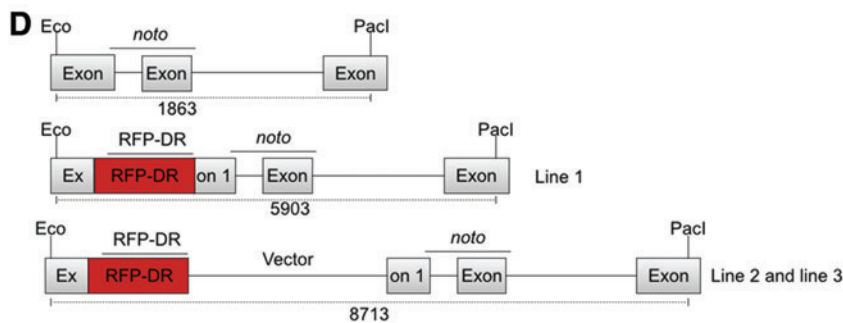


**C** *noto*:RFP-DR48 5' junction

*noto* <--- 24 bp homology ---> Vector  
 Precise junction - TACCGGAGCATAACCAACCAAACGTCTGTCCTTCCC  
 Line 1 - TACCGGAGCATAACCAACCAAACGTCTGTCCTTCCC  
 Line 2 - TACCGGAGCATAACCAACCAAACGTCTGTCCTTCCC  
 Line 3 - TACCGGAGCATAACCAACCAAACGTCTGTCCTTCCC

*noto*:RFP-DR48 3' junction

Vector <--- 24 bp homology ---> *noto*  
 Precise junction - AGGAAGTCCAGCTCTGCGCTCCCGCTTATTTACTCG  
 Line 1 - AGGAAGTCCAGCTCTGCGCTCCCGCTTATTTACTCG  
 Line 2 - ATTGATGGGAGCGCAGAGCTGGACAGGATCCAGCTCTGC-CTCCCGTTTATT  
 Line 3 - ATTGATGGGAGCGCAGAGCTGGACAGGATCCAGCTCTGCCCCCGCTTATT



**SUPPLEMENTARY FIG. S3.** Engineering *noto*:RFP-DR48. **(A)** Schematic showing RFP-DR48 integrated at *noto*. The NBM (yellow) contains both SpCas9 and ErCas12a PAMs for universal targeting using the underlined RNA cursor target site. Red is the engineered stop codon. **(B)** Southern blot of three *noto*:RFP-DR48 lines. Band intensity is roughly single copy based on the copy number controls in the *noto* probed blot (top). Gel shifts are present indicating precise (line 1) and linear vector integration (lines 2 and 3). **(C)** Junction fragment analysis of integrations showing precision at the 5' junction. Line 1 contains a precise 3' integration, while line 2 and line 3 contain differing NHEJ events. **(D)** Schematic of integrations events as determined by Southern blot and DNA sequencing analysis.