Restriction Enzymes Worksheet	
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Objective(s):

- Identify restriction sites.
- Show differences between blunt and sticky (cohesive) end cuts.
- Compare restriction enzyme differences on identical DNA.

Directions: Identify the <u>restriction sites</u> for each of the examples given. Show the cuts, <u>sticky</u> (<u>cohesive</u>) or <u>blunt</u>, number of DNA fragments produced and the number of base pairs in each (count the top row). (If there are three nucleotides on either side of the dash it is a blunt cut. If there are less than three on either side of the dash it is cohesive cut.)

- HpaI --- 5' GTT AAC 3'
 - 5' GGATGTTAACAATCTCTACGGGTTAACACCCTTGGGTTAACATCCGCGG 3'
 - 3' CCTACAATTGTTAGAGATGCCCAATTGTGGGAACCCAATTGTAGGCGCC 3'

Number of pieces of DNA _____

- Ssp1 --- 5' AAT ATT 3'
 - 5' GGATAATATT GTTAACAATCTCTACGGGTTAACACCCTTGGAATATTTTAA 3'
 - 3' CCTATTATAACAATTGTTAGAGATGCCCAATTGTGGGAACCTTATAAAATT 3'

Number of pieces of DNA _____

- 3. Psrl --- 5' CTGCA G 3'
 - 5' ACGCTGCA/GACGTATTATTAT CCGCCGCTGCA/GCC GTCATCA 3'
 - 5' TGCG/ACGTCTGCATAATAGGCGGCG/ACGTCGGCAGTAGT 3'

Number of pieces of DNA _____

- 4. HindII --- 5' GTC GAC 3'
 - 5' ACGACGTAGTCGACTTATTAT GTCGACCGCGCGTGTCGACCATCA 3'
 - 5 TGCTGCATCAGCTGAATAATACAGCTGGGCGCGCACAGCTGGTAGT 3*

Number of pieces of DNA _____

- 5. EcoRI --- 5' G AATTC 3'
 - 5' ACG ACGTATTAGAATTCTTAT CCGCCGCCGGAATTCT CATCA 3'
 - 5' TGC TGCATAATCTTAAGAATAGGCGGCGGCCTTAAGAGTAGT 3'

Number of pieces of DNA _____

Restriction Enzymes Worksheet Answers

Wei Ren

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Restriction Enzymes with Asymmetrical Recognition Sites S. P. Bennet, University of Bristol, 1987 Methods for Purification of Restriction Enzymes Thomas A. Bickle, Markus Streiff, 1983 Sure, cut buffers for restriction enzymes ,1992 Recognition Domains of Type I Restriction Enzymes Alexander Anthony Frank Gann, 1988 Three Exclusive Restriction Enzymes from Amersham ,1985* **Restriction Enzyme Analysis of Incomplete Parental Replicative Forms of ØX174** Alicia Morales Bogardus,1979 Production and Characterization of Recombinant Restriction Enzyme NgoMIV and Its Mutant Wei Ren, 2012 Discovery of restriction enzymes has led to the development of recombinant DNA technology and restriction enzymes are currently widely used in biotechnology medicine and research Till now thousands of restriction enzymes have been discovered purified characterized and commercialized As well as production of restriction enzymes has gained a huge profit in biotechnology industry Although restriction enzymes have been applied well in current molecular biology researches a lot of questions regarding their binding and catalytic mechanisms haven t been clearly explained or described by scientists NgoMIV is a tetrameric restriction enzyme which has been discovered and

purified about 20 years ago from Neisseria gonorrhoeae MS11 It belongs to the type II P and type II F subtypes The recognition site of NgoMIV is 5 G CCGGC 3 which is a palindromic sequence Also the tetrameric protein has two catalytic cores which means that it will bind with two cognate sites and cut them both NgoAIV which was discovered in another strain FA1090 of Neisseria gonorrhoeae is different from NgoMIV protein in two amino acid residues A hybrid protein called NgoIV hb was designed based on the differences In the thesis study here the expression of NgoMIV and its mutant was conducted To undercover the preference of the distance between two recognition sites digestion tests by applying a plasmid with four recognition sites as substrate were conducted At last several proposed mechanisms of catalysis based on the protein motion predicted by anisotropic elastic network model and current existing crystal structures were raised *Isolation of the Restriction Enzyme Sfa and Analysis of Its Recognition Sequence* Charles T. King,1975 *REBASE* New England Biolabs,1998 A collection of information about restriction enzymes methylases and the microorganisms from which they have been isolated isoschizomers recognition sequences cleavage sites methylation specificity commercial availability and references both published and unpublished Home page

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