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Title:	CONSTRUCTION AND CHARACTERIZATION OF A cDNA LIBRARY OF Eleusine coracana L.Gaertn
Publisher:	Birsa Agricultural University, Kanke, Ranchi, Jharkhand
Language:	en_US
Type:	Thesis
Pages:	35
Agrotags:	null
Keywords:	CONSTRUCTION AND CHARACTERIZATION OF A cDNA LIBRARY OF Eleusine coracana L.Gaertn

Abstract:	A cDNA library refers to a complete, or nearly complete set of all the mRNA contained within a cell or organism. cDNA libraries are prepared from total or enriched Poly(A)+ single stranded mRNA that is converted into a double-stranded DNA copy of the message using the enzyme reverse transcriptase. cDNA libraries are of great importance as: when the expression of the eukaryotic gene is required in a prokaryote (a bacterium) the sequence to be expressed must be free of introns as bacteria do not possess the enzymes necessary for removal of introns. The mRNA sequences from which cDNA libraries are prepared are mature, processed RNAs from which introns have been removed by eukaryotic enzymes. cDNA libraries have only expressed sequences so screening is easier for genes encoding polypeptides or proteins. Specific gene isolation is easier as tissue specific expression enriches the cDNA libraries for that gene. Comparison of a cDNA sequence with a genomic DNA sequence demarcates the position of the relevant gene and reveals the exon-intron boundaries. The present experiment entitled “Construction and characterization of a cDNA library of Eleusine coracana L.Gaertn.” was conducted in the molecular biology laboratory of College of Biotechnology, Birsa Agricultural University, Kanke, Ranchi. The work done has been summarised below: Total RNA was isolated using Plant RNA isolation kit (Bangalore Genei). From the total RNA, mRNA was isolated using oligo dT column. mRNA was used to set a RT-PCR with designed heterologous primer pairs. The products were in the range of 300 to 72bp, distinct amplification band was obtained only for primer pair 1 and it was between 70-100bp. Summary 35 The fragments were ligated with SmaI and EcoRV digested pBS separately. After ligation JM101 strain of E.coli was transformed with the recombinant plasmids. Blue/white screening of colonies was used to select 36 putative positive colonies. Colony PCR of the clones showed amplification of two distinct amplicons about 140 and 120 bp long. The 140 and 120 bp amplicons were being amplified in transformed and nontransformed E.coli JM101. This indicates that the primer pair 1 has homology to some sequences in the bacterial chromosome. FASTA analysis for homology showed that the sequences of primer pair 1 had no similarity to any reported bacterial sequences.
Description:	CONSTRUCTION AND CHARACTERIZATION OF A cDNA LIBRARY OF Eleusine coracana L.Gaertn
Subject:	Biotechnology
Theme:	CONSTRUCTION AND CHARACTERIZATION OF A cDNA LIBRARY OF Eleusine coracana L.Gaertn
These Type:	M.Sc
Issue Date:	2008
Appears in Collections:	Thesis (/handle/1/93550)

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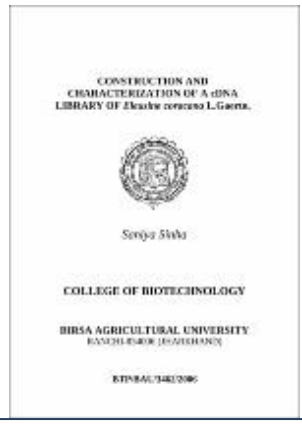
CONSTRUCTION AND
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