※ 注意：請於試卷內之「選擇題作答區」依序作答。

Part 1. Mary plans to isolate DNA from mouse liver tissues. Here is the protocol from Chapter 6 of Molecular Cloning that she will follow. Please help her answer the following questions.

Step 1. Dissect 10-20 mg of tissue and mince the tissue finely with a razor blade/scalpel.

Step 2. Transfer the minced tissue to a microfuge tube containing 600 µL of ice-cold cell lysis buffer. Homogenize the suspension quickly with 30-50 strokes of a microfuge pestle.

Step 3. Add 3 µL of 4 mg/mL DNase-free RNase. Incubate the digest for 15-60 minutes at 37°C.

Step 5. Allow the sample to cool to room temperature. Add 200 µL of potassium acetate solution and mix the contents of the tube by vortexing vigorously for 20 seconds.

Step 6. Pellet the precipitated protein/SDS complex by centrifugation at maximum speed for 3 minutes at 4°C in a microfuge.

Step 7. Transfer the **supernatant** to a fresh microfuge tube containing 600 µL of isopropanol. Mix the solution well and then recover the precipitate of DNA by centrifuging the tube at maximum speed for 1 minute at room temperature in a microfuge.

Step 8. Remove the **supernatant** by aspiration and add 600 µL of 70% ethanol to the DNA pellet. Invert the tube several times and centrifuge the tube at maximum speed for 1 minute at room temperature in a microfuge.

Step 9. Carefully remove the supernatant by aspiration and allow the DNA pellet to dry in air for 15 minutes.

Step 10. Redissolve the pellet of DNA in 100 µL of TE buffer (pH 7.6). The solubilization of the genomic DNA pellet can be facilitated by incubation for 16 hours at room temperature or for 1 hour at 65°C.

1. **According to this protocol, protein can be precipitated using _____. (3%)**
   (A) lysis buffer (B) TE buffer (C) potassium acetate (D) isopropanol

2. **At Step 7, what does the "supernatant" mainly contain? (3%)**
   (A) DNA (B) RNA (C) Protein (D) isopropanol

3. **At Step 8, what does the "supernatant" mainly contain? (3%)**
   (A) DNA (B) RNA (C) Protein (D) isopropanol

4. **According to this protocol, solubility of DNA can be improved by heat. (3%)**
   (A) True (B) False (C) Not mentioned in the protocol

Part 2. Please read the following article and answer the following questions [source: Modified from *Cell Metabolism*, 25 (1), 182–196 (2016)]

Exercise elicits several beneficial effects by acting on mitochondrial content/function, fatty acid oxidation, and glucose homeostasis. Indeed, muscle activity is important to counteract disease progression in diabetes, obesity, and metabolic syndrome. The signaling pathways that control the contraction-mediated beneficial effects on mitochondria and glucose/lipid homeostasis are **distinct** from insulin signaling and mainly rely on AMPK and PGC1α. We have recently found that exercise leads to nuclear translocation of the helix-loop-helix leucine zipper transcription factor EB (TFEB), an important regulator of lysosomal biogenesis and autophagy. Upregulation of TFEB has been found in several tissues after food deprivation, including liver and skeletal muscle. We have previously shown that in liver, TFEB regulates genes involved in lipid catabolism, fatty acid oxidation, and ketogenesis. Some of these effects are elicited by TFEB-mediated induction of PGC1α, a transcriptional coactivator, which interacts with and enhances the activity of transcription factors involved in mitochondrial biogenesis, glucose homeostasis, and lipid oxidation.

見背面

5. **Exercise can prevent the development of diabetes. (3%)**
   (A) True (B) False (C) Not mentioned in the article

6. **The word "distinct" is closest in meaning to (3%)**
   (A) dependent (B) disposed (C) despicable (D) different

7. **What is NOT true about TFEB? (3%)**
   (A) A transcription factor (B) only expressed in nucleus
   (C) controlling lysosomal biogenesis (D) regulating autophagy

8. **Which is TRUE about PGC1α? (3%)**
   (A) A transcriptional coactivator (B) can be regulated by TFEB
   (C) involved in mitochondrial biogenesis (D) All of the above

Part 3. Please read the following article and answer the following questions [source: Modified from *Nature Medicine*, 22, 1448-1455 (2016)]

Infection with Zika virus has been associated with serious neurological complications and fetal abnormalities. However, the dynamics of viral infection, replication and shedding are poorly understood. Here we show that both rhesus and cynomolgus macaques are highly susceptible to infection by lineages of Zika virus that are closely related to, or are currently circulating in, the Americas. After subcutaneous viral inoculation, viral RNA was detected in blood plasma as early as 1 d after infection. Viral RNA was also detected in saliva, urine, cerebrospinal fluid (CSF) and semen, but transiently in vaginal secretions. Although viral RNA during primary infection was cleared from blood plasma and urine within 10 d, viral RNA was detectable in saliva and seminal fluids until the end of the study, 3 weeks after the resolution of viremia in the blood. The control of primary Zika virus infection in the blood was correlated with rapid innate and adaptive immune responses. We also identified Zika RNA in tissues, including the brain and male and female reproductive tissues, during early and late stages of infection. Re-infection of six animals 45 d after primary infection with a heterologous strain **resulted in** complete protection, which suggests that primary Zika virus infection elicits protective immunity. Early invasion of Zika virus into the nervous system of healthy animals and the extent and duration of shedding in saliva and semen underscore possible concern for additional neurologic complications and nonarthropod-mediated transmission in humans.

9. **Zika virus infection is related to _____defects in babies. (3%)**
   (A) nephron (B) neutral (C) hepatic (D) brain

10. **How soon can Zika viral RNA be detected after subcutaneous inoculation? (3%)**
    (A) 1 day (B) 10 days (C) 3 weeks (D) 45 days

11. **Where can Zika virus be detected after subcutaneous inoculation? (3%)**
    (A) saliva (B) blood (C) semen (D) All of the above

12. **Zika virus can be permanently detected in vaginal secretions. (3%)**
    (A) True (B) False (C) Not mentioned in the article

13. **The phrase "result in" is closest in meaning to (3%)**
    (A) result from (B) due to (C) lead to (D) because of

接次頁

Part 4. Read the abstract of a Research paper below (*Science* 355 (6321), 198-201, 2017)

Prions are self-propagating protein aggregates that act as protein-based elements of inheritance in fungi. Although prevalent in eukaryotes, prions have not been identified in bacteria. Here we found that a bacterial protein, transcription terminator Rho of *Clostridium botulinum* (Cb-Rho), could form a prion. We identified a candidate prion-forming domain (cPrD) in Cb-Rho and showed that **it** conferred amyloidogenicity on Cb-Rho and could functionally replace the PrD of a yeast prion-forming protein. **Furthermore,** its cPrD enabled Cb-Rho to access alternative conformations in *Escherichia coli*—a soluble form that terminated transcription efficiently and an aggregated, self-propagating prion form that was functionally compromised. The prion form caused genome-wide changes in the transcriptome. Thus, Cb-Rho functions as a protein-based element of inheritance in bacteria, suggesting that the emergence of prions predates the evolutionary split between eukaryotes and bacteria.

14. The word "it" in line 4 of this abstract refers to (3%)
(A) cPrD (B) Cb-Rho (C) amyloidogenicity (D) PrD

15. The word "Furthermore" in line 6 of this abstract is closest in meaning to (3%)
(A) In addition (B) In between (C) In contrast (D) In detail

16. Which one from the following list of "Title" is most appropriate for this abstract (5%)
(A) A transcription terminator prion in fungi
(B) An evolutionary conserved role of Rho in eukaryotes
(C) A regulator forms a prion in bacteria

Part 5. Please read the following abstract of a review paper and answer questions 17-20. [Source: *Science Translational Medicine* 4(137), 137rv5 (2012)]

The connection between disease and the disruption of homeostatic interactions between the host and its microbiota is now well established. Drug developers and clinicians are starting to rely more heavily on therapies that directly target the microbiota and on the ecology of the microbiota to understand the outcomes of these treatments. The effects of those microbiota-targeted therapies that alter community composition range in scale from eliminating individual strains of a single species (for example, with antibacterial conjugate vaccines) to replacing the entire community with a new intact microbiota (for example, by fecal transplantation). Secondary infections linked to antibiotic use provide a cautionary tale of the unintended consequences of perturbing a microbial species network and highlight the need for new narrow-spectrum antibiotics with rapid companion diagnostics. Insights into microbial ecology will also benefit the development of probiotics, whose therapeutic prospects will depend on rigorous clinical testing. Future probiotics may take the form of a consortium of long-term community residents: "a fecal transplant in a capsule." The **efficacy** of microbiota-targeted therapies will need to be assessed using new diagnostic tools that measure community function rather than composition, including the temporal response of a microbial community to a defined perturbation such as an antibiotic or probiotic.

17. Which of the following could be used to replace the entire microbiota community with a new one? (3%)
(A) Antibacterial conjugate vaccines
(B) Fecal transplantation
(C) Narrow-spectrum antibiotics
(D) Probiotics

見背面

18. Which of the following is the most appropriate title of this article? (3%)
(A) Microbiota-Targeted Therapies: An Ecological Perspective
(B) Interactions Between the Microbiota and the Immune System
(C) Regulated Virulence Controls the Ability of a Pathogen to Compete with the Gut Microbiota
(D) Therapeutic Modulation of Microbiota-Host Metabolic Interactions

19. What should be measured to assess the efficacy of microbiota-targeted therapies? (3%)
(A) Body temperature
(B) Composition of microbiota community
(C) Biomass of microbiota
(D) Function of microbiota community

20. The word "efficacy" in line 12 is closest in meaning to (3%)
(A) Usefulness
(B) Inefficiency
(C) Effort
(D) Importance

Part 6. Please read the following abstract of a research paper and answer questions 21-24. [Source: *Science* 355(6322), 2017 Jan 20; DOI: 10.1126/science.aaf8399]

Immunotherapies based on immune checkpoint blockade are highly effective in a subset of patients. An ongoing challenge is the identification of biomarkers that predict which patients will benefit from these therapies. Aneuploidy, also known as somatic copy number alterations (SCNAs), is widespread in cancer and is posited to drive tumorigenesis. Analyzing 12 human cancer types, we find that, for most, highly aneuploid tumors show reduced expression of markers of cytotoxic infiltrating immune cells, especially CD8+ T cells, and increased expression of cell proliferation markers. Different types of SCNAs predict the proliferation and immune signatures, implying distinct underlying mechanisms. Using published data from two clinical trials of immune checkpoint blockade therapy for metastatic melanoma, we found that tumor aneuploidy inversely correlates with patient survival. Together with other tumor characteristics such as tumor mutational load, aneuploidy may thus help identify patients most likely to respond to immunotherapy.

21. Which of the following has the same meaning as "aneuploidy?" (3%)
(A) Immune checkpoint blockade
(B) Somatic copy number alterations
(C) Patient survival
(D) Tumor mutational load

22. What characteristics do highly aneuploidy tumors show? (3%)
(A) Reduced expression of markers of cytotoxic immune cells
(B) Reduced expression of cell proliferation markers
(C) Enhanced expression of cell death markers
(D) Enhanced expression of markers of cytotoxic immune cells

接次頁

23. Based on clinical trials of immune checkpoint blockade therapy for metastatic melanoma, tumor aneuploidy and patient survival show (3%)
(A) No correlation
(B) Positive correlation
(C) Negative correlation
(D) None of the above

24. Based on this study, what could serve as a new biomarker to predict which patients will benefit from immunotherapy? (3%)
(A) Metastatic melanoma
(B) Markers of cell proliferation
(C) Markers of cytotoxic immune cells
(D) Aneuploidy of tumors

Part 7. Please read the following abstract of a research paper and answer questions 25-28. [Source: *Nature* 541, 417-420 (2017)]

As malignant tumors develop, they interact intimately with their microenvironment and can activate autophagy, a catabolic process which provides nutrients during starvation. How tumors regulate autophagy in vivo and whether autophagy affects tumor growth is controversial. Here we demonstrate, using a well characterized *Drosophila melanogaster* malignant tumor model, that non-cell-autonomous autophagy is induced both in the tumor microenvironment and systemically in distant tissues. Tumor growth can be pharmacologically restrained using autophagy inhibitors, and early-stage tumor growth and invasion are genetically dependent on autophagy within the local tumor microenvironment. Induction of autophagy is mediated by *Drosophila* tumor necrosis factor and interleukin-6-like signaling from metabolically stressed tumor cells, whereas tumor growth depends on active amino acid transport. We show that dormant growth-impaired tumors from autophagy-deficient animals reactivate tumorous growth when transplanted into autophagy-proficient hosts. We conclude that transformed cells engage surrounding normal cells as active and essential microenvironmental contributors to early tumor growth through nutrient-generating autophagy.

25. What term is used to indicate a catabolic process which provides nutrients during starvation? (3%)
(A) Autophagy
(B) Tumor necrosis factor
(C) Apoptosis
(D) Active amino acid transport

26. What model organism was used in this study? (3%)
(A) Mouse (*Mus musculus*)
(B) Zebrafish (*Danio rerio*)
(C) Fruit fly (*Drosophila melanogaster*)
(D) Baker's yeast (*Saccharomyces cerevisiae*)

27. Which of the following statement is supported by the finding of this study? (3%)
(A) Autophagy and tumor growth are irrelevant.
(B) Autophagy inhibits tumor growth.
(C) Tumor growth can be promoted by blocking autophagy.
(D) Microenvironmental autophagy promotes tumor growth.

見背面

28. What happened when growth-impaired tumors from autophagy-deficient animals were transplanted into autophagy-proficient hosts? (3%)

(A) Tumors remained the same

(B) Tumors started to grow

(C) Tumor started to shrink

(D) None of the above

※ 注意：請於試卷內之「非選擇題作答區」標明題號依序作答。

Part 8. Please read the following abstract of a research paper and answer questions 29-31. [Source: *Nature* 540, 593-596 (2016)]

Cells grow well only in a narrow range of physiological conditions. Surviving extreme conditions requires the instantaneous expression of chaperones that help to overcome stressful situations. To ensure the preferential synthesis of these heat-shock proteins, cells inhibit transcription, pre-mRNA processing and nuclear export of non-heat-shock transcripts, while stress-specific mRNAs are exclusively exported and translated. How cells manage the selective retention of regular transcripts and the simultaneous rapid export of heat-shock mRNAs is largely unknown. In *Saccharomyces cerevisiae*, the shuttling RNA adaptor proteins Npl3, Gbp2, Hrb1 and Nab2 are loaded co-transcriptionally onto growing pre-mRNAs. For nuclear export, they recruit the export-receptor heterodimer Mex67–Mtr2 (TAP–p15 in humans). Here we show that cellular stress induces the dissociation of Mex67 and its adaptor proteins from regular mRNAs to prevent general mRNA export. At the same time, heat-shock mRNAs are rapidly exported in association with Mex67, without the need for adapters. The immediate co-transcriptional loading of Mex67 onto heat-shock mRNAs involves Hsf1, a heat-shock transcription factor that binds to heat-shock-promoter elements in stress-responsive genes. An important difference between the export modes is that adaptor-protein-bound mRNAs undergo quality control, whereas stress-specific transcripts do not. In fact, regular mRNAs are converted into uncontrolled stress-responsive transcripts if expressed under the control of a heat-shock promoter, suggesting that whether an mRNA undergoes quality control is encrypted therein. Under normal conditions, Mex67 adaptor proteins are recruited for RNA surveillance, with only quality-controlled mRNAs allowed to associate with Mex67 and leave the nucleus. Thus, at the cost of error-free mRNA formation, heat-shock mRNAs are exported and translated without delay, allowing cells to survive extreme situations.

Please answer the following questions in English.

29. Please describe the aim of this study (5%)

30. Can regular mRNAs be exported out of nucleus when cells are under stress? (1%) Why? (3%)

31. What is the take-home message of this article? (5%)

試題隨卷繳回