Generation of a useful roX1 allele by Targeted Gene Conversion


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Figure S1  Overview of roX1SMC17A creation. A) roX1mb710 is created by insertion of plArB, containing the roX1 promoter (white arrow) fused to LacZ, was moved into roX1 by targeted transposition. The resulting tandem insertion (roX1w+tandem) was the starting point for Hybrid Element Insertion mutagenesis that removed plArB and deleted 891 bp flanking the insertion site, producing roX1Δ891 (C). Mobilization of p[wmc roX1P-βgal] produced roX1SMC17A (D), and numerous identical rearrangements. The roX1SMC17A chromosome carries the fusion of LacZ with the roX1 promoter that is present in p[wmc roX1P-βgal]. All roX1 sequences between the promoter and the 5' P-end have been replaced with a full length LacZ gene. The 5' P-end has been replaced precisely with the 3' end. A complete list of the rearrangement classes produced by roX1Δ891 mobilization, and a model for the homology-dependent gene conversion event that likely produced roX1SMC17A, is presented in Supplementary Figure 2.
Figure S2  Rearrangements produced by roX1^{Δ891} mobilization.  

A) Four classes of rearrangements were present in white eyed offspring of dysgenic roX1^{Δ891} flies (top). The roX1 promoter is depicted by a white arrow. Imprecise excisions that remove all (class 1) or the 3’ end (class 2) of p[w^{+mc}roX1P-βgal] occurred in 4 flies. Rearrangements identical to roX1^{SMC17A} (class 3) were recovered 38 times. Rearrangements similar to roX1^{SMC17A}, but with the 3’ P-end missing, or inserted at a different location, account for 14 flies (class 4). A hypothetical mechanism for generating class 4 is presented in Supplemental Figure 3. 

B) Excision followed by resection reveals homology between the roX1 promoters on the chromosome and in p[w^{+mc}roX1P-βgal] (red arrow). Homology is also present at the 3’ P-end on the sister chromatid and at the site where the 5’ P-end excised (blue arrow). We postulate that these homologies support gap repair using a sister chromatid template. This will insert the full length LacZ gene into roX1 and substitute the 3’ P-end for the original 5’ end, the precise rearrangement found in roX1^{SMC17A} (bottom). Drawings not to scale.
Figure S3  Proposed mechanism capable of producing class 4 rearrangements. A) Hybrid Element Insertion (HEI) creates an inverted duplication of p[\text{w}^{mC}\text{roX1P-}$$\beta$$\text{gal}] on one chromatid, depicted in B. Red and blue arrows show the insertion sites of the 3' and 5' P-ends participating in HEI. Proximal (1,2) and distal (3,4) chromatid ends are labeled. HEI places a 3' P-end downstream from the 5' end in \text{roX1}^{\Delta891}. A green genomic fragment from \text{roX1}^{\Delta891} now appears in inverted orientation between the p[\text{w}^{mC}\text{roX1P-}$$\beta$$\text{gal}] elements in B. B) Chromatid arm 1 is resected to reveal homology to the \text{roX1} promoter. Broken arm 1 initiates recombinational repair with the \text{roX1} promoter in p[\text{w}^{mC}\text{roX1P-}$$\beta$$\text{gal}] (red arrow). C) Resolution produces a chromosome carrying the \text{roX1} promoter fused to LacZ. The 5' P-end has been replaced by a 3' P-end that is downstream from the insertion sites in \text{roX1}^{\Delta891} and \text{roX1}^{\text{SMC17A}}. This model is consistent with the structure of \text{roX1}^{\text{SMC20A,B}}, identical to \text{roX1}^{\text{SMC17A}} but with the 3' P-end moved 350 bp, creating a deletion of 1.25 kb. Twelve additional flies in this class also had the \text{roX1} promoter fused to LacZ, but no P-end could be detected using primers in \text{roX1}. We postulate that these rearrangements were similarly produced, but that the HEI insertion occurred distal to \text{roX1}.
Figure S4  Products of gap repair generated by mobilization of tandem insertion roX1[MS2-6]T4B. A) roX1[MS2-6]T4B. The roX1[MS2-6] insert (heavy gray line) is shown collinear to and below the corresponding genomic sequence. The MS2 loops are 430 bp from the plArB insertion site in roX1. Predicted products of homology-dependent gap repair presented in (B-D). Left panels depict short repair tracts (gray) that do not incorporate MS2 loops, right panels are longer tracts incorporating MS2 loops into the chromosome. B) Precise replacement by roX1[MS2-6] sequences. C) Repair is supported by homology in roX1 and at the 3’ P-end, leading to retention of a P-end and duplication of the 5’ roX1. D) Retention of plArB. E) Imprecise excision mutates mini-white, but leaves both P-elements in place.
Figure S5  Predicted products of gap repair upon mobilization of tandem insertion roX1[MS2-6]R36A. A) roX1[MS2-6]R36A has replaced pArB with p[w^mc GM roX1^MS2-6]. roX1^MS2-6 (heavy gray line) is shown collinear to and below the corresponding genomic sequence. The MS2 loops are 430 bp from the pArB insertion site in the roX1. Predicted products of homology dependent gap repair are depicted in B (precise repair of roX1) and C (retention of the 3’ P-end and duplication of 5’ roX1 sequences). Left panels depict short repair tracts that do not incorporate MS2 loops, right panels describe longer tracts incorporating MS2 loops into the chromosome.
Figure S6  Directing transposase-mediated gene conversion to a region lacking a P-element.  A) A double stranded break is introduced in a gene of interest by an engineered nuclease.  An oligonucleotide containing a landing site, such as attP, and homologous arms is introduced as a repair template.  B) A longer construct with engineered changes to the target gene (thick line), a visible marker (w+), and P-ends (black and gray arrowheads) is integrated into the landing site (C).  D) Mobilization with transposase creates a double stranded break.  Homology is revealed by resection of broken ends.  Gap repair using a sister chromatid template produces engineered chromosomes lacking the w+ marker.
File S1
MCP-GFP recruitment to a single domain within the male nucleus

File S1 A and B are available for download as .avi files at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.113.008508/-/DC1
Table S1  Primer sequences used for characterization of roX1 rearrangements (5’-3’)

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<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>Pry2</td>
<td>CTTGCCGACGGGACCACCTTATGTATT</td>
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<td>Pry4(+)</td>
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<td>pLac1(+)</td>
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