**10x ATAC Genomics Sample Processing**

1. **Scope and Applicability:** Allows for profiling all the open chromatin regions at a single nuclei level through the rapid generation of NGS-ready libraries from a pool of transposed nuclei.
2. **Materials:**
   1. Chromium Next GEM Single Cell ATAC Library Kit v1.1 (-20°C) (10x Genomics 1000163)
      1. ATAC Buffer B (2000193)
      2. ATAC Enzyme (2000123)
      3. 20X Nuclei Buffer (2000207)
      4. Barcoding Reagent B (2000194)
      5. Barcoding Enzyme (2000125)
      6. SI-PCR Primer B (2000128)
      7. Reducing Agent B (2000087)
      8. Amp Mix (2000047)
      9. Cleanup Buffer (2000088)
   2. DynaBeads MyOne Silane Beads (10x Genomics 2000048) (Stored at 4°C)
   3. Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1 (10x Genomics 1000159) (-80°C)
      1. Single Cell ATAC Gel Beads v1.1 (2000210)
   4. Chromium Next GEM Chip H Single Cell Kit v1.1 (10x Genomics 1000161) (RT)
      1. Next GEM Chip H (2000180)
      2. Gaskets (370017)
      3. Partitioning Oil (2000190)
      4. Recovery Agent (220016)
   5. Single Index Kit N Set A (10x Genomics 1000212) (Stored at -20°C)
      1. Single Index Plate N Set A (3000427)
   6. TempAssure PCR 8-tube strip (USA Scientific 1402-4700)
   7. 1.5 mL DNA LoBind Tubes (Eppendorf 022431021)
   8. 2.0 mL DNA LoBind Tubes (Eppendorf 022431048)
   9. SPRIselect Reagent Kit 60ml (Beckman Coulter B23318)
   10. 50% Glycerol (v/v) aqueous solution (Ricca Chemical Company 3290-32)
   11. Nuclease Free Water (Thermo AM9937)
   12. Low TE Buffer (Teknova T0221)
   13. Tween 20 (Millipore 655204-100mL)
   14. 100% Ethanol (AAPER/Pharmco E200G-P)
   15. Twin.Tek 96-well PCR Plate Semi-Skirted (Eppendorf 0030129326)
   16. Twin.Tek 96-well PCR Plate, Full-Skirted (Eppendorf 0030129300)
   17. BioRad Microseal ‘B’ (BioRad MSB1001)
   18. High Sensitivity NGS Kit (DNF-474-33)
   19. Quant-it PicoGreen Reagent Kit (Thermo P7589)
   20. 50 mL conical polypropylene Falcon tubes (Corning 352098)
   21. 15 mL conical polypropylene Falcon tube (Corning 430790)
   22. 10 mL serological pipette tip (Greiner 607180)
   23. 25 mL serological pipette tip (Greiner 357525)
   24. 25 mL divided reservoirs (VWR 41428-958)
   25. Qiagen Buffer EB (Qiagen 19086)
3. **Equipment:** 
   1. PCR Hood with UV for chip loading and processing
   2. 10x Genomics Chromium Controller
   3. BioRad C1000 Thermocycler with a deep-well block
   4. Rainin P1000, P20, P200, P10 single channel pipettes
   5. Rainin P200, P20, and P10 8-channel pipettes
   6. Rainin Pasteur P1000 and P200 low retention filtered tips
   7. Rainin 200 and 20 µL low retention filtered tips
   8. Rainin 1000, 200 and 20 µL tips filtered tips
   9. Eppendorf ThermoMixer C with 1.5 mL SmartBlock and heated lid (Eppendorf 2231000574)
   10. Vortex Mixer
   11. Ice Bucket
   12. Integra aluminum PCR block
   13. Aluminum 2 ml microtube rack
   14. MicroCentrifuge for both 1.5-2 mL tubes and 8-strips
   15. Plate Sealer
   16. Plastic spatula for sealing plates.
   17. 10x Vortex Adapter (10x Genomics 330002)
   18. 10x Chip Holder (10x Genomics 330019)
   19. 10x Magnetic Separator (2x) (10x Genomics 120250)
   20. Fragment Analyzer Instrument
   21. Post-amplification PCR Hood
   22. Post-amplification Vortex Mixer
   23. Post-amplification Microcentrifuge for both 1.5-2 mL tubes and 8-strips
   24. iPad for photo-documenting GEMs
   25. Serological pipette
   26. Lab timer with clock
4. **Safety:**
   1. Nitrile Gloves
   2. Eye protection
   3. Lab coat
   4. Disposable Lab Sleeves

**Warning: Personal Protective Equipment (PPE) should be used at all times while operating this protocol. If you are unsure what PPE you should be using, see your immediate supervisor.**

1. **Output:**
   1. Massively multiplexed transposed single nuclei NGS libraries.
2. **Reference Documents:** 
   1. MB0073 Fragment Analyzer Operation for PCR Products
      1. <https://www.protocols.io/view/fragment-analyzer-operation-for-pcr-products-ddvm2646>
   2. MB0194 384-Well PicoGreen
      1. <https://www.protocols.io/view/384-well-picogreen-ddvq265w>
3. **Setup (At Least One Hour Before Starting):** 
   1. Aliquot out 1 mL of 50% Glycerol into DNA LoBind tubes
      1. Store the aliquots at -20°C until needed.
   2. Prepare 50x 1 mL aliquots of 10% Tween 20 by adding 5 mL of Tween 20 to 45 mL of NFdH2O in a 50 mL falcon tube.
      1. Invert 20x to mix and let stand at room temperature for 5 minutes.
      2. Aliquot out 1 mL of the 10% Tween 20 into mL DNA LoBind Tubes.
      3. Store the aliquots at room temperature until needed.
      4. Dispose of the aliquots after 1 year.
   3. Fill the ice bucket with ice and place both the Integra aluminum PCR block and the aluminum 2.0 mL microtube rack onto the ice to cool.
4. **Methodology/Procedures:**
   1. **Transposition (prep at least 15 minutes before the sample hand off)**
      1. Pull out the following and allow to equilibrate to room temperature:
         1. ATAC Buffer B (2000193)
         2. 20X Nuclei Buffer (2000207) once thawed will need to be diluted 1:20 in nuclease-free water before use.
            1. For 1 mL of 1X Nuclei Buffer will need to add:

50 µL 20x Nuclei Buffer (2000207)

950 µL Nuclease-free water

* + - 1. Reducing Agent B (200087)
      2. This will be used for the GEM Generation and Barcoding step but needs at least 1 hour to thaw.
    1. Place ATAC Enzyme (2000123) on ice.
    2. Conduct the following steps in the designated PCR clean UV hood to prevent contamination and potential clogs or wetting failures.
    3. Following the table below, prepare enough Transposition mix for each reaction with 10% excess volume, vortex each reagent for 5s at max and adding reagents in the order listed to a 1.5 mL Eppendorf LoBind Tube (Eppendorf 022431021):

Graphical user interface, application, table

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* + 1. Vortex, spin briefly and add 61.6 µL of ATAC Buffer B (2000193) to the Transposition Mix tube using a P200 @ 61.6 µL.
    2. Vortex, spin briefly and add 26.4 µL of ATAC Enzyme (2000123) to the Transposition Mix tube using a P200 @ 26.4 µL.
       1. Vortex and briefly spin down the Transposition Mix.
    3. Using a P20, aliquot 10 µL of Transposition Mix into each well of a TempAssure PCR 8-strip. Centrifuge briefly and keep on ice
    4. Use the Nuclei Concentration Guidelines to calculate the volume of Nuclei stock and 1x nuclei buffer needed for a total volume of 5 µL.

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* + 1. Add the calculated volume of 1X Nuclei Buffer to Transposition mix and pipette mix to clean tip. Briefly centrifuge to collect at bottom of strip.
    2. Using a P200 set at 20 µL, gently pipette mix the nuclei stock solution approximately 10x in the middle of the solution. Then immediately switch to a P20 low-retention tip set at the calculated nuclei volume, and gently pipette mix the stock 10x in the middle of solution before aspirating the nuclei volume to transfer.
    3. Add the solution to the TempAssure 8-strip containing the Transposition mix. Gently pipette mix the solution 6x using a P20 @ 10 µL. Avoid introducing bubbles into the solution. **Do NOT vortex or centrifuge solution.**
    4. Load the strip onto the Thermocycler running the following program.

|  |  |  |
| --- | --- | --- |
| **Lid Temperature** | **Reaction Volume** | **Run Time** |
| ***50°C*** | ***15µL*** | ***60 min*** |
|  | | |
| **Step** | **Temperature** | **Time** |
| Incubate | 37°C | 60 min |
| Hold | 4°C | Hold |

* + 1. While program runs, prepare for **GEM Generation & Barcoding**.
  1. **GEM Generation & Barcoding**
     1. Prepare the following at least 30 minutes before thermocycler program ends.
        1. If processing fewer than 8 reactions, set aside 3 aliquots of 50% Glycerol and equilibrate to room temperature.
        2. Remove the Single Cell ATAC Gel Beads v1.1 (2000210) from the -80°C and allow them to equilibrate at room temperature for at least 30 minutes.
        3. Remove the Barcoding Reagent B (2000194) from kit to thaw and equilibrate to room temperature.
        4. Pull out the Barcoding Enzyme (2000125) from kit and keep on ice.
     2. Assemble the Chromium Next GEM Chip
     3. Close the Next GEM chip secondary holder lid and carefully attach the gasket (370017) onto the hooks starting from the left to the right. Make sure to do this before inserting the chip so that the gasket will align properly.
     4. Open the secondary holder with the gasket still attached. Careful not to touch the smooth side of the gasket.
     5. Using care to only handle the edges and avoiding contact with the ports or bottom surface, place a Chromium Next GEM Chip H (2000180) into the 10x Next GEM secondary chip holder (3000332).

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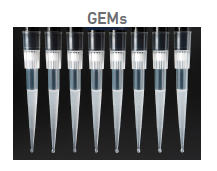
* + - 1. Cover both the gasket and the chip with a pipette tip box lid at all times. Only remove the tip box lid when you are actively adding solutions to the chip.
    1. Prior to chip loading, if fewer than 8 reactions are planned, add the following volumes of 50% Glycerol to each unused well, in the following order:
       1. 70 µL in labeled row 1 unused wells using a P200 @ 70 µL.
       2. 50 µL in labeled row 2 unused wells using a P200 @ 50 µL.
       3. 40 µL in labeled row 3 unused wells using a P200 @ 40 µL.
    2. Following the table below, prepare enough Master mix for each reaction with 10% excess volume, vortex each reagent for 5s at max and adding reagents in the order listed to a 1.5 mL Eppendorf LoBind Tube (Eppendorf 022431021):

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* + 1. Vortex, spin briefly and add 497.2 µL of Barcoding Reagent B (200194) to the Master Mix tube using a P1000 @ 497.2 µL.
    2. Vortex well, spin briefly and add 13.2 µL of Reducing Agent B (2000087) to the Master Mix tube using a P20 @ 13.2 µL.
    3. Vortex, spin briefly and add 17.6 µL of Barcoding Enzyme (2000125) to the Master Mix tube using a P20 @ 17.6 µL.
       1. Vortex the Master mix tube for 10 seconds at max speed.
       2. Briefly spin down the master mix tube to collect.
    4. Pull out the TempAssure PCR 8-strip from the thermocycler once the temperature reaches 4°C and place on block on ice.
    5. Aliquot 60 µL of Master mix into each well of a TempAssure PCR 8-strip containing the Transposed Nuclei using a P200 @ 60 µL for a total of 75 µL.
    6. Using an 8-channel P200 with low-retention tips set at 70 µL, gently pipette mix 10x the master mix + transposed nuclei solution. Then transfer the volume to row 1 of chip.
       - 1. Pipette slowly with the tips in the bottom of the central well depression, taking 5 seconds to depress the pipette plunger while raising the tips at the same rate the solution is filling the wells.
         2. Take care to not introduce air bubbles.
    7. Snap the Next GEM Single Cell ATAC Gel Beads v1.1 (2000210) into the 10x vortex adapter (330002) and vortex on max speed for 30 seconds.
    8. Remove the beads from the vortex adapter, snap off the tube strip holder, remove bead strip, and spin down for a full 5 seconds. Place the bead strip back, and snap into place with the tube strip holder.
    9. Pierce the foil on the bead strips using fresh P200 tips.
       1. Discard the tips after piercing.
    10. Using an 8-channel P200 @ 50 µL and low-retention tips, add 50 µL of Next GEM Single Cell ATAC Gel Beads v1.1 (2000210) to the labeled row 2 of the chip.
        1. Pipette slowly with the tips in the bottom of the central well depression taking 5 seconds to depress the pipette plunger while raising the tips at the same rate the solution is filling the wells.
        2. Take care to not introduce air bubbles.
        3. Allow the chip to sit and gravity prime for at least 30 seconds before adding any additional reagents.
    11. Add 40 µL of partitioning oil (2000190) to labeled row 3 using a P200 @ 40 µL and a low-retention tip. Pipette the oil slowly just above the bottom of the well, avoiding bubbles.
    12. Close the chip holder lid with the pre-attached gasket, careful not to touch the smooth side of the gasket.
        1. Do not tip the chip holder, keep the assembly horizontal to avoid any wetting of the 10x Gasket with partitioning oil.
        2. Do not press downwards on the gasket. This will lead directly to “wetting errors” that produce failed libraries.
    13. Place the assembly into the Chromium Controller.
        1. Press the large “Eject” button in the middle of the screen to load the chip.
        2. Press the large “Play” button on the screen to begin running the Chromium Controller.
        3. Runs will take around 18 minutes to finish.
    14. Return reagents to their proper storage locations.
        1. Note the freeze/thaw cycles of the beads as this may affect performance.
    15. Proceed directly to **GEM Transfer and Incubation** once the chip has finished running.
  1. **GEM Transfer and Incubation:**
     1. Label a semi-skirted Twin.Tek PCR plate (Eppendorf 0030129326) with the capture name according to the paperwork.
        1. Place this plate onto the Integra aluminum block in the ice bucket to chill.
     2. Set up the **GEM ATAC** protocol on the thermocycler.
     3. Upon completion of GEM formation on the Chromium Controller, press the large “eject” button on the screen.
     4. Remove the chip assembly from the Chromium Controller.
     5. Carefully remove and discard the 10x Gasket from the assembly.
     6. Open the 10x Chip holder (3000332) and fold the lid backwards until it clicks to create a 45 degree well angle.

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* + 1. Slowly aspirate 100 µL of GEMs from the lowest point of the recovery wells in the top row labeled with a 3 using a multichannel P200 @ 100 µL and low-retention tips.
       1. GEMs have high viscosity and need to be pipetted slowly.
       2. GEMs should appear even; excess partitioning oil suggests a clog occurred during the run.
    2. Photo document the GEMs, saving it in the load document. If there is obviously less than 100 µL of GEMs, take a picture of the chip. Note in the picture if there is leftover sample in the port and/or beads in the port, by circling the port in red.
    3. Dispense the GEMs into the labeled Twin.Tek PCR plate slowly over the course of several seconds.
       1. Pipette against the sidewalls of the wells, keeping the tips above liquid level to minimalize GEM loss.
    4. Seal the Twin.Tek PCR plate using BioRad microseal ‘B’ (BioRad MSB1001) and a plastic spatula.
    5. Load the plate onto the thermocycler running the following program.

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* + - 1. Run time is around 30 minutes.
    1. Upon completion, re-seal the plate with aluminum seal, and store at 15°C for up to 18 hours or at -20°C for up to one week.
    2. When ready, proceed directly to **Post GEM Incubation Cleanup**.
  1. **Post GEM Incubation Cleanup:** 
     1. Conduct the following steps in a Post-AMP PCR clean hood.
     2. Fill the ice bucket with ice and place both the aluminum PCR block and 1.5-2.0 mL aluminum microtube block on ice to cool.
     3. From the 10x Chromium Next GEM Single Cell ATAC Library Kit v1.1 (10x Genomics 1000163) stored at -20°C, remove the following items and allow them to thaw.
        1. Reducing Agent B (2000087)
        2. Cleanup Buffer (2000088) – Thaw at 65°C for 10 minutes in the Eppendorf ThermoMixer until crystals are no longer visible then allow to acclimate to room temperature.
     4. Remove the Dynabeads MyOne Silane beads (2000048) from the 4°C and allow them to equilibrate to room temperature.
     5. Prepare 15 mL of 80% Ethanol in a 15 mL conical Falcon tube (Corning 430790) by combining 12 mL of 100% Ethanol with 3 mL of water using two 10 mL serological pipettes, using new pipettes between reagents.
     6. Retrieve the RT-GEM plate from either -20°C or 4°C storage.
     7. Add 125 µL of Recovery Agent (220016) to each well using a single-channel P200 @ 125 µL.
        1. Use care not to touch the surface of the fluid to avoid complexity loss.
        2. Do not pipette mix or vortex this biphasic mixture.
     8. After 60s, transfer the biphasic mixture to a TempAssure PCR 8-strip (USA Scientific 1402-4700**)** using 2x P200 @ 115 µL and low-retention tips.
     9. Carefully pipette out the organic pink phase from the bottom of the tubes using an 8-channel P200 @ 115 µL and low-retention tips.
        1. Remove any significant volumes of organic pink phase using a single channel P20 with low-retention tips and volumes of close to 5 µL based off visual estimates.
        2. Do not remove all the organic phase since much of the cDNA exists at the interphase between layers. Leave between 5-10 µL of pink fluid in the bottom of every well of the sample strip.
           1. The strip should resemble the below image when finished. Take a photo of the strips for documentation.



* + 1. Vortex the DynaBeads MyOne Silane Beads (2000048) until fully re-suspended.
    2. Prepare DynaBeads cleanup mix by adding the following reagents, in order, to a 2.0 mL DNA LoBind Tubes (Eppendorf 022431048)

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* + - 1. Add 44 µL of NfdH2O to the DynaBeads Cleanup Mix 2 mL Eppendorf LoBind tube by using a P200 @ 44 µL.
      2. Vortex and spin down the room-temperature Cleanup Buffer (2000088).
         1. Add 1601.6 µL of Cleanup Buffer (2000088) to the 2 mL Eppendorf tube using a P1000 @ 1000 µL and a P1000 @ 601.6 µL.
      3. Add 70.4 µL of vortexed and manually tapped down tube of fully re-suspended DynaBeads MyOne Silane beads (2000048) to the DynaBeads Cleanup Mix 2 mL Eppendorf tube using a P200 @ 70.4 µL.
         1. Vortex thoroughly for >30s immediately before addition to the mix.
      4. Vortex and add 44 µL of Reducing Agent B (2000087) to the DynaBeads Cleanup Mix 2 mL tube using a P200 @ 44 µL.
    1. Vortex the DynaBeads Cleanup Mix 2 mL tube at max speed for 15 seconds.
       1. Do not spin down mix.
    2. Add 200 µL of DynaBeads Cleanup Mix to the sample strip using a P200 @ 200 µL using low retention tips.
       1. Do not touch the sample with the pipette tip or pipette mix the solution.
    3. Close the caps and briefly vortex the strip for 5 seconds at max speed.
    4. Allow the samples to incubate at room temperature for 10 minutes.
       1. Vortex the strip again for 5 seconds at max speed after the first 5 minutes of the incubation.
    5. During the 10-minute incubation, prepare Elution Solution 1 by adding the reagents in the order shown below to a 1.5 mL Eppendorf LoBind tube (Eppendorf 022431021):

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* + 1. Aliquot out 100 µL into each well of a new TempAssure PCR 8-strip using a P200 @ 100 µL.
       1. Label that strip “ES1” and set aside for now.
    2. Label a new TempAssure PCR 8-strip sample strip and set aside for now.
    3. Once the 10-minute incubation is complete, briefly vortex and spin down the tube strip and place it in the 10x Magnetic Separator (120250) in the “High” position.
    4. Wait 2 minutes or until the supernatant is clear.
    5. Free pour roughly 5 mL of 80% Ethanol into a 25 mL divided reservoir (VWR 41428-958) and set this aside.
    6. Once the sample strip is clear, carefully remove the supernatant using a P200 @ 150 µL (twice) equipped with low retention tips.
       1. Discard tips and waste into an appropriate waste container.
    7. Add 150 µL of 80% Ethanol **twice** (300 µL total) to each well of the sample strip using an 8-channel P200 @ 150 µL and low retention tips.
    8. Let this solution stand for 30 seconds.
    9. Carefully remove and discard the Ethanol wash using an 8-channel P200 @ 150 µL with low retention tips.
       1. Repeat with new tips to remove all the residual Ethanol.
    10. Carefully add 200 µL of 80% Ethanol to the 8-strip using an 8-channel P200 @ 200 µL with low retention tips.
        1. Let solution stand for 30 seconds.
    11. Remove the Ethanol wash using an 8-channel P200 @ 200 µL with low retention tips.
    12. Remove the strip from the 10x Magnetic Separator (120250) and briefly spin it down.
    13. Place the strip back into the 10x Magnetic Separator (120250) on the “Low” position.
    14. Remove any remaining Ethanol from the strips using an 8-channel P20 @ 10 µL with low retention tips.
    15. Quickly remove the strip form the 10x Magnetic Separator (120250) and immediately add 41 µL of Elution Solution 1 from the “ES1” strip using an 8-channel P200 @ 41 µL with low retention tips.
        1. **DO NOT THROW AWAY Elution Solution 1!**
    16. Close the strip caps and pulse vortex them at max speed for 15 seconds.
    17. Briefly spin the strip to collect the sample and incubate it off magnet at room temperature for 2.5 minutes.
    18. Return the strip to the 10x Magnetic Separator (120250) in the “Low” position.
        1. Leave the strip in the separator until it clears (around 1 minute).
    19. Transfer 40 µL of purified GEM products to pre-labeled TempAssure PCR 8-strip using a P200 @ 40 µL with low retention tips.
    20. Add 48 µL SPRIselect Reagent to each sample.
        1. Vortex and incubate at room temperature for 5 minutes
    21. Once the 5-minute incubation is complete, briefly spin down the tube strip and place it in the 10x Magnetic Separator (120250) in the “High” position.
    22. Wait 2 minutes or until the supernatant is clear.
    23. Free pour roughly 5 mL of 80% Ethanol into a 25 mL divided reservoir (VWR 41428-958) and set this aside.
    24. Once the sample strip is clear, carefully remove the supernatant using a P200 @ 200 µL equipped with low retention tips.
        1. Discard tips and waste into an appropriate waste container.
    25. Carefully add 200 µL of 80% Ethanol to the 8-strip using an 8-channel P200 @ 200 µL with low retention tips.
        1. Let solution stand for 30 seconds.
        2. Remove and discard EtOH.
        3. Repeat step **9.4.41.** one time.
    26. Remove the Ethanol wash using an 8-channel P200 @ 200 µL with low retention tips.
    27. Remove the strip from the 10x Magnetic Separator (120250) and briefly spin it down.
    28. Place the strip back into the 10x Magnetic Separator (120250) on the “Low” position.
    29. Remove any remaining Ethanol from the strips using an 8-channel P20 @ 10 µL with low retention tips.
    30. Quickly remove the strip from the 10x Magnetic Separator (120250) and immediately add 41 µL of Elution Solution 1 from the “ES1” strip using an 8-channel P200 @ 41 µL with low retention tips.
    31. Close the strip caps and pulse vortex them at max speed for 15 seconds.
    32. Briefly spin the strip to collect the sample and incubate it off magnet at room temperature for 5 minutes.
    33. Return the strip to the 10x Magnetic Separator (120250) in the “Low” position.
        1. Leave the strip in the separator until it clears (around 1 minute).
    34. Transfer 40 µL of purified GEM products to pre-labeled TempAssure PCR 8-strip using a P200 @ 40 µL with low retention tips.
    35. Samples may be stored at 4°C for up to 72 hours or at -20°C for up to two weeks.
    36. When ready, proceed directly to **Library Construction**.
  1. **Library Construction**
     1. From the 10x Chromium Next GEM Single Cell ATAC Library Kit v1.1 (10x Genomics 1000163) stored at -20°C, remove the following items and allow them to thaw.
        1. SI-PCR Primer B (2000128)
        2. Amp Mix (2000047) – Place on ice
     2. Take out the Single Index Plate N Set A (3000427) from -20°C to equilibrate to room temperature.
        1. Vortex and spin down once thawed.
     3. If done the same day **Post GEM Incubation Cleanup** then use the remaining 80% Ethanol. If not, create a fresh batch.
     4. Prepare Sample Index PCR mix by adding the following reagents, in order, to a 1.5 mL Eppendorf low-bind tube (Eppendorf 022431021).

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* + - 1. Vortex and add 440 µL of AMP Mix (2000047) to the Sample Index PCR Mix tube using a P1000 @ 440 µL.
      2. Vortex and add 66 µL of SI-PCR Primer B (2000128) to the Sample Index PCR Mix tube using a P200 @ 66 µL.
    1. Vortex the Sample Index PCR Mix for 5s on max.
    2. Spin down the master mix tube briefly to collect.
    3. Add 57.5 µL of Sample Index PCR mix to each 40 µL sample using a P200 @ 57.5 µL.
       1. Take care to not touch the pipette tip to the sample when pipetting. Do not pipette mix the sample.
    4. Add 2.5 µL of a pre-determined individual Single Index N Set A to the corresponding sample well using a P10 @ 2.5 µL.
    5. Close the caps on the sample tubes and briefly vortex for 5 seconds on max speed.
    6. Spin down the PCR tubes to collect the volume and return the tubes to the aluminum block in the ice bucket.
    7. To determine PCR cycling conditions, consult the following table. Use intermediate values for numbers near the borders.

|  |  |
| --- | --- |
| ATAC v1.1 LIBRARY | |
| Targeted Nuclei Recover | Index PCR Cycles |
| 500-2000 | 11 |
| 2001-6000 | 10 |
| 6001-10000 | 9 |

* + 1. Load the tubes containing sample and PCR mix onto a thermocycler and run the program below with the pre-determined PCR cycles.

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* + - 1. Upon completion of PCR, samples may either be stored at 4°C for up to 72 hours or proceed directly with SPRIselect cleanup.
    1. To begin cleanup, vortex the SPRIselect reagent on max speed until fully re-suspended.
    2. Free pour 5 mL of 80% Ethanol into the 25 mL divided reservoir.
    3. Label two TempAssure PCR 8-strips (USA Scientific 1402-4700), one for sample QC, and one for EB storage. Label one 96-well Eppendorf Twin.Tek Full-Skirted PCR plate (Eppendorf 0030129300) for the post-cleanup samples.
    4. Fill the 8-strip labeled for EB storage with 30 µL of Qiagen Buffer EB using a P200 @ 30 µL.
    5. Using a P200 @ 40 µL, transfer 40 µL of the homogenized SPRI select to each sample in the sample strip.
    6. Pulse vortex the 8-strip with PCR products and SPRI beads 15 times at max speed.
    7. Incubate the strip for 5-minutes at room temperature.
    8. Once the 5-minute incubation is over, place the 8-strip into the 10x Magnetic Separator (120250) using the “High” position.
       1. Leave the strips on the magnet with their lids closed until clear.
          1. This step generally takes between 2-5 minutes. Check every 2 minutes until clear.
    9. Taking care not to disturb the bead pellet, carefully transfer the supernatant using an 8-channel P200 @ 130 µL to a newly labeled strip.
       1. **Do not discard the supernatant as it contains the desired fraction of the sample.**
       2. Discard the strip sitting in the magnetic separator.
       3. Vortex to resuspend SPRIselect.
       4. Add 74 µL of the homogenized SPRIselect to each sample using a P200 @ 74 µL.
       5. Vortex and incubate at room temperature for 5 minutes.
    10. At the end of the 5 minutes incubation, quickly spin down sample then place on the 10x Magnetic Separator (120250) using the “High” position.
    11. Wait until solution clears in the strip tubes, usually takes 1-2 minutes.
    12. Carefully pipette and remove supernatant with a P200 @ 200 µL twice (solution at 204 µL).
        1. Dispose of tips and grab new ones.
    13. Carefully wash the beads by adding 200 µL of 80% Ethanol from the reservoir using a P200 @ 200 µL.
        1. Let the wash solution stand on the beads for 30 seconds.
    14. Carefully remove and discard the wash solution using an 8-channel P200 @ 200 µL.
    15. Repeat the wash step by adding a fresh aliquot of 80% Ethanol from the reagent reservoir using an 8-channel P200 @ 200 µL.
        1. Let the wash solution stand for 30 seconds.
    16. Remove and discard the Ethanol wash using an 8-channel P200 @ 200 µL.
    17. Remove the 8-strip from the magnet, and very briefly (<3s) spin the tube down.
    18. Immediately return the 8-strip to the magnet, this time in the “Low” position.
    19. Uncap the tubes, and remove any residual Ethanol using an 8-channel P20 @ 10 µL.
        1. It is very important not to over dry the samples, so small amounts of residual Ethanol are fine.
    20. Quickly remove the strip from the magnet and add 21 µL of Qiagen Buffer EB (Qiagen 19086) from the EB 8-strip using an 8-channel P200 @ 21 µL.
    21. Close the caps and pulse vortex the strip 10x at max speed until beads are fully resuspended.
    22. Let the strip incubate off magnet for 5 minutes at room temperature.
    23. Briefly spin down the strip to collect the volume.
    24. Upon completion of the 5-minute room temperature incubation, place the 8-strip back into the 10x Magnetic Separator in the “Low” position.
        1. Leave the sample on the magnet until it clears (around 2 minutes).
    25. Transfer 20µL of the cleaned products to the pre-labeled 96-well Eppendorf Twin.Tek Full-Skirted PCR plate using a P200 @ 20 µL, match the sample index to the plate wells.
    26. Add 9 µL of Qiagen Buffer EB (Qiagen 19086) from the EB 8-strip to the QC labeled 8-strip using an 8-channel P10 @ 9 µL.
    27. Transfer 1 µL of cleaned product to the QC strip using an 8-channel P10 @ 1 µL.
        1. Close and vortex the QC strip for 5 seconds at max speed then spin down to collect.
        2. Store the QC strip and 4°C until ready for **Post AMP 10X Quant and QC**.
    28. Store cleaned library samples at 4°C for up to 72 hours, or -20°C for long term storage.
    29. Proceed to **Library Quant and QC** using the 10-fold diluted 10x libraries in the QC strip.
  1. **Library Quant and QC**
     1. Follow MB0194: 384-well PicoGreen to quantify the sample, using 3 µL from the 10x diluted QC strip.
     2. Multiply the resulting concentration and yield by 10 to achieve the actual sample concentration and yields.
     3. Run 1 µL of the 10x diluted 10x QC sample on the FA using the FA SOP.
        1. Check the trace of the amplified cDNA. High quality cDNA should closely resemble the following:

Chart, histogram

Description automatically generated

* + - 1. There should be four distinct peaks as seen above. From left to right, nucleosome free peak, mono-nucleosome peak, di-nucleosome peak, and multinucleated fragments.
      2. Check for significant differences between the FA yield and PicoGreen yield.

1. **Take Down:** 
   1. Return any unused reagents.
   2. Clean the hood and ice bucket.