

## DNA EXTRACTION ON TISSUE, HOMEMADE RECIPE.

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### Abstract :

Molecular biology techniques are key tools for a better knowledge of the living, researchers at the level of some laboratories have developed kits to have a quality of the biological sample that meets these current requirements. Among these kits, DNA extraction kits from any type of source have been created and are generally required by different service providers in the field of molecular biology. That said, these requirements become a brake in the field of research for low-income laboratories, given their unavailability on the local market and their prices. In this sense our DNA extraction study was carried out on a camel brain tissue for a subsequent PCR study in order to have a fairly precise idea about the quality of a DNA extracted (by traditional process ) from a tissue without going through an extraction kit that costs more money, gives less DNA and with acceptable quality by the current tools of molecular biology. These new extraction methods are postulated in the form of property sold at high prices such as kits, but according to our study the technique that was used for the extraction of DNA from a tissue is reliable especially if it is used from the middle phase after grinding of the sample.

**Keywords:** DNA extraction, molecular biology, camelids, brain tissue.

### Introduction:

"Molecular biology" is an avatar in the history of biology, which in fact corresponds to the irruption of the techniques of chemistry and physics in the field of life sciences. The knowledge of the living has been strongly influenced by the vision and ideas of an Austrian physicist, Nobel Prize and specialist in quantum mechanics, Erwin Schrödinger, who published in 1944 a little book entitled What is life? This book will have a profound impact on several generations of biologists. (Schrödinger E, 1946.)

Schrödinger sees life as a very complex system, storing and transmitting huge amounts of information that could be compacted into a "hereditary code" in the molecules that make up the chromosomes. The old idea of a vital force that animates organic matter disappears. The path is traced. It is now necessary to understand the stratagems that live cells use to process information. The crucial question is to identify the molecules of the living allowing the treatment of this information. (Berche.P, 2016).

The eminent evolution of molecular genetics now offers new perspectives: the miracle molecule, both imagined and represented by several scientists, has finally been isolated. Today man has access to this molecule and has the ability to manipulate or even modify it. (Ghada Jebah Nouairia, 2010).

With the development of new machines for life analysis, service providers are increasingly demanding good quality samples for error-free results. Currently to meet these requirements, researchers at the level of some laboratory have developed kits to have a quality and quantity of the biological sample that meets these current requirements nevertheless a study revealed that there were significant differences between the methods (conventional and kits) used in terms of concentration, but not in terms of purity

of the DNA. The highest amount was obtained by the saline method (305 µg) and the lowest with commercial kits, particularly with extraction with the Invitrogen® kit (7.33 µg). (Ghada Jebah Nouairia et al,2014).

However, these requirements become a brake in the field of research for low-income laboratories (especially at the level of developing countries), given their unavailability in the local market and their prices (generally not affordable). would cost 2.5 to 5 times more than conventional methods.(Ghada Jebah Nouairia et al,2014).

In this sense our study (DNA extraction) was carried out on a camel brain tissue for a subsequent PCR study in order to have a fairly precise idea about the quality of a DNA extracted (by traditional process ) from a tissue without going through an extraction kit that costs more, gives less DNA and with acceptable quality by the current tools of molecular biology.

### **Materials and Methods:**

The tissue (brain) is thawed at room temperature. Grinding of a part of the brain infected with Prion. Washing the ground tissue with TE 10/10 to remove red blood cells, 3 phases were obtained after centrifugation 2500 rpm for 15 min. Coarse phase (large fragment) is at the bottom of the tube, it is directly added 90 µl of PK 100 mg / ml and 11 ml of SLB. Medium phase (in the middle of the other two phases) of white color. Clear phase (reddish) which is on the surface.

These last two phases were transferred to two other tubes in addition to TE 10/10 (up to 25ml) and then put in the freezer for 30 min then they are subjected to centrifugation at 2500 rpm.

The pellet (medium phase) is recovered for it added 90 µl of PK 10 mg / ml the supernatant is removed, the pellet of the medium phase is important (6 ml) is added to this pellet 10 ml SLB. The pellet of the clear phase (1 ml) is added 30 µl / ml PK 10 mg / ml and 1.5 ml SLB.

Then the three phases spend 1 hour at 56 ° C in a water bath. 0.5M NaCl 5M for the clear phase, 2M NaCl 5M for the coarse and medium phase is then added. This will allow a separation of two phases:

- A phase containing DNA.
- A phase containing the membrane debris of cells.

Thus many unwanted proteins (PK + cell debris) are removed from the solution after being driven to the bottom of the tube. The supernatant thus formed contains DNA. This is the Salting-Out phenomenon. After vigorous stirring followed by centrifugation at 4000 rpm for 15 minutes (so that the two phases are separated), the resulting supernatant is transferred to another tube, avoiding peeling off the pellet. Two volumes of absolute ethanol cold from that of the supernatant are added to the tube. It is noticed that as soon as the ethanol is added, the solution becomes whitish and the DNA starts to precipitate (the ethanol condenses the DNA).

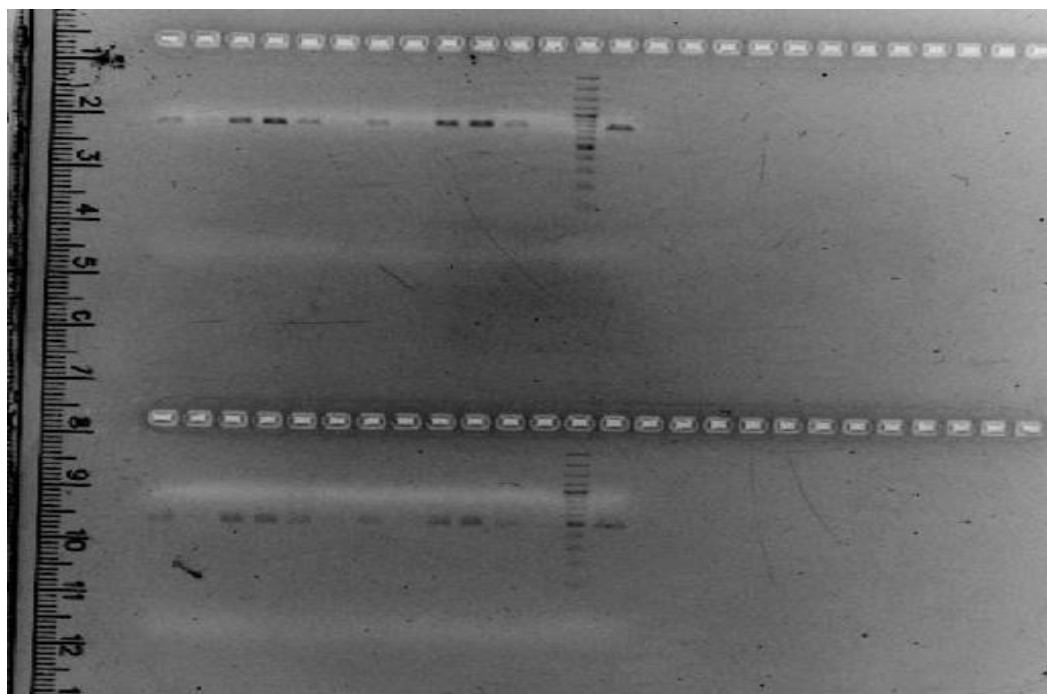
After gentle agitation, the DNA precipitates in the form of filaments which compact rapidly into a whitish mass visible to the naked eye called: jellyfish which will then be recovered in a sterile Eppendorf tube, then washed with cold ethanol at 70 ° C. % and 100% and dried. Dissolution of the jellyfish is carried out in 200 to 500 µl of TE buffer 10/1 (Tris / HCl: 10 mM, EDTA: 1 mM, pH = 8.0) according to the size of the jellyfish and gentle stirring at room temperature for at least 24h to finally have a completely dissolved DNA ready to be used (assay, PCR ...)

The DNAs obtained were diluted 1/100 to pass the PCR in the laboratory of Istituto Superiore di Sanità, Rome..

### **Results:**

The results of the PCR on the DNA extracted from the three phases are shown in the following figure (figure 1).

**Figure 1:** Results of the PCR on DNA extracted from the three phases of the dromedary brain.



*PC (coarse phase), PM (medium phase), PC (clear phase), The samples are deposited in the same order 4 times in a row.*

The results of PCR amplification of our DNA and agarose gel electrophoresis show that the DNAs extracted by our method are generally good and that the technique we used can replace the kits used in this type of extraction. Indeed, it is noted that all the DNA have passed the amplification test, apart the DNA extracted from the clear phase. It is also apparent from Figure 1 that the best samples are those extracted from the middle phase in terms of quality (reproducibility of the results at the four deposits) and quantity (density of the amplimers).

### **Conclusion:**

New extraction methods are applied in the form of property sold at high prices such as kits, But according to our study, the technique used for the extraction of DNA from a tissue (brain of a dromedary) is reliable especially if it is used from the middle phase after grinding of sample. It is also obvious that using such a technique is less expensive than using kits sold by international firms, which is a positive asset for laboratories that are mostly in developing countries.

It is obvious that for a better generalization of the technique the use of more samples and of different origin is desirable.

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