Supporting Information

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SI Text

SI Results

P Transposase-Induced Target Reduction. We attempted to improve reduction frequency without having to implement an extra step of FLP recombination. Preston *et al.* (1) showed that DSBs generated during P element transposition effectively induced recombination between two direct repeats. Both the 3′ and 5′ ends of a P element were present in our integration lines, although in an "inside-out" configuration different from the normal one (Fig. 1*B*). For M3 reduction, induction of P transposase in the soma did lead to higher degrees of eye mosaicism. We also recovered white-eyed progeny more frequently. However, subsequent molecular analyses indicated that most of the white-eyed events (16/23) still retained both *nbs* copies, and therefore did not represent recombinational events between the *nbs* copies (data not shown).

A Two-Step Recombineering Scheme for Landing Site Placement in Plasmids. The relatively large *att* sites (≈250 bp) that we and others used have to be strategically placed, such as in introns or intergenic regions, without interfering with target gene expression. This creates a challenge for traditional bacterial cloning using restriction enzymes and DNA ligase. We devised a two-step scheme using the "recombineering" method in bacteria (2) to efficiently modify various *nbs* fragments cloned into pTV2 (Fig. S1). We used long primer pairs to generate PCR products of a chloramphenicol-resistant gene (Cm), flanked by 50-bp *nbs* homologous arms. PCR products were transformed into recombineering competent cells that had been previously transformed with the *nbs* constructs. Recombination products between the linear PCR products and the circular vector, which carries an ampicilin resistant gene (Ap), were selected as Ap and Cm double resistant colonies. Using the condition outlined in *Materials and Methods*, we routinely recovered up to 50 colonies per transformation. We analyzed multiple colonies (usually 10) for every transformation. All checked colonies were the result of precise homologous recombination in bacteria. The Cm gene was excised by an 8-bp restriction enzyme, which cut site was embedded in the long PCR primers. The linearized plasmid was used either for ligation using the overhangs created by the eight cutter, or in a second round of recombineering with PCR-amplified *att* sequences flanked by the same *nbs* homology arms. Gap repair by recombination circularized the plasmid DNA, allowing efficient bacterial transformation.

SI Materials and Methods

Recombineering for *att* Placement. Recombineering was performed according to online protocols published at http://recombineering.ncifcrf.gov/. The bacterial strain SW102, kindly provided by Dr. Don Court (National Cancer Institute), was used for the entire study. A position between *nbs* and *CG14174* was selected for *att* placement. A Cm marker was first inserted at this position by recombineering. The Cm marker was amplified using primers CmFsenbs2998 and CmAscnbs2898 from a template in the plasmid pACYC184 (kindly provided by D. Chattoraj, National Cancer Institute). CmFsenbs2998 consists of a 50-bp *nbs*-homologous sequence to one side of the desired *att* insertion site, followed by the cut site for the 8-bp cutter FseI, then followed by 20 bp of sequence homologous to Cm. CmAscnbs2898 consists of a 50-bp *nbs* sequence homologous to the other side of the *att* insertion site followed by a cut site for the 8-bp cutter AscI, then followed by Cm homologous sequence. To eliminate the possibility of the Cm-resistant clones from the small amount of pACYC184 DNA as PCR templates, we diluted the PCR products at least 1,000-fold and used them for a second round of PCR amplification. Close to 1 μg of this second round of PCR products was cotransformed into SW102 cells with a few hundred nanograms of plasmid DNA from various *nbs* constructs in pTV2. We have also carried out recombineering by transforming smaller amounts of PCR products into SW102 cells that already had the *nbs* constructs. Cm and Ap double resistant clones were selected, plasmid DNA isolated, and retransformed into regular cloning strains to eliminate hitchhiking clones that were Cm sensitive. Once single correct clones were identified, plasmid DNA was cut with the 8-bp cutters and ligated with similarly digested PCR products of *attP* and *attB*. The primers attPend-AscI and attPhead-FseI were used to amplify *attP* from the plasmid pBCPB+ (kindly provided by Dr. Calos, Stanford University, Stanford, CA). The primers attBend2-AscI and attBhead-FseI were used for *attB* amplification from the same plasmid. These four primers were also used to verify the presence of *attR* and *attL* sites on integrants from microinjection experiments. A 40bp *attB* site was placed similarly in M40 using the annealed product of oligos FseattB40Ascplus and FseattB40Ascminus. An *attB* site was similarly placed into M7 using the primers nbs5499AscCm and nbs5600FseCm.

Additional Reagents for SIRT. Because the pTV2 vector was used for both the initial *attP* targeting step and all of the subsequent SIRT manipulations, we converted it into a Gateway vector using the conversion kit from Invitrogen to facilitate the cloning of large genomic fragments. The Gateway cassette was cloned into a blunted Acc65I in pTV2, creating vectors pTV2gw#4 and pTV2gw#10, in which the cassettes are in different orientations. The genomic clones can be PCR amplified and cloned by using the pCR8/GW/TOPO TA cloning kit from Invitrogen, and subsequently subcloned into pTV2gw vectors. We also created cassettes: FseI-Cm-FseI-attP and FseI-Cm-FseI-attB. In these cassettes, the Cm marker is flanked by two FseI sites, and at one side of Cm we placed either an *attP* or an *attB* site. These cassettes allow researchers to PCR amplify both Cm and *att* sites for recombineering. Once the correct clones are identified, the Cm marker can be excised by an FseI digestion followed by plasmid self-ligation.

1. Preston CR, Engels W, Flores C (2002) Efficient repair of DNA breaks in *Drosophila*: Evidence for single-strand annealing and competition with other repair pathways. *Genetics* 161:711–20.
2. Sawitzke JA, *et al* (2007). Recombineering: in vivo genetic engineering in E. coli, S. enterica, and beyond. *Methods Enzymol* 421:171–199.

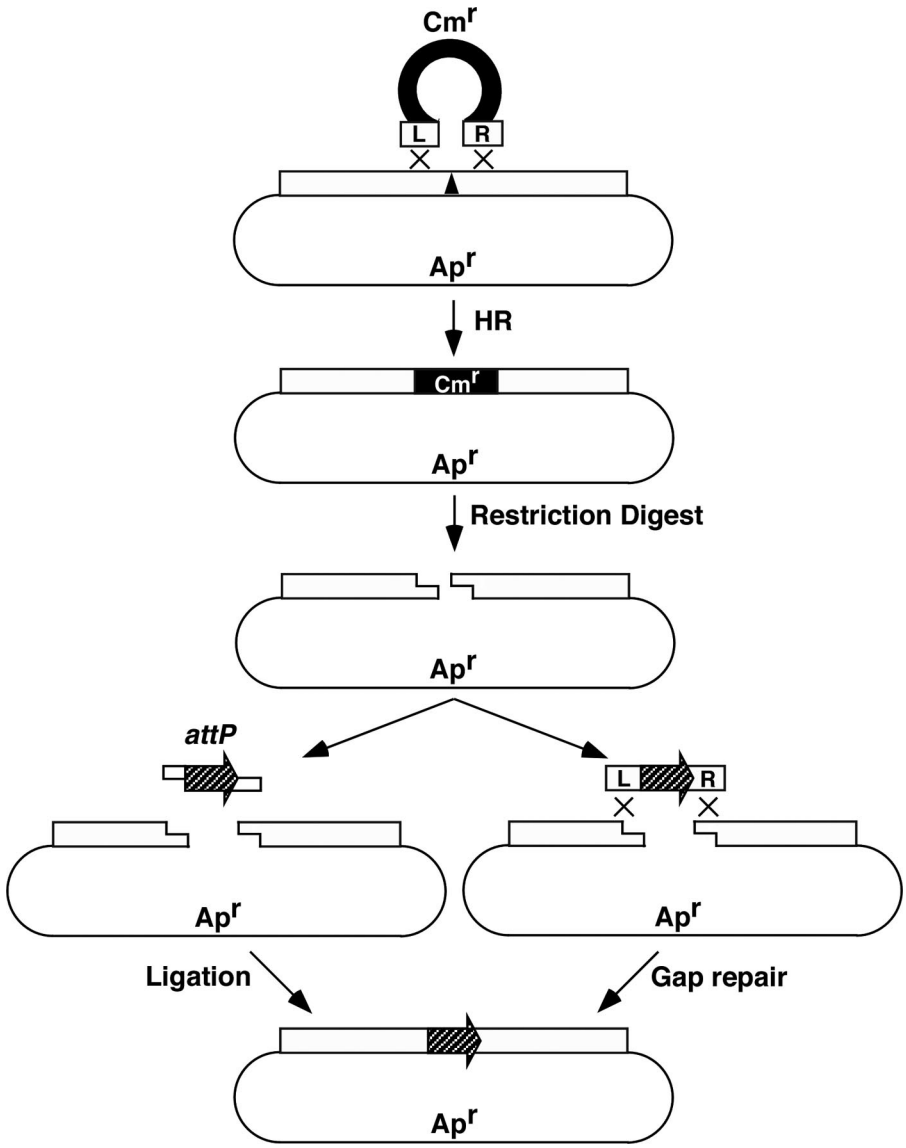

Fig. S1. A two-step recombineering scheme. (Top) The Cm^r gene is PCR amplified with primers carrying left and right arms of homology (L and R) to the insertion position (arrow head) on the plasmid vector, which carries Ap^r. Recombineering produces a plasmid carrying both Cm^r and Ap^r. The plasmid is digested with a rare-cutting enzyme, which recognition site was included in the PCR primers for Cm^r amplification. This cut plasmid can be either ligated to *att* fragments carrying similar overhangs (left), or used in a second round of recombineering with *att* fragments carrying the initial homology arms (right). The desired plasmid carries only Ap^r.

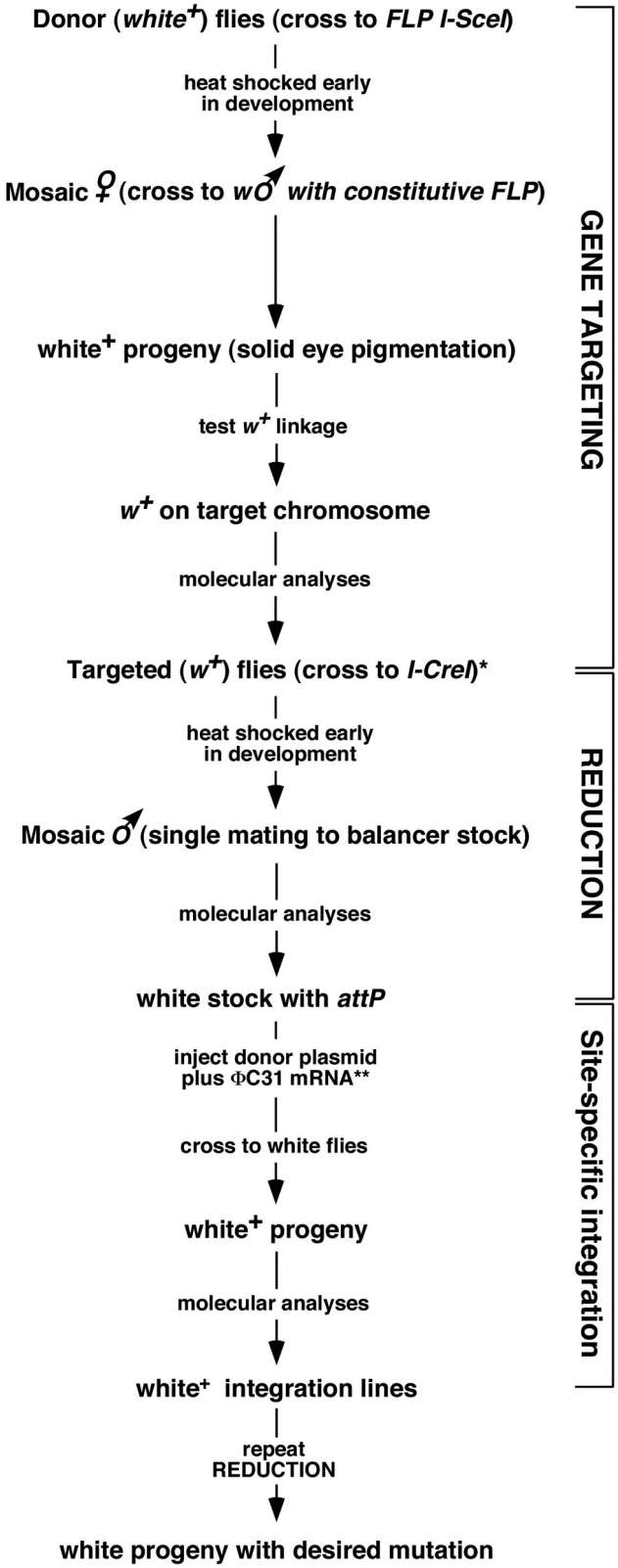

Fig. S2. A flow chart for carrying out SIRT experiments. (*) An optional step can be added to excise the vector backbone by FLP recombination for reduction after ϕC31-mediated plasmid integration. (**) Instead of using mRNA, one can construct a line with the targeted *attP* and a transgene expressing phiC31 endogenously.

Table S1. Primers

Name	Sequence (5′ to 3′)	Note
nbs2057d	TGG TAC CGA TTG GAC GAC TAA TGG CTC G	KpnI site underlined
nbs6819u	AGG TAC CGT CCT GCA TGA AAT CCA GCA G	
I-site-H3plus	AGC TGT AGG GAT AAC AGG GTA AT	I-SceI cut site underlined
I-Site-H3 minus	AGC TAT TAC CCT GTT ATC CCT AC	
nbs-V5-plus	TTA AAA GTC TTC GGC TGG CTG ATG GTA TGG TAA CCT GTG TTG GTA AGC CTA TCC CTA ACC CTC TCC TCG GTC TCG ATT CTA CGT AA	V5 tag underlined
nbs-V5-minus	TTA ATT ACG TAG AAT CGA GAC CGA GGA GAG GGT TAG GGA TAG GCT TAC CAA CAC AGG TTA CCA TAC CAT CAG CCA GCC GAA GAC TT	
nbs5447d	CTG CGC AAC AAT TAA ATC CGT	
nbs5398d	CAG CTG CTC ATC CAA ACG GA	
nbs4726d	GAT TCA GTG GCT TGG GCC TGA GCC GAT GCC ACA TAC TGT	
nbs4725u	AAT CAA TAG TAT ACA AGG TAG GTG	
nbs5148d	TAT GGG TTC AAG CAT CTT CGT G	
nbs5147u	CTA CTG CAC GCT ATG CTC GAG A	
nbs4630d	ATT CTG TTC CAG AAT ATC TAA	
nbs4629u	CTA ATA CAT TGC TCT ATA ACA G	
CmFsenbs2998	**GGC TGA TGG TAT GGT AAC CTG TGT TTA ATG TCG TGC CTA AAC GTA ATT AA**G GCC GGC *CAG CCA GTA TAC ACT CCG CTA*	*nbs* sequences in bold, FseI and AscI sites underlined, *Cm* sequence italicized
CmAscnbs2898	**TAT TTC GCA AGT TTA TTG TTA GCA AAA TAA AGT AAC TTT ACA AGC GAC GG**G GCG CGC *CCT GTG GAA CAC CTA CAT CTG*	
nbs5600FseCm	**TTG ACT AAA GGC ACG TAA AAA TGG TCT AAA ACA CAG AGA TTA TTA GTT TA**G GCC GGC *CAG CCA GTA TAC ACT CCG CTA*	
nbs5499AscCm	**CAG GAA ACA ACA CAA ACT TTT CGT CAT CTA TAA AGT ATT TAA AAA ATG AA**G GCG CGC *CCT GTG GAA CAC CTA CAT CTG*	
attPhead-FseI	AAT TAT GGC CGG CCG ACT AGT ACT GAC GGA CAC A	FseI site underlined
attBhead-FseI	ATT ATT GGC CGG CCT CGA CGA TGT AGG TCA CGG T	
attPend-AscI	TAT ATT GGC GCG CCT CTA GAA CTA GTC GCG CTC	AscI site underlined
attBend2-AscI	ATA ATA GGC GCG CCA TCG ATA CCG TCG ACA TGC C	
FseattB40Ascplus	CGG GTG CCA GGG CGT GCC CTT GGG CTC CCC GGG CGC GTA C	*attB* sequence underlined
FseattB40Ascminus	CGC GGT ACG CGC CCG GGG AGC CCA AGG GCA CGC CCT GGC ACC CGC CGG	
nbs2970d	AGG CAC GAC ATT AAA CAC	
nbs3942u	CCA CGA CTC AAT GGG AAA TC	
nbs4496d	GCC ATC GAG GAA GTT ACA GA	
nbs5467u	CAC GGA TTT AAT TGT TGC G	
nbs5275d	AGG TGA GAC ATG GTT ACA GG	
nbs6968u	AGG AAG AGG AAC TGC TCC TT	
FR6	GAT TGA TTC AGT GGC CTG CGA T	
FR7	GAT TGA TTC AGT GGC TTG GGC C	
nbs5663u	TCT GCT AAG GTC ACA CTG CT	
nbs4933d	GGT GAC ATC TAT GTT GGT GG	
nbs4535d	TCG GAG ATG AAC TTG TGC GTT GG	
nbs4766u	CAG TAC ACC ATA TGC AGC TGC TCA TCC AAA CGG	
nbs1991u	TGT TCG TGG TCA TCA ACA GG	
nbs2776d	CCA GGA AAC TGA ATC CTC CT	
nbs3169u	CGG CCG CAA GAA CTT TAA GA	