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(54) Title: GENETICALLY MODIFIED BACTERIA HAVING STABLE MUTATIONS WITHOUT THE REQUIREMENT OF AN-
TIBIOTIC MARKERS, AND SYSTEMS AND METHODS FOR GENERATING SAME

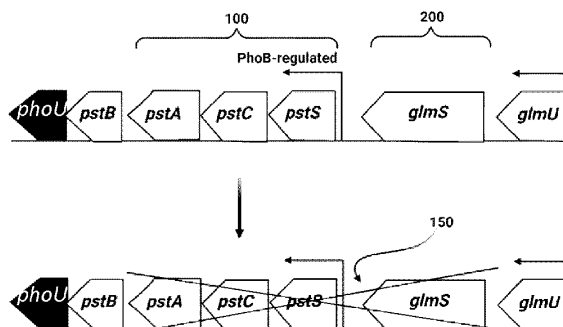


FIG. 1A

(57) Abstract: One drawback for using attenuated bacteria in cancer therapy and as vaccine vectors faces several challenges. These include ensuring safety and stability of the attenuated strains, achieving consistent colonization or delivery to the target site, and eliciting a controlled immune response. To address this, the present disclosure relates to systems, methods and genetically modified bacteria having reduced risk of reversing virulence without use of antibiotic markers. For example, the genetically modified bacteria comprises a chromosomal disruption in an auxotrophic gene and in one or more gene(s) involved in phosphate regulation, and a DNA rescue molecule comprising a functional copy of the auxotrophic gene. The genetically modified bacteria are obtained from virulent pathogenic bacteria containing a virulence plasmid.

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