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 ºC, o/n. Reagents are stable up to one week when stored at 4 ºC if not used immediately.

- Start machine wash (3x) w/ MqH_2O + 0.5% Tween-20

Run ID: Pioli_____

	REF	LOT	Exp. Date
Box 1 of 2			
Box 2 of 2 (optional)			
FC ID:			
PhiX control			

- 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 N of NaOH stock: -20 ºC Stock
  ⇨ Need 0.2 N NaOH (5x dilution) = 20 μL of 1 N NaOH + 80 μL of MqH_2O
  ⇨ **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:

  [Library 1] = _____ nM; need 10 μL of 4 nM => _____ μL + _____ μL of MqH_2O.

  [Library 2] = _____ nM; need 10 μL of 4 nM => _____ μL + _____ μL of MqH_2O.

  [PhiX] = 10 nM; need 5 μL of 4 nM => 2 μL + 3 μL MqH_2O.

- Denature a 4 nM Pooled Libraries:

  a. 5 μL of 4 nM Library 1 + 5 μL 4 nM Library 2 + 10 μL 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

	10 pM	*15 pM*	*18 pM*	*20 pM*
20 pM library/PhiX	400 μL	600 μL	720 μL	800 μL
Prechilled HT1	400 μL	200 μL	80 μL	0 μL

Mix well, then quick spin.

- Combine Library and PhiX

	1% Spike-In	*5% Spike-In*	*10% Spike-In*	*15% Spike-In*
15 pM denatured/diluted PhiX (stored up to 3 wks at -20 ºC)	7 μL	35 μL	70 μL	105 μL
15 pM denatured/diluted Library	693 μL	665 μL	630 μL	595 μL
Total	700 μL; 600 μL needed. Leave on ice until loading to cartridge			

- 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 uL 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_2O, **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_2O + 0.5% Tween-20.