

STOCK #: P-387A,B

SOURCE ORGANISM: *C. reinhardtii* 21 gr *mt+*

TYPE OF DNA: nuclear

INSERT: nuclear DNA, 14.5 kb Sal I/Bgl II genomic fragment

GENES PRESENT: *nit-1*

PLASMID NAME: pMN24 #1, #2

VECTOR: pUC119

HOST STRAIN: DH5

SELECTION: ampR, blue on X-gal

SOURCE: Lefebvre, August 1991

COMMENTS: See Fernández et al. PNAS 86, 6449-6453 (1989) and notes from Lefebvre on transformation.

Excerpts from letter from Pete Lefebvre August 14, 1991:

The plasmid is pMN24 precipitated in EtOH. It is described in the Fernández et al. PNAS paper initially describing nitrate reductase. It is a 14.5 kb Sal I - Bgl II fragment cloned into pUC119. This vector is a very high copy number vector, and small plasmid preps (50 ml) give lots of DNA.

A few caveats. We know from the primary sequence, which we hope to publish in the next couple of months, that NR contains some terrible stretches of GT repeats in different introns, and it can rearrange during plasmid growth if you are careless in choice of host strains for growth. We have used DH5 alpha as a reliably rec- host for growth, but any good recA- host should work. You should check your plasmid preps to make sure there are no rearrangements. Ideally, transformation should be used as the assay.

I am including two different cw-15 nit-1-305 strains: 5D is mt-, and is the strain that I have sent to more than 75 labs, and it is the best high efficiency transformation strain I know. The other one is from Rogene Schnell. It is B69, cw-15 nit-1-305 sr-1 mt+. We have less experience with transforming it....The 5D- strain clearly has problems during outcrosses with progeny viability. Mating and zygote germination are fine, but you can expect 80 or 90% progeny death in backcrosses. We have a lot of transformants we want to do genetics on, and we routinely have to recover rare F1 survivors and keep backcrossing to get them to behave. I am afraid there may be some sort of reciprocal translocation (for example) in the strain to explain this behavior. The B69 strain does not have this problem, but it also doesn't transform as well. That is a tricky assessment to make, given how incredibly variable transformation frequency is with beads. At times we reach levels of 200-500 colonies per  $10^7$  cells, and at other times under "identical" conditions we get 1% of that....