Supplementary Protocol November 2018

Nextera™ NGS Library Preparation from DNA/cDNA amplified with REPLI-g® Kits

This protocol links the amplification of DNA or RNA using the REPLI-g Advanced DNA Single Cell Kit (cat. nos. 150363, 150365), REPLI-g WTA Single Cell Kit (cat. nos. 150063, 150065), or REPLI-g Cell WGA & WTA Kit (cat. nos. 150052, 150054) to Nextera NGS library preparation according to the Nextera DNA Sample Preparation Guide (Illumina, October 2012), to easily go from single cells to sequencing-ready DNA and cDNA in one working day.

IMPORTANT: Please read the handbooks supplied with each required kit for general information on the handling and storage of kit components. Pay careful attention to the "Safety Information" and "Important Notes" sections before beginning this procedure.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Nextera DNA Sample Preparation Kit (Illumina, cat. no. FC-121-1030 or FC-121-1031)
- Nextera Index Kit (Illumina, cat. no. FC-121-1011 or FC-121-1012)

- Nextera DNA Sample Preparation Guide (October 2012); page numbers in this protocol refer to this version
- Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen, cat. no. P7581) or alternatively, Qubit® dsDNA BR Assay System (Invitrogen, cat. no. Q32850)
- MinElute® PCR Purification Kit (QIAGEN, cat. no. 28004 or 28006)
- Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880)
- Water bath, thermal cycler, or heating block
- Nuclease-free water

For NGS Library QC:

- Agilent® DNA Chip 7500 (Agilent, cat. no. 5067-150)
- GeneRead™ Library Quant Kit (QIAGEN, cat. no. 180612). Real-time PCR Kapa Kit (peqLab, cat. no. 07-KK4822) also provides adequate quantification.*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important points before starting

- This protocol is optimized for use with DNA or cDNA amplified using the REPLI-g Advanced DNA Single Cell Kit, REPLI-g WTA Single Cell Kit, or REPLI-g Cell WGA & WTA Kit. Use intact cells or nucleic acids for WTA or WGA reactions for highest sensitivity and reliability. Check the corresponding handbook for correct use and amount of cells.
- Because the REPLI-g amplification procedure ends in amplified DNA using either cellular RNA or DNA as starting material, the Nextera NGS library prep can be used for accurate RNAseq or DNAseq results.
- Purifying REPLI-g amplified DNA prior to Nextera NGS library preparation is neither necessary nor recommended.

- Use 50 ng REPLI-g amplified dsDNA. Therefore, concentration measurements should be done using quantification methods specifically for double stranded DNA, such as the Quant-iT PicoGreen dsDNA Reagent or the Qubit dsDNA BR Assay System. Avoid using other amounts of DNA because the enzymatic fragmentation method of Nextera NGS library preparation is more sensitive to input amount than other NGS library preparation methods based on mechanical fragmentation.
- This protocol follows the Nextera NGS library preparation procedure at all steps except for purification, where an easier cleanup method with MinElute kits is used. For clarity, wording from the Nextera Sample Preparation Guide was used at relevant steps.

Things to do before starting

All buffers and reagents should be vortexed before use to ensure thorough mixing.

Procedure

DNA tagmentation and clean-up

1. **Determine the concentration of the REPLI-g amplified DNA using the Quant-iT PicoGreen dsDNA Reagent or the Qubit dsDNA BR Assay System.**

   Note: Because the Nextera NGS library preparation method targets double-stranded DNA, perform DNA quantification with a method specific for double-stranded DNA, like Quant-iT PicoGreen dsDNA Reagent or the Qubit dsDNA BR Assay System. A simple OD measurement will lead to insufficient sequencing results.

   Note: The typical concentration of DNA or cDNA amplified by WGA or WTA is 250–850 ng/µl, depending on the protocol used.
2. **Dilute the REPLI-g amplified DNA with nuclease-free water to a concentration of 2.5 ng/µl.**
3. **Pipet 20 µl diluted REPLI-g amplified DNA (for a total of 50 ng DNA) into a fresh tube and perform the tagmentation reaction using the Nextera DNA Sample Preparation Kit.**

Carefully follow the protocol "Tagmentation of Genomic DNA" on page 21 of the Nextera DNA Sample Preparation Guide.

Note: Purifying the REPLI-g amplified DNA prior to tagmentation is not necessary. Residual primers or nucleotides do not affect the efficacy of the tagmentation reaction.

Note: The tagmentation reaction is very sensitive to the amount of input DNA. Make sure to use exactly 50 ng of REPLI-g amplified DNA.

4. **Clean up the tagmented DNA following the protocol "MinElute PCR Purification Kit protocol using a microcentrifuge." Elute in 25 µl total volume.**

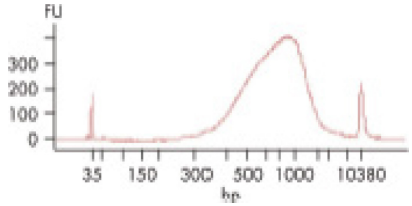
   Alternatively, follow the protocol "Cleanup of Tagmented DNA" on page 23 of the Nextera DNA Sample Preparation Guide using a different spin column.

DNA amplification and cleanup

5. Set up the PCR to index the library using index 1 primer (N7XX) and index 2 primer (N5XX) of the Nextera Index Kit. Carefully follow the protocol "PCR Setup" on page 25 of the Nextera DNA Sample Preparation Guide, with 5 cycles of limited-cycle PCR.
6. After the limited-cycle PCR, clean up the Nextera NGS library using AMPure XP beads. Carefully follow the "PCR Clean up" protocol on page 31 of the Nextera DNA Sample Preparation Guide.

Library quality control

7. Determine amplicon sizes by running 1 µl Nextera NGS library diluted 1:3 with water or TE on an Agilent DNA Chip 7500. The amplicon size distribution should be similar to the graph below. Follow any recommendations provided in the protocol "Quality Control" on page 34 of the Nextera DNA Sample Preparation Guide.



Note: Size distribution of Nextera NGS libraries typically range between 250–1500 bp.

8. Quantify the Nextera NGS library using a real-time PCR quantification method, like the GeneRead Library Quant Kit or the Real-time PCR Kapa Kit. Use 1 µl purified library and dilute according to the instructions provided with the quantification kit. Calculate the molarity of the Nextera NGS library.
9. Dilute the Nextera NGS Library as needed for sequencing on the MiSeq® instrument. See the table on page 35 of the Nextera DNA Sample Preparation Guide for instructions.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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