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Meat preparation protocol for meat identification V.1

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Based on https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3550954/

Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

This protocol is work in progress for now.

Abstract

Identifying species by identifying unique DNA sequences is common practice.
In this case we will identify the presence or absence of pork DNA in a meat sample.
We will use the alkaline method for DNA extraction and LAMP method for DNA amplification and detection.
This is not a new protocol but rather the detailing of a previously published protocol listed in the manuscript citation.

Guidelines

Follow the country specific guidelines.

Materials

Consumables: 15 ml falcon tube, 1.5 ml centrifuge tube or equivalent, 1 PCR tube 0.1 mL PCR tubes with flat caps, commonly known as low-profile PCR tubes that matches the device well size to store the ready to be tested sample (aliquot).
Labware: graduated cylinder
Devices: Mortar and pestle, LAMP/PCR/Water bath and thermometer, scale, pH meter, 1- 10 µl micropipette, 100-1000 µl micropipette and tips. Optional: vortex, LAMP or PCR device or water bath, scale and boat.
Reagents: NaOH, Tris, HCl, distilled-DNase free water, meat to be tested
Others: stir rod, spatula, nitrile gloves, pipette rack , tube rack, labeling pen

Safety warnings

Use appropriate PPE and follow Safe Work Practices.
NaOH (sodium hydroxide or caustic soda) needs to be handled with gloves because it's a highly corrosive alkali.
Seek and follow Safety Data Sheets for NaOH and HCl

Before start

Pork meat identification protocol. Meat Preparation protocol based on
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3550954/
"About 500 mg of meat was triturated in pestle & mortar with four volumes (4 ml) of 0.2N NaOH. 5 µl of the extract was again mixed with eight volumes (40 µl) of 0.2N NaOH in a sterile micro centrifuge tube. Mix was heated at 75 °C in water bath for 20 min and then added eight volumes (360 µl) of 0.04 M Tris HCl (pH 7.75) for neutralization of pH. 1 µl of final mix containing about 100 ng DNA was used for."

1 Prepare 5 ml of NaOH 0.2 mol/L (sodium hydroxide or caustic soda). This will be used twice, once for 4 mL and once for 40µL.

1.1 Label a new 15 ml falcon tube "NaOH 0.2 mol/L" and add 10 mL DNAfree water and 0.080 g of NaOH. Mix to dissolve, manually or using a vortex.

*For general molarity calculations see **Molarity calculation**.*
Molar concentration is the number of moles contained in one Liter of a substance. The concentration of a solution is expressed in mol/L C=(m/M)/V where V is the volume in liters m is mass in grams and M is molar weight? in grams/mol
https://www.alloprof.qc.ca/en/students/vl/sciences/calculating-molar-concentration-s1622
So:
*Molecular Weight of sodium hydroxide (NaOH) =40 g/mol: Na=22.989g/mol O=15.999g/mol H=1.008g/mol (**http://chembook.org/page-nonav.php?chnum=1§=5)***
so: 22.989g/mol+15.999g/mol+1.008g/mol=39.996g/mol.
-+
so m=C∗M∗V =0.2mol/L∗40g/mol ∗10 mL= 8 g/L∗10 mL=80 mg of NaOH

Meat Preparation

2 In a pestle and mortar add 0.5 g fresh pork meat without fat and 4 ml from the tube labelled "NaOH 0.2 mol/L" and grind (triturate) the mix. This will break the structure and results in a paste/liquid.

3 Measure 5 µL from previous step and add it to a new sterile PCR (100 µl) tube labeled "prepared sample". This is done preferably with a 1- 10 µl filtered micropipette.

4 Add 40µL from the tube labelled "NaOH 0.2 mol/L" to the "prepared sample" tube.

5 Heat the resulting mix (45 µL) at 75 Celsius for 20 minutes in either water bath, LAMP device or a PCR device.

6 Move the content of the "prepared sample" to a new 1.5 ml centrifuge tube and add 360 µl of 0.04 mol/L Tris HCl (pH 7.75) prepared using the https://www.protocols.io/view/prepare-tris-buffer-for-meat-identification-protoc-dndu5a6w protocol. !!! Check for the latest version. *This is done for for neutralization of pH.*

Note and warning. The paper specifies pH=7 while the referenced paper specifies pH=7.75
Note: We assume pH is measured at 25 Celsius and we will try with both options of pH-7 and 7.75

7 For samples prepare 1 µl in a PCR tube appropriate for your amplification device (like 100 µL PCR tube) and store at -20. That should normally contain about 100 ng of DNA according to the reference paper.

Protocol references

1 Girish et al: A rapid method for authentication of Buffalo (*Bubalus bubalis*) meat by Alkaline Lysis method of DNA extraction **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3550954/**

2 Girish et al: Rapid detection of pork using alkaline lysis- Loop Mediated Isothermal Amplification (AL-LAMP) technique. Published, journal pre-proof can be downloaded from Academia https://www.academia.edu/114013841/Rapid_detection_of_pork_using_alkaline_lysis_Loop_Mediated_Isothermal_Amplification_AL_LAMP_technique