MOLECULAR BIOTECHNOLOGY (SQG3213)

PRODUCTS FROM BIOTECHNOLOGY

ZAIDAH RAHMAT

- Various products including some everyday used items at home.
- Genes, enzymes, microorganisms are processed commercially.
- Modifications may have been done for improvement.

PRODUCTS FROM BIOTECHNOLOGY

Ice Forming Bacteria

- *Pseudomonas syringae (P. syringae)*:
  - Produce certain surface protein; Ina protein (ice-nucleation active protein)
  - Found on outer bacterial cell wall & functions as nucleating centers
  - Ice crystals formed on this centers
  - = “ice-plus”

- Variant/mutant – “ice-minus bacteria”:
  - Lacks genes for producing Ina protein
  - Less favorable for ice formation

- Both WT and variant/mutant occur naturally

PRODUCTS FROM BIOTECHNOLOGY

Ice Forming Bacteria

PRODUCTS FROM BIOTECHNOLOGY

Ice Forming Bacteria

- GE enables modification of such mutant to synthetically remove or alter the gene responsible for the surface protein

- Ice-minus strain competes with WT on plants surface:
  - If it dominates over WT – no ice will form on the surface
  - Frost development is lowered

PRODUCTS FROM BIOTECHNOLOGY

Blue Genes

- Blue jeans? Or blue dyes?

- Color blue – from marine / aquatic life (corals e.g. *Heliopora coerulea*)

- Nowadays – Indigo plant (*Indigofera*)

- Mostly used as dyes for jeans and food dye industry

PRODUCTS FROM BIOTECHNOLOGY

Blue Genes

PRODUCTS FROM BIOTECHNOLOGY

Biosynthetic Plastic vs. Biodegradable Plastic

- Biosynthetic - formation of chemical compound by living organism.

- Biodegradable – capable of decaying through the action of living organisms.

- Both are man-made from polymer that are lightweight, tough & transparent.

PRODUCTS FROM BIOTECHNOLOGY

Commercial Enzymes

- Natural occurring enzymes which are genetically modified to:
  - Be more effective at desired temperature, pH, etc.

- Used in various industry:
  - Food
  - Medical
  - Textile
  - Energy

PRODUCTS FROM BIOTECHNOLOGY

Commercial Enzymes

Examples:

- Detergent
  - lipases to dissolve fat stains and clean grease traps.
  - Tolerate hot and cold temperatures (thermotolerant and cryotolerant)

- Textiles
  - Replacement of harsh chemicals with enzymes
  - Enhance preparation of cotton
  - Reduce impurities
  - Minimize “pulls” in fabric
  - Improve color quality, etc.

PRODUCTS FROM BIOTECHNOLOGY

Commercial Enzymes

- Foods and beverages
  - Production of wine, beer, cheese and bread from yeast and bacteria
  - Specialized strains have been made to improve flavor and quality
  - Cheaper process and more predictable to ensure quality
  - E.g. starch is being converted into sugar by enzymes

PRODUCTS FROM BIOTECHNOLOGY

Commercial Enzymes

- Leather
  - Replacement of harmful chemicals with enzymes for tanning hides
  - Enzymes applied to remove hair & fat from hides
  - Enzymes used to rid of keratin & pigment, cleaning process and enhance softness
  - Prevent leather from rotting

- Biofuels
  - Using bioethanol from starchy plant materials
  - Presently using corn
  - Other possible candidate – wheat, bamboo, other grasses
  - Palm oil for biodiesel?
  - Debatable due to its cost effectiveness

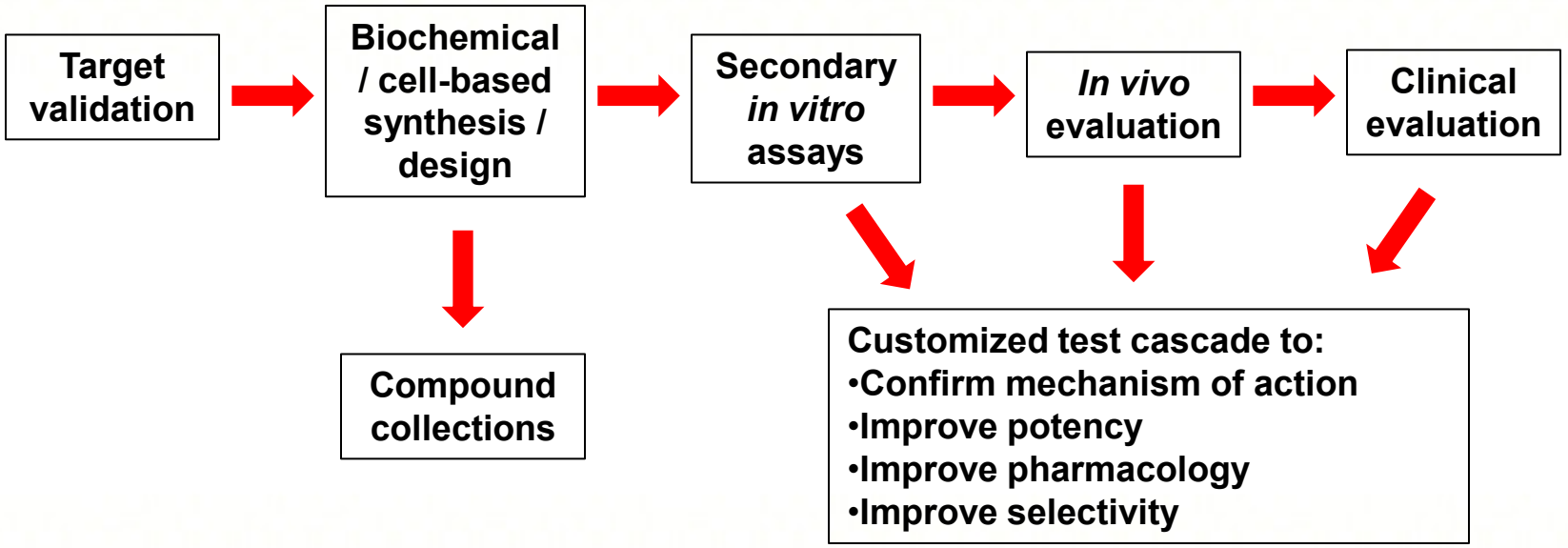
PRODUCTS FROM BIOTECHNOLOGY

Biopharmaceutical and Drug Development

- Production of medicinal drugs using biotechnology.
- Proteins, antibodies, nucleic acids, virus, bacteria (including virulant types)
- Used for therapeutic, *in vivo* diagnostic.
- E.g. Humulin (human insulin)
- Strict control over commercial distribution.

PRODUCTS FROM BIOTECHNOLOGY

Biopharmaceutical and Drug Development



PRODUCTS FROM BIOTECHNOLOGY

Biopharmaceutical and Drug Development

Preclinical	Phase I	Phase II	Phase III	Launch
Initial testing done in the lab or with animals	The first step in the development of a new drug. First studies with humans	Builds on results of Phase I	Large group, sometimes thousand of patients	
	Limited to patients, those who have cancer and limited treatment options	Involves more patients with a specific type of cancer	Participants are randomly assigned to either the new treatment or the standard treatment	
Goal: To test an initial idea to see if it has any effect	**Goal:** 1. To find the safest dose 2. To determine the most effective way to deliver a new drug 3. To identify the side effect	**Goal:** To see how well the treatment works against the disease	**Goal:** To see if the new treatment id better than the standard treatment for the cancer	**Goal:** To become the new standard for the cancer
		If treatment shows effect, and is shown to be safe enough, moves to Phase III	*Note: this is considered the most reliable and impartial way to test the new treatment*	

PRODUCTS FROM BIOTECHNOLOGY

Biocatalyst in Chemical Industries

- Enzymes – remarkable catalysts able to accept wide range of substrates (including complex molecules).

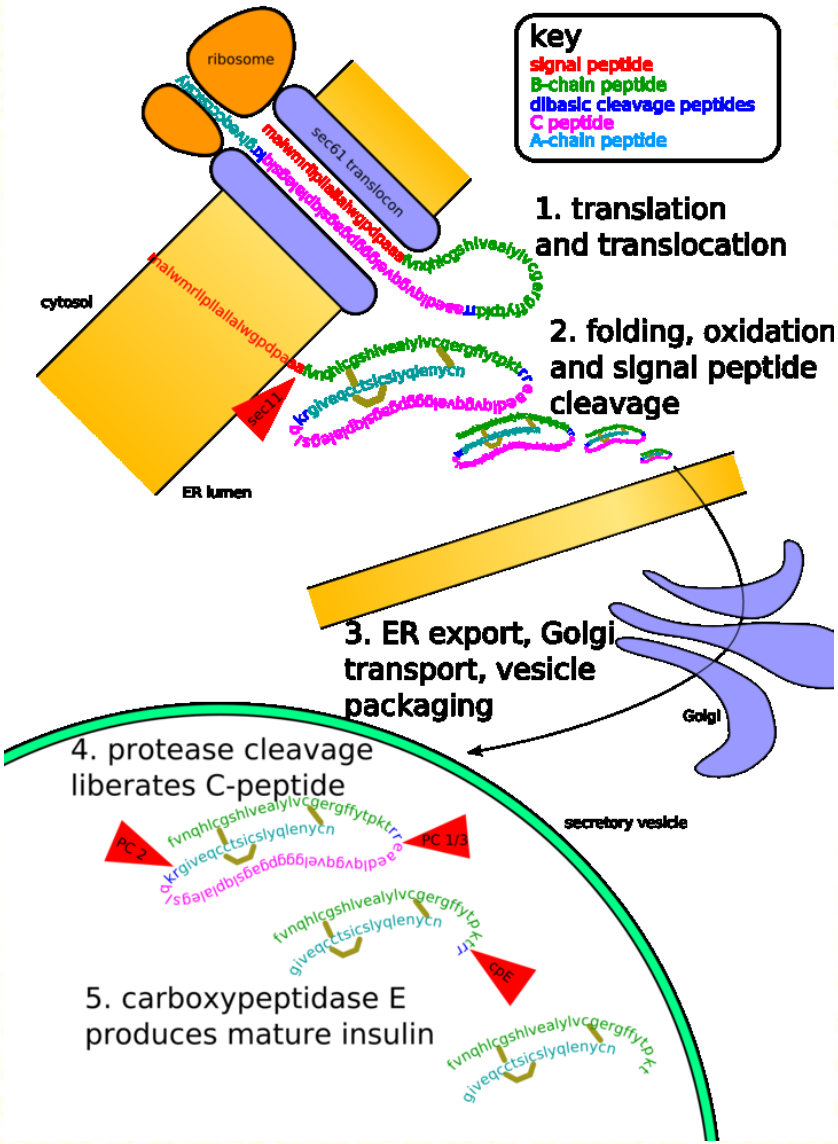
- Can be used in simple & complex transformations.

PRODUCTS FROM BIOTECHNOLOGY

Biocatalyst in Chemical Industries

- E.g.
  - Production of high-fructose corn syrup using xylose isomerase to isomerize D-glucose to D-fructose
  - Preparation of semisynthetic penicillins by penicillin amidase

PRODUCTS FROM BIOTECHNOLOGY



Medicinal Science - Insulin

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Antibiotics

- Produced with aerated vessels & agitation.

- Production process:

  - Culture preservation
  - Innoculum preparation
  - Seed stage
  - Production stage
  - Harvest, extraction and purification

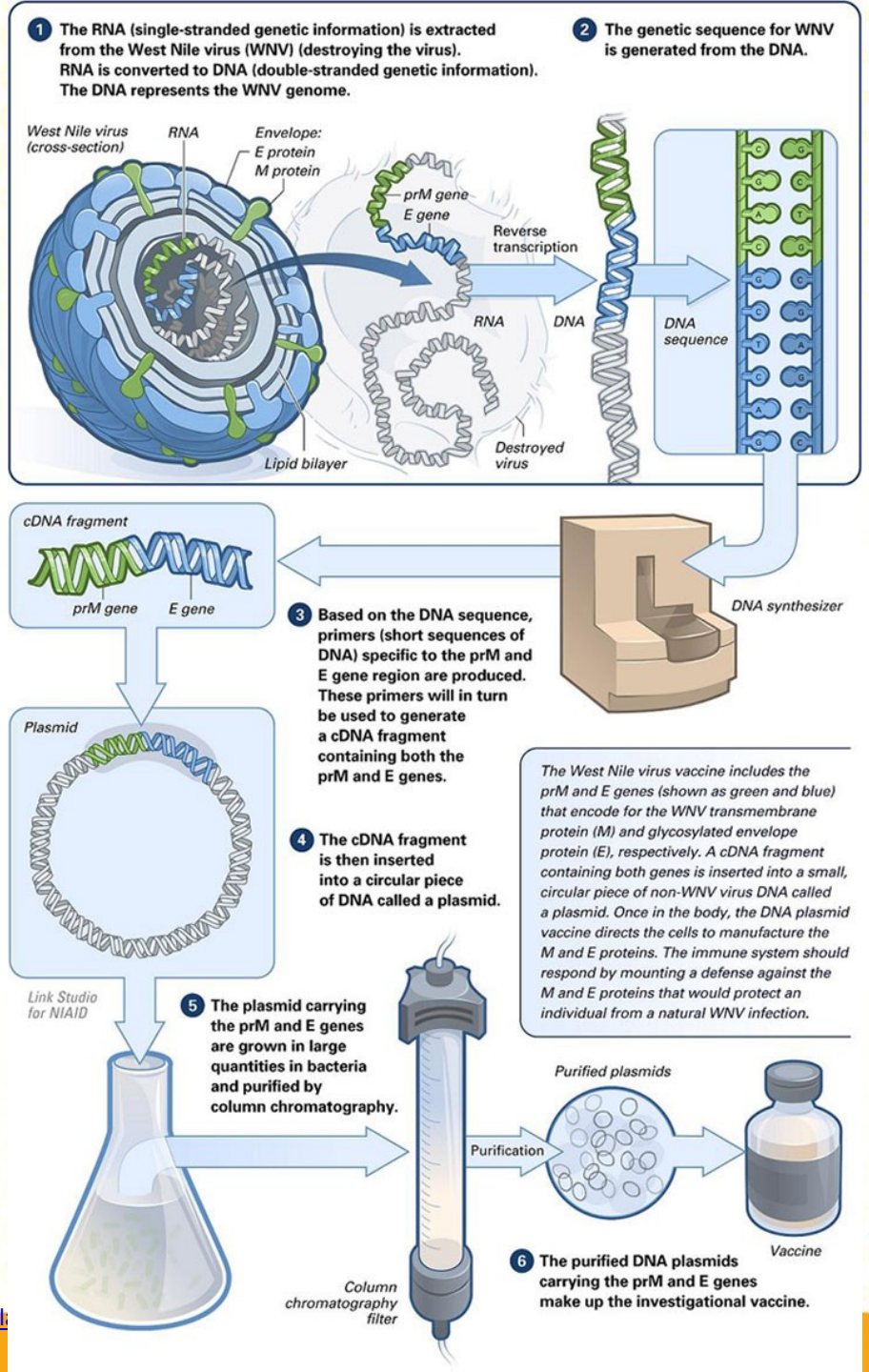
PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Antibiotics

- Various forms of antibiotics:
  - Pills
  - Gel capsules
  - Powders
  - Solutions for intravenous bags / syringes
- Strains used for production are not the original version of WT.

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines



http://www.youtube.com/watch?v=n6g76IbawWo&feature=pl

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines

- Making vaccines (general) & how it works

1. Vaccine made from an antigen isolated or produced from disease-causing microorganism.

2. Injected into blood stream.

3. B cells in blood stream respond to antigen by producing antibodies.

4. Antibodies bind o antigen to “neutralize” or inactivate it.

5. Memory cell are produced and remain ready.

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines

- Molecular approaches:

1. Delete virulence genes from pathogen.

- **Live-Attenuated Vaccines**
  - delete part of DNA coding the A1 peptide of toxin caused by cholera (*Vibrio cholerae).*
  - Most cloned DNA deleted using RE.
  - Cloned DNA conjugated to antibiotic resistance antibiotic, isolated and used as live-attenuated vaccine unable to revert to virulence.

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines

2. Express cloned antigen genes, purify & use.

- **Subunit Vaccines**
  - Derived from component of antigenic protein of pathogen.
  - Advantage : *may have lower side effects.
  - Disadvantages: *cost of purification.
    *may not provide protection against Pathogen.
  - E.g.: purified outer membrane protein of an animal virus.

- **Recombinant Subunit Vaccines**
  - Clone antigen gene not expressed at high levels in bacterial host.
  - E.g.: hepattis B surface antigen.

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines

3. Clone & express genes in nonpathogenic strain / vector.

- **Vector Vaccines**
  - Insert genes encoding virus antigen into harmless virus (vector) .
  - Vaccinate with recombinant virus to generate immune response which prevents infection.
  - Advantages: *resemble natural infection process of viral pathogen.
    *rapid recognition by immune system
    *after vaccination, amount of replicated viral vector of antigen increases
  - Disadvantage : *vector may cause serious infection in certain individuals.
  - E.g.: HBV core antigen / vaccinia virus vector.

PRODUCTS FROM BIOTECHNOLOGY

Medicinal Science - Vaccines

4. Recombinant plasmid vector containing gene for antigen.

- **DNA Vaccines**
  - genetic immunization.
  - Use *E. coli* plasmid vector with an animal virus promoter:
    *to clone gene for an antigen of a pathogen.
    *particle with plasmid DNA was gold-coated.
    *inject into subject under high pressure (biolistics).
    *expression of antigen gene induces formation of protective antibodies.

  - E.g.: mice vaccinated with influenza A DNA.

PRODUCTS FROM BIOTECHNOLOGY

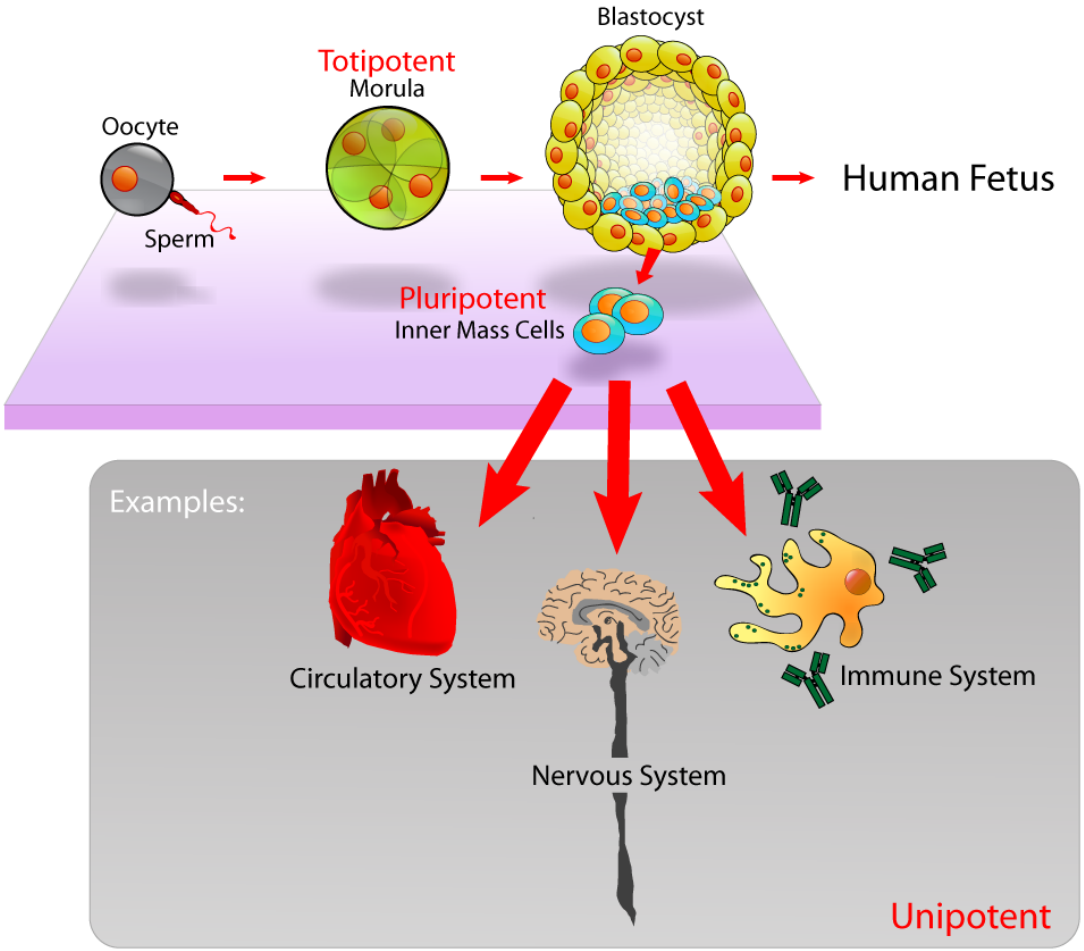
Medicinal Science - Vaccines

- Development of a DNA vaccine for Malaria.
  - Caused by intracellular parasite.
  - Effective vaccine to speed up formation of specific cytotoxic T cells – to kill infected cells before major infection occur.
  - Additional genes for other parasite antigens may be added to enhance immune response.
  - Vaccine – plasmid DNA containing gene encoding *P. falciparum* circumsporozoite protein (PfCSP).

Some scientists plan to genetically engineer mosquitoes incapable of carrying malaria parasite. Propose to replace or breed with existing mosquitoes. However, malaria parasite tends to adapt. Others fear the new mosquitoes could carry new viruses.

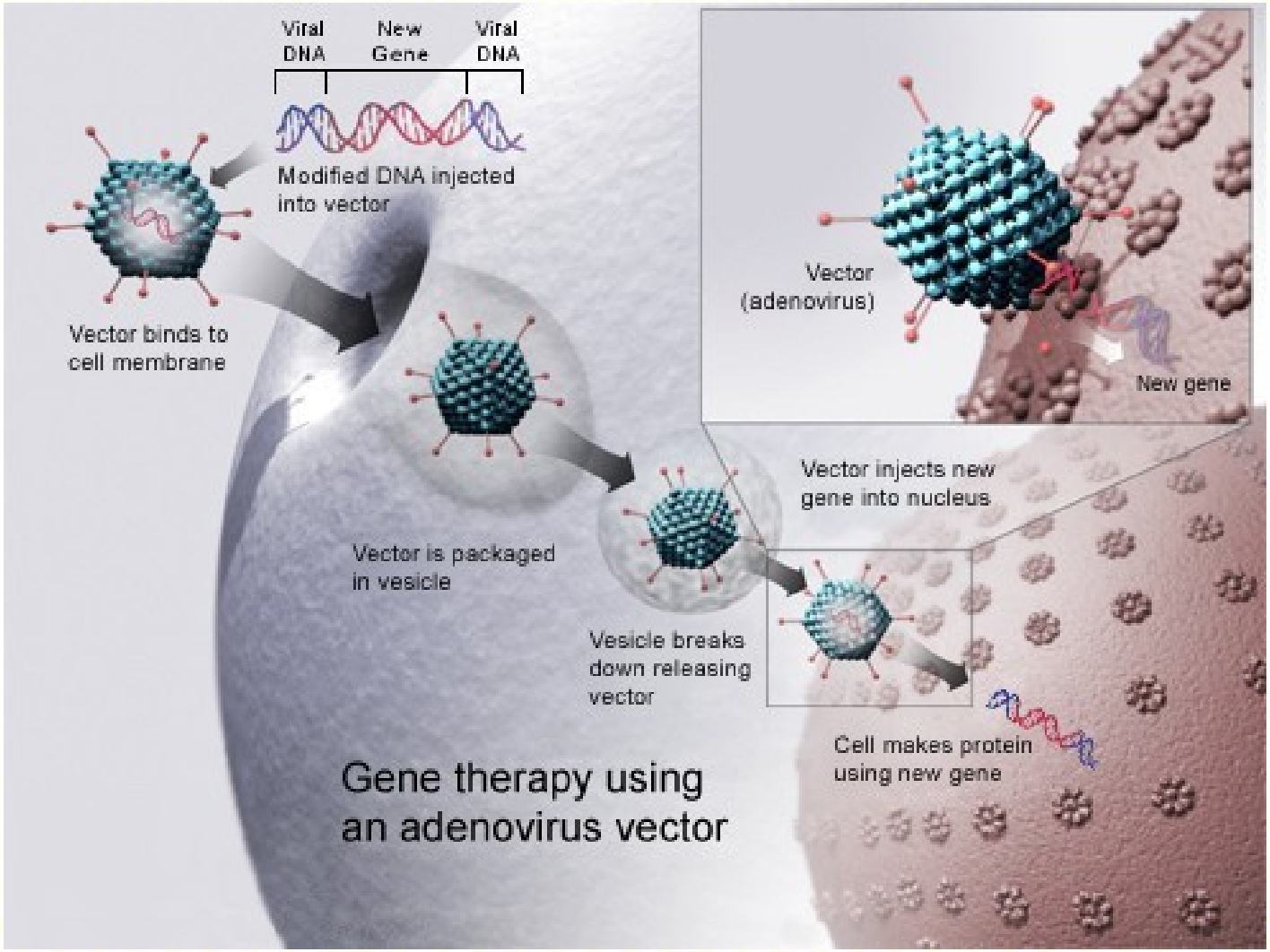
PRODUCTS FROM BIOTECHNOLOGY

Cell and Gene Therapy



PRODUCTS FROM BIOTECHNOLOGY

Cell and Gene Therapy



PRODUCTS FROM BIOTECHNOLOGY

Tissue Engineering & Regeneration Method

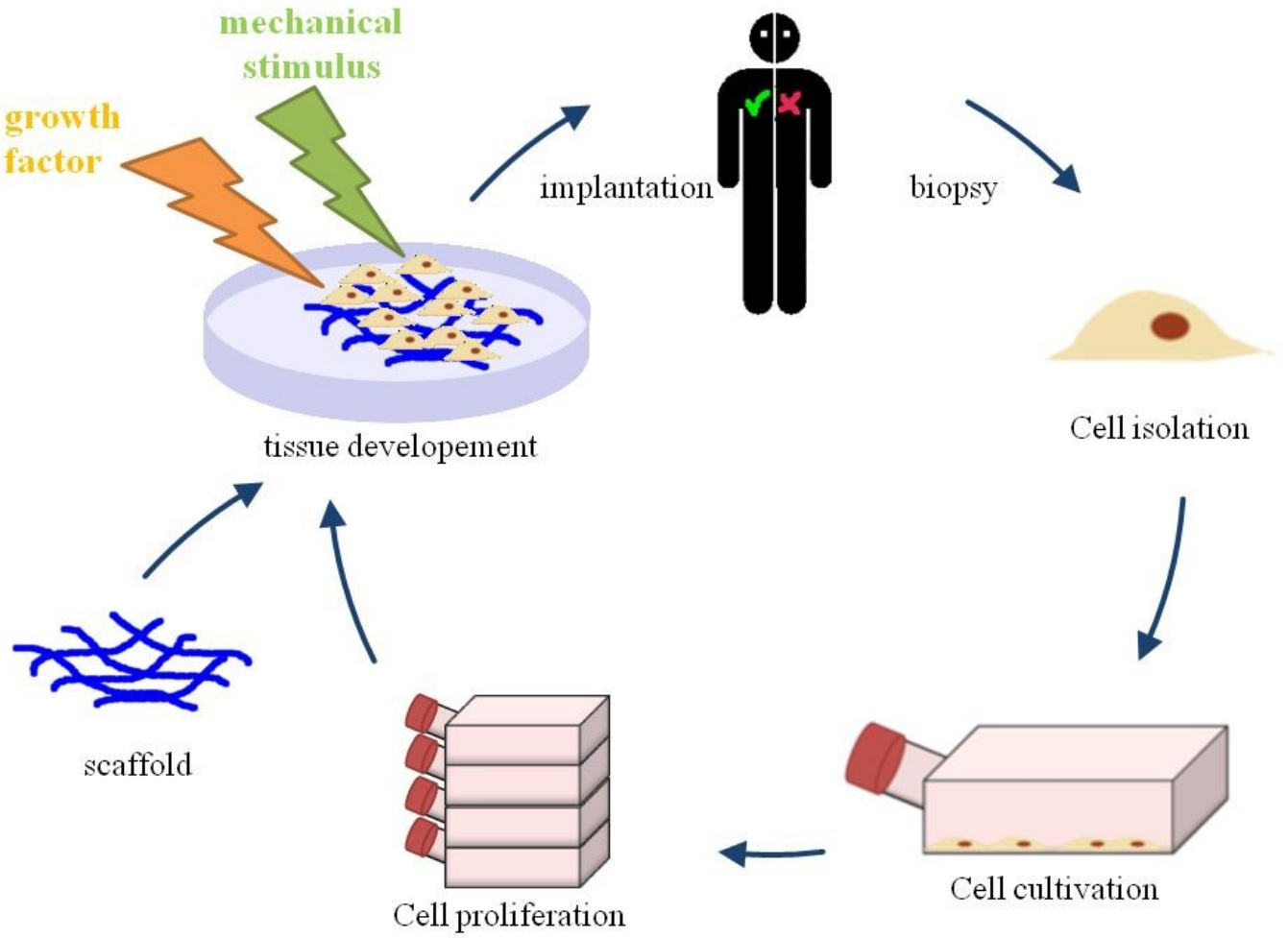
- Emerging multidisciplinary field comprising of biology, medicine and engineering.

- Therapeutic and diagnostic applications.

- Includes the field of:
  - Biomaterials
  - Cells
  - Biomolecules
  - Engineering design aspects
  - Biomechanical aspects of design
  - Informatics to support tissue engineering

PRODUCTS FROM BIOTECHNOLOGY

Tissue Engineering & Regeneration Method



- All of the products from biotechnology are made using Recombinant DNA Technology

Recombinant DNA technology

- Recombinant DNA technology & genetic engineering have changed molecular biology, basic science and medical research forever
- Provides a set of techniques for combining genes from different sources
- Applied to various industry

- Plasmids are key tools for DNA technology
  - Researchers use plasmids to insert genes into bacteria in a process known as cloning
- Bacteria take the recombinant plasmids and reproduce via transformation process
- Transformation - the taking up of DNA from the fluid surrounding the cell
- This clones the plasmids and the genes they carry
- The transferred DNA is then integrated into the recipient cell's chromosome
  - Products of the gene can then be harvested

Components of a cloning experiment

Cloning Component	Function
Donor DNA (insert)	Source of the DNA or gene to be cloned.
Restriction endonuclease	Enzyme used to cut both donor and vector DNA at specified locations, so that the donor DNA can be spliced into the vector.
Vector	Plasmid or bacteriophage used to introduce the gene to be cloned into a suitable host cell.
DNA ligase	Enzyme used to join the spliced ends of vector and donor DNA and thus form a recombinant vector.
Host cell	Usually a bacterium or a yeast cell. Recombinant vectors are introduced into host cells to obtain larger quantities of the recombinant DNA molecule.

References:

- Bernard R. Glick. (2008) Molecular Biotechnology: Principles & Applications of Recombinant DNA - John Wiley & Sons, Inc., USA.
- Acquaah, G. (2004) Understanding Biotechnology: An Integrated and Cyber-Based Approach. Pearson, Prentice Hall, New Jersey.