Protocol: Running Pooled 10x Genomics Libraries using Illumina MiSeq

- MiSeq Reagent Kit v3 supports 6-20 pM loading concentration. 75% of max capacity is recommended (~15 pM).
- Thaw the HT1 and Reagent Cartridge at 4 °C, o/n. Reagents are stable up to one week when stored at 4 °C if not used immediately.
- Start machine wash (3x) w/ MqH₂O + 0.5% Tween-20

Run ID: Pioli_____

<table>
<thead>
<tr>
<th>REF</th>
<th>LOT</th>
<th>Exp. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box 1 of 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Box 2 of 2 (optional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC ID: PhiX control</td>
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</table>

- Dilution of NaOH: Always prepare freshly diluted NaOH. And to prevent small pipetting error, prepare at least 1 mL of freshly diluted NaOH (within 12 hrs).
  \[1 \text{ N of } \text{NaOH stock: } -20 \text{ °C Stock}
  \Rightarrow \text{Need } 0.2 \text{ N NaOH (5x dilution)} = 20 \mu\text{L of } 1 \text{ N NaOH} + 80 \mu\text{L of MqH}_2\text{O}
  \Rightarrow \text{Mix well.}

- Inspect the Reagent Cartridge:
  a. Invert 10x to mix cartridge before use
  b. Check the position 1, 2 and 4 to make sure they are fully mixed and no precipitates.
  c. Tap the bottom of cartridge to get rid of bubbles if any.
- Re-boot the System.

- Making 4 nM Stock:
  \[\text{Library 1} = \text{_____ nM}; \text{ need 10 } \mu\text{L of 4 nM} \Rightarrow \text{_____ } \mu\text{L} + \text{_____ } \mu\text{L of MqH}_2\text{O}.
  \text{Library 2} = \text{_____ nM}; \text{ need 10 } \mu\text{L of 4 nM} \Rightarrow \text{_____ } \mu\text{L} + \text{_____ } \mu\text{L of MqH}_2\text{O}.
  \text{PhiX} = 10 \text{ nM}; \text{ need 5 } \mu\text{L of 4 nM} \Rightarrow 2 \mu\text{L} + 3 \mu\text{L MqH}_2\text{O}.

- Denature a 4 nM Pooled Libraries:
  a. 5 \mu\text{L of 4 nM Library 1} + 5 \mu\text{L 4 nM Library 2} + 10 \mu\text{L of 0.2 N NaOH}
  a. Vortex briefly and a quick spin (or … centrifuge at 280 x g for 1 min).
  b. Incubate at RT for 5 mins (exactly).
Note: Scale up accordingly if pooling >2 libraries. For example, pooling 3 libraries (5 µL of 4 nM each) would require 15 µL 0.2 N NaOH.

- Denature a 4 nM PhiX:
  a. 5 µL of 4 nM PhiX + 5 µL of 0.2 N NaOH
  c. Vortex briefly and a quick spin (or … centrifuge at 280 x g for 1 min).
  d. Incubate at RT for 5 mins (exactly).

- For a 20 pM Library or PhiX:
  ⇒ Add 990 µL of prechilled HT1 to 10 µL of denatured Pooled Libraries or PhiX.

- Dilute Denatured Library/PhiX to 15 pM; 75% of max capacity

<table>
<thead>
<tr>
<th></th>
<th>10 pM</th>
<th>15 pM</th>
<th>18 pM</th>
<th>20 pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 pM library/PhiX</td>
<td>400 µL</td>
<td>600 µL</td>
<td>720 µL</td>
<td>800 µL</td>
</tr>
<tr>
<td>Prechilled HT1</td>
<td>400 µL</td>
<td>200 µL</td>
<td>80 µL</td>
<td>0 µL</td>
</tr>
</tbody>
</table>

Mix well, then quick spin.

- Combine Library and PhiX

<table>
<thead>
<tr>
<th></th>
<th>1% Spike-In</th>
<th>5% Spike-In</th>
<th>10% Spike-In</th>
<th>15% Spike-In</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 pM denatured/diluted PhiX (stored up to 3 wks at -20 °C)</td>
<td>7 µL</td>
<td>35 µL</td>
<td>70 µL</td>
<td>105 µL</td>
</tr>
<tr>
<td>15 pM denatured/diluted Library</td>
<td>693 µL</td>
<td>665 µL</td>
<td>630 µL</td>
<td>595 µL</td>
</tr>
<tr>
<td>Total</td>
<td>700 µL; 600 µL needed.</td>
<td>Leave on ice until loading to cartridge</td>
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- Load sample library
  a. Clean the position 17 “Load Sample” with a Kimwipe®.
  b. Punch a hole on the foil seal with a clean 1-mL pipet tip.
  c. Load 600 µL of Sample-PhiX. Avoid touching the foil.

- Setup "Manual" run
  a. Run ID: Pioli_____
  b. Sample Index read (i7): 8, NNNNNNNN
  c. Paired-read
  d. Read 1 (10x Barcode UMI): 26
  e. Read 2 (Insert): 91

- Clean the Flow Cell
  a. Remove the Flow Cell from its container.
b. Rinse the Flow Cell with MqH$_2$O, **rinse well to get rid of excess salts**!
c. Be careful around the black Flow Cell port gasket!
d. Thoroughly dry with lint-free lens cleaning tissue.
e. (Optional) Clean the Flow Cell glass w/ alcohol wipe (not on the port gasket). Check the glass for streaks/fingerprints/lint/tissue fibers.
f. (Optional) Dry excess EtOH w/ lint-free lens cleaning tissue.
g. Visual check the cell port.

- Load the Flow Cell properly by holding the edges of Flow Cell. Wait for the RFID of Flow Cell identified! Close the Flow Cell compartment door.

- Raise the sipper handle for loading the PR2 (at 4 ºC) and check the Waste Bottle. Empty Waste Bottle if necessary, put back into position and then slowly lower the sipper handle. Wait for the RFID of the PR2 bottle.

- Load the Reagent Cartridge (w/ sample) and close the chiller door. Wait for the RFID of the Reagent Cartridge.

- Review the run parameters and perform a pre-run check before starting the run.

- Start Run.

- Perform a Post-Run Wash (1x) with MqH$_2$O + 0.5% Tween-20.