Part I (Questions 1-2) John was asked to transform and store his newly constructed plasmid into *E. coli* cells. He opened up a lab protocol book for both plasmid transformation and cell storage. **(Please answer the questions using English)**

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1 (12%). For plasmid transforamtion of *E. coli* system:

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Step 1. Culture *E. coli* in 250 ml of SOB medium in a 2-liter flask at 18 °C with vigorous shaking until an A_{600} of 0.6 is reached.

Step 2. Keep on ice for 10 min.

Step 3. Spin at 3000 x g (4000 RPM in Hitachi RPR-10 rotor) for 10 min at 4 °C.

Step 4. Resuspend the pellet in 80 ml of ice-cold TB. Keep in an ice-water bath for 10 min.

Step 5. Spin as above. Resuspend the pellet in 20 ml of TB. Add DMSO with gentle swirling to a final concentration of 7%. Keep the centrifugation bottle in ice-water bath for 10 min.

Step 6. Dispense by 1-2 ml into microfuge tubes. Freeze immediately in liquid nitrogen. Store the frozen competent cell at -80 °C.

Step 7. Before conducting transformation, take a tube of competent cell from -80 °C and thaw the competent cell at room temperature. Dispense by 200 μl into microfuge tubes on ice.

Step 8. Add plasmid solution (< 2 μl). Keep on ice for 30 min.

Step 9. Heat at 42 °C for 1 min without agitation. Transfer immediately onto ice.

Step 10. Add 0.8 ml of SOC. Incubate at 37 °C for 1 hr.

Step 11. Streak on plates containing appropriate antibiotics. Incubate the LB plates overnight at 37 °C.

TB: 10 mM HEPES, 55 mM $MnCl_2$, 15 mM $CaCl_2$, 250 mM KCl.
(Mix all components except for $MnCl_2$ and adjust pH to 6.7 with KOH. Then, dissolve $MnCl_2$. Sterilize the solution by filtration through a pre-rinsed 0.45 μm filter. Store the solution at 4 °C.)

SOB: 2% Bacto-trypton, 0.5% Bact-yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$
(Dissolve tryptone, yeast extract, NaCl and KCl in the purest water available. Autoclave for 30 min. Add 1/100 vol of filter-sterilized 1M $MgCl_2$, 1M $MgSO_4$.).

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SOC: Add 1/100 vol of filter-sterilized 2M glucose to SOB.

a. What is A_{600} in step 1?
b. According to the protocol, when John conducts heat-shock to the cells in 42 °C, should he swirl the sample tube to make the cells inside can evenly being heated?
c. According to this protocol, did John need to conduct autoclave procedure in his TB buffer preparation?

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2 (12%). Cell storage in glycerol Solution:

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Most strains of bacteria including *E. coli*, and fungi (spores is preferred) can be stored for one to two years in glycerol solution at -20 °C. At -80 °C they can be stored almost for lifetime.

Step 1. Prepare 0.5 ml of glycerol solution in 2.0 ml screw-cap vials, autoclave (15 min, 121 °C), cool, and store them at room temperature.
Step 2. Transfer 0.5 ml of an overnight-grown culture into a vial from step 1.
Step 3. Voltex, store at -20 or -80 °C (-80 °C storage is preferred for long-term maintenance).

a. What is the best temperature if John wants to store his plasmids for a long time?
b. What is the percentage of glycerol in the final vial for storage?
c. What does "Voltex" mean in step 3?

Part II (Questions 3-4). Read the article and answer the questions followed. **(Please answer the questions using English)**

3. The growing use of fluorescent biosensors to directly probe the spatiotemporal dynamics of biochemical processes in living cells has revolutionized the study of intracellular signaling. In this review, we summarize recent developments in the use of biosensors to illuminate the molecular details of G-protein-coupled receptor (GPCR) signaling pathways, which have long served as the model for our understanding of signal transduction, while also offering our perspectives on the future of this exciting field. Specifically, we highlight several ways in which biosensor-based single-cell analyses are being used to unravel many of the enduring mysteries that surround these diverse signaling pathways. (J Biol Chem.

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2015 Jan 20. pii: jbc.R114.616391.)

a (6%). What is the main technology this article tries to summarize?
b (6%). According to this article, what have long served as the model for our understanding of signal transduction?

4. The permafrost on the North Slope of Alaska is densely populated by shallow lakes that result from thermokarst erosion. These lakes release methane (CH_4) derived from a combination of ancient thermogenic pools and contemporary biogenic production. Despite the potential importance of CH_4 as a greenhouse gas, the contribution of biogenic CH_4 production in arctic thermokarst lakes in Alaska is not currently well understood. To further advance our knowledge of CH_4 dynamics in these lakes, we focused our study on (i) the potential for microbial CH_4 production in lake sediments, (ii) the role of sediment geochemistry in controlling biogenic CH_4 production, and (iii) the temperature dependence of this process. Sediment cores were collected from one site in Siqlukaq Lake and two sites in Sukok Lake in late October to early November. Analyses of pore water geochemistry, sedimentary organic matter and lipid biomarkers, stable carbon isotopes, results from CH_4 production experiments, and copy number of a methanogenic pathway-specific gene (mcrA) indicated the existence of different sources of CH_4 in each of the lakes chosen for the study. Analysis of this integrated data set revealed that there is biological CH_4 production in Siqlukaq at moderate levels, while the very low levels of CH_4 detected in Sukok had a mixed origin, with little to no biological CH_4 production. Furthermore, methanogenic archaea exhibited temperature-dependent use of *in situ* substrates for methanogenesis, and the amount of CH_4 produced was directly related to the amount of labile organic matter in the sediments. This study constitutes an important first step in better understanding the actual contribution of biogenic CH_4 from thermokarst lakes on the coastal plain of Alaska to the current CH_4 budgets. (Geobiology. 2015 Jan 22. doi: 10.1111/gbi.12124.)

a (6%). According to the authors, what is the purpose to detect "methanogenic pathway-specific gene (mcrA)" from the lake sediments?
b. (4%) In terms of the sources of the CH_4 detected from the lake, which lake the authors suggested to have more microorganism involved?
c. (4%) The author suggested that the amount of CH_4 produced was directly related to the amount of labile organic matter in the sediments. What is "labile organic matter"?

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Part III (Questions 5-12; **第 8, 10, 12 等題目請用流暢通順中文作答，無法翻譯之科學專有名詞可保留英文用字**)

5. Read the abstract of a Review paper below (J Exp Bot. 2014 Nov 4 ; 66(3):719-30.) and determine which one from the following lists of "Title" is most appropriate for this abstract. (5 %)

"Plants precisely time the onset of flowering to ensure reproductive success. A major factor in seasonal control of flowering time is the photoperiod. The length of the daily light period is measured by the circadian clock in leaves, and a signal is conveyed to the shoot apex to initiate floral transition accordingly. In the last two decades, the molecular players in the photoperiodic pathway have been identified in Arabidopsis thaliana. Moreover, the intricate connections between the circadian clockwork and components of the photoperiodic pathway have been unravelled. In particular, the molecular basis of time-of-day-dependent sensitivity to floral stimuli, as predicted by Bünning and Pittendrigh, has been elucidated. This review covers recent insights into the molecular mechanisms underlying clock regulation of photoperiodic responses and the integration of the photoperiodic pathway into the flowering time network in Arabidopsis. Furthermore, examples of conservation and divergence in photoperiodic flower induction in other plant species are discussed."

a) Time to flower: interplay between photoperiod and the circadian clock

b) Time to exhaustion at continuous and intermittent maximal lactate steady state during running exercise

c) Thyroid hormone and seasonal regulation of reproduction

6. Read the abstract of a Review paper below (Curr Opin Chem Biol. 2014 Apr;19:34-41) and determine which one from the following lists of "Title" is most appropriate for this abstract. (5 points)

"Iron is an essential nutrient for the survival of organisms. Bacterial pathogens possess specialized pathways to acquire heme from their human hosts. In this review, we present recent structural and biochemical data that provide mechanistic insights into several bacterial heme uptake pathways, encompassing the sequestration of heme from human hemoproteins to secreted or membrane-associated bacterial proteins, the transport of heme across bacterial membranes, and the degradation of heme within the bacterial cytosol to liberate iron. The pathways for heme transport into the bacterial cytosol are divergent, harboring non-homologous protein sequences, novel structures, varying numbers of proteins, and different mechanisms. Congruously, the breakdown of heme within the bacterial cytosol by sequence-divergent proteins releases iron and distinct degradation products."

a) Heme uptake in bacterial pathogens

b) Coupling heme and iron metabolism via ferritin H chain

c) X-ray structures of ferritins and related proteins

7. Read the abstract of a Research paper below (Mol Microbiol. 2015 Jan;95(2):209-30.) and determine which one from the following lists of "Title" is most appropriate for this abstract. (5 %)

"*Eubacterium rectale* is a prominent human gut symbiont yet little is known about the molecular strategies this bacterium has developed to acquire nutrients within the competitive gut ecosystem. Starch is one of the most abundant glycans in the human diet, and *E. rectale* increases in vivo when

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the host consumes a diet rich in resistant starch, although it is not a primary degrader of this glycan. Here we present the results of a quantitative proteomics study in which we identify two glycoside hydrolase 13 family enzymes, and three ABC transporter solute-binding proteins that are abundant during growth on starch and, we hypothesize, work together at the cell surface to degrade starch and capture the released maltooligosaccharides. EUR_21100 is a multidomain cell wall anchored amylase that preferentially targets starch polysaccharides, liberating maltotetraose, whereas the membrane-associated maltogenic amylase EUR_01860 breaks down maltooligosaccharides longer than maltotriose. The three solute-binding proteins display a range of glycan-binding specificities that ensure the capture of glucose through maltoheptaose and some α1,6-branched glycans. Taken together, we describe a pathway for starch utilization by *E. rectale* DSM 17629 that may be conserved among other starch-degrading Clostridium cluster XIVa organisms in the human gut."

a) Molecular details of a starch utilization pathway in the human gut symbiont *Eubacterium rectale.*

b) Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, Bacteroides thetaiotaomicron, to the suckling period.

c) A hybrid two-component system protein of a prominent human gut symbiont couples glycan sensing in vivo to carbohydrate metabolism.

8. Please translate the "Title" you choose in the Question 7 to Chinese. (Scientific terms used in the field can be written in English) (5%)

9. Read the abstract of a Research paper below (Proc Natl Acad Sci U S A. 2015 Jan 13;112(2):412-7) and determine which one from the following lists of "Title" is most appropriate for this abstract. (5 %)

"Diatoms are unicellular algae that accumulate significant amounts of triacylglycerols as storage lipids when their growth is limited by nutrients. Using biochemical, physiological, bioinformatics, and reverse genetic approaches, we analyzed how the flux of carbon into lipids is influenced by nitrogen stress in a model diatom, *Phaeodactylum tricornutum*. Our results reveal that the accumulation of lipids is a consequence of remodeling of intermediate metabolism, especially reactions in the tricarboxylic acid and the urea cycles. Specifically, approximately one-half of the cellular proteins are cannibalized; whereas the nitrogen is scavenged by the urea and glutamine synthetase/glutamine 2-oxoglutarate aminotransferase pathways and redirected to the de novo synthesis of nitrogen assimilation machinery, simultaneously, the photobiological flux of carbon and reductants is used to synthesize lipids. To further examine how nitrogen stress triggers the remodeling process, we knocked down the gene encoding for nitrate reductase, a key enzyme required for the assimilation of nitrate. The strain exhibits 40–50% of the mRNA copy numbers, protein content, and enzymatic activity of the wild type, concomitant with a 43% increase in cellular lipid content. We suggest a negative feedback sensor that couples photosynthetic carbon fixation to lipid biosynthesis and is regulated by the nitrogen assimilation pathway. This metabolic feedback enables diatoms to rapidly respond to fluctuations in environmental nitrogen availability."

a) Physiological and molecular analysis of carbon source supplementation and pH stress-induced lipid accumulation in the marine diatom *Phaeodactylum tricornutum.*

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b) Physiological and molecular evidence that environmental changes elicit morphological interconversion in the model diatom *Phaeodactylum tricornutum*.

c) Remodeling of intermediate metabolism in the diatom *Phaeodactylum tricornutum* under nitrogen stress

10. Briefly describes the background, major results, conclusion and significance of the paper described in the Question 9 using Chinese. (Scientific terms used in the field can be written in English) (10%)

11. Read the abstract of a Research paper below (J Nutr Biochem. 2015 Feb;26(2):112-9.) and determine which one from the following lists of "Title" is most appropriate for this abstract. (5%)

"Circadian rhythm plays an important role in maintaining homeostasis, and its disruption increases the risk of developing metabolic syndrome. Circadian rhythm is maintained by a central clock in the hypothalamus that is entrained by light, but circadian clocks are also present in peripheral tissues. These peripheral clocks are trained by other cues, such as diet. The aim of this study was to determine whether proanthocyanidins, the most abundant polyphenols in the human diet, modulate the expression of clock and clock-controlled genes in the liver, gut and mesenteric white adipose tissue (mWAT) in healthy and obese rats. Grape seed proanthocyanidin extracts (GSPEs) were administered for 21 days at 5, 25 or 50 mg GSPE/kg body weight in healthy rats and 25 mg GSPE/kg body weight in rats with diet-induced obesity. In healthy animals, GSPE administration led to the overexpression of core clock genes in a positive dose-dependent manner. Moreover, the acetylated BMAL1 protein ratio increased with the same pattern in the liver and mWAT. With regards to clock-controlled genes, Per2 was also overexpressed, whereas Rev-erbα and RORα were repressed in a negative dose-dependent manner. Diet-induced obesity always resulted in the overexpression of some core clock and clock-related genes, although the particular gene affected was tissue specific. GSPE administration counteracted disturbances in the clock genes in the liver and gut but was less effective in normalizing the clock gene disruption in WAT. In conclusion, proanthocyanidins have the capacity to modulate peripheral molecular clocks in both healthy and obese states."

a) Postnatal ontogenesis of the circadian clock within the rat liver.

b) Chronic consumption of dietary proanthocyanidins modulates peripheral clocks in healthy and obese rats.

c) Anthocyanins do not influence long-chain n-3 fatty acid status: studies in cells, rodents and humans

12. Briefly describes the background, major results, conclusion and significance of the paper described in the Question 11 using Chinese. (Scientific terms used in the field can be written in English) (10%)

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