Part 1. Bob has three dishes of mammalian cells, NTU cells, BST cells, and GREAT cells. He cultures them on 60-mm dishes, and he plans to isolate RNAs from each dish. Here is the protocol he found from Chapter 7 of Molecular Cloning. He will end up with three RNAs samples. Please answer the following questions.

Step 1. Remove the medium and rinse the cells on each dish once with 5 mL of sterile ice-cold PBS.
Step 2. Remove PBS and lyse the cells in 1 mL of monophasic lysis reagent per 90-mm culture dish (0.7 mL per 60-mm dish).
Step 3. Transfer the cell lysates to a polypropylene snap-cap tube. Homogenize the lysates with a polytron homogenizer for 15-30 seconds at room temperature.
Step 4. Incubate the homogenates for 5 minutes at room temperature to permit complete dissociation of nucleoprotein complexes.
Step 5. Add 0.2 mL of chloroform per milliliter of monophasic lysis reagent. Mix the samples by vigorous shaking or vortexing.
Step 6. Separate the mixture into two phases by centrifuging at 12,000 rpm for 15 minutes at 4°C. Transfer the upper aqueous phase to a fresh tube.
Step 7. Precipitate the RNA from the aqueous phase: For each initial milliliter of monophasic lysis reagent, add 0.25 volume of isopropanol and 0.25 volume of RNA precipitation solution. After thorough mixing, store the final solution for 10 minutes at room temperature.
Step 8. Collect the precipitated RNA by centrifugation at maximum speed for 10 minutes at 4°C in a microfuge. Wash the pellet twice with 75% ethanol, and centrifuge again. Remove any remaining ethanol with a disposable pipette tip. Store the open tube on the bench for a few minutes to allow the ethanol to evaporate. Do not allow pellet to dry completely.
Step 9. Add 50 μL of DEPC-treated H_2O. Addition of SDS to 0.5% followed by heating to 65°C may assist dissolution of the pellet. Store the RNA solution at -70°C.
Step 10. Estimate the concentration of the RNA by measuring the absorbance at 260 nm of an aliquot of the final preparation.

1. **How much TOTAL volume of monophasic lysis reagent will Bob use while performing RNA extraction? (2%)**
   (A) 1 mL (B) 0.7 mL (C) 3 mL (D) 2.1 mL
2. **How much TOTAL volume of isopropanol will Bob use while performing RNA extraction? (2%)**
   (A) 250 μL (B) 175 μL (C) 750 μL (D) 525 μL
3. **Bob would like to dissolve the RNA pellet with 100 μL DEPC-treated H_2O containing SDS as suggested by this protocol. He has a bottle of 10% SDS stock solution. How much DEPC-treated H_2O and 10% SDS, respectively will he need (total volume is 100 μL)? (4%)**
4. **Which instrument or device will Bob NOT need in this experiment? (2%)**
   (A) centrifuge (B) microwave (C) homogenizer (D) pipette

Part 2. Please read the following article and answer the following questions [source: Modified from *Cell Metabolism* 22(3) 367-380, 2015].

Preabsorptive nutrients trigger complex and integrative gut-brain negative feedback axes to prevent energy excess by suppressing food intake and endogenous nutrient production. Intestinal nutrients signal mainly through the release of GI peptides, which act on central targets in an endocrine fashion, or through local, paracrine action on nerve terminals innervating the gut. Alternatively, nutrients themselves act alone, or in cohort with gut peptides, to activate afferent neurons. Gut peptides are synthesized in enteroendocrine cells (EECs) of the gut epithelial mucosa. The EECs are exposed to the intestinal lumen on their apical sides and secrete peptides from their basolateral sides and are in close proximity to nerve endings expressing gut peptide receptors, supporting local, paracrine signaling. Classically, EECs are characterized by the peptides that they produce. The proximal small intestine contains I cells and K cells, which produce cholecystokinin (CCK) and glucose-dependent insulinotropic hormone (GIP), respectively, while the distal small intestine and colon contain L cells, which produce glucagon-like peptide-1/2 (GLP-1/2), oxyntomodulin and peptide

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YY (PYY). Nonetheless, EECs are activated by a variety of nutrient-dependent machineries that trigger membrane depolarization, second messenger cascades, and intracellular calcium elevation to stimulate gut peptide release.

5. **Preabsorptive nutrients stimulate the synthesis of endogenous nutrients. (2%)**
   (A) True (B) False (C) Not mentioned in the article
6. **Which one is gut peptides? (2%)**
   (A) CCK (B) GLP-1/2 (C) PYY (D) All of the above
7. **Which is NOT true about "enteroendocrine cells?" (2%)**
   (A) producing gut peptide (B) one of mucosa cells (C) making a contact to intestinal lumen (D) expressing gut peptide receptors.
8. **Which peptides do K cells produce? (2%)**
   (A) CCK (B) GIP (C) GLP-1/2 (D) PYY
9. **Which is NOT true about the mechanism by which enteroendocrine cells stimulate gut peptide release? (2%)**
   (A) trigger membrane depolarization (B) stimulate Ca^{2+} entry into cell (C) activate second messenger system (D) promote calcium release out of cell

Part 3. Please read the following article and answer the following questions [source: Am J Pathol 2015 Dec 24. pii: S0002-9440(15)00649-5].

Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in a human with severe pneumonia in 2012. Since then, infections have been detected in >1500 individuals, with disease severity ranging from asymptomatic to severe, fatal pneumonia. To elucidate the pathogenesis of this virus and investigate mechanisms underlying disease severity variation in the absence of autopsy data, a rhesus macaque and common marmoset model of MERS-CoV disease were analyzed. Rhesus macaques developed mild disease, and common marmosets exhibited moderate to severe, potentially lethal, disease. Both nonhuman primate species exhibited respiratory clinical signs after inoculation, which were more severe and of longer duration in the marmosets, and developed bronchointerstitial pneumonia. In marmosets, the pneumonia was more extensive, with development of severe airway lesions. Quantitative analysis showed significantly higher levels of pulmonary neutrophil infiltration and higher amounts of pulmonary viral antigen in marmosets. Pulmonary expression of the MERS-CoV receptor, dipeptidyl peptidase 4, was similar in marmosets and macaques. These results suggest that increased virus replication and the local immune response to MERS-CoV infection likely play a role in pulmonary pathology severity. Together, the rhesus macaque and common marmoset models of MERS-CoV span the wide range of disease severity reported in MERS-CoV-infected humans, which will aid in investigating MERS-CoV disease pathogenesis.

10. **Which system was NOT used in this study? (2%)**
    (A) autopsy data (B) rhesus macaques (C) marmosets (D) primate specie
11. **Dipeptidyl peptidase 4 is expressed in (A) heart (B) skin (C) lung (D) kidney. (2%)**
12. **Based on this abstract, the following statement of "human infected with MERS-CoV might not show any signs of being sick" is (A) True (B) False (C) Not mentioned in this abstract. (2%)**
13. **In terms of nonhuman primate species described in this abstract, which one shows severer clinical signs after inoculation? (2%)**
    (A) human (B) rhesus macaques (C) marmosets (D) Not mentioned in this abstract.
14. **Which one is NOT happening in marmosets inoculated with MERS-CoV? (2%)**
    (A) increased neutrophil infiltration (B) increased virus replication (C) increased expression of MERS-CoV receptor (D) increased immune response
15. **Which one of the following lists of "Title" fits the abstract best: (2%)**
    (A) MERS-CoV: A trigger for healthcare transformation (B) An acute immune response to MERS-CoV replication contributes to viral pathogenicity (C) Risk factors for MERS-CoV illness in primate species (D) MERS-CoV: diagnostics, epidemiology and transmission

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Part 4. Please read the following article and answer the following questions [source: http://www.the-scientist.com/?articles.view/articleNo/45035/title/Finding-Enhancers-with-CRISPR/].

While several groups are working to apply the CRISPR/Cas9 system for clinical purposes, some are using the tool to address fundamental questions about biology. Reuven Agami, a professor of genetics at the Netherlands Cancer Institute in Amsterdam, and his colleagues recently applied CRISPR to search for regulatory enhancer elements throughout the genome. They targeted the Cas9 nuclease to previously identified enhancer elements—transcription factor-binding DNA sequences that distally regulate gene expression—of two transcription factors: p53, and estrogen receptor alpha (ERα), which are both frequently mutated and deregulated in cancer. The team's findings, published today (January 11, 2016) in *Nature Biotechnology,* highlight key enhancer sequences of these two proteins, and demonstrate the utility of CRISPR for the systematic study of noncoding DNA sequences.

16. **"Enhancer" is a (A) transcription factor (B) DNA sequence (C) p53 (D) estrogen receptor alpha (2%)**
17. **Deregulated means (A) upregulated (B) downregulated (C) upregulated or downregulated (D) None of the above (2%)**
18. **"highlight" means (A) explore (B) demonstrate (C) study (D) emphasize (2%)**

Two other groups had previously used CRISPR to create knockout libraries of protein-coding genes in human cells for functional genetic screens. But the current work is the first genetic screen of regulatory elements using the CRISPR/Cas9 system, said Cecilia Moens of the Fred Hutchinson Cancer Research Center in Seattle who was not involved in the work. "This is a proof-of-concept study," she said.

"This [CRISPR-based approach] is really necessary because it is quite difficult to assess the function of regulatory elements in endogenous situations, without using reporter assays," said Ramin Shiekhattar, who studies the human epigenome and the molecular bases of cancer at the University of Miami Miller School of Medicine but was not part of the current work.

19. **Please translate the following two sentences "Many……challenge" in Chinese (4%).**

Many mutations in protein-coding genes are known to contribute to the growth of cancer. Finding mutations in noncoding regions that affect cancer growth has been a challenge.

"The proper function of enhancers is essential for maintaining healthy tissue and there are many hints that their malfunction in cancer may cause changes in gene expression that favor tumor development," said Agami. "What we needed was a tool to pinpoint which enhancers are potentially important in cancer because it is difficult to tell if a noncoding mutation in a tumor is causal for the tumor's growth, or a consequence of it."

20. **A noncoding mutation in a tumor _____ the tumor's growth. (2%)**
(A) results in (B) results from (C) could be the reason or the result of (D) is irrelevant to

Agami's team built a CRISPR single-guide RNA library targeted to 685 genomic sites, representing around 90 percent of the known enhancer elements of p53. Beyond the single-guide RNA, the human cells used for the screen also expressed p53 and an inducible oncogene, *Hras*. Inducing the aberrant expression of *Hras* causes p53-mediated cellular senescence. But if the team used CRISPR to knock out an enhancer essential for p53 function, expression of *Hras* enabled the cells to continue to grow uninhibited. In this way, the team identified three enhancer elements important for p53 function, including two just upstream of p21, also known as cyclin-dependent kinase inhibitor 1A. Apparently, p53 needs to bind at these two sites upstream of the p21 gene to fully activate cellular senescence.

21. **"fully" means (A) partially (B) entirely (C) suddenly (D) finally (2%)**

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"It is exciting to see this strategy work," David Root, director of the Broad Institute's Genetic Perturbation Platform who was not involved in the work, wrote in an email to *The Scientist*. "Next, it will be useful to learn how comprehensive this approach is. What fraction of all the candidate sites were accurately tested with a couple of sgRNAs [single-guide RNAs] apiece?"

Having classified DNA elements required for ERα activity, Agami's team plans to sequence these loci in breast tumor samples from patients that developed resistance to antiestrogen therapy. "We would like to know whether these tumor samples have accumulated mutations in these enhancer elements that may explain why the tumors became resistant to therapy," he said.

A useful extension of the current study, said Shiekhattar, would be to look at the enhancers active in specific contexts—such as precise times during development and in specific tumor types. "We can't quite recapitulate what happens *in vivo* with tissue culture," he said. "Now, we could be using CRISPR in mouse models."

22. "recapitulate" means (A) repeat (B) understand (C) sure (D) concern (2%)

In a second screen, the researchers built a different single-guide RNA library targeting 73 ERα enhancer binding sites. They identified three enhancer sequences that, when knocked out in human breast cancer cell lines, rely on ERα expression for growth.

"What CRISPR/Cas9 has done for us is to make targeting mutations really trivial," said Moens. "Applying this to [non-coding sequences] is clever, and it is surprising to me that it worked. But in principle, the idea is the same, to disrupt a regulatory element rather than the gene itself using a loss of function approach."

23. "in principle" means (A) logically (B) interestingly (C) mutually (D) generally (2%)

Part 5. Bob finished a polymerase chain reaction (PCR) of his DNA samples and conducted agarose gel electrophoresis to check the resulting products. However, Bob saw some unexpected results on the agarose gel and had no idea what went wrong. Below is the general troubleshooting guideline from JoVE [source: modified from J. Vis. Exp. (63), e3998, doi:10.3791/3998 (2012).]. Please read it and help Bob to resolve the problem.

I. First determine if any of the PCR reagents is catastrophic to your reaction. This can be achieved by preparing new reagents (e.g., fresh working stocks, new dilutions), and then systematically adding one new reagent at a time to reaction mixtures. This process will determine which reagent was the cause for the failed PCR experiment.
II. Primer dimers can form when primers preferentially self anneal or anneal to the other primer in the reaction. If this occurs, a small product of less than 100 bp will appear on the agarose gel. Start by altering the ratio of template to primer; if the primer concentration is in extreme excess over the template concentration, then the primers will be more likely to anneal to themselves or each other over the DNA template.
III. Lack of PCR products is likely due to reaction conditions that are too stringent (e.g., high annealing temperature or low PCR cycle number). Primer dimers and hairpin loop structures that form with the primers or in the denatured template DNA may also prevent amplification of PCR products because these molecules may no longer base pair with the desired DNA counterpart.
IV. Non-specific products are produced when PCR stringency is excessively low resulting in non-specific PCR bands with variable lengths. This produces a ladder effect on an agarose gel. It then is advisable to choose PCR conditions that increase stringency.

24. Which procedure won't you suggest that Bob should do if only a small band (~ 50bp) is observed? (4%)
(A) prepare fresh reagents (B) increase PCR cycle number (C) increase the annealing temperature (D) increase template amount.

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25. What procedure will you probably advise Bob to try if he sees four bands? (4%)
(A) increase the annealing temperature (B) redesign new primers with higher specificity (C) reduce the primer concentration (D) all of the above

26. What type of samples can't Bob use as the templates for PCR? (3%)
(A) genomic DNA from plants (B) cDNA from mammalian cell culture (C) plasmid DNA from bacteria (D) virus RNA

Part 6. Bob is asked to carry out a laboratory scale recombinant protein expression experiment using bacteria *E. coli* BL21DE3 via IPTG induction. The protocol is listed as followed:

I. Pick up a single colony from a fresh plate with bacterial cells and grow overnight in 5 mL LB broth with antibiotics at 37°C 250 rpm shaking.

II. Re-inoculate at 1% ratio (1:100 dilution) into a glass flask containing 250 mL LB and grow with 250 rpm shaking at 37°C for 4-6 hours.

III. Once the cell density (optical density at 600 nm; OD_{600}) reaches 0.4-0.8, add Isopropyl β-D-1-thiogalactopyranoside (IPTG) to working concentration of 1mM and then induce for 12-16 hours at 30°C with 250 rpm shaking.

IV. Pellet the cells by centrifugation at 6000 x g, for 20 min at 4°C and resuspend the pellet in phosphate buffer for lysis and protein purification.

27. At which cell density is bacterial culture not in good shape for IPTG induction? (3%)
(A) OD_{600} =0.4 (B) OD_{600}= 0. 7 (C) OD_{600}=2 (D) none of the above

28. How much volume of IPTG will Bob have to add roughly if he has the stock concentration of 1M? (5%)

Part 7. Please read the article and answer the following questions [source: Modified from Sci. Transl. Med. 7(289):289ra84, 2015].

Rapid advances in the forward engineering of genetic circuitry in living cells has positioned synthetic biology as a potential means to solve numerous biomedical problems, including disease diagnosis and therapy. One challenge in exploiting synthetic biology for translational applications is to engineer microbes that are well tolerated by patients and seamlessly integrate with existing clinical methods. We use the safe and widely used probiotic *Escherichia coli* Nissle 1917 to develop an orally administered diagnostic that can noninvasively indicate the presence of liver metastasis by producing easily detectable signals in urine. Our microbial diagnostic generated a high-contrast urine signal through selective expansion in liver metastases (106-fold enrichment) and high expression of a lacZ reporter maintained by engineering a stable plasmid system. The lacZ reporter cleaves a substrate to produce a small molecule that can be detected in urine. *E. coli* Nissle 1917 robustly colonized tumor tissue in rodent models of liver metastasis after oral delivery but did not colonize healthy organs or fibrotic liver tissue. We saw no deleterious health effects on the mice for more than 12 months after oral delivery. Our results demonstrate that probiotics can be programmed to safely and selectively deliver synthetic gene circuits to diseased tissue microenvironments in vivo.

29. What research topic is irrelevant to this article? (3%)
(A) synthetic biology (B) urine (C) diagnosis (D) bioenergy

30. According to this article, what potential toxicity does this novel platform show in vivo? (3%)
(A) no (B) lethal (C) not mentioned in this article

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Part 8. Please read the article and answer the following questions [source: Nat. Commun. 6: 8500 doi:10.1038/ncomms9500, 2015].

Advances in nanotechnology have provided unprecedented physical means to sample molecular space. Living cells provide additional capability in that they identify molecules within complex environments and actuate function. We have merged cells with nanotechnology for an integrated molecular processing network. Here we show that an engineered cell consortium autonomously generates feedback to chemical cues. Moreover, abiotic components are readily assembled onto cells, enabling amplified and 'binned' responses. Specifically, engineered cell populations are triggered by a quorum sensing (QS) signal molecule, autoinducer-2, to express surface-displayed fusions consisting of a fluorescent marker and an affinity peptide. The latter provides means for attaching magnetic nanoparticles to fluorescently activated subpopulations for coalescence into colour-indexed output. The resultant nano-guided cell network assesses QS activity and conveys molecular information as a 'bio-litmus' in a manner read by simple optical means.

31. Which title will fit the article best? (3%)
(A) Nano-guided cell networks as conveyors of molecular communication
(B) Synthetic mammalian gene circuits for biomedical applications
(C) Reprogramming microbes to be pathogen-seeking killers

32. When sensing the QS signals in the environment, what will the bacterial cells synthesize on cell surfaces for attaching to nanoparticles? Please answer in English (5%)

Part 9. Please read the article and answer the following questions [source: Modified from Nature 529:212-215, 2016].

The gut is home to trillions of microorganisms that have fundamental roles in many aspects of human biology, including immune function and metabolism. The reduced diversity of the gut microbiota in Western populations compared to that in populations living traditional lifestyles presents the question of which factors have driven microbiota change during modernization. Microbiota-accessible carbohydrates (MACs) found in dietary fibre have a crucial involvement in shaping this microbial ecosystem, and are notably reduced in the Western diet (high in fat and simple carbohydrates, low in fibre) compared with a more traditional diet. Here we show that changes in the microbiota of mice consuming a low-MAC diet and harbouring a human microbiota are largely reversible within a single generation. However, over several generations, a low-MAC diet results in a progressive loss of diversity, which is not recoverable after the reintroduction of dietary MACs. To restore the microbiota to its original state requires the administration of missing taxa in combination with dietary MAC consumption.

33. According to the article, what is main cause of the imbalance of the microbiota inside the gut? Please answer in English (5%)

34. What is the strategy that the authors indicated to tackle this issue? (3%)
(A) uptake probiotics (B) eat food with high fiber content (C) eat less fat (D) all of the above

Part 10. Please read the paragraph and answer the following questions [source: Modified from Nature 527:329-335, 2015]

Ever since Stephen Paget's 1889 hypothesis, metastatic organotropism has remained one of cancer's greatest mysteries. Here we "**demonstrate**" that exosomes from mouse and human lung-, liver- and brain-tropic tumour cells fuse preferentially with resident cells at their predicted destination, namely lung fibroblasts and epithelial cells, liver Kupffer cells and brain endothelial cells. We show that tumour-derived exosomes uptaken by organ-specific cells prepare the pre-metastatic niche. Treatment with

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exosomes from lung-tropic models redirected the metastasis of bone-tropic tumour cells. Exosome proteomics revealed distinct integrin expression patterns, in which the exosomal integrins α6β4 and α6β1 were associated with lung metastasis, while exosomal integrin αvβ5 was linked to liver metastasis. Targeting the integrins α6β4 and αvβ5 decreased exosome uptake, as well as lung and liver metastasis, respectively.

35. What does "demonstrate" mean in the paragraph? (3%)
(A) contribute (B) illustrate (C) design (D) invite

36. What is the take-home message that this paragraph tries to deliver? Please answer in English (6%)

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