



**STIKES NOTOKUSUMO
YOGYAKARTA**

BIOTEKNOLOGI

SISTEM PERKULIAHAN

Semester Gasal 2024 - 2025



BIOTEKNOLOGI

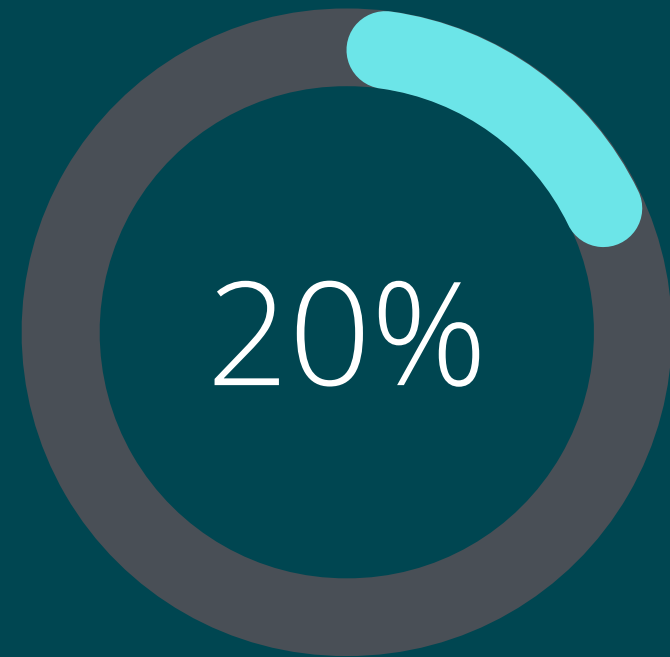
Deskripsi Mata Kuliah

Matakuliah bioteknologi menyajikan gambaran perkembangan bioteknologi yang digunakan sebagai pendekatan pengatasan permasalahan kesehatan, perkembangan bidang farmasi dan perkembangan metode penelitian. Materi yang dicakup dalam mata kuliah bioteknologi meliputi gambaran umum bioteknologi kultur jaringan tanaman, bioteknologi fermentasi, bioteknologi rekayasa genetika, rekayasa protein, rekayasa jaringan, bioteknologi dalam analisis, diagnostik dan penelitian, teknologi antibodi monoklonal, transgenik, dan bioteknologi dalam metode pengujian pra klinis (in vitro).

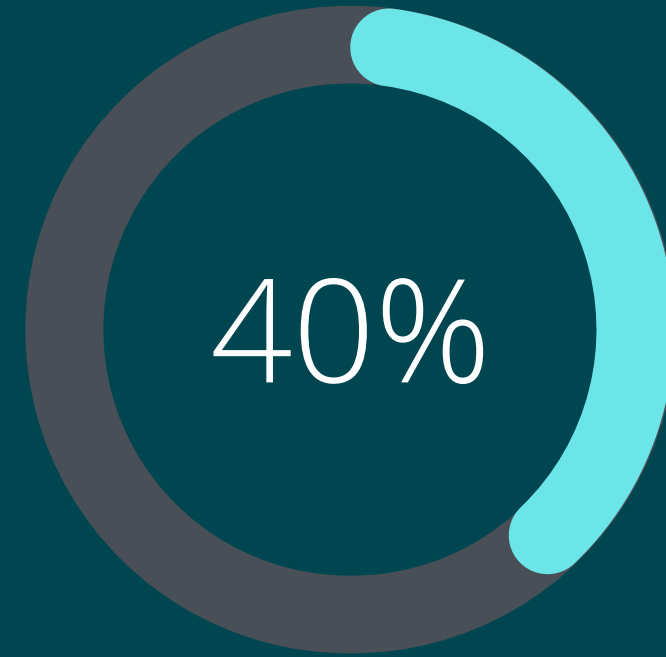


Ketentuan Perkuliahan

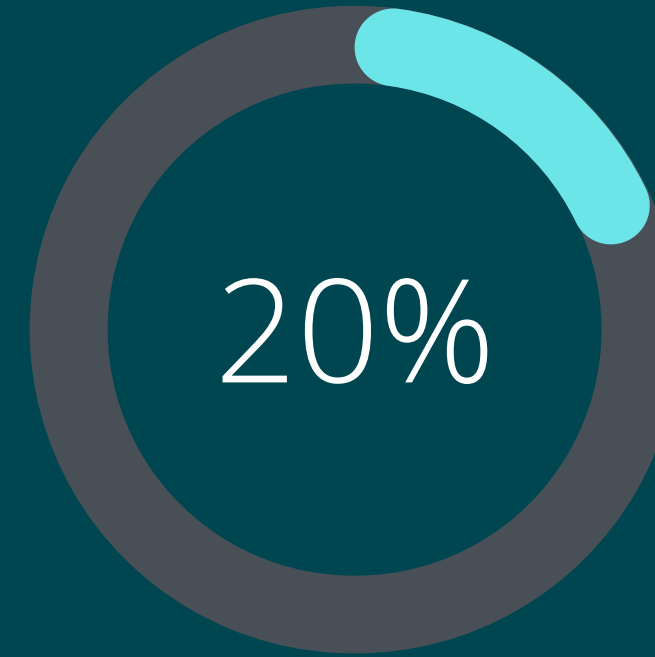
- Hadir tepat waktu. Toleransi keterlambatan 20 menit.
- Jumlah kehadiran 75% untuk mengikuti UTS dan UAS (maksimal 3x absen).
 - Melanggar = tidak boleh ikut UAS
- Tatap muka.



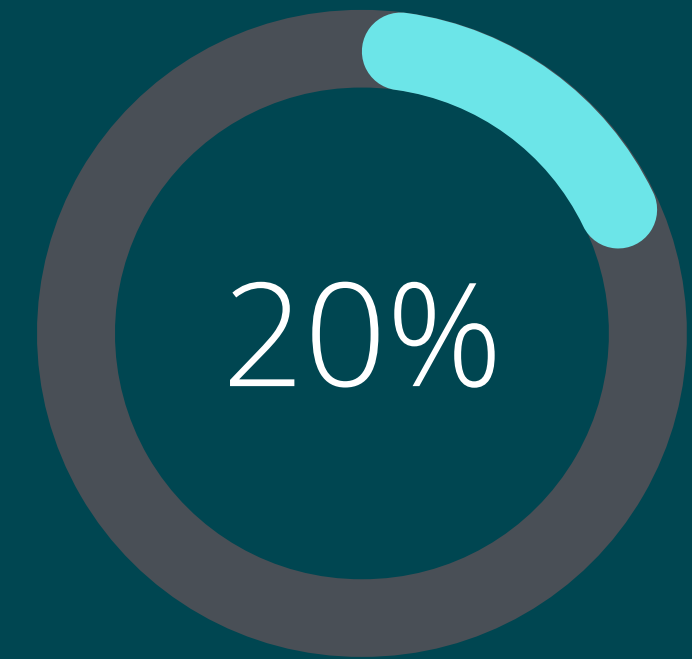
Keaktifan



Tugas



UTS



UAS

RENCANA PEMBELAJARAN SEMESTER

Jadwal Kuliah
Jumat : 13.00 - 14.40 WIB

Dosen Pengampu

- apt. Trifonia Rosa K, M.Biotech (Koordinator)
- apt. Perdana Haresmita, M.Pharm.Sci.

Rencana Pembelajaran Semester

MATERI UTS	DOSEN PENGAMPU
Peran bioteknologi dalam dunia farmasi	apt. Trifonia Rosa K, M.Biotech
Pendekatan bioteknologi kultur jaringan tanaman	apt. Trifonia Rosa K, M.Biotech
Pendekatan bioteknologi fermentasi	apt. Trifonia Rosa K, M.Biotech
Pendekatan bioteknologi rekayasa genetika	apt. Trifonia Rosa K, M.Biotech
Pendekatan bioteknologi rekayasa protein	apt. Perdana Priya Haresmita, M.Pharm.Sci
Pendekatan bioteknologi untuk analisis, diagnostik dan penelitian	apt. Perdana Priya Haresmita, M.Pharm.Sci
Pendekatan bioteknologi antibodi monoklonal	apt. Perdana Priya Haresmita, M.Pharm.Sci

Rencana Pembelajaran Semester

MATERI UAS	DOSEN PENGAMPU
Pendekatan bioteknologi transgenik	apt. Perdana Priya Haresmita, M.Pharm.Sci
Pendekatan bioteknologi untuk produksi biofarmasetikal	apt. Perdana Priya Haresmita, M.Pharm.Sci
Pendekatan bioteknologi rekayasa jaringan	apt. Perdana Priya Haresmita, M.Pharm.Sci
Studi jurnal tentang bioteknologi	apt. Perdana Priya Haresmita, M.Pharm.Sci
Studi jurnal tentang bioteknologi	apt. Perdana Priya Haresmita, M.Pharm.Sci
Studi jurnal tentang bioteknologi	apt. Perdana Priya Haresmita, M.Pharm.Sci
Studi jurnal tentang bioteknologi	apt. Perdana Priya Haresmita, M.Pharm.Sci

Referensi

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- Groves MJ, 2006, *Pharmaceutical Biotechnology*, 2nd ed., CRC, Taylor & Francis.
- Brown TA, 2006, *Gene Cloning & DNA analysis*, Blackwell Publ. Oxford
- Sven Frokjaer and Lars Hovgaard, 2000, *Pharmaceutical Formulation Development of Peptides and Proteins*, CRC Press
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- Saltzman W. M, *Tissue engineering: engineering principles for the design of replacement organs dan tissues*, Oxford university press, 2004
- W.W. Minuth, R. Strehl, K. Schumacher, *Tissue Engineering: Essentials for daily laboratory work*, Wiley- VCH, 2005



**STIKES NOTOKUSUMO
YOGYAKARTA**

PERTEMUAN 1

apt. Trifonia Rosa K, M.Biotech

BIOTECHNOLOGY

TOPIK BAHASAN

Peran bioteknologi dalam dunia farmasi

01

Pendahuluan

02

Ruang lingkup

03

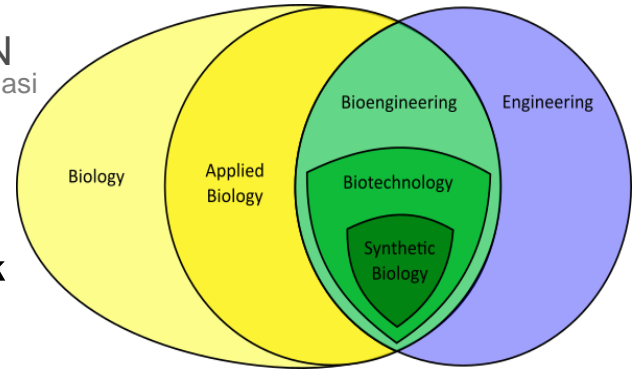
Produk farmasi
berdasar
bioteknologi

P E N D A H U L U A N

Peran bioteknologi dalam dunia farmasi

Bioteknologi merupakan gabungan dua kata yaitu bio dan teknologi.

Bio berarti makhluk hidup dan teknologi adalah cara untuk memproduksi barang atau jasa



Bioteknologi adalah cabang ilmu yang mempelajari pemanfaatan makhluk hidup (bakteri, fungi, virus, dan lain-lain) maupun produk dari makhluk hidup (enzim, alkohol) dalam proses produksi untuk menghasilkan barang dan jasa

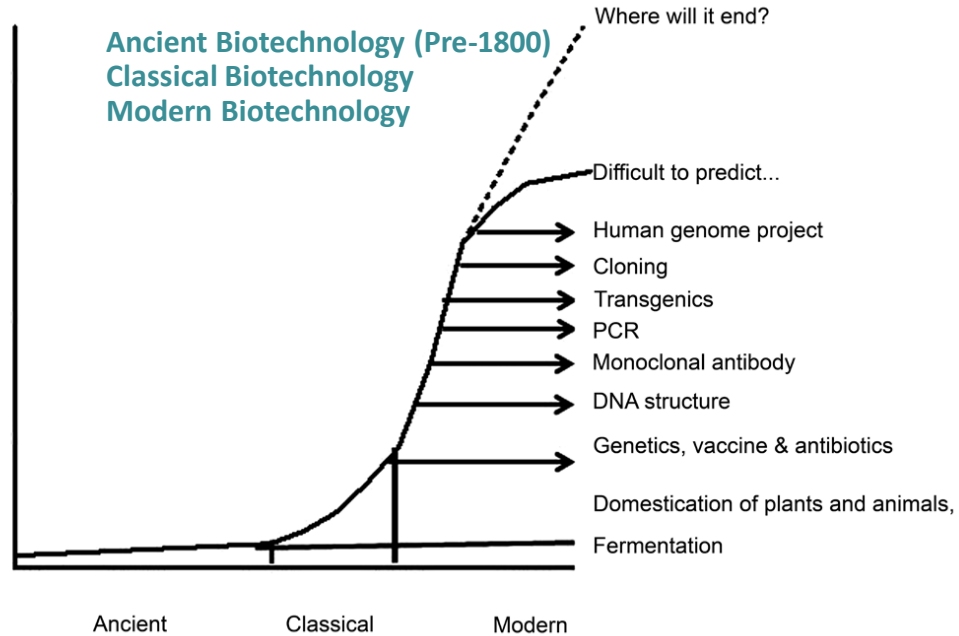


- European Federation of Biotechnology (1989) mendefinisikan bioteknologi sebagai perpaduan ilmu pengetahuan dan ilmu rekayasa yang bertujuan untuk meningkatkan aplikasi organisme hidup, sel, bagian dari organisme hidup dan analog molekular untuk menghasilkan produk dan jasa.
- Menurut Louchli (1987) bioteknologi didefinisikan sebagai segala teknik yang menggunakan organisme hidup atau bagian dari organisme untuk : (a) memperbaiki sifat tanaman atau hewan ; (b) mengembangkan mikroba untuk tujuan khusus atau ; (c) membuat / memodifikasi produk tanaman.

PENDAHULUAN

Peran bioteknologi dalam dunia farmasi

History of the development of biotechnology



Traditional Biotechnology

Traditional biotechnology includes tissue culture, mutagenesis.



Modern Biotechnology

Modern biotechnology includes DNA profiling, genome analysis, transgenics, DNA cloning.

The evolution of biotechnology over the last century

Created by Saveena Solanki



Year 2013

The **first bionic eye** is produced in the US giving hope to blind people worldwide.



Year 1998

A **draft of the human genome map** is created that locates more than 30,000 genes.



Year 1983

The **first genetically modified (transgenic) plant** is presented.



Year 1953

Biologists James Watson and Francis Crick describe the **double helix of DNA**.



Year 1928

Scottish bacteriologist Alexander Fleming discovers the **antibiotic use of penicillin**.

Year 2020



Biotechnology innovations lead the **fight against the SARS-CoV-2 pandemic**.

Year 2010



A group of researchers from the J.Craig Venter Institute creates the **first synthetic cell**.

Year 1997



Scientists introduce the world to Dolly the sheep, the **first clone of a mammal**.

Year 1969



An **enzyme is synthesized in vitro** for the first time in history.

Year 1943



Canadian scientist Oswald Theodore Avery discovers that **DNA is the carrier of genes**.

Year 1919



Hungarian agronomist Karl Ereky coins the **term biotechnology**.

History of the development of biotechnology

RUANG LINGKUP BIOTEKNOLOGI

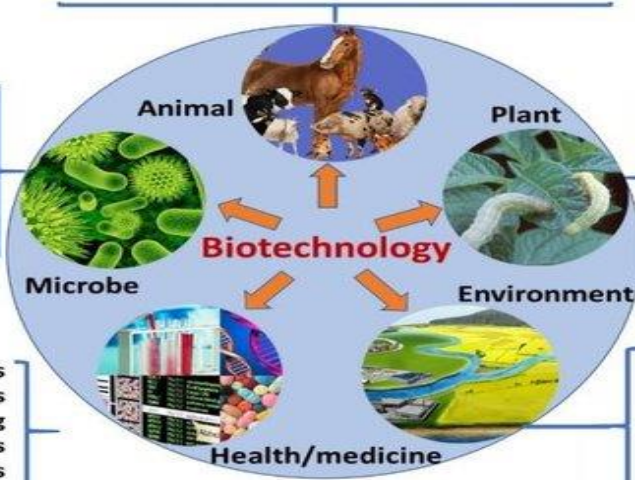
Peran bioteknologi dalam dunia farmasi

Major areas of biotechnology

Animal breeding/genetic improvement
Cryopreservation (gametes, embryos)
Artificial insemination and embryo transfers
Genetic diversity/heterozygosity of populations
Population structure analysis
Molecular diagnosis for diseases
Genetic engineering (transgenes)
Vaccines/pharmaceutical protein productions
Gene farming

Fermentation for enzymes/foods/vitamins/drugs
Genetic engineering/site directed mutations
Biofuels/biosynthesis and degradation
Bioconversion for natural compounds
Food and feed/brewing products
Pre/probiotic products
Whole cell biocatalysts

Genomics and drug production/personal medicines
Recombinant vaccines, pharmaceutical proteins
Molecular genotyping and phenotyping
Molecular mechanisms of diseases
Genome wide association studies
Genomes/ Diseasomes



Productivity/quality/tolerance and resistance
Micro-propagation
Molecular breeding
Genome editing
Transgenic plants
Edible vaccines

Renewable energy resources
Environmental biosensors
Treatment of wastes
Bioremediation
Pollution treatment
Biodegradation

Interdisciplinarity Fields of Biotechnology

Biotechnology is one of the most important applied interdisciplinary sciences of the **21st century**. It is the trusted area that enables us to find the beneficial way of life. Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.

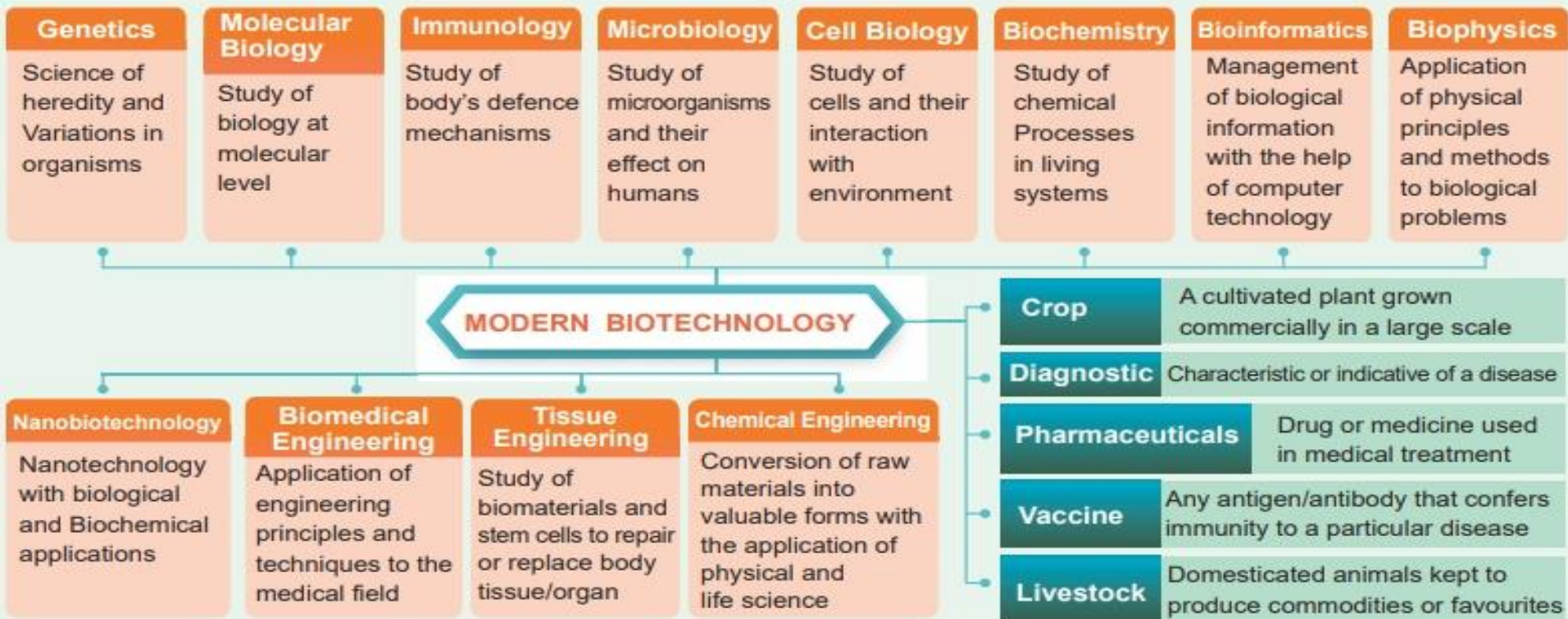


Figure 4.1: Interdisciplinarity Fields of Biotechnology

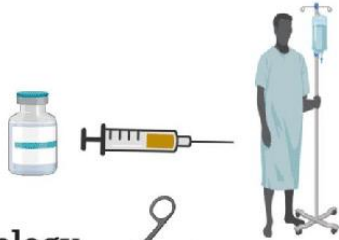
RUANG LINGKUP BIOTEKNOLOGI

Peran bioteknologi dalam dunia farmasi

A Blue biotechnology



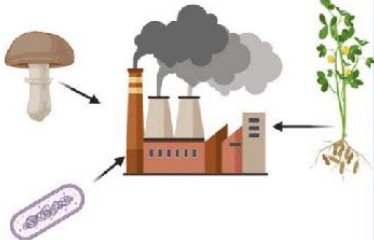
B Red biotechnology



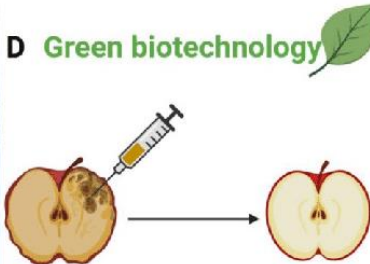
Biotechnology



C White biotechnology



D Green biotechnology



Type of Biotechnology

1. Blue biotechnology : Marine and aquatic applications of biotechnology
2. Red biotechnology : Medical applications
3. White biotechnology : Industrial applications
4. Green biotechnology : Agricultural applications

RUANG LINGKUP BIOTEKNOLOGI

Peran bioteknologi dalam dunia farmasi

Types of biotechnology



Red
medical and
pharmaceutical



White or gray
industrial



Green
agricultural



Gold
bioinformatics
and data



Blue
marine and
aquatic
environments



Yellow
food
production



Violet
governance
of ethical
considerations

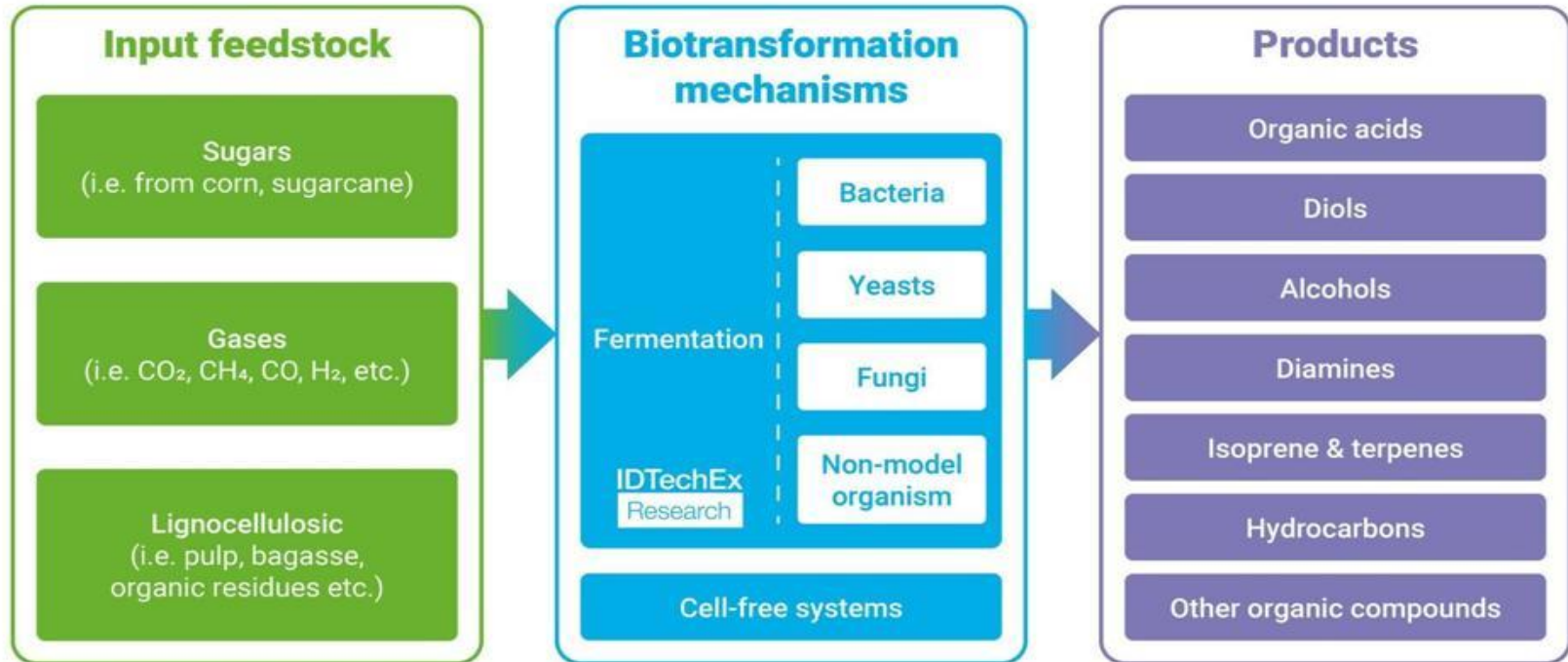


Dark
warfare

RUANG LINGKUP BIOTEKNOLOGI

Peran bioteknologi dalam dunia farmasi

Production of Biobased Chemicals using White Biotechnology



HOW does it **W**ORK?

What are...

Genetic Engineering?

The process of making changes directly to the DNA of an organism.

and

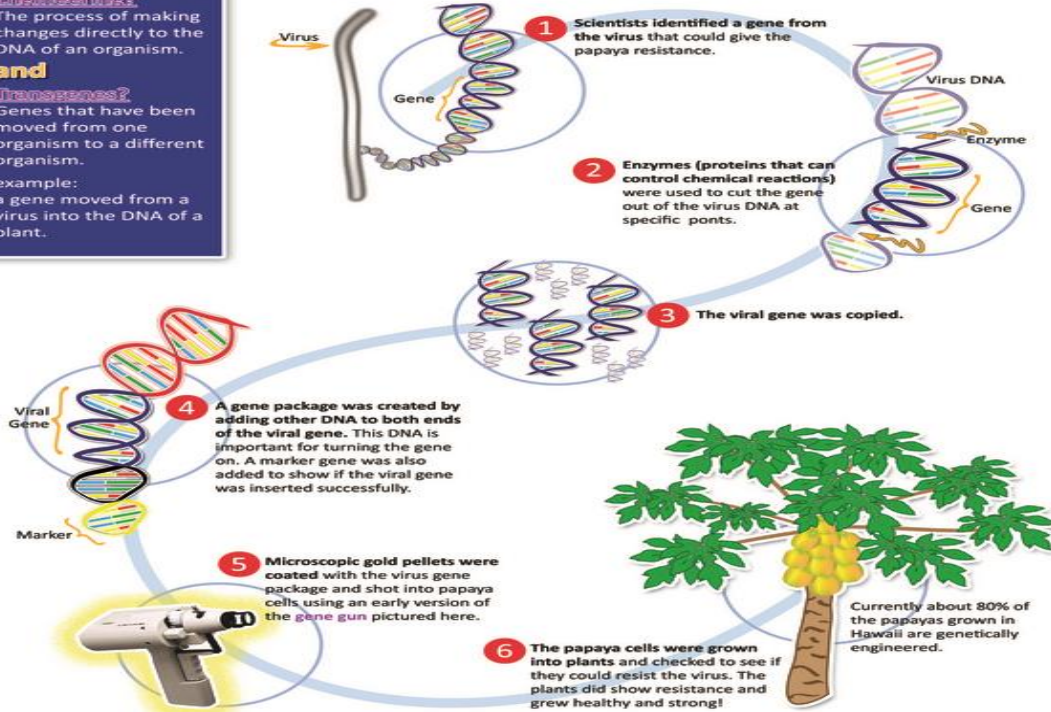
Transgenes?

Genes that have been moved from one organism to a different organism.

example:

a gene moved from a virus into the DNA of a plant.

Inserting a single gene from the ringspot virus into papaya DNA made the papaya resistant to the virus. Let's take a closer look at how papaya was genetically engineered...



The process of genetic engineering in papaya. This is the first fruit tree ever genetically engineered (for resistance to a virus that kills papaya plants), conducted by Land Grant Universities (University of Hawaii and Cornell University) in the USA

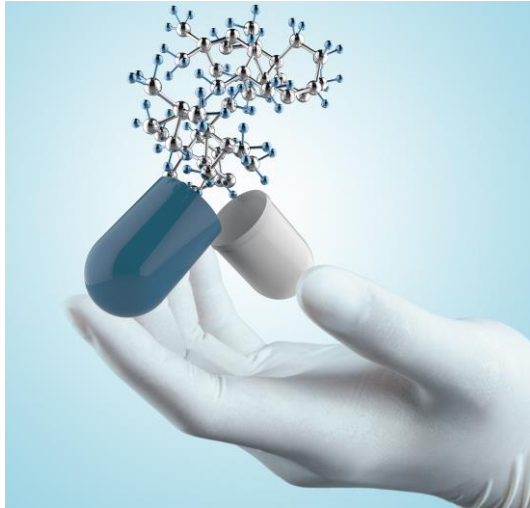
genetic
engineering

transgene

BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

BIOPHARMACEUTICAL



PHARMACEUTICAL BIOTECHNOLOGY

- **Bioteknologi farmasi** adalah bidang yang berkembang di mana prinsip-prinsip bioteknologi diterapkan pada pengembangan obat-obatan.
- Istilah "biofarmasi" diciptakan pada 1980-an dan mengacu pada **obat-obatan yang diproduksi dalam proses bioteknologi** menggunakan metode biologi molekuler.
- Mayoritas obat terapeutik di pasar saat ini adalah bioformulasi, seperti antibodi, produk asam nukleat, dan vaksin.

BIOPHARMACEUTICAL

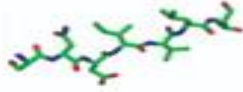
Peran bioteknologi dalam dunia farmasi

Small molecules



Salicylic acid
MW 138 Da

Peptides



Tiptorelin
MW 1311 Da

Proteins



Human growth hormone
MW 22,125 Da

mAbs



Rituximab
MW~150,000 Da

ADC



Brenduximab-vedotin
MW~150,000 Da

Analytical complexity

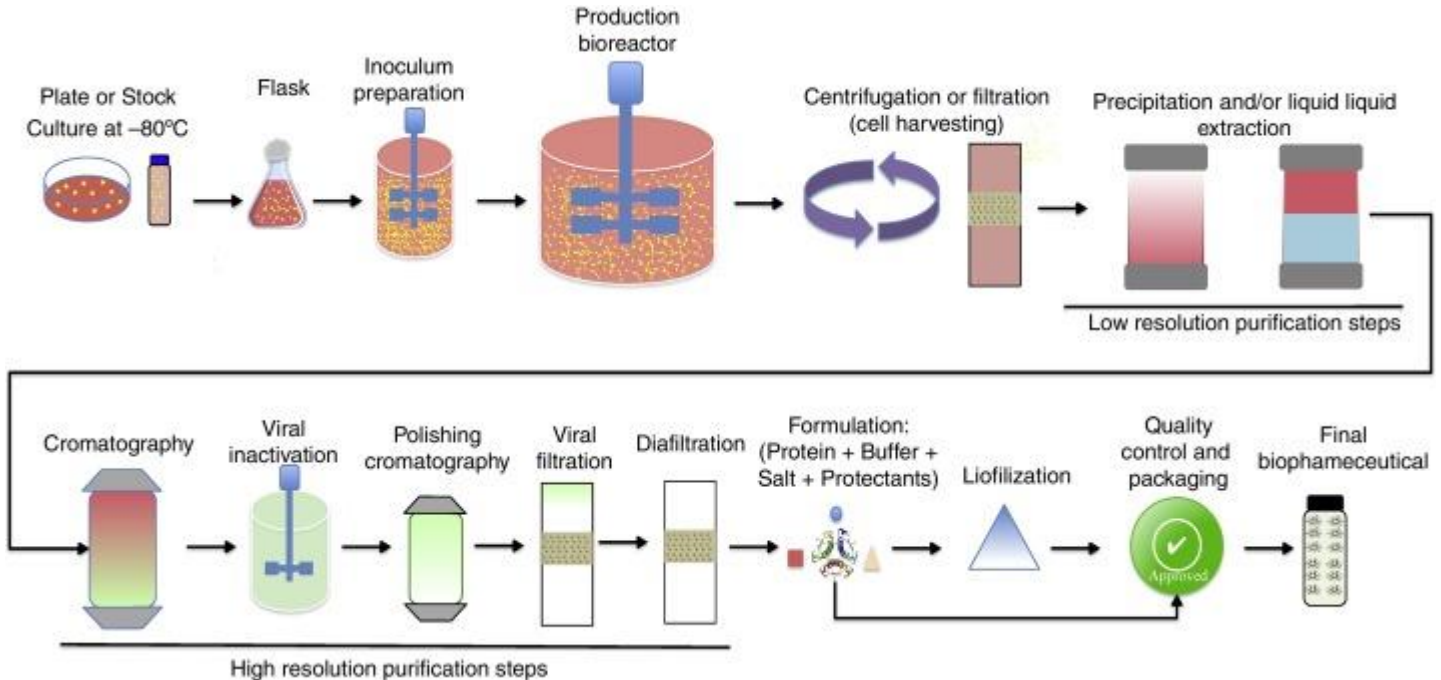


As illustrated, proteins, mAbs, and ADCs produced from living organisms have a complexity far exceeding that of small molecules and peptides produced from chemical synthesis.

BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

Biopharmaceuticals from microorganisms: from production to purification



The biopharmaceutical manufacturing technology flowchart exemplifying the upstream and the downstream bioprocess.

BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

BIOPHARMACEUTICALS

Blood clotting factors	Monoclonal antibodies	Thrombolytic agents
Colony stimulating factors	Growth factors	Neurotrophic factors
Enzymes	Polypeptide anticoagulants	Polypeptide hormones
Interferons	Interleukins	Vaccines

Biopharmaceutical class	Example
Blood factors	Factor VIII Factor IX
Thrombolytic agents	Tissue plasminogen activator
Hormones	Insulin Growth hormone Gonadotropins
Haematopoietic growth factors	Erythropoietin Colony stimulating factors
IFNs	IFN-alpha, -beta, -gamma
Interleukin-based products	Interleukin-2
Vaccines	Hepatitis B surface antigen

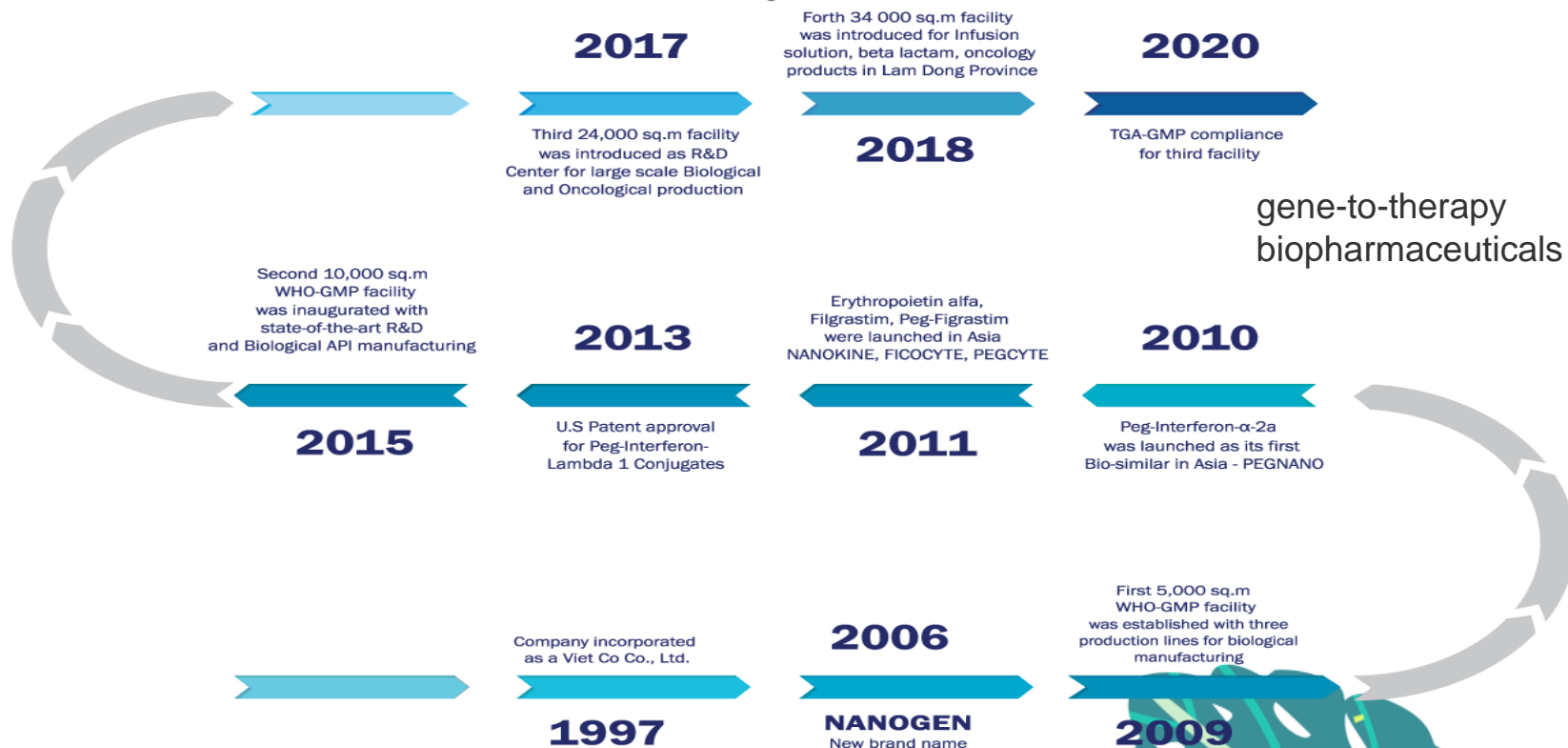
BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

<i>Biopharma product</i>	<i>Application</i>	<i>Companies</i>
Insulin	Diabetes	Eli Lilly, Novo Nordisk
Erythropoietin	Anemia	Amgen, Johnson & Johnson
Human growth hormone	Growth deficiency	Genentech, Pharmacia
Interferon α	Hepatitis	Schering, Roche
Interferon β	Multiple sclerosis	Chiron, Biogen
Factor VIII	Hemophilia	Bayer
Tissue plasminogen activator	Blood clot	Genentech
Glucocerebrosidase	Gaucher's disease	Genzyme
Therapeutic antibodies	Cancer	Glaxo, Amgen, Genentech
G-CSF	White blood cell	Amgen, Sankyo

BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi



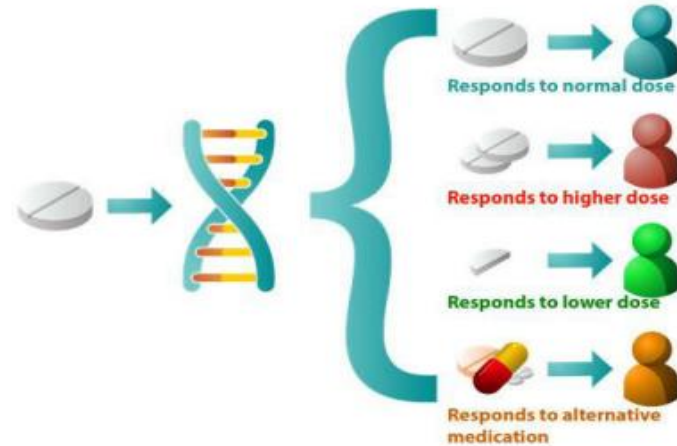
BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

"One size fits all"

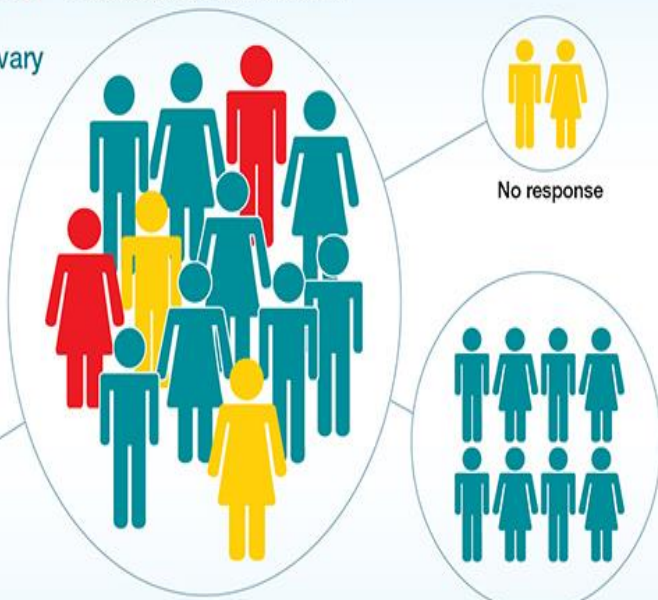


"Personalized Medicine"



Individual response to the same medication can vary

Patients taking same medication



No response

Desired response

Serious side effects

PHARMACOGENOMICS

BIOPHARMACEUTICAL

Peran bioteknologi dalam dunia farmasi

Drivers of change

Impact on biopharmaceutical manufacturing

Future proofing

- **Evolving expectations of the regulators** with regard to sterile manufacturing including emerging critical new technology areas like continuous bioprocessing
- **Flexible facilities** with greater flexibility, multi-product, hybrid, and completely modular facilities are being developed

Supply chain and quality

- Switch from **vials to pre-mixed bags** requires machinery and process changes
- Manufacturing parenteral 'Ready-To-Use' products require manufacturing to be **flexible to switch from one container to another** while maintaining sterility as well as set-up and manage a **manufacturing cold chain**

Data management

- Body sensors (e.g., wearable, implantable) require new manufacturing **capability to include chips** and new fill finish configurations to make these products
- Nano sensors, **regenerative medicines, and nano robotics** are concepts that possess great potential to transform pharmaceutical business in future

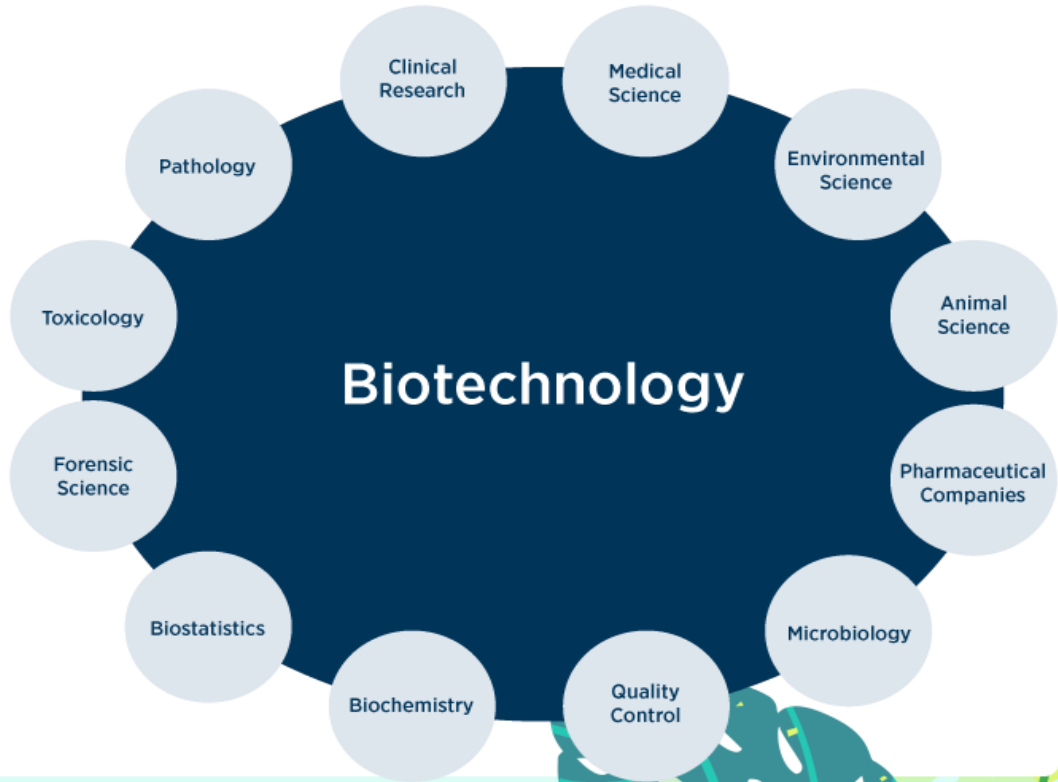
Regulatory and compliance

- **Serialization** and related changes in packaging and labeling are required and this would provide new information
- The tracking-tracing of internal movement between sites, including **automated handling processes**

Career Opportunities in Biotechnology

Peran bioteknologi dalam dunia farmasi

- Pharmaceutical Biotechnologist
- Molecular Biotechnologist
- Geneticist
- Environmental Biotechnologist
- Forensic Science Technicians





**Can pineapple
skins replace soap?**

<https://www.instagram.com/reel/CrFL0Rgopwm/?igshid=MzRIODBiNWFIZA==>

The background features a light mint green color with stylized tropical leaves in various shades of teal, lime green, and dark blue. The leaves are scattered across the page, with some in the top-left and bottom-right corners, and others in the center. The text "THANK YOU" is centered in a dark teal, sans-serif font. Below it, the word "TEMPLATE" is written in white, spaced-out letters inside a dark teal rectangular box.

THANK YOU

T E M P L A T E



BIOTEKNOLOGI

Pertemuan 2

apt. Trifonia Rosa Kurniasih, M.Biotech



**STIKES Notokusumo
Yogyakarta**

TOPIK BAHASAN : KULTUR JARINGAN

Pendahuluan

Manfaat

Faktor - faktor yang menunjang keberhasilan kultur jaringan.

Macam-macam teknik kultur jaringan





Pendahuluan

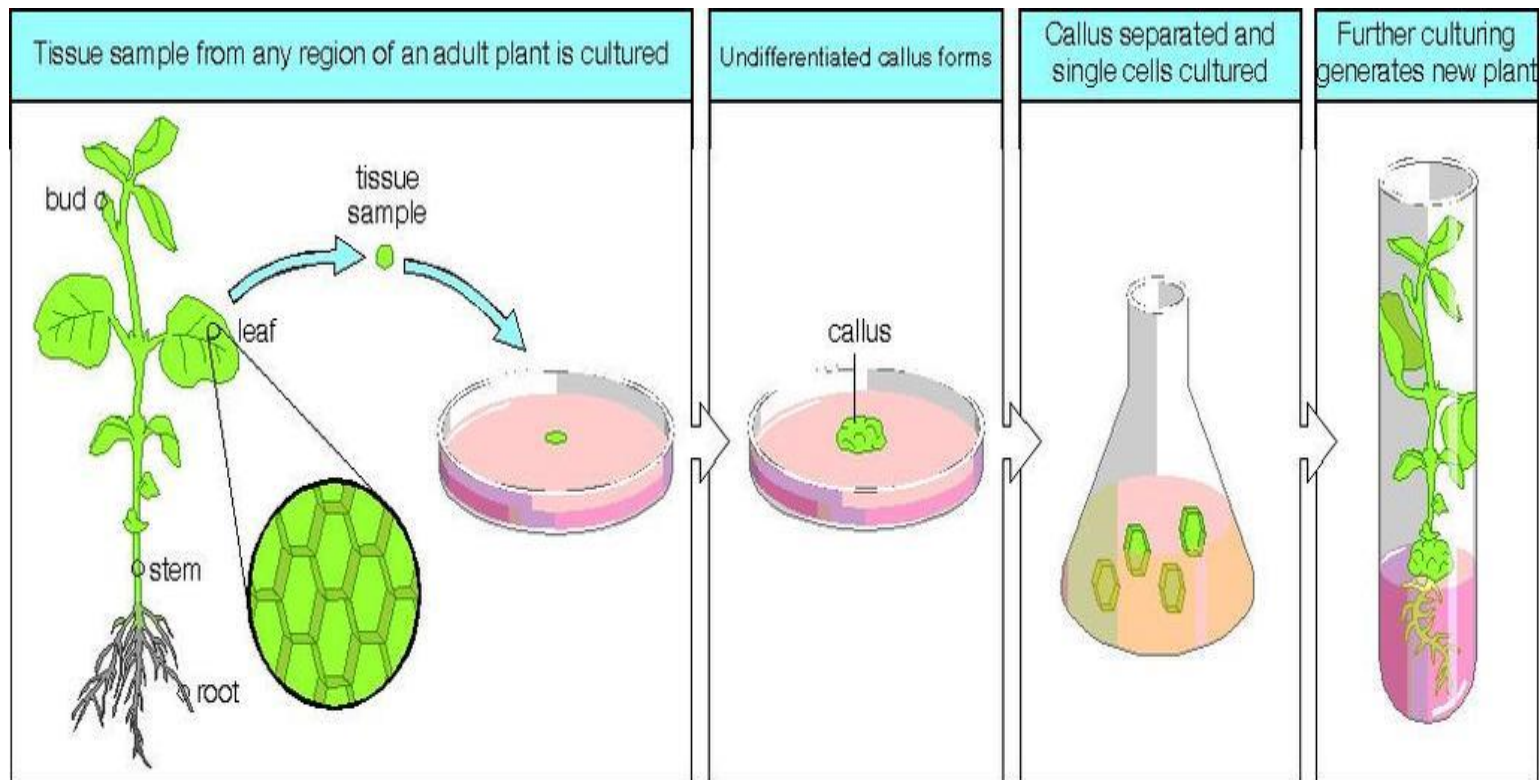
Beberapa istilah dalam kultur jaringan

- **Eksplan:** bagian tanaman untuk bahan awal inisiasi kultur
- **Kalus :** sekumpulan sel amorf yang terbentuk dari sel yang terus membelah
- **Planlet:** tanaman lengkap hasil regenerasi kultur jaringan
- **Subkultur:** pemindahan kultur ke media lain
- **Passage :** masa inkubasi
- **Inokulum:** bahan yang diambil pada setiap subkultur
- **Aseptik:** steril
- **Asenik:** satu macam organisme
- **Pucuk Adventif :** pucuk yang terbentuk pada tempat selain meristem (bukan jaringan asal), misal dari kalus, kotiledon, dll

- **Organogenesis :** pembentukan batang, daun, akar
- **Embrio somatik :** embrio yang bukan dari zigot
- **Embriogenesis :** pembentukan embrio
- **Androgenesis :** embrio terbentuk langsung dari kultur anther atau polen
- **Gynogenesis:** embrio terbentuk dari ovari yang belum dibuahi (fertilisasi)
- **Aklimatisasi:** masa adaptasi planlet
- **Variasi somaklonal :** variasi pucuk hasil subkultur
- **Hibridisasi genetik :** fusi protoplas sel yang berbeda
- **Heterokarion :** gabungan 2 atau lebih inti sel protoplasma
- **Cybrid:** gabungan sitoplasma 2 sel atau lebih



Pendahuluan



Kultur jaringan merupakan teknik perbanyakan sel, jaringan atau organ tanaman dengan pada medium buatan (*in vitro*) secara aseptik

Teknologi kultur *in vitro* dimulai dengan spekulasi ilmuwan dari Jerman bernama Haberlandt pada awal abad ke 20 tentang **teori totipotensi**.

Haberlandt menyatakan bahwa *setiap sel mampu tumbuh dan berkembang menjadi tanaman normal jika dikulturkan pada nutrisi dan lingkungan yang tepat*



Pendahuluan

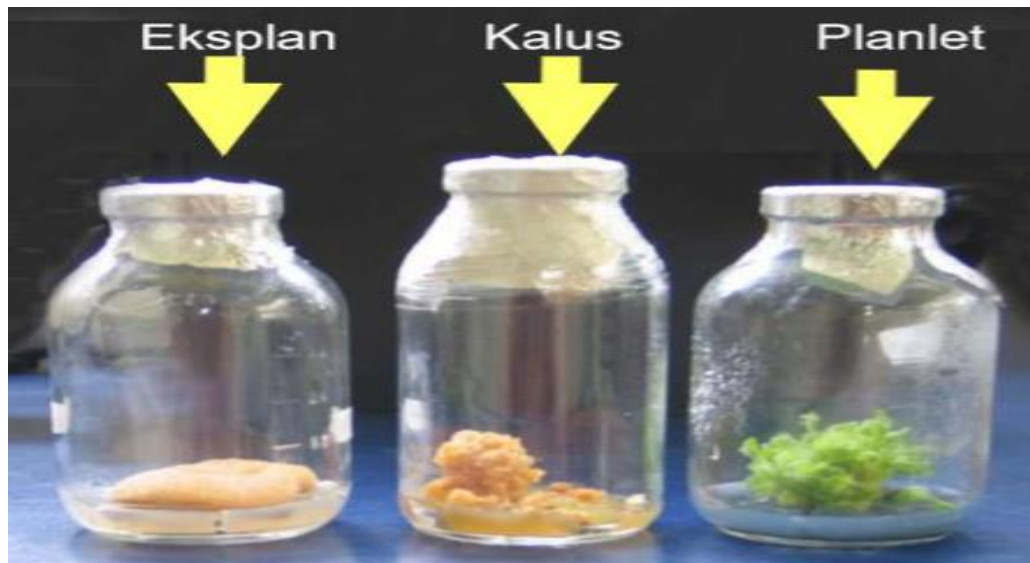
Landasan Kultur Jaringan

1. **Totipotensi** : adalah potensi atau kemampuan dari sebuah sel untuk tumbuh dan berkembang menjadi tanaman secara utuh jika distimulasi dengan benar dan sesuai.
2. **Rediferensiasi** : adalah kemampuan sel-sel masak (mature) kembali menjadi ke kondisi meristematik dan berkembang dari satu titik pertumbuhan baru yang diikuti oleh rediferensiasi yang mampu melakukan reorganisasi menjadi organ baru.
3. **Kompetensi** : menggambarkan potensi endogen dari sel atau jaringan untuk tumbuh dan berkembang dalam satu jalur tertentu.





Pendahuluan



Embriogenesis



- Embriogenesis: produksi embrio
- Embrio somatik: embrio dari somatik atau bukan sel kelamin
- Terjadi setelah pembentukan kalus

Organogenesis



- Produksi akar, batang atau daun
- Organ tumbuh dari perkembangan meristem atau dari diferensiasi jaringan
- Melalui pembentukan kalus tetapi kadang-kadang tanpa kalus



Pendahuluan

Different pathways of plant regeneration

Somatic embryogenesis.

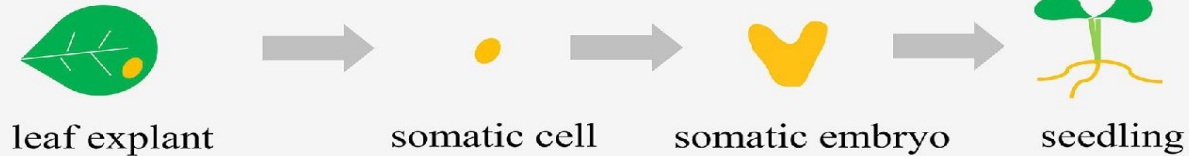
- In the **direct pathway**, the somatic cell originated from explants (e.g., a leaf) is induced to form the somatic embryo, which subsequently drives the development of the whole plant.
- In the **indirect pathway**, the explant (e.g., an immature embryo) is induced to initiate the embryonic callus, on which somatic embryos are formed to subsequently develop shoots and roots.

De novo organogenesis.

- In the **direct pathway**, shoots and roots are induced directly on the stem with pre-existing meristems.
- In the **indirect pathway**, pluripotent callus is produced around the wound in a leaf explant, with formation of shoots and roots subsequently induced.

A Somatic embryogenesis

direct

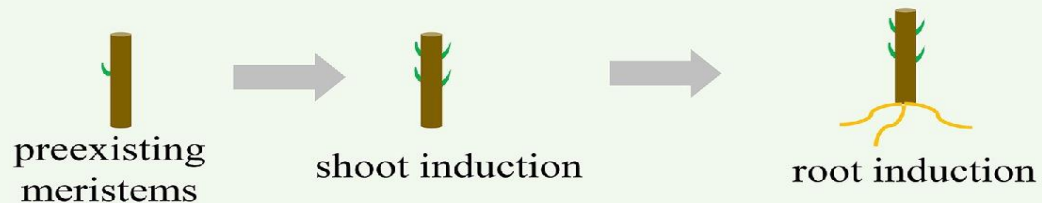


indirect

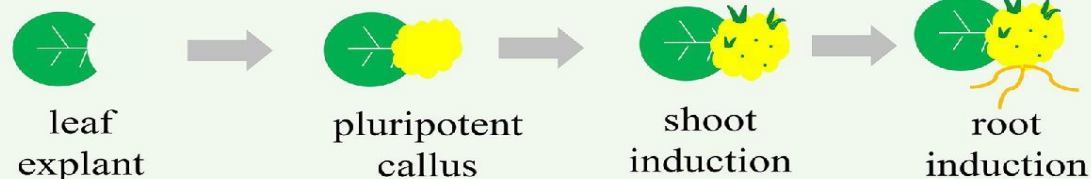


B *De novo* organogenesis

direct



indirect

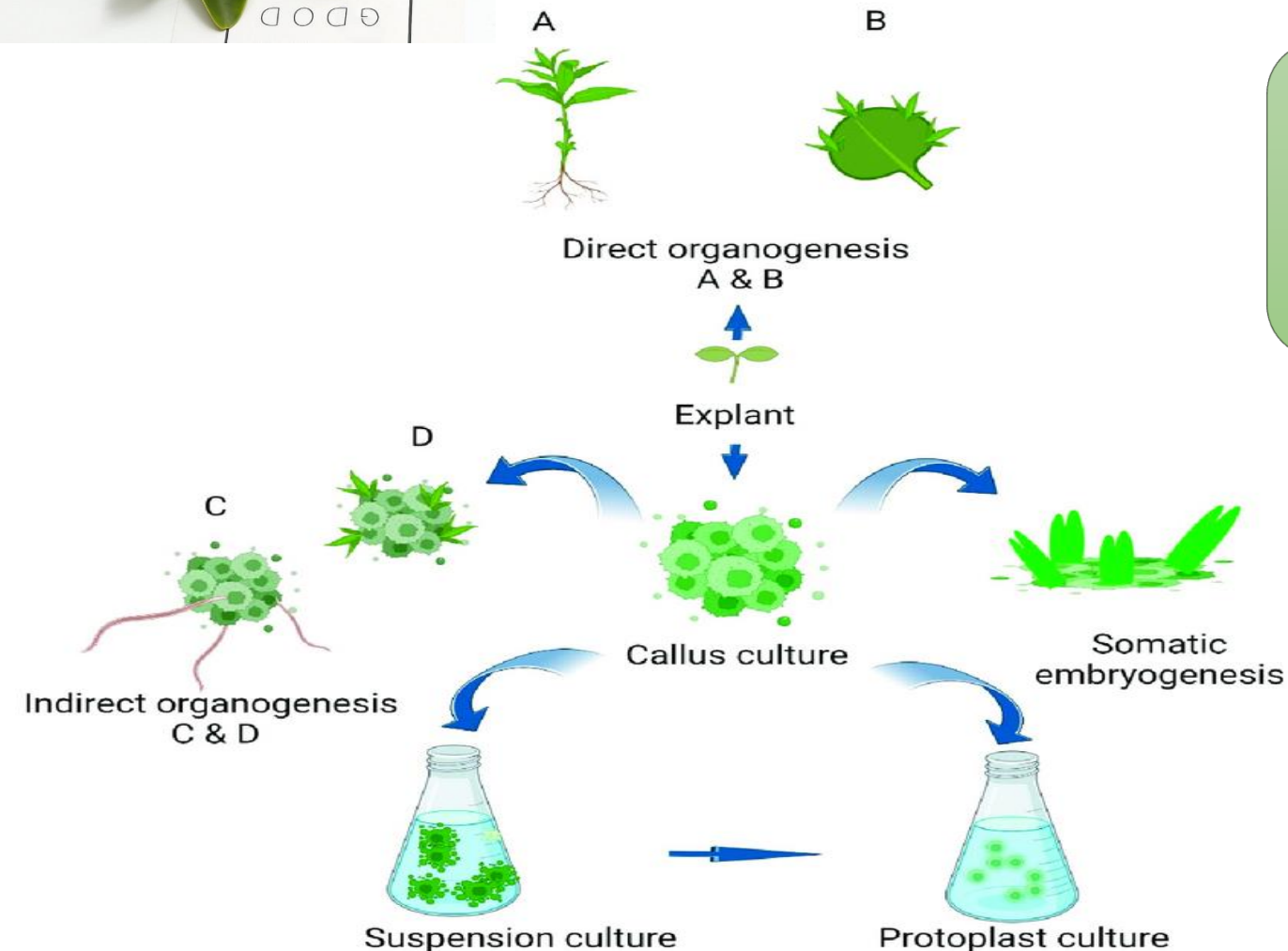




Pendahuluan

Different techniques in tissue culture for plant regeneration can be utilized for the selection and genetic transformation of plants.

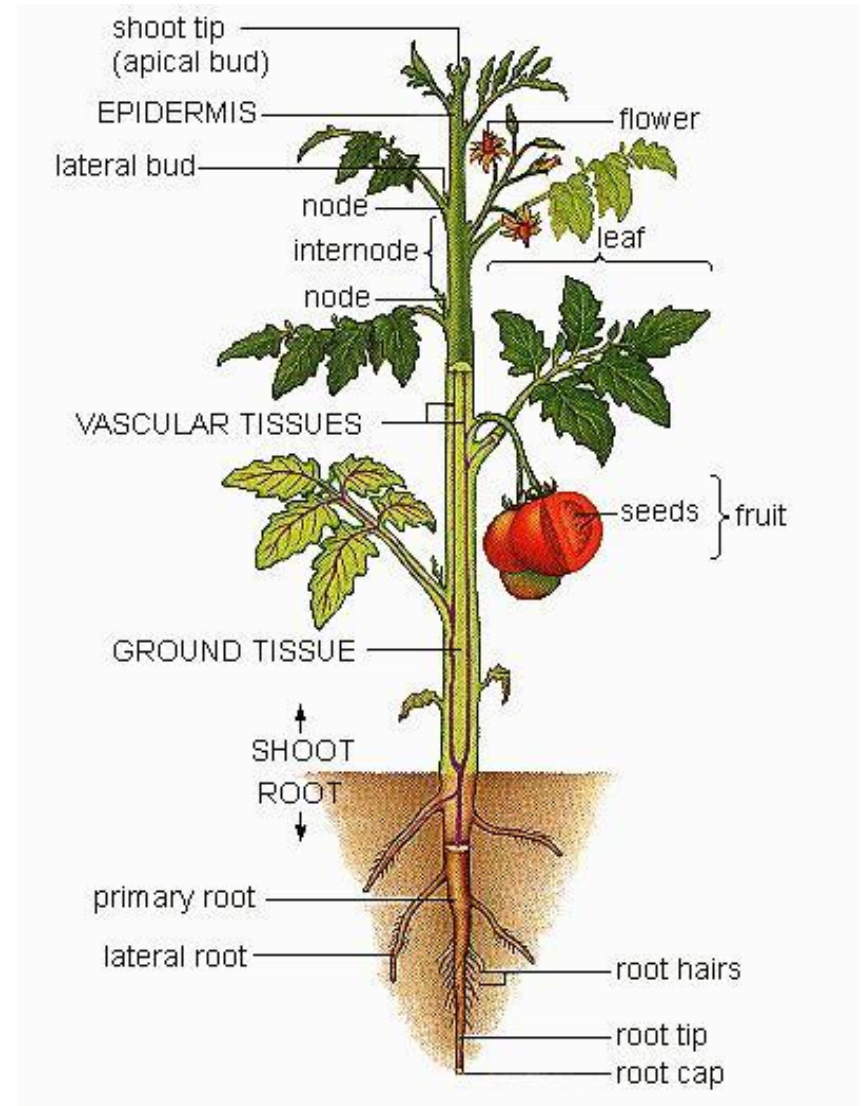
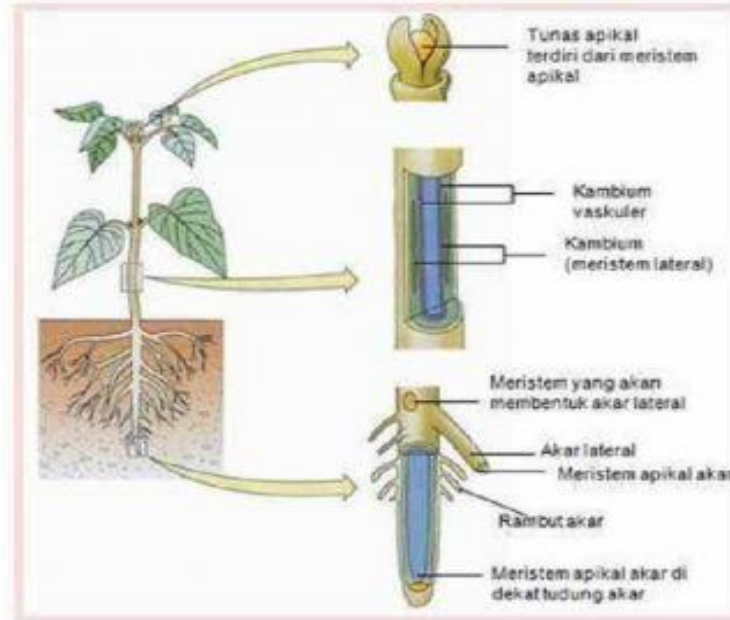
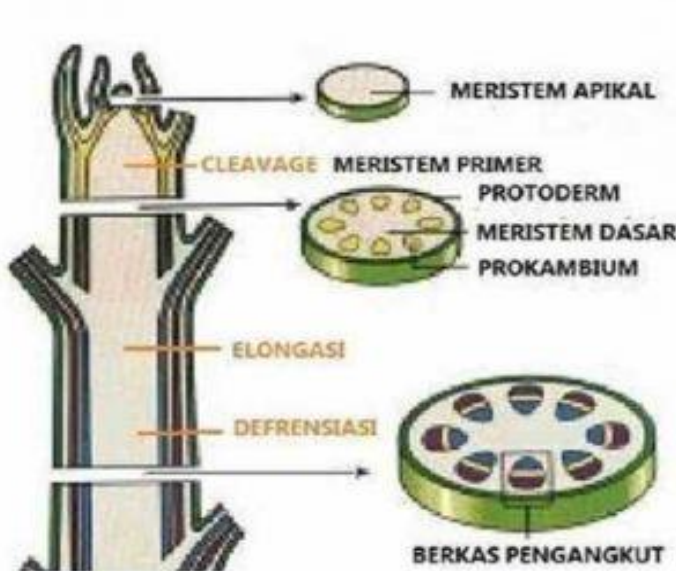
- Starting from explants under selection mediums, direct organogenesis can be achieved (A and B) or indirect organogenesis (C and D) through an intermediate callus phase.
- Further, callus can be used to form intact plantlets through an embryonic pathway or in suspension culture directly or via protoplast culture techniques in genetic transformation attempts





Pendahuluan

Jaringan Meristem



Jaringan meristem adalah jaringan tanaman yang memungkinkan tanaman untuk tumbuh



Manfaat Kultur Jaringan

Secara umum

- Perbanyak klon secara cepat
- Membentuk keragaman genetik somaklonal
- Mendapatkan tanaman bebas patogen
- Seleksi tanaman (kimia atau fisik)
- Pelestarian plasma nutfah (material genetik yang dapat diwariskan secara seksual atau somatik)
- Perbanyak tanaman yang sulit diperbanyak

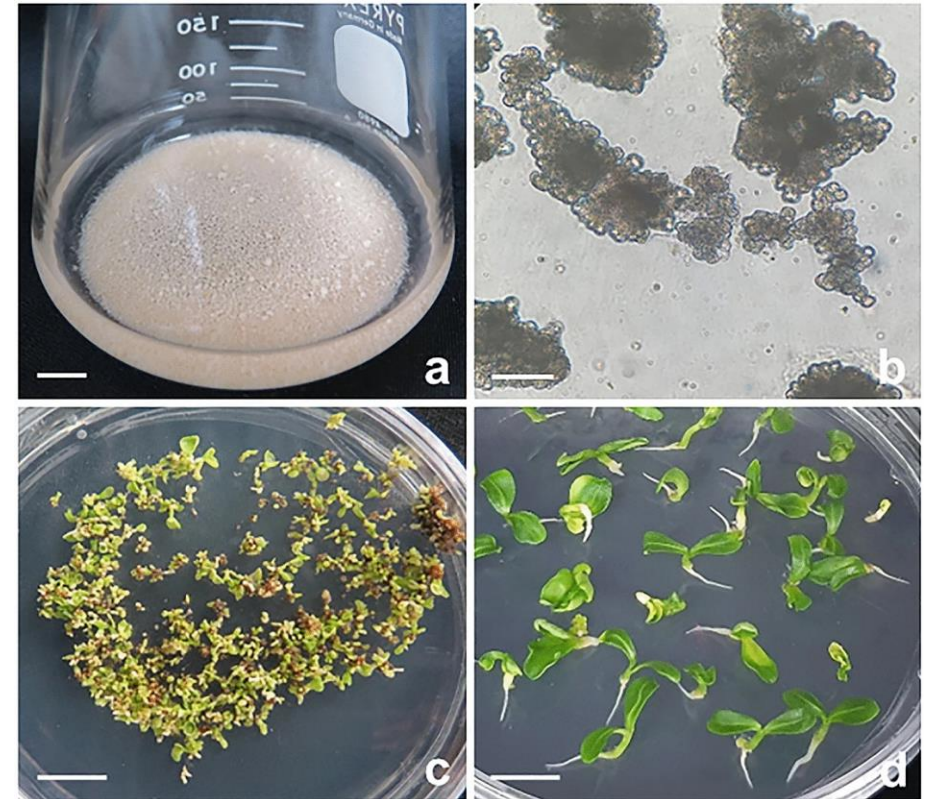




Manfaat Kultur Jaringan

Pemuliaan In Vitro

- Produksi tanaman haploid dan double haploid
- Hibridisasi somatik melalui fusi protoplas
- Seleksi keragaman alami
- Mutagenesis in vitro
- Pemuliaan Molekuler



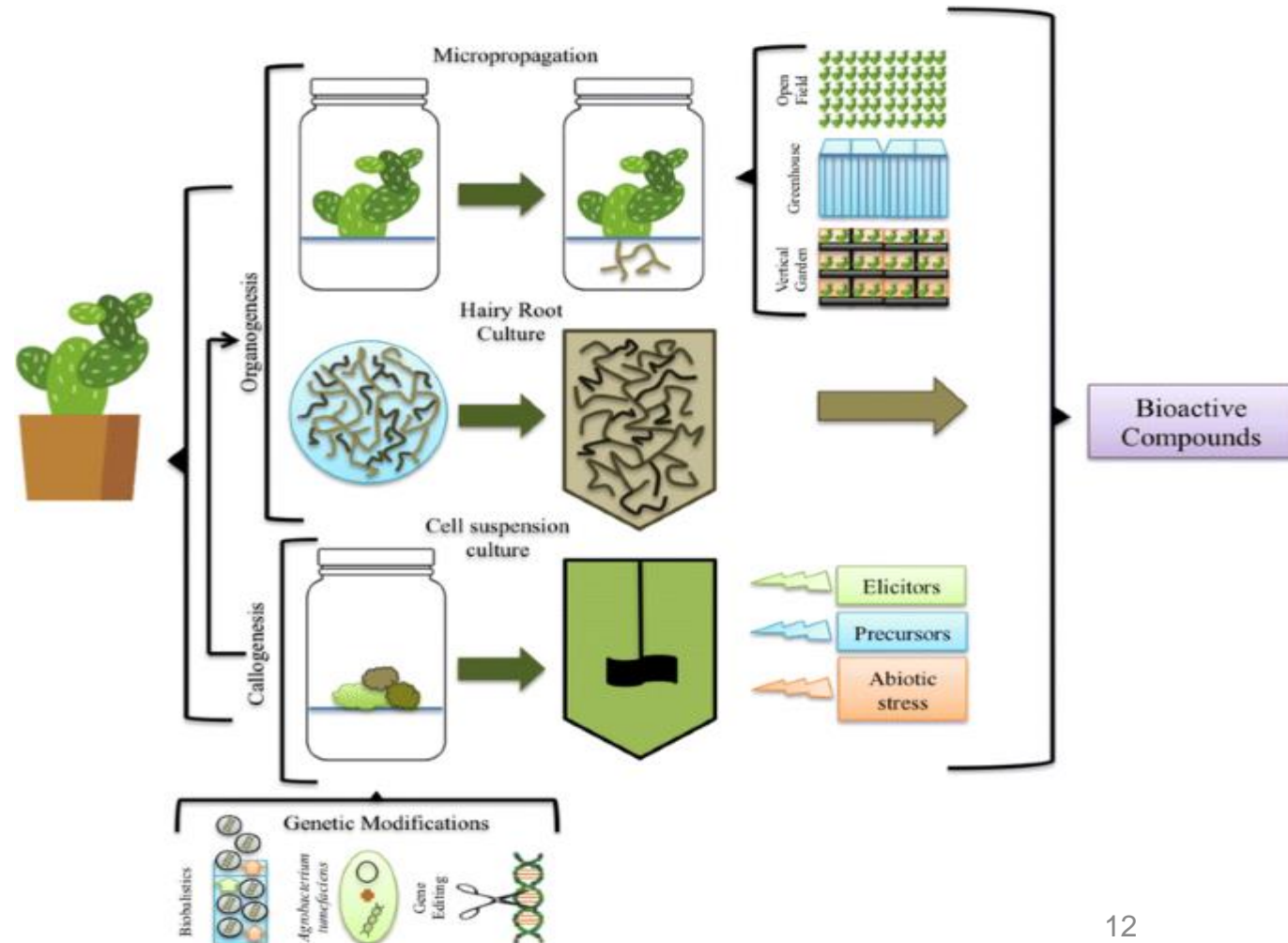
<https://doi.org/10.1038/s41598-021-94597-4>



Manfaat Kultur Jaringan

Untuk Tujuan Pengobatan :
metode alternatif untuk
memperoleh metabolit sekunder

- produksinya dapat diatur,
- kualitas dan hasil produksi lebih konsisten,
- biaya produksi lebih kecil,
- mengurangi penggunaan lahan

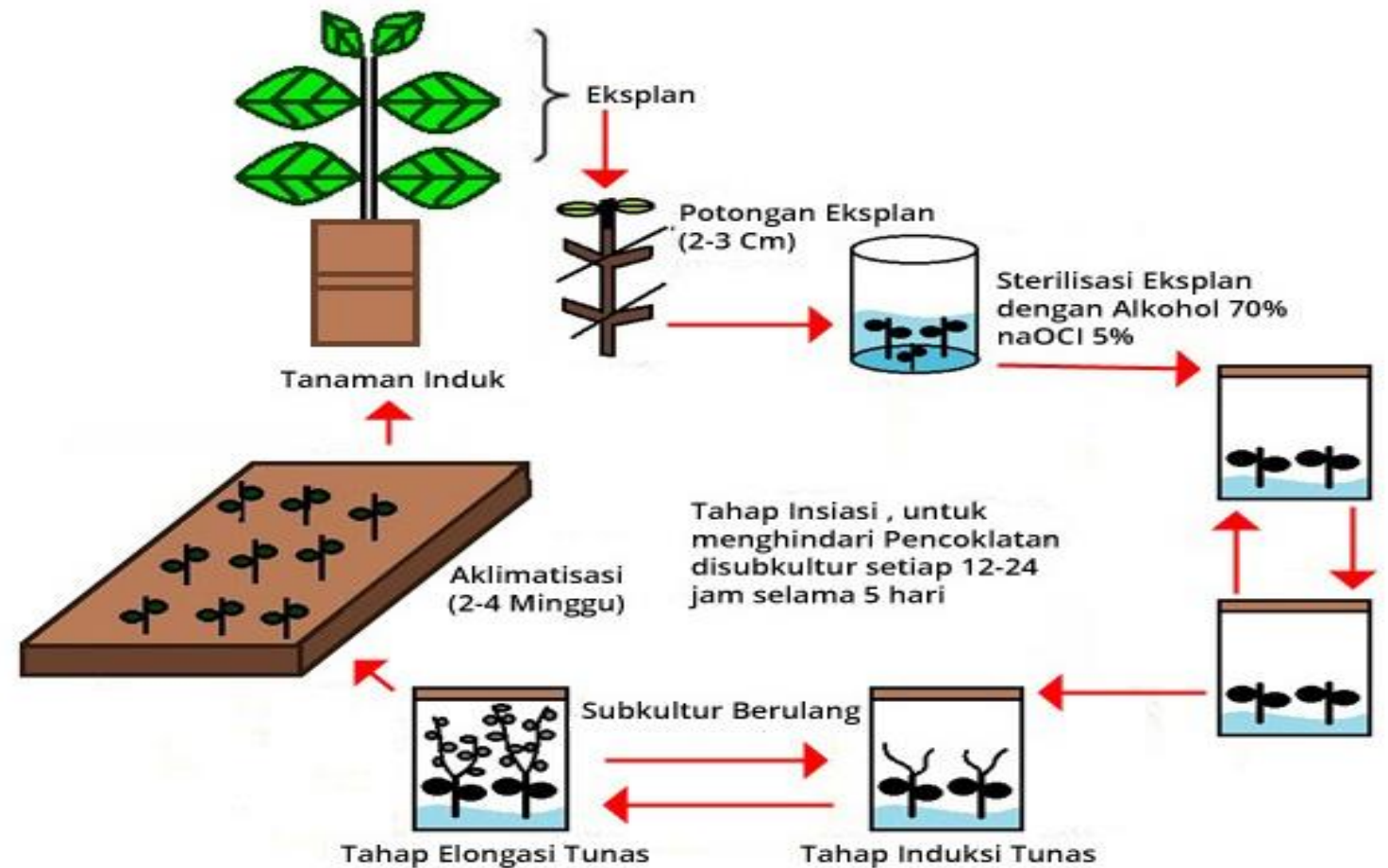




Manfaat Kultur Jaringan

Mikropropagasi :

Pengkulturan bagian tanaman yang sangat kecil (eksplan) secara aseptik didalam tabung kultur atau wadah serupa





Faktor penunjang

01

pemilihan eksplan
sebagai bahan
dasar untuk
pembentukan
kalus

02

penggunaan
medium yang
cocok

03

keadaan yang
aseptik

04

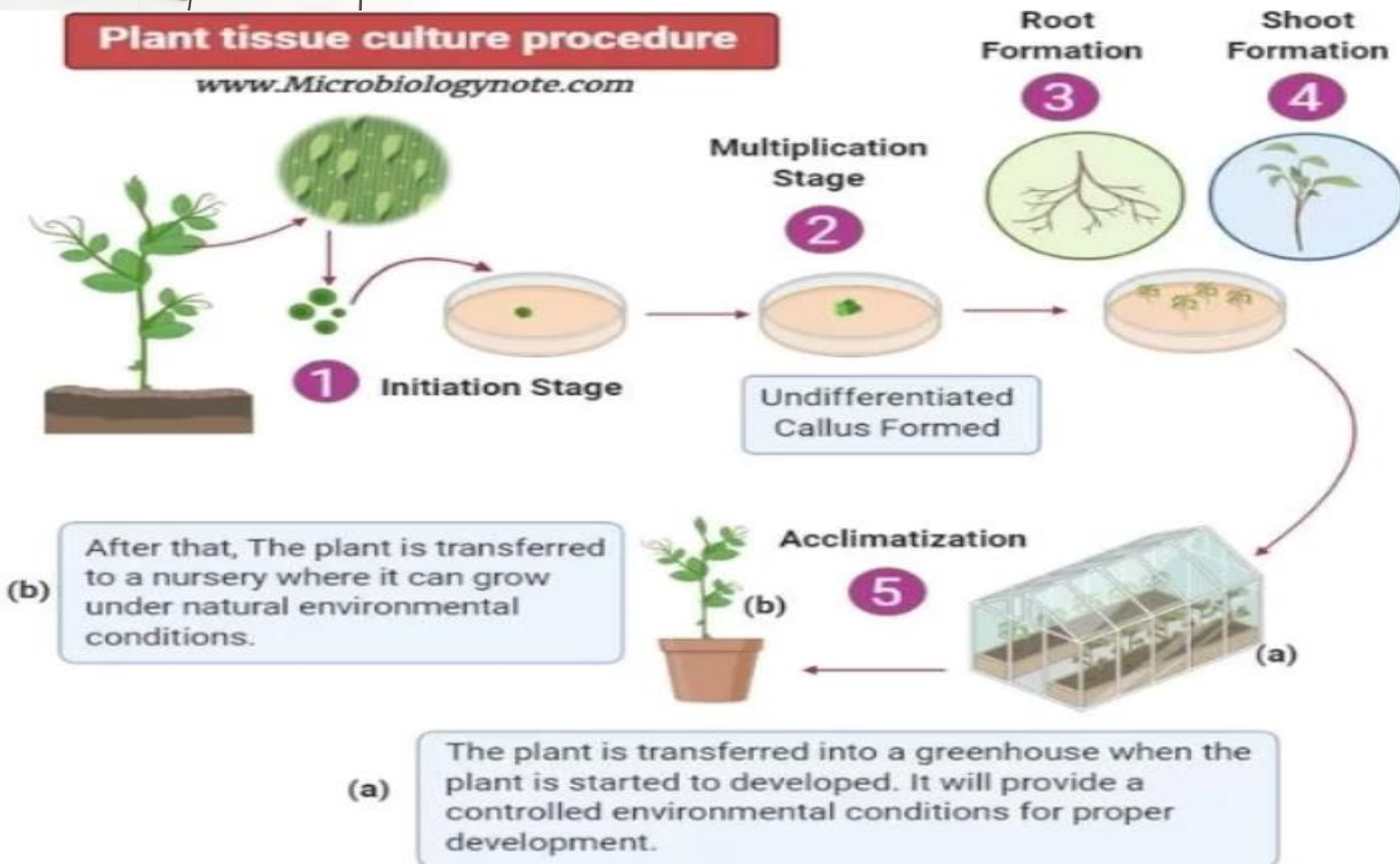
Lingkungan
(temperatur,
cahaya,
pengaturan udara
yang baik terutama
untuk kultur cair)



Faktor penunjang

Plant tissue culture procedure

www.Microbiologynote.com



Media tanam kultur jaringan merupakan media yang diperlukan agar sel atau jaringan tanaman yang diisolasi dapat tumbuh dan berkembang menjadi tanaman yang lengkap.

Media tersebut mempunyai komposisi nutrisi yang dapat mendukung pertumbuhan eksplan sesuai dengan yang diinginkan.

Media tanam pada kultur jaringan berisi kombinasi dari asam amino esensial, garam-garam anorganik, vitamin-vitamin, larutan buffer, dan sumber energi (glukosa)



Teknik kultur jaringan

KULTUR JARINGAN BERDASARKAN ASAL EKSPLAN



Kultur Sel

- Dilakukan isolasi sel tunggal yang diambil secara mekanik atau enzimatis atau dari kultur jaringan dipisahkan selnya. Ditujukan untuk seleksi mutan maupun industri (produksi metabolit sekunder atau induksi poliploid)

Kultur Meristem

- (daun, batang, akar, meristem) untuk membentuk tanaman utuh

Kultur Protoplas

- Yaitu sel telanjang yang tidak mempunyai dinding sel atau dinding selnya telah rusak

Proliferasi pucuk aksilar

- Pertumbuhan tunas terminal tertekan, sedangkan pertumbuhan tunas samping mengalami peningkatan

Induksi Tunas Adventif

- Inisiasi tunas adventif baik secara langsung pada permukaan eksplan yang dikulturkan atau secara tidak langsung pada permukaan kalus eksplan yang terbentuk



Teknik kultur jaringan

Kultur Organ

- Kultur semua bagian tanaman, untuk memperbanyak secara cepat, bebas penyakit

Kultur Kalus

- Kultur jaringan tanaman menggunakan eksplan jaringan akar

Kultur Suspensi Sel

- Kultur jaringan dengan menggunakan teknik bioreaktor

Kultur Protoplasma

- Shoot tip, tujuannya untuk memperbanyak vegetatif tanaman

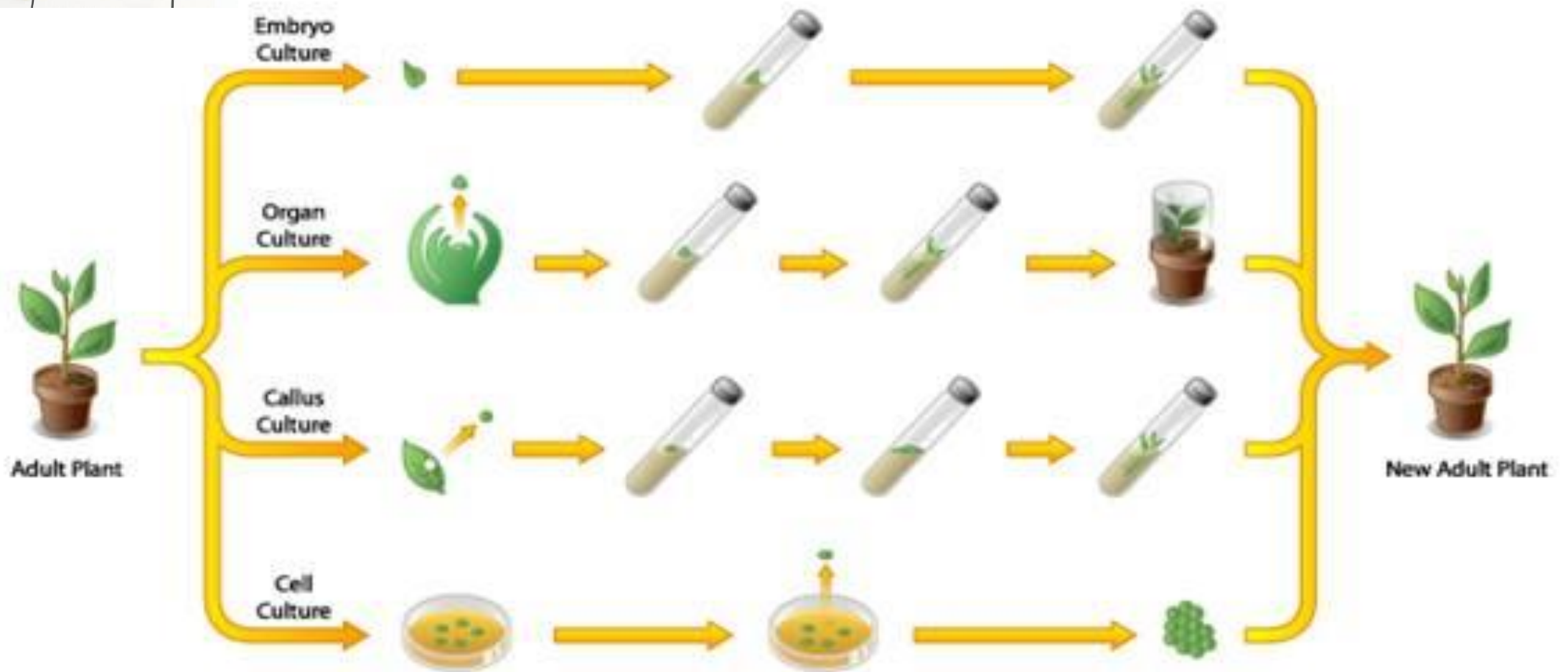
Kultur Haploid

- Bertujuan memperoleh kalus dari eksplan yang ditanam



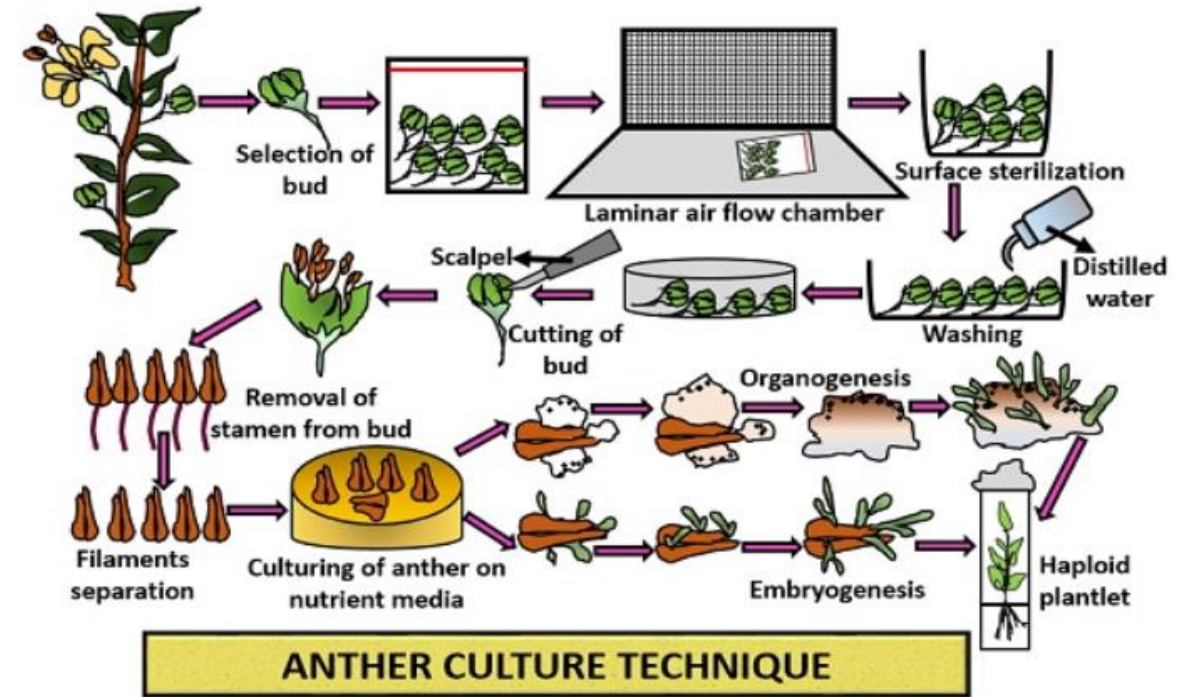
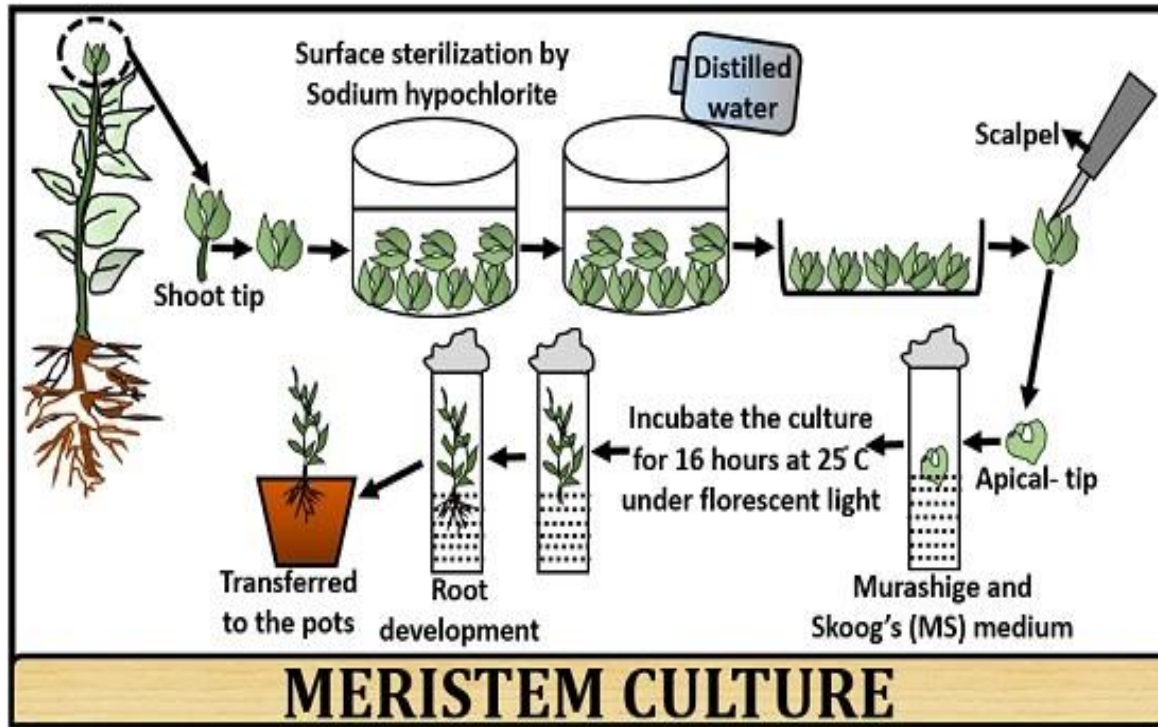


Teknik kultur jaringan





Teknik kultur jaringan



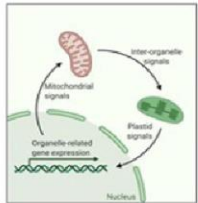


Teknik kultur jaringan



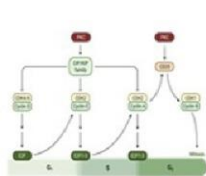
Protein function

Subcellular localization, protein-protein interaction, western blotting, protein secretion and transport, and protein activity analysis.



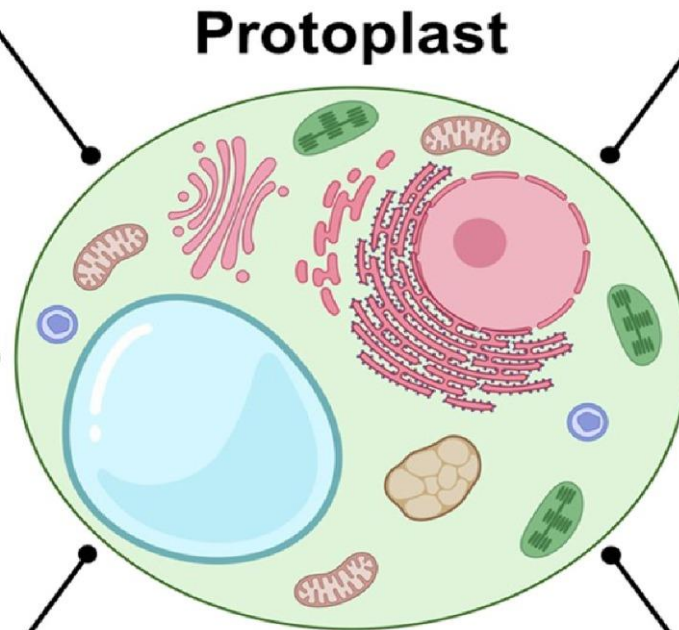
Signal transduction

Elucidating signal transduction pathways involved in plant growth, development, immunity and physiology.



Transcriptional regulation

Investigating gene promoter activity and gene expression level, and constructing transcriptional regulatory network.

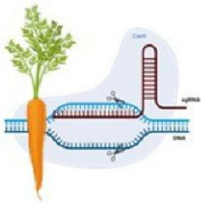


Protoplast

Protoplast culture refers to the process in which whole plants are developed from the culture of cells without cell wall

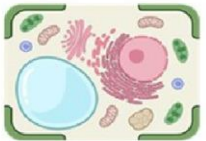
CRISPR/Cas9 editing

Estimating gene-editing efficiency, combining CRISPR/Cas9 with protoplast regeneration to develop plants with desired traits.



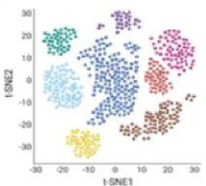
Cellular process

Cell wall synthesis, cell division, embryogenesis, cell differentiation and dedifferentiation, somatic hybridization, membrane transport.



Multi-omics analysis

Protoplast is a single-cell system to investigate genomics, transcriptomics, proteomics, metabolomics and epigenetics.





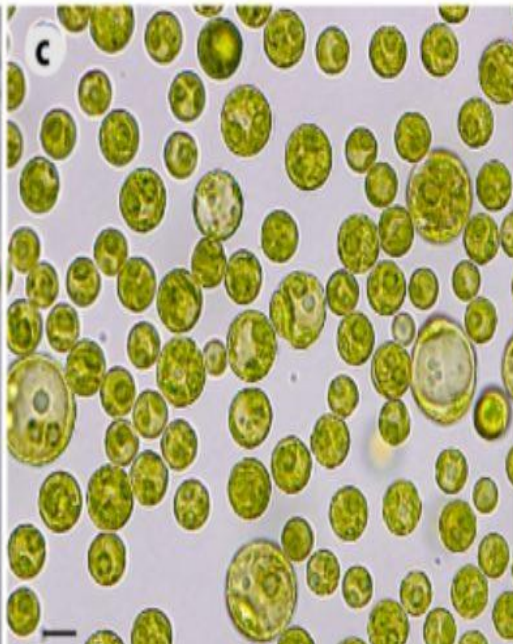
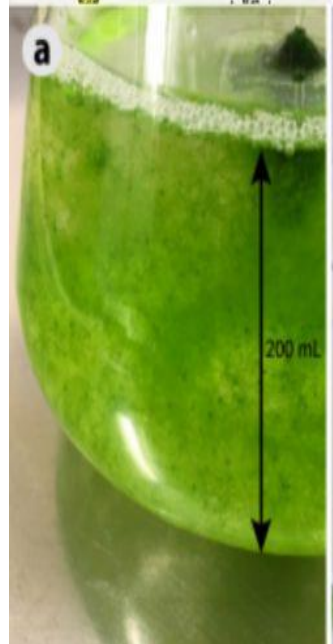
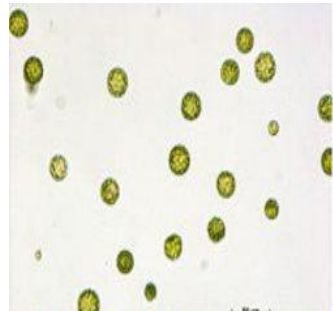
Teknik kultur jaringan

Protoplasts

In protoplast culture, protoplasts isolated from any plant part, including root, shoot, leaves, or embryo, is cultured in an artificial media under artificial conditions favoring cell division and plant regeneration

The mechanical method involves placing cells in a hypertonic solution, which induces the separation of the plasma wall, leading to the exudation of cellular material and the subsequent division of plant tissue, resulting in the release of protoplasts

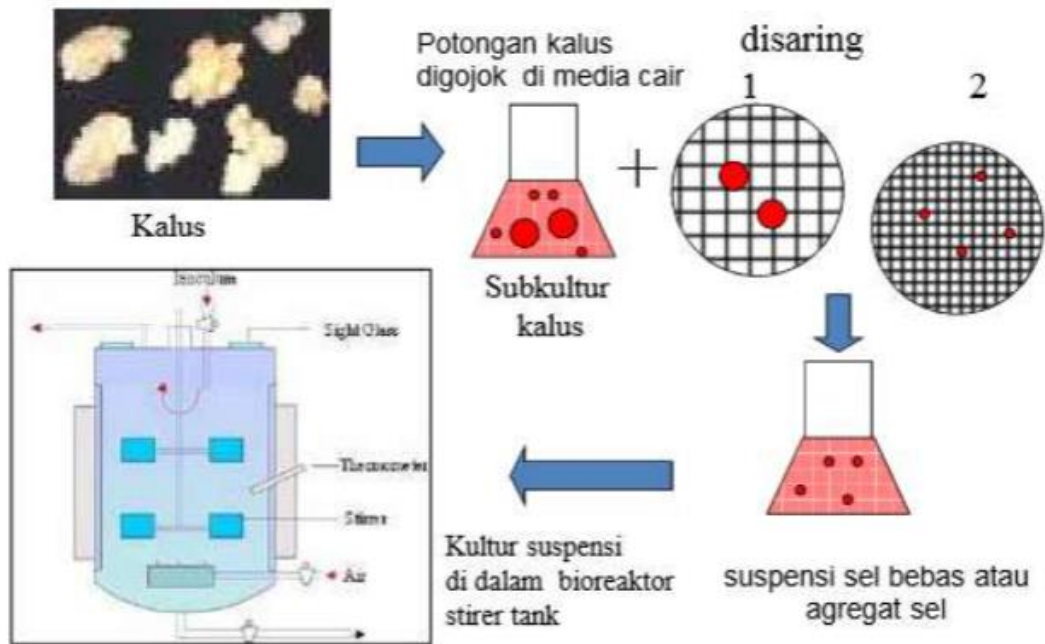
The enzymatic isolation of protoplasts represents a widely employed technique in protoplast extraction. It operates on the principle of employing enzymes, including cellulase, hemicellulose, dissociative enzymes, and pectinase, to facilitate the degradation of the plant cell wall, leading to the acquisition of protoplasts



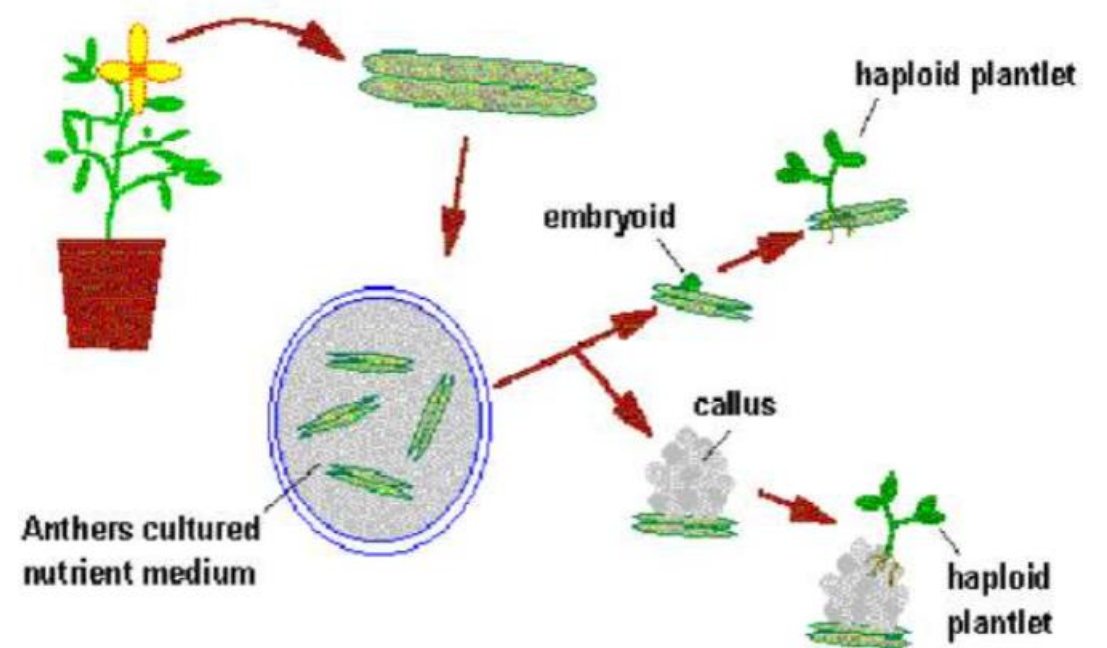


Teknik kultur jaringan

Kultur suspensi sel



Kultur Haploid (Anther/Mikrospora)





Kelebihan & Kekurangan



Kelebihan

- Jumlah bibit yang diproduksi bisa banyak
- Sehat, bebas dari virus dan penyakit lain
- Relatif cepat (rate 4-5 perbulan)
- Seragam
- Memudahkan dalam transportasi

Kekurangan

- Biaya lebih mahal
- Butuh skill/keahlian khusus
- Mutasi /off type akibat sub kultur berlebih variasi somaklonal



APLIKASI KULTUR JARINGAN





APLIKASI KULTUR JARINGAN



Planlet angrek Dendrobium NS 022/27 yang telah dikeluarkan dari berbagai komposisi media proliferasi (padat).



NS 02/21



NS 02/23



NS 02/27



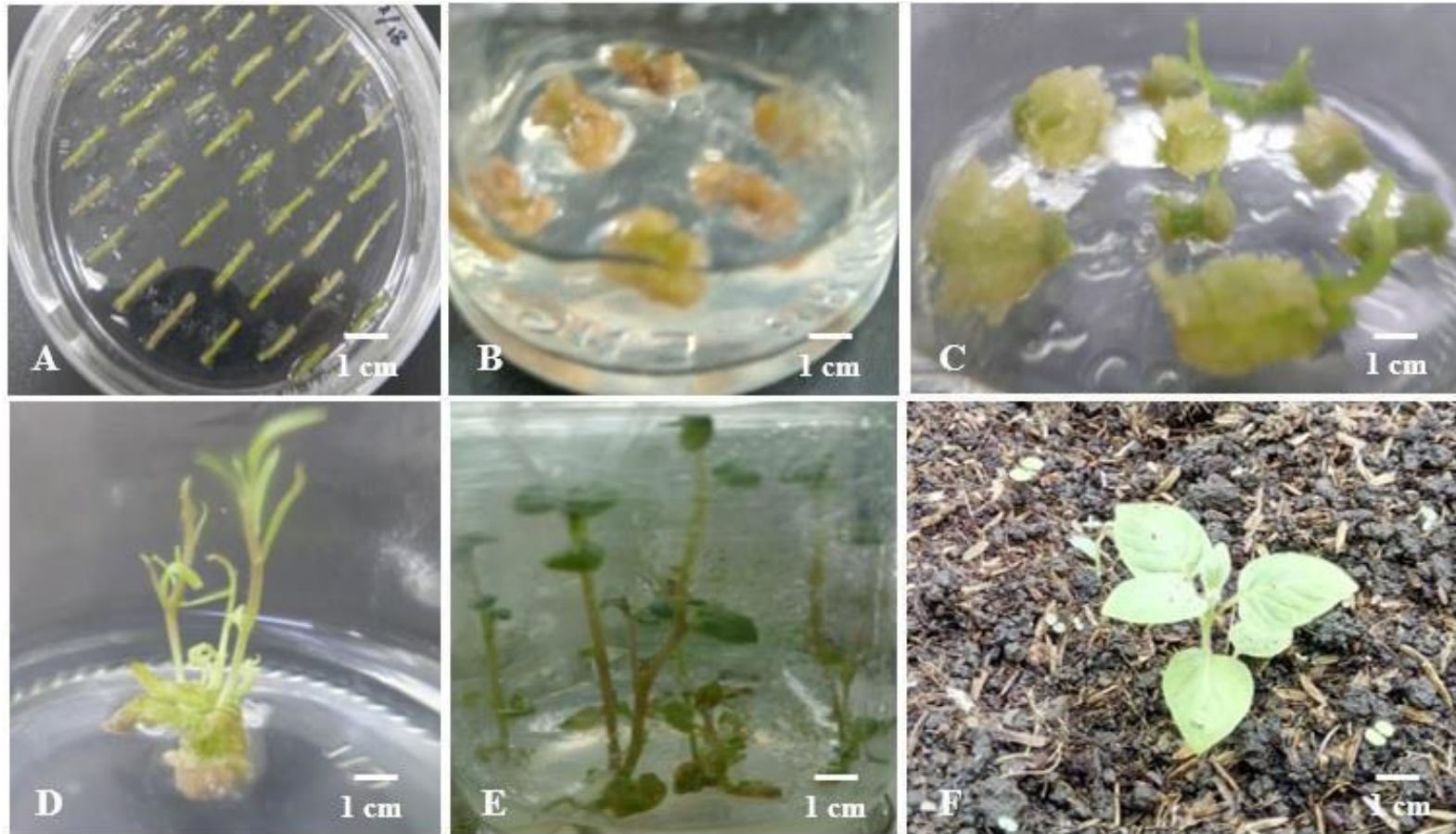
NS 022/62

Klon 5 nomor angrek Dendrobium hasil silangan Balithi dengan kode NS 022/21, NS 022/23, NS 022/27, dan NS 022/62



APLIKASI KULTUR JARINGAN

- tanaman kentang

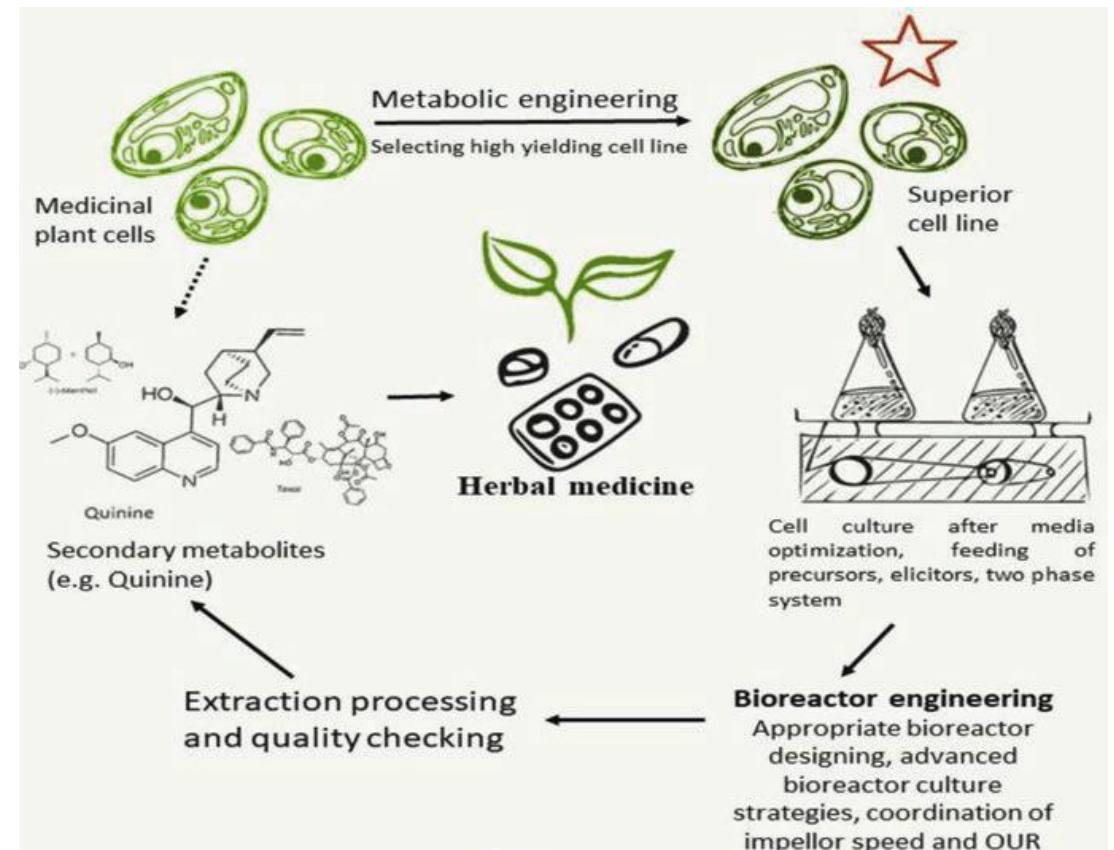




APLIKASI KULTUR JARINGAN

Tabel 1. Produksi metabolit sekunder melalui kultur jaringan

Nama Metabolit Sekunder	Nama tumbuhan penghasil
Vasine	<i>Adhatoda vasica</i>
Artemisinin	<i>Artemisia annua</i>
Azadirachtin	<i>Azadirachta indica</i>
Cathin	<i>Brucea javanica</i>
Capsiacin	<i>Capsicum annum</i>
Sennosides	<i>Cassia senna</i>
Ajmalicine, Secologanin, Indole alkaloids,	<i>Catharanthus roseus</i>
Vincristine	
Stilbenes	<i>Cayratia trifoliata</i>
Berberin	<i>Coscium fenestratum</i>
Sterols	<i>Hyssopus officinalis</i>
Shikonin	<i>Lithospermum erythrorhizon</i>
Ginseng saponin	<i>Panax notoginseng</i>
Podophyllotoxin	<i>Podophyllum hexandrum</i>
Taxane Paclitaxel	<i>Taxus chinensis</i>





APLIKASI KULTUR JARINGAN

Plant regeneration from protoplasts of *K. blossfeldiana*

'Charming Red Meadow'

a different types of isolated protoplast

- **a-I**: small protoplast without chloroplasts;
- **a-II**: dense protoplast with chloroplasts concentrated in the middle of the cell;
- **a-III**: dense protoplast with chloroplasts distributed homogenously throughout the cytoplasm;
- **a-IV**: vacuolated protoplast with chloroplasts concentrated in one zone of the cell;

b protoplast division at 3 days after culture initiation;

c microcolonies 2 weeks after culture initiation;

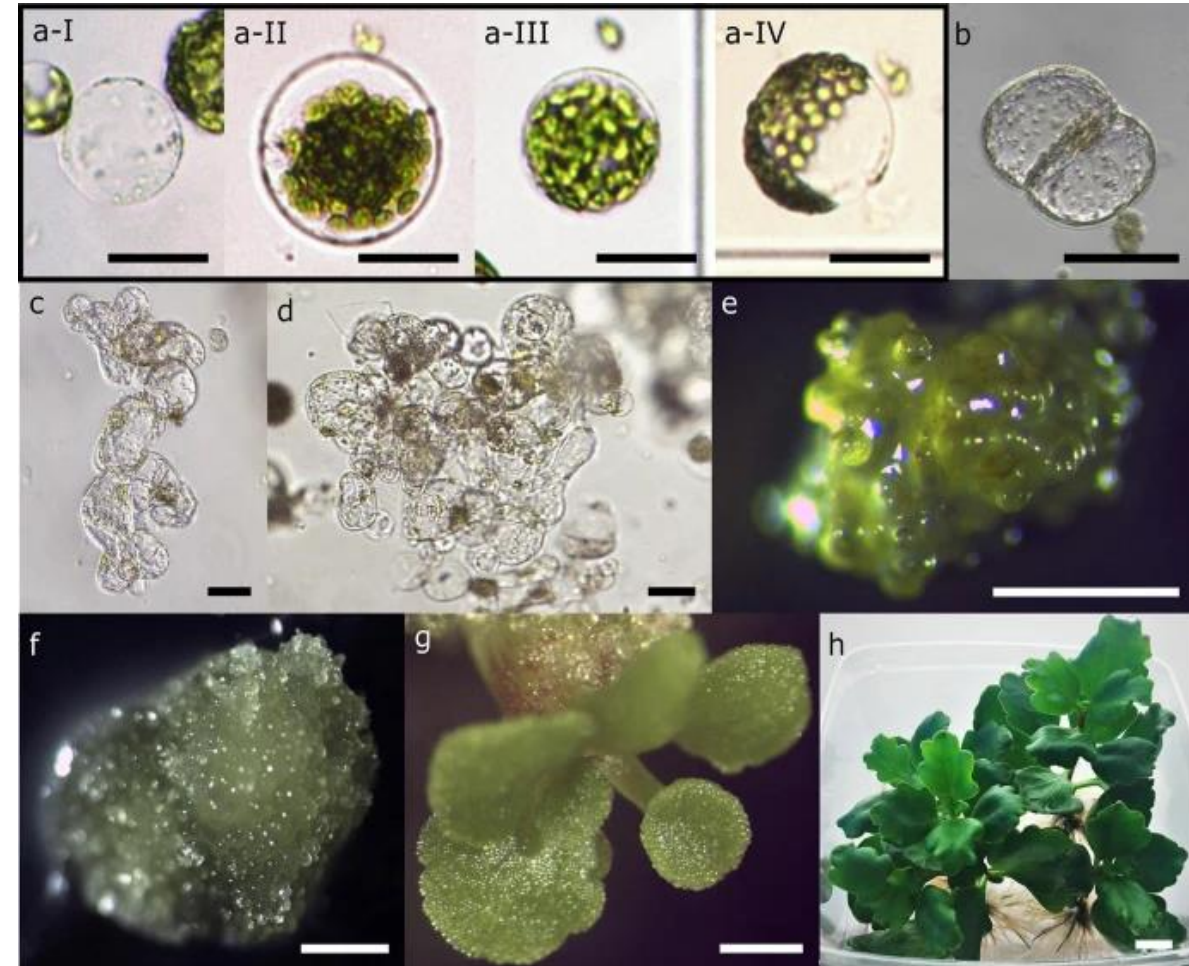
d microcolonies 4 weeks after culture initiation;

e green microcallus 8 weeks after culture initiation;

f yellow microcallus 8 weeks after culture initiation;

g shoot regenerated on the callus at 17 weeks;

h regenerated plants at 22 weeks



scale bars: **a, b** 25 μ m; **c, d** 50 μ m; **e-h** 1 mm 28



FEEL
GOOD

**THANK YOU FOR
YOUR
ATTENTION**

Link Video

- <https://youtu.be/xuwV3ywCxW8?t=2>
- <https://youtu.be/uPuxS1kxdVY?t=33>

**STIKES NOTOKUSUMO
YOGYAKARTA**



BIOTEKNOLOGI

Pertemuan 3 (September 2024)

apt. Trifonia Rosa Kurniasih, M.Biotech

Topik Bahasan

Pendekatan bioteknologi fermentasi dalam bidang Farmasi



Pendahuluan

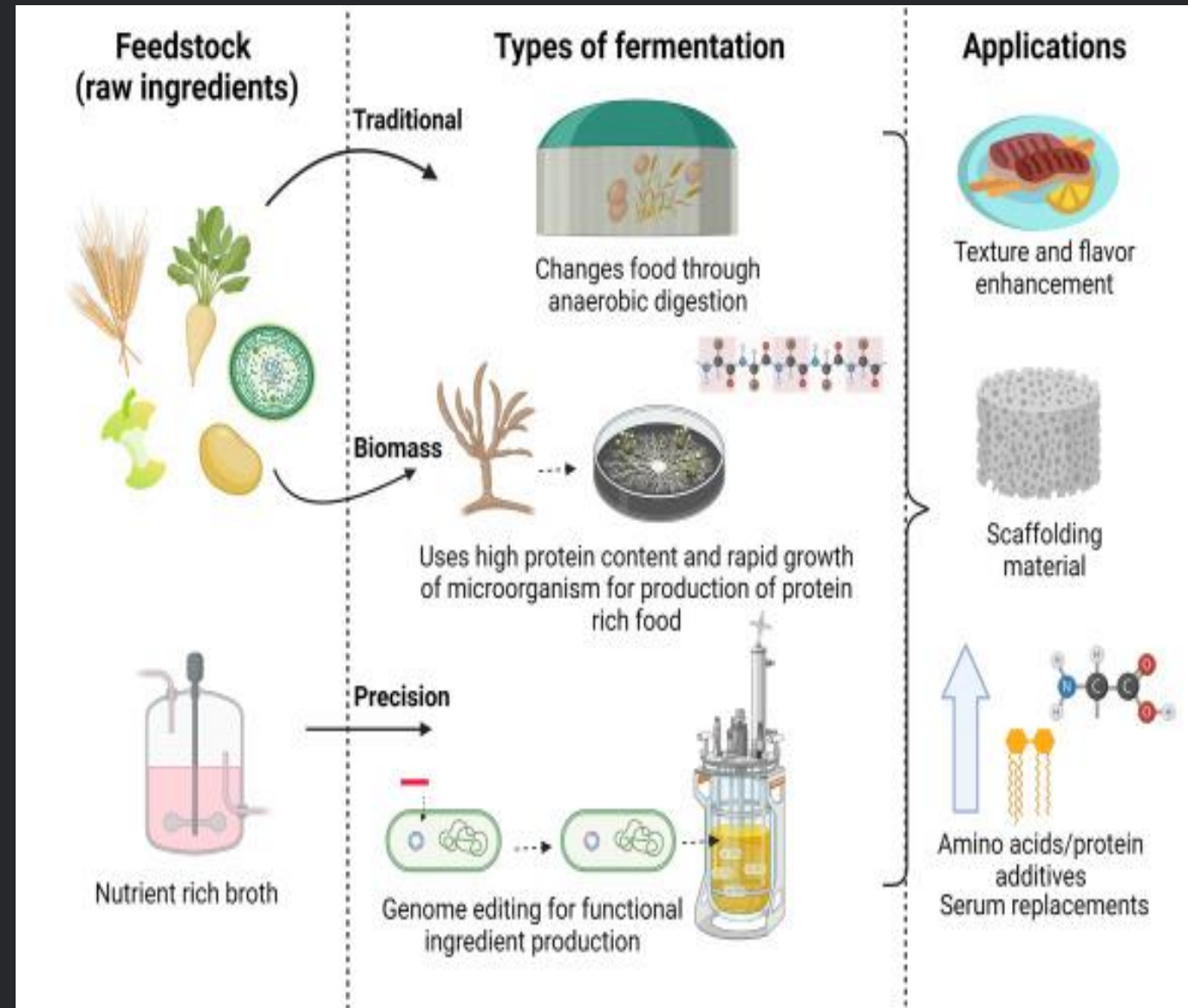
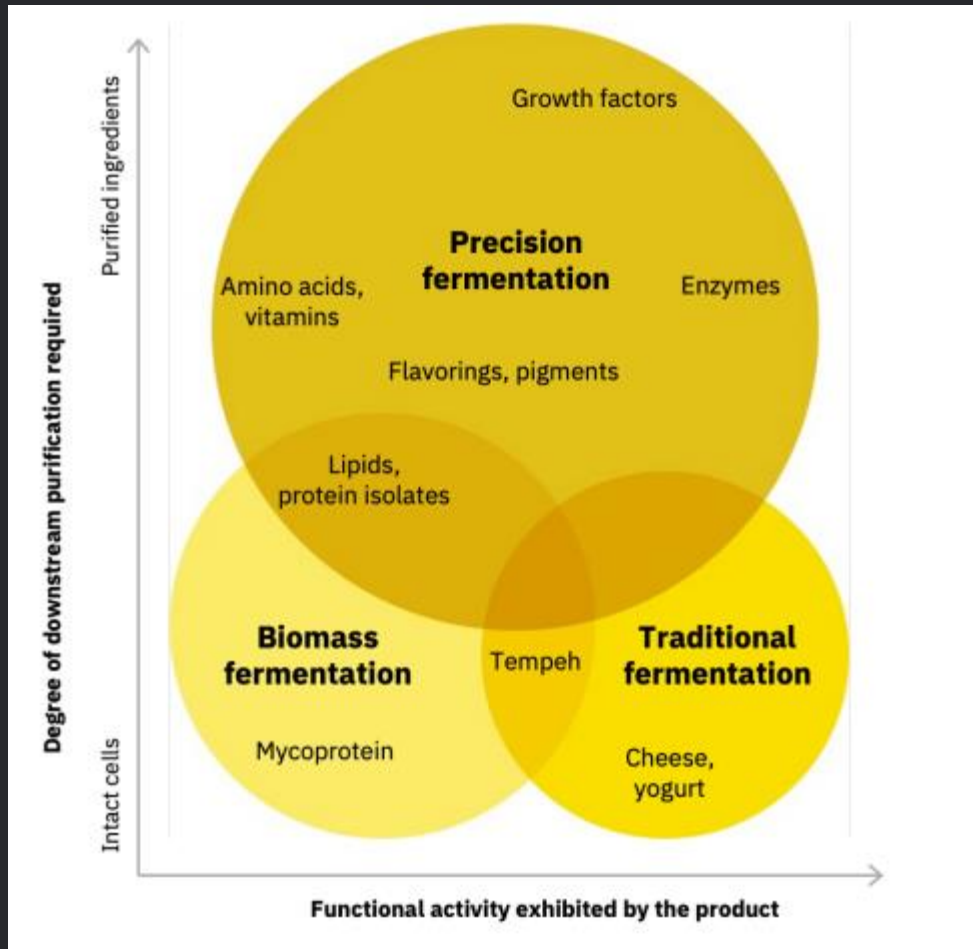
Proses fermentasi

Aplikasi teknologi fermentasi

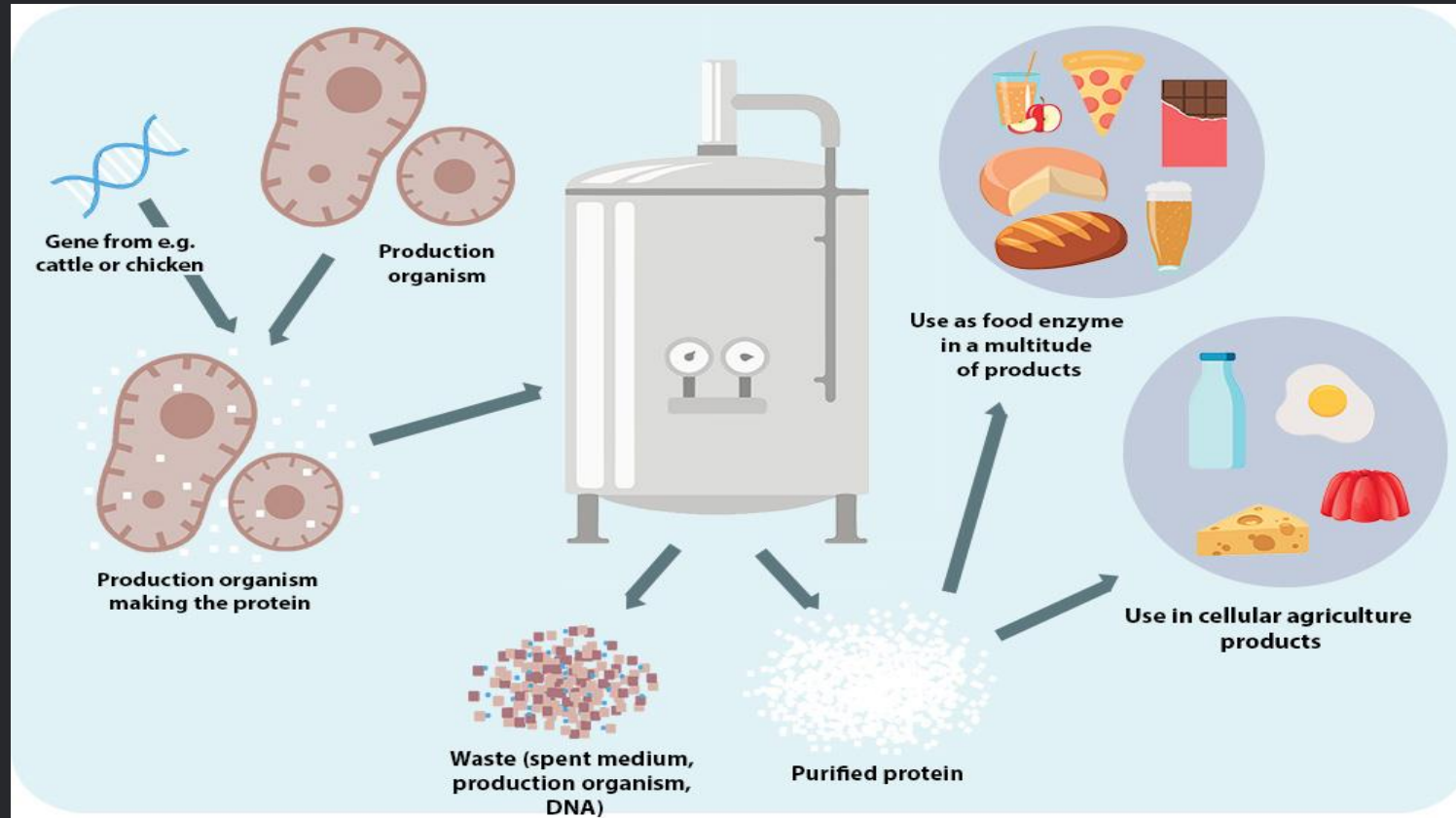
Fermentation: Humanity's Oldest Biotechnological Tool



Fermentasi ?



PENDAHULUAN

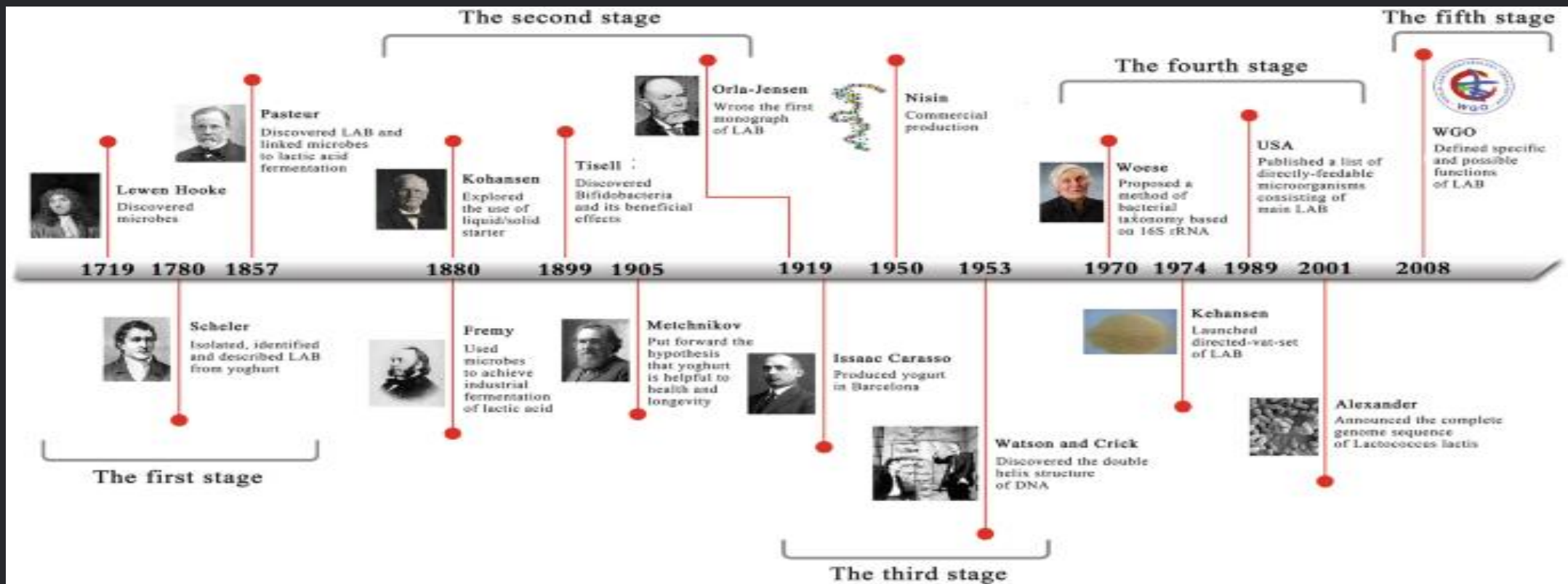


- Respirasi sel menghasilkan energi, tetapi merupakan proses aerobik (memerlukan oksigen)
- Selain molekul energi (seperti ATP), fermentasi menghasilkan berbagai molekul, termasuk etanol, karbon dioksida, asam laktat, metanol, hidrogen, metana, asam butirat, aseton, dan asam asetat.
- Contoh organisme yang melakukan fermentasi antara lain jamur (ragi), hewan (manusia, sapi), dan bakteri (*Clostridium*).

Fermentation, a chemical process by which molecules such as glucose are broken down anaerobically.

PENDAHULUAN

- Human-caused fermentation dates back to 10,000 BCE with the preservation of milk from camels, cattle, sheep, and goats.
- Dairy naturally ferments given the ideal climate and its essential microflora. In the searing temperatures of North Africa, milk spontaneously fermented, making the first documented yogurt

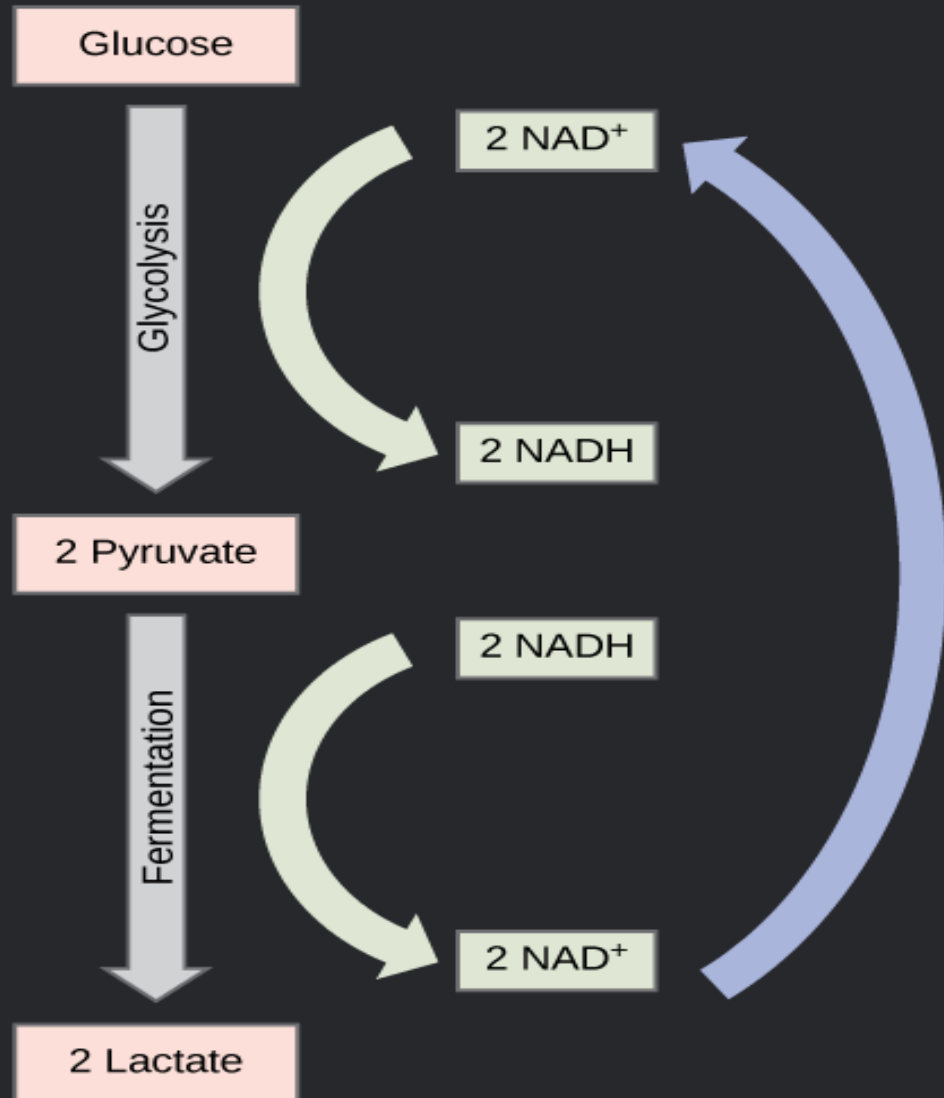


PENDAHULUAN



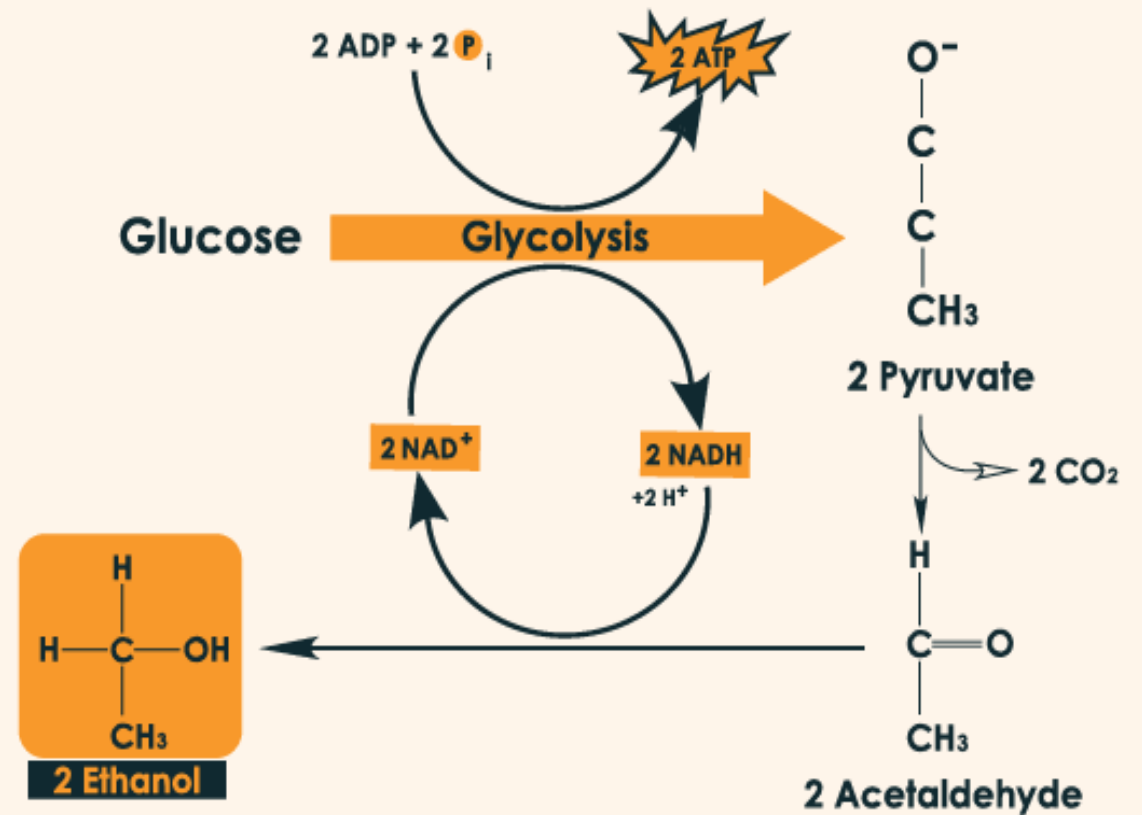
- Louis Pasteur in the 19th century used the term fermentation in a narrow sense to describe the changes brought about by yeasts and other microorganisms growing in the absence of air (anaerobically);
- He also recognized that ethyl alcohol and carbon dioxide are not the only products of fermentation.

Lactic Acid Fermentation



PENDAHULUAN

Alcoholic Fermentation



PENDAHULUAN

FERMENTATION...

Preserves
food

Enriches the
palette of
flavors

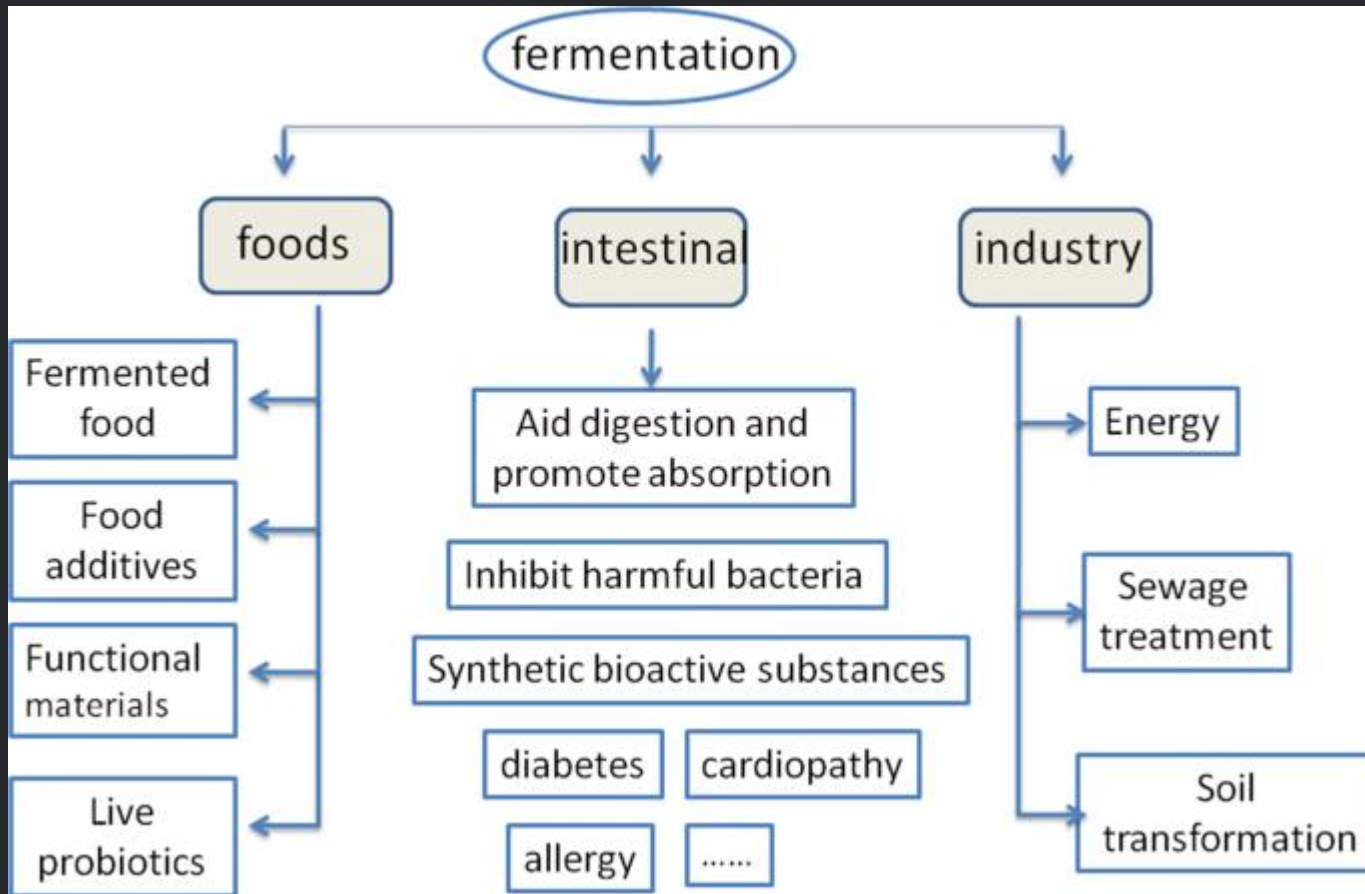
Allows a
better
assimilation
of food

Helps
digestion

Can **enrich**
intestines with
living
microorganisms



PENDAHULUAN



- Fermentation has currently evolved into a very important branch of bioengineering and has been a multidisciplinary focus in fields, such as microbiology, chemical engineering, genetic engineering, cellular engineering, mechanical engineering, and computer software and hardware engineering.
- With the development of modern biotechnology, fermentation has been applied to all aspects of life; microbes and metabolites are important in the production of fermented foods, in industry, and in human health

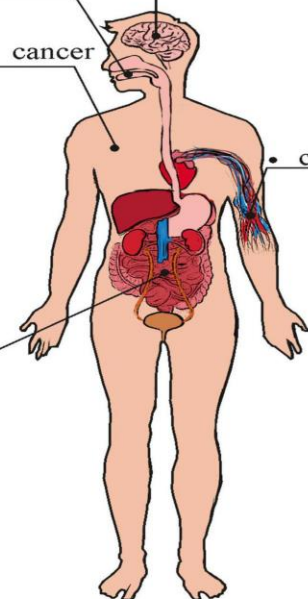
PENDAHULUAN

- breakdown of complex compounds and formation of beneficial catabolites (B vitamins, minerals or Omega-3 fatty acids)
- prevention of food spoilage
- inhibiting the growth of pathogenic microorganisms
- elimination of components toxic to humans (phytates, tannins)



FERMENTED FOOD

- treatment of halitosis and inflammation of the oral cavity
- improvement of the oral microbiota
- caries reduction
- mood improvement
- reduction of depression symptoms
- protection against cancer
- cholesterol reduction
- beneficial modification of the composition of intestinal microbiota
- inhibition of intestinal pathogens
- prevention of constipation
- shortening the duration of viral and bacterial diarrheas
- improving mineral absorption
- reducing the symptoms of lactose intolerance
- reducing the risk of obesity
- strengthening the immune system



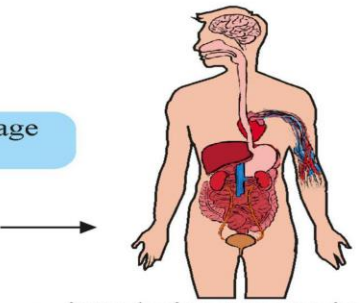
use of contaminated raw materials



contamination of products during the production process

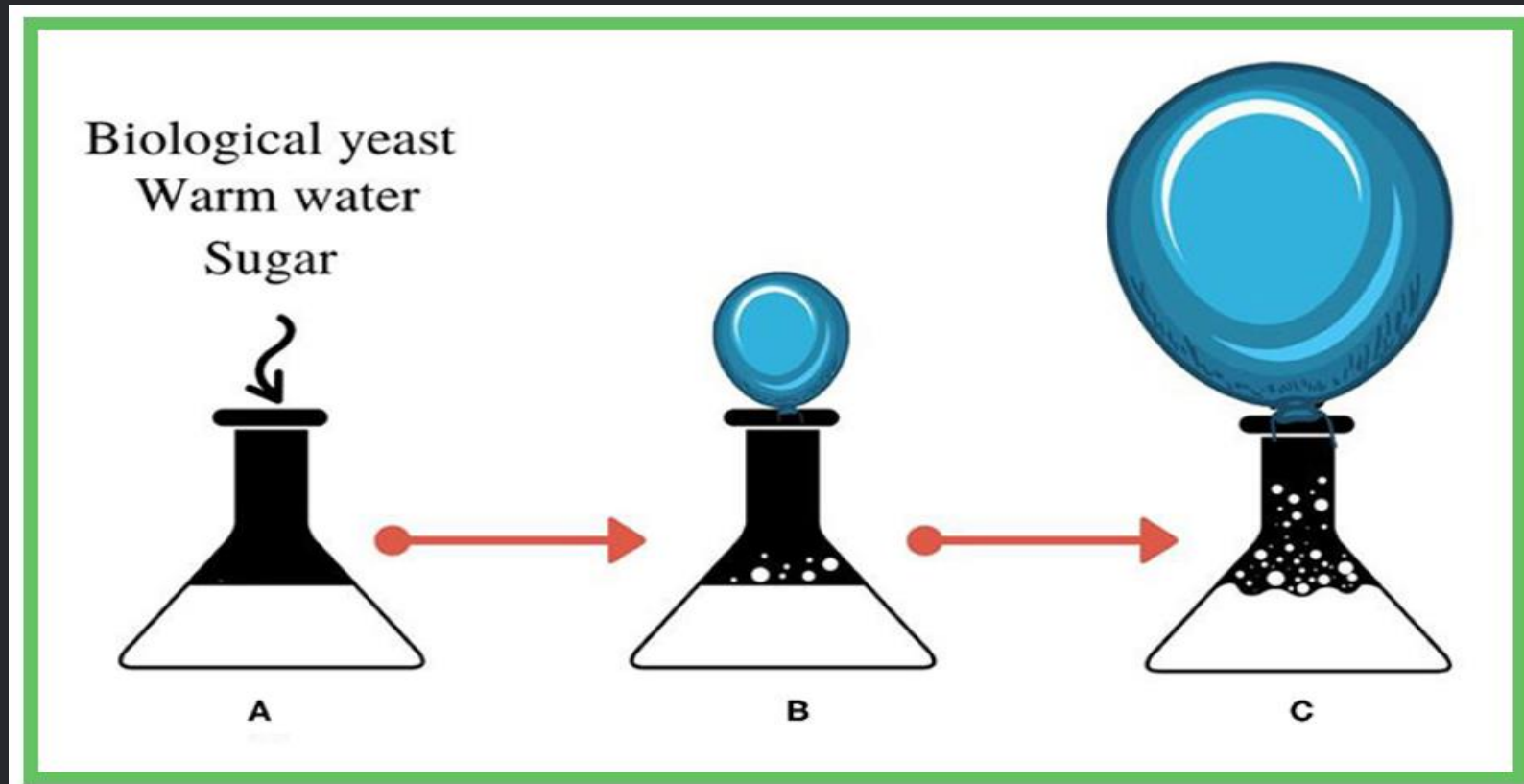


contamination at the distribution stage of fermented foods



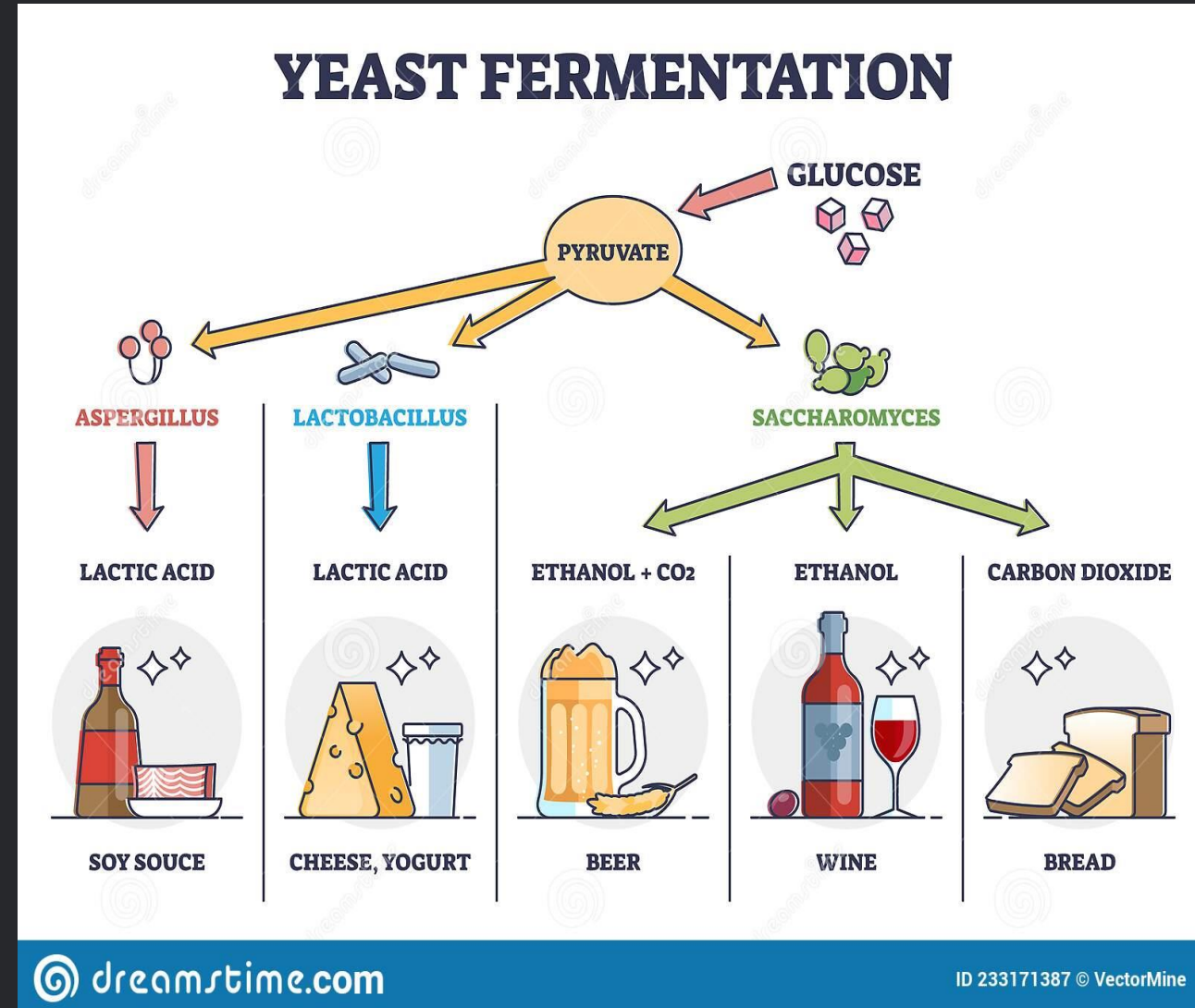
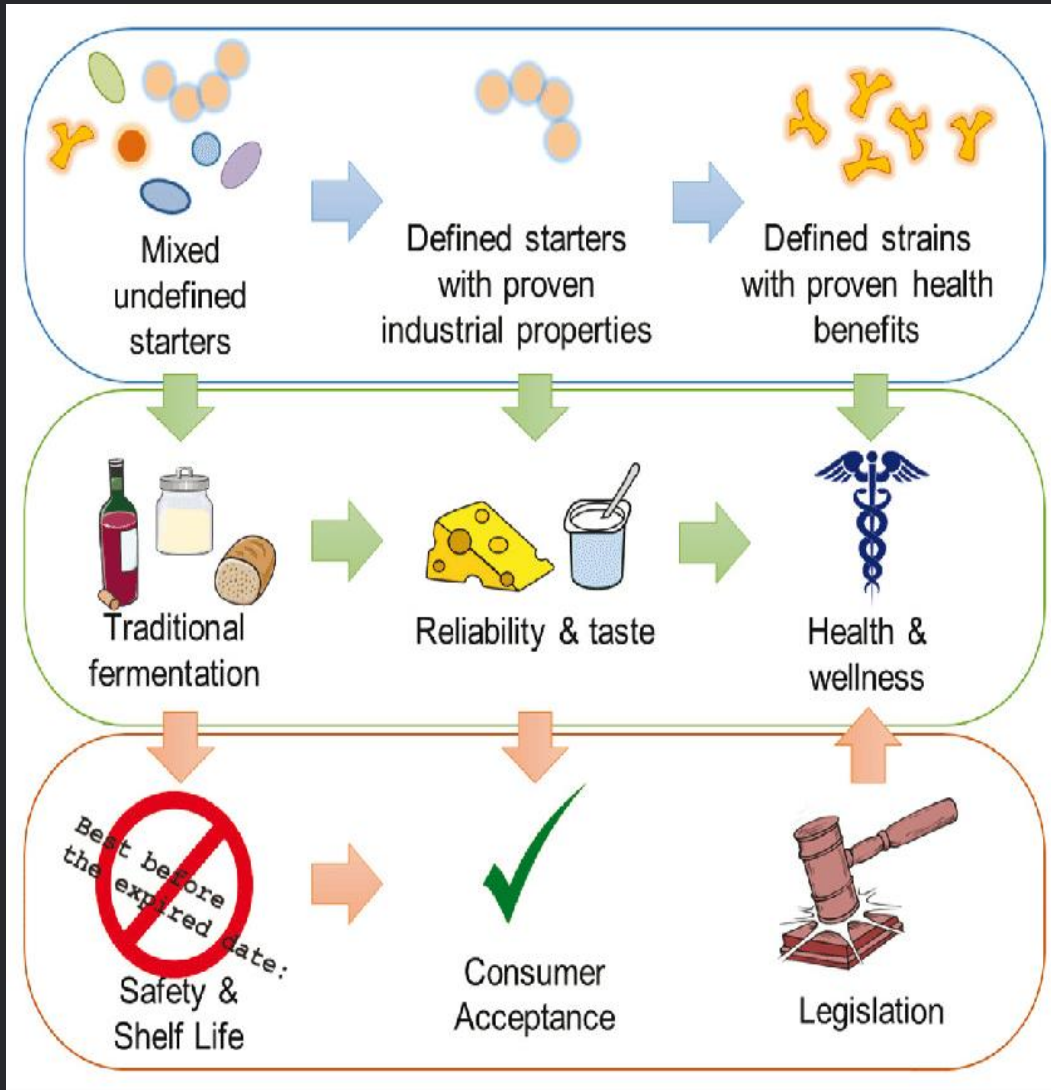
- introducing mycotoxins
- bacterial, viral, fungal infections
- formation of biogenic amines
- foods introducing drug resistance gene carriers

PROSES FERMENTASI



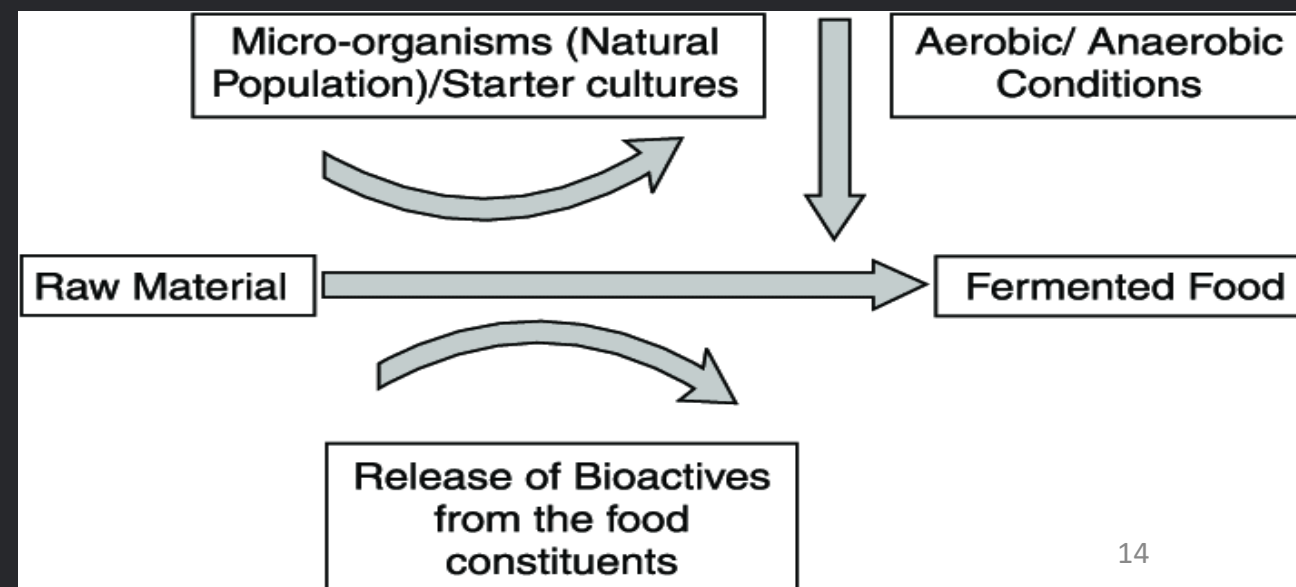
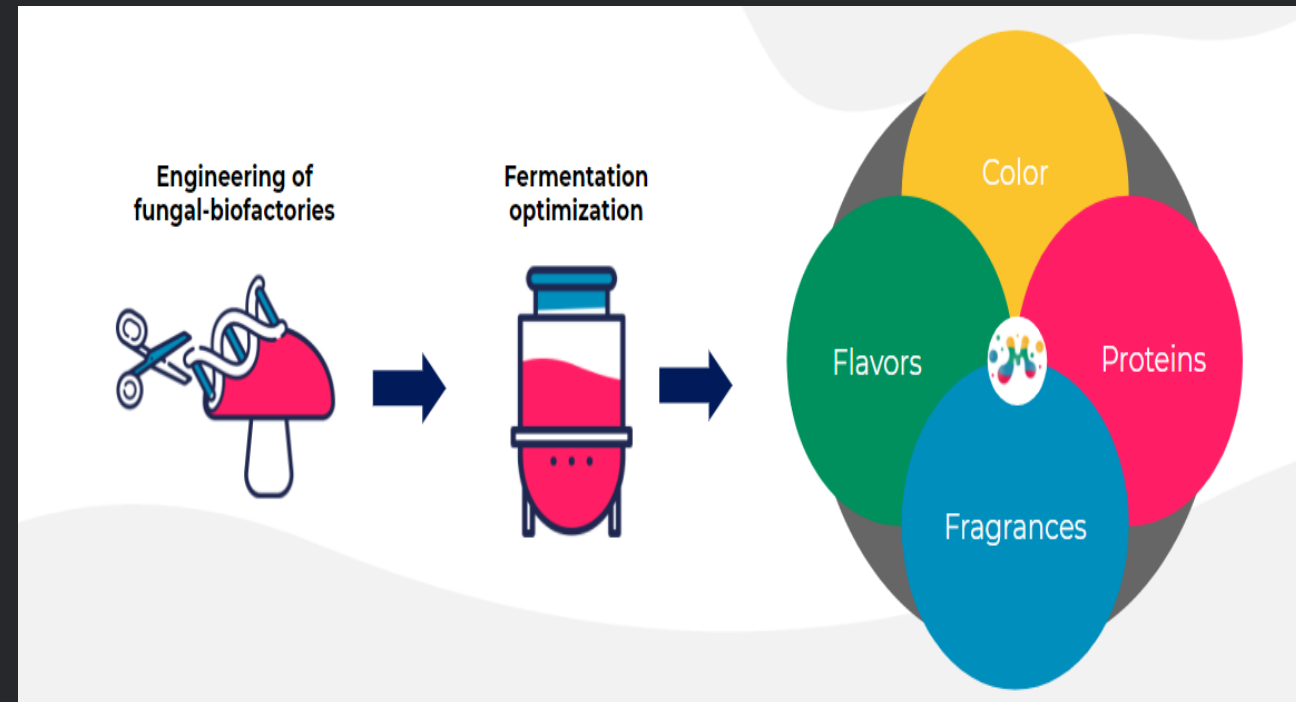
(A) Mix warm water, yeast, and sugar in a jar or flask with a small neck. (B) Place a balloon over the mouth of the flask. (C) After a few minutes, you should see the balloon inflate due to the formation of CO₂ gas via fermentation of the sugar by the yeast.

PROSES FERMENTASI



PROSES FERMENTASI

- The main principle of fermentation is to derive energy from carbohydrates in the absence of oxygen.
- Glucose is first partially oxidized to pyruvate by glycolysis.
- Then pyruvate is converted to alcohol or acid along with regeneration of NAD⁺ which can take part in glycolysis to produce more ATP.
- Fermentation yields only about 5% of the energy obtained by aerobic respiration.



PROSES FERMENTASI : TIPE FERMENTASI

Lactic acid homo-fermentation

- Glucose → Lactic acid
- Homolactic fermentation is carried out by bacteria belonging to the genera Lactococcus, Enterococcus, Streptococcus, and Pediococcus, and by some species of the genus Lactobacillus.
- Homofermentative LAB ferment glucose to lactic acid.
- Lactococcus spp. is used in the dairy starter culture.

Lactic acid hetero-fermentation

- Glucose → Lactic acid + Acetic acid + Ethyl alcohol + 2CO₂ + H₂O
- Heterolactic fermentation is carried out by bacteria of the genera Leuconostoc, Oenococcus, and Weissella, and by heterofermentative lactobacilli.
- Heterofermentative LAB ferment glucose with lactic acid, ethanol/acetic acid, and carbon dioxide (CO₂) as by-products.

Propionic acid fermentation

- Glucose → Lactic acid + Propionic acid + Acetic acid + CO₂ + H₂O
- Propionic acid fermentation is carried out by several bacteria that belong to the genus Propionibacterium and the species Clostridium propionicum.
- During propionic acid fermentation, both sugar and lactate can be used as the initial substrate.
- When sugar is available, these bacteria use the EMP pathway to produce pyruvate; the pyruvate is carboxylated to oxaloacetate and then reduced to propionate via malate, fumarate, and succinate.
- The other end products of propionic fermentation are acetic acid and CO₂.

PROSES FERMENTASI : TIPE FERMENTASI

Alcoholic fermentation

- Glucose → Ethyl alcohol
- Alcoholic fermentation is the best known of the fermentation processes.
- It is carried out by yeasts and some other fungi and bacteria.
- The first step of the alcoholic fermentation pathway involves pyruvate, which is formed by yeast via the EMP pathway, while it is obtained through the ED pathway in the case of *Zymomonas* (bacteria).
- The redox balance of alcoholic fermentation is achieved by the regeneration of NAD⁺ during the reduction of acetaldehyde to ethanol.

Butyric acid fermentation

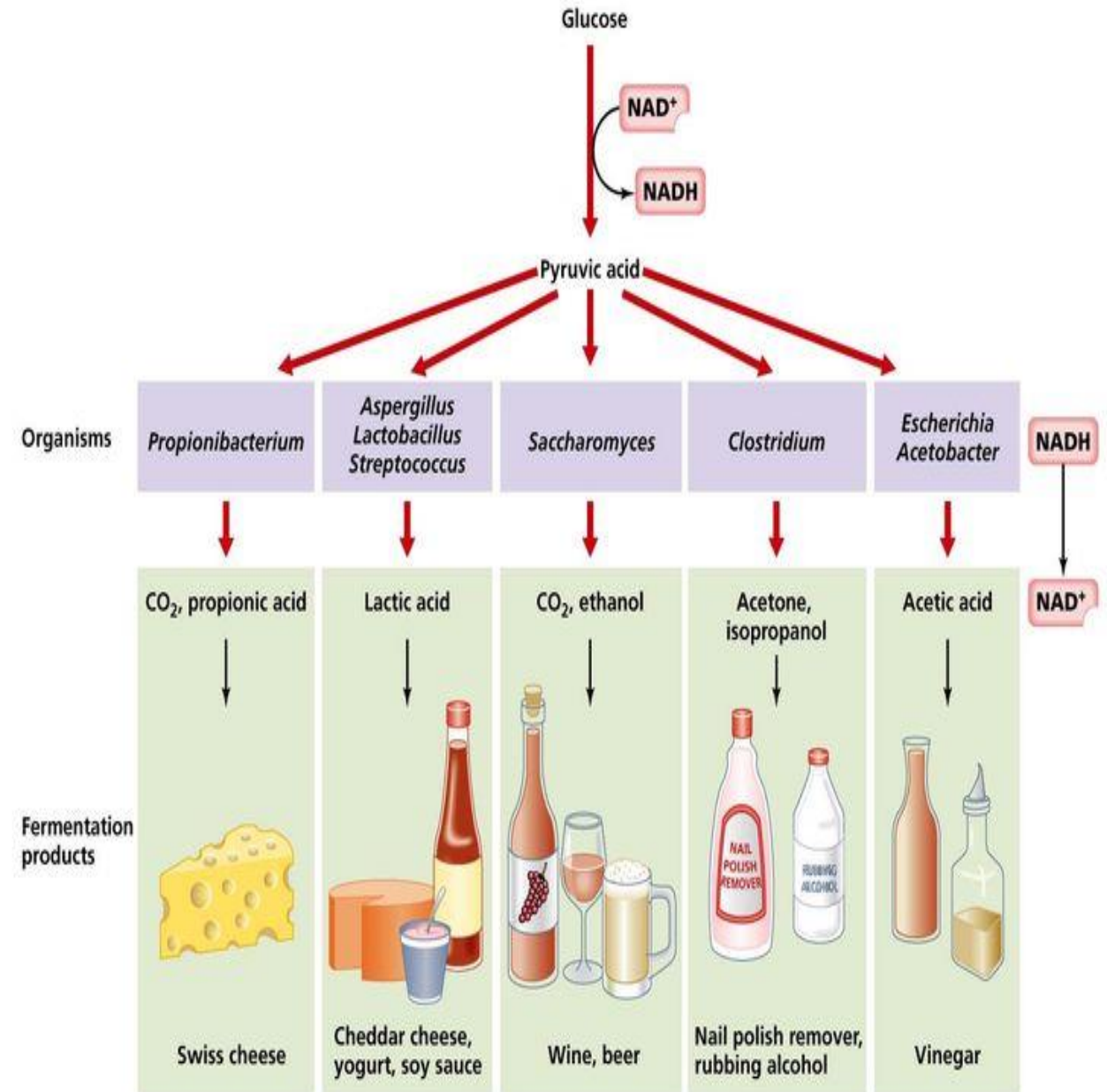
- Glucose → Acetic acid + Butyric acid
- Butyric acid fermentation is characteristic of several obligate anaerobic bacteria that mainly belong to the genus *Clostridium*.
- Pyruvate is in turn oxidized to acetyl-CoA, with the production of CO₂ and H₂.
- Part of the acetyl-CoA is converted into acetic acid, with ATP production.
- Some bacteria, such as *Clostridium acetobutylicum*, produce fewer acids and more neutral products, thus carrying out acetone butanol fermentation.

Diacetyl and 2,3-butylene glycol fermentation

- Citric acid → Pyruvic acid + Acetylmethylcarbon → Diacetyl ; 2,3-Butylene glycol
- Butanediol fermentation is carried out by members of the genera *Enterobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, and *Serratia*.
- The reactions that lead to the production of 2,3-butanediol involve a double decarboxylation step.

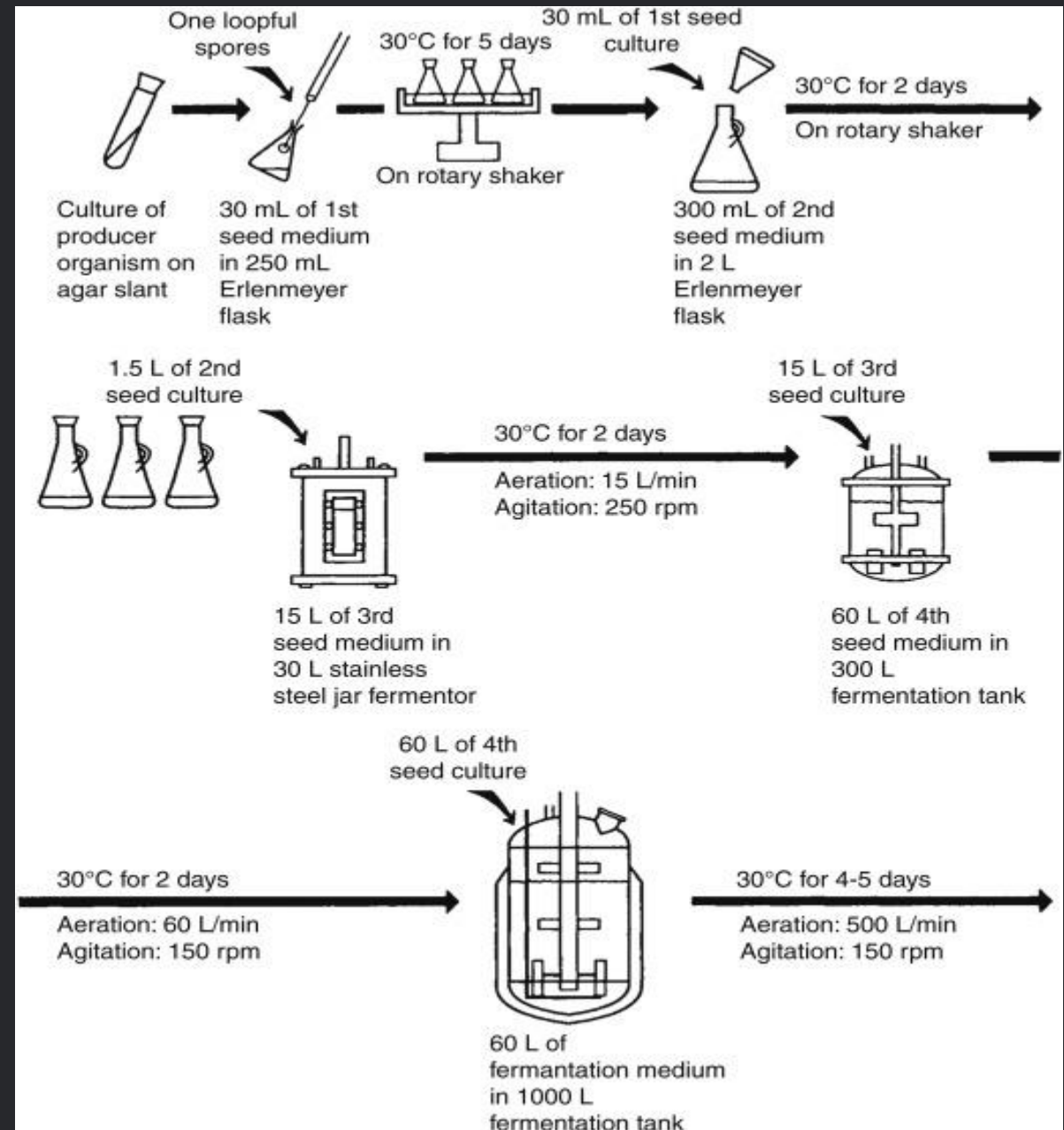
PROSES FERMENTASI

TIPE FERMENTASI



PROSES FERMENTASI

Product	Organism	Use
Ethanol	<i>Saccharomyces cerevisiae</i>	Industrial solvents, beverages
Glycerol	<i>Saccharomyces cerevisiae</i>	Production of explosives
Lactic acid	<i>Lactobacillus bulgaricus</i>	Food and pharmaceutical
Acetone and butanol	<i>Clostridium acetobutylicum</i>	Solvents
α -amylase	<i>Bacillus subtilis</i>	Starch hydrolysis



PROSES FERMENTASI

FERMENTED FOODS

PROBIOTICS

@MARIKADAY



SOURDOUGH BREAD



WINE & BEER



KIMCHI & SAUERKRAUT



SOY SAUCE & MISO



YOGHURT*



KOMBUCHA *

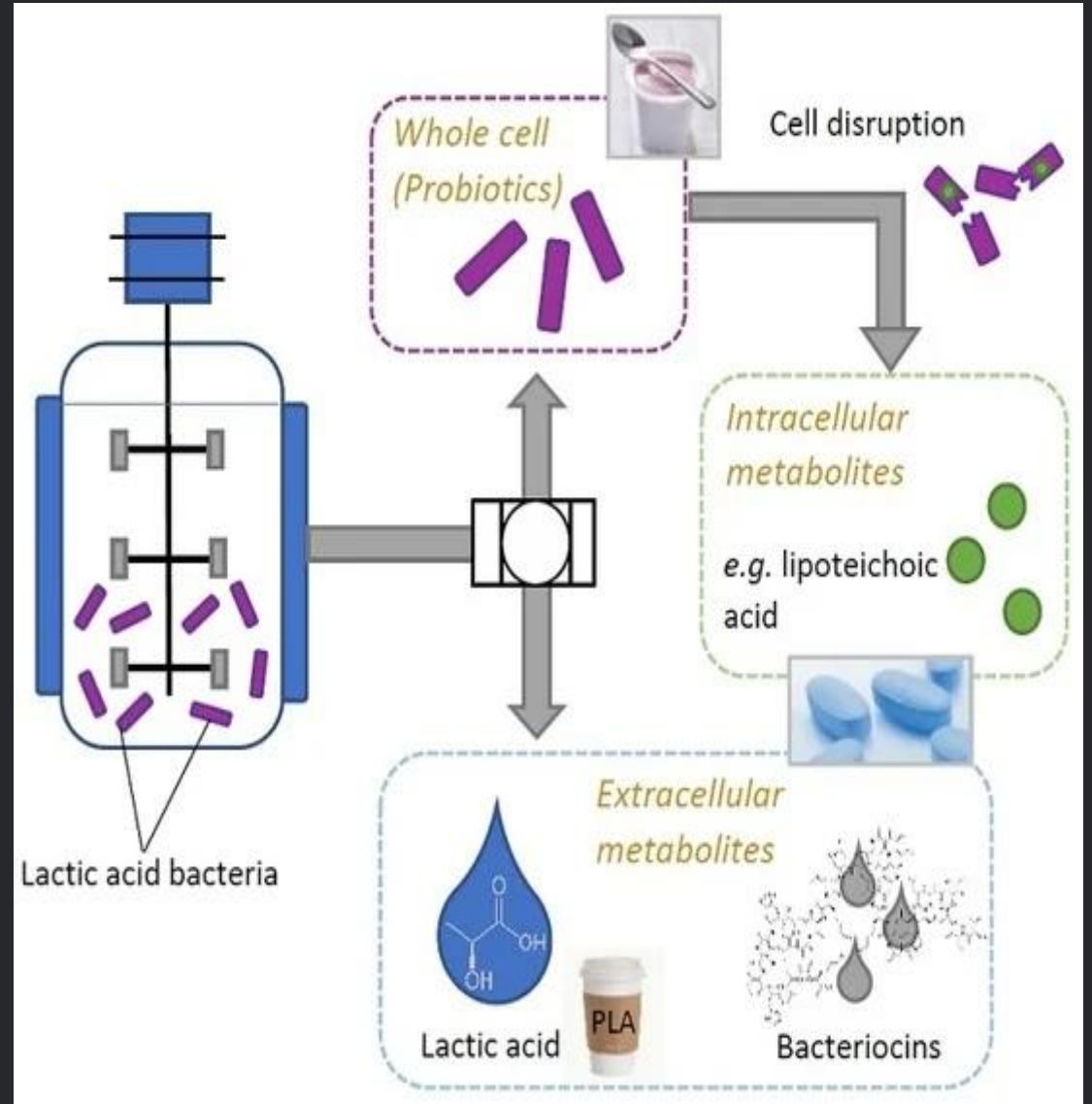


PROBIOTIC SUPPLEMENTS

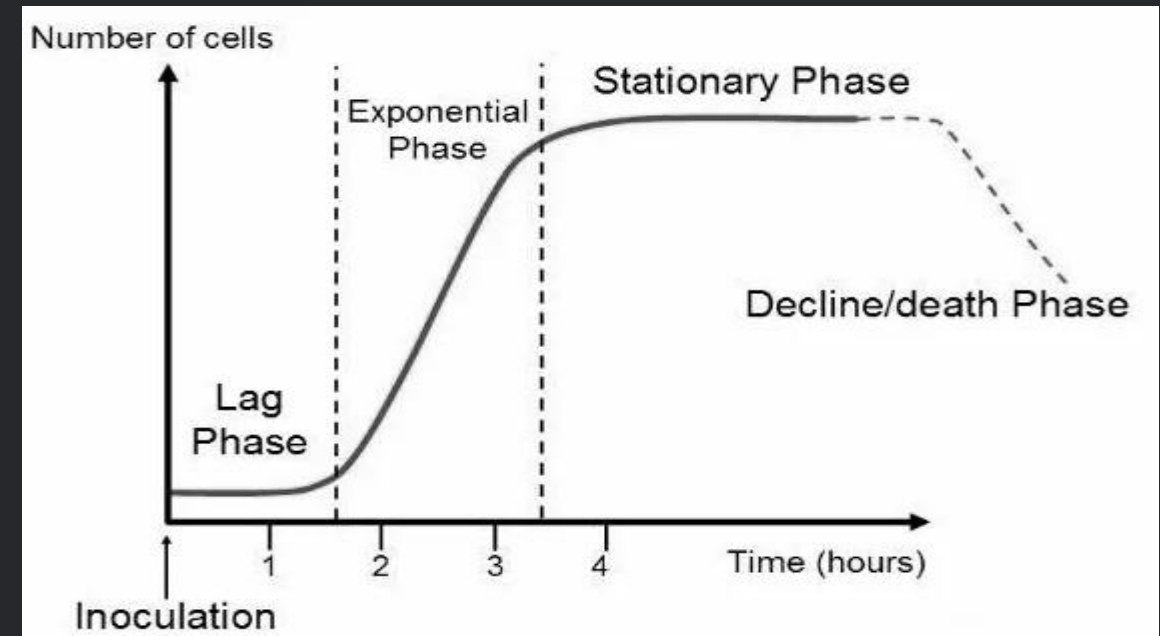


PROBIOTIC KEFIR

*** WHICH HAVE SPECIFIED BENEFICIAL MICROBES**

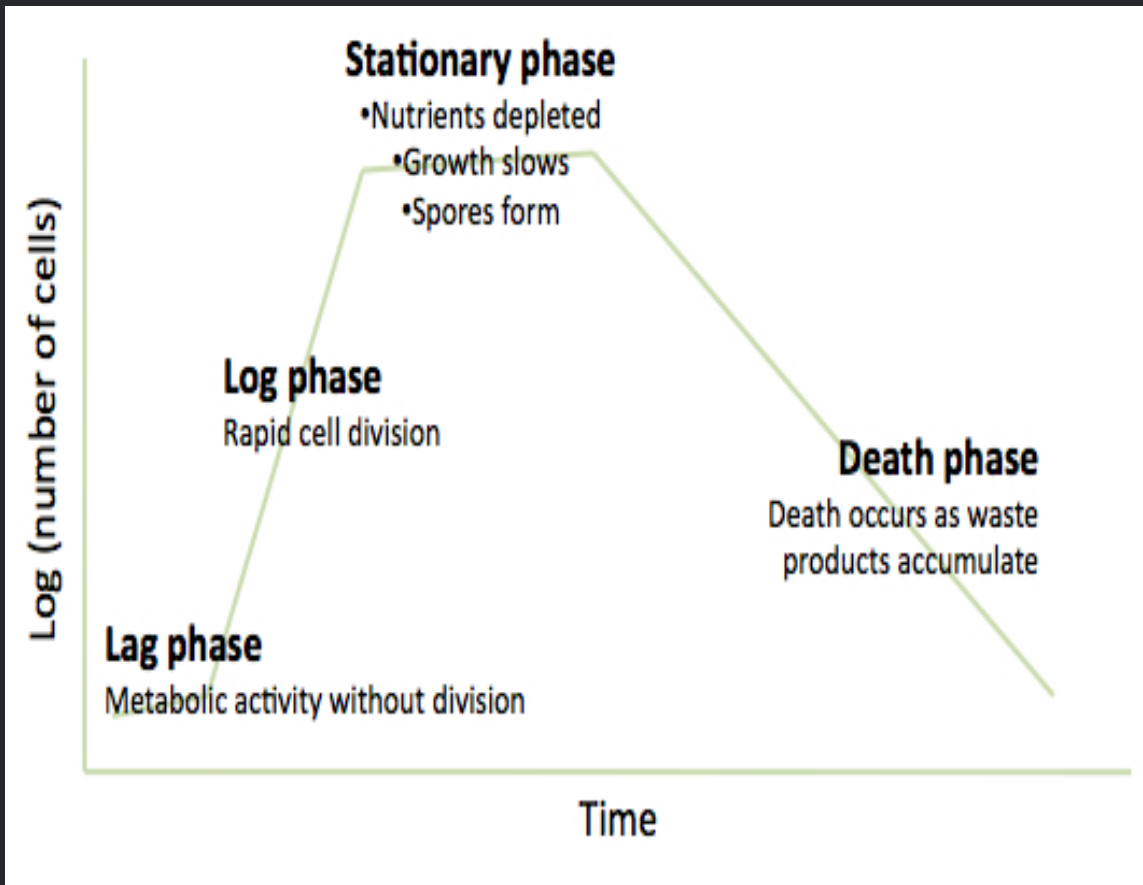


PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME



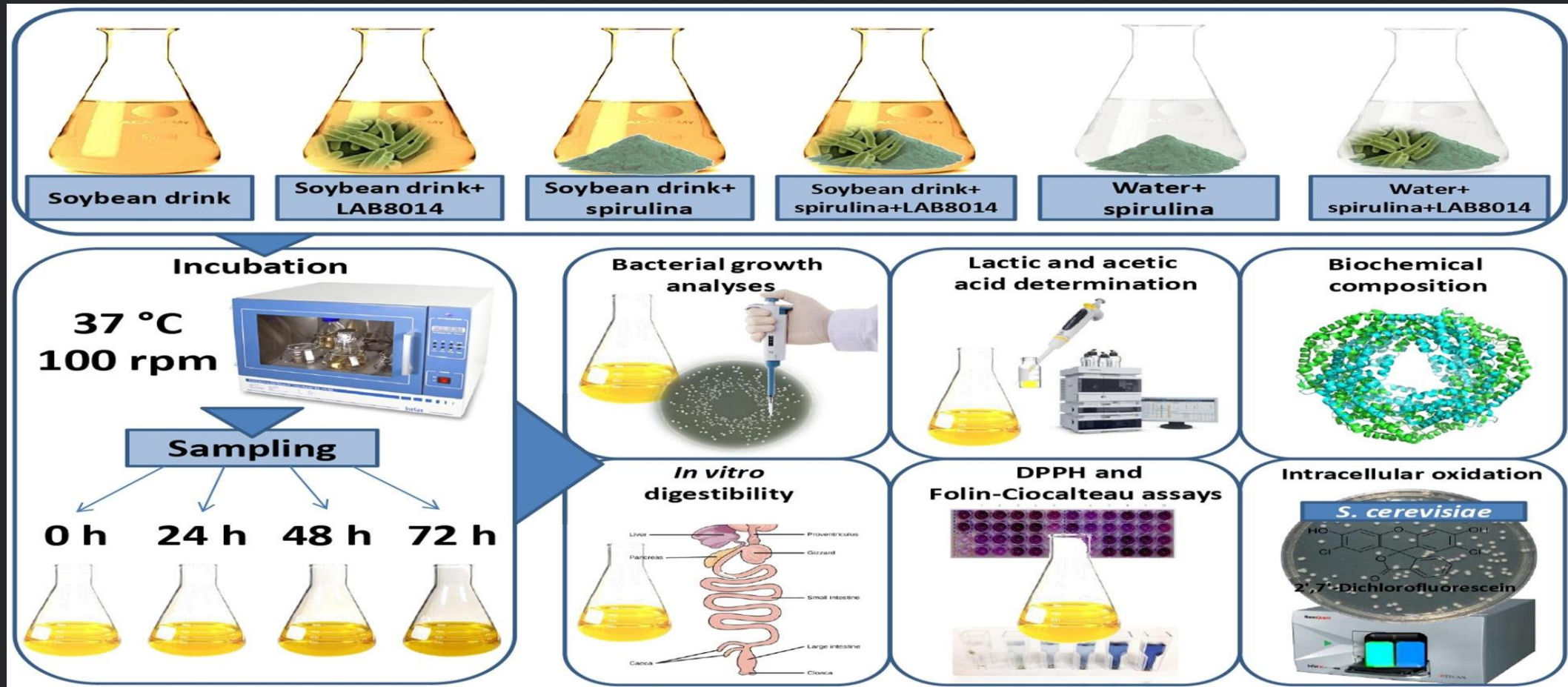
Growing phase	Microbes	Metabolisms	Metabolites
Lag phase	Individual growth	Anabolic metabolism dominates in cell, extracellular enzyme synthesis and transshipment, substrate begin to degrade	Enzymes
Logarithmic phase	Individual reproduction, population growth increasing geometrically	All kinds of metabolism are very high	Enzymes, functional polysaccharide, polypeptides, etc. Primary metabolites, such as alcohol, amino acid, pyruvate, and citric acid
Stationary phase	Population growth and the rate equal to the death	Accumulation of metabolites begins the secondary metabolism	Secondary metabolites, such as antibiotics, hormones, toxins, and pigments
Decline phase	Growth is inhibited by substrate, and the cells begin to autophagy	Old cell degradation, and new microbe population begins the new metabolism	Nucleotide, spore, sulfide, amino, monosaccharide, secondary metabolites

PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME

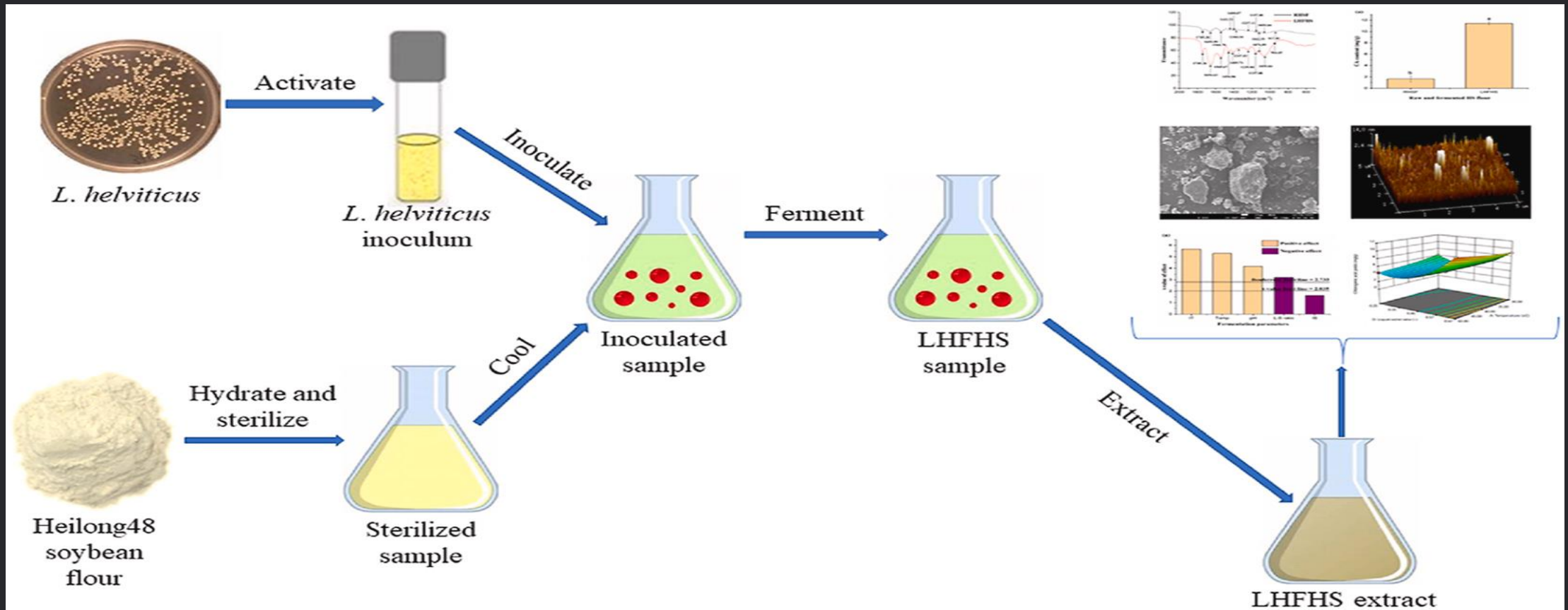


- For fermentation products (lactic acid, etc.) whose production depends on increasing bacterial growth or for other metabolic products whose production parallels bacterial growth, the stationary phase is the best time for harvest.
- To avoid the adverse effects of accumulating certain metabolic products during growth, some microbes begin using a type of metabolism conducive to survival; they start to synthesize secondary metabolites via complex secondary metabolic pathways.
- Secondary metabolites, such as antibiotics, hormones, pigments, and toxins, are low molecular mass, structurally complex organic compounds with diverse biological activities. Secondary metabolites are not directly involved in growth, development, or reproduction

PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME



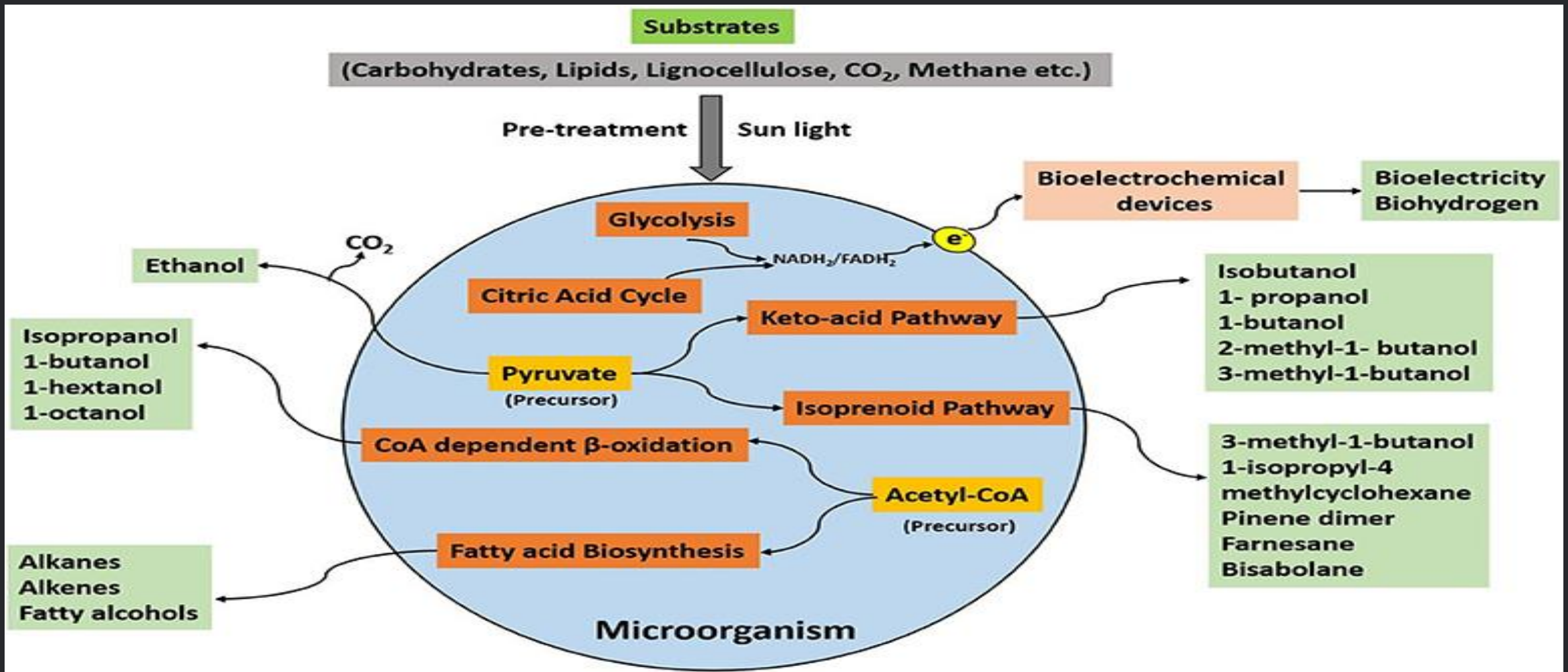
PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME



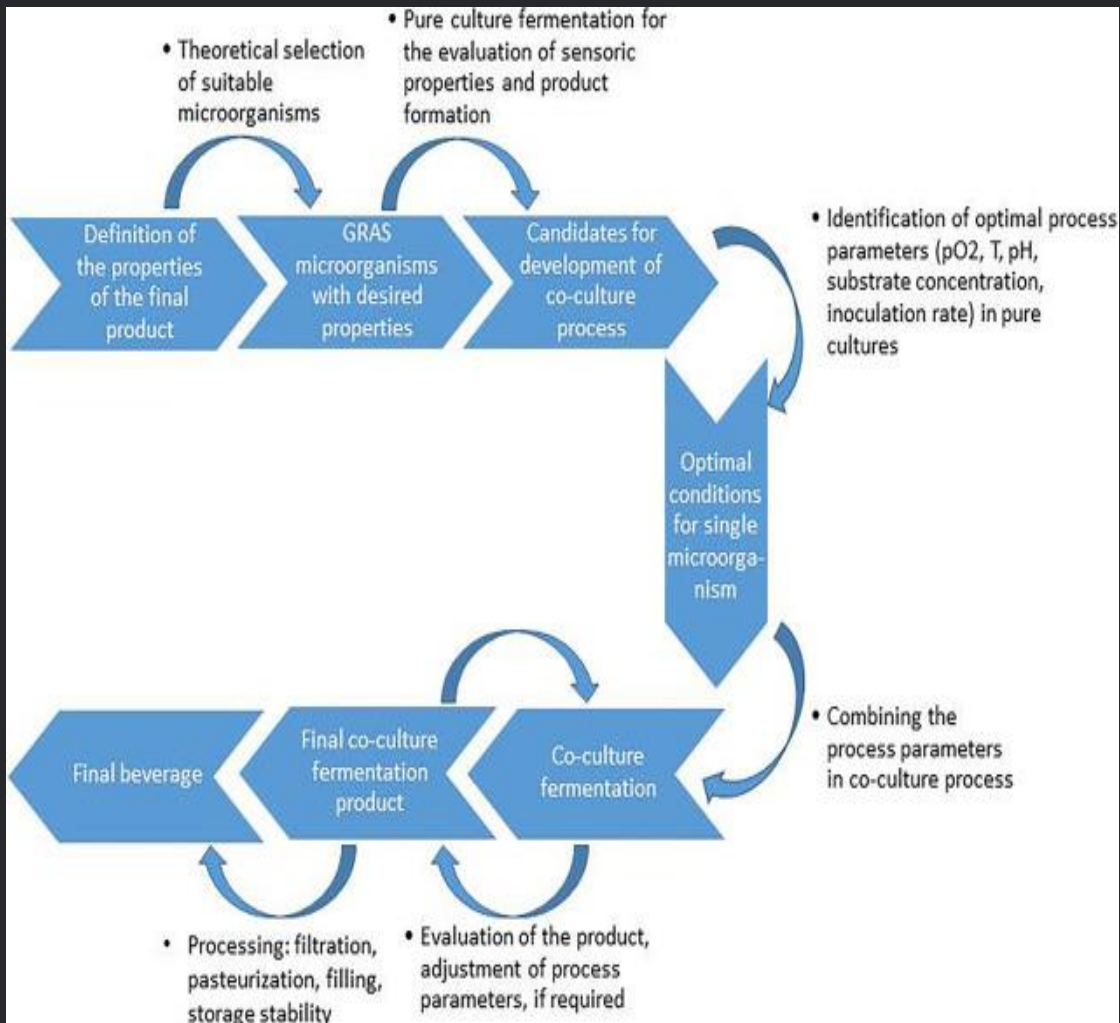
Novel solid-state fermentation extraction of 5-O-caffeoylquinic acid from heilong48 soybean using *Lactobacillus helveticus*. 5-CQA has tremendous application in food, pharmaceutical and cosmetic industries.

Chlorogenic acid (CGA), also known as 5-O-caffeoylquinic acid, is a polyphenol commonly found in human dietary products, especially in coffee beans.

PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME MENGHASILKAN BIOFUEL



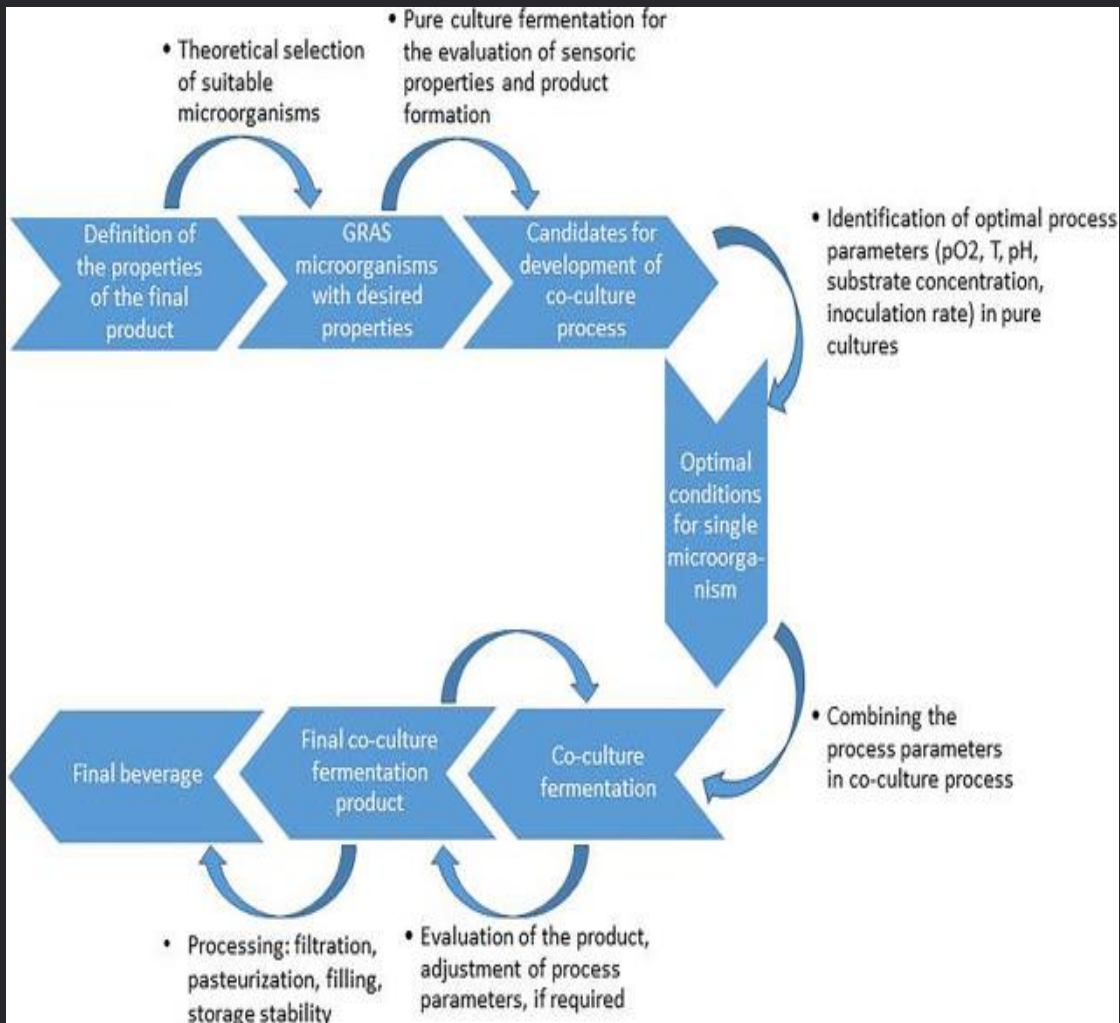
PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME



Mixed-culture fermentations offer a number of advantages over conventional single-culture fermentations:

- Product yield may be higher. Yogurt is made by the fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.
- The growth rate may be higher
- Mixed cultures are able to bring about multistep transformations that would be impossible for a single microorganism. Examples are the miso and shoyu fermentations in which *Aspergillus oryzae* strains are used to make koji.
- In some mixed cultures a remarkably stable association of microorganisms may occur.
- Compounds made by a mixture of microorganisms often complement each other and work to the exclusion of unwanted microorganisms.
- Mixed cultures permit better utilization of the substrate.
- Mixed cultures can be maintained indefinitely by unskilled people with a minimum of training. Mixed cultures offer more protection against contamination.
- Mixed-culture fermentations enable the utilization of cheap and impure substrates.
- Mixed cultures can provide necessary nutrients for optimal performance

PROSES FERMENTASI : PERTUMBUHAN MIKROORGANISME

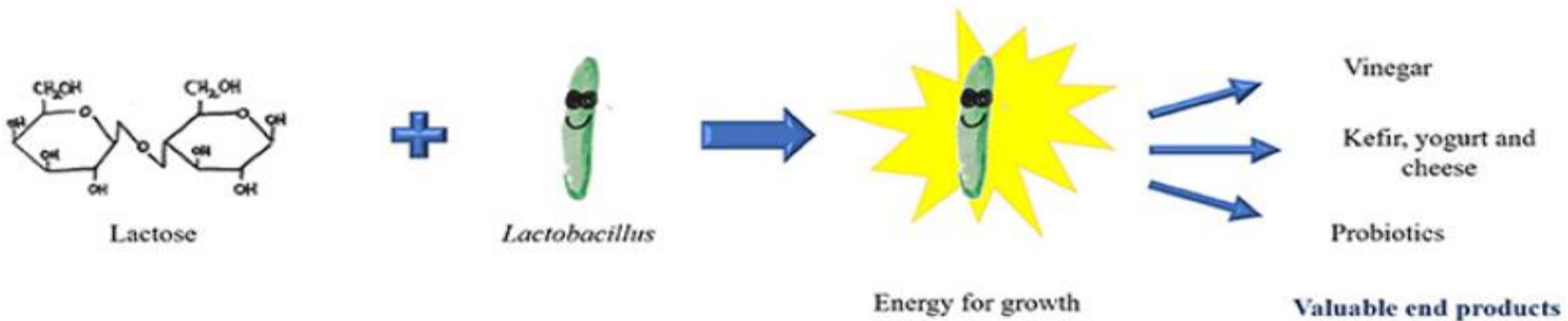


Mixed-culture fermentations also have some disadvantages

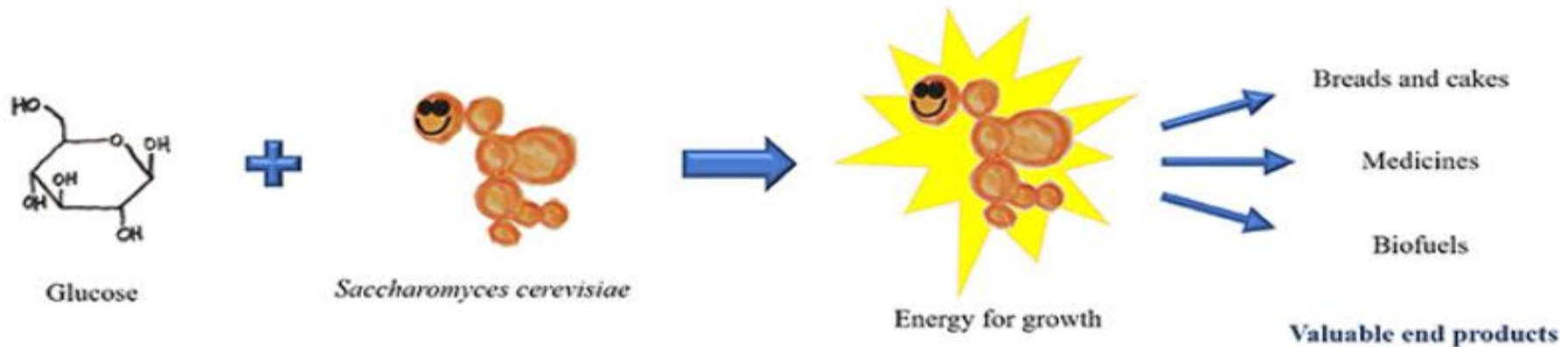
- Scientific study of mixed cultures is difficult
- Defining the product and the microorganisms employed becomes more involved in patent and regulatory procedures.
- Contamination of the fermentation is more difficult to detect and control.
- When two or three pure cultures are mixed together, it requires more time and space to produce several sets of inocula rather than just one.
- One of the worst problems in mixed-culture fermentation is the control of the optimum balance among the microorganisms involved. This can, however, be overcome if the behavior of the microorganisms is understood and this information is applied to their control.

APLIKASI TEKNIK FERMENTASI

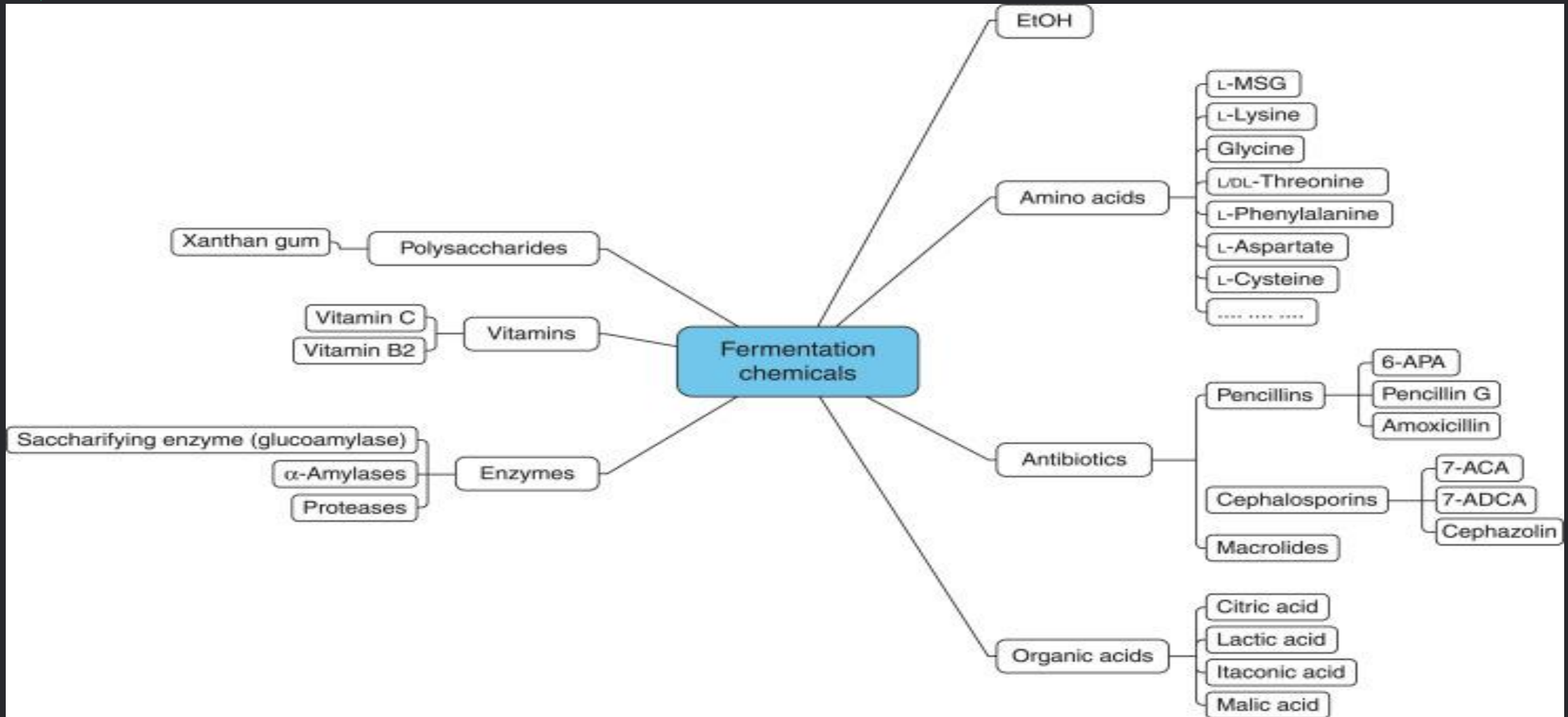
A) Lactic acid fermentation



B) Alcoholic fermentation



APLIKASI TEKNIK FERMENTASI

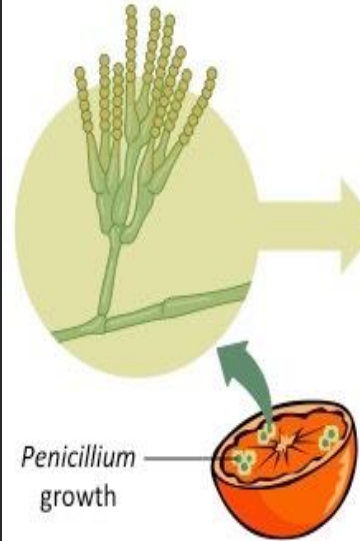


APLIKASI TEKNIK FERMENTASI

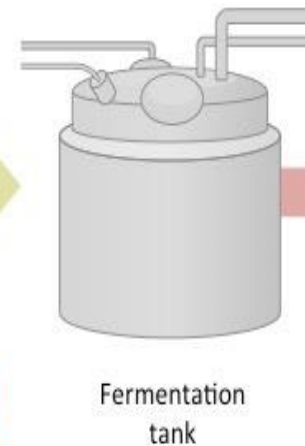
Application in medicine

- Production of antibiotics
- Production of insulin
- Production of growth hormones
- Production of vaccines
- Production of interferon

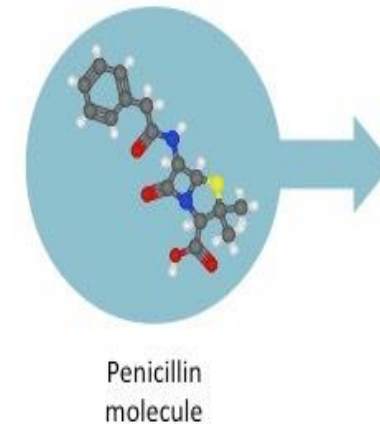
1 *Penicillium* mold produces the antibiotic penicillin



2 Scientists grow mold in deep batch fermenters by adding sugar and other key ingredients



3 Scientists separate the penicillin from the mold



4 Penicillin is purified for use as an antibiotic medicine

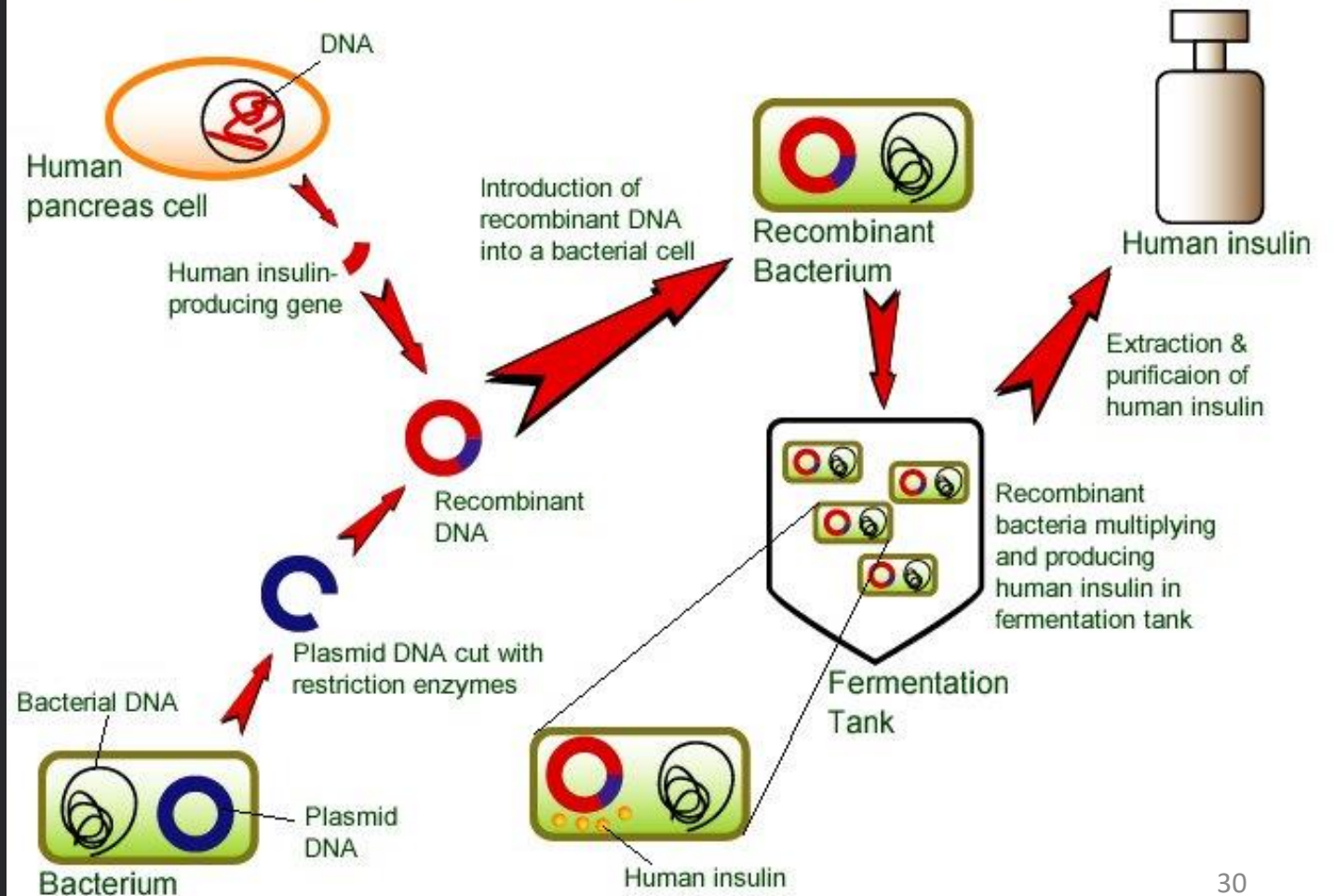


APLIKASI TEKNIK FERMENTASI

Application in medicine

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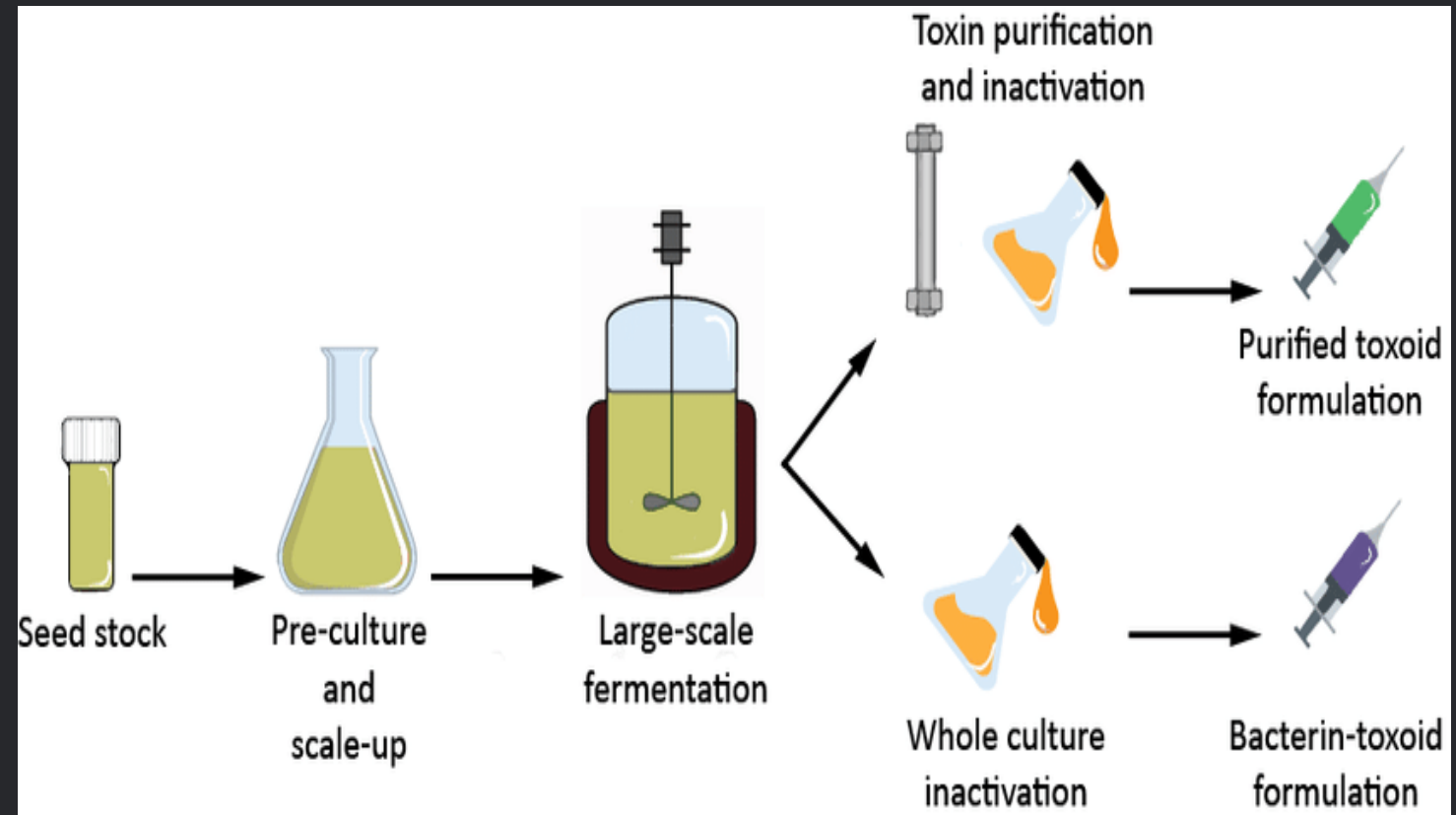
Human Insulin Production



APLIKASI TEKNIK FERMENTASI

Application in medicine

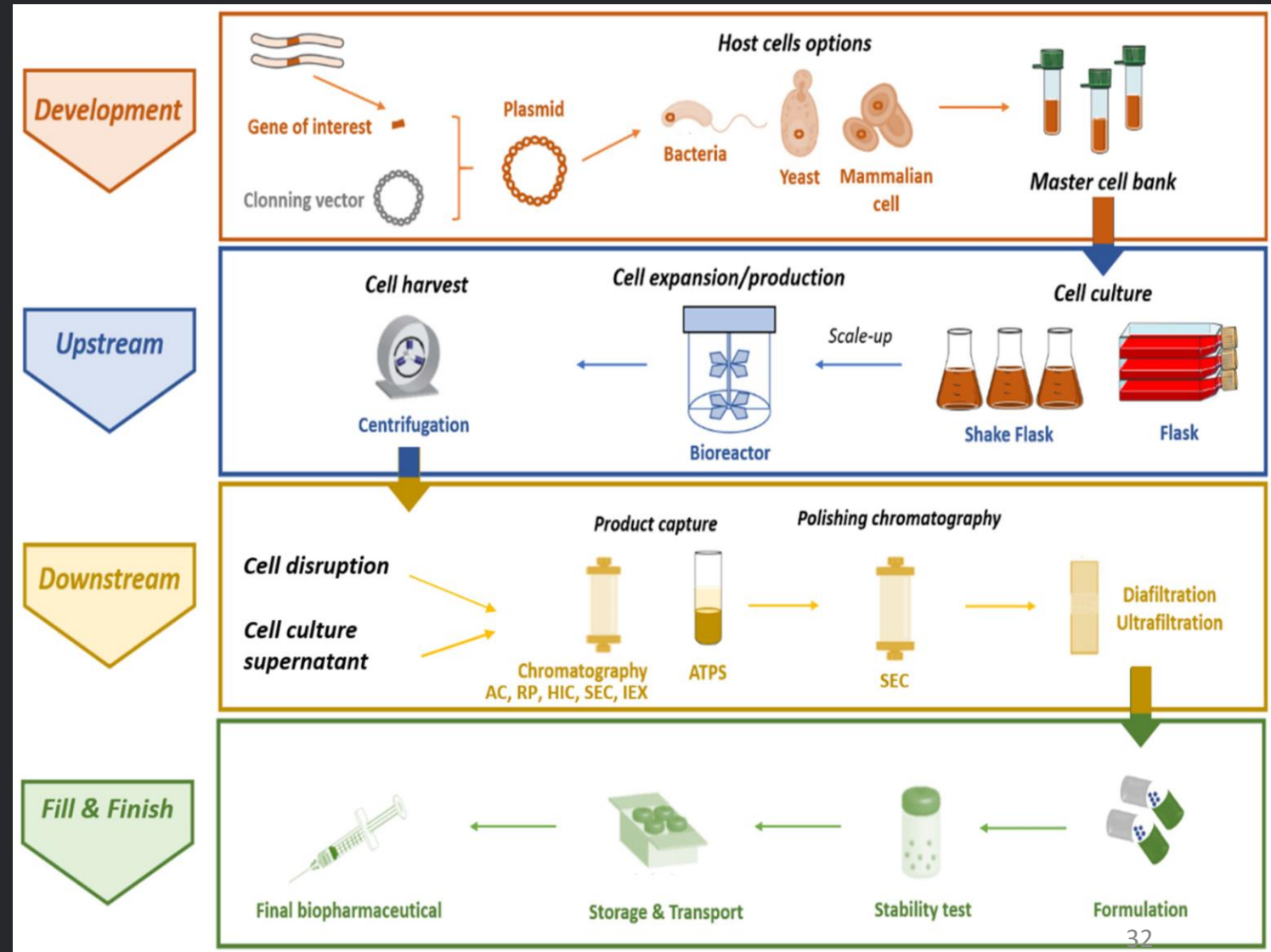
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APLIKASI TEKNIK FERMENTASI

Application in medicine

- Production of antibiotics
- Production of insulin
- Production of growth hormones
- Production of vaccines
- Production of interferon



APLIKASI TEKNIK FERMENTASI

Application in the food industry

- Production of fermented foods as cheese, wine, beer, and bread to high-value products
- Food grade bio preservatives
- Functional foods/Neutraceuticals
- Production of single-cell protein



THE BENEFITS OF FERMENTED FOODS

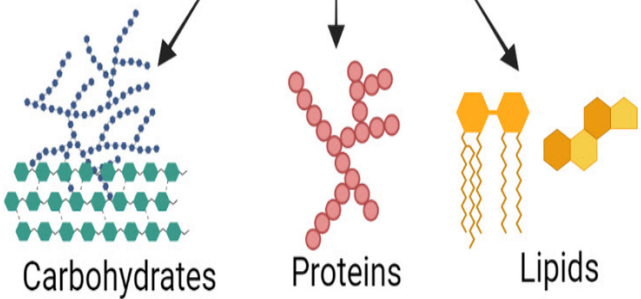
BY APAGE

WHY EAT FERMENTED FOODS?

 <h4>ENZYMES</h4> <p>Increased enzyme content helps you absorb nutrients, reducing the need for vitamins and supplements.</p>	 <h4>PROBIOTICS</h4> <p>These good bacteria help restore balance in the gut and aid digestion and immune health.</p>	 <h4>SAFETY</h4> <p>The lactic acid created during the fermentation process kills E. coli, making it safer to consume.</p>	 <h4>PRESERVATION</h4> <p>The lacto-fermentation process stores food longer than canning without depleting nutrients.</p>	 <h4>NUTRITION</h4> <p>The fermentation process increases the nutritional value by enriching certain nutrients.</p>
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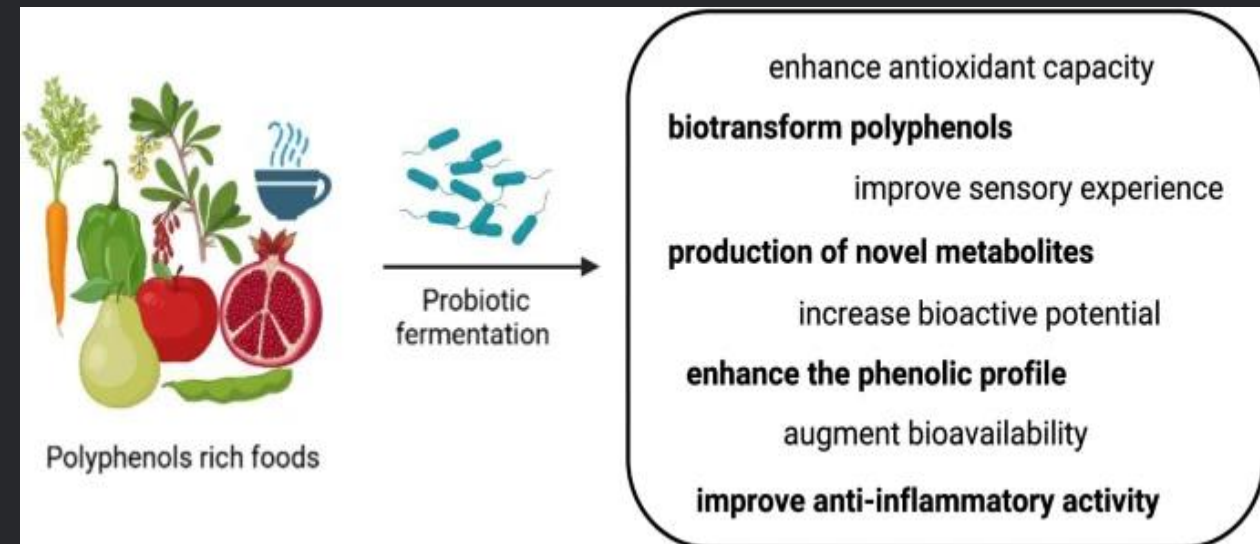
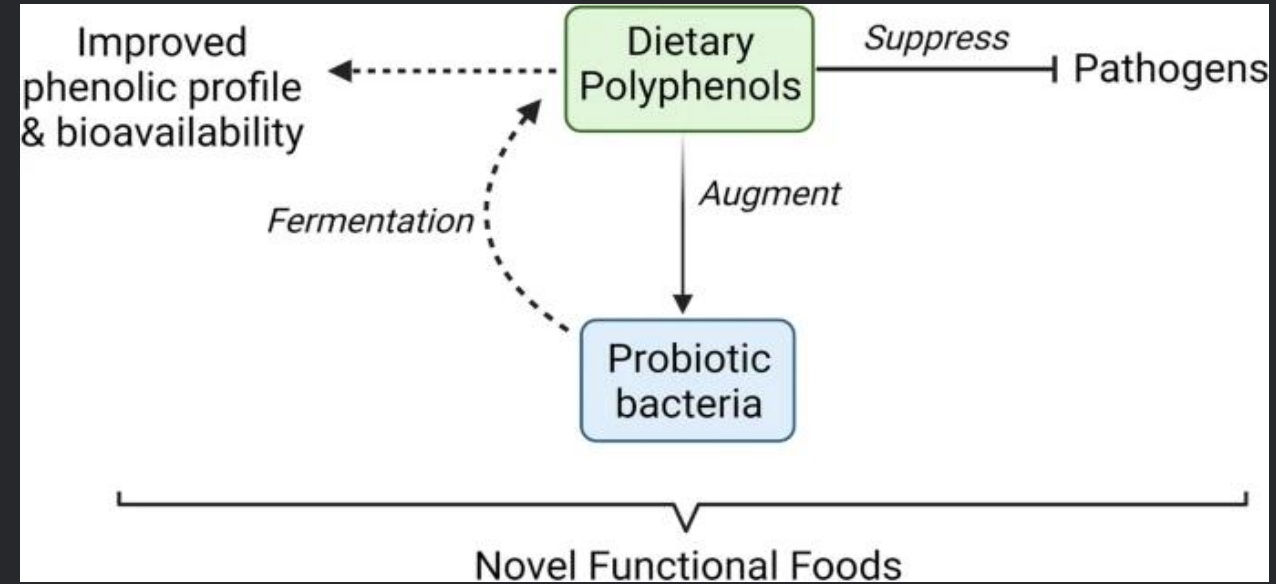
APLIKASI FERMENTASI

Probiotic microorganisms



Probiotic mediated fermentation

- Production of
 - Exopolysaccharides
 - Short chain fatty acids
 - Short digestible carbohydrates
- Synthesis of bioactive peptides
- Amino acid metabolism and absorption
- Improved protein digestion
- Improved fatty acid profile
- Enhanced short chain fatty acids profile
- Improved food flavour



PROBIOTIC FERMENTATION

S. no	Phenolic food source	Probiotic microorganisms used	Effect of fermentation	Reference
1	Tea extracts	Lactic acid bacteria	Production of novel compounds (quercetin and pyrogallol); increased cellular bioavailability (over 90%) for EGCG	Zhao & Shah 2016a
2	Kiwifruit juice	Lactic acid bacteria	Production of novel compounds- Protocatechuic acid and catechin; increased phenolic content and antioxidant activity	Wang et al. 2022
3	Apple juice	Six lactic acid bacterial strains	Enhanced the levels of caffeic acid and phlorizin; improved aroma; increased antioxidant activity (90%)	Wu et al. 2020
4	Soymilk	<i>Lactobacillus fermentum</i>	Increased free soy isoflavones content (up to 6 folds)	Zhou et al. 2021
5	<i>Capsicum annuum</i>	<i>Lactobacillus plantarum</i> , <i>Lactobacillus acidophilus</i> , and <i>Bacillus subtilis</i>	Production of novel metabolites- nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin	Liu et al. 2019
6	Vegetable juice from four crops	Lactic acid bacteria	Increased phenolic content (up to 24%); improved antioxidant activity	Lee et al. 2021

PROBIOTIC FERMENTATION

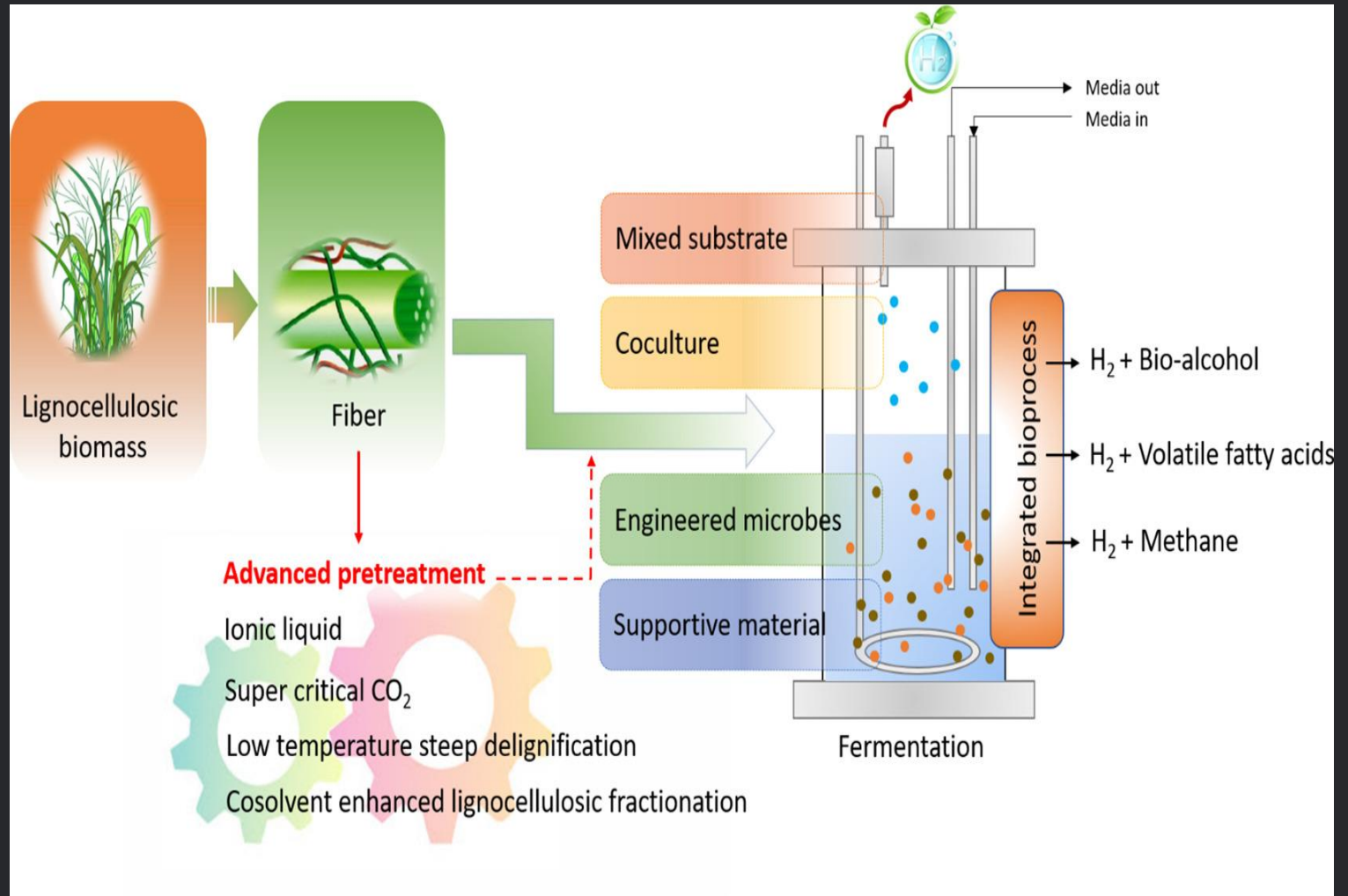
Fermented Food vs. Probiotic

Probiotic	Fermented Food	Probiotic Fermented Food
<ul style="list-style-type: none">▪ Must contain live microbes▪ Must be tested and shown to have health benefit▪ Must deliver level of live microbes shown to confer benefit	<ul style="list-style-type: none">▪ Made by live microbes▪ Live microbes might not survive into the food you consume (post-fermentation processing)▪ May not have been tested for health benefits beyond basic nutritional value▪ Likely healthy dietary components, but may not meet the bar to be called a probiotic	<ul style="list-style-type: none">▪ Must contain live microbes▪ Must be tested and shown to have health benefit▪ The health benefit must result, at least in part, from the live microbes present▪ The health benefit must go beyond meeting basic micro- and macro-nutrient nutritional needs▪ Must deliver level of live microbes shown to confer benefit

APLIKASI TEKNIK FERMENTASI

Other Applications

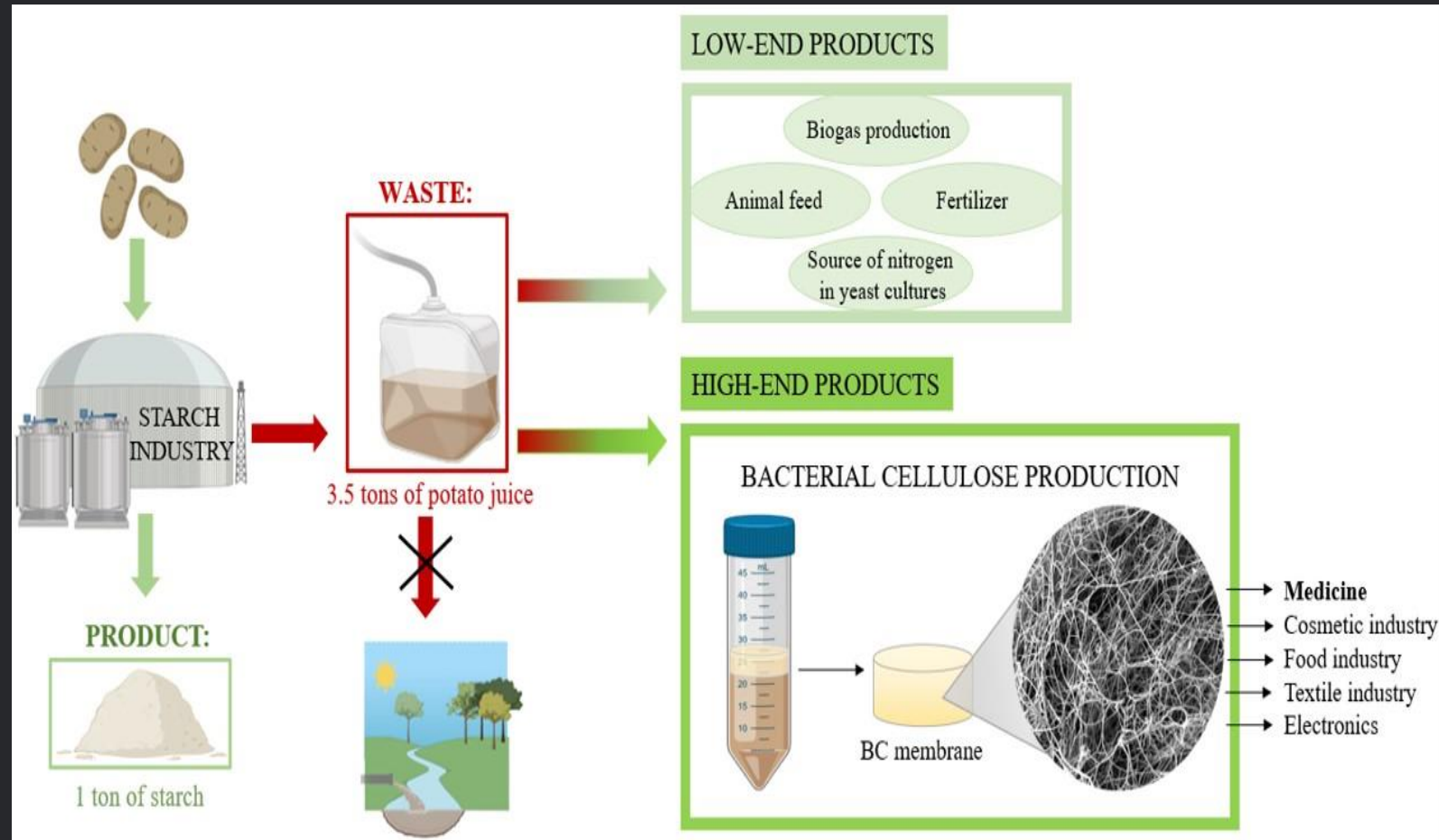
- It is also used for waste management such as biofuels production (biodiesels, bioethanol, butanol, biohydrogen, etc).
- It is also used to produce bio-surfactant, polymers production such as bacterial cellulose production.
- Development of bioremediation processes (involving microbes or their isolated enzymes) for soils and wastewater treatments.



APLIKASI TEKNIK FERMENTASI

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APLIKASI TEKNIK FERMENTASI

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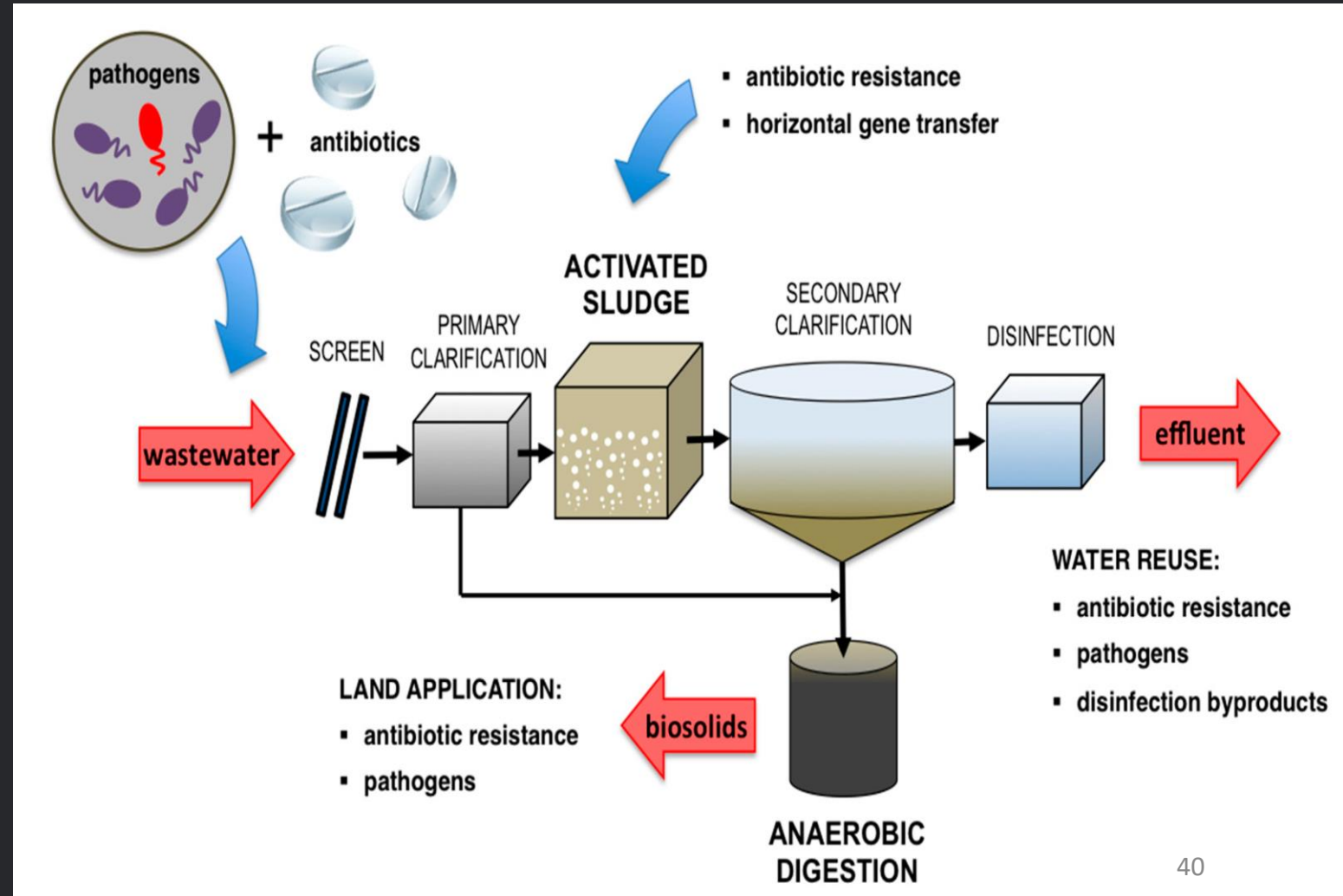
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HOW TO MAKE KOMBUCHA



7 cups water
½ cup sugar
4 bags tea

+



1 cup kombucha



1 to 4 weeks



SCOBY!

SCOBY

MAKE SCOBY



14 cups water
1 cup sugar
8 bags tea
1 SCOBY
2 cups kombucha



6 to 10 days



KOMBUCHA!

SCOBY KILLERS

decaf tea
flavored tea
honey
metal
refrigeration
antibacterial soap

2 cups

1st FERMENTATION



14 cups kombucha
favorite flavors



3 to 10 days



CARBONATION!

FAVORITE FLAVORS

ginger + lemon
mint + lime
pineapple + basil
mango + pepper
vanilla + orange
apple + cinnamon

the rest

2nd FERMENTATION

BREW BUCH



- Kombucha is a fermented beverage made from brewed tea and sugar.
- The kombucha process resembles Vinegar fermentation. Like vinegar, kombucha is a yeast fermentation of sugar to alcohol followed by a bacterial fermentation of alcohol to acetic acid.
- Ferment for 7 to 10 days: Keep the jar at room temperature, out of direct sunlight, and where it won't get jostled.
- Ferment for 7 to 10 days, checking the kombucha and the scoby periodically. It's not unusual for the scoby to float at the top, bottom, or even sideways during fermentation

Wheat fibre (WF)
or inulin (INU)
% 0 or 5 (w/w)



Pasteurization
at 75°C for 30 min

Yoghurt bacteria or
L. rhamnosus



Milk with or without
dietary fibre

Incubation at 37 °C
or 42 °C until pH 4.7



Fermented
milk

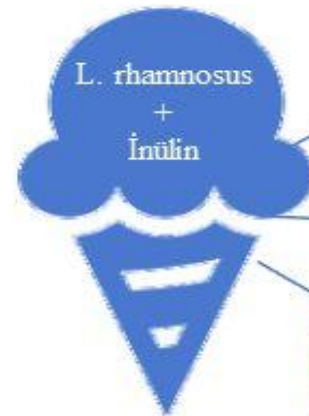
Preparation of final ice cream mix
(100%) by blending 30% fermented
milk and
70% ice cream mix

30%



70%

Preparation of
the 70% part
of the ice
cream mix



Best score of
general
acceptability

7.30 log cfu/g
on day 90

93.72%
viability on
day 90



Packaging and
storage at -25
°C for 90 days.

Referensi

1. Feng R, Chen L, Chen K. Fermentation trip: amazing microbes, amazing metabolisms. *Ann Microbiol.* 2018 Nov;68(11):717–29.
2. Bhatia SK, Jagtap SS, Bedekar AA, Bhatia RK, Rajendran K, Pugazhendhi A, et al. Renewable biohydrogen production from lignocellulosic biomass using fermentation and integration of systems with other energy generation technologies. *Science of The Total Environment.* 2021 Apr 15;765:144429.
3. Akpabli-Tsigbe NDK, Ma Y, Ekumah JN, Osabutey J, Hu J, Xu M, et al. Novel solid-state fermentation extraction of 5-O-caffeoylquinic acid from heilong48 soybean using *Lactobacillus helveticus*: Parametric screening and optimization. *LWT.* 2021 Sep 1;149:111809.
4. Sharma, R., Diwan, B., Singh, B.P. *et al.* Probiotic fermentation of polyphenols: potential sources of novel functional foods. *Food Prod Process and Nutr* **4**, 21 (2022). <https://doi.org/10.1186/s43014-022-00101-4>
5. Sezer, E.; Ayar, A.; Yilmaz, S.Ö. Fermentation of Dietary Fibre-Added Milk with Yoghurt Bacteria and *L. rhamnosus* and Use in Ice Cream Production. *Fermentation* **2023**, *9*, 3. <https://doi.org/10.3390/fermentation9010003>
6. Skowron K, Budzyńska A, Grudlewska-Buda K, Wiktorczyk-Kapischke N, Andrzejewska M, Wałęcka-Zacharska E and Gospodarek-Komkowska E (2022) Two Faces of Fermented Foods—The Benefits and Threats of Its Consumption. *Front. Microbiol.* 13:845166. <https://doi.org/10.3389/fmicb.2022.845166>
7. [Jurnal Fermentation : https://www.mdpi.com/journal/fermentation](https://www.mdpi.com/journal/fermentation)

Video :

<https://youtu.be/5eKdZ0dVCCo?t=299>

<https://youtu.be/bWxPpK7t5IE>

GLOSSARY

- **Microorganisms:** Organisms that cannot be seen with the naked eye, requiring a microscope.
- **Biotechnology:** The science of creating new or modified products using living organisms.
- **Anaerobic Pathway:** Biochemical reactions that do not require the presence of oxygen.
- **Lactic Acid Fermentation:** Biological process where sugar is converted into lactate and cellular energy (ATP).
- **Alcoholic Fermentation:** Biological process where sugar is converted to ethanol and cellular energy (ATP).
- **ATP:** Main energy coin used by living beings.
- **Ethanol:** It is the most common alcohol present in our lives.
- **Probiotic:** A usually dairy food or a dietary supplement containing microorganisms that replace or add to the beneficial bacteria normally present in the gastrointestinal tract.
- **Pathogen:** Capable of causing disease.
- **Biofuel:** A type of fuel made using living or once living material, such as plant material, which can serve as a replacement for petroleum-based fuels like gasoline.



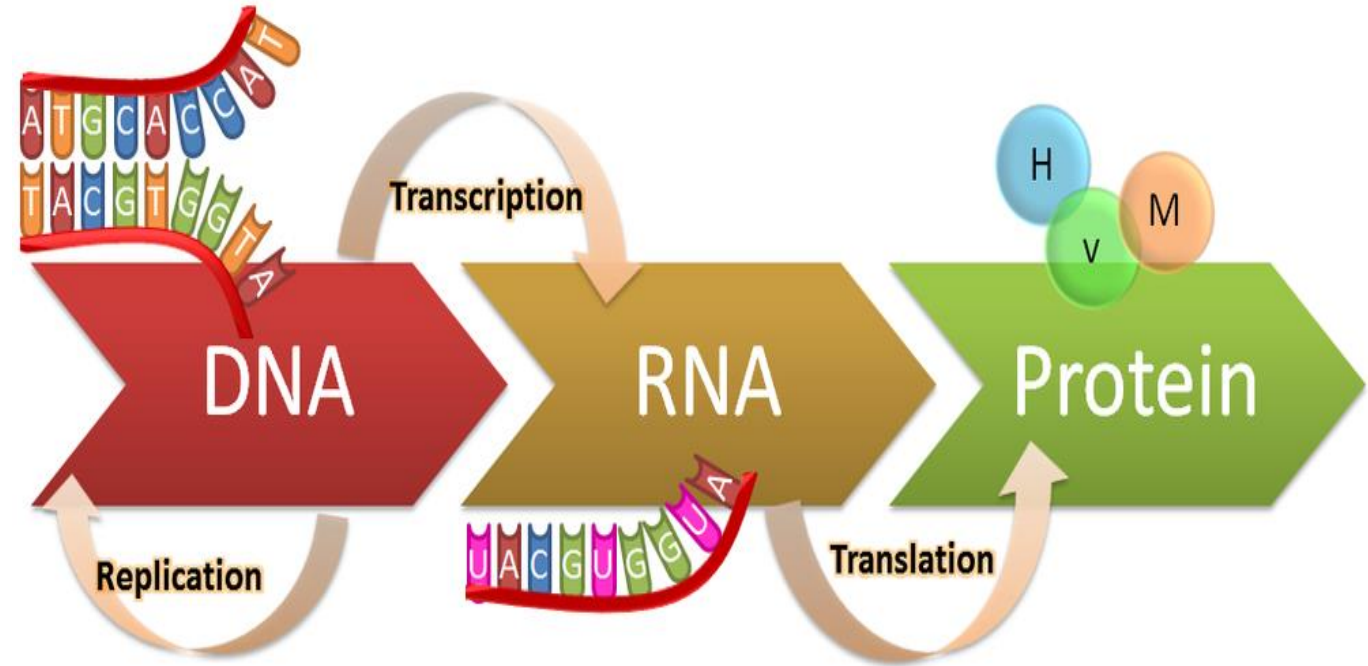
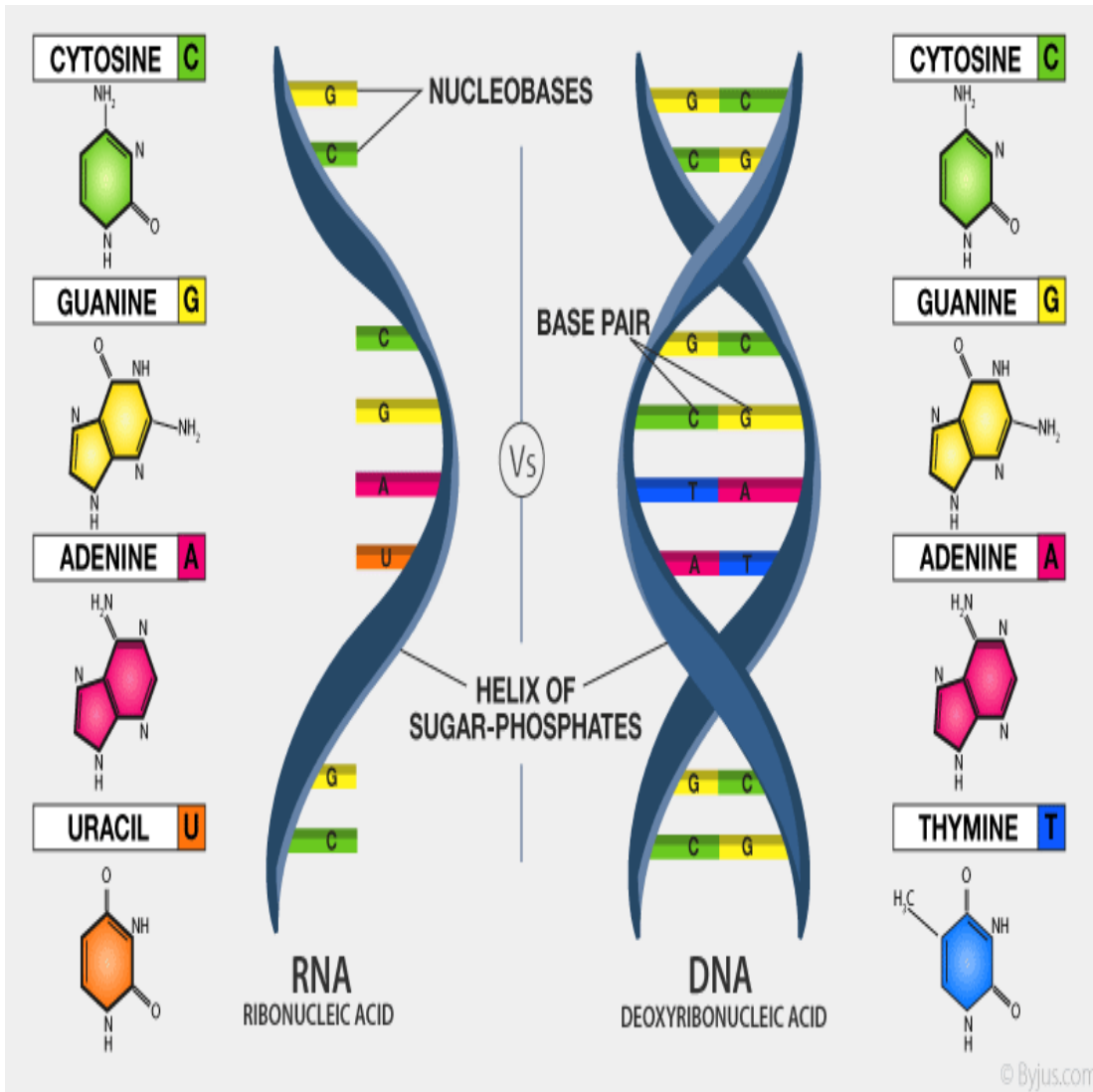
Thank you

See you next week



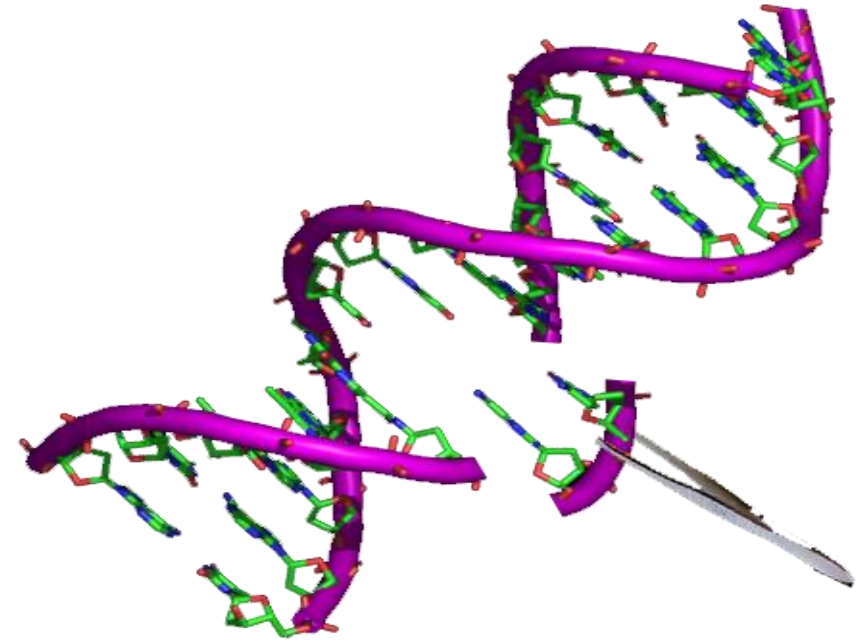
Rekayasa Genetika

- 1 Pendahuluan
- 2 Klasifikasi
- 3 Proses dan Teknik
- 4 Aplikasi



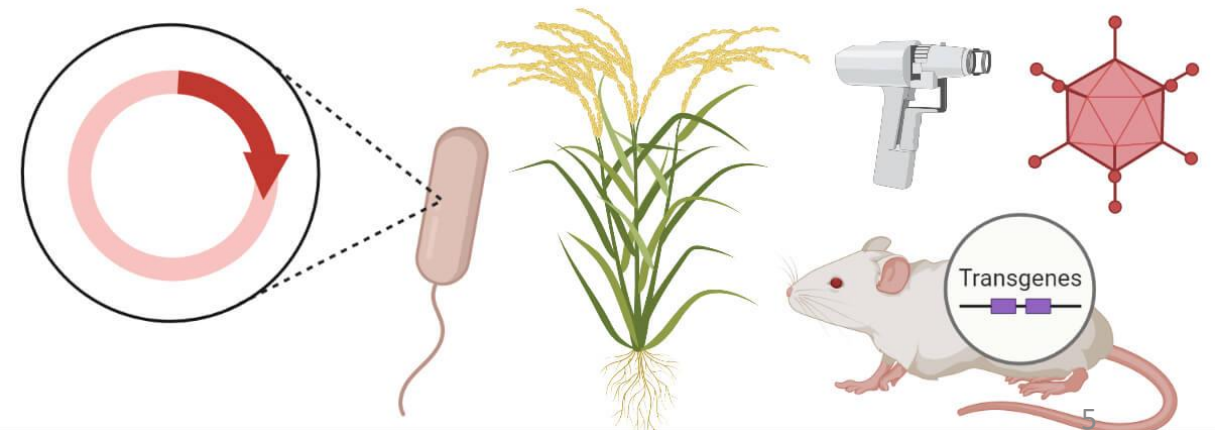
Genetic engineering (also called genetic modification) is a process that uses laboratory-based technologies to alter the DNA makeup of an organism. This may involve changing a single base pair (A-T or C-G), deleting a region of DNA or adding a new segment of DNA.

Organisms created by genetic engineering are called genetically modified organisms (GMOs).

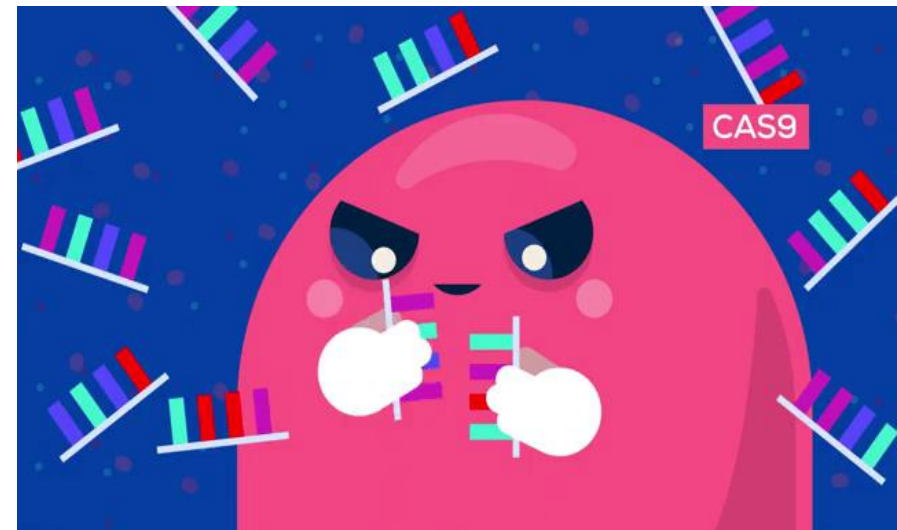
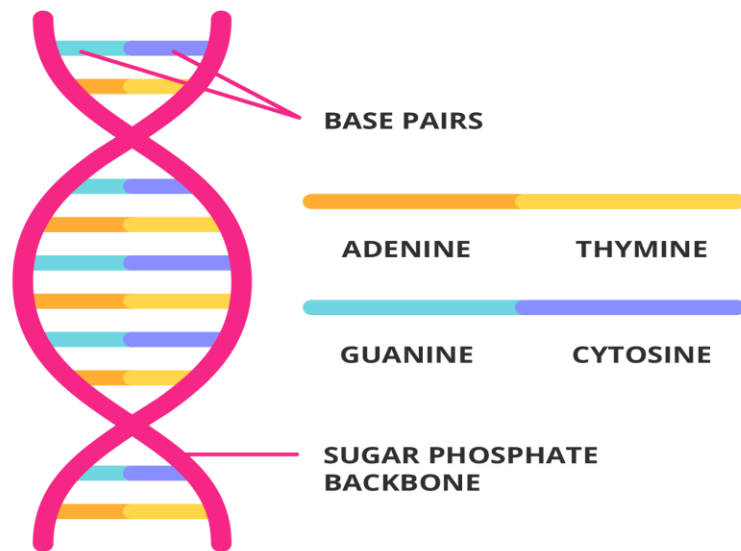


Genetically Modified Organism (GMO)

Bacteria, Plants, Animals, Viruses

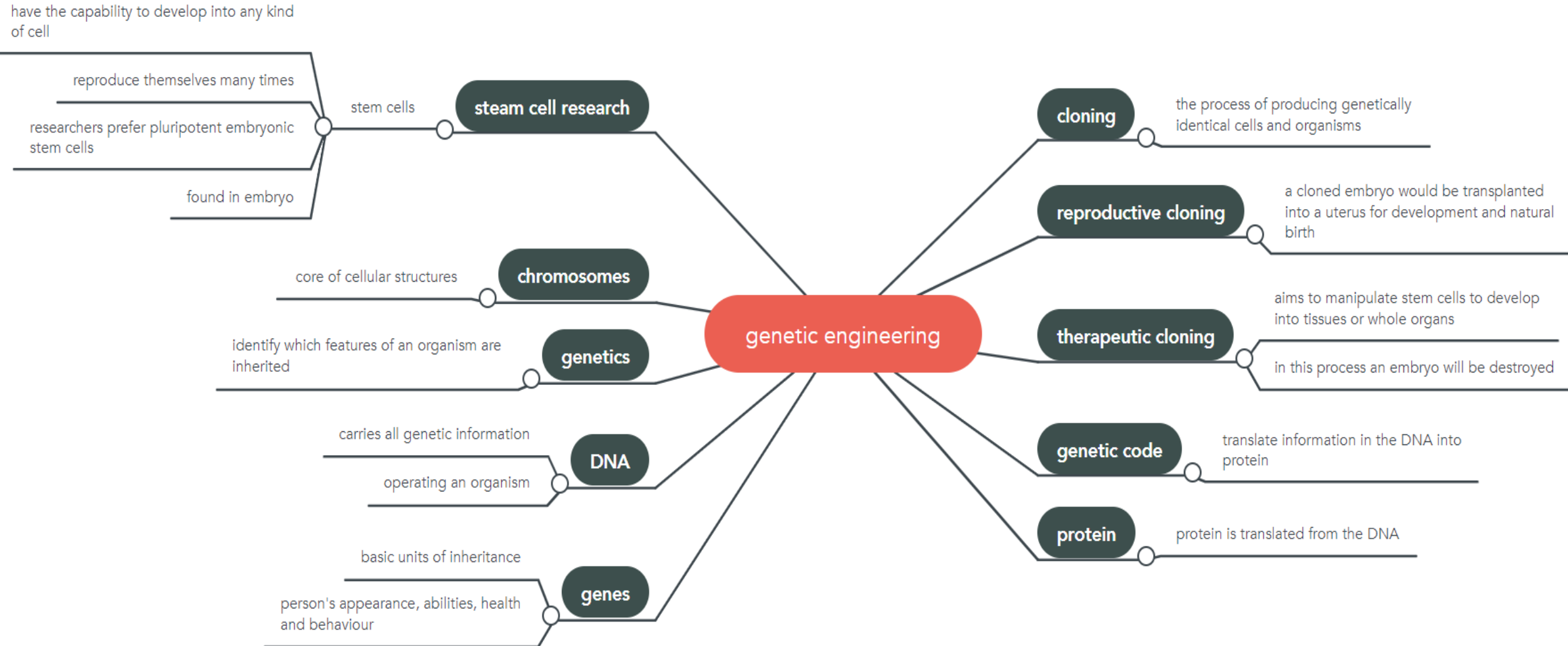


For example, genetic engineering may involve adding a gene from one species to an organism from a different species to produce a desired trait. Used in research and industry, genetic engineering has been applied to the production of cancer therapies, brewing yeasts, genetically modified plants and livestock, and more.



Source: (Mar, 2019). 'What is DNA?'.
Retrieved from U.S. National Library of Medicine.

REKAYASA GENETIKA



Recombinant DNA

- A recombinant DNA technology is a type of genetic engineering technology in which an artificial DNA molecule is constructed by ligating two different DNAs using physical methods. For that, the gene of interest is inserted into the plasmid vector and used for gene transfer experiments.

Gene delivering

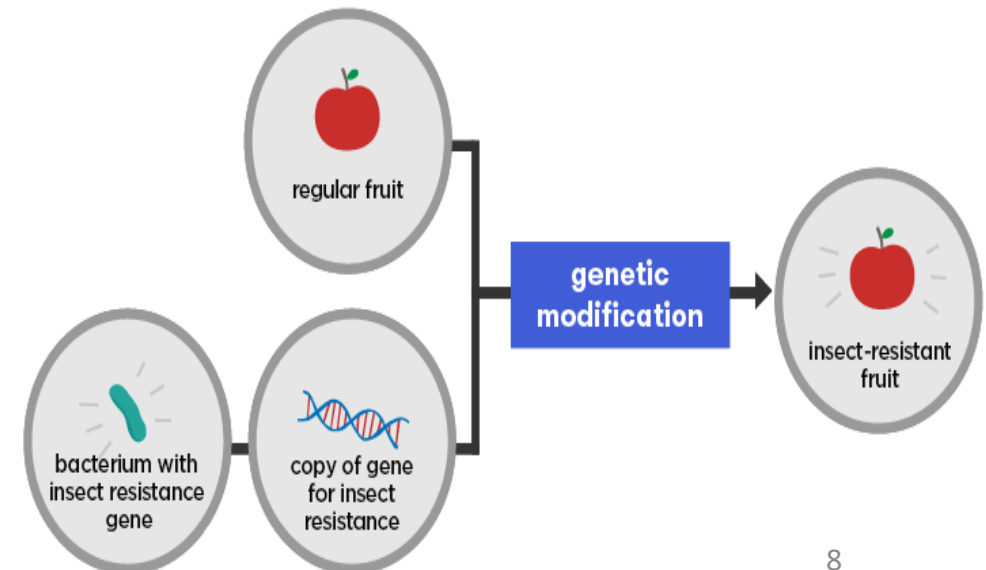
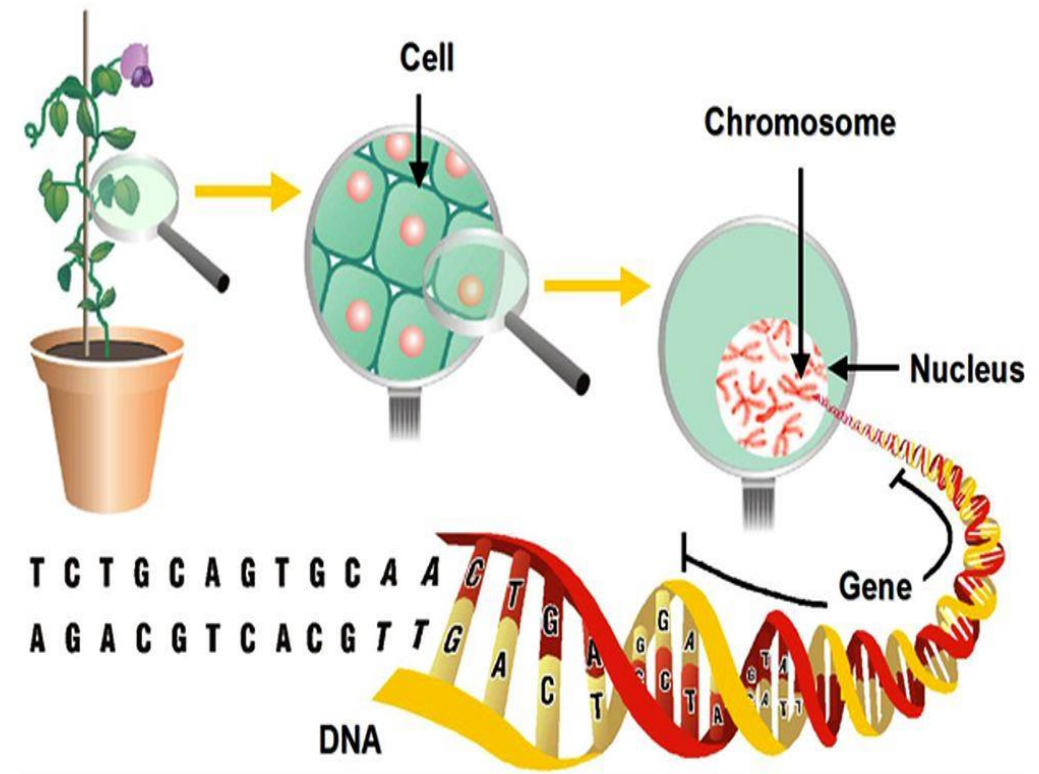
- Gene delivering technique is employed for the insertion of a gene of interest into the host genome.

Electrophoration, solicitation and viral vector

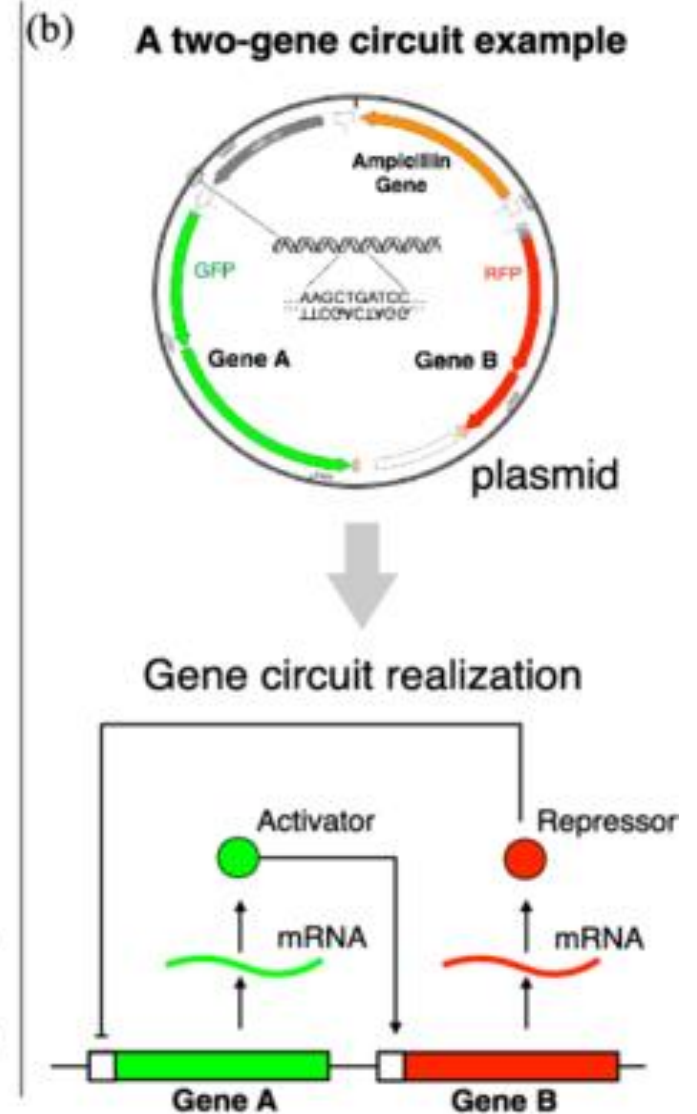
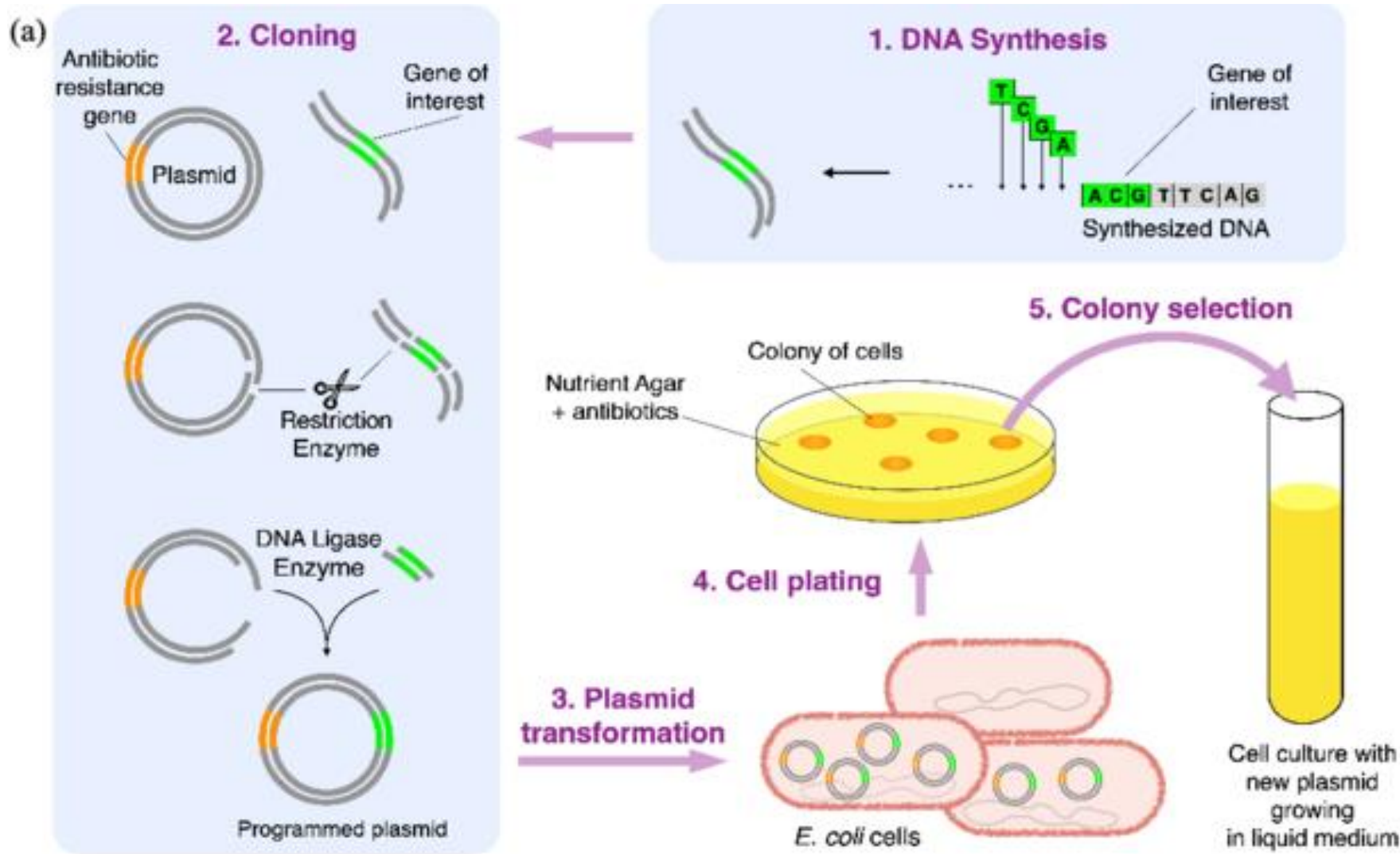
- mediated gene transfer, liposome-mediated gene transfer, transposon-mediated gene transfer are some of the methods used for that

Gene editing

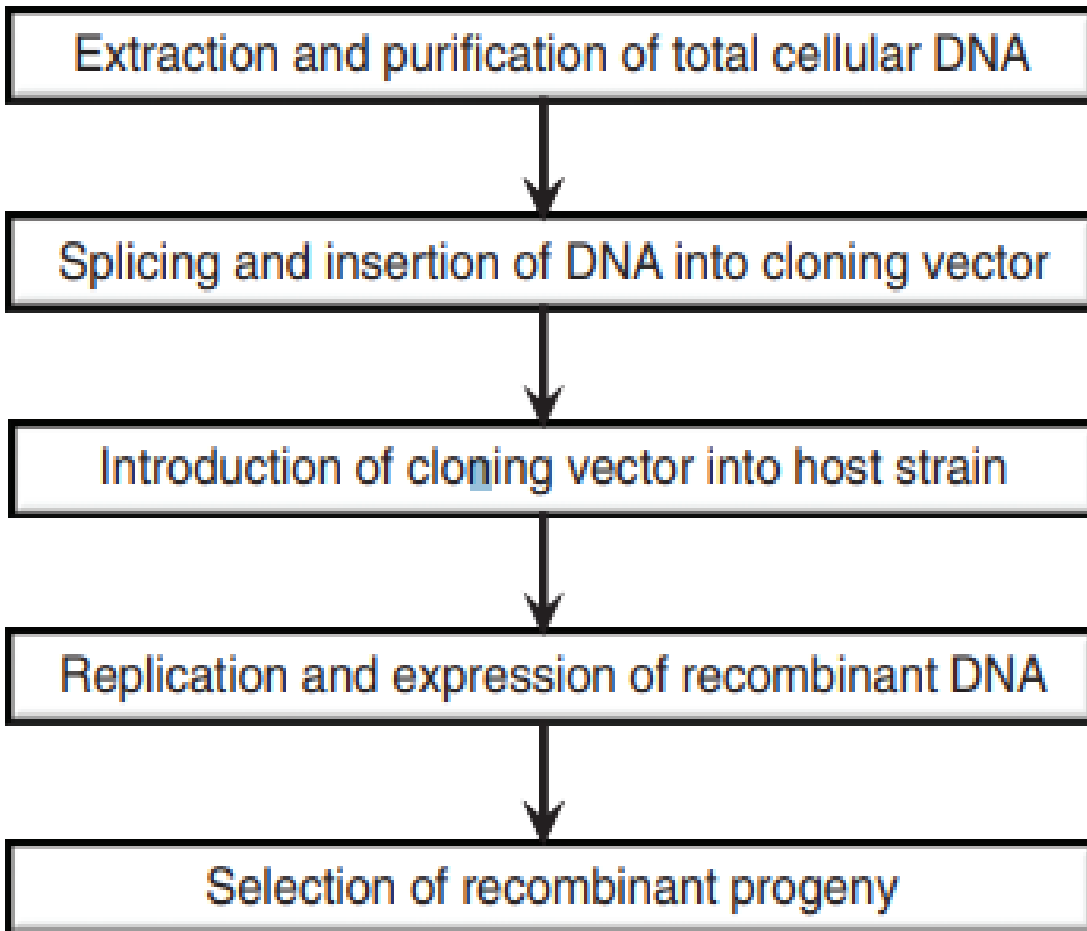
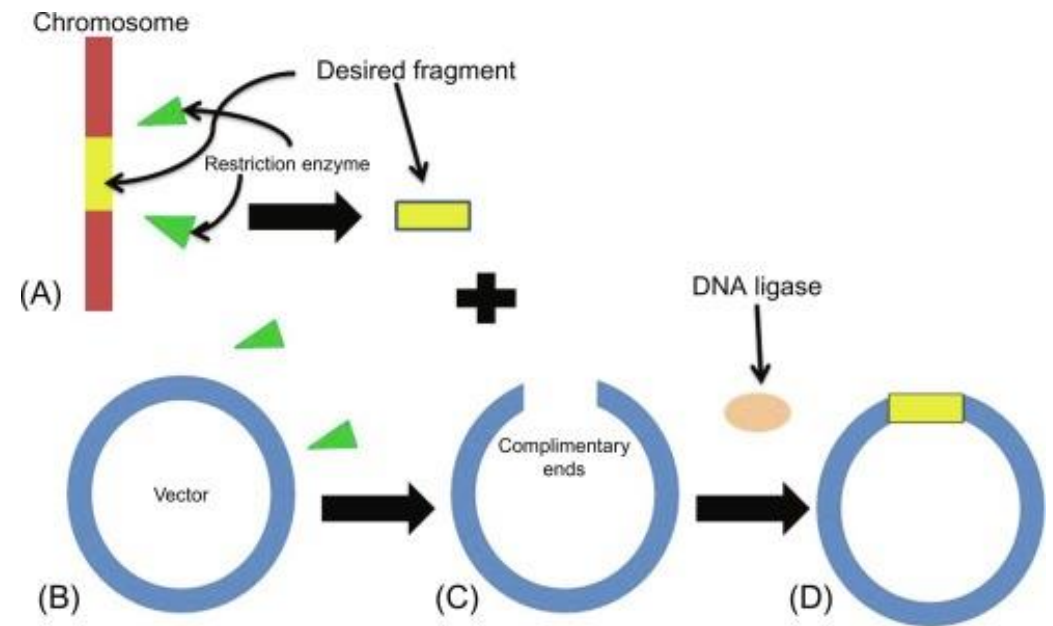
- A gene-editing technique is used to edit the genome in which an undesired DNA sequence is removed or a new gene can be inserted into the host genome. CRISPR-CAS9, TALEN and ZFN are some known gene-editing tools used in gene therapy experiments



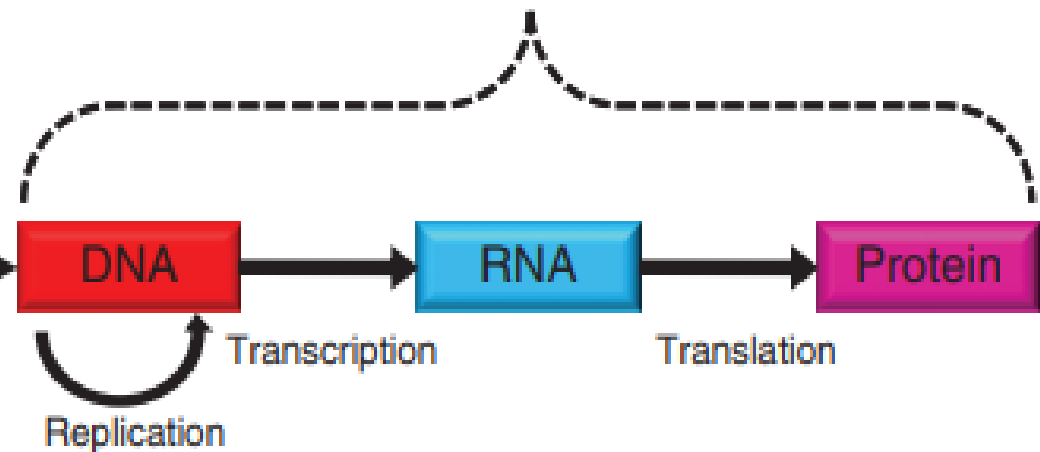
REKAYASA GENETIKA



REKAYASA GENETIKA

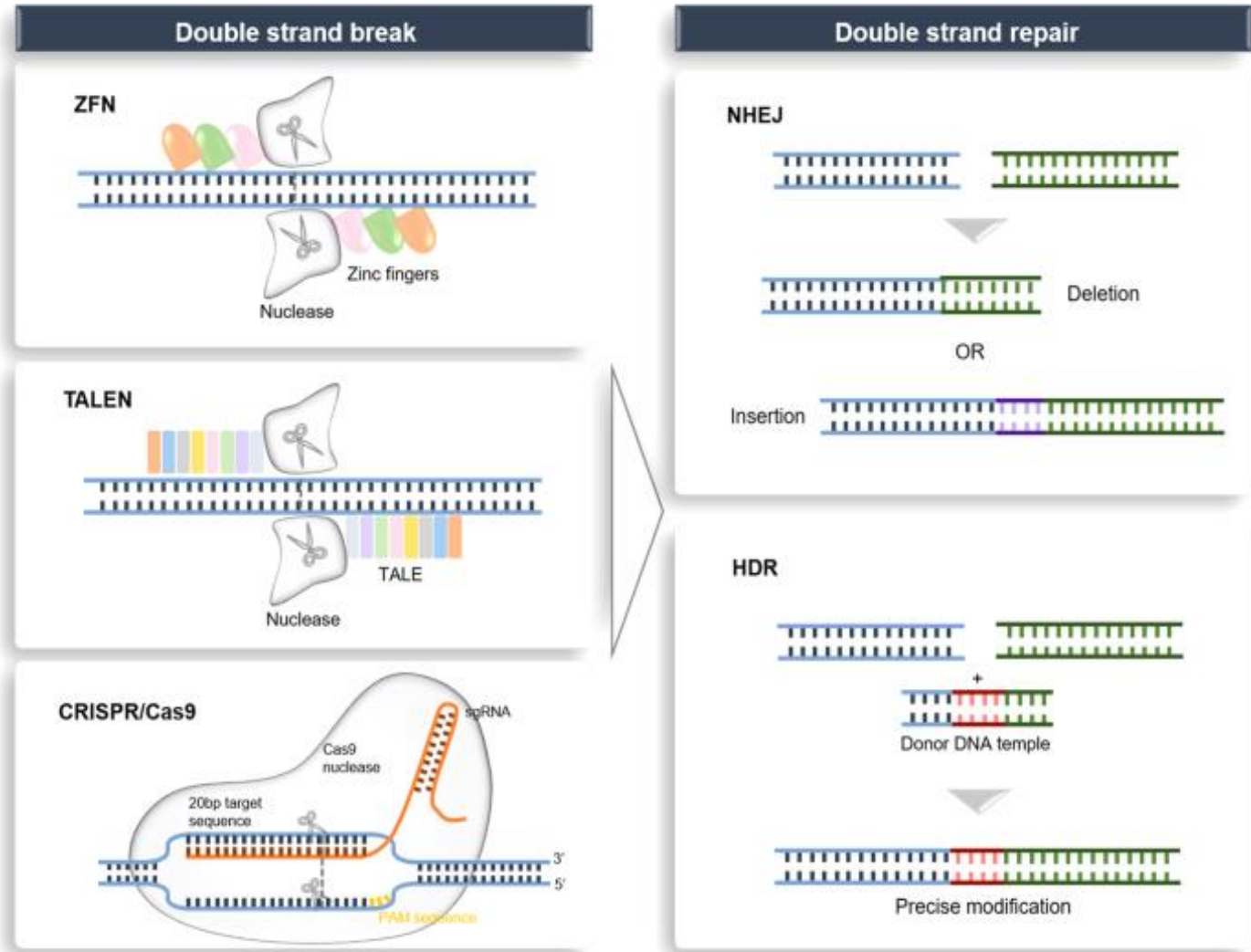
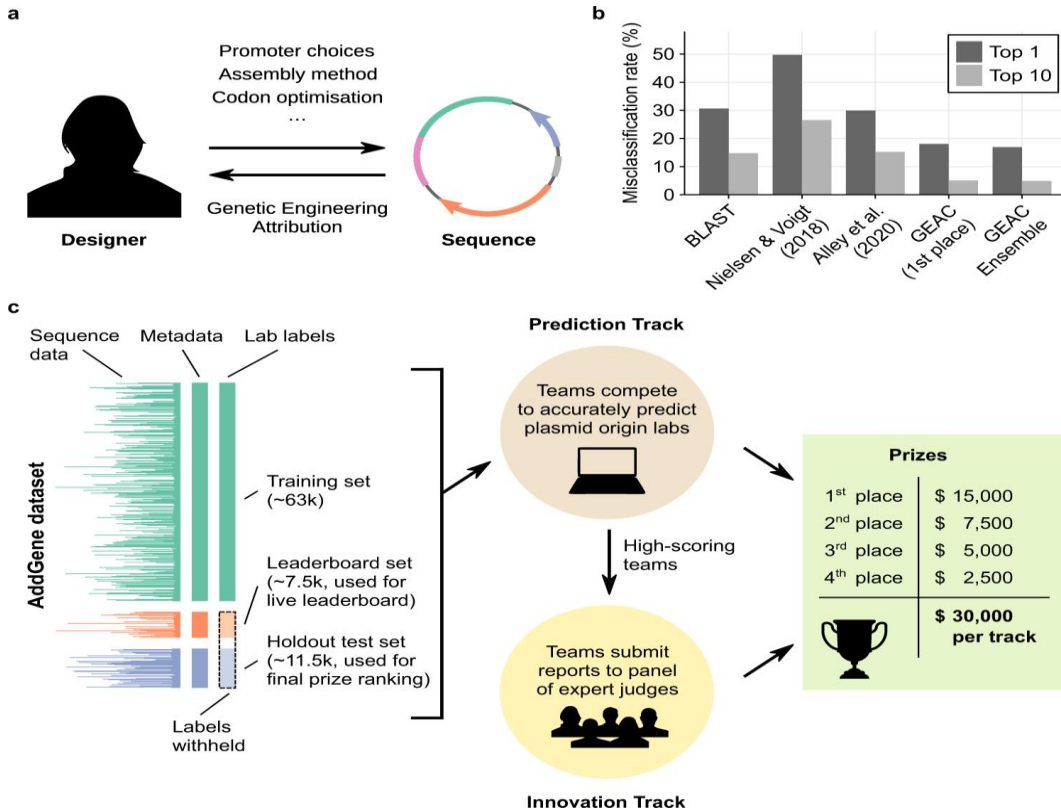


Central dogma of molecular biology



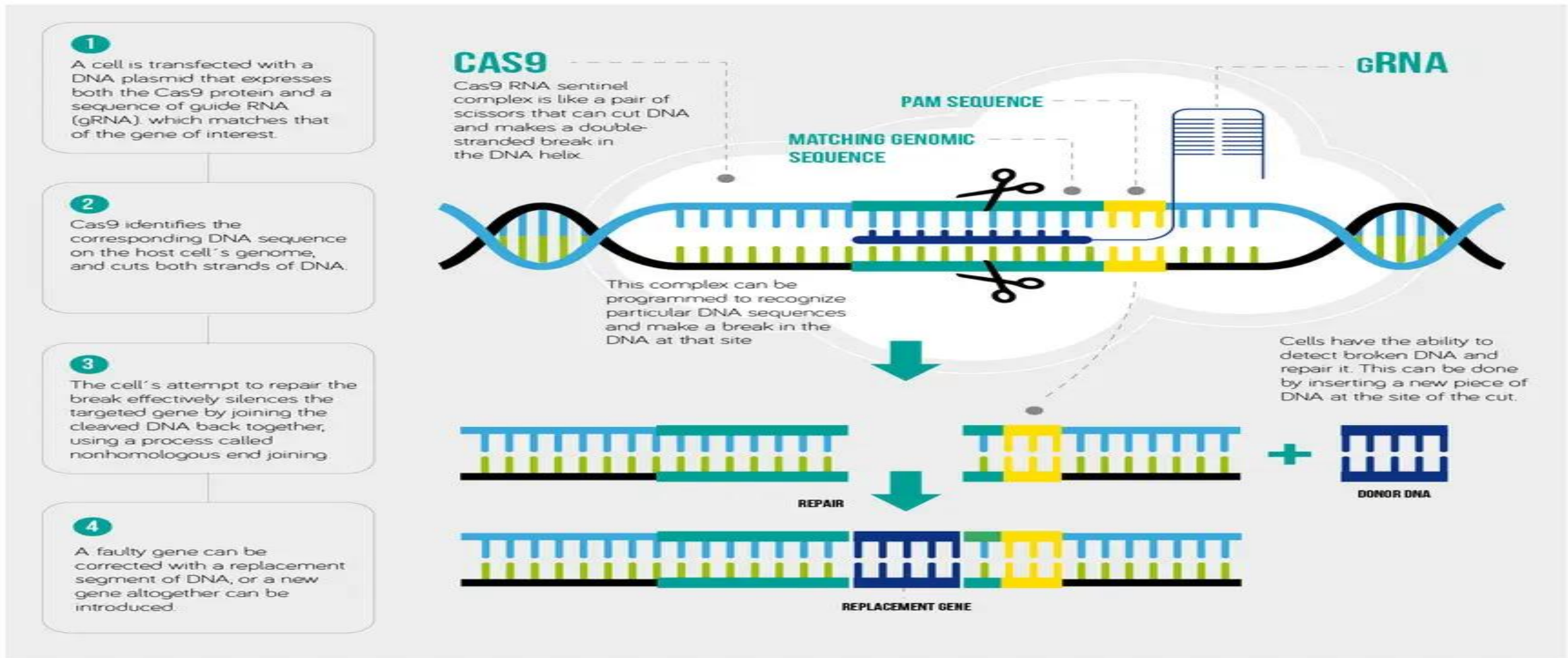
GENOME EDITING

- In the 1970s, the development of genetic engineering (manipulation of DNA or RNA) established a novel frontier in genome editing

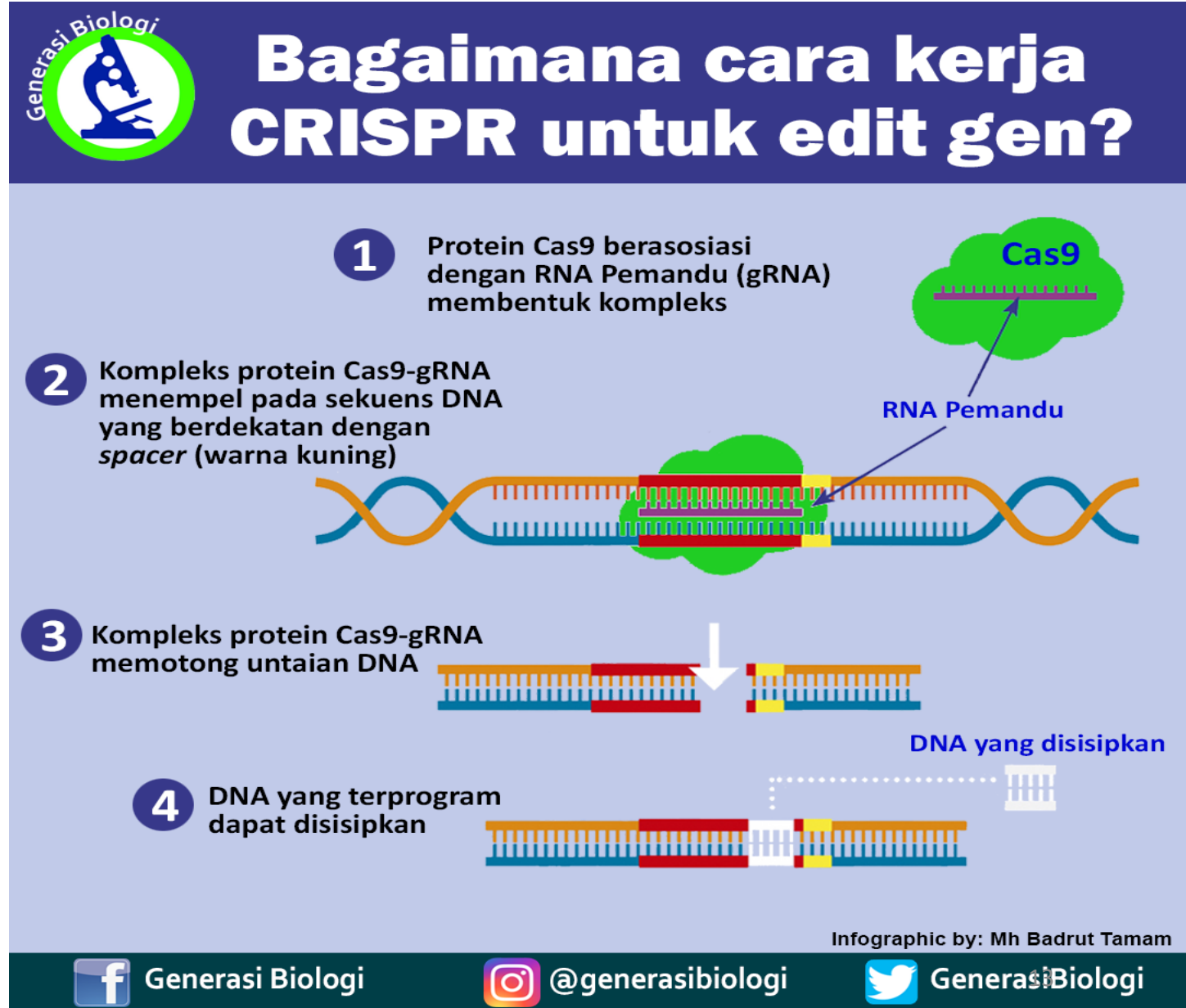


*ZFN Zinc-finger nuclease, TALEN Transcription activator-like effector nuclease, CRISPR Clustered regularly interspaced short palindromic repeat

CRISPR-Cas9

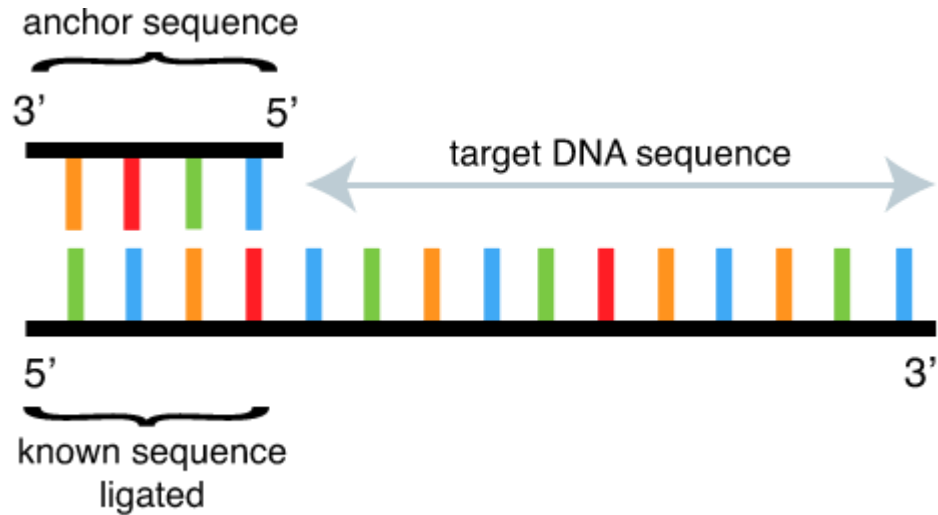


- **CRISPR** : Clustered Regularly Interspaced Short Palindromic Repeats
- **Cas9** : CRISPR associated protein 9
- CRISPR/Cas9 edits genes by precisely cutting DNA and then letting natural DNA repair processes to take over.
- The system consists of two parts: the Cas9 enzyme and a guide RNA
- A short DNA sequence, **the protospacer-adjacent motif (PAM)**, is frequently used to mark proper target sites. Cas proteins have evolved a multitude of PAM-interacting domains, which enables them to cope with viral anti-CRISPR measures that alter the sequence or accessibility of PAM elements.



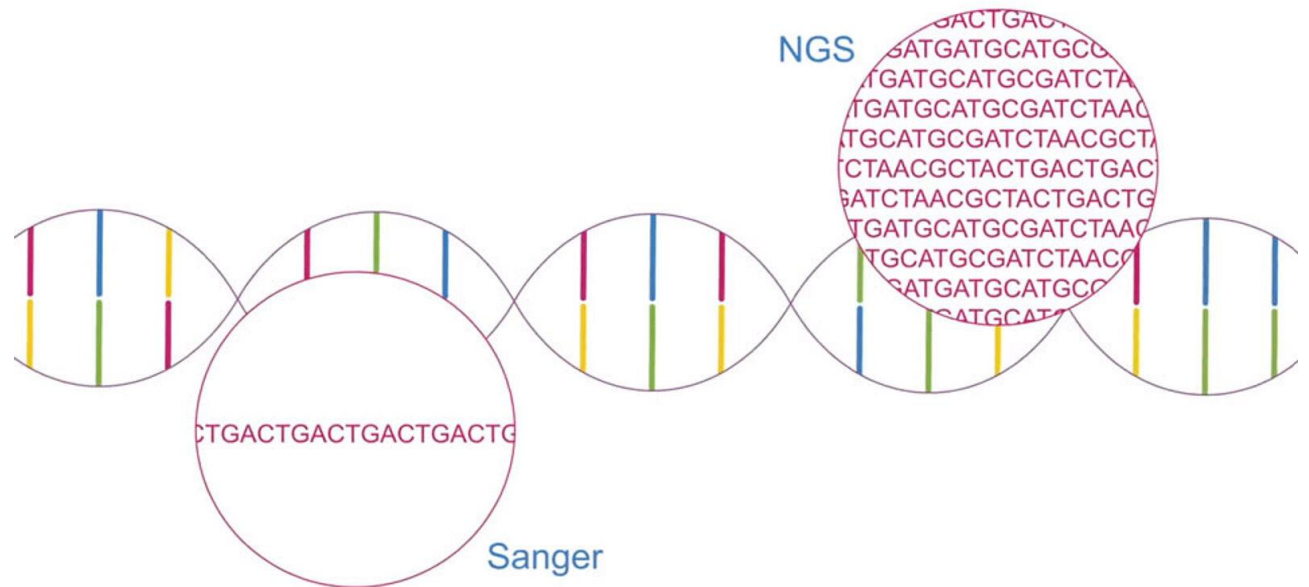
Approach	Characteristic	Advantages	Limitations
HR	Point mutation, insertion, deletion; Dividing cells	High specificity	Extremely low efficiency
ZFN-HDR	Point mutation, insertion, deletion; Dividing cells	High specificity	DSB dependent; Labor intensive cloning; Low efficiency
TALEN-HDR	Point mutation, insertion, deletion; Dividing cells	High specificity	DSB dependent; Labor intensive cloning; Low efficiency
Cas9-HDR	Point mutation, insertion, deletion; Dividing cells	Easy to engineer	DSB dependent; PAM site necessary; Off-target effects; Low efficiency
Cre-loxP	Excision, Inversion, translocation; Dividing and non-dividing cells	High specificity; High efficiency	Not useful for insertion or correction; Need prior insertion of loxP sites
HITI	Insertion; Dividing and non-dividing cells	Easy to engineer	DSB dependent; PAM site necessary; Off-target effects; Low efficiency
BE	Point mutation; Dividing and non-dividing cells	High efficiency; non-dividing cells	PAM site necessary; Off-target effects; Only conversion of C•G to T•A, A•T to G•C, or C•G-to-G•C
PE	Point mutation, small insertion, and deletion; Dividing and non-dividing cells	Non-dividing cells	PAM site necessary; off-target effects; low efficiency; limited to small edits.
CAST	Large DNA insertion	Large DNA insertions	Low efficiency

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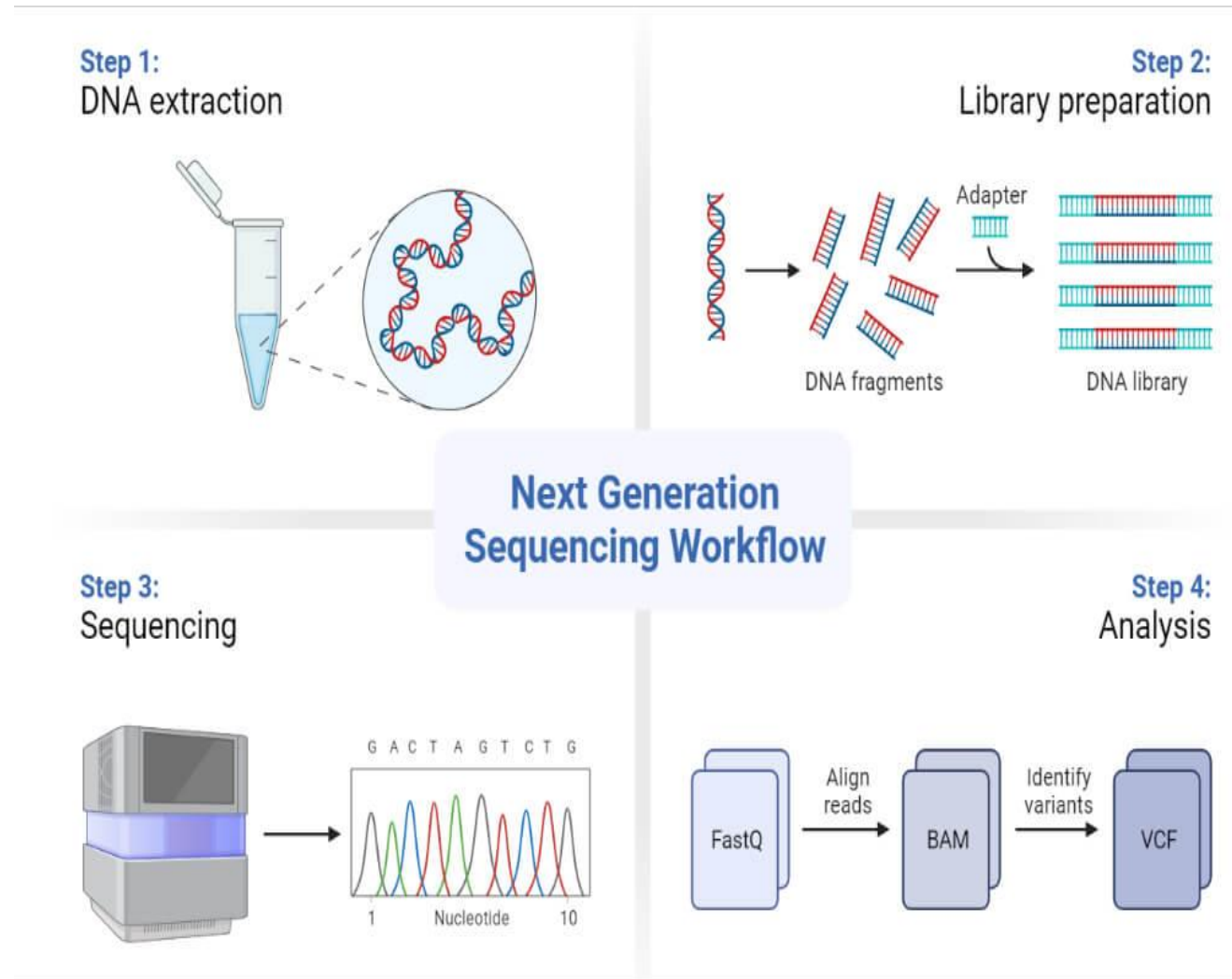
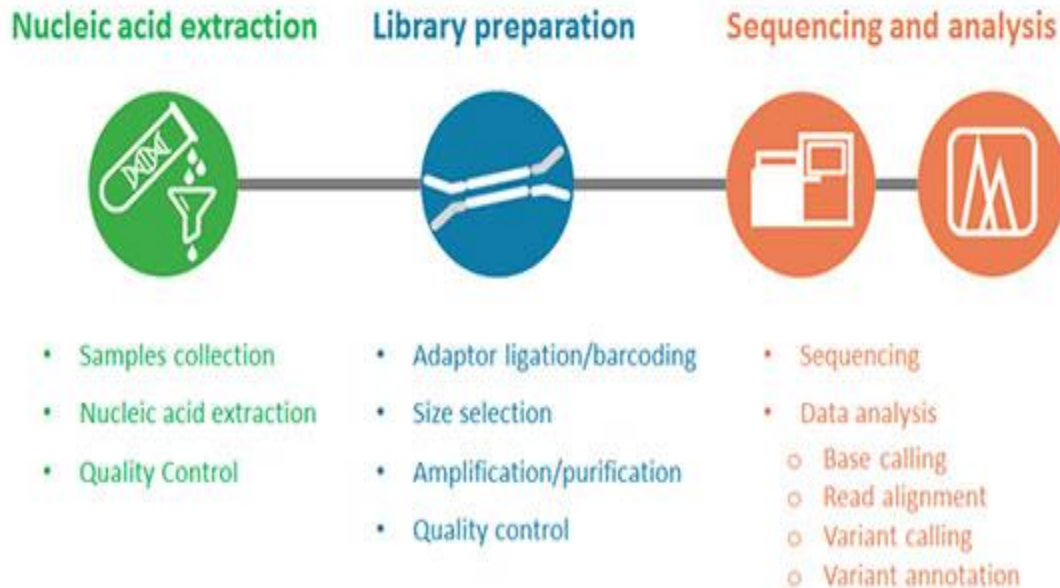
The gene must contain a sequence of DNA that we want to study and for that, a gene has some special characteristics. A candidate gene should have high GC content and a lower repetitive DNA sequence

DNA sequencing refers to the general laboratory technique for determining the exact sequence of nucleotides, or bases, in a DNA molecule. The sequence of the bases (often referred to by the first letters of their chemical names: A, T, C, and G) encodes the biological information that cells use to develop and operate

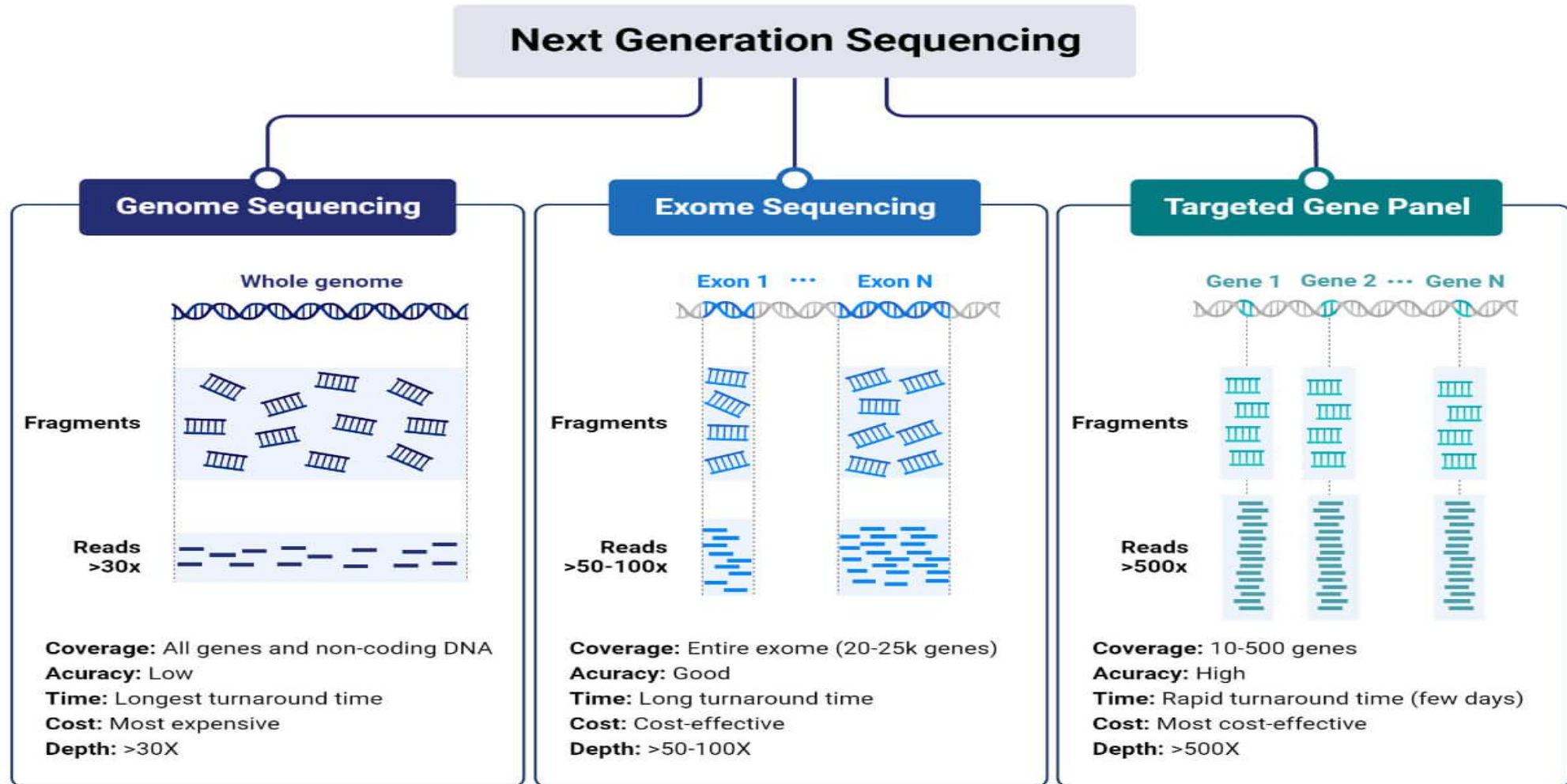


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- **Next-generation sequencing (NGS)** is a massively parallel sequencing technology that offers ultrahigh throughput, scalability, and speed.
- The technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA



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Template adapted from: Dr. Roshini Abraham
Clinical Immunologist at Nationwide Children's Hospital



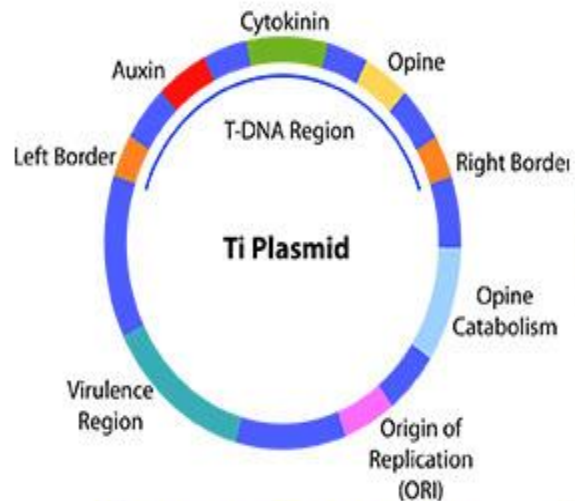
The
Biology
Notes

The
Chemistry
Notes

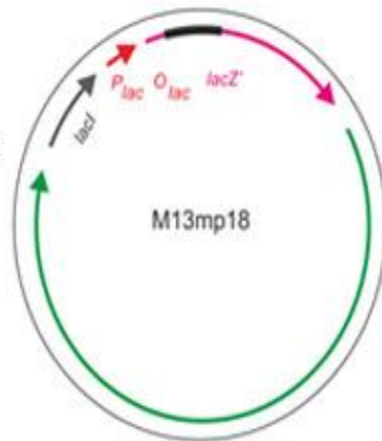
Created with
bio
RENDER
Designed By Sagar Aryal

Vectors act as vehicles to transfer genetic material from one cell to the other for different purposes like multiplying, expressing, or isolation

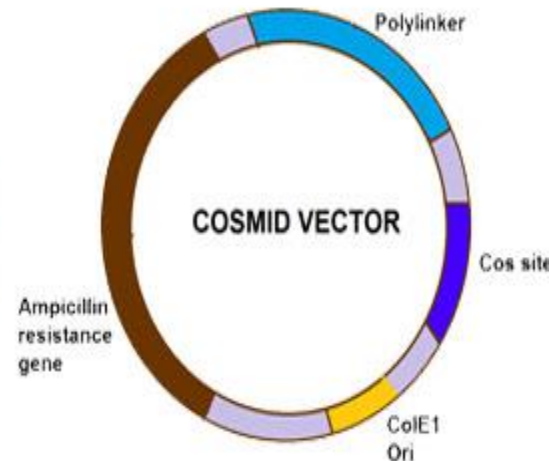
Types of Vectors



i) Plasmid Vector



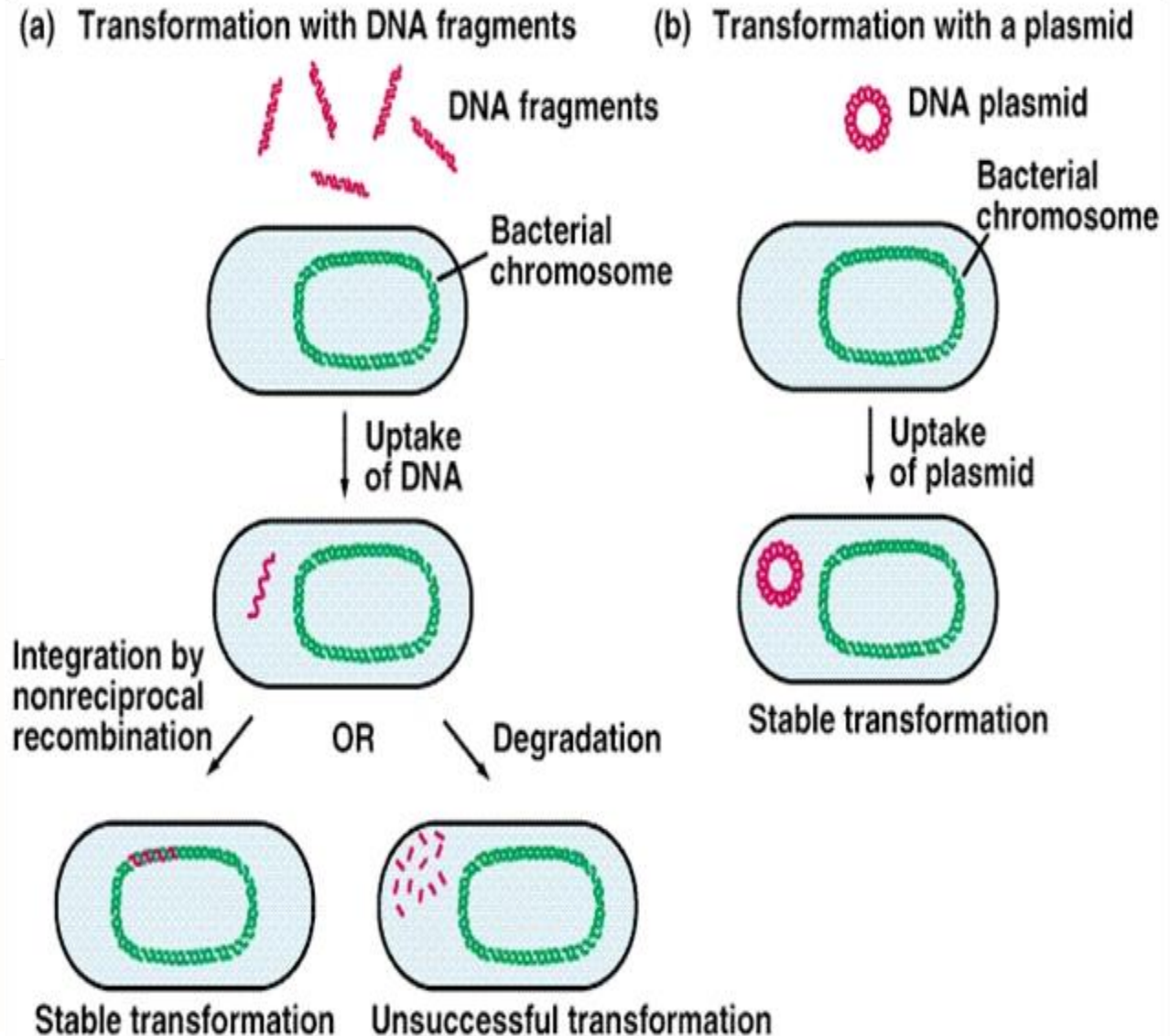
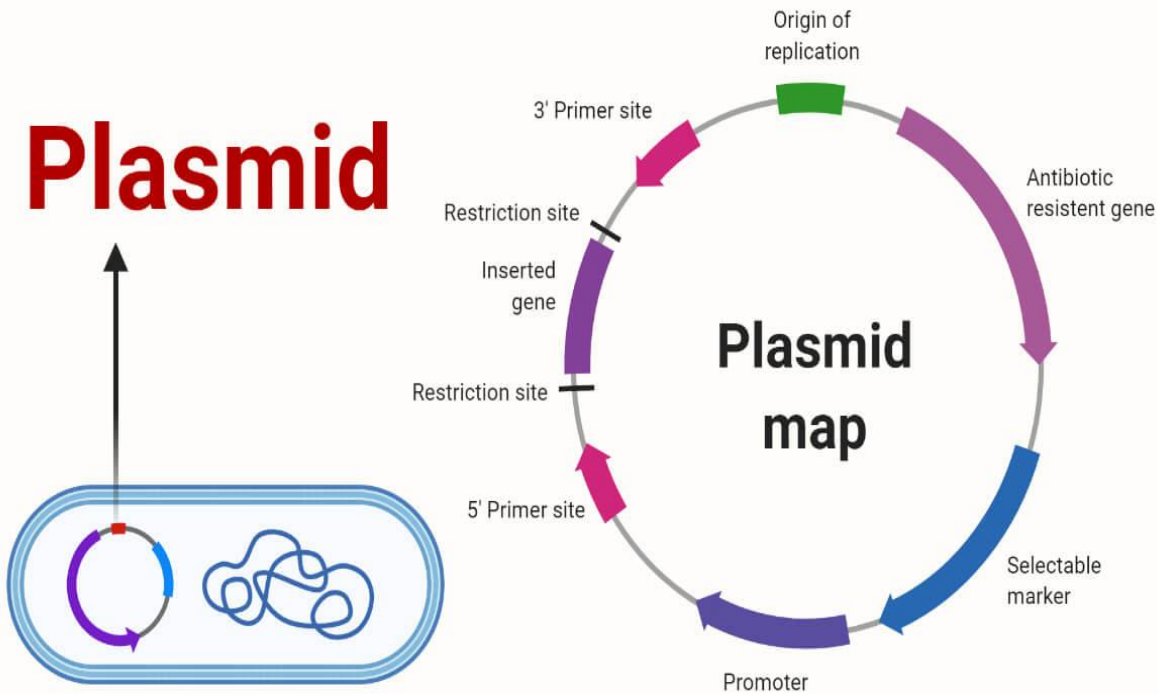
ii) Bacteriophages Vector



iii) Cosmids

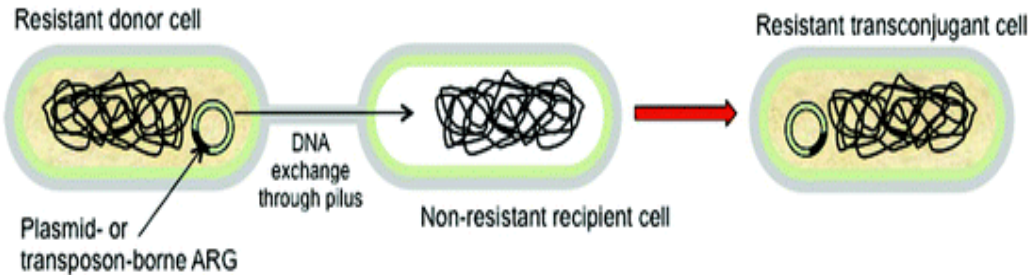
- a) Plasmids
- b) Cosmids
- c) Fosmids
- d) Phages
- e) Yeast artificial chromosomes (yacs)
- f) Transposons
- g) Bacterial artificial chromosomes (bacs)
- h) Viruses
 - Retroviruses
 - Adenoviruses
 - Adeno-associated viruses
 - Herpes simplex virus
 - Rhinoviruses
 - Human immunodeficiency virus (HIV)

Selecting plasmid for the genetic engineering experiment is one of the crucial steps in the entire experiment. Before selecting the plasmid, we must understand why the plasmid is used in the gene transfer experiments

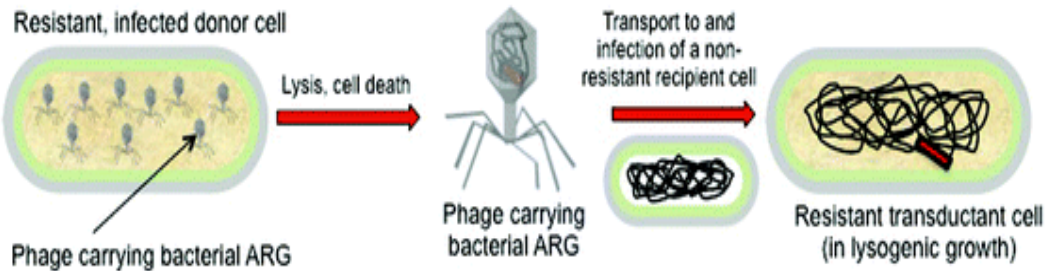


Transformation into the host genome

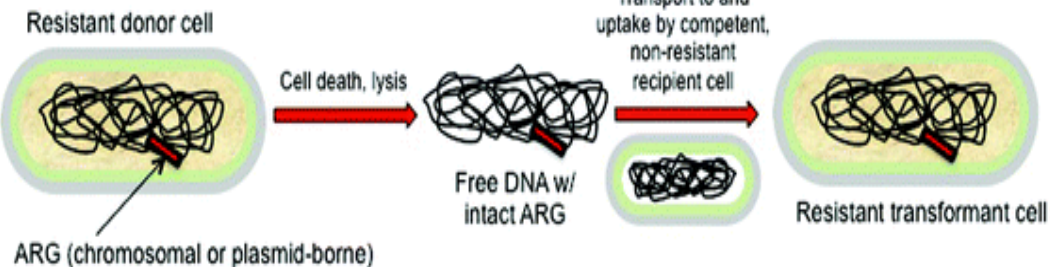
(a) Conjugation:



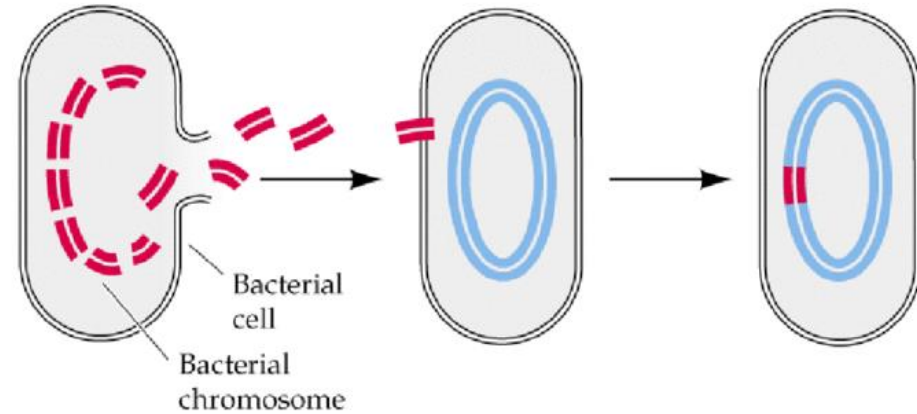
(b) Transduction:



(c) Natural transformation:



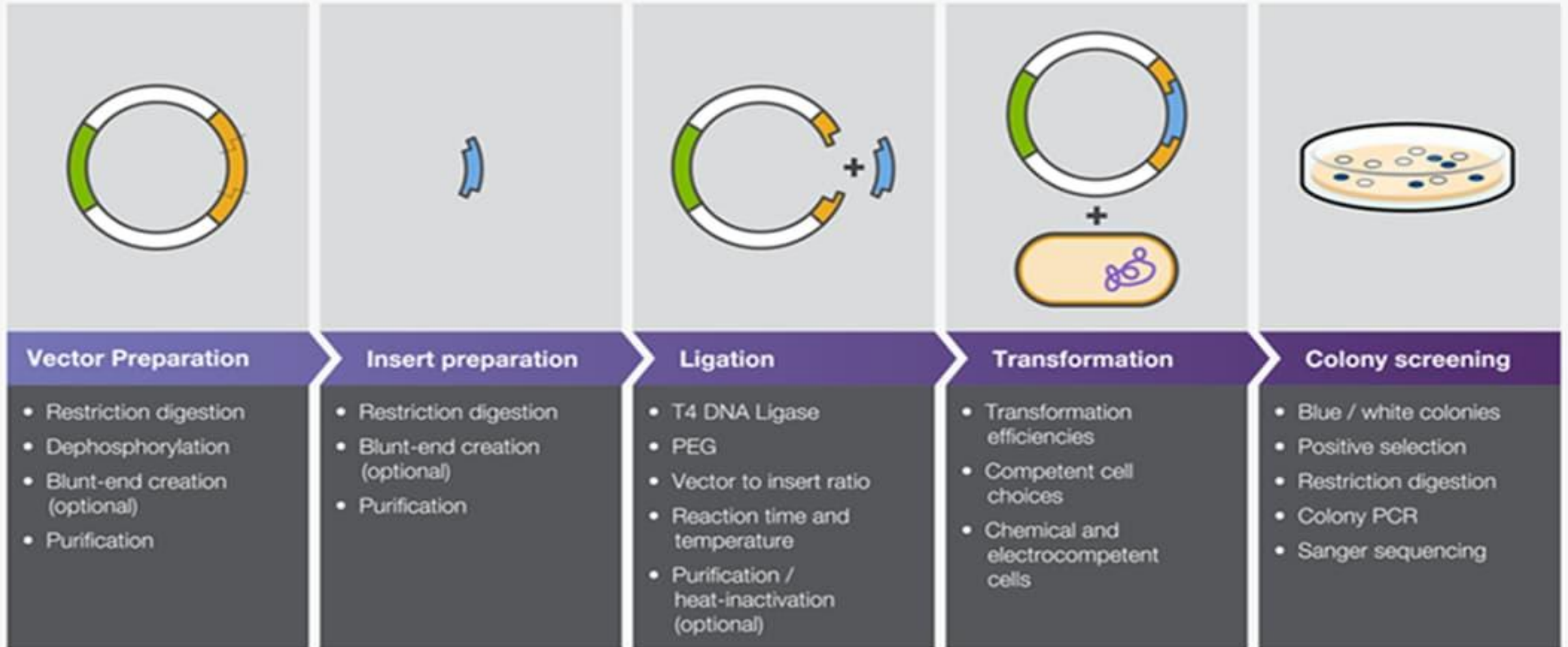
Transformation



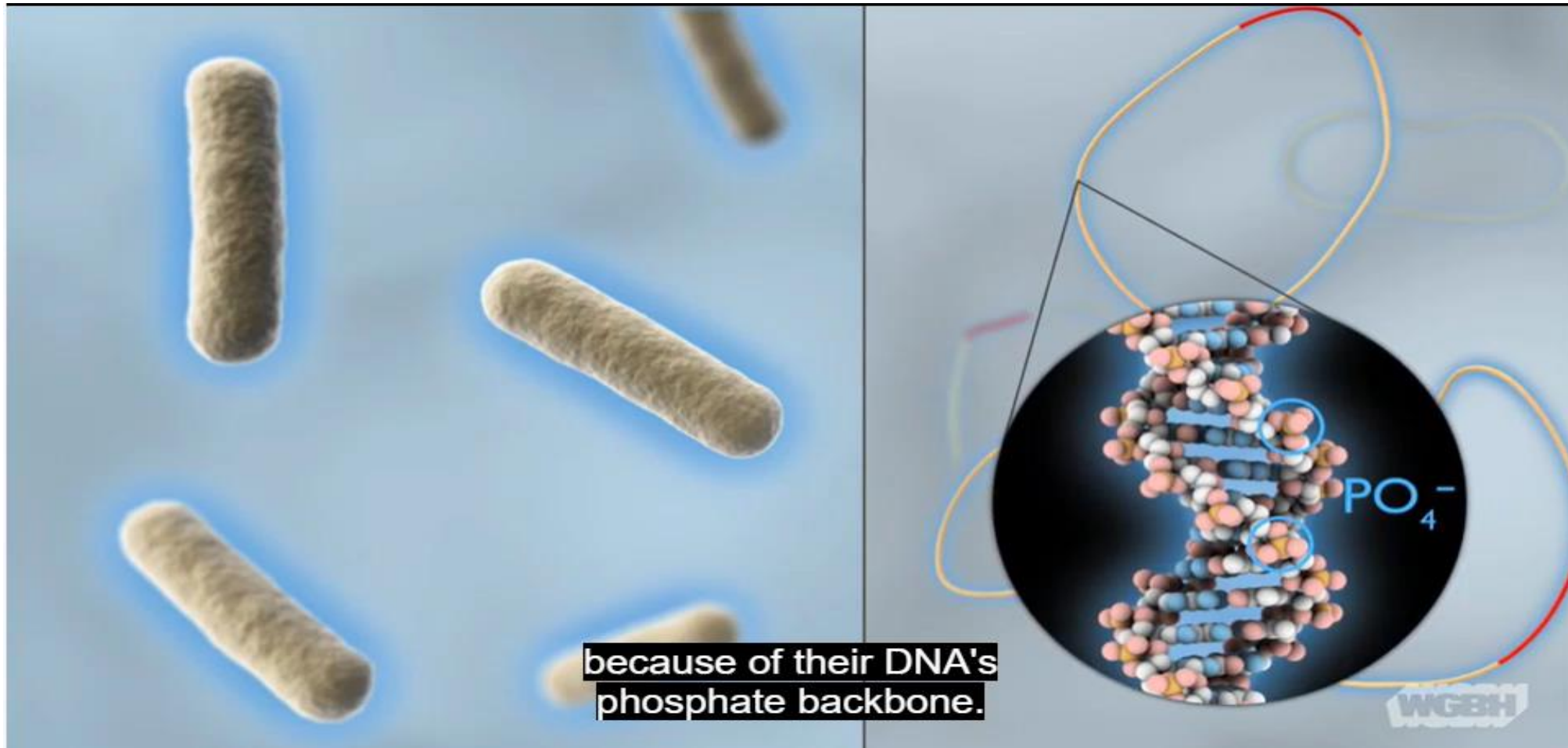
Permission pending from Sinaur Associates, Inc.

Conventional Gene Delivery Methods		Strengths	Limitations
Physical	Biostic Particle Delivery	<ul style="list-style-type: none"> • Easy • Transfers large sizes and amounts of DNA 	<ul style="list-style-type: none"> • Low integrity of delivered DNA • Short-term and low-level expression • Cell damage
	Electroporation	<ul style="list-style-type: none"> • Fast • Inexpensive 	<ul style="list-style-type: none"> • Limited range of plant species • Low integrity of delivered DNA • Toxicity
Chemical	PEG-Mediated Delivery	<ul style="list-style-type: none"> • High-efficiency protoplast transfection 	<ul style="list-style-type: none"> • No regeneration of protoplasts into whole and fertile plants
Biological	<i>Agrobacterium</i> -Mediated Delivery	<ul style="list-style-type: none"> • Low cost • High efficiency • Stable transformation 	<ul style="list-style-type: none"> • Limited host range

Traditional cloning



Bacteria transforming



Transformation into the host genome

	Advantages	Disadvantages
Naked DNA	No special skills needed Easy to produce	Low transduction efficiency Transient gene expression
Physical methods		
Microinjection	Up to 100% transduction efficiency (nuclear injection)	Requires highly specialized skills for delivery Limited to ex vivo delivery
Gene gun	Easy to perform Effective immunization with low amount of DNA	Poor tissue penetration
Electroporation	High transduction efficiency	Transient gene expression Toxicity, tissue damage Highly invasive
Sonoporation	Method well tolerated for other applications	Transient gene expression Toxicity not yet established
Laser irradiation	Can achieve 100% transduction efficiency	Special skills and expensive equipment necessary
Magentofection	Safety of method established in the clinic	Poor efficiency with naked DNA
Chemical methods		
Liposomes	Easy to produce Fusion liposomes improve transduction efficiency	Transient gene expression Toxicity, mildly immunogenic
Cationic polymers	Easy to manipulate for targeting	Transient gene expression Toxicity, mildly immunogenic

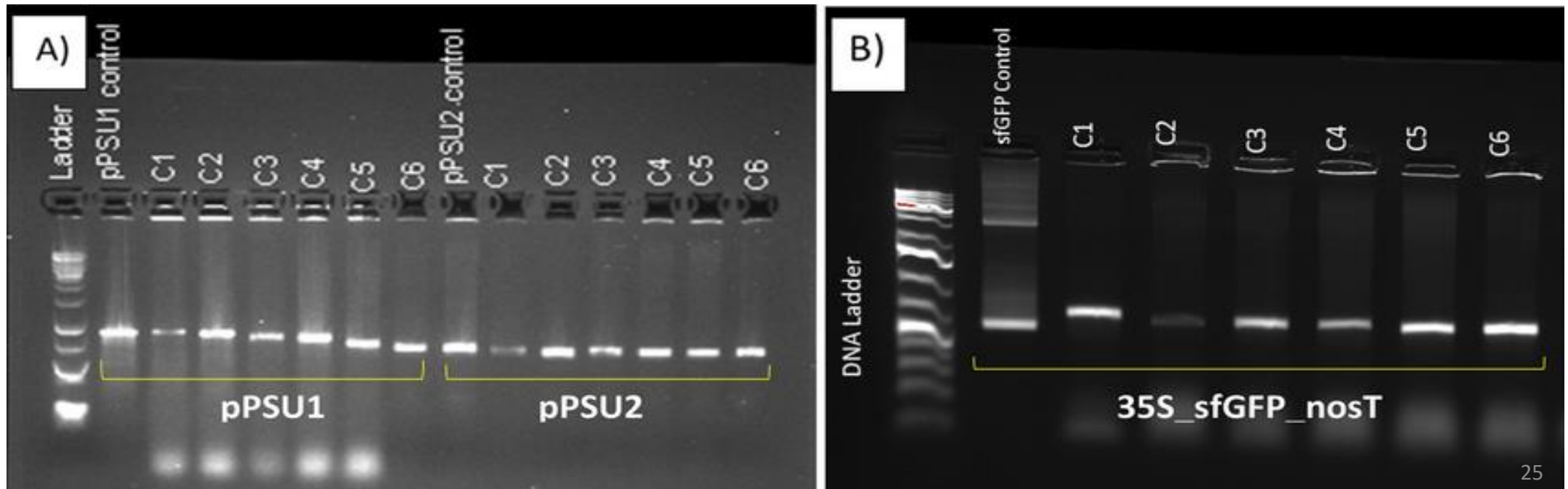
Source: From Mehier-Humbert and Guy, 2005; Conwell and Huang, 2005; Miyazaki et al., 2006.

Table 6 Summary of non-viral methods used for gene transfer.

Confirmation of insertion DNA

PCR- Metode deteksi berbasis reaksi berantai polimerase.

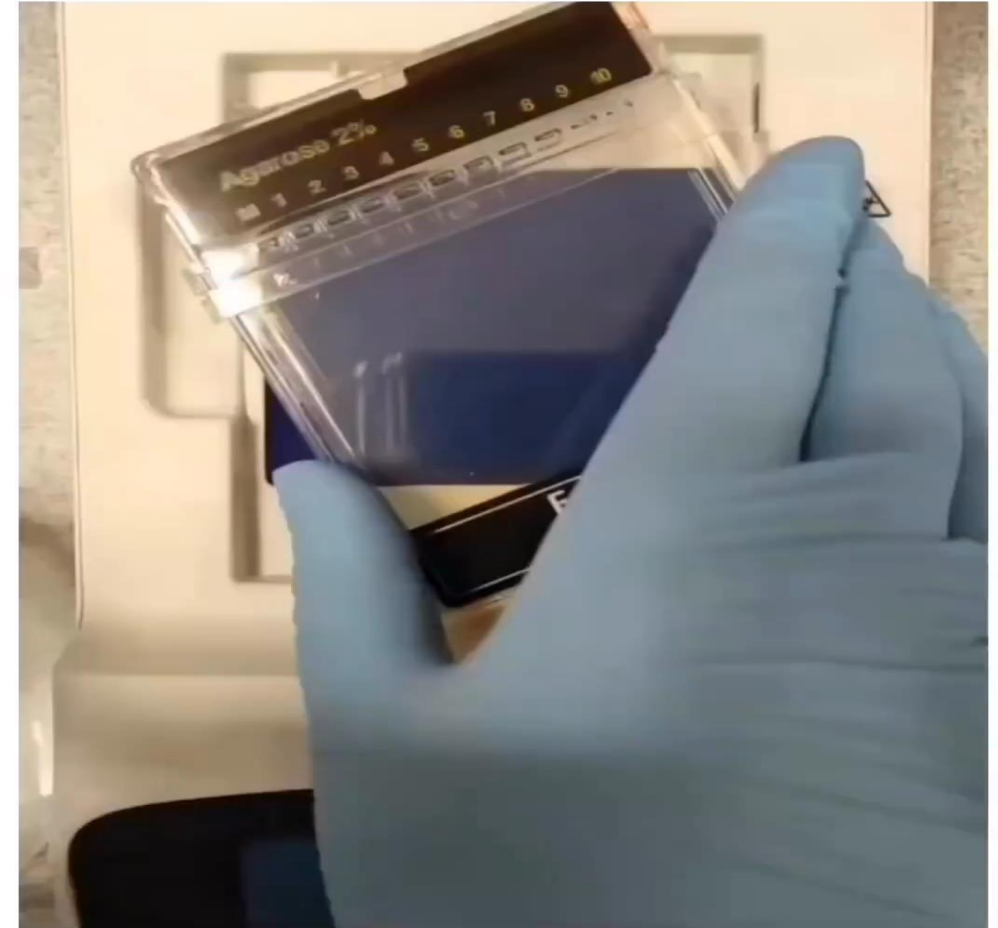
- DNA diekstraksi dari sel yang diubah dan diamplifikasi menggunakan primer yang melingkupi gen yang kita minati atau DNA rekombinan kita.
- Jika DNA rekombinan ada, pasti diperkuat, jika tidak, tidak ada amplifikasi yang diperoleh. Untuk konfirmasi dua faktor, satu set primer yang melingkupi DNA rekombinan spesifik dan satu set primer yang melingkupi urutan penanda yang dapat dipilih diambil dan PCR multipleks dilakukan.



Confirmation of insertion DNA



Fancy E-Gel electrophoresis system. Watch the DNA pieces separate by size in an elapsed 7 min.



<https://www.instagram.com/reel/CsNnFbjlOrD/?igshid=MzRIODBiNWFIZA==>
<https://www.instagram.com/reel/CvdyD1MISrc/?igshid=MzRIODBiNWFIZA==>

Rekayasa genetika memiliki nilai industri dan pertanian yang besar. Teknik ini dipraktekkan dalam kedokteran, penelitian genetik, pertanian, perbaikan tanaman, dan untuk produksi obat terapeutik

Genetic Engineering

Pros

- Fighting diseases
- Increase in life expectancy
- Increased variety of foods and drinks
- Nutritious food
- Decrease in the use of pesticides
- Medical foods
- Decrease in the use of resources
- Increase in growth rates of animals and plants
- Development of specific characteristics

Cons

- Religious and ethic concerns
- Genetic issues
- Health issues
- Allergies
- Resistant insects and pests
- Antibiotic resistance
- Reduction in genetic variety
- Effects on wildlife
- Soil pollution
- Displacement of natural species
- Influence of certain industries and interest groups

Genetic engineering, including gene editing, can have numerous benefits: faster and more precise breeding, higher crop yields, development of more nutritious food, and decreased need for herbicides and pesticides

Top 10 Genetically Modified Foods



Corn



Soy



Cotton



Papaya



Rice



Rapeseed
(Canola)



Potatoes



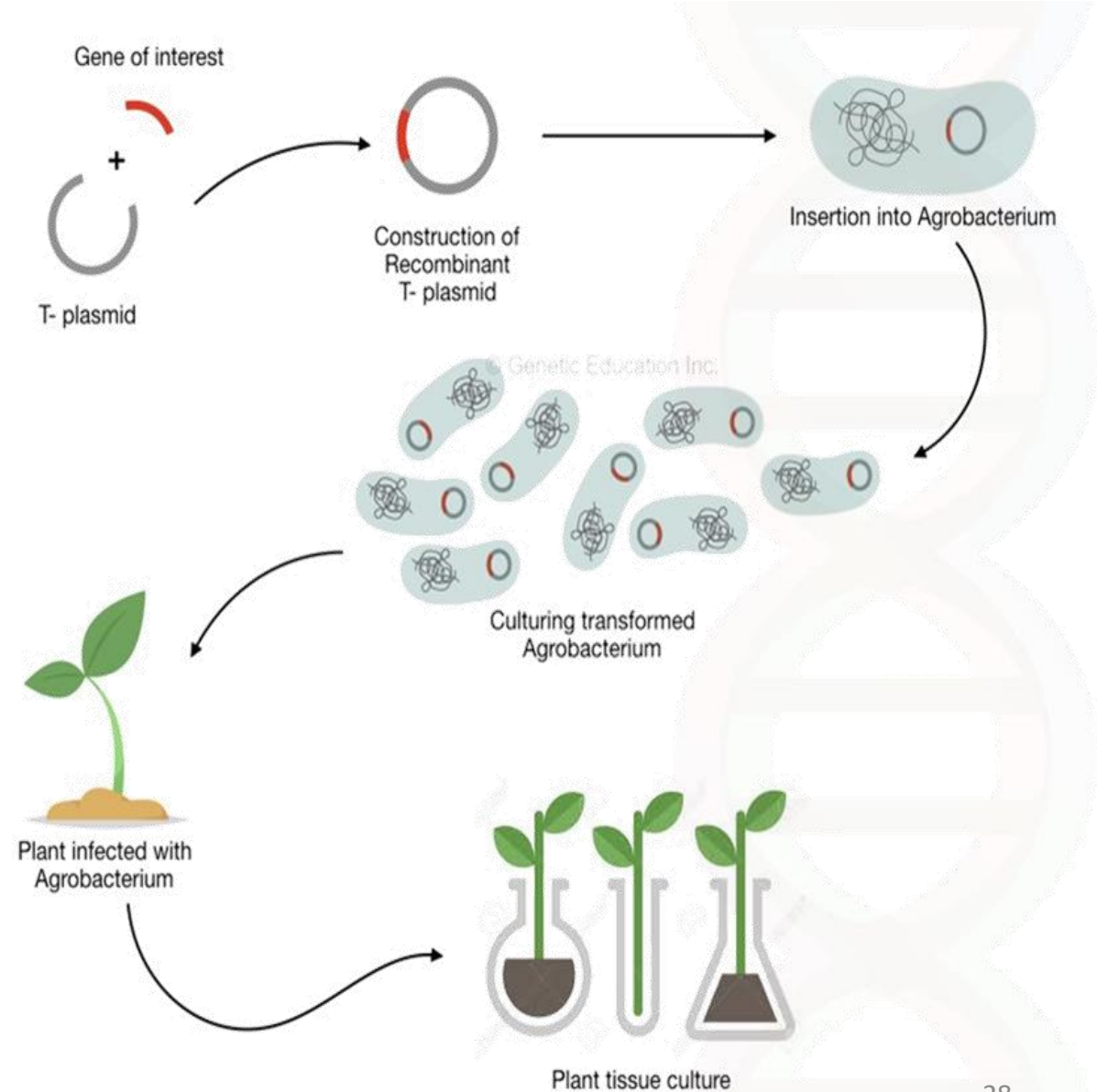
Tomatoes

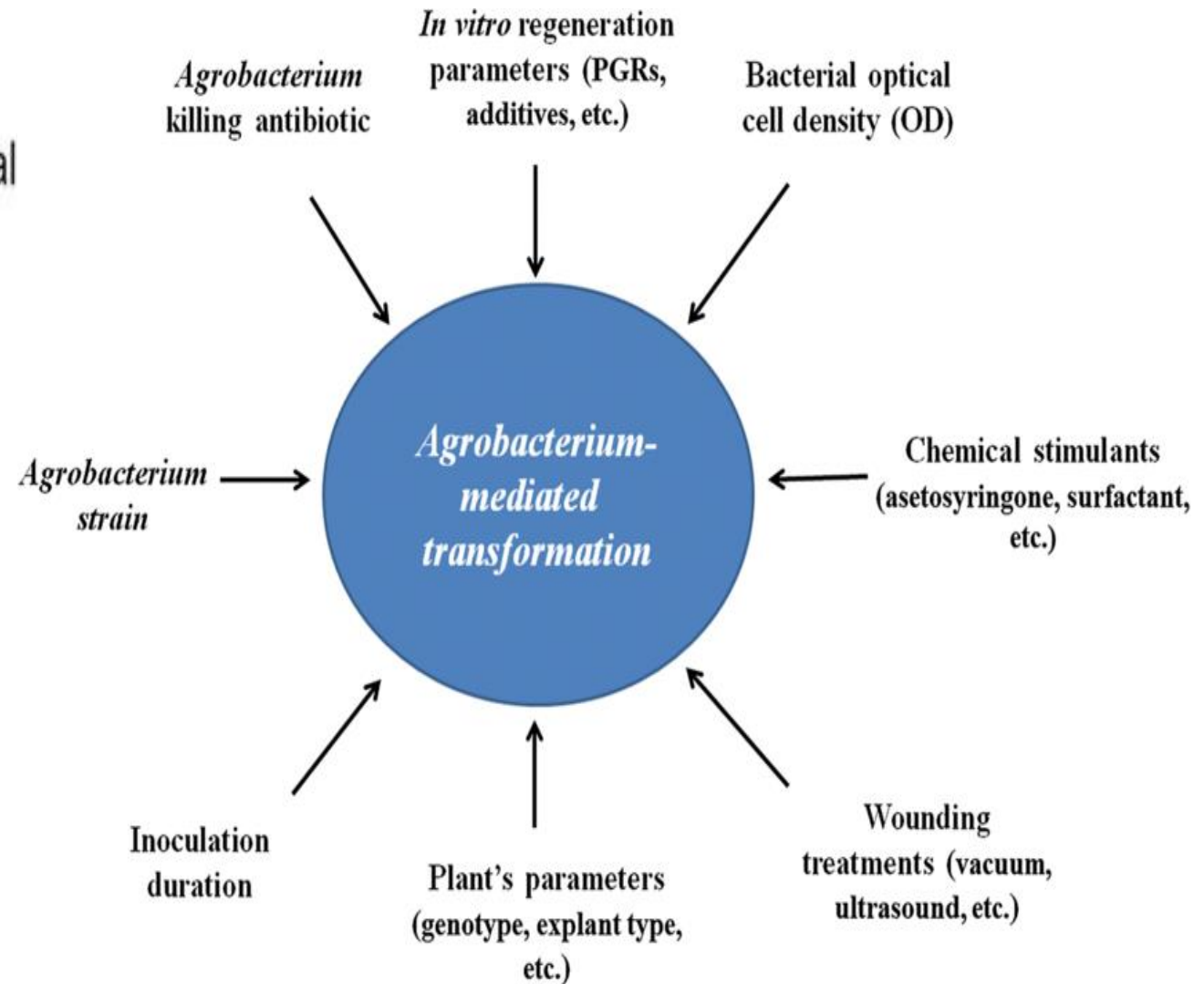
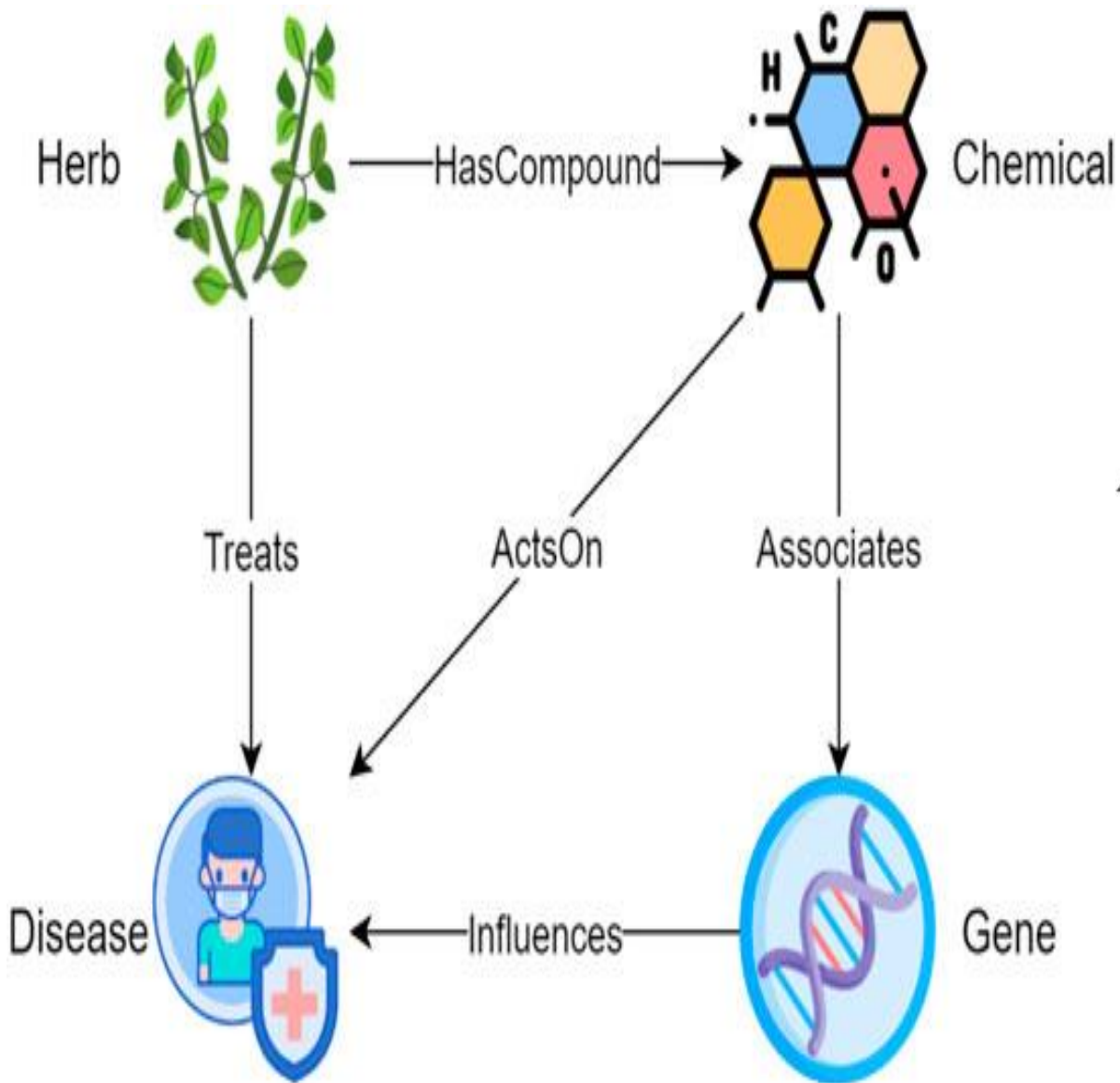


Dairy products



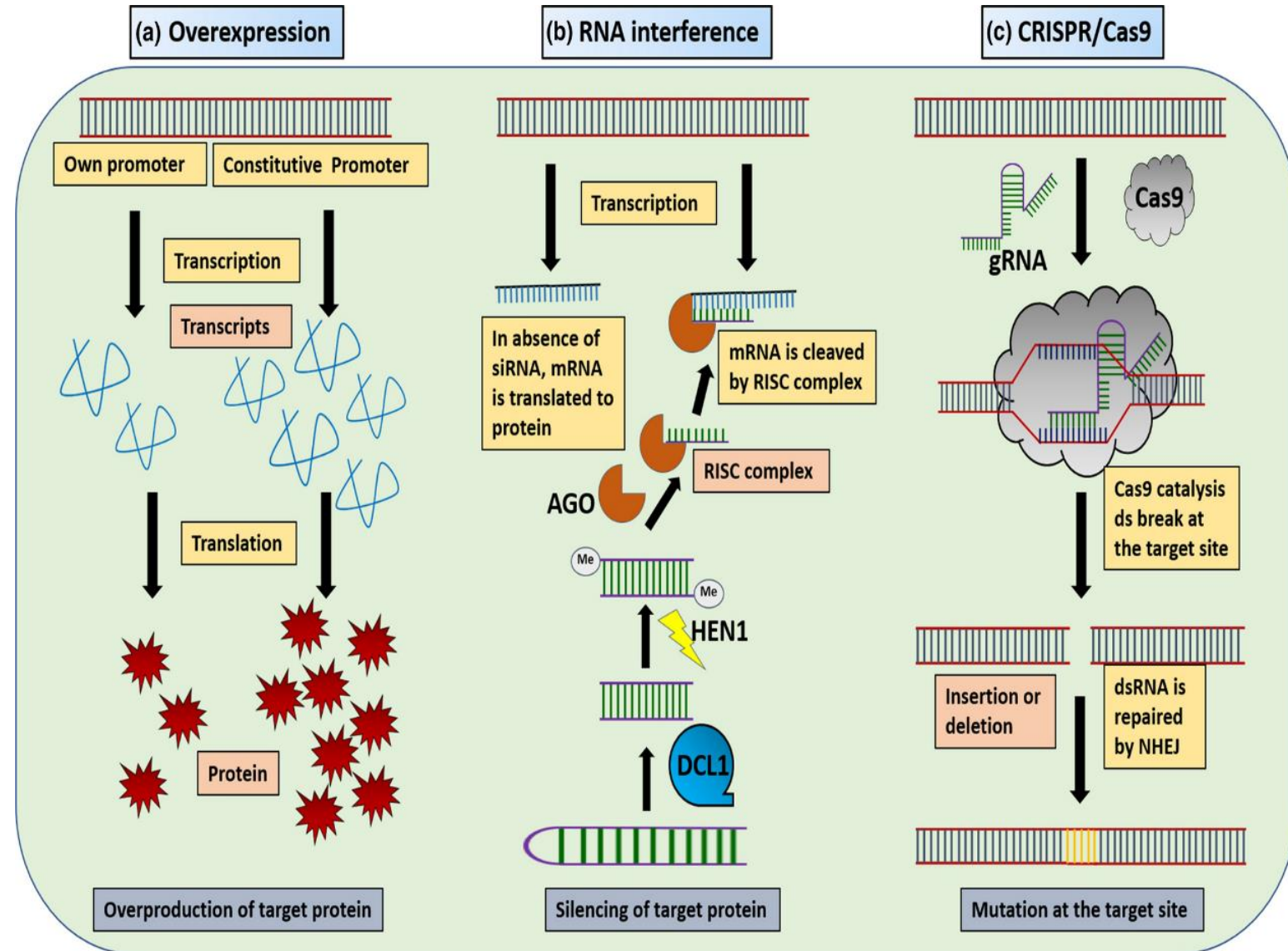
Peas



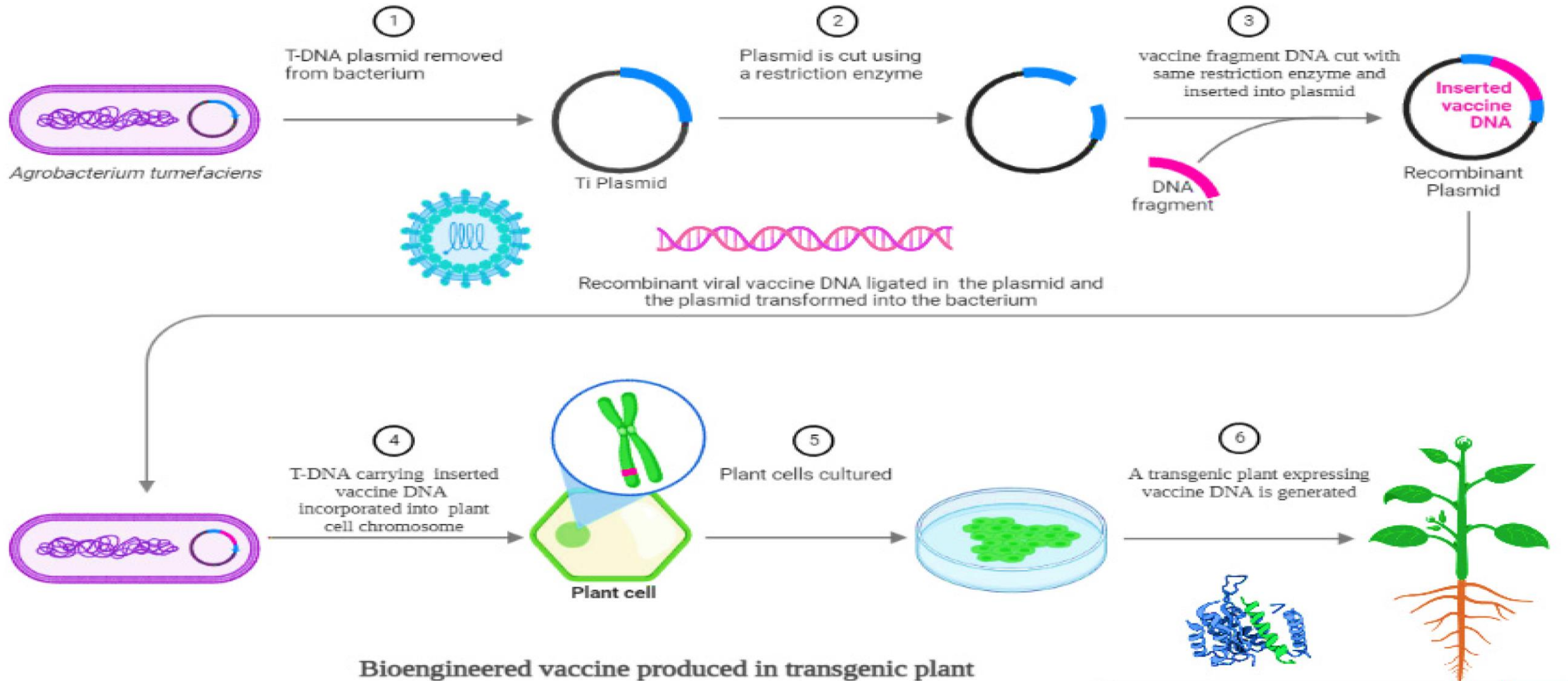


Strategies for crop improvement through biotechnological approaches.

- Overexpression leads to greater transcription of target gene which can be translated into protein
- RNA interference leads to downregulation of target gene;
- **Gene editing through CRISPR/Cas9** leads to insertions or deletions at target site which gives rise to mutations

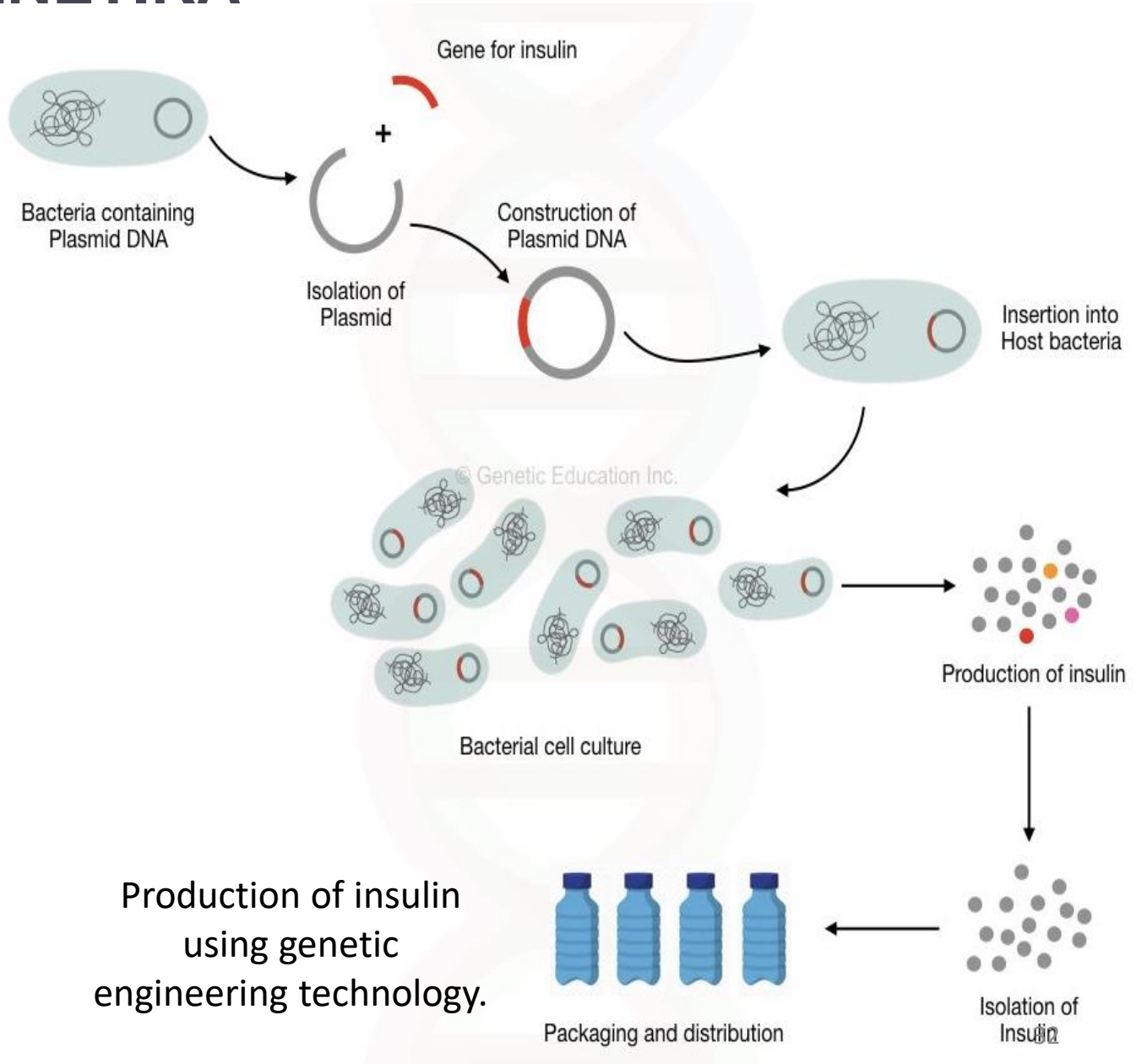


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REKAYASA GENETIKA

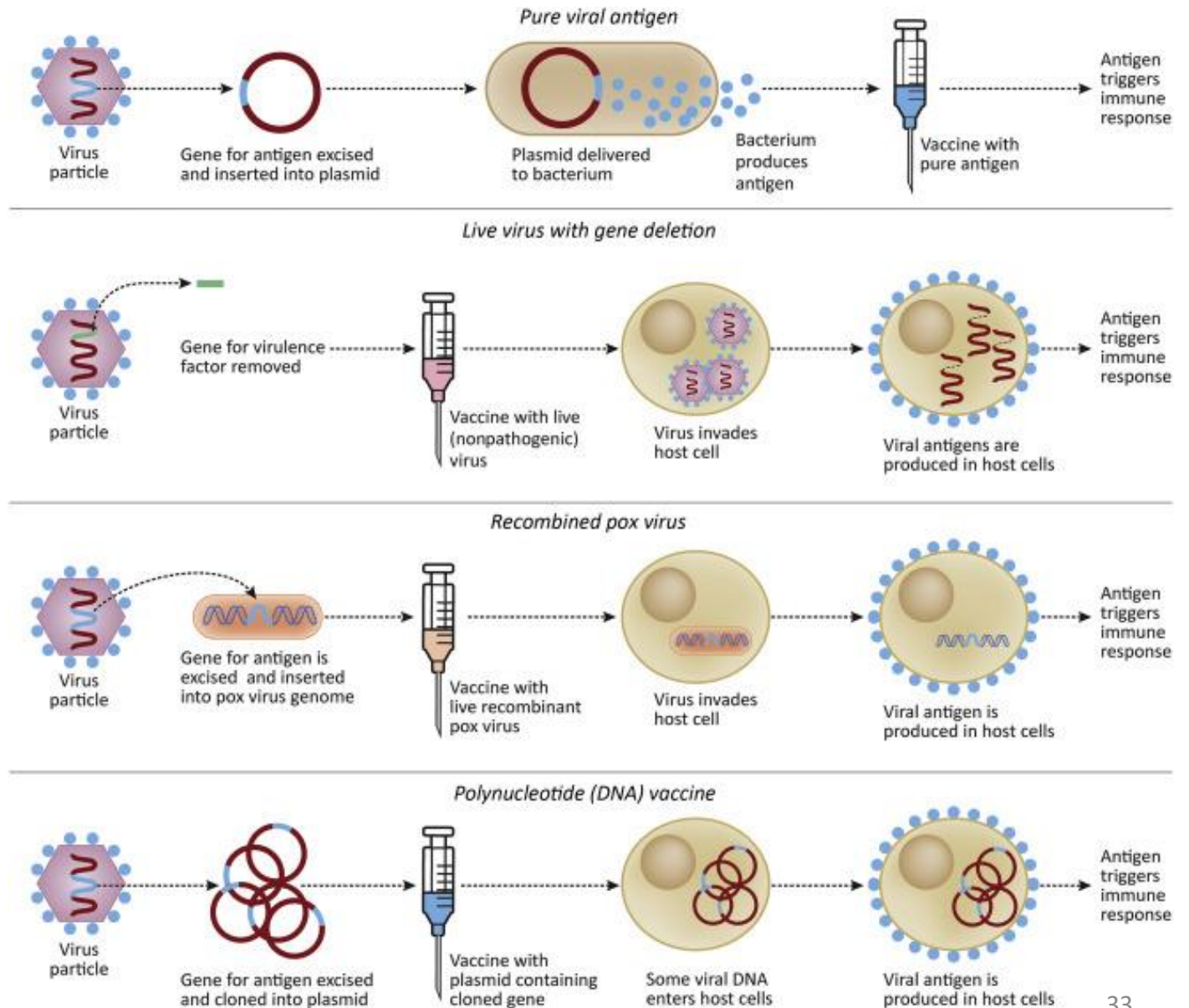
- Low-cost drugs, hormones, enzymes, and vaccines are created using genetic engineering tools
- A gene for insulin is isolated by restriction digestion or through PCR and inserted into the plasmid. The recombinant plasmid DNA is immediately inserted into the bacterial or yeast cell in which the plasmid is multiplying. As the microorganism starts dividing it starts making artificial insulin.



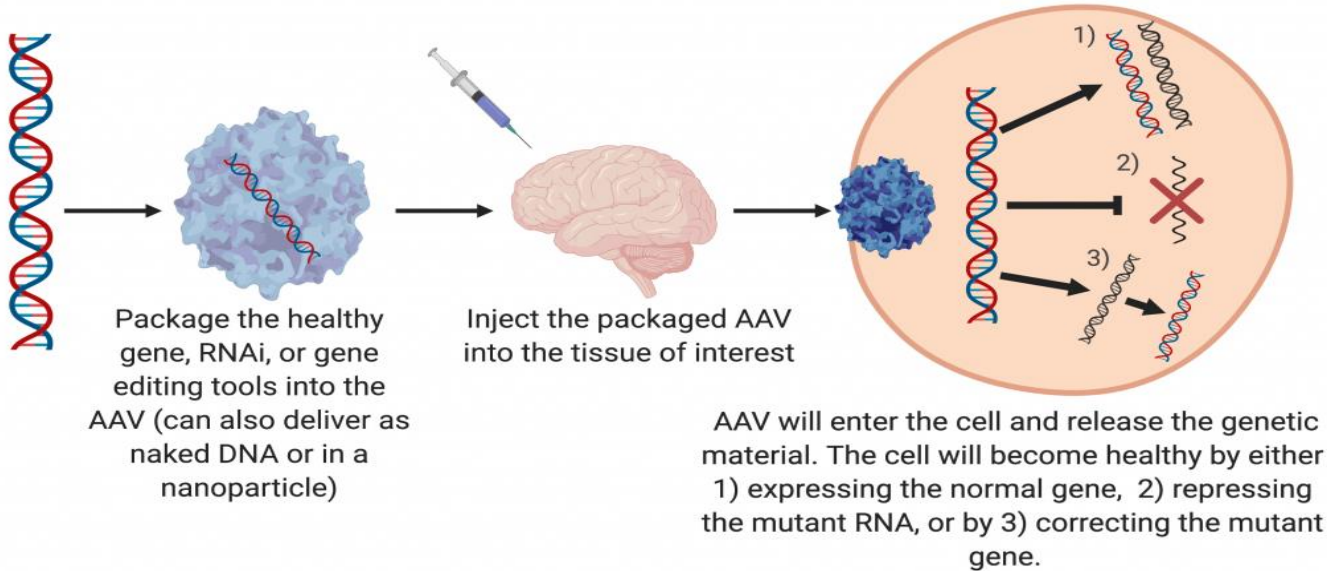
Production of insulin using genetic engineering technology.

Recombinant vaccines

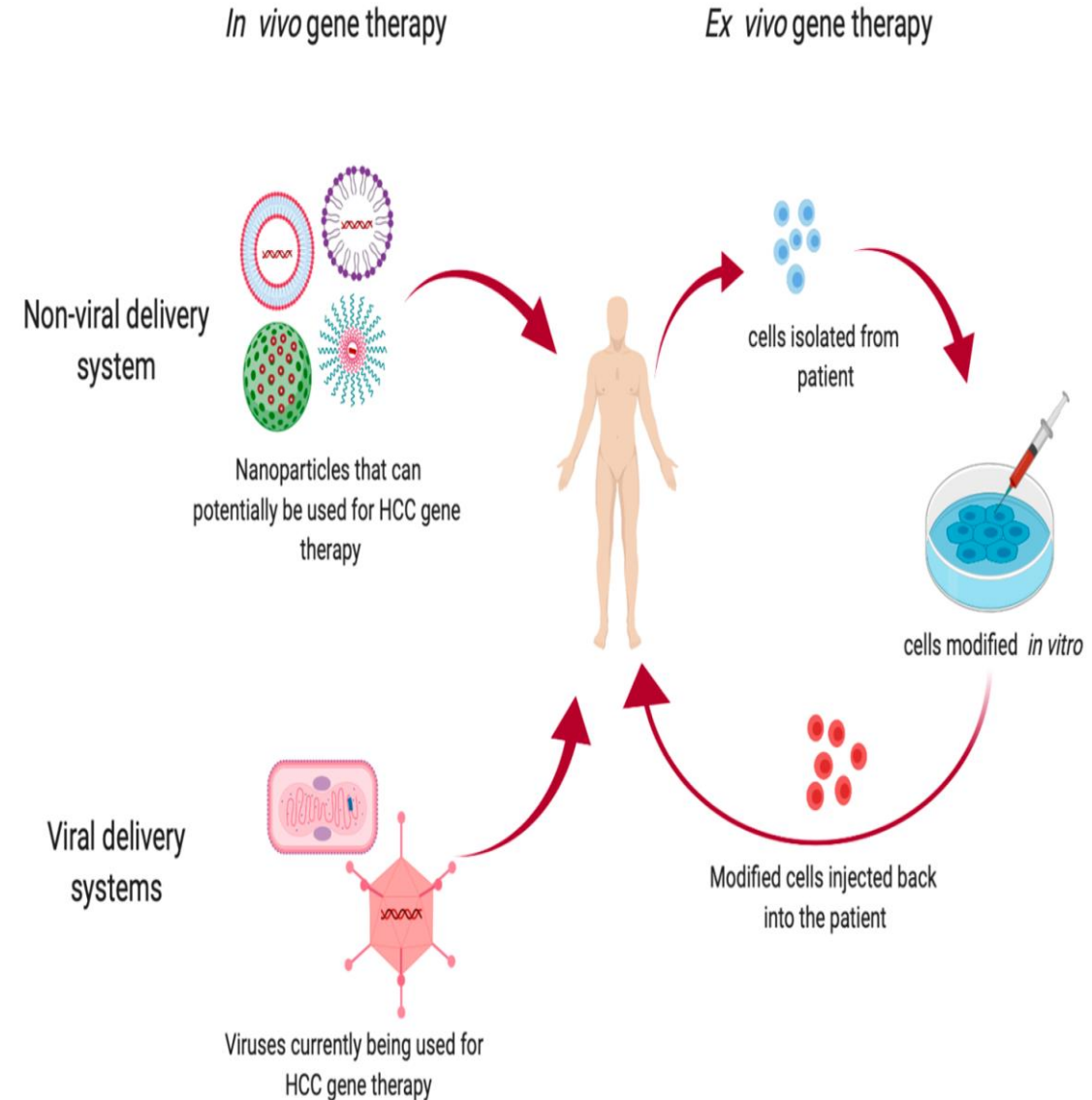
- Vaccines against smallpox, herpes simplex virus and hepatitis are produced using the genetic engineering technique. The vaccines are the inactivated viral particles used to induce an immune response against that pathogen, however, the chance of contamination is high in it.
- Using the recombinant DNA technology scientists has created a unique type of vaccines that only contains the DNA for viral coat protein thus the pathogen can never be activated again. The main advantage of it is that it is safer, contamination-free and more reactive

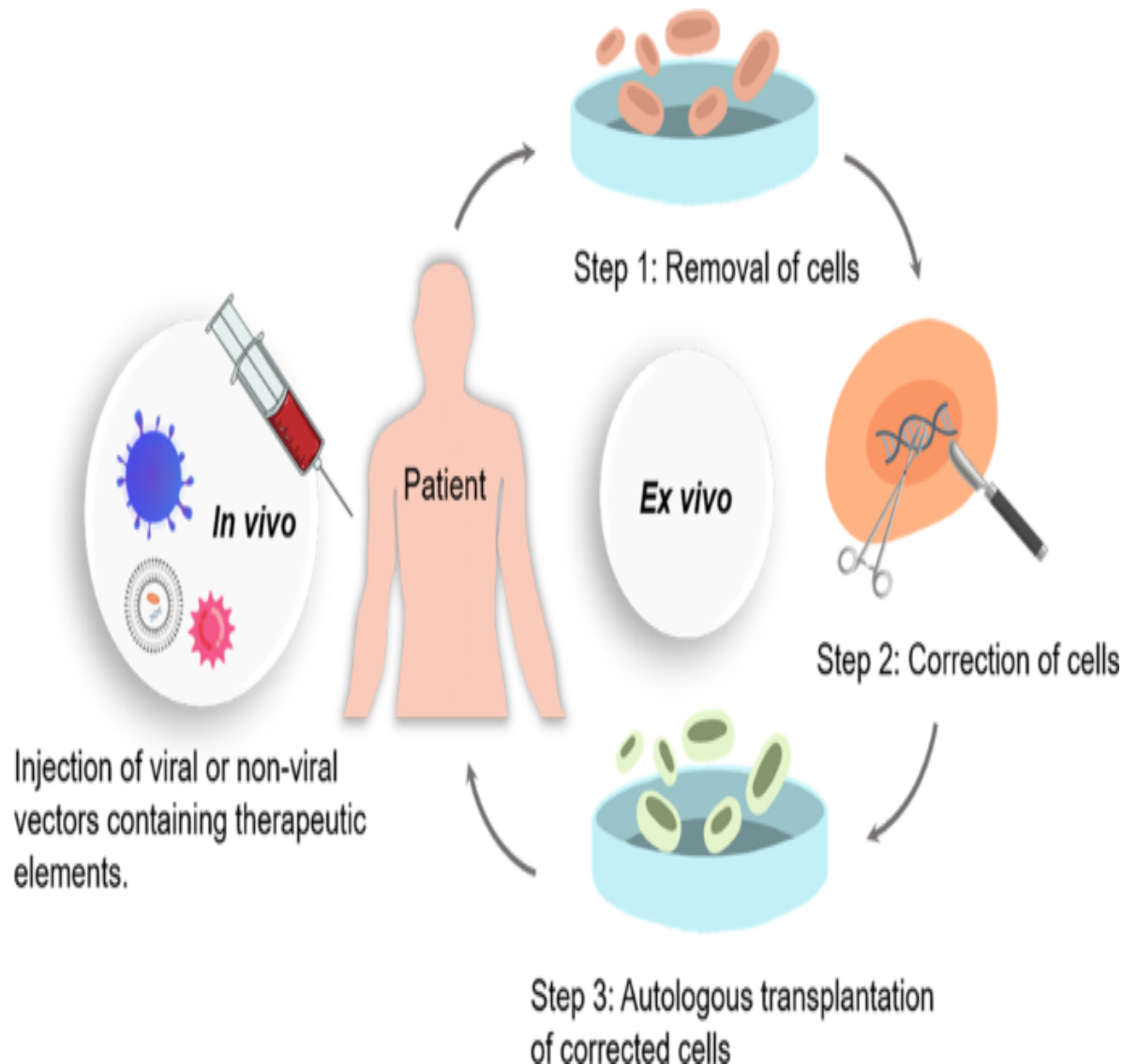


Gene Therapy



- Using the gene therapy or gene transfer technique, inherited genetic disorders can be cured. In the gene therapy, a faulty, non-function or mutated gene is replaced with the wild type one.
- Cystic fibrosis, Duchenne muscular dystrophy and sickle cell anemia like gene therapies are now under the final clinical trial phase and ready to use on patients.





Ex vivo and in vivo genome editing for clinical therapy

- **Right:** For in ex vivo editing therapy, cells are isolated from a patient to be treated, edited and then re-engrafted back to the patient. To achieve therapeutic success, the target cells must be able to survive in vitro and return to the target tissue after transplantation.
- **Left:** For in vivo editing therapy, engineered nucleases are delivered by viral or nonviral approaches and directly injected into the patient for systemic or targeted tissue (such as the eye, brain, or muscle) effect.



Factsinbrain 
@factsinbrain



Scientists in Taiwan added jelly-fish genes to carp fish DNA. This glowing fishes are the result!



Transgenic Fish

- Scientists in Taiwan created luminous fish by incorporating jellyfish genes into the DNA of carp fish. This innovative approach aims to enhance the understanding of how pollutants affect the organs of fish.

genetic engineering

new horizons in medicine

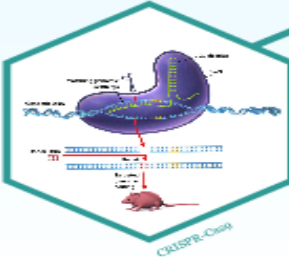
Since genetic engineering (also known as **recombinant DNA technology** or **genetic modification**) was first developed in the 1970s, scientists have discovered more and more ways in which the technology can be used in human medicine. Now techniques, including the gene editing tool known as CRISPR-Cas9, are opening up even more possibilities for us to change the DNA in the cells of bacteria, animals and plants – and potentially change medicine for ever.

Pharming



Microorganisms, animals and plants can be genetically modified to produce medically useful products. These transgenic organisms are already used regularly to produce substances such as human insulin, human growth hormone and blood clotting factors for haemophiliacs.

CRISPR-Cas9 technology

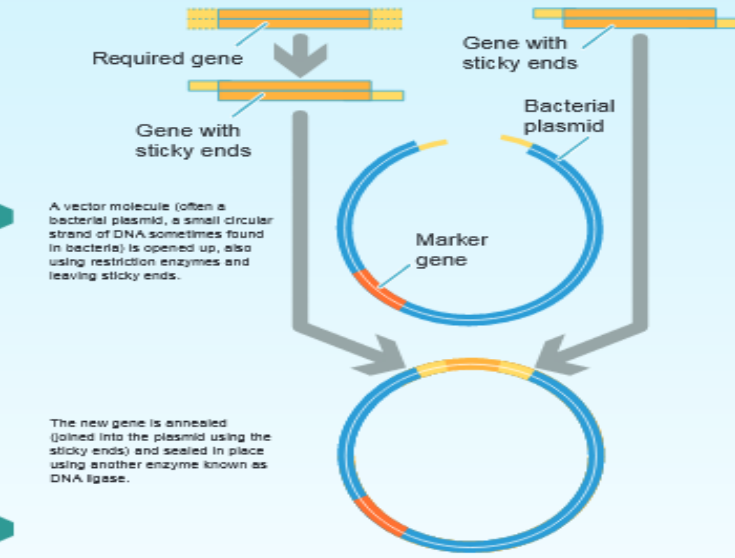


CRISPR-Cas9 is a genome editing tool which is changing the world of genetic engineering fast. It enables scientists to directly remove, add or change sections of the DNA sequence in a living cell. CRISPR-Cas9 is much faster, much cheaper and much more accurate than the traditional ways of editing DNA. Scientists think it has great potential for treating any diseases which involve the genome, including cancers, heart disease and even the high cholesterol levels which are a risk factor for heart disease.

The basic steps in traditional genetic engineering of a bacterium

The required gene is EITHER cut from the DNA of an organism using enzymes called restriction endonucleases which leave the gene with overlapping sections called sticky ends...

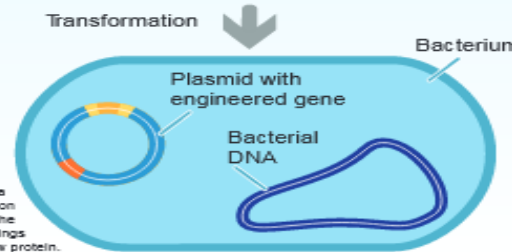
...OR ...synthesised specifically



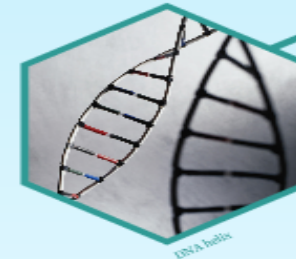
A vector molecule (often a bacterial plasmid, a small circular strand of DNA sometimes found in bacteria) is opened up, also using restriction enzymes and leaving sticky ends.

The new gene is annealed (joined into the plasmid using the sticky ends) and sealed in place using another enzyme known as DNA ligase.

The recombinant plasmid is inserted into a bacterial cell in a process known as transformation. It is then replicated whenever the bacterial cell replicates, and brings about the manufacture of a new protein.



Gene therapy



Gene therapy is still in its very early stages. It involves modifying human DNA either to repair or replace a faulty gene. The idea of gene therapy is to overcome the effects of a mutation which cause a genetic disease or tendency to a disease. Progress so far has been relatively slow, although there are early signs of success in treating some childhood leukaemias, HIV/AIDS and muscular dystrophy. The speed and precision of CRISPR-Cas9 gene editing technology gives scientists hope for the future.

Vaccines

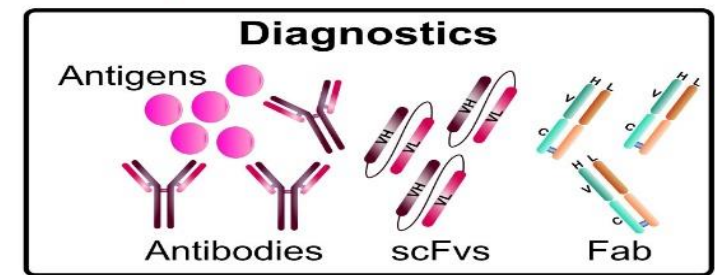
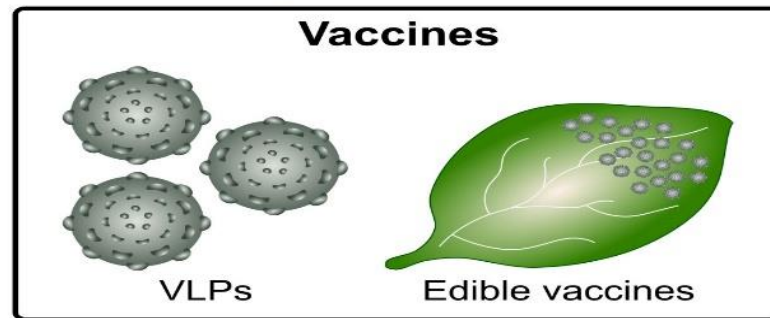
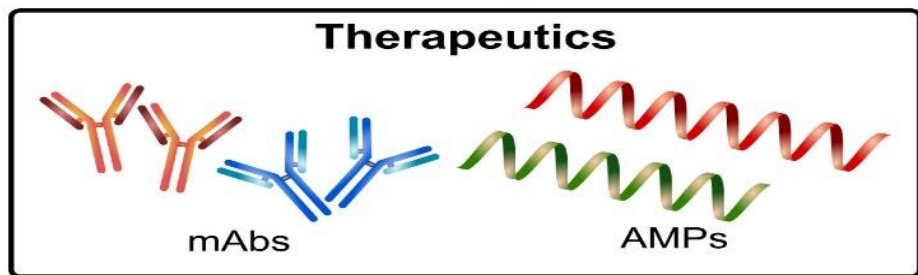
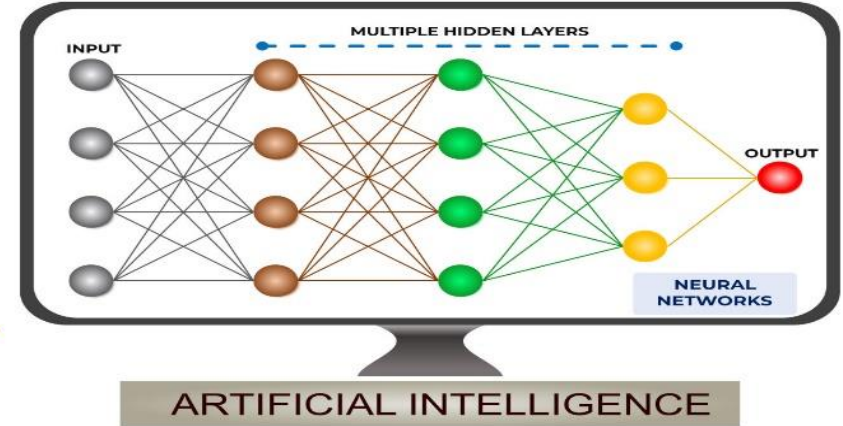
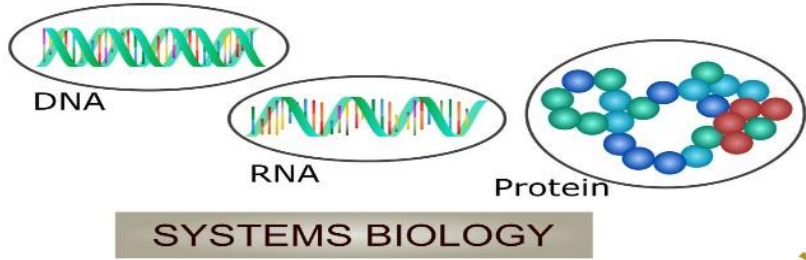


Some vaccines are very dangerous to make using conventional methods. Genetically engineered microbes can be used to produce the antigens needed in a safe and controllable way. The use of genetically modified yeast cells to produce a vaccine against the hepatitis B virus has been a major success story.

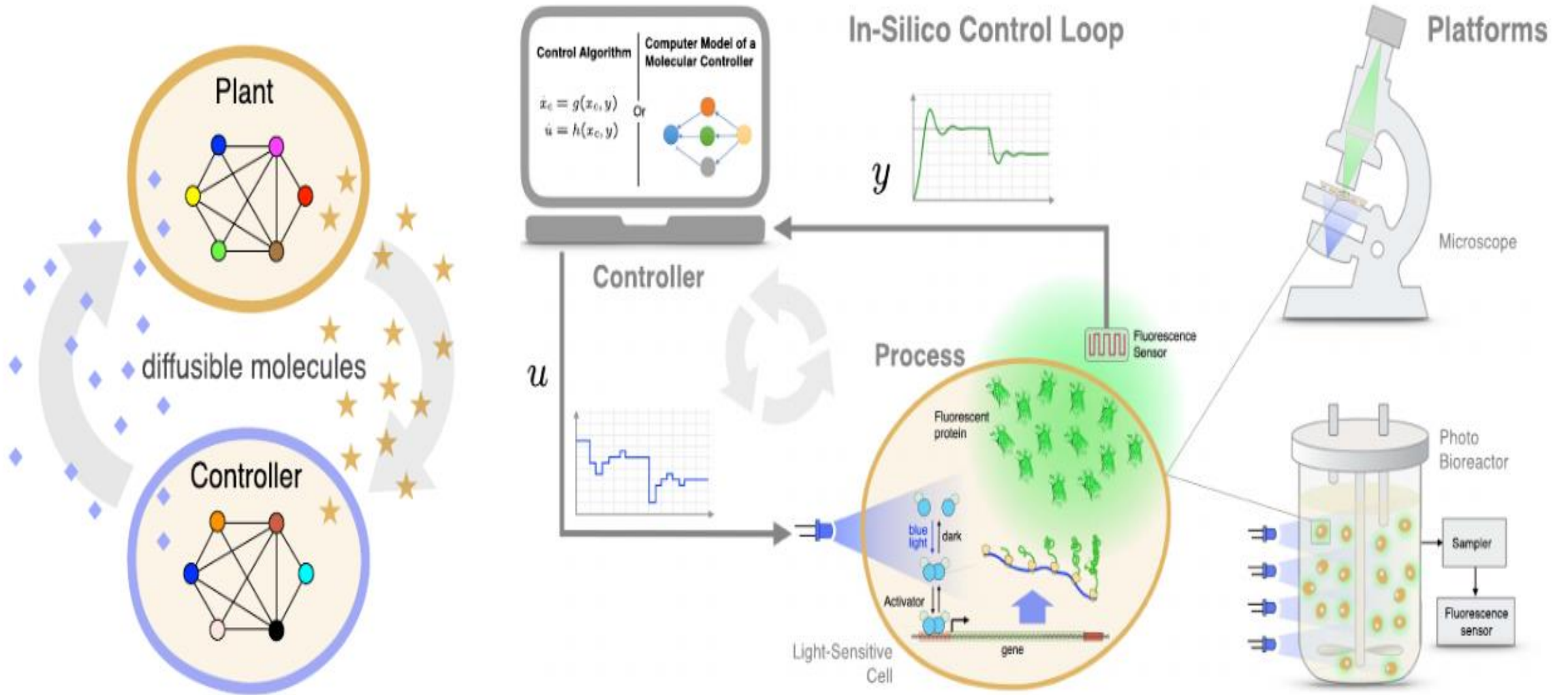
Xenotransplantation



The DNA of pigs has been modified using recombinant DNA technology so their cells develop without certain genes which trigger the human immune response. Other genes can be added which express human antigens. Work in this area has been slow, partly due to ethical and safety concerns, but interest is growing. Recent successes include German scientists using CRISPR-Cas9 to deliver multiple gene modifications in pigs, greatly reducing the human immune response to the pig cells.



Overview of AI Integration in Plant Molecular Pharming Pipeline



Computer control of living cells

Genetic Engineering | Genetics | Biology | Don't Memorise

- <https://youtu.be/4fBQ2umTaMA?t=55>

Genetic Engineering Will Change Everything Forever – CRISPR

- <https://www.youtube.com/watch?v=jAhjPd4uNFY>

Bacteria transforming

- <https://www.pbslearningmedia.org/resource/biot11.sci.life.gen.transbact/transforming-bacteria/>

Referensi ;

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- Crook, O.M., Warmbrod, K.L., Lipstein, G. *et al.* Analysis of the first genetic engineering attribution challenge. *Nat Commun* **13**, 7374 (2022). <https://doi.org/10.1038/s41467-022-35032-8>
- Khammash, M. H. (2022). Cybergenetics: Theory and Applications of Genetic Control Systems. *Proceedings of the IEEE*, 110(5), 631–658. <https://doi.org/10.1109/JPROC.2022.3170599>
- Parthiban S, Vijeesh T, Gayathri T, Shanmugaraj B, Sharma A and Sathishkumar R (2023) Artificial intelligence-driven systems engineering for next-generation plant-derived biopharmaceuticals. *Front. Plant Sci.* 14:1252166. doi: <https://doi.org/10.3389/fpls.2023.1252166>



THANKS