

Deletion Analysis of Tomato Leaf Curl Virus PRKT17 Promoter

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In order to define the minimal region of PRKT17 promoter previously isolated necessary for activity, deletions were carried out from 5' end of PRKT17 promoter based on the basic characteristics of promoters. Taking this as a reference A3 was designed. The *E.coli* clone pGBK111 containing A3 region could derive the resistance of *E. coli*. to level of 250µg per ml of chloramphenicol.

Key words: Deletion, Promoter, Region.

A promoter is a regulatory region of DNA generally located upstream (towards the 5' region) of a gene, providing a control point for regulated gene expression. Promoters are important elements for gene expression and regulates where, when and to what level a gene is expressed². The intricate pattern of gene regulation involves molecular signals that act on DNA sequences encoding protein products. *Cis*-acting molecules act upon and modulate the expression of physically adjacent, operably linked polypeptide-encoding sequences. *Trans*acting factors affect the expression of genes that may be physically located very far away, even on different chromosomes. The expression of a particular gene may be regulated by the concerted action of both *cis* and *trans*-acting elements⁷.

Promoters are generally characterized by the presence of TATA sequence (called the TATA box or Goldberg Hogness box) about 30 bp upstream from the site, where transcription begins,

as well as one or more promoter elements further upstream⁴. The functional anatomy of promoter region can be analyzed by determining which of its bases are necessary for efficient transcription. The CAAT and TATA boxes have been found to be critical elements in numerous eukaryotic promoters³.

Promoter cloning can be extensively applied for the systematic improvement of protein properties such as antibody binding affinity, enzyme regulation, increased or diverse substrate specificity, modification of other types of sequences *e.g.*, ribozymes¹

In an earlier study, two regions (intra and intergenic) of ToLCV (Tomato Leaf Curl Virus) promoters PRKT17 and PTOLP were isolated. They were further cloned into promoterless prokaryotic vector pKKLUX with *cat* (chloramphenical acetyl transferase) as reporter and promoterless yeast expression vector pYESSR with gene encoding protein resistance to zeocin as reporter. The expression studies showed that the two promoters regions could drive the reporter genes⁶. The two promoters being 600 and 300 bp long need to be analyzed for optimum/minimal region that give maximum promoter activity. These regions can then be functionally validated in prokaryotic and eukaryotic model systems.

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MATERIALS AND METHODS

In order to define the minimal region of PRKT17 promoter necessary for activity, deletions were made and they were placed upstream of the reporter gene. Deletions were carried out from 5' end of PRKT17 promoter based on the basic characteristics of promoters i.e., TATA box is absolutely essential to start transcription and the other sequence TTGACA (CAAT box) allows a very high transcription rate. Taking this as a reference A3 promoter sequences was amplified. Plasmid isolation from pKKLUX + PRKT17 was done. The alkaline lysis protocol of Birnboim and Doly (1979) with certain modifications was used for isolation of plasmids. The primers specific for A3 (578 bp) promoter fragments was designed based on the sequence using software Primer 3 and synthesized at Ocimum Biosolutions, Bangalore. The primer sequences designed were : F: 5' CTCTAGAAGGTACGCCGCGTCTCAA3' R: 5' TTCAAGCTTGAAGTTCCTAATTATAGCTGCTCCTT3'

Restriction sites *Xba*I in forward primer and *Hind*III in reverse primer were added to facilitate directional cloning in pKKLUX (*Bam*HI) vector for A3 cloning.

The purified PCR amplicons of promoter fragments were ligated to promoterless vector pKKLUX (*Bam*HI) after restriction with *Xba*I and *Hind*III for A3 to permit directional cloning. For ligation, an optimal molar ratio of 1:1 vector: insert was calculated. The ligation mixture along with linearised vector and amplicon DNA were mixed in 0.5 ml micro centrifuge tubes and incubated at 16°C for 16 hrs for ligation.

The competent cells of *E.coli* DH5 α were prepared and transformation of *E.coli* DH5 α was done. The confirmation of the presence of cloned insert was done by PCR amplification of recombinant vectors with respective primers. The confirmation was also done through restriction analysis.

Expression of cloned promoters were analysed by spotting the overnight grown cultures of A3, PRKT17 along with pBELO BACII (positive control), pKKLUX (*Bam*HI) vector (plain vector) and *E. coli* DH5 α . About 5 ml of Luria broth with chloramphenicol (25 μ g/ml) for pKKLUX + PRKT17, pKKLUX + A3, pBELO BACII vector,

ampicillin (100 μ g/ml) for promoterless pKKLUX (*Bam*HI) plain vector and Nalidixic acid (10 μ g/ml) for *E. coli* DH5 α was inoculated kept at 37°C with shaking. 15 μ l of cultures were taken and spotted on to the plates containing different concentrations of Chloramphenicol ranging from 5 μ g/ml to 500 μ g/ml. The plates were incubated at 37°C.

RESULTS AND DISCUSSION

A PCR product of A3 (578 bp) was amplified from pKKLUX + PRKT17 construct with specific primers carrying *Xba*I and *Hind*III sites and cloned into same sites of pKKLUX (*Bam*HI) and named as PGBK111. The recombinant construct PGBK111 was transformed to *E.coli* DH5 α using 30 μ l of ligation mixture. The clones were confirmed through PCR amplification by using specific primers. Confirmation was also done by restriction analysis using *Xba*I/*Hind*III which released 578bp (Figure 1 and 2). The promoter clones PGBK111, pKKLUX + PRKT17 along with pBELOBACII showed resistance upto 250 μ g per ml concentration of chloramphenicol (Figure 3). There are some internal sequences in the promoter, which are essential for the specificity of the promoter. The possible CAAT box of PRKT17 promoter, GGTC AATAT and GGCTACTGT are present at -89 to -81 bp and -161 to -153 bp, respectively⁶. In A3 (578 bp), both these elements are present. Hence it leads to transcription.

The importance of various portions of promoter regions for the strength of the promoter has been indicated in several studies. The manganese superoxide dismutase gene (*SodM*) is highly expressed in *Aspergillus oryzae*. To elucidate the basis for this high level expression, deletion analysis of the promoter was undertaken using β -glucuronidase (*GUS*) as reporter⁵. Deletion of a 63 bp sequence from -200 to -138 in the 1038 bp *Sod M* promoter caused a drastic decrease in *GUS* activity. The region from -209 to -178 contain cis acting elements and required for high level expression. In this region , two cis acting elements were found , the CAAT box and GC box⁵. The strong enhancing activity of this region is due to nearby arrangements of both the CAAT box and GC box. Thus, our studies have clearly indicated that promoter region A3 at -140 to -132 bp and at -112bp to -108bp containing the cis elements are

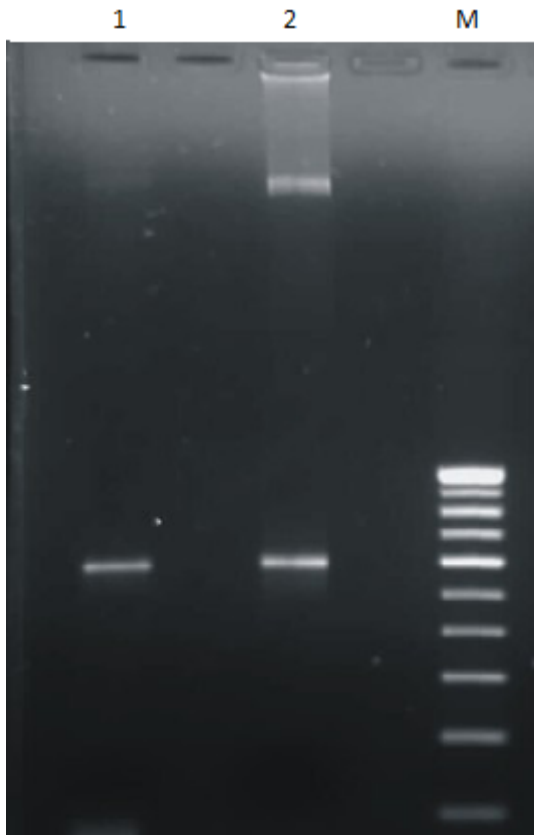


Fig. 1. PCR confirmation of pGBK111
 M. 100 bp Ladder
 1. pGBK111
 2. pGBK111

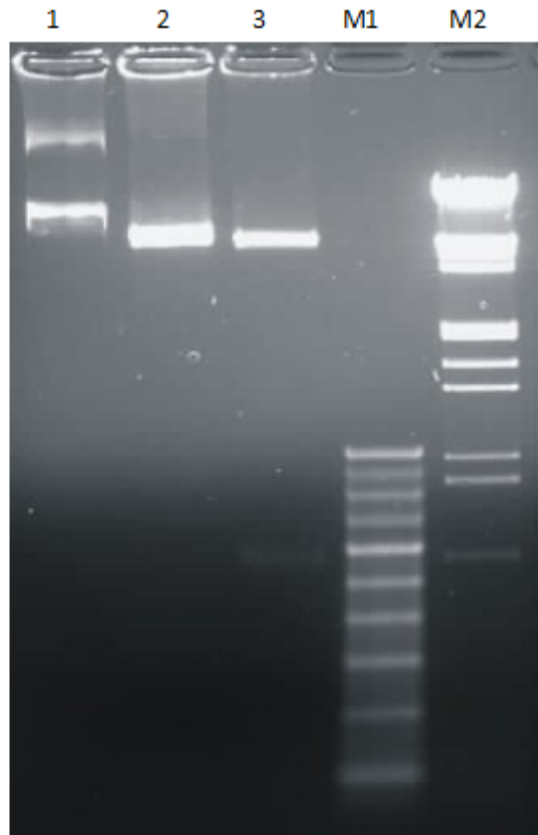


Fig. 2. Restriction confirmation of pGBK111
 M1. 100 bp ladder
 M2.Lambda DNA/*EcoRI*/*HindIII*/Double digest
 1. Uncut plasmid
 2. Cut with *HindIII*
 3. Cut with *XbaI* and *HindIII*

Fig. 1-2. PCR and restriction confirmation of pGBK111



Note : Growth of *E. coli* pKKLUX + A3 (pGBK111) on chloramphenicol plate

Fig. 3. Chloramphenicol plate assay

capable of driving the antibiotic resistance to chloramphenicol at 250 ppm. Analysis from the cister software showed that compared to the reference which showed the p[robability of 0.87 in PRKT17, the promoter sequence A3 (578 bp) showed the probability of 0.74. Deleted regions contains CAAT and TATA box at -40 and -34 bp and -27 to-22bp. Possibly, this has lead to drop in resistance to chloramphenicol as compared to original sequence PRKT17 which showed resistance to 350ppm concentration of chloramphenicol. Hence, we infer that these two elements are essential to drive higher transcription in PRKT17.

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