

**Genetic Modification
Genetics and Society
March 25, 2008**

Outline

Methods for making transgenic plants

Uses of modified plants

Methods for making transgenic and knockout animals

Outline

Methods for making transgenic plants

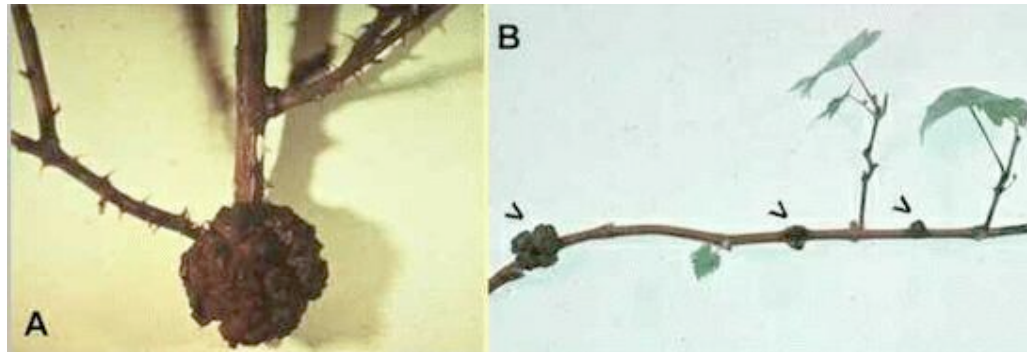
Uses of modified plants

Methods for making transgenic and knockout animals

How do you make transgenic plants?

- *Agrobacterium*
- Gene gun

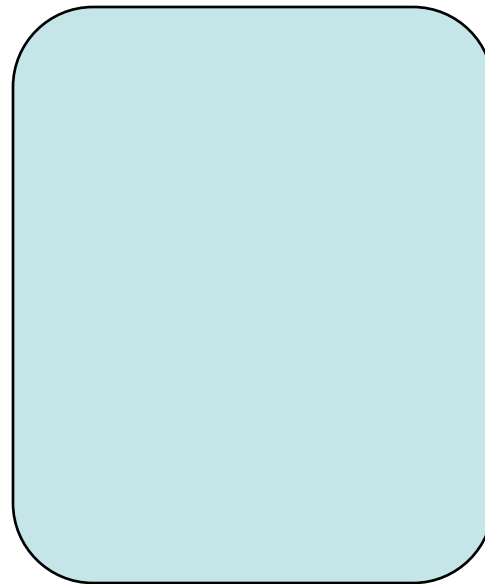
Agrobacterium tumefaciens



Agrobacterium tumefaciens is a bacterium that causes crown gall disease in plants

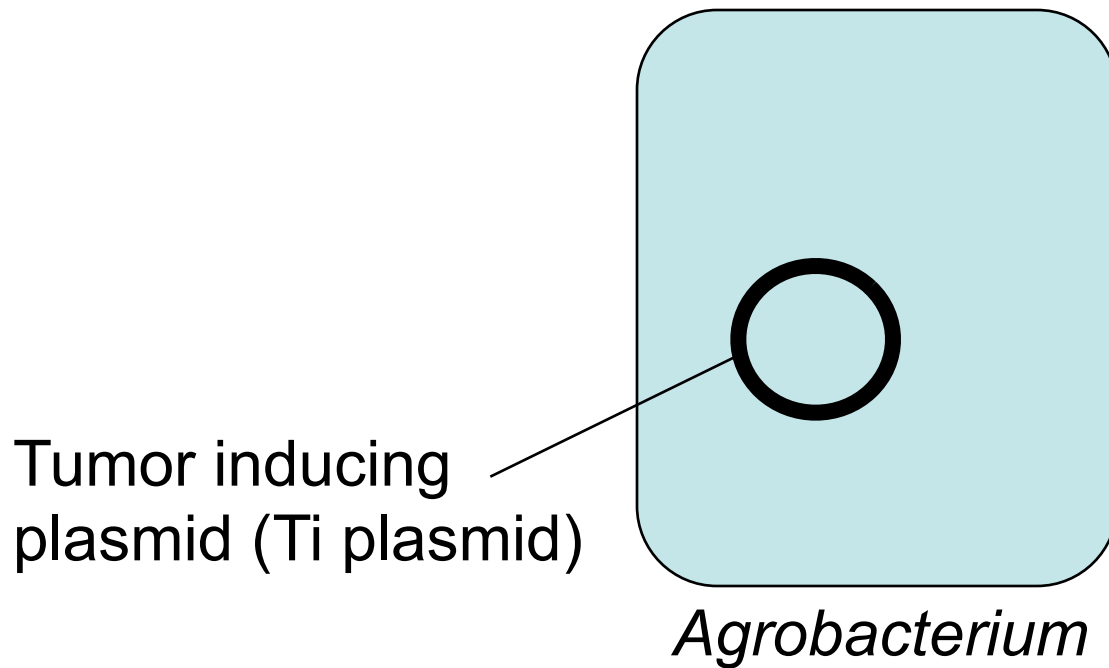
Crown gall disease causes a tumor-like swelling of the plant.

Tumor inducing plasmid



Agrobacterium

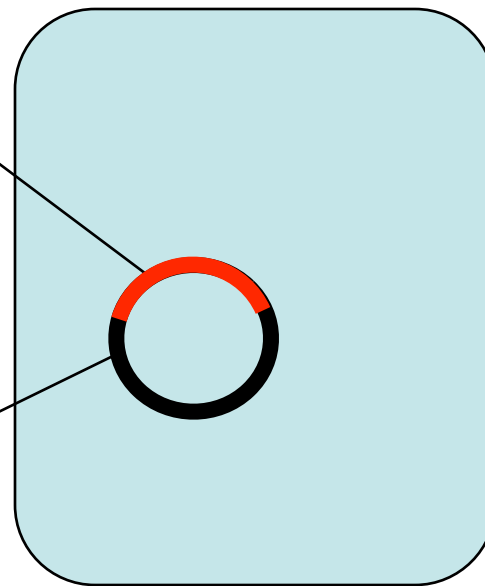
Tumor inducing plasmid



Tumor inducing plasmid

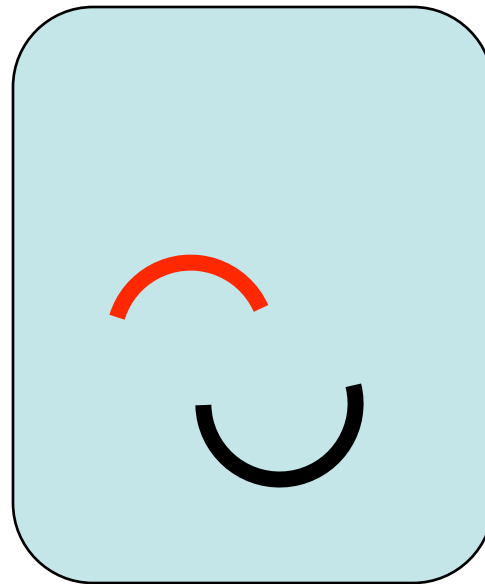
Part of the plasmid is
the **transferred DNA,**
or **T DNA**

Tumor inducing
plasmid (Ti plasmid)



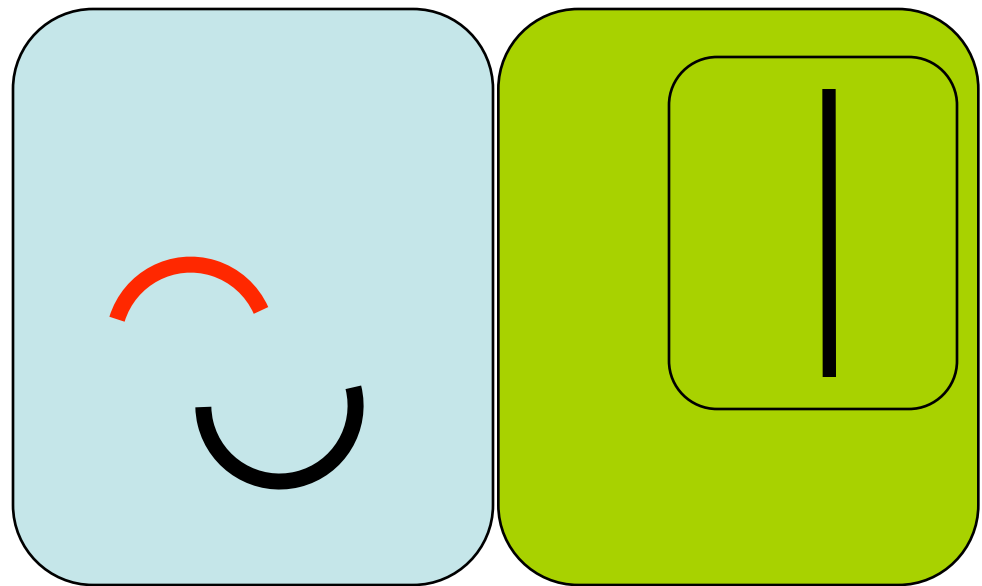
Agrobacterium

Tumor inducing plasmid



Agrobacterium

Tumor inducing plasmid

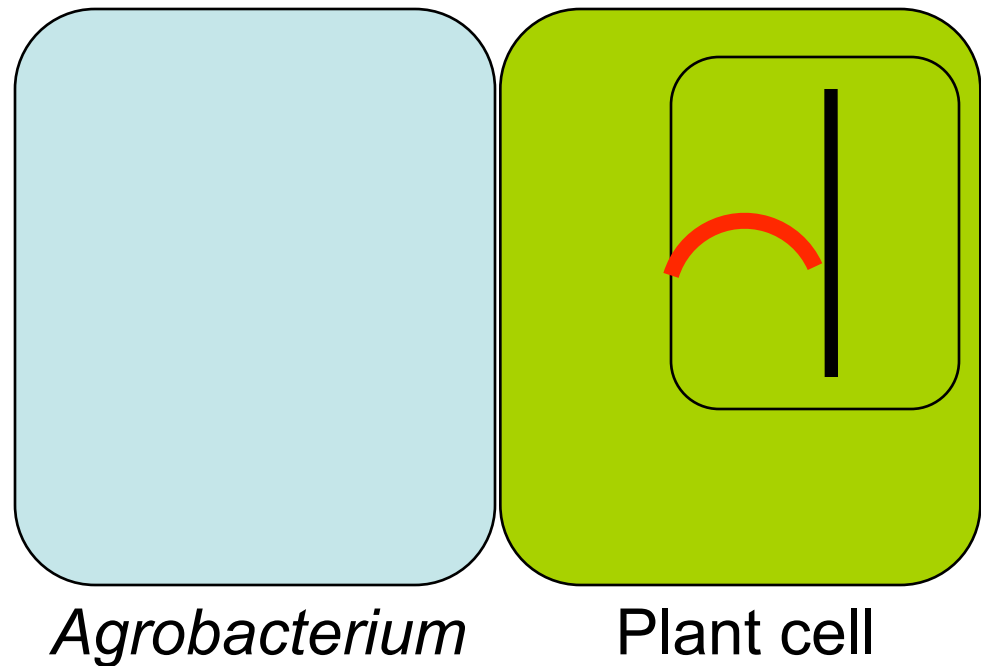


Agrobacterium

Plant cell

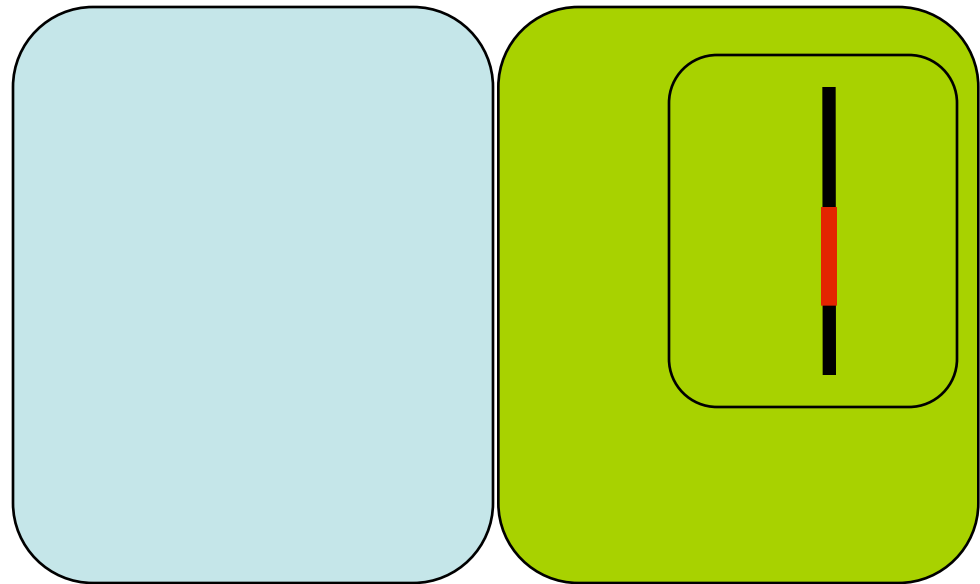
Tumor inducing plasmid

The bacterium has an unknown mechanism for transferring the T-DNA into a plant cell



Tumor inducing plasmid

The DNA incorporates randomly into the plant chromosome

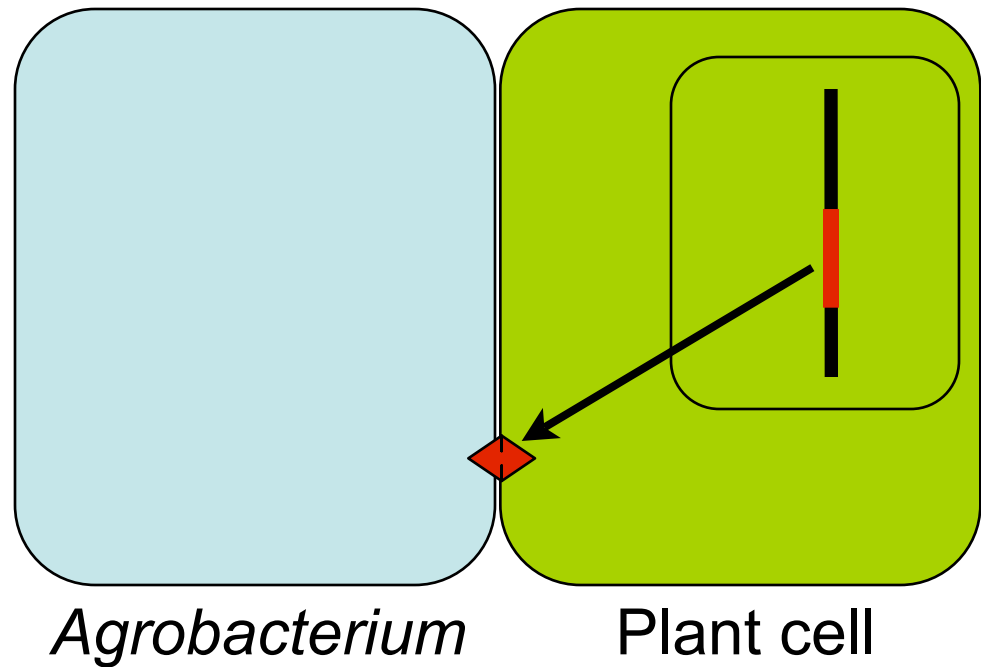


Agrobacterium

Plant cell

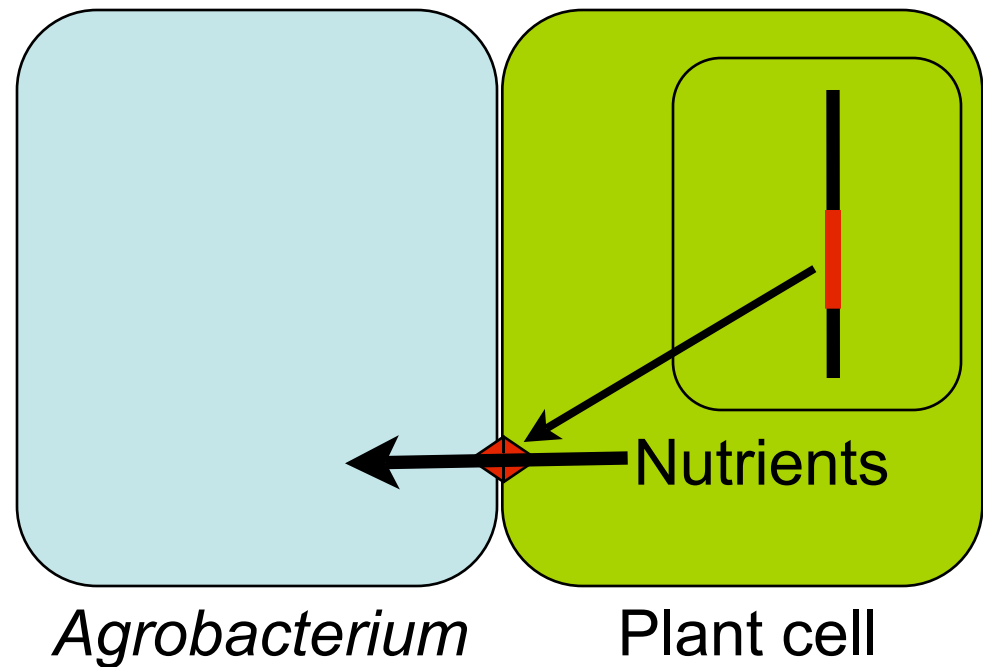
Tumor inducing plasmid

The DNA has genes that make nutrient transporters



Tumor inducing plasmid

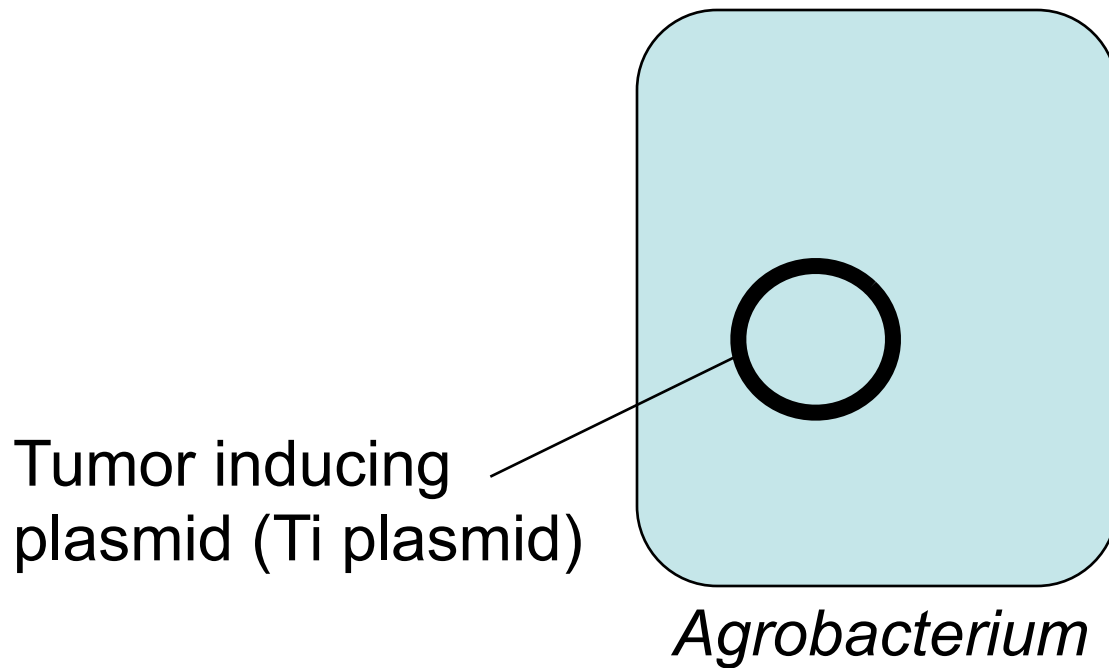
The DNA has genes that make nutrient transporters



Question

How can *Agrobacterium* be used to make transgenic plants?

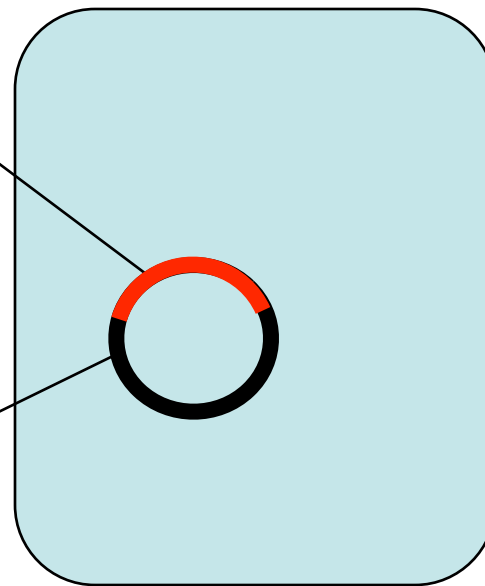
Tumor inducing plasmid



Tumor inducing plasmid

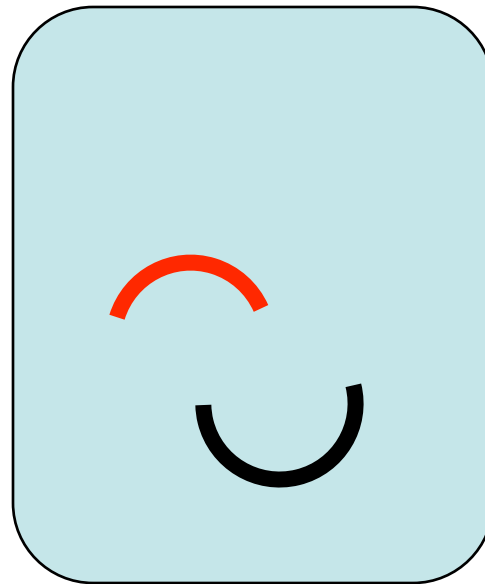
The T DNA can be replaced with any DNA

Tumor inducing plasmid (Ti plasmid)



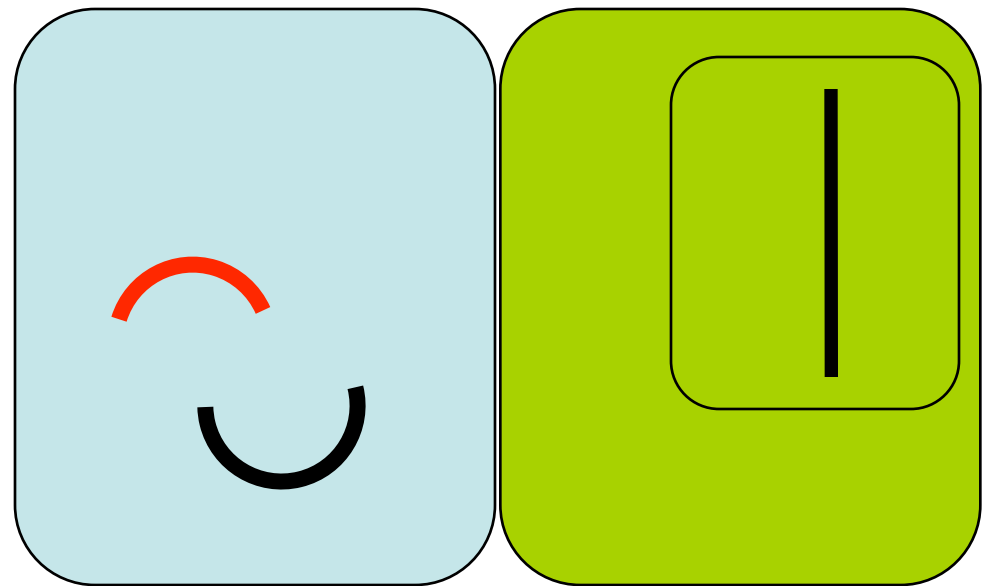
Agrobacterium

Tumor inducing plasmid



Agrobacterium

Tumor inducing plasmid

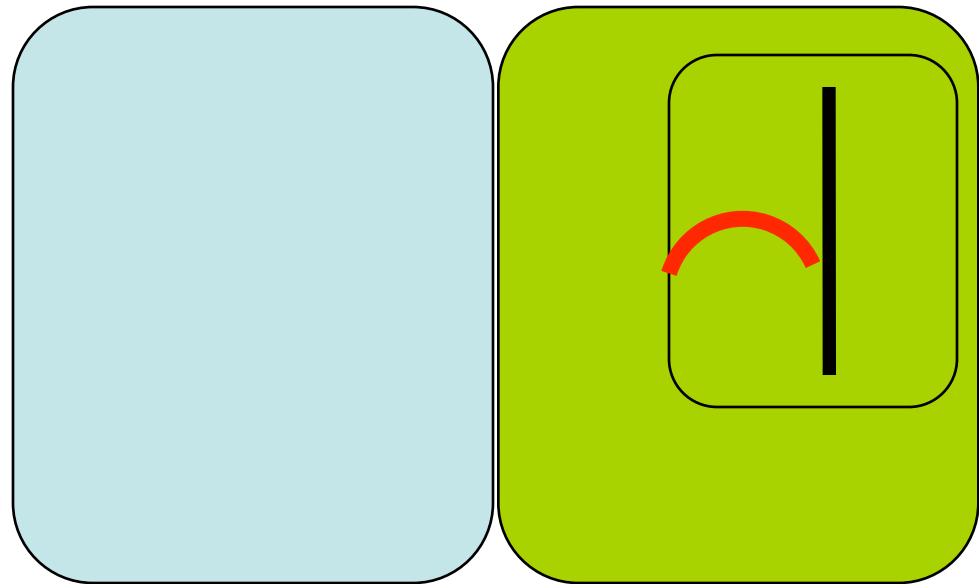


Agrobacterium

Plant cell

Tumor inducing plasmid

The bacterium transfers the DNA into a plant cell

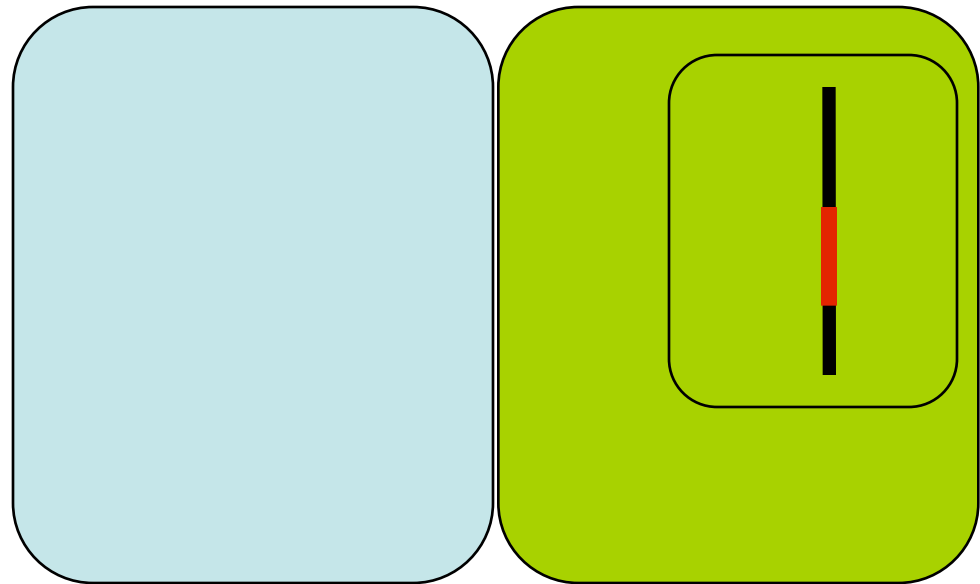


Agrobacterium

Plant cell

Tumor inducing plasmid

The DNA incorporates randomly into the plant chromosome

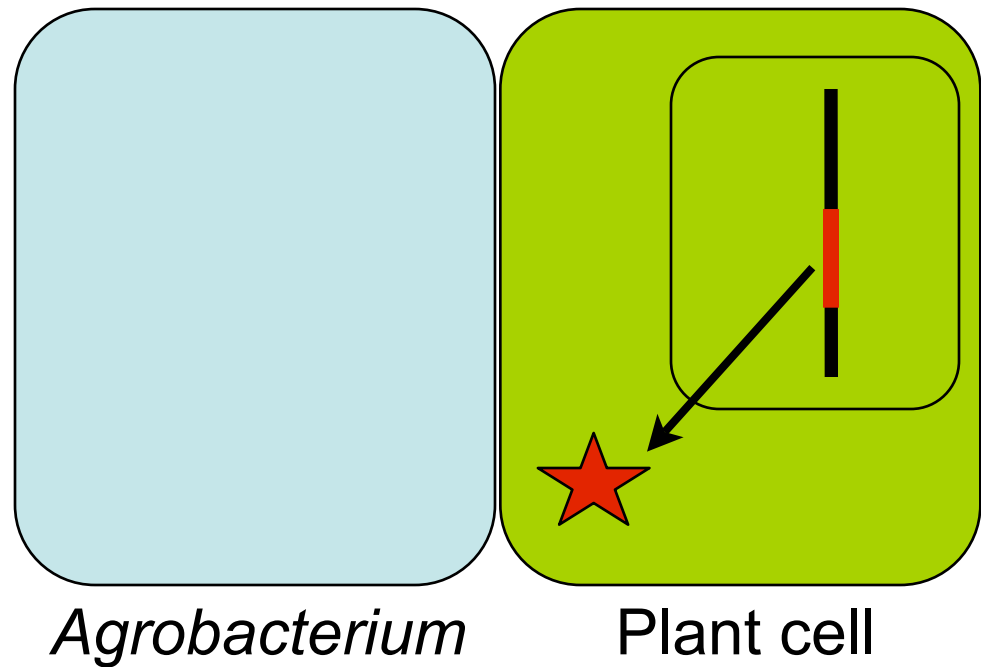


Agrobacterium

Plant cell

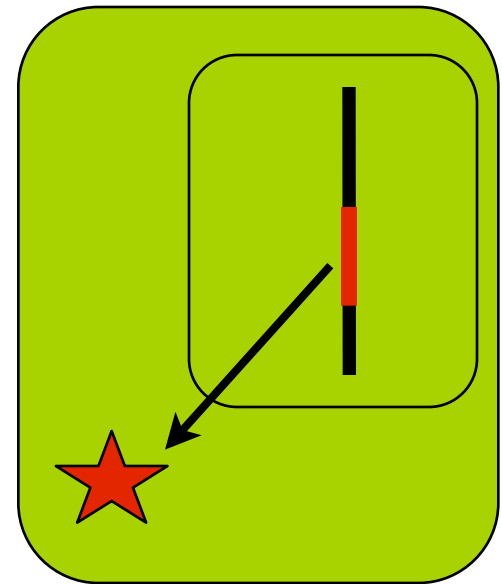
Tumor inducing plasmid

The DNA has genes that make anything you want



Tumor inducing plasmid

The DNA has genes that make anything you want



Plant cell

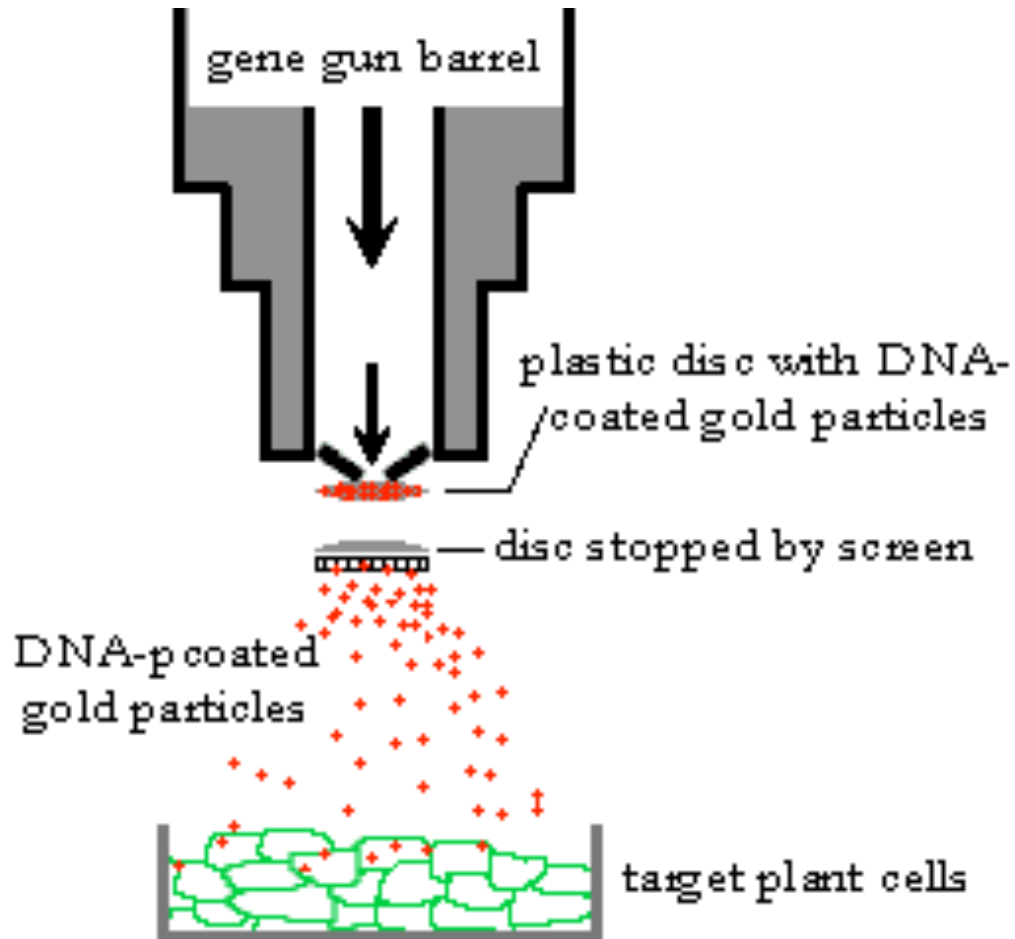
Gene gun

Gene gun

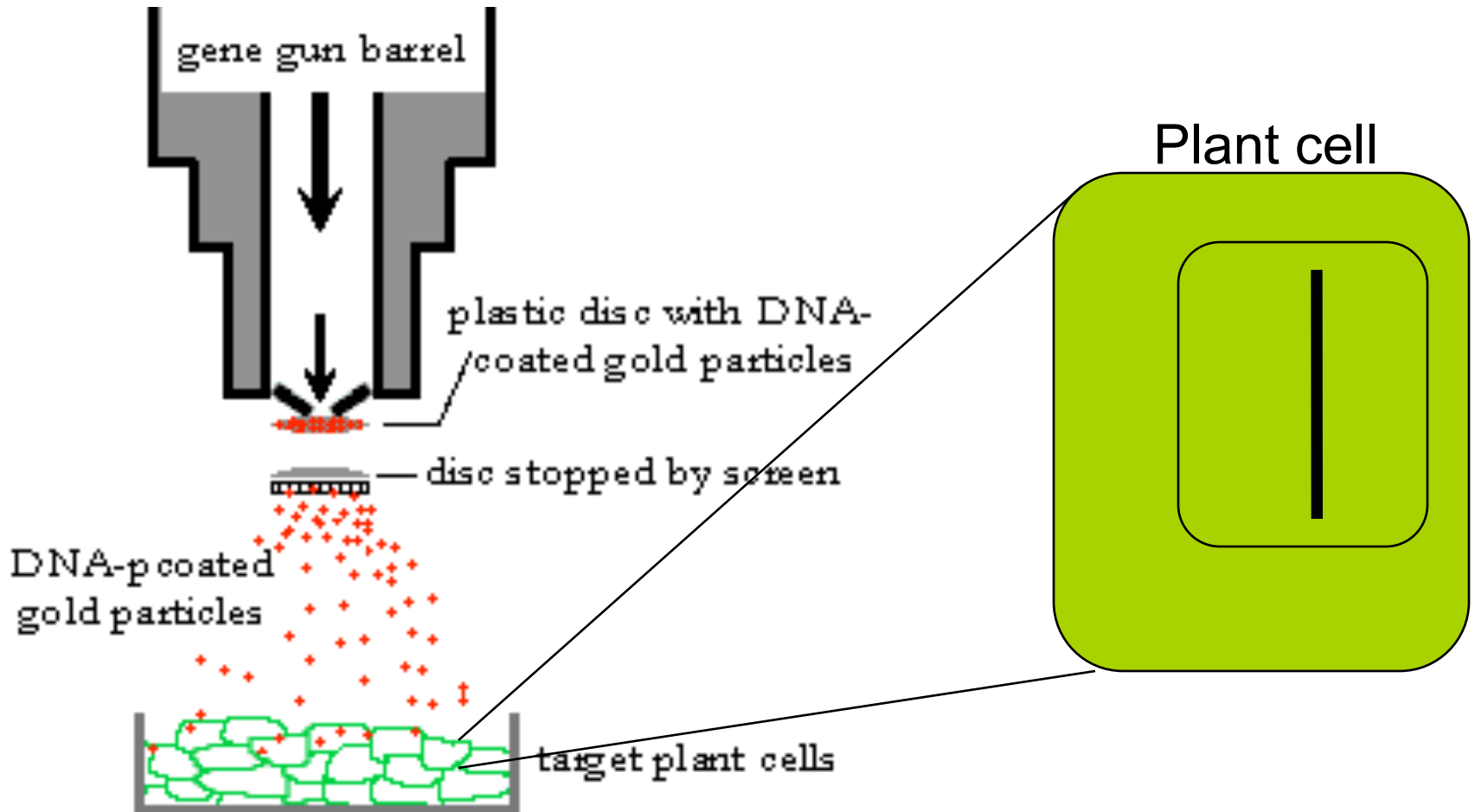


<http://science.marshall.edu/harrison/research.html>

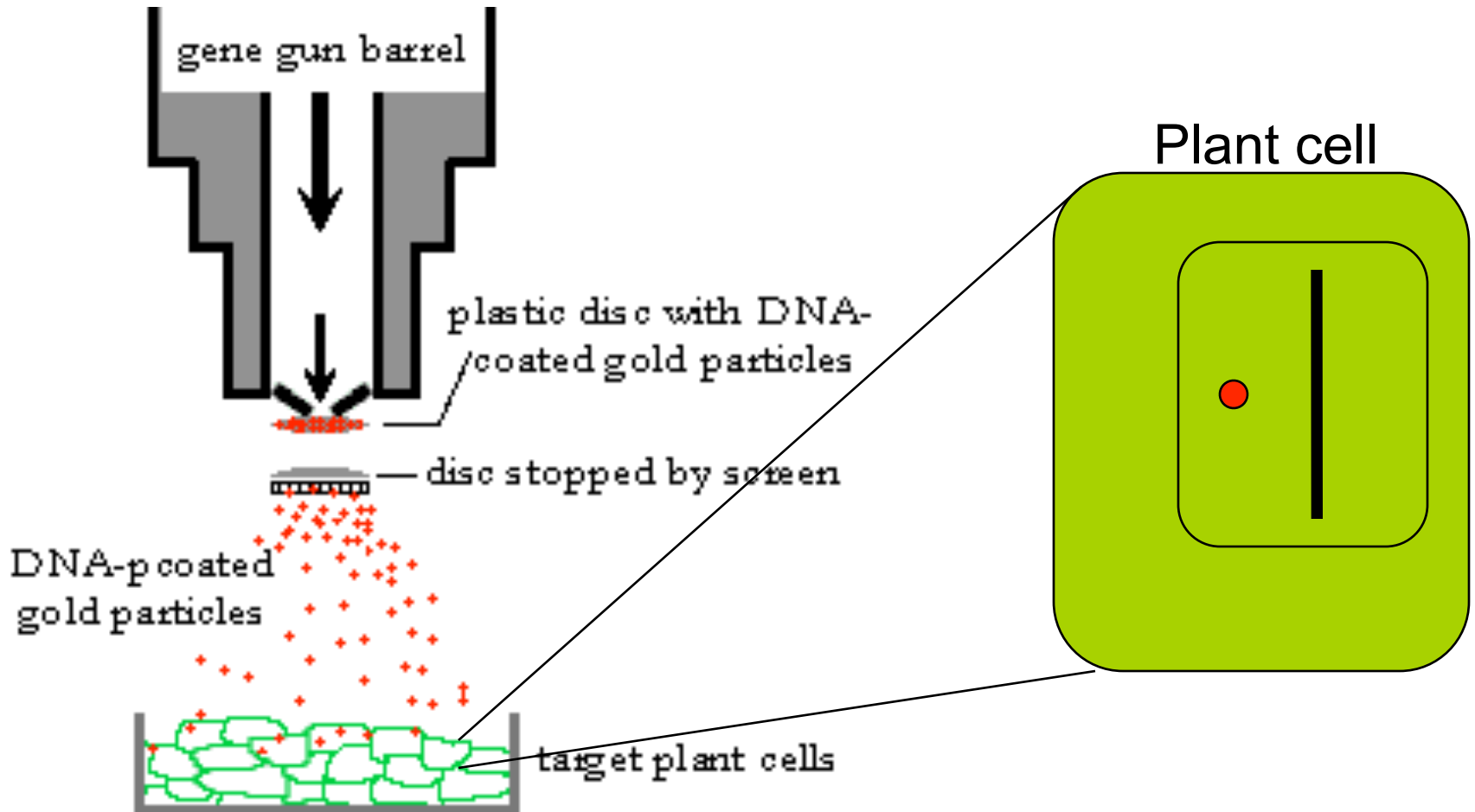
Gene gun



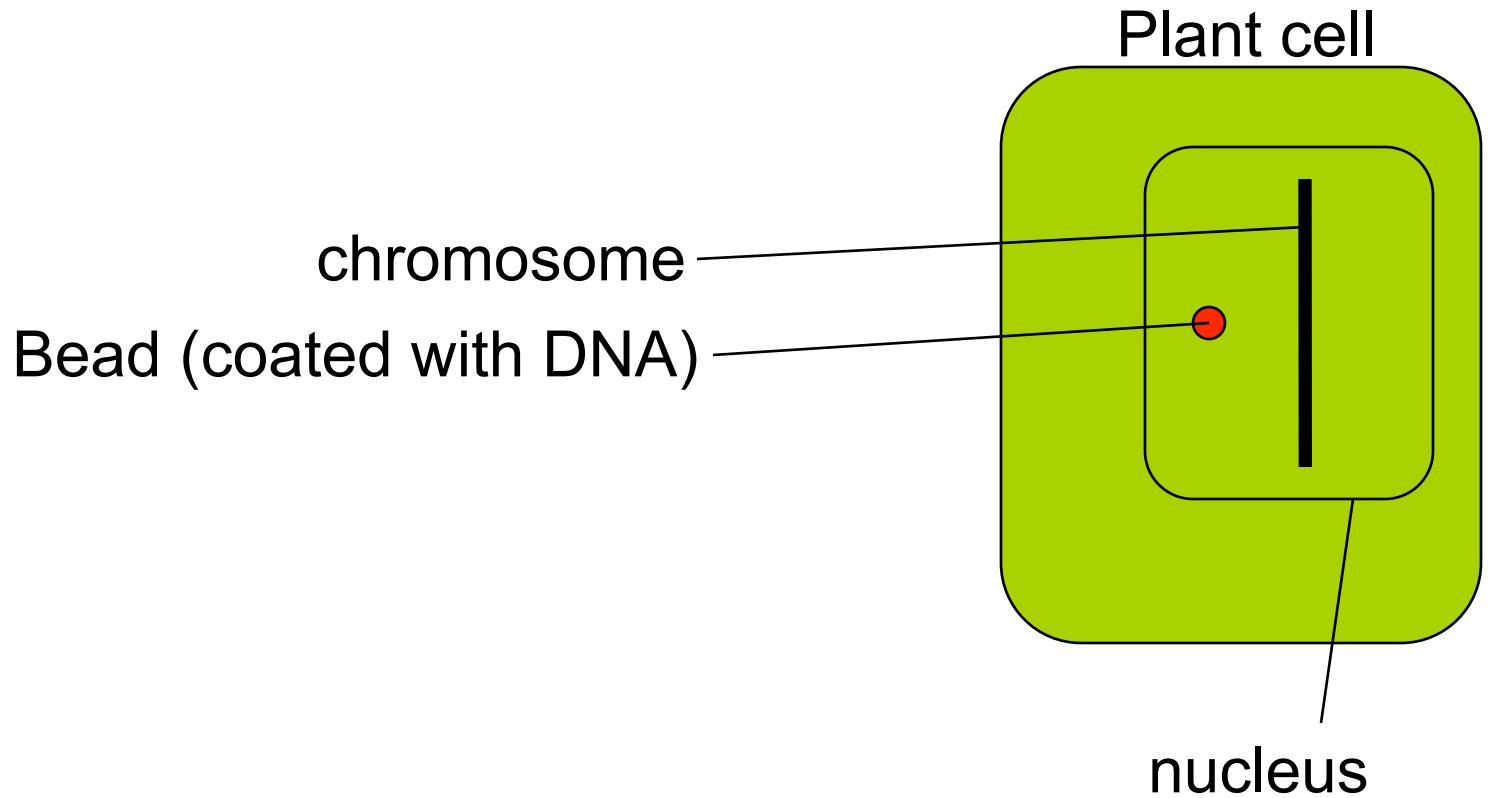
Gene gun



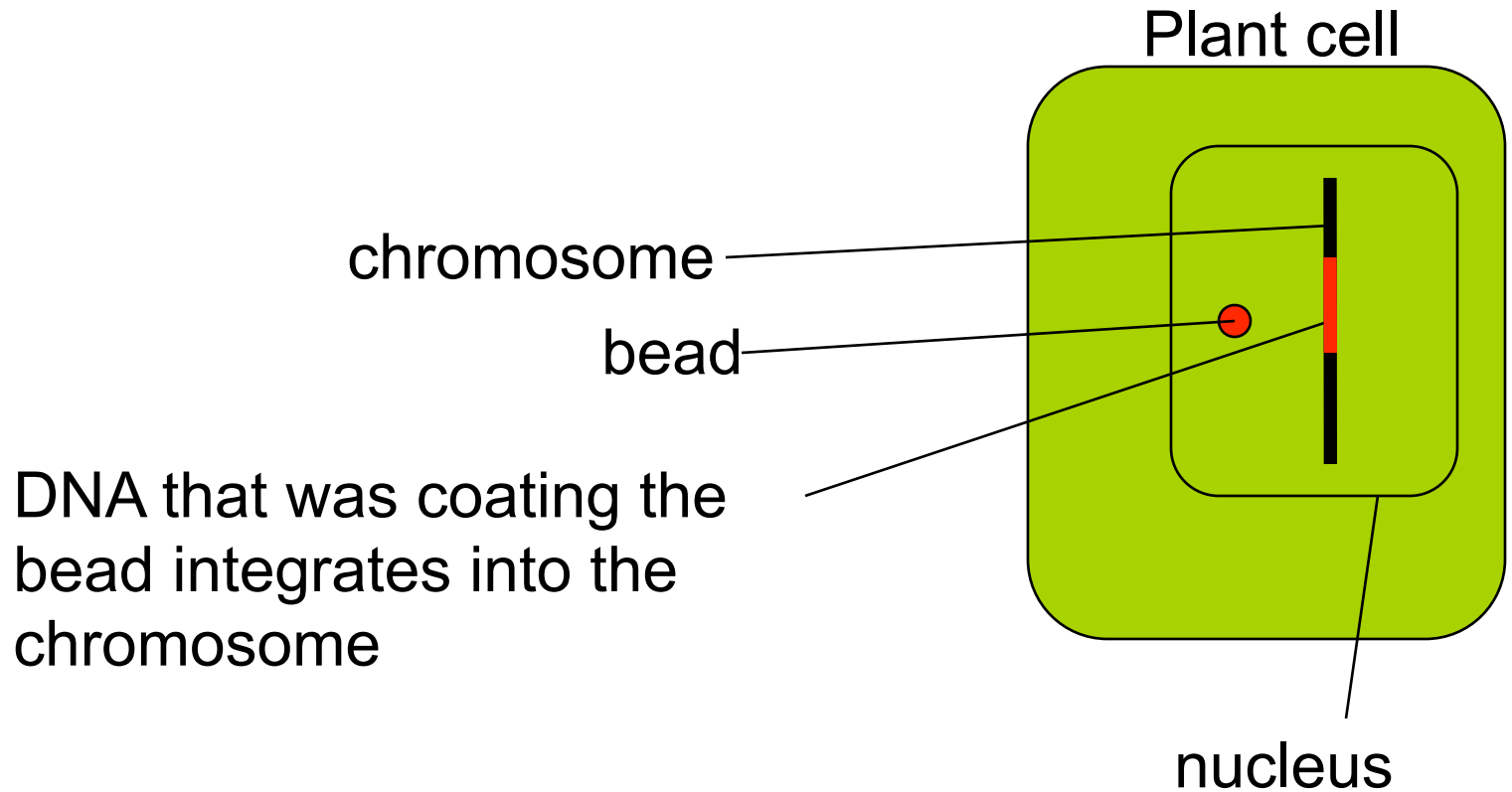
Gene gun



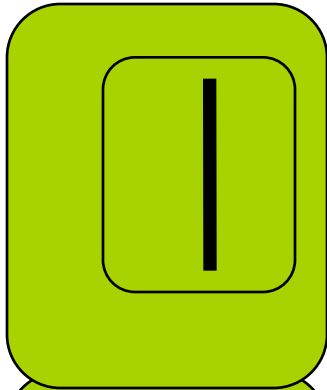
Gene gun



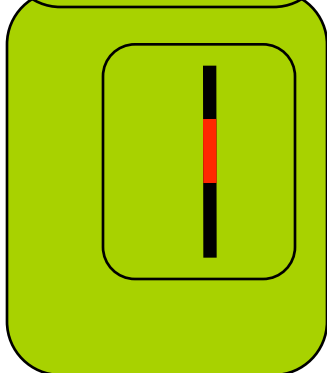
Gene gun



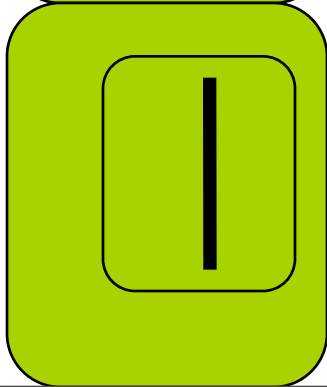
Growing a cell into a plant



Normal cell

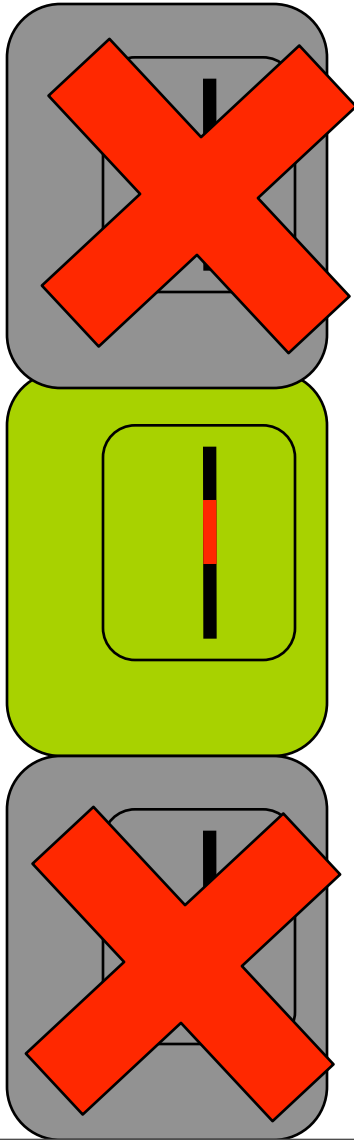


Transformed cell



Normal cell

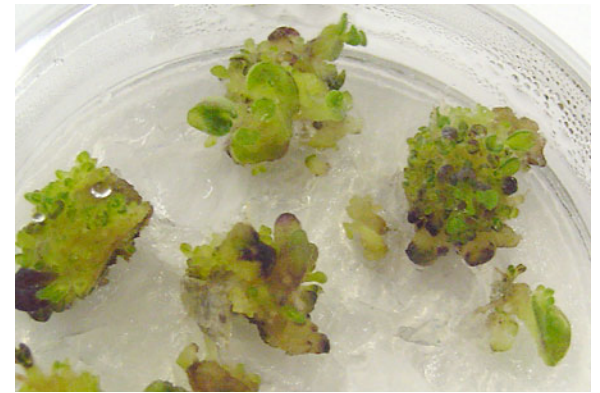
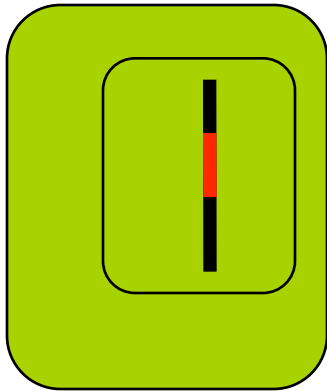
Growing a cell into a plant



Selection is used to kill the non-transformed cells

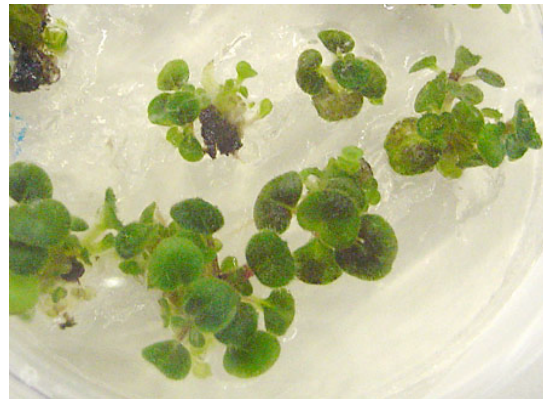
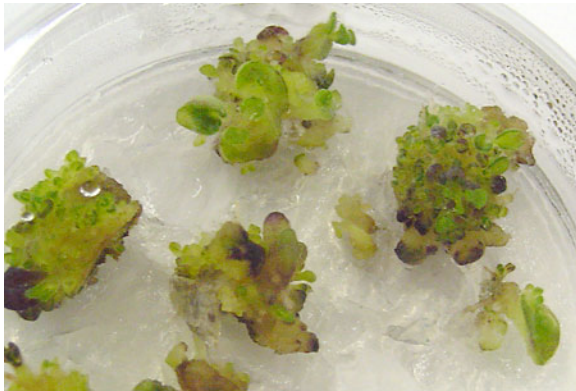
Growing a cell into a plant

Transformed cells are
grown in a special
media with plant
hormones



http://www.lau.edu.lb/news-events/news/plant_tissue_culture/photos.html

Growing a cell into a plant



http://www.lau.edu.lb/news-events/news/plant_tissue_culture/photos.html

The transgenic cells can be grown into transgenic plants

GM crops in the U.S.: 2005

Soybeans: 87% of 72 million acres

Corn: 52% of 74 million acres

Cotton: 79% of 14 million acres

National Agricultural Statistics Service (NASS), Agricultural Statistics Board,
U.S. Department of Agriculture

usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0605.pdf

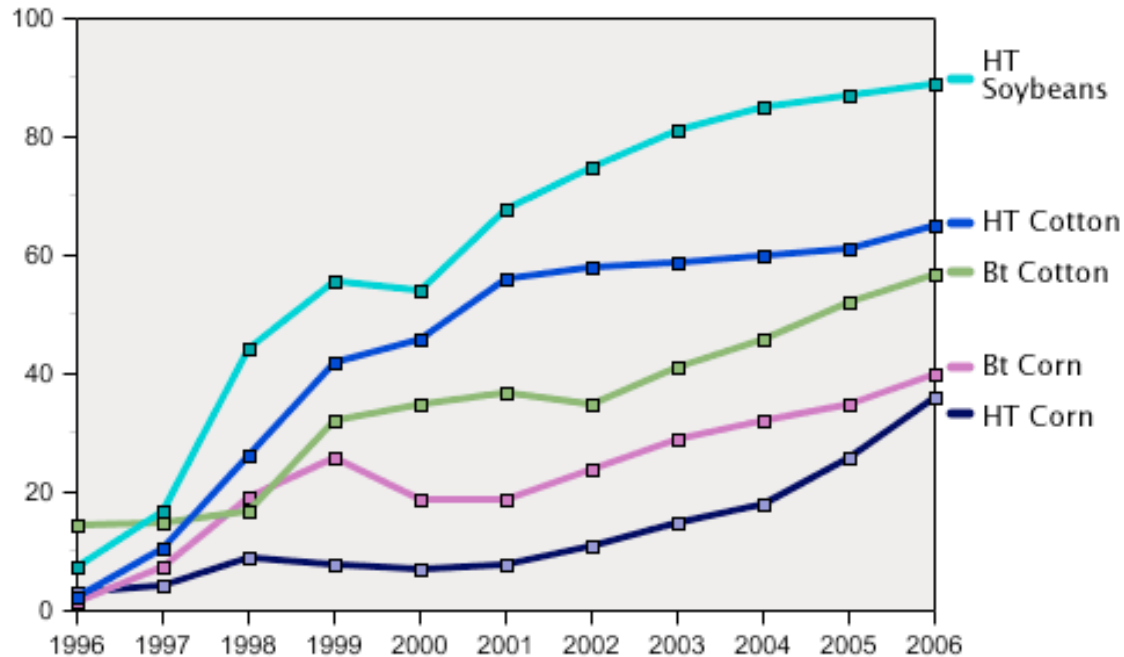
The United States accounts for nearly two-thirds of all genetically engineered crops planted globally.

<http://www.ens-newswire.com/ens/may2005/2005-05-13-03.asp>

GM crops in the U.S.: 10 years

Adoption of genetically engineered crops grows steadily in the U.S.

Percent of acres



Data for each crop category include varieties with both HT and Bt (stacked) traits.

Source: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-06 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Over 70 percent of processed foods on grocery store shelves in the U.S. contain ingredients and oils from biotech crops, according to an industry estimate.

www.usda.gov/2006/08/0306.xml

Outline

Methods for making transgenic plants

Uses of modified plants

Methods for making transgenic and knockout animals

Uses of Genetic Modification

Profit Friendly

- Herbicide resistance
- Insect resistance

Consumer friendly

- Extra nutrients



Herbicide resistance



Roundup-ready™ or LibertyLink™

- Transgenic plants are resistant to the herbicide (plant killer) glyphosate or glufosinate-ammonium.
- Weeds are not resistant.
- Allows spraying the weeds near the crops without worrying that the crops will die.

Roundup-ready™ Corn



<http://www.lsuagcenter.com/publications/agmag/archive/2001/summer/weed+control+with+roundup+ready+liberty+link+and+clearfield+corn.htm>

Insect resistance

Plants can also be made resistant to insects

Insect resistance

- The bacteria *Bacillus thuringiensis* (Bt) makes a protein that kills insects.
- Bt discovered in 1901 in Japan as a bacteria killing silkworm moths.
- Bt bacteria marketed as an insecticide in France in 1938
- In 1956, researchers, Hannay, Fitz-James and Angus found the insecticidal protein made by the bacteria.
- In the US, Bt was used commercially starting in 1958. By 1961, Bt was registered as a pesticide to the EPA.

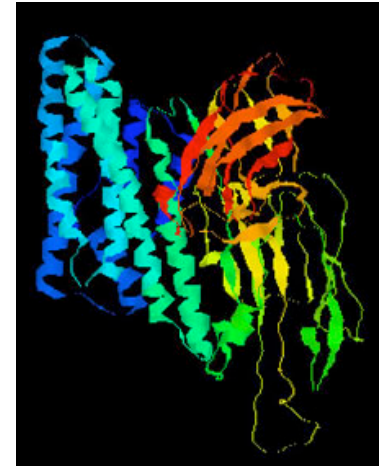
Bt toxin

There are thousands of known Bt-related bacteria

Each makes a protein that is toxic to a particular species of insect.

No toxicity to vertebrates has been found, even in large quantities of toxin.

Inserting the gene for the Bt toxin protein into a plant makes the plant resistant to insects.



Crops expressing Bt toxin

Corn



<http://generalhorticulture.tamu.edu/LearningCommunity/Assignments/TransgenicCrops/TransgenicCrops.htm>

Cotton



http://www.bt.ucsd.edu/crop_refuge.html

Advantages of Bt crops

- Less cost of insecticide
- Less health risk to farmers from insecticide
- Less chemical insecticide in/on food
- Greater yields with less labor
- Long-term advantages are unknown**

Nutrient Rich GM plants

Plants can be modified to contain higher levels of nutrients or nutrients that they don't usually contain.

Golden Rice

Adding two genes from other plants to make rice that produces vitamin A in the grain.



Other GM crops

Anti-nematode plants

Billions of people are infected with nematode worm parasites. Access to a constant supply of anti-nematode drugs is not available. Bt toxin can be used to kill parasites.

GM crops for ethanol production

Both the crop and the yeast used in fermentation can be improved by GM.

Summary

Agrobacterium and gene gun methods for making transgenic plants.

Herbicide (roundup-ready)

Insect resistant (Bt) plants

Improved nutrition of plants (golden rice)

Outline

Methods for making transgenic plants

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Transgenic and knockout animals

Gene gun aside



Fig. 3. In vivo bombardment of murine epidermis using the Helios® gene gun

The gene gun can also be used to transfer DNA into animal cells (skin cells).

THIS DOES NOT AFFECT THE SPERM OR EGG CELLS.

Simple transgenes in mice

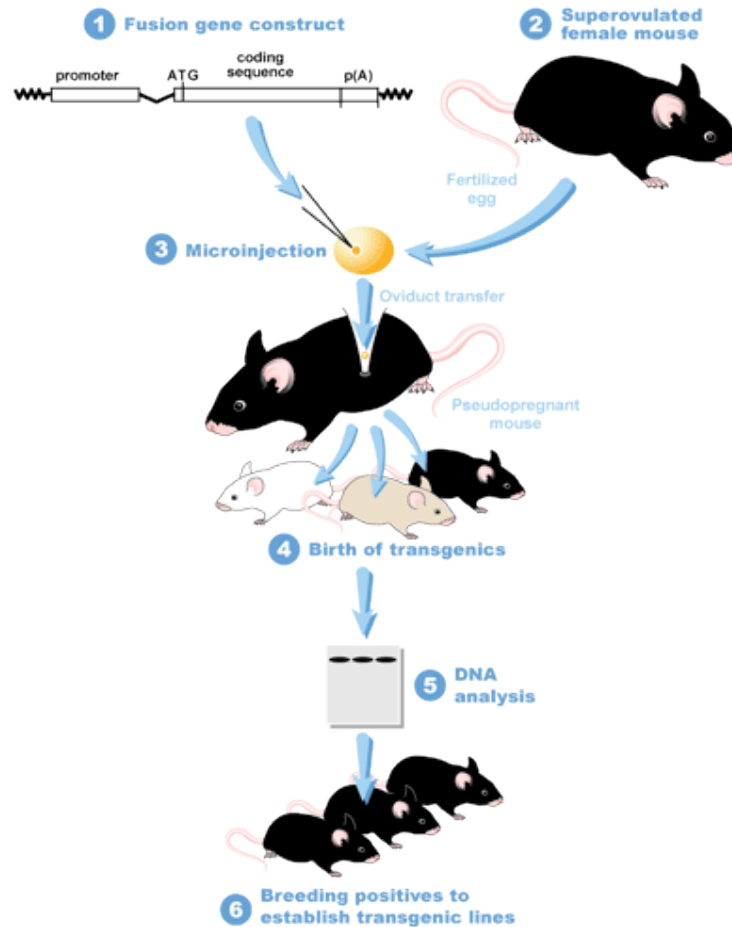


Plain DNA can be directly injected into *in vitro* fertilized eggs.

This is called **pronuclear injection.**

<http://www.montana.edu/wwwmri/transgenic.html>

Simple transgenes in mice



Simple transgenes in mice



chromosome

Simple transgenes in mice



The new DNA gets integrated into the genome at a random location.

Information can only be added by this technique

Simple transgenes in mice

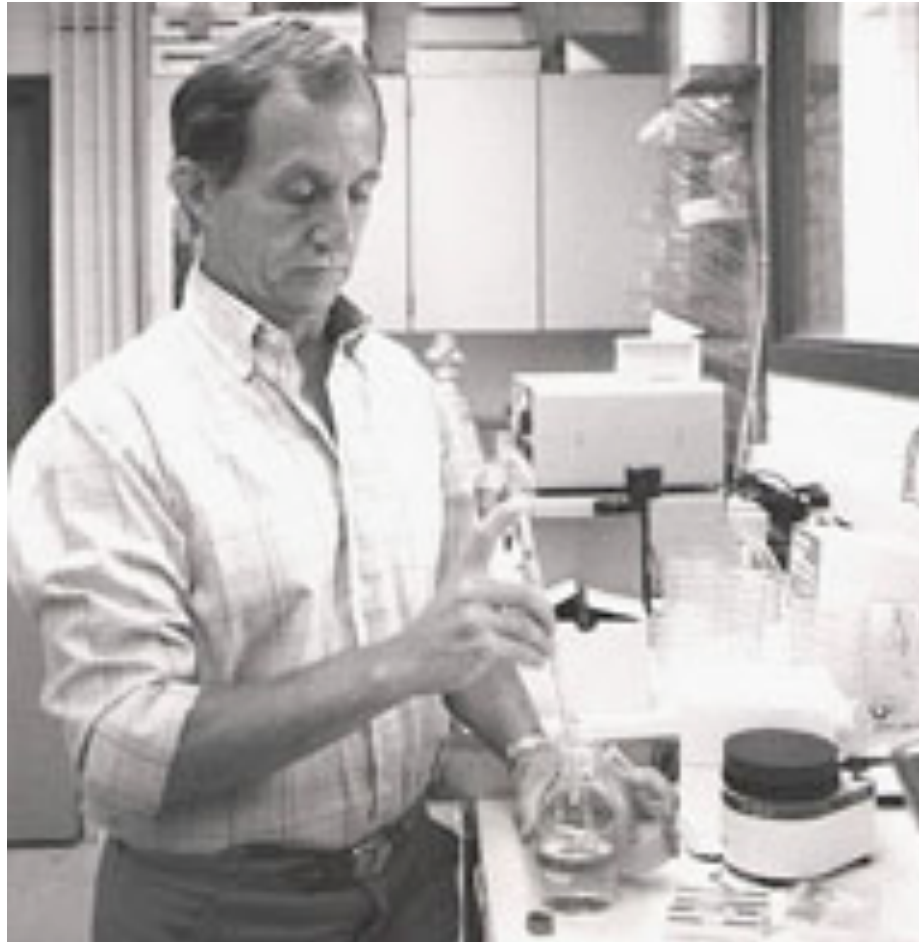


The new DNA gets integrated into the genome at a random location.

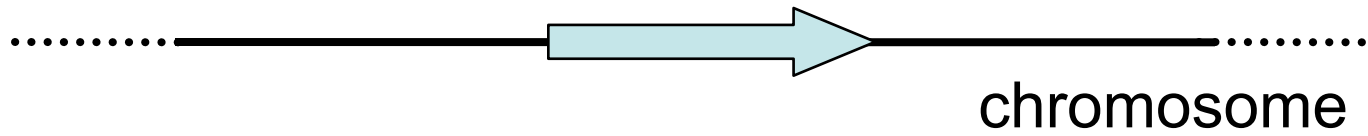
Information can only be added by this technique

That's how you add genes

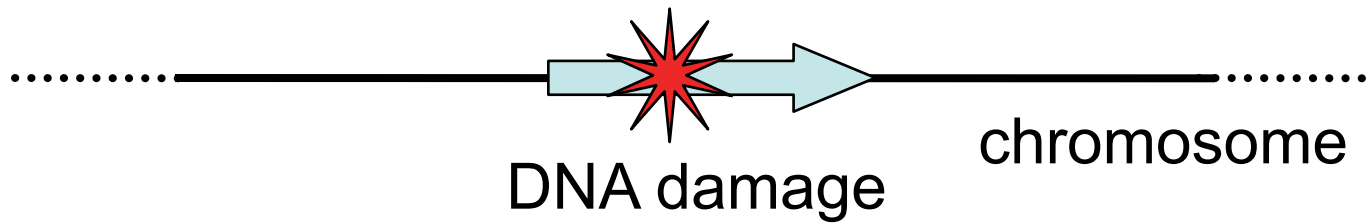
What if you want to take out a gene?



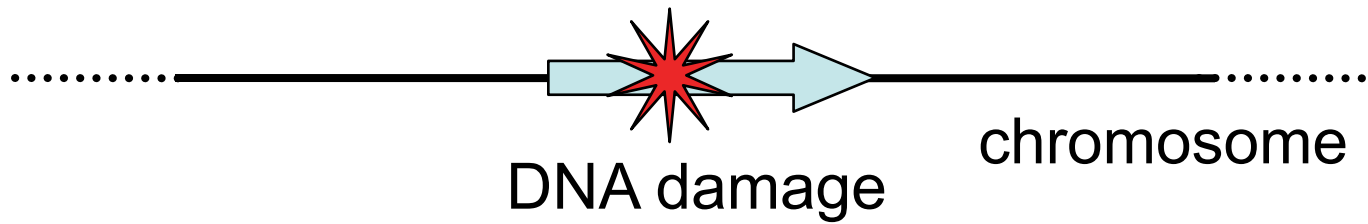
DNA repair



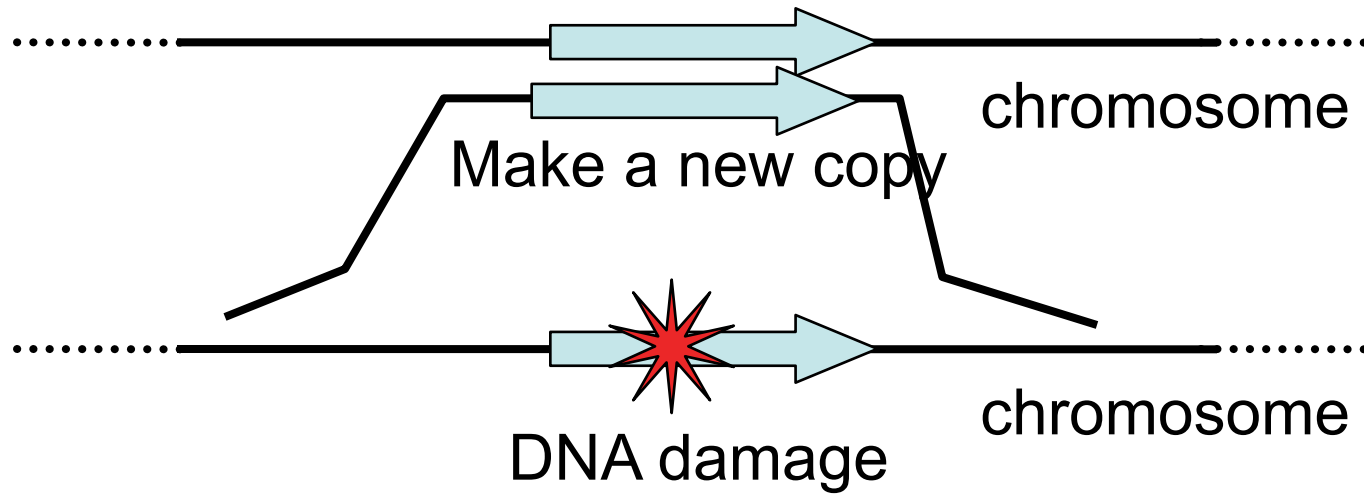
DNA repair



DNA repair



DNA repair

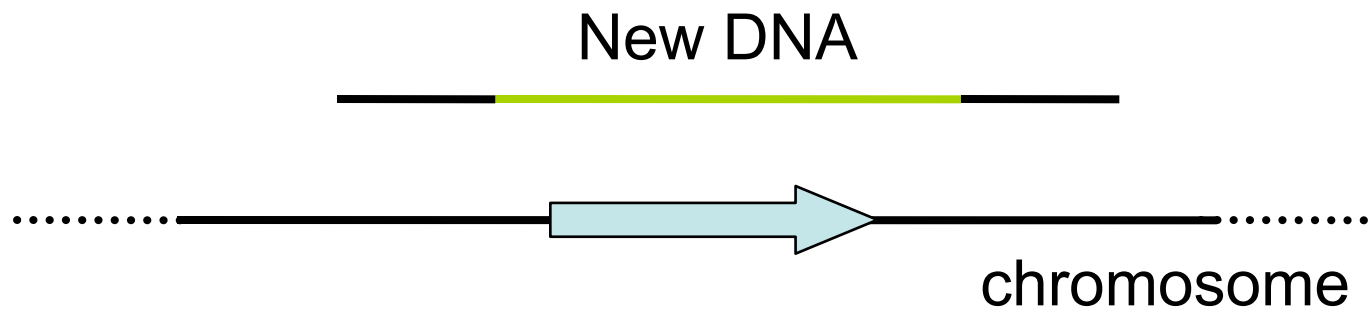


DNA repair

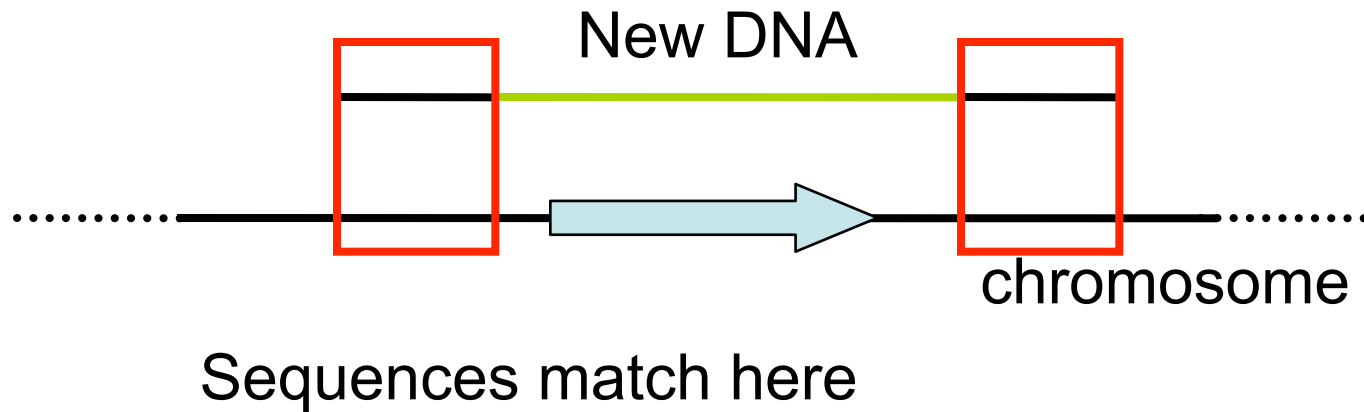


Replace damaged piece

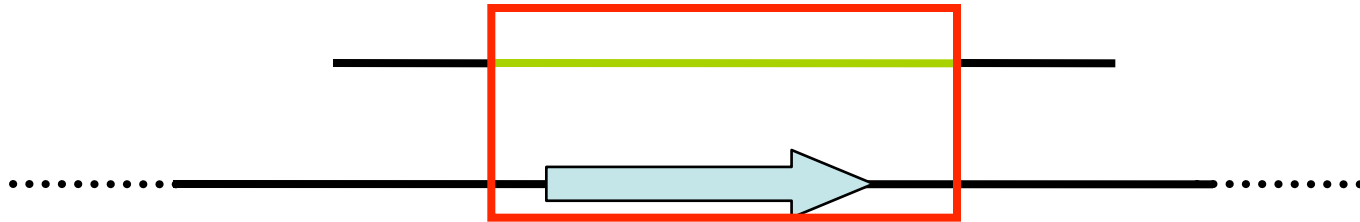
Knockout technology



Knockout technology

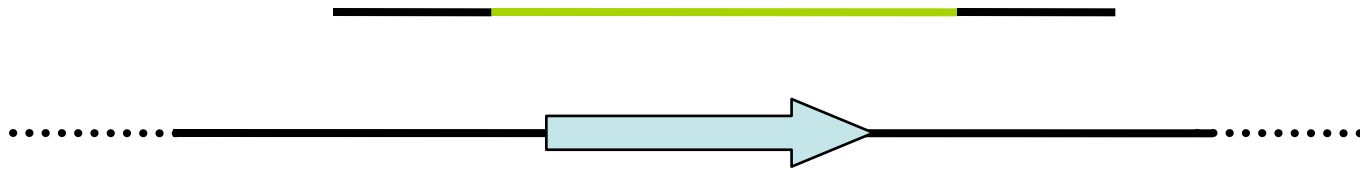


Knockout technology



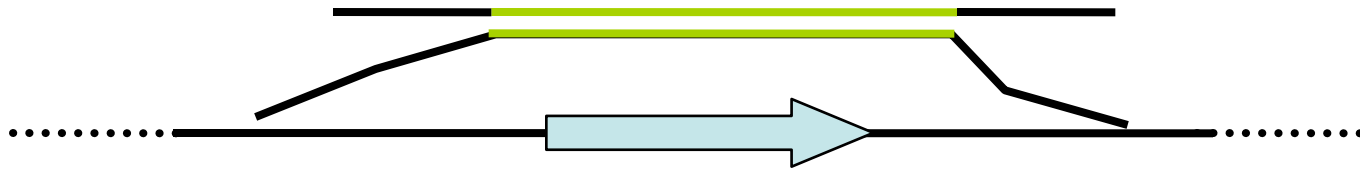
Sequences DON'T match here

Knockout technology



The cell will swap the pieces

Knockout technology



The cell will swap the pieces

Knockout technology



The cell will swap the pieces

This can add, delete or replace genes.

Knockout technology

The recombination reaction happens less than
1 in 10,000

How do we get this to work?

Knockout technology

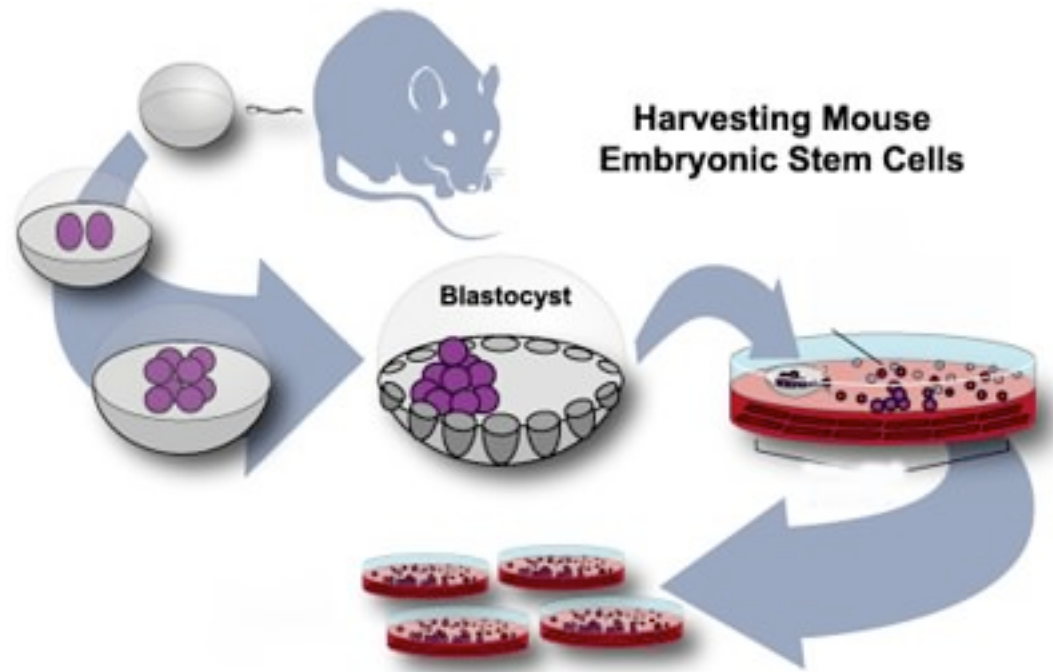
Embryonic stem cells

Knockout technology

Embryonic stem cells

What are ES cells?

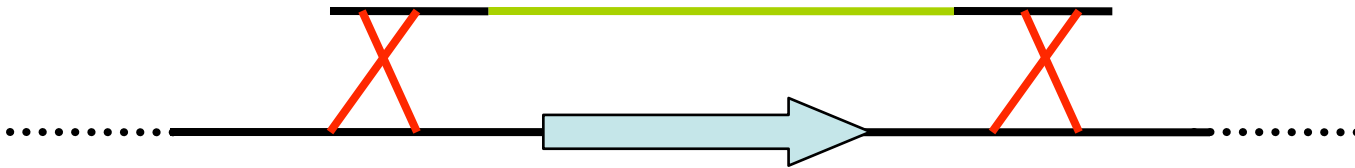
Knockout technology



http://www.openbiosystems.com/collateral/genomics/images/Cells%20and%20cell%20lines/ES_cell_isolation.jpg

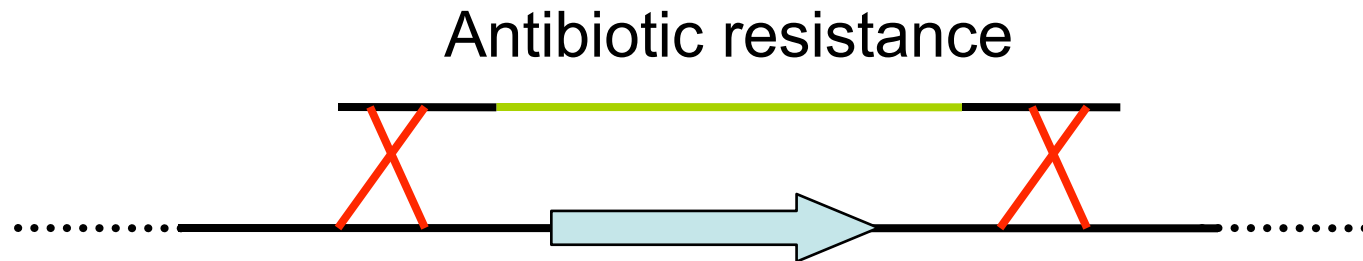
Knockout technology

How do we find the 1 in 10,000 cell?



Knockout technology

How do we find the 1 in 10,000 cell?



Only the one cell that got the new DNA will survive





Knockout technology

Now we have a cell with the new DNA.

How do we turn that cell into an animal?

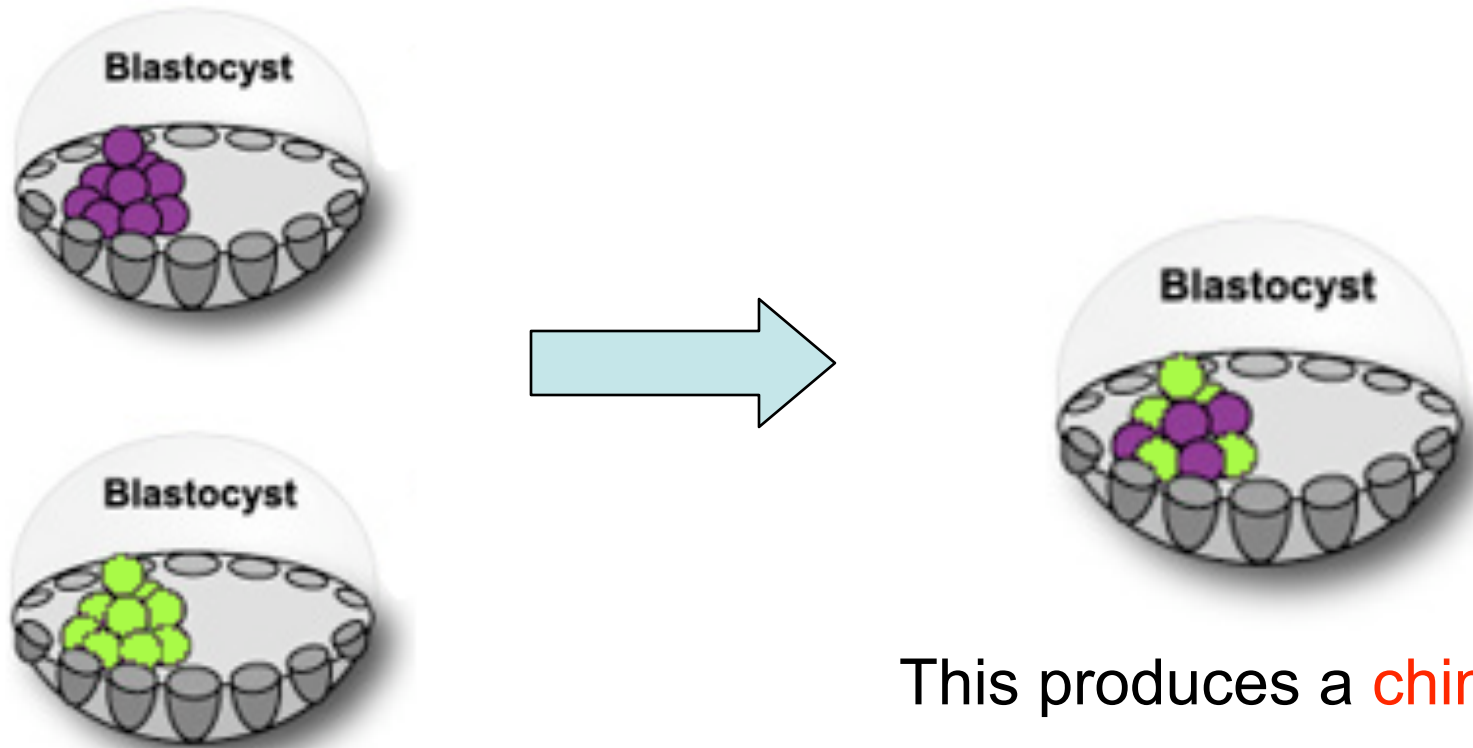
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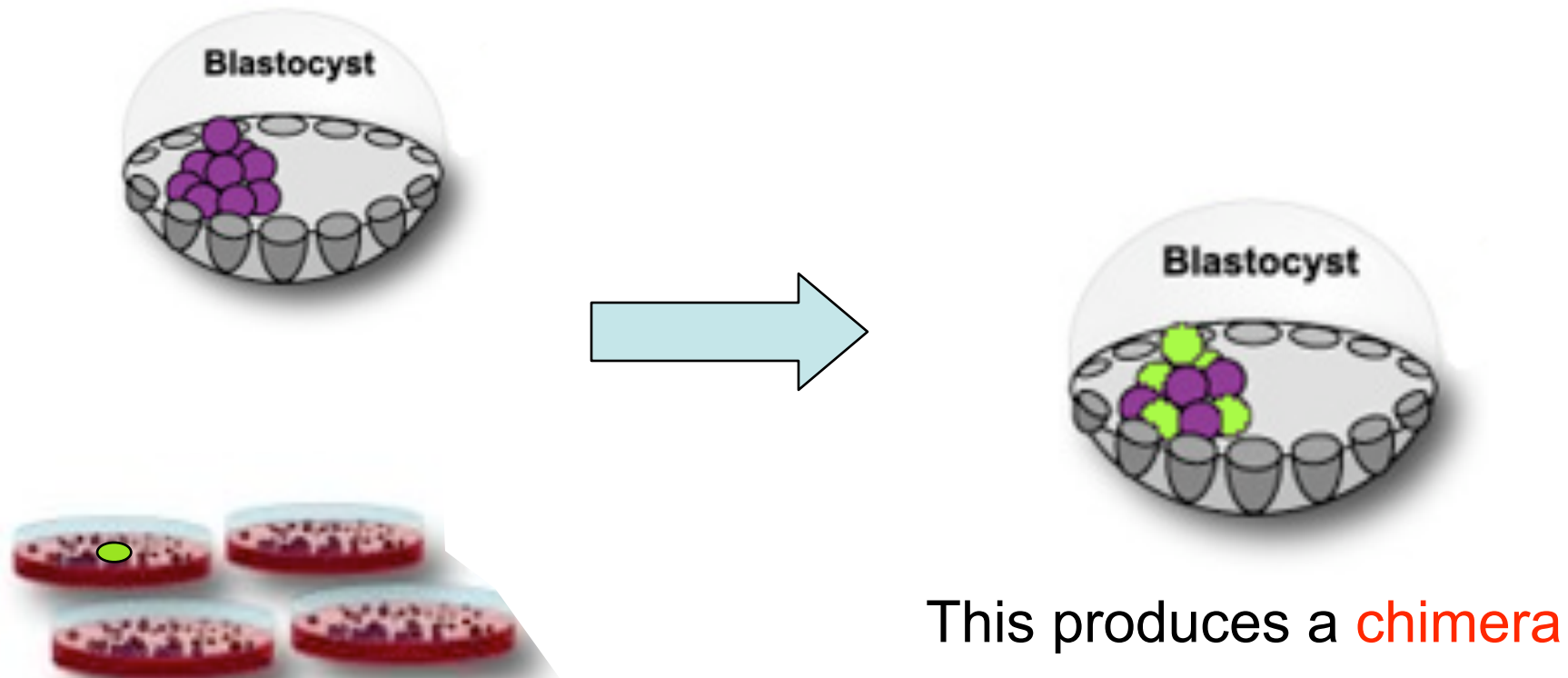
It's an embryonic stem cell

Cells from embryos can be mixed



This produces a **chimera**

Cells from embryos can be mixed



Chimeric mice



Summary

Transgenic animals can be made by injecting DNA into a fertilized egg.-DNA can only be added this way

Knockout technology can add, delete or replace genes

What can we do with
knockouts?

Knockout technology

Fel d1- is a protein made in cat skin

This single protein is the major allergen in cats

If you could knock-out the gene, you could make a hypoallergenic cat.

Allerca Inc. set out to do just that....



SUBSCRIBE TO
OUR NEWSLETTER 

ALLERCA has produced the world's first scientifically-proven hypoallergenic cats. These cats allow some of the millions of people with feline allergies to finally enjoy the love and companionship of a household pet without suffering from allergic symptoms.



CUSTOMER LOGIN

<http://www.allerca.com>:

Priced at US\$3,950*, the cost of an ALLERCA kitten is similar or less than some of the more exotic cat breeds available today. ALLERCA offers a number of payment options to facilitate the purchase of your ALLERCA GD kitten.

Organ Transplants

- Organ transplants can save lives (heart, lung, kidney, liver)
- Human organ donors are rare and often don't match tissue types.
- Organs from animals could solve the problem
 - Chimp
 - Baboon
 - Pigs

Organ Transplants

So far no animal to human transplant of a whole organ can be considered successful.

Basic problems

Chimps are endangered

Baboons work kind of, but are still hard to work with.

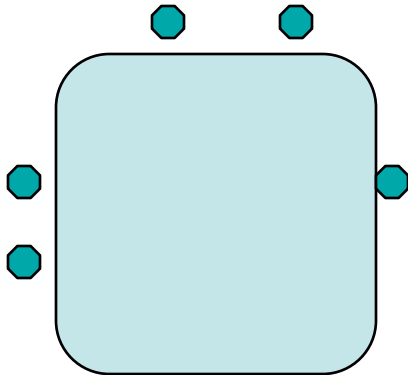
Pigs are easy and physiologically matched.

However: All animals except primates close to humans have a different sugar on their cells

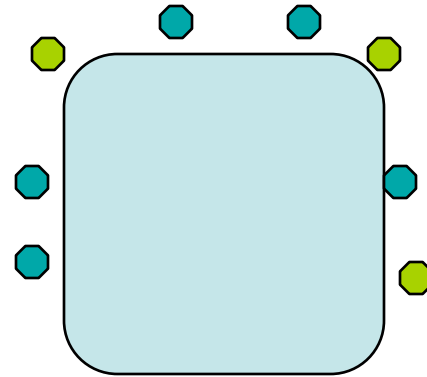
Similar but not the same as the B blood type.

Question

Why is it a problem that pig cells have a different sugar on their surface?



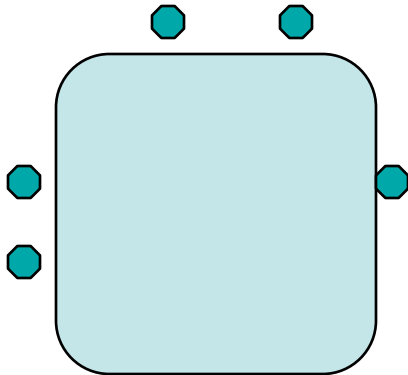
Human cell



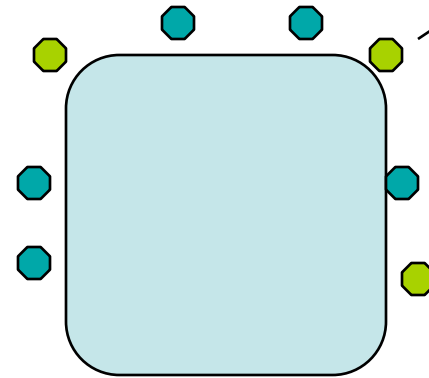
Pig cell

Question

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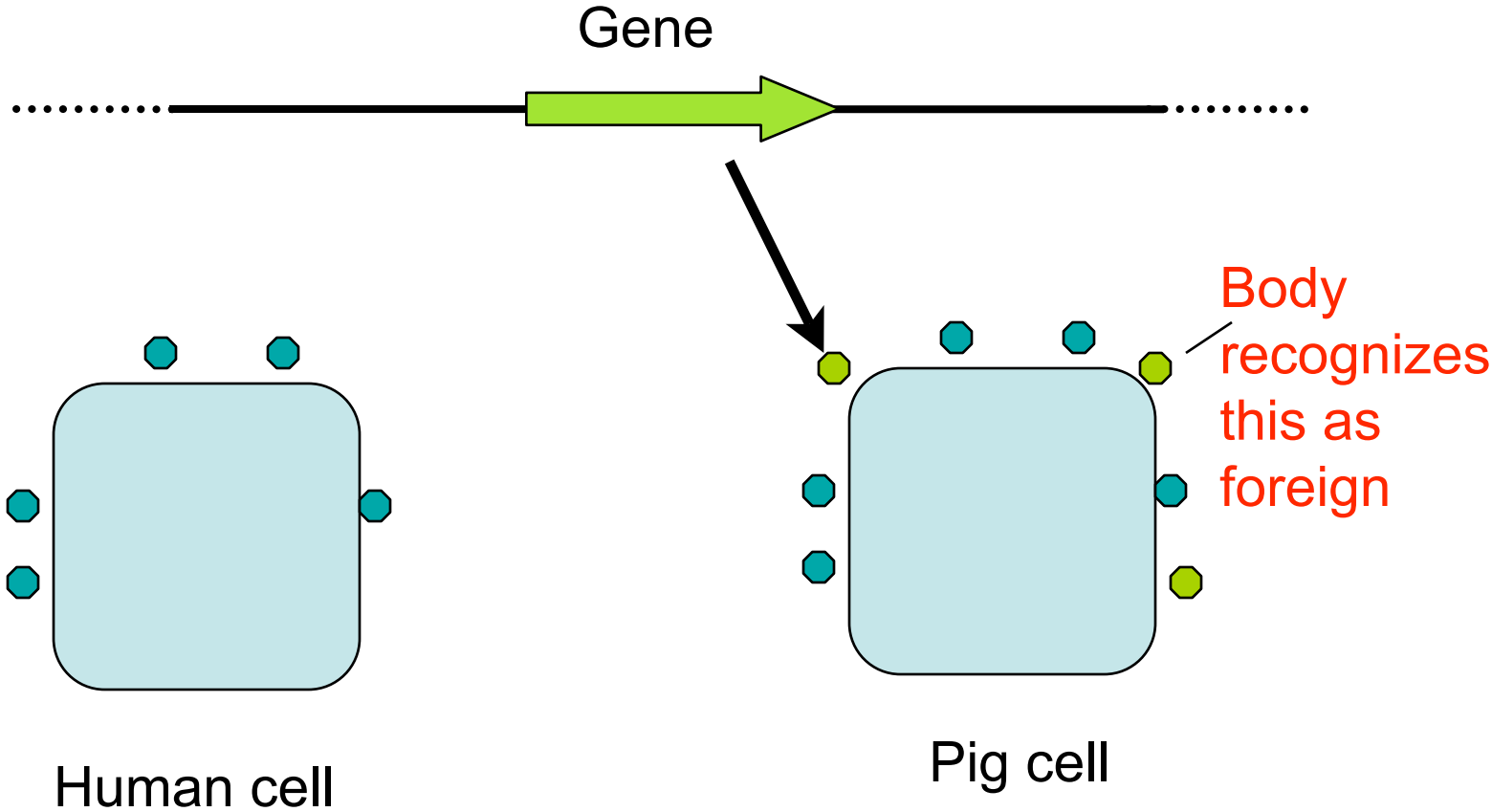
Human cell



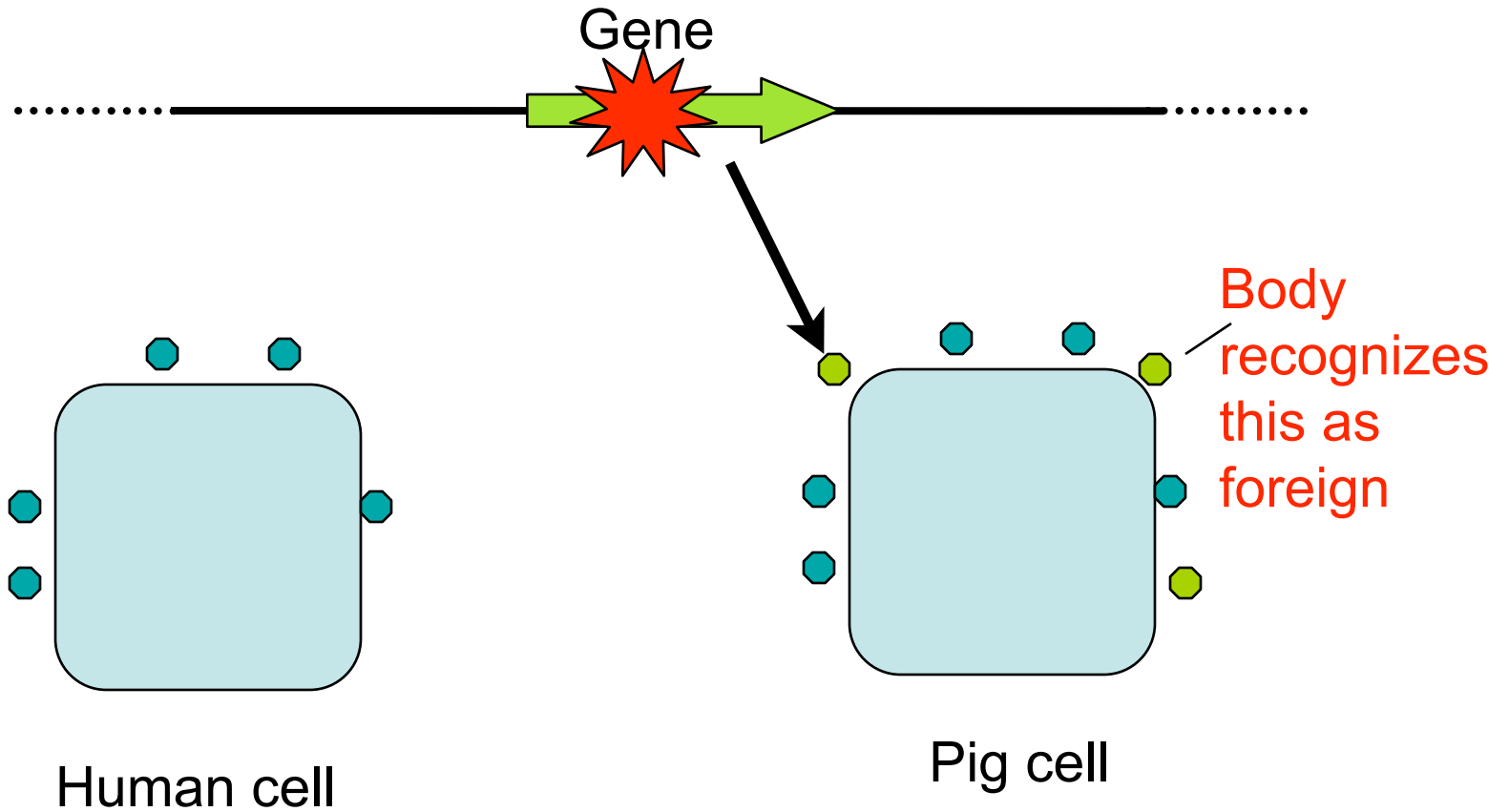
Pig cell

Body
recognizes
this as
foreign

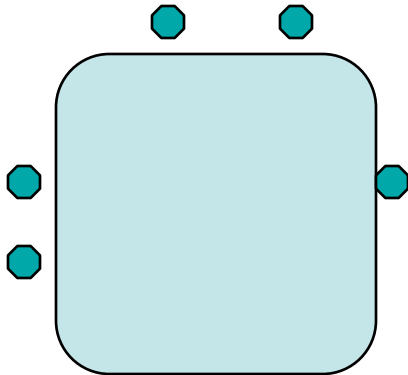
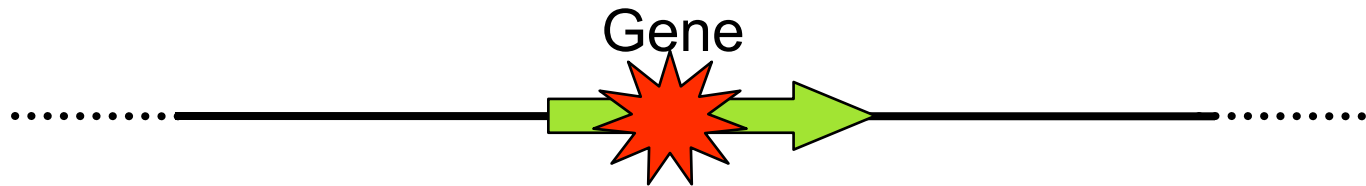
Question



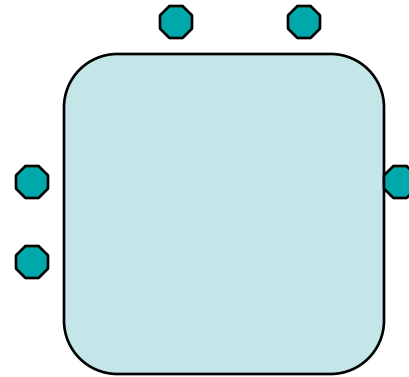
Question



Question



Human cell



Pig cell

Solutions?

- Nextran Inc in Princeton, New Jersey, a subsidiary of Baxter Healthcare;
- Imutran Ltd. of Cambridge U.K., a subsidiary of Novartis
- St. Vincent's Hospital (Melbourne) Ltd with Bresagen Ltd
- Alexion Pharmaceuticals, New Haven

Are researching triple transgenic pigs expressing various combinations of immune modifying and sugar modifying genes.

These still don't work in baboons quite yet- the organs last only a few weeks.

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Agrobacterium and gene gun methods for making transgenic plants.

Herbicide (roundup-ready)

Insect resistant (Bt) plants

Improved nutrition of plants (golden rice)