Group 1: HeLa Multiome (2 samples treated and untreated HeLa cells)
Group 2: SiHa Multiome (2 samples treated and untreated SiHa cells)
Group 3: HeLa Gene Expression (3 samples HeLa cells growing at different densities)
Group 3: SiHa Gene Expression (3 samples SiHa cells growing at different densities)

Multiome Protocol: (Groups 1 and 2)

1. Add Trypsin and incubate at 37 degrees until cells come off the plate
2. Add media to quench trypsin and transfer to new tube- spin down 500xg for 5 minutes
3. Resuspend in PBS+.04% BSA and count by adding 10 ul of cells to 10 uls of AOPI, mixing and then adding 10 ul to chamber of Countess Slide.
4. Transfer 500,000 cells to a 2mL eppendorf tube, spin down 500xg for 5 minutes
5. Remove all supernatant and resuspend cells in 500uL of EZ5 lysis buffer.
6. Incubate on ice for 7 minutes, then add 1.5 mL of staining buffer to quench
7. Centrifuge at 500xg for 5 minutes, then resuspend pellet in 25 uL of staining buffer
8. Add 1ul (.1ug) of the diluted hashing antibody to each tube. Make sure you keep track of which barcode was added to which sample.
9. Mix and incubate on ice for 10 minutes.
10. Add 1900 uL of staining buffer and spin at 500xg for 5 minutes
11. Remove supernatant and resuspend in 2 mL of staining buffer, pellet again at 500xg for 5 minutes.
12. Resuspend in 100 uL of staining buffer and count cells with countess.

Once cells have been counted Groups 1 and 2 will coordinate to add equal numbers of cells from each sample to a shared tube. The samples now each have their own barcode so we will be able to identify which sample they came from informatically.

13. Once cells have been mixed, add staining buffer to fill to 1 mL and then add 10 ul of 1% digitonin and 10 ul of tween 10% tween, mix with a pipet, then spin at 500xg for 5 minutes.
14. Remove supernatant and add 100 ul of 1x nuclei buffer
15. Count nuclei and add a maximum of 15,000 cells or 5 uL to the tagmentation reaction.
16. Incubate at 37 C for 1 hr
17. Add tagmented nuclei to barcoding mastermix and load chromium J chip for barcoding
18. After chromium run is complete, move to thermocycler for barcoding reaction.

Gene Expression Protocol: (Groups 3 and 4)

1. Add Trypsin and incubate at 37 degrees until cells come off the plate
2. Add media to quench trypsin and transfer to new tube- spin down 500xg for 5 minutes
3. Resuspend in PBS+.04% BSA and count by adding 10 ul of cells to 10 uls of AOPI, mixing and then adding 10 ul to chamber of Countess Slide.
4. Transfer 500,000 cells to a 1.5mL eppendorf tube, spin down 500xg for 5 minutes
5. Remove all supernatant and resuspend in 25 uL of staining buffer + 2.5ul FcX
6. Incubate on ice for 10 minutes
7. Add 1 uL (.025 ug) of hashing antibody to each tube and stain on ice for 20 minutes. Make sure you record which barcode is associated with each sample.
8. Add 1.5 mL of Staining Buffer and spin at 500xg for 5 minutes.
9. Remove supernatant and repeat steps 8 and 9 2 more times for a total of 3 washes
10. Resuspend in 100 uL of staining buffer and count cells with countess.

Once cells have been counted Groups 3 and 4 will coordinate to add equal numbers of cells from each sample to a shared tube. The samples now each have their own barcode so we will be able to identify which sample they came from informatically.

11. Add staining buffer to top to 1.5mL and spin down at 500xg for 5 minutes.
12. Remove supernatant and 200 uL resuspend in PBS + .04% BSA
13. Count with countess, dilute as necessary to 1000 cells/uL
14. Add ~13,000 cells to RT mix to and load chromium chip G
15. After chromium run is complete, move to thermocycler for barcoding reaction.