注意：考試開始鈴響前，不得翻閱試題，並不得書寫、畫記、作答。

國立清華大學 110 學年度碩士班考試入學試題

系所班組別：生命科學院
丁組(醫學生物科技學程)

科目代碼：0704

考試科目：分子生物學

一作答注意事項一

1. 請核對答案卷（卡）上之准考證號、科目名稱是否正確。
2. 考試開始後，請於作答前先翻閱整份試題，是否有污損或試題印刷不清，得舉手請監試人員處理，但不得要求解釋題意。
3. 考生限在答案卷上標記「▼ 由此開始作答」區內作答，且不可書寫姓名、准考證號或與作答無關之其他文字或符號。
4. 答案卷用盡不得要求加頁。
5. 答案卷可用任何書寫工具作答，惟為方便閱卷辨識，請儘量使用藍色或黑色書寫；答案卡限用 2B 鉛筆畫記；如畫記不清（含未依範例畫記）致光學閱讀機無法辨識答案者，其後果一律由考生自行負責。
6. 其他應考規則、違規處理及扣分方式，請自行詳閱准考證明上「**國立清華大學試場規則及違規處理辦法**」，無法因本試題封面作答注意事項中未列明而稱未知悉。

國立清華大學 110 學年度碩士班考試入學試題

系所班組別：生命科學院甲組、丁組

考試科目（代碼）：分子生物學(0404、0704)

 *請在【答案卡】作答

I. 選擇題 - multiple choice questions and select **One or More Answer Choices** according to the specific question directions (80 points, 2 points for each).

1. Enhancer function underlies regulatory processes by which cells establish patterns of gene expression. Which of the following descriptions about enhancer **is not correct**?
(A) Enhancer works as cis-regulatory elements to mediate spatiotemporal control of gene expression.
(B) Enhancers can be identified using a reporter gene. Once a reporter gene is integrated near an enhancer, its expression reflects the expression pattern.
(C) Next-generation sequencing (NGS)-based approach called RNase-seq enables identification of enhancers.
(D) Enhancer help recruit RNA polymerase II to promoters and can attract various chromatin-modifying enzymes to DNA.
(E) Enhancer is distal sequences that lie upstream or downstream of the core promoter and recruit transcription factors

2. The DNA polymerases are the workhorses for replication of genomic information in living cells. Which of the following descriptions about their biotechnological applications **is not correct**?
(A) During polymerase chain reaction (PCR), DNA polymerases often extend misprimed targets and primer-dimers, which are common sources of nonspecific amplification.
(B) During PCR, hot-start DNA polymerases reduce nonspecific amplification, increase yields, and allow convenient room temperature setup for high-throughput applications.
(C) Although archaeal DNA polymerases are extremely heat-stable, they may be slow in synthesizing DNA due to lower processivity.
(D) High-fidelity DNA polymerases are enzymes with strong proofreading activity based on their $3' \rightarrow 5'$ exonuclease activity.
(E) DNA polymerases can be engineered with high specificity by introducing a strong DNA-binding domain of another protein without compromising polymerase activity.

3. Which of the following components or events **is not** involved in epigenetic gene regulation?
(A) Histone acetylation
(B) Heavy methylation of promoter regions
(C) Ubiquitin-mediated degradation of transcription factors
(D) Nucleosome sliding
(E) Chromatin opening and closing

4. Agrobacterium-mediated T-DNA delivery of CRISPR/Cas9 expression cassettes is an advanced tool for genome editing in plants. Which of the following descriptions about this technique **is not correct**?
(A) Only the T-DNA region is transferred from agrobacterium into the host plant's nuclear DNA genome.
(B) The virulence region of the Ti plasmid encodes proteins to form a membrane-spanning DNA transporter called the type IV secretion system (T4SS).
(C) CRISPR/Cas9 cleaves foreign DNA via two components, Cas9 and sgRNA.
(D) The sgRNA is a synthetic RNA containing a 5′-end with a 20-nt sequence that acts as a guide sequence to identify the target sequence accompanied by a protospacer adjacent motif (PAM) sequence.
(E) When a template is present, nonhomologous end-joining (NHEJ) can be activated and results in gene replacement or knock-in.

5. Which of the following statements about small RNA **is not correct**?
(A) Small RNAs are short, non-coding RNA molecules that can regulate gene expression.
(B) Small RNAs can regulate gene expression in the nucleus via chromatin-dependent gene silencing.
(C) Most small interfering RNAs (siRNAs) are generated through multiple processing steps from longer primary transcripts produced by RNA polymerase II.
(D) Most microRNAs (miRNAs) form imperfectly complementary stem-loop structures, pair imperfectly with sites in the 3′ untranslated region of their target mRNAs.
(E) Both miRNAs and siRNAs are produced from longer RNA precursors through the activity of ribonuclease III–like nucleases called Dicer in animals and DICER-LIKE (DCL) in plants.

6. Which of the following descriptions about homeobox genes **is not correct**?
(A) They are a group of genes regulates development in multicellular organisms.
(B) The DNA binding motif in homeobox genes is typically a 180 base pair DNA sequence which encodes a 60 amino acid homeodomain that acts as transcriptional regulator.
(C) The use of chromatin immunoprecipitation sequencing can identify genes bound and modulated by homeodomain protein
(D) Yeast one-hybrid can be used for testing homeodomain protein-protein interactions.
(E) The homeobox Hox genes arrange on chromosomes in the same order as their expression domains along the body axis.

7. Which of the following descriptions about protein translation **is not correct**?
(A) Terminator is a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during translation
(B) Upstream open reading frames generally hinder translation of the main open reading frame (ORF)
(C) Ribosome density along ORFs can be studied with high resolution by sequencing of ribosome-protected mRNA footprints, a method known as ribosome footprinting.
(D) Mass spectrometry-based methods can provide estimates of synthesis and degradation rates for a substantial fraction of proteins.
(E) The direct method for analysis of the *de novo* protein synthesis is to detect the isotope-labeled amino acids incorporated into newly synthesized proteins.

8. Which of the following descriptions about electrophoretic mobility shift assay (EMSA) **is not correct**?
(A) It is used to detect protein complexes with nucleic acids.
(B) Solutions of protein and nucleic acid are combined and the resulting mixtures are subjected to electrophoresis under native conditions through polyacrylamide or agarose gel.
(C) The distribution of species containing nucleic acid is determined usually by autoradiography of ^{32}P-labeled nucleic acid
(D) Protein–nucleic acid complexes migrate more faster than the corresponding free nucleic acid.
(E) The electrophoretic mobility of a complex provides little direct information about the location of the nucleic acid sequences that are occupied by protein.

9. Which of the following sequence(s) in mRNA can form base-pairing with the 3' end of 16S rRNA?
(A) Kozak sequence
(B) Shine-Dalgarno sequence
(C) TATA Box sequence
(D) Internal Ribosome Entry sequence
(E) PolyA sequence

10. Which of the following sequence(s) contain AUG?
(A) Kozak sequence
(B) Shine-Dalgarno sequence
(C) TATA Box sequence
(D) Internal Ribosome Entry sequence
(E) PolyA sequence

11. _____ is the major factor promoting binding of fMet-tRNA to the 30S initiation complex?
(A) IF-2
(B) IF-3
(C) EF-Tu
(D) EF-G
(E) RRF

12. Which of the following factor(s) has an actual cap binding activity in translation initiation complex?
(A) eIF2
(B) eIF3
(C) eIF4A
(D) eIF4E
(E) eIF4G

13. Which of the following factor(s) is a GTP binding protein involved in protein translation?
(A) IF-2
(B) IF-3
(C) EF-Tu
(D) EF-Ts
(E) RF3

14. Quantitative real-time PCR (qPCR) can be used to detect and quantify SARS-CoV-2 virus in different samples. The cycle numbers for the measurement of viral RNA-dependent RNA polymerase (RdRp) expression in sample A and B are 30 and 25, respectively, and those of internal RNAse P control in sample A and B are 28 and 29, respectively. What is the fold-difference of the viral titers between sample A and B? The PCR efficiencies for gene RdRp and RNAse P are closed to 100%, and there is no sampling error.
(A) A is 6-folds higher than B.
(B) A is 32-folds higher than B.
(C) A is 64-folds higher than B.
(D) B is 6-folds higher than A
(E) B is 64-folds higher than A.

15. Which of the following descriptions are correct for BRCA1?
(A) It plays a role in double strand break repair through non-homologous end joining repair.
(B) It plays a role in double strand break repair through homology-directed repair.
(C) It controls mismatch repair (MMR).
(D) Its mutations contribute to microsatellite instability.
(E) Its mutations serve as a biomarker for PAPR inhibitors in cancer treatment.

16. Which of the following descriptions are correct for lentiviral vectors and their viral particles in gene therapy?
(A) They can infect dividing cells.
(B) They cannot infect non-dividing cells.
(C) They function by delivering RNA molecules in pair with reverse transcriptase enzymes.
(D) They can integrate their genome into cells.
(E) They may cause insertional mutagenesis due to the presence of robust enhancer-promoter sequences.

17. Which of the following is (are) involved in genome editing?
(A) Homologous recombination
(B) Zinc-finger nucleases (ZFNs)
(C) Transcription activator-like effector nuclease (TALENs)
(D) Clustered regularly interspaced short palindromic repeats (CRISPR)
(E) Histone acetyltransferase (HAT)

18. Which of the following is (are) involved in RNA editing?
(A) Watson-Crick base pairing
(B) Terminal uridylyltransferase (TUTase)
(C) Adenosine deaminases active on RNAs (ADARs)
(D) Cytidine deaminases acting on RNA (CDARs)
(E) DNA T4 ligase

19. Which of the following factor(s) affect post-transcriptional control of gene expression?
(A) mRNA stability
(B) RNA trans-splicing
(C) pre-mRNA cis-splicing
(D) mRNA polyadenylation
(E) Chromosome remodeling

20. Which of the following is (are) involved in RNA splicing?
(A) snRNPs
(B) ATP
(C) RNA polymerase II CTD
(D) SR protein
(E) Histone acetyltransferase

21. Which of the following is (are) involved in RNA self-splicing?
(A) Group I intron
(B) Group II intron
(C) SR proteins
(D) Sm proteins
(E) minor spliceosome

22. Which of the following is (are) involved in chromatin repression?
(A) open chromatin
(B) Histone methyltransferase-associated protein HP1
(C) Histone deacetylase (HDACs)
(D) telomere position effect (TPE)
(E) Silencing Information Regulator 3 (SIR3)

23. Which of the following event(s) is mediated by RNA polymerase II?
(A) capping
(B) exon definition
(C) intron definition
(D) formation of pre-mRNA precleavage complex
(E) polyadenylation

24. Which of the following description about RNA polyadenylation is (are) correct?
(A) transcription of eukaryotic genes ends before the polyadenylation site.
(B) polyadenylation efficiency varies upon the sequence of a polyadenylation signal
(C) both CPSF and poly(A) polymerase (PAP) are required for the initiation phase
(D) AAUAAA is required for the elongation phase
(E) polyadenylation only occurs in the nucleus

25. The new corona virus SARS-CoV-2 is an RNA virus. The replication of viral genome in the host cell requires
(A) RNA-dependent DNA polymerase
(B) DNA-dependent RNA polymerase
(C) DNA-dependent DNA polymerase
(D) RNA-dependent RNA polymerase
(E) Ribozyme

26. Upon SARS-CoV-2 virus infection, the viral orf1ab gene is transcribed into one single RNA molecule but translated into non-structural polyproteins. Which of the following statement is incorrect?
(A) Some non-structural proteins are produced by ribosomal frameshift
(B) Viral proteases may cut large polyproteins into small functional nonstructural proteins
(C) The large polyprotein itself can characterize viral genome replication
(D) Translation of orf1ab relies on host ribosome machinery
(E) Viral genome can be translated directly upon viral entry

27. Which of the following enzyme has the lowest copy fidelity?
(A) Pfu DNA polymerase
(B) Taq DNA polymerase
(C) HiFi DNA polymerase
(D) Phusion HiFi DNA polymerase
(E) DNA polymerase theta

28. Which of the following RNA molecule may guide Cas9 to the target genome loci?
(A) miRNA
(B) siRNA
(C) sgRNA
(D) lncRNA
(E) mRNA

29. Which of the following statement is not correct for restriction enzyme?
(A) A restriction enzyme may cut the DNA at specific site
(B) Restriction enzymes are mostly exonucleases
(C) A restriction enzyme may recognize a specific and short nucleotide sequence
(D) Restrictions enzymes cut only DNA substrates
(E) A restriction enzyme may cut DNA and generate sticky or blunt ends

30. The following DNA sequences are a part of the beginning sequence of gene A. Where do you think the transcription may begin?

(A) Before nucleotides 20
(B) Between nucleotides 20-50
(C) Between nucleotides 60-70
(D) Between nucleotides 70-90
(E) Somewhere behind nucleotide 90

```
    1         10        20        30        40        50        60        70
    I---------I---------I---------I---------I---------I---------I---------I
5'  ATCGGTCTCGGCTACTACATAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTAGGCTACTAC
3'  TAGCCAGAGCCGATGATGTATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGATCCGATGATG
                              Promoter

              80        90        100       110       120       130       140
    ----------I---------I---------I---------I---------I---------I---------I
5'  CAGGTATCGGTCTGATCTAGCTAGCTTCTCTTCTCTCTCTCCCCCGCGGGGGCTGTACTATCATGCGTCG
3'  GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGGGGGCGCCCCCGACATGATAGTACGCAGC
        RBS

              150       160       170       180       190       200       210
    ----------I---------I---------I---------I---------I---------I---------I
5'  TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAA
3'  AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCATTT
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31. According to the above sequence, which of the following is likely the source of this DNA?

(A) Human genome
(B) Mice genome
(C) Yeast genome
(D) E. Coli genome
(E) C. elegans genome

32. Which of the following is NOT an epigenetic modification?

(A) DNA methylation
(B) DNA phosphorylation
(C) Histone methylation
(D) Histone acetylation
(E) Histone phosphorylation

33. The lytic cycle is one of the two cycles of viral reproduction. Which of the following descriptions about the lytic cycle **is not correct**?
(A) With lytic phages, bacterial cells are broken open and destroyed after immediate replication of the virion.
(B) After the cell is destroyed, the phage progeny can find new hosts to infect.
(C) An example of a lytic bacteriophage is T4, which infects *E. coli* found in the human intestinal tract.
(D) Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high.
(E) As the lytic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring.

34. Non-coding RNA. Which of the following descriptions about the non-coding RNA **is not correct**?
(A) Non-coding RNAs are classified into two major categories: structural and regulatory non-coding RNAs.
(B) Structural non-coding RNAs comprise of siRNAs and miRNAs.
(C) Regulatory non-coding RNAs are further divided into three classes, small, medium and long non-coding RNAs.
(D) Short non-coding RNA with a size between 20–50 nucleotides. Medium non-coding RNA with a size between 50–200 nucleotides.
(E) Long non-coding RNA with maximum regulatory potency containing greater than 200 nucleotides.

35. Riboswitches are regulatory elements built into mRNA. Which of the following descriptions about the riboswitch **is not correct**?
(A) One common form of riboregulation in bacteria is the use of ribonucleic acid sequences encoded within mRNA that directly affect the expression of genes encoded in the full transcript.
(B) Riboswitches are trans-acting elements.
(C) Riboswitches are defined as mRNA elements that bind ligands and regulate mRNA expression by forming alternative structures in response to this ligand binding.
(D) Riboswitches are most often located in the 5' UTR of bacterial mRNA. There they regulate the occlusion of signals for transcription attenuation or translation initiation.
(E) However, in some eukaryotic mRNA, the thiamine pyrophosphate (TPP) riboswitch regulates splicing at the 3' end.

36. Spliceosome is found primarily within the nucleus of eukaryotic cells. Which of the following descriptions about the spliceosome **is not correct**?
(A) Spliceosomes splice the introns out of pre-mRNA and seal the exon ends together.
(B) The spliceosomes consist of DNA-protein complexes called small nuclear ribonucleoproteins (snRNPs).
(C) The five snRNPs involved in splicing are U1, U2, U4, U5, and U6. Together with some additional proteins, the snRNPs form the spliceosome.
(D) All the snRNPs except U6 contain a conserved sequence that binds the Sm proteins.
(E) Splicing factor – A protein component of the spliceosome that is not part of one of the snRNPs.

37. Nonsense-mediated decay (NMD) of eukaryotic mRNA. Which of the following descriptions about the NMD **is not correct**?
(A) NMD is triggered by the presence of premature stop codons.
(B) The exon junction complex (EJC) is bound to the mRNA upstream of each exon-exon junction.
(C) During splicing, the mRNA is loaded with EJC upstream of each exon-exon junction.
(D) If there is a premature stop codon, the ribosome finishes translating before all of the EJC complexes have been bumped off the mRNA, then nonsense-mediated decay is triggered by binding of initiation factors (IF) plus Upf1 to the remaining EJC.
(E) The next step of NMD is removal of the cap from the mRNA, then the mRNA is degraded from the exposed 5′ end.

38. RNA splicing can be regulated by exonic and intronic splicing enhancers and silencers. Which of the following descriptions about RNA splicing **is not correct**?
(A) Alternative splicing is often associated with strong splice sites.
(B) Specific exonic and intronic sequences can enhance or suppress splice-site selection.
(C) SR protein binds to exonic splicing enhancers and the heterogeneous nuclear ribonucleoproteins hnRNP A and B bind to exonic silencers.
(D) Other RNA-binding proteins can function as splicing regulators by binding to intronic splicing enhancers or silencers.
(E) Binding of both Nova and Fox to intronic sequences upstream of the alternative exon results in the suppression of exon.

39. Which of the following descriptions about 3′ mRNA end processing **is not correct**?
(A) The site of cleavage/polyadenylation is flanked by an upstream AAUAAA motif and a downstream U-rich or GU-rich element.
(B) The cleavage and polyadenylation specific factor (CPSE): One subunit binds to the AAUAAA motif and to the cleavage stimulatory factor (CstF). One binds to downstream GU-rich sequence.
(C) CPSF and CstF enhance each other.
(D) The enzyme 73-kDa CPSF subunit cleaves the RNA.
(E) Poly(A)-binding protein synthesizes the poly(A) tail.

40. Novel amino acids can be inserted at certain stop codons. Which of the following descriptions **is not correct**?
(A) Rare amino acids are not one of the standard amino acid.
(B) Selenocysteine (Sec) is encoded by UGA, one of the stop codon.
(C) The choice between "stop" and Sec depends on the selenocysteine insertion sequence (SECIS element).
(D) In bacteria, SECIS element forms a stem and loop just after the UGA.
(E) In mammals, SelB protein recognizes both tRNA-Sec and SECIS element. The Sec is delivered to the right place and then inserted as part of the growing polypeptide.

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II. 簡答題 - essay questions (20 points, 4 points for each)

41. Western Blot result depends on the whole system including antigen content, sensitivity of primary antibody, sensitivity of secondary antibody, sensitivity of substrate, efficiency of color development and photographic fixing. Please provide the Western Blot troubleshooting to solve the following unsatisfactory results:
(a) No signal or weak signal (2 points)
(b) Non-specific bands (2 points)

42. 5'-untranslated region of E. coli thiM mRNA contain a riboswitch, which can be used to control translation initiation via mRNA secondary structure.
(a) What is "aptamer" in thiM mRNA? (2 points)
(b) How does the change of thiM mRNA secondary structure affect translation initiation? (2 points)

43. What is a protospacer adjacent motif (PAM) sequence? Why is it important in designing a CRISPR/Cas experiment?

44. The "UK variant"—lineage B.1.1.7 of SARS-CoV-2 has raised widespread concern these days. In the UK variant, several nucleotide mutations were found on the receptor-binding domain (RBD) of viral spike protein, but only one amino acid change N501Y was characterized. Please explain why other RBD nucleotide mutations are silent?

45. All eukaryotes have three different RNA polymerases. Match the name of each RNA polymerases in Column I to its correct description in Column II. Options from Column I can be used more than once.
Column I
(1) RNA polymerase I
(2) RNA polymerase II
(3) RNA polymerase III

Column II
A. Transcribing the genes for the two large rRNA molecules.
B. Transcribing most eukaryotic genes that encode proteins.
C. Transcribing the genes for 5S rRNA and other small RNA molecules.
D. Transcribing the genes for tRNA.