File S1

Supporting Text

Cloning of the 82kb insert by recombineering-mediated gap repair

Primers bacL-f and bacL_NotI-r (with a 3’ NotI site) were used to amplify a 500bp left arm of homology, and primers bacR_NotI-f (with a 5’ NotI site) and bacR-r were used to amplify a 500bp right arm of homology. The two arms were ligated after NotI digestion, and AscI and EcoRI sites were added by a second round of PCR with bacL_AscI-f and bacR_EcoRI-r. The resulting 1000 bp product, flanked by AscI and EcoRI, was cloned into pWalkman and used for gap repair. To verify the integrity of the BAC fragment throughout the construction, 10 PCR primer pairs spaced evenly throughout the 80 kb were used (see Table S1C for their sequences). The final construct was also verified by restriction digest analyses with several enzymes: SalI, KpnI, NotI, BamHI and sequencing of cloning junctions.

Insertion of DNA elements by recombineering

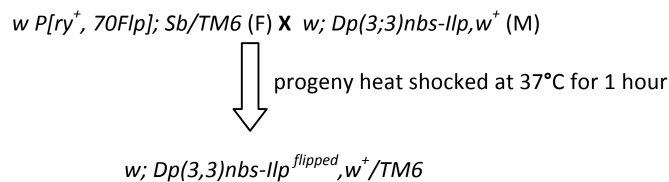
The insertion of *attB@nbs* and *attB@Ilp* were accomplished with bacterial recombineering using the *galK* positive-negative selection system (WARMING *et al.* 2005) essentially as described on the website http://redrecombineering.ncifcrf.gov/. Briefly, we generated the *galK* insert by PCR, using primers with flanking homology to the *nbs* region – nbsGalk-f and nbsGalk-r. This fragment was inserted at *nbs* at the position identical to that of the *attP* in the nbs-attP line (GAO *et al.* 2008) in the construct pWalkman{nbs-Ilp}, and clones were selected on galactose plates. Subsequently, *galK* was replaced by an *attB* insert with *nbs* flanking homology. Primers nbs2776-f and nbs3169-r were used to amplify *attB@nbs* from an existing construct (GAO *et al.* 2008). After the second round of recombineering, cells that retained *galK* were selected against on deoxygalactose plates. A similar procedure was followed for *attB@Ilp,* which was placed within the intergenic region between CG8177 and CG14168. The following primers were used to insert GalK: bac810Galk-f and bac810Galk-r; and attB: bac810_attB-end with bac810_attB-head. The success of *attB* placement in each case was verified by sequencing.

For I-SceI cut site insertion at position IS2, the I-SceI cut site at IS1 (described previously) was first removed by *galK* selection and replaced with original sequences in pP[acman]. Subsequently, a cut site was inserted at the new position, again using *galK* selection. The primers used were: up7993Galk-f and dn7993Galk-r for inserting *galK* at IS1; acman7929-f and acman8029-r for restoring IS1 site to pP[acman] sequences; up8150Galk-f and dn8150Galk-r for inserting *galK* at IS2. To insert the cut site at IS2, oligos up8150IS2-f and dn8150IS2-r were annealed and converted to dsDNA by a single round of DNA synthesis. This DNA was then used as template in a PCR with primers up8150IS2plus-f and dn8150IS2plus-r to generate PCR product used to replace *galK* at IS2.

File S2
Crossing schemes for induced reduction

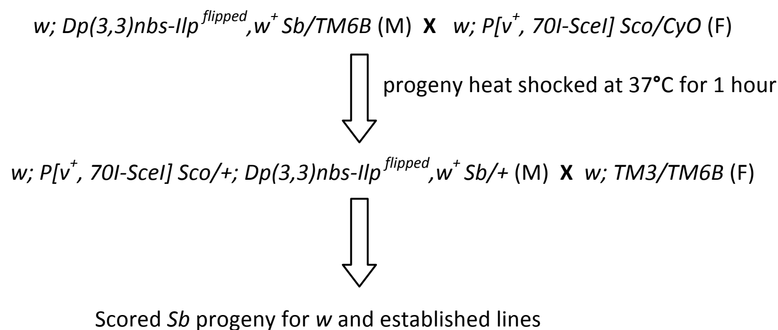
I. Reduction by ISceI

1. FLP-mediated excision of vector sequences

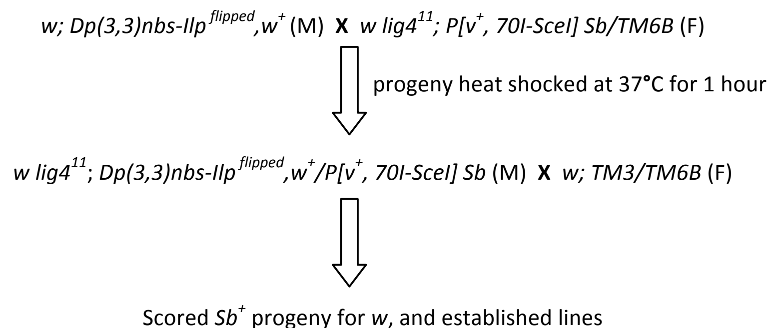


2. Reduction by I-SceI cutting

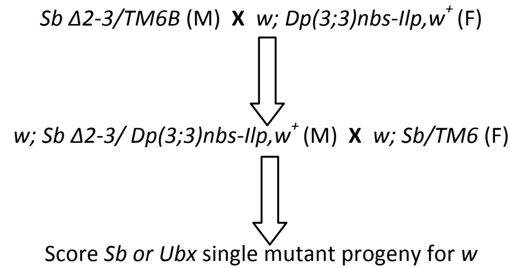
2.1. *I-SceI* on chromosome 2



2.2. *I-SceI* on chromosome 3 (with *ligase 4* mutation)



II. Reduction by P transposase



Legend:

M and F stand for male and female respectively. A heat shock treatment to the progeny was administered on day 3 after the cross had been set up. *Dp(3,3)nbs-Ilp,w^+* indicates the 82kb duplication (*Dp*) of the *nbs-Ilp* region marked with *white*$^+$. *Dp(3,3)nbs-$Ilp^{flipped}$,w^+* denotes the same duplication but with vector sequences excised by FLP recombinase. We did not verify the excision of the vector nor established an "excised" stock as FLP has been shown to function at virtually 100% efficiency under these conditions (GOLIC and LINDQUIST 1989, WEI and RONG 2007). p*P[ry^+, 70FLP]* is a *hsp70* promoter-driven *FLP* transgene on the X chromosome (GOLIC and LINDQUIST 1989). p*P[v^+, 70I-SceI]* is a *hsp70* promoter-driven *I-SceI* tansgene (RONG and GOLIC 2003). We used one insertion on chromosome 2 marked with *Sco* and one on chromosome 3 marked with *Sb*. The *$lig4^{11}$* mutation was previously described (WEI and RONG 2007). A P transposase source is denoted as *Δ2-3*.

References

GAO, G., C. MCMAHON, J. CHEN and Y. S. RONG, 2008 A powerful method combining homologous recombination and site-specific recombination for targeted mutagenesis in Drosophila. Proc Natl Acad Sci U S A **105:** 13999-14004.

GOLIC, K. G., and S. LINDQUIST, 1989 The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell **59:** 499-509.

RONG, Y. S., and K. G. GOLIC, 2003 The homologous chromosome is an effective template for the repair of mitotic DNA double-strand breaks in Drosophila. Genetics **165:** 1831-1842.

WARMING, S., N. COSTANTINO, D. L. COURT, N. A. JENKINS and N. G. COPELAND, 2005 Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res **33:** e36.

WEI, D. S., and Y. S. RONG, 2007 A genetic screen for DNA double-strand break repair mutations in Drosophila. Genetics **177:** 63-77.

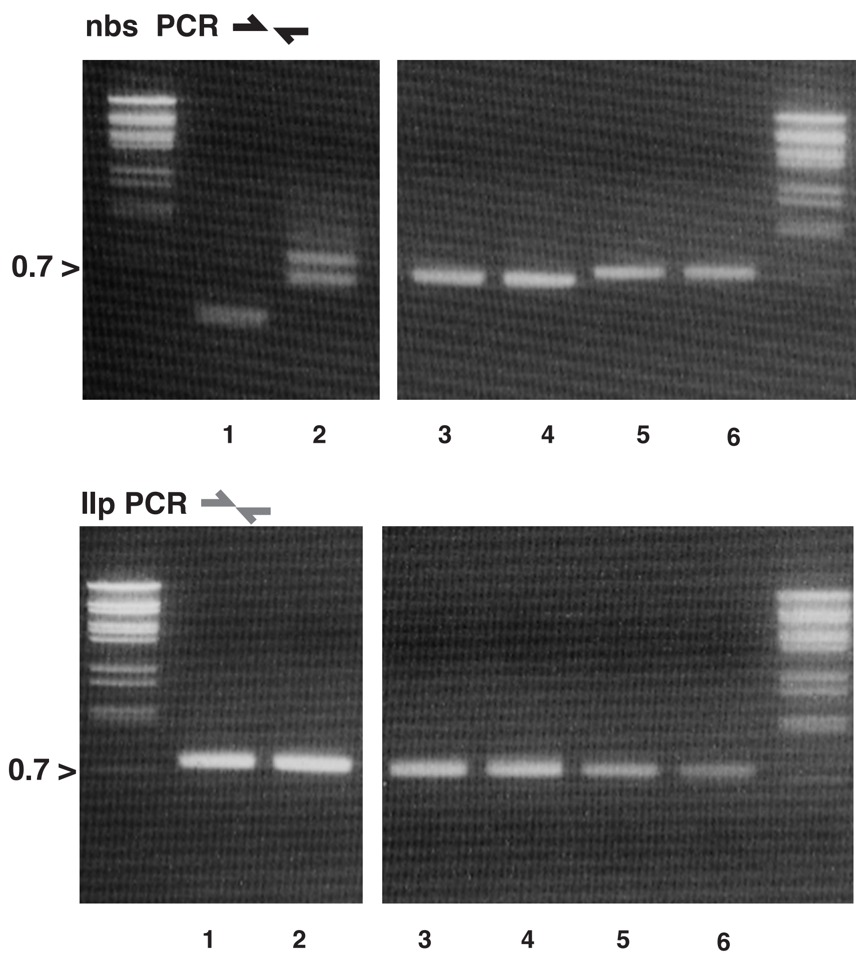
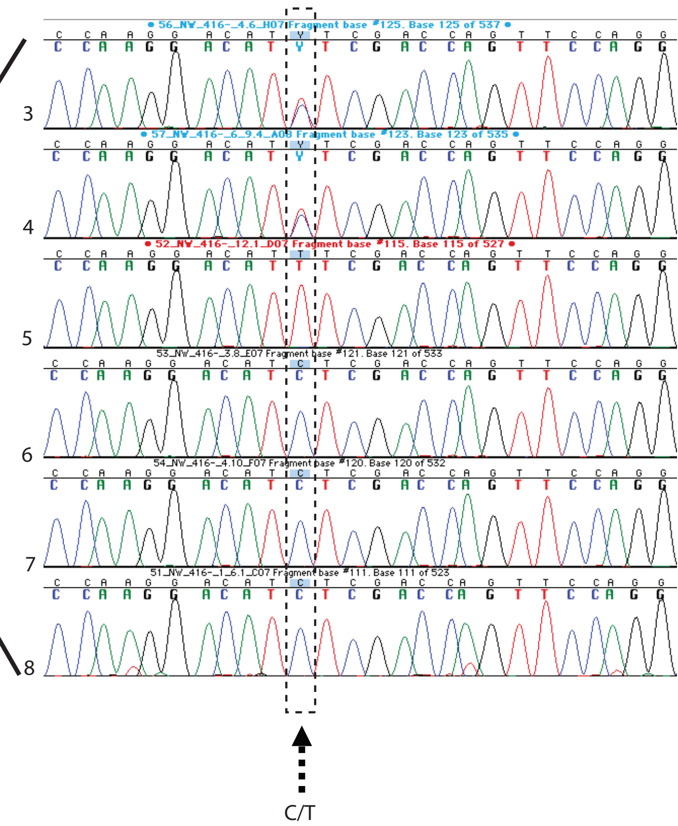
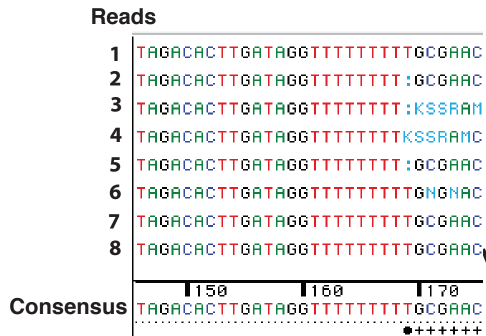


Figure S1 Integration lines with possible rearrangement of the *nbs-Ilp* region. Representative results for diagnostic PCR tests of integration lines showing abnormal patterns. Marker size is indicated to the left of the gel pictures in kb. At the top is the PCR test for the *nbs* region (for primer locations see Figure 1). At the bottom is the PCR test for the *Ilp* region. Lane 1: *wt*; lane 2: a duplication line from a previous integration at *attP@nbs*; lanes 3-6: additional integration lines from pWalkman{nbs-Ilp}IS1 integration. Note that the absence of the *attB@Ilp* band in the lanes 3-6 suggests that the *attB* might have participated in an additional round of phiC31-mediated recombination.

SNP at p35
PCR with primer pair bac416
sequenced with primer bac416r



indel at p44
PCR with primer pair bac502
sequenced with primer bac502f



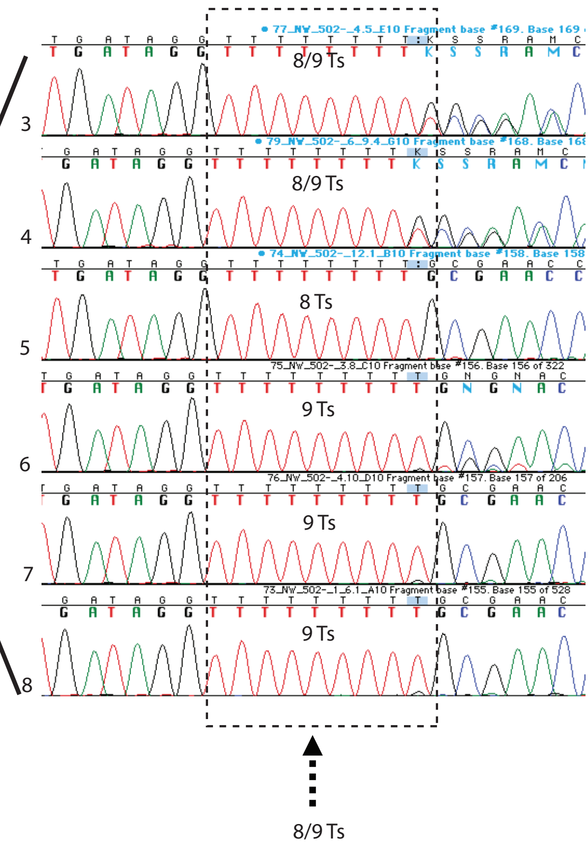


Figure S2 Genotyping SNPs in reduction events. Genotyping of the C/T SNP at position p35 is shown at the top. Sequences around the SNP region are shown for eight samples. Sample 1 was PCR amplified from pWalkman{nbs-Ilp} plasmid DNA showing a "C" at p35. Sample 2 was PCR amplified from genomic DNA of the starting line with *attP@nbs* showing a "T" at position p35. Samples 3 and 4 were amplified from genomic DNA of two NHEJ type lines (Figure 2). The sequencing data shown at the right displays a C/T double peak suggesting that both *nbs-Ilp* copies are present, which is consistent with NHEJ type events not being reduced. Samples 5-8 were derived from four lines that have been classified as "reduced" based on the PCR tests shown in Figures 1 and 2. Either a "C" or a "T" single peak was displayed in the sequencing data, consistent with the presence of a single *nbs-Ilp* copy. Genotyping of the T indel SNP at position p44 is shown at the bottom. The samples are the same as the ones used for genotyping p35. At this position, the *nbs-Ilp* region derived from the BAC clone (sample 1) has a string of nine "Ts", while the chromosomal copy has eight (sample 2). For samples 3 and 4 (NHEJ type), the deletion of a single "T" resulted in the appearance of a double peak for all bases downstream of the indel, due the presence of both *nbs-Ilp* copies. For reduced samples (samples 5-8), the indel is homozygous, again consistent with the presence of only one *nbs-Ilp* copy.

Table S1 Primers used for plasmid construction, gap repair, verification PCRs and PCR assays

A. Primers used for plasmid construction

primer name	**sequence**
ScBaNhSpMlBsSb+	tagggataacagggtaatggatccgctagcatgcacgcgtacgcctgcagg
ScBaNhSpM1BsSb-	cctgcaggcgtacgcgtgcatgctagcggatccattaccctgttatcccta
NheFRT48-plus	ctaggaagttcctatactttctagagaataggaacttcggaataggaacttc
NheFRT48-minus	ctaggaagttcctattccgaagttcctattctctagaaagtataggaacttc
SphFRT48-plus	gaagttcctatactttctagagaataggaacttcggaataggaacttccatg
SphFRT48-minus	gaagttcctattccgaagttcctattctctagaaagtataggaacttccatg

B. Primers used for gap repair

primer name	**sequence**
bacL-f	GCGGAGTTTCGATAAACAAAG
bacL_**NotI**-r	ctcaa**gcggccgc**CTACGATAACAAACACCTGCCC
bacL_**AscI**-f	gtcaa**ggcgcgcc**GCGGAGTTTCGATAAACAAAG
bacR-r	CTCGGCTATCTCGACTATCTCG
bacR_**NotI**-f	gacaa**gcggccgc**CTCGAGGTCGTTTGTGGTTTGTG
bacR_**EcoRI**-r	cagtt**gaattc**CTCGGCTATCTCGACTATCTCG

C. Primers used for verification of nbs-llp region integrity during cloning

primer name	**sequence**
bac074-f	gtatcgggatgcatcgaagt
bac074-r	agagaaaatggccggagaat
bac064-f	gctccaatcgtcctcatcat
bac064-r	caggtgtgttgggggaatac
bac165-f	AGCCGGTTTGTTTTTGTGTC
bac165-r	CTCGACGGTATCGTGGCTAT
bac267-f	TCTACGTCATGGAGCACGAG
bac267-r	ATTCATGGTTCCAGCGTCTC
bac320-f	TCTTGAACTGCAGCACATCC
bac320-r	CAACAACATGGGGCACTAGA
bac416-f	CTCCCGACTCAACACCGTAT
bac416-r	CCAGCTATGCCAAGTTCACA
bac502-f	AGCAATACCAGGGGACAGTG
bac502-r	AACGAAACGTAACCGACAGG
bac600-f	TCGTCGATCTCTGGGTCTTT
bac600-r	AGGCGTGAATAATGCGATTC
bac729-f	ACGAGAAGCACGTCCAGTTT
bac729-r	TCTGTTCATTGGGGCTAAGG
bac835-f	AGGATGGAGGCTTGAGGATT

bac835-r	TTATCCCGGAATGCTTTGAG
D. Primers used for verification of gap repair junctions	
primer name	**sequence**
bacLj-f	GATTGGGTATCATCGTTGGG
acman_7520d	GGATCAAGAGCTACCAACTG??
acman_8363u	CGCTGACTTTGAGTGGAATG
bacRend-f	CTCCTCGTTCTCCCTGCACATG
acman7929-f	gtgtaaaacgacggccagtg
acman8261-r	gttcaatgatatccagtgcag
E. Primers used for positioning attachment sites (attB) at a) nbs locus and b) Ilp locus	
a) attB at nbs	
primer name	**sequence**
nbsGalk-f	ggctgatggtatggtaacctgtgtttaatgtcgtgcctaaacgtaattaaCCTGTTGACAATTAATCATCGGCA
nbsGalk-r	tatttcgcaagtttattgttagcaaaataaagtaactttacaagcgacggTCAGCACTGTCCTGCTCCTT
nbs2776-f	ccaggaaactgaatcctcct
nbs3169-r	cggccgcaagaactttaaga
b) attB at Ilp	
bac810Galk_f	cacaatgaaatgatactaacgatacttaaaaacgtgttcaaactcttgtcaatattgtaaaaaacctgttgacaattaatcatcggca
bac810Galk_r	caaatggtcatctgaccaaattcctaaatgaaagagaatgttgtttgcctttttgaatatcagcactgtcctgctcctt
bac810_att-end	cacaatgaaatgatactaacgatacttaaaaacgtgttcaaactcttgtcaatattgtaaaaaaatcgataccgtcgacatgcc
bac810_att-head	caaatggtcatctgaccaaattcctaaatgaaagagaatgttgtttgcctttttgaatattcgacgatgtaggtcacggt
F. Primers used for a) removing I-SceI at IS1 and b) placing a new one at IS2	
a) removing I-SceI at IS1	
primer name	**sequence**
up7993Galk-f	ccagtgagcgcgcgtaatacgactcactatagggcgaattggagctcgttt **cctgttgacaattaatcatcggca**
dn7993Galk-r	gaccgctcgaggtttcctgcaggcgtacgcgtgcatgctagcggatcc **tcagcactgtcctgctcctt**
acman7929-f	gtgtaaaacgacggccagtg
acman8029-r	gccggcgcgccatcgataac
a) placing I-SceI at IS2	
primer name	**sequence**
up8150Galk-f	GCGATAGAATGAGTGCGAGGCCGAGATAGTCGAGATAGCCGAGATACAATAC **cctgttgacaattaatcatcggca**
dn8150Galk-r	ctactttccgcaaaaatgggttttattaacttacatacatactagaattc **tcagcactgtcctgctcctt**
up8150IS2-f	GCGATAGAATGAGTGCGAGGCCGAGATAGTCGAGATAGCCGAGATACAATAC**ATTACCCTGTTATCCCTA**

dn8150IS2-r	ctactttccgcaaaaatgggttttattaacttacatacatactagaattc **TAGGGATAACAGGGTAAT**
up8150IS2plus-f	*CTAGATATCGCCCGATTCAGATGCGGATATGGGAATGTG***GCGATAGAATGAGTGCGAGG**
dn8150IS2plus-r	ctactttccgcaaaaatggg

G. Primers used for attachment site PCR assay

primer name	sequence
nbs2858	caatcgcaagttgtccaagg
nbs3169	cggccgcaagaactttaaga
bac810L-f	cgataatcgaatgcactgaag
bac810R-r	ccagtcactgcaatcgtag