SAFETY NOTE:

Wear gloves when handling acrylamide.

Even after polymerisation, some un-polymerised acrylamide may be present.

WHAT YOU NEED
Horizontal electrophoresis unit, large; Cleaver Scientific
Gel mould, glass plates 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, Ethanol, 50 mL tubes & lint-free wipes
Acrylamide-bis acrylamide solution 19:1, 40%
APS-TEMED sachets (Severn Biotech) or ammonium persulphate and TEMED to use to make up APS-TEMED solution (fresh).
TAE Buffer

For the hPAGE protocol video, see: https://www.kirkhousetrust.org/hpagevideo

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.

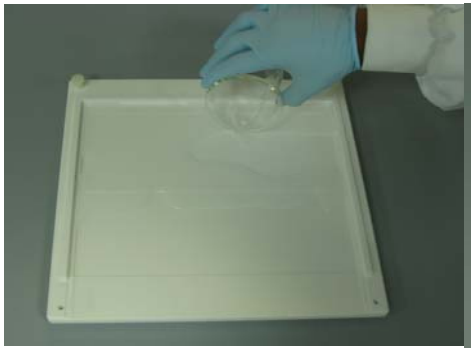
3. Prepare the gel mix using APS-TEMED Sachets

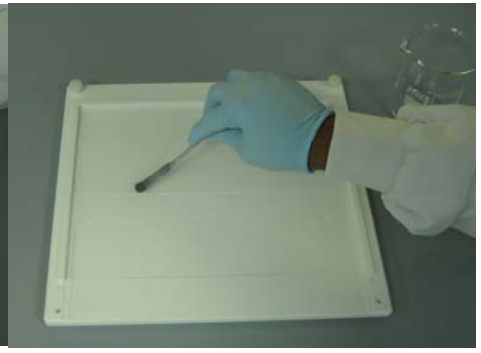
1. The hPAGE mould requires 125 mL gel solution (see the table on page 4) for volumes measured for different sizes of gel plate. For a 6% gel, add the following into a glass conical flask or beaker: 18.8 mL acrylamide-bis 40% gel solution, 2.5 mL TAE buffer, 53.7 mL water. Use 50 mL water to re-suspend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

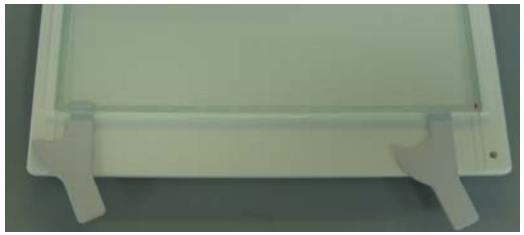
2. Take the glass plate, ***silanised face down***, slot into the top of the mould and lower it carefully making sure that no bubbles form in the gel solution.

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 (continued):*

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 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 (continued):*

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 then 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

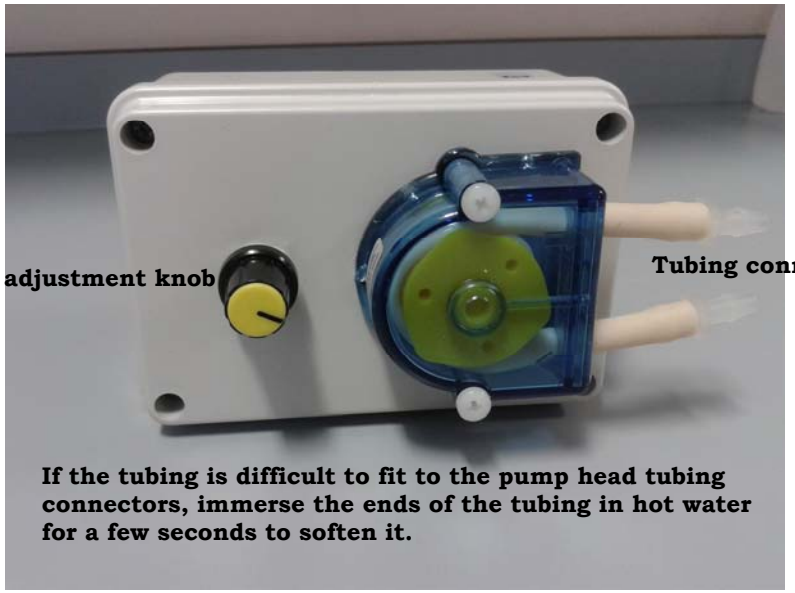
10. Use of peristaltic pump with large gel tank:

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.

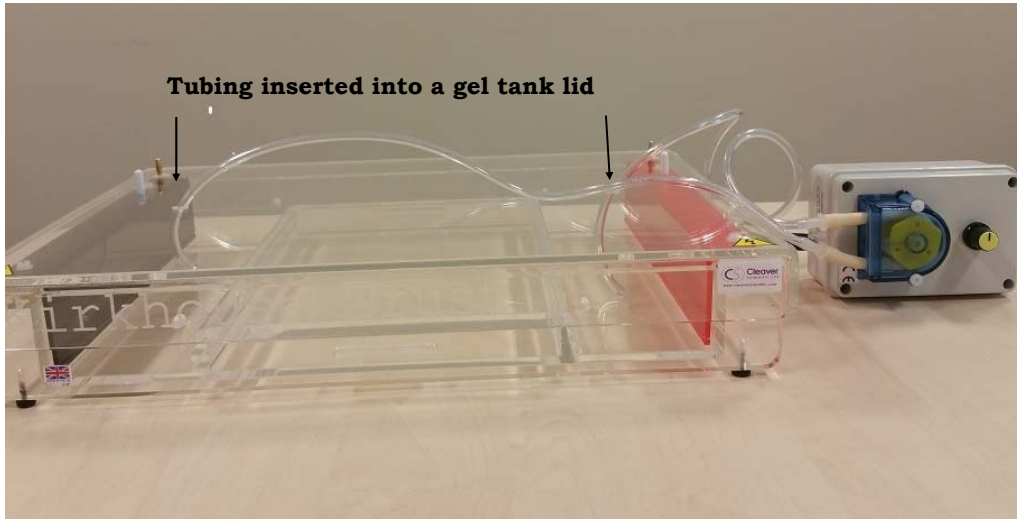
2. The electrophoresis power supply can be set at about 90 V for long runs, for example overnight runs.

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.