USER GUIDE

applied biosystems® by life technologies™

StockMarks® Kits for Horses, Cattle, and Dogs

Equine, Bovine, and Canine Genotyping Kits

Catalog Numbers 4336405, 4307480, 4307481

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Revision F

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life technologies™

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About this guide

Revision history

Revision	Date	Description
F	April 2014	• Removed references to discontinued products. • Updated document template with associated updates to the front and back covers, limited license information, warranty information, trademark statement, and safety statements. • Updated company name to Thermo Fisher Scientific.
E	June 2010	Baseline for revision history.

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Background information

Humans have been breeding animals selectively for centuries. Animals with superior traits, such as speed and strength in the case of horses, or high milk production and lean carcasses in the case of cattle, are used as breeding stock for subsequent generations. This classical method is based on physical observation of such traits in adult animals and the careful maintenance of lineage records by breeding organizations. Historically, serological typing has confirmed an offspring's parentage.

Recently, breeders have turned to molecular biology and the use of DNA markers for parentage verification. Microsatellite markers, also referred to as short tandem repeats (STRs), are evenly distributed across genomes. They are highly polymorphic, and they can be identified within DNA samples using PCR.

With the StockMarks® animal genotyping kits, we have combined the advantages of PCR-based tests with the informativeness of STRs to provide an automated approach to genotyping animals for parentage verification.

Available products

The following kits are available:

- StockMarks® Kit for Horses (Equine genotyping kit): Cat. no. 4336405
- StockMarks® Kit for Cattle (Bovine genotyping kit): Cat. no. 4307480
- StockMarks® Kit for Dogs (Canine genotyping kit): Cat. no. 4307481

Product description

StockMarks® for Horses, StockMarks® for Cattle, and the StockMarks® for Dogs are part of a complete solution for animal parentage typing. These kits employ a proprietary PCR process for amplification of STRs using fluorescent dye-labeled primers. StockMarks® for Horses and StockMarks® for Cattle amplify STRs recommended by the International Society of Animal Genetics (ISAG), while StockMarks® for Dogs amplifies STRs recommended by the American Kennel Club (AKC).

Kit contents and storage

Each StockMarks® animal genotyping kit contains enough reagents to test 100 animals.

Component	Description	Storage conditions
Amplification primer mix†	Containing a balanced amount of each dye-labeled forward primer with its corresponding reverse primer	Store at –15 to –25°C for long-term storage.‡ Protect from light.
AmpliTaq Gold® DNA Polymerase	5 U/µL	Store at –15 to –25°C.
Control DNA	• StockMarks® for Horses, 20 ng/µL • StockMarks® for Cattle, 20 ng/µL • StockMarks® for Dogs, 10 ng/µL	Store at –15 to –25°C for long-term storage.‡
dNTP mix	100 tests	
StockMarks® PCR Buffer	100 tests	
25 mM $MgCl_2$	Supplied only with StockMarks® for Dogs	

† For a description of the animal-specific loci amplified by the primers, expected size range, and associated dye label refer to: Table 4 on page 21 for equine-specific loci, Table 5 on page 28 for bovine-specific loci, and Table 6 on page 34 for canine-specific loci.

‡ When the kit is in frequent use (on a daily basis), minimize freeze-thaw cycles by storing all components at 2–8°C.

Note: We do not support diluted reactions or guarantee the performance of the StockMarks® kits when reactions are diluted.

AmpliTaq Gold® DNA Polymerase

AmpliTaq Gold® DNA Polymerase is a modified version of recombinant AmpliTaq® DNA Polymerase. It is provided in an inactive state and activated through a pre-PCR step of 9–12 minutes at 95°C. Once activated, AmpliTaq Gold® DNA Polymerase has the same enzyme unit activity, thermal stability, and half-life at 95°C as AmpliTaq® DNA Polymerase. You can substitute AmpliTaq Gold® DNA Polymerase unit-for-unit for AmpliTaq® DNA Polymerase in any optimized PCR system, generally without further optimization.

Because the enzyme is supplied in an inactive state, you can prepare complete reaction premixes in advance. You can then transfer the reaction mix into 96-well microplates or reaction tubes with a multi-channel pipettor. This is a very important point for high-throughput applications: the enzyme does not become active until the temperature is well above primer annealing, minimizing primer-dimer formation (primer oligomerization).

Control DNA

Control DNA is provided with each kit to verify the correct amplification and detection of the animal-specific loci. Chapter 3 contains Genotyper™ software analysis plots of results from each type of animal control DNA.

Primers

The kits provide premixed forward and reverse primers. PCR conditions are optimized so that the loci can be amplified as multiplex reactions, and then loaded with the GeneScan™ 350 ROX™ or the GeneScan™ 500 LIZ® internal size standard into one of the Applied Biosystems® Genetic Analyzers.

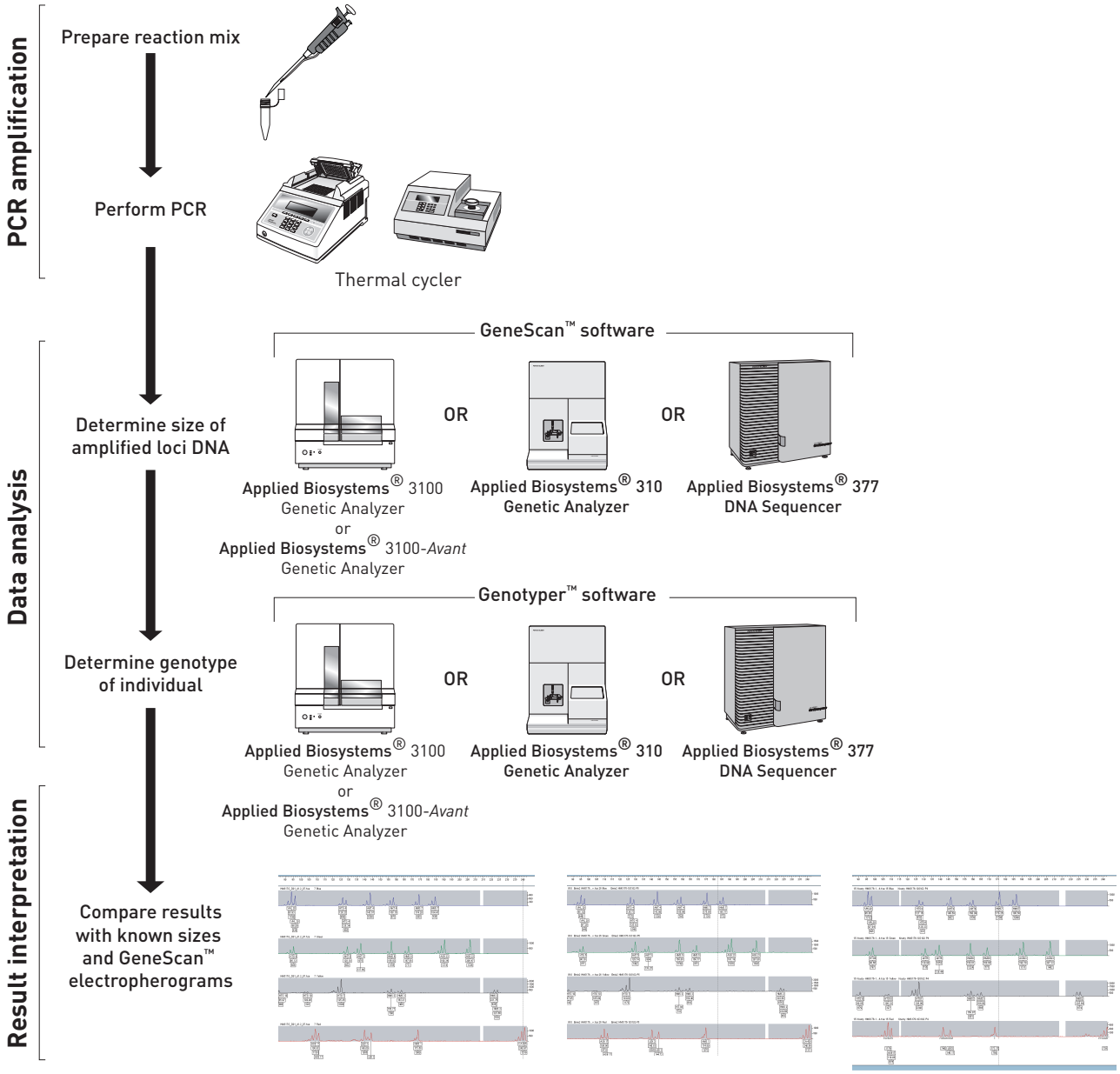
Required materials and equipment

Unless otherwise indicated, all materials are available from Life Technologies. MLS: Major laboratory supplier.

Materials	Source
Instruments	
One of the following genetic analyzers: • Applied Biosystems® 3100 Genetic Analyzer • Applied Biosystems® 3100-*Avant* Genetic Analyzer • Applied Biosystems® 310 Genetic Analyzer	Contact your local Life Technologies sales office.
GeneAmp® PCR System 9700	
For the 3100 and 3100-*Avant* Instruments	
POP-4® Performance Optimized Polymer	Cat. no. 4352755
DS-33 Dye Primer Matrix Standard Set (StockMarks® for Horses only)	Cat. no. 4345833
DS-32 Dye Primer Matrix Standard Set (StockMarks® for Cattle and StockMarks® for Dogs only)	Cat. no. 4345831
For the 310 Instrument	
POP-4® Performance Optimized Polymer	Cat. no. 402838
DS-33 Dye Primer Matrix Standard Set (StockMarks® for Horses only)	Cat. no. 4318159
DS-32 Dye Primer Matrix Standard Set (StockMarks® for Cattle and StockMarks® for Dogs only)	Cat. no. 4312131

Materials	Source
For the 377 Instrument	
DS-33 Dye Primer Matrix Standard Set (StockMarks® for Horses only)	Cat. no. 4318159
DS-32 Dye Primer Matrix Standard Set (StockMarks® for Cattle and StockMarks® for Dogs only)	Cat. no. 4312131
5% denaturing gel (36-cm or 12-cm well-to-read) for the Applied Biosystems® 377 instrument	MLS
For the 9700 Instrument	
GeneAmp® Thin-Walled Reaction Tubes with Flat Cap, 0.5-mL	Cat. no. N8010737
For the 9700 or 9600 Instrument	
MicroAmp® Reaction Tubes, 0.2-mL with Caps	Cat. no. N8010540
MicroAmp® Reaction Tubes, 0.2-mL without Caps	Cat. no. N8010533
MicroAmp® 8-Strip Reaction Tubes, 0.2-mL without Caps	Cat. no. N8010580
MicroAmp® Caps, 12 caps/strip or 8 caps/strip	Cat. no. N8010534 or N8010535
For all instruments	
GeneScan™ 350 ROX™ Size Standard	Cat. no. 401735
GeneScan™ 500 ROX™ Size Standard	Cat. no. 401734
GeneScan™ 500 LIZ® Size Standard	Cat. no. 4322682
Hi-Di™ Formamide	Cat. no. 4311320
Disposable gloves	MLS
Microcentrifuge	MLS
Pipet tips, aerosol resistant	MLS
Pipettors	MLS
Sterile deionized water	MLS
Vortexer	MLS

Procedure overview



2 Amplification of short tandem repeat (STR) loci

The determination of parentage and genotype begins with PCR amplification of the STRs using fluorescent dye-labeled primers. This chapter describes how to prepare the reactions for PCR amplification and provides the kit-specific conditions to program the thermal cycler.

Equine loci amplification

Prepare the 17-plex PCR

The amplification primer mix for the StockMarks® for Horses contains 17 primer sets, one for each of the STRs. Prepare one 17-plex PCR in a 15-µL reaction volume for each animal to be tested.

1. Combine the following reagents in a 0.2- or 0.5-mL tube to prepare a master mix for the 17-plex reactions:

Component	Volume (µL) for 1 sample	Volume (µL) for 10 samples†
StockMarks® PCR Buffer	2.5	27.5
dNTP mix	4.0	44.0
AmpliTaq Gold® DNA Polymerase	0.5	5.5
Amplification primer mix	4.0	33.0
Deionized water	3.0	44.0
Total volume	**14.0**	**154.0**

† Includes volume for one additional sample to account for losses that occur during reagent transfers.

2. Vortex the master mix.
3. For each animal to be tested, transfer 14 µL of master mix to a separate thin-walled PCR tube.

IMPORTANT! Use the appropriate PCR tube for the thermal cycler that you will be using to perform PCR (see "Required materials and equipment" on page 9).

4. Dilute control and sample DNA in Nuclease-free Water:
   a. Dilute the Control DNA 1:8.
   b. Dilute the DNA sample to 1–10 ng/µL.
5. Add 1 µL of diluted control or sample DNA to each PCR tube, and cap the tube.
6. Vortex the tubes briefly.
7. Spin the tubes briefly in a microcentrifuge to ensure that all contents are collected in the bottoms of the tubes.

Run the 17-plex reactions

See the instrument user guide for help with programming the thermal cycler.

1. Place the reaction tubes in the thermal cycler.
2. Use the following thermal-cycling conditions:

Table 1 Thermal-cycling conditions for amplification of equine loci.

Temperatures and times					
Initial step†	**Melt**	**Anneal**	**Extend**	**Final extension**	**Final hold**
1 Cycle	**30 Cycles**			**1 Cycle**	**Hold**
95°C 10 min	95°C 30 sec	60°C 30 sec	72°C 60 sec	72°C 60 min	4°C ∞‡

† The initial 10-minute, 95°C heating step is required to activate the AmpliTaq Gold® DNA Polymerase.
‡ Up to 24 hours.

Note: If you are using the GeneAmp® PCR System 9700, program it in 9600 Emulation Mode.

3. Start the run.
4. Store the PCR products at 2–6°C until analysis (Chapter 3 on page 19).

Bovine loci amplification

Prepare the 11-plex PCR

The amplification primer mix for the StockMarks® for Cattle contains 11 primer sets, one for each of the STRs recommended by the ISAG. Prepare one 11-plex PCR in a 15-µL reaction volume for each animal to be tested.

1. Combine the following reagents in a 0.2- or 0.5-mL tube to prepare a master mix for the 11-plex reactions:

Component	Volume (µL) for 1 sample	Volume (µL) for 10 samples†
StockMarks® PCR Buffer	3.0	33.0
dNTP mix	4.0	44.0
AmpliTaq Gold® DNA Polymerase	0.5	5.5
Amplification primer mix	5.5	60.5
Deionized water	1.0	11.0
Total volume	**14.0**	**154.0**

† Includes volume for one additional sample to account for losses that occur during reagent transfers.

2. Vortex the master mix.
3. For each animal to be tested, transfer 14 µL of master mix to a separate thin-walled PCR tube.

   IMPORTANT! Use the appropriate PCR tube for the thermal cycler that you will be using to perform PCR (see "Required materials and equipment" on page 9).

4. Dilute control and sample DNA in Nuclease-free Water:
   a. Dilute the Control DNA 1:8.
   b. Dilute the DNA sample to 1–10 ng/µL.
5. Add 1 µL of diluted control or sample DNA to each PCR tube, and cap the tube.
6. Vortex the tubes briefly.
7. Spin the tubes briefly in a microcentrifuge to ensure that all of their contents are collected in the bottoms of the tubes.

Run the 11-plex reactions

See the instrument user guide for help with programming the thermal cycler.

1. Place the reaction tubes in the thermal cycler.
2. Use the following thermal-cycling conditions:

Table 2 Thermal-cycling conditions for amplification of bovine loci

Thermal cycler	Temperatures and times						
	Initial step†	Melt	Anneal	Extend	Final extension	Final step	Final hold
	1 Cycle	31 Cycles			1 Cycle	1 Cycle	Hold
GeneAmp® PCR System 9700‡	95°C 10 min	94°C 45 sec	50% ramp 61°C 45 sec	80% ramp 72°C 60 sec	72°C 60 min	25°C 2 hr	4°C ∞§
GeneAmp® PCR System 9600	95°C 10 min	94°C 45 sec	30 sec ramp 61°C 45 sec	30 sec ramp 72°C 60 sec	72°C 60 min	25°C 2 hr	4°C ∞§

† The initial 10-minute, 95°C heating step is required to activate the AmpliTaq Gold® DNA Polymerase.

‡ Program the 9700 System in 9600 Emulation Mode.

§ Up to 24 hours.

3. Start the run.
4. Store the PCR products at 2–6°C until analysis (Chapter 3 on page 19).

Canine loci amplification

Prepare the 10-plex PCR

The amplification primer mix for the StockMarks® for Dogs contains 10 primer sets, one for each of the STRs recommended by the AKC. Prepare one 10-plex PCR in a 10-µL reaction volume for each animal to be tested.

1. Combine the following reagents in a 0.2- or 0.5-mL tube to prepare a master mix for the 10-plex reactions:

Component	Volume (µL) for 1 sample	Volume (µL) for 10 samples†
StockMarks® PCR Buffer	1.4	15.3
25 mM $MgCl_2$	0.36	4.0
dNTP mix	2.2	24.2
AmpliTaq Gold® DNA Polymerase	0.36	4.0
Amplification primer mix	2.8	30.8
Deionized water	1.9	20.8
Total volume	**9.0**	**99.0**

† Includes volume for one additional sample to account for losses that occur during reagent transfers.

2. Vortex the master mix.

3. For each animal to be tested, transfer 9 µL of master mix to a separate thin-walled PCR tube.

   IMPORTANT! Use the appropriate PCR tube for the thermal cycler that you will be using to perform PCR (see "Required materials and equipment" on page 9).

4. Dilute control and sample DNA in Nuclease-free Water:
   a. Dilute the Control DNA 1:8.
   b. Dilute the DNA sample to 1–10 ng/µL.
5. Add 1 µL of diluted control or sample DNA to each PCR tube, and cap the tube.
6. Vortex the tubes briefly.
7. Spin the tubes briefly in a microcentrifuge to ensure that all of their contents are collected in the bottoms of the tubes.

Run the 10-plex reactions

See the instrument user guide for help with programming the thermal cycler.

1. Place the reaction tubes in the thermal cycler.
2. Use the following thermal-cycling conditions:

Table 3 Thermal-cycling conditions for amplification of canine loci

Thermal cycler	Temperatures and times								
	Initial step†	Melt	Anneal	Extend	Melt	Anneal	Extend	Final extension	Final hold
	1 Cycle	20 Cycles			15 Cycles			1 Cycle	Hold
GeneAmp® PCR System 9700‡	95°C 10 min	95°C 30 sec	50% ramp§ 58°C 30 sec	80% ramp 72°C 60 sec	95°C 30 sec	50% ramp 56°C 30 sec	80% ramp 72°C 60 sec	72°C 30 min	4°C ∞††
GeneAmp® PCR System 9600	95°C 10 min	95°C 30 sec	58°C 30 sec	72°C 60 sec	95°C 30 sec	56°C 30 sec	72°C 60 sec	72°C 30 min	4°C ∞††

† The initial 10-minute, 95°C heating step is required to activate the AmpliTaq Gold® DNA Polymerase.
‡ Program the 9700 System in 9600 Emulation Mode.
§ Decreases the temperature by 0.1°C per cycle to 56°C.
†† Up to 24 hours.

3. Start the run.
4. Store the PCR products at 2–6°C until analysis (Chapter 3 on page 19).

3 Data analysis

After the amplification process, determine the size of the dye-labeled PCR products by running them through one of several Applied Biosystems® genetic analyzers.

This chapter explains how to prepare PCR products for analysis. It also lists the expected size ranges (in base pairs) of the various equine, bovine, and canine loci.

Data analysis when using StockMarks® for Horses

Prepare equine PCR products for analysis

Product preparation depends on the instrument used to analyze the samples, as described in the following table. See the appropriate instrument user guide for instructions on using the various Applied Biosystems® genetic analyzers.

To prepare PCR products for analysis on this instrument...	Use the procedure on page...	Use this Filter Set...	Use this Matrix File...
Applied Biosystems® 3100 *or* Applied Biosystems® 3100-*Avant*	20	Filter Set G5	DS-33 Dye Primer Matrix Standard Set (Cat. no. 4345833)
Applied Biosystems® 310	20	Filter Set G5	DS-33 Dye Primer Matrix Standard Set (Cat. no. 4318159)
Applied Biosystems® 377	20	Filter Set G5†	DS-33 Dye Primer Matrix Standard Set (Cat. no. 4318159)

† Filter Set G5 is not supported on the Applied Biosystems® 377 Instrument with Macintosh® OS-based Data Collection Software. Requires the Basic Bundle for Applied Biosystems® 377 DNA Sequencer, including data collection software (Cat. no. 4327420).

Preparation for the Applied Biosystems® 3100 or 3100-*Avant* instruments:

1. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the undiluted 17-plex reaction product
   - 20 µL Hi-Di™ Formamide
   - 0.5 µL GeneScan™ 500 LIZ® Size Standard

   IMPORTANT! Do not use the GeneScan™ 500 ROX™ and GeneScan™ 350 ROX™ size standards with the equine kit.

   Note: For each animal tested, you may need to adjust the amounts of PCR products or deionized water depending on how much starting DNA you used.
2. Spin the mixtures briefly in a microcentrifuge.
3. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
4. Place the samples into the Applied Biosystems® 3100 or Applied Biosystems® 3100-*Avant* Genetic Analyzer. Use the default module.

   Note: You can decrease the run time to 1000 seconds.

Preparation for the Applied Biosystems® 310 instrument:

1. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the undiluted 17-plex reaction product
   - 11.5 µL Hi-Di™ Formamide
   - 0.5 µL GeneScan™ 500 LIZ® Size Standard

   IMPORTANT! Do not use the GeneScan™ 500 ROX™ and GeneScan™ 350 ROX™ size standards with the equine kit.

   Note: For each animal tested, you may need to adjust the amounts of PCR products or deionized water depending on how much starting DNA you used.
2. Spin the mixtures briefly in a microcentrifuge.
3. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
4. Place the samples into the Applied Biosystems® 310 Genetic Analyzer.
   - With a 1-mL polymer syringe, use the GS STR Pop 4 (1 mL) G5 module
   - With a 2.5-mL syringe, use the GS STR Pop 4 (2.5 mL) G5 module

Preparation for the Applied Biosystems® 377 Instrument:

1. Prepare a 1X loading stock using the following reagents in the ratios shown:
   - 2.9 µL Hi-Di™ Formamide
   - 0.5 µL Loading Buffer (50 mm EDTA, 50 mg/mL blue dextran)
   - 0.6 µL GeneScan™ 500 LIZ® Size Standard

   IMPORTANT! Do not use the GeneScan™ 500 ROX™ and GeneScan™ 350 ROX™ size standards with the equine kit.

2. In one 0.5-mL tube for each animal, combine 0.4 µL of the undiluted PCR products with 2.0 µL of 1X loading stock. Cap the tubes.

   Note: For each animal tested, you may need to adjust the amounts of PCR products or deionized water depending on how much starting DNA you used.

3. Spin the mixtures briefly in a microcentrifuge.

4. Heat the tubes at 95°C for 2 minutes with the caps off, then immediately place the tubes on ice for 3 minutes.

5. Load the entire volume onto a 66-lane, 36-cm, 5% Long Ranger gel. Use 0.33-mm spacers and 1X TBE buffer.

6. Depending on the length of the gel used, select the appropriate run module as follows:

Plate size, well-to-read (cm)	Plate check module	Pre-run module	Run module
12	G	GS PR 12G 2400	GS Run 12G 2400
36	G	GS PR 36G 2400	GS Run 36G 2400

Determine the size of amplified equine DNA

The expected size ranges for equine-specific loci in Table 4 are based on empirical observations of allele sizes from genotyping studies on an Applied Biosystems® 377 DNA Sequencer.

Occasionally, you may observe an allele that falls outside of its expected size range and that overlaps with the expected size range of another locus. Such alleles may be present at extremely low frequencies in the population. They can be observed as the number of individual animals in the genotyped population increases and/or when different breeds are included in the population.

Table 4 Expected size ranges for equine-specific loci

Locus	Dye	Color	Expected size range (bp)†
VHL20	6-FAM™	Blue	83–102
HTG4	6-FAM™	Blue	116–137
AHT4	6-FAM™	Blue	140–166
HMS7	6-FAM™	Blue	167–187
HTG6	VIC®	Green	74–103
AHT5	VIC®	Green	126–147
HMS6	VIC®	Green	154–170
ASB23	VIC®	Green	176–212
ASB2	VIC®	Green	237–268
HTG10	NED™	Yellow	83–110

Table 4 Expected size ranges for equine-specific loci *(continued)*

Locus	Dye	Color	Expected size range (bp)†
HTG7	NED™	Yellow	114–128
HMS3	NED™	Yellow	146–170
HMS2	NED™	Yellow	215–236
ASB17	PET®	Red	104–116
LEX3	PET®	Red	137–160
HMS1	PET®	Red	166–178
CA425	PET®	Red	224–247

† The expected size range may be from 1–6 bp smaller when separated on either an Applied Biosystems® 310, Applied Biosystems® 3100, or Applied Biosystems® 3100-*Avant*™ Genetic Analyzer.

Amplification of the 17 equine-specific loci using the equine control DNA as the sample should provide results similar to those illustrated in Figures 1, 2, and 3. These figures show Genotyper™ software plots of GeneScan™ software results when equine control DNA is separated on an:

- Applied Biosystems® 3100 Genetic Analyzer (Figure 1 on page 23)
- Applied Biosystems® 310 Genetic Analyzer (Figure 2 on page 24)
- Applied Biosystems® 377 DNA Sequencer (Figure 3 on page 25)

As described in Chapter 4, "Interpretation of results," plots of the 17 amplified loci can be compared to GeneScan™ software electropherograms to determine whether the horse tested is homozygous or heterozygous at each locus.

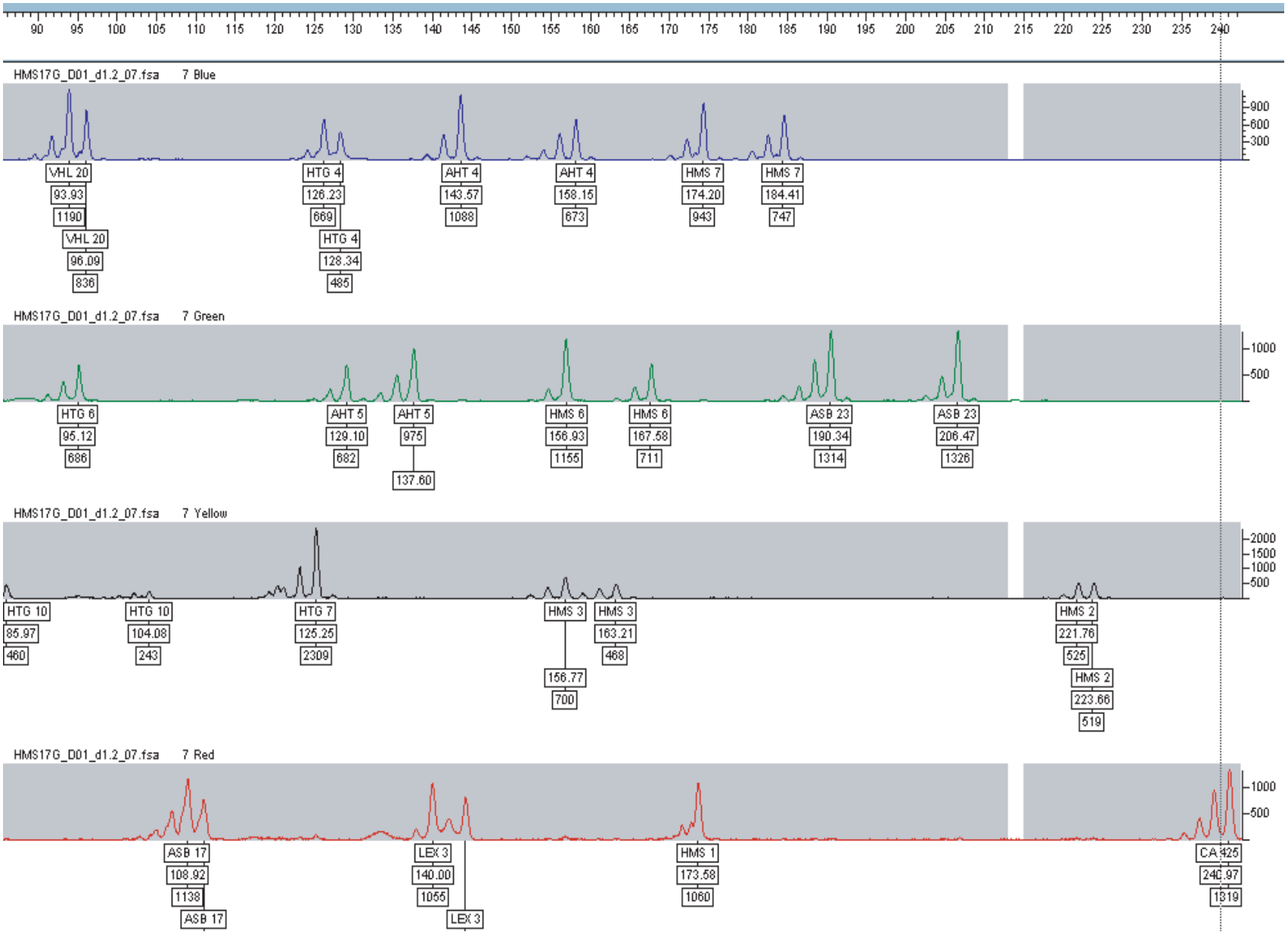


Figure 1 Genotyper™ software analysis of PCR amplification products using equine control DNA separated on the Applied Biosystems® 3100 Genetic Analyzer

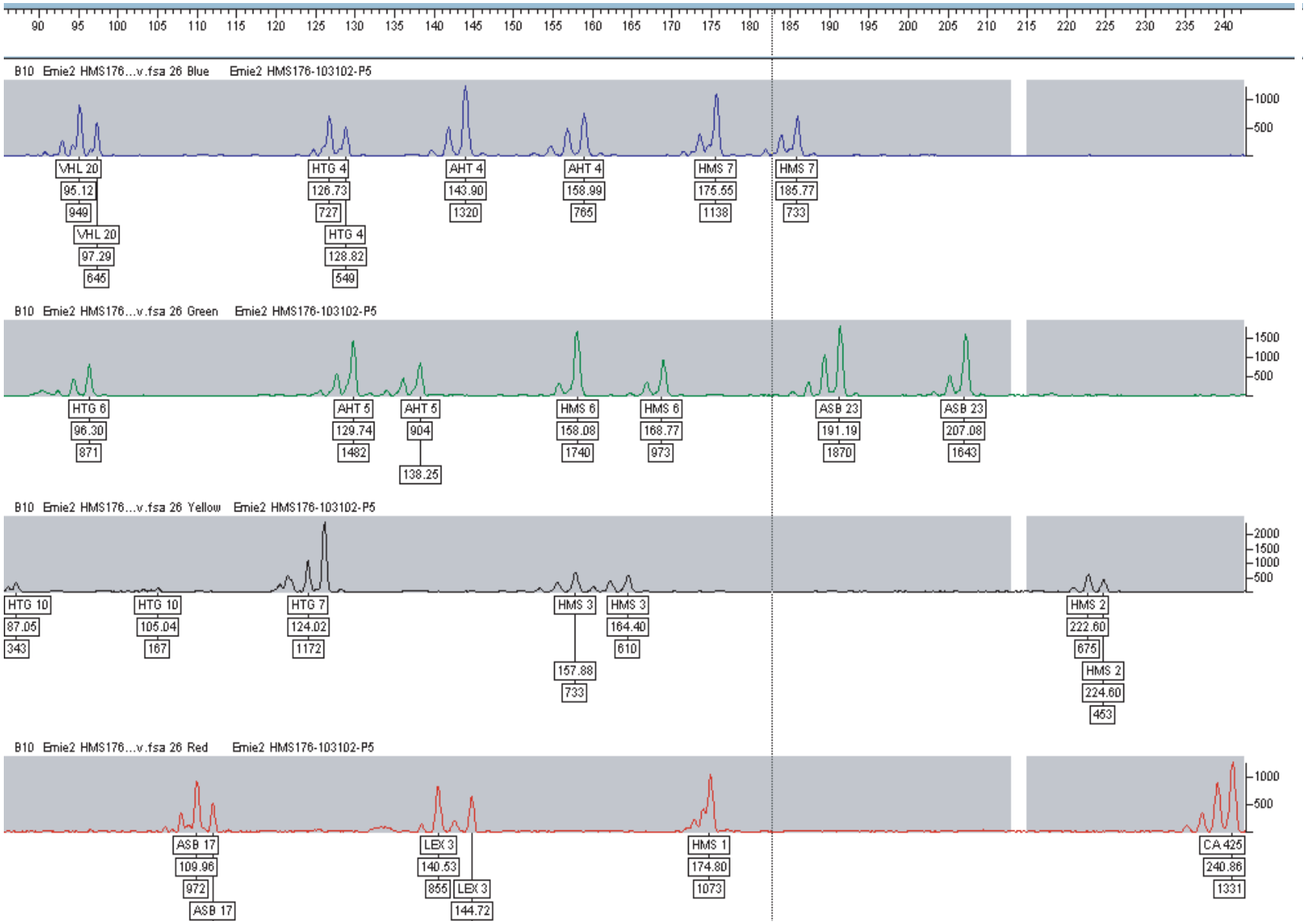


Figure 2 Genotyper™ software analysis of PCR amplification products using equine control DNA separated on the Applied Biosystems® 310 Genetic Analyzer

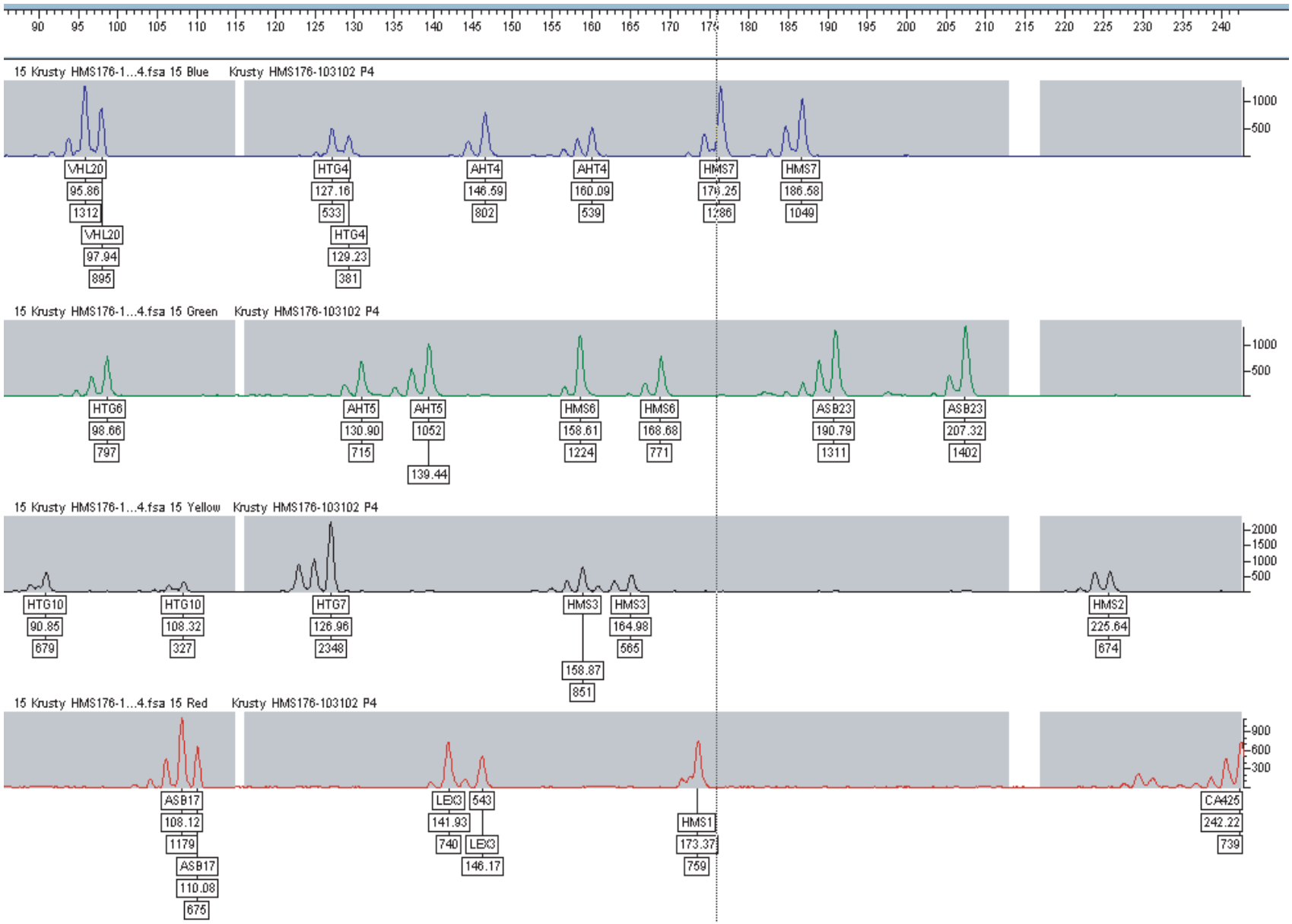


Figure 3 Genotyper™ software analysis of PCR amplification products using equine control DNA separated on the Applied Biosystems® 377 DNA Sequencer

Data analysis when using StockMarks® for Cattle

Prepare bovine PCR products for analysis

Product preparation depends on the instrument used to analyze the samples, as described in the following table. See the appropriate instrument user guide for instructions on using the various Applied Biosystems® genetic analyzers.

To prepare PCR products for analysis on this instrument...	Use the procedure on page...	Use this filter set...	Use this matrix file...
Applied Biosystems® 3100 *or* Applied Biosystems® 3100-*Avant*	26	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4345831)
Applied Biosystems® 310	26	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4312131)
Applied Biosystems® 377	27	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4312131)

Preparation for the Applied Biosystems® 3100 or 3100-*Avant* instruments:

1. For each animal tested, dilute each PCR amplification reaction with 150 µL of deionized water.

   Note: You may need to adjust the amounts of PCR products or deionized water depending on how much starting DNA you used.

2. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the diluted 11-plex reaction product
   - 11.5 µL Hi-Di™ Formamide
   - 0.5 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.

3. Spin the mixtures briefly in a microcentrifuge.
4. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
5. Place the samples into the Applied Biosystems® 3100 or Applied Biosystems® 3100-*Avant* Genetic Analyzer. Use the default module.

   Note: You can decrease the run time to 1000 seconds.

Preparation for the Applied Biosystems® 310 instrument:

1. For each animal tested, dilute each PCR amplification reaction with 90 µL of deionized water.

   Note: You may need to adjust the amounts of PCR products or deionized water depending on how much starting DNA was used.

2. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the diluted 11-plex reaction product
   - 11.5 µL Hi-Di™ Formamide
   - 0.5 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.
3. Spin the mixtures briefly in a microcentrifuge.
4. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
5. Place the samples into the Applied Biosystems® 310 Genetic Analyzer.
   - With a 1-mL polymer syringe, use the GS STR Pop 4 (1 mL) F module
   - With a 2.5-mL syringe, use the GS STR Pop 4 (2.5 mL) F module

Preparation for the Applied Biosystems® 377 instrument:

1. Prepare a 1X loading stock using the following reagents in the ratios shown:
   - 2.9 µL Hi-Di™ Formamide
   - 0.5 µL Loading Buffer (50 mm EDTA, 50 mg/mL blue dextran)
   - 0.6 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.
2. For each animal tested, dilute the PCR amplification reaction with 90 µL deionized water.

   Note: You may need to adjust the amounts of the PCR products or deionized water depending on how much starting DNA was used.
3. In one 0.5-mL tube for each animal, combine 0.4 µL of the diluted PCR products prepared in step 2 with 2.0 µL of 1X loading stock. Cap the tubes.
4. Spin the mixtures briefly in a microcentrifuge.
5. Heat the tubes at 95°C for 2 minutes with the caps off, then immediately place the tubes on ice for 3 minutes.
6. Load the entire volume onto a 66-lane, 36-cm, 5% Long Ranger gel. Use 0.33-mm spacers and 1X TBE buffer.
7. Depending on the length of the gel used, select the appropriate run module as follows:

Plate size, well-to-read (cm)	Plate check module	Pre-run module	Run module
12	F	GS PR 12F 2400	GS Run 12F 2400
36	F	GS PR 36F 2400	GS Run 36F 2400

Determine the size of amplified bovine DNA

The expected size ranges for bovine-specific loci in Table 5 are based upon empirical observations of allele sizes from genotyping studies on an Applied Biosystems® 377 DNA Sequencer.

Occasionally, you may observe an allele that falls outside of its expected size range and that overlaps with the expected size range of another locus. Such alleles may be present at extremely low frequencies in the population. They can be observed as the number of individual animals in the genotyped population increases and/or when different breeds are included in the population.

Table 5 Expected size ranges for bovine-specific loci

Locus	Dye	Color	Expected size range (bp)†
TGLA227	FAM™	Blue	64–115
BM2113	FAM™	Blue	116–146
TGLA53	FAM™	Blue	147–197
ETH10	FAM™	Blue	198–234
SPS115	FAM™	Blue	235–265
TGLA126	JOE™	Green	104–131
TGLA122	JOE™	Green	134–193
INRA23	JOE™	Green	193–235
ETH3	NED™	Yellow	90–135
ETH225	NED™	Yellow	136–165
BM1824	NED™	Yellow	170–218

† The expected size range may be from 1–6 bp smaller when separated on either an Applied Biosystems® 310, an Applied Biosystems® 3100, or an Applied Biosystems® 3100-*Avant* Genetic Analyzer.

Amplification of the 11 bovine-specific loci using the bovine control DNA as the sample should provide results similar to those illustrated in Figures 4, 5, and 6. These figures show Genotyper™ software plots of GeneScan™ software results when bovine control DNA is separated on an

- Applied Biosystems® 3100 Genetic Analyzer (Figure 4 on page 29)
- Applied Biosystems® 310 Genetic Analyzer (Figure 5 on page 30)
- Applied Biosystems® 377 DNA Sequencer (Figure 6 on page 31)

As described in Chapter 4, "Interpretation of results," plots of the 11 amplified loci can be compared to GeneScan™ software electropherograms to determine whether the cow tested in homozygous or heterozygous at each locus.

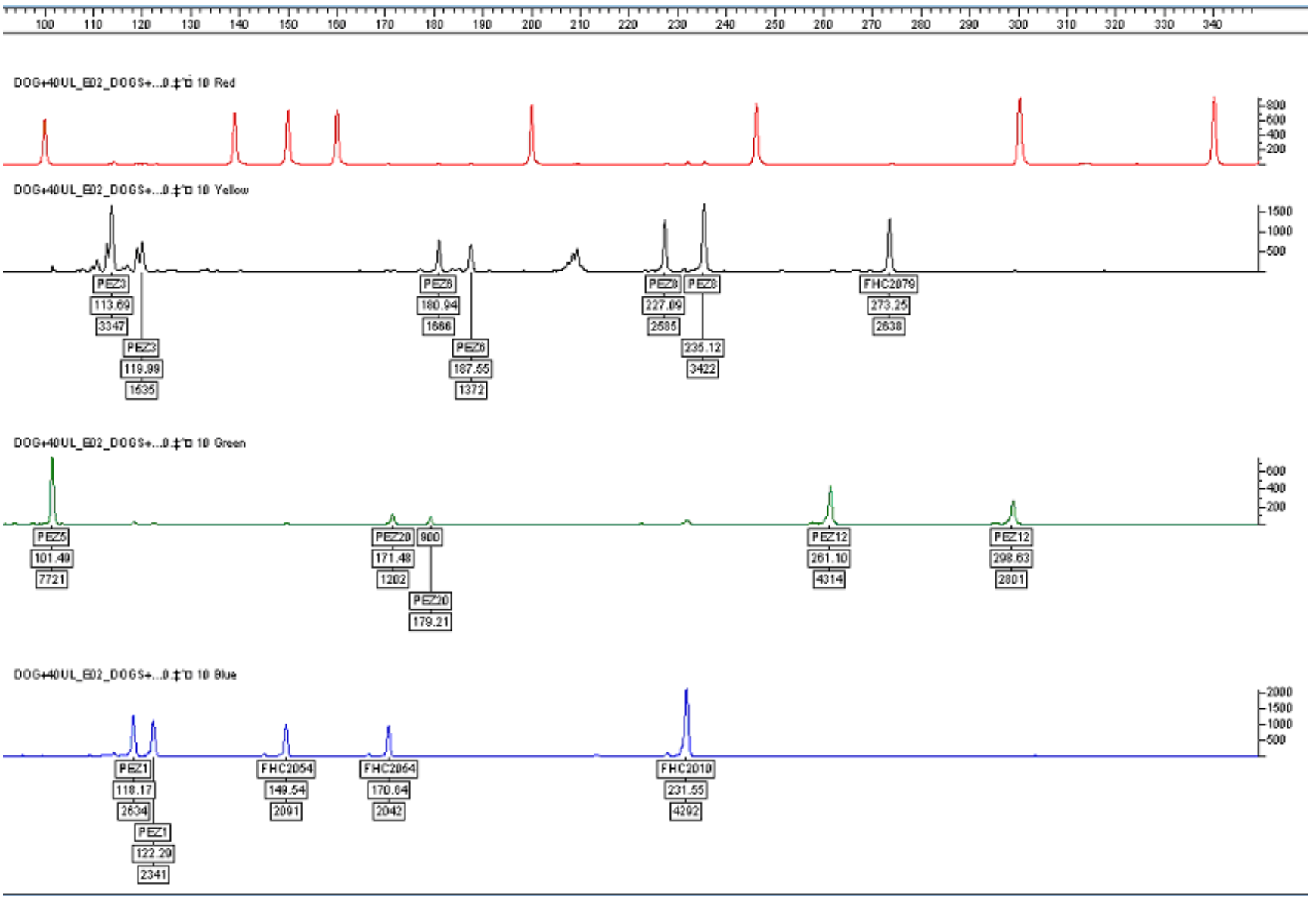


Figure 4 Genotyper™ software analysis of PCR amplification products using bovine control DNA separated on an Applied Biosystems® 3100 Genetic Analyzer

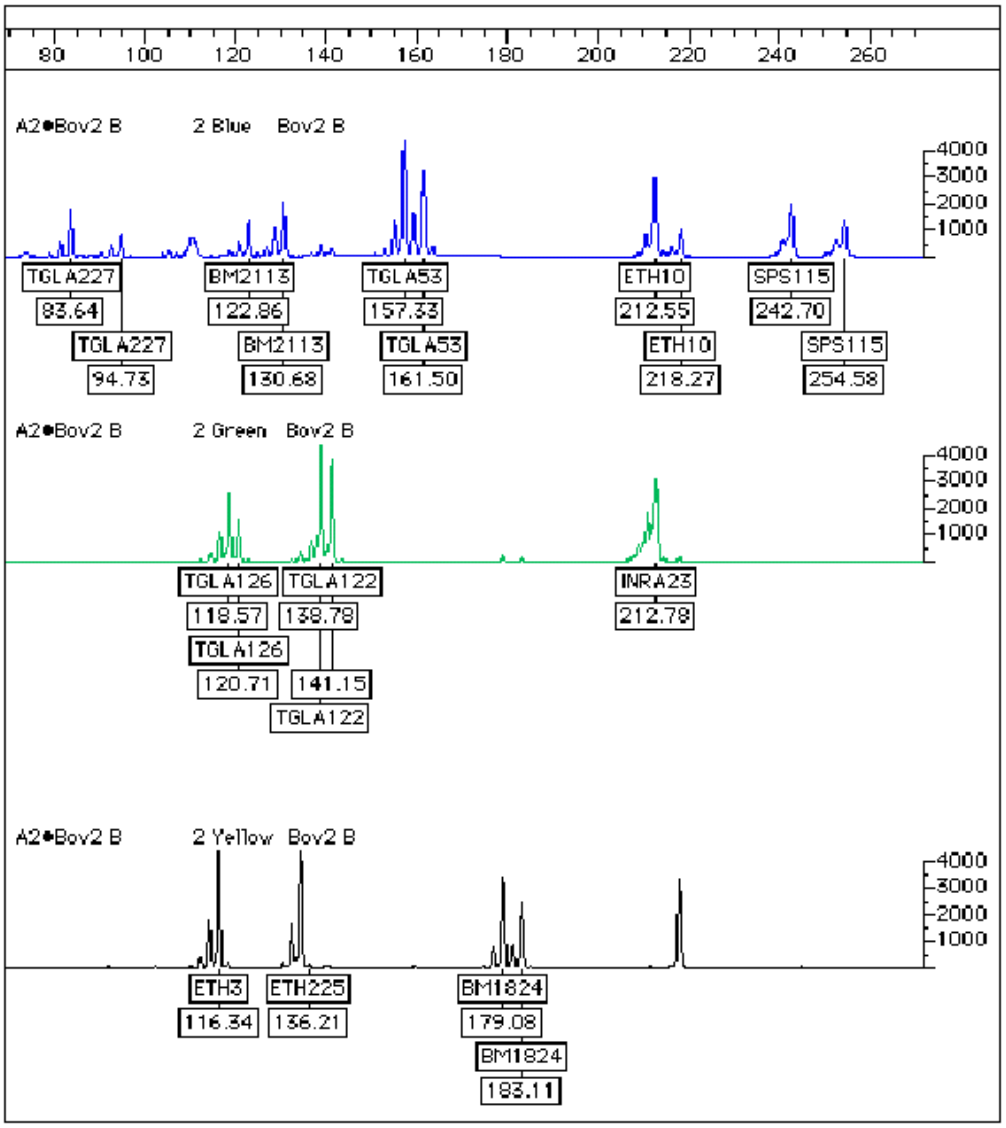


Figure 5 Genotyper™ software analysis of PCR amplification products using bovine control DNA and separated on an Applied Biosystems® 310 Genetic Analyzer

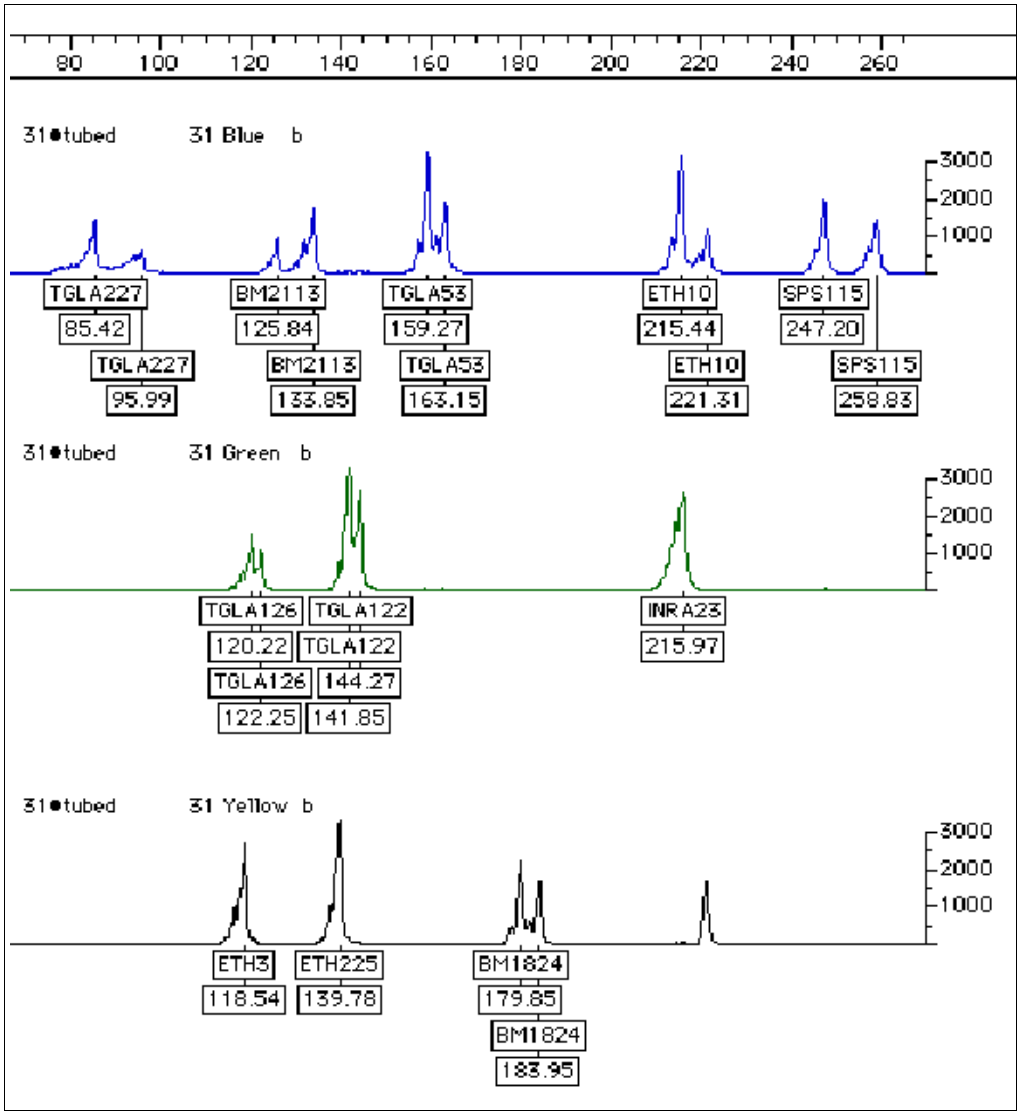


Figure 6 Genotyper™ software analysis of PCR amplification products using bovine control DNA and separated on an Applied Biosystems® 377 DNA Sequencer

Data analysis when using StockMarks® for Dogs

Prepare canine PCR products for analysis

Product preparation depends on the instrument used to analyze the samples, as described in the following table. See the appropriate instrument user guide for instructions on using the various Applied Biosystems® genetic analyzers.

To prepare PCR products for analysis on this instrument...	Use the procedure on page...	Use this filter set...	Use this matrix file...
Applied Biosystems® 3100 *or* Applied Biosystems® 3100-*Avant*	32	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4345831)
Applied Biosystems® 310	32	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4312131)
Applied Biosystems® 377	33	Filter Set F	DS-32 Dye Primer Matrix Standard Set (Cat. no. 4312131)

Preparation for the Applied Biosystems® 3100 or 3100-*Avant* instruments:

1. For each animal tested, dilute each PCR amplification reaction with 20 µL of deionized water.

   Note: You may need to adjust the amounts of the PCR products or deionized water depending on how much starting DNA was used.

2. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the diluted 11-plex reaction product
   - 11.5 µL Hi-Di Formamide
   - 0.5 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.

3. Spin the mixtures briefly in a microcentrifuge.
4. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
5. Place the samples into the Applied Biosystems® 3100 or 3100-*Avant* Genetic Analyzer. Use the default module.

   Note: You can decrease the run time to 1000 seconds.

Preparation for the Applied Biosystems® 310 instrument:

1. For each animal tested, dilute each PCR amplification reaction with 20 µL of deionized water.

   Note: You may need to adjust the amounts of the PCR products or deionized water depending on how much starting DNA was used.

2. Combine the following in a 0.5-mL tube:
   - 1.0 µL of the diluted 11-plex reaction product
   - 11.5 µL Hi-Di™ Formamide
   - 0.5 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.
3. Spin the mixtures briefly in a microcentrifuge.
4. Heat at 95°C for 2 minutes, then immediately place the tubes on ice for 3 minutes.
5. Place the samples into the Applied Biosystems® 310 Genetic Analyzer.
   - With a 1-mL polymer syringe, use the GS STR Pop 4 (1 mL) F module
   - With a 2.5-mL syringe, use the GS STR Pop 4 (2.5 mL) F module).

Preparation for the Applied Biosystems® 377 instrument:

1. Prepare a 1X loading stock using the following reagents in the ratios shown:
   - 2.9 µL Hi-Di™ Formamide
   - 0.5 µL Loading Buffer (50 mm EDTA, 50 mg/mL blue dextran)
   - 0.6 µL GeneScan™ 350 ROX™ Size Standard

   Note: Alternatively, you can use GeneScan™ 500 ROX™ Size Standard to obtain a greater sizing precision of the higher molecular weight fragments.
2. For each animal tested, dilute the PCR amplification reaction with 20 µL deionized water.

   Note: You may need to adjust the amounts of the PCR products or deionized water depending on how much starting DNA was used.
3. In one 0.5-mL tube for each animal, combine 0.4 µL of the diluted PCR products prepared in step 2 with 2.0 µL of 1X loading stock. Cap the tubes.
4. Spin the mixtures briefly in a microcentrifuge.
5. Heat the tubes at 95°C for 2 minutes with the caps off, then immediately place the tubes on ice for 3 minutes.
6. Load the entire volume onto a 66-lane, 36-cm, 5% Long Ranger gel. Use 0.33-mm spacers and 1X TBE buffer.
7. Depending on the length of the gel used, select the appropriate run module as follows:

Plate size, well-to-read (cm)	Plate check module	Pre-run module	Run module
12	F	GS PR 12F 2400	GS Run 12F 2400
36	F	GS PR 36F 2400	GS Run 36F 2400

Determine the size of amplified canine DNA

The expected size ranges for canine-specific loci in Table 6 are based upon empirical observations of allele sizes from genotyping studies on an Applied Biosystems® 377 DNA Sequencer.

Occasionally, you may observe an allele that falls outside of its expected size range and that overlaps with the expected size range of another locus. Such alleles may be present at extremely low frequencies in the population. They can be observed as the number of individual animals in the genotyped population increases and/or when different breeds are included in the population.

Table 6 Expected size ranges for canine-specific loci

Locus	Dye	Color	Expected size range (bp)†
PEZ 1	FAM™	Blue	92–136
FHC 2054	FAM™	Blue	140–183
FHC 2010	FAM™	Blue	210–260
PEZ 5	JOE™	Green	97–121
PEZ 20	JOE™	Green	170–201
PEZ 12	JOE™	Green	250–320
PEZ 3	NED™	Yellow	95–154
PEZ 6	NED™	Yellow	164–214
PEZ 8	NED™	Yellow	222–260
FHC 2079	NED™	Yellow	263–299

† The expected size range may be from 1–6 bp smaller when separated on either an Applied Biosystems® 310, an Applied Biosystems® 3100, or an Applied Biosystems® 3100-*Avant* Genetic Analyzer.

Amplification of the ten canine-specific loci using the canine control DNA as the sample should provide results similar to those illustrated in Figures 7, 8, and 9. These figures show Genotyper™ software plots of GeneScan™ software results when canine control DNA is separated on an

- Applied Biosystems® 3100 Genetic Analyzer (Figure 7 on page 35)
- Applied Biosystems® 310 Genetic Analyzer (Figure 8 on page 36)
- Applied Biosystems® 377 DNA Sequencer (Figure 9 on page 37)

As described in Chapter 4, "Interpretation of results," plots of the ten amplified loci can be compared to GeneScan™ electropherograms to determine whether the dog tested in homozygous or heterozygous at each locus.

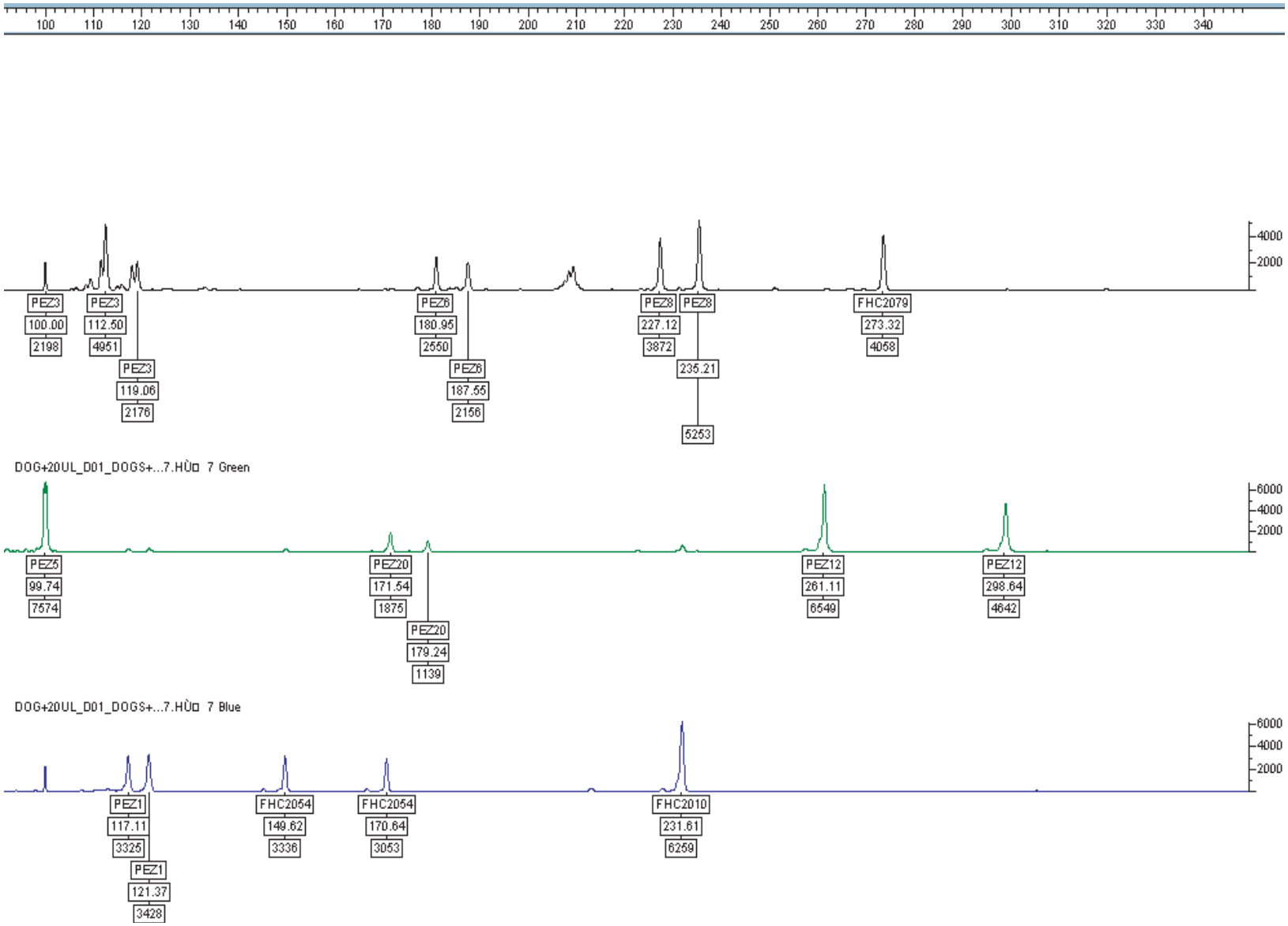


Figure 7 Genotyper™ software analysis of PCR amplification products using canine control DNA and separated on an Applied Biosystems® 3100 Genetic Analyzer

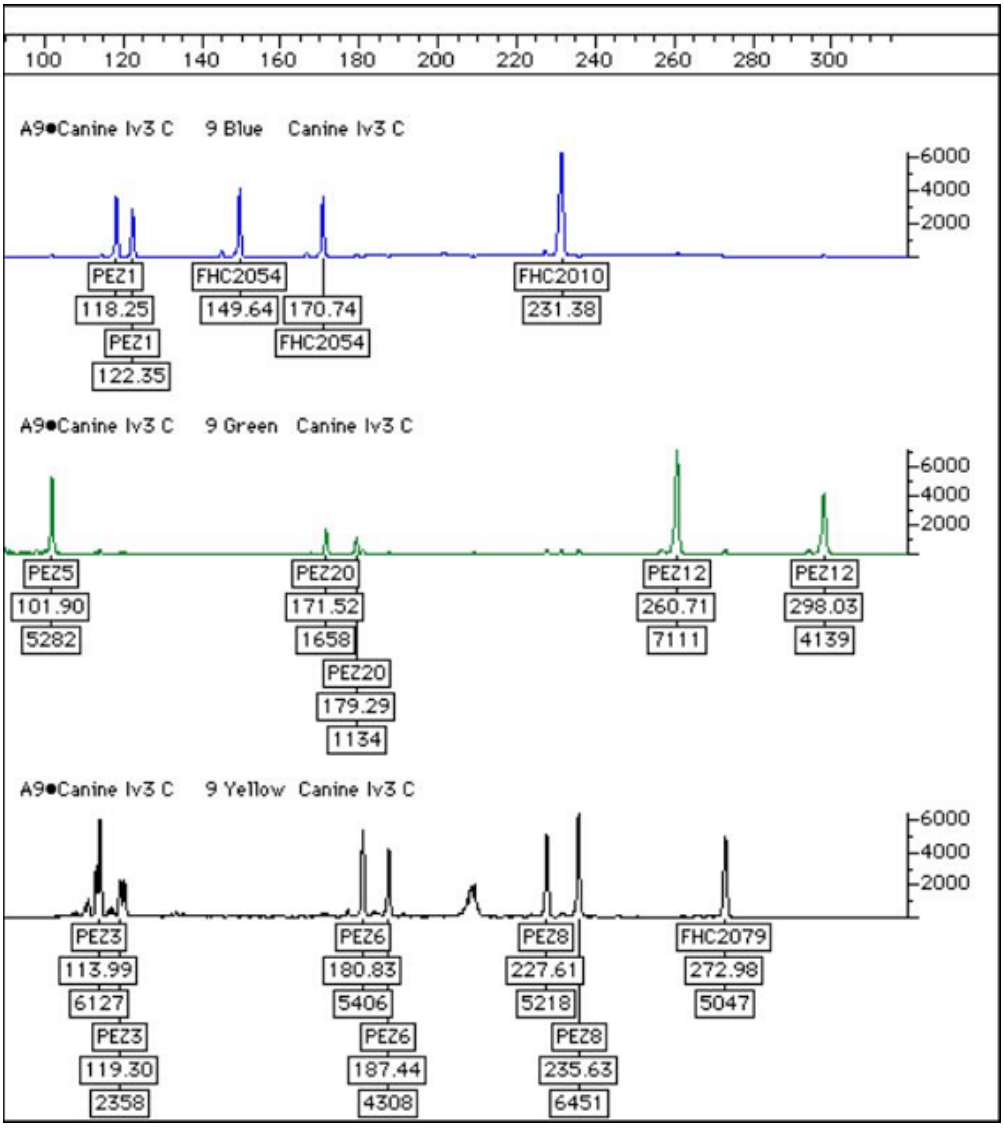


Figure 8 Genotyper™ software analysis of PCR amplification products using canine control DNA and separated on an Applied Biosystems® 310 Genetic Analyzer

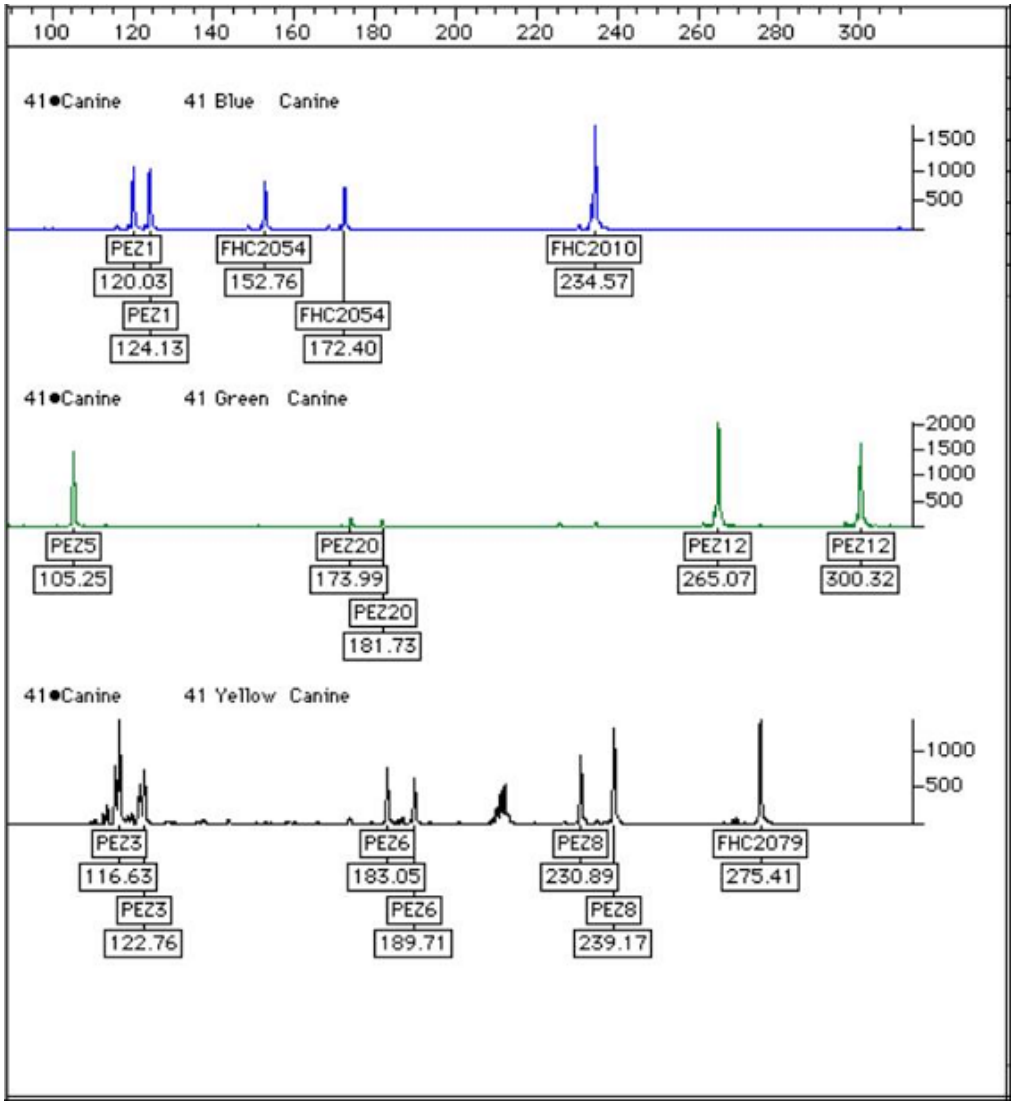


Figure 9 Genotyper ™software analysis of PCR amplification products using canine control DNA and separated on an Applied Biosystems® 377 DNA Sequencer

4 Interpretation of results

As the samples migrate past the fluorescence detector in the instrument, the GeneScan™ software collects the signal and assigns a base pair size for each sample. You then export GeneScan™ data directly to Genotyper™ or GeneMapper® software for automated genotyping.

By comparing test data to control data presented in this chapter, you can determine whether the animal tested is homozygous or heterozygous at each locus. Animals can then be compared to each other to include or exclude possible parents.

Successful amplification using StockMarks® for Horses or Cattle

Number of allele peaks indicates genotype

Successful amplification yields allele peaks with the associated PCR stutter bands within a maximum range of 8 base pairs from the allele peak. The number of allele peaks depends on whether the individual tested is a heterozygote or homozygote.

For the StockMarks® for Horses and StockMarks® for Cattle, all loci are dinucleotide repeats. Dinucleotide repeats give specific stutter patterns that are illustrated in Figures 10 through 14 on pages 39 through 41.

Homozygous individual: 2-bp stutter

The GeneScan™ software electropherogram of a dinucleotide repeat marker from a homozygous individual (genotype: 118.6 bp, 118.6 bp) is shown in Figure 10.

The peaks at 116.6 bp, 114.6 bp and 112.6 bp are the typical 2-bp stutter pattern seen with dinucleotide repeats. They represent the –2 bp, –4 bp, and –6 bp stutters from the true 118.6-bp allele.

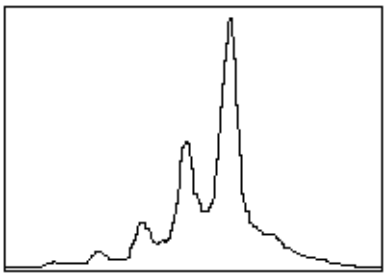


Figure 10 Typical pattern for dinucleotide repeat homozygote

Heterozygous individual: 2-bp stutter

The GeneScan™ software electropherogram of a dinucleotide repeat marker from a heterozygous individual (allele 1: 90 bp, allele 2: 98 bp) is shown in Figure 11. Allele sizes differ by 8 bp.

The 2-bp stutter peak to the left of each allele peak is always of lower intensity than the allele peak itself. The larger 98-bp allele peak is of lower intensity than the smaller 90-bp allele. In heterozygotes, the higher molecular weight allele often produces a fluorescent signal of lower intensity than the lower molecular weight peak, suggesting a less efficient amplification of the larger fragment.

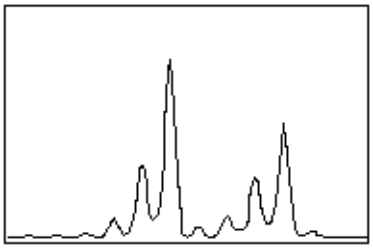


Figure 11 Typical pattern for dinucleotide repeat heterozygote.

Heterozygous individual: 4-bp stutter

The GeneScan™ software electropherogram from a dinucleotide repeat marker of a heterozygous individual (allele 1: 86 bp, allele 2: 90 bp) is shown in Figure 12. Allele sizes differ by 4 bp.

When the difference between the allele sizes is 4 bp or less, a shift occurs in the height ratio between the two allele peaks (compare with Figure 11). The fluorescent signal from the –4 bp stutter of the 90-bp allele is added to the signal from the 86-bp allele.

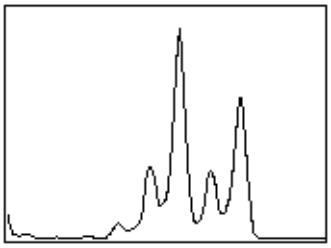


Figure 12 Typical pattern for dinucleotide repeat heterozygote.

Heterozygous individual: 2- and 4-bp stutter

The GeneScan™ software electropherogram from a dinucleotide repeat marker of a heterozygous individual (allele 1: 92 bp, allele 2: 94 bp) is shown in Figure 13. Allele sizes differ by 2 bp.

The fluorescent signal from the –2 bp stutter of the 94-bp allele is added to the signal of the 92-bp allele. The signal from the –4 bp stutter band of the 94-bp allele is added to the signal from the –2 bp stutter band of the 92-bp allele.

A dinucleotide repeat marker for a heterozygous individual shows this typical “triangle pattern” when the alleles differ by 2 bp.

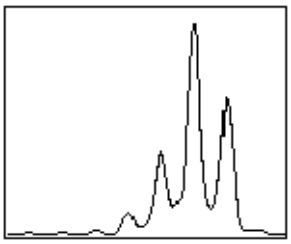


Figure 13 Typical pattern for dinucleotide repeat heterozygote.

Heterozygous individual: 1-bp stutter

A GeneScan™ software electropherogram for a dinucleotide repeat marker, where peaks are seen at 1-bp intervals, is shown below in Figure 14. AmpliTaq® DNA Polymerase tends to add a nontemplated A to the end of a PCR product during amplification. Thus, a ladder of peaks differing by 1 bp may be seen for PCR products when both the true allele and allele-plus-A products show 2-bp stutter bands. This phenomenon tends to be locus-specific.

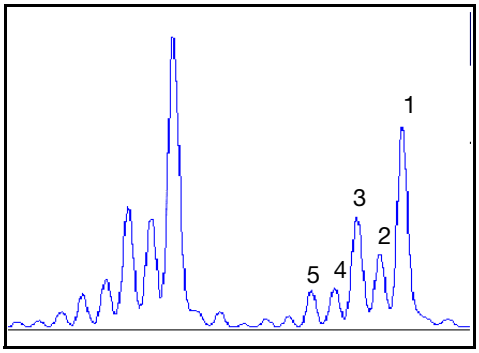


Figure 14 Typical pattern for dinucleotide repeat heterozygote showing 1-bp stutter

One allele in Figure 14 above is labeled to indicate the origin of the peaks seen. The pattern produced is a combination of both the 2-bp stutter peaks from the true allele, and the allele plus the nontemplated A. The resulting peaks differ by 1 bp (see table).

Peak	Origin
1	+A product of allele peak
2	True allele peak based on DNA sequence
3	–2 bp stutter of +A peak
4	–2 bp stutter to true allele peak
5	–4 bp stutter of +A peak

Successful amplification using StockMarks® for Dogs

Stutter products

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (n–4) than the corresponding main allele peak. This is referred to as the stutter peak or product. Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996).

Following are some general conclusions from these measurements and observations:

- For each locus, the percent stutter generally increases with allele length. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- For the alleles within a particular locus, the percent stutter is generally greater for the longer allele in a heterozygous sample (this is related to the first point above).
- Each allele within a locus displays percent stutter that is reproducible.
- The percent stutter does not change significantly with the quantity of input DNA, for on-scale data.

Addition of 3′ A nucleotide

AmpliTaq Gold® enzyme, like many other DNA polymerases, can catalyze the addition of a single nucleotide (predominately adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*, 1996). This non-template addition results in a PCR product that is one base pair longer than the actual target sequence, and the PCR product with the extra nucleotide is referred to as the "+A" form.

The efficiency of "A addition" is related to the particular sequence of the DNA at the 3′ end of the PCR product. Also, the AmpliTaq Gold® DNA Polymerase generally requires extra time to complete the A nucleotide addition at the 3′ end of the PCR products.

The kit includes 2 main design features that promote maximum A addition:

- The primer sequences have been optimized to encourage A addition.
- The final extension step is 60°C for 60 minutes.

The final extension step gives the AmpliTaq Gold® DNA Polymerase extra time to complete A addition to all double-stranded PCR product. STR systems that have not been optimized for maximum A addition may have "split peaks," where each allele is represented by 2 peaks that are 1 base pair apart.

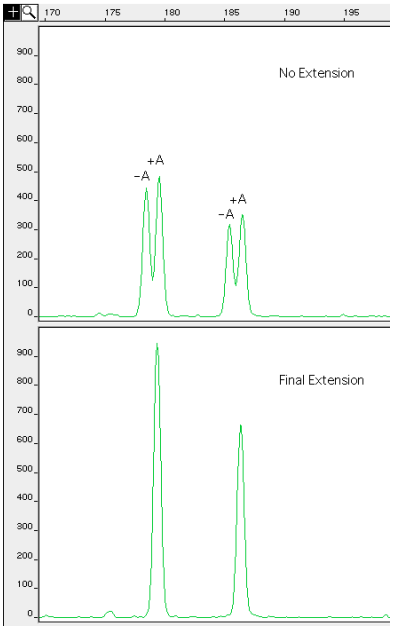


Figure 15 Split peaks resulting from incomplete A nucleotide addition due to omission of the 60-minute extension step

Lack of full A nucleotide addition may be observed in kit results when the amount of input DNA is greater than recommended protocols. The reason is that more time is needed for AmpliTaq Gold® DNA Polymerase to add the A nucleotide to all molecules as more PCR product is generated. Amplification of too much input DNA will also result in off-scale data.

Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram displays. Several causes for the appearance of extra peaks, including the stutter product (found at the n–4 position), incomplete 3′ A nucleotide addition (found at the n–1 position), artifacts, and mixed DNA samples.

A

Good PCR practices

Introduction

PCR techniques require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these techniques can lead to amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution, followed by a 70% ethanol rinse to remove residual bleach.

IMPORTANT! To avoid false positives due to cross-contamination:

- Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps come in strips of 8 or 12.

B Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: **www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**.
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**.

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Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe, A.L., Ghosh, S., and F.S. Collins (1996) Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: Implications for PCR-based genotyping and cloning. *BioTechniques* 21:700–709.

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol*. 155:335–350.

Saiki, R.K., Scharf, S., Faloona, F., *et al*. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

Walsh, P.S., Fildes, N.J., and Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res*. 24:2807–2812.

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

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