Mystery Meat Workshop

The following is based on the protocol of Manchester Digital Laboratory, which is based on a protocol by La Paillasse in Paris, France and the scientific paper by Matsunaga et al. (1999)

Introduction

What's in your food? Can you trust what you're eating? In the spring of 2013, consumers across Europe were rocked with news that meat packers were surreptitiously slipping horse meat into their beef products. Contamination ranged from a few percentage points to as much as 80 percent. Other undeclared meats, such as pork, were also discovered by food inspectors.[1]

Many are disgusted by the idea of eating horse, and others avoid meats like pork for religious reasons. Consumers should be able to trust their food, but many feel like the system is completely outside their control.

Not so! With this protocol, you will be able to differentiate between several common livestock species in order to discover what's on your plate. In the process, a powerful technique used in molecular biology called polymerase chain reaction or PCR.

Equipment, consumables, reagents

The DNA extraction is fast enough that you will want to have the PCR ready to go immediately.

Consumables
Your sample, control samples (optional)
200 mM KOH (in dH_2O), dissolve 1.1 gr KOH in 100 mL
200 mM HCl (in dH_2O), dilute standard 10% (3 M) HCl 15x so 7 mL in 93 mL
Micropipette tips (200 uL)
Thin permanent markers
Microfuge tubes (1.5 or 2 mL), two for each sample

Equipment
Micropipetters (200 uL)
Sharp knifes + cutting boards, one for each sample
Hotplate
Pot with boiling water *Have ready!*
Floating tube racks
Vortexer (optional)
Microcentrifuge

[1] http://www.theguardian.com/uk/2013/may/10/horsemeat-scandal-timeline-investigation

A. Dirty DNA Extraction

Total time: 30 minutes

Cut samples of about 50 mg (if you do not have a precision balance, it's about 1 – 3 mm^3) and place them individually in labeled microtubes (1.5 or 2 ml). Note what number is what. Use a separate knife for each sample. It is very important to avoid contamination between samples.

Pipette 100 to 200 uL solution of 200 mM KOH (sodium hydroxide) into your microtubes. SAVE THE TIP.
Purpose: the soda will burst the lipid membranes of cells and allow the DNA to be extracted

Crush the meat in the tube with your pipette tip or a toothpick then shake with a vortexer (if available).

Boil a pot of water, place the tubes in a tube floating foam and leave the tubes in boiling water for 10 minutes, but not too long as the DNA itself may degrade
Purpose: denature (destroy) the proteins of the sample that may disturb / degrade DNA and inhibit the PCR reaction.

Shake (using a vortex if you have one available).

Pipette 100 to 200 uL (*imperative to use the same volume as in step 2!*) of 200 mM HCl (hydrochloric acid) into each tube.
Purpose: neutralize the pH.

Shake (using a vortex if you have one available).

Centrifuge the tubes at high speed (at least 10,000 g) to 14000 rev / min** in a small bench top centrifuge for 5 minutes.
Safety: be very careful to distribute tubes such that the centrifuge remains balanced, each opposite another. An imbalanced centrifuge rotating at a high speed, like 14000 rev / min, is a serious dangerous, capable of doing severe damage to the machine and you. Goal: the cell debris should pellet at the bottom of the tube due to the centrifugation, whereas the DNA should remain in the aqueous phase as it is soluble in water.

Directly transfer supernatant to a fresh tube, taking care to handle it with care to avoid resuspension of the pellet. It is better to lose some supernatant than have debris in your supernatant. Again, you don't want any debris.

B. Polymerase Chain Reaction of Species-Specific Mitochondrial Regions
Total time: 20 minutes of preparation and 2 hours for the PCR

Consumables
PCR tubes 0.2 mL
Primers
Sopachem PCR mastermix
Nuclease free water

Equipment
PCR machine
P20 pipette & yellow tips
P2 pipette & white tips

Ensure that you have a primer master mix: UNIV:G:C:B:S:P:H – 1 : 0.2 : 3 : 0.6 : 3 : 0.6 : 2 at 10x concentration of 4.0 uM (for ratio 1, adjusted for others), ideally in nuclease-free water. This makes the final PCR concentration for the ratio 1 equal to 0.4 uM. Therefore, a 10x working mix should, in uM, be 4.0 : 0.8 : 12.0 : 2.4 : 12.0 : 2.4 : 8.0 for UNIV:G:C:B:S:P:H.

Make 250 ul of 10x primer mix, start with 146 uL dH2O.

Abbrev.	**Amount**	**Name**	**Sequence**
UNIV	10.0 uL	Meat Universal F	5'-GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3'
G	2.0 uL	Goat R	5'-CTCGACAAATGTGAGTTACAGAGGGA-3'
C	30.0 uL	Chicken R	5'-AAGATACAGATGAAGAAGAATGAGGCG-3'
B	6.0 uL	Cattle R	5'-CTAGAAAAGTGTAAGACCCGTAATATAAG-3'
S	30.0 uL	Sheep R	5'-CTATGAATGCTGTGGCTATTGTCGCA-3'
P	6.0 uL	Pig R	5'-GCTGATAGTAGATTTGTGATGACCGTA-3'
H	20.0 uL	Horse R	5'-CTCAGATTCACTCGACGAGGGTAGTA-3'
	To make a final solution of 50 ul:		

Label 0.2 mL PCR tubes for each sample.

Add 14 uL dH_2O.

Add 20 uL of 2x VWR Red Taq DNA polymerase Master Mix to each tube.

~~Add 10 uL 20 mM $MgCl_2$.~~ (already in our red taq DNA Master Mix)

Add 4 uL primer master mix.

Add 2 uL sample DNA (always add the DNA the last)

Tightly close the tubes and mix the contents by gently tapping with your finger. Give a 2 second centrifuge (in 1.5 mL centrifuge tube holder so the tube doesn't slip away) if drops get up on the side of the tube.

Start the following PCR program, adapted for these specific primers:

94°C	30 s repeat x35
60°C	45 s repeat x35
72°C	45 s repeat x35
4°C	hold forever

7. Prepare the gel while the PCR is running.

C. Gel electrophoresis and analysis
30 minutes to 1 hour

Consumables
Agarose
Erlenmeyer flask
Invitrogen SYBR Safe
TAE buffer 1x
Loading dye
Low molecular weight DNA ladder
Gloves

Equipment
Gel tray
Gel comb
Gel tank
Power supply
Transilluminator
Camera
P20 Pipette

Prepare the TAE 1x final concentration.

Weigh enough agarose gel to achieve a 2% (2 g per 100mL) agarose and mix with TAE in a glass container (flask). We use a high concentration agarose as fragments DNA that we analyze are small.

Melt mixture microwaved until all the agarose is dissolved. Let cool on the bench a while (about 5 minutes)

Once you can comfortably touch the flask, add the dye. For Invitrogen SYBR Safe, we add 4 to 6 uL for a 100 mL gel. Swirl carefully to avoid bubbles.

Prepare the electrophoresis tank, then pour the still-liquid gel into the mold (do not forget to use the comb to form wells). Wait for the solidification of the gel. Remove the combs.

Add ~~5 uL of 6x loading dye to your PCR product and pipette them in the wells in order.~~ *(loading dye is already in the polymerase solution)*
We've had the best results loading the full PCR product + dye (20 to 30 uL). You may want to load less based on your DNA stain sensitivity and visualization system.

Add your low molecular weight DNA ladder.

Connect the electrodes to the generator - use about 100V.

Migrate about 30 minutes until the DNA fragments are well separated from each other.

Turn off the generator and remove the gel. Be wary of self-electrocution!

Visualize the gel with a blue light / orange filter.

Take a picture and analyze band migration distances.