INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO



Q1-1

English (Official)

35th International Biology Olympiad

Practical Exam: Molecular Biology

General Instructions

Overview of the Practical Exam

The molecular biology practical exam is divided into five key sections, each focusing on different aspects of molecular biology and cellular techniques. This exam is a blend of theoretical knowledge assessments and hands-on experimental tasks.

In this practical exam, you have 90 minutes to complete **FIVE INDEPENDENT tasks.** You can perform the tasks in any order. Here is a brief overview of what to expect:

Part A: *Fundamental Mechanisms Underlying the Foreign Body Response* (12 points)

This section is theoretical and involves analyzing data from experiments on the foreign body response in mice. You will evaluate cytokine profiles and their impact on the formation of multinucleated giant cells from macrophages.

Part B: *Plasmid Purification* (34 points)

This is an experimental section where you will perform steps to purify a plasmid using the alkaline lysis method. This part requires practical work, including using spectrophotometry for quantitative and qualitative DNA analysis.

Part C: *Advanced Imaging Techniques* (14 points)

This section is theoretical. You will match complex biological scenarios with appropriate advanced imaging techniques, demonstrating your understanding of the capabilities and applications of different microscopy methods.

Part D: *Numerical Aperture in Microscopy* (12 points)

This is a theoretical part focused on the concept of numerical aperture and its importance in microscopy. You will solve problems related to the numerical aperture and its effect on image resolution and depth of field.

Part E: *Approaches of Studying Actin Filaments in Macrophage Fusion*(7 points)

This part involves both theoretical knowledge and predictions about experimental setups. You will assess various molecular and cellular techniques for studying actin dynamics during macrophage fusion.

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Keep in mind:

- An answer sheet will be provided. **Write your answers in the answer sheet. Only answers provided in the answer sheet will be scored.**
- Please remember to write your name and your student code in the given boxes.
- Check the equipment and materials provided according to the table below. If anything from the table is absent, please raise a question "?" mark.
- During experiments, ensure that you wear gloves, and handle the equipment and samples carefully.
- Make sure to manage your time effectively to complete all sections within the 90-minute duration.
- Stop answering as soon as you hear the stop signal at the end of the exam.
- Materials, papers, or equipment should not be taken out of the examination room.
- DISCARD LIQUID WASTE INTO A 250-mL PLASTIC BEAKER (liquid waste).
- DISCARD USED TIPS, TUBES, TISSUES IN THE YELLOW BIN (solid waste)
- Any spilled solutions, samples or equipment damaged by you will not be replaced. Use the following cards to ask for water/washroom/help.

Drinking water card	**Washroom card**	**Other queries card**
		

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Equipment and Materials

Please ensure that all necessary reagents and materials are available on your bench.

Name	**Quantity**
Drinking water (Blue) card	1 piece
Other Queries (Red) card	1 piece
Washroom (Yellow) card	1 piece
Centrifugation cards (1-7)	7 pieces
Digital clock	1 piece
Timer	1 piece
Safety goggles	1 piece
Tissue paper	1 box
Wash bottle with water (dH_2O)	1 piece
250-mL plastic beaker for liquid waste	1 piece
Yellow bin for solid waste (tips, tubes, tissue, gloves)	1 piece
Ice bin (under the desk)	1 piece
Pen, pencil, ruler	1 piece of each
Resuspension Buffer B1 (red label)	300 µL
Lysis Buffer B2 (blue label)	300 µL
Neutralization Buffer B3 (yellow label) (on ice)	500 µL
Wash Buffer 1 W1 (green label)	300 µL
Wash Buffer 2 W2 (orange label)	500 µL

Equipment and Materials

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Please ensure that all necessary reagents and materials are available on your bench.

DNA Elution Buffer **E** (white label)	70 µL
Spin Column with the collection tube without lid	1 piece
1.5-mL microcentrifuge tube for elution	1 piece
2-mL microcentrifuge tube for plasmid dilution	1 piece
Water (ddH_2O) in 15-mL tube will be used for plasmid dilution	1 piece
Quartz spectrophotometer cuvettes (1 cm – path length) on white holder	2 pieces
UV/V spectrophotometer	1 piece
Overnight bacterial culture for plasmid isolation (labeled **PI**)	1 tube
100-1000 µL Micropipette	1 piece
20-200 µL Micropipette	1 piece
0.5-10 µL Micropipette	1 piece
Tips for 100-1000 µL micropipette	1 box
Tips for 20-200 µL micropipette	1 box
Tips for 0.5-10 µL micropipette	1 box
LB culture broth for blanking (labeled Blank)	1 tube
Bacterial culture for OD measurements (labeled with numbers 1-5)	5 tubes

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PART A. Fundamental Mechanisms Underlying the Foreign Body Response (12 points)

A young researcher is studying the formation of multinucleated giant cells (MGCs) from macrophages, a crucial part of the foreign body response to implanted materials. MGCs tend to form constantly during the first month after implantation accompanied by significant increase in cytokine amount that triggers the fusion. To identify the key cytokines involved, he used C57BL/6J mice (wild type) and implanted four types of biomaterials into their peritoneal cavities. Over five weeks, he collected cytokines from the implantation sites, producing preliminary results shown in Figure 1. Further, the cells on the surface of each implant has been stained with nuclear and cytoplasmic dyes to calculate the fusion index (the ratio of nuclei within MGC divided to the total number of macrophage and MGC nuclei represented in percentage). Additionally, similar experiments were conducted with IL-6, IL-4, TNF and IFN-γ knockout (KO) mice models. The fusion index data is depicted in Table 1.

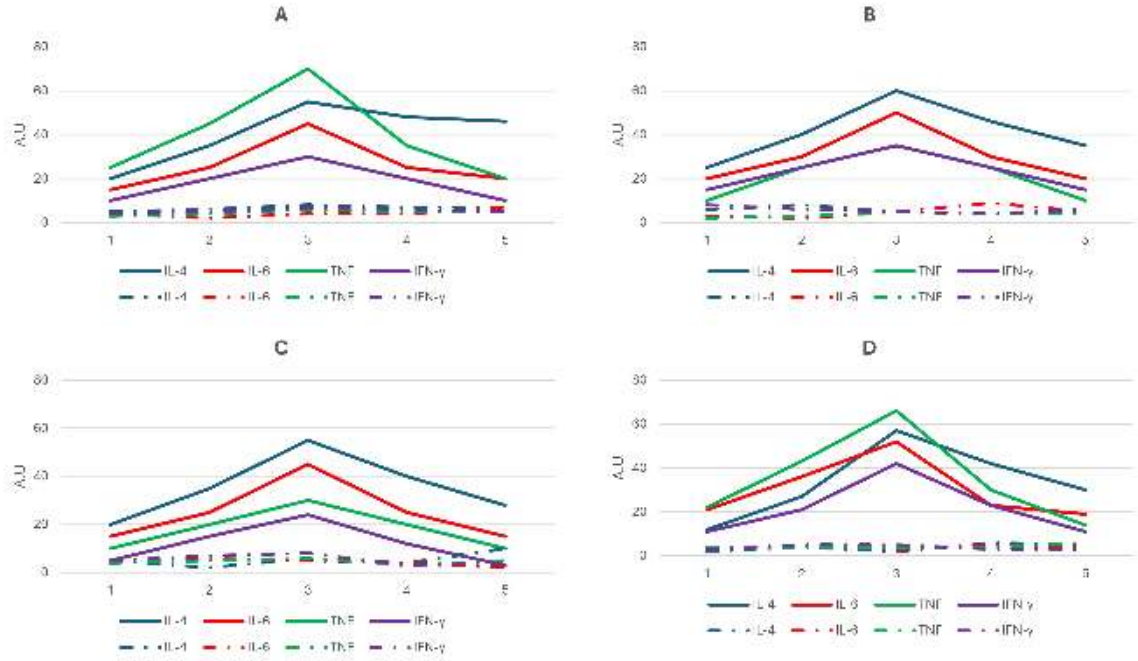


Figure 1. The results of cytokines produced as a response to the different types of implanted biomaterials. The dashed lines represent the control group where only a buffer solution was added. A.U. - Arbitrary Unit of cytokine produced. Weeks are labeled with numbers 1-5. The implanted materials are labeled with the letters A-D (A: Plastic, B: Metal, C: Meshed Polymer, D: Silicon).

Table 1. Fusion index (%) data based on different implant materials and mouse genotypes.

	Mouse genotype				
Implant material	**IL-6$^{(KO)}$**	**IL-4$^{(KO)}$**	**TNF$^{(KO)}$**	**IFN-γ$^{(KO)}$**	**Wild type**
Plastic	27.8	6.8	17.9	33.9	29.6
Metal	36.9	9.1	23.7	21.8	34.9
Meshed Polymer	15.8	2.9	28.9	19.7	27.6
Silicon	19.7	8.7	32.1	16.8	30.5

Question 1.1: Based on the comparative analysis of cytokine concentration profiles over 5 weeks, which cytokine profile is the most indicative of a potent candidate contributing to multinucleated giant cell formation from macrophages? 3.0pt

A) The cytokine with the highest peak concentration within the first week of implantation across different materials.

B) The cytokine with the lowest overall concentration increase.

C) The cytokine that shows the most rapid post-implantation concentration increase.

D) The cytokine that remains elevated for the longest duration post-peak concentration.

Question 1.2. For further studies, the researcher needs to decide which cytokine would be the most promising candidate to use for *in vitro*-based experiments. Which of the following cytokines should the researcher use? 3.0pt

A) IL-4

B) IL-6

C) TNF

D) IFN-γ

Question 1.3. The researcher studies macrophage fusion by isolating pro-inflammatory macrophages from thioglycolate-treated mice and culturing them on various substrates to form multinucleated giant cells. After adding cytokines and incubating for 24 hours, the cells are fixed and stained with Wright's stain to visualize cell boundaries and nuclei, as shown in Figure 2. The Principal Investigator questioned if the observed phenomena were due to fusion or mitosis without cytoplasmic division. To confirm fusion, which imaging tool would be the most appropriate to use? 3.0pt

A) Transmission electron microscopy

B) Fluorescent imaging on fixed macrophages

C) Phase contrast live-cell imaging on live macrophages

D) Time-lapse imaging on fixed macrophage sample

Question 1.4. Based on Figure 2, the researcher is required to select only the two most suitable candidate surface for subsequent experimentation (Solvent option was not considered as a control for this particular question). Fortuitously, he has access to a chart (Table 2) detailing the characteristics of various glass-coating surfaces concerning their imaging capabilities. Utilizing the information from the provided table, identify two plating substrates that would optimize the range of potential variations in his future experimental configurations. Among these selections, one substrate should serve as a negative control. Put a corresponding letter into each selection. 3.0pt

1.Clean glass 2.Petrolatum 3.Oleamide 4.Paraffin 5.Permanox (use 1-5 labels for answer)

1.4.1 Substrate for fusion ____

1.4.2 Substrate as a negative control ____

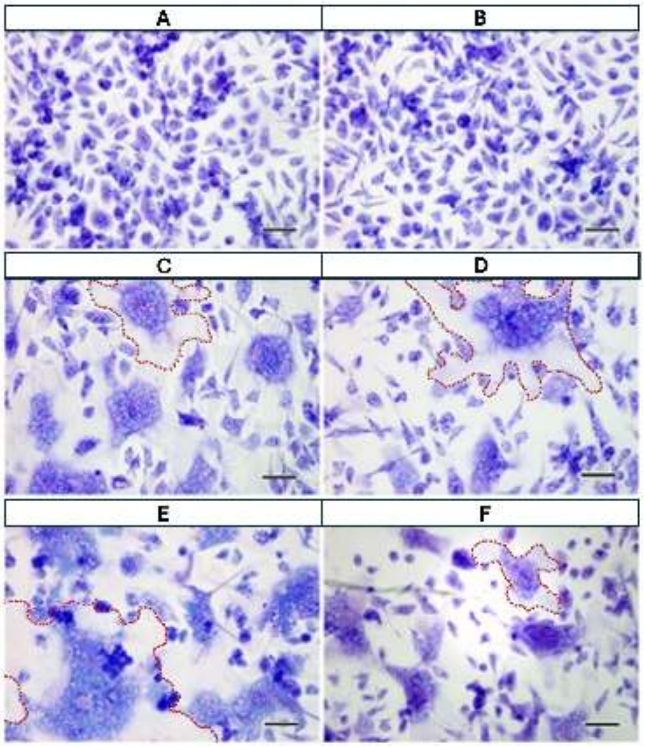


Figure 2. Stained and fixed macrophages seeded on different surfaces. A - Clean glass, B - Solvent, C - Petrolatum, D - Oleamide, E - Paraffin, F - Permanox. The representative multinucleated giant cell is labeled with a dashed line.

Table 2. Imaging capabilities of different glass coating types.

Imaging techniques	Paraffin	Oleamide	Petrolatum	Permanox	Clean glass
Phase contrast	**+**	**+**	**+**	**"+"**	**+**
Brightfield	**+**	**+**	**+**	**"+"**	**+**
DIC	**+**	**+**	**N/T**	**-**	**+**
Widefield	**+**	**+**	**+**	**"+"**	**+**
Confocal	**+**	**+**	**+**	**"+"**	**+**
TIRF	**+**	**+**	**N/T**	**-**	**+**
PALM	**+**	**+**	**N/T**	**-**	**+**
SIM	**N/T**	**N/T**	**N/T**	**N/T**	**N/T**

+ - suitable

- - unsuitable

DIC - differential interference contrast

TIRF - total internal reflection fluorescence

PALM - photo-activated localization microscopy

SIM - structured illumination microscopy

"+" - only with low magnification objective

N/T - Not tested

PART B. Plasmid purification (34 points)

The researcher observed macrophage fusion via membrane protrusions driven by cytoskeletal changes, especially in the actin network. To study actin's role, he planned live-cell imaging with GFP-tagged actin (GFP -Green Fluorescent Protein), requiring macrophage transfection with an eGFP-Actin plasmid. After ordering a bacterial culture with this plasmid, he needs to increase and purify it for transfection. In this session, you'll purify the plasmid.

Quantitative and Qualitative Analysis of DNA via Spectrophotometry

Spectrophotometers are instruments engineered to measure the extent of light absorption by molecules (Figure 4).



Figure 4. The spectrophotometer SP-UV1000 is used in the experiment.

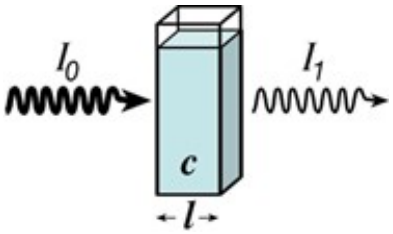
Beer-Lambert law

When a beam of incident light of intensity (I_0) passes through a solution of concentration (*c*), and the path length traveled by the light beam through the sample is *l*, the intensity of the transmitted light beam is I_1. The Beer-Lambert law establishes that the absorbance (A_λ) at wavelength (λ) is directly proportional to the concentration (*c*) of the solution and the path length (*l*) through which the light travels:

$$\mathbf{A_\lambda = \varepsilon c l}$$

where:

- A_λ is the absorbance at wavelength λ.
- ε is the molar extinction coefficient mL (µg cm)$^{-1}$.
- *c* is the concentration of the compound in solution µg (mL)$^{-1}$.
- *l* is the path length in centimeters (cm).



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DNA exhibits maximal absorption at 260 nm. In contrast, proteins have an absorption peak at approximately 280 nm, principally arising from the aromatic amino acids tryptophan, tyrosine, and phenylalanine, as well as from disulfide bonds.

Absorbance is a dimensionless quantity that indicates how much light is absorbed by the solution. The extinction coefficient (ε) is a measure of how strongly a chemical species absorbs light at a given wavelength.

Utilizing the Beer-Lambert law, the concentration of DNA in a solution can be determined by measuring the OD at the absorption peak (λ = 260 nm). For double-stranded DNA, the molar extinction coefficient is (ε = 0.02 mL (µg cm)$^{-1}$) at this wavelength. Hence, an A260 reading of 1 corresponds to a 50 µg/mL concentration of double-stranded DNA in a standard cuvette with a path length l of 1 cm.

Before starting the plasmid purification process, you will receive five Eppendorf tubes, labeled 1 through 5, each containing *E. coli* cultures at various OD (A600). These densities correspond to distinct stages of bacterial growth. Your task is to graph OD values from each tube to visualize the growth curve. From this analysis, determine which tube's contents are most suitable for plasmid purification. Please, plot the curve on a graphing paper given on the answer sheet.

Part B1. Bacterial OD (A600) measurement (12 points)

To measure the optical density (OD) of a bacterial culture using a spectrophotometer, follow these step-by-step instructions:

1. Important, before you start, wash the cuvettes provided for you thoroughly.
2. The cuvette has 2 sides: one is clear (do not touch this side with your fingers), and the other one is frosted (this is the side where you can hold the cuvette). The light should path through the clear side of the cuvette.
3. To set up and work on the spectrophotometer use the following buttons on the keypad

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The function required	The button on the keypad	Operation
Set the wavelength		Press this button to enter the wavelength required
Multipurpose button, which functions according to what is on the display screen.		When setting up the wavelength: select “ok” by pressing “left” button to choose the number and “enter” the wavelength by “right” button to start measurements with the entered wavelength
Roll up the menu options		The button is used to change the values, when setting up the wavelength
Roll down the menu options		The button is used to change the values, when setting up the wavelength
Calibrate 100% T/0Abs		This one is pressed once you insert your “blank” solution to calibrate the spectrophotometer

Setting the wavelength:

1. Press the **GOTO**λ button to start entering the wavelength.
2. Choose **Roll Up** or **Roll Down** buttons to move between the numbers.
3. When setting up the wavelength: select “ok” button (as shown on the display) by pressing “left” button to choose the number.
4. Press “enter” (as shown on the display) button on the right to set the chosen wavelength.

Calibration/Blanking:

1. Set the wavelength to 600 nm following the instructions above;
2. Using a pipette, fill a cuvette with the entire volume of blank solution (sterile broth used for growing the bacteria, without any cells) labeled **Blank**. Note: you will only have **2 quartz cuvettes**, please use one for calibration only, and the other one for measuring bacterial samples 1-5;
3. Insert the cuvette into the spectrophotometer's cuvette holder (number 1; the one closest to you), ensuring it's oriented correctly according to the direction of light. The cuvette has 2 sides: one is clear (do not touch this side with your fingers), and the other one is frosted (this is the side where you can hold the cuvette). The light should pass through the clear side of the cuvette.

4. Close the lid of the spectrophotometer.
5. Press the "ZERO" button to set the absorbance to zero with the blank solution. Wait until you see a smiling face on the screen of the spectrophotometer.
6. Take off the cuvette and use it to blank the spectrophotometer before measuring each tube (1-5).

Measurement:

1. Mix your bacterial culture gently by inverting tube number 1 to ensure an even distribution of cells.
2. Using a pipette, transfer the entire volume of a sample of the bacterial culture into a clean cuvette. Ensure no bubbles are formed as they can affect the accuracy of the measurements.
3. Insert the cuvette with the bacterial sample into the spectrophotometer into cuvette holder (number 1), maintaining the same orientation as used for the blank. Use the same cuvette holder position throughout the experiment.
4. Close the lid of the spectrophotometer. Wait until you see a smiling face on the screen of the spectrophotometer.
5. Record the optical density (OD) value to **three decimal places** displayed by the spectrophotometer into the **table provided in the answer sheet (Question 2.1)**.

Cleaning:

1. After you record the absorbance, remove the cuvette. You can discard the bacterial culture into a LIQUID WASTE beaker, and wash the cuvette 2-3 times thoroughly with water (dH_2O) in wash bottle. Let water drain from the cuvette by gently tapping on a tissue paper.

Repeat Measurements:

1. Apply **Measurement** and **Cleaning** procedures for tubes 2-5.
2. Always recalibrate the spectrophotometer with the blank solution.

	Question 2.1. Use the OD600 values obtained to plot the growth curve of your bacterial culture in the **answer sheet**. The **x-axis values** of the plot correspond to the tube numbers.	5.0pt

	Question 2.2. Identify which tube number would be suitable to use for plasmid purification purposes. Put a checkmark on the **answer sheet**. (Keep in mind that you will be given another tube (PI) for plasmid purification that has already been prepared)	4.0pt

Final wash

1. Discard liquids from cuvettes and wash both cuvettes thoroughly with water (dH2O) in wash bottle. Invert them on tissue paper to let the water drain. You will use them in the Part B2 of the experiment.

Part B2. Detection of Nucleic Acids Using Absorption Spectroscopy (23 points)

In this experiment, you will extract and purify plasmid DNA from the *E. coli* culture that is provided for you. **The tube that you use for plasmid purification is labeled PI,** which stands for plasmid isolation**.**

Important Notes Before Beginning

• It is VERY important for you to label your tubes with your **student ID** with a marker because there will be **one** centrifuge for every **3 people**. You should be able to identify your tube!

• All centrifugation steps will be carried out by volunteers at 15,000 x g (~15,000 RCF). When you are ready to centrifuge just raise the card, which corresponds to the centrifugation step you are performing now (there are in total 7 cards for centrifugation), and a nearby volunteer will come and take your tube **labeled with your student ID.**

• The **ice bin** is located under your desk.

Please be aware that once the volunteer has started the centrifugation you will need to wait until it finishes.

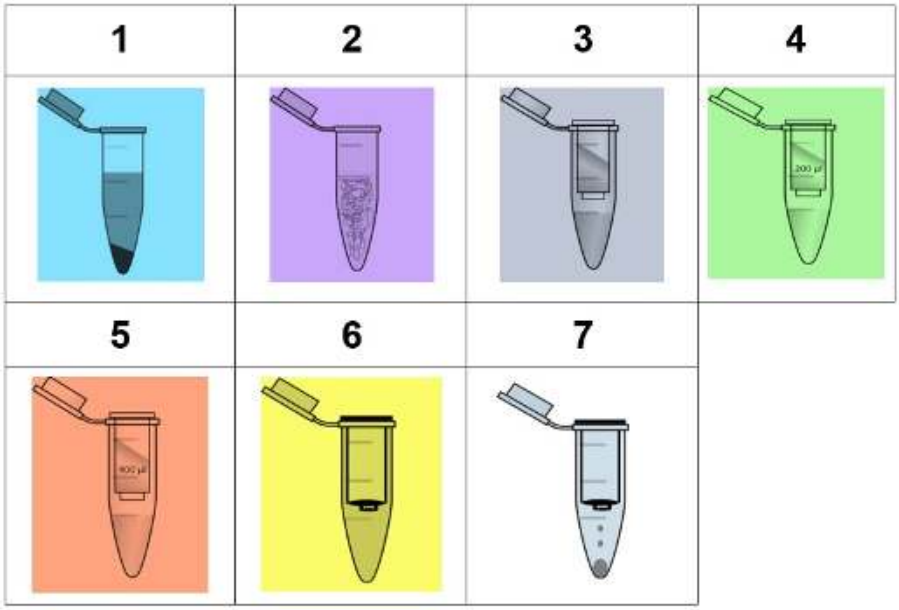


Figure 5. Centrifugation cards, which will be provided to you for plasmid purification. Please note, the spin column and the tube from step 1 will look different to the tubes shown in the picture.

Plasmid Isolation Protocol

1. Pellet 2 mL bacterial culture by centrifugation for 30 seconds (raise BLUE card № 1). Discard supernatant into a 250-mL plastic beaker labeled LIQUID WASTE.

2. Resuspend the pellet in 200 µL Plasmid Resuspension Buffer (**B1**) (tube is labeled red). Pipet to ensure cells are completely resuspended. There should be no visible clumps.

3. Lyse cells by adding 200 µL Plasmid Lysis Buffer (**B2**) (tube is labeled blue). Gently invert the tube immediately 5–6 times until the color changes to dark pink and the solution is clear and viscous. Incubate for 1 minute at room temperature.

4. Neutralize the lysate by adding 400 µL of Plasmid Neutralization Buffer (**B3**) in ice bin under your desk (tube is labeled yellow). Gently invert the tube until the color is uniformly yellow and a precipitate forms. Incubate for 2 minutes at room temperature.

5. Clarify the lysate by centrifuging for 3 minutes (raise PURPLE card № 2).

6. Carefully transfer the supernatant to the spin column which is inserted into the collection tube without a lid (labeled with your student-ID), and centrifuge for 1 minute (raise GRAY card № 3). Discard flow-through.

7. Re-insert the column into the SAME collection tube without lid and add 200 µL of Plasmid Wash Buffer 1 (**W1)** (tube is labeled green). Centrifuge for 1 minute (raise GREEN card № 4). Discard flow-through.

8. Re-insert the column into the SAME collection tube without lid and add 400 µL of Plasmid Wash Buffer 2 (**W2)** (tube is labeled orange) and centrifuge for 1 minute (raise ORANGE card № 5). Discard flow-through.

9. Insert the column into the SAME collection tube, and re-spin the column for 1 minute (raise the YELLOW card № 6).

10. Transfer the column to a clean 1.5 mL microcentrifuge tube with removable lid. Label with your student ID.

11. Add 40 µL DNA Elution Buffer **E** (labeled white) to the center of the matrix in the column. Wait for 1 minute, then spin for 1 minute (raise WHITE card № 7) to elute plasmids.

12. At this point, you should have purified **at least** 30 µL of plasmid.

Nucleic Acid Absorbance Measurements

Step 1. Before determining the purity and the concentration of the plasmid you isolated, you are required to dilute the plasmid with ddH_2O water provided in a 15mL tube. How much of each do you need to make a 1:70 dilution to the final volume of 2000 µL? **Make sure to write the values to one decimal in the answer sheet**

Question 2.3. Volume of ddH_2O required (µL):	2.0pt

Question 2.4. Volume of the eluted plasmid (µL):	2.0pt

Using the calculation above to dilute the plasmid in the clean 2mL tube provided.

Step 2. Set the wavelength at 260 nm on the spectrophotometer.

Step 3. Calibrate the instrument by filling the cuvette with 2 mL of ddH_2O water (your "blank") and inserting it into the spectrophotometer. Close the lid of the spectrophotometer. Then press "Zero" on the keypad. Once the absorbance is set to 0, and a smiling face appears on the screen, take off the cuvette with water.

Step 4. Place the cuvette containing the entire volume of your diluted plasmid. Record the absorbance value for 260 nm.

Step 5. Repeat steps 2-4 using the 280 nm wavelength now. Put it in the same cuvette holder position as before.

Question 2.5. Based on the absorbance value at 260nm calculate DNA concentration (in µg/mL), using the formula: $A_\lambda = \varepsilon c l$ and extinction coefficient, $\varepsilon = 0.02\ (\mu g/mL)^{-1} cm^{-1}$). **Please mind the dilution factor when calculations are made. Write the calculation values to two decimal places.** 6pt

Question 2.6. Calculate A260/A280. Write the ratio value to two decimal places. Based on your A260/A280 ratio, the purity of your plasmid will be assessed and corresponding points will be granted. 8pt

Step 6. Wash the cuvettes thoroughly with water (dH2O) in a wash bottle, then invert them on a clean tissue to dry. They will be used by other students from your country. So their results will depend on how well you clean the cuvettes!

PART B3. Bacterial Plasmid Analysis (12 points)

Having successfully purified the plasmids, the researcher now wonders about the behavior of these plasmids of different conformations. Using gel electrophoresis, the researcher wants to check the plasmid that he isolated.

Question 2.7. In agarose gel electrophoresis, supercoiled plasmids generally migrate faster through the gel compared to linear or relaxed circular forms due to their compact structure. 2.0pt

A) True

B) False

Question 2.8. Which statement best describes why supercoiled DNA migrates differently than its linear counterpart in agarose gel electrophoresis? 2.0pt

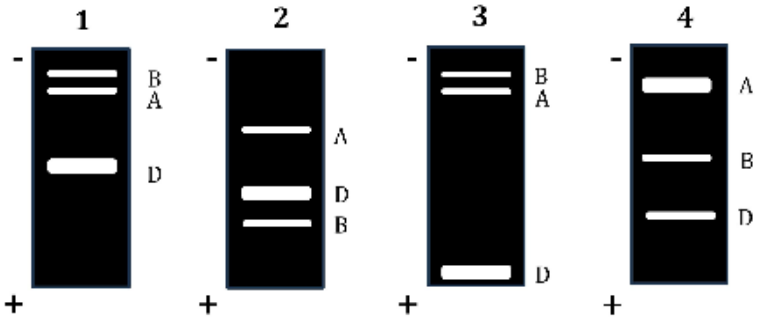
A) Supercoiled DNA is smaller in size (molecular weight).

B) Supercoiling increases the DNA's overall negative charge.

C) The compact structure of supercoiled DNA reduces frictional forces.

D) Supercoiled DNA is more positively charged, attracting it to the negative pole faster.

Question 2.9. Once the researcher has purified the plasmid, he wants to run a gel-electrophoresis to check the molecular weight of the plasmid**.** When uncut plasmid DNA is isolated and run on an agarose gel, he may observe several bands. Hopefully, most of his isolated DNA will be supercoiled, but other forms can also crop up. One of the diagrams below shows how these forms will show up on an agarose gel (in terms of relative migration speeds). From the gel (1-4) select the one with the most possible band position. Put the answer (1-4) in the answer sheet. 3pt



Legends: A: Linear, B: Nicked/Relaxed, D: Supercoiled

PART C. Advanced Imaging Techniques (14 points)

With the plasmid analysis underway, the researcher shifts his focus to a more detailed exploration of the macrophage fusion mechanism. He decides to use various microscopy techniques to study macrophage fusion, understanding that each technique offers unique insights into the microscopic world.

Your first task is to match each **imaging type** to the **description** given in the table. Further, assess each **experimental scenario** and match them with corresponding **imaging type** (not description).

Imaging Type	Description	Experimental Scenario
1.1 Phase Contrast Microscopy with Time-Lapse Capabilities	A. Visualizes specific cellular components using fluorescence. Cells or parts are labeled with fluorescent dyes or proteins, emitting light under specific wavelengths. It allows the observation of two different targets simultaneously, each with a distinct fluorophore.	A. 3D Imaging of Macrophage Fusion: Generating detailed three-dimensional reconstructions of fusing macrophages to analyze the structural changes during the fusion process.
1.2 Fluorescent Microscopy with Dual-Color Fluorescence	B. Enhances contrast in transparent specimens without staining, converting phase shifts in light into image brightness changes. Time-lapse capabilities capture dynamic processes in living cells over time.	B. Surface Examination of Macrophage Fusion Events: Visualizing the events at the plasma membrane interface during macrophage fusion with high axial resolution.
1.3 Confocal Microscopy with Live-Cell Imaging Adaptations	C. Exceeds the diffraction limit of light microscopy, offering higher resolution. Adapted for dynamic imaging to capture fast-moving cellular processes at a nanoscale.	C. Monitoring Macrophage Fusion Over Time: Observing the dynamics of the macrophage fusion process, focusing on changes in cell morphology and fusion frequency in response to various stimuli.
1.4 Total Internal Reflection Fluorescence (TIRF) Microscopy with Multi-Angle Viewing	D. Utilizes an evanescent wave for selective illumination of fluorophores near the coverslip. Allows enhanced observation from different orientations.	D. Visualization of Macrophage Membrane Proteins During Fusion: Studying the co-localization and interaction of two membrane proteins that facilitate the fusion process between macrophages.

1.5 Super-Resolution Microscopy adapted for Dynamic Imaging	E. Uses a laser and a pinhole to provide clear, detailed images of thick specimens, eliminating out-of-focus light. Adapted for live-cell imaging with controlled temperature and CO_2 to maintain cell viability.	E. Quantitative Morphometric Analysis of Fused Macrophages: Using computational analysis to measure and quantify the morphological changes of macrophages post-fusion, including cell size, number of nuclei, and cytoplasmic complexity.
1.6 Transmission Electron Microscopy (TEM) with Cryo-Preparation	F. Applies traditional staining, like Hematoxylin and Eosin (H&E), for contrast in tissue sections. The image analysis uses methods for enhanced accuracy and efficiency in feature analysis and quantification.	F. Ultrastructural Analysis of Macrophage Fusion: Examining the detailed ultrastructure of macrophage membranes and cytosolic components in the fusion process at high resolution.
1.7 Histological Staining with Automated Digital Image Analysis	G. Uses electron transmission through a specimen to create images. Involves rapid freezing of samples to preserve their natural state and prevent dehydration or staining artifacts.	G. High-Resolution Mapping of Fusion Sites: Identifying and characterizing the precise locations and molecular arrangements at the sites of macrophage fusion with resolution beyond the diffraction limit of light.

Question 3.1. Match the imaging techniques number to the correct description letter using the table below. **Do not** forget to transfer your answers to the answer sheet 7.0pt

Imaging techniques	1.1	1.2	1.3	1.4	1.5	1.6	1.7
Description							

Question 3.2. Next, your task is to assess each experimental scenario and determine which imaging technique, based on its described capabilities and modifications, would be the most effective and appropriate for the specific research requirements presented. **Do not** forget to transfer your answer to the answer sheet. 7.0pt

Imaging techniques	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	**1.6**	**1.7**
Experimental scenario							

PART D. Numerical Aperture (NA) in Microscopy (12 points)

Numerical Aperture (NA) is a critical concept in microscopy, as it directly influences the resolving power and brightness of the images produced. NA is a dimensionless number that characterizes the range of angles over which the system can accept or emit light. Essentially, it measures the ability of a microscope lens to gather light and resolve fine specimen detail at a fixed object distance. Importance of NA: A higher NA indicates a wider cone of light that can enter the lens, which translates to a greater ability to capture fine details (higher resolution) and improved brightness in the image. However, high NA lenses usually have shorter working distances and a shallower depth of field.

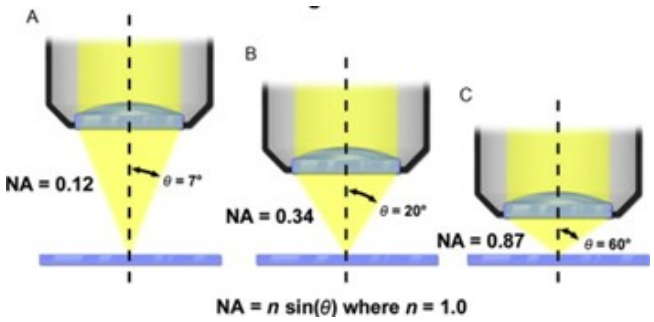


Figure 6. The diagram of the objective and the factors that affect numerical aperture.

The NA is influenced by two main factors:

1. The refractive index of the medium between the specimen and the objective lens. Using immersion oil can increase this index, allowing a higher NA as shown in Figure 6.
2. The half-angle (θ) of the maximum cone of light that can enter the lens. Larger angles result in a higher NA.

The NA of a microscope objective is calculated using the formula:

$$NA = n \times \sin(\theta)$$

where:

- n is the refractive index of the medium between the specimen and the objective (e.g., air, water, oil).
- (θ) is the half-angle of the maximum cone of light that can enter the objective lens. This angle is measured from the optical axis of the lens to the edge of the light cone.

Question 4.1. A microscope objective lens is used to observe a specimen through different media: air (n = 1.0), water (n = 1.33), and immersion oil (n = 1.515). If the half-angle of the light cone for this lens is 55 degrees, calculate the NA for each medium. 6.0pt

Question 4.2. Match the medium to the appropriate scenario based on **hypothetical** numerical aperture (NA) values for three objective types: Air: **0.45**, Water:**0.85** and Immersion oil: **1.4**. Fill in the "Suitable Medium" column with the letter corresponding to the Medium (**A**-Air, **B**-Water, or **C**-Immersion oil) that best fits the scenario described. 6.0pt

Scenario	Suitable Medium
1. Observing a delicate fresh-water living organism and needing moderate enhancement without harming it.	
2. Requiring the utmost detail to study cellular structures on a prepared slide.	
3. Needing to observe a sample where maintaining a greater distance between the lens and the sample is crucial to avoid damage.	

PART E. Approaches of Studying Actin Filaments in Macrophage Fusion (7 points)

The role of actin filaments in macrophage fusion is a critical aspect of understanding the cytoskeletal rearrangements required for this process. Here is the list of molecular and cellular techniques and experimental designs that could be performed to test the involvement of actin specifically in macrophage-macrophage fusion.

Question 5.1. Please, identify if each experimental approach is suitable to determine the involvement of actin specifically in macrophage fusion by putting a checkmark in corresponding **YES** or **NO** section. 7pt

Experimental Approach	YES	NO
5.1.1 Implementing GFP labeled F-actin filaments in macrophages, allowing live-cell fluorescence microscopy to visualize the actin cytoskeleton. Time-lapse imaging observes actin behavior before, during, and after macrophage fusion.		
5.1.2 Co-immunoprecipitation (Co-IP) with mass spectrometry can identify actin-binding proteins involved in forming membrane protrusion.		
5.1.3 Northern blot analysis to detect mRNA levels, providing insights into actin protein dynamics or polymerization during macrophage fusion.		
5.1.4 FRET biosensors for actin monitor filament dynamics in living cells. These biosensors have fluorescent proteins that change emission properties upon binding to F-actin, enabling real-time visualization of actin dynamics.		
5.1.5 A regular Western blot analysis to detect actin presence during macrophage fusion.		
5.1.6 Treating macrophages with actin-disrupting drugs like cytochalasin D or latrunculin A that inhibits actin polymerization, allowing study of its effects on macrophage fusion.		
5.1.7 Performing the assay to measure the rate of actin polymerization and depolymerization *in vitro* using purified actin from macrophages.		

IBO 2024 Kazakhstan

A1-1

English (Official)

35th International Biology Olympiad

Practical Exam: Molecular Biology

Part A – Fundamental Mechanisms Underlying the Foreign Body Response (12 points)

1.1 (3.0 pt)
Question 1.1. Write the correct option (A-D):

1.2 (3.0 pt)
Question 1.2. Write the correct option (A-D):

1.3 (3.0 pt)
Question 1.3. Write the correct option (A-D):

1.4 (3.0 pt)
Question 1.4. Write the correct option (1-5):
1.4.1 ____

1.4.2 ____

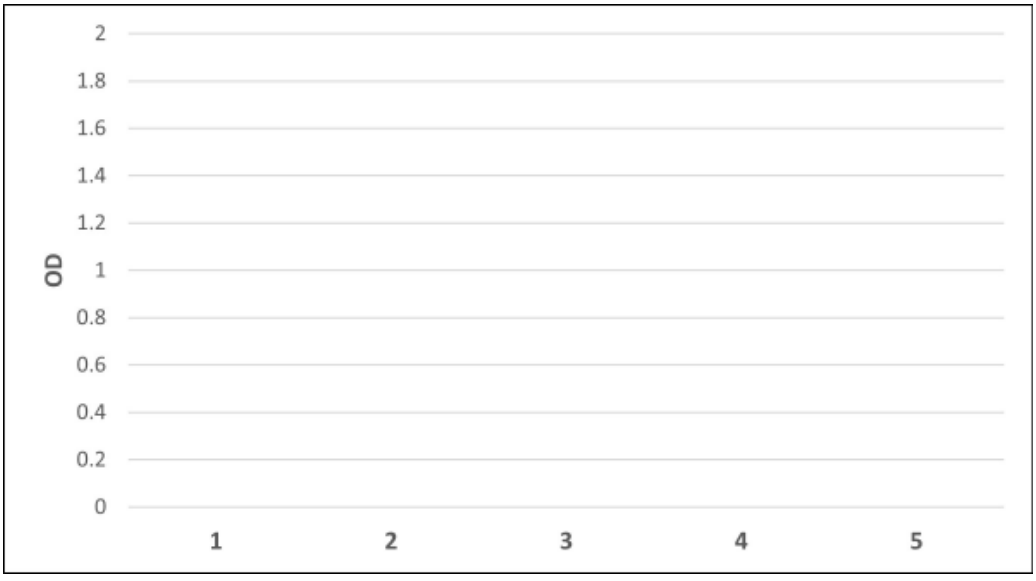
35th International Biology Olympiad

Practical Exam: Molecular Biology

Part B – Plasmid Purification (34 points)

2.1 (5.0 pt)
Question 2.1. Fill in the OD values to **three decimal places** into the following table and plot the curve on a corresponding field provided below:

Tube number/Time point	OD value
1	
2	
3	
4	
5	



2.2 (4.0 pt)
Question 2.2.

Tube number/Time point	1	2	3	4	5
Checkmark (√)					

2.3 (2.0 pt)
Question 2.3. Volume of ddH_2O required (µL):

2.4 (2.0 pt)
Question 2.4. Volume of the eluted plasmid (µL):

2.5 (6 pt)
Question 2.5. DNA concentration (µg/mL) :

2.6 (8 pt)
Question 2.6.

A260:_____

A280:_____

A260/A280:_____

2.7 (2.0 pt)
Question 2.7. Write the correct option (A-B):

2.8 (2.0 pt)
Question 2.8. Write the correct option (A-D):

2.9 (3 pt)
Question 2.9. Write the correct option (1-4):

Part C – Advanced Imaging Techniques (14 points)

3.1 (7.0 pt)
Question 3.1. Determine imaging techniques based on their descriptions and match them in the following table:

Imaging techniques	1.1	1.2	1.3	1.4	1.5	1.6	1.7
Description							

3.2 (7.0 pt)
Question 3.2. Match the imaging techniques with the corresponding scenarios in the following table:

Imaging techniques	1.1	1.2	1.3	1.4	1.5	1.6	1.7
Scenarios							

Part D – Numerical Aperture in Microscopy (12 points)

4.1 (6.0 pt)
Question 4.1.

(A) NA (air):

(B) NA (water):

(C) NA (oil):

4.2 (6.0 pt)
Question 4.2. Match the medium with appropriate scenario in the following table:

Scenario	**Medium (A-C)**
1	
2	
3	

Part E – Experimental Approach to Study Actin Filaments in Macrophage Fusion (7 points)

5.1 (7 pt)
Question 5.1. Put **checkmarks (√)** to YES or NO column based on the validity of experimental approach.

Statement	YES	NO
Q5.1.1		
Q5.1.2		
Q5.1.3		
Q5.1.4		
Q5.1.5		
Q5.1.6		
Q5.1.7		

PART A

		points
Q.1.1	D	3
Q.1.2	A	3
Q.1.3	C	3
Q.1.4.1	4 or E	1,5
Q.1.4.1	1 or A	1,5

PART B

Q.2.1	Full point (0.4)	Half point (0.2)
1	0 - 0.04	
2	0.124-0.152	0.11-0.17
3	0.97-1.19	0.86-1.30
4	1.26-1.55	1.12-1.69
5	1.36-1.67	1.21-1.82

Q.2.1	Plot points	1,5
	Curve	1,5
	Line	0
	no curve	0
	bar graph	0
Q.2.2	3-5 correct	3
Q.2.3	Full point	
	1971.43 (1971.4) or 1971.83 (1971.8)	2
Q.2.4	28.57 (28.6) or 28.17 (28.2)	2

Q.2.5 Based on A260 calculation and used dilution factor (70 or 71) (give full points if calculation is correct)

Q.2.6	A260: 0.080-0.123	2
	A280: 0.040-0.070	2
	A260/A280	
	1.63-1.99 Full point	4
	1.45-2.17 Half point	2
Q2.7	A	2
Q2.8	C	2
Q2.9	1	3

PART C

Q3.1 — 7 ponits

1,1	1,2	1,3	1,4	1,5	1,6	1,7
B	A	E	D	C	G	F

Q3.2 — 7 ponits

1,1	1,2	1,3	1,4	1,5	1,6	1,7
C	D	A	B	G	F	E

PART D

Q.4.1	Range	Points
Air	0.81-0.83	2
Water	1.07-1.09	2
Oil	1.23-1.25	2

Q.4.2

Scenario	Medium	Points
1	B	2
2	C	2
3	A	2

PART E

Q.5.1

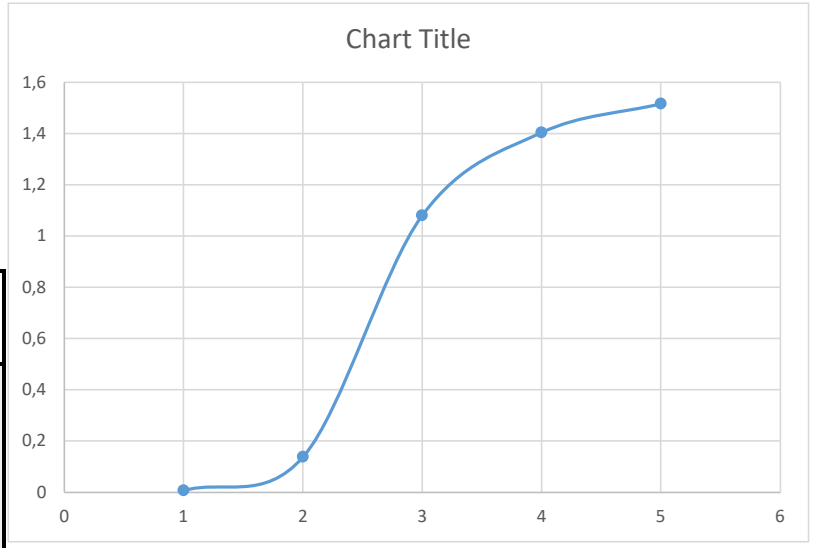
	YES	NO	Points
5.1.1	v		1
5.1.2	v		1
5.1.3		v	1
5.1.4	v		1
5.1.5		v	1
5.1.6	v		1
5.1.7		v	1

	1	2	3	4	5	6	average	DNA concentration	±10%		±20%	
260	0,1	0,104	0,098	0,103	0,084	0,12	0,1015	355,25	319,73	390,78	284,20	426,30
280	0,054	0,06	0,054	0,058	0,043	0,07	0,0565			Was not used for evaluation purpose		
Ratio	1,85	1,73	1,81	1,78	1,95	1,71	1,807273	1,63	1,99		1,45	2,17
	Referfence measurements							full point for ratio			half point	

Accepted ranges for DNA concentration

The concentration was evaluated based on **A260** of a student and **dilution factor the student used Q.2.3 and Q.2.4**

	input 260 A	DNA conc.
DNA conc. Calc.	0,211	738,5



Bacterial OD

Time point	Group 1	Group 2	Group 3	Group 4	Average	full point		half point	
1	0,007	0,004	0,002	0,015	0,007	0	0	0	0
2	0,142	0,141	0,135	0,136	0,1385	0,12465	0,15235	0,1108	0,1662
3	1,076	1,078	1,085	1,086	1,08125	0,973125	1,189375	0,865	1,2975
4	1,406	1,404	1,405	1,403	1,4045	1,26405	1,54495	1,1236	1,6854
5	1,509	1,524	1,527	1,506	1,5165	1,36485	1,66815	1,2132	1,8198