OSUCCC LTB Laboratories Procedure Quality Control of DNA using agarose gel electrophoresis with post-run staining			Effective: 1/01/2014
Written by: Robin Taxier, Clinical Laboratory Technologist		Reviewed by: Donna Bucci, Laboratory Manager	
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1.0 PRINCIPAL

This step is essential for checking that the isolated DNA is of high molecular weight. For adequate resolution of RFLPs, native DNA should migrate as a tight band of molecular weight > 40 Kb. However, degradation of part of the isolated DNA is inevitable, and the protocol below is designed to run the DNA under optimal conditions for ascertaining the relative amounts of degraded and high molecular weight DNA.

2.0 SPECIMEN

DNA samples isolated from mouthwash and buccal samples.

3.0 MATERIALS AND REAGENTS

DNA samples
Gel: Agarose
- 20x TAE Buffer
- ddH2O
- Ethidium bromide (see EtBr protocol for safe handling)
- Molecular weight marker (lambda scale)
- Agarose gel loading dye

4.0 EQUIPMENT

Microwave
Electrophoresis Unit
Imaging Unit
Erlenmeyer flask

5.0 SAFETY

Ethidium Bromide is a known intercalating agent. Use gloves and extra precaution. Waste product containing Ethidium Bromide (gel, staining liquid) is to be disposed of in a specially marked container.

6.0 PROCEDURE

6.1 CASTING THE GEL

6.1.1. Prepare an agarose gel using the proportions listed here.

6.1.2. Combine the agarose and water in a Erlenmeyer flask of at least twice the volume of the gel recipe.

6.1.3 Heat the agarose and water in a microwave for one-minute intervals, removing the flask to swirl the agarose and water, until the agarose is dissolved into the water. The solution should not be cloudy. Watch closely to ensure that the mixture does not boil over.

	0.7%	1.0%	2.0%
Agarose	1.05 g	1.5 g	3.0 g
20X TAE	7.5 ml	7.5 ml	7.5 ml
ddH2O	142.5 ml	142.5 ml	142.5 ml
total volume	150 ml	150 ml	150 ml

Note: Half of a recipe is sufficient for an 8x8x2.5 cm plate

6.1.4. Add 20X TAE swirl to mix, and pour the gel onto a taped plate with casting comb in place. Allow 20-30 minutes for solidification.

6.2 LOADING AND RUNNING THE GEL

6.2.1. Carefully remove the tape and the gel casting combs and place the gel in a horizontal electrophoresis apparatus. Add 1X TAE electrophoresis buffer to the reservoirs until the buffer just covers the agarose gel.

6.2.2. Add 10 µl of the diluted and dyed molecular weight marker (as per manual instructions for the specific product) in the first lane. Add 10 µl of at least one-tenth volume of 10X agarose gel loading dye to each DNA sample, mix, and load into the rest of the wells.

6.2.3. Electrophorese the gel at 85-120 mA until the required separation has been achieved.

6.3 STAINING AND IMAGING THE GEL

6.3.1. Prepare a solution of .5 µg/mL EtBr in gel running buffer or ddH2O. Submerge the gel in the staining bath for 20 minutes, with occasional gentle agitations. After this point, use extreme caution when handling the gel.

6.3.2. Submerge the stained gel in a bath of ddH20 – enough to completely cover the gel –for 10 minutes, with occasional gentle agitations to reduce background staining.

7.0 REFERENCES

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2.http://ww2.chemistry.gatech.edu/~lw26/bCourse_Information/4581/techniques/gel_elect/agarose.html

3. "Imaging Ethidium Bromide Stained Nucleic Acid Gels using the KODAK Image Station 2000R" from www.carestreamhealth.com/go/molecular

4. https://www.nationaldiagnostics.com/electrophoresis/article/ethidium-bromide-staining