

Figure S11 - *C. posadasii* Optical Map

Genomic DNA was prepared in agarose plugs using a modification of the Scharz protocol (Pan and Cole 1992a; Schwartz and Cantor 1984). The mycelial phase of *C. posadasii* C735 was grown in liquid medium which contained 1% glucose plus 0.5% yeast extract (GYE) at 30°C for 48 h in a gyratory shaker-incubator. Approximately 40 mg (wet weight) of mycelia was resuspended in 1.0 ml of 125 mM EDTA-50 mM sodium citrate to which 10 mg of chitinase (200 to 600 units of activity per g; Sigma) was added. The suspension was immediately mixed with 1.5 ml of molten 1% low-melting-point agarose (at 55°C in 125 mM EDTA-50 mM sodium citrate), which was added to plug molds and cooled to 4°C for 20 min. Spheroplasts were produced in the agarose by incubation of the plugs in 50 mM sodium citrate (pH 5.7)-0.4 M EDTA (pH 8.0)-1% 2-mercaptoethanol at 37°C for 24 h. The plugs were placed in fresh solution as described above and incubated under the same conditions for an additional 24 h. To lyse the spheroplasts, plugs were washed three times with 50 mM EDTA (pH 8.0) and then incubated in NDS buffer (0.5 M EDTA [pH 8.0], 10 mM Tris-HCl [pH 9.5], 1% lauroylsarcosine) which contained 2 mg of proteinase K per ml at 50°C for 24 h. The plugs were subsequently rinsed three times with 50 mM EDTA (pH 8.0) and stored at 4°C in 0.5 M EDTA. The plugs were shipped to OpGen, Inc. (Madison, WI) where a *Mlu*I optical restriction map (Schwartz et al. 1993) was prepared.

In the resulting map (below), the chromosomes are wide bars bounded above and below by sequence assembly contigs. Restriction cut sites are vertical black bars and homologous regions are shaded in green. Lines connecting contigs to chromosomes illustrate the directionality of the sequence contig relative to the chromosomal restriction map.

