Outline of construction and features of pBS-*GMR-eya*(shRNA)

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The pBS-GMR-eya(shRNA) was constructed by Yong-Jae (James) Kwon and Greg J. Beitel (Department of Molecular Biosciences, Northwestern University, Evanston USA) as detailed in:

> Adaptable and Efficient Genome Editing by sgRNA-Cas9 Protein Co-injection into *Drosophila*
> Kevin G. Nyberg, Joe Nguyen, Yong-Jae Kwon, Shelby Blythe, Greg J. Beitel, Richard W. Carthew
> **doi:** https://doi.org/10.1101/2020.05.07.080762

Purpose:

The primary use of the vector is for Drosophila genome editing by homologous repair, such as CRISPR editing. The pBS-*GMR-eya*(shRNA) vector serves as backbone into which templates for homologous repair are inserted, typically by Gibson assembly into the EcoRV site. During editing, homologous repair events only copy the template and the backbone is not incorporated into the genome. However, in the event of an integration rather than a repair event, or an aberrant repair event, the backbone will be inserted into the genome which will cause an easily scorable dominant small-eye phenotype. Notably, enough eye tissue is present in *GMR-eya*(shRNA) heterozygotes that eye color and/or fluorescent markers can be scored.

Additional uses of the vector include using it as a dominant marker in making other genetic tools such as balancer chromosomes, and as a template to PCR amplify the *GMR-eya*(shRNA) cassette as a dominant marker for other constructs.

Use:

While any sites in the MCS can in priniciple be used for cloning or Gibson assembly, the EcoRV site has been tested to work well for Gibson assembly.

Optimally positioned sequencing primers for sequences inserted into the EcoRV site:

pBSII_seq_F2 5'-ggcggccgctctagaactag-3' for the SacI/NotI side
pBSII_seq_R2 5'-actgggctcgaggcgatc-3' for the GMR side

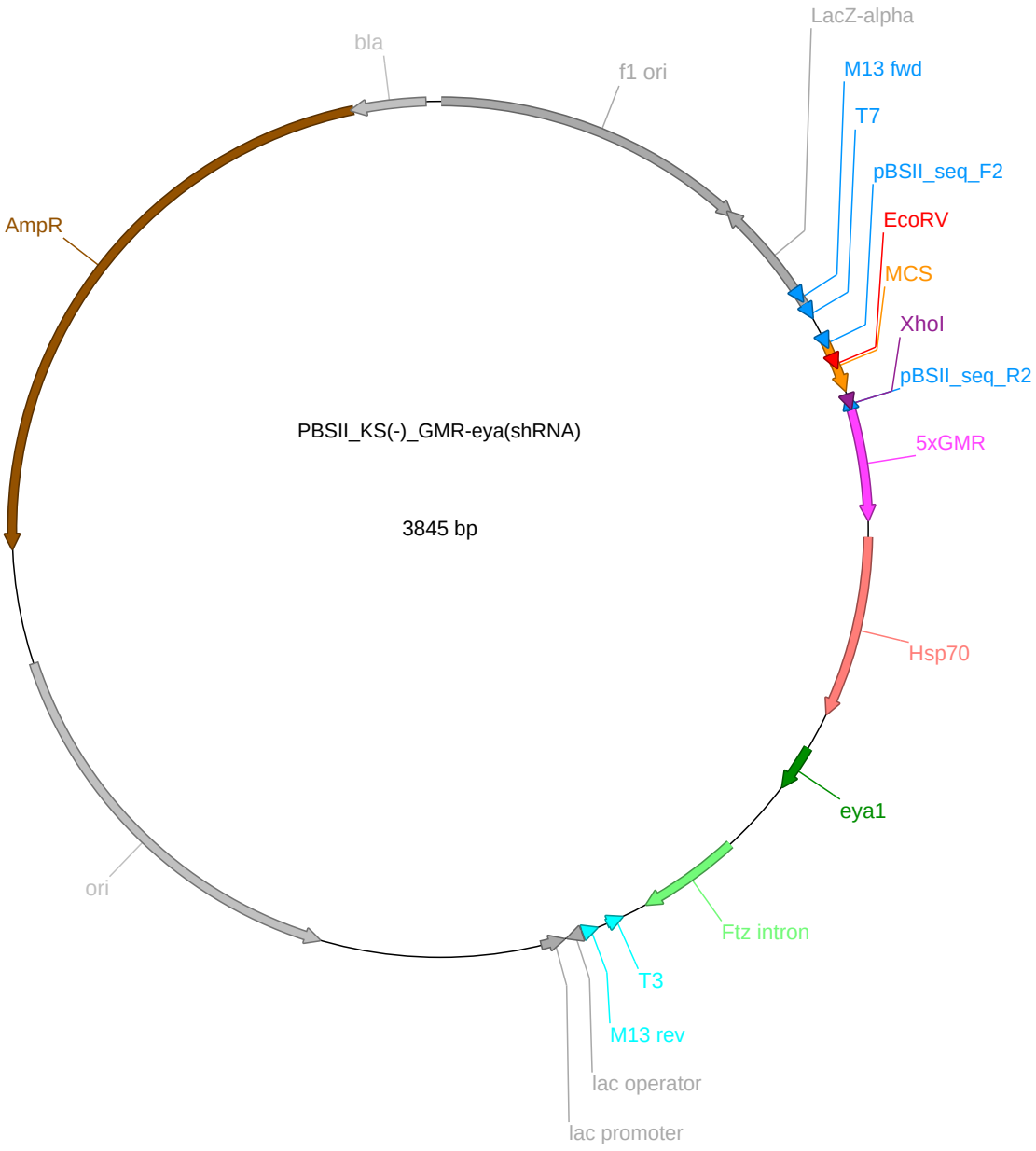
Construction:

The pBS-*GMR-eya*(shRNA) vector is a standard pBSII-KS(-) vector containing a dominant *Drosophila* eye marker comprised of the synthetic *glass* multiple reporter (GMR) enhancer driving the *eya*(shRNA) from the Transgenic RNAi Project (TRiP) expression construct HMS04515 that targets all three transcripts of the *eyes absent* gene. GMR is a synthetic eye-specific enhancer composed of five tandem repeats of a 29 bp element from the *Rh1* gene. Tests using a *GMR-Gal4* line to drive the UAS-*eya*(shRNA) constructs

in the TRiP lines HMS04515 and HM05716 revealed that HMS04515 produced a stronger eye phenotype than HM05716. Therefore, the *eya*(shRNA) sequence from HMS04515, tagttatattcaagcata, was used to construct the GMR-*eya*(shRNA) dominant marker cassette. A PCR fragment containing the GMR enhancer, the *hsp70* minimal promoter and a TATA box was amplified from pGMR to add flanking XhoI and XbaI sites. This fragment was inserted into a version of pKanC5 (Le T., et al., 2007 BioTechniques, **42**, 164-166. PMCID: PMC1955475) in which a linker containing XhoI and AvrII (XbaI compatible) sites had been inserted between the BbvI and AscI sites. This GMR cassette was then used to replace the UAS enhancer and upstream Gypsy insulator of the HMS04515 Valium20 *eya*(shRNA) construct by inserting the GMR cassette between the StuI and XbaI sites of HMS04515 using Gibson assembly. The ~440 bp 5' Gypsy element normally present in the Valium20 vector was eliminated to minimize the final size of the 820 bp GMR-*eya*(shRNA) marker. The efficacy of the resulting GMR-*eya*(shRNA) construct was tested by integrating this Valium20-based construct into the genome by ΦC31 mediated-recombination (Groth AC, et al., Genetics. 2004 166:1775-82). Once validated, the GMR-*eya*(shRNA) cassette was amplified by PCR and inserted in the KpnI site of pBSII-KS(-) using Gibson assembly.

Sequences inserted into the EcoRV site in the MCS can be efficiently sequenced using the custom primer pBSII_seq_R2 5'-actgggctcgaggcgatc-3' on GMR side, and the custom primer pBSII_seq_F2 5'-ggcggccgctctagaactag-3' or the standard M13-forward or T7 primers the SacI/NotI side.

Map


LacZ-alpha
bla
f1 ori
M13 fwd
T7
pBSII_seq_F2
EcoRV
MCS
XhoI
pBSII_seq_R2
AmpR
PBSII_KS(-)_GMR-eya(shRNA)
3845 bp
5xGMR
Hsp70
eya1
ori
Ftz intron
T3
M13 rev
lac operator
lac promoter