8 Appendix

8.1 Appendix B: Abbreviations

Table A.1: List of abbreviations

bp	Base pair	PBS	Phosphate buffered saline
BSA	Albumin from bovine serum	PCR	Polymerase chain reaction
CHD	Congenital heart disease	PMSF	Phenylmethanesulfonyl fluoride
ChIP	Chromatin immunoprecipitation	Pol II	RNA polymerase II
ChIP-chip	Chromatin immunoprecipitation followed by microarray analysis	qPCR	Quantitative real-time PCR
CNB	Conserved non-coding block	RNAi	RNA interference
DEPC	Diethyl pyrocarbonate	RNA	Ribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium	RNase	Ribonuclease
DMSO	Dimethylsulfoxid	RT-PCR	Reverse Transciption PCR
DNA	Desoxyribonucleic acid	sec	Second
DNase	Desoxyribonuklease	SDS	Sodiumdodecylsulfate
dNTP	Desoxyribonukleosidtriphosphate	siRNA	Short interfering RNA
DTT	1,4-Dithiothreit	TBE	Tris-borate-ETDA (buffer)
Dpc	Days post coitum		
EDTA	Ethylenediaminetetraacetic acid	TE	Tris-EDTA (buffer)
EGTA	Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid	TF	Transcription factor
g	Gram	TFBM	Transcription factor binding motif
GO	Gene Ontology	TFBS	Transcription factor binding site
h	Hour	Tris	Tris(hydroxymethyl)-aminomethane
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid	TSS	Transcription start site
HRP	Horse radish peroxidase	U	Unit of enzyme activity
kb	Kilo base	UV	Ultra violet light
l	Liter	v/v	Volume per volume
m	Meter	W	Watt
M	Mol/l, molar	w/v	Weight per volume
min	Minute	IVT	In vitro transcription
Mol. biol.	Molecular biology grade		
o.n.	Over night		
p	p-value		
p.a.	pro analysis		
Hpf	Hours post fertilization		

8.2 Appendix C: siRNAs

Table A.2: List of siRNAs used in mammalian cells.

Company	Name	Target	Accession	Sequence
Qiagen	siDpf3	Dpf3a/ Dpf3b	NM_058212/ AK039011	CGGGACAGTCATTCCTAATAA
Invitrogen	siDpf3a	Dpf3a	NM_058212	TGACTCTGGTCATTGTTCTAGTTCT
Qiagen	siMef2a-1	Mef2a	NM_194070	CACATTCTGCTGAATTATTTA
Qiagen	siMef2a-2	Mef2a	NM_194070	AAGTAATTATTAGGAATATAA
Qiagen	siNon	Synthetic	Synthetic	Unknown
Invitrogen	si_FITC	Synthetic	Synthetic	Unknown

8.3 Appendix D: Lists of Primers

8.3.1 Human primers

Table A.3: Primers used for human *DPF3* promoter cloning.

Gene name	Primer name	Primer sequence	Acc. number
DPF3	Hu_H1_fwd_0_K	ATAGGTACCTGTGATTGGCCCGTCGCTTCCC	NM_012074
DPF3	Hu_H1_fwd_0_J	ATAGGTACCACCTAGCTACCAGCAGCGCCGA	NM_012074
DPF3	Hu_H1_fwd_0_I	ATAGGTACCAAAGGGAGTGACCTCTGGGCTT	NM_012074
DPF3	Hu_H1_fwd_0_H	ATAGGTACCATAATACTCTCCCGTGCGTCTC	NM_012074
DPF3	Hu_H1_fwd_0_F	ATAGGTACCGAGCGCAAACTCTTCTGCAGT	NM_012074
DPF3	Hu_H1_fwd_0_G	ATAGGTACCGCCAAAGGCTAATGAATAGGAT	NM_012074
DPF3	Hu_H1_fwd_0_D	ATAGGTACCGCAGAGCACATGTAGTAATTTG	NM_012074
DPF3	Hu_H1_fwd_0_B	ATAGGTACCCAAGATGCTATGCAAAGGATT	NM_012074
DPF3	Hu_H1_fwd_0_A	ATAGGTACCATTATGCTGGTTTCTGCATCCA	NM_012074
DPF3	Hu_H1 rev1E	ATAGCTAGCAACAGAATATTGTCTGTCTCAGA	NM_012074

8.3.2 Mouse primers

Table A.4: Real-time PCR primers used for mouse expression profiling.

Gene name	Primer name	Primer sequence	Acc. number
Dpf3a		CAGACGGGACAGTCATTCCTAAT	NM_058212
Dpf3a		CTCCCAAATGAGCAGAGCGT	NM_058212
Dpf3b		CCTCATTTCTACCAGCGGGA	AK039011
Dpf3b		GCAACACACGAGTGGTTGATG	AK039011
Mef2a		ATGGTTGTGAGAGCCCTGATG	NM_001033713
Mef2a		AGAAGTTCTGAGGTGGCAAGC	NM_001033713
Hprt		AAACAATGCAAACTTTGCTTTCC	NM_013556
Hprt		GGTCCTTTTCACCAGCAAGCT	NM_013556

8.3.3 Zebrafish primers

Table A.5: Primers used for zebrafish *dpf3* (NM_001111169) cloning.

Gene name	Primer name	Primer sequence	Restriction site
dpf3	zDPF3_ATG_CDS_BamHI_f	AGAGGGATCCATGGCGACTGTCATTCAGAATC	BamHI
	zDPF3_STOP_CDS_EcoRI_r	AGAGGAATTCCTACCGATTACCCAGATGTGTT	EcoRI

Table A.6: Primers used for zebrafish *dpf1* (NM_001030204), *dpf2* (NM_212696), *dpf3* (NM_001111169) *in situ* probe cloning.

Gene name	Primer name	Primer sequence	Probe name	IVT primer	linearized
dpf1	zdpf1_ISH_f	TATCAAAATGGCCACAGTGGTC	zdpf1-ISHb	T7	HindIII
	zdpf1_ISH_r_T7	TGTAATCCGTTGTTGAGGCTGA			
dpf2	zdpf2_ISH_f	GGAGAATATCGTTAAAGTGCTCG	zdpf2-ISHa	T7	HindIII
	zdpf2_ISH_r_T7	GCCATTTGGTCCTTTCTTAGGG			
dpf3	zdpf3_ISH2b_f	GCAGCAGGTCAGATGTACACCTA	zdpf3-ISH2b	Sp6	EcoRV
	zdpf3_ISH2b_r_Sp6	GCAGGATTTACACTCGATGCACT			

Table A.7: Real-time PCR primers used for Zebrafish GeneChip Genome Array confirmation.

Gene name	Primer name	Primer sequence	Acc. number
zgc:110715	zgc:110715_RT_f	GGAAGATGCACCAGCAGATTG	ENSDARG00000035958
	zgc:110715_RT_r	TGTCTGACTTGGCCACTTTGG	
cmya1	cmya1_RT_f	AATAGCTCAGCCAGCCACATTT	ENSDARG00000030722
	cmya1_RT_r	TGGGACCTCAGGAACTCGCT	
mybbp1a	mybbp1a _RT_f	CCCGTGTTGTGTGTGAATCTGA	ENSDARG00000028323
	mybbp1a _RT_r	GAAACATGCCTGACCCTGCT	
calm3a	calm3a _RT_f	GTGATGCGCTCATTGGGTC	ENSDARG00000037014
	calm3a _RT_r	TTCCATTGCCATCAGCGTC	
fn1b	fn1b _RT_f	CCAATTCAATGGAACGCTCC	ENSDARG00000006526
	fn1b _RT_r	ACGTCAAACCTGCTTTGAGGC	
irx1b	irx1b _RT_f	CCTCACCTTCAACCCATCCTG	ENSDARG00000056594
	irx1b _RT_r	TCGCCCAATTGTGTAGTTTCC	
mmp9	mmp9_RT_f	AGAGAGGAAAAGGCAAGGTGCT	ENSDARG00000042816
	mmp9_RT_r	CAGCTGAGCCTTTACATCAAGTCT	
akt1s1	akt1s1_RT_f	CTGAATGAATCGGCAGGTGTC	ENSDARG00000060779
	akt1s1_RT_r	CCCATCAGACTCAAAGAAGGGC	
pik3r3	pik3r3_RT_f	ACCCGAGGACTCAGACCATGTA	ENSDARG00000034409
	pik3r3_RT_r	TCACGTCTCTCCAGTCGTCATC	
zgc136930	zgc136930_RT_f	ACATCAGCCAGGAAAGAGCTG	ENSDARG00000055192
	zgc136930_RT_r	AACTCTGCTCCAGACTCATGCTT	
flncb	flncb _RT_f	GGCAACCGAATGTACAACGTC	ENSDARG00000018820
	flncb _RT_r	ACCATTTCTTCACCCCATTTGA	
hsp70	hsp70_RT_f	TCCCTGGAGTCTTACGCCTTC	ENSDARG00000055723
	hsp70_RT_r	CTCGCTGATCTTGCCTTTCAG	
gamma-crystallin	gamma-crystallin_RT_f	ATGTCTCACTGCCAGTCCTGC	ENSDARG00000057515
	gamma-crystallin_RT_r	TGCCACATCCTGCCTCTGTAG	
prmt1	prmt1_RT_f	AGGCTTCTCTACCAGCCCAGA	ENSDARG00000010246
	prmt1_RT_r	CGTTGGGCTTCATGCTGATAG	
zgc:101755	zgc:101755_RT_f	CATGGAGATCTGGGTCAGGG	ENSDARG00000056090
	zgc:101755_RT_r	CGGAGGTGATCGAAACGAA	
crabp1a	crabp1a _RT_f	ATGTGGAGATTCGACAGGACG	ENSDARG00000045926
	crabp1a _RT_r	CGCGAAAGTTGATTTCAGTGG	

pitx2a	pitx2a _RT_f	TCCGAATCCCAGGAACAAGAG	ENSDARG00000036194
	pitx2a _RT_r	TGGACGCGTTTCAGTGGTTT	
zgc:55491	zgc:55491_RT_f	CGCGAGTGCATTAGGAAGTTTC	ENSDARG00000005948
	zgc:55491_RT_r	CCAATTTCCACTGGAGCGATA	
myst1	myst1_RT_f	AAGGCTCGTTTGGCGTTGA	ENSDARG00000027187
	myst1_RT_r	TCTGGCTGGTCCACAAGTTCT	
irx4a	irx4a _RT_f	AAGGATCTGCAGCTGAGCGA	ENSDARG00000035648
	irx4a _RT_r	TGGAAGCGGTGCTTGAGTTC	
tmsb	tmsb _RT_f	AAGAACCCTTTGCCAACCAA	ENSDARG00000054911
	tmsb _RT_r	AGACGTCATTCACGGTGTGG	
zgc:64199	zgc:64199_RT_f	TGGCCAGTAAGAACGCTTCACT	ENSDARG00000042027
	zgc:64199_RT_r	CACGATAGGCTGTTCCACTGCT	
dlx4a	dlx4a _RT_f	ACCGCAATAGAGGCCTTCAA	ENSDARG00000011956
	dlx4a _RT_r	CTGTAGCGCTCCTGTGTGTGA	
tmod4	tmod4_RT_f	TCGTTCCTAAACCAGGCTCG	ENSDARG00000020890
	tmod4_RT_r	TTCTTAGCGCTTCCTCCAGC	
hand2	hand2_RT_f	TCAGGGAGCAGCAATGACAAG	ENSDARG00000008305
	hand2_RT_r	AGCTCCAATGCCCAAACATG	
epoR	epoR _RT_f	CGCTGGAGGTGTTATCTGAGG	ENSDARG00000043609
	epoR _RT_r	CGAGTCTGAACGCTGGTCCT	
gapdh	gapdh _RT_f	ATGGCAAGCTTACTGGTATGGC	ENSDARG00000043457
	gapdh _RT_r	AGGTTTCTCAAGACGGACTGTCA	

8.4 Appendix E: Zebrafish Expression arrays

8.4.1 Array quality control
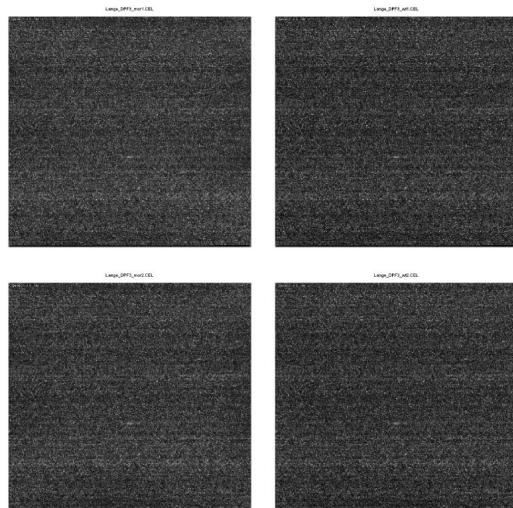
**Figure 8.1: Raw images of Zebrafish Expression arrays.**
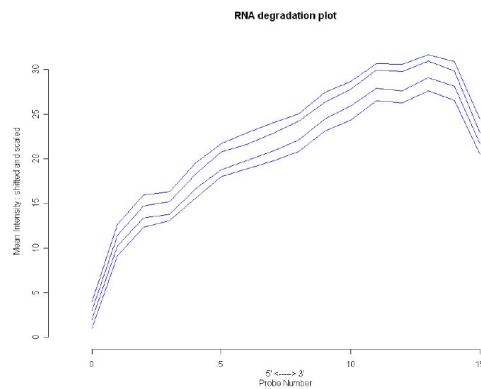
Figure 8.2: Zebrafish RNA degradation plots.

8.4.2 Data analysis

The data for analysis of no background correction (noBG) and MAS background correction (MASBG) are summarized below. In the analysis, the data was normalized with Qspline normalization (abbreviated as qspline).
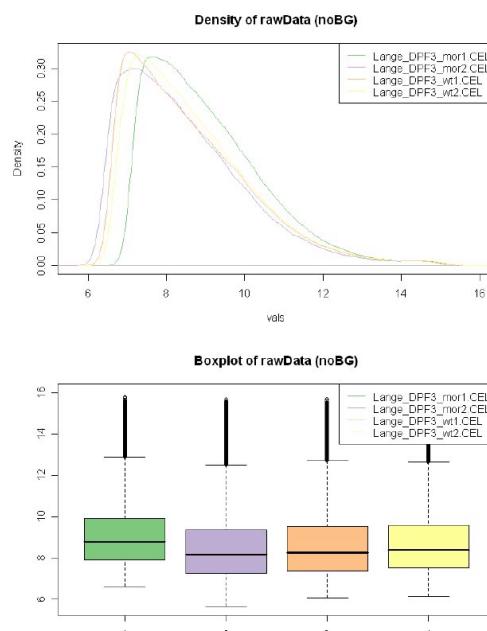

**Figure 8.3: Boxplot and densities of intensities for rawData noBG.**
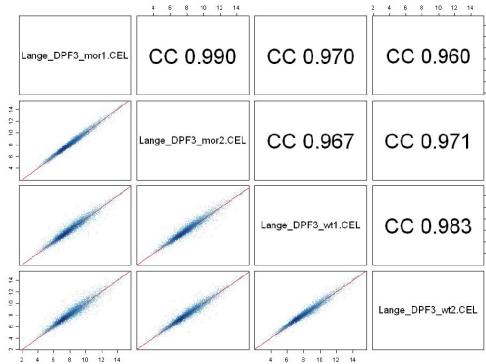
**Figure 8.4: Correlation plots of intensities for rawData noBG.**
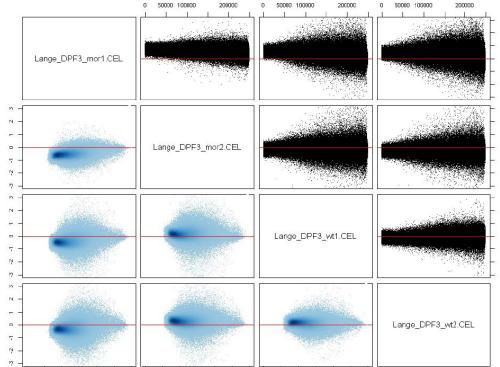
Figure 8.5: MA-plots and M vs. rank (A) of intensities for rawData noBG.
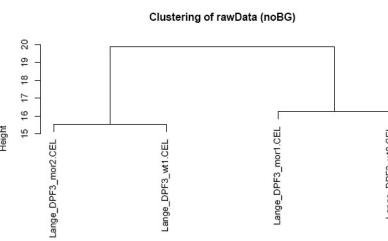

Figure 8.6: Clustering of intensities for rawData noBG.
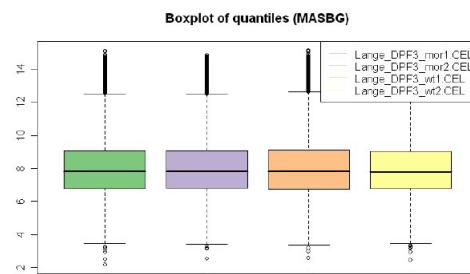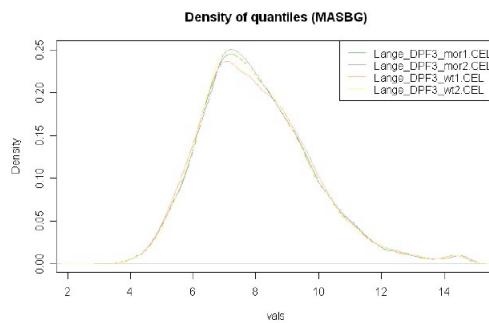
**Figure 8.7: Boxplot and densities of intensities for quantiles MASBG.**

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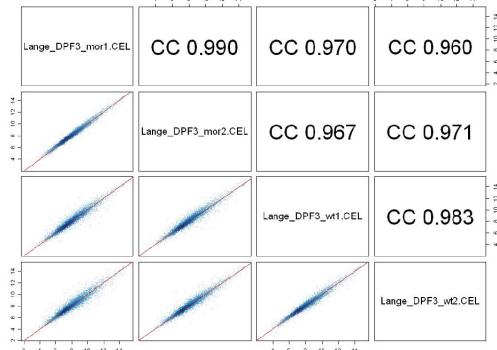
**Figure 8.8: Correlation plots of intensities for quantiles MASBG.**
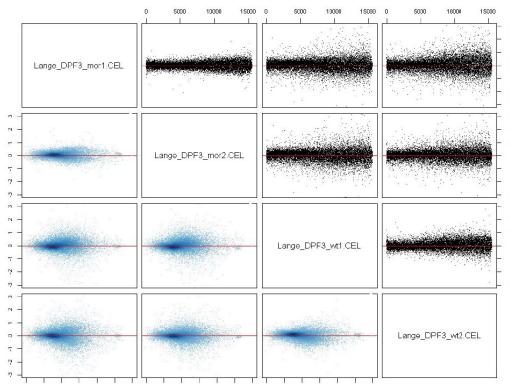
**Figure 8.9: MA-plots and M vs. rank(A) of intensities for quantiles MASBG.**
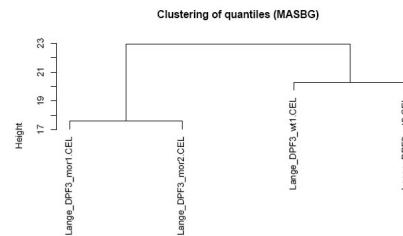
Figure 8.10: Clustering of intensities for quantiles MASBG.

9 Publications

Li H, **Lange M**, Sperling S, Patel DJ. Structural basis for modified histone tail readout by the tandem plant-homeodomain of DPF3. In preparation.

Lange M, Kaynak B, Forster UB, Tönjes M, Fischer JJ, Grimm CH, Schlesinger J, Just S, Dunkel I, Krueger T, Mebus S, Lehrach H, Lurz R, Gobom J, Rottbauer W, Abdelilah-Seyfried S, Sperling S. Regulation of Muscle Development by DPF3, a Novel Histone Acetylation and Methylation Reader of the BAF Chromatin Remodelling Complex. Genes Dev., resubmitted.

Hammer S*, Toenjes M*, **Lange M**, Fischer JJ, Dunkel I, Mebus S, Grimm CH, Hetzer R, Berger F, Sperling S. Characterization of TBX20 in human hearts and its regulation by TFAP2. J. Cell. Biochem., 2008 Feb 14; [Epub ahead of print]. * Equal contribution.

Elliott DA, Solloway MJ, Wise N, Biben C, Costa MW, Furtado MB, **Lange M**, Dunwoodie S, Harvey RP. A tyrosine-rich domain within homeodomain transcription factor Nkx2-5 is an essential element in the early cardiac transcriptional regulatory machinery. Development, 2006 Apr;133(7):1311-22.

Berriel Diaz M, **Lange M**, Heldmaier G, Klingenspor M. Depression of transcription and translation during daily torpor in the Djungarian hamster (*Phodopus sungorus*). J Comp Physiol [B]. 2004 Aug;174(6):495-502.

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Selbstständigkeitserklärung

Hiermit erkläre ich, daß ich diese Arbeit selbst verfaßt habe sowie keine anderen als die angegebenen Quellen und Hilfsmittel in Anspruch genommen habe. Die Chromatin-Immunpräziptation wurde von Dr. Christina Grimm und Jenny J. Fischer innerhalb der Arbeitsgruppe durchgeführt. Die bioinformatische Auswertung der Microarray Daten wurde von Tammo Krüger innerhalb der Arbeitsgruppe durchgeführt. Die massenspektroskopische Auswertung, *in situ* Hybridisierung im Seeigel sowie die elektronenmikroskopischen Aufnahmen von C2C12 Zellen wurden je von Dr. Johan Gobom, Alexander Kühn und Rudi Lurz am Max-Planck-Institut für molekulare Genetik durchgeführt. Morpholino-Injektionen sowie funktionelle Analysen im Zebrabärbling wurden von Dr. Steffen Just und Dr. Wolfgang Rottbauer an der Universität Heidelberg initiiert und von Dr. Ulrike B. Förster und Dr. Salim Abdelilah-Seyfried am Max-Delbrück Zentrum für molekulare Medizin in Berlin-Buch fortgeführt. Ich versichere, daß diese Arbeit in dieser oder anderer Form keiner anderen Prüfungsbehörde vorgelegt wurde.

Martin Lange   Berlin, Juni 2008