1. Protocol for DNA isolation from food products

DNA from food products such as cheese and processed meat products will be isolated with the commercially available kit “Nucleospin® Food” from Macherey-Nagel.

Follow the protocol described in the kit’s manual with the following modifications:

1. Step 1 “Homogenize sample”: Cut and grind with a scalpel more than 200 mg from at least 3 points of the food product. Grind in as smaller pieces as you can and mix well all pieces from the 3 different points. Weight 200 mg in an eppendorf-like tube and continue with the kit’s protocol.

2. After preparing your samples also prepare an “extraction control sample”. This sample contains all the buffers that will be used during DNA isolation, but no meat product (of any origin). This control will ensure that your buffers are not contaminated with animal DNA or inter-samples contamination during DNA isolation. Treat this sample as any of the other samples. This sample should always be treated last, in order to accomplish its purpose.

3. Step 2” Lyse cells: Incubate with proteinase K for 1 hour.

4. Step 3 “Adjust DNA binding conditions”: Use 500 µl sample, 500 µl Buffer C4 and 500 µl ethanol.

5. Step 6 “Elute DNA”: Elute with 70 µl of pre-heated buffer CE. Split your samples in 2 aliquots of 20 µl and 50 µl respectively. Do not split the “extraction control” sample. Keep this sample aside solely for PCR. Do not measure absorbance at 260 and 280 nm in this sample.

Institute of Applied Biosciences, Center For Research and Technology Hellas, 6th km Charilaou – Thermis, 570 01, Thessaloniki, Greece ☎+ 30 2310 498 272 Fax: +30 2310 498 270

Keep the 50 μl aliquot in the freezer in order to use solely for qPCR purposes.

Using the 20 μl aliquot define the DNA concentration in your samples using a spectrophotometer by measuring absorbance at 260 and 280 nm. Calculate the appropriate dilution for your samples so as to have a final concentration of **25 ng DNA/μl**. Dilute with PCR water. Keep the water aliquot in order to use it in the PCR master mix. This will verify the presence or absence of contamination during samples' dilution.

Prepare samples' dilutions using the 50 μl aliquot of each sample.

2. Preparation of DNA standards for horse DNA detection

1. After having isolated DNA from horse meat and measured its concentration with a spectrophotometer, calculate the appropriate dilutions so as to have the desired standards concentration (eg. 0.1%, 1%, 5%).

Dilute horse DNA in the above described concentrations with **other meat products' DNA that have been previously tested and found negative for horse DNA** (eg. pork salami, chicken nuggets). Make the appropriate calculation so as your final DNA mix has also a concentration of **25 ng DNA/μl**.

These are the DNA standards that will be used for the quantification of horse DNA in your samples.

2. Make a mixture of DNA samples derived from different meat products (eg. pork salami, chicken nuggets) with a final concentration of 50 ng DNA/μl, 25 ng DNA/μl and 10 ng DNA/μl.

These are the DNA standards that will be used to verify that your sample contains **amplifiable DNA from animal origin** (see below).

Institute of Applied Biosciences, Center For Research and Technology Hellas, 6th km Charilaou – Thermis, 570 01, Thessaloniki, Greece ☎+ 30 2310 498 272 Fax: +30 2310 498 270

3. qPCR for the detection of horse DNA

Prepare your master mix according to the kit's manual that will be used for qPCR (eg Kapa SYBR Fast – Kapa Biosystems, SYBR Fast Green Mastermix - Applied Biosystems). Prepare a volume 10% greater than the volume that will be needed (remember to take into account that you will also be using a no template control).

Set up your PCR reactions with the following sets of primers:

1) One set of primers specific for horse DNA. Set up your reactions with your samples, horse DNA standards, extraction control sample and no template control (PCR water).

2) One set of universal primers for common animal species. Set up your reactions with your samples, meat products' DNA standards, extraction control sample and no template control (PCR water).

Use 1 μl of all samples and standards in the concentrations described above!!!

Use the same PCR water that you used for sample dilutions in your master mix and no template control!!!

Set up the real time instrumentation according to the real time kit's manual and run your qPCR accordingly.

Good luck!!!

Institute of Applied Biosciences, Center For Research and Technology Hellas, 6th km Charilaou – Thermis, 570 01, Thessaloniki, Greece ☎+ 30 2310 498 272 Fax: +30 2310 498 270