Jan 07, 2026

Xenium Dual Chemistry (Prime 5K + Custom V1)

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvodwm7g4o/v1

Arianna Williams-Katek[1], Nicholas Banovich[1,2,3]

[1]Translational Genomics Research Institute (TGen); [2]Department of Veterans Affairs Medical Center; [3]Biodevelopmental Origins of Lung Disease (BOLD) Center, Vanderbilt University School of Medicine

Arianna Williams-Katek

DOI: https://dx.doi.org/10.17504/protocols.io.5qpvodwm7g4o/v1

Protocol Citation: Arianna Williams-Katek, Nicholas Banovich 2026. Xenium Dual Chemistry (Prime 5K + Custom V1). **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvodwm7g4o/v1

Manuscript citation:
TBA

Protocol status: Working
We use this protocol and it's working. Only tested on human lung samples.

Created: October 20, 2025

Last Modified: January 08, 2026

Protocol Integer ID: 230340

Keywords: 10x genomics xenium v1 custom panel, xenium dual chemistry, v1 probe, dual chemistry, gene, decoding chemistry, broad coverage of the prime, custom v1, prime, xenium

Funders Acknowledgements:
Nicholas E. Banovich
Grant ID: U01HL175444

Disclaimer

Abstract

We developed a 'dual chemistry' method that combines the high sensitivity of a 10X Genomics Xenium V1 custom panel (up to 480 genes) with the broad coverage of the Prime 5K panel (5001 genes) on a single tissue section. This involved co-hybridizing Prime and V1 probes and sequentially running the V1 and Prime decoding chemistries.

FFPE Tissue Micro-Array (TMA) Construction - Human Lung

1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

2 Protocol adapted with modification from

Citation

Wisner L, Larsen B, Maguire A (2022)
. Manual Construction of a Tissue Microarray using the Tape Method and a Handheld Microarrayer.
Journal of visualized experiments : JoVE.

https://doi.org/10.3791/63086 LINK

3 Our punch is a custom square 3mm tip used on the commercially available Tissue-Tek Quick-Ray TMA System (IHC World IW-UT06).

4 Mold Preparation:

4.1 Pour a couple drops of paraffin wax to thinly coat the bottom of a metal mold and allow to solidify at room temperature.

4.2 Use a finger to scrape away the wax around the outer edges of the mold to leave a rectangle of wax approximately the size of the a paper guide (~12 × 24mm).

4.3 Place a cut piece of paper (~12 × 24mm) with sample placement guidelines and place it in the center of the mold. Press onto the wax.

4.4 Cut a piece of double-sided gorilla tape (Amazon B01MG65C24) and place over the paper. Ensure that the tape piece is large enough to stick to the exposed metal of the mold beyond the paper edges.

5 Donor Block Preparation:

5.1 Acclimate FFPE blocks to room temperature if coming from 4 °C for at least 30 minutes.

5.2 Warm each block at 35 °C for 15 minutes prior to punching.
You may wish to warm the blocks in batches as you work through the TMA. Blocks that are too cold may be more likely to crack under the pressure of the punch. Blocks that are too warm may result in sample collapse or deformation due to loss of wax integrity.

6 Sample Punching:

6.1 Clean the tissue punch and forceps with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol between each sample punch.

6.2 Punch straight down into block based on prior H&E area selection.
Sometimes the cassette grid backing may break. This is not a problem unless it leaves plastic that indents into the core or block.

6.3 After punching, gently depress the punch plunger to expel the core.

6.4 Place the core, tissue-side down against the tape, with clean forceps onto one of the grid squares according to your predesigned TMA map.

6.5 Press core gently to ensure firm adhesion to the tape.

6.6 Repeat process for the number of cores in your TMA.
Samples in this paper are 17 3mm punches in a 3×6 array. One corner core is left empty to allow for block orientation determination before and after sectioning.

7 TMA Block Completion:

7.1 With all cores tissue-side down against the tape in the mold, warm the entire mold at 37 °C for 10 minutes.

7.2 Fill an empty embedding mold with molten paraffin wax (Fisher Scientific NC1180943) and keep on a heated embedding station (or hot plate) at 60 °C .

7.3 Use a P1000 pipette set to 400uL and repeatedly aspirate/dispense the molten wax from/into the heated mold to warm the plastic of the pipette tip.

7.4 After both the TMA and the pipette tip are warmed, aspirate 400uL of molten wax and quickly inject the wax between the cores by bringing the pipette tip directly above the point where four cores meet and, with some force, dispense the wax.
Shortening the time between wax aspiration and dispensing prevents the wax from cooling in the pipette tip and improves permeation between the cores.

7.5 Repeat step 7.4 until wax is between as many of the cores as possible while still liquid.

7.6 Tap the mold on a hard flat surface to try to dislodge any hidden air trapped in the wax.

7.7 Before the wax can solidify completely, place a cassette back over the cores and slowly pour molten wax onto/through the cassette mesh to complete the block.

7.8 Allow the new block to cool at room temperature for at least an hour. After that time, it can be moved to 4 °C .

7.9 After at least 1 hour at 4 °C , carefully pop the completed TMA out of the mold and store at 4 °C .

7.10 Because of the addition of the double-sided tape, these blocks can be harder to pop out of their molds than traditional blocks. You can hit the mold on a hard surface to help dislodge the block. Having the block cold also helps this process.

FFPE Sectioning and Sample Placement

8 Protocol adapted from 10X Genomics Demonstrated Protocol CG000578 Rev F. Microtome used was a Leica RM2135 with DURAEDGE Low-Profile PTFE blades (VWR 89508-406).

8.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

9 Fill a water bath with nuclease-free water (Thermo Fisher 4387936) and set temperature to 38 °C .

9.1 A different optimal temperature may be necessary for other tissue types.

10 Allow Xenium slides (10X Genomics 3000941) to come to room temperature for at least 30min prior to sample placement.

11 Face and rehydrate block in chilled nuclease-free water.

11.1 If the block has been cut before, discard at least 50um into the sample (can be used for adjacent H&Es or other staining) to ensure that the Xenium section will contain RNA that has not been exposed during storage.

12 Cut a 5um section from the sample block and transfer to the water bath. Allow the section to float for ~30seconds. Dip the Xenium slide into the water and use a probe or forceps to guide the sample section into the bordered capture area. Pull the slide up diagonally out of the bath, allowing excess water to drain off, and set aside.

12.1 A different optimal float time may be necessary for other tissue types.

12.2 For our purposes in this paper, we took three adjacent 5um sections and placed each onto a Xenium slide (3 total).

13 Allow slides to dry overnight at Room temperature .

Critical step to promote proper drying of TMAs and help avoid tissue detachment through the assay.

14 The next day, bake the slides in a rack for 3 hours at 42 °C in an incubator.

15 After drying, slides can be stored at Room temperature in a desiccator for up to 4 weeks before starting deparaffinization.

15.1 The slides in this paper were stored as above for 14 days.

Dual Chemistry FFPE Xenium Deparaffinization & Decrosslinking

16 Protocol adapted with modifications from 10X Genomics Demonstrated Protocol CG000578 Rev F.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000578 Rev F.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000578 Rev F.

16.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

16.2 Thermocycler used was a Bio-Rad C1000.

17 Prepare buffers.

17.1 1X PBS:
18mL nuclease-free water
2mL RNase-free PBS, 10X, pH 7.4 (Thermo Fisher AM9624)

17.2 PBS-T:
9,950uL 1X PBS (from previous step)
50uL 10% Tween-20 (Fisher Scientific NC2401211)

17.3 8 Coplin Jars:
x2 Xylene (Millipore Sigma 534056) or Neo-Clear (Millipore Sigma 1098435000)
x2 100% Ethanol (Millipore Sigma E7023)
x2 96% Ethanol (diluted in nuclease-free water)
x1 70% Ethanol (diluted in nuclease-free water)
x1 Nuclease-free water (Thermo Fisher 4387936)

18 Turn on heatblock to 95 °C to begin heating.

19 Preheat thermocycler to 60 °C with a Xenium thermocycler adaptor (10X Genomics 3000954 v1, 3002207 Dual + Prime) then place slides, tissue-side up, on the adaptor with the lid open.

20 Incubate slides on thermocycler for 30 minutes.

21 At the end of the incubation, remove slide from thermocycler and allow to come to room temperature for 7 minutes.

21.1 During this time, thaw Xenium FFPE Tissue Enhancer (10X Genomics 2000798) according to manufacturer's CG000578 guidelines:
"Thaw in a thermomixer for 30 min at 37°C, 300 rpm with shaking. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly. Alternatively, thaw in a water bath for 30 min at 37°C. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly. Perform thaw and cools steps in the dark."

22 Gently immerse slide in Xylene/Neo-Clear Jar 1 and incubate for 10 minutes.

23 Gently immerse slide in Xylene/Neo-Clear Jar 2 and incubate for 10 minutes.

23.1 During this time, thaw Perm Enzyme B (10X Genomics 3000553) accoding to manufacturer's CG000578 guidelines:
"Maintain at room temperature. Pipette mix, centrifuge briefly. DO NOT vortex."

24 Gently immerse slide in 100% Ethanol Jar 1 for 3 minutes.

25 Gently immerse slide in 100% Ethanol Jar 2 for 3 minutes.

26 Gently immerse slide in 96% Ethanol Jar 1 for 3 minutes.

27 Gently immerse slide in 96% Ethanol Jar 2 for 3 minutes.

28 Gently immerse slide in 70% Ethanol Jar for 3 minutes.

29 Gently immerse slide in Nuclease-free Water Jar for 20 seconds.

30 Assemble slide into Xenium v1 cassettes (10X Genomics 3000951). Use a lint-free laboratory wipe (Fisher Scientific 18323A) to dry the back of the slide.

31 Add 500uL 1X PBS to the cassette well.

32 Prepare Decrosslinking Buffers.

32.1 Diluted Perm Enzyme B:
998uL 1X PBS (from previous step)
2uL Perm Enzyme B (10X Genomics 3000553)

Mix thoroughly with a 1-ml pipette set to 600 ul. Maintain at room temperature.

32.2 Decrosslinking Buffer (amount below for one slide):
508.8uL Xenium FFPE Tissue Enhancer (10X Genomics 2000798)
34.4uL 8M Urea (Millipore Sigma 51457)
6.9uL Diluted Perm Enzyme B (from previous step)

Pipette mix thoroughly. Maintain at room temperature.

33 Set thermal cycler and start the following program:
Lid Temp = 80 °C
Reaction Volume = 100uL

22 °C - Infinite Hold (for preheating)
80 °C - 30 minutes
22 °C - 10 minutes
22 °C - Infinite Hold

34 Remove 1X PBS from cassette well.

35 Add 500uL Decrosslinking Buffer to the cassette well.

36 Apply a new Xenium Cassette Lid to the cassette and place on the thermocycler adaptor. Close the thermal cycler lid.

37 Skip the first hold step of the thermal cycler program to begin the Decrosslinking.

38 At the end of the program, remove the cassettes from the thermal cycler. Remove the cassette lid and remove the Decrosslinking Buffer from the slide.

39 Three PBS-T washes.

39.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

39.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

39.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

40 Add 500uL PBS-T to the cassette well and proceed to the next step of the protocol.
This is not a safe stopping point.

Dual Chemistry Xenium Priming Hybridization

41 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

41.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

41.2 Thermocycler used was a Bio-Rad C1000.

42 Prepare buffers.

42.1 1X PBS:
31.5mL nuclease-free water
3.5mL RNase-free PBS, 10X, pH 7.4 (Thermo Fisher AM9624)

42.2 PBS-T:

24.88mL 1X PBS (from previous step)
0.125mL 10% Tween-20 (Fisher Scientific NC2401211)

42.3 0.5X SSC-T:
1,600uL nuclease-free water
41.2uL SSC Buffer, 20X (Millipore Sigma S6639)
8.2uL 10% Tween-20 (Fisher Scientific NC2401211)

43 Thaw Xenium 5K Hu PTP Priming Oligos (10X Genomics 2001224) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature."

44 Thaw Priming Hyb Buffer (10X Genomics 2001228) according to manufacturer's CG000760 guidelines:
"Thaw in a thermomixer (300 rpm shaking) at 37°C for 5 min or until completely thawed/clear. Check for precipitate and invert until clear. Maintain at room temperature after thawing."

45 Incubate 52.5uL per slide of Xenium 5K Hu PTP Priming Oligos on the preheated 95 °C heatblock for 2 minutes.

46 Immediately transfer heated Xenium 5K Hu PTP Priming Oligos to ice and incubate for 1 minute, then maintain on ice.

47 Prepare Priming Hybridization Mix:
94.5uL Priming Hyb Buffer (10X Genomics 2001228)
10.5uL TE Buffer (Fisher Scientific BP24731)
52.5uL Xenium 5K Hu PTP Panel Priming Oligos (10X Genomics 2001224)

Pipette mix and centrifuge briefly, then maintain at room temperature.

48 Set thermal cycler and start the following program:
Lid Temp = 50 °C
Reaction Volume = 100uL

50 °C - Infinite Hold (for preheating)
50 °C - 1.5 hours (Priming Hybridization)
50 °C - Infinite Hold
50 °C - 30 minutes (Post Priming Hybridization Wash)
50 °C - Infinite Hold

49 Remove all PBS-T from Xenium cassette.

50 Place a Xenium Cassette Insert according to manufacturer's guidelines in CG000760 Rev C, pg. 53.

51 Add 150uL Priming Hybridization Mix into the cutout corner of the Xenium Cassette Insert, so that the reagent flows evenly over the sample.

52 Apply a new Xenium Cassette Lid v2 to the cassette.

53 Incubate cassettes on the prepped thermal cycler, tightly close the lid, and skip the initial hold phase.

54 Thaw Post-Priming Wash Buffer (10X Genomics 2001229) according to manufacturer's CG000760 guidelines:
"Thaw at 37°C for 5 min or until completely thawed/clear. Vortex and centrifuge briefly. Maintain at room
temperature."

55 After Priming Hybridization is finished and the second hold step is reached, remove the cassette from the thermal cycler. Remove the Cassette Lid and add 200uL PBS-T to the cutout corner of the Xenium Cassette Insert.

56 Use forceps to gently lift the floated insert and then remove the buffer from the cassette well.

57 Two PBS-T washes.

57.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

57.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

58 Add 500uL Post-Priming Wash Buffer to the cassette well.

59 Apply previously used Xenium Cassette Lid v2. Incubate cassettes on the prepped thermal cycler, tightly close the lid, and skip the second hold phase.

59.1 During this time, thaw 2X RNase Buffer (10X Genomics 2000411) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Vortex and centrifuge briefly. Maintain at room temperature after thawing."

59.2 During this time, thaw RNase Enzyme (10X Genomics 3000593) according to manufacturer's CG000760 guidelines:
"Pipette mix, centrifuge briefly. Maintain on ice until ready to use."

60 Once incubation is complete, immediately proceed to the next step. Final hold time after the 30 minutes has elapsed should not exceed 2-3 minutes.
This is not a safe stopping point.

Dual Chemistry Xenium RNase Treatment and Polishing

61 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

61.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

61.2 Thermocycler used was a Bio-Rad C1000.

62 Set thermal cycler and start the following program:
Lid Temp = 37 °C
Reaction Volume = 100uL

37 °C - Infinite Hold (for preheating)
37 °C - 20 minutes (RNase Treatment)
37 °C - Infinite Hold

63 Prepare buffers.

63.1 RNase Mix:
269.5uL nuclease-free water
275.0uL 2X RNase Buffer (10X Genomics 2000411)
5.5uL RNase Enzyme (10X Genomics 3000593)

Pipette mix. Maintain on ice.

64 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

65 Three PBS-T washes.

65.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

65.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

65.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

66 Add 500uL RNase Mix to the cassette well. Apply the cassette lid and place on the preheated thermal cycler.

67 Close the lid and skip the initial hold.

67.1 During this time, thaw Polishing Buffer (10X Genomics 2001231) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Vortex and centrifuge briefly. Maintain at room temperature after thawing."

67.2 During this time, thaw Polishing Enzyme (10X Genomics 2001230) according to manufacturer's CG000760 guidelines:
"Pipette mix, centrifuge briefly. Maintain on ice until ready to use."

68 Prepare buffers.

68.1 Polishing Reaction Mix:
495.0uL Polishing Buffer (10X Genomics 2001231)
27.5uL nuclease-free water
27.5uL Polishing Enzyme (10X Genomics 2001230)

Pipette mix. Maintain on ice.

69 Once incubation is complete, immediately proceed to the next step.

70 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

71 Set thermal cycler and start the following program:
Lid Temp = 37 °C
Reaction Volume = 100uL

37 °C - Infinite Hold (for preheating)
37 °C - 1 hour (Polishing)
37 °C - Infinite Hold

72 Three 0.5X SSC-T washes.

72.1 Add 500uL 0.5X SSC-T to the cassette well. Incubate for 1 min at Room temperature . Remove all 0.5X SSC-T.

72.2 Add 500uL 0.5X SSC-T to the cassette well. Incubate for 1 min at Room temperature . Remove all 0.5X SSC-T.

72.3 Add 500uL 0.5X SSC-T to the cassette well. Incubate for 1 min at Room temperature . Remove all 0.5X SSC-T.

73 Add 500uL Polishing Reaction Mix. Apply a cassette lid and place on the preheated thermal cycler.

74 Close the lid and skip the initial hold.

74.1 During this time, prep Probe Hybridization reagents according to manufacturer's CG000760 guidelines.

75 Once incubation is complete, immediately proceed to the next step.

Dual Chemistry Xenium Probe Hybridization

76 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

76.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

76.2 Thermocycler used was a Bio-Rad C1000.

77 Prepare Xenium Prime 5K human panel probes.

77.1 Thaw Probe Hyb Buffer B (10X Genomics 2001232) according to manufacturer's CG000760 guidelines:
"Thaw at 37 °C for 5 minutes or until completely thawed. Check for precipitate and invert until clear. Maintain at room temperature after thawing."

77.2 Thaw Xenium 5K Hu PTP Panel Probes (10X Genomics 2001225) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Maintain at room temperature after thawing."

77.3 Briefly centrifuge Xenium 5K Hu PTP Panel Probes and then remove 52.5uL into a new 1.5mL tube.

77.4 Preheat aliquoted probes by incubating at 95 °C heatblock for 2 minutes, followed by immediate transfer to ice for 1 min. Then maintain on ice until use.

78 Prepare Xenium v1 custom probes for the spike-in.

78.1 For the samples in this paper, we use a custom 380 gene panel (6ZAAWG) combined with two 50 gene add-on panels (6ZX7JP and 43R3RN), all at 16rxn quantities. This is a custom design setup that we worked directly with 10X Genomics to construct. To use a traditional single design custom panel, follow the steps outlined for 6ZAAWG only and then increase the amount spiked in to a full 10.5uL in step 76.1.

78.2 Centrifuge lyophilized custom probe panel tubes briefly.

78.3 Resuspend custom probes in TE buffer:
6ZAAWG (custom v1 base panel) - resuspend in 233.33uL (⅓ of the normal volume)
6ZX7JP (custom v1 add-on 50 gene) - resuspend in 116.67uL (⅙ of the normal volume)
43R3RN (custom v1 add-on 50 gene) - resuspend in 116.67uL (⅙ of the normal volume)

78.4 Vortex tubes twice for 15 seconds each, then incubate at room temperature for 5 minutes.

78.5 Centrifuge tubes briefly and maintain at room temperature prior to hybridization.

78.6 Aliquot probes into new individual 1.5mL tubes at the following volumes:
6ZAAWG (custom v1 base panel) - 5.5uL
6ZX7JP (custom v1 add-on 50 gene) - 2.7uL
43R3RN (custom v1 add-on 50 gene) - 2.7uL

78.7 Preheat aliquoted probes by incubating at 95 °C heatblock for 2 minutes, followed by immediate transfer to ice for 1 min. Then maintain on ice until use.

79 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

80 Set thermal cycler and start the following program:
Lid Temp = 50 °C
Reaction Volume = 100uL

50 °C - Infinite Hold (for preheating)
50 °C - Overnight, 16 - 24 hours(Probe Hybridization)
50 °C - Infinite Hold

81 Three PBS-T washes.

81.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

81.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

81.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .

82 Prepare buffers.

82.1 Probe Hybridization Mix:
94.5uL Probe Hyb Buffer B (10X Genomics 2001232)
54.5uL Xenium 5K Hu PTP Panel Probes (10X Genomics 2001225)
5.25uL 6ZAAWG custom v1 base panel (10X Genomics varies)
2.63uL 6ZX7JP custom v1 add-on panel (10X Genomics varies)
2.63uL 43R3RN custom v1 add-on panel (10X Genomics varies)

Pipette mix and centrifuge briefly. Maintain at room temperature but use as immediately as possible.

83 Remove PBS-T from cassette well.

84 Place a new Xenium Cassette Insert according to manufacturer's guidelines in CG000760 Rev C, pg. 53.

85 Add 150uL Probe Hybridization Mix into the cutout corner of the Xenium Cassette Insert, so that the reagent flows evenly over the sample.

86 Apply a Xenium Cassette Lid v2 to the cassette.

87 Incubate cassette on the prepped thermal cycler, tightly close the lid, and skip the initial hold phase.

88 Once incubation is complete, immediately proceed to the next step.

Dual Chemistry Xenium Probe Hybridization Wash

89 Protocol adapted with modifications from 10X Genomics Demonstrated Protocol CG000760 Rev C.

"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

89.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

89.2 Thermocycler used was a Bio-Rad C1000.

90 Thaw Post Hybridization Wash Buffer (10X Genomics 2000395) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature for 30 min or until thawed completely. Vortex and centrifuge briefly. Keep the buffer at room temperature after thawing."

91 After Probe Hybridization is finished, remove the cassette from the thermal cycler. Remove the Cassette Lid and add 200uL PBS-T to the cutout corner of the Xenium Cassette Insert.

92 Use forceps to gently lift the floated insert and then remove the buffer from the cassette well.

92.1 Do not allow cassette to cool down before beginning the PBS-T washes.

93 Set thermal cycler and start the following program:
Lid Temp = 35 °C
Reaction Volume = 100uL

35 °C - Infinite Hold (for preheating)
35 °C - 15 minutes (Post Hybridization Wash)
35 °C - Infinite Hold

94 Two PBS-T washes.

94.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

94.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

95 Add 500uL Post Hybridization Wash Buffer to the cassette well. Apply a cassette lid and place on the preheated thermal cycler.

96 Close the lid and skip the initial hold.

96.1 During this time, prep Ligation reagents

97 Once incubation is complete, immediately proceed to the next step. Final hold time after the 15 minutes has elapsed should not exceed 2-3 minutes.
This is not a safe stopping point.

Dual Chemistry Xenium Ligation

98 Protocol adapted with modifications from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

98.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

98.2 Thermocycler used was a Bio-Rad C1000.

99 Prepare buffers.

99.1 Thaw Xenium Ligation Buffer (10X Genomics 2000391) according to manufacturer's CG000749 guidelines:
"Thaw at room temperature for 15 min or until completely thawed. Pipette mix and centrifuge briefly. Maintain at room temperature after thawing."
Note that this is the v1 chemistry ligation buffer. Do not use the Prime 5K ligation buffer.

99.2 Thaw Ligation Enzyme A (10X Genomics 2000397) and Ligation Enzyme B (10X Genomics 2000398) according to manufacturer's CG000760 guidelines:
"Pipette mix and centrifuge briefly. Maintain on ice until ready to use."

99.3 Ligation Mix:
481.5uL Xenium Ligation Buffer (10X Genomics ***2000391***)
13.5uL Ligation Enzyme A (10X Genomics 2000397)
55.0uL Ligation Enzyme B (10X Genomics 2000398)

Pipette mix and centrifuge briefly. Maintain on ice.

100 Set thermal cycler and start the following program:
Lid Temp = 42 °C
Reaction Volume = 100uL

42 °C - Infinite Hold (for preheating)
42 °C - 30 minutes (Ligation)
42 °C - Infinite Hold

101 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

102 Three PBS-T washes.

102.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

102.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

102.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

103 Add 500uL Ligation Mix to the cassette well. Apply the cassette lid and place on the preheated thermal cycler.

104 Close the lid and skip the initial hold.

104.1 During this time, prep Amplification reagents.

105 Once incubation is complete, immediately proceed to the next step.

Dual Chemistry Xenium Amplification

106 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

106.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

106.2 Thermocycler used was a Bio-Rad C1000.

107 Prepare buffers.

107.1 Thaw Amplification Enhancer Buffer (10X Genomics 2001234) according to manufacturer's CG000760 guidelines:
"Thaw at 37 °C for 5 min or until completely thawed. Vortex and centrifuge briefly. Maintain at room temperature."

107.2 Thaw Amplification Enhancer (10X Genomics 2001235) according to manufacturer's CG000760 guidelines:
"Transfer to ice immediately before use. Pipette mix 10X (pipette set to 75uL) and centrifuge briefly. Maintain on ice until ready to use."

107.3 Amplification Enhancer Master Mix:
495.0uL Amplification Enhancer Buffer (10X Genomics 2001234)
55.0uL Amplification Enhancer (10X Genomics 2001235)

Pipette mix 10X and centrifuge briefly. Maintain on ice.

108 Set thermal cycler and start the following program:
Lid Temp = Off
Reaction Volume = 100uL

4 °C - Infinite Hold (for preheating)
4 °C - 2 hours (Amplification Enhancer)
4 °C - Infinite Hold

109 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

110 Three PBS-T washes.

110.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

110.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

110.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature .
Remove all PBS-T.

111 Add 500uL Amplification Enhancer Master Mix to the cassette well. Apply the cassette lid and place on the preheated thermal cycler.

112 Close the lid and skip the initial hold.

113 Prepare buffers.

113.1 During this time, thaw Amplification Enhancer Wash Buffer (10X Genomics 2001236) according to manufacturer's CG000760 guidelines:
"Thaw at 37 °C for 5 min or until completely thawed. Vortex and centrifuge briefly. Maintain at room temperature."

113.2 During this time, thaw Amplification Mix (10X Genomics 2000392) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Vortex and centrifuge briefly. Maintain at room temperature until ready to use."

113.3 Amplification Master Mix:
495.0uL Amplification Mix (10X Genomics 2000392)
55.0uL nuclease-free water

Pipette mix 10X and centrifuge briefly. Maintain on ice.

114 Once incubation is complete, immediately proceed to the next step.

115 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

116 Add 500uL Amplification Enhancer Wash Buffer (10X Genomics 2001236) to the well.

117 Incubate 1 minute at room temperature.

118 Set thermal cycler and start the following program:
Lid Temp = 30 °C
Reaction Volume = 100uL

30 °C - Infinite Hold (for preheating)
30 °C - 2 hours (Amplification)
30 °C - Infinite Hold

119 Remove all wash buffer and add 500uL Amplification Master Mix to the well. Apply the cassette lid and place on the preheated thermal cycler.

120 Close the lid and skip the initial hold.

120.1 During this time, prep Cell Segmentation Staining reagents.

121 Once incubation is complete, immediately proceed to the next step. Final hold time after the 15 minutes has elapsed should not exceed 2-3 minutes.
This is not a safe stopping point.

122 Remove Xenium cassette from thermal cycler, remove lid, and remove buffer from the cassette well.

123 Three TE Buffer, pH 8.0 washes.

123.1 Add 500uL TE Buffer, pH 8.0 to the cassette well. Incubate for 1 min at Room temperature . Remove all TE Buffer.

123.2 Add 500uL TE Buffer, pH 8.0 to the cassette well. Incubate for 1 min at Room temperature . Remove all TE Buffer.

123.3 Add 500uL TE Buffer, pH 8.0 to the cassette well. Incubate for 1 min at Room temperature .

Dual Chemistry Xenium Cell Segmentation Staining

124 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

124.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

124.2 Thermocycler used was a Bio-Rad C1000.

125 Prepare buffers.

125.1 Thaw Xenium Block and Stain Buffer (10X Genomics 2001083) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature for 30 min. Vortex and centrifuge briefly. Maintain on ice."

125.2 70% Ethanol:
10.5mL 100% Ethanol (Millipore Sigma E7023)
4.5mL nuclease-free water

125.3 1X Diluted Xenium Block and Stain Buffer:
990.0uL nuclease-free water
330.0uL Xenium Block and Stain Buffer (10X Genomics 2001083)

Vortex and centrifuge briefly. Maintain on ice.

126 Remove buffer from the cassette well.

127 Four Ethanol washes.

127.1 Add 1000uL 70% Ethanol to the cassette well. Incubate for 2 min at Room temperature . Remove all ethanol.

127.2 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at Room temperature . Remove all ethanol.

127.3 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at Room temperature . Remove all ethanol.

127.4 Add 1000uL 70% Ethanol to the cassette well. Incubate for 2 min at Room temperature . Remove all ethanol.

128 Add 1000uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

129 Add 500uL 1X Diluted Xenium Block and Stain Buffer to the cassette well. Apply cassette lid.

130 Incubate for 1 hour at room temperature.

130.1 During this time, prep Xenium Multi-Tissue Stain Mix (10X Genomics 2000991).

130.2 Centrifuge tube for 5 seconds.

130.3 Add 220uL 1X Diluted Xenium Block and Stain Buffer to the Xenium Multi-Tissue Stain Mix tube. Pipette mix 15X (pipette set to 175uL) and centrifuge briefly.

130.4 Incubate resuspension for 30 minutes at room temperature.

130.5 Centrifuge Xenium Multi-Tissue Stain Mix for 10 minutes at 14,000 rcf at 4 °C , then maintain on ice.

131 Remove 1X Diluted Xenium Block and Stain Buffer from cassette well.

132 Place a new Xenium Cassette Insert according to manufacturer's guidelines in CG000760 Rev C, pg. 53.

133 Add 100uL Xenium Multi-Tissue Stain Mix into the cutout corner of the Xenium Cassette Insert, so that the reagent flows evenly over the sample.

134 Apply a Xenium Cassette Lid v2 to the cassette.

135 Incubate the cassette overnight (16-24 hours) at 4 °C .

136 The following day, prepare Xenium Stain Enhancer (10X Genomics 2000992).

136.1 Thaw Xenium Stain Enhancer at room temperature for 10 minutes. Centrifuge briefly and ensure white powder is at the bottom of the tube.

136.2 Add 1100uL 1X PBS to the Xenium Stain Enhancer tube. Pipette mix 5X and centrifuge for 5 seconds.

137 Remove the Cassette Lid and add 200uL PBS-T to the cutout corner of the Xenium Cassette Insert.

138 Use forceps to gently lift the floated insert and then remove the buffer from the cassette well.

139 Three PBS-T washes.

139.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

139.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

139.3 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

140 Add 500uL Xenium Stain Enhancer buffer to the cassette well.

141 Apply the Xenium cassette lid and incubate at room temperature for 20 minutes.

141.1 During this time, thaw Autofluorescence Quenching reagents according to manufacturer's CG000760 guidelines.

142 Remove cassette lid and remove buffer from the cassette well.

143 Two PBS-T washes.

143.1 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

143.2 Add 500uL PBS-T to the cassette well. Incubate for 1 min at Room temperature . Remove all PBS-T.

144 Add 500uL PBS-T to the cassette well.

Dual Chemistry Xenium Autofluorescence Quenching and Nuclei Staining

145 Protocol adapted from 10X Genomics Demonstrated Protocol CG000760 Rev C.
"V1 Only Slide" was prepared without alteration according to the v1 directions in 10X Genomics Demonstrated Protocol CG000749 Rev B.
"Prime Only Slide" was prepared without alteration according to the Prime directions in 10X Genomics Demonstrated Protocol CG000760 Rev C.

145.1 Ensure that all work station areas and equipment have been cleaned with RNase AWAY (VWR 53225-514) followed by 70% Isopropanol. Ensure all consumables are nuclease-free.

145.2 Thermocycler used was a Bio-Rad C1000.

146 Prepare buffers.

146.1 Thaw Autofluorescence Mix (10X Genomics 2000753) according to manufacturer's CG000760 guidelines:
"Thaw in a thermomixer (with 2.0mL thermoblock) for 15 minutes at 37 °C , 300 rpm with shaking. Cool to room temperature for 5 minutes. Vortex for 30 seconds and centrifuge briefly. Alternatively, thaw in a waterbath for 15 minutes at 37 °C . Cool to room temperature for 5 minutes. Vortex for 30 seconds and centrifuge briefly."

146.2 Thaw Reducing Agent B (10X Genomics 2000087) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Vortex and centrifuge briefly."

146.3 Thaw Nuclei Staining Buffer (10X Genomics 2000762) according to manufacturer's CG000760 guidelines:
"Thaw at room temperature. Vortex and centrifuge briefly. Maintain at room temperature in the dark until use."

146.4 Diluted Reducing Agent B:
544.4uL 1X PBS
5.5uL Reducing Agent B (10X Genomics 2000087)

Vortex and maintain at room temperature.

146.5 Autofluorescence Solution:
544.4uL 100% Ethanol (Millipore Sigma E7023)
5.5uL Autofluorescence Mix (10X Genomics 2000753)

Vortex and centrifuge briefly. Maintain at room temperature in the dark until use.

147 Remove Xenium cassette lid and remove buffer from the cassette well.

148 Add 500uL Diluted Reducing Agent B to the cassette well. Incubate for 10 min at room temperature.

149 Remove Xenium cassette lid and remove buffer from the cassette well.

150 Three Ethanol washes.

150.1 Add 1000uL 70% Ethanol to the cassette well. Incubate for 1 min at room temperature. Remove all ethanol.

150.2 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at room temperature. Remove all ethanol.

150.3 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at room temperature. Remove all ethanol.

151 Add freshly mixed 500uL Autofluorescence Solution to the cassette well. Apply a Xenium Cassette Lid v2 to the cassette.

152 Incubate for 10 minutes at room temperature in the dark.

153 Set thermal cycler and start the following program:
Lid Temp = 37 °C
Reaction Volume = 100uL

37 °C - Infinite Hold (for preheating)
37 °C - 5 minutes (Drying)

154 Remove Xenium cassette lid and remove buffer from the cassette well.

155 Three Ethanol washes.

155.1 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at room temperature. Remove all ethanol.

155.2 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at room temperature. Remove all ethanol.

155.3 Add 1000uL 100% Ethanol (Millipore Sigma E7023) to the cassette well. Incubate for 2 min at room temperature. Remove all ethanol.

156 Place cassette without lid nor buffer on the preheated thermal cycler. Skip initial hold and initiate drying.
Do not close the thermal cycler lid and promptly remove the slides after 5 minutes.

157 After drying, immediately add 1000uL 1X PBS and incubate for 1 minute at room temperature in the dark. Remove all PBS.

158 Add 1000uL PBS-T and incubate for 2 minutes at room temperature in the dark. Remove all PBS-T.

159 Add 500uL Nuclei Staining Buffer (10X Genomics 2000762) and incubate for 1 minute at room temperature in the dark. Remove all buffer.

160 Three PBS-T washes.

160.1 Add 1000uL PBS-T to the cassette well. Incubate for 1 min at room temperature in the dark. Remove all PBS-T.

160.2 Add 1000uL PBS-T to the cassette well. Incubate for 1 min at room temperature in the dark. Remove all PBS-T.

160.3 Add 1000uL PBS-T to the cassette well. Incubate for 1 min at room temperature in the dark. Remove all PBS-T.

161 Add 1000uL PBS-T to the cassette well.

162 Slides can then proceed immediately to instrument run or be stored (Dual Chemistry Xenium Slide Storage).

Dual Chemistry Xenium Slide Storage

163 For short-term storage, slides can be placed at 4 °C in the dark for up to 1 week prior to instrument run. Ensure that slide is hydrated with 1000uL PBS-T and sealed with a cassette lid.

164 The slides in this paper used the long-term option of storage at -20 °C in the dark for up to 1 month following the steps below.
v1 slide was stored for 18 days between sample prep completion and instrument loading. Prime slide was stored for 22 days between sample prep completion and instrument loading.

Dual slide was loaded at both of these dates and was stored at 4 °C during the ~12-16 hours between completion of the v1 run and start of the prime run.

165 Long-term Xenium slide storage:

165.1 Prepare Cryoprotectant: 30% glycerol solution in PBS
3mL 99.5% Glycerol (Fisher Scientific 327255000)
7mL 1X PBS

Ensure solution is mixed thoroughly and maintain at room temperature. Place 10mL in a slide mailer (or whatever volume is needed) to sufficiently cover the Xenium slide at least up to the bottom of the label when placed with the label up.

165.2 Remove all PBS-T from the cassette well.

165.3 Add 1000uL 70% Ethanol and incubate for 2 minutes at room temperature. Remove all ethanol.

165.4 Add 1000uL 100% Ethanol (Millipore Sigma E7023) and incubate for 2 minutes at room temperature. Remove all ethanol.

165.5 Add 1000uL 100% Ethanol (Millipore Sigma E7023) and incubate for 2 minutes at room temperature. Remove all ethanol.

166 Remove slide from the cassette and submerge slide in the cryoprotectant slide mailer.

166.1 Store slide at -20 °C in the dark until instrument run (up to 1 month).

166.2 Clean slide cassette with water and 70% isoproanol. Allow them to air dry and then store until instrument run.

167 Running a slide from long-term storage:

167.1 Remove slide mailer from -20 °C and allow to come to room temperature for 30 minutes.

Cryoprotectant and slides must thaw completely before slide removal from the mailer to avoid any ice crystal scratching on the slide/sample surface.

167.2 Remove slide from slide mailer and submerge fully in slide mailer containing fresh PBS-T.

167.3 Submerge slide in at least three additional slide mailers containing fresh PBS-T to rise off the glycerol.

167.4 Assemble slide into stored slide cassette and add 1000uL PBS-T prior to run.

v1 Chemistry Xenium Run

168 Dual chemistry slide was run alongside a normal v1 slide in a single Xenium run. Ensure that Dual chemistry slide is in a v1 Xenium slide cassette (1000566).

169 Run setup was followed without alteration as described for "Reagent Preparation & Loading for Xenium v1
Gene Expression Workflows" in 10X Genomics User Guide CG000584, Rev K, page 42.

170 At the end of the run, the slides were removed from the instrument. Post-run buffer was removed from the slides and 1000uL fresh PBS-T was added to each slide. Slides were then stored at 4 °C until Prime run (Dual slide) or post-run H&E (v1 slide).

170.1 You may switch the Dual slide into a Xenium cassette v2 during the reagent exchange or at a later time.

Prime Chemistry Xenium Run

171 Dual chemistry slide was run alongside a normal Prime slide in a single Xenium run. Ensure that Dual chemistry slide is in a Xenium cassette v2 (1000723).

172 Run setup was followed without alteration as described for "Reagent Preparation & Loading for Xenium Prime Gene Expression Workflow" in 10X Genomics User Guide CG000584, Rev K, page 53.

173 At the end of the run, the slides were removed from the instrument. Post-run buffer was removed from the slides and 1000uL fresh PBS-T was added to each slide. Slides were then stored at 4 °C until post-run H&E.

Post-Xenium H&E Staining

174 Protocol adapted with modifications from 10X Genomics Demonstrated Protocol CG000613 Rev B.

175 Prepare quencher removal reagents.

175.1 Quencher Removal Solution:
10mL Milli-Q Water
17.4mg Sodium Hydrosulfite (Millipore Sigma 157953)

Only prepare solution once you are immediately ready to place slides. Solution begins to lose performance immediately and will decline even after 10 minutes. Each batch must be made fresh. Combine in fume hood and vortex to mix. Maintain at room temperature.

175.2 Prepare three slide mailers with 10mL Milli-Q water each.

175.3 Prepare staining containers with the following reagents if not already ready:
x1 Hematoxylin Solution (Millipore Sigma MHS16)
x5 Milli-Q Water
x1 Bluing Solution (Agilent CS70230-2)
x2 95% Ethanol
x1 Eosin Solution (Fisher Scientific NC2101164)
x2 100% Ethanol (Millipore Sigma E7023)
x2 Xylene (Millipore Sigma 534056)

176 Remove all PBS-T buffer from cassette well. Then remove cassette.

177 Autofluorescence Quenching Removal:

177.1 Immerse slide in Quencher Removal Solution for 10 minutes at room temperature.

177.2 Immerse slide in Milli-Q Water slide mailer #1 and incubate for 1 minute at room temperature.

177.3 Immerse slide in Milli-Q Water slide mailer #2 and incubate for 1 minute at room temperature.

177.4 Immerse slide in Milli-Q Water slide mailer #3 and incubate for 1 minute at room temperature.

178 H&E Staining:

178.1 Immerse slide in Milli-Q water container #1 for 2 minutes at room temperature.

178.2 Immerse slide in Hematoxylin Solution container for 2 minutes at room temperature.

178.3 Immerse slide in Milli-Q water container #2 for 1 minute at room temperature.

178.4 Immerse slide in Milli-Q water container #3 for 1 minute at room temperature.

178.5 Immerse slide in Milli-Q water container #4 for 1 minute at room temperature.

178.6 Immerse slide in Bluing Solution container for 1 minute at room temperature.

178.7 Immerse slide in Milli-Q water container #5 for 1 minute at room temperature.

178.8 Immerse slide in 95% Ethanol container #1 for 3 minutes at room temperature.

178.9 Immerse slide in Eosin container for 45 seconds at room temperature.

178.10 Immerse slide in 95% Ethanol container #2 for 1 minute at room temperature.

178.11 Immerse slide in 100% Ethanol container #1 for 30 seconds at room temperature.

178.12 Immerse slide in 100% Ethanol container #2 for 30 seconds at room temperature.

178.13 Immerse slide in Xylene container #1 for 3 minutes at room temperature.

178.14 Immerse slide in Xylene container #2 for 3 minutes at room temperature.

179 Coverslip slide using ~150uL Leica Mounting Media (Fisher Scientific NC1109222) and #1.5 50×24mm cover glass (VWR 16002-264).

180 Allow coverslip to set for at least 30 minutes and then image slide.

180.1 Slides in this paper were imaged on an Aperio CS2.

Citations

Step 2

Wisner L, Larsen B, Maguire A. Manual Construction of a Tissue Microarray using the Tape Method and a Handheld Microarrayer.
https://doi.org/10.3791/63086