

No	Subject : Cellular	Lecturer	Note
1.	Introduction-Recent dev of . Anim Biotech:Mol-Cell	GC	Class Discussion
2	Basic Mol-Cell Laboratory Practice, In vitro.	GC	Class Discussion
3	Chromosome Bio-Cell Analysis		
3.	In vitro- Cell culture (IVM, IVF, IVG)	GC	Class Discussion
4.	Nuclear Cell – Gene (Transgenic Animal-Cloning: Cell communication and Cell Reprogramming) Basic Theory : Part 1	GC	Class Discussion
5.	Nuclear Cell – Gene (Transgenic Animal-Cloning) Technical Aspect: Part 2.	GC	Class Discussion
6.	Software analysis of Chromosome Cells	GC+ Lab. Tech.	Lab works/Visit/LSIH
7.	Nuclear Cells Transfer /Gen Transfer	GC + Lab. Tech	Lab work/Visit/LSIH (Jika Lab-tidak sedang digunakan)

Biotech : Basic Mol-Sel Laboratory- *in Vitro*

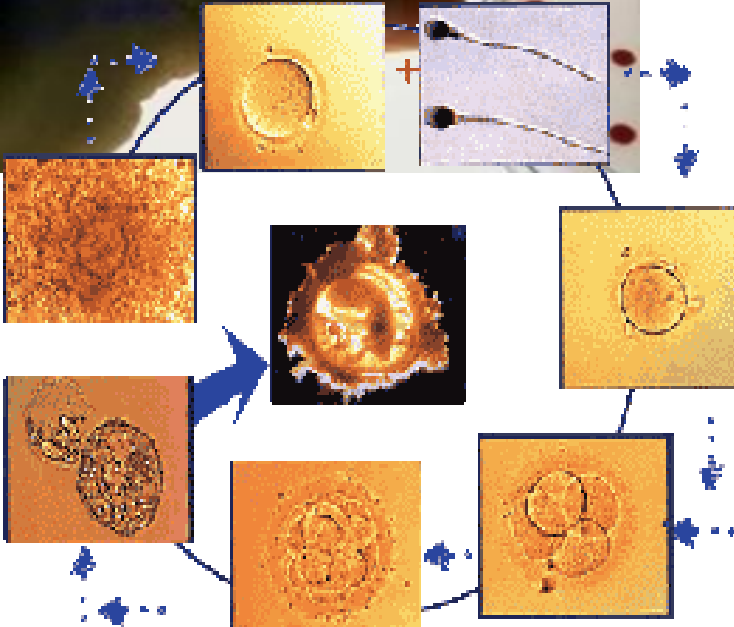


- Most widely used biotech products are recombinant proteins (produced by gene cloning in cell culture)

- **Cell culture** refers to the technique of growing cells in a lab under controlled conditions; similar to *in vitro*

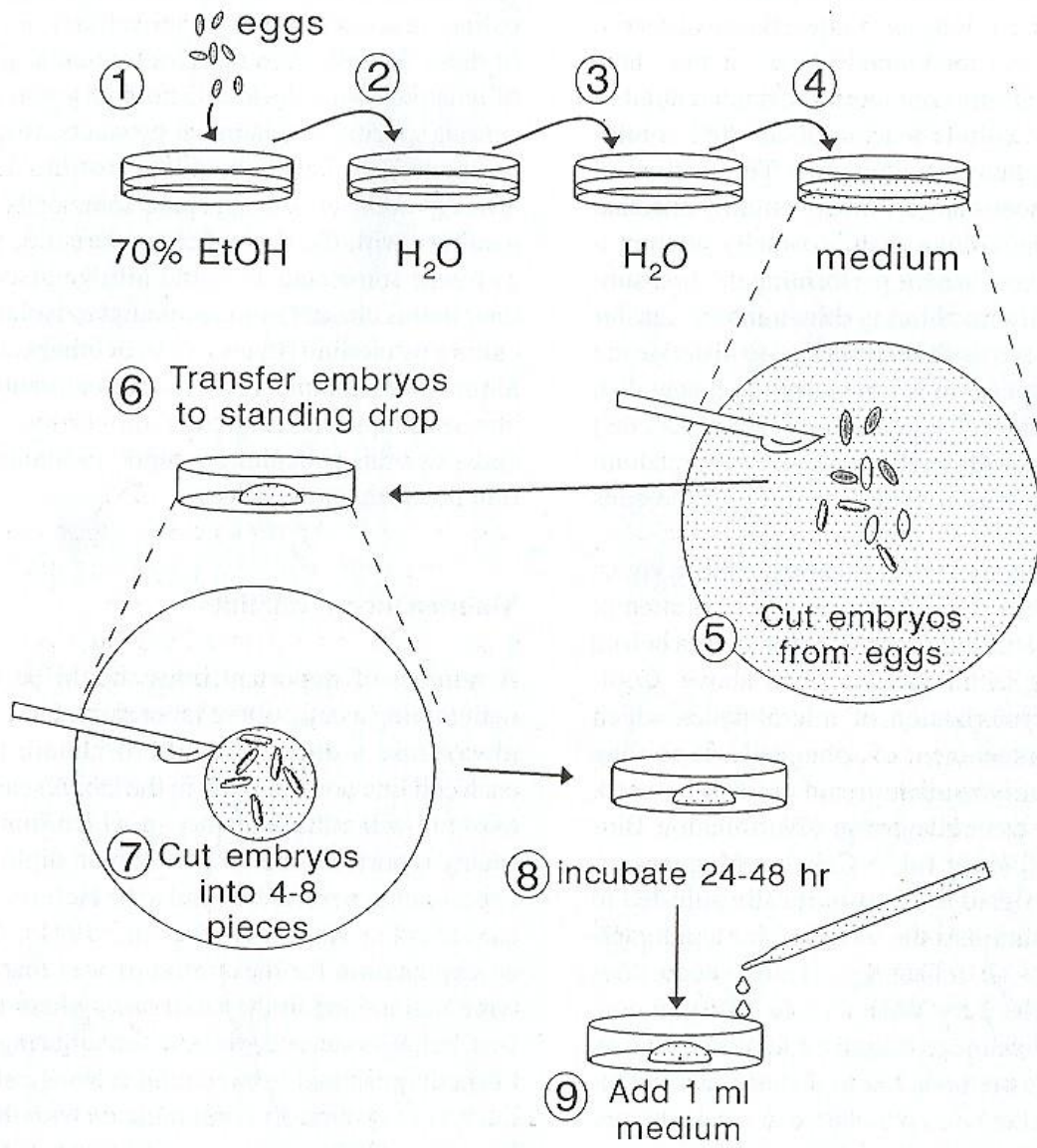
- “*in vitro*” refers to working in a controlled environment outside of a living organism

- **Bioreactors** (large culturing “containers” where DNA of interest can be mass produced) are also used



Animal Cell Biotechnology:

Primary Culture Procedure



Desinfection step

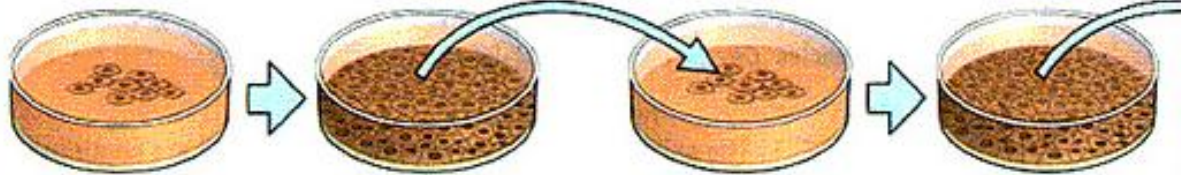
Tissue isolation

Incubation & growth

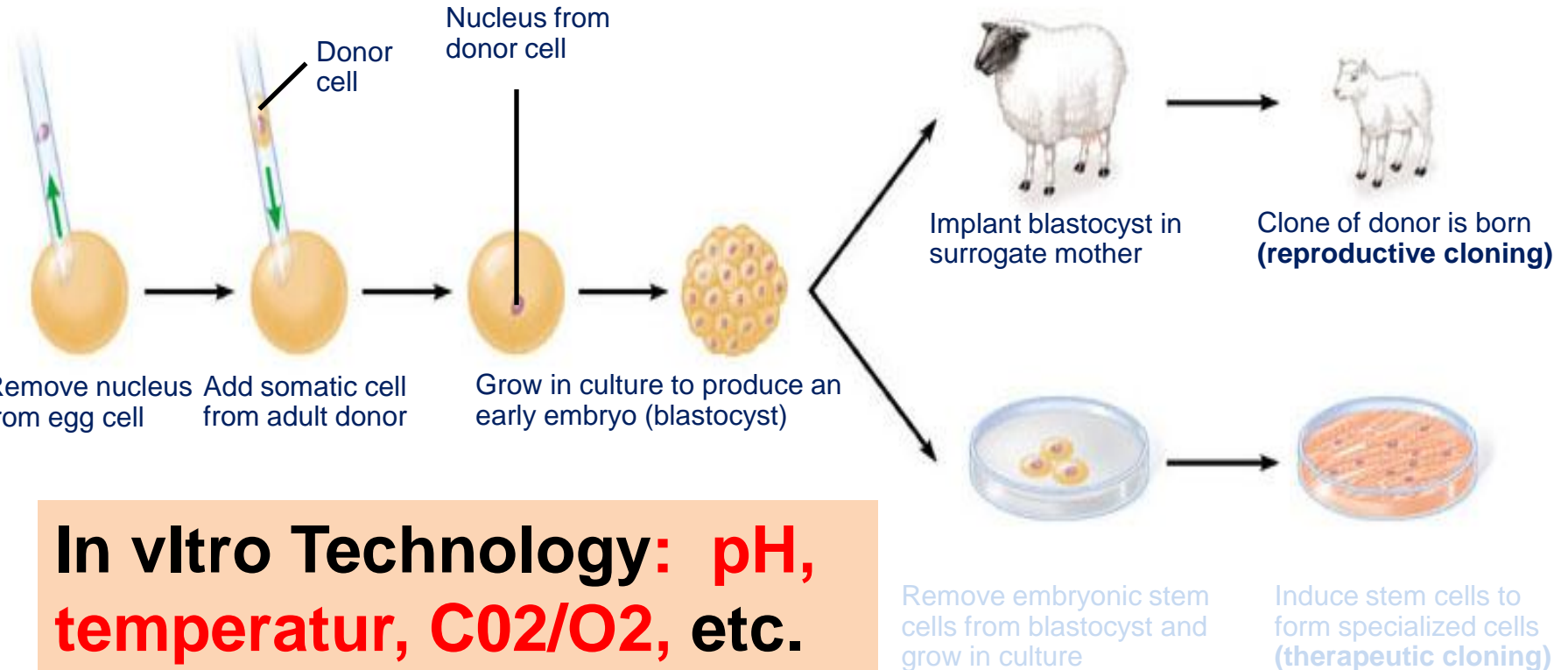
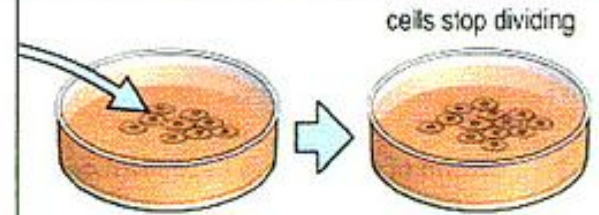
Primary cells

Animal Cell Culture: Biotechnology

Cells divide until they completely cover the dish and continue to divide when placed in fresh culture medium



After a finite number of cell multiplications, cells stop dividing



In vitro Technology: pH, temperatur, CO₂/O₂, etc.

Kultur Sel : 1. In Vitro Maturation (IVM) of Oocyte 2. In Vitro Fertilization and Development 2. In Vitro Growth (IVG) : Kultur Folikel

Memahami : Cell development (folikulogenesis)

Material : Oocyte.-Cumulus-Granulosa Cell Complexes
from large antral follicles (4 – 6 mm)

Medium : TCM199, (10 % FCS, 0.1 ng/ml Na-
pyruvate, Antibiotics.

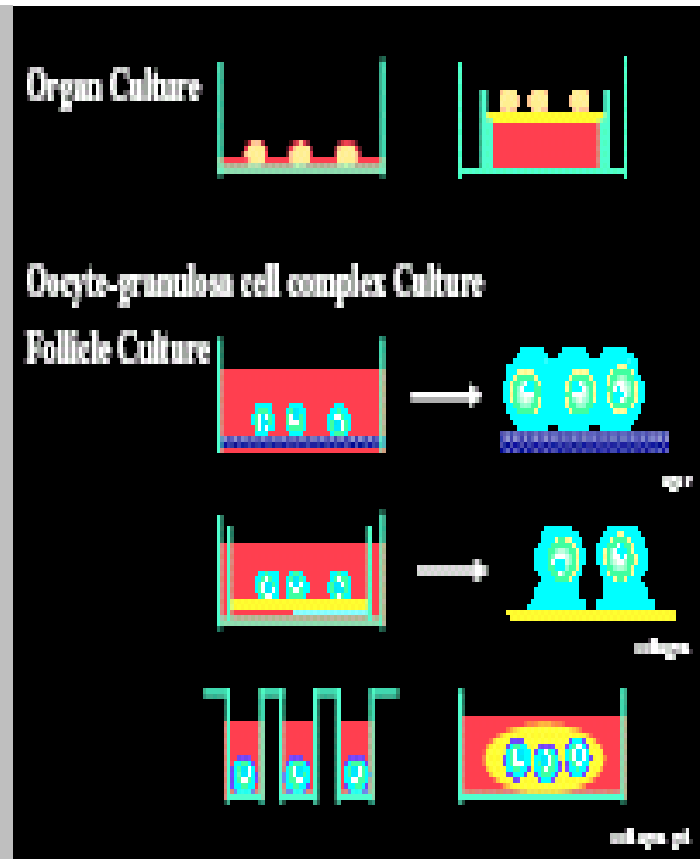
Hormon : FSH and LH

Temperatur : 38.5 – 39 0C

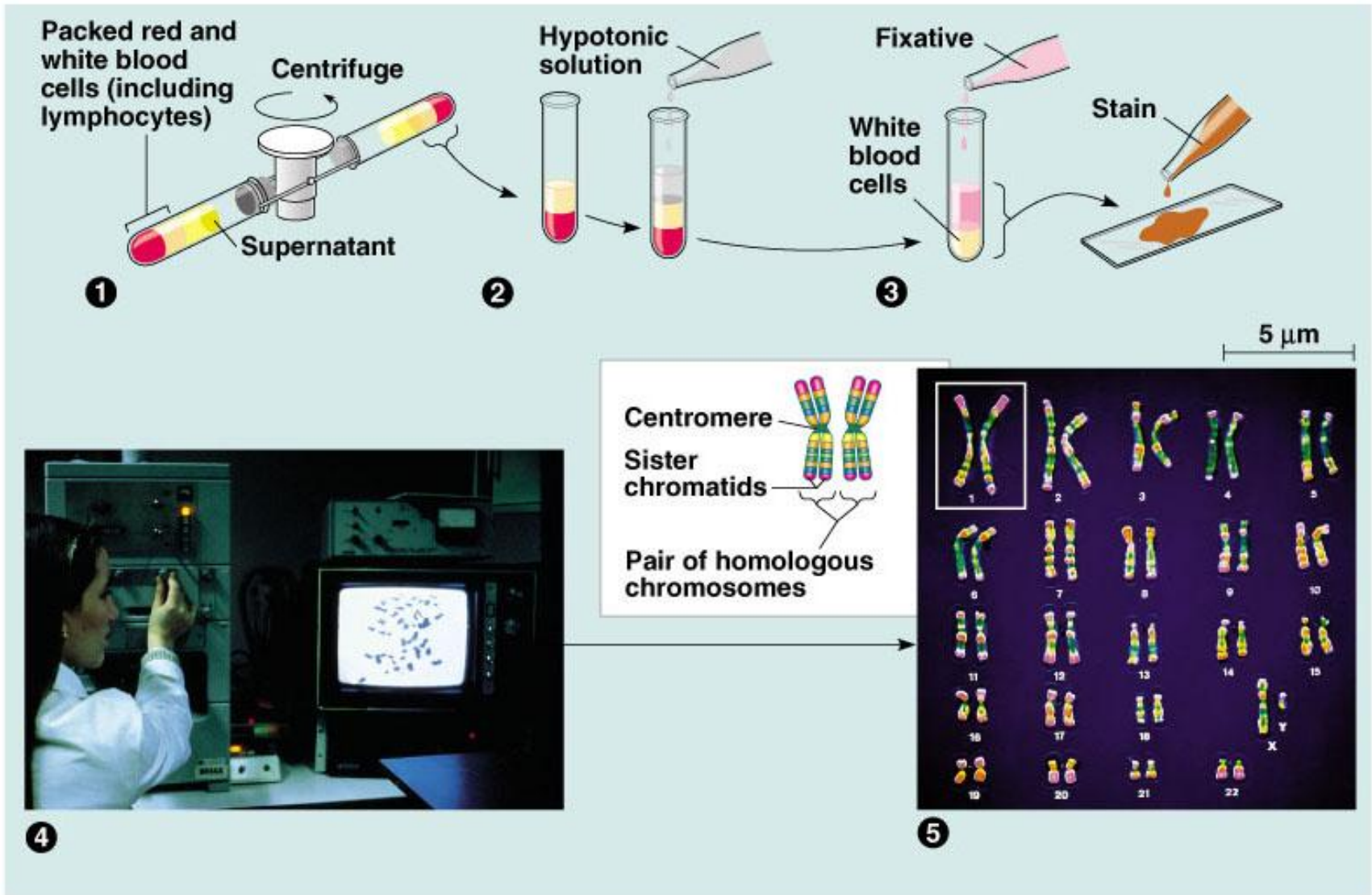
Gas % : 5 % CO2 in air

Dish : Falcon 35 mm

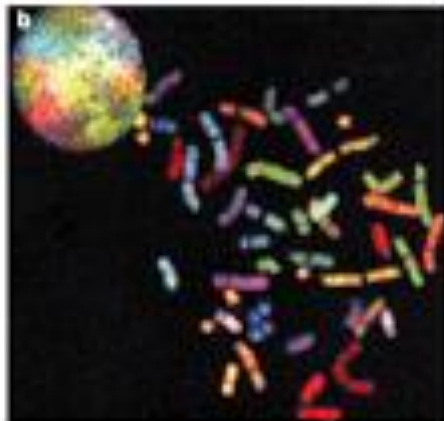
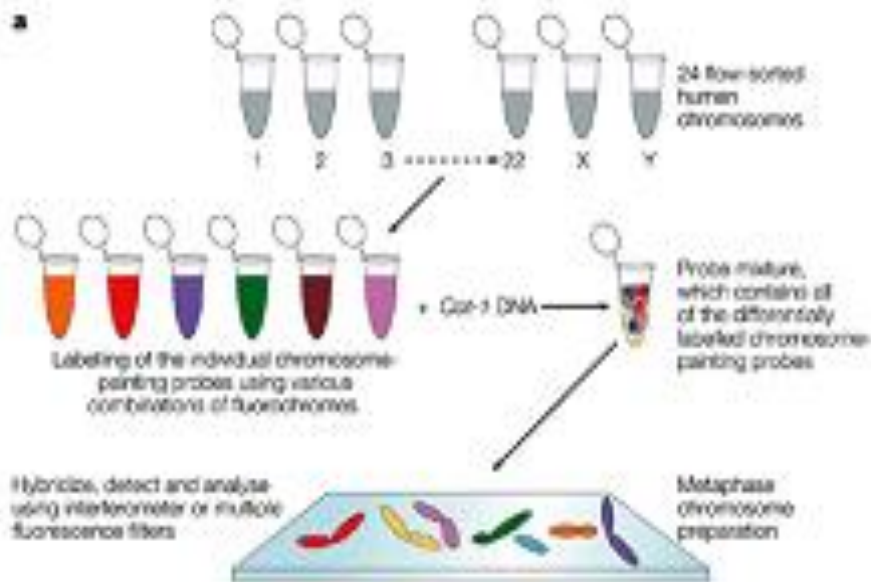
Drop : 100 ul (10 ul/oocyte), covered with
mineral oil/ parafin oil



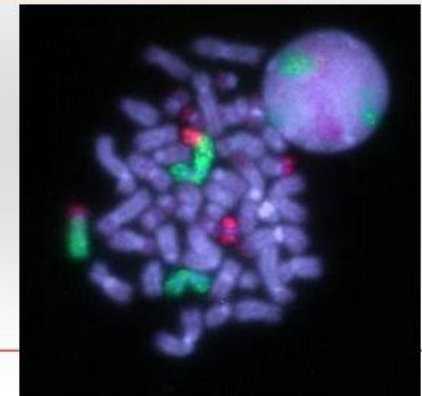
Cellular Analysis : Karyotyping



Molecular karyotyping



Chromosome painting



Identifikasi Gen Pada posisi Kromosom

Terapi Gen

Biotech Treatments

- In the near future, it may be commonplace for treatments to include the use of **gene therapy** (attempt to replace “defective” gene with “normal” gene) and **tissue engineering** (designing & growing tissues for use in regenerative medicines).

- 1st Genetically Modified Organism (GMO) to produce human protein was ***E. coli*** (pictured right) that was given DNA to produce *somatostatin* (hGH -human growth hormone - 1977)



Molecular Pharming

- **Molecular pharming** is the use of genetically modified plants (**or animals**) as a source of pharmaceutical products.
- These are usually recombinant proteins with a therapeutic value.



- This is an emerging but very challenging field that requires:
 - manipulation (at the genetic engineering level) of protein **glycosylation** (addition of polysaccharide chain)
 - subcellular protein targeting in plant cells

Animal Applications

- Animals can be used as *bioreactors*!
- Many human therapeutic proteins are needed in massive quantities (>100s of kgs), so scientists create female **transgenic animals** to express therapeutic proteins in milk.



- Goats, cattle, sheep, & chickens are sources of **antibodies** (protective proteins that recognize & destroy foreign material)
- **Transgenic** refers to containing genes from another source

Knock Outs



- Basic research in biotech uses *knock-out* experiments, which are very helpful for learning about the function of a gene.
- A **knock-out** is created when an active gene is replaced with DNA that has no functional information.
- Without the gene present, it may be possible to determine how the gene affects the organism (its function)

Dolly

In 1996, Dolly the sheep became the first cloned animal created by the somatic cell nuclear transfer process.



- Born: July 5, 1996
- Announced: February 22, 1997
- Died: February 14, 2003
- Dolly was cloned from a cell taken from a six-year-old ewe
- She became the center of much controversy that still exists today

Forensic Applications

- **DNA fingerprinting** is the classic example of a forensic application. It is used most commonly for law enforcement and crime scene investigation (CSI).
- It was first used in 1987 to convict a rapist in England.



- Other applications of DNA fingerprinting include
 - identifying human remains
 - paternity tests
 - endangered species (reduces poaching)
 - **epidemiology** (spread of disease)

Implementasi: Paternity Test (Human)

- Who's the father?
- Ternak: Similaritas DNA

