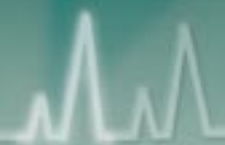


Instruction Manual

Canine Genotypes™

Panel 1.1, F-860S/L

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DIAGNOSTICS

Canine Genotypes™ Panel 1.1

Microsatellite Assay for Canine Parentage Testing and Identification

Instruction Manual

F-860S Canine Genotypes™ Panel 1.1 Kit for 100 Reactions

F-860L Canine Genotypes™ Panel 1.1 Kit for 500 Reactions

Introduction.....	4
Parentage Testing and Individual Identification	
Using Short Tandem Repeat (STR) Loci.....	4
Canine Genotypes™ Panel 1.1 Kit Overview	4
Canine Genotypes™ Panel 1.1 Kit Performance Characteristics	6
Kit Components and Storage Conditions.....	6
User-Supplied Equipment and Consumables.....	8
DNA Extraction	8
PCR Amplification	8
Electrophoresis	9
Samples and DNA Extraction	9
PCR Amplification Protocol	10
Electrophoresis Protocol	12
Electrophoresis Using ABI PRISM® 310 Genetic Analyzer	12
Electrophoresis Using ABI PRISM® 3100 Genetic Analyzer or ABI PRISM® 3100- <i>Avant</i> Genetic Analyzer	13
Electrophoresis Using ABI PRISM® 3130 XL Genetic Analyzer or ABI PRISM® 3130 Genetic Analyzer	14
Analysis and Interpretation of the Results	15
Representative Results	15
Allele Distributions.....	19
Allele Calling and Stutter Peaks.....	19
Plus-A Peaks	21
References.....	22
Appendix I: Avoiding PCR Carryover Contamination	22
Appendix II: Troubleshooting.....	23
Appendix III: Warranty	24

Introduction

Parentage Testing and Individual Identification Using Short Tandem Repeat (STR) Loci

Short Tandem Repeat (STR) loci, i.e. microsatellites, are a class of nuclear DNA markers consisting of tandemly repeated sequence motifs of two to seven base pairs in length. Alleles of STR loci vary by the number of times a given sequence motif is repeated. STR alleles are detected using Polymerase Chain Reaction (PCR) and by separating the amplification products using electrophoresis. Due to their high level of polymorphism (informativeness) and Mendelian inheritance, microsatellites have become the markers of choice for parentage testing and individual identification.

Canine Genotypes™ Panel 1.1 Kit Overview

The Canine Genotypes™ Panel 1.1 kit encompasses the following 19 loci: AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030, Amelogenin, FH2848, AHT121, FH2054, REN162C04, AHTh171 and REN247M23 (Table 1). These markers are included in the 'core panel' of loci recommended by the Applied Genetics Committee of Companioning Animals of the International Society for Animal Genetics (ISAG).

The Canine Genotypes™ Panel 1.1 kit allows co-amplification of the above markers in a single multiplex PCR reaction. One primer from each primer pair is end-labeled with a fluorescent dye. Following PCR, the fragments are separated and detected in a single electrophoresis injection, using an automated electrophoresis instrument, e.g. ABI PRISM® 310 Genetic Analyzer (Applied Biosystems), ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) or ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems).

The Canine Genotypes™ Panel 1.1 kit provides all of the reagents necessary for amplification of the 19 loci. In addition, the kit includes canine control DNA, originating from an ATCC cell line, for verification of acceptable PCR and electrophoresis conditions.

Table 1. Locus descriptions for the Canine Genotypes™ Panel 1.1 kit microsatellites and the amelogenin marker.

Locus Name	Chromosome	Repeat Motif	Size Range (bp) ¹	Dye Color ²
AHTk211	26	di	79-101	Blue
CXX279	22	di	109-133	Blue
REN169O18	29	di	150-170	Blue
INU055	10	di	190-216	Blue
REN54P11	18	di	222-244	Blue
INRA21	21	di	87-111	Green
AHT137	11	di	126-156	Green
REN169D01	14	di	199-221	Green
AHTTh260	16	di	230-254	Green
AHTk253	23	di	277-297	Green
INU005	33	di	102-136	Black
INU030	12	di	139-157	Black
Amelogenin	X	-	174-218	Black
FH2848	2	di	222-244	Black
AHT121	13	di	68-118	Red
FH2054	12	tetra	135-179	Red
REN162C04	7	di	192-212	Red
AHTTh171	6	di	215-239	Red
REN247M23	15	di	258-282	Red

¹ Size ranges are based on information provided by ISAG and data generated by Finnzymes Diagnostics. The data represents a large selection of dog breeds. However, some breeds may have alleles outside the ranges provided.

² Dye colors are listed as they appear following electrophoresis with Filter Set G5.

Canine Genotypes™ Panel 1.1 Kit Performance Characteristics

The Canine Genotypes™ Panel 1.1 delivers optimal results when 1.0-2.0 nanograms of high quality genomic DNA is applied in the kit's total PCR reaction volume of 20 µl. The reagents and reaction protocols of the Canine Genotypes™ Panel 1.1 kit have been optimized to deliver similar amplification yields (peak sizes) for alleles within and between loci, when an appropriate amount of high quality DNA is applied. The kit employs a proprietary Phusion™ Hot Start DNA Polymerase of Finnzymes Oy (Wang *et al.* 2004), providing the following features:

- Due to proofreading activity (3'-to-5' exonuclease activity) of the Phusion™ Hot Start DNA Polymerase, the results are not impaired by the normal tendency of DNA polymerases to add an extra nucleotide (most often adenine) to the end of the amplification products. Therefore, allele callings using the kit represent the true alleles of an individual, instead of 'plus-A peaks' or 'split peaks' typically interpreted when using e.g. normal *Taq* DNA polymerase.
- The Phusion™ Hot Start DNA Polymerase has the highest processivity of all known DNA polymerases. This high processivity results in robust and high-yield amplification of all target loci. In STR multiplexing, high processivity enables reliable amplification of even the longest fragments and avoids allele 'drop-out' occurrences, which can present a problem with difficult templates and/or low genomic DNA copy numbers, when using a normal *Taq* DNA polymerase.

Kit Components and Storage Conditions

The Canine Genotypes™ Panel 1.1 kit contains all reagents necessary to co-amplify the 18 microsatellites and the amelogenin locus (please see Table 1 for locus descriptions). The kit components are:

- **F-861: Canine Genotypes™ Panel 1.1 Master Mix.** A PCR master mix in an optimized buffer containing MgCl₂, deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP) and Phusion™ Hot Start DNA Polymerase with an activity of 0.05 U/µl.

- **F-862: Canine Genotypes™ Panel 1.1 Primer Mix.** An optimized PCR primer mix in buffer, including forward and reverse primers for the AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030, Amelogenin, FH2848, AHT121, FH2054, REN162C04 AHTh171 and REN247M23 loci. One primer from each primer pair is end-labeled with a fluorescent dye.
- **F-863S/L: Canine Genotypes™ Control DNA001.** Canine genomic DNA in 1.0 ng/μl concentration for verification of acceptable PCR and electrophoresis conditions. The genomic DNA has been extracted from an ATCC 'MDCK01' canine cell line.

All kit components should be stored at -20°C. Repeated freezing and thawing of the components will affect the performance of the kit and must be avoided. The kit is stable for six months from the date of packaging when stored and handled properly. The kit components and storage conditions are listed in Table 2.

Table 2. Canine Genotypes™ Panel 1.1 kit components and storage conditions for product codes: a) F-860S (sufficient for 100 reactions); and b) F-860L (sufficient for 500 reactions).

a)

Kit Component	Description	Storage Conditions
Canine Genotypes™ Panel 1.1 Master Mix (F-861)	1 tube (blue cap) 1.1 ml	-20 °C ¹
Canine Genotypes™ Panel 1.1 Primer Mix (F-862)	1 tube (red cap) 1.1 ml	-20 °C ¹ . Store protected from light at all times.
Canine Genotypes™ Control DNA001 (F-863L)	1 tube (green cap) 30 μl	-20 °C ¹

b)

Canine Genotypes™ Panel 1.1 Master Mix (F-861)	5 tubes (blue cap) 1.1 ml each	-20 °C ¹
Canine Genotypes™ Panel 1.1 Primer Mix (F-862)	5 tubes (red cap) 1.1 ml each	-20 °C ¹ . Store protected from light at all times.
Canine Genotypes™ Control DNA001 (F-863L)	1 tube (green cap) 150 μl	-20 °C ¹

¹ Repeated freezing and thawing of the components will affect the performance of the kit and must be avoided.

User-Supplied Equipment and Consumables

In addition to the Canine Genotypes™ Panel 1.1 kit, the equipment and consumables listed below are required for dog parentage testing and identification.

DNA Extraction

- DNA extraction consumables. DNA extraction can be performed using various methods. The specific equipment and consumables are not listed in this Instruction Manual, except for the details provided in Samples and DNA Extraction section.

PCR Amplification

- Sterile deionized water.
- Disposable gloves.
- Microcentrifuge.
- Vortex.
- Pipettes.
- Aerosol-resistant pipette tips.
- 1.5 ml microcentrifuge tubes.
- 0.2 ml PCR reaction vessels (tubes and caps, strips and strip caps or plates and plate sealers).
- Thermal cycler. The Canine Genotypes™ Panel 1.1 kit has been optimized for PCR using the following thermal cyclers: ABI GeneAmp PCR System 2400® (Applied Biosystems), ABI GeneAmp PCR System 7900® (96-well; Applied Biosystems), ABI GeneAmp PCR System 9600® (Applied Biosystems), ABI GeneAmp PCR System 9700® (384-well; Applied Biosystems), DNA Engine® (PTC-200™; Bio-Rad Laboratories), DNA Engine Tetrad® (Bio-Rad Laboratories), DNA Engine Tetrad 2® (Bio-Rad Laboratories), Piko™ Thermal Cycler (Finnzymes Instruments) and PTC-100® (Bio-Rad Laboratories). Use of the Canine Genotypes™ Panel 1.1 kit with a thermal cycler having similar performance characteristics as the instruments listed above is likely to deliver similar results.

Electrophoresis

- Electrophoresis instrument. The Canine Genotypes™ Panel 1.1 kit has been optimized for electrophoresis using the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems), ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems), ABI PRISM® 3100-Avant Genetic Analyzer, ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems) and ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems).
- GeneScan™ -500[LIZ]® Size Standard (Applied Biosystems). The Canine Genotypes™ Panel 1.1 markers have been optimized for allele calling using the GeneScan™ -500[LIZ]® Size Standard.
- DS-33 Dye Primer Matrix Standard Set (Applied Biosystems). The end-labeled primers of the Canine Genotypes™ Panel 1.1 kit are compatible with Filter Set G5, requiring the use of the DS-33 Dye Primer Matrix Standard.
- POP-4™ Performance Optimized Polymer (Applied Biosystems).
- Deionized formamide.
- Genetic Analyzer vessels and septums (Applied Biosystems).
- Additional electrophoresis consumables are required. Please refer to the ABI PRISM® User Guides for further details.

Samples and DNA Extraction

The Canine Genotypes™ Panel 1.1 kit has been optimized for use with dog hair, cheek swab and blood samples. However, application of any tissue providing high quality genomic DNA is possible.

The Canine Genotypes™ Panel 1.1 kit delivers optimal results when 1.0-2.0 nanograms of high quality genomic DNA is applied in the kit's total PCR volume of 20 µl. However, acceptable results can be obtained with as little as 0.5 ng and as much as 10 ng of genomic DNA. Following these recommendation guidelines is important: application of too little or too much template DNA can result in compromised amplification of some/all markers, undesired 'overshoot' of some/all markers and/or undesired occurrence of non-specific amplification products.

In addition to DNA yield, DNA purity and concentration of PCR inhibitors present may vary among extracts from different DNA extraction protocols. If the template/inhibitor ratio differs much from an optimum, the user may need to dilute the extracts and/or apply less/more of the extracts into PCR. It is therefore recommended that, when beginning to use the Canine Genotypes™ Panel 1.1 kit, the user first verifies the correct amount of template DNA to be used, by applying a dilution series of the extracted template DNA into the PCR reactions.

The Canine Genotypes™ Panel 1.1 kit delivers high quality and uniform results e.g. with Chelex® - proteinase K DNA extraction protocol (Figure 1; Walsh *et al.* 1991) or DNA IQ™ System (Promega Corporation).

PCR Amplification Protocol

The Canine Genotypes™ Panel 1.1 kit utilizes a DNA polymerase that is inactive at room temperature. Nevertheless, in order to maximize the specificity and uniformity of the amplification products, and to minimize cross-contaminating aerosols, it is recommended that PCR reactions are always set up on ice.

1. Prepare a reaction mix on ice for PCR by combining the following into a 1.5 ml microcentrifuge tube:
 - Number of samples x 10 µl of **Canine Genotypes™ Panel 1.1 Master Mix (F-861)**.
 - Number of samples x 10 µl of **Canine Genotypes™ Panel 1.1 Primer Mix (F-862)**.

Remember to allocate at least one sample in the above calculation for a positive control DNA and at least one sample for a negative control. The total volume of the PCR reaction mix is enough to account for possible volume losses due to reagent pipetting. A single 1.5 ml microcentrifuge tube and the above formulation can be used for up to ~70 samples.

2. Close the microcentrifuge tube and vortex it at full speed for 5 sec. Spin the tube briefly to remove possible liquid from the cap.
3. Label PCR reaction vessels and transfer 18 µl of the PCR reaction mix into each vessel.
4. Add 2 µl of sample DNA extract or positive control DNA (1.0 ng/µl) into each vessel. Allocate at least one vessel for a negative control and, instead of DNA, add 2 µl of dH₂O into that vessel.
5. Close the reaction vessels, vortex them gently and spin them briefly to remove possible liquid from the caps or sealers.
6. Immediately place the reaction vessels into a thermal cycler. Start the PCR program provided in Table 3.

Table 3. Thermal cycling programs of the Canine Genotypes™ Panel 1.1 kit for different PCR instruments.

PCR Instrument	Cycling Profile	Noteworthy Instrument Settings
<ul style="list-style-type: none"> • ABI GeneAmp PCR System 2400® • ABI GeneAmp PCR System 7900® (96-well) • ABI GeneAmp PCR System 9600® (384-well) • ABI GeneAmp PCR System 9700® (384-well) 	<ol style="list-style-type: none"> 1. 98°C for 3 min 2. 30 cycles of <ul style="list-style-type: none"> 98°C for 15 s 60°C for 75 s 72°C for 30 s 3. 72°C for 5 min 	<ul style="list-style-type: none"> • Ramping speed: 100%
<ul style="list-style-type: none"> • Piko™ Thermal Cycler 	<ol style="list-style-type: none"> 1. 98°C for 3 min 2. 30 cycles of <ul style="list-style-type: none"> 98°C for 15 s 60°C for 75 s 72°C for 30 s 3. 72°C for 5 min 	<ul style="list-style-type: none"> • Default settings
<ul style="list-style-type: none"> • DNA Engine® (PTC-200™) • DNA Engine Tetrad® • DNA Engine Tetrad® 2 • PTC-100® 	<ol style="list-style-type: none"> 1. 98°C for 3 min 2. 98°C for 15 s 3. 60°C for 75 s 4. 72°C for 30 s Repeat 2. - 4. for additional 29 cycles 5. 72°C for 5 min 	<ul style="list-style-type: none"> • Control method: block

Electrophoresis Protocol

The Canine Genotypes™ Panel 1.1 kit has been optimized for electrophoresis using the ABI PRISM® 310 Genetic Analyzer, ABI PRISM® 3100 Genetic Analyzer, ABI PRISM® 3100-Avant Genetic Analyzer, ABI PRISM® 3130 XL Genetic Analyzer and ABI PRISM® 3130 Genetic Analyzer. In addition to the instructions outlined below, please refer to the respective ABI PRISM® User Guides for electrophoresis details.

The Canine Genotypes™ Panel 1.1 kit is compatible with Filter Set G5, requiring matrix files generated with the DS-33 Dye Primer Matrix Standard Set. The matrix file values will vary between instruments and electrophoresis conditions. A matrix file must therefore be generated separately for each instrument.

The quantity of the PCR products will vary depending on the DNA template amount and quality applied in the PCR reactions. We therefore recommend that, when beginning to use the Canine Genotypes™ Panel 1.1 kit, the user first performs electrophoresis for a dilution series of the PCR products to verify that electrophoresis peak sizes are optimal. For this experiment, we recommend to use undiluted PCR products and 1:5, 1:10, 1:20 and 1:40 PCR product dilutions into dH₂O.

Electrophoresis Using ABI PRISM® 310 Genetic Analyzer

1. Prepare a reaction mix for electrophoresis by combining the following into a 1.5 ml microcentrifuge tube:
 - Number of samples x 11 µl of deionized formamide.
 - Number of samples x 0.3 µl of GeneScan™ -500[LIZ]® Size Standard.

The total volume of the mix is enough to account for possible volume losses due to reagent pipetting.

2. Close the microcentrifuge tube and vortex it at full speed for 5 sec. Spin the tube briefly to remove possible liquid from the cap.
3. Label 0.5 ml Genetic Analyzer tubes and transfer 10 µl of the mix into each tube.
4. Add 1.5 µl of PCR product (or PCR product diluted into dH₂O; see above) from the PCR Amplification Protocol into each tube. Mix the solutions by pipetting. Seal the tubes with septums.

5. Heat the tubes at 95°C for 3 min to denature the samples and immediately chill them on ice (e.g. crushed ice or ice-water bath) for at least 3 min.
6. Place the tubes in an auto-sampler tray, place the tray in an ABI PRISM® 310 Genetic Analyzer and close the instrument doors.
7. Select the GS STR Pop 4 (1-mL) G5 module or GS STR Pop 4 (2.5-mL) G5 module for 1 ml and 2.5 ml polymer syringes, respectively. Use the following (default) values for other injection list parameters:
 - Inj. Secs: 5
 - Inj. kV: 15.0
 - Run kV: 15.0
 - Run °C: 60
 - Run Time: 28
8. Begin electrophoresis according to the ABI PRISM® User Guide instructions.

Electrophoresis Using ABI PRISM® 3100 Genetic Analyzer or ABI PRISM® 3100-Avant Genetic Analyzer

1. Prepare a reaction mix for electrophoresis by combining the following into a 1.5 ml microcentrifuge tube:
 - Number of samples x 11 µl of deionized formamide.
 - Number of samples x 0.3 µl of GeneScan™ -500[LIZ]® Size Standard.

The total volume of the mix is enough to account for possible volume losses due to reagent pipetting.

2. Close the microcentrifuge tube and vortex it at full speed for 5 sec. Spin the tube briefly to remove possible liquid from the cap.
3. Transfer 10 µl of the mix into each vessel of a 96-well plate suitable for use in an ABI PRISM® 3100 Genetic Analyzer or ABI PRISM® 3100-Avant Genetic Analyzer.
4. Add 1.5 µl of PCR product (or PCR product diluted into dH₂O; see above) from the PCR Amplification Protocol into each well. Mix the solutions by pipetting. Seal the plate.
5. Heat the plate at 95°C for 3 min to denature the samples and immediately chill them on ice (e.g. crushed ice or ice-water bath) for at least 3 min.

6. Place the plate in an auto-sampler tray and close the instrument doors.
7. Select the GeneScan 36_Pop4 module. Use the following values for injection in combination with 36 cm capillaries:
 - Inj. Secs: 22.0
 - Inj. kV: 1.0
 - Run kV: 15.0
 - Run °C: 60
 - Run Time: 1200 sec
8. Begin electrophoresis according to the ABI PRISM® User Guide instructions.

Electrophoresis Using ABI PRISM® 3130 XL Genetic Analyzer or ABI PRISM® 3130 Genetic Analyzer

1. Prepare a reaction mix for electrophoresis by combining the following into a 1.5 ml microcentrifuge tube:
 - Number of samples x 11 µl of deionized formamide.
 - Number of samples x 0.3 µl of GeneScan™ -500[LIZ]® Size Standard.

The total volume of the mix is enough to account for possible volume losses due to reagent pipetting.

2. Close the microcentrifuge tube and vortex it at full speed for 5 sec. Spin the tube briefly to remove possible liquid from the cap.
3. Transfer 10 µl of the mix into each vessel of a 96-well plate suitable for use in an ABI PRISM® 3130 XL Genetic Analyzer or ABI PRISM® 3130 Genetic Analyzer.
4. Add 1.5 µl of PCR product (or PCR product diluted into dH₂O; see above) from the PCR Amplification Protocol into each well. Mix the solutions by pipetting. Seal the plate.
5. Heat the plate at 95°C for 3 min to denature the samples and immediately chill them on ice (e.g. crushed ice or ice-water bath) for at least 3 min.
6. Place the plate in an auto-sampler tray and close the instrument doors.

7. Select the Fragment Analysis 36_Pop4 module. Use the following values for injection in combination with 36 cm capillaries:
 - Inj. Secs: 12
 - Inj. kV: 1.2
 - Run kV: 15.0
 - Run °C: 60
 - Run Time: 1500 sec

8. Begin electrophoresis according to the ABI PRISM® User Guide instructions.

Analysis and Interpretation of the Results

Representative Results

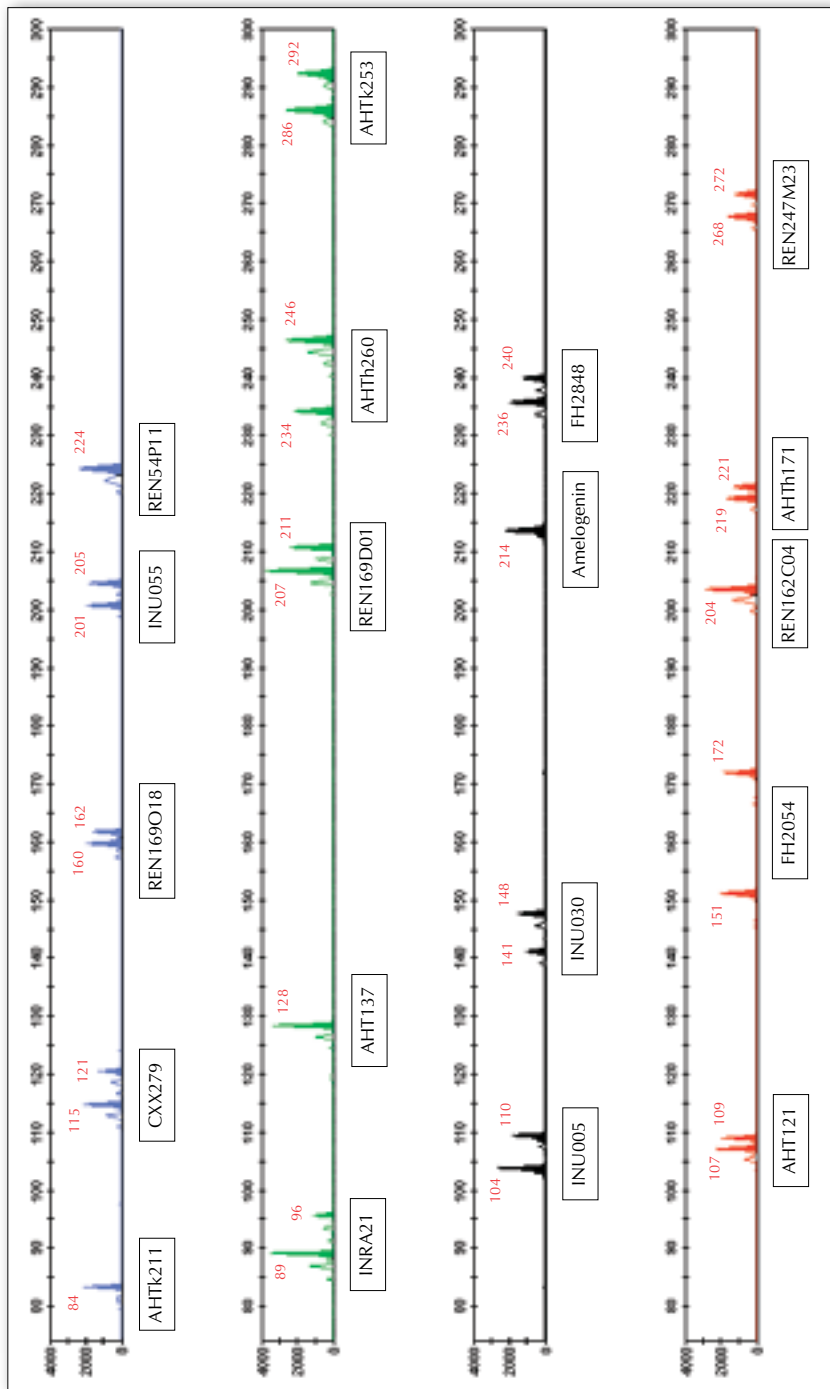
The reagents and protocols of the Canine Genotypes™ Panel 1.1 kit have been optimized to deliver similar peak sizes within and between loci, when applying an appropriate amount of high quality genomic DNA. PCR and electrophoresis conditions are acceptable when the fluorescent intensities of the Canine Genotypes™ Control DNA001 alleles fall between 1000 and 4000 Relative Fluorescence Units (RFU). Variation within this range is acceptable and can occur due to specific performance characteristics of the applied PCR or electrophoresis instruments.

Finzymes Diagnostics recommends that the user optimizes the DNA template amounts applied in PCR and PCR product dilutions applied in electrophoresis to deliver allele fluorescence intensities between ~1000-4000 RFU. Peaks lower than ~300 RFU and higher than ~6000 RFU should be interpreted with caution.

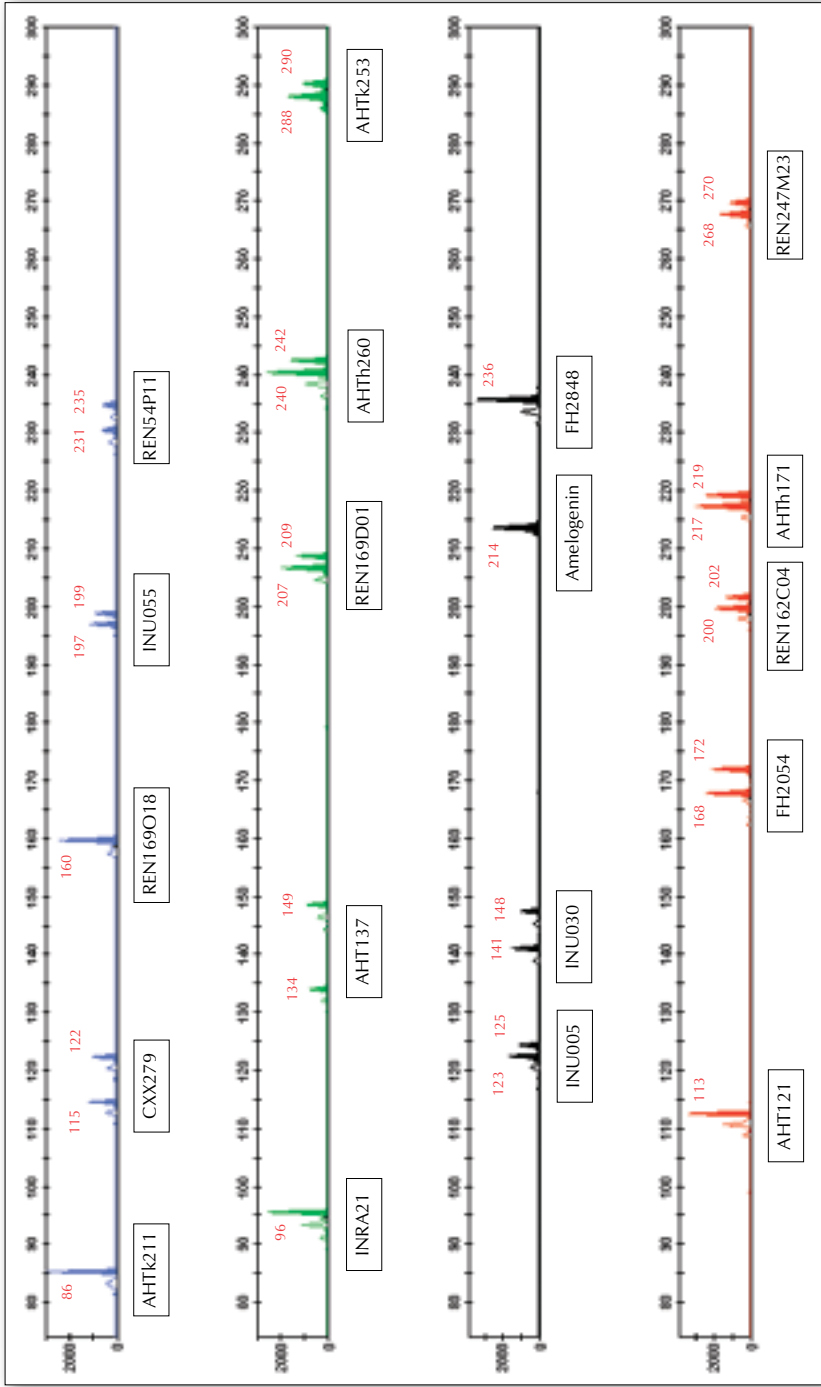
Representative results using 1.0 ng of Canine Genotypes™ Control DNA001, DNA extracted from a hair sample with Chelex® - proteinase K protocol (Walsh *et al.* 1991) and DNA from an ISAG 2006 comparison test sample are shown in Figures 1. a), b) and c), respectively. The PCR reactions were carried out using a DNA Engine® (PTC-200™) thermal cycler and the amplification products were separated on an ABI Prism® 310 Genetic Analyzer.

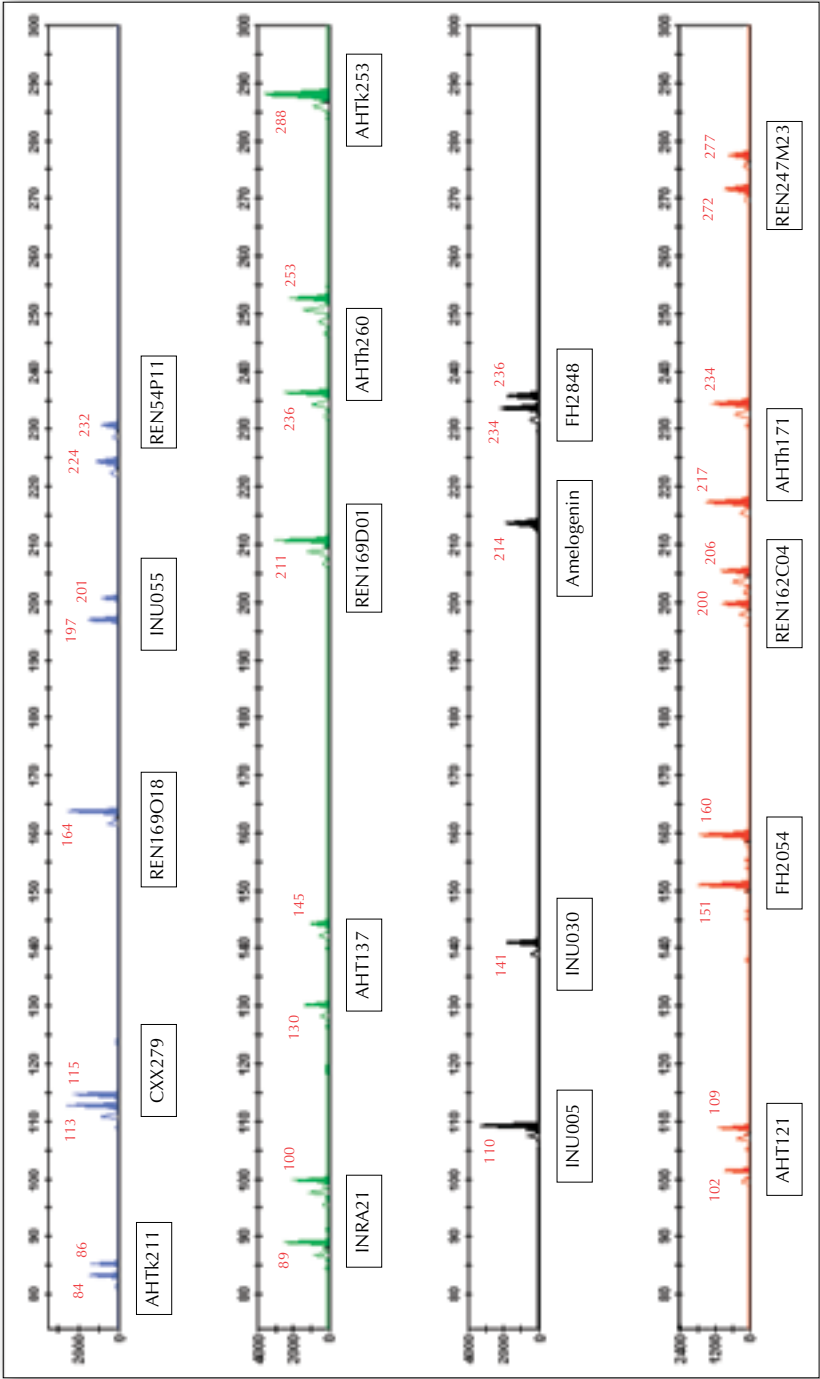
Figure 1. Canine Genotypes™ Panel 1.1 kit results using a) 1.0 ng of Canine Genotypes™ Control DNA001; b) template DNA extracted from a hair sample with a Chelex® -proteinase K protocol (Walsh *et al.*, 1991) protocol; and c) 1 µl of DNA from an ISAG 2006 comparison test sample. The allele sizes indicated in red were rounded to the closest integer number from the ABI PRISM® 310 Genetic Analyzer data.

a)



b)





Allele Distributions

The allele size ranges of the STR loci included in the Canine Genotypes™ Panel 1.1 kit (Table 1) are based on information provided by ISAG, as well as genotyping studies including a large selection of dog breeds. Nevertheless, some dog breeds may have alleles that fall outside the ranges provided. Such alleles are expected to occur at very low frequencies.

Majority of the STR loci included in the Canine Genotypes™ Panel 1.1 kit have alleles occurring in 2 bp or 4 bp intervals. However, some loci can have alleles occurring less than 2 bp apart. This is due to some dog breeds having insertions or deletions in the microsatellite regions or sequences flanking them. Furthermore, some dog breeds may have an altered microsatellite base composition, while the sequence length remains the same, resulting in slightly shifted fragment migration during electrophoresis.

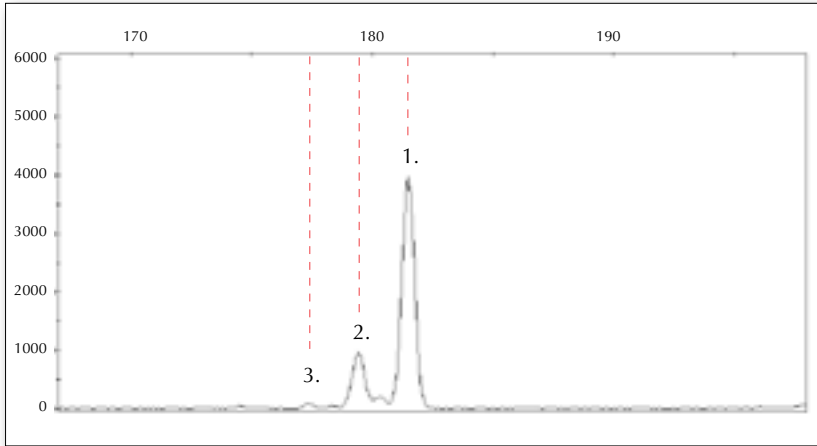
Allele Calling and Stutter Peaks

Microsatellite amplification can result in one or more stutter peak, arguably due to a phenomenon known as slipped strand mispairing (e.g. Goldstein & Schlötterer 1999). The stutter peaks typically lack one repeat unit relative to the true allele. Hence, for di- and tetranucleotide repeat motifs, they are typically 2 bp or 4 bp shorter than the true alleles, respectively. A total of 17 markers of the Canine Genotypes™ Panel 1.1 kit are dinucleotide microsatellite loci (Table 1), i.e. their repeat motifs are two base pairs in length. For these loci, the stutter peaks are typically 2 bp shorter than the true alleles. For the tetranucleotide locus FH2054, the stutter peaks are typically 4 bp shorter than the true alleles.

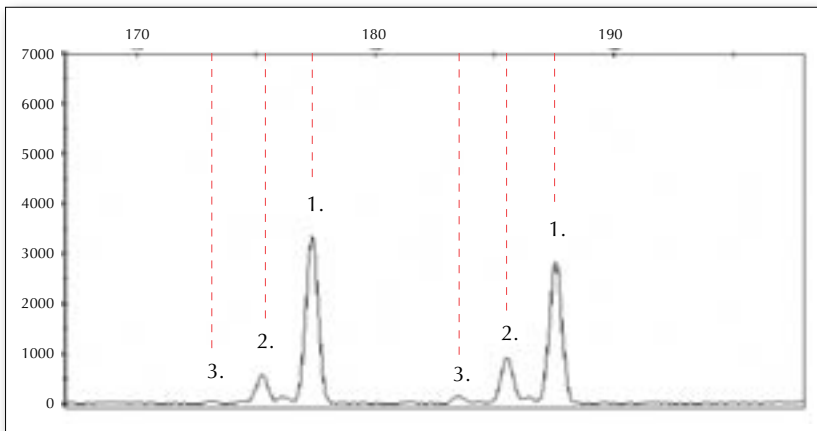
When interpreting the results, it is noteworthy that within one locus the longer alleles may have smaller amplification yields (peak sizes) than the shorter alleles. In addition, the stutter peaks are much smaller than the true allele peaks. Further, within some loci, the longer alleles may show more significant stuttering than the shorter alleles.

Typical peak profiles for homozygous individuals, heterozygous individuals with the two alleles > 2 bp apart and heterozygous individuals with the two alleles exactly 2 bp apart are exemplified in Figures 2. a), b) and c), respectively.

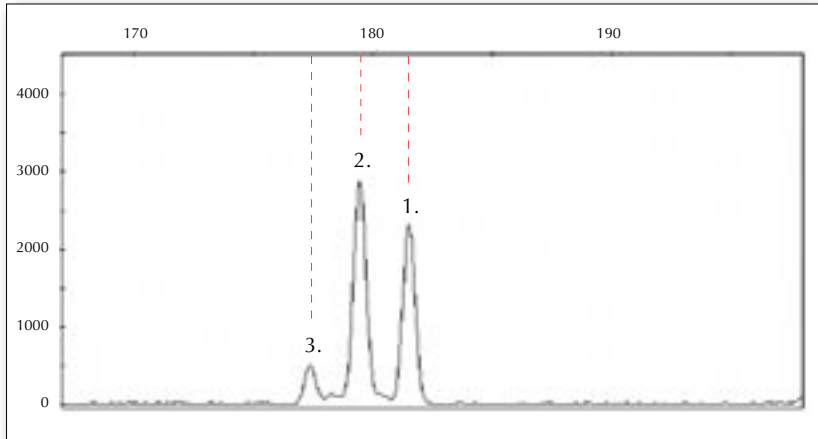
Figure 2.



- a) A typical dinucleotide microsatellite peak profile for a homozygous individual. The numbers correspond to the following PCR amplicons: 1. the true allele based on its complete DNA sequence; 2. the -2 bp stutter peak of the true allele; 3. the -4 bp stutter peak of the true allele.



- b) A typical dinucleotide microsatellite peak profile for a heterozygous individual with the two alleles > 2 bp apart. The numbers correspond to the following PCR amplicons: 1. the true alleles based on their complete DNA sequences; 2. the -2 bp stutter peaks of the true alleles; 3. the -4 bp stutter peaks of the true alleles.



- c) A typical dinucleotide microsatellite peak profile for a heterozygous individual with the two alleles exactly 2 bp apart. The numbers correspond to the following PCR amplicons: 1. the true longer allele based on its complete DNA sequence; 2. the true shorter allele and the -2 bp stutter peak of the longer allele; 3. the -4 bp stutter peak of the true longer allele and the -2 bp stutter peak of the shorter allele.

Plus-A Peaks

Due to the proofreading activity (3'-to-5' exonuclease activity) of the Phusion™ Hot Start DNA Polymerase, plus-A peaks (A-activity peaks) are non-existent in the Canine Genotypes™ Panel 1.1 kit results. Therefore, allele callings using the kit always represent the true alleles of an individual, instead of the plus-A peaks typically interpreted when using e.g. a normal *Taq* DNA polymerase.

References

Goldstein DB & Schlötterer C (1999) *Microsatellites: Evolution and Applications*. Oxford University Press, Oxford.

Walsh PS, Metzger DA & Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10: 506-13.

Wang Y, Prosen DE, Mei L, Sullivan JC, Finney M & Vander Horn PB (2004) A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro. *Nucleic Acids Res.* 32: 1197-207.

Appendix I: Avoiding PCR Carryover Contamination

Due to extreme sensitivity of PCR assays, they are susceptible to carryover contamination by previously amplified PCR products. A single molecule of amplified DNA may influence the results by contaminating the reaction mixture before PCR.

Along with other precautions mentioned in this Instruction Manual, we strongly recommend the following general guidelines, in order to further reduce the risk of carryover contamination:

- Set up physically strictly separated working places for (1) DNA extraction and sample preparation before PCR, (2) setting up PCR reactions, and (3) preparing electrophoresis reagent mixes and performing electrophoresis. Workflow in the laboratory should always proceed unidirectionally from (1) to (3) and traffic from the electrophoresis working place to the other separated working places during the same day should be avoided.
- Use different laboratory equipment (disposable gloves, micropipettes, pipette tip boxes, laboratory coats etc.) in each working place.
- Change gloves frequently.
- Use aerosol-resistant pipette tips.
- Use new and/or sterilized glassware and plasticware.

Appendix II: Troubleshooting

Problem	Possible Explanation	Recommended Actions
Faint or no signals from the test sample for all loci, but normal signals for all loci from the Canine Genotypes™ Control DNA001.	DNA quantity of the test sample is below the assay's level of sensitivity.	Measure the DNA concentration and add sample DNA into PCR in the quantity recommended in this Instruction Manual.
	PCR inhibitor concentration of the test sample is too high.	Dilute the sample DNA extract into dH ₂ O (e.g. 1:2, 1:5 and 1:20 dilutions) and repeat the protocol.
Faint or no signals from both the test sample and the Canine Genotypes™ Control DNA001 for all loci.	There has been a user error in the PCR or electrophoresis setup.	Repeat the protocol.
	The cycling profile applied is not optimal for the Canine Genotypes™ Panel 1.1 kit.	Check the PCR program.
Overshoot for all or some loci and occurrence of non-specific amplification products from the test sample, but normal signals for all loci from the Canine Genotypes™ Control DNA001.	The sample DNA quantity added into PCR is too high.	Measure the DNA concentration and add sample DNA into PCR in the quantity recommended in this Instruction Manual. Alternatively repeat the protocol for a dilution series of the sample DNA into dH ₂ O (e.g. 1:2, 1:5, 1:10 and 1:20 dilutions)
Overshoot for all or some loci and occurrence of non-specific amplification products from both the test sample and the Canine Genotypes™ Control DNA001.	There has been a user error in the PCR or electrophoresis setup.	Repeat the protocol.
	The cycling profile applied is not optimal for the Canine Genotypes™ Panel 1.1 kit.	Check the PCR program.

Appendix III: Warranty

Finnzymes Oy warrants that its products will meet the specifications stated on the Instruction Manual, and Finnzymes Oy agrees to replace the products free of charge if the products do not conform to the specification s. Notice for replacement must be given within 60 days of receipt. In consideration of the above commitments by Finnzymes Oy, the buyer agrees to and accepts the following conditions:

1. That this warranty is in lieu of all other warranties, express or implied;
2. That ALL WARRANTIES OF MERCHANT ABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE ARE HEREBY EXCLUDED AND WAIVED;
3. That the buyer's sole remedy shall be to obtain replacement of the product free of charge from Finnzymes Oy; and
4. That this remedy is in lieu of all other remedies or claims for damages, consequential or otherwise, which the buyer may have against Finnzymes Oy.

Exclusive Terms of Sale

Finnzymes Oy does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of Finnzymes Oy. Prices are subject to change without notice.

Recommended Guidelines for Safe Use of the Products

Finnzymes Oy recommends that the buyer and other persons using the products follow the N.I.H. guidelines published in the Federal Register, Volume 41, No. 131, July 7, 1976, and any amendments thereto. Finnzymes Oy disclaims any and all responsibility for any injury or damage which may be caused by the failure of the buyer or any other person to follow said guidelines.

Research Use Only

For research use only. Since these products are intended for research purposes by qualified persons, the Environmental Protection Agency does not require us to supply Premanufacturing Notice.

Notice to User

The information presented here is accurate and reliable to the best of our knowledge and belief, but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary.

Notice to Purchaser: Limited License

These products are sold under licensing agreements of Finnzymes Oy with F. Hoffman-La Roche Ltd. The purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front fee, either by a payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. These products are licensed under U.S. Patents Number 5,075,217 and 5,582,979 and are manufactured and sold to end-users in kits for use in academic research under an agreement between Finnzymes Oy and Marshfield Clinic. In the U.S. the products are licensed for internal use by the end user solely for academic scientific research. Applications for which a fee or other consideration is directly or indirectly charged to a customer of the end user in the U.S. requires a separate license from Marshfield Clinic. In the U.S. no right to perform or offer commercial services of any kind is hereby granted by implication or estoppel.

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