



S5 Fig. Maps of vectors used in *in vitro* translational and ribosomal methylase inhibition analysis. (A) pCR-XL-TOPO NLuc contains the nanoluciferase gene (NLuc) located downstream of both lac and T7 polymerase promoters and was used for analysis of inhibition of prokaryotic translation by S-F, S-D and nourseothricin. (B) pT7CFE1-NLuc contains the NLuc gene located downstream of a T7 polymerase promoter and IRES sequence and was used for analysis of inhibition of eukaryotic translation by streptothricins. (C) pBAD-NpmA contains a codon-optimized npmA gene (S17 Fig) that encodes a 16S rRNA A1408 methylase that blocks activity of all known 2-deoxystreptamine-based aminoglycosides including apramycin. (D) pBAD-ArmA contains a codon-optimized armA gene (S17 Fig) that encodes a 16S rRNA G1405 methylase that blocks activity of 4,6-disubstituted deoxystreptamine (DOS) aminoglycosides such as gentamicin and does not block the activity of apramycin. Constructs were confirmed by Sanger sequencing.