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Extraction of bacterial DNA using ChargeSwitch gDNA Mini Bacteria kit on KingFisher Flex robot

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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 the ChargeSwitch gDNA Mini Bacteria 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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In the whole procedure, need wear PPE

Bacterial culture isolates
ChargeSwitch™ gDNA Mini Bacteria Kit (CS11301)

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

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At the first time use, add RNase A to Resuspension Buffer (R4).

1 Sample processing

1.1 Bacterial Broth Culture

If using broth, pick single colony and grow in 5 ml Trypticase Soy Broth (TSB), overnight grow at 37 degree without shaking. Use 0.4 ml of the bacterial culture and centrifuge for 4min at 8000 rpm, discard supernatant and resuspend the cell pellet in 100 µL of Resuspension Buffer (R4) containing RNase A, then add 5 ul of lysozyme solution (50 mg/ML). Mix by vortexing. After adding RNase A to Resuspension Buffer (R4), store the buffer at 4 degrees Celsius.

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1.2 Bacterial Colonies in Plate

If using bacterial colonies, scoop 3 colonies into 100 µL of Resuspension Buffer (R4) containing RNase A, and then add 5 ul of lysozyme solution (50 mg/ML). Mix by vortexing.

2 Prepare plates for Robot

2.1 Load all resuspension solution prepared in 1.1. or 1.2 into 2.4 mL 96 deep well Sample plate.

2.2 Prepare Elution Plate by adding 100 ul of Elution buffer to each well of the 0.5ml 96-well plate.

2.3 Prepare 3 Wash Plates (Wash Plate 1, Wash Plate 2 and Wash Plate 3) by adding 650 ul Wash Buffer (W12) to each well of 2.4 mL 96 deep well plates.

3 Operate on KingFisher Flex machine

3.1 Install script of ChargeSwitch gDNA Mini Bacteria kit on KingFisher Flex by requesting the script through ThermoFisher and installing it using BindIt software

3.2 Select the right script/method and then click start.

3.3 Load 0.5ml 96-well plate containing the Tip comb.

3.4 Load Elution plate.

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3.5 Load Wash Plate 1, Wash Plate 2, and Wash Plate 3 one by one.

3.6 Load Sample plate. The instrument will automatically start processing the sample plate.

(While the instrument is processing the sample plate, prepare a premix of 500 ul Lysisi Buffer (L14) and 10 ul of Proteinase K solution).

3.7 6) After 10 mins of sample processing, there is the **first pause**. Once the Instrument pauses, pick up the sample plate from the instrument and add 500 ul of the premix prepared in step 5 (500 ul Lysis Buffer/Proteinase K mixture) to each sample well of Sample plate.

3.8 Then load Sample plate back into the instrument and click Start.

3.9 After 10 mins, there is a **second pause**, take the sample plate off the instrument and add 40 ul of Charge Switch Magnetic Beads and 300 ul Binding Buffer (B8) to each sample well, and then load the Sample plate back into the instrumentand click Start. The machine will do the remaining steps until it is done.

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