

Appendix for

A versatile toolbox for determining IRES activity in cells and embryonic tissues

Philipp Koch^{1*}, Zijian Zhang^{2*}, Naomi R. Genuth^{2,3*}, Teodorus Theo Susanto^{2,4}, Martin Haimann¹, Alena Khmelinskaia^{5,6,7}, Gun Woo Byeon^{2,8}, Saurabh Dey¹, Maria Barna^{2‡} and Kathrin Leppek^{1‡}

¹ Institute of Clinical Chemistry and Clinical Pharmacology, Biomedical Center II (BMZ II), Venusberg-Campus 1, University Hospital Bonn, University of Bonn, 53127 Bonn, Germany

² Department of Genetics, Stanford University, Stanford, California 94305, USA

³ Present address: Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA 94720, USA

⁴ Present address: Epigenetic and Epitranscriptomic Systems, Genome Institute of Singapore, A*STAR, Singapore 138672, Singapore

⁵ Transdisciplinary Research Area "Building Blocks of Matter and Fundamental Interactions", University of Bonn, 53113 Bonn, Germany

⁶ Life and Medical Sciences Institute, University of Bonn, 53121 Bonn, Germany

⁷ Present address: Department of Chemistry, Ludwig-Maximilians-Universität München, 81377 München, Germany

⁸ Present address: Department of Electrical and Computer Engineering, University of Washington, Seattle, WA, USA 98195, USA

Contact: Maria Barna: mbarna@stanford.edu; Kathrin Leppek: kleppek@uni-bonn.de

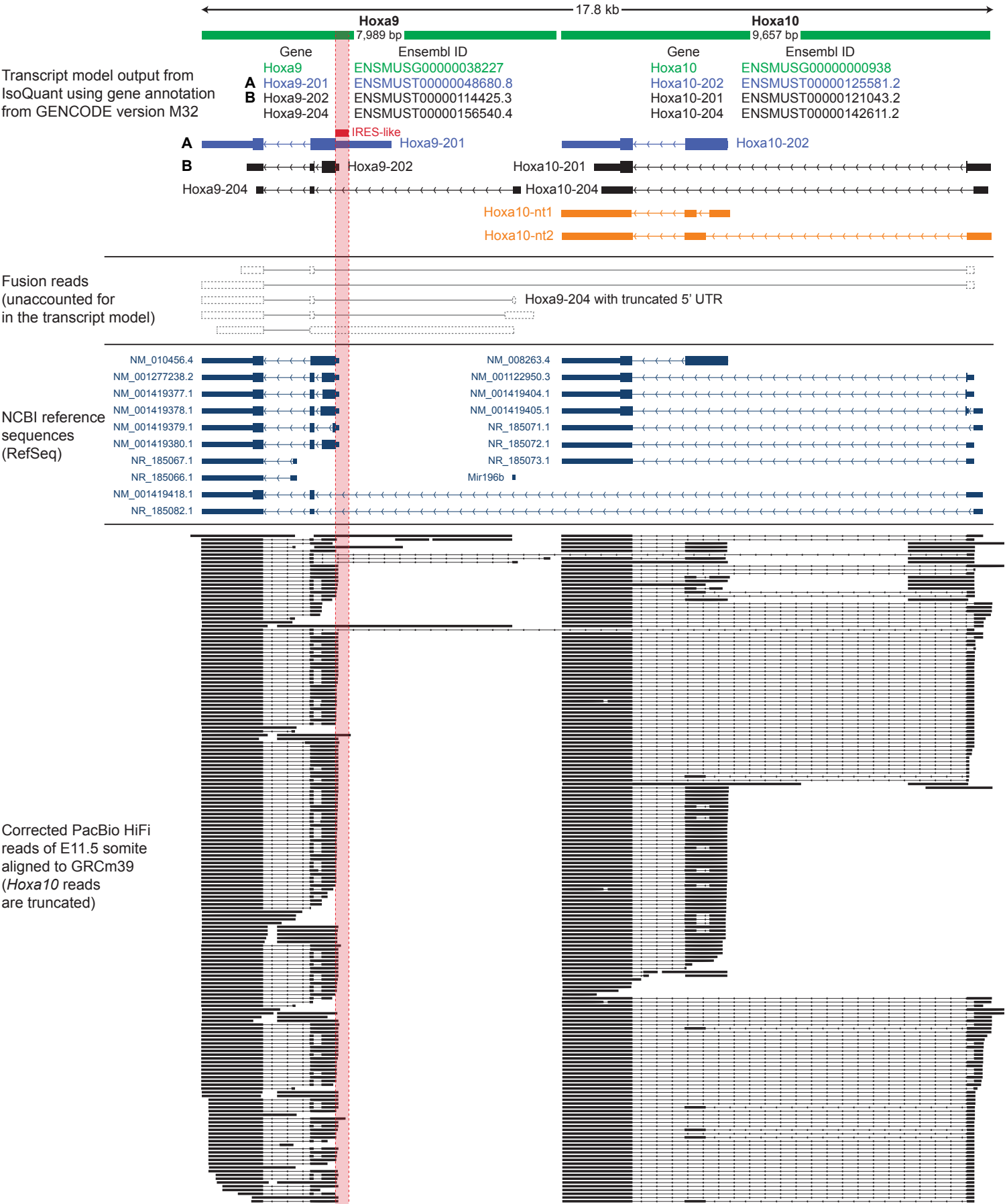
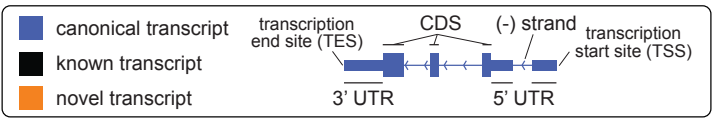
* authors contributed equally

‡ co-corresponding authors

Table of Contents

This document includes:	page
Appendix Figure S1 to S6	2-13
Appendix Table S1	14
Appendix Table S2	16

PacBio long-read sequencing
(neural tube and somite RNA):
Hoxa cluster: ***Hoxa9***, ***Hoxa10***



(continued in Fig. S2)

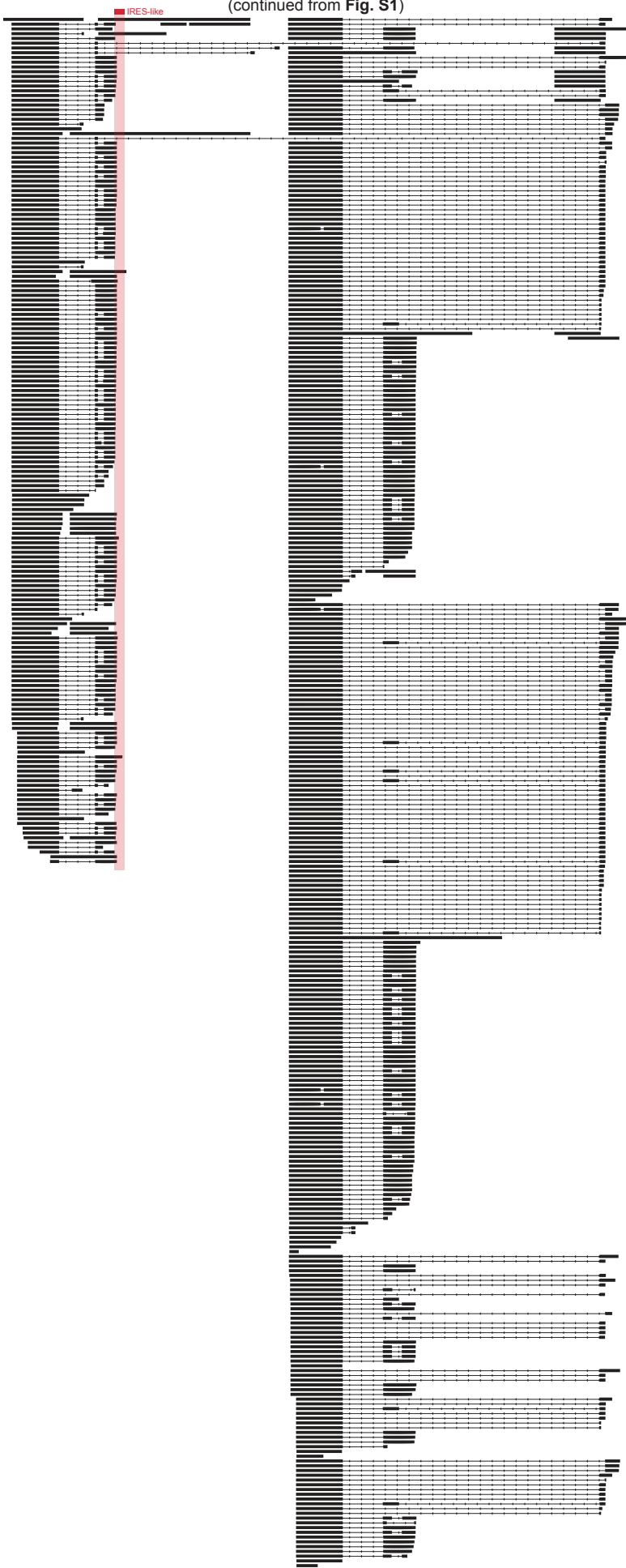
Appendix Figure S1. *Hoxa9* and *Hoxa10* mRNA expression in mouse embryo somite and neural tube tissues with complete corrected PacBio HiFi reads.

Illustration of a genome browser snapshot showing the transcript models predicted by IsoQuant, NCBI RefSeq, and corrected HiFi reads from the IsoQuant output around *Hoxa9* and *Hoxa10* loci (chr6:52,200,050-52,217,850). The 5' UTR IRES-like element tested is indicated by the red box. Despite 5' UTR truncations, reads of identical intron chains to isoform A were assigned to Hoxa9-201 IRES-containing reference transcript. Similarly, reads of identical intron chains to isoform B but with an extended 3' UTR were assigned to Hoxa9-202. Only two novel transcripts were detected for the *Hoxa10* gene. The previously reported *Hoxa9*/Mir196b fusion transcript is similar to Hoxa9-204 with a truncated 5' UTR. Otherwise, there was no novel isoform identified that indicates any fusion transcripts. The full list of the corrected reads is shown in **Appendix Fig. S2**.

Hoxa cluster: ***Hoxa9***, ***Hoxa10***

Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39

(continued from Fig. S1)



Appendix Figure S2. Complete Hoxa9 and Hoxa10 corrected PacBio HiFi reads (continued reads).

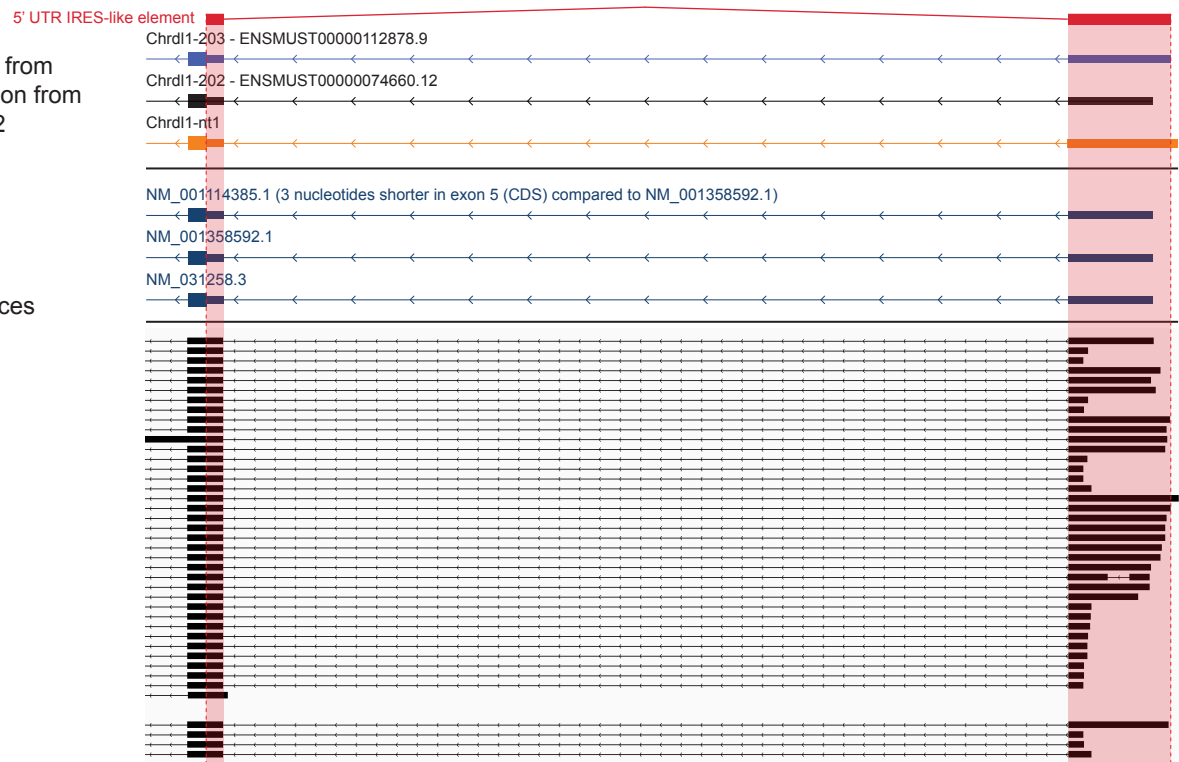
Full display, and continuation from **Appendix Fig. S1**, of corrected PacBio HiFi reads from IsoQuant output that are aligned to Hoxa9 and Hoxa10 locus. Red bar corresponds to the 5' UTR IRES-like construct tested in this paper.

A *Chrdl1*

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

NCBI reference sequences
(RefSeq)

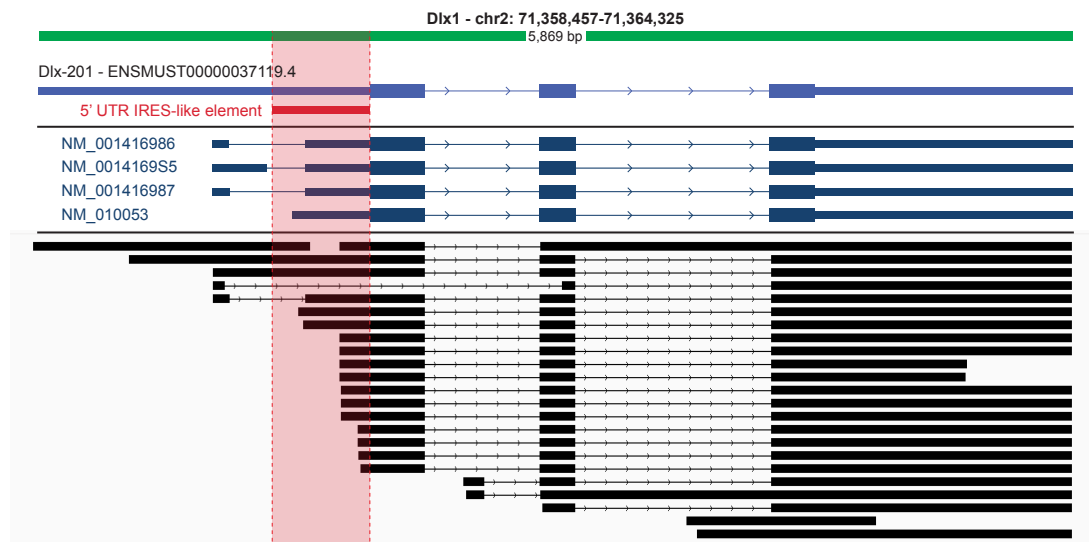
Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39

**B** *Dlx1*

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

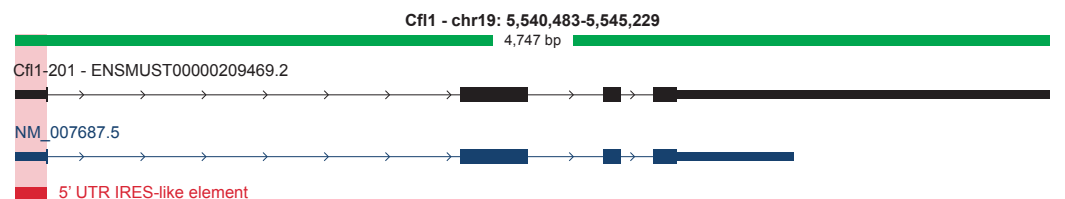
NCBI reference sequences
(RefSeq)

Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39

**C** *Cofilin*

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

NCBI reference sequences
(RefSeq)



Appendix Figure S3. Cellular IRES-like RNA expression in mouse embryo somite and neural tube tissues by PacBio HiFi sequencing.

(A) Illustration of a genome browser snapshot showing the transcript models predicted by IsoQuant, NCBI RefSeq, and corrected HiFi reads from the IsoQuant output of the first two exons of *Chrdl1* gene which contains the 5' UTR region. The 5' UTR IRES-like element tested is indicated by the red box. A complete view of the whole gene is shown in **Appendix Fig. S4**.

(B) Same genome browser snapshot as in (A) for the complete *Dlx1* gene instead. The *Dlx1* canonical transcript was not detected in this dataset.

(C) Same genome browser snapshot as in (A) for the complete *Cofilin* (*Cfl1*) gene instead, without the corrected reads being displayed, due to the extremely large number of reads that are largely uniform.

Chrdl1 (FL mRNA)

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

NCBI reference sequences
(RefSeq)

Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39



Appendix Figure S4. A genome browser view showing the complete Chrdl1 locus.

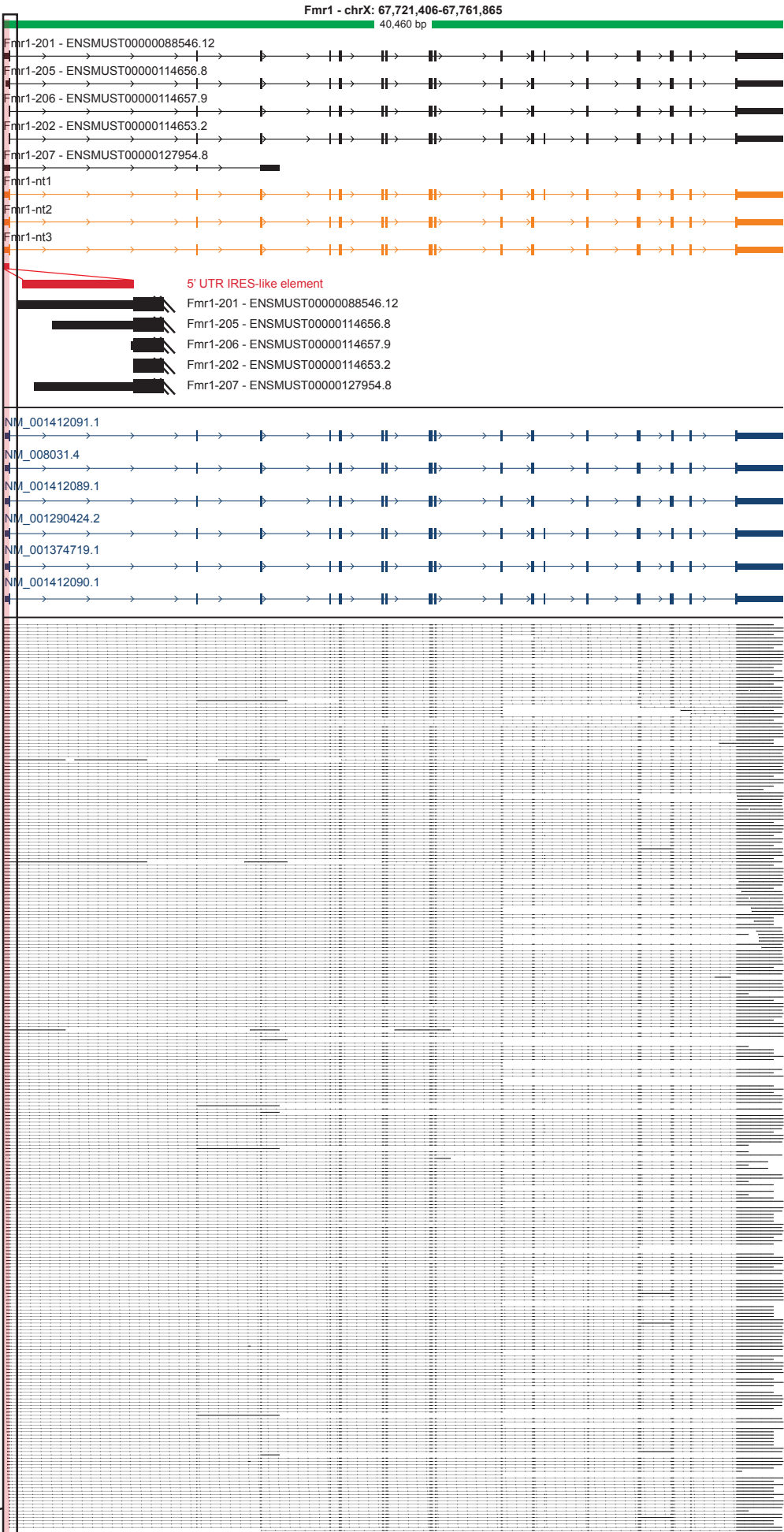
Illustration of a genome browser snapshot showing the transcript models predicted by IsoQuant, NCBI RefSeq, and corrected HiFi reads from IsoQuant output. Only one novel isoform was detected, with a different transcription start site and transcription end site compared to *Chrdl1-203*. Red region corresponds to the 5' UTR IRES-like element tested.

Fmr1 (FL mRNA)

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

NCBI reference sequences
(RefSeq)

Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39



Appendix Figure S5. A genome browser view showing the complete Fmr1 locus.

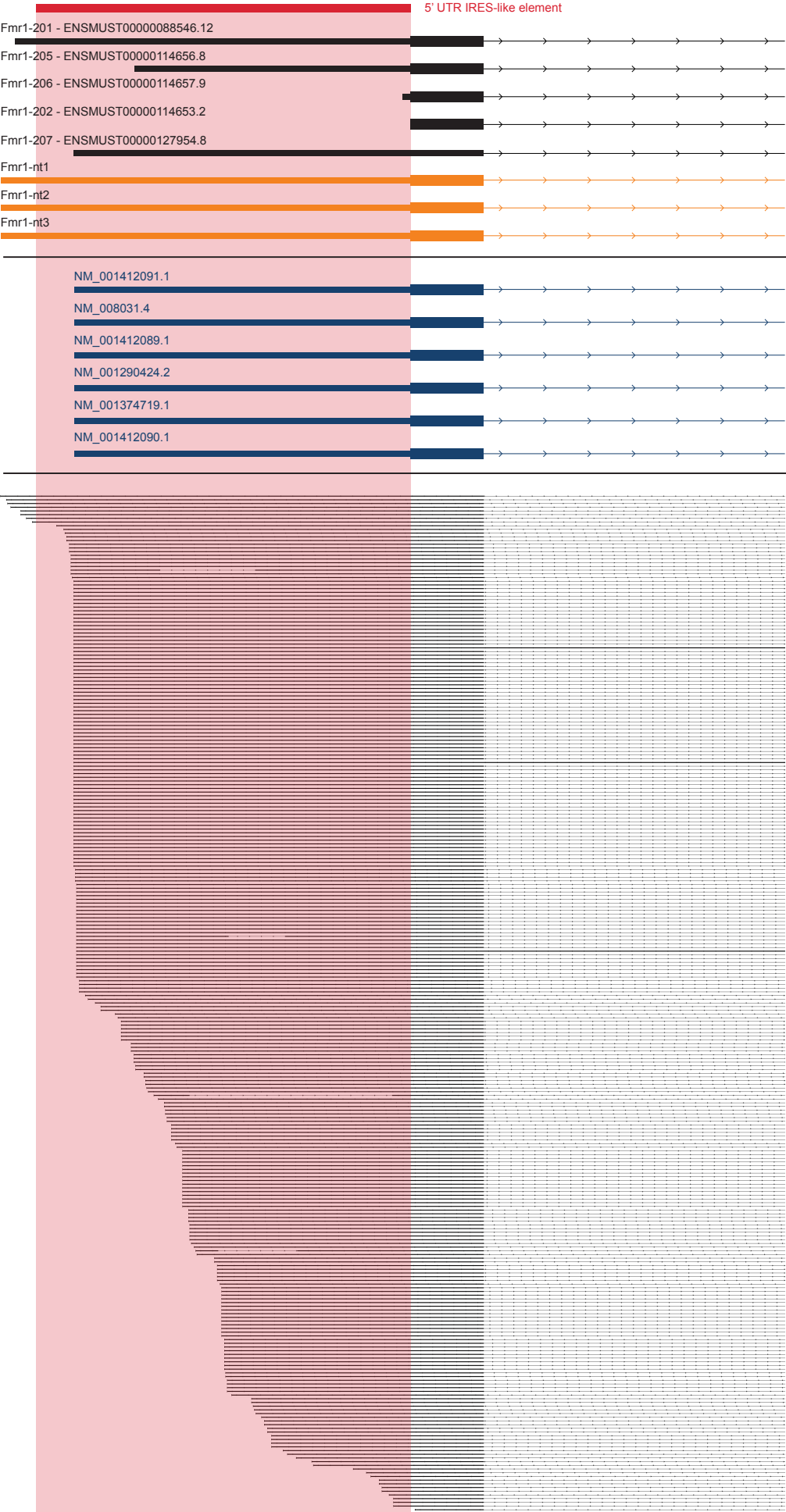
Illustration of a genome browser snapshot showing the transcript models predicted by IsoQuant, NCBI RefSeq, and corrected HiFi reads from IsoQuant output of the full FMR1 gene. Red bar corresponds to the 5' UTR IRES-like element tested. Zoom-in view of the region in the black box is presented in **Appendix Fig. S6**.

Fmr1 (5' UTR zoom-in view)

Transcript model output from
IsoQuant using annotation from
GENCODE version M32

NCBI reference sequences
(RefSeq)

Corrected PacBio HiFi
reads of E11.5 somite
aligned to GRCm39



Appendix Figure S6. A genome browser view focusing on 5' UTR of *Fmr1*.

A zoomed-in view of **Appendix Fig. S5**, highlighting *Fmr1* 5' UTR region. Red bar corresponds to the 5' UTR IRES-like element tested.

APPENDIX TABLES

Appendix Table S1: Plasmids used in this study.

All plasmids used for mammalian transient transfection are listed in the table.

Appendix Table S1. List of plasmids		
Plasmid	Notes	Reference
Mammalian cells		
Expression constructs		
pRF-HCV IRES	pKL033, kindly provided by D. Ruggero	(Yoon et al. 2006)
mRuby3-ZK-spEGFP	pKL477, kindly provided by C. K. Chen	(Chen et al. 2021)
mRuby3-ZK-spEGFP-IRES2(EMCV)	pKL479, kindly provided by C. K. Chen	(Chen et al. 2021)
pcDNA3.1-5'UTR-3xHA-Nluc	pKL401, kindly provided by C. Howard	(Osuna et al. 2017)
pGL3	pKL037, SV40 promoter, Firefly luciferase	Promega
pGL3-HBB	pKL039	(Xue et al. 2015)
pGL3-FLB-stop (Fluc- β -globin)	pKL079	(Leppek et al. 2020)
pGL3-FLB-fusion-HBB5'	pKL082	This study
pCR-Blunt-II-TOPO-HoxA9-3'UTR	pKL090/pK240, kindly provided by K. Fujii	This study
pGL3-NLB-fusion-HBB5'	pMH001	This study
pGL3-Nluc-HBB5'	pMH002	This study
pGL3-NLB-hHBB3'-HBB5'	pMH023	This study
SV40-promoterless constructs and controls		
pGL3-SV40minus-NLB-HBB5'-HBB3'	pLL015	This study
pGL3-SV40minus-NLB-empty5'-HBB3'	pLL016	This study
pGL3-SV40minus-NLB-HCV-HBB3'	pLL017	This study
pGL3-SV40minus-NLB-EMCV(IRES2)-HBB3'	pLL018	This study
pGL3-SV40minus-NLB-P4native-HBB3'	pLL019	This study
pGL3-SV40minus-NLB-A3(IRES)-HBB3'	pLL020	This study
pGL3-SV40minus-NLB-A5(IRES)-HBB3'	pLL021	This study
pGL3-SV40minus-NLB-A9(IRES)-HBB3'	pLL022	This study
pGL3-SV40-NLB-empty5'-HBB3'	pLL026	This study
pGL3-SV40-NLB-HCV-HBB3'	pLL027	This study
pGL3-SV40-NLB-EMCV(IRES2)-HBB3'	pLL028	This study
pGL3-SV40-NLB-A3(IRES)-HBB3'	pLL030	This study
pGL3-SV40-NLB-A5(IRES)-HBB3'	pLL031	This study
pGL3-SV40-NLB-A9(IRES)-HBB3'	pLL032	This study
pGL3-SV40minus-NLB-A9(IRES)-M2-HBB3'	pLL036	This study
pGL3-SV40minus-NLB-A9(IRES)-M5-HBB3'	pLL037	This study
pGL3-SV40minus-NLB-A9(IRES)-M12-HBB3'	pLL038	This study
pGL3-SV40minus-NLB-A9(IRES)-M13-HBB3'	pLL039	This study
circRNA split-EGFP constructs		

mRuby_ZKSCAN_spEGFP_HBB	pLL008	This study
mRuby_ZKSCAN_spEGFP_HCV	pLL009	This study
mRuby_ZKSCAN_spEGFP_CVB3	pPK008	This study
mRuby_ZKSCAN_spEGFP_P4native	pLL010	This study
mRuby_ZKSCAN_spEGFP_a3(IRES)	pLL011	This study
mRuby_ZKSCAN_spEGFP_a5(IRES)	pLL012	This study
mRuby_ZKSCAN_spEGFP_a9(IRES)	pLL013	This study
mRuby_ZKSCAN_spEGFP_a9(IRES)-M2	pLL040	This study
mRuby_ZKSCAN_spEGFP_a9(IRES)-M5	pLL041	This study
mRuby_ZKSCAN_spEGFP_a9(IRES)-M12	pLL042	This study
mRuby_ZKSCAN_spEGFP_a9(IRES)-M13	pLL043	This study
mRuby_ZKSCAN_spEGFP_invHBB	pPK009	This study
mRuby_ZKSCAN_spEGFP_invHCV	pPK010	This study
mRuby_ZKSCAN_spEGFP_invEMCV	pPK011	This study
mRuby_ZKSCAN_spEGFP_invA9	pPK012	This study
mRuby_ZKSCAN_spEGFP_invA5	pPK013	This study
mRuby_ZKSCAN_spEGFP_invCVB3	pPK015	This study
mRuby3_ZKSCAN_spEGFP_Chrdl1(IRES)	pPK026	This study
mRuby3_ZKSCAN_spEGFP_Dlx1(IRES)	pPK027	This study
mRuby3_ZKSCAN_spEGFP_Gdf5(IRES)	pPK028	This study
mRuby3_ZKSCAN_spEGFP_Sema3a(IRES)	pPK029	This study
mRuby3_ZKSCAN_spEGFP_Zfx(IRES)	pPK030	This study
mRuby3_ZKSCAN_spEGFP_c-Myc(IRES)	pPK031	This study
mRuby3_ZKSCAN_spEGFP_CACNA1A(IRES)	pPK032	This study
mRuby3_ZKSCAN_spEGFP_Cofilin(IRES)	pPK033	This study
mRuby3_ZKSCAN_spEGFP_FMR1(IRES)	pPK034	This study
mRuby3_ZKSCAN_spEGFP_c-Myc(IRES) Inv	pPK037	This study
mRuby3_ZKSCAN_spEGFP_Chrdl1(IRES) Inv	pPK038	This study
mRuby3_ZKSCAN_spEGFP_Dlx1(IRES) Inv	pPK039	This study
mRuby3_ZKSCAN_spEGFP_FMR1(IRES) Inv	pPK040	This study
mRuby3_ZKSCAN_spEGFP_Sema3a(IRES) Inv	pPK041	This study
mRuby3_ZKSCAN_spEGFP_Bcl2(IRES)	pPK042	This study
mRuby3_ZKSCAN_spEGFP_Bcl2(IRES) Inv	pPK043	This study
mRuby3_ZKSCAN_spEGFP_Cofilin(IRES) Inv	pPK044	This study

Appendix Table S2: DNA Oligonucleotides used in this study.

All DNA oligonucleotides used for cloning, RT-PCR, and RT-qPCR are listed in the table. F, forward primer; R, reverse primer.

Appendix Table S2. DNA oligonucleotides		
Name	Sequence	Description
qPCR primer		
KL050	TGGAGAATAACTTCTTCGTGGA	Rluc qPCR F
KL051	TTGGACGACGAACTTCACC	Rluc qPCR R
KL052	AAGAGATACGCCCTGGTTC	Fluc qPCR F
KL053	TTGTATTTCAGCCCATATCGTTTC	Fluc qPCR R
KL056	GCCAACCGTGAAAAGATGAC	mouse β -actin F
KL057	CATCACAATGCCTGTGGTAC	mouse β -actin R
KL075	GAAGGCTCATGGCAAGAAGG	rabbit β -globin qPCR F
KL076	ATGATGAGACAGCACAATAACCAG	rabbit β -globin qPCR R
KL412	CATGGCTGCAACACTTACACAGCA	mouse <i>Nupl1</i> qPCR F
KL413	ATTGCAAGCCAGTGCCAATACCTG	mouse <i>Nupl1</i> qPCR R
KL109	AAAAACAACCCAGCGAAGGC	mouse <i>Hoxa9</i> qPCR F (CDS2 ampl.)
KL110	ATCGCTTCTTCCGAGTGGAG	mouse <i>Hoxa9</i> qPCR R (CDS2 ampl.)
KL585	CCGTATGAAGGTCTGAGCGG	Nanoluc qPCR F
KL586	CAGTGTGCCATAGTGCAGGA	Nanoluc qPCR R
KL596	CTTCGTTGGCCACAATTAAACAAACCAG	a9 IRES primer (mouse) F (IRES1)
KL597	GCCCAGCAGGAAGGAGTC	a9 CDS primer (mouse) R (IRES1&4)
JS98	GCGCGATCCCTTTGCATAAA	<i>Hoxa9</i> IRES2 amplicon F
JS99	CCGACCCGCCGAAATTATGA	<i>Hoxa9</i> IRES2 and IRES3 amplicons R
JS104	TGCAAAACACCGGGCCATTA	<i>Hoxa9</i> IRES3 and IRES4 amplicons F
NG771	TGGTGCCACCAAGTTGTTACATG	85 nt 5' UTR <i>Hoxa9</i> amplicon F
NG761	TGCAGTAGCCCGCGCCTG	85 nt 5' UTR <i>Hoxa9</i> amplicon R
NG808	GCCCTGGGCAACTACTATGT	<i>Hoxa9</i> CDS 5' amplicon F
NG809	GGACTGAAGTCGGGGTGTTC	<i>Hoxa9</i> CDS 5' amplicon R
SV40-promoterless plasmids and +SV40 controls		
LL042	CGCGTGCTAGCCCGGGCTCGAGATCTAAGCTTactttgtcttctgacacaac	SV40minus-oligo-1 F
LL043	gttgtgtcagaagcaaatgtAAGCTTAGATCTCGAGCCCGGGCTAGCACGCG	SV40minus-oligo-1 R
LL044	CGCGTGCTAGCCCGGGCTCGAGATCTGCCACCATGGCCGTTTACCC	SV40minus-oligo-2 F
LL045	GGGTAAACGGCCATGGTGGCAGATCTCGAGCCCGGGCTAGCACGCG	SV40minus-oligo-2 R
LL046	CTAGCCCGGGCTCGAGATCTTTGGGGGCGACACTCC	SV40minus-HCV Gibson F
LL047	TCGTATGGGTAAACGGCCATGGTGGCTTTACCAACGAATTC	SV40minus-HCV Gibson R
LL048	CTAGCCCGGGCTCGAGATCTccctctctccctcccc	SV40minus-EMCV Gibson F
LL049	TCGTATGGGTAAACGGCCATGATggttgtggccatattatcatcgt	SV40minus-EMCV Gibson R
LL052	CTAGCCCGGGCTCGAGATCTtggcggcgagtgctc	SV40minus-A3 Gibson F
LL053	TCGTATGGGTAAACGGCCATggcgcgatgtttcacgacgc	SV40minus-A3 Gibson R
LL054	CTAGCCCGGGCTCGAGATCTatcaggcaggatttacgactg	SV40minus-A5 Gibson F
LL055	TCGTATGGGTAAACGGCCATggtgcttgatttggtgctcg	SV40minus-A5 Gibson R
LL056	CTAGCCCGGGCTCGAGATCTttgatcttttaattcttctgttgcca	SV40minus-A9 Gibson F
LL057	TCGTATGGGTAAACGGCCATggtgcagtagcccgcc	SV40minus-A9 Gibson R
LL058	TAGGCTTTTGCAAAAAGCTTTTGGGGGCGACACTCC	SV40-HCV Gibson F
LL059	TAGGCTTTTGCAAAAAGCTTccctctctccctcccc	SV40-EMCV Gibson F
LL061	TAGGCTTTTGCAAAAAGCTTtggcggcgagtgctc	SV40-A3 Gibson F
LL062	TAGGCTTTTGCAAAAAGCTTatcaggcaggatttacgactg	SV40-A5 Gibson F

LL063	TAGGCTTTTGCAAAAAGCTTttgatcttttaatcttcgttgcca	SV40-A9 Gibson F
LL064	GAGGCCCTAGGCTTTTGCAAAAAGCTTGCCACCATGGCCGTTTACCC	SV40-oligo-3 F
LL065	GGGTAAACGGCCATGGTGGCAAGCTTTTGCAAAAAGCCTAGGCCTC	SV40-oligo-3 R
LL068	CTAGCCCGGGCTCGAGATCTAAGCTTtacatttgcttctgacacaa	SV40minus-HBB Gibson F
LL069	TCGTATGGGTAAACGGCCATGGTGGCGATATCggtgtctgtttga	SV40minus-HBB Gibson R
LL070	CGCGTTATTGTTCTGCCGGGCGTTGACGTGACGCG	SV40minus-A9-M2 Gibson F
LL071	CGCGTTATTGTTCTGCCGGGCGGACACTATTTCGCG	SV40minus-A9-M5 Gibson F
LL072	CGCGTTATTGTTCTGCCGGGCGGTTACGTGACGCG	SV40minus-A9-M12 Gibson F
LL073	CGCGTTATTGTTCTGCCGGGCGGACACGTGTGCCG	SV40minus-A9-M13 Gibson F
LL074	CGCGTCACGTCAACGCCCGGCAGAACATAACGCG	SV40minus-A9-M2 Gibson R
LL075	CGCGAATAGTGTCCGCCCGGCAGAACATAACGCG	SV40minus-A9-M5 Gibson R
LL076	CGCGTCACGTGAACGCCCGGCAGAACATAACGCG	SV40minus-A9-M12 Gibson R
LL077	CGGCACACGTGTCCGCCCGGCAGAACATAACGCG	SV40minus-A9-M13 Gibson R
<i>Cloning of mRuby-ZSCAN-splitEGFP reporter plasmids</i>		
LL017	ACGAGCTGTACAAGTAAGATacatttgcttctgacacaact	ZK-spEGFP HBB F
LL018	TCCTCGCCCTTGCTCACCATTggtgtctgtttgaggttg	ZK-spEGFP HBB R
LL019	ACGAGCTGTACAAGTAAGATTGGGGCGGACACT	ZK-spEGFP HCV F
LL020	TCCTCGCCCTTGCTCACCATTGGTGGCTTTACCAACGAAT	ZK-spEGFP HCV R
LL021	ACGAGCTGTACAAGTAAGATgctgttattgttctgtcgcg	ZK-spEGFP P4native F
LL022	TCCTCGCCCTTGCTCACCATTggtgcagtagcccgcg	ZK-spEGFP P4native R
LL023	ACGAGCTGTACAAGTAAGATtggcgcgcgagtg	ZK-spEGFP A3(IRES) F
LL024	TCCTCGCCCTTGCTCACCATTggcgcgatgtttcacgatcg	ZK-spEGFP A3(IRES) R
LL025	ACGAGCTGTACAAGTAAGATatcaggcaggatttacgac	ZK-spEGFP A5(IRES) F
LL026	TCCTCGCCCTTGCTCACCATTggtgcttgattgtggctcg	ZK-spEGFP A5(IRES) R
LL027	ACGAGCTGTACAAGTAAGATttgatcttttaatcttcgttg	ZK-spEGFP A9(IRES) F
LL028	TCCTCGCCCTTGCTCACCATTggtgcagtagcccgcg	ZK-spEGFP A9(IRES) R
PK100	TTAAACAGCCTGTGGGTTGATCCACCCACAGGCCATTGGGCGCTAGCACTCTGGTAT	ZK-spEGFP CVB3_F
PK101	TGGGGGAGGGGGTATAAAACAGGCGCACAAAGGTACCGTGATACCAGAGTGCTAGCGCCC	ZK-spEGFP CVB3_R
PK102	GTTTTATACCCCTCCCCAACTGTAACCTAGAAGTAACACACACCGATCAACAGTCAGC	ZK-spEGFP CVB3_F
PK103	GTAACAGAAGTGCTTGATCAAAACGTGGCTGGTGTGCCACGCTGACTGTTGATCGGTGTG	ZK-spEGFP CVB3_R
PK104	TGATCAAGCACTTCTGTACCCCGGACTGAGTATCAATAGACTGCTCAGCGGTTGAAGG	ZK-spEGFP CVB3_F
PK105	AGGTTTTTCGAAGTAGTTGGCCGGATAACGAACGCTTCTCCTTCAACCGCGTGAGCAGT	ZK-spEGFP CVB3_R
PK106	CCAATACTTTCGAAAAACCTAGTAACACCGTGGAAGTTGCAGAGTGTTTCGCTCAGCACT	ZK-spEGFP CVB3_F
PK107	GGGGAATGCGGTGACTCATCGACCTGATCTACACTGGGGTAGTGCTGAGCGAAACACTCT	ZK-spEGFP CVB3_R
PK108	GATGAGTCACCGCATTTCCACGGGCGACCGTGGCGGTGGCTGCGTTGGCGGCTGCCCCA	ZK-spEGFP CVB3_F
PK109	CGCACCATGTCTGTATTAGAGCGTCCCATGGGTTTCCCATGGGCAGGCCGCCAACGCAG	ZK-spEGFP CVB3_R
PK110	TCTAATACAGACATGGTGCAGAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCCTGA	ZK-spEGFP CVB3_F
PK111	GGCTTGAGGGTGTGTGCTCCGAGTTAGGATTAGCCGCATTCAGGGGCCGGAGGACTACC	ZK-spEGFP CVB3_R
PK112	GGAGCACACACCTCAAGCCAGAGGGCAGTGTGTCGTAACGGGCAACTCTGCA GCGGAAC	ZK-spEGFP CVB3_F
PK113	GTATAGGAATAAAATGAAACACGGACACCCAAAGTAGTCGGTTCCGCTGCAGAGTTGCC	ZK-spEGFP CVB3_R

PK114	GTTCATTTTATTCCTATACTGGCTGCTTATGGTGACAATTGAGAGATCGTTA CCATATA	ZK-spEGFP CVB3_F
PK115	ATAATAGCTCTATTAGTCACCGGATGGCCAATCCAATAGCTATATGGTAACGA TCTCTCA	ZK-spEGFP CVB3_R
PK116	GTGACTAATAGAGCTATTATATATCCCTTTGTTGGGTTTATACCACTTAGCTT GAAAGAG	ZK-spEGFP CVB3_F
PK117	GGTTTGCTGTATTCAACTTAACAATGAATTGTAATGTTTAACTCTTTCAAG CTAAGTGGTA	ZK-spEGFP CVB3_R
PK118	CGAGCTGTACAAGTAAGATTTAAACAGCCTGTGGGTGATCC	ZK-spEGFP GACVB3_F
PK119	CCCTTGCTCACCATGATGGTTTGCTGTATTCAACTTAACAATGAATTGTAATG	ZK-spEGFP GACVB3_R
PK130	CGAGCTGTACAAGTAAGATggtgtctgtttgaggttgctagtga	ZK-spEGFP invHBB F
PK131	CCCTTGCTCACCATacatttgccttctgacacaactgtgt	ZK-spEGFP invHBB R
PK132	CGAGCTGTACAAGTAAGATGGTGGCTTTACCAACGAATTCG	ZK-spEGFP invHCV F
PK133	CCCTTGCTCACCATTTGGGGGCGACACTCCAC	ZK-spEGFP invHCV R
PK134	CGAGCTGTACAAGTAAGATggttgtggccatattatcatcgtgt	ZK-spEGFP invEMCV F
PK135	CCCTTGCTCACCATGATccccctctcccccccccc	ZK-spEGFP invEMCV R
PK136	CGAGCTGTACAAGTAAGATggtgcagtagccccg	ZK-spEGFP invA9 F
PK137	CCCTTGCTCACCATttgatcttttaatcttcgttggccacaattaaaac	ZK-spEGFP invA9 R
PK138	CCCTTGCTCACCATttgatcttttaatcttcgttggccacaattaaaac	ZK-spEGFP invA5 F
PK139	CCCTTGCTCACCATatcaggcaggatttacgactggac	ZK-spEGFP invA5 R
PK142	CGAGCTGTACAAGTAAGATGGTTTGCTGTATTCAACTTAACAATGAATTGTAA TG	ZK-spEGFP invCVB3 F
PK143	CCCTTGCTCACCATGATTTAAACAGCCTGTGGGTGATCC	ZK-spEGFP invCVB3 R
PK175	ACGAGCTGTACAAGTAAGATACTAGAACTCGTGTAATTCAGC	ZK-spEGFP cMyc_F
PK176	TCCTCGCCCTTGCTCACCATggTCGCGGGAGGCTGC	ZK-spEGFP cMyc_R
PK177	ACGAGCTGTACAAGTAAGATCAGGCGTTGGGGGCG	ZK-spEGFP Dlx1_F
PK178	TCCTCGCCCTTGCTCACCATggCTCTCTCGCGGGGTCTG	ZK-spEGFP Dlx1_R
PK179	ACGAGCTGTACAAGTAAGATGAAAGTCATGGCTTTTGGGATTCTGAA	ZK-spEGFP CACNA1A_F
PK180	TCCTCGCCCTTGCTCACCATggACGGTGAGGTCCGTGGAC	ZK-spEGFP CACNA1A_R
PK181	ACGAGCTGTACAAGTAAGATGCCGGAAGGCCGCC	ZK-spEGFP Cofilin_F
PK182	TCCTCGCCCTTGCTCACCATggGTTTCCGGAACGAAAGGGAGAC	ZK-spEGFP Cofilin_R
PK183	ACGAGCTGTACAAGTAAGATGTGTGGTGGGGGCGC	ZK-spEGFP Chrdl1_F
PK184	TCCTCGCCCTTGCTCACCATggCTTCTACTTTTTTCTCCTTCGAGCTACTG	ZK-spEGFP Chrdl1_R
PK187	ACGAGCTGTACAAGTAAGATTTCAAGCCCTCAGTCAGTTGTGC	ZK-spEGFP Gdf5_F
PK188	TCCTCGCCCTTGCTCACCATggCCTCTGGCCAGCCGC	ZK-spEGFP Gdf_R
PK189	ACGAGCTGTACAAGTAAGATGTGACAAGAGGAAGGGGAGT	ZK-spEGFP Sema3a_F
PK190	TCCTCGCCCTTGCTCACCATggGCTGCAGACGCTGGAGG	ZK-spEGFP Sema3a_R
PK191	ACGAGCTGTACAAGTAAGATACGGAACCTCGGGCCG	ZK-spEGFP Zfx_F
PK192	TCCTCGCCCTTGCTCACCATggGGCCTTTAAATTCTCATCAGCCAGAAC	ZK-spEGFP Zfx_R
PK193	ACGAGCTGTACAAGTAAGATAGGAGGCGCAGCGG	ZK-spEGFP FMR1_F
PK194	TCCTCGCCCTTGCTCACCATggCTTCTCGTCCGTCTCTCGC	ZK-spEGFP FMR1_R
PK215	ACGAGCTGTACAAGTAAGATCTTCTACTTTTTTCTCCTTCGAGCTACTG	ZK-spEGFP Chrdl1_Fo
PK216	TCCTCGCCCTTGCTCACCATGggTGTGGTGGGGGCGC	ZK-spEGFP Chrdl1_Re
PK217	ACGAGCTGTACAAGTAAGATTCGCGGGAGGCTGC	ZK-spEGFP CMyc_F
PK218	TCCTCGCCCTTGCTCACCATggACTAGAACTCGTGTAATTCAGC	ZK-spEGFP CMyc_R
PK219	ACGAGCTGTACAAGTAAGATCTTCTCTCGCGGGGTCTG	ZK-spEGFP Dlx1_F
PK220	TCCTCGCCCTTGCTCACCATggCAGGCGTTGGGGGCG	ZK-spEGFP Dlx1_R
PK221	ACGAGCTGTACAAGTAAGATCTTCTCGTCCGTCTCTCGC	ZK-spEGFP FMR_F
PK222	TCCTCGCCCTTGCTCACCATggAGGAGGCGCAGCGG	ZK-spEGFP FMR_R
PK223	ACGAGCTGTACAAGTAAGATGCTGCAGACGCTGGAGG	ZK-spEGFP Sema3a_F
PK224	TCCTCGCCCTTGCTCACCATGggTGACAAGAGGAAGGGGAGT	ZK-spEGFP Sema3a_R

PK268	ACGAGCTGTACAAGTAAGATGCGCCCCCCCT	ZK-spEGFP Bcl2_F
PK269	TCCTCGCCCTTGCTCACCATggCCTTCCCAGAGGAAAAGCAACG	ZK-spEGFP Bcl2_R
PK270	ACGAGCTGTACAAGTAAGATCCTTCCCAGAGGAAAAGCAACG	ZK-spEGFP Bcl2 inv_F
PK271	TCCTCGCCCTTGCTCACCATggGCGCCCGCCCT	ZK-spEGFP Bcl2 inv_R
PK272	ACGAGCTGTACAAGTAAGATGTTTCCGGAAACGAAAGGGAGAC	ZK-spEGFP Cofilin inv_F
PK273	TCCTCGCCCTTGCTCACCATGGGCCGGAAGCCGCC	ZK-spEGFP Cofilin inv_R
	qPCR Primer for linear/circRNA quantification	
LL034	Atgaagtggtcatggaagg	mRuby3-RTqPCR-F
LL035	Tccctcgatgactttgatcc	mRuby3-RTqPCR-R
LL036	CAGAAGAACGGCATCAAGGT	Circ-junction-RTqPCR-F
LL037	GGGGGTGTTCTGCTGGTAG	Circ-junction-RTqPCR-R
	Cloning of mRuby-ZSCAN-splitEGFP mutation plasmids	
LL070	CGCGTTATTGTTCTGCCGGGCGTTGACGTGACGCG	SV40minus-A9-M2 Gibs F
LL074	CGCGTCACGTCAACGCCCGGCAGAACAATAACGCG	SV40minus-A9-M2 Gibs R
LL071	CGCGTTATTGTTCTGCCGGGCGGACACTATTGCGG	SV40minus-A9-M5 Gibs F
LL075	CGCGAATAGTGTCGCCCGGCAGAACAATAACGCG	SV40minus-A9-M5 Gibs R
LL072	CGCGTTATTGTTCTGCCGGGCGTTACGTGACGCG	SV40minus-A9-M12 Gibs F
LL076	CGCGTCACGTGAACGCCCGGCAGAACAATAACGCG	SV40minus-A9-M12 Gibs R
LL073	CGCGTTATTGTTCTGCCGGGCGGACACGTGTGCCG	SV40minus-A9-M13 Gibs F
LL077	CGGCACACGTGTCCGCCCGGCAGAACAATAACGCG	SV40minus-A9-M13 Gibs R
	Cloning of pGL3-Nluc and -NLB plasmids	
MH001	aacctcaaacagacaccGATATCGCCACCATGGCCGTTTACCCATACGAT	pMH001/002 Nluc insert part 1 F
MH002	ctggacagatgcacGCCCATCTGCTGGCCCGCGTCATGCTGGCCATggtgga tGATCCGAGCTCGGTACCCGCCAGAATGCGTTCG	pMH001 Nluc insert part 2 R
MH003	GCCGGCCGCCCCGACTCTAGAATTACGCCAGAATGCGTTCG	pMH002 Nluc insert part 2 R
MH008	GCGAACGCATTCTGGCGTAAGCTCGCTTCTTGCTGTCC	pMH023 HBB3' insert F
MH009	GCCGGCCGCCCCGACTCTAGATTGCAATGAAAATAAATGTTTTTATTAGGCA GAATCC	pMH023 HBB3' insert R
MH055	CGAACGCATTCTGGCGGGTACCGAGCTCGGATCatccaccATGGCCAGCATGA CC	pMH001 Nluc insert part 1 R
MH056	GGTACCCGCCAGAATGCGTTCG	pMH001 Nluc insert part 2 F