Prova orale A

- 1. Ci parli della sua esperienza lavorativa, inerente le tematiche del bando.
- 2. Descriva i controlli qualitativi che si effettuano a valle della preparazione di una libreria di seguenziamento.
- 3. In Excel cosa permette di fare il comando "Filtro"?
- 4. Possono esserci, nella stessa cartella, due file con lo stesso nome?
- 5. Ci legga ad alta voce e traduca il testo evidenziato.

Library preparation is a key step in sequencing. For RNA sequencing there are advantages to both strand specificity and working with minute starting material, yet until recently there was no kit available enabling both. The Illumina TruSeq stranded mRNA Sample Preparation kit (TruSeq) requires abundant starting material while the Takara Bio SMART-Seq v4 Ultra Low Input RNA kit (V4) sacrifices strand specificity. The SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Pico) by Takara Bio claims to overcome these limitations. Comparative evaluation of these kits is important for selecting the appropriate protocol. We compared the three kits in a realistic differential expression analysis. We prepared and sequenced samples from two experimental conditions of biological interest with each of the three kits. We report differences between the kits at the level of differential gene expression; for example, the Pico kit results in 55% fewer differentially expressed genes than TruSeq. Nevertheless, the agreement of the observed enriched pathways suggests that comparable functional results can be obtained. In summary we conclude that the Pico kit sufficiently reproduces the results of the other kits at the level of pathway analysis while providing a combination of options that is not available in the other kits.

Prova orale B

- 1. Ci parli della sua esperienza lavorativa, inerente le tematiche del bando.
- 2. Descriva i controlli quantitativi che si effettuano a valle della preparazione di una libreria di sequenziamento.
- 3. Cosa permette di fare PowerPoint?
- 4. Cosa indica il campo "Ccn" nell'invio di una email?
- 5. Ci legga ad alta voce e traduca il testo evidenziato.

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RNA-Sequencing (RNA-Seq) analysis has become the *de facto* method for measuring gene expression genome wide. However, when designing an experiment, the investigator is faced with the task of making many decisions, including choice of platform and library preparation protocol, which can involve considerable trade-offs. We focus here on the Illumina and Takara Bio kits and investigate the differential effect of library prep protocol. The

Prova orale C

- 1. Ci parli della sua esperienza lavorativa, inerente le tematiche del bando.
- 2. Nello studio del trascrittoma, quali tipologie di librerie conosce?
- 3. In Word come posso creare una tabella?
- 4. Quali sono le estensioni più utilizzate per i file immagine?
- 5. Ci legga ad alta voce e traduca il testo evidenziato.

Abstract

Nearly all infectious agents contain DNA or RNA genomes, making sequencing an attractive approach for pathogen detection. The cost of high-throughput or next-generation sequencing has been reduced by several orders of magnitude since its advent in 2004, and it has emerged as an enabling technological platform for the detection and taxonomic characterization of microorganisms in clinical samples from patients. This review focuses on the application of untargeted metagenomic next-generation sequencing to the clinical diagnosis of infectious diseases, particularly in areas in which conventional diagnostic approaches have limitations. The review covers (a) next-generation sequencing technologies and common platforms, (b) next-generation sequencing assay workflows in the clinical microbiology laboratory, (c) bioinformatics analysis of metagenomic next-generation sequencing data, (d) validation and use of metagenomic next-generation sequencing infectious diseases, and (e) significant case reports and studies in this area. Next-generation sequencing is a new technology that has the promise to enhance our ability to diagnose, interrogate, and track infectious diseases.

Prova orale D

- 1. Ci parli della sua esperienza lavorativa, inerente le tematiche del bando.
- 2. Nello studio del genoma, quali tipologie di librerie conosce?
- 3. Cosa permette di fare Excel?
- 4. Nella posta elettronica cosa si intende per "spam"?
- 5. Ci legga ad alta voce e traduca il testo evidenziato.

INTRODUCTION

Overview of Next-Generation Sequencing

Next-generation sequencing (NGS), also termed high-throughput or massively parallel sequencing, is a genre of technologies that allows for thousands to billions of DNA fragments to be simultaneously and independently sequenced. The applications of NGS in clinical microbiological testing are manifold and include metagenomic NGS (mNGS), which allows for an unbiased approach to the detection of pathogens. This review focuses on using mNGS methods to identify pathogens directly from clinical samples from patients (1–13). Untargeted mNGS approaches use what is known as shotgun sequencing of clinical samples or pure microbial cultures in which random samples of analyte DNA or RNA are surveyed en masse, in contrast to targeted approaches that utilize singleplex or multiplex polymerase