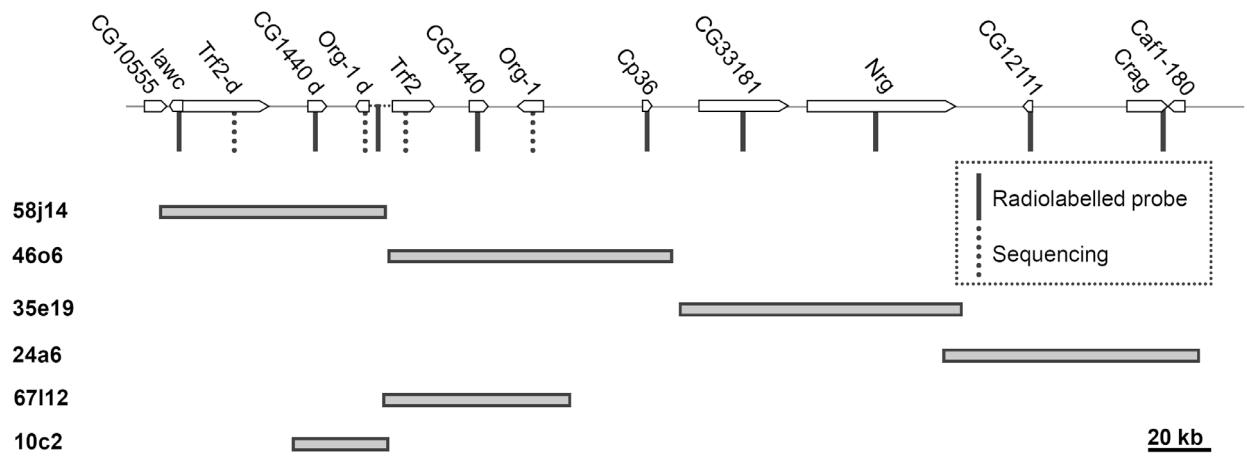


A



B

Locus	Name	Primers	Size bp
Radiolabelled probes			
<i>lawc</i>	DMCG32711ex1-1	TGTGTGAGTCGCGATAAACG	738
	DMCG32711_1_5	TCAGCTCTGGTAGCGAAACA	
<i>CG1440</i>	DMCG1440ex35-1	GCGTTATGTTAAGCCGCATT	598
	DMCG1440ex35-2	GGCGTTCATCACCAGATAGC	
<i>IST-Trf2</i>	inter43_44-1	AACCGGTTATGATGCGAAAG	936
	Inter43_44-2	CCGTATAGCTCAACCGATTTG	
<i>Cp36</i>	CP36-F1	CAGACAGCGAGCAGTAGACG	802
	CP36-R1	GTGGAGCCTCGATCTTCTTG	
<i>CG33181</i>	DMCG33181-3	TTCTACGGAATGCAAACGTG	694
	DMCG33181-4	TTGAGCGATACAATCGAACG	
<i>Nrg</i>	NRGintron1F	GCAGTGCAGAAAGCAAAGGT	350
	NRGintron1R	CCGGTTGTTTGGTGATTCTT	
<i>CG12111</i>	DSC12111-F1	TCTGCTGTTAATCCTGACGA	614
	DSC12111-R1	TACCACTTAATGTAGGTGAACTTGA	
<i>Crag</i>	DMCRAG7	GTACAGATCGAACGGCAGGT	805
	DMCRAG8	CATACTCGCGATGGAATGTG	
Sequencing probes			
<i>Trf2</i>	DMTRF2-1	GCCATCCATACACCACTGC	530
	DMTRF2-2	GAACGTTGCTTGCGGAAA	
<i>Org-1</i>	OrgF1	AGCCAATTTGCACTTTCAT	1054
	OrgR1	GTGCCATCACATCCCGAAAG	

Figure S1 Selection of the clones used to sequence the *sex-ratio* region on X^{SR6} . (A): Schematic representation of the *sex-ratio* region on the X chromosome and position of the clones. For the sake of simplicity, we only represented the genes used to design probes for the selection of clones and two genes adjacent to the region under study. The open boxes represent the genes. The grey boxes represent the clones, with their names on the left. The vertical bars show the fragments used as probes to position the clones on the X chromosome (see materials and methods), the dotted vertical lines represent the fragments that were sequenced to discriminate between the proximal and distal copies of the duplication. (B): PCR primers to produce the fragments used as probes (ordered from left to right).

D. melanogaster

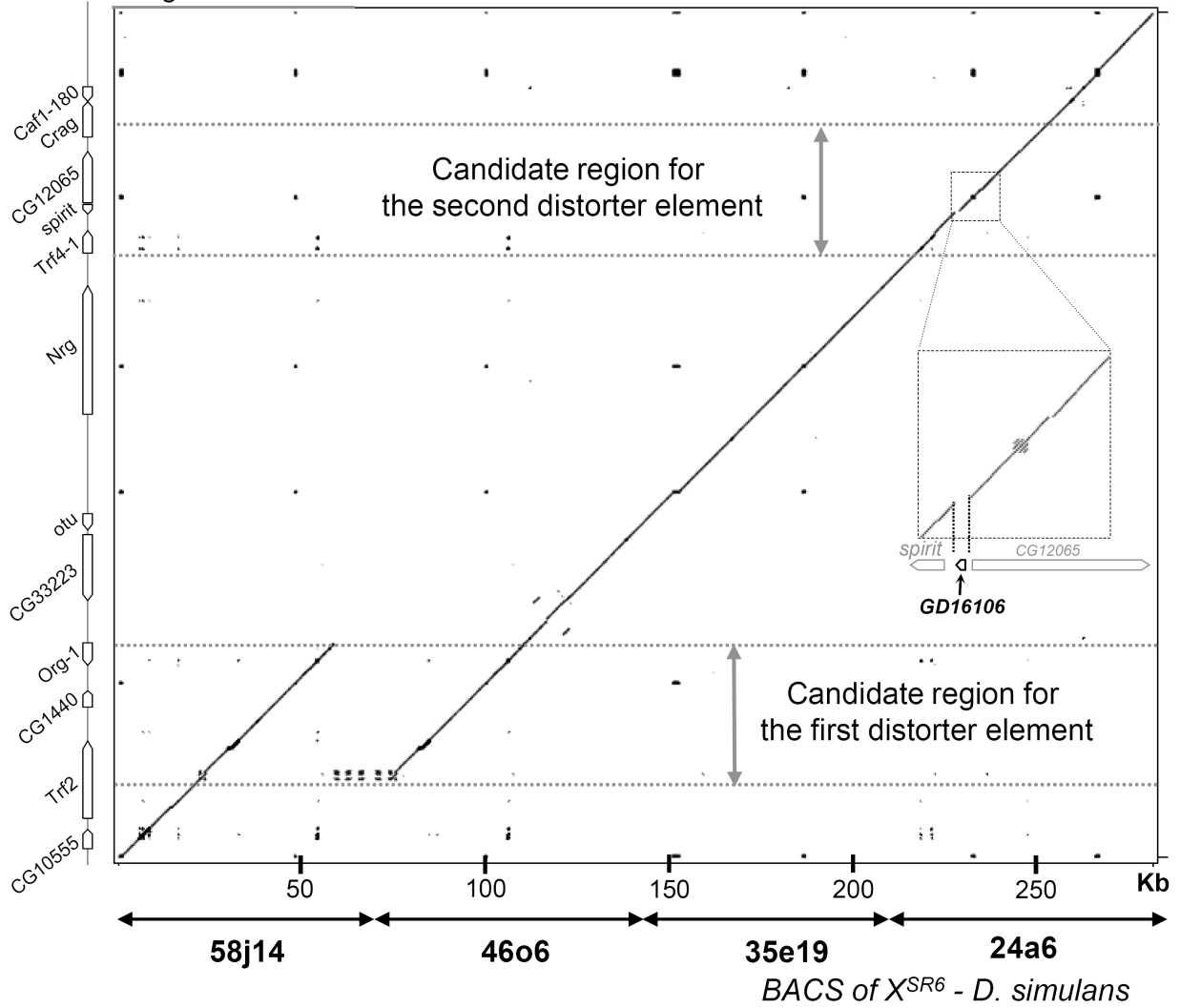


Figure S2 Dot plot comparison of the *sex-ratio* region on the X^{SR6} chromosome of *D. simulans* (below) with the homologous region in *D. melanogaster* (left). Boxes along the vertical axis represent the genes annotated in *D. melanogaster* genome. The limits of the candidate region for the second element involved in drive are those determined in Montchamp-Moreau *et al* (2006).

DDSA traces interpretation			
Site	Position	DDSA type of trace	Deduction of the neo-synthesized copy
1	5838	Tandem repeat	Proximal copy (4606)
2	5949	Micro-homology traces	Proximal copy (4606)
3	6198	Tandem repeat	Distal copy (58j14)
4	6992	Micro-homology traces	Proximal copy (4606)
5	8787	Micro-homology traces	Proximal copy (4606)

Legends:
tandem repeat
micro-homology traces

Site 1: Position: 5838 bp

```

XSR6d-58j14  CCGGCATTTATCAAACGGCAATGTCG-----GTGGAATCTTTAACTAGAAACAAC
XSR6p-4606  CCGGCATTTATCAAACGGCAATGTCGTGGAAATCGTGTGGAATCTTTAACTAGAAACAAC
*****
Dsim        CCGGCATTTATCAAACGGCAATGTCG-----GTGGAATCTTTAACTAGAAACAAC
Dsec       CCGGCATTTATCAAACGGCAATGTCG-----GTGGAATCTTTAACTAGAAACAAC
Dmel       GAGGCATTTATCAAATCGGCAATGTCG-----GGGGAATCTTCAACTAGAAACAAC

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Site 2: Position: 5949 bp

```

XSR6d-58j14  ATAGATCTCTTTGGCATTAAAT-----GAGTGAAGCTCCTAAATGTACATATGCAATAT
XSR6p-4606  ATAGATCTCTTTGGC-----TCCATAATGTACATATGCAATAT
*****
Dsim        ATAGATCTCTTTGGCATTAAAT-----GAGTGAAGCTCCTAAATGTACATATGCAATAT
Dsec       ATAGATCTCTTTGGCATTAAATTTAAATGAGTGAAGCTCCTAAATGTACATATGCAATAT
Dmel       ATAGATCTTTTTGGCATTAAAT-----GAGTGAAGCTCCTAAACATACATATGCAATAT
Dyak       ATAGACCTTTTTGGTATTAAAT-----GAGAGAAGCTCCTAAATGTACATTTGAAATAT

```

Site 3: Position: 6198 bp

```

XSR6d-58j14  TCGATGTTGGTGCATAACTGTTGTTGTTGCATAACTGTTGTTGTTGCTGTTGCTCGATTT
XSR6p-4606  TCGATGTTGGTGCATAACTGTTGTTGTTGCTGTTGCTCGATTT
*****
Dsec       TCGATGTTGGTGCATAACTGTTGTTGTTGCTGTTGCTCGATTT
Dsim       TCGATGTTGGTGCATAACTGTTGTTGTTGCTGTTGCTCGATTT
Dmel       TCGATGTTGGTGCATAACTGTTGTTGTTGCTGTTGCTCGATTT

```

Site 4: Position: 6992 bp

```

XSR6d-58j14  AATTTGCGTGTTCTTATTTTCCCAAATGTCAACTTTTCCAAATTATTTATTTACTTTTAT
XSR6p-4606  AATTTACGTGTTCTTATTTTCCCAA-----TTATTTATTTACTTTTAT
*****
Dsim-a     AATTTGCGTGTTCTTATTTTCCCAAATTTCAACTTTTCCAAATTATTTATTTACTTTTAT
Dsim-b     AATTTGCGTGTTCTTATTTTCCCAAATGTCAACTTTTCTAATTATTTATTTACTTTTAT
Dsec       AATTTGCATGTTCTTATTTTCCCAAATGTCAACTTTTCCGATTATTTATTTACTTTTAT
Dmel       AATTTGCATGTTTCTATTTTCCCAAATGTCAACTTTACGA-----TTATTAACTTTAT

```

Site 5: Position: 8787 bp

```

XSR6d-58j14  GAGTACCAGGATTCAAATCACGAGTACCAGGACTTGAATCACGAGTATCAGGACTCAACA
XSR6p-4606  GAGTACCAGGATTCAA-----ATCAGGAGTATCAGGACTCAACA
*****
Dsim       GAGTACCAGGATTCAAATCACGAGTACCAGGACTTGAATCACGAGTATCAGGACTCAACA

```

Figure S3 Signatures of the DDSA model. Several traces of the DDSA model have been detected when aligning the duplicated fragments according to Fiston-Lavier *et al* (2007): XSR6d-58j14 = distal copy on X^{SR6}, XSR6p-58j14 = proximal copy on X^{SR6}. The stars show the position identical between the copies. Homologous sequences in other species were found in flybase. Dsim: *D. simulans*, Dsec: *D. sechellia*, Dmel: *D. melanogaster*, and Dyak: *D. yakuba*. The positions of sites correspond to those in abscissa of Figure 2.

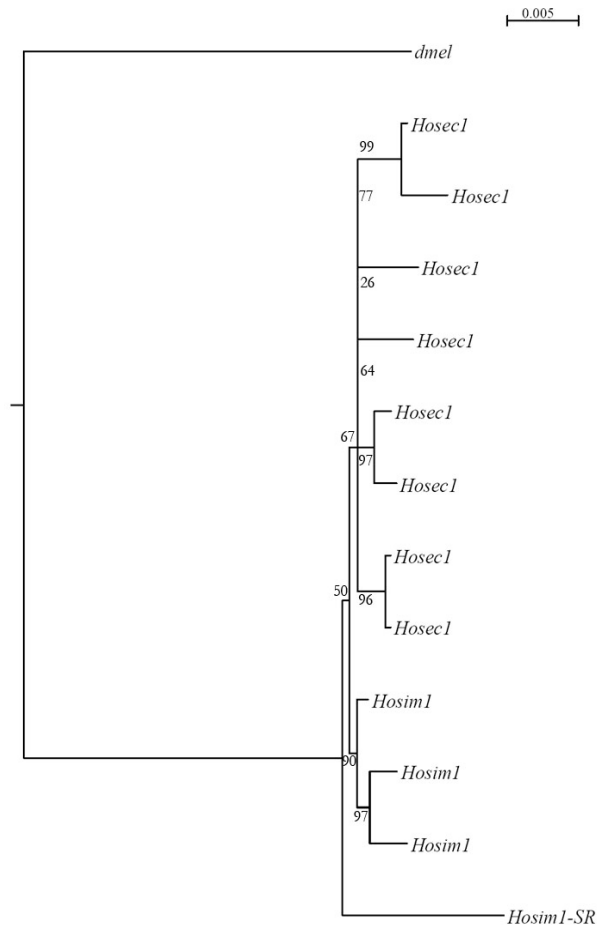


Figure S4 Maximum likelihood tree of *Hosim1* homologous sequences (HKY85 model) rooted with *D. melanogaster* sequence (*dmel*). *Hosec1* : sequence found in *D. sechellia* (DE FREITAS ORTIZ and LORETO 2009). Numbers are the bootstrap values (100 repetitions).

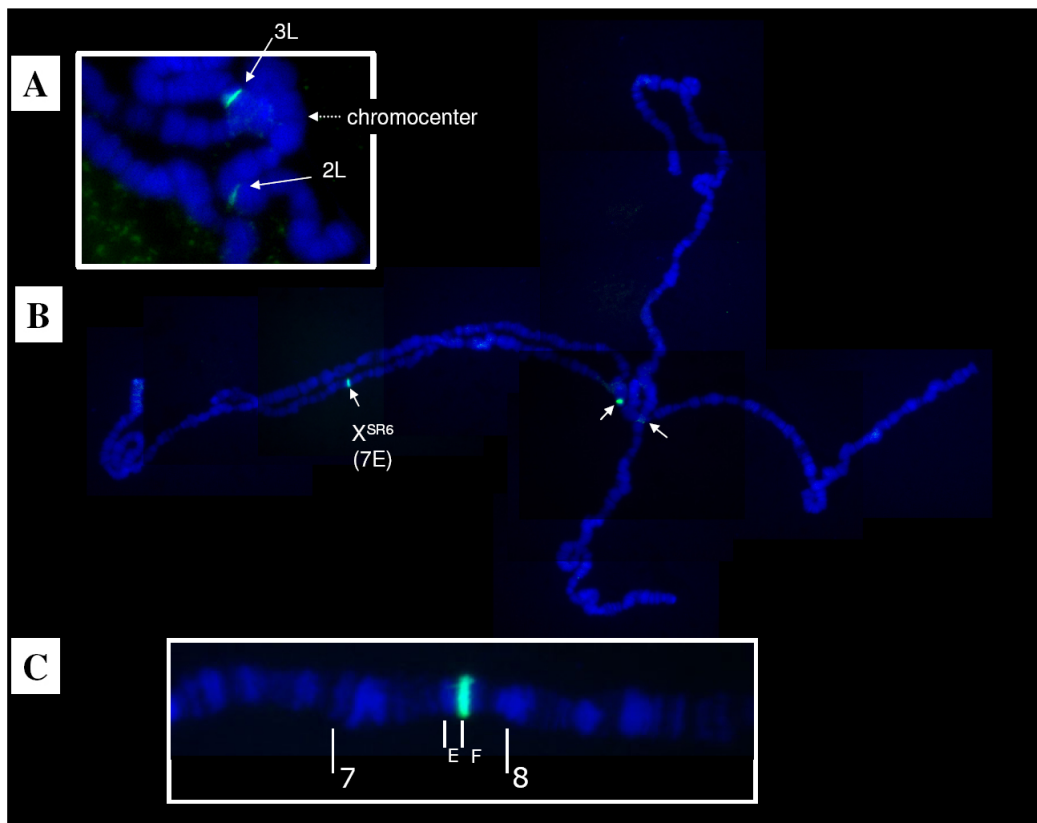
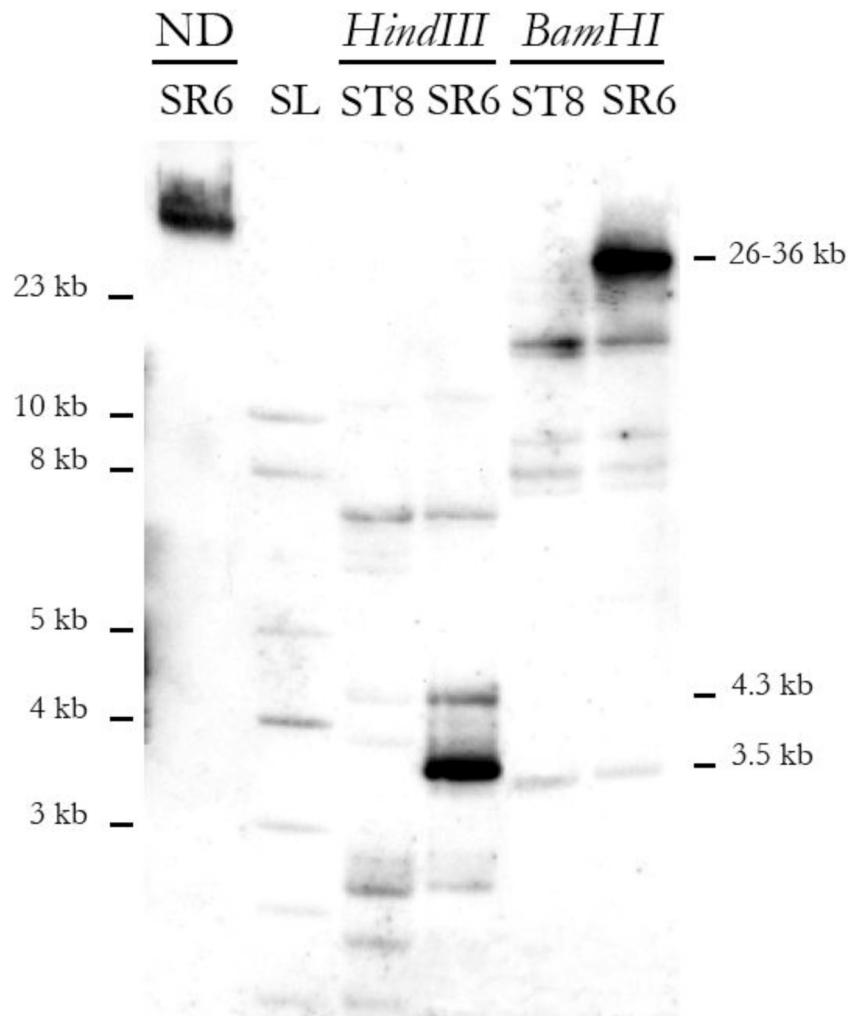


Figure S5 Visualization of *Hosim1* insertion sites by *in situ* hybridization on polytene chromosomes. (A) Two autosomal pericentromeric sites are visible in $(X^{ST8})_{ST8}$ males (3L and 2L arms). (B) In $(X^{SR6})_{ST8}$ males, an additional signal is visible on the X^{SR6} chromosome. It co-localizes with the supplementary band induced by the duplication (see Figure 2 in Montchamp-Moreau *et al.*, 2006). C. Focus on the X^{SR6} specific insertion site.

A



B

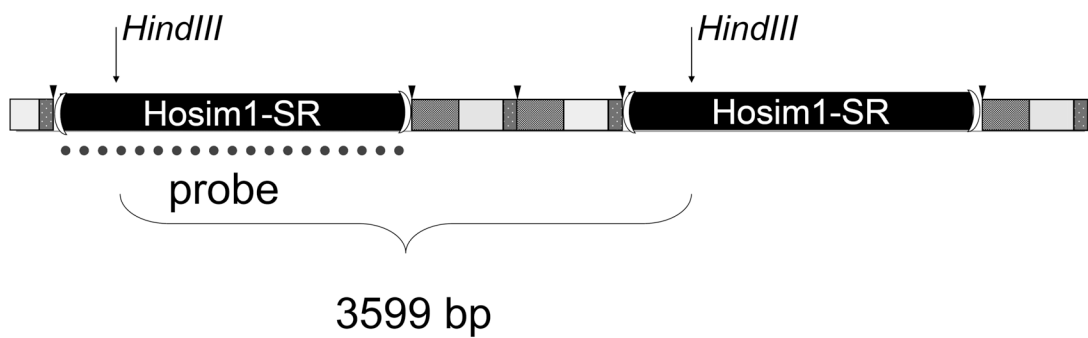


Figure S6 Control of the organization of the junction region. (A) Southern blot with DNA of $(X^{SR6})_{ST8}$ males digested with *BamHI* or *HindIII* and probed with *Hosim1-SR*. ND: Non Digested. ST8= $(X^{ST8})_{ST8}$ males, SR6 = $(X^{SR6})_{ST8}$ males. SL = Smart Ladder. (B) Position of *Hosim1* probe and *HindIII* sites in the basic module of the junction.

```

Hosim1-3L GAGTGCCTAAATGCAAACTACTGCAAAACTGTGCTTAGTGTGGTGGTGGATCGCAAGGAAACTTATCGCGTCACCTTAAAAATAAGCACCCAGCAGCTTTG
Hosim1-2L .....
Hosim1-U .....
Hosim1-SR .....
cDNA .....

Hosim1-3L GAGGAAACAAAGCGCCAAACCAATGAAAGCCGACAGGAAGCAAAATGTAACATAATATTTGATTTATTCATTTAAGTAGGCTTACTAAATTCCTGATT
Hosim1-2L .....
Hosim1-U .....
Hosim1-SR .....
cDNA .....

Hosim1-3L CTTTTAACATAGACTCCTCAGATAATTGGACCCCAACAGCAAGTAATAACTTCGTTCAITTCACGACC---GGCTGCCGGGAAAGCTGAGAAAA
Hosim1-2L .....
Hosim1-U .....
Hosim1-SR .....
cDNA .....

```

Figure S7 Identification of an intron in *Hosim1-SR*. The shorter cDNA fragment of Figure 8 have been sequenced and aligned with the sequences of *Hosim1* (DE FREITAS ORTIZ and LORETO 2009) and *Hosim1-SR* . Comparing the sequences shows that the sequenced cDNA came from *Hosim1-SR* form. The part highlighted in yellow shows an intron delimited by the characteristic GT-AG nucleotides, which is close to the deletion of 79nt specific of *Hosim1-SR* (Figure 5).

Table S1 PCR primers used in the study.

Locus	Name	Primers	Used for	DNA size (bp)	cDNA size (bp)
<i>GAPDH</i>	GAPDH-q1	AAGGCTGGCATTTCGCTGAACG AATTGCGCCCTTGCGGATTATG	quantification	198	198
	hAT1-F hAT1-R	CGCCCCGGATACAAGATGCCCA CTGCAACAAGTGCAGCAACACA	probe for FISH	850	850
<i>Hosim1</i>	Hosim1-q1-F Hosim1-q1-R (c)	GGAGCTGGCATCTGCAGTTTGT CTGCTCAACGAATGCCTCGCA	quantification	148	148
	Hosim1ST-q-F Hosim1ST-q-R (b)	TTCACCAAAGTGAACGAACG TAAGTGAACAACGAACGAACG	quantification	150	150
	hAT2-F hAT2-R (a)	ATGGCTTCAAACGAAAGCA CATTATCGGAAACACAGCA	Length polymorphism	Hosim1-ST : 784 Hosim1-SR : 699	Hosim1-ST : 784 and 717 Hosim1-SR : 699 and 632
	Hosimbam-F Hosimbam-R	GGATCCCCGAGTGCAGGGAAGTT GGATCCGGCACAGAAAATACGCAAGGAAGCA	probe for Southern blot		
<i>RPL17</i>	RPL17-q2-F RPL17-q2-R	CCCTCCTTTTCGTTTTCGTT GTGTTGTTCGGCACAGTTCAT	quantification	345	161
<i>RPII140</i>	RPII140-q1-F RPII140-q1-R	ATGGTGGCTTGCGTTTCGGTG ATTGTTGCGCAGATTGGCGATGG	quantification	158	158
<i>light</i>	Light410-F Light535-R	CCGATTCCAAAGCTCACATT TTGACAAAACACTGCCTTCG	quantification	194	126
<i>Trf2</i>	DMTRF2-1 DMTRF2-2	GCCATCCATACACCACTGC GAACGTTGCTTGCGGAAA	control of cDNA	526	462
<i>IST</i>	motif1-q2-F motif1-q2-R	AGTTGCCTTTGAGTTTTCTG ACTGATTTTGTTCACCTGACT	expression of <i>rISTs</i>	79	79
junction distal (a)	jct-F jct-orgLongR2	GTCCTTGCAGGCAGACAAA TGCGCACTCGGGGTCACGAT	control of duplication organization	757	-
junction proximal (c)	hosimSR1768-F jctTRF2-R	ACATCAGTTCCTTGCGAACGCCT AGAGAGTACGTGCCAGTGTGTA	control of duplication organization	1907	-
junction internal (b)	Jct-oranbleu-F hosimSR247-R	AGGGTACGGCAACGGTCACA TCCTCAAAGCTGCTGGGTGC	control of duplication organization	627	-