

Gel migration for DNA Typing

1) Prepare migration buffer.

- The migration buffers can vary and be **TBE** or **TAE** solutions.

TAE buffer is a buffer solution containing a mixture of Tris base, acetic acid and EDTA.

TBE or Tris/Borate/EDTA, is a buffer solution containing a mixture of Tris base, boric acid and EDTA.

In molecular biology, TBE and TAE buffers are often used in procedures involving nucleic acids, the most common being electrophoresis. Tris-acid solutions are effective buffers for slightly basic conditions, which keep DNA deprotonated and soluble in water. EDTA is a chelator of divalent cations, particularly of magnesium (Mg^{2+}). As these ions are necessary co-factors for many enzymes, including contaminant nucleases, the role of the EDTA is to protect the nucleic acids against enzymatic degradation.

- 50ml to make the gel + 50ml of migration buffer => 100ml/gel.

2) Prepare the gel.

- Prepare gel at 1.2% agarose (Agarose Electrophoresis Grade In vitrogen, Ref: 15510-027) in the chosen buffer => 0.6g/50ml/gel.

- Dilute in a beaker, and microwave: 4x 30sec. Make sure it does not boil too much. Keep an eye on it, stop and shake it often.

- When everything is well dissolved, put it under cold water until you can hold the beaker without burning yourself.

- Add the safe DNA (Cambridge Biosciences GelRed 10,000X in water 0.5ml, Ref: IBRBT41003), $d=1/10000$.

- Pour about 50ml in the plastic tank. Wait 10-15min for the gel to set. Remove the well-makers and add the migration buffer.

3) Load the gel.

- 5ul DNA Ladder
- 10ul PCR product

Run at 100V for 60min. Check black negative on top, and always check there are bubbles after plugging the cover.