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Cloning of the rDNA repeat unit: An EcoRI fragment spanning the entire non-transcribed spacer region of *Neurospora crassa* wild type strain 74A.

Nontranscribed spacer regions are known to be variable between species, and thus may be used to identify species differences of the genus Neurospora. This led us to clone this region from a known 74A wild type *N. crassa* strain. pMF2, a plasmid containing the coding sequences for 17S-5.8S-26S rRNA with additional flanking sequences, was constructed by Free et al. in 1979. We used their clone as a probe to clone adjacent sequence. Initial cloning experiments were conducted using pBR325. A clone of 3400 bp containing most of the nontranscribed spacer region of Neurospora crassa rDNA sequences was inserted into the chloramphenicol gene EcoRI site. The clone may include the promoter, as well as the termination sites, for transcription of rRNA 17S, 5.8S and 26S in *Neurospora crassa* 74A wild type.

To achieve this, plasmid pBR325 was restricted with EcoRI and then treated with bacterial alkaline phosphatase (BAP) at 65° C for 1h. The preparation was then shaken with an equal amount of phenol saturated with 0.1 M Tris-HCl, pH 9.0, followed by the addition of an equal volume of chloroform, shaken again, and then centrifuged at 15,000 rpm for five min. The upper aqueous solution was removed, brought to 1M with NaCl EtOH precipitated in dry ice for 15 min, then centrifuged again. The pellet was vacuum dried and resuspended in distilled water. Total nuclear DNA isolated from wild-type *N. crassa* strain 74A (FGSC #987) was restricted with EcoRI. The *N. crassa* nuclear EcoRI-digested DNA was subjected to electrophoresis on 0.7% agarose gels and the band which carresponded to the nontranscribed spacer sequences was cut out of the gels, electroeluted, and run over a DE-52 column. This DNA was ligated with the BAP-treated EcoRI-digested pBR325 DNA in a ration of 1O:1 to lOO:1, and then incubated at 4° C for 1 to 2 days. *Escherichia coli* strain LE 392 was used for the transformation experiments.

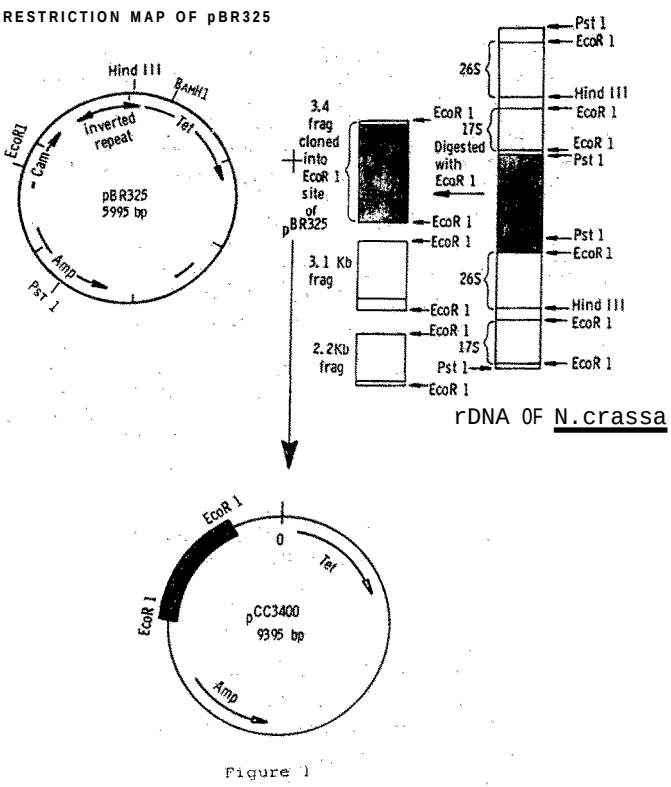

Figure 1

Figure 1 explains the cloning strategy and gives the restriction map of both pBR325 and the new plasmid pCC3400 which was made after inserting the rDNA sequences of *N. crassa* into the EcoRI site of pBR325.

The colonies whose DNA generated the appropriate restriction fragment sizes were rechecked and the DNA blotted onto nitrocellulose paper, then hybridized to an rDNA 32P-labeled probe. This probe contained approximately 600 bps in common,with the spacer region which was being cloned. The probe was derived from pMF2 DNA (Free et al. 137: 1219-1226, 1979). When the plasmid pMF2 DNA is restricted with Pst I two fragments are generated - a 4.36 kb fragment (the cloning vector, p8R322) and a 6 kb fragment (the Neurospora rDNA insert), The 6 kb fragment contains the entire coding regions for 17S to 5.8S to 26S rRNA and additional sequences approximately 1 kb which are portions of the spacer regions.

The EcoRI fragment inserted into the EcoRI site of pBR325 is approximately 3.4 kb in size and contains mostly the nontranscribed spacer region, plus external transcribed spacer region, and additional coding sequences approximately 150 bps near the 3' end of 26S rDNA. The desired clone contains the 3.4 kb insert, as well as the 6 kb piece of pBR325 DNA. When this DNA is restricted it generates the following fragment sizes: using Hind III → 5.5 kb, 2.9 kb, 900 bp; using Pst I → 4.9 kb, 2.8 kb, 1.6 kb; using EcoRI → 5.9 kb, 3.4 kb; using BamHI → 9.3 kb; using XhoI → 8.9 kb, 400 bp; and using SmaI 9.3 kb. (Supported in part by a Department of Energy Grant to SKD.) - - - Molecular Genetics Laboratories of Howard University, Botany Department, Washington, D.C. 20059, and the National Institutes of Health, Bethesda, MD 20014.