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Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability

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We have constructed pCL1920 and pCL1921, a pair of low copy number plasmids which contain a 580 bp *Bst*UI fragment that carries the *lac* promoter/operator, multiple cloning sites and *lacZ* fragment of pUC19 (1) cloned in place of the polylinker region in pGB2 (2), a pSC101 derived plasmid which confers spectinomycin (50 μg/ml) and streptomycin (100 μg/ml) resistance in *Escherichia coli*. All multiple cloning sites indicated are unique except for an additional *Eco*RI site as shown in the figure. pCL1920 and pCL1921 contain the *Bst*UI fragment in opposite orientations with respect to the pGB2 sequences. In the absence of inducer the pCL1920/21 vectors do not produce detectable levels of β-galactosidase in JM105 (*lacI*^{q} *lacZ*ΔM15) (1) cells (less than 2 Miller units) (3). In the presence of 2 mM IPTG (isopropyl-β-D-thiogalactopyranoside) the β- galactosidase levels of the pCL1920/21 [JM105] transformants rose to 11 units, while the pUC19 [JM105] transformants produced 470 units; a 43 fold increase. These results are consistent with the expected 40 fold difference in plasmid copy number between pCL1920/21 (5 copies per cell) compared to that of the pUC vectors (200 copies per cell). Thus the pCL1920 and pCL1921 vectors allow regulated low-level expression of genes inserted downstream of the *lac* promoter-operator when transformed into strains containing the *lacI*^{q} gene. They should also be useful for cloning genes which may be deleterious at high copy number. Since the pCL1920/21 vectors are compatible with ColE1 derived plasmids they can be used to form stable co-transformants together with pBR322 or pUC derived plasmids. For blue/white screening of inserts competent host cells with the *lacZ*ΔM15 gene are used, and the transformation mixture is plated on LB, spectinomycin plates pre-spread with 5 μl of 0.2 M IPTG and 25 μl 40 mg/ml X-gal (5-bromo-4-chloro-indolyl-β-D-galactopyranoside) per plate. We will provide these plasmids in CL83, a recA$^-$ (*recA*56) derivative of JM83 (1).

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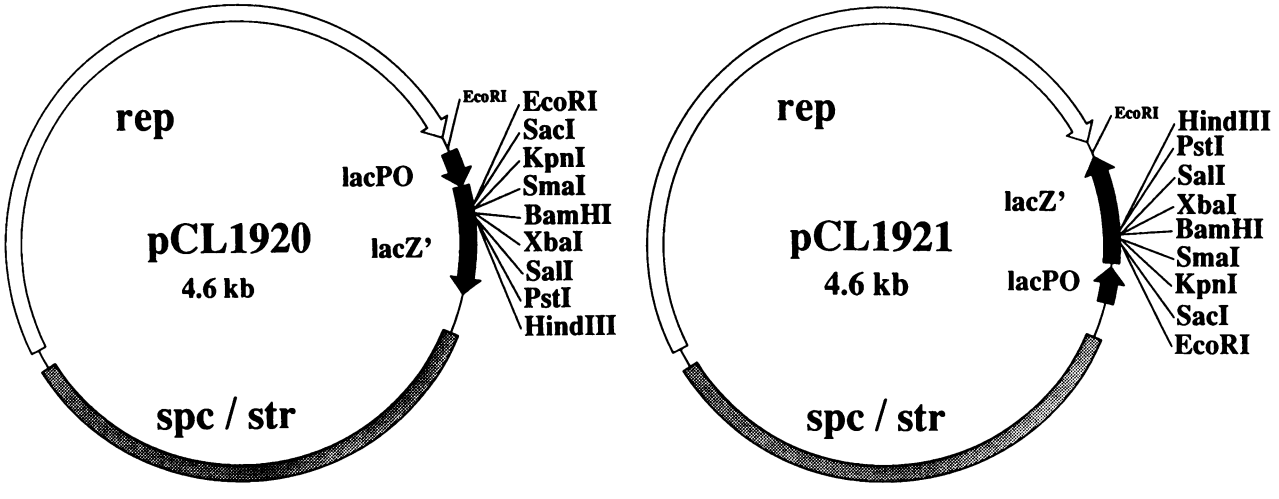

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