1. The ant is a social insect _____.
   (A) that live in an organized colony
   (B) that was organized in living colony
   (C) it lived in an organized colony
   (D) what lives in an organized colony

2. Most psychologists deeply believe that it is just as difficult to change a person’s way of thinking _____ to rectify his deep-rooted habits.
   (A) as it does
   (B) as it is
   (C) than is it
   (D) than it does

3. Weather is usually determined by _____.
   (A) there are various atmospheric conditions
   (B) atmospheric conditions are various
   (C) various atmospheric conditions
   (D) atmospheric conditions vary

4. _____ depends on our ideas of the human-nature relationship.
   (A) What do we do with nature
   (B) What we do with nature
   (C) We do what with nature
   (D) Do what with nature

5. Koala bears are _____ marsupials.
   (A) leaf-eating
   (B) eating leaves
   (C) they eat leaves
   (D) leaves-eating

6. Flying saucers _____ because of its saucer-like shape and its capacity to fly.
   (A) is so called
   (B) called so is
   (C) so called is
   (D) so is called

7. _____ us the ability to resist distance, but also helps us build our body tissues.
   (A) Vitamin C not only provides
   (B) Not only does vitamin C provide
   (C) Not only vitamin C provides
   (D) Vitamin C, which not only provides
8. Metal factors may result in _____ physiological disorders.
   (A) and
   (B) among
   (C) where
   (D) severe

9. Blood pressure _____ by the force that blood exerts against the walls of the arteries.
   (A) determined
   (B) being determined
   (C) is determined
   (D) which it is determined

10. Unlike other mammals, _____ in the pouch of the mother’s body.
    (A) the bodies of the marsupial are raised
    (B) the marsupial raises its babies
    (C) the raise of the marsupial’s babies is
    (D) raised are the babies of the marsupial

11. While _____ on the freeway, all drivers must keep alert all the time on the journey.
    (A) drive
    (B) they driving
    (C) are driving
    (D) driving

12. Our teacher recommended that everyone _____ a composition as homework.
    (A) writes
    (B) wrote
    (C) writing
    (D) write

13. Only when a monkey is mature enough _____ to develop its independence from its mother.
    (A) does it begin
    (B) it begins
    (C) it had begun
    (D) it will begin

14. The doctor _____ said she had a fever.
    (A) whom she went
    (B) that she went
    (C) to whom she went to
    (D) that she went to

15. Anyone _____ receiving information about the club should write to the secretary.
    (A) interesting to
    (B) interested in
    (C) is interesting about
    (D) who interested of
TRIZOL® Reagent
Cat. No. 15596-026  Size: 100 ml
Store at 2 to 8°C.
WARNING: Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice (show label where possible). Phenol (108-95-2) and Other Components (NJTSRN 80100437-5000p).
TRIZOL has demonstrated stability of 12 months when stored at room temperature. However, we recommend storage at 2 to 8°C for optimal performance.
Description:
TRIZOL Reagent (U.S. Patent No.5,346,994) is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent is a mono-phasic solution of phenol and guanidine isothiocyanate, an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1). During sample homogenization or lysis, TRIZOL Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropanol alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation (2). Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropanol alcohol yields proteins from the organic phase (2). Copurification of the DNA may be useful for normalizing RNA yields from sample to sample.

This technique performs well with small quantities of tissue (50-100 mg) and cells (5 × 10⁶), and large quantities of tissue (≥1 g) and cells (≥10⁷), of human, animal, plant, or bacterial origin. The simplicity of the TRIZOL Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by TRIZOL Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly (A)+ selection in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR*), treatment of the isolated RNA with amplification grade DNase I (Cat. No. 18068) is recommended when the two primers lie within a single exon.

TRIZOL Reagent facilitates isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from rat liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, (composed of mRNA’s and hnRNA’s) two predominant ribosomal RNA bands at ~5 kb (28S) and 18S, and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an A260/A280 ratio ≥1.8 when diluted into TE.

Precautions for Preventing RNase Contamination:
RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.
- Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
- Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.
- In the presence of TRIZOL Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150°C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, rinsed thoroughly with water, and autoclaved.

Other Precautions:
- Use of disposable tubes made of clear polypropylene is recommended when working with less than 2-ml volumes of TRIZOL.
- For larger volumes, use glass (Corning) or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 x g with TRIZOL Reagent and chloroform. Do not use tubes that leak or crack.
- Carefully equilibrate the weights of the tubes prior to centrifugation.
- Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugation.
INSTRUCTIONS FOR RNA ISOLATION:

Caution: When working with TRIZOL Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

Unless otherwise stated, the procedure is carried out at 15 to 30°C, and reagents are at 15 to 30°C.

Reagents required, but not supplied:
- Chloroform, Isopropyl alcohol, 75% Ethanol (in DEPC-treated water)
- RNase-free water or 0.5% SDS solution [To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved waters.]

1. HOMOGENIZATION (see notes 1-3)
   a. Tissues
      Homogenize tissue samples in 1 ml of TRIZOL Reagent per 50-100 mg of tissue using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for homogenization.
   b. Cells Grown in Monolayer
      Lyse cells directly in a culture dish by adding 1 ml of TRIZOL Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIZOL Reagent added is based on the area of the culture dish (1 ml per 10 cm²) and not on the number of cells present. An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.
   c. Cells Grown in Suspension
      Pellet cells by centrifugation. Lyse cells in TRIZOL Reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 × 10⁶ of animal, plant or yeast cells, or per 1 × 10⁷ bacterial cells. Washing cells before addition of TRIZOL Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

OPTIONAL: An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberculous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 × g for 10 minutes at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2. PHASE SEPARATION
   Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRIZOL reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 × g for 15 minutes at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIZOL Reagent used for homogenization.

3. RNA PRECIPITATION
   Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000 × g for 10 minutes at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA WASH
   Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 × g for 5 minutes at 2 to 8°C.

5. REDISSOLVING THE RNA
   At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redisolved in 100% formamide (deionized) and stored at -70°C (5).
RNA Isolation Notes:
1. Isolation of RNA from small quantities of tissue (1 to 10 mg) or Cell (102 to 104) Samples: Add 800 μl of TRIZOL to the tissue or cells. Following sample lysis, add chloroform and proceed with the phase separation as described in step 2. Prior to precipitating the RNA with isopropyl alcohol, add 5-10 μg RNA-free glycogen (Cat. No 10814) as carrier to the aqueous phase. To reduce viscosity, shear the genomic DNA with 2 passes through a 26 gauge needle prior to chloroform addition. The glycogen remains in the aqueous phase and is co-precipitated with the RNA. It does not inhibit first-strand synthesis at concentrations up to 4 mg/ml and does not inhibit PCR.
2. After homogenization and before addition of chloroform, samples can be stored at -60 to -70°C for at least one month. The RNA precipitate (step 4, RNA WASH) can be stored in 75% ethanol at 2 to 8°C for at least one week, or at least one year at -5 to -20°C.
3. Table-top centrifuges that can attain a maximum of 2,600 × g are suitable for use in these protocols if the centrifugation time is increased to 30-60 minutes in steps 2 and 3.

INSTRUCTIONS FOR DNA ISOLATION:
After complete removal of the aqueous phase, as described in the RNA isolation protocol, the DNA in the interphase and phenol phase from the initial homogenate may be isolated. Following precipitation and a series of washes, the DNA is solubilized in 8 mM NaOH. Full recovery of DNA from tissues and culture cells permits the use of TRIZOL Reagent for the determination of the DNA content in analyzed samples (2). Simultaneous extraction of genomic DNA allows for normalization of the results of Northern analysis per genomic DNA instead of the more variable total RNA or tissue weight. (Depending on the source, the DNA pellet obtained may require additional purification (e.g., phenol extraction) prior to other applications.

Unless otherwise stated, the procedure is carried out at 15 to 30°C.

1. DNA PRECIPITATION
Remove the remaining aqueous phase overlying the interphase, and precipitate the DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 1 ml of TRIZOL used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15 to 30°C for 2-3 minutes and sediment DNA by centrifugation at no more than 2,000 × g for 5 minutes at 2 to 8°C.

Careful removal of the aqueous phase is critical for the quality of the isolated DNA.

2. DNA WASH
Remove the phenol-ethanol supernatant, and if desired, save it for protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol. Use 1 ml of the solution per 1 ml of TRIZOL Reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × g for 5 minutes at 2 to 8°C. Following these two washes, suspend the DNA pellet in 75% ethanol (1.5-2 ml of 75% ethanol per 1 ml TRIZOL Reagent), store for 10-20 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × g for 5 minutes at 2 to 8°C.

An additional wash in 0.1 M sodium citrate-10% ethanol solution is required for large pellets containing > 200 μg DNA or large amounts of a non-DNA material.

3. REDISSOLVING THE DNA
Air dry the DNA 5 to 15 minutes in an open tube. (DO NOT DRY UNDER CENTRIFUGATION; it will be more difficult to dissolve.) Dissolve DNA in 8 mM NaOH such that the concentration of DNA is 0.2 – 0.3 μg/μl. Typically add 300 – 600 μl of 8 mM NaOH to DNA isolated from 107 cells or 50 – 70 mg of tissue. Resuspending in weak base is HIGHLY recommended since isolated DNA does not resuspend well in water or in Tris buffers. The pH of the 8 mM NaOH is only ~9 and should be easily adjusted with TE or HEPES once the DNA is in solution. At this stage, the DNA preparations (especially from tissues) may contain insoluble gel-like material (fragments of membranes, etc.) Remove the insoluble material by centrifugation at >12,000 × g for 10 minutes. Transfer the supernatant containing the DNA to a new tube. DNA solubilized in 8 mM NaOH can be stored overnight at 4°C; for prolonged storage, samples should be adjusted with HEPES to pH 7-8 (see table) and supplemented with 1 mM EDTA. Once the pH is adjusted, DNA can be stored at 4°C or ~20°C.

Quantification and Expected Yields of DNA
Take an aliquot of the DNA preparation solubilized in 8 mM NaOH, mix it with water and measure the A260 of the resulting solution. Calculate the DNA content using the A260 value for double-stranded DNA. One A260 unit equals 50 μg of double-stranded DNA/mL. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1 × 106 diploid cells of human, rat, and mouse origin equals: 7.1 μg, 6.5 μg, and 5.8 μg, respectively (3).
Applications:

Amplification of DNA by PCR:

After redissolving the DNA in 8 mM NaOH, adjust the pH to 8.4 with 0.1 M HEPES (see table). Add 0.1 to 1.0 μg of the DNA sample to your PCR reaction mixture and perform the standard PCR protocol.

Restriction endonuclease reactions:

Adjust the pH of the DNA solution to a required value using HEPES (see table). Alternatively, samples may be dialyzed against 1 mM EDTA, pH 7 to pH 8.0. Use 3-5 units of enzyme per microgram of DNA. Use the conditions recommended by the manufacturer for the particular enzyme, and allow the reaction to proceed for 3 to 24 h. In a typical assay, 80-90% of the DNA is digestible.

pH Adjustment of DNA Samples Dissolved in 8 mM NaOH:

(For 1 ml of 8 mM NaOH use the following amounts of 0.1 M or 1 M HEPES, free acid.)

<table>
<thead>
<tr>
<th>Final pH</th>
<th>0.1 M HEPES (μl)</th>
<th>Final pH</th>
<th>1 M HEPES (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.4</td>
<td>86</td>
<td>7.5</td>
<td>159</td>
</tr>
<tr>
<td>8.2</td>
<td>93</td>
<td>7.2</td>
<td>23</td>
</tr>
<tr>
<td>8.0</td>
<td>101</td>
<td>7.0</td>
<td>32</td>
</tr>
<tr>
<td>7.8</td>
<td>117</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DNA Isolation Notes:

1. The phenol phase and interphase can be stored at 2 to 8°C overnight.
2. Samples suspended in 75% ethanol can be stored at 2 to 8°C for months.
3. Samples dissolved in 8 mM NaOH can be stored overnight at 2 to 8°C. For long-term storage, adjust the pH to 7-8, and adjust the EDTA concentration to 1 mM.

INSTRUCTIONS FOR PROTEIN ISOLATION:

Proteins are isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol (step 1, DNA PRECIPITATION). The resulting preparation can be analyzed for the presence of specific proteins by Western blotting (2).

Reagents required, but not supplied:

- Isopropyl alcohol
- 0.3 M Guanidine hydrochloride in 95% ethanol
- Ethanol
- 1% SDS

1. PROTEIN PRECIPITATION

Precipitate proteins from the phenol-ethanol supernatant (approximate volume 0.8 ml per 1 ml of TRIZOL Reagent) with isopropyl alcohol. Add 1.5 ml of isopropanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Store samples for 10 minutes at 15 to 30°C, and sediment the protein precipitate at 12,000 x g for 10 minutes at 2 to 8°C.

2. PROTEIN WASH

Remove the supernatant and wash the protein pellet 3 times in a solution containing 0.3 M guanidine hydrochloride in 95% ethanol. Add 2 ml of wash solution per 1 ml of TRIZOL Reagent used for the initial homogenization. During each wash cycle, store the protein pellet in the wash solution for 20 minutes at 15 to 30°C and centrifuge at 7,500 x g for 5 minutes at 2 to 8°C. After the final wash, vortex the protein pellet in 2 ml of ethanol. Store the protein pellet in ethanol for 20 minutes at 15 to 30°C and centrifuge at 7,500 x g for 5 minutes at 2 to 8°C.

3. REDISSOLVING THE PROTEIN PELLET

Vacuum dry the protein pellet for 5-10 minutes. Dissolve it in 1% SDS by pipetting. Complete dissolution of the protein pellet may require incubating the sample at 50°C. Sediment any insoluble material by centrifugation at 10,000 x g for 10 minutes at 2 to 8°C, and transfer the supernatant to a fresh tube. The sample is ready for use in Western blotting or may be stored at -5 to -20°C for future use.

Protein Isolation Notes:

1. The protein pellet suspended in 0.3 M guanidine hydrochloride-95% ethanol or in ethanol can be stored for at least one month at 2 to 8°C, or for at least one year at -5 to -20°C.
2. The following protocol is an alternative approach that allows for more efficient recovery of proteins. Dialyze the phenol-ethanol supernatant against three changes of 0.1% SDS at 2 to 8°C. Centrifuge the dialyzed material at 10,000 x g for 10 minutes. Use the clear supernatant for Western blotting.
3. Proteins may be quantified by the Bradford method as long as the concentration of SDS is low enough (<0.1%) so that it will not interfere. Methods that do not have detergent-interface problems, and that do not rely on A260/A280 measurements may be used (traces of phenol may cause overestimation of protein concentrations).
Troubleshooting Guide:

RNA ISOLATION

- Expected yields of RNA per mg of tissue or $1 \times 10^6$ cultured cells
  - Liver and spleen, 6-10 µg
  - Skeletal muscles and brain, 1-1.5 µg
  - Epithelial cells ($1 \times 10^6$ cultured cells), 8-15 µg
  - Kidney, 3-4 µg
  - Placenta, 1-4 µg
  - Fibroblasts, ($1 \times 10^6$ cultured cells) 5-7 µg

- Low yield
  - Incomplete homogenization or lysis of samples. Final RNA pellet incompletely redissolved.
  - $A_{260}/A_{280}$ ratio <1.65
  - RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase absorbance at 280 nm (6,7).
  - Sample homogenized in too small a reagent volume.
  - Following homogenization, samples were not stored at room temperature for 5 minutes.
  - The aqueous phase was contaminated with the phenol phase.
  - Incomplete dissolution of the final RNA pellet.

- RNA degradation
  - Tissues were not immediately processed or frozen after removal from the animal.
  - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
  - Cells were dispersed by trypsin digestion.
  - Aqueous solutions or tubes were not RNase-free.
  - Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

- DNA contamination
  - Sample homogenized in too small a reagent volume.
  - Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.

  - Proteoglycan and polysaccharide contamination
    - The following modification of the RNA precipitation (step 3) removes these contaminating compounds from the isolated RNA. Add to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of TRIZOL Reagent used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. A combination of the modified precipitation with an additional centrifugation of the initial homogenate (note 2, RNA isolation protocol) is required to isolate pure RNA from plant material containing a very high level of polysaccharides.

DNA ISOLATION

- Expected yields of DNA per mg of tissue or $1 \times 10^6$ cultured cells
  - Liver and kidney, 3-4 µg
  - Cultured human, rat, and mouse cells ($1 \times 10^6$), 5-7 µg
  - Skeletal muscles, brain, and placenta 2-3 µg
  - Fibroblasts, 5-7 µg

- Low yield
  - Incomplete homogenization or lysis of samples. Final DNA pellet incompletely redissolved.

- $A_{260}/A_{280}$ ratio <1.70
  - DNA sample was diluted in water instead of TE prior to spectrophotometric analysis.
  - Phenol was not sufficiently removed from the DNA preparation. Wash the DNA pellet an additional time with 0.1 M sodium citrate in 10% ethanol.

- DNA degradation
  - Tissues were not immediately processed or frozen after removal from the animal.
  - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
  - Samples were homogenized with a Polytron or other high speed homogenizer.

- DNA contamination
  - Incomplete removal of aqueous phase.
  - DNA pellet insufficiently washed with 0.1 M sodium citrate in 10% ethanol.

- Other applications
  - Prior to use in PCR amplification, adjust the pH to 8.4.
  - For digestion of the DNA with restriction endonucleases, adjust the pH to the desired value, use 3-5 units of enzyme per µg of DNA, and allow the reaction to go for 3-24 hours under optimal conditions for the particular enzyme. Typically 80-90% of the DNA is digested.
PROTEIN ISOLATION

Low yield
- Incomplete homogenization or lysis of samples.
- Final DNA pellet incompletely redissolved.

Protein degradation
- Tissues were not immediately processed or frozen after removing from the animal.
- Band deformation in PAGE
- Protein pellet insufficiently washed.

References:

Teflon® is a registered trademark of E. I. Du Pont de Nemours & Co.
TISSUMIZER® is a registered trademark of Tekmar Co.
TRIZOL® is a registered trademark of Molecular Research Center, Inc.
*PCR is covered by a patent held by Hoffinan LaRoche Corporation.

看完上文後，請回答下列問題，並將答案寫在答案卷上。回答答案時，請盡量以中文回答，但若遇到不易翻譯的專有名詞(如 RNA, DNA, 0.5 M NaOH, isopropyl alcohol, trypsin, phenol, chloroform, formamide, SDS, PCR 等等)，可直接用英文表示。

16. According to this article, what is the right way(s) to store Trizol? (including temperature and other factors) (10分)

17. According to this article, what will you do to prevent RNase contamination? (10分)

18. Please write a step-by-step protocol about how to isolate RNA from 75mg of tissue. (30分)
(限500字以內，以條列式，一步一步依序說明較佳)

19. What can you do to improve your skill or solve problem if you got RNA contamination in DNA product? (10分)

20. According to this article, what will you do (for performing PCR) after you have DNA product? (10分)
A: Choose the one best response to each following questions. (50 points)

1. The following sugars are not reducing sugar:
   (A) 1 and 2; (B) 2 and 3; (C) 1, 2, and 3; (D) only 2; (E) only 5.

2. Which of the following compounds is an anomer with α-D-glucose?
   (A) β-D-glucose; (B) β-D-fructose; (C) α-D-galactose; (D) α-L-glucose;
   (E) α-D-mannose.

3. Which of the following statements is not true for glycosaminoglycans?
   (A) are the major fraction of proteoglycans; (B) are acid polysaccharides; (C)
   present repeating disaccharide units; (D) exhibit many β-1,2 glycosidic linkage;
   (E) present on the animal cell surface and in the extracellular matrix.

4. Which membrane lipid is absent from prokaryotes and it make the fluidity of
   animal cellular membrane less sensitive to temperature changes?
   (A) phospholipids; (B) glycolipids; (C) cholesterol; (D) sphingomyelin; (E)
   cerebroside.

5. Which ion is required for synaptic vesicle to attach and fuse with presynaptic
   membrane during nerve impulse?
   (A) Na⁺; (B) Cl⁻; (C) K⁺; (D) Ca²⁺; (E) Mg²⁺.

6. Which vitamin is the component of coenzyme A?
   (A) niacin; (B) riboflavin; (C) thiamine; (D) pantothenate; (E) folate.

7. What kind of kinase domain present within the intracellular domain of epidermal
   growth factor?
   (A) protein kinase A; (B) protein kinase C; (C) serine kinase; (D) tyrosine kinase;
   (E) phosphatidylinositol 3-kinase.

8. If glucose radiolabeled on carbon 3 is converted to pyruvate by the glycolysis
   pathway, on which of following labeled pyruvate (bold C*) is right?
   (A) C\text{H}_3\text{COCOOH}; (B) CH_3C\overset{\text{C}}{\text{O}}\text{COOH}; (C) CH_3COC\overset{\text{C}}{\text{O}}\text{OH}; (D)
   C\text{H}_3\text{C}\overset{\text{C}}{\text{O}}\text{COOH}; (E) CH_3C\overset{\text{C}}{\text{O}}\text{C}\overset{\text{O}}{\text{OH}}.

9. Which of the following cycle is related to the production of lactate from glucose in
   active muscle with resynthesis of glucose from lactate in liver?
   (A) Cori cycle; (B) futile cycle; (C) glyoxylate cycle; (D) Citric acid cycle; (E) Q
   cycle.
(10) During Q-cycle of the electron transport of mitochondria, QH₂ transfer its one electron to FeS center. Which compound accepts the other electron of QH₂?
(A) Cytochrome a₃; (B) Cytochrome b₅₆; (C) Cytochrome b₅₇; (D) Cytochrome b₆₇; (E) Cytochrome c.

(11) In the carbon assimilation reactions of photosynthesis, how many molecules of ATP and NADPH are used to bring 1 molecule carbon dioxide to hexose?
(A) 3 molecules of ATP and 3 molecules of NADPH; (B) 2 molecules of ATP and 2 molecules of NADPH; (C) 3 molecules of ATP and 2 molecules of NADPH; (D) 2 molecules of ATP and 3 molecules of NADPH; (E) none of above.

(12) Which of the following statements about reduced glutathione is incorrect?
(A) is a tripeptide with a cysteine residue; (B) can maintain the normal structure of red blood cells; (C) can keep hemoglobin in the ferric state; (D) can detoxify hydrogen peroxide and organic peroxide; (E) in normal red blood cells, the ratio of the reduced to oxidized forms of glutathione is 500.

(13) The substrate of glycogen synthase is:
(A) ADP-glucose; (B) GDP-glucose; (C) UDP-glucose; (D) ADP-galactose; (E) GDP-galactose.

(14) The following metabolic mutants are active in glycogen degradation:
1. mutation of Ser 14 to Ala 14 in liver phosphorylase,
2. overexpression of phosphorylase kinase in the liver,
3. loss of GTPase activity of G protein α subunit,
4. loss of the gene that encodes inhibitor 1 of protein phosphatase,
5. loss of phosphodiesterase activity.
(A) 1, 2, and 3; (B) 1, 3, and 5; (C) 2, 3, and 5; (D) 2 and 3; (E) only 3.

(15) If malonyl CoA is synthesized from ¹⁴C-labeled carbon dioxide and unlabeled acetyl CoA, and the labeled malonate is then used for fatty acid synthesis, the final product of fatty acid will have radioactive carbon in:
(A) every carbon; (B) every odd-numbered carbon; (C) every even-numbered C; (D) only the farthest carbon from C-1; (E) no part of the molecule.

(16) In DNA ligation proceeds, which of the following statements is wrong?
(A) NAD⁺ is the energy source in eukaryotes; (B) It seals breaks in dsDNA molecules; (C) Adenylation is involves in the reaction; (D) Ligase catalyzes the formation of a phosphor- diester bond; (E) None of above.

(17) Which of the following statements is correct during bacterial protein synthesis?
(A) The Met residue found at carboxyl-terminal is usually modified; (B) The initiator tRNA is the tRNAₘ₃s; (C) The activated formyl donor is N⁰-formyltetrahydrofolate; (D) Transformylase modified free methionine then
attached to tRNA; (E) None of above.

(18) Which of the following statements about tRNA is wrong?
(A) Contains a 5'-ACC-3' in the C-terminal; (B) Methylation imparts a hydrophobic character to some regions of tRNAs; (C) The 5' end of a tRNA is phosphorylated; (D) They contain many unusual bases; (E) None of above.

(19) Which of following states is wrong?
(A) Restriction enzymes split DNA into specific fragments; (B) Southern blotting can be used to detect the copy number of a gene; (C) Gene disruption provides clues to gene expressing pattern; (D) Ti plasmid can be used as vectors to deliver foreign genes into plant cells; (E) None of above.

(20) Enzyme can be inhibited by specific molecules, which of following pairing is not match?
(A) Chymotrypsin/TPCK; (B) Glycopeptidase transpeptidase/penicillin; (C) HIV protease/Crixivan; (D) Acetylcholinesterase/DIPF; (E) None of above.

(21) Which of following states is wrong?
(A) K_M is the concentration of substrate at which half the active sites are filled; (B) K_M is equal to the substrate concentration at which the reaction rate is half its maximal value; (C) The value of K_M is dependent of enzyme and substrate concentrations; (D) A high K_M indicates weak binding; (E) None of above.

(22) Which of the following statements is wrong?
(A) Transfer RNA carries amino acids in an activated form for peptide-bond formation; (B) DNA polymerase catalyzes phosphodiester-bond formation from 3'-4to-5' direction; (C) RNA polymerase uses DNA as the template to synthesize RNA; (D) All cellular RNA is synthesized by RNA polymerases; (E) None of above.

(23) Which of the following amino acid contains thioether group?
(A) Met; (B) Val; (C) Pro; (D) Thr; (E) Ser

(24) Seven of the 20 amino acids have readily ionizable side chains. Which of the following amino acid is not belonging of them? (A) His; (B) Cys; (C) Lys; (D) Arg; (E) Ala.

(25) Which of the following protein purification methods is depending in protein size?
(A) Gel-filtration chromatography; (B) Salting out; (C) Affinity chromatography; (D) IEF; (E) DEAE chromatography.
B. Please answer the following questions (50 points)

1. Yeast two-hybrid system is a common method that is used to studies the protein-protein interaction. If you can not use this technique now, please choose another method that can do the same works and describe the principle and processes. (15 points)

2. Please write the cellular localization of the following metabolic pathway. (10 points)
   1. TCA cycle   2. Ketone-body formation   3. Fatty acid synthesis   4. Pentose phosphate pathway

3. After a meal rich in carbohydrates, blood-glucose levels rise. What strategies for liver lead to restore a normal range of blood-glucose levels? Including the mechanisms of nonhormone and hormone, such as glucose and insulin, respectively. (15 points)

4. Please explain many bacteria and plants, but not animals, are able to subsist on the environment containing only acetate? (10 points)
一. 是非題 ( 每題 1 分，共 10 題，請使用 ○ 或 X 作答，10%)

1. When specific transcription factors bind to enhancers only RNA polymerase can bind to the promoter of a gene so that it can be activated.

2. Protein binding to DNA occurs through hydrogen bond donors and acceptors as well as hydrophobic patches mostly in the major groove, but these types of contacts also occur with the minor groove.

3. Positive regulation of gene transcription can work when a ligand activates an activator or when a ligand inactivates an activator.

4. RNA polymerase, just like primase and DNA polymerase, always synthesizes in the 5'-to-3' direction and always requires a primer before it can initiate synthesis.

5. Large eukaryotic chromosomes have one or few replication origins while circular prokaryotic chromosomes have many replication origins.

6. After cleavage of DNA, Topoisomerase I remains covalently link to the DNA in order to facilitate reformation of the phosphodiester bond.

7. Histones serve to package DNA in manner that allows long chromosomes to fit into a small nucleus while keeping DNA accessible to non-histone binding proteins.

8. The RNA part of snRNP provide most if not all of the structural information required for splicesome activity, but the catalytic function of the splicesome is in large part in the splicesome proteins.

9. A promoter sequence can be symmetrical or asymmetrical depending on whether it works on two genes or one gene.

10. A gene transcript can be DNA or RNA or a protein.

二. 單選題 ( 每題 2 分，共 35 題，請使用大寫 ABCDE 作答，70%)

1. Which of the following DNA polymerases is responsible for most of the DNA replication in *E. coli*? (A). DNA Polymerase I (B).DNA Polymerase II (C).DNA Polymerase III (D).DNA Polymerase (E).DNA Polymerase

2. Which of the following features are not found in any eukaryotic chromosomes? (A).Telomeres (B).Linear chromosomes (C).Circular chromosomes (D).Has nucleosomes (E).Chromolmeres

3. DNA polymerases require a template and a primer. Where can the primer come from? (A).Primase (B). a gap in DNA (C).RNA polymerase (D). All of the above (E).Both of (B) and (C) above.

4. Which of the following are not true for tRNAs? (A).The amino acid is attached to the 5' end. (B). The tRNA has four stems and three loops. (C).The anticodon loop binds to the mRNA. (D).The tRNA is charged with an amino acid by a specific synthetase enzyme. (E).The charged tRNA is bound by an elongation factor and escorted to the ribosomal entry site during translation.
5. Which of the following histone proteins in not found in nucleosome cores?
   (A) H1       (B) H2a      (C) H2b       (D) H3       (E) H4

6. DNA transcription and replication are similar in many ways, such as:
   (A) Both require a primer in order to initiate the process. (B) Both processes
       synthesize a new strand of nucleic acid complementary to a DNA strand (C) Both
       use ribonucleotides to synthesize a new strand of nucleic acid (D) Both are
       exceptionally accurate, with error rates of 1/10,000,000 (E) None of the above

7. The amount of protein expressed from a particular gene may be influenced by:
   (A) The stability of the mRNA. (B) The interaction of Rho with the promoter.
       (C) The efficiency of transcriptional termination. (D) The inhibition of translation
       via sense strand RNA (E) All of the above

8. Transcription of the lac operon always requires: (A) A complex of ATP and CRP.
       (B) The lac repressor. (C) RNA polymerase holoenzyme. (D) A novel sigma (σ)
       factor. (E) All of the above

9. Transcriptional termination via a Rho-independent mechanism relies upon:
   (A) The secondary structure of the RNA polymerase to terminate elongation.
       (B) The cleavage reaction requiring ATP to terminate elongation (C) The
       secondary structure of the DNA to termination elongation (D) The secondary
       structure of the mRNA to terminate elongation (E) None of the above

10. Which of the following individuals received the Nobel Prize in Chemistry for the
    discovery of PCR? (A) James Watson (B) Barbara McClintock (C) Francis Crick
    (D) Kary Mullis (E) Craig Venter

11. Which of the following is NOT an inverted repeat? (A) AGCGCT (B) ATATAT
    (C) TTCGAA (D) TAGGAT (E) CCCGGG

12. Which of the following restriction enzyme cuts in the DNA would produce "sticky
    ends"? (A) ATTT ↓ AAAT (B) AT ↓ TAAT (C) AGC ↓ GCT
    (D) CCC ↓ GGG (E) CC ↓ GG

13. Which of the following has the least to do with RT-PCR? (A) Restriction enzyme
    (B) mRNA (C) cDNA (D) Reverse transcriptase (E) DNA polymerase

14. Which of the following has the least to do with Western blotting? (A) DNA
    (B) Protein (C) Electrophoresis (D) Nitrocellulose filter (E) Antibody

15. Which of the following would not be possible to address using a Northern blot?
    (A) Location of restriction sites in a particular gene. (B) Spatial (ie. tissue-specific)
    expression of a particular gene. (C) Temporal (ie. developmental) expression of a
    particular gene. (D) mRNA size. (E) Number of splice-variant types of RNA.

16. T7 and T3 promoters that are found in some cloning vectors (eg. plasmids) are
    used to generate which of the following? (A) DNA (B) RNA (C) RNA
    polymerase (D) Beta lactamase (E) Beta galactosidase
17. Which of the following is NOT usually found in plasmid cloning vectors?
   (D). reporter gene such as lacZ (E). multiple cloning site

18. Electrophoresis is utilized to separate DNA based on differences in: (A). shape
   (B). charge and nucleotide sequence (C). charge (D). nucleotide sequence
   (E). length

19. The large (Klenow) fragment of E. coli DNA polymerase I contains which of the
   following activities? (A). reverse transcriptase activity (B). polymerase activity
   and 3'-5'-exonuclease activity (C). nick-translation activity (D). polymerase activity
   and nick-translation activity (E). 5'-3'-exonuclease activity

20. Which of the following statements are true about double-strand DNA:

21. With what mRNA codon would the tRNA in the diagram be able to form a
codon-anticodon base pairing interaction? (anticodon: 5'-UAC-3')
   (A). 5'-UAC-3' (B). 5'-UAG-3' (C). 5'-CAU-3' (D). 5'-AUG-3' (E). 5'-GUA-3'

22. The primary RNA transcript of the chicken ovalbumin gene is 7700 nucleotides
   long, but the mature mRNA that is translated on the ribosome is 1872 nucleotides
   long. This size difference occurs primarily as a result of: (A). capping (B). splicing
   (C). removal of poly A tails (D). cleavage of polycistronic mRNA (E). reverse
   transcription

23. RNAs that catalyze biological reactions, such as self-splicing introns, are known
   as: (A). lariats (B). enzymes (C). mature RNAs (D). ribozymes
   (E). spliceosomes

24. Which of the following is NOT involved in regulating the synthesis of RNA in the
   eukaryotic nucleus? (A). amplification of some genes such as rRNA genes
   (B). spliceosomes that stimulate synthesis of intron-containing hnRNAs (C). use of
   different RNA polymerases to transcribe different classes of RNA (D). enhancers
   that can stimulate specific promoters (E). activate genes in euchromatin, and
   inactivate genes in heterochromatin

25. Which of the following features would you NOT expect to find in heterogeneous
   nuclear RNA (hnRNA)? (A). 5'-cap structure (B). polycistronic coding (C). intron
   (D). polyadenylation at 3' end (E). U nucleotides

26. An E. coli strain is F- lac Z- met+ bio+. Cells from this strain are mixed with an E.
   coli strain that is lac Z+ met+ bio- and carrying an F episome with the P_{lac} O+ lac
   Z+ DNA sequence on the episome, and cultured for several hours. Then cells were
   removed, washed, and transferred to minimal media containing lactose as the only
   sugar source. A few cells were able to grow on minimal media with lactose, and
   formed colonies. How did these few cells become lac Z+ met+ bio+?
27. A key feature of insertional mutagenesis for the identification of plasmids containing recombinant plasmids is: (A) the DNA sequencing of recombinant DNA (B) the disruption of a gene on the plasmid by the inserted recombinant DNA (C) the production of nutritional auxotrophs (D) the production of restriction endonuclease maps of recombinant plasmids (E) introns can be moved to new locations within the gene

28. The role of the inducer of the lac operon. The inducer: (A) binds to the operator and prevents the repressor from binding at this site (B) binds to the termination codons and allows protein synthesis to continue (C) binds to the promoter and prevents the repressor from binding to the operator (D) combines with a repressor and prevents it from binding to the promoter (E) combines with a repressor and prevent it from binding to the operator

29. The short interfering RNA (siRNA) (A) leads to translational repression of target messenger RNAs (B) is synthesized by RNA polymerase I (C) is able to amplify in all eukaryotic cells (D) usually hybridizes to its target mRNA perfectly (E) is a single-stranded RNA containing approximately 21-26 nucleotides in length.

30. RNA editing is a type of RNA processing in which the sequence of a RNA molecule is altered. Which molecule is responsible for the nucleotide alternation during RNA editing? (A) Small nuclear RNA (B) Micro RNA (C) tRNA (D) Guide RNA (E) mRNA

31. Transposons of some eukaryotes may occupy more than half the genome. One could characterize transposons in the following manner: (A) short DNA sequences about 1 kb in length that have the ability to move in the genome (B) relatively long DNA sequences >10 kb in length that have the ability to move in the genome (C) relatively short DNA sequences about 1 kb in length that have stable positions in the genome (D) moderately repetitive DNA segments that are found mainly around the centromere (E) moderately repetitive DNA segments that are found mainly around the telomere

32. One plausible explanation for the observation of more types of proteins in a cell than apparent coding regions in the DNA is that (A) mutations cause alterations in the translation process. (B) there are many different promoter sites for a given gene in eukaryotes. (C) alternative splicing allows for multiple proteins from a single transcript. (D) conservation of intron/exon splicing junctions enable intron fusion. (E) there are many cases in which different classes of introns constitute a common protein.

33. Coupled transcription and translation does not occur in eukaryotes because
(A). of slower rates of transcription in eukaryotes relative to bacteria. (B). of slower rates of translation in eukaryotes relative to bacteria. (C). in eukaryotes, the rate of translation far exceeds the rate of transcription, but these rates are balanced in bacteria. (D). in bacteria, the rate of translation far exceeds the rate of transcription, but these rates are balanced in eukaryotes. (E). transcription and translation occur in one cellular compartment in bacteria, but in separate cellular compartments in eukaryotes.

34. Which one of the following structures is coded for by the shortest (or smallest) sequence of DNA? (A). transfer RNA having 75 nucleotides (B). mRNA having 75 codons (C). a polypeptide composed of 75 amino acids (D). a protein composed of 2 polypeptides, each 35 amino acids long (E). a protein composed of 60 amino acids

35. Researchers have created a gene for very high-level expression of a mutated form of ASF/SF2 (an SR protein that binds near 5’-splice junctions). This mutant form produces a protein without the RS domain. Expression of this protein could possibly produce what effect? (A).Cause increased splicing of all transcripts containing introns. (B).Prevent association of snRNP U2 with introns, but allow all other splicing factors to bind. (C). Inhibit removal of all introns from all pre-mRNA molecules. (could block normal ASF/SF2 binding) (D).Increase binding of snRNP U1 to 5’-splice junctions. (E).Block snRNP U1 from binding but would still allow all other snRNPs to associate with the introns.

三. 問答題 (20%)
1. Please describe the basic structure of an ordinary cloning plasmid, and explain the function of each component? If PCR uses Taq but not Pfu enzyme, Why its PCR products can directly ligate into certain plasmid (TA cloning vector) without the digestion of restriction enzyme? (5%)
2. If X protein has certain biological activity (e.g. enzyme or hormone), from the viewpoint of Mol Biol central dogma, please describe the possible control mechanisms on its biological activity? (7%)
3. What is the function of a nuclear receptor? Explain the difference between type I and type II nuclear receptors and give an example of each. (8%)
1. The ant is a social insect _____.
   (A) that live in an organized colony
   (B) that was organized in living colony
   (C) it lived in an organized colony
   (D) what lives in an organized colony

2. Most psychologists deeply believe that it is just as difficult to change a person’s way of thinking _____ to rectify his deep-rooted habits.
   (A) as it does
   (B) as it is
   (C) than is it
   (D) than it does

3. Weather is usually determined by _____.
   (A) there are various atmospheric conditions
   (B) atmospheric conditions are various
   (C) various atmospheric conditions
   (D) atmospheric conditions vary

4. _____ depends on our ideas of the human-nature relationship.
   (A) What do we do with nature
   (B) What we do with nature
   (C) We do what with nature
   (D) Do what with nature

5. Koala bears are _____.
   (A) leaf-eating
   (B) eating leaves
   (C) they eat leaves
   (D) leaves-eating

6. Flying saucers _____ because of its saucer-like shape and its capacity to fly.
   (A) is so called
   (B) called so is
   (C) so called is
   (D) so is called

7. _____ us the ability to resist distance, but also helps us build our body tissues.
   (A) Vitamin C not only provides
   (B) Not only does vitamin C provide
   (C) Not only vitamin C provides
   (D) Vitamin C, which not only provides
8. Metal factors may result in _____ physiological disorders.
   (A) and
   (B) among
   (C) where
   (D) severe

9. Blood pressure _____ by the force that blood exerts against the walls of the arteries.
   (A) determined
   (B) being determined
   (C) is determined
   (D) which it is determined

10. Unlike other mammals, _____ in the pouch of the mother’s body.
    (A) the bodies of the marsupial are raised
    (B) the marsupial raises its babies
    (C) the raise of the marsupial’s babies is
    (D) raised are the babies of the marsupial

11. While _____ on the freeway, all drivers must keep alert all the time on the journey.
    (A) drive
    (B) they driving
    (C) are driving
    (D) driving

12. Our teacher recommended that everyone _____ a composition as homework.
    (A) writes
    (B) wrote
    (C) writing
    (D) write

13. Only when a monkey is mature enough _____ to develop its independence from its mother.
    (A) does it begin
    (B) it begins
    (C) it had begun
    (D) it will begin

14. The doctor _____ said she had a fever.
    (A) whom she went
    (B) that she went
    (C) to whom she went to
    (D) that she went to

15. Anyone _____ receiving information about the club should write to the secretary.
    (A) interesting to
    (B) interested in
    (C) is interesting about
    (D) who interested of
TRIZOL® Reagent
Cat. No. 15596-026  Size: 100 ml
Store at 2 to 8°C.
WARNING: Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice (show label where possible). Phenol (108-95-2) and Other Components (NJTSRN 80100437-5000p).
TRIZOL has demonstrated stability of 12 months when stored at room temperature. However, we recommend storage at 2 to 8°C for optimal performance.
Description:
TRIZOL Reagent (U.S.Patent No.5,346,994) is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1). During sample homogenization or lysis, TRIZOL Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation (2). Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropl alcohol yields proteins from the organic phase (2). Copurification of the DNA may be useful for normalizing RNA yields from sample to sample.
This technique performs well with small quantities of tissue (50-100 mg) and cells (5 x 10^6), and large quantities of tissue (>1 g) and cells (>10^7), of human, animal, plant, or bacterial origin. The simplicity of the TRIZOL Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by TRIZOL Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly (A)^+ selection, in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR®), treatment of the isolated RNA with amplification grade DNase I (Cat. No. 18068) is recommended when the two primers lie within a single exon.
TRIZOL Reagent facilitates isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from rat liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, (composed of mRNA's and hnRNA's) two predominant ribosomal RNA bands at ~5 kb (28S) and at ~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an A260/A280 ratio ≥1.8 when diluted into TE.
Precautions for Preventing RNase Contamination:
RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.
• Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
• Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.
• In the presence of TRIZOL Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150°C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, rinsed thoroughly with water, and autoclaved.
Other Precautions:
• Use of disposable tubes made of clear polypropylene is recommended when working with less than 2-ml volumes of TRIZOL.
• For larger volumes, use glass (Corning) or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 x g with TRIZOL Reagent and chloroform. Do not use tubes that leak or crack.
• Carefully equilibrate the weights of the tubes prior to centrifugation.
• Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugation.
INSTRUCTIONS FOR RNA ISOLATION:

Caution: When working with TRIZOL Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

Unless otherwise stated, the procedure is carried out at 15 to 30°C, and reagents are at 15 to 30°C.

Reagents required, but not supplied:
- Chloroform, Isopropyl alcohol, 75% Ethanol (in DEPC-treated water)
- RNase-free water or 0.5% SDS solution [To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved waters.]

1. HOMOGENIZATION (see notes 1-3)
   a. Tissues
      Homogenize tissue samples in 1 ml of TRIZOL Reagent per 50-100 mg of tissue using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for homogenization.
   b. Cells Grown in Monolayer
      Lyse cells directly in a culture dish by adding 1 ml of TRIZOL Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIZOL Reagent added is based on the area of the culture dish (1 ml per 10 cm²) and not on the number of cells present. An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.
   c. Cells Grown in Suspension
      Pellet cells by centrifugation. Lyse cells in TRIZOL Reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 x 10⁶ animal, plant or yeast cells, or per 1 x 10⁷ bacterial cells. Washing cells before addition of TRIZOL Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

OPTIONAL: An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 x g for 10 minutes at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2. PHASE SEPARATION
   Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRIZOL reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 x g for 15 minutes at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIZOL Reagent used for homogenization.

3. RNA PRECIPITATION
   Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA WASH
   Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at 2 to 8°C.

5. REDISSOLVING THE RNA
   At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70°C.
RNA Isolation Notes:
1. Isolation of RNA from small quantities of tissue (1 to 10 mg) or Cell (102 to 104) Samples: Add 800 μl of TRIZOL to the tissue or cells. Following sample lysis, add chloroform and proceed with the phase separation as described in step 2. Prior to precipitating the RNA with isopropyl alcohol, add 5-10 μg RNAse-free glycogen (Cat. No 10814) as carrier to the aqueous phase. To reduce viscosity, shear the genomic DNA with 2 passes through a 26 gauge needle prior to chloroform addition. The glycogen remains in the aqueous phase and is co-precipitated with the RNA. It does not inhibit first-strand synthesis at concentrations up to 4 mg/ml and does not inhibit PCR.
2. After homogenization and before addition of chloroform, samples can be stored at -60 to -70°C for at least one month. The RNA precipitate (step 4, RNA WASH) can be stored in 75% ethanol at 2 to 8°C for at least one week, or at least one year at -5 to -20°C.
3. Table-top centrifuges that can attain a maximum of 2,600 × g are suitable for use in these protocols if the centrifugation time is increased to 30-60 minutes in steps 2 and 3.

INSTRUCTIONS FOR DNA ISOLATION:
After complete removal of the aqueous phase, as described in the RNA isolation protocol, the DNA in the interphase and phenol phase from the initial homogenate may be isolated. Following precipitation and a series of washes, the DNA is solubilized in 8 mM NaOH. Full recovery of DNA from tissues and culture cells permits the use of TRIZOL Reagent for the determination of the DNA content in analyzed samples (2). Simultaneous extraction of genomic DNA allows for normalization of the results of Northern analysis per genomic DNA instead of the more variable total RNA or tissue weight. (Depending on the source, the DNA pellet obtained may require additional purification (e.g., phenol extraction) prior to other applications.

Unless otherwise stated, the procedure is carried out at 15 to 30°C.
1. DNA PRECIPITATION
Remove the remaining aqueous phase overlaying the interphase, and precipitate the DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 1 ml of TRIZOL used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15 to 30°C for 2-3 minutes and sediment DNA by centrifugation at no more than 2,000 × g for 5 minutes at 2 to 8°C.

Careful removal of the aqueous phase is critical for the quality of the isolated DNA.

2. DNA WASH
Remove the phenol-ethanol supernatant, and if desired, save it for protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol. Use 1 ml of the solution per 1 ml of TRIZOL Reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × g for 5 minutes at 2 to 8°C. Following these two washes, suspend the DNA pellet in 75% ethanol (1.5-2 ml of 75% ethanol per 1 ml TRIZOL Reagent), store for 10-20 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × g for 5 minutes at 2 to 8°C.

An additional wash in 0.1 M sodium citrate-10% ethanol solution is required for large pellets containing > 200 μg DNA or large amounts of a non-DNA material.

3. REDISSOLVING THE DNA
Air dry the DNA 5 to 15 minutes in an open tube. (DO NOT DRY UNDER CENTRIFUGATION; it will be more difficult to dissolve.) Dissolve DNA in 8 mM NaOH such that the concentration of DNA is 0.2 – 0.3 μg/μl. Typically add 300 – 600 μl of 8 mM NaOH to DNA isolated from 107 cells or 50 – 70 mg of tissue. Resuspending in weak base is HIGHLY recommended since isolated DNA does not resuspend well in water or in Tris buffers. The pH of the 8 mM NaOH is only ~9 and should be easily adjusted with TE or HEPES once the DNA is in solution. At this stage, the DNA preparations (especially from tissues) may contain insoluble gel-like material (fragments of membranes, etc.) Remove the insoluble material by centrifugation at >12,000 × g for 10 minutes. Transfer the supernatant containing the DNA to a new tube. DNA solubilized in 8 mM NaOH can be stored overnight at 4°C; for prolonged storage, samples should be adjusted with HEPES to pH 7-8 (see table) and supplemented with 1 mM EDTA. Once the pH is adjusted, DNA can be stored at 4°C or – 20°C.

Quantitation and Expected Yields of DNA
Take an aliquot of the DNA preparation solubilized in 8 mM NaOH, mix it with water and measure the A260 of the resulting solution. Calculate the DNA content using the A260 value for double-stranded DNA. One A260 unit equals 50 μg of double-stranded DNA/ml. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1 × 106 diploid cells of human, rat, and mouse origin equals: 7.1 μg, 6.5 μg, and 5.8 μg, respectively (3).
Applications:

**Amplification of DNA by PCR:**
After redissolving the DNA in 8 mM NaOH, adjust the pH to 8.4 with 0.1 M HEPES (see table). Add 0.1 to 1.0 μg of the DNA sample to your PCR reaction mixture and perform the standard PCR protocol.

**Restriction endonuclease reactions:**
Adjust the pH of the DNA solution to a required value using HEPES (see table). Alternatively, samples may be dialyzed against 1 mM EDTA, pH 7 to pH 8.0. Use 3-5 units of enzyme per microgram of DNA. Use the conditions recommended by the manufacturer for the particular enzyme, and allow the reaction to proceed for 3 to 24 h. In a typical assay, 80-90% of the DNA is digestible.

**pH Adjustment of DNA Samples Dissolved in 8 mM NaOH:**
(For 1 ml of 8 mM NaOH use the following amounts of 0.1 M or 1 M HEPES, free acid.)

<table>
<thead>
<tr>
<th>Final pH</th>
<th>0.1 M HEPES (μl)</th>
<th>Final pH</th>
<th>1 M HEPES (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.4</td>
<td>86</td>
<td>7.5</td>
<td>159</td>
</tr>
<tr>
<td>8.2</td>
<td>93</td>
<td>7.2</td>
<td>23</td>
</tr>
<tr>
<td>8.0</td>
<td>101</td>
<td>7.0</td>
<td>32</td>
</tr>
<tr>
<td>7.8</td>
<td>117</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DNA Isolation Notes:**
1. The phenol phase and interphase can be stored at 2 to 8°C overnight.
2. Samples suspended in 75% ethanol can be stored at 2 to 8°C for months.
3. Samples dissolved in 8 mM NaOH can be stored overnight at 2 to 8°C. For long-term storage, adjust the pH to 7-8, and adjust the EDTA concentration to 1 mM.

**INSTRUCTIONS FOR PROTEIN ISOLATION:**
Proteins are isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol (step 1, DNA PRECIPITATION). The resulting preparation can be analyzed for the presence of specific proteins by Western blotting (2).

**Reagents required, but not supplied:**
- Isopropyl alcohol
- 0.3 M Guanidine hydrochloride in 95% ethanol
- Ethanol
- 1% SDS

1. **PROTEIN PRECIPITATION**
Precipitate proteins from the phenol-ethanol supernatant (approximate volume 0.8 ml per 1 ml of TRIZOL Reagent) with isopropyl alcohol. Add 1.5 ml of isopropanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Store samples for 10 minutes at 15 to 30°C, and sediment the protein precipitate at 12,000 × g for 10 minutes at 2 to 8°C.

2. **PROTEIN WASH**
Remove the supernatant and wash the protein pellet 3 times in a solution containing 0.3 M guanidine hydrochloride in 95% ethanol. Add 2 ml of wash solution per 1 ml of TRIZOL Reagent used for the initial homogenization. During each wash cycle, store the protein pellet in the wash solution for 20 minutes at 15 to 30°C and centrifuge at 7,500 × g for 5 minutes at 2 to 8°C. After the final wash, vortex the protein pellet in 2 ml of ethanol. Store the protein pellet in ethanol for 20 minutes at 15 to 30°C and centrifuge at 7,500 × g for 5 minutes at 2 to 8°C.

3. **REDISSOLVING THE PROTEIN PELLET**
Vacuum dry the protein pellet for 5-10 minutes. Dissolve it in 1% SDS by pipetting. Complete dissolution of the protein pellet may require incubating the sample at 50°C. Sediment any insoluble material by centrifugation at 10,000 × g for 10 minutes at 2 to 8°C, and transfer the supernatant to a fresh tube. The sample is ready for use in Western blotting or may be stored at -5 to -20°C for future use.

**Protein Isolation Notes:**
1. The protein pellet suspended in 0.3 M guanidine hydrochloride-95% ethanol or in ethanol can be stored for at least one month at 2 to 8°C, or for at least one year at -5 to -20°C.
2. The following protocol is an alternative approach that allows for more efficient recovery of proteins. Dialyze the phenol-ethanol supernatant against three changes of 0.1% SDS at 2 to 8°C. Centrifuge the dialyzed material at 10,000 × g for 10 minutes. Use the clear supernatant for Western blotting.
3. Proteins may be quantified by the Bradford method as long as the concentration of SDS is low enough (<0.1%) so that it will not interfere. Methods that do not have detergent-interface problems, and that do not rely on A260/A280 measurements may be used (traces of phenol may cause overestimation of protein concentrations).
Troubleshooting Guide:

**RNA ISOLATION**

- Expected yields of RNA per mg of tissue or 1 x 10^6 cultured cells
  - Liver and spleen, 6-10 μg
  - Skeletal muscles and brain, 1-1.5 μg
  - Epithelial cells (1 x 10^6 cultured cells), 8-15 μg
  - Kidney, 3-4 μg
  - Placenta, 1-4 μg
  - Fibroblasts, (1 x 10^6 cultured cells) 5-7 μg

- Low yield
  - Incomplete homogenization or lysis of samples. Final RNA pellet incompletely redissolved.

- A260/A280 ratio <1.65
  - RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase absorbance at 280 nm (6,7).
  - Sample homogenized in too small a reagent volume.
  - Following homogenization, samples were not stored at room temperature for 5 minutes.
  - The aqueous phase was contaminated with the phenol phase.
  - Incomplete dissolution of the final RNA pellet.

- RNA degradation
  - Tissues were not immediately processed or frozen after removal from the animal.
  - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
  - Cells were dispersed by trypsin digestion.
  - Aqueous solutions or tubes were not RNase-free.
  - Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

- DNA contamination
  - Sample homogenized in too small a reagent volume.
  - Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.

- Proteoglycan and polysaccharide contamination
  - The following modification of the RNA precipitation (step 3) removes these contaminating compounds from the isolated RNA. Add to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of TRIZOL Reagent used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. A combination of the modified precipitation with an additional centrifugation of the initial homogenate (note 2, RNA isolation protocol) is required to isolate pure RNA from plant material containing a very high level of polysaccharides.

**DNA ISOLATION**

- Expected yields of DNA per mg of tissue or 1 x 10^6 cultured cells
  - Liver and kidney, 3-4 μg
  - Cultured human, rat, and mouse cells (1 x 10^6), 5-7 μg
  - Skeletal muscles, brain, and placenta 2-3 μg
  - Fibroblasts, 5-7 μg

- Low yield
  - Incomplete homogenization or lysis of samples. Final DNA pellet incompletely redissolved.

- A260/280 ratio <1.70
  - DNA sample was diluted in water instead of TE prior to spectrophotometric analysis.
  - Phenol was not sufficiently removed from the DNA preparation. Wash the DNA pellet an additional time with 0.1 M sodium citrate in 10% ethanol.

- DNA degradation
  - Tissues were not immediately processed or frozen after removal from the animal.
  - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
  - Samples were homogenized with a Polytron or other high speed homogenizer.

- RNA contamination
  - Incomplete removal of aqueous phase.
  - DNA pellet insufficiently washed with 0.1 M sodium citrate in 10% ethanol.

- Other applications
  - Prior to use in PCR amplification, adjust the pH to 8.4.
  - For digestion of the DNA with restriction endonucleases, adjust the pH to the desired value, use 3-5 units of enzyme per μg of DNA, and allow the reaction to go for 3-24 hours under optimal conditions for the particular enzyme. Typically 80-90% of the DNA is digested.
PROTEIN ISOLATION

- Low yield
- Incomplete homogenization or lysis of samples.
- Final DNA pellet incompletely redissolved.
- Protein degradation
  - Tissues were not immediately processed or frozen after removing from the animal.
- Band deformation in PAGE
  - Protein pellet insufficiently washed.

References:

Teflon® is a registered trademark of E.I. Du Pont de Nemours & Co.
TISSUMIZER® is a registered trademark of Tekmar Co.
TRIZOL® is a registered trademark of Molecular Research Center, Inc.
*PCR is covered by a patent held by Hoffman LaRoche Corporation.

看完上文後，請回答下列問題，並將答案寫在答案卷上。回答答卷時，請盡量以中文回答，但若遇到不易翻譯的專有名詞（如 RNA, DNA, 0.5 M NaOH, isopropyl alcohol, trypsin, phenol, chloroform, formamide, SDS, PCR 等等），可直接用英文表示。

16. According to this article, what is the right way(s) to store Trizol? (including temperature and other factors) (10分)

17. According to this article, what will you do to prevent RNase contamination? (10分)

18. Please write a step-by-step protocol about how to isolate RNA from 75mg of tissue. (30分)
(限500字以內，以條列式，一步一步依序說明較佳)

19. What can you do to improve your skill or solve problem if you got RNA contamination in DNA product? (10分)

20. According to this article, what will you do (for performing PCR) after you have DNA product? (10分)
(Atomic mass: He= 4.00; C= 12.01; O= 16.00; F= 19.00; Ar= 39.95; Fe= 55.85; Br= 79.90; Rh= 102.9; Ag= 107.9; Re= 186.2; Pt= 195.1 g/mol)

1) The density of a liquid is determined by successively weighing 25, 50, 75, 100 and 125 mL of the liquid in a 250 mL beaker. If volume of liquid is plotted along the horizontal axis, and total mass of beaker plus liquid is plotted on the vertical axis, then
   (a) the x, or horizontal, intercept is the negative value of the weight of the beaker.
   (b) the y, or vertical, intercept is the weight of the empty beaker.
   (c) The slope of the line is 1.0.
   (d) the line will pass through the origin.
   (e) the slope of the line is independent of the identity of the liquid.

2) The rate constant, k, for the thermal decomposition of acetaldehyde is 0.10 M$^{-1}$s$^{-1}$ at 500 K and 0.30 M$^{-1}$s$^{-1}$ at 469 K. What is the value of the activation energy? ($R = 8.314$ J/K mol)
   (a) 70.3 kJ/mol  (b) $-70.3$ kJ/mol  (c) 0.693 kJ/mol
   (d) $-0.693$ kJ/mol  (e) 32.0 kJ/mol

3) Beta decay of thorium-234 is a first order reaction. Its rate constant is $2.88 \times 10^{-2}$/day. What is the half-life of the nuclide? (Ln 2 = 0.693)
   (a) 53.1 days  (b) 1.22 days  (c) 0.693 days  (d) 24.1 days  (e) 101 days

4) Assume 12500 J of energy is added to 2.0 mole (36 grams) of H$_2$O as an ice sample at 0°C. The molar heat of fusion is 6.02 kJ/mol. The specific heat of liquid water is 4.18 J/mol K. The molar heat of vaporization is 40.6 kJ/mol. The resulting sample contains which of the following?
   (a) only ice  (b) ice and water  (c) only water  (d) water and water vapor
   (e) only water vapor

5) A crude type of disappearing ink is based on the following endothermic equilibrium:
   [Co(H$_2$)$_3$Cl$_2$(aq) [colorless] $\rightarrow$ [CoCl$_2$(H$_2$)$_2$](aq) [blue] + 2 H$_2$O
   If the reactant solution was used to write on a piece of paper and the paper is allowed to partially dry, what can be done to bring out the colored handwriting?
   (a) Add water  (b) Decrease the volume  (c) Put the paper in the freezer
   (d) Put the paper in the oven

6) How many electrons can be described by the quantum numbers $n=3$, $l=3$, $m_l=1$?
   (a) 0  (b) 2  (c) 6  (d) 10  (e) 14

7) Which reactions will not produce a precipitate from aqueous solution?
   (a) Hg$_2$NO$_3$$_2$ + KI  (b) FeSO$_4$ + Ba(OH)$_2$  (c) Pb(NO)$_3$ + NaCO$_3$
   (d) ZnCl$_2$ + (NH$_4$)$_2$S  (e) NaBr + Al$_2$(SO$_4$)$_3$
8) Calculate the concentration of free zinc ion, [Zn^{2+}], in a solution that is initially 0.10 M Zn(NO_3)_{2+} and 0.20 M Na_4EDTA. K_f [Zn(EDTA)]^{2-} = 3.0
   (a) 0.10  (b) 3.3 x 10^{-17}  (c) 0  (d) 3.0 x 10^{-16}  (e) 1.8 x 10^{-9}

9) Acetic acid (HC_2H_3O_2) is a weak acid (K_a = 1.8 x 10^{-5}). Calculate the pH of a 17.6 M\text{C}_2\text{H}_3\text{O}_2 solution. (You need only estimation.)
   (a) 4.3  (b) 6.4  (c) 1.7  (d) 0.97  (e) 7.4

10) How many moles of solid NaF would have to be added to 1.0 L of 1.90 M HF solution to achieve a buffer of pH 3.35? Assume there is no volume change. (K_a for HF = 7.2 x 10^{-4}).  
\(10^{-3.35} = 0.000447\)
   (a) 3.1  (b) 2.3  (c) 1.6  (d) 1.0  (e) 4.9

11) Which of the following has the highest total concentration of ions?
   (a) 0.05 M HBr  (b) 0.1 M CH_3OH  (c) 0.04 M (NH_4)_2SO_4  
   (d) 0.1 M NaCl  (e) 0.03 M Al_2(SO_4)_3

12) Using the activity series of redox reaction, determine which reaction will occur.
   (a) Cu(s) with Zn^{2+}(aq) and Zn(s) with Mg^{2+}(aq)
   (b) Mg(s) with Cu^{2+}(aq) and Zn^{2+}(aq) with Mg^{2+}(aq)
   (c) Mg(s) with Cu(s) and Zn(s) with Cu(s)
   (d) Mg(s) with Zn^{2+}(aq) and Zn(s) with Cu^{2+}(aq)

13) Which of the following is the strongest acid?
   (a) HF  (b) KOH  (c) HClO_4  (d) HClO  (e) HBrO

14) H_2O_2(aq) + H^+(aq) + Cl^-(aq) \rightarrow Cl_2(g) + H_2O(l)  (Balance the equation by yourself.)
   A cell based on the reaction above has a standard potential of +0.42V at 25°C. If all of the species are at standard conditions except [H^+], at what pH will the cell have a potential of zero?  
\(F = 96485 \text{ C/mol}\)
   (a) 3.55  (b) 7.09  (c) 10.6  (d) 14.2  (e) 1.53

15) A chemist is given a white solid that is suspected of being pure cocaine. When 1.22 g of the solid is dissolved in 15.60 g of benzene the freezing point is lowered by 1.32°C. Calculate the molar mass of the solid. The molal freezing point constant \(K_f\) for benzene is 5.12°C/m. (Molecular weight C_8H_18 = 78 g/mol)
   (a) 303 g/mol  (b) 605 g/mol  (c) 251 g/mol  (d) 856 g/mol  (e) 1252 g/mol

16) Which of the following is optically active (i.e., chiral)?
   (a) HN(CH_3)_2  (b) CH_3Cl_2  (c) 2-chloropropane  (d) 2-chlorobutane  
   (e) 3-chloropentane

17) Inside human body, which is the most abundant element in terms of atomic percentage and mass percentage, respectively?
   (a) H, C  (b) O, N  (c) Si, O  (d) H, O  (e) O, Ca

18) The complementary nucleic acid sequence for the following DNA sequence
\(-GAC\text{TAC\text{GTT\text{AGC}}\) is
   (a) GAC TAC GTT AGC  (b) TCA GCA TGG CTA  (c) CGA TTG CAT CAG  
   (d) CTG ATG CAA TCG  (e) none of these
19) When heat is added to proteins, the hydrogen bonding in the secondary structure breaks apart. What are the algebraic signs of $\Delta H$ and $\Delta S$ for the denaturation process?
   (a) Both $\Delta H$ and $\Delta S$ are positive
   (b) Both $\Delta H$ and $\Delta S$ are negative
   (c) $\Delta H$ is positive and $\Delta S$ is negative
   (d) $\Delta H$ is negative and $\Delta S$ is positive
   (e) $\Delta H$ is positive and $\Delta S$ is zero

20) Iron is biologically important in the transport of oxygen by red blood cells from the lungs to the various organs of the body. In the blood of an adult human, there are approximately $2.60 \times 10^{13}$ red blood cells with a total of 2.90 g of iron. On the average, how many iron atoms are present in each red blood cell?
   (a) $8.33 \times 10^{-10}$  (b) $1.20 \times 10^9$  (c) $3.12 \times 10^{22}$  (d) $2.60 \times 10^{13}$  (e) $5.19 \times 10^{-2}$

(II) 計算題

21) A lungful of air (500 mL) contains 4.1% CO$_2$ by volume. How many grams of KO$_2$(s) are needed to remove the CO$_2$ from a lungful of air at STP according to the following reaction (you need to balance the equation)?
   (5%) $\text{KO}_2(s) + \text{CO}_2 \rightarrow \text{K}_2\text{CO}_3(s) + \text{O}_2(g)$ (Balance the equation by yourself)

22) An unknown gas contains 83% C and 17% H by mass. It effuses at 0.87 times the rate of CO$_2$ gas under the same conditions. What is molecular formula of gas? (10%)

23) You are given a small bar of an unknown metal, X. You find the density of the metal to be 10.5 g/cm$^3$. An X-ray diffraction experiment measures the edge of the unit cell as 490 pm and identifies the metal crystal in a face-centered cubic lattice structure.
   (a) Please calculate the atomic mass of X; (1 pm = $10^{-10}$ cm) (8%)
   (b) What is X most likely to be? (你可以參考本書卷開頭處一些金屬原子量) (4%)

24) Hemoglobin (血紅素) (abbreviated Hb) is a protein that is responsible for the transport of oxygen in the blood of mammals 哺乳動物. Each hemoglobin molecule contains four iron atoms that are the binding sites for O$_2$ molecules. The oxygen binding is pH dependent.
   The relevant equilibrium reaction is
   $\text{HbH}_4^{4+}(aq) + 4 \text{O}_2(g) \rightleftharpoons \text{Hb}(\text{O}_2)_4(aq) + 4 \text{H}^+$
   $\text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^-(aq) + \text{H}^+(aq)$
   $K_a = 4.3 \times 10^{-7}$
   (a) What is the concentration ratio of CO$_2$ (usually written H$_2$CO$_3$) to HCO$_3^-$ in blood at pH = 7.40 and pH = 6.70, respectively? Why carbonate buffers are important in regulating pH of blood? (10$^{-7.4}$ = 3.98 x 10$^{-8}$, 10$^{-6.7}$ = 2.0 x 10$^{-7}$) (4%)
   (b) What form of hemoglobin, HbH$_4^{4+}$ or Hb(O$_2$)$_4$, is favored in the lungs 肺臟?
   What form is favored in the cells 輔胞? (4%)
   (c) When a person hyperventilates 循環過快, the concentration of CO$_2$ in the blood is decreased. How does this affect the oxygen-binding equilibrium? How does breathing into a paper bag to counteract this effect? (3%)
   (d) When a person has suffered a cardiac arrest 心跳停止, injection of a sodium bicarbonate solution is given. Why is this necessary? (2%)
A: Choose the one best response to each following questions. (50 points)

(1) The following sugars are not reducing sugar:
   1. maltose, 2. sucrose, 3. trehalose, 4. glucose, 5. xylose.
   (A) 1 and 2; (B) 2 and 3; (C) 1, 2, and 3; (D) only 2; (E) only 5.

(2) Which of the following compounds is an anomer with \( \alpha \)-D-glucose?
   (A) \( \beta \)-D-glucose; (B) \( \beta \)-D- fructose; (C) \( \alpha \)-D- galactose; (D) \( \alpha \)-L-glucose;
   (E) \( \alpha \)-D-mannose.

(3) Which of the following statements is not true for glycosaminoglycans?
   (A) are the major fraction of proteoglycans; (B) are acid polysaccharides; (C)
   present repeating disaccharide units; (D) exhibit many \( \beta \)-1,2 glycosidic linkage;
   (E) present on the animal cell surface and in the extracellular matrix.

(4) Which membrane lipid is absent from prokaryotes and it make the fluidity of
   animal cellular membrane less sensitive to temperature changes?
   (A) phospholipids; (B) glycolipids; (C) cholesterol; (D) sphingomyelin; (E)
   cerebroside.

(5) Which ion is required for synaptic vesicle to attach and fuse with presynaptic
   membrane during nerve impulse?
   (A) \( \text{Na}^+ \); (B) \( \text{Cl}^- \); (C) \( \text{K}^+ \); (D) \( \text{Ca}^{2+} \); (E) \( \text{Mg}^{2+} \).

(6) Which vitamin is the component of coenzyme A?
   (A) niacin; (B) riboflavin; (C) thiamine; (D) pantothenate; (E) folate.

(7) What kind of kinase domain present within the intracellular domain of epidermal
   growth factor?
   (A) protein kinase A; (B) protein kinase C; (C) serine kinase; (D) tyrosine kinase;
   (E) phosphotidyl inositol 3-kinase.

(8) If glucose radiolabeled on carbon 3 is converted to pyruvate by the glycolysis
   pathway, on which of following labeled pyruvate (\textbf{bold \( C^* \)}) is right?
   (A) \( \text{C}^*\text{H}_2\text{COCOOH} \); (B) \( \text{CH}_3\text{C}^*\text{OCOOH} \); (C) \( \text{CH}_2\text{COC}\text{OOH} \); (D)
   \( \text{C}^*\text{H}_3\text{C}^*\text{OCOOH} \); (E) \( \text{CH}_3\text{C}^*\text{OC}\text{OOH} \).

(9) Which of the following cycle is related to the production of lactate from glucose in
   active muscle with resynthesis of glucose from lactate in liver?
   (A) Cori cycle; (B) futile cycle; (C) glyoxylate cycle; (D) Citric acid cycle; (E) Q
   cycle.
(10) During Q-cycle of the electron transport of mitochondria, QH₂ transfer its one electron to FeS center. Which compound accepts the other electron of QH₂?
(A) Cytochrome a; (B) Cytochrome a₃; (C) Cytochrome b₁; (D) Cytochrome b₄; (E) Cytochrome c.

(11) In the carbon assimilation reactions of photosynthesis, how many molecules of ATP and NADPH are used to bring 1 molecule carbon dioxide to hexose?
(A) 3 molecules of ATP and 3 molecules of NADPH; (B) 2 molecules of ATP and 2 molecules of NADPH; (C) 3 molecules of ATP and 2 molecules of NADPH; (D) 2 molecules of ATP and 3 molecules of NADPH; (E) none of above.

(12) Which of the following statements about reduced glutathione is incorrect?
(A) is a tripeptide with a cysteine residue; (B) can maintain the normal structure of red blood cells; (C) can keep hemoglobin in the ferric state; (D) can detoxify hydrogen peroxide and organic peroxide; (E) in normal red blood cells, the ratio of the reduced to oxidized forms of glutathione is 500.

(13) The substrate of glycogen synthase is:
(A) ADP-glucose; (B) GDP-glucose; (C) UDP-glucose; (D) ADP-galactose; (E) GDP-galactose.

(14) The following metabolic mutants are active in glycogen degradation:
1. mutation of Ser 14 to Ala 14 in liver phosphorylase,
2. overexpression of phosphorylase kinase in the liver,
3. loss of GTPase activity of G protein α subunit,
4. loss of the gene that encodes inhibitor 1 of protein phosphatase,
5. loss of phosphodiesterase activity.
(A) 1, 2, and 3; (B) 1, 3, and 5; (C) 2, 3, and 5; (D) 2 and 3; (E) only 3.

(15) If malonyl CoA is synthesized from ¹⁴C-labeled carbon dioxide and unlabeled acetyl CoA, and the labeled malonate is then used for fatty acid synthesis, the final product of fatty acid will have radioactive carbon in:
(A) every carbon; (B) every odd-numbered carbon; (C) every even-numbered C; (D) only the farthest carbon from C-1; (E) no part of the molecule.

(16) In DNA ligation proceeds, which of the following statements is wrong?
(A) NAD⁺ is the energy source in eukaryotes; (B) It seals breaks in dsDNA molecules; (C) Adenylate is involved in the reaction; (D) Ligase catalyzes the formation of a phosphodiester bond; (E) None of above.

(17) Which of the following statements is correct during bacterial protein synthesis?
(A) The Met residue found at carboxyl-terminal is usually modified; (B) The initiator tRNA is the tRNAₘ₃₅; (C) The activated formyl donor is N⁰-formyltetrahydrofolate; (D) Transformylase modified free methionine then
attached to tRNA; (E) None of above.

(18) Which of the following statements about tRNA is wrong?
(A) Contains a 5’-ACC-3’ in the C-terminal; (B) Methylation imparts a
hydrophobic character to some regions of tRNAs; (C) The 5’ end of a tRNA is
phosphorylated; (D) They contain many unusual bases; (E) None of above.

(19) Which of following states is wrong?
(A) Restriction enzymes split DNA into specific fragments; (B) Southern blotting
can be used to detect the copy number of a gene; (C) Gene disruption provides
clues to gene expressing pattern; (D) Ti plasmid can be used as vectors to deliver
foreign genes into plant cells; (E) None of above.

(20) Enzyme can be inhibited by specific molecules, which of following pairing is not match?
(A) Chymotrypsin/TPCK; (B) Glycopeptide transpeptidase/penicillin; (C) HIV
protease/Crixivan; (D) Acetylcholin esterase/DIPF; (E) None of above.

(21) Which of following states is wrong?
(A) K_M is the concentration of substrate at which half the active sites are filled; (B)
K_M is equal to the substrate concentration at which the reaction rate is half its
maximal value; (C) The value of K_M is dependent of enzyme and substrate
concentrations; (D) A high K_M indicates weak binding; (E) None of above.

(22) Which of the following statements is wrong?
(A) Transfer RNA carries amino acids in an activated form for peptide-bond
formation; (B) DNA polymerase catalyzes phosphodiester-bond formation from
3’-to- 5’ direction; (C) RNA polymerase uses DNA as the template to synthesizes
RNA; (D) All cellular RNA is synthesized by RNA polymerases; (E) None of
above.

(23) Which of the following amino acid contains thioether group?
(A) Met; (B) Val; (C) Pro; (D) Thr; (E) Ser

(24) Seven of the 20 amino acids have readily ionizable side chains. Which of the
following amino acid is not belonging of them? (A) His; (B) Cys; (C) Lys;
(D) Arg; (E) Ala.

(25) Which of the following protein purification methods is depending in protein size?
(A) Gel-filtration chromatography; (B) Salting out; (C) Affinity chromatography
(D) IEF; (E) DEAE chromatography.
B. Please answer the following questions (50 points)

1. Yeast two-hybrid system is a common method that is used to studies the protein-protein interaction. If you can not use this technique now, please choose another method that can do the same works and describe the principle and processes. (15 points)

2. Please write the cellular localization of the following metabolic pathway. (10 points)
   1. TCA cycle
   2. Ketone-body formation
   3. Fatty acid synthesis
   4. Pentose phosphate pathway

3. After a meal rich in carbohydrates, blood-glucose levels rise. What strategies for liver lead to restore a normal range of blood-glucose levels? Including the mechanisms of nonhormone and hormone, such as glucose and insulin, respectively. (15 points)

4. Please explain many bacteria and plants, but not animals, are able to subsist on the environment containing only acetate? (10 points)