

Bandyopadhyay, Douglass, et al.
Figure S5.

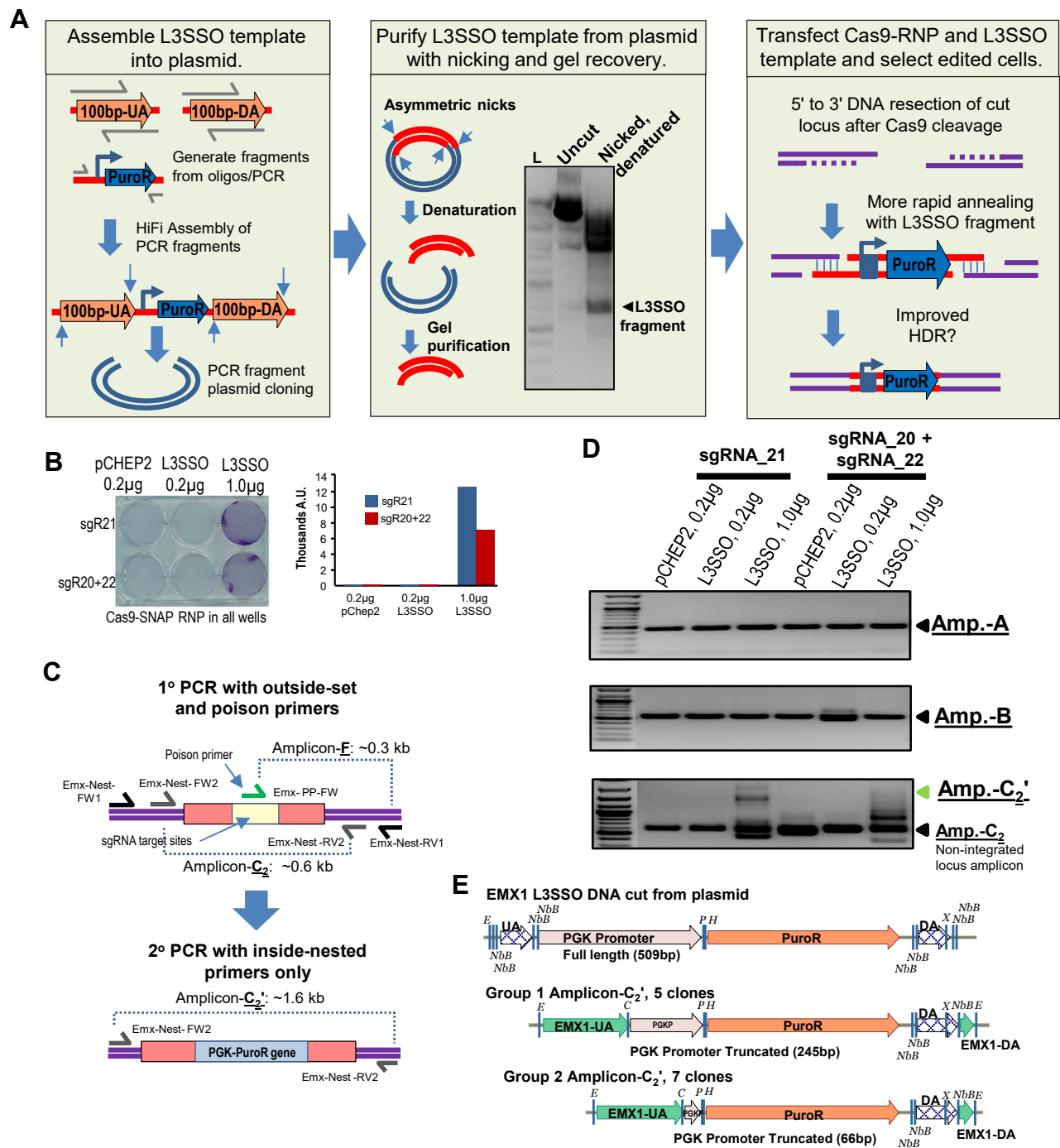


Figure S5. Improved Cas9-stimulated HDR at EMX1 with Long 3' Single-Stranded Overhang (L3SSO) DNAs.

A) Schematic of method to generate L3SSO DNAs from multiple nicking of plasmid and denaturing gel electrophoresis. Final panel shows the model for improved HDR. B) Representative experiment of Crystal violet staining and quantitation of puromycin-selected cells nucleofected with Cas9SNAP-RNPs and either the intact pCHEP2 plasmid or the L3SSO cut and purified from pCHEP2. C) Schematic of poison-primer approach to detect proper HDR event at EMX1. D) Genomic PCR analysis of the EMX1 locus for integration of L3SSO. Format for detecting Amplicons –A and –B is the same as in Fig. 2C. Gel for Amplicon-C₂' are only showing the products of the 2^o PCR while the 1^o PCR with poison primers is not shown. E) Diagrams of the sequenced Amplicon-C₂' products compared to the L3SSO DNA repair template. UA: Upstream arm, DA: Downstream arm, E: EcoRI, C: ClaI, P: PstI, H: HindIII, NbB: Nb.BbvCI, X: XmaI restriction sites. Patterned arrows represent L3SSO segments, while green arrows represent the endogenous EMX1 locus sequence.