

Construction of plasmids that express *E.coli* β -galactosidase in mammalian cells

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We have constructed pUC19 based plasmids capable of expressing *E.coli* beta-galactosidase (β -Gal) under the control of different viral promoters in mammalian cells. In addition, each vector contains an RNA splice signal, a polyadenylation signal and a unique *Not* I site from which the β -Gal gene may be excised and into which alternative DNA coding sequences may be cloned.

These vectors generated between 7.3 u/mg cell extract (see (1) for unit def.) (pAd β) and 2530.0 u (pCMV β) of β -Gal activity following transfection into human 293 cells and between 11.8 u (pAd β) and 116.1 u (pSV β) following electroporation of human Raji lymphoblastoid cells.

These vectors have utility - as reference plasmids when transfecting other reporter gene constructs (1); in optimising conditions for electroporation of cells and analysis of gene expression *in vivo* when used in conjunction with a histochemical stain (2); as "enhancer-trap" vectors and in analysing the effect of *cis* acting elements and *trans* acting factors on these and alternative promoter elements when used in conjunction with a viable stain for β -Gal (3) and a fluorescence activated cell sorter (FACS).

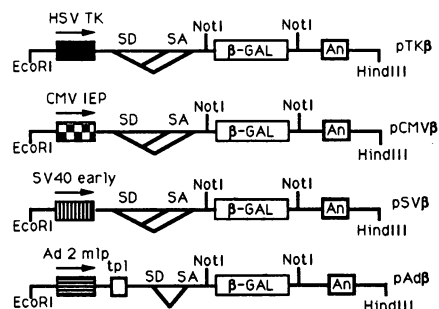


Fig.1 β -Gal expression vectors.

Key : HSV TK - Herpes simplex virus thymidine kinase promoter. CMV IEP- human cytomegalovirus immediate early promoter / enhancer. SV40 early - SV40 early promoter / origin. Ad2mlp- Adenovirus 2 major late promoter. tpl- fused tripartite leader. An- SV40 late polyadenylation signal. SD/ SA- RNA splice donor and acceptor sequence. β -Gal- *E.coli* beta-galactosidase gene. All vectors are based in pUC19. The figure shows the polylinker region of the vector only.

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References

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