

# RECOMBINANT DNA TECHNOLOGY

By;

Dr. Adeel Chaudhary

**2<sup>nd</sup> yr Molecular Genetics**

**Medical Technology**

**College of Applied Medical Sciences**

Recombinant DNA is a form of artificial DNA that is made through the combination or insertion of **one** or **more** DNA strands, therefore combining DNA sequences as per your requirement, within different species i.e. DNA sequences that would not normally occur together

- **In order to understand this you must remember how a fragment of DNA, representing a genetic code is involved in proteins synthesis.**
- **i.e. mRNA transcription from this DNA fragment followed by translation involving rRNA and tRNA that carries the amino acid.**

- **Therefore if you need to produce large quantities of a functional protein artificially you must first clone the DNA fragment corresponding to the gene using recombinant technology.**
- **This protein may be responsible for giving fluorescent color to small fishes or making plants resistant to pesticides etc**

**In genetics recombinant DNA technology has many uses e.g:**

**Agriculture:** growing crops of your choice (GM food), pesticide resistant crops, fruits with attractive colors, all being grown in artificial conditions.

**Pharmacology:** artificial insulin production, drug delivery to target sites

**Medicine:** gene therapy, antiviral therapy, vaccination, synthesizing clotting factors

**Other uses:** fluorescent fishes, glowing plants etc



## **Advantages of Recombinant technology:**

- Provide substantial quantity
- No need for natural or organic factors
- Tailor made product that you can control
- Unlimited utilizations
- Cheap
- Resistant to natural inhibitors

## **Disadvantages of Recombinant technology:**

- Commercialized and became big source of income for businessmen
- Effects natural immune system of the body
- Can destroy natural ecosystem that relies on organic cycle
- Prone to cause mutation that could have harmful effects
- **Major international concern:** manufacturing of biological weapons such as botulism & anthrax to target humans with specific genotype
- Concerns of creating super-human race

## Cloning;

The term 'clone' means, **exact copy** of the **parent**. A **duplicate** or a **look alike** carrying the same genetic signature or genetic map.

Cloning is the best application of recombinant DNA technology and could be applied to something as simple as DNA fragment or a larger, sophisticated mammalian specie such as humans.

**Molecular cloning** is carried out *in-vitro* where a specific fragment of **DNA** is isolated from an organism **'donor'** and introduced into a **'plasmid'** that replicates in a **'host'** cell making multiple copies of that DNA fragment.



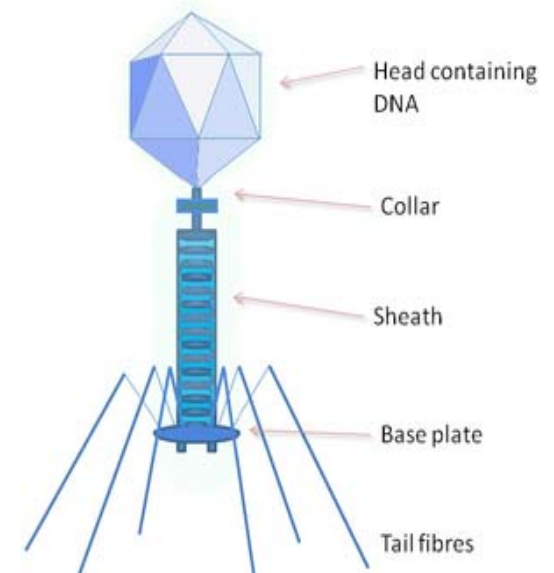
## Plasmids

These are *double stranded DNA* that are usually *circular* and mostly found inside certain bacterial specie *e.g. E.coli*

However most plasmids are now commercially available, ready to be used, providing specific fragment insertion sites.

Plasmids in genetic engineering are also known as 'vectors'

**Vectors** also include viruses known as bacterio-phage that use bacteria as their host to replicate. Hence a bacterio-phage can be used to transfect and create several copies of the DNA fragment of interest by replicating several times in a bacteria.



## Types of Vectors Used

### Plasmids (5-10 kb)

- pUC18

2686 bp, in host it replicates 500 copies per cell and contains several RE sites

### Bacteriophage (10-15 kb)

- Lambda (able to replace 1/3 of the chromosome with large pieces of foreign DNA)

- M13

### Cosmids (50 kb)

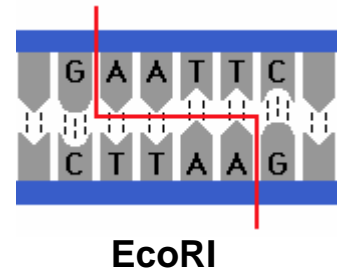
- Hybrid vectors, part plasmid – part lambda

### BACs & YACs (300 kb, up to 1,000 kb)

- Artificial chromosomes

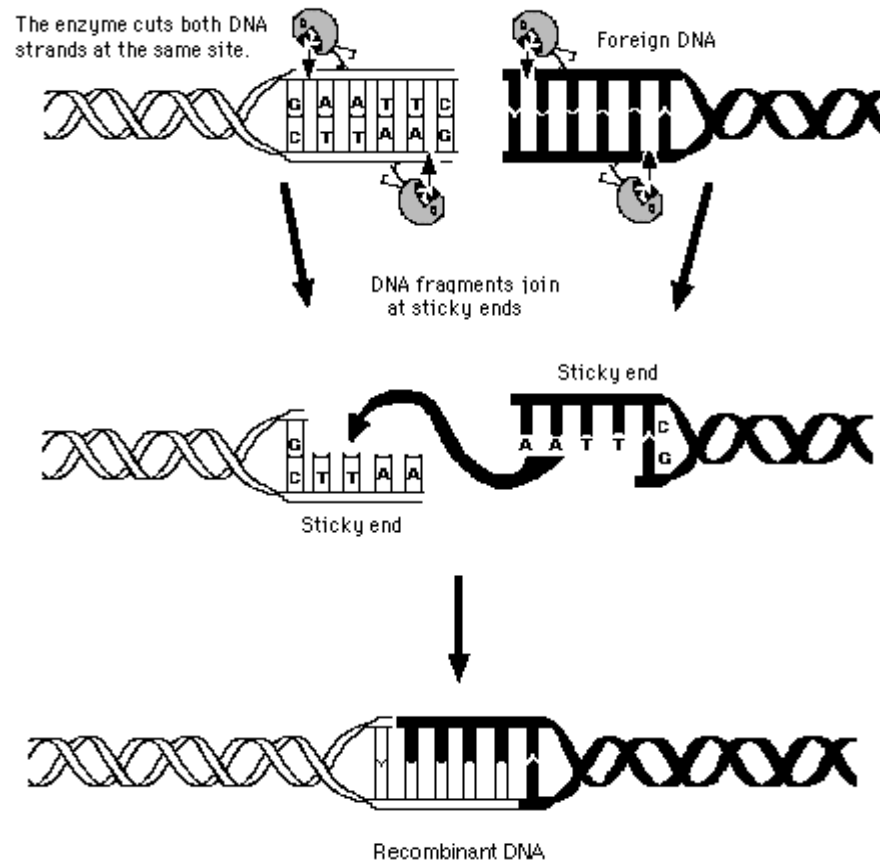
## MAIN STEPS INVOLVED IN MOLECULAR CLONING USING RECOMBINANT TECHNOLOGY

- 1) DNA from donor is isolated and purified
- 2) Restriction Enzymes (endonucleases) generate fragments of purified DNA by cutting the DNA at recognition site. There are well over a hundred restriction enzymes, each cutting in a very precise way a specific base sequence of the DNA molecule not exceeding 4-6 bp.
- 3) Fragments are inserted, pasted or spliced into plasmid
- 4) Plasmid transferred to host cell
- 5) As host cell replicates, recombinant molecules are passed on to progeny known as 'clones'
- 6) Cloned DNA can be recovered & analyzed from the host cells after processing.

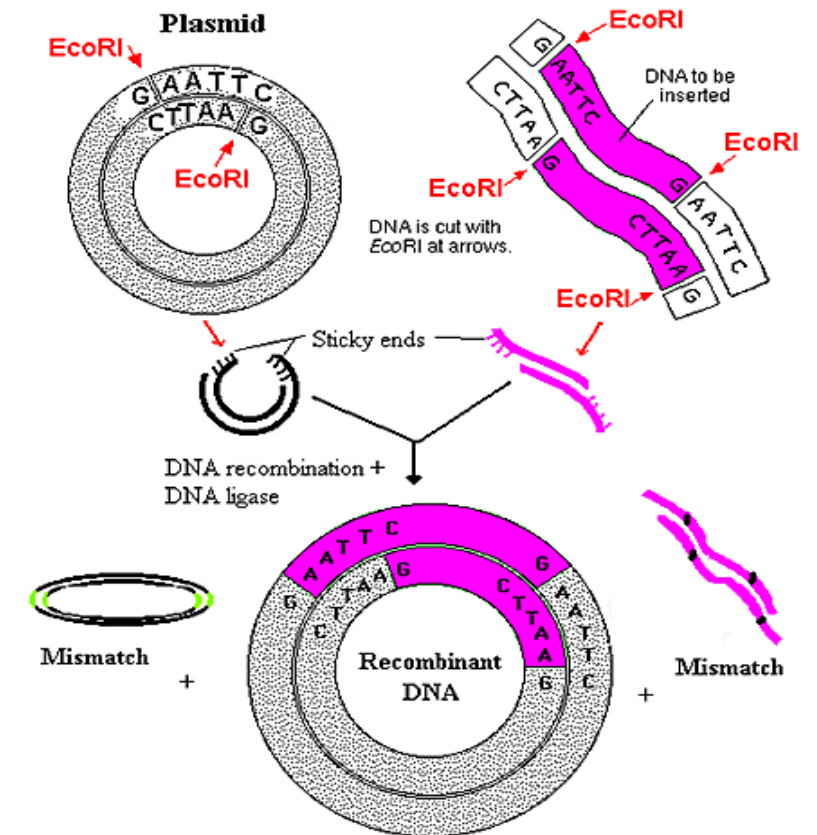
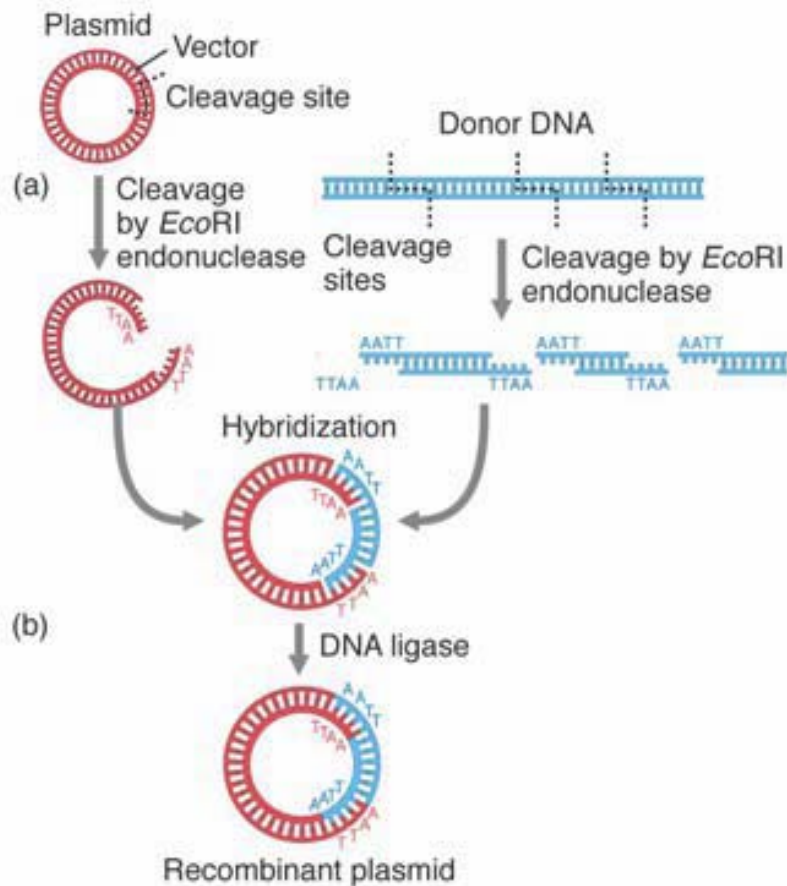


# CUTTING AND LIGATION OF FRAGMENTED DNA FROM DONOR AND FROM THE PLASMID..... STEPS IN DETAIL

## Restriction Enzyme Action of EcoRI



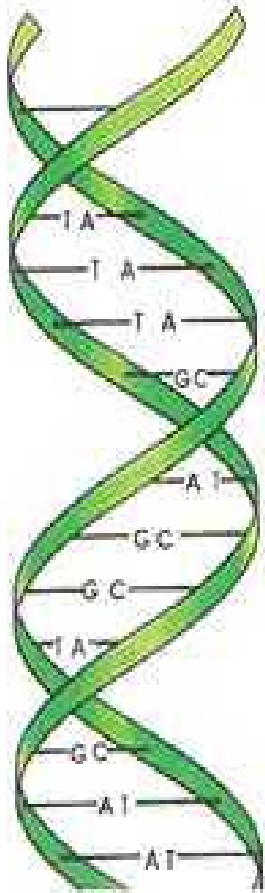
This "sticky ends" from two different DNA molecules can hybridize together; then the nicks are sealed using **ligase**. The result is **recombinant DNA**. When this recombinant vector is inserted into *E. coli*, the cell processes the instructions and by translation & transcription, it assembles the amino acids forming the protein product of interest. More importantly, the new instructions are passed along to the next generation of *E. coli* cells forming **'recombinant clones'** on the culture agar.



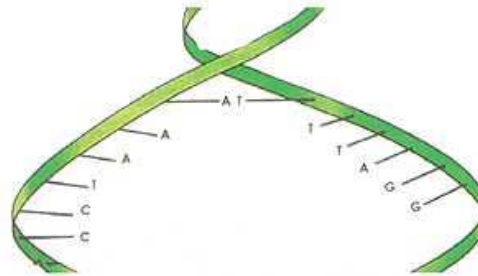
**Inserting a DNA Sample into a Plasmid**

# Cloning Insulin A & B chains using recombinant DNA technology

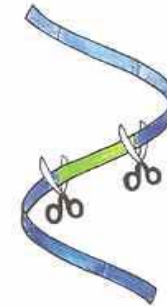
1- DNA strand with the specific nucleotide sequence for Insulin chain A and chain B



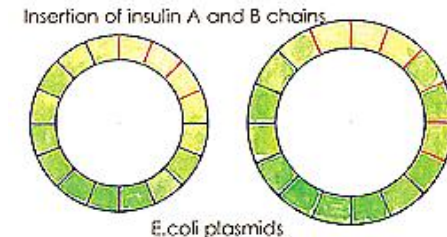
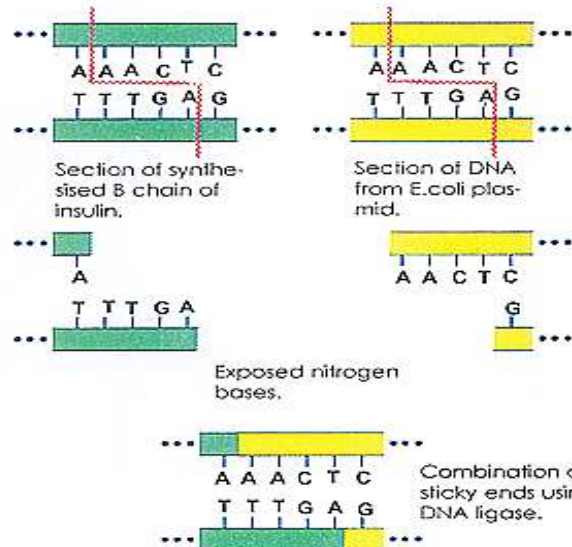
2- Unraveling strand of the DNA of chromosome 11, with the exposed nucleotides coding for the A & B chain of Insulin



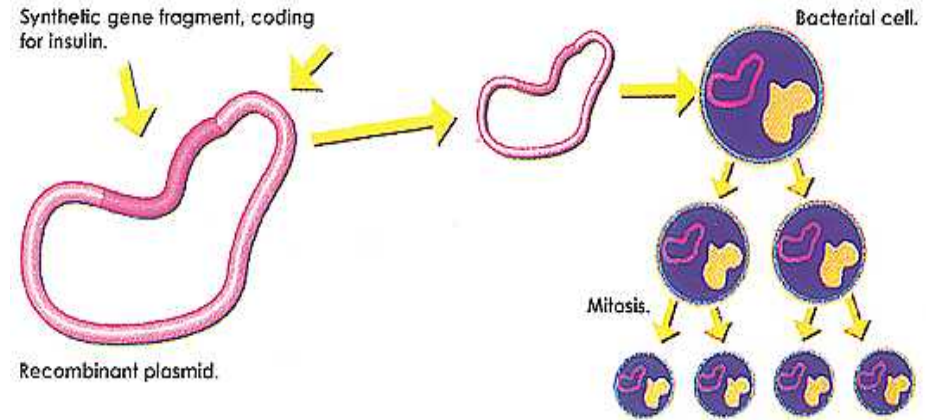
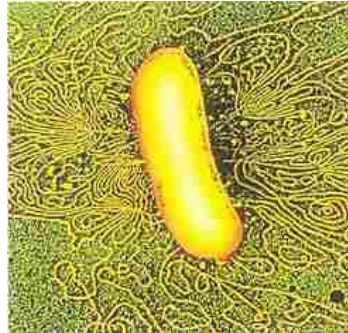
3- Restriction endonuclease (natural scissors) cut the insulin gene fragment by recognizing particular stretches of nucleotides.



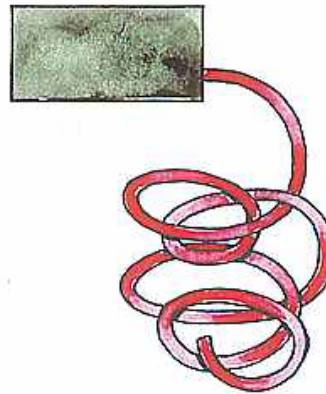
4- The synthetic A and B chain 'genes' are then separately inserted into the gene for a bacterial enzyme, B-galactosidase, which is spliced into and carried by the plasmid following complete compatibility.



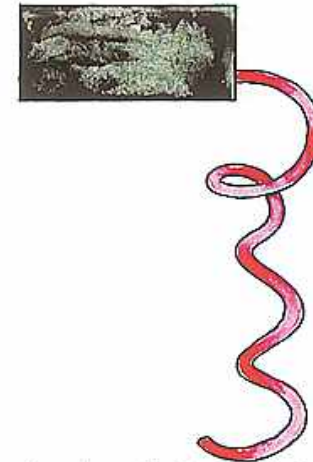
The *recombinant plasmids* are then introduced into E. coli cells. Practical use of Recombinant DNA technology in the synthesis of human insulin requires *millions of copies of the bacteria* whose plasmid has been combined with the insulin gene in order to yield insulin. The insulin gene is expressed as it replicates with the B-galactosidase in the cell undergoing mitosis



The protein which is formed, consists partly of B-galactosidase, joined to either the A or B chain of insulin. The A and B chains are then extracted from the B-galactosidase fragment and purified



$\beta$  - gal protein fused with A chain insulin protein.



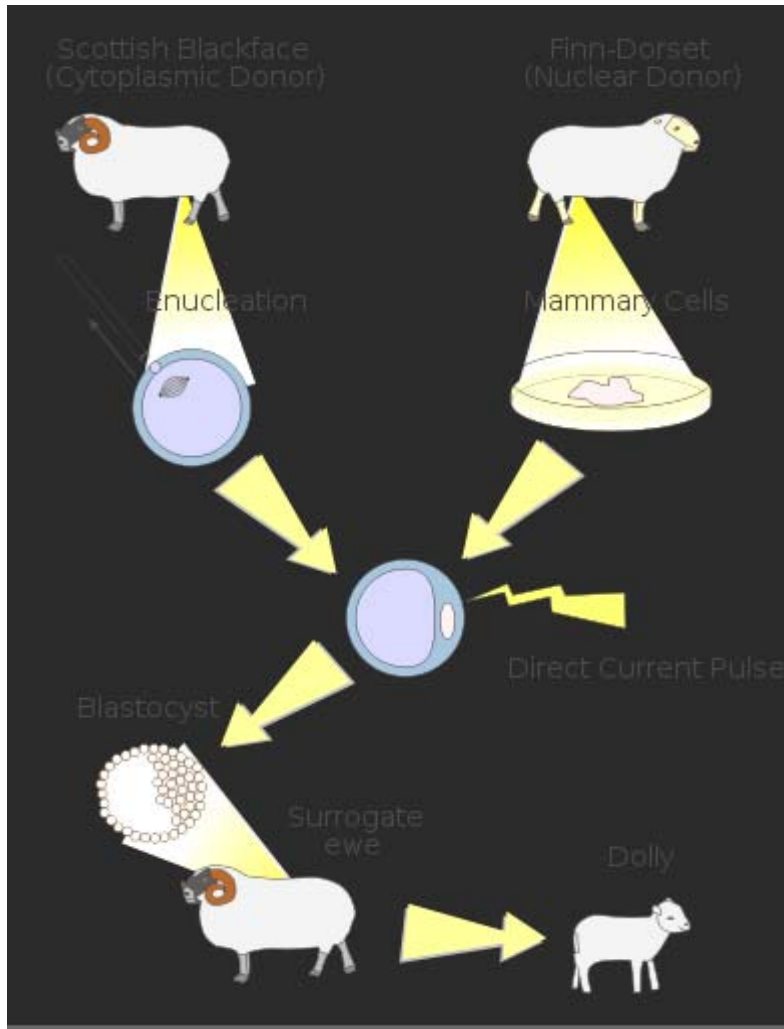
$\beta$  - gal protein fused with B chain insulin protein.

## Colony selection

- Colonies carrying the recombinant clones are differentiated from normal colonies using colony selection. As in case of recombinant insulin, three types of *E.coli* colonies grow on agar: normal colonies, colonies with A chain Insulin protein and colonies with B chain insulin protein.
- Certain antibiotics could be added to the agar that would inhibit the growth of normal colonies with normal plasmid inserts.
- The recombinant colonies are purified after collection. Then SDS is used to lyse the bacterial membranes. The resulting protein is checked using ELISA.
- Or DNA could be extracted from these recombinant colonies, followed by RE digestion and after hybridizing them with biotin-labeled probe the product size is determined by electrophoresis.



**The first complete mammalian clone using 'somatic cell'. Cloning of 'Dolly' the sheep in 1996.**



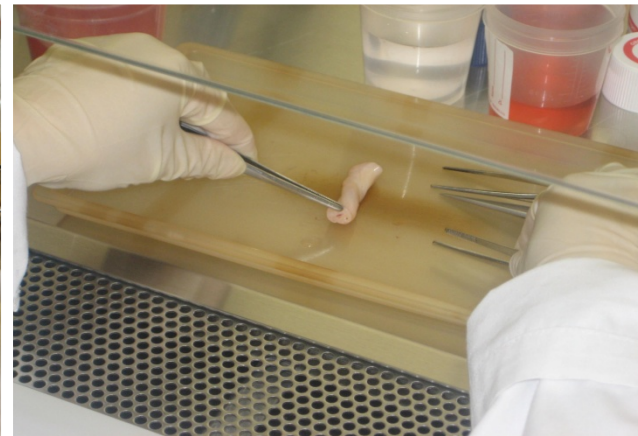
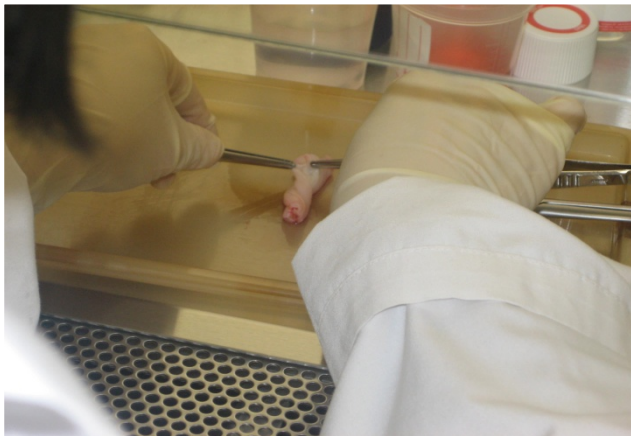
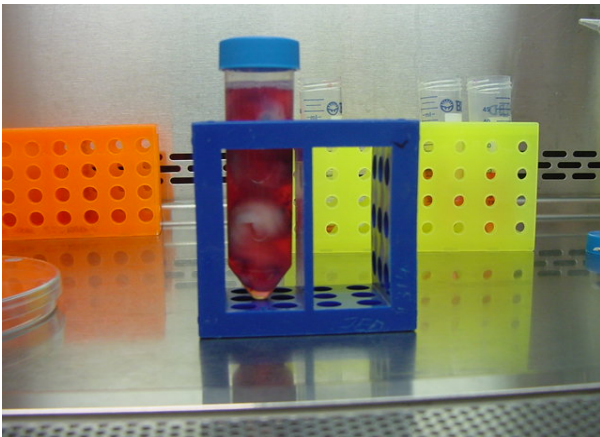
Dolly was suffering from lung cancer and early aging. She was put down in 2003. She left behind three siblings

# Stem cells

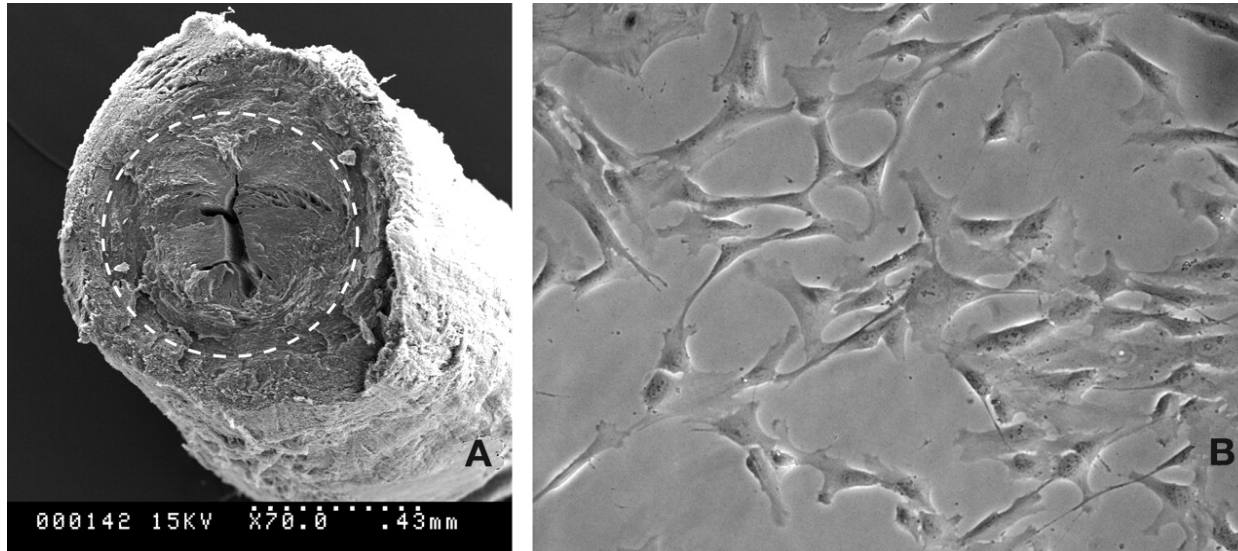
**Embryonic stem cells:** They have the ability to differentiate into all cell lineages (pluripotent), an early cell showing potential advantages but ethics and its ability to cause cancer limit its usage

**Adult stem cell:** Hematopoietic stem cells have been in use for sometime now. They can be used to synthesize blood cells but recipient has to be immunocompromised.

**Mesenchymal Stem Cells** can be obtained from cord blood or umbilical cords. They have the ability to be programmed using certain growth factors to give specific cell lineage of choice (multipotent).  
Has great potential for various utilization in future.



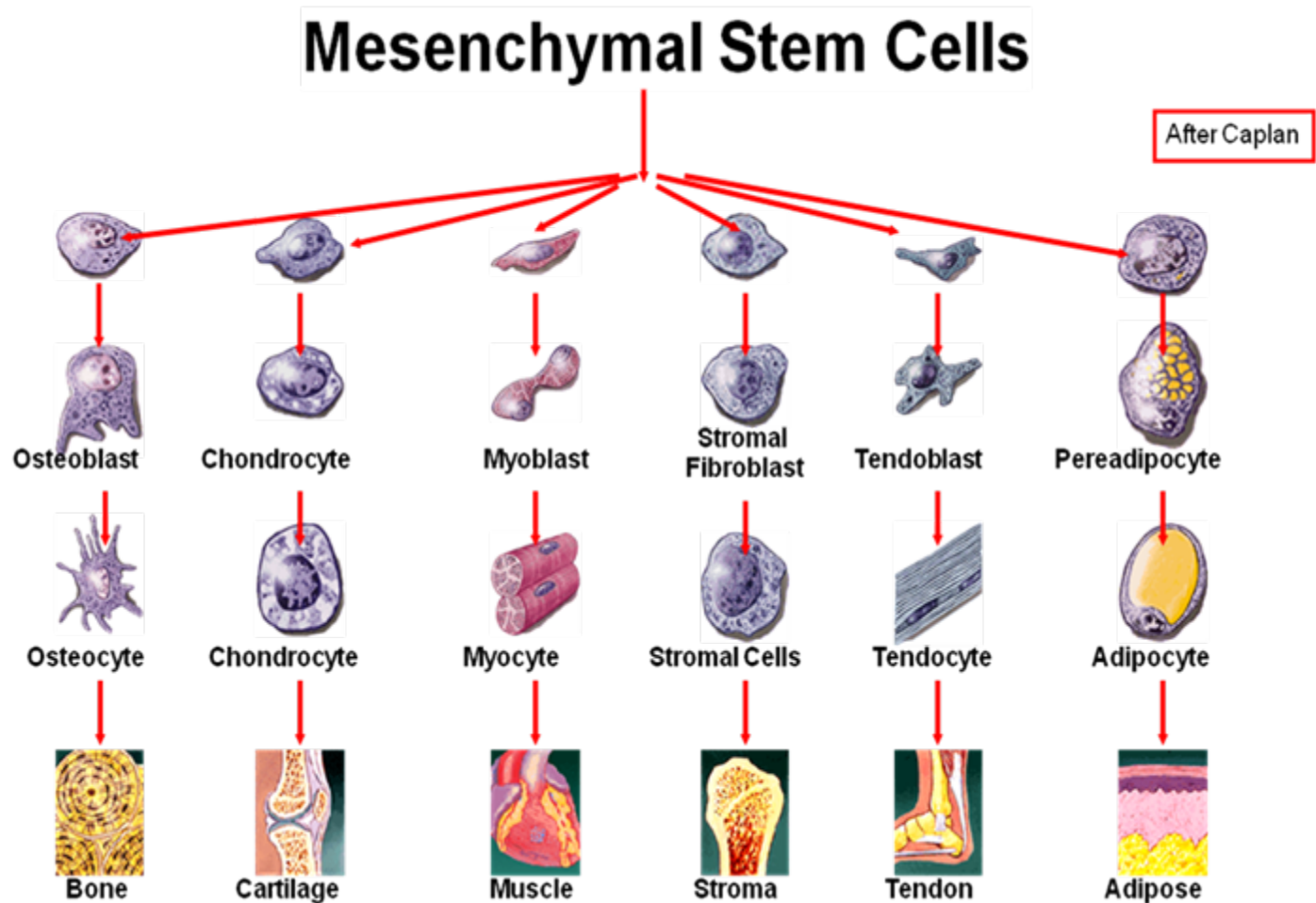
Technologist handling the human umbilical cord and dissecting the perivascular tissue. A patented technique where  $1 \times 10^6$  Mesenchymal cells can be harvested and stored. *(Prof. John Davies group at UFT, Canada-Aug 2008)*



**(A):** Scanning electron microscopy of an umbilical artery that has been excised from a human umbilical cord as part of the HUCPV cell harvesting procedure. The white dotted line represents the outer margin of the vessel and thus illustrates the perivascular tissue from which the HUCPV cells are harvested.

**(B):** HUCPV cells display a fibroblastic morphology (field width = 660 μm). Abbreviation: HUCPV, human umbilical cord perivascular. *(Prof. John Davies group at UFT, Canada-Aug 2008)*

**MSCs obtained from HUPVCs are multipotent and can give rise to several cell lineages of choice**



## Related Questions:

- Why do you need specific restriction enzyme in recombinant technology?
- Selection of a vector depends upon?
- What are the main steps of cloning?
- What is the shape of plasmid?
- How do you carryout colony selection?
- Why is colony selection important?
- Can you think of other applications of cloning related to mammals excluding DNA fragment and mammalian cloning?
- Do you support GM food or advocate against it? Give reasons for your choice.
- Do you think, as muslims, our ethics allow us to clone similar to the way 'Dolly' was cloned?