PerkinElmer Life Sciences, Inc.

AcycloPrime™-FP SNP Detection Kit

Catalog Numbers

R110/TAMRA	1K SNPs	10K SNPs	100K SNPs
G/A	ACP101A	ACP101B	ACP101C
G/C	ACP102A	ACP102B	ACP102C
G/T	ACP103A	ACP103B	ACP103C
C/A	ACP104A	ACP104B	ACP104C
C/T	ACP106A	ACP106B	ACP106C
A/T	ACP109A	ACP109B	ACP109C
Combo*	-	ACP113B	ACP113C

***ACP113B contains 3 x 1K G/A, 3 x 1K C/T, 1 x 1K G/C, 1 x 1K G/T, 1 x 1K C/T and 1 x 1K A/T**
ACP113C contains 3 x 10K G/A, 3 x 10K C/T, 1 x 10K G/C, 1 x 10K G/T, 1 x 10K C/T and 1 x 10K A/T

NEW! **PCR Clean-Up Reagents now included.** **NEW!**

For Laboratory Use

Caution: Research chemicals for research purposes only.

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I. PRODUCT NAME

AcycloPrime™-FP SNP Detection Kit.

II. INTENDED USE

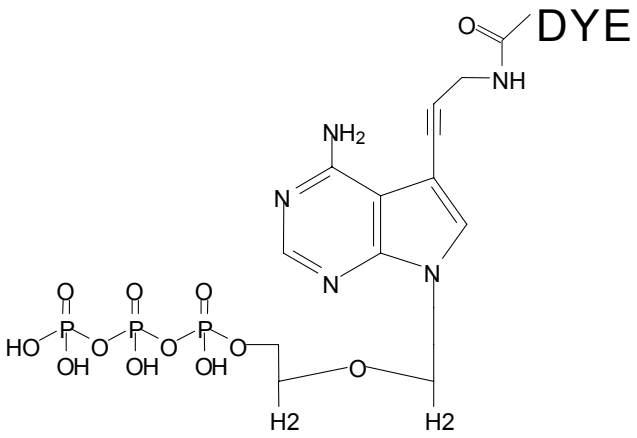
The AcycloPrime™-FP SNP detection kit is designed to determine the base at a SNP location in an amplified DNA sample by a modification of Template-directed Dye-terminator Incorporation with Fluorescence Polarization detection (TDI-FP) (1, 2).

III. INTRODUCTION

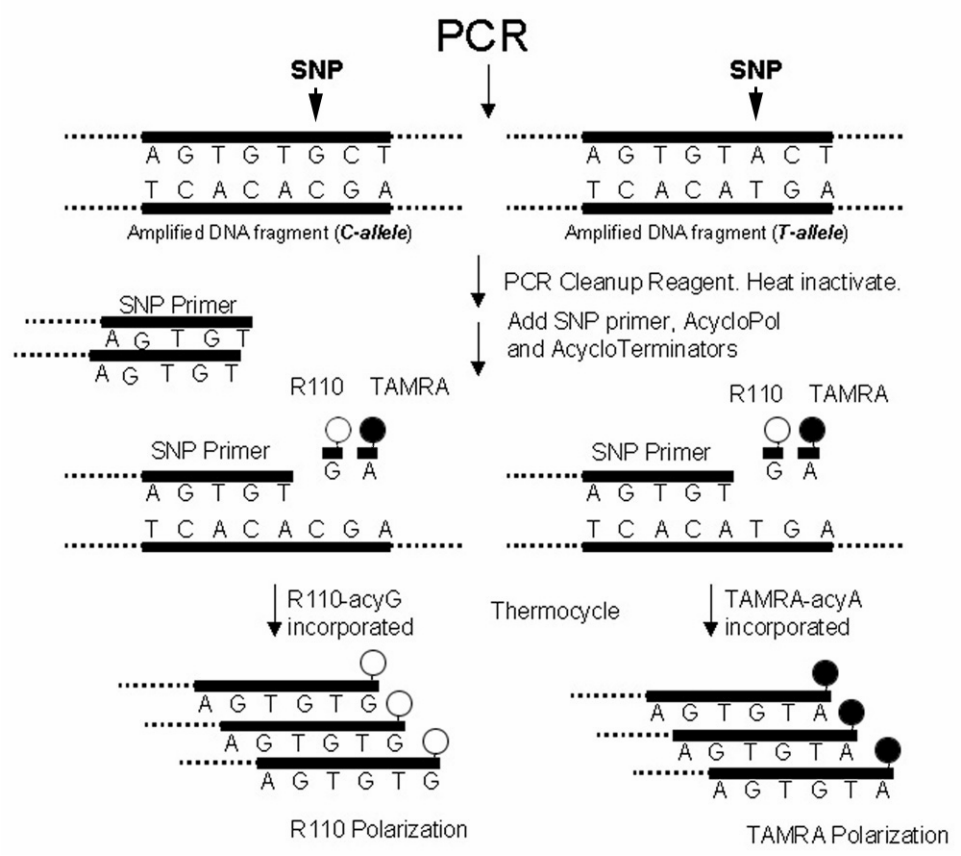
Single nucleotide polymorphisms (SNPs) are the most common genetic variations between individuals of a species and are therefore thought to be responsible for a large part of individual phenotypic variation. Research on human SNPs is expected to facilitate genetic mapping studies that may lead to a better understanding of the genetic basis for complex diseases and individual variation in drug metabolism.

IV. PRINCIPLES OF THE PROCEDURE

The AcycloPrime-FP process uses a thermostable polymerase to extend by one base an oligonucleotide primer that ends immediately upstream of the SNP position, using one of two fluorescent dye-labeled terminators. The identity of the base added is then determined by the increased fluorescence polarization (FP) of its linked dye. The process uses AcycloTerminators™ and AcycloPol™, a novel mutant thermostable polymerase from the Archeon family. 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 (3). The structure of a representative dye-labeled AcycloTerminator, dye-acyATP, is shown below:



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 AcycloTerminator into a primer oligonucleotide increases its polarization. This is used to determine which of the two labeled terminators has been incorporated.
The AcycloPrime-FP process is illustrated schematically below:



V. KIT COMPONENTS

Reagents supplied are intended FOR LABORATORY USE. Kits are shipped on dry ice. For maximum stability, all reagents should be stored at -20°C as described below and on the vial labels. If desired for convenience, the AcycloTerminator Mix, 10X Reaction Buffer and PCR Clean-Up Reagent Diluent Buffer may be stored at 4°C for up to one month.

NOTE: The AcycloTerminator Mix, particularly if it contains R110-acyGTP (ACP101, ACP102, ACP103, and ACP113), is sensitive to light. Under ordinary circumstances, the brown bottle is sufficient to protect the solution from light effects. If the solution is removed from the brown bottle it is shipped in, take precautions to minimize prolonged light exposure. Exposure to normal laboratory lighting during routine assay operations is not detrimental to subsequent stability.

1. 10X Reaction Buffer

2 vials, 1.1 mL (1K SNPs) or
1 bottle, 21 mL (10K SNPs) or
1 bottle, 204 mL (100K SNPs)

Tris-HCl containing $MgSO_4$ and detergent. Store at -20°C upon receipt.

2. AcycloPol™

1 vial, 0.055 mL (1K SNPs) or
1 vial, 0.525 mL (10K SNPs) or
1 vial, 5.1 mL (100K SNPs)

Contains a mutant thermostable polymerase in buffer with salts, reducing agent and glycerol. Store at -20°C upon receipt.

3. AcycloTerminator™ Mix

1 bottle, 1.1 mL (1K SNPs) or

1 bottle, 10.5 mL or
10 bottles, 1.1 mL (10K SNPs) or

1 bottle, 102 mL or
10 bottles, 10.5 mL (100K SNPs)

Each mix contains one R110-labeled AcycloTerminator, one TAMRA-labeled AcycloTerminator and two unlabeled terminators. The vial label specifies which labeled AcycloTerminators are in the particular AcycloTerminator Mix. The first base listed is labeled with R110 and the second base with TAMRA; *e.g.* the G/A AcycloTerminator Mix contains R110-acyGTP and TAMRA-acyATP. Different AcycloTerminator Mixes contain different pairs of labeled AcycloTerminators and are used for different SNPs. Verify that you have ordered and received the correct AcycloTerminator Mix for the reactions to be performed.

On choosing the correct kit:

Which bases are incorporated depends upon which orientation is chosen to prepare the SNP primer. To illustrate, consider the following sequence for two heterozygous chromosomes containing a G/A SNP (bold) on the upper strands. Notice that this is a C/T SNP on the lower strands.

```
5'-ACTTAGGCCGACCGCTAGCGTACAGAGGCCTTAGACATGCATGCAGT-3'
3'-TGAATCCGGCTGGCGATCGCATGTCTCCGGAATCTGTACGTACGTCA-5'

5'-ACTTAGGCCGACCGCTAGCGTACGGAGGCCTTAGACATGCATGCAGT-3'
3'-TGAATCCGGCTGGCGATCGCATGCCTCCGGAATCTGTACGTACGTCA-5'
```

If the SNP primer has the sequence underlined on the upper strands, the next base added will be either an A or a G, so use the G/A kit. If the SNP primer has the sequence underlined on the lower strands, the next base added will be either a C or a T, so use the C/T kit. Notice this means that two different kits are required to perform the assay with both forward and reverse SNP primers unless the SNP is either G/C or A/T.

4. PCR Clean-Up Reagent, 10X

This is a custom formulation of Exo-SAP-IT™ sold under agreement with USB® Corporation. It is optimized to degrade primers and dNTPs from the amplification steps to prevent interference with primer extension. This material is significantly different from Exo-SAP-IT and is used differently as described in section VII, Processing of PCR Product.

1 vial, 0.2 mL (1K SNPs) or
1 bottle, 2.0 mL (10K SNPs) or
1 bottle, 20.0 mL (100K SNPs)

Contains a mixture of exonuclease I and shrimp alkaline phosphatase in buffer with glycerol. Store at -20°C on receipt.

5. PCR Clean-Up Dilution Buffer

1 bottle, 2.0 mL (1K SNPs) or
1 bottle, 20.0 mL (10K SNPs) or
1 bottle, 200 mL (100K SNPs)

Contains a buffer. Store at -20°C on receipt.

6. Instruction Manual

Includes performance data, instructions for reagent preparation and a protocol for primer extension.

VI. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

1. Thermocycler

Preferred models can utilize a skirted, PCR-compatible microplate.

2. Microplates, 384 or 96 Wells, Black

Microplates from a number of manufacturers have been used satisfactorily in the assay. For PCR amplification, it is convenient to use a black 384-well PCR plate such as catalog number MSP-3862 from MJ Research. These plates can be used directly in MJ Research and some other thermocyclers, and all of the subsequent assay steps can be performed directly in the well with no sample transfer steps. Similarly designed black 96-well PCR plates (HSP-9666, MJ Research) can be used in the same way with no modifications to the assay protocol. If evaporation is found to be a problem when using 96-well plates, some users have found it helpful to add 1 μL of extra water to each well. To work well with most FP readers, a skirted plate must be used.

3. Fluorescence Polarization Reader

The assay has been shown to work well with several instruments including the PerkinElmer Victor[2], the Tecan *Ultra* and the Molecular Devices Analyst™. To ensure optimal assay performance, **it is essential to use the appropriate spectral filters, and dichroic mirrors if applicable.** These may not be provided as 'standard' components with particular FP readers.

Victor Filters

Recommended Filters for the Victor[2]:

Dye	Excitation	Emission
R110	485(30)	535(40)
TAMRA	544(15)	595(60)

G-factor Calibration

The instrument G-factor should be adjusted to facilitate comparisons between various instruments or different sites and to help with trouble-shooting. On the Victor, the two polarization directions are called S and P. With the G-factor set to 1.0, read the S and P intensities for a 20 µL of a solution containing 1 µL of any Terminator Mix and 2 µL of 10X Reaction Buffer. It is useful to average the results for several wells to get an accurate measurement. Then use the equation below to calculate the G-factor that will give a value of 50 mP for the unincorporated terminators:

$$G = [(950*S) / (1050*P)]$$

After adding the new G factor into the Victor software, reading the mP again should give a result near 50 mP. This must be done twice, once for R110 and once for TAMRA.

4. Reagents for Amplification of Target DNA

If PCR is used, purchase a licensed PCR kit. Polymerase Chain Reaction patents are owned by Roche Molecular Systems, Inc. and Hoffmann-La Roche, Inc. and are licensed to various suppliers of these reagents.

5. SNP Primer(s)

A SNP primer is a synthetic oligonucleotide complementary to the sequence adjacent to the SNP site. The next base added to the primer is therefore complementary to the base at the SNP location on the target DNA strand. Although limitations are imposed by the specific sequence around the SNP site, the best approach to primer design is to use standard criteria to ensure a melting temperature (T_m) in the range of 60°C-80°C. It is helpful to select sequences that minimize secondary structure (*e.g.*, hairpin loops) and that have approximately a 50% G+C content. The length should be kept short (usually 20-30 bases). An important control is to design primers complementary to both the sense and anti-sense strands, and to run the assay in both the "forward" and "reverse" directions.

6. DNA

This is the target DNA containing the SNPs of interest. The DNA quality must be such that it is compatible with chosen method of amplification, *e.g.*, PCR.

7. Allele-calling Software

A series of Excel-based workbooks for data analysis and allele calling are available for download from http://lifesciences.perkinelmer.com/products/snp.asp. For the Victor, files allowing automatic data transfer into the workbook are: SNP macro Victor 96 well, SNP macro Victor 384 well, or SNP macro Victor 384 4x96 well. The latter file enables four different genotypes to be analyzed in a single 384-well plate. For manual data input from the Victor, use Victor 96 well or Victor 384 well. For those with other instruments, use GENERIC 96 well or GENERIC 384 well. For those using the Molecular Devices Analyst or Tecan Ultra, similar software is available on request. To work efficiently with the workbooks, the instrument protocol should be set up so that R110 polarization is read before TAMRA polarization.

8. Other Equipment

Pipettors and/or pipettes that can accurately and precisely deliver required volumes. Low retention pipette tips are recommended.
Laboratory vortex mixer
Test tube rack
Polypropylene tubes

VII. REAGENT PREPARATION AND ASSAY PROTOCOL

As described in detail below, the 20 µL final AcycloPrime-FP reaction volume consists of:

5 µL of amplified DNA sample
2 µL of PCR Clean-Up Reagent, 1X
13 µL of AcycloPrime Mix

Each analysis uses a single microplate well, with each step performed sequentially by adding the next set of reagents. To avoid sample transfer steps, the microplate must be compatible with both the thermocycler and the FP reader.

Amplification of Target DNA

1. The sequence containing the SNP of interest must be amplified, both to increase its quantity (for sensitivity) and to reduce the complexity of the target (for specificity). Although other amplification methods can be used, these instructions assume amplification by AmpliTaq Gold® PCR in a total volume of 5 µL. Even if larger amplification reactions are performed, only 5 µL of the solution should be used in subsequent steps.

2. Most assay problems result from issues in the PCR reaction. The amount of PCR reaction product desired for subsequent steps is 50-500 fmol in 5 µL. For a 200 bp

amplicon, this will be 6-60 ng of product in a 5 µL PCR reaction. The best results will be obtained if the amount of product in each sample is consistent.

3. If too little PCR product is produced, insufficient signal will be produced to see the polarization; if too much PCR product is produced, misincorporation errors can sometimes result. If multiple bands are produced, the amount of specific target sequence available for primer extension is decreased and there is a possibility that non-specific sites may be represented which can bind the SNP primer. We recommend the following PCR conditions as robust and reliable for most assays.

PCR Conditions	Recommended	Maximum Limit
Primers	100-200 nM	1 µM
dNTPs	100-150 µM	200 µM
Genomic DNA	5-10 ng/5 µL	20 ng/5 µL
Amplicon Size	80-200 bp	1000 bp
Cycles	35	45

Typical PCR cycling conditions are as follows: an initial denaturation and enzyme activation step at 95°C for 10 minutes, followed by 25-45 cycles consisting of 94°C for 15 seconds (denaturation), 55°C for 30 seconds (annealing of primer), and 72°C for 60 seconds (extension). After a final primer extension step at 72°C for 10 minutes, the reactions are cooled to 4°C until further use.

Processing of PCR Product

1. Primers and dNTPs used in the PCR reaction must be eliminated because they will interfere with the AcycloPrime-FP reaction. This is accomplished by enzymatic digestion using the PCR Clean-Up Reagent, 10X and the PCR Clean-Up Dilution Buffer.

2. Dilute the PCR Clean-Up Reagent 10-fold with the PCR Clean-Up Dilution Buffer provided in the kit to prepare PCR Clean-Up Reagent, 1X.

   NOTE: The dilution step should be performed just prior to use. If it is necessary to dilute sooner, keep the diluted reagent cold until use to avoid deterioration of the enzymes. The diluted reagent is stable for 1-2 days at 4°C, but loses up to 30% of its activity in 4 hours at room temperature.

3. Add 2 µL PCR Clean-Up Reagent, 1X to each 5 µL amplification reaction. Incubate at 37°C for 60 minutes.

4. Inactivate the enzymes in the PCR Clean-Up Reagent by heating at 80°C for 15 minutes.

AcycloPrime-FP Mix

1. For storage, SNP primers should be dissolved at 100 µM in TE Buffer (10 mM Tris-HCl, pH 7.6 containing 0.1 mM EDTA). Before use in the AcycloPrime-FP reaction, dilute the 100 µM SNP primer 1/10 with water to give a concentration of 10 µM. If a different primer concentration is used, appropriately adjust the quantities of primer and water in the AcycloPrime Mix.

2. For each SNP primer used, prepare the appropriate amount of AcycloPrime-FP Mix as described in the table below (some excess allowed for losses in liquid transfer):

Reagent	µL/Single Reaction	µL/96 wells	mL/384 wells	mL/1K Reactions	mL/10K Reactions
AcycloPol	0.05	6	0.022	0.055	0.525
10X Reaction Buffer	2	240	0.88	2.2	21
AcycloTerminator Mix	1	120	0.44	1.1	10.5
SNP Primer (10 µM)	0.5	60	0.22	0.55	5.25
Water	9.45	1134	4.158	10.395	99.225
Total Volume:	13 µL	1560 µL	5.72 mL	14.3 mL	136.5 mL

AcycloPrime-FP Protocol

1. Add 13 µL of AcycloPrime-FP Mix to each well containing 7 µL of amplified and processed target DNA.
2. After an initial denaturation at 95°C for 2 minutes, perform 10-30 thermal cycles* consisting of: 95°C for 15 seconds and 55°C for 30 seconds.
3. Read the fluorescence polarization in an appropriate instrument.

***NOTE:** The AcycloPrime-FP reaction is very stable. This property can be used to establish the optimum conditions for a particular assay by for example, reading the result after 10 cycles, and then performing additional cycles before reading the plate again, until a sufficient difference between the negative controls and the samples is achieved. Many users find that 25 cycles is an optimum number for many assays. When finished, bring to room temperature and read. If not reading immediately, hold at 4°C. If desired, finished plates may be stored at 4°C in the dark for at least several weeks without significant loss of signal.

Calculations

1. The FP value is calculated in the instrument software by following formula:

$$mP = 1000 \times [I_{vv} - I_{vh}]/[I_{vv} + I_{vh}]$$

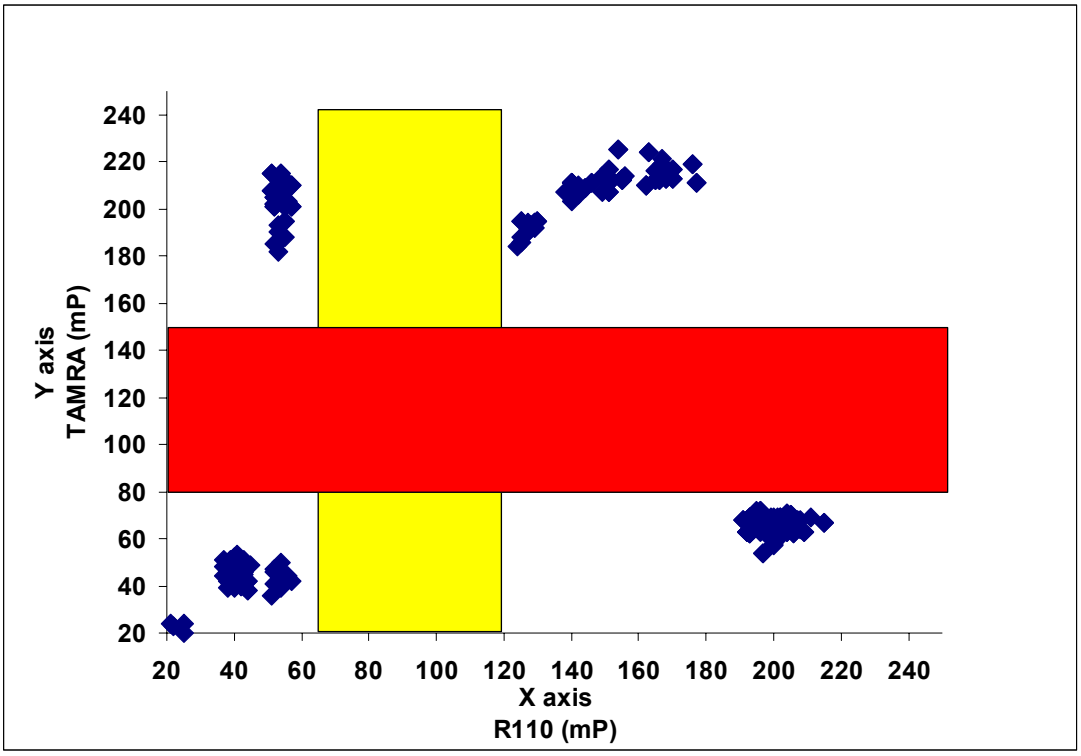
where I_{vv} is the emission intensity measured when the excitation and emission polarizers are parallel and I_{vh} is the emission intensity measured when the emission and excitation polarizers are oriented perpendicular to each other.

2. Allele scoring: As shown in the next section, a plot of the polarization of R110 vs. the polarization of TAMRA should show four data clusters representing the two homozygotes, the heterozygotes and the negative controls. The separation between the clusters should be at least 50 mP to determine the genotypes.

3. Note that the genotype on the target strand is the complement of the labeled terminator incorporated into the SNP primer.

VIII. REPRESENTATIVE RESULTS

This graph is from an Excel workbook and shows the clustering of AcycloPrime-FP data obtained by plotting the TAMRA polarization vs. the R110 polarization. The homozygous TAMRA genotypes appear as the cluster in the upper left. Similarly, the homozygous R110 genotypes are represented by the cluster in the lower right. Heterozygotes appear in the upper right cluster. Negative controls and failed PCR reactions are in the lower left cluster.



IX. SAFETY CONSIDERATIONS

10X Reaction Buffer contains tris(hydroxymethyl)aminomethane which can be irritating to eyes and skin. AcycloPol and the PCR Clean-Up Reagent, 10X contain glycerol which can be irritating to eyes and skin. Target organ: kidney.

X. TROUBLESHOOTING

Amplification Problems

Many of the problems encountered in performing AcycloPrime-FP occur in the sample DNA amplification steps. It is important to follow accepted guidelines for the selection of PCR primers. Best results are obtained with short amplicons of 200 bp or less. This minimizes the likelihood of a cross-reacting sequence in the PCR product and improves the PCR yield.

It is usually sufficient to begin the amplification with 5 ng of high-quality genomic DNA. If the DNA is of lower quality, it may be necessary to start with 10 ng or more in order to achieve the necessary PCR yield.

The PCR conditions recommended in section VII will limit the total productivity of the PCR reaction to a level that is unlikely to permit misincorporation. Because limiting conditions will reduce the PCR efficiency, it is recommended that at least 35 PCR cycles be performed to assure that sufficient product is made.

Normally it is not necessary to analyze the products of a PCR reaction. For trouble-shooting purposes, it is helpful to demonstrate the production of the right amount of product (50-500 fmol/5 µL PCR reaction) is present by running a gel. About 0.5 ng of product will give a detectable band. There also should be only a single predominant band.

AcycloPrime-FP

1. It is desirable to run several controls in each reaction. Replacing DNA in some reactions with water provides negative controls. Other desirable controls include known homozygous and heterozygous samples for the SNP of interest. It is also possible to use synthetic oligonucleotide templates with a corresponding SNP primer to demonstrate appropriate behavior of the AcycloPrime-FP reaction.

2. For particular types of sequences, it may be necessary to modify the recommended primer extension conditions. For example, if the SNP sequence being studied is particularly high in AT, it may not be possible to design a SNP primer with a T_m of 60°C or greater. In such a case, it may be necessary to lower the annealing temperature to a value 5 or more degrees below the T_m. Due to the high representation of the target sequence in the PCR reaction product, the reduced hybridization specificity caused by decreasing the annealing temperature does not cause a corresponding loss of specificity in the overall reaction.

3. A set of 40-mer synthetic oligonucleotide templates derived from reference SNP rs656 has been used in our laboratories and their sequences are shown in the table below. Using the complements of the chosen template sequences, it is possible to create

dsDNA targets and/or evaluate the reverse primer reaction. Many alternative sequences should work just as well as these.

Synthetic Oligo Template and Primer Sequences

Oligo	Sequence (5' – 3')
A Template	ATTGGATTATTTGTAACTC**A**AAGGATAAGTGCATAAGGGG
C Template	ATTGGATTATTTGTAACTC**C**AAGGATAAGTGCATAAGGGG
G Template	ATTGGATTATTTGTAACTC**G**AAGGATAAGTGCATAAGGGG
T Template	ATTGGATTATTTGTAACTC**T**AAGGATAAGTGCATAAGGGG
SNP Primer	CCCCTTATGCACTTATCCTT
reverse SNP Primer	ATTGGATTATTTGTAACTC

3. FP is a calculated value that includes the signals from both the free and the incorporated labeled AcycloTerminators. FP will reach its largest value when all of the labeled AcycloTerminator is incorporated into SNP primer. Unincorporated labeled AcycloTerminators contribute to the background signal. Therefore, it is desirable to push the reaction to completion.

   However, when the correctly base-paired AcycloTerminator is exhausted, pushing the reaction too much can lead to the non-specific incorporation of whatever labeled AcycloTerminators remain. Misincorporation typically results in poor separation of data clusters and can result in all samples appearing to be heterozygotes.

   Reading plates after various numbers of AcycloPrime-FP cycles allows stopping the process before misincorporation becomes a problem. It has occasionally been found useful to double the AcycloTerminator Mix in the primer extension reaction for a difficult SNP. If the suggested PCR conditions are used, the likelihood of misincorporation is reduced because the amount PCR product produced is automatically limited to the useful concentration range.

4. We do not encourage the practice of adding unlabeled terminators to effectively dilute or compete with the labeled AcycloTerminators in the reaction as has been suggested by others. In general, we urge the optimization of the process by controlling the extent of amplification in the PCR by appropriate assay composition and in the AcycloPrime-FP reactions through optimizing the number of thermal cycles.

XI. REFERENCES

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

2. Hsu, T.M., Chen, X., Duan, S., Miller, R.D., and Kwok, P-Y., "Universal SNP genotyping assay with fluorescence polarization detection", BioTechniques (2001) 31, 560-570.

3. Gardner, A.F. and Jack, W.E. "Acyclic and dideoxy terminator preferences denote divergent sugar recognition by archaeon and *Taq* DNA polymerases", Nucleic Acids Res. 30, 605-613.

XII. LICENSING

The AcycloPol mutant DNA polymerase is sold under license from New England BioLabs®, Inc. and is covered by U.S. Patents 5,352,778, 5,500,363 and 5,756,334. Purchase of this kit does not include a license to perform PCR, but purchase of a product from an approved vendor includes such a user license. The methods of use for the PCR Clean-Up Reagent are covered under U.S. Patents 5,741,676 and 5,756,285 issued to USB as well as other patents pending.

End User License

The purchase price of this product includes a limited, non-transferable license under U.S. Patents 5,888,819, 6,004,744 and their foreign counterparts owned by Orchid BioSciences, Inc. of Princeton, New Jersey, to perform the number of genotypes (for purposes of this End User License, genotype means the detection or quantification of an individual SNP within a single sample) indicated on the kit label, solely for the detection and analysis of SNPs in samples for research purposes only and only on instruments using fluorescence polarization or fluorescence energy transfer detection platforms. This license specifically excludes performing services for a third party and any and all diagnostic, clinical or therapeutic uses. Information about purchasing extended licenses to practice primer extension technology beyond the scope of this limited end-user license using the FP methodology may be obtained by contacting Business Development at PerkinElmer Life Sciences, Inc., Boston, Massachusetts, U.S.A., at (617) 482-9595 and using primer extension technology covered by Orchid BioSciences, Inc. by contacting the Senior Director for Business Development at Orchid BioSciences, Inc., Princeton, New Jersey, U.S.A., at (609) 750-2200.

Trademark Citations

AcycloPrime, AcycloTerminator, and AcycloPol are trademarks of PerkinElmer Life Sciences, Inc.

USB is a registered trademark of USB Corporation.

Exo-SAP-IT, patent pending, is a trademark of USB Corporation.

BioLabs is a registered trademark of New England BioLabs, Inc.

AmpliTaq Gold is a registered trademark of Roche Molecular Systems, Inc.

Analyst is a trademark of Molecular Devices Corporation.

XIII. NAME AND PLACE OF MANUFACTURE

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Netherlands	0800 02 23 042
Norway	800 11 947
Spain	900 873 255
Sweden	020 79 07 35
Switzerland	0800 55 50 27
United Kingdom	0800 89 60 46

XIV. ORDERING INFORMATION

NOTE: The letter designations in the kit names refer to the labeled AcycloTerminators included in the kit. The first letter refers to the AcycloTerminator labeled with R110 and the second letter to the AcycloTerminator labeled with TAMRA. Note that the genotype on the target strand is the complement of the labeled terminator incorporated into the SNP primer.

Catalog No. ACP101A	AcycloPrime-FP SNP Detection Kit **G/A**, 1K SNPs
Catalog No. ACP101B	AcycloPrime-FP SNP Detection Kit **G/A**, 10K SNPs
Catalog No. ACP101C	AcycloPrime-FP SNP Detection Kit **G/A**, 100K SNPs
Catalog No. ACP102A	AcycloPrime-FP SNP Detection Kit **G/C**, 1K SNPs
Catalog No. ACP102B	AcycloPrime-FP SNP Detection Kit **G/C**, 10K SNPs
Catalog No. ACP102C	AcycloPrime-FP SNP Detection Kit **G/C**, 100K SNPs
Catalog No. ACP103A	AcycloPrime-FP SNP Detection Kit **G/T**, 1K SNPs
Catalog No. ACP103B	AcycloPrime-FP SNP Detection Kit **G/T**, 10K SNPs
Catalog No. ACP103C	AcycloPrime-FP SNP Detection Kit **G/T**, 100K SNPs
Catalog No. ACP104A	AcycloPrime-FP SNP Detection Kit **C/A**, 1K SNPs
Catalog No. ACP104B	AcycloPrime-FP SNP Detection Kit **C/A**, 10K SNPs
Catalog No. ACP104C	AcycloPrime-FP SNP Detection Kit **C/A**, 100K SNPs
Catalog No. ACP106A	AcycloPrime-FP SNP Detection Kit **C/T**, 1K SNPs
Catalog No. ACP106B	AcycloPrime-FP SNP Detection Kit **C/T**, 10K SNPs
Catalog No. ACP106C	AcycloPrime-FP SNP Detection Kit **C/T**, 100K SNPs
Catalog No. ACP109A	AcycloPrime-FP SNP Detection Kit **A/T**, 1K SNPs
Catalog No. ACP109B	AcycloPrime-FP SNP Detection Kit **A/T**, 10K SNPs
Catalog No. ACP109C	AcycloPrime-FP SNP Detection Kit **A/T**, 100K SNPs
Catalog No. ACP113B	AcycloPrime-FP SNP Detection Kit **Combination***, 10K SNPs
Catalog No. ACP113C	AcycloPrime-FP SNP Detection Kit **Combination***, 100K SNPs

*ACP113B contains 10 of the 1K Terminator Mixes: 3 x G/A, 3 x C/T, 1 x G/C, 1 x G/T, 1 x C/T and 1 x A/T
ACP113C contains 10 of the 10K Terminator Mixes: 3 x G/A, 3 x C/T, 1 x G/C, 1 x G/T, 1 x C/T and 1 x A/T

Catalog No. 1420-042, -043	Victor^2 Instrument
Catalog No. 1420-5700	filter 480(30)
Catalog No. 1420-5710	filter 535(40)
Catalog No. 1420-503	filter 544(15)
Catalog No. 1420-5690	filter 595(60)

XV. DATE OF ISSUANCE

February 2002

PC4038A-0202

APPENDIX. SIMPLIFIED ASSAY PROTOCOL

Amplify Genomic DNA containing SNP site of interest
(Use 5 µL reaction volume)

↓

Degrade Primers and dNTPs
(Add 2 µL of PCR Clean-Up Reagent to the PCR wells. Incubate at 37°C for 1 hour)

↓

Inactivate PCR Clean-Up Reagent - Heat to 80°C for 15 min.

↓

Primer Extension
(To existing 7 µL, add 13 µL of the components listed below. Make a master mix as indicated below to arrive at volumes sufficient for the number of wells. Make extra to account for pipetting losses)

Reagent	µL/Single Reaction	µL/96 wells	mL/384 wells	mL/1K Reactions	mL/10K Reactions
AcycloPol	0.05	6	0.022	0.055	0.525
10X Reaction Buffer	2	240	0.88	2.2	21
AcycloTerminator Mix	1	120	0.44	1.1	10.5
SNP Primer (10 µM)	0.5	60	0.22	0.55	5.25
Water	9.45	1134	4.158	10.395	99.225
Total Volume:	13 µL	1560 µL	5.72 mL	14.3 mL	136.5 mL

↓

Thermal Cycling - (1) Denature for 2 min at 95°C for 2 minutes.
(2) Perform 10-40 cycles of 95°C for 15 seconds and 55 °C for 30 seconds.
(3) At end of cycles ramp down to 15°C for 2 minutes then to 4°C.

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Spin Plate for 30 Seconds in Centrifuge

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Read on Fluorescence Polarization-Capable Instrument – Victor^2

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SNP Data Analysis