**Extraction of WGS Bacterial Isolates Using MagMAX CORE**

1.0 Purpose

The purpose of this procedure is to define a uniform method of extraction of DNA and RNA from different sample type using an in-house bead loading system and MagMAX CORE Nucleic acid purification kit (cat.#A32700 or A32702).

2.0 Responsibility

It is the responsibility of all laboratory technicians to follow this procedure. It is the responsibility of the laboratory management to ensure that this procedure is accurate and up-to-date.

3.0 Scope

This procedure is applicable to samples types listed in step 5.0 below requiring DNA and RNA extraction. This procedure utilizes the MagMAX CORE Nucleic Acid Purification Kit (Thermo, cat.#A32700 or A32702).

# **4.0 Reagent preparation**

Note: Throughout this procedure, 1x PBS pH=7.4 is referred to simply as PBS.

4.1 All components of this kit are stored at room temperature.

4.2 Make Lysis solution as per MagMAX CORE Lysis Buffer and Bead Mix recipe, which includes 450 µl/reaction of MagMAX Core Solution and 2.05 µl/reaction of Reagent DX (Qiagen, cat.# 19088). After the addition of each component of the Lysis Buffer, be sure to thoroughly vortex the solution. Keep the solution at room temperature.The prepared lysis buffer can be stored at room temperature for up to 24 hours.

4.3 Prepare Bead/Proteinase K Mix as per recipe, 20 µl/reaction of MagMAX CORE Magnetic Beads and 10 µl/reaction of MagMAX CORE Proteinase K. It’s recommended to prepare Bead/Proteinase K mix daily but this can be stored at 4°C for up to one week.

* 1. **Sample preparation**
  2. All samples are prepared in a biological safety cabinet, using sleeves and double gloves. Use freshly prepared 10% bleach solution (0.525% NaOCL) to decontaminate inbetween samples. Do not votex or centrifuge samples to reduce risk of contamination.
  3. Plate or Slant for Intra- and Extracellular Bacteria
     1. Add 200 µl of PBS to a labeled 1.5 ml microcentrifuge tube.
     2. Using a disposable loop, take 1 loop-full of bacteria from the plate or slant. Gently swirl the loop in the PBS to dislodge the bacteria.
     3. Proceed to step 6.1.
  4. Culture, solid subsample for Intra- and Extracellular Bacteria
     1. Add 200 µl of PBS to the subculture and gently pipette the mixture up and down to resuspend the pellet.
     2. Proceed to step 6.1.
  5. Culture, liquid subsample for Intra- and Extracellular Bacteria
     1. Shake the culture bottles in the biological safety cabinet for 2 to 3 min using the VXR basic Vibrax shaker (IKA, cat.# 0002819001). After shaking, tap them on the bench to remove liquid from the inner cap before opening.
     2. Draw 200 μl from each bacterial liquid subsample and add it to the bead tubes as described.

1. **Plate set up and sample Transfer From Tube to Lysis Plate**
   1. Plate setup:

|  |  |  |  |
| --- | --- | --- | --- |
| **Plate ID** | **Plate Type** | **Reagent** | **Volume per well** |
| Tip Comb | Standard plate | Place a tip comb in the plate.  Be sure the comb is flat | |
| Sample plate | Deep well | Bead mix,lysate and CORE binding Solution | |
| **Wash 1** | Deep Well | MagMAX CORE Wash Solution 1 | **500 l** |
| **Wash 2** | Deep Well | MagMAX CORE Wash Solution 2 | **500 l** |
| **Elution**(label with worklist date) | Standard | MagMAX CORE Elution Buffer | **90 l** |

Deep well plates (Axygen, cat.# P-2ML-SQ-C), standard plates (Thermo, cat.# 1900110), plate covers (Corning, cat.# 3931), and deep-well plate comb (Thermo, cat.# 97002534).

* 1. Add 0.5 mm diameter Zirconia/Silica beads (Biospec, cat.# 11079105z) to the polypropylene cluster tubes (Axygen, cat.# MTS-11-8-C-R) using the bead dispensing apparatus.
  2. Add **450 l of lysis buffer** to each bead tube corresponding to a sample.
  3. Pipet to mix and transfer **200 l of sample** (or PBS for NECs) to the lysis buffer in a biological safety cabinet.
  4. After all samples and controls have been added to the bead tubes, seal the bead tubes with a cap cluster mat (USA Scientific, cat.# 1775-3007). Be sure that all caps are inserted into the tubes to the same depth.
  5. Place the compression mat over the sealed bead tubes to prevent unnecessary movement and leaking when it is placed into the Mini-Beadbeater-96 (BioSpec, cat.#1001). Place the box lid over the compression mat.
  6. Place the bead tube rack in the Beadbeater with the rack lid facing towards you.
  7. Bead beat the samples for 2.5 min, rest for 5 min, and bead beat for an additional 2.5 min. Be sure to switch off and unplug the Beadbeater.
  8. Centrifuge samples at 2,500 rpm for 5 min.
  9. Using the decapper (Micronic, cat.# MP54001), remove the caps from the tubes. Discard the caps into the waste container.
  10. Make Bead/Proteinase K mix as per recipe printed in step 4.3. Keep at room temperature.
  11. Prepare wash and elution plates as per step 6.1.
  12. Invert the tube of Bead/Proteinase K and vortex to mix several times to resuspend the beads, then add **30 µl** of **Bead/Proteinase K mix** to the required wells on the deep well plate labelled “Sample”.
  13. Transfer **500 µl of lysate** from bead tubes to the deep well plate with bead mix. Be careful not to transfer any zirconia/silica beads.
  14. Using BioShake XP (QInstruments, cat.# 1808-0505) shake sample/bead mix at 1800 rpm for 2 min.
  15. Add **350 µl of MagMAX CORE Binding Solution** to each sample well. Immediately proceed to magnetic particle processore (KingFisher Flex).
  16. Turn on the KingFisher Flex. Push the right arrow to select “user” then the down arrow to select “DNA” and then toggle down to the “MagMAX\_Core\_Flex” program and then push start.
  17. Load each of the plates according to the display’s instructions, pressing start after each plate is loaded. Be sure that the orientation of the plate is correct (A1 is in the A1 location).
  18. Press start and record usage in the log book.
  19. The program runs approximately 27 minutes. The first plate to be removed is the Elution plate, which now contains the eluted DNA. Place a lid over this plate, label it with the worklist date, and seal it with parafilm. Place the plate with the eluted DNA on ice. All other plates can be placed in a ziplock bag and discarded in the biohazard waste, with the exception of the unlabeled plate that held the comb, which can be reused.
  20. Turn off the KingFisher Flex and wipe down with DRNaseFree solution (Argos, cat.# EW-04397-24).
  21. The elution plate can now be used for bacterial whole genome sequencing methods.

1. References

Applied Biosystems, Instruction Manual for MagMAX CORE Nucleic acid purification kit (cat. #A32700 or A32702)

AAVLD Section 5.4 Test Methods

ISO 17025 Section 5.4 Test Methods

AHDC QM Section 5.4 Test Methods and Method Validation

8.0 Changes from Previous Version