All. 1

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Elenco di domande per colloquio orale n.1

1) Quale tecnica suggerirebbe per valutare l'interazione tra un recettore di membrana cellulare con una libreria di piccole molecole e perché.
2) Quali sono le principali criticità nella gestione di una facility multiutenza?

Accertamento lingua inglese (leggere e tradurre):

Beginning sometime in the late 1980s a combination of technological improvements made it possible to screen compounds in a kind of automated, industrialized way. Rather than assaying just a few compounds at a time by hand, robotic systems became available that were designed to operate on 96-well plates, often measuring fluorescence as the readout. In this format, the first and last columns on the plate are usually reserved for controls, so that 80 new data points per plate can be obtained. Steady improvements in speed, reliability, and accuracy were followed by further miniaturization so that eventually 384- and even 1536-well plates could be used. Proponents and proud managers could, and did, toss around ever-increasing numbers of compounds that could be or that had been screened.

Selezione pubblica n. 2024N9

Elenco di domande per colloquio orale n.2

1) Che strategia suggerirebbe per uno screening di una libreria di piccole molecole per identificare un inibitore di un enzima con attività proteasica?
2) Nell'eventualità che la presenza del tecnico non sia richiesta dagli utenti, quali sono le necessarie procedure organizzative e gestionali per l'uso della strumentazione in una facility di ricerca?

Accertamento lingua inglese (leggere e tradurre):

In the technique called fluorescence polarization (FP), polarized light is used to excite the fluorophore. If fluorophore isn't bound to a receptor but is free in solution, it tends to be tumbling rapidly and fluorescence emission will therefore have low polarization. But binding to a macromolecular target will reduce the rotation rate and increase the measured polarization. Fluorophores based on the fluorescein or BODIPY, linked to ligands or substrates are frequently used. In this way, for example, a fluorescein-labelled DNA substrate was used to establish an HTS assay to identify small-molecules interfering with its interaction with the potential anti-cancer target, replication protein A (RPA), which is involved in nucleotide excision repair.

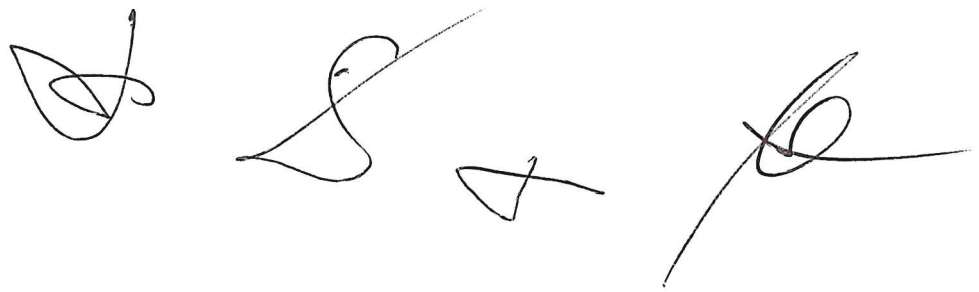
Selezione pubblica n. 2024N9

Elenco di domande per colloquio orale n.3

1) Che strategia suggerirebbe per uno screening di una libreria di piccole molecole per inibire un'interazione proteina-proteina?
2) Come organizzerebbe la procedura di abilitazione alla strumentazione a utenti in modo che possano adoperare la strumentazione in modo autonomo?

Accertamento lingua inglese (leggere e tradurre):

In fluorescence resonance energy transfer, also called Forster resonance energy transfer (FRET), an excited fluorophore in one part of a molecule (the donor) has its fluorescence internally transferred to a second label attached somewhere else on the molecule (the acceptor). The acceptor is often fluorescent itself, with its emission at a longer wavelength than that of the donor so that the two are easy to tell apart. As long as the donor and acceptor are located within 10–75 angstroms of each other the transfer is efficient and relatively little donor fluorescence will be detectable. But if cleavage occurs anywhere in between, as in Figure 6.6, where the ring-opening action of the bacterial enzyme b-lactamase has separated the two, donor fluorescence will be observed. In this case, observing the change in fluorescence emission from 520nM to 447nM would indicate cellular expression of the enzyme, a reporter gene of interest for cellular high-throughput screens.



Selezione pubblica n. 2024N9

Elenco di domande per colloquio orale n.4

1) Nella progettazione di una campagna di screening basata sulla fluorescenza quali tipologie di misure suggerirebbe per ottenere un saggio il più affidabile possibile?
2) Se la facility possedesse delle librerie di composti da screening, come gestirebbe l'utilizzo della libreria quando richiesta dagli utenti nelle loro campagne di screening?

Accertamento lingua inglese (leggere e tradurre):

Another bead-based method for detecting molecular interactions that doesn't involve radioisotopes is called AlphaScreen, which stands for Amplified Luminescent Proximity Homogeneous Assay. This uses two specialized types of bead, a donor and an acceptor. The donor bead, to which one molecule is conjugated, contains a photosensitizer (phthalocyanine) that produces large amounts of singlet oxygen upon laser excitation. Because $^1O_2$ has a very short lifetime, it can diffuse out only about 200 nM, so an acceptor bead, to which the second component is conjugated, will emit light if and only if it's been drawn close to the donor bead by binding interactions between the two components, as shown in Figure 6.8. This technique has found broad applicability to areas like second messengers, kinases, and protein–protein interactions.

Selezione pubblica n. 2024N9

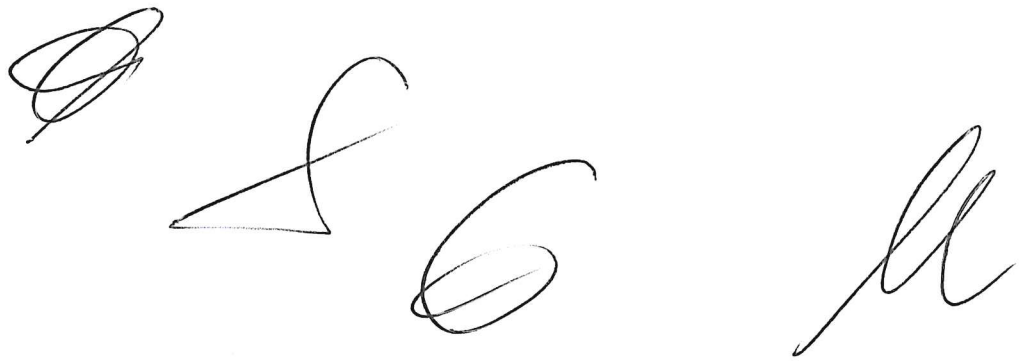
Elenco di domande per colloquio orale n.5

1) Nella progettazione di una strategia di screening di una libreria di piccole molecole basata su due tecniche ortogonali fra loro (un saggio primario e un saggio di conferma secondario) quali aspetti considererebbe importanti e perché?
2) In caso di un'alta richiesta per l'utilizzo di una specifica strumentazione, che misure adotterebbe per un equo e giusto utilizzo da parte degli utenti?

Accertamento lingua inglese (leggere e tradurre):

The publication of Lipinski's 'Rule of Five' (ROF) without a doubt improved screening libraries in a major way. Most libraries nowadays are designed from the outset to be "rule of five compliant". Gone are the days when a combi compound might have two n-octyl groups and a MW of 700 Da. Without the emphasis Lipinski's rule placed on small, reasonable molecules, the screening of combi libraries would have resulted in many fewer clinical candidates than it has to date.

One needs to keep in mind, though, that there are cases where the famous rule admittedly doesn't apply, is too strict, or isn't strict enough. Natural products, as we'll see, have always been given a free-pass based on the empirical observation that some of them violate the rule but are still orally bioavailable. It wouldn't be appropriate to deselect compounds from natural product libraries because they violate the rule of five.



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Elenco di domande per colloquio orale n.6

1) Che strategia suggerirebbe per uno screening di una libreria di piccole molecole per identificare inibitori ATP-competitivi in una protein-chinasi?
2) In una facility che produce molti dati digitali che procedura suggerirebbe per la conservazione e condivisione dei risultati con gli utenti?

Accertamento lingua inglese (leggere e tradurre):

A study at Novartis compared hits obtained by screening 30,000 compounds for tyrosine kinase activity in three different assays: a scintillation proximity assay (SPA), a homogeneous time-resolved fluorescence energy transfer assay (HT-FRET), and a fluorescence polarization (FP) assay. Library compounds were screened as mixtures of five in a well, which meant that after active wells were identified, the five constituents would be deconvoluted by screening each compound individually or using other methods to establish which of the compounds was active. When this was done SPA, HT-FRET, and FP turned up 30, 59, and 64 hits respectively. A low degree of overlap was observed between the sets. Only four or seven compounds, depending on where the activity bars were set, would have been identified as hits in all three assays, had they been run independently.