Multple-choice Questions

1. How do you detect homologous genes in the mouse and human genomes?

   a. Use the BLAST algorithms on both human and mouse genomes
   b. align the human and mouse genomes
   c. align the himan and mouse genomes
   d. consult the UNIPROT library

2. What is Gag?

   a. A gene that contains the lentivirus
   b. A polyprotein that contains the core structural elements of retroviruses
   c. A protein that produces the viral envelope
   d. A gene essential for the life of eukaryotes

3. CA domain–containing proteins:

   a. Cannot form high molecular weight oligomers
   b. Are conserved between human and mouse and have detectable levels of mRNA in adult human tissues
   c. Are species-specific and have detectable levels of mRNA in adult tissues
   d. Are conserved between human and mouse and have detectable levels of mRNA only in developing tissues

4. Fig 1G shows a result obtained by:

   a. Immunoprecipitation
   b. Western blot
   c. RT-qPCR
   d. Mass spectrometry

5. Two orthologous genes:

   a. are in the same genome
   b. have necessarily the same function
   c. are in different genomes
   d. have the same coding sequence

6. To avoid using transfected plasmids for the expression of Gag homologs, the authors:

   a. Performed mRNA sequencing only on the VLP fraction
   b. Performed mRNA sequencing only on the whole-cell lysate fraction

c. Used CRISPR activation to silence the expression of endogenous genes in mouse N2a cells
d. Used CRISPR activation to induce expression of endogenous genes in mouse N2a cells

7. How does PEG10 work?

a. It binds and helps secrete its own RNA without forming capsids
b. It helps secrete exogenous RNA cargoes
c. It is naturally inactive
d. It forms virus-like capsids and binds and helps secrete its own RNA

8. SEND platform combines:

a. Gag, cargo mRNA, and fusogen
b. Gag, cargo mRNA, VSVg
c. Endogenous Gag homolog, cargo mRNA, and fusogen
d. Endogenous Gag homolog, cargo mRNA

9. The manuscript points out how retroviral LTRs are integrated into mammalian genomes. Which is the main role of proviral LTRs during productive viral infection?

a. The integrated provirus utilizes the promotor elements in the 5' LTR to drive transcription
b. The provirus does not need the LTRs to replicate its genome
c. LTRs allow transcription of *gag* only
d. LTR are also present in retroviral genomes

10. According to the paper, where you can find the virus-like particle fraction?

a. In the supernatant of every cell type
b. In the supernatant of cells that overexpress some CA-containing gene
c. In the whole lysate of HEK 293 FT cells
d. In the whole lysate of HEK 293 FT cells that overexpress some CA-containing gene

11. How do the authors assess the immunogenicity of SEND?

a. Exploiting gene expression data demonstrating that HsPEG10 is highly expressed in the thymic epithelium
b. They assessed SEND immunogenicity by using *in vivo* models
c. They used other pseudotyped vectors containing the ectodomain of PEG10 for testing human PBMC activation *in vitro*
d. They use vectors expressing PEG10 homologs

12. In which organisms are retrotrasposons found?

    a. In all prokaryotes but not eukaryotes
    b. In all eukaryotes but not in prokaryotes
    c. In both eukaryotes and prokaryotes
    d. Only in mammals

13. Where do the authors describe the production of oligomeric forms of CA-containing proteins in *Escherichia coli*?

    a. Figure 1D
    b. Figure 1F
    c. Figure 1B
    d. Figure 1G

14. Western blot is an analytical technique used for detecting…

    a. RNA
    b. DNA
    c. Proteins
    d. Antibodies

15. In Fig 2 the authors use CRISPR to:

    a. Perform genome editing
    b. Find and block retrotransposon elements
    c. Downregulate exogenous CA domain-containing genes
    d. Overexpress endogenous genes

16. How the authors find the PEG10 region responsible for RNA binding?

    a. Making target deletions in PEG mRNA and evaluating protein enrichment
    b. Making target deletions in PEG protein and evaluating mRNA enrichment
    c. Thanks to computational survey
    d. Using CRISPR

17. What is a knock-in mouse?

    a. A mouse in which an endogenous gene is deleted from the genome
    b. A mouse in which an exogenous sequence is added to change a specific gene in the genome
    c. A mouse in which an endogenous gene is overexpressed in the genome
    d. A mouse in which an exogenous sequence is overexpressed in the genome

18. Why do the authors need a knock-in mouse in Fig 2I?

a. They need *Peg10* overexpression
b. They need a tag in the *Peg10* mRNA
c. They need a tag in the PEG10 protein
d. They need *Peg10* downregulation

19. In Fig 2, which panel demonstrates directly that PEG10 bins its own UTRs?

a. Panel c
b. Panel F
c. Panel H
d. Panel I

20. In Fig 3C the authors show a nuclear staining using DAPI. Using this reagent, what is it possible to detect in a cell culture?

a. Ferroptosis
b. Necrosis
c. Apoptosis
d. Pyroptosis

21. Figure 3H shows a result obtained by:

a. Flow cytometry
b. Transcriptomics
c. Chromosome capture
d. Mass spectrometry

22. Figure 4 contains panels, A and B, showing the delivery of gene editing tools into human and mouse cells. How do the authors evaluate the delivery?

a. immunoprecipitation
b. mRNA sequencing
c. ELISA
d. immunofluorescence

23. For the identification of RNA species in the VLP fraction the authors used:

a. DNA sequencing
b. Chip sequencing
c. mRNA sequencing
d. Single cell sequencing

24. Which is the “fusogen” used by the authors in the SEND modular system?

    a. vesicular stomatitis virus
    b. VZV
    c. MLV
    d. vesicular stomatitis virus envelope protein

25. The Authors repeated co-packaging strategy with the human SEND system, generating indels in HEK293FT cells at the HsVEGFA locus. Which percentage of indels did they obtain?

    a. 20%
    b. 30%
    c. 40%
    d. 26%

26. The authors characterized indels at the HsVEGFA locus in HEK293FT cells treated with SEND containing either SpCas9 mRNA or cargo and an unmodified sgRNA. How many replicates do the authors performed for the determining indels by NGS?

    a. 5 replicates
    b. a single analysis
    c. 6 replicates
    d. 3 replicates

27. Which is one of the functions of MmPEG10 *in vivo*?

    a. To silence gene expression in neurodevelopment
    b. To bind tRNA
    c. To bind siRNA
    d. to stabilize mRNAs with fundamental roles in neurodevelopment

28. How do the authors determine the main role of MmPEG10 NC domain in mRNA export?

    a. They performed deletions in RT domain
    b. They performed deletions in NC domain
    c. They performed deletions both in RT and NC domains
    d. They evaluated mRNA export without performing any deletion

29. What is the Cre-LoxP system?
    a. A tool for genetic manipulation based on the action of a recombinase on its target sites
    b. A transgenic mouse line
    c. A transgenic cell line

d. A tool for genetic manipulation at the basis of the CRISPR technology

30. One of the statistical tests used by the Authors is the two-way ANOVA test. This statistical test determines the staistical significance of:
a. the differences among multiple groups, evaluating 2 independent variables
b. the differences between 2 independent groups, where the variable is binary
c. the differences between 2 independent groups, where the variable is continuous
d. the differences among 2 groups, evaluating multiple independent variables.

Open questions

1. The authors employ different version of the CRISPR technology along the paper. Describe briefly what is the CRISPR/Cas system in general

2. To demonstrate mRNA delivery into cells, the authors tested the delivery of a mRNA coding for Cre. Why is this approach very sensitive?

Titolo ed abstract:

1. Proponi un abstract per l'articolo, di meno di 1500 caratteri inclusi gli spazi
2. Proponi un titolo per l'articolo, di meno di 150 caratteri inclusi gli spazi

Answers

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