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Meat identification protocol

This protocol is a draft, published without a DOI.

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Manuscript citation:
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: 3 x 1.5 ml Centrifuge tubes or equivalent, 1 PCR tube that matches the device well size to store the ready to be tested sample (aliquot).
Devices: Pestle and mortar, LAMP/PCR/Water bath and thermometer, scale, pH meter, 1- 10 µl micropipette, 100- 1000 µl micropipette. Optional: Vortex.
Reagents: NaOH, Tris, HCl, Dnase free water, meat to be tested

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."

Meat Preparation

1 Measure 500 mg pork meat without fat. This is our sample (aliquot.

2 Grind (triturate) the meat in a pestle and mortar. This will break the structure and results in a paste/liquid.

3 Measure 1 ml of the liquid meat and add it to a sterile centrifuge tube labeled "raw sample". This is done with a 100- 1000 µl micropipette.

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

4.1 In a new centrifuge tube labelled "NaOH 0.2 mol/L" add 5 mL DNAfree water and 40 mg or NaOH. Mix to dissolve manually or using a vortex.

*For general molarity calculations see **Molarity calcualation***
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
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*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 for us: m=C*M*V =0.2mol/L*40g/mol *5mL= 8 g/L*5 mL=40 mg of NaOH*

5 Add 4 ml from the tube labelled "NaOH 0.2 mol/L" to the "raw sample" tube. *This will break down the cell membranes freeing the DNA and also break down (denaturate) some of the proteins.*

6 Measure 5 µL from previous step and add it to a new sterile centrifuge tube labeled "prepared sample". This is done with a 1- 10 µl micropipette.

7 Add in the "prepared sample" new tube 40µL from the tube labelled "NaOH 0.2 mol/L".

8 Heat the resulting mix at 75 Celsius for 20 minutes in a water bath or using the LAMP device or a PCR device. *This will further break down the cell membranes freeing the DNA.*

9 Prepare (360 µl) of 0.04 mol/L Tris HCl (pH 7.75).
This will be done by mixing Tris with HCl

MW for TRIS Hydrochloride (C4H11NO3 ·HCl) 157.6 mol/Liter

0.04 M TRIS TRIS Hydrochloride: m=C*M*V =0.04mol/L*157.6g/mol *360uL=2.26 mg of TRIS Hydrochloride

Normally we make half the solution and titrate with HCl
https://www.reddit.com/r/labrats/comments/193ei6n/trying_to_prepare_trishcl_buffer_ph_seems/?rdt=42146

https://www.cshlpress.com/pdf/sample/2014/LabRefV1/LabRefV1Ch1S1.pdf has collection of reagents
pH calculations= https://www.youtube.com/watch?v=387t-nh0OMg
https://www.cusabio.com/m-296.html#a21 for calculating Tris HCL pH from Tris and Hydrocloric acid

10 Add 360 µl of the 0.04 mol/L Tris HCl. This is done for for neutralization of pH.

11 Sample 1 µl in a PCR tube and store at -20.

LAMP

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Protocol references

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