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Rational design for further advancement of *E. coli* expression systems

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In view of a continuously growing number of hosts used for production of recombinant proteins *E. coli* is still one of the most frequently used microbial expression systems. Beside its approved regulatory status, this is mainly due to continuous advancements in host cell and process engineering. The rapidly growing knowledge on *E. coli´s* cell physiology and new molecular technologies represent the basis for rational and efficient molecular modification. Consequently we see increasing product yields, improved quality and a steadily expanding range of proteins which can economically be produced with *E. coli*. However, there is still potential for further improvements especially in context with the sy nchronization/ harmonization of host cell capabilities, recombinant protein production and fermentation process design. Another, c urrently not sufficiently considered field is the negative impact of supplementary elements in expression systems on product yield and quality. For instance, the constitutive expressions of antibiotic resistance mediating proteins (beta-lactamase or neomycin phosphotransferase II) are not even required during production. Unrecognized malfunctions in systems, for instance limited termination efficiency to read-through transcription and additional metabolic load on host cells. Open to question is also the fact that systems equipped with a broad set of special functions (folding chaperones, rare tRNAs, inhibitors etc.) are used in a preventive unquestioned way even though these functions are not required for production of the target protein. We propose to reduce the recombinant system to the absolute required minimum in order to avoid nonessential burden in expression systems and to follow a minimal invasive host cell design approach. .

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