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DNA Profiling. An Approach of Systemic Characterization, Classification, and Comparison of Genomic DNAs

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A new method to obtain profiles of giant genome DNAs is described. It is based on two key techniques: Random PCR using oligonucleotides and temperature gradient gel electrophoresis. The experiments applied to the DNAs of 3 strains of E.coli demonstrated that this technique is informative and useful. The random PCR which uses various combinations of oligonucleotides as primers is effective to extract specific DNAs from a giant genome DNA.

Taxonomy, developmental biology, genetics and the other fields related to the function of DNAs are becoming more dependent on the information obtained directly from DNAs. DNA profiling introduced here is useful to extract the information which characterizes the whole genome. It is based on a technique of sampling of specific regions and a technique of denaturing gradient gel electrophoresis,[1,2] especially temperature gradient gel electrophoresis (TGGE)[3]. The procedure of DNA profiling is schematically shown in Fig. 1.

Once DNA is prepared at the amount of 10^3 molecules or more, random-PCR-based DNA profiling is ready to be attained via three steps shown in Fig.1. At the first step, one an obtain enough amount of specific DNA fragments used for TGGE. Random PCR is also useful to generate a limited number of and suitably sized DNA fragments which are neither too much nor too big to be analysed by TGGE. The size of a DNA which shows highly informative mobility transitions in the denaturing gradient gel techniques is limited to 10^{2-3} bp.[2] Moreover, such desirable number of DNA fragments could not be obtained by restriction enzymes since a 4-cutter one, which produce suitably sized DNA fragments of 10^{2-3} bp, generates about 18000 fragments provided that the genomic DNA analysed is the size of E.coli DNA; 4.7×10^6 bp. Obviously, the gel electrophoresis of such a great number of DNA fragments results only in a smear. Here, we try to exemplify the feasibility of random-PCR-based DNA profiling. The most essential part of this method is the sampling process, random PCR, which has a significance of extracting a specified partial information from a whole genome. The suitable size of oligonucleotides used for random PCR, n (in nucleotide), could be roughly estimated from the probabilistic point of view to be; $n=\log_4 N$, where N is the DNA size in nucleotide of a genome

concerned. Note that instead of lengthening the size of oligonucleotides, the equivalent effect can be obtained by increasing the PCR cycle in which DNAs are amplified in an exponential mode. It can be easily performed by restarting the second PCR process using a tiny portion of the first PCR product as template DNAs. Through the competition between DNAs in exponential-mode replication, a small number of DNAs are expected to become the majorities. With a genome size becoming great, the number of tough competitors becomes larger. Therefore, it needs a long-termed selection process.[5] Stoichiometry between a template and oligonucleotides, thermodynamic stabilities of those complexes, and the others should be also taken into account.[6] The content of standard oligomers are arbitrary to researchers so far as they apply the same subsets of oligomers to a series of comparison experiments. However, it will be reasonable to define a fixed set of oligonucleotides for the sake of universality (to be discussed in detail elsewhere).

The result obtained from the experiment with E.coli DNAs is shown in Fig.2. Highly similar patterns were obtained between the DNAs of different strains. When the other oligonucleotides were applied to the random PCR with those bacterial DNAs, quite similar inclinations were observed (data not shown). It demonstrates the effectiveness of random PCR for extracting specific subset sequences from giant genomic DNAs under the direction of oligonucleotides. Therefore, though the choice of oligonucleotides may be at random, the amplified DNAs are not so but deterministic depending on the oligonucleotides used.

The DNAs thus prepared were subjected to TGGE for DNA profiling. In Fig.3, shown are characteristic mobility profiles corresponding to the sequences of DNAs.[2,6] Even a single-base substitution in DNA often results in a different mobility profile[7] and the mobility transitions are characteristic to each DNA, reflecting its nucleotide sequence.[2,6] Among the information obtained by this method there are knowledges on the relative amount of the DNAs amplified and the length and characteristics-in-sequence of those DNAs. Since each

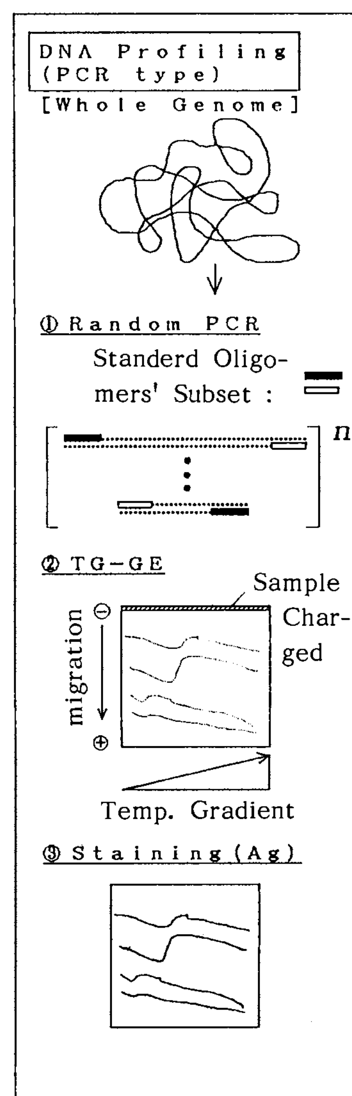


Fig.1. Procedure of random-PCR-based DNA profiling. In random PCR, various DNA fragments were shown to be amplified and in TGGE, those DNAs migrate perpendicularly to the direction of temperature gradient and express characteristic mobility profiles after staining with silver. The dotted lines represent DNA bands which are not visible.

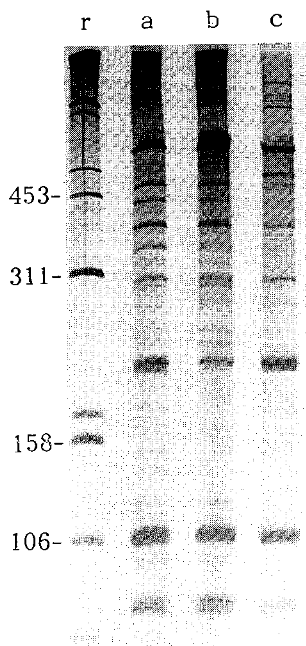
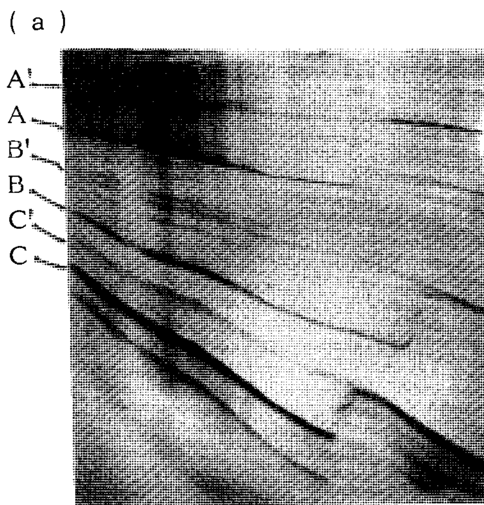
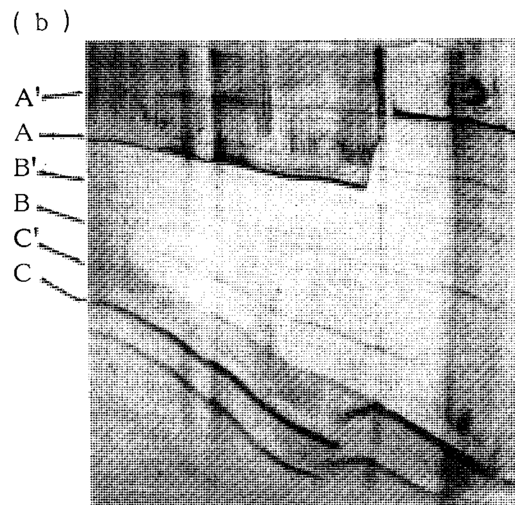


Fig.2. DNA fragments obtained by random PCR. The used primers were d(GpCpGpCpApTpTpApGpApCpG) and d(CpGpGpTpGpCpTpG) and the templates, and a genomic DNA of E.coli S26 (a), X4-4 (b), LE392 (c) as a template. The reaction mixture contained a template DNA, 5×10^{-17} mol; oligonucleotides, 3.5×10^{-10} mol (each); each of dNTPs, 200 uM; AmpliTaq (Perkin-Elmer Cetus), 1.25 Unit; and gelatin, 0.01 %(w/v) in 500 ul of PCR buffer (10 mM Tris-HCl (pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$). PCR was performed after preincubation at 94 °C for 5 min. The PCR cycle adopted consists of 94 °C-2 min denaturation, 30 °C-5 min annealing, and 45 °C-4 min polymerization, which was repeated 40 times, and was followed by an incubation of 45 °C-5 min. The gel electrophoresis was performed in 8 M urea at 60 °C. Lane r contains size markers (expressed in nucleotides).





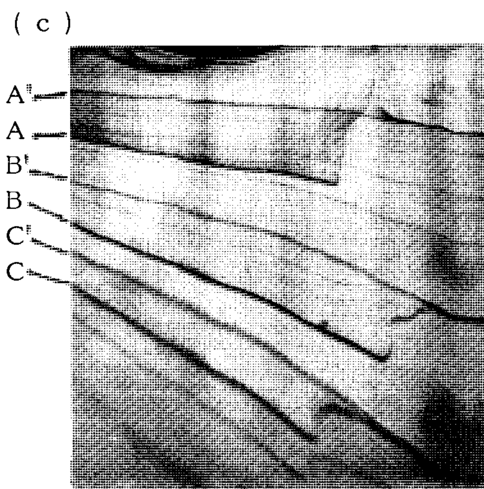


Fig.3. DNA profiling of 3 strains of E.coli. The DNA samples from E.colis (a) S26, (b) X4-4, and (c) LE392 were prepared as described in the legend of Fig.2 and were subjected to TGGE under the following conditions: gels, 4% (w/v) polyacrylamide containing 8 M urea; and temperature gradients, 19 °C upto 70 °C, generated by a homemade apparatus. The temperature gradient linearly rises left to right and the direction of migration is top to bottom. The major bands denoted by A, A', B, B', C, and C' are shown to draw the similar mobility profiles against the difference of strains. The bands, A', B', and C', are single-stranded DNAs corresponding to the double-strands, A, B, and C, respectively.

combination of oligonucleotides can offer a different group of DNA fragments, a decade of oligonucleotides, if used in various combination, will give us multiple information. This method is more useful than RFLP for a genomic analysis since the latter can hardly deal with a giant DNA at the fragment size of 10^{2-3} bp with a help of DNA probes (DNA fingerprinting[8]). RFLP offers only the knowledge about the distribution of the restriction sites and does not utilize sequence information except DNA fingerprinting which therefore more informative but rather laborious.[9]

The popularity of this method will depend on the goodness of the design of standard oligonucleotides.

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