[Recombinant DNA technology and anti-viral drug]

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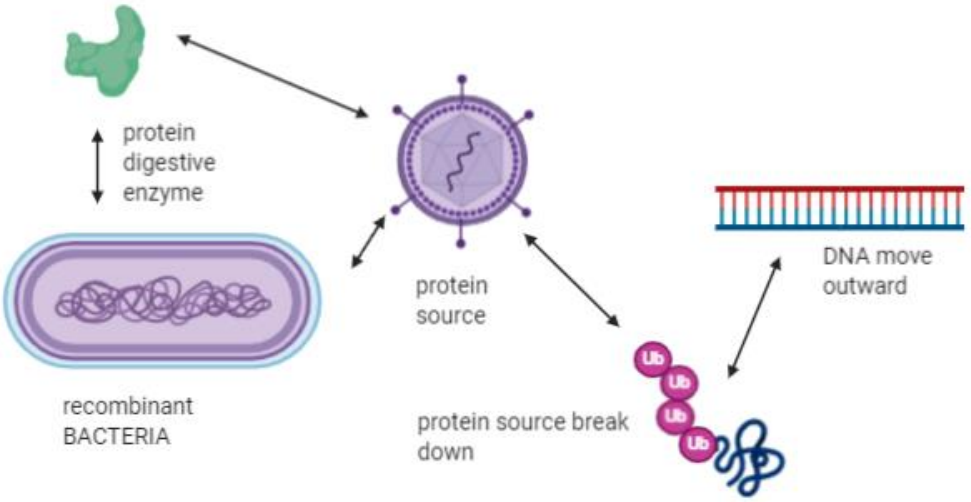
Recombinant DNA technology and anti-viral drug:

Abstract:

A virus is a small transmissible partial that replicates only inside the living cells of an organism. All viruses carry nucleic acid (DNA or RNA) and a protein coat, which enclose the nucleic acid. once a protein source reaches our body recombinant bacteria can produce a protein digestive enzyme against protein source(virus) they cause Inhibition of protein coating and genetic material move outward. There is a various factor which cause denaturation of protein. some of them are following: Heat, UV radiation, pressure, organic solvent, salt and heavy metals, altered PH, detergents etc.

UV radiation causes the creation of a mutation. heavy metals can damage the functions of organs such as the brain, kidney, lungs, live. organic solvents benzene, carbon tetrachloride, and trichloroethylene cause the creation of toxicity.



Advantages of recombinant DNA technology against a protein source:

- Target protein source inside the cell
- Act as a vaccination
- Protein source cannot be replicate
- Short half-life of enzyme 4-7 hour

Protocol of recombinant DNA:

- RNA ISOLATION
- Cdna synthesis
- restriction digestion and ligation
- Transformation

RNA ISOLATION:

- assemble the cells into 2 ml tubes
- rotate the cell suspension at high speed for 1 min using a centrifuge, reject the supernatant and resuspend in 1 ml of lysis buffer prewarmed at 65 ºC
- put-on 900 µl of acid phenol: chloroform and vortex for 10 sec
- Permit the 2 ml tube in the bench for 10 min at room temperature.
- rotate at high speed for 10 min at 4 °C, shift the supernatant to new 2 ml tube
- put-on 0.3 volume of 5 M sodium acetate and 0.7 capacity of acid phenol: chloroform blend slowly the tube and incubate on ice for 10 min.
- Top rotation for 10 min at 4 °C ,shift the supernatant to fresh 2 ml tubes.
- Put-on 0.1 volume of 3 M sodium acetate and the identical volume of isopropanol Incubate the tubes at -20 °C for 1 hour
- rotation at high speed for 10 min at 4 °C and throwaway the supernatant. clean the pellet with 500 µl ethanol (70 %)
- Centrifuge at 7,500 g for 5 min at 4 °C. Air dry the pellet for 5–10 min
- diffuse in 25 µl RNase free water. Put into a 1ml tube 10ug of RNA
- put-on 5 µl of DNase buffer, Put-on 1 µl of DNase and totally the capacity up to 50 µl with RNase free water.
- Incubate at 37 ºC for 30 m Put-on 500 µl ethanol (70 %)
- Centrifuge at 7,500 g for 5m at 4 °C. Air dry the pellet for 5–10 min

- utilize the nanodrop equipment to approach RNA concentration and standard

CDNA synthesis protocol:

- blend RNA sample and primer d(T)23VN in two sterile RNase-free microfuge tubes

Total RNA	1–6 µl
d(T)23VN (50 µM)	2 µl
nuclease-free H20	variable
Total Volume	8 µl

- Denature RNA for 5m at 70°C, Put-on the following ingredient to one tube

M-MuLV Reaction Mix	10 µl
M-MuLV Enzyme Mix	2 µl

- Incubate the 20 µl Cdna synthesis reaction at 42°C for 1h, deactivate the enzyme at 80°C for 5m. diluted reaction to 50 µl with 30 µl H2O for PCR

restriction digestion:

- defrost all reagents on ice. collect the reaction mix into 50 µL volume in a microfuge tube
- Put-on reagents in following sequence: water, buffer, BSA, DNA template, restriction enzyme
- slowly mix by tapping the tube. in a short centrifuge to settle tube contents. Produce negative control reaction without template DNA.
- Produce positive control reaction with template of known cutting site corresponding to the restriction enzyme of option representative Incubation time and temperature is 37°C for 1h
- . Incubation time temperature is 65°C for 20m, examine the results of your PCR reaction via gel electrophoresis.

water	50 µL
buffer	1X
DNA template	0.05 units/µL
Restriction enzyme	5-10 U per µg of DNA template (should not be over 10% of reaction capacity)

ligation protocol:

- Defrost all reagents on ice
- collect reaction blend into 10 µL volume in a microfuge tube
- Put-on reagents in following sequence: water, buffer, insert, vector, T4 ligase.

- slowly blend by stirring gently with the pipette tip, representative Incubation time and temperature is 15°C for at a minimum 4h

T4 DNA ligase	0.1 to 1 Weiss unit
Ligase buffer (with ATP)	1X
Vector	25 ng
Insert	75 ng

- Add 10 µL of water

DNA transformation protocol:

- defrost all reagents completely on ice, Put-on 1 µL of ligation reaction to defrost competent cells.
- slowly blend by tapping a tube of competent cells. incubate reaction on ice for 30m
- Heat shock the capable cell fusion by incubation for 30 to 60s in a 42°C water bath. Incubate tubes on ice for 2m
- Put-on 250 to 500 µL, SOC or LB media. Incubate at 37°C and spinning at 250 rpm
- hot selection plates to 37°C. layout 10, 50, and 100 µL of transformed cells on selection plates
- Incubate plates at 30°C whole night.

Competent cells	to 50 µL
Ligation reaction	1-5 µL
SOC or LB media	950 ml

Conclusion:

After performing Recombinant experiment, locate injection site and Inject the bacteria. the bacteria would start transcribing the gene to produce protein digestive enzyme against the virus. By injecting antigens into the body, the immune system can learn to accept them as invaders, produce antibodies, and remember them for the future. By injecting recomb bacteria into the body, they can help immune system. And bacteria are directly digest the virus protein.