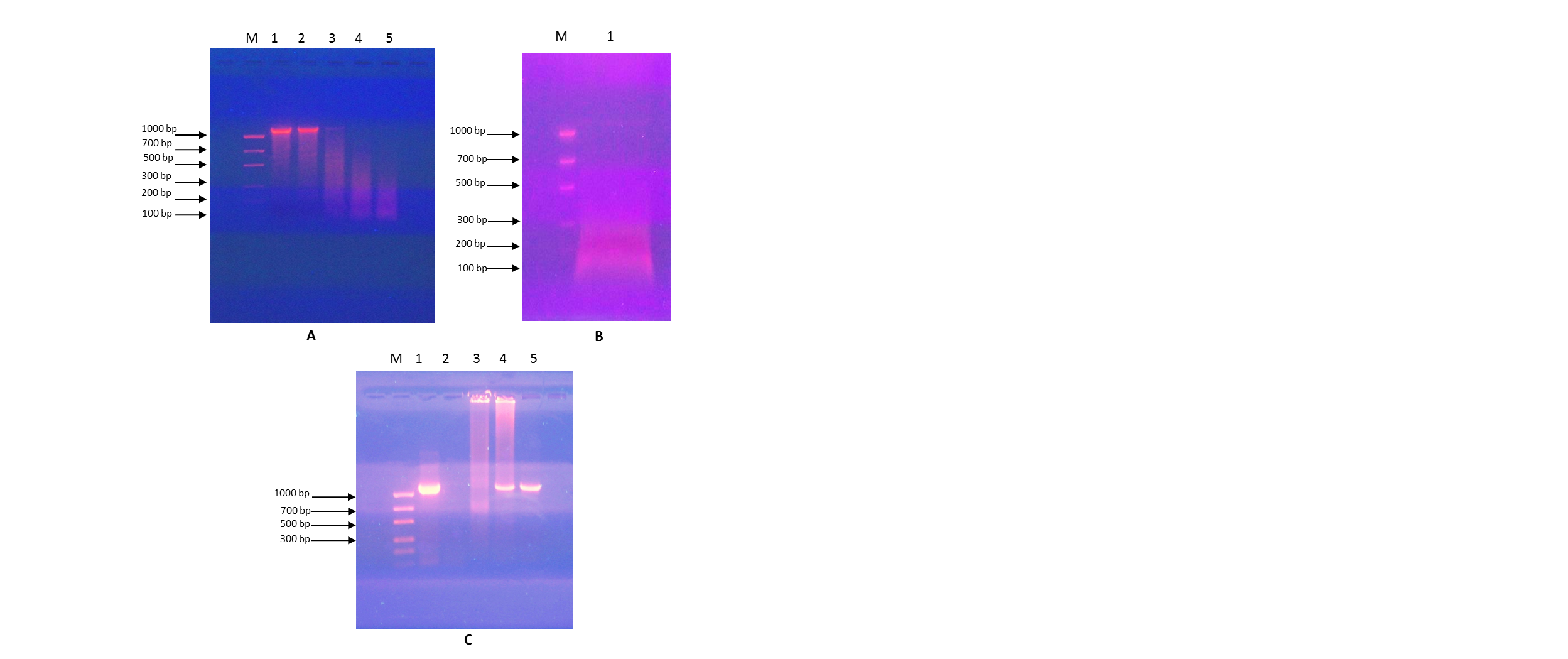
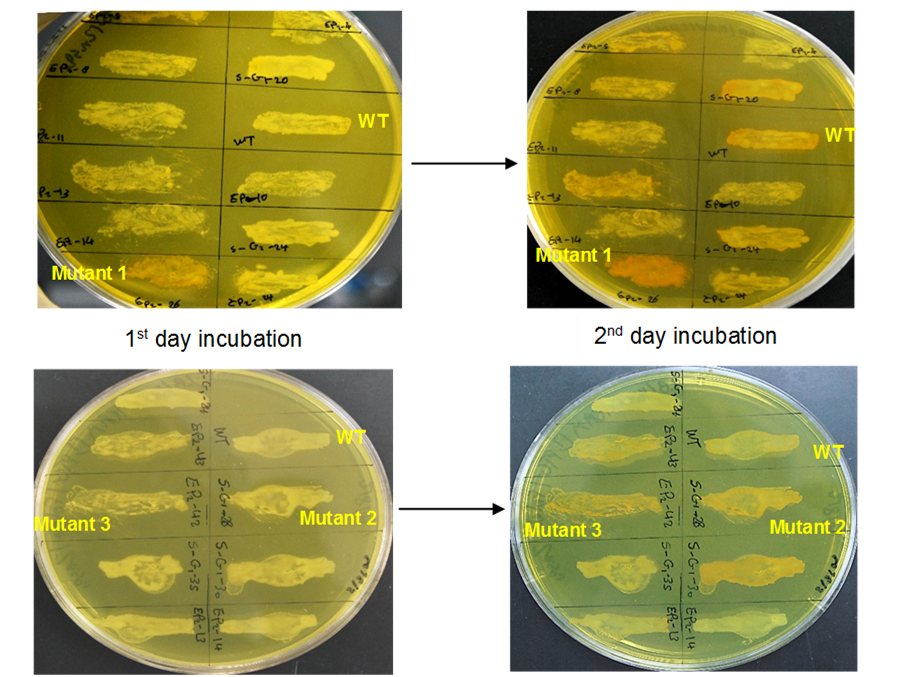
**Appendix A. Supplementary data**

**The following is Supplementary data to this article:**



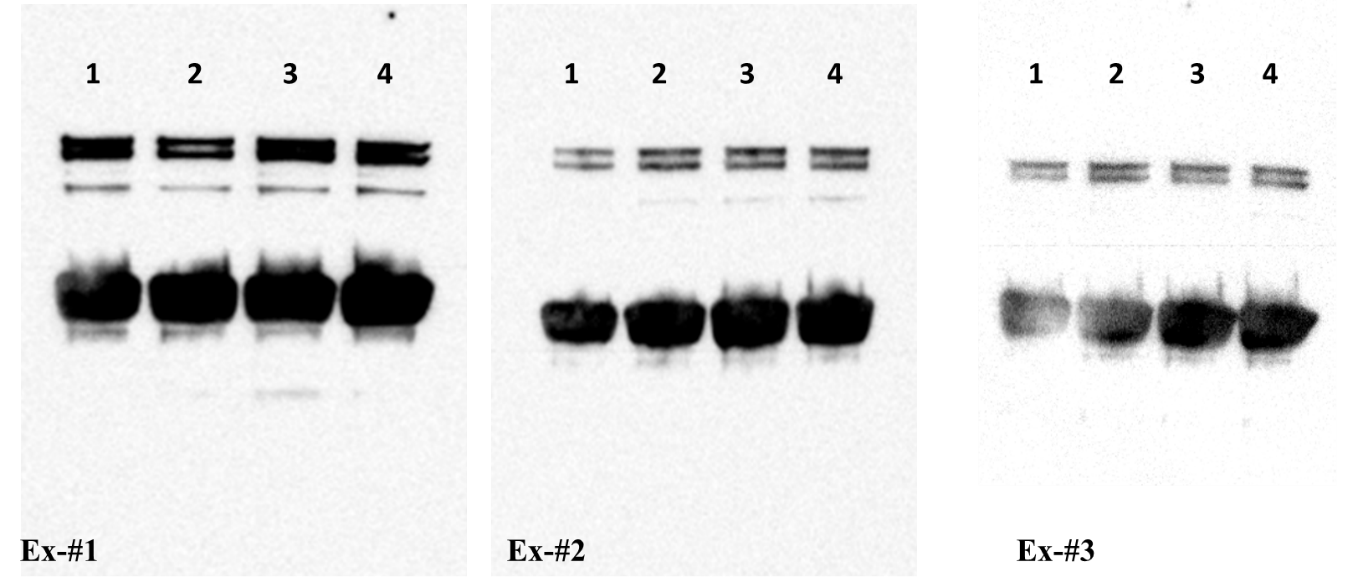
**Supplemental Figure S1*.*** DNA shuffling of glucarpidase**. A.** Time course of DNase digestion of the purified error-prone CPG2 DNA analysed by 2% agarose gel electrophoresis. M is the MassRuler™ Express DNA Ladder LR Forward (100-1000 bp); lanes 1-5 contain samples digested with DNase I for 30- sec, 1, 2, 3 and 5 min. (see experimental section for more details), **B.** Large scale DNase digestion of the error-prone PCR product where the 200-300bp size fragments were cut, and the DNA fragments were eluted, **C.** An overall summary of the entire DNA mutagenesis process, showing different stages in the production of variants of the CPG2 gene. M is the MassRuler™ Express DNA Ladder LR Forward (100-1000 bp); lane 1, error-prone PCR product; lane 2, DNase I fragments; lane 3, self-reassembled (primerless) PCR; lane 4, amplified PCR product obtained using specific primers for CPG2; lane 5 , purified shuffled PCR product ready for cloning.

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**Supplemental Figure S2*.*** Screening of the mutant CPG2 constructs in *E. coli* BL21(DE3)RIL by growth on LB plates supplemented with kanamycin, chloramphenicol, folate and IPTG. The arrow indicates an isolate that shows significantly darker coloration after two days of incubation relative to cells harboring the original pET28a-CPG2 construct. The higher the activity of glucarpidase, the more insoluble material will be produced and hence the darker the color formed.



**Supplemental Figure S3*.*** Multiple alignments of the amino acid sequences of the active glucarpidase gene mutants - CPG2I100T, CPG2G123S, and CPG2T329A - relative the wild type. Substituted amino acids, produced by shuffling, are underlined in red.



**Supplemental Figure S4.** Ex-#1, Ex-#2, and Ex-#3 are three independent western blot replicates. Lanes 1, 2, 3 and 4 are WT CPG2, CPG2 I100T, CPG2 T329A and CPG2 G123S, respectively