Supplemental Information

Table 1s. Effect of copy number of the donor plasmid on gap repair

Partner plasmid	Gap repair, % (fold-increase)[1]	Nucleotide opposite lesion		
		-1	BS[2]	Total
None	1.4±0.4	23	-	24
High copy				
Homologous (pFGP20/T-amp)	71.6±8.2 (37)	1	35T	36
Heterologous (pUC18)	1.9±0.2	14	-	14
Low copy				
Homologous (pLH500)	11.5±3.4 (5)	2	7T	9
Heterologous (pACS)	2.3±0.5			
Single copy				
Homologous (pSCGH)	3.9±0.6 (3)	nd	nd	nd
Heterologous (pFSO1)	1.3±0.32			
Origin-less synthetic plasmid[3]				
Homologous (pOFGP20C/A)	4.5±2 (2.6)	25	42 C,T	67
Heterologous	1.7±0.4			

The efficiency of gap repair via HR increases with increasing copy number of the donor plasmid. This may be explained by the fact that in this two-plasmid system HR repair is a bi-molecular reaction.

[1]Fold-increase in plasmid gap repair due to the presence of the homologous partner plasmid relative to the heterologous plasmid. Notice that the background gap repair is very similar regardless of the copy number of the heterologous partner plasmid, and averages 1.7±0.4%. It is composed mainly of background constitutive TLS by polIII holoenzyme, which produces -1 frameshifts.

[2]BS, base substitutions. The nucleotides inserted opposite the lesion are indicated.

[3]The origin-less plasmid pOFGP20C/A (see also Table 2) cannot replicate, and therefore is similar to a single-copy plasmid as long as it survives in the cell.

E. coli AB1157 cells were transformed with the indicated partner plasmid. Cells harboring the partner plasmid were then transformed again either with the gap-lesion plasmid GP21, or with GP20 without the abasic site, and plated on kanamycin-LB plates. The efficiency of gap repair was calculated by dividing the number of colonies obtained with the gap-lesion plasmid, by the number of colonies obtained with GP20. Typically plates with 50-200 colonies were counted, except for the background plates,

where colony counts were lower. Each experiment was repeated 4-6 times, and the average is presented.

Table 2s. Sequence analysis of the nucleotide opposite the lesion in plasmid obtained from HR gap repair experiments performed in recombination deficient mutants

Relevant genotype	Nucleotide present opposite lesion site					Total no of sequences	Gap-repair, %	Fraction of gap-repair attributed to HR, %
	A	T	C	G	-1			
ΔrecA		1			19	20	2.8±0.2	5
ΔrecF		10			3	13	12.7±3.5	77
recB21		7				7	84±29	> 86
ΔruvC		10			0	10	68±4	> 90
*ΔrecG**			7		0	7	47±8	> 86
ΔrusA		10				10	95±6	>90
*ΔruvAC**			7		1	8	56±6.5	88
ΔruvAC, recG		5				5	34±4	> 89
Wild-type		35			1	36	83±8	97

Plasmids were extracted from Kan^R colonies obtained in the HR gap repair experiments described in Fig. 2, and subjected to DNA sequence analysis. The homologous donor plasmid contained a T opposite the site corresponding to the lesion, except in the experiments with the *ΔrecG* and *ΔruvAC* strains (marked with asterisks), where it contained a C at that location. The extent of gap repair was taken from Fig. 2. The fraction of gap repair attributed to HR (last column) was calculated by dividing the number of base substitution mutation (markers of HR) by the total number of sequences analyzed.