LSOP Title	Gel electrophoresis and imaging
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Location	UQ Node/Centre-wide
Policy/Procedure Link	
Risk Assessments	
Approved by	Akila Wijerathna-Yapa
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Contact for Assistance	plantsuccess@uq.edu.au

1.0 Scope

This procedure covers the creation of a gel, loading the gel, and imaging of the gel. It also includes creation of the TAE buffer.

This LSOP does not cover how to create samples for loading, how to analyse gel imagining results, or how to use gels with ethidium bromide.

2.0 Definitions

Min – minutes

mL – milli litres

uL – micro litres

TAE – Tris base/acetic acid/EDTA (ethylenediaminetetraaceticacid) buffer

TBE – tris/borate/EDTA buffer

PCR – polymerase chain reaction (samples)

3.0 Materials and Equipment

1. Microwave
2. Magnetic mixer and stirring rod
3. Gel cast + combs
4. Redsafe staining dye
5. TAE/TBE buffer
6. Pipette and pipette tips
7. Loading dye
8. DNA ladder

9. Gel tank + power supply
10. Agarose gel
11. MilliQ water
12. Gel visualiser
13. Paper towel
14. Glass beaker

4.0 Prescribed Actions

Gel:

1. Measure out required agarose gel powder:
    a. There are three volumes for gels: 50ml (small), 100ml (medium), 250ml (large).
    b. Red safe nucleic acid staining solution for gel 5ul for 100ml gel.
    c. To calculate required agarose gel powder.
        i. % gel conc wanted x gel volume.
        ii. Usually 1.2-1.5% gel conc. Used.

2. Fill beaker with TAE/TBE buffer (see appendix) and heat in the microwave to mix.
3. Cool down mixture slightly and place on top of magnetic mixer with stirring rod inside beaker.
4. Add in appropriate amount of redsafe staining dye to beaker and allow to mix.

5. Place mixture into gel box that has gel combs already placed in and allow gel to set (~20min).
6. Replace/remove combs after gel is set and place gel box in electrophoresis tank filled with TAE/TBE buffer.

    N.B. if using 6X loading dye, add 5ul dye to every 25ul of sample.
7. Prepare PCR samples by adding in 4uL dye so that each reaction has 20uL total volume.
8. Pipette in DNA ladder and prepped PCR solutions into the combs.
9. Place electrophoresis tank lid with connected electrodes on top of tank and connect wires to power supply.
10. Set to 150V and run for 20min.
11. Once gel is run, visualise in glei mating system and save image file.

Gel visualisation:

1. Stop gel run by pressing stop button on the power supply button.
2. Turn on gel visualiser machine screen/computer and login by clocking on “lab” option.
3. Remove gel cast container from chamber and either place the gel + gel cast in doc or remove gel from cast and place in doc.

4. Wipe up any excess TAE buffer before closing gel doc.
5. Press camera icon found in lower left hand of screen and wait for image to be produced.

6. Edit image using “transform” option in bottom of screen.
7. Save image.

8. Remove gel/gel+gel cast and throw away.
9. Wipe excess TAE buffer on doc and close.
10. Turn off visualiser screen.

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5.0 Appendix

TAE buffer:

1. Take the 50xTAE buffer
    a. Above the sink on the shelf above the TAE buffer dispenser
2. 100mL of 50XTAE buffer
3. Top up to 5L with MilliQ water
4. Make sure to label the bottle with date, name, conc and chemical information,