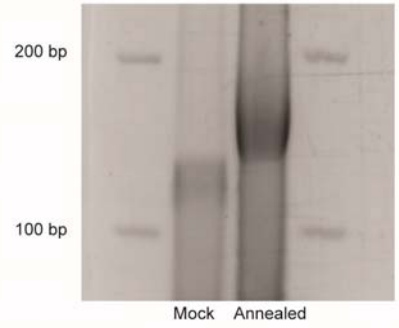
A



B

nhr-23::2xFLAG oligo	Viable injected P0	Rescued F1	P0 with rescued F1	PCR hits	Precise Knockins	% Knockins/ rescued F1	% Knockins/ P0
sense 200mer	102	27	n/a	10	7	25.9	6.9
sense 200mer mock annealed	20	5	2	3	1	20.0	5.0
sense+antisense 200mer mock annealed	32	9	2	1	1	11.1	3.1
sense+antisense 200mer annealed	24	4	4	0	0	0.0	0.0

Figure S4 dsDNA is not a more effective template than ssDNA for introduction of a *2xFLAG* epitope at the 3' end of *nhr-23*. (A) 50 ng/µl of sense of and antisense *nhr-23::2xFLAG* oligos in annealing buffer (TE buffer with 50 mM NaCl) were either annealed by heating to 95ºC for two minutes and then slowly cooling to 25ºC over 30 minutes in a thermocycler, or mock annealed (kept at 25ºC). Annealing was confirmed by resolving the annealed and mock annealed oligos on a 4% TAE-agarose gel and staining with GelRed. The 1KB+ (Invitrogen) size standard is provided. (B) Table comparing the knock-in efficiencies of sense oligos, and either mock annealed or annealed sense+antisense *nhr-23::2xFLAG* 200mers. The sense 200mer data is pooled from all experiments using *pha-1(ts)* sense oligos and *nhr-23::2xFLAG* sense 200mers (Figures 1 and 3). A control where the sense oligo was injected in annealing buffer was performed to ensure that the buffer did not affect knock-in efficiency.