

Recombinant DNA  
Genetics and Society  
March 12, 2009

# Recombinant DNA

- Basic recombinant DNA
- Transgenic animals
- DNA Sequencing

# Complementarity is the secret of life.

- We can **read** it.
- We can **change** it.

# Basic recombinant DNA

# Recombinant

**Recombinant** DNA is DNA that is a mixture of DNA from multiple sources.

# Transgenic

A **transgenic** organism is one which contains a gene taken from another organism.

# Vector

A **vector** is an agent that carries DNA from one organism to another.

# Vector

Some examples:

Virus

Plasmid

# Vector

## **A vector must have:**

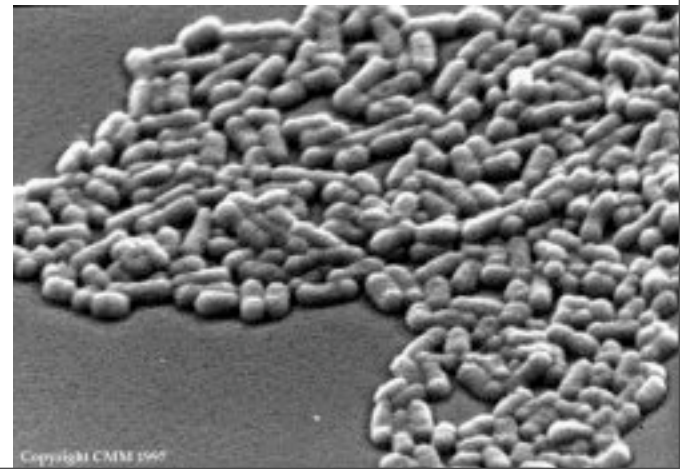
- A mechanism to get into the organism.
- A mechanism for reproducing (replicating).
- A way to know that the vector is present (this is called a **selectable marker**).

# *E. coli*

*E. coli* is a species of bacteria that live in human intestines.

Most humans have *E. coli* in their gut, however in very rare cases there are forms of *E. coli* that can cause illness or death.

Scientists use the non-dangerous forms, usually further mutated so that they can't escape into the wild.



# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

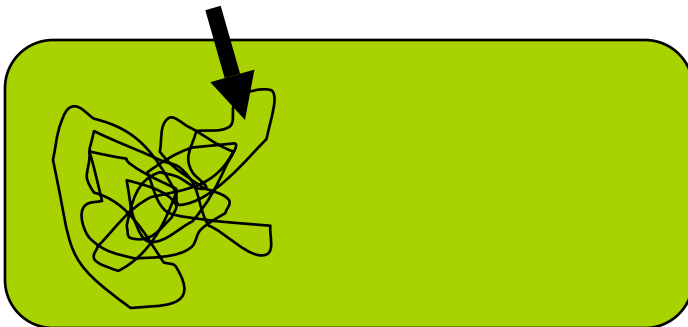


*E coli.*

# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

chromosome

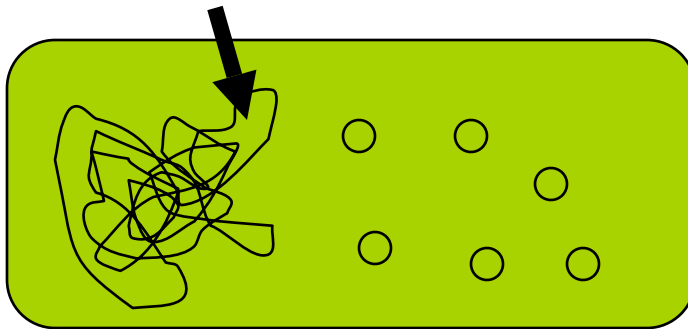


*E coli.*

# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

chromosome

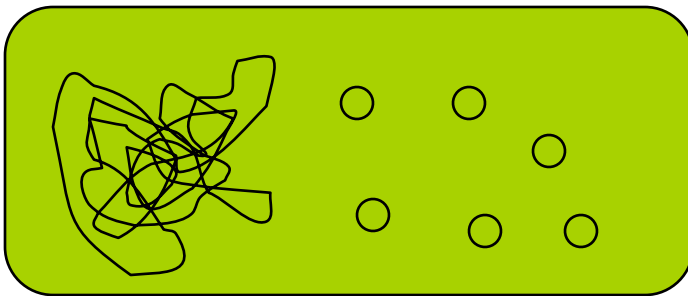


plasmids

*E coli.*

# Plasmids

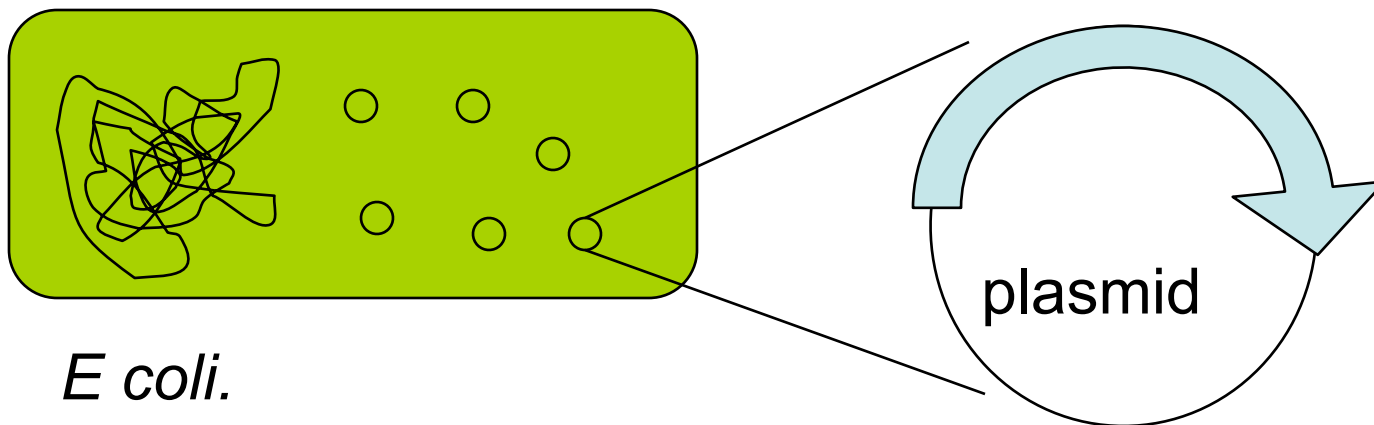
Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes



*E coli.*

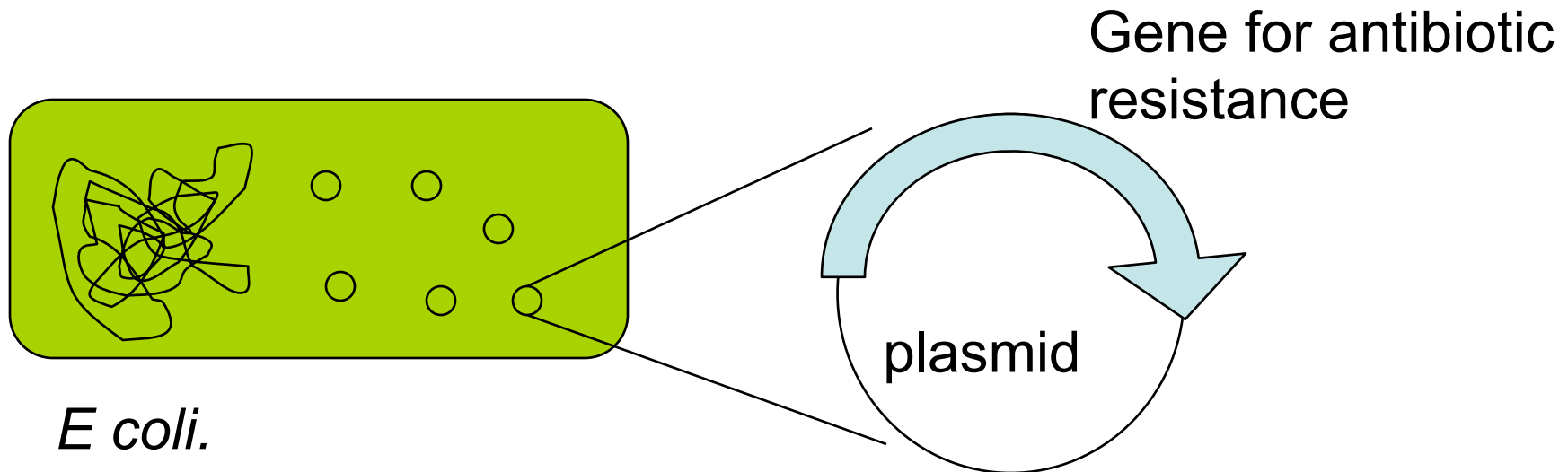
# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes



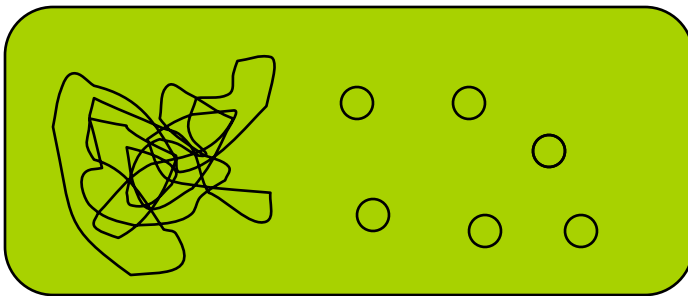
# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes



# Plasmids

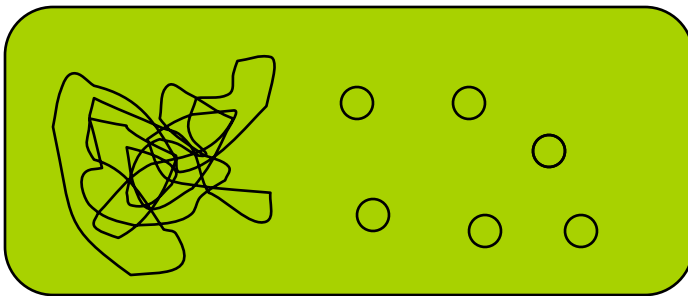
Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes



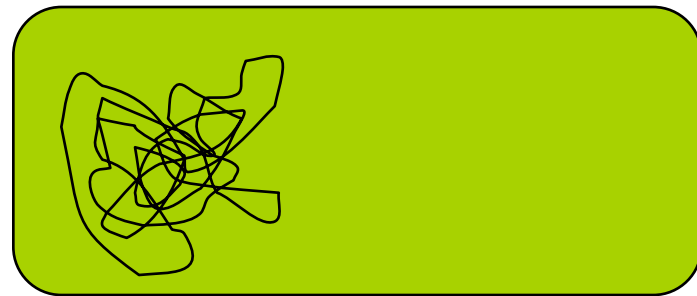
*E coli.*

# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

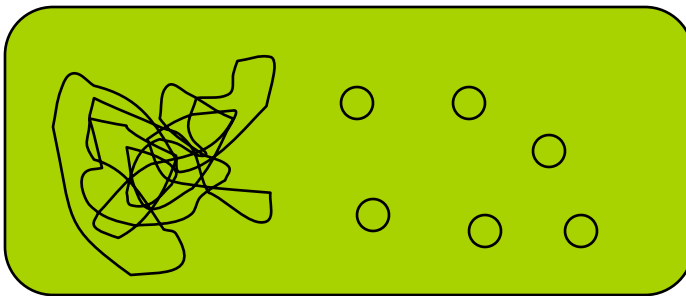


*E coli.*

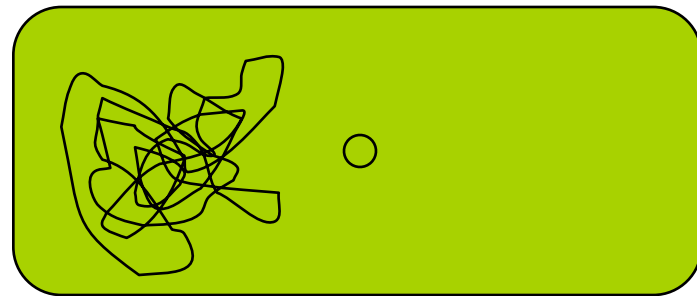


# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

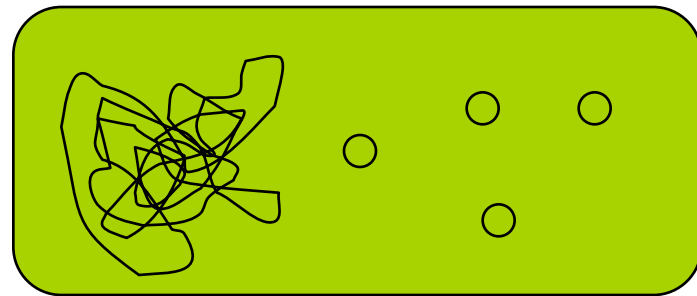
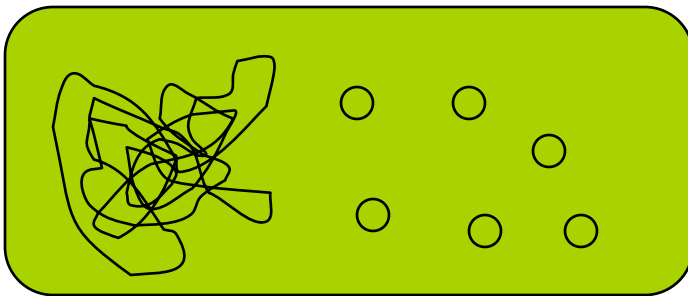


*E coli.*



# Plasmids

Plasmids are naturally occurring circular pieces of DNA  
In the wild they get into bacteria by natural competence.  
They carry antibiotic resistance genes

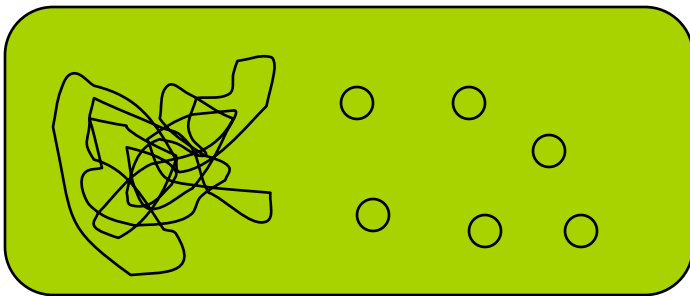


*E coli.*

# Plasmids

In the lab plasmids can be easily added to *E. coli*.

DNA from any other source can be added to the plasmid

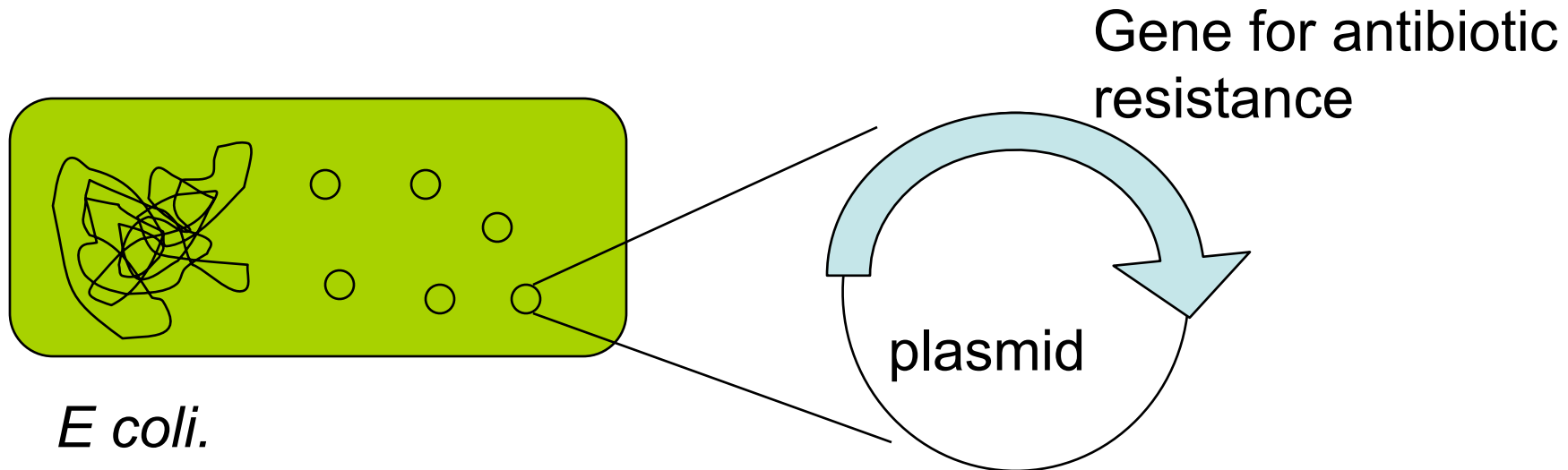


*E coli.*

# Plasmids

In the lab plasmids can be easily added to *E. coli*.

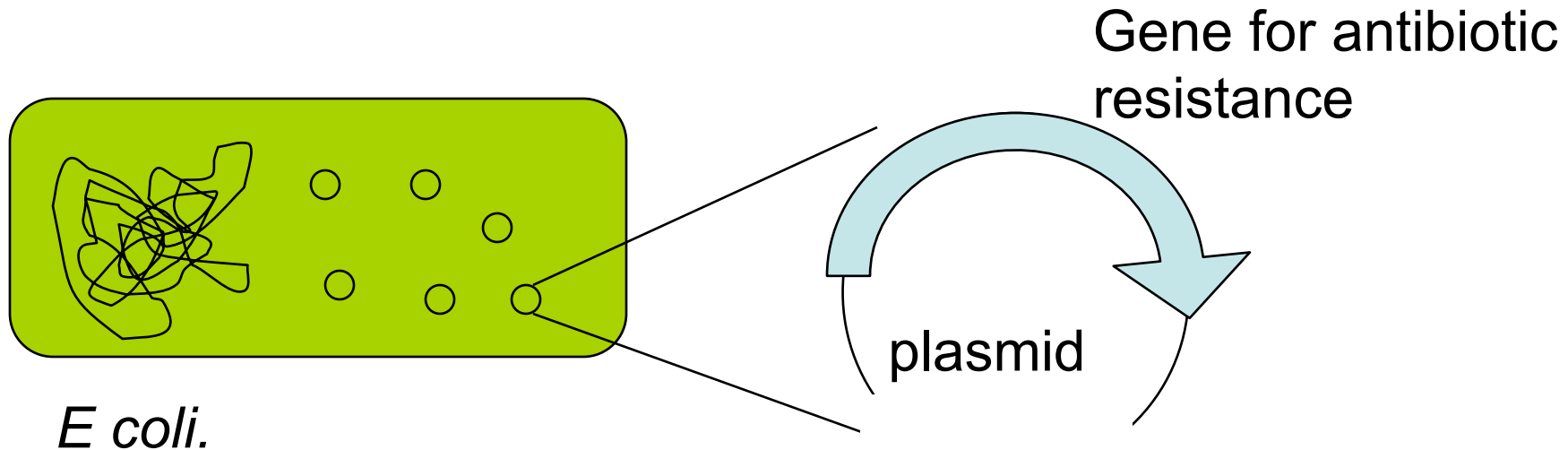
DNA from any other source can be added to the plasmid



# Plasmids

In the lab plasmids can be easily added to *E. coli*.

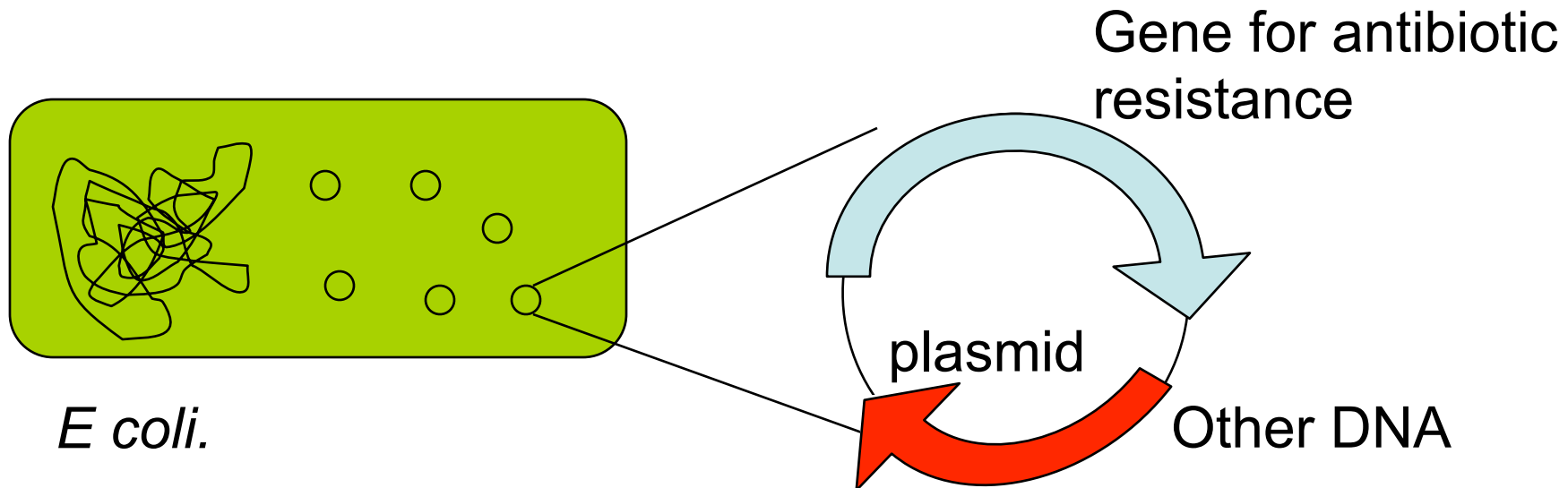
DNA from any other source can be added to the plasmid



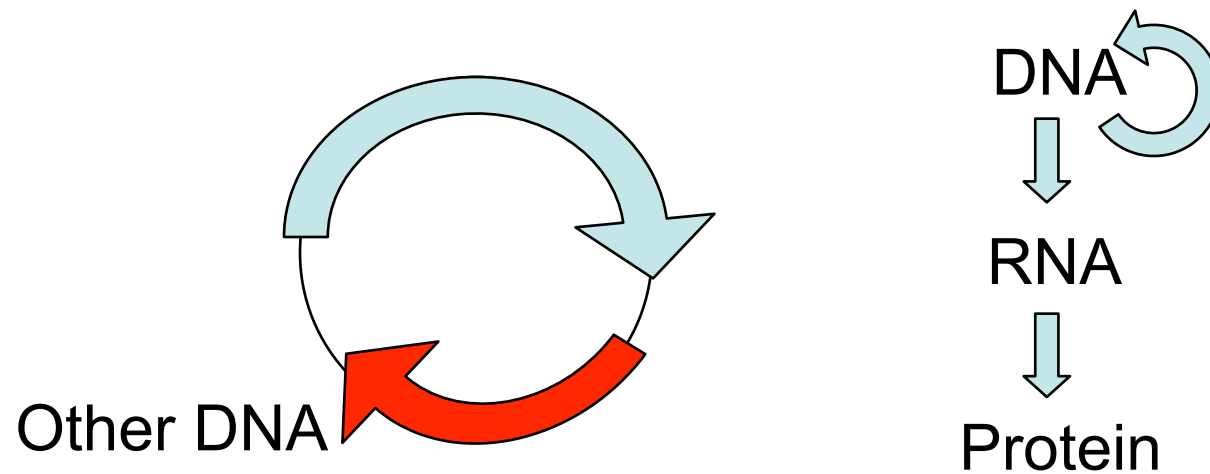
# Plasmids

In the lab plasmids can be easily added to *E. coli*.

DNA from any other source can be added to the plasmid

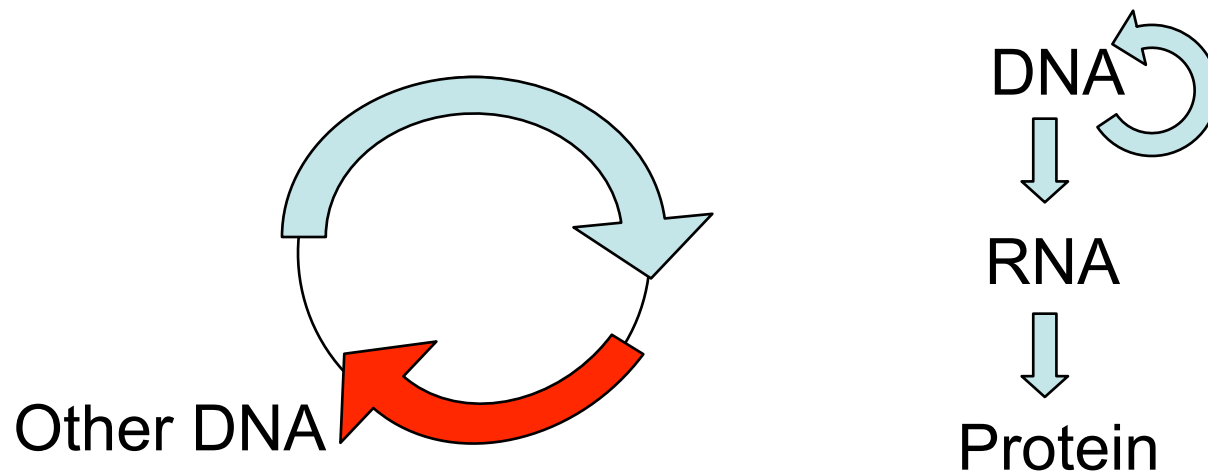


# What can we do with plasmids?



# What can we do with plasmids?

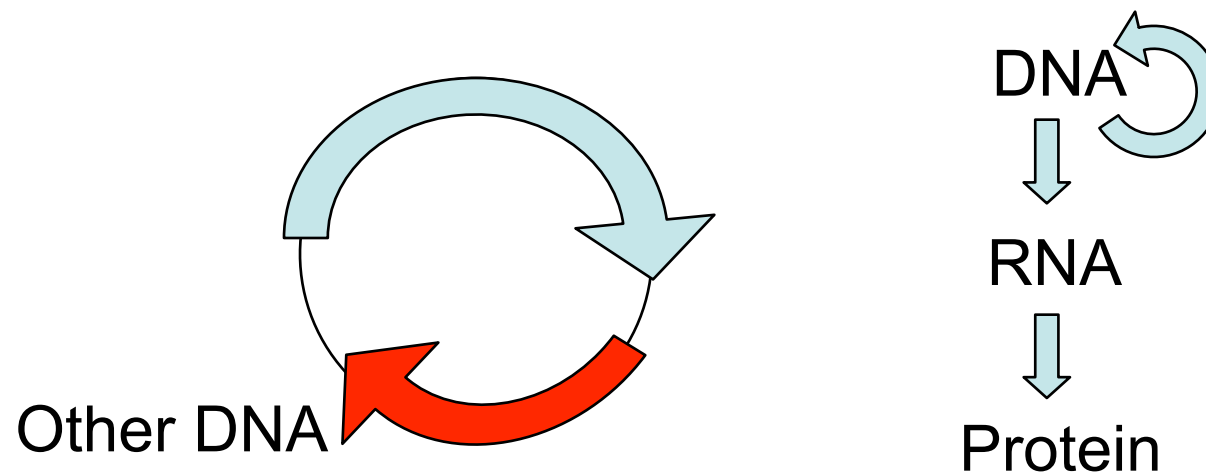
Use them to hold DNA



# What can we do with plasmids?

Use them to hold DNA

Use them to make proteins

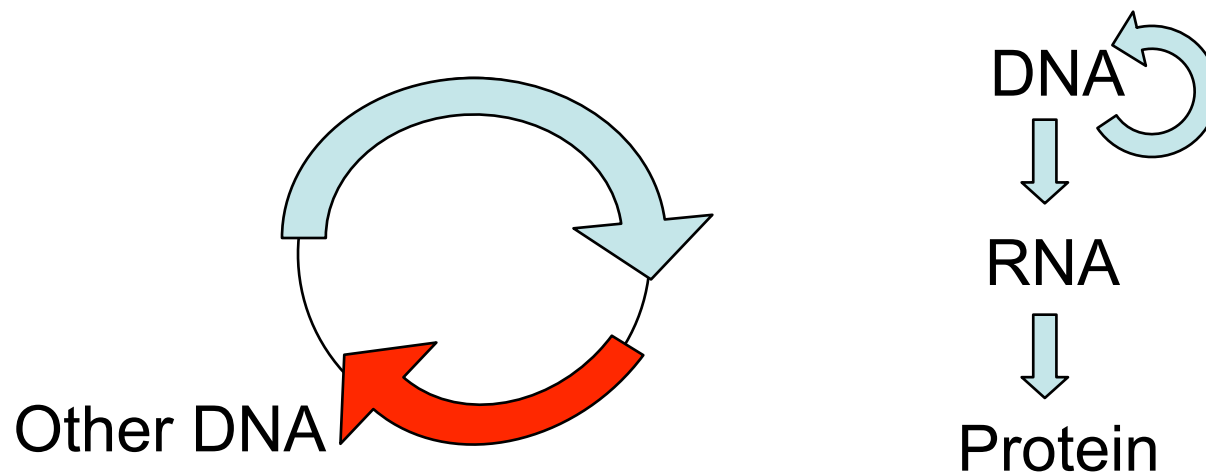


# What can we do with plasmids?

Use them to hold DNA

Use them to make proteins

Use them to make proteins that make drugs



# Recombinant protein

The first recombinant protein drug on the market was human insulin.

Prior to this, insulin had to be extracted from pigs at great cost, and with some risk to the patients.

Genentech released recombinant human insulin in 1982.

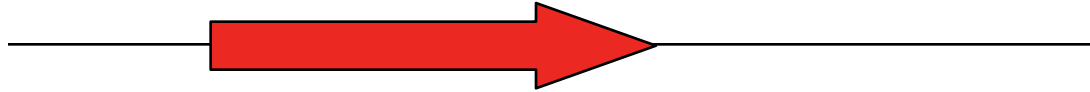
# Plasmids

Human DNA

---

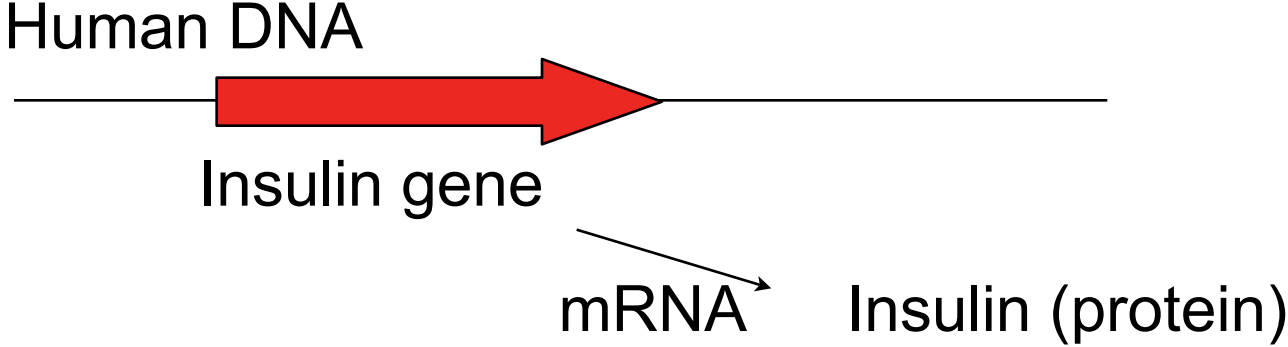
# Plasmids

Human DNA

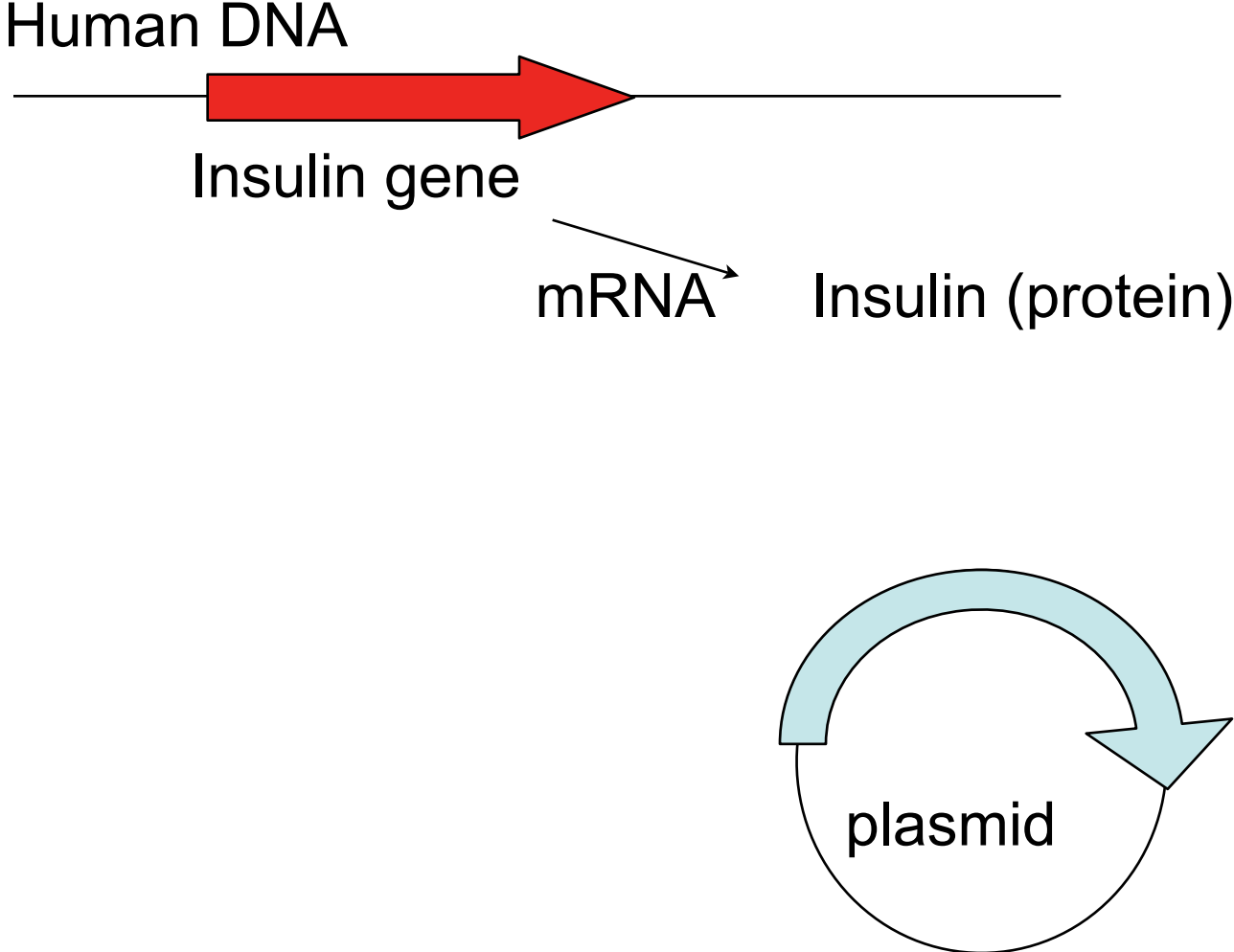


Insulin gene

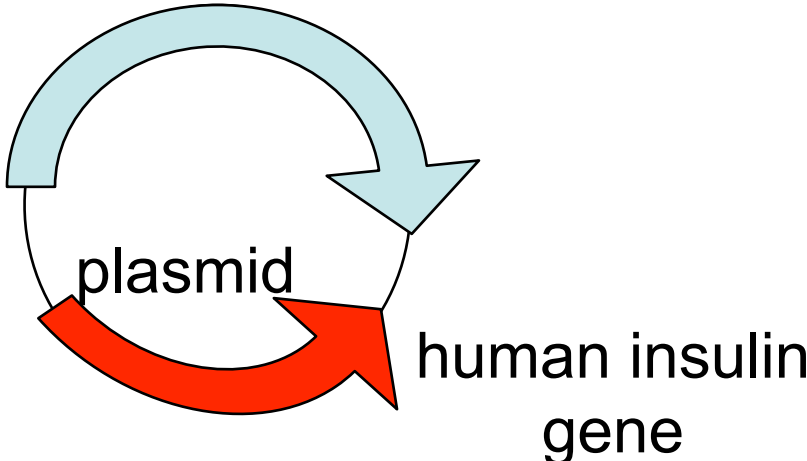
# Plasmids



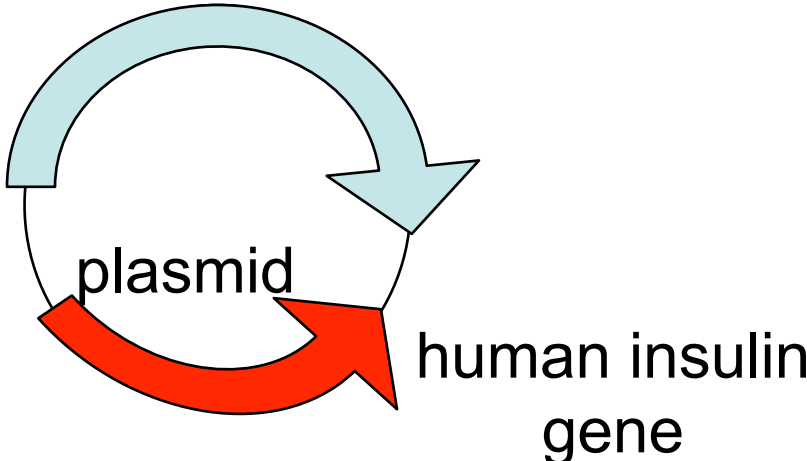
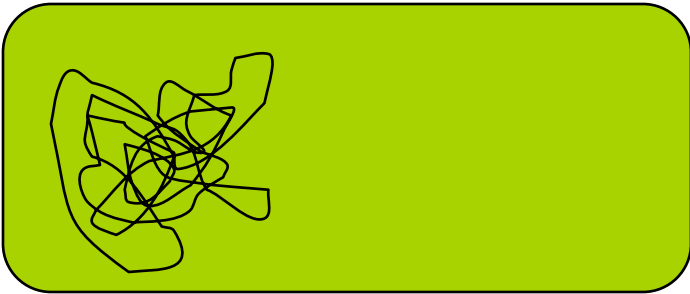
# Plasmids



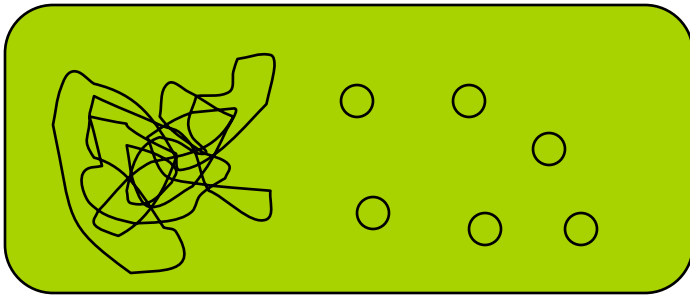
# Plasmids



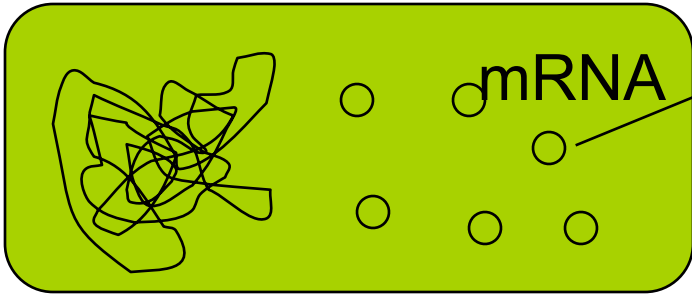
# Plasmids



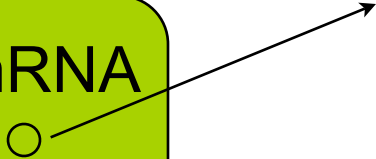
# Plasmids



# Plasmids



Insulin (protein)



# Some other recombinant drugs:

Blood clotting factors (hemophilia)

Interferon (leukemia)

vaccines

tPA (clot-buster, heart attacks and stroke)

Human growth hormone

# Growth hormone (humatrope)

In 2003 the FDA approved Eli Lilly's drug humatrope for use in children of short stature (shortest 1.2%).

This would correspond to heights of less than 5' 3" and 4' 11" in adult men and women, respectively.

Drug trials showed increases of 3" in final height.

Estimated costs: \$20-40K per year.

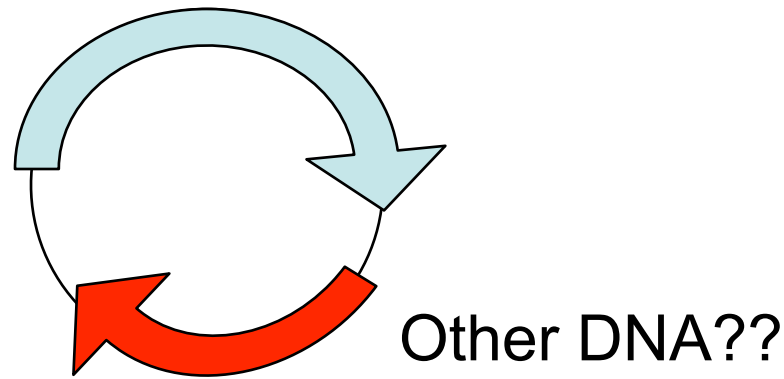
# Summary

**Plasmids** can be used to hold DNA in bacteria.

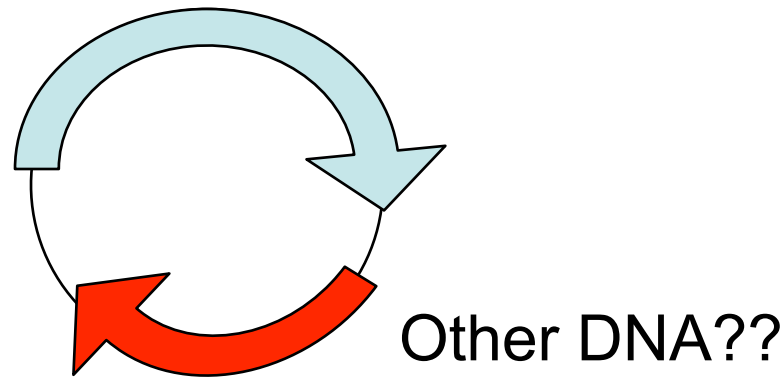
**Recombinant DNA** is a combination of DNA from multiple sources.

**Human proteins can be made in bacteria, and many other organisms**

# How do we get DNA from organisms?



# How do we get DNA from organisms?



If you can't get enough tissue sample to grind up, you can use PCR

# Other transgenic organisms used to make proteins

Yeast

Plants

Dairy animals

Human cancer cells, grown in a dish

# Transgenic animals

# Simple transgenes in mice

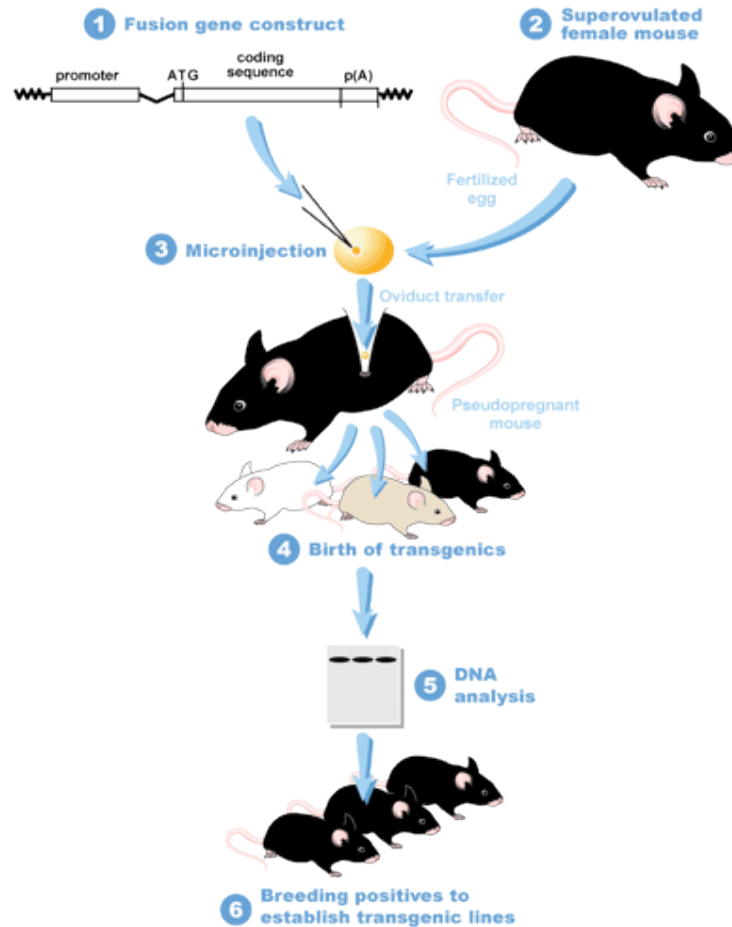


Plain DNA can be directly injected into *in vitro* fertilized eggs.

This is called **pronuclear injection.**

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

# Simple transgenes in mice



# Simple transgenes in mice

New DNA



chromosome

# Simple transgenes in mice



# Simple transgenes in mice



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

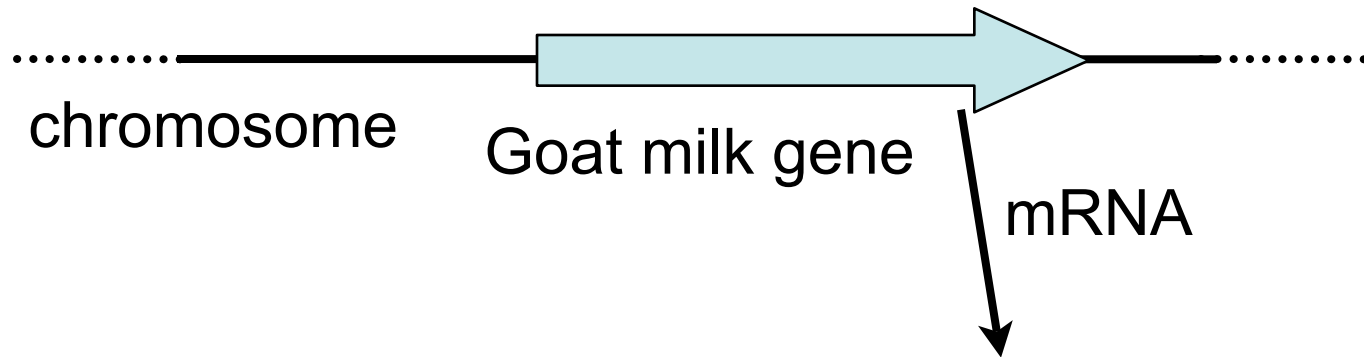
Information can only be added by this technique

How can we use this?

# Dairy transgenes



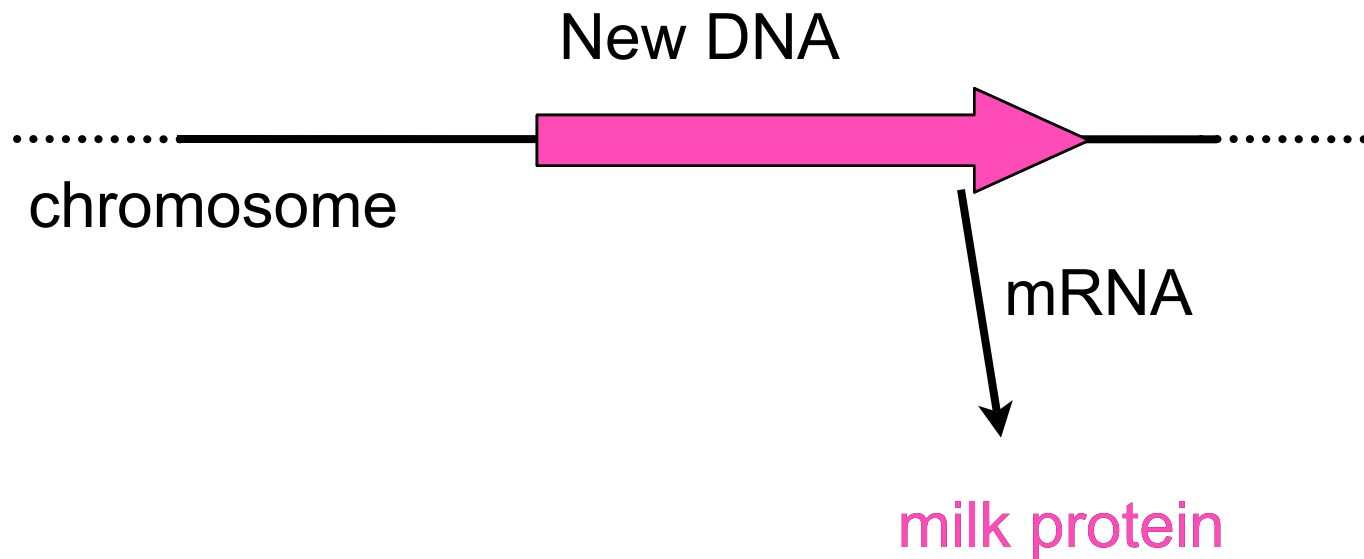
# Dairy transgenes



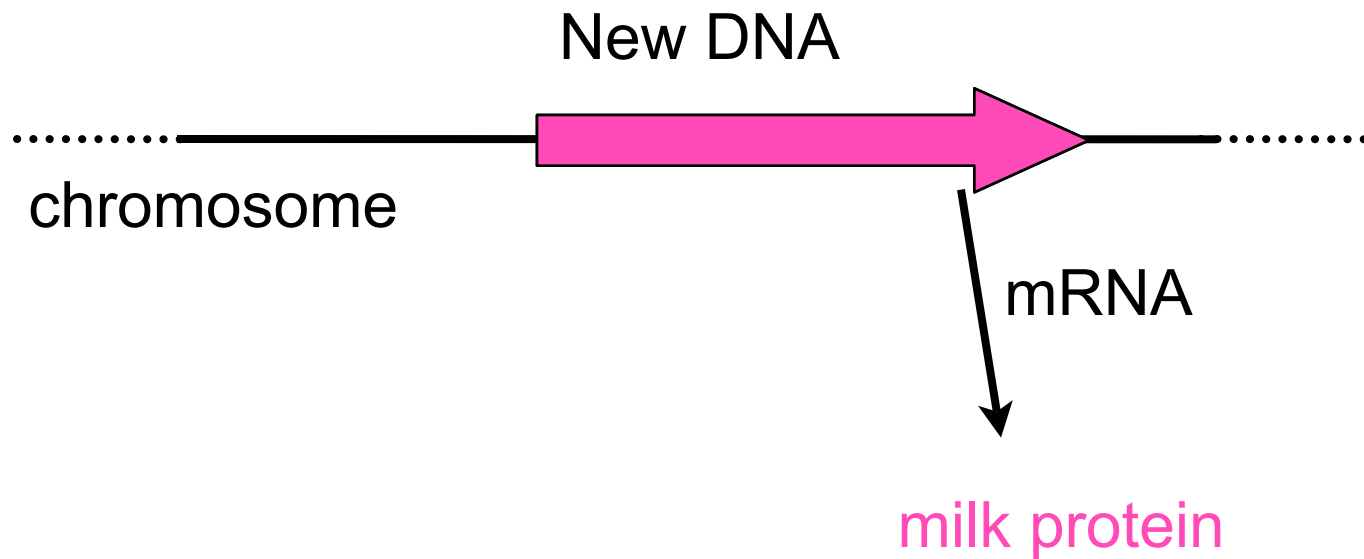
# Dairy transgenes



# Dairy transgenes



# Dairy transgenes



# Dairy animals

- Antithrombin is a protein produced in the blood that prevents blood from clotting
- Some people lack the AT gene
- ATyrn is a transgenically produced form of AT made in goats.
- GTC Biotherapeutics got European approval in 2006, FDA approved 2009

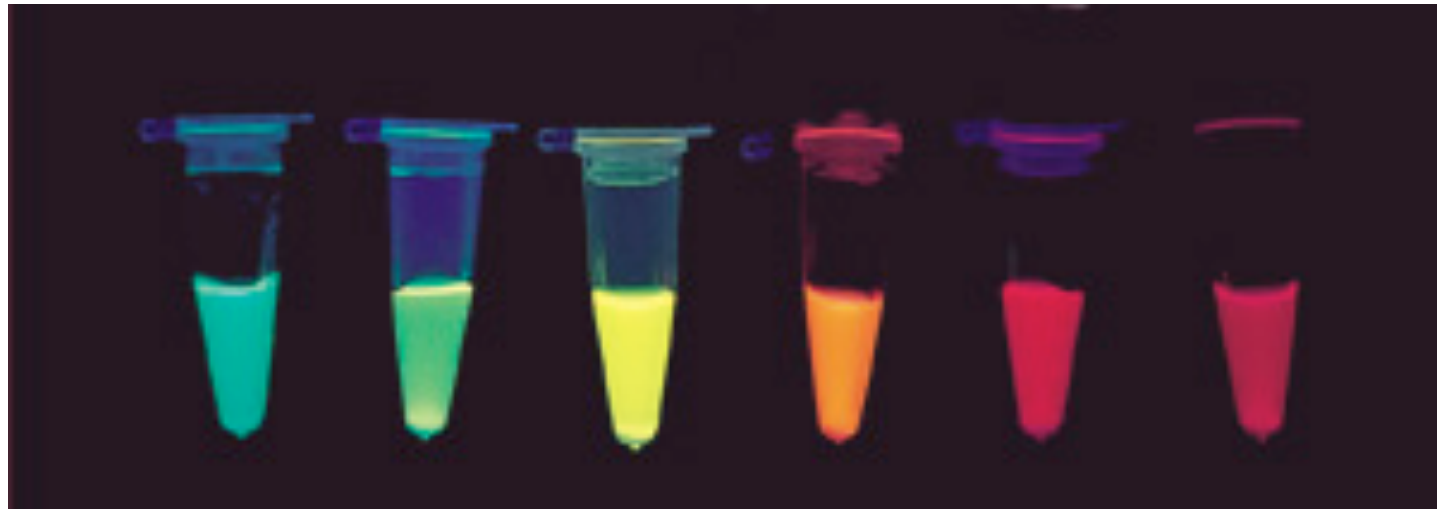
# Green Fluorescent Protein (GFP)



[http://en.wikipedia.org/wiki/Aequorea\\_victoria](http://en.wikipedia.org/wiki/Aequorea_victoria)

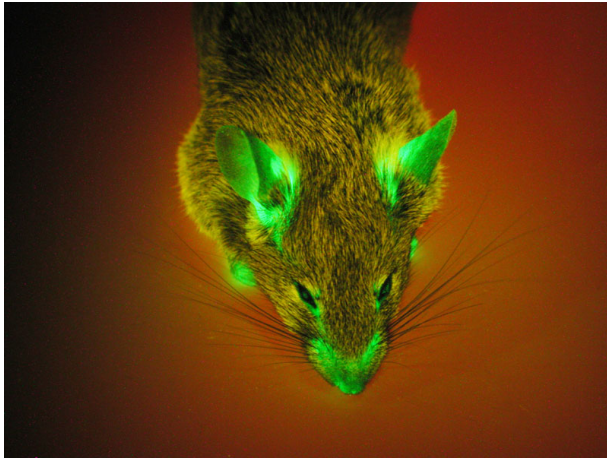
Aequorea victoria jellyfish from the pacific northwest

# GFP comes in other colors

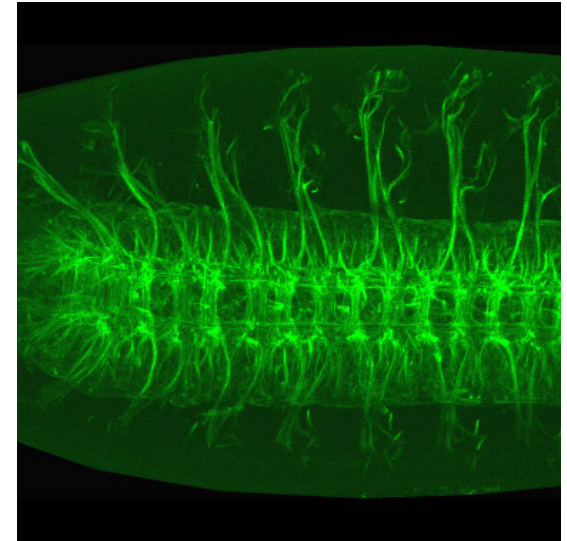


<http://www.clontech.com/clontech/gfp/index.shtml>

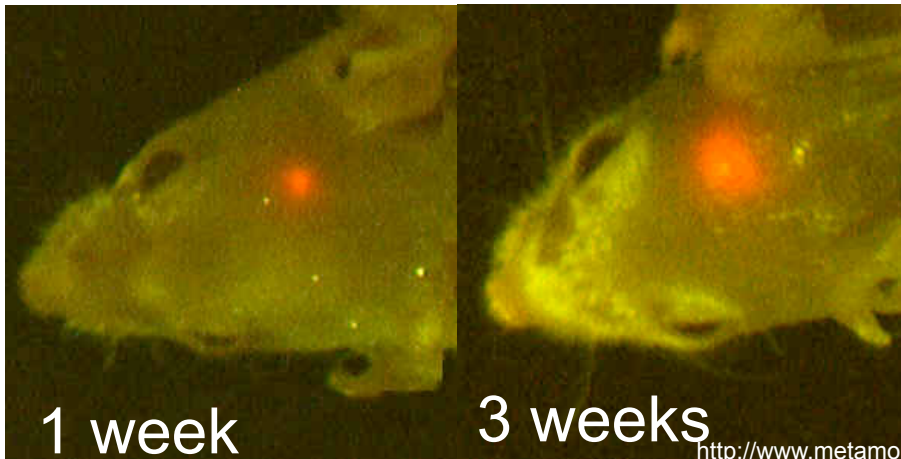
# GFP can be used in research



<http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE%20-%20GFP%20Mouse%20-%20800X600.jpg>



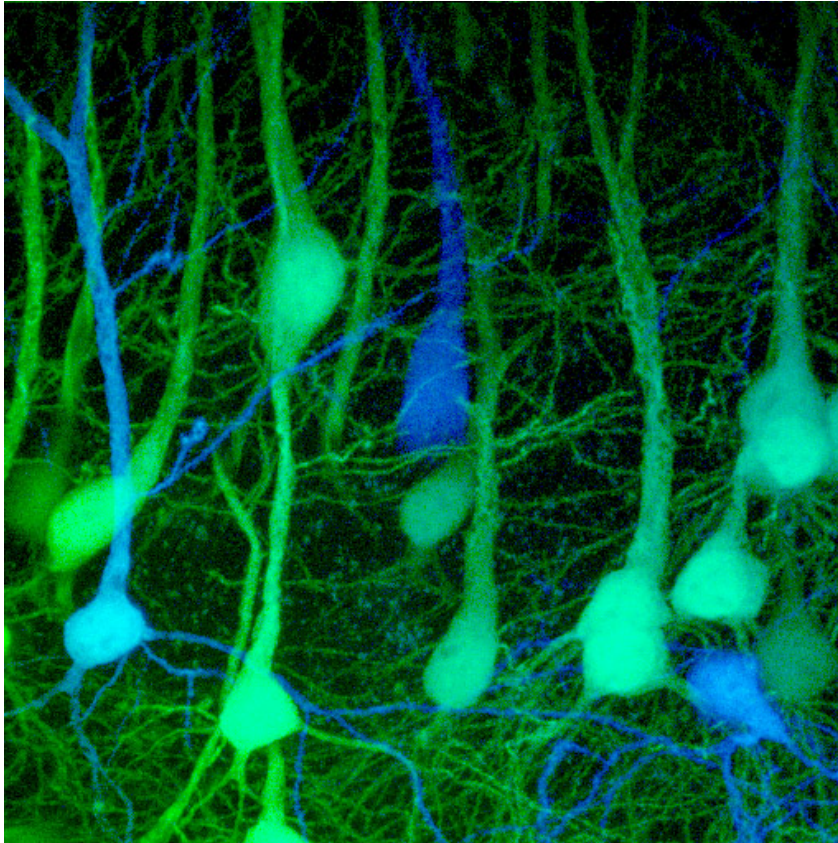
<http://microscopy.bio-rad.com/moviesandimages/Multiphoton.htm>



Red-fluorescent brain tumor cells were injected into a mouse, where they grew into a brain tumor.

<http://www.metamou>

# Different colors can be used to mark different cells



# GFP pets



<http://zoologicalsciences.com/>



<http://www.azoo.com.tw/>

Taiwaneese company Taikong: Fluorescent Fish has become the representative of Taikong's achievement of Bio-Technologies. Since the first Fully-Fluorescent Transgenic fish announced in 2001, there are 10 more new species Fluorescent Fish be announced continuously. In 2005, Taikong will announce 5 new species Fluorescent Fish at the same time. They are TK1 diamond series – Emerald night Pearl, Ruby night Pearl, Golden Night pearl and TK2 Platinum series – Platinum red Leopard and Platinum green Leopard.

<http://www.azoo.com.tw/>

# DNA Sequencing

# How does DNA sequencing work?

GGATCTCCTCTGTATGAACGCGG  
DNA



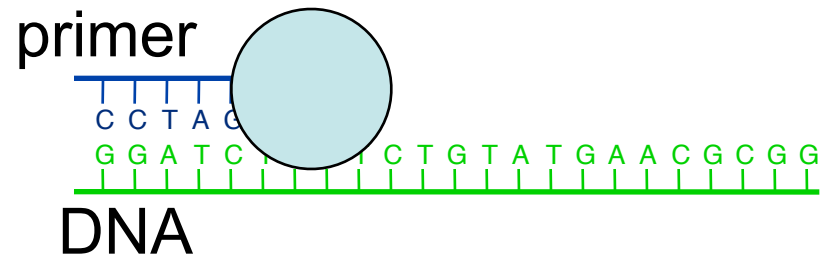
# How does DNA sequencing work?

primer

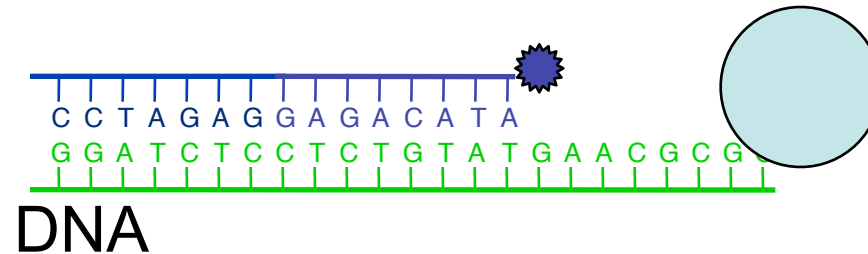


DNA

# How does DNA sequencing work?

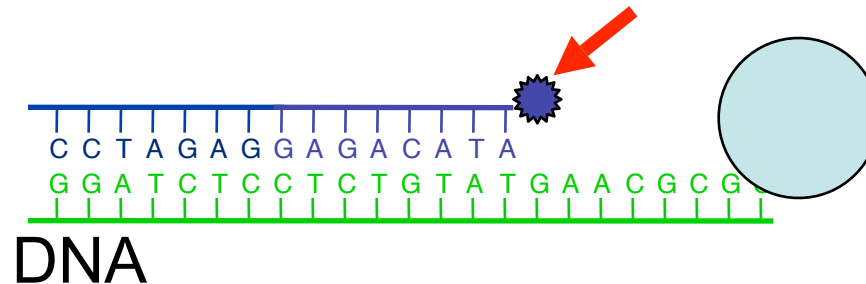


# How does DNA sequencing work?

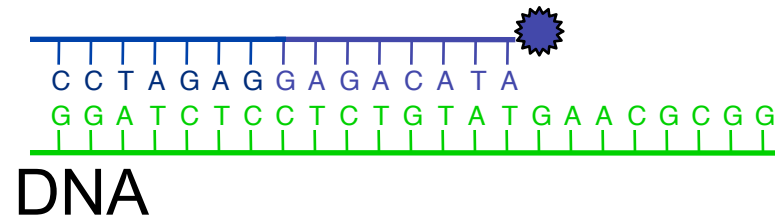


# How does DNA sequencing work?

This base has been dyed blue.  
It can't be added to.



# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?

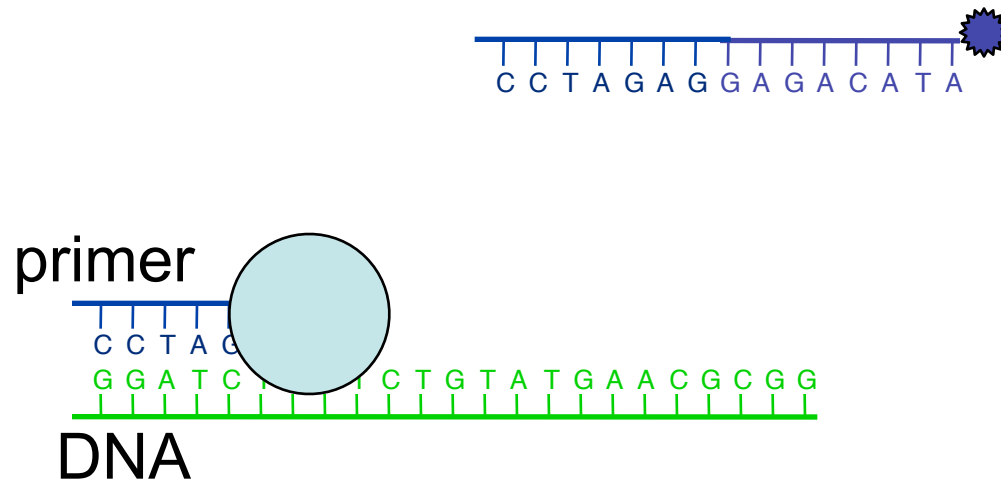


primer

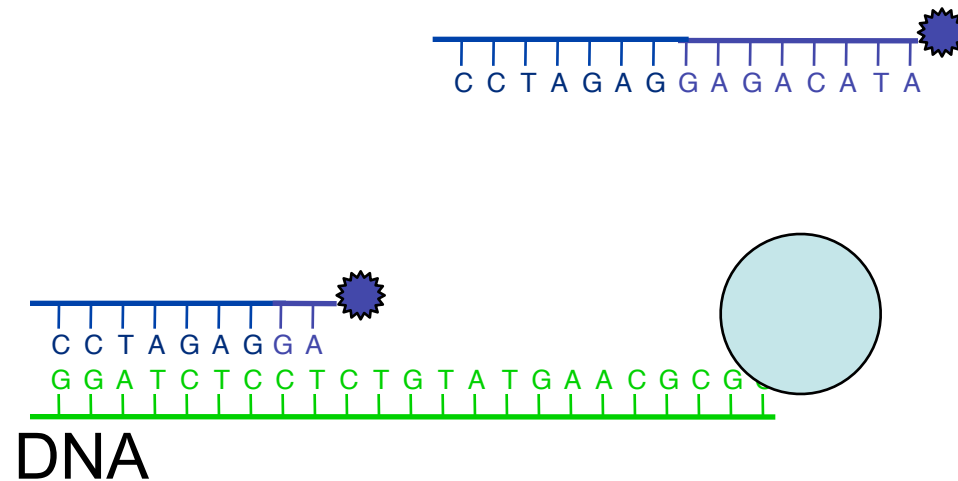


DNA

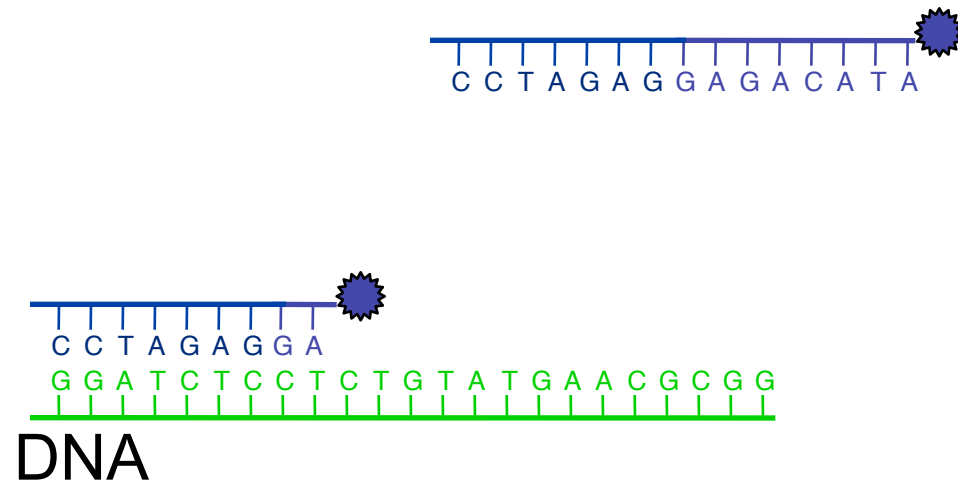
# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?

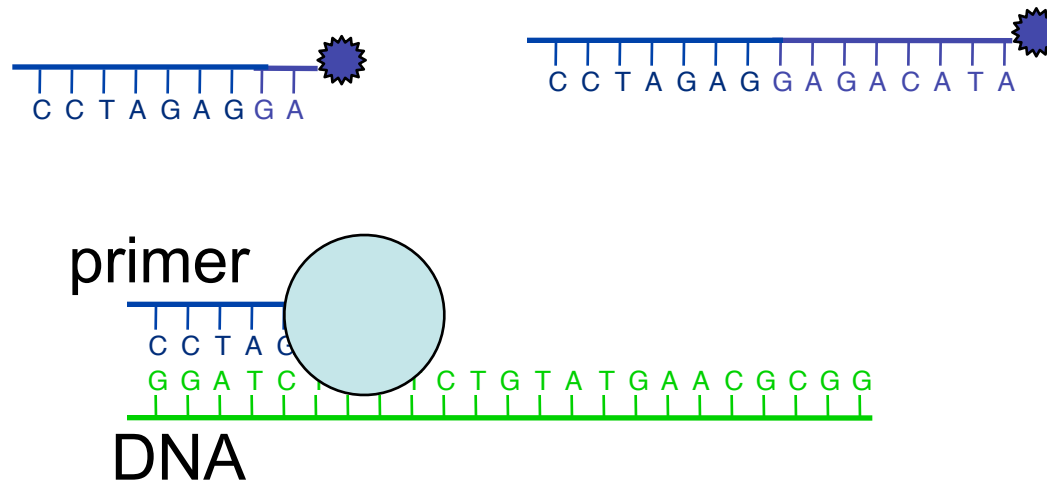


primer

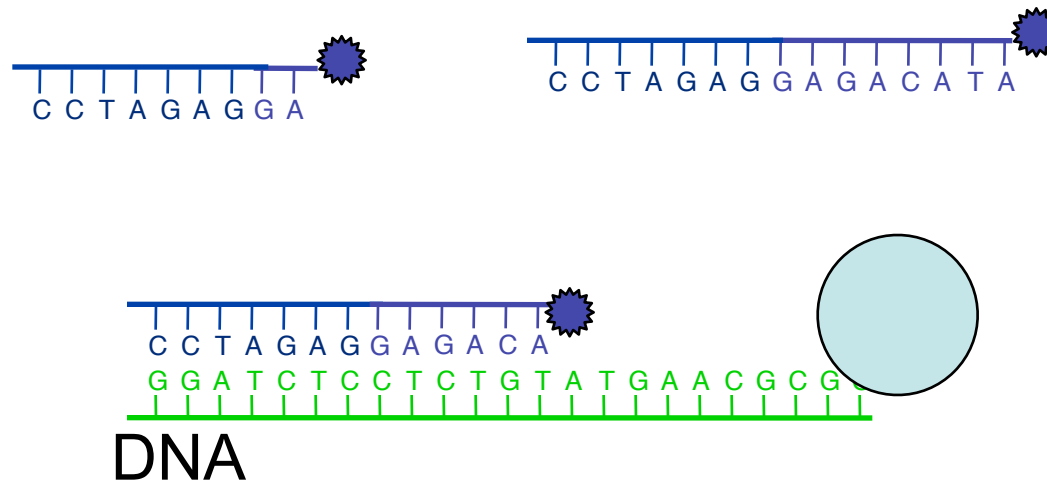


DNA

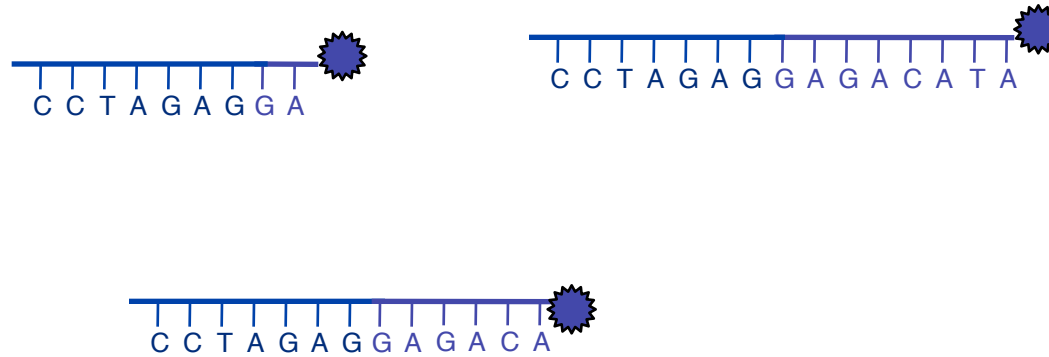
# How does DNA sequencing work?



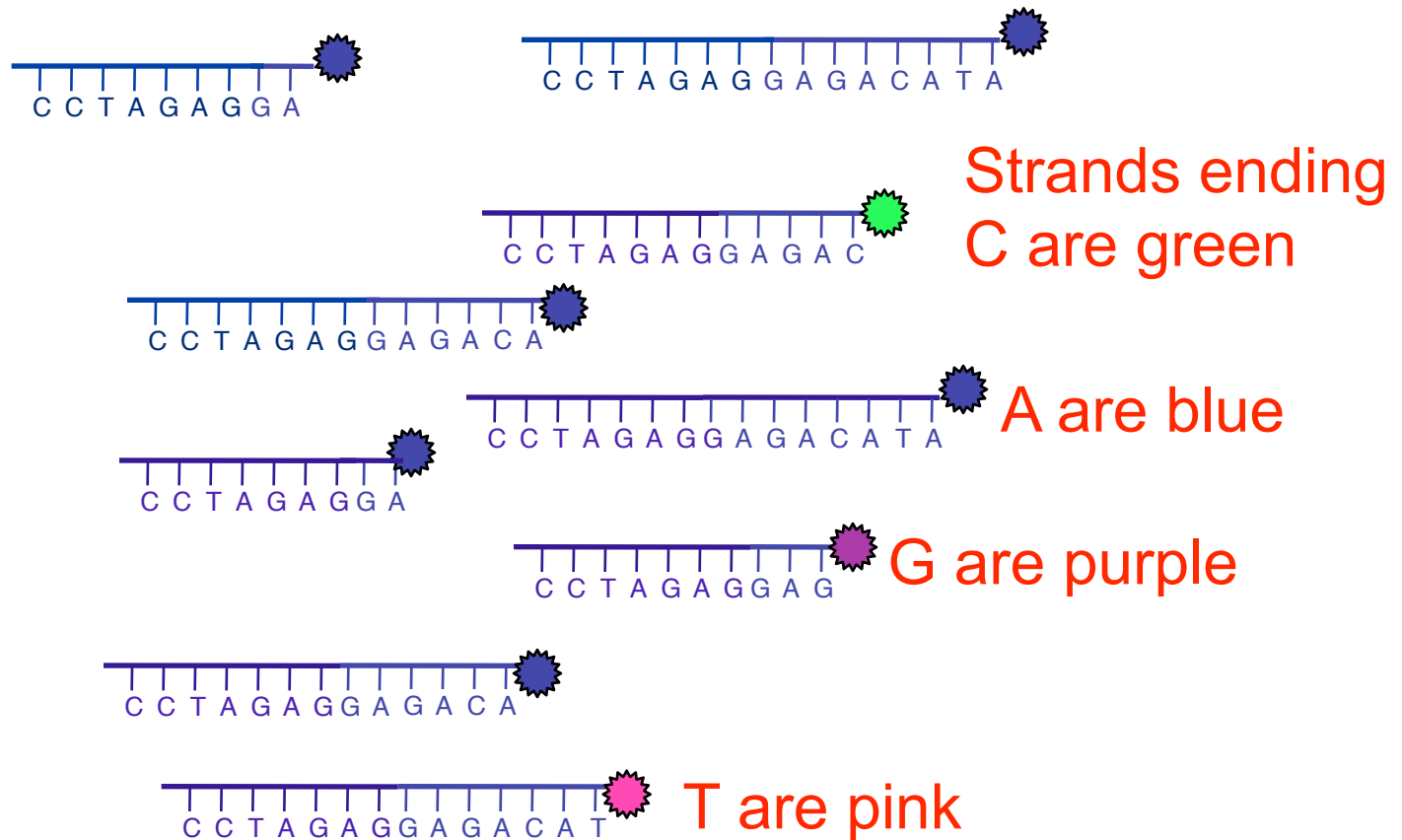
# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?





# How does DNA sequencing work?



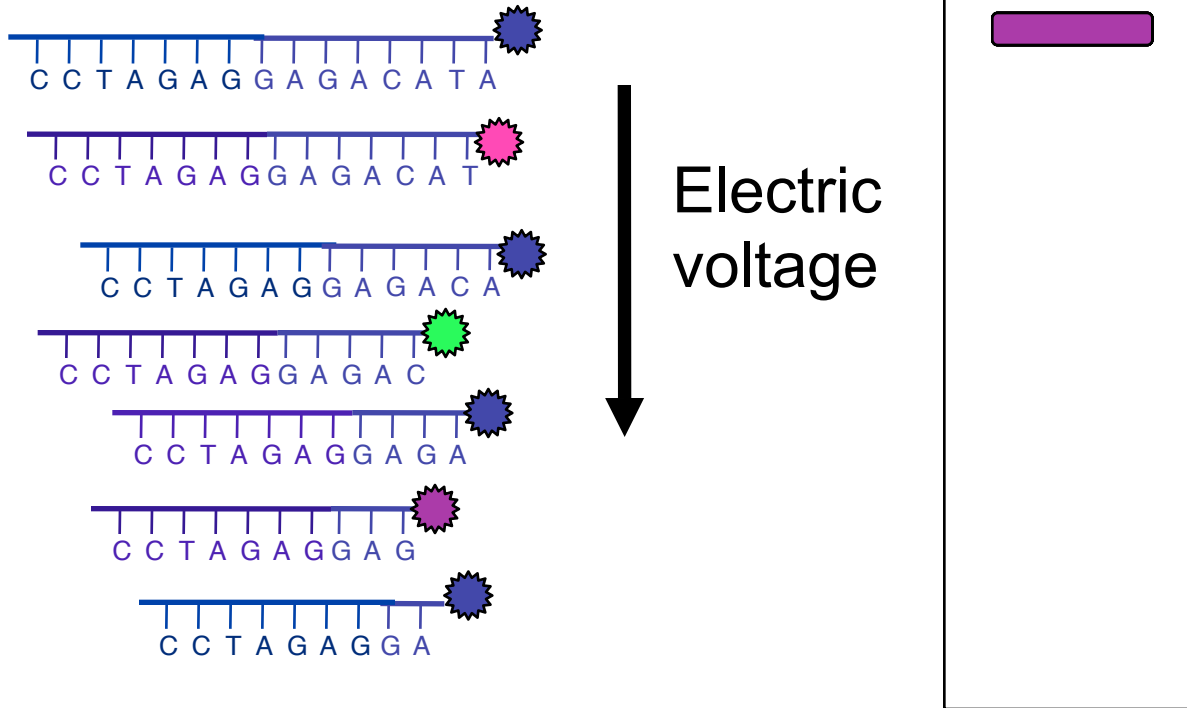
# How does DNA sequencing work?



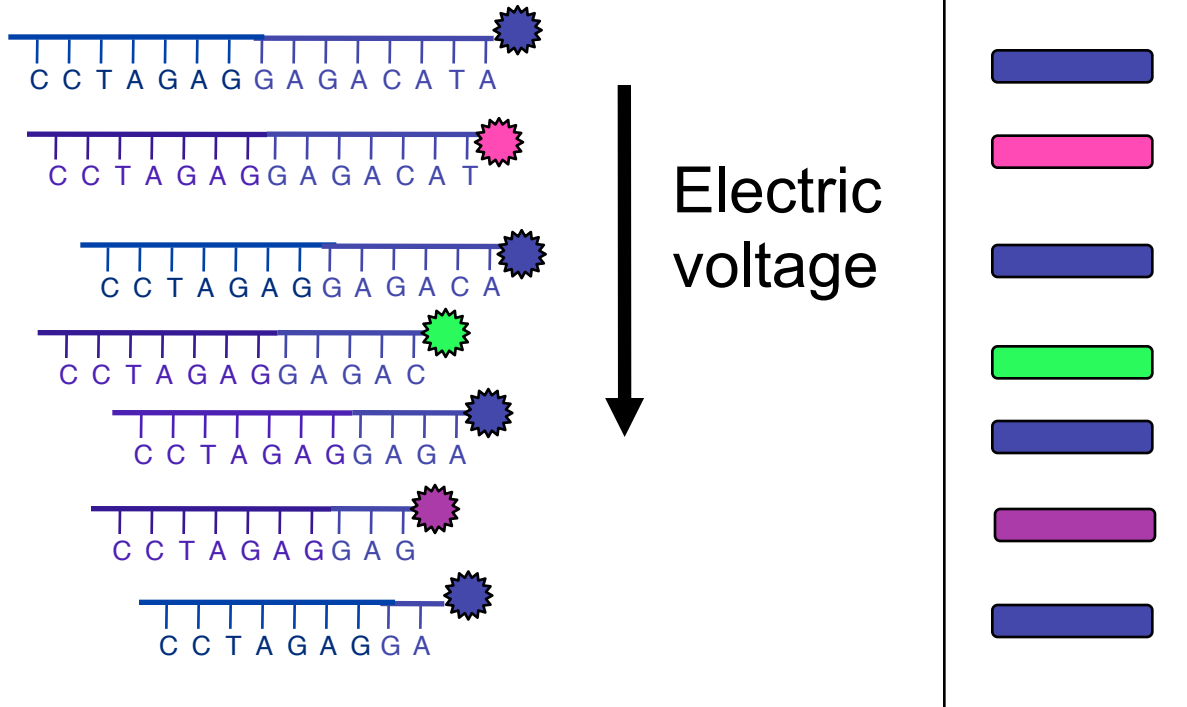
Electric  
voltage



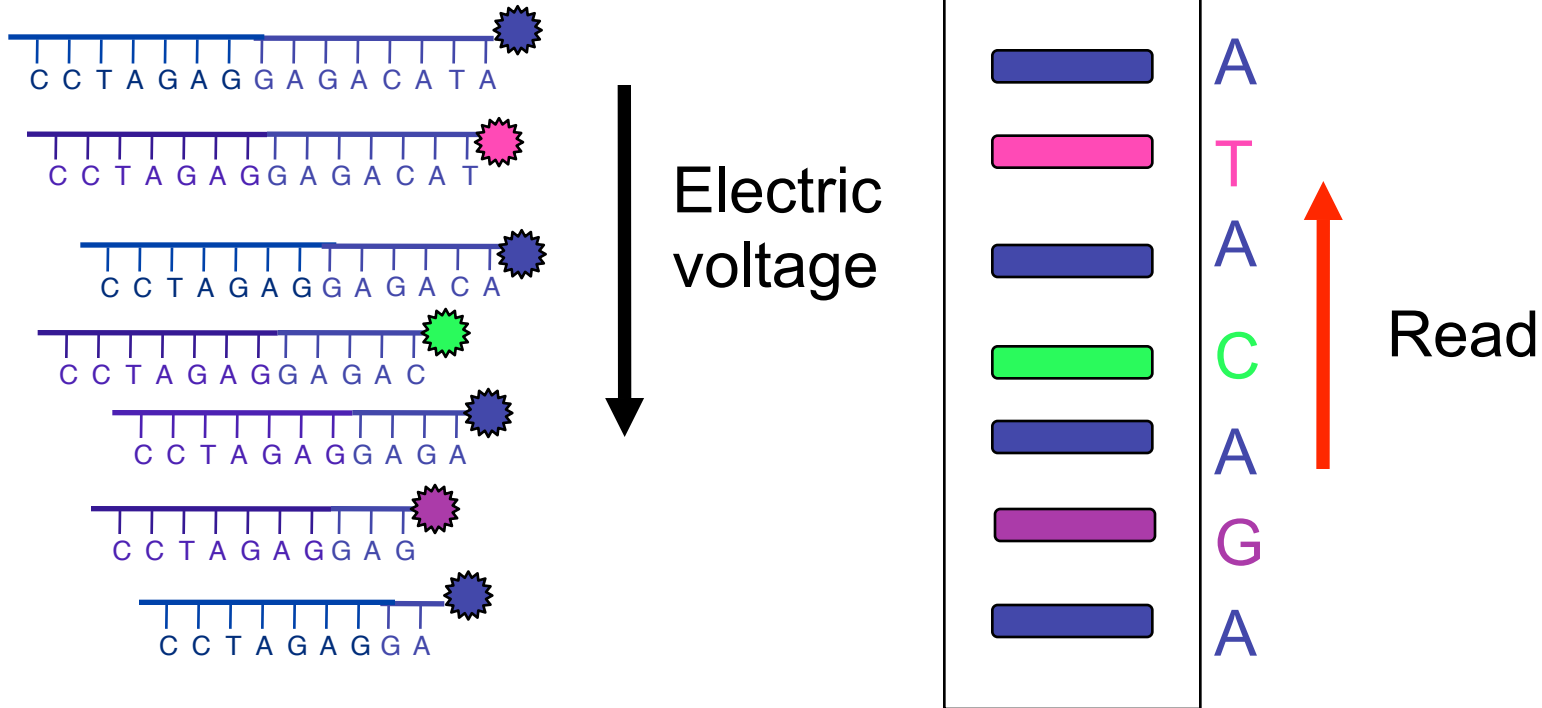
# How does DNA sequencing work?



# How does DNA sequencing work?



# How does DNA sequencing work?





What can we do with DNA  
sequencing?

# >90% of bacteria in the environment can't be cultured in the lab

- Human gut
- Sargasso sea
- Acid mine drainage
- Alaskan soil
- New York Air
- New Orleans floodwater



# Tyrolean Ice man

- Sequenced his DNA (Central European human), and DNA from his gut (red deer and ibex).



<http://www.ncbi.nlm.nih.gov/Web/Newsltr/V14N1/iceman.html>  
<http://www.pbs.org/wgbh/nova/icemummies/iceman.html>

# Sequencing Extinct Species

- Woolly mammoth
- Neanderthals
- Cave bears
- Saber tooth tigers
- Dodo

<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=extinct>

# New sequencing technologies

- Solexa (Illumina Inc.)
- 454 Life Sciences (a division of Roche)

New technologies that sequence 1-4 billion base pairs per day per machine

# Colony Collapse Disorder

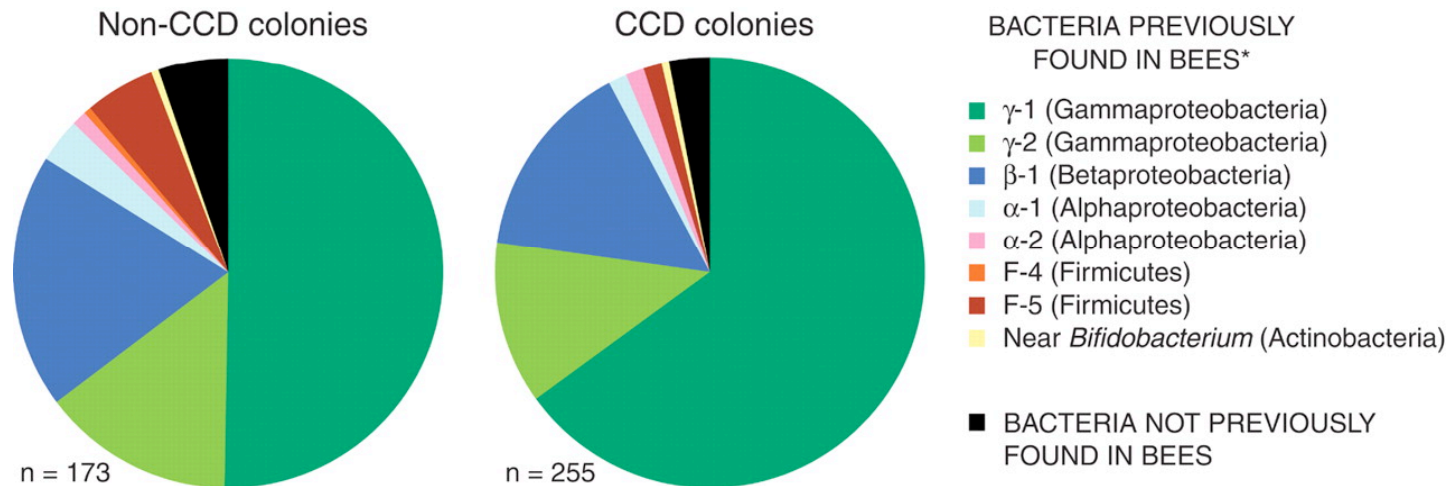
# Colony Collapse Disorder

- In 2006 beekeepers notice some hives where the workers leave and never return
- The disorder is spreading around the country. Average 45% loss of bees.
- \$15 billion U.S. crops depend on bee pollination annually
- Cause is unknown

# Colony Collapse Disorder

- In collaboration with 454, bee researchers sequenced DNA and mRNA from CCD and non-CCD hives.
- From the sequences they identified all of the bacteria, fungi, mites, and viruses present in the hives.

# Colony Collapse Disorder



# Colony Collapse Disorder

Agent	CCD (30)	Non-CCD (21)
IAPV	25 (83.3%)	1 (4.8%)
KBV	30 (100%)	16 (76.2%)
N. apis	27 (90%)	10 (47.6%)
N. ceranae	30 (100%)	17 (80.9%)
All four agents	23 (76.7%)	0 (0%)

# Colony Collapse Disorder

Agent	CCD (30)	Non-CCD (21)
IAPV	25 (83.3%)	1 (4.8%)
KBV	30 (100%)	16 (76.2%)
N. apis	27 (90%)	10 (47.6%)
N. ceranae	30 (100%)	17 (80.9%)
All four agents	23 (76.7%)	0 (0%)

# Transplant death

# Transplant death

- Three women in Australia who were 63 years of age, 64 years of age, and 44 years of age received a liver transplant or kidney transplants from one male donor who was 57 years of age.

# Transplant death

- Three women in Australia who were 63 years of age, 64 years of age, and 44 years of age received a liver transplant or kidney transplants from one male donor who was 57 years of age.
- The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas.

# Transplant death

- Three women in Australia who were 63 years of age, 64 years of age, and 44 years of age received a liver transplant or kidney transplants from one male donor who was 57 years of age.
- The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas.
- All three died 4 to 6 weeks after transplantation.

# Transplant death

- Three women in Australia who were 63 years of age, 64 years of age, and 44 years of age received a liver transplant or kidney transplants from one male donor who was 57 years of age.
- The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas.
- All three died 4 to 6 weeks after transplantation.

# Transplant death

- Three women in Australia who were 63 years of age, 64 years of age, and 44 years of age received a liver transplant or kidney transplants from one male donor who was 57 years of age.
- The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas.
- All three died 4 to 6 weeks after transplantation.
- Bacterial and viral cultures and PCR assays were done to look for:
  - herpesviruses 1 through 8, lyssavirus, influenza A and B viruses, respiratory syncytial virus, picornavirus, adenovirus, human parainfluenza virus, flavivirus, alphavirus, hantavirus, polyomavirus, Crimean–Congo hemorrhagic fever virus, Rift Valley fever virus, toxoplasma, Mycobacterium tuberculosis, and Mycoplasma pneumoniae

# Transplant death

# Transplant death

- 454 sequencing was done on preserved tissues from two recipients.

# Transplant death

- 454 sequencing was done on preserved tissues from two recipients.
- 15 million base pairs of sequence were analyzed

# Transplant death

- 454 sequencing was done on preserved tissues from two recipients.
- 15 million base pairs of sequence were analyzed
- A previously undiscovered old world arena virus was discovered.

# Transplant death

- 454 sequencing was done on preserved tissues from two recipients.
- 15 million base pairs of sequence were analyzed
- A previously undiscovered old world arena virus was discovered.
- The virus was then confirmed by culturing it from the tissues and detecting antibodies in the donor.

# Maternal blood sampling

- Small amounts of fetal DNA is present in a mother's blood stream.
- 10 billion sequence reads were done from maternal blood (14 weeks).
- For trisomy 21 fetuses, there was a detectable increase in sequences from chromosome 21
- Not yet cost effective (>\$1000/ test)

# New sequencing technologies

- Each new sequencing machine produces the same amount of sequence that a sequencing center produced 3 years before.
- Sequencing centers are replacing their old machines with new ones





# Human genome

- 2000: worldwide effort, 10 years,  
• \$3 billion
  
- 2009: 9 machines, 8 days,  
• \$5000

# Summary

- **Recombinant** DNA is DNA that is a mixture of DNA from multiple sources.
- **Plasmids** are small circles of DNA in bacteria.
- This is one example of a **vector**.
- Many **protein drugs** are made via recombinant techniques.
- **DNA sequencing** can be used for many things