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A minichromosome of LGVI from crossing two quasi-terminal reciprocal translocations

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Abstract

Non-reciprocal translocations, in which one chromosome is a pure donor and another is a pure recipient, have found abundant uses in genetics, molecular biology, and cytology (Perkins 1997Advances in Genetics 36:239-398). Our original aim was to prepare a strain carrying a chromosome truncated in both arms, with the idea that such a small chromosome would be easily purified by pulsed field electrophoresis and would be a good preliminary substrate for genomic sequencing.

A minichromosome of LGVI from crossing two quasi-terminal reciprocal translocations

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Non-reciprocal translocations, in which one chromosome is a pure donor and another is a pure recipient, have found abundant uses in genetics, molecular biology, and cytology (Perkins 1997Advances in Genetics 36:239-398). Our original aim was to prepare a strain carrying a chromosome truncated in both arms, with the idea that such a small chromosome would be easily purified by pulsed field electrophoresis and would be a good preliminary substrate for genomic sequencing. While current strategies have bypassed this approach, the minichromosome strain, and others which could be constructed in a similar manner, may yet be useful for study of chromosome pairing and segregation in meiosis. We therefore describe its preparation.

The minichromosome carries the centromere and linked markers from LG VI, but with both arms very much abbreviated. Clearly such a chromosome can result from crossingover in a cross between two quasi-terminal translocations, one of which transfers much of the left arm of LGVI to a second chromosome, and another which transfers a substantial piece of the right arm to yet a third chromosome. Preliminary identification of the desired product is much simplified by the inclusion of genetic markers. The translocation T(VILÆI)T39M777 trp-2; ad-8 a (prepared from strains kindly furnished by David Perkins) was crossed to prototrophic T(VIRÆIII)OY329 A. Candidates from the crossover class, ad-8 but tryptophan-independent, could be either the desired euploid strains carrying the minichromosome, or partial diploids. The candidates were screened for fertility on fluffy lawns and the Barren partial diploids were discarded. The fertile strains, which predictably produced many white deficiency spores in these testcrosses, were examined for the possible presence of a minichromosome. Representative examples carried a chromosome in the size range of 1.6-2.0 megabase pairs, as judged from examination of intact chromosomes by the CHEF gel technology. Acriflavin-stained pachytene chromosomes from a homozygous cross of two of these of the opposite mating type were examined by N. B. Raju, who observed paired minichromosomes of a size consistent with the expected double translocation and inconsistent with either parental single translocation.

Cultures of each mating type have been deposited in the FGSC as #8320 and #8404 (A and a, respectively) under the category "Heteromorphic Strains".

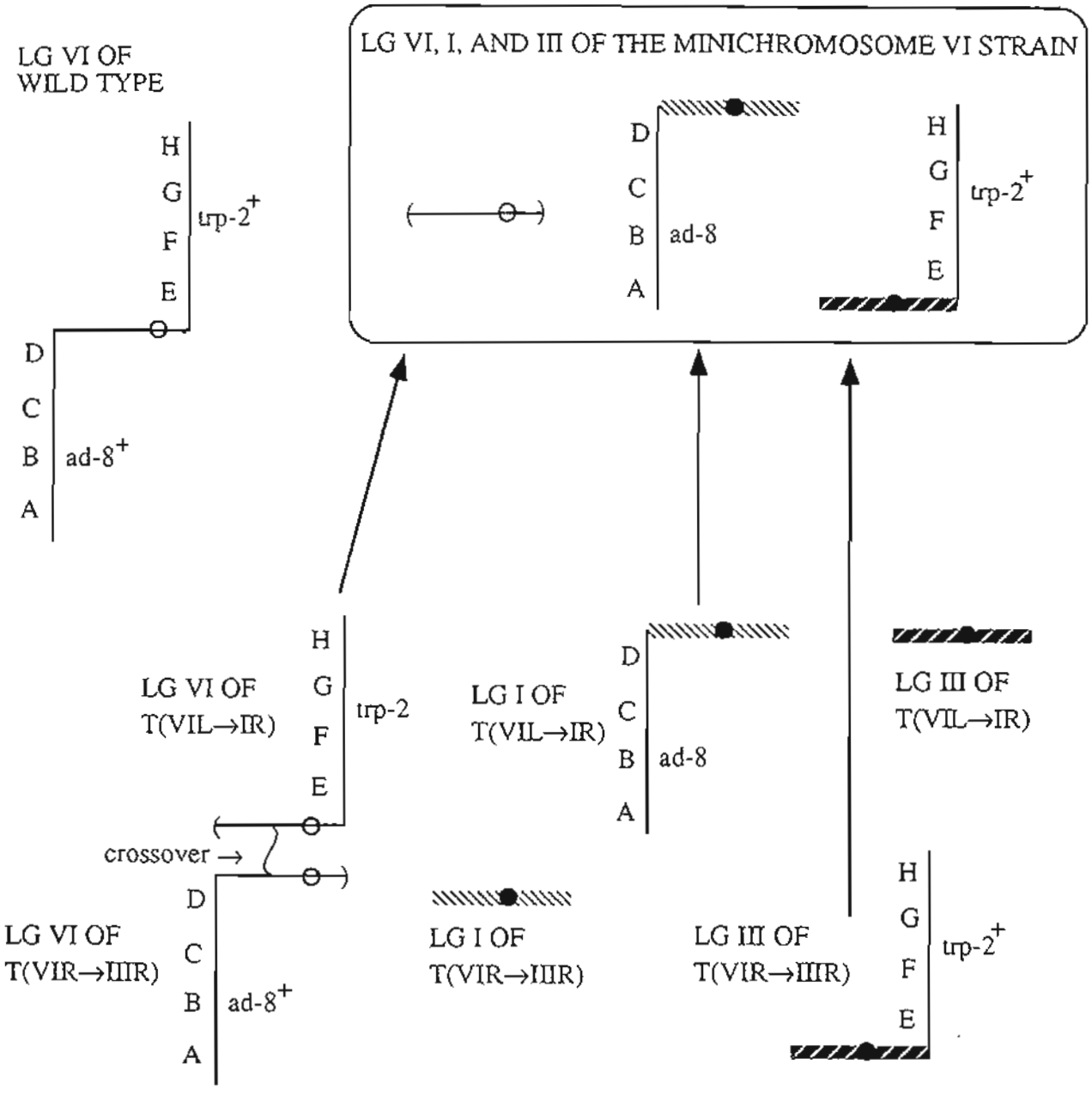

Figure 1. Construction of a euploid strain with a minichromosome of LG VI, showing the relevant three linkage groups. The chromosomes are not drawn to scale. Only one chromatid of each bivalent is shown. T(VIL →IR) and T(VIR→IIIR) are *T39M777 trp-2; ad-8* and prototrophic *OY329* , respectively. The wild type LG VI is also diagrammed for comparison.