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Poster Presentation

Biochemical assay development for drug discovery: a sequential optimization from protein expression to enzymatic activity

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Background

The drug discovery process based on high-throughput screening (HTS) requires highly demanding efforts to comply with the strict criteria of homogeneity, sensitivity, processivity, reproducibility and miniaturization. For enzymatic targets, the ability to screen in parallel multiple parameters is a prerequisite to identify the permissive conditions to develop functional assays and to upscale the protein production to entirely support an HTS campaign, which generally requires several milligrams of pure protein.

Results

To fulfill the requirements of the enzymatic assay configuration for HTS, we have developed a streamlined process for the sequential optimization of recombinant expression, purification and activity of therapeutically-relevant enzymes, applying automation and miniaturization.

Concerning recombinant expression, we routinely challenge in parallel three to five chimerical tagged versions of the target enzyme in 4 different insect cell lines. Insect cell cultures have been optimized to effectively support recombinant protein expression in 24-deep-well format, allowing the simultaneous screening of 36 different conditions for each version of the target.

Tag-based purification by affinity chromatography undergoes a robot-assisted optimization in 96-well format, where 8–16 different buffer conditions are screened in parallel. Selection of the best-supporting purification conditions is determined by integrating data on specific activity of the enzyme, degree of purity and final production yield. The identified parameters for expression and purification are applied to a large-scale FPLC-based production, which usually provide the entire amount of enzyme requested for the assay development.

The enzymatic activity is optimized by screening in 384-well format pre-assembled plates covering over 250 different conditions using HTS-compatible fluorescent or luminescent readout. In particular, 18 buffers ranging from pH 4.5 to 9.5, 20 monovalent and divalent cations, and 40 additives, including detergents, reducing agents, chelators, organic solvents, stabilizers, and phospholipids, are challenged in a dose-dependent manner, to determine the best working conditions for the target enzyme.

Conclusion

The overall optimization on protein expression, purification and enzymatic activity for potential drug targets tests over 800 different conditions, with dramatic impact on the final assay configuration. We can demonstrate that the specific activity of the enzyme is increased on average by 200-fold at the end of the process, with cases reaching up to 600-fold. This result has two direct valuable consequences. First, the assay is configured with maximally active enzyme preparations, which is considered a hall-

mark for potentially successful screenings. Second, the enzyme concentration in the reaction is reduced at its minimal amount, keeping unaltered the robustness of the assay. This implies that the sensitivity to potential inhibitors is significantly increased, and screening of large libraries with difficult-to-express enzymes is made realistic.