Evaluation of Hereditary Distance by Restriction Landmark Genomic Scanning (RLGS)

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The characteristics of RLGS

" Genome scanning" is defined as a high-speed survey for the simultaneous detection of signals from many loci throughout a genome by one process. By southern hybridization or polymerase chain reaction (PCR) only one locus can be detected in one process. By the fingerprint method or Alu-L1-PCR, some loci can be detected in one process but scanning is insufficient. To make up for this shortage in genome scanning, the restriction landmark genome scanning (RLGS) method has been developed. RLGS employs direct-end labeling of the genome digested with a restriction enzyme which high resolution two-dimensional electrophoresis is then carried out. In this method there is no hybridization procedure. After the first enzyme digestion (this will be used as a landmark), labeling is achieved. After the initial labeling, the second digestion is brought about by a second enzyme and then one-dimensional electrophoresis by agarose gel is carried out. After the one-dimensional electrophoresis, a third digestion is brought about by a third restriction enzyme and then two-dimensional electrophoresis by polyacrylamide gel is carried out. RLGS has the following advantages for genome scanning. (a) It has a speed-scanning ability. (b) The scanning field can be extended by the use of different kinds of landmarks in an additional series of electrophoreses. (c)This method can be applied to any organism. (d)The intensity of a spot reflects the copy number of the restriction landmark. Haploid and diploid genomic DNAs can be distinguished. (e)Using a methylation-sensitive enzyme, the methylated state of genomic DNA can be screened. (f)After finding spots of interest, the DNA fragments of those spots can be cloned from punched out gel. Figure 1 shows the principles and the entire procedure of the RLGS method.

Protocol

The Procedure of the RLGS method consists of 8 steps.
(i)Blocking: This is done to reduce the background generated by the incorporation of radioactivity in the nonspecifically damaged sites. The sample DNA was treated with 10 units of E. coli DNA polymerase I in the presence of 0.33mM dGTPaS, 0.33mM[α-^{32}P] dCTP and 0.33mM [α-^{32}P] dGTP.
(ii)Landmark cleavage by restriction enzyme A: This step is for cleavage of genomic DNA at the restriction landmarks. The blocked DNA is digested by 20 units of restriction enzyme A, whose site is used as a restriction landmark.
(iii)Labeling: The labeling method depends on the shape of the end of the restriction fragment. For a 5' protruding end, the reaction of [α-^{32}P] deoxy-nucleotide with E.coli DNA polymerase I is used. For a 3' protruding end or blunt end, the reaction of [α-^{32}P] dideoxy-nucleotide with deoxynucleotidyl terminal transferase is used.
(iv)Fragmentation of labeled DNA with restriction enzyme B: The samples were incubated with 20 units of restriction enzyme B, ddGTP, ddCTP and $MgCl_2$ at 37°C for 60 minutes.
(v)First fractionation by agarose gel electrophoresis: DNA restriction fragments were fractionated in one dimension by 1% agarose gel electrophoresis.

(vi)Fragmentation of labeled DNA with restriction enzyme C: A strip of the one-dimensional gel was treated with 1500 units of restriction enzyme C.
(vii)Second fractionation by polyacrylamide gel: The second fractionation was done in a two-dimensional 6% polyacrylamide gel electrophoresis after connection the agarose strip to the two-dimensional polyacrylamide gel.
(viii)Autoradiography: The final gel samples were dried and auto-radiographed.

Purpose

Polymorphism shows the diversity of form and characteristics within a species. Genetic polymorphisms are not often recognized visually. Polymorphism is the most important basis of variety in living organisms. The RLGS method is suitable for screening the physical state of genomic states at high speed. It has the best detection ability of all genome scanning methods. It can be effectively applied to the detection of DNA polymorphisms in mammals. In this report we have tried to evaluate the hereditary distance of the intersubspieces by the RLGS method.

Materials and Methods

Mice was examined for the calculation the DNA polymorphic rates as a means of evaluating the intraspieces hereditary distances: mice (B6, D2, M.spretus, M.m. domestics and M.m.molossinus). All tissues came from the liver and were immediately frozen in liquid nitrogen and genomic DNA extraction was performed.
The combination of three restriction enzymes are NotI, PvuII and PstI. The extracted DNA was treated using this combination of three restriction enzymes following the above-mentioned protocol of the RLGS method. After autoradiography, the polymorphic ratios were calculated by a comparison of two strains. The sum of the number of specific polymorphic spots and non-specific polymorphic spots of one strain was divided by the total number of countable polymorphic & non-polymorphic spots.

Results

The results are shown in Table 1 and Figure 1. In addition, The ratio of polymorphic spots between B6 and D2 was 13.4% and that between B6 and M.spretus was 50.9%. As you can see, the polymorphic rate between laboratory mouse B6 and wild mouse M. spretus was higher than that between laboratatory mice B6 and D2. Table 1 and Figure 1 show the same result of the intersubspieces. From this result, the polymorphic rate determined by the RLGS method seems to correlate with hereditary distance.

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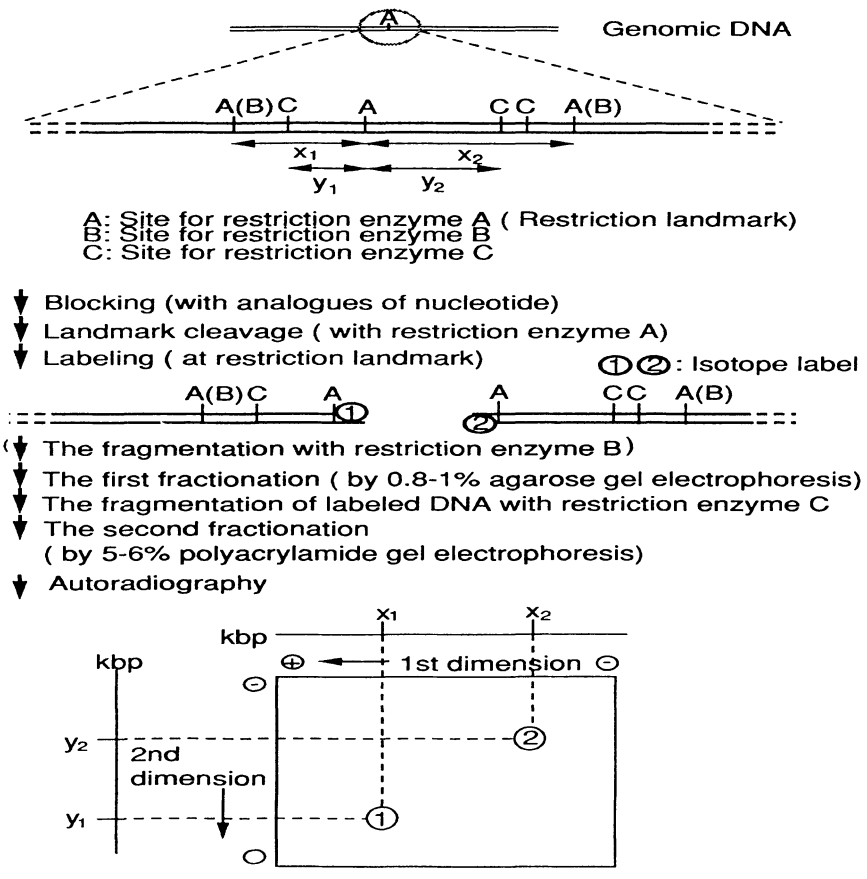

Figure1. Principle of RLGS method

Table 1. RLGS polymorphism among three Mus species

	M. m. domestics	M. m. molossinus	M. spretus
Mus musculus domestics (C3H/HeN)	-	33%	56%
Mus musculus molossinus	-	-	40%
Mus spretus	-	-	-