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# Application of AFLP Markers to DNA Fingerprinting in Rainbow Trout (*Oncorhynchus mykiss*)

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## INTRODUCTION

The most important of various merits are the capacity to investigate an total genome for polymorphism and AFLP is superior to any other systems in terms of the number of sequences amplified per reaction and its reproducibility(Vos et al., 1995). The AFLP technique provides a novel and very powerful DNA fingerprinting technique for DNAs of origin or complexity (Vos et al., 1995). In this study, genomic DNAs isolated from rainbow trout were digested by restriction enzymes, ligated by adapters and amplified by selective primers in order to identify the AFLP variations, to determine genetic distances and to detect the intra-population genetic markers.

## MATERIALS AND METHODS

### Rainbow trout collection and sources of genomic DNA

AFLP-PCR analysis was performed on genomic DNA samples from a total of 23 individuals from rainbow trout (*Oncorhynchus mykiss*) collected from a aquaculture facility in the Kangwon-do, Korea. Purity and concentration of DNA purified were estimated by calculating the ratio of $A_{260}/A_{280}$ measured with a spectrophotometer (Shimadzu, Australia).

### Genomic DNA digestion and adapter ligation, AFLP primers and amplification conditions

The original AFLP protocol developed by Zabeau and Vos (European Patent Application No. 0534858 A1, 1993) was followed with the minimum modifications. Eight selective primers were synthesized to be complementary to the adapter/restriction-site sequences and to carry selective 3′ nucleotides. Selective primer pairs included two EcoR I + 4 primers and two HindIII + 4 primers. Amplification was performed in a DNA Thermal Cycler (Perkin Elmer Cetus, USA). The gels were illuminated with UV light and photographed by UV DNA photographic system (Seoulin Co. Korea).

### Data analytical methods

An average of whin-population similarity is calculated across all pairwise comparisons among individuals within a population. Single linkage cluster analysis was performed on the similarity matrices in order to generate a dendrogram using pc-package program

Systat version 10 (SPSS Inc., USA).

## RESULTS AND DISCUSSION

Considering the resolution of two gel electrophoresis, it is possible to construct a high resolution with the large number of primers available in AGE and PAGE in rainbow trout. While minor band, less than 150 bp, unidentified in agarose gel, AFLP fragments ranged from approximately 100 bp were detected in sequencing gel electrophoresis. A total of fragments, an average of fragments per primer and polymorphic DNA bands identified in polyacrylamide gel electrophoresis were much more than those in agarose gel electrophoresis. As calculated by bandsharing analysis in AGE, the average level of genetic difference was approximately 0.590 ± 0.125 in the individuals of this population. Also, in PAGE, the average level of genetic difference was approximately 0.654 ± 0.081 in the individuals of this population. On the whole, the similarity index using PAGE dat was higher than that obtained from AGE.

Table 4. Similarity matrix including bandsharing values and genetic differences calculated using Nei and li's index of similarity for rainbow trout (O. *mykiss*) obtained in PAGE data.

| | | Bandsharing values of individuals | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Genetic differences of individuals | 1 | - | 0.770 | 0.602 | 0.585 | 0.654 | 0.596 | 0.613 | 0.615 | 0.676 | 0.590 | 0.727 | 0.642 | 0.687 | 0.720 | 0.686 | 0.635 |
| | 2 | 0.230 | - | 0.630 | 0.625 | 0.634 | 0.591 | 0.648 | 0.676 | 0.654 | 0.608 | 0.636 | 0.556 | 0.569 | 0.613 | 0.608 | 0.417 |
| | 3 | 0.398 | 0.370 | - | 0.633 | 0.664 | 0.572 | 0.683 | 0.628 | 0.664 | 0.612 | 0.528 | 0.592 | 0.712 | 0.589 | 0.665 | 0.473 |
| | 4 | 0.415 | 0.375 | 0.367 | - | 0.581 | 0.561 | 0.625 | 0.595 | 0.599 | 0.590 | 0.545 | 0.608 | 0.650 | 0.597 | 0.578 | 0.591 |
| | 5 | 0.346 | 0.366 | 0.336 | 0.419 | - | 0.728 | 0.774 | 0.737 | 0.651 | 0.585 | 0.652 | 0.611 | 0.679 | 0.607 | 0.659 | 0.620 |
| | 6 | 0.404 | 0.409 | 0.428 | 0.439 | 0.272 | - | 0.616 | 0.732 | 0.564 | 0.731 | 0.488 | 0.723 | 0.660 | 0.664 | 0.676 | 0.561 |
| | 7 | 0.387 | 0.352 | 0.317 | 0.375 | 0.226 | 0.384 | - | 0.720 | 0.685 | 0.853 | 0.694 | 0.706 | 0.828 | 0.743 | 0.664 | 0.592 |
| | 8 | 0.385 | 0.324 | 0.372 | 0.405 | 0.263 | 0.368 | 0.280 | - | 0.717 | 0.741 | 0.689 | 0.771 | 0.821 | 0.681 | 0.703 | 0.606 |
| | 9 | 0.324 | 0.346 | 0.336 | 0.401 | 0.349 | 0.436 | 0.315 | 0.283 | - | 0.701 | 0.591 | 0.631 | 0.689 | 0.574 | 0.541 | 0.609 |
| | 10 | 0.410 | 0.392 | 0.388 | 0.410 | 0.415 | 0.269 | 0.147 | 0.259 | 0.299 | - | 0.687 | 0.816 | 0.833 | 0.734 | 0.658 | 0.560 |
| | 11 | 0.273 | 0.364 | 0.472 | 0.455 | 0.348 | 0.512 | 0.306 | 0.311 | 0.409 | 0.313 | - | 0.841 | 0.792 | 0.718 | 0.661 | 0.645 |
| | 12 | 0.358 | 0.444 | 0.408 | 0.392 | 0.389 | 0.273 | 0.294 | 0.229 | 0.369 | 0.184 | 0.159 | - | 0.825 | 0.789 | 0.700 | 0.758 |
| | 13 | 0.313 | 0.431 | 0.288 | 0.350 | 0.321 | 0.340 | 0.172 | 0.179 | 0.311 | 0.167 | 0.208 | 0.175 | - | 0.762 | 0.680 | 0.634 |
| | 14 | 0.280 | 0.387 | 0.411 | 0.403 | 0.393 | 0.336 | 0.257 | 0.319 | 0.426 | 0.266 | 0.282 | 0.211 | 0.238 | - | 0.709 | 0.526 |
| | 15 | 0.314 | 0.392 | 0.335 | 0.422 | 0.341 | 0.324 | 0.336 | 0.297 | 0.459 | 0.342 | 0.339 | 0.300 | 0.320 | 0.291 | - | 0.504 |
| | 16 | 0.365 | 0.583 | 0.527 | 0.409 | 0.380 | 0.439 | 0.408 | 0.394 | 0.391 | 0.440 | 0.355 | 0.242 | 0.366 | 0.474 | 0.496 | - |

## REFERENCES


Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23 (21): 4407-4414.

Zabeau, M. and P. Vos. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application Number: 9240269. 7, Publication Number: 0534 858 A1.