

Appendix 8.

Protocol used for the experimental verification of putative strong promoters in *Thermotoga maritima* genome

In the context of a former study, the activity of thirteen putative strong promoters harbouring an UP element has been measured in *E. coli* cell free extracts (Sakanyan *et al.*, 2003). The promoters were fused to the *Bacillus stearothermophilus argC* reporter-gene, which codes for N-acetyl glutamylphosphate reductase. Then, ArgC synthesis was performed in the coupled *E. coli* transcription-translation system. The Ptac promoter was used as a reference to compare protein yields. It is a strong hybrid promoter consisting of the -35 region of the *trp* promoter and the -10 region of the *lacUV5* promoter/operator (de Boer *et al.*, 1983). In all assays, the optimal Shine-Dalgarno sequence GGAGG was associated to the promoter.

References

de Boer, H.A., Comstock, L.J. and Vasser, M. (1983) The tac promoter: a functional hybrid derived from the trp and lac promoters. *Proc. Natl. Acad. Sci. USA.*, **80**, 21-25.