

* we as human have been utilizing microbes in old practices of biotechnology sometimes without knowing that we are using it

Ex → Many food products are made by using microbes

- Mining → Releasing precious metal from mountain or air using microbes even without knowing that they are using it

- 50% of earth living matter made of bacteria

give living organism is micro

less than 1% of all bacterial species around us because we can only grow in the lab less than 1%, the other 99% we didn't find away to grow them in lab if we do exp. we can detect their present

Because we can detect their genome

- scientist try to find new microbes because it can be used in biotechnology application

* yeast produce antibiotic

* Group of drugs called statins that some of it is produced in yeast

* Many protein drugs are being produced in yeast that could be used for therapeutic

- Pichia pastoris → very important type of yeast because it's used to produce many type of proteins that are used in various biotech application

Why because this type of yeast can grow like bacteria in large quantity & have strong promoters that could be induce.

• All DNA poly. are actually from bacteria archaea that live in extreme hot condition

- Many bacteria & archaea can live in very hot condition which means the enzymes they have inside them can well-stand hot temperature

- Cellulase → from microbes degrade cellulose
animal food consist mainly of plant waste

* Subtilisin → protease

↓ used in laundry why?

because cloth stain might have protein, lipid, carbohydrates, so we use proteases, ligases, amylases that must well stand high temperature or extreme ph's, extreme alkaline condition

* Bio prospecting → discovery of new genes, enzymes, protein in new organism that could be used new biotech. application

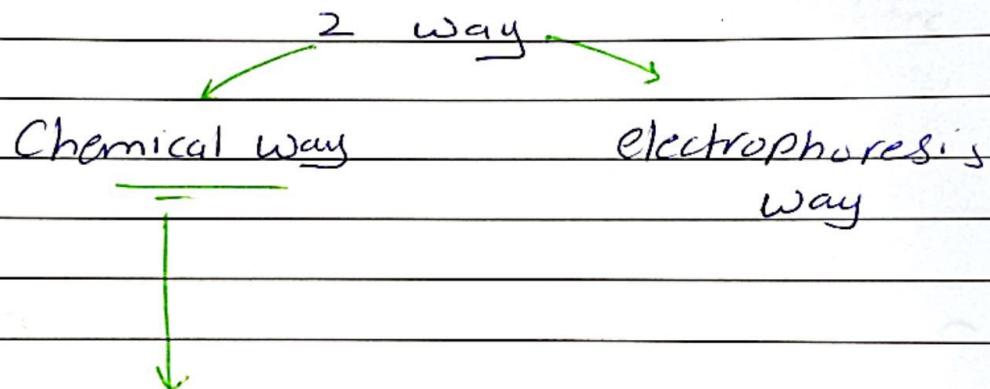
• We could use micro-organisms as tools to produce proteins
→ bacteria Ecoli, to do cloning, transformation

- Some bacteria naturally take DNA from surrounding to change their phenotype genotype or use DNA as a source of food

* Bacteria that is commonly used for cloning it's not naturally taking their DNA that's why we use methods to force the cell take the DNA outside

- Some cells considered naturally competent, but the bacteria that we use for cloning we have to make them competent artificially or force them to take DNA by process called electrophoresis

● Transformation could be done by



Rely on using CaCl_2 , RbCl_2 .

the cells are treated by these salt, & then cells can be heat shock to allow plasmid to enter the cell

* Basically competent cell treated with CaCl_2 , RbCl_2 are mixed with the DNA that we want to insert inside the cell.

Then → we subject the cell to heat shock for 1 minute, during it transient pores will be opened to allow the plasmid insert inside the cell

& following transformation, we have to do selection

→ Based on selective markers that are found on the plasmid we insert inside bacteria

Once → plasmid is inserted the next step is to express the marker gene that will allow the cell to be resistance for ex. to antibiotic if we did the antibiotic selection

* electroporation require sophisticated instrument, could be used to introduce different cell type mammalian, fungi, ... & the cells are subjected to high voltage electric pulse that cause formation of transient pores

→ which the DNA can enter the cell

* Cells before electroporation they must be repaired by washing them extensively to remove any salts

↳ Might carry electric current & causes a close secret

• After removing all salt from bacteria, we put the bacteria & plasmid inside a chamber called cubic & this chamber added to device & this chamber connected to 2 wires connected to device called electroporator.

↳ Generate electric pulse

→ So we have a brief electrical shock (If the duration of electric pulse is higher, the bacteria will die)

Once electric pulse is applied, next step is to do selection

* Micro organisms could be used to produce protein by recombinant DNA technology.

We could express certain protein inside fungi, bacteria, yeast & many of protein expressed inside fungi, bacteria, they are expressed as Fusion protein.

C. How *

So you clone the Gene of interest with coding sequence for a tag protein or fusion partner.

So in this ex.

we have expression vector, in which the coding sequence of the gene we want to express is fused with the sequence of tag partner & this plasmid is inserted inside cell.

So whenever we use this plasmid we have a Fusion protein made

Contain a.a of your protein & a.a of the fusion partner

? Fusion partner ~~partner~~ *

- 1 → For purification, make it happen easily
- 2 → Make protein fold correctly
- 3 → prevent formation of Inclusion Body
- 4 → increase stability, solubility inside cell
- 5 → Make your protein secreted outside the cell

→ Using affinity column how?

your protein of interest is fused to another protein called maltose binding protein

Now → this fusion protein can be purified by a single step using affinity column

Why?, they consist of beads from the surface of the bead there is maltose

↓ wash

If this maltose binding protein can bind to maltose, which mean that your protein will bind to the bead & another protein will pass through the column

next step → separate your protein from the maltose binding protein

how this happen? we add proteases

↳ beginning is important

we need to put some aa between your protein & fusion partner & this aa they are the target sequence of proteases

So → after purifying your protein, you cut it with appropriate protease that will release your protein from the fusion partner & now you have your protein purified

* Some genes from microbes they could be used as reporters

- we have some gene for ex: lux genes

Reporterpoin
Reporter gene
lacZ gene

→ originates from bacteria called **vibrio** that live on the surface of the fish & these genes can produce light by a process called Bioluminescence

- involves the production of light by a chemical reaction (happen inside a biological organism)

- we have a colorless substrate called the luciferin + ATP + O₂ & one of the lux genes encode enzyme called luciferase (convert luciferin to oxyluciferin)

Chemiluminescence

Production of light because of chemical reaction

* lux genes could be used to study gene expression how?

They could be used as reporter genes

↳ a gene will tell u sth

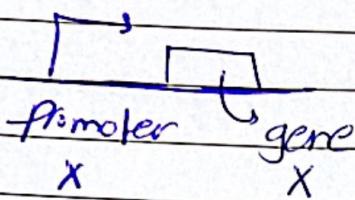
- lux gene can tell if we had a gene expressed or not

? expresstion

Clone promoter of the Gene I want to study with lux gene

because promoter will determine whether your gene will be expressed or not.

ON → exp. , OFF → not exp.



let's say we have gene X, is gene X expressed or not?

We add lux gene that we clone it (on plasmid) downstream of promoter X, & then you insert the plasmid to cell Y & add to cell Y luciferin, if light produced lux gene is expressed.

Transcription Factor can bind to promoter Y & stimulate transcription

indirect way of knowing if gene is expressed or not

Direct way to detect gene exp. by looking mRNA or trying to find protein encoded

Reverse PCR, Real time PCR, Illumina, in situ hybridization, northern blotting

* lux gene ^{use} indicator of the presence of certain type of bacteria or contaminants in the environment

* Bioassay → Find a certain type of organism in a sample (blood test, corona virus test)

- Fluorescent bioassay mean we rely on lux gene to detect the presence of bacteria causes tuberculosis.

↳ caused by micro bacteria lungs ~~Aspergillus~~

express Lux, lux Lux 313!

↙ so it glows *

scientist fused lux gene with a promoter that could be activated only if there is type of chemical contaminant

so some promoters can only be activated if there is certain type of heavy metal (silver, brass, zinc)

e. lux gene detect heavy metal in sample ↳

↳ By fusing the lux gene with a promoter that could only be activated in the presence of heavy metal

* Microorganisms as tools

↳ yeast two-hybrid system

used for research & detect
protein-protein interaction

- imagine you discover a new protein
~~what is its function?~~
what is its binding?

is to know which protein
might bind to it

* protein are instruments of the cell,
most of the function carry out of the
cell, & most of the function of protein
involves binding to sth else

- protein bind to DNA → Expression factor
- " " " small molecules → Enzyme
- protein to other protein

* Is protein A interacts with protein B?

First thing you do you fuse the coding
sequence of protein A with the coding sequence
of DNA binding domain of a transcription
factor

* Transcription Factor → in general they have
2 domains
1 DBD 2 AD

* Domain →

- protein have more than 1 domain
 - incoded by different exons
 - Represent structural & functional units of a protein
 - Regions in proteins could be separated from each other & still maintain their structure & function
- so → proteins might have different functions because they have different domain

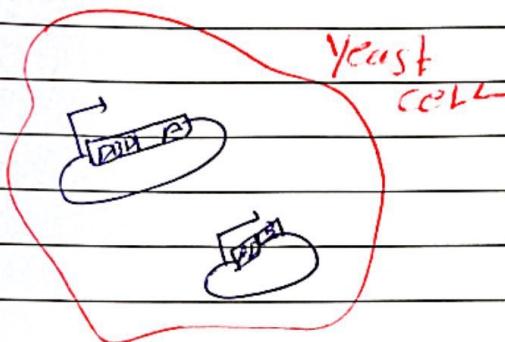
DBD → this structural part of the protein allow the transcription factor to bind to DNA (promoter region)

AD → promoting transcription, important for the binding of RNA poly.

DBD, AD → could separate physically & still carry out their function

* Coding sequence DBD is fused to the coding sequence of protein A

so basically you will have 2 plasmids & these 2 plasmid inserted inside yeast cell



so basically inside the yeast 2 fusion protein will be produced

- 1 → consist of DBD & A
- 2 → " " " AD & B

- Now inside the yeast there will be a reporter gene (*lacZ*, *lux*, GFP)

& the promoter of this reporter gene could bind by DBD, so DBD can bind upstream the reporter gene

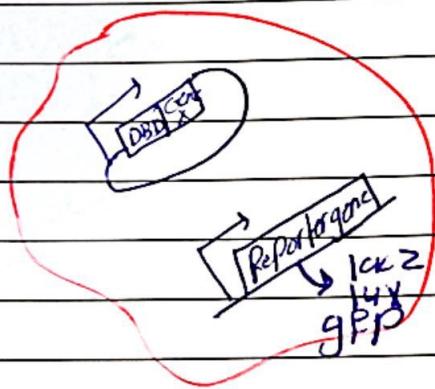
• expression of reporter gene require DBD & AD, but they are separated from each other, if they can only come together if A & B are interacted to each other

normally they are connected interaction of A & B ends AD closer to the promoter which causes the transcrip. of reporter gene & the production of mRNA

* yeast two hybrid →

many interactions between thousands of protein interact
utilizes hybrid

e.g. → Protein X



- put coding sequence

- put in yeast cell & there will be also a reporter gene

library in which you will fuse the coding sequence of every protein with a AD



library inside a test tube & then you introduce them to thousands of yeast cells each cell will have gene X

So basically you transform million of yeast cell carrying gene X with millions of copies of plasmid in library

effectively you will grow yeast cell on plate, & you have yeast colony (can grow like bacteria)

- Each colony consists of cell & each cell with the same colony have 2 plasmid

Genes  no. of library

plate λ -gal zip, Reporter is λ acZ cistron
also λ -galactose operon has λ -gal m^r & colony yes
it transcribes reporter into β -gal. enzymes interact β -gal

cell types library with different genes
using cell extract to measure activity
of sequence

Genes that was added from library,
so I could determine type of gene,
so I determine the protein

But after i do that i will repeat the 2 hybrid screen once again using your protein X & the protein you discovered to confirm it's a stable interaction

Because you might get false positive blue colony

? Real note is

Sometimes X-gal might be degraded & convert to blue product even without the presence of enzyme, sometimes their will be transient exp. from the lacZ even without interaction.

Sometimes R1D come closer to D13D without interaction for reason we don't know

Application one of the first protein produced by Recombinant DNA tech. is chymosin

protease used to make cheese produce inside bacteria

Before it was from rennin enzyme obtained from the stomach of animal

* we have been using microbes in Fermentation because Fermentation can be used to make yogurt, ...

- partial degradation of sugars to produce energy & it happen in the absence of O_2 during Fermentation

• some microbes could be used to produce therapeutic proteins ex insulin

The active insulin require two chains (α | β), & the first insulin produced was in E.coli

so the coding sequence of the α chain was cloned into a plasmid & this plasmid contained the β -gal enzyme ($lacZ$)

& the β chain was also cloned into another plasmid along to β -gal

As both were separately introduced into E.coli & it grow in large scale & then E.coli cell produce a fusion protein consist of β -gal & chain α & another β -gal & chain β

* So β -gal was used as a tag to allow the purification of the α & β chain using Affinity chromatography

$\xrightarrow{\text{So}}$ an antibody against β -gal was add to affinity column & this allow the capture of β & α proteins

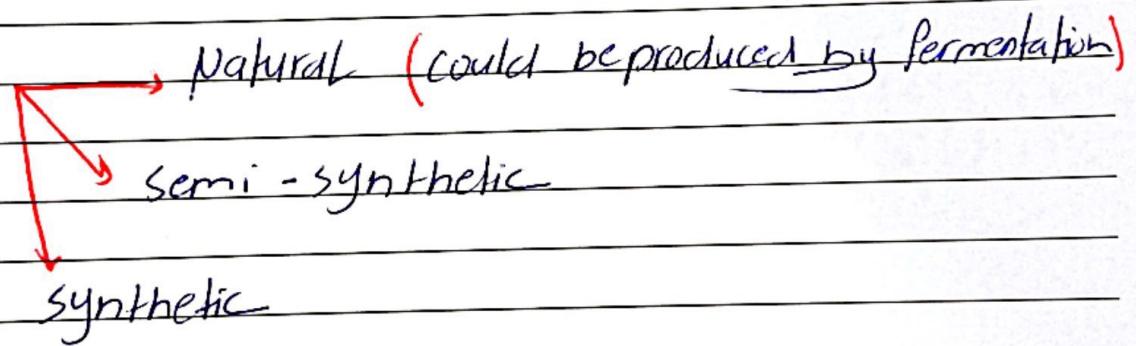
* β -gal. was removed with CNBr & then α chain & β chain were mixed, folded together & the cysteine were oxidize to form a diserhybridge between 2 chains & now we have mature active insulin.

Applications

↳ Antibiotics:

- For bacterial infection
- Molecules produced by microbes (bacteria, fungi) & it could kill other microbes

* Alexander discovered antibiotic by chance, fungus that can produce penicillin & it could kill bacteria



- Most we use is semi-synthetic & synthetic
- Some antibiotics they are just organic molecules, some are peptides

* Most of antibiotic inhibit cell growth, bacterial growth. Most of antibiotics don't kill bacteria, they stop bacterial growth to give more chance for immune system to fight bacteria

- Once bacteria enter our body because there are a lot of food, they start multiplying very quickly.

→ The killing actually happens from immune system

* There are some effects of certain antibiotics still unknown

But we know that some antibiotics can stop transcription, replication, cell wall synthesis in bacteria, some block enzymatic activity, inhibit some chemical reaction required for growth, stop translation because it can bind to ribosome, some damage plasma membrane

Resistance to antibiotic

& Resistance to antibiotic could happen because bacteria can have mutation in certain genes, or it could obtain resistant gene by horizontal gene transfer

Q. Resistance ~~will not go~~ -

over use & mis-use

infection ~~susceptible~~ ↴
bacterial vs viral illness
is viral illness gets antibiotic ious

* Where is the most antibiotic resistance bacteria found?

In hospital

superbug
highly
resistance

* Many scientists are trying to do sth called bioprospecting

Finding new

strains of bacteria that might be producing new type of antibiotics

* because of superbug, scientist trying to find another way in fighting bacteria

One of these ways is to use Phage therapy

virus ↓ that can kill bacteria

* Acinetobacter baumannii

No antibiotic was basically able to kill this bacteria

Mixture of phages (different phages that can bind & kill this bacteria)

* Bacteria can also become resistance to phages they already have defense mechanism like CRISPR

- 2 Major advan. of phage therapy

1, phages can kill bacteria with mechanisms different than antibiotics

2, species-specific which mean each bacteria has its own unique phage (we can engineer phages easily to target specific type of bacteria)

* Inside our bodies we have more bacterial cell microbial cell compared to human cell (10:1)

But most of these microbes are considered Good for us

* Pathogen → Organism that cause disease to its host
Bacteria, viruses, fungi, protozoa

jingle jingle it's antibiotic JESSE! -
Good bacteria

* So using phages as a therapy insures that you will only be able to use phages to target bad bacteria

Inside our guts we have bacteria helps us to digest our food & protect us against pathogenic & produce some Vitamins for us

- some antibiotic can effect gram + not gram - of bacteria

gram +, gram -, gi

certain type of bacteria

← w7 up asym *

we use a mixture of phages because maybe bacteria can be resistance to one phage, so you hope other phages will kill the bacteria

* some scientists are trying to join CRISPR with phages, so basically cloning cas & CRISPR guide RNA into phage genome & allow phages to infect bacteria delivering the CRISPR-cas system into bacteria & it will target antibiotic resistance bacteria

So → CRISPR designed to target gene responsible for the resistance

↳ the idea of this treatment is to design guide RNA that will be complementary to a sequence within antibiotic resistance gene, so that antibiotic resistance gene can be disrupted

↳ now bacteria becomes sensitive to antibiotic & could be killed easily by antibiotic treatment

* Vaccines → Pathogens, fragments of pathogens, genes, nucleic acid from patho. that are injected inside our body to try to teach our immune system about these pathogens so that in the future if the real pathogen comes & infect us, our immune system can respond quickly, more efficiently

* Cow pox → virus infect cows

* Small pox → similar virus infect humans

* Antigens → Foreign substances that enter our body, stimulate our immune response

* Antibodies → Proteins that can bind to antigens & it can prevent antigens pathogenes to do harm for us by several ways

* Antibodies → can produce by a type of lymphocytes called B lymphocytes

↓
They have on their surface antigen binding receptors

When the receptor

(Y) ~~binds to~~

binds to antigens

B-lymphocytes get activated

& they start proliferating (cell division)

Thousands of activated B-cells generated from a single B-cell, some of the B cells become plasma cells

which mean basically antibodies.

Some become Memory cell

- During vaccination we are trying to generate Memory B cell or Memory T cell

↓
↓
↓
↓
↓
↓

Responsible for the

immune system response
which called secondary immune

response (produce more antibody)

more quickly & concentration in blood will be high
for long of time)

* vaccines are used to stimulate immune system to produce antibodies & memory cells

- **Antibodies** → proteins that can bind molecules on the surface of the pathogens & they can protect by different mechanisms

1 → Agglutination

- spike proteins used by corona to bind to our cells, production of antibodies after the injection of the vaccine against spike proteins

antibody → ~~just like a pulp~~ binds

→ binds

2 → opsonization

- Antibody bind to the surface of the pathogen enhancing **phagocytosis** of the pathogen for ex) Macrophage

→ ~~it's a way of attacking it, so it's a way of attacking it~~

3 neutralization

- prevent the bind to our cell
- or sometimes diseases are caused by the releasing of **toxins** of bacteria, so some antibodies can produce against toxins preventing the toxin from doing damage to our cells or tissue

4 Activation of complement

- antibodies bind to the surface of the cells activating the complement system which consist of proteins that are normally found in the blood & once they are activated they will form pores in the membrane of bacteria

& through this pores water ions will rush to bacteria causing the swelling of bacteria & finally the lysis of bacteria

5) antibodies can activate some types of immune system cells like nature killing cells & once they are activated they will recognize cells like viruses infected cells & cancerous cells

they bind on their surface of antigens & these antigens could be bound by antibodies.

so Nature killer cells they come to recognize the infected cells or the cancerous cell with antibody binding to its surface & the nature killer will mediate cytotoxicity - killing of cells -

* Types of vaccines

It vaccine could consist of a whole pathogen injected inside our body

1) live attenuated bacteria

weak, can't cause disease & all the bacteria is injected

2 \rightarrow **toxoids**

- Some vaccines protect us from toxins produced by bacteria

3 \rightarrow **pathogen-derived antigens**

- fragment from pathogen like membrane, carbohydrates

Injecting live ~~vaccine~~ \rightarrow *
attenuated bacteria
~~vaccine~~ \rightarrow ~~attenuated~~

multiple injections \leftarrow so dead or inactivated bacteria is more safe but less immunogenic (the response is less than the live)

- Many vaccines are considered subunit vaccines which mean are made of proteins
 \downarrow \rightarrow derived from pathogens (surface proteins)

* Hepatitis B virus vaccine is made of subunit vaccine & it made by Recombinant DNA tech.

One of the surface proteins of hepatitis B was cloned into plasmid & the plasmid was introduced to a host cell - yeast - & in the yeast was purified & then it could be used as vaccine

- Some subunit vaccines protect against some certain types of cancer.

• **Cervical** cancer which affect females could be caused by HPV

- certain types of cancer are caused by viral infections

• **Gardasil** is a subunit vaccine protect against HPV & cervical cancer in females

SARS-CoV-2 ~~is my virus~~ *

consist of + sense
RNA Genome

on the surface of the virus
we have spike protein & this spike
protein is used by the virus to bind
a receptor on the surface of respiratory
system cells called ACE2 that's why
most corona virus vaccine they involve the
use of spike protein as an antigen

- Microbiota

all the microbes inside
or on human body

- microbiome

genomes of
microbiota

* Why we study Microbiota through
microbiome?

Most of the microbiota we can't
grow them in the lab so in order
to study them we can only sequence their
genome

* Microbiota

↳ consist of fungi, viruses, helminths
bacteria & archaea

- Scientist found out that microbiome changes in many types of diseases like diabetist

- Scientist thought that microbiome found in the gut but it turned out there is a respiratory system microbiome

* Cancer, allergy, autoimmunity ...

↳ Might causes changes in the microbiome or might be infected by microbiome

- Many communication happen between our cells & the cells of microbiome

- Diet, genetics, drugs, environment

↳ Microbiome → jīv

* In general Microbiota they are harmless, they protect us from against pathogenic microbes, synthesis some useful molecules for us help in the process of digesting food or stimulate our immune response

- Because the genome of bacteria is small they could be synthesized in the lab

So in one exp. scientist designed a synthetic genome for bacteria called M. mycoides

They prepared hundreds of DNA fragments together inside E. coli because these hundreds of fragments have overlapping sequence so they were able to connect them together & the final size of the genome 1.8 Mb

- 10,000 DNA Fragment each
Fragment 1080 bp

→ ~~is it possible to construct~~
construct new types of bacteria carrying special genes so that we use this bacteria for specific purpose

- & they did cloning for all these fragments in E. coli & then they assembled the genome inside yeast

bigger
cell, S. cerevisiae

Then it was isolated & added to bacteria called M. capricolum

- Metagenomics

sequencing genome of entire communities of Microbes

→ soil sample (1000's of bacterial species) & you will extract DNA then you do sequencing

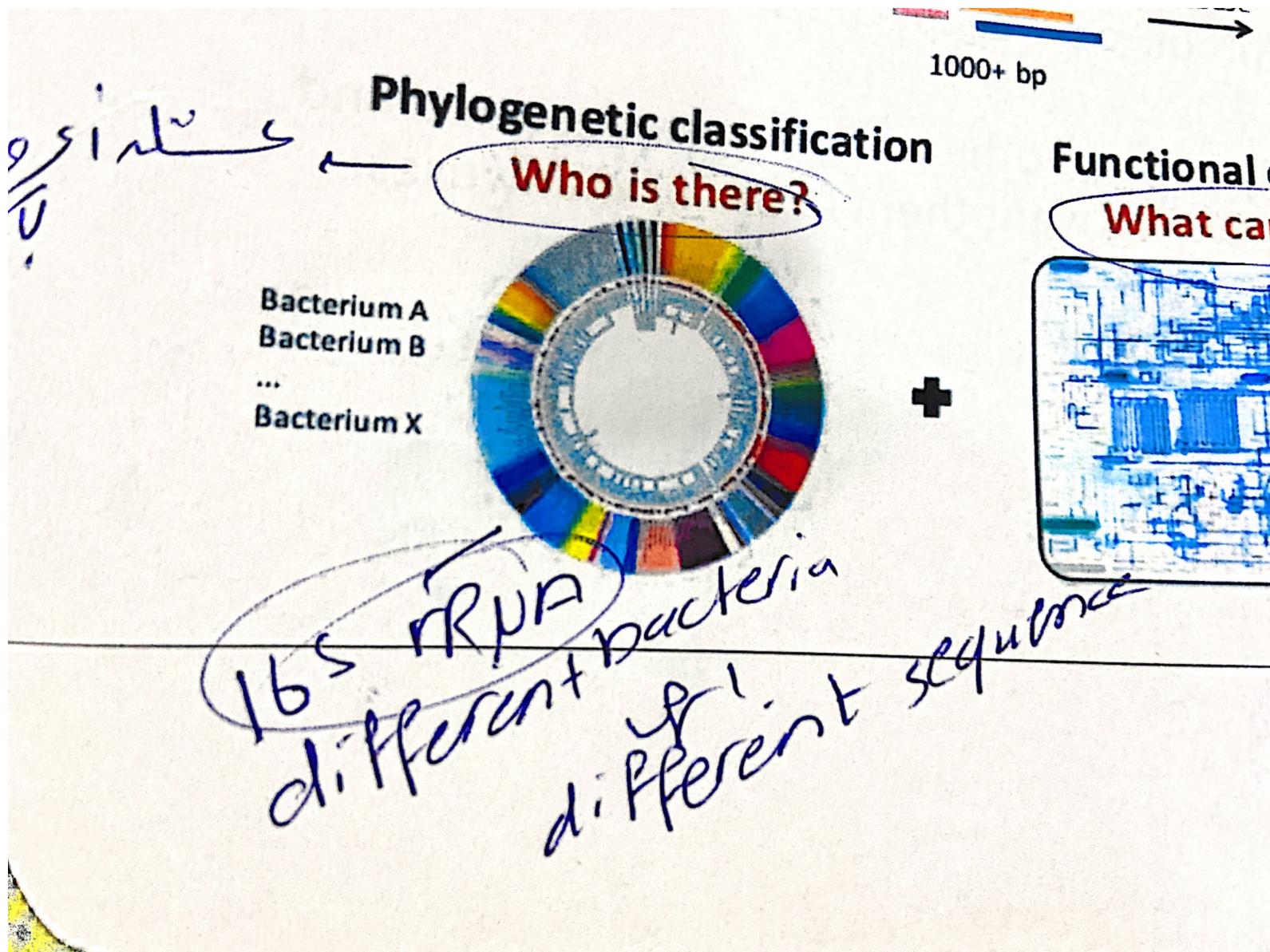
↓
Read

↓

contig

↓

assemble genome then you analyze all the assembled genome



Inc.

Microalgae

produce
biofuel

Synthesis
lipids