

Table S1. Piwi gene Identification codes.

Gene	Species	Strain	Gene ID	Tree Identifier
Ago3	Anopheles stephensi	Indian strain, Astel 2.1 geneset	ASTEI04992	AsAgo3
		SDA-500 strain, AteS 1.0 geneset	ASTE009599	AsAgo3
	Anopheles gambiae	PEST, Agamp3.7 geneset	AGAP008862	AgAgo3
	Aedes aegypti	Liverpool, AaegL 1.4 geneset	AAEL007823	AaeAgo3
	Drosophila melanogaster		3355150	DmAgo3
	Anopheles albimanus	STECLA, AalbS1.1 geneset	AALB001790	AA1
	Anopheles arabiensis	Dongola, AaraD1.1 geneset	AARA009401	AR2
	Anopheles christi	ACHKN1017, AchrA1.1 geneset	ACHR007454	AC2
	Anopheles darlingi	Coari, AdarC2.2 geneset	ADAC008631	AD1
	Anopheles dirus	WRAIR2, AdirW1.1 geneset	ADIR009721	AI1
	Anopheles epiroticus	Epiroticus2, AepiE1.1 geneset	AEPI002451	AP1
	Anopheles funestus	FUMOZ, AfunF1.1 geneset	AFUN000985	AF1
	Anopheles minimus A	MINIMUS1, AminM1.1 geneset	AMIN001620	AM1
			AMIN001621	AM2
Anopheles quadriannulatus A	SANGWE, AquaS1.1 geneset	AQUA007856* AQUA007857	AQ1	
Aubergine	Anopheles stephensi	Indian strain, Astel 2.1 geneset	ASTEI03833	AsAub
		SDA-500 strain, AteS 1.0 geneset	ASTE011384	AsAub
	Anopheles gambiae	PEST, Agamp3.7 geneset	AGAP009509	AgAub
	Aedes aegypti	Liverpool, AaegL 1.4 geneset	AAEL008076	AaePiwi1
			AAEL008098	AaePiwi2
			AAEL013692	AaePiwi3
			AAEL007698	AaePiwi4
	Drosophila melanogaster		34524	DmAub
	Anopheles arabiensis	Dongola, AaraD1.1 geneset	AARA011871	AR3
	Anopheles christi	ACHKN1017, AchrA1.1 geneset	ACHR004324	AC3
	Anopheles darlingi	Coari, AdarC2.2 geneset	ADAC006051	AD3
	Anopheles dirus	WRAIR2, AdirW1.1 geneset	ADIR006020	AI4
	Anopheles epiroticus	Epiroticus2, AepiE1.1 geneset	AEPI004893	AP3
	Anopheles funestus	FUMOZ, AfunF1.1 geneset	AFUN005296	AF3
Anopheles minimus	MINIMUS1, AminM1.1 geneset	AMIN001881	AM4	
Anopheles quadriannulatus A	SANGWE, AquaS1.1 geneset	AQUA005807	AQ3	
Piwi	Anopheles stephensi	Indian strain, Astel 2.1 geneset	ASTEI06803	AsPiwi
		SDA-500 strain, AteS 1.0 geneset	ASTE008262	AsPiwi
	Anopheles gambiae	PEST, Agamp3.7 geneset	AGAP011204	AgPiwi
	Aedes aegypti	Liverpool, AaegL 1.4 geneset	AAEL013233	AaePiwi5
			AAEL013227	AaePiwi6
			AAEL006287	AaePiwi7
	Drosophila melanogaster		34521	DmPiwi
	Anopheles albimanus	STECLA, AalbS1.1 geneset	AALB010564	AA2
			AALB010570	AA3
	Anopheles ariabiensis	Dongola, AaraD1.1 geneset	AARA000129	AR1
	Anopheles christi	ACHKN1017, AchrA1.1 geneset	ACHR009351	AC1
	Anopheles darlingi	Coari, AdarC2.2 geneset	ADAC008732	AD2
	Anopheles dirus	WRAIR2, AdirW1.1 geneset	ADIR000087	AI2
			ADIR000092	AI3
	Anopheles epiroticus	Epiroticus2, AepiE1.1 geneset	AEPI006652	AP2
	Anopheles funestus	FUMOZ, AfunF1.1 geneset	AFUN004060	AF2
Anopheles minimus	MINIMUS1, AminM1.1 geneset	AMIN008377	AM3	
Anopheles quadriannulatus A	SANGWE, AquaS1.1 geneset	AQUA005063	AQ2	

*Gene model in VectorBase missassigned this ortholog as two genes.

Table S1: p-values from statistical analysis using two-sided t-tests of tissue and time point expression as measured by qPCR.

						Female Carcass PBM					Female Ovaries PBM					
		Embryo	Larvae	Pupae	Male	0 hr	2 hr	24 hr	48 hr	72 hr	0 hr	2 hr	24 hr	48 hr	72 hr	
Ago3	Female Carcass PBM	Embryo														
		Larvae	0.0031													
		Pupae	0.5915	0.0988												
		Male	0.1256	0.0561	0.1274											
		0 hr	0.0006	0.1580	0.0381	0.0472										
	Female Ovaries PBM	2 hr	0.0034	0.7066	0.0954	0.0691	0.3396									
		24 hr	0.0003	0.0487	0.0270	0.0448	0.6071	0.1411								
		48 hr	0.9310	0.0403	0.5937	0.0411	0.0156	0.0416	0.0114							
		72 hr	0.0045	0.9389	0.1117	0.0568	0.1952	0.6896	0.0810	0.0441						
		0 hr	0.0130	0.8574	0.1559	0.0589	0.2992	0.6880	0.1860	0.0566	0.9061					
	Female Carcass PBM	2 hr	0.8558	0.0891	0.8016	0.1222	0.0399	0.0897	0.0303	0.8047	0.0977	0.1263				
		24 hr	0.7325	0.0872	0.5364	0.1672	0.0494	0.0931	0.0409	0.7608	0.0924	0.1088	0.6752			
		48 hr	0.0023	0.0008	0.0031	0.1450	0.0006	0.0014	0.0006	0.0001	0.0008	0.0009	0.0022	0.0034		
		72 hr	0.0000	0.0000	0.0000	0.9517	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0011	0.0670	
Embryo																
Aub	Female Carcass PBM	Larvae	0.0003													
		Pupae	0.0002	0.6728												
		Male	0.0034	0.6012	0.4092											
		0 hr	0.0000	0.1562	0.3397	0.1368										
		2 hr	0.0001	0.3059	0.5855	0.2340	0.5657									
	Female Ovaries PBM	24 hr	0.0000	0.0165	0.0457	0.0419	0.1027	0.0146								
		48 hr	0.0000	0.6404	0.3642	0.8096	0.0647	0.1524	0.0081							
		72 hr	0.0000	0.0231	0.0670	0.0458	0.2126	0.0415	0.4714	0.0102						
		0 hr	0.8853	0.1226	0.1000	0.1840	0.0646	0.0952	0.0536	0.0450	0.0456					
		2 hr	0.5716	0.0537	0.0445	0.0790	0.0301	0.0469	0.0283	0.0098	0.0222	0.5828				
	Female Carcass PBM	24 hr	0.4040	0.0351	0.0293	0.0518	0.0199	0.0321	0.0196	0.0045	0.0148	0.4399	0.8225			
		48 hr	0.0067	0.0004	0.0003	0.0006	0.0002	0.0006	0.0003	0.0000	0.0002	0.0088	0.0313	0.0512		
		72 hr	0.0030	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1141	0.4935	0.7207	0.0549	
		Embryo														
Larvae		0.0000														
Piwi	Female Carcass PBM	Pupae	0.0000	0.0818												
		Male	N/A	N/A	N/A											
		0 hr	0.0000	0.0304	0.0003	N/A										
		2 hr	N/A	N/A	N/A	N/A	N/A									
		24 hr	0.0000	0.0110	0.0001	N/A	0.2045	N/A								
	Female Ovaries PBM	48 hr	N/A	N/A	N/A	N/A	N/A	N/A	N/A							
		72 hr	0.0000	0.5909	0.0222	N/A	0.0671	N/A	0.0253	N/A						
		0 hr	0.0000	0.7588	0.0885	N/A	0.0023	N/A	0.0005	N/A	0.3449					
		2 hr	0.0009	0.0113	0.0769	N/A	0.0023	N/A	0.0009	N/A	0.0057	0.0126				
		24 hr	0.0000	0.0110	0.0001	N/A	0.2045	N/A	N/A	N/A	0.0253	0.0005	0.0009			
	Female Carcass PBM	48 hr	0.0514	0.0005	0.0007	N/A	0.0012	N/A	0.0005	N/A	0.0004	0.0005	0.0017	0.0011		
		72 hr	0.0000	0.0000	0.0000	N/A	0.0000	N/A	0.0000	N/A	0.0000	0.0000	0.0000	0.0000		
		Embryo														
		Larvae	0.0000													
Pupae		0.0000	0.0818													

P-values less than 0.05 and 0.001 are highlighted in light and dark orange respectively. N/A indicates little or no amplification of the target gene in one of the compared samples in technical and biological replicates, which prohibited performance of the test. This occurred for Piwi amplified from male samples and female carcass samples.

Table S3. List of primers.

Primers	5'-3' Forward	5'-3' Reverse
5' RACE		
Ago3	CGCGCGCACACTCGCCAACG	CGGCCACCCCGTCGCGGAAT
Ago3 Rev 2		TGCGGGTGCAGCGCCTGA
Ago3 Rev 2		GCTGCGGCACGATGCGGGCCTGT
Aub	TGGCACGCGGCTTCAGGGGGA	CGGGCTGGGACACGCCGGGA
Aub Rev 1		CGGGCTGGGACACGCCGGGACA
Aub Rev 2		TGCGCCATGCCGGGCGACGCT
Piwi	CCGCCCGGTCTGTGTAATCCGATGC	ATGACGGGGCCCTGCGTGCGG
Piwi Rev 1		TGCGCCGGTATCGCCGCTCCGT
Piwi Rev 2		GCTGGGGCGCGGCAGAATGCGGT
3' RACE		
Ago3	TACTCGCGGGCCACGATCCA	CCGGCACACGAATGGTCCCC
Aub	GCCGCCCGTGGCATGCGGTT	GCCACCGCTGCGATACACACCCCG
Aub For1	TGCAAGCTCGGCGGCGTACCGT	
Aub For 2	CGGCCGGCATCGAGCAGGAGGTGC	
Aub For 4	GCAACATGCTCGAAAAGAACTTACT	
Piwi	ACCGCACACGCGGGGCGAAGA	ACTGGGCGGTGAGGAAGGCCAGC
Piwi For 1	GGCGATGGGCAGCTGCGCGGTGT	
Piwi For 2	ACCGCAATCCGCCGCCCGGCAC	
Quantitative PCR		
Ago3	AGCGTGCTCGATCATACCGT	CGTCCCGCAGCACAATGTAG
Aub	CCGGGCTGACGGATTCAATG	CGATCCGGATTGAGACGGGT
Piwi	GGTTCAAATCGGTGCCGCTG	CATTCACGTACGCGTTGCCT
rpS7	GGTGACCTGGATAAGAACCA	CGGCCAGTCAGTTCTTGTAC
Determination of cDNA sequence		
Ago3 1	CCTCTGCCAACCGGGACAGT	CACCATTGCTCCTGCGAGA
Ago3 2	TGTTCCGGCGCGTCATGTAT	GTTGGCAACCTTGCCTCCT
Ago3 3	ACAAGGAAACGCCGAGGAG	GTTGGCAACCTTGCCTCCT
Ago3 4	GGCGATCAAACGGCTGCTCT	AGCCGGAGCATTCCCTAGCC
Aub 1	GGAGGTTATCCACCGCAAGG	ACAGAAGCGGTTGCCAGTC
Aub 2	GACCCCTGGCGGATACACTG	TACGATCGTGCCGCTGACAT
Aub 3	GGCAGCACGAAAACGATGTG	TCGACGAGACGACGATCGAG
Aub 4	GGAGCTCGATCGTCTGCTCG	ATCGCACCGAACGATTTGCT
Aub 5	AAATCGTTCCGGTGCATGGT	TCGTGCCGGACCAATTGTAG
Piwi 1A	GGCAACACAGCTTGCGAAGA	GCTTGTGGGTTTGCATCTGCT
Piwi 1B	GCTGCTGGGTGCGATTCTTT	GCTTGTGGGTTTGCATCTGCT
Piwi 2	GCAGCAGATGCAAACCCACA	GGATCAGTGCTGCATGACG
Piwi 3	TTGAGCCAACGGTGGAGGAT	GACGAACCGTCCTTCGTGCT
Piwi 4	CAGGTCTCGGTGCACAGGT	AGCGGCACCGATTTGAACAT
Piwi 5	GCACCGGAACAGATGTTCA	TGCTTCGACGCGTACATGGT
Piwi 6	CGAAGCAGACCGAACCGAAG	GTCCGCCGTGAGACCACTCT