Application of the RLGS Image Analysis Tool (RAT) to the Construction of a Genetic Linkage Map of Recombinant Inbred Strain SMXA

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1 Introduction

The characterization of genomic DNA is essential for establishing a genetic basis of biological properties. Recently, much effort has been made to determine the complete genomic sequence of several organisms. Despite the methodological and strategic improvements of mapping and sequencing as well as progress in automation, the genome is still so large and many species of genomes have yet to be mapped. Under such circumstances, the restriction landmark genome scanning method (RLGS), which was originally developed to scan the whole genome, is a promising tool as it can be effectively applied to any organism including mammalian species. The power of the RLGS method has been demonstrated in various lines of genetic analysis, including the construction of a genetic linkage map, identification of the methylation status of a genome, and detection of the loss of heterozygosity in the genome of cancers [1].

In constructing a genetic map by the RLGS method, the presence or absence of hundreds of spots must be scored in many progeny. It should be possible to conduct this scoring step automatically with the assistance of a computer-based image analysis system. Although there are several types of commercially available software for the analysis of 2-D protein electrophoresis, they are not suitable for analysis of the RLGS profile. Therefore, the RLGS profile has been mainly analyzed by human visual observation, this being a laborious and time-consuming procedure. To overcome this drawback, we developed a novel image analysis system, RAT (RLGS Analysis Tool) [2].

In this study, we assessed the accuracy of RAT by scoring the matches and discordance between the analyses of RAT and human observation. RAT could reproduce almost all data obtained by the observation. We then applied RAT to practical analysis for the construction of a genetic linkage map using the recombinant inbred strain SMXA. We were able to identify 162 RLGS spots/loci, 121 of which were correctly mapped to a specific chromosome.

2 Methods and Results

RAT was developed to fully account for the characteristics of RLGS profile and can be used to automatically construct a genetic linkage map. RAT consists of four processes. The first three are used to construct the master profile, in which all spots are classified into paternal-specific polymorphic, maternal specific polymorphic, or non-polymorphic spots. In the fourth process, the RLGS profile of each offspring is compared to the master profile, and the presence or absence of polymorphic spots is scored to prepare the strain distribution pattern (SDP). The resultant SDP can be directly used for linkage analysis.

The first analysis process of RAT is background subtraction. In this process, the spots are discriminated from the background of the x-ray film. The x-ray film is initially subjected to digitalization by scanner. The digitized film image is then transformed into a binary image, in which the spots are represented as a white area (groups of white pixels) and the background is represented as a black area. In the second process, RAT compares F_1 and parental binary images, and determines the spot correspondences between two images by using a template matching technique. In the third process, RAT recognizes the origin of spots on F_1 film. The spots on F_1 are inherited from paternal and/or maternal progenitors, and RAT classifies the spots into paternal-specific polymorphic, maternal-specific polymorphic, and non-polymorphic (common) spots. This process is performed basically to evaluate the degree of overlap between the corresponding spots, and the final results are transmitted into the master profile. In the last process, RAT compares the RLGS profiles of each offspring with the master profile, scores the presence or absence of polymorphic spots, and returns a list of SDPs.

To assess the accuracy of RAT, we compared the results from RAT and human observation by using RLGS profiles of Syrian hamster. Without low quality part of RLGS film, RAT could reproduce almost all data obtained by the observation and we concluded that the RAT program was feasible for identifying polymorphic spots and scoring them in backcross progeny. We then used RAT to construct the genetic linkage map of the recombinant inbred strain SMXA. A total of 162 novel RLGS spots/loci (72 SM/J-specific and 90 A/J-specific) were identified. We transmitted the resultant SDPs of novel spots to linkage analysis software Map Manager, and finally 121 of 162 polymorphic RLGS spots/loci were chromosomally mapped.

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References

[1] Hayashizaki, Y. et al., Restriction landmark genomic scanning method and its various applications, *Electrophoresis*, 14:251–258, 1993.

[2] Sugahara, Y. et al., An automatic image analysis system for RLGS films, *Mammalian Genome*, 9:643–651, 1998.