

¹ Supplementary Materials: Elucidating the Transcriptome of
² Turkey Hemorrhagic Enteritis Virus

³

⁴ **Running Title:** Novel Insights into Turkey Hemorrhagic Enteritis Virus Transcriptome

⁵ Abraham Quaye^{1*}, Bret Pickett^{*}, Joel S. Griffitts^{*}, Bradford K. Berges^{*}, Brian D. Poole^{†*}

⁶ *Department of Microbiology and Molecular Biology, Brigham Young University

⁷ ¹First-author

⁸ [†] Corresponding Author

⁹ **Corresponding Author Information**

¹⁰ brian_poole@byu.edu

¹¹ Department of Microbiology and Molecular Biology,

¹² 4007 Life Sciences Building (LSB),

¹³ Brigham Young University,

¹⁴ Provo, Utah

¹⁵

¹⁶ **Supplementary Table 1A**

Table 1: Table 1a: Most Transcriptionally Active Regions of THEV at 12h.p.i

Time	Region	Strand	Total Reads	Percentage
12hpi	MLP	+	235	38.8%
12hpi	E2	-	161	26.6%
12hpi	E3	+	104	17.2%
12hpi	E4	-	40	6.6%
12hpi		-,+/-	40	6.6%
12hpi	E1	+	20	3.3%
12hpi	IM	-	5	0.8%

¹⁷ **Supplementary Table 1B**

Table 2: Table 1b: Most Transcriptionally Active Regions of THEV at 24h.p.i

Time	Region	Strand	Total Reads	Percentage
24hpi	MLP	+	52,589	45.7%
24hpi	E3	+	29,209	25.4%
24hpi	E2	-	27,833	24.2%
24hpi	E1	+	2,724	2.4%
24hpi		-,+/-	1,312	1.1%

Time	Region	Strand	Total Reads	Percentage
24hpi	IM	-	744	0.6%
24hpi	E4	-	664	0.6%

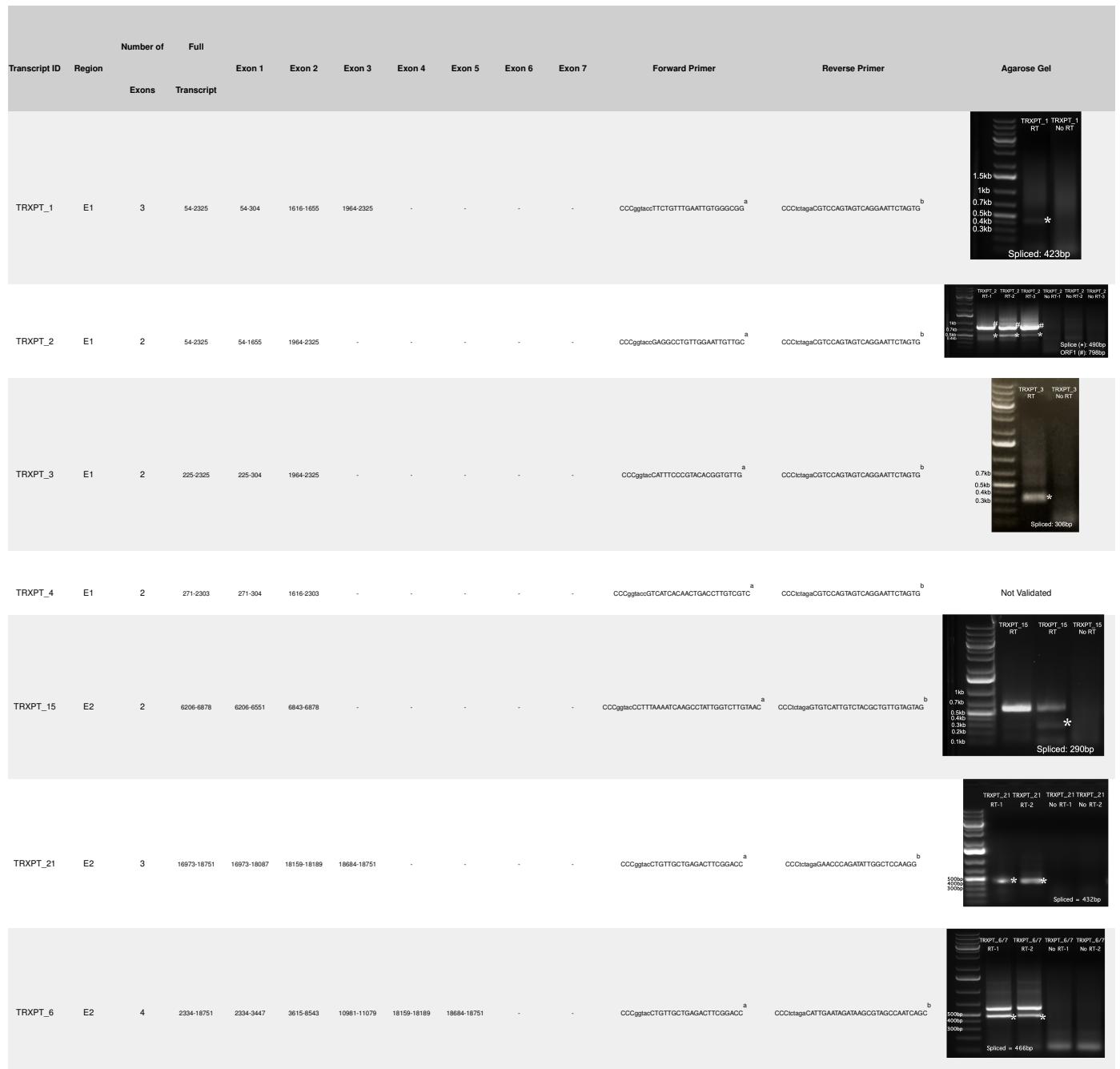
¹⁸ **Supplementary Table 1C**

Table 3: Table 1c: Most Transcriptionally Active Regions of THEV at 72h.p.i

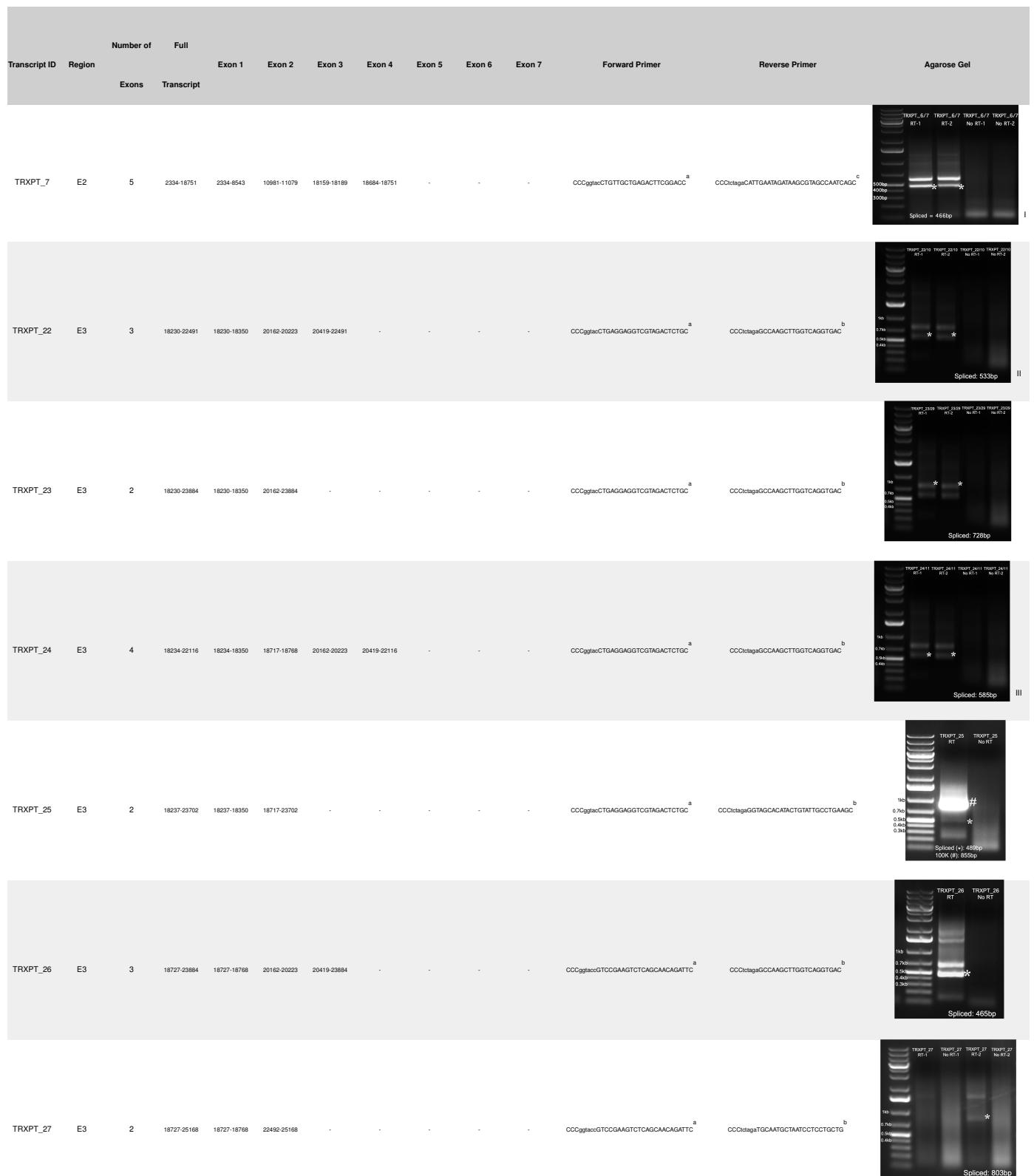
Time	Region	Strand	Total Reads	Percentage
72hpi	MLP	+	1,437,273	67.4%
72hpi	E2	-	304,274	14.3%
72hpi	E3	+	271,392	12.7%
72hpi	E1	+	74,135	3.5%
72hpi		-,+/-	27,680	1.3%
72hpi	IM	-	14,484	0.7%
72hpi	E4	-	3,568	0.2%

19 **Supplementary PCR Methods**

Table 4: Table 1: Agarose Gels Showing PCR Amplification of THEV cDNA With Gene-Specific Primers



^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_6 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;



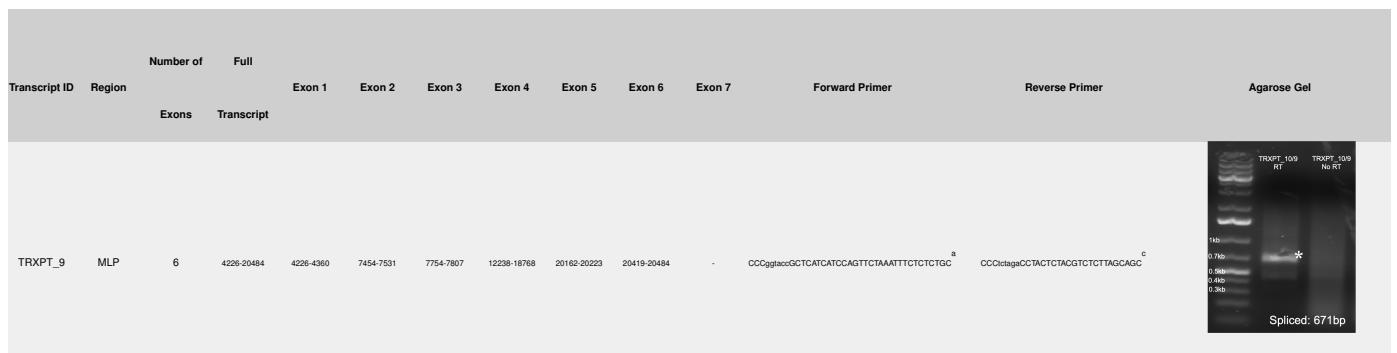
^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_6 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;

Transcript ID	Region	Number of Exons		Full Transcript							Forward Primer	Reverse Primer	Agarose Gel
		Exon	Transcript	Exon 1	Exon 2	Exon 3	Exon 4	Exon 5	Exon 6	Exon 7			
TRXPT_29	E3	2	18230-20732	18230-18350	20162-20732	-	-	-	-	-	CCGgtacCTGAGGAGGTCTGTAAGCTCTGC ^a	CCClctagaGCCAAGCTGGTCAGGTGAC ^b	
TRXPT_28	E4	2	25192-26247	25192-25701	26055-26247	-	-	-	-	-	CCGgtacGGACACGTGTTCTGTTAGAGAAC ^a	CCClctagaCAGTCAATCCGACCGCTG ^b	
TRXPT_5	IM	2	2334-3678	2334-3447	3615-3678	-	-	-	-	-	CCGgtacTCTGGTAGATCTTCAAACAGAAAG ^a	CCClctagaGCCAACCTGTAGGTCGATTAC ^b	
TRXPT_10	MLP	7	4226-22116	4226-4360	7454-7531	7754-7807	12238-18350	20162-20223	20419-22116	-	CCGgtacGCTCATCATCCAGTTCTAAATTCTCTCTGC ^a	CCClctagaCTACTCTACGTCCTTAGCAGC ^b	
TRXPT_11	MLP	6	4226-22116	4226-4360	7454-7531	7754-7807	13610-18350	18717-18768	20162-20223	20419-22116	CCGgtacGCTCATCATCCAGTTCTAAATTCTCTCTGC ^a	CCClctagaGCTTCAGTATTAGCAGCTCACAAACC ^c	
TRXPT_12	MLP	4	4226-25168	4226-4360	7454-7531	7754-7807	22492-25168	-	-	-	CCGgtacGCTCATCATCCAGTTCTAAATTCTCTCTGC ^a	CCClctagaTTTCCAGCTGAACCTGGAG ^b	
TRXPT_13	MLP	6	4279-22116	4279-4360	7454-7531	7754-7807	18717-18768	20162-20223	20419-22116	-	CCGgtacGCTCATCATCCAGTTCTAAATTCTCTCTGC ^a	CCClctagaGCCAAGCTGGTCAGGTGAC ^b	

^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_6 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;



^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_6 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;



^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_6 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;

- 20 In the table above, the restriction sites in the primer tails are shown in lowercase letters. All the primer
 21 melting temperatures (TMs) are 58-60°C using a hot start Taq DNA polymerase. The PCR reaction mix
 22 was done per manufacturer's instructions. The PCR cycling conditions were as follows: Initial denaturation
 23 – 95°C for 1 minute; cyclical denaturation – 95°C for 30 seconds, annealing – variable temperature (at least
 24 53°C) for 30 seconds, primer extension – 68°C for variable time, and final elongation – 68°C for 5 minutes.
 25 We used 35 cycles of amplification.

26 Supplementary Computational Analysis

- 27 Our trimmed RNA-seq reads were mapped to the genome of *M. gallopano* (with THEV's genome as one of
 28 its chromosomes) using Hisat2 to generate the alignment (BAM) files and StringTie used to assemble the
 29 transcriptome with a GTF file containing the predicted THEV ORFs as a guide. StringTie was also used to
 30 estimate the normalized expression levels (FPKM) of all the transcripts and Ballgown was used to perform
 31 statistical analysis and comparisons of the transcript expression levels, which instructive in understanding
 32 the temporal regulation THEV genes. We used RegTools to extract and analyze the splice junctions in
 33 the BAM files. The command regtools junctions extract provides a wealth of information about all the
 34 splice sites in the BAM file provided such as: the start and end positions, the strand, and number of reads
 35 supporting the splice junctions. The command regtools junctions annotate gives even more informa-
 36 tion such as: the splice site donor-acceptor sequences and transcripts/genes that overlap the junction.
 37 These information was the basis for estimating and comparing the splicing activity of different regions (TUs)
 38 of THEV over time.

³⁹ The entire analysis pipeline was managed with `Snakemake`, from which we generate the below diagram of
⁴⁰ the major steps of the analysis pipeline.

