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Analysis of the Genetic Diversity of Neisseria Meningitidis in South Africa

Coulson, Garry Brian

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APPENDIX A

Pulsed-Field Gel Electrophoresis (PFGE)

1. Cell Suspension Buffer (100mM Tris, 100mM EDTA pH 8.0)

    - 50ml 1M Tris* (pH 8.0)
    - 100ml 0.5M EDTA* (pH 8.0)

    Dilute to a final volume of 500ml with sterile water and autoclave.

2. Cell Lysis Buffer (50mM Tris, 50mM EDTA pH 8.0 and 1% sarcosyl)

    - 50ml 1M Tris* (pH 8.0)
    - 100ml 0.5M EDTA* (pH 8.0)
    - 10g Sarcosyl* powder

    Dilute to a final volume of 1000ml with sterile water and autoclave.

3. Proteinase K (20mg/ml stock solution)

    - 100mg Proteinase K powder (Roche Diagnostics, Mannheim, Germany)
    - 5ml Sterile water

    Aliquot into 500µl amounts and store frozen.

4. 1% Seakem Gold® - 1% SDS Agarose Gel

    - 0.5g Seakem Gold® Agarose
    - 47ml TE Buffer
    - 2.5ml 20% SDS* solution

    Dissolve agarose completely in the TE buffer and place flask in 55°C water bath for 5mins. Add 2.5ml SDS and mix well. Return to the 55°C water bath.

* Sigma Chemical Co, St. Louis, MO, USA.

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5. TE buffer (10mM Tris, 1mM EDTA pH 8.0)

- 10ml 1M Tris* (pH 8.0)
- 2ml 0.5M EDTA* (pH 8.0)

Dilute to 1000ml with sterile water and autoclave.

6. 1% Seakem Gold® Agarose

- 1.5g Seakem Gold® Agarose (Cambrex Bio Science, Rockland, USA)
- 150ml 0.5X TBE Buffer

Dissolve agarose completely in the TBE buffer and place the flask in a 55°C water bath.

7. 5X TBE Buffer Stock

- 54g Tris powder*
- 27.5g Boric acid powder*
- 20ml EDTA (0.5M, pH 8.0)

Make up to 1000ml with sterile water and dissolve completely.

* Sigma Chemical Co, St. Louis, MO, USA.

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APPENDIX B

Serogroup-Specific PCR (SS-PCR)

1. 2% Agarose Gel

- 0.6g Agarose powder
- 30ml 1X TAE buffer

Dissolve the agarose completely by boiling, allow to cool slightly and add 2µl ethydium bromide (10mg/ml). Pour into gel tray and allow to polymerize for 30mins.

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APPENDIX C

Multi-Locus Sequence Typing (MLST)

1. 5M Sodium Chloride (NaCl)

    - 29.22g NaCl powder*

    Dissolve NaCl powder completely in 100ml sterile water and autoclave.

2. 10% CTAB/0.7M NaCl

    - 4.1g NaCl *
    - 10g CTAB (Cetyltrimethylammonium bromide)*

    Dissolve NaCl and CTAB completely in 80ml sterile water. Heat to 65°C if necessary to assist with dissolving. Adjust the volume to 100mls with sterile water.

3. 6M Sodium Iodide (NaI)

    - 135g NaI*

    Dissolve the NaI completely in 150ml sterile water and autoclave. Store at 4°C.

4. NEW Wash Buffer (0.1M NaCl, 10mM Tris pH 8.0, ethanol, water)

    - 1ml 1M Tris* (pH 8.0)
    - 2ml 5M NaCl*
    - 200µl 0.5M EDTA (pH 8.0)

    Make up to 100ml with sterile water and autoclave. Add 100ml reagent grade Ethanol and store at -20°C.

* Sigma Chemical Co, St. Louis, MO, USA.

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