Screening through the PLICable promoter toolbox enhances protein production in *Escherichia coli*

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The PLICable pET-promoter toolbox enables with a decent throughput and minimized experimental efforts increased functional protein production in E. coli.

Escherichia coli is a common host for recombinant protein production in which production titers are highly dependent on the employed expression system. Promoters are thereby a key element to control gene expression levels. In this study, a novel PLICable promoter toolbox was developed which enables in a single cloning step and after a screening experiment to identify out of ten IPTG-inducible promoters (T7, A3, *lpp*, *tac*, *pac*, Sp6, *lac*, *npr*, *trc* and *syn*) the most suitable one for high level protein production (Figure 1). The target gene is cloned under the control of different promoters in a single and efficient cloning step using the ligase-free cloning method PLICing (phosphorothioate-based ligase-independent gene cloning). The promoter toolbox was firstly validated using three well producible proteins (a cellulase from a metagenome library, a phytase from *Yersinia mollaretii* and an alcohol dehydrogenase from *Pseudomonas putida*) and then applied to two enzymes (3D1 DNA polymerase and glutamate dehydrogenase mutant) which are poorly produced in *E. coli*. By applying our PLICable pET-promoter toolbox, we were able to increase production by 2 fold for 3D1 DNA polymerase (*lac* promoter) and 29 fold for glutamate dehydrogenase mutant H52Y (*trc* promoter).

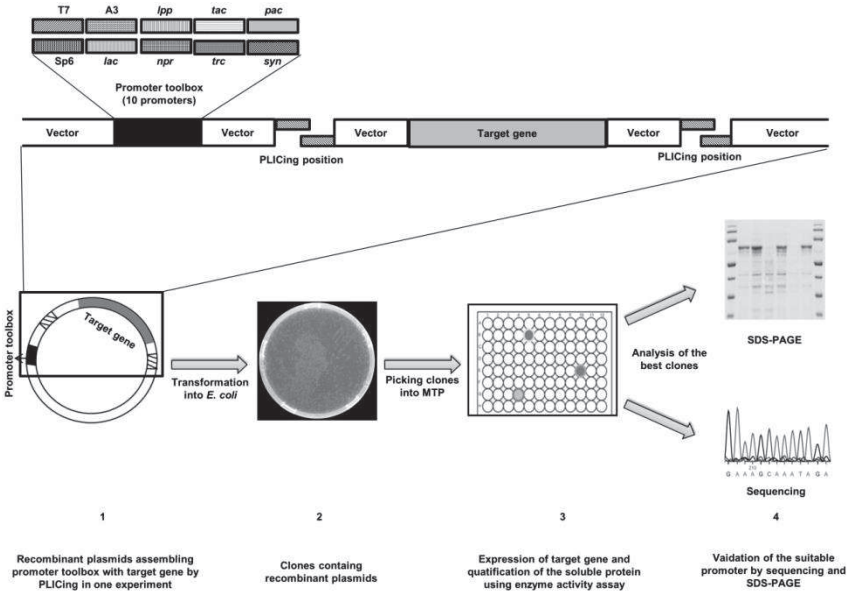

Figure 1 Construction and validation of PLICable pET-promoter toolbox. (1) Cloning of 10 IPTG-inducible promoters (T7, A3, *lpp*, *tac*, *pac*, Sp6, *lac*, *npr*, *trc* and *syn*) upstream different target genes (*celA2*: cellulase gene from a metagenome library, *phy*: phytase gene of *Yersinia mollaretti*, *xylB*: alcohol dehydrogenase gene of *Pseudomonas putida*, DNA polymerase gene (*3D1*) generated by recombining different DNA polymerases from *Thermus aquaticus*, *Thermus thermophiles*, *Thermus flavus* by staggered extension process, and codon optimized GDH gene mutant (*gdh mutant*) from bovine brain) by PLICing. (2) Transformation of plasmids into *E. coli*. (3) Transfer of colonies and expression in 96-well MTP followed by quantification of the soluble protein production amounts by enzyme activity. (4) Analysis of clones which showed high protein production by sequencing the promoter as well as determination of soluble protein production levels by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).