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Allelic Segregation of PCR-amplified DNA of Tandem Repeats
(PCR로 증폭된 연쇄반복 대립형의 분리 보합)

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The human genome contains numerous tandemly repeated sequences. These tandem repeats (TR) exhibit extreme polymorphism due to the wide variation of the copy number of unit repeats. The TRs served as a powerful tool in various fields of genetics; in forensic identification of criminals, in determining paternity, in mapping disease genes, not to mention the Human Genome Project.

The STRs with unit-repeats of only a few nucleotides long were particularly useful. They are ubiquitous and could be amplified by PCR. Although the alleles are different by a few nucleotides, they could be discretely identified on a denaturing acrylamide gel. However, the resolution of STR alleles by denaturing acrylamide gel electrophoresis is also a serious setback for STR typing system. The denaturing gel electrophoresis is laborious and requires either silver-staining or use of isotopes. We have been developing an alternative approach for STR typing based on our discovery of allele-specific hybridization of the amplified TR.

The PCR-amplified alleles of TR has unique sequences on both ends of the tandemly repeated sequence. Alleles are different only by the number of the repeated sequences. When two different allelic DNAs are denatured together, only original duplexes made of singlestrands with exactly matching number of repeat unit were formed. The alleles could then be identified by simply allowing hybridization of an unknown DNA to a set of known standard alleles. We cloned a range of alleles for various TRs and prepared purified allelic DNA for standards. On each well of an ELISA plate, each standard allelic DNA was harnessed and labeled amplified sample DNA was applied to allow hybridization between the sample and standards. We could observe distinct color development demonstrating allele-specific hybridization on solid support.