Birzeit University

**Biology and Biochemistry Department**

**BIOL 244**

**Cell Biology lab**

**Experiment 5: Analysis of subcellular fraction II.**

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**Title:** **Analysis of subcellular fractionsII.**

**Objectives: measurement the DNA concentration in nucleus from Previously Experiment (#Experiment 3), nuclear fraction examination under a microscope and measurement size of nucleus and get to know her.**

**Introduction:**

**The accuracy of estimates of DNA concentration are importance factors for efficient use of DNA samples in high-throughput genotyping and sequencing analysis, also it important for many applications in molecular biology. Therefore, it is important to identify the DNA in the cells and study it, each of cells has a special DNA containing genetic information. We have two optical technologies used quantify nucleic acids: UV-Vis measurement and fluorescence measurement. In this experiment, we chose a UV technique and used a spectrophotometers like a method to determine and measuring nucleic acid purity and protein ratio, the nucleic acid and protein have a spectrophotometric absorbance of a sample at 260nm compared to the value measured at 280nm and the number was closer to 1.8 – 2.0 .We used A260/230 Nucleic Acid Ratios (This is a secondary measure of nucleic acid purity).**

**Materials:**

**Spectrophotometer- Micropipettes- 18 test tubes- 0.25M sucrose- Micropipettes – Cuvettes- Aceto-orcein - All fraction from experiment 3**

**Methods:**

For this experiment was isolated The homogenate fraction, resuspend fraction, mitochondria fraction, and lysosomes fraction, from the rat liver by killed a rat and removed its liver and put it in the ice-cold, we was cut 1.2g from the liver, we added amount of HB-buffer to the liver(that help to remove the free fatty and lipids) and chop it into small pieces with scalpels, then homogenized by Place the mixture in a homogenizer tube and homogenize with a motor homogenizer (up to 10-15 strokes) while keeping it chilled, then centrifuged the homogenate at 400xg for 5 minutes that to remove the cell debris and unbroken cells, after that in the centrifuge tubes appeared pellet( un broken cell ) we got rid of it, and the supernatant(SN1) that what we need this is the whole homogenate, we collected it volume **(v\*)** and talked 2ml and saved it on ice on tube H; to use it later, we repeat the centrifuge for the rest of the homogenate(SN1) for 15 minute at 4000xg, in the centrifuge tube we had a pellet (nuclei) we resupended it in the same volume of **v\*** of H-buffer and saved it on ice in tube N, and supernatant(SN2) we placed into a clean centrifuge tube to centrifuge it at 12000xg for 15 minute the pellet which resulted is the mitochondrial fraction we was resuspend it in the same volume of **v\*** of H-buffer and saved it on ice in tube M, and the supernatant(SN3) recorded its volume, to (SN3) we added 80 mMCaCl2 to yield final concentration of 8mM of CaCl 2 in the **sample 1 ml of CaCl per 9 ml of supernatant**, stirred gently and centrifuged at 20000xg for 25 minute at 4 C , this give a microsomal fraction enriched in lysosomes we resuspend the pellet in the same volume of **v\***of 150 mM KCL in 10 mM HEPES buffer, PH7 saved it on ice in tube L,we removed the supernatant, to determination the DNA concentration we was prepared 18 tube for the whole fraction ,6 tubes labelling for Homogenate , 6 tubes for Mitochondrial fraction, and 6 tubes for Nuclear fraction, tube number 1 from all was the stock tjat we was used to do serial dilution of each fraction by increased ratio by 5 as how it shows in table 1 when do that we mixed the fraction well, the spectrophotometer was turned on and the wavelength was set at **A260** to measure the absorbance for all 24 tubes , for the absorbance between 1.5 and 2 we was re measured it at **A280** to determine the dilution factor for it by used this law :

**DNA concentration (µg/ml)=A260\*50 µg/ml\*dilution factor**

**Results:**

* Determine the DNA concentration and take readings and determine the size of it.

Absorption of dilute tubes of nuclear fraction, homogeneous and mitochondria at 260n to 280n for some tubes of them:

**Table 5.1: Serial dilution for each fraction.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tube** | **0.25M**  **Sucrose(ml)** | **Previous tube (ml)** | **Vfinal (ml)** | **Final dilution ratio** |
| **1** |  | 1.25ml stock solution |  |  |
| **2** | 1 | 0.25 | 1.25 | 1:5 |
| **3** | 1 | 0.25 | 1.25 | 1:25 |
| **4** | 1 | 0.25 | 1.25 | 1:125 |
| **5** | 1 | 0.25 | 1.25 | 1:625 |
| **6** | 1 | 0.25 | 1.25 | 1:3125 |

**Table 2: Absorbance for Nuclear fraction.**

|  |  |  |
| --- | --- | --- |
| Tube Number | Absorbance | DNA concentration |
| N1 | 20.02 |  |
| N2 | 1.442 | 14.42 |
| N3 | 0.495 | 9.9 |
| N4 | 0.280 | 0.112 |
| N5 | 0.239 | 0.0192 |
| N6 | 0.236 | 0.00377 |

**Table 3: Absorbance for Homogenate fraction**

|  |  |  |
| --- | --- | --- |
| Tube number | Absorbance | DNA concentration |
| H1 | 20.02 |  |
| H2 | 3.565 | 35.65 |
| H3 | 1.100 | 22 |
| H4 | 0.429 | 0.1716 |
| H5 | 0.273 | 0.0218 |
| H6 | 0.232 | 0.00371 |

**Table 4. Absorbance for mitochondrial fraction.**

|  |  |  |
| --- | --- | --- |
| Tube number | Absorbance | DNA concentration |
| M1 | 2.310 |  |
| M2 | 0.722 | 2.888 |
| M3 | 0.336 | 6.72 |
| M4 | 0.262 | 0.1048 |
| M5 | 0.253 | 0.0202 |
| M6 | 0.251 | 0.0040 |

**Discussion:**

**To determine the Nucleic acid concentrations (DNA,RNA) and protein we was measured the absorbance of ultraviolet light (maxima at 260-280nm).We could use to estimate the concentration of dsDNA in a fraction assuming A260 of 1 meaning that the fraction contains 50 μg / mL of dsDNA, in this experiment we determined conc. for each tube of the fractions.**

**We noticed from tubes 2,3 and 4 when the absorbance in the tubes decrease the dilution ratio was increase.**

**We showed the lowest concentration of DNA was in Mitochondrial, the second concentration was in the Nuclear fraction and in Homogenate fraction include the highest value. (Because it have all the organelles)**

**Questions:**

**Q1: What does endosymbiotic theory suggest?**

**The Endosymbiotic theory one organism that able to live in another organism and grow a symbiotic relationships inside it such as mitochondria and chloroplast, they think it develop from prokaryotes (mitochondria and chloroplasts it will be phagocytosed and it found inside host cells but no digested)**

**Endosymbiotic theory goes back over 100 years.**

**Q2: What is the aceto-orcein and what does it do?**

**purple-red color stain.**

**Q3:** **what was the reason that we have used plastic cuvettes in previous experiments and quarts cuvettes in this experiment?**

**Quartz cuvettes use a UV on (260-280) nm.**

**Plastic cuvettes use a visible spectrophotometer on 600 nm**

**Both naturally occurring and artificial quartz crystal contain at least ninety-nine percent silicone dioxide, while cut glass crystal only consists of up to eighty percent silicone dioxide.**

**Glass has a random molecular structure but quartz have a symmetrical structure and naturally occurring so the chemical structure of quartz is stronger than glass making it able to handle a greater extend of chemicals.**

**Quartz has a higher temperatures. This makes quartz a valuable material that can be used as protective covering due to its resistance to high pressure. It can also be utilized in harsh environments with high temperatures as a substitute for glass**

**Quartz has a bigger transmission range than glass.**

**References:**

[**https://www.qsiquartz.com/difference-between-glass-and-quartz/**](https://www.qsiquartz.com/difference-between-glass-and-quartz/)

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[**https://www.thermofisher.com/ps/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-quantitation.html?gclid=EAIaIQobChMImfH4rdm\_8AIVD7LtCh2bgQdaEAAYASAAEgJib\_D\_BwE&ef\_id=EAIaIQobChMImfH4rdm\_8AIVD7LtCh2bgQdaEAAYASAAEgJib\_D\_BwE%3AG%3As&s\_kwcid=AL!3652!3!393988169001!b!!g!!%2Bnucleic%20%2Bacid%20%2Bpurity&cid=bid\_pca\_aqb\_r01\_co\_cp1359\_pjt0000\_bid00000\_0se\_gaw\_nt\_pur\_con&fbclid=IwAR0r\_OpFim\_R-\_OpGxxeiGJc-wyCZujNuQMTRNrIzSKn6N97Fio6foybGWc**](https://www.thermofisher.com/ps/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-quantitation.html?gclid=EAIaIQobChMImfH4rdm_8AIVD7LtCh2bgQdaEAAYASAAEgJib_D_BwE&ef_id=EAIaIQobChMImfH4rdm_8AIVD7LtCh2bgQdaEAAYASAAEgJib_D_BwE%3AG%3As&s_kwcid=AL!3652!3!393988169001!b!!g!!%2Bnucleic%20%2Bacid%20%2Bpurity&cid=bid_pca_aqb_r01_co_cp1359_pjt0000_bid00000_0se_gaw_nt_pur_con&fbclid=IwAR0r_OpFim_R-_OpGxxeiGJc-wyCZujNuQMTRNrIzSKn6N97Fio6foybGWc)

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