Algal Research 31 (2018) 167–172

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Short communication

High-throughput system for quantifying and characterizing homologous recombination in *Chlamydomonas reinhardtii*

Hussam Hassan Nour-Eldin[1], Elizabeth A. Specht[2], Joseph Ostrand, Kevin T.D. Hoang[3], Prema S. Karunanithi[4], Stephen P. Mayfield*

California Center for Algae Biotechnology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, United States

ARTICLE INFO

Keywords:
Chlamydomonas reinhardtii
Nuclear genetic engineering
Homologous recombination
High-throughput screen
Split marker system
Intron homology sequence

ABSTRACT

In the green alga *Chlamydomonas reinhardtii*, introduced DNA fragments predominantly insert randomly into the nuclear genome by non-homologous recombination (NHR), often resulting in highly variable phenotypes between transformants. Homologous recombination (HR) can occur in *C. reinhardtii* but at very low frequency, and is often accompanied by insertions, deletions, and/or rearrangements at the recombination site. To benchmark the frequency and characterize the nature of HR integrations in *C. reinhardtii*, we developed a system for detecting and characterizing HR events that includes three intact markers and one split marker as well a novel design element: utilizing a long intron as the homology region. In this study we demonstrate that this system meets the following criteria: accommodates high-throughput screening; provides a high-fidelity phenotype for detecting HR without false positives from reversion or locus heterogeneity; allows and captures both single- and double-crossover HR events; reports HR and NHR rates from a single transformation; and allows characterization of imprecise recombination or rearrangement at the integration site. Using this system we reproducibly determined the HR rate in our recipient strain of *C. reinhardtii* and characterized a number of recombinants by restriction digests and sequencing of PCR amplified recombination junctions to show that both double and single crossover events were recovered and that integration occurred both via perfect and imperfect (i.e. accompanied by insertions, deletions, and rearrangements) HR. This system is valuable for systematically testing approaches for increasing HR efficiency and accuracy.

1. Introduction

Integration of linear DNA fragments into the nuclear genome of *C. reinhardtii* can occur via homologous recombination with very low frequency and is typically accompanied by insertions and/or deletions at the recombination site (referred to from hereon as imperfect HR) [1–4]. With the intent of developing methods for improving the efficiency and accuracy of HR in *C. reinhardtii*, we desired a standardized, robust, and high-throughput HR screening approach that addresses shortcomings of existing approaches. In the following, we describe its design and demonstrate its robust ability to measure consistent HR:NHR frequencies across independent transformations, and to characterize features of discrete recombination events. The properties of our approach are discussed in comparison to existing approaches.

2. Results

2.1. System design

We developed a two-step recombination detection system that allows HR and NHR frequency to be measured from the same transformation. First, a recipient strain was created using the pHR18 plasmid. From the 5′ to 3′ end, the functional part of pHR18 consists of a complete *ARS2* cassette (periplasmic arylsulfatase) [5], a complete *ARG7*

Abbreviations: HR, homologous recombination; NHR, non-homologous recombination; NHEJ, non-homologous end-joining

* Corresponding author.
E-mail addresses: huha@plen.ku.dk (H.H. Nour-Eldin), elizabeth.specht@colorado.edu (E.A. Specht), jostrand@ucsd.edu (J. Ostrand), kevin_hoang@nymc.edu (K.T.D. Hoang), pkarunan@ucdavis.edu (P.S. Karunanithi), smayfield@ucsd.edu (S.P. Mayfield).
[1] Current affiliation: DynaMo Center, Dept of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark; Copenhagen Plant Science Center, Dept of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark.
[2] Current affiliation: The Good Food Institute, 1380 Monroe St NW, #229 Washington, DC 20010, United States.
[3] Current affiliation: New York Medical College, School of Medicine, United States.
[4] Current affiliation: University of California Davis, College of Biological Sciences, United States.

https://doi.org/10.1016/j.algal.2018.02.005
Received 8 August 2017; Received in revised form 29 January 2018; Accepted 6 February 2018

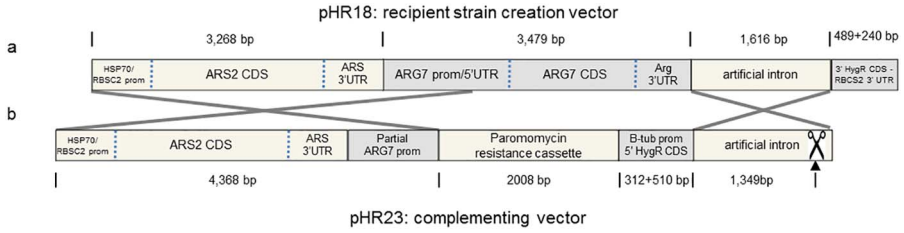

Fig. 1. Schematic of vectors comprising the HR detection system. a) pHR18 is the vector used to create the recipient strains, and integrates at random into the nuclear genome. b) pHR23 is the complementing vector designed to recombine at the pHR18 locus within the nuclear genome to confer hygromycin resistance. Diagonal lines indicate regions of homology suitable for recombination. The arrow below pHR23 indicates the *Eco*RV site, where the plasmid was linearized prior to electroporation. Sizes of individual elements is provided above and below each construct, respectively.

cassette (expressing the CDS of argininosuccinate lyase from native promoter and 3′UTR) [6], and a 1.6 kb intron (see M&M) followed by the 3′ half of a Hygromycin B phosphotransferase expression cassette (last 489 bp of Hyg CDS followed by 240 bp of RBCS2 3'UTR) (Fig. 1a). The *ARS2* cassette encodes the arylsulfatase reporter under the control of a constitutive promoter and serves as a semi-quantitative reporter for gene expression [5]. The *ARG7* cassette in pHR18 enables recovery of transformants of arginine auxotrophic strains [6]. The 1.6 kb intron was introduced to increase the length of homology between two non-functional halves of the hygromycin resistance gene (the 5′ half being located in pHR23; see below). Additionally, including an intron into a broken marker system enables capturing and characterization of recombination events that include insertions and deletions.

To screen for integration via HR, the recipient strain is transformed with pHR23. From the 5′ to 3′ end, pHR23 consists of a region identical to 4.3 kb of the 5′ end of the pHR18 vector, a complete paromomycin resistance cassette, and the 5′ half of the hygromycin resistance cassette (β-tub promoter followed by first 510 bp of Hyg CDS) followed by the intron which is also in pHR18 (Fig. 1b). Homologous recombination between the intron sequences reconstitutes a complete (intron-containing) hygromycin resistance cassette (Fig. 2a). By plating an aliquot of the pHR23 transformation on paromomycin we can estimate total number of pHR23 transformants. Comparison between the number of colonies obtained on hygromycin to those obtained on paromomycin allows calculation of an HR:NHR ratio from a single transformation. By including a region identical to the 4.3 kb at the 5′ end of the pHR18 vector, both single and double recombination events can occur and be captured (Fig. 2). By extending this region of homology into the ARG7 cassette, recombination within this region disrupts the *ARG7* cassette found in pHR18 (see below). Thus, the system is designed such that four types of integration events can be detected: 1) random integration of pHR23 results in a strain that is resistant to paramomycin (PAR^+), expresses ARS2 ($ARS2^+$), is arginine prototroph (ARG^+) but is sensitive to hygromycin (HYG^-); 2) HR mediated integration via double crossover (wherein recombination occurs in both regions of homology) results in a strain that is $ARS2^+$, ARG^-, PAR^+ and HYG^+ (Fig. 2a); 3) a single crossover within the intron after which the recombination branch migrates to the 5′ end of the pHR23 template and then recombines (non-homologously) results in a strain that is $ARS2^+$, ARG^+, PAR^+ and HYG^+ (Fig. 2b); and 4) a single crossover within the 4.3 kb 5′ homology region after which the recombination branch migrates to the 3′ end of the pHR23 template and then recombines (non-homologously) results

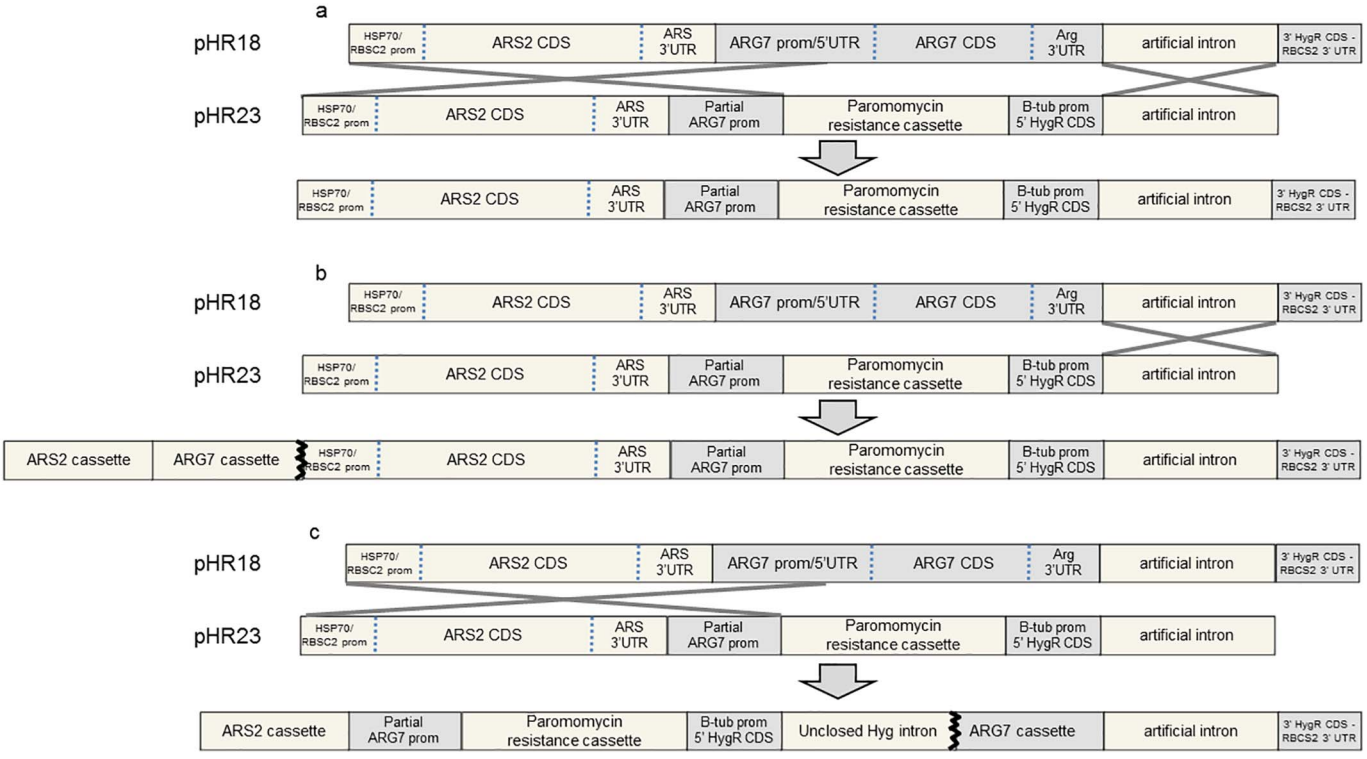

Fig. 2. Predicted outcomes for achieving hygromycin-resistant transformants. a) Schematic of the expected recombination site after a perfect double recombination event. b) Schematic of the expected recombination site after a single homologous recombination (crossover) within the 3′ homology region (artificial intron). The jagged border between the ARG7 cassette and the 5′ homology indicates that this junction is formed non-homologously after branch migration reached the end of the pHR23 template. Bacterial plasmid backbone sequence (not pictured) may also be incorporated at this site. c) Schematic of the expected recombination site after a single homologous recombination within the 5′ homology region of pHR18. Again, the jagged border indicates a region of non-homologous junction. The schematics are generally drawn to scale (1 kb = 1.8 cm). Due to space constraints the ARS and ARG7 cassettes are not this scale in the outcome schematic in b) and c). “Closed” vs “Unclosed Hyg intron” relates to whether the intron contains its closing splice site. Prior to transformation, pHR23 is linearized within the Hyg intron 265 bp upstream of the closing splice site resulting in an “unclosed intron”. In pHR18, the intron is complete and contains the closing splice site.