Fungal Genetics Reports

Volume 32 Article 7

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Recommended Citation

Verma, M., B. Ghosh, C. Chambers, R.J. Crouch, and S.K. Dutta (1985) "A new rDNA clone isolated from Neurospora crassa wild type strain 74A containing all coding regions," *Fungal Genetics Reports*: Vol. 32, Article 7. https://doi.org/10.4148/1941-4765.1574

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Abstract

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The pMF2 rDNA clone of Neurospora isolated from the slime mutant (Free et al., J. Bact., 137:1214, 1979) has been used extensively This clone contains a 5.9 kbp Pst I fragment that includes 17s, 5.85, 26s rDNA, a 331 bp internal transcribed spacer and 700 bp external spacers (Chambers, Crouch and Dutta, 1964 unpublished). Recently a comparison of the nucleotide sequences of rDNA of Neurospora crassa 74A strain (FGSC 987) and Saccharomyces carlsbergemis using a computer program of Wilbur and Lippman (PNAS 8:726, 1983) has been performed by Chambers, Crouch and Dutta (Paper in preparation, 1985). The alignments indicates the general lack of homology of the internal transcribed spacer regions in N, crassa and S. carlsbergensis.

The general procedure for cloning using pBR322 has been described by us earlier (Neurospora Newsl. 31: 20-21, 1984). Using a Pst I fragment of Neurospora crassa wild-type strain 74A (FGSC 987), we have constructed a clone (namely pCC103) which has 960 bp additional rDNA sequences than pMF2. Plasmid pBR322 was restricted with Pst I and treated with bacterial alkaline phosphatase. The preparation was extracted with phenol and chloroform and precipitated with alcohol. Total nuclear DNA isolated from wild type N. crassa strain 74A was restricted with Pst I and a fragment containing the entire rDNA was isolated (using Pst I 5.9 kbp pMF2 fragment as probe) and ligated to Pst I-digested pBR322. The product was transformed into Escherichia coli strain LE392 and clone pCC103 was isolated which was resistant to tetracycline and sensitive to ampicillin. These results were confirmed by restriction endonuclease analysis (Table If and hybridization of Southern blots of pCClO3 and pMF2 using nick-translated pMF2 as probe,

We have performed a thorough restriction analysis of these two clones and our important inference is as follows: The size of the insert in pCC103 is 960 bp larger (6.86 kb) than that of pMF2 (5.9 kb) and thus it seems likely that pCClO3 contains some additional sequences. Since the rRNA coding sequences are conserved it is likely that these additional sequences are present in the nontranscribed and/or external spacer regions,

Restriction analysis of pCC103 and pMF2 indicates that the pattern of digestion of DNAs from these two clones is different and the size of the fragments (base pairs) produced as a result of digestion with Pst I, Hind III, Him II, Bam HI, Sst II, Sma I and Xba I are shown in Table I.

The comparison of the digestion patterns of pCClO3 and pMF2 (Table I) suggests the existence of one additional site for Bam HI and Hind III in pCC103. The detailed analysis of the exact locations of these restriction sites is in progress. This analysis which is part of our ongoing work will help to determine the initiation and termination sites and processing sites in this clone.

This clone should be useful in screening and identification of rDNA clones for a variety of Neurospora species.

TABLE I

Restriction patterns of pCC103 N. crassa 74A rDNA cloned in pBR322 plasmid in comparison to that of pMF2 rDNA clone of N. crassa slime mutant

Pst I		Hind III		Bam HI		Hinc II		Sma I		Sst II		Xba I	
pCC103	pMF2	pCC103	pMF2	pCC103	pMF2	pCC103	pMF2	pCC103	pMF2	PCC103	pMF2	pCC103	pMF2
6860	5900	5680	6200	5110	5200	3340	5200	7900	7500	6500	6700	11250	10300
4326	4326	4700	4100	3780	4700	3250	1700	1650	950	3050	1900		
		800		2000	300	2250	1560	780	860	1700	1700		
				300		1400	1100	630	760				
						300	537	260	260				

The numbers indicate the sizes (nucleotide base pairs) of DNA fragments generated after digesting the clone with various restriction endonuclease. 0.5 to 1 µg of DNA was digested with the restriction enzyme (the supplier's conditions of digestion were strictly followed) and the digested product was analyzed on agarose (0.6-1.2%) or polyacrylamide (4-6X) gels. Lambda Hind III and pBR322 HaeIII digested products were taken as the molecular weight (nucleotide base pairs) size markers.

(Supported in part by a DOE grant to SKD)- - - Molecular Genetics Laboratories, Botany Department, Howard University, Washington, DC 20059, and the National Institutes of Health, Bethesda, MD 20014.