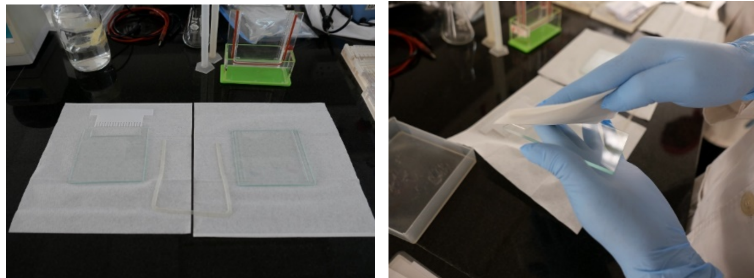
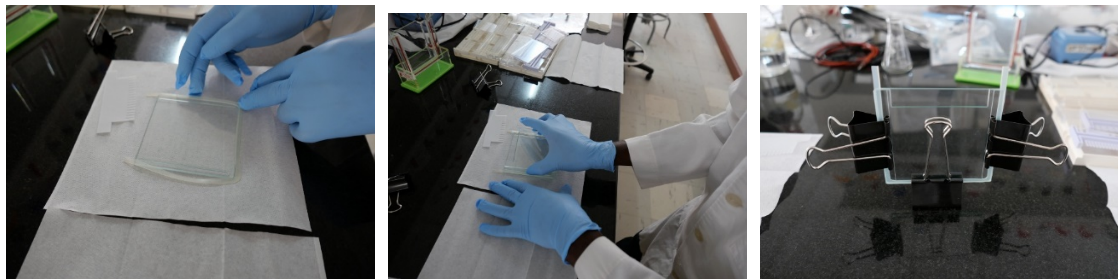
Polyacrylamide Gel Electrophoresis

Gel preparation and electrophoresis for slab (vertical) mini gel

1. Wear gloves. Clean the glass plates and spacers thoroughly using EtOH and paper towel. Hold the plates by the edges with wear gloves, do not touch on the working surfaces (inside) of the plates. Wipe the plates with EtOH and set them aside to dry. The glass plates must be free of tiny dusts to prevent air bubbles in the gel.



2. Assemble the glass plates with spacers with binder clip.

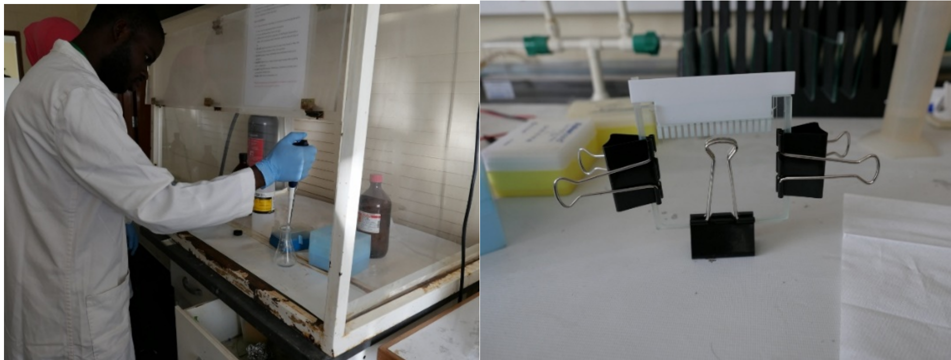


3. Wear gloves. Prepare the gel solution with the desired polyacrylamide percentage according to the table below, which gives the amount of each component required to make ca.10 mL (for a 100mm (H) x 100mm (W) x 1mm (thickness) mini gel. Generally, 8% of gel concentration is recommend. If you want to see more slight difference, you can change to 10% or 12%. Work quickly after addition of TEMED to complete the gel before the acrylamide polymerizes (before start gelation). Pour the gel solution into the gel plate form the upper slot.

Polyacrylamide gel (for 1 mini-gel plate*)			
Gel concentration (Up to PCR product size)	8%	10%	12%
DW	5.6mL	5.0mL	4.4mL
30% acrylamide solution (29:1)**	2.4mL	3.0mL	3.6mL
10×TBE	880μL	880μL	880μL
10% APS	30.8μL	30.8μL	30.8μL
TEMED	15μL	15μL	15μL

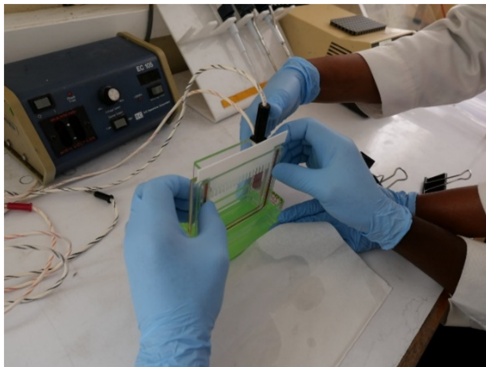
4. Work quickly after addition of TEMED to complete the gel before the acrylamide polymerizes (before start gelation). Pour the gel solution into the gel plate form the upper slot.

5. Immediately insert the appropriate comb into the gel, being careful not to allow air bubbles to become trapped under the teeth.



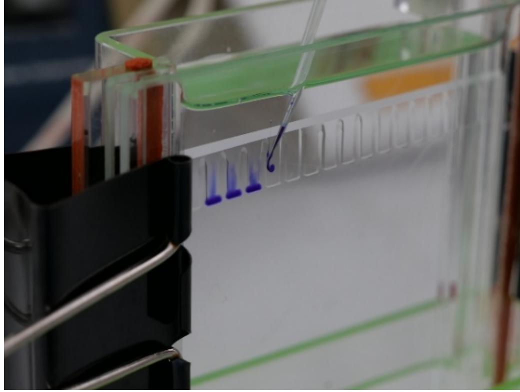
6. Allow the acrylamide to polymerize for 1hr at room temperature.

7. After polymerization is complete and when ready to proceed with electrophoresis, set the plate to electrophoresis chamber with binder clip. Add electrophoresis buffer (0.5 x TBE) and carefully pull the combs from the gel.



8. Use a pipette or a syringe to flush out the wells once more with Electrophoresis buffer.

9. Mix the DNA samples with the appropriate amount of gel loading buffer (according to the manufacture's protocol). We strongly recommend to use the loading dye with DNA-binding fluorescent materials** instead of using ethidium bromide (EtBr). EtBr has been commonly used for band detection of electrophoresed gel, however, it may cause cancer (carcinogen) and tend to less use for lab safety, recently.
Load the mixture into the wells using a micropipette.



10. Connect the electrodes to a power pack, turn on the power, and begin the electrophoresis. Running condition is 100V (to 150V) constant for 1 to 1.5 hrs (depend on the migration of dye).

11. After electrophoresis, remove the gel from gel plate and detect PCR fragment under UV transilluminator and capture photo images.

List of materials

Acrylamide (mono: bis =29:1) (30% w/v)
Ammonium persulfate (10% w/v)
TEMED
10x or 5x TBE buffer
Mini-gel plate set
Electric power supply
** loading dye such as SafeGreen (GeneCopoeia, Inc.), EZ-VISION (VWR)