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MUTATER: Tool for the Introduction of Custom Position Based Mutations in Protein and Nucleotide Sequences

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Abstract

MUTATER is an innovative computer based tool that has been designed to create custom mutations in Protein and Nucleotide sequences. It has the ability to work on both protein and nucleotide sequences, and in both cases, inputs are given by the user in RAW format. Then user has to define the position of Mutation and the amino acid / nucleotide change that is required at that specific position(s) in the sequence files. The output is been shown in the box with an option to save newly formed sequence file and also displays the changes that are being made in a separate Change Box. Thus it serves as a Basic but completely Novel and unique tool when it comes to create Mutations and changes in Sequence files. MUTATER has been developed in C # and is fully compatible with Windows based PCs and is freely available at the website. http://sites.google.com/site/mutater/

Keywords: Amino acid; FASTA; RAW; Protein; Nucleotide; Mutation tool; Sequence analysis

Introduction

Sudden permanent change is an inherited characteristic of an organism. [http://www.encyclopedia.com/doc/ 1O142-mutation.html].Protein design and analysis techniques widely incorporate point mutations (Parthiban et al., 2006). Point mutations, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another (Fersht, 1997). The production of point mutation in a sequence is now routinely performed in molecular biology laboratories since the development of protein-engineering techniques (Freese and Ernst, 1959).

In the field of Bioinformatics, Genomics, Genetics and wherever Sequences are used in the form of text files for computer based Mutational Studies and other calculations, one of the basic steps is always to obtain a Sequence file and then produce changes in it. Normally sequence files are of length Thousand plus in terms of alphabets denoting Amino acids and Nucleotide bases and it's a time consuming and Error prone job when a researcher has to manually go through the text files looking for the position where he/she can perform changes in order to proceed Mutational studies. Since data in text files has no numbering pattern, there is always chance of error present due to repetitive occurrence of Amino acids and Bases.

Therefore keeping this in mind MUTATER has been developed. It is very basic yet useful tool. Beta versions of this had been developed in C++ and then implemented with Graphical User Interface (GUI) using C Sharp. This is a windows compatible software and very light on resources.

It takes input in the form of sequence files for both protein and nucleotide sequences and then efficiently produce desired sequence mutations. Mutations can be created at any position inside the sequence file(s). Users only have to enter the position number and the new amino acid or nucleotide one letter code into the tool Mutation Box

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and new sequence file generates. Sequence mutation rate isn't limited and one can enter or create as many mutations as required into their sequence files. Changes always appear in Bold and Highlighted in both original and mutated sequence for easier readability and the Results Box also display changes that have been produced and with the option to save mutated sequences in default format.

Implementation

MUTATER has been made to create custom mutations in nucleotide and protein sequences. A brief overview of how MUTATER works on sequence files and the logic that has been implemented is as under:

Upon executing the exe file of MUTATER, it displays GUI of the program (Figure 1). There are separate tabs to work on protein and nucleotide sequences. GUI is presented with "browse" button to locate the sequence file and upload it into the MUTATER. Input files are given in Text document format sequence file as Input from the hard drive, flash drive or user specified position and then display inside the input box.

User is facilitated with two choices, Protein Mutate and Nucleotide Mutate tabs. After uploading protein sequence in the protein mutate tab, user is given two choices: 1st to

ng MUTATER V 3.0		
MUTATER		
Protein Mutate Nucleotide Mutate Gene to Protein		
Browse File		
Original Sequence		
, Mutation Box		
Enter The Mutation		
Position Amino Acid OK Cancel		
Mutated Sequence	Amino Count	length
	Sava Aa	Cancel
	Save As	Cancer
Change Box		
		*
		-
Developed By: Azeem Butt ©.2007 - 2008. All Rights Reserve	ed	V 3.0

Figure 1: MUTATER GUI.

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MUTATER V 3.0
MUTATER
rotein Mutate Nucleotide Mutate Gene to Protein
Browse File C:\Users\Zammy\Desktop\Protein.bt
Original Sequence
MEDGKPVWAPHPTDGFQMGNIVDIGPDSLTIEPLNQKGKTFLALINQVFPAEEDSKKDVEDNCSLMYLNEATLLHNIKVRYSKDRIYTVV ANILIAVNPYFDIPKIYSSEAIKSYQGKSLGTRPPHVFAIADKAFRDMKVLKMSQSIIVSGESGAGKTENTKFVLRYLTESYGTGQDIDDRIV EANPLLEAFGNAKTVRNNNSSRFGKFVEIHFNEKSSVVGGFVSHYLLEKSRICVQGKEERNYHIFYRLCAGASEDIREKLHLSSPDNFRYL NRGCTRYFANKETDKQILQNRKSPEYLKAGSMKDPLLDDHGDFIRMCTAMKKIGLDDEEKLDLFRVVAGVLHLGNIDFEEAGSTSGGCN LKNKSAQSLEYCAELLGLDQDDLRVSLTTRVMLTTAGGTKGTVIKVPLKVEQANNARDALAKTVYSHLFDHVVNRVNQCFPFETSSYFIG VLDIAGFEYFEHNSFEQFCINYCNEKLQQFFNERILKEEQELYQKEGLGVNEVHYVDNQDCIDLIEAKLVGILDILDEENRLPQPSDQHFTS AVHQKHKDHFRLTIPRKSKLAVHRNIRDDEGFIIRHFAGAVCYETTQFVEKNNDALHMSLESLICESRDKFIRELFESSTNNNKDTKQKAG KLSFISVGNKFKTQLNLLLDKLRSTGASFIRCIKPNLKMTSHHFEGAQILSQLQCSGMVSVLDLMQGGYPSRASFHELYNMYKKYMPDKL ARLDPRLFCKALFKALGLNENDYKFGLTKVFFRPGKFAEFDQIMKSDPDHLAELVKRVNHWLTCSRWKKVQWCSLSVIKLKNKIKYRAE
Enter The Mutation
Position 10 Amino Acid D OK Cancel
Mutated Sequence Amino Count 1285
MEDGKPVWALHPTDGFQMGNIVDIGPDSLTIEPLNQKGKTFLALDNQVFPAEEDSKKDVEDNCSLMYLNEATLLHNIKVRYSKDRIY TYVANILIAVNPDFDIPKIYSSEAIKSYQGKSLGTRPPHVFAIADKAFRDMKVLKMSQSIIVSGESGAGKTENTKFVLRYLTESYGTGQDI DDRIVEANPLLEAFGNAKTVRNNNSSRFGKFVEIHFNEKSSVVGGFVSHYLLEKSRICVQGKEERNYHIFYRLCAGASEDIREKLHLSS PDNFRYLNRGCTRYFANKETDKQILQNRKSPEYLKAGSMKDPLLDDHGDFIRMCTAMKKIGLDDEEKLDLFRVVAGVLHLGNIDFEE AGSTSGGCNLKNKSAQSLEYCAELLGLDQDDLRVSLTTRVMLTTAGGTKGTVIKVPLKVEQANNARDALAKTVYSHLFDHVVNNRVN QCFPFETSSYFIGVLDIAGFEYFEHNSFEQFCINYCNEKLQQFFNERILKEEQELYQKEGLGVNEVHYVDNQDCIDLIEAKLVGILDILDE ENRLPQPSDQHFTSAVHQKHKDHFRLTIPRKSKLAVHRNIRDDEGFIIRHFAGAVCYETTGFVEKNNDALHMSLESLICESRDKFIREL FESSTNNNKDTKQKAGKLSFISVGNKFKTQLNLLLDKLRSTGASFIRCIKPNLKMTSHHFEGAQILSQLQCSGMVSVLDLMQGGYPSR ASFHELYNMYKKYMPDKLARLDPRLFCKALFKALGLNENDYKFGLTKVFFRPGKFAEFDQIMKSDPDHLAELVKRVNHWLTCSRWK
Save As Cancel
Change Box
Normal Amino Acid At Position= 10 is Proline AND After Mutation New Amino Acid At Position= 10 is Aspartic Acid
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Figure 2: MUTATER GUI. Amino acid sequence is showing along with mutated sequence with other details including sequence length, Position and mutated amino acid.

enter position or location of amino acid in numbers where mutation is required and 2nd choice requires the input of the Alphabet, the letter code of Amino acid (Figur 2). MUTATER then creates a new text file containing a "change "with the option to save the newly formed sequence. In addition, MUTATER displays New Sequence file as well as Mentioning "before" and "after" conditions of the Sequence file and also emboldens the 'Change 'positions in Both Original and mutated sequences in the display box to make them eye-catching and flashy. Nucleotide Mutate (Figure 3) works in same manner as that of protein mutate except that its subject area is Nucleotide sequences.

Results and Discussion

The objective of developing the MUTATER is to allow researchers and users to quickly work on, modify and qualify sequence files, and ultimately saving the time during research. MUTATER has been successfully executed and both the Protein Mutate and Nucleotide Mutate tabs were successfully implemented and developed.

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			MUTATER		
otein Mutate Nu	cleotide Mutate	Gene to Protein			
Browse File	C:\Users\Zamr	ny\Desktop\Protein	ı.bd		
Original Seq	uence				
MEDGKPVWAPHI ANILIAVNPYFDIPI EANPLLEAFGNAK VRGCTRYFANKE JKNKSAQSLEYCA /LDIAGFEYFEHN AVHQKHKDHFRL KLSFISVGNKFKT(ARLDPRLFCKALF Mutation Box	PTDGFQMGNIV KIYSSEAIKSYQ (TVRNNNSSRF TDKQILQNRKS VELLGLDQDDLF SFEQFCINYCNE TIPRKSKLAVHF QLNLLLDKLRST KALGLNENDYK	DIGPDSLTIEPLNQ GKSLGTRPPHVFA GKFVEIHFNEKSSV PEYLKAGSMKDPL RVSLTTRVMLTTA KLQQFFNERILKE RNIRDDEGFIIRHF/ GASFIRCIKPNLKM FGLTKVFFRPGKF	KGKTFLALINQVFPAEEDSK IADKAFRDMKVLKMSQSIIV VGGFVSHYLLEKSRICVQG LDDHGDFIRMCTAMKKIGL GGTKGTVIKVPLKVEQANNV EQELYQKEGLGVNEVHYVD AGAVCYETTQFVEKNNDALI MTSHHFEGAQILSQLQCSGI AEFDQIMKSDPDHLAELVKI	KDVEDNCSLMYLNEATLLHNI SGESGAGKTENTKFVLRYLTE KEERNYHIFYRLCAGASEDIRE DDEEKLDLFRVVAGVLHLGNII NQDCIDLIEAKLVGILDILDEN NQDCIDLIEAKLVGILDILDEEN HMSLESLICESRDKFIRELFES IVSVLDLMQGGYPSRASFHEL RVNHWLTCSRWKKVQWCSL	KVRYSKDRIYTYV SYGTGQDIDDRIV EKLHLSSPDNFRYL DFEEAGSTSGGCN NQCFPFETSSYFIG IRLPQPSDQHFTS STNNNKDTKQKAG YNMYKKYMPDKL SVIKLKNKIKYRAE
Enter The I	Autotion				
	viutauon				
Position	10 An	nino Acid D	OK	Cancel	
Mutated Se	quence			Amino C	ount 1285
MEDGKPVWAD TYVANILIAVNP DDRIVEANPLLE PDNFRYLNRGC AGSTSGGCNLK QCFPFETSSYFI ENRLPQPSDQF FESSTNNNKDT ASFHELYNMYK	HPTDGFQMGN DFDIPKIYSSEAI EAFGNAKTVRN CTRYFANKETDI (NKSAQSLEYCA GVLDIAGFEYFE IFTSAVHQKHKI 'KQKAGKLSFIS' (KYMPDKLARLE	IVDIGPDSLTIEPLN KSYQGKSLGTRPF NNSSRFGKFVEIH (QILQNRKSPEYLK ELLGLDQDDLRVS HNSFEQFCINYCN DHFRLTIPRKSKLA /GNKFKTQLNLLLE)PRLFCKALFKALG	NQKGKTFLALDNQVFPAEED PHVFAIADKAFRDMKVLKMS FNEKSSVVGGFVSHYLLEKS AGSMKDPLLDDHGDFIRM SLTTRVMLTTAGGTKGTVIK IEKLQQFFNERILKEEQELYC IVHRNIRDDEGFIIRHFAGAN DKLRSTGASFIRCIKPFRPGK	DSKKDVEDNCSLMYLNEATLLI QSIIVSGESGAGKTENTKFVLF RICVQGKEERNYHIFYRLCAG TAMKKIGLDDEEKLDLFRVV/ VPLKVEQANNARDALAKTVYS IKEGLGVNEVHYVDNQDCIDLI CYETTQFVEKNNDALHMSLE SHHFEGAQILSQLQCSGMVS' FAEFDQIMKSDPDHLAELVKR	HNIKVRYSKDRIY RYLTESYGTGQDI ASEDIREKLHLSS GVLHLGNIDFEE SHLFDHVVNRVN EAKLVGILDILDE SLICESRDKFIREL VNDLMQGGYPSR VNHWLTCSRWK
MEDGKPVWAD TYVANILIAVNP DDRIVEANPLLE PDNFRYLNRGC AGSTSGGCNLK QCFPFETSSYFI ENRLPQPSDQF FESSTNNNKDT ASFHELYNMYK	HPTDGFQMGN DFDIPKIYSSEAI ZAFGNAKTVRN CTRYFANKETDI (NKSAQSLEYCA GVLDIAGFEYFE IFTSAVHQKHKI 'KQKAGKLSFIS' (KYMPDKLARLE	IVDIGPDSLTIEPLN KSYQGKSLGTRPF NNSSRFGKFVEIH KOLGLDQDDLRVS ELLGLDQDDLRVS HNSFEQFCINYCN DHFRLTIPRKSKLA /GNKFKTQLNLLLD PRLFCKALFKALG	NQKGKTFLALDNQVFPAEED PHVFAIADKAFRDMKVLKMS FNEKSSVVGGFVSHYLLEKS AGSMKDPLLDDHGDFIRM SLTTRVMLTTAGGTKGTVIK EKLQQFFNERILKEEQELYG VHRNIRDDEGFIIRHFAGAN XLRSTGASFIRCIKPFRPGK	OSKKDVEDNCSLMYLNEATLLI QSIIVSGESGAGKTENTKFVLI IRICVQGKEERNYHIFYRLCAG CTAMKKIGLDDEEKLDLFRVV/ VPLKVEQANNARDALAKTVY IKEGLGVNEVHYVDNQDCIDLI CYETTQFVEKNNDALHMSLE ISHHFEGAQILSQLQCSGMVS' FAEFDQIMKSDPDHLAELVKR	HNIKVRYSKDRIY RYLTESYGTGQDI ASEDIREKLHLSS GVLHLGNIDFEE SHLFDHVVNRVN EAKLVGILDILDE SLICESRDKFIREL VLDLMQGGYPSR VNHWLTCSRWK ~
MEDGKPVWAD TYVANILIAVNP DDRIVEANPLLE PDNFRYLNRGC AGSTSGGCNLK QCFPFETSSYFI ENRLPQPSDQF FESSTNNNKDT ASFHELYNMYK	HPTDGFQMGN DFDIPKIYSSEAI ZAFGNAKTVRN CTRYFANKETDJ (NKSAQSLEYCA GVLDIAGFEYFE IFTSAVHQKHKI 'KQKAGKLSFIS') (KYMPDKLARLE	IVDIGPDSLTIEPLN KSYQGKSLGTRPF NNSSRFGKFVEIH (GILQNRKSPEYLK ELLGLDQDDLRV3 HNSFEQFCINYCN DHFRLTIPRKSKLA /GNKFKTQLNLLLD)PRLFCKALFKALG	NQKGKTFLALDNQVFPAEED PHVFAIADKAFRDMKVLKMS FNEKSSVVGGFVSHYLLEKS (AGSMKDPLLDDHGDFIRM SLTTRVMLTTAGGTKGTVIIK EKLQQFFNERILKEEQELYC (VHRNIRDDEGFIIRHFAGA))KLRSTGASFIRCIKPNLKM LNENDYKFGLTKVFFRPGK	DSKKDVEDNCSLMYLNEATLLI QSIIVSGESGAGKTENTKFVLI RICVQGKEERNYHIFYRLCAG CTAMKKIGLDDEEKLDLFRVV/ VPLKVEQANNARDALAKTVY KEGLGVNEVHYVDNQDCIDLI CYETTQFVEKNNDALHMSLE ISHHFEGAQILSQLQCSGMVS FAEFDQIMKSDPDHLAELVKR	HNIKVRYSKDRIY RYLTESYGTGQDI ASEDIREKLHLSS GGVLHLGNIDFEE SHLFDHVVNRVN EAKLVGILDILDE SLICESRDKFIREL VLDLMQGGYPSR VNHWLTCSRWK AS Cancel
MEDGKPVWAD TYVANILIAVNP DDRIVEANPLLE PDNFRYLNRGC AGSTSGGCNLK QCFPFETSSYFI ENRLPQPSDQF FESSTNNNKDT ASFHELYNMYK	HPTDGFQMGN DFDIPKIYSSEAI SAFGNAKTVRN CTRYFANKETDI (NKSAQSLEYCA GVLDIAGFEYFE HFTSAVHQKHKI 'KQKAGKLSFIS' (KYMPDKLARLE K Acid At Pos Acid	IVDIGPDSLTIEPLN KSYQGKSLGTRPF NNSSRFGKFVEIH WILGNRKSPEYLK ELLGLDQDDLRVS HNSFEQFCINYCN DHFRLTIPRKSKLA /GNKFKTQLNLLLD PRLFCKALFKALG	VQKGKTFLALDNQVFPAEED PHVFAIADKAFRDMKVLLKMS FNEKSSVVGGFVSHYLLEKS AGSMKDPLLDDHGDFIRM SLTTRVMLTTAGGTKGTVIK EKLQQFFNERILKEEQELYG VHRNIRDDEGFIIRHFAGAN XLRSTGASFIRCIKPNLKM LNENDYKFGLTKVFFRPGK	SKKDVEDNCSLMYLNEATLLI QSIIVSGESGAGKTENTKFVLI IRICVQGKEERNYHIFYRLCAG CTAMKKIGLDDEEKLDLFRVV/ VPLKVEQANNARDALAKTVY IKEGLGVNEVHYVDNQDCIDLI CYETTQFVEKNNDALHMSLE ISHHFEGAQILSQLQCSGMVS FAEFDQIMKSDPDHLAELVKR Save	HNIKVRYSKDRIY RYLTESYGTGQDI ASEDIREKLHLSS GVLHLGNIDFEE SHLFDHVVNRVN EAKLVGILDILDE SLICESRDKFIREL VLDLMQGGYPSR VNHWLTCSRWK ~ As Cancel

Figure 3: MUTATER GUI. Nucleotide sequence is showing along with mutated sequence with other details including sequence length, Position and mutated nucleotide in position.

Protein Mutate

In this tab, Protein sequence files were uploaded through Browse button and MUTATER was able to read one letter amino codes successfully. Amino Count which counts the length of the sequence also showed correct number for different sequence files. Desired position and amino acid were added in Mutation Box and the Mutated file was successfully acquired along with complete account of Changes in the sequence file before and after the mutation display inside the "Change box". (Figure 2).

Nucleotide Mutate

Second tab of the program works on Nucleotide sequences. It read the sequence file precisely as an input, successfully calculated Nucleotide count and produced mutated file with the desired amendments. Again all the changes were displayed in the "Change Box" with the option to save newly created file (Figure 3).

The experimental sequence files of Proteins and Nucleotides were taken randomly from the Different Databases e.g. NCBI [http://www.ncbi.nlm.nih.gov/], Expasy [http:/

/www.expasy.ch/]. MUTATER appeared to have almost 99% success rate during the testing. It successfully read the sequence files from the specified locations, counted total sequence lengths and produced mutated files with the option to save the new file so it can be used afterwards.

The GUI of the program is also very simple, unproblematic and self explanatory. This is completely new and simple tool which will replace the traditional Manual method used currently that has high ratio of errors.

With MUTATER, users can easily create multiple mutations, count the sequence length, save new sequence files and see changes in Real Time Environment and save lot of precious time during different research projects.

Current version of this software is available for download in exe format, next step which is already in progress is to make it as an online tool with more accuracy and functionality and also able to read sequence files in FASTA and other formats including currently supported RAW format and also to make it compatible for Linux environment. Future development will also include ability to distinguish between types of mutations for example missense , non sense, point mutations etc and to work on multiple sequence files at a time and numbering of whole sequence files of amino acids and nucleotides in both input and

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output text boxes for easy readability.

Availability and Requirements

- **Project name:** MUTATER
- Current Version : 3.0
- **Project home page:** http://sites.google.com/site/ mutater/
- **Operating system(s):** Windows based PC
- **Programming language:** C++/ C #

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