Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Microbiological Methods 64 (2006) 305–315
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Hybridization monitor: A method for identifying differences between complex genomes

Yueqing Cao, Zhengbo He, Zhongkang Wang, Youping Yin, Guoxiong Peng, Yuxian Xia*

Genetic Engineering Research Center, Chongqing University; Institute of Bioengineering, Key Laboratory of Biomechanics and Tissue Engineering, Ministry of Education, Chongqing University; Chongqing 400030, PR China

Received 25 April 2005; received in revised form 20 May 2005; accepted 20 May 2005
Available online 6 July 2005

Abstract

We have developed a method to identify and amplify differential fragments between two complex genomes. This technique, named hybridization-monitored genome differential analysis (HMDA), incorporates a monitor system into a PCR-based solid subtraction hybridization that tracks the entire hybridization process. This is achieved by monitoring the subtraction progress using PCR analysis of the conserved sequence of 18S rDNA in the tester sample after each round of subtraction. Homologous fragments can then be eliminated when bound to the driver DNA immobilized on a solid membrane. The hybridization continues until the conserved DNA sequence of 18S rDNA can no longer be detected, and most of the unbound DNA fragments left in the liquid were mainly the tester-specific fragments, thus greatly decreasing the complexity of DNA template of PCR amplification, increasing the amplification efficiency of differences accordingly, and ensuring high positive efficiency and coverage across the tester genome. We have applied the technique in a comparison between the genomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which are two completely sequenced organisms. Results indicated that 95% of the subtracted clones have been confirmed to be different to the driver analyzed using the BLASTN homology alignment. With this technique, 240-fold enrichment of differences is obtained, and the coverage of the difference is up to 79%. These results indicate that HMDA can efficiently identify sequences that differ between two complex genomes.

Keywords: Genome difference; Subtractive hybridization; Hybridization monitor; Driver; Tester

1. Introduction

The ability to identify and analyze species-specific sequences within a genome will assist in elucidating the evolutionary pattern of the species and have profound benefits for the development of evolutionary

* Corresponding author. Tel.: +86 23 65120486; fax: +86 23 65120490.
E-mail address: yuxianxia@cqu.edu.cn (X. Yuxian).

0167-7012/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2005.05.010

biology. Identifying the differences between the genomes of two species helps to clarify how genes are related to particular attributes and elucidate their involvement in different biological mechanisms. For distantly related organisms, different sequences can serve as probes for intermediate species and can aid in the construction of phylogenetic trees (Calia et al., 1998), or can be used as a diagnostic marker for DNA-based detection (Agron et al., 2001; Radnedge et al., 2001). Once the genomic sequences of organisms have been established, computer-based comparisons can analyze genomic differences between species. However, with the techniques currently available, the genomes of eukaryotic organisms are too massive and complex to be sequenced efficiently without great expense.

During the last few decades, various methods have been developed to identify and isolate unique sequences present in one genome but absent in another. Most of these techniques are based on the subtraction hybridization technique, which enriches the target-specific DNA fragments and removes any common sequences (Lamar and Palmer, 1984; Straus and Ausubel, 1990; Lisitsyn et al., 1993a,b; Diatchenko et al., 1996; Akopyants et al., 1998). An important factor in the subtraction process is the ability to determine whether the subtraction is complete. In general, high false positive efficiency results from insufficient subtraction (Sagerstrom et al., 1997), and yet there is currently no appropriate strategy to determine the extent of the subtraction process. Representational difference analysis (RDA) incorporated "kinetic enrichment" into PCR-based subtractive hybridization, but this method is labor-intensive and prone to high rate of false positives and negatives if not properly performed (Hubank and Schatz, 1999). In addition, the genomes to be analyzed must be nearly identical with polymorphism limited to the region around the locus of interest (Baldocchi and Flaherty, 1997). Modifications of this method have not yet solved these defects (Allen et al., 2003). Suppression subtractive hybridization (SSH), a subtractive method based on suppression PCR, is considered the most effective way to analyze genomic differences, but a lack of appropriate controls precludes its use with complex genomes. Though the genomic subtraction kit from CLONTECH can control for analysis of the DNA hybridization process, the subtraction efficiency of the control in this kit does not adequately reflect the hybridization status of the samples' reaction conditions, such as the sequence complexity, concentration and quality of DNA sample. These shortcomings make it difficult to estimate the subtraction process, thus resulting in increased false positives (Rebrikov et al., 2000). The inherent deficiencies of these techniques exclude them from being applied to eukaryotic organisms with large and highly variable genomes.

In this report, we describe a novel method to compare sequences between two complex genomes. This method utilizes a hybridization monitor system called hybridization-monitored differential analysis (HMDA), which monitors the entire hybridization process. To demonstrate the efficiency and feasibility of the method, we utilized the technique to evaluate genomic differences between two well-known yeast species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

2. Materials and methods

2.1. Strains, vector and cultural conditions

Model strains, *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Schizosaccharomyces pombe* (*S. pombe*), were from the Institute of Microbiology, Chinese Academy of Sciences. *S. cerevisiae* and *S. pombe* were grown in Yeast Peptone Dextrose (YPD) at 30 °C with constant shaking. The vector used for differential cloning was pGEM-T (Promega).

2.2. Extraction of DNAs

S. cerevisiae and *S. pombe* genomic DNAs were prepared as described previously (Straus and Ausubel, 1990). The concentration of genomic DNA was determined by spectrophotometric analysis. Genomic DNA for subtraction is expected to have an A260/A280 absorbance ratio range of 1.8–1.9.

2.3. Preparation of adaptor

The adaptor was prepared by annealing the synthetic oligonucleotides, 5′-CGG GTA CCG AGC TCG-3′ and 5′-AAT TCG AGC TCG GTA CCC G-3′ in annealing buffer (10 mmol/L Tris, pH 7.5–8.0,