

Table S3. Validation of expression of *Anopheles gambiae* midgut novel transcripts by qRT-PCR.

Validation of transcript expression by qRT-PCR was done in an independent *A. gambiae* midgut sample. The primers used, amplicon size, Cufflinks class code, FPKM and detection results are indicated.

Transcript ID	Primer F	Primer R	Amplicon Size	Class code	FPKM	Detected?
TCONS_00006299	CAATTTGCACACAAATAAAC	TAAGCAGACAAGTGACATTAG	150	o	0.306869	Yes
TCONS_00003756	ATAAACACATACGAACAGAGAGAC	TTGTGAGCTCTTTGTTGAC	150	u	0.393754	Yes
TCONS_00011544	TCTCTCTCTCTCTCTCTTTAC	ACTACCCTGTAGAAGTCAACTAA	150	o	0.461712	Yes
TCONS_00024341	ATTTGGATGGTAATACTGCATAC	TGTTATACATGTTTCATTTGCGT	150	u	0.463521	Yes
TCONS_00005711	GAAAAATGAAGAAGGTGAAGAAC	GACAATCGGTTCTCTCAG	150	e	0.506611	Yes
TCONS_00009632	CAGCGTCAATTACGATACG	GATAGTCCGATAATATTTCTCT	150	u	0.530405	Yes
TCONS_00019129	TAAAAACGGAAGTGAATGAG	GAACGATAAACTACGATACGAG	150	u	0.55613	Yes
TCONS_00000582	ACTACAAAATGCATCTCTATCTG	TTCTTTGTATTACGCTACTTTACG	150	e	0.559452	Yes
TCONS_00011333	GGTGTGTATGTGTGAGTATTTG	GTATTTAAGCCCTTTGTTAACTTG	150	u	0.592527	No
TCONS_00005591	TATATACTGTGCGATGATTATATGG	ACAAAAGATATACTCCTTTAGATG	150	p	0.808216	No
TCONS_00005686	TTCCATTTACTAAACAACTGTAACCT	TATATGAAGTCCAACTCCAGTG	150	p	0.86968	Yes
TCONS_00024038	CATATACAGTCTCAGATGATAAAAC	AGTGTCACTAGAGACGAAG	150	u	0.884429	No
TCONS_00017003	AAAACTTAATCGAAATCGTCTC	GATTCTGTACTGATTTTGACTC	150	p	0.936899	Yes
TCONS_00021911	GTAATCTGTACTGTACTCGACTG	AGTTCATCTTAAACACATACAC	149	u	0.989295	Yes
TCONS_00024695	TTAATAAAACACACACACACAC	TATACTGGGTAATGCACAG	151	u	0.992318	Yes
TCONS_00007720	AATCTAGAGAGTACAATCGAAAC	CGTAGTCGAGAAATAGATTTAG	148	u	1.019166	Yes
TCONS_00024309	ACTCGTATGACTTAAACAACATGAAC	AAATGTCTCAATATGCTGGTTAC	150	u	1.05593	Yes
TCONS_00024266	CATACCTACGAAAAACCGATG	GATGTCAAAATGTTTTACTTCCAC	150	p	1.146248	Yes
TCONS_00012845	TAATTACGTTTATAAGCTAACAC	CGGTGTATAGTGTGATGATG	150	u	1.180639	Yes
TCONS_00007648	GTGTAGAATGTGTTGTTTATTG	CTTAGGGTAACCTAACGCTATC	150	u	1.197861	Yes
TCONS_00005769	TTTTAGGGTTTTTTGTTGTTGTTG	TATGTTCCGTTGATTTATAATCCC	150	i	1.333727	Yes
TCONS_00024315	CTACGGTTCATCAAGTGC	ATAACACTCCACCACATCC	150	u	1.384962	Yes
TCONS_00009216	ACTAGCAAAACACAATCGCTC	GATTTGTTTTCTTTTGCTG	150	u	1.407028	Yes
TCONS_00021375	AACACGTAGAATAAACCAAGG	GCTGTAACGTAAAGTTGACTC	150	u	1.496962	Yes
TCONS_00023257	GACATCTTGAATGTGATTATGTTG	CTCTCTCTCTTTCTGTCTCTC	150	u	1.521614	No
TCONS_00034103	TTTCTACGGTAGCTTTACTTC	ACATGAACATGATTGTATCC	150	u	1.602879	Yes
TCONS_00017437	GGAAATAGCGTTGATTTAGC	CACTGATATGTAGAGTGTGTAGA	149	u	1.633662	Yes
TCONS_00013184	AACTCACACATACACTCAAAAG	AAACTGATAAGAGACTGCTACTAC	600	j	1.645551	Yes
TCONS_00005060	ATGAGTTAAACATGGAAAGAC	GTTATCTGTATACATTTACTTTTC	149	u	1.648681	No
TCONS_00005000	TGAGCTTATACTAGTTCTTTTGG	GGTTAGTTTAAATACATACCAACATC	150	u	1.652122	Yes
TCONS_00005001	TTCTACTGGTTCTATTATTACCG	ATCTGTCTTTCTGATCTTTAC	150	u	1.702571	Yes
TCONS_00018857	GTATCGCATTACTCGAAAAATAAG	TCAGCTGTCTTATTCTTCTCTC	600	j	1.715457	Yes
TCONS_00009945	CTGAAGCATCTGATGAACACTACTAC	GACGTACTCTCGTTGAGG	600	j	1.813174	Yes
TCONS_00028506	TAACAAAGCACTACCTCAAGC	AGGTGACTTCACACTGTCC	600	=	1.836078	Yes
TCONS_00010621	CTATTGCAAACTCGTGTAGTATATG	GTGTCCCACTAGTCTTACAAAATC	599	i	1.870046	Yes
TCONS_00036217	GATGAAGAGGAAGATGACGAG	GAACAGGACAGGACCGAG	600	=	1.968085	Yes
TCONS_00030238	GTTTCAGATTTCTTTCTCATT	GTCTGAAATTAACGAGCTGTAG	600	=	2.033674	Yes
TCONS_00014056	GAGATAATGGACACATATCAGAAG	ACTTTCACCATCTGAAGCTATAC	601	=	2.064097	Yes
TCONS_00022107	GATCGCTTTATATATTGTTAGCAC	TATTTTATAACGACGATGTGTTG	599	p	2.175829	No
TCONS_00016970	TGTAGTCTAAATCTCTTTCCCTTAG	GTCTGCTACATAAAAACAAAACCTG	600	j	2.221265	Yes
TCONS_00001231	TCGTTTTGATAAACTCGATAAC	TATCTGTTAAGAACGTTTCCAG	601	=	2.320936	Yes
TCONS_00007415	GAGAAGTACATATATTGCATACC	CATTGTTTACCATCTGTACC	150	u	2.328564	Yes
TCONS_00016414	CTAAAAATGCTTCCCAATG	ATACATTGGTAGCGAAATGAC	600	=	2.431768	No
TCONS_00032866	GGCAGATATAAGATGATAACG	GCCCTGTACATTTCAAAC	149	u	2.465282	Yes
TCONS_00022447	TCTAGGAGGAGATGACCAG	CCAGTAATGGTCTAATAAATTG	149	u	2.495375	Yes
TCONS_00016620	ATTGCGATAAAAGGCAAC	ATCTTAATGATGCTTGAACCT	604	j	2.552828	Yes
TCONS_00005525	ATGCTATCGATTTATGTTAGTC	GTACGATGTACAGCATAAGAG	151	u	2.55412	No
TCONS_00005695	GTTAGATTTGTAATGTTGACTC	GAGAGAGAGAGAGAGAGAGAGA	150	u	2.641962	No
TCONS_00025373	ATTCAGTGTGACATACATATCC	GTTCTGTTTCAATTTGTCG	154	u	2.673924	Yes
TCONS_00016462	CGACGGCTTTGATTTGTA	GGAAAATCAGGTGTGCCAGT	425	i	2.733234	Yes
TCONS_00022002	GTAGGAAAAAGGGGTGAGC	CCGCAATCAATTTGGAGTC	226	u	3.052776	Yes
TCONS_00006269	GCCTTGCTCGAGTATGTACGA	GTCCAATACCGCCGAGAGT	304	u	3.310948	Yes
TCONS_00017751	AAGTCGACGGATTACCACGA	CCGTTCAATTTGATCAACC	203	u	3.495017	Yes
TCONS_00032244	GATTGCAGCAACAGAGATAGC	AGAACGCAACACGCATACAG	205	u	3.692191	Yes
TCONS_00021987	CCGCTATCGAATCTTTGGAG	GCTACGCTGACGAAAAAGTACA	226	u	3.733785	Yes
TCONS_00027665	GCGGTTATCTTTGTCGTTG	GCAGAATGGTTGGGATATTG	203	u	4.170971	Yes

* Cufflinks class codes:

- = Complete match of intron chain.
- c Contained.
- j Potentially novel isoform (fragment): at least one splice junction is shared with a reference transcript.
- e Single exon transfrag overlapping a reference exon and at least 10 bp of a reference intron, indicating a possible pre-mRNA fragment.
- i A transfrag falling entirely within a reference intron.
- o Generic exonic overlap with a reference transcript.
- p Possible polymerase run-on fragment (within 2Kbases of a reference transcript).
- r Repeat. Currently determined by looking at the soft-masked reference sequence and applied to transcripts where at least 50% of the bases are lower case.
- u Unknown, intergenic transcript.
- x Exonic overlap with reference on the opposite strand.
- s An intron of the transfrag overlaps a reference intron on the opposite strand (likely due to read mapping errors).
- . tracking file only, indicates multiple classifications.