IDENTIFICATION AND CHARACTERIZATION OF NUCLEAR MATRIX ATTACHMENT REGIONS IN THE ADP-GLUCOSE PYROPHOSPHORYLASE LARGE SUBUNIT GENE ISOLATED FROM *SORGHUM BICOLOUR*

Pulak Das[1] & Indrani Chandra[2]
[1]Scientist, Department of Biotechnology, The University of Burdwan, Burdwan-713104, West Bengal, India.
[2]Teacher in-Charge, Department of Biotechnology, The University of Burdwan, Burdwan-713104, West Bengal, India.

Received: September 14, 2018 Accepted: November 04, 2018

ABSTRACT: *ADP-glucose pyrophosphorylase (ADPGlcPPase) controls the first committed step of starch synthesis by catalyzing the biosynthesis of ADP-glucose from glucose-phosphate and ATP. It is a tetrameric protein consisting of two small and two large subunits. The small subunits have a catalytic function, while the large subunits regulate the enzyme activity. These types of region are involved in the structural and functional organization of all eukaryotic genomes. Such sequences are known as matrix attachment regions or MARs. DNA replication and transcription of genes take place primarily in regions in contact with the internal matrix. In this study, the gene encoding the ADPGlcPPase large subunit (ADPGlcPPase β) in Sorghum bicolour was cloned and characterized.DNA sequencing indicate that ADPGlcPPase β had a 63% AT content. It contain T box (TTWTWTTWTT), DNA unwinding sequence (AATATT), weak and stronger matrix binding sequences.*

Key Words: *ADP-glucose pyrophosphorylase,Large subunit,Gene cloning,Polymerase chain Reaction, Matrix attachment region.*

INTRODUCTION

Eukaryotic chromatin is organized into a series of individual loops or domains (Paulson *et al.,* 1977). The bases of these loops seem to be defined by specific DNA elements that are involved in attachment of the chromatin fiber to a scaffold of non-histone chromosomal proteins that is called the nuclear matrix. These specific DNA elements have been termed scaffold-associated regions, matrix-associated regions or matrix-attachment regions (MARs) (Cockerill *et al.,* 1986).

MARs are defined and identified by *in vitro* biochemical tests. Specifically, MARs are genomic DNA fragments that have the ability to bind to isolated nuclear matrices *in vitro*.Nuclear matrices are particulate protein structures that are prepared by histone depletion of isolated nuclei (Gasser *et al.,* 1986). MARs generally bind to nuclear matrices of diverse species (Izaurralde *et al.,* 1988& Cockerill *et al.,*1986).In a few instances MARs have been shown to co-localize with the limits of DNase I-sensitive domains of tissue-specific genes (Phi-Van,L. *et al.,* 1988), suggesting that MARs may function as structural boundaries of individual chromatin domains (Dillon *et al.,* 1994). Consistent with this view, human MARs can function as insulator elements in *Drosophila*, shielding integrated transgenes from chromosomal position effects *in vivo* (Fournier *et al.,* 1998). Other MARs have been found in promoter regions or within introns, where they may function in the regulation of transcription, and some MARs seem to stimulate expression of heterologous reporters in stably but not transiently transfected cells (Bode *et al.,* 1996). However, the functions of MARs as assessed by transfection experiments remain equivocal. Finally, MARs may be *cis* elements of chromosome dynamics, regulating chromosome shape and maintenance (Hart *et al.,* 1998).

Although MARs tend to be AT-rich (Laemmli *et al.,* 1992), they lack readily defined consensus motifs that mediate matrix binding. This raises the possibility that the matrix-binding activity of these elements may be encoded by complex sequence motifs that may vary between and within genomes. These kinds of motifs may affect higher order DNA structure. On the other hand, a number of simple sequence motifs have been reported to be enriched in DNAs with matrix-binding activities (Boulikas,T. *et al.,* 1995). These include various binding sites for topoisomerase II (Spitzner *et al.,* 1998), DNA unwinding motifs (Bode *et al.,* 1992) and simple sequence motifs called the A, T and H-boxes (Dickinson *et al.,* 1992). In this paper we explored the MARs properties of ADP-glucose pyrophosphorylase β subunit.

ADP-glucose pyrophosphorylase (ADPGlcPPase) catalyzes the synthesis of starch in plants and glycogen in bacteria, respectively; in these reactions, ADP-glucose (ADPGlc) is synthesized from glucose-phosphate (Glc-1-P) and ATP, which is then utilized as the glycosyl donor for elongation of α-1,4-glucosidic chains (Ballicora

et al., 2004, Preiss, 2009). This reaction is the first committed step of starch synthesis (Preiss, 1982); hence, ADPGlcPPase plays a pivotal role in the carbon metabolism in plants. The physiological importance of ADPGlcPPase in plants has been illustrated by numerous studies (Preiss *et al.,* 1991, Smith *et al.,* 1995, Kavakli *et al.,* 2000; Slattery *et al.,* 2000).ADPGlcPPases identified from higher plants are all hetero tetramers consisting of two α- and two β-subunits (Thorbjornsen *et al.,* 1996, Burgess *et al.,* 1997, Chen *et al.,* 1998, Beckles *et al.,* 2001, Burton *et al.,* 2002). The α-subunit, commonly known as the small subunit, is the catalytic center that interacts with the substrates (Glc-1-P and ATP). The β-subunit, or the large subunit, plays a regulatory role (Ballicora *et al.,* 2004). However Cross *et al.,* (2004) suggested that both subunits of ADPGlcPPase played an equally important regulatory role in potato and maize. These types of region are involved in the structural and functional organization of all eukaryotic genomes.In this study we isolate, clone and characterize large subunit of ADP-glucose pyrophosphorylase *from Sorghum bicolour*.

2. MATERIALS & METHODS

2.1Plant Materials

The plant material for this study was taken from Ajeet Seeds Pvt Ltd, Maharashtra. Fresh young leaves from *Sorghum bicolor* plants were collected for DNA extraction.

2.2 DNA isolation

Genomic DNA was isolated according to a modified CTAB method (Zhu *et al.,* 2010). The concentration and quality of the obtained genomic DNA samples were estimated by measuring O.D. at 260/280 nm in UV spectrophotometer. Finally, all the genomic DNA samples were diluted to a final concentration of 40ng/μl with 1X TE buffer (10mM Tris-HCL; pH 8.0; 1mM EDTA). Intactness of genomic DNA was checked by agarose gel electrophoresis. DNA samples were stored at -20ºC for further use.

2.3 Designing of oligonucleotide primer PCR (DOP-PCR)

The following ADPGlcPPase β gene sequences publically accessible from the National Center for Biotechnology Information were aligned to generate a ADPGlcPPase β nucleotide consensus sequence. The alignment of these nucleotide sequences indicated that large stretches of the nucleotides are highly conserved and thus could be used as primer targets. The consensus regions were used to design primers.

2.3Amplification of ADPGlcPPase β by PCR

The ADPGlcPPase β gene was amplified from DNA using *ADP* β specific primers(Forward: 5' AAG CTT GAA TTC GGT TCC ATT 3' & Reverse: 5' GAA TTC AAT TTT GAA CAA TAC 3').PCR reaction was performed using 40ng of DNA along with forward and reverse primers(10pmol each),200μM of dNTP's, 1.5mM Mgcl2 and 3U of high fidelity DNA polymerase(Promega, USA).The amplification cycle was initial 5 minutes denaturation at 94ºc, after that 35 cycles of denaturation at 94ºc for 20sec, annealing at 58ºc for 30sec and extension 72ºc for 1.30min.Final extension 72ºc for 7min.The PCR amplified product was analysed on 1% agarose gel along with DNA molecular weight marker.

2.4 Cloning and characterization of ADPGlcPPase β gene

The amplified ADPGlcPPase β gene fragment was gel eluted and ligated into pGEMT easy vector (Promega, USA).The recombinant plasmid *(pGEMT-ADP)* was characterized using E.coRI restriction digestion and analysed on 1% agarose gel for the insert. The gene in recombinant plasmid *pGEMT-ADP* was sequenced in Eurofins Genomics, Bangalore and sequence was blast in NCBI.

2.5 Sequence analysis

Nucleotide sequence analysis was done using software pDRAW 32 and online bioinformatics analysis facility available at www.justbio.com, www.expasy.org and www.ncbi.nlm.nih.gov.

3. RESULTS AND DISCUSSION

3.1DNA isolation

DNA isolated from leaf material of *Sorghum bicolor* presented in Figure 3.

3.2 Designing of oligonucleotide primer PCR

Nucleotide sequences of ADPGlcPPase βgenes available from NCBI Gen-Bank database were aligned using multiple sequence alignment program Clustal W 1.8 (www.bioedit.com).Two sequences with high degree of sequence similarity were selected for the design and synthesis of primers from highly conserved region
Multiple sequence alignment for forward primer

Sorghum bicolor /AF010283 5' AAGCTTGAATTCGGTTCCATT 3'
Sorghum bicolor / XM002455967 5' AAGCTTGAATTCGGTTCCATT 3'
****

Designed Forward Primer
ADPF' 5'**AAG CTT GAA TTC GGT TCC ATT** 3'

Multiple sequence alignment for reverse primer
Sorghum bicolor / AF010283 5' GAATTCAATTTTGAACAATAC 3'
Sorghum bicolor / XM002455967 5'GAATTCAATTTTGAACAATAC 3'
****

Designed Reverse Primer
ADP R 5'**GAA TTC AAT TTT GAA CAA TAC** 3'

Fig. 1: Clustal W 1.8 multiple sequence alignment of the ADPGlcPPase β gene sequences: *Sorghum bicolor* (AF010283 & XM002455967), The region selected and is conserved for designing primers are in bold letters. Only the 5' and the 3' regions of the gene coding sequences are shown.

3.3amplification of ADPGlcPPase β by PCR

The ADPGlcPPase β gene of the length of about 1494 bp was amplified from DNA using ADPGlcPPase β specific primer by using PCR in a reaction catalyzed by the enzyme Taq polymerase is presented in Figure 4.

3.4 The construction and identification of ADPGlcPPase β gene.

ADPGlcPPase β gene was amplified. The gene was then cloned in *pGEMT easy* vector (Promega, USA). Plasmid DNA were isolated and digested with *Eco*R I . The digestion pattern revealed presence of insert (Figure 5).The clone was designated as pADP as sequenced. The sequencing analysis confirmed the amplified gene to be 95-98 % identical to the target gene (GenBank Accession no. AF010283,XM002455967). Sequence was deposited in the NCBI Gen Bank (ID KM236585).

3.5 Characterization of ADPGlcPPase β:

1494 bp DNA fragment was isolated, cloned and sequenced. This fragment named ADPGlcPPase β, had a 63% AT content. It contain T box (TTWTWTTWTT), DNA unwinding sequence (AATATT), weak and stronger matrix binding sequences .One stronger matrix binding sequences, two T box, two DNA unwinding sequence, two A/T/C stretch of 25 nt were found in the ADPGlcPPase β DNA sequences(Fig 2)

```
   1 GGTTCCATTC TTTGGTACCT GTAAACCCAT ACAGCATAAC TTGAAGATGA
  51 ACAGCAGTGC AGGGATAACT GAAGCCTAGG AGAAAATAAA AGGGATATGG
 101 ACGTTTATAA GAAGCATGTA TTCTAACAGA CTACCTAATA TTATTCAGAT
 151 TGTGTGACAT TCTATAAAAG ACCCAAATGC TTATAAAGAT ATATCATGTT
 201 GTAAAAATTT GATCTGCATT TCCTTTCGTA AGCAATAAAA CAAGTCTCTT
 251 TTGGGAGTTA CCATGATGAA AAAGTTAAAA TATAATCTCA ATAATAACTC
 301 TGCATAGGGA AAACTTATCC TTCTGCTTAT GATTAATTTT TTTTTTCTAT
 351 TACAAGAACC ACTGTAGAAT GGGAAAATCA CTATCTTAAA TTTAAATCTA
 401 CAAGAACCTG GAATAATTAG TGCTACATTT CAATGTCTAA TGAGAACAGT
 451 TTCTTTTGGA CATTCCAATT CTCCTTTGAA AATGATATAT AGTTCCTGCT
 501 GTGATATAAT TCATTGTATC TGTGAGTGGT ACCATGGCCA AGGAAGTAAA
 551 AAGAGATGCA TAGAGGTTCT GGAATGACTT ACATTTAGAG AATAGTTAAA
 601 AAGGCTAAAA CAACCAGTAG GGGGGTGGGG GGGAAAGGGG ACAGAAATTT
 651 TCATGAGGGA CCATGATTGA ACTATTGAGG GTGCAAAAAA TATTTTTTTT
 701 CAAATGTGGT GAAATACCTA AGAGATGTGC ACCTAGCATA GTTTTTTCTT
 751 TGGGCTCCCC TTGGCCTCTC CTTTCCTCGT CCCCTGAAGT ATCCGATTTT
 801 TTTCCTGGGG AAGGAAGGTG ATCTGGGAAA CGGTAATCAT AAGAGGGAGA
 851 GCTATGCGGC ATATGCCCTC AAAGGCACTT TGGCATTATC GAAGCAATAT
 901 CGATCTCTGT TAGTTTATCT TGCGTGAACG TTTGTGGAAA CTACTACCTT
 951 ACAAGCATTA GTGACAGCTC AGAAAGTTAT TTCTGAAAGG TTTCATGTGT
1001 ACCGTGGGAA ATGTTGCCAA CTCAAACACC TTCAATATGT TGTTTGCACG
1051 CAAATTCTTC TGGAAGAAAG GTGTCTAAAA CTATGAGCGG GTTACAGAAA
1101 GGTACAAACC ACGGCTGTGC ATTTTGGAAG TATCATCTAT AGATGTTTGT
1151 TGAGGGGAAA GCCGCACGCC AAAGTTATTT ACTCAGAAAC AGCTTCAACA
1201 CACAATTGTC TGTGATATGA TGGCATCTCT CACGCACCAT CACCTCTCTC
1251 TCATGTCTGT TTATTTTATT GCCCTTTCTG CTCATAAAAA TCATTAAGAG
1301 TTTATAAATA TGCATAGGCA TACCAATATG CACATCTATT AATTAGCCAG
1351 AAGATCATCT TCTTATTCTT TAGTTTAGTT ATTGTTTGAA AAATTTGTCC
1401 AGGCCTAGGG AGCTCGTGCA CAGTACTAAT GCATCTTCAT CAAATGTGAA
1451 TTTCAAAAAG GAAGTAGGAA CCTATGAGAG TATTGTTCAA AATT
```

1 - 514: weak matrix binding
137 - 142: DNA unwinding sequence (AATATT)
275 - 299: A/T/C stretch of 25 nt
337 - 346: T box (TTWTWTTWTT)
374 - 398: A/T/C stretch of 25 nt
515 - 1495: stronger matrix binding
689 - 694: DNA unwinding sequence (AATATT)
1261 - 127: T box (TTWTWTTWTT)

Fig. 2: Sequence properties of ADPGlcPPase β (Gene bank no . KM236585)

Figures

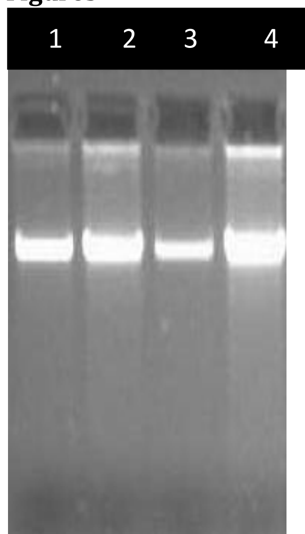

Fig 3 Isoalation of DNA

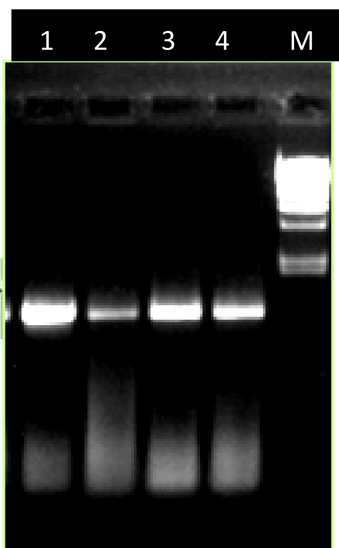

Fig 4: ADPGlcPPase β amplification by PCR

Upon completion of DNA isolation & PCR , agarose gel (1%) was run at constant volt (100amp) for 1hr. **(Fig3)**Lane 1-4- Genomic DNA isolated from *Sorghum bicolour* leaf sample**(Fig 4)**PCR amplified from DNA of *Sorghum bicolor* using ADPF and ADPR primers,Lane 1-4- ADPGlcPPase β gene amplified from DNA, Lane M- HindIII Maker .

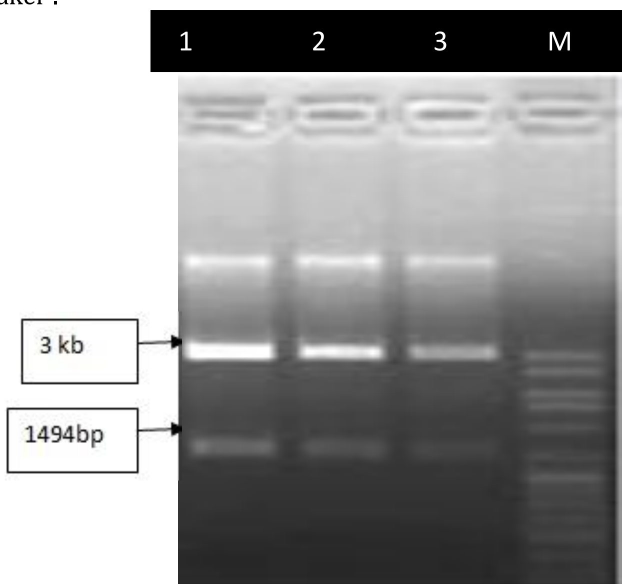

Fig 5. Characterization of recombinant plasmids.

Fig 5 Restriction digestion of *pGEMT*- ADPGlcPPase β clone by *E.coRI,* Lane 1-3-clone which contain ADPGlcPPase β gene, Lane M-100bp Marker (100bp- 3kb**).**

CONCLUSIONS

ADP-glucose pyrophosphorylase catalyzes the synthesis of starch in plants and glycogen in bacteria. It is hetero tetramers consisting of two α- and two β-subunits. The α-subunit, commonly known as the small subunit, is the catalytic center that interacts with the substrates (Glc-1-P and ATP). The β-subunit, or the large subunit, plays a regulatory role (ADPGlcPPase β) These types of region(ADPGlcPPase β) are involved in the structural and functional organization of all eukaryotic genomes. In this paper, genomic DNA sequences of the ADPGlcPPase β gene from *Sorghum bicolour* was cloned and analyzed. One stronger matrix binding sequences, two T box, two DNA unwinding sequence, two A/T/C stretch of 25 nt were found in the ADPGlcPPase β DNA sequence.These results could provide valuable information to elucidate the molecular characteristics of ADPGlcPPase β gene and facilitate further investigation of the biological function of ADPGlcPPase β in *Sorghum bicolour*.

ACKNOWLEDGMENT

I would like to thanks Mr.Sameer Mulay,MD ASPL for providing me an opportunity to work in PBRC and compile the work for publication. The help rendered by Dr.S.K Rawal for his technical suggestion during the preparation of the manuscript is gratefully acknowledged. I am also thankful to Govt. of India, in the Department of Biotechnology, The University of Burdwan.

REFERENCES

1. Ballicora MA, Iglesias AA and Preiss J (2004). AGP;ADP-glucose pyrophosphorylase; a regulatory enzyme for plant starch synthesis. Photosynth. Res. 79: 1-24.
2. Ballicora MA, Laughlin MJ, Fu Y, Okita TW, et al. (1995). Adenosine 5'-diphosphate-glucose pyrophosphorylase from potato tuber. Significance of the N terminus of the small subunit for catalytic properties and heat stability. Plant Physiol. 109: 245- 251.
3. Beckles DM, Smith AM and ap Rees T (2001) A cytosolic ADP-glucose pyrophosphorylase is a feature of graminaceous endosperms, but not of other starch-storing organs. Plant Physiol. 125: 818-827.
4. Bode J, Kohwi Y, Dickinson L, Joh T, Klehr D, Mielke C, Kohwi-Shigematsu T (1992) Biological significance of unwinding capability of nuclear matrix associating DNAs. Science 255:195–197
5. Bode J, Stengert-Iber, M. Schlake T, Kay V, Dietz-Pfeilstetter A (1996) Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. Crit Rev Eukaryot Gene Exp 6:115–138
6. Boulikas, T. 1995. Chromatin domains and prediction of MAR sequences. Int. Rev. Cytol. 162A: 279–388.
7. Burton RA, Johnson PE, Beckles DM, Fincher GB, et al. (2002) Characterization of the genes encoding the cytosolic and plastidial forms of ADP-glucose pyrophosphorylase in wheat endosperm. Plant Physiol. 130: 1464-1475.
8. Burgess D, Penton A, Dunsmuir P and Dooner H (1997). Molecular cloning and characterization of ADP-glucose pyrophosphorylase cDNA clone isolated from pea cotyledon. Plant Mol. Biol. 33: 431-444.
9. Chen B-Y, Janes HW and Gianfagna T (1998). PCR cloning and characterization of multiple ADP-glucose pyrophosphorylase cDNAs from tomato. Plant Sci. 136: 59-67.
10. Cockerill, P. N., and Garrard, W. T. (1986). Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44:273-82.
11. Denyer K, Dunlap F, Thorbjornsen T, Keeling P, et al. (1996). The major form of ADP-glucose pyrophosphorylase in maize endosperm is extra-plastidial. Plant Physiol. 112: 779-785.
12. Dickinson, L.A., Joh, T., Kohwi, Y., and Kohwi-Shigematsu (1992) A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. Cell.70:631–645
13. Dillon, N. and F. Grosveld. 1994. Chromatin domains as potential units of eukaryotic gene function. Curr. Opin. Genet. Dev.4:260–264.
14. Fournier,R Namciu,S.J., and Blochlinger, (1998) Human Matrix Attachment Regions Insulate Transgene Expression from Chromosomal Position Effects in Drosophila melanogaster Mol. Cell.Biol.18:2382–2391.
15. Gasser, S.M. and Laemmli, U.K. (1986).Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of D. melanogasterCell. 46: 521–530.
16. Hart, C. M. and Laemmli, U. K. (1998). Facilitation of chromatin dynamics by SARs. Curr. Opin. Genet. Dev.8:519–525.
17. Huang B, Hennen-Bierwagen TA and Myers AM (2013). Functions of multiple genes encoding ADP-glucose pyrophosphorylase subunits in maize endosperm, embryo, and leaf. Plant Physiol. 164: 596-611.
18. Izaurralde, E., Mirkovitch, J. and Laemmli, U. K. (1988). Interaction of DNA with nuclear scaffolds in vitro. Mol. Biol.200:111–125.
19. Kavakli IH, Slattery CJ, Ito H and Okita TW (2000). The conversion of carbon and nitrogen into starch and storage proteins in developing storage organs: an overview. Aust. J. Plant Physiol. 27: 561-570
20. Laemmli, U.K., Kas, E., Poljak, L. and Adachi Y. (1992) Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr. Opin.Genet. Dev.2:275–285.
21. Paulson, J.R. and Laemmli, U.K. (1977) The structure of histone-depleted metaphase chromosomes. Cell. 12: 817–828

22. Phi-Van, L. and Strätling, W. H. (1988). The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J.7:655–664.
23. Preiss J (1982) Regulation of the biosynthesis and degradation of starch. Annu. Rev. Plant Physiol. 54: 431-454.
24. Preiss J, Ball K, Hutney J, Smith-White B, et al. (1991) Regulatory mechanisms involved in the biosynthesis of starch. Pure Appl. Chem. 63: 535-544.
25. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning; A Laboratory Manual, 2nd ed., New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor
26. Slattery CJ, Kavakli IH and Okita TW (2000). Engineering starch for increased quantity and quality. Trends Plant Sci. 5: 291-298.
27. Smith AM, Denyer K and Martin CR (1995) What controls the amount and structure of starch in storage organs? Plant Physiol. 107: 673-677.
28. Spitzner,J.R. and Muller,M.T. (1988) Eukaryotic topoisomerase 11 preferentially cleaves alternating purine-pyrimidine repeats.Nucleic Acids Res. 16:5533–5556.
29. Thorbjornsen T, Villand P, Kleczkowski LA and Olsen OA (1996). A single gene encodes two different transcripts for the ADP-glucose pyrophosphorylase small subunit from barley (Hordeum vulgare). Biochem. J. 313: 149-154.