Thermo Scientific Canine Genotypes Panel 2.1

F- 864S 100 reactions

Technical Manual

Product Description

Parentage testing, individual identification and investigation of forensic cases using short tandem repeat (STR) loci.

Short Tandem Repeat (STR) loci, or microsatellites, are a class of nuclear DNA markers consisting of tandemly repetitive 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 and Mendelian inheritance, microsatellites have become the markers of choice for parentage testing and individual identification.

The use of STRs for the characterization of dogs' biological evidence became commonly used in forensic cases as well. Canine Genotypes Panel 2.1 is an easy to use STR genotyping kit that has been validated for use in canine forensics (see references). The kit is compatible with genomic DNA extracted from various types of dog biological samples: liquid or dried blood, saliva stains, buccal cells, hair and blood-saliva mixture samples.

Kit overview

Thermo Scientific Canine Genotypes Panel 2.1 encompasses the following 19 loci: PEZ02, ZFX/Y, PEZ17, FH2017, FH2309, PEZ05, FH2001, FH2328, FH2004, FH2361, PEZ21, FH2054, FH3377, FH2107, FH2088, vWF.X, FH2010, PEZ16, FH3313 (Table 1). These markers were developed and validated for use in forensics (see references). Validation study assessed both the robustness and reliability of the markers, and the sensitivity and reproducibility of multiplex PCR assay.

The Canine Genotypes Panel 2.1 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

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in a single electrophoresis injection, using an automated electrophoresis instrument, such as ABI PRISM 3130 Genetic Analyzer, ABI PRISM 3130*xl* Genetic Analyzer, ABI PRISM 3500 Genetic Analyzer or ABI PRISM 3500*xl* Genetic Analyzer (all Applied Biosystems).

The Canine Genotypes Panel 2.1 provides all of the reagents necessary for amplification of the 19 loci. In addition, the kit includes canine control DNA, originating from an MDCK.1 cell line, for verification of PCR and electrophoresis conditions.

The Canine Genotypes Panel 2.1 delivers optimal results when 1–2 ng of high quality genomic DNA is applied in PCR reaction volume of 20 µL. The reagents and reaction protocols of the Canine Genotypes Panel 2.1 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 Thermo Scientific Phusion Hot Start DNA Polymerase providing the following features:

- Allele callings represent the true alleles of an individual, instead of 'plus-A' peaks or 'split peaks' typically encountered when using e.g. *Taq* DNA polymerase. This is due to the proofreading activity of the Phusion Hot Start DNA Polymerase. The results are not impaired by the tendency of non-proofreading DNA polymerases to add an extra nucleotide (most often adenine) to the end of the amplification products.
- The high processivity of Phusion Hot Start DNA Polymerase allows for robust and high-yield amplification of all target loci. High processivity enables reliable amplification of even the longest fragments and avoids allele 'drop-out' occurrences during multiplex PCR, which can present a problem with difficult templates and/or low genomic DNA copy numbers, when using a *Taq* DNA polymerase.

Table 1. Locus descriptions for the Canine Genotypes Panel 2.1 markers.

Locus	Chromosome	Repeat Motif	Size range (bp)	Dye color[1]
PEZ02	17	GGAA	104-145	blue
ZFX/Y	X/Y	-	159-164	blue
PEZ17	4	GAAA	190-225	blue
FH2017	15	$AGGT_{(m)}AGAT_{(n)}GATA_{(o)}$	256-276	blue
FH2309	1	GAAA	339-428	blue
PEZ05	12	TTTA	92-117	green
FH2001	23	GATA	118-160	green
FH2328	33	GAAA	171-213	green
FH2004	11	AAAG	232-326	green
FH2361	29	GAAA	322-439	green
PEZ21	2	AAAT	83-103	yellow
FH2054	12	GATA	139-177	yellow
FH3377	3	GAAAA	183-305	yellow
FH2107	3	GAAA	291-426	yellow
FH2088	15	$(TTTA)_m(TTCA)_n$	94-138	red
vWF.X	27	AGGAAT	151-187	red
FH2010	24	ATGA	221-243	red
PEZ16	27	GAAA	280-332	red
FH3313	19	GAAA	340-446	red

[1]Dye colors are listed as they appear in electrophoresis with filter set G5: 6FAM (blue), VIC (green), NED (yellow), PET (red). Size standard LIZ appears in orange color.

Kit components and storage conditions

The Canine Genotypes Panel 2.1 kit contains all reagents necessary to co-amplify 18 STR loci and one gender determination locus (see Table 1 for locus descriptions). Composition of the kit:

- Canine Genotypes Panel 2.1 Master Mix. A PCR master mix in an optimized buffer containing $MgCl_2$, deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP) and Phusion Hot Start DNA Polymerase (0.05 U/µL).
- Canine Genotypes Panel 2.1 Primer Mix. An optimized PCR primer mix in a buffer, including forward and reverse primers for the PEZ02, ZFX/Y, PEZ17, FH2017, FH2309, PEZ05, FH2001, FH2328, FH2004, FH2361, PEZ21, FH2054, FH3377, FH2107, FH2088, vWF.X, FH2010, PEZ16, FH3313 loci. One primer from each primer pair is end-labeled with a fluorescent dye.
- Canine Genotypes Panel 2.1 Control DNA. Canine Control DNA at 0.25 ng/µL concentration is used for verification of PCR and electrophoresis conditions. Genomic DNA is derived from ATCC 'MDCK.1' 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 2.1 kit components and storage conditions for F-864S (sufficient for 100 reactions).

Kit Component	Description	Storage conditions
Canine Genotypes Panel 2.1 Master Mix	1 tube (blue cap) 1.1 mL	-20 ºC[1]
Canine Genotypes Panel 2.1 Primer Mix	1 tube (red cap) 1.1 mL	-20 ºC[1]. Store protected from light at all times.
Canine Genotypes Panel 2.1 Control DNA	1 tube (green cap) 30 µL	-20 ºC[1]

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

Important Note! The primer mix should be protected from light all the times as fluorescent dyes in the primer mix are light sensitive.

Materials needed but not supplied

Additional equipment and consumables required:

- DNA extraction can be performed using various methods. The specific equipment and consumables recommended for DNA purification are listed in **Samples and DNA extraction**.

PCR

- Water nuclease free
- 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 2.1 kit has been optimized for PCR using the following thermal cyclers: Eppendorf Mastercycler gradient (Eppendorf), ABI Veriti® 96-Well Thermal Cycler (Applied Biosystems), ABI GeneAmp PCR System 9700® (96-well; Applied Biosystems), Eppendorf™ Mastercycler™ pro S (Eppendorf), LabCycler (SensoQuest), Arktik Thermal Cycler and Piko Thermal Cycler (Thermo Scientific).

Electrophoresis

- Electrophoresis instrument. The Canine Genotypes Panel 2.1 has been optimized for electrophoresis using the ABI PRISM 3100 Genetic Analyzer, ABI PRISM 3100-*Avant* Genetic, Analyzer, ABI PRISM 3130 Genetic Analyzer, ABI PRISM 3130*xl* Genetic Analyzer, ABI 3500 Genetic Analyzer and ABI 3500*xl* Genetic Analyzer (all Applied Biosystems). The use of Canine Genotypes Panel 2.1 in other genetic analyzers is likely to deliver similar results.
- GeneScan™ 500 LIZ® Size Standard (Applied Biosystems). The Canine Genotypes Panel 2.1 markers have been optimized for allele calling using the GeneScan 500 LIZ Size Standard.
- **Note:** If you use the GeneScan™ 600 LIZ® Size Standard v2.0 as an alternative, please see Control DNA allelic size Table 5 for ABI 3500 Genetic Analyzer. For other analyzers perform the appropriate optimization studies to support the use of this size standard with the Canine Genotypes Panel 2.1 kit.
- DS-33 Dye Primer Matrix Standard Set (Applied Biosystems). The end-labeled primers of the Canine Genotypes Panel 2.1 kit are compatible with Filter Set G5, requiring the use of the DS-33 Dye Primer Matrix Standard.
- POP-7™(for ABI 3500) and POP-4™ (for others) - 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 2.1 has been optimized for use with dog blood, hair, cheek swab, saliva stains and saliva-blood mixed samples. However, use of high-quality genomic DNA isolated from other tissue is possible.

The Canine Genotypes Panel 2.1 delivers optimal results when 1–2 ng of high quality genomic DNA is applied in PCR volume of 20 µL. However, the kit delivers acceptable results with genomic DNA amounts ranging from 0.25 ng to 10 ng. Following these recommendation guidelines is important: application of too little or too much template DNA can result in compromised amplification of some/all microsatellites, undesired 'overshoot' of some/all markers and/or undesired occurrence of non-specific amplification products.

DNA yield, DNA purity and the amount of PCR inhibitors may vary due to different DNA extraction protocols. When you first start using the Canine Genotypes Panel 2.1, we strongly recommend preparing dilution series of the extracted DNA in order to optimize the amount of template DNA needed for PCR.

The Canine Genotypes Panel 2.1 delivers high-quality results when DNA purified with Thermo Scientific DNA purification kits is used (see Table 3).

Table 3. Thermo Scientific genomic DNA purification kits for different canine samples.

Sample type	Recommended genomic DNA purification kit
Liquid blood (EDTA, citrate) Dried blood spot	GeneJET Whole Blood Genomic DNA Purification Mini Kit #K0781, #K0782
	MagJET Whole Blood Genomic DNA Kit #K2741, #K2742
Buccal cells Saliva stains Hair follicle	MagJET Genomic DNA Kit #K2721, #K2722
	GeneJET Genomic DNA Purification Kit #K0721, #K0722
Mixed samples of blood and saliva	MagJET Genomic DNA Kit #K2721, #K2722

Note: For more details about genomic DNA purification protocols please contact technical support: Europe ts.molbio.eu@thermofisher.com or US ts.molbio@thermofisher.com.

PCR

The Canine Genotypes Panel 2.1 utilizes Phusion Hot Start 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, we strongly recommend that PCR reactions are always set up on ice.

1. Prepare a reaction mix for PCR on ice by combining the following into a 1.5 mL microcentrifuge tube:

   - Volume of Canine Genotypes Panel 2.1 Master Mix = **N** × 10 µL
   - Volume of Canine Genotypes Panel 2.1 Primer Mix = **N** × 10 µL

   N = Number of samples

   Include the following controls:
   - positive control (Canine Genotypes Panel 2.1 Control DNA)
   - negative control (H_2O)

   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 at full speed for 5 s. Spin the tube briefly to remove any liquid remaining in 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 (0.25 ng/µL) into each vessel. Allocate at least one vessel for a negative control and, instead of DNA, add 2 µL of H_2O into that vessel.
5. Close the reaction vessels, vortex gently and spin briefly to remove possible liquid from the caps or sealers.
6. Immediately place the reaction vessels into a thermal cycler. Start the PCR program.

Table 4. Thermal cycling programs of the Canine Genotypes Panel 2.1 for different PCR instruments.

PCR instrument	Cycling profile	Noteworthy instrument settings
Piko® Thermal Cycler Arktik Thermal Cycler Eppendorf Mastercycler gradient Eppendorf™ Mastercycler™ pro S SensoQuest LabCycler	1. 98°C for 3 min 2. 30 cycles of 98°C for 15 s 60°C for 75 s 72°C for 30 s 3. 72°C for 5 min	Default settings
ABI GeneAmp PCR System 9700 (96-well) ABI Veriti® 96-Well Thermal Cycler	1. 98°C for 3 min 2. 30 cycles of 98°C for 15 s 60°C for 75 s 72°C for 30 s 3. 72°C for 5 min	Ramping speed: 100 %

Electrophoresis

The Canine Genotypes Panel 2.1 has been optimized for electrophoresis using the ABI PRISM 3100 Genetic Analyzer, ABI PRISM 3100-*Avant* Genetic Analyzer, ABI PRISM 3130 Genetic Analyzer, ABI PRISM 3130*xl* Genetic Analyzer, ABI PRISM 3500 Genetic Analyzer and ABI PRISM 3500*xl* Genetic Analyzer (all Applied Biosystems). In addition to the instructions outlined below, please refer to the instrument User Manual for electrophoresis details.

The Canine Genotypes Panel 2.1 is compatible with Filter Set G5, requiring matrix files generated with the DS-33 Dye Primer Matrix Standard Set. The matrix file values vary between instruments and electrophoresis conditions. A matrix file must therefore be generated separately for each instrument.

The quantity of the microsatellite PCR products varies depending on the amount and quality of the DNA template used for the PCR reactions. When you first start using the Canine Genotypes Panel 2.1, we strongly recommend preparing a dilution series of the PCR products and running electrophoresis in order to optimize the allele fluorescence intensities (for the recommended range, see Representative

results). For this experiment, use undiluted PCR products and 1:5, 1:10, 1:20 and 1:40 PCR product dilutions in H_2O.

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

1. Prepare a reaction mix for electrophoresis by combining the following into a 1.5 mL microcentrifuge tube:

   - Number of samples × 11 µL of deionized formamide.
   - Number of samples × 0.3 µL of GeneScan 500 LIZ Size Standard.

   An excess volume to compensate for volume losses due to reagent pipetting is already included.

2. Close the microcentrifuge tube and vortex it at full speed for 5 s. Spin the tube briefly to collect liquid from the tube walls.
3. Transfer 10 µL of the mix into each well of a 96-well plate compatible with the instrument.
4. Add 1.5 µL of PCR product (or PCR product diluted into H_2O; see Electrophoresis) 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 the plate on ice (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: 10.0
   - Inj. kV: 3.0
   - Run kV: 15.0
   - Run °C: 60
   - Run Time: 1200 s

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

Electrophoresis Using ABI PRISM® 3130 Genetic Analyzer or ABI PRISM® 3130*xl* 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 formulas provide excess volume to collect liquid from the tube walls.

2. Close the microcentrifuge tube and vortex it at full speed for 5 s. Spin the tube briefly to remove possible liquid from the cap.
3. Transfer 10 µL of the mix into each well of a 96-well plate compatible with the instrument.

4. Add 1 µL of PCR product (or PCR product diluted into H_2O; see Electrophoresis) 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 the plate 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 s
8. Begin electrophoresis according to the ABI PRISM User Guide instructions.

7.3 Electrophoresis Using ABI PRISM® 3500 Genetic Analyzer or ABI PRISM® 3500*xl* Genetic Analyzer

1. Prepare a reaction mix for electrophoresis by combining the following into a 1.5 ml microcentrifuge tube:

   - Number of samples × 11 µL of deionized formamide.
   - Number of samples x 0.2 µL of GeneScan 500 LIZ Size Standard or GeneScan™ 600 LIZ Size Standard v2.0.

   The formulas provide excess volume to compensate for volume losses due to reagent pipetting.

2. Close the microcentrifuge tube and vortex it at full speed for 5 s. Spin the tube briefly to collect liquid from the tube walls.
3. Transfer 10 µL of the mix into each well of a 96-well plate compatible with the instrument.
4. Add 1 µL of PCR product (or PCR product diluted into H_2O; see Electrophoresis) 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 the plate on ice (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 50_Pop7 module. Use the following values for injection in combination with 50 cm capillaries:
   - Inj. Secs: 8
   - Inj. kV: 1.5
   - Run kV: 19.5
   - Run °C: 60
   - Run Time: 1330 s
8. Start electrophoresis according to the ABI PRISM User Guide instructions.

Table 5. Average allele sizes of all 19 loci for Canine control DNA using LIZ 500 size standard with ABI 3130*xl* and ABI 3500 genetic analyzers and LIZ 600 size standard with ABI 3500.

	LIZ 500				LIZ 600	
	ABI 3130		ABI 3500		ABI 3500	
Locus	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
PEZ02	130.9 (130.7-131.2)	130.9 (130.7-131.2)	130.5 (130.4-130.7)	130.5 (130.4-130.7)	129.5 (129.4-129.6)	129.5 (129.4-129.6)
ZFX/Y	161.1 (161-161.2)	161.1 (161-161.2)	160.7 (160.6-160.8)	160.7 (160.6-160.8)	158.0 (157.9-158.0)	158.0 (157.9-158.0)
PEZ17	202 (201.8-202.2)	214.1 (213.9-214.3)	201.8 (201.7-201.9)	213.9 (213.8-214.0)	199.6 (199.5-199.8)	212.0 (212.0-212.1)
FH2017	263.5 (263.3-263.8)	267.5 (267.2-267.8)	263.1 (263.10-263.2)	267.1 (267.0-267.2)	262.1 (262.0-262.2)	266.1 (266.0-266.2)
FH2309	394.7 (394.5-394.9)	394.7 (394.5-394.9)	394.9 (394.9-395.0)	394.9 (394.9-395.0)	395.0 (394.8-395.1)	395.0 (394.8-395.1)
PEZ05	103 (102.8-103.2)	103 (102.8-103.2)	102.7 (102.5-102.8)	102.7 (102.5-102.8)	101.6 (101.2-101.9)	101.6 (101.2-101.9)
FH2001	129.4 (129.2-129.6)	146.8 (146.5-147)	128.9 (128.8-129.1)	146.6 (146.5-146.8)	128.0 (127.9-128.1)	144.2 (144.1-144.2)
FH2328	171.1 (170.8-171.4)	206.2 (205.8-206.6)	170.6 (170.5-170.7)	205.7 (205.6-205.8)	167.9 (167.8-168.0)	203.7 (203.6-203.8)
FH2004	233.6 (233.4-233.8)	241.8 (241.5-242)	233.3 (233.1-233.4)	241.4 (241.3-241.6)	231.8 (231.7-231.9)	240.0 (240.0-240.1)
FH2361	345.5 345.2-345.7)	347.4 (347.2-347.6)	344.9 (344.7-345.1)	346.9 (346.7-347.1)	343.1 (343.1-343.1)	345.1 (345.0-345.2)
PEZ21	89.1 (88.9-89.3)	97.1 (96.9-97.3)	89.2 (89.0-89.3)	97.0 (96.8-97.1)	87.8 (87.4-88.3)	95.6 (95.2-96.0)
FH2054	150.2 (150-150.4)	170.9 (170.7-171.1)	149.9 (149.8-150.1)	170.4 (170.2-170.6)	147.2 (147.1-147.3)	167.7 (167.6-167.8)
FH3377	198.9 (198.6-199.2)	198.9 (198.6-199.2)	198.5 (198.4-198.6)	198.5 (198.4-198.6)	196.2 (196.1-196.4)	196.2 (196.2-196.4)
FH2107	367.9 (367.1-368.7)	386.3 (385.4-387.2)	367.3 (367.2-367.4)	385.7 (385.5-385.8)	366.4 (366.3-366.6)	385.6 (385.4-385.8)
FH2088	123.9 (123.7-124.1)	127.8 (127.6-127.9)	123.3 (123.2-123.4)	127.2 (127.1-127.4)	122.6 (122.5-122.7)	126.5 (126.4-126.6)
VWF.X	158.5 (158.3-158.6)	158.5 (158.3-158.6)	158.2 (158.1-158.3)	158.2 (158.1-158.3)	155.4 (155.3-155.5)	155.4 (155.3-155.5)
FH2010	233.6 233.4-233.8)	233.6 (233.4-233.8)	233.2 (233.1-233.4)	233.2 (233.1-233.4)	231.8 (231.7-231.9)	231.8 (231.7-231.9)
PEZ16	300.3 (299.8-300.7)	304.4 (303.8-304.9)	299.5 (299.5-299.6)	303.6 (303.4-303.8)	298.7 (298.6-298.8)	302.6 (302.4-302.7)
FH3313	414.4 (413.4-415.3)	419.9 (418.9-420.8) 4	413.7 (413.6-413.8)	419.1 (418.9-419.2)	413.7 (413.5-413.9)	419.1 (418.9-419.3)

Analysis and interpretation of the results

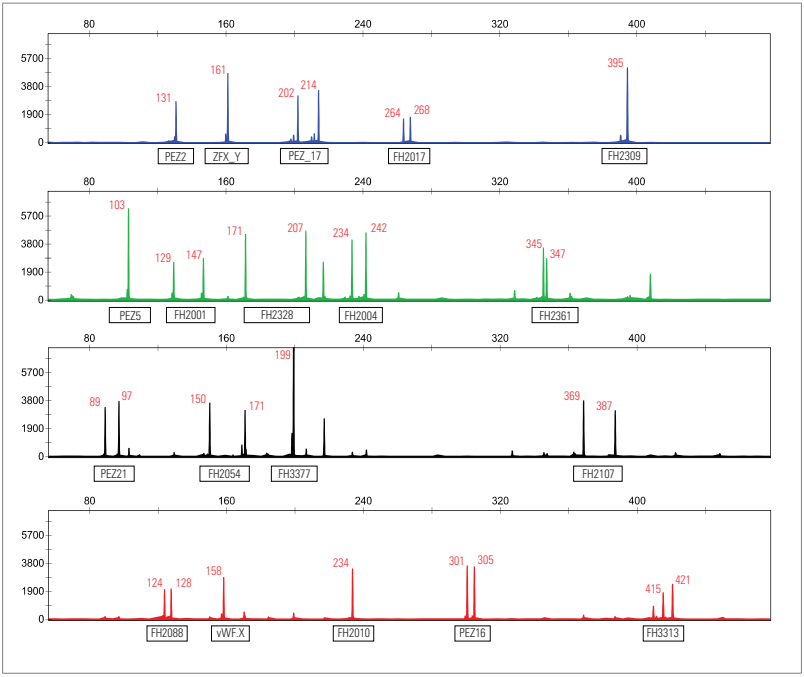
Representative results

The reagents and protocols of the Canine Genotypes Panel 2.1 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 DNA 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.

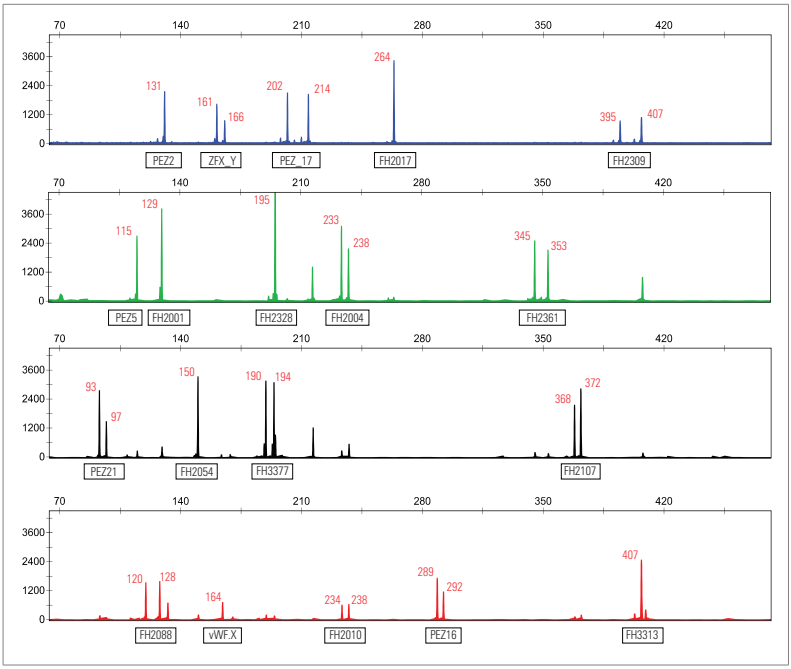
We recommend optimizing both the DNA template amount for PCR and the amount of PCR product dilutions used for electrophoresis so that the allele fluorescence intensities are between 1000 and 4000 RFU. Peaks lower than 300 RFU and higher than 6000 RFU should be interpreted with caution.

Figures 1 A, B and C show genotyping results with Canine Genotypes Panel 2.1 using 0.5 ng of Canine Control DNA, DNA from buccal swab purified using GeneJET Genomic DNA Purification Kit and DNA from human blood- dog saliva mixed sample purified using MagJET Genomic DNA Purification Kit, respectively. The PCR were carried out using Arktik Thermal Cycler and the amplification products were separated on an ABI PRISM 3130 *xI* Genetic Analyzer.

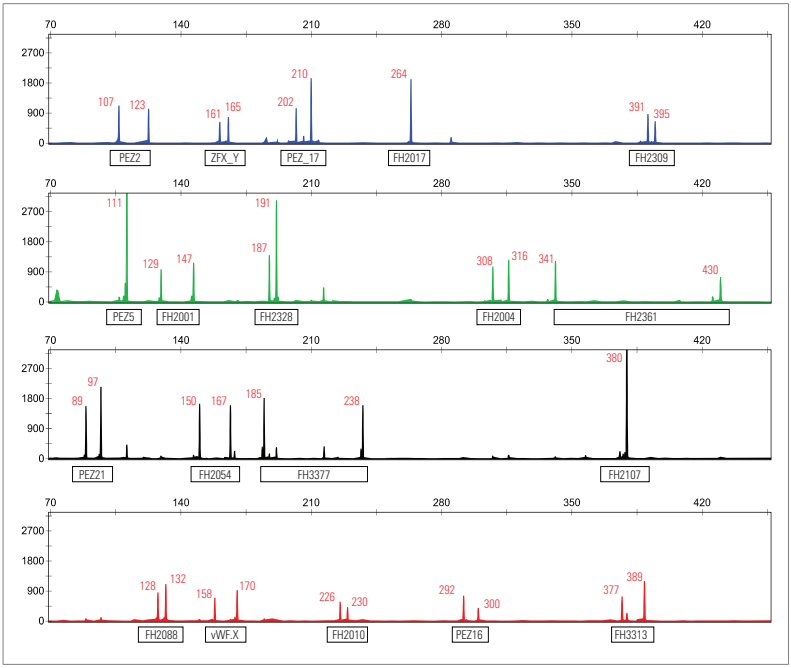
Figure 1.



A. Canine Genotypes Panel 2.1 control DNA genotyping profile.



B. Canine Genotypes Panel 2.1 - genotyping profile of canine DNA extracted from buccal swab. DNA was extracted using GeneJET Genomic DNA Purification Kit.



C. A mixed sample containing both canine and human DNA was genotyped using Canine Genotypes Panel 2.1 kit. DNA was extracted from a mixture of dog saliva and human blood

Stutter peaks and allele calling

Microsatellite amplification can result in one or more stutter peak, arguably due to a phenomenon known as slipped strand mispairing (Goldstein and Schlötterer, 1999). The stutter peaks typically lack one repeat unit relative to the true allele. Hence, for tetranucleotide repeat motifs, they are typically 4 bp shorter than the true alleles. A total of 16 markers of the Canine Genotypes Panel 2.1 are tetranucleotide microsatellite loci (Table 1). The PCR amplification of tetranucleotide short tandem repeat (STR) loci typically produces a minor product band 4 bp shorter than the corresponding main allele band.

PCR amplification results from tetranucleotide repeat loci are easier to interpret because only a single stutter band is typically observed, in a position four bases shorter than allele band.

When interpreting the results, it is noteworthy that within one locus the longer alleles may display smaller amplification yields (peak sizes) than the shorter alleles. Moreover, within some loci, the longer alleles may display more significant stuttering than the shorter alleles.

A separate developmental validation study that assessed stutter percentages of Canine Genotypes Panel 2.1 kit has been conducted and published by M. Dayton *et al*., 2009.

Plus-A peaks

Due to the proofreading activity (3'-to-5' exonuclease activity) of the Phusion Hot Start DNA Polymerase, the Canine Genotypes Panel 2.1 results do not contain plus-A peaks. Therefore, allele callings using the kit always represent the true alleles of an individual, instead of the plus-A peak typically interpreted when using e.g. a *Taq* DNA polymerase.

Species specificity

The Canine Genotypes Panel 2.1 is specific to canine DNA and full 19 locus profiles were not observed while testing DNA samples from chicken, mouse, rat, horse, cow, pig, cat, fish, monkey and human (Dayton *et al*., 2009). Most peaks fall outside of the canine allele size ranges with a few peaks close to canine allele positions. However, none of the peaks, exhibited in any of the non-canine species, appeared to exhibit the morphology of STR products (all peaks above 500 RFU lack stutter peaks), which is helpful for evaluation if non-canine species amplification has occurred in a DNA mixture (Dayton *et al*., 2009).

The Canine Genotypes Panel 2.1 kit is also suitable for STR genotyping of wolf (Canis lupus) DNA. The peaks obtained using wolf DNA are concordant with the allele spectrum of the domestic dog for each locus (Dayton *et al*., 2009).

Note on possible nonspecific artifacts

As determined by many independent amplifications of different dog DNA samples (including mixed human – dog DNA samples) prepared using various DNA extraction methods, including control DNA samples, some constant nonspecific amplifications in particular loci were observed. Although the signals from nonspecific products are quite low compared to specific signals of amplified microsatellites, attention should be paid when interpreting the results. The nonspecific products might be produced following amplification in locus FH2361 (328 and 407 bp), FH3377 (217 bp), FH3313 (409 bp), FH2107 (327 bp). It is highly probable that these peaks are PCR artifacts generated during 19-plex PCR. One could expect to see single peaks common across most genotypes, which are always identical and do not cause data misinterpretation.

References

1. Dayton, M., *et al*. (2009) Developmental Validation of Short Tandem Repeat Reagent Kit for Forensic DNA Profiling of Canine Biological Material, *Croatian Medical Journal*., vol 50, no 3, pp. 268-285.

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Troubleshooting

Problem	Cause	Recommended Actions
Faint or no signals from the test sample for all loci, but normal signals for all loci from the Control DNA.	DNA quantity of the test sample is below the assay sensitivity.	Measure the DNA concentration and add sample DNA into PCR in the quantity recommended in this Instruction Manual.
	DNA concentration of the test sample is too high or DNA purity is suboptimal.	Dilute the sample DNA into dH_2O (e.g. 1:2, 1:5 and 1:20 dilutions) and repeat the protocol. Repeat DNA extraction procedure.
Faint or no signals from both the test sample and the Control DNA for all loci.	There has been a user error in the PCR or electrophoresis setup.	Repeat the protocol. Check if LIZ size standard is present.
	The cycling profile applied is not optimal for the Canine Genotypes Panel 2.1.	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 Control DNA.	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_2O (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 Control DNA.	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 2.1.	Check the PCR program.

Appendix I: Avoiding carryover contamination

Due to their high sensitivity, PCR assays 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. The following general guidelines should be followed, in addition to other precautions mentioned in this Technical Manual, in order to minimize the risk of carryover contamination:

- Set up physically and strictly separate working places for (1) DNA extraction and sample preparation before PCR, (2) setup of the PCR reactions, and (3) preparing electrophoresis reagent mixes and performing electrophoresis. Workflow in the laboratory should always be unidirectional 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 and always before leaving an area.
- Use aerosol-resistant pipette tips.
- Use new and/or sterilized glassware and plasticware.

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