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# “Application of microarray technology for the detection of changes in gene expression after doping with recombinant human growth hormone”

(Pilot project, funded by WADA 2004)



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## Ultimate Goal:



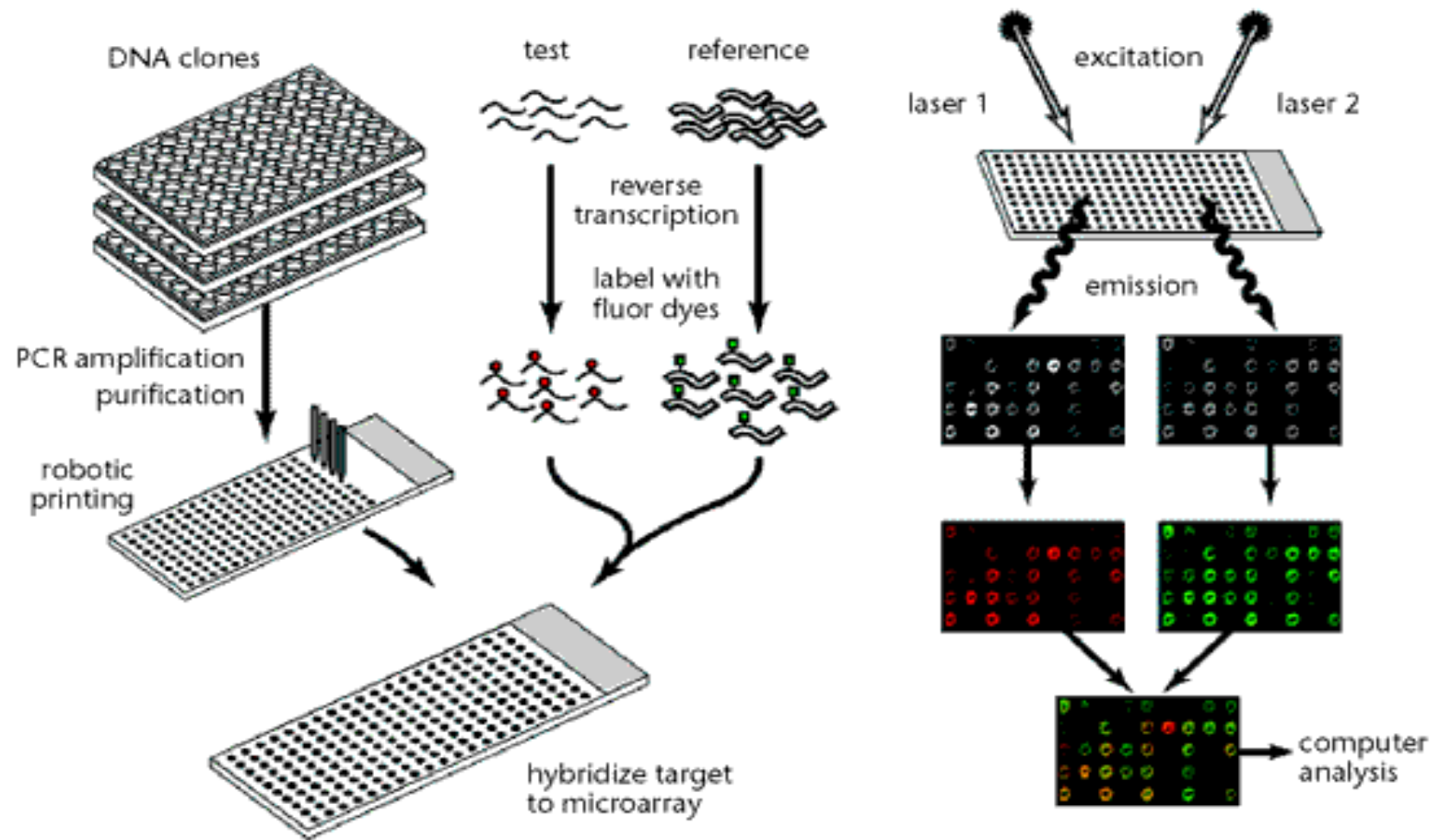
Development of a targeted DNA microarray to identify specific changes in blood cell gene expression related to the administration of human growth hormone

## Present project:

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

# Microarray procedure



Cell lines  
in cell culture

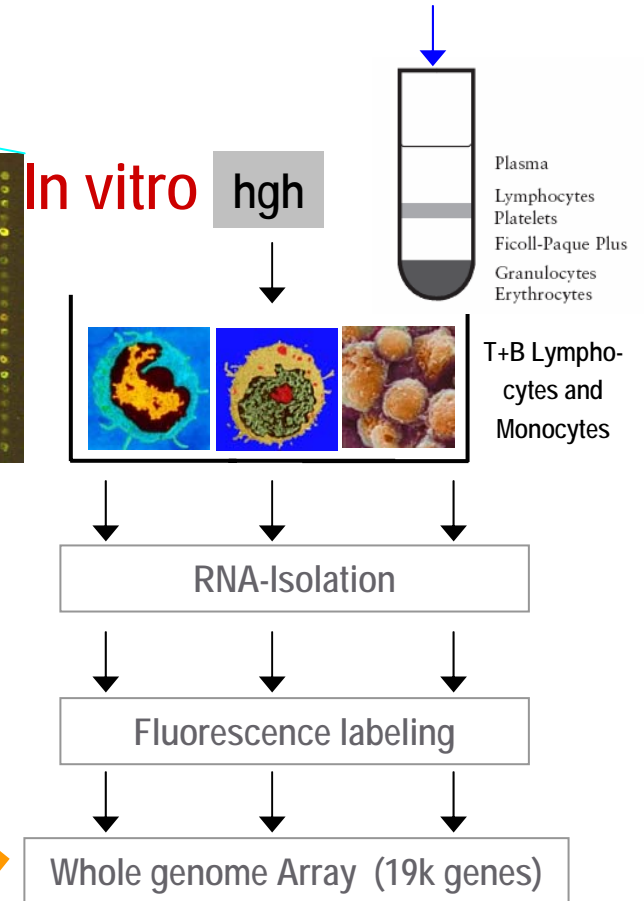
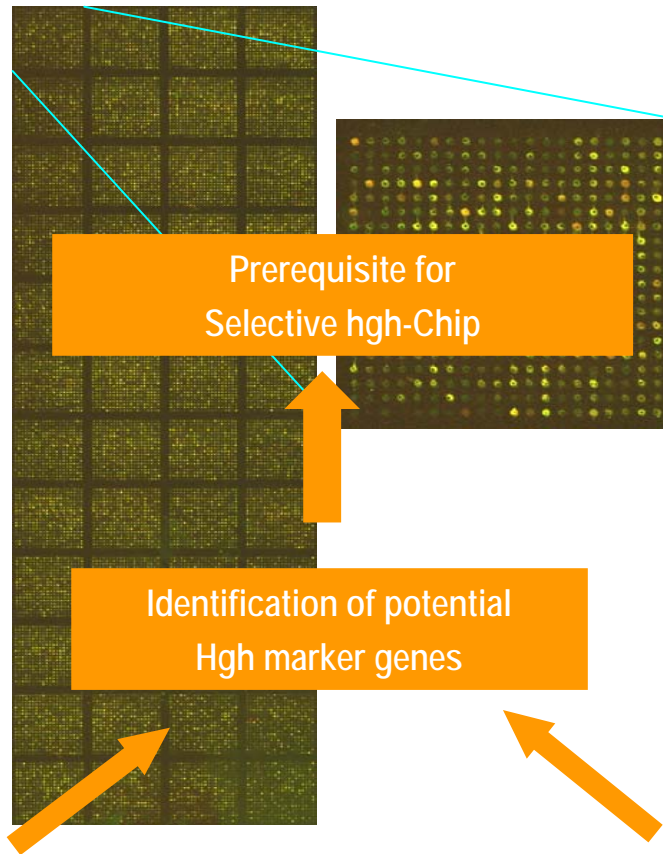
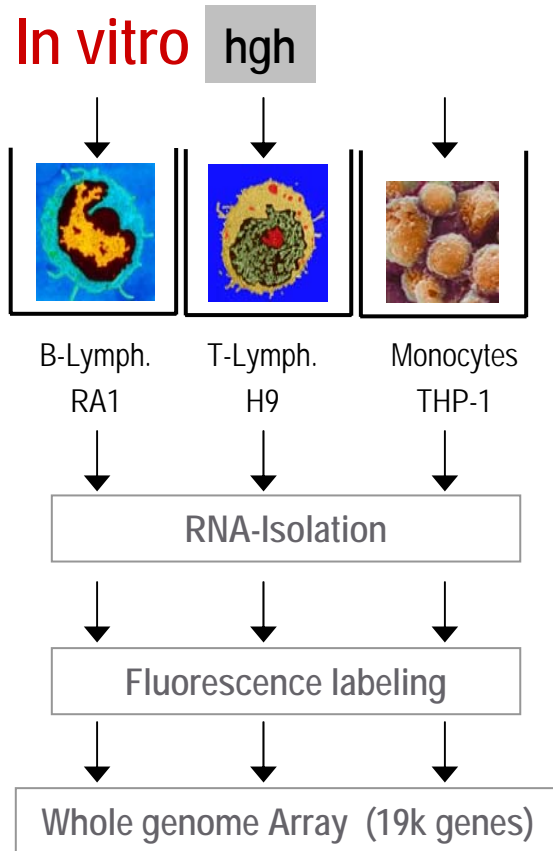
1. ←

# Approach Year 1 (2004)

→ 2.

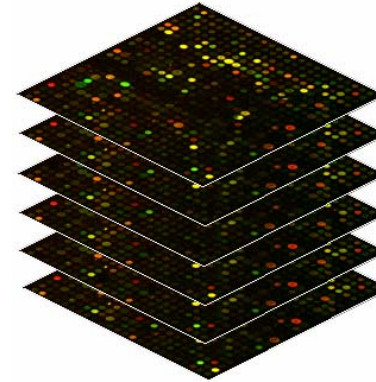
Lymphocytes/Monocytes

Obtained from blood donors by  
gradient centrifugation (Ficoll)



## Experimental design etc.

- Sample = hgh-treated cells, Reference = untreated cells
- High concentrations: 2  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$
- biological replicates, dye swaps
- BASE (BioArray Software Environment)
- Limma package
  - R-based package (Bioconductor)
  - QC plots
  - Data normalisation (Lowess)
  - Forward and backward reactions analysed separately
  - Generation of top-tables

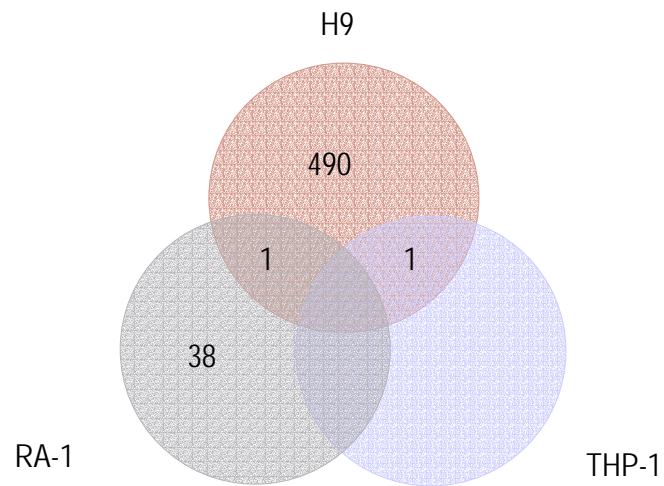


Name	Labeled extract	Owner	(A/N) Group	World	High date	Array slide	Protocol	Log scale	Images	Result	Blank
T0	T0.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-07-29	none	Hybridization D10 EasyH40	1	2	1	
R4	R4.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-07-29	none	Hybridization D10 EasyH40	1	2	1	
R5	R5.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-07-29	none	Hybridization D10 EasyH40	1	2	1	
R6	R6.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-07-29	none	Hybridization D10 EasyH40	1	2	1	
R9	R9.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-07-29	none	Hybridization D10 EasyH40	1	2	1	
T11	T11.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	
T12	T12.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	
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H1	H1.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	
H2	H2.a1.J1 (Cy3), Reference RNA.a5.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	
2A	2A1.a1.J1 (Cy3), 2A2.a1.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	
2B	2B1.a1.J1 (Cy3), 2B2.a1.J1 (Cy5)	rene	none (0)	none	2004-08-13	none	Hybridization D10 EasyH40	1	2	1	

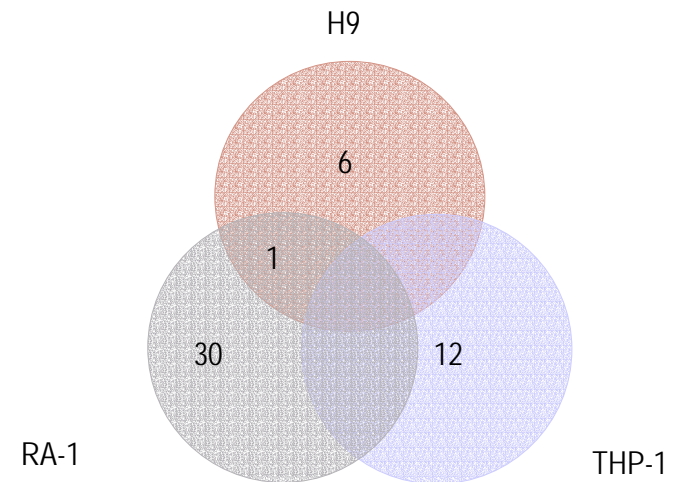
## Results of the gene expression study



## Differentially expressed genes in the three cell lines



Up-regulated genes



Down-regulated genes

## Dosage related and cell-type specific differences in hgh-response

Culture	Hgh-concentrations			
	2 µg hgh/mL		20 µg hgh/mL	
	Gene-regulation			
	up	down	up	down
H9	491	7	298	5
RA-1	39	31	266	15
THP-1	1	12	82	1

## H9 (T lymphocytes)

- Up-regulated genes

cell adhesion

DNA replication

polyamine biosynthesis

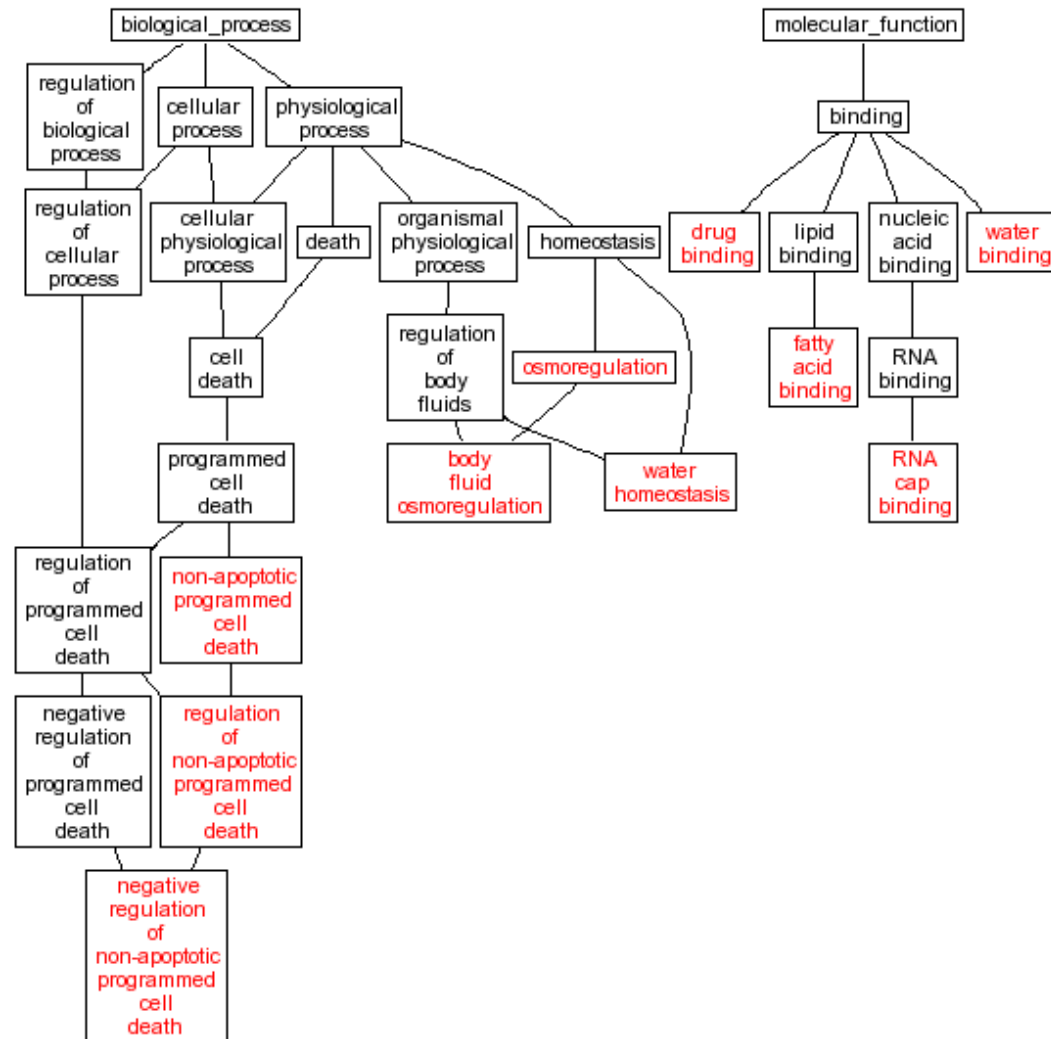
spermidine biosynthesis

aspartyl-tRNA aminoacylation

fatty acid beta-oxidation

# H9 (T lymphocytes)

- Down-regulated genes





## Conclusions

- Increasing prevalence of growth hormone abuse
- Need for a test → introduction of DNA microarrays in doping control (proof-of-concept)
- Anabolic effects reflected in gene expression patterns
- Effects detected in accordance with literature
- New growth hormone responsive genes identified
- Potential of a selective growth hormone chip

## Outlook Project continuation (supported by WADA)

- in vivo studies
- Time course studies on volunteers (athletes) receiving hgh
- Gene expression analysis using whole genome chips
- Marker gene identification (also via suppression subtractive hybridization, SSH)
- Establishment and evaluation of selective hgh-chip

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