Rates of *in situ* transcription and splicing in large human genes

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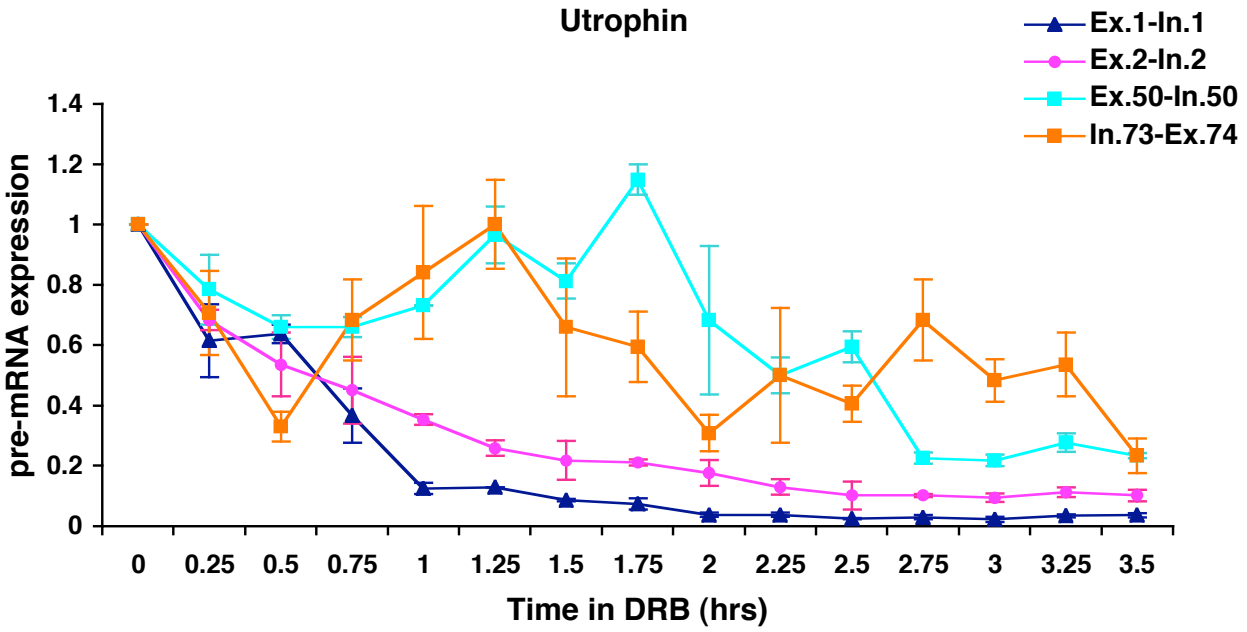
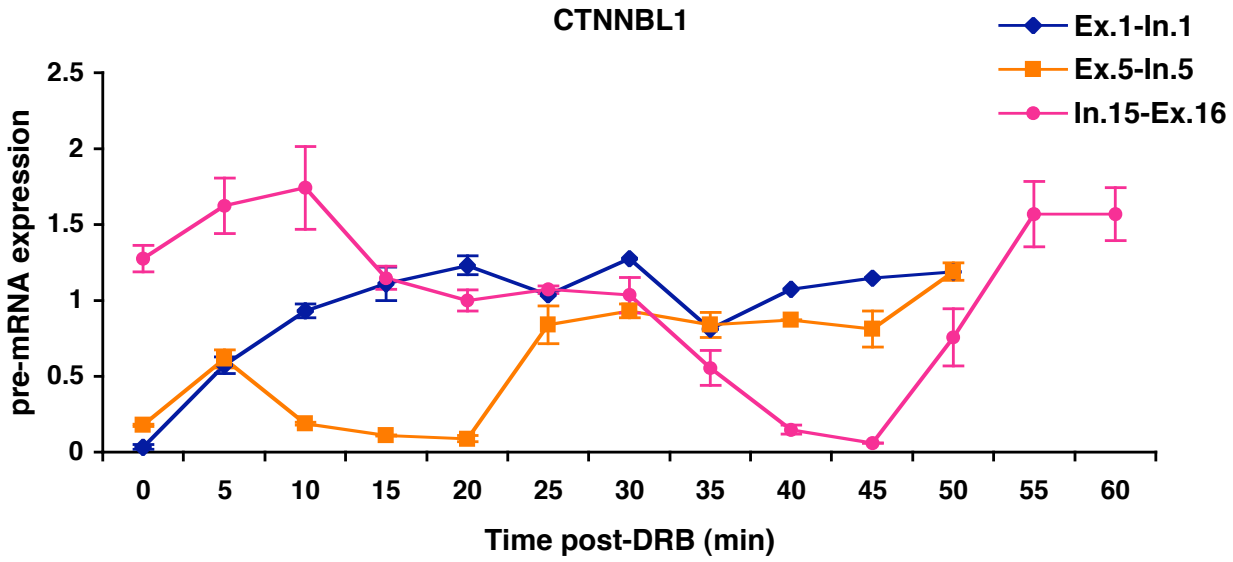
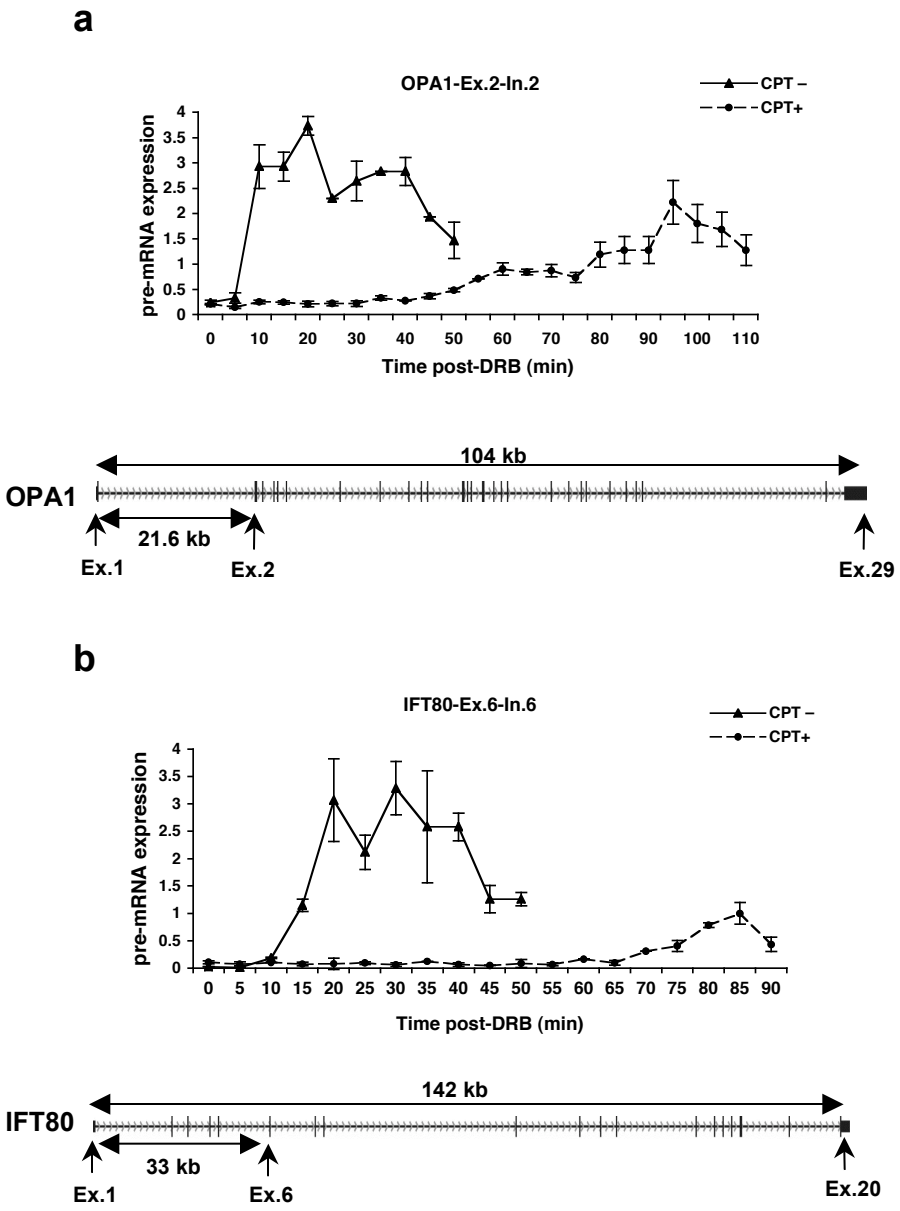


Supplementary Figure 1: Kinetics of DRB dependent inhibition of pre-mRNA transcription. Tet21 cells were treated with 100μM of DRB for the indicated times and then harvested for isolation of total RNA. Shown are the levels of pre-mRNA as determined by qRT-PCR for various regions of the Utrophin gene.



Supplementary Figure 2: Rate of elongation by RNAPII after 30 minutes of DRB treatment. Tet21 cells were treated with 100μM of DRB for 30 minutes and then the cells were washed twice with PBS and further incubated in fresh medium. Cells were harvested at 5 minute intervals for isolation of total RNA. Shown are the levels of pre-mRNA as determined by qRT-PCR for various regions of the CTNNBL1 gene.

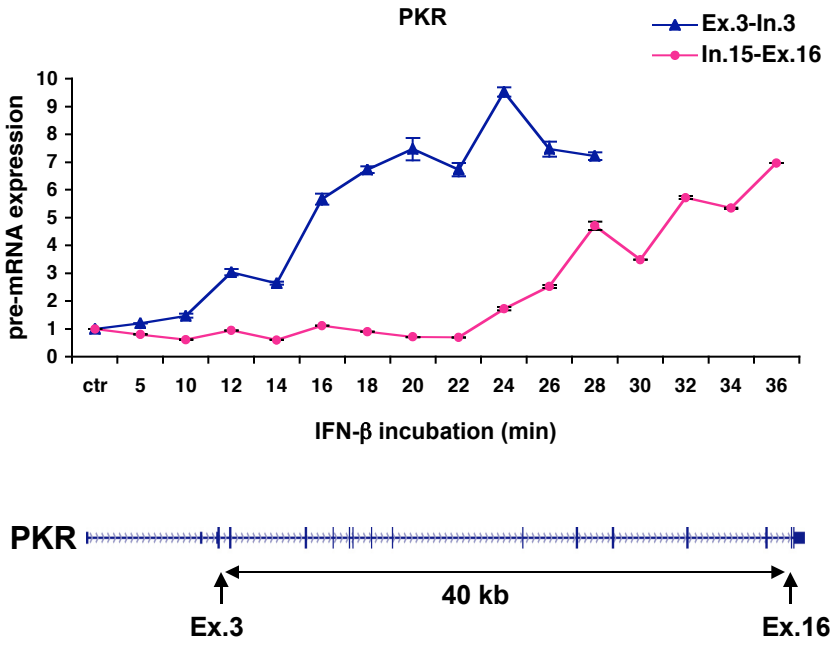


Supplementary Figure 3: The topoisomerase inhibitor camptothecin delays expression of downstream gene regions following DRB treatment.

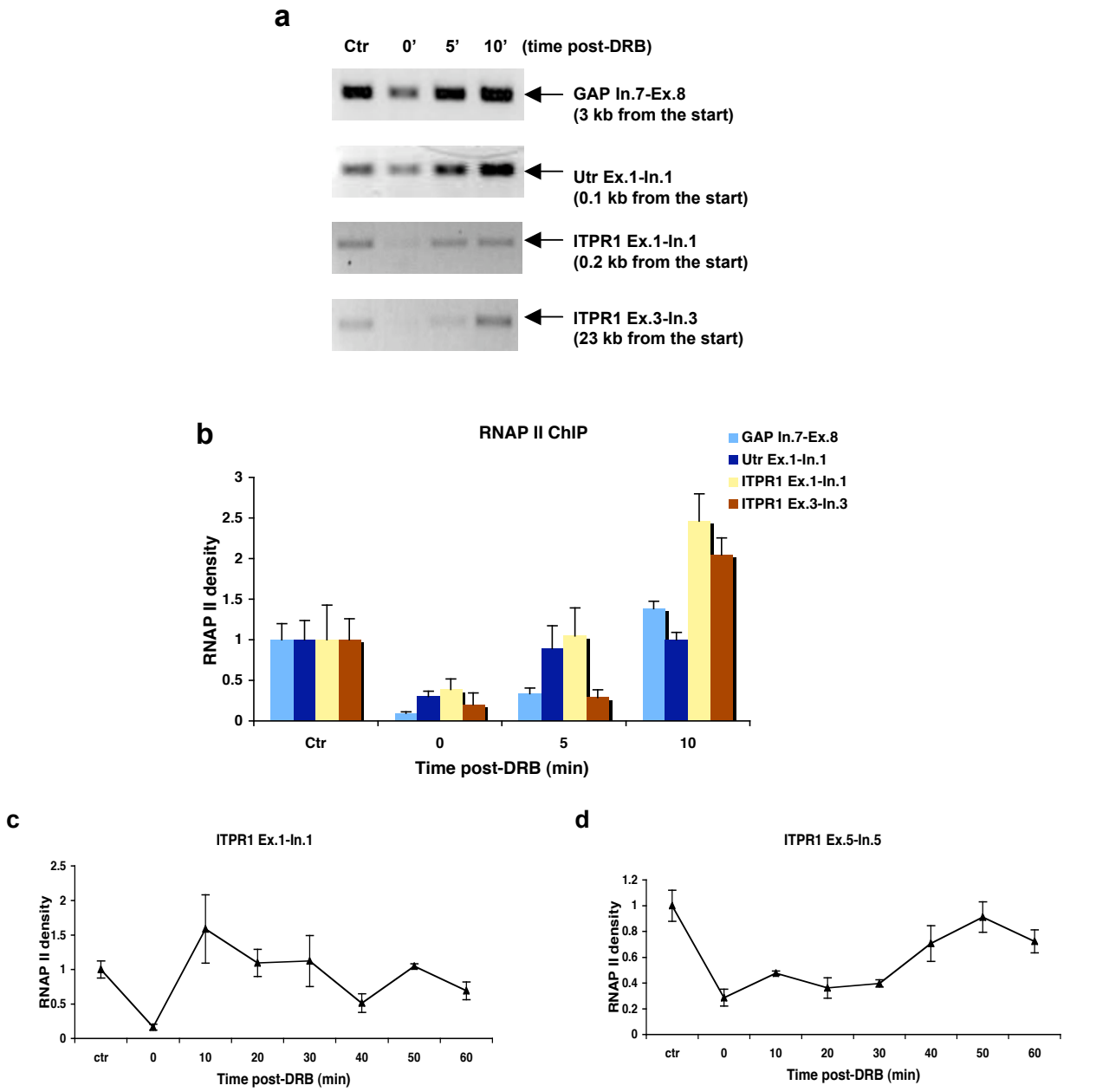
Camptothecin (CPT, 15μM) was added to cells 15 minutes before the end of the 3hr DRB incubation and then fresh medium was added containing CPT. Cells were harvested for RNA isolation and qRT-PCR at 5 minute intervals.

a) Expression levels for the exon 2-intron 2 region of the OPA1 gene located 21.6 kb downstream of the transcription start site in the presence or absence of CPT.

b) Expression levels for the exon 6-intron 6 region of the IFT80 gene located 33 kb downstream of the transcription start site in the presence or absence of CPT. Gene structures are shown below each graph.

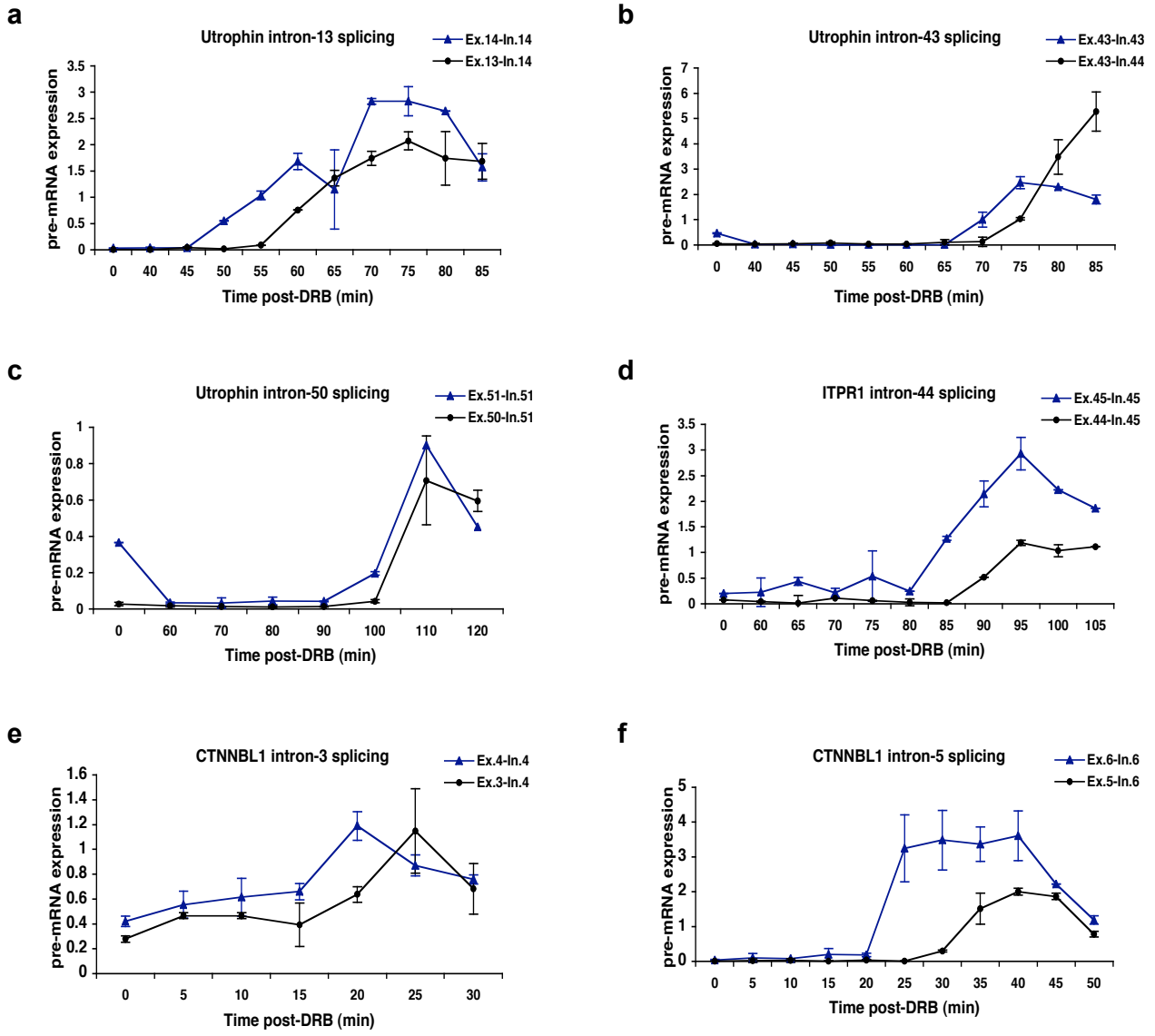


Supplementary Figure 4: Interferon-β induced gene expression in HT1080 cells.
The human HT1080 fibrosarcoma cell line was used to study the rate of transcription of interferon-β (INF-β) induced genes. Briefly cells were treated with INF-β at 1000 units/ml in DMEM supplemented with 10% Fetal Bovine Serum for the times indicated. Total RNA was isolated from cells and qRT-PCR was used to determine the pre-mRNA expression of the INF-β−induced PKR gene. Shown are the kinetics of pre-mRNA accumulation of exon 3 (Ex.3-In.3) and exon 16 (In.15-Ex.16).



Supplementary Figure 5: ChIP analysis of the distribution of RNAPII after DRB inhibition and release.

Cells treated with or without DRB were fixed and harvested for chromatin immunoprecipitation (ChIP) using RNAPII specific antibodies. The precipitated DNA was PCR amplified to detect the presence of RNAPII in various regions of the genes. a) Standard PCR and gel analysis of the RNAPII ChIP samples. The identical primer sets were used for 35-42 cycles. Ctr-untreated cells; 0'-cells treated with DRB for 3 hrs; 5' and 10'-cells treated with DRB for 3 hrs and released for 5 and 10 minutes respectively. b) Real time qPCR analysis of the samples used in panel (a). c and d) Real time PCR analysis of RNAPII ChIP of cells treated with DRB for 3 hrs and released for the indicated times. Two regions of the ITPR1 gene were examined: c) the exon 1-intron 1 region and d) the exon 5-intron 5 region located 133 kb from the transcription start site. The results are plotted relative to the ChIP signal in untreated cells which is set to 1.



Supplementary Figure 6: Kinetics of splicing of selected U2-dependent introns.
RNA samples from DRB treated cells were analyzed by qRT-PCR using primer pairs that detect the transcription of the exon downstream of the indicated intron or the ligated exon product of splicing (see Fig. 3 in main text). a) Transcription of exon 14 (Ex.14-In.14) and splicing of intron 13 (Ex.13-In.14) of Utrophin gene. b) Transcription of exon 43 (Ex.43-In.43) and splicing of intron 43 (Ex.43-In.44) of the Utrophin gene. c) Transcription of exon 51 (Ex.51-In.51) and splicing of intron 50 (Ex.50-In.51) of the Utrophin gene. d) Transcription of exon 45 (Ex.45-In.45) and splicing of intron 44 (Ex.44-In.45) of the ITPR1 gene. e) Transcription of exon 4 (Ex.4-In.4) and splicing of intron 3 (Ex.3-In.4) of the CTNNBL1 gene. f) Transcription of exon 6 (ex.6-In.6) and splicing of intron 5 (Ex.5-In.6) of the CTNNBL1 gene.

Supplementary Table 1: Sequences of the primer sets used in this study.

GAPDH-F	GAAACTGTGGCGTGATGGC
GAPDH-R	CACCACTGACACGTTGGCAG

GAPDH-F1	GGGACTGGCTTTCCCATAATTTCCT
GAPDH-R1	TAGAGGCAGGGATGATGTTCTGGA

Utr-Ex-1F	GGCAAGATGGCCAAGTATGGAG
Utr-In-1R	GCTTTCTTGAGCTTCCTTTACCTACCAG

Utr-In-1F	CCATTCCACAGATGAACACAATGACG
Utr-Ex-2R	GCGGCATCTGAACCATCGAAGT

Utr-Ex-3F	CAGAGTGGGAAACCACCCATCA
Utr-In-3R	GGTACCCACACTGGGTCATCAA

Utr-Ex-13F	GTGGCAGGAATTATTGGAAGAACA
Utr-In-13R	CTGTGAATAAGCTTCATGATCAGTT

Utr-Ex-14F	TCGACGTCTGGCTGTAAGTGATGG
Utr-In-14R	CAGGGCAGCAGTCTAATGCTAGGT

Utr-Ex-43F	GCAGGTGATGAAGTACAGGCAT
Utr-In-43R	GAGACACGCAGTATGTGACTCTG

Utr-In-44R	CATGATCCCACATCTCTGACAAC

Utr-Ex-50F	TGGATGCCTCTCATCGGGAGAA
Utr-In-50R	GCACACAGGGCAAACACAGGTA

Utr-Ex-51F	GGCTACTATGCTTCAACATCGACTGG
Utr-In-51R	GTGGTAAGGCTGCGCTTTCTCT

Utr-In-73F	GGGACCTCATTGCAGGAAGCAA
Utr-Ex-74R	CAGGAAACATTGGTTGGCCGGA

Bcl2-Ex2F	TGACTGAGTACCTGAACCGGCA
Bcl2-In2R	TCAGCCCAGACTCACATCACCA

Bcl2-In2F	CCCAACTGCAGGATGCCTTTGT
Bcl2-Ex3R	GGGCAGGCATGTTGACTTCACT

ITPR1-Ex-1F	TCTTCGCGGACATGGGATTACC
ITPR1-In-1R	GCATGCACATCCATCAAGATCTCCC

ITPR1-Ex-3F	TGTACGCGGAGGGATCGACAAA
ITPR1-In-3R	CAACCGCACTTAGAAAGCCAAG

ITPR1-Ex-5F	AGTTCTGGAAAGCCGCTAAGCC

ITPR1-In-5R	ACCAAGGCAGCCACTCACTACT

ITPR1-Ex-40F	CAGGCAAGTTCTGGTCAACCGT
ITPR1-In-40R	ACACCACAAAGCGTACCTCCTC

ITPR1-Ex-44F	TCATCACAGCCCTGATCCTCAA
ITPR1-Ex-45F	CATCATGGAAAGCAGGCACGACAG
ITPR1-In-45R	GCAGATCCTCTGCTCTGTCCCAAA

EFNA5-EX1F	GTGCTCTGGATGTGTGTGTTCAGC
EFNA5-IN1R	TCTCCGCCTGTTATCGGCTTACCT

EFNA5-EX2F	AGAGATGGGAATGTAACCGGCCTC
EFNA5-IN2R	GTTCTTGCAGCTGCCCTACAACAC

OPA1-EX1F	GGCCTGGTAAGTGCAGGCTCTAAT
OPA1-IN1R	TCTGGGTCCTCAAGAACATCTGGG

OPA1-EX2F	AAATATGGCTACCAGCCTCGCA
OPA1-In2R	ACCAGGAGGAATGTCAAGTTCACC

OPA1-EX18F	GGAACAGCTCTGAAAGCATTG

OPA1-EX19F	GGACAAGCATGCTAAAGGCACACC
OPA1-IN19R	TCGTATGGATGCCAAAGATTGCCAG

OPA1-In28F	CCCTGACCTCAGTTTGTTAAATGGG
OPA1-EX29R	CAGCTGATGGTTAAAGCGCCCGTA

IFT80.Ex.1F	AGAAGTATCGCGGGAAGAGGAA
IFT80.In.1R	AAGATGGGATCCAGTGATGGCT

IFT80.Ex.2F	TGCTGGAAGTGGAGTCATGAGA
IFT80.In.2R	AGGTAAACAGCCACTAAAGGAATGA

IFT80.Ex.3F	GGCAGAAAGCTTTGTCCTCACAAG
IFT80.In.3R	AGCCCATACACAAACCAAGTGAC

IFT80.In.19F	TGGAAGTCACACAGTCTATCTGTT
IFT80.Ex.20R	TGCTGGATTGGCTGCTTGATGA

CTNNBL1.Ex.1F	AGTGCAGGGAAGTGGAGTATTTGC
CTNNBL1.In.1R	AGAGGAGGTGAGATGAAAGGGCT

CTNNBL1.Ex.3F	GGAGCCATTGGATGAAAGCTCA

CTNNBL1.Ex.4F	TACCACCTTCTGGTGGAGCTGAAT
CTNNBL1.In.4R	GGAGAAAGAGTAACAGCACTTCCC

CTNNBL1.Ex.5F	TGAAGAGGGAGCAGAAGTGCTCAT
CTNNBL1.In.5R	TATTCACTCCAGCCTCACCACACT

CTNNBL1.Ex.6F	TATTCTTGCAGGTGGATGGGCA
CTNNBL1.In.6R	CAAAGCAACTCAGGCAATGGCA

CTNNBL1.In.15F	TTCTGGGAAAGGTATGAAGCGACG
CTNNBL1.Ex.16R	TAGAAGTTCTCCAGCAAGCCCA

KIFAP3.Ex.1F	AAATAACCGCGCCTGCCTCTCAA
KIFAP3.In.1R	AAACTAGCGTTGCCCAGTGACA

KIFAP3.Ex.7F	GAGCTTTGGCAAGAAGAACTCTC

KIFAP3.Ex.8F	ACAGGAACAGCTATTACGAGGT
KIFAP3.In.8R	CCCATGCTAAAGACAGACGAAC

KIFAP3.In.19F	CCCTGCTAGGAAGAGAATCTTGGT
KIFAP3.Ex.20R	TGGTTGGCCAAAGCCATCCATT

SLC9A9.Ex.1F	TGGAGGAGCAATGGTGTATGGT
SLC9A9.In.1R	TTCGTTACGCTGCAGCACCTTT

SLC9A9.Ex.7F	TTTGATGCCGCAGCATTCTTCCAG

SLC9A9.Ex.8F	TGCTTTCTTGGAGTGCCTTCCTGT
SLC9A9.In.8R	AGAGGCTGATTCTGACCCAAACCA

SLC9A9.In.15F	AGACAGGGCAGTTACAGAAAGG
SLC9A9.Ex.16R	GGAGGCTTGCTCCTGGTAATTT

PKR.Ex-3F	ACCGTCAGAAGCAGGGAGTAGT
PKR.In-3R	GGTTTCCAGCTTCATCCATGTCCC

PKR.In-15F	GGAATCCTGCTTGCTTCTTGGC
PKR.Ex-16R	TGCCATCCCGTAGGTCTGTGAA