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| **Description** |
| This method sheet describes the procedure for the separation and visualization of DNA fragments by agarose gel electrophoresis using SYBR® Safe DNA gel stain. Protocol from the Gnatwork Bangladesh workshop, September 3-6th 2018. |
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| Updated version of: Harrup, L.E. (2014). The Pirbright Institute *Culicoides* DNA Barcoding Protocols, Version 2, available at www.ibvnet.com |

# GN_11: Agarose Gel Electrophoresis using SYBR Safe DNA gel stain

Citation: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3

## A. Introduction

This method sheet describes the procedure for the separation and visualization of DNA fragments by agarose gel electrophoresis using SYBR® Safe DNA gel stain.

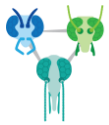
## B. Materials

### Equipment

- Pipettes (0.2-2 µl, 2-20 µl)
- 50 ml conical flask
- 100 ml measuring cylinder
- Microwave
- Balance (≤0.01 g accuracy)
- Electrophoresis gel tank with power pack
- Electrophoresis gel comb
- Electrophoresis gel tray
- 500 ml wash bottle
- 10 litre carboy or 1 litre glass or plastic bottle for 1x TAE solution
- Transilluminator (both UV transillumination and blue-light transillumination is suitable for use with SYBR® Safe DNA Gel Stain)
- Safe Imager™ viewing glasses (ThermoFisher Scientific: S37103) (or equivalent) (optional if dedicated gel documentation system is used)
- Image capture device
- Heat-proof glove or similar
- Fridge (+4°C)
- Freezer (-20°C)
- Sterile disposable pipette tips containing hydrophobic filters (0.2-2 µl, 2-20 µl)
- Parafilm® or 200 µl reaction tubes (not required if PCR product already contains a loading dye e.g. Qiagen CoralLoad Concentrate)
- Autoclave tape or gel tray end blocks
- Blue roll
- Nitrile Gloves

### Reagents

- 50x tris-acetate-EDTA (TAE) (2 M tris-acetate, 0.05 M EDTA, pH 8.3, nuclease free)
- Deionised water (or equivalent) for buffers
- DNA loading dye (e.g. Qiagen Gel Pilot Loading Dye 5x (Qiagen 239901) or Invitrogen™ BlueJuice™ Gel Loading Buffer (10X) (ThermoFisher Scientific: 10816015) (not required if PCR product already contains a loading dye e.g. Qiagen CoralLoad Concentrate)
- DNA Ladder (e.g. E-Gel® Low Range Quantitative DNA Ladder (ThermoFisher Scientific: 12373-031); ThermoFisher Scientific: TrackIt™ 50 bp DNA Ladder (ThermoFisher Scientific: 10488-043); Qiagen GelPilot 100bp Plus DNA Ladder (Qiagen: 239045))
- Agarose for electrophoresis
- SYBR® Safe DNA Gel Stain (ThermoFisher Scientific: S33102)
- DNAZap™ Solutions (ThermoFisher Scientific: AM9890) (or equivalent)



## C. Method

C.1 Put on nitrile gloves.

C.2 Use DNAZap™ (or equivalent) to clean all working areas and equipment to be used e.g. pipettes where relevant, to reduce/remove any potential DNA contamination.

C.3 Discard used gloves and put on a new pair of nitrile gloves and wear throughout the remainder of the procedure.

C.4 Prepare 1x tris-acetate-EDTA (TAE) buffer (20 ml 50x TAE per 980 ml deionized water).

C.5 Fill gel tank with sufficient 1x TAE to cover gel.

C.6 Fill labelled wash bottle with 1x TAE buffer.

C.7 Add 3 µl SYBR® Safe DNA Gel Stain to the positive end of the gel tank.

C.8 Select an appropriately sized and percentage gel (see Table 1) to cast.

C.9 Prepare casting tray by sealing ends using autoclave tape (or use appropriate gel tray blocks) and fit the gel comb(s) to the appropriate position(s) in the tray.

C.10 Add the required amount of agarose and 1x TAE to a conical flask (see Table 1) and heat to dissolve agarose in a microwave oven (beware of boiling agarose it can easily overflow and burn, use a heat proof glove or similar when handling the hot conical flask).

C.11 Allow the agarose to cool to approximately 60˚C (so that the conical flask feels warm but not hot to the touch).

C.12 Add SYBR® Safe DNA Gel Stain to a 1x concentration to the melted agarose (e.g. 3 µl SYBR® Safe DNA Gel Stain to 30 ml agarose) and gently swirl solution to mix (avoid making bubbles in the solution).

C.13 Gently pour melted agarose into the prepared casting tray (pop any bubbles in the poured agarose using a pipette tip).

C.14 Allow the gel to set at room temperature for approximately 30 minutes or until set.

C.15 If the PCR product does not already include a loading dye (e.g. Qiagen CoralLoad Con-centrate), add an appropriate amount to a subsample of the PCR product either in separate drops on a sheet of parafilm or in new 200 µl reaction tubes (if the concentration of PCR product is too high reduce the volume added to the dye and replace volume with nuclease free water). For example:

- Qiagen Gel Pilot Loading Dye (contains xylene cyanol (light blue), bromophenol blue (dark blue and Orange G (orange)): add 1 volume Qiagen Gel Pilot Loading Dye 5x to 4 volumes PCR product i.e. for 10 µl wells add 2 µl Qiagen Gel Pilot Loading Dye 5x to 8µl PCR product, to-tal volume 10 µl.
- BlueJuice™ Gel Loading Buffer (10x): add 1 µl BlueJuice™ Gel Loading Buffer (10x) to 9 µl PCR product, total volume 10 µl.

**Table 1** Agarose gel concentrations

| Percentage of Gel (*w/v*) | Efficient Range of Separation of Linear DNA molecules (base pairs (bp)) | Volume of 1x TAE (ml) | Amount of Agarose (g) |
|---|---|---|---|
| 0.5 | 1000-30,000 | 30 | 0.15 |
| | | 50 | 0.25 |
| | | 100 | 0.50 |
| 0.7 | 800-12,000 | 30 | 0.21 |
| | | 50 | 0.35 |
| | | 100 | 0.70 |
| 1.0 | 50-10,000 | 30 | 0.30 |
| | | 50 | 0.50 |
| | | 100 | 1.00 |
| 1.2 | 400-7,000 | 30 | 0.36 |
| | | 50 | 0.60 |
| | | 100 | 1.20 |
| 1.5 | 200-3,000 | 30 | 0.45 |
| | | 50 | 0.75 |
| | | 100 | 1.50 |
| 2.0 | 50-2,000 | 30 | 0.60 |
| | | 50 | 1.00 |
| | | 100 | 2.00 |

**Table 2** Loading dye mobility rates (values indicate the size of DNA fragments with which the dye will co-migrate at that particular gel concentration)

| Agarose Gel Concentration (%) | Xylene Cyanol FF | Bromophenol Blue | Cresol Red | Orange G |
|---|---|---|---|---|
| 0.7 | 8000 | 600 | 3000 | 100 |
| 1.0 | 4000 | 400 | 1500 | 50 |
| 1.5 | 2000 | 250 | 900 | 20 |
| 2.0 | 900 | 120 | 300 | <10 |
| 3.0 | 400 | 50 | >100 | <10 |

C.16 Reseal tubes containing remaining PCR product immediately and store if required for further applications at 4˚C if to be used with 24 hours otherwise store at -20˚C.

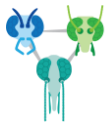
C.17 Remove the autoclave tape or gel tray end blocks from the end of the gel tray and then carefully remove the comb from the gel itself.

C.18 Load the gel with ladder and PCR product by either:

i. Placing the gel on a sheet of blue roll and using the wash bottle wash a small amount of 1x TAE buffer over the surface of the gel to fill the wells. Add an appropriate volume of DNA ladder to at least one well on each roll of the gel (volume dependent upon comb size). Then load one sample per well (use a new tip for each well to prevent cross-contamination) (no not load a sample into the well containing the DNA ladder). Then carefully lower the gel into the electrophoresis tank with the wells at the negative (black) end of the tank (DNA fragments will migrate from negative to positive.

Or

ii. Lower the gel into the electrophoresis tank with the wells at the negative (black) end of the tank (DNA fragments will migrate from negative to positive). Add an appropriate volume of DNA ladder to at least one well on each roll of the gel (volume dependent upon comb size). Then load one sample per well (use a new tip for each well to prevent cross-contamination) (no not load a sample into the well containing the DNA ladder).



C.19 Place the lid on the gel tank and connect all wires required between the power pack and the gel tank.

C.20 Run the gel at approximately 5 V/cm (distance as measured between the gel tank electrodes) for 45-60 minutes or as required (monitor the progress based on the migration distance of the gel tracking dyes in the loading dye (see Table 2 for examples).

C.21 When sufficient time has elapsed turn off and disconnect the power supply and carefully remove the gel from the electrophoresis tank.

C.22 Visualize the gel on UV transilluminator or blue-light transilluminator.

C.23 Save a copy of an image of the gel and record details in the relevant laboratory notebook including the agarose concentration used, the DNA ladder used, which wells correspond to which samples, the presence and size of bands present and details of any wells where bands are not present, ensure copy of an unedited gel is saved i.e. no contrast enhancements, cropping etc.

C.24 Unlike ethidium bromide-based gels, SYBR® Safe gels require no specialist disposal. Dispose of gel as per local rules.

C.25 Use DNAZap™ (or equivalent) to clean all surfaces used to reduce/remove any potential DNA contamination.

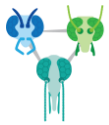
C.26 Remove and discard gloves.

## D. Results

Appropriate details of all samples used with this protocol including details of their location on the gel should be recorded in the appropriate lab book in addition to details of what percentage agarose gel was used, which DNA ladder was used, how the gel was interpreted referencing other method sheets where appropriate, and if an image capture device is available, a copy of an image of the gel, including an unedited copy i.e. no contracts enhancements, no cropping.

## E. Tips and troubleshooting

- SYBR® Safe DNA Gel Stain is a highly sensitive stain for visualization of DNA in agarose or acrylamide gels. SYBR® Safe DNA gel stain is specifically formulated to be a less hazardous alternative to ethidium bromide that can utilize either blue light or UV excitation. While SYBR® Safe DNA Gel Stain is considered safer/less mutagenic than either ethidium bromide and its alternatives such as Biotium GelRed™ it is still a DNA-binding material, and appropriate personnel protective equipment must be used (as detailed in this protocol) with reference to the appropriate local risk assessments.
- When bound to nucleic acids, the SYBR® Safe DNA gel stain has a fluorescence excitation maxima at 280 and 502 nm, and an emission maximum at 530 nm. When used with blue light illumination, SYBR® Safe DNA gel stain has less background fluorescence than ethidium bromide–stained gels when illuminated with UV light.
- SYBR® Safe DNA gel stain should ideally be stored at 4°C, however, can be stored at between 2°C to 25°C. Undiluted SYBR® Safe in DMSO freezes at low temperatures; therefore, if frozen the product must be completely thawed and mixed before using. Repeated freeze-thawing has minimal impact on product performance.



- If step C.7 was missed and the SYBR® Safe DNA Stain was not added prior to the gel before setting and being run, the gel may be stained post-electrophoresis. Add 3 µl of SYBR® Safe DNA stain to 50 ml of 1x TAE (50 ml of agarose is sufficient to cover most minigel trays, adjust volumes as required for larger gels), place the gel to be stained in a plastic container (do not use a glass container, as the dye in the staining solution may adsorb to the walls of the container, resulting in insufficient gel staining). Then gently pour sufficient 1x TAE with SYBR® Safe DNA Stain into the plastic contained such that the gel is fully immersed. Cover the container with aluminium foil and incubate at room temperature for 30 minutes, placing the container on an orbital shaker at ~50 rpm to agitate the solution. After the 30 minutes has elapsed, turn off the shaker and remove the gel, continuing with the protocol at step C.22 no destaining is required.
- If the agarose gel is observed to melt during an electrophoretic separation it is a sign that either the electrophoresis buffer has been omitted in the preparation of the gel or has become exhausted during the course of the run. For high-voltage electrophoresis over long time periods, Tris-Borate-EDTA (TBE) buffer should be used instead of TAE buffer as it has a greater buffering capacity. However, borate is an enzyme inhibitor hence using TBE is not recommended if you will be isolating the DNA for downstream enzymatic steps (e.g. via gel excision). For example, borate carry-over can affect ligations, therefore use TAE in preference for these samples.
- If poor resolution of DNA fragments are observed following electrophoresis the most frequent cause is an inappropriate choice of agarose concentration. Low percentage agarose gels should be used to resolve high-molecular-weight DNA fragments and high percentage gels for low-molecular-weight DNA (see Table 1). Low melting point agarose may be more appropriate to low-molecular weight DNA fragments, check agarose supplier's recommendation. Fuzzy bands, encountered particularly with small DNA fragments, result from diffusion of the DNA through the gel, this is especially true when gels are run for long periods of time at low voltages. Reassess the choice of agarose, gel time, running voltage and time the gel is run for and repeat as required.
- Trailing/smearing of DNA bands is most frequently observed with high-molecular-weight DNA fragments (or degraded DNA), this is often caused by overloading the DNA sample or running gels at high voltages. DNA samples loaded into torn sample wells will also cause extensive smearing, as the DNA will tend to run in the interface between the agarose and the gel support. Do not use torn gels, if trailing/smearing of band is observed in an untorn gel reassess the volume of DNA sample added per well and the voltage the gel is run at, and re-run a new gel as required.
- The dyes in the DNA loading buffers migrate at different rates depending on the dye and the concentration of the agarose gel (see Table 2). Loading buffers typically contain two or three different dyes, allowing the user to monitor DNA migration as the gel is running. The loading buffer are available with a range of different combinations of dyes with different migration rates (Table 2). Loading buffer containing dyes with migration rates most suited to monitoring the size of DNA fragment(s) to be analysed/visualised should be selected for use.