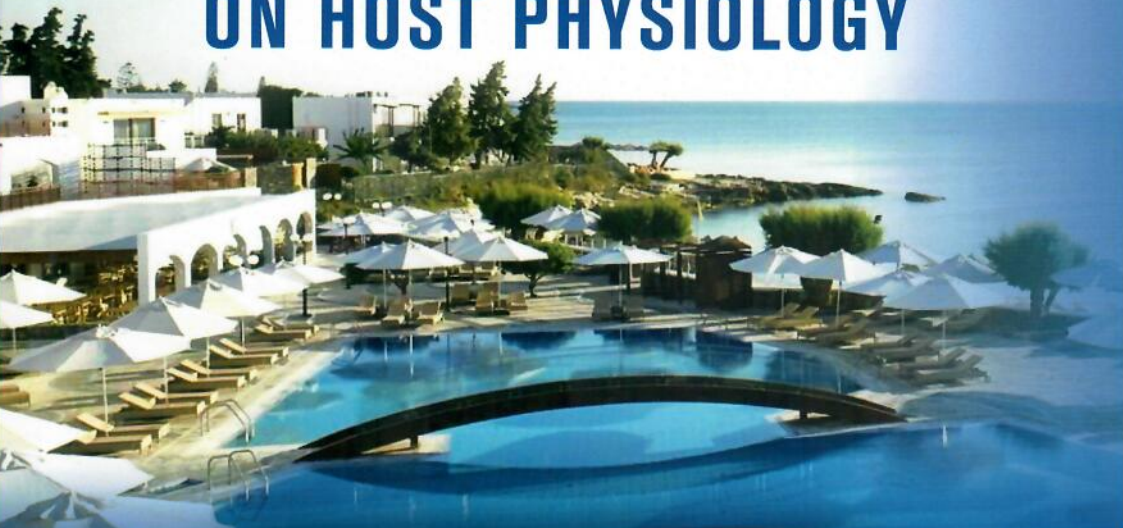
RPP10

A COMPARATIVE VIEW ON HOST PHYSIOLOGY



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Quality control of recombinant proteins: from molecular design to application

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The obtainment of high-quality pure protein samples is a major goal in recombinant protein production. Indeed, correct execution and interpretation of biophysical/structural assays relies on the use of soluble and homogeneous pure protein samples, natively active at well-known concentrations in suitable buffers [1]. However, quality control is very often overlooked, while it is essential for a rational scale-up and successful downstream application. The quality level of a recombinant product depends on the production process, which is determined by the host, and, principally, on the purification procedure [1]. Thus, it is fundamental to design the purification strategy from the very beginning of the molecular cloning project. In this respect, protein fusion technology is a valuable toolbox that can be considered to increase yield and purity. To validate the production and purification process, it is mandatory to assess, by well-established techniques, protein quality in terms of purity (e.g. denaturing protein gel electrophoresis, SDS-PAGE), homogeneity (e.g. dynamic light scattering, DLS; size exclusion chromatography, SEC), and accurate concentration (e.g. UV spectroscopy) [1]. This presentation will focus on the production and quality control in *Escherichia coli* of two difficult-to-express proteins of biomedical interest, the plant lectin frutalin [2] and human P53 [3], which were selected as examples. The distinctiveness of the production and purification strategy to obtain high yields of pure frutalin and P53 mutants will be discussed, along with their quality evaluation through the above mentioned techniques.

References: [1] Oliveira C. & Domingues L. (2018) Guidelines to reach high-quality purified recombinant proteins. Applied Microbiology and Biotechnology, 102: 81-92. [2] Oliveira C., Teixeira J.A., Domingues L. (2014) Recombinant production of plant lectins in microbial systems for biomedical application – the frutalin case study. Frontiers in Plant Science, 5(390). [3] Gomes A.S., Trovão F., Andrade Pinheiro B., Freire F., Gomes S., Oliveira C., Domingues L., Romão M.J., Saraiva L., Carvalho A.L. (2018) The crystal structure of the R280K mutant of human p53 explains the loss of DNA binding. International Journal of Molecular Sciences, 19(4): 1184.

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