

Bandyopadhyay, Douglass et al.
Figure S4.

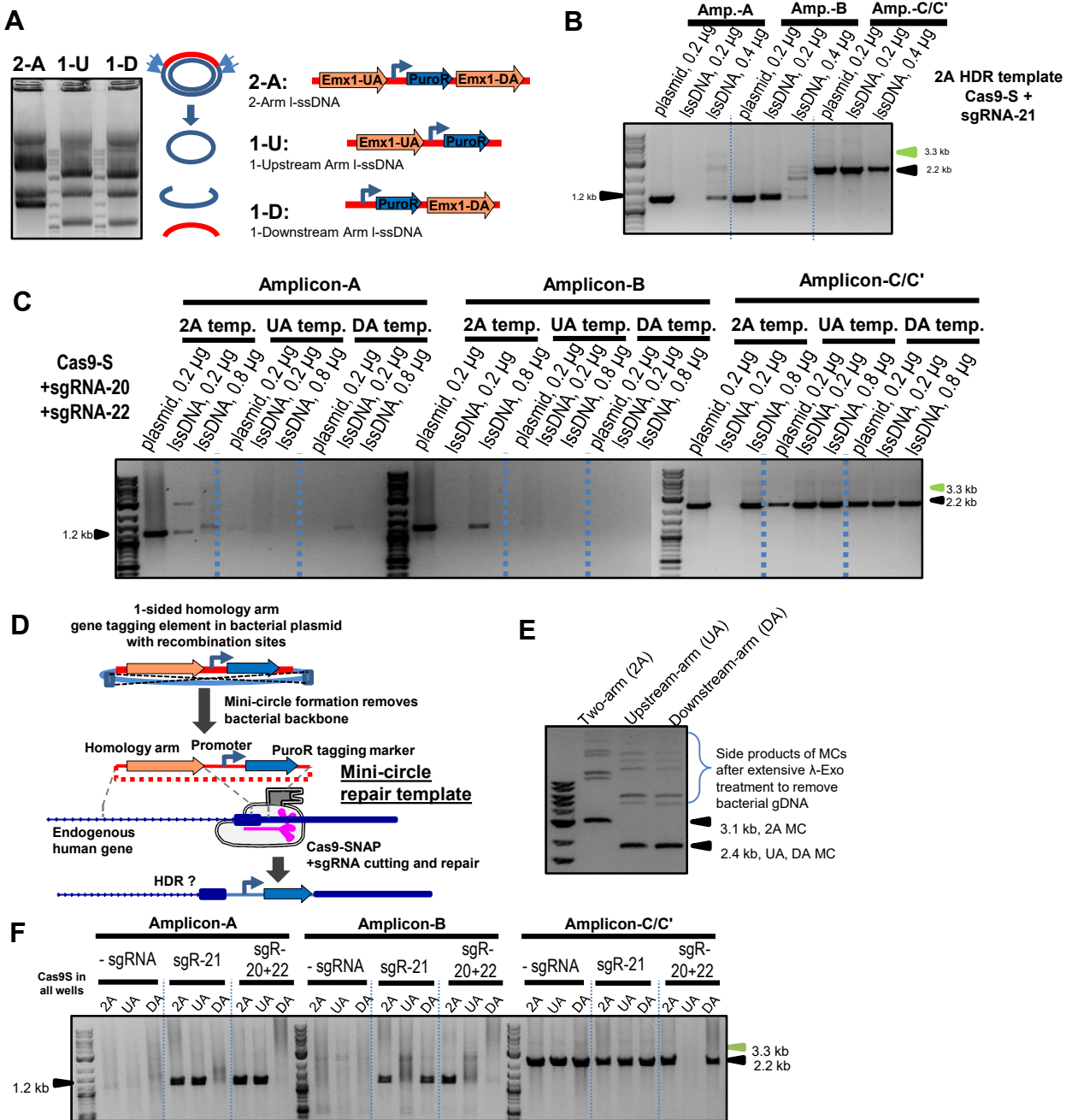


Figure S4. Long single-stranded DNAs (IssDNAs) and Mini-circles (MC) also underperform in Cas9-stimulated HDR.

A) Schematic of generating IssDNAs from nicking of plasmids and denaturing and resolving smallest linear fragment on an agarose gel. Left panel shows the constructs with both homology arms (2-A) or a single homology arm. B) Genomic PCR of puromycin-selected cells after transfecting just the 2-Arm IssDNA, Cas9-SNP and sgRNA₂₁. The respective Amplicons- A, -B, and -C are the same as in Fig. 3 and 4, where the black arrowheads point to the 1.2 kb size of Amplicon-A and -B and the 2.2 kb size of Amplicon-C from the endogenous EMX1 locus. A desired HDR event generating a 3.3 kb Amplicon-C' is not observed (green arrowhead). C) Genomic PCR analysis of IssDNA templates using two sgRNAs, with format of assay similar to B). D) Diagram of initial concept to generate MC repair templates where vector backbone bacterial sequences are recombined out to yield a MC DNA, and in this case a single homology arm. The formats of the Two-arm (2A), Upstream-arm (UA) and Downstream-arm (DA) constructs are as detailed in A). E) Gel showing efficiency of generating the MCs after the 4.4 kb plasmid backbone is removed. Although the MC is the dominant product, larger contaminating recombinants still persist in the purification. F) Genomic PCR of puromycin-selected cells after transfecting just the 2A, UA, and DA MCs along with Cas9-SNP and sgRNAs. The respective Amplicons- A, -B, and -C are the same as in Fig. 2, where the black arrowheads point to the 1.2 kb size of Amplicon-A and -B and the 2.2 kb size of Amplicon-C from the endogenous EMX1 locus. A desired HDR event generating a 3.3 kb Amplicon-C' is not readily observed (green arrowhead).