

A Novel Method for SNP Analysis Using Fluorescence Polarization

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Introduction

Single nucleotide polymorphisms (SNPs) are the most common genetic variations between individuals of a species and are therefore thought to be responsible for a large part of individual phenotypic variation. It has been estimated that an SNP occurs every 100–300 bases in the human genome. Research on human SNPs is expected to facilitate genetic mapping studies that may lead to a better understanding of the genetic basis for complex diseases and individual variation in drug metabolism.

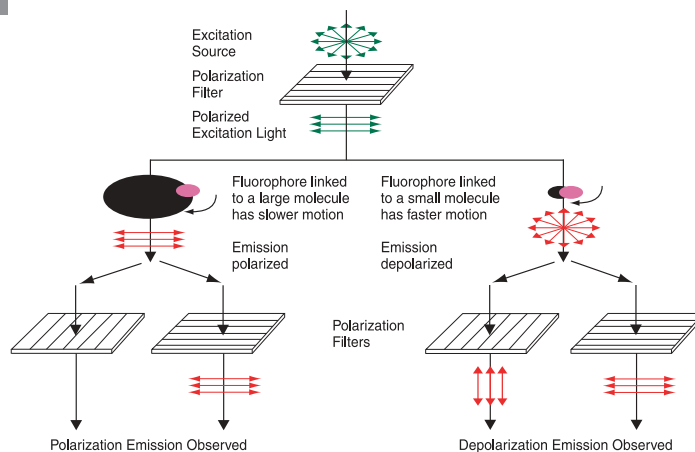
We have developed a novel assay, called the AcycloPrime™-FP SNP Detection Kit, for the identification of known SNPs based on fluorescence polarization (FP) detection. The kit is designed for performing an assay to determine the base at an SNP location in an amplified DNA sample by a modification of Template-directed Dye-terminator Incorporation with Fluorescence Polarization detection (TDI-FP) (1). The assay is homogeneous, inexpensive, very accurate and readily adapted to automation.

Methods

FP is an empirical technique that measures the vertical and horizontal components of the fluorescence emission produced after excitation by plane polarized light. Polarization values (in mP units) are inversely related to the speed of molecular rotation of the fluorescent target. Since molecular rotation is inversely related to the molecular volume, incorporation of a fluorescent terminator into a primer oligonucleotide increases its polarization. This is used to determine which labeled terminator has been incorporated.

1

Principle of Fluorescence Polarization (FP)

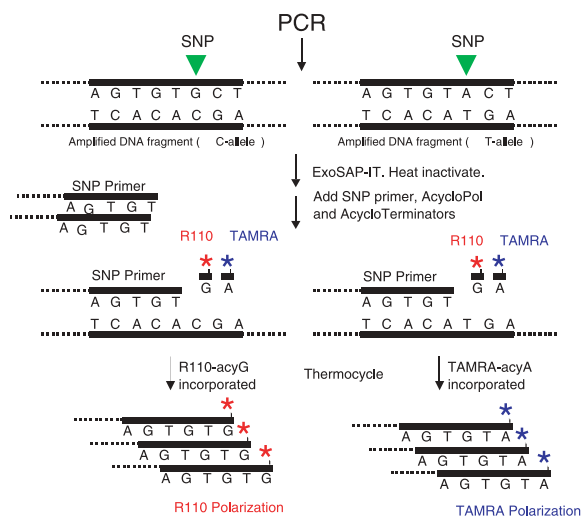


Chen, et al., Genome Research 9, 492-498 (1999)

FP occurs when a fluorescent dye is excited by plane-polarized light. It is independent of fluorescence intensity. Since a large molecule has slower rotation than a small molecule, a dye-labeled terminator attached to a primer will show a higher level of polarization than a free dye-labeled terminator.

2

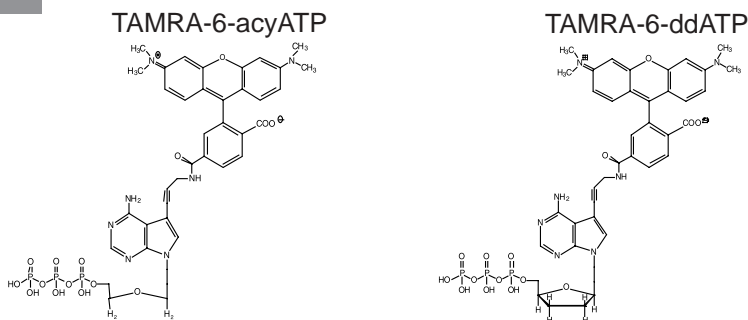
AcycloPrime Reactions



Following PCR amplification of the sequence containing the SNP of interest, excess primer and dNTPs are removed through incubation with shrimp alkaline phosphatase (SAP) and exonuclease I. Once the enzymes are heat inactivated, the AcycloPrime-FP process uses a thermostable polymerase to add one of two fluorescent terminators to a primer that ends immediately upstream of the SNP site. The terminator(s) added are identified by their increased FP and represent the allele(s) present in the original DNA sample.

3

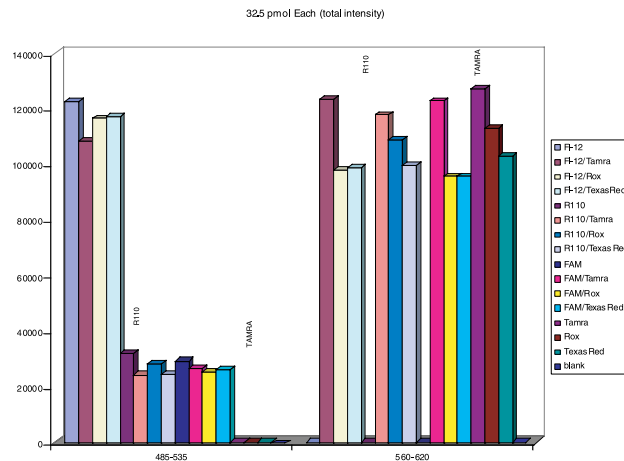
AcycloTerminator and Dideoxyterminator Structures



The AcycloPrime process uses AcycloPol™, a novel mutant thermostable polymerase from the Archeon family, and a pair of AcycloTerminators™ labeled with R110 and TAMRA, representing the possible alleles for the SNP of interest. AcycloTerminator™ non-nucleotide analogs are biologically active with a variety of DNA polymerases. Similarly to 2',3'-dideoxynucleotide-5'-triphosphates, the acyclic analogs function as chain terminators. The analog is incorporated by the DNA polymerase in a base-specific manner onto the 3'-end of the DNA chain, and since there is no 3'-hydroxyl, is unable to function in further chain elongation. It has been found that AcycloPol has a higher affinity and specificity for derivatized AcycloTerminators than various Taq mutants have for derivatized 2',3'-dideoxynucleotide terminators.

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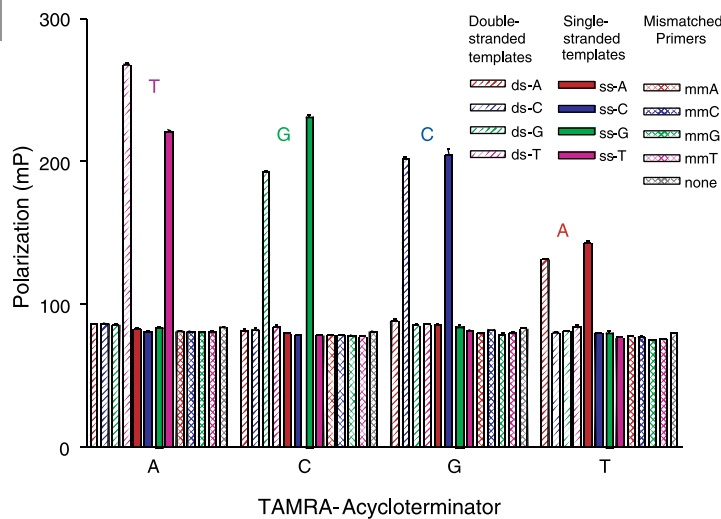
Selection of Dyes



Solutions containing 32.5 pmol of each dye were read on an FP instrument using excitation/emission filters of 485 nm/535 nm or 560 nm/620 nm. The goal was to identify a pair of fluorescent dyes showing no overlap between the filter sets. The analysis of spectral properties of various dyes showed no overlaps between two groups: fluorescein/FAM/R110 & TAMRA/ROX/Texas Red. For development of a 2-color assay method, the dyes R110 and TAMRA were chosen from these groups.

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Assay Precision

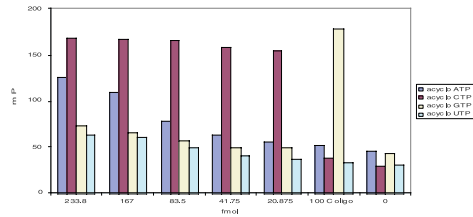


Synthetic 40-mer oligo templates containing each of the possible bases at the SNP location were used to demonstrate the specificity of terminator incorporation using AcycloPrime reagents. No misincorporation was found using either single-stranded or double-stranded templates. Error bars(!) indicate 1σ .

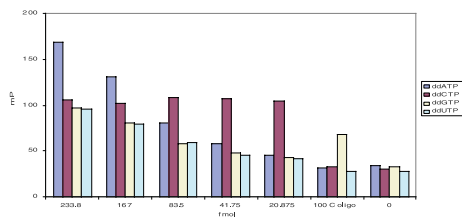
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Misincorporation with Excess Target DNA

AcycloPol, Acyclos



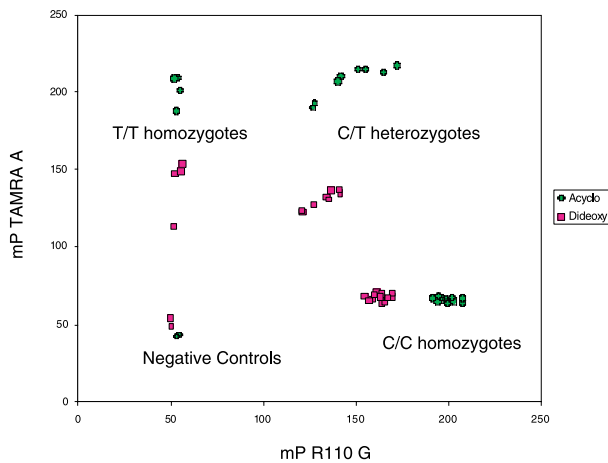
ThermoSequenase, Dideoxys



Overproduction of target DNA in the amplification steps can lead to misincorporation during primer extension when the reaction is pushed further after all of the “correct” terminator is incorporated. The high specificity and affinity of AcycloPol for labeled AcycloTerminators makes the AcycloPrime reactions more resistant to misincorporation caused by excess target DNA than is the combination of ThermoSequenase and dideoxyterminators. In this experiment, each group of data represents 4 labeled terminators. Starting at the right, the incubation was with no template, 100 fmol of a synthetic oligo containing a C at the SNP location, or increasing amounts of a target DNA template with a G at the SNP position.

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AcycloPol vs. ThermoSequenase



The same samples were run using AcycloPrime kits (AcycloPol and AcycloTerminators) or ThermoSequenase and dideoxyterminators. For each condition, a plot of the polarization of the two dyes shows four data clusters representing the two homozygotes, the heterozygotes and the negative controls. The affinity and specificity of AcycloPol for labeled AcycloTerminators results in more complete AcycloTerminator incorporation and therefore larger FP values. This results in improved cluster separation.

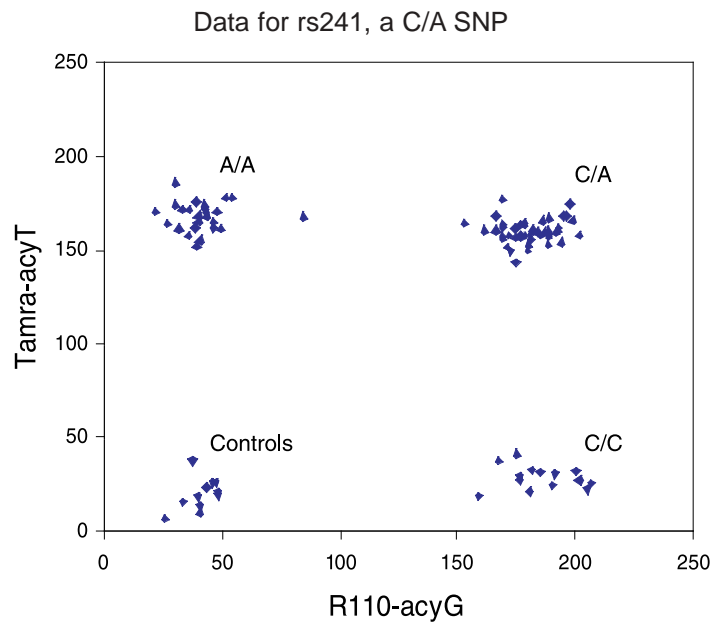
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The enhanced performance of the AcycloPrime kits has been verified by independent researchers (next 2 figures).



Data using AcycloPrime kits courtesy Dr. Steven Hamilton, New York State Psychiatric Institute and Dr. James Knowles, Columbia University.

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Data using AcycloPrime kits courtesy Dr. Pui Kwok, Washington University, St. Louis.

Conclusions

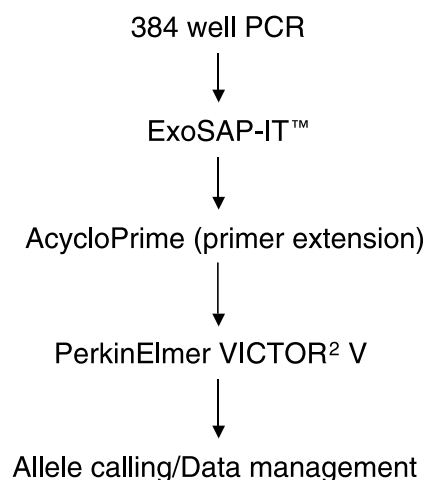
AcycloTerminators have been combined with a mutant thermostable polymerase, AcycloPol, in a specific chain termination assay for SNPs. The combination of AcycloTerminators and AcycloPol has allowed the development of a streamlined, inexpensive assay procedure with improved accuracy relative to dideoxynucleotides and Thermosequenase. The method is a simple, homogeneous assay for SNP analysis that requires no centrifugation, washing, separation or transfer steps, resulting in minimum hands-on time.

The complete AcycloPrime assay is conveniently performed in black 384-well PCR plates, reading the FP using a Victor² V instrument with FP capabilities.

Literature Cited

1. Chen, X., Levine, L. and Kwok, P-Y, "Fluorescence polarization in homogeneous nucleic acid analysis", *Genome Res.* 9:492-498 (1999).

SNP Assay Workflow





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