



VERYfinder

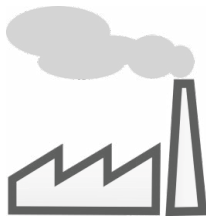
Ingredient Authenticity
Real-Time PCR kits

In 2013 horse meat scandal has highlighted the need to have the scientific means to check the components of our foods. As our population grows, increasing amounts of food are produced in our respective countries and also imported from overseas. The ingredients of an individual food product may be sourced from a single farm or from a wide range of producers and manufacturers worldwide. The authenticity and origin of these ingredients must be labelled so that this information can be included on the food label of the final product, thus creating a 'paper trail' which follows ingredients as they are exported around the globe. However, as highlighted by the horse meat scandal, there is room for error or intentional adulteration of ingredients during this process.

Identity Preservation Tracking



Primary
Producer



Processing
Company



Distributor



Retailer

Consumer

Traceability

Adulteration can occur for a variety of reasons, often linked to financial gain. Food industry and consumers share then a common interest in the possibility to have a fast and accurate method determining the authenticity of the ingredient used for food preparation.

Food industry often makes use of products, where it is difficult to verify the real content in raw material stated by the supplier. This problem translates into economic and reputation risks for the company. On the other side consumers want to be sure the products they eat: deserve the price; don't contain risks for health; are not infringing religious rules.

DNA testing allows an efficient and sensitive identification of plant and animal derivatives, easily detecting accidental contaminations or potential frauds related to false declaration on the label of the species constituting the food.

Real-Time PCR is the most sensitive method for the detection and quantification of DNA sequences of different contaminating species. The method, combined with an appropriate nucleic acid extraction system, allows the analysis of raw materials, semi-finished and finished products as well.

VERYfinder is the product line developed by **Generon** with premium quality molecular biology reagents to provide the customer a turn-key solution for the detection of a wide selection of vegetable and animal contaminating ingredients in raw and processed food using Real-Time PCR. The portfolio is complemented by kits for the extraction of nucleic acids developed by **Generon R&D**:

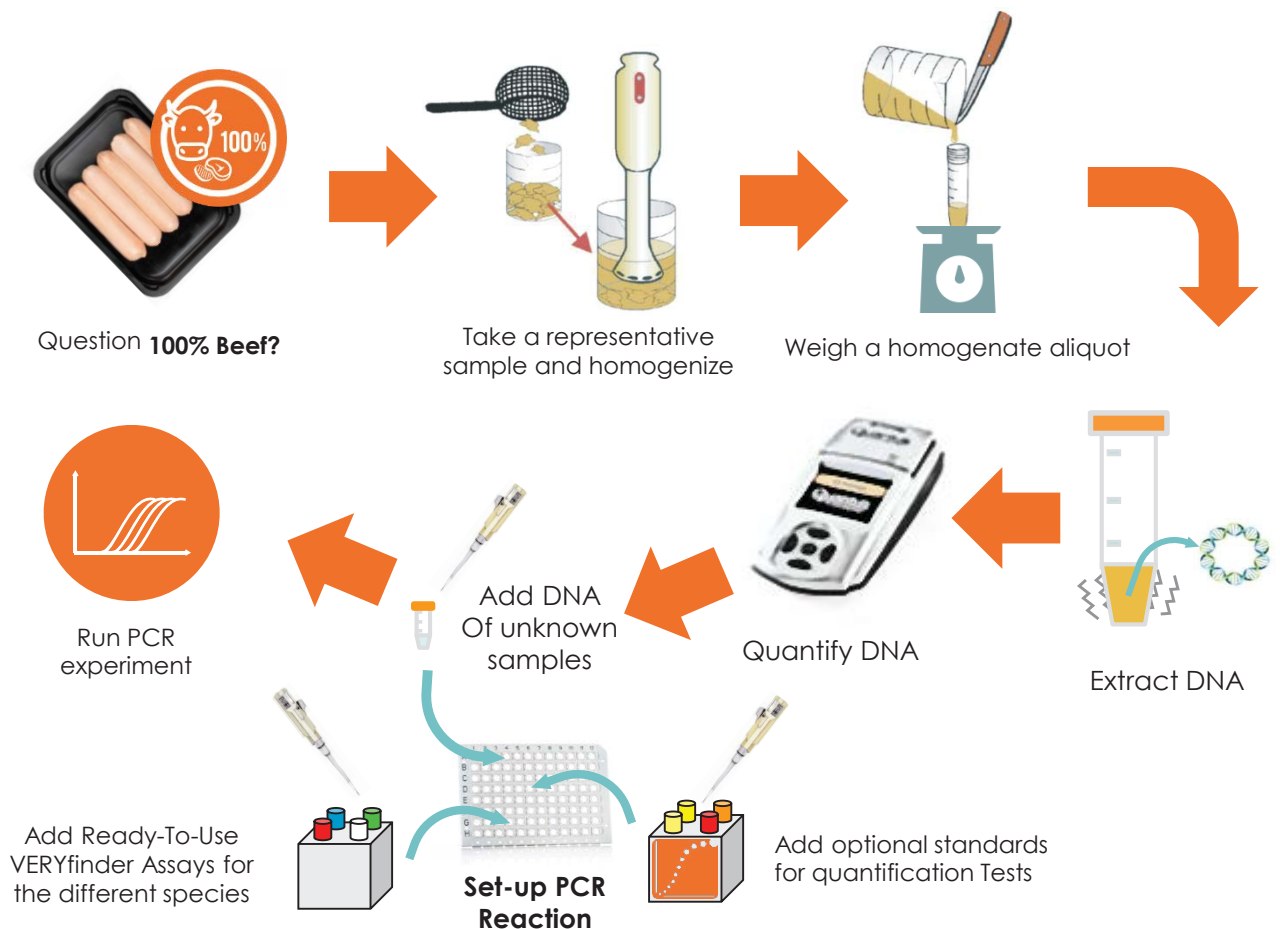
Ion Spin DNA Prep One-for-All is specifically indicated when extracting from very homogeneous matrices where small sampling units are not impacting mass representativeness.

Ion Force DNA Extractor FAST is a kit featuring the peculiar advantage of a scalable protocol allowing for the extractions from large and representative sampling units.

Both the extraction kits:

- allow the extraction of **DNA from any matrix** either of animal or vegetal origin
- have a proven capability in **recovering fragmented DNA** thus allowing extraction from processed matrices
- allow removal of PCR inhibitors from complex matrices through a **progressive clean-up**.

Workflow for the detection of undeclared meat species



A specific portfolio of **VERYfinder** products was developed to assess meat authenticity. The portfolio includes:

- **Semi-Q Detection Assays**
- **Total Meat Content Assay**
- **DNA Standards in Water**
- **Customized DNA Standards**
- **Pure Meat DNA Extracts**

The combined use of the different components allows the user to perform very targeted experiments taking into consideration specific matrices and manufacturing processes. This is of peculiar importance as the DNA extracted from the ingredients present in the matrix and the physical treatments typical of the food/feed industry, deeply impact on the detection of the contaminant DNAs. A collaboration with Generon ensures the best reliability in the results obtained through PCR analysis through a very accurate training and constant support of the lab staff involved in the analysis.

An extremely valuable point for choosing Generon is also our capacity to perform custom validations on specific matrices evaluating all the details of clients scenario e.g. animal anatomical part involved, ingredients included in the finished products, heat treatments during processing.

VERYfinder™ Meat Testing Products

A. Semi-Quantitative Meat-ID Assays

Description	50 Reactions kit
VERYfinder Swine Semi-Q Assay	PMA01S-50
VERYfinder Bovine Semi-Q Assay	PMA02S-50
VERYfinder Turkey Semi-Q Assay	PMA03S-50
VERYfinder Poultry Semi-Q Assay	PMA04S-50
VERYfinder Sheep Semi-Q Assay	PMA05S-50
VERYfinder Goat Semi-Q Assay	PMA06S-50
VERYfinder Buffalo Semi-Q Assay	PMA07S-50
VERYfinder Equine Semi-Q Assay	PMA08S-50
VERYfinder Equine (According to EURL) Semi-Q Assay	PMA11S-50
VERYfinder Rabbit Semi-Q Assay	PMA12S-50
VERYfinder Duck Semi-Q Assay	PMA13S-50
VERYfinder Goose Semi-Q Assay	PMA14S-50
VERYfinder Cat and Dog Semi-Q Assay	PMA96S-50
VERYfinder Veggie Semi-Q Assay	PMA99S-50

Each kit box contains target specific reagents (enzyme, primers and probes), negative control, 2 vials of positive control (120 µL Target DNA at a concentration 8 ng/µL; 1% target DNA – 99% bovine/swine DNA) extracted from raw material or heat treated material respectively. Heat treatment performed in autoclave at 121°C for 20'.

B. VERYfinder Standard Materials

Description	Quantiscale	Quanticust	Pure DNA Extract
VERYfinder Swine	PMA01W	PMA01X	PMA01R
VERYfinder Bovine	PMA02W	PMA02X	PMA02R
VERYfinder Turkey	PMA03W	PMA03X	PMA03R
VERYfinder Poultry	PMA04W	PMA04X	PMA04R
VERYfinder Sheep	PMA05W	PMA05X	PMA05R
VERYfinder Goat	PMA06W	PMA06X	PMA06R
VERYfinder Buffalo	PMA07W	PMA07X	PMA07R
VERYfinder Horse	PMA08W	PMA08X	PMA08R
VERYfinder Donkey	PMA11R		
VERYfinder Rabbit	PMA12W	PMA12X	PMA12R
VERYfinder Duck	PMA13W	PMA13X	PMA13R
VERYfinder Goose	PMA14W	PMA14X	PMA14R
VERYfinder Total Meat	PMA99W	PMA99X	PMA99R

VERYfinder Quantiscale

Each box includes 4 vials containing 120 µl of standard of target DNA (extracted from raw material) at scalar concentration (8 ng/µl; 800 pg/µl; 80 pg/µl; 8 pg/µl) dispersed in water. PMA99W can be customized but normally contains 50% each of the following DNA: Swine, Bovine.

VERYfinder Quanticust

Each box includes 1 set of customized DNA:

- Or 5 vials containing 120 µl of standard target DNA dispersed at various customizable concentration (e.g. 100%; 50%; 10%; 1%; 0,1%) in a background DNA extracted from on-demand matrices (e.g. swine/bovine DNA) at a total concentration of 8 ng/µl.
- Or 1 vials containing 300 µl of standard target DNA dispersed at customizable concentration (e.g. 1% equine in 99% bovine) at a total concentration of 8 ng/µl.

DNA can be extracted from raw material or heat treated material respectively (Heat treatment performed in autoclave at 121°C for 20') upon customer request.

VERYfinder Pure DNA Extract

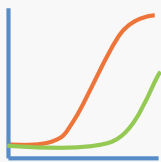
Each box includes a vial containing 1200 ng of ION-Force purified target DNA at a concentration 8 ng/µl extracted from raw material or heat treated material respectively (Heat treatment performed in autoclave at 121°C for 20') upon customer request. PMA99R contains 60 ng of DNA from each meat kind and bovine DNA to bring total DNA amount to 1200 ng.

Each **VERYfinder Assay** has been in-silico designed with short amplicons allowing the detection also of DNA fragmented by the manufacturing processes, therefore they can be used on both raw and processed food. This design was then thoroughly tested on real samples to determine the specificity and the sensitivity of target detection.

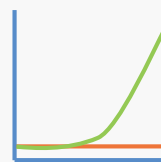
All **VERYfinder assays** include in each Real-Time PCR reaction an inhibition control, the so called **Internal Amplification Control (IAC)**, to demonstrate that PCR inhibitors or impurities that interfere with DNA polymerase are not lowering PCR reaction efficiency. IAC is a non-target DNA sequence present in the very same sample tube (as it is introduced through the assay mastermix), which is co-amplified simultaneously with the target sequence. In a PCR with an IAC, a control signal should always be produced even though there is no target sequence present.

Internal Amplification Control

Target — (red line)
IAC — (green line)



Sample is positive

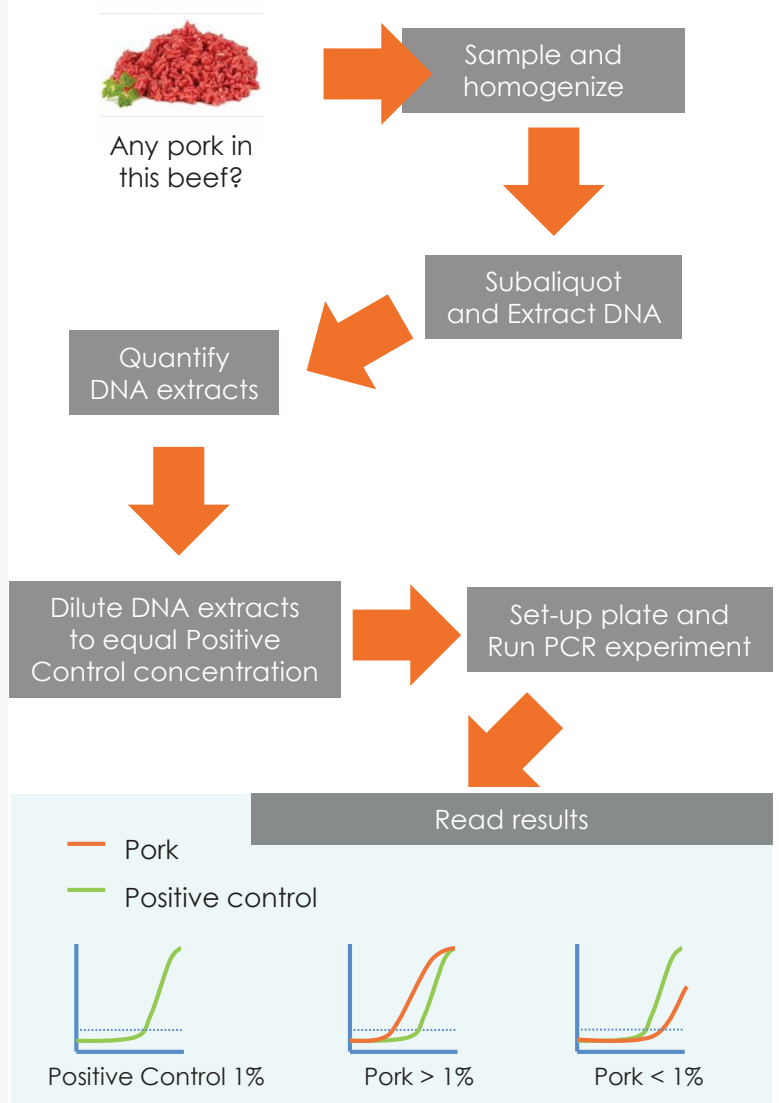


Sample is negative



PCR inhibition

Performing a semi-Q test in matrices containing meat only



— (red line) Pork
— (green line) Positive control

Positive Control 1%

Pork > 1%

Pork < 1%

When testing products containing ingredients other than meat (for example Bolognese lasagne) the DNA extracted is a complex mixture where the DNA of meat contributes only in part. The percentage of contamination of an undeclared meat type must be referenced not to the total DNA but only to the meat derived DNA portion. **VERYfinder Total Meat Assay**, when used with one or more of the **VERYfinder Meat ID kits** and **VERYfinder Quantiscale/Quanticust Standard Materials**, enables the simple and reliable determination of the percentage of a target meat species with respect to total meat in food samples.

VERYfinder™ Meat Testing Products

Description

VERYfinder Total Meat Assay

50 Reactions kit

PMA99A-50

Each box contains target specific reagents (enzyme, primers and probes), negative control. The assay needs to be used along with a Quantiscale or Quanticust product.

Performing a quantitative test in products containing ingredients other than meat



Any pork in this lasagne?



Unknown Sample	Std 100% Swine	Std 10% Swine	Std 1% Swine	Std 0,1% Swine	Negative Control	Using VERYfinder Swine
Unknown Sample	Std 100% Swine	Std 10% Swine	Std 1% Swine	Std 0,1% Swine		
Unknown Sample	Std 100% Meat	Std 10% Meat	Std 1% Meat	Std 0,1% Meat	Negative Control	Using VERYfinder Total Meat
Unknown Sample	Std 100% Meat	Std 10% Meat	Std 1% Meat	Std 0,1% Meat		



Calculate Pork Meat % relative to the Total Meat Content

Example: Measured Total Meat Content = 20%; Measured Swine Content = 0.4%; Swine Content = 2%

It's worth to note that while detection of undeclared meat in products is relatively straightforward (present or not present) problems arise as with quantification. There is a tendency to focus on meat content expressed as weight/weight (w/w). DNA measurements used in quantification of species are however hardly correlated to meat content (w/w) for several reasons, for example:

- Differences in species genome size
- Tissue cell size (i.e. cell number per gram meat) and mitochondrial distribution
- DNA extractability
- DNA degradation
- Tissue fat and water content

Therefore all the results obtained using VERYfinder kits shall be intended as percentages of DNA copies and not percentages in meat weight.

VERYfinder™ Vegetal Testing Products

VERYfinder Quantitative Assays

VERYfinder Soft/Durum Wheat Contamination

PSV02Q-50

First test in the market using real-time qPCR to measure the presence of soft wheat (*Triticum aestivum*) in durum wheat (*Triticum durum*). Italian regulations (D.P.R. 187/01; D.M. 12/1/96, 26/4/02) forbid to manufacture pasta with soft wheat, however a trace presence of soft wheat up to 3% is tolerated.

Each kit box contains: target specific reagents for the detection of *Total Wheat* (enzyme, primers and probes) and *Soft Wheat* (enzyme, primers and probes); two sets of pre-extracted DNA standards for creation of calibration curves for *Total wheat* and *Soft Wheat* respectively; negative control.

VERYfinder™ Fish Testing Products

VERYfinder Atlantic Cod (*Gadus morhua*) Assay

PFA01A-50

First test in the market using real-time qPCR to verify the identity of fillets samples as *Gadus morhua* vs other *Gadidae* and *Merluccidae* family members.

Each kit box contains target specific reagents (enzyme, primers and probes), negative control, positive control.

Thanks to the strong expertise matured on assay design Generon is also able to develop authenticity tests on-demand through progressive phases of a R&D project agreed with the clients.

- Definition of the scope and of the requested technical specifications
- In-silico feasibility study
- Primary tests on pure ingredients
- Cross-Reactivities analysis on pure ingredients
- Performance analysis on complex matrices

An extremely valuable point for choosing Generon is our capacity to perform custom validations taking into consideration client's specific matrices and manufacturing processes (physical/chemical treatments). This is of peculiar importance as these factors deeply impact on the detection of contaminant DNAs. A collaboration with Generon then ensures the best reliability in the results obtained through PCR analysis.



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