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Methods for single/low-copy integration by ultraviolet and trimethylpsoralen treatment in *Caenorhabditis elegans*

Eriko Kage-Nakadai [a,b], Rieko Imae [a], Sawako Yoshina [a], Shohei Mitani [a,*]

[a] Department of Physiology, Tokyo Women's Medical University, School of Medicine, 8-1, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
[b] The OCU Advanced Research Institute for Natural Science and Technology, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

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ABSTRACT

Single/low-copy transgene integration is essential for avoiding overexpression, ectopic expression and gene silencing in the germline. Here, we present an overview of a method that uses ultraviolet and trimethylpsoralen (UV/TMP) to generate single/low-copy gene integrations in *Caenorhabditis elegans*. Single/low-copy transgenes from extrachromosomal arrays are integrated into the genome using positive selection based on temperature sensitivity with a *vps-45* rescue fragment and negative selection based on benzimidazole sensitivity with a *ben-1* rescue fragment. The copy number of the integrated transgenes is determined using quantitative PCR. Our UV/TMP integration method, which is based on familiar extrachromosomal transgenics, provides a simple approach for generating single/low-copy gene integrations.

1. Introduction

Transgenic technologies have allowed for diverse molecular and genetic analyses in many organisms. In the nematode *Caenorhabditis elegans*, transgenic animals are typically obtained using DNA microinjection into the syncytial germ cells of the hermaphrodite gonad, which generates multi-copy extrachromosomal arrays [1]. These transgenes are semi-stable, i.e., transgenic animals are mosaic, in which some cells lose the extrachromosomal (Ex) array, and the arrays are partially transmitted to the next generation [2]. Ex arrays typically contain hundreds of copies of the injected DNA [1], resulting in overexpression, ectopic expression and silencing of the expression of the transgene in the germline [3]. Ex arrays can be integrated into the chromosomes using gamma-ray or ultraviolet (UV, 254 nm) irradiation [4,5]. However, integrated arrays still contain a high copy-number of transgenes.

Methods using microparticle bombardment create low-copy chromosomal integrated strains (Is) [6]. The biolistic technique allows for direct integration of small amounts of exogenous DNA into the chromosomes while avoiding the formation of Ex arrays. Praitis et al. adopted *unc-119* as a positive selection marker and *sup-7* as a negative selection marker as needed [6]. More recently, Mos1-mediated single-copy insertion (MosSCI) based on homologous recombination was developed [7]. In MosSCI methods, *unc-119* and *twk-18*(*gf*) are used as positive and negative selection markers, respectively [7].

We have developed an alternative single/low-copy integration method [8] based on random integrations of transgenes into the chromosomes from multi-copy Ex arrays using ultraviolet and trimethylpsoralen (UV/TMP) [9–11]. The combination of long-wavelength UV irradiation (wavelength 365 nm) and TMP treatment has a higher mutation frequency and less rearrangement of chromosomes, such as inversion and translocation, compared to sole short-wavelength UV irradiation (wavelength 254 nm) [5,11,12]. Our positive–negative selection strategies are as follows. Positive selection was based on rescue of the *vps-45* mutant phenotype. Because *vps-45* mutants are unable to grow and reproduce normally at 20 °C or higher [13], only *vps-45* mutants carrying the positive selection marker, the *vps-45* mini gene, grow and reproduce, which allows for easy identification of the transformants. On the other hand, negative selection was based on rescue of the *ben-1* mutant phenotype. *ben-1* mutants are resistant to an anti-tubulin drug benzimidazole [14], thus, *ben-1* mutants not carrying the negative selection marker, the *ben-1* gene, predominantly grow and reproduce on the selection media, which enables differentiation of low-copy integrants from Ex arrays and multi-copy integrants that are highly likely to have the *ben-1* gene (Fig. 1). Here, we provide a detailed protocol and tips for the establishment of single/low-copy integrated strains.

* Corresponding author. Fax: +81 3 5269 7414.
E-mail addresses: nakadai@life.osaka-cu.ac.jp (E. Kage-Nakadai), rimae@research.twmu.ac.jp (R. Imae), yoshina@research.twmu.ac.jp (S. Yoshina), mitani1@research.twmu.ac.jp (S. Mitani).

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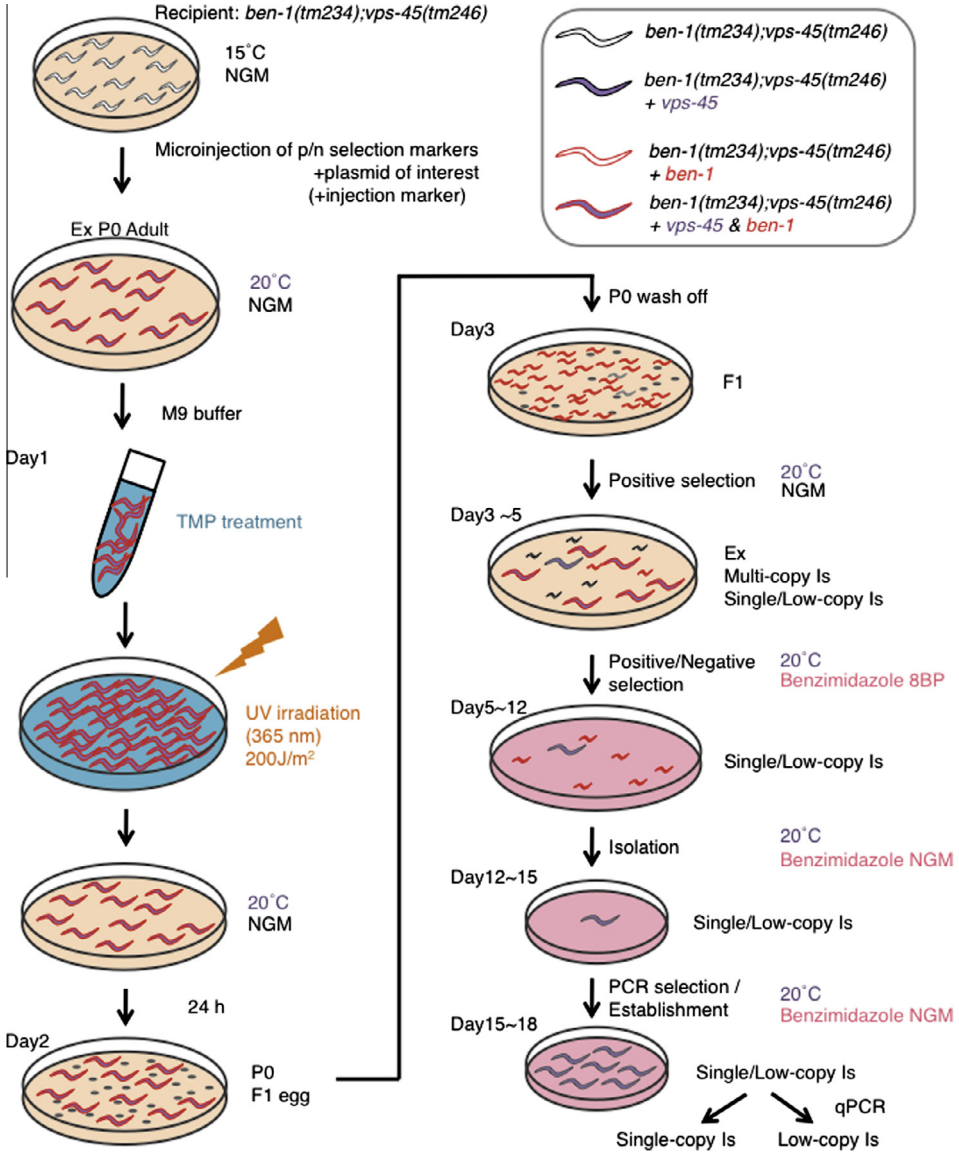

Fig. 1. Schematic overview of single/low-copy integration methods using UV/TMP. The recipient *ben-1(tm234);vps-45*(*tm246*) animals are cultured at 15 °C on NGM. The positive selection marker (*vps-45* mini gene), the negative selection marker (*ben-1* gene), a plasmid of interest and an injection marker (as needed) are co-injected into the recipient strain to generate transgenic strains carrying Ex arrays. P0 animals of the parental Ex strain are cultured at 20 °C on NGM and treated with TMP and UV irradiation. F1 animals are first selected using the positive selection (cultured at 20 °C). Under the selection conditions, animals with incomplete- or no-integration do not survive because their *ts* phenotype is not rescued by the *vps-45* transgene. *ts*-rescued animals that are selected using the positive selection are then selected using the positive/negative selection (cultured at 20 °C on benzimidazole-containing media). Under these conditions, single- or low-copy integrants survive, but multi-copy integrants or Ex array-carrying animals do not survive because they are rescued by the negative selection marker, i.e., the *ben-1* transgene, which has high chance of being included in the multi-copy transgenes. Single/low-copy Is animals are PCR-selected and further tested using quantitative PCR (qPCR) to determine the copy number.

2. Protocols

2.1. Plasmid preparation

Any plasmids that contain sequences to be integrated can be used. Here, we used *pFX_HBG_Lw_dpy-30p_NLS_GFP*, which was constructed as follows: approximately 1.2 kbp of the upstream genomic region of the *dpy-30* gene was subcloned into the HindIII/BamHI sites of *pPD96.04*, and the $P_{dpy\text{-}30}$*::NLS::GFP::LacZ* sequence was amplified from the plasmid and then subcloned into the HindIII/NotI sites of *pFX_HBG_Lw_vps-45* [8]. For the positive selection marker, *pFX_HBG_Lw_vps-45* or *Peft-3::vps-45* could be used. The former contains a floxed *vps-45* mini gene (*eft-3p::vps-45cDNA::unc-86 3′-UTR*), which was designed to be excised by Cre recombinase, and the latter contained only the sequence of the *vps-45* mini gene. We used *Peft-3::vps-45* in the present study. The negative selection marker plasmid (*pGEMT_ben-1*(+)) was constructed as described in our previous study [8]. The positive and the negative selection marker plasmids can be provided upon request.

2.2. Generation of parental Ex transgenic strain

The recipient strain *tm234*(*ben-1*);*tm246*(*vps-45*) is distributed by National BioResource Project (NBRP) [15], and *tm234;tm246* should be cultured at 15 °C. The positive selection marker, the negative selection marker and a plasmid of interest are co-injected at 60 ng/μl each along with an injection marker, if needed (20 ng/μl), into the *tm234;tm246* strain. The F1 transformants are cultured at 15 °C until the F2 eggs hatch, and this is followed by a shift up to