A plasmid vector for isolation of strong promoters in Escherichia coli.

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In order to isolate very strong promoters from bacteria and bacteriophage a plasmid named pProm was constructed. It possesses an origin (ORI) for replication in Gram-negative bacteria, an ORI for replication in Gram-positive bacteria, a promoterless ampicillin resistance gene with a multiple cloning site (MCS) in the position formerly occupied by the ampicillin promoter, a tetracycline resistance gene for selection in Gram-negative bacteria and a chloramphenicol resistance gene for selection in Gram-positive bacteria. Insertion in the MCS of DNA fragments of Staphylococcus aureus bacteriophages resulted in isolation of several clones very resistant to ampicillin. The DNA fragments inserted in these recombinant plasmids were sequenced and all of them contained putative promoter motifs. Direct measurement of the penicillinase activity indicated that one of the isolated promoters could be included within a group of the stronger known prokaryotic promoters. According to these results pProm is a powerful tool to perform studies on promoter strength and for industrial applications.