The Gnatwork
Intended use of resource / data

Open access resources and data provided by The Gnatwork should be used for the intended purpose only, as specified below.

Title of resource
GN_12: Agarose Gel Electrophoresis using Pre-Cast High Throughput SYBR Safe E-Gels
Authored by
When using this protocol, the following should be referenced: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3.
DOI
10.13140/RG.2.2.14879.41129
Description
This method sheet describes the procedure for the separation and visualization of DNA fragments using a pre-cast E-Gel® 96 gels containing SYBR® Safe DNA gel stain (ThermoFisher Scientific) and the High-Throughput E-Gel® Electrophoresis System (ThermoFisher Scientific). Protocol from the Gnatwork Bangladesh workshop, September 3-6th 2018.
Intended use
Scientific research use and training purposes.
Restrictions on use
Content is not to be redistributed in the public domain (e.g. presentation, lecture, online or in publications).
Resource history
Updated version of: Harrup, L.E. (2014). The Pirbright Institute *Culicoides* DNA Barcoding Protocols, Version 2, available at www.ibvnet.com

GN_12: Agarose Gel Electrophoresis using Pre-Cast High Throughput SYBR Safe E-Gels

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 using a pre-cast E-Gel® 96 gels containing SYBR® Safe DNA gel stain (ThermoFisher Scientific) and the High-Throughput E-Gel® Electrophoresis System (ThermoFisher Scientific).

B. Materials

Equipment

- Single channel pipette (2-20 µl)
- Multichannel pipette (5-50 µl 12-channel multichannel) (optional)
- Invitrogen™ E-Gel® High-Throughput DNA Electrophoresis System E-Base™ Integrated Power System (EBM03)
- Transilluminator (both UV transillumination and blue-light transillumination is suitable for use with SYBR® Safe DNA Gel Stain)
- Gel documentation system
- Computer with Invitrogen™ E-Editor™ Software installed
- Fridge (+4°C)
- Freezer (-20°C)
- Sterile disposable pipette tips containing hydrophobic filters (2-20 µl)
- Blue roll
- Nitrile Gloves
- Parafilm® or 200 µl reaction tubes (not required if PCR product already contains a loading dye e.g. Qiagen CoralLoad Concentrate)

Reagents

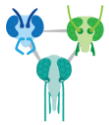
- Nuclease free water
- DNAZap™ Solutions (ThermoFisher Scientific: AM9890) (or equivalent)
- DNA Ladder (e.g. E-Gel® Low Range Quantitative DNA Ladder (ThermoFisher Scientific: 12373-031))
- E-Gel® sample loading buffer (ThermoFisher Scientific: 10482055) (ThermoFisher Scientific: 10482-055) (not required if PCR product already contains a loading dye e.g. Qiagen CoralLoad Concentrate or if the optimised run time is already known for the PCR product)
- E-Gel® 96 gels with SYBR® Safe (ThermoFisher Scientific)

C. Method

C.1 Put on nitrile gloves.

C.2 Use DNAZap™ solutions to clean all working surfaces 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 Unseal package and remove the E-Gel®, carefully remove the comb from the gel and place E-Gel® on the white back of the packet (to aid visualisation when loading the gel.

C.5 Add 10 µl of nuclease free water and 10 µl DNA ladder to each marker lane.

C.6 If no loading buffer is required (i.e. optimised run time for PCR product is already known, or the PCR product already contains a loading buffer e.g. Qiagen CoralLoad Concentrate) add 15 µl of nuclease free water into each lane a sample is to be run in, then add 5 µl of sample per well. Reseal tubes containing remaining PCR product and store at 4˚C. Then proceed to step C.8, otherwise proceed to step C.7

C.7 If the PCR product does not already include a loading dye e.g. Qiagen CoralLoad Concentrate and/or the optimised run time for PCR product is not already known add E-Gel® Sample Loading Buffer to a subsample of the PCR product in new reaction tubes or a PCR plate such that the total volume is 20 µl i.e. 5 µl PCR product, 15 µl E-Gel® sample loading buffer. Reseal tubes containing remaining PCR product and store at 4˚C.

C.8 Add 20 µl nuclease free water into all remaining unused wells.

C.9 Plug in the E-Base™ and select the EG program (The EG program is to run E-Gel® 96 and 48 gels, while the EP program is to run the E-PAGE® 96 and 48 gels) then adjust the run time setting as required. Maximum run time for E-Gel® 96 well gels is 12 minutes.

C.10 Clip the loaded gel into the E-Base™, if the gel is properly inserted the fan in the E-Base™ will start to run and the red-light illuminates.

C.11 To begin electrophoresis press the pwr/prg button on the E-Base™, the red light will change to green and the time start counting down.

C.12 When the gel has finished running the E-Base™ will beep and the green light will change to a red flashing light.

C.13 Turn off the E-Base™ and remove the E-Gel®.

C.14 Visualize the gel on UV Transilluminator or blue-light transilluminator.

C.15 Save copy of an image of the gel, then align and save a copy of the aligned gel image using E-Editor™ software. Record details in the relevant laboratory notebook including agarose concentration used, 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 both the aligned and unedited gel is saved i.e. no contrast enhancements, cropping etc.

C.16 Unlike ethidium bromide-based gels, SYBR® Safe gels require no specialist disposal. Dispose of gel as per local rules.

C.17 Discard PCR product if no longer required for further downstream applications, otherwise store PCR product at 4°C if to be used in the next 24 hours, or store at -20°C.

C.18 Use DNAZap™ (or equivalent) to clean all surfaces used to reduce/remove any potential DNA contamination.

C.19 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.

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 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 so should be regarded as potentially mutagenic, 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 illuminated with UV light.
- If poor resolution of DNA fragments are observed following electrophoresis the most frequent cause is an inappropriate choice of agarose concentration or insufficient running time to allow bands to separate. Low percentage agarose gels should be used to resolve high-molecular-weight DNA fragments and high percentage gels for low-molecular-weight DNA. Reassess the choice of gel concentration and time the gel is run for and repeat as required. E-Gel® with SYBR® DNA Stain are suitable for the resolution of 100 bp to 2 kb fragments dependent upon the E-Gel® choose (see suppliers recommendation). The detection sensitivity is 1ng per band of DNA.
- Trailing and smearing of DNA bands is most frequently observed with high-molecular-weight DNA fragments, 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.
- Each E-Gel® should be loaded within 30 minutes of removing the gel from the package and run within 15 minutes of loading.
- Adjust the run time for the E-Gel® as appropriate for the fragments being used such that they are well distributed across the length of the run but not such that they overrun into the next well.
- The E-Gel® 96 loading format is compatible with multi-channel pipettors, and the most commonly used 8-, 12-, and 96-pin liquid handling robots.
- See the E-Gel® Technical Guide for further information on the E-Gel® system or contact a ThermoFisher Scientific technical representative.

F. References

E-Gel® Technical Guide