| SOP Title: illumina TruSeq DNA Sample Preparation | Version 1.1, Page 1 |
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**Staff Able to Perform Procedure:** Research Technician and higher

**Principle of the Method:**
**This protocol explains how to prepare 12 pooled indexed paired-end libraries of genomic DNA for subsequent cluster generation and DNA sequencing using illumina reagents. The purpose of this protocol is to add adapter sequences onto the ends of DNA fragments to generate multiplexed single read or paired end sequencing libraries.**

**Sample Type:** 1 ug of high quality DNA

**Equipment Requirements:**
- Agilent 2100 Bioanalyzer (Agilent)
- GeneChip Hybridization Oven 640(Affymetrix)
- PicoTiter Plate Shaker (Fischer)
- Bioanalyzer Chip Vortex (IKA)
- Fluorometer (TBS)
- Covaris S2 or E210 System (Covaris)
- Dark reader transilluminator(Clare Chemical Research)
- Electrophoresis power supply
- Thermo Scientific Owl B2 EassyCast Mini Gel system (Thermo Scientific)
- Thermal cycler (Applied Biosystems)
- Vortex (VWR)
- Microcentrifuge (VWR)
- Magnetic Stand (Ambion)
- Timer (1 hour)

**Reagents & Material Requirements:**
- TruSeq DNA Sample Preparation Kit (illumina)
- 50X TAE (BioRad)
- 100 bp DNA Ladder (Roche)
- Covaris 100 ul (6 X 16 mm) round bottom tubes with AFA fiber (KBiosciences)
- Agencourt AMPure XP 60 ml kit (Beckman Coulter)
- Certified low-range ultra Agarose (BioRad)
- Agilent RNA 6000 Nano Bioanalyzer Chip Kit (Agilent)
- 96-well storage plates, round well, 0.8ml (MIDI plate) (Fisher Scientific)
- 100% Ethanol (VWR)
- Razor Blades (VWR)
- RNAse- free centrifuge tubes 1.5ml, 200 ul (USA Scientific)
- Pipette Man 2ul, 20ul, 200ul, 1000ul (Rainin)
- Filter Pipette Tips (Rainin)

**Preparation of Fragment DNA**
- Remove one tube of Resuspension Buffer from storage and thaw it at room temperature.
- Turn on the Covaris instrument at least 30 minutes before starting.
- De-gas and pre-chill the water to a temperature of 3° to 6°C. You can start the fragmentation procedure at 6°C.

**Make CFP**
- Normalize the gDNA samples to 55 µl at 20 ng/µl into each well of the new CFP plate.

**Fragment DNA**

- Shear 1 µg of gDNA sample by transferring 52.5 µl of each DNA from the CFP plate to each Covaris tube.
- Fragment the DNA using the following settings:
  - Duty cycle-10%
  - Intensity-5.0
  - Bursts per second-200
  - Duration-120 seconds
  - Mode-Frequency sweeping
  - Power-23W
  - Temperature-5.5° to 6°C
- Seal the Covaris tube and briefly centrifuge to 600 xg for 5 seconds.
- Transfer 50 µl of fragmented DNA from the Covaris tube to each well of the new 0.3 ml PCR plate (the IMP plate).

**Preparation for End Repair**

- Remove one tube of End repair Mix and one tube of End Repair Control per 48 reactions and thaw to room temperature.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.

**Make IMP**

- Briefly centrifuge the thawed End Repair Control tube to 600 xg for 5 seconds.
- Add 10 µl of thawed End Repair Control to each well of the IMP plate.
- Add 40 µl of End Repair Mix to each well of the IMP plate containing the fragmented DNA.
- Adjust the pipette to 100 µl, and pipette each sample up and down 10 times to mix.
- Seal the IMP plate with a Microseal 'B' adhesive seal.

**Incubate 1 IMP**

- Incubate the IMP plate on the thermal cycler, with the lid closed, for 30 minutes at 30°C.

**Clean up IMP**

- Vortex the AMPure XP Beads, then add 160 µl of well-mixed beads to each well.
- Adjust the pipette to 200 µl, and pipette each sample up and down 10 times to mix.
- Incubate the IMP plate at room temperature for 15 minutes.
- Place the plate on the magnetic stand at room temperature for 15 minutes or until the liquid appears clear.
- Remove and discard 127.5 µl of the supernatant from each well of the plate. Repeat once.
- **Leave the IMP plate on the magnetic stand while performing the following 80% Ethanol wash steps**
- Add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads.
- Incubate at room temperature for 30 seconds, then remove and discard the supernatant from each well.
- Repeat last 2 steps for a total of two 80% EtOH washes.
- Remove plate from magnetic stand and let the plate stand at room temperature for 15 minutes.
- Resuspend in 17.5 µl Resuspension buffer. Pipette up and down 10 times to mix.
- Incubate plate at room temperature for 2 minutes.
- Place the plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.
- Transfer 15 µl of the clear supernatant from each well to a new 0.3 ml PCR plate (the ALP plate).
- ***Safe Stopping Point; store at -15° to -25°C for up to seven days.

**Preparation for Adenylate 3' Ends**

- Remove A-Tailing Mix and A-Tailing Control from storage and thaw them at room temperature.
- Remove the ALP plate from storage and let stand to thaw at room temperature.
- Briefly centrifuge the thawed ALP plate to 280 xg for 1 minute, then remove seal.

**Add ATL**

- Add 2.5 µl of A-Tailing Control to each well.
- Add 12.5 µl of A-Tailing Mix to each well.
- Adjust the pipette to 30 µl, and pipette each sample up and down 10 times to mix.
- Seal the ALP plate with a Microseal 'B' adhesive seal.

**Incubate 1 ALP**

- Incubate the ALP plate on the thermal cycler for 30 minutes at 35°C with the lid closed.
- Immediately remove plate and immediately proceed to Ligate Adapters.

**Preparation of Ligate Adapters**

- Remove the appropriate DNA adapter Index tubes and one tube of Ligase Control, and one tube of Stop Ligase Mix, and thaw them at room temperature.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes at room temperature.

**Add LIG**

- Briefly centrifuge the thawed DNA Adapter Index tubes, Ligase Control, and Stop Ligase Mix tubes to 600 xg for 5 seconds.
- Remove DNA Ligase Mix immediately before use.
- Remove seal from ALP plate.
- Add 2.5 µl of Ligase Control to each well.
- Add 2.5 µl of DNA Ligase Mix to each well.
- Return the DNA Ligase Mix back to -15° to -25°C storage.
- Add 2.5 µl of each thawed DNA Adapter Index to each well.
- Adjust the pipette to 37.5 µl, and pipette each sample up and down 10 times to mix.
- Seal the ALP plate with a Microseal 'B' adhesive seal.

**Incubate 2 ALP**

- Incubate the ALP plate on the thermal cycler for 10 minutes at 30°C.
- Remove the ALP plate from the thermal cycler.

**Add STL**

- Remove seal from plate.
- Add 5 µl of Stop Ligase Mix to each well.
- Adjust the pipette to 42.5 µl, and pipette each sample up and down 10 times to mix.

**Clean Up ALP**

- Vortex the AMPure XP Beads, then add 42.5 µl of mixed beads to each well.
- Adjust pipette to 85 µl, and pipette each sample up and down 10 times to mix.
- Incubate the plate at room temperature for 15 minutes.
- Place the ALP plate on the magnetic stand at room temperature for 2 minutes or until liquid appears clear.
- Remove and discard 80 µl of the supernatant from each well.
- **Leave the ALP plate on the magnetic stand while performing the following 80% Ethanol wash steps**
- Add 200 µl of freshly prepared 80% EtOH to each well without disturbing beads.
- Incubate plate at room temperature for at least 30 seconds, then remove and discard supernatant from each well.
- Repeat last two steps for a total of two 80% EtOH washes.
- Let the ALP plate stand at room temperature for 15 minutes, then remove plate from magnetic

stand.
- Resupsend each well with 52.5 µl Resuspension Buffer. Gently pipette each sample up and down 10 times to mix.
- Incubate the ALP plate at room temperature for 2 minutes.
- Place the ALP plate on the magnetic stand at room temperature for 2 minutes or until liquid appears clear.
- Transfer 50 µl of the clear supernatant from each well to a new 0.3 ml PCR plate (the CAP plate).
- Vortex the AMPure XP Beads, then add 50 µl of mixed beads to each well of the CAP plate.
- Adjust the pipette to 100 µl, and pipette each sample up and down 10 times to mix.
- Incubate the CAP plate at room temperature for 15 minutes.
- Place the CAP plate on the magnetic stand at room temperature for 2 minutes or until liquid appears clear.
- Remove and discard 95 µl of the supernatant from each well.
- **Leave the CAP plate on the magnetic stand while performing the following 80% Ethanol wash steps**
- Add 200 µl of freshly prepared 80% EtOH to each well.
- Incubate the CAP plate at room temperature for at least 30 seconds, then remove and discard all of the supernatant from each well.
- Repeat last two steps for a total of two 80% EtOH washes.
- Let the CAP plate stand at room temperature for 15 minutes, then remove plate from the magnetic stand.
- Resuspend the dried pellet in each well with 22.5 ml Resuspension Buffer. Pipette entire volume up and down 10 times.
- Incubate the CAP plate at room temperature for 2 minutes.
- Place the CAP plate on the magnetic stand at room temperature for 2 minutes or until liquid appears clear.
- Transfer 20 µl of the clear supernatant from each well of the CAP plate to a new 0.3 ml PCR plate (the SSP plate).
- ***Safe Stopping Point; store at -15° to -25°C for up to seven days.

**Preparation for Purify Ligation Products**
- Prepare 1X TAE buffer.
- Remove the SSP plate from storage, thaw to room temperature, centrifuge the thawed SSP plate to 280 xg for 1 minute.

**Size Separate SSP**
- Prepare a 150 ml, 2% agarose with SyBr Gold gel using 1 X TAE Buffer as follows:
  - Add 3 g of agarose powder in 150 ml of 1X TAE buffer.
  - Microwave the gel buffer until the powder is completely dissolved.
  - Cool the gel on the bench for 5 minutes and then add 15 µl of SyBr Gold.
  - Pour the entire gel buffer to the gel tray.
- Add 7 µl of 4X Loading Buffer to each well of the SSP plate.
- Add 17 µl Resuspension Buffer and 7 µl of 4X Loading Buffer to 3 µl of DNA Ladder.
- Fill the tank with 1X TAE Buffer.
- Load ladder onto one lane of the gel.
- Load the samples from each well of the SSP plate onto the other lanes, leaving a gap of one empty lane between samples and ladders.
- Run the gel at 120 V for 120 minutes.
- View the gel on a Dark Reader transilluminator.
- Excise a band from the gel spanning the width of the lane and ranging in size from 400-500 bp using a clean scalpel.

- If proceeding with the TruSeq Exome Enrichment protocol, excise a band ranging in size from 300-400 bp.

**Size Separate Gel**

- Follow instructions in the MinElute Gel Extraction Kit to purify each sample. Incubate the gel slices in the QG solution at room temperature (NOT at 50°C as instructed) until gel slices completely dissolve, vortexing every two minutes.
- Follow the instructions in the MinElute Gel extraction Kit to purify on one MinElute spin column, eluting in 25 µl of QIAGEN EB.
- Transfer 20 µl of each sample from the MinElute collection tube to the new 0.3 ml PCR plate (the PCR plate).
- ***Safe Stopping Point; store at -15° to -25°C for up to seven days.

**Preparation of Enrich DNA Fragments**

- Remove one tube each of PCR Master Mix and PCR Primer Cocktail from -15° to -25°C storage and thaw them at room temperature.
- Briefly centrifuge the thawed PCR Master Mix and PCR Primer Cocktail tubes to 600 xg for 5 seconds.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.
- Remove the PCR plate from -15° to -25°C storage and let stand to thaw at room temperature. Briefly centrifuge the thawed PCR plate to 280 xg for 1 minute. Remove the adhesive seal from the thawed PCR plate.
- Pre-program the thermal cycler as follows:
  - 98°C for 30 seconds
  - 10 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 4°C

**Make PCR**

- Add 5 µl of thawed PCR Primer Cocktail to each well of the PCR plate.
- Add 25 µl of thawed PCR Master Mix to each well of the PCR plate. Pipette entire volume up and down 10 times to mix.

**Amp PCR**

- Amplify the PCR plate in the pre-programmed thermal cycler (see above), with the lid closed.

**Clean Up PCR**

- Vortex the AMPure XP beads, then add 50 µl of the mixed beads to each well of the PCR plate. Pipette entire volume up and down 10 times to mix.
- Incubate the PCR plate at room temperature for 15 minutes.
- Place the PCR plate on the magnetic stand at room temperature for 2 minutes or until liquid appears clear.
- Remove and discard 95 µl of the supernatant from each well.
- **Leave the PCR plate on the magnetic stand while performing the following 80% Ethanol wash steps**
- Add 200 µl of freshly prepared 80% EtOH to each well.
- Incubate the PCR plate at room temperature for at least 30 seconds, then remove and discard all of the supernatant from each well.
- Repeat the last two steps for a total of two 80% EtOH.
- Remove the PCR plate from magnetic stand, and let plate stand at room temperature for 15

minutes to dry.
- Resuspend with 32.5 µl Resuspension Buffer. Pipette the entire volume up and down 10 times.
- Incubate the PCR plate at room temperature for 2 minutes.
- Place the PCR plate on the magnetic stand at room temperature for 2 minutes or until the liquid appears clear.
- Transfer 30 µl of the clear supernatant from each well to a new 0.3 ml PCR plate (the TSP1 plate).
- ***Safe Stopping Point; store at -15° to -25°C for up to seven days.

**Quantify Libraries**
- Quantify libraries using qPCR according to the Illumina qPCR Quantification Protocol Guide.
- If performing exome enrichment, proceed to the TruSeq Exome Enrichment Guide
- For all other sequencing applications, proceed to Pool Libraries.

**Quality Control**
- Gel: Load 10% of the volume of the library on a gel and check that the size range is as expected: a narrow smear similar in size to the DNA excised from the gel after the ligation.
- Agilent Bioanalyzer with a high sensitivity DNA chip: make a 1:100 dilution of the library using water and load 1 µl of the diluted library on the DNA chip.

**Preparation for Pool Libraries**
- Remove the TSP1 plate from -15° to -25°C storage and let stand to thaw at room temperature.
- Briefly centrifuge the thawed TSP1 plate to 280 xg for 1 minute.
- Remove the adhesive seal from the thawed TSP1 plate.

**Make DCT**
- Transfer 10 µl of sample library from each well of the TSP1 plate to the new MIDI plate.
- Normalize the concentration of sample library in each well of DCT plate to 10 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.
- Pipette entire library volume up and down 10 times to mix.
- Do one of the following:
  - For non-multiplexed libraries: Proceed to cluster generation or seal DCT plate with a Microseal 'B' adhesive and store at -15° to -25°C.
  - For multiplexed libraries: Proceed to Make PDP.

**Make PDP**
- **Do not make a PDP plate if there is no pooling.**
- Determine the number of samples to be combined in each pool.
- Transfer 10 µl of each normalized sample library to be pooled from the DCT plate to a new 0.3 ml PCR plate (the PDP plate).
- Pipette the entire volume up and down 10 times to mix.
- Do one of the following:
  - Proceed to cluster generation OR
  - Seal the PDP plate with a Microseal 'B' adhesive seal and store at -15° to -25°C.