

S3 Table

Primer name	Primer sequence (5'- 3')	Gene	Purpose	Reference
Masc_F1	TGTCGCGAYTATGTTTGGGGW	<i>EkMasc/EkMascB</i>	Identification <i>EkMasc/EkMascB</i>	This study
			3' RACE-PCR (first round PCR)	
			Genetic sexing PCR	
Masc_R1	GGTAGCCTCCTTATTGAGCCAT	<i>EkMasc/EkMascB</i>	Identification <i>EkMasc/EkMascB</i>	This study
			5' RACE-PCR (second round PCR)	
			Genetic sexing PCR	
Adapter Primer (AP)	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT	-	RACE-PCR cDNA synthesis First round RACE-PCR for 5'RACE	Invitrogen 3' RACE System for Rapid Amplification of cDNA Ends kit (Thermo Fisher Scientific, Waltham, MA, USA)
Abridged Universal Adapter Primer (AUAP)	GGCCACGCGTCGACTAGTAC	-	First round RACE-PCR (3'RACE) Second round RACE-PCR (3'RACE and 5'RACE)	Invitrogen 3' RACE System for Rapid Amplification of cDNA Ends kit (Thermo Fisher Scientific, Waltham, MA, USA)
Masc_VII_F1	ACGYGCTGAATGATCCCGAT	<i>EkMasc/EkMascB</i>	3' RACE-PCR (second round PCR)	This study
Masc_V_R1	CGGAGGTTTGGTAGTGTAAT	<i>EkMasc/EkMascB</i>	5' RACE-PCR (cDNA primer)	This study
Masc_IV_R1	ACAGGCCTTGATGGTGAAT	<i>EkMasc/EkMascB</i>	5' RACE-PCR (first round PCR)	This study
Masc_Sb_F	TGTTTCAGGTCCAAGCAATGT	<i>EkMasc/EkMascB</i>	Southern blot	This study
Masc_Sb_R	TCAGTCTTACTCGCAGTCTCC	<i>EkMasc/EkMascB</i>	Southern blot	This study
Masc_F_Via	ACTGAAACAACCACCTGCGA	<i>EkMasc/EkMascB</i>	qPCR Z-linkage	This study
Masc_R_VI	ATTTGTTTCGCGCACCAAATGT	<i>EkMasc/EkMascB</i>	qPCR Z-linkage	This study
Ek_Ace2_F	CCCGCCAACACCTAAGAGTCC	<i>Ace2</i>	qPCR Z-linkage	This study
Ek_Ace2_R	TGTACAAGTCGAGTGTGGCC	<i>Ace2</i>	qPCR Z-linkage	This study

rp49_deg_F1	ATTGACAACAGAGTSCGCAG	<i>rp49</i>	Isolation rp49	This study
rp49_deg_R1	CTGATGCTGAGCTGCTGGGC	<i>rp49</i>	Isolation rp49	This study
qrp49_F2	GGTTACGGATCAAACAAGAAGAC	<i>Ek</i> rp49	qRT-PCR	This study
qrp49_R2	GATTTCCAGCTCACGTACATTG	<i>Ek</i> rp49	qRT-PCR	This study
qMasc_F2	GCTTGGTCGGTTGAGTTTG	<i>Ek</i> Masc	qRT-PCR	This study
qMasc_R2	GCCCTGTGCATTATAACCTTG	<i>Ek</i> Masc	qRT-PCR	This study
qMascB_F1	GCTGGAACCTTTGATGCAGAAC	<i>Ek</i> MascB	qRT-PCR	This study
qMascB_R1	CCCAACCTCTTAGCGTACTTAAC	<i>Ek</i> MascB	qRT-PCR	This study
Masc_bmd_qF1	TAGACATTGACACTACCAAACC	<i>Ek</i> Masc/ <i>Ek</i> MascB	Splicing	This study
Masc_R_X	ACGTTGGTGGAATGACGGAA	<i>Ek</i> Masc/ <i>Ek</i> MascB	Splicing	This study
qPiMasc_F1	AGAAAGCAGTGTAAGTACTTCACGAA	<i>Pi</i> Masc	Splicing	This study
qPiMasc_R2	GGTCTCATTTGAACTCGAAGTTCCA	<i>Pi</i> Masc	Splicing	This study
CpMasc_F4	TCCGTTTCTCAACTTCGCCC	<i>Cp</i> Masc	Splicing	This study
CpMasc_R2	TTAAAAGACACCGAATTATCCAGTTGT	<i>Cp</i> Masc	Splicing	This study
dsx_dR_F2	CTCTAGTCCTCGTCATCCTCAA	<i>Ek</i> dsx	Determine RNAi effects on sexual development	This study
dsx_dR_R2	CGACATGCTGTACTCCTTTCTC	<i>Ek</i> dsx	Determine RNAi effects on sexual development	This study
siRNA name	siRNA sequence (5'-3')	Gene	Purpose	Reference
siMasc_II_s	CCAGGAAGAUAGAAACUGAAA	<i>Ek</i> Masc/ <i>Ek</i> MascB	RNAi	This study
siMasc_II_as	UCAGUUUCUAUCUCCUGGUU	<i>Ek</i> Masc/ <i>Ek</i> MascB	RNAi	This study
siMasc_VII_s	UAGUAUGUCAAGAGAAGAUUC	<i>Ek</i> Masc/ <i>Ek</i> MascB	RNAi	This study
siMasc_VII_as	AUCUUCUCUUGACAUACUAAU	<i>Ek</i> Masc/ <i>Ek</i> MascB	RNAi	This study
siGFP gui-1	AUAGACGUUGUGGCUGUUGUA		RNAi (negative control)	Kiuchi et al. (2014) ^a
siGFP pas-1	CAACAGCCACAACGUCUAAUUU		RNAi (negative control)	Kiuchi et al. (2014) ^a

^a Kiuchi T, Koga H, Kawamoto M, Shoji K, Sakai H, Arai Y, et al. A single female-specific piRNA is the primary determiner of sex in the silkworm. *Nature*. 2014; 509(7502):633–636. doi: 10.1038/nature13315