Identification of a cosmid clone containing the Neurospora crassa lys-5 and un-4 genes, isolation of a partial lys-5 cDNA and associated chromosome walking.

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**Recommended Citation**  
[https://doi.org/10.4148/1941-4765.1238](https://doi.org/10.4148/1941-4765.1238)
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Abstract
The un-4 gene of Neurospora crassa was cloned to determine the limits of a chromosome walk on linkage group VI (LGVI) and to allow analysis of un loci on LGVI. Subsequent analysis identified the lys-5 locus on the same cosmid clone as un-4. We have isolated and sequenced a partial lys-5 cDNA clone and initiated a chromosome walk from the lys-5, un-4 cosmid clone.

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Identification of a cosmid clone containing the *Neurospora crassa* *lys-5* and *un-4* genes, isolation of a partial *lys-5* cDNA and associated chromosome walking.

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The *un-4* gene of *Neurospora crassa* was cloned to determine the limits of a chromosome walk on linkage group VI (LGVI) and to allow analysis of *un* loci on LGVI. Subsequent analysis identified the *lys-5* locus on the same cosmid clone as *un-4*. We have isolated and sequenced a partial *lys-5* cDNA clone and initiated a chromosome walk from the *lys-5*, *un-4* cosmid clone.

A chromosome walk from the *epc-1* locus has been extended 420 kb towards the left telomere of linkage group VI (LGVI) (36). One of three heat-sensitive loci of unknown function on LGVI, *un-13*, was found in the *epc-1* walk. The *un-4* locus maps to LGVI. Three rounds of transformation using sib-selection with cosmid DNA pools from the Orbach/Sachs *Neurospora crassa* genomic library identified an *un-4* cosmid, G13:8:G, by selection for transformants able to grow at the restrictive temperature of 34°C. A 1.2 kb cDNA isolate from a cDNA library (based on mRNA isolated from dormant conidia and kindly provided by M. Sachs), designated pYW19-2, was identified using a G13:8:G insert probe.

DNA sequence analysis of pYW19-2 identified an open reading frame encoding a deduced polypeptide with strong similarity to homocitrate synthases and isopropylmalate synthases from other organisms (Figure 1). *Neurospora* *lys-5* mutants lack homocitrate synthase activity. G13:8:G DNA complements *lys-5* spheroplasts allowing growth on minimal medium. *lys-5* maps 2% away from *un-4* and *un-4*, by definition, is irreproducible by supplementation at the restrictive temperature. Thus, *un-4* and *lys-5* are separate loci and both are present in G13:8:G. pYW19-2 likely represents a partial *lys-5* cDNA clone. The partial deduced *Lys-5* polypeptide has highest similarity to the homocitrate synthase of *Penicillium chrysogenum* with 80% identity in an optimized alignment (Figure 2).

1 CGTATTTGAG TATGCAGGTC CAAAGGAGGG CATTGCC TCTTCTCCGC AAGATTTGCT
V I E Y V K S I G L E V R P S S E D S P
1 CCGCTCCGAT CTCCGTCAT CTCCGTCAT TTCCGTCAT GCTGACAGG GCGCGTTCCAA
D L R S L Y L R A V D K V G H V H
1 CGCTCTGGAT ATCCGCGGA CTGGCTGCGG CCGTCTCCGA CCGAGACGTG AGTGACCTGT
R V G I A D T V G C A S P R Q V Y D L V
185 CGTACCGCT CCGCGCGCCG TTTGCGCGCA TATGCGCGCA CATTCCACAG AGCGACACCG
R T L R G V V S C D I E T H F H D D T G
211 CTGGCGCGTGT GACCAAGCCT CCTGCGCCGTG CGGCCCAGCA TGCGCCGCTC
C A X A N A Y C A L E A G A T H I D T S
301 CGTCTGCTCT ATGCGGGACG GTAAGGCTGT CACCCCGCTCT GCGGCCCTGA TGCGCTGCGT
V L C I G E R N G I T P L G G L M A R M
361 GCTGCTGAGT AGCCGGCGCAT AGCTGAGACG TGGCTGGTAC GCTGACACCT CCGCGCGCCG
I U T S P D V Y V K S I G L E V H V H
421 CGCGAGATGG GTTGGCGCCG CGTGCTGAGT CCAACGCGCC CCGCCCGGAC GCGCGCGGGCC
E D L V B A V E N T P T F N N P J T G
481 TTTCTGCACCC TCTACCCCGA AGCCAGCGCC CCGAGCGGCC GCGGCCCGCG GTACCTCCA ACAAACCCCG
3 C A S P T H K A C G I H A K A I L N N P S
541 CACCCCTGAA AATGCCTAGCC CTGGCGCGGT CCGTCTCCAC GTGCTGGCGC TCTGGCGCGT
E Y I L N P A D F G L T R Y V H F A S
601 GGCTGGCTAC GGTGCGAAGG GCGTGCGAGC GGTGCGGCGC TCTTGTGCGG TCTTGTGCGG
R L G W N A V K T R V G Q L G L E M T
661 CGCGAGCA CTGAGCGAGT GTCGCGCGC GACGAGCGCC CGCGGCGCGC GGCGGCGCGC
D D Q V K E C T A K I K A L A D V R P I
723 CGCCATGGAG CAGCGGGGAT CGATCTGAGG TACCTGCACC CTTGCTGGCG CTTGCTGGCG
A I D D A S I R T P H L G L H E Q N
781 CAAAGCGGCG CGTCGCGCTG TTTGCGGAA CTAAGCGGAA CGCGGCGGTT CGCGGCGCG
K V Q P P A V V E N *
841 ATGCACCGTT TGGAGTGGG GCGAAATGAC CAGATTGTTT ACGGAGGAGG AATCCATGGAG
901 CGCTGATGTT CTCTTTTCTC GGTTATAATT CGCTTATGCT CCGAGCTTTT CTGGCGGCTG
961 TTAGCTGCTA TAATCTGCTC GCTGCGCGGA CAAACTGCGG GTCTGGCGGA GTTGAACGGG
1021 ATGGCGCAGG AGGCGGATAT ATCCGCGCGC GTCTTATCTC AGGGGAGGAGT CTGGCGGAA
1081 ATATGCGGCA AGATAGAGAA AATCAGCGGG AGACAGAAGT ACAAGCTGCA CTTGCTGGC
1141 CTTGCGGCGG AAAAAAAAAA AAAAAAAAA AA

Figure 1. Nucleotide sequence of the cDNA insert of pYW19-2 and the deduced polypeptide product (GenBank AF142777). The stop codon is indicated by a *. Several isolates, including NC4A2-T7, from the Neurospora Genome Project, University of New Mexico, overlap pYW19-2 from position 549 to the polyadenylation site.
The pYW19-2 insert was used to probe a Southern blot of G13:8::G restriction digests. Results suggest that an approximately 6.3-kb EcoRI fragment contains the lys-5 gene. As cosmid G13:8::G was not identified in the cpe-1 walk we initiated a chromosome walk from the lys-5/un-4 region in an attempt to link up to our cpe-1 walk. A G13:8::G based probe identified cosmid X6:6. A X6:6::F based probe identified cosmid X22:2:B. No new cosmid clones were identified with a X22:2:B based probe.

*Penicillium* c. HC Synthase 181 *IEVIEFVKSK GIEIRFSSED*

*Neurospora* Lys-5

| Pc HC | SFRSDLVLDDL SIYSAVDKVG VNRVGIAADTV GCASPRQVYE LVRVLRGVG |
|-------|---------------|--------------------------|--------------------------|--------------------------|
|       | |||||+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | SFRSDLVLDDL SLYRAVDKGV VGHRVGIADTV GCASPRQVYD LVRVLRGVS |

| Pc HC | CDIETHPHND TGCAIanAF C L EA GATHID TSVLGIGERN GITPLGGLMA |
|-------|---------------|--------------------------|--------------------------|--------------------------|
|       | |||||+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | CDIETHPHND TGCAianAYC LEA CAYHID TSVLGIGERN GITPLGGLMA |

| Pc HC | RMNADREYV KS K YKLEKLK EI EDLVAEAV EVNI PFFNNYI TGFCAGTHKA |
|-------|---------------|--------------------------|--------------------------|--------------------------|
|       | |||||+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | RMIVTSPPDV KS K YKLEKLK EI EDLVAEAV EINTPFNNFI TGFCAGTHKA |

| Pc HC | GIAKAILNN PSTEINPDA DFGMSYVHF ASRGTGWNAI KSRAQQKLE |
|-------|---------------|--------------------------|--------------------------|--------------------------|
|       | |||||+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | GIAKAILNN PSTEINPDA DFGSTRYVHF ASRGTGWNAI KTUGQLGL |

| Pc HC | MTDQYKEKCT A W I KAMADR I PAVD DADI SI IRAYHRNLKS G ENKPLDLI |
|-------|---------------|--------------------------|--------------------------|--------------------------|
|       | |||||+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | MTDQYKEKCT A W I KALADVIR PIAVDA DASI IRTFHLGLHL QNKVQPAAV |

| Pc HC | AEEQAAFAAK E KELLEAQAA GLPV |
|-------|--------------------------|--------------------------|--------------------------|--------------------------|
|       | |+|+|+| |+|+| |+|+| |+|+| |+|+| |+|+| |+| |
| Lys-5 | EN |

Figure 2. Comparison of the amino acid sequence of the homocitrate synthase of *Penicillium chrysogenum* (Gene Bank AJ223630) and Lys-5. Identical residues are indicated by a vertical line. Residues of similar chemical properties are indicated by a +. A motif conserved in all known homocitrate synthases is underlined.