Supplementary Materials:

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Materials and Methods:

Yeast strains used in this report have the haploid S288c background and are derivatives of yRA strains reported in Anand *et. al* [15] *(ho*Δ *mat*Δ*::hisG hml*Δ*::hisG hmr*Δ*::ADE3 ura3*Δ*851 trp1*Δ*63 leu2*Δ*::KAN ade3::GAL10::HO).* A complete list of strains and plasmids used in this study is found in Table S1. The recipient sequences for strain yRA111 (*can1Δ::UR 5′ intron HOcs::NAT*) contain the 5' sequences from the *URA3* gene (*UR),* an artificial split-intron with only the splice-donor site (5′ SD) and the HO recognition site (HOcs) was built using a combination of Gibson assembly (NEB), *in vivo* plasmid-based recombination in yeast and plasmid rescue. The recipient cassette was PCR-amplified from the respective plasmid and integrated at the *CAN1* locus on Ch V by standard yeast transformation. Complete sequence information of the recipient and donors are presented in Supplementary Table 2. Various 108-bp donors, first designed *in silico,* were obtained as synthetic G-blocks (IDT) and assembled into an existing pRS314-based (*CEN4; TRP1*) plasmid containing the 3′ splice-acceptor of the intron, the 3′ sequence from the *URA3* gene (*A3*) and the *TRP1* marker, using *in vivo* recombination and plasmid rescue from a yeast host strain. The desired donor cassettes were amplified from the plasmid using Phusion (NEB) and integrated at the *FAU1* locus on Ch. V as described in Anand et al. [15] by standard yeast transformation. The list of recipient and donor sequences used in this study is listed in Table S2. Mutants were constructed using standard yeast gene deletion protocols. Pol3-01 and Pol2-4 mutants were created using Cas9 mediated gene targeting. A detail protocol for gene targeting with Cas9 is described at http://www.nature.com/protocolexchange/protocols/5611

BIR assay: BIR efficiencies were determined as described previously[15]. Briefly, cells were plated for individual colonies on YEPD + clonNat to retain the HOcs (which is marked with *NAT*). Approximately one million cells from individual colonies were appropriately diluted and simultaneously plated on YEPD plates to get the total cell count and on YEP-Gal plates for the induction HO of endonuclease. Cells that grew on YEP-Gal plates (DNA break-survivors) were counted and replica plated to plates lacking uracil to determine BIR frequencies. For each replicate, Ura^+ frequencies were calculated as the total Ura^+ cells that grew on plates lacking uracil over the total cells on YEPD. For measuring BIR, at least 2 replicates that comprise a few hundred colonies (ranging from ~100 to ~300) were analyzed. For those constructs in which viability (and therefore BIR) was less than 1 %, the number of cells plated was appropriately scaled up from a few thousand to about a few million. Total cells plated were deduced from cells growing on serially diluted down plates. The average of the replicates constituted mean BIR for that experiment. Experiments were independently repeated 3 times and averaged to arrive at experimental mean. Experimental means were statistically compared by one-tailed student's t-test and the standard error bars graphed using the inbuilt graphing software available in Graphpad Prism. * indicate p value < 0.01 and ** indicate p value < 0.05. NHEJ efficiencies were determined by replica plating cells that grew on YEP-Gal plates onto clonNAT plates. Experiments in which Cas9 was used to

Cas9 was used to induce a chromosomal break were done as follows. First, a DSB was induced with HO by plating on YEP-Gal plates. Survivors that repaired their break by NHEJ were screened by replica plating on clonNAT plates. Individual survivors were sequenced and those that showed "CA" sequence insertion were selected for the Cas9 experiment. Galactose inducible Cas9 plasmids were transformed into the above NHEJ survivors. BIR assay were done essentially as described for HO except that cells were always maintained on 300ug/mL of hygromycin to retain the plasmid.

Analysis of recombination junctions: Sequence analyses to determine mismatch corrections were carried out using the alignment feature contained in the freely available DNA analyses software Serial Cloner 2.6.1 (Franck Perez, Serialbasics). First, the reconstituted *URA3* gene formed after BIR was PCR-amplified using primers that were positioned at the start and end of the *URA3* gene (Supplementary Methods). Individual PCR reactions were sequenced (Eton Bioscience Inc.) and aligned with the 108-bp recipient strand. If there were no changes to the recipient strand during BIR, the PCR product should show a perfect alignment with the recipient strand. Mismatch correction was manually curated for each of the individual sequences. For determining % correction, about 50 samples were sequence analyzed for each of the construct.

The fraction of allowable binding sites was calculated using custom written MATLAB program *allowable_config* (See supplementary methods). *allowable_config* takes as its input the window size, period of mismatch and total number of basepairs and calculates the fraction of binding sites without any mismatches and the fraction of allowable binding sites under the criteria in which the binding window is broken up into a region that cannot tolerate any mismatches (first 5 basepairs) and a region that can tolerate up to one mismatch (last 4 basepairs). We used a window size of w=9, period of mismatch of 1 to 60 and total number of basepairs of n=100,000 for our calculations. *allowable_config* generates a column vector of n zeroes and introduces ones corresponding to the mismatch period. The zeroes represent correct basepairings while the ones represent mismatches. *allowable_config* considers every window of size w and assigns a pass or fail rating for each window. The program assigns pass or fail ratings for the case where there cannot be any mismatches in the window and for the case where there can be up to one mismatch in the window. The fraction of allowable binding sites is simply the ratio of the windows that pass to the total number of windows.

Table S1

strain #	genotype	notes on donor
yRA111	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT	Donor less strain
yRA253	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	0 bp mismatch (100% identical donor)
yRA254	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	76% identical donor
yRA269	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	68% identical donor
yRA270	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	85% identical donor
yRA271	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	95% identical donor
yRA272	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	63rd bp mismatched
yRA273	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	9th bp mismatched
yRA274	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	4th bp mismatched
yRA275	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 9th bp mismatched
yRA278	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3	every 7th bp mismatched

Table S1

	ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	
yRA279	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 8th bp mismatched
yRA280	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 10th bp mismatched
yAB277	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	54th bp mismatch
yAB278	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	36th and 72nd mismatch
yAB279	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	27th 54th and 81st mismatch
yAB280	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	22nd, 44th, 66th and 88th mismatch
yAB281	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	Every 11th bp mismatched
yAB282	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	Every 12th bp mismatched
yAB283	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	Every 13th bp mismatched
yAB284	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	Every 9th with transition mutation
yRA317	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3	every 2nd bp mismatched

Table S1

	ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	
yRA318	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 3rd bp mismatched
yRA319	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 4th bp mismatched
yRA320	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 5th bp mismatched
yRA321	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 6th bp mismatched
yRA322	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 9th bp mismatched starting at 5th nucleotide
yRA323	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 9th bp mismatched and tripple mismatches at 9th, 10th and 11th
yRA324	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	every 9th bp mismatched and tripple mismatches at 18th, 19th and 20th
yRA325	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	1 nt tail
yRA326	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	2nt tail
yRA327	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT,	3 nt tail

Table S1

	intron_SA_A3::TRP1	
yRA340	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	donor with 54 bp homology
yRA341	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	donor with 27 bp homology
yRA342	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	donor with 54 bp homology; mismatched at every 9th
yRA343	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	donor with 27 bp homology ; mismcatched at every 9th
yRA344	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	yRA254 strain with "CA" NEJ insertion at the Hocs
yRA345	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	3 nt insertion at 9th position
yRA346	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	3 nt deletion at 9th position
yRA347	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	1 nt insertion at 9th position
yRA348	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	1 nt insertion at 9th position
yRA349	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT, intron_SA_A3::TRP1	3 nt insertion at 54th position
yRA350	MATa::DEL.HOcs::hisG ura3D851 trp1DEL.63 leu2DEL.::KAN hmlDEL.::hisG HMR::ADE3 ade3::GAL::HO can1DEL::UR intron_SD :: HOcs::NAT,	3 nt deletion at 54th position

Table S1

	intron_SA_A3::TRP1	
yRA288	yRA253; mlh1::LEU2	
yRA289	yRA254; mlh1::LEU2	
yRA290	yRA273; mlh1::LEU2	
yRA291	yRA274; mlh1::LEU2	
yRA292	yRA275; mlh1::LEU2	
yRA293	yRA253; pms1::LEU2	
yRA294	yRA254; pms1::LEU2	
yRA295	yRA273; pms1::LEU2	
yRA296	yRA274; pms1::LEU2	
yRA297	yRA275; pms1::LEU2	
yAB256	yRA253 ; msh2::HPH	
yAB257	yRA274 ; msh2::HPH	
yAB258	yRA275 ; msh2::HPH	
yAB259	yRA253 ; rad51::HPH	
yAB260	yRA274 ; rad51::HPH	
yAB261	yRA275 ; rad51::HPH	
yAB271	yRA319 ; rad51::HPH	
yAB272	yRA320 ; rad51::HPH	
yAB273	yRA321 ; rad51::HPH	
yAB274	yRA322 ; rad51::HPH	
yAB268	yRA253 ; rad1::LEU2	
yAB269	yRA274 ; rad1::LEU2	
yAB270	yRA275 ; rad1::LEU2	
yAB285	yRA253 ; pol3-01	
yAB286	yRA274 ; pol3-01	
yAB287	yRA275 ; pol3-01	
yAB292	yRA253 ; pol2-4	
yAB293	yRA274 ; pol2-4	

Table S1

yAB294	yRA275 ; pol2-4	
yAB317	yRA253 ; exo1::HPH	
yAB318	yRA274 ; exo1::HPH	
yAB319	yRA275 ; exo1::HPH	
yRA310	yRA253 selected for "CA" NEJ Insertion at HO cut site (yRA300); transformed the bRA74	
yRA311	yRA274 selected for "CA" NEJ Insertion at HO cut site (yRA301); transformed the bRA74	
yRA312	yRA275 selected for "CA" NEJ Insertion at HO cut site (yRA302); transformed the bRA74	
yAB321	yRA253 msh2::HPH selected for "CA" NEJ Insertion at HO cut site (yRA331); transformed with plasmid bRA134	
yAB322	yRA274 msh2::HPH selected for "CA" NEJ Insertion at HO cut site (yRA332); transformed with plasmid bRA134	
yAB323	yRA275 msh2::HPH selected for "CA" NEJ Insertion at HO cut site (yRA333); transformed with plasmid bRA134	
yAB328	yRA253 ; msh6::LEU	
yAB329	yRA274 ; msh6::LEU	
yAB330	yRA275 ; msh6::LEU	

plasmids	**features**	**repair template**
bRA74	Cen vector HPH:pGAL1-Cas9 (guide CTGGTTTTGGTTTTGTAGAG); cutting yields a 34 nt tail	NA
bRA134	Cen vector LEU2:pGAL1-Cas9 (guide CTGGTTTTGGTTTTGTAGAG); cutiing yileds a 34 nt tail	NA
bRA114	Cas9 vector used to make Pol3-01 (guide TCCTTTGATATCGAGTGTGC)	CAGCTCCATTGCGTATCATGTCCTTTGCTATCGCGTGTGCTGGTAGGATTGGCGTCTTTCCGGAACCTGAATACGATCCC

Table S1

bRA124	Cas9 vector used to make pol2-4 allele (guide TATCAAATGCCATTACCACA)	TTCATTGAAGATACTAGGAAAATTGCATTTGCCGATCCAGTAGTGATGGCTTTCGCAATAGCTACCACGAAGCCGCCTTTAAAATTCCCGGATTCCGC

Table S2

Recipient sequence	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
100% homology donor	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
68% homeology donor	TATTATCGACGTAACCAGGCTTTTCACCAGGTCAATCTAAATGGGTTGCTTCTGG ATTATAAAGTTATAGTAGTCAGGTAGGTCCGTGAGTTTCAGCCCACCCCAACA
76% homeology donor	TATTATCGACTTAAGCAGGCTTTTCAGCAGGCGGATCTAATAAAATTCGTTCGCA ATGATAAAAATAGCATACTCGGGCAGGTCCGTGAGTTTCTGCCCACCCCAACA
85% homeology donor	TAATATTGACTAAATGAGTCTTTTCAGCTGGTCGATCGAAAGAAATTCGGTTTCAA TTATTAGAATAGCATTGTCGGGTTATTCTTTGAGTTTGAGCTTACCTCAACA
95% homeology donor	TAATATGGACTAAAGGAGGCTTTTCAGCAGGTCGATCTAAATAAATTCGTTCTCA ATTATTAAAATAGCATAGTCGGGTTTTTCTTTGAGTTTCAGCTTACCGCAACA
1 mismatch at the 63rd position	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAAGTCGTTTTCA ATGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
1 mismatch at the 9th position	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTGCCGCAACA
1 mismatch at the 4th position	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCGACA
every 2nd position mismatched	AATTTTCGTCAATACGTGCCATATGTCCTGCTGGTTGTTATTTATTACCTATACTA AGTTAATATTTGGAAACTGGCGATATACATATTGATACTGGTATGCCCTAGA
every 3rd position mismatched	AAAAATCGAGTATAGCAGCCTATTGTGGAGCTCCATGTATATTAAATCCTTATCTA TCATAAATATTGCTTACTCCGGATTATCATTAAGATTGAGGTTACCCCATCA
every 4th position mismatched	AAATTTGGTCTATAGGTGGCATTTGTGCTGGTGGATGTAATTAATTTCCTTTACAA AGATAAAATTAGGATACTCGCGTTATTCATTTTGTTACAGGTTTGCGCTACA
every 5th position mismatched	TAAAATGGTCTAATGGAGCCTTTACTGCTGGTCCATCTTAATATATTCCTTTTGAA TGTTTAATATAGGATAGACGGGATTTTGTTTTTGTTTGAGCTATCCGGAACA
every 6th position	AAATATCGACTATAGGAGCCTTTTGTGCAGCTCGATGTAAATTAATTCCTTTTCTA TGATAAAAATTGCATACTCGGGATTTTCATTTAGATTCAGGTTTCCCCAACA

Table S2

mismatched	
every 7th position mismatched	TAAAATGGACAAAAGGACGCTTTTGTGCAGGACGATCTTAATAAAATCGTTTACA ATGAATAAAATTGCATAGACGGGTTATTCTTTAAGTTTCTGCTTTCGGCAACA
every 8th position mismatched	TAATTTGGACTATAGGAGGCATTTCTGCTGGTCGATGTAAATAATTTCGTTTACAA TGATAAAAATAGGATAGTCGCGTTTTTCATTTAGTTACAGCTTTGCGCAACA
every 9th position mismatched	AAATATGGAGTAAAGGAGCCTTTTCTGGAGGTCGATGTAAATAAAATCGTTTTCT ATGATTAATATAGCATACTCGGGTTTATCTTTTAGATTCAGCTTACCGCAACA
every 10th position mismatched	TAATATGGTCTAAAGGAGCCTTTTCTGCTGGTCGATCTTAATAAATTCCTTTTCAA TGTTTAAAATAGGATAGTCGGGATTTTCTTTTTGTTTCAGCTATCCGCAACA
every 11th position mismatched	TAATATGGAGTAAAGGAGGCATTTCTGCAGGACGATCTAAATTAATTCGTTTTGA ATGATTAAATTAGCATAGTCCGGTTTTTCTTATAGTTTCAGCATTCCGCAACA
every 12th position mismatched	TAATATGGACTATAGGAGGCTTTTGTGCAGGTCGATGTAAATAAATTCCTTTTCAA TGATAAAAATAGCATACTCGGGTTTTTCATTTAGTTTCAGGTTTCCGCAACA
every 13th position mismatched	TAATTTGGACTAAAGGACGCTTTTCTGCAGCTCGATCTAAATATATTCGTTTTCAA AGATTAAAATAGCTTAGTCGGGTTTTACTTTTAGTTTCACCTTTCCGCAACA
every 9th position mismatched starting at the 5th	TAATTTGGACTAATGGAGGCTTATCTGCAGGACGATCTAATTAAATTCGATTTCAA TGTTTAAAATACCATAGTCGCGTTTTTCTATTAGTTTCTGCTTTCCGGAACA
every 9th position mismatched, and triple mismatches at 9th, 10th, and 11th	AAATATGGAGTAAAGGAGCCTTTTCTGGAGGTCGATGTAAATAAAATCGTTTTCT ATGATTAATATAGCATACTCGGGTTTATCTTTTAGATTCAGCAAACCGCAACA
every 9th position mismatched, and triple mismatches at 18th, 19th, and 20th	AAATATGGAGTAAAGGAGCCTTTTCTGGAGGTCGATGTAAATAAAATCGTTTTCT ATGATTAATATAGCATACTCGGGTTTATCTTTTTCATTCAGCTTACCGCAACA
every 9th position mismatched	CAATATGGATTAAAGGAGACTTTTCTGTAGGTCGATTTAAATAAACTCGTTTTCGA TGATTAAGATAGCATAATCGGGTTTCTCTTTTAGCTTCAGCTTCCCGCAACA

Table S2

(transition)	
1 bp tail (mismatch at the 1st position)	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACT
2 bp tail (mismatches at the 1st and 2nd position)	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAAGT
3 bp tail (mismatches at the 1st , 2nd and 3rd position)	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCATGT
1 mismatch at the 54th position	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCTA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
2 mismatches at 36th and 72nd	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATGTAAATAAATTCGTTTTCAA TGATTAAAATAGCATACTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
3 mismatches at the 27th 54th and 88th	TAATATGGACTAAAGGAGGCTTTTCTGGAGGTCGATCTAAATAAATTCGTTTTCTA TGATTAAAATAGCATAGTCGGGTTTATCTTTTAGTTTCAGCTTTCCGCAACA
4 mismatches at the 22nd, 44th, 66th and 88th	TAATATGGACTAAAGGAGGCATTTCTGCAGGTCGATCTAAATTAATTCGTTTTCAA TGATTAAATTAGCATAGTCGGGTTTTTCTTATAGTTTCAGCTTTCCGCAACA
3 bp insertion at the 9th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTCTTTCCGCAACA
3 bp deletion at the 9th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGTCCGCAACA
1 bp insertion at the 9th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTTCCGCAACA
1 bp deletion at the 9th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCAA TGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTCCGCAACA
3 bp insertion at the 54th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTTTCCT TAATGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA

Table S2

3 bp deletion at the 54th	TAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCGTTAATGA TTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCAACA
URA3 promoter	CTTTTTTTTGATTTCGGTTTCTTTGAAATTTTTTTGATTCGGTAATCTCCGAACAGA AGGAAGAACGAAGGAAGGAGCACAGACTTAGATTGGTATATATACGCATATGTA GTGTTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAA AACCTGCAGGAAACGAAGATAAATC
UR	ATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTGCTG CCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCATTGGAT GTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTT GTTTACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGCACAGT TAAGCCGCTAAAGGCATTATCCGCCAAGTACAATTTTTTACTCTTCGAAGACAGA AAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTACTCTGCGGGTGTATACA GAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCCAGGT ATTGTTAGCGGTTTGA
5' splice donor (SD)	GTATGTTAATATGGACTAAAGGAGGCTTTTCTGCAGGTC
Hocs	GATCTAAATAAATTCGTTTTCAATGATTAAAATAGCATAGTCGGGTTTTTCTTTTAG TTTCAGCTTTCCGCAACAGTATAATTTTATAAACCCTGGTTTTGGTTTTGTAGAGT GGTT
3' splice acceptor (SA)	CCCGGGTACCGAGCTCGAATTTTTACTAACAAATGGTATTATTTATAACAG
A3	AGCAGGCGGCAGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAG AATTGTCATGCAAGGGCTCCCTATCTACTGGAGAATATACTAAGGGTACTGTTGA CATTGCGAAGAGCGACAAAGATTTTGTTATCGGCTTTATTGCTCAAAGAGACATG GGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGGTTTA GATGACAAGGGAGACGCATTGGGTCAACAGTATAGAACCGTGGATGATGTGGTC TCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGG ATGCTAAGGTAGAGGGTGAACGTTACAGAAAAGCAGGCTGGGAAGCATATTTGA GAAGATGCGGCCAGCAAAACTAA
URA3 terminator	AAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAAT TTAATTATATCAGTTATTACCC
recombining block (recipient sequence)	GTATGTTAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGATCTAAATAAATTCG TTTTCAATGATTAAAATAGCATAGTCGGGTTTTTCTTTTAGTTTCAGCTTTCCGCA ACA