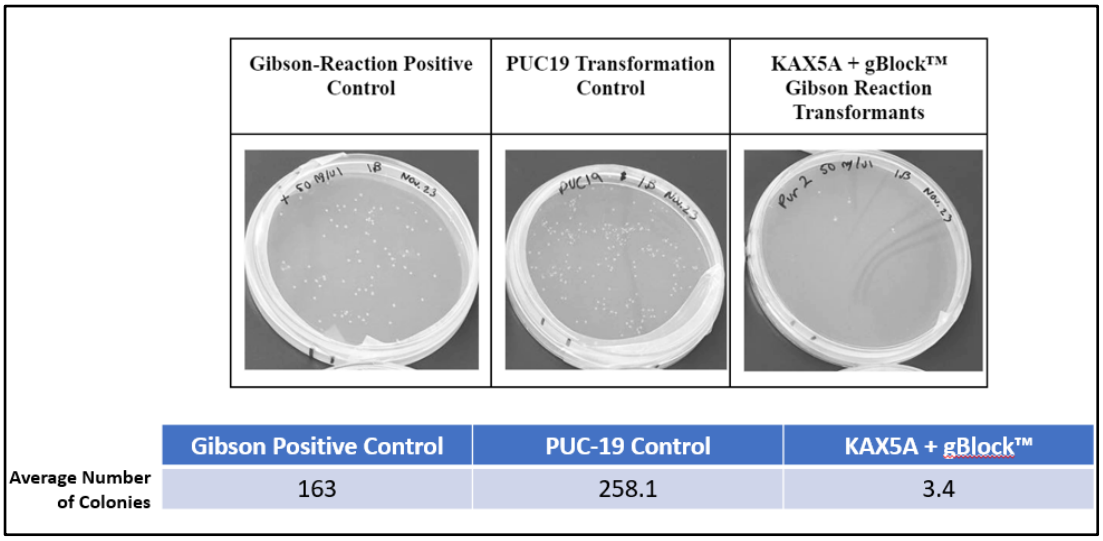
SUPPLEMENTARY MATERIAL

TABLE. S1 Properties of Primer Sequences for gBlock™ Amplification and pKAX5A Inverse PCR. Forward and Reverse primers, along with the associated melting temperature(s) were determined using SnapGene® (v 7.0).

Primer ID & Type (Forward or Reverse)	Nucleotide Sequence (5' to 3')	Melting Temperature Range (°C)
gBlock Forward	GCCCGGCTGGCGCCC	66
gBlock Reverse	GGTCAGGGTCTTGTAAGAGGCC TCGG	65
pKAX5A Backbone Forward	GAGGCCTCTTACAAGACCCTGA CCCTGCAAACCCTG	70-71
pKAX5A Backbone Reverse	CGCCGCCGCGGGCGC	70-71



	Gibson Positive Control	PUC-19 Control	KAX5A + gBlock™
Average Number of Colonies	163	258.1	3.4

FIG. S1 Colony Quantification of Transformants. Representative images of colonies observed on transformed plates (top) and the average number of colonies quantified per condition (bottom). Top10 E.coli transformed with the positive control from GeneArt (10 ng of a 1.5 kb kanamycin cassette and 30 ng of a 2.7 kb vector with an ampicillin resistance gene), 10 pg/µl of PUC19 control and 50 ng/µl of purified pKAX5A and gBlock™ Gibson reaction mix were plated on Ampicillin-resistant LB plates. n=3.

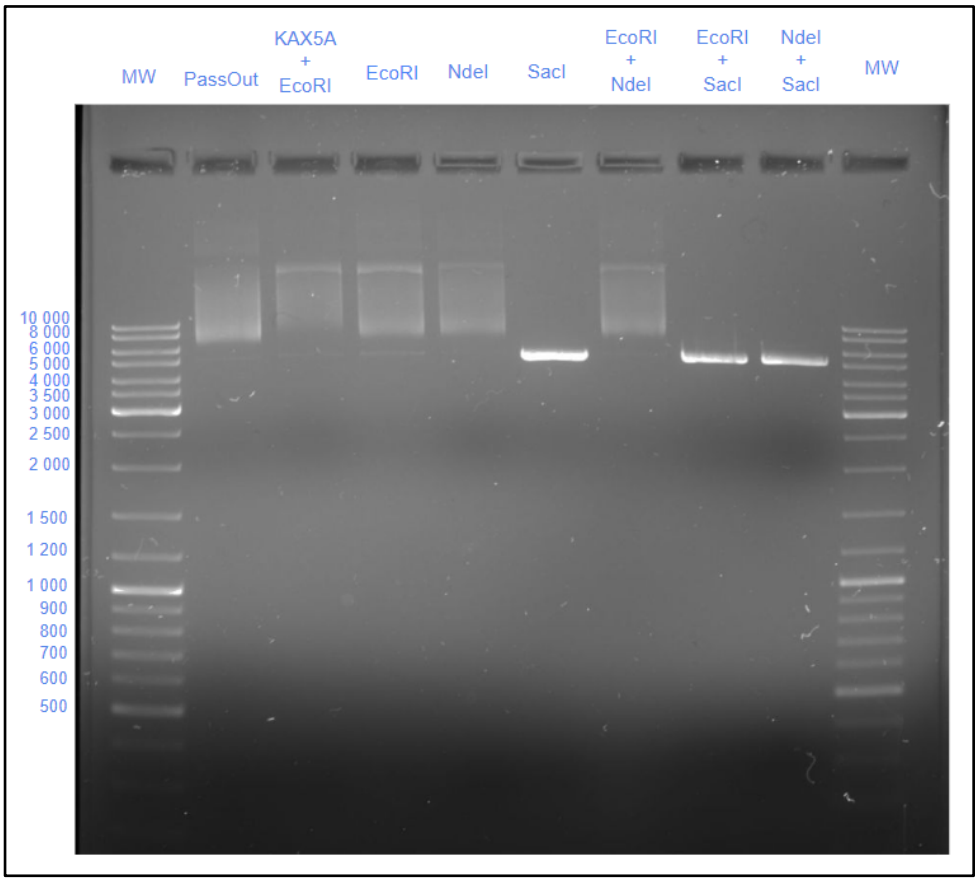


FIG. S2 Restriction Digest on Isolated Transformant Plasmid DNA. Plasmid isolated from suspected "PassOut" transformants were digested using EcoRI and NdeI, which were encoded in the gBlock™ insert, as well as SacI which is only present in the pKAX5A backbone. Suspected PassOut construct was digested with each enzyme separately (lanes 4, 5, 6) and together (lane 7, 8, 9). Undigested PassOut construct was run as a control (lane 2) to assess plasmid recircularization, and EcoRI digest of pKAX5A template to assess the template validity (lane 3).

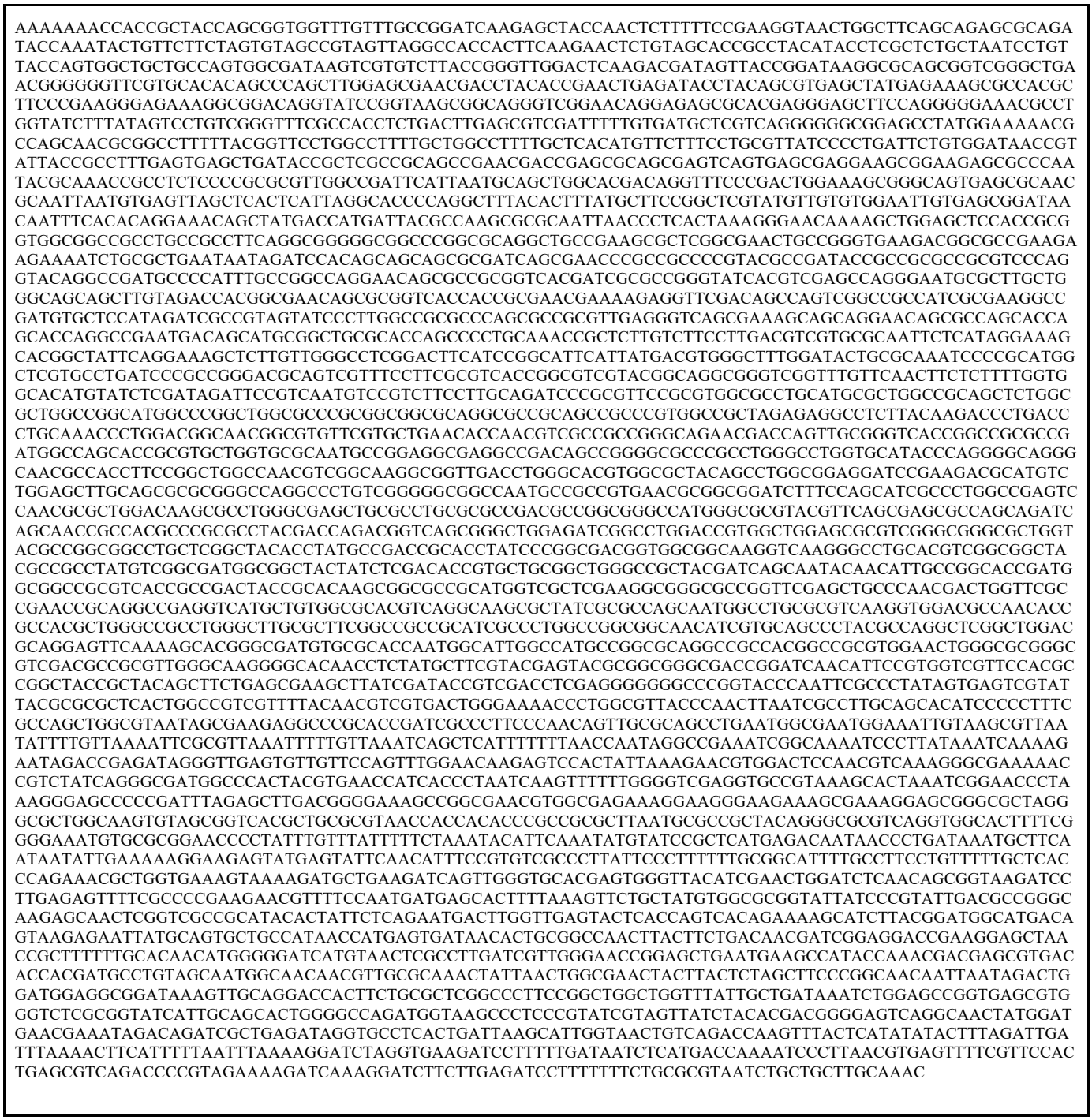


FIG. S3 Sequence Results of Extracted Plasmid. Whole plasmid Nanopore sequencing (Plasmidsaurus) was used to determine the nucleotide sequence of the extracted plasmid from suspected PassOut transformants. The obtained sequence exactly corresponds to the known sequence of pKAX5A, displayed in a 5’ to 3’ direction.