Description

We developed GetX, a stand-alone python script that allows the user to design mutation cassettes for scarless genome editing in bacteria using our previously described two-step recombination method. GetX allows the user to design synthetic DNA fragments required to perform bacterial genome editing with simple inputs of 1) genome information from the target strain and 2) information for the desired mutation. GetX designs DNA fragments for introduction of sequences for protein tagging, deletions, insertions, and point mutations. It also designs synonymous codon fragments required to manipulate essential target genes to enable survival of the strain during the genome editing procedure. GetX provides genbank and fasta format files for a mutation template plasmid, a mutation cassette, and a sequence around the mutation site to guide the user through the design of the materials required for the scarless bacterial genome editing method.

getx.py generates mutation fragments and provides files useful to perform the facile genome modification method described in "A versatile and highly efficient method for scarless genome editing in Escherichia coli and Salmonella enterica (2014), BMC Biotechnology 14:84, DOI: 10.1186/1472-6750-14-84."

CITATION

Please cite the original manuscript below for genome editing methods and procedure. "A versatile and highly efficient method for scarless genome editing in Escherichia coli and Salmonella enterica (2014), BMC Biotechnology 14:84, DOI: 10.1186/1472-6750-14-84." Please refer to the following link to cite for the python script.
"https://sourceforge.net/projects/getx/"

INSTALLATION AND RUNNING THE SCRIPT

getx is written and tested with python 2.7.6 and biopython 1.63 on OSX 10.6.8. Standalone executable versions were tested on Mac OS X 10.6 (64 bit), Ubuntu 12.04.4 (64 bit), and on Windows 7 (32 bit). Current version may not work with other version of python or biopython. Copy the getx.py script and the following genbank files (pHA1887_4.gb, T2ISceI_C_2.gb, T2ISceI_K_2.gb, T2ISceI_T_2.gb, and T2ISceI_C2_2.gb) in a folder. Download a genbank or a fasta file of the target genome that the user wish to modify. Run the script by executing the script by python, “python getx.py”.

USE OF OUTPUT FILES

getX will generate seven files into the same folder where the user runs the script including three individual fasta files for a mutation cassette template plasmid, for a mutation cassette, and a small region of genome surrounding the mutation, three individual genbank files for the same above, and a “.tab” file including the 5’-mutation fragment and 3’-mutation fragment sequences, sequences of PCR primers required to amplify a mutation cassette from its template plasmid. We recommend the user to examine three genbank files to confirm the design using a genbank file viewer. We have been using CLC Main workbench (available from Qiagen Bioinformatics) successfully to examine the genbank files and later to confirm the sequence of plasmids and to confirm sequence surrounding the part of the genome manipulated. We also recommend to

design a set of PCR primers surrounding the region manipulated manually to confirm the mutants created. The script is not designed to generate the latter primers.

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