overnight. The temperature for prehybridization and hybridization for screening sh1,bz-m4 cDNA library was 55°C rather than 67°C since a heterologous probe was used. The temperature for screening bt2-7503 and bt2-C cDNA library was 67°C. Hybridization was done in a hot air incubator with rotation. Washing condition was a 15-minute wash at room temperature in 40 mM Na₂HPO₄(pH7.2) and 5% SDS, two 50-minute washes at 67°C in 40 mM Na₂HPO₄(pH7.2) and 1% SDS and two 20-minute washes at 67°C in 40 mM Na₂HPO₄(pH7.2) and 1% SDS [Church and Gilbert, 1984]. After washing, filters were exposed to Kodak X-OMAT AR film for various times at -80°C with two intensifier screens. The autoradiographs were developed using a Konica QX-60A developer.

3.4 Subcloning into Plasmids

Lambda DNA of purified clones was prepared by scraping off the top agarose from two petri plates exhibiting confluent lysis. The top agarose was cut into pieces and was vigorously shaken at room temperature in 25 ml of SM [50 mM Tris-HCl (pH7.5), 100 mM NaCl, 8 mM MgSO₄ and 0.01% gelatin] for longer than 3 hours [Ingham, 1990]. At this point conventional technology was followed [Maniatis et al., 1982]. Purified lambda DNA was cut with EcoRI. Restriction enzyme reaction was done according to the suppliers procedure(BRL). These inserts were subcloned into pUC19 using conventional technique [Maniatis et al., 1982]. Plasmid preparation of cDNA clones