SAFETY NOTE:

Wear gloves! Acrylamide is a neurotoxin

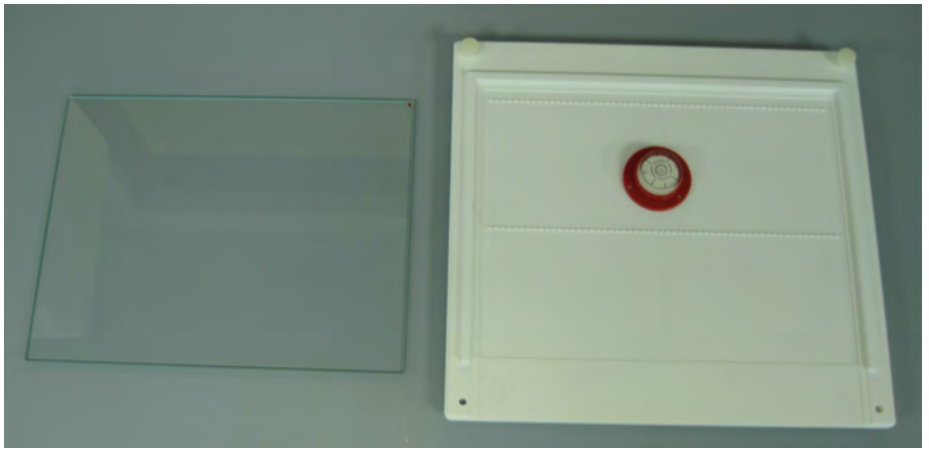
After polymerisation, some unpolymerised acrylamide may be present

WHAT YOU NEED
Horizontal electrophoresis unit, large; Cleaver Scientific
Gel mould, glass plates of different sizes and levelling bubble
Gel wedge for lifting the glass plate from the mould (casting unit)
Peristaltic pump and tubing to recirculate buffer for long runs
Silane 3-(Methacryloyloxy)propyltrimethoxysilane)
Acetic acid (glacial); Ethanol, 50 mL tubes & lint-free wipes
Acrylamide-bis acrylamide solution 19:1, 40%
APS-TEMED sachets (Severn Biotech)
Buffer Stock 50X TAE

1. Prepare the silane solution and glass plate:

1. Prepare the silane solution in a 50 mL tube by adding 8 mL ethanol, 1.8 mL distilled water, 200 µL acetic acid and 10 µL silane (3-trimethoxysilyl-propyl-methacrylate). Mix the solution gently. Any remaining silane solution should be stored in the fridge (4°C) until the plate needs to be re-silanised.
2. Pipette about 1 mL of the silane solution onto a surface of a full plate. For the half and quarter plate, reduce the volume of silane applied proportionately.
3. Spread the solution equally over the glass plate using a lint-free wipe.
4. Cover the glass plate with the lint-free wipe (to prevent dust from settling on the plate as it dries). Leave to air dry for 1 hour.
5. Mark the glass plate with a permanent marker to identify which side is silanised.
6. After 1 hour, polish the glass plate with a lint-free wipe moistened with a small amount of ethanol.

2. Level the gel mould:



1. Position the gel mould on a lab tray and place the level bubble provided in the mould.
2. Turn the screws at the top of the mould until the bubble is centred.

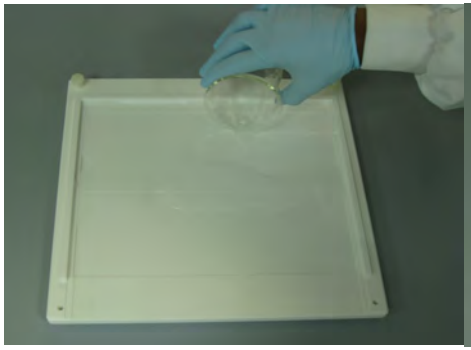
3. Prepare the gel mix:

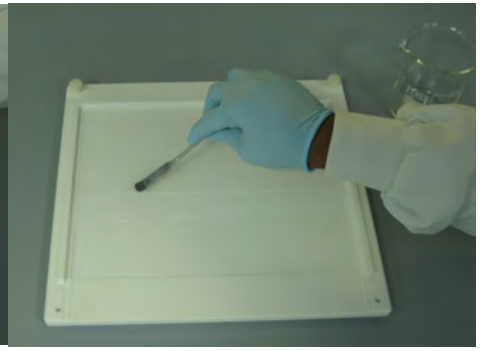
1. The hPAGE mould requires 125 mL gel solution (see the table on page 4) for volumes measured for different sizes of gel plate. For a 6% gel, add the following into a glass conical flask or beaker: 18.8 mL acrylamide-bis 40% gel solution, 2.5 mL 50X TAE buffer, 53.7 mL water. Use 50 mL water to re-suspend the APS-TEMED in the sachet (total 125 mL).

2. Immediately before pouring the gel, prepare the APS-TEMED solution. Put 50 mL of water into a centrifuge tube. Cut open the sachet and add some of this water to the sachet to dissolve the APS-TEMED. Continue to rinse the sachet until all crystals have dissolved. Add this 50 mL of APS-TEMED solution to the gel solution in a beaker and gently swirl so that the gel solution is well mixed.

4. Pour the gel mix into the mould:

1. Quickly, but carefully, pour the gel mix into the mould. Pour first around the gel well notches. To ensure that no bubbles are caught around these, a spatula tip must be run 2-3 times along each row of gel well notches.





4. Pour the gel mix into the mould (cont'd):

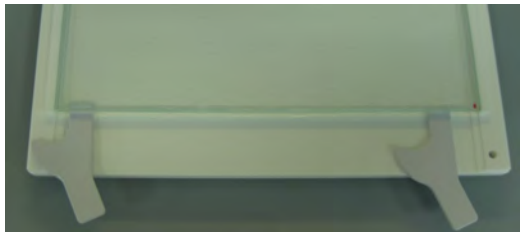
2. Take the glass plate, ***silanised face down***, slot into the top of the mould and lower it carefully making sure that no bubbles form in the gel solution.

3. To monitor the time taken for the gel solution to set: Approximately 1 mL of solution should be pipetted into a microfuge tube.

4. The gel should start to polymerise after 5—10 minutes[1] depending on ambient temperature. Leave 60 minutes to ensure complete polymerisation before loading samples.

5. Run the gel:

1. Use the two gel wedges provided (one either side of the glass plate) to gently lift the glass plate from the mould.



2. Place the glass plate, ***gel side up***, onto the support of the gel tank.

3. Carefully pour additional running buffer[2] into the tank so that it covers the gel plate to a maximum depth of **1-2 mm**.

Notes:

[1] This allows time to lift the plate and remove any bubbles. However, it is still necessary to position the plates and combs as quickly as possible otherwise the gel may polymerise unevenly.

[2] Running buffer can be diluted with bottled drinking water.

5. *Run the gel (cont'd):*

4. To facilitate sample loading, the gel wells can be visualised by placing a black mat in the space underneath the gel tank.

5. Apply the samples in loading buffer (maximum 4 µL[3]).

6. Set the power pack to constant voltage at 120 V (or ~3-5 V/cm) and run the blue dye marker to about two thirds of the gel below the wells or longer for large fragments.

6. *Stain and photograph the affixed gel:*

WHAT YOU NEED
EtBr in water or electrophoresis buffer (0.5 µg/mL or 0.1 µg/mL)[4]
Staining tray
UV Transilluminator, hood and camera unit
UV Mask or UV Safety Spectacles

1. When electrophoresis is completed, remove the gel plate from the tank and wipe off excess buffer.

2. Place gel plate into the staining tray and pipette onto the gel surface the minimum amount of EtBr solution so that the whole gel is covered in staining solution.

3. Stain for 30-60 minutes[5] and then remove the gel plate from the staining tray by allowing the stain solution to run off the gel into the tray. Wipe off excess stain from the base of the plate.

Notes:
[3] The gel well volume is = 4.5 µL
[4] From 10 mg/mL stock: 0.5 µg/mL is 1:20,000 dilution = 5 µL/100 mL; 0.1 µg/mL is 1:100,000 = 1 µL/100 mL.
[5] De-staining for an hour or more in water or buffer can reduce high or uneven background.

6. *Stain and photograph the affixed gel (cont'd):*

4. Turn the gel plate, with the ***gel face down*** on the UV transilluminator. **Note:** this will reverse the order of the gel lanes.

5. Photograph, stain and dispose of the EtBr solution[6] (see page 9).

6. Load the gel images onto the computer. Flip the image to reflect the loading sequence using the editing software.

7. *Removal of gel from the silanised plate:*

1. Scrape the polymerised gel from the glass plate using the scraper and discard the gel in laboratory waste.

2. Scrape the plate a second time removing all traces of residual gel.

3. Clean the plate by wiping it with 70% ethanol and a lint free wipe.

8. *Cleaning the glass plate before re-silanising:*

1. After the plate has been used for approximately 5 times it will need to be re-silanised.

2. Prepare a solution of 2 N NaOH.

3. Plate the glass plate in a tray and then pipette a few mL of 2 N NaOH over the silanised surface of the glass plate. Use a lint-free wipe or tissue **(wear gloves!)** and spread evenly over the plate. Leave for 30 minutes then wash the plate with plenty of water.

Notes:
[6] This protocol reduces the volume and amount of EtBr to a minimum, alleviating the problem of disposal: the concentration of EtBr remaining is lower than 0.5 µg/mL, the level that most regard as safe to dispose of without special precautions.

9. Use of various sizes of glass plate supplied:

1. A set of plates is supplied; a full plate, half plate and 4 quarter plates for use with the large horizontal gel unit. ***Note:*** to polymerise acrylamide solution use one sachet of APS/TEMED for a full plate, and use the appropriate proportion for smaller plates.

2. Separation of PCR products of the same DNA sample can be compared by using a different gel concentration on each of the 4 quarter plates. 35 mL of acrylamide solution is required for each quarter plate, therefore, if only one quarter plate is being prepared reduce APS/TEMED proportionately.

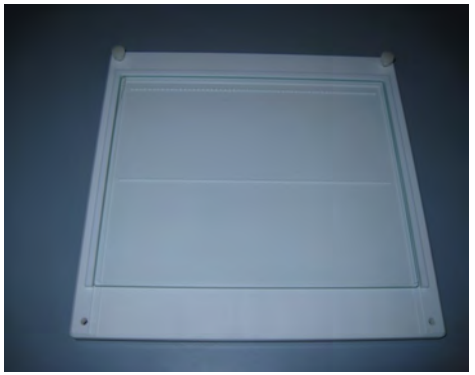
3. When running only a small number of samples, use the quarter plate with the medium size electrophoresis unit supplied.

4. When running a gel for a long time, e.g. overnight, use the peristaltic pump with tubing placed through the two port holes in the large gel tank lid to circulate the buffer during the run.

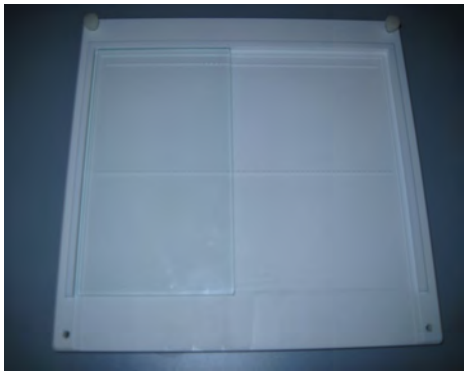
5. See the video: 'The Kirkhouse Trust Horizontal Polyacrylamide Gel Electrophoresis System' at: www.kirkhousetrust.org, go to 'Resources → Training Videos → hPAGE system'.

For a full plate (125 mL)	**4 % Acrylamide solution (mL) for gel**	**6 % Acrylamide solution (mL) for gel**	**8 % Acrylamide solution (mL) for gel**	**10 % Acrylamide solution (mL) for gel**
Acrylamide-bis 40% gel solution	12.5	18.8	25	31.3
Water (mL)	60	53.7	47.5	41.2
TAE Buffer (mL)	2.5	2.5	2.5	2.5
Water to dissolve APS-TEMED	50	50	50	50

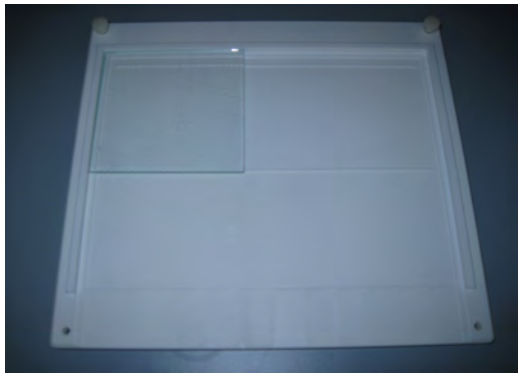
Full plate
27 x 21 cm
125 mL



Half plate used in horizontal or vertical orientation
13.5 x 21 cm
60 mL



Quarter Plate
13.5 x 12.5 cm
35 mL



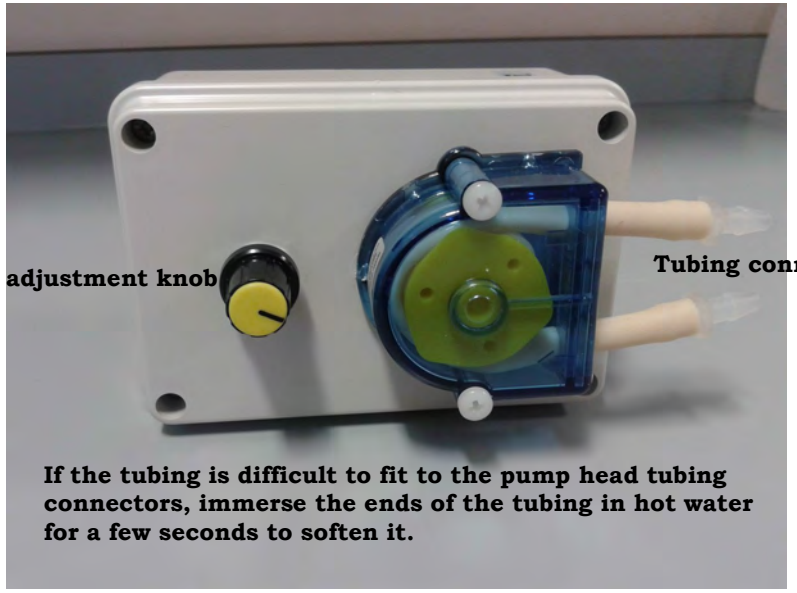
10. Use of peristaltic pump with large gel tank:

1. An Aquadoser 24 V peristaltic pump and tubing is supplied to use with the large horizontal gel tank when performing long runs. The purpose is to minimise changes in buffer performance, i.e. buffer temperature and pH, therefore improving the quality of gel bands produced.

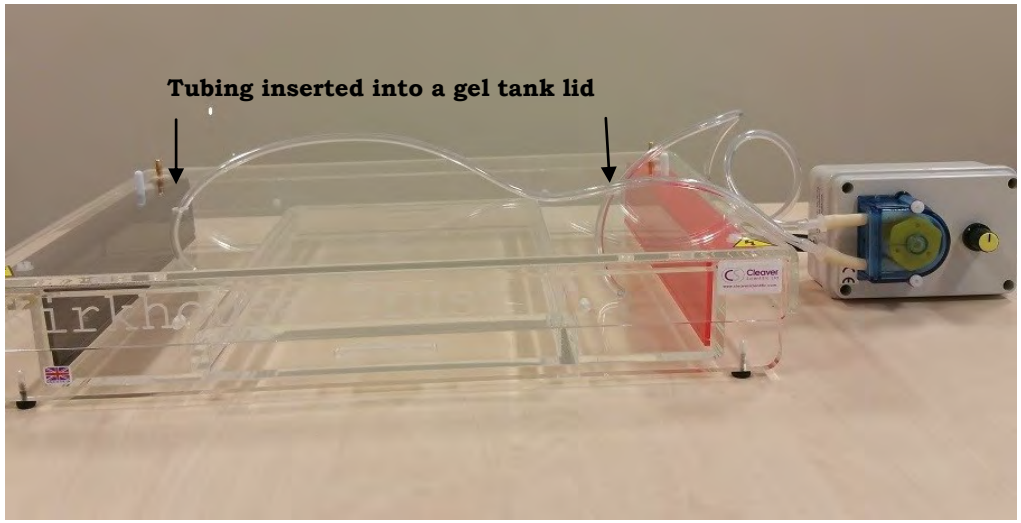
2. The electrophoresis power supply can be set at about 90 V for long runs, for example overnight runs.

3. Set the Aquadoser pump to circulate at about 16 rpm. This can be manually timed by observing the pump head revolutions and timing.

4. It may be necessary to prime the tubing before use; put the ends of the tubing in the gel tank so they are immersed in the buffer but not touching the base of the gel tank. Turn on the pump and allow it to run until bubbles are no longer seen in the tubing. You may need to increase the speed of the pump to clear the bubbles then set it back to 16 rpm.



If the tubing is difficult to fit to the pump head tubing connectors, immerse the ends of the tubing in hot water for a few seconds to soften it.



Peristaltic pump in use, showing tubing route through each of the two port holes in the lid.
Feed the tubing through the each port until the tubing end is below the surface of the buffer but not touching the base of the tank.

1. Cleaver Scientific horizontal electrophoresis units:

In the exceptional circumstance that the platinum electrode is broken (**A**) a new positive (red) or negative (black) electrode for the Cleaver Scientific horizontal electrophoresis unit should be requested from Kirkhouse Trust.

It is important to specify the size of horizontal electrophoresis unit (small, medium or large) and whether the **RED** or **BLACK** electrode is required as each is supplied as a separate cartridge.

2. How to replace the electrode:

1. Loosen the two white plastic screws with a flat head screw driver (**B**). Use your fingers to completely remove the screws (**B** and **C**).

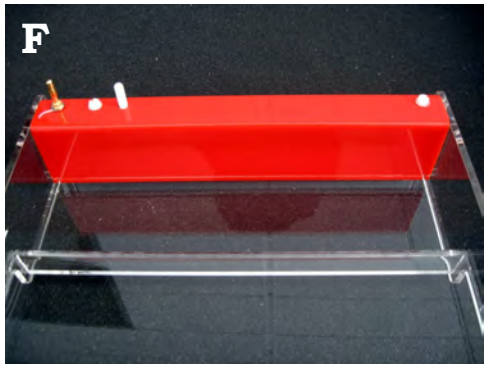
2. Lift the faulty unit (in this example, the negative electrode) from the electrophoresis tray and replace with the new unit (**E**) .

3. Replace the two screws with fingers and only ***tighten very gently*** with the screwdriver:

****CAUTION ****

There is a danger of stripping the screw thread if it is tightened too much.

4. The same procedure is followed to replace the positive electrode (**F**).



1. Preparation of the agarose gel solution:

WHAT YOU NEED
Agarose
50X TAE buffer
Ethidium bromide solution
Microwave

1. Choose a glass bottle or conical flask that is 2-4 times the volume of the solution because agarose boils over easily.
2. Sprinkle pre-measured agarose powder onto room temperature 1X buffer and swirl gently to mix. Weigh the bottle or flask to note the initial weight.
3. Cover with microwaveable plastic wrap and pierce.
4. Heat the container on HIGH power for about 45 seconds or until bubbles appear. ****Caution**** The solution can become superheated and boil over on your hands. As you remove the container, give it a gentle swirl **Wear Gloves** and **Hold at arms length**.
5. Reheat on HIGH power until the solution comes to the boil. Remove, gently swirl and check to see that all the particles are dissolved and the solution is mixed thoroughly.
6. Check the final weight is the same as the initial volume – add distilled water to make up losses.

2. Pouring the gel:

WHAT YOU NEED
Cleaver Scientific horizontal electrophoresis unit
DNA loading buffer / dye solution
50 bp or 100 bp DNA ladder
50X TAE buffer
Power pack

1. Allow the agarose solution to cool to 50-60°C[1]. If an 'in-gel' staining method is to be used, add the nucleic acid stain and swirl gently to mix thoroughly.
2. Various sizes of the horizontal gel electrophoresis units have been supplied. To cast a gel, the trays do **not** need to be taped. An electronic copy of the instruction manual can be found at www.kirkhousetrust.org, go to 'Resources → Research Resources → Equipment Manuals'.
3. Gel thickness has a profound effect on the resolution of smaller fragments – for optimal resolution cast horizontal gels **3-4 mm thick**.
4. After pouring gel leave to set for 1 hour (with the gel unit lid on if possible).

Notes:
[1] If the agarose solution is hotter than 50-60°C it will cause the Perspex tray to warp.

3. Loading and running the gel:

1. Add 1 µL of DNA dye loading buffer[1] per 5 µL of sample and mix thoroughly.
2. DNA ladders (50 bp, 100 bp and 1 kb) are provided ready-to-use in loading dye
3. Buffer Depth should be 3-5mm.

   For <1 Kb voltage should be 5 V/cm[2].

   Gel should be run until the band of interest has migrated 40-60% down the length of the gel.

4. Photographing the gel:

See Gel Photography Protocol — page 9.

Notes:

[1] Thermo Scientific 6X DNA Loading Dye is supplied in a 1.0 mL vial . Use with samples on agarose or polyacrylamide gels. It contains two dyes, bromophenol blue and xylene cyanol FF, for easy visual tracking of DNA migration during electrophoresis at 6X concentration. Composition 10 mM Tris-HCl (pH 7.6) 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol 60 mM EDTA. Add 1/6 volume of 6X DNA Loading Dye to your DNA sample.

Notes:

[2] Power Supply: Voltage which is too high will cause band streaking and smearing, if the voltage is too low, it will cause band broadening due to diffusion.

WHAT YOU NEED
Gel hood with Canon PowerShot (or similar) camera
UV transilluminator
UV facemask (IMPORTANT)
Fluorescent ruler

Image of a gel stained with EtBr

1. Set up camera and gel:

1. Switch on the camera.
2. Set to Tv (shutter priority) and use the wheel (just below the zoom) to set 1 sec exposure.
3. Set to close up (press the control ring on the right of the camera; a flower icon).
4. Disable flash (by pressing the right side of the control ring)
5. Place the gel plate face down in a UV transparent Perspex tray in the centre of the transilluminator and also for polyacrylamide gels attached to a glass plate[1].

Notes:

[1] Glass is ***not*** UV transparent.

1. Set up camera and gel (cont'd):

6. Lay the fluorescent rule alongside the gel.
7. Place the camera hood over the gel dish.

2. Photograph the gel:

SAFETY NOTE:
Make sure your eyes are protected from the UV lamp!

1. Switch the transilluminator to full power.
2. Select a zoom setting to cover the selected area of gel – you may not be able to focus if you zoom in too close.
3. Depress the shooting button halfway to focus the camera.
4. Depress completely to take the shot.
5. Check the exposure and adjust the exposure time if necessary to obtain better shots.
6. Transfer the image to a computer and store in a folder[2,3]

Notes:

[2] The default resolution setting produces files which are unnecessarily large for most purposes. To create smaller files, press 'FUNC SET' and step down ten places. Step right to 'S'. Alternatively, you can reduce the size of the file using a program such as Paint.net. In addition, you can also download a free program such as Image J : **http://imagej.nih.gov/ij/**
For information on file compression go to www.kirkhousetrust.org →Resources → Help & Advice → File Compression.

[3] You will need a USB cable or a card reader for this.

Author: ed.southern@bioch.ox.ac.uk; October 2016

Kirkhouse Trust Policy:

The Kirkhouse Trust makes no recommendation for disposal of Ethidium Bromide. You should follow the regulations that pertain to your institution.

However, we do recommend that you use low concentrations, 0.5 µg/mL or lower, of EtBr for staining gels. There are two reasons for this. First, higher levels will introduce high background in the gel photograph. Second, if there is a hazard, the lower the concentration, the lower the risk. So we recommend staining in 0.1 µg/mL [1] for 1 hour or longer, or staining in 0.5 µg/mL[2] for 15 to 30 min and de-staining, if necessary, for 30 min.

Also see the video: Kirkhouse Trust Horizontal Polyacrylamide Gel Electrophoresis System at www.kirkhousetrust.org, go to: Resources → Training Videos → hPAGE system.

Notes:

[1] 0.1 µg/mL is a 1:100,000 dilution in TBE of 10 mg/mL EtBr stock (e.g. 0.1 µL in 10 mL).

[2] 0.5 µg/mL is a 1:20,000 dilution (e.g. 0.5 µL in 10 mL).

What others say:

If no regulation is in place in your institute, you may wish to see what others recommend. Practices vary widely:

1. Princeton University and the University of Pennsylvania recommend that levels below 10 µg/mL can be disposed of without special precautions.
   http://web.princeton.edu/sites/ehs/chemwaste/etbr.html#gel
   http://www.ehrs.upenn.edu/resources/waste/chem/ebpolicy.html

2. The University of Birmingham recommends that levels below 1.0 µg/mL can be disposed of without special precautions.
   http://www.biosciences.bham.ac.uk/safety/Disposal%20of%20Ethidium%20Bromide%20Waste%200905.doc

3. Columbia University recommends decontamination treatments for solid waste and for solution concentrations above 0.5 µg/mL.
   http://ehs.columbia.edu/etbr.html

4. Here is a blog with multiple opinions:
   http://rrresearch.blogspot.com/2006/10/heresy-about-ethidium-bromide.html

ITEMS SUPPLIED
Ethidium bromide de-staining bag
Beaker, plastic 1 or 2 Litre (label 'Ethidium Bromide Waste')
Gloves/laboratory coat/safety glasses
Pair of forceps

Disposal of ethidium bromide solution with a de-staining bag

1. Add one bag to your ethidium bromide waste solution from the staining of a gel (agarose or hPAGE).

2. A single de-staining bag will remove **5 mg** of ethidium bromide. Calculate the amount of ethidium bromide in your solution and change the bag after approximately **20—30 gels**.

3. The de-staining bag works by adsorption of the stain and will completely remove the pale orange colour due to the ethidium bromide.

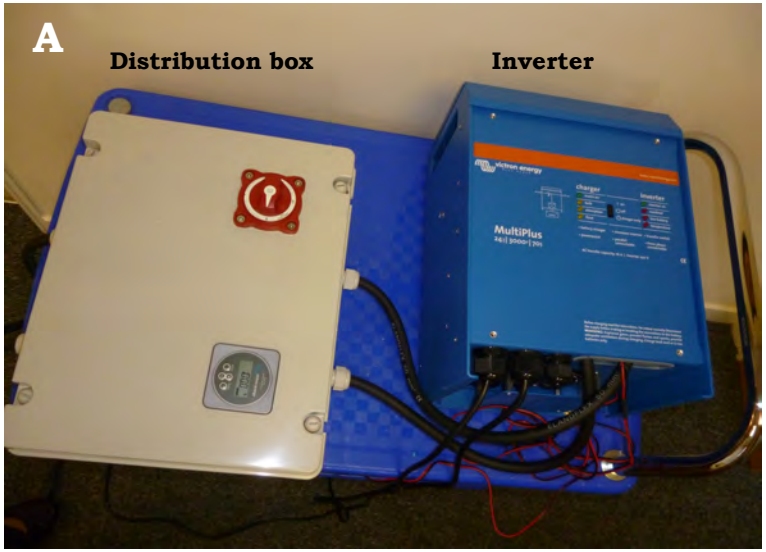
4. When solution is colourless, dispose of the liquid in the normal way. Do not discard the de-staining bag, continue to use it until the 5 mg ethidium bromide limit or 20-30 gels have been de-stained.

5. Replace with a new de-staining bag after 20-30 gels have been de-stained and dispose of the used bag in accordance with your institution's regulations for hazardous waste.

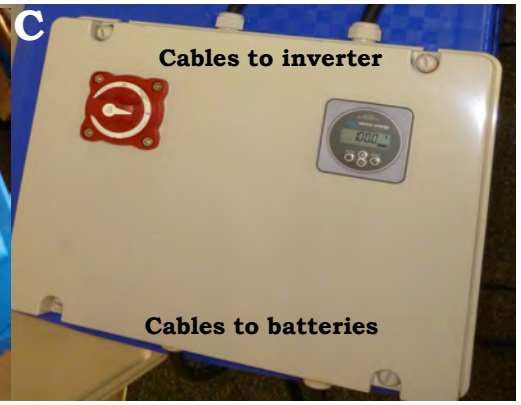
WHAT YOU NEED
Inverter, battery cables and battery linker cable, distribution box with battery monitor supplied by KT
10 mm Spanner / Adjustable Spanner / 10 mm Socket / Screw driver
2 Deep Cycle Batteries: We recommended Victron AGM Battery 12V 220Ah Deep Cycle C-20

1. Connect the inverter to the distribution box

1. For the **Victron 24/3000/70-16 Inverter model**, unpack the blue inverter and white distribution box and lay out the Inverter as shown in **(A),** next to a mains electricity socket and the equipment to be used with the battery back-up (also known as UPS or Inverter).



2. Unscrew the inverter face plate **(B)**. Connect the two battery cables which come from the 'top' end of the distribution box as shown in **(C).** Pass the cables through the aperture. Loosen the large nuts and attach the cable with a red sleeve to the (+) positive terminal and the cable with the black sleeve to the (-) negative terminal. Tighten the nuts.

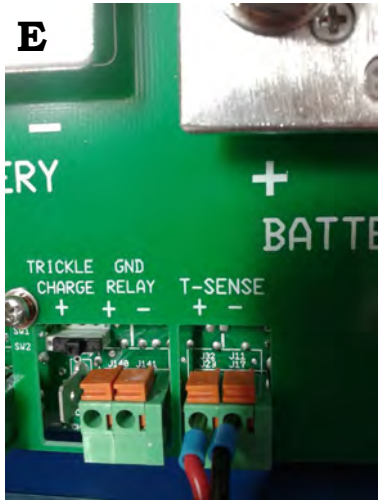


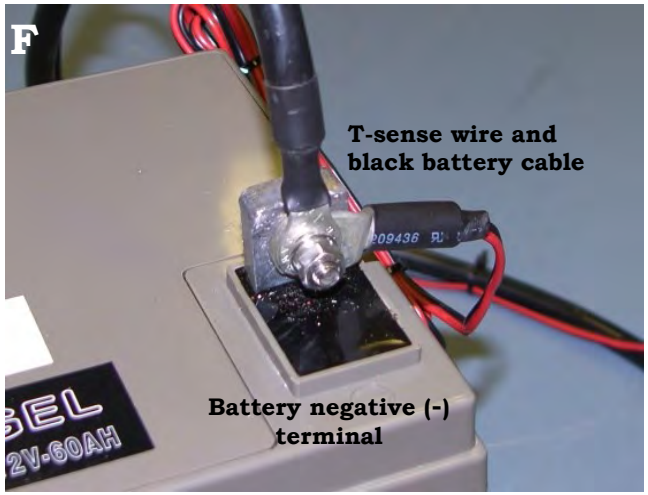
2. Connect the batteries with battery linker cable

1. Connect the two 12 volt batteries **(D)** together with the short black linking lead with a **red end sleeve.** Connect the red (+) positive terminal of one battery to the black (-) negative terminal of the other.

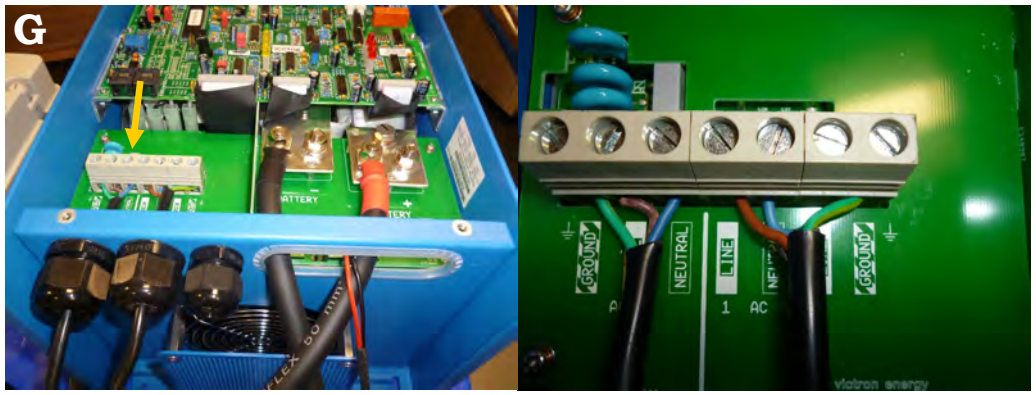
3. Connect the distribution box cables to the batteries; the T-sense cable and the two AC cables

1. Connect the battery cable with the **red end sleeve** from the bottom of the distribution box to the **positive (+)** terminal of the battery.
2. A thin red/black T-sense cable with metal terminal pins is supplied with the inverter (in the blue box the inverter is packed in). Connect the terminal pins into the inverter—two green sockets with orange clips marked 'T-sense' and (+) and (-) **(E)**. Connect the red cable to the (+) and the black cable to the (-). Then attach the opposite end of this cable to the (-) negative terminal of the battery **(F).**



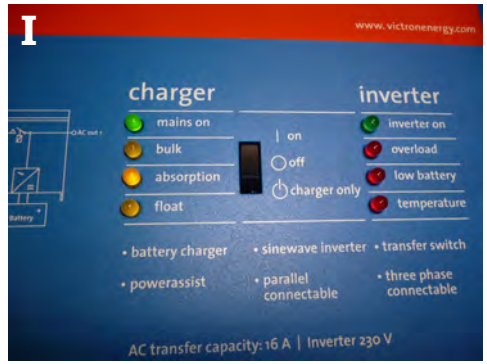


3. Once the final battery connection is made, the battery monitor display will be activated but will need to be set with the correct values (see Section 5 or the Victron Battery Monitor Instruction Manual).
4. Connect the AC-IN and AC-OUT cables to the inverter **(G)**. Ensure that the plug on the AC-IN cable has a 13 amp fuse. The AC-OUT cable can have either a 3-pin kettle plug to connect with equipment or a regular plug with a 13 amp fuse to connect with a surge protector.



4. Switch on and set battery monitor

1. Plug the Inverter power AC-IN lead into the mains electricity and turn the red master switch on the distribution box clockwise to the 'ON' position **(H).** On the inverter, press the rocker switch to 'ON' **(I).** The Inverter is now 'live' and connected to the batteries.
2. Connect the Inverter output lead (AC-OUT) with appropriate plug to equipment or a surge protector**.** Press the Inverter switch to ON. The Inverter LED will light up briefly but if the mains power is on, the Inverter will automatically revert to charging mode and charge the batteries **(I)**.



5. Switch on and set battery monitor (continued..)

3. To set the battery monitor use the + or — buttons to scroll through to see the set values for battery voltage, current, power, ampere-hours consumed and % battery charge.
4. The settings depend on the total number of amp hours available from the batteries. It is strongly advised not to use more than 50% of the total amp hours available.

Press SETUP for two seconds to access these functions and use the **+** and **–** buttons to browse through the options.

Press SELECT to access the desired parameter.

Use SELECT and the **+** and **–** buttons to customize. A short beep confirms the setting.

Press SETUP at any time to return to the scrolling text, and press SETUP again to return to normal mode.

Setting 01	Battery capacity. Set the total amp hours of the batteries at 50% e.g. 120 Ah total, set battery capacity at 60 Ah.
Setting 02	Charged voltage. Set at 28.4 V
Setting 03	Tail current. Set at 1%
Setting 04	Charged detection time. Leave this at the default (3 minutes)
Setting 05	Peukert exponent. Leave at the default (1.25)
Setting 06	Charge efficiency factor. Set at 83%

5. For further information consult the BM700 Instruction Manual pages 17-21. This manual and the Instruction Manual for the Inverter can be found at www.kirkhousetrust.org. Go to 'Resources → Research Resources → Equipment Manuals'.
6. When the Inverter is switched from OFF to CHARGER ONLY the Charger LED will light **(I)** and send power into the batteries.
7. If the power supply fails, the Inverter will automatically switch from CHARGER to INVERTER mode and supply power to the equipment from the stored energy in the batteries. The digital display will show less than 24 volt **(J).** When mains power is restored the inverter will return to CHARGE mode after 30 seconds.

1. Horizontal polyacrylamide gel:

This space has been left for you to add your own notes.
Use the permanent marker pen supplied.

2. Agarose gel:

This space has been left for you to add your own notes.
Use the permanent marker pen supplied.

1. Kirkhouse Trust policy on economical use of FTA:

The Kirkhouse Trust provides GE Whatman FTA technology for its funded projects. However, for the supply to be replenished the Trust has set the following conditions:

1. FTA cards, FTA solution and the Harris punch/cutting mat must only be used for the projects KT funds and for no other research work without prior approval.

2. FTA Technology is expensive and it is expected that the user will employ it in the most economical way. Staff on the project should be instructed to use the FTA cards economically.

3. Each FTA card has four sample sections, but ***with careful and judicious use each sample section can be further sub-divided.*** (Please see page 17 for a visual example).

2. Storage of FTA Cards:

1. Cards must be archived and stored properly. Place a card in a clip-top plastic bag and store in a desiccator containing active desiccant. The desiccant is self-indicating so that it can be renewed when inactive.

2. A regime should be in place with someone formally designated to be responsible to ensure that these instructions are being followed and, in particular, that the desiccant is checked regularly and remains active.

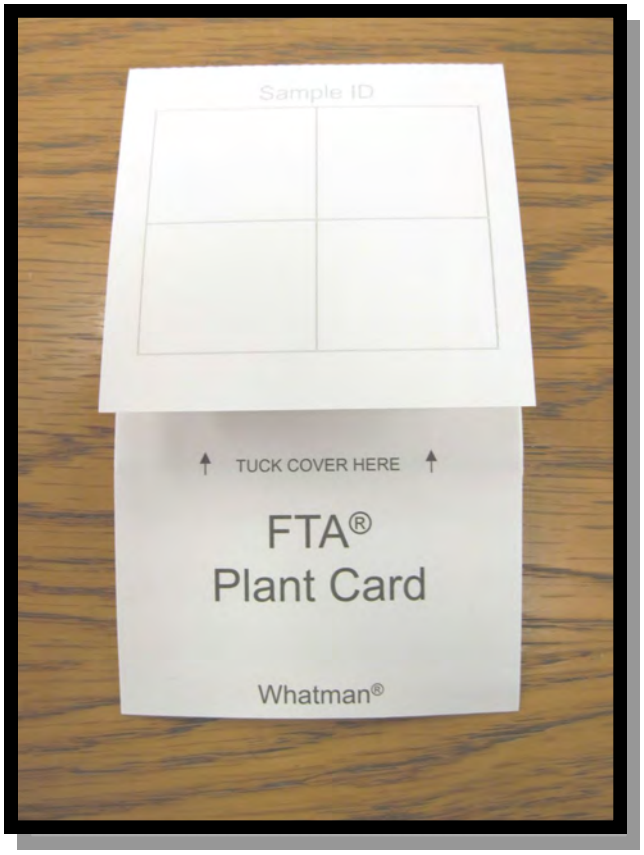
3. FTA Card Protocols can be downloaded at www.kirkhousetrust.org Go to 'Resources → Research Resources → Equipment Manuals'.

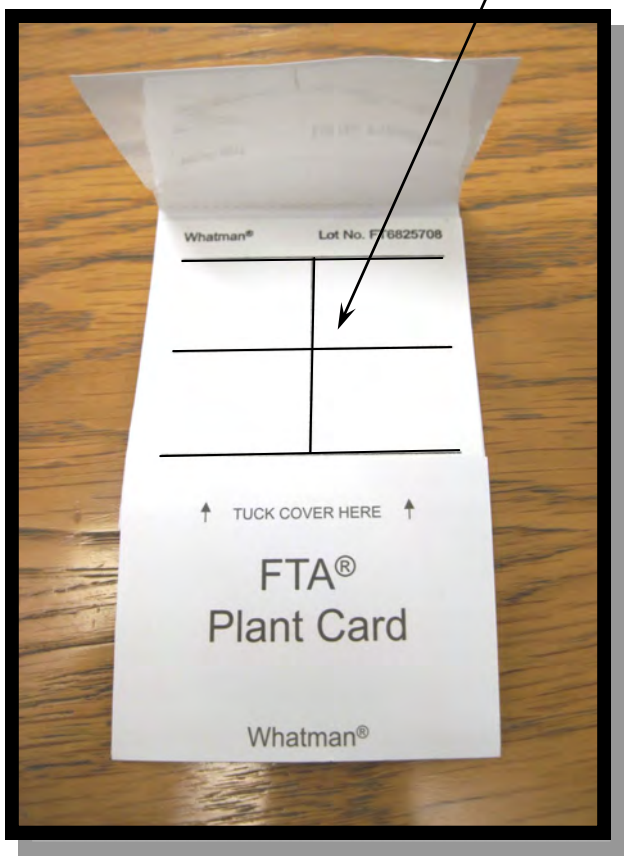
1. Sub-dividing of FTA Cards:

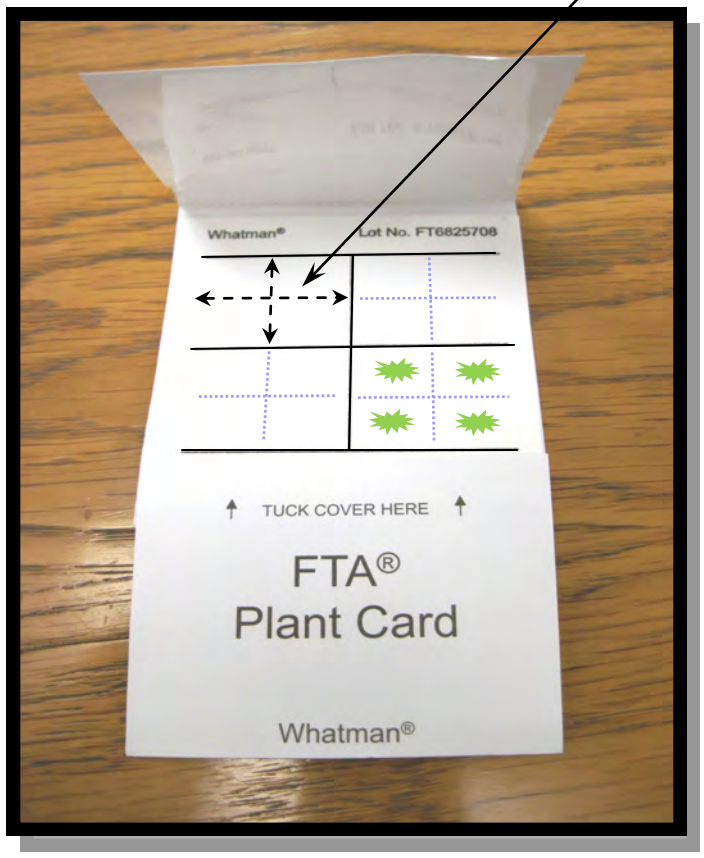
New Card

Mark the new card into four sample areas

Each of the four sample areas should be sub-divided into four to produce a sixteen square grid, use a pencil to draw sub-divisions







This system allows adequate space to accommodate one leaf sample within each square of the newly divided sixteen square grid.
A box of 100 FTA Plant Cards will allow 1,600 plant samples to be collected.

1. Sample collection by direct leaf press:

WHAT YOU NEED
GE Whatman FTA PlantSaver card
Parafilm
Pestle
Plastic clip top bag, desiccator and silica gel desiccant

Always wear gloves when handling FTA PlantSaver Cards to avoid contamination of the cards.

Store unused FTA PlantSaver Cards in a desiccator. (Avoid light and excessive humidity).

1. Label the FTA PlantSaver Card with the appropriate sample identification. Divide each of the four sample areas on the card into 2 or 4 sections so that the card can accommodate 8-16 samples (see page 15 Use of Whatman FTA Plant Cards).
2. Place the leaf over the marked area (the underside of the leaf facing down) on top of the FTA matrix card.
3. Overlay the leaf, either with Parafilm or replace the cover sheet.

1. Sample collection by direct leaf press (cont'd):

4. Using a heavy blunt object (such as a small porcelain pestle, tack hammer or screwdriver handle) pound each sample area with moderate force for 15 seconds[1]. This will burst the cell walls of the plant tissue.
5. It is important that the samples be pressed by a smooth surface so that the filter matrix is not damaged.
6. Verify that sufficient plant material has been transferred to the paper by checking the back of the FTA card: plant tissue should be visible on the other side of the matrix.
7. **Use great care not to damage the matrix.**
8. Ensure that no large pieces of plant tissue remain adhered to the FTA card as this may interfere with later processing.
9. When samples have been transferred to the paper allow the FTA card to air dry for a minimum of one hour at room temperature.
10. If sample is to be archived, place in a clip top bag and store in the desiccator with active silica gel (all items supplied by KT).

Notes

[1] A quality leaf press is the most important factor for obtaining good quality plant DNA sample on the FTA card. A leaf press made with too little force will not transfer enough DNA to the card. For the best results it is important to apply quick, strong pressure to the Parafilm/leaf/card sandwich. This will provide enough momentum to break the cell walls. Applying too much or too little pressure or pressure that is uneven is not likely to work well. Also, hitting the card too strongly will damage the matrix making it too fragile for processing. When applying force, do not use a rubbing motion.

2. Sample collection of plant homogenate:

WHAT YOU NEED
GE Whatman FTA PlantSaver card
Parafilm
Pestle
Phosphate-buffered Saline (PBS) buffer
Plastic clip top bag, desiccator, and silica gel desiccant

1. Label the FTA PlantSaver card with the appropriate sample identification.
2. Use a minimum of 10 mg of young plant tissue. Add 1 part plant tissue to 5 parts PBS and using a mortar and pestle grind leaf material to a smooth homogenate (if preferred use a micropestle and microfuge tube). The ratio of 1 part plant material and 5 parts PBS is critical for good results. [For soybean and some species of cereal it may be necessary to add dithiothreitol (DTT) to improve the amount of DNA that binds to the FTA].
3. Apply the homogenate to the FTA PlantSaver card matrix inside the marked circle using a wide mouth pipette or a pipette tip that has been cut to give a 1.5 to 2.0 mm opening (the sample will likely be too viscous to use pipette tips with narrow openings). Allow the sample to air dry on the FTA card for a minimum of 2 hours at room temperature.
4. If all of the plant tissue cannot be homogenised completely, the semi -homogenised tissue can be pressed against the card and then discarded.
5. If the sample is to be archived, place in a clip-top bag or store in a desiccator with active silica gel (all items supplied by KT).

3. Removing a sample disc from an FTA Card for analysis:

WHAT YOU NEED
Whatman FTA PlantSaver card
Harris 2 mm punch or similar
Cutting mat

1. To prevent carry-over between samples, always ensure that the sample applied is dry before taking a punch.
2. Place the FTA PlantSaver card on a cutting mat. For cards with outer paper layers, ensure that the mat is directly beneath the FTA card with no paper layer in between.
3. Place the tip of the coring punch, e.g. a Harris 2 mm micropunch, over the area to be sampled. Do NOT depress the ejection plunger at this time.
4. Press down firmly on barrel of the coring device and twist one quarter turn to cut the disc out of the card.
5. Once the disc is in the corer, transfer the disc to the desired PCR tube or tray by depressing the ejection plunger and ejecting the disc.
6. Care should be taken when handling the dry FTA discs because the static charge that can develop on some plastic labware can cause the disc to be ejected from the tubes and adhere to other surfaces.
7. In order to ensure there is no cross-contamination between samples, the coring device can be cleaned using one of the two methods described below. Use the method which fits best your laboratory workflow.

4. Cleaning the corer tip:

1. Rinse the tip with ethanol between samples and dry with a sterile wipe.

   OR

2. Take one punch from blank filter paper or an unspotted area of the FTA Plant Card between samples.

5. Preparing the FTA for DNA analysis:

WHAT YOU NEED
FTA Purification Reagent - TE buffer pH 8 1X with 1% Triton X
Microfuge tubes
TE^{-1} pH 8.0 buffer

1. Take a sample disc from the dried spot (follow the instructions in Protocol 3). For plant samples a 2 mm disc is recommended.
2. Place disc in a 0.5 mL or 1.5 mL micro-centrifuge tube.
3. Add 200 µL of FTA Purification Reagent to the tube.
4. Incubate for 5 minutes at room temperature with moderate manual mixing.

5. Preparing the FTA for DNA analysis (cont'd):

5. Remove and discard the used FTA Purification Reagent with a pipette.
6. Repeat steps 3-5 once, for a total of 2 washes with FTA Purification Reagent.
7. At this stage the FTA disc should be white (i.e. no chlorophyll). If the disc is still very green, repeat steps 3-5 with an additional FTA Reagent wash.
8. Add 200 µL of TE^{-1} Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).
9. Incubate for 5 minutes at room temperature.
10. Remove and discard all used TE^{-1} Buffer with a pipette.
11. Repeat steps 8-10 once for a total of 2 washes with TE^{-1} Buffer.
12. Ensure that all the liquid has been removed before performing analysis. The disc maybe allowed to dry[1].

Notes:
[1] It is recommended that analysis be conducted within 3 hours of the disc washing. If this is not possible, the punch can be stored at 4 °C or -20 °C in a dark environment for up to 1 week.

6. Modified protocol for enhanced sample washing:

With some plant species removal of chlorophyll from the punch becomes difficult. This may interfere with downstream analysis. The following protocol is an enhanced washing method developed to remove the chlorophyll from such samples:

1. After washing with the FTA Purification Reagent, wash the punches with 200 µL iso-propanol.
2. Incubate for 2 minutes, pipette up and down a couple of times and discard. Repeat for a total of 2 washes with isopropanol.
3. Dry the punches at room temperature to ensure that the isopropanol has been completely removed.

7. PCR of washed punch:

1. The washed and air-dried disc is now ready for analysis by PCR using standard protocols.
2. The disc is included in the PCR reaction.
3. There is no need to change reaction volume or PCR conditions due to the presence of the disc.
4. For the PCR it can be safely assumed that the punch + DNA constitutes zero added volume.
5. Recommended reaction volume of the PCR analysis of plant DNA is between 25-50 µL.
6. For some species of plant a 2 mm punch may contain too much DNA causing the PCR reaction to be inhibited. In this situation a smaller 1.2 mm punch or similar can be used in as much as 50 µL of PCR reaction mix.

1. CTAB DNA extraction from plant tissue: Use only when the FTA method is inappropriate e.g. for bulk segregant analysis

WHAT YOU NEED
Plant tissue - frozen or fresh
Mortar & pestle OR 2 mL centrifuge tubes, and micro-pestles (all kept at –20°C for ~1 hour)
Microcentrifuge and dry block set at 65°C
Solvents—isopropanol, ethanol, Chloroform-isoamyl alcohol (24:1)
Buffers and solutions prepared beforehand (see page 23)
Stuart Tube Rotator (or you can mix by hand)

1. After sampling from the plant, immediately wash the fresh plant leaf with distilled water and blot dry with paper towel. Surface sterilise the leaf with 70% ethanol.
2. Grind 200–500 mg plant tissue in either a mortar or 2 mL tube until all the tissue is disrupted. With larger leaves, roll into a tube-shape and place into the 2 mL tube before macerating.
3. Add 400 µL of hot (65°C) CTAB extraction buffer to each tube.
4. Mix well by flicking the tube (do not vortex)[1] and incubate the samples for ~20 minutes at 65°C (ideally, shake the samples every 5 minutes or so during the incubation to keep the tissue saturated).
5. After incubation add 400 µL of chloroform-isoamyl alcohol (24:1) to each tube. Perform this using a fume hood or similar.
6. Shake the tubes using a rotary shaker at room temperature for 15 minutes.
7. Centrifuge the samples at ~12,000 x g for 5 minutes.
8. Transfer the upper, clear aqueous layer of each sample to a new tube (avoiding plant debris and chloroform layers) and add 400 µL of chloroform-isoamyl alcohol (24:1) (equal volume), then repeat steps 5 and 6.
9. Transfer the upper aqueous layer to a new tube (avoiding taking any of the chloroform layer) and add an equal volume of isopropanol (~400 µL).
10. Mix the samples (by gentle inversion) and incubate at room temperature for 15–30 minutes.
11. Centrifuge the samples at maximum speed in the micro centrifuge for 5 minutes.
12. Discard the supernatant. This can be done with care by aspirating off the supernatant with a 1 mL pipette.
13. Add 400 µL DNA washing solution (kept on ice) to each sample and agitate gently. Leave for 5 minutes before centrifuging for 3 minutes. Remove supernatant. If the pellet of the sample is not clean, then repeat this step again[2].
14. This is a good stopping point in the protocol if you find that time is limited. Add the DNA washing solution to each sample and leave overnight at 4°C.
15. Place the open tubes on a clean, absorbent surface inverted for 10 minutes to dry the pellet or until most of the reagent has evaporated from the tube.
16. Resuspend the pellet in 100 µL TE buffer and 6 µL RNAse A (10 mg/mL).
17. Incubate the samples at room temperature for 15-30 minutes.
18. Precipitate the DNA by adding 1 mL cold 100 % ethanol (kept on ice) and incubate at room temperature for 30 minutes.
19. Centrifuge the samples for 5 minutes and discard the supernatant. Allow the pellet to air dry for ~ 2 hours.
20. Re-suspend the pellet in 100 µL TE overnight at 4°C.

Notes:

1. Never vortex genomic DNA too vigorously as it shears it.

Note:

2. The pellet might not stick tightly to the tube after this step so care is advised.

2. Stock solutions of buffers and reagents:

1. 10 % hexadecyltrimethylammonium bromide (CTAB): Add 10 g CTAB to 80 mL H_2O. Heat to 65 °C to dissolve and adjust the volume to 100 mL with H_2O. Store at room temperature.
   *** Do NOT autoclave CTAB solution ***
2. 5 M sodium chloride (NaCl).
3. 1 M tris-hydrochloric acid (Tris-HCl) pH 8.0.
4. 0.5 M ethylenediaminetetraacetic acid (EDTA) pH 8.0[3].
5. DNA washing solution stored at 4°C: 70 % ethanol , 0.77 g/ L ammonium acetate.
   *** Do NOT autoclave ammonium acetate solution[4] **.*
   Filter solution using a 10 mL syringe through a 0.2 µm filter unit.
6. Dithiothreitol (DTT): Weigh out just before use and add to CTAB buffer.
7. RNase A (10 mg/mL).
8. Proteinase K (20 mg/mL).

3. CTAB working solution:

CTAB Extraction Buffer: 2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0 and finally add DTT to 1% just before use.

Reagent	Final concentration	Stock concentration	To prepare 5 mL
CTAB	2 %	10 %	1 mL
NaCl	1.4 M	5 M	1.4 mL
EDTA pH 8.0	20 mM	0.5 M	200 µL
Tris-HCl pH 8.0	100 mM	1 M	500 µL
DTT	1 %	-	50 mg
dH_2O	-	-	1.85 mL

4. Stuart Tube Rotator (mixer):

1. Place tubes with lids firmly closed and facing inwards. Balance the tubes so the disc rotates evenly.
2. Switch the unit on using the green on/off switch. The unit will rotate at a fixed speed of 20rpm.
3. Once mixing is complete switch the unit off using the green on / off switch and unload the tubes.

Stuart Tube Rotator
Position the tubes on the disc so they are balanced, for example place tubes opposite each other.

Note:
[3] EDTA will not go into solution below pH 8.0. Add solid NaOH sparingly and allow the solution to equilibrate between additions to avoid over-shooting the final pH.
[4] Ammonium acetate decomposes in hot water and should not be autoclaved.

WHAT YOU NEED
Lyophilised oligonucleotides (Primers)
1X TE buffer pH 8.0
Sterile screw cap 2 mL tubes

1. Resuspension of lyophilised oligonucleotides:

1. Oligonucleotides supplied by KT are lyophilised. The oligo pellet may become dislodged in transit and end up in the lid of the tubes / plate wells. Before opening the tube, it is important to spin down every oligonucleotide tube in the centrifuge or agitate the plate to dislodge stuck pellets.
2. Dried DNA is usually very easy to re-suspend in aqueous solution but some oligos need more time to go into solution than others. Re-suspend in TE buffer pH 8.0 (1X).
3. To reconstitute, use the nanomole quantity for each specific oligo shown on the datasheet. For example, to make a 100 µM concentration stock solution: Take the number of nmoles in the tube and multiply that by 10. This will be the number of microlitres of buffer to add to get a 100 µM solution.
4. Once reconstituted[1], divide the remaining stock solution into several small aliquots of oligos and store the stock solution at 4°C in a fridge if you are going to use it soon, but freeze at –20°C. Avoid too many freeze-thaw cycles.

Notes:

[1] Oligos can be stored at different concentrations but concentrations <1 µM may change over time as some of the oligo can adhere to the plastic of the tube.

2. Use this area to calculate your oligo concentration:

This space has been left for you to work out your own oligo concentration.

3. Cowpea plates, sets 1 and 2

The Cowpea primer sets are supplied with the forward and reverse primers mixed together. The oligos have a final concentration of 100 µM. The plates are supplied dried (lyophilised) and have been standardized to be made up to 200 µl. Add 200 µl of 1X TE buffer pH 8.0 to each well or tube to reconstitute the primers. You will have to agitate them a bit to get them to reconstitute in the buffer liquid.

4. Properties of oligonucleotides:

You may need to calculate the molecular weight, melting temperature or some other property of an oligonucleotide which depends on its base sequence. Programs are available to help you do this. For example:

http://www.basic.northwestern.edu/biotools/oligocalc.html

1. GE Illustra puReTaq™ Ready-To-Go™ PCR beads:

WHAT YOU NEED
Thermal cycler (PCR machine)
GE Illustra PCR beads[1] (dried as a single bead in tube)
Primers—1 or 2 µL of each primer
Template DNA in FTA punch or other sample
Molecular Biology grade water
Additional PCR tubes to divide the pre-mix into two

1. GE PCR beads are supplied in a sealed pouch as they are sensitive to moisture. Once a pouch is opened, any unused tubes must be stored in a desiccator containing active desiccant (silica gel—see page 38).

2. Each tube contains a single bead and is made up to a 25 µL reaction mix[2]. The pre-mix can be divided into two PCR tests using the additional tubes supplied. There is no loss of resolution.

3. Add 1 or 2 µL each of forward and reverse primers to the tube.

4. Mix the contents of the tube gently. The reaction is fully dissolved and mixed when the solution appears clear.

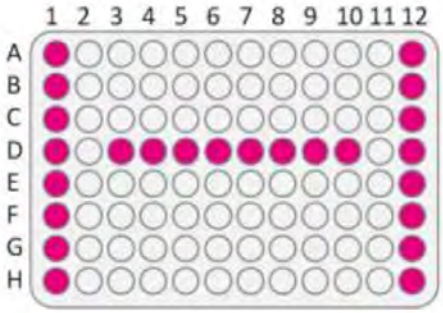
5. Load tubes onto thermal cycler and run chosen PCR program.

Notes:
[1] Composition of a GE PCR bead: Stabilisers; BSA; dNTPs; 2.5 units puReTaq DNA polymerase and reaction buffer.
[2] When a bead is reconstituted to a final volume of 25 µl, the concentration of each dNTP is 200 µM in 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM $MgCl_2$.

2. ABI 2720 or SimpliAmp Thermal Cyclers and BioRad MyCycler:

PCR Protocols for the ABI thermal cyclers are found in the Instruction Manual CD provided and PDF versions of Instruction Manuals can be found on the Trust website: www.kirkhousetrust.org, go to:
'Resources →Research Resources → Equipment Manuals'.

Hints & Tips: For all thermal cyclers, ensure that all consumables used in the block are of the same height and are spread evenly across the block. Insert empty "dummy" tubes if necessary in each corner to spread the pressure of the heated lid evenly.



Example of the placement of PCR tubes to ensure the heated lid applies even pressure on the tube lids. Some of the tubes can be "dummy" tubes filled with just water or buffer.

WHAT YOU NEED
DNA ladder that contains fragments of known concentration appropriate to unknown sample.
Agarose or hPAGE gel after electrophoresis with unknown sample(s) and dilutions of DNA ladder.

1. Preparation of DNA ladder and samples

1. For an hPAGE gel with a 55 well gel comb, each well can be loaded with a maximum volume of 4.5 µL. Load 1 µL, 2 µL and 4 µL of an appropriate DNA ladder.

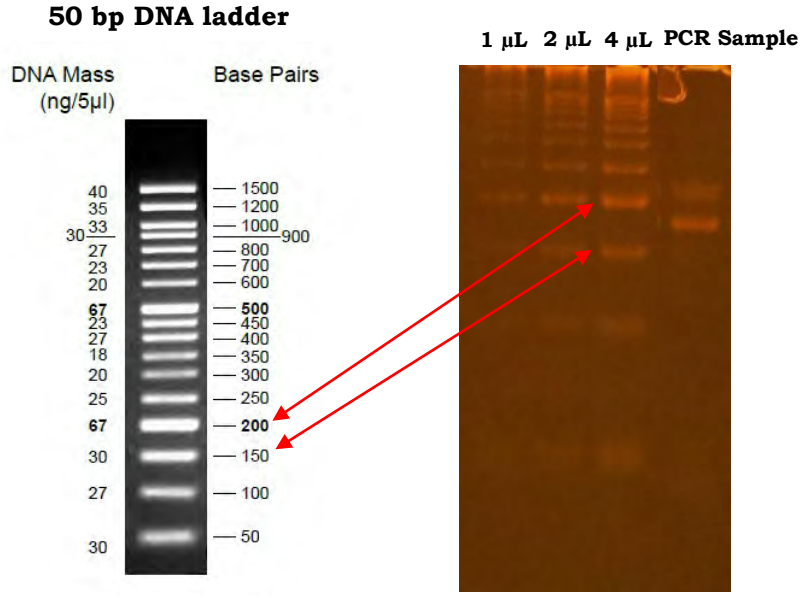
2. The unknown sample (either from a genomic DNA extraction or PCR product) will require the addition of loading buffer. Note the volume of sample and loading buffer added for final calculation.

2. Determining the approximate DNA concentration post-electrophoresis:

1. After ethidium bromide staining, the DNA concentration of the unknown sample can be estimated by visualisation and comparison with the intensity of the unknown 'band' and the intensity of 'bands' of either DNA ladder dilutions (e.g. 50 bp, 100 bp) or DNA Lambda (48.5 Kb).

2. DNA ladders contain fragments of known concentrations listed in the datasheet e.g. 67 ng/5 µL. Recalculate the fragment concentration for the volume that has been loaded onto the gel.

3. After recalculation, the approximate concentration of the unknown sample can be estimated. If the sample has been diluted when loaded, this will need to be taken into account. See the following example opposite.



A PCR sample (4 µL) was run on a 6 % gel (hPAGE) next to 4 µL, 2 µL and 1 µL dilutions of a 50 bp ladder. The gel was post-stained with EtBr.

To estimate the concentration of the unknown PCR sample, visually compare the intensity of the band with the 50 bp DNA ladder dilutions as follows: Looking at the 4 µL ladder lane, the intensity of the unknown is less than the 200 bp band but greater than the 150 bp band.

The 200 bp and the 150 bp bands have a concentration of 67 and 30 ng/5 µL respectively. Since 4 µL of ladder was loaded onto the gel, the concentrations of these bands can be calculated to be about 53 ng and 24 ng/4 µL respectively. As the intensity of the unknown sample is closer to that of the 53 ng/4 µL it is estimated that the concentration of the unknown PCR sample is 44 ng/4 µL or 11 ng/µL.

3. Estimation of genomic DNA

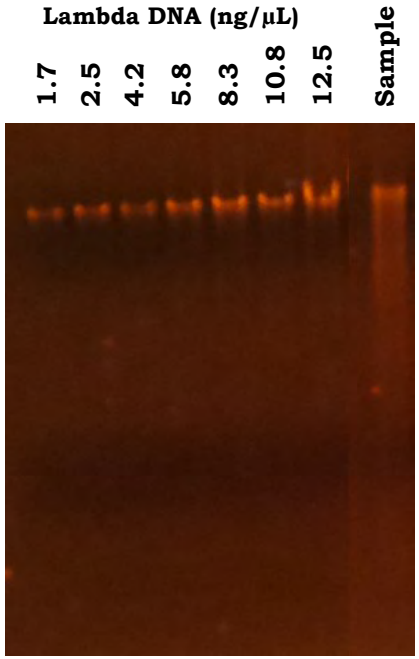
1. The amount of genomic DNA obtained from a CTAB extraction can be estimated by comparison with a standard solution of DNA Lambda[1]. DNA Lambda will show one band on an agarose or hPAGE gel.

2. Prepare dilutions of DNA Lambda and the unknown sample(s) as for a DNA ladder (Section 2).

3. The approximate concentration of the unknown sample can be estimated. If the sample has been diluted when loaded, this will need to be taken into account. See the example opposite:

Notes:

[1] DNA Lambda (uncut) is supplied in a solution and as a consequence must be shipped at -20°C. If this standard is required please contact Kirkhouse Trust.



A unknown genomic DNA sample (diluted 1:10) was run on a 0.8% agarose gel next to various dilutions of lambda DNA. The gel post-stained with EtBr.

To estimate the concentration of the unknown genomic DNA sample, visually compare the intensity of the band with the lambda DNA dilutions.

The intensity of the unknown genomic DNA sample appeared brighter than the lambda DNA band for 5.8 ng/µL , but less than the band for 8.3 ng/µL

The unknown genomic DNA sample can be estimated as follows:

The two corresponding lambda DNA bands have a concentration of 5.8 and 8.3 ng/µL respectively. Therefore, the approximate concentration of the diluted unknown genomic DNA sample is 7 ng/µL or undiluted sample of 70 ng/µL.

Make all buffers and solutions with distilled or bottled water:

1. TE pH 8.0 buffer[1]:

10 mM Tris-HCl pH 8.0.

1 mM EDTA-Na pH 8.0.

To prepare 100 mL of TE buffer pH 8.0:
Add 10 mL of 100 mM Tris-HCl pH 8.0 and 2 mL of 50 mM EDTA-Na and make up to 100 mL with molecular biology water.

Some protocols use TE 10:0.1 with 0.1 mM EDTA to reduce the interaction of the EDTA in downstream applications.

2. Tris-Borate-EDTA (TBE) buffer:

	grams for 10X TBE	grams for 5X TBE
89 mM Tris base (FW 121.1)	108 g	54 g
89 mM Boric acid (FW 61.8)	55 g	27.5 g
2 mM EDTA disodium salt (FW 372.2)	7.4 g	3.7 g

Add bottled or distilled water to 1 litre.

Notes:
[1] Tris has a large temperature co-efficient (-0.028 pH/°C) which means that the pH of a Tris buffer will increase with decreasing temperature. Therefore, it is important to adjust the pH at the same temperature at which the buffer will be used. Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.

3. Tris Acetate (TAE) buffer:

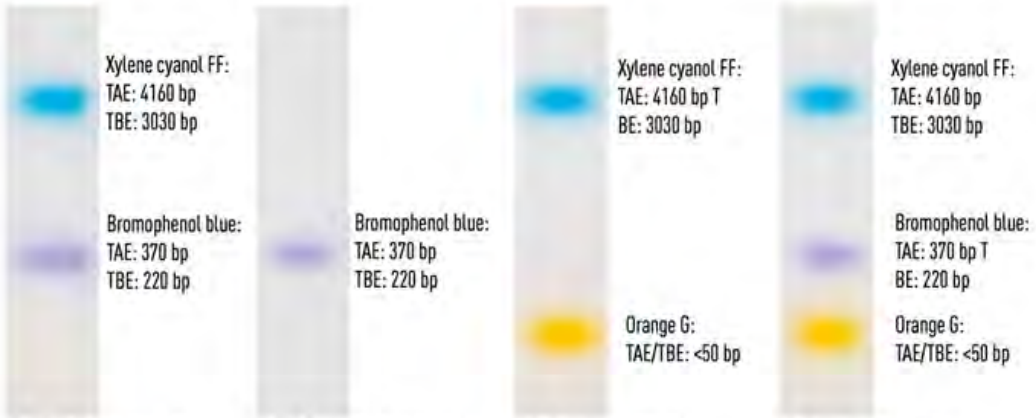
For a final concentration :	To prepare 1 litre 50X TAE
40 mM Tris base (FW 121.1)	242 g
20 mM glacial acetic acid (FW 61.8)	57.1 g
1 mM EDTA— make 500mM with 186.1 g EDTA disodium salt (FW 372.2) and correct pH to 8.0	100 mL

Add bottled or distilled water to 1 litre.

To make 1X TAE from 50X TAE stock, dilute 20ml of stock into 980 mL of water.

1. DNA Loading Dye / Buffer:

1. Add 1 µL of DNA loading dye[1] per 5 µL of sample and mix thoroughly.
2. The dyes separate in agarose as approximate bp size markers as shown below:



Notes:

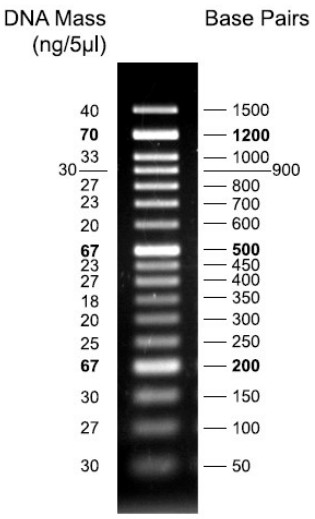
[1] DNA loading dye 6X is usually supplied in a 1 mL vial containing 10mM Tris-HCl pH 7.6, 0.03% **bromophenol blue**, 0.03% **xylene cyanol FF**, 60% glycerol, 60mM EDTA. It is a non-denaturing loading buffer for native polyacrylamide and agarose gel applications.

In 1% agarose gels bromophenol blue co-migrates with ~300 bp fragment and xylene cyanol FF – with ~4000 bp fragment. Add 1/6 volume of 6X DNA Loading dye to the DNA sample

2. DNA Ladders:

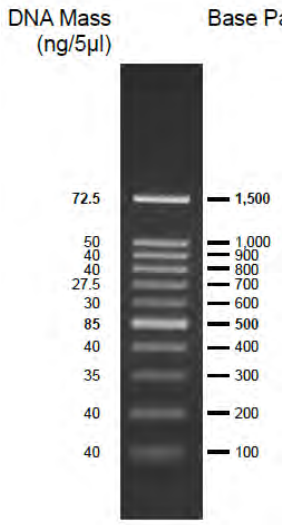
1. DNA ladders (50 bp, 100 bp or 1 Kb) are provided ready-to-use in loading dye .
2. The 50 bp ladder usually contains fragments ranging from 50 bp to 1,500 bp in 50 bp increments with double intensity reference bands at 200 bp, 500 bp and 1,200 bp. Tracking dye is orange G.
3. The 100 bp DNA ladder usually contains fragments ranging from 100 bp to 1,500 bp, with a high intensity reference band at 500 and 1,500 bp. Tracking dyes are orange G & xylene cyanol FF.
4. The 1Kb DNA ladder usually contains ranging from 250-10,000 bp with high intensity reference bands at 1K and 3K. Tracking dyes are bromophenol blue & xylene cyanol FF.

<u>50 bp DNA ladder</u>



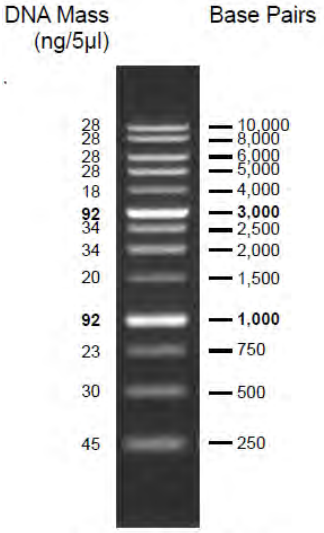
2% TAE agarose gel

<u>100 bp DNA ladder</u>



1.7 % TAE agarose gel

<u>1 Kb DNA ladder</u>



1 % TAE agarose gel

1. Yeast Dextrose Chalk (YDC) agar medium:

WHAT YOU NEED FOR 1 LITRE	
Agar	15 g
Yeast Extract	10 g
$CaCO_3$ (light powder)	20 g
D-Glucose (Dextrose)	20 g

1. Weigh out all ingredients into a suitable oversize container.
2. Add 750 mL distilled or bottled water. Bring to boil to dissolve. ($CaCO_3$ will not completely dissolve).
3. Make up to 1 litre volume with water.
4. Dispense into bottles (300 mL or 500 mL medical flats supplied), with constant swirling to ensure even distribution of $CaCO_3$. Fill bottle half to two thirds full.
5. Autoclave at 121°C, 115 psi for 15 minutes.
6. Allow medium to cool to approximately 50°C.
7. Swirl to ensure even distribution of $CaCO_3$ and avoid air bubbles. Pour into sterile petri plates (22 mL per 9.0 cm plate).
8. Leave plates to set in a laminar flow cabinet or similar, before use.
9. Store prepared plates inverted in polythene bags at room temperature. Prepared plates can be stored for several months provided they do not dry out.

2. MXP medium:

To prepare medium for *Xanthomonas Campestris pr phaseoli* (MXP) see the instructions in the article provided in the Lab Manager's Manual:

Claflin, L.E., Vidavar, A.K. and Sasser, M. (1987).
MXP, a semi-selective medium for *Xanthomonas campestris* pv. Phaseoli.
Phytopathology 77:730-734.

3. Directions for cornmeal agar & other media supplied:

Product information from manufacturers can be found online, for example Oxoid brand product information can be found at www.oxoid.com or ask the P&D team for a Technical Data Sheet if one is not provided.

Corn Meal Agar:
Suspend 17g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Potato Dextrose Agar:
Suspend 39 g of powder in 1 litre of purified water and bring to the boil. Distribute into suitable containers and sterilise in the autoclave at 121°C

Malt Extract Agar:
Suspend 50 g in 1 litre of distilled water and bring to the boil to dissolve. Sterilize by autoclaving at 115°C for 10 minutes. in order to suppress the bacterial growth. For fungi a pH value of 3.5 is recommended, but this depends on the microorganisms.

1. Standard Units and Prefixes:

In 1960, the 11th CGPM adopted a first series of prefixes and symbols of prefixes to form the names and symbols of decimal multiples and submultiples of SI units. Over the years, the list has been extended as summarized in the following table.

factor	prefix	symbol	factor	prefix	symbol
10^{24}	yotta-	Y	10^{-1}	deci-	d
10^{21}	zetta-	Z	10^{-2}	centi-	c
10^{18}	exa-	E	10^{-3}	**milli-**	**m**
10^{15}	peta-	P	10^{-6}	**micro-**	**µ**
10^{12}	tera-	T	10^{-9}	**nano-**	**n**
10^{9}	giga-	G	10^{-12}	**pico-**	**p**
10^{6}	mega-	M	10^{-15}	femto-	f
10^{3}	kilo-	k	10^{-18}	atto-	a
10^{2}	hecto-	h	10^{-21}	zepto-	z
10^{1}	deca-	da	10^{-24}	yocto-	y

2. Volumes, concentrations etc.:

The mole is the SI unit for the amount of a substance and one of the seven fundamental SI units. It is defined as the amount of substance of a system that contains as many elementary entities as there are atoms in 0.012 kilograms of carbon-12 (BIPM 1998, p. 97). It is abbreviated "mol," and the number of entities in a mole of substance is given by Avogadro's number (6.023×10^{23}).
Thus, mmol = 10^{-3} moles, µmol = 10^{-6} moles, etc.

Note that some authors use upper case 'm', thus, pMol = 10^{-12} moles. This may lead to confusion as it is conventional to use uppercase 'M' for molarity (I.e. concentration in moles per litre). Thus, 0.1 M = 0.1 moles per litre = 0.1 mol/l = 100 mM = 100 mmol/l etc.

Volume units are fractions of a litre. The more correct symbol for litre is a capital 'L', but lower case 'l' is more common. Thus ml = mL = millilitre = 10^{-3} litre

Note that a space should be introduced between the number and the symbol for units. It is not necessary to place a stop after the symbol and it is not necessary to pluralise. Thus, 10ml, 10 ml. and 10 mls are incorrect; 10 ml and 10 mL are correct.

1. Alcohol Burner—Operation:

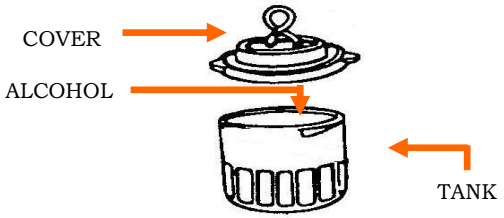


Fig. 1

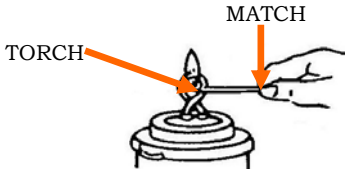


Fig. 2

1. Take off the cover, fill the tank with alcohol (max 100 mL) and then close the cover (Fig. 1). Note: No wick is required. Fuel consumption approx. 1 mL per min.
2. Clean up the fuel if there is any on the outside of the tank.
3. Heat the torch by a glowing match until a flame lights over the torch (Fig. 2).
4. The flame is large at the beginning and then becomes small and stable.
5. To turn off the flame, just put the cap on the burner.
6. 100 mL fuel will last for about 1.5 hours.

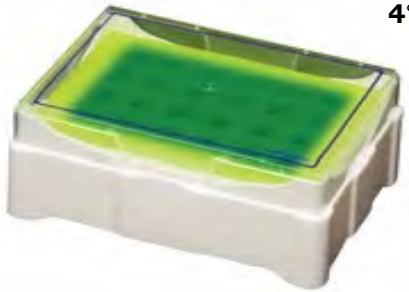
2. Safety:

1. Use either ethanol or methanol. If methanol is used, it should be coloured, so that the flame is clearly visible.
2. Use a needle to clean the holes in torch ring if clogged.
3. After use, put the attached rubber plug into the ring of torch in order to prevent fuel loss.
4. Fuel will not leak even if burner is turned upside down as long as the cover is well secured.

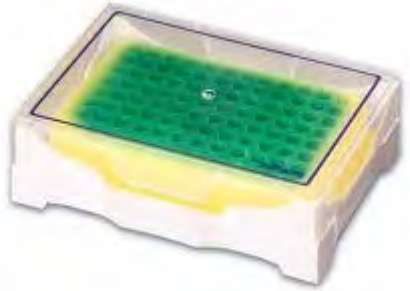
2. Bench Top Coolers for micro-centrifuge or PCR Tubes

1. The MCT rack holds 20 micro-tubes (1.5 mL) and the PCR rack holds 96 (0.2 mL) tubes. Depending on the ambient temperature, both racks should maintain a sample temperature of around 4°C for approximately 2 hours. The rack will change from **green** to **yellow** as the temperature increases.
2. Place in freezer for at least 24 hours before use; the racks will then turn green.
3. Keep the lid on when in use. The inside rows keep cooler longer than the outside rows.

4°C MCT COOLER



4°C PCR TUBE COOLER



Kirkhouse Trust

AUTOCLAVE—DIXON'S ST1528, 15 LITRE

October 2016

WHAT YOU NEED
Dixon's Autoclave ST1528
Operator's Manual
Sterilisation bags and autoclave indicator tape

1. Locate the instruction manual supplied and follow the installation procedure. Operation of the autoclave is described in the manual and it is important to read this carefully. An electronic copy for the Dixon's ST1528 manual can be found on the Kirkhouse Trust website, under 'Resources—Equipment Manuals'.

2. Make sure that the lid, nuts and bolts are secured in the correct manner as described in the Manual. Fill the chamber with water. Place the lid, rotate clockwise until it hits the stops and the drop bolts line up, tighten the wing nuts in opposite pairs to ensure an even pressure; tighten to finger tightness.

3. Do not pack the items to be sterilised too tightly; there must be enough room for steam to circulate freely around the items. . Any lids, such as on bottles, need to be separate or very loosely placed on so pressure does not build inside the container.

4. The autoclave works like a pressure cooker. The air in the chamber is expelled as the water boils and steam fills the chamber. A clean jet of steam and not 'spluttery water droplets' should be visible through the air cock before it is closed off. This is important as it means all air has been expelled.

5. When the sterilisation period is complete, turn off the red switch and allow the autoclave to cool. Do not use the air-cock to release pressure. Allow the autoclave to cool to 85°C or less.

6. When the autoclave has cooled, open the air-cock (6 turns). Check the pressure gauge reads zero. It is important to unscrew the nuts in the following sequence: unscrew the 6 wing nuts in opposite pairs, one in each hand (2 full turns). This allows the lid to rise to a safe position, then unscrew them fully and swing down the drop bolts. Finally, unscrew the 2 collared wing nuts as a pair, one in each hand. Remove the lid.

A	Draw-off Cock	E	Thermometer
B	Aircock	F	Wing Nut (6)
C	Pressure Gauge	G	Drop Bolt (6)
D	Safety Valve	H	Switch

ITEMS SUPPLIED
Balance; 2 place
Weighing dishes; small, medium and large

1. Make sure the balance is levelled. Use the two front feet of the balance for levelling until the air bubble is centred in the level indicator.
2. Press the 'On/Off' pad to turn the balance on.
3. Place a weighing dish of appropriate size on the balance.
4. Zero the balance by pressing the 'Tare' button.
5. **REMOVE THE WEIGHING DISH FROM THE BALANCE TO THE BENCH**

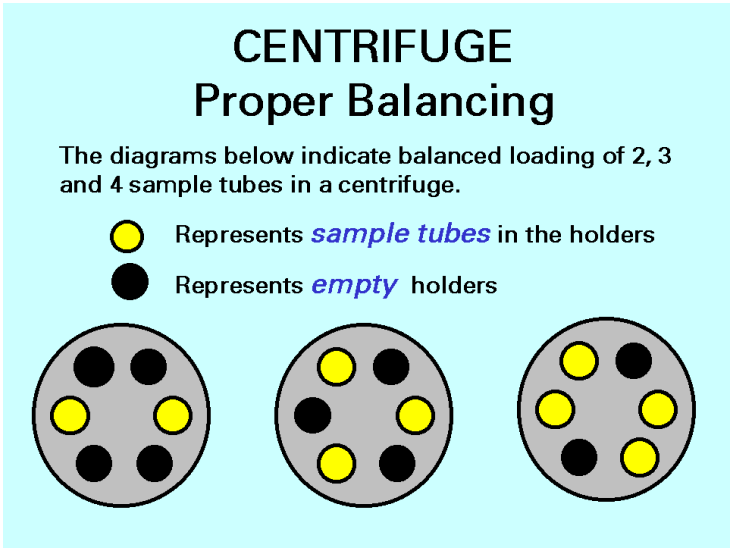
PROPER CARE OF THE BALANCE IS ESSENTIAL TO KEEP IT IN GOOD WORKING ORDER

6. Carefully transfer the material to be weighed into the weighing dish on the bench.
7. Replace weighing dish with contents on the balance and record mass.
8. Press ON/OFF pad to turn the balance off.

CLEAN UP SPILLS ON OR NEAR THE BALANCE AS SOON AS THEY OCCUR

ITEMS SUPPLIED
Benchtop Microfuge, 24 place rotor
Microfuge tubes

1. Microfuge tubes of the same weight must be placed opposite each other in the rotor, or follow the scheme in 'Centrifuge Proper Balancing' diagram.
2. Ensure plastic rotor cover is in place before centrifugation.
3. A centrifuge user manual is available at www.kirkhousetrust.org Go to 'Resources → Research Resources → Equipment Manuals'.



KEEP ROTOR CLEAN
WIPE UP ANY SPILLS IMMEDIATELY!

WHAT YOU NEED
Neubauer Improved Haemocytometer
Cell suspension suitably diluted
Tally counter
Pasteur pipettes
Microscope (compound or stereo)

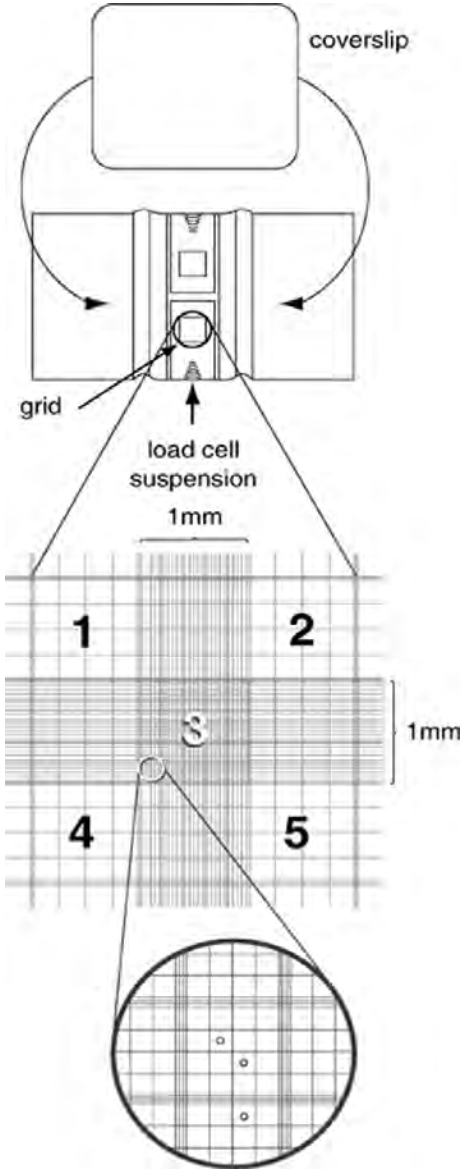
1. Preparation before using a counting chamber:

1. The haemocytometer is an expensive piece of equipment that must be handled carefully. To avoid getting fingerprints on the ruled areas, the counting chamber should be held by the sides and bottom only.

2. **DO NOT USE A REGULAR COVERSLIP AS THIS WILL NOT GIVE ACCURATE RESULTS.**

3. Take extra care when focusing the microscope as the counting chamber is much thicker than a conventional slide. **The microscope objective, as well as the chamber and cover glass, may be damaged beyond repair if the user is not careful.**

4. Clean the counting chamber gently with 70% alcohol and a lint-free wipe



Note:
The figure opposite is reprinted with the kind permission of Current Protocols in Cell Biology, John Wiley & Sons Inc. www.currentprotocols.com/protocol/cb0101

2. Protocol for using a counting chamber:

1. Moisten the shoulders of the haemocytometer and affix cover glass using gentle pressure and small circular motions. This ensures that the depth of the chamber is correct (0.1 mm).

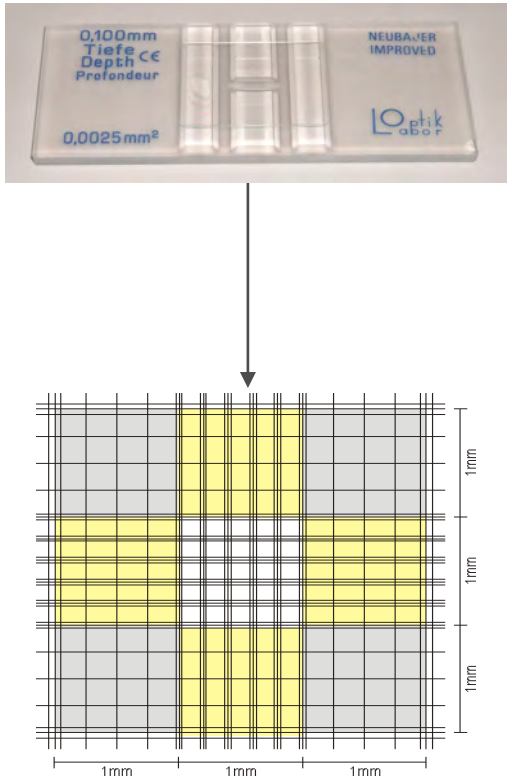
2. Using a Pasteur pipette, place a drop of the cell suspension at the edge of the 'V' shape of the chamber. Allow the suspension to be drawn into the chamber by capillary action. ****CARE**** Do not over-fill or under-fill the chamber. Fill the opposite chamber in the same manner.

3. The rulings cover 9 mm^2 in a 3x3 square. The central square of the grid is ruled into 25 groups of 16 smaller squares (area of each smaller square is 0.0025 mm^2) with each group separated by triple lines, the middle one of which is the boundary.

   The area of the central square is: 25 x 16 x 0.0025 = 1 mm^2 and the volume is: 1 mm^2 x 0.1 mm = 0.1mm^3 or 0.1 µL (1 mL = 1000 mm^3).

   The number of cells counted per mL = number of cells counted per square mm x dilution x 10,000.

4. To ensure accuracy of counts, a specific counting pattern must be determined. See: http://www.ruf.rice.edu/~bioslabs/methods/microscopy/cellcounting.html
   (Reproduced with permission, a pdf 'Using a Counting Chamber' can be found at www.kirkhousetrust.org, go to: Resources → Research Resources → Equipment Manuals → Haemocytometer.
   Counting Fungal Spores: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC202674/pdf/aem00111-0126.pdf



ITEMS SUPPLIED
Desiccator
Active silica gel, orange colour

Regeneration of colourless silica gel

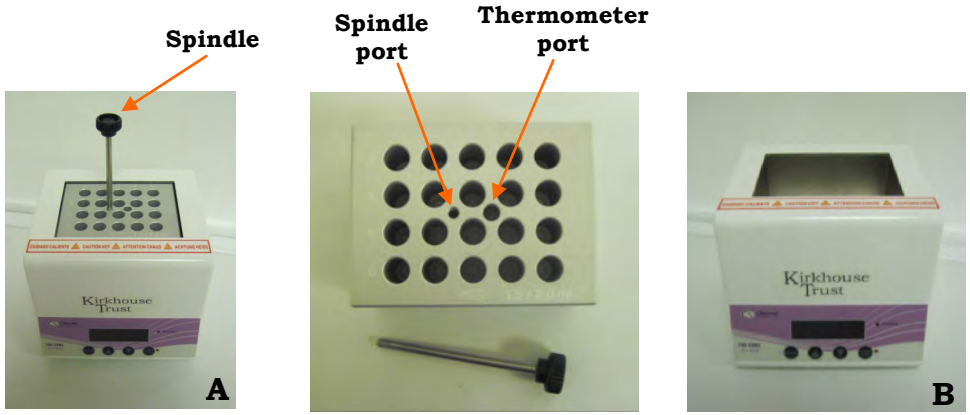
1. Do **not** discard colourless silica gel. It can be regenerated and used many times.

2. Silica gel adsorbs water vapour and maintains a dry environment for your samples. When the gel's adsorption capacity is exhausted the colour changes from orange to colourless.

3. When colourless, the silica gel can be regenerated by heating in a drying oven at 120 to 140°C for approximately 3 hours. The colour changes back to orange.

4. The silica gel is suitable for numerous drying applications although it is not recommended for strongly acidic or strongly alkaline compounds.

ITEMS SUPPLIED
Cleaver dry block unit supplied as a single reversible metal heating block for 0.5 mL and 1.5/2 mL tubes
Tubes 1.5 mL, 2 mL



1. Use as a dry block:

1. For optimum temperature accuracy OR when using a different type of block, the unit needs to be calibrated. Consult the Instruction Manual supplied. A copy of the manual can be found as a pdf at www.kirkhousetrust.org, in the Resources section.

2. The dry block heater can be used as either a heating block (A) or, if required, as a mini water bath (B).

3. Make sure that the unit is plugged into the surge protector (supplied by KT) which then connects to either the KT battery back -up unit or the mains electricity (if the supply is stable).

4. Screw the spindle into the spindle port when inserting or removing a heating block.

   NOTE: Keep the spindle in a safe place! Perhaps tape it to the rear of the dry block or keep it in the lab tool box.

2. Use as a mini-water bath:

1. If the unit needs to be used as a water bath then take care not to overfill it. *Remember:* when tubes are placed in the cavity, the level of water will increase.

2. The approximate water bath volume is:
   Single block (Cleaver) 350 to 400 mL

   Take care not to overfill the dry block cavity.

3. ***Do not leave water in the dry block. Always dry after use***.

The laptop computer supplied by Kirkhouse Trust has a Microsoft operating system and is pre-loaded with the software listed below. The laptop can also be used for gel documentation with the Canon camera.

The 17.3" Genesis V Specification Snapshot:

Powerful Intel® CPUs
8Gb memory
Windows 10
Intel Integrated Graphics
SuperSpeed USB 3.0
HDMI Output Connection
VGA Output Connection
1.0MP Webcam
Wireless & Bluetooth
USB ports
Memory Card Reader

1. Setting up the Motic BA210 microscope:

1. Carefully unpack the microscope and place it onto a level surface.
2. Plug the microscope into the surge protector (supplied by KT) which then connects to the mains electricity.
3. On the lower right hand side of the microscope there is a knob which controls the amount of light, (the rheostat control **(1)**. Turn this fully anti-clockwise (low light setting).
4. It is essential, ***before*** turning on the microscope, to turn the rheostat control to the lowest level. Then, gradually increase the light to the level required to illuminate the specimen. Too much light can damage the specimen and blow the bulb.
5. Turn on by pressing the green light switch on the right hand side of the microscope **(2)**.

Key:

1	Light control — Rheostat	5	Diopter Adjustment Ring
2	Power on button	6	Control for fine focus (inner)
3	Y-axis control	7	X-axis control
4	Coarse focus height stopper	8	Control for coarse focus (outer)

Note: model shown *without* trinocular camera port

2. Viewing a specimen:

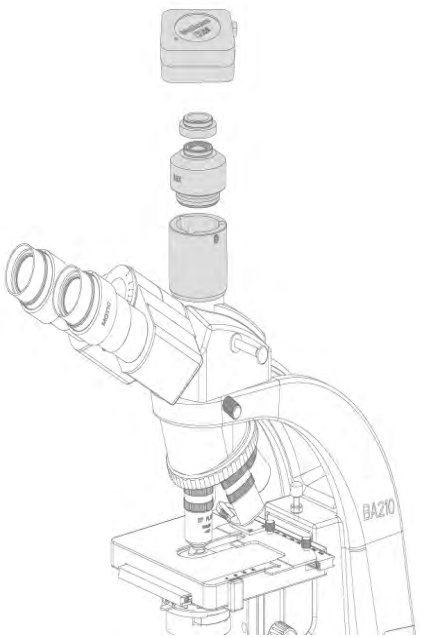
1. Make sure that the eye piece inter-pupillary distance is adjusted for each microscope user. Focus a specimen slide using the 10X objective. Adjust the inter-pupillary distance so that both the right and left field of view becomes one.
2. To compensate for the differences in vision between the left and right eyes, the diopter must be adjusted. Using the right eye and viewing through the right-hand eyepiece, adjust the focus with the fine or coarse adjustment until the image of the sample is sharp. Then use your left eye only and again view through the left -hand eyepiece with its independent diopter focusing ring, focus until the sample is sharp **(5)**.
3. The coarse focus height stopper marks the stage position at which the sample is in focus. When the sample is in focus, turn the coarse focus stopper screw **(4)** anti-clockwise until it reaches the stop. The stage is now locked and cannot be moved upward. The fine focus can move the stage but only lowers it.
5. Place the specimen onto the microscope stage and secure in place by using the clip.
6. Use either the x-axis or y-axis controls **(7 & 3 respectively)** to directly align the specimen so it is underneath the objective.
7. Focus the specimen with the 10X objective. Use the coarse and fine focus knobs **(6 & 8)** until the image of the specimen is sharp.
8. The 100X objective to view for bacteria etc., must be used with immersion oil to fill the space between the microscope cover slide and objective lens. After use clean the objective thoroughly.

3. Care of the microscope:

1. Care should be taken when removing the dust cover from the microscope in order to avoid dislodging an eyepiece; dropping an eyepiece can damage it beyond repair.
2. Eyepieces should be kept clean using lens wipes or lint free wipes. Fingerprints can blur images. A fresh piece of lens wipe should be used to clean each eyepiece. Gently 'fog' the eyepiece with your breath before wiping to remove fingerprints.
3. It is best not to wear mascara when using a microscope. When mascara drops onto the eyepiece lens, rubbing it off can etch the glass. Blow off as much of the mascara as possible before wiping the lens.
4. The light bulb is a 3W LED and can be replaced. Consult the Motic Instruction Manual for the BA210 found at: www.kirkhousetrust.org, go to Resources → Research Resources → Equipment Manuals.

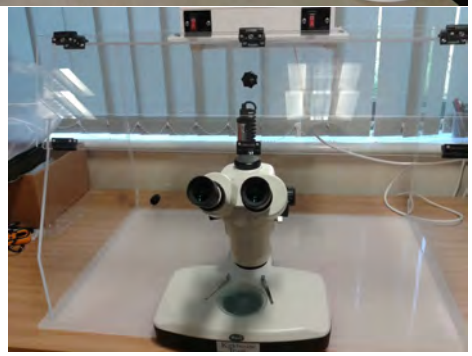
4. *Attaching a camera:*

1. The BA-210 trinocular microscope models come with an image port on the head to which a camera may be attached using a C mount adapter.
2. To connect the camera to the microscope, remove the front lens of the camera and replace with the adapter; see the camera's instruction manual.
3. Loosen the knurled screw and remove the protective cover from the trinocular port on the microscope.
4. Insert the adapter with the camera attached to the vertical port. It should insert easily but if it does not, unscrew the knurled screw until it fits.
5. Re-tighten the knurled screw until the camera is securely in place.



5. *Dust hood*

1. The KT dust hood with HEPA filter-fan, light and hinged sloping front can be used to provide a cleaner environment when using the microscope.
2. The microscope can be moved forward and the hinged door raised when viewing specimens or moved further back into the hood for sample preparation.



1. *Setting up the Motic SMZ-168 microscope:*

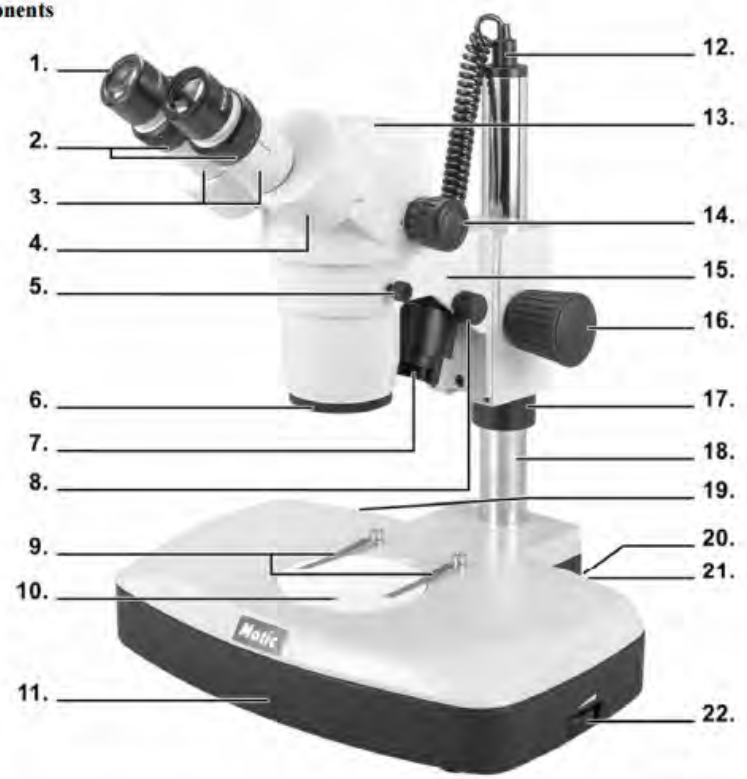
1. Carefully unpack the microscope and place it onto a level and stable surface.
2. Before connecting the microscope to a power source, adjust the light intensity to its minimum level using the light intensity control **(22)**. This step is to be repeated every time the microscope is turned on and off to prolong the life of the bulb.
3. Plug the microscope into the surge protector (supplied by KT) which then connects to the mains electricity.
4. To change a stage, use your fingers to remove the stage. To insert the glass stage, place the blue filter into the centre of the filter receptacle (frosted surface facing down) and replace the glass stage.
5. Turn on the main switch **(19)**.
6. Turn on the incident illumination or transmitted illumination **(20 or 21)** or both at the same time depending on the specimen being observed.

WARNING:
Transmitted illumination can <u>ONLY</u> be used with the frosted glass stage.

The heated generated by the transmitted illumination can melt or damage the black and white stage.

7. The angle of incident illumination is adjusted using the adjustment screw **(8)** which also varies the orientation of the lens.
8. Make sure that the inter-pupillary distance is adjusted for each microscope user. Look through the eye pieces and move the prism housing outwards or inwards. Do this until the two fields of view are as one through both eye pieces.

Components



SMZ-168-BL

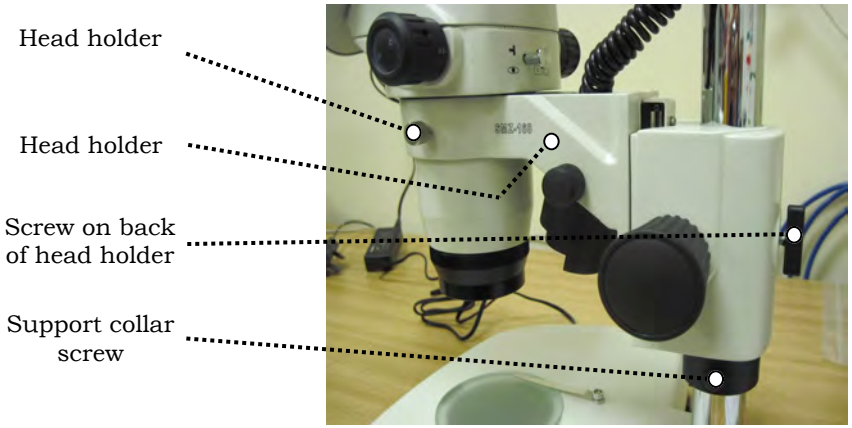
1. Eyepieces	9. Slide stage clips	17. Support collar
2. Diopter adjuster	10. Stage	18. Column
3. Eyepiece tubes	11. Base	19. On/Off switch
4. Prism housing	12. Power cord for incident illumination	20. Incident illumination switch (I)
5. Head holder locking screw	13. Head	21. Transmitted illumination switch (T)
6. Objectives	14. Zoom knob	22. Light intensity control
7. Incident illumination	15. Head holder	
8. Incident light adjustment screw	16. Focus knob	

2. *Viewing a specimen:*

1. Turn the zoom control **(14)** to the lowest magnification of 0.75x.
2. Place the specimen onto the centre of the microscope stage and secure in place using the slide stage clips.
3. Turn the focusing control **(16)** to mid-focus range.
4. The head holder **(15)** is mounted on a column **(18)** which can be moved up and down depending on the size of the object being focused.
5. To change magnification, turn the zoom control **(14)** to the lowest magnification and focus the image, then switch to the highest magnification and focus the image. The stereo microscope is now "parfocal".
6. To compensate for the differences in vision between the left and right eyes, the diopter must be adjusted. Using the right eye, look through the right-hand eyepiece, and then adjust the focus.
   Using your left eye, look through the left-hand eyepiece and adjust the focus by turning the diopter adjuster on the left-hand tube until the image appears sharp.
7. If depth of field is required to work with a sample e.g. single spore isolation from a fungal culture, the 2X objective should be unscrewed—this leaves the 1X objective in place. Replace the 10X eyepieces with 15X eyepieces.

3. *Changing the height of head holder:*

1. Support the head holder **(15)** with one hand (without touching any lenses) and the other loosen the screw on the support collar **(17).** The head holder can now be moved down to the base **(11).**
2. Supporting the head, loosen the head holder locking screw.



3. Looking through the eyepieces, move the head holder up or down until the specimen comes into focus. Tighten the head holder locking screw and do not let go of the head.
4. Slide the security collar up to the head holder and tighten the support collar screw and the screw on the back of the head holder. Now the head can be released.
   It is not necessary to adjust the head every time the specimen is changed, only when unable to bring the image into focus.
5. Adjust the focus control **(16)** until the image appears sharp.

4. Attaching a camera:

1. The SMZ-168-TL model microscope comes with an image port on the head, (pictured below) to which a camera may be attached using a C mount adapter.
2. To connect the camera to the microscope, remove the front lens of the camera and replace with the adapter; see the camera's instruction manual.
3. Loosen the knurled screw (see the arrow on the figure below) and remove the protective cover.
4. Insert the adapter with the camera attached to the vertical port. It should insert easily but if it does not, unscrew the knurled screw until it fits.
5. Re-tighten the knurled screw until the camera is securely in place.

For a video with instructions for attaching a camera, go to Motic's website:

http://www.motic.com/As_Moticam_CMOS/product_459.html

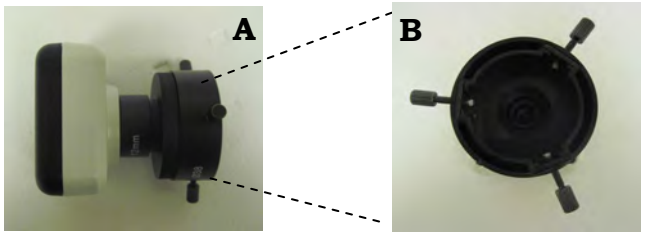
WHAT YOU NEED
Motic microscope (compound or stereo)
Motic Moticam X or Moticam 2 camera and accessories
Motic Images Plus software CD
Laptop
Microscope slide with samples for viewing

1. Load the Moticam software onto the laptop:

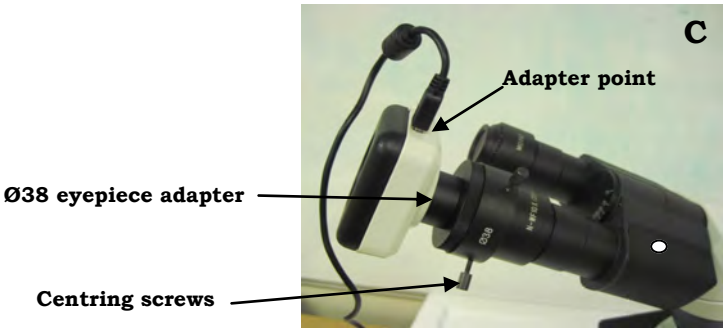
1. Insert the Moticam software CD into the laptop.
2. The software will run automatically upon loading.
3. Install the software for the program “Motic Images Plus”.

2a. To attach the Motic Moticam X camera to the microscope eyepiece:

1. Remove the dust cap from the Moticam case.
2. Screw the 12 mm focusable lens to the Moticam case.
3. Attach the Ø38 eyepiece adapter to the 12mm focusable lens making sure that the three centring screws are loosened (A and B).



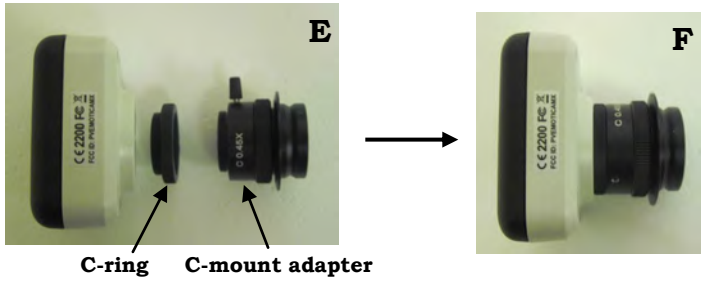
4. Place the camera assembly onto the microscope eyepiece (C) by attaching the Ø38 eyepiece adapter and tightening the three centring screws. Connect the USB power supply from the computer (or via the plug) to the adapter point (on the Moticam case). The light will flash and turn solid blue when the camera is ready.



2b. To attach the Moticam camera to the trinocular head (stereo or compound):

1. Remove the dust cap from the Moticam camera and screw in the C-ring (E).

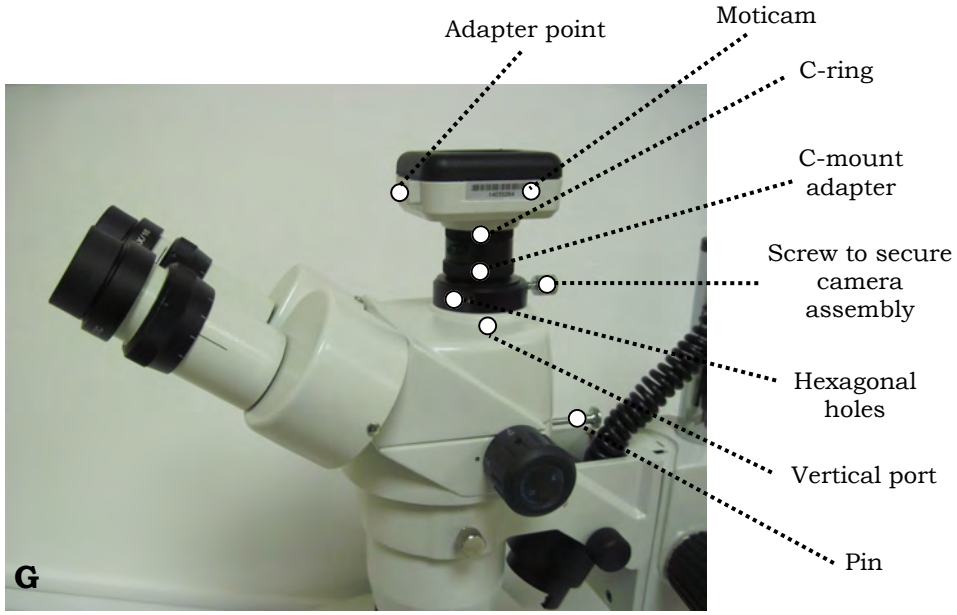
2. For the stereo microscope use the 0.45X C-mount camera adapter. For the compound microscope use the 0.5X C-mount adapter. Screw the C-mount adapter to the C-ring (F).



3. Loosen the screw and place the camera assembly onto the vertical port . Tighten up the screw to secure the camera assembly in place. In addition, there are small hexagonal holes that can be tightened with a hexagonal key to further secure the camera assembly **(G).**

4. Connect the USB power supply from the computer (or via the plug) to the adapter point (on the Moticam case). The light will flash and turn solid blue when the camera is ready.

5. **Pull out the pin located downwards from the vertical port (G) in order for the image to be visible on the laptop. When you are viewing the specimen through the microscope only, push the pin back in.**



3. To view and save images with the laptop

1. Turn on the power to the microscope.
2. Place your specimen onto the microscope stage (and position it using the x and y adjustment controls if necessary). **Start by using the lowest objective on the microscope to visualise your sample.**
3. On the laptop, connect to the Motic Moticam X WiFi by clicking on the wireless network and double clicking to connect to the MC_WiFi Wireless network. The password is 12345678. The Moticam 2 camera connects directly to the laptop via a USB port.
4. Open up the Motic Images Plus programme, and click on the capture tab. This will open up a new window entitled "Motic Live Imaging Module". On the Motic Live Imaging Module click the open button and you will see the live image being broadcast from the microscope eye piece.

Motic Images Plus software programme opening screen

5. Use the different objectives on the microscope to further zoom in on your specimen. For sharper focusing use the fine focusing control.
6. To capture your image, click on the  tab, in the Motic Live Imaging Module.
7. On this tab, you can select from the drop down menu, the format that you wish your image to be captured in. The chosen format for the captured image will depend on how much detail is required and file size (the larger the format numbers, the bigger the file size and the more detail captured).
8. You can save (as different file types) and edit the image by referring back to the Motic Images Plus window in which the image will appear on the right-hand side of the screen.

4. Disassembly:

1. Turn off the power to the microscope.
2. Remove the USB cable from the laptop (or plug) and from the adapter point on the Moticam case.
3. Unscrew the camera assembly in the reverse order that you constructed it, and replace the dust cap back onto the Motic camera.
4. Store the Motic Moticam camera and its accessories in a safe place.

5. To edit images:

1. Image editing software ("Paint.net") has been preloaded into the laptop. Images can be edited including file size reduction in this programme.

   In addition, you can also download a free program such as Image J : http://imagej.nih.gov/ij/

1. Standardising the Jenway pH meter:

ITEMS SUPPLIED
pH meter with Tris compatible electrode
pH 4.0, 7.0 and 10.0 standard buffers
Beakers
Electrode storage solution

1. Remove electrode from the electrode storage solution and rinse with distilled water, pat dry with a tissue.

2. Follow the Operating Instructions for the Jenway 3510 pH Meter. The instructions can be found at: www.kirkhousetrust.org, go to 'Resources → Research Resources → Equipment Manuals'.

3. Press the Mode button until the display shows pH mode. Clear previous buffer information by pressing Setup and Enter buttons.

4. Place electrode in the standard pH solution and press the 'Standardise' button. When the signal stops flashing or you press enter, the buffer is stored. It is best to do this with two pH standard buffers which range between the pH you wish to measure

   e.g. pH 4.0 and 7.0 or pH 7.0 and 10.0.

5. Rinse the electrode with part of the solution to be measured, pat dry with tissue. Place the electrode in the solution to be measured.

2. Care of pH meter electrode (probe):

ITEMS SUPPLIED
pH meter
Electrode storage solution
Beaker

1. Always keep your pH electrode moist. It can be kept with the end in a beaker of electrode storage solution.

2. When the pH meter is not being used for a pH measurement, the electrode should be immersed in the electrode storage solution provided. Alternatively use either a solution of 4 M KCl or a 1:1 solution of a pH 4 or 7 buffer and saturated KCl solution.

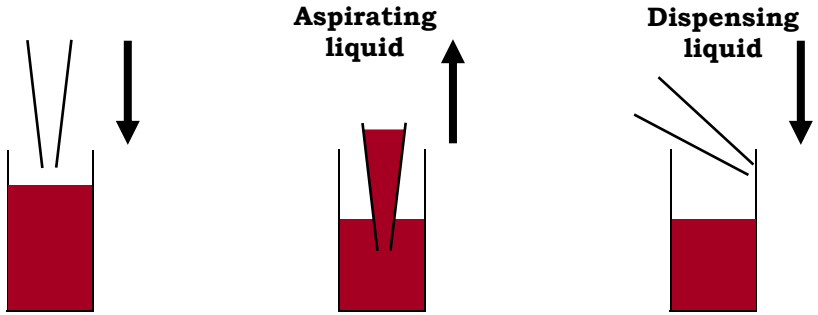
3. You may notice white KCl crystals forming on the outside of the electrode. You can rinse the electrode to remove the KCl crystals and blot dry before use.

DO NOT STORE ELECTRODE IN DISTILLED OR DEIONIZED WATER
This will cause ions to leach out of the glass bulb and render your electrode useless
Electrode storage solution is provided

Notes:
Temperature can affect the measured pH value. Please note that Tris has a large temperature co-efficient and the pH of a Tris solution should be adjusted at the same temperature at which it will be used.

ITEMS SUPPLIED
Pipette stand
Single channel manual pipettes – maximum volume 2 µL, 20 µL, 200 µL and 1 mL
Multichannel pipette – 8 channel
Pipette tips loose 10 µL, 200 µL, 1 mL
Pipette tips racked 10 µL, 200 µL, 1 mL
Note: 10 µL tips may be used for 2 and 20 µL pipettes

1. Correct pipetting:



1. Set the volume required.
2. Press down the plunger to the first stop (step 1). The pipette should be held vertically during aspiration.
3. When aspirating the liquid, the tip should only be immersed a few millimetres into the liquid; optimum immersion depth for a 10 µL tip is 1 mm; for a 200 µL tip it is 2 to 3 mm and for a 1 mL tip it's 2 to 4 mm. Release the plunger slowly and evenly. The tip will then fill up smoothly. If the solution is viscous, allow the pipette tip to fill to final volume before removing it from the solution. The filled tip should be moved up against the wall of the receptacle to avoid residues of liquid on the outside of the tip.
4. Dispense the liquid by pressing down the plunger to the first stop, then eject the remaining liquid by pressing the plunger down to the second stop. Move the tip against the wall of the vessel (step 3).
5. Remove the tip into a waste vessel by pressing down on the tip discarder.
6. Remember to change tips between solutions to avoid mixing or contaminating the solutions used.

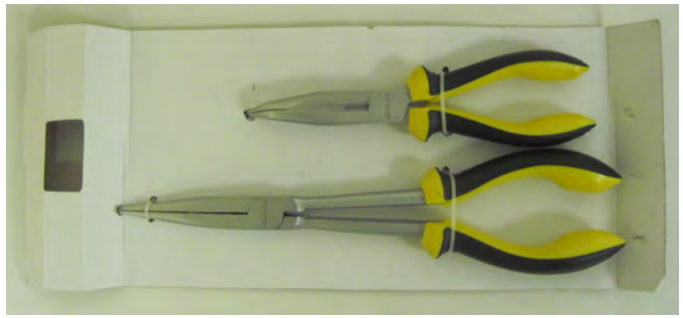
2. Care of Pipettes:

1. Do not invert the pipette with solution in the tip – the liquid will contaminate and eventually damage the piston.
2. Wipe the pipette over with a damp cloth from time to time.
3. Occasionally check that the pipette is delivering the correct volume by pipetting a known volume of water and weighing the dispensed volume.
4. Pipettes require servicing and recalibration on a regular basis. The Trust has a system whereby the pipette set supplied and used in the lab for the previous year should be exchanged for a recalibrated set of pipettes at the KT Annual Meeting.

General toolset

15 piece screwdriver set

Spanner 150 mm adjustable



Pliers—long reach narrow nose and bent nose

PLEASE KEEP THE TOOLKIT IN THE LABORATORY

ALWAYS RETURN TOOLS AFTER USE!

Protocols

Equipment: Instructions & Care