Laboratory Operating Procedure

DNA extraction (bacteria)

28/09/17

Prepared by: *Yue Tang*

Contact: *Yue.tang@apha.gsi.gov.uk*

Institution: *APHA, UK*

LOP-Version: 1

Attachments:

Introduction

This protocol describes an automated method to extract DNA from bacterial cells. The isolation procedure is based on magnetic-bead technology. The samples are lysed by incubation with a special buffer containing chaotropic salts and Proteinase K. Magnetic Glass Particles are added and the DNA is bound to their surfaces. Unbound substances are removed by several washing steps, then the purified DNA is eluted. The MagNA Pure LC automatically performs all isolation and purification steps such as addition of Lysis/Binding buffer and magnetic glass particles (MGPs), binding of DNA to the MGPs, washing steps, elution of the pure DNA, and transfer to a cooled storage cartridge.

Sample Material

Bacterial culture

Equipment and Reagents

Equipment

- Heating block capable of maintaining 65°C
- Microcentrifuge tubes
- Vortex Mixer
- MagNA Pure LC 2.0 Instrument for 8-32 samples per run

Reagents

- MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)

Literature

- Application note: *https://lifescience.roche.com/wcsstore/RASCatalogAssetStore/Articles/MP96_App_Note_5_2015.pdf*

-

1. 1.5 ml over-night cultures in LB broth from a single colony.

2. - Spin to collect bacterial cells.

3. - Wash cells with 500 µl TE buffer

3 - Re-suspend cells in 100 µl TE buffer

4 - Add 130 µl Bacterial Lysis Buffer and 20 µl Proteinase K

5 - Incubate at 65 °C for 10 minutes

6 - Place 100 µl sample mix in a sample cartridge

7 - Perform automated DNA extraction according to manufacturer's instructions

8 - Store DNA samples at -20°C until required