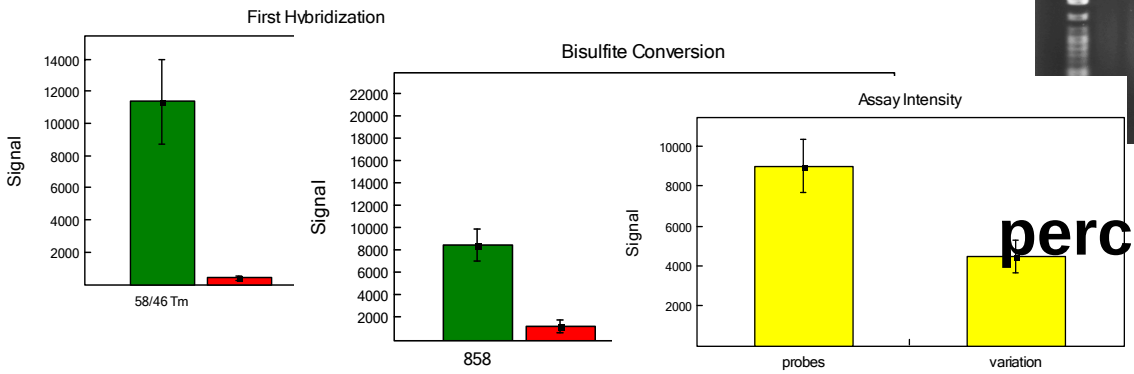
Methylation Analysis Pipeline





Excel-like Output with Links

SYMBOL	GeneCards-L	Entrez-Link	DIS	CP	ANNOTATION	(%	(%	(%	(%	(%	(%	(%	(%	(%
CDKN1A	GC_CDKN1A	NM_000389.2	-242	Y	Homo sapiens cyclin-dependent kinase inhibitor 1A	93	28	87	89	93	93	89	78	0
MAGEL2	GC_MAGEL2	NM_019066.2	-170	Y	Homo sapiens MAGE-like 2 (MAGEL2), mRNA.	97	98	97	98	97	91	97	98	24
MAP3K9	GC_MAP3K9	NM_033141.2	17	Y	Homo sapiens mitogen-activated protein kinase kina	85	8	62	64	88	96	69	47	3
PDGFB	GC_PDGFB	NM_002608.1	25	Y	Homo sapiens platelet-derived growth factor beta pol	89	29	78	83	91	91	83	64	0
PLXDC2	GC_PLXDC2	NM_032812.7	-914	Y	Homo sapiens plexin domain containing 2 (PLXDC2)	85	18	75	73	84	89	69	42	4



percent methylation

Summary: Methylation Analysis

- Personal
  - Roger Fischer
- Turn-around Time
  - 3-4 Weeks
- Quality
  - Input 1 µg genomics DNA (>100 ng/µl)
  - Quality control at all levels (bisulfite treatment, labeling, hybridization, scanning, analysis)
- Data Return
  - Raw Data
  - Interpreted Data (MS Office compatible)
  - Personal Meeting
- Cost
  - 305 € for 13.000 promoter per sample

Vector & Clone Repository

André Leischwitz

Genomics & Proteomics Core Facilities, DKFZ

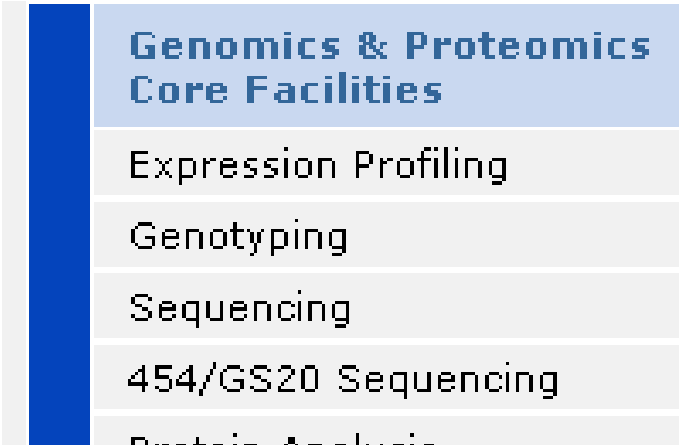
dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

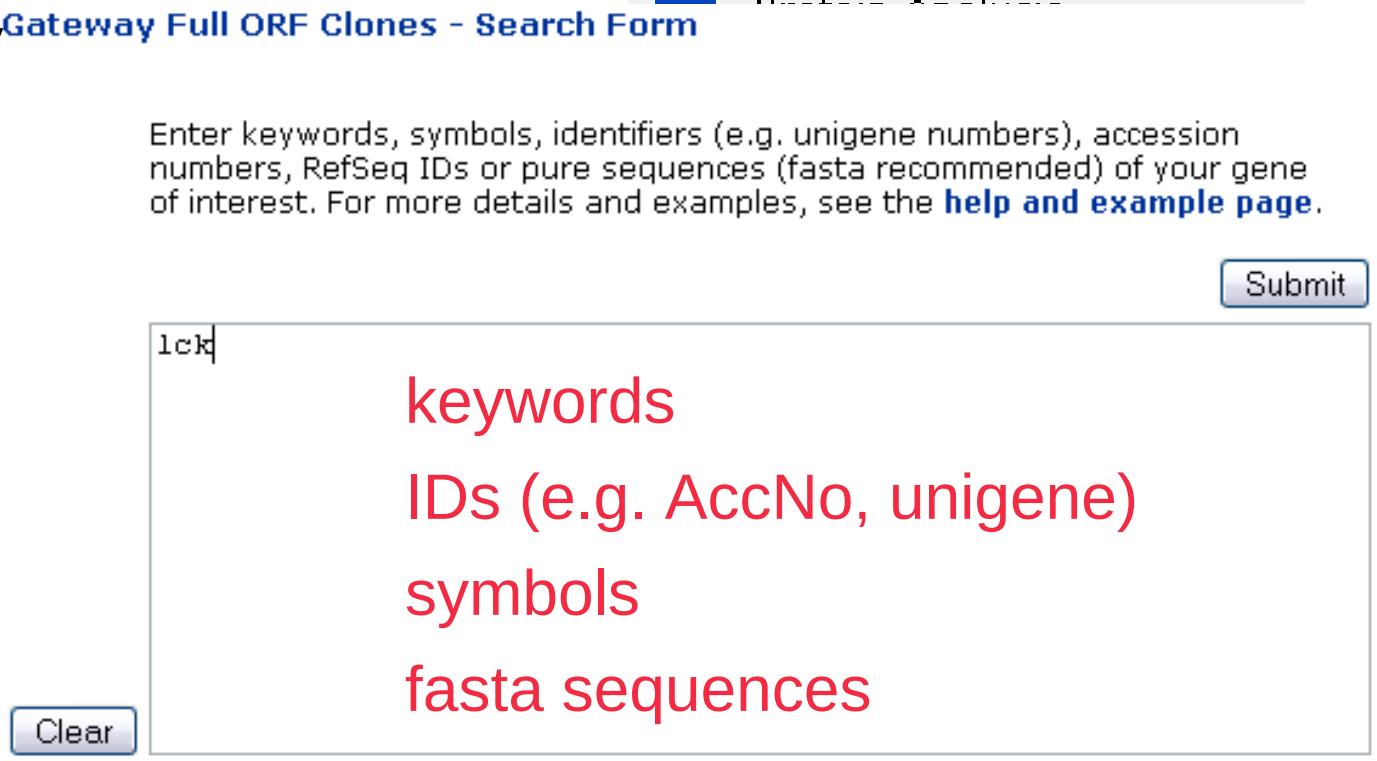
Overview: Clone Repository

- Contact
  - A. Leischwitz (a.leischwitz@dkfz.de, -4734)
- Offer
  - Full Open Reading Frame (ORF) Clones
    - Gateway format, with/without stop codon
    - Partially or fully sequenced (majority in EMBL/Genbank)
    - >20.000 clones (mainly human)
    - Collab. Harvard Institute of Proteomics
  - Vector repository
    - Commercial and home-made vectors
    - *E.coli* strains
  - Interactive search interface (Keyword, IDs, blast, vector features)

How to obtain Clones

1. Login at www.dkfz.de/gpcf
2. Navigate to
   "Vector & Clone Repository"
3. Open "Search for
   Gateway Full ORF clones"
4. Enter Query





How to obtain Clones

5. Check results
6. Verify information

Clone search results

Your queries:
lck

Query results:

2 hits for keyword: lck

Rank	Order	Gene	Organism
1000	☑	LCK	homo sapiens Lymphocyte-specific
1000	☐	LCK	homo sapiens Lymphocyte-specific

Clone 161228636

Symbol: LCK
Description: Lymphocyte-specific protein tyrosine kinase
Organism: homo sapiens
ORF Length: 1620
Amino-Acid-Exchange: none
Cloning System: gateway, pDONR221
Stopcodon: closed (incl. stop codon)
Genbank: DQ891675
Genbank Hit: BC013200
LocusLink/Entrez: 3932
Source (Stanford): Hs.470627
Unigene (NCBI): Hs.470627
Golden Path: DQ891675
Aliases: "LCK; ONCOGENE LCK; LYMPHOCYTE-SPECIFIC PROTEIN-TYROSINE KINASE; Lymphocyte-specific protein tyrosine kinase"
Potential Protein: NP_005347
Sequence:

```
tgtacaaaaaagcaggctccaccATGGGCTGTGGCTGCAGCTCACACCCGGAAGATGACT
GGATGGAAAACATCGATGTGTGTGAGAACTGCCATTATCCCATAGTCCCACTGGATGGCA
AGGGCACGCTGCTCATCCGAAATGGCTCTGAGGTGCGGGACCCACTGGTTACCTACGAAG
GCTCCAATCCGCCGGCTTCCCCACTGCAAGACAACCTGGTTATCGCTCTGCACAGCTATG
AGCCCTCTCACGACGGAGATCTGGGCTTTGAGAAGGGGGAACAGCTCCGCATCCTGGAGC
AGAGCGGCGAGTGGTGGAAGGCGCAGTCCCTGACCACGGGCCAGGAAGGCTTCATCCCCT
TCAATTTTGTGGCCAAAGCGAACAGCCTGGAGCCCGAACCCTGGTTCTTCAAGAACCTGA
GCCGCAAGGACGCGGAGCGGCAGCTCCTGGCGCCCGGGAACACTCACGGCTCCTTCCTCA
TCCGGGAGAGCGAGAGCACCGCGGGATCGTTTTCACTGTCGGTCCGGGACTTCGACCAGA
```

Find your vector of interest

Genomics & proteomics core facility - Vectors and Strains from GPCF - Mozilla Firefox

Datei Bearbeiten Ansicht Chronik Lesezeichen Extras Hilfe

http://www.dkfz.de/gpcf/vector_search.html?vector=161925158

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Genomics & Proteomics Core Facilities
Sequencing
454/GS FLX Sequencing
Expression Profiling
Methylation Analysis
Genotyping
Protein Analysis
Peptide Synthesis
Yeast Two-Hybrid
Contamination Control
Flow Cytometry
miRNA Profiling
Vector & Clone Repository
Vectors & Strains
Gateway ORF Clones
PlasMapper
Equipment
How to find us
WIKIs
Your Downloads
Tools
Pricelist

Username: korn
Logout

Vectors and Strains - Search

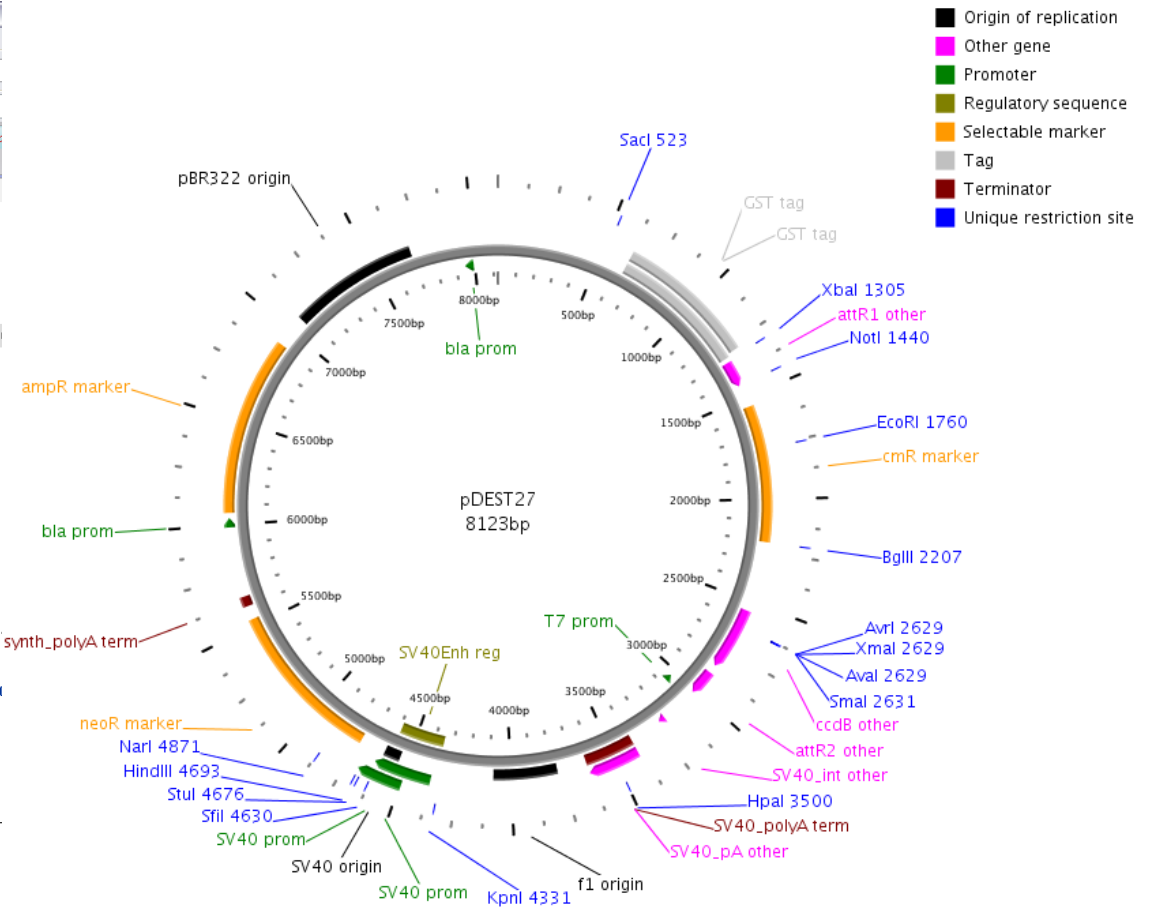
Vector details

Vector pDEST27

DKFZ ID: V000144 Map
Name: pDEST27
Sequence: **pDEST27**
Description: N-terminal GST fusion expression vector for mamm compatible
Type: Mammalian expression vector;Gateway-compatible
Manual: **pDEST12_pDEST27_pDEST26_pDEST8_pDEST2**
Accno:
Host Strain: DB3.1
Vector: pDEST27
Origin of Replication: pUC
Resistance: Amp (100ng/µl); Cm (30ng/µl); Neo (100-1000ng/
Promoter: CMV
Operator:
Tag: N-term. GST
Protease:
Restriction Site: Gateway
Source: Invitrogen
Comment: The chloramphenicol resistance is lost after cloning. Therefore plate clones at 100 µg ampicillin / ml LB, use gentinicin at a conc. of 100-1000 µg/ml, depending on the activity of the drug
PubMed:

Please contact **Oliver Heil** in case of errors or discrepa

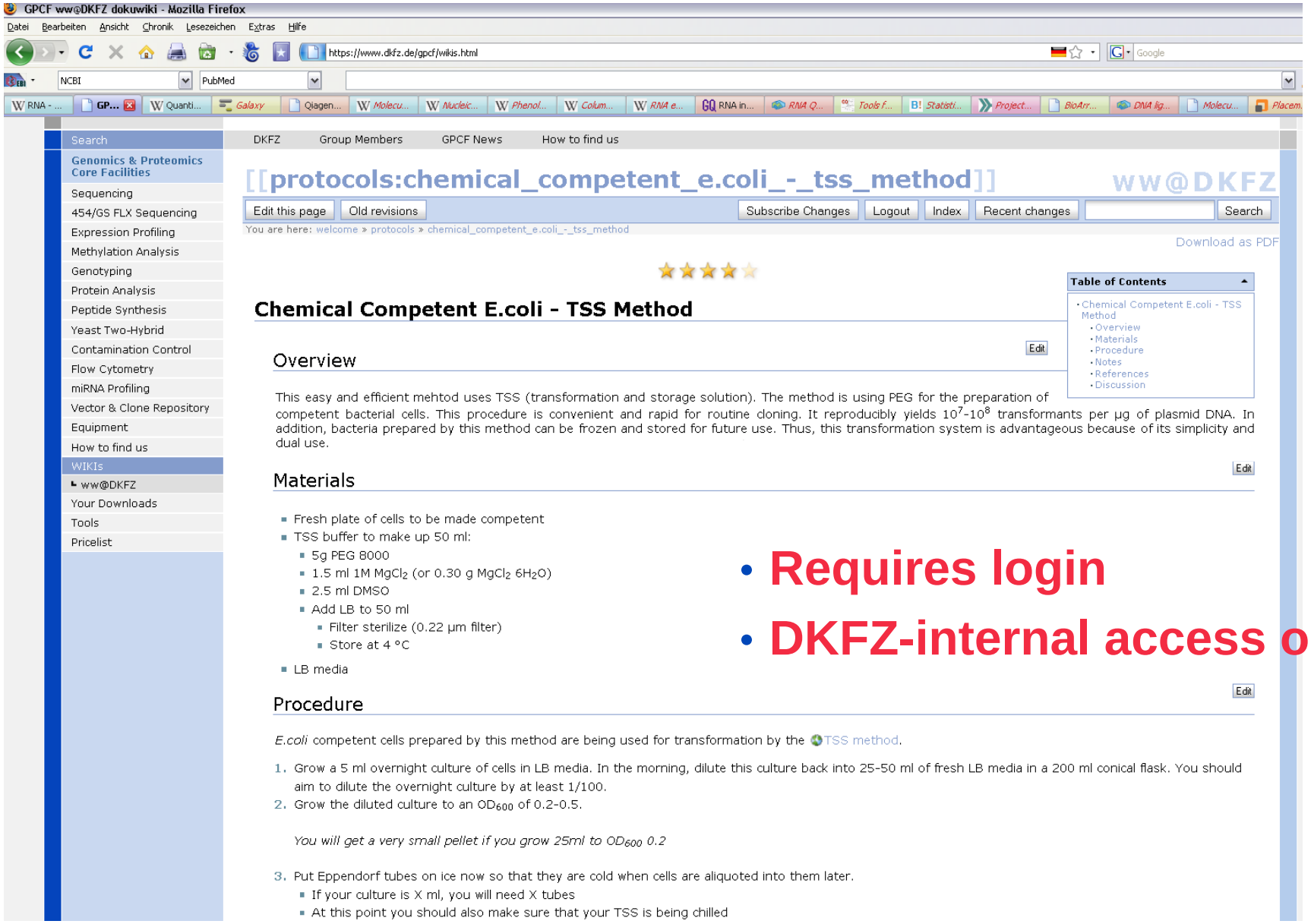
```
>pDESTT27
cattagatgcatgtcgttacataacttacggtaaatggcccgcctggctgaccgcccaac
gacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggact
ttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaa
gtgtatcatatgccaagtacgcccccctattgacgtcaatgacggtaaatggcccgcctgg
cattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtatta
```



Summary: Vector & Clone Repository

- Turn-around Time
  - <1 Week
- Quality
  - All clones partially or fully sequenced
  - Biannual Annotation update
- Material/Information Return
  - *E.coli* strains, to be picked up INF515
  - Manuals available for download
  - Vector maps an sequences online
- Cost
  - 30 €
- Limitations
  - No transfer to collaborators outside of DKFZ

wetlab wiki (ww@DKFZ)



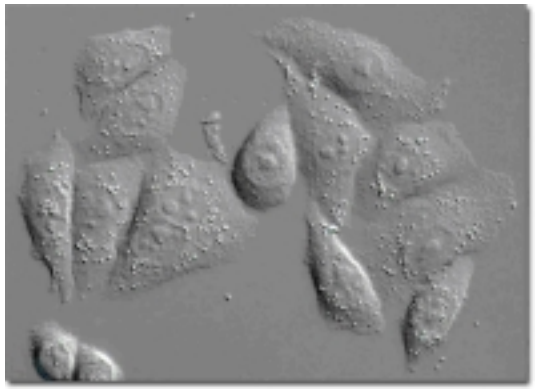
- **Requires login**
- **DKFZ-internal access only**

Summary: Equipment/ Assisted Access & wetlab wiki

- Personal
  - Matthias Schick
- Support
  - Introduction to systems
  - System care
  - Online booking
- Turn-around Time, Quality & Data
  - Do-it-yourself
- Cost
  - wetlab wiki – ww@DKFZ (free)
  - Silverquant scanning (free)
  - qPCR (25 € per run)
  - QiaCube (10 € per run)
  - Ingenuity (250 € per year and Kostenstelle)

Multiplex Cell Culture Contamination Test (McCT)

Markus Schmitt / Michael Pawlita



Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview

- **Contact**
  - **Markus Schmitt (Markus.Schmitt@dkfz.de -4937)**
  - **Michael Pawlita (M.Pawlita@dkfz.de -4645)**
- **Offer**
  - **Cell culture contamination test**
    - **Multiplex PCR + bead-based hybridisation (Luminex)**
    - ***Viruses*: SMRV, HPV18 (HeLa cells), SV40, Adenoviruses, HBV, all human herpesviruses**
    - ***Mycoplasma:* genus + 11 species + Acholeplasma Laidlawii**
    - ***Species*: Human, monkey, mouse, rat, chinese hamster, dog, cat, rabbit, guinea pig**
    - ***Y-chromosome*: Human, monkey, rat, mouse**
  - **Cell lysate (>10^6 cells, heat-treated in PBS) to be supplied by user**
- **Outlook**
  - **Human cell identity verification by multiplex SNP-typing**

Results

711 cell lines (3-7/2008)

SMRV: **18 (2.5%):**
- **Namalwa, BL60: human Burkitt-Lymphoma**
- **HEK293**
- **CEM, Jurkat: human T-cell**
- **B95-8: marmoset EBV producer line**
- **HeLa**

***Mycoplasma*:** **156 (21.9%)**

Inter-species: **45 (6,4 %)**

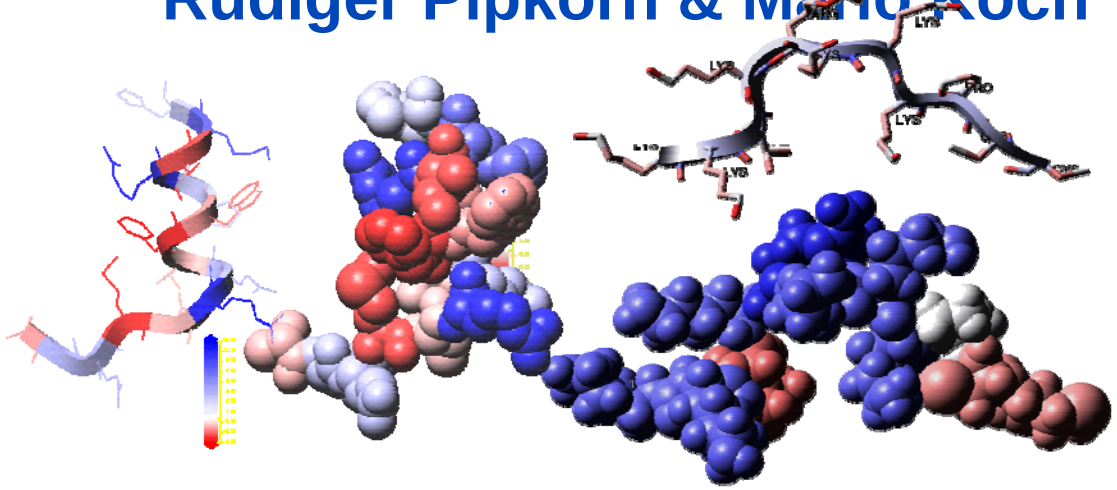
HeLa (HPV18): **7 (1%)**

Summary

- Personel
  - Saskia Ziegler
- Turn-around Time
  - Max. 2 weeks
- Quality
  - External controls and standards
  - Internal PCR/DNA control (mammalian a-pol)
- Cost
  - 25 €/lysate, bulk rates negotiable

Central Peptide Synthesis Unit

Rüdiger Pipkorn & Mario Koch



Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview:
Syntheses of complex functional Peptides

- Contact
  - R. Pipkorn (r.pipkorn@dkfz.de, Tel.: 42-2487, FAX: 42-2486)

- **Special Syntheses for:**
  - **Structural studies** (Phosphorylation)
  - **Drug Delivery** (Membrantransport facilitat. Peptides, DNA-Binding Peptides)
  - **Subcellular Address Peptides (**e.g. NLS, MLS**)**
  - **Peptide- and DNA-Analoga (**PNA**)**

- **Non-Standart Syntheses for**
  - **Analytics and Diagnostics**
  - **Peptides -and PNA-Products for Chip-Technology**
  - **Peptide-Chimeres with Nucleic Acids and it‘s Derivatives**

    (PNA/Methoxy-RNA – Opener /Closer)

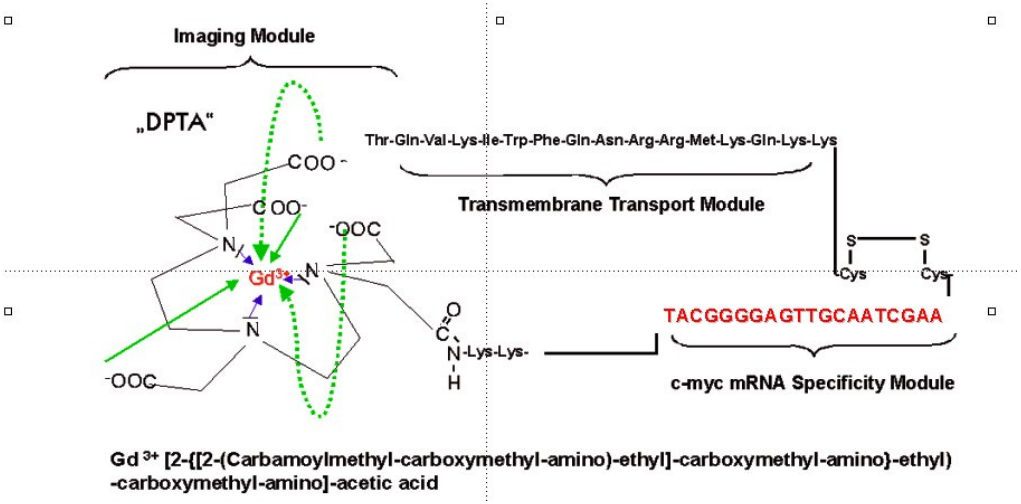
Summary: Expression Profiling

- **Personal**
  - Rüdiger Pipkorn
  - Mario Koch
- **Turn-around Time**
  - 1-3 weeks
- **Material Supply**
  - pMol - mMol
- **Quality**
  - QC at all levels (educts, intermediates & chemical compounds)
  - MS
  - HPLC
- **Cost**
  - Starting at ~8€ / amino acid

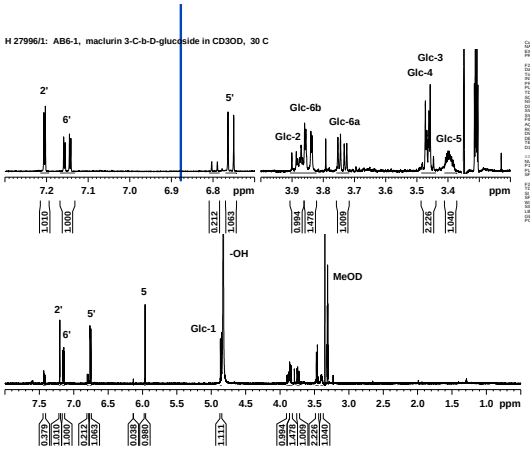
Outlook

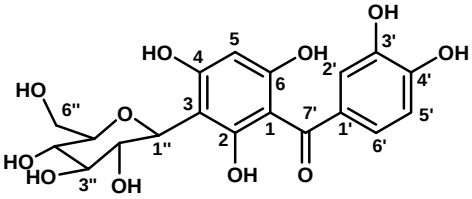
- Funktional Peptides for **Molecular Imaging in the MRT / PET** /SPECT

- Syntheses of peptide-based **intravasal Contrast Agents for High Field MRT**



- Synthese of **Peptides for tight Cluster Spotting** to Membranes of Carbon Origin appropriate to high Throughput Proteom Analyses

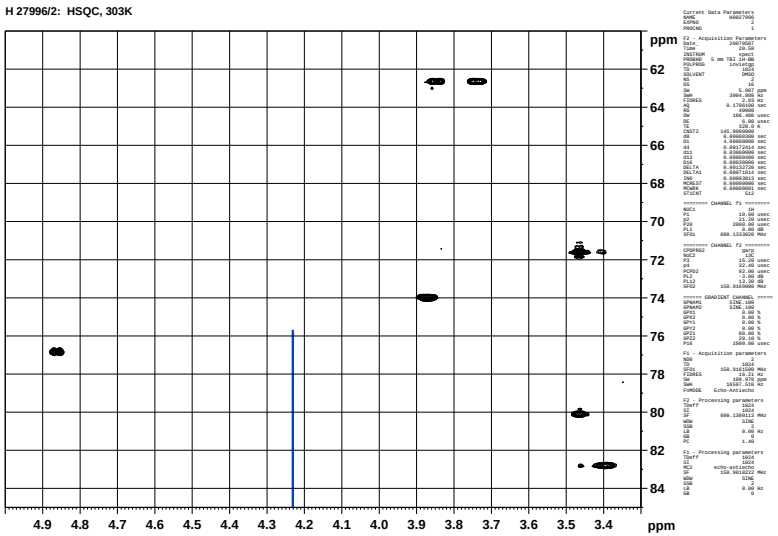


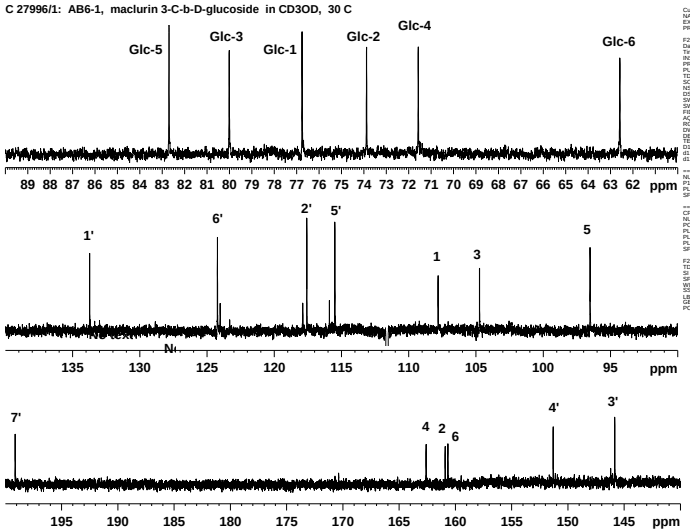


maclurin 3-*C*-β-D-glucopyranoside
$C_{19}H_{20}O_{11}$
Exact Mass: 424.1006

Molecular Structure Analysis

NMR Spectroscopy





Genomics & Proteomics
Core Facilities

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NMR Spectroscopy

Who does the work and where …

Gabriele Schwebel, technician for NMR
INF 280, H2.02.029 Lab
H2.02.035 NMR Tel. 4544
Email: g.schwebel@dkfz.de

Dr. William E. Hull (PhD), specialist for NMR, Dept. Head
INF 280, H2.02.079 Buro, Tel. 4515
H2.02.035 NMR Tel. 4544
Email: w.hull@dkfz.de

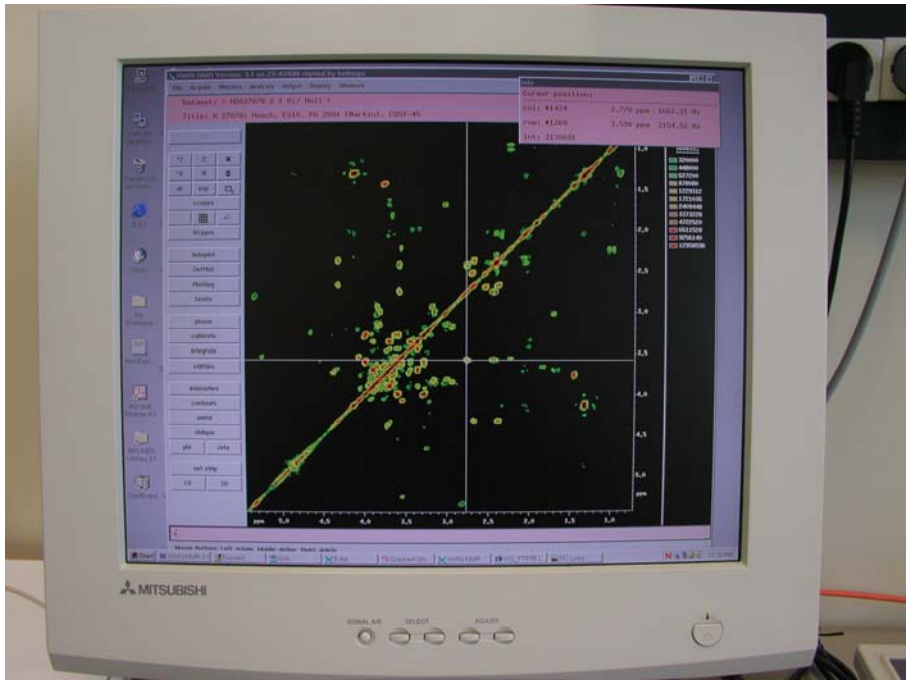
What instrumentation do we have …

Avance Spectrometers from Bruker BioSpin GmbH, Karlsruhe
AV-600 (14.1 T, 600 MHz for ^{1}H, 54 mm bore, 4 RF channels, 2001)
AV-400 (9.4 T, 400 MHz, 54 mm bore, 3 RF channels, **Oct. 2008**)
Both instruments use same type of *Windows* workstation (dual-core Pentium) and same ***TopSpin*** software version; offer same palette of experiments.

NMR Spectroscopy

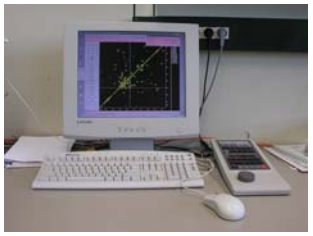
600 MHz NMR system

The 14.1 Tesla magnet
with active shielding



Examination of a
2D H,H-COSY
experiment

NMR Spectroscopy



Services available

- ***Routine 1D and 2D NMR at 400 MHz …***

Quality control and structure elucidation of synthetic compounds and natural products (MW < 2000)

- all standard 1D and 2D NMR techniques for **^{1}H, ^{13}C, ^{19}F, ^{31}P,**
- other nuclei available upon request,
- direct X-nucleus detection or inverse ^{1}H / X-nuc 2D with ^{1}H detection,
- homo- and heteronuclear decoupling, water suppression,
- nuclear Overhauser effects, mixture analysis, quantitative analysis,
- structure-based spectrum prediction using PERCH.
- A3 spectra plots (laser printer) and emf graphic files,
- peak and integral lists as A4 printout and ascii text files,
- raw and processed data permanently archived on CD, DVD,
- upon request: transfer of data sets to experienced customer via house network (off-line analysis using *WIN-NMR* or *TopSpin*).

NMR Spectroscopy

Services available

- ***Advanced 1D - 4D NMR at 600 MHz …***

  Quality control, structure elucidation, stereochemistry, conformation and dynamics of synthetic compounds, natural products, metabolites, carbohydrates, peptides, lipids, proteins, nucleic acids, (MW < 20 kD)

  - all modern direct and inverse techniques for ^{1}H,^{19}F,^{13}C,^{31}P,^{15}N,^{2}H,
  - micro-coil probehead for nmol sample quantities in small volumes,
  - long-term projects in metabolomics:
    analysis of bio-fluids, tissue extracts, biopsies;
  - long-term projects with biopolymers:
    conformation, dynamics, ligand-receptor interactions;
  - same analysis, archiving, data output and transfer capabilities as for 400 MHz.

NMR Spectroscopy

- ***Sample Submission Forms***

  available in room H2.02.029 and soon via Intranet

  bookkeeping info, sample info, expected structure,
  nuclei and techniques required (following consultation).

- ***YOU supply …***

  typ. 1 - 10 mg purified or isolated solid or liquid samples (solvent-free);
  samples will be kept in a refrigerator or freezer until measurement.

- ***WE supply …***

  5-mm sample tubes, ca. 0.5 ml deuterated solvent, and *much know-how!*

- ***WE return …***

  - measured sample solution and any unused sample
  (NMR is nondestructive, no sample lost !!),
  - NMR spectra and evaluation printouts in paper form;
  upon request: raw data, plots as digital graphics (.emf), data tables as text files; fully assigned and interpreted spectra with structure elucidation.

NMR Spectroscopy

➢ *Measurement Time in the Spectrometer*

routine 1D and 2D NMR at 400 MHz

typ. 15 - 60 min for ^{1}H, ^{19}F, ^{31}P; 1 - 12 h for ^{13}C; 1 - 24 h for 2D exper.
NOTE: *measurement time decreases with the square of substance concentration*.

advanced 1D and 2D NMR at 600 MHz

typ. 1 h to several days, depending on required information, techniques and sample quantities.

➢ *Response Time: Return of samples and results …*

routine NMR at 400 MHz

typ. 0.5 - 2 days, depending on backlog, nuclei requested and sample quantity; *appointments can be made for immediate measurement of unstable samples.*

advanced NMR at 600 MHz

can be 1 day or several weeks, depending on required services and work load (consultation, project planning necessary)

NMR Spectroscopy

- ***Cost Structure*** (fixed + variable terms, calculations in progress)

Basic or Fixed cost per sample and instrument:
mean values for *operating costs* (cryogens, electricity, *without instrument depreciation*), *consumables* and *personnel*: sample preparation, thermal equilibration in spectrometer and experiment set-up (ca. 15 min).

Variable cost for typical standard 1D experiments:
personnel and *operating costs* for measurement times of 10 - 60 min with standard plots, depends on nucleus and experiment.

Surcharge for extended data acquisition (1D or 2D experiments):
proportional to operating cost per hour
(spectrum quality is proportional to square-root of time).

Estimated Costs (Euro)	**400 MHz**	**600 MHz**
Fixed cost per sample with ^{1}H spectrum	25	60
Cost per additional nucleus (std. exper)	10	20
Surcharge *per hour* additional meas. time	1	2
Analysis & Interpretation (per hour)	30	60

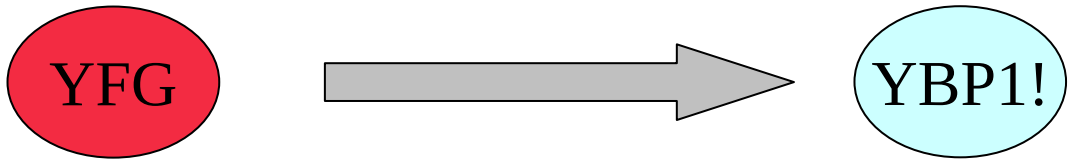
Protein Interaction Services

Dr. Manfred Koegl

dkfz.
GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

What is the Yeast Two Hybrid-System?

- A genetic method to identify potential protein-protein interactions. Works in yeast.

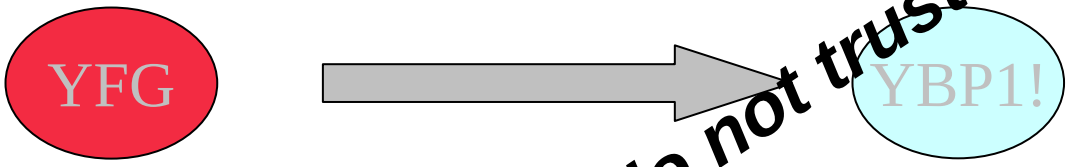


Your favorite protein *A protein binding to YFG!*

- Widely used.
- Can be automated.

What is the Yeast Two Hybrid-System?

- A genetic method to identify potential protein-protein interactions. Works in yeast.



Your favorite protein *A protein binding to YFG!*

Many people do not trust it...

- Widely used.

- Can be automated.

The Y2H Predjudice

"All you ever get is a lot of false positives!"

The Truth

- Yes, the Y2H system does produce a lot of false positives.
  - There a way to deal with th
- False negatives are
- Y2H screenir

Don't do Yeast Two-Hybrid screens!

Let us do them!

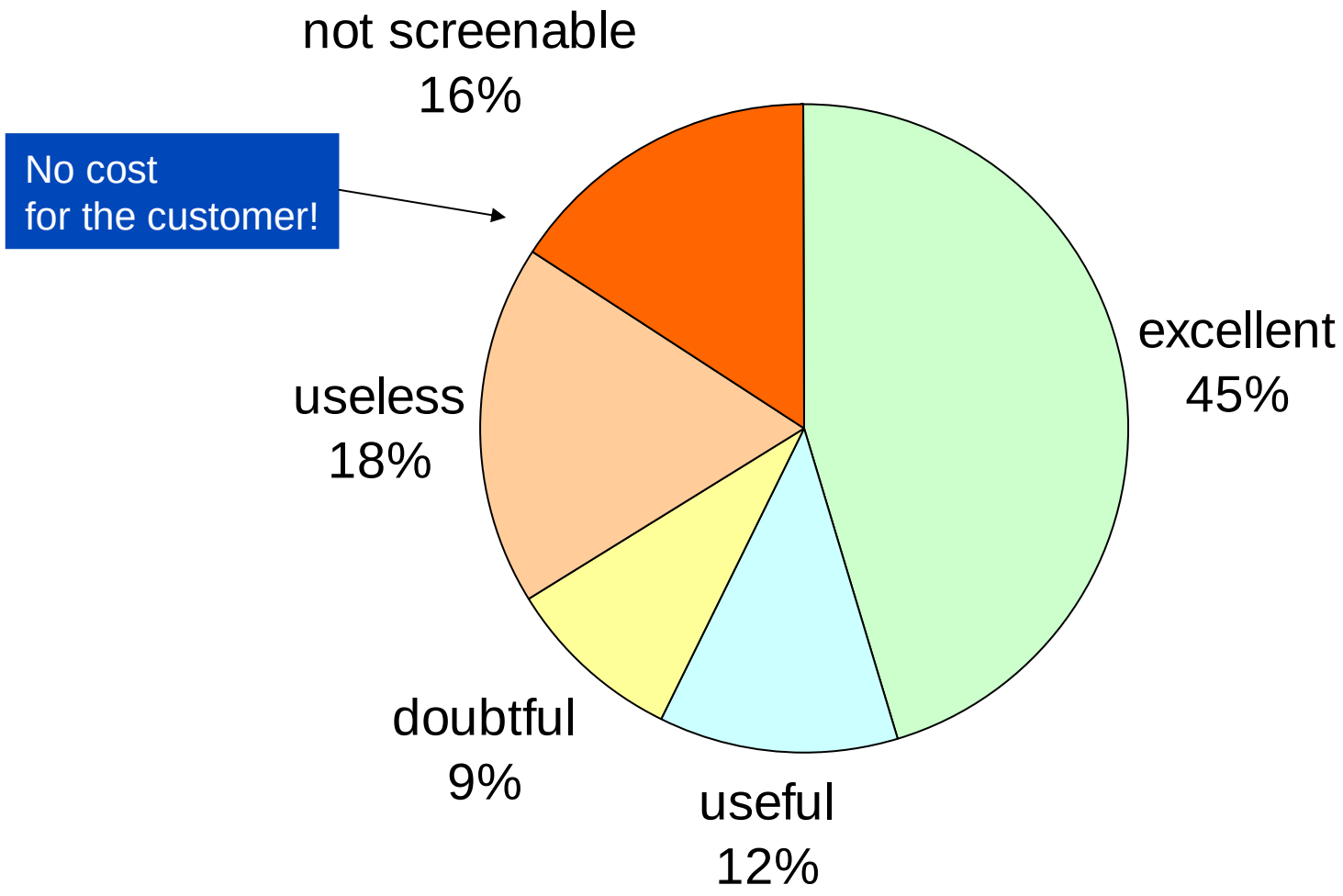
tills

2300 screens done since 2006

The Service

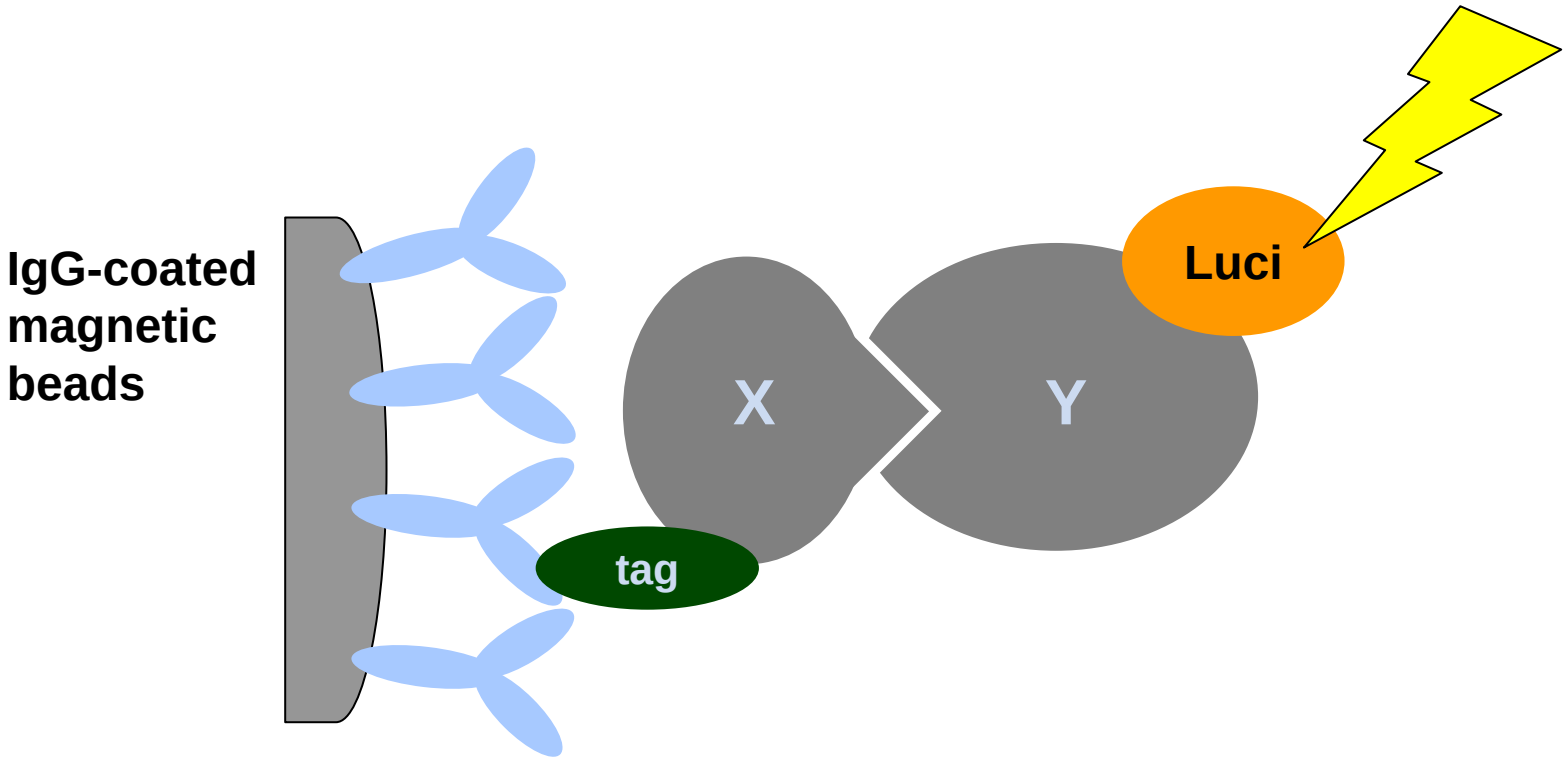
- You send us the plasmid
- 7 – 12 weeks later, we send back the data
- Cost: 1000.- €

Screen Quality



Confirming Yeast Two-Hybrid Data: LUMIER-Assays

Tagged proteins, transiently expressed in mammalian cells

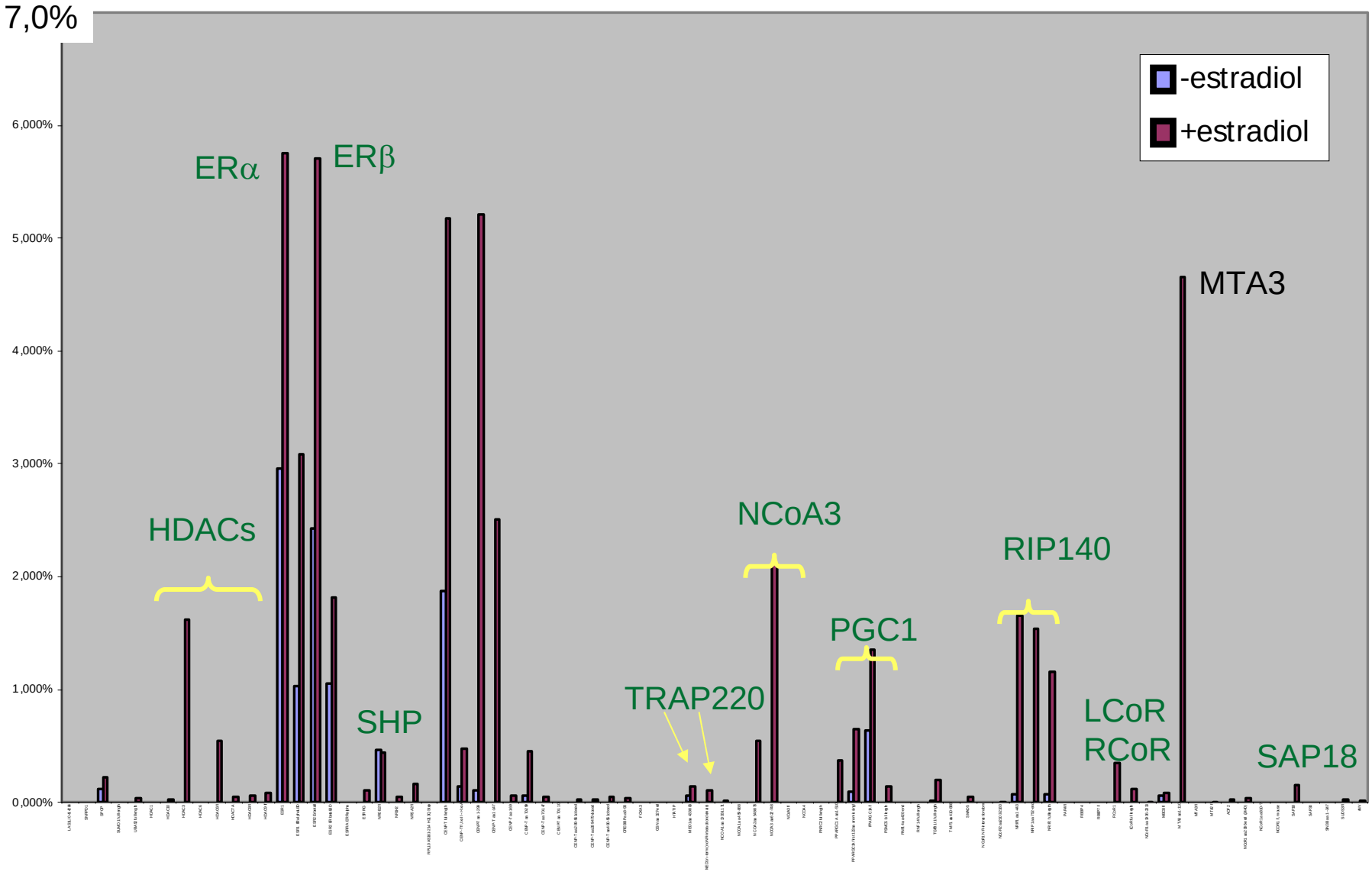


*"LUMIER"´, Idea by Barrios-Rodiles et al., (2005) Science 307:1621-5.

Advantages

- Works in mammalian cells! In 96-well plates!
- Sensitive, fast
- Allows to look at external triggers

Estrogen Receptor α + 80 Proteins



Shared Equipment/Assisted Access, wetlab wiki

Matthias Schick

Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview: Equipment/ Assisted Access & wetlab wiki

- Contact
  - M. Schick (m.schick@dkfz.de, -4703)
- Offer
  - Shared Resources (INF515)
    - Ingenuity Software access
    - Real time PCR system (Lightcycler480, 96 and 384well)
    - Sample preparation robot for DNA and RNA (QiaCube)
    - Silverquant Chipscanner (Eppendorf)
    - Full online booking
    - Introduction to systems
  - wetlab wiki
    - Lab protocols
    - Discussion threads

Ingenuity Pathway Analysis 6.5

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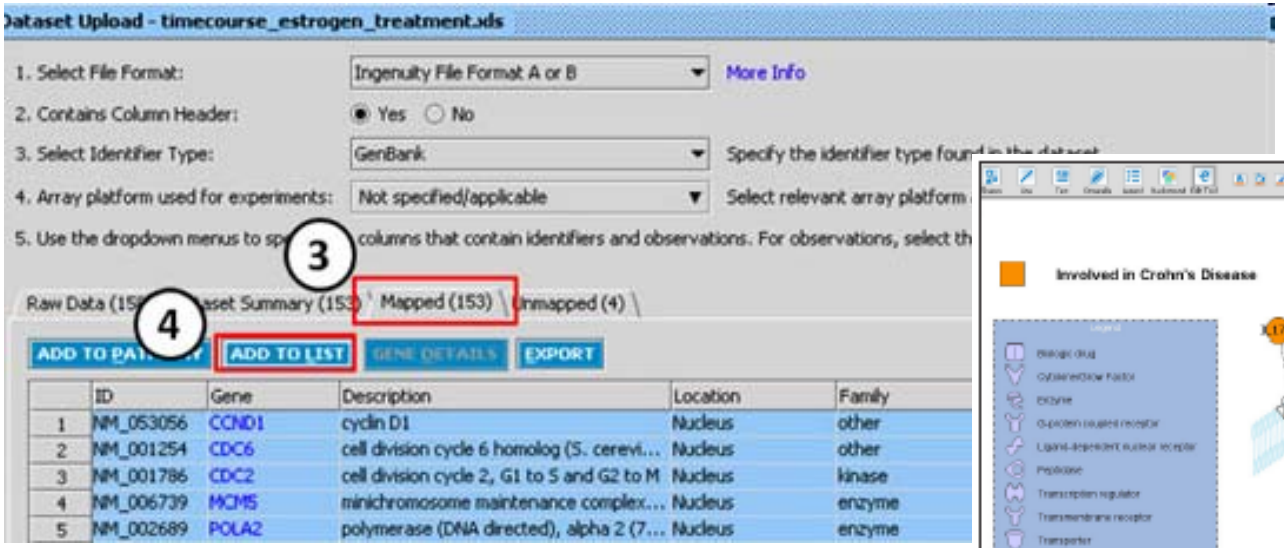
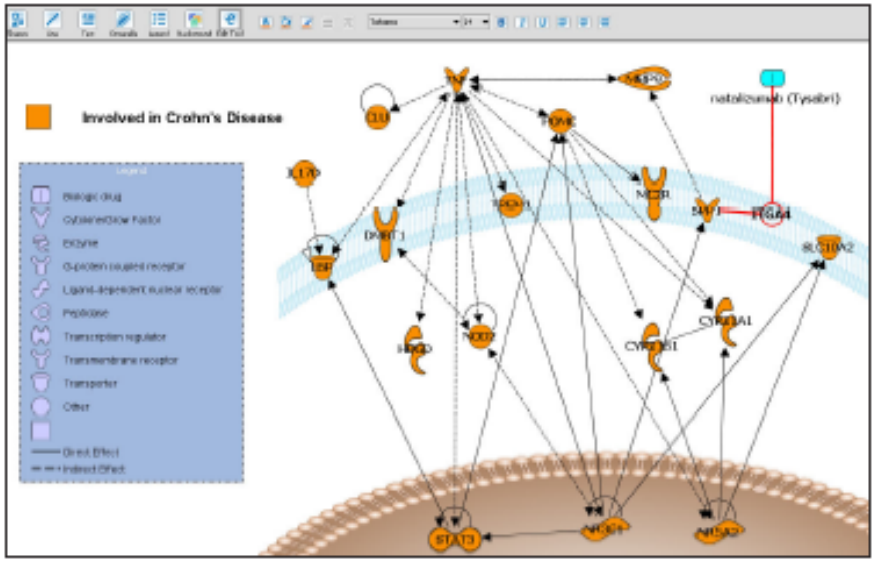




Use PC and license at GPCF (INF515)

Lightcycler480

- SYBR Green assays
- Fluorescent probe assays
- Taqman
- UPL assays
- LightCycler® 480 Genotyping Master

- Multiwell Plates 384 and 96

QiaCube

- DNA isolation (plasmid or genomics)
- RNA isolation
- PCR product clean-up

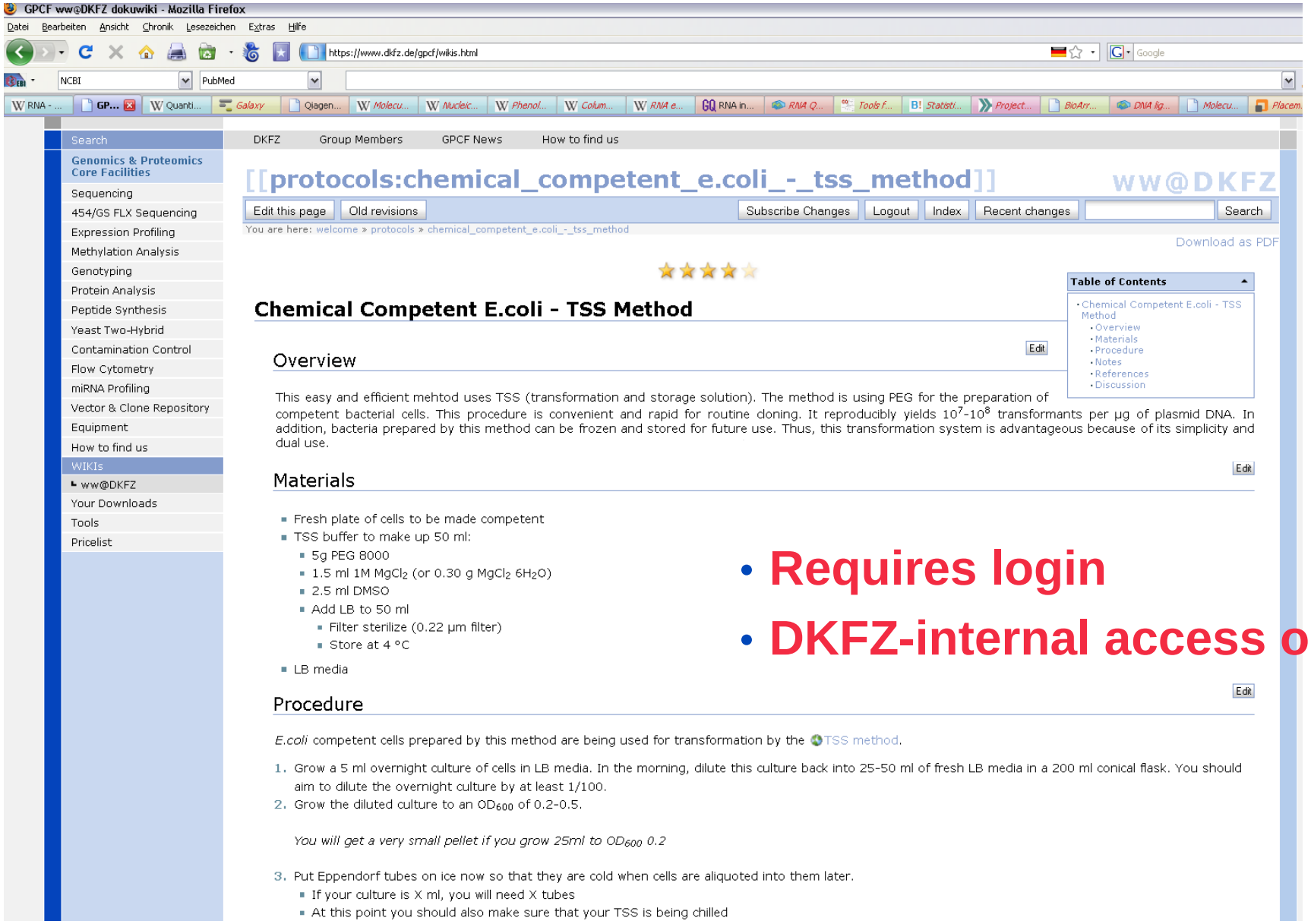
- Up to 12 samples
- Standard Qiagen spin kits
- Fully automated

- Receive an introduction, book the robot and purify your samples

SilverQuant Scanner

- Highly sensitive silver detection on microarrays
  - Expression chips
  - Transcription Factor chips
- Receive an introduction, book the system and scan your chips

wetlab wiki (ww@DKFZ)



- **Requires login**
- **DKFZ-internal access only**

Summary: Equipment/ Assisted Access & wetlab wiki

- Personal
  - Matthias Schick
- Support
  - Introduction to systems
  - System care
  - Online booking
- Turn-around Time, Quality & Data
  - Do-it-yourself
- Cost
  - wetlab wiki – ww@DKFZ (free)
  - Silverquant scanning (free)
  - qPCR (25 € per run)
  - QiaCube (10 € per run)
  - Ingenuity (250 € per year and Kostenstelle)

HUSAR Bioinformatics Lab

Karl-Heinz Glatting

Agnes Hotz-Wagenblatt

Molecular Biophysics, not yet Genomics & Proteomics Core Facilities, DKFZ

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

Overview: HUSAR Bioinformatics Lab

- Contact
  - K.-H. Glatting, A.Hotz-Wagenblatt (genome@dkfz.de, -2334, -2349)
  - Located at TP3, 4th floor, Room 4.201
- Offer
  - HUSAR Analysis Package with Web Interface W2H
  - SRS Sequence Retrieval System with more than 600 databases
  - STADEN Sequence Assembly and Analysis
  - TRANSFAC Transcription Factor Binding Site and Promoter Analysis
  - Customized Analysis Pipelines
  - Support in Data analysis
  - Courses on Sequence Analysis with HUSAR
- Collaboration with ZDV (Tobias Reber, Holger Haas)

Overview: HUSAR Bioinformatics Lab

- Requirements using the HUSAR analysis package, TRANSFAC
  - Userid and Password for the HUSAR machine (apply via the ZDV portal – Unix/Husar/HPC)
- Requirements for Bioinformatic Support
  - none

- Input from User
  - Any Problem in the Bioinformatics Area
  - Sequences for Analysis
  - Any Questions Regarding Bioinformatics Results

20 years HUSAR – Heidelberg Unix Sequence Analysis Resource

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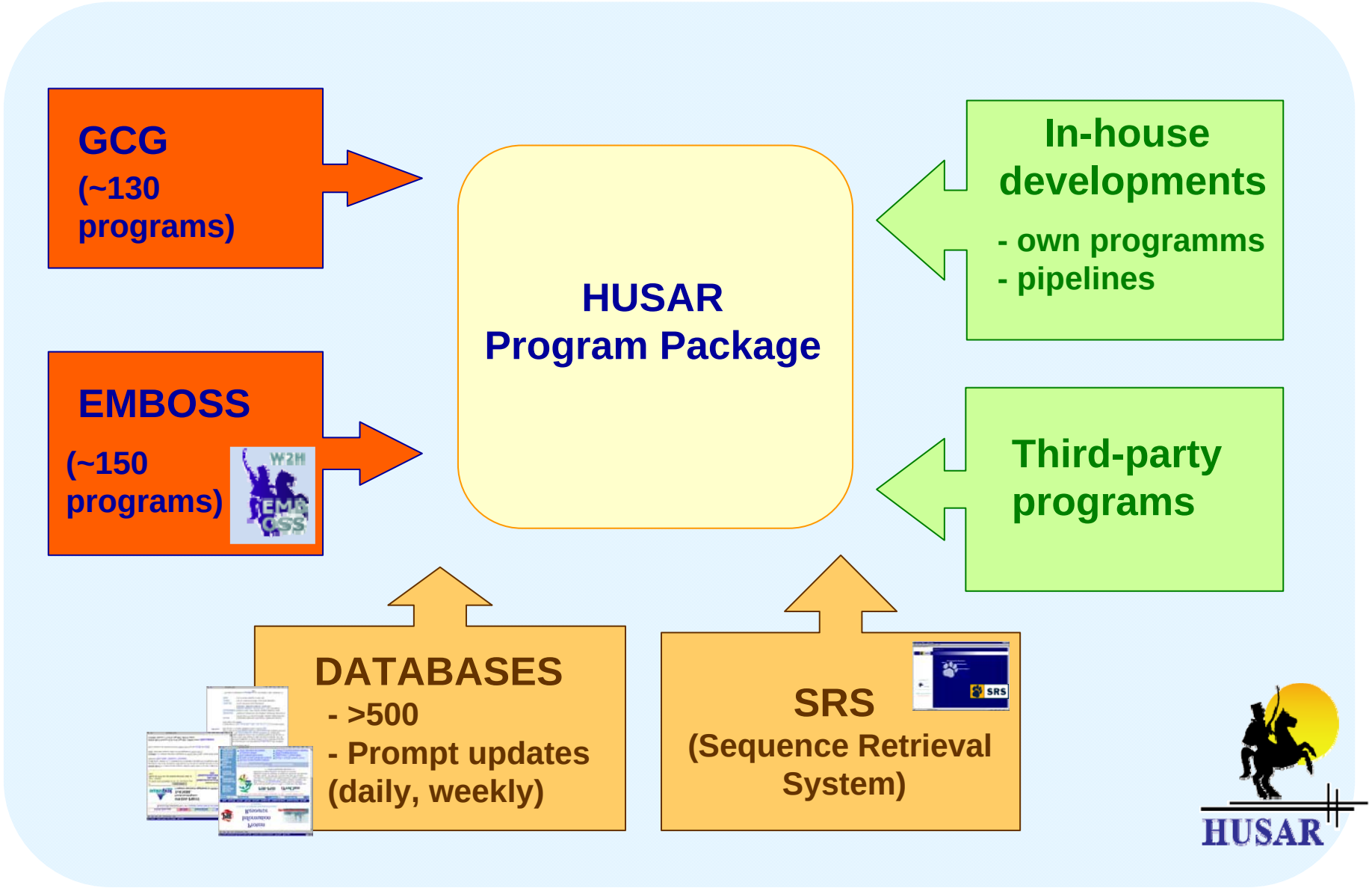
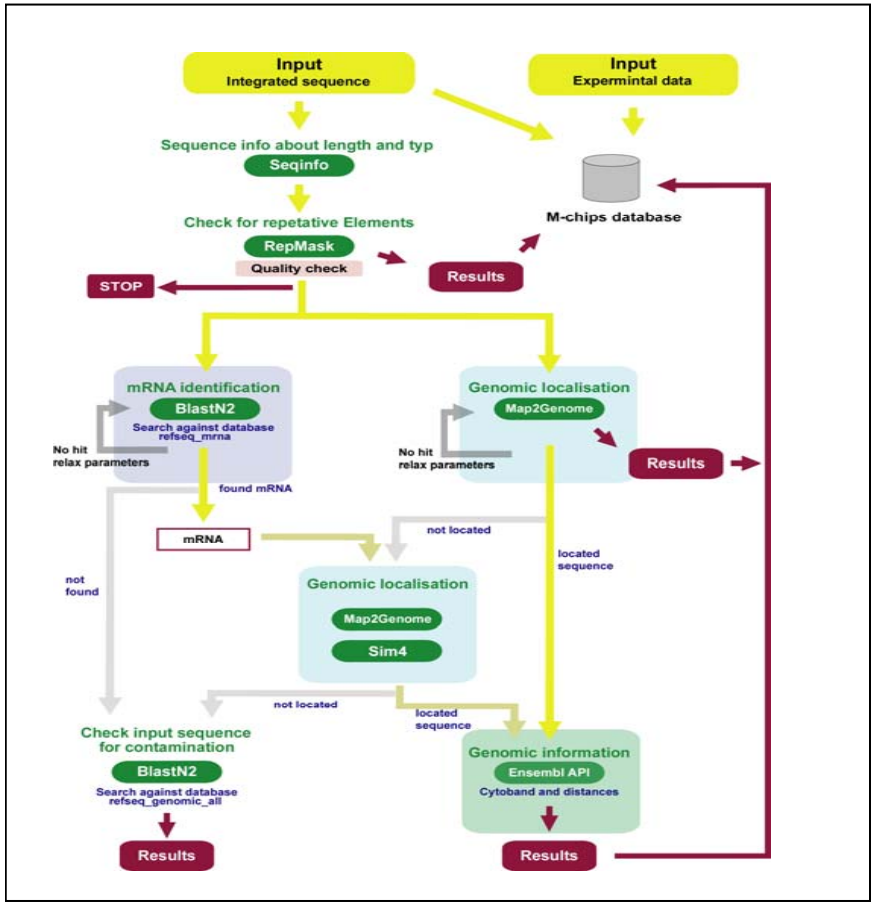
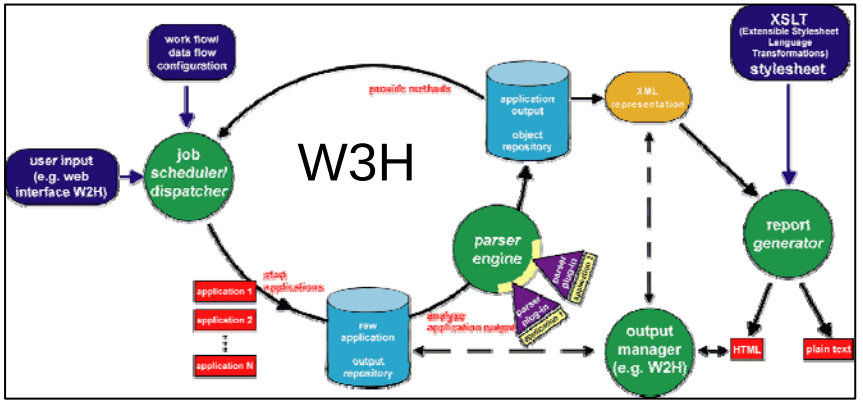


Tailor-made analysis pipelines – WHY?

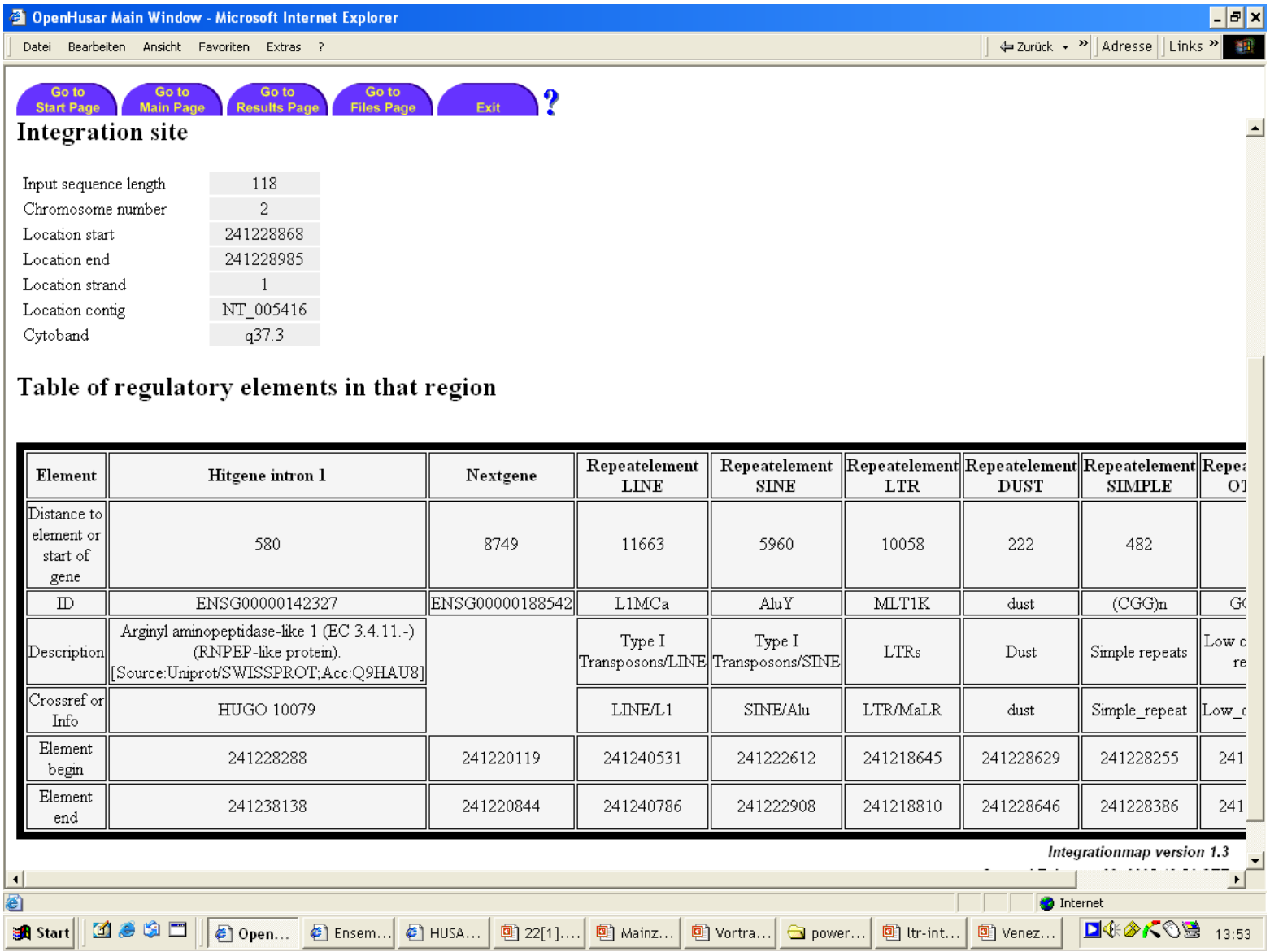
- **Problems are complex; one program is not enough.**
- **Users need compact presentable reports on analysis results**



High-throughput analysis by tailor-made analysis pipelines (Example IntegrationMap)



Pipeline Output: Example IntegrationMap



Integration site

Input sequence length	118
Chromosome number	2
Location start	241228868
Location end	241228985
Location strand	1
Location contig	NT_005416
Cytoband	q37.3

Table of regulatory elements in that region

Element	Hitgene intron 1	Nextgene	Repeatelement LINE	Repeatelement SINE	Repeatelement LTR	Repeatelement DUST	Repeatelement SIMPLE	Repea OT
Distance to element or start of gene	580	8749	11663	5960	10058	222	482	
ID	ENSG00000142327	ENSG00000188542	L1MCa	AluY	MLT1K	dust	(CGG)n	G(
Description	Arginyl aminopeptidase-like 1 (EC 3.4.11.-) (RNPEP-like protein). [Source:Uniprot/SWISSPROT;Acc:Q9HAU8]		Type I Transposons/LINE	Type I Transposons/SINE	LTRs	Dust	Simple repeats	Low c re
Crossref or Info	HUGO 10079		LINE/L1	SINE/Alu	LTR/MaLR	dust	Simple_repeat	Low_c
Element begin	241228288	241220119	241240531	241222612	241218645	241228629	241228255	241
Element end	241238138	241220844	241240786	241222908	241218810	241228646	241228386	241

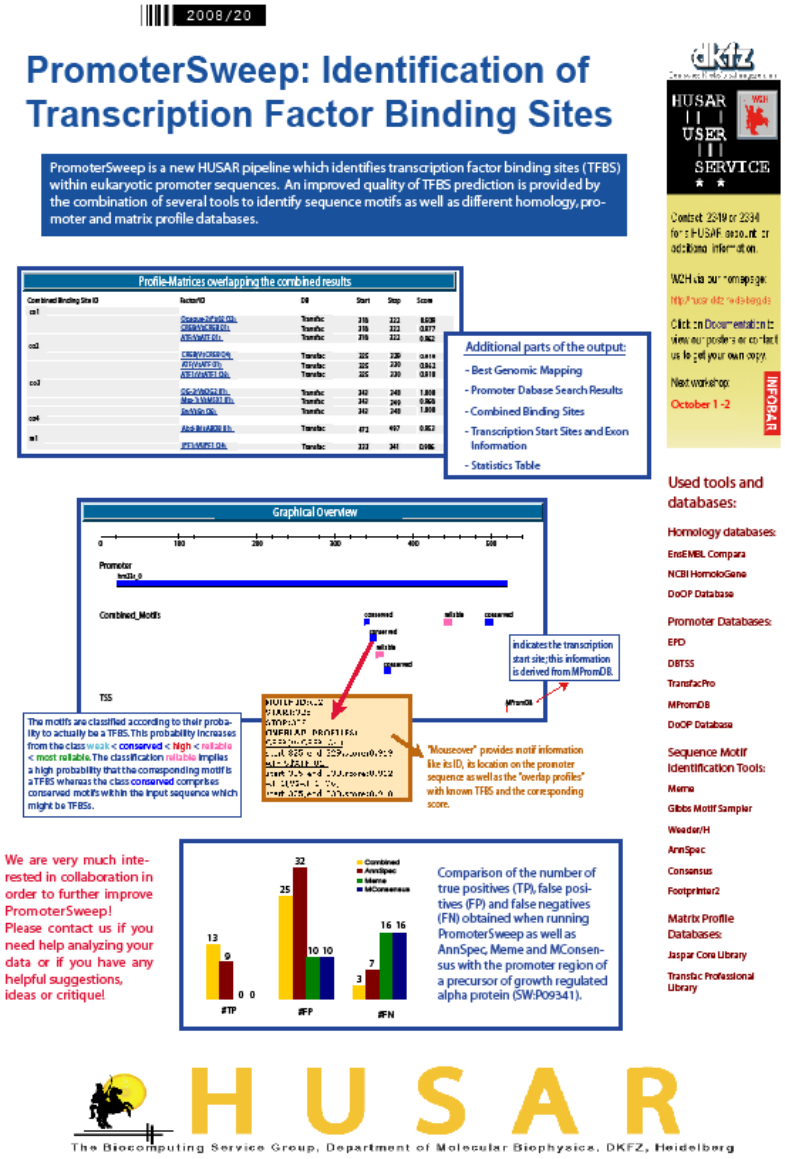
Integrationmap version 1.3

Selection of Available Pipelines

- **cDNA2Genome** Tool for mapping CDNAs
- **ESTAnnotator** EST Identification Tool
- **DomainSweep** Protein Family Search Tool
- **ProtSweep** Protein Identification Tool
- **2DSweep** Secondary Structure Prediction Tool
  - Collaboration with Stefan Wiemann/Poustka
- **GOPet** cDNA and Protein Function Annotation
  - Collaboration with Rainer König/Eils
- **IntegrationMap** Mapping of LTR integration sites
- **IntegrationSeq** Removal of primer and adapter sequences
  - Collaboration with Steffanie Laufs/Zeller
- **miRpredict** miRNA prediction
  - Collaboration with Frank Westermann/Schwab

HUSAR

Outlook: PromoterSweep



Summary: HUSAR Bioinformatics

dkfz.

- Personal
  - Karl-Heinz Glatting
  - Agnes Hotz-Wagenblatt
  - IT Specialist Volker Rössler und 2 Apprentices
  - 4 HIWIs for programming and documentation
- Turn-around Time
  - Depending on problem, from some hours (just running some programs) up to 6 month (for the development of a new task)
- Quality
  - Quality control of program results
  - Testing the scripts and pipelines before release
- Data Return
  - Depending on problem, very often tab delimited files
  - Instructions how to use scripts and pipelines
  - Personal Meeting
- Cost
  - None at the moment

Expression Profiling Webpage

dkfz.

Genomics & Proteomics Core Facilities
Expression Profiling
- Affymetrix GeneChips®
- Affymetrix Technology
- Illumina BeadChips®
- Illlumina Technology
- Illumina Analysis

submission form

454/GS20 Sequencing
Protein Analysis
Peptide Synthesis
Yeast Two-Hybrid
Vector & Clone Repository
Pricelist
Equipment
Downloads
Tools

Username: korn
Logout

Expression Profiling Service

Welcome to the DKFZ Microarray Core Facility. We provide access to full service of state-of-the-art molecular profiling based on **Affymetrix** and **Illumina** technology for members of DKFZ, NCT and their collaborators (via DKFZ accounts).

We support Affymetrix expression profiling using whole genome **GeneChips®**

- **Human U133Plus 2.0**
- **Mouse 430 2.0**
- **Rat 230 2.0**
- Please **enquire** for additional GeneChips®

We support Illumina expression profiling using whole genome **BeadChip®** Sentrix arrays

- **Human Sentrix-6 V2**
- **Human Sentrix-8 V2**
- **Mouse Sentrix-6 V1.1**
- **Mouse Sentrix-8 V1.1**
- **Rat Sentrix-12**

Input from you

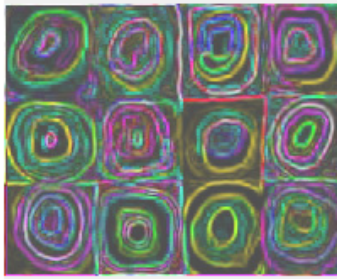
As starting material we take **total RNA** that is quality checked and has a defined concentration. You may provide the samples together with the signed expression profiling sample **submission form**, after logging into the GPCF web (see left menu).

What we do

We perform incoming QC for quality and concentration of all samples (Nanodrop ND-1000, Agilent 2100 Bioanalyzer).
Upon acceptation of samples we perform labeling and hybridization to the microarrays you request, monitoring the quality at all steps.
Image acquisition, single chip analysis as well as normalization across all of your samples is performed, and in depth analysis support via practical software training is provided

Output to you

- All RNA and labeling QC data (conc. of input RNA, quality quality of cRNA)
- Raw data of microarrays



Contact

Dr. Bernhard Korn
German Cancer Research Center
Im Neuenheimer Feld 515
69120 Heidelberg
Germany

Fon: +49 6221 42 4700
Email: **contact form**

www.dkfz.de/gpcf

Access to Services for ZMBH

- Conditions for access
  - Service exchange on internal conditions
- DKFZ account (userID) ?
  - Login!
- Kostenstellen ?
  - Fee for service
  - Identity of „internal user“
  - To be worked on
- Mode for immediate access
  - See the person responsible for the service needed
  - Requests on paper

Acknowledgements

- Agnes Hotz-Wagenblatt, Karl-Heinz Glatting
- Andreas Hunziker
- Manfred Kögl
- Martina Schnölzer
- Michael Pawlita
- Rüdiger Pipkorn
- Stephan Wolf
- Wolf Lehmann
- William Hull

... and the complete GPCF team

Alliance work: Axel Szabowski & Ralf Tolle

www.dkfz.de/gpcf