***Visium Total*: spatial total RNA sequencing via in situ polyA-tailing**

Last Edits Made: DWM, 6/29/2021

Materials/Reagents

- Yeast PAP
- 5X Yeast PAP reaction buffer
- 20U/ul SUPERase in RNAse Inhibitor

Buffers

***Yeast PAP Reaction Mix**:*

	Final Conc.	**Volume to add**			
		1 RXN	4.4 RXNs	8.8 RXNs	
5X Yeast PAP reaction buffer	**1X**	15ul	66ul	132ul	
Yeast PAP	**0.25M**	3ul	13.2ul	26.4ul	
25mM ATP	**1%**	1.5ul	6.6ul	13.2ul	
SUPERase	**0.8U/ul**	3ul	13.2ul	26.4ul	
H_2O	**--**	52.5ul	231ul	462ul	
Total Volume	**--**	**75ul**	**330ul**	**660ul**	

***Yeast Reaction Buffer**:*

	Final Conc.	**Volume to add**			
		1 RXN	4.4 RXNs	8.8 RXNs	
5X Yeast PAP reaction buffer	**1X**	50ul			
SUPERase	**0.2U/ul**	2.5ul			
H_2O	**--**	197.5ul			
Total Volume	**--**	**250ul**			

Protocol

1. Section onto Visium slide according to protocols from 10x Genomics.
2. Fix, stain, and image tissue according to Visium protocol ("Step 1: Tissue Staining & Imaging").
3. Prepare appropriate volumes of **Yeast PAP Reaction Mix** and **Yeast Reaction Buffer**.
4. Set the thermocycler to 37°C (Step 2.1.a)
5. Place the slide into the Slide Cassette (See Visium protocol for "Tips and Best Practices").
6. Add **75ul** of **Yeast Reaction Buffer** to each well to equilibrate tissue. Incubate at room temperature for 30sec.
7. Remove and discard buffer from each well.
8. Add **75ul** of **Yeast PAP Enzyme Mix**.
9. Place slide onto the Thermocycler Adaptor and incubate at 37°C for 25min.
10. Remove and discard enzyme mix from each well. Proceed directly to Step 2.1.c to permeabilize tissue, and follow the standard Visium protocol to completion.