Nextera™ DNA Flex Dried Blood Spot Extraction

Protocol Guide

Introduction

The following protocol demonstrates how to prepare a dried blood spot extraction and proceed directly to Tagmentation as described in the *Nextera DNA Flex Library Prep Reference Guide (document # 10000000025416)*.

Consumables

- Dried Blood Spots (DBS) card (GE Healthcare, catalog # 10534320)
- SPB (Sample Purification Beads)
- BLB (Blood Lysis Buffer)
- PK1 (Proteinase K)
- 96-well PCR plate
- 1.5 ml tube
- 80% ethanol
- Nuclease-free water
- [Optional] Microseal 'B' adhesive seal

About Reagents

- BLB is shipped frozen but stored at room temperature. Keep at room temperature for optimal use.
- For SPB, use Agencourt® AMPure® XP beads from Beckman Coulter Life Sciences (Catalog #A63880).

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.

Equipment

Equipment	Supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
Eppendorf Thermomixer Compact with 1.5 ml block	Sigma-Aldrich, catalog # T1317
Harris Uni-Core Punch, 3.0 mm	Sigma-Aldrich, catalog # WHAWB100039

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
BLB	15°C to 30°C	Check for any precipitates. If present, heat at 37°C for 10 minutes and vortex to resuspend.
DBS	Room temperature	Add EDTA-stablized blood (70 µl per shot) or add a finger prick sample.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Keep at room temperature.
PK1	-25°C to -15°C	Place on ice.

2 Preheat the thermal mixer to 56°C.

Dried Blood Spot Extraction

1. Prepare 5 × 3 mm^2 punches from a DBS card and add them to a 1.5 ml tube.
2. Combine the following reagents per reaction to create a lysis master mix.
   - BLB (20 µl)
   - PK1 (2 µl)
   - Nuclease-free water (178 µl)
3. Vortex and centrifuge the lysis master mix briefly.
4. Add 200 µl master mix to each sample.
5. Pipette to mix.
6. Shake on the preheated thermal mixer at 56°C for ten minutes.
7. Briefly centrifuge the sample.
8. Without removing the punches, transfer all of the supernatant (~190 µl) to a new 1.5 ml tube.
9. Vortex and invert SPB multiple times until resuspended.
10. Add 90 µl of SPB to the lysed sample.
11. Using a pipette set to 200 µl, thoroughly mix SPB and sample.
12. Incubate at room temperature for five minutes.
13. Place on the magnetic stand and incubate for five minutes.
    The lysis reaction turns brown, so the SPB are not visible.
14. Without disturbing the bead pellet, pipette to remove the supernatant.
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 once 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. Using a P20 pipette, remove any residual EtOH.
19. Air-dry on the magnetic stand for five minutes.
20. Remove from the magnetic stand.
21. Resuspend SPB in 30 µl of nuclease-free water. Pipette to resuspend.
22. Transfer the resuspended beads to a 96-well PCR plate.
23. 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.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'B' adhesive seal, and store at 2°C to 8°C for up to 3 days.