APPENDIX

ACKNOWLEDGEMENT

LIST OF FIGURES

LIST OF TABLES

PUBLICATIONS

SELBSTÄNDIGKEITSERKLÄRUNG

ACKNOWLEDGMENTS

This dissertation represents the effort of many years of work and would not have been possible to complete without the cooperation and assistance of various people who deserve my acknowledgement.

I would like to express my sincere gratitude to my advisor, Prof. Dr. Hans Lehrach, for the opportunity of preparing my thesis work in his department, for the guidance and for reviewing this thesis. I also thank Prof. Dr. Gerd Multhaup for reviewing this thesis.

I am exceptionally grateful to Dr. Michal Janitz, my group leader, for the interesting topic he offered me, his gentle guidance during my work and the inspiring, pleasant working environment.

My very special thanks go to Dr. Dominique Vanhecke for her encouragement, patience and her all-embracing inspiration at any step of my work. I also thank my mentor, Dr. Sylvia Krobitsch for her support, her feedback and the interesting conversations and my colleague Markus Ralser for his assistance with several software and plasmid problems.

Sabine Thamm of the group of Dr. Janitz, equipped with the right touch for plasmid preparations, cells and Ph.D. students, was always very helpful and cooperative such as all others in the group and in the institute, including the administration staff. I always felt very welcome. Thank you all for that!

Particular thanks go to Dr. Lajos Nyarsik and his biotechnology engineers Regine Schwarz and Tillmann Wegwerth for the spotting jobs with the SciFlexArrayer. I also thank Dr. Florian Wagner and his assistant staff from the RZPD for spotting samples with their robot as well as Dr. Klaus Hultschig and his coworkers for giving me the chance to test different spotting parameters.

I am indebted to Dr. Bernhard Haendler from Schering-AG Berlin, particularly for the plasmids and androgenic products he placed at my disposal and for the cooperative correspondence with him. The members of the Student Association (STA) provided me with the enzyme "motivase" and offered a pleasant and helpful platform not only for technical conversations. Especially in the year when I took the chair with my two colleagues Andreas Dahl and Marc Sultan we set a lot in motion, e.g. built up an annual scientific Ph.D. retreat and a contact point for newbies and foreigner students. I learned a great deal about organising and scheduling during this time.

I sincerely thank my family, especially my dear mum and dad who nurtured my curiosity and provided all options to me. My very special thanks goes to my husband Dirk, who supported me during the whole time of my work and was always patient particularly in software- and hardware problems, and to my son Jannik for clearing up my mind every time I am with him. Thank you for being at my side! Furthermore sincere thanks go to my friends for their support.

Last but not least I want to thank the HEK 293T and all other cells for growing...

This work was supported by the European Commission (integrated project MolTools) and the German Federal Ministry of Education and Research (grant no 0313068, 01GS0416, 01GR0414, 31P3674).

LIST OF FIGURES

Number	Titel	Page
Figure 1	Suitable containers for reverse transfection	15
Figure 2	Manual spotting	23
Figure 3	Automated spotting using the sciFlexArrayer	23
Figure 4	The CAPPIA process	32
Figure 5	Monolayer of transfected HEK 293T cells on different slide types	34
Figure 6	Fluorescent signals from detection of expressed proteins on different slide	
	types	35
Figure 7	Reverse transfection on a 4-well Lab-Tek™ Chamber Slide™	36
Figure 8	Different final concentrations of DNA in samples prepared by LD-method with	
	three plasmids ("triple-transfection")	38
Figure 9	Different concentrations of gelatine in samples prepared by LD-method	39
Figure 10	Different DNA concentrations in samples prepared by gelatine method	
	compared to samples prepared by LD-method	40
Figure 11	Expression of fluorescent ZsGreen protein in comparison with EGFP	41
Figure 12	Signals of red reporter GAL4-Red and green reporter GAL4-pZsGreen	41
Figure 13	Comparison of different spotting pins used for automated spotting	43
Figure 14	Repetitive robot spotting for amplification of fluorescent signal	44
Figure 15	Dispensing of different sample volumes using the sciFlexArrayer	
	piezodispenser S5	45
Figure 16	Storing slides does not lessen fluorescent signals on a robotically spotted slide	46
Figure 17	Effect of rehydration on robotically spotted slides before transfection	47
Figure 18	Comparison of different transfection reagents for reverse transfection	48
Figure 19	Different ratios of transfection mix components	50
Figure 20	Different protocols to fix cells after reverse transfection	52
Figure 21	Different treatment of the slides after cells fixing	53
Figure 22:	Different methods to detect signal of non-autofluorescent lacZ	55
Figure 23	Reverse transfection of fibroblast cell line Hekl	58
Figure 24	Cell array based PPI screens in different cell lines	59
Figure 25	Application of CAPPIA for the detection of hormone-dependent interactions	61
Figure 26	Dose response of AR-LBD and AR-NTD interactions to androgenic and	
	anti-androgenic compounds	63
Figure 27	High-throughput screens for PPI using PR-stable and PR-trans bait cell arrays	65

LIST OF TABLES

Number	Titel	Page
Table 1	Tested slide and their sources	14
Table 2	List of the components of the mammalian two-hybrid kits from Invitrogen™ and	
	Stratagene	17
Table 3	List of preys (samples A-Q)	20
Table 4	Primary antibodies	26
Table 5	Secondary antibodies	26
Table 6	Different pins from TeleChem tested for CAPPIA experiments	43

ABBREVIATIONS

Degree Celsius
Micro
Activation domain
Androgen receptor
Bait
Binding domain
Bi-distillated water
Base pair
Bovine serum albumin
Caenorhabditis elegans
Cell array based protein-protein-interaction assay
Complementary DNA
African green monkey kidney cell line
Fluorescent cyanine dye 3
Drosophila melanogaster
4`,6-diamidino-2`-phenylindole dihydrochlorid
DNA binding domain
5a-dihydrosterone
Dulbecco's Modified Eagle Medium
Dimethyl sulfoxide
Desoxyribonucleic acid
Red fluorescent protein from Discosoma sp. reef coral
Escherichia coli
Enhanced green fluorescence protein
Fetal calf serum
Fluorescein isothiocyanate
Fluorescence resonance energy transfer
Gamma amino propyl silane coated slide
Glucocorticoid receptor
Red fluorescent protein from Heteractis Crispa reef coral
Human embryonic kidney
Human skin fibroblast
Human epithelial cells, fatal cervical carcinoma

HEPES	4-2-hydroxyethyl-1-piperazineethanesulfonic acid
HepG2	Human hepatocellular carcinoma
HPRD	Human Protein References Database
HSP	Heat-shock protein
lκB	Inhibitor -kappaB
lacZ	ß-D-galactosidase gene
LBD	Ligand-binding domain
LD	Lipide DNA
Luc	Luciferase
Μ	Moles per liter
mM	Milimoles per liter
MPA	Medroxyprogesterone acetate
MPIMG	Max Planck Institute for Molecular Genetics
n	nano
NCoR	Nuclear hormone receptor corepressors
NF-κβ	Nuclear factor -kappaB
NTD	N-terminal activation domain
OH-Flu	2-hydroxyflutamid
Р	Prey
PBS	Phosphate buffered saline
PBS PC-3	Phosphate buffered saline Human prostate epithel
PBS PC-3 PCR	Phosphate buffered saline Human prostate epithel Polymerase chain reaction
PBS PC-3 PCR PEI	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine
PBS PC-3 PCR PEI pLacZ	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ
PBS PC-3 PCR PEI pLacZ PPI	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction
PBS PC-3 PCR PEI pLacZ PPI PRB-slide	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/IacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc RNA	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase Ribonucleic acid
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc RNA RNAi	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase Ribonucleic acid RNA interference
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc RNA RNAi rpm	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase Ribonucleic acid RNA interference Revolutions per minute
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc RNA RNAi RNAi RNAi	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase Ribonucleic acid RNA interference Revolutions per minute Ras Recruitment System
PBS PC-3 PCR PEI pLacZ PPI PRB-slide PR-slide R R1881 RLuc RNA RNAi RNAi RNAi RNAi RNAi RRS RZPD	Phosphate buffered saline Human prostate epithel Polymerase chain reaction Polyethylenimine pcDNA3.1D/V5-His/lacZ Protein-protein-interaction Prey + Reporter + Bait spotted slide Prey + Reporter spotted slide Prey + Reporter spotted slide Reporter Methyltrienolone Renilla luciferase Ribonucleic acid RNA interference Revolutions per minute Ras Recruitment System

SH3 domain	Src homology 3 domain
siRNA	Small interfering RNA or short interfering RNA
SMRT	Silencing mediator of retinoid and thyroid receptor
SNP	Single nucleotide polymorphism
SRS	SOS Recruitment System
STEP	Surface transfection and expression protocol
SV40T	Simian virus 40 large T antigen
TAD	Transactivation domain
TNF	Tumour necrosis factor
TRAF	TNF receptor-associated factor
VP16	Etoposide phosphate
VPL slide	VECTABOND™ Reagent + Poly-L-Lysine coated slide
WI-38	Human lung fibroblast

PUBLICATIONS

Articles

<u>A. Fiebitz</u>, L. Nyarsik, B. Haendler, Y.H. Hu, F. Wagner, S. Thamm, H. Lehrach, M. Janitz, D. Vanhecke (2006): High-throughput mammalian twohybrid screening for protein-protein interactions using transfected cell arrays Submitted: Nature Methods

Y.H. Hu YH, D. Vanhecke, H-J. Warnatz, F. Wagner, <u>A. Fiebitz</u>, S. Thamm, P. Kahlem, M.L. Yaspo, H. Lehrach, M. Janitz: Cell array-based intracellular localization screening reveals novel functional features of human chromosome 21 proteins, BMC Genomics 2006, 7:155 (16 June 2006)

Poster presentations

Berlin, 6. – 9.9.2006 Neurodegenative Diseases: Molecular Mechanismus in a Functional Genomics Framework

Berlin, 18. - 21.4.2005 GBM Annual Fall Meeting

Berlin, 4. - 7.4.2004 Human Genome Meeting

Paris, 15. - 19.5.2003 FOCIS 3rd Annual Meeting

Berlin, 17. - 19.11.2002 Symposium of the NGFN and DHGP

SELBSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass ich die vorliegende Arbeit eigenständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ich versichere, dass diese Arbeit in dieser oder anderer Form noch keiner anderen Prüfungsbehörde vorgelegt wurde.

Berlin, den

(Andrea Fiebitz)