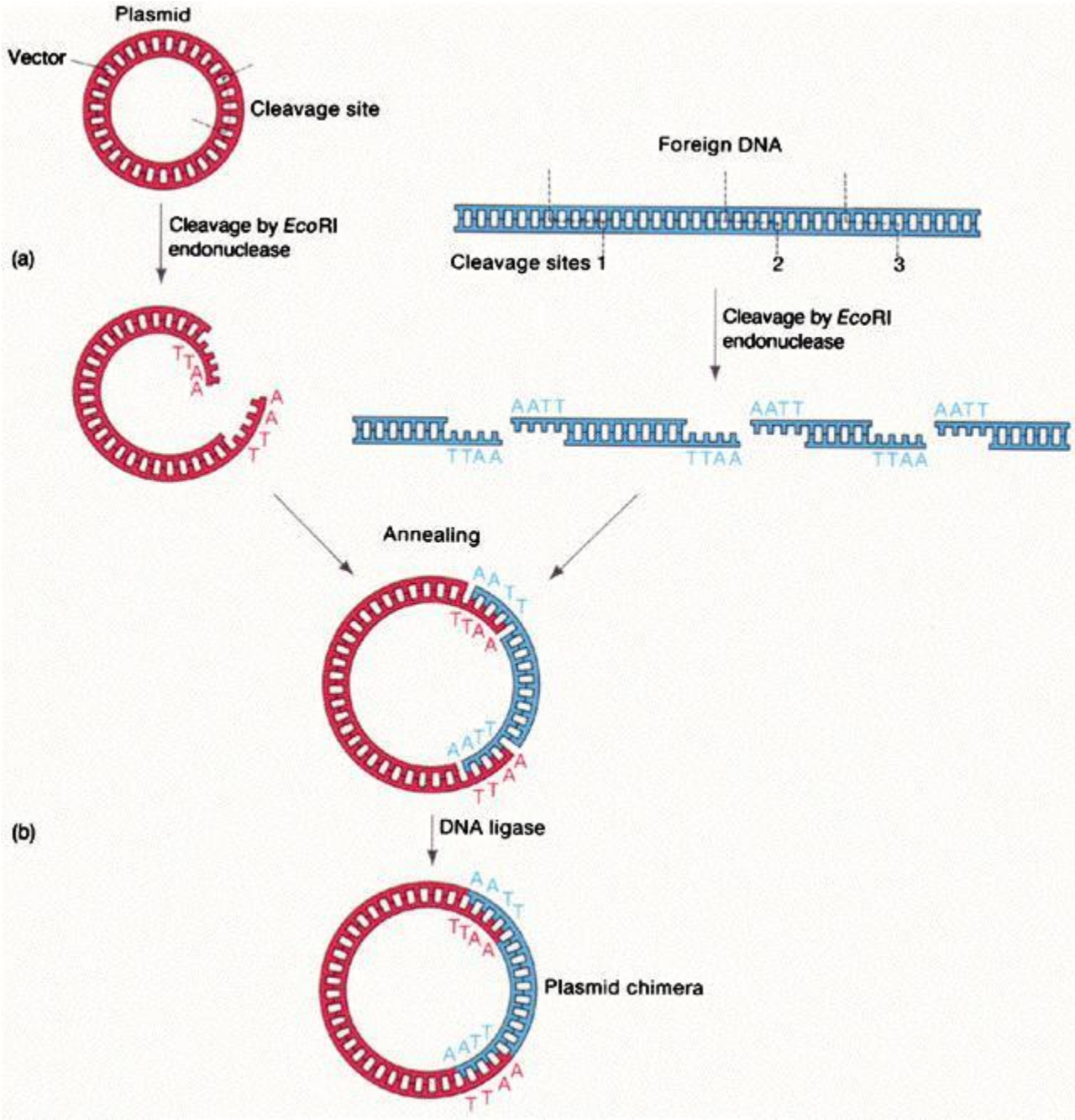
Making Recombinant DNA

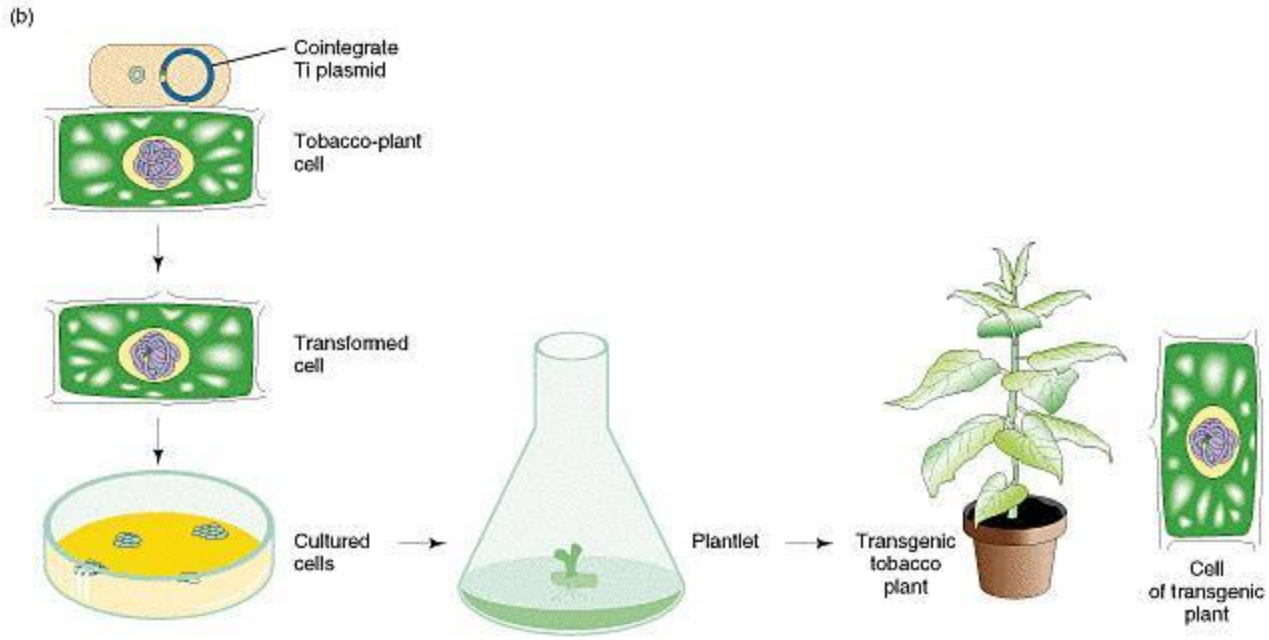
There are four main steps involved in making recombinant DNA:

- isolating DNA
- cutting DNA
- joining DNA
- amplifying recombinant DNA

Restriction enzymes:



- Amplified genes can be used for research, industrial, medical, or agricultural purposes.



3. Cloning a Specific Gene

- **Choosing a cloning vector:**

plasmids - usually < 10,000 bp (10 kbp) inserts

lambda phage - 10 - 15 kbp inserts

cosmids - combination of plasmids + phage; around 45 kbp inserts

SS phages - good for sequencing (N.B. - I just add NaOH)

Expression vectors - **used for expressing proteins**

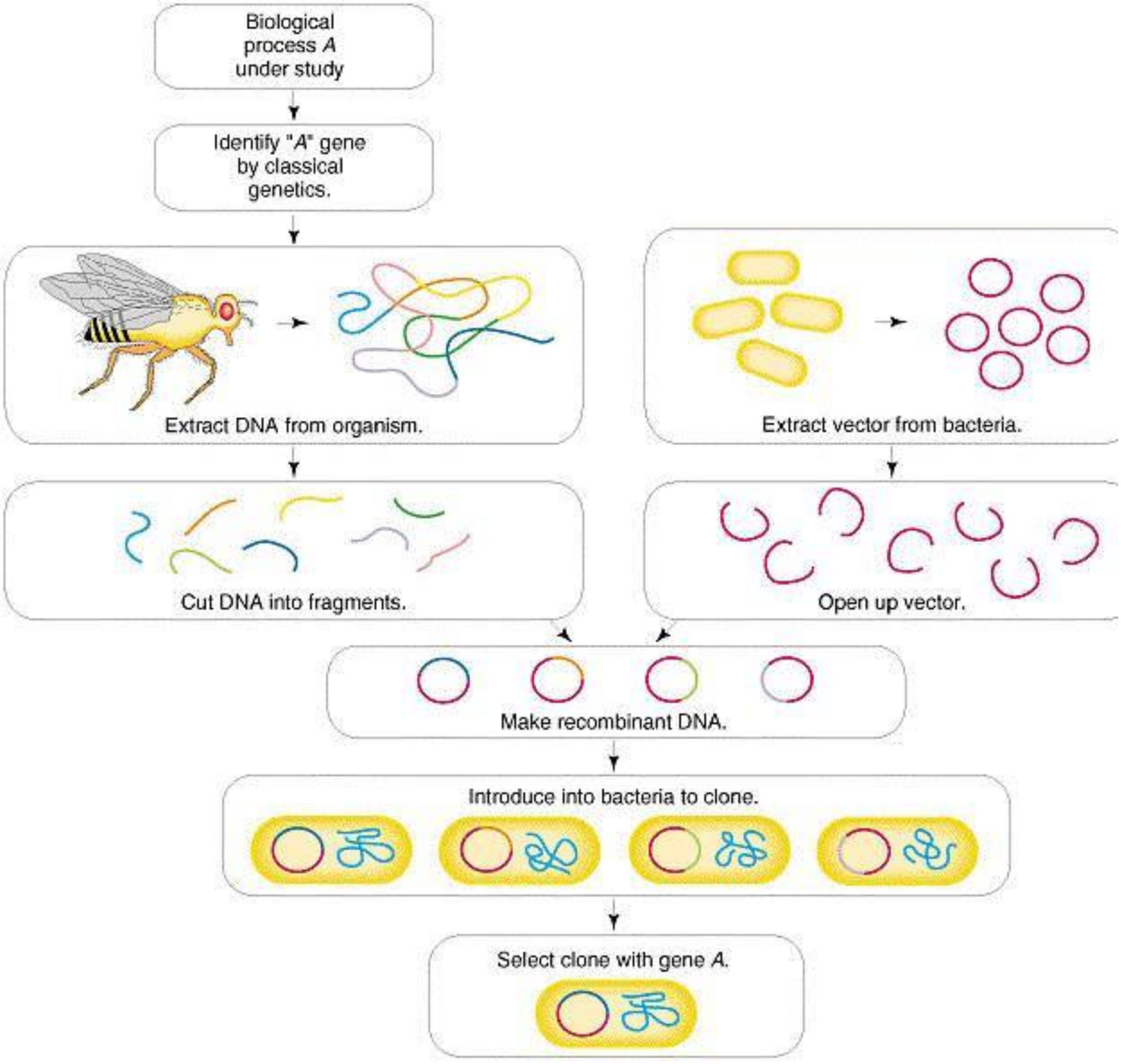
BACs **B**acterial **A**rtificial **C**hromosome**s** - up to 300,000 bp

YACs **Y**east **A**rtificial **C**hromosome**s** - up to 1,000,000 bp or so.

HACs **H**uman **A**rtificial **C**hromosome**s** - up to 20,000,000 bp (so far!)

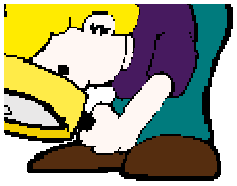
Making a DNA library

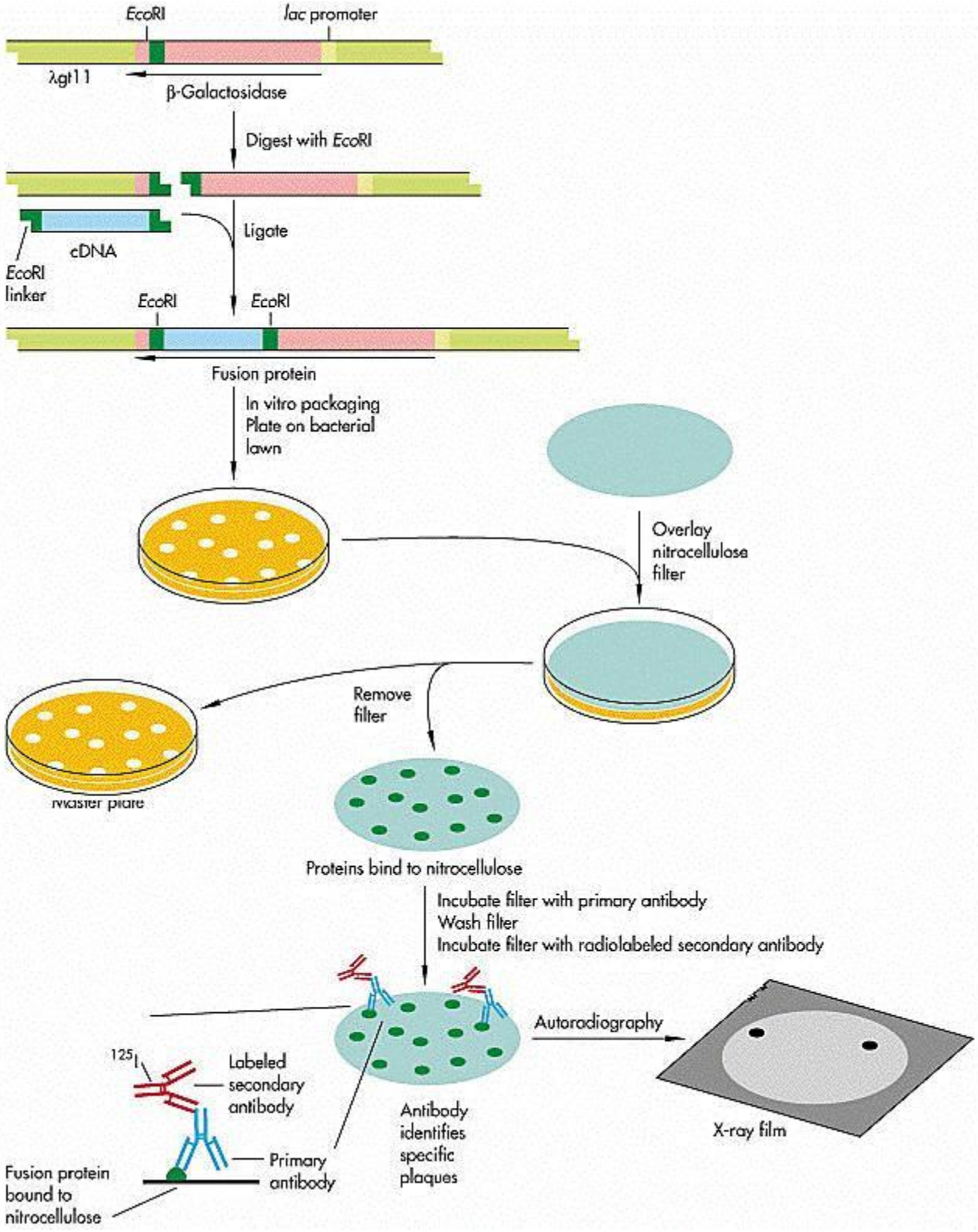
- A DNA library consists of DNA from a particular organism inserted into bacterial plasmids.



- The genes of interest must be identified in the DNA library

There are several different ways of doing this. Here's one method, using antibodies:



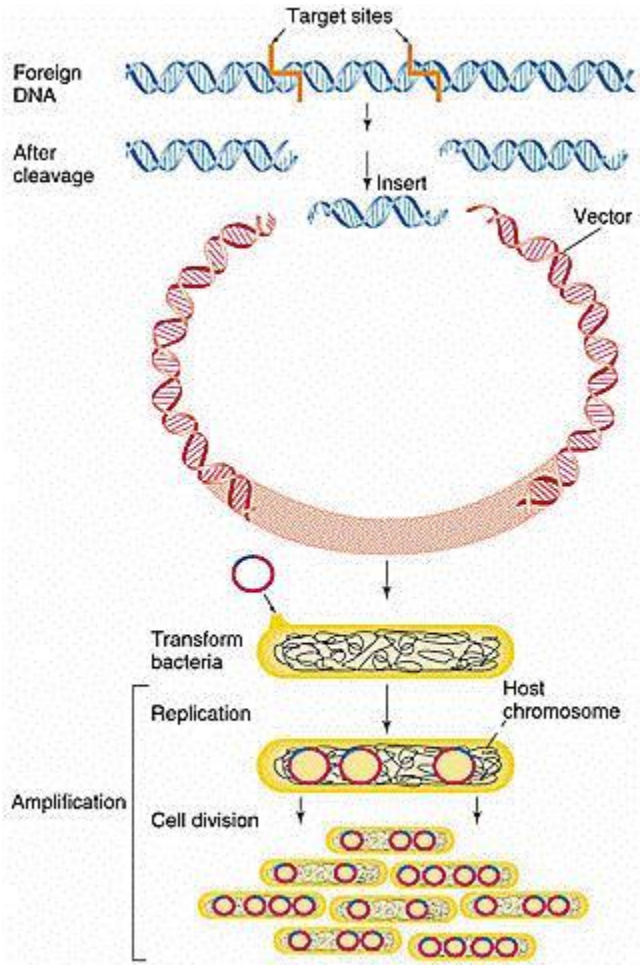


- **Finding specific clones using probes**

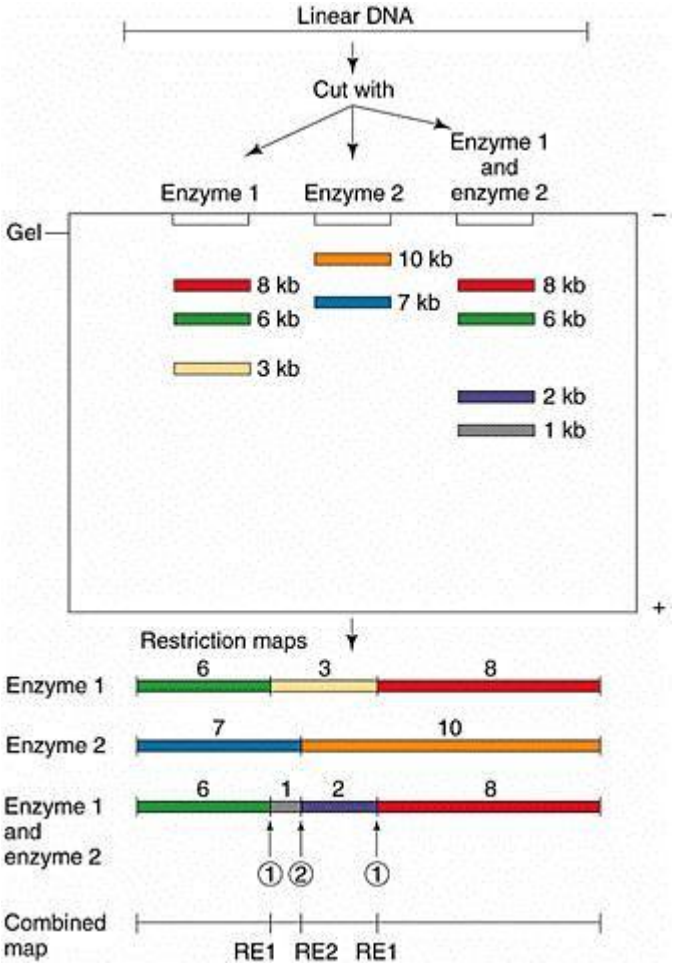
probes to find **DNA** (Southerns)
probes to find **RNA** (Northerns)
probes to find **proteins** (Westerns)

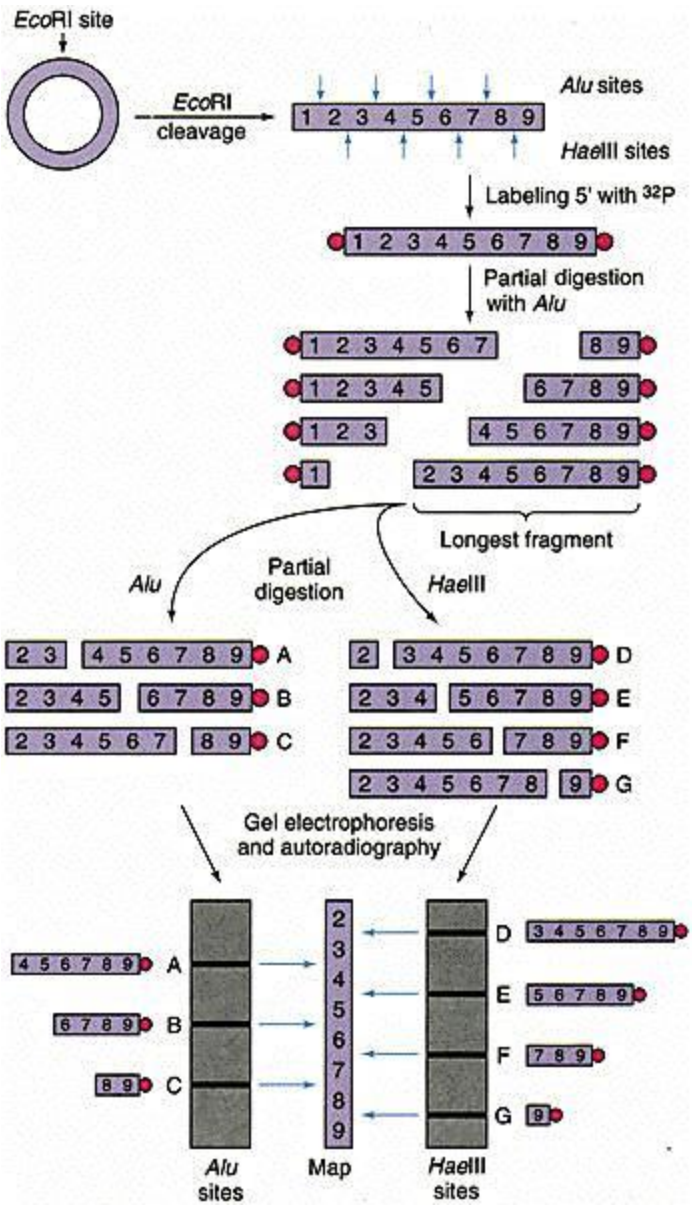
- **Finding specific clones by functional complimentation**
- **Positional cloning**
- **Cloning a gene by tagging**

- Selected DNA sequences in the library can be amplified

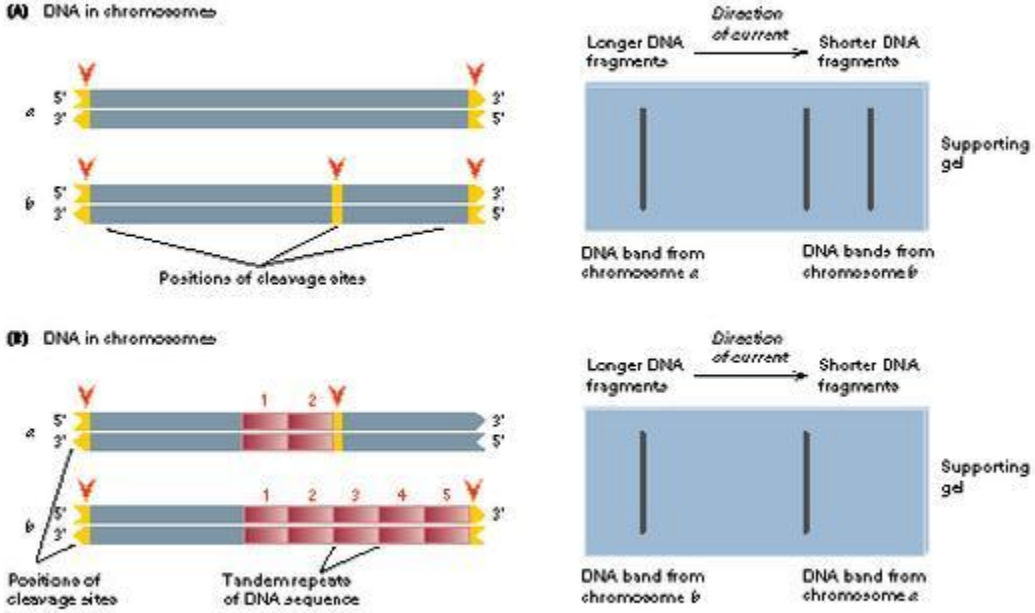


- Restriction enzymes can be used to provide markers on a chromosome





- Restriction Fragment Length Polymorphisms (RFLPs) can be used to locate a gene


(A) DNA in chromosomes
a
b
5'
3'
Positions of cleavage sites
Direction of current
Longer DNA fragments
Shorter DNA fragments
Supporting gel
DNA band from chromosome a
DNA bands from chromosome b
(B) DNA in chromosomes
1
2
3
4
5
Positions of cleavage sites
Tandem repeats of DNA sequence
DNA band from chromosome b
DNA band from chromosome a