New recombinant linear DNA-elements derived from *Kluyveromyces lactis* killer plasmidsJörg Kämper, Friedhelm Meinhardt^{*,†}, Norio Gunge¹ and Karl Esser

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The cytoplasmically localized linear killer plasmids pGKL1 and 2 from *Kluyveromyces lactis* carry terminal inverted repeats (TIRs) and 5' covalently linked proteins. pGKL1 (8.8 kb) encodes the killer toxin, whereas pGKL2 (13.4 kb) is responsible for the maintenance of both linear plasmids (1). After integration of a nuclear gene (*leu2* from *Saccharomyces cerevisiae*) via *in vivo* recombination into pGKL1, nuclear associated linear plasmids with telomere structures were obtained, indicating that nuclear genes cannot be expressed in the cytoplasm under control of their own promoters (2). Thus the promoter and terminator regions of ORF2 from pGKL1 (killer toxin) were fused to the *leu2* gene (Fig.1), ensuring the cytoplasmic expression of the gene.

Fig. 1: *Leu2*-gene flanked by promoter and terminator sequences of pGKL1. The ATG-

startcodon of ORF2 from pGKL1 is fused in phase to the second codon of *leu2*; this codon was converted from Ser to His by creation of a new *Nsi*I-site via *in vitro* mutagenesis. The boxed sequences indicate the upstream conserved sequences (ucs) of pGKL1 genes (3). ■ = pGKL1 derived sequence □ = *leu2* sequence — = coding region.

Normal printed numbers: nucleotide position corresponding to pGKL1-sequence (3,4,5). Numbers in *italics*: corresponding to *leu2*-sequence (6).

Fig. 2 (right side): DNA-preparations of the strain F102-2, carrying the plasmids pGKL1 and 2 and of a transformant (T43) with the additional plasmid pJKL1.

In order to obtain recombination between the homologous regions of the DNA-fragment and pGKL1

(black boxes in Fig. 1), the linear fragment was transferred to the strain F102-2 of *Saccharomyces cerevisiae*

(7), which carries both killer plasmids.

Results:

- 1.) 95% of the transformants carry a new 7.3 kb linear plasmid, pJKL1 (Fig. 2).
- 2.) Treatment with λ -Exonuclease and Exonuclease III indicates that the 5'ends of plasmid pJKL1 are protected (Method: 8).
- 3.) Extraction of protein-associated DNA suggests covalently bound proteins (Method: 9).
- 4.) The physical map and the position of the *leu2* gene, determined by Southern-hybridizations, are exactly as expected.
- 5.) The end-fragments of pJKL1 hybridize with an oligonucleotide-probe specific for the TIRs.

Thus, the constructed plasmid pJKL1 has the same physical structure as the native linear plasmids (TIRs, covalently bound proteins), but ORF2 of the native plasmid pGKL1 was replaced with *leu2*. *pJKL1* represents a new class of recombinant molecules based on cytoplasmically localized linear DNA elements.

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