Figure 43. The position of primers and the *Bam*HI restriction site (Bold) on the *5NU* cDNA sequence.

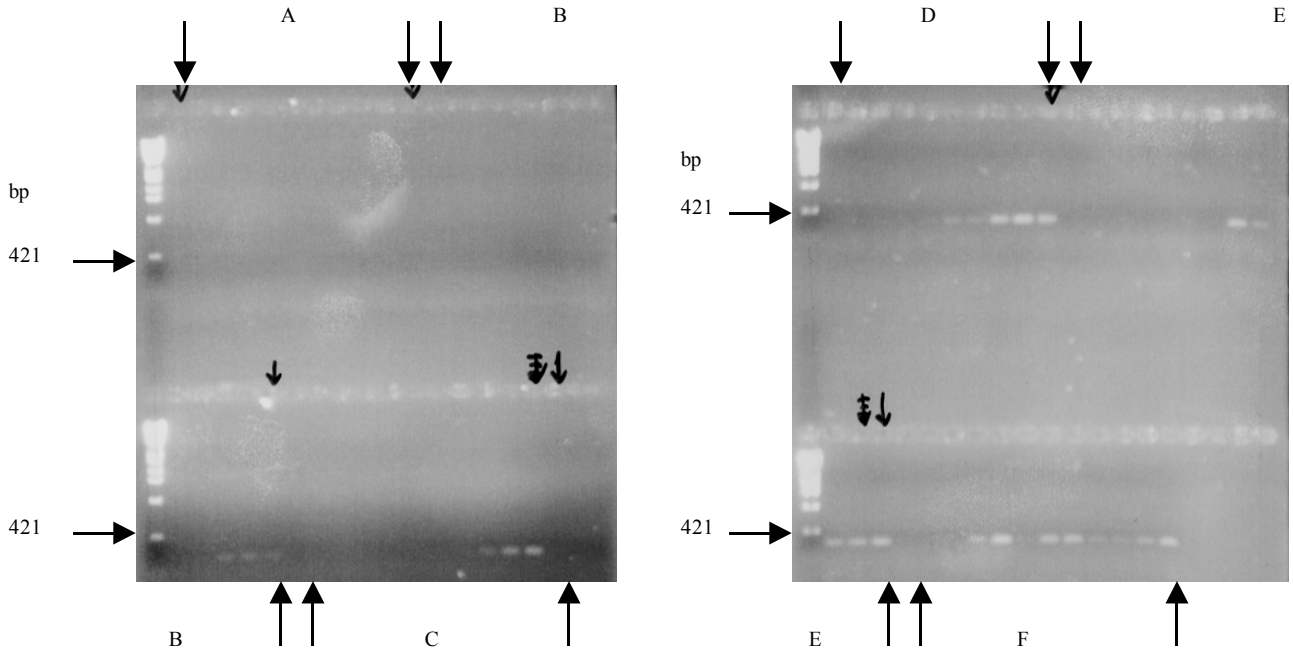

Figure 44. Determination of optimum conditions for CC1 and CC2 primers. Genomic DNA (100 ng) was used as template. Primers were designed from the *5NU* cDNA. A-F were the PCR reactions contained 0.5, 0.8, 1, 1.25, 1.5 and 2 mM $MgCl_2$, respectively. Temperature of each $MgCl_2$ conc varied from 58-47^{o}C (left to right on the figure). The expected size of 270 bp PCR product was observed under the correct conditions.

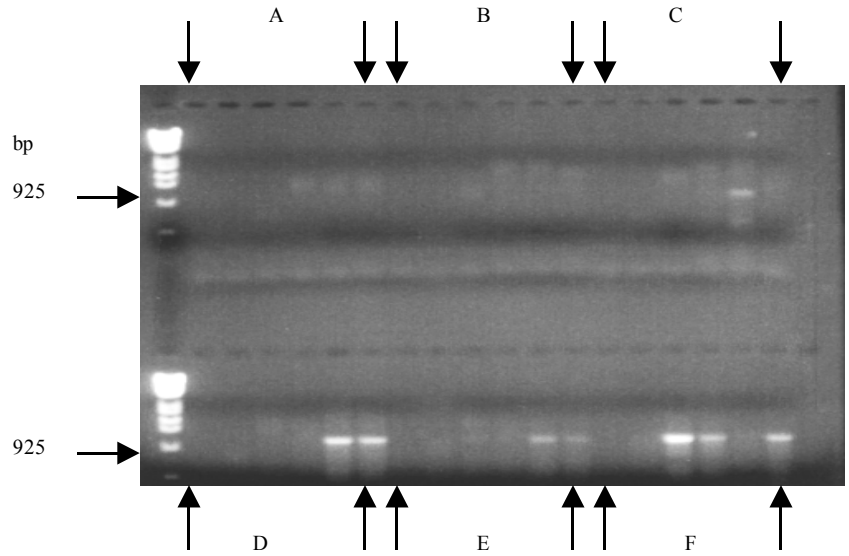

Figure 45. Optimization of PCR condition for CC1 and K1 primers. Genomic DNA (100 ng) was used as template. A-F were the PCR reactions contained 0.5, 0.8, 1, 1.25, 1.5 and 2 mM $MgCl_2$ conc, respectively. Temperature of each $MgCl_2$ conc varied from 64-44^{o}C (left to right on the figure). The expected size of 1,187 bp PCR product was observed under the correct conditions.

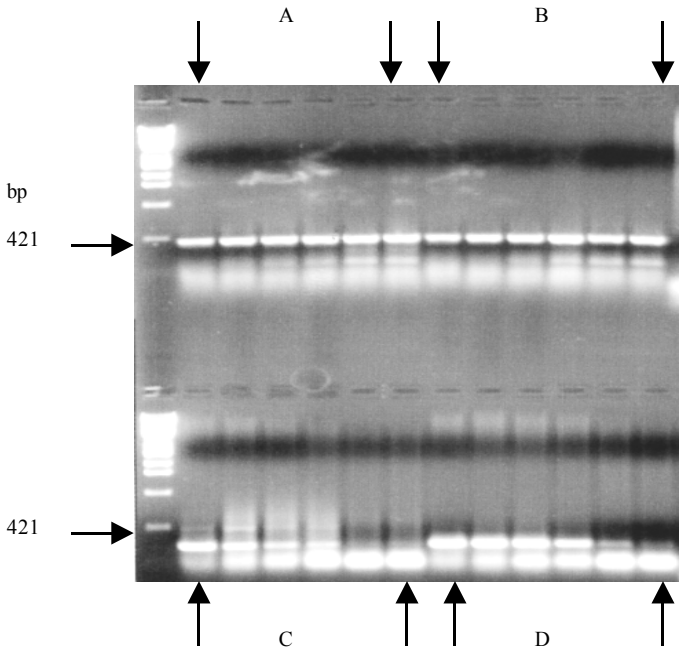

Figure 46. Determination of optimum conditions for CC1 and NU1R (top); R1 and NU1R (bottom) primers. Genomic DNA (100 ng) was used as template. A-D were the PCR reactions at 42, 48, 43 and 52°C, respectively. $MgCl_2$ conc of each temperature varied from 0.5, 0.8, 1, 1.25, 1.5 and 2 mM (left to right on the figure). The expected size of 409 and 254 bp PCR products were observed under the correct conditions.

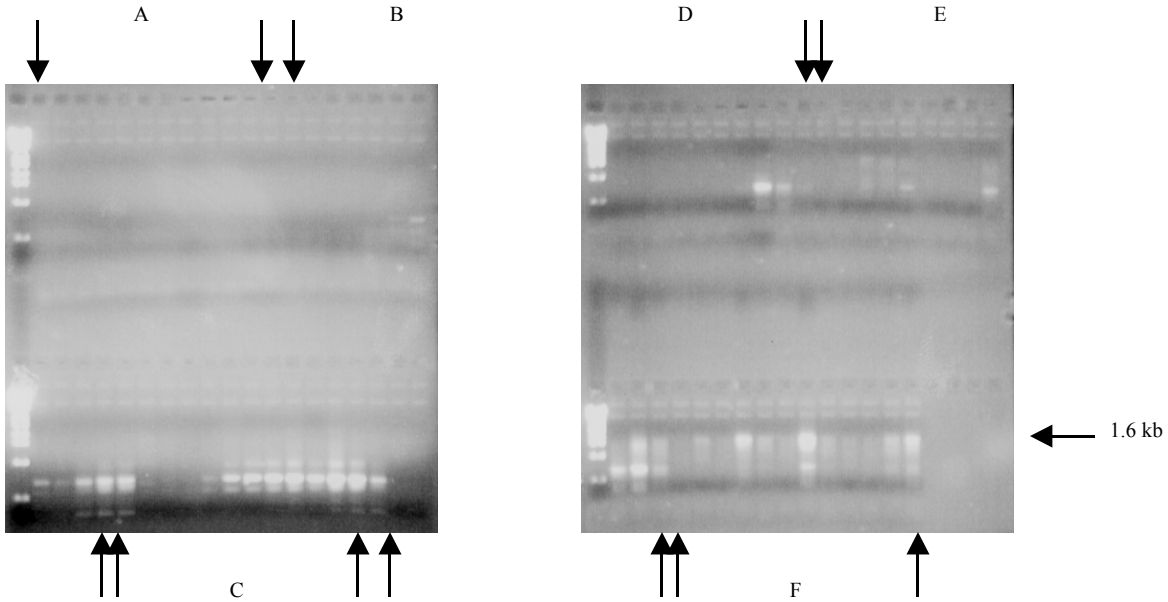

Figure 47. Assay of optimum conditions for amplification the SSK273 plasmid containing BSR cassette as template and CC1 and CC2 as primers. A-F are the PCR reactions containing 0.5, 0.8, 1.0, 1.25, 1.5 and 2.0 mM $MgCl_2$ conc, respectively. For each $MgCl_2$ conc, the temperature varied from 58-47°C (left to right). The expected PCR product of 1,646 bp was produced in the right conditions (F). Two micrograms Lambda StyI is the marker.

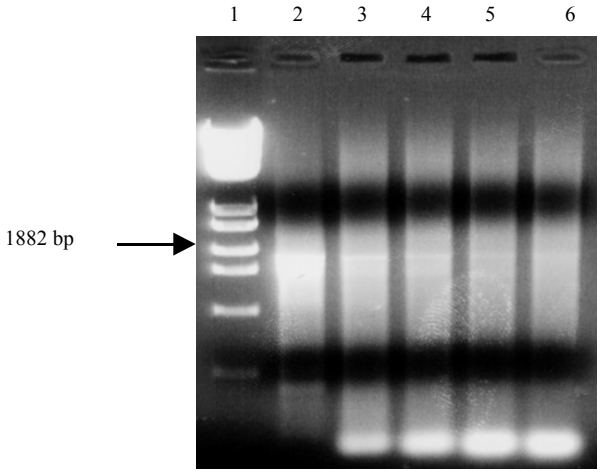

Figure 48. Optimum PCR conditions for CC3 and NU1R primers. SSK273 plasmid containing BSR cassette (200 ng) was used as template. The PCR reaction was prepared in 0.5, 0.8, 1.0, 1.25 and 1.5 mM $MgCl_2$, final conc (lane 2-5, respectively). The PCR conditions were 94°C for 2.5 min, 34 cycles of 94°C for 30 sec, 42.4°C for 30 sec and 72°C for 3 min and followed by a final extension of 72°C for 10 min.

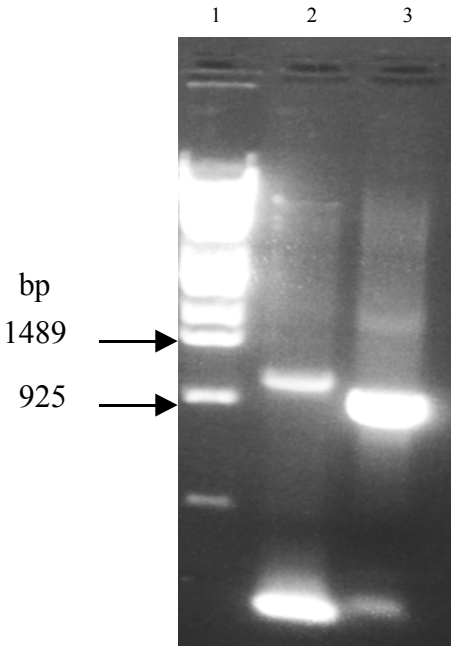

Figure 49. Amplification of *5NU* fragments from genomic DNA of non-transformant cells. Genomic DNA was isolated as described by Noegel *et al*, 1996. Lane 1, 2 micrograms Lambda StyI as marker. Lane 2, CC1 and K1 as primers, lane 3, R1 and K1 as primers. The expected size of 1,187 and 1,032 bp were seen, respectively.

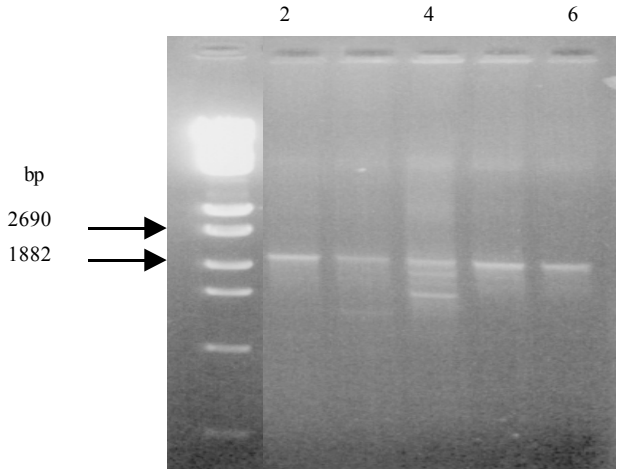

Figure 50. PCR product of SSK273 plasmid containing BSR cassette. CC1 and K1 primers were used as 5' and 3' primers, respectively. These primers were designed to surround the *Bam*HI site on *5NU* cDNA. The expected size of about 2.5 kb band was produced, indicating that the BSR cassette was inserted into the gene (lane 2-6). Lane 1 contained 2 micrograms Lambda *Sty*I as marker.

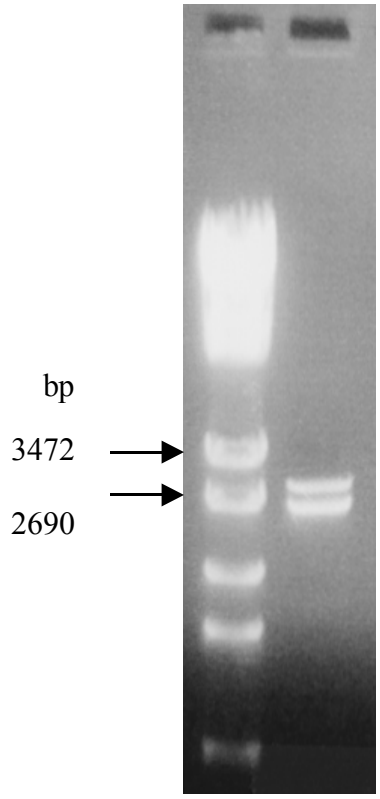

Figure 51. The SSK273 plasmid containing BSR cassette digested with *Dra*II and *Sac*II. After digestion with both enzymes, the DNA was treated with CIAP to prevent self-ligation. Two bands of 3.3 kb *5NU* cDNA containing BSR cassette and 2.9 kb pBS were seen on the 1% TAE agarose gel. The upper band was cut, gene cleaned and transformed into *Dictyostelium* cells for knock out mutagenesis. Lane 1 contained 2 µg lambda *Sty*I as marker.

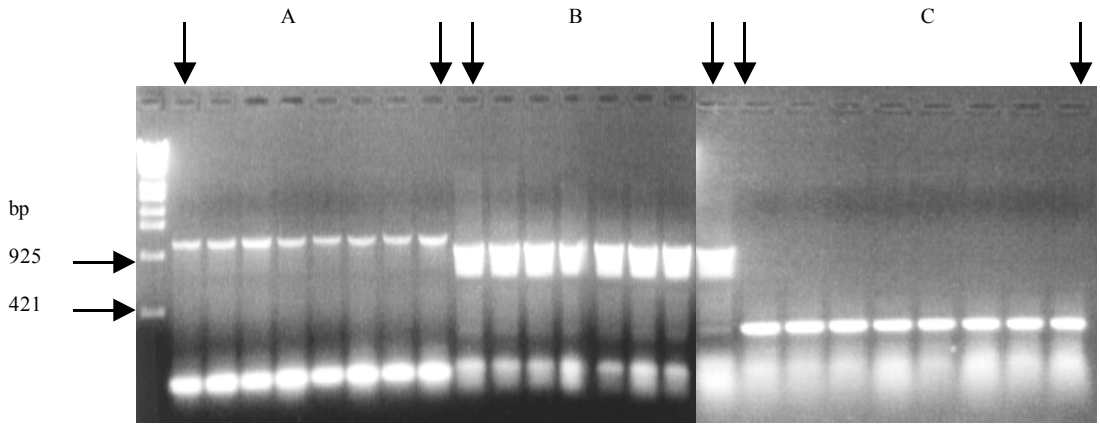

Figure 52. PCR analysis of genomic DNA from transformant *Dictyostelium* cells. The DNA was isolated from Noegel *et al,* 1996. CC1 and K1 were used in (A); R1 and K1 in (B) and CC1 and CC2 in (C).