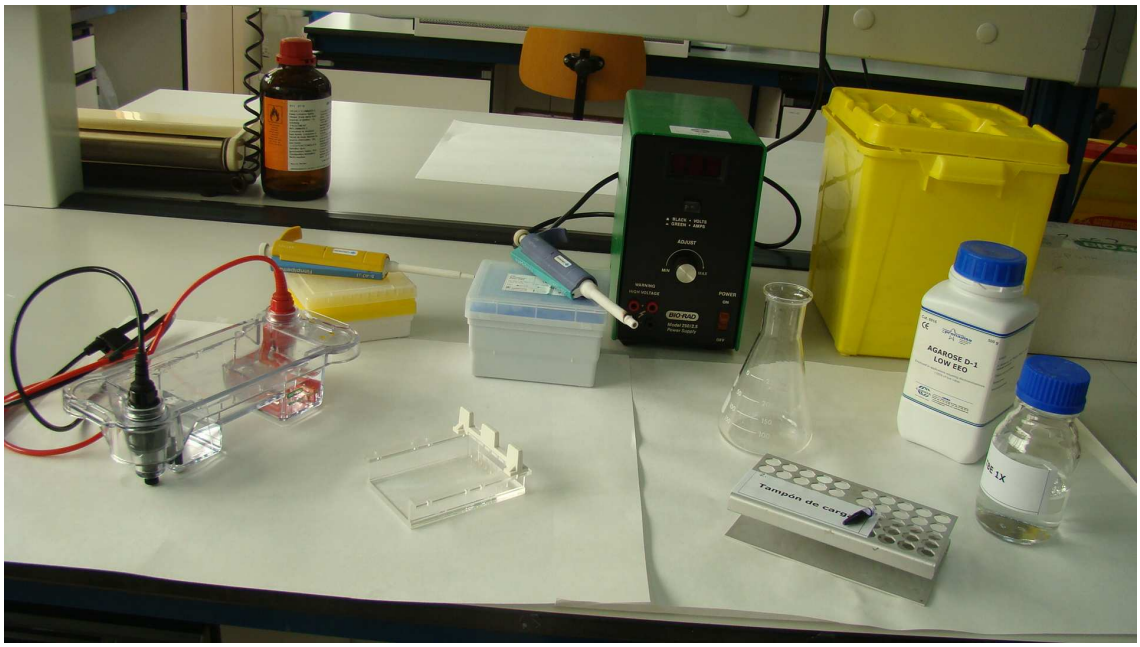
PROTOCOL 2. ANALYSIS OF DNA BY AGAROSE GEL ELECTROPHORESIS

Materials

- Flasks
- Micropipettes and sterile tips
- Agarose
- Microwave oven
- Electrophoresis chamber and gel trays
- UV transilluminator
- Gel analysis computer system

Solutions

- TBE 1X: 0,9 M Tris, 0,9 M Boric acid, 20 mM EDTA (pH=8,0). Prepare a stock solution 10 X.
- Loading buffer: 0.25 % bromophenol blue, 10% glycerol and 40% sacarose in 1X TBE. Keep at 4 ºC protected from light.
- DNA staining Solution: Gel Red
- Molecular Weight Marker: commercial MWM (eg 100 bp ladder for endonuclease digestions and PCR experiments), plasmid DNAs from control strains for plasmid analysis…..



Procedure

1. Select type and concentration of agarose required (usually from 0.7 to 1.5% w/v) which is DNA-size dependent. As a rough guide:

    fragments of > 15 kb, use 0.3% agarose

    fragments of 2-15 kb, use 0.7-1.0% agarose

    fragments of 0.1-2 kb, use 1.0-2.0% agarose

    fragments of 20-300 bp, use 3-9% NuSieve agarose

- Weigh out agarose in a Flask

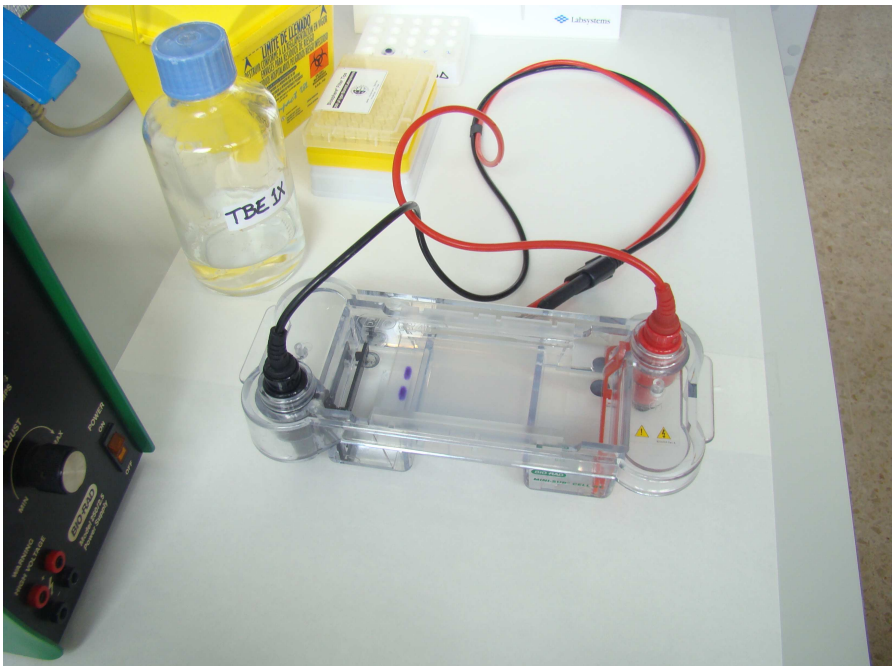
- Dissolve agarose in TBE 1X by heating in a microwave oven. Add Gel Red when the agarose is warm.

- Prepare gel mould, place the comb at one end of the tray (previously sealed with masking tape), pour agarose into mould (to cover the lower third of the comb) and allow to set at room temperature or at 4ºC. Cooling to 4ºC reduces the risk of tearing the bottoms of the wells on removing the comb.

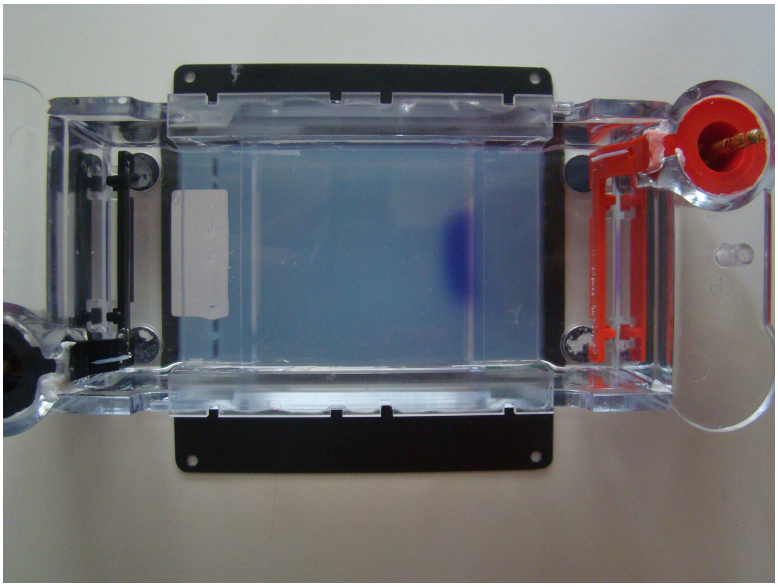
- Remove the tape from the gel tray without damaging the ends of the gel. Do not remove the comb at this point. Place the gel tray into the electrophoresis chamber.

2. Fill the electrophoresis chamber with 1X TBE buffer. The buffer must completely cover the gel and electrodes. Carefully remove the comb from the gel, leaving the wells that you will fill with your DNA samples.

3. Mix samples with 1-5 µl loading buffer and load DNA into the appropriate well.



4. Connect tank to power suply (DNA runs towards the positive (+, red) electrode. Run at 75-100 V until the bromophenol blue is about ¾ of the way down the gel.



5. Examine and photograph under UV Light.

WARNING: transilluminators emit high intensity UV light: the safe time of exposure for avoidance of extremely painful conjunctivitis is 8-10 sec per day. Always use a UV-blocking face shield.

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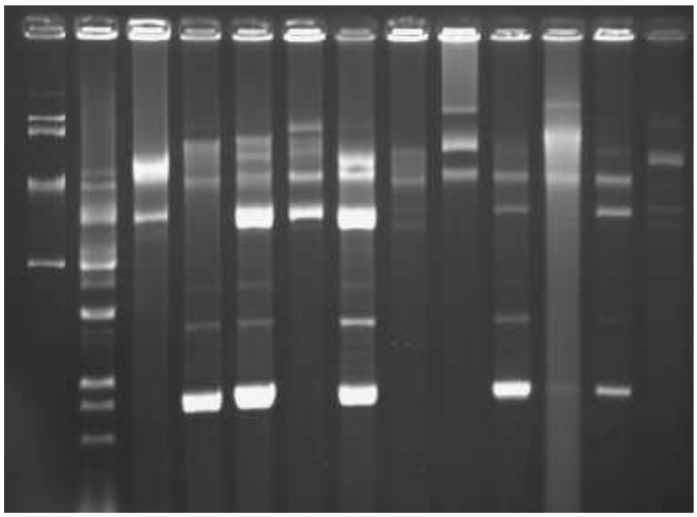

Computer-assisted analysis equipment: