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Extraction of bacterial DNA using MagMAX™ CORE Nucleic Acid Purification Kit on KingFisher™ Flex Instrument

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Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

This procedure is used to extract genome DNA of bacteria isolates using theMagMAX™ CORE Nucleic Acid Purification Kit on the KingFisher Flex Robot. The process consists of Sample processing, Prepare plates for Robot, and Operate on KingFisher Flex machine.

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Robot, KingFisher Flex, MagMAX CORE, DNA Extraction

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In the whole procedure, need wear PPE

Bacterial culture isolates
MagMAX™ CORE Nucleic Acid Purification Kit (A32702 or A32700)

Need work in the biosafety level 2 hood to scoop the bacteria in the buffer tube

:

1 Prepare plates for Robot

1.1 Prepare Wash Plate 2 by adding 500 µL of MagMAX™ CORE Wash Solution 2

1.2 Prepare Wash Plate 1 by adding 500 µL of MagMAX™ CORE Wash Solution 1

1.3 Prepare Elution Plate by adding 100 µL of MagMAX™ CORE Elution Buffer

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1.4 Set Tip Comb in a 0.5 ml 96-Well Plate

A	B	C	D
Plate setup of Processing Plates: KingFisher™ Flex instrument			
Plate ID	**Plate Type**	**Reagent**	**Volume Per Well**
Wash Plate 1	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
Wash Plate 2	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
Elution Plate	Standard	MagMAX™ CORE Elution Buffer	100 µL
Tip Comb Plate	Standard	Place a tip comb in the plate	Place a tip comb in the plate

1.5 Prepare Bead/PK mix by adding 20 µL of MagMAX™ CORE Magnetic Beads and 10 µL of MagMAX™ CORE Proteinase K for the required number of samples plus 10% overage.

1.6 Prepare Lysis/Binding solution by adding 350 µL of MagMAX™ CORE Lysis Solution and 350 µL of MagMAX™ CORE Binding Solution for for the required number of samples plus 10% overage. Mix by inverting the tube or bottle at least 10 times.

1.7 Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the required wells in the 2.4 mL 96- deep well sample plate.

1.8 Transfer 200 µL sample to a well with 30 µL Bead/PK mix in Sample plate. Shake vigorously for 2 minutes on a plate shaker at room temperature, or pipette mix them and incubate for 2 mins at room temperature.

1.9 Add 700 µL of Lysis/Binding Solution to each sample-containing well.

2 Sample processing

From plate, scoop a full loop of 1-µl loop to 200 µL of PBS or molecular grade water solution. Mix the sample by vortexing.

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3 Operate on KingFisher Flex machine

3.1 Select the right script on the instrument or load the script if it was not already in the instrument.

Download kit script KingFisher Flex heated script: MagMAX CORE_Flex.bdz at https://www.thermofisher.com/order/catalog/product/A32700#/A32700, and then use BindIt sofware to transfer this script from a computer to the machine with the connection line.
BindIt software can be requested and downloaded at the website https://www.thermofisher.com/us/en/home/global/forms/life-science/download-bindit-software-kingfisher-instruments.html

3.2 Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The instrument will direct the user as to which plate to load in what position.

3.3 Load the Tip Comb Plate

3.4 Load the Elution Plate

3.5 Load the Wash Plate 1

3.6 Load Wash Plate 2

3.7 Lastly, load the sample plate

3.8 Click Start Button, and the machine will process and complete in around 20 mins

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4 Laboratory specific variations from protocol

Ohio ADDL: use molecular grade water to resuspend the colonies.

Illinois VDL, Mississippi VRDL, and Oklahoma ADDL: use PBS to resuspend the colonies.

Oklahoma ADDL: uses Dry Garnet 0.7mm beads (component of QiAMP fecal kit) to lyse the colony solution on Qiagen Tissuelyzer II (Cat. No. / ID: 85300) for 90seconds at 30Hz.

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