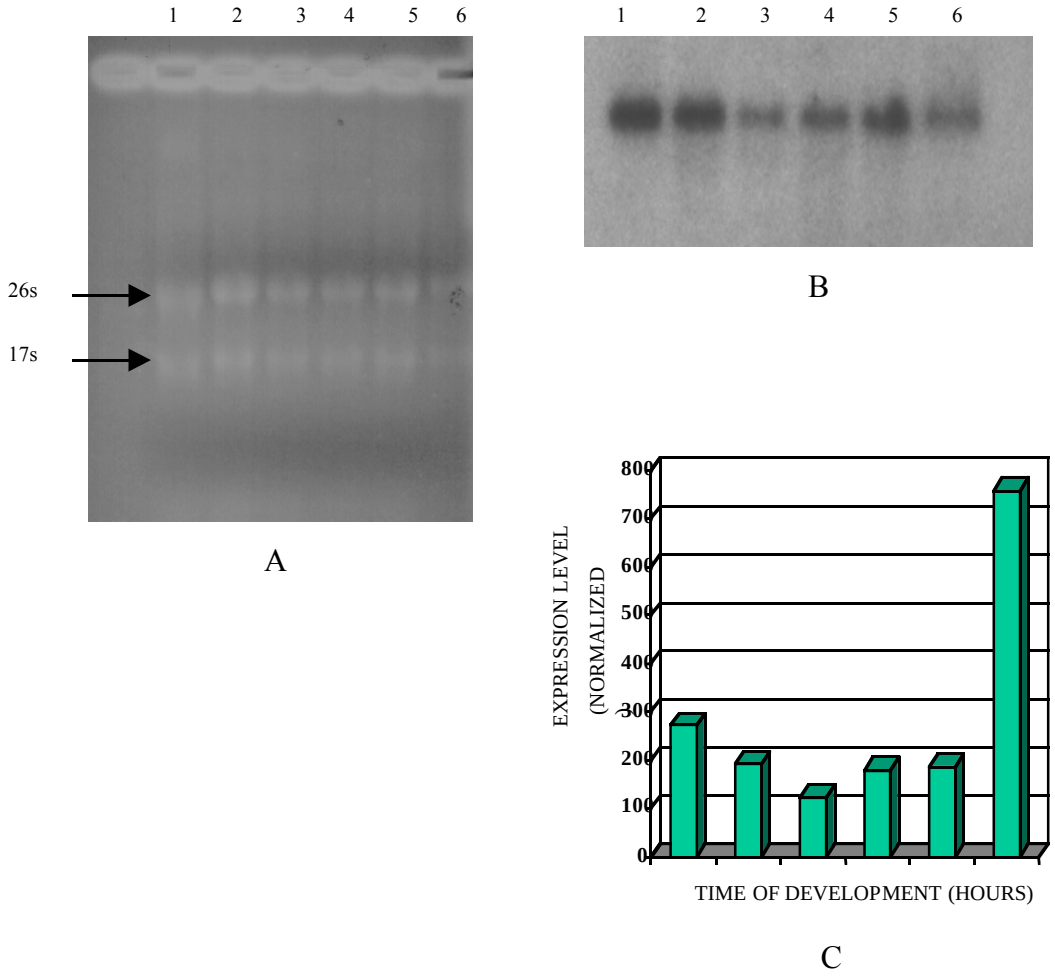

Figure 53. Northern analysis of Blasticidin transformants, and non-transformed cells (WT). Figure 53A shows two bands of rRNA on the formaldehyde gel from WT (lane 1) and all transformant cells (lane 2-6). After vacuum blotting to transfer the RNA onto a nylon membrane, the blot was hybridized with random primed *5NU* probe. After overnight exposure on film, bands were visualized in all RNA (B). The result showed that the BSR cassette was not inserted into the *5NU* gene. Figure 53C shows the expression level normalized to rRNA bands.

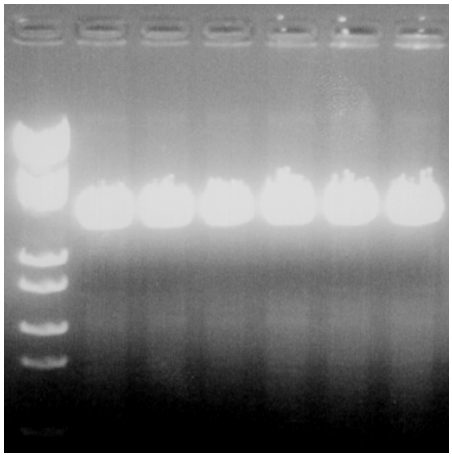

Figure 54. Digestion of SLA872 (*AP*) plasmid with *Nsi* and *Mun*I. Ten micrograms of the plasmids were double digested with *Nsi* and *Mun*I at 37°C for at least 1 h. The digested sample was treated with CIAP to prevent self-ligation of the plasmid. After isopropanol precipitation and electrophoresis, the cut form was seen on 1% TAE agarose gel. The bands were cut out of the gel, gene cleaned and quantitated by EtBr.

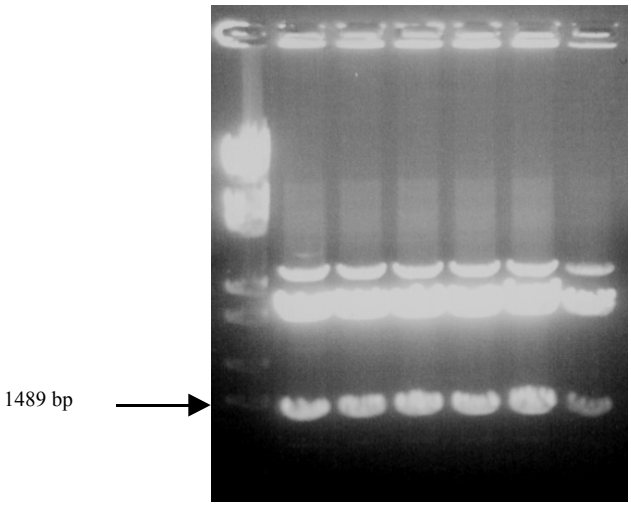

Figure 55. Double digestion of pBSR19 with *Eco*RI and *Pst*I. To release a BSR cassette, double digestion was performed at 37°C for at least 1 h. After electrophoresis on 1% TAE agarose gel, the 1.4 kb band of BSR cassette was cut out of the gel, gene cleaned and quantitated by EtBr dot assay.

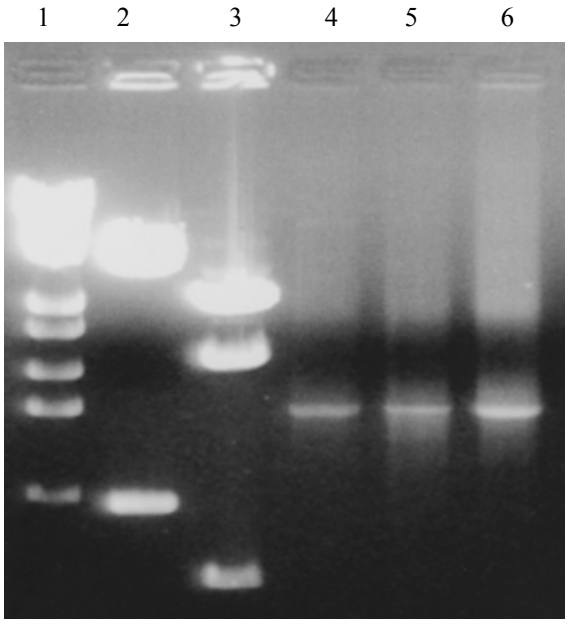

Figure 56. Digestion of boiling plasmid prep of SLA872 clones at the *Nsi* and *Mun*I sites. The positive clone was digested with *Kpn*I (lane 2), *Sal*I and *Not*I (lane 3) to release BSR cassette. Also, AP2F and K1 primers were used to amplify the positive. The expected size of about 1.5 kb was observed (lane4-6).

AAAAAAACAAAATAAAAAAACGTTGTTTTGTTTGAAATTATTATTTTTTT 50
TTTGTTAAAGAAACTATTAGGATTAATAAAGCACAATTACAAATCCCAGT 100
AACATTACAAATAATTCTACTTCTTCAAAAGTAAATTATACAAAGATGGA 150 AP1F
ACAAGCACCAAATTCATCATCAATCCGTGAATCGGGTATTGGCGGTGGTG 200
GTGGTGGTGGTGGTGGTGGTAATGATAAAGATGAAATGGATG**GATCC**ACT 250 *Bam*HI
GCAATTCCAATGGAGCCAATGACATCATCATCAGGTGTAAGTAGTGGTGG 300
TGGTGGTAAAGTTGTATCCCACTTTTTATTAGAAGAAGATTTTGATAGCA 350
AGAAAAGATTCACAAGTACACATAAGAGAATTTTTTACATATTGACATCA 400
TTGGCAATACTAGTGACTGTTGTGTTATTGATTGTGTTTTTATTACCAAG 450 R1
AGGATGGGAAGGCCCAAAGAAAAAGACAAATATAATTATGATGATTGGTG 500
ATGGTATGGGACCAGCAGCGTTGACCATGGCAAGAGTATGTTTTCATACA 550
AAAGGTGAGAGTACAAGTCAGGCACATTTACATTTAGACCCATATATAGT 600 AP1R
GGGCACAGTGAAGACATACTCAAGCAACAGTGTGGTTACCGACAGTGCCG 650
CAGCGGCAACGGCCTATGCAAGTGGTGTCAAGACGTATAATAATGCAGTG 700
GGTGTCGATGCAAATGGCAAGCCAGCAGGCACAATCATTGAGGCAGCTAA 750
AAAGTTGGGTATGAAGACAGGATTGGTCGTCACAACTCGTATCTCTGATG 800 AP2F
CGACACCAGCATGCTATTTCGCACACAGCGCAACGCGTCATGACGAGGCA 850
TTCATTATCGAT**CAATTG**TTGGACAAGGAGATCGATGTGATATTGGGTGG 900 *Mun*I
TGGTAAACAATTCTTTAGCAATAAAACACTTCAAGATGCTGTATCAAGCA 950 AP2R
AATACAACTACTCGTATGTTGAGAGTAAACAAGAGATGGAACAAGTTGAA 1000
GCAGGTAGAATTTTAGGACTCTTTGCAGATTACAACATACCTTGGGAAAT 1050
CGATCGTTTAAGAGATCCAACATTACTTTCAACCAAACCATCCCTACAGG 1100
AGATGACCACCAAAGCATTGAATTTAATCTCACAAAACAATGAAAATGGT 1150
TTCTTTTTAATGGTGGAAGGCTCAAAGATTGATGTGGCAGCTCATATCAA 1200
TGACGCACCCACTCAGATTTGGGAGACTG**ATGCAT**TTGATCAAACTTTTA 1250 *Nsi*
ATCTAGTTAGAGAATGGGCAGAGAAGGATGGTAATACCATTGTAATTGTG 1300 K1
ACAGCAGATCATGAAACTGGTGGTCTTACATTAGCAAATCAAATGGTTAT 1350
CGATGGTAATCCAAAATATTCATGGTCACCAGAGACATTATTGACAGTGA 1400
ATAAATCTGCTGATCTCATGGCAGAACTTATCAAAGGTGGTGCTGATCCA 1450
ACGAAATTGATTTTCGATAATACAGGTTACACATTAACATCAGATGATTT 1500
AAAAGAAATCAATAGAACCAAGTCTGCCTATTATCTCAATCAAGTGATTG 1550
GTCGTATCGTTTCAAACTATGCTGATATTGGTTTCACCACTGGTGGTCAT 1600
ACTGGTGAGGATGTCAATCTCTACACCTTTGGTGATACAATCTCTGAAGG 1650
TAAATTTGAAACTCGTCTAAAAGATCATCAAACTGAAATAGTTGATAATT 1700
ATATTGATCCAATCTTAAGAGGTAATATAAATAATATAGATATTGCTTCA 1750
TTTATCATTAAAACTTTAAATTTAGATATTCAATCAATTACAGAAYCATT 1800
AAAAGATTTTATTCCTAAACCATAAAA 1827

Figure 57. The position of primers and the *Bam*HI, *Nsi* and *Mun*I restriction sites (Bold) on the *AP* cDNA sequence.

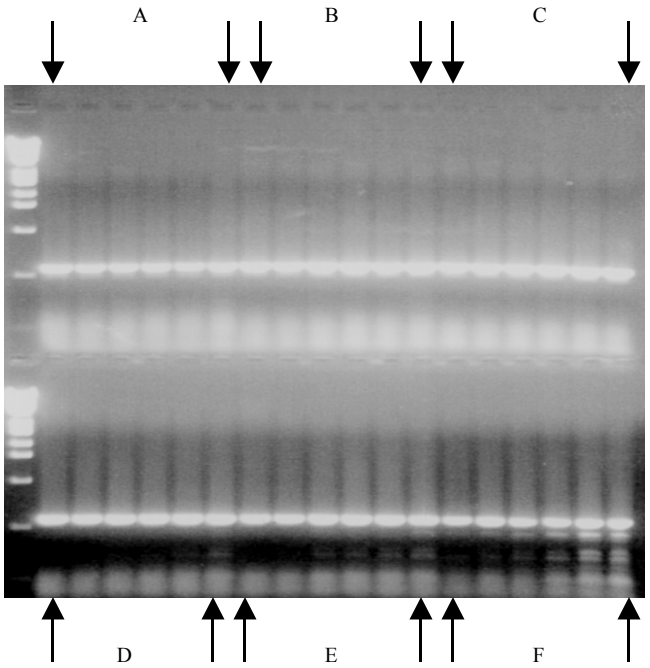

Figure 58. Determination of optimum conditions for AP1R and AP1F primers. Genomic DNA (100 ng) was used as template. Primers were designed from the *AP* cDNA sequence. A-F were the PCR reactions contained 0.5, 0.8, 1.0, 1.25, 1.5 and 2.0 mM $MgCl_2$, respectively. Temperature of each $MgCl_2$ conc varied from 47-37°C (left to right on the figure). The expected size of 490 bp PCR product was observed under the correct conditions.

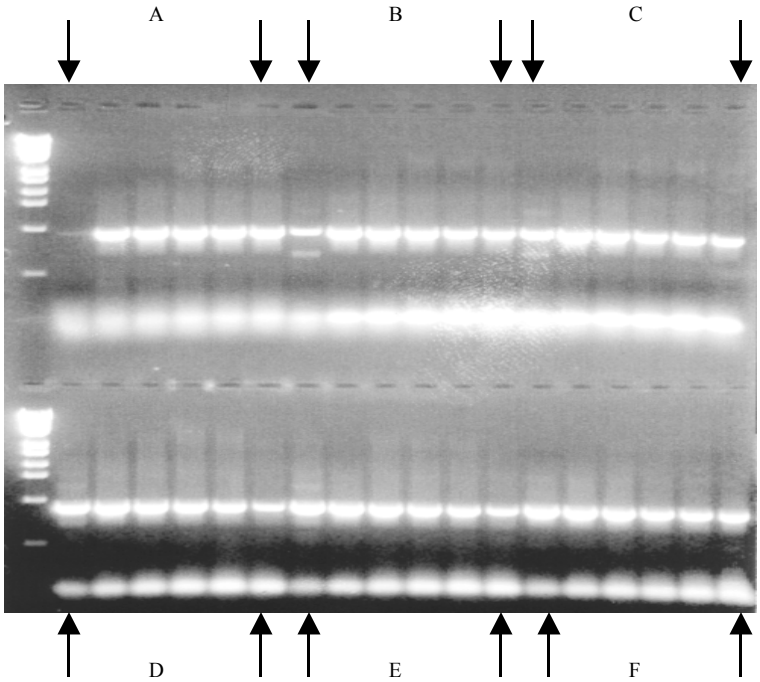

Figure 59. Determination of optimum conditions for SLA872 R1 and SLA872 K1 primers. Genomic DNA (100 ng) was used as template. A-F were the PCR reactions contained 0.5, 0.8, 1.0, 1.25, 1.5 and 2 mM $MgCl_2$, respectively. Temperature of each $MgCl_2$ conc varied from 66-44^{o}C (left to right on the figure). The expected size of 870 bp PCR product was observed under the correct conditions.