A. **pEGAD**

↓ *Age*I/*Bam*HI digestion to remove the *EGFP-(Ala)*$_{10}$ fragment

↓ Ligation with a polylinker

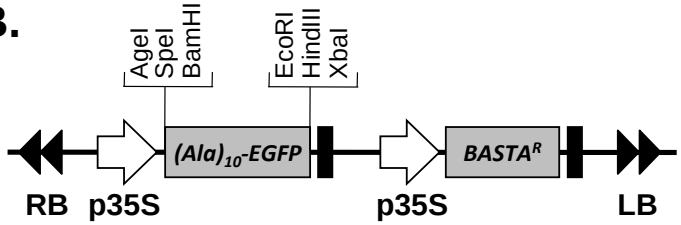
pBASTArd

↓ *Bam*HI/*Eco*RI digestion

↓ Ligation with the *(Ala)*$_{10}$*-EGFP* fragment from pEZT-NL

pB-CG

B.



S8 Fig. Preparation of pB-CG.

(a) A schematic showing the construction of pB-CG. The fragment encoding EGFP-(Ala)$_{10}$ in pEGAD plasmid [Cutler, S. R., Ehrhardt, D. W., Griffitts, J. S. and Somerville, C. R. (2000) Random GFP::cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. Proc. Natl. Acad. Sci. U S A. **97**, 3718-3723] was removed by digestion with *Age*I and *Bam*HI followed by ligation to a polylinker cassette made of two oligonucleotides with a *Bam*HI site (double underlined) (5'-CCGGTACTAGTGGATCCCCCGGGGAATTCAAGCTTA-3' and 5'-GATCTAAGCTTGAATTCCCCGGGGGATCCACTAGTA-3'), yielding pBASTArd. During this ligation, the *Bam*HI site (GGATCC) 3' to the EGFP-(Ala)$_{10}$ coding sequence in pEGAD was replaced by GGATCT. A sequence encoding (Ala)$_{10}$-EGFP was amplified by PCR using pEZT-NL (http://deepgreen.stanford.edu/) and the following set of primers (the forward primer including a *Bam*HI site as underlined: 5'-AAGGATCCGCTGCTGCCGCTGCCGCTGCGGCAGCGGCCGGACCGGTCGCCACCATG-3'; and the reverse primer including *Eco*RI site as underlined 5'-AAGAATTCTTACTTGTACAGCTCGTCCATGCC-3'). The resultant fragment was subcloned into pBASTArd using the *Bam*HI and *Eco*RI sites, yielding pB-CG.

(b) The T-DNA region of pB-CG. Right and left borders (RB and LB), CaMV35S promoters (p35S), the basta herbicide resistance locus (BASTA^R), and the terminator (black box), all of which originated from pEGAD, are indicated.