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# MTX5: a cell line expressing biologically active HSV-1 Vmw65 protein

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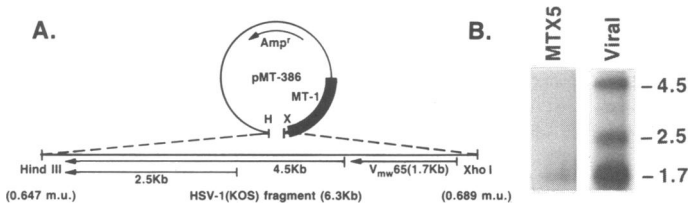
We report the construction of a cell line expressing the HSV-1 trans -acting virion factor, Vmw65. A 6.3 Kb DNA fragment containing the Vmw65 gene and its promoter (1) was cloned 3' of the mouse MT-1 gene enhancer creating plasmid pMT-386 (Fig. 1A). L Tk- cells were cotransformed with pMT-386 and pDG504 (HSV-2 Tk gene; 2). Northern blot analysis of Tk+ clones revealed that MTX5 cells encode a 1.7 Kb RNA species, similar in size and sequence to viral Vmw65 mRNA (relative abundancy, Fig. 1B; probe = pMT-386 viral DNA). Determination of Vmw65 biological activity used plasmid constructs containing Vmw65-responsive (HSV ICP4; 3) or Vmw65-nonresponsive (HSV Tk; 4) promoter regions fused immediately 5' of the bacterial lac Z gene. Detection of beta galactosidase activity in transfected cells required the activation of plasmid-borne promoters. The level of ICP4 promoter activity (pON 105) in transfected MTX5 cells was 60 fold higher than background and was not increased by HSV infection. The specificity of trans-activation was validated by the absence of Tk promoter activity (pON 245) in uninfected MTX5 cells. The use of the MTX5 cell line provides an effective, virus-free method for obtaining high levels of HSV alpha gene expression.

| DNA transfected | promoter present | Relative beta galactosidase levels cell line | | |
|---|---|---|---|---|
| | | L Tk- | MTX5 | MTX5(+HSV-2) |
| pON 105 | ICP4 | 1 | 60 | 60 |
| pON 245 | Tk | 1 | 1 | 80 |
| pON 1 | none | 1 | 1 | 1 |

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