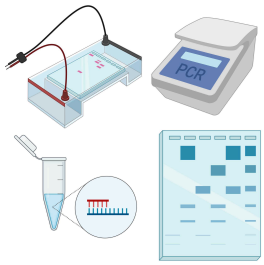
Feb 07, 2024

DNA Quality Control by Agarose Gel Electrophoresis



Forked from DNA Quality Control by Agarose Gel Electrophoresis

DOI
dx.doi.org/10.17504/protocols.io.kqdg3xzb1g25/v1

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Protocol Citation: Benjamin Schwessinger 2024. DNA Quality Control by Agarose Gel Electrophoresis. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3xzb1g25/v1**

Protocol status: Working
We use this protocol and it's working

Created: February 07, 2024

Last Modified: February 07, 2024

Protocol Integer ID: 94792

Keywords: quality control by agarose gel electrophoresi, dna quality via agarose gel electrophoresi, agarose gel electrophoresis dna, agarose gel electrophoresi, different wheat pathogen, wheat pathogen, uninfected wheat plant, plant pathogen diagnostic, wheat plant, dna quality, old wheat plant, genomic, total dna stock sample, week old wheat plant, pathogen, dna concentration, whole dna extract, infective agent for each treatment group, dna size, dna length, relative dna concentration, dna, experiment, dna length of molecule, dna size of ones dna, ones dna, major pathogen, standard dna ladder with band, size of dna molecule, standard dna ladder

Abstract

This protocols is part of the ANU Biosecurity mini-research project #1 "Plant Pathogen Diagnostics: Visuals, subcultures, and genomics".

You will be provided four pots of 3-4 week old wheat plants that have been infected with different wheat pathogens. Each pot has been infected with one major pathogen. You will not know which pot has been infected with which pathogen. However, you will be provided a compendium of 10-15 wheat pathogens that will guide you to identify the infective agent for each treatment group. The fifth treatment group will be uninfected wheat plants which will be clearly identified. You can use treatment group #5 as negative control for your experiments.

In total, each group will obtain five pots each:

	A	B
	Treatment group 1	Unknown infective agent
	Treatment group 2	Unknown infective agent
	Treatment group 3	Unknown infective agent
	Treatment group 4	Unknown infective agent
	Treatment group 5	Uninfected control

This specific protocol is a step by step guide to assess DNA quality via agarose gel electrophoresis. Agarose gel electrophoresis lets you assess the size of DNA molecules. In addition, you can estimate DNA concentrations and impurities of RNA.

During agarose gel electrophoresis DNA gets linearised. Larger molecules take longer to migrate through the gel when migration is driven by a electric potential at the specific pH of the buffer. When running a standard DNA ladder with bands of known molecular size, one can compare the DNA size of ones DNA or PCR sample with the known standards.

The final goal is to achieve the following:

- To assess DNA length of molecules in total DNA stock samples and for all PCR reactions.
- To approximately estimate relative DNA concentrations.
- To test if negative PCR controls did not amplify anything as appropriate.
- To test if whole DNA extracts contain RNA in addition to DNA.

- Select samples from three research groups to be sequenced in the practical of week 6.

This protocol is applicable for week 5.

Protocols progress overview:

- Week 5 Run a 1% Agarose Gel electrophoresis for all samples of each research group.

Some useful explainers and resources:
- **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846332/**
- https://en.wikipedia.org/wiki/Agarose_gel_electrophoresis
- https://www.youtube.com/watch?v=ZDZUAleWX78

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Guidelines

You must have read, understood, and follow the health and safety instructions provided in the "Overview Mini-Research Project #1 BIOL3106/6106" provided on Wattle (ANU learning portal).

You must have signed and returned one copy of the "Student Safety Declaration Form For Practical Class Work" before starting any laboratory work.

You must have read and understood the Hazard Sheets (Risk assessment) of all chemicals listed bellow in the "Safety Warnings" section. These Hazard Sheets are provided on Wattle as part of the "Overview Mini-Research Project #1 BIOL3106/6106" document.

Materials

As always you need to bring a lab notebook, a printed version of this protocol, and a pen to record your adventures in the lab.

Consumables and culture material needed:

- Pre-cast 1% Agarose Gel in TBE
- PCR strip tubes
- Gel Loading Dye, Purple (6X); 20 uL total. https://www.nebiolabs.com.au/products/b7024-gel-loading-dye-purple-6x#Product%20Information
- DNA ladder; 12 uL total. https://www.nebiolabs.com.au/products/n0550-quick-load-purple-1-kb-plus-dna-ladder#Product%20Information

Equipment needed:

- Mini benchtop spinner for PCR strip tubes of 8.
- Voltage block.
- DNA gel electrophoresis chamber

Troubleshooting

Safety warnings

This protocol does not require any hazardous substances.

You need to wear safety equipment at all times including lab coats, gloves, and safety goggles when handling chemicals and biological agents. While the major biological agents used for the wheat infection are pathogens commonly found in Australia, you must treat them as they were infective agents of general concern. Treat them with care. Do not remove them from the laboratory. Do not spread them via clothing. Use a dedicated notebook and pen to make notes during the mini-research project. Do not put anything into your mouth while in the laboratory. Wash your hands each time you leave the laboratory.

Before start

You must study the protocol carefully before you start. If anything is unclear post questions directly here on protocols.io.

Week 5: DNA agarose gel electrophoresis

1h 48m

1 You will load all DNA and PCR samples into one lane of a 1% agarose gel each. You will share your gel with another research group as each gel has two rows.

2 You will receive your ITS PCR strip tubes of 8 with PCR reaction from last week 4. The strip will contain the 5 reaction plus negative control for the ITS reaction. 5m

3 You will receive your diluted DNA stock solutions @ 10ng/ul for TG1 to TG5 from last week. 2m

4 Now you will prepare all samples to be ready to be loaded onto the agarose gel.

4.1 Label a two new strip tubes of 8 with PCR or DNA and the respective sample names TG1-TG5 + neg or TG1-TG5. 5m

4.2 Pipette 5 uL of each PCR reaction or DNA stock [10ng/ul] solution into the correctly labelled strip tube of 8. 5m

4.3 Add 1 uL of gel loading dye to each sample. This will be 11 samples in total. Six PCR samples and five DNA samples.
Close all tubes carefully once you added the loading dye. 5m

4.4 Now mix the samples with the gel loading dye by flicking them briefly with your finger. Make sure all tubes are closed properly before flicking. 2m

4.5 Spin down the content of the PCR strip tubes in the small benchtop spinners that take PCR strip tubes of 8. Collect all liquids at the bottom of the tube. 2m

5 Now you can proceed to loading the samples onto the agarose gel

5.1 Note down the sample order number on how you indent to load the samples on the agarose gel. For example, DNA ladder; ITS TG1 to TG5, negC; DNA TG1 to TG5; DNA ladder. 5m

5.2 You will share one gel with another group. Each group will load their samples either in the top or the bottom row. Note down which row you are using and co-ordinate with your paired research group.

5.3 Load 5 ul the DNA ladder and your samples as noted down. 10m

6 Once all samples are loaded from both research groups, add the lid, connect the power block and set for 1hr @ 100 V. 1h

7 After 1hr, open the lid and take out the gel.

8 Image the gel as instructed by the demonstrator. 5m

9 Make sure to label your pictures as follows so we can share them online in class and you can clearly identify your pictures. 2m

YYYYMMDD_ResearchGroupName_PCRGel_PictureNumber

For example:

20230206_BSPS_PCRGel_2.jpg

Pictures that do not follow this naming convention will not be shared with the class.

10 Now you have captured the DNA quality and size distribution. We will discuss results in class and select the samples of three research groups for metabarcode sequencing and total DNA sequencing in week 6.