DNA inserted two bases down from the initiation site of a SP6 polymerase transcription vector is transcribed efficiently *in vitro*

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We describe here a SP6 RNA polymerase transcription vector that can be used for synthesis of RNAs containing, at most, two extra G's at the 5' end. A plasmid designated pJMSP-0 was obtained by insertion of a *NaeI*/*SacI* pGEM3 (Promega-Biotec) fragment between the *AvaII* and *SacI* sites of the M13 vector mp19. Deletion of 15 bases in the multiple cloning region of pJMSP-0 by *S1* nuclease treatment, generated a plasmid designated pJMSP-8 containing the new *StuI* site (Fig.1A). When several DNA sequences were introduced at this site, transcription proceeded at a rate comparable to transcription from pJMSP-0 and pJMSP-8 (Fig.1B). Many viral RNA genomes possess a 5' terminal G, and for these, transcripts with but a single extraneous 5' G can be made readily. It was found previously that the presence of a longer heterologous 5' proximal sequence can greatly diminish the biological activity of transcribed viral RNAs (2,3). This research was supported by USDA grant 86-CRCR-1-1940 and NIH grants A101466, A115342, AI21942, and AI23742. We thank Ola Dzionott-Bujarska for excellent technical assistance.

Figure 1. A. Polylinker sequences in original pGEM3 (and pJMSP-0) and in the new pJMSP-8 plasmids. Vector sequences are in bold letters; the first transcribed base is underlined. B. Electrophoretic analysis (1.5% agarose) of SP6 RNA polymerase transcripts (their 5' end sequences are shown in brackets): lane 1. Plasmid pJMSP-8, linearized with *BglII* (GGCCTGC); lane 2. Plasmid pJMSP-0, linearized with *BglII* (GAATACA); lanes 3-6. Plasmid pJMSP-8, having, respectively, the following brome mosaic virus cDNA1 (4) fragments inserted between the *StuI*-*EcoRI* sites and linearized with *EcoRI*: *SnaBI*/*EcoRI* (GGGTAGG), *EcoRV*/*EcoRI* (GGATCAC), *BalI*/*EcoRI* (GGCCAAC) and *BclI*/*EcoRI* (GGGATCA).

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