DEMONSTRATED PROTOCOL

Visium Spatial Slide Reset

Overview

This protocol outlines the steps necessary to reset Visium Spatial slides containing incorrectly placed tissue for reuse with 10x Genomics Visium Spatial protocols. This protocol is compatible with Visium Spatial Tissue Optimization slides, Visium Spatial Gene Expression slides, and Visium Gateway Gene Expression slides. Slides must be reset prior to tissue permeabilization; once permeabilization has been started, slides can no longer be reused.

Slide resetting may be necessary in the following situations:

- Tissue was not placed correctly within a Capture Area on a Visium slide, which can lead to suboptimal analysis
- Tissue sectioned onto a Visium slide did not contain the expected region of interest
- Damaged or folded tissue was placed on a Visium slide

This protocol should only be performed prior to tissue permeabilization and should not be used as a substitute for practicing tissue sectioning and placement. Resetting Visium Spatial or Gateway Gene Expression slides may result in a slight decrease in sensitivity compared to a new slide (see Results) and should be used only if absolutely necessary. Slides should only be reset once.

This protocol was demonstrated with:

- Slides containing mouse eye, small intestine, testes, and kidney that were reset and reprocessed with human heart
- Slides containing human breast, heart, and brain that were reset and reprocessed with mouse brain

Results are expected to be similar across other human and mouse tissues, although some tissues may require additional optimization.

Additional Guidance

Consult the Visium Spatial Protocols - Tissue Preparation Guide (Document CG000240) for Tips & Best Practices on freezing, embedding, and cryosectioning tissue and placing sections on Visium Spatial slides.

Preparation-Buffers

Tissue Lysis Buffer *Prepare fresh, invert 10x to mix, maintain at 50°C*	**Stock**	**Final**	**30 ml**
SDS	20%	10 %	15 ml
Trizma Hydrochloride, pH 9	1000 mM	10 mM	300 µl
Qiagen Proteinase K	20 mg/ml	3.3 mg/ml	5 ml
Ultrapure Water	-	-	9.7 ml

0.08 M KOH mix *Prepare fresh, Maintain at room temperature*	**Stock**	**Final**	**60 ml**
Potassium Hydroxide Solution, 8 M	8 M	0.08 M	600 µl
Ultrapure Water	-	-	59.4 ml

10 mM Tris-HCl, pH 7 *Maintain at room temperature*	**Stock**	**Final**	**30 ml**
Tris 1M, pH 7.0, RNase-free	1000 mM	10 mM	300 µl
Ultrapure Water	-	-	29.7 ml

Prepare Tissue Lysis Buffer and 10 mM Tris-HCl in 50-ml centrifuge tubes.

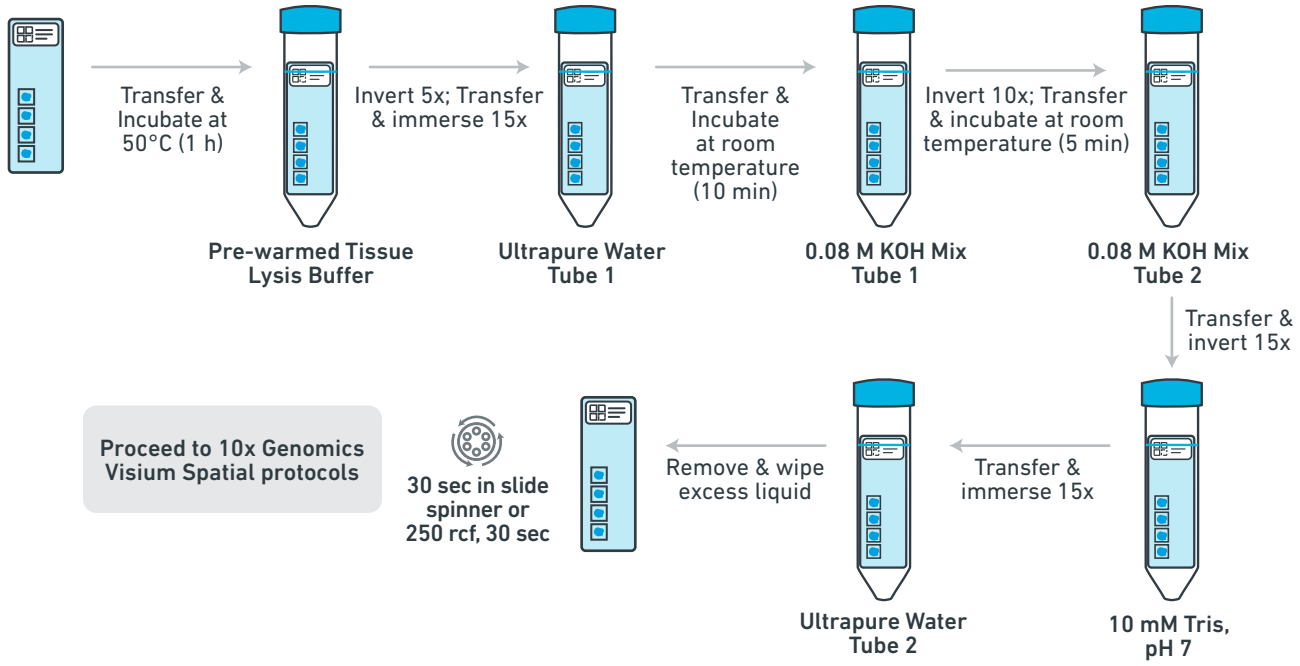
Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	SDS, 20% Solution, RNase-free	AM9820
Millipore Sigma	Potassium Hydroxide Solution, 8M	P4494-50ML
	Sodium Dodecyl Sulfate Solution, BioUltra, for Molecular Biology Alternative to Thermo Fisher product	05030-500ML-F
Qiagen	Qiagen Proteinase K	19133
Corning	Self-Standing Polypropylene Centrifuge tubes (50 ml), sterile	430921
-	UltraPure DNase/RNase-Free Distilled Water	-
-	Tris 1M, pH 7.0, RNase-free	-
-	Trizma Hydrochloride Solution, 1M pH 9	-
Specific Equipment*		
LabNet	Slide Spinner	C1303-T
-	Heat Block for 50-ml Centrifuge Tubes Alternatively, a water bath can be used in place of a heat block	-

*This list may not include some standard laboratory equipment.

Protocol Overview

Slide Reset ~2h



Protocol

This protocol is compatible with:

- Tissue sections that have not been fixed and stained
- Tissue sections that have been methanol fixed, and H&E or immunofluorescence stained

For optimal results, new tissue sections should be placed onto reset slides within a week. If a coverslip was placed on the slide, remove coverslip as outlined in the Visium Demonstrated Protocols (see References) prior to beginning the reset protocol.

Tissue Lysis Buffer:

Preheat a water bath or heat block to **50°C**. Pre-warm the 50-ml centrifuge tube containing **30 ml** Tissue Lysis Buffer and maintain at **50°C**.

0.08 M KOH Mix:

Label two 50-ml centrifuge tubes as Tube 1 and 2. Dispense the following amount of 0.08 M KOH Mix into the tubes and maintain at **room temperature**:

- 30 ml in Tube 1
- 30 ml in Tube 2

Ultrapure Water:

Label two 50-ml centrifuge tubes as Tube 1 and 2. Dispense the following amount of ultrapure water into the tubes and maintain at **room temperature**:

- 45 ml in Tube 1
- 45 ml in Tube 2

When transferring slide, ensure that the active surface of the slide faces up and is never touched. When immersing the slide in a solution, ensure that the slide is completely immersed and each immersion is ~3 sec.

a. Transfer slide to the 50-ml centrifuge tube containing pre-warmed Tissue Lysis Buffer.

b. Incubate for **1 hr** at **50°C**.

c. Slowly invert 50-ml centrifuge tube with Tissue Lysis Buffer 5x. Some tissue may remain on the slide.

d. Remove slide from the 50-ml centrifuge tube. Wipe excess liquid from the back of the slide using a laboratory wipe and without touching the active surface of the slide.

e. Immerse slide 15x in a new 50-ml centrifuge tube containing ultrapure water (Ultrapure Water Tube 1).

f. Remove the slide and wipe excess liquid from the back of the slide using a laboratory wipe and without touching the active surface of the slide.

g. Transfer slide to a 50-ml centrifuge tube containing 0.08 M KOH Mix (KOH Mix Tube 1).

h. Incubate for **10 min** at **room temperature**.

i. Gently invert 50-ml centrifuge tube containing 0.08 M KOH Mix (KOH Mix Tube 1) and slide 10x.

j. Transfer slide to a new 50-ml centrifuge tube containing 0.08 M KOH Mix (KOH Mix Tube 2).

k. Incubate for **5 min** at **room temperature.**

l. Remove slide from KOH Mix Tube 2 and wipe excess liquid from the back of the slide without touching the active surface.

m. Transfer slide to the 50-ml centrifuge tube containing 10 mM Tris, pH 7 and invert slowly 15x.

n. Remove slide and wipe excess liquid from the back of the slide without touching the active surface.

o. Immerse slide 15x in a 50-ml centrifuge tube containing fresh ultrapure water (Ultrapure Water Tube 2).

p. Remove slide from ultrapure water and wipe excess liquid from the back of the slide using a laboratory wipe and without touching the active surface of the slide.

q. Centrifuge for **30 sec** in a slide spinner. Alternatively, place the slide in a 50-ml centrifuge tube and centrifuge at **250 rcf** for **30 sec** in a swinging bucket centrifuge.

r. Visually check the slide for presence of any remaining tissue. If the tissue is not removed completely, reset protocol should be repeated within **7 days**.

s. Slide can be stored in a sealed container in a desiccator at **room temperature** or at **4°C** for up to **7 days** before tissue section placement.

Results

Assay Sensitivity

Resetting Visium Spatial or Gateway Gene Expression slides will likely result in a decrease in the number of unique transcripts detected for many tissue types. Similarly, resetting Visium Spatial Tissue Optimization slides may cause a reduction in cDNA footprint fluorescence intensity for certain tissue types; however, this should be uniform across the entire slide and should not affect determination of optimal permeabilization time.

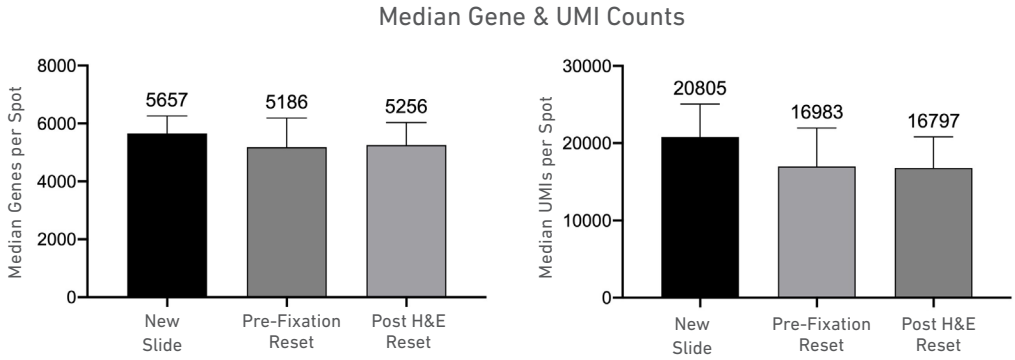


Figure 1. Median gene and UMI counts per spot between new and reset slides. Visium Spatial Gene Expression slides containing human breast tissue sections that were either not fixed or fixed and stained with H&E were reset with mouse brain tissue sections. Values indicate the average of 8 replicates per condition. Median Gene and UMI metrics are based on samples downsampled to 50K raw reads per spot.

Visium Spatial Slide Reset Example

Visium Spatial Gene Expression Slide Reset	
Before Reset	
Original section	Human breast cancer tissue
Protocol performed	Demonstrated Protocol - Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols (Document CG000312)
After Reset	
New section	Mouse brain tissue
Protocol performed	Demonstrated Protocol - Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols (Document CG000312 User Guide - Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)

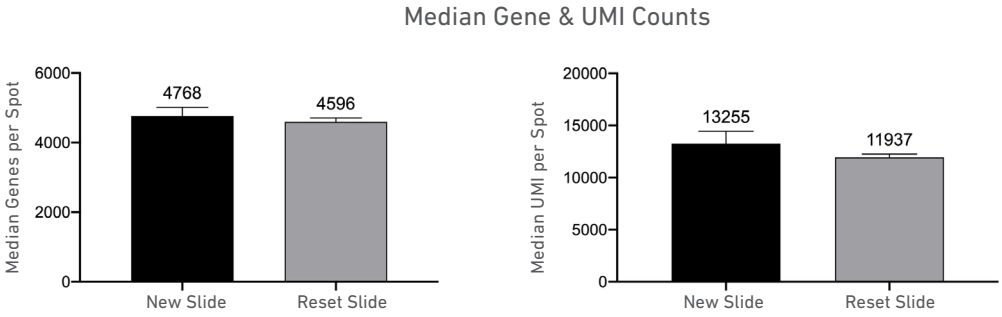


Figure 2. Median gene and UMI counts per spot between new and reset slide. Visium Spatial Gene Expression slides containing immunofluorescence stained human breast tissue sections were reset with mouse brain tissue sections. Values indicate the average of 4 replicates per condition. Median Gene and UMI metrics are based on samples downsampled to 50K raw reads per spot.

Contamination Assessment

Visium Spatial Gene Expression slides containing human breast tissue sections were reset with mouse brain tissue sections, processed through the Visium Spatial Gene Expression workflow, and sequenced. Contamination level was assessed by determining how many transcripts from the original human tissue remained in the reset mouse brain sample.

Percent contamination was subsequently calculated by dividing the number of UMI counts uniquely aligned to the human reference genome (GrCh38) by the number of total UMI counts uniquely aligned to either the mouse (mm10) or human reference genome. UMI counts were calculated across the entire capture area (4992 spots). Values are based on the average of two replicates per condition.

Condition	GrCh38 UMI counts per million total UMIs	Percent Contamination
Pre-Fixation Reset	109.45	0.0109%
Post H&E Reset	123.87	0.0124%

References

- Methanol Fixation, H&E Staining & Imaging for Visium Spatial Protocols (CG000160)
- Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols (CG000312)
- Visium Spatial Gene Expression Reagent Kits User Guide (CG000239)

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