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Abstract

Chromosome walks from the *cpc-1* and *Bml* loci were extended and the *cys-2* locus cloned by sib-selection using a subset of the Orbach/Sachs *Neurospora crassa* genomic library.

Chromosome walking and gene cloning using a *Neurospora crassa* Linkage Group VI-specific library.

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Chromosome walks from the *cpc-1* and *Bml* loci were extended and the *cys-2* locus cloned by sib-selection using a subset of the Orbach/Sachs *Neurospora crassa* genomic library.

A 222 clone sub-library of the Orbach/Sachs *Neurospora crassa* genomic library was assembled in three microtiter dishes representing cosmids with inserts specific to LGVI based on preliminary assignment of Neurospora cosmid clones to specific linkage groups by the University of Georgia Neurospora genome project (J. Arnold, personal communication). A sub-library adds efficiency to chromosome walking and to sib-selection of genes; in this example 222 clones were blotted for hybridization or prepared for transformation versus 4,800 for the original library. The LGVI library was used to extend chromosome walks that were initiated to clone the *ylo-1* and *vvd* loci. The chromosome walks originated at the *cpc-1* locus and the *Bml* locus and represented approximately 420 kb and 110 kb, respectively, of Neurospora sequences on LGVIL (Figure 1; Wan *et al.* 1997 Fungal Genet. Biol. 21:329-336). The *cpc-1* and *Bml* walks had stopped due to apparent gaps encountered when screening the entire Orbach/Sachs genomic library. Using our sub-library we were able to identify cosmids extending the *cpc-1* and *Bml* walks that were not identified in our screen of the entire Orbach/Sachs library although the filters containing these clones appeared to be in good condition for hybridization. The LGVI sub-library allowed extension of the *cpc-1* walk, from cosmid G13:4:D, towards the LGVI left telomere by seven "steps". One new step has been taken from one end of the *Bml* walk using a cosmid G12:10:C based probe (Figure 1). No new cosmids have been found to extend the other end of the *Bml* walk or of the *cpc-1* walk. Several additional overlapping cosmids were identified in the extension of the *cpc-1* walk. The X24:12:C probe also identified cosmids G8:8:C and G9:1:B, the X25:9:C probe also identified cosmid X9:12:G. We have not determined the size of the additions to the *cpc-1* and *Bml* walks.

The LGVI library was divided into 28 primary pools by pooling cosmid DNA representing one or two rows from each microtiter dish. Each primary pool has from six to twelve cosmid clones.

Two rounds of transformation into *cys-2* spheroplasts identified a *cys-2*$^+$ cosmid, X11:5:C. A probe based on X11:5:C identified one additional cosmid, X17:4:F, which is also *cys-2*$^+$ (Figure 1). Cloning of *cys-2*$^+$ establishes that the walk from the *cpc-1* locus towards the LGVIL telomere has not reached the *cys-2* locus (Figure 1).

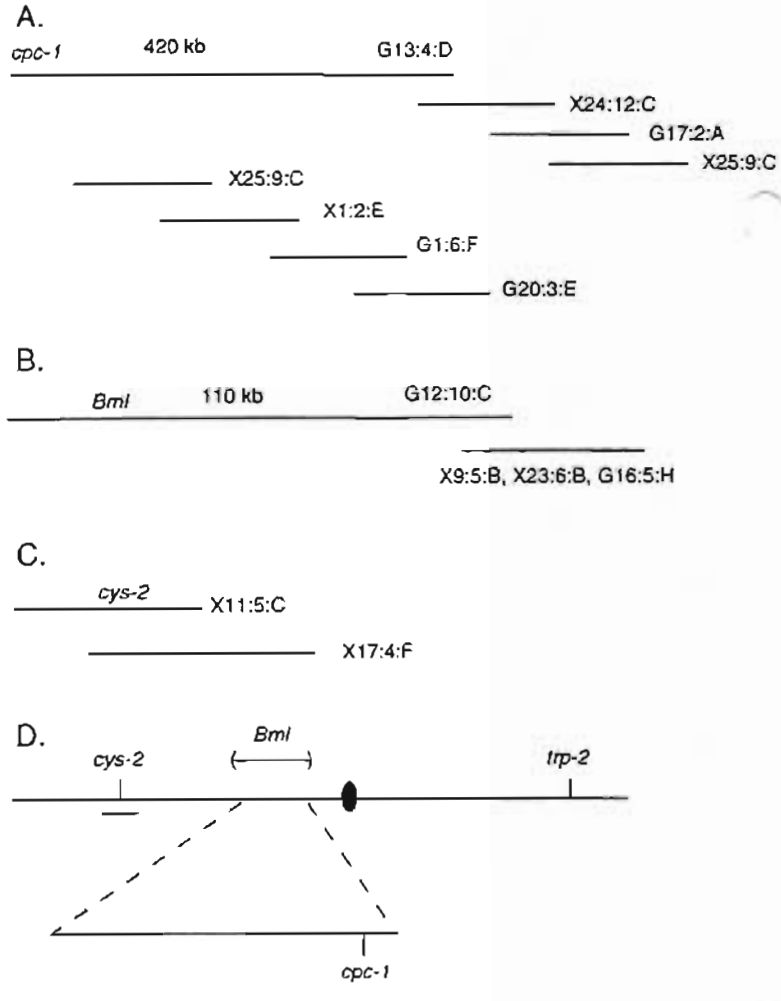

Figure 1. Chromosome walks on Neurospora LGVI. The figure is not to scale.
(A) Seven steps added to the *cpc-1* walk. (B) Three cosmids identified using a G12:10:C probe fr the *Bml* walk. (C) Two *cys-2*$^+$ cosmids. (D) Relative locations of the *cpc-1* and *cys-2* walks on LGVIL. The location of the *Bml* walk on LGVIL has not been established with respect to the *cpc-1* walk.