***CrkdsRed*4th chromosome balancer**

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**Description:**

*CrkdsRed* (also known as *Crk[dsRed]*) is recessive lethal mutation in the *Crk* gene (CG1587) that is marked by the 3XP3-dsRed marker in the Scarless targeting CRISPR system (<https://flycrispr.org/scarless-gene-editing> , Bier et al., 2018). Adult *CrkdsRed/*+ flies are easily scored for dsRed eyes using appropriate illumination and filters, even without magnification by a dissecting microscope.

The *CrkdsRed* chromosome serves as a 4th chromosome balancer because there is essentially no recombination on the fourth chromosome, the *CrkdsRed* is a dominant scorable marker and the mutation is recessive lethal (1,435/1,435 adults in a stock of *CrkdsRed*/*Gateya* flies were dsRed, small-eyed heterozygotes).

The *CrkdsRed* mutation was created by targeting the *Crk* locus with CRISPR and an HDR template constructed by Gibson assembly of the Scarless 3XP3 dsRed marker (Addgene #64703, (Bier et al., 2018)) flanked by *Crk* 5’ and 3’ homology arms (see below) in the pBS*-GMR-eya*(shRNA) backbone (Addgene # , (Nyberg et al., 2020), https://doi.org/10.1101/2020.05.07.080762). Homologous repair from the HDR template should result in the replacement of almost the entire ORF region of the *Crk* gene with the Scarless dsRed construct, from 48 bp 5’ of the ATG to 121 bp 5’ of the TAA stop codon. The *GMR-eya(shRNA)* served as a counter selection marker for screening for potential repair events that could have resulted in integration of the vector instead of homologous repair from the HDR template. Two dsRed, WT-eye positive lines were identified in progeny of embryos (BDSC stock #55821 y[1] M{GFP[E.3xP3]=vas-Cas9.RFP}ZH-2A w[1118] ) injected with the *Crk*/Scarless dsRed HDR template. One dsRed line was established from a single male to found the *CrkdsRed* chromosome. The precise nature of the repair event that created the *CrkdsRed* mutation has not been investigated. The *vas-Cas9* and potential off-target mutations were removed from the background by backcrossing the *CrkdsRed* allele against *w1118* for five generations.

**Methods for construction of *Crk* HDR Repair template and sgRNA plasmids**

*Crk* HDR template

Assembled order of the circular *Crk*/Scarless dsRed HDR template construct:

*pBS eya(shRNA)* backbone…*Crk*5’ homology…Scarless dsRed marker…*Crkt* 3’ homology…*pBS* *eya(shRNA)* backbone

The sequences of the primers used to create that *Gat* targeting construct were (*Gat* sequences underlined):

Crk 5’ homology arm forward: 5’ – CCGGGCTGCAGGAATTCGATATTTTTGATCCTAGCTTCAAAATCT – 3’

Crk 5’ homology arm reverse: 5’ – CTTTAACGTACGTCACAATATGATTATCTTTCTAGGGATAAATAGAAATTATGTGATATAATGCAAATATA – 3’

Crk 3’ homology arm forward:
5’ – GAGCAATATTTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGAATTGGAAATAGGTGACATTATTAAAGTCA – 3’

Crk 3’ homology arm reverse: 5’ – CGACGGTATCGATAAGCTTGATAGAAGCACTAACTAACTATTGATCTAAAGAT– 3’

Primers for amplifying the Scarless dsRed marker:

Scarless dsRed forward: 5’ – ATATTGTGACGTACGTTAAAGAT – 3’

Scarless dsRed reverse: 5’ – GCATTCTTGAAATATTGCTCTCT – 3’

*Crk* sgRNA plasmids

The following oligonucleotides were annealed and ligated into pU6-BbsI-chiRNA (Addgene #45946; RRID:Addgene\_45946) as describe in <https://flycrispr.org/scarless-gene-editing> to create two plasmids that produce sgRNAs targeting the 5’ and 3’ regions of the *Crk* gene:

*Crk* sgRNA1 forward: 5'- CTTCGAATTTCTATTTATTTAATC -3'

*Crk* sgRNA1 reverse: 5'- AAACGATTAAATAAATAGAAATTC -3'

*Crk* sgRNA2 forward 5'- CTTCGGATAAGACTGCATTAAAAT -3'

*Crk* sgRNA2 Reverse 5'- AAACATTTTAATGCAGTCTTATCC -3'

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**References:**

Bier, E., Harrison, M.M., O'Connor-Giles, K.M., Wildonger, J., 2018. Advances in Engineering the Fly Genome with the CRISPR-Cas System. Genetics 208, 1-18.

Nyberg, K., Nuyen, J., Kwon, Y., Blythe, S., Beitel, G.J., Carthew, R.W., 2020. Adaptable and Efficient Genome Editing by sgRNA-Cas9 Protein Co-injection into Drosophila. BioRxiv 2020.05.07.080762.