BIOINFORMATICS: LECTURE 4: Molecular Techniques - DNA FOOTPRINTING

Course name: Bioinformatics and Computer Application

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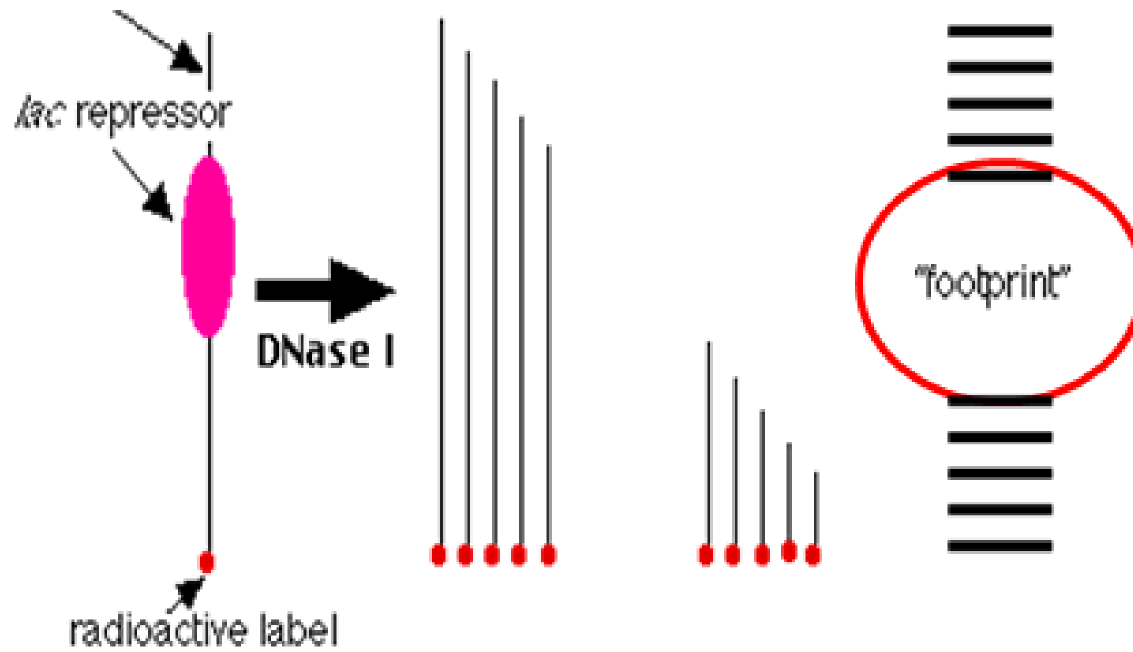
INTRODUCTION

- DNA footprinting is a method of investigating the sequence specific of DNA – binding protein in vitro.
- This technique can be used to study protein – DNA interactions both outside and within cell.
- Techniques like DNA footprinting help elucidate which protein bind to these associated region of DNA and unravel the complexities of transcriptional control.

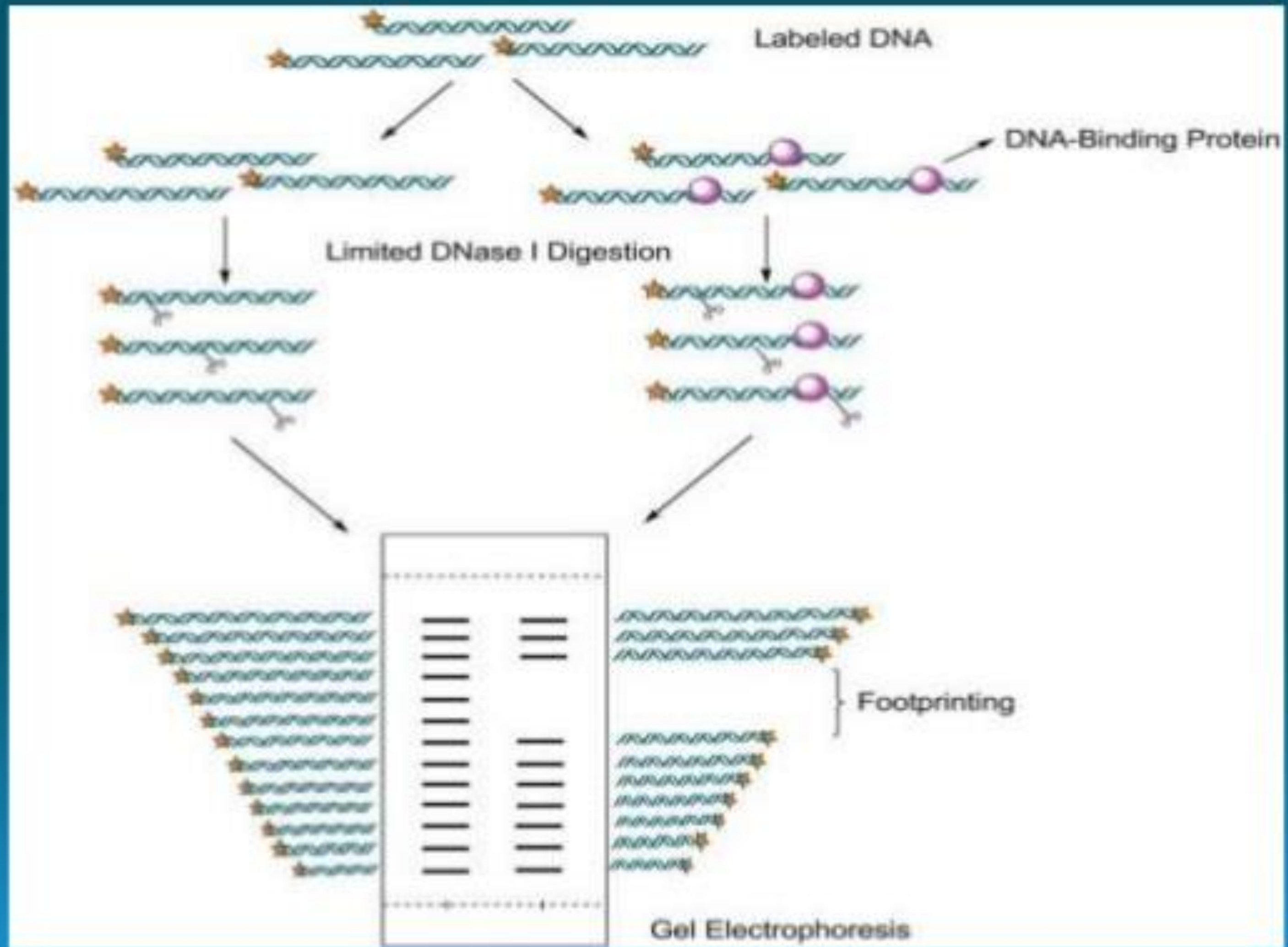
HISTORY

- In 1978,David galas and Albert Schmitz developed the DNA footprinting technique to study the binding specificity of the lac repressor protein .
- It was originally a modification of the maxam – gilbert chemical sequencing technique .

Principle



Procedure:



Labeling

- The DNA template can be labeled at the 3′ or 5′ end , depending on the location of binding sites .
- Labels that can be used are : radioactivity and fluorescence .
- Radioactivity has been traditionally used to label DNA fragments for footprinting analysis.
- Radioactive labelling is very sensitive and is optimal for visualising small amount of DNA .
- Fluorescence is a desirable advancement due to the hazards of using radio-chemicals .

- However, it has been difficult to optimize because it is not always sensitive enough to detect the low concentrations of the target DNA strands used in a DNA footprintings experiments.
- Electrophoretic sequencing gels or capillary electrophoresis have been successfully in analysing foot printing of fluorescent tagged fragments .

Cleavage agent

- A variety of cleavage agent can be chosen .
- Ideally a desirable agent is one that is sequence neutral , easy to use , and is easy to control .
- The following cleavage agent are described in detail : **DNase I** is a large protein that function as a double – strand endonuclease .
- It binds the minor group of DNA and cleaves the phospodiester backbone .it is good cleavage agent for footprinting because its size makes it easily physically hindered .thus is more likely to have to its action blocked by a bound protein on a DNA sequence In addition , the **DNase I** enzyme is easily controlled by adding EDTA to stop the reaction .

- **Hydroxyl radicals** are created from the Fenton reaction , which involves reducing Fe2+ with H2o2 to form free hydroxyl molecules .These hydroxyl molecules react with the DNA backbone resulting in a break. Due to their small size , the resulting DNA footprint has a high resolution .
- **Ultraviolet irradiation** can be used to excite nucleic acid and create photoreactions , which result in damaged bases in a DNA strand. Advantages of uv are that it reacts with very quickly and therefore capture the interactions that are only momentary .

Applications

- **DNA footprinting is often used to identify the binding sites of proteins in a DNA molecule. Researchers often use this technique to identify whether a particular protein can activate or inhibit transcription. In addition, scientists also use this method to detect where proteins bind to DNA in a living cell.**

Thank You!