

Figure 33. Northern analysis of RNA from 0-24 h of development using the *actin* probe. Lane 1-7 (A) contained 10 micrograms of RNA from 0, 4, 8, 12, 16, 20 and 24 h, respectively. The blot was first probed with *5NU*, stripped and reprobated with *actin* probe. The result shows that mRNA was not degraded from any stage of development. Figure 33B shows the expression level normalized to rRNA bands.

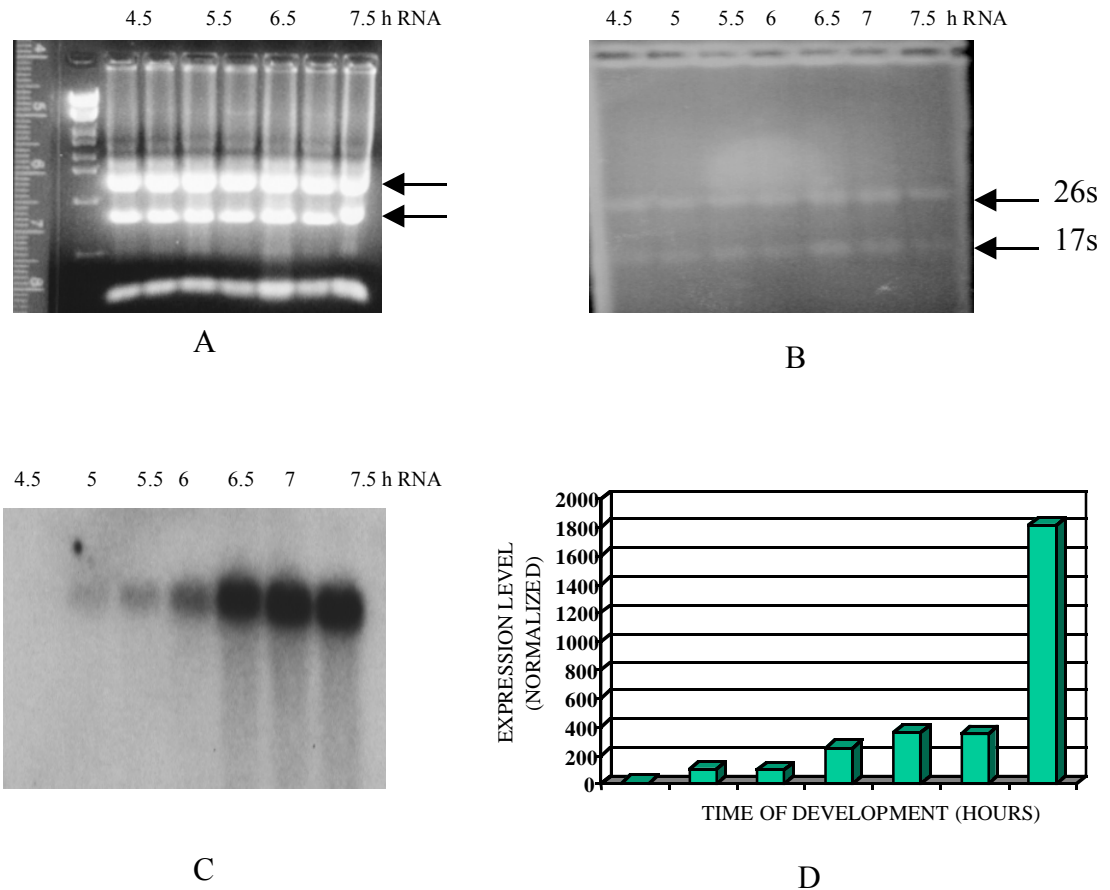


Figure 34. Northern analysis using *5NU* as the probe. Lane 1-7 contained 10 micrograms RNA of 4.5, 5, 5.5, 6, 6.5, 7 and 7.5 h of development, respectively. Figure 34A shows RNA on 1% TBE agarose gel. Figure 34B shows the same RNA on the formaldehyde gel. 17s and 26s rRNA were seen on both gels. Figure 34C shows an autoradiography of RNA with the *5NU* probe. Figure 34D shows the expression level normalized to rRNA bands.

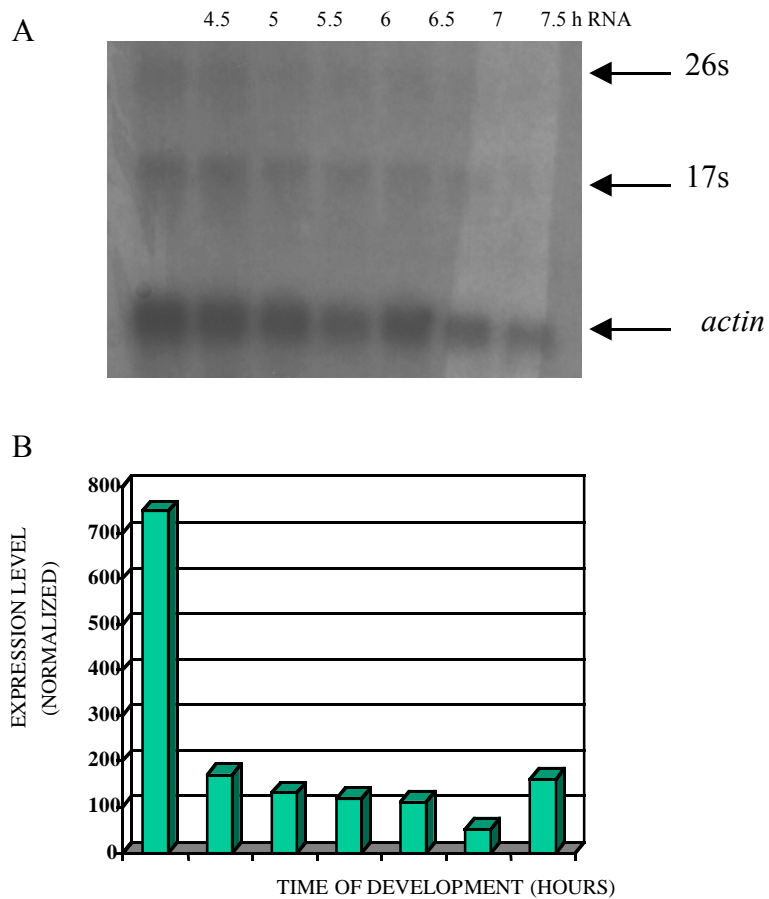


Figure 35. Northern analysis with an *actin* probe. The blot was the same as the one hybridized with *5NU* probe. The blot was stripped and reprobated with *actin* to see the abundance of genes on the same blot. Lane 1-7 (A) contained RNA of 4.5, 5, 5.5, 6, 6.5, 7 and 7.5 h of development. Figure 35B shows the expression level normalized to rRNA bands.

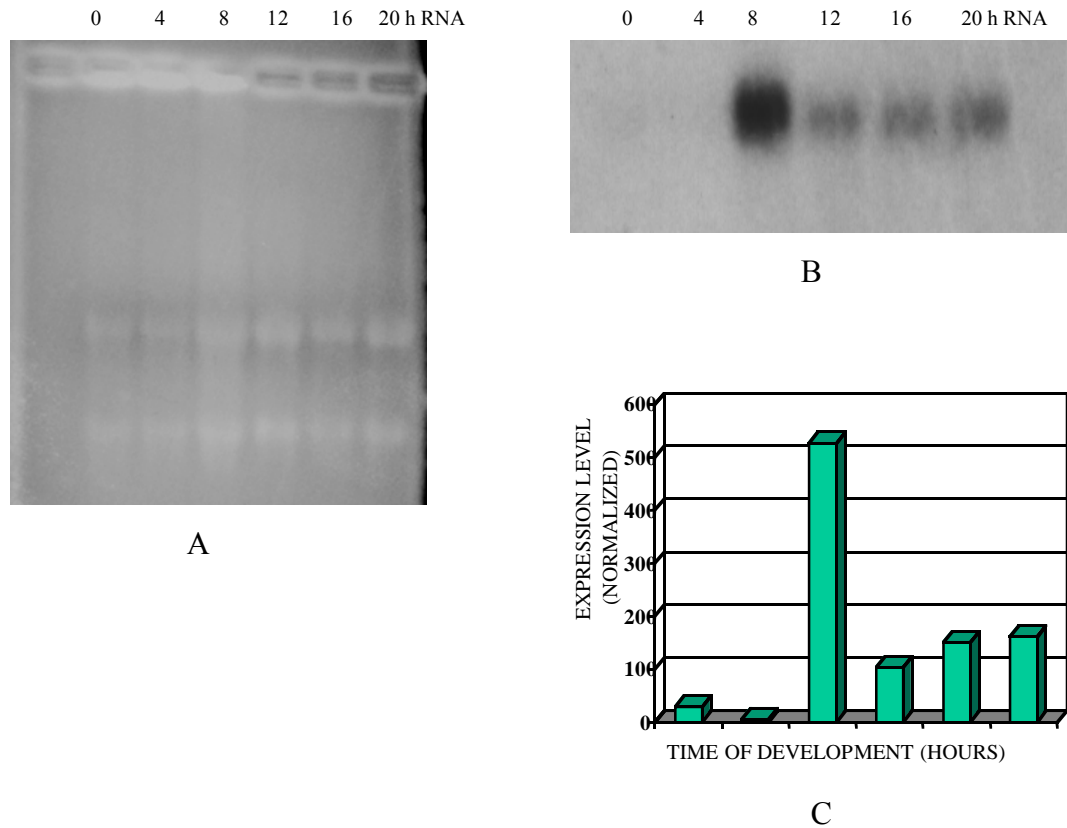


Figure 36. Northern analysis of RNA from 0-20 h of development with a Transcription Factor II probe. Lane 1-6 contained 10 micrograms RNA of 0, 4, 8, 12, 16 and 20 h of development, respectively. Figure 36A shows RNA on a formaldehyde gel after stained with EtBr. 17s and 26s rRNA was seen on the gel. Figure 36B shows the hybridized band of RNA with *TFII* probe after overnight exposure. The results show that the *TFII* gene is developmentally regulated. Figure 36C shows the expression level normalized to rRNA bands.

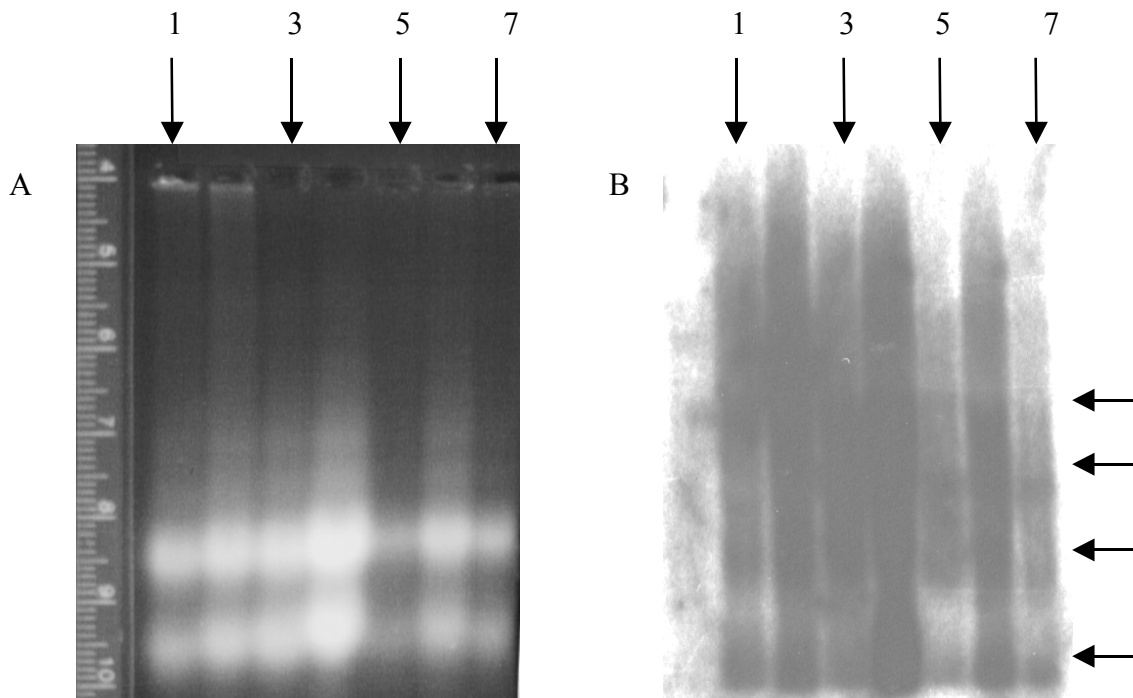


Figure 37. Northern analysis of RNA from developmental stages with *AP* probe. Lane 1-7 contained 10 mg RNA from 0, 4, 8, 12, 16, 20 and 24 h of development. Figure 37A showed two rRNA bands of 17s and 26s on the formaldehyde gel after staining with EtBr. Figure 37B showed 4 hybridized RNA bands with *AP*. The top two bands are probably rRNA of 17s and 26s. The bands at the bottom are putative *AP* mRNA. The result shows that *AP* is expressed at all stages of development.

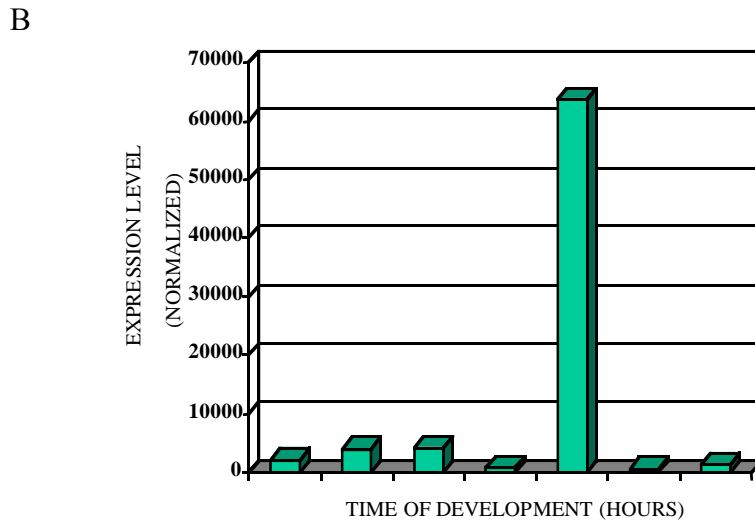
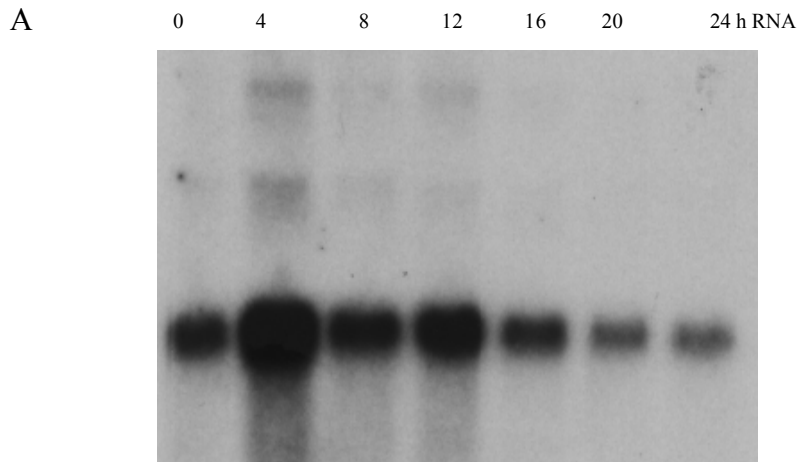
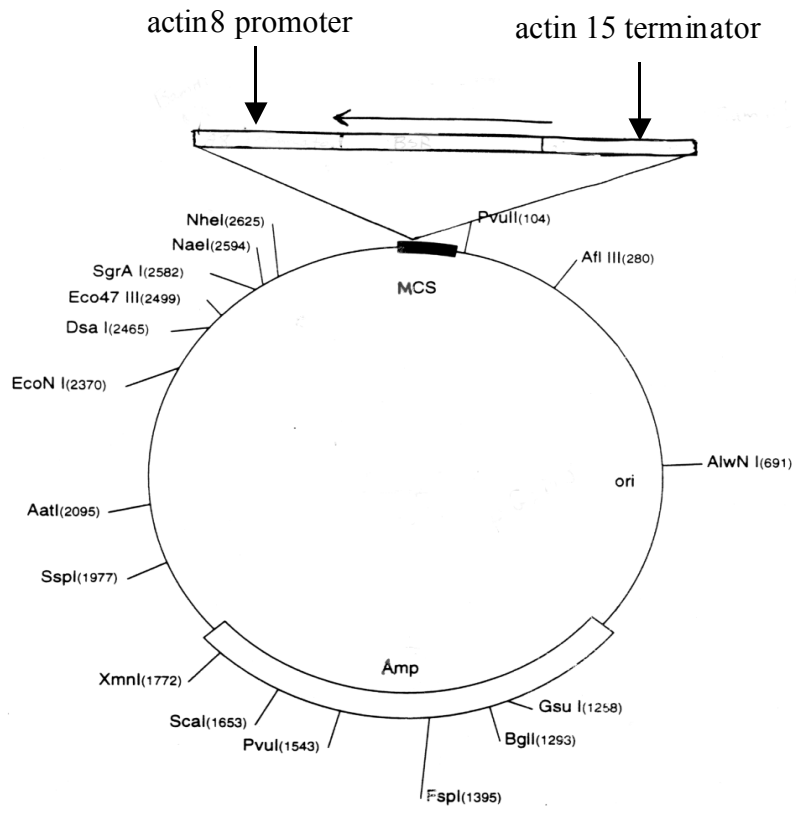


Figure 38. Northern analysis of developmental RNA with *actin* probe. Lane 1-7 (A) contained 10 micrograms RNA of 0, 4, 8, 12, 16, 20 and 24 h of development. The blot was first probed with *AP* and then reprobated with *actin* probe. Figure 38B shows the expression level normalized to rRNA bands.



MCS:

ATTTA GGTGA CACTA TAGAA TACAA GCTTGCATGC CTGCA GGTCG ACTCT

SP6 Promoter



AGAGG ATCCC CGGGT ACCGA GCTCG AATTC CGGTC TCCCT ATAGT GAGTC

GTATT A

T7 Promoter

restriction sites: HindIII 7; SphI 17; PstI 23; Sall 25; Accl 26; HincII 27; XbaI 32; BamHI 37;

AvaI 42; SmaI 44; KpnI 50; SacI 56 EcoRI 58.

Figure 39. Map of pBSR19.

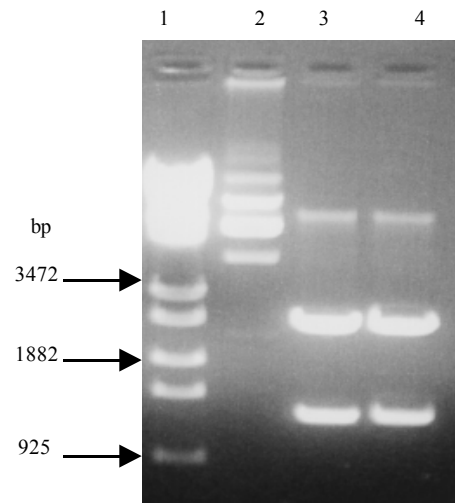


Figure 40. Release of the BSR cassette from the pBSR19 plasmid. The plasmid contained blasticidin resistant cassette in pGEM3 vector. Lane 1 contained 2 micrograms lambda StyI. Lane 2 contained undigested pBSR19 while lane 3 and 4 contained pBSR19 digested with *Bam*HI. The 1.4 kb band of BSR cassette was seen on the 1% TAE agarose gel.

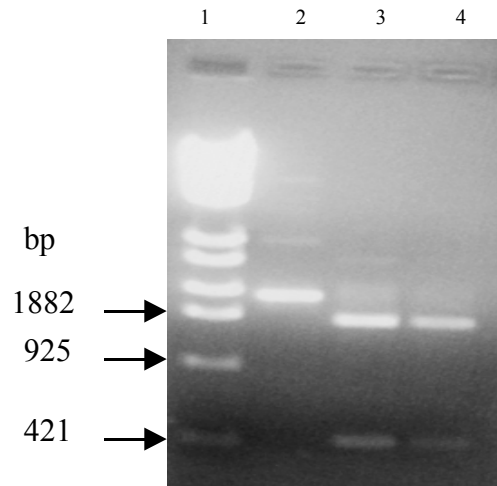


Figure 41. *5NU* cDNA. Lane 1 contained 2 micrograms Lambda *Sty*I as marker. Lane 2 contained the undigested cDNA. Lane 3 and 4 contained the cDNA digested with *Bam*HI. Two bands of 0.4 and 1.5 kb were seen on the 1% TBE agarose gel. The result shows that *Bam*HI digests the *5NU* cDNA only once.

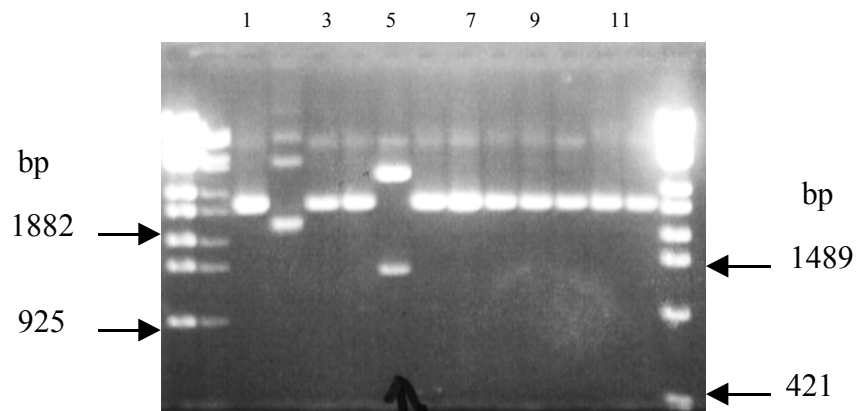


Figure 42. Digestion of boiling plasmid prep of SSK273 clones at the *Bam*HI site. Ten white colonies (from the plate treated with IPTG and X-gal) were picked and digested with *Bam*HI to release the BSR cassette (lane 3-12). Lane 1 contained a plasmid prep of blue colonies digested with *Bam*HI. Lane 2 contained uncut plasmid prep from the clone used in lane 3. Standard markers were 2 micrograms of Lambda *Sty*I. The digestions showed that the plasmid prep in lane 5 contained a positive clone.