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.