An Automatic Image Analysis System for RLGS Films

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Abstract

We developed an automatic image analysis system for RLGS films, RAT(RLGS Analysis Tool). RAT identifies the parental alleles by comparing the RLGS profile of F_1 with those of the parents. We tested the feasibility of RAT and obtained results were satisfactory for the initial screening of RLGS films.

1 Introduction

The RLGS (Restriction Landmark Genome Scanning) method was originally developed as a powerful method for scanning restriction landmarks throughout the genome of any organism. It has been effectively applied to various lines of genetic analyses, especially map-based identification of the important genes, which requires a genetic linkage map, and identification of closely linked DNA markers to a mutant locus. It also provides a method of analyzing differential methylation of CpG-rich landmark sites associated with genomic imprinting and the changes in CpG methylation of specific loci.

In the RLGS procedure, genomic DNA is cleaved with a landmark enzyme such as NotI and the cleavage site is end-labeled with radioisotope. The DNA fragments are then subjected to high-resolution two-dimensional electrophoresis. The single RLGS profile displays 2,000 or more restriction landmarks as spots. In order to identify the chromosomal location of spots using the RLGS spot mapping method, many sets of corresponding spots in two RLGS profiles need to be compared. The polymorphic spots are listed and their presence and absence are scored for each individual in the pedigree for genetic analysis. Despite the wide applicability and usefulness of RLGS-based genetic study, the processes of reading and scoring the RLGS profiles are very laborious and have been mainly dependent on human visual observation. To resolve the problem of these time-consuming steps, we developed a novel automatic image analysis system for RLGS spot mapping.

2 Materials and Methods

Computer analysis: All computing was performed with a Power Macintosh 9500/132. The computer program was developed with the environment provided by IDL software. RLGS X-ray films were scanned and transformed to digitized images by Lumiscan75 which has 140×140 dpi resolution and 4096 gray level.

The overall procedures for analyzing RLGS images consist of the following four steps.

1. **Background subtraction:** Original scanned images are transformed to binary images, representing the spots as white areas and the background as black.

2. **Separation of white area into individual spots:** In the binary images, the white area is not always composed of one spot but often of two or more spots. In this step, a white area composed of two or more spots is separated into the individual spots.

3. **Matching:** The correspondences of all spots are calculated from the binary image of F_1 and the parental images.

4. **Judgment of the strain specificity of a spot on the F_1 image:** By comparing the F_1 and parental binary images, the spots are classified into three categories: paternal- or maternal-specific spot or non-polymorphic (shared in common) spot.

3 Summary

We evaluated the accuracy of *RAT* in identifying the polymorphic and non-polymorphic spots. The RLGS profiles used for this evaluation were obtained from the laboratory mouse C3H, wild mouse Mus musculus mollosinus (MSM) and their (MSM×C3H) F_1 progeny, and all three film images were clear. The finally obtained correspondence rate of *RAT* to the human visual observation is about 85%, which is considered to be feasible for the initial screening.

RAT was developed using clear image films. In order to extend the usefulness of *RAT*, we are applying *RAT* to many films including the lower quality ones, and accumulating data.

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