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Mystery meat protocol

Horse meat scandal 2013

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Dirty DNA extraction

Equipment, consumables, reagents

(~30 mins)

Consumables

- Distilled water (dH_2O)
- Your sample
- Optional: control samples
- 200 mM KOH (in dH_2O),

  dissolve 1.1 gr NaOH in 100 mL
- 200 mM HCl (in dH_2O),

  dilute standard 10% (3 M) HCl 15x; so 7 mL in 93 mL
- Pipette tips (200 uL)
- Microfuge tubes (1.5 or 2 mL)

Equipment

- Thin permanent markers
- Sharp knife (one per type of meat)
- Cutting boards / plates
- Hotplate
- Pot with boiling water, have ready!
- Micropipette (200 uL)
- Microcentrifuge
- Vortexer (optional)

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Dirty DNA extraction

Protocol

dirty DNA extraction

Cut samples of about 50 mg
(if you do not have a precision balance, it's about 1 – 3 mm^3)

dirty DNA extraction

Use a separate knife for each sample. It is very important to avoid contamination between samples.

dirty DNA extraction

Place them individually in labeled microtubes (1.5 or 2 ml).

Pipette 100 to 200 uL solution of 200 mM KOH (potassium hydroxide) into your microtubes.

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).

dirty DNA extraction

- Place the tubes in floating foam in the pot with hot boiling water

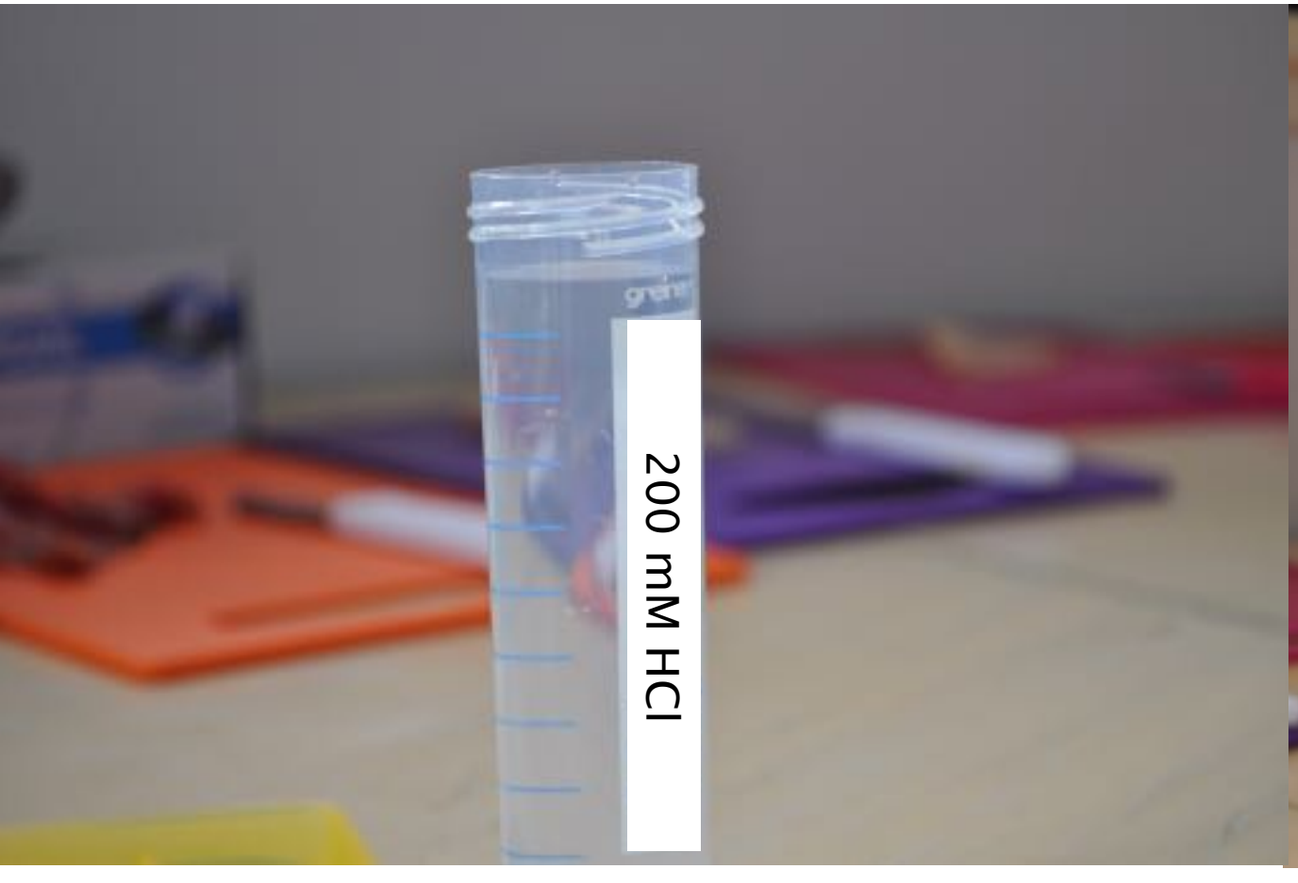
- Leave the tubes in boiling water for 10 minutes, but not too long as the DNA itself may degrade

Purpose: denature (destroy) the proteins of the sample that may disturb / degrade DNA and inhibit the PCR reaction.

dirty DNA extraction

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.



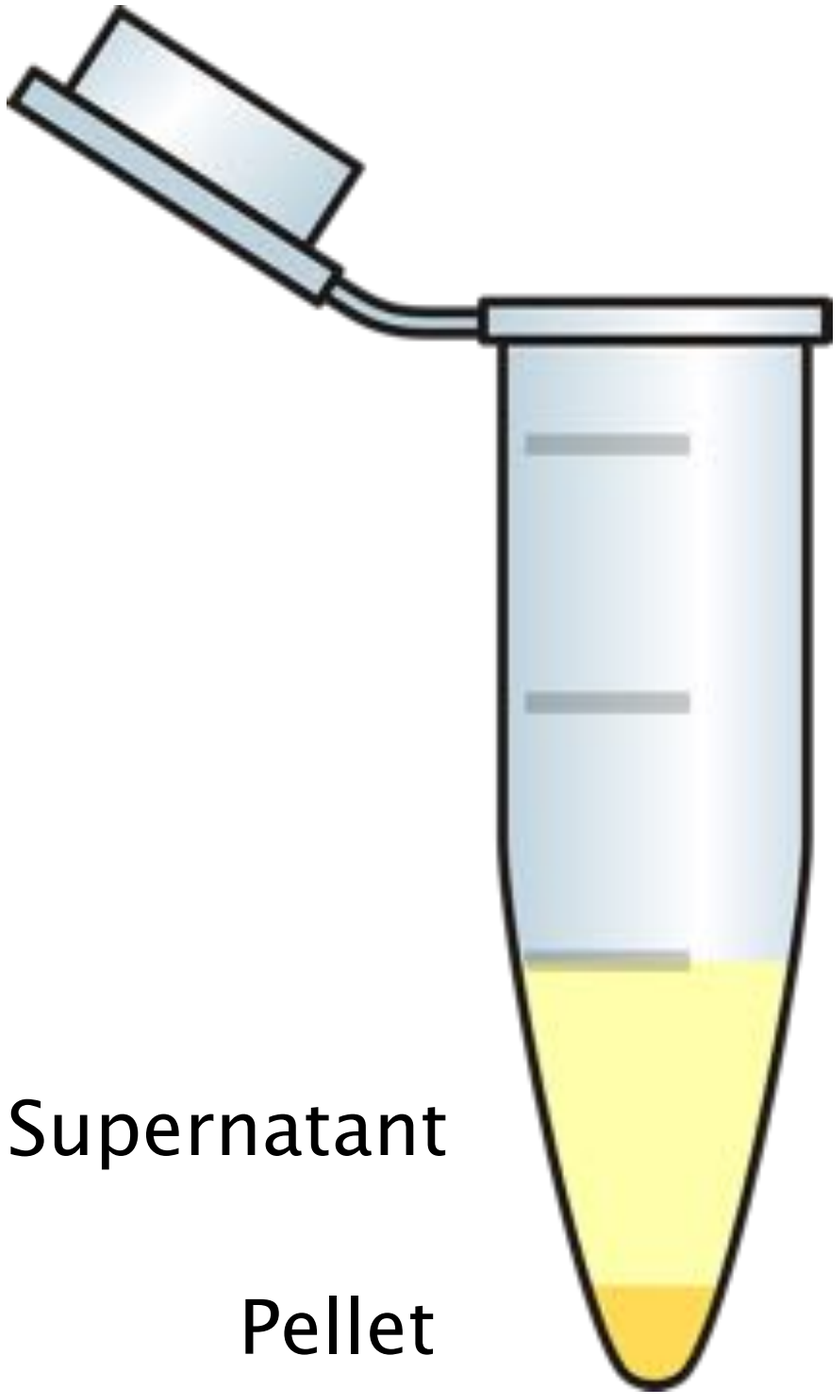
dirty DNA extraction

- Centrifuge the tubes at high speed (10,000 g / 14000 rpm) in a small bench top centrifuge for 10 minutes.
- Safety: be very careful to distribute tubes such that the centrifuge remains balanced i.e. each opposite another. An imbalanced centrifuge rotating at a high speed, is a serious danger.
- Purpose: 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.

dirty DNA extraction

- Directly transfer the supernatant to a PCR 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.



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Polymerase Chain Reaction of Species-Specific Mitochondrial Regions

Equipment, consumables, reagents

~30 mins prep, 3 hours to run the PCR

Consumables

- PCR tubes 0.2 mL
- Primers
- Sopachem PCR master mix
- Nuclease free water
- Pipette tips

Equipment

- PCR machine
- Pipettes P2 + P20

Polymerase Chain Reaction of Species-Specific Mitochondrial Regions

Protocol

PCR for mitochondrial region

Make a 10x (4.0uM) primer master mix in the following ratio:

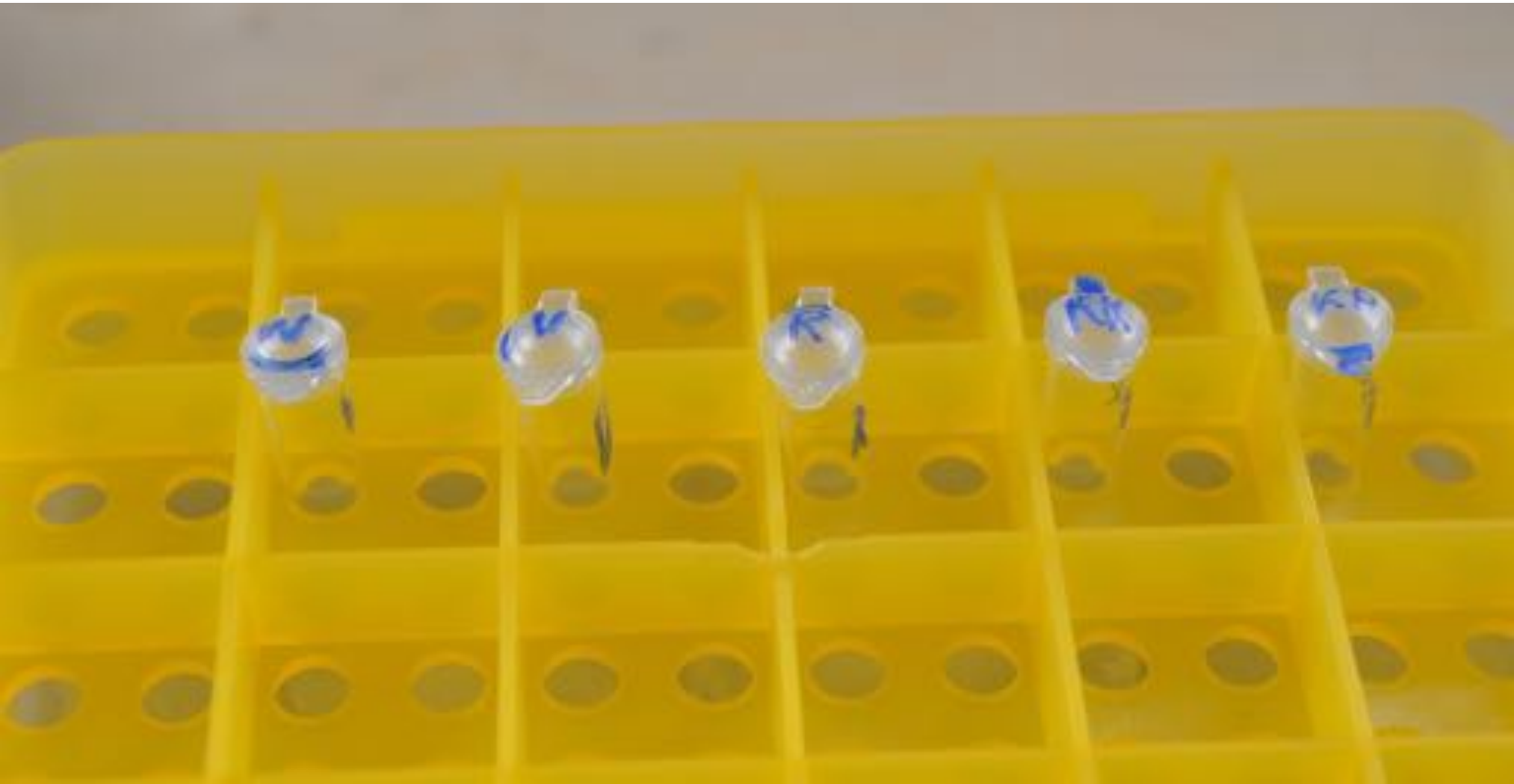
UNIV:G:C:B:S:P:H – 4.0 : 0.8 : 12.0 : 2.4 : 12.0 : 2.4 : 8.0

(your 10x primer master mix makes up 1/10th of the final PCR sample. Therefore, your working solution contains a concentration of 0.4uM primer mastermix)

Abbrev.	Amount (uL)	Name	Sequence
UNIV	1.60	Meat Universal F	
B	0.96	Cattle R	5’-CTAGAAAAGTGTAAGACCCGTAATATAAG-3’
P	0.96	Pig R	5'-GCTGATAGTAGATTTGTGATGACCGTA-3’
C	4.80	Chicken R	5’-AAGATACAGATGAAGAAGAATGAGGCG-3’
S	4.80	Sheep R	5’-CTATGAATGCTGTGGCTATTGTCGCA-3’
G	0.32	Goat R	5’-CTCGACAAATGTGAGTTACAGAGGGA-3’
H	3.20	Horse R	5’-CTCAGATTCACTCGACGAGGGTAGTA-3’

PCR

Label 0.2 mL PCR tubes for each sample.



PCR

Keep the tubes on ice

PCR

Add 11.5 uL of PCR grade dH_2O.

PCR

Add 2.5 uL primer master mix.

PCR

Add 25 uL of VWR Red Taq DNA polymerase Master Mix to each tube.

PCR

Put the tubes into your own build thermocycler

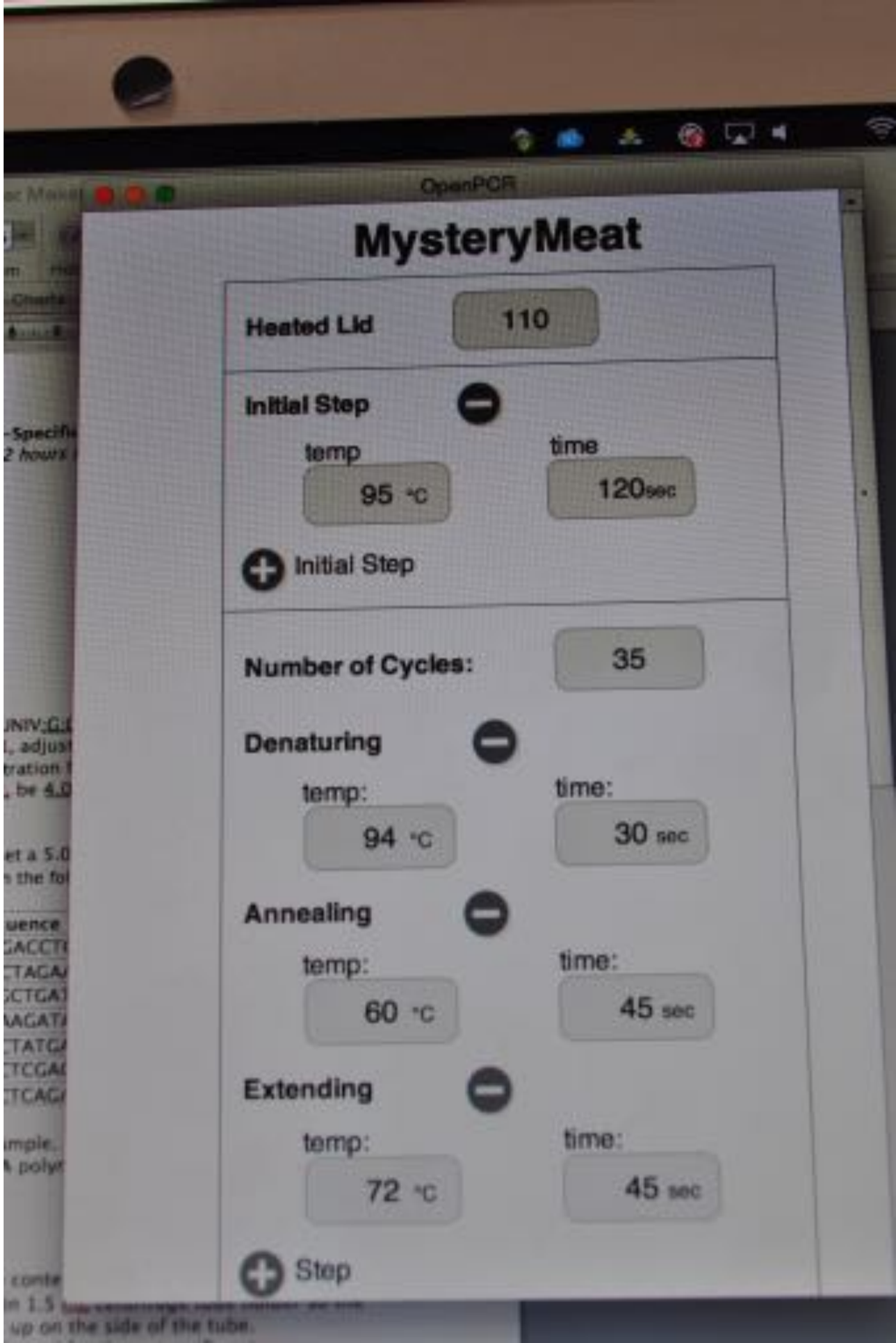
Start the following PCR programme, adapted for these specific primers

94ºC – 30 s repeat x35

60ºC – 45 s repeat x35

72ºC – 45 s repeat x35

4ºC – hold forever



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Gel electrophoresis

Consumables and Reagents
(2 - 3 hours)

Consumables

- Agarose
- Invitrogen SYBR Safe
- TAE buffer 1x
- Loading dye
- Low molecular weight DNA ladder

Equipment

- P20 Pipette
- Erlenmeyer flask (twice as big as your gel volume)
- Gel tray
- Gel comb
- Gel tank
- Power supply
- Blue LED transilluminator
- Camera

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Gel electrophoresis

Gel electrophoresis

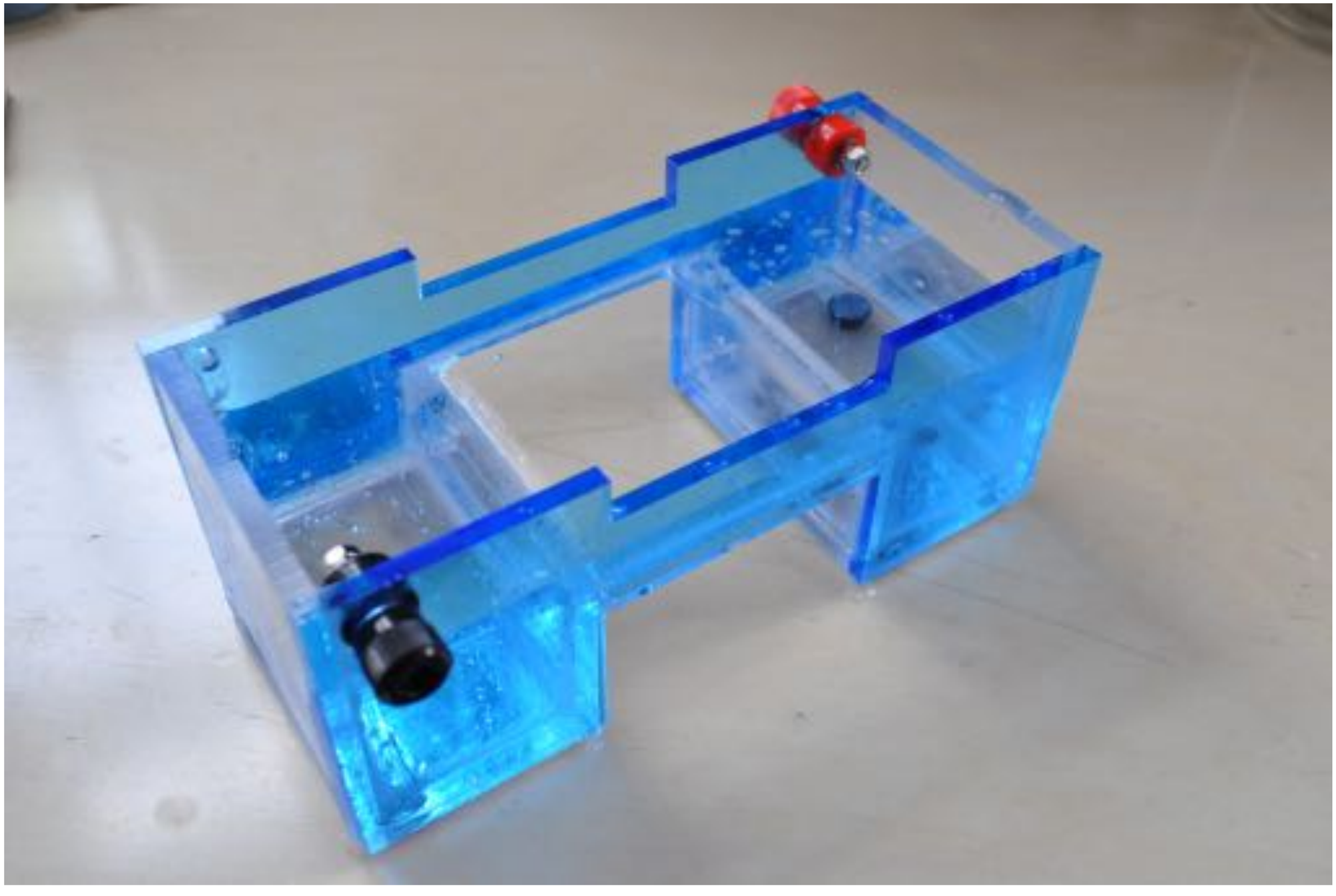
Prepare the 1x TAE final concentration

Gel electrophoresis

Tape your gel tray very well

Gel electrophoresis

Check whether your gel box is water proof



- Weigh enough agarose gel to achieve a 2% agarose gel (2 g per 100mL)

(We use a high concentration agarose as fragments DNA that we analyze are small.)

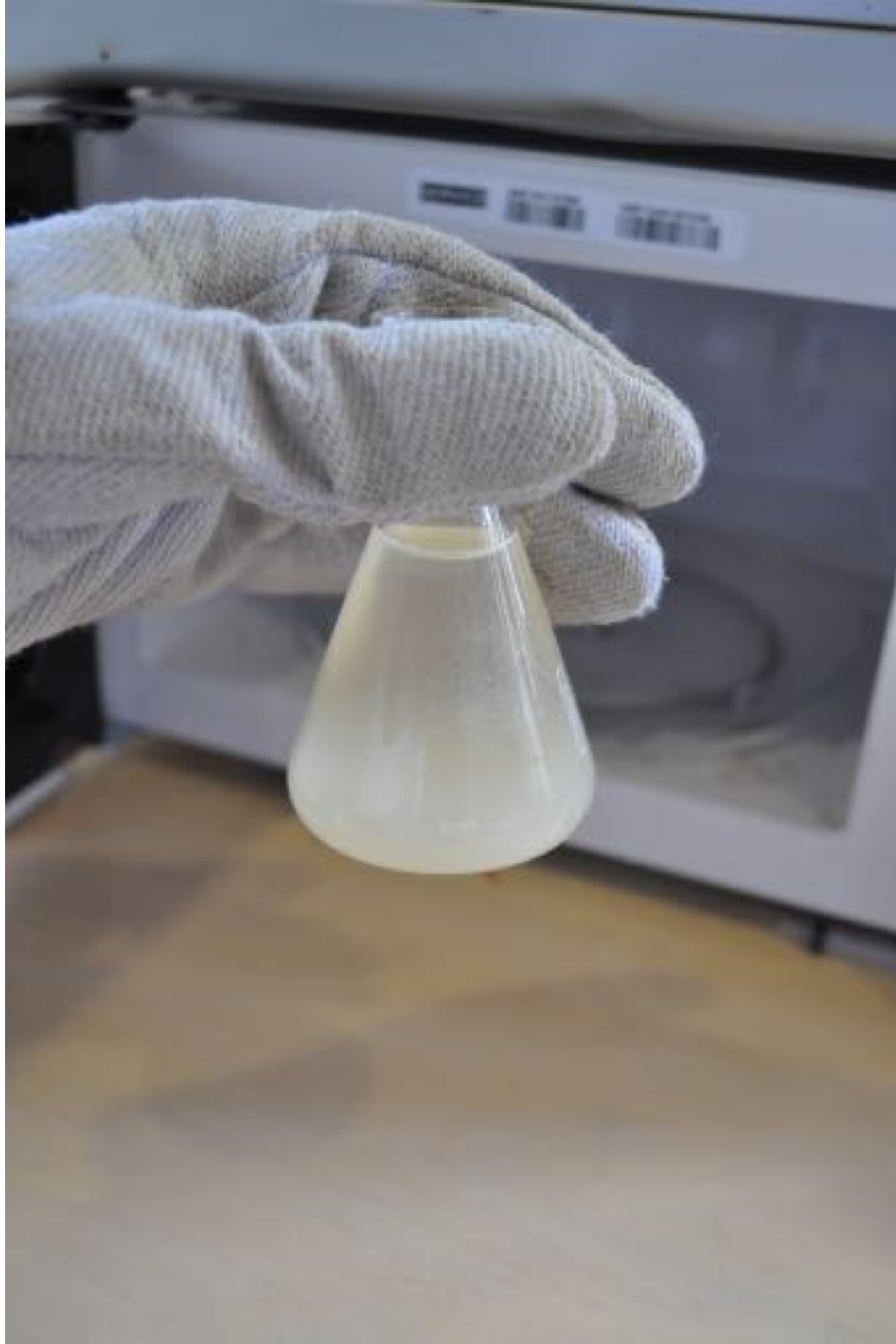
Mix with TAE in a glass erlenmeyer (flask).

Gel electrophoresis

Melt the mixture in the microwave until all the agarose is dissolved

Swirl in between

When dissolved, let the gel cool down on the bench (~5 minutes)



- Once you comfortably touch the flask, add 4- 6 ul SYBR safe.
- Swirl carefully to avoid bubbles.
- Pour the still-liquid gel into the mould
- Do not forget to use the comb to form wells).
- Wait for the solidification of the gel. (~30mins)

Gel electrophoresis

Use cling film to prevent the gel of touching surfaces.
In case of a leaking gel tray you can just throw away the cling film containing the toxic gel.

- We do not add loading dye to our samples as the polymerase master mix already contains a dye

- Load your PCR product onto the gel (15 – 30 ul)

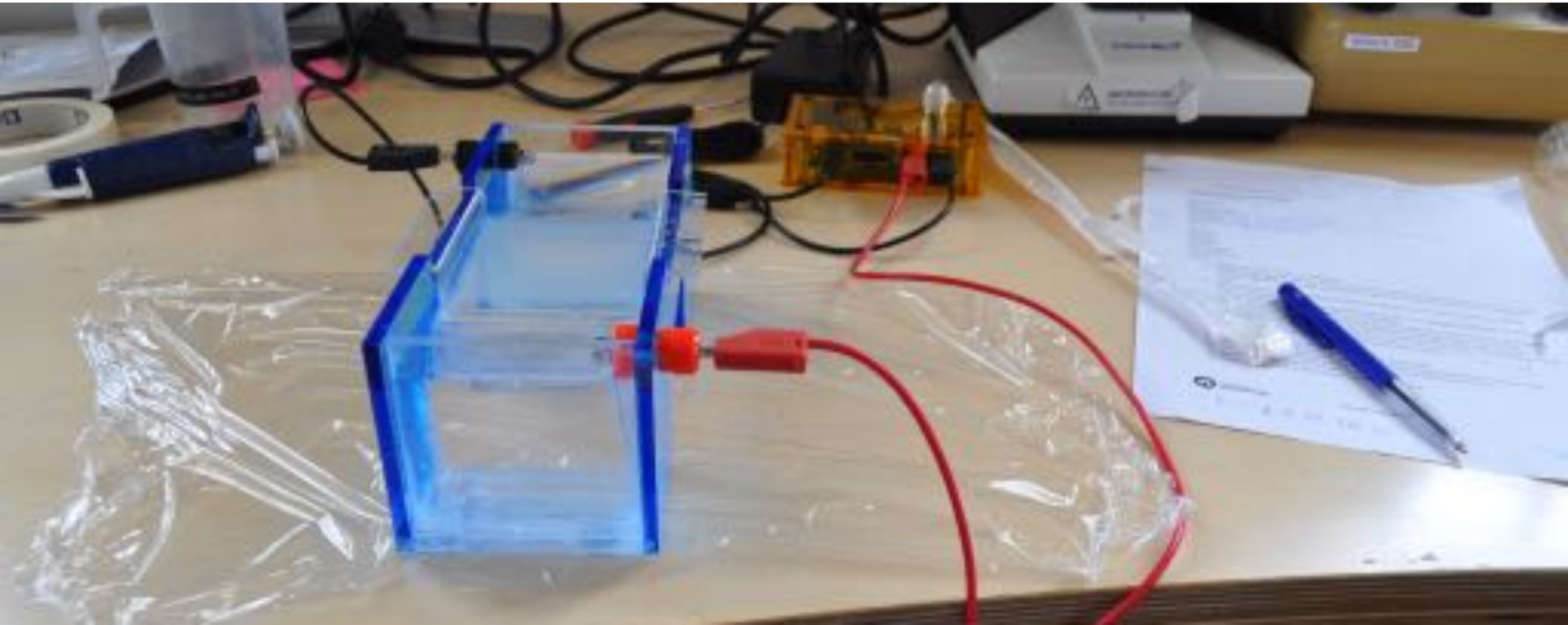
- Add your low molecular weight DNA ladder to the gel

Note: make sure you stick you pipette tips into the wells and pipette out slowly. You do not want your PCR product floating around in the liquid

Gel electrophoresis

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

Note: make sure the positive electrode is the one the DNA is moving to!



Gel electrophoresis

- 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.