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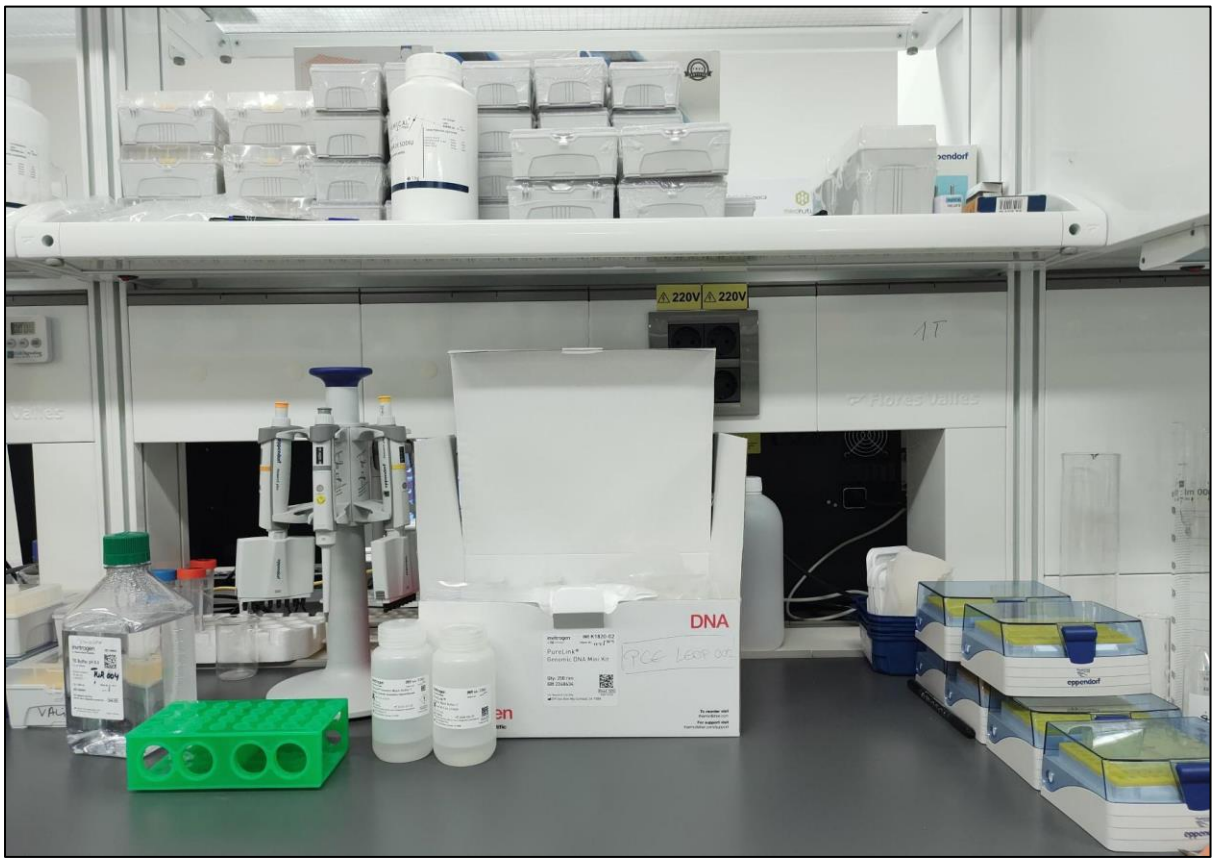
The Education, Scholarships, Apprenticeships and Youth Entrepreneurship Programme – EEA Grants 2014-2021

#7 DNA EXTRACTION FROM CELL CULTURE, WHOLE BLOOD OR TISSUE

Research Center for Advanced Medicine – Medfuture (UMFIH) can perform molecular assays based on RNA, DNA or proteins in order to evaluate the expression and the modification of these molecules after different treatments or stimuli or as potential biomarkers for diseases. More info can be found at: https://medfuture.umfcluj.ro/.



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DNA extraction from biological samples (e.g. cell and tissue samples) is the incipient protocol in all assays involving DNA analysis between different types of samples (e.g. normal and pathological tissue, control and treatment samples) in order to evaluate the molecular changes that take place at the genomic level. The proper execution of this protocol is vital for the ongoing of the next analysis, the majority of them requiring strict sample standards like DNA integrity and purity.

IMPORTANT!

- DNA extraction is usually made with commercial kits (e.g. Invitrogen) that use separation columns based on silicon dioxide for DNA capture at alkaline pH.
- In the final step, the DNA is resuspended in ultrapure water in a maximum volume of 100ul. The final volume will dictate the amount of DNA for each microliter. If you suspect low concentrations of DNA try using a small volume of dilution water as DNA protocols that follow DNA extraction usually require a small amount of sample. Even so, in case of a diluted sample it is posible to use evaporation tehniques, but is better to be avoided.

a. 200 uL sample (cells in PBS1X; homogenized tissue piece in PBS1X; or 200uL whole blood) is transferred to a sterile 1.5 mL tube
b. Add 20 µL of Proteinase K and vortex the sample
c. Add 20 uL of RNase, homogenize with a pipette and incubate for 5 minutes at room temperature
d. Add 200 uL of Lysis Solution and homogenize by vortexing for 5 seconds
e. The samples are incubated for 10 minutes at 55°C
f. Add 200 uL of 100% Pure Ethanol, and vortex for 5 seconds
g. The 640 uL mixture is transferred to a silica filter column and the sample is centrifuged at 12.000xg for 2 minutes at room temperature
h. The liquid in the collector tube is discarded and the column with the filter is placed back on that collector tube
i. Add 500 uL of washing detergent 1 (rich in Guanidine HCl) and centrifuge the samples at 12.000xg for 2 minutes, at room temperature
j. Throw away the liquid from the collecting tube and put the column back in its place
k. Add 500 uL of washing detergent 2 (rich in pure ethanol) and centrifuge at maximum speed (17895xg) for 2 minutes, at room temperature
l. The collected liquid is discarded and the column is placed back on the collection tube

m. The washing step with detergent 2 is repeated
n. After washing, change the collection tube and centrifuge at maximum speed (17895xg) for 2 minutes, at room temperature to remove the ethanol residue from the column
o. The silica column is transferred to a sterile 1.5 mL eppendorf tube
p. Carefully add at least 30 uL of ultrapure water (maximum 100 uL) over the filter and incubate the sample for 2 minutes at room temperature. This allows the DNA to detach from the filter.
q. Centrifuge at maximum speed (17895xg) for 2 minutes, at room temperature; the obtained solution is analyzed spectrophotometrically using a small volume to obtain data about DNA concentration and purity
r. Samples are stored at -80°C.

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