Lab Protocols

Running an Agarose Gel

1-2% Agarose Gel works well with DNA fragments from 100 bp – 2 Kb

1 – Using EtBr*

*Handed carefully, carcinogenic! Use gloves and do not allow any contact with your skin.

1% Agarose Gel

20 ml TBE Buffer (0.5X)
0.20 g Agarose
0.3 µl EtBr

- Add buffer and agarose in an appropriate container and microwave it until mixture is completely clear (~ 1 min)
- Leave in flask to cool down until it is no longer hot to the touch (but before it hardens)
- Add EtBr to the gel mix
- Set the gel tray inside the electrophoresis chamber, with the open sides facing the walls of the chamber.
- Pour into gel tray and set the comb
- Leave to set in an horizontal surface for approx. 30 mins
- Remove comb pick up the gel tray and place it again in running position in the electrophoresis chamber

Preparing samples:

- Place 3 ul DNA in parafilm
- Add 3 ul Loading Dye (6X)

Note: Always run a DNA ladder with samples to be able to determine the size of your amplified fragment. Use 100 bp DNA ladder or 1 KbPlus according to the size fragment that you are expecting. Loading the gel:

- Fill electrophoresis chamber with TBE Buffer (0.5X); until red line
- Load DNA ladder, at least one per row
- Load all 6 ul sample/loading dye mix in in one of the gel wells (one sample per well) using a 10 µl pipette
- Run gel at 100V for ~30 min
- Bands can be visualized and photographed using the photodocumentation system
2 –No EtBr: GelRed

1% Gel

20 ml TBE buffer (0.5X)

0.2 g Agarose

- Add buffer and agarose in an appropriate container and microwave until mixture is completely dissolved and clear (~ 1 min)
- Leave in flask to cool down until it is no longer hot to the touch
- Set the gel tray inside the electrophoresis chamber, with the open sides facing the walls of the chamber
- Pour into gel tray and set the comb
- Leave to set for approx. 30 min
- Remove comb pick up the gel tray and place it again in running position in the electrophoresis chamber

Preparing samples:

- Place 3 ul of DNA in parafilm
- Add 3 ul of Loading Dye Mix (stock solution: GelRed + Loading Dye (6X), dilution 10:1000)

Note: Always run a DNA ladder with samples to be able to determine the size of your amplified fragment. Use 100 bp DNA ladder (mix with Loading Dye Mix; 3:3) or 1 KbPlus according to the size fragment that you are expecting.

Loading the gel:

- Fill electrophoresis chamber with TBE Buffer (0.5X); until red line
- Load DNA ladder, at least one per row
- Load all 6 ul sample/loading dye mix in one of the gel wells (one well per sample) using a 10 µl pipette
- Run gel at 100V for ~30 min
- Bands can be visualized and photographed using the photodocumentation system. Always take a picture of your gel. Always keep a record.

Using GelRed with PCR Products generated with GoGreenTaq:

For products that have already Dye on it there is a slight modification of the protocol:

- Add buffer and agarose in an appropriate container and microwave until mixture is completely dissolved and clear (~ 1 min, do not let it boil)
- Leave in flask to cool until it is no longer hot to the touch
- **Add 3 ul of GelRedMix diluted in water** (working stock solution: GelRed + ddH₂O, dilution 1:100) to the gel mix
- Pour into gel box and set comb
- Leave to set for approx. 30 mins
- Remove comb and place gel box in gel tray

Loading the gel:

- Fill gel tray with TBE Buffer (0.5X); until red line
- Load your samples into gel using a 10 µl pipette
- Load DNA ladder, at least one per row
- Run gel at 100V for ~30 mins
- Bands can be visualized and photographed using the photodocumentation system. Always take a picture of your gel. Always keep a record.

**ALL AGAROSE GELS SHOULD BE DISPOSED OF IN HAZAROUS WASTE**