Bandyopadhyay, Douglass, et al.
Figure S2.

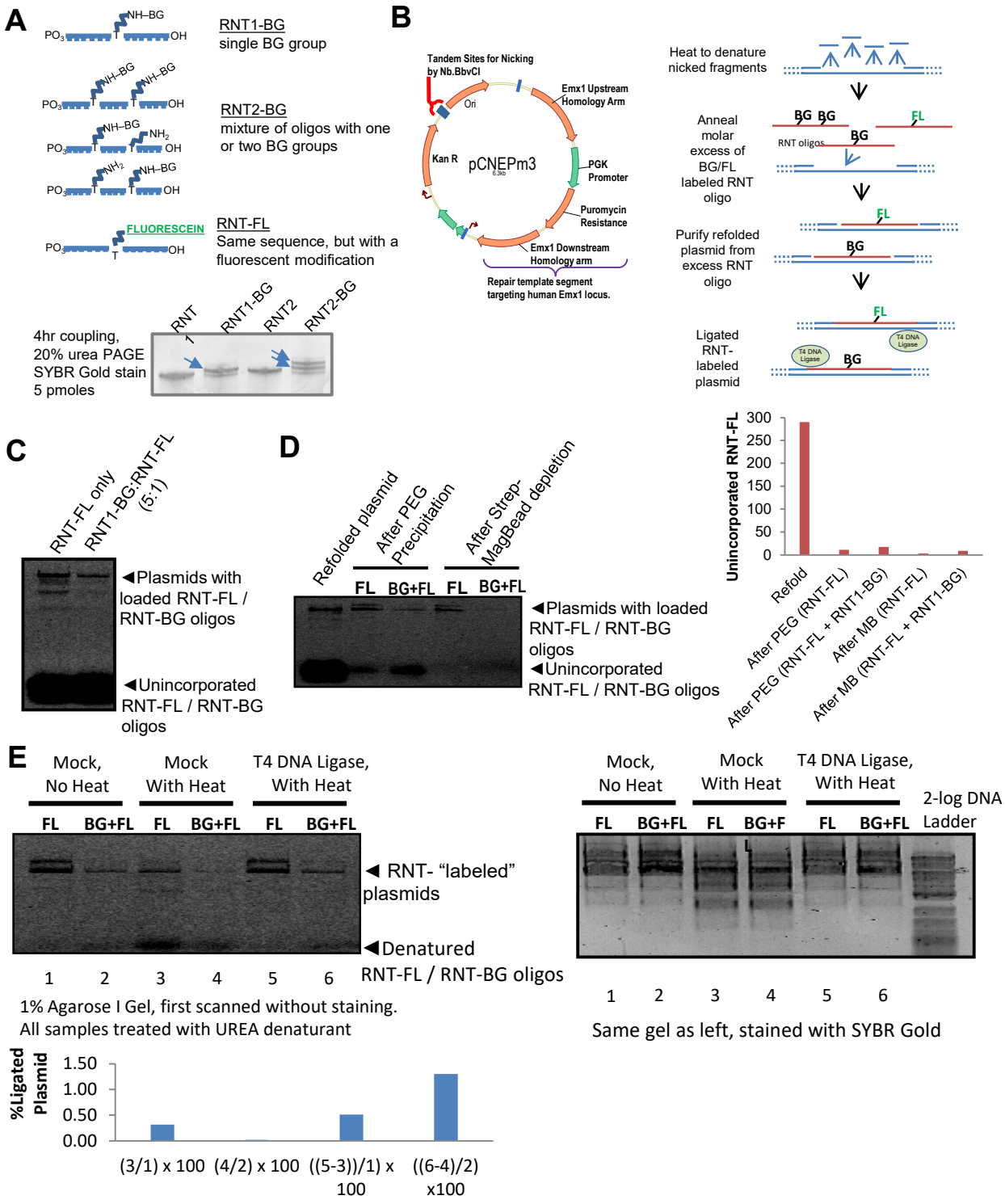

Figure S2. Procedure to label plasmids with a BenzylGuanine (BG) coupled oligonucleotide.
(A) Chemical coupling of BG-NHS ester to the primary amine of an internal dT with a C6-amino group. (B) The pCNEPm3 plasmid has homology arms to EMX1 flanking a PGK promoter and puromycin acetyltransferase. A tandem array of Nb.BbvCI sites in the backbone is nicked and heated to liberate the cut fragments on one strand, which can then be quantitatively replaced with an oligonucleotide that is coupled with BG or a tracer amount of Fluorescein (FL) for tracking the labeling reactions. (C) Retention of RNT-FL and RNT-BG oligos onto nicked and refolded plasmids. The fluorescent RNT-FL oligo serves as a proxy for plasmid labeling with RNT-BG. (D) Removal of unincorporated oligo by precipitating plasmids with PEG followed by additional magnetic bead based antisense oligo depletion. Quantitation of fluorescent signal of gel scan on the right. (E) Demonstration of covalent linkage of RNT-BG + RNT-FL oligos to plasmids after T4 DNA ligase reaction, because ligated oligos do not get denatured away from plasmid during heating with urea. Gel on the left is a fluorescence scan for the RNT-FL tracer oligo, while the right gel is later stained with SYBR gold to image total plasmids. Graph is quantitation of gel on the left.