
USAGodEaterBurstDLCPack14rar

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also, it does its work without using any command line switches and without being tied to a filename. it just treats everything as a template, and then processes it according to user-specified information. in this case some procedure has to be followed in order to get the files stored in the right location. thus, at the first launch of the executable file a dialog window that the necessary data is to be downloaded and then the configuration routine begins. once recombinants had been identified then ~2l of the culture was streaked onto lb+ cm6.25/kan22.5 plates and incubated overnight at 42c. the next day a few colonies were picked from each sector and patch plated onto lb+cm/kan and lb+amp plates and incubated overnight at 30c. the vast majority of the cmr or kanr recombinants were phenotypically amps suggesting that the resident plasmid psim6 had been cured from

the recombinants. while the genome of *e. albertii* has been sequenced, to the best of our knowledge, we have not encountered any reports demonstrating chromosomal modifications in *e. albertii*. here we describe a simple modified protocol using the lambda red recombineering system to generate chromosomal mutations in *e. albertii*, which appears to be genetically recalcitrant. using this protocol we successfully deleted the lee-encoded genes, *ler* and *grlra*, and the non-lee encoded gene *hfq*, albeit at frequencies lower than those reported in *e. coli*. this protocol will aid pathogeneticists to rapidly construct knockout, knockin, epitope-tagged, transcriptional, translational, and other subtle mutations in *e. albertii* in order to comprehensively characterize its molecular mechanisms of virulence to successfully counteract this emerging broad host pathogen.

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