Cambridge Assessment International Education

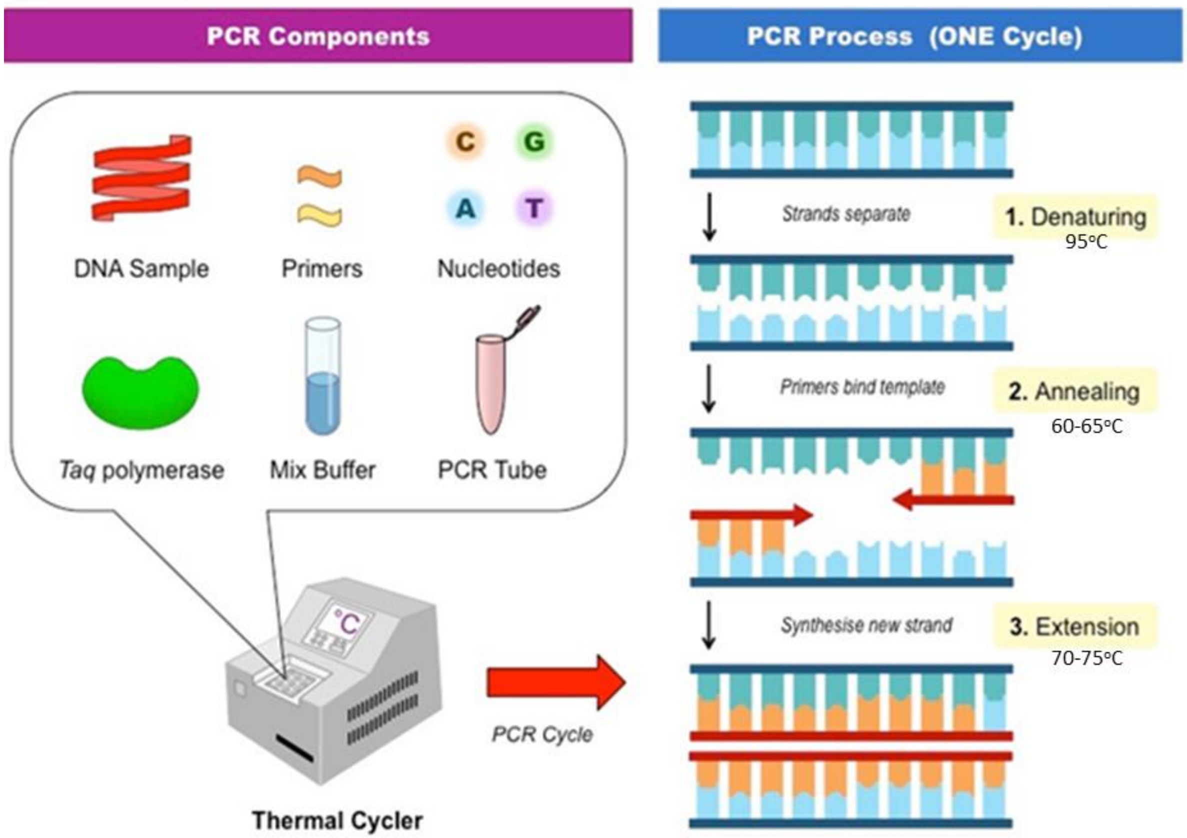
A2 LEVEL

Chapter 19

Genetic Technology

Chapter Outline

Technology	Function	Application
1. Polymerase Chain Reaction (PCR) • Role of *Taq* polymerase	**Clone and amplify DNA**	Too many…



Chapter Outline

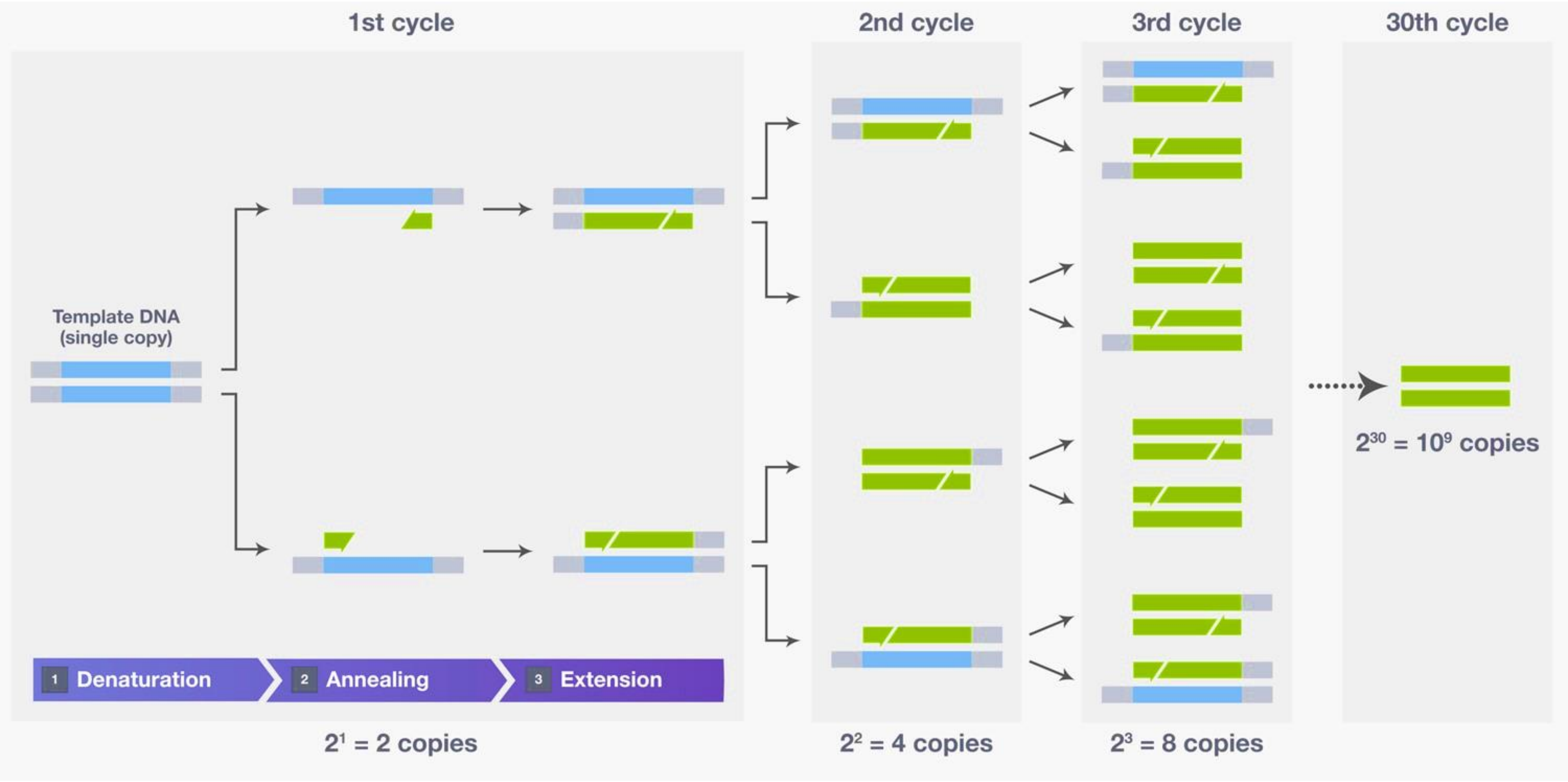
Technology	Function	Application
2. Recombinant DNA technology / Gene Cloning • Properties of plasmids • Promoters • Marker genes (e.g. fluorescent genes / genes coding for easily stained substances) • Role of restriction endonucleases, reverse transcriptase and ligase	**To make many copies of a gene or protein**	**Large-scale production of human proteins as drugs** E.g. insulin, factor VIII for haemophilia, adenosine deaminase for SCID **Genetic engineering of crop plants and livestock** • GM salmon • Insect resistance E.g. Bt maize, Bt cotton • Herbicide resistance E.g. maize, cotton, tobacco, oil seed rape • Ethical and social implications of using GMOs in food production

Chapter Outline

Technology	Function	Application
3. Gel electrophoresis • For protein and for DNA • Probes	**To separate fragments of DNA according to length** OR **To separate diff proteins according to mass/charge** Distinguish btwn alleles of a gene	**Genetic fingerprinting / DNA profiling** • Paternity testing • Criminal Investigations **Genetic screening** • Breast cancer (*BRCA1*, *BRCA2*) • Genes for haemophilia, SCA, Huntington's Disease and CF **Gene Therapy** • Vectors (viruses, liposomes, naked DNA) • SCID, inherited eye disease, CF • Social and ethical considerations

Chapter Outline

Technology	Function	Application
4. Microarrays	**Identify expressed genes by detecting mRNA** OR **Distinguish btwn alleles of a gene**	• **Genetic screening** • Drug testing • Identify genes that are switched on/off in diseases
5. Bioinformatics and DNA sequencing (Extra! Not in syllabus)	Collection, processing and analysis of biological info and data using computer software Allows comparison of DNA and protein sequences	• Role in drug development E.g. against *Plasmodium* • Protein and DNA seq provide evidence for evolution
6. CRIPSR-Cas9 (Extra! Not in syllabus)	Cut DNA at specific loci and allow genome editing	Cure viral diseases and genetic diseases, even cancer!



Genetic Technology 1

POLYMERASE CHAIN REACTION (PCR)

Function: Clone and amplify DNA

Updated on 12/8/21 by Beh SJ @behlogy

Chapter Outline

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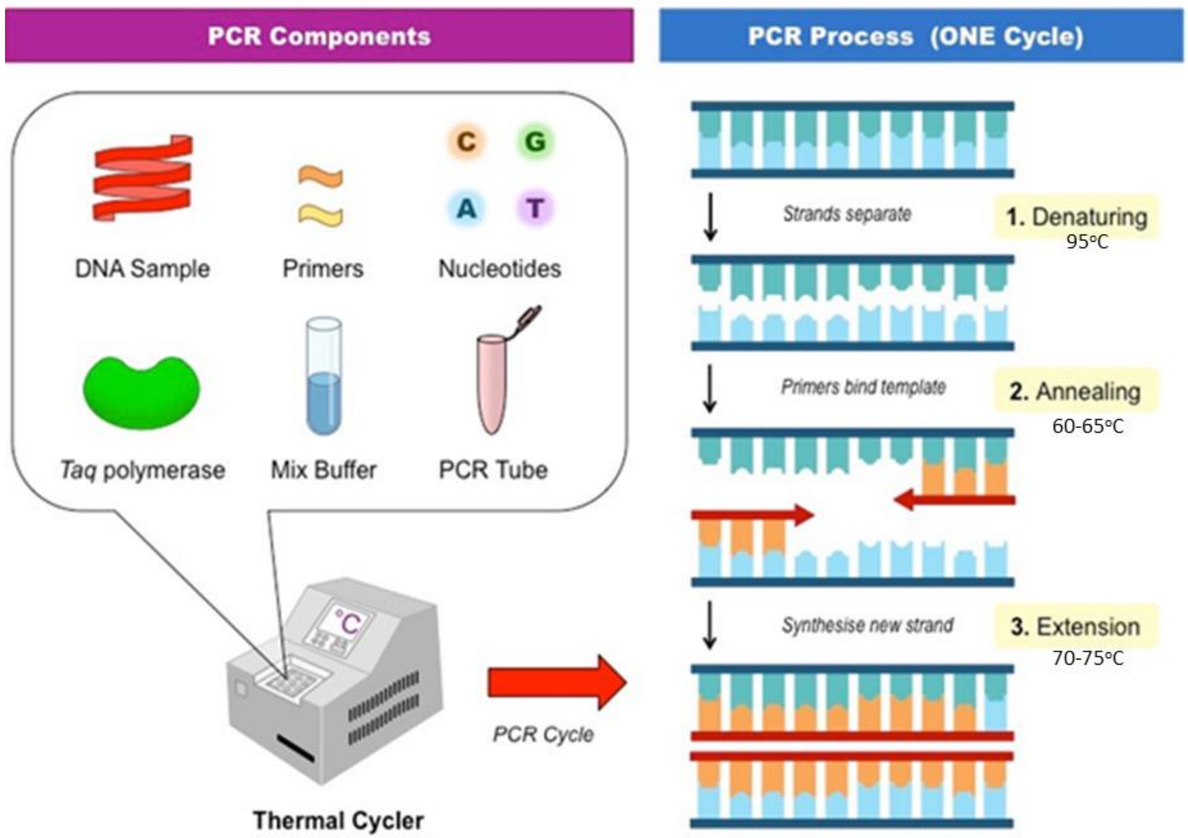
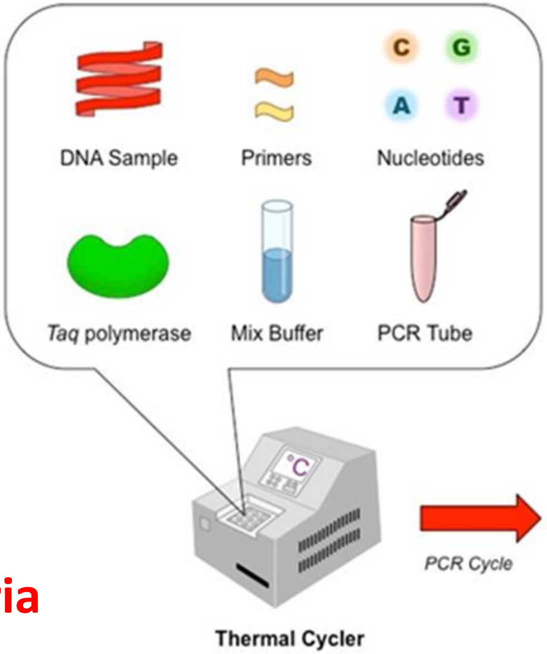


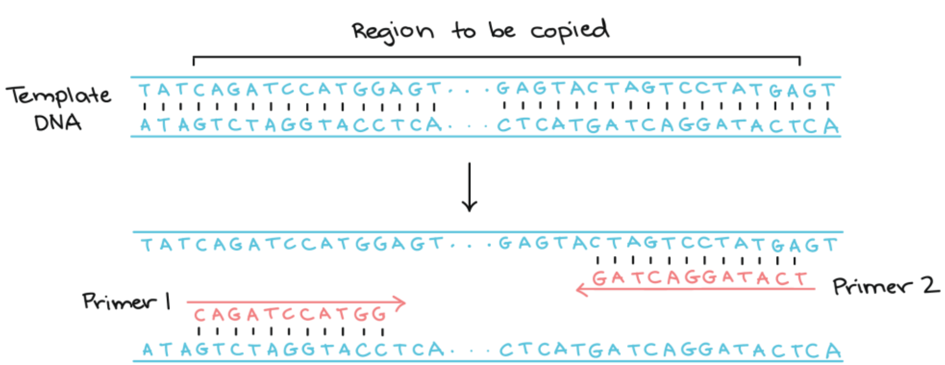
Polymerase Chain Reaction (PCR)

- **Rapid and efficient process**
- **Function: clone and amplify DNA**
- Amplify = production of many copies of a length of DNA
- Only small sample of DNA needed



Components needed:

1. **Template DNA**
2. ***Taq* polymerase**
   - **Heat-stable DNA pol** from *Thermus aquaticus* **bacteria**
3. **Buffer**
   - Contains KCl and $MgCl_2$
4. **4 types of nucleotides** (A, T, C, G)
5. **2 Primers**
   - Short sequence of ~20bp of single-stranded RNA/DNA
   - **Complementary base pair to start and end of target region for amplification**



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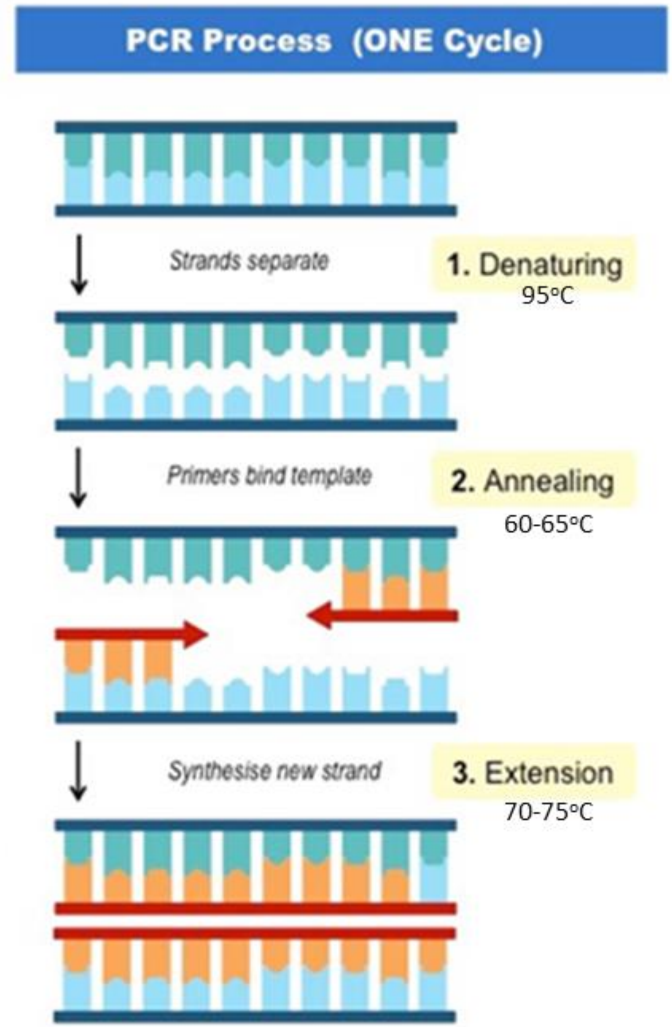
Polymerase Chain Reaction (PCR)

1. Denaturation (95°C)

- **DNA strands separate / denature** into 2 strands by heat
- **Hydrogen bonds** between DNA strands **breaks**
- Bases are exposed
- Produce template strands for copying



Polymerase Chain Reaction (PCR)

2. Annealing (60-65 °C)

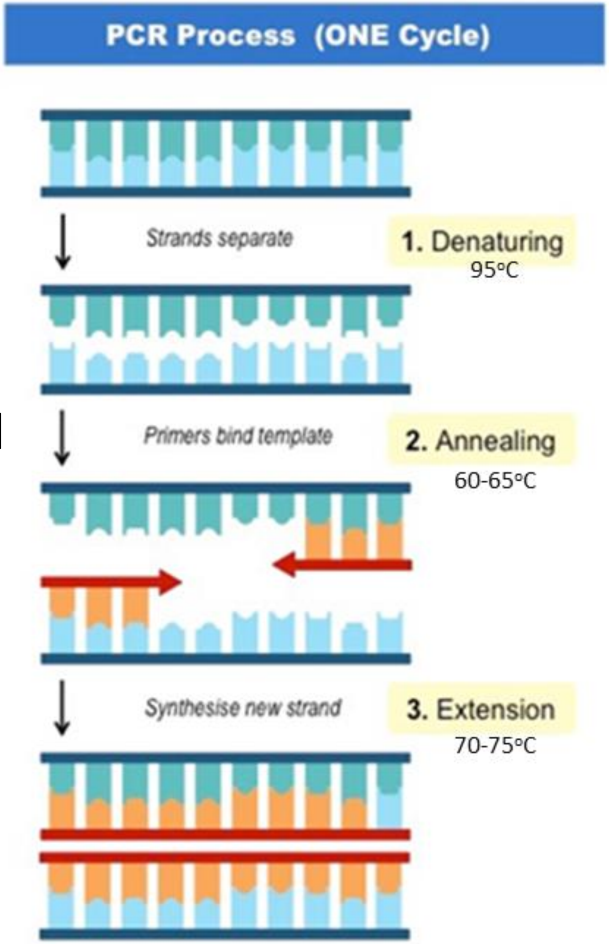
- **Primer added**
- Primers anneal / bind to specific section of DNA
- Via complementary base pairing
- New hydrogen bonds form

Role of primers:

- **Bind to target region for amplification**
- Acts as a starting point for for *Taq* polymerase to bind

→ *Taq* polymerase only binds to double-stranded DNA and add new nucleotides to an existing strand

- Reduce reannealing of separated strands

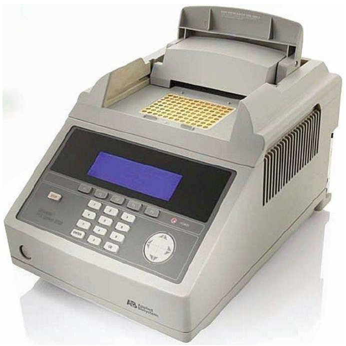


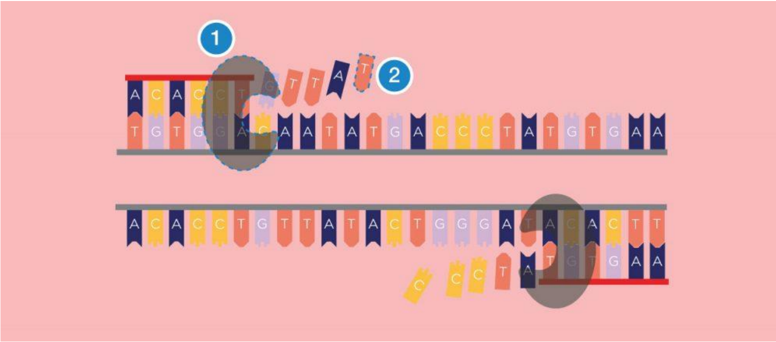
Polymerase Chain Reaction (PCR)

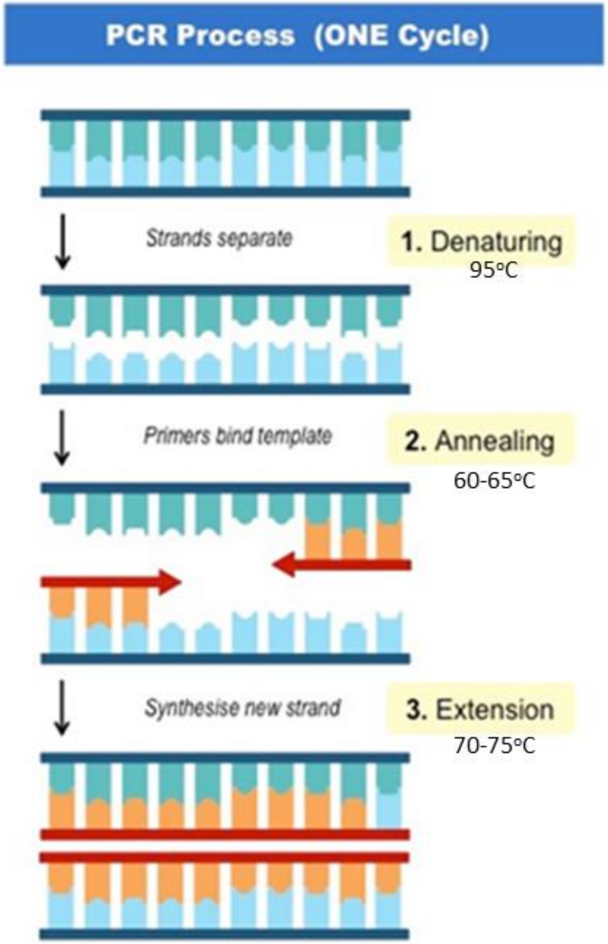
3. Extension (70-75 °C)

- ***Taq* polymerase** binds to primer
- **Synthesises new DNA strands**
- Complementary to the DNA template strands

- *Taq* polymerase has a high optimal temperature / is **heat stable**
- Does not need replacing each cycle







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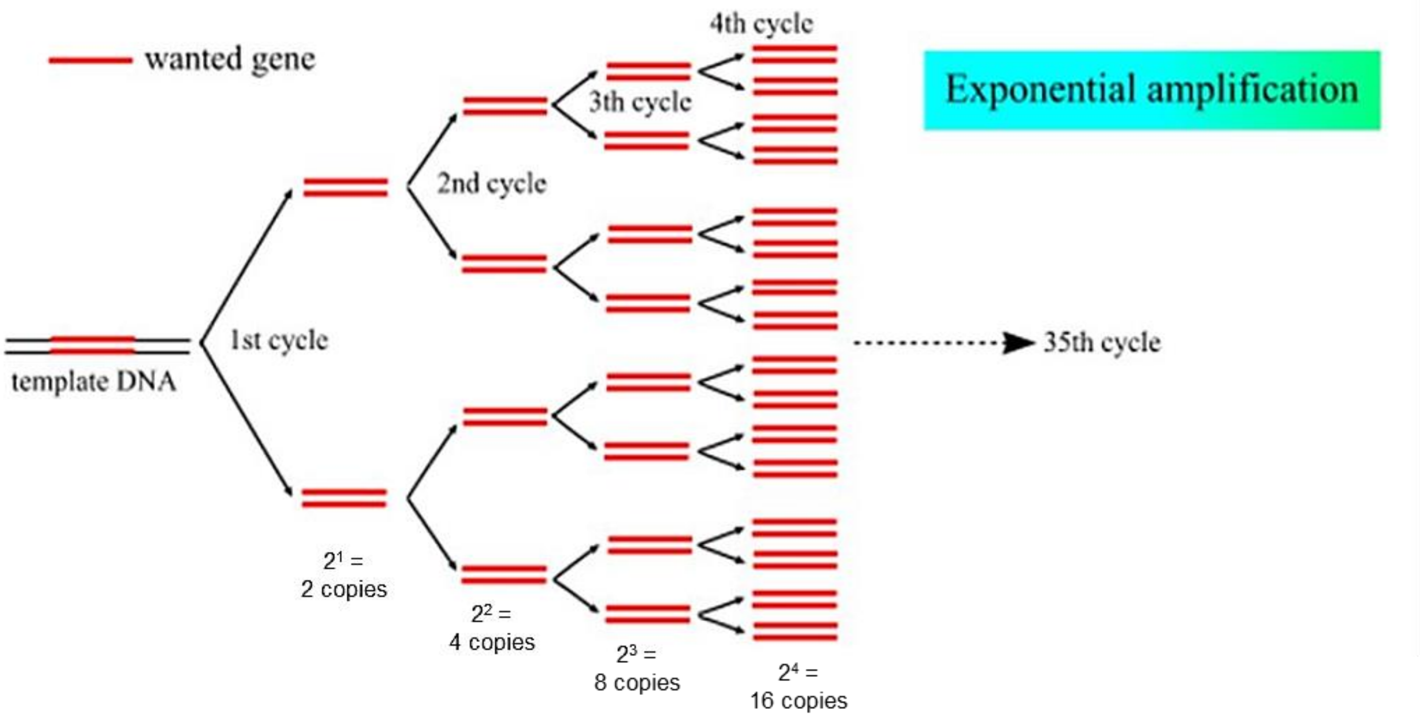
Polymerase Chain Reaction (PCR)

- DNA is heated again to separate strands
- **The process is repeated** until sufficient DNA is produced (usually ~30 cycles)
- The number of DNA molecules **doubles every cycle** = efficient process!
- **Number of DNA double helix copies made from one starting molecule after n cycles of PCR = 2^n**



PCR simulation = http://learn.genetics.utah.edu/content/labs/pcr/
Currently not available ☹ Watch it here = https://www.youtube.com/watch?v=0jlWKw5gEP8

Polymerase Chain Reaction (PCR)

Advantages:

- **Rapid, efficient**
- **Only small sample of DNA needed** for amplification of DNA
- Process is **automated** in a thermal cycler

Disadvantages:

- Need to know the precise DNA sequence beforehand to design primers
- DNA amplification have to be in shorter fragments than gene cloning in bacteria (Genetic Technique 2)

Applications of Polymerase Chain Reaction (PCR)

Function: clone and amplify DNA

Applications:

- **DNA sequencing**

→ Able to ***amplify small amount of DNA*** extracted for sequencing (E.g. in fossils, blood sample)

- **DNA profiling**

→ Able to ***amplify small amounts of DNA*** extracted (E.g. at crime scene)

- **Recombinant DNA Technology**

→ ***Amplify DNA/gene*** needed for insertion into plasmid

- **Genetic screening**

→ To identify mutations/disease genes/DNA from pathogens

→ Use primers complementary to target gene

→ To identify and ***amplify target gene in specific***

→ Gel electrophoresis used to isolate gene

Genetic Technology 2

RECOMBINANT DNA TECHNOLOGY / GENE CLONING

Function: to make many copies of one gene / protein

Updated on 12/8/21 by Beh SJ @behlogy

Chapter Outline

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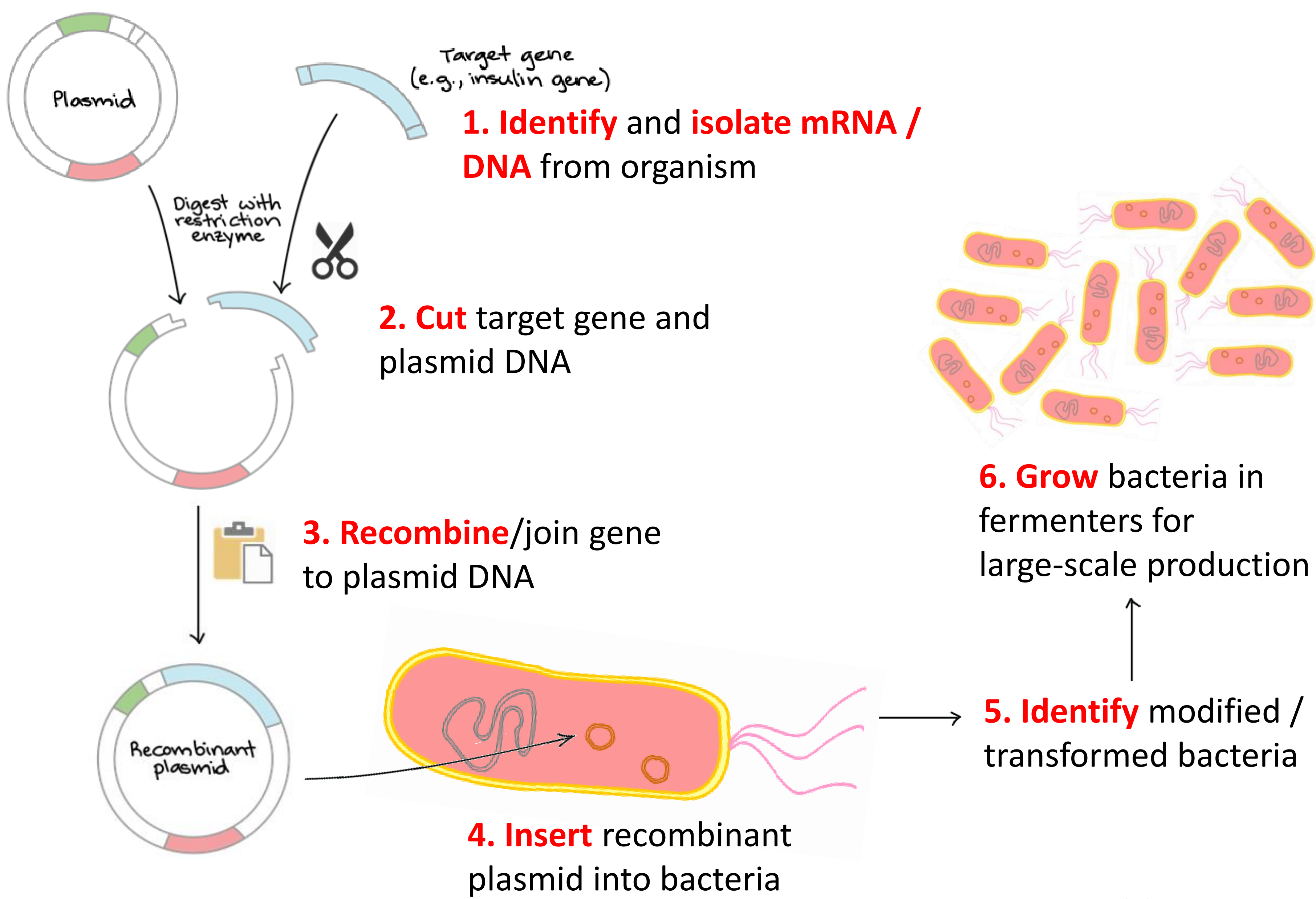
Recombinant DNA Technology

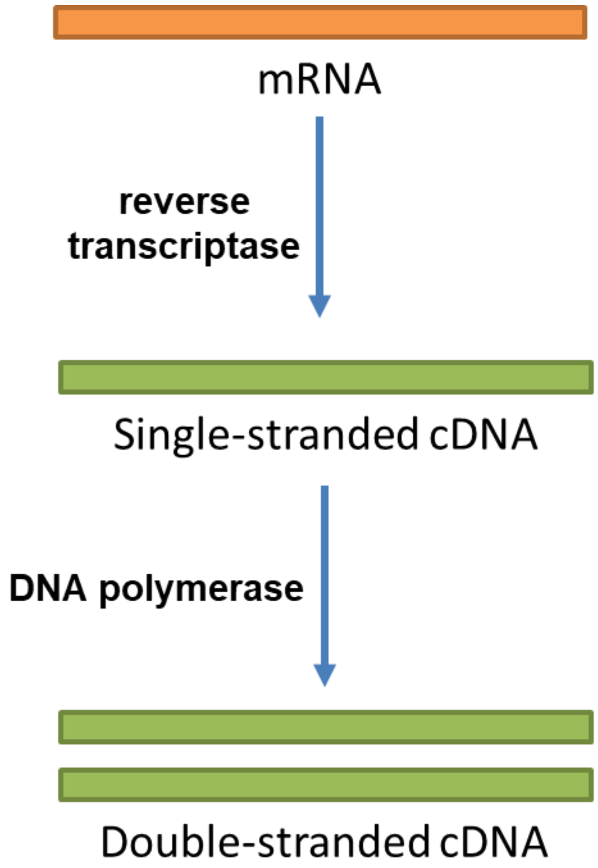


E.g. Production of human insulin

1. Obtain mRNA for human insulin

- From β cells of islets of Langerhans of pancreas
- **Reverse transcriptase** to make **cDNA** (single-stranded complementary DNA) **from mRNA**
- **DNA polymerase** used to make **double-stranded cDNA** from ssDNA
- Final result: gene with no introns, shorter DNA

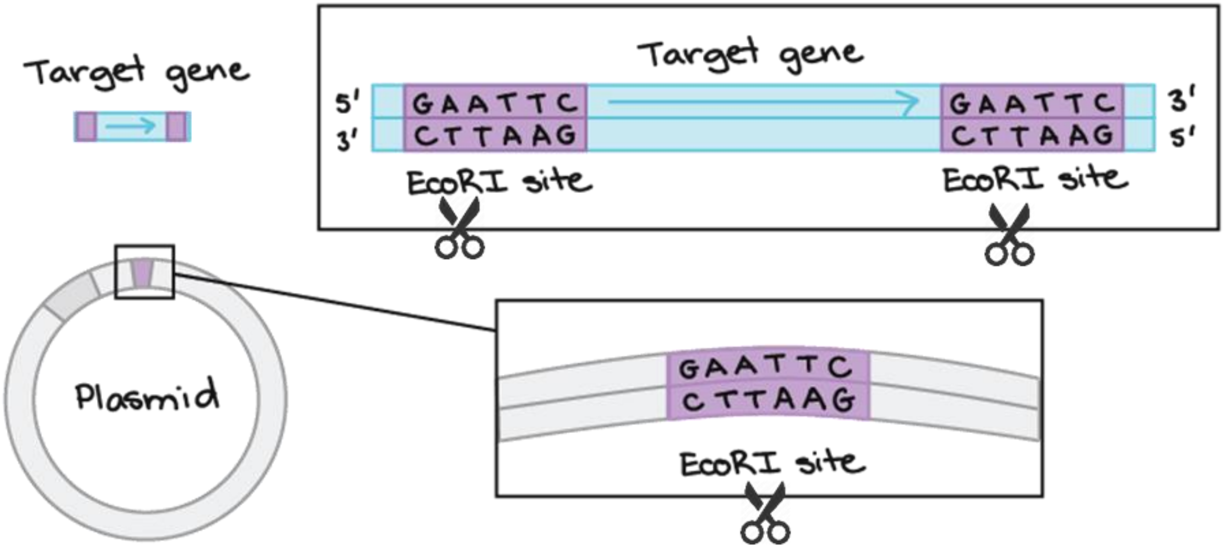
- DNA may be amplified using **Polymerase Chain Reaction (PCR)**



2. Cut target gene and plasmid DNA

- Use **restriction enzyme** to cut the gene
- Restriction sites should be present at both sides of gene

- Obtain **plasmids** from bacteria
- Cut plasmid at 1 restriction site **using SAME restriction enzyme**
- **Complementary sticky ends** produced



Restriction Enzymes / Endonucleases

- From bacteria
- **Recognize, bind and cut DNA** at a specific sequence, called a **restriction site**
- Hydrolyses/cleaves the phosphodiester bond btwn nucleotides
- Diff kind of RE cuts at a diff specific seq

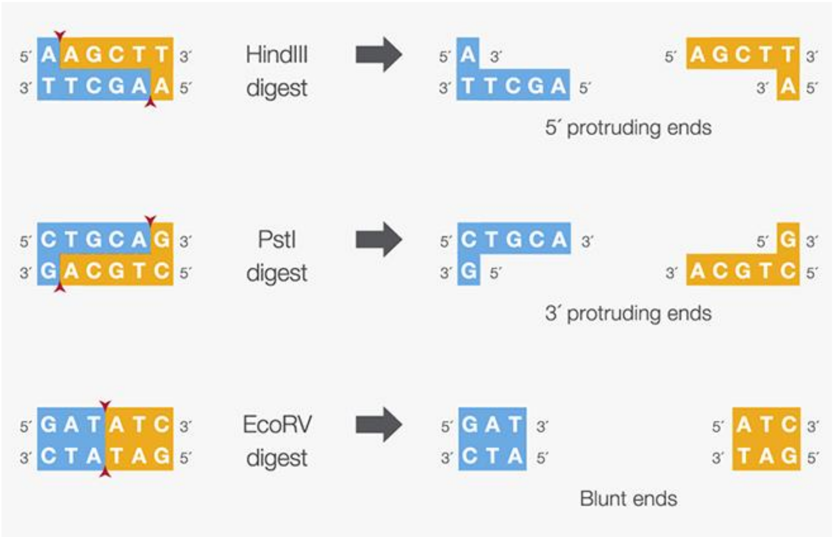
- Most restriction sites are **palindromic sequences**
- Restriction enzymes produces **sticky ends** or **blunt ends**

At the '**sticky ends**':

- Result of a **staggered cut**
- A few **unpaired nucleotides at ends**
- Able to easily form H bonds / **complementary base pair**

At '**blunt ends**'

- NO unpaired nucleotides

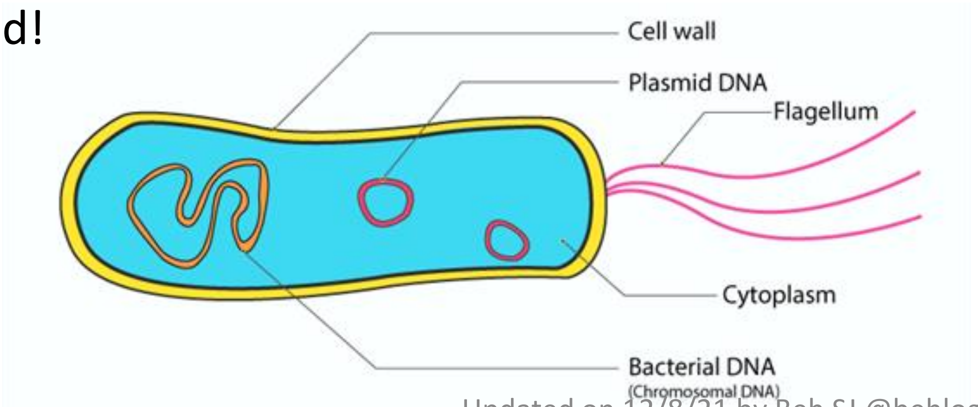


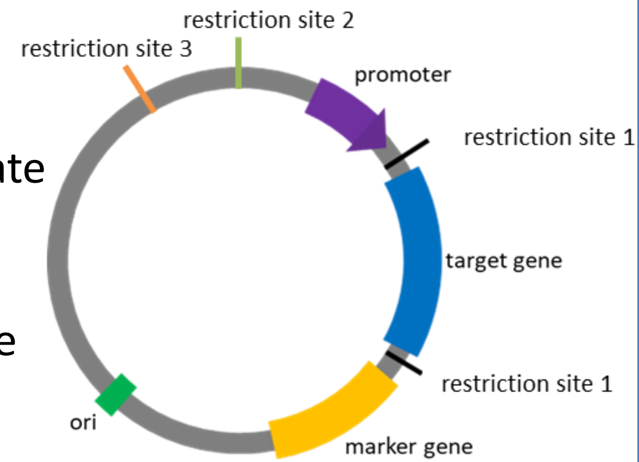
Plasmids

- Small, circular, double-stranded DNA
- Found in bacteria, but now usually artificially made in lab

Frequently used in gene technology because:

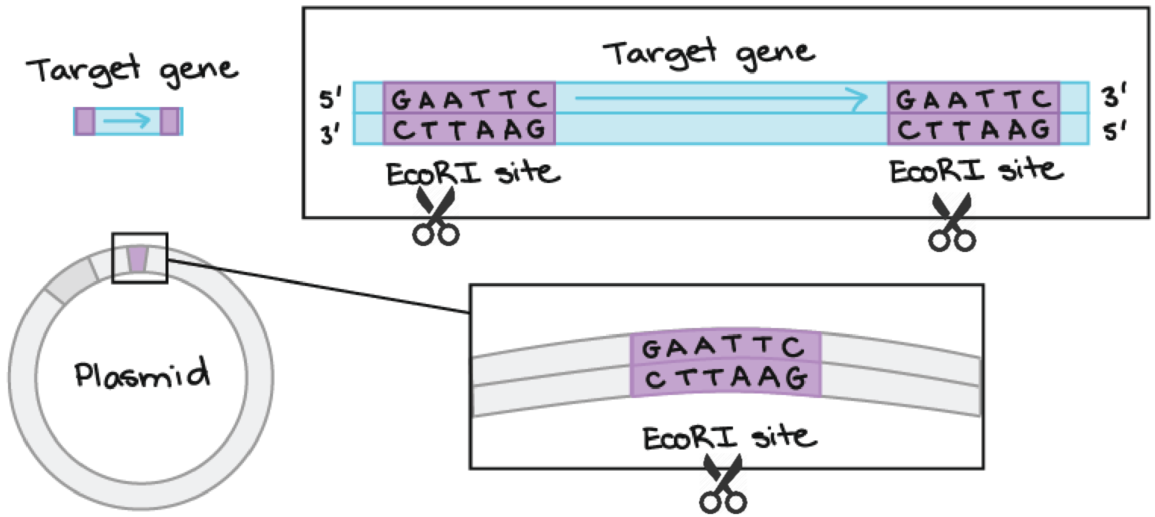
- **Small** so can be inserted into cells
- Circular so more stable / not damaged by host cell enzymes
- Plasmids act as **vector** to deliver desired genes to bacteria
- **Easy to extract** from bacteria
- **Can be taken up** by bacteria due to low molecular mass/small
- Has **high copy number**
  – many copies can be present in one bacterial host cell
- Replicate independently within bacteria
  – able to clone/replicate any genes inserted into them
- Has **specific DNA sequences** needed!

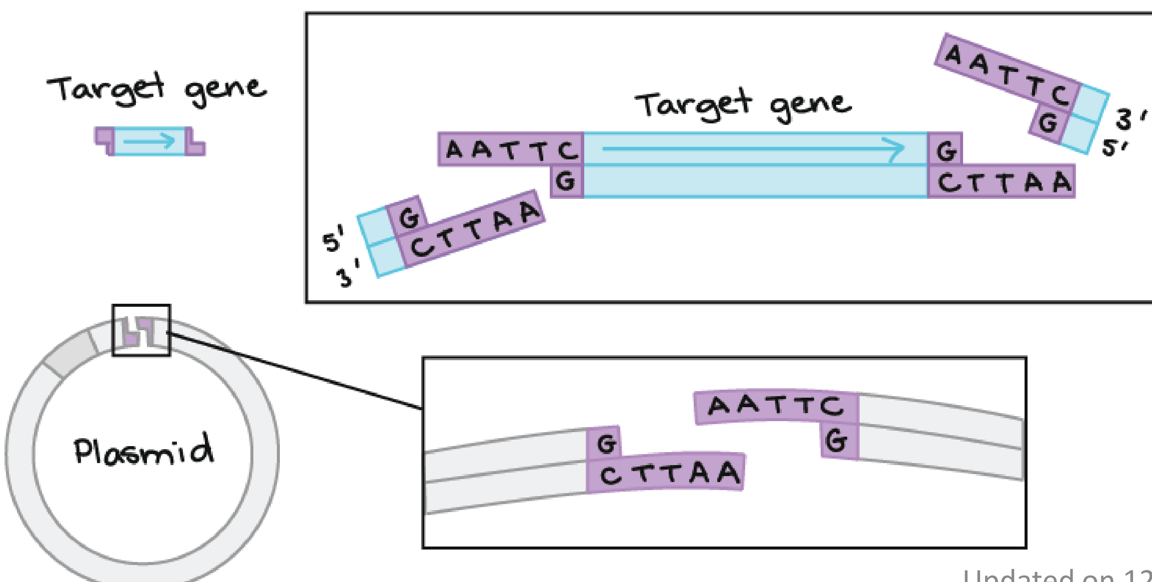


DNA Sequence	Function
Origin of replication	• Allows bacterial DNA polymerase to bind and **replication to be initiated**
Restriction site	• For restriction enzyme to cut and produce sticky ends, so **gene of interest can be inserted** • Can have multiple to be cut by diff RE → has multiple cloning sites
Promoter	• **Initiates transcription** • Allows binding of **RNA polymerase** / • transcription factors • Ensures correct strand is used as template • Diff promoters determine diff level of expression and where it is expressed • E.g. use promoter upstream of *lacZ* gene in lac operon – target gene will be expressed in the presence of lactose 
Marker Gene	• Gene of interest is inserted close to / into marker gene • Both the target gene and marker gene are expressed • Helps to **recognise recombinant plasmids / modified bacteria / transgenic organisms** • E.g. genes for **antibiotic resistance, genes for fluorescent / easily stained substances**

Plasmids

Restriction Enzymes





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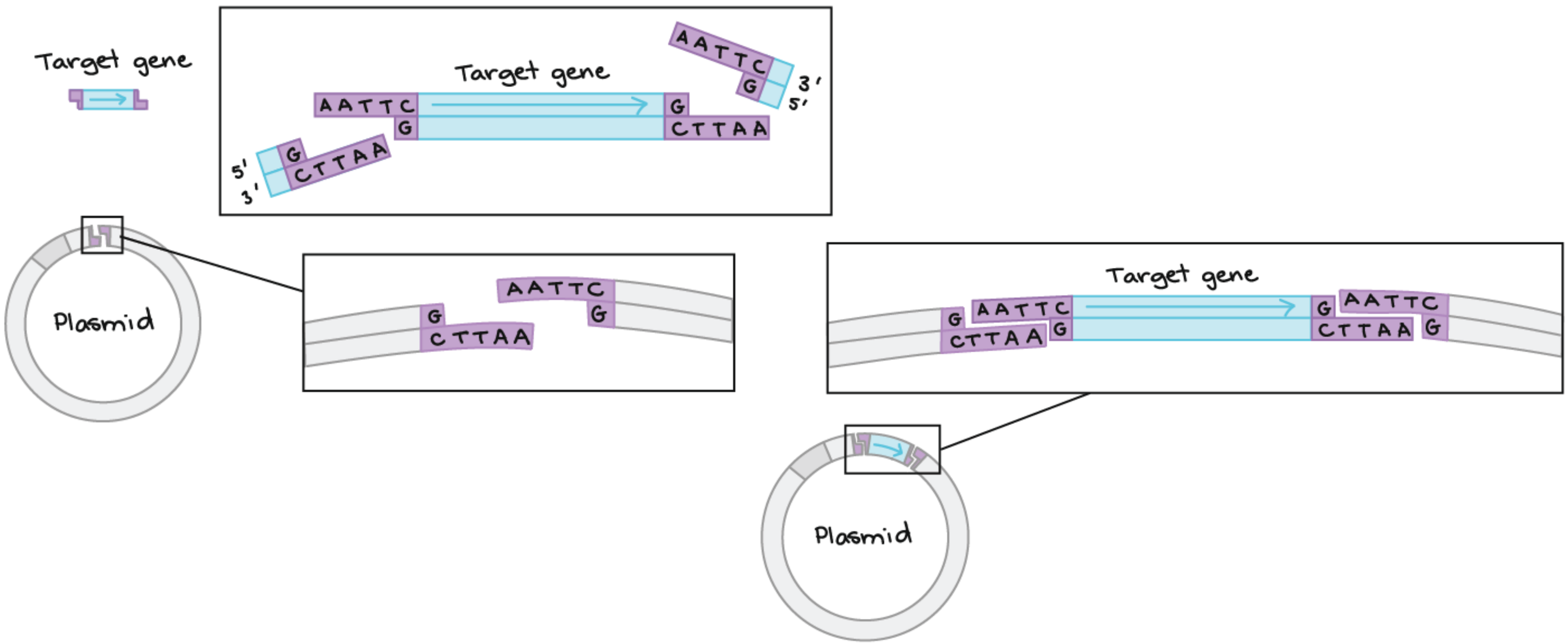
3. Recombine/join gene to plasmid DNA

- **Mix** cDNA / insulin gene with plasmid
- **DNA ligase seals nicks in sugar-phosphate backbone**

→ Catalyses the formation of phosphodiester bond

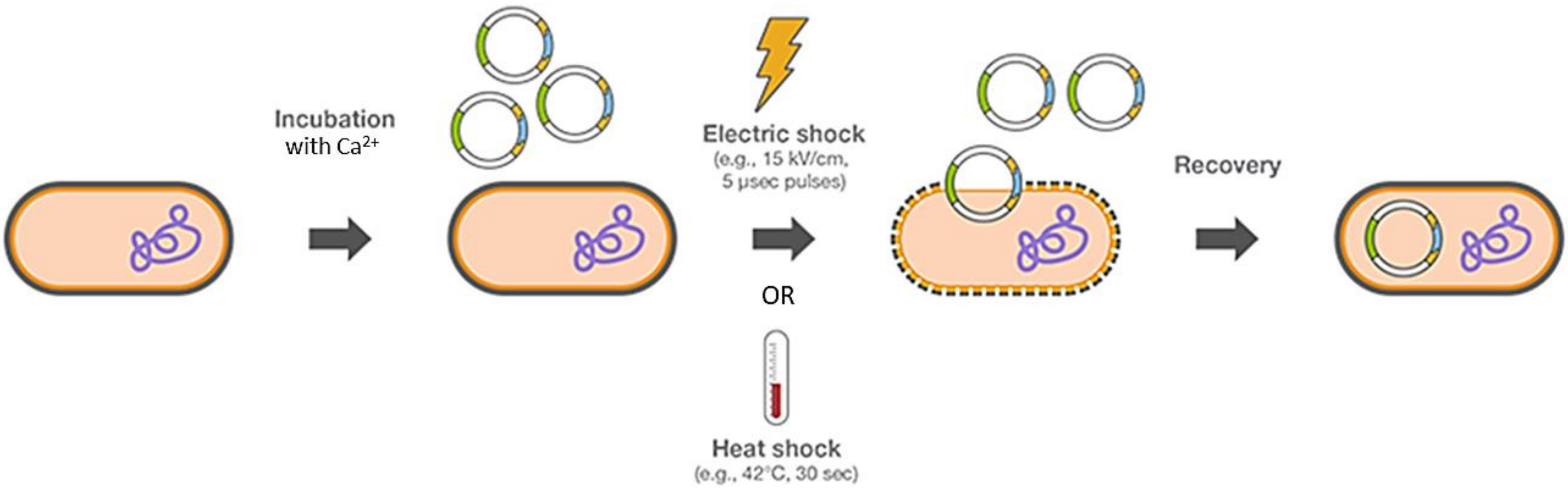
- To form **recombinant DNA**



4. Insert recombinant plasmid into bacteria

1. Recombinant plasmids **mixed** with bacteria
2. Treat bacteria with a solution of **Ca^{2+} ions** and allow to cool
3. Apply **heat shock** (~42°C) or use **electroporation** to increase chances of plasmids passing through cell surface membrane

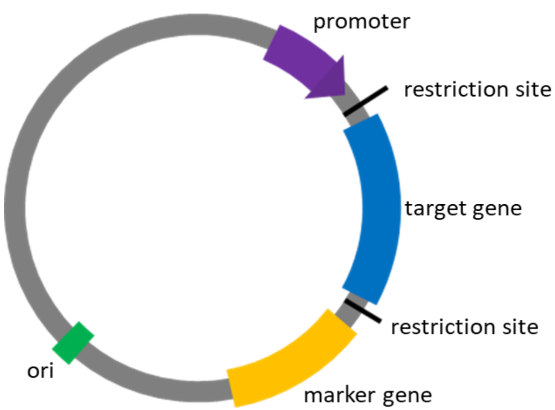
Only ~1% bacteria will take up recombinant plasmids = **transformed**



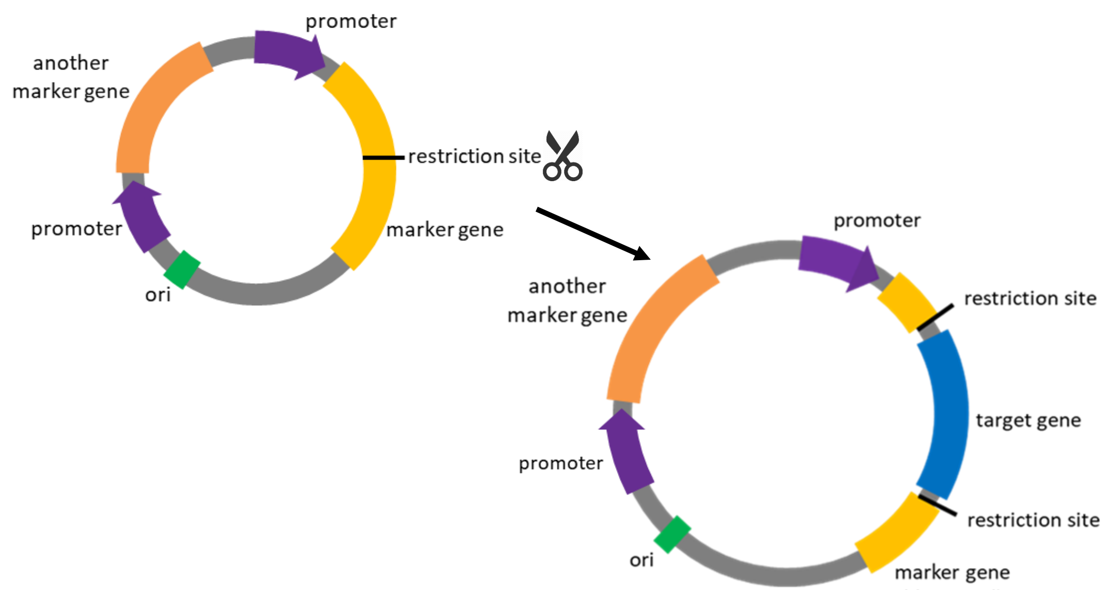
5. Identify modified / transformed bacteria

- **Marker genes** in the plasmid helps to **recognise recombinant plasmids / modified bacteria / transgenic organisms**
- E.g genes for **antibiotic resistance, genes for fluorescent / easily stained substances**
- Gene of interest is inserted **close to** OR **into** marker gene
- Both target gene and marker gene are expressed

Gene of interest is inserted **close to** marker gene
Downstream of same promoter



Gene of interest is inserted **into** marker gene
Marker gene is disrupted!
There is another marker gene that is expressed together
Extra function: easy to identify recombinant plasmid



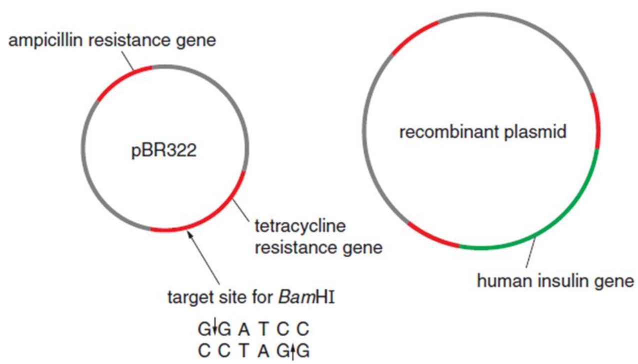
5. Identify modified / transformed bacteria

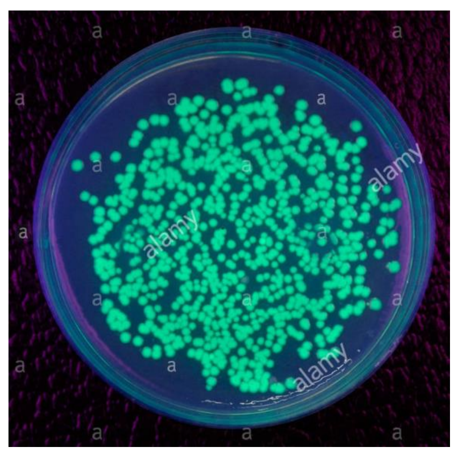
Ways things can go wrong:

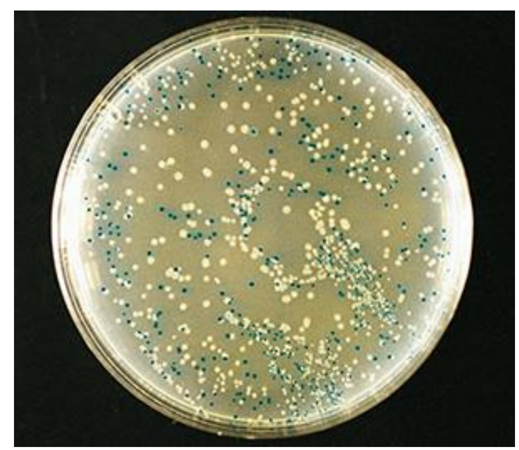
1. Bacteria did not take up recombinant plasmid
2. Gene of interest did not join with plasmid…
   Cut plasmid just rejoined with itself

Ways to identify transformed bacteria:

a) **Use antibiotic selection**
b) **Use green fluorescent protein (GFP)**
c) **Use an easily stained substance (β galactosidase, GUS)**







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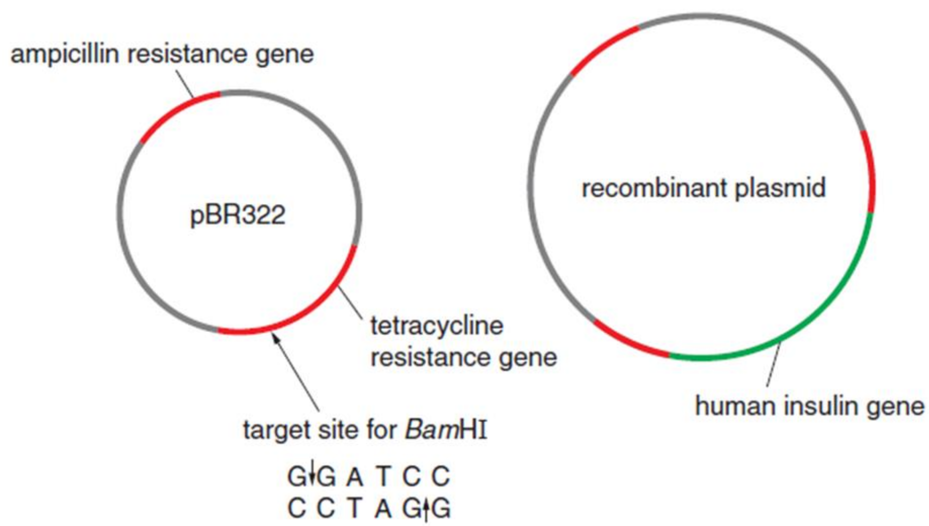
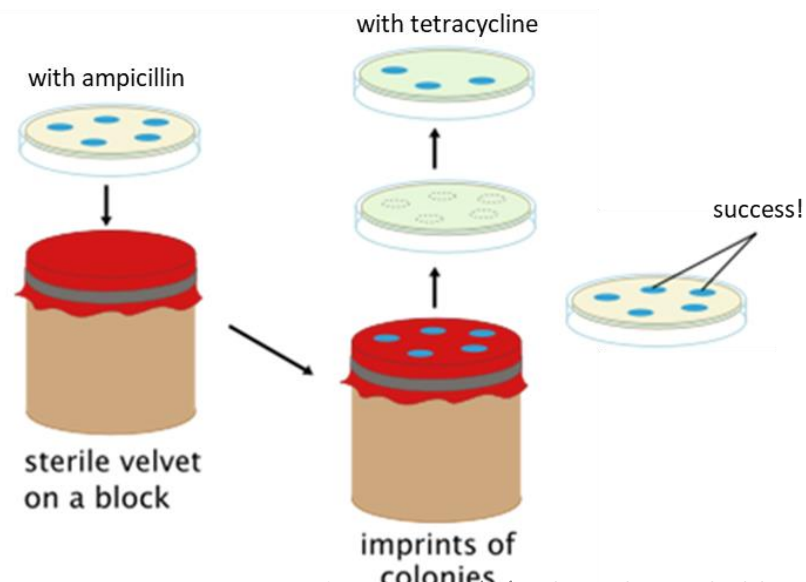
a) Antibiotic Selection

- **Genes for antibiotic resistance** used as markers
  E.g. ampicillin resistance gene (**amp**^{r}) , tetracycline resistance gene (**tet**^{r})

- Grow on agar containing the antibiotic (ampicillin)
- **Bacteria with plasmid will be able to survive**
- Bacteria without the plasmid dies

- Then make a **replica plate** by using sponge/velvet pad
- Grow bacteria on agar containing **2**nd **antibiotic** (tetracycline)
- **Bacteria with recombinant plasmid will die**



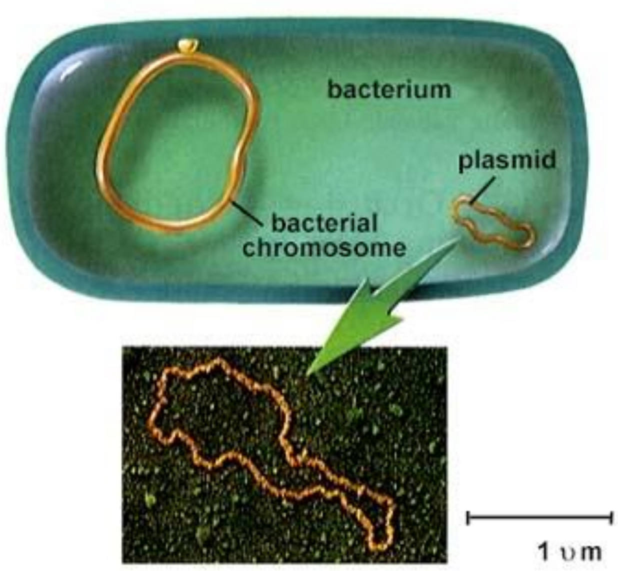


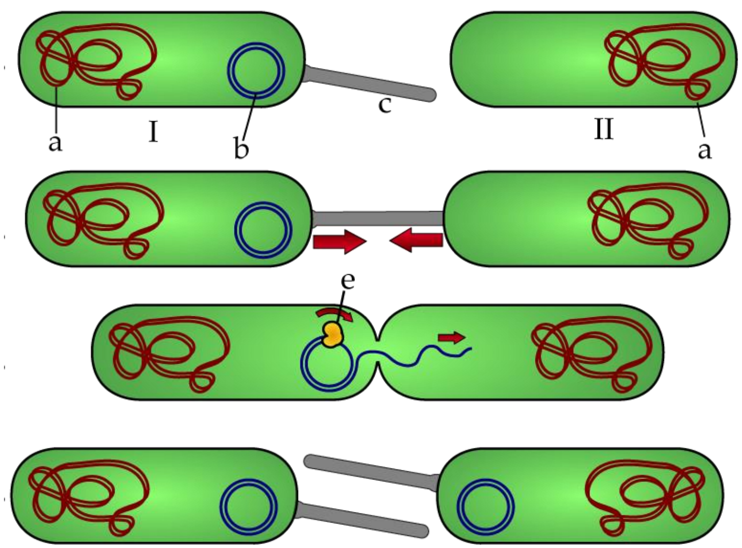
a) Antibiotic Selection

BUT PROBLEM!

- **Risk of spread of antibiotic resistance to other bacteria** of same / diff species
- Plasmids are easily transferred between bacteria via conjugation
- This **makes the use of antibiotics less effective** in disease-causing bacteria
- Slower process for identification of transformed bacteria as well

→ So… use other methods to identify modified bacteria



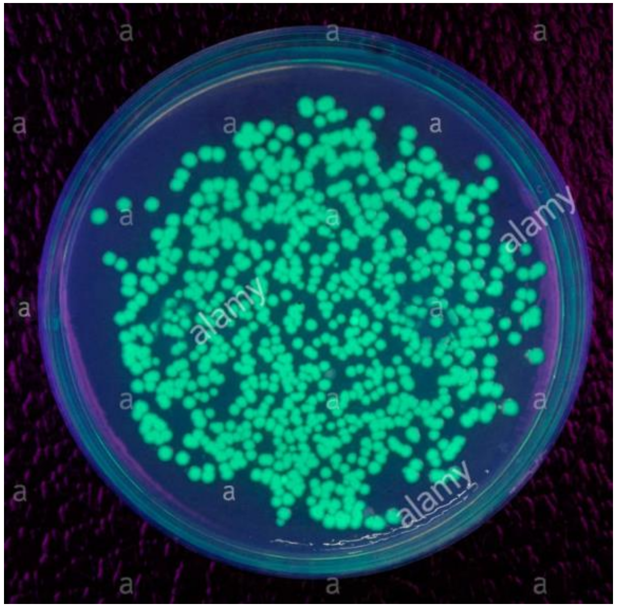


b) Use Green Fluorescent Protein (GFP)

- Gene for fluorescent substances used as marker
  E.g. gene for Green fluorescent protein (**GFP**)

- From jellyfish
- **GFP emits bright green light**
- **When exposed to UV**

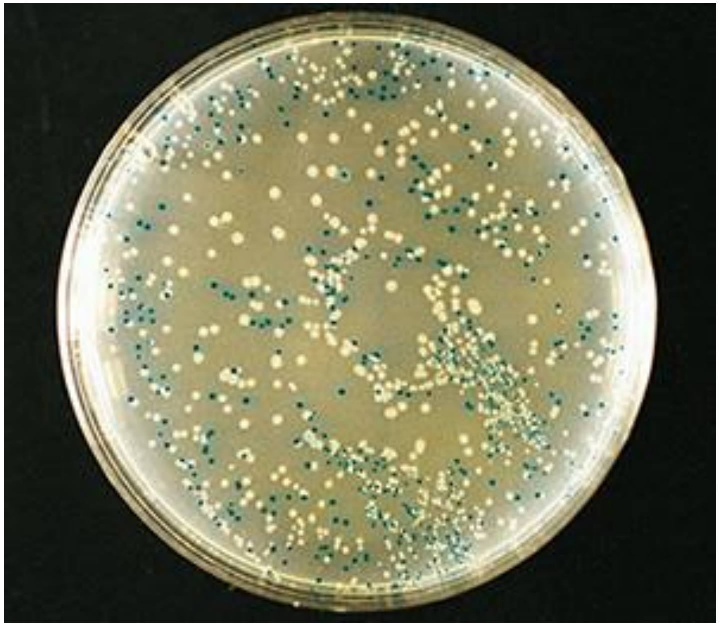
- **Bacteria with plasmid will express GFP and will appear green**

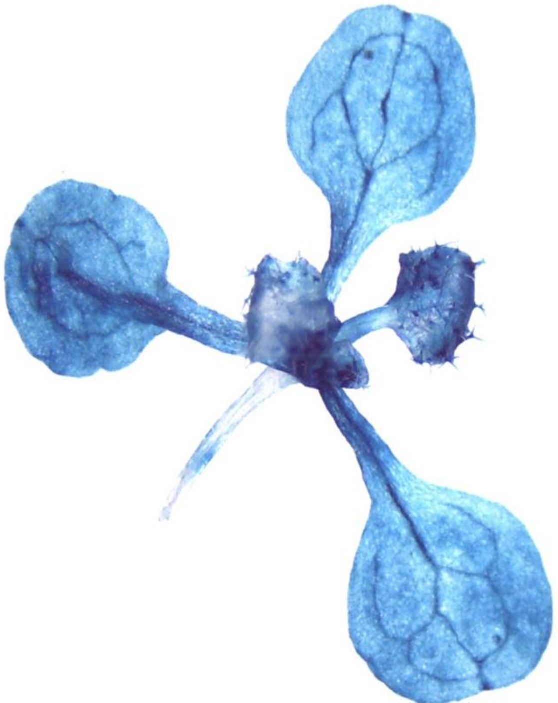


c) Use an easily stained substance

- Use gene that codes for easily stained substances as marker
  E.g. gene for **β galactosidase** aka *lacZ* gene
  E.g. gene for **GUS** enzyme

- Enzyme splits a special non-blue substrate into **product that is blue**
- **Bacteria with plasmid will become blue**





Advantages of using genes for fluorescent / easily stained substances as markers

- **Avoid use of antibiotics**
- More **economical** / time saving / labour saving
- **Visible colour** is easy to identify / detect
- Enable identification of transformed cells AND **transgenic organisms**
- **No known risk** / ill effect on GM organism

6. Grow bacteria in fermenters for large-scale production

- Allow transformed bacteria cells to **multiply/clone**
- **Grow in large-scale culture / fermenter**
- *E. coli* can divide once every 20mins

- Bacteria produces multiple copies of the **gene / protein product** (in this case, human insulin)
- Bacteria has **replication, transcription and translation machinery** to copy the gene and express the protein product
  (e.g. DNA polymerase, RNA polymerase, ribosomes)

- Insulin **extracted and purified** to be sold on market

Advantages of Recombinant DNA Technology

- **Conventional method** = extract insulin from pancreas of pigs or cattle

Advantages of producing human insulin by gene technology:

1. **Chemically identical** to human insulin
   → exact fit to insulin receptors on target organs
   → does not stimulate the immune system
   → faster response
   → fewer side affects
   → less / no risk of allergic reaction
2. **Effective in people who have developed tolerance** to animal-derived insulin
3. **Avoid ethical issues** related to religion & use of animal products
   → no killing of animals
4. **Lower cost** of purification and processing
5. **Mass production** = **large** and **constant reliable supply** all year round
6. **Less risk of contamination/infection**
   → no risk of transfer of disease
7. Potential to engineer / improve recombinant proteins

Applications of Recombinant DNA Technology

- **Production of pharmaceuticals**

→ **No modifying of protein in bacteria** (bcs no membrane-bound organelles)

→ Can **genetically modify eukaryotic cells** to produce human proteins
E.g. yeast cells, insect larvae cells, mammalian cells

Human Protein	Treatment for	Produced in
Human insulin	Diabetes	Recombinant bacteria
Factor VIII	Helps blood clot in haemophilia patients	GM hamster kidney and ovary cells (in fermenter)
Adenosine deaminase (ADA)	Help in development of B & T cells in severe combined immunodeficiency disorder (SCID) patients	GM insect larva of the cabbage looper moth
Antithrombin	Stop blood coagulation	Milk of GM goats
Alpha-antitrypsin	Emphysema	Milk of GM sheep

- **Used in genetic engineering to produce GMOs in agriculture**

Genetic Engineering

- Manipulation of genetic material to modify specific characteristics of an organism
- **Extraction of genes from one organism** / synthesis of genes
- In order to **place them in another organism**
- Of the same or diff species

New hosts:

- Have **recombinant DNA (rDNA)** = combination of DNA from two or more sources
- Expresses the new **gene product / protein**

- **Genetically modified (GM) / transgenic organisms**
  = organisms with any altered DNA

Gene Editing

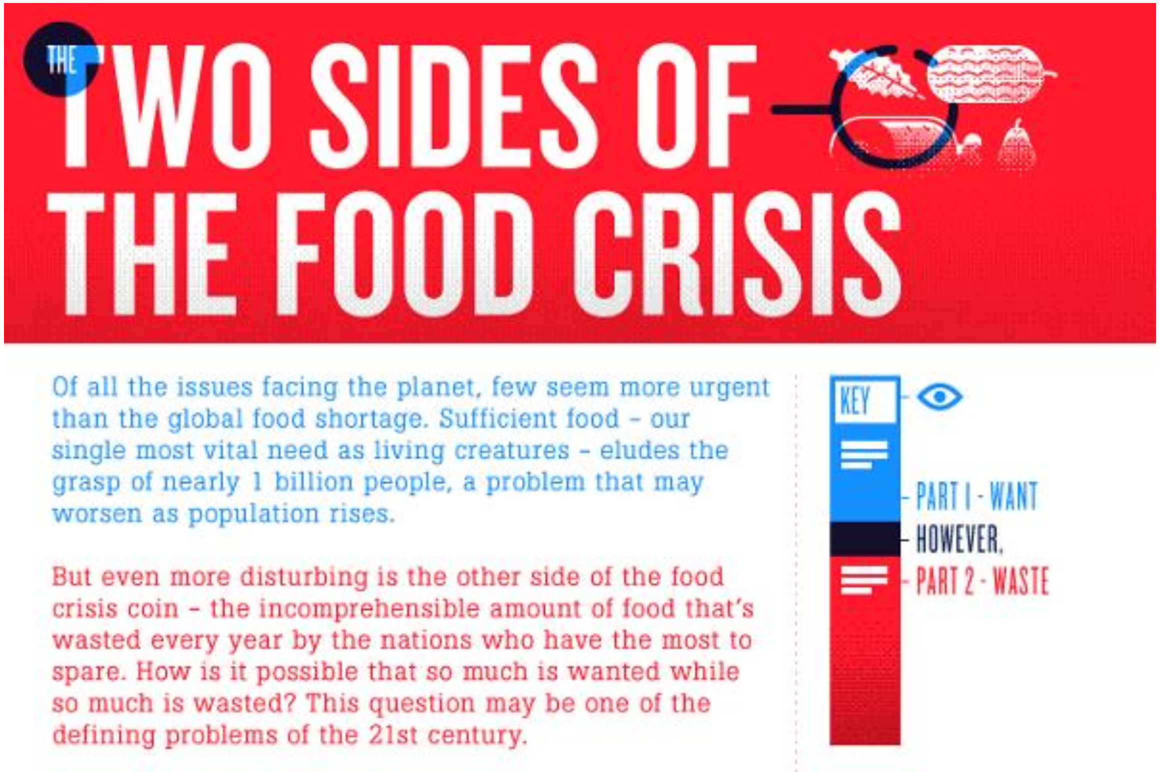
- Form of genetic engineering
- Involving the **insertion, deletion or replacement of DNA**
- **At specific sites** in the genome

GMOs in Agriculture

Aims of using genetic engineering to produce GMOs in agriculture:

- **improve quality** of crop plants and livestock
- **Increase yield** of crop plants and livestock

→ **Solve world food demand**



GMOs in Agriculture

Livestock can be engineered to:

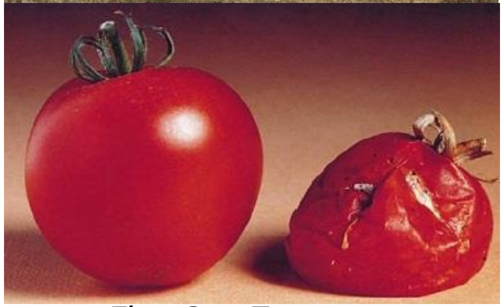
- Have high growth rate
- Grow larger
- Have higher yield (e.g. milk, meat)

Crop plants can be engineered to:

- Have higher yield
- Better quality / taste
- Delayed ripening of fruits (to increase shelf life)
- Additional nutritional benefits
- Resist disease/pests/insects (so less pesticides used)
- Resist herbicides (to reduce competition from weeds)
- Grow in adverse conditions / more tolerant to climate change (e.g. high salinity, dry, hot, cold climates)
- Grow in poor quality land, require less fertilizer

→ More cost effective / have health benefits

→ Less effect on food chain / pollinators



Flavr Savr Tomatoes

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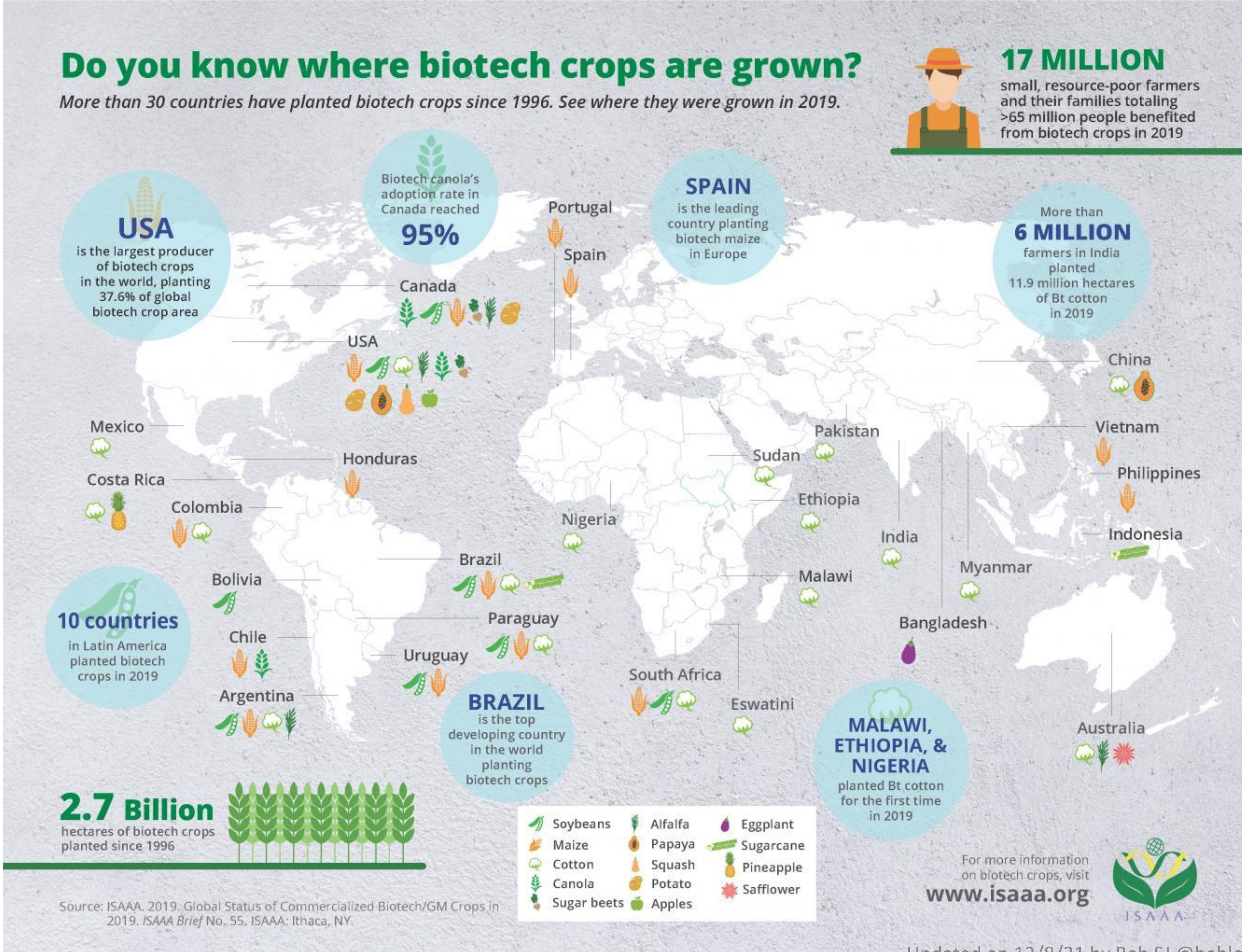

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GMOs in Agriculture

Why use GMOs instead of artificial selection?

- Much **faster** results
- Allows the **retention of other desirable characteristics** of best varieties of species
- Able to use best genes from other species of **plants and even non-plants**

But…

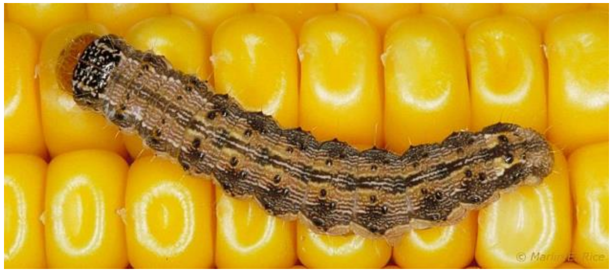
- Complicated process
- Expensive
- Not always successful

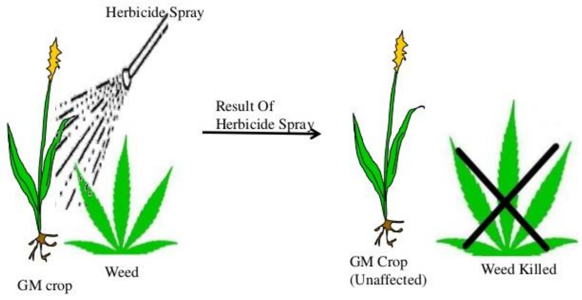
GMOs in Agriculture

Important examples:

- **GM salmon**
- Vitamin A enhanced rice (**Golden rice™**)
- **Insect resistance**
  E.g. Bt maize, Bt cotton
- **Herbicide resistance**
  E.g. maize, cotton, tobacco, oil seed rape

Ethical and social implications of using GMOs in food production

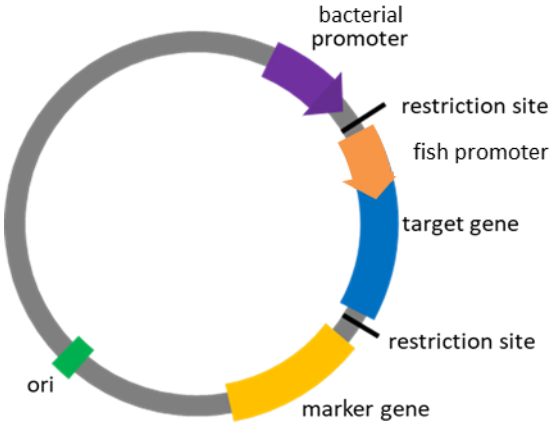




GM Atlantic Salmon

- AquAdvantage® salmon
- Grows to market weight in about 16-18 months
- Normal salmon needs 32-36 months

- Genes for growth hormone regulator transferred from **diff species** of salmon
- Genes for promoter from another **diff species**



Benefits:

- **High yield**
- **Consistent** yield all-year round
- **Conserve wild fish populations**
- All modified salmon eggs are triploid and sterile

→ so impossible for them to breed among themselves and with other salmon

→ eliminate impact to wild population

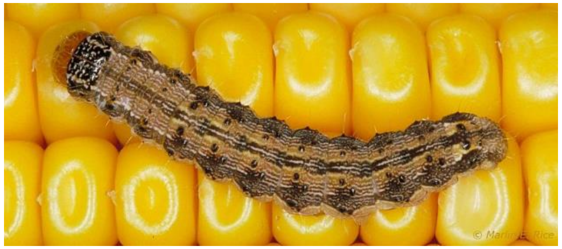
Insect-Resistant Crops

Bt Crops

E.g. Bt cotton, Bt maize

- **Bt cotton** protected against **boll weevils**

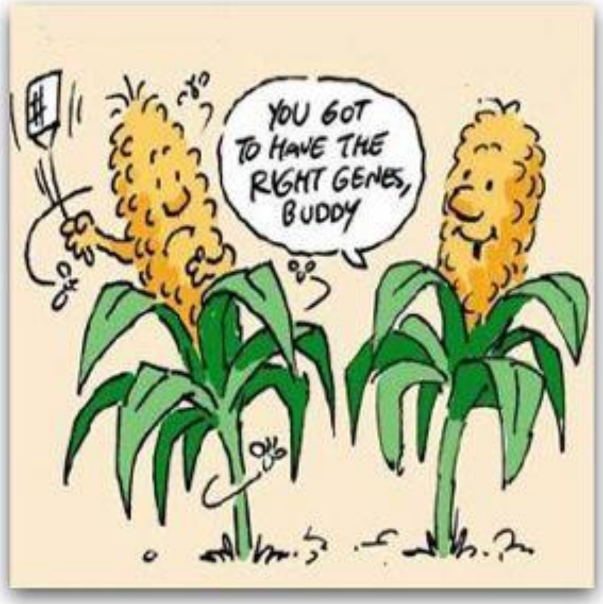
- **Bt maize** protected against **corn borer** caterpillars
- Corn borer eats leaves and burrows into stalk
  → plant cannot support the ears of corn



- Gene for **Bt toxin** from bacteria ***Bacillus thuringiensis*** inserted into maize / cotton
- **Lethal to insects** but not other animals
- Crop able to produce own insecticide

Insect-Resistant Crops

Bt Crops



Benefits of Bt Crops:

- **Increased yields**

- **Only kills specific insects that eats it** and does not kill beneficial insects
  E.g. pollinators / bees / predators of pests

→ Conserve biodiversity / food web

- **Less pesticide used**

→ Reduce risk of pesticide affecting other non-target species in the same environment

- Less risk of harm to humans from spray drift / pesticide residues on food

Herbicide-Resistant Crops

Glyphosate-resistant crops

E.g. tobacco, oil seed rape, Roundup Ready soybean

- Genetically modified to be **resistant to herbicides containing glyphosate** (e.g. Roundup)
- Glyphosate inhibits enzymes involved in amino acid synthesis

- Gene from bacterium *Agrobacterium* coding for enzymes with same function that are not affected by glyphosate
- Herbicide resistant gene acts as marker gene

→ **Herbicides will have no effect on plant, only the weeds**

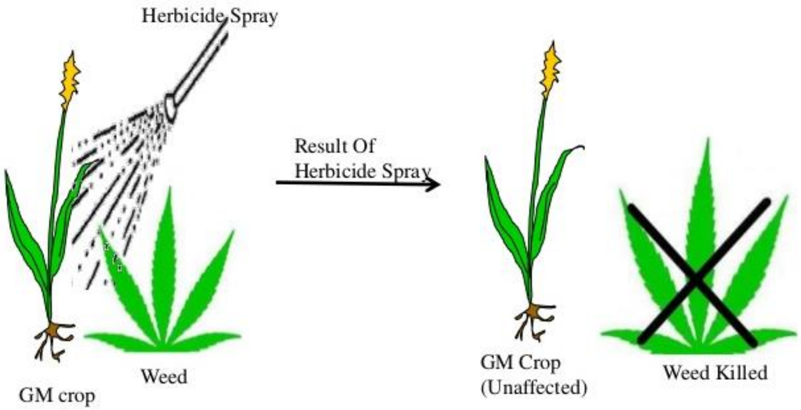
Advantages:

- Can control/kill weeds

→ reduce competition

→ increase yield

- Less manual weeding needed



Herbicide-Resistant Crops

Glyphosate-resistant crops

Disadvantages:

Environmental

1. Intensive use of herbicides

→ High toxicity of more herbicide left after use

→ harmful to humans

2. Cross-pollination with wild plants / organic crops

→ Result in new more resistant weeds = "**superweeds**"

→ Superweeds are selected for by herbicide use

→ Outcompete and lose traditional varieties of plants

→ **Loss of genetic diversity**

→ Cause effect on rest of food chain = less food or shelter for other species

https://www.thestar.com.my/news/nation/2018/01/16/farmers-go-against-the-grain-weed-interbreeding-sees-clearfields-future-grow-cloudy/

Solution: Use **sterile seeds/plants** to avoid superweeds

Herbicide-Resistant Crops

Glyphosate-resistant crops

Disadvantages:

Economic

- **High cost of GM seeds**

→ Seeds are sterile and cannot use own seed

→ Too expensive for people to buy / farmers to sell

→ Difficult for farmers in developing countries to obtain GM seed

→ Might reduce efforts to relieve poverty

→ Under-developed countries become more dependent on other counties

- **Cost of herbicide**

→ cost of problems with **pollution**

→ cost of **human health problems**

→ loss due to contaminated crops of organic farms

Other ethical and social implications of using GMOs in food production

- **GM production cost is expensive**

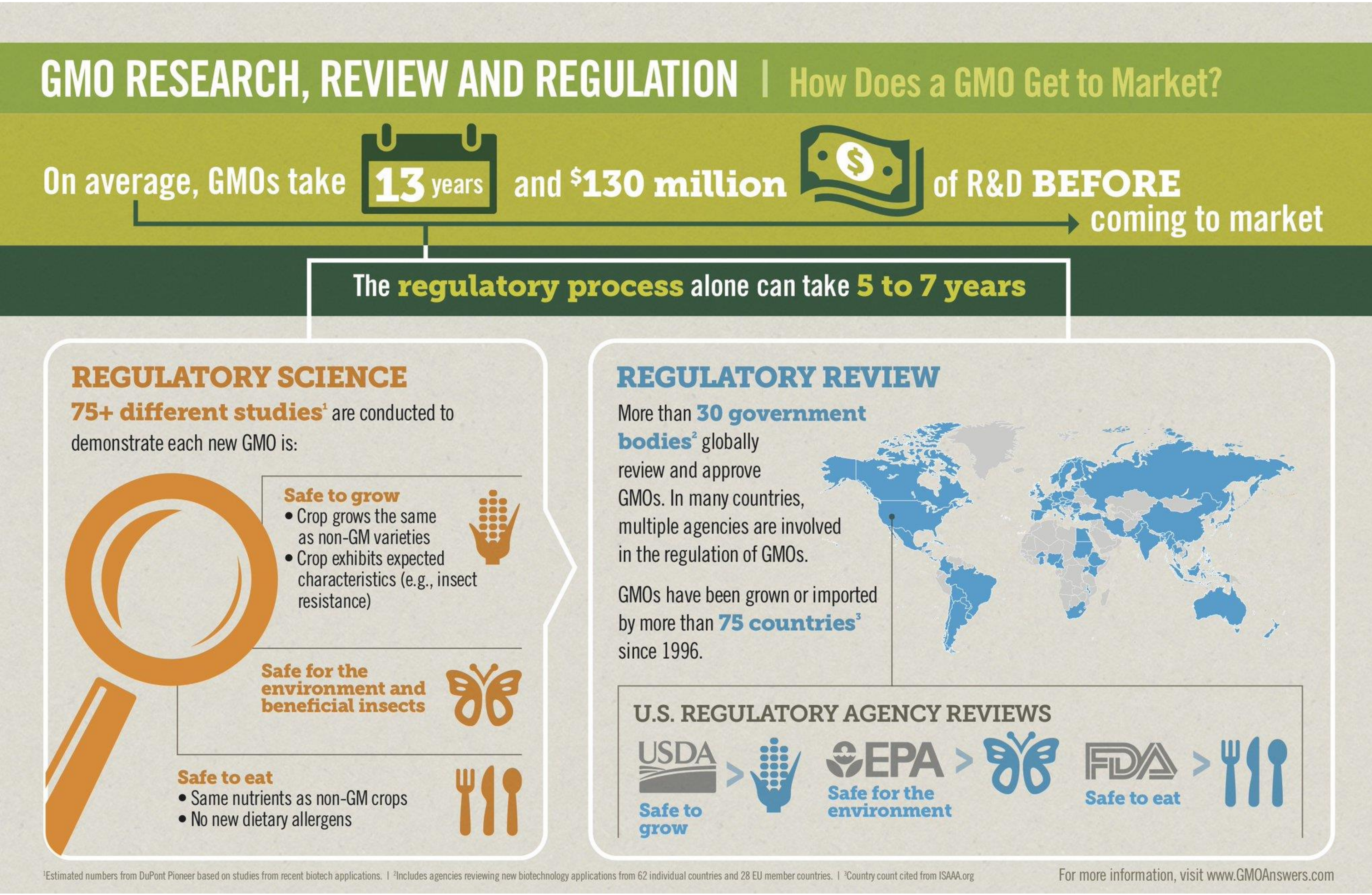
→ Does it outweigh benefits? It has only 1% success…

- **Monopoly** / too much power held by multinational companies

- People may avoid / refuse to buy for GM food

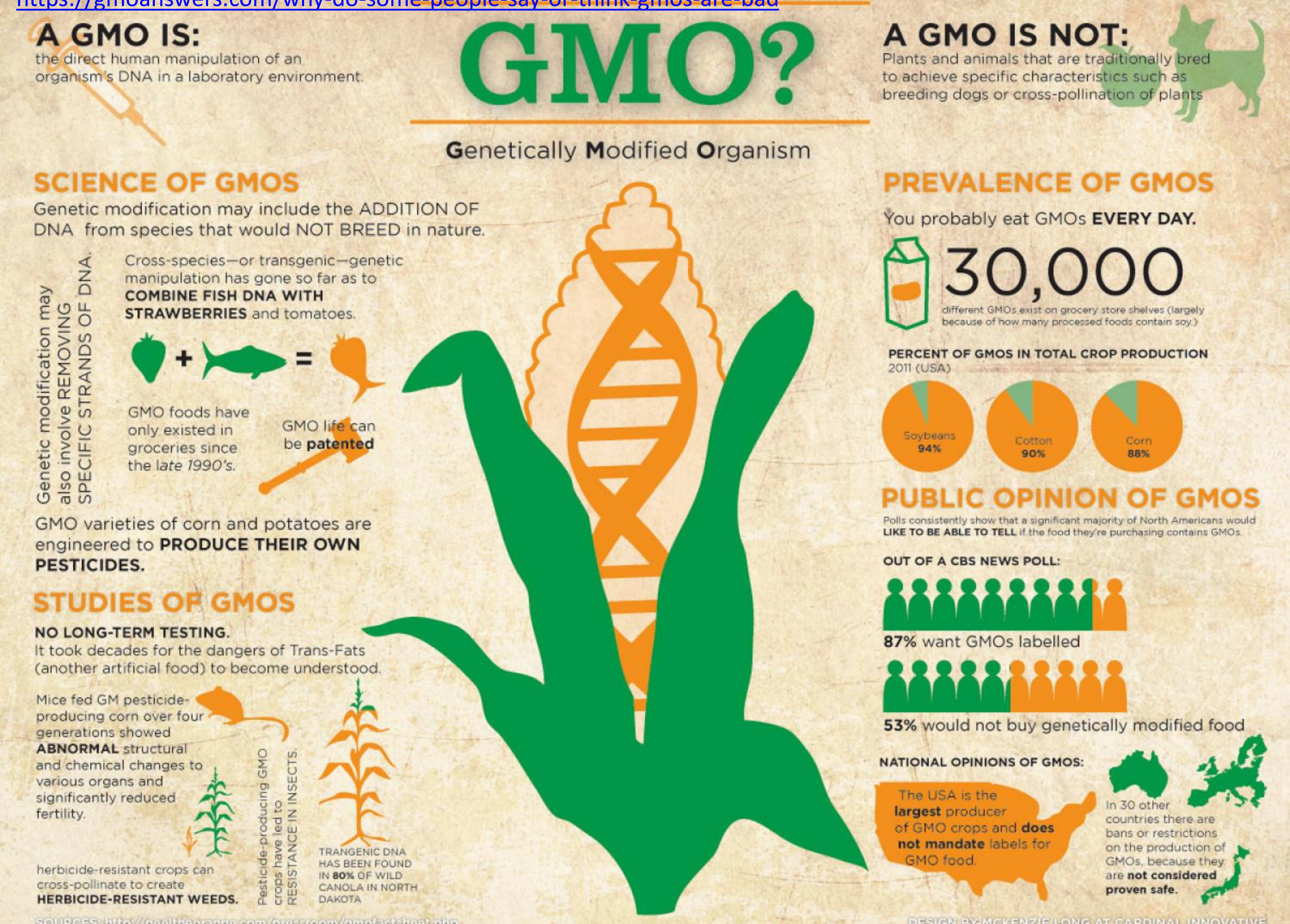
- No long-term studies done on effects on **human health**
- Possible **allergic** reactions in humans / adverse effects on the immune system

https://www.youtube.com/watch?v=7TmcXYp8xu4



Updated on 12/8/21 by Beh SJ @behlogy

https://gmoanswers.com/why-do-some-people-say-or-think-gmos-are-bad



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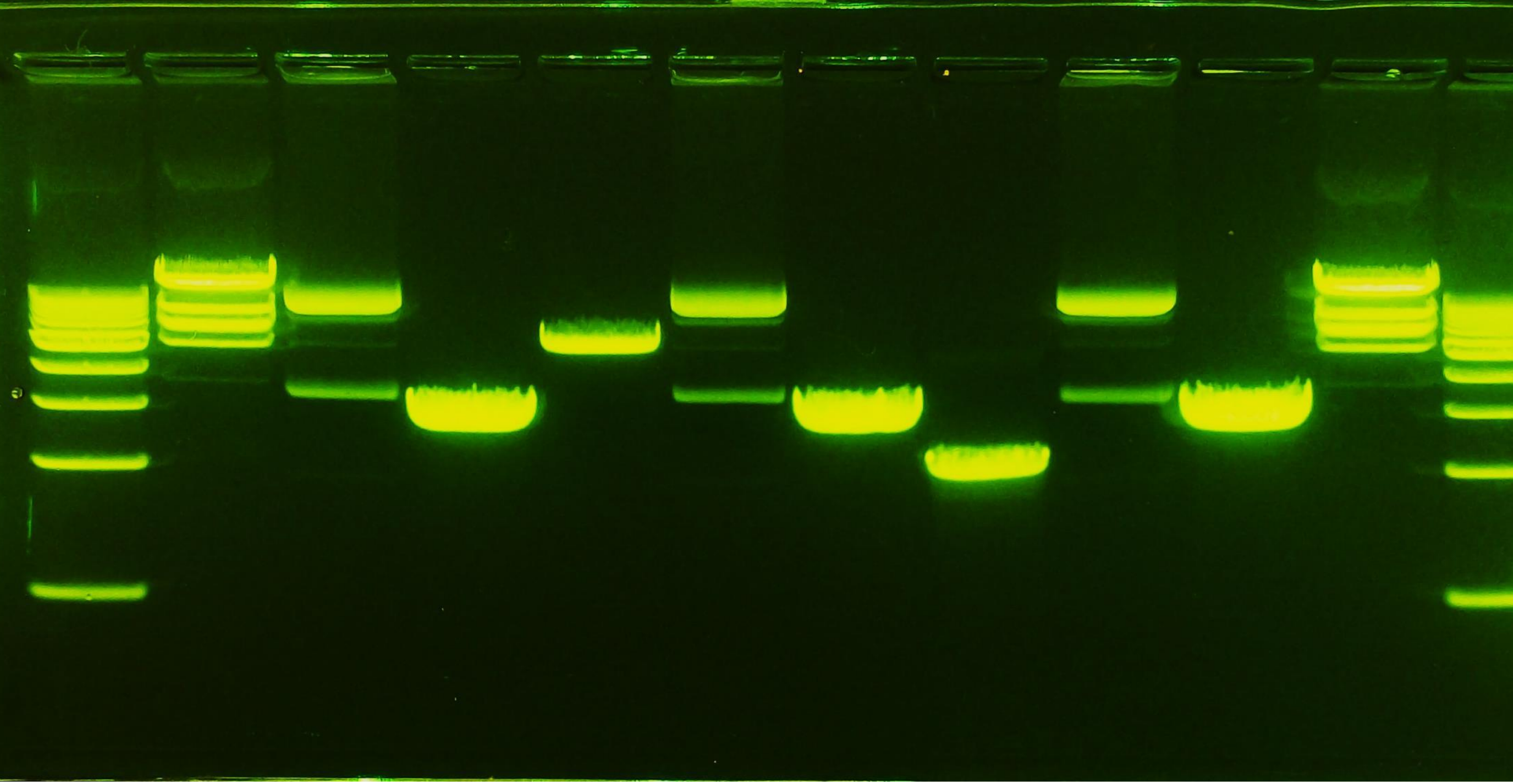

Genetic Technology 3

GEL ELECTROPHORESIS

Function: To separate fragments of DNA according to length OR

To separate diff proteins according to mass/charge

Updated on 12/8/21 by Beh SJ @behlogy

Chapter Outline

Technology	Function	Application
3. Gel electrophoresis • For protein and for DNA • Probes	**To separate fragments of DNA according to length** OR **To separate diff proteins according to mass/charge** Distinguish btwn alleles of a gene	**Genetic fingerprinting / DNA profiling** • Paternity testing • Criminal Investigations **Genetic screening** • Breast cancer (*BRCA1*, *BRCA2*) • Genes for haemophilia, SCA, Huntington's Disease and CF **Gene Therapy** • Vectors (viruses, liposomes, naked DNA) • SCID, inherited eye disease, CF • Social and ethical considerations

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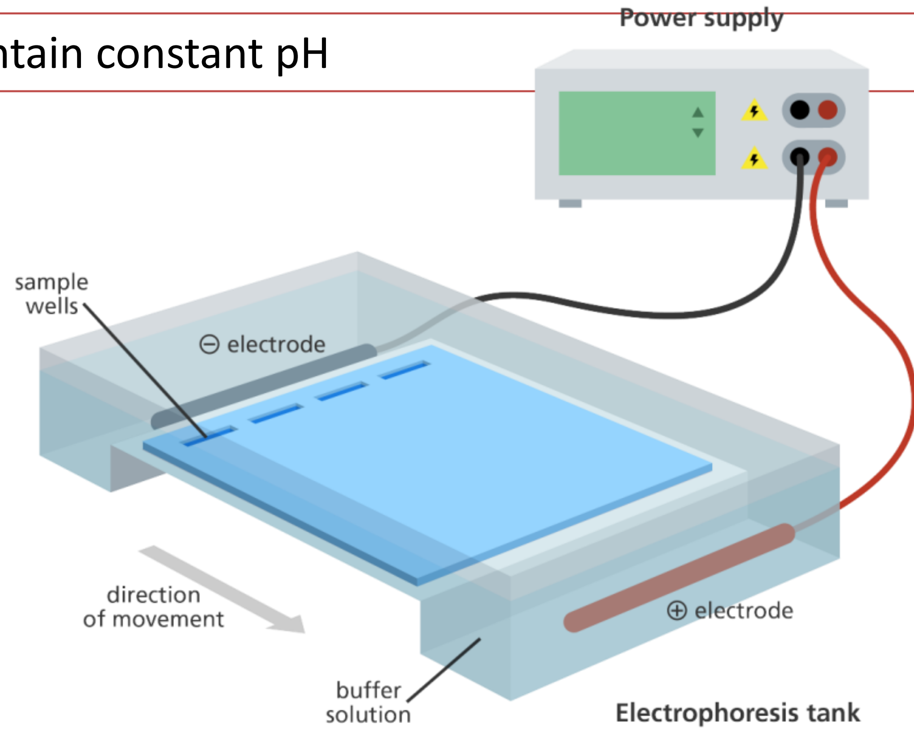
Gel Electrophoresis

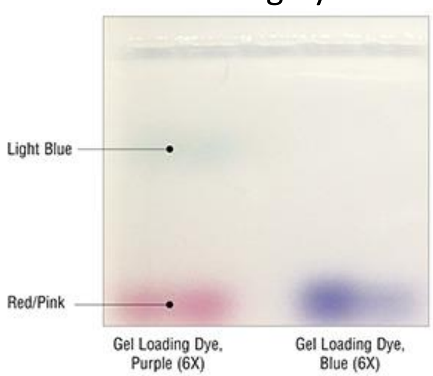
Components	Functions
Agarose gel (DNA) or Polyacrylamide gel (proteins) with wells	Diff gel, diff impedance Even spaces between the gel molecules so DNA / protein can move within gel properly Wells to load DNA / protein
Electrodes + electric field	Molecules with net negative charge will move towards positive electrode
Buffer solution	Maintain constant pH



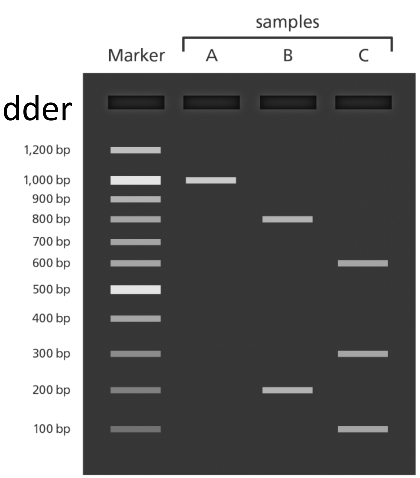
Gel Electrophoresis

Components	Functions
Loading dye	Visible dye to track DNA migration → Ensure electrophoresis is stopped before DNA / protein runs off the gel
Staining agent	Dye that stains the colourless DNA / protein E.g. Ethidium bromide causes DNA to fluoresce under UV light
DNA ladder or protein ladder	Has DNA fragments or substances of known lengths / mass → Can estimate the length / mass of samples by comparing them to the ladder
Gene probes (DNA)	To locate a specific DNA sequence

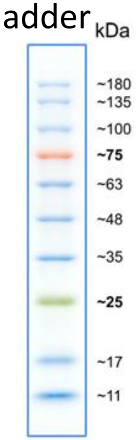
Loading dye



DNA ladder



Protein ladder

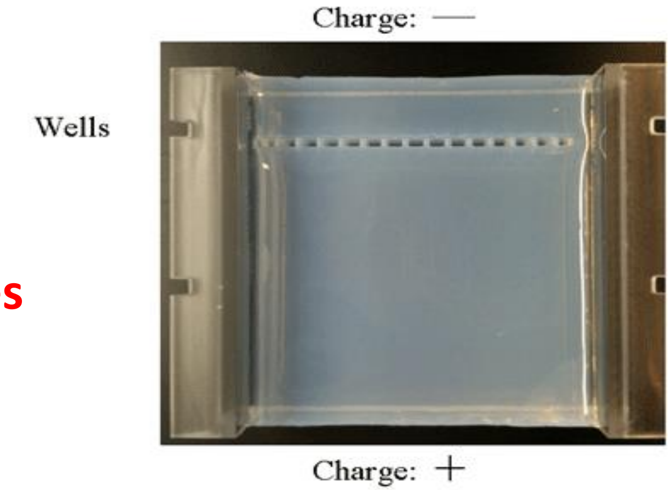


Gel Electrophoresis of DNA

Procedure:

1. **Obtain sufficient quantity of DNA**

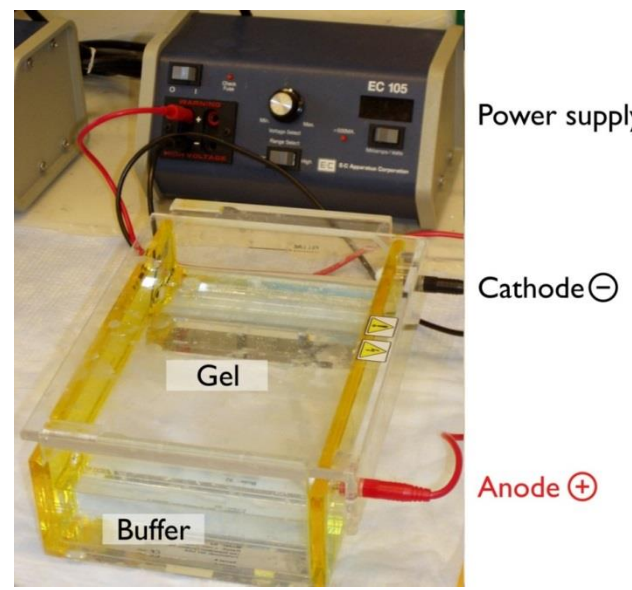
- DNA can amplified using PCR or gene cloning in bacteria



2. Cut DNA into fragments using **restriction enzymes**

- Many DNA fragments of diff lengths produced

3. DNA samples are mixed with **loading dye** and **staining agent**



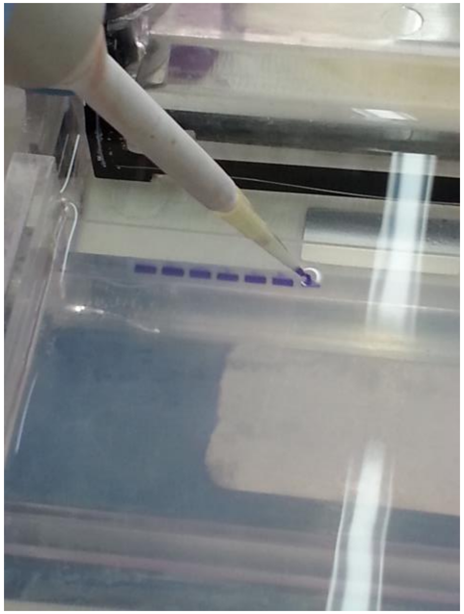
4. Gel is covered with **buffer** solution

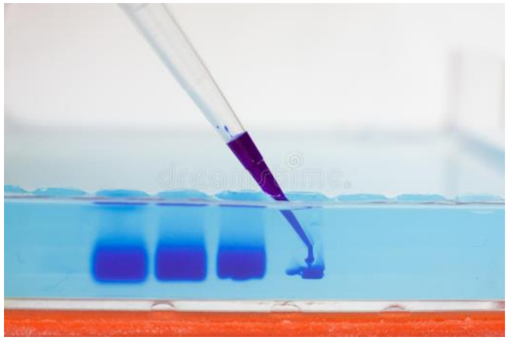
Gel Electrophoresis of DNA

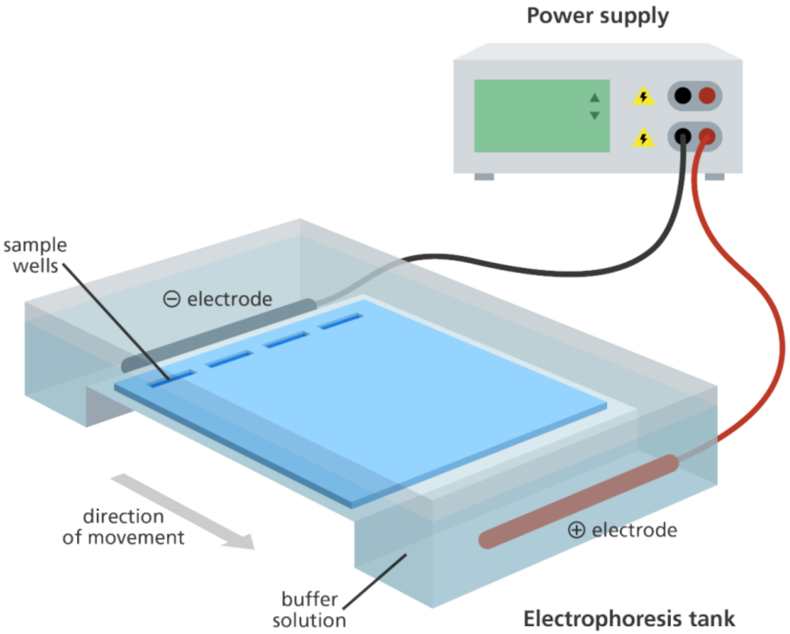
5. DNA fragments are loaded into **wells at the negative end** of the gel (cathode)

- **DNA ladder** with DNA fragments of known lengths is usually run through the gel at the same time

6. A **direct current** is applied through the gel





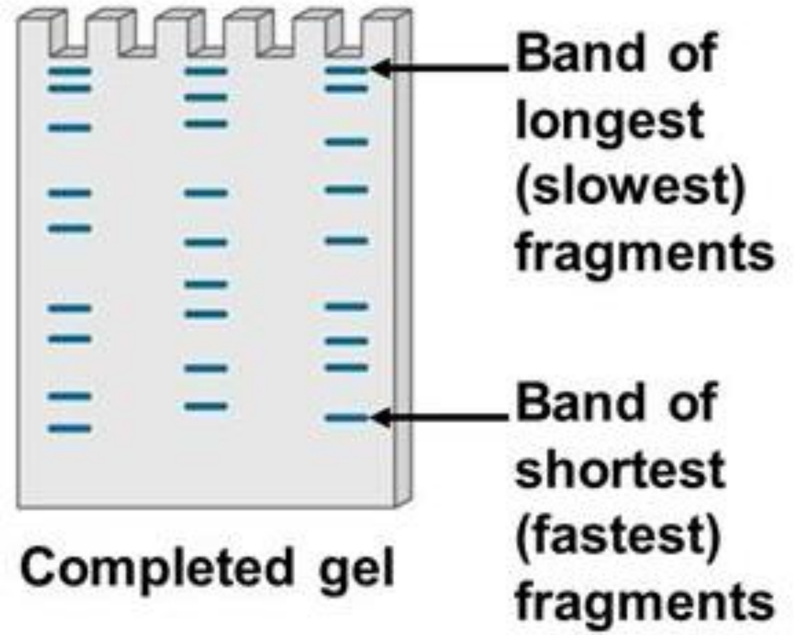


Gel Electrophoresis of DNA

7 . DNA is attracted **towards the positive electrode** (anode)

- Separation due to electric field / potential difference
- Bcs DNA is **negatively charged** due to the phosphate groups

- Gel acts a molecular sieve
- **Shorter fragments of DNA move faster and further per unit time through the gel than larger fragments**
- Due to less impedance of gel
- DNA fragments separate and arranged in order of size

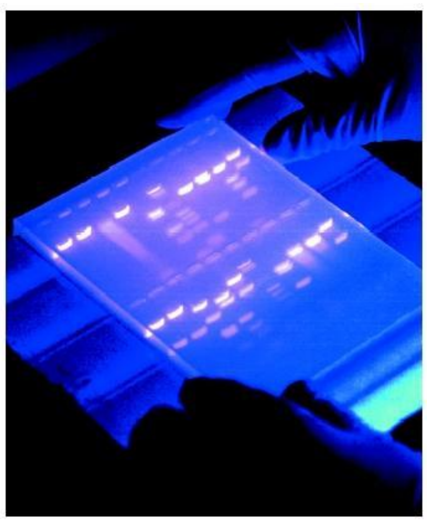


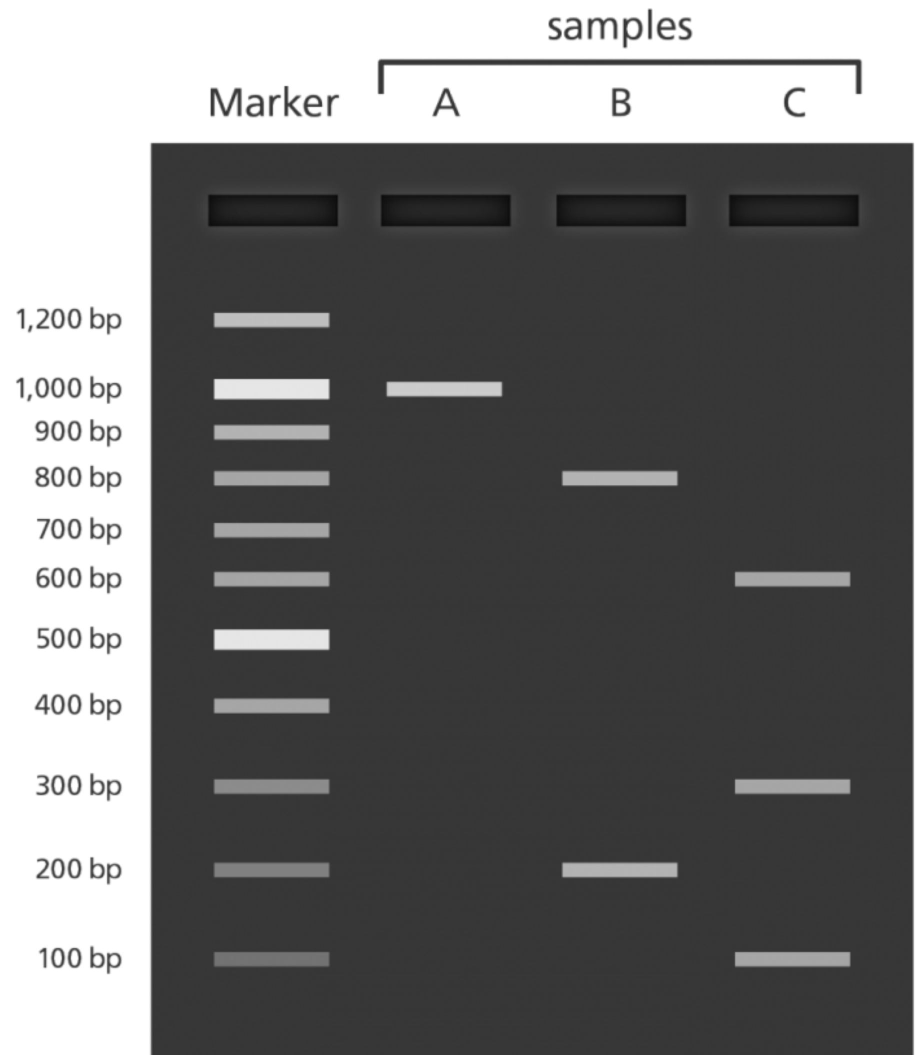
Gel Electrophoresis of DNA

8. **Visualise bands under UV light**

- Staining agent causes DNA to fluoresce
- Each band is millions of DNA pieces of the same size

- Compare position with reference DNA to estimate length of fragments





Simulation = http://learn.genetics.utah.edu/content/labs/gel/

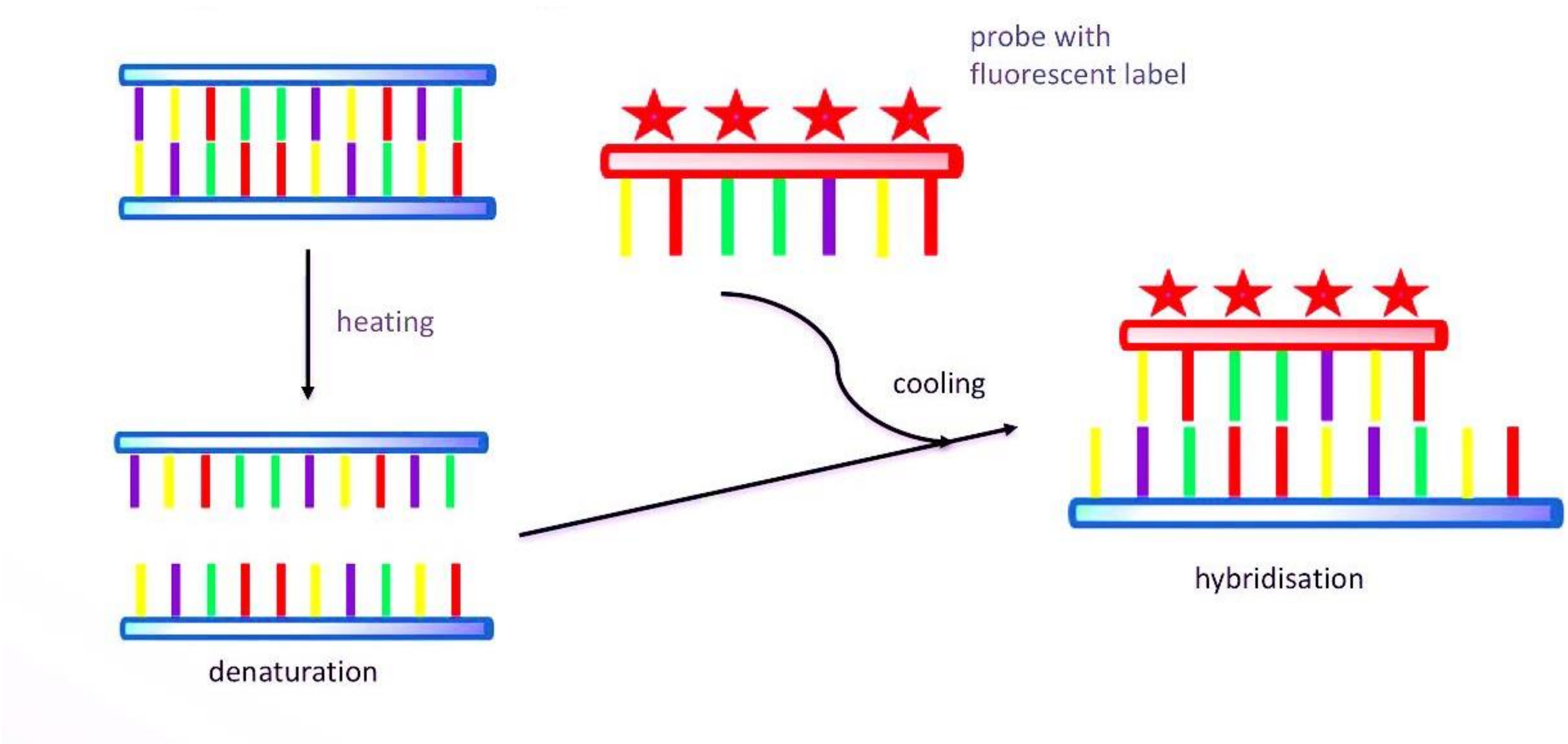
Gel Electrophoresis of DNA

P5

9. **DNA pieces transferred to membrane** / nylon / nitrocellulose / absorbent paper

- This is called Southern blotting

10. DNA **heated** to separate strands



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Gel Electrophoresis of DNA

11. DNA cooled and **gene probes** are added

- Gene probes are used **to locate a specific DNA sequence or gene**
- Gene probes = **short, single stranded DNA (ssDNA)**
- ‘Labelled’ / attached with **radioactive / fluorescent** substance
- Can **complementary base pair** with **specific** DNA fragments

12. View DNA fragments **under UV light / X-ray film**

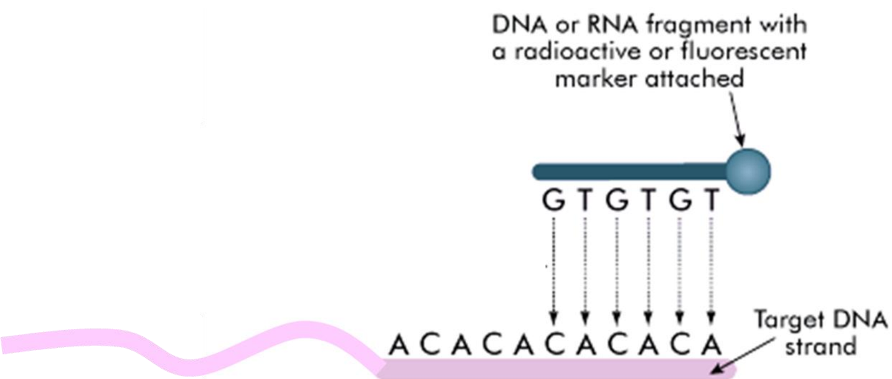
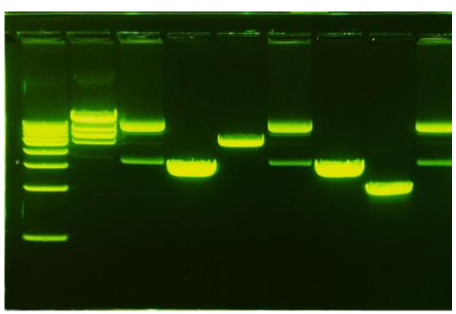


Diagram of a gene probe

Radioactive Phosphorus Isotopes Probes on an X-ray film



Fluorescent probes under UV light

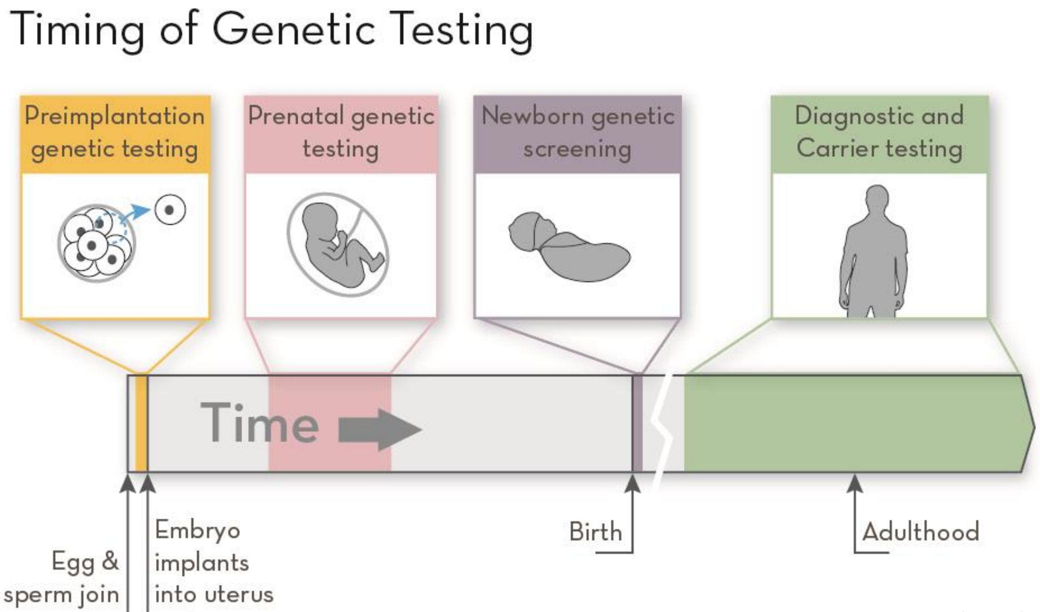
Applications of DNA Gel Electrophoresis

- Can use to **distinguish between the alleles of a gene**
- **Genetic fingerprinting / DNA profiling**
  - Paternity testing
  - Criminal Investigations
- **Genetic screening**
  - Breast cancer (*BRCA1*, *BRCA2*)
  - Genes for haemophilia, SCA, Huntington's Disease and CF
- **Gene Therapy**
  - Vectors (viruses, liposomes, naked DNA)
  - SCID, inherited eye disease, CF
  - Social and ethical considerations

Genetic Screening

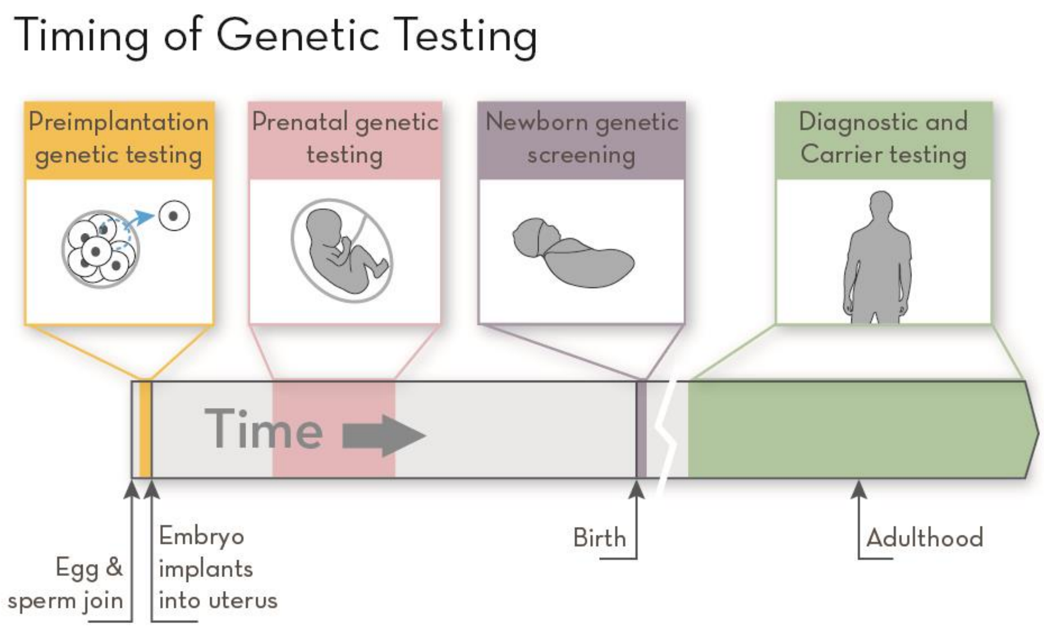
- Aka genetic testing
- **Analysis of a person's DNA to check for presence of a particular allele**
- DNA obtained from **tissue samples** (e.g. blood)
- Can be carried out on embryo, fetus, newborn or adults

- Important examples: breast cancer, haemophilia, sickle cell anaemia, Huntington's disease, cystic fibrosis (CF), Down syndrome



Genetic Screening

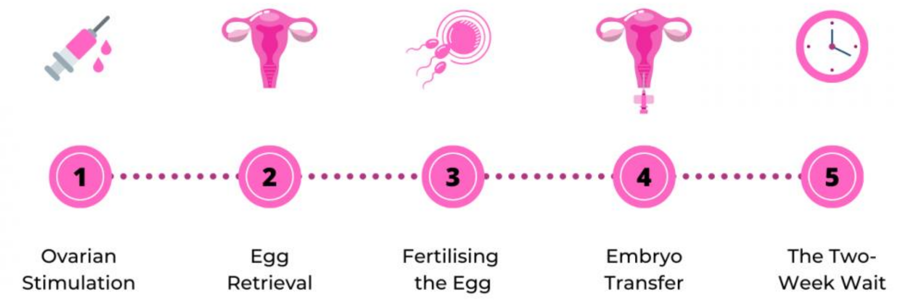
Type of Genetic Screening	Who is it for?
1. Pre-implantation genetic diagnosis (PGD)	Newly formed embryo from IVF Before implantation into uterus of mother
2. Prenatal screening	Unborn children / fetus
3. Newborn screening	Newborn babies
4. Carrier screening	Individuals with a family history of a genetic condition / cancer Potential parents



In vitro fertilisation (IVF)

Chap 18 Recap

1. **Hormone** treatment used to induce **superovulation**
2. Many oocytes harvested from female
3. Obtain fresh / frozen sperm
4. **Oocytes mixed with sperm**
   – Ideally genetically different
5. Conduct genetic test and select embryos that are most likely to survive
6. **Embryo transfer!**
   – Embryo implanted in uterus of **donor female**
   – OR may use similar species / non-rare breed as **surrogate mother**
   – OR **freeze embryos** and store for long time in frozen zoo



E.g. IVF + Frozen Zoos: https://www.youtube.com/watch?v=0UsoDqECYb8

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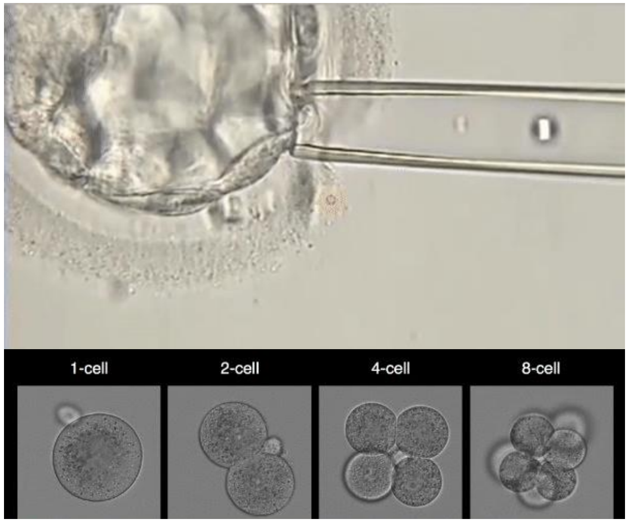
Genetic Screening

6. Pre-implantation genetic diagnosis (PGD) and preselection of embryos

1. Pre-implantation genetic diagnosis (PGD)

- Newly formed embryo from ***in vitro* fertilisation (IVF)**
- Tested before implantation into uterus of mother



Procedure

1) **Embryo biopsy** = removing a cell from an embryo
- At 8-cell stage (day 4/5)

2) **PCR** to amplify DNA

3) **Gel electrophoresis**
- Analyse DNA for faulty alleles to determine if embryo has genetic disease
- E.g. haemophilia, Huntington's disease, cystic fibrosis

4) **Pre-selection** = select only embryo without faulty alleles for implantation

1. Pre-implantation genetic diagnosis (PGD)

Social or ethical implications:

- Identify whether **embryos from IVF** have a genetic condition

→ Avoid implantation of embryos with faulty alleles

→ Allows couples to have children who would otherwise choose not to

BUT…
****It's controversial!****

Ethical concerns:

- **Embryos might be destroyed** if not pre-selected for implantation
- Could lead to selection based on gender or specific traits ("designer babies")
- Contrary to beliefs / values
- Genetic disease may not develop

2. Prenatal screening

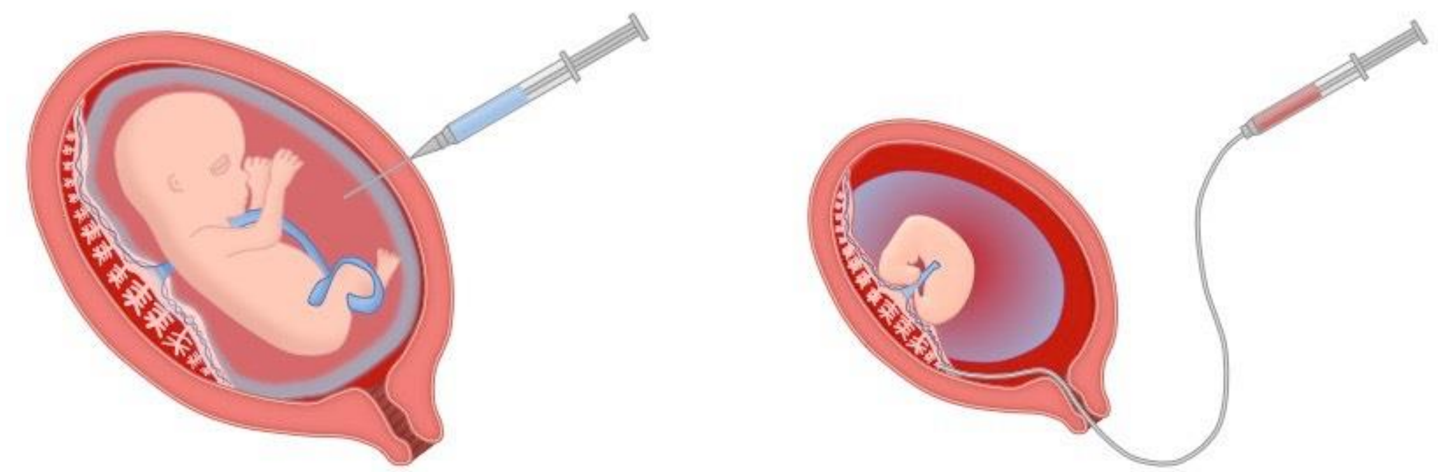
Methods to obtain tissue samples:

(1) amniocentesis

https://www.webmd.com/baby/video/amniocentesis

(2) chorionic villus sampling (CVS)

https://www.mayoclinic.org/tests-procedures/chorionic-villus-sampling/multimedia/chorionic-villus-sampling-video/vid-20121056



Amniocentesis
Needle used to withdraw
foetal cells from *amniotic fluid*

Chorionic Villi Sampling
Suction tube used to remove foetal cells
from *chorion* (where placenta will develop)

2. Prenatal screening

Advantages

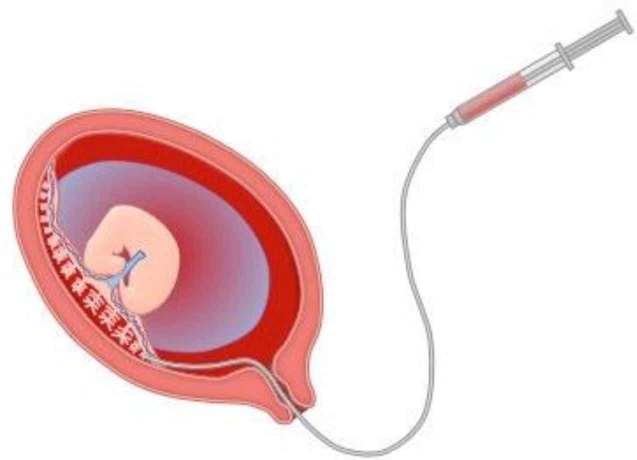
- Helps to provide **early diagnosis** for **fetuses in utero**

→ So can give **early treatment** when born

→ Allows parents to **prepare** for the birth of a child who will need treatment for a considerable time or even throughout life

→ Women can avoid late **therapeutic abortions** = terminate pregnancy for medical reasons

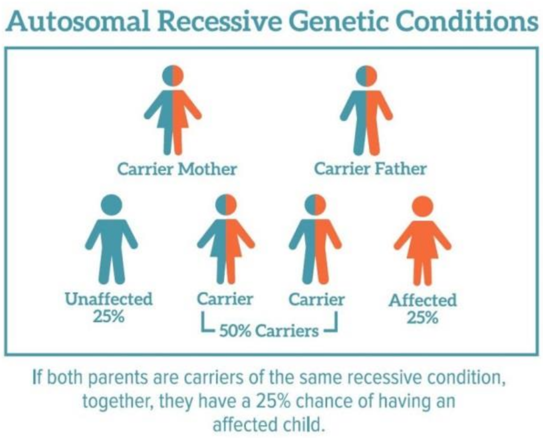
Amniocentesis
Needle used to withdraw foetal cells from *amniotic fluid*



Chorionic Villi Sampling
Suction tube used to remove foetal cells from *chorion* (where placenta will develop)

3. Newborn screening / 4. Carrier Screening

E.g. haemophilia, sickle cell anaemia, cystic fibrosis (CF), Huntington's disease, Down syndrome

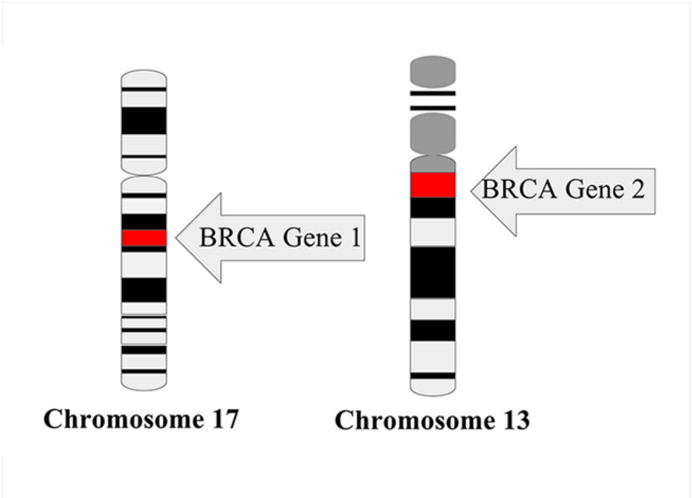


E.g. breast cancer

- Faulty alleles of the ***BRCA1*** and ***BRCA2* genes**
- Increase the **chance of developing breast cancer**
- High risk

If tested positive for the faulty alleles:

- Increased testing to detect cancer early
- Elective mastectomy = removal of breast(s) before the occurrence / diagnosis of cancer

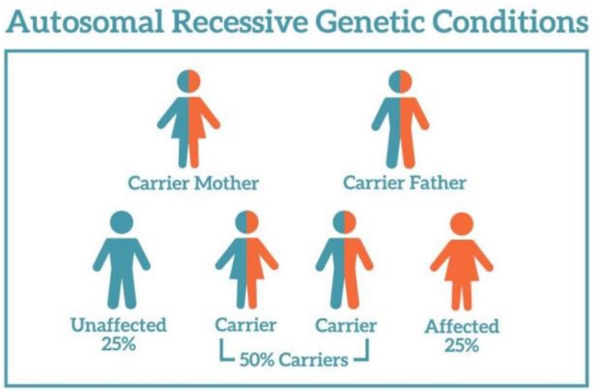


3. Newborn screening / 4. Carrier Screening

Advantages

- Can **identify carriers** of genetic conditions

→ Allows couples who are both carriers of a genetic condition to **choose not to have children**



If both parents are carriers of the same recessive condition, together, they have a 25% chance of having an affected child.

- Helps to provide **early diagnosis**
  e.g. Huntington's disease (symptoms only occur later in life)

→ can prepare for the future:

1. if present, enables **lifestyle change / early treatment / regular check-ups**
2. **preventative treatment** may be cheaper than treating disease itself

→ if tested negative, genetic screening **removes anxiety**

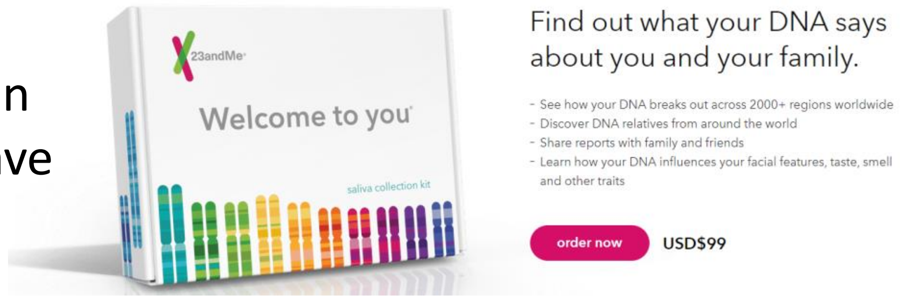
3. Newborn screening / 4. Carrier Screening

Disadvantages

- Test is expensive
- Many mutations still unknown so may still not lead to positive diagnosis

If person tested <u>positive</u>:

- For some diseases, **no treatment** is possible
- May lead to **anxiety**
- May experience **social / financial discrimination** (e.g. life insurance refusal)
- May still not develop diseases even though mutation is present
- Couple may decide to not have children OR couple may be tested *after* they have children



https://www.vox.com/recode/2019/12/13/20978024/genetic-testing-dna-consequences-23andme-ancestry

Genetic Counselling

Couple are referred to genetic counsellors if they:

- both have genetic disease (in family) or are carriers
- have history of recurrent miscarriages
- female is an older woman

Genetic counsellor can:

- run pedigree analysis + genetic screening
- explain results of tests / estimates chances of having affected child
- an provide couple with information on the risk of genetic diseases

Genetic counsellor may discuss/advice on options:

- termination or therapeutic abortion
- therapies / treatments (e.g. gene therapy)
- financial implications of having affected child
- the effect of having affected child on existing siblings
- other ethical issues

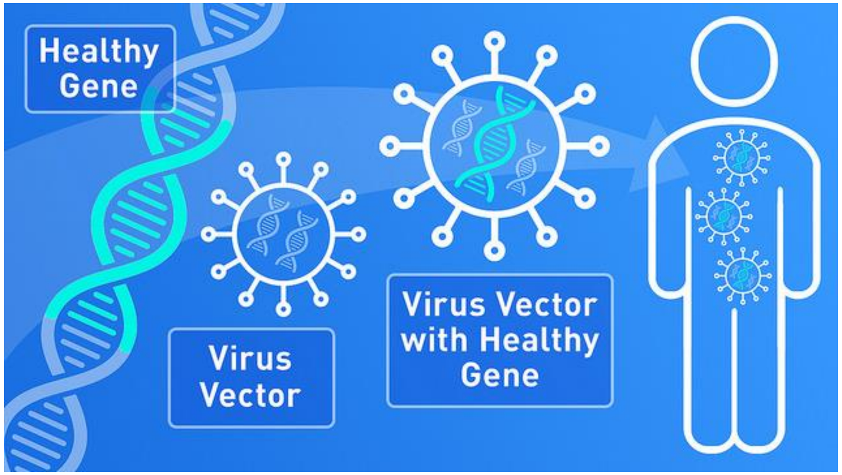
Gene Therapy

- To treat serious, common genetic diseases
- Caused by a ***single* faulty, recessive allele**
- By inserting **normal, dominant allele**
- Into affected cells of individual
- Via a **vector** (e.g. virus, liposome, or just naked DNA)
- Result = Host will have **recombinant DNA**

Aim:

- To obtain **functional normal polypeptide**
- Reduce symptoms of disorder
- Restore / enhance cellular functions
- **Increase quality of life / survival**

Note: Gene editing in gametes or embryos is illegal in humans! However, we can do it to somatic cells / animal gametes and embryos to make GMOs



e.g. SCID, cystic fibrosis, Leber's congenital amaurosis (LCA), thalassaemia, haemophilia

Gene Therapy

If faulty allele was **dominant**, gene therapy is **very, very difficult** because….

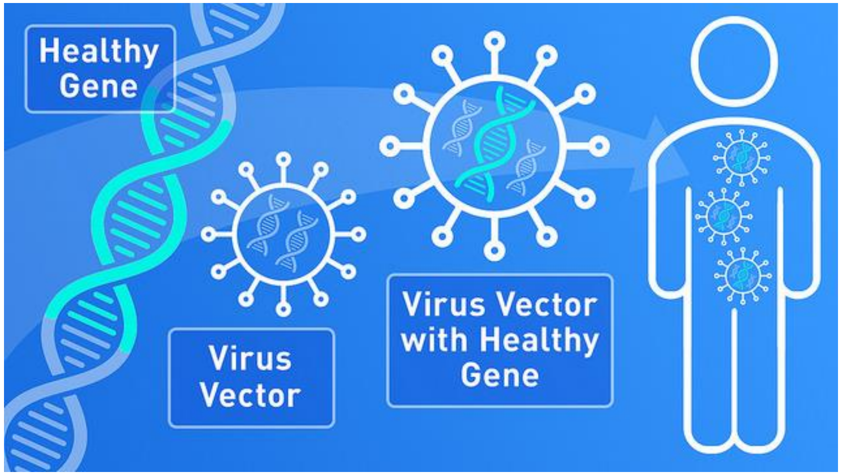
- It will still be **expressed even when the normal, recessive allele** is present
- We'll need to knock out / remove / replace the faulty allele

→ Extremely difficult bcs it **requires insertion of DNA into precise location in genome** (But we have a solution now! Refer to Extras – not in syllabus)

E.g. Huntington's disease

- Caused by a faulty, autosomal dominant allele of the *huntingtin* gene
- Dominant allele also affects tissues in many parts of the body

→ Gene therapy only alters genotype of a few targeted cells



Gene Therapy

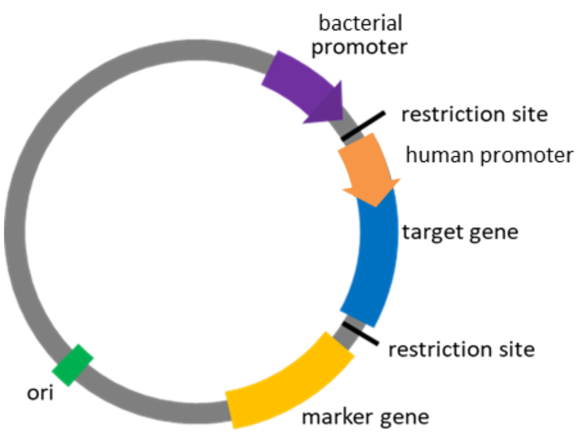
Procedure:

1. **Obtain normal, dominant allele** / cDNA from **mRNA** in cells of healthy person
2. Use **gel electrophoresis + gene probe** for identification
3. Use **PCR** to amplify DNA

4. Make **recombinant DNA**
- Use **restriction enzyme** to cut DNA and form complementary sticky ends
- Use **DNA ligase** to join cDNA with promoter / vector DNA
- Add **human promoter** upstream of target allele to ensure transcription in host

5. Insert normal allele into **vector**
- **Virus vector / Liposome vector / naked DNA**

6. Inject / spray into host

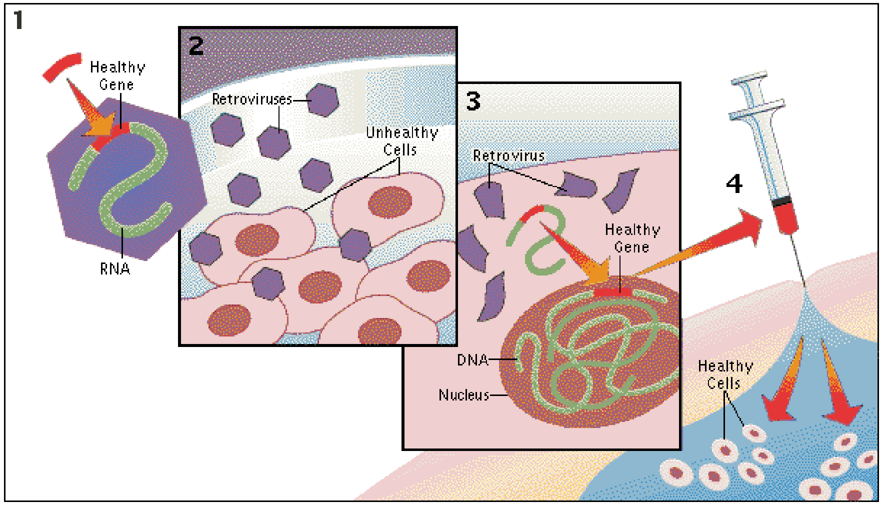


Gene Therapy

Types of Vectors

1. Virus vector

- Usually retroviruses
- **Non-pathogenic**
- **Naturally inserts its viral DNA into host genome**
- Recognises specific cells



Problems:

- May cause **side effects / allergies**
- May be **removed by immune system** before it reaches target cells
- The virus may **trigger an immune response** which destroys the infected cells
- Most non-pathogenic viruses are not very good at getting into cells, so very few cells receive the allele

- **Short term** effect as host cells have short lifespan
- Repeat treatments needed

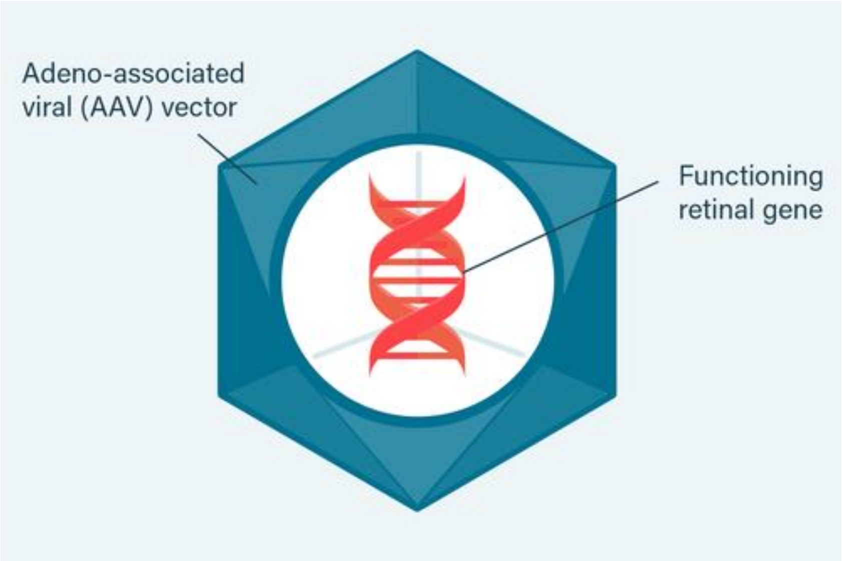
Gene Therapy

Problems:

- **Retroviruses** also cause **random insertion** of genes into host's genome
- May insert its genes within another gene or within regulatory sequence
- May activate oncogenes / switch off tumour suppressor genes → cancer
- May have **uncontrolled viral replication**

Solution:

- **Adeno-associated virus (AAV)** does not insert genes into host's genome, not passed on to daughter cells
- Lentiviruses can be modified to have no uncontrolled viral replication
- Or use different vector!



Gene Therapy

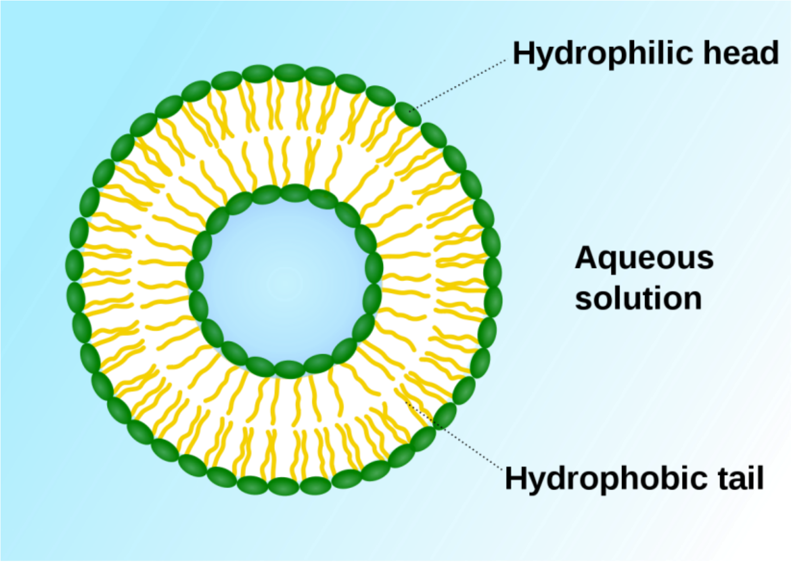
Types of Vectors

2. Liposome vectors

- **Small spheres of phospholipid**
- Sprayed as an **aerosol** / Delivered using **inhaler**
- Liposome **fuses with host cell surface membrane**
- Trigger the immune response less than viruses

Problems:

- Not as effective in insertion as viruses
- **Short term effect** as host cells have short lifespan
- **Repeat treatments needed**



Gene Therapy

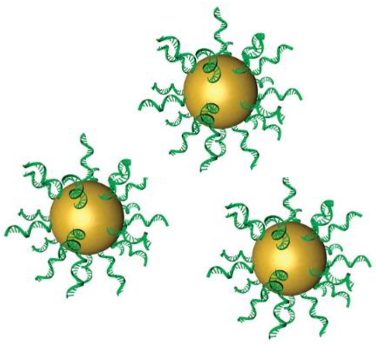
Types of Vectors

3. Naked DNA

- Very cheap
- Can be delivered via **direct injection** or **gene gun**
- No problems associated with using vectors
- Does not trigger immune response

Problems:

- Easily degraded
- Gene expression is very low



Gene Therapy: SCID

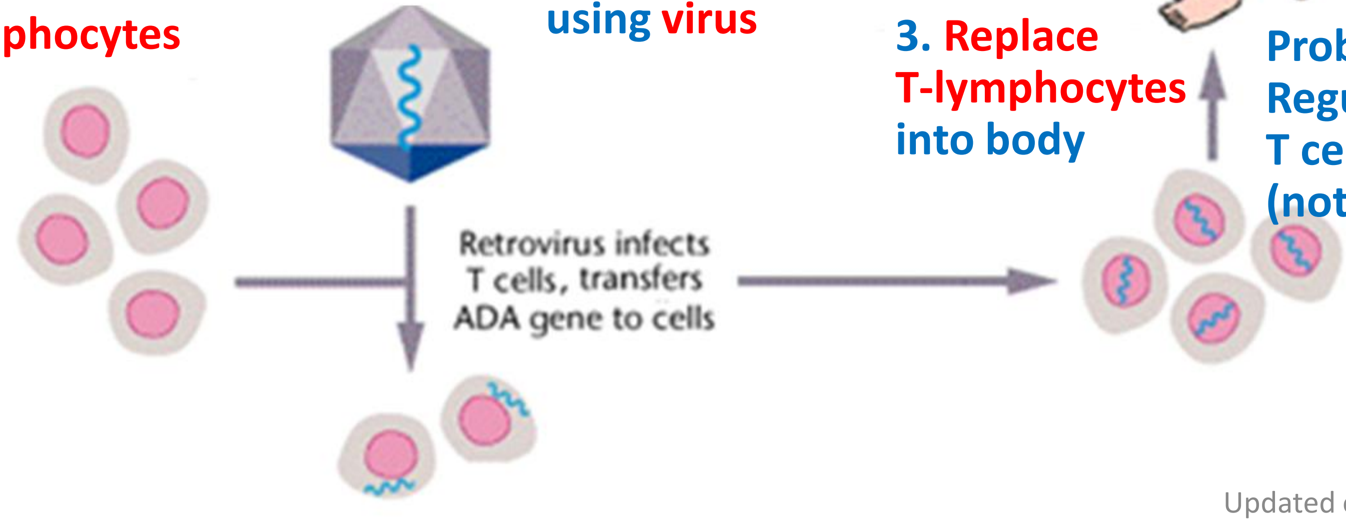
- Severe combined immunodeficiency (SCID)
- Immune cells do not function properly
- **Highly susceptible to infections**
- B and T cells unable to make **adenosine deaminase (ADA)**
- Due to faulty allele coding for the enzyme
- X-linked recessive allele

"Bubble boy"

https://www.youtube.com/watch?v=pJa6KVLwl9U

1. Remove T-lymphocytes

2. Introduce normal allele for ADA enzyme using virus



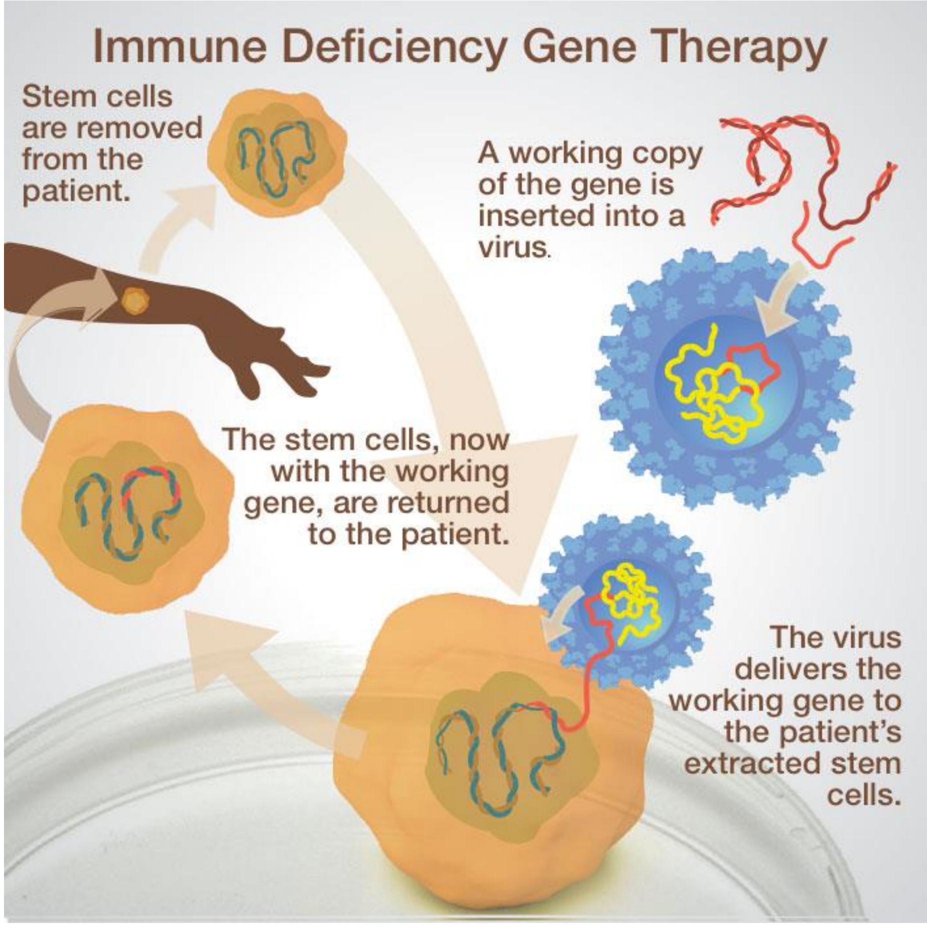
3. Replace T-lymphocytes into body

Problem:
Regular transfusions of T cells are needed (not a permanent cure)

Gene Therapy: SCID

Alternative, longer lasting method:

- **Remove stem cells** from bone marrow
- Insert normal alleles into stem cells using **retroviruses**
- **Return stem cells** into patient



Problem:

- Side effect = leukemia
- Due to random insertion of alleles into cell's genome

Solution:

- Use **Adeno-associated viruses (AAV)**

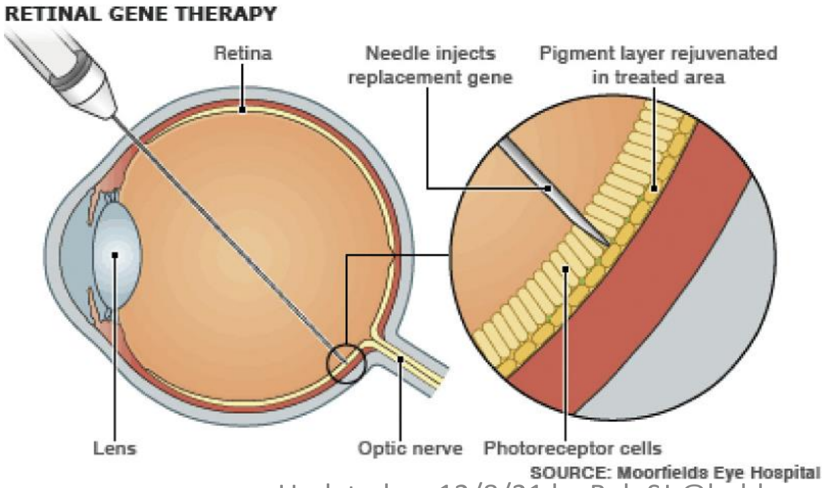
Gene Therapy: LCA

- Leber's congenital amaurosis (LCA)
- **Autosomal recessive eye disease**
- **Retina** cells die off gradually
- Severe loss of vision at birth

- Delivery of dominant, normal allele using **adeno-associated virus (AAV)**
- Vectors are injected directly into the retina
- So retina cells can make functional protein and restore vision

Suitable for treatment using gene therapy bcs:

- Caused by recessive allele of a single gene
- **Only need to get allele into a few cells**
- **Ease of access** to affected area
- Only targets eye / no surgery needed
- Serious so worth the risk



Gene Therapy: Cystic Fibrosis

Chap 16 Recap

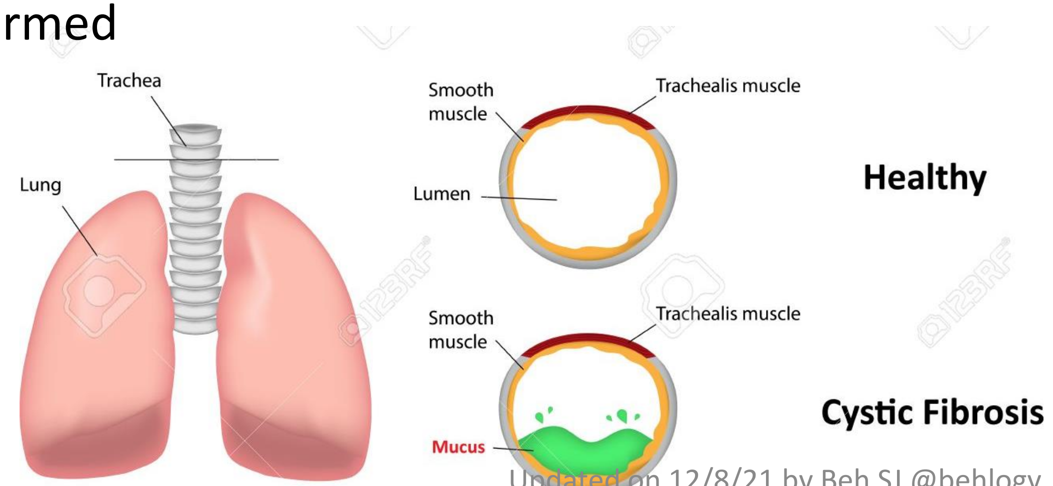
- Inherited genetic disease
- Faulty, autosomal recessive allele of the ***CFTR* gene**

Normal CFTR protein :

- Transmembrane protein at cell surface membrane
- Acts as **chloride channel**
- Has binding site for ATP
- **Cl^- moves out of cell via active transport**
- Water is drawn out from cell

→ **Normal / less viscous mucus** formed

→ Easy removal by cilia



Gene Therapy: Cystic Fibrosis

Chap 16 Recap

Faulty *CFTR* allele:

a) Base deletion → faulty CFTR (most common)

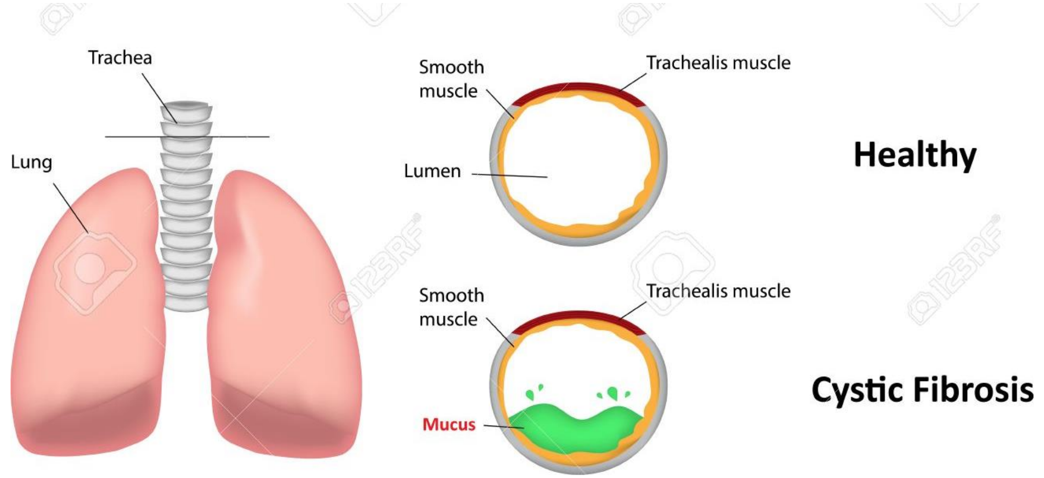
b) Base substitution → STOP codon → incomplete CFTR

Faulty CFTR protein:

- No functional channels for Cl^- ions
- **Cl^- ions do not move out**
- **Less water leaves cell**

→ Formation of **thick, sticky mucus** on cell surface membrane

→ Cannot be removed by cilia

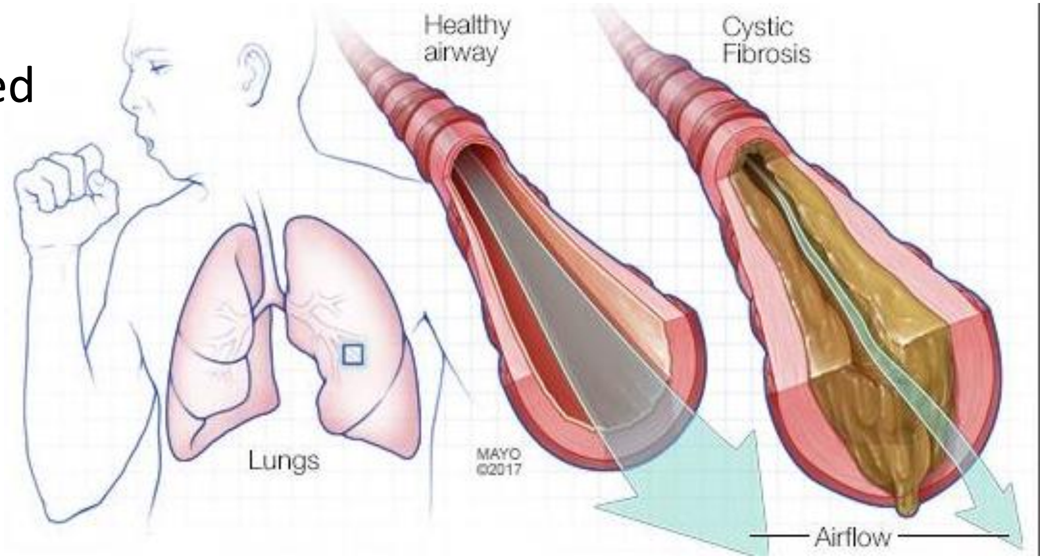


Gene Therapy: Cystic Fibrosis

Chap 16 Recap

Symptoms:

- **thick and sticky mucus** produced at **lungs**
- mucus not moved effectively by **cilia** → mucus accumulates
- mucus traps bacteria → **more infections**
- **reduced gaseous exchange** → due to longer diffusion pathway
- difficulty in breathing, wheezing
- coughing → cause lungs to be scarred
- blocked **pancreatic duct** → reduced digestion, damage of pancreatic tissues causes diabetes
- blocked **sperm ducts / oviducts** → reduced fertility



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Gene Therapy: Cystic Fibrosis

Traditional treatments

- Deals with symptoms only not causes

1. Thick mucus in lungs → physiotherapy, percussion therapy to loosen & remove mucus easily
2. Recurrent bacterial infections → antibiotics
3. Reduced digestion → enzyme supplements

Gene therapy

- Insert normal allele for CFTR into lungs cells

→ Usually inhaled or sprayed

- Treats the cause rather than the symptoms
- No physiotherapy, antibiotics etc. needed
- Less time consuming than other treatments
- Effects are short-lived (few days) and treatment needs repeating
- May have side-effects

Gene Therapy: Cystic Fibrosis

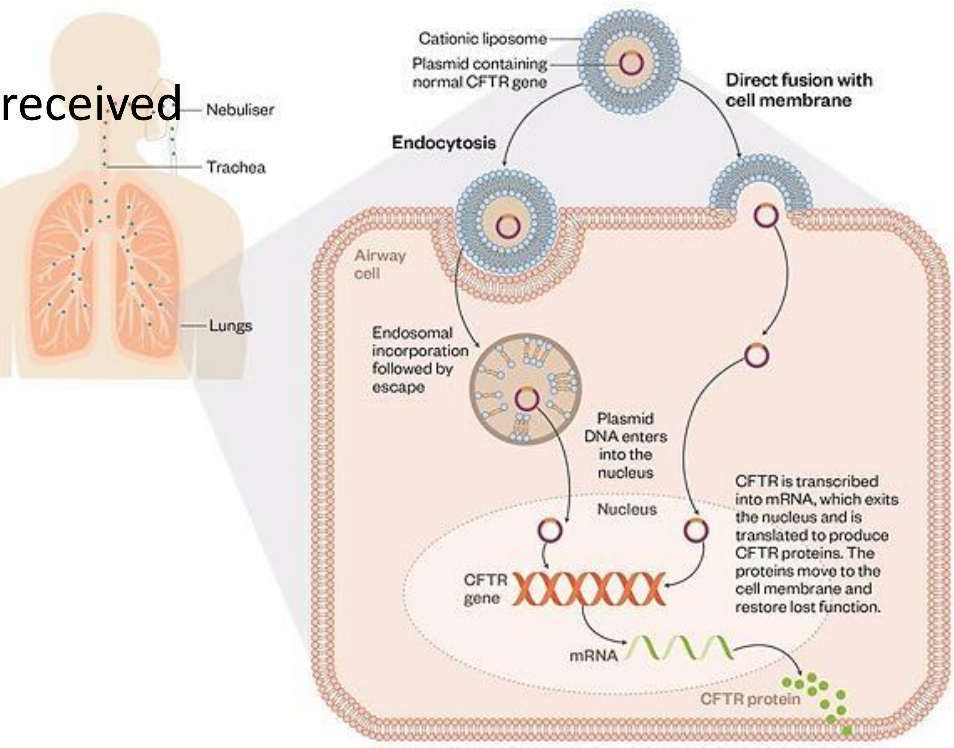
Vectors commonly used:

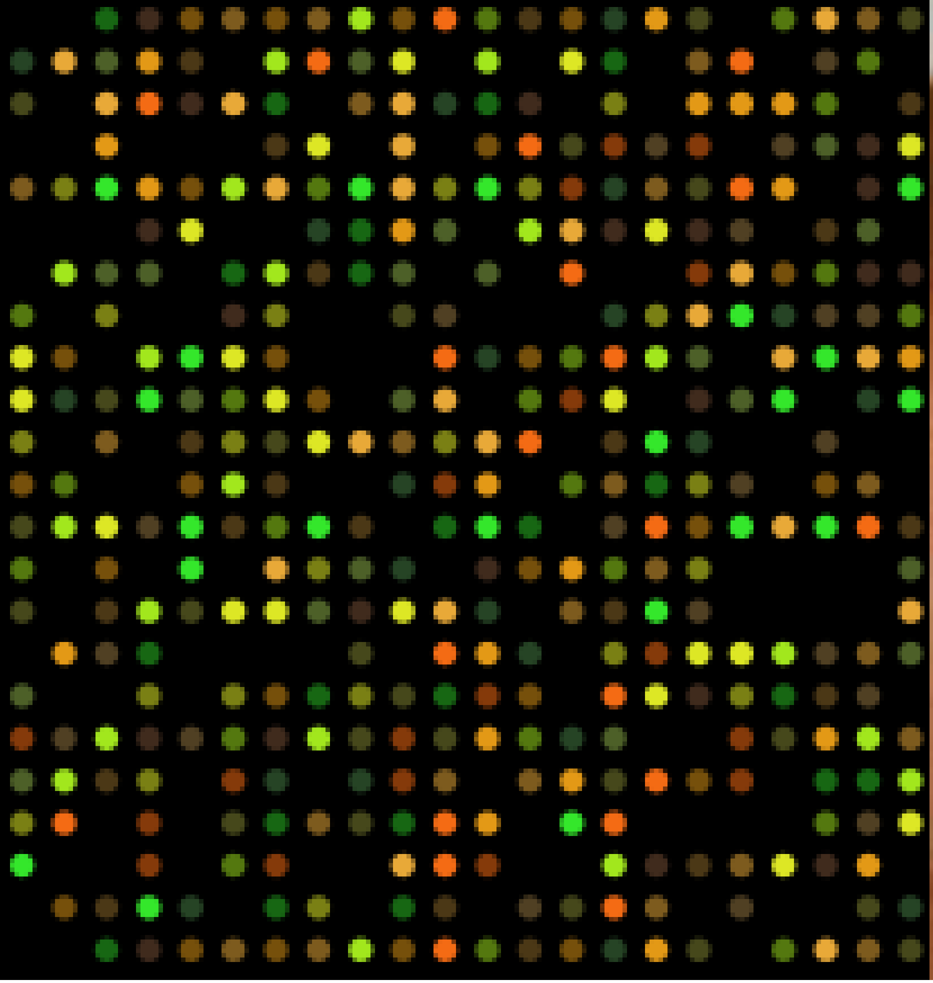
1) Liposomes

- Sprayed into nose
- Not long lasting & only a few cells received the normal allele

2) Adenoviruses

- Normal harmless
- Used to infect respiratory cells
- Not all cells take up virus
- Side effects due to viral infection





Genetic Technology 4

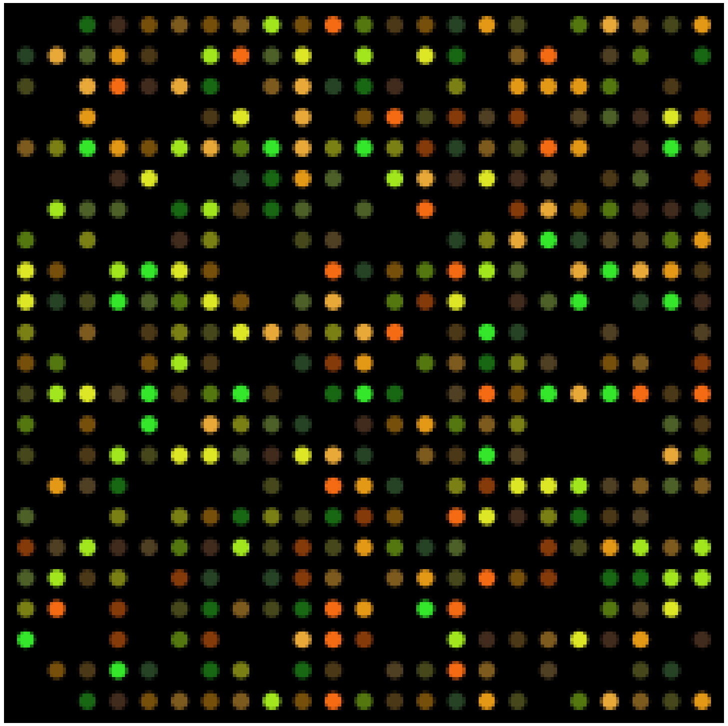
MICROARRAYS

Function: Identify expressed genes by detecting mRNA

+ Distinguish btwn alleles of a gene

Chapter Outline

Technology	Function	Application
4. Microarrays	**Identify expressed genes by detecting mRNA** OR **Distinguish btwn alleles of a gene**	• **Genetic screening** • Drug testing • Identify genes that are switched on/off in diseases



Microarrays

- **Function:**

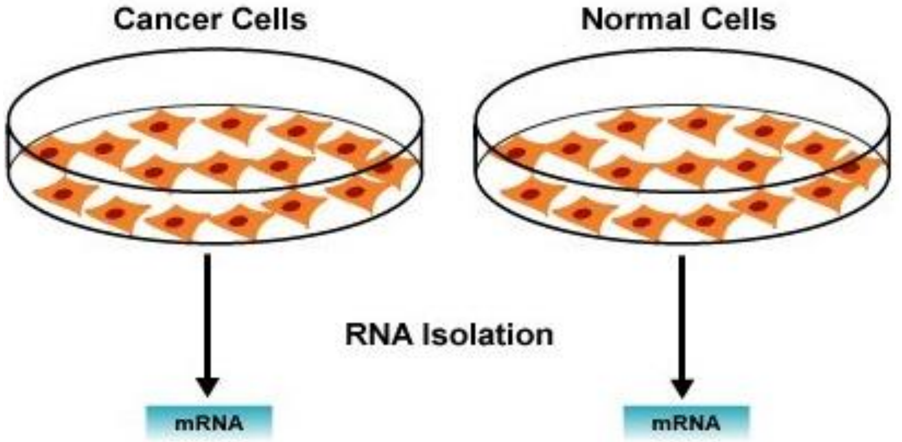
1) To distinguish btwn alleles of a gene

- Use **DNA** microarray
- Compares alleles found in genomes of 2 individuals/species

2) To identify expressed / transcribed genes

- Transcription of a gene produces **mRNA**
- So can assess gene expression by **measuring mRNA levels**

- Compares relative mRNA levels **between 2 samples**
- One acts as a control (normal cells)

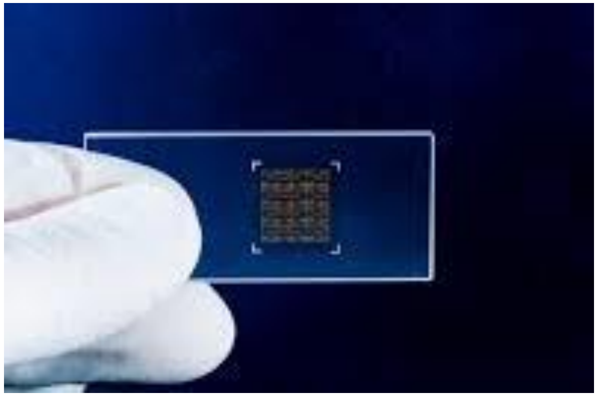


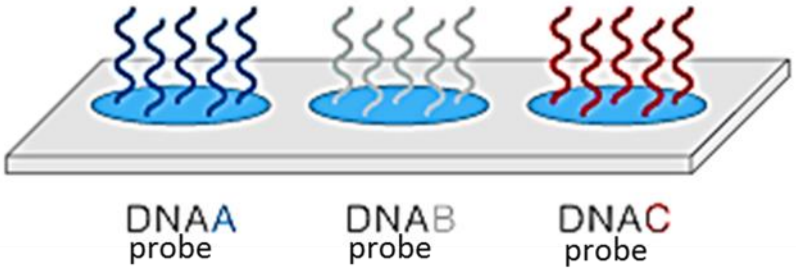
Microarrays

Components: A microarray chip

- Involves a "chip" with has >10 000 cells
- Gene probe is bound at known positions to a chip
- **Gene probe** = **short lengths of single-stranded DNA (ssDNA) complementary to allele/gene**

- **Many copies of one type of probe placed in one cell of the microarray**
- **Diff cells has a diff type of gene probe** complementary to specific allele/gene
- So many genes can
  be assessed at the same time!





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Microarrays

1A. **To distinguish btwn alleles of a gene**

Extract **DNA** from 2 samples

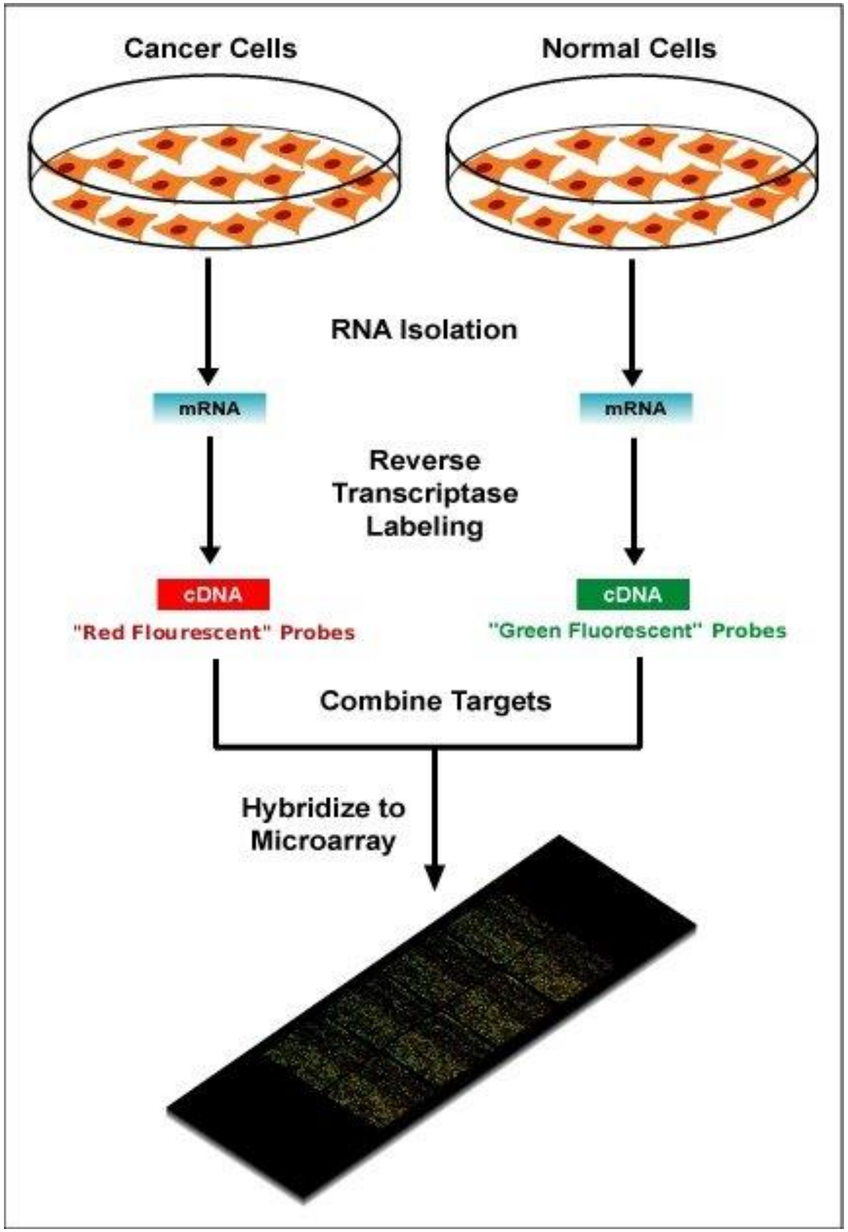
- Cut into fragments
- Denature DNA into **ssDNA** by heating it

OR

1B. **To identify expressed / transcribed genes**

Isolate all **mRNA** of cells of 2 samples

- Use mRNA as a template to form **single-stranded complementary DNA (cDNA)**
- Use reverse transcriptase



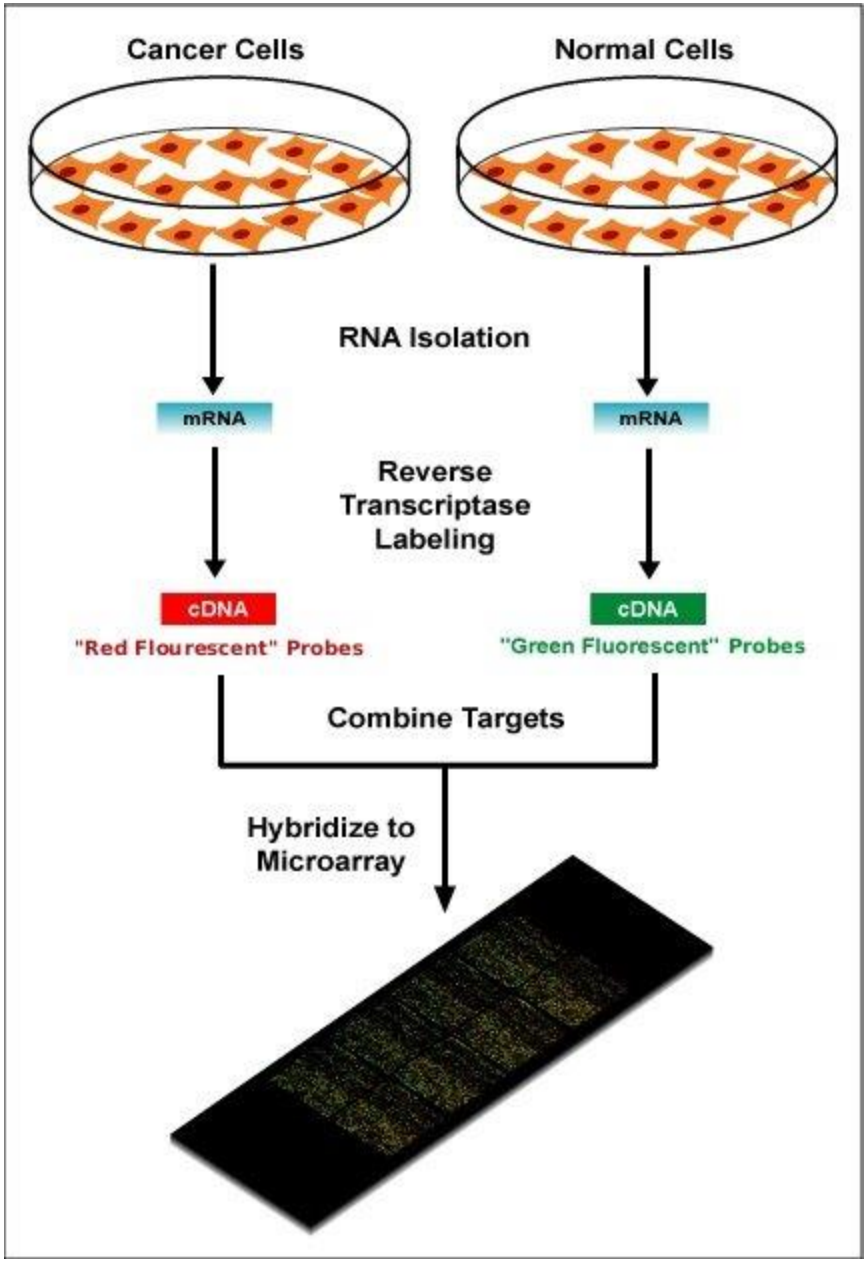
Microarrays

2. Label ssDNA using **fluorescent 'tags'**

- One sample red, one sample green

3. Labelled ssDNA from both samples are washed over microarray

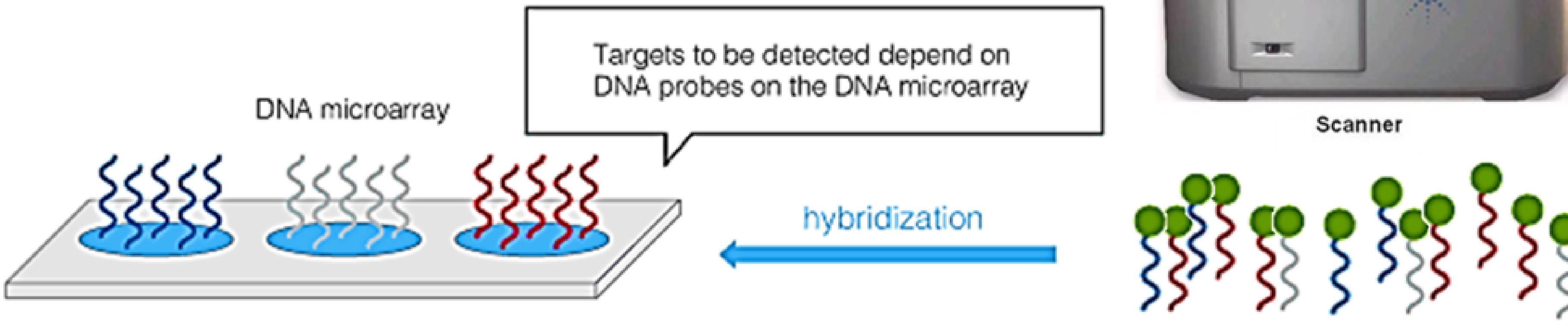
- Allow ssDNA to **hybridise / bind** to the complementary gene probes
- Unbound ssDNA will be washed off
- Bound DNA will not be washed off

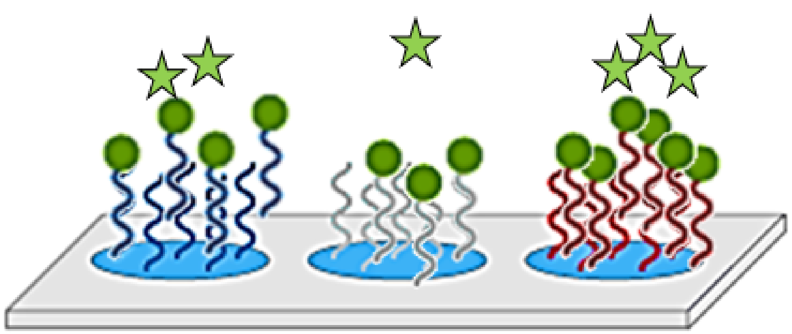


Microarrays

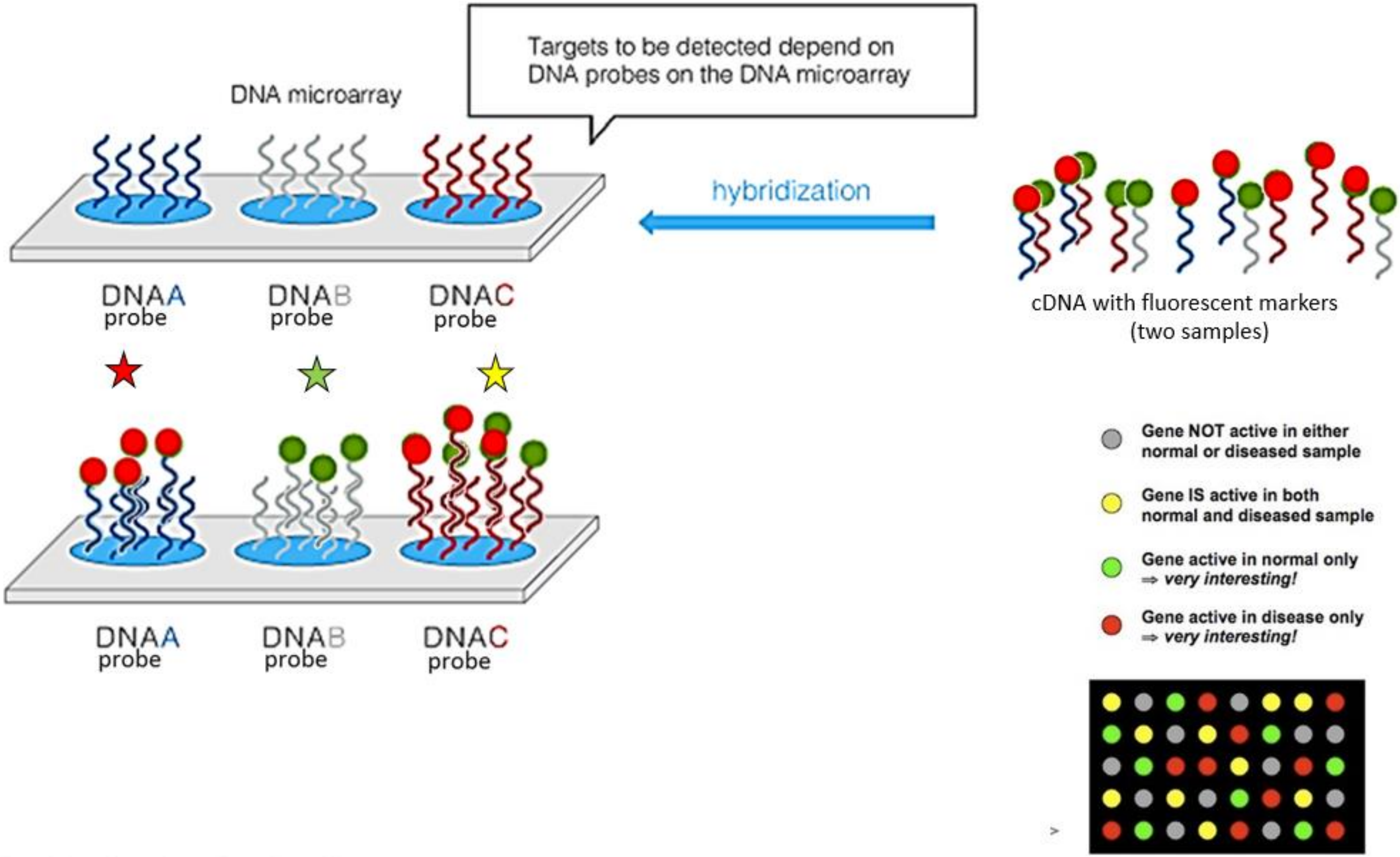
4. Use **laser / UV light** to identify fluorescent spots

- Fluorescence = hybridised probes with ssDNA = gene is expressed
- Position and intensity is recorded by scanner → positions are then identified as named genes
- **Intensity proportional to gene expression**





Microarrays



Microarrays

Applications:

- **Genetic screening** – can distinguish btwn alleles of a gene
- **Drug testing** – identify which genes the drugs have acted on
- Identify genes that are overexpressed/not expressed in **diseases**

Genetic Technology 5 (Extra! Not in syllabus)

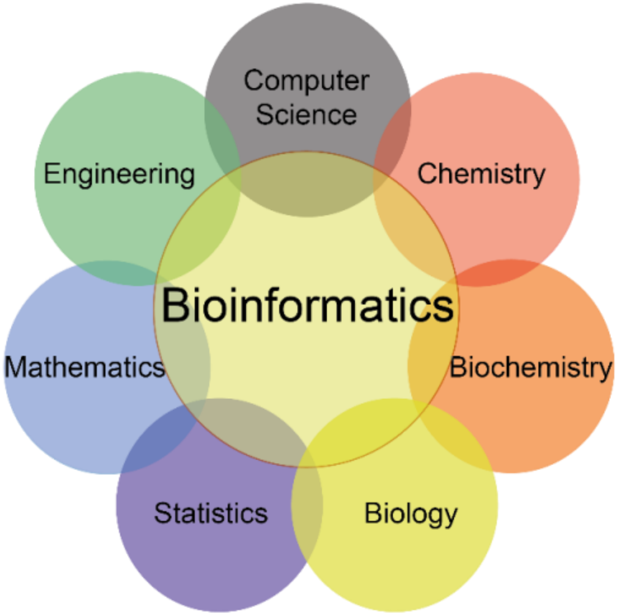
BIOINFORMATICS

Function: Collection, processing and analysis of biological info and data using computer software

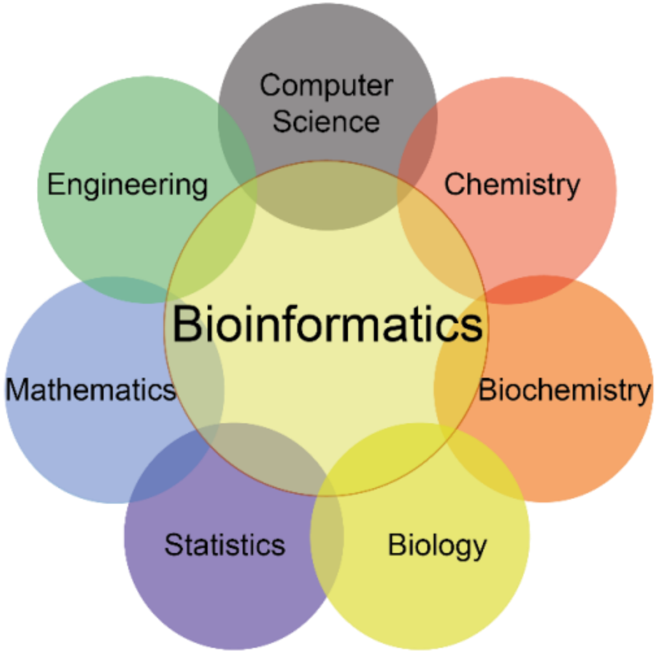
Updated on 12/8/21 by Beh SJ @behlogy

Chapter Outline

Technology	Function	Application
5. Bioinformatics • DNA sequencing (details not required)	**Collection, processing and analysis of biological info and data using computer software** Allows comparison of DNA and protein sequences	• **Role in drug development** E.g. against *Plasmodium* • Protein and DNA seq provide **evidence for evolution**



Bioinformatics



- Interdisciplinary field
- **Collection, processing and analysis of biological information & data**
- Using **computer programs / software**

- It is a store / database

→ Collection of huge-quantities of data ("big data") from all over the world

→ From **DNA sequencing**, **microarrays**, other techniques

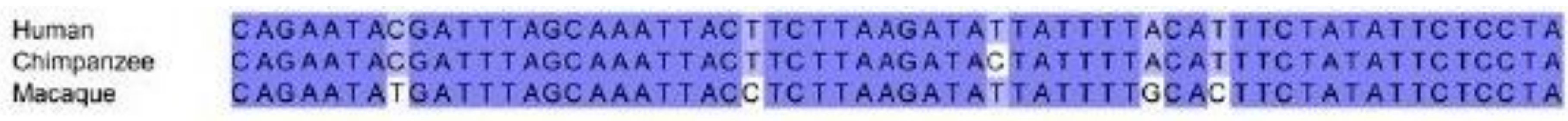
→ Can **search online for DNA and amino acid sequences**

→ And use that to **model / predict tertiary structure of protein**

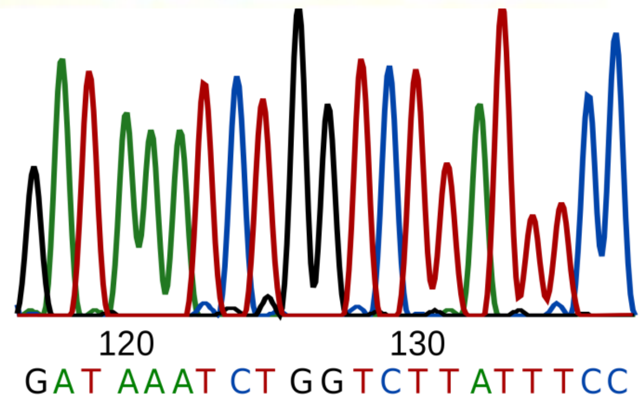
- Function: **To allow comparison of DNA and protein sequences**

DNA sequencing

- **Determine the exact sequence of bases in genes**



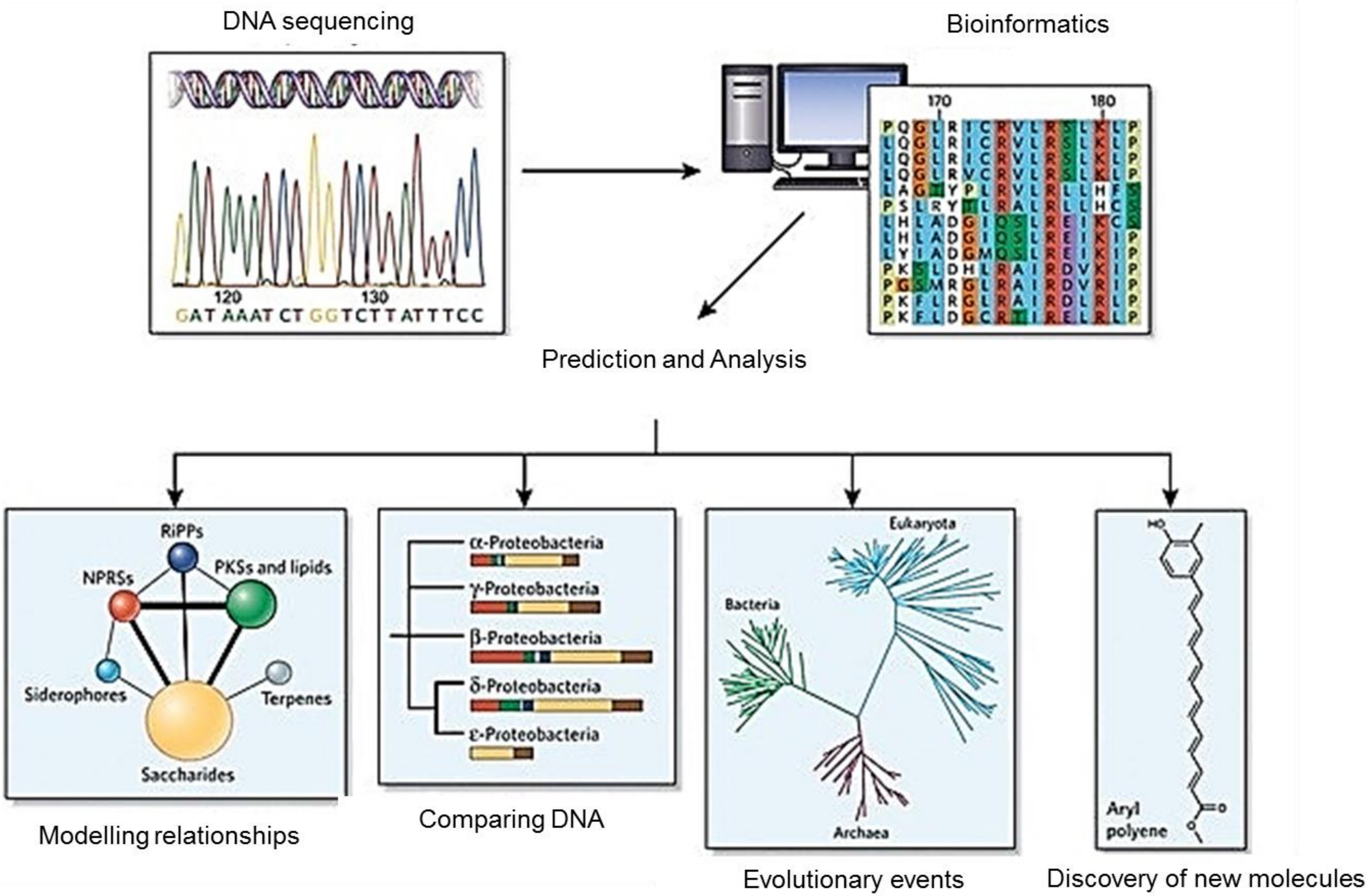
- Fully automated process
- Genomes of many species have been published
- Gives information about the **location of genes**
- Provides **evidence for the evolutionary links** between organisms



E.g. Human Genome Project

- All 22+XY chromosomes have been sequenced
- Gives info about the location of genes

Bioinformatics



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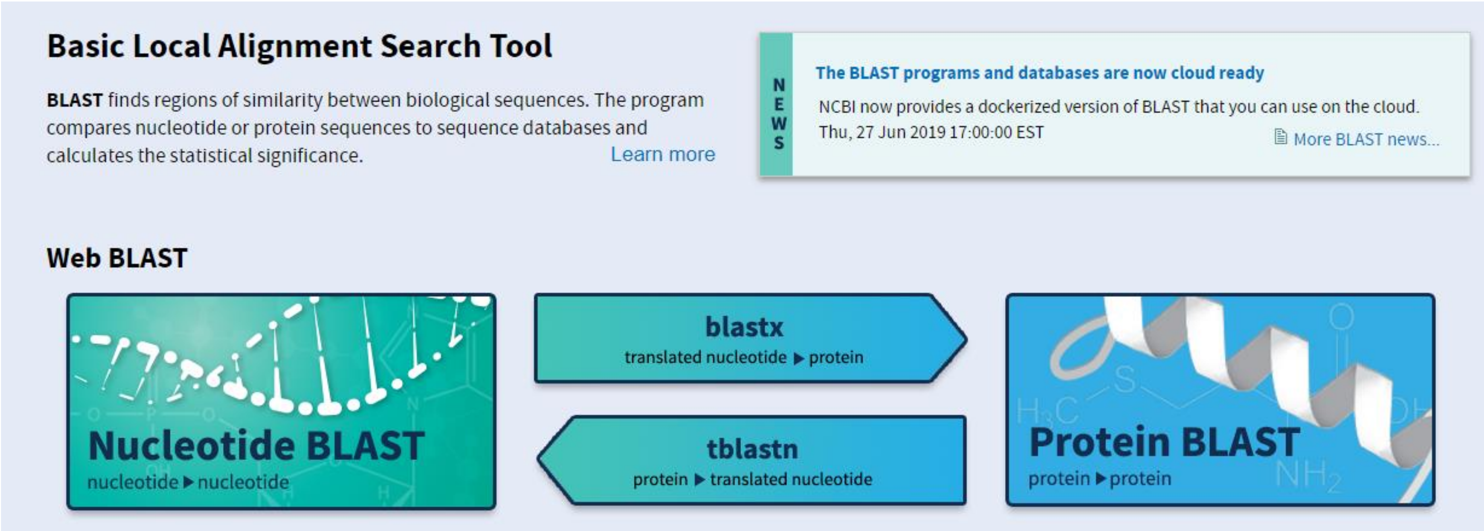
Bioinformatics

Example of Databases available:

i) Ensembl – genomes of vertebrates
https://asia.ensembl.org/index.html

ii) UniProt – primary sequences & functions of proteins
https://www.uniprot.org/

iii) BLAST search tool – an algorithm to search the database for target DNA or protein sequence
https://blast.ncbi.nlm.nih.gov/Blast.cgi



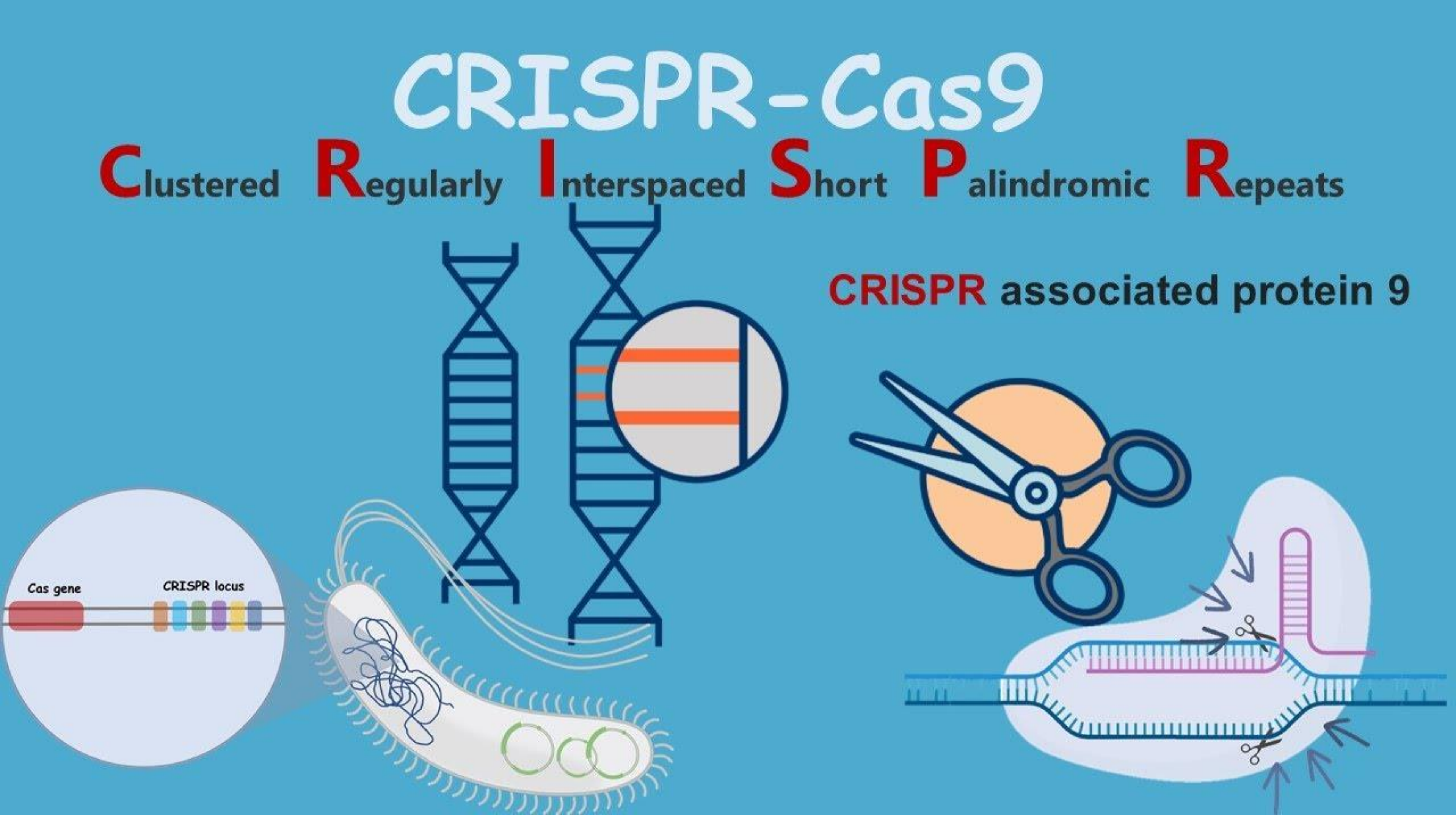
Bioinformatics

Applications:

- Identify **evolutionary relationships / genetic relatedness**
  - By comparing between genomes/protein seq of diff species
  - Close similarities = recent common ancestry

- Compare between genes / proteins of humans and model organisms
  - E.g. mouse, *Drosophila* fruit flies, yeast, *C. elegans* roundworm
  - If target gene have similar sequence and protein have similar structure
  - **Model organism can be used to investigate when and where genes have effect in humans**

- Identify genome seq and proteins of pathogens
  - E.g. *Plasmodium*, *Staphylococcus areus*
  - Can help in **vaccine/drug development**



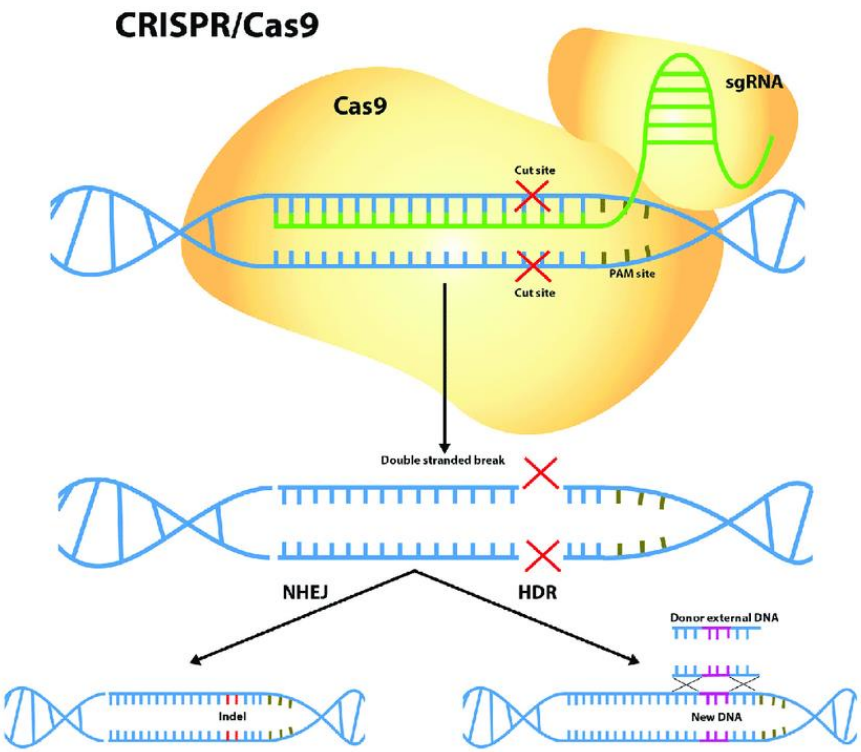
Genetic Technology 6 (Extra! Not in syllabus)

CRISPR-Cas9

Function: cut DNA at specific loci and allow genome editing

Chapter Outline

Technology	Function	Application
6. CRIPSR-Cas9 (Extra! Not in syllabus)	Cut DNA at specific loci and allow genome editing	Cure viral diseases and genetic diseases, even cancer!



CRISPR-Cas9

- **Inexpensive, much easier and very effective**
- Bacterial system to protect itself against virus

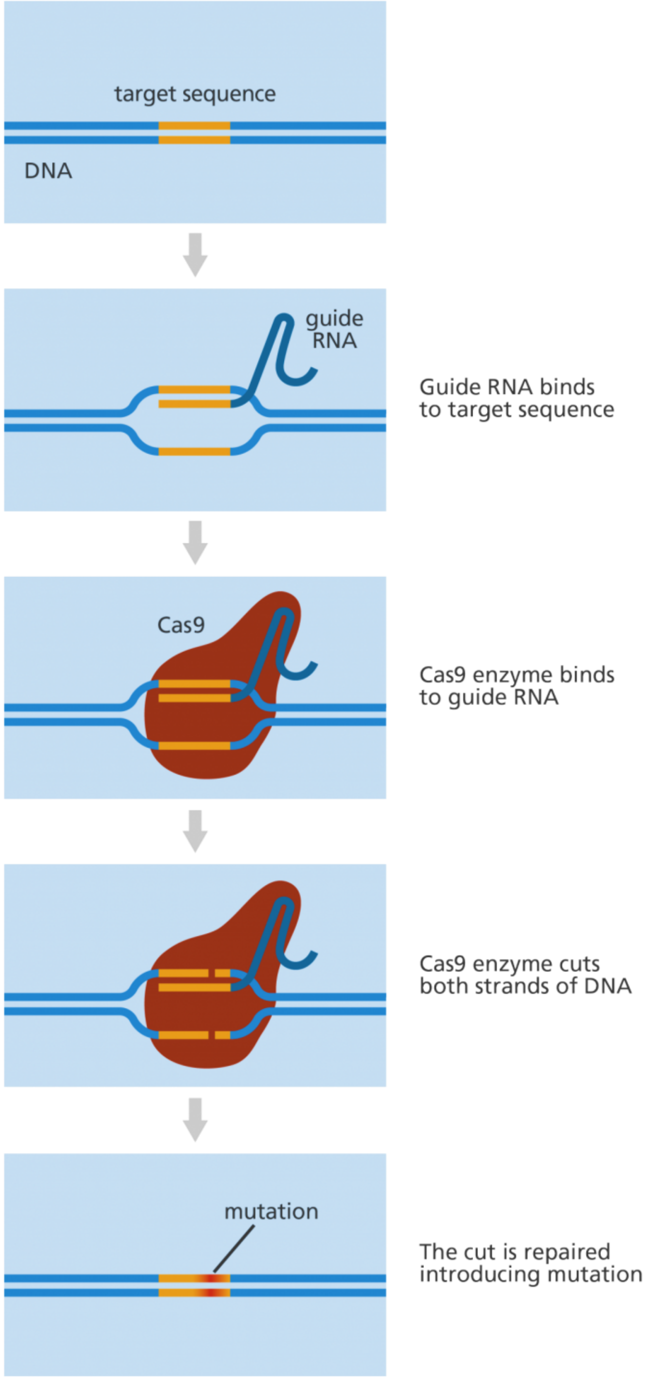
Components:

- **Cas9** = enzymes that cuts DNA
- **gRNA** = guide RNA that be complementary to target DNA

Result: A double-stranded break is introduced at specific loci

- Cell will try to repair the double-stranded break but repair is error prone
- Extra bases are included → **gene is disrupted and deactivated**

https://www.youtube.com/watch?v=4YKFw2KZA5o



CRISPR-Cas9

Potential:

- Technology is versatile and improving

→ Can combine with other enzymes in order to introduce specific mutations / increase transcription / insert gene at specific location etc.

→ Can combine with GFP and act like a gene probe *in situ*

- Very cool experiments and research

→E.g. The bold plan to end malaria with a gene drive https://www.youtube.com/watch?v=P0HPHUzsHbI

- **Cure viral diseases and genetic diseases, even cancer!**

→ Can just inject CRISPR into body

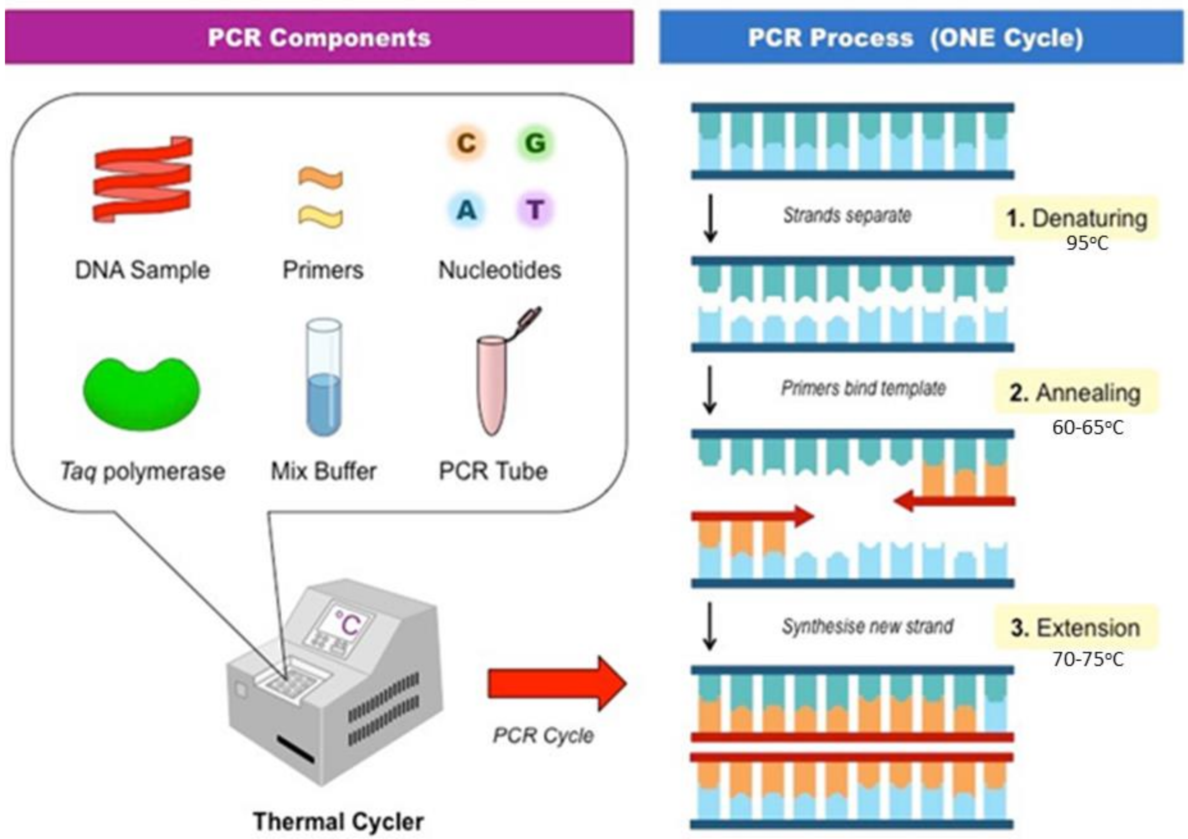
→ Extend life expectancy

Potential Issues / Problems: https://www.youtube.com/watch?v=jAhjPd4uNFY

- Increased preselection → Designer babies
- Super soldiers and biological weapons

Chapter Outline

Technology	Function	Application
1. Polymerase Chain Reaction (PCR) • Role of *Taq* polymerase	**Clone and amplify DNA**	Too many…



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Chapter Outline

Technology	Function	Application
2. Recombinant DNA technology / Gene Cloning • Properties of plasmids • Promoters • Marker genes (e.g. fluorescent genes / genes coding for easily stained substances) • Role of restriction endonucleases, reverse transcriptase and ligase	**To make many copies of a gene or protein**	**Large-scale production of human proteins as drugs** E.g. insulin, factor VIII for haemophilia, adenosine deaminase for SCID **Genetic engineering of crop plants and livestock** • Golden rice™ • GM salmon • Insect resistance E.g. Bt maize, Bt cotton • Herbicide resistance E.g. maize, cotton, tobacco, oil seed rape • Ethical and social implications of using GMOs in food production

Chapter Outline

Technology	Function	Application
3. Gel electrophoresis • For protein and for DNA • Probes	**To separate fragments of DNA according to length** OR **To separate diff proteins according to mass/charge** Distinguish btwn alleles of a gene	**Genetic fingerprinting / DNA profiling** • Paternity testing • Criminal Investigations **Genetic screening** • Breast cancer (*BRCA1*, *BRCA2*) • Genes for haemophilia, SCA, Huntington's Disease and CF **Gene Therapy** • Vectors (viruses, liposomes, naked DNA) • SCID, inherited eye disease, CF • Social and ethical considerations

Chapter Outline

Technology	Function	Application
4. Microarrays	**Identify expressed genes by detecting mRNA** OR **Distinguish btwn alleles of a gene**	• **Genetic screening** • Drug testing • Identify genes that are switched on/off in diseases
5. Bioinformatics and DNA sequencing (Extra! Not in syllabus)	Collection, processing and analysis of biological info and data using computer software Allows comparison of DNA and protein sequences	• Role in drug development E.g. against *Plasmodium* • Protein and DNA seq provide evidence for evolution
6. CRIPSR-Cas9 (Extra! Not in syllabus)	Function: cut DNA at specific loci and allow genome editing	Cure viral diseases and genetic diseases, even cancer!