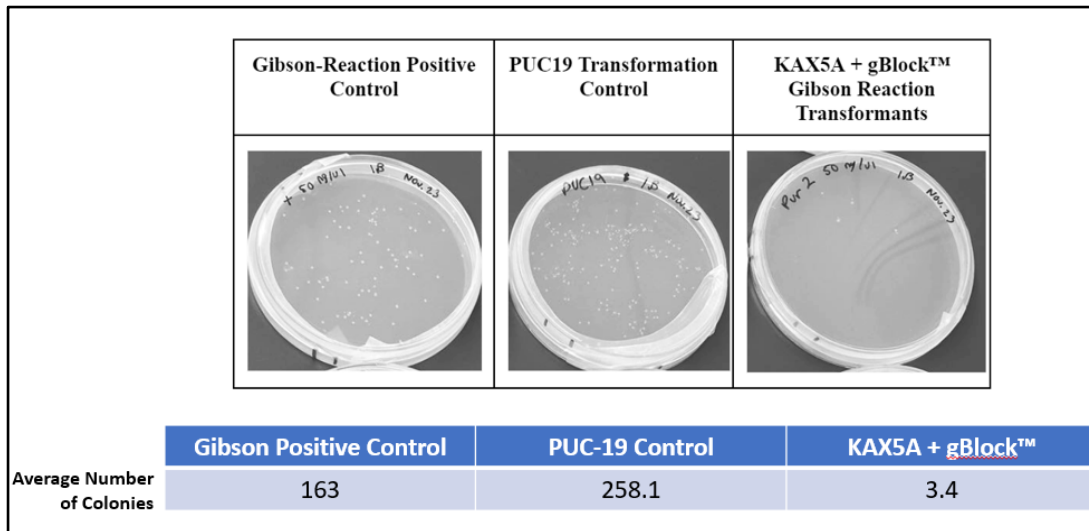


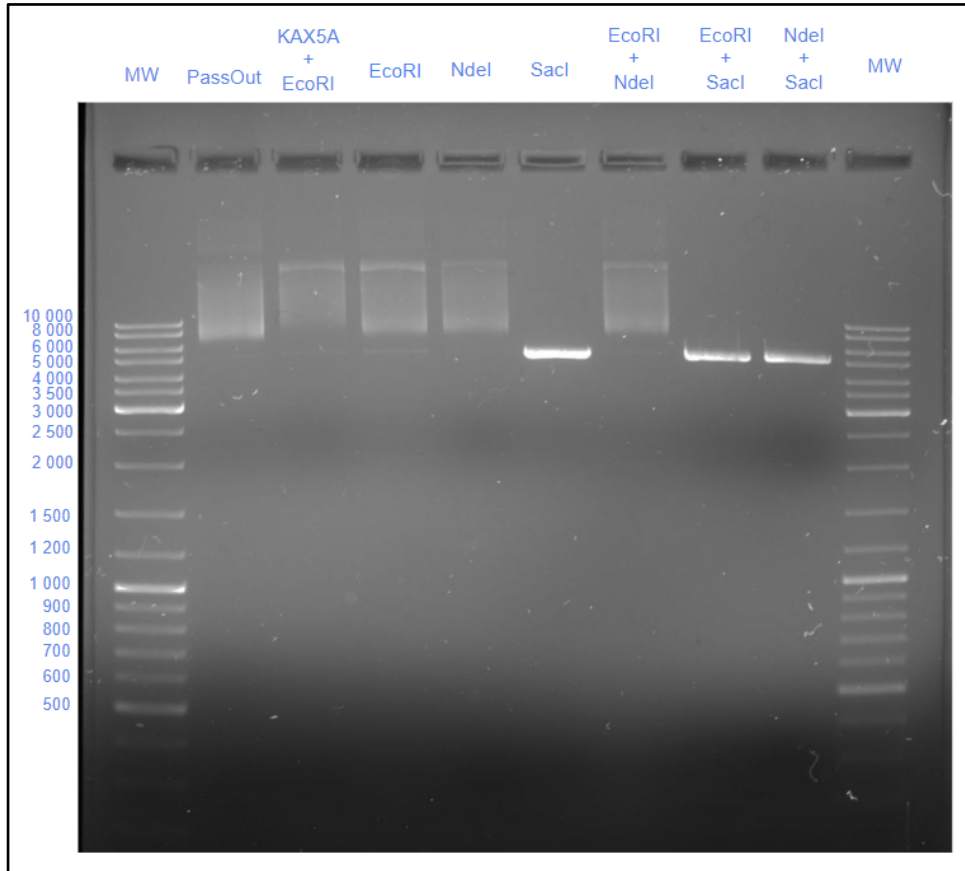
## SUPPLEMENTARY MATERIAL

**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).

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



**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).

AAAAAAAAACCACCGCTACCAGCGGTGGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGA  
TACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGT  
TACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGA  
ACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGC  
TTCCCAAGGGAAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCT  
GGTATCTTTAGTCCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCAATTTTGTGATGGTCTGTCAGGGGGCGGAGCTTGGAAAAACG  
CCAGCAACGCGCCTTTTTACGGTTCTCGCCTTTTGTGGCCTTTTGTCTACATGTTCTTCTCGCTTATCCCCTGATTCTGTGGATAACCGT  
ATTACCGCCTTTGAGTGAAGTGTATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAAGTGAAGCGGAAGAGCGCCCAA  
TACGCAAACCGCCTCTCCCCGCGCTTGGCCGATTCAATATGACGCTGGCAGCAGAGTTTCCCGACTGGAAAGCGGGCAGTGAAGCGCAAC  
GCAATTAATGTGAGTTAGTCACTCATTAGGCACCCACGGCTTTACACTTTATGCTTCCCGCTCGTATGTTGTGGAAATTTGAGCGGATAA  
CAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGC  
GTGGCGGCCGCTGCCGCTTACAGGCGGGGGCGGCCCGCGCAGGCTGCCGAAGCGCTCGCGAACTGCCGGTGAAGACGGCGCCGAAGA  
AGAAAATCTGCGTGAATAATAGATCCACAGCAGCAGCGCATCAGCGAACCCCGCCCGTACGCCGATAACCGCGCGCCGCTCCAG  
GTACAGGCCGATGCCCCATTTGCCGGCAGGAACAGCGCCGCTACGATCGCGCCGGTATCACGTGAGCGAGGAAATGCGTTGCT  
GGCAGCAGCTTGTAGACCACGGCGAACAGCGCGGTACCACCGCAACGAAAAGAGGTTGACAGCCAGTGGCGCCATCGGAAGGCC  
GATGTGCTCCATAGATCGCCGTAGTATCCCTTGGCCGCGCCAGCGCCGCTTGGAGGTCAGCGAAAGCAGCAGGAACAGCGCCAGCACA  
GCACAGGCCGAATGACAGCATGGCGTGCACACAGCCCTGCAACCGCTCTTGTCTTCTTGCAGTGTGCGCAATTTCAATGAAAG  
CACGGTATTACGAAAGCTTGTGGGCTCGGACTTACCCGCTTCAATATGACGTTGGGCTTGGATACTCGCAAAATCCCCGATGG  
CTCGTCTGATCCCGCGGGACGCTGTTCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCT  
GCACATGTATCTCGATAGATTCCGTCATGTCGCTTCTTGCAGATCCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCTTCCGCT  
GCTGGCCGCTATGGCCCGCTGGCGCCCGCGCGCGCAGGCGCCGACGCGCCGCTGGCCGCTAGAGAGGCCCTTTACAAGACCTGACC  
CTGCAAACCTGGACGGCAACGGCGTGTTCGTGCTGAACACCAACGTCGCGCCGGGCGAGAACGACAGTTGCGGGTACCAGCCGCGCCG  
ATGGCCAGCACCGCGTGTGGTGCATGCGGAGGCGAGGCGGACAGCGGGGCGCCCGCTGGGCTGGTGCATACCCAGGGCGAGG  
CAACGCCACCTTCCGGCTGGCAACGTCGGCAAGGCGGTTGACCTGGGCAGTGGCGCTACAGCCTGGCGGAGGATCCGAAGACGCATGTC  
TGGAGCTTGCAGCGCGCGGGCCAGGCCCTGTCGGGGCGGCCAATGCCCGCTGAACCGCGGGATCTTCCAGCATCGCCTGGCCGAGTC  
CAACGCGCTGGACAAGCGCCTGGCGGAGCTGCGCTGCGCGCCGACGCGCGGGCCATGGGCGGTACGTTACAGCAGCGCCAGCAGATC  
AGCAACCGCCACGCCCGCCTACGACCAGACGGTACGCGGGTGGAGATCGGCTGGACCGTGGTGGAGCGCTCGGGCGGGCGTGGT  
ACGCGGGCGGCTGCTCGGCTACACCTATGCCGACCGCACCTATCCCGGCGACGGTGGCGGCAAGGTCAAGGGCCTGCAGTGGCGGCTA  
CGCCGCTATGTCGGCGATGGCGGCTACTATCTCGACACCGTGTGCGGCTGGGCCGCTACGATCAGCAATAACAATTGCCGGCACCGATG  
GCGGGCGGTCACCGCGACTACCGCACAAGCGGCGCCGATGGTCTGCTCGAAGGCGGGCGCCGGTTCGAGCTGCCAACGACTGGTTGCG  
CGAACCGCAGGCCGAGGTCATGCTGTGGCGCACGTACGCAAGCGCTATCGCGCCAGCAATGGCCTGCGCGCTAAGGTGGACGCCAACCC  
GCCACGCTGGCCCGCTGGCTTGGCTTCCGCGCCGCTACGCGCTTGGCGCGCAACATCGTGCAGCCATACGCCAGCTCGGCTGGAC  
GCAGGATTTAAAAGCACGGGCGATGTGCGCACAATGGCATTGGCCATGCCGGCGCAGGCCGCCACGGCCGCTGGAATGGGCGCGGGC  
GTCGACGCGCGTGGGCAAGGGGCAACCTCTATGCTTCTGACGAGTACGCGCGGGCGACCGGATCAACATTCCGTTGCTTCCACGC  
CGGTACCGCTACAGCTTCTGAGCGAAGCTATCGATAACCGTCAACCTCGAGGGGGGCGCCGGTACCAATTCCGCTATAGTGAAGTGT  
TACGCGGCTACTGGCCGTTTTACAACGTCGTAAGTGGGAAAACCTGGCGTTACCCAATTAATCGCCTTACGACACATCCGCTTTC  
GCCAGTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAAATTTGAAGCGTTAA  
TATTTGTAAAATTCGCGTTAAATTTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAG  
AATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAAGCTGGACTCCAACGTCAAAGGGCGAAAAAC  
CGTCTATCAGGGCGATGGGGATCATGTAACCTCGCCTTATCCCTTTTTTGGGGTTCGAGGTGCCGTAAGCACTAAATCGGAACCTA  
AAGGGAGCCCCGATTTAGAGCTTGCAGGGGAAAGCCGGCGAACGTTGGCGAGAAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGTAGG  
GCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACACCGCCGCGCTTAAATGCGCCGCTACAGGGCGCGTCAAGTGGCACTTTTCG  
GGAAATGTGCGCGGAACCCCTATTTGTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCA  
ATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTTGTCGCTTATCCCTTTTTTGGCGCATTTTGCCTTCTGTTTTTGTCTAC  
CCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCC  
TTGAGATTTTCCGCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGCGTATTATCCCGTATTGACCGCGGC  
AAGAGCAACTCGTCCGCGATACACTATTCTCAGAATGACTTGGTTGAGTACTACACAGTACAGAAAAGCATCTTACGGATGGCATGACA  
GTAAGAGAATTATGACGTGCTGCCATAACCATGAGTGAACACTGCGGCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAA  
CCGCTTTTTTGCACAACATGGGGATCATGTAACCTCGCCTTATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAACGACGAGCGTGAC  
ACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTAGCTTCCCGCAACAATTAATAGACTG  
GATGGAGGGCGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTCCGGCTGGCTGGTTATTGCTGATAAATCTGGAGCCGGTGAAGCGTG  
GGTCTCGCGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCGATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGAT  
GAACGAAATAGACAGATCGATAGGTGCCTCACTGATTAAGCATGGTAACTGTCAGACCAAGTTACTCATATATACTTATGATTTGATTA  
TTTTAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTAACGTGAGTTTTCTGTTCCAC  
TGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGGTAATCTGCTGCTTGGCAAAC

**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.