

APPENDIX

Appendix A: Chemicals Used in ChromSTEM Staining

<i>Reagent</i>	<i>Formula</i>
<i>Washing solution</i>	Hank's balanced salt solution without calcium and magnesium
<i>Fixation solution</i>	2.5% EM grade glutaraldehyde 2% paraformaldehyde 2 mM CaCl ₂ 0.1 M sodium cacodylate buffer, pH = 7.4
<i>Blocking solution</i>	10 mM glycine 10 mM potassium cyanide 0.1 M sodium cacodylate buffer, pH = 7.4
<i>DNA staining solution</i>	10 μM DRAQ5 0.1% saponin 0.1 M sodium cacodylate buffer, pH = 7.4
<i>Bathing solution</i>	2.5 mM 3,3'-diaminobenzidine tetrahydrochloride (DAB) 0.1 M sodium cacodylate buffer, pH = 7.4
<i>Reduced osmium staining solution</i>	2% osmium tetroxide 1.5% potassium ferrocyanide 2 mM CaCl ₂ 0.15 M sodium cacodylate buffer, pH = 7.4

<i>DurcupanTM resin mixture</i> 1	10 mL Durcupan TM ACM single component A, M, epoxy resin 10 mL Durucupan TM ACM single component B, hardener 964 0.15 mL Durcupan TM ACM single component D
<i>DurcupanTM resin mixture</i> 2	10 mL Durcupan TM ACM single component A, M, epoxy resin 10 mL Durucupan TM ACM single component B, hardener 964 0.2 mL Durcupan TM ACM, single component C, accelerator 960 0.15 mL Durcupan TM ACM single component D
<i>1:1 infiltration mixture</i>	10 mL 100% ethanol 10 mL Durcupan TM resin mixture 1
<i>2:1 infiltration mixture</i>	5 mL 100% ethanol 10 mL Durcupan TM resin mixture 1

Appendix B: Sample Preparation for ChromSTEM for Cell Cultures

Fixation:

1. Wash the cells in the petri-dish in the washing solution for 3 times, 2 minutes each.
2. Fix the cells with the fixation solution for 5 minutes at room temperature.
3. Continue to fix the cells with fresh fixation solution for an additional 1 hour on ice.

The following steps before the last ethanol dehydration are either on ice or on a cold stage. All reagents must be chilled to 4°C prior to use.

DNA Staining:

4. Wash the cells with 0.1M sodium cacodylate buffer for 5 times on the ice, 2 minutes each.
5. Block the cells with blocking solution for 15 minutes.
6. Stain the cells with DNA staining solution for 10 minutes.
7. Wash the cells with the blocking solution for 3 times, 5 minutes each.

Photo-bleaching:

8. Bath the cells in the bathing solution prior to photo-bleaching
9. Photo-bleach the cells using continuous epi-fluorescence illumination (150 W Xenon Lamp) with Cy5 red filter and a 100x objective for 7 minutes for each spot on the cold stage.
10. Replace the bathing solution in the petri-dish with a fresh bathing solution every 15 minutes (roughly two spots).

Heavy metal staining:

11. Rinse the cells with 0.1 M sodium cacodylate buffer for 5 times, 2 minutes each.
12. Stain the cells with reduced osmium staining solution for 30 minutes.
13. Wash the cells with double distilled water for 5 times, 2 minutes each.

Dehydration and Resin embedding:

14. Dehydrate the cells with serial ethanol (30%, 50%, 70%, 85%, 95%, 100% twice) on ice, 2 minutes each.
15. Wash the cells with 100% ethanol at room temperature for 2 minutes.
16. Infiltrate the cells with 1:1 infiltration mixture at room temperature for 30 minutes.
17. Infiltrate the cells with 2:1 infiltration mixture at room temperature for 2 hours.
18. Infiltrate the cells with DurcupanTM resin mixture 1 at room temperature for 1 hour.
19. Infiltrate the cells with DurcupanTM resin mixture 2 at 50 °C in the dry oven for 1 hour.
20. Flat embed the cells with fresh DurcupanTM resin mixture 2 in Beem capsule and cure at 60 °C in the dry oven for 48 hours.

Appendix C: Sample Preparation for ChromSTEM for Tissue Biopsies

Harvest mouse ovary (Performed by PDX tumor core in Northwestern University)

1. Euthanize the mice and harvest the ovary
2. Dissect the ovary with ideal orientation using a double-edged platinum knife to smaller pieces ($\sim 4^3$ mm³ cubes).
3. Store the tissue biopsies on ice-cold 0.1 M PBS for no more than 30 minutes before the sectioning.

Section the tissue with a vibratome

4. Embed the tissue cubes in 5% low-temperature agarose and chill at 4°C for 5 minutes
5. Section the agarose-tissue sample in ice-cold 0.1 M PBS using a vibratome (Leica) to 40 μ m (roughly 3 layers of cells) slices.
6. Deposit the thin slices onto a plasma treated glass-bottom petri-dish (MatTek) coated with poly-L-lysine, and cover with ice-cold 0.1 M PBS. The tissue slice will adhere to the bottom of the dish due to gravity and poly-L-lysine. DO NOT leave the tissue in PBS for more than 5 minutes before chemical fixation.

ChromSTEM tissue sample preparation

7. Treat the tissue slice in the petri-dish as the cell culture and repeat S1 Protocol for the rest of ChromSTEM sample preparation.

Appendix D: Sample Preparation and Imaging for Photon Localization Microscopy of

A549

Photon Localization Microscopy sample preparation

1. Wash cells with phosphate-buffered saline (PBS) for 2 minutes
2. Fix cell with a solution of 3% Paraformaldehyde and 0.1% Glutaraldehyde in PBS for 10 minutes.
3. Wash cells for 5 minutes in PBS.
4. Quenched cells in 0.1% sodium borohydride in PBS for 7 minutes.
5. Wash cells 3 times in PBS for 5 minutes each
6. Permeabilize cells in blocking buffer (0.2% Triton X-100 and 3% Bovine serum albumin (BSA) in PBS) for 20 minutes.
7. Add the primary antibodies to target heterochromatin (anti-H3K9me3, Abcam and anti-H3K27me3, Abcam) to the blocking buffer to a concentration of 2.5 $\mu\text{g}/\text{mL}$ and incubated for 2 hours.
8. Wash cells in washing buffer (0.1% Triton X-100 and 0.2% BSA in PBS) 3 times for 5 minutes.
9. Incubate cells with the secondary antibody (Alexa Fluor 647, Thermo Fisher Scientific) at a concentration of 2.5 $\mu\text{g}/\text{mL}$ in blocking buffer for 40 minutes.
10. Wash cells two times in PBS for 5 minutes each.
11. Image cells in standard imaging buffer with an oxygen scavenging system containing 0.5 mg/mL glucose oxidase (Sigma-Aldrich), 40 $\mu\text{g}/\text{mL}$ catalase (Roche or Sigma-Aldrich),

143 mM 2-hydroxy-1-ethanethiol, and 100 mg/mL glucose in TN buffer (50 mM Tris (pH 8.0) and 10 mM NaCl)

Appendix E: Sample Preparation for Whole Cheek Cell STEM Tomography

Cheek Cell Collection:

1. Volunteer needs to rinse their mouth before sample collection with water.
2. Gently brush the inner cheek with a CytoBrush from top to bottom in one direction without rotation for 5 times.
3. Rinse the CtyoBrush with cells in PBS (1x) in 1mL vial thoroughly. Rotating the brush gently release the cheek cells into the solution.
4. Concentrate the cell solution by centrifuging at 2000 rpm, dispose of the supernatant carefully, and resuspend the cell pellet in 1 mL fresh PBS
5. Concentrate the cell solution again by centrifuging at 2000 rpm, dispose of the supernatant, and resuspend the cell pellet in 100 uL fresh PBS

Cell attachment:

6. Carefully deposit 30 μ L cell suspension onto a hydrophobic surface (ideally the cap of the petri-dish, a droplet will form.
7. Place the TEM grid with carbon/formvar film on the droplet, and the plasma-treated side in touch with the liquid.
8. Flip the petri-dish cap and the grid will hang freely because of the surface tension of the droplet. Place the cap onto a wet chamber and leave the sample in a cell incubator at 37°C for 30 min.

Fixation:

9. Carefully flip the cap again so the TEM grid is on top of the droplet. Deposit 5 100 μ L 1x PBS droplets onto a hydrophobic surface (parafilm). Carefully rinse the TEM grid by floating it on the 100 μ L droplet for 2 min each. Transfer the grid with a loop tool or carefully with a pair of tweezers.
10. Deposit 100 μ L droplet of EM fixative (same as the ChrmSTEM fixative in Appendix A). Float the TEM grid on top of the droplet for 20 min at room temperature.
11. Deposit 5 100 μ L DI droplet onto a hydrophobic surface (parafilm) Carefully rinse the TEM grid by floating on the 100 μ L droplet for 2 min each. Transfer the grid with a loop tool or carefully with a pair of tweezers.

Dehydration (Freeze-dry method):

12. Plunge freeze the sample in liquid ethane with Vitrobot. Blot the sample once for 1s before freezing
13. Transfer the frozen sample into a turbo freeze dryer, follow the protocol for cell sample.

Dehydration (Critical point dry method):

12. Transfer the frozen sample into a turbo freeze dryer, follow the protocol for cell sample.
13. Dehydrate the sample in ethanol with progressively higher concentration (30%, 50%, 70%, 85%, 95%, 100% x 2 times) in a foam capsule for 10 min in each concentration.