

May 29, 1997

Kevin McCluskey, Curator
Fungal Genetics Stock Center
Department of Microbiology
University of Kansas Medical Center
Kansas City, KS 66103

Dear Kevin,

I'm depositing five Neurospora crassa strains (MB 5, 17, 19, 26 and 27) that are all RIP'd mutants of a fatty acid synthase (FAS) gene. The proposed designation for these mutants is cel-2. The particular RIP'd gene is the one showing homology to the fatty acid synthase (beta subunit) gene of Saccharomyces cerevisiae (FAS1) and other fungi.

To RIP the gene, I used a plasmid provided by Dorsey Stuart's lab. I assume it is the same one identified out of the Stock Center's lambda-ZAP library that is mentioned by R. Yamashita and D. Stuart in the Fungal Genetics Newsletter (#43, pp. 66-67). The plasmid contains a portion of the gene that is about 3 kb of the 3' end (the complete S. cerevisiae gene is about 6 kb; Kottig et al., Mol. Gen. Genet. 226: 310-314). I cotransformed this plasmid into wild type with pBARMTE1 and selected for Ignite resistance, then checked stable transformants for the presence of a second copy of the beta-FAS gene by Southern blotting.

A transformant having a second copy was crossed to wild type, and single ascospores from the cross were grown on VMN + 1% Tween 40. Tween 40 provides palmitate, the optimal supplement for the cel strain, which synthesizes little fatty acid de novo. The cel strain, if it has a defective FAS, should have a defect in the alpha subunit (based on its low level of 4-phosphopantetheine in FAS, which is bound to the acyl carrier protein region of this subunit). Beta-subunit mutants were expected to have a phenotype similar to cel. Each of these 5 lines has a Tween 40 or palmitate supplementation requirement, showing virtually no growth without the supplement. The requirement is thus tighter than for cel, which grows very slowly without supplementation.

The ability of these lines to synthesize fatty acids de novo was determined by feeding them 30 μ M labelled (deuterated) palmitate, which we detect by GC/MS. These lines synthesize less than 10% of their total