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A simple method for isolating disomic strains of *Saccharomyces cerevisiae*

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Abstract

A simple method to select disomic ($N + 1$) strains that should be applicable for almost any chromosome in *Saccharomyces cerevisiae* is presented. A diploid heterozygous for a *KanMX* knock-out mutation in an essential gene is sporulated and viable geneticin (G418)-resistant colonies selected. Disomic products of a missegregation or non-disjunction event containing a copy of both the wild-type essential gene and its complementary *KanMX* knock-out allele make up most of the viable colonies. This method has been used to isolate disomic haploids for a variety of chromosomes. It is appropriately named MARV (for missegregation-associated restoration of viability) and is easily adaptable to virtually any strain.

Keywords

Yeast; aneuploid

Introduction

Disomic strains, haploids containing an extra copy of one chromosome ($N + 1$), have proved useful in genetic mapping, investigating chromosome stability, isolating recombination mutants and studying aneuploid physiology (Ajimura *et al.*, 1993; Campbell *et al.*, 1981; Mortimer and Hawthorne, 1973; Torres *et al.*, 2007). However, disomic strain isolation can be laborious, involving multiple crosses, sporulation, dissection of many asci and selection of candidate strains, followed by time-consuming screenings that might also entail additional ascus dissections. To facilitate the isolation of disomic strains for virtually any chromosome in any strain background, a method was developed that involves only two polymerase chain reaction (PCR)-based DNA transformations, mating, sporulation and a single selection for disomic candidates.

The method involves the construction and sporulation of a starter diploid strain containing a heterozygous essential gene (ESG) inactivated with *KanMX* (*esg* : *KanMX/ESG*), which confers resistance to geneticin (G418) (Wach *et al.*, 1994). When this strain is sporulated

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and chromosomes have undergone proper disjunction, all spores are non-viable on G418-containing medium. Missegregation or non-disjunction of the *KanMX*-containing chromosome or its homologue produces disomic spores that contain both *esg::KanMX* and the essential gene (*ESG*) and are viable on G418-containing medium (Figure 1A). In addition, a heterozygous *can1::STE2pr-HIS3* gene is employed to facilitate selection of haploid canavanine-resistant (Can^{R}) meiotic products using random spores (Tong and Boone, 2007). As di- some production using this method involves chromosome non-disjunction or missegregation, the method has been aptly termed missegregation-associated restoration of viability (MARV).

Materials and Methods

Yeast cell growth, sporulation and ascus dissection

All strains were maintained, grown and sporulated on standard media and asci were dissected and analysed as previously described (Sherman *et al.*, 1986).

Yeast transformation and DNA analysis

DNA molecules were introduced into yeast by one-step gene replacement (Rothstein, 1983a). Intact chromosomes were separated by pulsed-field gel electrophoresis, as previously described (Guerra and Kaback, 1999). Integrative transformation was confirmed by DNA blot hybridization (Southern, 1975) and colony polymerase chain reaction (PCR; Liang and Richardson, 1992). Gels were stained with ethidium bromide and fluorescent gel bands were quantitated using Image Quant 5.2 (Molecular Dynamics, University of Virginia, USA).

Selection of disomic strains

Appropriately marked diploids were sporulated on 2% KAc plates for 5 days at 30 °C. The cells were harvested using a sterile spatula and suspended in 1 ml 0.05 mg/ml Zymolyase 20-T (MP Biomedical, Solon, OH, USA) dissolved in sterile distilled water and incubated for 3 h at 30°C on a shaker. The treated spores were then sonicated for 60 s using a Microson ultrasonic cell disruptor (Misonic Inc., Farmingdale, NY, USA) set at 100 W, and plated on $\text{His}^- \text{Arg}^-$ synthetic dropout medium containing 0.8 mg/ml G418 (MP Biomedical) and 60 $\mu\text{g/ml}$ canavanine (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 2 days at 30 °C.

Results

To construct the strains used in all studies presented here, PCR-amplified *can1::STE2pr-HIS3* was introduced into a *MAT α* haploid from which a bona fide $\text{Can}^{\text{R}}\text{His}^+$ transformant was selected and crossed to a *MAT α* strain to produce starter diploid DZ10 (genotype *MAT α /MAT α , *can1::STE2pr-HIS3/CAN1*, *his3-11,15/his3-11, 15*, *ade1/ADE1*). Next, a centromere-linked essential gene from the desired chromosome was chosen and *esg::KanMX* copies of it PCR-amplified from the appropriate yeast gene knock-out strain (Winzeler *et al.*, 1999). The PCR product was introduced into strain DZ10 and a bona fide G418-resistance (G418^{R}) transformant selected (Figure 2). To isolate disomic strains, the*

starter diploid was sporulated and the resultant asci treated with zymolyase, which kills most unsporulated cells (Herman and Rine, 1997). Spores were then dissociated by sonication and plated on synthetic His⁻Arg⁻ medium containing G418 and canavanine and colonies arising after 2 days analysed.

Strains disomic for chromosome I, which were expected to be obtained easily (Tauro *et al.*, 1968), were the first isolated using diploid strain DZ11, which contains the heterozygous chromosome I essential gene mutation *efb1::KanMX/EFB1* (Figure 2). Colony PCR (not shown) and blot hybridization of pulsed-field gel electrophoresis (PFGE)-separated chromosomes confirmed proper integration of *efb1::KanMX* on chromosome I (Figure 3A). In addition, ascus dissection demonstrated 2 : 2 segregation of spore viability on YEPD medium, where all viable spores were G418-sensitive (Figure 1B). DZ11 is also heterozygous for *ADE1*, which is tightly linked to *EFB1*, and all viable spores were Ade⁺, confirming this linkage in the transformant shown. Microscopic observation of YEPD dissection plates showed that non-viable spores did not produce microcolonies.

To isolate chromosome I disomes, ca. 10⁶ spores were plated, producing ca. 200 G418^RCan^RHis⁺ colonies. 10 colonies were randomly selected and all were *MATa* and none sporulated. All contained a disomic copy of chromosome I, as demonstrated by PFGE, which showed an increased intensity of the chromosome I band (Figure 3B). PFGE using 60 s pulses indicated that all other resolved chromosomes were present at ca. 1 copy/cell (Figure 3C). Disomic haploids for chromosome I were also isolated with similar frequency, using three other essential gene knock-out mutations, *cdc24::KanMX*, *pta1::KanMX* and *tfc3::KanMX*.

To determine whether this method could be used to isolate strains disomic for other chromosomes, diploid strains DZ12, DZ13 and DZ14, each heterozygous for an essential gene *KanMX* knock-out mutation on chromosomes IX, X and XIV, respectively, were constructed. Colony PCR (not shown) and blot hybridization of PFGE-separated chromosomes confirmed proper integration of *esg::KanMX* constructs (Figure 3A). Dissection of ca. 20 asci from each showed 2 : 2 segregation for spore viability on YEPD, where all viable spores were G418-sensitive (not shown). To isolate disomes, diploids were sporulated and treated as described above. All G418^RCan^RHis⁺ colonies tested were *MATa* and did not sporulate. Singly disomic strains were found for each chromosome, as demonstrated by PFGE (Figure 3C). 90% (9/10) of DZ12 spores were singly disomic for chromosome IX, 50% (4/8) of DZ13 spores were singly disomic for chromosome X and 22% (2/9) of DZ14 spores were singly disomic for chromosome XIV. Remaining colonies were either disomic for several chromosomes or homozygous *MATa* diploids. Based on these studies, every essential gene tried produced disomic products, making it likely that most essential genes in the yeast genome knock-out collection can be used. However, it should be noted that this method cannot be employed as described above for chromosomes III and V, which contain *MATa* and *CAN1*, respectively.

Discussion

A new method called MARV was developed for isolating haploids that are disomic for almost any chromosome. Since this method involves only two transformations, sporulation and selection, it is quick and easily adaptable to virtually any strain. While the method described excludes chromosomes III and V, transplanting *MATa* to a different chromosome (Wu *et al.*, 1997) should enable selection of chromosome III disomes, while substituting *lyp1::MFA1pr-LEU2* (Tong and Boone, 2006) for *can1::STE2pr-HIS3* and selecting for thialysine rather than canavanine resistance should enable selection of chromosome V disomes.

Disomic haploids can arise due to non-disjunction during mitosis, meiosis I or meiosis II. It appears that most of the disomes isolated here were from meiosis I non-disjunction. Selection of disomes that arose during meiosis II requires a prior crossover between the centromere and the heterozygous *esg::KanMX* gene. Most of the heterozygous *esg::KanMX* genes used here were tightly linked to centromeres, limiting the frequency of disomes that arose during meiosis II. When either *cdc24::KanMX* or *pta1::KanMX* which are not tightly linked to the chromosome I centromere was used, the frequency of disome isolation increased by ~30%, a fraction consistent with the contribution of additional meiosis II non-disjunction events (data not shown). In addition, mitotic non-disjunction is too rare in yeast to account for the frequency of disomic colonies isolated (Chan and Botstein, 1993). Accordingly, meiosis I non-disjunction is likely to be responsible for most of the disomes selected using this method.

Strains isolated for the two smaller chromosomes tested were almost always singly disomic. In contrast, single disomes for the two larger chromosomes were not found 100% of the time and required screening of multiple colonies. Furthermore, it appears that the overall frequency of disome isolation decreased as chromosome size increased. This observation is in agreement with prior results suggesting that small chromosomes segregate less efficiently during meiosis (Goldway *et al.*, 1993).

In summary, MARV has been shown to be useful for isolating haploids that are singly disomic for a desired chromosome. It appears to work using many different essential genes and indeed might be useful in genomic screens using all essential genes. Once stable disomes are isolated, they can be used as is or the *esg::KanMX* mutation can be removed, using crosses or DNA transformation. This method should be applicable to a wide variety of studies where disomic strains are required.

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References

- Ajimura M, Leem S, Ogawa H. Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics*. 1993; 133(1):51–66. [PubMed: 8417989]
- Campbell D, Doctor J, Feuersanger J, Doolittle M. Differential mitotic stability of yeast disomes derived from triploid meiosis. *Genetics*. 1981; 98(2):239–255. [PubMed: 7035289]
- Chan S, Botstein D. Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. *Genetics*. 1993; 135(3):667–691.
- Goldway M, Arbel T, Simchen G. Meiotic non-disjunction and recombination of chromosome III and homologous fragments in *Saccharomyces cerevisiae*. *Genetics*. 1993; 133(2):149–158. [PubMed: 8436265]
- Guerra CE, Kaback DB. The role of Centromere Alignment in Meiosis I Segregation of Homologous Chromosomes in *Saccharomyces cerevisiae*. *Genetics*. 1999; 153:1547–1560. [PubMed: 10581265]
- Herman P, Rine J. Yeast spore germination: a requirement for Ras protein activity during re-entry into the cell cycle. *EMBO J*. 1997; 16(20):6171–6181. [PubMed: 9321396]
- Liang Q, Richardson T. A simple and rapid method for screening transformant yeast colonies using PCR. *BioTechniques*. 1992; 13:730–732. 735. [PubMed: 1418974]
- Mortimer R, Hawthorne D. Genetic mapping in *Saccharomyces* IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. *Genetics*. 1973; 74(1):33–54. [PubMed: 17248609]
- Rothstein R. One-step gene disruption in yeast. *Methods Enzymol*. 1983; 101:202–211. [PubMed: 6310324]
- Sherman, F.; Fink, G.; Hicks, J. *Methods in Yeast Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1986.
- Southern E. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*. 1975; 3:503–517. [PubMed: 1195397]
- Tauro, P.; Rodarte-Y-Ramon, U.; McKey, T.; Mortimer, R. *Donner Laboratory Semiannual Report*. Berkeley, CA: Lawrence Radiation Laboratory; 1968. Isolation of diomics in *Saccharomyces cerevisiae*; p. 19-24.
- Tong A, Boone C. Synthetic genetic array analysis in *Saccharomyces cerevisiae*. *Methods Mol Biol*. 2006; 313:171–192. [PubMed: 16118434]
- Tong A, Boone C. High-throughput strain construction and systematic synthetic lethal screening in *Saccharomyces cerevisiae*. *Methods Microbiol*. 2007; 36 (in press).
- Torres E, Sokolsky T, Tucker C, et al. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science*. 2007a; 317(5840):916–924. [PubMed: 17702937]
- Wach A, Brachat A, Poehlmann R, Philippsen P. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*. 1994; 10:1793–1808. [PubMed: 7747518]
- Winzeler E, Shoemaker D, Astromoff A, et al. Functional characterization of the *S.cerevisiae* genome by gene deletion and parallel analysis. *Science*. 1999; 285(5429):901–906. [PubMed: 10436161]
- Wu X, Wu C, Haber J. Rules of donor preference in *Saccharomyces* mating-type gene switching revealed by a competition assay involving two types of recombination. *Genetics*. 1997; 147(2): 399–407. [PubMed: 9335581]

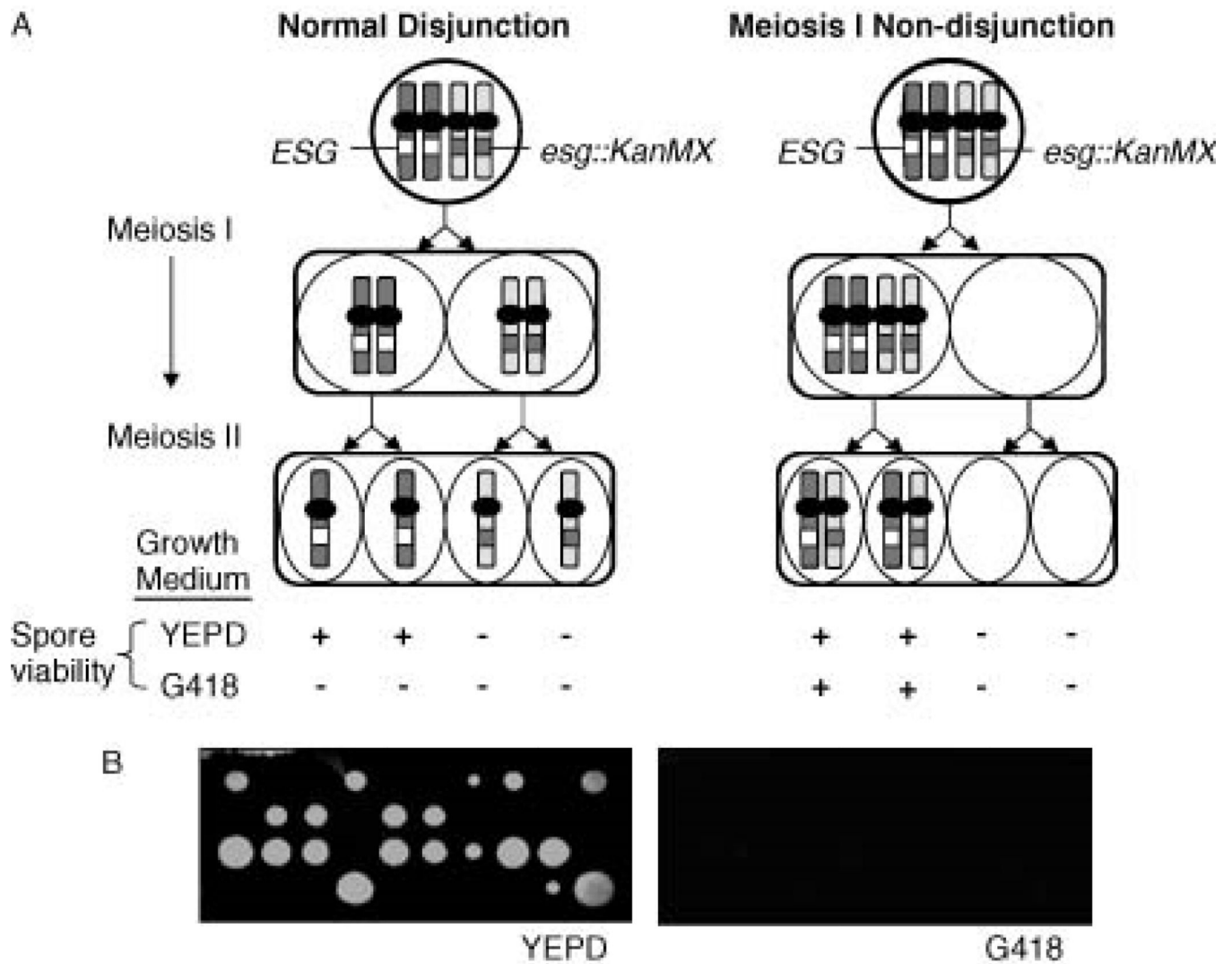
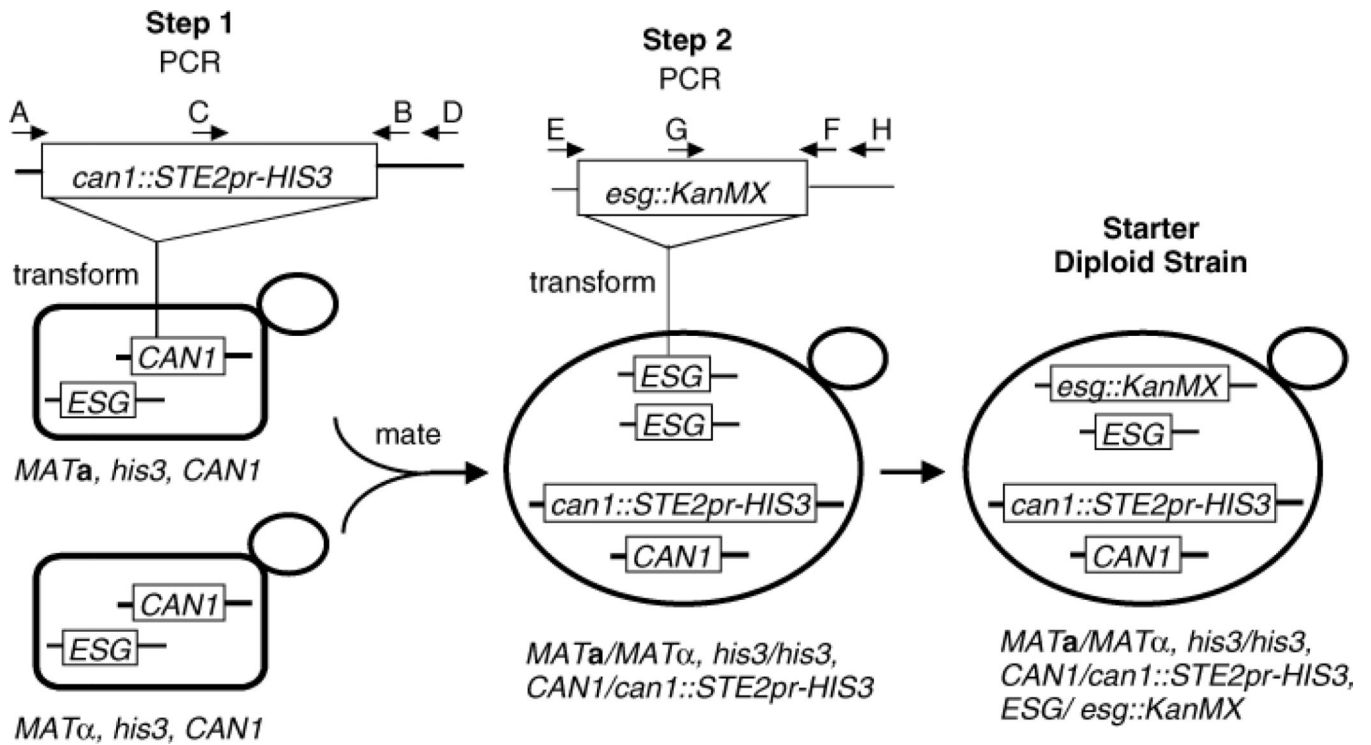


Figure 1.

(A) Meiotic disjunction and non-disjunction of a heterozygous *KanMX* insertion mutation in an essential gene. The dominant selectable marker *KanMX* (G418 resistance) inactivates an essential gene (*ESG*) in a diploid (*esg::KanMX*). Normal meiosis produces 2 : 2 (*esg::KanMX* : *ESG*) segregation of spore viability on YEPD media and 0 : 4 segregation of spore viability on G418-containing media. Meiosis I non-disjunction results in co-segregation of *ESG* and *esg::KanMX* producing two disomic spores that are viable on G418 medium. (B) Asci from starter diploid strain DZ11 that were dissected onto YEPD medium show normal chromosome I disjunction: 2 : 2 segregation of viability and 0 : 4 segregation of viability when replica-plated onto G418-containing media

**Figure 2.**

Construction of strains used to generate disomes. In Step 1, PCR-amplified *can1::STE2pr-HIS3* templated from *S. cerevisiae* strain Y7029 (courtesy of Charles Boone), using primers A (5'-ATCAGGGAATCCCTTTTTCG) and B (5'-ACTCCGTCTGCTTTCTTTTCG) was integrated in place of *CAN1* in strain DZ3-4A by one-step gene replacement. Proper integration in His⁺Can^R transformants was verified by colony PCR, using primers C (5'-GAACTTGTTTTGCAAAGGGA) and D (5'-AAAGAGTTCTGCCCTTGGCTT) and a transformant was mated as shown. In Step 2, PCR-amplified *esg::KanMX* for the noted chromosome (*efb1::KanMX*, chromosome I; *sqt1::KanMX*, chromosome IX; *mpp10::KanMX*, chromosome X; and *rlp7::KanMX*, chromosome XIV) templated from Yeast Gene Knock-out Strains 20404, 22348, 26799 and 25330 (Open Biosystems, Huntsville, AL, USA), using primers E and F (see below), was integrated in place of one copy of the wild-type *ESG* by one-step gene replacement and G418^R transformants selected. Integration was verified by colony PCR, using primers G and H (sequence listed below). PCR primers used: E, (*EFB1*, 5'-TTCCGCCATTTTCAACCA; *SQT1*, 5'-TGCTGGGTTTAAAACCCCAT; *MPP10*, 5'-TTCCACATCTCGTCATGAAT; *RLP7*, 5'-TTCATCACGCAGTACGGATT). F, (*EFB1*, 5'-ACACCGCGTATTTTCGACAA; *SQT1*, 5'-ATCCCCAAAGAAC TTACCGGA; *MPP10*, 5'-TTCCACATCTCGTCATGAAT; *RLP7*, 5'-AAGCGAGGAAAAAAGAGGC). G, (5'-TGATTTT GATGACGAGCGTAAT). H, (*EFB1*, 5'-TGTCCTTTTTCACGCAGGTA; *SQT1*, 5'-CCAACGGAACAGCCTTCAA; *MPP10*, 5'-CAGATCTTGCTGAAAGTGACG; *RLP7*, 5'-TTTGCTGATATATGGCCTTGA)

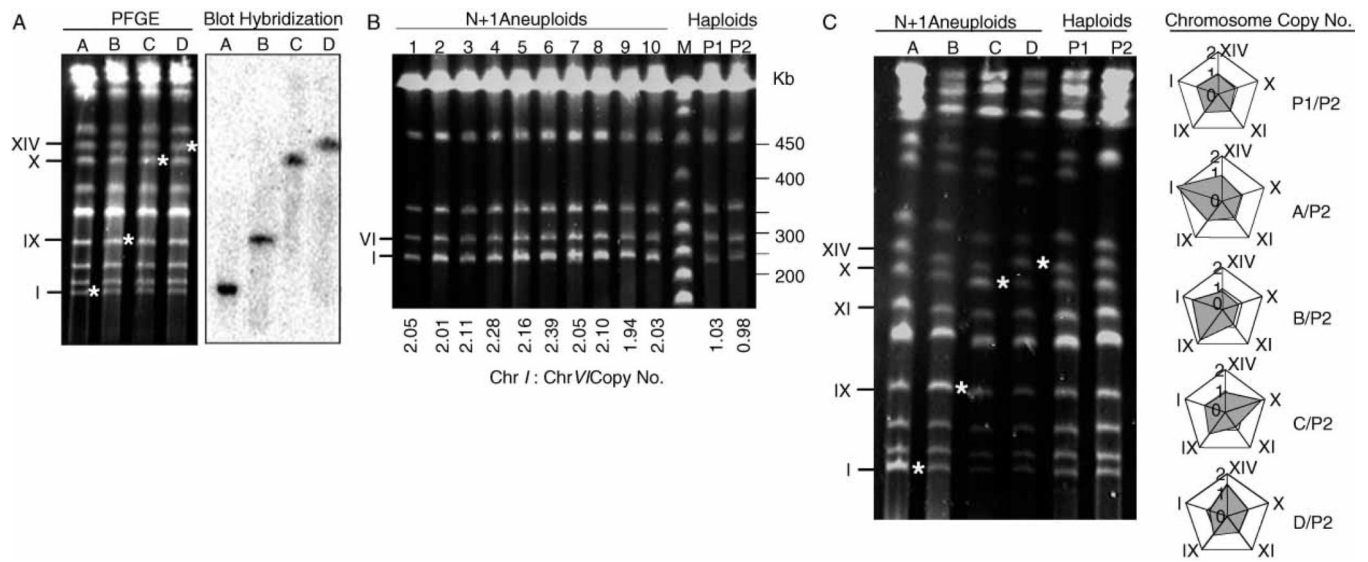


Figure 3.

PFGE karyotypes of marked diploids, haploid parents and disomic strains selected by MARV. (A) Proper localization of the *KanMX* marker in heterozygous *esg::KanMX/ESG* starter diploids for chromosomes I, IX, X or XIV. Intact chromosomes were separated using 60 s pulses and stained with ethidium bromide (left). The gel was blotted and hybridized to ^{32}P multi-primeTM (GE Healthcare, Piscataway, NJ, USA) -labelled *KanMX* DNA probe (right). Probe DNA was amplified from plasmid PJT, using forward 5'-TCAGGTGCGACAATCTATCGAT and reverse 5'-CTCACCGAGGCAGTTCCATAG primer sequences. Lane A, strain DZ11 (Chr. I); lane B, strain DZ12 (Chr. IX); lane C, strain DZ13 (Chr. X); lane D, strain DZ14 (Chr. XIV). Asterisks denote *esg::KanMX* targeted chromosomes. (B) PFGE analysis of Chr. I disomic strains. Intact chromosomes from G418^RCan^RHis⁺ spore colonies were separated using 25 s pulses and the fluorescent band intensities quantitated. Chr. I copy number shown below each lane was determined by comparing the relative band intensities of Chr. I and VI within the same strain and correcting for the size difference between the two chromosomes. Lanes 1–10, MARV-selected Chr. I disomes; lane M, Lambda Ladder PFG Marker (New England BioLabs, Ipswich, MA, USA); lanes P1 and P2, haploid parents of starter diploid DZ10, showing euploid karyotypes. (C) PFGE analysis of Chr. I, IX, X and XIV disomic strains. Intact chromosomes from G418^RCan^RHis⁺ spore colonies were separated using 60 s pulses and the fluorescent band intensities quantitated. Lane A, Chr. I; lane B, Chr. IX; lane C, Chr. X; lane D, Chr. XIV. P1 and P2 are the same as in lane B. Determination of chromosome copy number: for each aneuploid (lanes A–D), band intensities of five chromosomes (I, IX, XI, X, XIV) were quantitated and the results normalized against the corresponding band intensities in the haploid parent control (lane P2). Band intensities of the two haploid parents (P1 and P2) were similarly compared to ensure euploidy P1/P2. The adjacent Radar chart constructed using Excel (Microsoft, Redmond, WA, USA) shows the ratio of each quantitated chromosome for aneuploids (A–D) and haploid (P1) with respect to the haploid control (P2).