Qexp-FS Meat 5-species Kit

Direct multiplex PCR testing for the detection of 5-species meat

Operation Manual

Ver. EN-B

Research Use Only

REF C900200-24 & C900200-48

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Intended Use

DNA barcoding is a method for identifying species by analyzing specific gene sequences. Usually, the extraction or purification of the nucleotide of the samples to be analyzed is performed first. Second, the amplification of the target sequence is performed by PCR and then performed DNA sequencing. The results of sequencing will be aligned with the gene database to identify what species it is. The candidate gene for DNA barcoding is different for different species, mainly choosing the conserved sequence as a candidate. The commonly used gene for plants is plastid or nuclear ribosomal DNA; mitochondrial DNA for animals, e.g., mitochondrial cytochrome oxidase 1 gene, CO1.[1]

People have recently paid more attention to food safety, and the food source or problem of food fraud under the carpet comes to the fore. According to the reports from the European Commission, food has an issue more easily on food fraud are olive oil, milk, honey, wine, fish, etc. People with bad intentions will mix them with cheaper ingredients or imitate them with cheaper ingredients with a similar appearance for profiteering.[2]

The *Qexp*-FS Meat 5-species Kit is designed for source tracking of meat samples with the concept of DNA barcoding but without DNA extraction. Laboratorians can amplify the mitochondrial DNA of the 5 species (pig, chicken, cattle, goat, sheep) through direct PCR. Then analyzing the target sequences by fragment analyzer like capillary gel electrophoresis is performed to identify what species are in the meat samples. The *Qexp*-FS Meat 5-species Kit is intended for molecular biology application testing. This product is not intended for the diagnosis, prevention, or treatment of a disease. Results are for identification of the 5-species DNA in food or related products. Testing with the *Qexp*-FS Meat 5-species Kit is intended for use by trained laboratory personnel in the techniques of molecular biology.

Product Information

***Qexp*-FS Meat 5-species Kit**

Product Description

The *Qexp*-FS Meat 5-species Kit (cat# C900200-24, C900200-48) includes the assay and positive controls for direct multiplex PCR testing to detect the 5-species gene in meat products. The *Qexp*- FS Meat 5-species Kit includes the following components:

- **Direct Lysis assay**
  Meat lysis assay contains DNA Lysis Buffer, DNA binding Membrane, and Wash Buffer.
- **Direct Meat Kit assay**
  Direct multiplex PCR assay – multiplexed assays contain five primer sets specific to the goat, chicken, cattle, sheep, and pig genes respectively.
- **Positive Control-Meat**
  The positive control includes the plasmids containing 5-species genes as mentioned.

Contents and Storage

Table 1 Direct Meat Kit, 24 and 48 reactions

Label	Component	Amount (μL)		Storage
		24 rxns	48 rxns	
Direct Lysis assay				
L1	DNA Lysis Buffer	12 mL	24 mL	-20℃
W1	Wash Buffer	4.8 mL	9.6 mL	-20℃
	DNA binding Membrane	24 pcs	48 pcs	RT
Meat multiplex PCR assay				
1	Primer mix-Meat	36	72	-20℃
A1	Direct PCR PreMix	300	600	-20℃
PC	Positive Control-Meat	10	20	-20℃
W	Nuclease-Free Water	300	600	-20℃

- All components should be stored at -20℃ immediately after unpacking, except the DNA binding Membrane (stored at room temperature, RT).
- It is recommended to aliquot the reagents to avoid the risk of efficiency decrease by being thawed and frozen more than 2 times.

Required materials not supplied in kit

Table 2 Sample collection tool (Not Provided)

Item	Source
Collection tool	
Thin tip tweezers	any supplier*

Table 3 Required Materials for Direct Meat 5-Species Kit (Not Provided)

Item	Source
Consumables	
Disposable powder-free gloves and lab coats	any supplier*
Disposable pipette tips with the aerosol barrier	any supplier*
10% bleach or other appropriate cleaning solution that degrades nucleic acids	any supplier*
75% ethanol for bench cleaning	any supplier*
PCR tubes, plates, or strip tubes for the thermocycler being used	any supplier*
1.5 mL microcentrifuge tubes, nuclease-free	any supplier*
Equipment	
Several micropipettes capable of pipetting volumes from 2µL to 1000µL	any supplier*
Vortex and centrifuge	any supplier*
PCR workstation, for master mix preparing and setup	any supplier*
Qamp$_{mini}$ Thermal cycler	BiOptic Cat. C310200
Qsep$_{400}$™ Bio-Fragment Analyzers *Qsep*$_{100}$™ Bio-Fragment Analyzer *Qsep*$_{1}$™ Bio-Fragment Analyzer *Qsep*$_{1}$-Lite™ Bio-Fragment Analyzer	BiOptic Cat. C400100 BiOptic Cat. C100100 BiOptic Cat. C100001 BiOptic Cat. C100001-L
Qairbox	BiOptic Cat. C104301-00
Qsep series cartridge kits-S1 high resolution cartridge	BiOptic

*The required materials not provided in this kit can be ordered from another major laboratory supplier.

Assay limitations

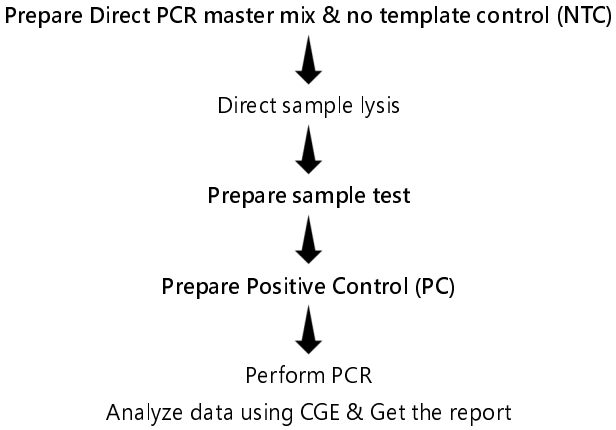
- This kit is performed for testing whether the food, feeds or related products contain the 5-species meat. There may be no signal if tested with overprocessing products or overcooked foods, since the DNA template may be destroyed.
- The storage of the samples must follow the appropriate procedures and conditions. Inappropriate uses may cause a decrease in the efficiency and sensitivity of the assay.
- Failed results may be caused by:
  - Improper sample collection or storage
  - Degradation of the DNA templates in the procedure
  - The presence of the PCR inhibitors
- Positive no template control (NTC) results may be caused by:
  - Cross-contamination during sample collection or preparation
  - DNA contamination between samples
  - Cross-contamination by PCR amplicon
  - Contamination by improper preparation of positive control

Warning and precautions

To avoid contamination results, the Direct Meat Kit workflow should use different areas or rooms. A dedicated PCR workstation for the preparation of the direct PCR assay would be ideal, and the analysis of the PCR product should be carried out in a separate area. The tube with positive control should be handled with great care to avoid contamination problems.

- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Always use pipette tips with aerosol barriers. Tips should be sterile and DNases- and RNases-free.
- Reagents must be stored and handled as specified in the content and storage section of the kits (please find them on Table 1).
- To prevent contamination results, be sure to use different pipettes for the preparation of direct PCR reagents and master mixes, for the preparation of Meat Lysates, and for pipetting the positive control.
- Modifications of assay reagents or assay protocols are not permitted.
- Do not use the kit after the expiration date.
- Dispose of waste in compliance with local, state, and federal regulations.

Workflow



※ IMPORTANT

- To avoid contamination results, always use different areas and pipettes for preparing reagents, adding positive control, adding sample lysate, and analyzing PCR amplicon.
- Always use tips with the aerosol barrier to avoid contamination.
- For preparing the reactions every time, include the following controls:
  One positive control
  One no template control (NTC)

The Direct PCR PreMix can be stored at 4℃ for a week. It is recommended to prepare an appropriate amount of the above reagents, then do the Direct Meat test immediately. The Direct PCR master mix could be prepared fresh at room temperature before use.

The DNA from samples could be released by using the required DNA Lysis Buffer, then amplified by the Direct PCR assay. In the process, the primers anneal to the specific sequences of 5-species genes.

The PCR product is analyzed by the *Qsep* series Bio-Fragment Analyzers, then the report will be provided automatically by the software *Q-Analyzer* (not required in the kit).

Preparation of Direct PCR Reaction

Prepare Direct PCR master mix

1. Thaw the kit at room temperature.
2. Gently vortex the Primer mix-Meat, Direct PCR PreMix, and Positive Control-Meat.
3. Centrifuge briefly to collect the liquid at the bottom of the tube.
4. Prepare the Direct PCR master mix in the PCR tube or 1.5 mL microcentrifuge tube:

Table 4 Preparation of Direct PCR master mix

Component	Volume per sample	Volume for n samples plus NTC	Volume for 46 samples plus NTC	Positive control*
Primer mix-Meat	1.5	1.5 x (n+1)	70.5	1.5
Direct PCR PreMix	12.5	12.5 x (n+1)	587.5	12.5
Nuclease-Free Water	11	11 x (n+1)	517	9
TOTAL(µL)	25	25 x (n +1)	1175	23

*Positive control is prepared individually in a PCR tube.

5. Mix by pipetting or gently inverting the Direct PCR master mix, then centrifuge briefly to collect the liquid at the bottom of the tube.
6. Transfer every 25 µL Direct PCR master mix to a PCR tube for samples and NTC.

Guidelines for Meat Sample Processing

IMPORTANT

- Before sample processing, clean the thin-tip tweezers with diluted bleach then rinse with clean water several times to remove the bleach.
- Ensure that the thin tip tweezers are cleaned with bleach before usage If many samples need to be processed and tested.
- Ensure that there is no cross-contamination during sample processing.

Meat sample processing and lysate preparation

1. Prepare a clean 1.5 mL microcentrifuge tube and aliquot 500 µL L1 DNA Lysis Buffer in it for a sample.
2. Prepare two clean 1.5 mL microcentrifuge tubes and aliquot 100 µL W1 Wash Buffer in every tube, then label as W1-A, W1-B.
3. Mash the appropriate amount of meat sample with a thin-tip tweezer.
4. Take about $3mm^3$ mashed meat sample with the thin tip tweezer into 500 µL L1.
5. Clean the thin tip tweezer with diluted bleach then rinse with clean water and dry it with clean tissue paper.
6. Take a DNA Binding Membrane carefully with the thin tip tweezer into L1 & mashed meat mixture.
7. Mix well by pulse-vortexing or inverting then incubate at room temperature for at least 5 minutes.

8. After incubation, take out the membrane with a thin tip tweezer then wash it in 100 µL W1-A for about 10-15 seconds.
9. Take out the membrane with a thin tip tweezer then wash it in 100 µL W1-B for about 10-15 seconds.
10. Transfer the membrane with the thin tip tweezer into the prepared 25 µL Direct PCR master mix. (as the following section)

Prepare Direct PCR reaction

Table 5 The preparation list of all RT-PCR reaction

Component	Volume per reaction		
	NTC	Sample reaction	Positive control
Direct PCR master mix	25	25	23
DNA binding Membrane	-	+	-
Positive Control-Meat	-	-	2
TOTAL	25	25	25

※ Do not use the same pipette to prepare Direct PCR master mix and add positive controls. Do not add positive control in the same area as reagent preparation. Avoid producing bobbles in the PCR tube.

※ Always prepare the NTC first, and the positive control last to avoid cross-contamination.

1. For NTC, do not need to add anything to Direct PCR master mix.
2. For sample reaction, transfer the membrane to the prepared Direct PCR master mix.
3. For positive control, transfer 2µL Positive Control-Meat in the prepared Direct PCR master mix.
4. Mix by inverting briefly or vortex gently after addition, then centrifuge briefly to collect the liquid at the bottom.

Perform Direct PCR using the Thermal Cycler

Before using the thermal cyclers, please read the operation manual that the manufacturer provided and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure safe operation.

Setup and Run

1. Confirm the thermal protocol as below:

Table 6 The PCR program of the Direct Meat assay

Step	Number of cycles	Temperature (℃)	Time
Activation	1	95	10 minutes
Denaturation	40	95	30 seconds
Anneal		55	30 seconds
Extension		72	30 seconds
Extension	1	72	1 minute
	1	4	hold

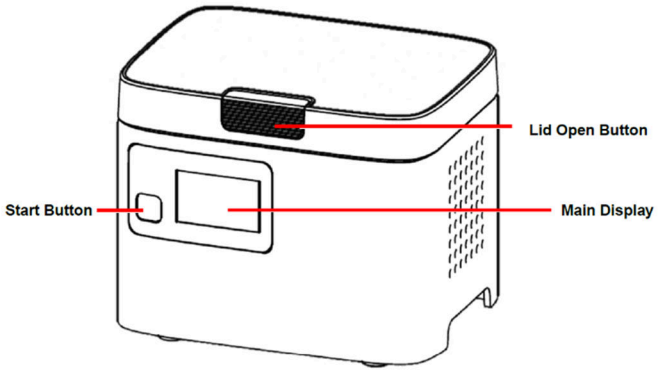
2. After the PCR is complete, transfer the PCR tube to the CGE system for analysis.

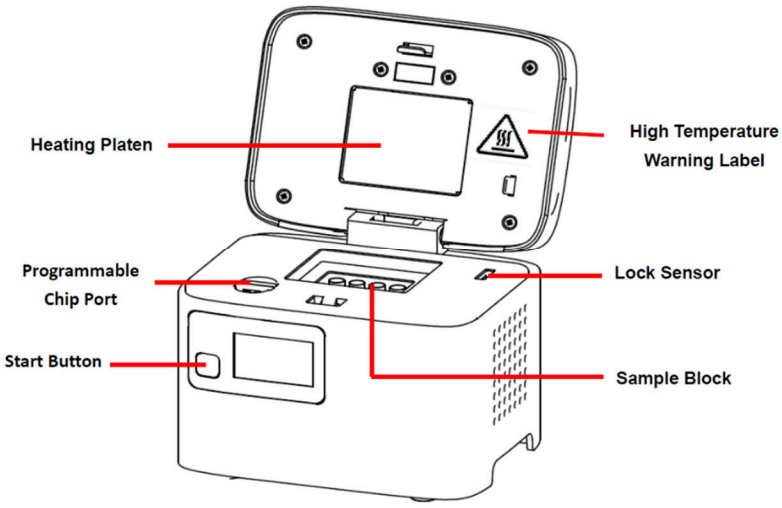
Perform PCR using *Qamp*$_{mini}$™ Thermal Cycler

General Description

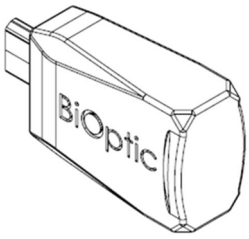
Before using the *Qamp*$_{mini}$™, please read this operation manual carefully and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure the safe operation of the *Qamp*$_{mini}$™.

Qamp$_{mini}$™ is a portable PCR thermocycler. It contains a centrally positioned Peltier heating & cooling module for 1-8 samples. This design leads to accuracy in analysis and cost efficiency without sacrificing performance and quality. With the compact size and one start button to operate design, *Qamp*$_{mini}$™ is the ideal instrument for laboratories or classrooms and in the fields of epidemiology, veterinary, food testing, pathogen detection, ecology, archaeology research, and others.





Programmable Chip is for saving PCR programs according to applications. The program details can be edited by the $Qamp_{mini}$™ writer and will be recognized automatically when the Programmable Chip is inserted into the port.



For more detailed information on the instruments, please find them on our website.

Setup and Run

1. Confirm or edit the thermal protocol following Table 6 by the $Qamp_{mini}$™ writer.
2. Insert Programmable Chip into the port, then turn on the power switch.
3. Open the lid and load the reaction vessel. (only 0.2 mL tubes are available)
4. Close the lid and wait until the lid is heated to 60℃, the machine will make two beeps.
5. Press the start button to proceed with the program.
6. After the PCR is complete, press the start button for 3 seconds to stop the program. Then transfer the PCR tube to the CGE system for further analysis.

Analysis and Results

For better sensitivity and resolution, please use capillary gel electrophoresis (CGE) systems, such as BiOptic *Qsep* series Bio-Fragment Analyzers, to analyze the Direct PCR amplicon. It is available to perform with any brand's CGE system. Before using the capillary gel electrophoresis system, please read the operation manual which the manufacturer provided and pay attention to the safety information.

Instrument and Software

Table 7 Instrument and software compatibility

Instrument	*Q-Analyzer*™
$Qsep_{400}$™ Bio-Fragment Analyzer	Version 4.0.1 or later
$Qsep_{100}$™ Bio-Fragment Analyzer	
$Qsep_{1}$™ Bio-Fragment Analyzer	
$Qsep_{1}$-Lite™ Bio-Fragment Analyzer	

Table 8 Software Requirements

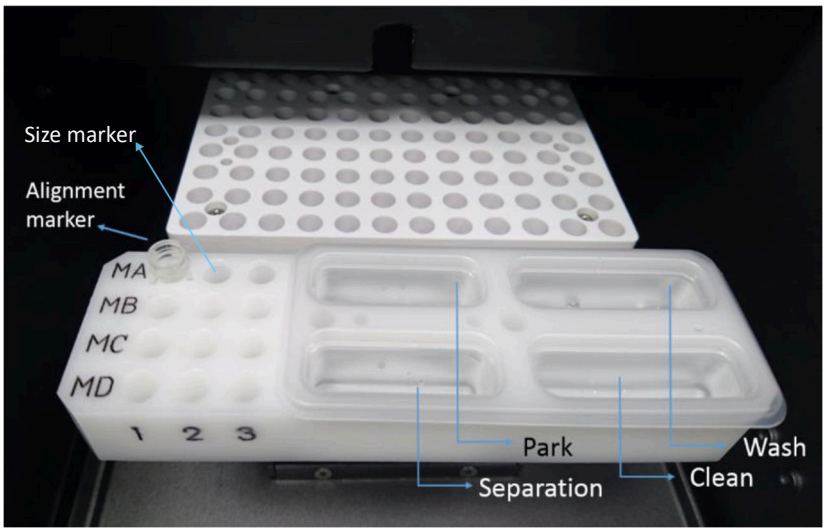
	Minimum	Recommended
OS	Windows 10 32 bit	Windows 10 64 bit
CPU	Intel Core i5	Intel Core i7
RAM	8G RAM	16G RAM
HDD	500 GB	1 TB
PORT	RJ45 x 1 USB 3.0 x 1	RJ45 x 2 USB 3.0 x 2
Screen Resolution	1280 x 800	1920 x 1080

Perform PCR using $Qsep_{100}$™ Bio-Fragment Analyzer

Setup and Run

1. "**Connect**" $Qsep_{100}$.

   "**Change Buffer**" to place Buffer Tray (filled up with water and Separation Buffer in the right position), Alignment Marker 20 bp & 1000 bp (C109100) at MA1, Size Marker (C109200) at MA2.

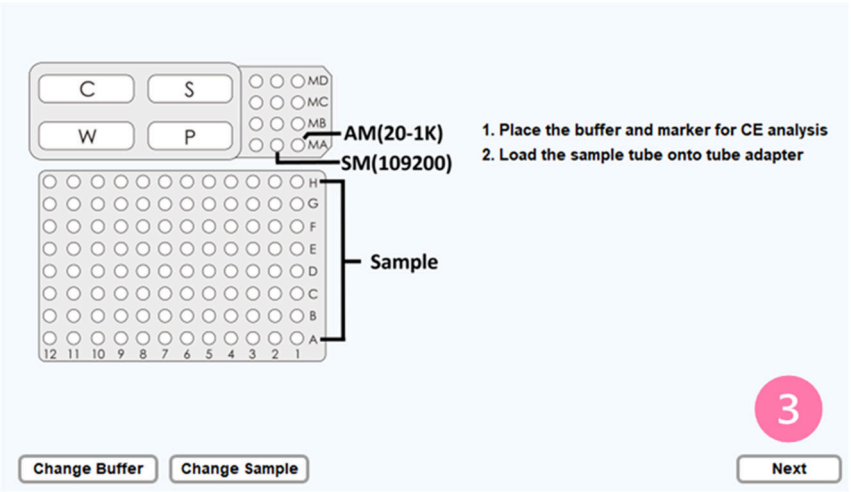


   "**Change Sample**" to place the samples. Remember to remove the cap of PCR tube. (For more information, please refer to *Qsep* Series Operation Manual)

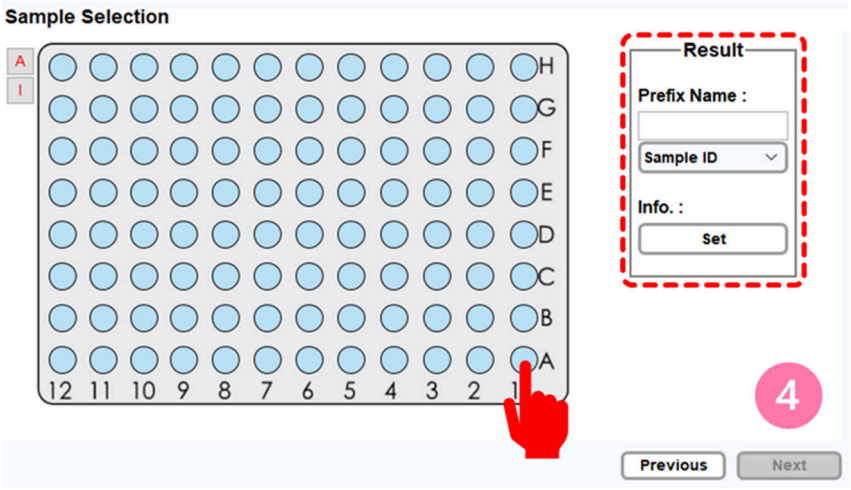
2. "**Latch**" the S1 cartridge. (For more information, please refer to the F0060-S1_ High Resolution Cartridge User Guide)

3. Switch to "**Assay**" and select the icon in the following order: "**Food**" → "**Meat Species**" → "**Cattle, Chicken, Sheep, Goat, Pig**".

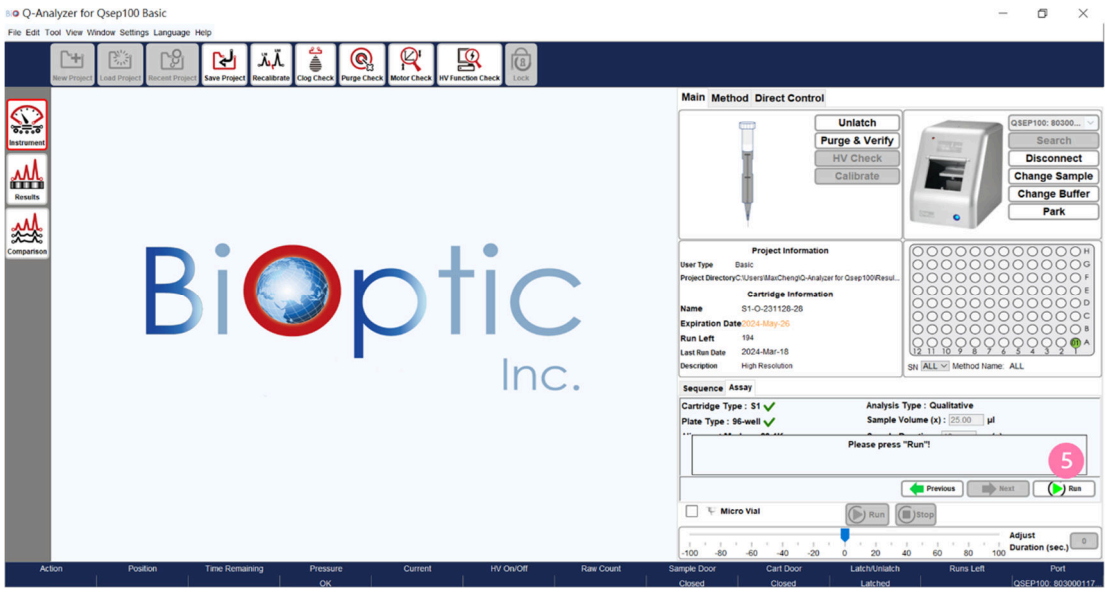
4. Click "**Next**" to select the sample position.



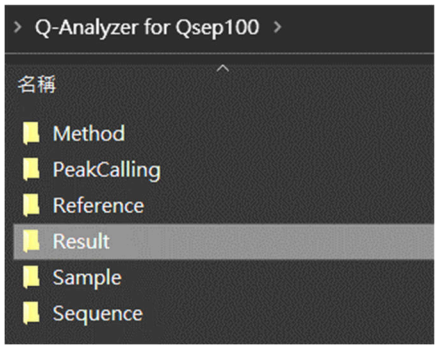
5. Select the sample position and click "**Next**".
6. (Optional) Type the result name in the field of "**Prefix Name**" and click "**set**" to type the desired information of the sample.



7. Click "**Run**" to start the analysis.



4. The PDF report can be found in the "**Result**" folder with the date of the test.



The results of quality control (NTC and Positive Control)

- Ensure that there is no signal of the target sequences revealed in the NTC.
  - ➢ If the NTC has any signal, the reagents must be contaminated in the procedure.
- Ensure that all signals of the target sequences are shown in the positive control.
  - ➢ If the positive control did not show any signal, it may be caused by the degradation of the reagents. Please contact the distributor in your area.

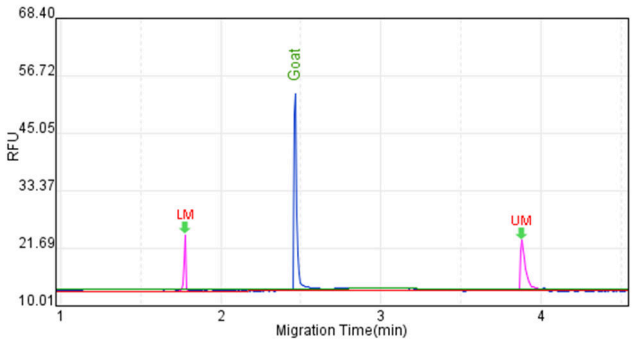
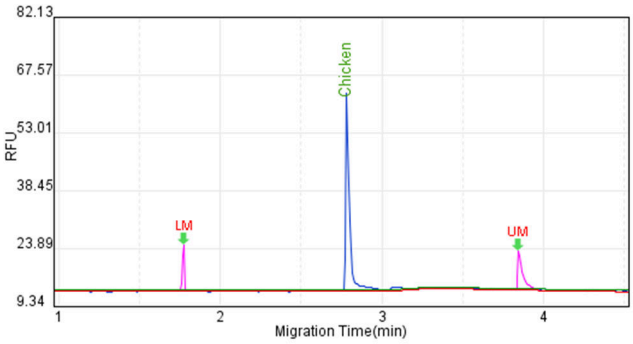
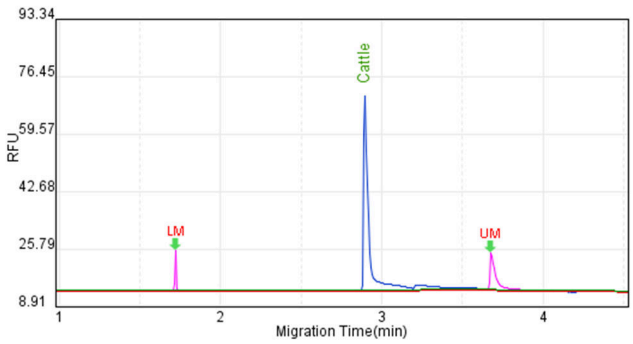
The result of Direct Meat assay

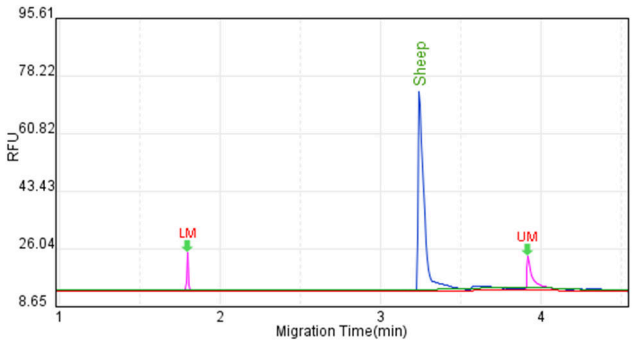
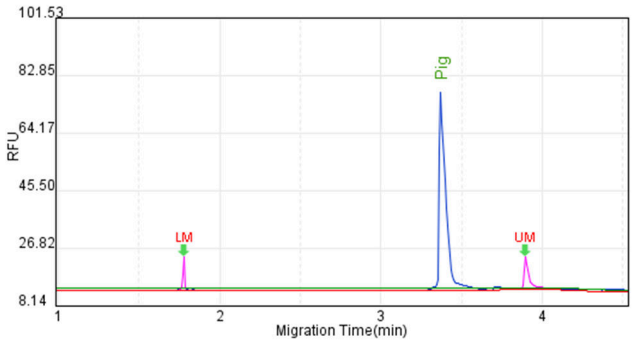
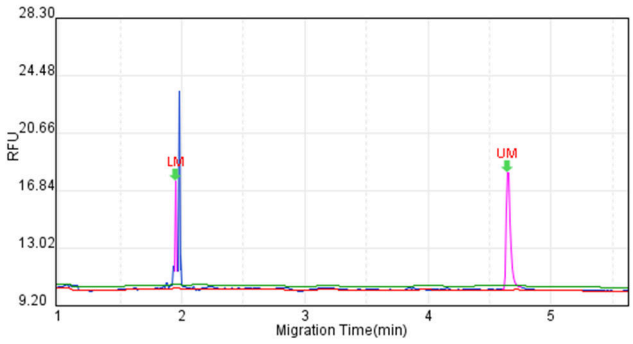
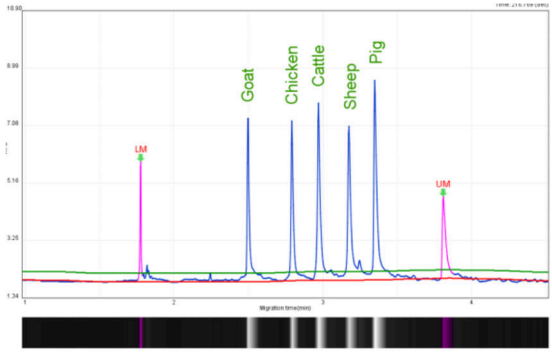
The data shown below were run by BiOpitc's Capillary Gel Electrophoresis system, $Qsep_{100}$ fragment analyzer with S1 cartridge.

Table 9 The size of the target sequences

	Goat	Chicken	Cattle	Sheep	Pig
Target size	159 bp	228 bp	273 bp	334 bp	396 bp

Table 10 The CGE result of Direct Meat assay

Type	Result data
Goat	
Chicken	
Cattle	

Sheep	
Pig	
NTC	
Positive Control (Multi-Results)	

Documentation and Support

Related documentation

For other related documents of the PCR kits, *Qsep* series instruments, BiOptic Catalog, etc., please visit the Download page on BiOptic's website.

Customer and technical support

For more information about the detection kit or instruments, please visit BiOptic's website or contact the Distributors in your area.

Reference

1. Illuminate Biodiversity-International Barcode of Life (https://ibol.org/)
2. European Commission-Knowledge Centre for Food Fraud and Quality (https://knowledge4policy.ec.europa.eu/food-fraud-quality/topic/food-fraud_en)

Symbol Explanation

Table11 Explanation of the symbols used in the labeling

Symbol	Symbol Title	Explanatory Text
	Manufacturer	Indicates the medical device manufacturer
	Catalog number	Indicate the manufacturer's catalog number to identify the medical device