

A reduced *Escherichia coli* lacking mobile genetic elements

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Scarless genomic surgery was used to delete 43 genomic regions from the sequenced *Escherichia coli* strain, MG1655. Regions deleted include large K-islands, prophages, phage remnants, restriction modification genes, flagellar and chemotaxis related genes and all transposable elements including IS and RHS (recombination hot spot) elements. The resulting multiple deletion strain MDS43 has a 15.27% reduction in its genome size from 4,639,221 to 3,930,956 bp. It is also missing endonuclease A, the lac operon and is T1 phage resistant. Strain MDS43 along with intermediates MDS41 and 42, were characterized for growth on standard microbiological media, and for auxotrophy using the Biolog panel of 384 substrates. Transformation efficiency, global gene expression, and mutation rates were compared with the parent MG1655. As expected for strains lacking transposable elements, the measured frequency of mutation by IS hopping dropped to zero. Point mutation rates were virtually unchanged. Unexpectedly we found that several practical properties of the strains were improved. In particular, some gene sequences that were unclonable in wild type *E. coli* were able to be cloned in MDS strains. The reason for this unclonability was found to be hyperactive IS hopping into the target plasmid which was, of course, not possible in our construct. Reintroduction of the IS-free plasmid into DH10B or C600 resulted in immediate introduction of IS elements of a variety of types into the cloned gene. We conclude that the stress of producing a toxic protein induces transposition. Consistent with this hypothesis, increases in the IS transposition rate were measured in wild type *E. coli* cells subjected to heat shock, cold shock or upon induction of an expression plasmid for chloramphenicol acetyl transferase. The reduced genome *E. coli* strains can be efficiently transformed. Excellent yields of recombinant proteins have also been obtained. This demonstration of strain improvement through genome reduction provides further incentive for constructing additional reduced genome strains which will provide a “clean background” for functional genomics studies, a more efficient platform for biotechnology applications as well as a unique tool for studies of genome stability and evolution.