※ 注意：請於試卷內之「選擇題作答區」依序作答。

Part 1. Bob plans to prepare the TWO kinds of competent cells, DH5α and DH10B, for plasmid transformation. Here is the protocol he found from cDNA Libeary Protocols, 69.129-137 (1997). He will follow the protocol exactly. Based on the protocol, please answer the following questions.

Step 1. Pick a single colony from a plate of cells that have been freshly grown for 16–20 h at 37°C, and transfer it to 50 mL of LB medium in a sterile 300-mL flask. Incubate the culture for 16–20 h at 37°C with moderate shaking.

Step 2. Inoculate 1 mL of the culture into 100 mL of LB medium in a sterile 500-mL flask. Grow cells at 37°C for about 3 h with vigorous shaking (300 cycles/min on a rotary shaker).

Step 3. Transfer the cells aseptically to two 50-mL prechilled, sterile polypropylene tubes. Leave the tubes on ice (0°C) for 10 min.

Step 4. Centrifuge cells at 2400*g* for 10 min at 4°C.

Step 5. Pour off the supernatant, resuspend each pellet of cells in 10 mL of ice-cold 0.1M $CaCl_2$, and store on ice.

Step 6. Recover the cells by centrifugation at 2400*g* for 10 min at 4°C. Discard the supernatant, and resuspend each pellet in 10 mL of an ice-cold solution of 0.1M $CaCl_2$. Keep the resuspended cells on ice for 30 min.

Step 7. Centrifuge cells at 2400*g* for 10 min at 4°C. Discard the supernatant, and resuspend each pellet in 2 mL of the ice-cold solution of 0.1M $CaCl_2$

Step 8. Dispense cells and aliquot them (250 µL/tube) into prechilled, sterile polypropylene tubes. Freeze immediately at −70°C.

1. **How many flasks will Bob use while preparing TWO competent cells? (3%)**
   (A) 2 (B) 4 (C) 6 (D) 8
2. **How much TOTAL volume of ice-cold 0.1M $CaCl_2$ will Bob use while preparing competent cells? (3%)**
   (A) 22 mL (B) 44 mL (C) 66 mL (D) 88 mL
3. **How many maximum polypropylene tubes (Step 8) will Bob end up have? (3%)**
   (A) 8 (B) 16 (C) 32 (D) 64
4. **Which instrument or device will Bob NOT need in this experiment? (3%)**
   (A) centrifuge (B) microwave (C) shaker (D) pipette

Part 2. Please read the following article and answer the following questions [source: Modified from *Nature* 588, 693–698(2020)]

Despite its success in achieving the long-term survival of 10–30% of treated individuals, immune therapy is still ineffective for most patients with cancer. Many efforts are therefore underway to identify new approaches that enhance such immune 'checkpoint' therapy. Here we show that inhibiting PCSK9—a key protein in the regulation of cholesterol metabolism—can boost the response of tumors to immune checkpoint therapy, through a mechanism that is independent of PCSK9's cholesterol-regulating functions. Deleting the *PCSK9* gene in mouse cancer cells substantially **attenuates** their growth in mice in a manner that depends on cytotoxic T cells. **It** also enhances the efficacy of immune therapy that is targeted at the checkpoint protein PD1. Furthermore, clinically approved PCSK9-neutralizing antibodies synergize with anti-PD1 therapy in suppressing tumor growth in mouse models of cancer. Inhibiting PCSK9—either through genetic deletion or using PCSK9 antibodies—increases the expression of major histocompatibility protein class I (MHC I) proteins on the tumor cell surface, promoting robust intratumoral infiltration of cytotoxic T cells. Mechanistically, we find that PCSK9 can disrupt the recycling of MHC I to the cell surface by associating with it physically and promoting its relocation and degradation in the lysosome. Together, these results suggest that inhibiting PCSK9 is a promising way to enhance immune checkpoint therapy for cancer.

5. **PCSK9 modulates immune checkpoint therapy through regulating cholesterol level. (3%)**
   (A) True (B) False (C) Not mentioned in the article
6. **The word "attenuates" is closest in meaning to (3%)**
   (A) accelerates (B) enhances (C) advance (D) reduces

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7. **The word "it" in line 6 of this abstract refers to (3%)**
   (A) cytotoxic T cell (B) mouse cancer cell
   (C) deleting the *PCSK9* gene (D) PCSK9-neutralizing antibody
8. **What is NOT true about PCSK9? (3%)**
   (A) It regulates cholesterol metabolism.
   (B) It enhances the response of tumors to immune checkpoint therapy.
   (C) It can associate with MHC I.
   (D) It decreases MHC I expression on the tumors.
9. **MHC I can be relocated and degraded in the lysosome. (3%)**
   (A) True (B) False (C) Not mentioned in the article

Part 3. Please read the following article and answer the following questions [source: Modified from *Nature* December 18 (2020)]

Moderna's vaccine works in the same way as the one produced by Pfizer and BioNTech. Both consist of RNA molecules encased in lipid nanoparticles. The RNA in both vaccines encodes a slightly modified form of the SARS-CoV-2 protein known as spike, which enables the virus to infect human cells.

Once taken up by cells, the RNA is used to produce the protein, which then triggers an immune response. The RNA does not enter the nucleus where the cell's genome resides, and is degraded by the cell within a day of the injection.

Like Pfizer's vaccine, Moderna's seems to be highly effective — about 94% — at preventing symptomatic SARS-CoV-2 infections. Its safety profile is also similar to Pfizer's, with fatigue, headaches and pain at the site of injection among the most often cited side effects.

Since the Pfizer vaccine was rolled out in the United Kingdom and the United States, there have been occasional reports of recipients experiencing severe allergic reactions called anaphylaxis after being injected. There have been no signs of such reactions so far in the Moderna trial, which excluded people who have had anaphylactic reactions to previous vaccines, but not those with other allergies, such as reactions to food.

The two vaccines differ in the composition of the lipid nanoparticle that encases the RNA, and Moderna's formulation allows the vaccine to be stored at higher temperature than Pfizer's, which must be kept at −70 °C. Moderna's vaccine can be stored in a −20 °C freezer for 6 months, and at about 4 °C for 30 days. This promises to streamline the logistics of deploying the vaccine, particularly in rural areas and in countries with limited health-care infrastructure.

10. **Spike protein enables the vaccine to be taken up by cells. (3%)**
    (A) True (B) False (C) Not mentioned in the article
11. **RNA vaccines are effective because RNA itself trigger an immune response. (3%)**
    (A) True (B) False (C) Not mentioned in the article
12. **Moderna vaccines have longer shelf life than Pfizer vaccines. (3%)**
    (A) True (B) False (C) Not mentioned in the article
13. **Moderna announces no cases of anaphylaxis in its trial so far. (3%)**
    (A) True (B) False (C) Not mentioned in the article
14. **What is NOT true about Moderna and Pfizer vaccines? (3%)**
    (A) Both are RNA-based vaccines
    (B) Both are highly effective
    (C) Both are encased in the same composition of lipid nanoparticle
    (D) Both RNAs encodes spike protein

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Part 4. Please read the following article and answer the following questions [source: Modified from *Nucleic Acids Research*, 49 (1), 177–189 (2021)]

Short-chain acylations of lysine residues in eukaryotic proteins are recognized as essential posttranslational chemical modifications (PTMs) that regulate cellular processes from transcription, cell cycle, metabolism, to signal transduction. Lysine butyrylation was initially discovered as a normal straight chain butyrylation (Knbu). Here we report its structural isomer, branched chain butyrylation, i.e. lysine isobutyrylation (Kibu), existing as a new PTM on nuclear histones. Uniquely, isobutyryl-CoA is **derived from** valine catabolism and branched chain fatty acid oxidation which is **distinct from** the metabolism of *n*-butyryl-CoA. Several histone acetyltransferases were found to possess lysine isobutyryltransferase activity *in vitro*, especially p300 and HAT1. Transfection and western blot experiments showed that p300 regulated histone isobutyrylation levels in the cell. We resolved the X-ray crystal structures of HAT1 in complex with isobutyryl-CoA that gleaned an atomic level insight into HAT-catalyzed isobutyrylation. RNA-Seq profiling revealed that isobutyrate greatly affected the expression of genes associated with many pivotal biological pathways. Together, our findings identify Kibu as a novel chemical modification mark in histones and suggest its extensive role in regulating epigenetics and cellular physiology.

15. What is NOT regulated by posttranslational chemical modifications (PTMs)? (3%)

(A) acylations of lysine residues **(B) signal transduction**

(C) cell cycle **(D) gene expression**

16. What is NOT true about isobutyrylation? (3%)

(A) It affects gene expression. **(B) It is a new PTM of histone.**

(C) It can be regulated by p300 **(D) It is derived from butyrylation.**

17. The phrase "derived from" is NOT closest in meaning to? (3%)

(A) originated from **(B) resulted in**

(C) arose from **(D) risen from**

18. The phrase "distinct from" is closest in meaning to? (3%)

(A) similar to **(B) different from**

(C) in contrast **(D) compatible with**

Part 5. Please read the following article and answer the following questions [source: Modified from *Nature Metabolism* 2, 1248–1264(2020)]

In addition to fatty acids, glucose and lactate are important myocardial substrates under physiologic and stress conditions. They are metabolized to pyruvate, which enters mitochondria via the mitochondrial pyruvate carrier (MPC) for citric acid cycle metabolism. In the present study, we show that MPC-mediated mitochondrial pyruvate utilization is essential for the partitioning of glucose-derived cytosolic metabolic intermediates, which modulate myocardial stress adaptation. Mice with cardiomyocyte-restricted deletion of subunit 1 of MPC ($cMPC1^{-/-}$) developed age-dependent pathologic cardiac hypertrophy, transitioning to a dilated cardiomyopathy and premature death. Hypertrophied hearts accumulated lactate, pyruvate and glycogen, and displayed increased protein O-linked *N*-acetylglucosamine, which was prevented by increasing availability of non-glucose substrates in vivo by a ketogenic diet (KD) or a high-fat diet, which reversed the structural, metabolic and functional remodelling of non-stressed $cMPC1^{-/-}$ hearts. Although concurrent short-term KDs did not rescue $cMPC1^{-/-}$ hearts from rapid decompensation and early mortality after pressure overload, 3 weeks of a KD before transverse aortic constriction was sufficient to rescue this phenotype. Together, our results highlight the centrality of pyruvate metabolism to myocardial metabolism and function.

19. The phrase "in addition to" is closest in meaning to? (3%)

(A) Unlike

(B) As long as

(C) Besides

(D) On the contrary

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20. This study highlights the potential role of dietary interventions in enhancing fatty acid metabolism to reverse cardiac dysfunction. (3%)

(A) True (B) False (C) Not mentioned in the article

21. This study reveals a central role of mitochondrial pyruvate utilization in regulating cardiac metabolism and function. (3%)

(A) True (B) False (C) Not mentioned in the article

Part 6. Please read the following article and answer the following questions [source: Modified from *Advance Science* 8, 2001121(2021)]

Polymers shape human life but they also have been identified as pollutants in the oceans due to their long lifetime and low degradability. Recently, various researchers have studied the impact of (micro)plastics on marine life, biodiversity, and potential toxicity. Even if the consequences are still heavily discussed, prevention of unnecessary waste is desired. Especially, newly designed polymers that degrade in seawater are discussed as potential alternatives to commodity polymers in certain applications. Biodegradable polymers that degrade *in vivo* (used for biomedical applications) or during composting often exhibit too slow degradation rates in seawater. To date, no comprehensive summary for the degradation performance of polymers in seawater has been reported, **nor** are the studies for seawater-degradation following uniform standards. This review summarizes concepts, mechanisms, and other factors affecting the degradation process in seawater of several biodegradable polymers or polymer blends. As most of such materials cannot degrade or degrade too slowly, strategies and innovative routes for the preparation of seawater-degradable polymers with rapid degradation in natural environments are reviewed. It is believed that this selection will help to further understand and drive the development of seawater-degradable polymers.

22. Which one is "unlikely" the keyword of the abstract? (3%)

(A) biodegradable polyesters

(B) seawater-degradable polymers

(C) marine plastic pollution

(D) natural environment

23. Which title will fit the article best? (3%)

(A) Seawater-degradable polymers –Shaping human life

(B) Seawater-degradable polymers -Fighting the marine plastic pollution

(C) Seawater-degradable polymers –Slow degradation in natural environments

24. What is synonymous to "nor" ? (3%)

(A) neither (B) or (C) additional (D) either

Part 7. Please read the following article and answer the following questions [source: Modified from https://www.sciencedaily.com/releases/2021/01/210111115747.htm]

The coralberry currently again adorns many living rooms: In winter it bears bright red fruits, which make it a popular ornamental plant at this time of year. For pharmacists, however, it is interesting for a different reason: It contains an active substance that has emerged in recent years as a beacon of hope against asthma and certain types of cancer.

Unfortunately, obtaining the substance with the cryptic name FR900359 (abbreviated: FR) in larger quantities is **rather** laborious. Cultivating the plants in greenhouses takes many weeks; moreover, the yield can vary enormously depending on the specimen. Incidentally, they do not produce the active ingredient themselves, but have bacteria in their leaves that do it for them. "However, these only grow in the coralberry and cannot be cultivated in the laboratory," explains Dr. Max Crüsemann of the Institute of Pharmaceutical Biology at the University of Bonn.

Manufacturing FR is a complex undertaking. The bacteria have a special assembly line for this purpose, in which a number of

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enzymes work hand in hand. The bacterial genetic makeup specifies how this assembly line must be set up. "We have now searched huge databases for other microorganisms that also have these genes for FR synthesis." Crüsemann explains. "In the process, we came across another bacterium. Unlike its coralberry relative, it does not grow in plants, but in soil and is easily propagated in culture media." This finding should greatly facilitate the production of FR in the future.

25. Which statement is incorrect? (3%)

(A) The coralberry has been shown to carry therapeutic powers

(B) The coralberry plant is found to cause asthma and cancer

(C) FR is an active ingredient associated with the ornamental plant coralberry

(D) FR can be produced by certain microorganisms

26. What's the main breakthrough described in this article? (3%)

(A) Chemically synthesized FR has become the main source for large scale production

(B) The advancement of cultivating coralberry plants can fulfill the demand of FR.

(C) The microorganisms identified on the leaves of coralberry plants are now in large scale laboratory cultivation for FR synthesis.

(D) A certain type of bacteria isolated from soil can be the potential candidate for expediting FR synthesis.

27. What is the meaning of "rather" in the context? (3%)

(A) quite (B) insignificantly (C) partially (D) specifically

Part 8. Please read the following article and answer the following questions [source: Modified from Molecular Cell (2021), doi: https://doi.org/10.1016/j.molcel.2020.12.017]

The series of RNA folding events that occur during transcription can critically influence cellular RNA function. Here, we present reconstructing RNA dynamics from data (R2D2), a method to uncover details of cotranscriptional RNA folding. We model the folding of the *Escherichia coli* signal recognition particle (SRP) RNA and show that it requires specific local structural fluctuations within a key hairpin to **engender** efficient cotranscriptional conformational rearrangement into the functional structure. All-atom molecular dynamics simulations suggest that this rearrangement proceeds through an internal toehold-mediated strand-displacement mechanism, which can be disrupted with a point mutation that limits local structural fluctuations and rescued with compensating mutations that restore these fluctuations. Moreover, a cotranscriptional folding intermediate could be cleaved *in vitro* by recombinant *E. coli* **RNase P**, suggesting potential cotranscriptional processing. These results from experiment-guided multi-scale modeling demonstrate that even an RNA with a simple functional structure can undergo complex folding and processing during synthesis.

28. What does "engender" indicate in the paragraph? (3%)

(A) interrupt (B) cure (C) dull (D) promote

29. What is the major role of RNase P in the context? (3%)

(A) to facilitate the RNA folding

(B) to evaluate the RNA folding process

(C) to synthesize RNA elements

(D) to maintain the structure of SRP

30. Which technology is the least possible to be directly involved in this research (3%)

(A) computational modelling

(B) Western blotting

(C) transcriptional assay

(D) mutagenesis assay

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Part 9. Please read the following article and answer the following questions [source: Modified from Cell (2021), doi: https://doi.org/10.1016/j.cell.2020.12.011]

The microbiota shields the host against infections in a process known as colonization resistance. How infections themselves shape this fundamental process remains largely unknown. Here, we show that gut microbiota from previously infected hosts display enhanced resistance to infection. This long-term functional remodeling is associated with altered bile acid metabolism leading to the expansion of taxa that utilize the sulfonic acid taurine. Notably, supplying exogenous taurine alone is sufficient to induce this alteration in microbiota function and enhance resistance. Mechanistically, taurine **potentiates** the microbiota's production of sulfide, an inhibitor of cellular respiration, which is key to host invasion by numerous pathogens. As such, pharmaceutical sequestration of sulfide perturbs the microbiota's composition and promotes pathogen invasion. Together, this work reveals a process by which the host, triggered by infection, can deploy taurine as a nutrient to nourish and train the microbiota, promoting its resistance to subsequent infection.

31. Which one is NOT among the main findings in this study? (3%)

(A) prior gut infection increases the microbiota's resistance to subsequent infection

(B) infection induces host taurine production and the expansion of taurine utilizers

(C) the gut microbiota converts taurine to sulfide, inhibiting pathogen invasion

(D) taurine is found to be synthesized in gut microorganisms

32. What is antonymous to "potentiate"? (3%)

(A) empower (B) elicit (C) attenuate (D) raise

33. What will be the most relevant title of the research? (4%)

(A) infection trains the host for microbiota-enhanced resistance to pathogens

(B) taurine as the essential nutritional supplementation

(C) taurine synthesis via the gut bacteria to fight against pathogen infection

(D) a balanced diet can enhance the health of gut microorganisms

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