A

PRIMA DOMANDA
Descriva sinteticamente (10 minuti) le sue esperienze indicate nel curriculum, i rapporti tecnico / gestionali, pubblicazioni e/o brevetti presentati.

SECONDA DOMANDA
Si vuole studiare la co-localizzazione di una proteina umana di interesse con un complesso multiproteico e la sua localizzazione sub-cellulare avendo a disposizione un mab (anticorpo monoclonale) di topo. Come procederebbe?

TERZA DOMANDA
Da una diluizione seriale di una coltura batterica di E.coli, sono state conteggiate 50 colonie su piastra di terreno solido LB, nella quale furono piastrate 200µl di una coltura diluita 10^{-6}. Determinare la concentrazione batterica per ml [CFU/ml] della coltura madre.

CONOSCENZA DI INFORMATICA DI BASE
Si vuole modificare una immagine digitale acquisita in microscopia che software utilizzerebbe?

PROVA DI CONOSCENZA DI LINGUA INGLESE

Abstract 6

Osteosarcoma (OS) is still a disorder threatening children life. A growing number of evidences highlights the role of circular RNAs (circRNAs) during OS malignancy. Herein, we aimed to address the pathological contribution of the unrecognized circ_0020378 to OS progression. Analysis of the expression of circ_0020378, miR-556-5p, and MAPK1 in OS tissues and cells was performed using RT-qPCR or western blotting. CCK8, colony formation assays, and Tranwell migration assays were adopted to assess the OS cell viability, clone formation ability and migration. Tumor xenograft mouse model was used to assess the in vivo function of circ_0020378. The relationship between miR-556-5p and circ_0020378 or MAPK1 was discovered using luciferase reporter assays and RNA binding protein immunoprecipitation tests. In OS tissues and cells, circ_0020378 and MAPK1 were significantly elevated, although miR-556-5p expression exhibited a different pattern. Circ_0020378 silence attenuated OS cell proliferation, colony formation ability and migration in vitro, and retarded tumor growth in vivo. MiR-556-5p was targeted by circ_0020378. Furthermore, miR-556-5p inhibitor promoted the OS cell proliferation and migration, while this promoted malignant actions of OS cells were abrogated by circ_0020378 silence. Additionally, miR-556-5p directly bound to MAPK1, and MAPK1 silence exerted its inhibitory effect on OS cell proliferation and migration, and yet the inhibition was offset by miR-556-5p inhibitor. Circ_0020378 acts as a novel tumor promoter that controls OS growth by miR-556-5p/MAPK1 axis, suggesting circ_0020378/miR-556-5p/MAPK1 might be a novel target for OS intervention.

B

PRIMA DOMANDA
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SECONDA DOMANDA
Partendo da un lisato cellulare si vogliono determinare le quantità relative di una proteina di interesse nel citoplasma e nel nucleo. Come procederebbe?

TERZA DOMANDA
Avendo a disposizione una confezione di tripsina si richiede di preparare una soluzione di 100 ml allo 0.5% da utilizzare per l'allestimento di colture primarie a partire da una biopsia. Come procede?

CONOSCENZA DI INFORMATICA DI BASE
Si vuole allestire un istogramma a colonne che software utilizzerebbe?

PROVA DI CONOSCENZA DI LINGUA INGLESE

Abstract 1

Membrane proteins play an essential role in all living organisms. Although there have been numerous efforts in the past to elucidate the structure and function of eukaryotic primary active transporters, knowledge about the majority of these membrane proteins is still minimal. This is often due to their low availability and complex handling. In this study, we homologously expressed three ATP-dependent transport proteins, STE6-2p, NEO1-p, and YPK9-p, in Pichia pastoris and subsequently optimized the solubilization and purification processes. Sequential use of different mild detergents and utilization of hydrophilic matrices in the purification procedure allowed us to obtain all three transporters monodisperse and in high purity, enabling initial structural analysis by cryo-electron microscopy. Using the respective substrates, we determined the specific activity of all target proteins using an ATPase assay. This study opens the door to further functional and structural studies of this pharmacologically important class of membrane proteins.

C

PRIMA DOMANDA
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SECONDA DOMANDA
Si vuole inserire in un cDNA codificante una proteina di interesse un epitope tag all'estremità 3' del CDS e quindi clonarlo in vettori plasmidici di espressione per cellule di mammifero, per baculovirus, per E.coli e per saggi di traduzione in vitro. Come procederebbe?

TERZA DOMANDA
Nel foglio di accompagnamento dell'oligonucleotide liofilizzato sono riportati i seguenti parametri:

Yield [OD]	11.6
Yield [µg]	316
Yield [nmol]	30.9
Vol for 100 pmol/µl	309 →

Prepari 400 µl di una soluzione 0.5 µM di oligonucleotide in acqua o 0.1xTE

CONOSCENZA DI INFORMATICA DI BASE
Si vuole allestire una tabella contenente Nomi e Dati che software utilizzerebbe?

PROVA DI CONOSCENZA DI LINGUA INGLESE

Abstract 2

Cisplatin-induced ototoxicity is caused by reactive oxygen species. It has been recognized that estradiol (E2) regulates redox balance. However, little is known about the protective mechanisms of E2 against cisplatin-induced ototoxicity. In this study, we investigated the effect of E2 on nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated hair cell protection using the organ of Corti isolated from mice. The organ of Corti collected from C57BL/6 mice at 3-5 postnatal days was used in all experiments. The organ of Corti was exposed to 20 μM cisplatin with/without 100 nM E2 to examine the effect of E2 on cisplatin-induced hair cell loss. The mRNA expression of Nrf2 and the phase II detoxification gene after E2 and cisplatin treatment was analyzed using quantitative real-time PCR. E2 significantly reduces cisplatin-induced cochlear hair cell death. In addition, 100 nM E2 increased the mRNA expression of Nrf2 and phase II detoxification genes in the organ of Corti under cisplatin treatment. Our results suggest that E2 activates Nrf2, phase II detoxification enzymes and exerts a protective effect against cisplatin-induced ototoxicity.

D

PRIMA DOMANDA
Descriva sinteticamente (10 minuti) le sue esperienze indicate nel curriculum, i rapporti tecnico / gestionali, pubblicazioni e/o brevetti presentati.

SECONDA DOMANDA
Vogliamo misurare le quantità relative di un trascritto isolato da 2 linee cellulari. Come procederebbe e con quali tecniche?

TERZA DOMANDA
Si hanno a disposizione:
1 Lt di soluzione contenente 1 M Tris-HCl pH 8.0 / 0.4 M NaCl
1 Lt di soluzione contenente 1 M Tris-HCl pH 8.0
Acqua distillata q.b.

Si richiede di preparare:
0.4 Lt di soluzione contenente 250 mM Tris-HCl pH 8.0 / 25 mM NaCl
Quanti ml delle soluzioni madre sono richiesti e quanti di acqua?

CONOSCENZA DI INFORMATICA DI BASE
Si vuole inserire un file *name.jpg* in una presentazione *name.ppt* che comando utilizzerebbe?

PROVA DI CONOSCENZA DI LINGUA INGLESE

Abstract 5

Tracing and manipulating cells in embryos are essential to understand development. Lipophilic dye microinjections, viral transfection and iontophoresis have been key to map the origin of the progenitor cells that form the different organs in the post-implantation mouse embryo. These techniques require advanced manipulation skills and only iontophoresis, a demanding approach of limited efficiency, has been used for single-cell labelling. Here, we perform lineage tracing and local gene ablation using cell-permeant Cre recombinase (TAT-Cre) microinjection. First, we map the fate of undifferentiated progenitors to the different heart chambers. Then, we achieve single-cell recombination by titrating the dose of TAT-Cre, which allows clonal analysis of nascent mesoderm progenitors. Finally, injecting TAT-Cre to Mycnflox/flox embryos in the primitive heart tube revealed that Mycn plays a cell-autonomous role in maintaining cardiomyocyte proliferation. This tool will help researchers identify the cell progenitors and gene networks involved in organ development, helping to understand the origin of congenital defects.

E

PRIMA DOMANDA
Descriva sinteticamente (10 minuti) le sue esperienze indicate nel curriculum, i rapporti tecnico / gestionali, pubblicazioni e/o brevetti presentati.

SECONDA DOMANDA
Si intende esprimere stabilmente un GOI (gene of interest) in una linea cellulare di mammifero e in una coltura di cellule primarie. Come procederebbe?

TERZA DOMANDA
Avendo una soluzione di 1 lt di acido cloridrico 1 M, si vuole preparare 300ml di una soluzione 0.05 N

CONOSCENZA DI INFORMATICA DI BASE
Riceve un file *NAME.pdf* che software utilizzerebbe per leggerlo e modificarlo?

PROVA DI CONOSCENZA DI LINGUA INGLESE

[redacted]

Abstract 4

Advances in DNA sequencing technology and bioinformatics have revealed the enormous potential of microbes to produce structurally complex specialized metabolites with diverse uses in medicine and agriculture. However, these molecules typically require structural modification to optimize them for application, which can be difficult using synthetic chemistry. Bioengineering offers a complementary approach to structural modification but is often hampered by genetic intractability and requires a thorough understanding of biosynthetic gene function. Expression of specialized metabolite biosynthetic gene clusters (BGCs) in heterologous hosts can surmount these problems. However, current approaches to BGC cloning and manipulation are inefficient, lack fidelity, and can be prohibitively expensive. Here, we report a yeast-based platform that exploits transformation-associated recombination (TAR) for high efficiency capture and parallelized manipulation of BGCs. As a proof of concept, we clone, heterologously express and genetically analyze BGCs for the structurally related nonribosomal peptides eponemycin and TMC-86A, clarifying remaining ambiguities in the biosynthesis of these important proteasome inhibitors. Our results show that the eponemycin BGC also directs the production of TMC-86A and reveal contrasting mechanisms for initiating the assembly of these two metabolites. Moreover, our data shed light on the mechanisms for biosynthesis and incorporation of 4,5-dehydro-l-leucine (dhL), an unusual nonproteinogenic amino acid incorporated into both TMC-86A and eponemycin.

F

PRIMA DOMANDA
Descriva sinteticamente (10 minuti) le sue esperienze indicate nel curriculum, i rapporti tecnico / gestionali, pubblicazioni e/o brevetti presentati.

SECONDA DOMANDA
Si vogliono produrre 2 proteine umane ricombinanti, la cui attività funzionale di una di esse richiede modificazioni post-traduzionali. Inoltre serve determinare la quantità prodotta e purificare solo le forme delle proteine full-lenght. Come procederebbe?

TERZA DOMANDA
L'agente selettivo G418 è fornito in polvere alla potenza (purezza) del 50% preparare 1000 ml di terreno sterile contenente 400µg/ml di G418. Quanti grammi occorrono?

CONOSCENZA DI INFORMATICA DI BASE
Riceve un file *NAME.docx* con quale software lo legge

PROVA DI CONOSCENZA DI LINGUA INGLESE

Abstract 3

As a sign of chronic kidney disease (CKD) progression, renal fibrosis is an irreversible and alarming pathological change. The accurate diagnosis of renal fibrosis depends on the widely used renal biopsy, but this diagnostic modality is invasive and can easily lead to sampling error. With the development of imaging techniques, an increasing number of noninvasive imaging techniques, such as multipara meter magnetic resonance imaging (MRI) and ultrasound elastography, have gained attention in assessing kidney fibrosis. Depending on their ability to detect changes in tissue stiffness and diffusion of water molecules, ultrasound elastography and some MRI techniques can indirectly assess the degree of fibrosis. The worsening of renal tissue oxygenation and perfusion measured by blood oxygenation level-dependent MRI and arterial spin labeling MRI separately is also an indirect reflection of renal fibrosis. Objective and quantitative indices of fibrosis may be available in the future by using novel techniques, such as photoacoustic imaging and fluorescence microscopy. However, these imaging techniques are susceptible to interference or may not be convenient. Due to the lack of sufficient specificity and sensitivity, these imaging techniques are neither widely accepted nor proposed by clinicians. These obstructions must be overcome by conducting technology research and more prospective studies. In this review, we emphasize the recent advancement of these noninvasive imaging techniques and provide clinicians a continuously updated perspective on the assessment of kidney fibrosis.