Course :Genomics

GENOME MAPPING

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Genetic and Physical Maps

- **Genetic mapping** is based on the use of genetic techniques to construct maps showing the positions of genes and other sequence features on a genome. Genetic techniques include cross-breeding experiments or, in the case of humans, the examination of family histories (pedigrees).

- **Physical mapping** uses molecular biology techniques to examine DNA molecules directly in order to construct maps showing the positions of sequence features, including genes. **Physical mapping**

Drawbacks of using gene as marker

- Genes are very useful markers but they are by no means ideal. One problem, especially with larger genomes such as those of vertebrates and flowering plants, is that a map based entirely on genes is not very detailed

DNA Markers

- Mapped features that are not genes are called **DNA markers**. As with gene markers, a **DNA marker** must have at least two alleles to be useful.
- There are three types of DNA sequence feature that satisfy this requirement:
  - Restriction fragment length polymorphisms (**RFLPs**),
  - Simple sequence length polymorphisms (**SSLPs**),
  - Single nucleotide polymorphisms (**SNPs**).

DNA Molecular Markers

First Generation DNA Markers

YEAR		NAME
1974	RFLP	Restriction Fragment Length Polymorphism

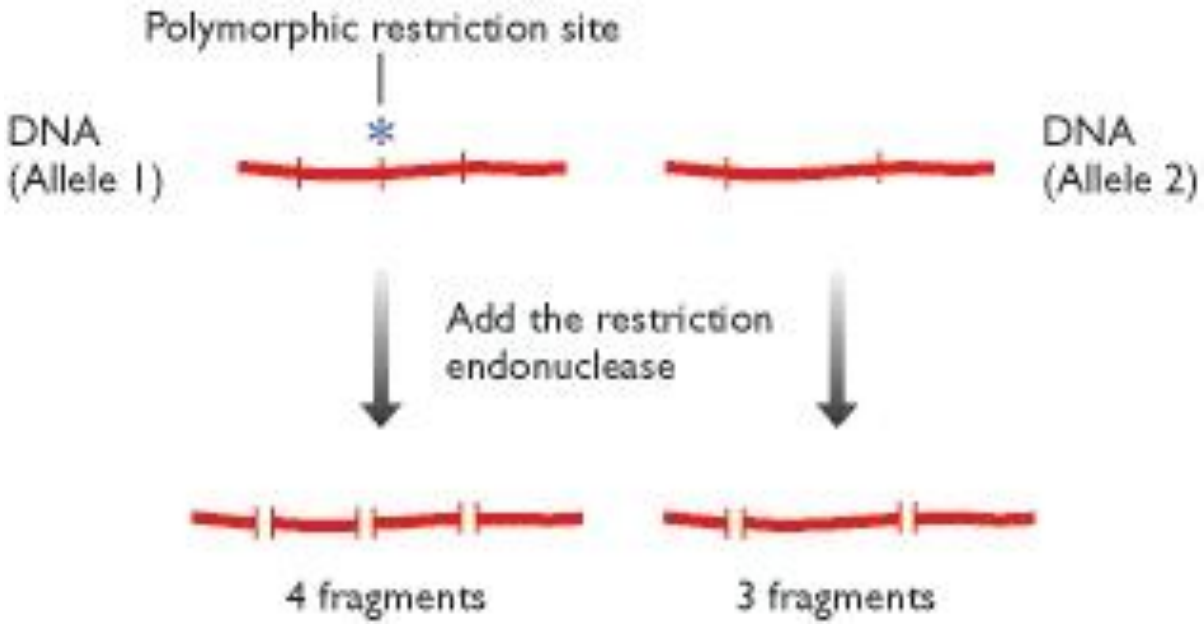
Second Generation DNA Markers

1990	RAPD	Randomly amplified Polymorphic DNA
1992	SSR	Simple sequence Repeats

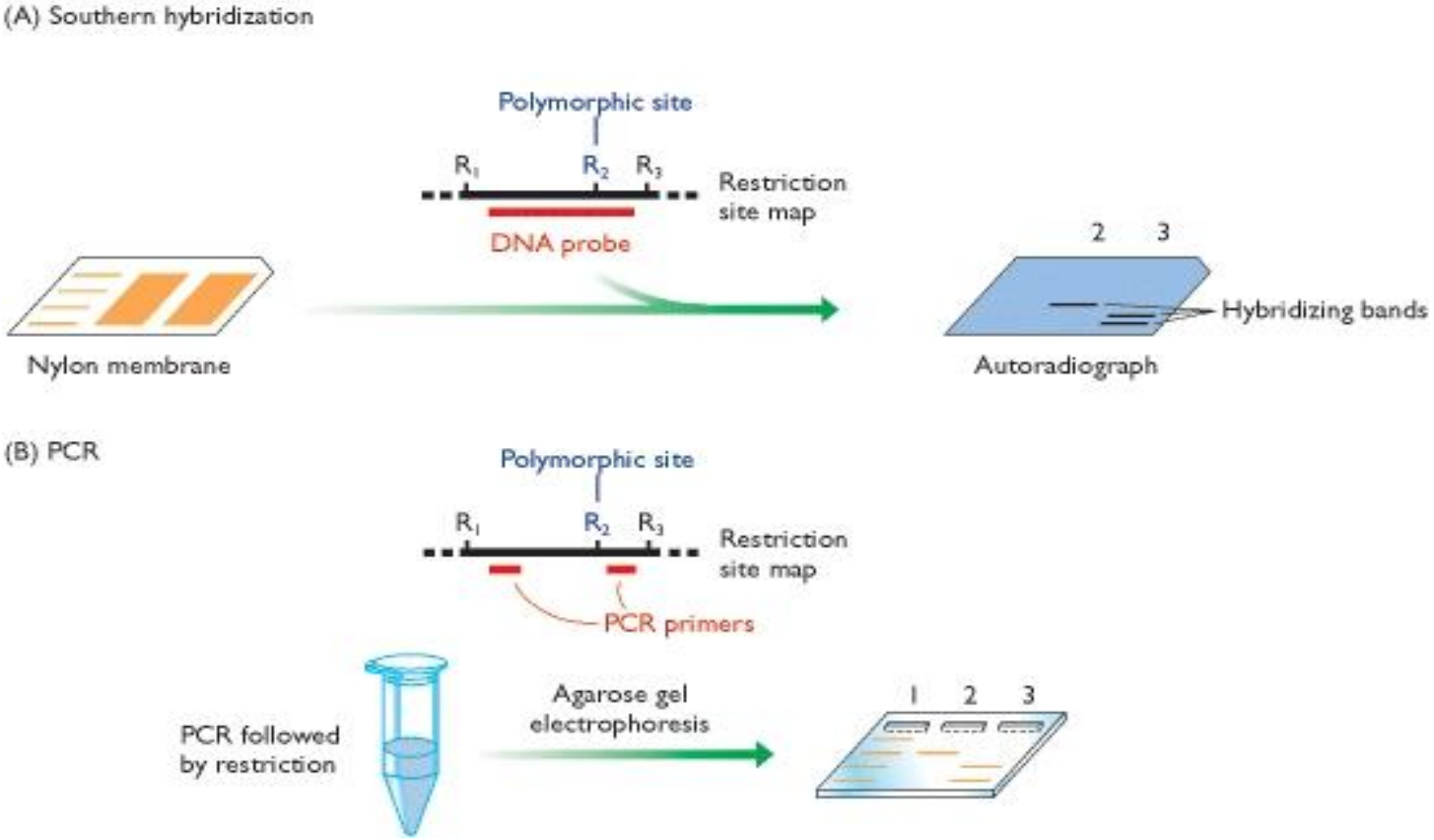
New Generation DNA Markers

1994	SNP	Single Nucleotide Polymorphism
1995	AFLP	Amplified Fragment Length Polymorphism

Restriction fragment length polymorphisms (RFLPs)



Two methods of scoring RFLP



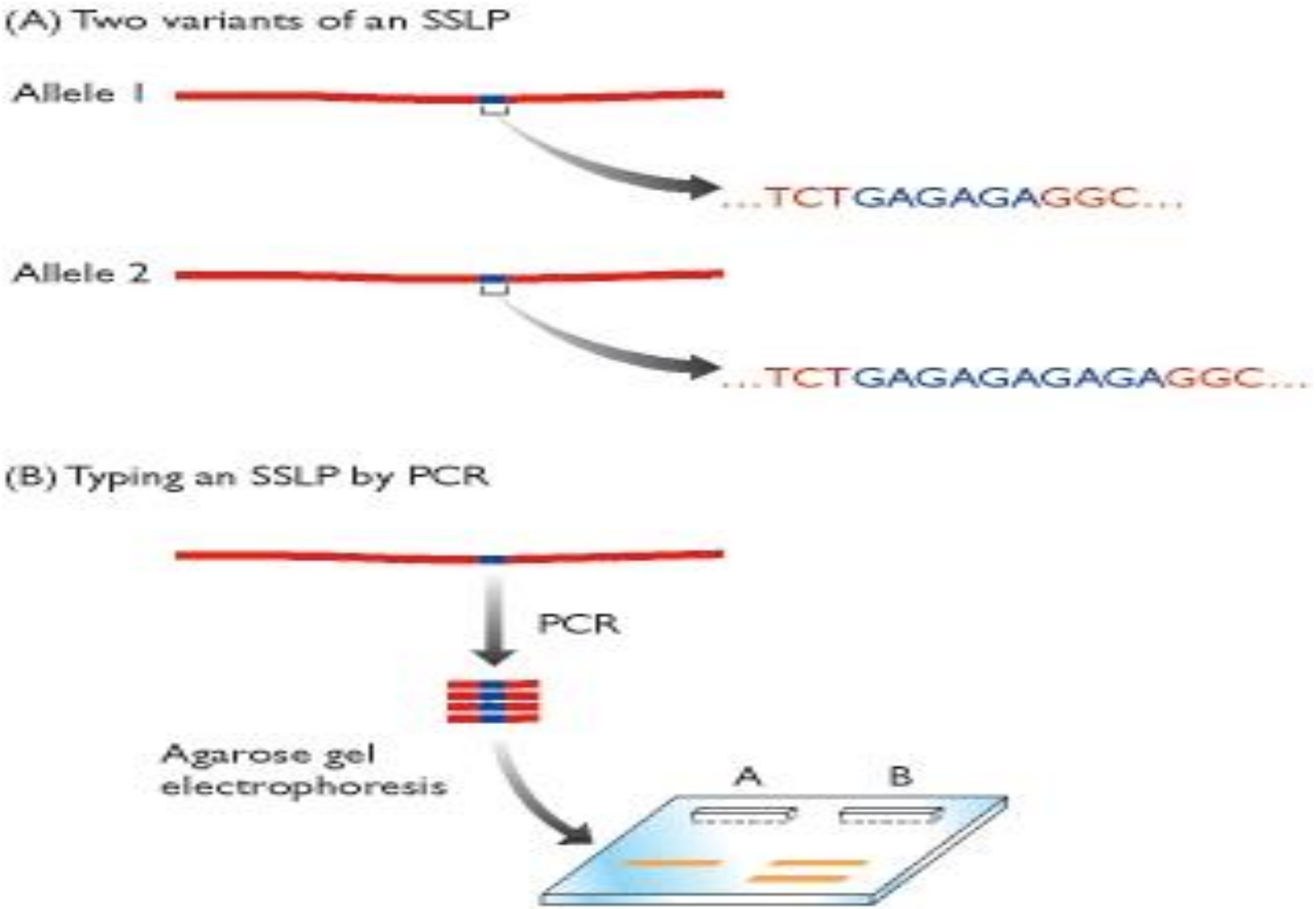
Simple sequence length polymorphisms (SSLPs)

- **SSLPs** are arrays of repeat sequences that display length variations, different alleles containing different numbers of repeat units

Two types :

- **Minisatellites**, also known as **variable number of tandem repeats** (**VNTRs**), in which the repeat unit is up to 25 **bp** in length;

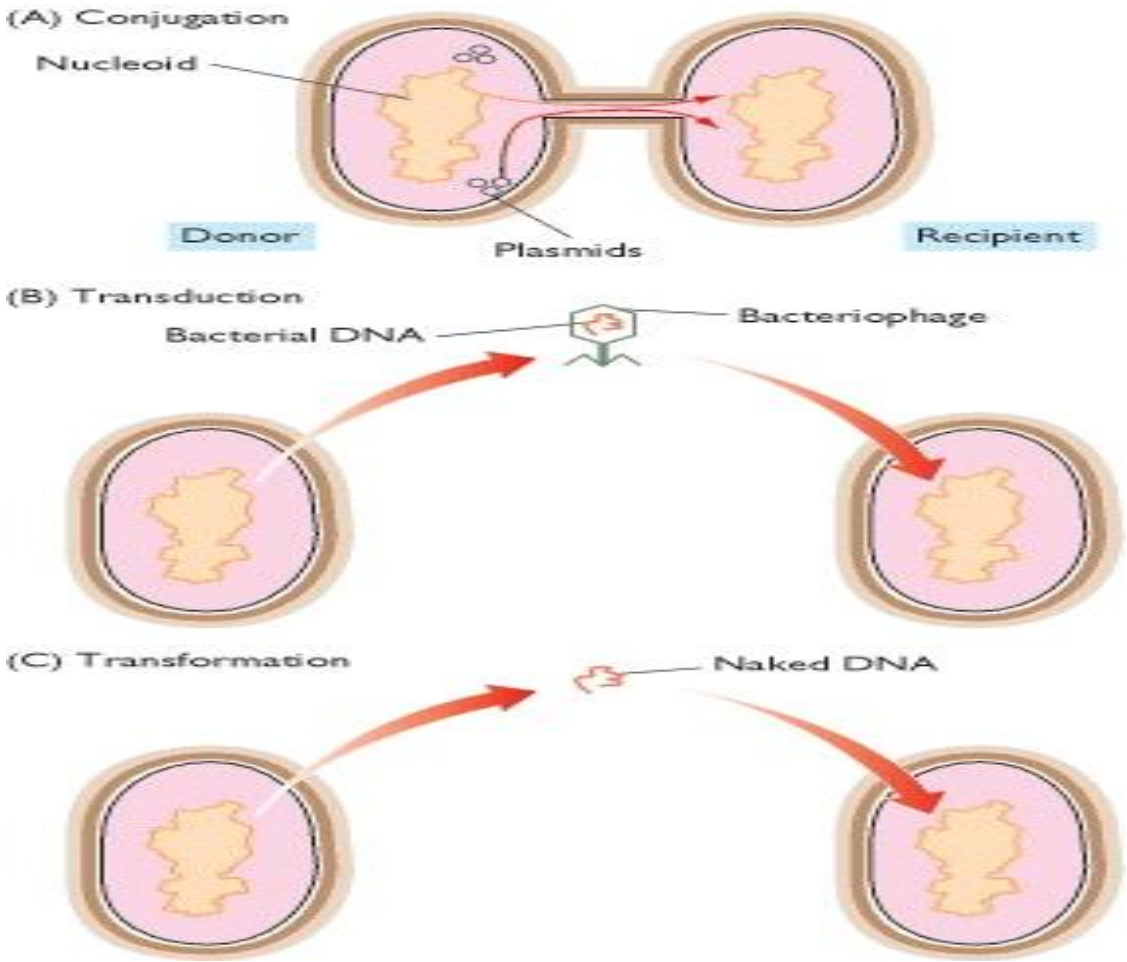
- **Microsatellites** or **simple tandem repeats** (**STRs**), whose repeats are shorter, usually dinucleotide or tetranucleotide units.


(A) Two variants of an SSLP
Allele 1
...TCTGAGAGAGGC...
Allele 2
...TCTGAGAGAGAGAGGC...
(B) Typing an SSLP by PCR
PCR
Agarose gel electrophoresis
A
B

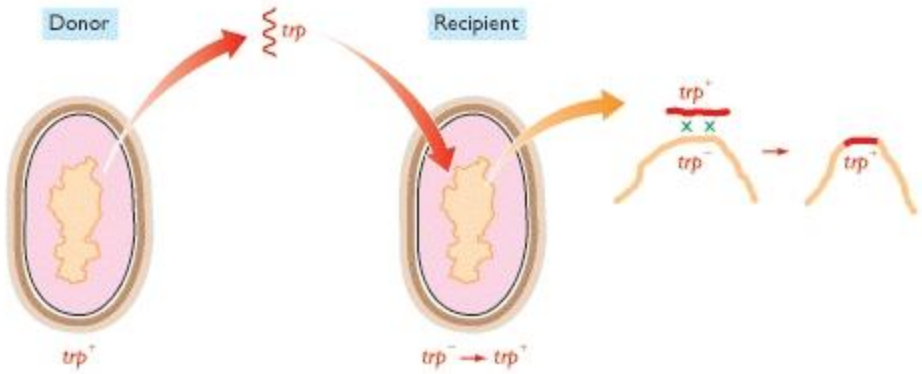
Microsatellites are popular than Minisatellites

- Minisatellites are not spread evenly
- But found at more telomeric regions
- PCR typing is easy
- 10 -30 copies of minisatellites ,not longer than 4bp

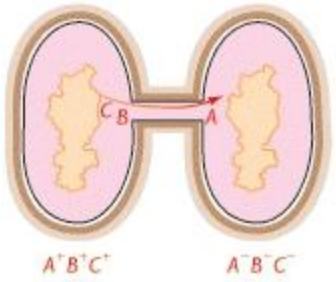
Genetic mapping in bacteria

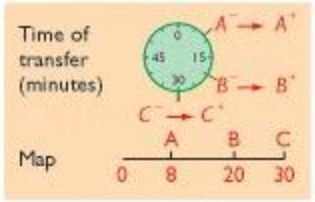


(A) Transfer of DNA between donor and recipient bacteria

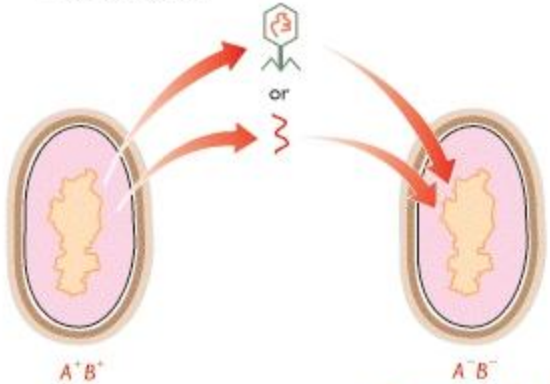


(B) Sequential transfer of markers during conjugation





(C) Co-transfer of closely linked markers during transduction or transformation



Frequency with which $A^-B^- \rightarrow A^+B^+$ depends on how close together *A* and *B* are on the chromosome

Physical Mapping

- ***The resolution of a genetic map depends on the number of crossovers that have been scored*** **.**

- ***Genetic maps have limited accuracy***

Physical Mapping Types

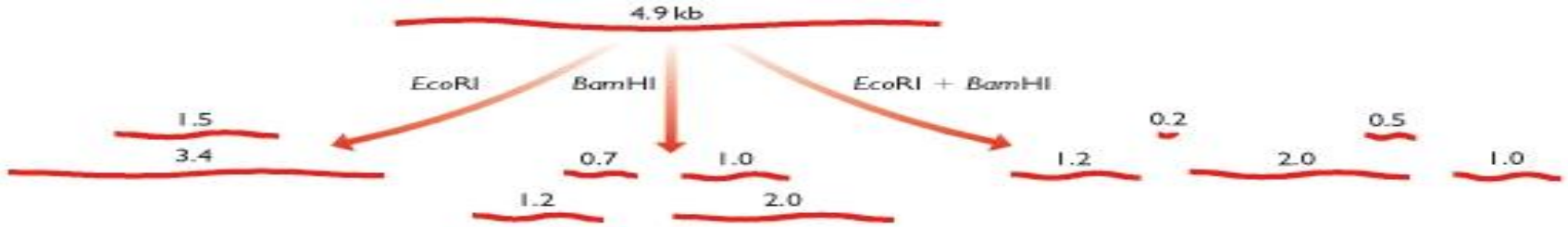
- **Restriction mapping**, which locates the relative positions on a DNA molecule of the recognition sequences for restriction endonucleases;

- **Fluorescent *in situ* hybridization (FISH)**, in which marker locations are mapped by hybridizing a probe containing the marker to intact chromosomes;

- **Sequence tagged site (STS) mapping**, in which the positions of short sequences are mapped by **PCR** and/or hybridization analysis of genome fragments.

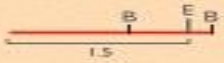
Restriction mapping

- **Genetic mapping** using **RFLPs** as **DNA markers** can locate the positions of polymorphic restriction sites within a genome , but very few of the restriction sites in a genome are polymorphic, so many sites are not mapped by this technique



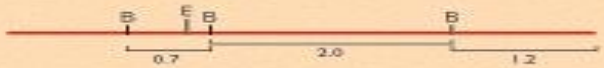
INTERPRETATION OF THE DOUBLE RESTRICTION

Fragments	Conclusions
0.2 kb, 0.5 kb	These must derive from the 0.7 kb *Bam*HI fragment, which therefore has an internal *Eco*RI site:
1.0 kb	This must be a *Bam*HI fragment with no internal *Eco*RI site. We can account for the 1.5 kb *Eco*RI fragment if we place the 1.0 kb fragment thus:
1.2 kb, 2.0 kb	These must also be *Bam*HI fragments with no internal *Eco*RI sites. They must lie within the 3.4 kb *Eco*RI fragment. There are two possibilities:



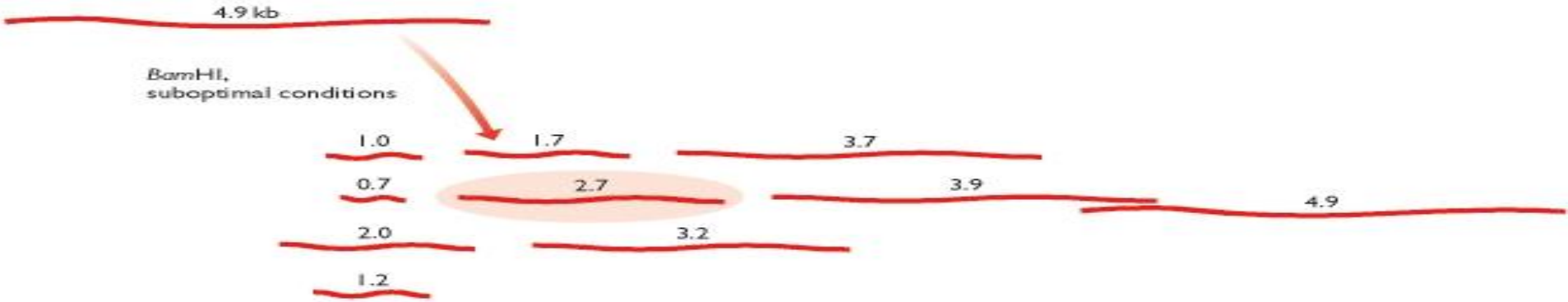
MAP I

MAP II



PREDICTED RESULTS OF A PARTIAL *Bam*HI RESTRICTION

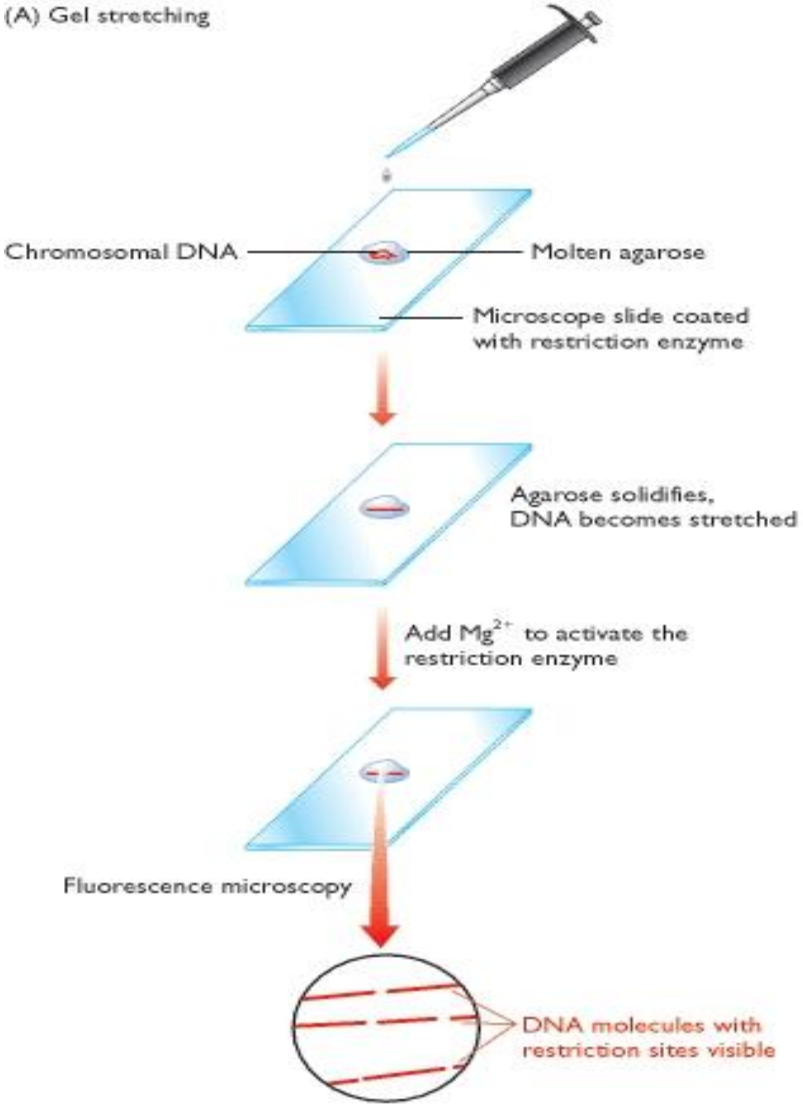
If Map I is correct, then the partial restriction products will include a fragment of 1.2 + 0.7 = 1.9 kb
If Map II is correct, then the partial restriction products will include a fragment of 2.0 + 0.7 = 2.7 kb

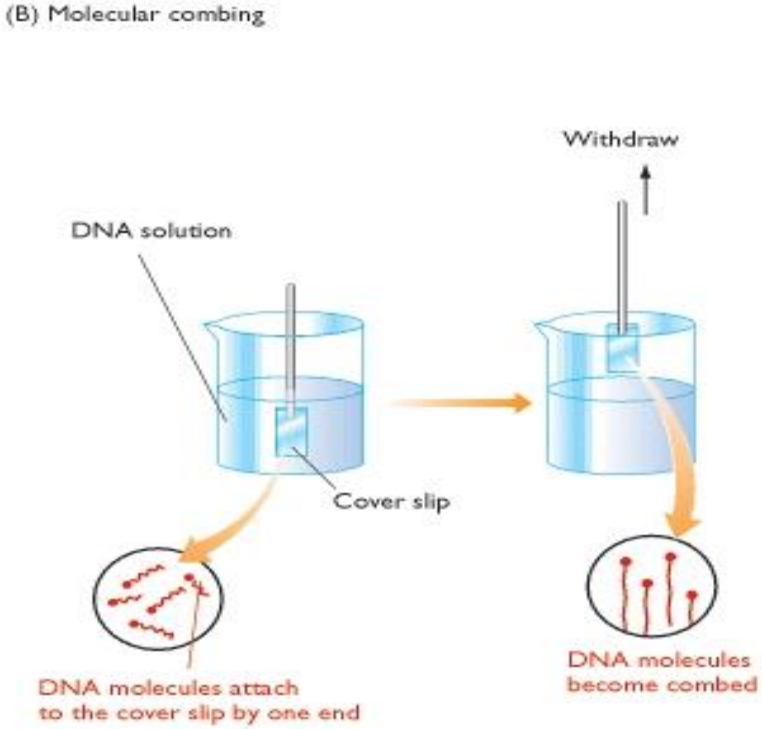


CONCLUSION
Map II is correct

Direct examination of DNA molecules for restriction sites

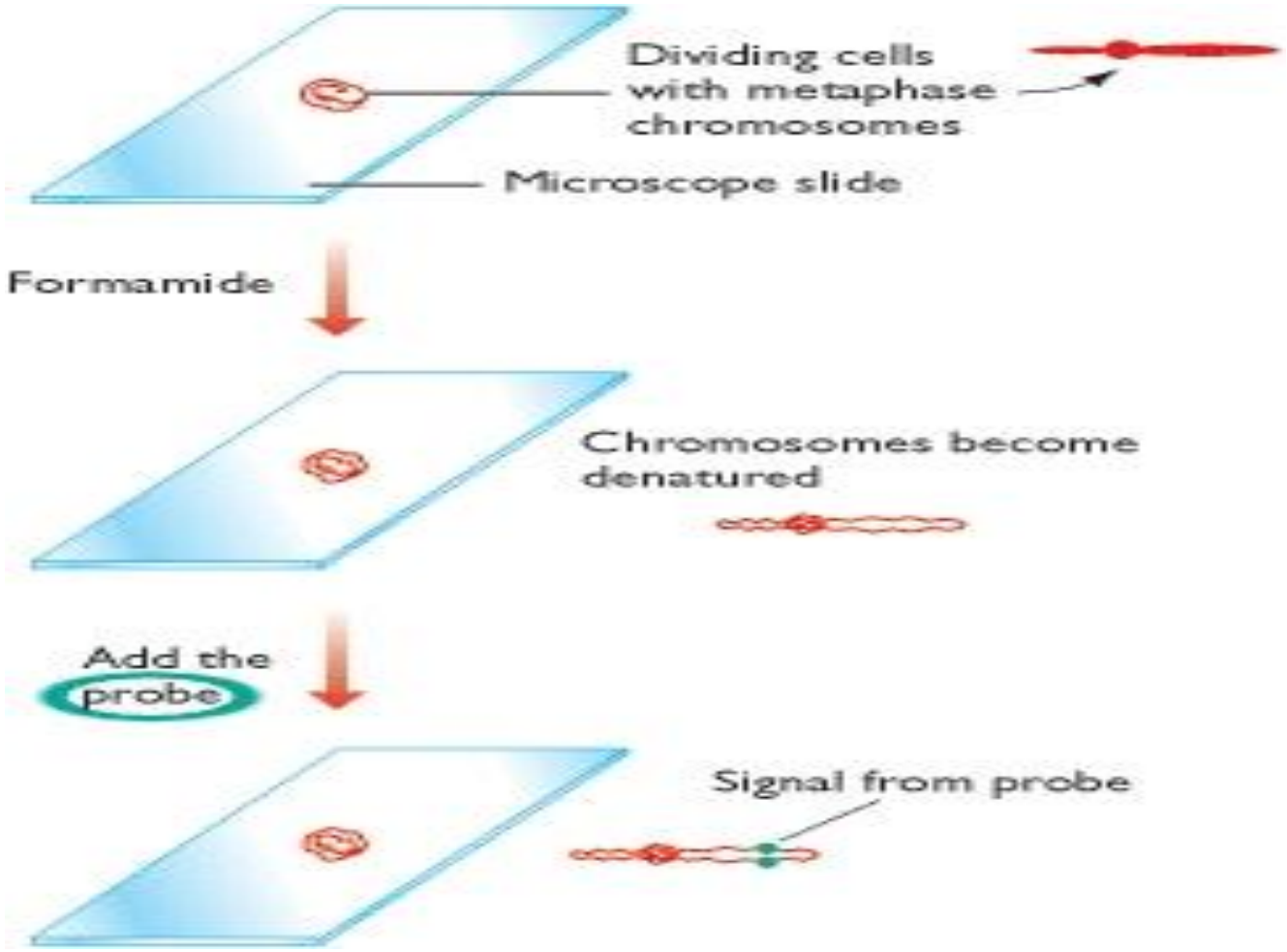
- Optical mapping: Restriction sites are directly located by looking at the cut DNA molecules with a microscope. The DNA must first be attached to a glass slide in such a way that the individual molecules become stretched out, rather than clumped together in a mass. There are two ways of doing this: **gel stretching** and **molecular combing**.


(A) Gel stretching
Chromosomal DNA
Molten agarose
Microscope slide coated with restriction enzyme
Agarose solidifies, DNA becomes stretched
Add Mg^{2+} to activate the restriction enzyme
Fluorescence microscopy
DNA molecules with restriction sites visible

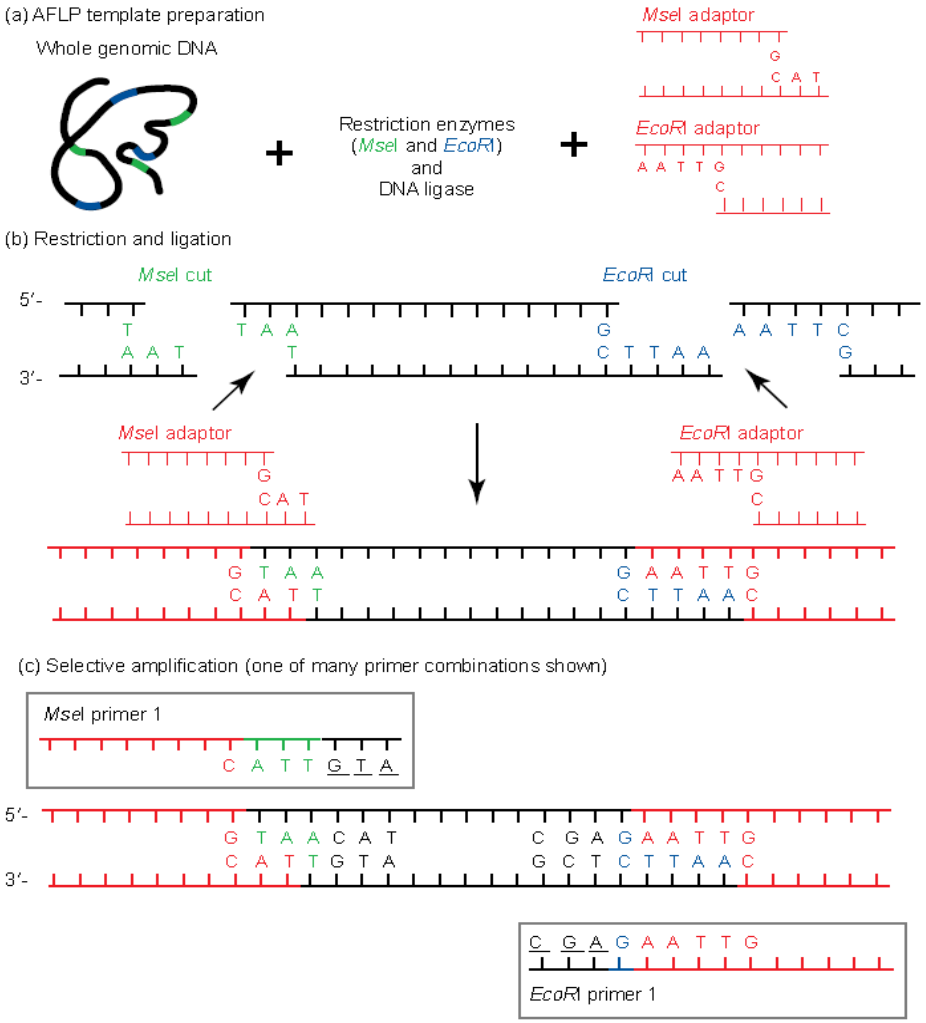

(B) Molecular combing
Withdraw
DNA solution
Cover slip
DNA molecules attach to the cover slip by one end
DNA molecules become combed

Fluorescent *in situ* hybridization (FISH)

- *In situ* hybridization is a version of hybridization analysis in which an intact chromosome is examined by probing it with a labeled DNA molecule. The position on the chromosome at which hybridization occurs provides information about the map location of the DNA sequence used as the probe


Dividing cells
with metaphase
chromosomes
Microscope slide
Formamide
Chromosomes become
denatured
Add the
probe
Signal from probe

AFLP (Amplified Fragment Length Polymorphism)



Sample AFLP Profile