PCR analysis

gene	forward primer sequence 5´-3´	reverse primer sequence 5´-3´	amplicon size	gene bank accession number
mu p62	CAT CAA ACA GCT GGC GAG AT	GTG CCC GAT AAT TCT GAC GA	342 bp	AF057352
LT2	CTT AAT GAG GTC GGA ATC GA	GCT TGT CGT AAT AAT GGC GGC ATA C	500 bp	DL242837
D7 Mit140	GGA AGT TGT GGG ACC TTT AGG	CCT CTT CTG GCC TGT GAG GG	137 bp/125 bp	AC133502
D7 Mit12	GCT GGG TTT ATT CAT TGA AA	TCC AGC TCA TGG GTA GAA GA	197 bp/220 bp	AC122001
SnuPE IGF2	TCA GTG AAT CAA ATT A			NM_010514.2
SnuPE H19	CTC AGA CGG AGA TGG A			NR_001592
hu-H19	TTC AAA GCC TCC ACG ACT CT	CTG AGA CTC AAG GCC GTC TC	100 bp	NR_002196
hu-β-actin	TGC GTG ACA TTA AGG AGA AG	GTC AGG CAG CTC GTA GCT CT	106 bp	NM_001101
hu-IGF2	GGA CTT GA TCC CTG AAC CA	TGA AAA TTC CCG TGA GAA GG	100 bp	NM_000612
mu-β-actin	GCT GTG CTA TGT TGC TCT AGA CTT C	CTC AGT AAC AGT CCG CCT AGA AGC	500 bp	NM_007393
mu-H19 cDNA	TAA GTC GAT TGC ACT GGT TTG GAG T	TGA TGG AAC TGC TTC CAH ACT AG	188 bp	NR_001592
mu-Igf2 cDNA	GGC CCC GGA GAG ACT CTG TGC	TGG GGG TGG GTA AGG AGA AAC CT	600 bp	NM_010514
hu p62	GTT CCC GCA TCA TCA CTC TTA T	GAA TCT CGC CAG CTG TTT GA	117 bp	AF057352
mu Igf2	GGA AGT CGA TGT TGG TGC TTC TC	CGA ACA GAC AAA CTG AAG CGT GT	186 bp	NM_010514
mu H19	CAG AGG TGG ATG TGC CTG CC	CAG AGG TGG ATG TGC CTG CC	80 bp	NM_023123
mu 18S	GCG CTT CTC TTT CCG CCA	AGC TCT CCG ACA CCT CTC TT	149 bp	NM_003278
mu cyclophilin	GGC CGA TGA CGA GCC C	TGT CTT TGG AAC TTT GTC TGC	63 bp	NM_0089707
mu PTEN	GTG AGG ATG GTA GGG GAA TC	AGA GGA CTC AAA GGG GTG ACC	133 bp	NM_008960
mu Aire	CGG AGC TAC CTG CAG AGA C	GTG ACA GCA GCA TCA GAG C	106 bp	NM_009646
tTA	GTG CAG AGC CAG CCT TCT TA	CCT CGA TGG TAG ACC CGT AA	129 bp	U89929

Taq-Man probe sequences

probe name	sequence 5´- 3´	Taq Man probe	annealing temperature
hu-p62	6-FAM d(TGT GAA TCT CTT CAT CCC AAC CCA GGC T) BHQ-1	1.5 pmol	60°C
Igf2	6-FAM d(CCT TCG CCT TGT GCT GCA TCG CTG CT) BHQ-1	1.5 pmol	60°C
H19	6-FAM d(TCA CTG AAG GCG AGG ATG ACA GGT GTG G) BHQ-1	2.5 pmol	60°C
18S	6-FAM d(CCA CGC CAA CCC ACC GCC CTG TG) BHQ-1	2.5 pmol	60°C
cyclophilin	6-FAM d(TGG GCC GCG TCT CCT TCG A) BHQ-1	1.5 pmol	60°C

PCR conditions

gene	initial denaturation (94°C)	denaturation (94°C)	annealing (60°C)	elongation (72°C)
PTEN	10 min	15 sec	15 sec	15 sec
mu 18S	8 min	15 sec	15 sec	15 sec
Igf2 (SNuPE)	5 min	30 sec	60 sec	60 sec
H19 (SNuPE)	5 min	60 sec	60 sec	30 sec

mRNA stability

Experiments were performed the day after cell isolation. Therefore, medium was replaced by FCS-free William´ s medium E (Gibco, Invitrogen, Karlsruhe, Germany) two hours before the experiments started and cells were maintained under serum-free conditions for up to 10 hours until harvest.

For transcription block in murine hepatocytes, 1.0 mg/ml Act D was diluted in cell culture medium to a final concentration of 10 µg/ml. All cells were treated at different time points, but harvested simultaneously (0/4/6/8/10 hours) after Act D incubation. Therefore, the cell culture medium was removed, cells were washed twice with 1x PBS and Qiazol lysis reagent was added to each well.