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Nextera DNA Library Prep Checklist

For Research Use Only. Not for use in diagnostic procedures.

For Research, Forensic, or Paternity Use Only

Tagment Genomic DNA

- ☐1 Label a new 96-well TCY plate NSP1.
- ☐2 Save the following program as TAG NSP1 on a thermal cycler with a heated lid.
  - Choose the preheat lid option and set to 55°C
  - 55°C for 5 minutes
  - Hold at 10°C
- ☐3 Add 20 µl of genomic DNA at 2.5 ng/µl (50 ng total).
- ☐4 Add 25 µl of TD Buffer
- ☐5 Add 5 µl of TDE1
- ☐6 Pipette up and down 10 times to mix.
- ☐7 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐8 Place on the preprogrammed thermal cycler and run the TAG NSP1 program.

Clean Up Tagmented DNA

- ☐1 Label a new midi plate NSP2.
- ☐2 Label a new TCY plate NSP3.
- ☐3 Add 180 µl Zymo DNA binding buffer to the NSP2 plate.
- ☐4 Transfer 50 µl from NSP1 to the NSP2. Pipette up and down 10 times to mix.
- ☐5 Place the Zymo-Spin I-96 Plate on the Collection Plate.
- ☐6 Transfer sample mixture from NSP2 to the Zymo-Spin I-96 Plate.
- ☐7 Centrifuge at 1300 × g at 20°C for 2 minutes.
- ☐8 Discard the flow-through.
- ☐9 Wash 2 times with 300 µl Zymo wash buffer.
- ☐10 Centrifuge at 1300 × g for 2 minutes.
- ☐11 Place the Zymo-Spin I-96 Plate on NSP3.
- ☐12 Add 25 µl of RSB directly to the column matrix in each well.
- ☐13 Incubate for 2 minutes at room temperature.
- ☐14 Centrifuge at 1300 × g at 20°C for 2 minutes.

Amplify Tagmented DNA

- ☐1 Label a new 96-well microplate NAP1.
- ☐2 Save the following program as PCR AMP on a thermal cycler with a heated lid.
  - Choose the preheat lid option and set to 100°C
  - 72°C for 3 minutes
  - 98°C for 30 seconds
  - 5 cycles of:
    - 98°C for 10 seconds
    - 63°C for 30 seconds
    - 72°C for 3 minutes
  - Hold at 10°C
- ☐3 Arrange Index 1 (i7) adapters as follows:
  - 24 libraries—Columns 1–6
  - 96 libraries—Columns 1–12
- ☐4 Arrange Index 2 (i5) adapters as follows:
  - 24 libraries—Rows A–D
  - 96 libraries—Rows A–H
- ☐5 Place the plate on the TruSeq Index Plate Fixture.
- ☐6 Add 5 µl of each Index 1 adapter down each column.
- ☐7 Add 5 µl of each Index 2 adapter across each row.
- ☐8 Add 15 µl NPM.
- ☐9 Add 5 µl PPC.
- ☐10 Transfer 20 µl from NSP3 to NAP1. Pipette up and down 3–5 times to mix.
- ☐11 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐12 Transfer the NAP1 plate to the post-amplification area.
- ☐13 Place on the preprogrammed thermal cycler and run the PCR AMP program.

Clean Up Libraries

- ☐1 Prepare fresh 80% ethanol from absolute ethanol.
- ☐2 Label a new midi plate NAP2.
- ☐3 Label a new TCY plate NLP.
- ☐4 Centrifuge NAP1 at 280 × g at 20˚C for 1 minute.
- ☐5 Transfer the contents of NAP1 to NAP2.
- ☐6 Vortex AMPure XP beads for 30 seconds. Add beads to a trough.
- ☐7 Add 30 µl AMPure XP beads to NAP2.
- ☐8 Add 30 µl AMPure XP beads to NAP2.
- ☐9 Pipette up and down 10 times to mix.
- ☐10 Incubate at room temperature for 5 minutes.
- ☐11 Place on a magnetic stand until liquid is clear. Keep on magnetic stand until step 16.
- ☐12 Remove and discard supernatant.
- ☐13 Wash 2 times with 200 µl 80% EtOH.
- ☐14 Remove residual EtOH.
- ☐15 Air-dry the beads for 15 minutes.
- ☐16 Remove from the magnetic stand.
- ☐17 Add 32.5 µl RSB.
- ☐18 Pipette up and down 10 times to mix.
- ☐19 Incubate at room temperature for 2 minutes.
- ☐20 Place on a magnetic stand until liquid is clear.
- ☐21 Transfer 30 µl supernatant from NAP2 to NLP.

Check Libraries

- ☐1 Quantify your libraries. Convert library concentration using the formula 1 ng/ul = 3 nM.
- ☐2 Run 1 µl of 1:3 diluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.

Normalize and Pool Libraries

- ☐1 Apply the NDP barcode label to a new 96-well midi plate.
- ☐2 Apply the NPP barcode label to a new 96-well midi plate (for indexed libraries).
- ☐3 If NLP was stored frozen, thaw at room temperature and then centrifuge at 280 × g for 1 minute.
- ☐4 Transfer 10 µl library from NLP to NDP.
- ☐5 Normalize to 2 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.
- ☐6 Shake at 1000 rpm for 2 minutes.
- ☐7 Centrifuge at 280 × g for 1 minute.
- ☐8 Transfer 5 µl from each well in column 1 of NDP to column 1 of NPP.
- ☐9 Repeat step 8 for the remaining columns of NDP until samples are pooled in column 1 of NPP.
- ☐10 Combine the contents of column 1 into A2 of NPP.
- ☐11 Shake at 1800 rpm for 2 minutes.
- ☐12 Denature and dilute pooled libraries to the loading concentration for the instrument you are using. See the denature and dilute libraries guide for your instrument.

Acronyms

Acronym	Definition
NAP1	Nextera Amplification Plate 1
NAP2	Nextera Amplification Plate 2
NDP	Nextera Dilution Plate
NLP	Nextera Library Plate
NPM	Nextera PCR Master Mix
NSP1	Nextera Sample Plate 1
NSP2	Nextera Sample Plate 2
NSP3	Nextera Sample Plate 3
NPP	Nextera Pooled Plate
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme