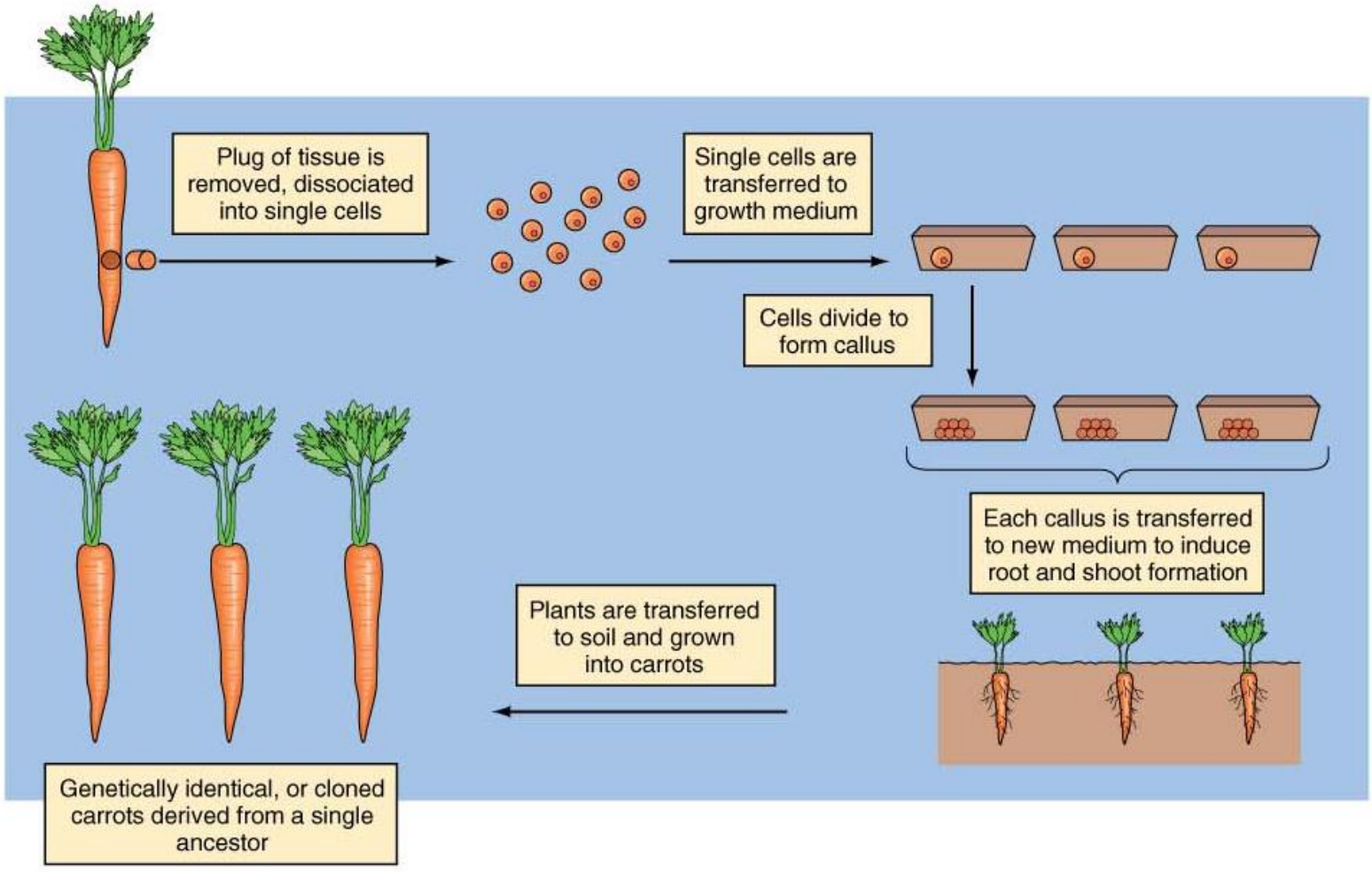
CLONES

- GENETICALLY IDENTICAL ORGANISMS OR MOLECULES DERIVED FROM A COMMON ANCESTOR

CLONING PLANTS FROM SINGLE CELLS



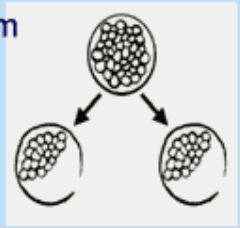
CLONING ANIMALS

- ANIMALS WERE CLONED MORE THAN 20 YEARS AGO

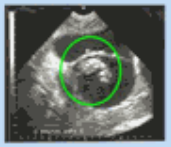
- TWO TECHNIQUES
  - EMBRYO SPLITTING
  - NUCLEAR TRANSFER

Cloning by Embryo Splitting

Embryo is split to form two half-embryos



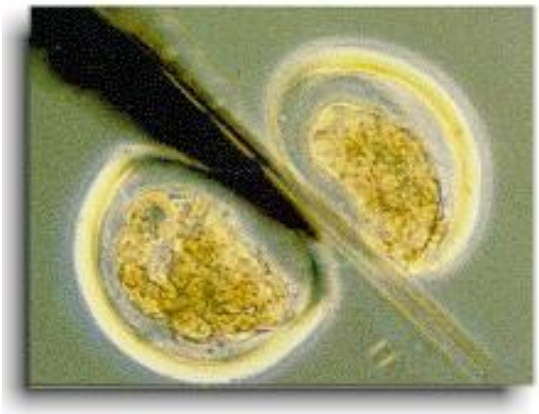
Embryos are transferred to an unrelated surrogate mother



Pregnancy is monitored by ultrasound

Sheep gives birth to identical twins

animalscience.ucdavis.edu

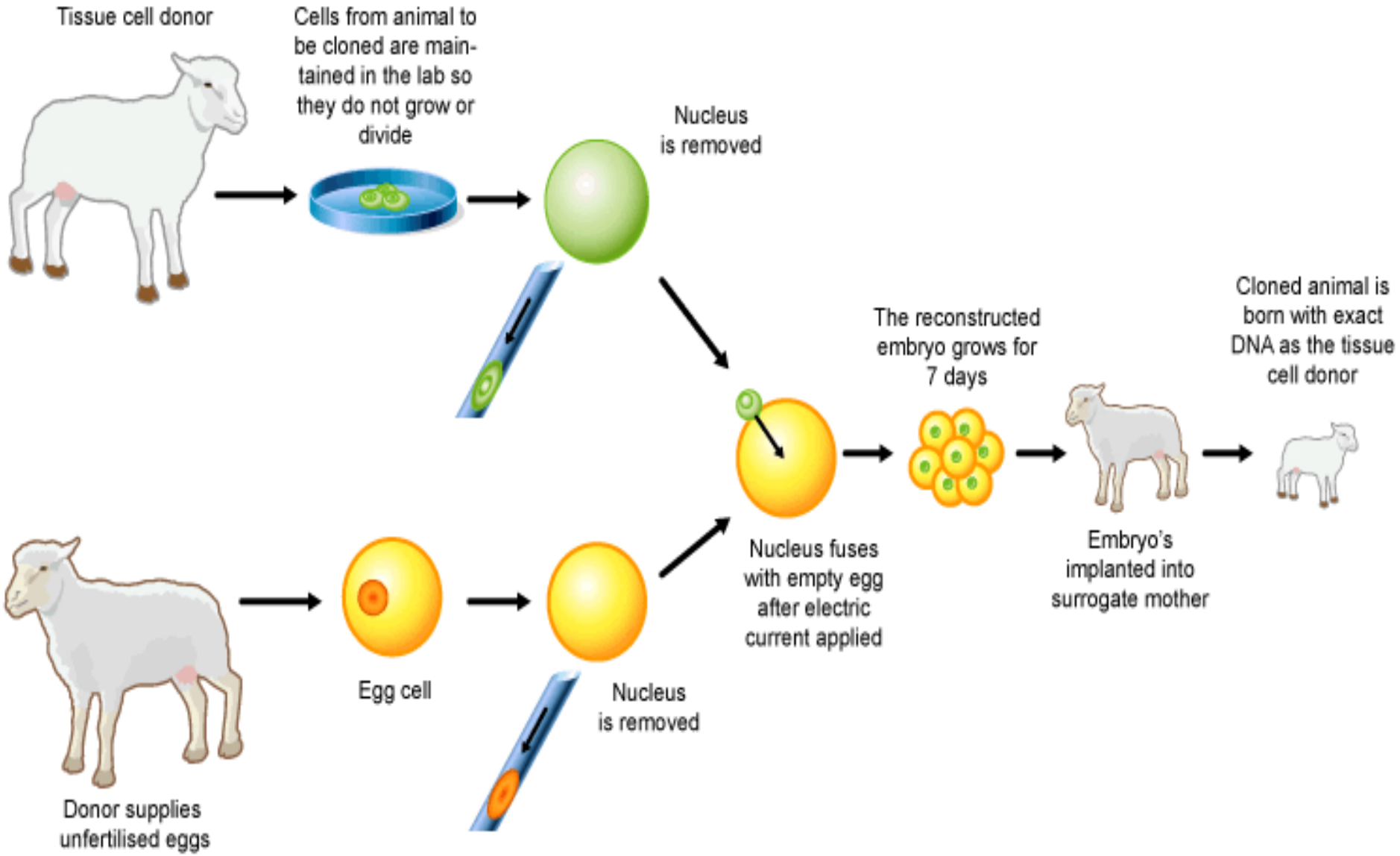


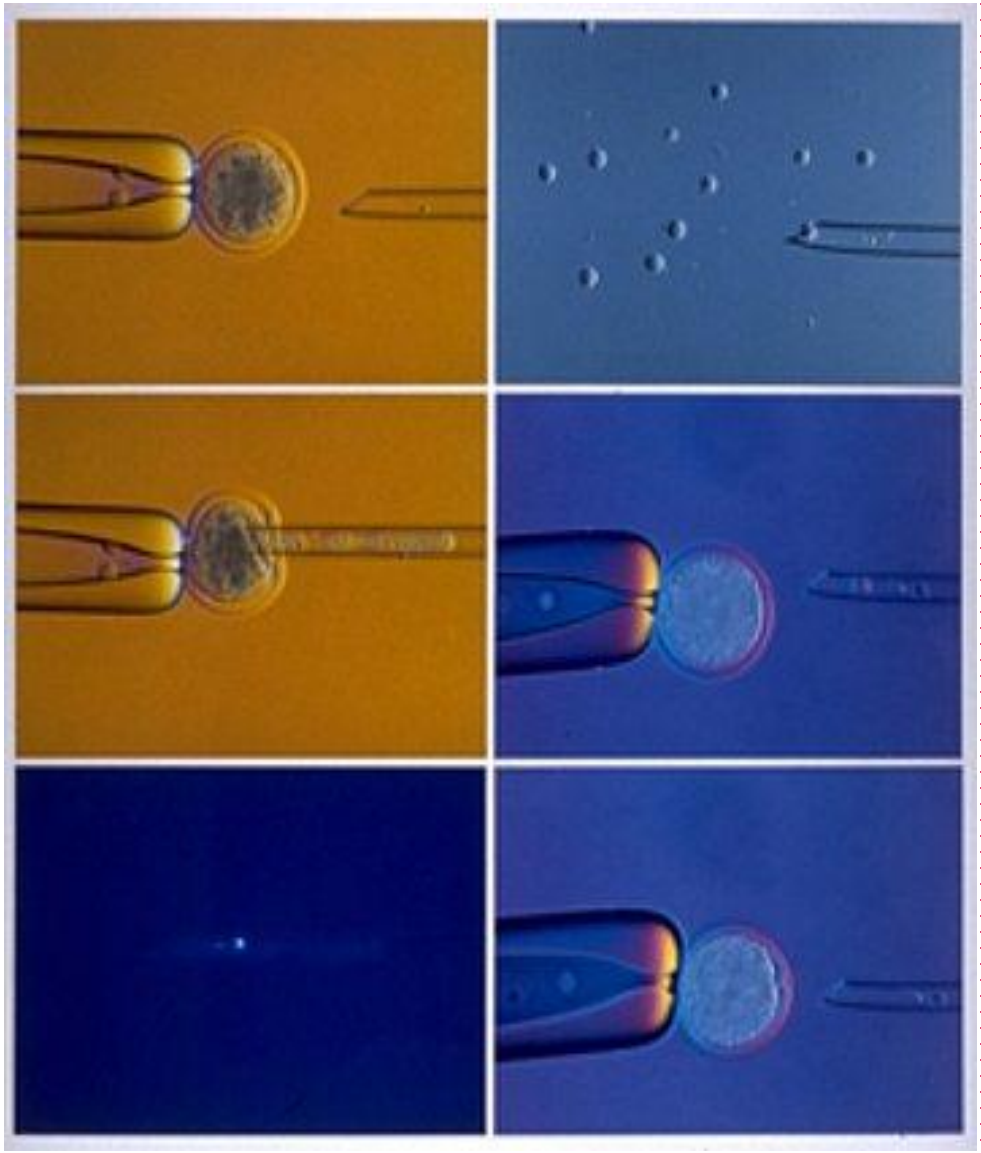
library.thinkquest.org

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





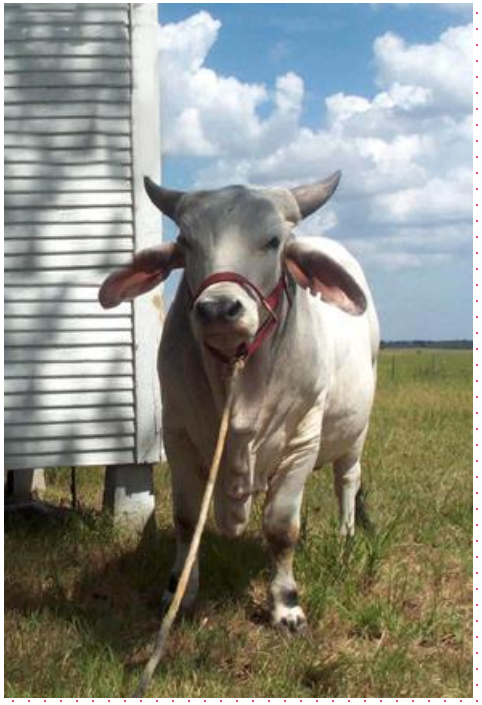
Cloning by nuclear transfer

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



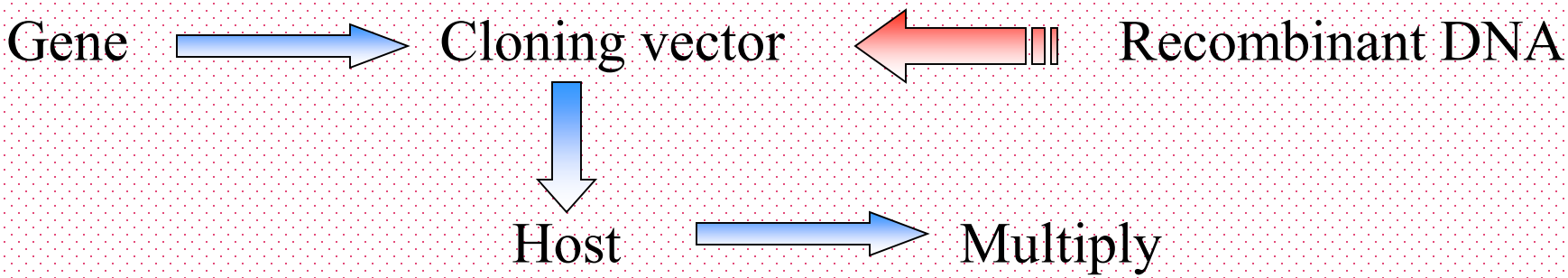
Second chance

Also cloned animals about to go extinct - gaur etc

at Texas A&M

Gene Cloning

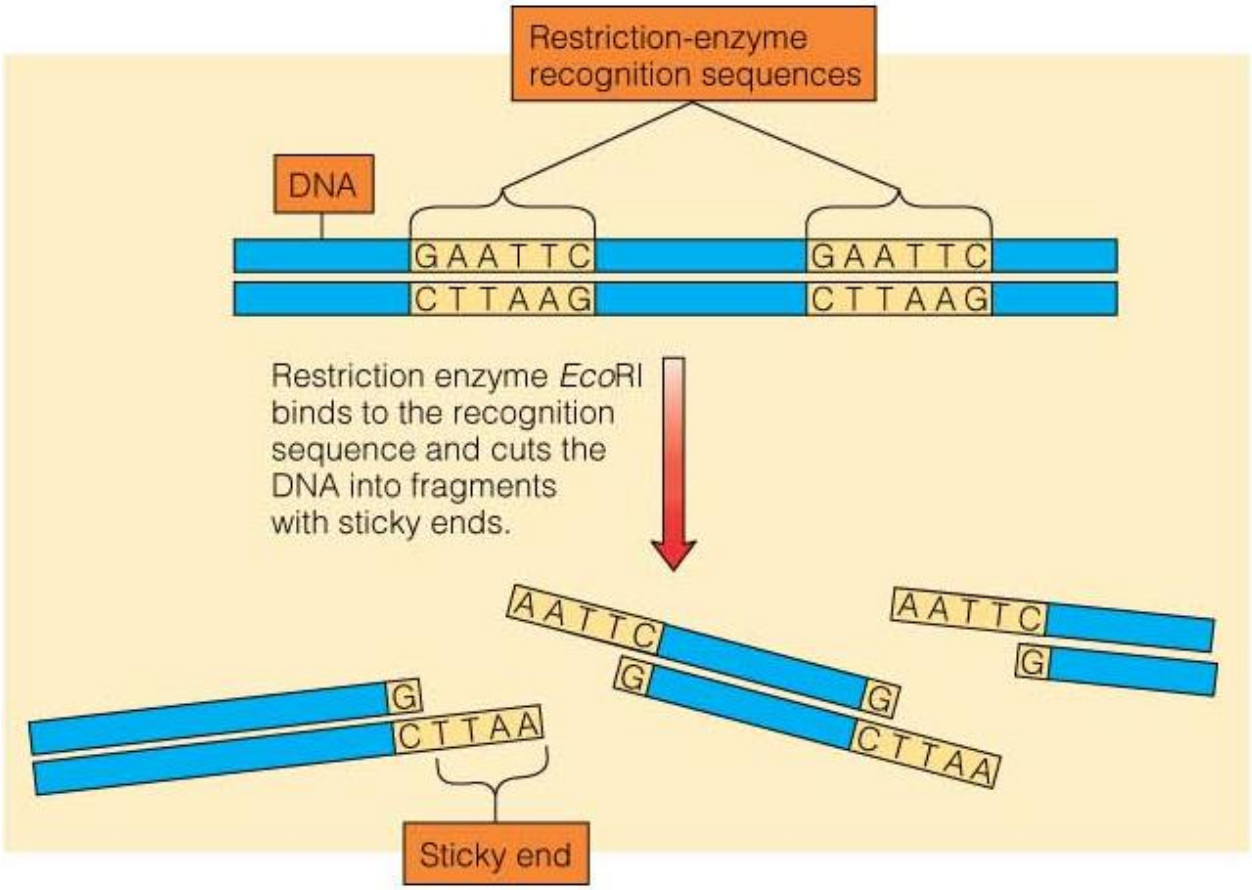
GOAL: To get enough copies of the gene to manipulate



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

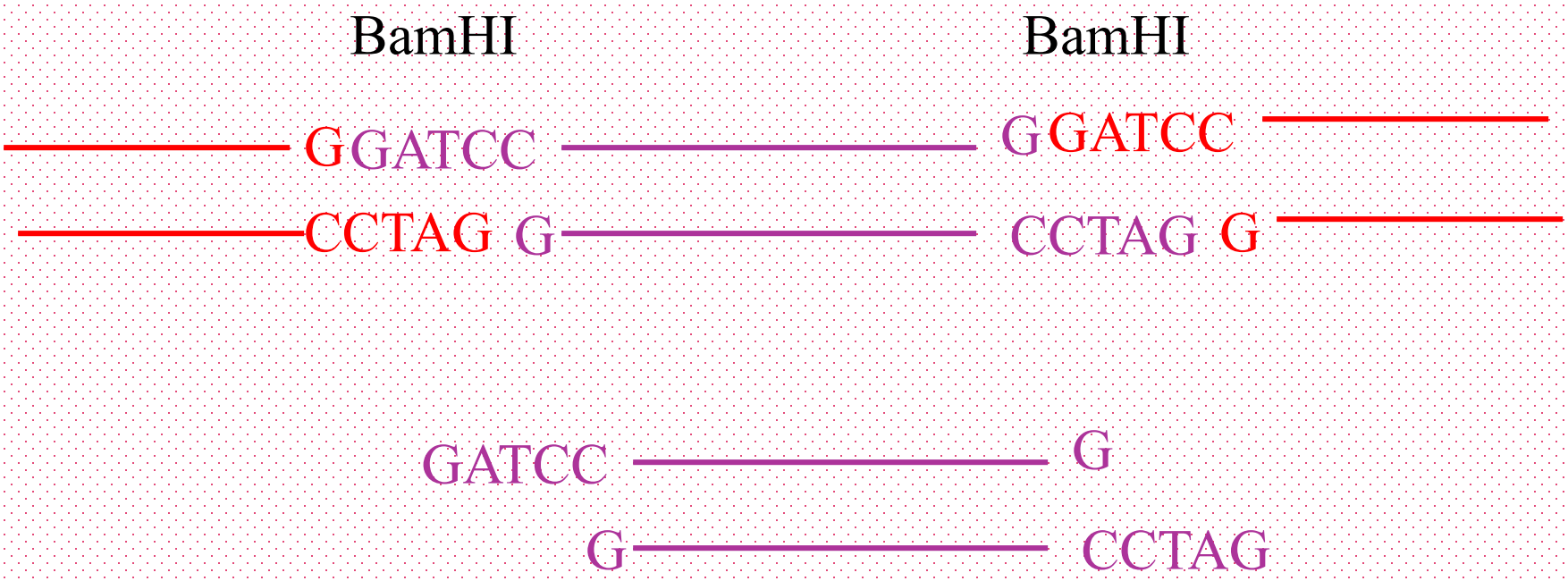
All identical to starting gene - CLONES

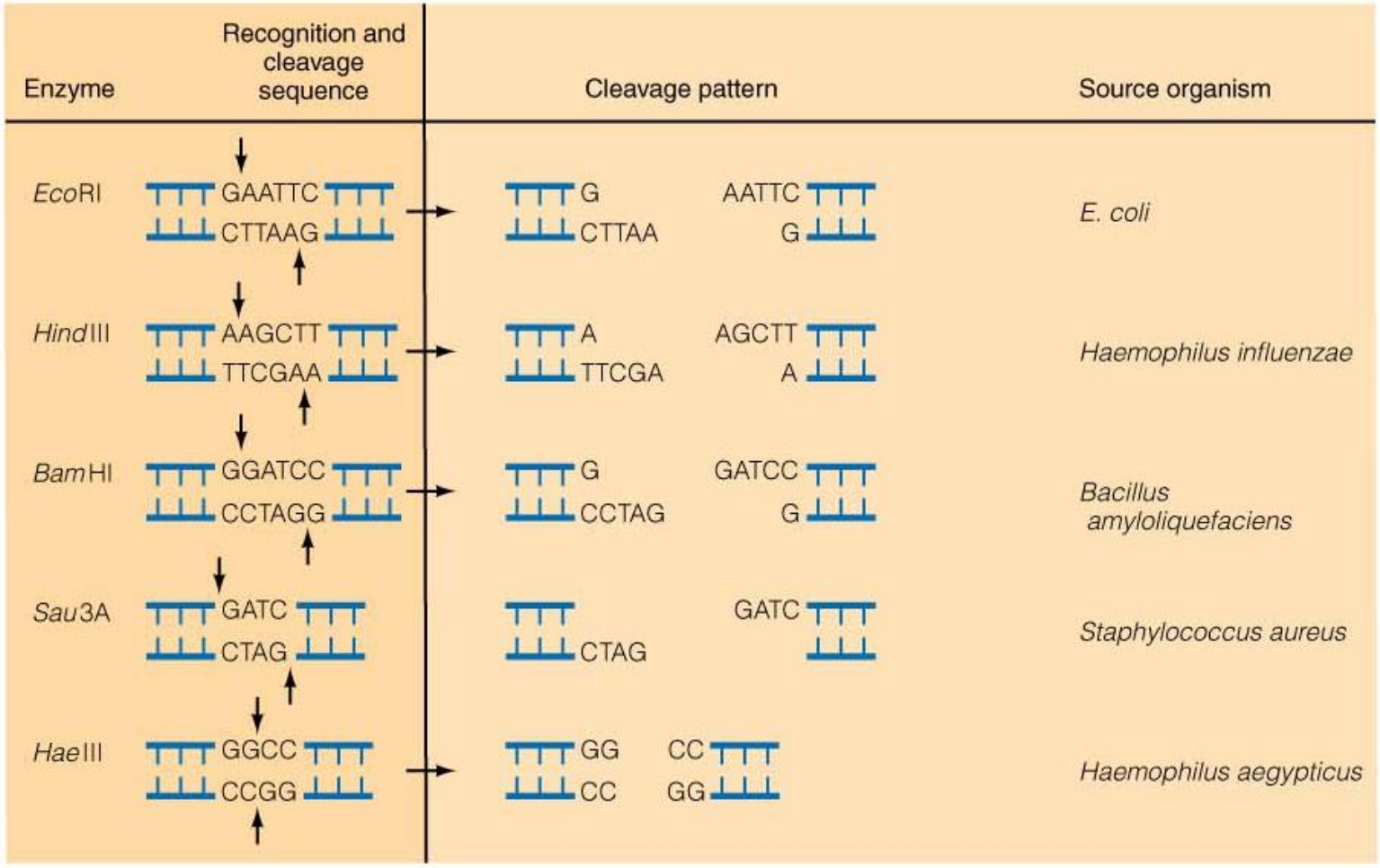
RESTRICTION ENZYMES



Inserting foreign DNA using restriction enzymes

Ligase




Enzyme
Recognition and cleavage sequence
Cleavage pattern
Source organism
EcoRI
GAATTC
CTTAAG
G
CTTAA
AATTC
G
E. coli
HindIII
AAGCTT
TTCGAA
A
TTCGA
AGCTT
A
Haemophilus influenzae
BamHI
GGATCC
CCTAGG
G
CCTAG
GATCC
G
Bacillus amyloliquefaciens
Sau3A
GATC
CTAG
CTAG
GATC
Staphylococcus aureus
HaeIII
GGCC
CCGG
GG
CC
CC
GG
Haemophilus aegypticus

CLONING DNA IN PLASMID VECTORS

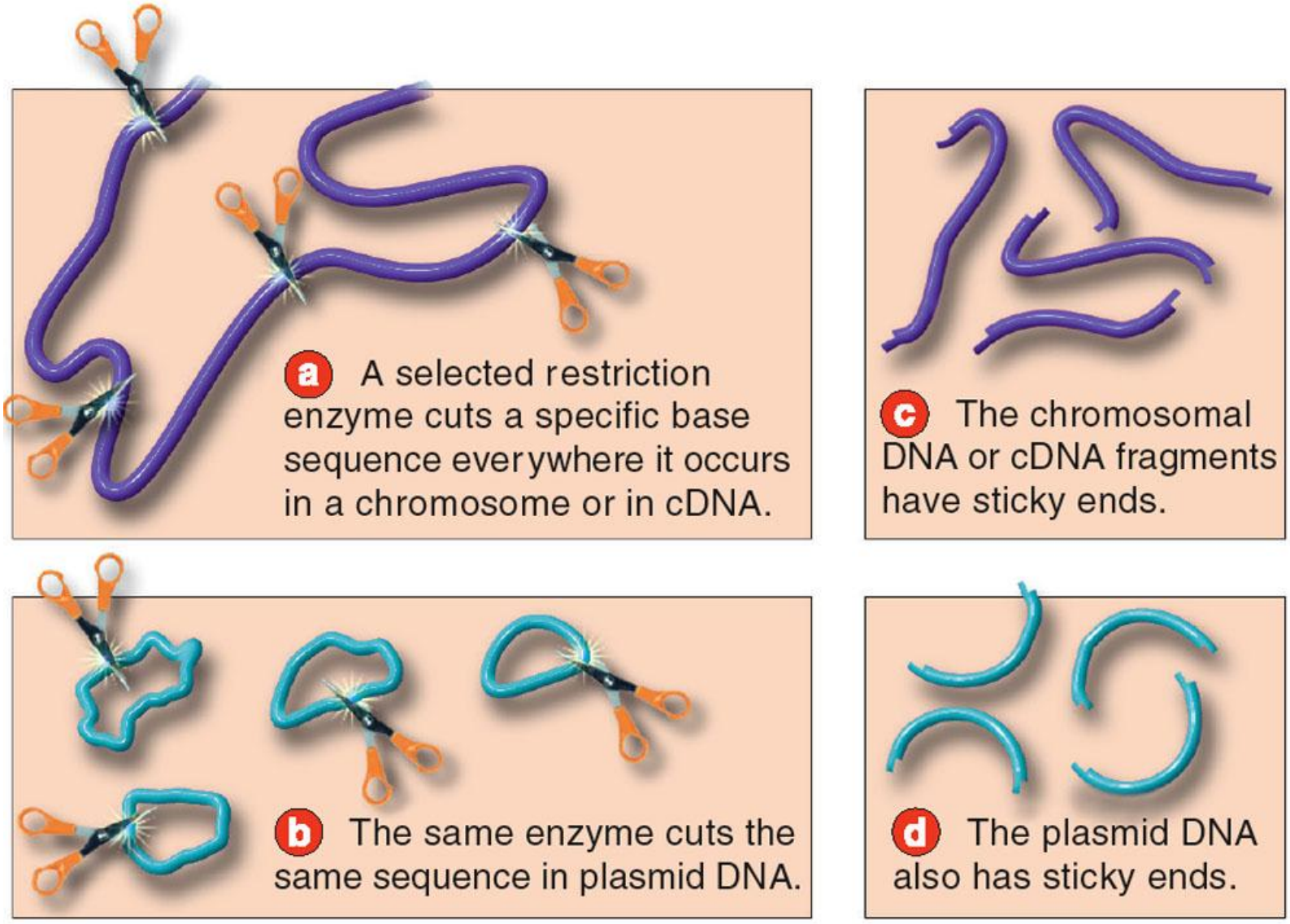


Fig. 13.11a-d

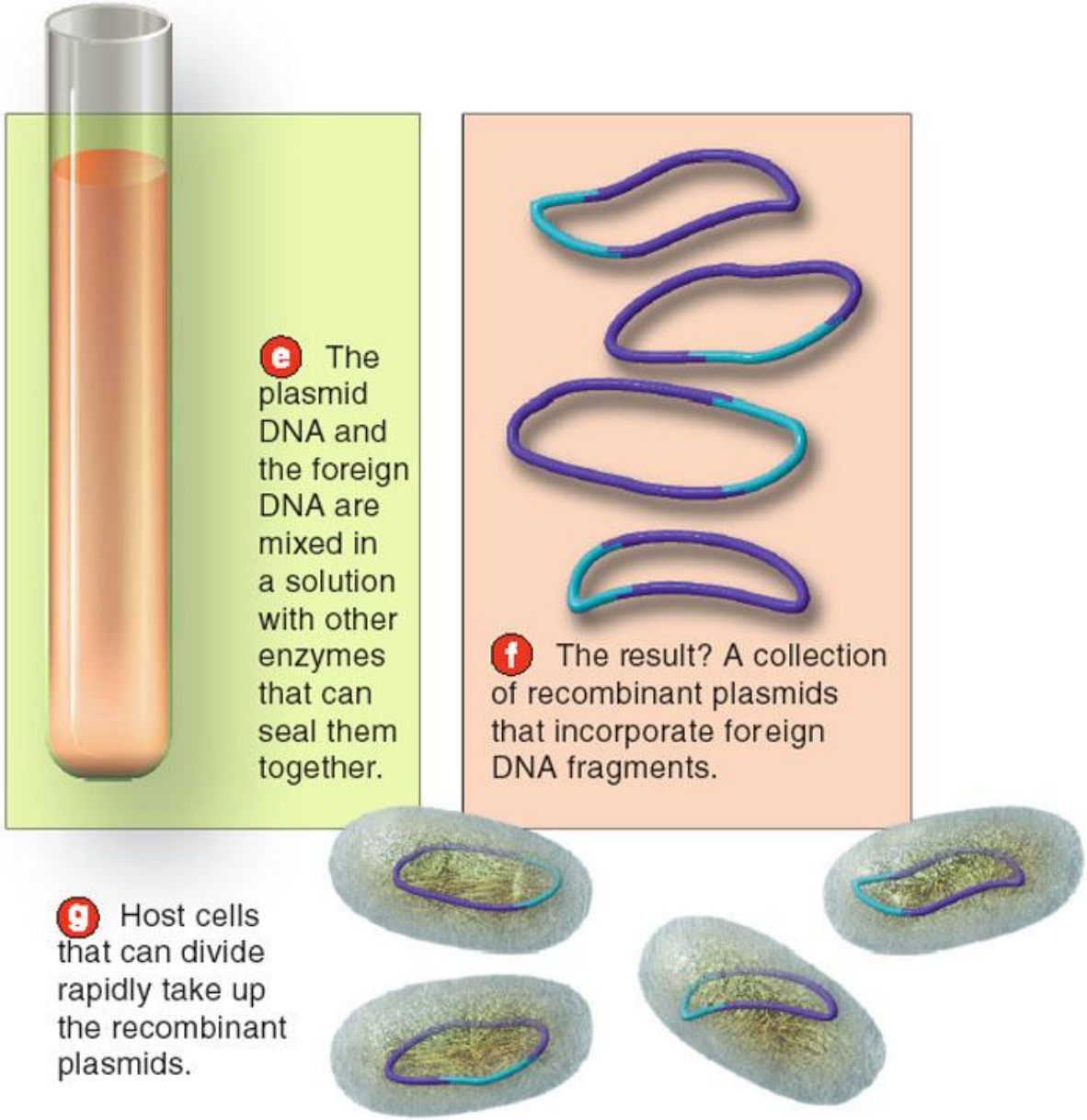
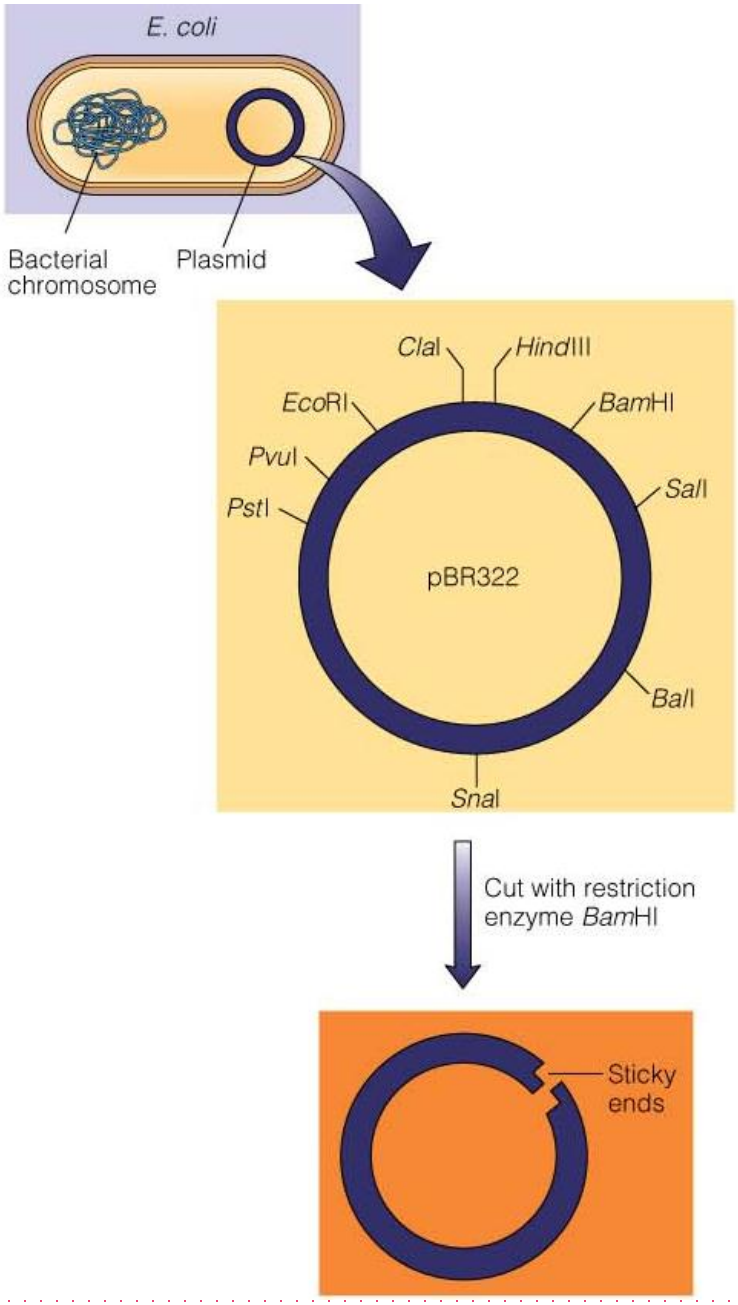

e The plasmid DNA and the foreign DNA are mixed in a solution with other enzymes that can seal them together.
f The result? A collection of recombinant plasmids that incorporate foreign DNA fragments.
g Host cells that can divide rapidly take up the recombinant plasmids.

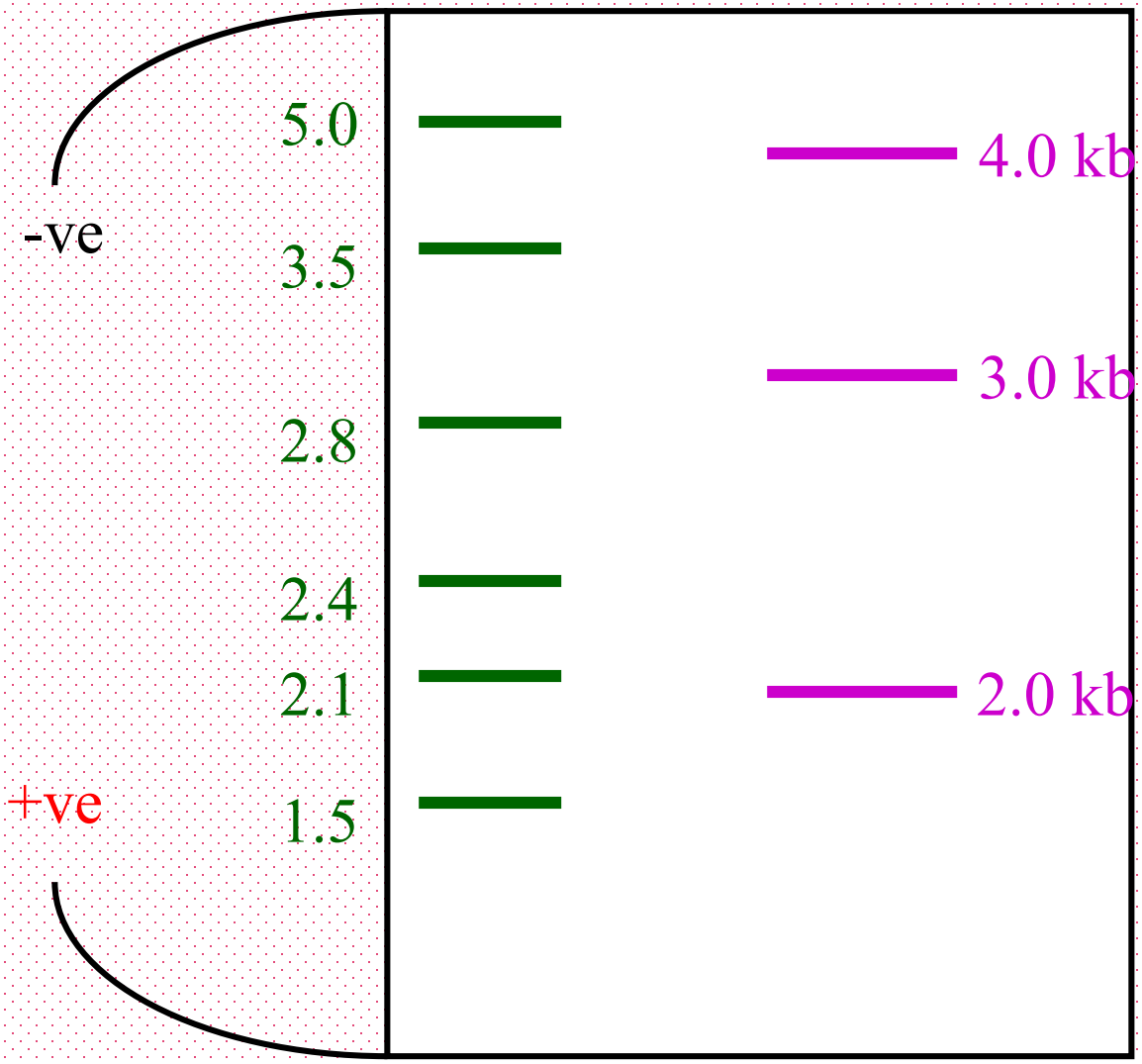
Fig. 13.11e-g


E. coli
Bacterial chromosome
Plasmid
ClaI
HindIII
EcoRI
BamHI
PvuI
SalI
PstI
pBR322
BalI
SnaI
Cut with restriction enzyme BamHI
Sticky ends

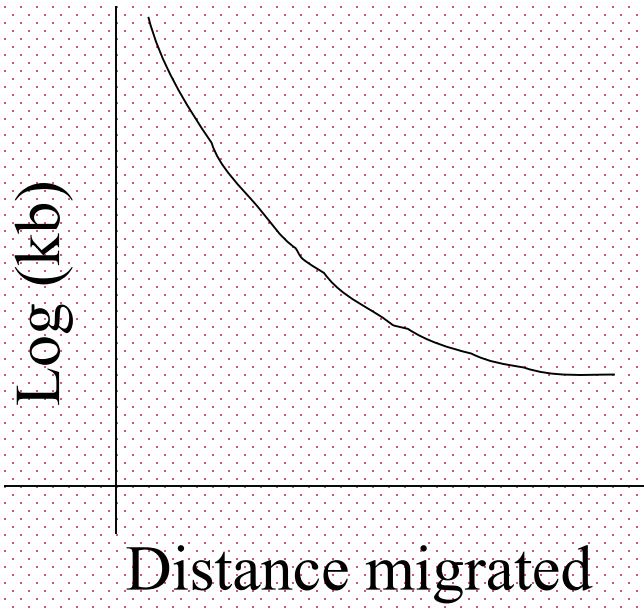
Steps in cloning a single piece of DNA

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.

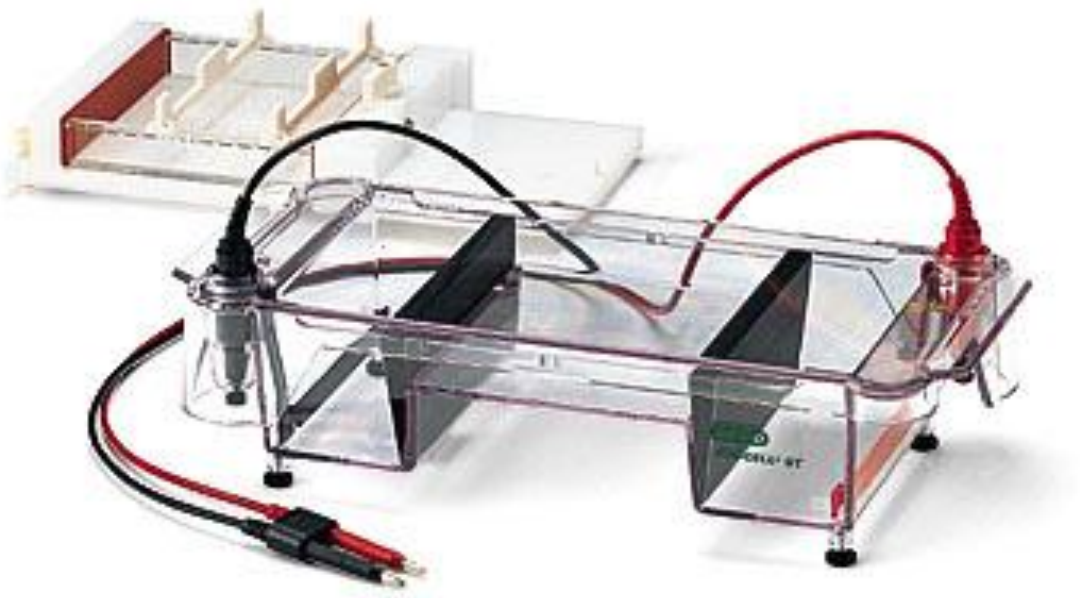
Gel electrophoresis

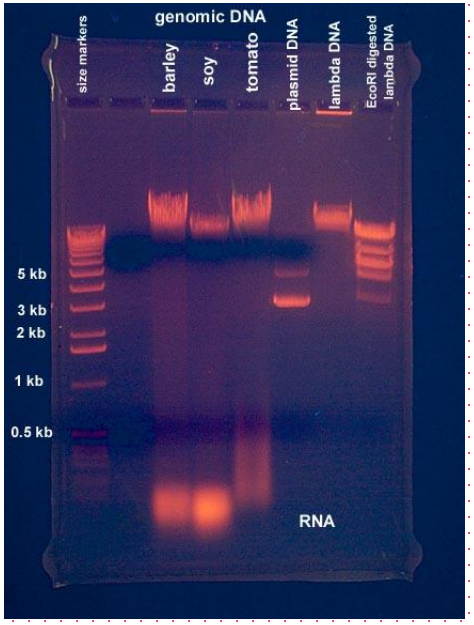


Size separation



Gel electrophoresis system or "gel box"





gel stained with ethidium bromide

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

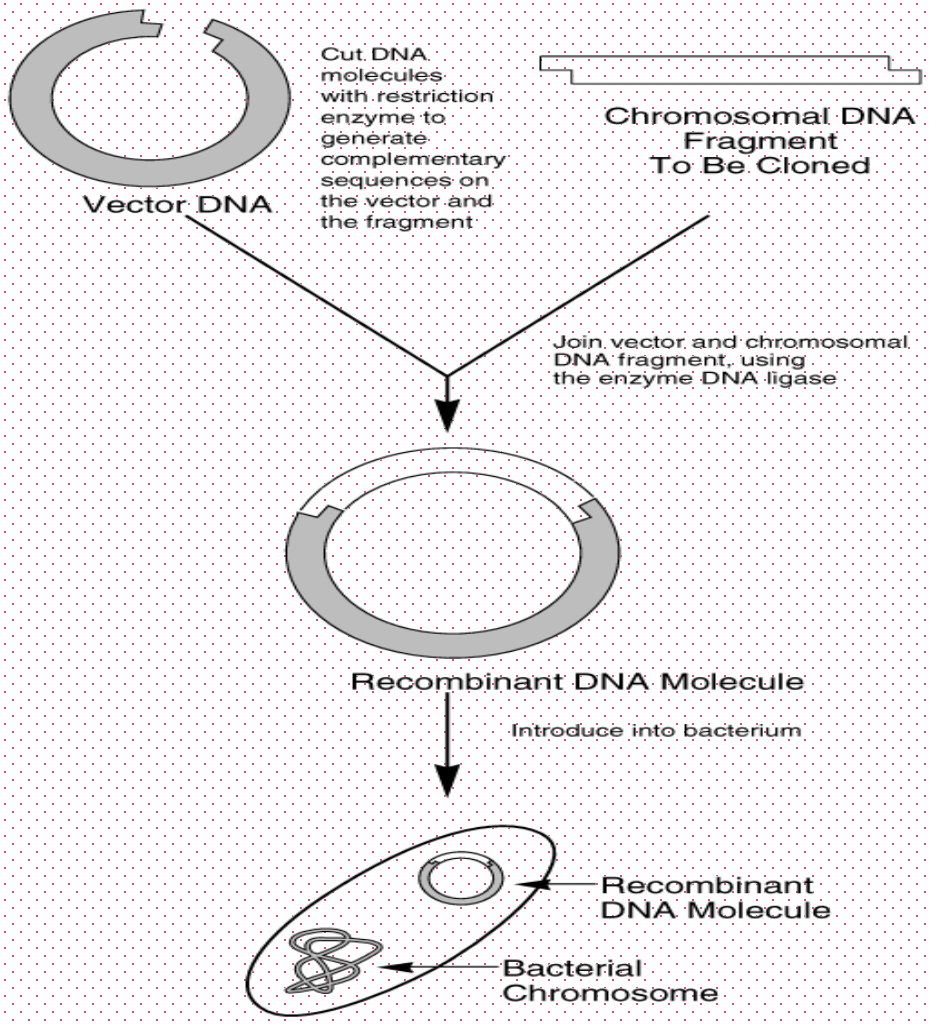
- While inserting the donor DNA, an existing gene in the vector is inactivated
- OR
- In addition to the Donor gene a marker gene is added

DNA CLONING

- MASSIVE AMPLIFICATION OF DNA SEQUENCES
- STABLE PROPAGATION OF DNA SEQUENCES
- A SINGLE DNA MOLECULE CAN BE AMPLIFIED ALLOWING IT TO BE:
  - STUDIED - SEQUENCED
  - MANIPULATED - MUTAGENISED OR ENGINEERED
  - EXPRESSED - GENERATION OF PROTEIN

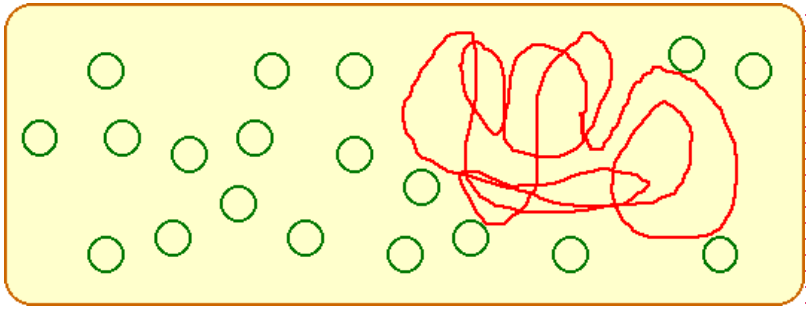
CLONING PROCESS

- GENE OF INTEREST IS CUT OUT WITH RE
- HOST PLASMID IS CUT WITH SAME RE
- GENE IS INSERTED INTO PLASMID AND LIGATED WITH LIGASE
- NEW PLASMID INSERTED INTO BACTERIUM (TRANSFORM)



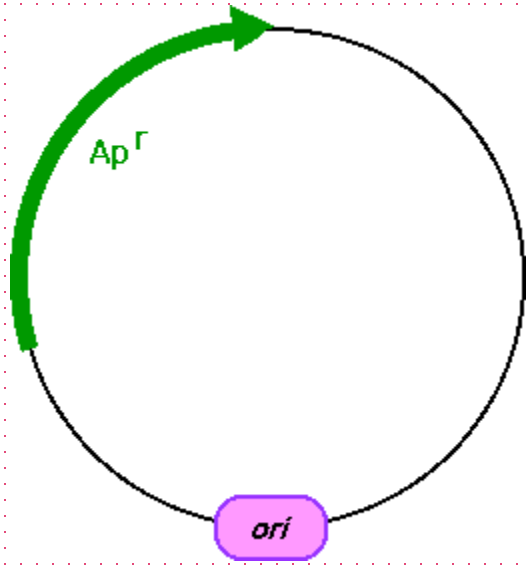
PLASMIDS

- BACTERIAL CELLS MAY CONTAIN EXTRA-CHROMOSOMAL DNA CALLED PLASMIDS.
- PLASMIDS ARE USUALLY REPRESENTED BY SMALL, CIRCULAR DNA.
- SOME PLASMIDS ARE PRESENT IN MULTIPLE COPIES IN THE CELL



SELECTIVE MARKER

- SELECTIVE MARKER IS REQUIRED FOR MAINTENANCE OF PLASMID IN THE CELL.
- BECAUSE OF THE PRESENCE OF THE SELECTIVE MARKER THE PLASMID BECOMES USEFUL FOR THE CELL.
- UNDER THE SELECTIVE CONDITIONS, ONLY CELLS THAT CONTAIN PLASMIDS WITH SELECTABLE MARKER CAN SURVIVE
- GENES THAT CONFER RESISTANCE TO VARIOUS ANTIBIOTICS ARE USED.
- GENES THAT MAKE CELLS RESISTANT TO AMPICILLIN, NEOMYCIN, OR CHLORAMPHENICOL ARE USED



PLASMID CLONING STRATEGY

- **INVOLVES FIVE STEPS:**

  ENZYME RESTRICTION DIGEST OF **DNA SAMPLE.**

  ENZYME RESTRICTION DIGEST OF **DNA PLASMID VECTOR.**

  LIGATION OF DNA SAMPLE PRODUCTS AND PLASMID VECTOR.

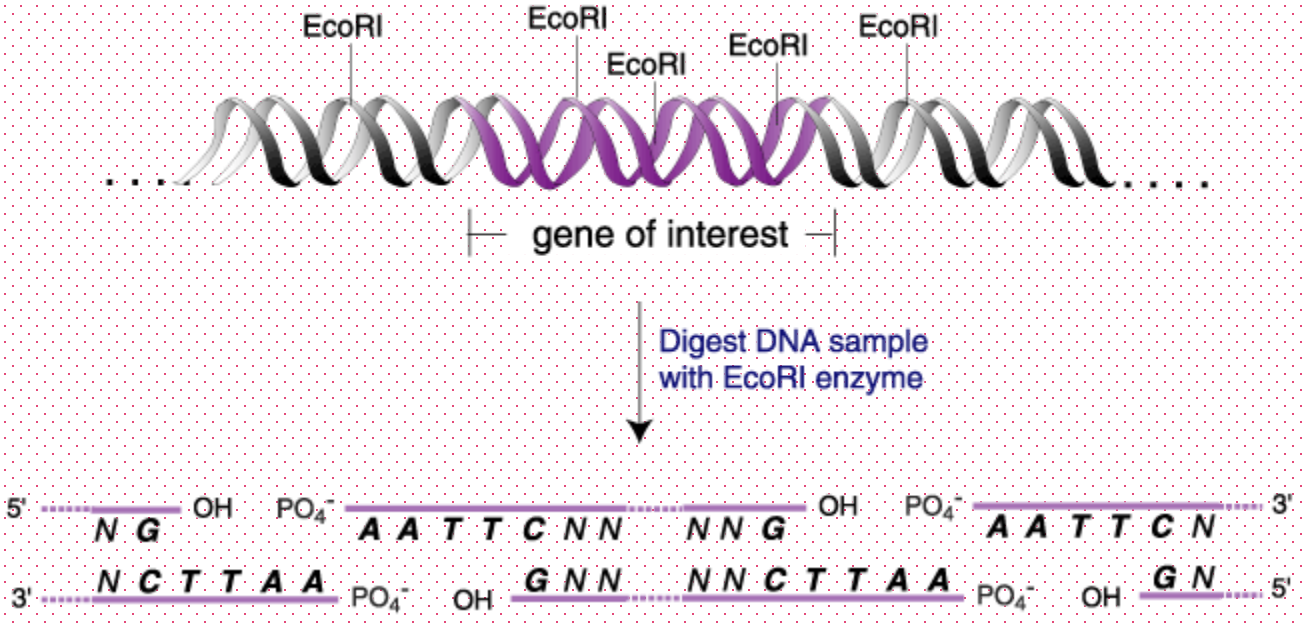
  TRANSFORMATION WITH THE LIGATION PRODUCTS.

  GROWTH ON AGAR PLATES WITH SELECTION FOR **ANTIBIOTIC RESISTANCE.**

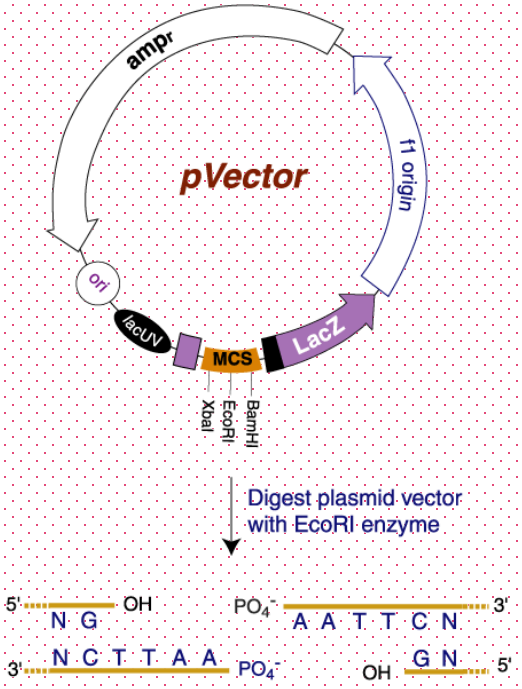
STEP 4. TRANSFORMATION OF LIGATION PRODUCTS

- THE PROCESS OF TRANSFERRING EXOGENOUS DNA INTO CELLS IS CALL "TRANSFORMATION"
- THERE ARE BASICALLY TWO GENERAL METHODS FOR TRANSFORMING BACTERIA. THE FIRST IS A CHEMICAL METHOD UTILIZING CACL2 AND HEAT SHOCK TO PROMOTE DNA ENTRY INTO CELLS.
- A SECOND METHOD IS CALLED ELECTROPORATION BASED ON A SHORT PULSE OF ELECTRIC CHARGE TO FACILITATE DNA UPTAKE.

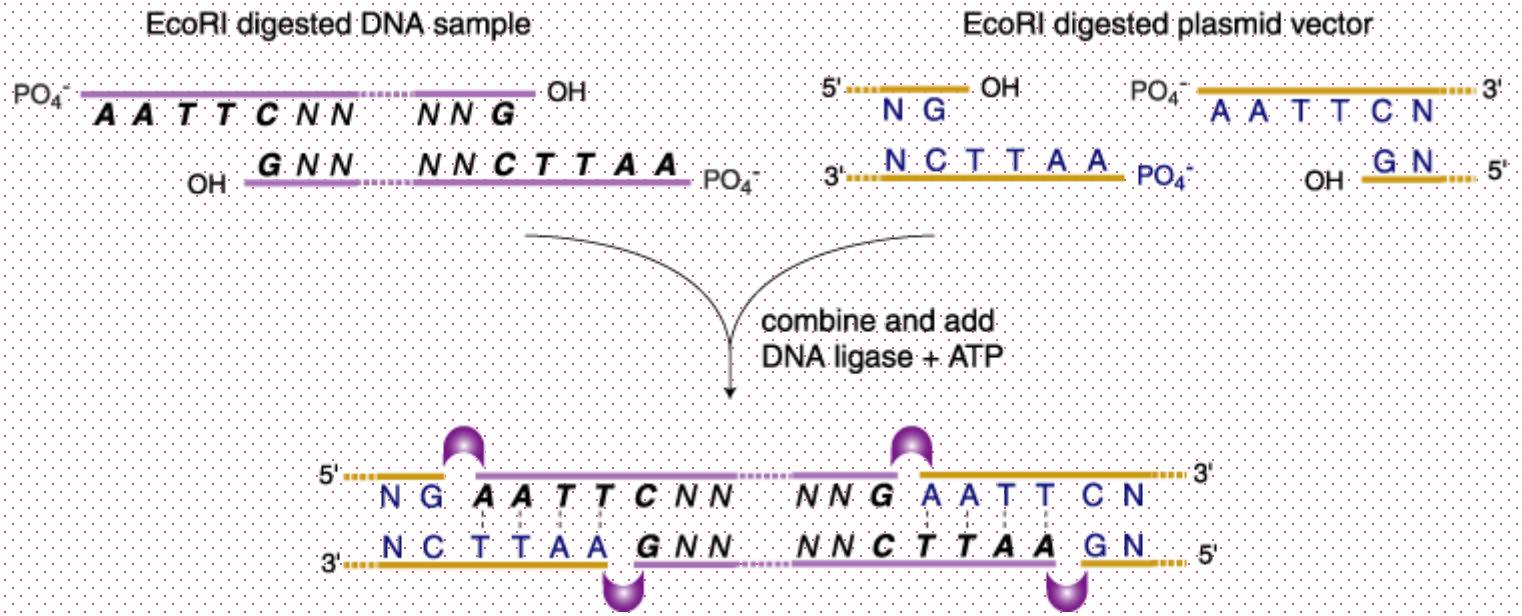
STEP 1. RE DIGESTION OF DNA SAMPLE



STEP 2. RE DIGESTION OF PLASMID DNA



STEP 3. LIGATION OF DNA SAMPLE AND PLASMID DNA



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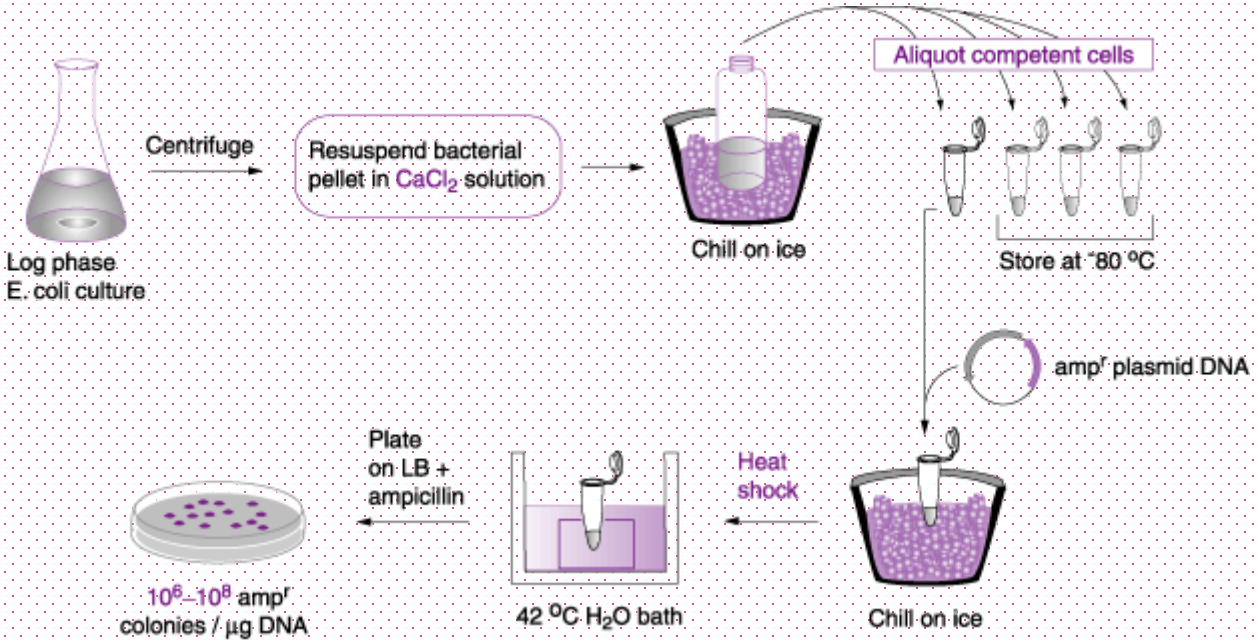
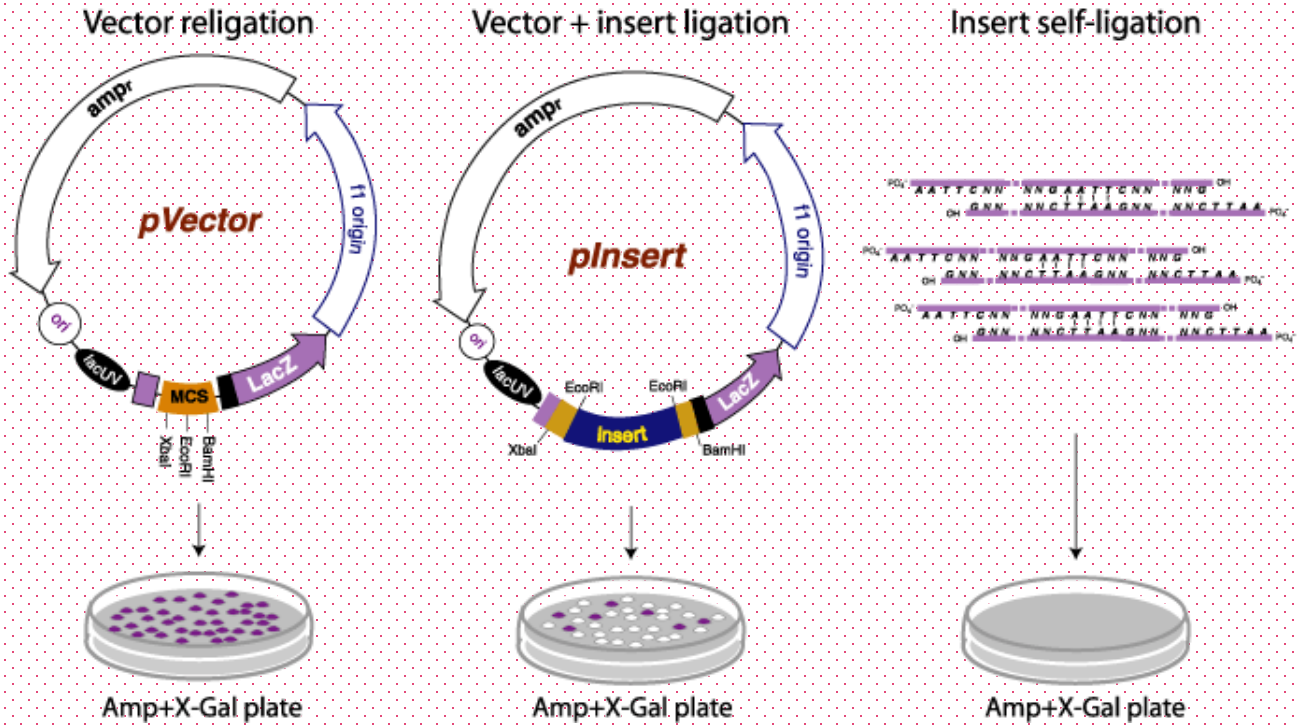
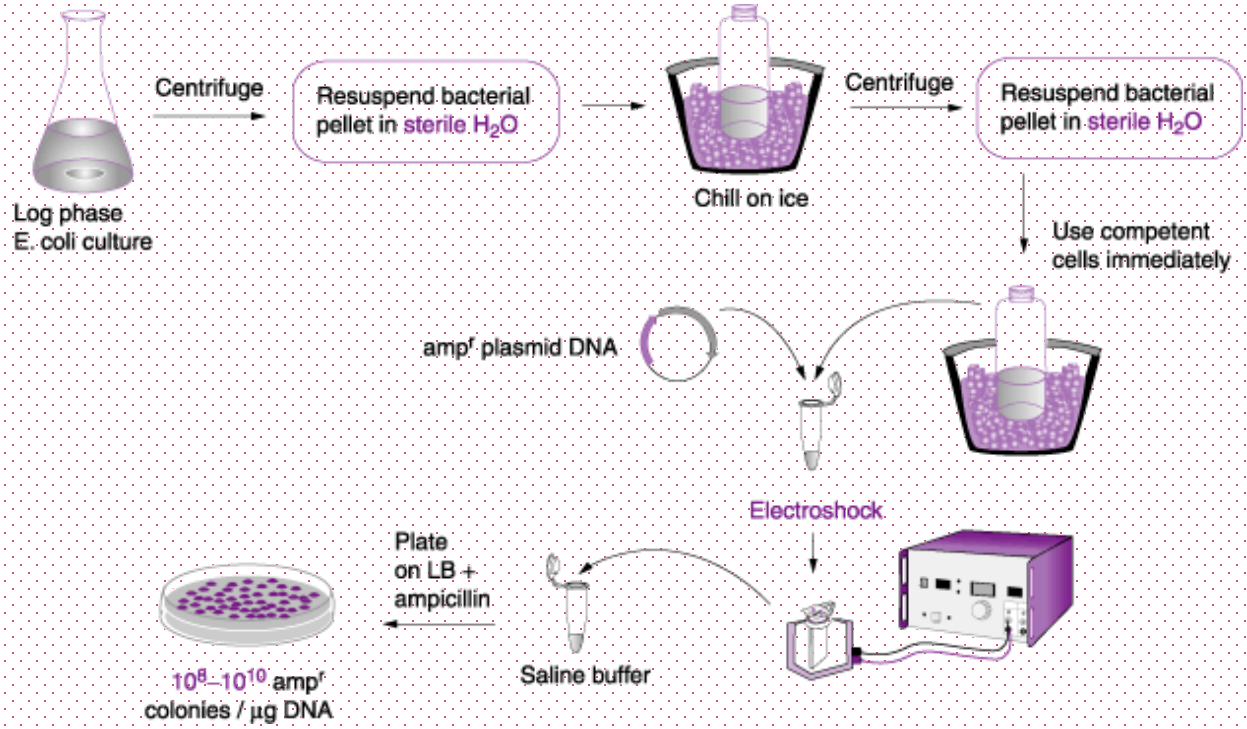
CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE



STEP 5. GROWTH ON AGAR PLATES



TRANSFORMATION BY ELECTROPORATION



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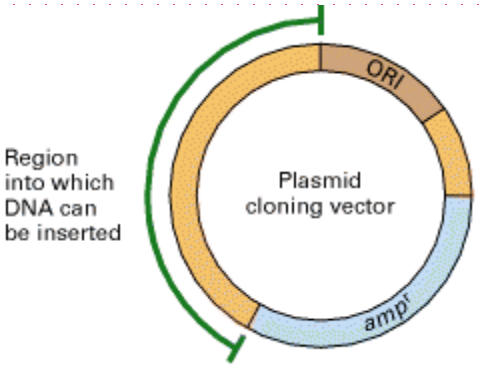
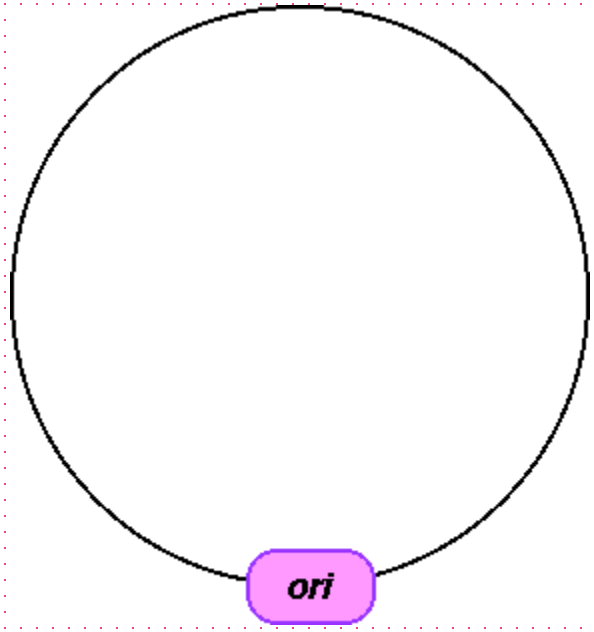
PLASMID VECTORS

- PLASMID VECTORS ARE ≈1.2–3KB AND CONTAIN:
- REPLICATION ORIGIN (ORI) SEQUENCE
- A GENE THAT PERMITS SELECTION,
- HERE THE SELECTIVE GENE IS *AMP*R; IT ENCODES THE ENZYME B-LACTAMASE, WHICH INACTIVATES AMPICILLIN.
- EXOGENOUS DNA CAN BE INSERTED INTO THE BRACKETED REGION .



ORIGIN OF REPLICATION

- **ORIGIN OF REPLICATION** IS A DNA SEGMENT RECOGNIZED BY THE CELLULAR DNA-REPLICATION ENZYMES.
- WITHOUT REPLICATION ORIGIN, DNA CANNOT BE REPLICATED IN THE CELL.



MULTIPLE CLONING SITE

- GENE TO BE CLONED CAN BE INTRODUCED INTO THE CLONING VECTOR AT ONE OF THE RESTRICTION SITES PRESENT IN THE POLYLINKER