

Illumina DNA Prep Microbial Colony Extraction

Demonstrated Protocol

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IMPORTANT
NOTICE

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Introduction

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

- IPB (Illumina Purification Beads) Illumina catalog # 20060057 or 20060058
- 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

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
IPB	Room temperature	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.

* IPB is included in the Illumina DNA Prep kit or may be purchased separately.

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 IPB several times to resuspend.
- 10 Add 20 µl IPB 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 IPB 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 μ l 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 IPB in 32.5 μ l 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 μ l to a new 1.5 ml tube.
- 25 Add 20 μ l of nuclease-free water to a 96-well PCR plate.
- 26 Transfer 10 μ l 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 *Illumina DNA 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 v02	October 2023	Updated kit name from Nextera DNA Flex to Illumina DNA Prep. Updated Sample Purification Beads SBP to Illumina Purification Beads IPB.
Document # 10000000332941 v01	February 2018	Update to preparation step for preparation of PowerBead Tubes.
Document # 1000000035294 v00	October 2017	Initial release.