Thermo Scientific Bovine Genotypes Panel 2.2

F- 847S 100 reactions
F- 847L 500 reactions

Technical Manual

Product Description

Parentage testing and individual identification using short tandem repeat (STR) loci

Short Tandem Repeat (STR) loci, or 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.

Kit overview

The Thermo Scientific Bovine Genotypes Panel 2.2 encompasses the following six STR loci: SPS113, RM067, CSRM60, MGTG4B, CSSM66 and ILSTS006 (Table 1). These are among the list of loci recommended by the International Society for Animal Genetics (ISAG) and the Food and Agriculture Organization of the United Nations (FAO) for genetic studies of domestic animals.

The Bovine Genotypes™ Panel 2.2 allows co-amplification of the six microsatellites 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, such as ABI PRISM 310 Genetic Analyzer or ABI PRISM 3100 Genetic Analyzer (both Applied Biosystems).

The microsatellite alleles of the Bovine Genotypes Panel 2.2 can be separated and detected in the same single electrophoresis injection with the microsatellite alleles of the Bovine Genotypes Panel 1.2 (Thermo Scientific Cat #F-904).

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The Bovine Genotypes Panel 2.2 provides all the necessary reagents for amplification of the six microsatellite loci. In addition, the kit includes bovine control DNA for verification of acceptable PCR and electrophoresis conditions.

Kit performance characteristics

The Bovine Genotypes Panel 2.2 delivers optimal results when 1–2 nanograms of high-quality genomic DNA is applied in the PCR reaction volume of 20 µL.

The reagents and reaction protocols of the Bovine Genotypes Panel 2.2 have been optimized to deliver similar amplification yields (peak sizes) for alleles within and among loci, when an appropriate amount of high-quality DNA is applied.

The kit employs Thermo Scientific Phusion Hot Start DNA Polymerase. Allele callings obtained with this kit represent the true alleles of an individual, instead of 'plus-A' peaks or 'split peaks' typically interpreted when using a *Taq* DNA polymerase. This is due to the proofreading (3'→5' exonuclease) activity of the Phusion™ Hot Start DNA Polymerase. The results are not impaired by the tendency of DNA polymerases to add an extra nucleotide (most often adenine) to the end of the amplification products.

Table 1. Locus descriptions for the Bovine Genotypes Panel 2.2 microsatellites.

Locus name	Chromosome	Repeat motif	Size range (bp)	Dye color[1]
SPS113	10	di	279–307	Blue
RM067	4	di	83–101	Green
CSRM60 (D10S5)	10	di	79–115	Red
MGTG4B	4	di	129–153	Red
CSSM66 (D14S31)	14	di	171–209	Red
ILSTS006 (D7S8)	7	di	277–309	Red

[1] Dye colors are listed as they appear after electrophoresis with Filter Set G5 (Applied Biosystems).

Kit components and storage conditions

The Bovine Genotypes Panel 2.2 contains all reagents necessary to co-amplify the six microsatellites (see Table 1 for locus descriptions). The kit components are:

- F-841: Bovine Genotypes 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).
- F-849: Bovine Genotypes Panel 2.2 Primer Mix. A PCR primer mix in buffer, including forward and reverse primers for SPS113, RM067, CSRM60, MGTG4B, CSSM66 and ILSTS006 microsatellite loci. One primer from each primer pair is end-labeled with a fluorescent dye.
- F-843: Bovine Genotypes Control DNA001. Bovine genomic DNA in 0.5 ng/µL concentration for verification of acceptable PCR and electrophoresis conditions.

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 until the expiry date when stored and handled properly.

Table 2. Bovine Genotypes Panel 2.2 components and storage conditions for: F-847S (sufficient for 100 reactions) and F-847L (sufficient for 500 reactions).

Kit Component	Description	Storage conditions
Bovine Genotypes Master Mix (F-841)	1 tube (blue cap) 1.1 mL	-20 °C
	5 tubes (blue cap) 1.1 mL each	
Bovine Genotypes Panel 2.2 Primer Mix (F-849)	1 tube (red cap) 1.1 mL	-20 °C. Store protected from light at all times.
	5 tubes (red cap) 1.1 mL each	
Bovine Genotypes Control DNA001 (F-843)	1 tube (green cap) 30 µL	-20 °C
	1 tube (green cap) 150 µL	

Materials needed but not supplied

In addition to the Bovine Genotypes Panel 2.2 kit, the equipment and consumables listed below are required for cattle 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 Sample and DNA extraction.

PCR

- 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 Bovine Genotypes Panel 2.2 has been optimized for PCR using most commercially available thermal cyclers.

Electrophoresis

- Electrophoresis instrument. The Bovine Genotypes Panel 2.2 has been optimized for electrophoresis using the ABI PRISM 310 Genetic Analyzer, ABI PRISM 3100 Genetic Analyzer, ABI PRISM 3100-Avant Genetic Analyzer and 3130 Genetic Analyzer (all Applied Biosystems). Use of the Bovine Genotypes Panel 2.2 in other analyzers is likely to deliver similar results.

- GeneScan™ 500 LIZ® Size Standard (Applied Biosystems)
- DS-33 Dye Primer Matrix Standard Set (Applied Biosystems). The end-labeled primers of the Bovine Genotypes Panel 2.2 are compatible with Filter Set G5, requiring the use of the DS-33 Dye Primer Matrix Standard POP™ Performance Optimized Polymer (Applied Biosystems)
- Deionized formamide
- Genetic Analyzer tubes and septums (Applied Biosystems)
- Additional electrophoresis consumables are required. Please refer to the User Guide of your electrophoresis instrument for further details.

Samples and DNA extraction

The Bovine Genotypes Panel 2.2 has been optimized for use with cattle hair and blood samples. However, any tissue providing high-quality genomic DNA is applicable.

The Bovine Genotypes Panel 2.2 delivers optimal results when 1–2 nanograms of high-quality genomic DNA is applied in a PCR reaction volume of 20 µL. However, the kit delivers acceptable results with genomic DNA amounts ranging from ~ 0.5 to 10 ng. Following these recommendation guidelines is important: application of too little or too much template DNA can result in compromised amplification, 'overshoot' of microsatellites or in non-specific amplification products.

DNA yield, DNA purity and the amount of PCR inhibitors may vary among extracts from different DNA extraction protocols. When you first start to use the Bovine Genotypes Panel 2.2, we recommend preparing a dilution series of the extracted DNA in order to optimize the amount of template DNA needed for PCR.

The Bovine Genotypes Panel 2.2 delivers high-quality and uniform results, such as with Chelex® - proteinase K DNA extraction protocol (Walsh et al., 1991; Figure 1) or DNA IQ™ System (Promega Corporation).

General laboratory guidelines and precautions

The following general guidelines and precautions should be followed at all times when applying the protocols presented in this instruction manual:

- Use protective gloves and clothing throughout the protocols.
- Mix all solutions well before use.
- Follow the guidelines listed in Appendix I for reducing PCR carryover contamination risks.
- Prepare all reactions on ice.

PCR

The Bovine Genotypes Panel 2.2 utilizes Phusion Hot Start DNA Polymerase that is inactive at room temperature. Nevertheless, in order to maximize the specificity and uniformity of the PCR, and to minimize cross-contaminating aerosols, we recommend that PCR reactions are always set up on ice.

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

- Volume of Bovine Genotypes Master Mix (F-841) = **N** x 10 µL
- Volume of Bovine Genotypes Panel 2.2 Primer Mix (F-849) = **N** x 10 µL

N = Number of samples

Include the following controls:
- positive control (Bovine Genotypes Control DNA001)
- negative control (H_2O)

The formulas provide excess volume to compensate for 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 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 to each vessel. Allocate one vessel for a positive control and add 2 µL of Bovine Genotypes Control DNA001 to that vessel. Furthermore, allocate at least one vessel for a negative control and, instead of DNA, add 2 µL of H_2O to 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.

Table 3. Thermal cycling programs of the Bovine Genotypes Panel 2.2 for different PCR instruments.

PCR instrument	Cycling profile	Noteworthy instrument settings
• Piko® Thermal Cycler • DNA Engine® (PTC-200™) • DNA Engine Tetrad® • DNA Engine Tetrad® 2 • PTC-100®	1. 98°C for 60 s 2. 98°C for 20 s 3. 60°C for 75 s 4. 72°C for 30 s Repeat the steps 2–4 for additional 29 cycles 5. 72°C for 5 min	Control method: block
• GeneAmp® PCR System 2400 • GeneAmp® PCR System 7900 (96-well) • GeneAmp® PCR System 9600 • GeneAmp® PCR System 9700 (384-well)	1. 98°C for 60 s 2. 30 cycles of 98°C for 20 s 60°C for 75 s 72°C for 30 s 3. 72°C for 5 min	None

Electrophoresis

The Bovine Genotypes Panel 2.2 has been optimized for electrophoresis with the ABI PRISM 310 Genetic Analyzer, ABI PRISM 3100 Genetic Analyzer, ABI PRISM 3100-Avant Genetic Analyzer and 3130 Genetic Analyzer (all Applied Biosystems). In addition to the instructions outlined below, please refer to the instrument User Guides for electrophoresis details.

The Bovine Genotypes Panel 2.2 is compatible with Filter Set G5, requiring matrix files generated with the DS-33 Dye Primer Matrix Standard Set. The matrix file values vary among 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 template DNA used for the PCR reactions. When you first start to use the Bovine Genotypes Panel 2.2, we 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.

The microsatellite alleles of the Bovine Genotypes Panel 2.2 can be separated and detected in the same single electrophoresis injection with the microsatellite alleles of the Bovine Genotypes Panel 1.2, as instructed below.

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

   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 remove any liquid remaining in the cap.
3. Label 0.5 mL Genetic Analyzer tubes and transfer 10 µL of the mix into each tube.

   a) For combined electrophoresis of the Bovine Genotypes Panel 2.2 and Bovine Genotypes Panel 1.2 microsatellites, add 1.5 µL and 1.0 µL of PCR products from the PCR runs of the Bovine Genotypes Panel 2.2 and Bovine Genotypes Panel 1.2, respectively, to each tube (or PCR products diluted into H_2O; see Electrophoresis). Mix the solutions by pipetting. Seal the tubes with septums.

   b) For electrophoresis of the Bovine Genotypes Panel 2.2 microsatellites, add 1.5 µL of PCR product (or PCR product diluted into H_2O; see Electrophoresis) to each tube. Mix the solutions by pipetting. Seal the tubes with septums.
4. Heat the tubes at 95 °C for 3 minutes to denature the samples and immediately chill the plate on ice (for example crushed ice or ice-water bath) for at least 3 minutes.
5. Place the tubes in an auto-sampler tray, place the tray in an ABI PRISM 310 Genetic Analyzer and close the instrument doors.
6. 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 values for other injection list parameters:
   - Inj. Secs: 8 for combined electrophoresis of the Bovine Genotypes Panel 2.2 and Bovine Genotypes Panel 1.2 microsatellites
   - Inj. Secs: 5 for electrophoresis of the Bovine Genotypes Panel 2.2 microsatellites
   - Inj. kV: 15.0
   - Run kV: 15.0
   - Run °C: 60
   - Run Time: 28
7. Begin electrophoresis according to ABI PRISM User Guide instructions.

Electrophoresis with ABI PRISM 3100 Genetic Analyzer, ABI PRISM 3100-Avant Genetic Analyzer or 3130 Genetic Analyzer

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

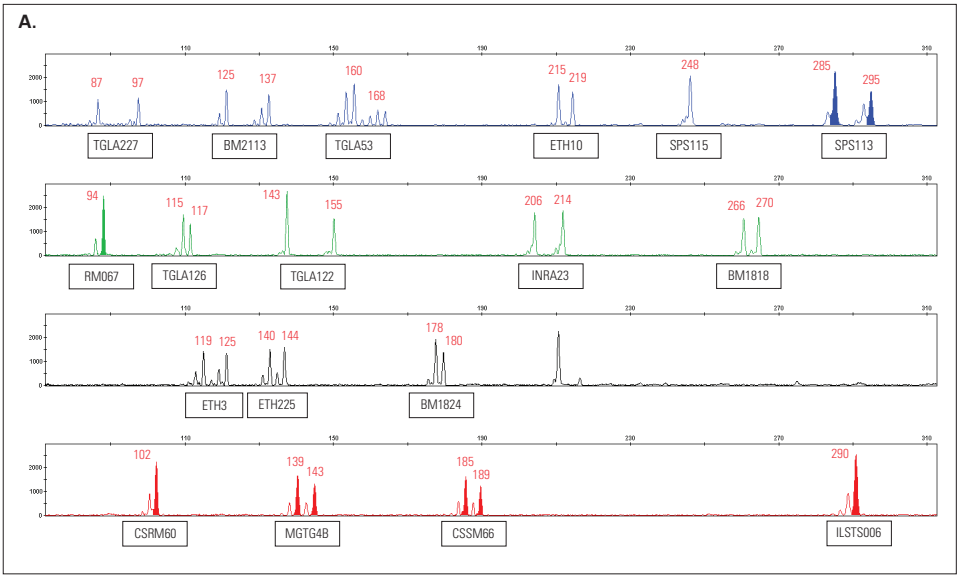
   • Number of samples × 11 µL of deionized formamide.
   • Number of samples × 0.3 µL of GeneScan 500 LIZ Size Standard.

   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 remove any liquid remaining in the cap.
3. Transfer 10 µL of the mix into each well of a 96-well plate compatible with
   the instrument.

   a) For combined electrophoresis of the Bovine Genotypes Panel 2.2 and Bovine Genotypes Panel 1.2 microsatellites, add 1.5 µL and 1.0 µL of PCR products from the PCR runs of the Bovine Genotypes Panel 2.2 and Bovine Genotypes Panel 1.2, respectively, to each well (or PCR products diluted into H_2O; see Chapter 7). Mix the solutions by pipetting. Seal the plate.

   b) For electrophoresis of the Bovine Genotypes Panel 2.2, add 1.5 µL of PCR product (or PCR product diluted into H_2O; see Electrophoresis) to each well. Mix the solutions by pipetting. Seal the plate.
4. Heat the plate at 95 °C for 3 minutes to denature the samples and immediately chill the plate on ice (for example crushed ice or ice-water bath) for at least 3 minutes.
5. Place the plate in an auto-sampler tray and close the instrument doors.
6. Select the GeneScan 36_Pop4 module. Use the following values for injection in combination with 36 cm capillaries.

   • Inj. Secs: 22
   • Inj. kV: 1.0
   • Run kV: 15.0
   • Run °C: 60
   • Run Time: 1200 s
7. Begin electrophoresis according to ABI PRISM User Guide instructions.



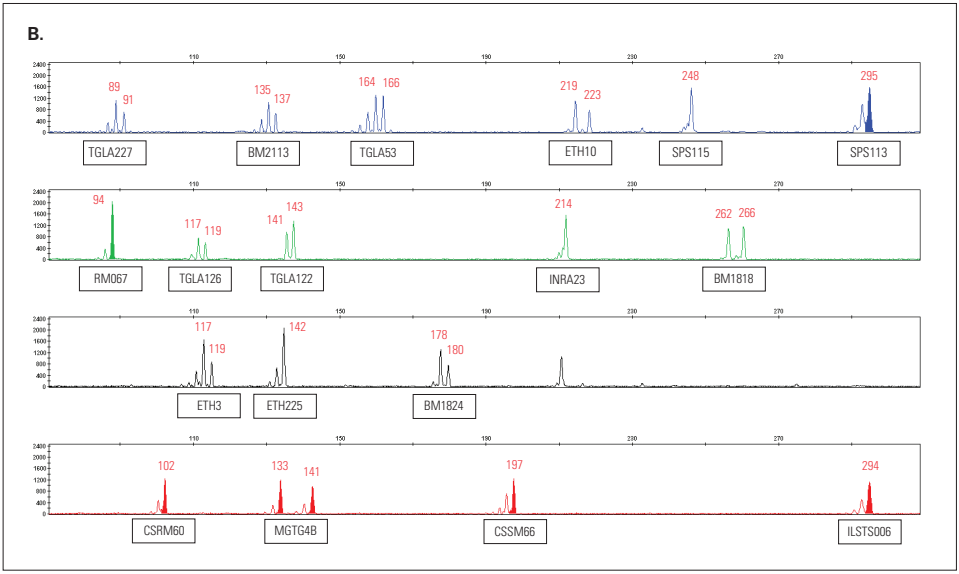


Figure 1. Results obtained with the Bovine Genotypes Panel 2.2 when analyzed in the same electrophoresis injection with the Bovine Genotypes Panel 1.2 microsatellites using: **A.** 1.0 ng of Bovine Genotypes Control DNA001, and **B.** template DNA extracted from a hair sample with a Chelex — proteinase K protocol (Walsh et al., 1991). The allele nomenclature is based on ISAG guidelines.

Analysis and interpretation of the results

Representative results

The reagents and protocols of the Bovine Genotypes Panel 2.2 have been optimized to deliver similar peak sizes within and among loci, when applying an appropriate amount of high-quality genomic DNA. PCR and electrophoresis conditions are acceptable when the fluorescent intensities of the Bovine 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.

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

Figure 1 shows Bovine Genotypes Panel 2.2 results when analyzed in the same electrophoresis injection with the Bovine Genotypes Panel 1.2 microsatellites. Figure 1A and B on page 8 shows results using Bovine Genotypes Control DNA001 and using Chelex - proteinase K extracted DNA from hair samples (Walsh et al., 1991), 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.

ISAG nomenclature

For the locus RM067, the Bovine Genotypes Control DNA001 genotype follows the nomenclature recommended by ISAG (Table 1). For the remaining five loci, we recommend the allele calls as listed in Table 4.

Table 4. Bovine Genotypes Control DNA001 genotype following ISAG allele nomenclature (RM067) or allele calls recommended by Thermo Scientific (SPS113, RM067, CSRM60, MGTG4B, CSSM66 and ILSTS006).

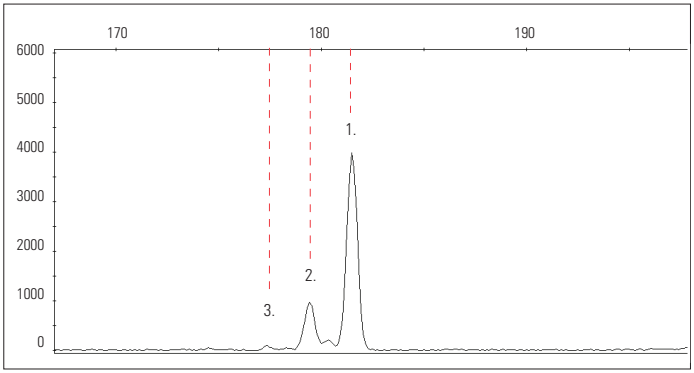
Locus	Allele 1	Allele 2
SPS113	285	295
RM067	94	94
CSRM60 (D10S5)	102	102
MGTG4B	139	143
CSSM66 (D14S31)	185	189
ILSTS006 (D7S8)	290	290

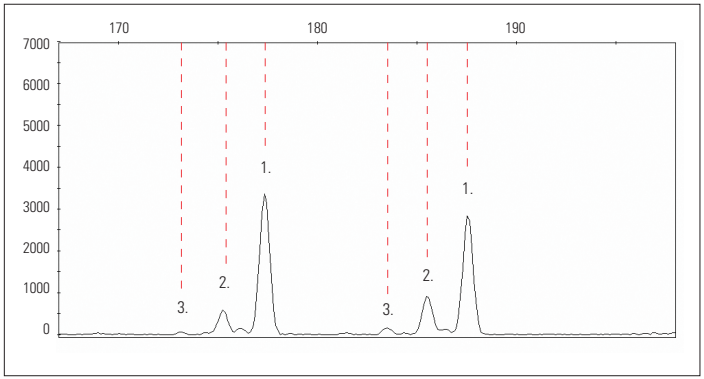
Allele calling and stutter peaks

All six markers of the Bovine Genotypes Panel 2.2 are dinucleotide microsatellite loci (their repeat motifs are two base pairs in length). PCR of dinucleotide STR loci typically results in one or more stutter peaks, arguably due to a phenomenon

known as slipped strand mispairing (Goldstein & Schlötterer 1999). Using the Bovine Genotypes Panel 2.2, the stutters appear two base pairs apart.

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. In addition, the stutter peaks are normally much smaller than the true allele peaks. Further, within some loci, the longer alleles may display more significant stuttering than the shorter alleles.





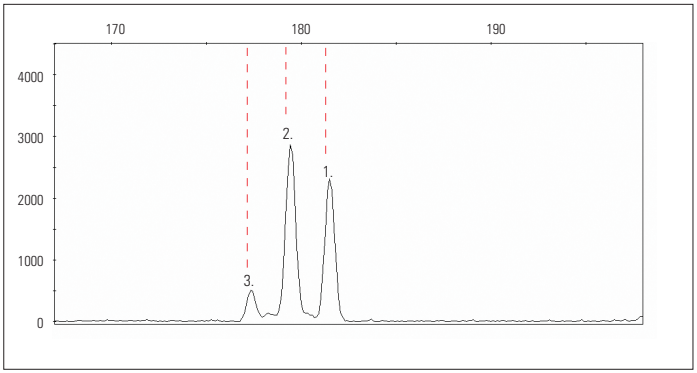


Figure 2.

A. A typical 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 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 peak profile for a heterozygous individual with the two alleles 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 (3'→5' exonuclease) activity of the Phusion Hot Start DNA Polymerase, the Bovine Genotypes Panel 2.2 results are not hampered by plus-A peaks (A-activity peaks). The allele callings using the kit represent the true alleles of an individual, instead of the plus-A peaks typically interpreted when using such as a *Taq* DNA polymerase.

References

1. D. B. Goldstein, C. Schlötterer, Microsatellites: Evolution and Applications (Oxford University Press, Oxford, 1999).
2. P. S. Walsh, D. A. Metzger, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*. 10, 506-513 (1991).

Troubleshooting

Problem	Possible explanation	Recommended action
Faint or no signals from the test sample for all loci, but normal signals for all loci from the Bovine 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 to PCR in the quantity recommended in this Technical Manual.
	PCR inhibitor concentration of the test sample is too high.	Dilute the sample DNA extract into H_2O (for example 1:5, 1:10 and 1:20 dilutions) and repeat the protocol.
Faint or no signals from both the test sample and the Bovine Genotypes Control DNA001 for all loci.	There has been a error in the PCR or electrophoresis setup.	Repeat the protocol checking the setup.
	The cycling profile applied is not optimal for the Bovine Genotypes Panel 2.2.	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 Bovine Genotypes Control DNA001.	The sample DNA quantity added to PCR is too high.	Measure the DNA concentration and add sample DNA to PCR in the quantity recommended in this Technical Manual. Alternatively, dilute the sample DNA extract into H_2O (for example 1:5, 1:10 and 1:20 dilutions) and repeat the protocol.
Overshoot for all or some loci and occurrence of non-specific amplification products from both the test sample and the Bovine Genotypes Control DNA001.	There has been a error in the PCR or electrophoresis setup.	Repeat the protocol checking the setup.
	The cycling profile applied is not optimal for the Bovine Genotypes Panel 2.2.	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) set up of the 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 and always before leaving an area.
- Use aerosol-resistant pipette tips.
- Use new and/or sterilized glassware and plasticware.

thermoscientific.com/onebio

Europe
Customer Service
cs.molbio.eu@thermofisher.com
Technical Support
ts.molbio.eu@thermofisher.com
Tel 00800 222 00 888
Fax 00800 222 00 889

United States
Customer Service
cs.molbio@thermofisher.com
Technical Support
ts.molbio@thermofisher.com
Tel 877 661 8841
Fax 800 292 6088

Canada
Customer Service
cs.molbio@thermofisher.com
Technical Support
ts.molbio@thermofisher.com
Tel 800 340 9026
Fax 800 472 8322

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