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Restriction mapping

Aim:- To construct restriction maps using NEB cutter version 2.0

Query:- P

Introduction:-

This tool will take a DNA sequence & find the large non-overlapping open reading frames using the E. coli genetic code & sites for all Type II & Commercially available type III restriction enzymes that cut sequences just once. By default only enzymes available from NEB are used. But other sets may be chosen. Just enter your sequence & submit. Further option will appear with output. The maximum size of the input is 1 Mbyte & the maximum sequence length is 300 kbases.

Query:- PBR322

URL:- www.ncbi.nlm.nih.gov/
nc2.neb.com/neb cutter 2/

Procedure:-

→ Log on to NCBI (www.ncbi.nlm.nil.gov/)
→ Retrieve a nucleotide sequence of your own interest in FASTA format.

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-> Log on to (nc2.neb.com/NEB cutter 2.1)
-> Paste the retrieved nucleotide seq in the space given in NEB cutter welcome page.
-> Click on either Linear / Circular option.
-> Click on either NEB enzyme / commercial enzyme option.
-> Click on submit option.
-> Phase I result will be displayed.

Procedure for phase II Results:
-> Click on the option called 2 Cutters / 3 Cutters on phase I result page.
-> On clicking on to 2 Cutters & 3 Cutter the tool shows about the no. of times a restriction enzyme cuts.
-> Select two points on sequence obtained in phase I results & click on flanking enzyme option.
-> Thus phase II result are obtained.

Procedure for phase III Results:
-> Click on option called custom digest
-> Then select the enzyme of your interest & click on to option called custom digest.
-> In order to view gel electrophoresis which on a option 'view gel' that gives the gel electrophoresis diagram.
-> In the gel electrophoresis diagrammatic page. User has an option to given their interest

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of maker agarose %.
Thus phase III results is obtained.

Result & Discussion:

A restriction mapping is done with the help of NEB cutter V2.0 tool.
This tool not only used to obtain the restriction map but also action / the user to obtain the following data.

i) This tool allow the user to obtain the result in both linear & Circular pattern.
ii) 2 Cutter :- A particular restriction enzyme which has two restriction sites on DNA.
iii) 3 Cutter: A particular restriction enzyme cleans the DNA thrice.
iv) Flanking enzymes.
v) Custom digest [This option allow the user to select main interested restriction enzymes list. in order to clean the given DNA query].
vi) View gel [This option allow the user to view electrophoresis pattern of the restriction maps which have been obtained custom digest].

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