

SP6 CELL-FREE TRANSCRIPTION

1.1 Introduction

This is a method for preparing RNA transcripts of cloned DNA behind the SP6 promoter. SP6 is a Salmonella phage whose polymerase is found to be very active in vitro and will only transcribe off its own promoter. The promoter has been cloned and the polymerase is commercially available, making it a powerful system for preparing artificial mRNAs and RNA hybridization probes. For translation purposes, transcript is often prepared in the presence of a capped nucleotide analog in order to generate capped transcript that will be used with higher efficiency for translation both in vitro and in vivo. For transcripts to be used in the wheat germ system, capped mRNA have been shown to translate more efficiently, therefore, both capped and uncapped G nucleotides have been optimized to allow maximum initiation with the capped analog with enough uncapped present to allow elongation of transcripts (capped analog can only be incorporated in the initial position, since it has no free 5' position). For translations in the rabbit reticulocyte lysate the cap-structure is not necessary. The T7 phage polymerase and promoter represent a similar system reputed to be even more active. However, since we have hundreds of SP6 plasmids, we have opted to stick with SP6.

The transcription conditions have recently (Sept. 1991) been reviewed and revised based on the publication by Gurevich et al. (1991) *Anal. Biochem.* 195, 207-213. Note that there are different CB5XSP6T1 for WG and RRL transcripts.