SBL100-Lecture

Introduction to genetic engineering Part III

“genetic engineering” is a techniques in which DNA may be cut, rejoined, its sequence determined, or the sequence of a segment altered to suit an intended use.

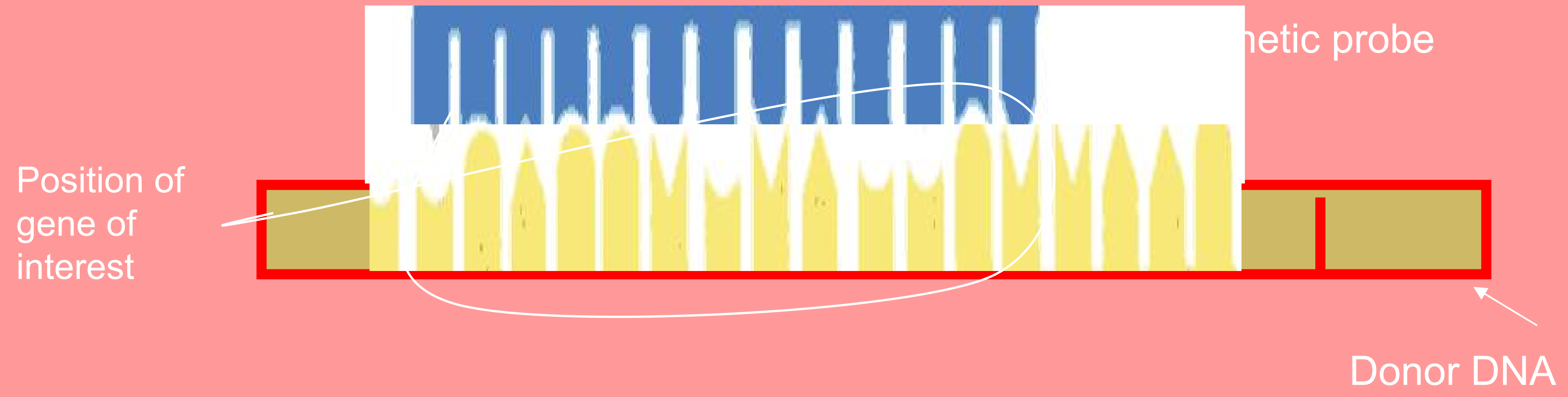
5 Stages involved in GE

1. Isolation
2. Cutting
3. Ligation and Insertion
4. Transformation
5. Expression

1. Isolation

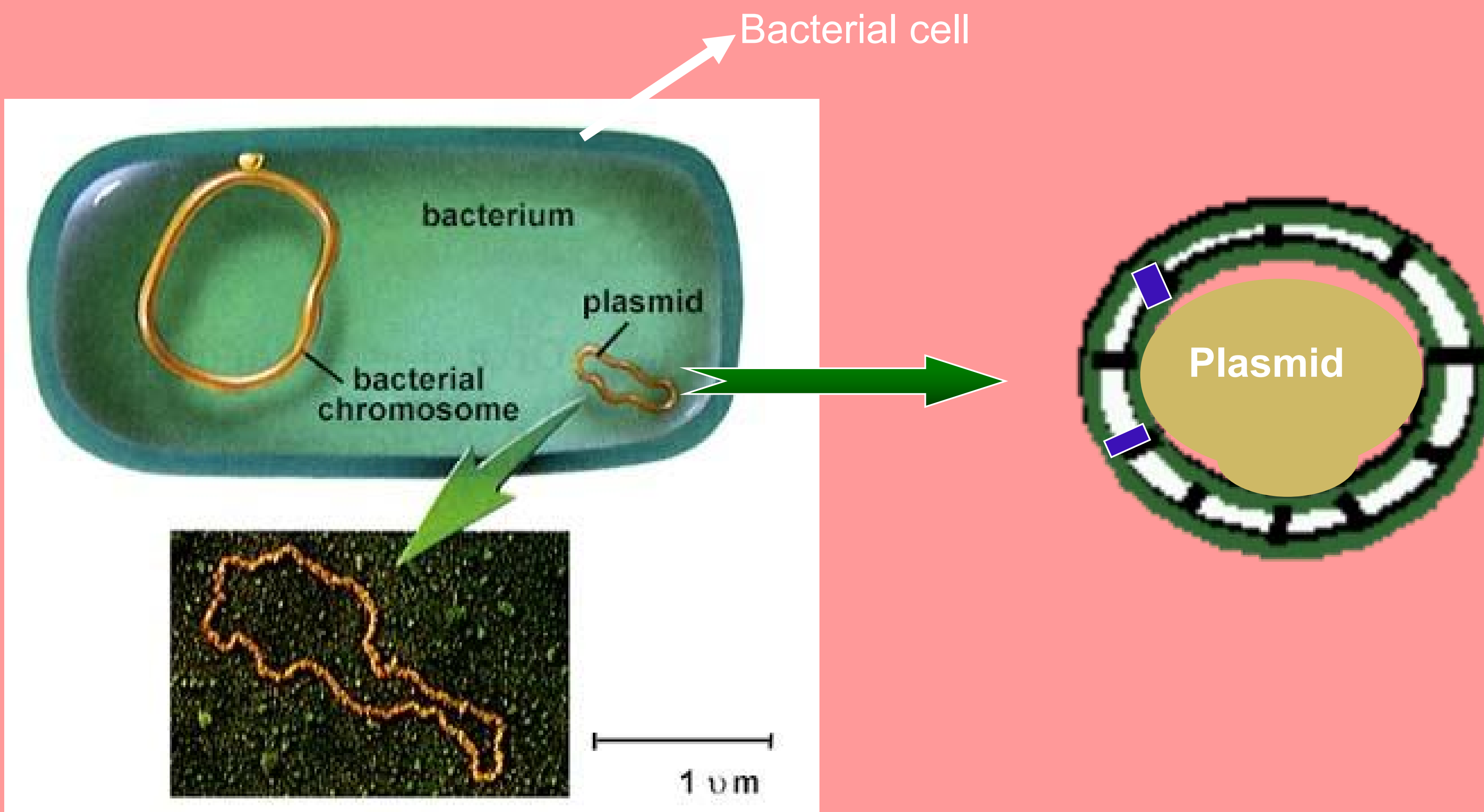
(a) **Isolation** of a specific gene from donor e.g. human

- Cells broken open
- Genetic probe added
- Reveals position of the gene of interest

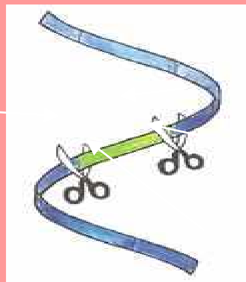


1. Isolation

(b) **Isolation** of plasmid from a bacterial cell



2. Cutting



Restriction site

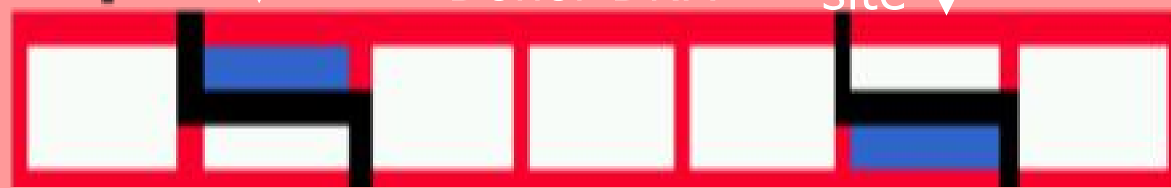
Restriction enzymes

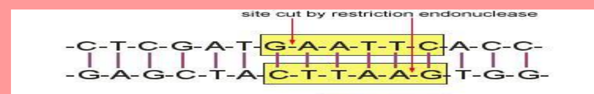
Restriction site

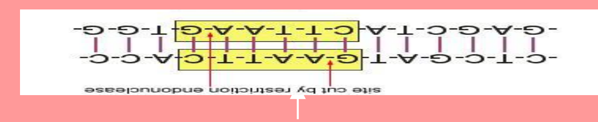
Restriction site

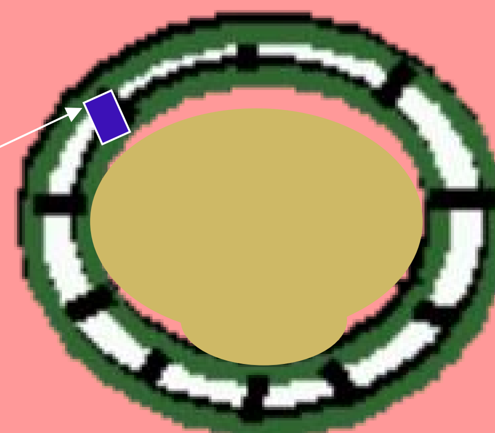
Donor DNA

Restriction site





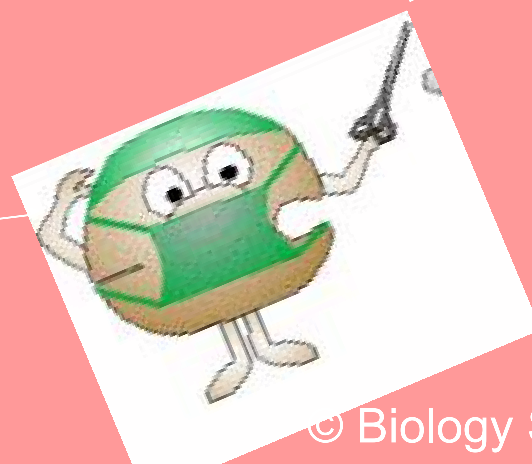




Plasmid

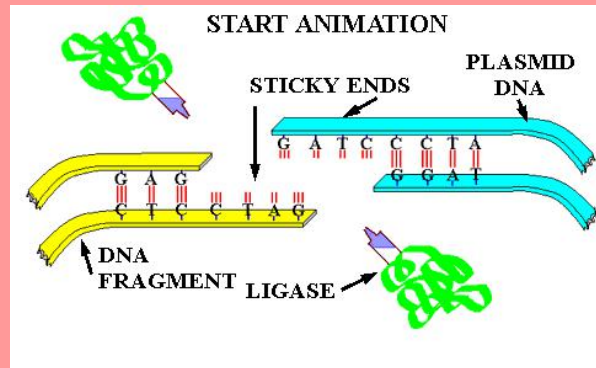
- Restriction enzymes act as molecular scissors and cut DNA at specific sites called restriction sites

Restriction enzymes

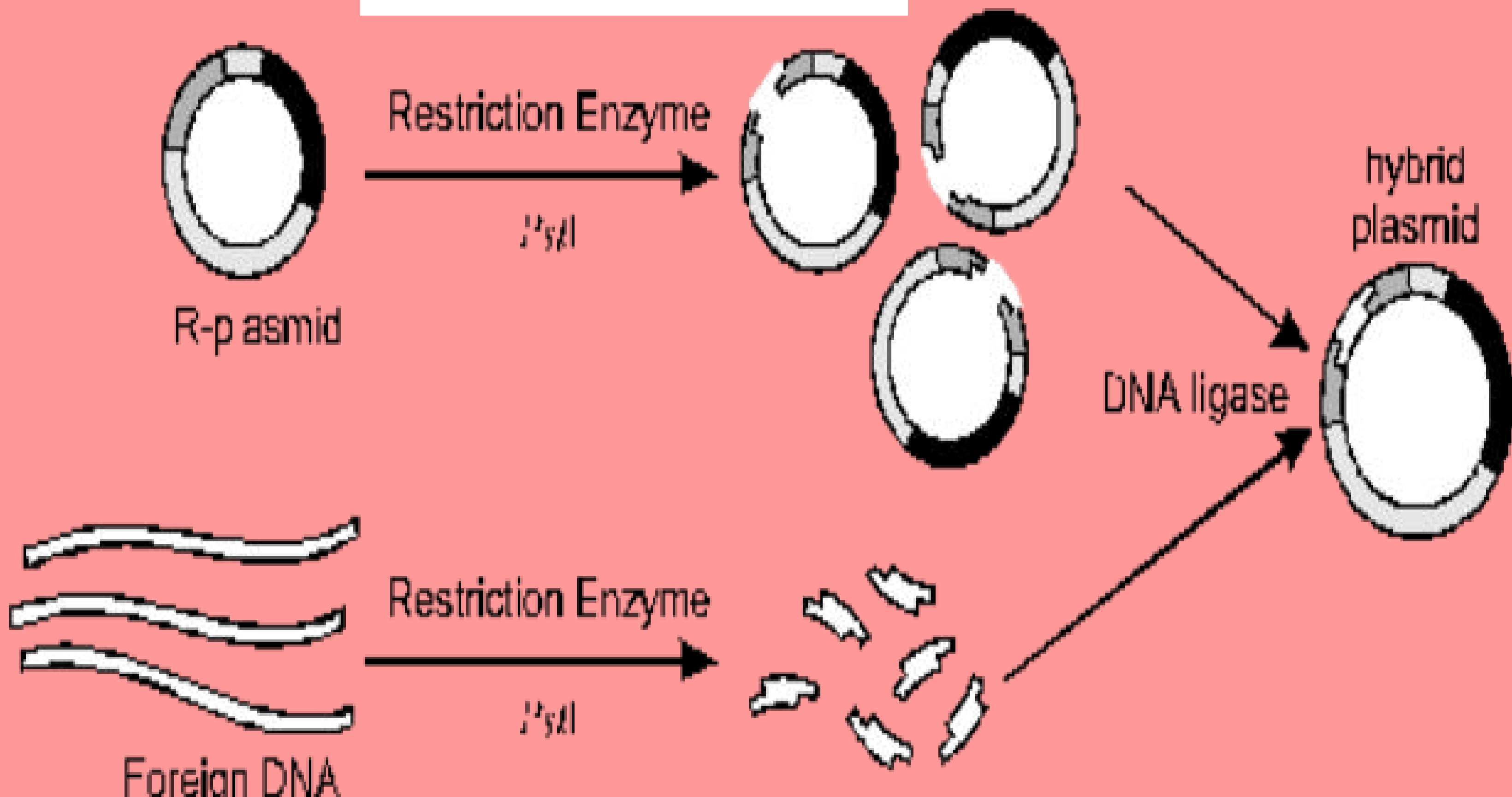


3. Ligation and Insertion

DNA Ligase

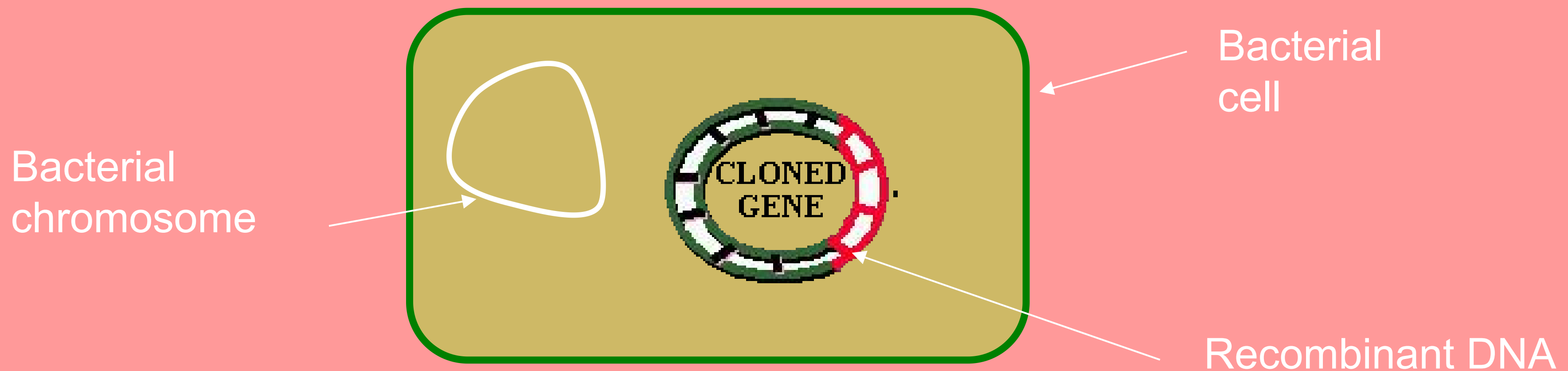


Ligation –rejoining cut fragments of DNA and forming artificial recombinant molecules



4. Transformation

Recombinant DNA introduced into bacterial cell

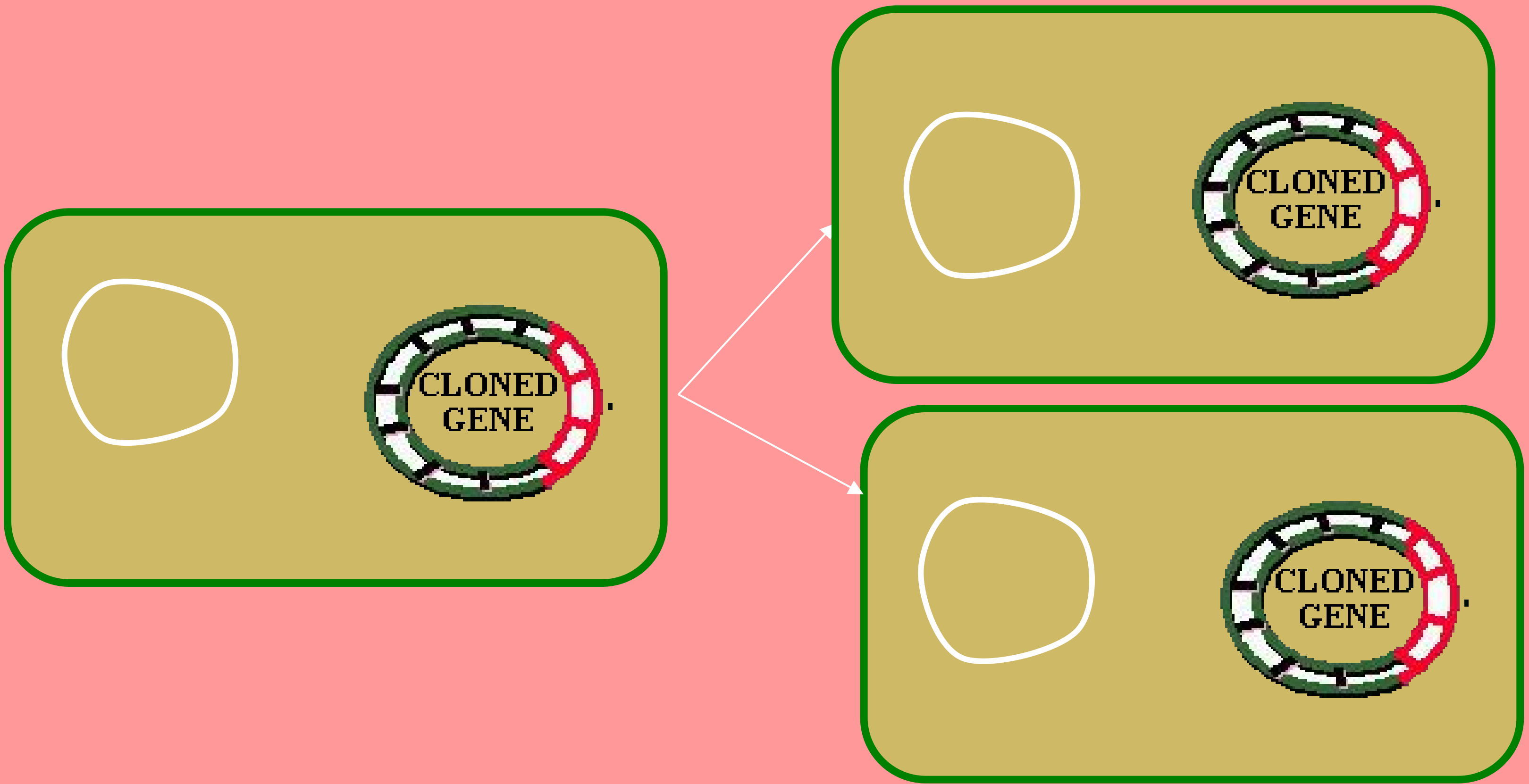


5. Expression

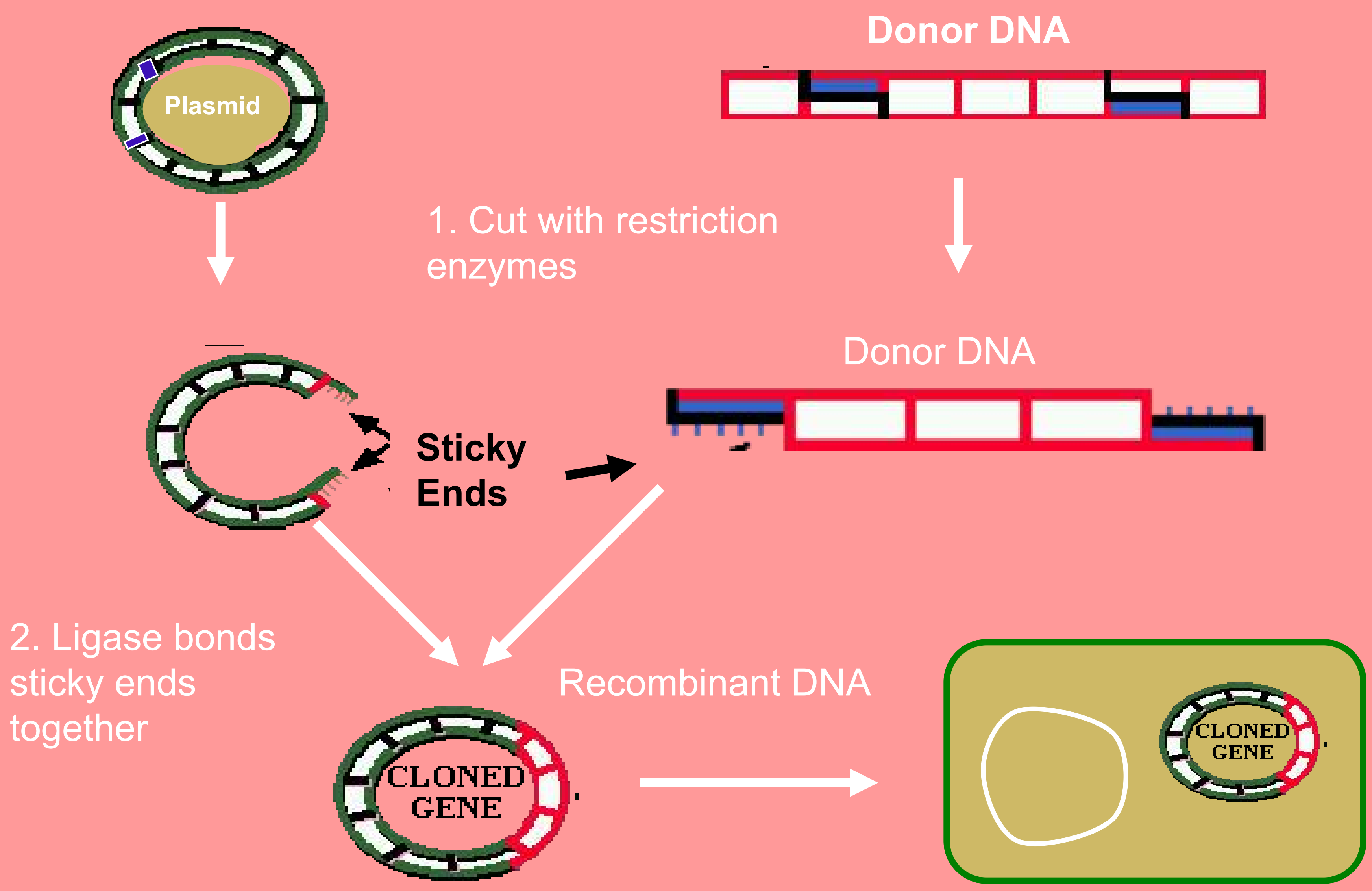
Bacterial cell reproduces by Binary Fisson

Bacterial cell produces the polypeptide

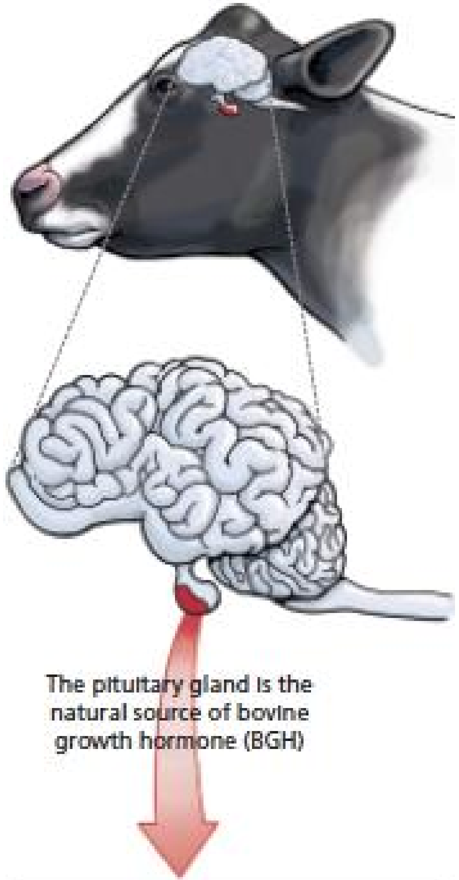
Coded for by the donor DNA

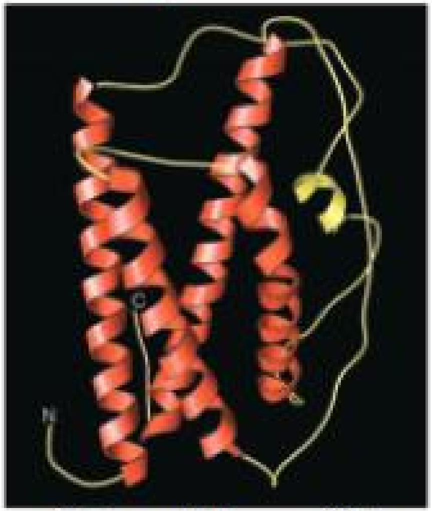


Summary of steps



Approaches of genetic engineering




Bovine growth hormone (BGH)

During 1980s, genetic engineers at the Monsanto Corporation began to produce recombinant bovine growth hormone (rBGH) by manipulating the DNA sequence (gene) that carries the instructions for, or encodes, the growth hormone protein.

Growth hormone acts on many different organs to increase the overall size of the body.

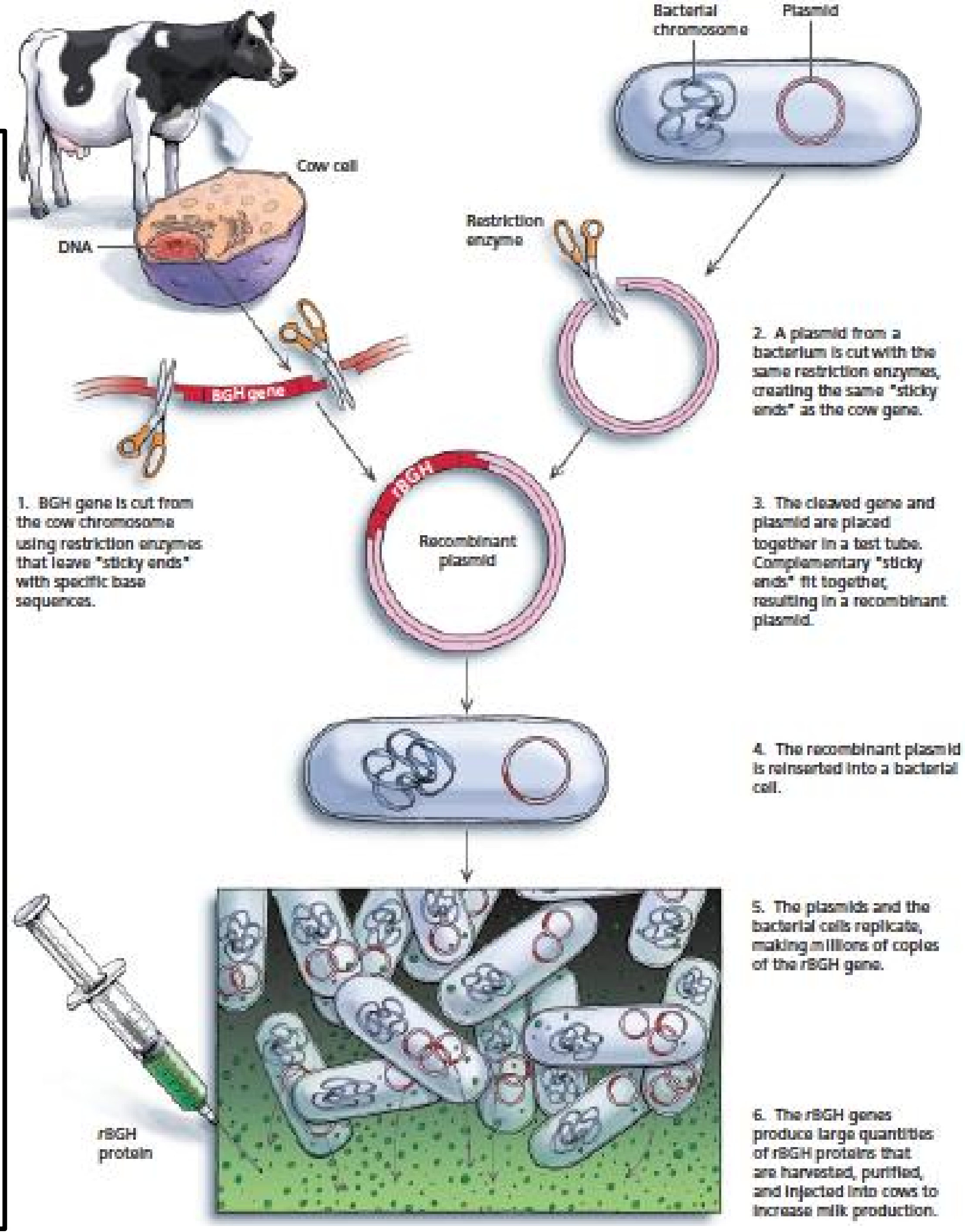
Before the advent of genetic technologies, growth hormone was procured from the pituitary glands of slaughtered cows and then injected into live cows.

However, harvesting the growth hormone from the pituitary glands of cows and humans is laborious, and many cadavers are necessary to obtain small amounts of the protein.

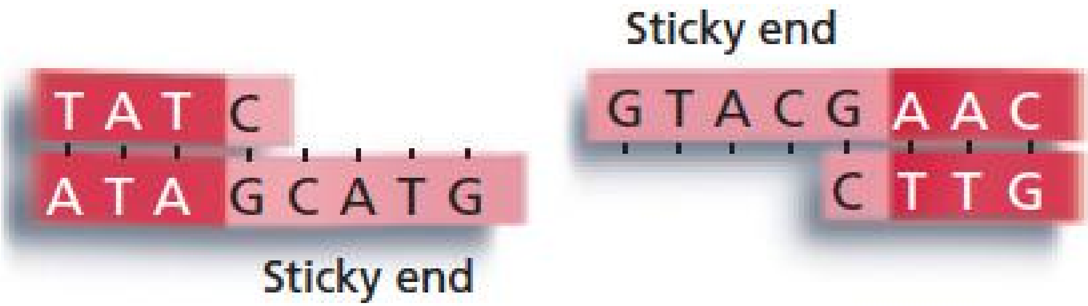
Genetic engineers at Monsanto realized that they could produce large quantities of bovine growth hormone in the laboratory, inject dairy cows, and increase milk production.

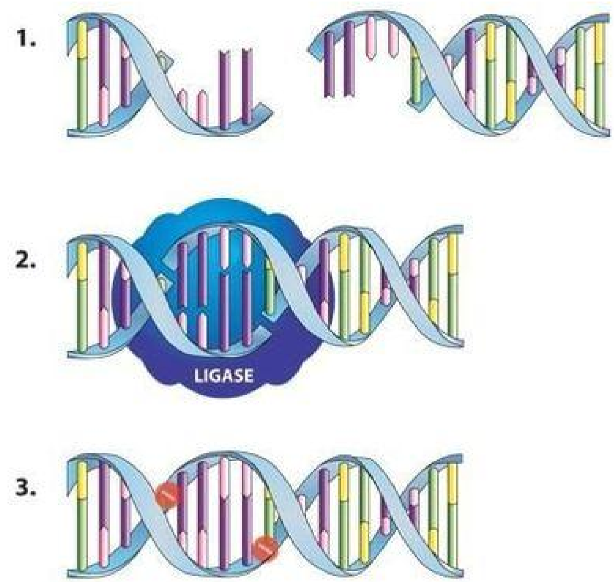
A case study: How engineers produced

- The gene is sliced from cow chromosome on which it resides by exposing the cow DNA.
- Once the gene is removed from the cow genome it is inserted into a bacterial structure called a plasmid .
- A plasmid is a circular piece of DNA that can replicate itself.
- When the cut plasmid and the cut gene are placed together in a test tube they reform into a circular plasmid with the extra gene incorporated.
- The bacterial plasmid has now genetically engineered to carry a cow gene.
- rBGH is called a recombinant gene because removed from its original location cow genome and



restriction enzymes are highly specific molecular scissors. cut DNA at specific sequences:

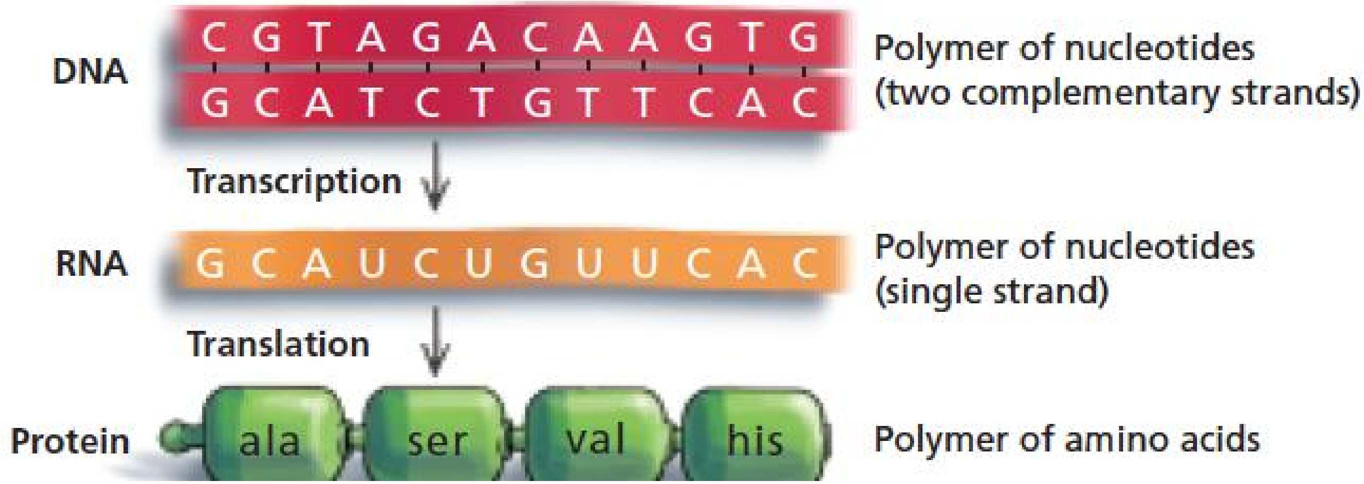




The first step in the production of the rBGH protein is to transfer the BGH gene from the nucleus of a cow cell into a bacterial cell.

Bacteria with the BGH gene will then serve as factories to produce millions of copies of this gene and its protein product—making many copies of a gene is called cloning the gene.

Once inside the cell, plasmids replicate, themselves, as does the bacterial cell, making thousands of copies of rBGH gene. Using this procedure, scientists grow large amounts of bacteria



The cloned BGH gene into bacterial cells, now produce the protein encoded by the gene.

The process of protein synthesis is also referred to as gene expres

proteins are synthesized when the genes that encode them are turn

The scientists were then able to break open the bacterial cells, isolate the BGH protein, and inject it into cows.

Close to one-third of all dairy cows in the USA now undergo daily injections with rBGH, produced 20-30% more milk.

FDA Regulations

FDA a governmental organization which ensures the safety of all domestic and imported foods and food ingredients.

The manufacturer of any new food that is not Generally Recognized As Safe (GRAS) must obtain FDA approval before marketing its product.

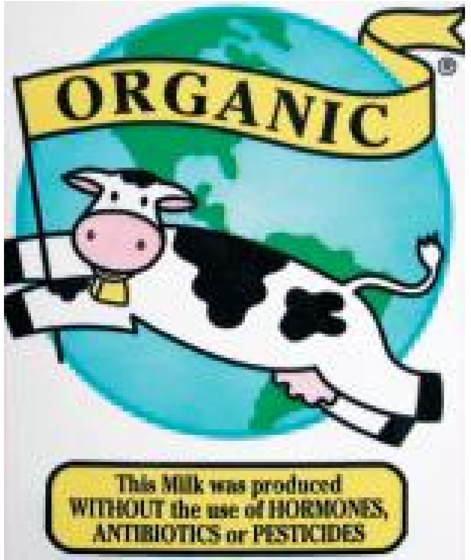
Adding substances to foods also requires FDA approval.

According to both the FDA and Monsanto, there is no detectable difference between milk from treated and untreated cows and no way to distinguish between the two.

milk from r BGH treated cows was deemed safe for human consumption by FDA in 1993.

distributors of milk from untreated cows label their milk as " hormone free"

In humans, studies indicate milk from cows treated with rBGH may contain elevated levels of insulin-like growth factor-1 (IGF-1), which can increase the risk of breast cancer , colon cancer and other types of cancer.

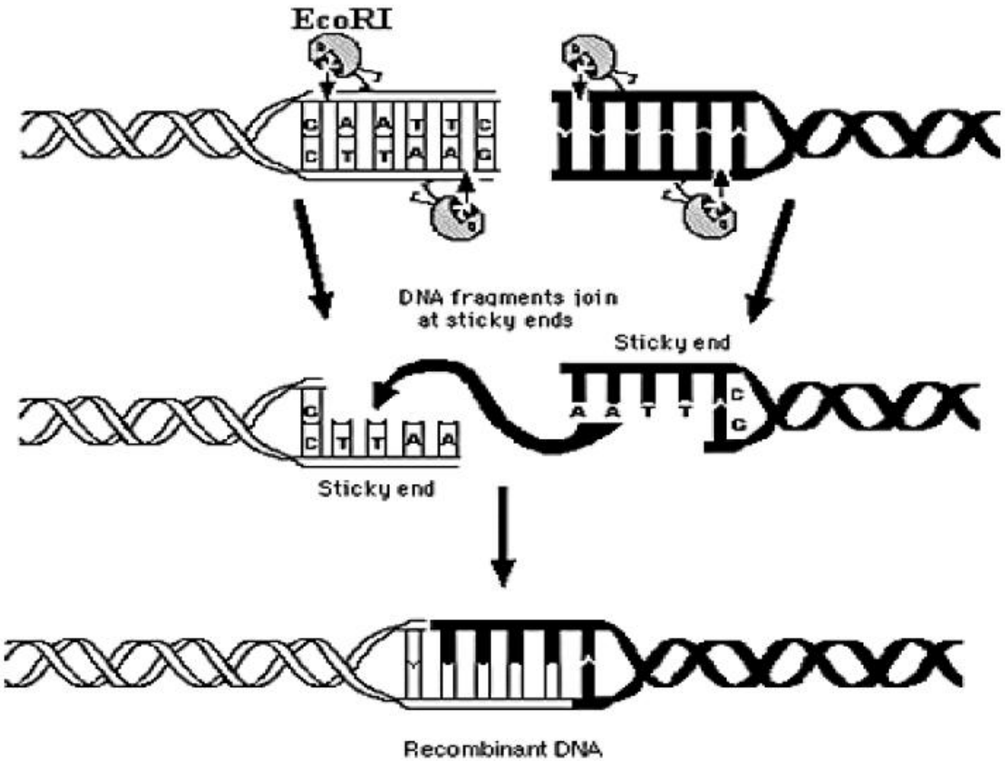


10.04.23

SBL100-Lecture

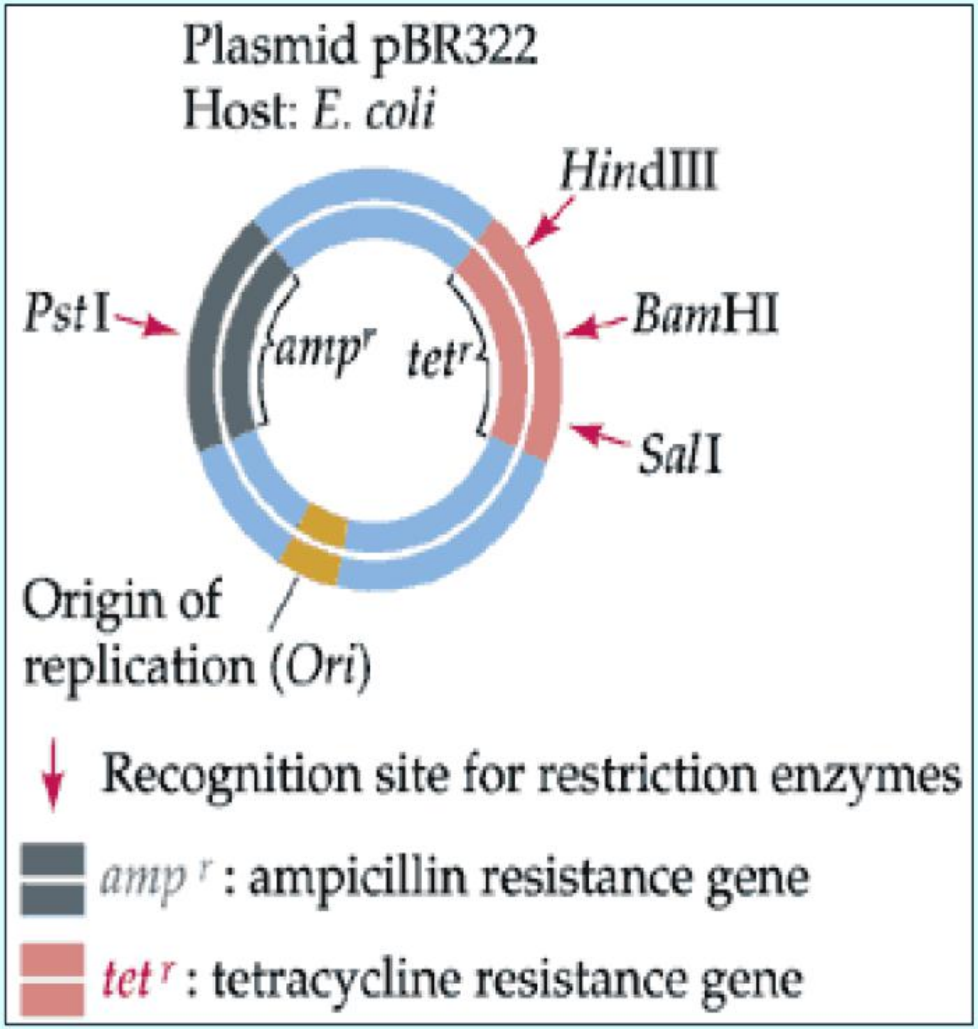
Introduction to genetic engineering Part IV

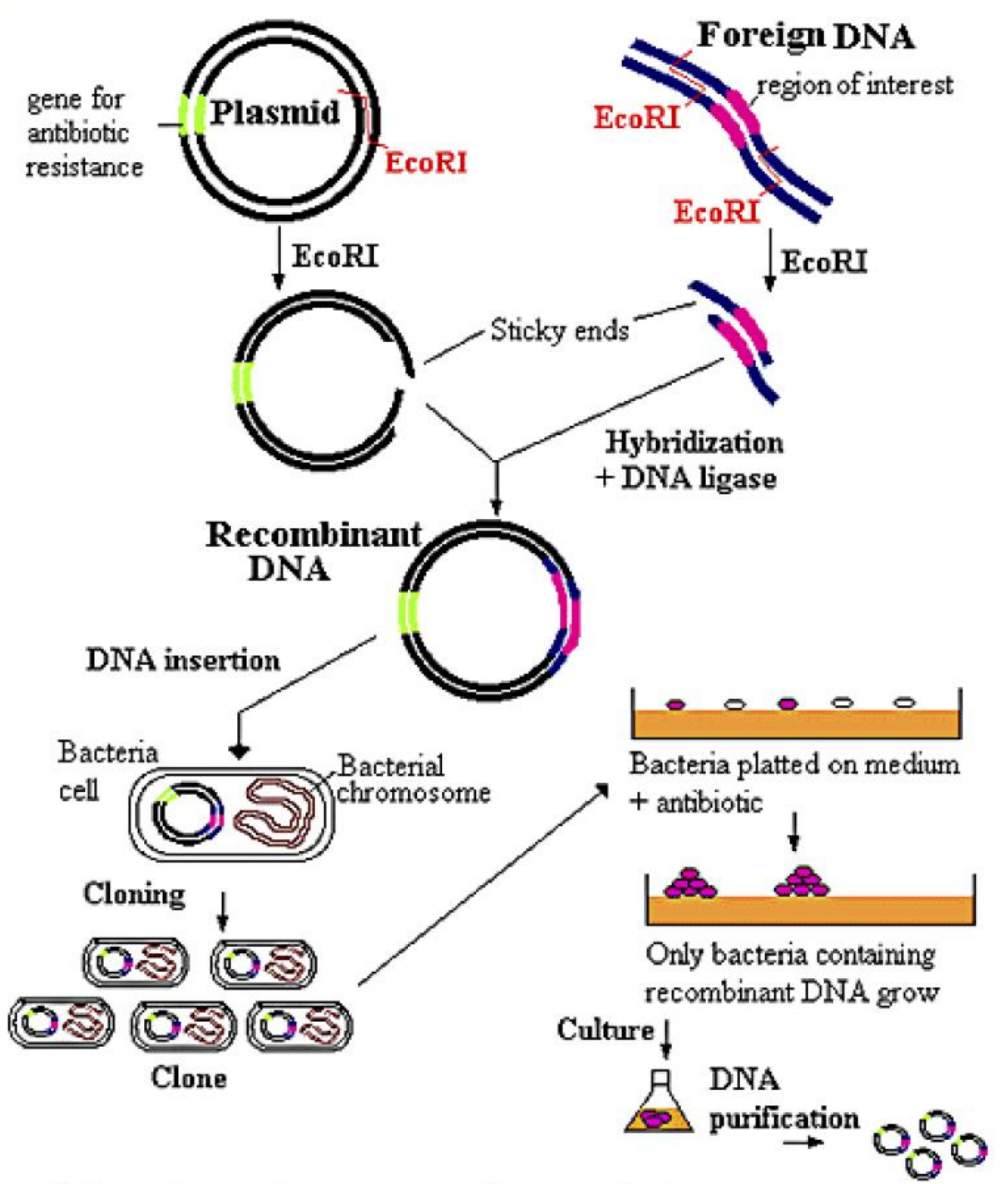
Restriction Enzyme



Restriction Enzyme
Action of EcoRI

Vector - Plasmid

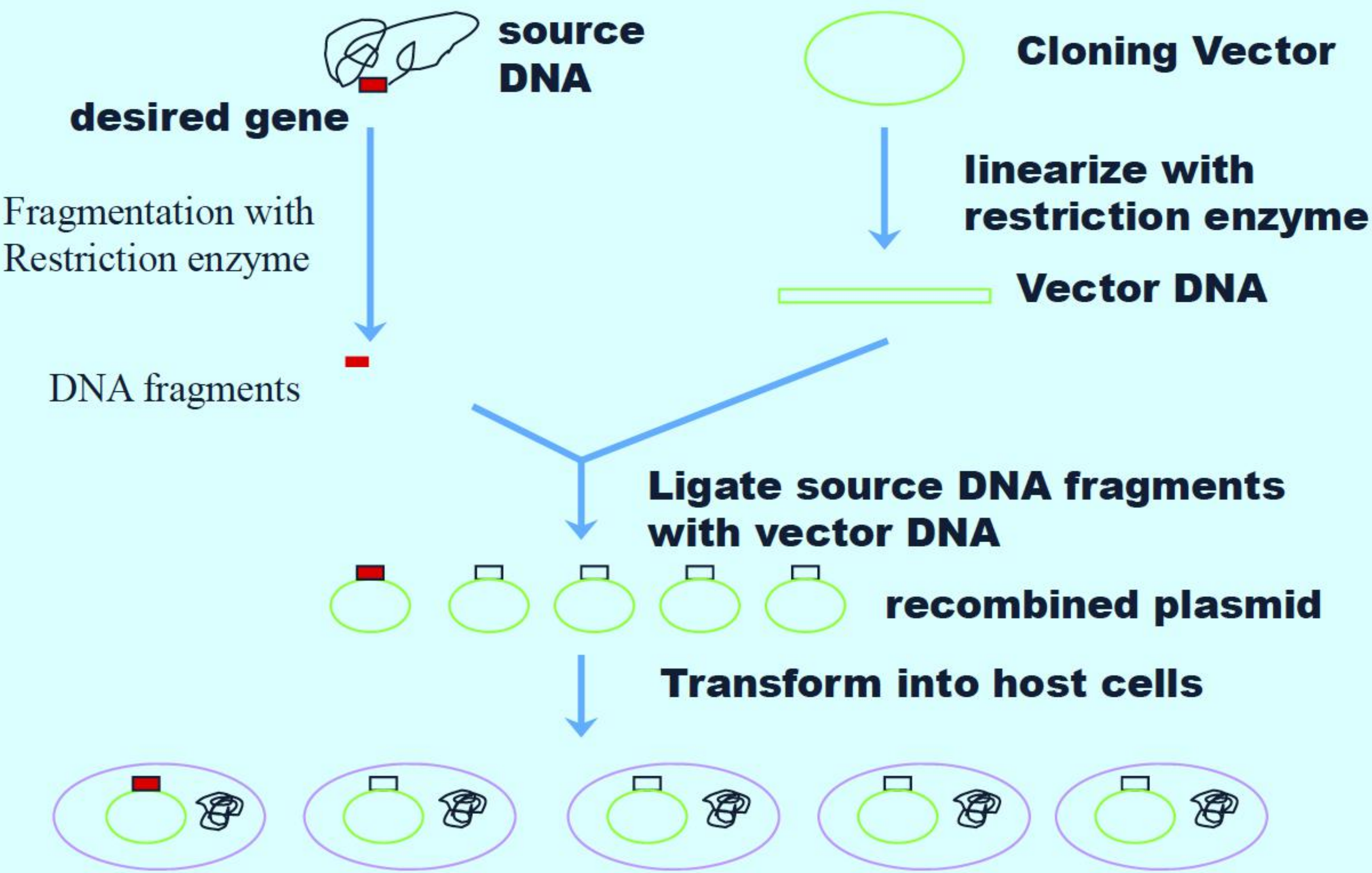


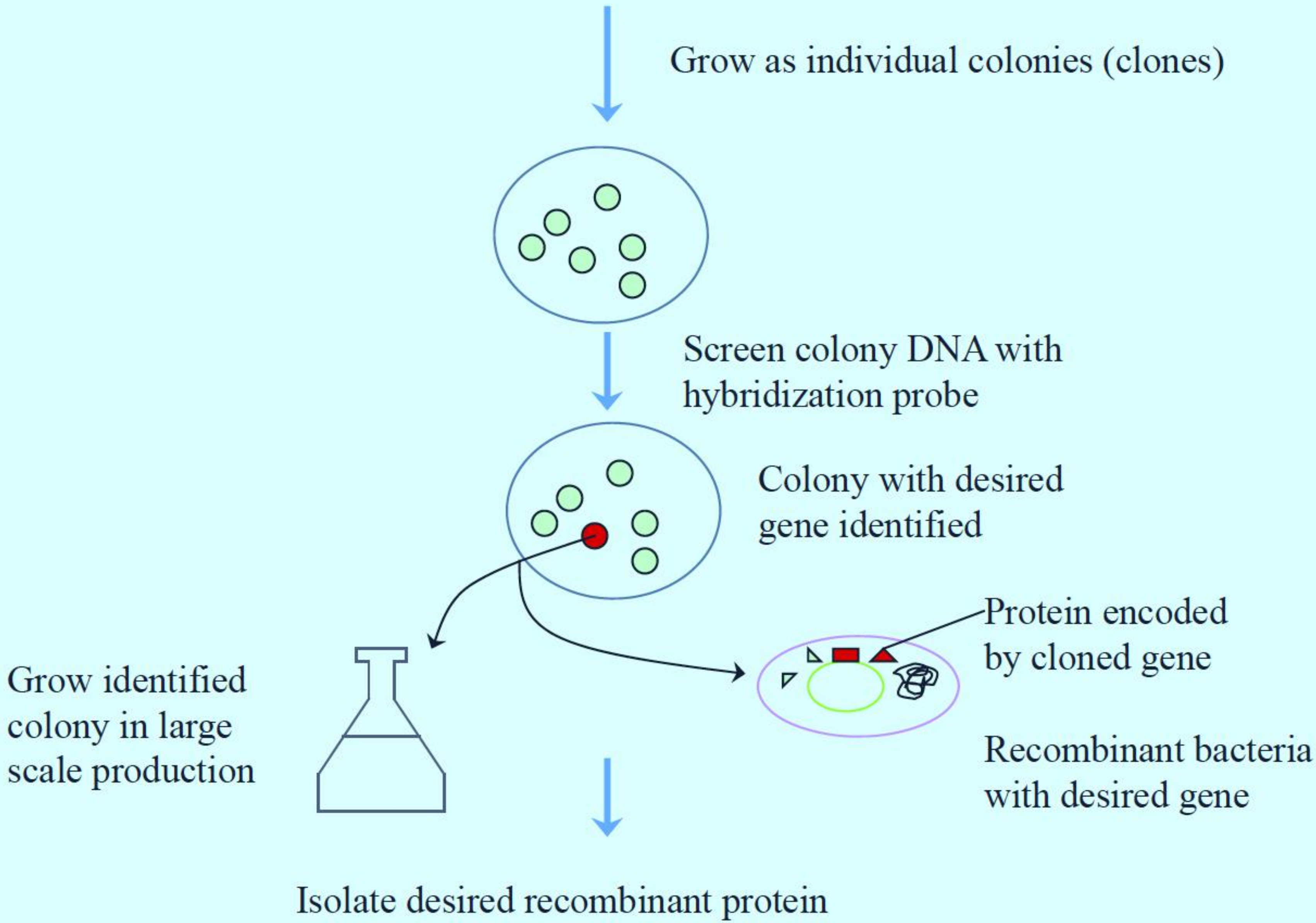


Cloning into a plasmid

Clone = A group of cells that contain the same recombinant DNA molecule

Overall Cloning Process




Grow as individual colonies (clones)
Screen colony DNA with hybridization probe
Colony with desired gene identified
Protein encoded by cloned gene
Grow identified colony in large scale production
Recombinant bacteria with desired gene
Isolate desired recombinant protein

Production of Recombinant Proteins and Peptides

Generic Name	Brand Name	Therapeutic Use
Human insulin	Humulin (Lilly) Novolin (Novo Nordisk)	Insulin dependent diabetes
Human growth	Protopin (Genentech) Humatrope (Lilly) Nutropin (Genentech)	Growth hormone deficiency in children; growth retardation in chronic renal disease
Hepatitis B vaccine	Engerix-B (SmithKline Beecham) Recombivax HB (MSD)	Hepatitis B prevention
Interferon alfa-2a	Roferon-A (Hoffman)	Hairy cell leukemia; AIDS related Kaposi's sacroma

Production of Recombinant Proteins and Peptides

Generic Name	Brand Name	Therapeutic Use
Muromonab-CD3	Orthoclone OKT 3 (Ortho)	Acute allograft rejection in renal and cardiac transplant patients
Epoetin alfa	Epogen (Amgen)	Anemias of chronic renal disease, AIDS and cancer chemotherapy
Interferon beta-1b	Betaseron (Bertex)	Multiple sclerosis
Dornase alfa	Pulmozyme (Genetech)	Cystic fibrosis
Imiglucerase	Cerezyme (Genzyme)	Type 1 gaucher's disease

Genetic Engineers Can Modify Foods

Whether you realize it or not, you have been eating genetically modified foods now.

- Improving yield of crop plants is the driving force behind majority of genetic engineering.
- Yield can be increased when plants are engineered to be resistant to pesticides and herbicides, drought, and freezing.
- People believe that improving farmers' yields may help decrease world hunger problems
- Crop plants are genetically modified to increase their shelf life, yield, and nutritive value.
- The first genetically engineered fresh produce was tomatoes, in store in 1994.
- These tomatoes were engineered to soften and ripen more slowly.
- It taste better and increases selftime in grocery without overripe and mushy.
- An enzyme called pectinase mediates the ripening process in tomatoes.
- This enzyme breaks down pectin, a naturally occurring substance found in plant cells.

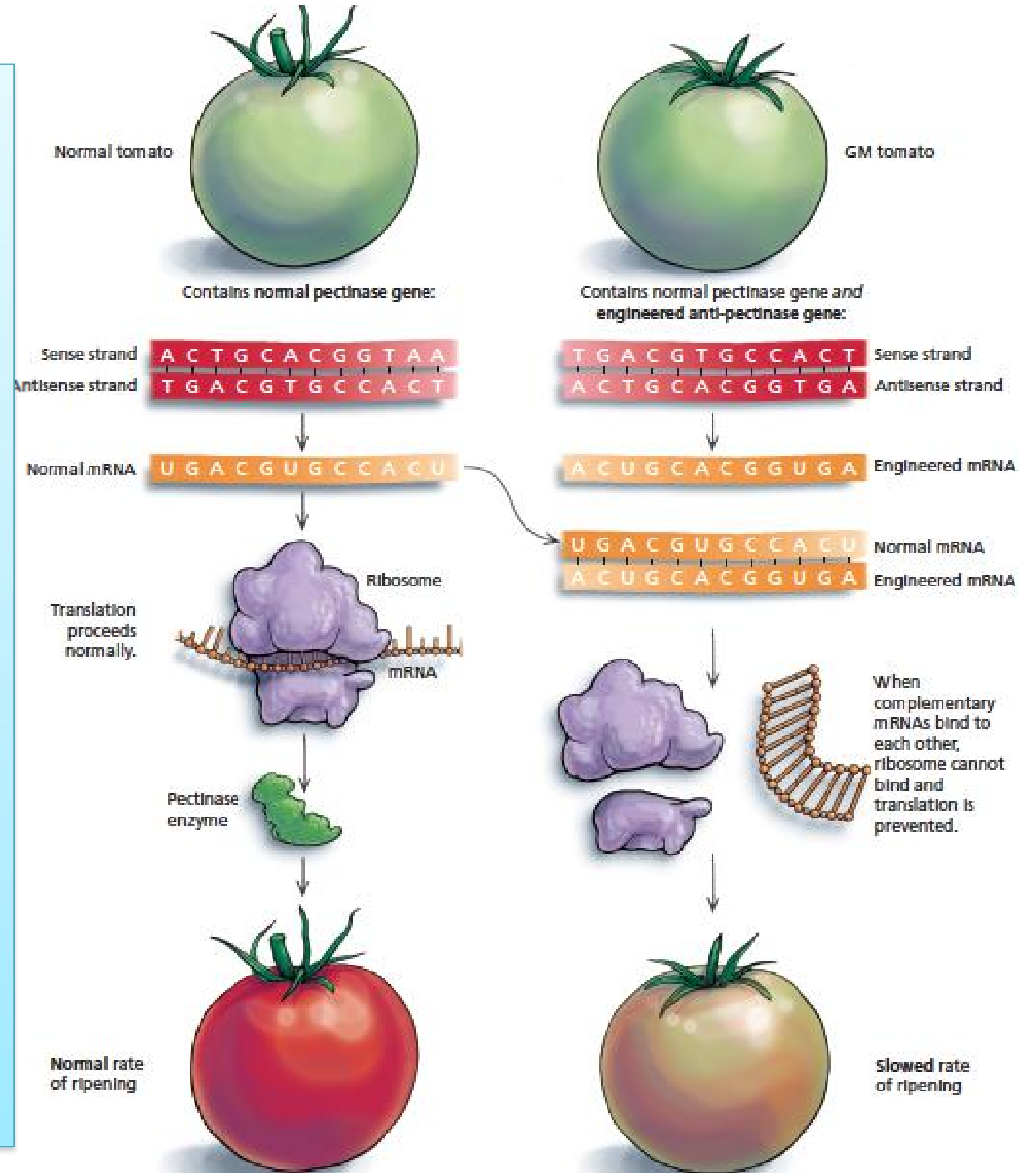
- In tomatoes, engineers insert a gene that produces an mRNA transcript complementary to the mRNA produced by transcription of a pectinase gene.

- In dsDNA, the strand that codes for a protein is called the sense, and its complement is called the antisense.

- When antisense version of the pectinase gene is transcribed, it produces an mRNA that is complementary to the mRNA from the normally transcribed pectinase gene.

- When GE antisense gene base pairs with its naturally occurring pectinase
 complement, ripening is slowed.

- Thus, less of the pectinase enzyme is produced and ripening occurs more slowly.

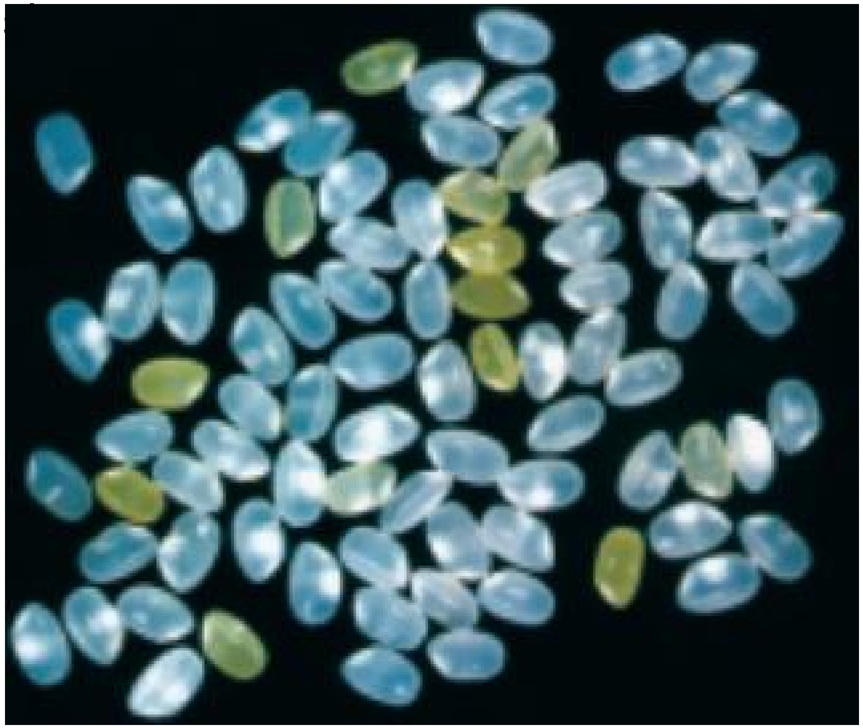


due to uncontrolled population increase, it will demand an increase in yield of crop plants in order to feed all the world' s people.

- Genetic engineers are able to increase the nutritive value of crops.
- engineers have increased amount of β-carotene in rice, a staple food for world' s people.
- this Golden rice will help decrease blindness because cells require β-carotene in order to synthesize vitamin A, required for vi

When a gene from one organism is incorporated into the genome of another organism, a transgenic organism is produced.

A transgenic organism is commonly referred to as a genetically modified organism or GMO .



Golden Rice has been genetically engineered to produce more β-carotene

How Are Crops Genetically Modified?

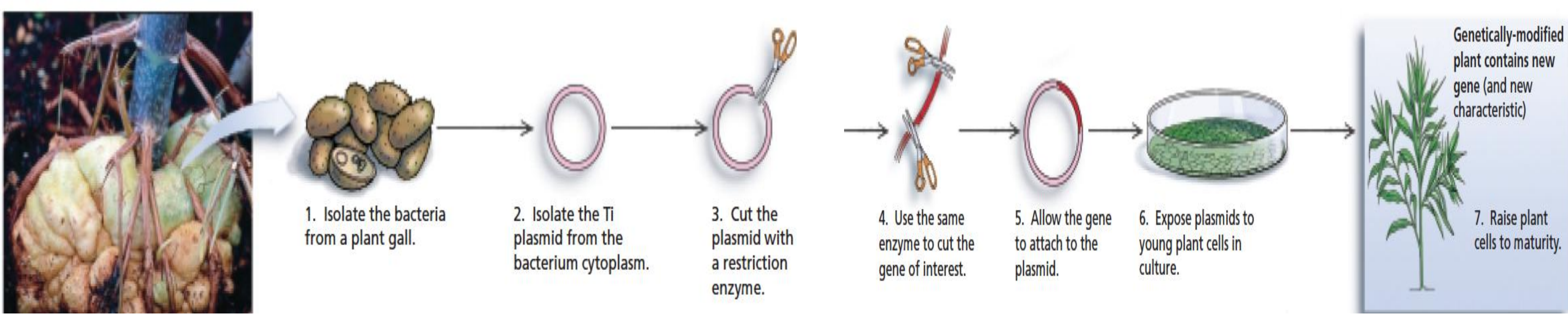
To modify crop plants, the gene must be able to gain access to the plant cell, which means it must be able to move through the plant's rigid outer cell wall.

In nature, Agrobacterium tumefaciens bacterium infects plants and causes tumors called galls. The tumors are induced by a plasmid, called Ti plasmid (for Tumor inducing).

Moving genes into other agricultural crops such as corn, barley, and rice are done by using a device called a gene gun .

A gene gun shoots tungsten-coated pellets covered with foreign DNA into plant cells .

A small percentage of these DNA genes may be incorporated into the plant' s genome.



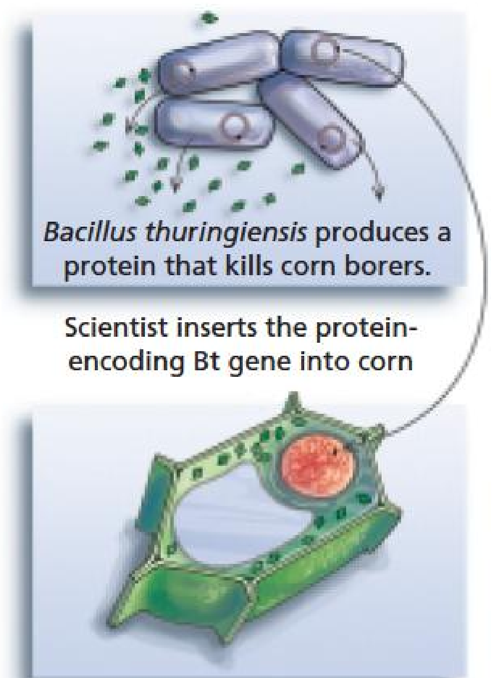
To remove farmer’s reliance on pesticides, agrobusiness companies have engineered plants that are genetically resistant to pests.

For example, corn plants have been engineered to kill the corn borer.

(a) Corn plants have been engineered to kill the insects that eat them.

Corn borer

(b) How it works:



(a) corn borer damages corn and decreases yields.

(b) gene present in the bacterium Bacillus thuringiensis produces a protein that is toxic to the corn borer.

When this gene is inserted into corn DNA, the plant produces the protein that kills the corn borer, thereby providing resistance to the pest.

Scientists transferred a gene from the soil bacterium Bacillus thuringiensis (Bt) into corn.

The Bt gene encodes proteins that are lethal to corn borers but not to humans

close to one-half of all corn currently grown in the United States is engineered with this gene

Some examples of Genetic engineering

Flavr Savr Tomato

Normally, tomatoes are picked while green and transported many miles before being sprayed with ethylene to ripen them.

This prevents damage and perishing on

The Flavr Savr tomato is a genetically engineered tomato which has a gene inserted to extend shelf-life by slowing down the rotting process.

The Flavr Savr tomato was the first GM fruit to be sold in the World.

Is it better to spray tomatoes with ethylene than genetically engineer them?

Some examples of Genetic engineering

Golden Rice

Genetically engineered rice which contains a gene from carrots (or other vegetable) which causes the rice to contain the building blocks for vitamin A production in the body.

Vitamin A deficiency causes blindness and death.

125 million children suffer from vitamin A deficiency. Most of these children live in developing countries where rice is the staple food.

Too much vitamin A causes other health problems.

Some people think that GM crops like this one promote the use of GM foods to people that are not in the position to say no.

Some examples of Genetic engineering

Superspuds

The 'Protato' contains 60% more protein per gram than a 'normal' potato, it also has a larger yield.

A gene was used from the grain Amaranth which codes for storage protein.

In tests with rats and rabbits there have been no side effects and no allergic affects.

Could the Protato face the same opposition as Amflora?

<u>Amflora</u>

Potato created for the starch industry.

Used antibiotic resistance marker gene.

Fear that the genes could escape into the environment.

It was proposed that the waste potato was fed to livestock.

This caused outrage from some European countries, why?

Some examples of Genetic engineering

GloFish

Originally created in an attempt to show levels of pollutions in rivers.

Native to India and Bangladesh. None have survived in American rivers.

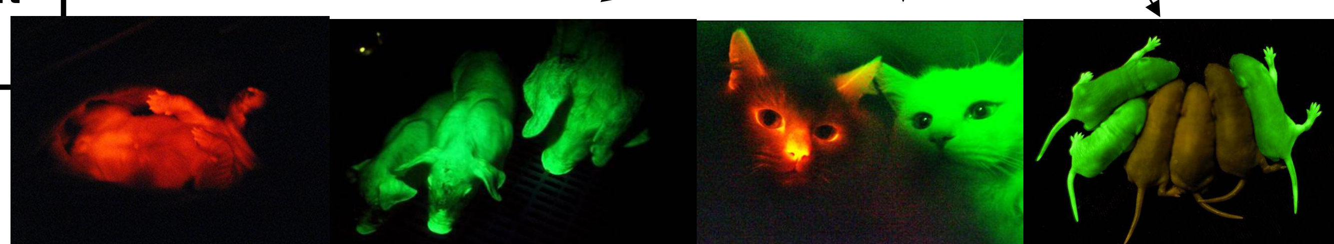
Zebra fish with a gene inserted from jellyfish or coral to make them fluoresce.

The first genetically engineered organism to be sold as a pet.

They can reproduce, but it is illegal to do so!

How does this fish benefit us?

Other fluorescent organisms



Some examples of Genetic engineering

Spider-Goat

Goats which produce spider silk in their milk!

The gene transferred from a spider causes the goats to produce an extra protein in their milk which can be extracted and spun into spider silk thread.

Spider silk is stronger than steel, lightweight, and very elastic.

It holds it's strength between -40˚C and 220˚C.

Spider silk could be used to manufacture;

- replacement ligaments
- wound covering (it has antiseptic properties and vitamin K which helps with blood clotting!)
- optical communications
- bullet proof clothing
- waterproof clothing!!

Spiders cannot be farmed as they are cannibalistic –

they eat each other!

About 75% of spider goats are euthanised as there are strict controls meaning that they cannot leave the facility where they are created.

Why create a life to destroy it?

Some examples of Genetic engineering

AquAdvantage Salmon

This fish has not been consumed by humans yet – is it safe?

A gene which controls the growth hormone from one breed of salmon is inserted into the DNA of another.

This causes it to grow much quicker than 'normal' salmon.

What affect could the AquAdvantage salmon have on wild salmon if it escaped?

Could this gene be transferred to humans if we eat it?

What could happen if this occurred?

AquAdvantage salmon

Normal salmon

Some examples of Genetic engineering

Venomous cabbage

Cabbage which has been genetically engineered to include the gene for Scorpion venom.

This reduces the use of chemical pesticides sprayed on crops.

The venom is poisonous to caterpillars – it acts as a pesticide.

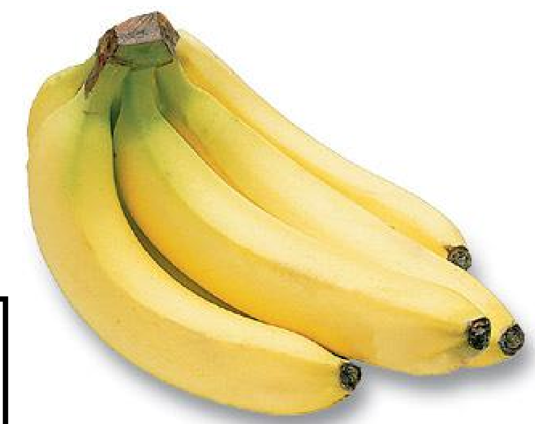
The toxin has been altered - it does not kill human cells

What if the toxin mutates and alters again?

What affect will the toxin have on the biodiversity of the area?

Some examples of Genetic engineering

Banana Vaccine



Bananas, carrots, potatoes and lettuce have all been genetically engineering to deliver vaccines for diseases.

The banana has been the most successful in testing.

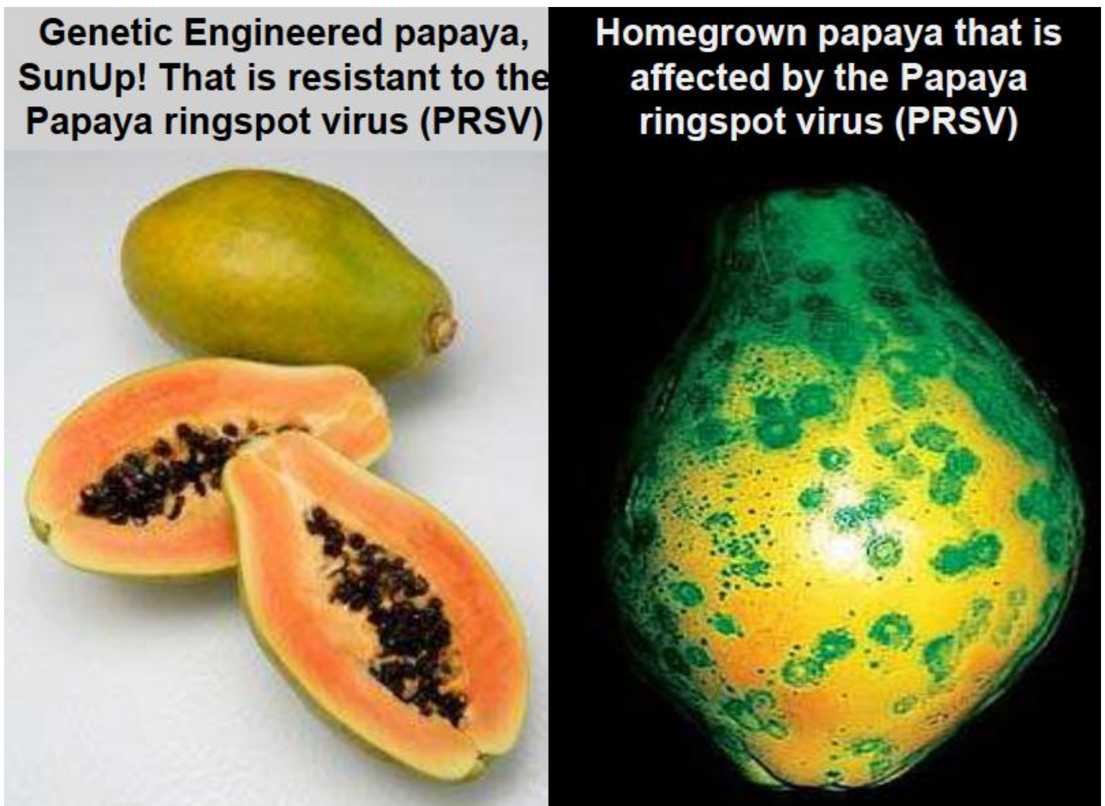
The virus' genes are transferred to the banana cells and become a permanent part of that banana's genetic code.

When the gene has been inserted into the banana, it's cells produces virus proteins (not the infectious part).

When you eat the banana you ingest these proteins and the body produces antibodies against them – this is exactly h

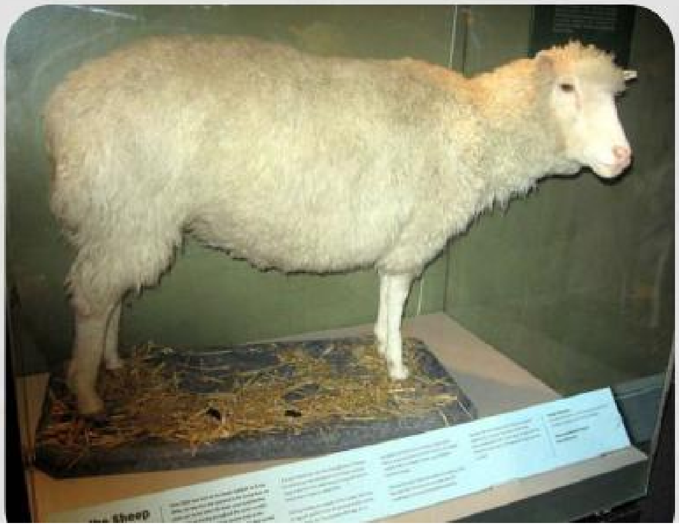
Is there a risk of people taking the vaccine without realising they are doing so?

- **A Blue Rose is a genetically modified Rose.**



- Transgenic fruit obtained from pear and apple.

Using Modern DNA and cross fertilization techniques; the Dolion a cross between a lion and a dog.



- Dolly the sheep is the world's most famous clone.
- Dolly was born 5 July 1996 to three mothers (one provided the egg, another the DNA and a third carried the cloned embryo to term).

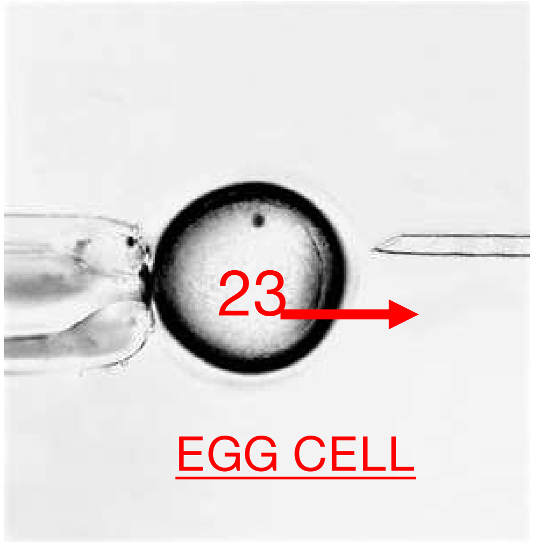
Genetic Engineers Can Modify Humans

The genetic modifications may one day include replacing defective or nonfunctional alleles of a gene with a functional copy of the gene.

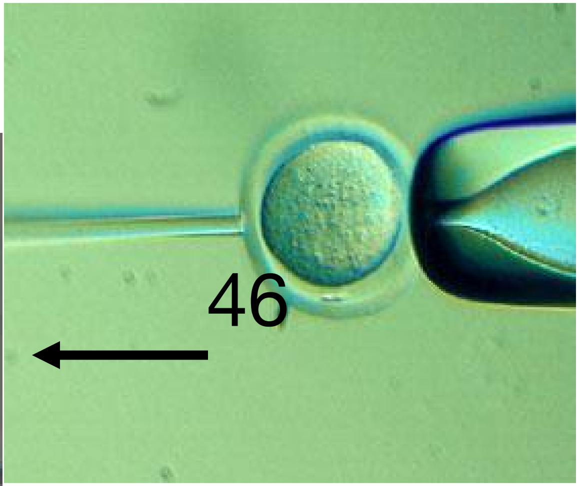
If this happens, it might be possible for physicians to diagnose genetic defects in early embryos and fix them, allowing the embryo to develop into a disease-free adult

How could you clone a human?

- Step 1: An egg is removed from a female human
- Eggs are haploid: 23 chromosomes.
- The nucleus of the egg is removed and is thrown away.



- Step 2: A body cell is removed from another person.
- The nucleus of the body cell is removed
- Body cells are diploid: 46 chromosomes.
- Step 3:
- The nucleus of the diploid body cell is put into the egg.
- This egg no longer needs to be fertilized since it has all 46 chromosomes.



Body Cell

- Step 4: The egg is then charged with electricity to start mitosis.

- Step 5: Its then put into a surrogate mother so it can grow.

- Its going to be genetically identical to the parent of the body cell.
- But it will be a baby.

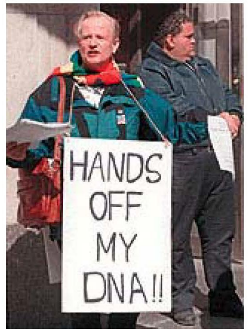
- Any Plants and animals can be cloned.

Some reasons why the work of genetic engineers is important	Some reasons why the work of genetic engineers is controversial
• GM animals and crops may make farms more productive. • GM crops may be made to taste better, last longer, or contain more nutrients. • Genetic engineers hope to cure diseases and save lives.	• GM crops encourage agribusiness, which may close down some small farms. • GM animals and crops may cause health problems in consumers. • GM crops might have unexpected adverse effects on the environment. • Present research might lead to the unethical genetic modification of humans.

Are genetic engineers doing more good than harm? This chart lists
some of the pros and cons of genetic engineering

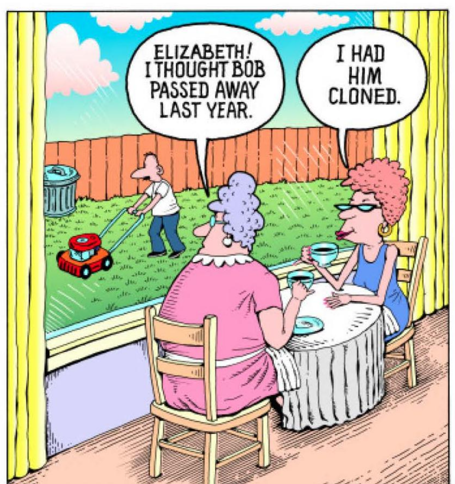
Ethical issues raised by genomics (ELSI) (Ethical legal, societal implications)

- Individual's genome holds key to disease susceptibility
- Potential for misuse recognized by founders of Human Genome Project



Genetic modification of humans

- Once we know the genes responsible for particular diseases, should we "cure" the diseases?
- Should we also modify genes responsible for traits such as height or beauty?
- Should we allow the cloning of human beings?



Protesters at a World Trade Organization meeting in Seattle. These people are concerned about how GMOs
may affect humans and the

References:

•Lehninger g Principles of Biochemistry. 5th Edition. 2008. David L. Nelson and Michael M.Cox.
•Molecular Cell Biology. 5th Edition. 2004. Lodish, Berk, Matsudaira, Kaiser, Krieger, Scott,Zipursky and Darnell.
•Biology: Science for Life, 4/E Colleen M. Belk, University of Minnesota, Virginia Borden Maier, ISBN-10: 0321767829 • ISBN-13: 9780321767820 ©2013
•An Introduction to Genetic Engineering, Third edition,2008, By Desmond S. T. Nicholl
•Biology, by Raven and Johnson, sixth edition 2002
•Nickell, J. and Fischer, J.F. (1999). Crime Science: Methods of Forensic Detection. Kentucky: The University Press of Kentucky.
•Riciu , A. (2011, November 26). Pituitary Dwarfism Causes, Symptoms, Diagnosis and Treatment. Retrieved from http://www.doctortipster.com/6928-pituitary-dwarfism-causes-symptoms-diagnosis-and-treatment.html

•Acknowledgement:
•Internet sources, teaching slides on slideshare, scitable and others ppts from Prof Ashok and Prof CS Dey

Today:
We learnt about genetic engineering implications

Home assignment:
Advantages and disadvantages of genetic engineering

Next class:
Cancer biology