Nextera Protocol for multiplexed genomic DNA libraries

Overview

This protocol is derived from Sergey Kryazhimskiy’s (Desai Lab and now his own lab) modifications of Illumina’s Nextera Protocol. It uses 1/20th the reaction volume specified by Illumina. The protocol was published in Baym, Kryazhimskiy et al., 2015 (PLoS One) although the protocol described below differs in a number of steps from the published protocol.

The protocol is very straightforward and easily produces 96 libraries for multiplexed whole genome sequencing in ~4 hours.

Related documentation

1. Original Illumina protocol
2. Desai and Kishony lab paper describing protocol
3. Analysis of data from first attempt in the Gresham lab to use the protocol

Consumables

<table>
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<tr>
<th>Product</th>
<th>Supplier</th>
<th>Catalogue #</th>
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Nextera DNA Sample Preparation Kit (96 or 24 samples)  |  Illumina  |  FC-121-1031  
Nextera Index Kit (96 indices, 384 samples)  |  Illumina  |  FC-121-1012  
Microseal ‘A’ film  |  Biorad  |  MSB-5001  
Microseal ‘B’ film  |  Biorad  |  MSB-1001  
AMPure XP beads  |  Beckman Coulter  |  A63880  
Primer P1 (AATGATACGGCGACCACCGA), purified with HPSF, at 10M  
Primer P2 (CAAGCAGAAGACGGCATACGA), purified with HPSF, at 10M  
Library Amplification Kit  |  KAPA Biosystems  |  KK2611/KK2612  

Labware

- PCR plates. Use PCR plates that do not have a rim. On some thermocyclers (e.g., Biorad) a rim can prevent the lid from pressing tightly and uniformly on the plate which can cause evaporation and partial or complete loss of samples, especially in edge wells. VWR® 96-Well Thermal Cycling Plate from VWR (Cat. # 89049-178) seem to work fine for us
- 200l and 10l multichannel pipettes
- 96-well plate magnetic stand (e.g., Life Technologies, Cat. #123-31D)

Step 1. Tagmentation of genomic DNA Preparation

1. Prepare gDNA at concentration at or below 2.5ng/l Note: Producing libraries from below 2.5ng of material has proven to be difficult. Also accurate quantification of input DNA is nessasary, avoid freeze thaws between measuring the DNA and running the Nextera Tagmentation –Nathan
2. Confirm the concentration by HS Qubit assay
3. Remove the TD, TDE1 and gDNA from the –20°C and thaw on ice
4. After thawing, mix all reagents and gDNA by gently vortexing

Procedure (\( n \) samples = \( r \) rows, \( c \) columns)

1. Make the Tagmentation Master Mix (TMM) by mixing \( n \times 1.02 \times 1.25 \)l of TD Buffer and \( n \times 1.02 \times 0.25 \)l of TDE1 in a PCR tube. Mix thoroughly by gently pipetting the mixture up and down 20 times
2. Distribute TMM into \( r \) tubes (or a PCR strip), \( c \times 1.02 \times 1.51 \) into each tube
3. With a multichannel pipette, distribute TMM into all wells of a fresh plate ("tagmentation plate"), 1.5l per well
4. With a multichannel pipette, transfer 1l of gDNA into the tagmentation plate (total volume = 2.5l per well). Mix by gently pipetting up and down 10 times. Change tips after every transfer.
5. Cover plate with Microseal ‘B’
6. Give the plate a quick spin to collect all liquid at the bottom (Sorvall or Allegra centrifuges, 1000 rpm for 1 min). Do not forget to balance the centrifuge.
7. Place the plate in the thermocycler and run the following program:
   - 55°C for 5 min
   - Hold at 10°C

NOTE: ensure that the lid is tight and that it is heated during incubation

Step 2. PCR (with reconditioning)

Preparation

1. Remove the KAPA polymerase mix (KAPA amplification kit KK2611/KK2612) and the indices from the –20°C and thaw at room temperature. I recommend storing the indices in aliquots in tube strips, so that they are accessible with a multichannel pipette.
2. After thawing, mix reagents and indices by vortexing.
**Procedure (n samples = r rows, c columns)**

1. Make r Row Master Mixes (RMMs), one for each row, by mixing c \(x 1.02 \times 1.88\) l (full plate = 23 l) of 2x KAPA master mix and c \(x 1.02 \times 0.625\) l (full plate = 7.65 l) of each of the N50x indices in each of r PCR tubes. Make sure to note which tube receives which index. Mix thoroughly by gently pipetting the mixture up and down 20 times.
2. Make c Column Master Mixes (CMMs), one for each column, by mixing r \(x 1.02 \times 1.88\) l of 2x KAPA master mix and r \(x 1.02 \times 0.625\) l (full plate = 5.1 l) of each of the N7xx indices into c PCR tubes. Make sure to note which tube receives which index. Mix thoroughly by gently pipetting the mixture up and down 20 times.
3. Arrange the CMMs in a plate holder just above or below the PCR plate with tagmented genomic DNA. With a multichannel pipette, transfer 2.5 l of CMMs into each well of the plate, so that each column receives the same N7xx index. Make sure that the column number corresponds to the N7xx index. Change tips after every transfer.
4. Arrange the RMMs strip on a plate holder to the left or to the right of the plate containing tagmented DNA. With a multichannel pipette, transfer 2.5 l of the RMMs into each well of the plate, so that each row receives the same N5xx index (final total volume per well = 7.5 l). Make sure that the row number corresponds to the N5xx index. Mix by gently pipetting up and down 10 times. Change tips after every transfer.
5. Cover plate with Microseal 'A'. Make sure to press well on each well, especially edge wells.
6. Give the plate a quick spin to collect all liquid at the bottom at 1000 rpm for 1 min.
7. Place the tubes in the thermocycler and run the following program:
   - 72°C for 3 min
   - 98°C for 2:45 min
   - 98°C for 15 sec
   - 62°C for 30 sec
   - 72°C for 1:30 min
   - Repeat steps (3–5) 8 times
   - Hold at 4°C

   NOTE: Ensure that the lid is tight and that it is heated during incubation

10. Make Reconditioning PCR Master Mix (RMM), by mixing n \(x 1.02 \times 8.5\) l of KAPA polymerase mix, n \(x 1.02 \times 0.5\) l of primer P1, and n \(x 1.02 \times 0.5\) l of primer P2. Mix thoroughly by gently pipetting the mixture up and down 20 times.
11. Distribute RMM into r tubes (or a PCR strip), c \(x 1.02 \times 9.5\) l into each tube
12. With a multichannel pipette, transfer 9.5 l of RMM into each well of the plate (final PCR volume 17 l). Mix by gently pipetting up and down 10 times. Change tips after every transfer.
13. Cover plate with Microseal 'A'
14. Give the plate a quick spin to collect all liquid at the bottom at 1000 rpm for 1 min.
15. Place the tubes in the thermocycler and run the following program:
   - 95°C for 5 min
   - 98°C for 20 sec
   - 62°C for 20 sec
   - 72°C for 30 sec
   - Repeat steps (2–4) 4 times
   - 72°C for 2 min
   - Hold at 4°C

   NOTE: Ensure that the lid is tight and that it is heated during incubation

**Step 3. PCR Clean-up and size selection**

**Preparation**

1. Bring AMPure XP beads to room temperature
2. Prepare fresh 70% ethanol from absolute ethanol in a sterile reservoir. You will need n \(x 400\) l of 70% ethanol for washing (full plate = 50ml). Prepare that by adding either 35ml 100% EtOH to 15 ml of H20 or 37ml of 95% EtOH to 13ml H20.

**Procedure (n samples = r rows, c columns, PCR volume V )**

1. Centrifuge the plate to collect all liquid (1000 rpm for 1 min)
2. Vortex beads for 30 sec to ensure that they are evenly dispersed
3. Transfer cx1.05 \(x 1 \times V\) l (full plate,171 rxn = 214 l) of beads into r PCR tubes or a PCR strip
4. Using a multichannel pipette, transfer V l (full plate, 171 rxn = 17 l) of beads into each well containing the PCR product. Mix well by gently pipetting up and down 20 times. The color of the mixture should appear homogeneous after mixing. Change tips between columns
5. Incubate at room temperature for 5 min. DNA is now on the beads
6. Place the plate on the magnetic stand and incubate for about 1 min to separate beads from solution. Wait for the solution to become clear
7. While the plate is on the magnetic stand, aspirate clear solution from the plate and discard. Do not disturb the beads. If beads are accidentally pipetted, resuspend them back, wait for the solution to clear up, and repeat
8. While the plate is on the magnetic stand, dispense 200l of 70% ethanol into each well and incubate for 30 seconds at room temperature.
   - Aspirate out ethanol without disturbing the beads and discard. Repeat for a total of 2 washes
9. Remove the remaining ethanol with P10 pipette.
10. Let the plate air dry for approximately 5 min. Do not overdry the beads.
11. Take the plate off the magnetic stand. Add 33µl of 10mM Tris-HCl (pH 8) to each well of the plate. Carefully resuspend the beads by mixing 10-15 times. Incubate for 2 min at room temperature. DNA is now in the solution.
12. Place the plate back onto the magnetic stand and incubate for about 1 min to separate beads from solution. Wait for the solution to become clear.
13. While the plate is on the magnetic stand, aspirate clear solution from the plate and transfer to a fresh plate. Do not disturb the beads. If beads are accidentally pipetted, resuspend them back, wait for the solution to clear up, and repeat.