

Additional File 1

1) doublesex sequence information

NCBI GenBank deposited sequence as female *dsx* of *Anopheles gambiae*

>KM978938.1 *Anopheles gambiae* female-specific doublesex mRNA, complete cds, alternatively spliced

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ATCTCCCAAACCCCTAGCACCATCGCTTCCCTCGACCAACCAATACCGACAGCCCGAGAACGATCGTACCCGAGTGGAAAAC
ACATTGTATTTTTCGCAGCAAAAACAACACAGAAATCTTTAAATATTTTAAGATAAACTCCATGTCCCGACAAATCTGCTT
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GTATTTCGTAAGTGCACAAGCCC
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CGAAGCGTACCTGGAGTCGGTGC GGCGGAGTAAAAAGTGTTCGCGCTCAAGGATAGCGAGGCGCCGGGCGAGGAGCCGA
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VectorBase sequence matching female specific dsx of Anopheles gambiae (Exon 5)

>AGAP004050-RB AGAP004050-RB-E5 exon:protein_coding
 GTCAAGCGGTGGTCAACGAATACTCAGATTGCATAATCTGAACATGTTTGTATGGCGTGGAGTTGCGCAATACCACCCGT
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 ACGAATTC AATAGTTCTGTGCTATTTTAAATCAAGCCGCGTGC GCAACTGATGCCGATAAGTTCAAAC TAGTGTTTAAGG
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 AAACGGCCTGTACACACACACAGAAAACACTGCAGCATGTTTGTACATAGTAGATCCTAGAGCAGGTGGTTCGTTGCTCCT
 CGAACGCTCTGGACGCACGGCTTCGCGCGTATTTGCGTAGCGTTCCGCCGATCGTGGGTATTCGTA CTGCCACAAGCCCG
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 TCTGGAAC TACCCCGTCCAGGGATGCACCGGCTCGGCTCATGCCCTGCAAAACAGTCCGGGCTCCACTGTAGTAGCTCC
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 ATCCTTGC GCTACTACTACCGTTTGC GCGGTGCTGATCTAGAGCAGACCCTTTCCACTCCACTCTACAATCAATCA
 TCTGTGCAGAAAG

Alignment of KM978938.1 and AGAP004050 Exon5

KM978938.1	--CAAGCGGTGGTCAACGAATACTCAGATTGCATAATCTGAACATGTTTGTATGGCGTGG	58
AGAP004050_E5	GTCAAGCGGTGGTCAACGAATACTCAGATTGCATAATCTGAACATGTTTGTATGGCGTGG	60

KM978938.1	AGTTGCGCAATACCACCCGTCAGAGTGGATGATAAACTTCCGCACCCTGTAACCTGTCC	118
AGAP004050_E5	AGTTGCGCAATACCACCCGTCAGAGTGGATGATAAACTTCCGCACCCTGTAACCTGTCC	120

KM978938.1	GTATCTTTGTATGTGGGTGTGTGTATGTGTGTTTGGTGAAACGAATTC AATAGTTCTGTG	178
AGAP004050_E5	GTATCTTTGTATGTGGGTGTGTGTATGTGTGTTTGGTGAAACGAATTC AATAGTTCTGTG	180

KM978938.1	CTATTTTAAATCAAGCCGCGTGC GCAACTGATGCCGATAAGTTCAAAC TAGTGTTTAAGG	238
AGAP004050_E5	CTATTTTAAATCAAGCCGCGTGC GCAACTGATGCCGATAAGTTCAAAC TAGTGTTTAAGG	240

KM978938.1	AGTGGAGCGAGAGAGCCGCACCACGGTACAGAAGGGCAGCAGAATGGGTGCGCAGCCTAG	298

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AGAP004050_E5      AGTGGAGCGAGAGCCGCACCACGGTACAGAAGGGCAGCAGAATGGGTCGGCAGCCTAG 300
*****

KM978938.1        CTGCACTGGTGC GG TGC GTTCCGGCATCTCGGGGAGAGGGCGGGGAAATTCTAGTGT TAAA 358
AGAP004050_E5     CTGCACTGGTGC GG TGC GTTCCGGCGTCTCGGGGAGAGGGCGAGGAAATTCTAGTGT TAAA 360
*****

KM978938.1        TCGGAGCAGCAAAAACAAAACAGTGGTTCGTCGCCGTTCAAGAAACGGCCTATACACA--CA 416
AGAP004050_E5     TCGGAGCAGCAAAAACAAAACAGTGGTTCGTCGCCGTTCAAGAAACGGCCTGTACACACACA 420
*****

KM978938.1        CAGAAAACACTGCAGCATGTTTGTACATAGTAGATCCTAGAGCAGGTGGTTCGCTGCTCCT 476
AGAP004050_E5     CAGAAAACACTGCAGCATGTTTGTACATAGTAGATCCTAGAGCAGGTGGTTCGCTGCTCCT 480
*****

KM978938.1        CGAACGCTCTGGACGCACGACTTTGCGCGTACTTTCGCTAGCGTTCCGCCGATCGTGGGTA 536
AGAP004050_E5     CGAACGCTCTGGACGCACGACTTTGCGCGTACTTTCGCTAGCGTTCCGCCGATCGTGGGTA 540
*****

KM978938.1        TTCGTA CTGCCACAAGCCC----- 555
AGAP004050_E5     TTCGTA CTGCCACAAGCCC----- 555
*****

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Primers used for Real Time PCR (yellow) and dsRNA production (cyan)

Table 1. Sequences of PCR primers.

Gene	Vector Base ID	Primer name	Primer sequence	Efficiency (%)
<i>Female doublesex</i>	AGAP004050	newDSX-f	AGAGGGCGGGGAAATTCTAGT	111.19
		newDSX-r	GGGCTTGTGGCAGTACGAATA	
<i>Female doublesex</i>	AGAP004050	dsRNA_dsx-f2 (dsx1586)	CAAGCGGTGGTCAACGAATA	
		dsRNA_dsx-r3 (dsx1589)	GCCCACTCCTAAACTACTT	
<i>Female doublesex</i>	AGAP004050	T7dsRNA_dsx-f2	TAATACGACTCACTATAGGGCAAGCGGTGGTCAACGAA TA	
		T7dsRNA_dsx-r3	TAATACGACTCACTATAGGGGCCCACTCCTTAACACTA CTT	

Confirmation of sequence specificity in the *A. gambiae* genome

Your search is limited to records that include: *Anopheles gambiae* (taxid:7165) [Full Entrez Query](#)

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report description](#)

Job title: Nucleotide Sequence (557 letters)

RID [56JNXF08015](#) (Expires on 02-02 02:21 am)

Query ID [Icl|Query_214823](#)

Description None

Molecule type nucleic acid

Query Length 557

Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.8.1+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#) [MSA viewer](#)

Graphic Summary

Distribution of the top 5 Blast Hits on 5 subject sequences

Mouse over to see the title, click to show alignments

Color key for alignment scores

- <40
- 40-50
- 50-80
- 80-200
- >=200

Query

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Anopheles gambiae str. PEST AGAP004050-RD (AgaP_AGAP004050). mRNA	1029	1029	100%	0.0	100%	XM_003436650.1
<input type="checkbox"/> Anopheles gambiae str. PEST AGAP004050-RB (AgaP_AGAP004050). mRNA	1029	1029	100%	0.0	100%	XM_309601.5
<input type="checkbox"/> Anopheles gambiae female-specific doublesex protein (DSX) mRNA, complete cds	977	977	100%	0.0	98%	AY903308.1
<input type="checkbox"/> Anopheles gambiae female-specific doublesex mRNA, complete cds, alternatively spliced	972	972	100%	0.0	98%	KM978938.1
<input type="checkbox"/> Anopheles gambiae female-specific doublesex protein mRNA, complete cds	172	172	19%	4e-42	96%	DQ137802.1

Results

Job 275851

Description No description available

Submitted Thursday, January 31st, 2019 14:31:57 -0500

Compute Time 3 seconds

[CLEAR RESULTS](#) [EXPAND ALL](#)

Checked Hits

[Quick align](#) [Pass to ClustalW](#) [Download](#)

include query

Organism	Database	HSPs
<i>Anopheles gambiae</i>	(Transcripts) PEST strain transcript sequences, AgamP4.10 geneset	10

Show Query/Hit Numbers

Hit	Gene Name	Description	Query	Aln Length	E-value	Score	Identity	Query Hit	DB Sequence Hit
<input type="checkbox"/>	AGAP004050-RB	Doublesex female isoform	557	0.0	1114	100%	>		
<input type="checkbox"/>	AGAP007546-RA	4'-phosphopantetheinyl transferase...	32	0.007	44	87.5%			
<input type="checkbox"/>	AGAP008368-RA	TEP14 thioester-containing protein 14	25	0.090	40	92%			
<input type="checkbox"/>	AGAP009016-RA	LIX1-like protein	22	0.32	38	95.5%			
<input type="checkbox"/>	AGAP000046-RA		28	1.1	36	85.7%			
<input type="checkbox"/>	AGAP007502-RA	kinesin family member 11	23	1.1	36	91.3%			
<input type="checkbox"/>	AGAP013082-RA		21	1.1	36	95.2%			
<input type="checkbox"/>	AGAP028433-RA		23	1.1	36	91.3%			
<input type="checkbox"/>	AGAP029050-RA	leucine-rich repeat flightless-inte...	23	1.1	36	91.3%			
<input type="checkbox"/>	AGAP029050-RB	leucine-rich repeat flightless-inte...	23	1.1	36	91.3%			