Clones

- Genetically identical organisms or molecules derived from a common ancestor

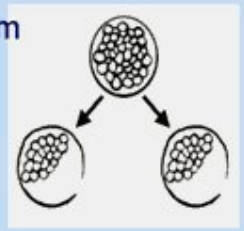
By
Ram Balak Mahto
Guest faculty
Zoology department
V.S.J College Rajnagar Madhubani

Cloning Animals

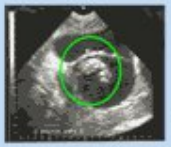
- Animals were cloned more than 20 years ago
- Two techniques
  - Embryo splitting
  - Nuclear transfer

Cloning by Embryo Splitting

Embryo is split to form two half-embryos



Embryos are transferred to an unrelated surrogate mother



Pregnancy is monitored by ultrasound

Sheep gives birth to identical twins

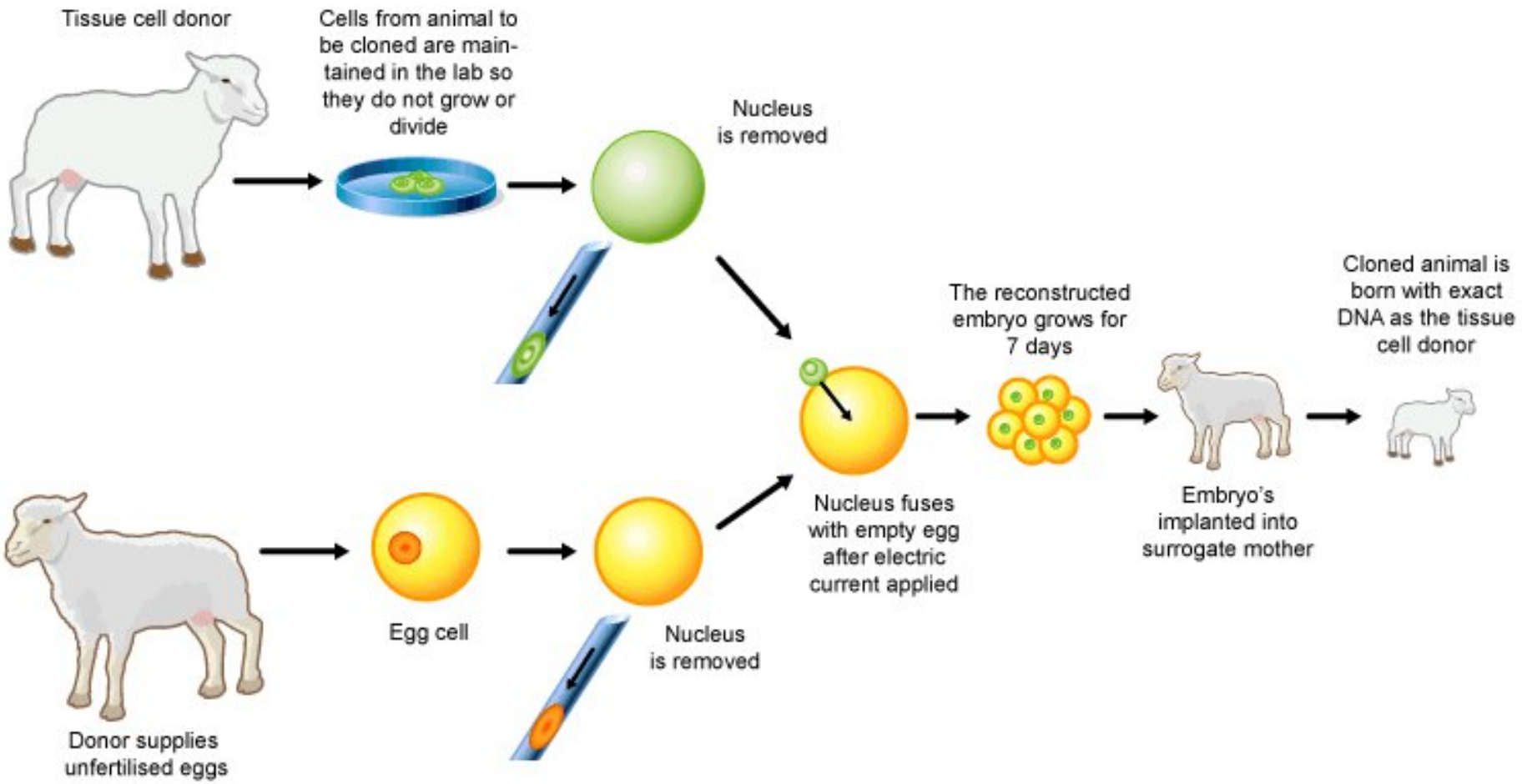
animalscience.ucdavis.edu

library.thinkquest.org

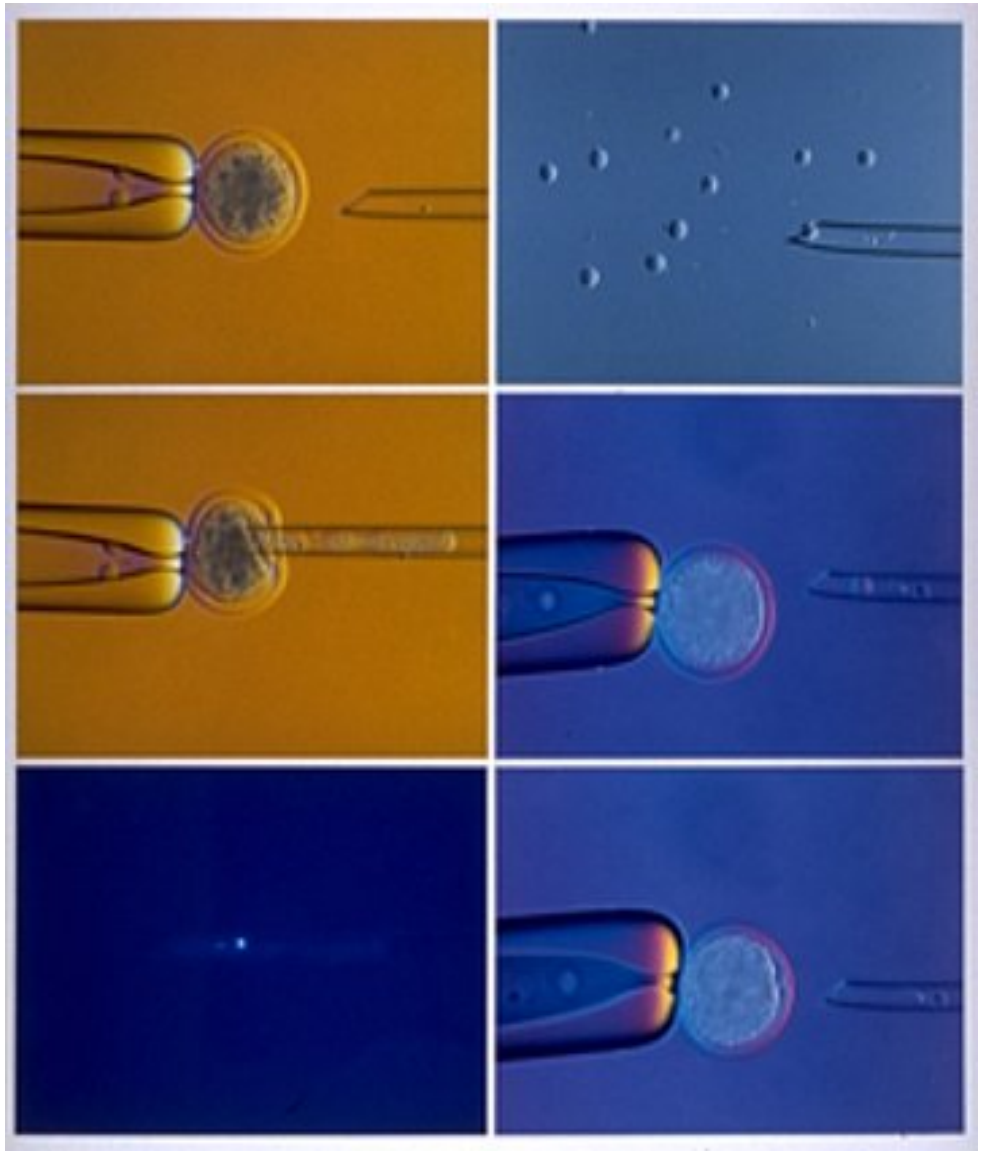
Embryo Splitting

- Egg collected
- Fertilized by *in vitro* fertilization (IVF)
- Embryo is grown to 8–16 cells
- Cells are separated
- Separated cells grown into separate embryos
- Embryos transplanted into surrogate mothers
- May be used to clone any mammalian embryos, including humans

Cloning by nuclear transfer



www.biotechnologyonline.gov.au



Cloning by nuclear transfer

www.pnas.org

Nuclear Transfer

- First done in 1986
- More difficult
- Nucleus is removed from an egg
- Enucleated eggs are fused with other cells
- Embryos are transplanted into a surrogate mother
- In 1997, Dolly the sheep was the first mammalian clone from an adult donor cell

Cloned animals

Second addition

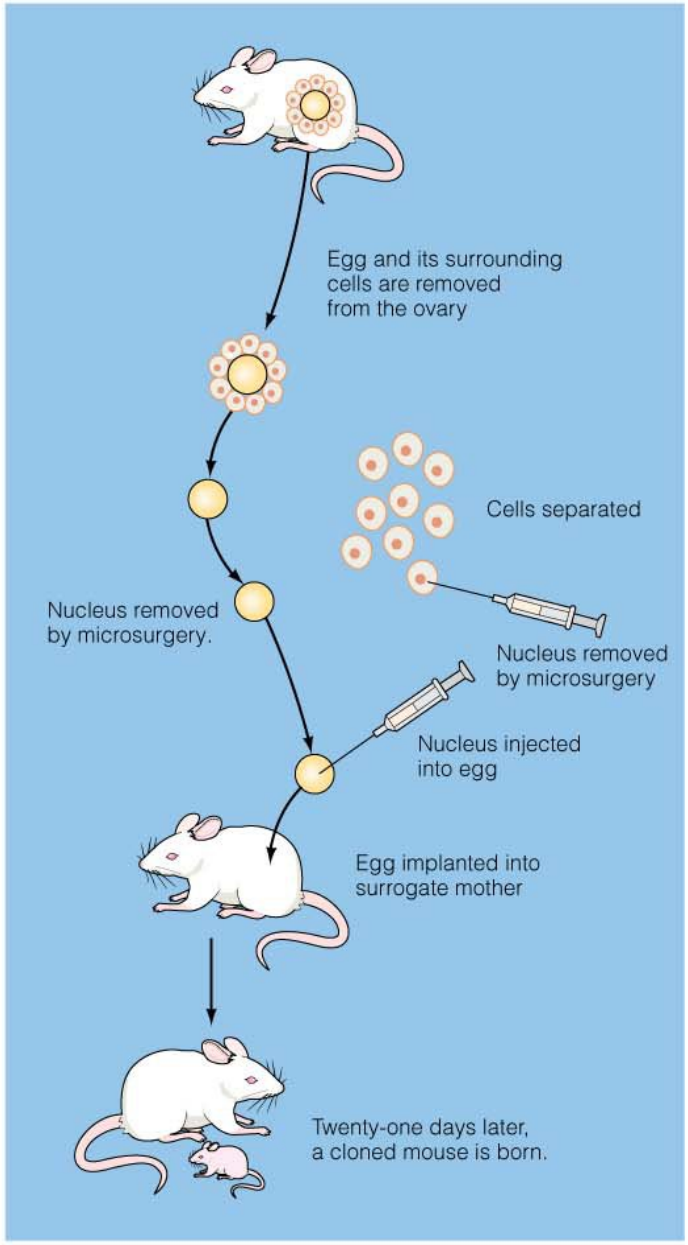
Second chance

Also cloned animals about to go extinct - gaur etc

at Texas A&M

Fig. 13.5

Cloning Mice by Injection of Nuclei from Adult Cells



Problems -

don’t live as long

not carbon copies/identical

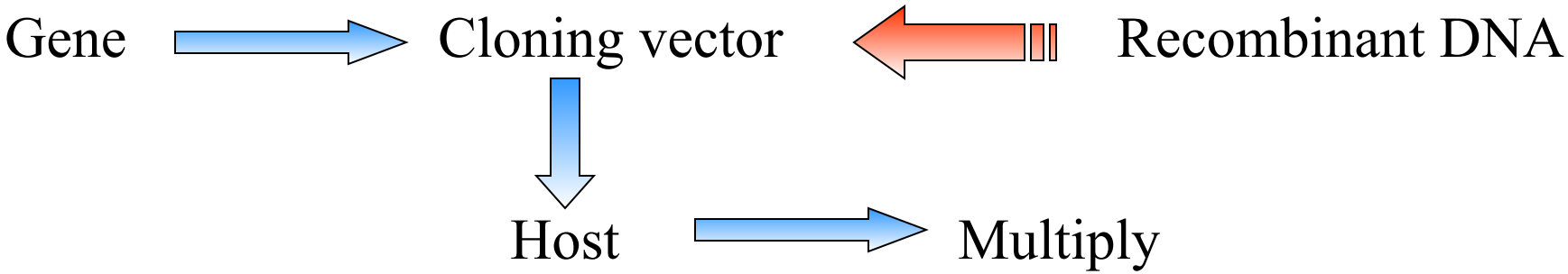
develop diseases early

very low success rate - 0.1 - 3%

Dedifferentiation/reprogramming may not be complete or accurate

Gene Cloning

GOAL: To get enough copies of the gene to manipulate



Started with: few copies → Ended with: Many copies.

All identical to starting gene - CLONES

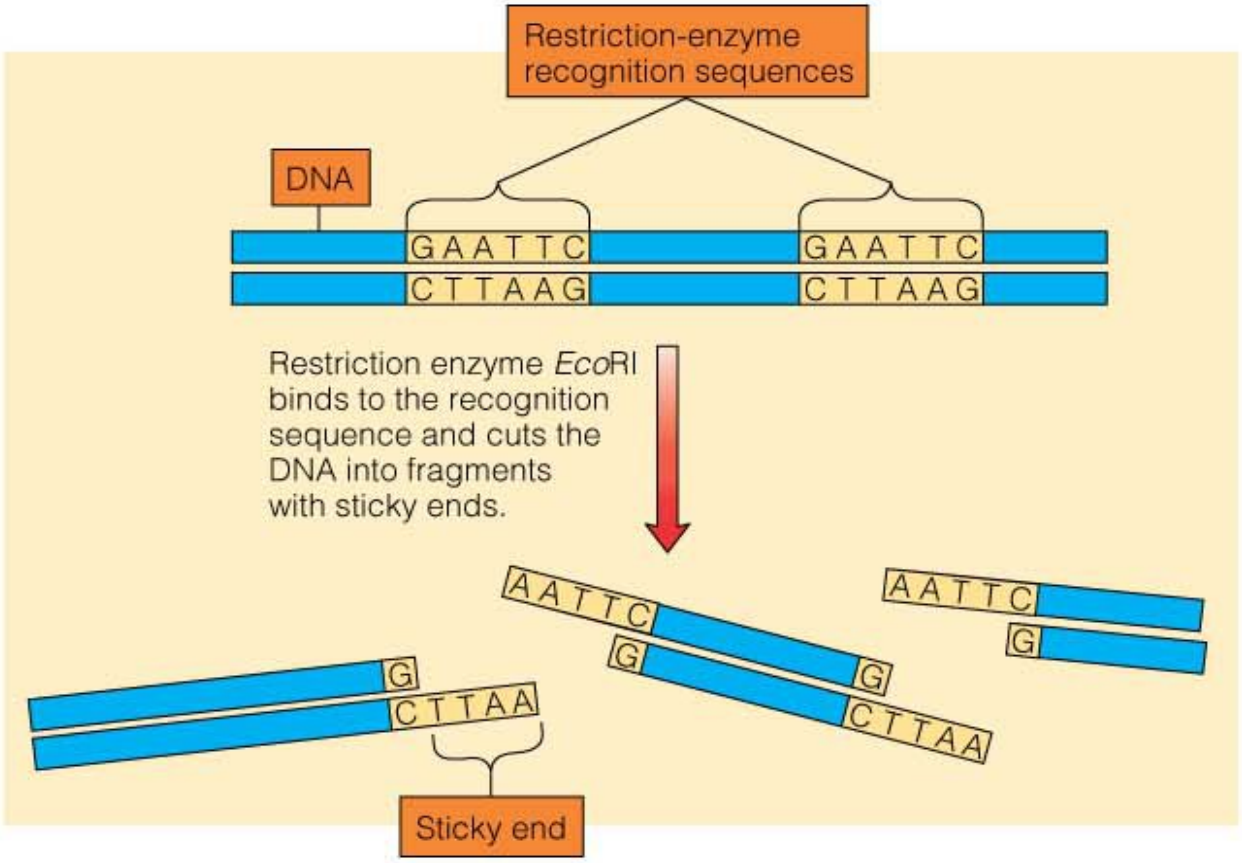
Restriction enzymes

Nobel Prize

Werner Arber, Daniel Nathans and Hamilton O. Smith

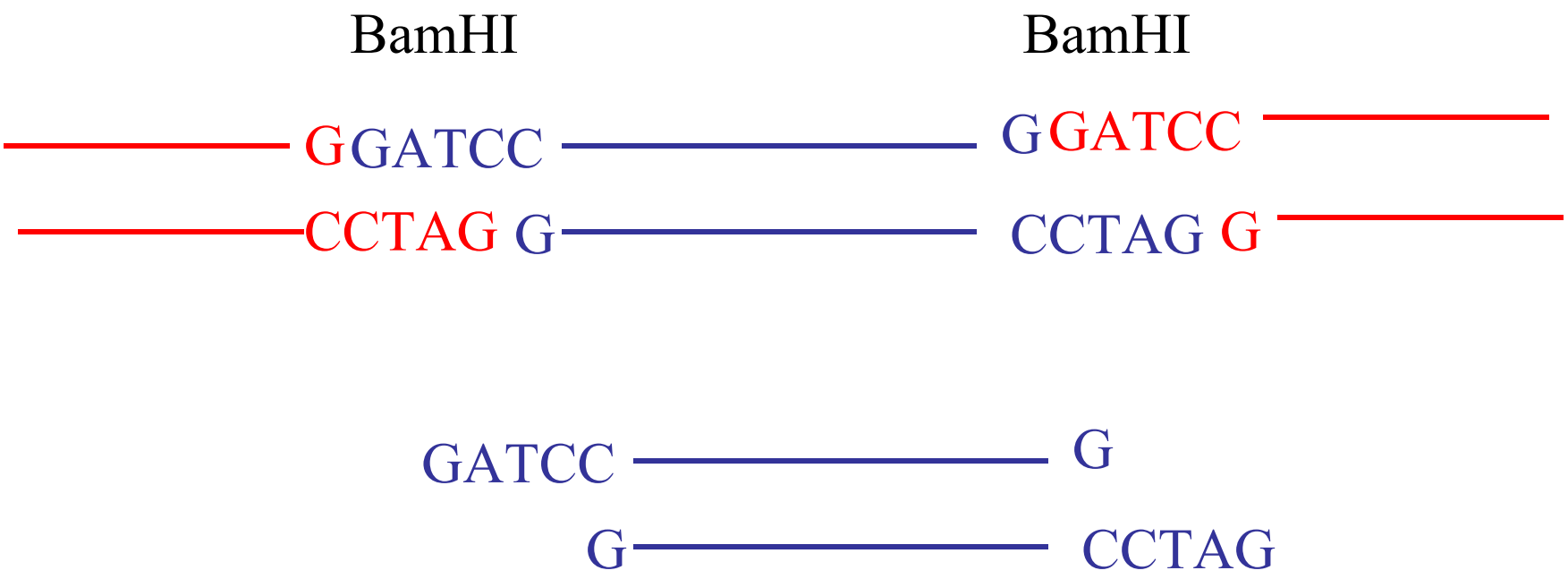
1978

Restriction Enzymes



Inserting foreign DNA using restriction enzymes

Ligase



Frequency of occurrence of restriction sites

If DNA sequence has equal amounts of each base

If bases are distributed randomly

6 base cutter $(1/4)^6 = 1$ site in ~4000 bp

4 base cutter $(1/4)^4 = 1$ site in 256 bp

Common Restriction Enzymes

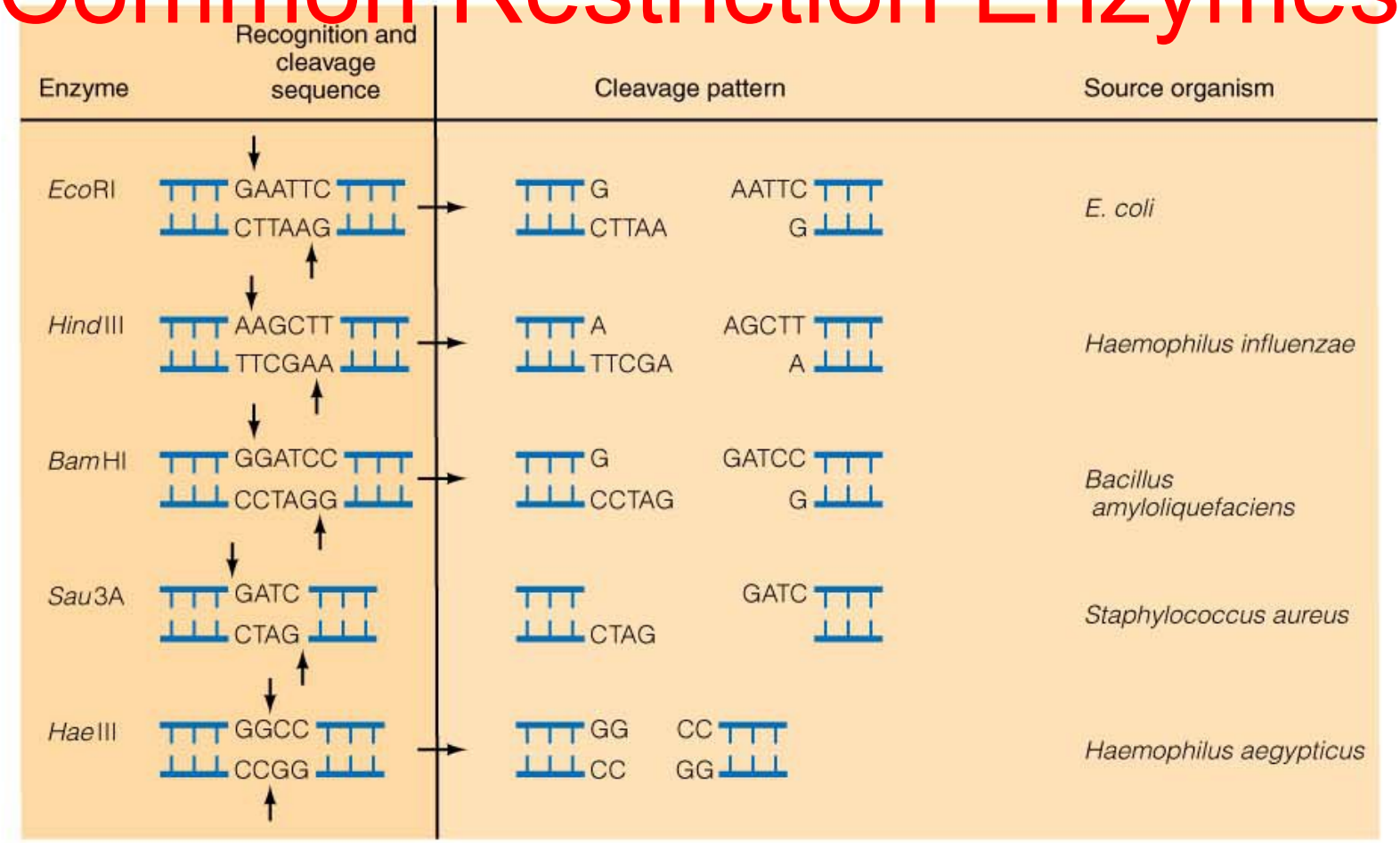


Fig. 13.8

Cloning DNA in Plasmid Vectors

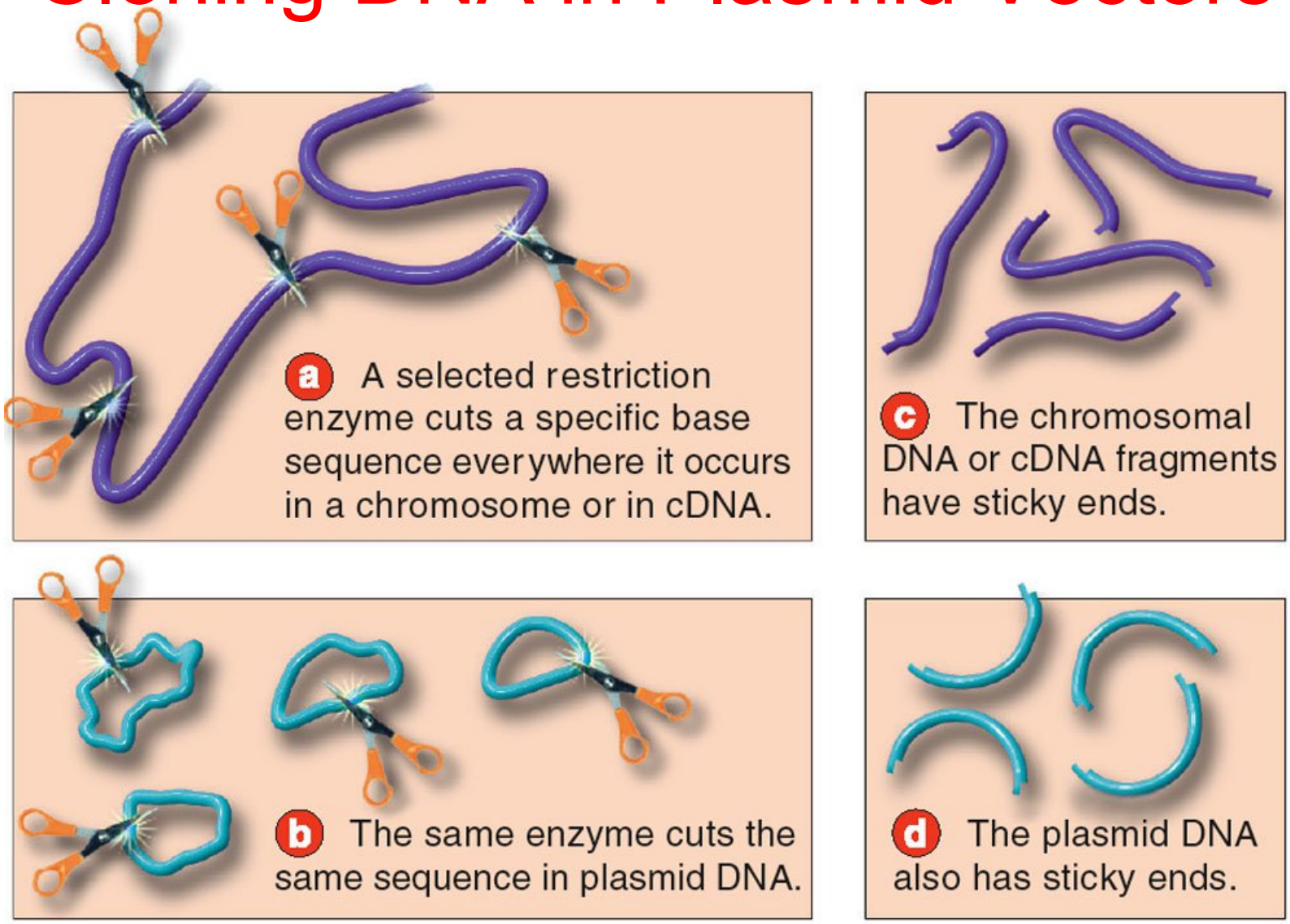


Fig. 13.11a-d

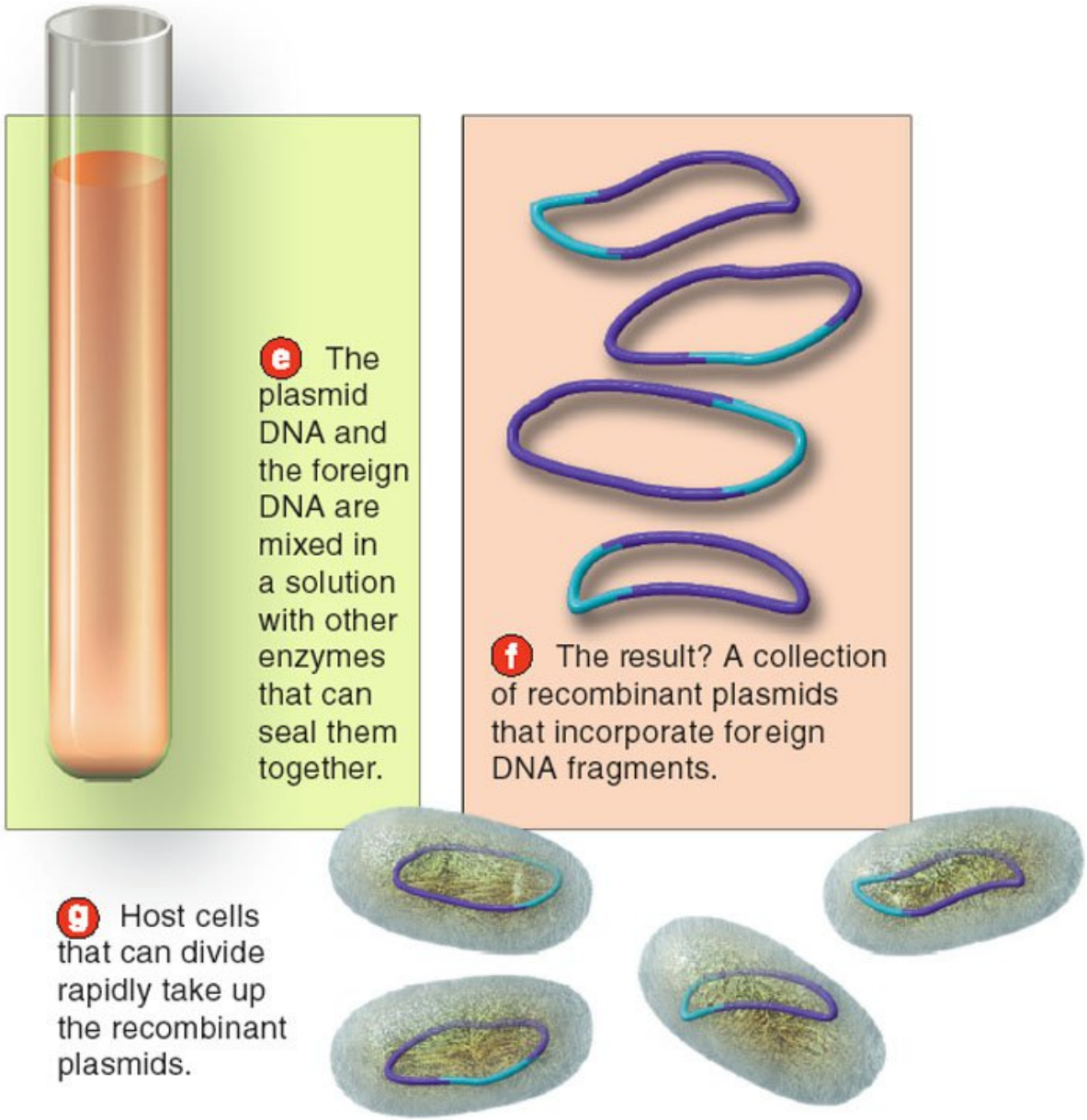

e The plasmid DNA and the foreign DNA are mixed in a solution with other enzymes that can seal them together.
f The result? A collection of recombinant plasmids that incorporate foreign DNA fragments.
g Host cells that can divide rapidly take up the recombinant plasmids.

Fig. 13.11e-g

Plasmid Use
to Carry DNA
Fragments

= Vectors

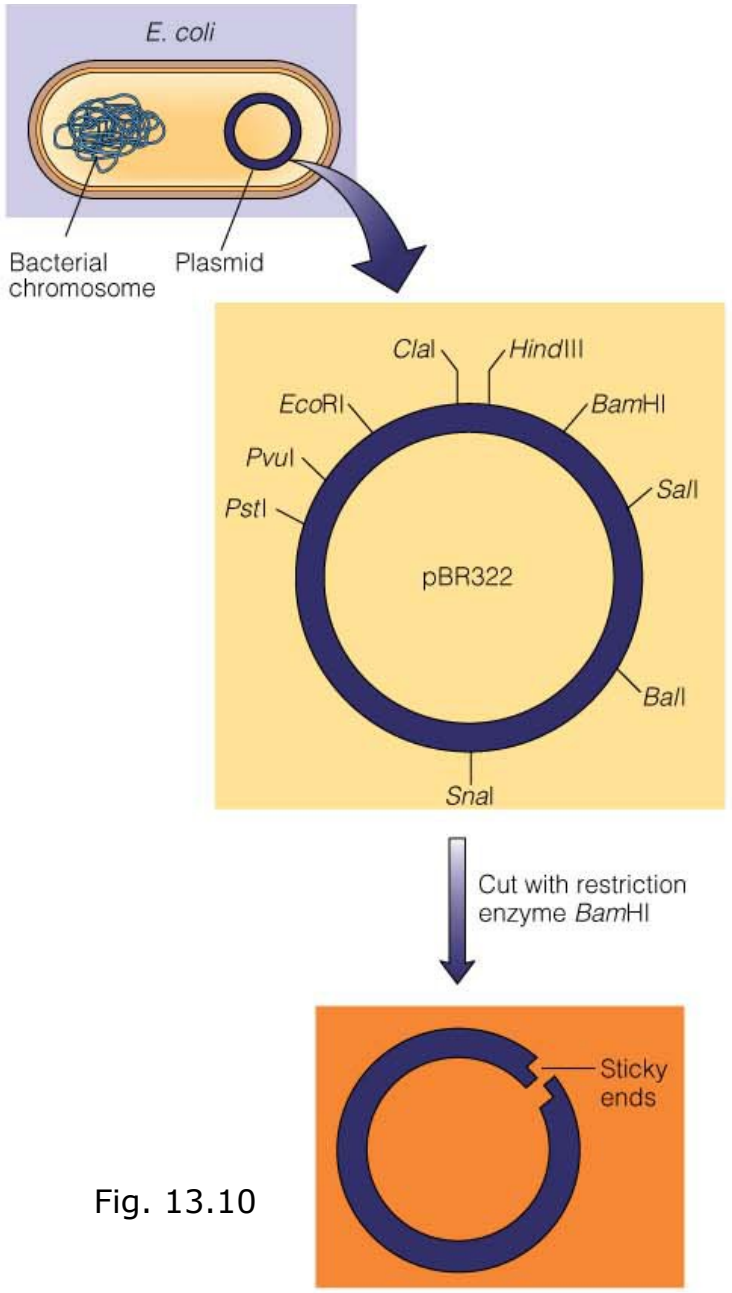
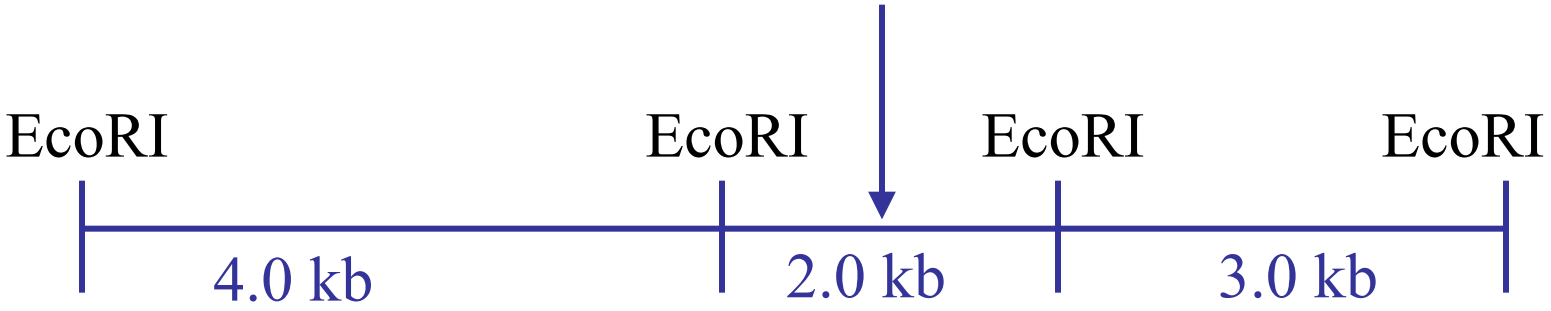


Fig. 13.10

Cloning Vectors

Vector	Size of Insert Accepted (kb)
Plasmid	up to 15
Bacteriophage	up to 90
Bacterial artificial chromosome (BAC)	100–500
Yeast artificial chromosome (YAC)	250–2,000



Problem: How to get the 2.0 kb piece to subclone into vector

Randomly

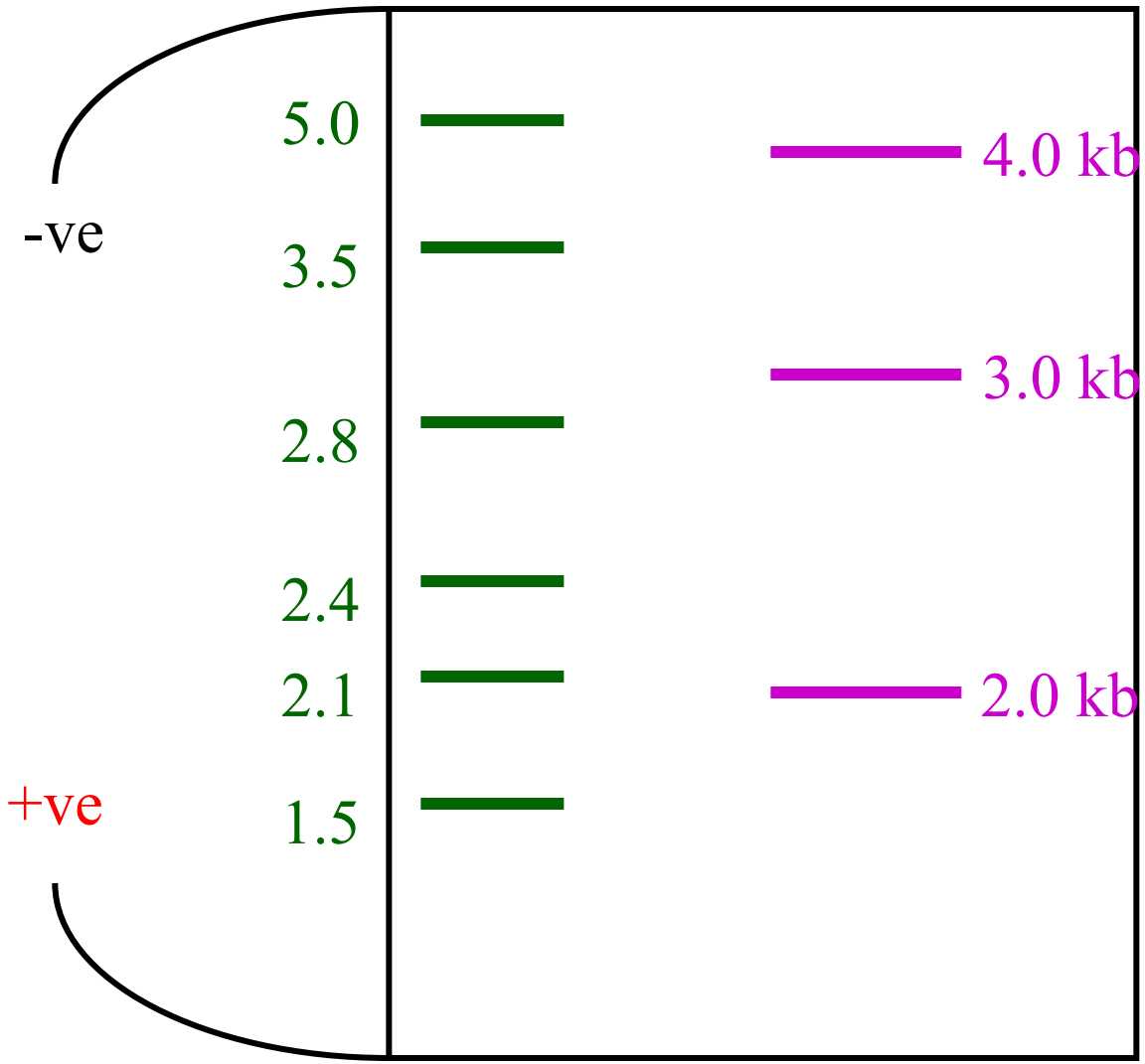
Shotgun cloning

Isolate specific fragment

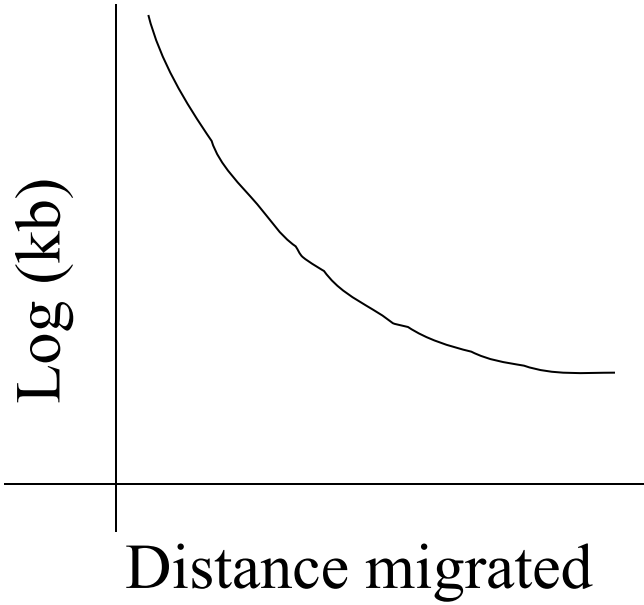
Steps in cloning a single piece of DNA

1. Appropriate restriction sites
2. Cut vector and foreign DNA with RE
3. Run on gel to separate fragments
4. Isolate specific fragment
5. Ligate with cut vector
6. Transform host bacteria. Selection.
7. Grow up colonies.
8. Isolate plasmid DNA.
9. Cut with RE to confirm presence of foreign DNA.
10. Run on gel to identify recombinant plasmids.

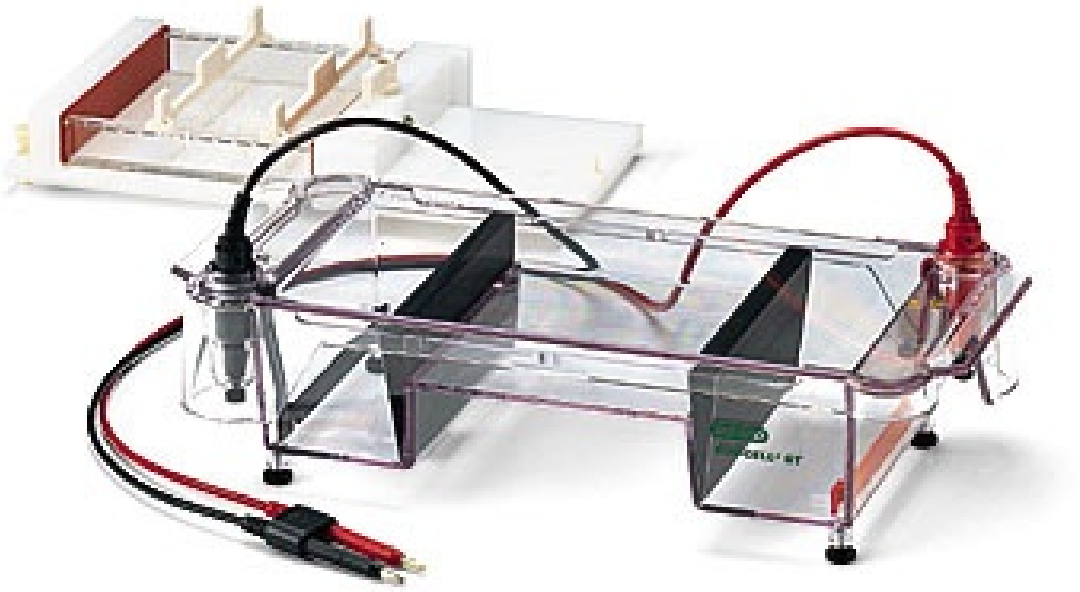
Gel electrophoresis

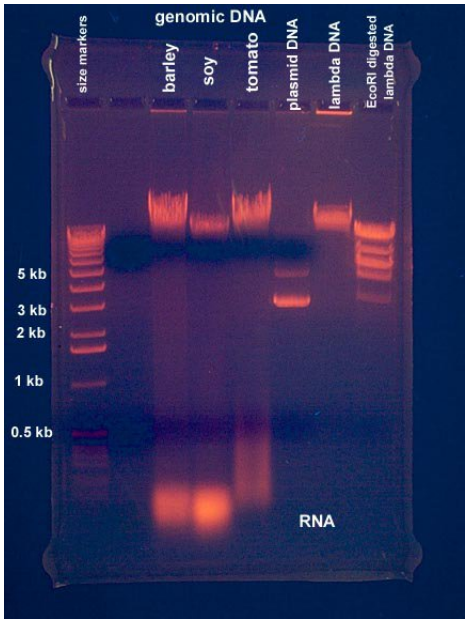


Size separation

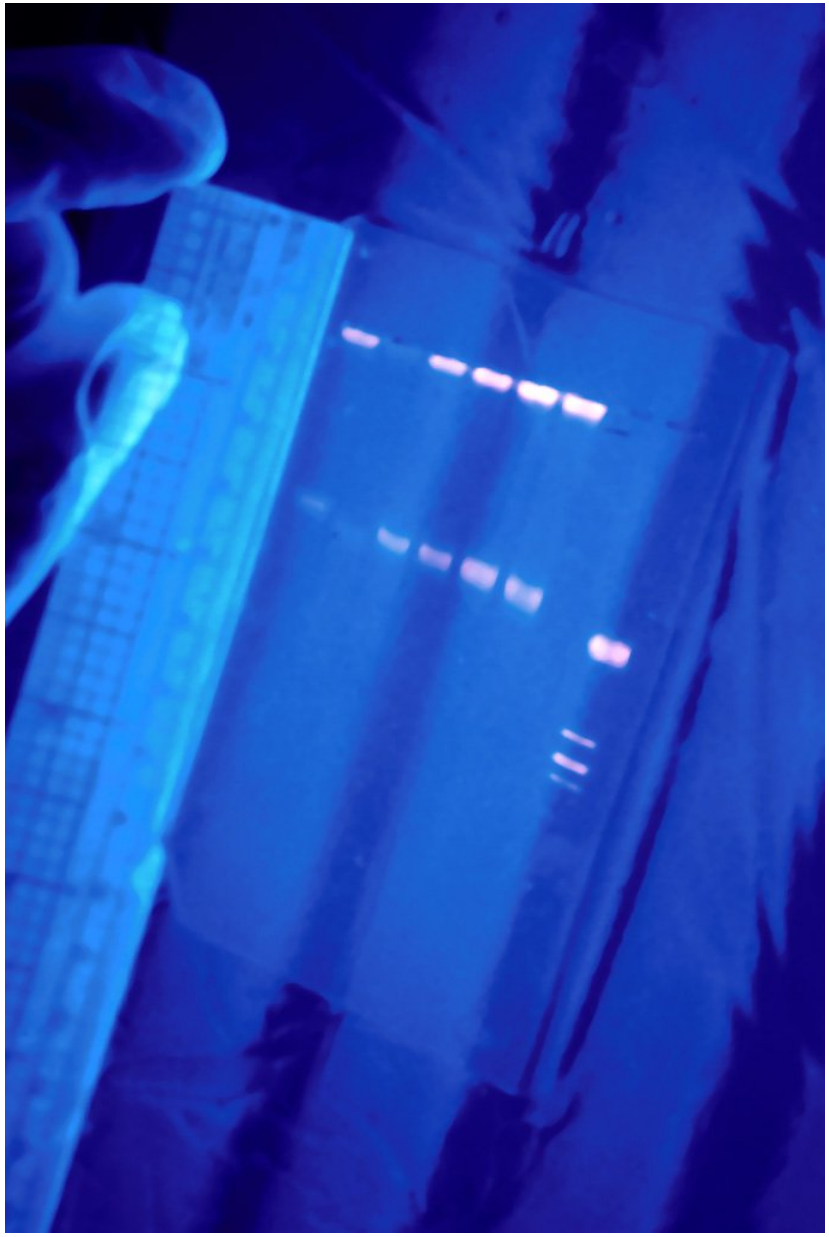


Gel electrophoresis system or "gel box"





gel stained with ethidium bromide



Credit: © Michael Gabridge/Visuals Unlimited

34173

UV illumination of stained DNA fragments separated in an agarose gel by electrophoresis.

Selecting Cells with Vectors

- Vectors carry antibiotic resistance genes
- Growing antibiotic-sensitive cells on media with antibiotics ensures that all growing cells must carry the vector
- Selecting Cells with Recombinant Vectors
- While inserting the donor DNA, an existing gene in the vector is inactivated
- OR
- In addition to the Donor gene a marker gene is added

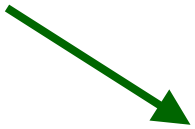
How to tell that plasmid now contains insert

Original vector - 4 kb with one RE (EcoRI) site

DNA to be inserted - 2 kb, flanked by same RE

Cut plasmids isolated from colonies. Run gel

Vector alone (no insert) - 1 band
4 kb



Vector + insert - 2 bands
4 kb AND 2 kb