AMMINISTRAZIONE CENTRALE
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UNIVERSITÀ
DEGLI STUDI
DI PADOVA

SELEZIONE PUBBLICA N. 2023T32, PER TITOLI ED ESAMI, AL FINE DI REPERIRE N. 1 TECNOLOGO DI RICERCA, DI PRIMO LIVELLO (CATEGORIA STIPENDIALE "EP1"), DA ASSUMERE MEDIANTE CONTRATTO DI LAVORO A TERMINE, A TEMPO PIENO, PER N. 60 MESI, AI SENSI DELL'ART. 24-*BIS* DELLA LEGGE 30.12.2010, N. 240, E DEL C.C.N.L. DEL 19.04.2018, IN QUANTO COMPATIBILE, PRESSO IL DIPARTIMENTO DI MEDICINA - DIMED.

QUESITI COLLOQUIO

BUSTA N. 1

Domanda: Estrazione di DNA da diverse matrici biologiche e analisi genetica.

Bioinformatica: Cosa è un file Fastq?

Inglese: The concept of gene therapy.
Gene therapy is based on the principle of the genetic modification of living cells for use in treating various disorders. The final goal of gene therapy is to cure patients who suffer from genetic disorders, including congenital diseases, infectious diseases, and cancer.
This concept has existed for several decades; however, in practice, "genetic modification" exclusively refers to the transfer of therapeutic genes into living cells or the human body.
Multigenic disorders are too complex to tackle; however, monogenic disorders, particularly those caused by small mutations, are likely to be cured by the supplementation or augmentation of non-mutated genes.
Traditional gene therapy approaches for treating congenital disorders primarily rely on gene transfer, which introduces one or, at most, a few transgenes to compensate for the gene function.
Therefore, the key to performing successful gene therapy depends on the use of efficient transduction techniques in human cells.

BUSTA N. 2

Domanda: Droplet Digital PCR: principi e applicazioni.

Bioinformatica: Breve descrizione di un software bioinformatico.

Inglese: Targeted genome editing techniques.
Gene targeting is a well-established technique used in model organisms to manipulate the genomic sequence by using the host homologous recombination system.
Since gene targeting mediates target sequence-specific recombination events through homology-arms, it is an ideal technique for correcting certain genetic mutations for gene therapy. In principle, gene targeting is mediated by the introduction of a donor vector containing homology sequence arms in order to mediate site-specific strand exchange. In model organisms, such as bacteria, and certain cell types, such as chicken DT40 cells or mouse ES cells, exhibit a relatively high homologous recombination efficiency that is practical enough

for laboratory use, although most human primary cells are not. In order to facilitate the delivery of the donor vector into human cells, several methods mediated by viral vectors have been developed historically; for example, integration-defective retroviral vectors, adenoviral vectors and AAV vectors.

BUSTA N. 3

Domanda: Estrazione di RNA da diverse matrici biologiche e analisi di espressione genica.

Bioinformatica: Che cos'è la pipeline per l'analisi genetica.

Inglese: Genome editing to treat haemophilia.
One of the disadvantages of in vivo and ex vivo gene therapy using viral and non-viral vectors for gene delivery is its insertion into any part of the genome, which can cause undesired mutations. Therefore, it is crucial to generate a strategy for site-specific gene integration into the genome. Currently, procedures using site-directed endonucleases performing specific breaks in the genome to integrate complete genes into safe sites of the genome are being conducted.
Correction of specific mutations or the insertion of complete genes is possible using site-oriented endonucleases. For several years, endonucleases, including zinc finger nucleases (ZFN), transcriptional activator-like nucleases (TALEN), and, more recently, the CRISPR/Cas9 system, have been used in gene therapy for hemophilia. These endonucleases break specific sites in DNA and activate intrinsic repair mechanisms in which desired sequences can be inserted or deleted. Different studies have reported successful in vitro correction of two of the main mutations that cause hemophilia A.

BUSTA N. 4

Domanda: Analisi di mutazioni genetiche mediante NGS.

Bioinformatica: Che cos'è il coverage del sequenziamento NGS?

Inglese: Gene Therapy for Hemophilia B.
Adeno-associated virus (AAV) is a non-pathogenic single-stranded DNA parvovirus that is naturally replication deficient in the absence of helper virus co-infection. Recombinant AAV (rAAV) vectors lack sequences encoding Rep, Cap, and AAP (for replication, capsid structure, and promoting capsid assembly, respectively) and generally elicit only mild and transient innate immune responses compared to other viral vectors. Also, AAV integration into the host genome occurs inefficiently in the absence of the rep gene, reducing risks of genotoxicity. Efficient in vivo gene transfer, various viral capsids with strong tropism for the liver, and the ability to confer long-term gene expression in hepatocytes has made AAV the vector of choice for most hemophilia gene therapy clinical trials.
Currently, AAV vectors are produced using two vastly different manufacturing platforms: HEK293 cells or Sf9 insect cell-based baculovirus expression vector system. The vector in all current clinical trials is given intravenously, and the transgene is expressed from a liver-specific promoter.

BUSTA N. 5

Domanda: La piattaforma NGS: principali caratteristiche e caricamento dei campioni.

Bioinformatica: Cosa significa l'Allineamento delle reads.

Inglese: Whole blood flow cytometry.
Whole blood flow cytometric analysis of platelets that allows detailed evaluation of platelet function using multiple fluorochrome-antibody conjugates has gained attention in the recent years. Some of the advantages of this methodological approach include: minimal blood volume requirement (e.g. 5 uL); platelets are analyzed in their physiological environment with the presence of erythrocytes and leukocytes; minimal sample manipulation that may pre-activate or result in the loss of platelets; and the ability to determine both the activation state and reactivity of circulating platelets to agonists.
Due to its multiple advantages, flow cytometric analysis of platelet function has been widely used in multiple clinical settings. These include the diagnosis of inherited platelet disorders, and the measurement of circulating platelet activation and/or reactivity in acute myocardial infarction, diabetes mellitus, and pre-eclampsia. In addition, platelet flow cytometry can also be used to monitor disease progression and therapeutic interventions (e.g. anti-thrombotic agents), identify a patient's risk for thrombotic disorders in various clinical conditions and assess the quality of stored platelet concentrates.

BUSTA N. 6

Domanda: I vantaggi del sequenziamento NGS rispetto al sequenziamento Sanger.

Bioinformatica: Esempi di Software per l'analisi delle varianti genetiche.

Inglese: mRNA-Based Approaches to Treating Liver Diseases.
The potential efficacy of in vitro-transcribed mRNA used to treat liver diseases is based upon their ability to encode proteins that replace impaired hepatic functions using the translational machinery of the target cells, i.e., hepatocytes.
Therapeutic mRNA offers a distinct advantage over protein replacement or enzyme replacement therapy used to restore the functional proteins that are otherwise deficient or abnormal: mRNA delivered and expressed intracellularly allows post-translational modifications of the encoded protein by the host cells.
The utility of RNA expression constructs to stimulate protein production was first described in 1990 in mice injected intramuscularly. For decades since then, however, the use of RNA for therapy was considered impractical due to the following: (1) inherent instability and vulnerability to nuclease digestion, (2) tendency to induce inflammation and strong innate immune responses, and (3) inability to readily cross the cell membrane and enter the cytoplasm.
Recent technical advances that circumvent these obstacles have optimized mRNA molecules and maximized their therapeutic potential by engineering them to display low immunogenicity, prolonged stability, and potent translation efficiency.