Nextera™ DNA Flex Microbial Colony Extraction

Demonstrated Protocol

Introduction

The following protocol demonstrates how to proceed from plated microbial cultures directly to Tagmentation as described in the *Nextera™ DNA Flex Library Prep Reference Guide (document # 10000000025416)* without upstream quantitation. This protocol provides greater than 100 ng input at its conclusion.

DISCLAIMER

The information in this Illumina Demonstrated Protocol is being provided as a courtesy. In some cases, reagents are required to be purchased from non-authorized third-party suppliers. Illumina does not guarantee or promise technical support for the performance of our products used with any reagent purchased from a non-authorized third-party supplier.

Consumables

- SPB (Sample Purification Beads)
- RSB (Resuspension Buffer)
- PowerBead tubes, glass (0.5 mm) (QIAGEN, catalog # 13116-50)
- Disposable inoculation loop (10 µl) (Fisher, catalog # 12870155)
- 96-well PCR plate
- Blood agar plate
- 1.5 ml tube
- 80% ethanol
- Nuclease-free water

About Reagents

- For SPB, use Agencourt® AMPure® XP beads from Beckman Coulter Life Sciences (Catalog #A63880).

Bacterial Colonies

The bacteria are grown overnight at 37°C on a blood agar plate. This protocol assumes sufficient colonies to fill half of 10 µl loop from each bacterial culture plate.

This protocol is validated for the following bacteria:

- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Enterobacter cloacae*
- *Escherichia coli*
- *Acinetobacter baumannii*
- *Enterococcus faecalis*
- *Streptococcus agalactiae*
- *Staphylococcus aureus*

Equipment

Equipment	Supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
Vortex-Genie 2	Sigma, catalog # Z258423
Vortex Adapter for 1.5–2.0 ml tubes (24)	QIAGEN, catalog # 13000-V1-24

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
SPB	2°C to -8°C*	Let stand for 30 minutes to bring to room temperature. Keep at room temperature.
PowerBead Tubes	Room temperature	Add 300 µl of nuclease-free water to the PowerBead tubes containing 0.5mm glass beads.

* SPB is included in the Nextera DNA Flex.

Colony-based Microbial Extraction

1 Using a 10 µl disposable inoculation loop, pick a half loopful of colonies from the bacterial culture plate.
2 Resuspend colonies in the PowerBead tube containing glass beads and nuclease-free water.
3 Fit a Vortex-Genie 2 with a vortex adapter.
4 Vortex at speed 6 for five minutes.
5 Centrifuge at 20,000 × g for two minutes. Make sure that the glass beads are at the bottom of the PowerBead tube.
6 Transfer all supernatant (~150 µl) ***without*** the glass beads to a new 1.5 ml tube.
7 Add 30 µl nuclease-free water to a new 1.5 ml tube.
8 Transfer 20 µl supernatant to the 1.5 ml tube containing nuclease-free water. Pipette to mix.
9 Vortex and invert SPB several times to resuspend.
10 Add 20 µl SPB to the 1.5 ml sample tube containing supernatant and water.
11 Using a pipette set to 50 µl, mix ten times to thoroughly mix the beads and sample.
12 Incubate at room temperature for five minutes.
13 Place on the magnet until the beads have fully migrated to the side of the tube (~5 minutes).

NOTE
The color of the bacteria can make the SPB difficult to see.

14 Remove the supernatant without disrupting the bead pellet.
15 Make sure that a bead pellet is at the bottom of the tube before discarding the supernatant.
16 If the beads are accidentally aspirated:

a Return the sample to the tube and allow it to settle.
b Remove and discard the supernatant.

17 Wash two times as follows.

   a Add 200 µl fresh 80% EtOH to each tube.
   b Incubate on the magnetic stand for 30 seconds.
   c Remove and discard all supernatant from each tube.

18 Remove any residual EtOH using a P20 pipette.

19 Air-dry the tubes on the magnetic stand for five minutes.

20 Remove the tubes from the magnetic stand.

21 Resuspend SPB in 32.5 µl of RSB. Pipette to mix.

22 Incubate at room temperature for 2 minutes.

23 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

24 Transfer 30 µl to a new 1.5 ml tube.

25 Add 20 µl of nuclease-free water to a 96-well PCR plate.

26 Transfer 10 µl of sample to the 96-well PCR plate containing water. Pipette to mix.

27 Proceed directly to the Tagment Genomic DNA procedure described in the *Nextera™ DNA Flex Library Prep Reference Guide (document # 10000000025416)*. Start at the step adding tagmentation master mix to the sample well.

Revision History

Document	Date	Description of Change
Document # 10000000332941 v01	February 2018	Update to preparation step for preparation of PowerBead Tubes.
Document # 1000000035294 v00	October 2017	Initial release.