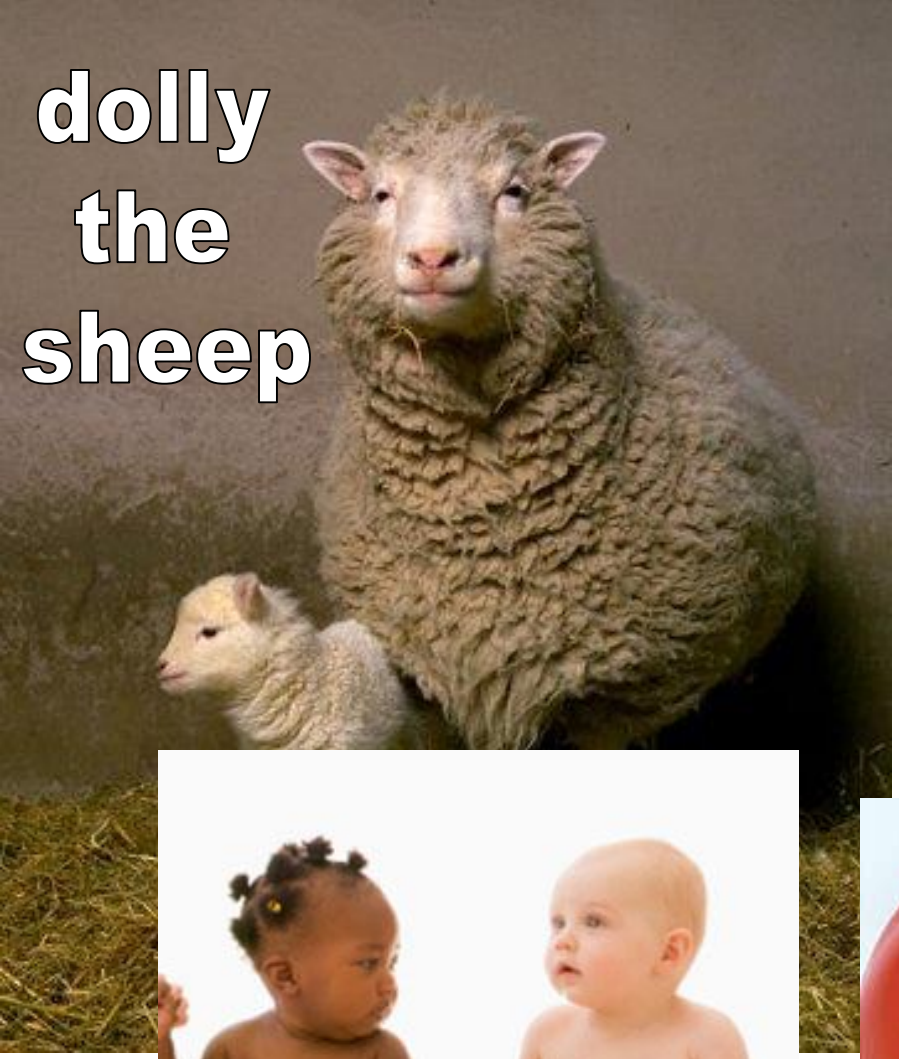
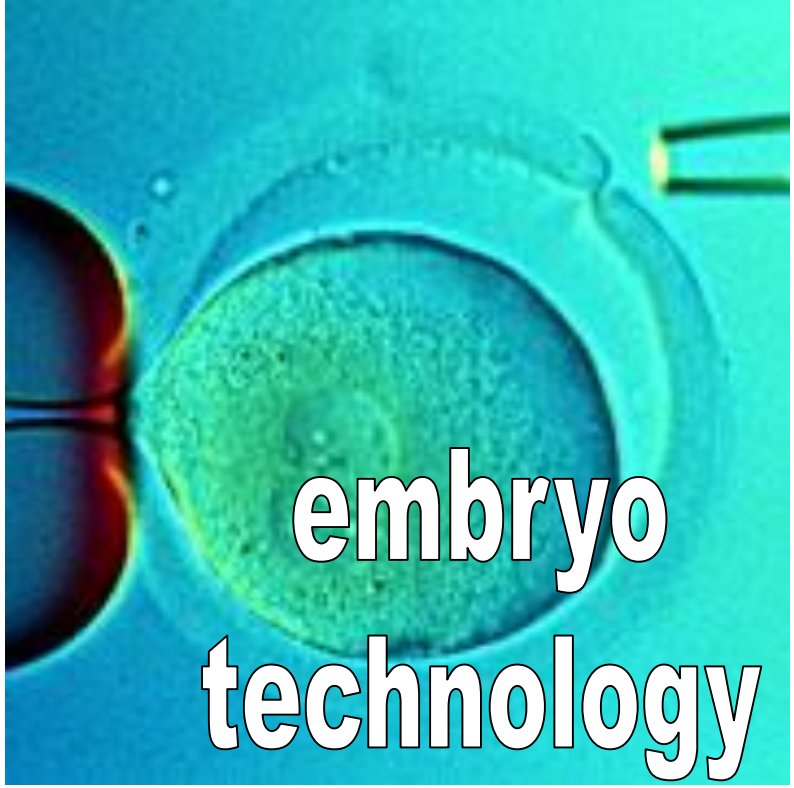
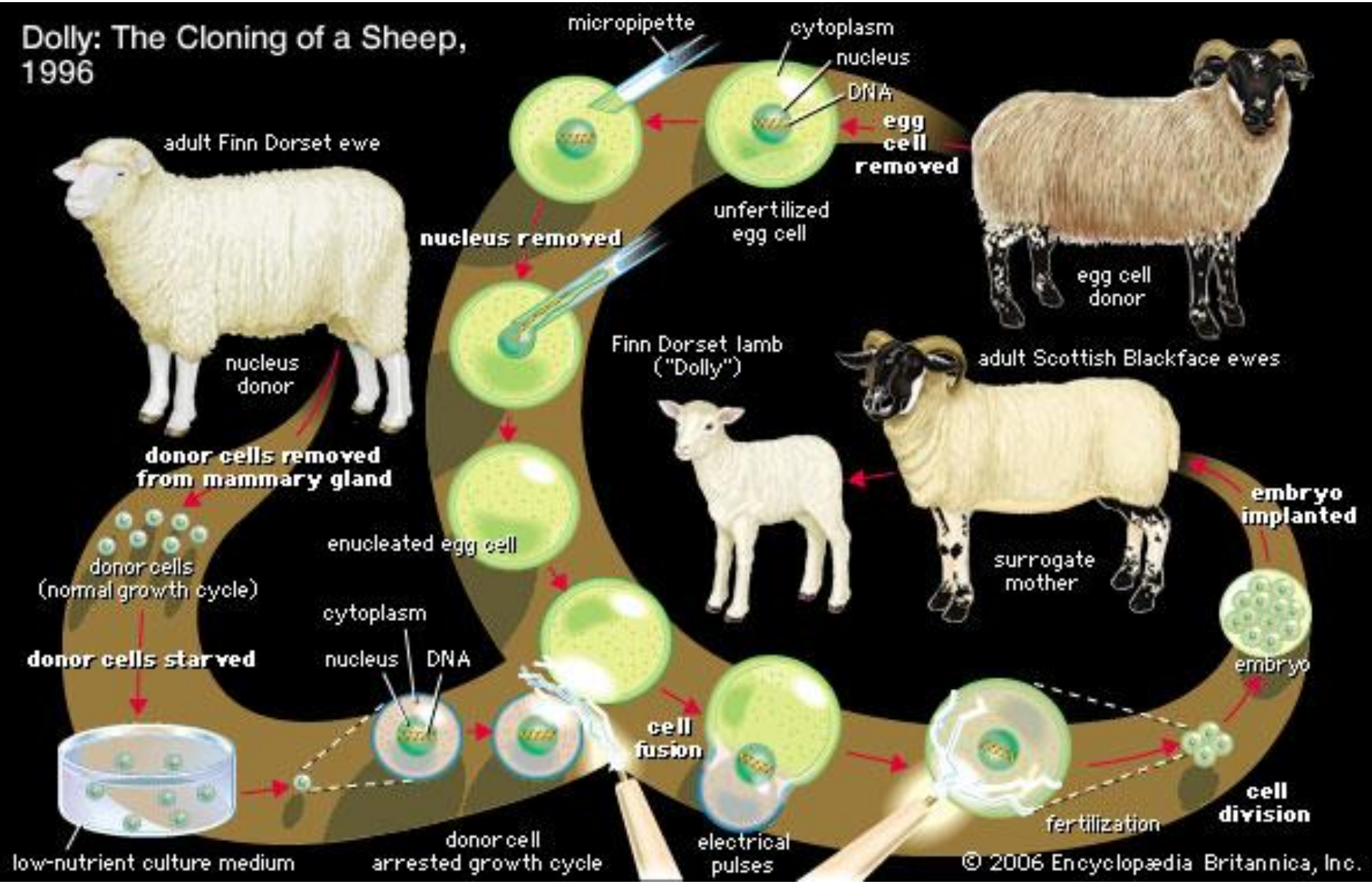
GENE TECHNOLOGY


dolly
the
sheep

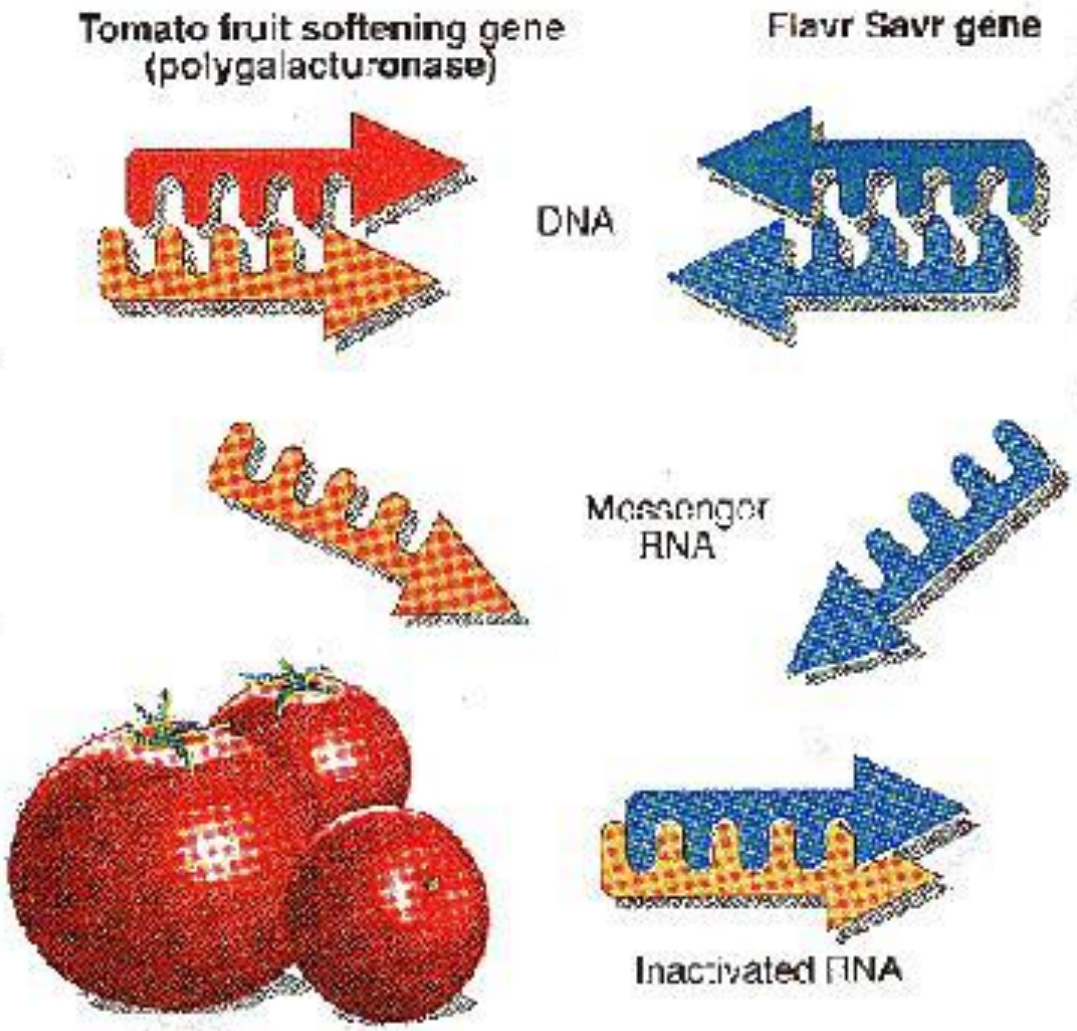

embryo
technology


designer
babies


flavr savr
tomatoes
GROWN FROM
84805
GENETICALLY MODIFIED SEEDS

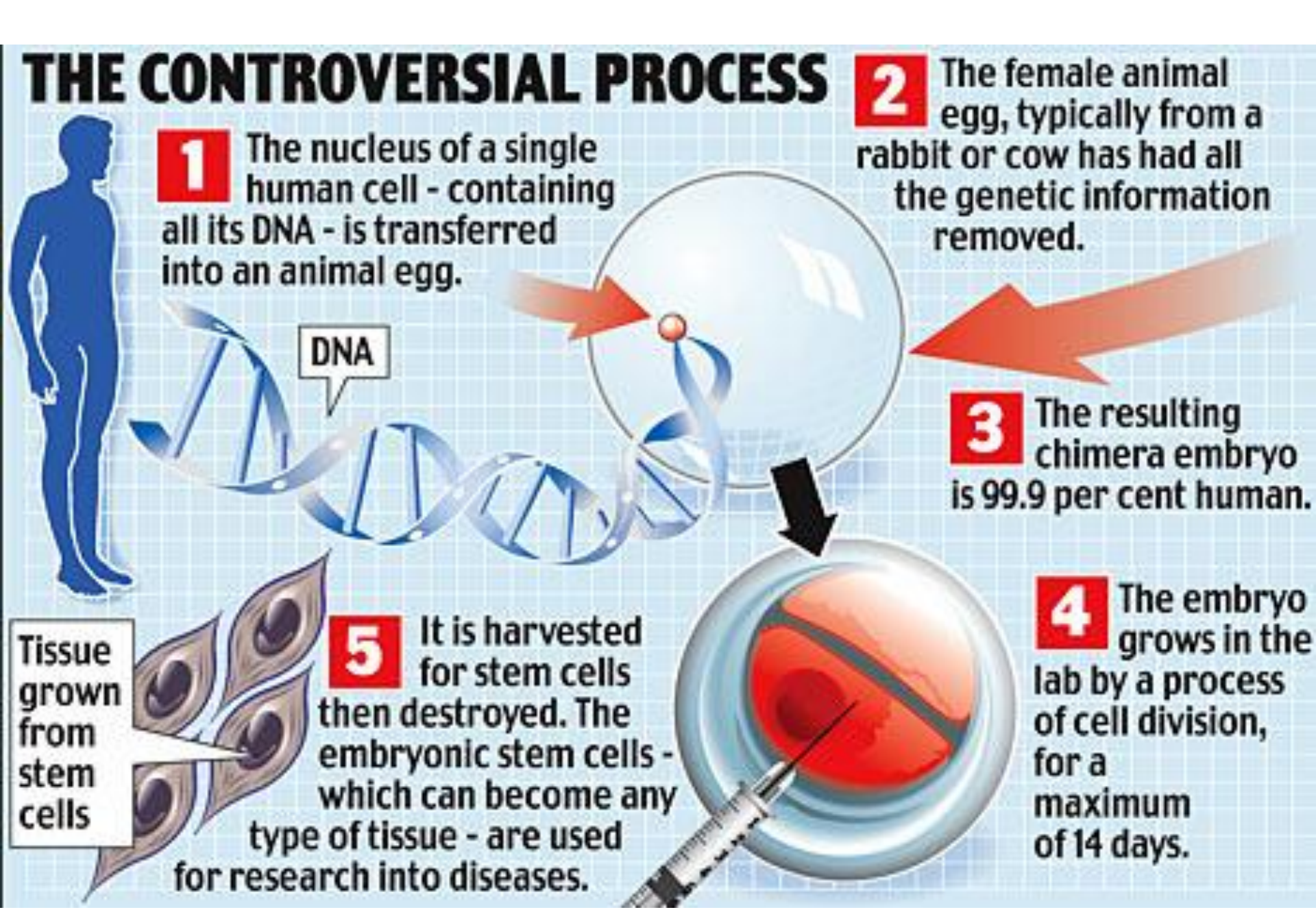

Dolly: The Cloning of a Sheep, 1996
micropipette
cytoplasm
nucleus
DNA
egg cell removed
adult Finn Dorset ewe
unfertilized egg cell
nucleus removed
egg cell donor
Finn Dorset lamb ("Dolly")
adult Scottish Blackface ewes
nucleus donor
donor cells removed from mammary gland
embryo implanted
enucleated egg cell
surrogate mother
donor cells (normal growth cycle)
cytoplasm
nucleus
DNA
donor cells starved
embryo
cell fusion
cell division
fertilization
low-nutrient culture medium
donor cell arrested growth cycle
electrical pulses
© 2006 Encyclopædia Britannica, Inc.

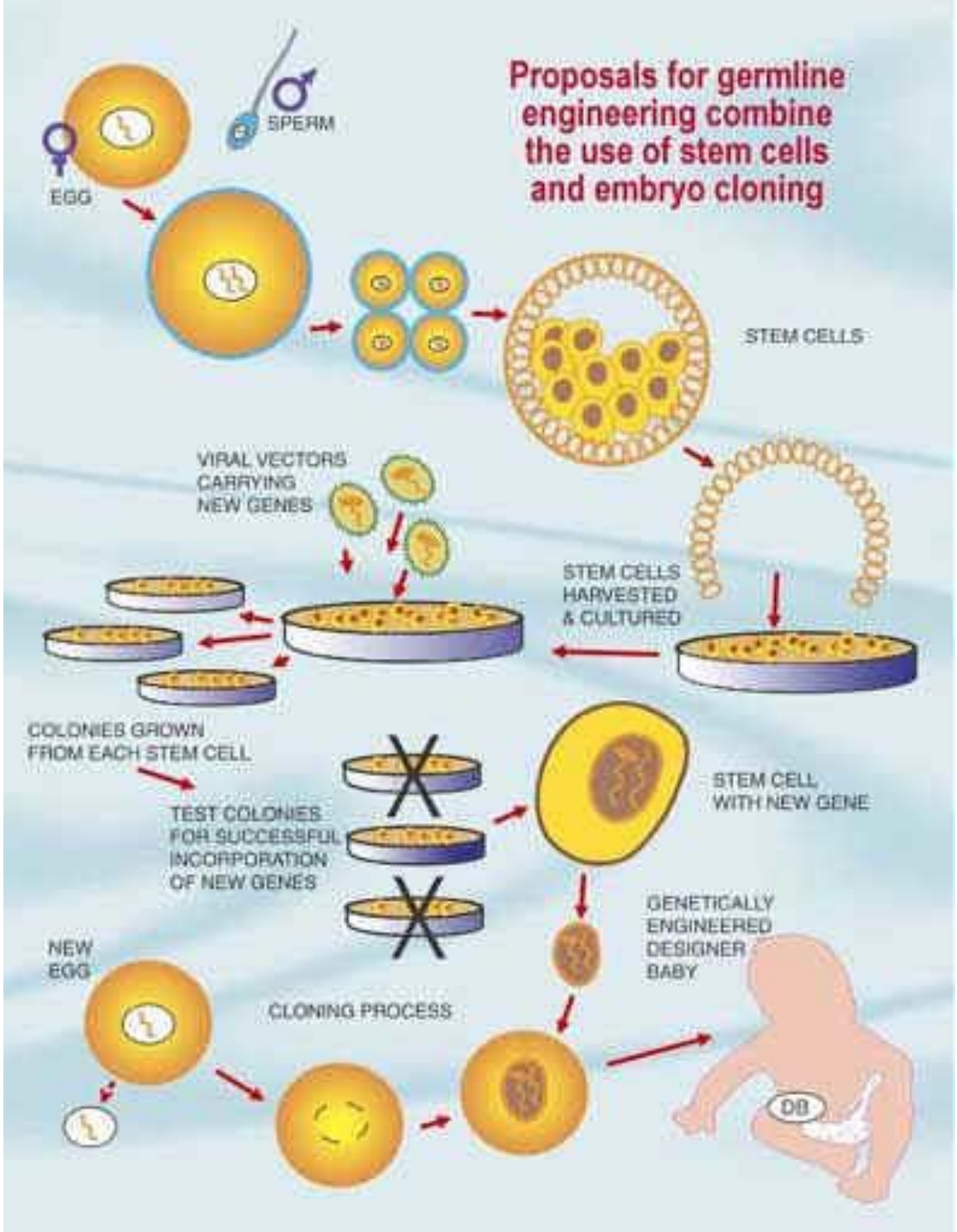
How the Flavr Savr stops the rot



Food chain: in ordinary tomatoes, messenger RNA from the softening gene is translated into the protein polygalacturonase. In the Flavr Savr, this RNA is blocked by RNA from the antisense gene

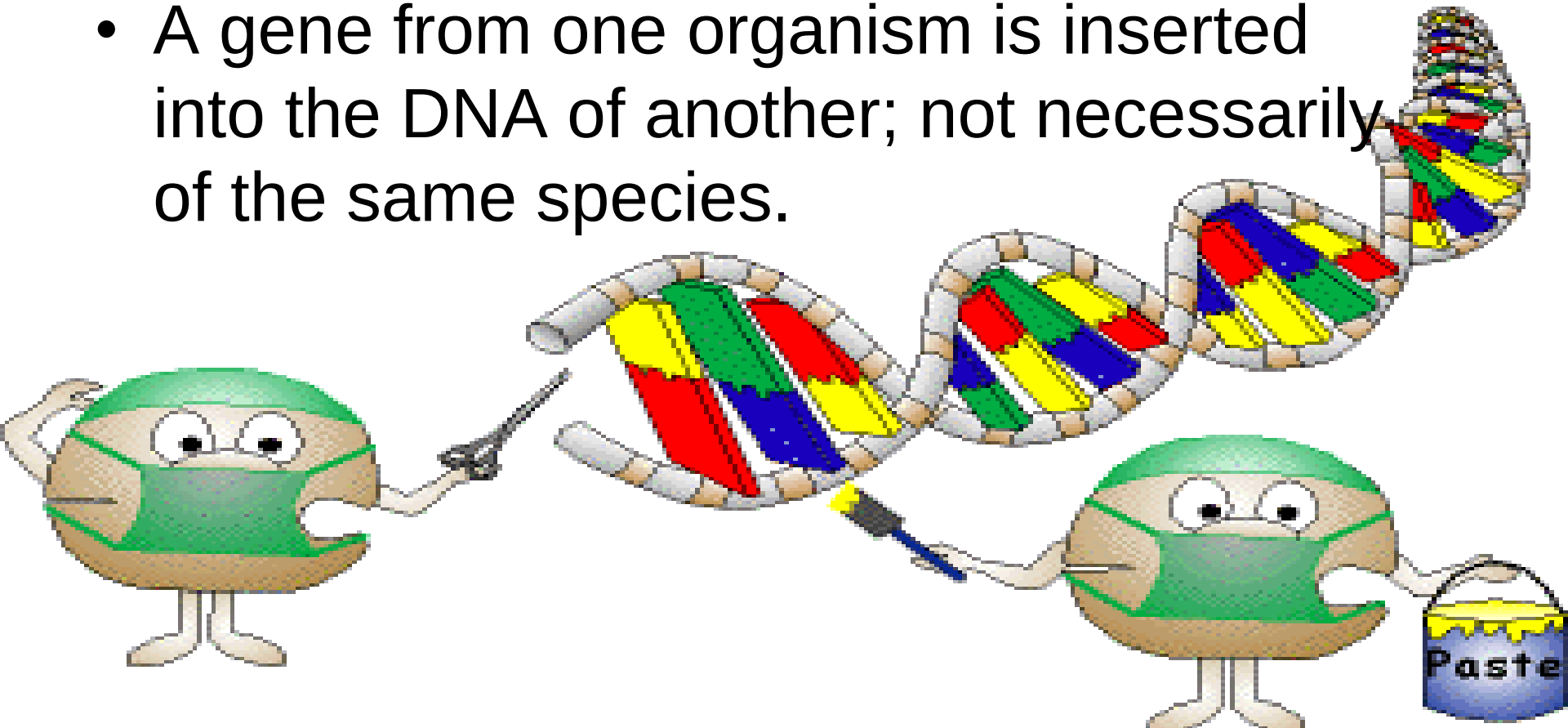
THE CONTROVERSIAL PROCESS




Proposals for germline engineering combine the use of stem cells and embryo cloning
SPERM
EGG
STEM CELLS
VIRAL VECTORS CARRYING NEW GENES
STEM CELLS HARVESTED & CULTURED
COLONIES GROWN FROM EACH STEM CELL
TEST COLONIES FOR SUCCESSFUL INCORPORATION OF NEW GENES
STEM CELL WITH NEW GENE
GENETICALLY ENGINEERED DESIGNER BABY
NEW EGG
CLONING PROCESS
DB

GENE TRANSFER

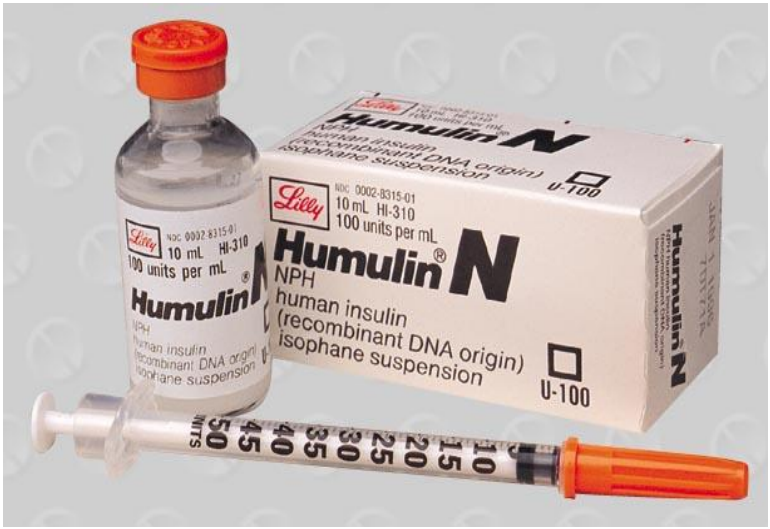
- One method of gene technology that is commonly used is gene transfer.
- A gene from one organism is inserted into the DNA of another; not necessarily of the same species.



- DNA that contains genetic material from TWO different organisms is called **recombinant DNA**.
- An organism that contains recombinant DNA is called a **transgenic organism.**

WHY TRANSFER GENES?

- The organism receiving the gene (host) benefits from it in some way
  - Flavr Savr tomatoes have a longer shelf life as they are less likely to rot

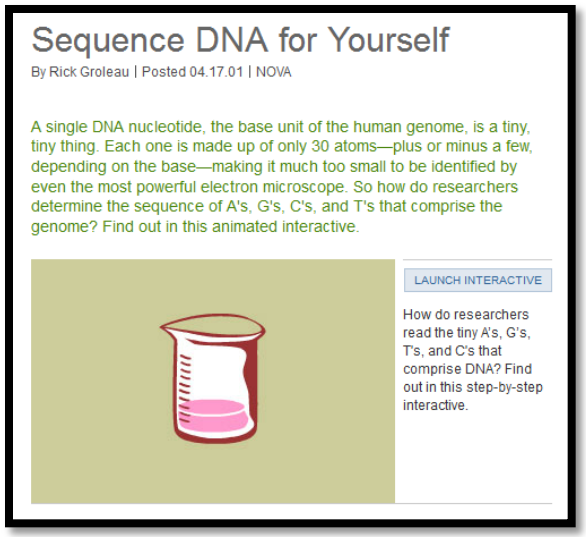


- The organism is used to produce a protein which is beneficial to humans
  - Bacteria used to produce human insulin
  - Sheep used to produce Alpha-1-antitrypsin in their milk

SOME USEFUL WEBLINKS

- recombinant DNA technology; creating a GEM (genetically engineered microorganism)
  - http://webapps.css.udel.edu/Biotech/rDNA.html

- NOVA Online | how to sequence DNA – read through this v good animation slowly and carefully!
  - http://www.pbs.org/wgbh/nova/genome/sequ_flash.html



RECOMBINANT DNA TECHNOLOGY

Involves:

- Isolation of the gene using enzymes
- Location of the DNA fragment using DNA probes
- Insertion of donor gene into a vector
- Transformation of recipient cell
- Check recipient cells contain recombinant DNA using genetic markers
- Multiplication of host cells

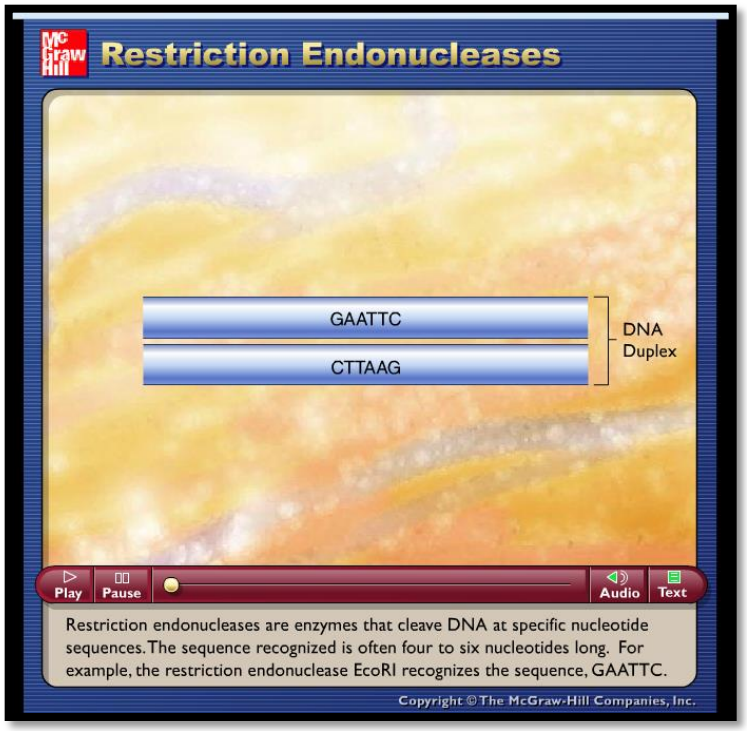
USING BACTERIA TO PRODUCE USEFUL PROTEINS

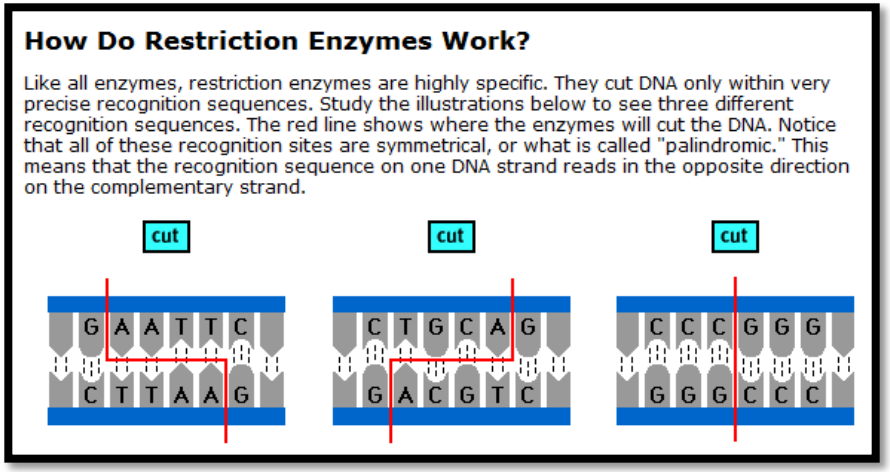
METHOD

The first step in the process is obtaining donor DNA. The most common techniques used are:

- cutting the DNA from donor chromosomes using **restriction endonuclease enzymes**

- using **reverse transcriptase** enzyme to make cDNA from donor mRNA





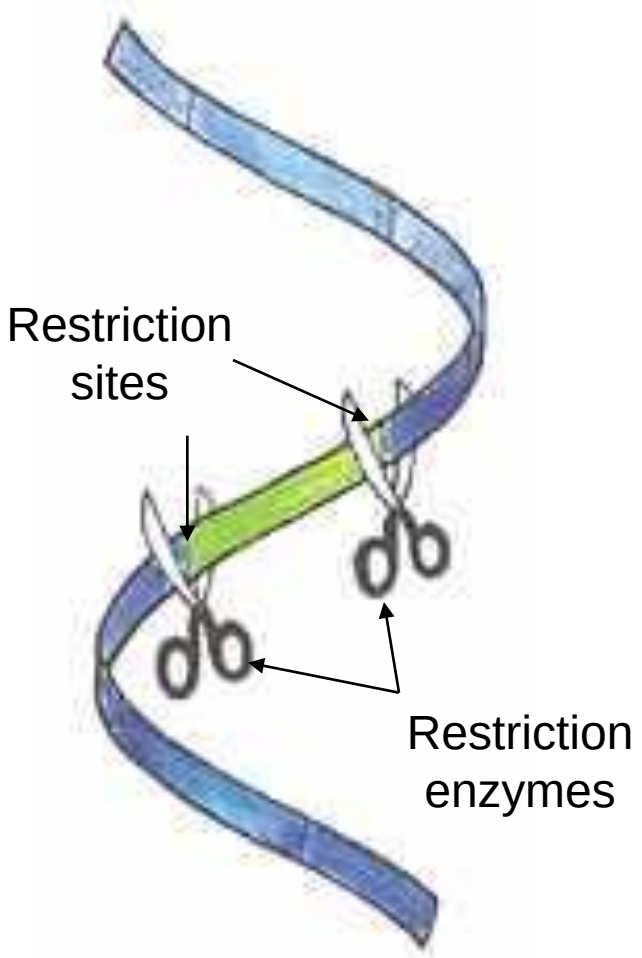
restriction endonucleases

http://highered.mcgraw-hill.com/olc/dl/120078/bio37.swf

http://www.phschool.com/science/biology_place/labbench/lab6/enzwork.html

Cutting DNA using restriction enzymes

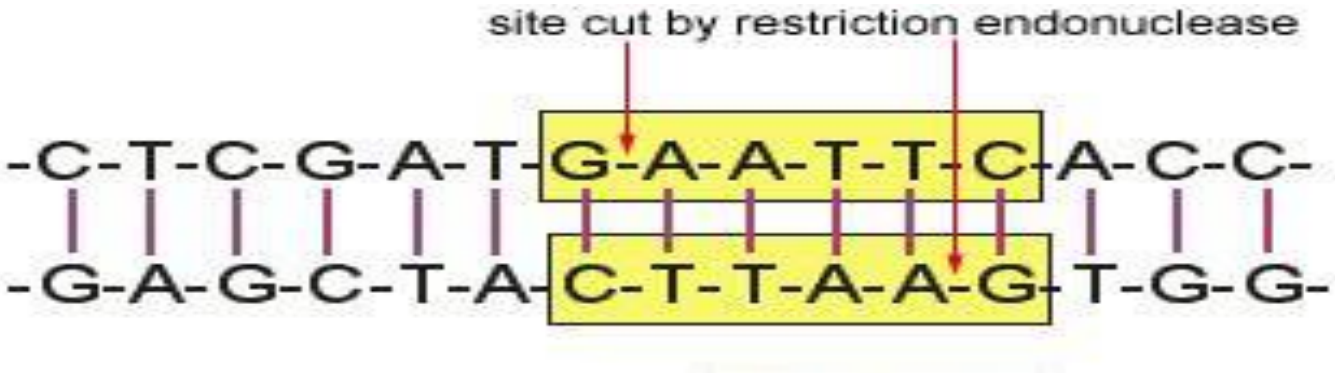
- REs cut donor DNA at specific points called **recognition sites**.
- These are specific base sequences found at the start and end of the required gene
- The enzymes make staggered cuts, called **sticky ends**.

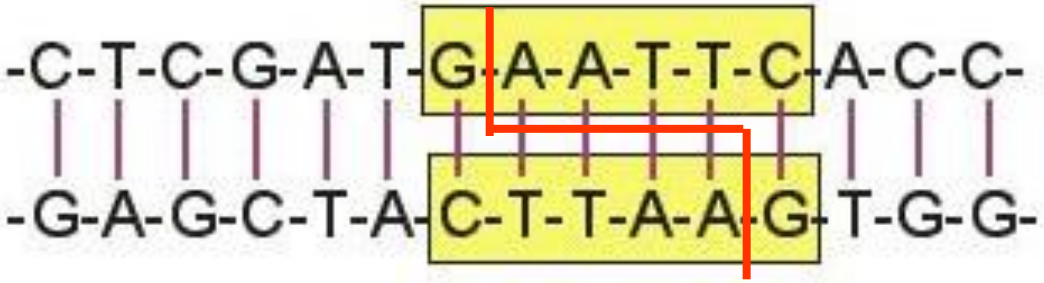


EcoR1

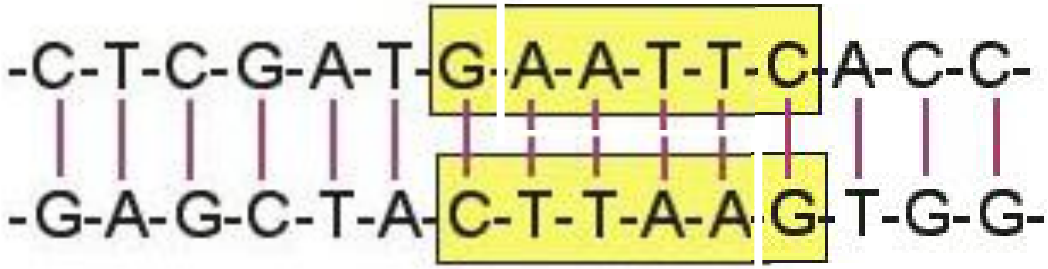
- Is a restriction enzyme made by E coli

  (many bacteria make restriction enzymes to cut invading DNA preventing infection)
- It recognises the base sequence GAATTC and cuts both the DNA backbone and the hydrogen bonds between the base pairs.

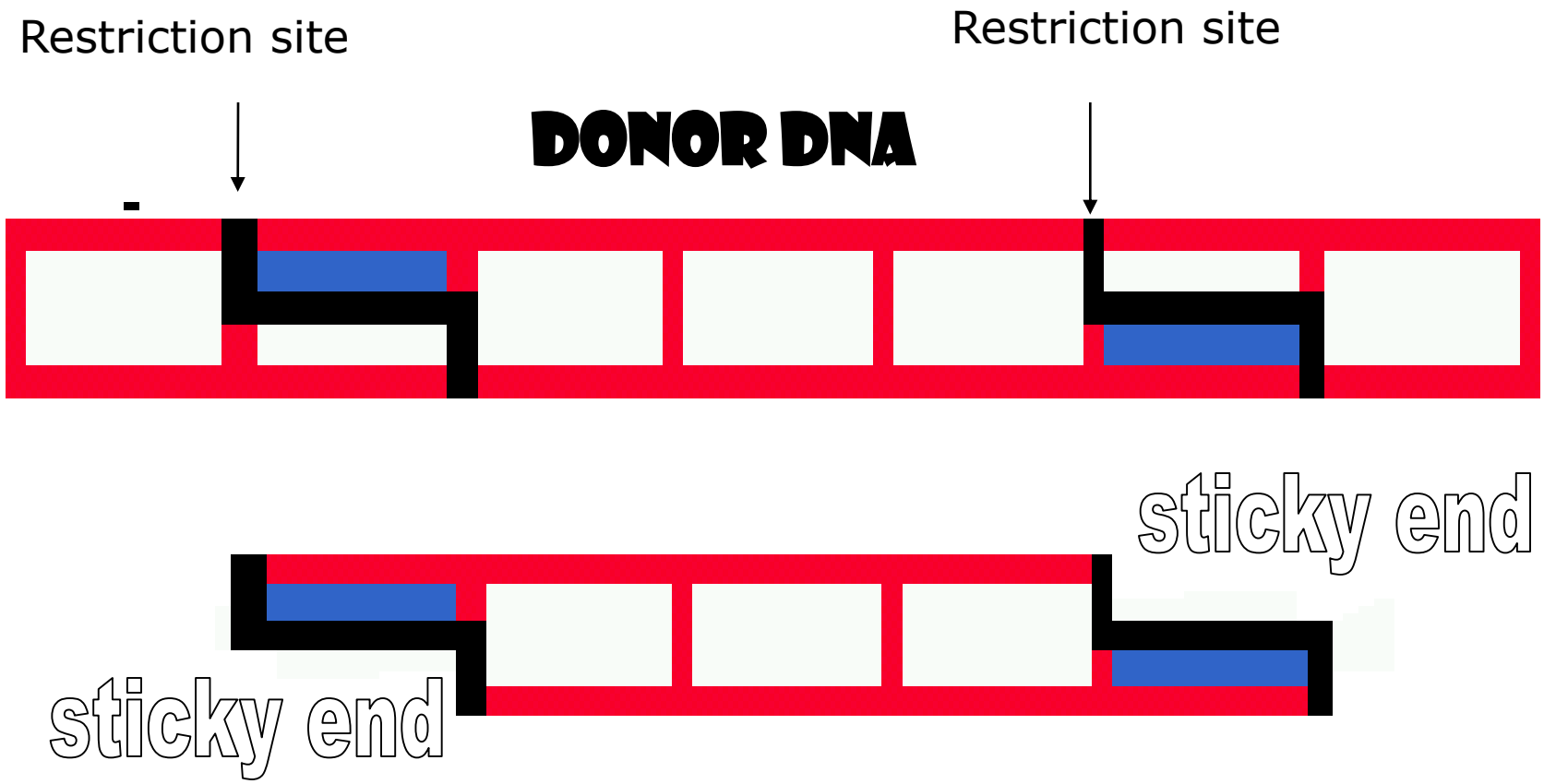




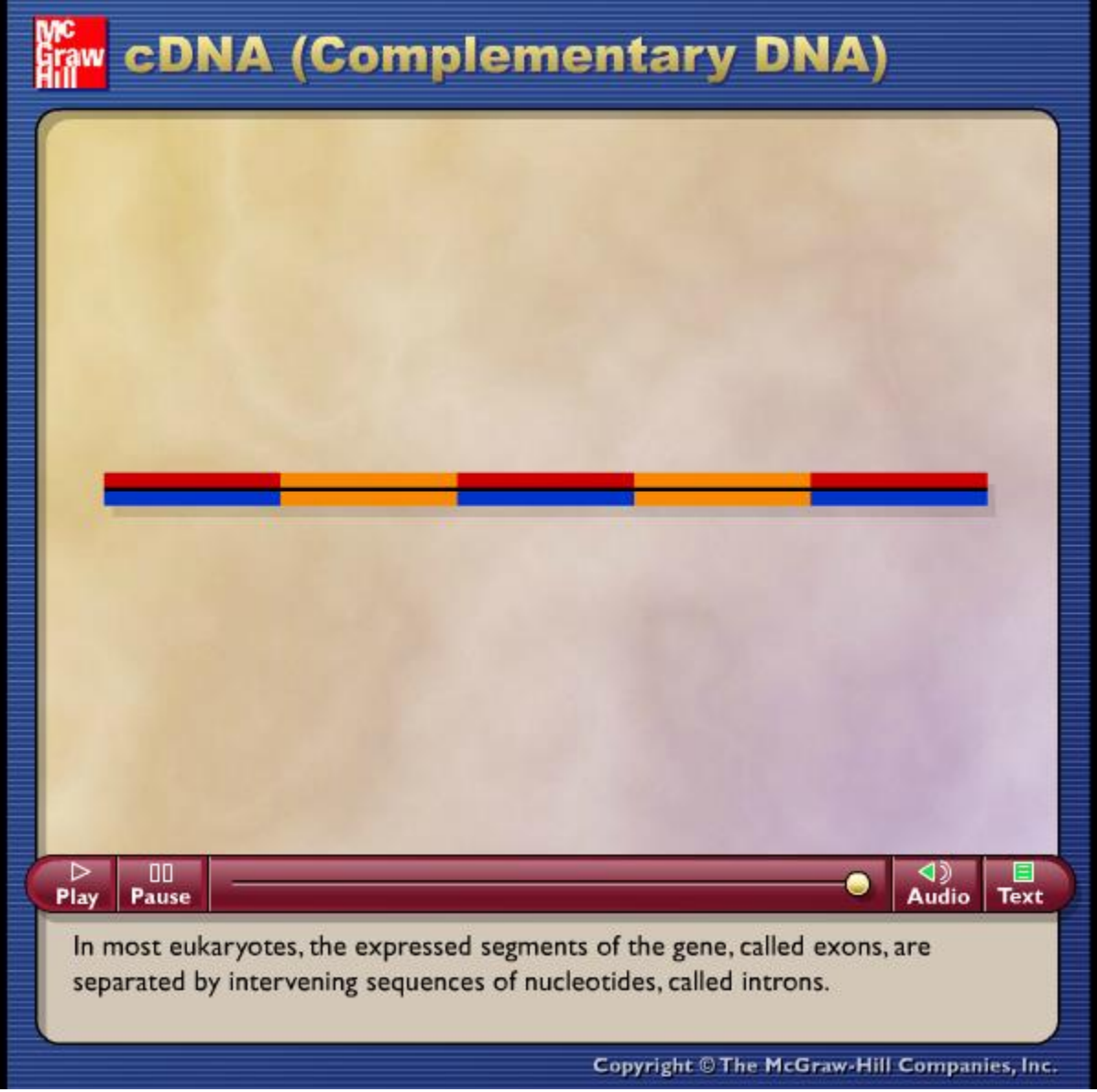
sticky end



sticky end



This happens at both ends of the gene forming sticky ends which can be used to insert the gene into the recipient DNA



reverse transcriptase and cDNA

http://highered.mcgraw-hill.com/olc/dl/120078/bio_h.swf

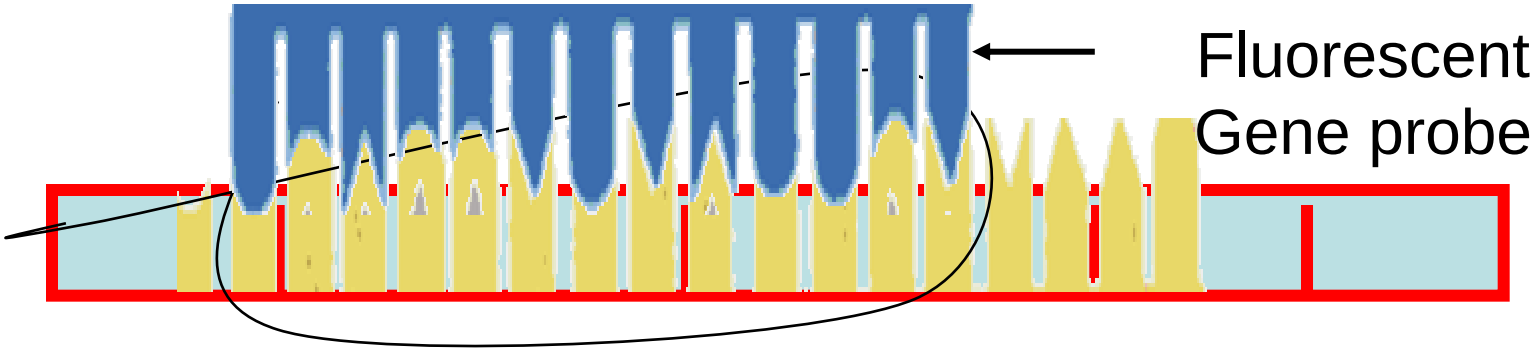
reverse transcriptase and cDNA

- Copy DNA (cDNA) can be made from mRNA using the enzyme **reverse transcriptase;**
- This is the reverse of transcription in which mRNA is made from DNA
- mRNA for the desired gene is removed from donor cells, (the advantage of this is that introns have been removed)
- Single stranded mRNA is mixed with free DNA nucleotides and reverse transcriptase; a single strand of DNA is made.
- The single stranded DNA is used as a template to make double stranded DNA using **DNA polymerase enzymes**.

USE DNA PROBES TO LOCATE THE DNA FRAGMENT WITH THE DESIRED GENE

- When the gene has been cut from the DNA it will be mixed with other fragments of DNA that have been cut using the same restriction enzyme
- To separate the required gene from the mixture a specific **DNA probe** is added
- The probe will have a specific base sequence which will bind to a specifically selected **complementary base sequence** found only in the required gene
- The probe is usually **fluorescent** revealing the position of the required gene

DNA fragment containing gene



The genetic probe binds to the gene because it has a complementary DNA sequence to part of the gene.

INCORPORATE DONOR GENES INTO A VECTOR

- DNA cannot enter the bacterial cell on its own
- So must be carried into the cell using a vector, such as

Bacteriophage

Bacterial plasmid

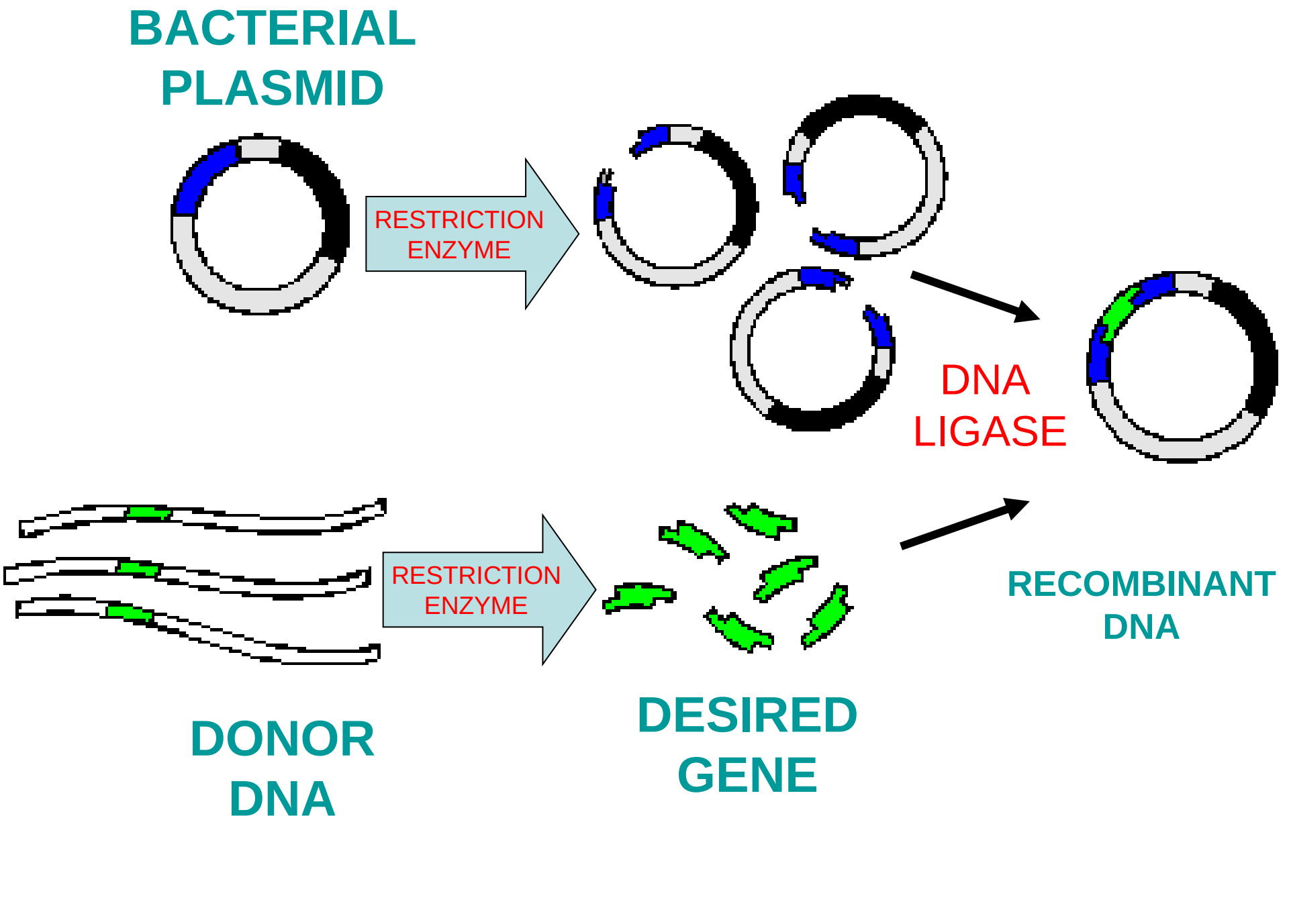
- Bacteria contain small circles of DNA – **plasmids**
- Which are separate from bacterial chromosomes
- Plasmids can be moved easily in and out of cells so make useful **vectors** for genes

- The plasmid is opened (cleaved) by the same restriction endonuclease used to remove the donor gene, leaving sticky ends

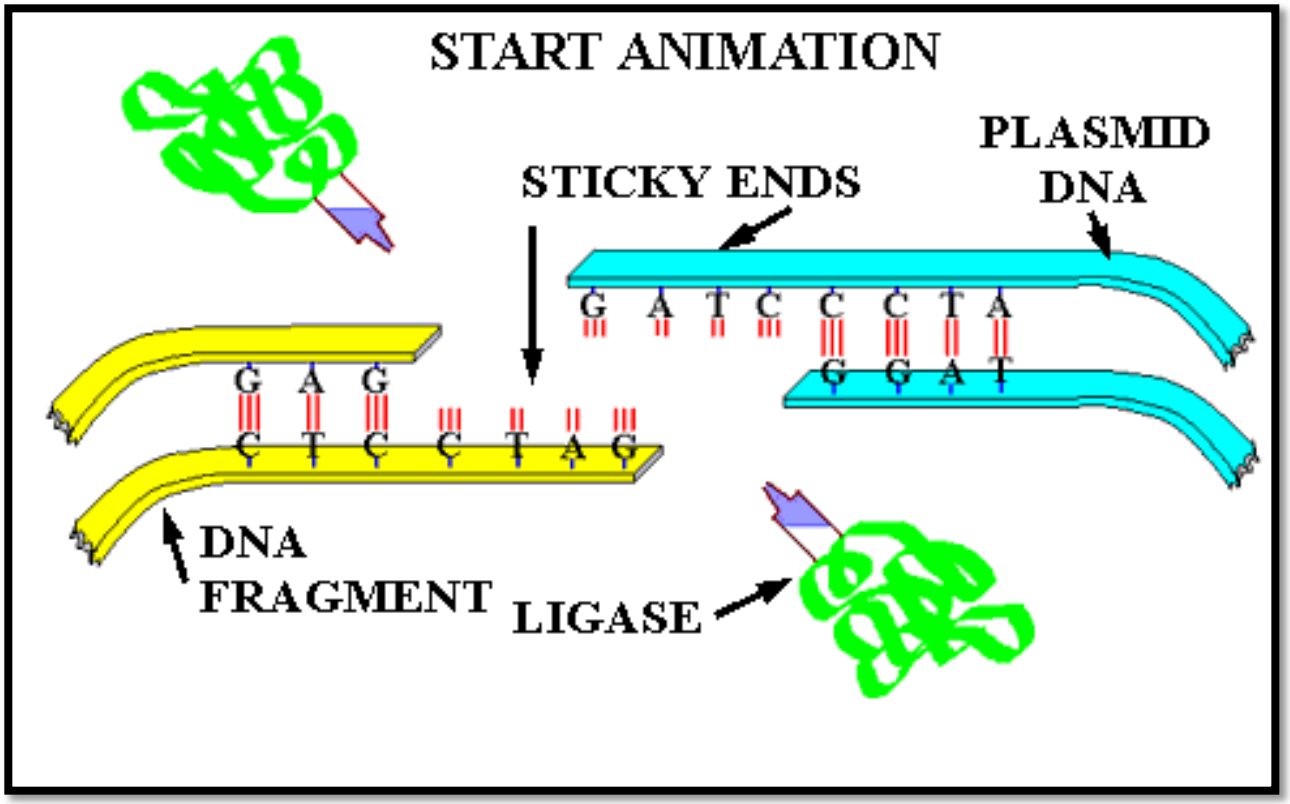
- The donor gene (formed using restriction endonucleases or reverse transcription) is mixed with the open plasmids
- The complementary sticky ends of the some of the donor genes and plasmids join by H bonds

- The phosphate sugar backbone of the plasmid is closed (annealed) using the enzyme DNA ligase

- Recombinant DNA has been formed.


BACTERIAL PLASMID
RESTRICTION ENZYME
DNA LIGASE
RESTRICTION ENZYME
RECOMBINANT DNA
DONOR DNA
DESIRED GENE

Ligation –rejoining cut fragments of DNA and forming artificial recombinant molecules (more detailled website...)

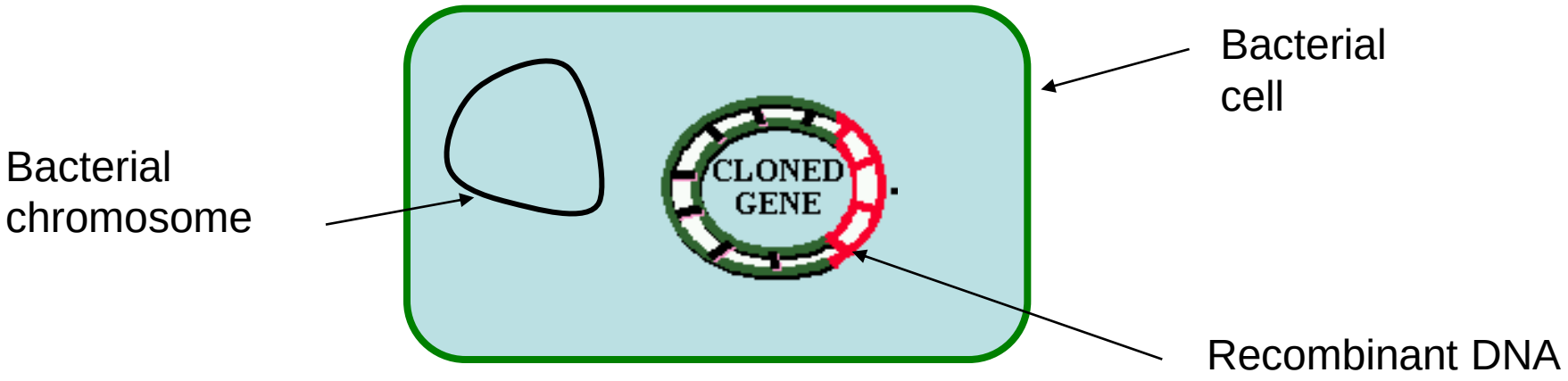


http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap10.html#Sticky_ended_cut

TRANSFORMATION OF RECIPIENT CELL

- Inserting the recombinant DNA into the host cell is called **transformation**
- The plasmids are placed in a culture of the host cells (**Escherichia coli** or **Sacchromyces cerevisae**; these organisms have a very rapid life cycle)
- Some of the cells take up the plasmids, a process aided by either using a brief **electric current** or subjecting them to **ice shock treatment**.

Recombinant DNA introduced into bacterial cell



Check recipient cell contains the recombinant DNA

- Not all host cells take up the recombinant DNA plasmid, so will not contain the gene required to make the protein
- Cells are identified using **marker genes** (antibiotic resistance)
- These marker genes are inserted into the bacterial plasmid at the same time as the donor gene
- Marker genes are usually resistant to antibiotics

- The bacteria can be grown on plates of agar containing the antibiotic

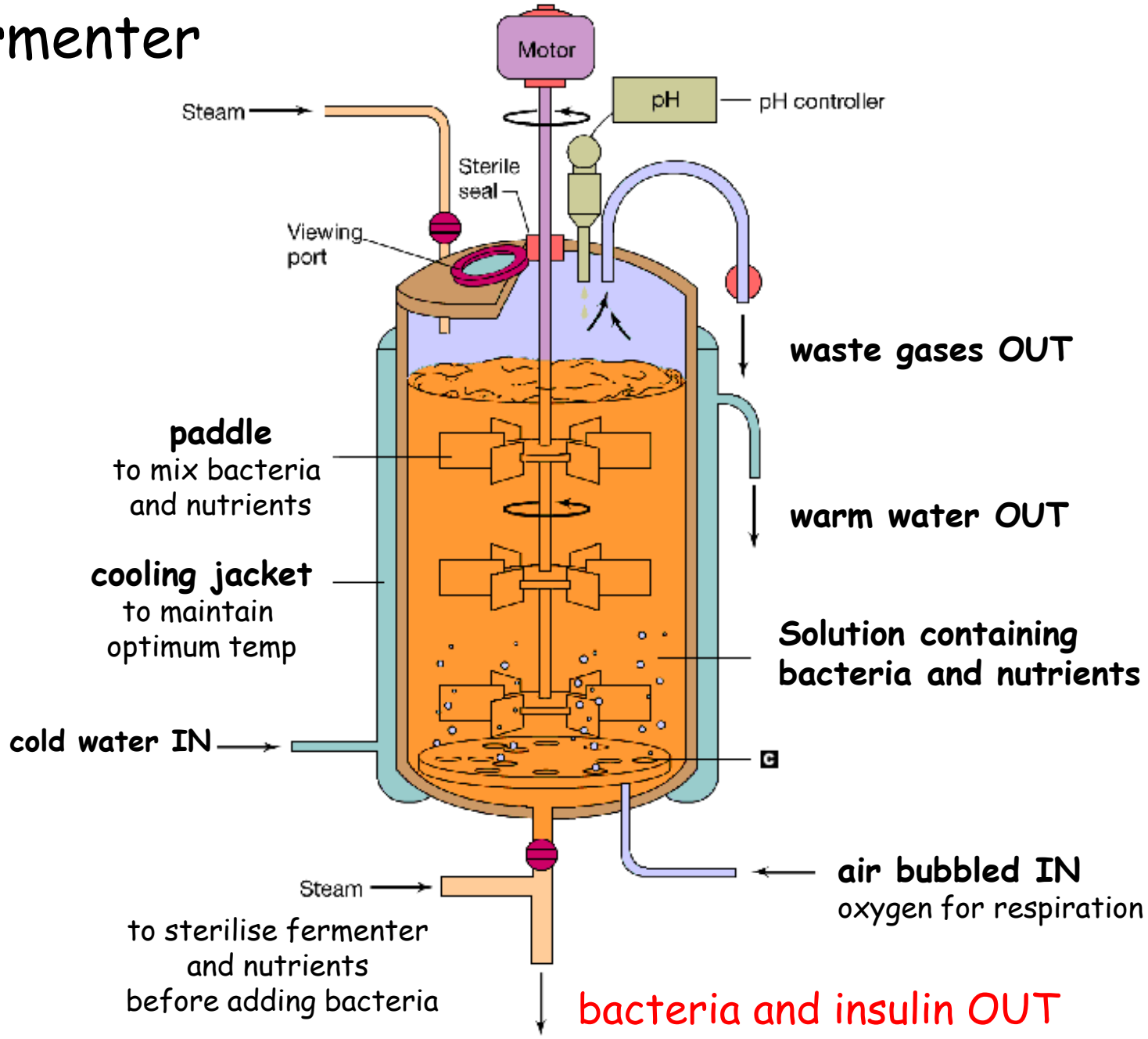
- Cells into which the gene has been inserted can be identified as the host cell will be resistant to the antibiotic.

bath figure 29.4
page 638

MULTIPLICATION OF HOST CELLS

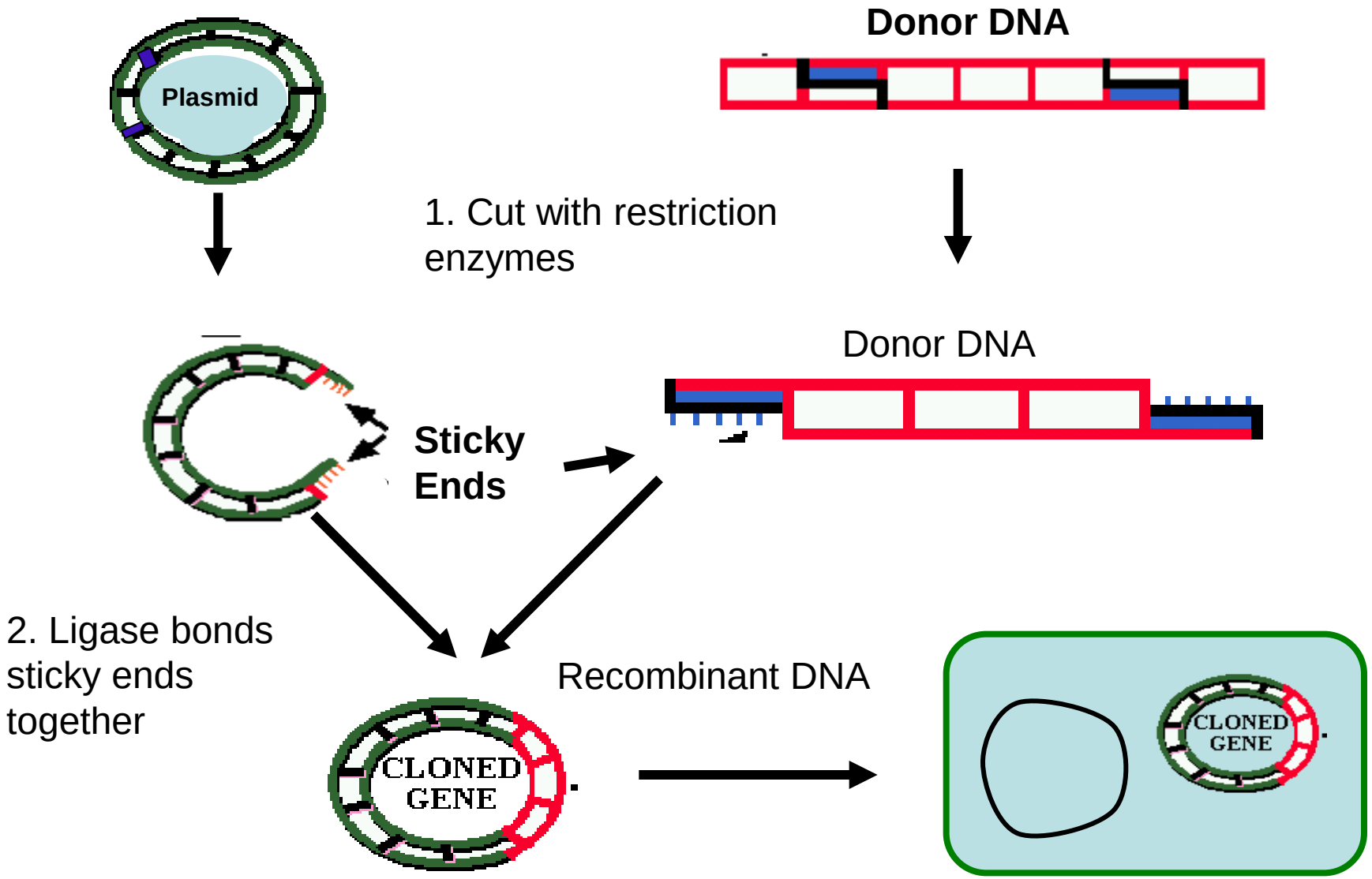
- Cells are grown in industrial **fermenters** where they multiply rapidly, so increasing the amount of recombinant DNA
- The fermenter allows **optimum growth conditions** to be maintained; temperature, pH, nutrient requirements, oxygen and carbon dioxide levels
- The cells transcribe the gene, translate the mRNA and so produce the protein
- The protein is removed, purified, processed and packaged for sale.

A fermenter


Motor
pH
pH controller
Steam
Sterile seal
Viewing port
waste gases OUT
paddle
to mix bacteria and nutrients
warm water OUT
cooling jacket
to maintain optimum temp
Solution containing bacteria and nutrients
cold water IN
G
Steam
air bubbled IN
oxygen for respiration
to sterilise fermenter and nutrients before adding bacteria
bacteria and insulin OUT

BATCH CULTIVATION	CONTINUOUS CULTIVATION
The bacteria and substrates are placed in the fermenter for approximately 6 days to allow growth to reach stationary phase, for maximum product formation, after which it is emptied, cleaned and sterilised for the next batch	Production occurs for several weeks, with new raw materials being added throughout the process and the products being continuously removed
Used to produce secondary metabolites; substances not needed for the growth of the organism, so not produced until after the exponential growth phase is completed	Used to produce primary metabolites; substances essential for the life of the organism, so produced continuously

Summary of steps



Micro organisms

- Bacteria can make human insulin
- This prevented many diabetics from getting an allergic reaction to animal insulin
- Bacteria make interferon which can fight virus infections and some cancers

ADVANTAGES

- Cost-effective and efficient production of useful substances and desirable traits

PRODUCTS OF GENETICALLY ENGINEERED MICRO-ORGANISMS

- Insulin
- Human growth hormone (HGH)
- Enzymes
- Adhesives
- Lung surfactant protein
- interferon

TRANSGENIC ORGANISMS

- Organisms into which one or more genes from another organism have been artificially inserted

TRANSGENIC ANIMALS

Genes may be introduced into animals to Improve:

- Growth rate
- Milk yield and quality
- Meat production and quality

However they may also be used to produce substances of **medical value**:

- Interferon
- Blood-clotting factors
- Alpha-1-antitrypsin
- Human serum albumin
- Haemoglobin
- vaccines

Animals used in GE

- The human gene to clot blood has been inserted into the DNA of sheep. They produce blood clotting factor in their milk which is used by Haemophiliacs.

- Goats produce the protein Alpha-1-antitrypsin which is used to treat emphysema and Cystic Fibrosis

Vaccines

- Genetically engineered microbes can be used to produce the antigens needed in a safe and controllable way.
- The use of genetically modified yeast cells to produce a vaccine against the hepatitis B virus has been a major success story.

Genes are introduced in plants to:

- Improve crop yields
- Increase variety
- Prolong shelf-life by controlled ripening
- Increase protein content
- Improve texture
- Improve flavour
- Give cultivated plants increased resistance to pests, diseases and unfavourable environmental conditions

WORK SHEETS
Examples of GM Crops
Advantages & Disadvantages

GENE THERAPY

- Genetic disease may be caused by an absent or faulty gene
- Gene therapy involves an absent or faulty gene being replaced with the introduction of a functional gene to restore normal metabolism and eliminate a disease

SOMATIC CELL GENE REPLACEMENT THERAPY

- This involves inserting the gene into somatic cells of the body; lungs, muscles, liver etc,

- Germ cell gene replacement therapy involves inserting the genes into ova or sperm cells and is currently under strict governmental control

Advantages of Somatic Cell Gene Tharapy

- A gene can be ‘silenced’, so that the disease does not develop
- A specific tissue can be targeted, ensuring the treatment is restricted to where it is required
- Fewer drugs required (which usually target all cells of the body)
- Cells that take up the gene pass it to new cells during mitosis

Disadvantages of Somatic Cell Gene Tharapy

- As sex cells are not involved the genes are not passed on to the offspring
- It is often difficult to get human cells to take up the gene
- Short term nature of some gene therapy requires repeated treatment
- Immune response - Genes injected with a virus may trigger an immune response against the virus.
- Problems with viral vectors (once inside the patient, the viral vector could recover its ability to cause disease).
- The genetic material might not get into the right place in the cell's DNA

Gene therapy is a possible cure for single gene disorders

sickle-cell anaemia

cystic fibrosis

muscular dystrophy

worksheets

Disease	Target organ	Method of delivering gene	Success
Cystic fibrosis	Lungs	Lipid droplets inhaled into lungs	Short term in humans
Sickle cell anaemia	Bone marrow	Virus inserted into blood making cells in bone marrow	Mice only
Muscular dystrophy	Skeletal muscle	Virus injected into muscle	Permanent effects in humans

Moral and Ethical Issues

- Gene technology gives much greater control over genetic manipulation; any organism could be made transgenic
- Benefits

  Faulty genes identified and replaced; Increased crop yield; Make specific medicines
- Concerns

  Foreign genes enter the wrong organism; Accidental transfer of wrong genes; Health concerns; Designer babies; Religious concerns

Really good website about DNA, the genome, genes and gene technology, with animations too!

- http://www.yourgenome.org/

SECTIONS OF GENE TECHNOLOGY

1. Obtaining a gene – restriction enzymes and reverse transcriptase (Mills and McAllister)
2. DNA probes and electrophoresis (Lee and Kerr)
3. Gene transfer – getting a gene into a plasmid (Cooke and Russell)
4. Gene transfer – getting the plasmid taken up (Milliken and Palmer)
5. Genetically Engineered Microorganisms, transgenic plants (not case study) and transgenic animals (Blackmore and Kennedy)
6. Gene therapy (not case study) (Burrows and Connolly)
7. Genome sequencing (before case study) (Withers and Clarke)
8. Genetic mapping (after case study) and knockin knockout technology (Morrison and Stewart)
9. Ethics of gene technology (before case study) Ethics of gene technology (after case study) (Paul and French)

CHALLENGE

ON ONE A4 SIDE:

1. **CREATE A SUMMARY OF YOUR SPECIAL EXPERT AREA OF KNOWLEDGE**
2. **COLOURFUL**
3. **BASED ON THE SPEC.**
4. **USE YOUR TEXTBOOK!**
5. **YOU ARE TEACHING YOUR PEERS ON THIS…**